An ecological study of waste stabilization ponds in Werribee, Australia, with special reference to nutrient and plankton dynamics

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Patrick Chuen-chi Lai

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Contents

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1. The Werribee Treatment Complex: A combination of environmental conservation and waste management

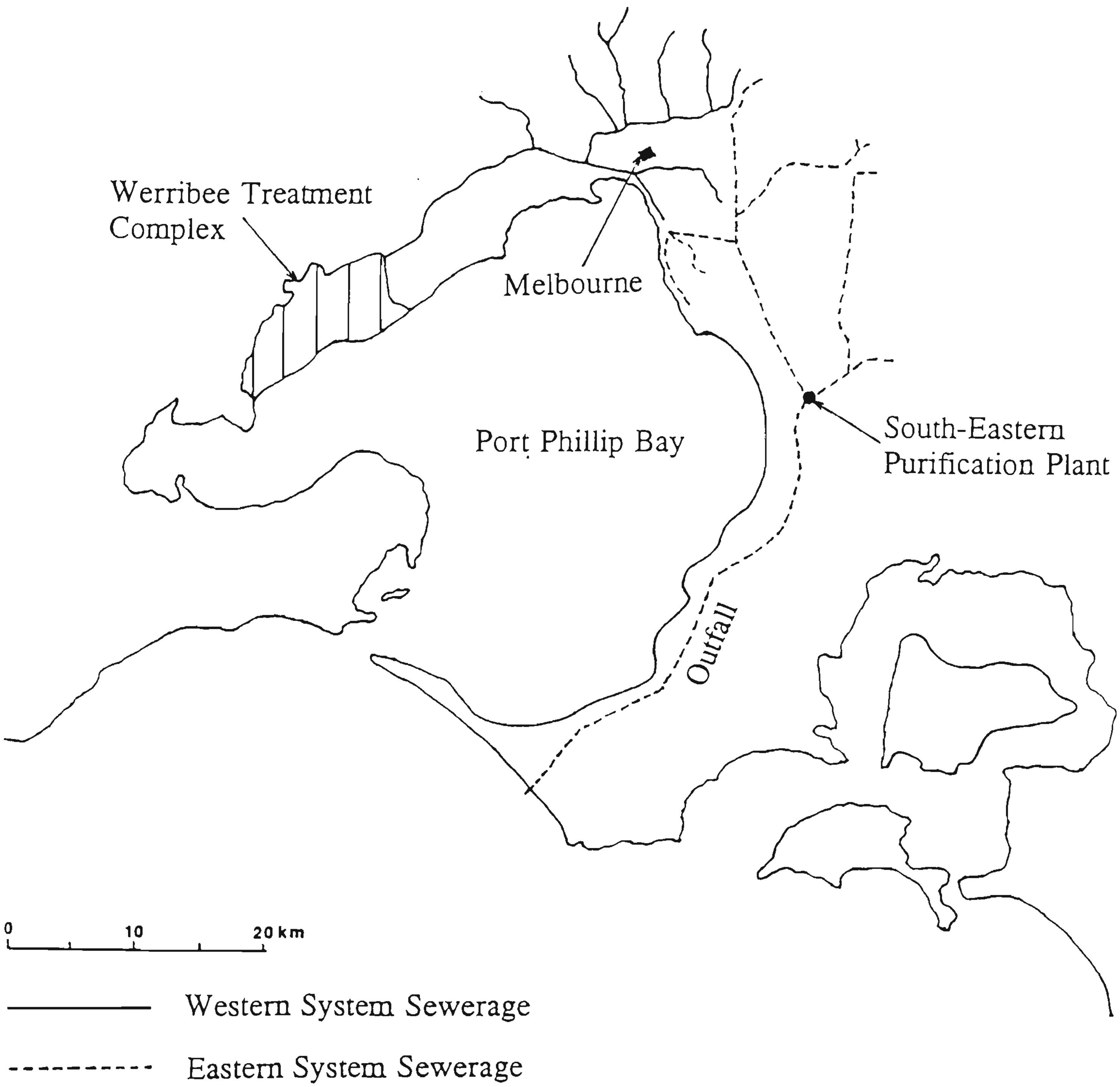
1.1 INTRODUCTION

Proper disposal of domestic sewage is one of the major challenges facing environmental managers in large urban centres. The discharge of untreated or inadequately treated sewage into inland waterways and coastal waters is an important cause of environmental pollution in the receiving systems.

This chapter examines the Werribee Treatment Complex (WTC), a specific waste water treatment facility in Melbourne, Australia. Three treatment methods, namely stabilization pond treatment, land filtration and grass filtration are described in detail. The extensive wetland systems created as a result of the treatment processes provide important habitats for wildlife, resulting in the declaration of the area as a "Wetland of International Importance". Economic benefits derived from livestock grazing, crop cultivation and methane production are also important components of this sustainable development. This chapter hopes to demonstrate how waste management may be combined with environmental conservation.

The Werribee Treatment Complex (37°49'S 144°58'E) is located 35 km south-west of Melbourne covering an area of 108.5 km^2 (Fig. 1.1). The local climate type is marine with mild winters and warm summers, all seasons are moist. The mean maximum and minimum January temperatures are 26 and 13°C respectively, while those recorded in July

Fig. 1.1 Location of the Werribee Treatment Complex and the Sewerage Systems in Melbourne.



Werribee Treatment Complex
Melbourne
Port Phillip Bay
South-Eastern Purification Plant
Outfall
0
10
20 km
Western System Sewerage
Eastern System Sewerage

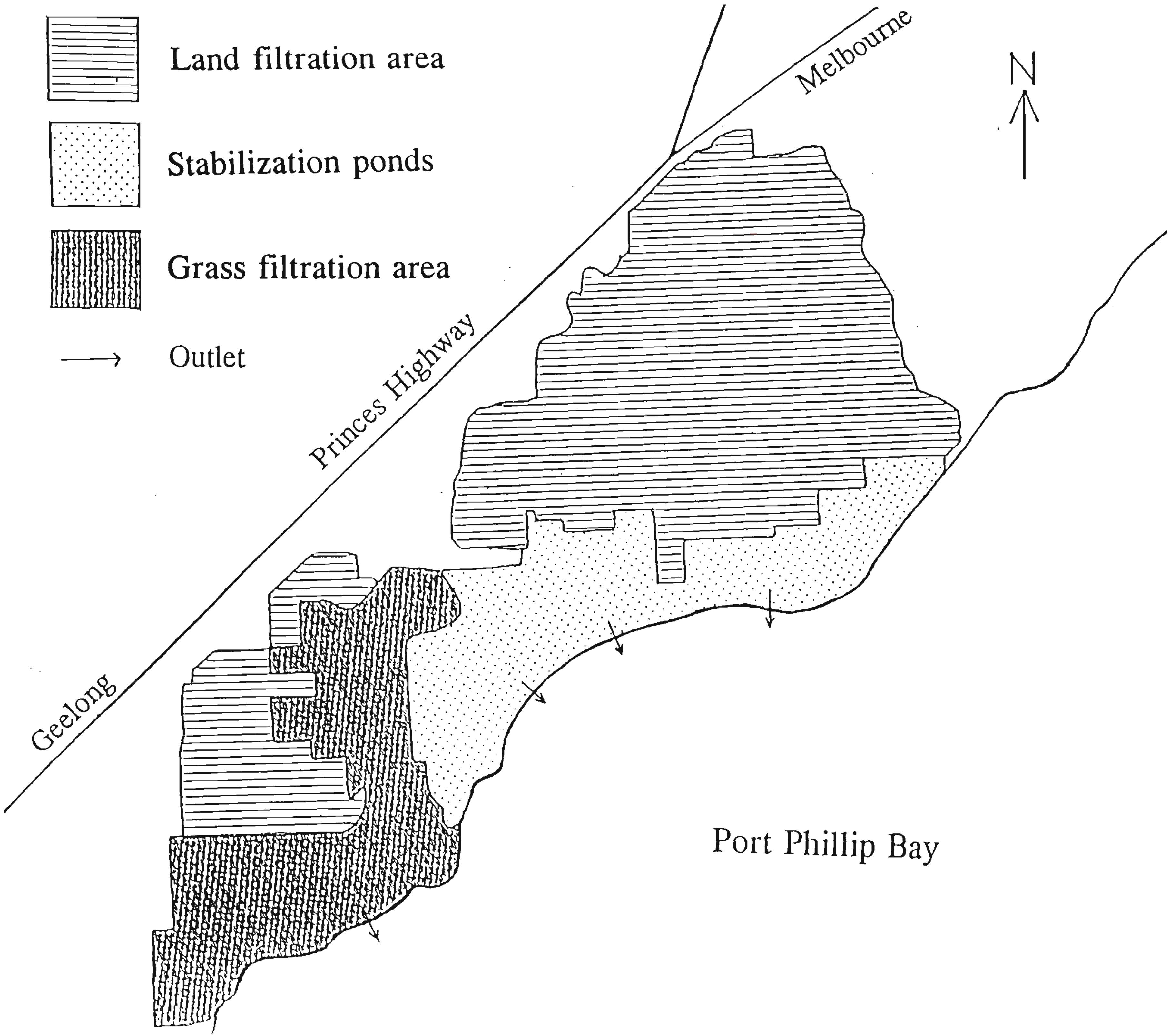
are 14 and 6°C. Two or three times each summer there are hot and dry spells when temperatures reach over 35°C for consecutive days. Annual rainfall totals are approximately 660 mm and usually evenly distributed throughout the year (Linacre & Hobbs, 1977).

The Complex is managed by a semi-government instrumentality, Melbourne Water Corporation, which has responsibilities such as town planning, water supply, management of sewerage and drainage. Apart from these, Melbourne Water is also responsible for the collection, treatment and proper disposal of Melbourne's waste water. The sewerage system in Melbourne is currently divided into two parts namely eastern and western systems (Fig. 1.1). The western system, now serving the central, northern and western suburbs of Melbourne, was established in 1892 and was the first waste water treatment system in Melbourne. To date, the western sewerage system covers an area of about 860 km^2 and delivers approximately 50% of Melbourne's waste water to the Werribee Treatment Complex for purification (Bissett & Pace, 1993). The eastern sewerage system which serves the southern and eastern suburbs was established in 1975, when the Werribee Treatment Complex appeared to be approaching its carrying capacity, to cope with the majority of population growth in the eastern and south-eastern regions. Today, the Werribee Treatment Complex treats the domestic sewage of 1.5 million people and 80% of the industrial waste water in Melbourne. Of the 520 million litres of waste water it handles daily, 80% comes from domestic sources and 20% from industry. Treated waste water is carried by drains and discharged via 4 outlets (+ one additional outlet during winter) to Port Phillip Bay under licence from the Environmental Protection Agency of Victoria.

1.2 DEVELOPMENT OF THE WERRIBEE TREATMENT COMPLEX

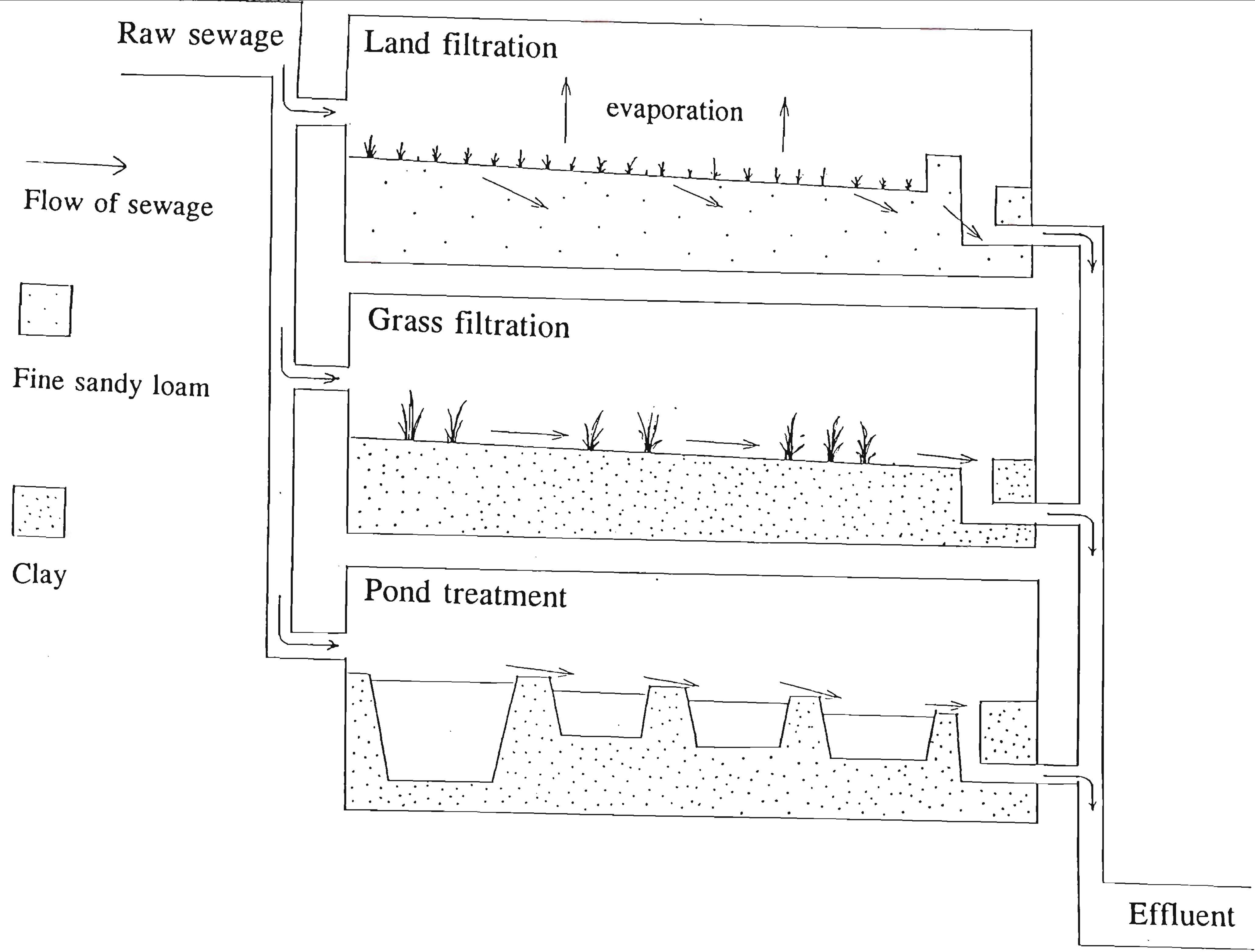
The Werribee Treatment Complex uses three treatment systems: land filtration, grass filtration and stabilization ponds (Fig. 1.2). The use of land treatment systems in Werribee was first proposed in 1890. The first site (35.8 km^2) was chosen for its low rainfall, high evaporation rate as well as moderate permeable soil type (fine sandy loams), which are all essential factors for the successful operation of the land-filtration treatment process. Operation began in 1897 with an area of 5.15 km^2, while the rest was leased to farmers for dairy farming and vegetable cultivation. In 1900, that piece of farm land was converted for waste water irrigation in the wet winter months to reduce the load of land filtration which was less efficient during periods of low evaporation. In the following summer, sheep were acquired to graze on the paddocks of high-quality pasture resulting from irrigation by the nutrient-rich waste water. Cattle and goats were subsequently introduced to the Complex in the following ten years. Subsequent development of the Complex has been achieved through successive acquisition of land and application of new treatment processes to cope with the increasing waste water loading. In 1930, grass filtration commenced on the western side of the complex with heavier clays and loams as a permanent winter treatment facility. Seven years later, stabilization pond treatment was introduced at the lower foreshore area of the complex to handle peak daily and wet weather flows which exceeded the capacity of the land and grass filtration systems (Croxford, 1978). It is important to note that, in all of these treatment processes, efforts are made to ensure that energy input and operational costs are minimized while recycling of useful resources is maximized. Indeed, the Complex, with the largest scale of slow-rate irrigation system in the world (Seabrook, 1975) and stabilization pond treatment in

Fig. 1.2 Physical layout of the three treatment methods in the Werribee Treatment Complex.


Land filtration area
Stabilization ponds
Grass filtration area
Outlet
Melbourne
N
Princes Highway
Geelong
Port Phillip Bay

Australia (Australian Environment Council, 1987), is highly regarded in the development of "environmentally-friendly" land and pond treatment technologies. The operational principles of the three treatment processes are summarized in Fig. 1.3 and described in section 1.3.

Fig. 1.3 Diagrammatic representation of the three treatment methods in the Werribee Treatment Complex.



Raw sewage
Land filtration
evaporation
Grass filtration
Pond treatment
Flow of sewage
Fine sandy loam
Clay
Effluent

1.3 PRINCIPLES OF TREATMENT PROCESSES

1.3.1 Land filtration

Land treatment of waste water was first applied in the sixteenth century and became commonly used in Europe in the nineteenth century. Nowadays, its application in Europe is less common because of the large land requirement. However, it is still widely practised in the United States and Australia (Gray, 1992). The important factors in the design of land treatment systems are land availability, permeability and depth of soil, precipitation level and evapo-transpiration rate (Johnson, 1973).

Basically, the waste water is purified as it passes through soil with moderate permeability. Suspended solids, including micro-organisms are filtered out physically by the top layers of the soil. Organic matter trapped in the soil is then metabolised by a wide range of soil heterotrophs such as fungi, bacteria and actinomycete. Nutrients released by the decomposition process are subsequently taken up by the plants which have to be harvested regularly to maintain a high nutrient-removal efficiency (Gray, 1992). It is also claimed that the humus, silt and clay particles can provide a large adsorption surface for heavy metals and inorganic nutrients, thus removing them from the waste water (Gray, 1992). Significantly, about half of the waste water is lost to the atmosphere through evapo-transpiration, while the rest is collected by sub-surface drains.

Land filtration in WTC is operated during the summer months (October to April) when the evaporation rate is high (Croxford, 1978). The treatment process involves flooding the paddocks with waste water to a depth of 10 cm for one to two days. The paddocks are surrounded by elevated banks to prevent the overflow of untreated sewage directly into nearby drains. The land is then allowed to dry for five days, followed by a 14-day grazing period by livestock to remove the rapidly-growing pasture. Around 10 to 11 rotations can be achieved each season. Dominant pasture species found in the paddock areas include Prairie Grass (*Bromus catharticus*) and Sea Barley-grass (*Hordeum marinum*) on the rides and perennial Rye Grass (*Lolium perenne*), White Clover (*Trifolium repens*) and Water Couch (*Paspalum distichum*) in the wetter areas. Weed is usually not common in these areas (Cropper & Calder, 1987).

Land filtration occupies the largest proportion of land amongst the three treatment processes but treats only 15% of the total amount of waste water annually. Although land filtration is considered to be the least efficient treatment method, the quality of the effluent is the best among the treatment processes.

1.3.2 Grass filtration

Grass filtration (also known as overland flow) refers to the treatment of waste water as it flows across the land by gravity (Dinges, 1982). It is similar to land filtration except that the waste water flows across the land surface rather than percolates through the soil. Consequently, a specific site with relatively low permeability, such as areas with underlying clay or clay loam soil, has to be chosen (Gray, 1992). The land should be prepared to provide a slope between two to six degrees. Suitable grass species should also be planted as purification is achieved by the filtering and microbial actions as the waste water flows through the vegetation. During the purification process, suspended solids are settled and retained at the top end of the irrigation bay where anaerobic conditions are created for initial degradation. The organic matter is decomposed biologically by an active film of micro-organisms built up on the stems of the vegetation. Nutrients released and accumulated in the system are taken up by the plants, which have to be removed regularly to maintain optimal operational conditions. Heavy metals are precipitated as sulphides in the anaerobic regions.

Grass filtration in WTC is used from May to September when land filtration is impracticable because of low evaporation rates. Although Rye grasses (*Lolium* spp.) are grown in these filtration areas, weed is a serious problem in many paddocks (Cropper & Clader, 1987). Rye grasses are chosen because they grow in a slender erect form rather than tufts, and provide better waste water seeping (Croxford, 1978). Furthermore, these grasses can grow under prolonged wet conditions. Prior to passing the waste water through the grass beds, preliminary sedimentation is carried out to prevent clogging. An average of 36 to 48 hours are required for the waste water to travel through the filtration area and the effluent is collected in open drains and discharged to Port Phillip Bay. At the end of September, the grass filtration area is let dry and cattle are allowed onto the paddocks to graze on the grasses after the grasses have shed their seeds. Seeds left in the soil are important for the regeneration of the grass beds at the first irrigation in the following season. The establishment of the filtration areas in the next season is carried out in early autumn. Seed germination is first promoted by one or two normal irrigation bouts. Then, a small quantity of sedimented waste water is allowed to flow through the vegetation to allow the building up of the bacterial film. After two to three weeks, the flow rate can be increased to the maximum and maintained until the end of the season.

1.3.3 Stabilization pond treatment

Porges and Mackenthun (1963) defined a waste stabilization pond as a basin, natural or artificial, designed or used to treat organic wastes by natural biological, biochemical and physical processes, commonly referred to as self-purification. Pond application as a means of waste water treatment was discovered accidentally in ponds formed by the clogging of a prepared gravel seepage in California in 1924 (Pearse *et al.*, 1948). Following that, the use of waste stabilization ponds as well as research into the chemical, physical and biological aspects of these systems have been intensely and rapidly developed (Rohlich, 1976). Nowadays, they are recognised as a major primary, secondary and tertiary treatment process, and are used throughout the world, serving populations ranging from 1,000 to 1,000,000 (Gloyna, 1971).

Many different terms and names have been applied to the man-made artificial basins in which organic wastes are treated by naturally occurring biological and physical processes. These names included sewage lagoons, oxidations ponds, as well as waste stabilization ponds. Originally, a sewage lagoon refers to one which receives raw sewage and an oxidation pond receives partially treated sewage, while waste stabilization pond is an exclusive term which describes all types of ponds or lagoons for biological waste water treatment (Gloyna, 1971; Benefield & Randall, 1980).

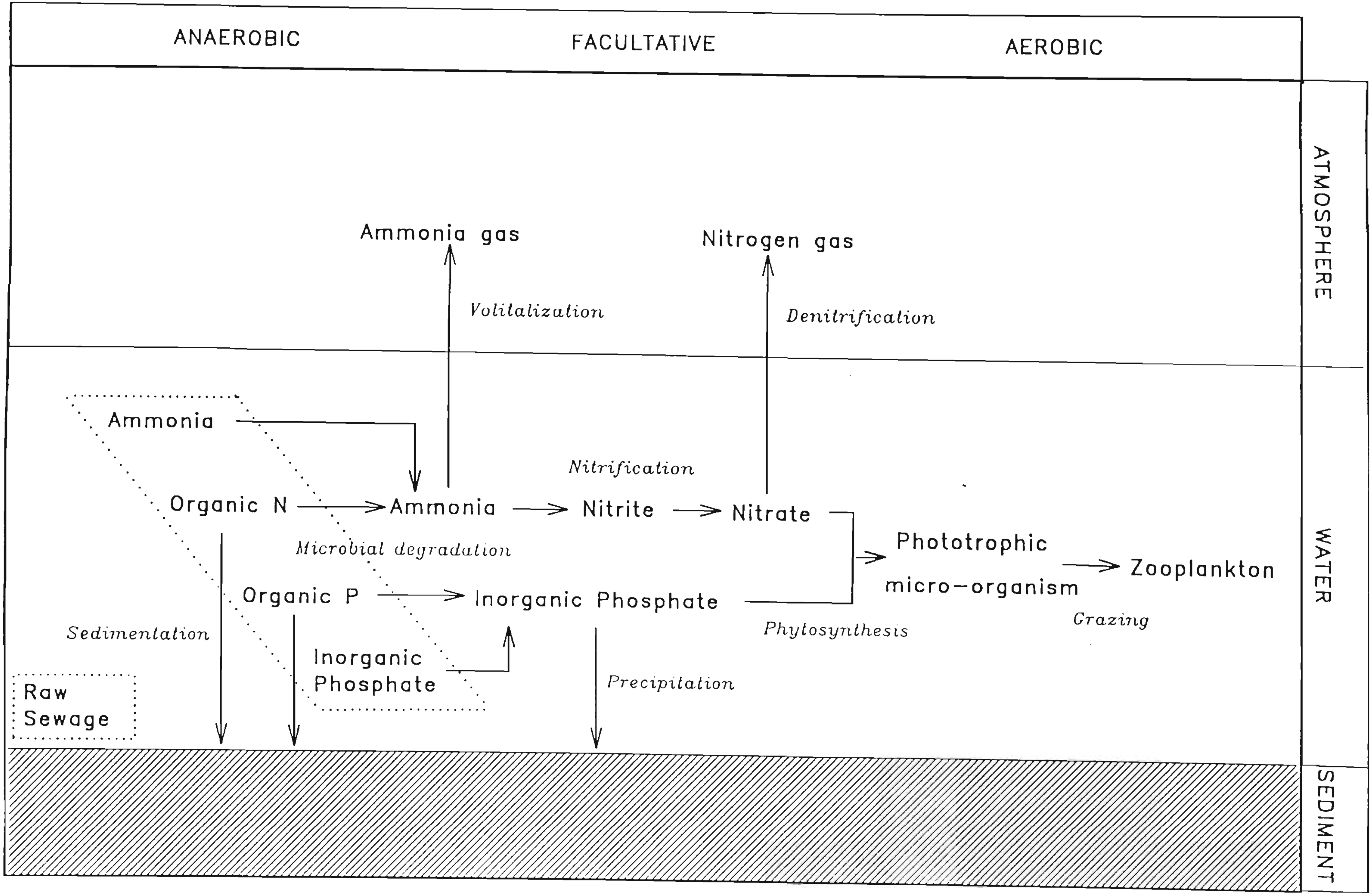
Stabilization ponds can be divided functionally into anaerobic, facultative and aerobic sections with respect to their oxygen content (Metcalf & Eddy, 1991). Moreover, they can also be classified by the stages of the treatment process. A primary stabilization pond is one which receives raw sewage while a secondary pond receives primarily treated effluent from sedimentation ponds. A maturation (or polish) pond is at the last stage of pond treatment and is designed to improve the effluent quality by reducing the levels of nutrients, suspended solids and pathogenic micro-organisms. It is a common practice to treat waste water by passing it through a number of ponds connected in a series (Horan, 1990). Due to the high organic loading of untreated sewage, the first few ponds are always anaerobic throughout the entire water column. The purification mechanisms occur in these anaerobic ponds involve sedimentation and anaerobic bacterial breakdown of organic matter into methane, carbon dioxide and organic acids. The proper functioning of the anaerobic ponds depends on the development of a bottom sludge layer and a upper scum layer which provide an active biological film and a heat insulation layer respectively (Gray, 1992). Failure in scum layer formation usually leads to serious odour problems or low efficiency due to heat loss.

The organic loading gradually decreases as the waste water passes through the succeeding facultative ponds which are characterized by an anaerobic bottom layer and an aerobic upper layer. In the facultative ponds, the settleable organic matter is degraded anaerobically at the bottom while the soluble fraction is broken down aerobically. Nutrients and carbon dioxide released from the decomposition processes are then consumed by the algae which produce oxygen via photosynthesis. The oxygen is utilized by the aerobic bacteria for further decomposition of the organic matter, thereby completing the "symbiotic cycle" (Gray, 1992). The last section of the series is aerobic which mainly functions as polishing ponds. At this stage, the algal biomass is often significantly reduced as a result of grazing by zooplankton such as copepods and cladocerans.

Nutrients can also be significantly reduced by the pond treatment. The removal mechanisms of the key-nutrients in stabilization ponds are summarized in Fig. 1.4. Nitrogenous waste in raw sewage is mainly composed of human excreta and proteins. The organic nitrogen is decomposed by the bacteria into ammonia, which is either taken up by phytoplankton (Ferrara & Avci, 1982) or lost to the atmosphere in form of ammonia gas as a result of the elevated pH due to increased photosynthetic activities (Pano & Middlebrooks, 1982). Ammonia can also be removed by nitrification during which ammonia is reduced to nitrite and then nitrate by nitrifying bacteria in aerobic condition (Painter, 1977). Nitrate is then denitrified to nitrogen gas at the sediment-water interface or absorbed into algal cells. Phosphorus in raw sewage presents mainly in the form of inorganic phosphate which comes from soaps and detergents. A small amount of phosphorus exists in organic forms. Organic phosphorus is converted to inorganic phosphate which is then precipitated to the sediment in form of a complex with calcium and hydroxide ions at high pH. Some phosphate is also lost through cell uptake (Toms *et al.*, 1975).

Waste stabilization ponds are predominantly used to serve small populations in rural areas (Gray, 1992). However, in Melbourne, a metropolitan city with a population of over three million, they are used as a major waste water treatment method. The pond series handle about 60% of the total volume of sewage distributed to WTC annually. The

Fig. 1.4 Environmental fate of the key nutrients in the waste stabilization ponds.


ANAEROBIC
FACULTATIVE
AEROBIC
ATMOSPHERE
WATER
SEDIMENT
Ammonia gas
Nitrogen gas
Volitalization
Denitrification
Ammonia
Nitrification
Organic N
Ammonia
Nitrite
Nitrate
Phototrophic micro-organism
Zooplankton
Microbial degradation
Organic P
Inorganic Phosphate
Sedimentation
Phytosynthesis
Grazing
Inorganic Phosphate
Precipitation
Raw Sewage

pond treatment process in WTC is operated throughout the year to handle the peak daily flow and the wet weather flow. Each pond system consists of 8 to 12 ponds connected in series, of which the first pond is anaerobic. The anaerobic pond is followed by a few facultative ponds and the effluent is polished in the last few maturation ponds before being discharged to Port Phillip Bay. Each pond has an area of between 40,000 to 80,000 m^2. The depth of the ponds is approximately 1 m, except for the first pond of each series which is usually 2 m deep to allow for the sedimentation of raw sewage. The detention time for each pond series is between 50 to 80 days (Bissett & Pace, 1993). The operational characteristics of various treatment processes in WTC are summarized in Table 1.1.

Table 1.1 Summary of treatment processes in Werribee (91-92) (Bissett and Pace 1993)

Treatment	Operating season	% of sewage treated	Annual flow (million litre)	Area (km^2)
Stabilization ponds	all year	60%	109169	16.54
Grass filtration	winter	25%	46438	14.43
Land filtration	summer	15%	28321	32.39

1.4 ECONOMIC BENEFITS FROM THE TREATMENT OPERATIONS

1.4.1 Livestock production

Raw sewage contains high amounts of nitrogen and phosphorus which are essential for plant growth. In the Werribee Treatment Complex, the raw sewage has an average of 56 mg/L of total nitrogen and 9 mg/L of total phosphorus (Hussainy, 1979). Once the organic sewage is degraded by micro-organisms, the inorganic nutrients are released in forms suitable for the uptake by plants. These nutrients are essential in supporting high primary productivity in the systems. Fast-growing, high-quality pasture in the land and grass filtration areas provides excellent feed for the livestock which convert the primary production into secondary production without any significant extra energy input into the system.

In 1991-92, there were 13,879 cattle, 14,138 sheep and 873 goats in the Complex (Bissett & Pace, 1993). Sheep are purchased in the spring and summer, and are sold in the autumn after being fattened. Cattle are sold and replaced by the new calves that are bred naturally in the Complex. The main cattle herds are Herefords and Aberdeen Angus while the sheep are composed of Border Leicester Cross, Corriedale and Corriedale Cross (Croxford, 1978). These breeds are chosen because they are all hardy and less susceptible to foot rot. Lice control and immunization are also practised in the Complex. Moreover, livestock are subjected to routine test for parasites and possible toxic contaminants such as pesticides and heavy metals before they are sold to the market. The income from selling of the livestock alone was A$3.0 million in 1991-92, which covered

20% of the total expenditure of the whole operation at WTC (Bissett & Pace, 1993). It is recognised that the carrying capacity of the Complex in terms of livestock grazing has not been reached, further development is still possible.

1.4.2 Methane production

Methane (CH_4) is produced during the process of anaerobic degradation of organic matter at the anaerobic ponds. By constructing a flexible plastic cover on top of the anaerobic ponds, methane gas can be collected. The methane produced can be used to generate electricity to run the aerators to expand the aerobic sections of the pond series or sell to the State Electricity Commission of Victoria. In addition to methane collection, covering the anaerobic ponds can also control the emission of odorous and greenhouse gases, and retain the heat produced by the degradation processes to increase the rate of microbial action. At the moment, methane collection is only at an experimental stage, it is applied on the newly constructed pond systems which have larger surface areas. In 1991-92, 14000-16000 m^3 of methane was produced per day (Bissett & Pace, 1993).

1.5 CONSERVATIONAL VALUES OF THE COMPLEX - A WETLAND OF INTERNATIONAL IMPORTANCE

The wide range of habitats found in and around the Werribee Treatment Complex, including artificial stabilization ponds, grass and land filtration paddocks as well as natural salt marshes, coastal wetlands, beaches and spits, offer an important sanctuary to a diversity of wildlife. The prohibition of hunting and the constant water supply also render the wetlands a valuable drought and hunting refuge for waterfowl in Victoria. Moreover, the constant input of nutrients and energy sources from the sewage treatment processes also helps to maintain the productivity of the system.

In addition to providing grazing, the grass and land filtration areas also support a wide range of organisms because of the high levels of primary productivity resulting from the nutrient-rich irrigant. In particular, these areas provide an important feeding ground for wildfowl which often forage and rest in nearby stabilization pond areas. Moreover, the large populations of insects supported by the system in turn attract a great number of insectivorous birds.

Theoretically, as the sewage progresses through the stabilization pond series, the ammonia content is reduced while the levels of dissolved oxygen and inorganic nutrients increased (Gray, 1992). This enables the bacteria to be replaced by the algae, which are then consumed by zooplankton. This section of the pond area should provide favourable habitats for many waterbirds. Herbivorous birds typically feed on the vegetation and the macrophytic algae along the pond banks. Filter-feeding ducks appear in thousands to graze on the zooplankton in the ponds. Diving ducks which feed on the benthic invertebrates such as insect larvae and worms are also common (Lane & Peake, 1990). Significantly, fish and other aquatic vertebrate are not present in the ponds (Lewis, 1987).

In 1983, the International Union for the Conservation of Nature (IUCN) designated the Complex as a "Wetland of International Importance" under the Rasmar Convention (Lane & Peake, 1990). Moreover, due to its significant location for migratory birds in the southern hemisphere, two bilateral treaties, namely Japan-Australia Migratory Bird Agreement and China-Australia Migratory Bird Agreement, were signed in 1975 and 1984 respectively to protect the migratory bird species and their habitats. It became increasingly apparent that the management strategies of the Complex required a comprehensive input of environmental and conservational aims in addition to the waste water management objectives. In 1985, the Melbourne and Metropolitan Werribee Treatment Complex Wildlife Consultative Committee was formed to consider these issues.

1.5.1 Avifauna

The Werribee Sewage Treatment Complex is internationally famous for its birdlife of which 252 species have been recorded (Cropper, 1987). Permanent water supply, abundant food sources resulting from the sewage treatment processes, roosting and nesting sites provided by the dead trees in the ponds and reduced human disturbance, are all important factors that have attracted numerous bird species to inhabit the Complex. Among these birds, some are of particular importance. For example, the Complex supports up to 50% of the total population of the Orange-bellied Parrot which is one of the most threatened bird species in Australia (Kennedy, 1990). Significant numbers of waders (18,000 to 32,000), of which 75% are migratory, also visit the Complex. They feed on the intertidal mudflats along the foreshore at low tides and roost in the inland areas. A copious quantity of invertebrates is available at the foreshore area because of the nutrient enrichment from the effluent. Several rare species such as Cox's Sandpiper (*Calidris paramelanotus*), Asian Dowitcher (*Limnodromus semipalmatus*) and Buff-breasted Sandpiper (*Tryngites subruficollis*) have been recorded at the Complex, while the most common species is the Red-necked Stint. Waterfowl such as swans and ducks are common all over the stabilization pond areas. Cropper (1987) reported that the pond areas can support up to 65,000 individuals, and the common species are Australian Shelduck, Grey Teal, Chestnut Teal, Pink-eared Duck, Australasian Shoveler and Black Swan. Some rare and vulnerable species such as Fairy Tern (*Sterna nereis*) and Lewin's Water Rail (*Rallus pectoralis*) are also present in the Complex. A list of common bird species is given in Appendix 1. A complete species list can be found in Cropper (1987).

1.5.2 Mammals

Schulz (1987) identified a total of 24 species of mammals in the Complex, three of which are of state or regional significance. The small mouse-like nocturnal insectivorous marsupial, Fat-tailed Dunnart (*Sminthopsis crassicaudata*) is found in the dry grazing paddocks in the Complex. They utilize the surface basalt rocks and cracks in the soil for shelter, nesting and foraging. Along the banks of the drain network that carries purified effluent, Water Rats (*Hydromys chrysogaster*) burrow and feed on aquatic insects and crustaceans. The world famous Platypus (*Ornithorhynchus anatinus*) also inhabits the Werribee River at the eastern boundary of the Complex. Unfortunately, introduced species such as feral cats, black rats, rabbits and foxes are extremely abundant in the Complex, posing a serious threat to the survival of many native species.

1.5.3 Reptiles and amphibians

At least 17 species of reptiles occur in the Complex, of which 11 species are lizards, five snakes and one tortoise (Lane & Peake, 1990). One of Australia's rarest and most vulnerable grassland inhabitants, the Striped Legless Lizard (*Delma impar*), has been recorded in the dry grassland areas. However, the frequent inundation of grasslands during the sewage treatment operations would probably render the paddocks unsuitable for the lizards.

Seven species of frogs have been recorded in the swampy areas and water courses in the WTC (Schulz, 1987). Two of them, namely the Green and Golden Bell Frog (*Litoria rainformis*) and Spotted Burrowing Frog (*Neobatrachus sudelli*), are of regional importance.

1.5.4 Flora

At present, the most significant conservation value of the diverse plant communities in the Complex is their provision of a wide range of microhabitats to different wildlife (Cropper & Calder, 1987). Nevertheless, several rare and vulnerable plant species have been identified. One of them is a low saltmarsh plant, the Grey Glasswort (*Halosarcia halocnemoides*), which is the critical food plant for the endangered Orange-bellied Parrots. The endangered Little Dumpy Orchid (*Pterostylis truncata*) occurs exclusively in the dry grazing paddocks. Patches of Kangaroo Grass (*Themeda triandra*) which remain on the Complex represent the very few isolated remnants of the native grasslands in Victoria.

1.6 FUTURE PROSPECTS OF THE COMPLEX

1.6.1 Upgrading treatment processes

At present the most pressing task of the Complex is to upgrade and further develop the treatment processes to cope with the increasing sewage loading. Recent development in the land and grass filtration areas has involved laser grading to improve the gradient of the bay and the sewage flows (Lane & Peake, 1990). Larger and deeper stabilization ponds are constructed and mechanical aeration devices introduced in the anaerobic ponds to expand the proportion of aerobic sections in the series. Emergent aquatic macrophytes have also been planted along the banks of these new ponds to improve the habitat quality for birds. Significantly, small islands have also been constructed in the middle of these ponds to provide the birds with a habitat protected from the disturbances of predators such as foxes and feral cats. Future options for upgrading the system, including the incorporation of activated sludge treatment prior to discharging, conversion of the grass and land filtration areas to treatment ponds, have also been considered (Lane & Peake, 1990). Notwithstanding the importance of improving the waste water treatment capacities of WTC, it is acknowledged that the impacts of any augmentation and/or refinement of the systems on local wildlife have to be assessed carefully to ensure that the conservation goals are not undermined.

1.6.2 Tree plantation

Four species of eucalypt (*Eucalyptus* spp.), She-oak (*Casuarina* spp.), poplar

(*Omalanthus* spp.) and pine trees (*Callitris* spp.) have been selected for planting as a commercial forestry trail in the Complex using sedimented waste water as irrigant (Bissett & Pace, 1993). It is believed that tree plantations will take up a much larger amount of water per unit area as compared with the pasture in the land filtration areas. Recent studies show that trees can grow from 10 cm to over 4 m in two years, with the Mahogany Gum (*Eucalyptus robusta*) and Flooded Gum (*Eucalyptus grandis*) showing exceptionally fast growth.

1.6.3 Harvesting of zooplankton

A company (Zootech) from Tasmania has been successful in harvesting the zooplankton mainly rotifers, copepods (e.g. *Mesocyclops australiensis*) and cladocerans (e.g. *Daphnia carinata*) from the aerobic ponds, and marketing them as feed for aquarium fish. Further work is being carried out to investigate the possibility of formulating species-specific feed for native fish [e.g. Golden Perch (*Macquaria ambigua*) and Silver Perch (*Bidyanus bidyanus*)] by altering the relative proportion of different types of zooplankton in the manufacturing process. This technology is currently being transferred to other countries including Indonesia and Kenya (B. Quin, personal communication). It is worth noting that the removal of zooplankton from stabilization ponds is likely to be beneficial to the system as nutrients and total suspended solids will be removed in the process. However, the long-term impacts of this practice on the treatment processes and the ecological effects on the local biota in the Complex and in Port Phillip Bay have to be examined carefully.

1.6.4 Reuse of treated waste water for agriculture

Agricultural experiments using treated waste water from the stabilization ponds have been conducted in WTC. Test species include a variety of cereal, oilseed, summer grain and forage crops with sunflowers and winter sown barley showing encouraging results. The possibility of using chlorinated secondary treated effluent as an alternative water source for irrigation is currently being explored.

1.6.5 Gas collection

Methane gas collection has been carried out in the anaerobic ponds of the new pond series. The feasibility and cost justification of this exercise is currently under review. It is clear, however, that the emission of odorous and greenhouse gases can be reduced significantly.

1.7 CONCLUDING REMARKS

The Werribee Treatment Complex in Melbourne, Australia, is an example of a sustainable development which is technologically efficient, economically viable and environmentally acceptable. Such a development could well be a model for some less developed nations in regard to sewage treatment and disposal as it does not require as many advanced technological facilities, well-trained personnel and energy inputs as other treatment methods (Lai & Lam, in press). Indeed, this system should be particularly appropriate for fast developing countries where natural habitats have largely been destroyed, so that artificial wetlands of this type can be used for the conservation of natural wildlife. Clearly, a good understanding of the prevailing physical conditions and cultural characteristics of the local communities is crucial to its success. In particular, a number of key elements are essential:

1) Availability of land with lower priority to urban development.

2) Site with an appropriate topography, soil characteristics and a buffer zone to the urban development.

3) Good urban planning so that sewerage systems can be established and treatment facilities set in place prior to major urban developments.

4) Availability of local biota that are suitable for use in treatment processes (e.g. grass species in the grass and land filtration systems)

5) Good understanding of the characteristics of the nature and volume of sewage loading in the area.

6) Regulations of proper disposal of hazardous materials should be set up and strictly enforced as illegal discharges of toxicants into the system may render the system inoperable and/or cause contamination of the local biota and livestock.

7) Acceptability of local people to consuming crops and livestock that have been "exposed" to sewage.

8) Compatibility with the environmental and conservation aims of local governments.

2. Performance of waste stabilization ponds (145W) in Werribee Treatment Complex with particular reference to nutrient removal

2.1 INTRODUCTION

Previously, biochemical oxygen demand, suspended solids and faecal coliform bacterial count have commonly been used as criteria for assessing the efficiency of sewage treatment facilities, while nutrient parameters have largely been overlooked (Toms *et al.*, 1975; Hussainy, 1979). Recently, more attention has been paid to the eutrophication of receiving water bodies which is mainly caused by nutrient enrichment from the effluent of waste treatment facilities. To date, nutrient parameters have become an integral part of effluent discharge guildlines set by environmental protection agencies.

Although waste stabilization ponds have been widely used over the world and proved to be an economical way of sewage treatment (Gloyna, 1971), waste stabilization ponds often do not have special design configurations for nutrient removal. Furthermore, the biological and chemical mechanisms involved in waste stabilization ponds have not been well studied as compared with other conventional sewage treatment processes such as activated sludge systems. In particular, most studies concerned with nutrient removal in waste stabilization ponds adopt an engineering approach. The processes are often modelled mathematically while individual biological and chemical mechanisms have largely been ignored (e.g. Ferrara & Harleman, 1980). This sometimes led to different conclusions even though the same set of data was used. For example, there is no consensus with regard to the detailed mechanisms of nitrogen removal among studies on the same waste stabilization ponds in

United States (DiGiano, 1982; Ferrara & Avci, 1982; Pano & Middlebrooks, 1982). Moreover, most studies on phosphorus removal have focussed mainly on removal efficiencies (by comparing the amount of phosphorus in the influent and effluent), rather than the actual biological and chemical mechanisms involved (e.g. Narasiah & Morasse, 1984; Lakshmi & Pitchai, 1991; Kandadai, 1992).

In this chapter, I will describe the nutrient dynamics of a series of waste stabilization ponds at Werribee, and investigate the possible nutrient-removal mechanisms in the pond system. Various factors that may influence removal efficiencies, as well as the role of phytoplankton and zooplankton in the treatment processes are also studied.

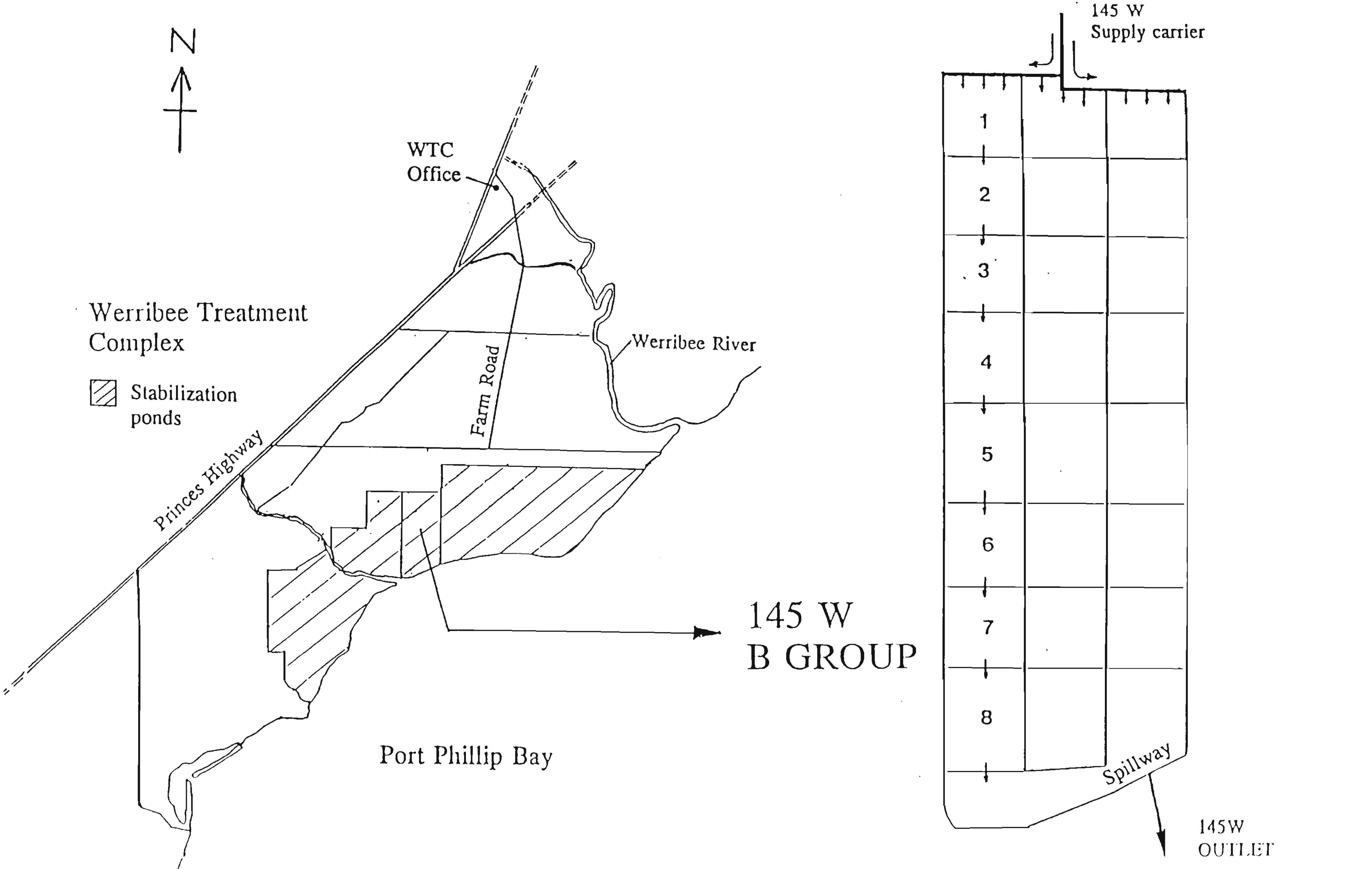
2.2 STUDY AREA

2.2.1 General description

The study was carried out in a series of waste stabilization ponds (145W Group B) at the Werribee Treatment Complex from January 1993 to March 1994 (refer to chapter 1 for a detailed description of the complex). The system consists of three series of ponds in a parallel setting (Fig. 2.1). The series basically function separately except that they are inter-connected by drainage structures for emergency in case of overloading. One of the series was chosen for this study and the study ponds were named pond 1 to pond 8 here (and in the following chapters) where pond 1 refers to the first pond that receives the raw sewage from the carrier and pond 8 is the last pond of the series where treated sewage is discharged into Port Phillip Bay via an outlet.

The ponds are all roughly square in shape with the banks lined with stones. The ponds vary in size with areas ranging from 43800 to 62100 m^2. The depth of the ponds is around 1 m except for pond 1 which is 2 m deep to allow for the sedimentation of sewage. The water in pond 1 is usually black in colour. Subsequent ponds are usually brown in colour due to the high density of the photosynthetic sulphur bacteria (Gray, 1992), and are followed by ponds with high phytoplankton biomass which are green in colour. Water in the ponds at the end of the series is usually clearer as a result of reduced phytoplankton abundance due to zooplankton grazing. The area is directly exposed to Port Phillip Bay and often windy.

Fig. 2.1 Location and physical layout of the study pond system at the Werribee Treatment Complex.


N
Werribee Treatment Complex
Stabilization ponds
WTC Office
Farm Road
Werribee River
Princes Highway
Port Phillip Bay
145 W B GROUP
145 W Supply carrier
1
2
3
4
5
6
7
8
Spillway
145W OUTLET

2.2.2 Operational characteristics

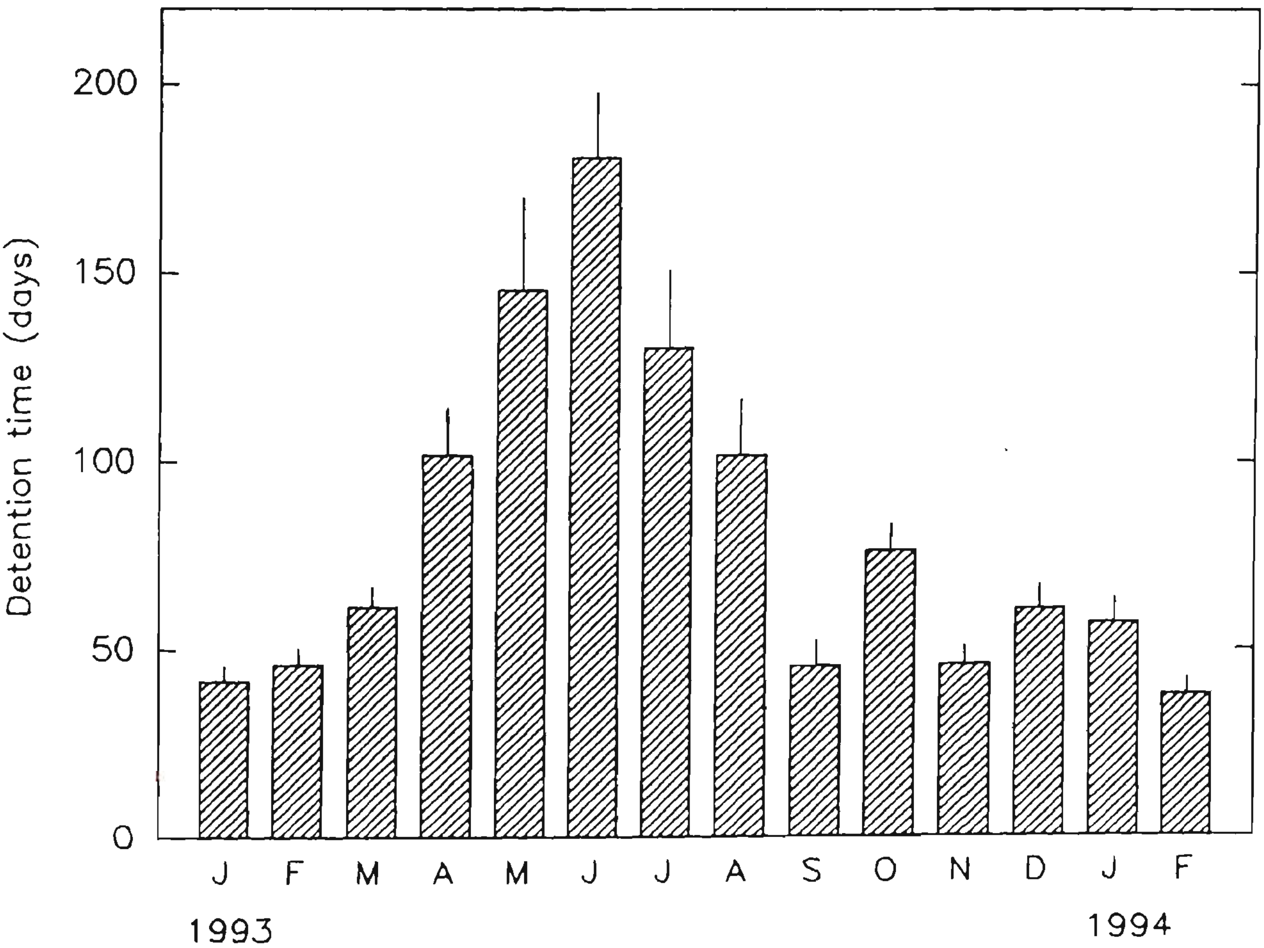
The system receives unsedimented raw sewage after primary screening. Sewage passes through the pond series by gravitation; sewage fills up the first pond and then enters the second pond through a number of outflows. The outflow of pond 1 is equipped with scum boards which are used to screen out the scum and large particles. Raw sewage is usually fed to the lagoons during the night. This time lag from the peak daily usage of water is due to the distance between the complex and Metropolitan Melbourne.

The large variations in the volume of sewage distributed to 145W lagoon series resulted in a marked variation in the detention time of the system during the study period. Fig. 2.2 shows the average monthly detention time of the 145W stabilization pond system based on the total volume of the 145W system divided by the daily flow rate of sewage delivered to the system. It is clear that the detention time was much longer in the late autumn and winter than in the summer during the study period. As the sewage flow is only regulated by gravity, different series may not always receive the same volume of sewage. Consequently, there may be minor differences between the detention time of individual pond series and the average detention time of the 145W series presented in Fig. 2.2. Nevertheless they should share the same general temporal pattern.

2.2.3 Biological characteristics

A general description of the flora and fauna in the Werribee Treatment Complex has been given in Lai and Lam (In press) and chapter 1. In the study site, the dominant

Fig. 2.2 Monthly detention time of the 145W stabilization pond series of the Werribee Treatment Complex over the period January 93 to February 94. Vertical lines are 1 S.E..


Detention time (days)
200
150
100
50
0
J
F
M
A
M
J
J
A
S
O
N
D
J
F
1993
1994

vegetation along the banks of the ponds are Sea Club-rush [*Bolboschoenus caldwellii* (V. Cook) Sojak] and Canary-grass (*Phalaris aquatica* L.), while trees and shrubs are absent. Phytoplankton abundances increase significantly in pond 2 and/or pond 3 and vary in the later section (refer to later sections and chapter 3 for details of phytoplankton dynamics and composition). Preliminary studies on the water samples collected at the banks showed that zooplankton including cladocerans, copepods and rotifers are mainly restricted to the last few ponds. Notwithstanding, blooming of rotifers had been observed in pond 4 on one occasion in October 93. Dynamics of zooplankton were studied in pond 7 and pond 8, and will be discussed in chapters 3 and 4. There is no aquatic vertebrate in the ponds, however, waterfowl are abundant (refer to chapter 5 for details of the avifaunal dynamics). Rabbits, foxes and snakes have been commonly sighted in the study site. Cattle were sometimes allowed to graze in the pond area as a means of biological weed control.

2.3 MATERIALS AND METHODS

2.3.1 Sample collection

Samples were taken monthly between 1000 and 1400 hrs over the period from March 1993 to January 1994 in the stabilization pond series under investigation (see section 2.2) at Werribee. Water samples were collected in the middle of each of the four banks of pond 1 through pond 8. Samples were chilled with an ice-bath while being transported to the laboratory, and kept at 4 °C for subsequent analyses. During each field visit, dissolved oxygen and temperature of the surface water were monitored at each sampling point using a Dissolved Oxygen Meter (YSI Model 58). Preliminary monitoring of temperatures and dissolved oxygen contents at different depths revealed no obvious stratification in the ponds throughout the year. This may be attributed to the shallowness of the pond and the often windy conditions prevailing at the site which could have enhanced vertical mixing. Monthly maximum and minimum water temperatures (at 30 cm depth) were recorded at pond 8 with a submerged maximum/minimum thermometer. Two surface water samples, each of 250mL, were also collected at each sampling point for laboratory analyses of pH, total phosphorus, phosphate, Kjeldahl nitrogen, ammoniacal nitrogen, nitrite, nitrate, total suspended solids and chlorophyll *a* content.

2.3.2 Laboratory investigation

Chemical analyses were carried out as soon as possible upon return to the laboratory. Unless stated otherwise, all analytical procedures followed the Standard Methods for The Examination of Water and Wastewater (APHA AWWA WPCF, 1989). Nutrient concentrations of unfiltered water samples were determined using an automatic flow injection analyzer (Aquatec 5400 Analyzer) following the Aquatec Instruction Manual (Tecator, 1990). pH was measured in the laboratory with a bench-top pH meter (Orion model SA520).

Chlorophyll a content

Chlorophyll *a* contents of the water samples were determined to provide an indirect measure of phytoplankton abundance. 100 mL of water sample was filtered through a glass-fibre filter (Whatman GF/C) which was then macerated in 90% acetone with a tissue grinder at 500 rpm for 1 minute. After a 24-hour extraction by 90% acetone at 4 °C in the dark, the sample was centrifuged and the absorbance of supernatant measured with a spectrophotometer at 750 nm and 664 nm. Absorbances at 750 nm and 665 nm were measured again after the sample had been acidified with 0.1 M hydrochloric acid to determine the exact content of chlorophyll *a* in the presence of phaeophytin *a* using the following equation:

$$\text{Chlorophyll } a\text{, mg/m}^3 = [26.7 \times (664_b - 665_a) \times V_1] / (V_2 \times L)$$

where:

V_1 = volume of extract, mL,

V_2 = volume of sample, L,

L = light path length or width of cuvette, cm, and

664_b, 665_a = optical density of 90% acetone extract before and after acidification, respectively.

Total suspended solids

100 mL of water sample was filtered through a pre-weighed glass-fibre filter (Whatman GF/C), and the filter paper was dried to a constant weight at 103 to 105 °C. The filter paper was then weighed again and the increase in weight was taken as the total suspended solids.

Ammoniacal nitrogen and Kjeldahl nitrogen

In the flow-injection analyses, the water sample containing ammoniacal nitrogen (ammonium ions and aqueous ammonia) was first mixed with sodium hydroxide. The increase in pH in the solution resulted in the formation of ammonia gas which diffused through a gas permeable membrane into an indicator (Nred) stream that was composed of a mixture of acid-base indicators. The ammonia gas altered the pH of the solution, and thus the colour of the indicator. The colour change was measured spetrophotometrically at 590 nm. For the analysis of kjeldahl nitrogen, the sample was digested with the Kjeldahl method to convert ammoniacal and organic nitrogen into ammonium using selenium as a catalyst. Kjeldhal nitrogen was determined by the amount of ammonium in the digested sample and the difference between the Kjeldahl and ammoniacal nitrogen represented the amount of organic nitrogen in the sample.

Nitrite and nitrate

Nitrite in the water sample was reacted with sulphanilamide under acidic conditions to form a diazo compound which was then coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) and resulted in the formation of a reddish-purple azo dye. The concentration of nitrite was then determined by measuring the absorbance of the dye at 540 nm. To determine the nitrate concentration, a cadmium reductor column was used to reduce the nitrate into nitrite in the water sample and the concentration of nitrite in the reduced water sample was measured as above. The concentration of nitrate was then determined by the difference in the nitrite concentrations between the reduced and the original water samples.

Orthophosphate and total phosphorus

The concentration of orthophosphate was determined with the stannous chloride method. Orthophosphate in the water sample reacted with ammonium molybdate to form the heteropoly molybdophosphoric acid, which was subsequently reduced by stannous chloride in a sulphuric acid medium to form a blue complex. The absorbance of the blue complex formed was then measured spectrophotometrically at 690 nm. Persulphate digestion of the water sample was carried out to convert all the phosphorus species into orthophosphate. Total phosphorus was subsquently determined by the amount of orthophosphate in the digested sample.

2.4 RESULTS

2.4.1 Temperature

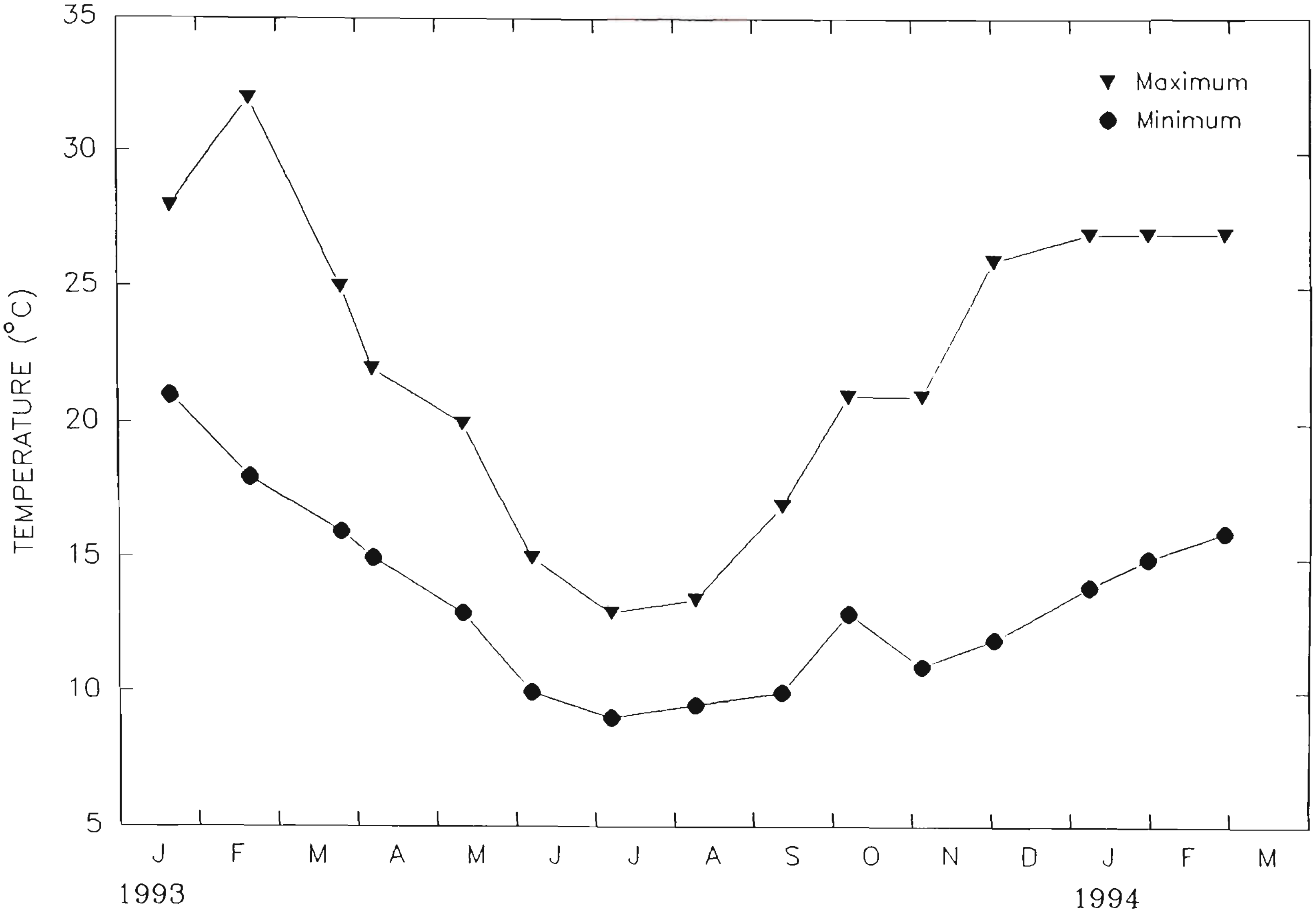
Temperatures varied significantly between the ponds (two-factor ANOVA, $F = 2.32$, d.f. = 7, 248, $P < 0.05$) and in different months (two-factor ANOVA, $F = 859.880$, d.f. = 11, 248, $P < 0.001$). However when pond 1 was excluded from the analysis, no significant difference in temperature was observed among the remaining seven ponds (pond 2 to pond 8) (two-factor ANOVA, $F = 0.516$, d.f. = 6, 226, $P > 0.5$). Temperature in pond 1 was significantly higher than that of the other ponds. Seasonal variations in the maximum and minimum temperatures of the surface water in pond 8 are shown in Fig. 2.3. Lowest water temperatures were recorded in June, July and August while the warmest months were December, January and February.

2.4.2 Dissolved oxygen, pH and chlorophyll *a* content

Variations of dissolved oxygen concentration, pH and chlorophyll *a* content of the water from pond 1 to pond 8 during the study period are shown in Figs 2.4, 2.5 and 2.6 respectively. Pond 1 was strictly anaerobic in all seasons while there was a general increase in dissolved oxygen contents from pond 1 to pond 8 (Fig. 2.4). Raw sewage in pond 1 was more or less neutral in pH, but the treated sewage became alkaline as it passed through the ponds (Fig. 2.5).

Chlorophyll *a* contents generally exhibited two peaks across the eight ponds (Fig. 2.6). Phytoplankton was practically absent in pond 1, while the first peak in chlorophyll *a*

Fig. 2.3 Monthly maximum and minimum water temperatures in pond 8 over the period January 93 to February 94.



Maximum
Minimum
TEMPERATURE (°C)
35
30
25
20
15
10
5
J
F
M
A
M
J
J
A
S
O
N
D
J
F
M
1993
1994

Fig. 2.4 Variations of the dissolved oxygen level from pond 1 to pond 8 over the period March 93 to January 94. Vertical lines are 1 S.E..

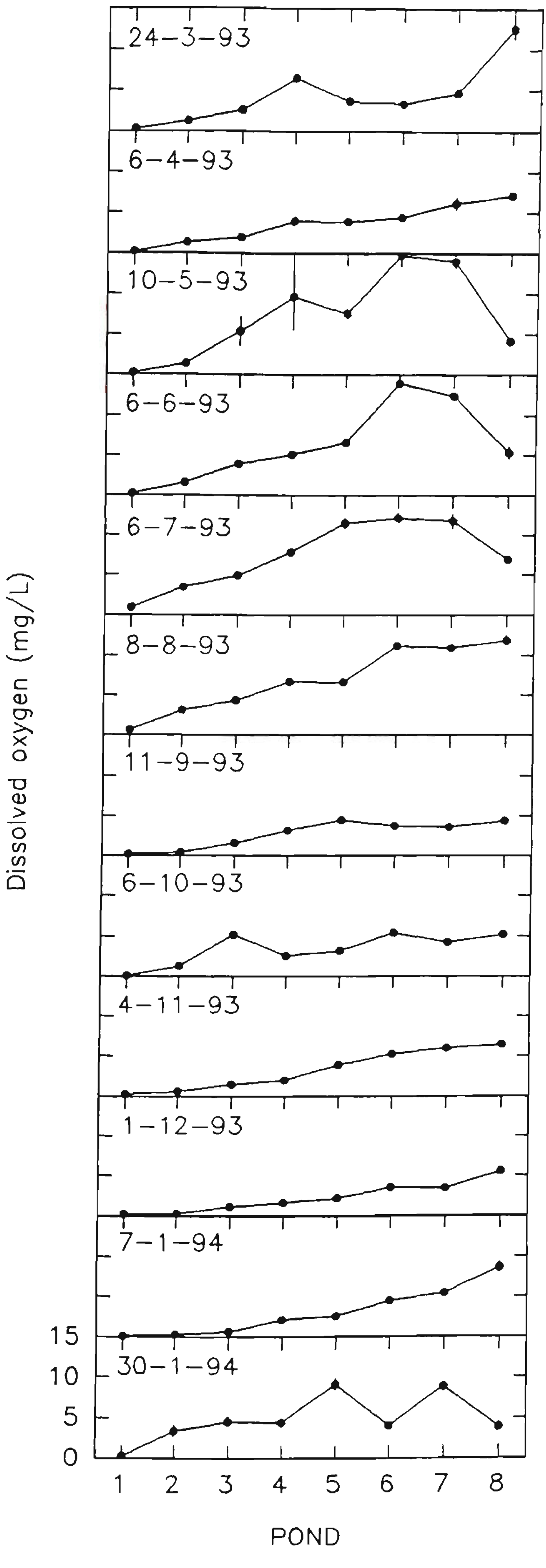

24-3-93
6-4-93
10-5-93
6-6-93
6-7-93
8-8-93
11-9-93
6-10-93
4-11-93
1-12-93
7-1-94
30-1-94
15
10
5
0
1
2
3
4
5
6
7
8
Dissolved oxygen (mg/L)
POND

Fig. 2.5 Variations of the pH level from pond 1 to pond 8 over the period March 93 to January 94.

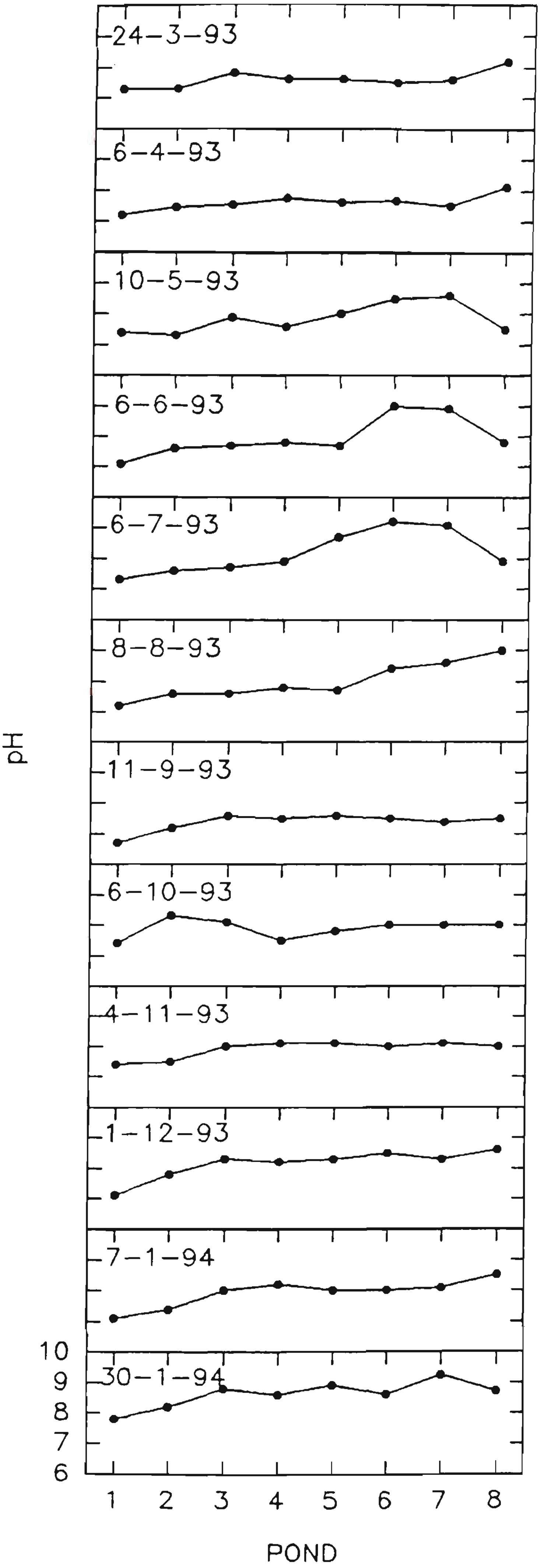
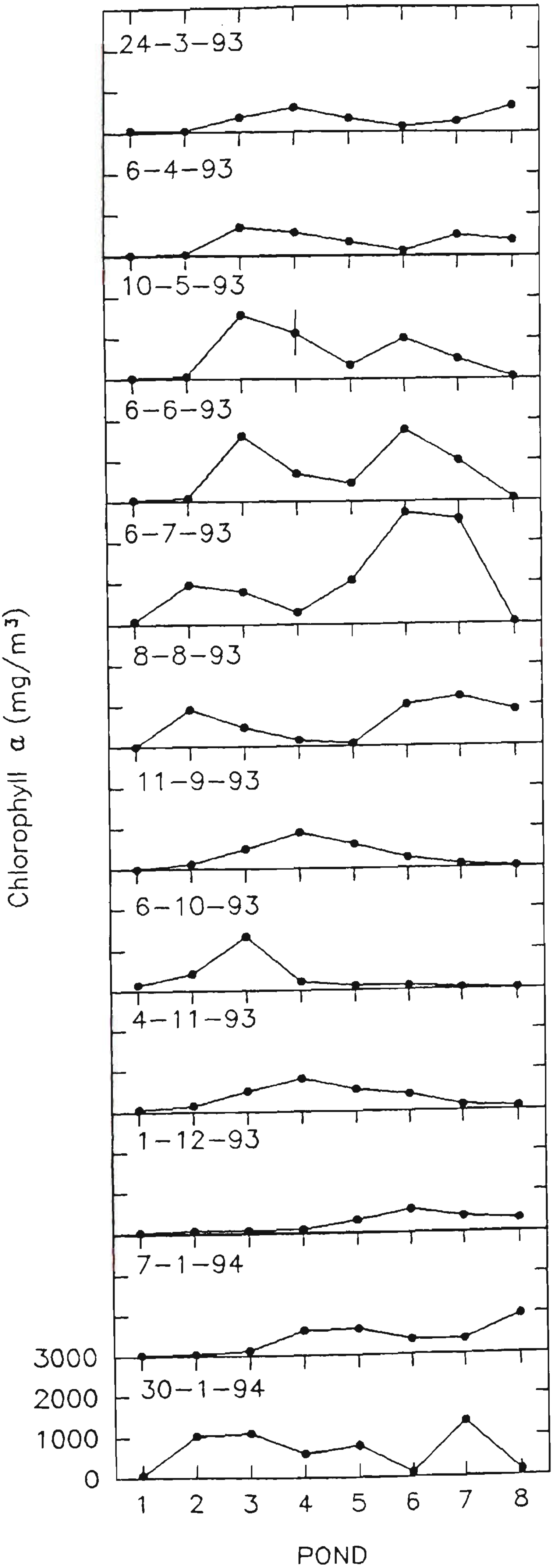

24-3-93
6-4-93
10-5-93
6-6-93
6-7-93
8-8-93
11-9-93
6-10-93
4-11-93
1-12-93
7-1-94
30-1-94
pH
10
9
8
7
6
1
2
3
4
5
6
7
8
POND

Fig. 2.6 Variations of the chlorophyll *a* content from pond 1 to pond 8 over the period March 93 to January 94. Vertical lines are 1 S.E..


24-3-93
6-4-93
10-5-93
6-6-93
6-7-93
8-8-93
11-9-93
6-10-93
4-11-93
1-12-93
7-1-94
30-1-94
Chlorophyll a (mg/m3)
3000
2000
1000
0
1
2
3
4
5
6
7
8
POND

content occurred in pond 2 or pond 3. The second peak appeared in the last few ponds (pond 6 to pond 8) of the series. The high chlorophyll *a* contents in pond 6 and pond 7 from May to July coincided with the period during which the longest detention time was observed in the system (cf. Fig. 2.2). However, this peak of chlorophyll *a* content disappeared during September and October when the population densities of the zooplankton were highest (see chapter 3). Chlorophyll *a* content was positively correlated with dissolved oxygen ($r = 0.60$, $n = 96$, $P < 0.001$) and pH ($r = 0.61$, $n = 96$, $P < 0.001$).

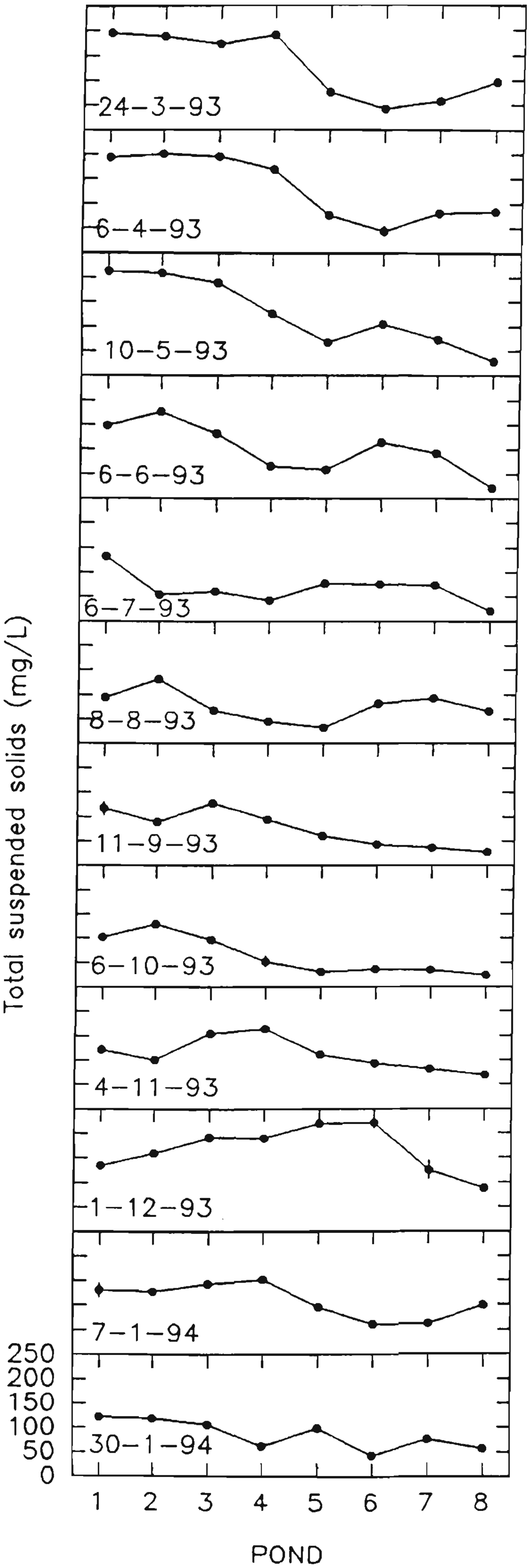
2.4.3 Total suspended solids

Concentrations of total suspended solids were generally high in the first pond during all seasons (Fig. 2.7). These were mainly composed of organic debris, such as food residues or human waste. Although the concentrations of total suspended solids generally decreased from pond 1 to pond 8, they did not exhibit a monotonic decline pattern. In fact, the spatial variations of total suspended solids in the last few ponds of the series were similar to those exhibited by chlorophyll *a* content. Indeed, there was a significant correlation between chlorophyll *a* content and total suspended solids when pond 1 and pond 2 were excluded form the analysis ($r = 0.23$, $n = 72$, $P < 0.05$). This relationship was particularly evident when phytoplankton were abundant; more than 60% of the variations in total suspended solids could be explained by the chlorophyll *a* content ($r > 0.79$, $n = 6$, $P < 0.01$).

2.4.4 Nitrogen

Variations of the concentration of Kjeldahl nitrogen, ammoniacal nitrogen, nitrite and

Fig. 2.7 Variations of total suspended solids from pond 1 to pond 8 over the period March 93 to January 94. Vertical lines are 1 S.E..


24-3-93
6-4-93
10-5-93
6-6-93
6-7-93
8-8-93
11-9-93
6-10-93
4-11-93
1-12-93
7-1-94
30-1-94
Total suspended solids (mg/L)
250
200
150
100
50
0
1
2
3
4
5
6
7
8
POND

nitrate of the water in different ponds during the study period are shown in Fig. 2.8. Variations of Kjeldahl nitrogen shared a similar pattern with ammoniacal nitrogen although there was a general reduction in the difference between the two parameters (i.e. organic nitrogen) from pond 1 to pond 8. In most cases, the variations in the organic nitrogen concentration were closely related to those of the chlorophyll *a* content. Particularly, the low concentrations of organic nitrogen recorded in pond 8 between September and November coincided with the period of low phytoplankton abundance.

Dynamics of different nitrogen species exhibited marked seasonal variations in the pond system. From April 93 to August 93 when the detention time was the longest, the concentrations of ammoniacal nitrogen were reduced to very low levels, while concentrations of nitrite and nitrate increased. Indeed, there is a signifcant negative correlation between the concentration of ammoniacal nitrogen in pond 8 and the detention time of the system ($r = -0.55$, $n = 12$, $P < 0.05$). By contrast, no obvious reduction in ammoniacal nitrogen was observed between September 93 and December 93 and the concentrations of nitrite and nitrate remained low throughout the pond series. This was particularly evident during September and October when phytoplankton abundance was extremely low.

2.4.5 Phosphorus

Variations of the concentration of total phosphorous and phosphate in different ponds during the study period are shown in Fig. 2.9. Concentrations of total phosphorus remained stable across different ponds except for the sudden rise in pond 8 during the period with long detention time. The variations of the total phosphorus from pond 3 to pond 8 were

Fig. 2.8 Variations of the concentration of Kjeldahl nitrogen, ammoniacal nitrogen, nitrite and nitrate from pond 1 to pond 8 over the period March 93 to January 94. Vertical lines are 1 S.E..

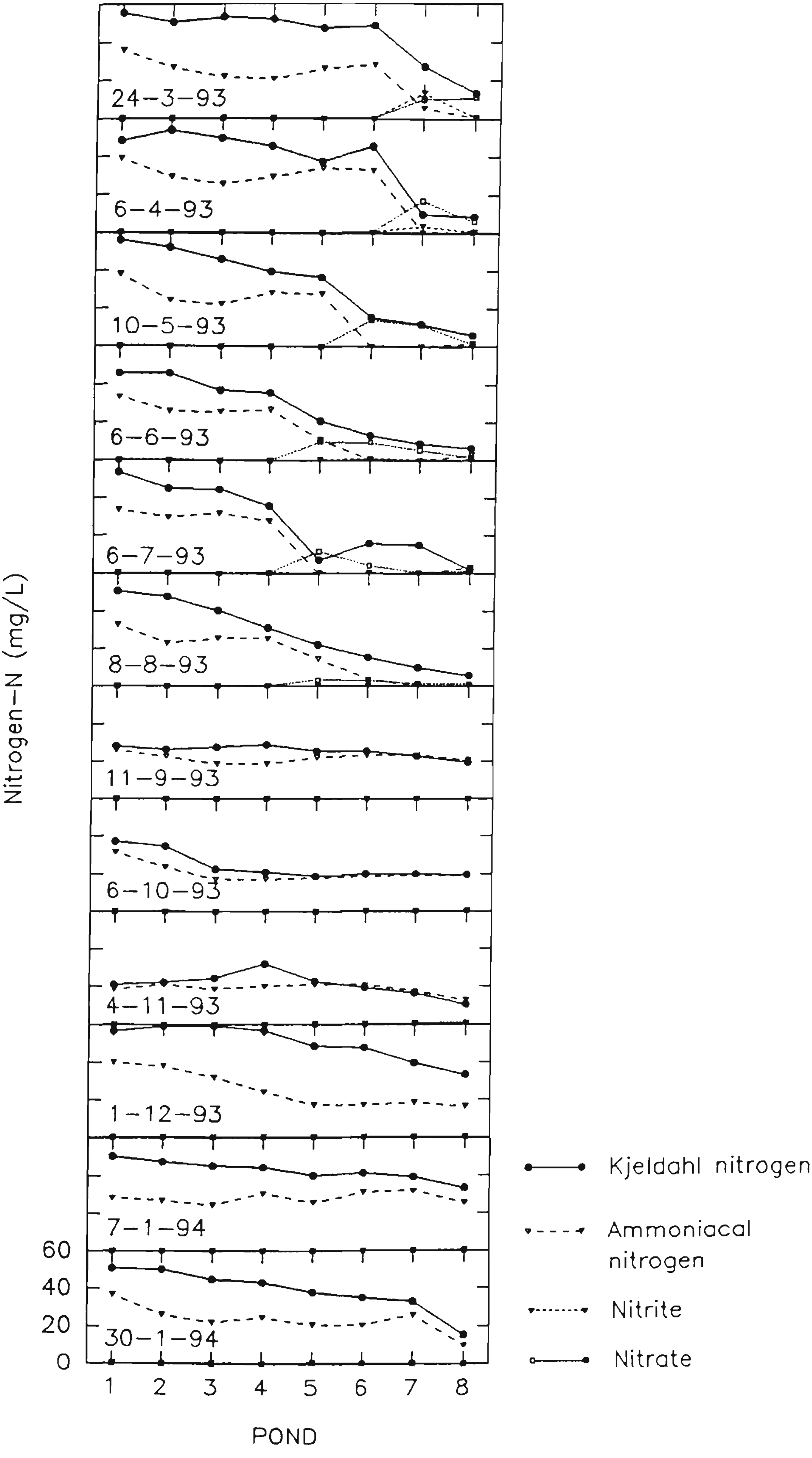
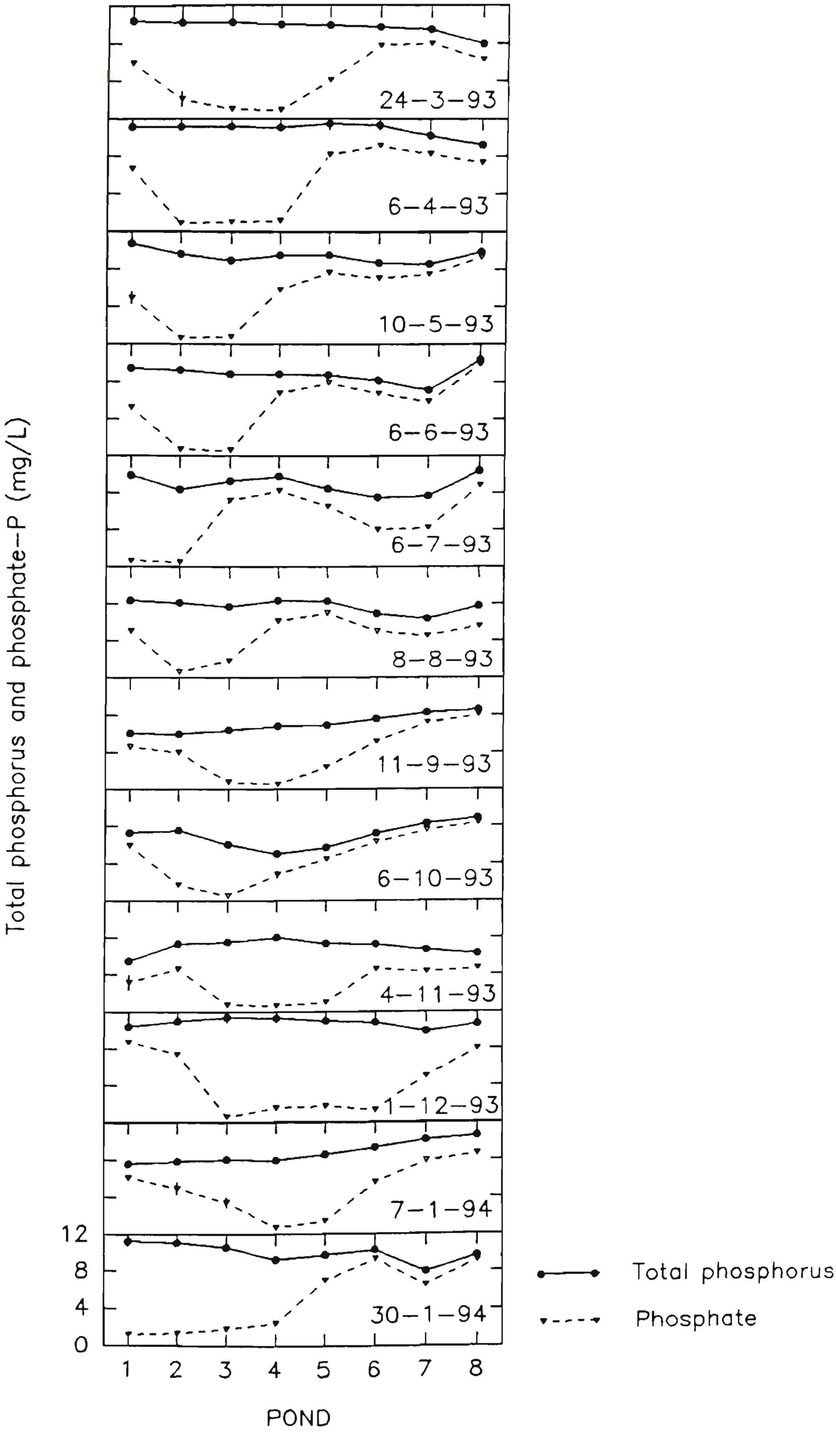

24-3-93
6-4-93
10-5-93
6-6-93
6-7-93
8-8-93
11-9-93
6-10-93
4-11-93
1-12-93
7-1-94
30-1-94
Nitrogen-N (mg/L)
60
40
20
0
1
2
3
4
5
6
7
8
POND
Kjeldahl nitrogen
Ammoniacal nitrogen
Nitrite
Nitrate

Fig. 2.9 Variations of the concentration of total phosphorus and phosphate from pond 1 to pond 8 over the period March 93 to January 94. Vertical lines are 1 S.E..



24-3-93
6-4-93
10-5-93
6-6-93
6-7-93
8-8-93
11-9-93
6-10-93
4-11-93
1-12-93
7-1-94
30-1-94
12
8
4
0
1
2
3
4
5
6
7
8
POND
Total phosphorus and phosphate-P (mg/L)
Total phosphorus
Phosphate

negatively correlated with pH during months of marked pH changes in response to a sharp increase in phytoplankton abundance in different ponds ($r < -0.73$, $n = 6$, $P < 0.05$).

Spatial variations in the concentration of phosphate across the ponds exhibited similar patterns throughout the year. Phosphate concentrations of the raw sewage dropped markedly in the first few ponds, followed by a gradual increase in subsequent ponds until all the phosphorus in the later section of the pond system was present almost entirely in the form of phosphate. Correlations between variations in phosphate concentration and chlorophyll *a* content from pond 3 to pond 8 during months of high phytoplankton abundance were significant ($r > 0.73$, $n = 6$, $P < 0.05$).

The physico-chemical characteristics of the water in pond 1 (influent) and pond 8 (effluent) of the pond system during the study period are summarized in Table 2.1.

Table 2.1 Mean values of physico-chemical parameters of the influent (Pond 1) and effluent (pond 8) of the system over the period from March 93 to January 94. Concentrations are in mg/L. Ranges are given in parentheses.

	Pond 1	Pond 8
pH	7.3 (6.7 - 7.8)	8.2 (7.5 - 9.0)
Temperature (°C)	16.6 (10.0 - 23.1)	15.7 (10.0 - 22.9)
Dissolved oxygen (mg/L)	0.25 (0.10 - 0.90)	6.78 (3.90 - 12.57)
Chlorophyll *a* content (mg/m^3)	43.0 (0 - 144.2)	294.4 (15.7 - 957.5)
Total suspended solids (mg/L)	142.7 (95.2 - 211.5)	56.0 (23.8 - 98.3)
Kjeldahl nitrogen-N (mg/L)	45.64 (20.9 - 55.9)	14.3 (2.1 - 33.0)
Ammoniacal nitrogen-N (mg/L)	32.3 (25.8 - 40.0)	9.3 (0 - 21.0)
Nitite-N (mg/L)	0.193 (0.104 - 0.336)	0.352 (0.123 - 0.908)
Nitrate-N (mg/L)	0.003 (0 - 0.025)	2.0 (0.032- 1.102)
Total phosphorus (mg/L)	8.9 (6.0 - 12.2)	9.1 (6.2 - 10.6)
Orthophosphate (mg/L)	4.8 (0.6 - 8.716)	7.8 (4.7 - 9.8)

2.5 DISCUSSION

Temperature has been regarded as the most important physical factor influencing the efficiency of waste stabilization ponds as it affects the metabolic rate of the micro-organisms in the system, and thus the rate of degradation of organic matter and subsequent stabilization of inorganic nutrients (Gray, 1992). Since stabilization ponds usually operate in a relatively uncontrolled environment, water temperature and hence the efficiency of the system changes with the weather. The higher temperatures recorded in the first anaerobic pond as compared to other ponds in the series could be attributed to the increased metabolic activities of bacteria associated with the high organic loading of raw sewage. Previous studies in Europe and America have shown that the nutrient removal efficiency by stabilization ponds was higher in summer than in winter (Van Der Post & Engelbrecht, 1973; Toms *et al.*, 1975; Pano & Middlebrooks, 1982; Santos & Oliveira, 1987). Similarly, Hussainy (1979) reported that the removal efficiency of ammoniacal nitrogen, and the rate of nitrification in particular, was higher during summer at Werribee.

Contrary to the above findings, removal of nitrogen in the present study was more efficient during autumn and early winter as compared with summer during which nitrogen removal was incomplete. The complete removal of ammoniacal nitrogen in the study system during the colder months was mainly attributed to the longer detention time which allowed nitrification to occur. In this process, ammoniacal nitrogen is oxidized to nitrite, and then nitrate, by nitrifying bacteria (Painter, 1977; Sharma & Ahlert, 1977). During that period of long detention time, growth of phytoplankton in the ponds exceeded the loss due to flushing through the outflow, and the phytoplankton abundance increased significantly (Toms *et al.*, 1975). High photosynthetic activities of the phytoplankton not only increased the dissolved oxygen level, but also elevated the pH by consuming the acidic carbon dioxide in the ponds. Both of these conditions are optimal for the nitrifying bacteria and could have speeded up the rate of nitrification (Wild *et al.*, 1971). Nitrification does not occur commonly in facultative ponds due to the low density of nitrifying bacteria in the aerobic zone of the waste stabilization ponds. The bacteria tend to adsorb onto the surface of particles which settle to the bottom anoxic sludge layer where the process of the nitrification is arrested (Ferrara & Avci, 1982). However, in the Werribee ponds, the high intensity of photosynthetic action might have created an aerobic condition throughout the depth of the pond allowing nitrification to take place down to the water-sludge interface.

Since ammonium is the most preferentially utilized form of nitrogen for phytoplankton (Boney, 1989), uptake of ammoniacal nitrogen by the abundant phytoplankton might have contributed to the removal of ammonium from the waste water (Ferrara & Avci, 1982). The increase of organic nitrogen (the difference between Kjeldahl and ammoniacal nitrogen) at the last few ponds coincided with the increase of chlorophyll *a* content during May and July, suggesting that some inorganic nitrogen might have been converted to organic nitrogen in the algal cells through phytoplankton uptake and growth. Indeed, estimates of the total suspended solids revealed that the dry weight of phytoplankton in the study ponds could be as high as 100 mg/L. This could represent 4 to 7.5 mg/L of nitrogen depending on the species composition of the phytoplankton (Hemens & Stander, 1969).

Pano & Middlebrooks (1982) suggested that another mechanism namely ammonia volatilization/ammonia stripping may also be important in the removal of ammoniacal nitrogen in stabilization ponds. It is proposed that carbon dioxide consumed by actively photosynthesising algae exceed that supplied by organic degradation, resulting in an increase in pH. Ammoniacal nitrogen in water exists in an equilibrium of dissolved ammonia (NH_3) and ammonium ion (NH_4^+), and alkaline pH shifts the equilibrium towards ammonia. The volatilization of ammonia to the atmosphere depends on the mass transfer coefficient which is enhanced by the mixing effect of wind action and high temperature. Although this mechanism is unlikely to be the major ammonia removal pathway in the Werribee system, it helps to explain the minor reduction of ammoniacal nitrogen concentration when there are no noticeable increases in phytoplankton biomass nor nitrate and nitrite.

In view of the low nitrate and nitrite concentrations recorded in the pond system studied, it is generally believed that nitrification-denitrification is unlikely to be a principal mechanism of nitrogen removal (Toms *et al.*, 1975; Ferrara & Avci, 1982; Pano & Middlebrooks, 1982; Reed, 1985). However, the observation that a reduction in the concentration of ammoniacal nitrogen was accompanied by a significant increase in nitrite and nitrate levels in the present study suggest that nitrification can be an important mechanism in nitrogen removal if the detention time is long enough to allow significant growth of phytoplankton. Permanent removal of nitrogen from the system can be achieved through denitrification during which nitrate is reduced to nitrite and then to elemental nitrogen, and the nitorgen gas is ultimately exported to the atmosphere. Although denitrification is an anoxic process and may not prevail in aerobic ponds, Hussainy (1979) showed that there was diurnal variations in dissolved oxygen which was particular apparent during phytoplankton blooms in the Werribee ponds, and that dissolved oxygen could drop to a very low level during night time which might permit denitrification to take place. This alternation of aerobic and anaerobic conditions during day and night time respectively resembles the controlled environment in the activated sludge reactor for nitrogen removal (Horan, 1990). Notwithstanding, the decrease in nitrite and nitrate concentrations might also be due to direct uptake by phytoplankton when the more preferred ammonium was at a low concentration level (Fitzgerald & Rohlich, 1964).

Phosphorus can exist in the raw sewage as orthophosphate, condensed phosphorus originated from detergent, and organic phosphorus in food residue and human wastes. As phosphorus does not exist in gaseous form, it is often regarded as the nutrient more amenable to control (Kandadai, 1992). However, unlike carbon that can be exported permanently to the atmosphere in form of methane and nitrogen in form of ammonia or nitrogen gas, various forms of phosphorus will remain in the system either in the water column or in the sludge. Removal can only be accomplished by physical separation of the stabilized phosphorus from the system. In the present study, there was no significant removal of phosphorus form the system under all the different temperature and/or detention time regimes. Sedimentation of phosphorus from the raw sewage was not evident in the system as the phosphorus in the water column remained at similar levels across the pond series. This observation, however, cannot exclude the possibility that sedimentation could have occurred mainly in pond 1, or the sedimentation of phosphorus was balanced by a release of phosphorus from the bottom sludge. Indeed, an increase in total phosphorus concentration was observed in the final ponds in some months although the loss of pond water through evaporation may also contribute to such an increase in total phosphorus concentration.

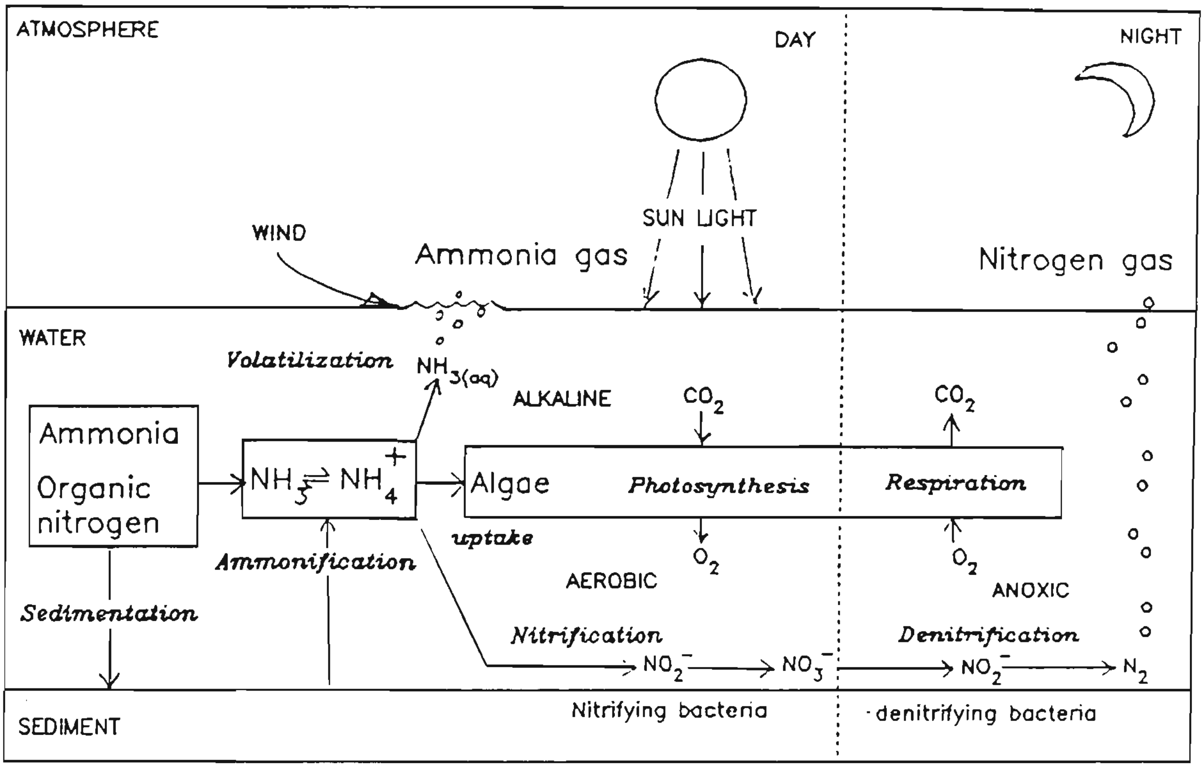
Total phosphorus measured in this study included all forms of phosphorus present in the water column including as dissolved phosphate as well as organic-bound phosphorus in bacteria or algae. Despite the consistency between the occurrence of the high phytoplankton abundance and the decrease in total phosphorus concentration, the decrease could not be due to phytoplankton uptake as the phosphate taken up by the algae should be represented in total phosphorus analyses. One possible reason for the decrease in total phosphorus from the water column during periods of high phytoplankton abundance might be the precipitation of hydroxyapatite [$Ca_5(PO_4)_3OH$] (Toms *et al.*, 1975). Increase in photosynthetic activities removes carbon dioxide from the water, and results in a higher pH. Dissolved phosphate then reacts with calcium ions and hydroxide ions to form hydroxyapatite. Hydroxyapatite, which settles down to the sludge layer, is likely to be released back to the water column at a lower pH after the algal bloom has subsided or during night time when there was no photosynthesis (Fitzerald & Rohlich, 1964; Hemens & Mason, 1968; Lakshmi & Pitchai, 1991).

Indeed, previous studies have suggested two principal mechanisms for phosphorus removal in stabilization ponds (Toms *et al.*, 1975; Lakshmi & Pitchai, 1991). Firstly, phosphate could be removed from the water by phytoplankton consumption and this is supported by the negative correlation between phosphate concentration and chlorophyll *a* content observed in this study. Nevertheless the reduction of phosphate due to algal growth is not as apparent as that of nitrogen as nitrogen is usually more limiting for algal growth than phosphorus in the sewage (Fitzgerald & Rohlich 1964; Hemens & Stander, 1969). Secondly, the increase of pH due to the photosynthetic activity of phytoplankton could result in the precipitation of phosphate with calcium and hydroxide ions (see above). However, the sudden decrease in phosphate concentration in the first few ponds of the series cannot be adequately explained by the two mechanisms as phytoplankton abundance was generally low in those ponds. Importantly, this sudden drop in phosphate content was not accompanied by a drop in the total phosphorus indicating that the phosphate had merely been converted into another form, but still remained in the water column.

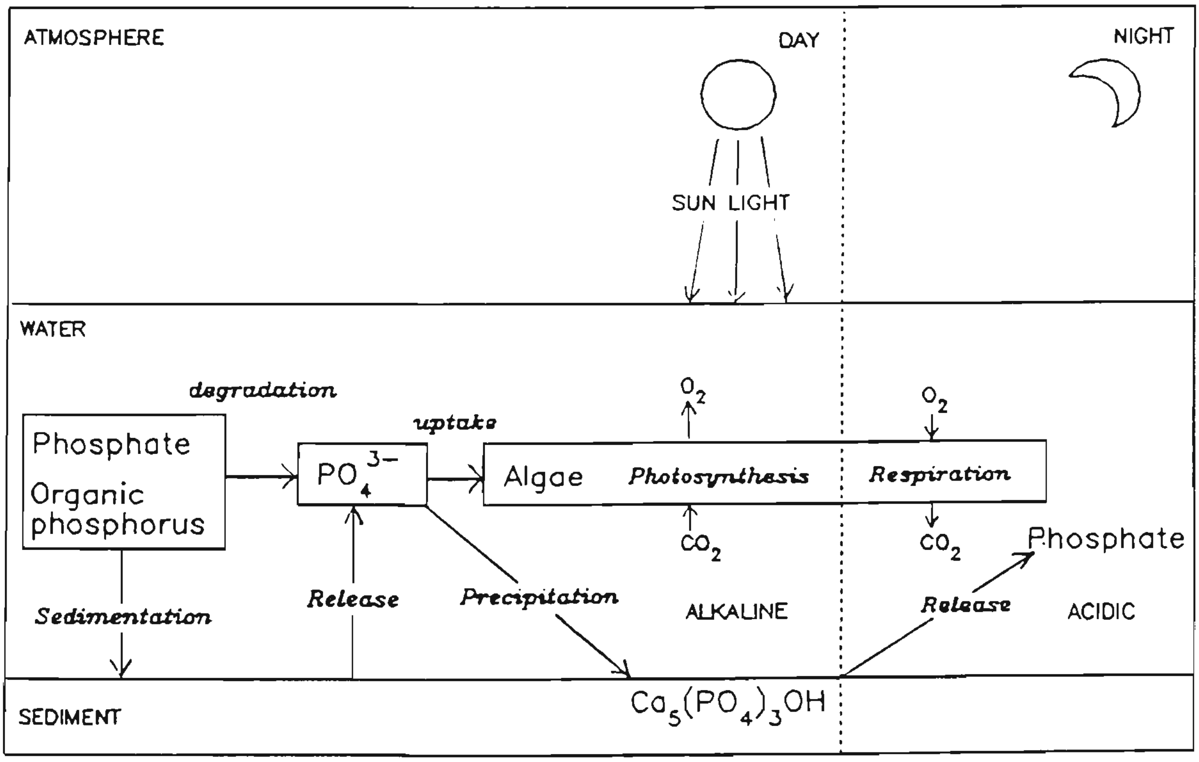
Dramatic initial uptake of phosphate by bacteria under aerated conditions has been reported in activated sludge system, and this "luxury uptake" of phosphate into bacterial cells is much higher than what is required for the growth of the bacteria (Levin & Shapiro, 1965). A subsequent "bleed back" of the phosphate from the bacteria into the water column may occur under extended aeration (Vacker *et al.*, 1967; Wells, 1969). Brar & Tollefson (1975) later found that the increased phosphate uptake under aeration was due to the turbulence created rather than the rise in dissolved oxygen. In the Werribee system, pond 1 is much higher than pond 2, and the rapid flow of water from pond 1 to pond 2 created by gravity may have provided sufficient turbulence for the "luxury uptake" to occur which causes a sudden drop in phosphate content in pond 2. The subsequent increase in phosphate concentration may be due to the endogenous cell destruction and/or the predation of bacteria by protozoans which release the phosphate back to the water. However, these hypotheses need to be tested by examining the density of *Actinetobacter* bacteria, the genus associated with "phosphate uptake" in the stabilization ponds (Horan, 1990). In addition to the function of algae as an oxygen-producing source for aerobic bacterial decomposition (Bartsch, 1961), the results of this study concur with previous findings that algae play a direct role in nutrient removal in waste stabilization ponds (Toms *et al.*, 1975; Ferrara & Avci, 1982; Houng & Gloyna, 1984; Santos & Oliveira, 1987). The principal biochemical and physical pathways of the different species of nitrogen and phosphorus are summarized in Fig. 2.10.

Fig 2.10 Principal biochemical and physical pathways for nitrogen and phosphorus removal in waste stabilization ponds.

NITROGEN



PHOSPHORUS



With regard to water-quality improvement, an increase in phytoplankton biomass would nevertheless result in an increase in total suspended solids in the effluent. Indeed, in the later sections of the pond series, the prevailing high ammoniacal nitrogen concentrations did not seem to suppress the growth of algae (cf. Konig *et al.*, 1987), and the suspended solids mainly consisted of phytoplankton. The potential impact of an effluent containing high phytoplankton biomass would depend on the size of the receiving water body, and whether the algae can survive in the receiving system (Toms *et al.*, 1975; Mithchell, 1980). In Werribee, the freshwater algae in the effluent are unlikely to survive in Port Phillip Bay which is a marine environment. The dead algal cells will increase the biological oxygen demand of the bay in the vicinity of the outlet.

In the Werribee ponds, zooplankton appeared in the aerobic ponds in high densities and played a significant role in the reduction of the total suspended solids. These animals grazed on the phytoplankton and then produced compact faecal pellets which settled to the pond bottom (Loedolff, 1965). Consequently, levels of total suspends solids were low in the effluent, particularly in September and October when zooplankton abundance was highest. However, the inorganic nutrients regenerated by zooplankton through their grazing activities on phytoplankton could be significant (Moegenburg & Vanni, 1991), and in this study, high amounts of unstabilized inorganic phosphate and ammoniacal nitrogen were observed in the last few ponds in the series. Indeed, Mitchell and Williams (1982a) reported that the zooplankton could only account for less than 6% of total phosphate and nitrogen in the pond system. Through grazing, the stabilized nutrients in algal cells are released back into the water column in inorganic forms which may cause eutrophication in the receiving water body. Intense grazing on the phytoplankton which are essential in nutrient removal may also be detrimental to the treatment process. Indeed, no significant reduction of ammoniacal nitrogen was observed during periods of high zooplankton and low phytoplankton abundances. The seasonal changes in plankton communities in the Werribee ponds will be examined in chapter 3.

In summary, although the study ponds in Werribee can produce effluent with low levels of suspended solids, the objectives in relation to nutrient removal have not been achieved. During most part of the year, detention time is not long enough to allow complete removal of nitrogen. Aerators can be incorporated into the system to increase the dissolved oxygen level mechanically and enhance nitrification for ammoniacal nitrogen removal. In the case of phosphorus, there is merely a change from organic phosphorus in the raw sewage to a soluble form of inorganic phosphate, with no significant removal of phosphorus from the system. Addition of lime may be an option to increase the rate of precipitation of the phosphate to the bottom sludge. However, desludge has to be carried out regularly to remove the settled phosphate, which can be released back into the water column at lower pH. In addition, the elimination of bacteria from the water following phosphate uptake may also result in significant phosphate reduction.

3. Seasonal changes in plankton communities in waste stabilization ponds: biotic and abiotic influences

3.1 INTRODUCTION

Waste stabilisation ponds have long been employed in the treatment of waste water (Gloyna, 1971; Rohlich, 1976; Lai & Lam, in press). Although the role of zooplankton in the process of self-purification has been well documented (Loedolff, 1965; Kryutchkova, 1968; Andronikova, 1978; Mitchell, 1980), the conventional bacteria-algae model of waste stabilization ponds (e.g. Glyoyna, 1971) pays little attention to the contributions of these organisms in the treatment processes. Moreover, zooplankton communities can influence the phytoplankton abundance directly through grazing and indirectly through nutrient regeneration (Vain & Temte, 1990; Moegenburg & Vain, 1991). These trophic interactions can have a significant impact on the composition and abundance of phytoplankton which are crucial to the treatment processes of the stabilization ponds (see chapter 2). Clearly, a better understanding of the ecological interactions between the zooplankton and phytoplankton communities in the pond systems is essential for better pond management.

The zooplankton communities in most waste stabilization ponds reported in the literature from different geographical regions have remarkably similar compositions, being dominated by cladocerans (mainly *Daphnia* and *Moina*), copepods and rotifers (e.g. Loedolff, 1965; Kryutchkova, 1968; Daborn *et al.*, 1978; Mitchell, 1980; Dinges, 1982; Mishra & Saksena, 1990). The composition of these communities and abundance of individual species often show marked seasonal patterns, and these seasonal changes have been attributed to mainly physical factors such as temperature (e.g. Loedolff, 1965; Mitchell & Williams, 1982a; 1982b) and photoperiod (e.g. Dinges, 1982). Despite the apparent importance of physico-chemical parameters in regulating zooplankton communities, it is conceivable that biotic factors, e.g. food availability and interspecific interactions, may also be important. The possible role of these factors is underscored by the fact that the majority of the zooplankton species appeared to have similar niches and thus may compete with each other for food and/or other resources (DeMott, 1989). The zooplankton communities inhabiting the Werribee ponds provide an unique opportunity to examine the ecological interactions of sympatric zooplankton under conditions of high nutrient inputs and little or no predation.

In this chapter, I examine the temporal variations in the abundance of the dominant phytoplankton and zooplankton species in two waste stabilization ponds at Werribee and their relationships with physical environmental factors. I will also explore the possible role of biotic influences, in particular intraspecific and interspecific competition for food, in shaping the seasonal patterns of the zooplankton. Impact of the zooplankton on the phytoplankton communities will be investigated.

3.2 MATERIALS AND METHODS

The study was undertaken in the last two ponds (pond 7 and pond 8) in a stabilization pond series (145W Group B) at Werribee Treatment Complex between March 93 and February 94. A general description of the Complex and the details of the study site are given in chapters 1 and 2 respectively.

3.2.1 Physico-chemical characteristics

Physico-chemical parameters including temperature, pH and dissolved oxygen of the study ponds were monitored monthly between 1000 and 1400 hrs during the study period. Surface and bottom (just above the sludge layer) dissolved oxygen levels and temperatures were measured in the middle of the pond using a dissolved oxygen meter (YSI Model 58) on board a rubber dingy. Water samples were collected at a depth of 50mm for pH measurement with a bench-top pH meter (Orion model SA520).

3.2.2 Chlorophyll *a* content

Chlorophyll *a* contents in pond 7 and pond 8 were measured monthly to provide an indirect measure of the amount of food available to the zooplankton (details of the methodologies are given in chapter 2). However, it is important to note that chlorophyll contents can only give a rough estimate of the amount of food available to zooplankton as different phytoplankton species may have different nutritive value, palatability and digestibility (Boney, 1989). Moreover, some zooplankton can switch from being a phytoplankton grazer to a detritus browser under low concentrations of phytoplankton (Horton *et al.*, 1979).

3.2.3 Sampling procedure

Monthly samples were collected between 1000 and 1400 hrs from March 1993 to February 1994. Each pond was divided into eight roughly equal sections and phytoplankton and zooplankton samples were taken from a randomly-chosen station in each of the eight sections on board of a rubber dingy. At each station, 50 mL of water was collected and preserved with Lugol's iodine for subsequent phytoplankton analyses. Zooplankton were harvested with a plankton net (diameter: 125 mm, mesh size: 158 μm) hauled vertically from a depth of 0.5 m. Three replicate zooplankton samples were collected from each of the eight stations in a pond.

3.2.4 Laboratory investigation

Phytoplankton samples collected were placed in a 1 mL Sedgwick-Rafter under a compound microscope for identification and numeration following Michael (1984). Phytoplankton specimens were identified to genus level according to Prescott (1978). Abundance level of phytoplankton was determined subjectively to evaluate the relative importance of various phytoplankton groups. All individuals in each zooplankton sample were identified and counted under a stereomicroscope. In cases where the zooplankton numbers were too high, the animals were subsampled volumetrically following Michael (1984).

3.3 RESULTS

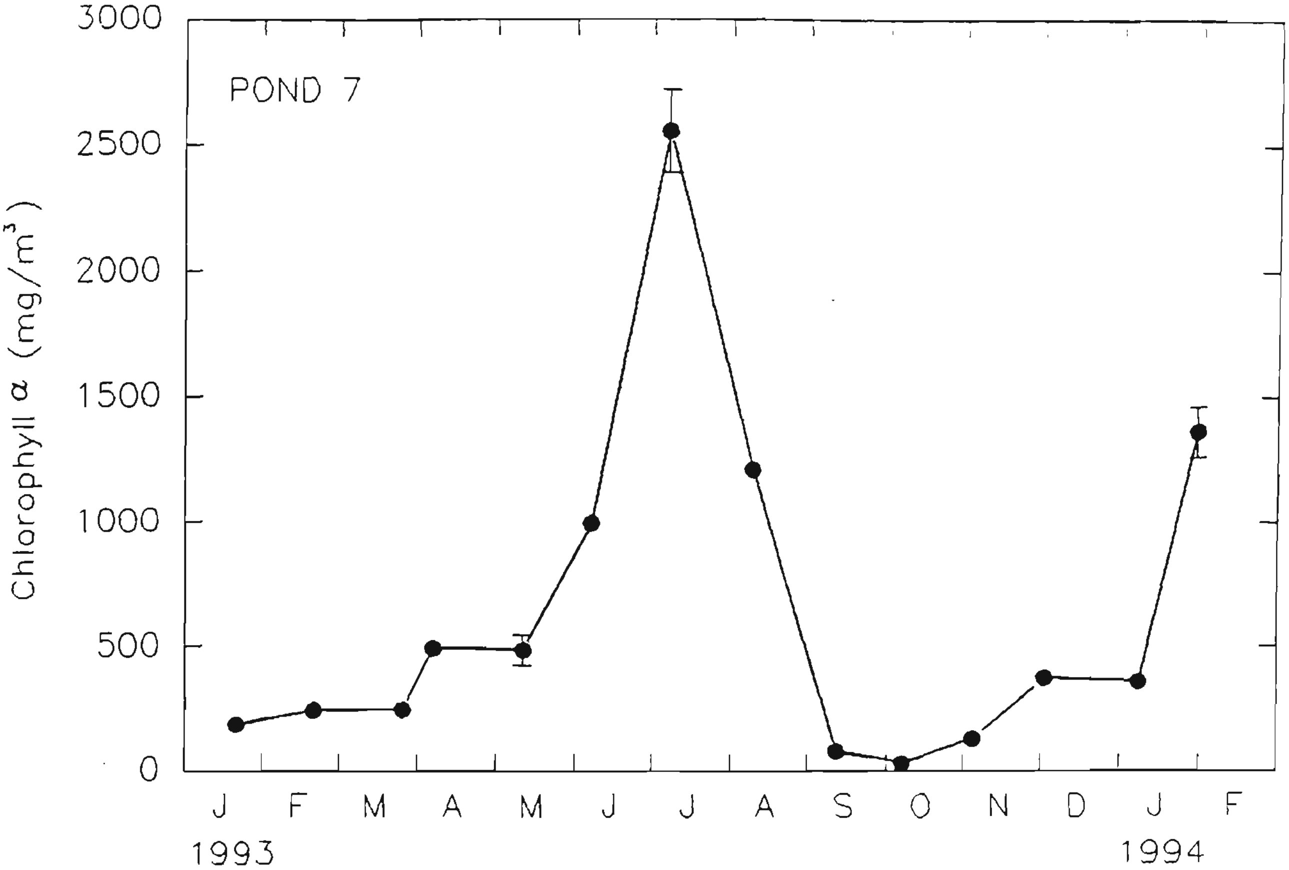
3.3.1 Phytoplankton

High concentrations of chlorophyll *a* were recorded in July/August (winter) and January (summer) in both ponds (Fig. 3.1). The peak of the chlorophyll *a* content in the winter coincided with the period of the longest detention time (cf. Fig. 2.2). The phytoplankton taxa recorded during the study period include Cyanophyceae (*Microcystis*, *Gloeocapsa*), Bacillariophyceae (*Tabellaria*, *Navicula*), Euglenophyceae (*Euglena*) and Chlorophyceae (*Closterium*, *Stylosphaeridium*, *Scenedesmus*, *Ankistrodesmus*, *Chlorella*, *Oocystis*). The occurrence of the phytoplankton showed clear seasonal patterns in both pond 7 and pond 8 (Table 3.1).

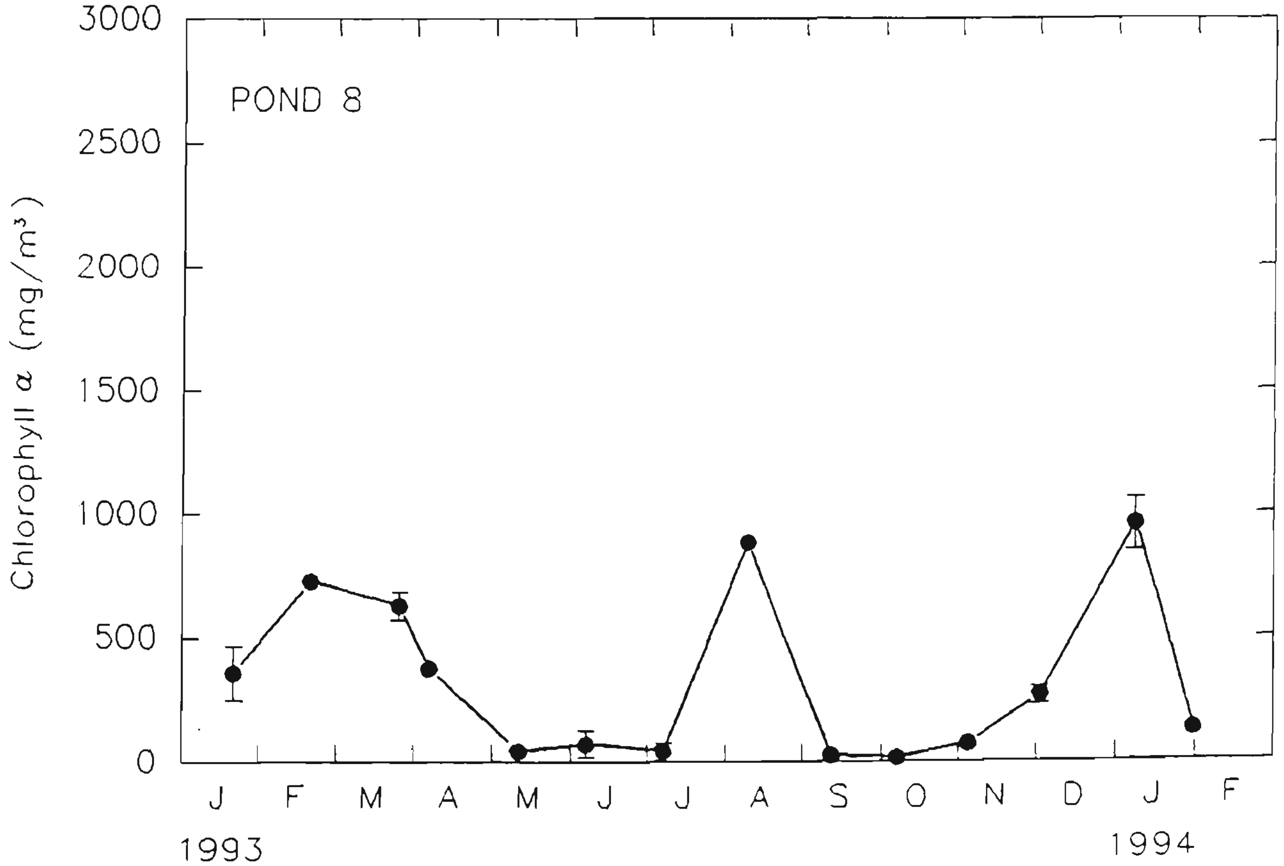
Table 3.1 A summary of the seasonal variations of various phytoplankton groups in sewage ponds at Werribee. * = rare, ** = common, *** = abundant.

Pond 7												
Taxa	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Cyanophyceae	*	*	*	*	*	*	*	*	*	*	*	*
Bacillariophyceae	*		*									
Euglenophyceae		*	**	***	***	***			*	*	*	
Chlorophyceae	***	***	**		*	*			*	*	***	***
Pond 8												
Taxa	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Cyanophyceae	*	*	*	*			*	*	*	*		*
Bacillariophyceae	**								*			
Euglenophyceae				*	**	***			*	**	**	*
Chlorophyceae	***	***			*				*	*		***

Fig. 3.1 Seasonal variations of chlorophyll *a* content (mg/m^3) in pond 7 and pond 8 over the period January 1993 to January 1994. Vertical lines are 1 S.E..



POND 7
3000
2500
2000
1500
1000
500
0
Chlorophyll α (mg/m³)
J F M A M J J A S O N D J F
1993
1994



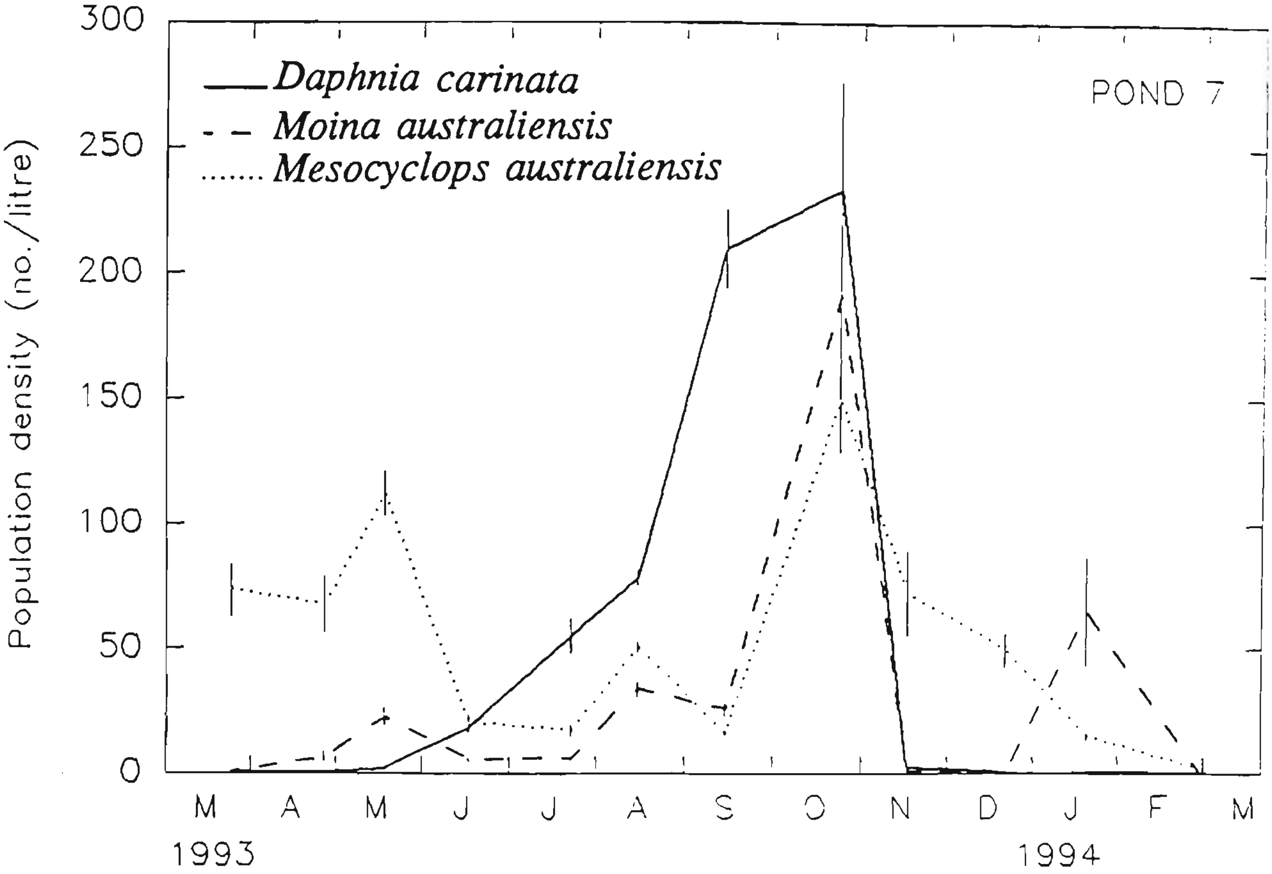
POND 8
3000
2500
2000
1500
1000
500
0
Chlorophyll α (mg/m³)
J F M A M J J A S O N D J F
1993
1994

In general, phytoplankton abundance was higher in pond 7 than pond 8 although both ponds exhibited similar seasonal patterns (Fig. 3.1). Euglenophyceae abundance peaked in the winter which coincided with a marked increase in chlorophyll *a* concentration when the detention time was longer and ammoniacal nitrogen lower (cf. Figs 2.2 & 2.8). In the summer when the ammoniacal nitrogen was high, Euglenophyceae were replaced by Chlorophyceae as the dominant phytoplankton. Importantly, they were both absent in September and October when the cladoceran population densities were highest (see section 3.3.2). In pond 7, Cyanophyceae occurred throughout the year even in September and October when all other phytoplankton taxa were absent (Table 3.1).

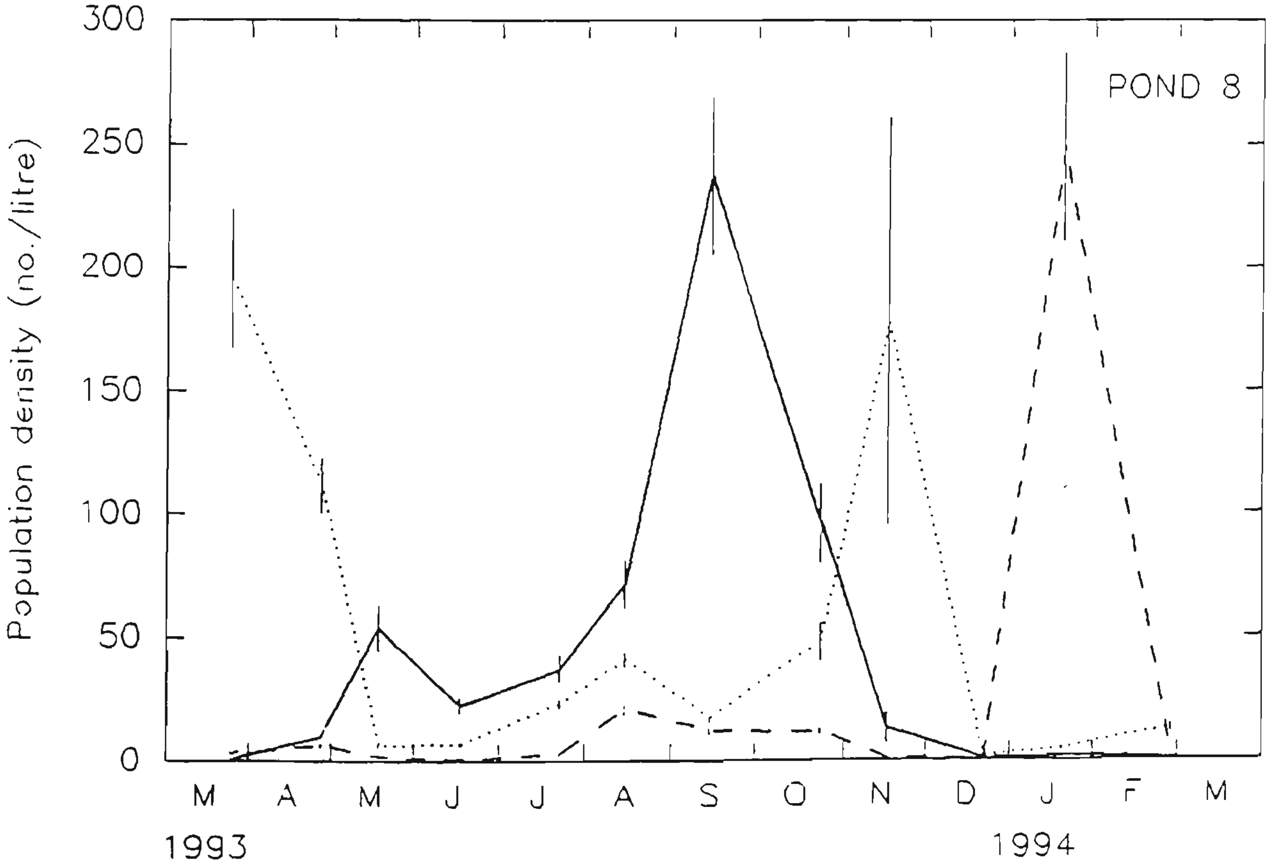
3.3.2 Zooplankton

A list of zooplankton species recorded during the study period is given in Table 3.2. Seasonal variations of the population densities of *Daphnia carinata, Moina australiensis* [*M. tenuicornis* reported in Hussainy (1979) probably refers to this species] and *Mesocyclops australiensis* [This species is very common in south-eastern Australia and has been wrongly identified as *Mesocyclops leuckarti* which in fact does not occur in Australia (D. Morton personal communication)] in pond 7 and pond 8 are shown in Fig. 3.2. Population densities of the dominant rotifers, *Brachionus calyciflorus* and *Asplanchna* cf. *sieboldi*, are shown in Fig 3.3. Other rotifer species recorded were generally rare (Table 3.2).

Fig. 3.2 Seasonal variations of the population densities of *Daphnia carinata*, *Moina australiensis* and *Mesocyclops australiensis* in pond 7 and pond 8 over the period March 1993 to February 1994. Vertical lines are 1 S.E..

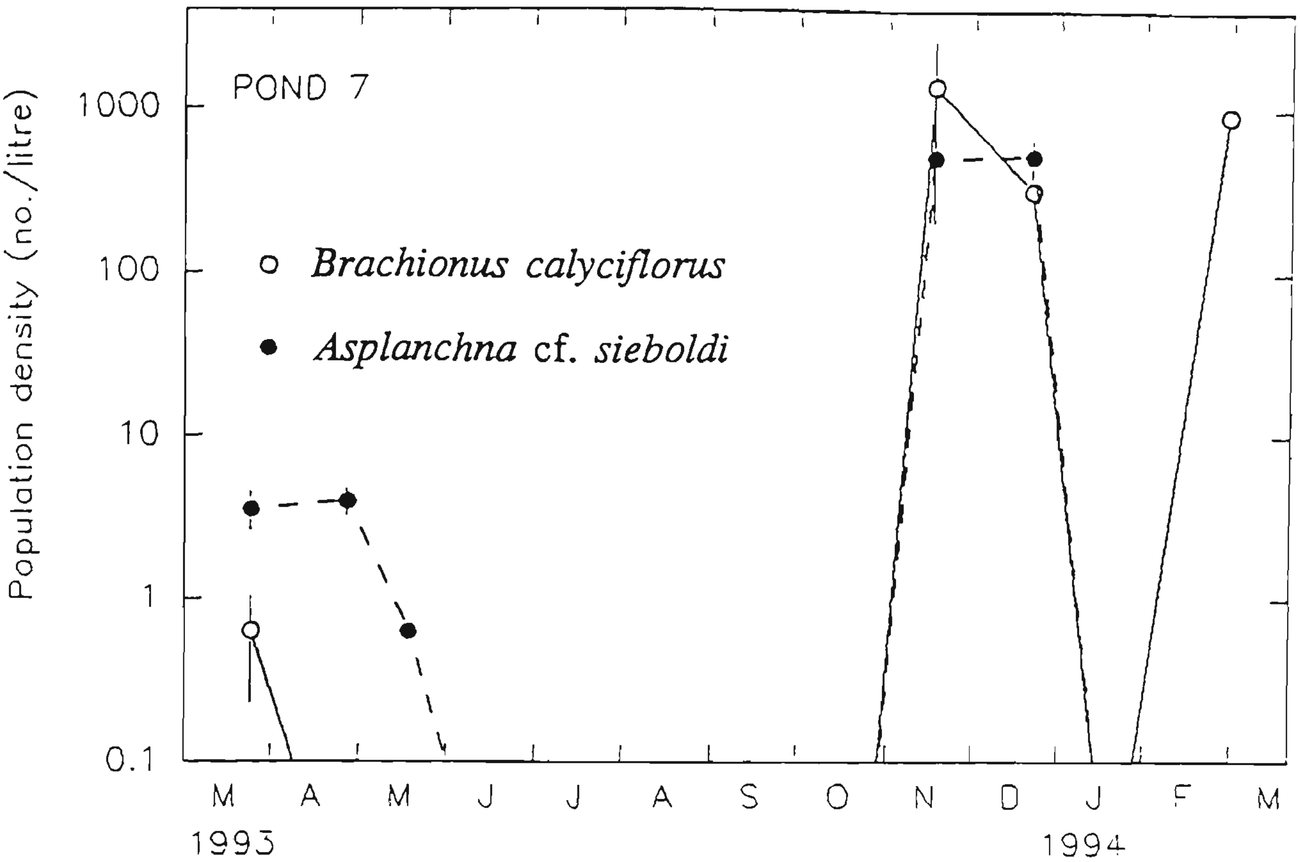


Daphnia carinata
Moina australiensis
Mesocyclops australiensis
POND 7
Population density (no./litre)
300
250
200
150
100
50
0
M A M J J A S O N D J F M
1993
1994



POND 8
Population density (no./litre)
300
250
200
150
100
50
0
M A M J J A S O N D J F M
1993
1994

Fig. 3.3 Seasonal variations of the population densities (logarithmic scale) of *Brachionus calyciflorus* and *Asplanchna* cf. *sieboldi* in pond 7 and pond 8 over the period March 1993 to February 1994). Vertical lines are 1 S.E..


POND 7
Population density (no./litre)
1000
100
10
1
0.1
Brachionus calyciflorus
Asplanchna cf. sieboldi
M A M J J A S O N D J F M
1993
1994

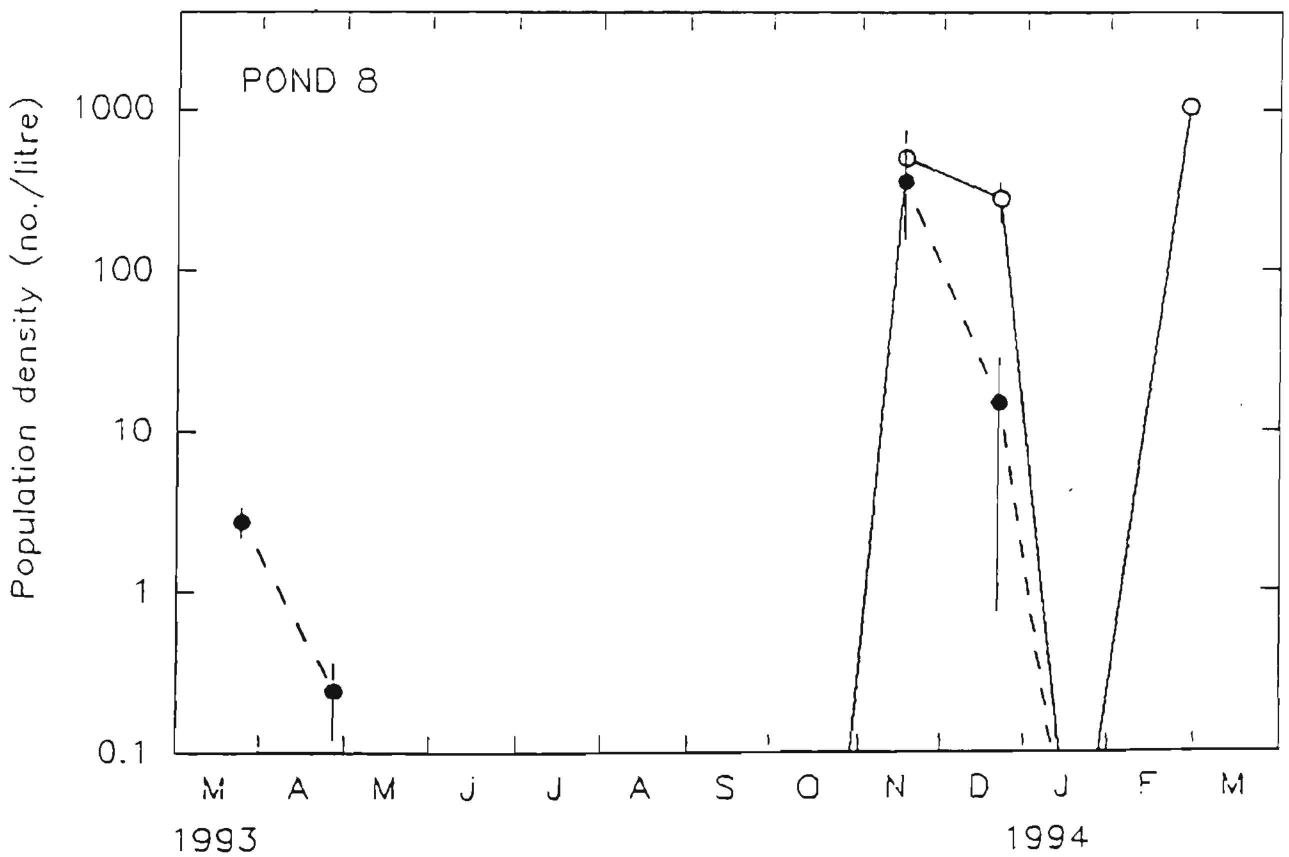

POND 8
Population density (no./litre)
1000
100
10
1
0.1
M A M J J A S O N D J F M
1993
1994

Table 3.2 A list of zooplankton species recorded in Pond 7 and pond 8 during the study period.

Cladocera
    Daphnidae
        Daphnia carinata King, 1853

    Moinidae
        Moina australiensis Sars, 1896

    Chydoridae
        Chydorus sp.

Copepoda
    Cyclopidae
        Mesocyclops australiensis Sars, 1908

Rotifera
    Brachioninae
        Brachionus calyciflorus Pallas, 1766
        Brachionus angularis Gosse, 1834

    Testudinellidae
        Filinia cf. *longiseta* Ehrenberg, 1834

    Asplanchnidae
        Asplanchna cf. *sieboldi* Leydig, 1854

The correlation coefficients between the population density of each species and the physical parameters (dissolved oxygen level, temperature and pH) in pond 7 and 8 are shown in Table 3.3. In most cases, no significant correlation between the population densities of different species and dissolved oxygen, temperature and pH was observed except for *Asplanchna* cf. *sieboldi* which was positively correlated with temperature in pond 7.

Table 3.3 Correlation coefficients between the zooplankton population densities and the physical parameters (pH, dissolved oxygen and temperature) in pond 7 and pond 8 from March 93 to February 94.

	Pond 7			Pond 8		
	pH	D.O.	Temp.	pH	D.O.	Temp.
Daphnia carinata	-0.35	-0.29	-0.32	-0.52	-0.07	-0.43
Moina australiensis	-0.22	-0.18	0.06	0.19	0.04	0.19
Mesocyclops australiensis	-0.29	-0.26	0.32	0.21	0.30	0.38
Brachionus calyciflorus	0.17	-0.39	0.38	0.29	-0.43	0.26
Asplanchna cf. *sieboldi*	-0.10	-0.26	0.61*	-0.12	-0.14	0.34

n = 12 in all cases, significance level: * $P < 0.05$

Densities of *D. carinata* started to increase in the autumn and were highest in the spring. The population started to decline in late spring and virtually disappeared during the summer. Similar patterns were observed in both ponds. No distinct morphological differences were observed for *D. carinata* in all the samples examined. Despite the absence of *D. carinata* from the ponds when water temperatures were higher than 20 °C, no significant correlation between temperature and abundance of *D. carinata* was observed (Table 3.3). Interestingly, when *D. carinata* was present there were significant correlations between *D. carinata* abundance and the chlorophyll *a* content of previous months when the influence of other physical factors were removed (Partial correlation between *D. carinata* and chlorophyll a content while controlling for pH, temperature and dissolved oxygen). Strongest correlations were observed between *Daphnia* abundance and chlorophyll *a* content three months previously for pond 7 (r =

0.80, d.f. = 6, $P < 0.05$) and one month previously for pond 8 ($r = 0.85$, d.f. = 6, $P < 0.05$). Generally, highest chlorophyll *a* contents occurred before the density peaks of *D. carinata* which coincided with a drop in chlorophyll *a* content.

The seasonal pattern of *Moina australiensis* in pond 7 and pond 8 was less obvious than *D. carinata* and the correlation between the density of *Moina australiensis* and temperature was not significant (Table 3.3). In pond 7 when the influence of the physical factors was removed from the analysis, the abundance of *Moina australiensis* was found to be positively correlated with chlorophyll *a* content recorded three months previously in pond 7 ($r = 0.78$, d.f. = 6, $P < 0.05$) and that recorded in the same month in pond 8 ($r = 0.75$, d.f. = 7, $P < 0.05$). Interestingly, in pond 7 where chlorophyll *a* content was generally high, the abundance of *Moina australiensis* peaked in October 93 and there was a significant correlation between the abundances of *Moina australiensis* and *D. carinata* ($r = 0.70$, $n = 12$, $P < 0.01$). However, the correlation became insignificant when the influence of chlorophyll *a* content was removed from the analysis ($r = 0.18$, d.f. = 8, $P > 0.5$). This indicates that the population densities of the two cladocerans varied in parallel with the fluctuations in the chlorophyll *a* content. In contrast, density of *Moina australiensis* in pond 8 peaked in January when *D. carinata* was completely absent, and when the two cladoceran species coexisted, *Moina australiensis* was usually rare.

The copepod *Mesocyclops australiensis* occurred in the ponds throughout the year and exhibited a bimodal pattern with peaks in the autumn and spring. Similar density patterns were observed in both ponds and these were not related to temperature (Table 3.3). In general, density peaks of *Mesocyclops australiensis* coincided with those of rotifers, except for the spring peak in pond 7 which coincided with that of the cladocerans.

Rotifers were absent from the ponds for most of the year, but their densities could reach as high as 1,000 individuals per litre during late spring and summer when the densities of the cladocerans were low. The sudden drop in the population density of both species in January coincided with the peak in *Moina australiensis* densities in both ponds. Although no significant correlation was found between chlorophyll *a* content and the algivorous rotifer *B. calyciflorus* in both ponds ($r < 0.23$, $n = 12$, $P > 0.1$), it is worth noting that the appearence of the rotifers followed the replacement of Euglenophyceae by the Chlorophyceae and the disappearance of the cladocerans.

3.4 DISCUSSION

3.4.1 Seasonal changes in phytoplankton communities

The phytoplankton communities in this study shared a similar composition with other waste stabilization ponds studied elsewhere (e.g. Singh & Saxena, 1969; Patil *et al.*, 1975). Indeed, Benson-Evans and Williams (1975) pointed out that the algal composition was usually very similar among waste stabilization ponds, with *Chlorella, Scenedesmus, Chlamydomonas* and *Euglena* as the most common and cosmopolitan algal genera.

Despite the general consistency of algal communities in waste stabilization ponds in different geographical areas, the composition and abundance of the phytoplankton varied widely in the Werribee pond system throughout the year. During winter (July and August), a peak in chlorophyll *a* content was observed and the phytoplankton collected in that period were dominated by Euglenophyceae. This increase in phytoplankton abundance was attributed to the prolonged detention time (see chapter 2). During periods of low light intensity, Euglenophyceae, being able to migrate vertically with the flagella, could have stayed at the surface zone with strongest irradiation, and thus blocked the light penetration for the other phytoplankton group. These rendered them more competitive than the other phytoplankton, particularly during periods with low photosynthetic rate. It is worth noting that despite the mixing effect of wind action, the chlorophyll *a* content recorded in this study could have been overestimated because of the migration of the Euglenophyceae to the surface at day time.

The marked increase in phytoplankton abundance was followed by a peak in zooplankton density in September, presumably due to the increased food availability. The increase in grazing pressure resulted in a sudden drop in the chlorophyll *a* content. It is believed that grazing pressure on the phytoplankton population will select for the large and inedible algae as they are not as heavily grazed as the smaller species (Porter, 1973; McCauley & Briand, 1979; Lynch & Shapiro, 1981). It is particularly evident in Cyanophyceae (blue-green algae) which are unpalatable to zooplankton due to their toxicity, nutritional inadequacy and existence in large filamentous colonies (Hanazato, 1991). In Werribee, when the grazing pressure was highest in September and October, the phytoplankton biomass became lowest. During this time, Cyanophyceae (mainly *Microcystis*) was the only algal group present, indicating that they were able to escape grazing by the zooplankton. However, the phytoplankton community became rapidly dominated by Chlorophyceae which mainly consisted of *Chlorella* and *Scenedesmus* following a decline in the grazing pressure in the summer. The dominance of Chlorophyceae during the summer was mainly attributed to their tolerance to ammonia. Indeed, Konig *et al.* (1987) found that *Chlorella* was more tolerant to ammonia than *Euglena* in laboratory growth studies. During the summer, the ammoniacal nitrogen in the study ponds could be as high as 30mg/L which excluded the phytoplankton species with low ammonia tolerance.

It is possible that in stabilization ponds where *in situ* nutrient concentrations are high and the nutrients regenerated by the zooplankton excretion may be less significant, competition among inorganic nutrients may not be an important determinant for the phytoplankton changes. For instance, the Euglenophyceae has a smaller area to volume ratio than the Chlorophyceae and this should be a disadvantage for competing for the ambient inorganic nutrients. However, this did not seem to limit their proliferation during the winter. Similarly, Cyanophyceae did not gain much advantage from their ability to fix atmospheric nitrogen.

3.4.2 Seasonal changes in zooplankton communities

The zooplankton communities in the study ponds at Werribee were dominated by cladocerans (particularly *Daphnia, Moina)*, cyclopoid copepods and rotifers (e.g. *Brachionus* and *Asplanchna*). Calanoid copepods were not recorded from the samples taken in the stabilization ponds at Werribee (cf. Hussainy, 1979), and this is consistent with a general decline in the relative abundance of these animals in natural water bodies as nutrient levels increase (McNaught, 1975).

Temperature has been considered as the single most important factor regulating zooplankton population in waste stabilization ponds. It may affect individual animals directly or indirectly through its influence on pH, food availability and dissolved oxygen levels (Loedolff, 1965). Moreover, differences in temperature adaptation among various species could cause a reversal in competitive ability which may account for the seasonal changes in zooplankton communities [see review by DeMott (1989)]. Previous studies in Australia suggested that temperature was the major factor shaping seasonal patterns of *Daphnia carinata* (e.g. Timms, 1970; Mitchell & Williams, 1982a, 1982b; Kobayashi, 1992). Significantly, Mitchell & Williams (1982a) observed higher mortality for *D. carinata* when the animals were cultured at 24.5 °C in the laboratory as compared with those kept at 10, 14.5 and 18 °C. Moreover, a significant correlation between water temperature and population density of *D. carinata* has been reported by Mitchell & Williams (1982b). In this study, despite the apparent absence of *D. carinata* at higher temperatures, no direct negative correlation between water temperature and *Daphnia* abundance was observed, suggesting that temperature may only be important in setting a upper tolerance limit (around 20 °C). This phenomenon might be attributed to the influence of temperature on the feeding rates or the balance between energy intake and metabolic demands of zooplankton (Hebert, 1978). Particularly, previous studies have shown that the metabolic requirements increase more rapidly than feeding rates in *Daphnia pulex* when the temperature is higher than 20 °C, and that larger individuals are more adversely affected (Lampert, 1977; Lynch, 1977). Indeed, *D. carinata* has been regarded as a 'cool water' species (*sensu* Hebert, 1977a), typically with highest abundances in autumn, winter or early spring. These animals are usually absent in the summer and they re-emerge in the autumn from ephippial eggs.

Contrary to the seasonal patterns of *Daphnia carinata* described above, Huang & Hu (1984) reported a summer peak in the population densities of *D. carinata* ssp. in Donghu Lake, central China, when the temperatures were as high as 30 °C. Furthermore, *D. carinata* was also recorded in a seasonal pond in India where temperatures ranged from 20 to 37 °C (Venkataraman, 1981). These observations suggest that tropical *D. carinata* might have evolved different adaptations to temperature stress as compared to its temperate counterpart.

Similar to the present study, Dinges (1982) found that populations of *Daphnia* in stabilization ponds in Texas also appeared in early winter and disappeared in late spring, but concluded that the dominant environmental factor affecting the abundance of *Daphnia* was photoperiod. It was suggested that the longer period of bright sunlight in the summer would enhance photosynthesis and result in elevated pH and increased levels of ammonia (NH_3) which could be detrimental to the cladocerans.

Despite the important influence of physical factors on the seasonal pattern of *Daphnia* populations recorded in previous studies, it is apparent that in the stabilization ponds at Werribee where food concentration varied widely throughout the year, availability of food might play a significant role in controlling the abundance of *D. carinata*. This hypothesis is supported by the strong correlations between the population density of *D. carinata* and chlorophyll *a* content in both pond 7 and pond 8. Higher phytoplankton abundance in July and August provided better food conditions for *D. carinata* and resulted in an increase in the mean brood size of the reproductive females (see chapter 4). Consequently, population density peaked in the following months as a result of pronounced juvenile recruitments. The intense grazing during September and October reduced substantially the phytoplankton biomass and led to a drastic decline in *D. carinata* density in November.

Although *D. carinata* is a facultative browser (see Mitchell & Williams, 1982a) and can switch from phytoplankton grazing to browsing or foraging on sedimented detritus, this detrital material is generally less suitable for promoting cladoceran growth (Horton *et al.*, 1979). Indeed, the close association between the population density of

D. carinata and phytoplankton abundance observed in the present study indicates that phytoplankton might be the more important food source than the virtually unlimited sedimented detritus in the stabilization ponds. Thus, it is likely that despite the high nutrient levels in the stabilization ponds, the zooplankton population at Werribee could still at times be food-limited (cf. Daborn *et al.*, 1978; Mitchell & Williams, 1982a). It is also worth noting that the sampling method employed in this study, which did not include the water layer immediately above the sediment (cf. Mitchell & Williams, 1982a), might have underestimated the population density of the zooplankton during periods of low phytoplankton biomass as some cladocerans might have shifted to the bottom of the ponds to feed on the sedimented detritus (Horton *et al.*, 1979). However, as the density peak of the cladocerans was observed during periods of low phytoplankton biomass, even if the sampling method included the water stratum just above the sediment, only a higher density peak of cladocerans might have been obtained. It is unlikely that a different seasonal pattern of cladoceran abundance would have been resulted.

The other sympatric cladoceran *Moina australiensis* did not exhibit marked seasonal fluctuations in the stabilization ponds at Werribee and its abundance was not related to temperature. Indeed, the population density of this filter feeder was closely associated with chlorophyll *a* content as revealed by correlation analyses. For instance, the results show that the population densities of *Moina australiensis* and *D. carinata* in pond 7 fluctuated in parallel with the variation in food availability. Conversely, in pond 8 where food density was relatively low, abundance of *Moina australiensis* was suppressed by *D. carinata* suggesting that the two species might have competed for the same resource. Possible niche overlap between these two species has been examined by comparing their reproductive patterns and will be described in chapter 4. Interestingly, unlike the pattern in pond 7, the density peaks of the two cladocerans in pond 8 did not coincide with each other, but appeared in sequence. This pattern may be an example of temporal niche partitioning exhibited by competing species under conditions of low food availability (Pennak, 1957; Hutchinson, 1961), thus lending support to the hypothesis that interspecific interactions in general, and competition in particular, could be important in determining the seasonal patterns of different zooplankton species in the present study.

Bayly & Williams (1973) found no consistency in the seasonal variations of *Mesocyclops australiensis* across different water bodies in Australia. Similarly, our data revealed no correlation between *Mesocyclops australiensis* abundance and temperature. Instead, the seasonal pattern of these raptorial copepods was closely related to the availability of their food resources. Density peaks of *Mesocyclops australiensis* generally coincided with those of rotifers in both ponds indicating that the copepods might prey on the rotifers which are small in size (Bayly & Williams, 1973). Significantly, the density peak of *Mesocyclops australiensis* in October 93 coincided with that of the two cladocerans, suggesting that juvenile cladocerans might also be an important food source for the predatory copepods (Lynch, 1979).

At Werribee, *Brachionus calyciflorus* was extremely abundant in the summer. By contrast, Daborn *et al.* (1978) reported that *B. calyciflorus* was numerous in spring and was rare or absent in the summer when cladocerans were abundant in a Canadian oxidation pond. The same negative relationship between the population densities of the cladocerans and the rotifers was also observed in this study, suggesting that interspecific interactions may, again, be more important than temperature in controlling the abundance of these animals. In late autumn and winter when Euglenophyceae (size 30-50 μm) were the dominant phytoplankton group, the large filter feeders (*D. carinata* and *Moina australiensis*) were abundant and the small particle feeders (*B. calyciflorus*) were rare. *B. calyciflorus* feeds mainly on small algae such as *Chlorella* and *Scenedesmus* (Hutchinson, 1967) and may not be able to handle the larger euglenoids. Significantly, Doohan (1975) reported that, for brachionids, the size of the food particles reaching their jaws was limited to less than 12 μm due to the weak centrifugal action of the corona. As a consequence, the rotifers could be starved and replaced by the large cladocerans during the period when Euglenophyceae were the dominant phytoplankton. Large cladocerans in this case possess the competitive advantages not only in faster filtering rates (Brooks & Dodson, 1965) but also in the ability to consume food particles of a wider size spectrum (Burns, 1968; Kobayashi, 1991). Indeed, the dominance of large filter feeders such as *D. carinata* in the stabilization ponds at Werribee supported the 'size-efficiency hypothesis' of Brooks & Dodson (1965) which states that without the planktivorous fish to prey on the larger and more conspicuous zooplankton, the larger-bodied zooplankton, being more efficient filter feeders, would out-compete their smaller-bodied counterparts. Interestingly, Romanovsky (1984a, 1984b, 1985) proposed that small zooplankton which require a lower threshold food level should be more competitive as compared with large individuals when food is limiting. It is worth noting that results of this study do not contradict Romanovsky's hypothesis as food supply in the stabilization ponds is usually non-limiting. Moreover, the removal of the smaller species by the predatory copepods might also lead to the dominancy of the larger species in the present system (Confer, 1971; Kerfoot, 1977).

As mentioned earlier, the rotifers were highly abundant during the summer when the cladocerans were absent. This increase in the density of *B. calyciflorus* could be attributed to a change in the dominant phytoplankton groups (from the larger Euglenophyceae to the smaller Chlorophyceae such as *Chlorella* and *Scenedesmus*), coupled with the subsequent decline in the number of larger-bodied competitors (see above). This change reflects perfectly a specialized tracking of phytoplankton succession by the zooplankton (DeMott, 1989). Carnivorous *A.* cf. *sieboldi* appeared in high densities when the density of *B. calyciflorus* peaked, and this suggests that *A.* cf. *sieboldi* might have been preying on the smaller *B. calyciflorus* (Lynch, 1979). It is worth noting that, during the decline of the cladoceran population in late spring, attachment of *B. calyciflorus* on the antennae and the bodies of cladocerans was commonly observed. Such epizoic infestations could have also played a significant role in controlling the cladoceran numbers.

4. Factors influencing reproductive characteristics of sympatric zooplankton in waste stabilization ponds

4.1 INTRODUCTION

In chapter 3, I described the seasonal changes of zooplankton communities in the two waste stabilization ponds at Werribee. It was suggested that high temperature limits the occurrence of the dominant cladoceran, *Daphnia carinata*. Below this limit, the seasonal changes in the abundance of *D. carinata* were mainly attributed to the variations in phytoplankton abundance. Similarly, the fluctuations in the abundances of other common zooplankton species, namely *Moina australiensis* and *Mesocyclops australiensis*, were attributed to biotic factors such as variations in food availability, and intraspecific and interspecific interactions.

Green (1966) suggested that food, temperature, dissolved oxygen as well as the population density were the major factors which influence the reproductive dynamics of natural zooplankton communities. Hebert (1977b) proposed that an examination of the reproductive characteristics of zooplankton communities would provide useful insights into the possible agents which regulate their seasonal changes in abundance. Here, in an attempt to elucidate how various biotic and abiotic factors may influence the temporal and spatial patterns of the zooplankton species, I examine the effects of environmental factors (e.g. temperature) and ecological circumstances (e.g. food availability, and intraspecific and interspecific interactions) on some key fitness traits, including body size, brood size and the reproductive status of mature females, of three common zooplankton species namely *Daphnia carinata, Moina australiensis* and *Mesocyclops australiensis* inhabiting the Werribee stabilization ponds. I will also explore the possible mechanisms which may have allowed zooplankton species with similar niches to co-exist in a nutrient-rich environment.

4.2 MATERIALS AND METHODS

The study was undertaken in the last two maturation ponds (pond 7 and pond 8) of an eight-pond treatment series (145W) at the Werribee Treatment Complex. Zooplankton samples were collected monthly between March 1993 and February 1994. All individuals in the zooplankton samples were identified and enumerated under a dissecting microscope. Details of the study area and the sampling procedure are given in chapters 2 and 3 respectively.

4.2.1 Measurement of body size

For each of the two cladoceran species (*Daphnia carinata* and *Moina australiensis*), one hundred individuals chosen randomly from the pooled sample on each sampling date were used for size-frequency analyses. The size of each individual, defined as the distance between the crown of the head to the posterior border of the carapace excluding the spine if present (cf. Green, 1966; Smirnov & Timms, 1983; Benzie, 1988), was measured to the nearest 0.1 mm with a graticuled eyepiece on a stereomicroscope. Fifty brooding females were randomly chosen for measurement of body size and brood size (see section 4.2.2). All individuals were used when the sample size was less than 50.

Since there is no visible morphological change at the acquisition of reproductive maturity, mature adults are defined as individuals larger than the smallest reproductive females observed in the population (Hebert, 1978)

4.2.2 Investigation of reproductive characteristics

The number of brooding females, ephippial females and males were counted for the cladocerans. Brooding cladocerans preserved in formalin for an extended period of time often have ruptured brood chambers which may lead to an underestimation of the number of brooding females and their brood size (Prepas & Rigler, 1978). Consequently, fresh samples were kept at 4 °C and processed as soon as possible after they were killed in 5% formalin. The brood chambers of individual females were dissected under a stereomicroscope and the brood size, defined as the number of eggs or embryos in the brood chamber of a mature female, determined. Only females with closed (intact) carapace were used in this analysis. The percentage of gravid females was also recorded for the copepod, *Mesocyclops australiensis*.

It is conceivable that variations in mean brood size of cladocerans could be closely related to their body sizes. To compare the brood size between the individual brooding females of *Daphnia carinata* and those of *Moina australiensis*, it is necessary to remove the confounding effect due to the differences in body size among the individuals. To achieve this, the relationships between female size and brood size were examined and the mean brood size for a standard-sized female determined following Hebert (1977b).

4.3 RESULTS

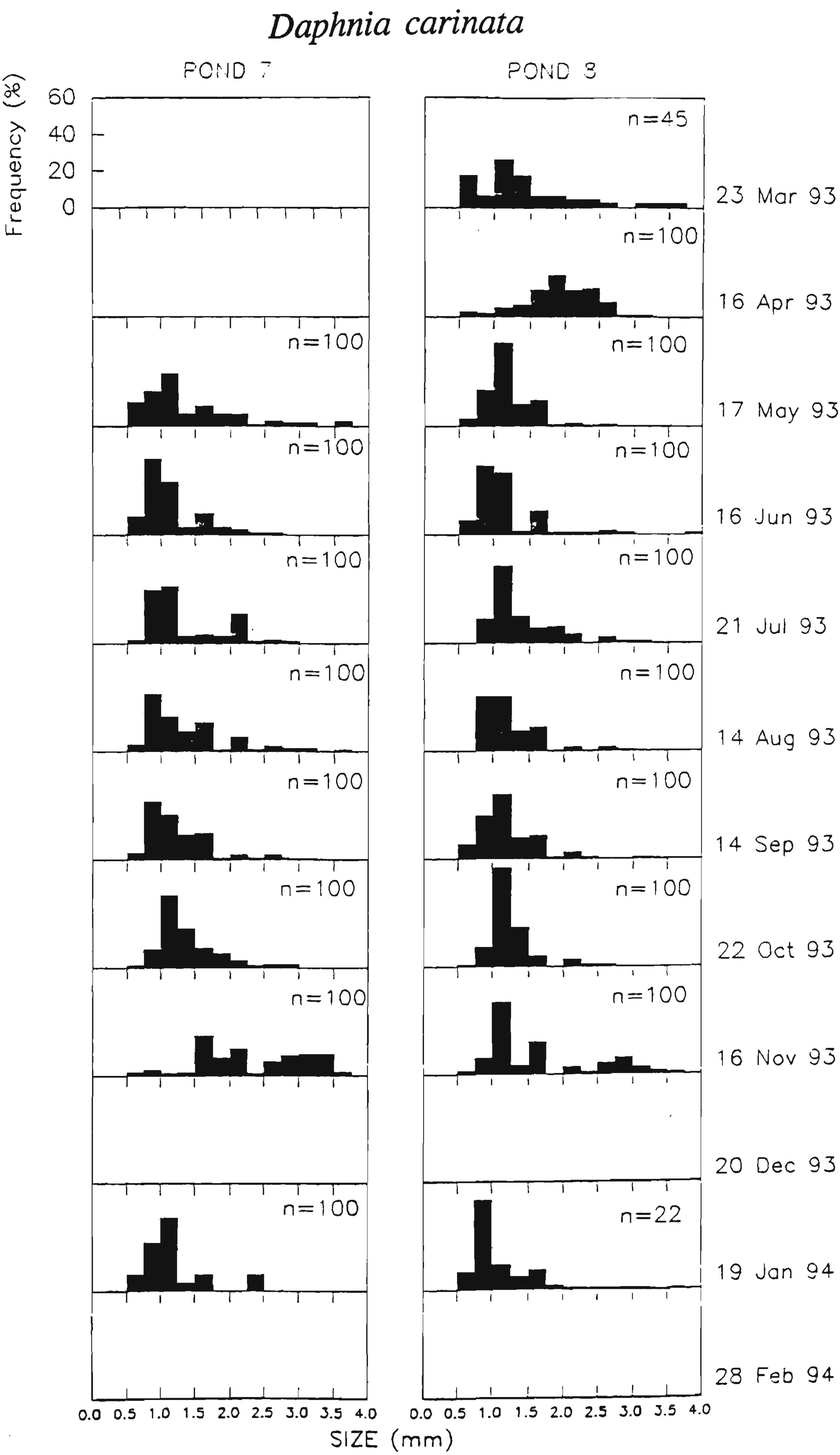
4.3.1 Body size of *Daphnia carinata* and *Moina australiensis*

The observed minimum lengths of brooding females for *D. carinata* and *M. australiensis* were 2.1 mm and 1.1 mm respectively, thus animals greater than these sizes were considered as mature adults.

Size-frequency histograms for *D. carinata* are shown in Fig. 4.1. Although individual cohorts cannot be identified clearly from the histograms, our data revealed that recruitment for *D. carinata* was almost continuous, with no distinct breeding seasons. March samples collected from pond 8 at the re-emergence of the populations contained a high percentage of large individuals. A similar population structure was observed when *D. carinata* first appeared in pond 7 in May. As the population grew, the percentage of juveniles increased as a result of pronounced recruitment, and mature adults were relatively small at that time. As the *Daphnia* populations started to decline in November, they were again consisted of a high proportion of relatively large individuals.

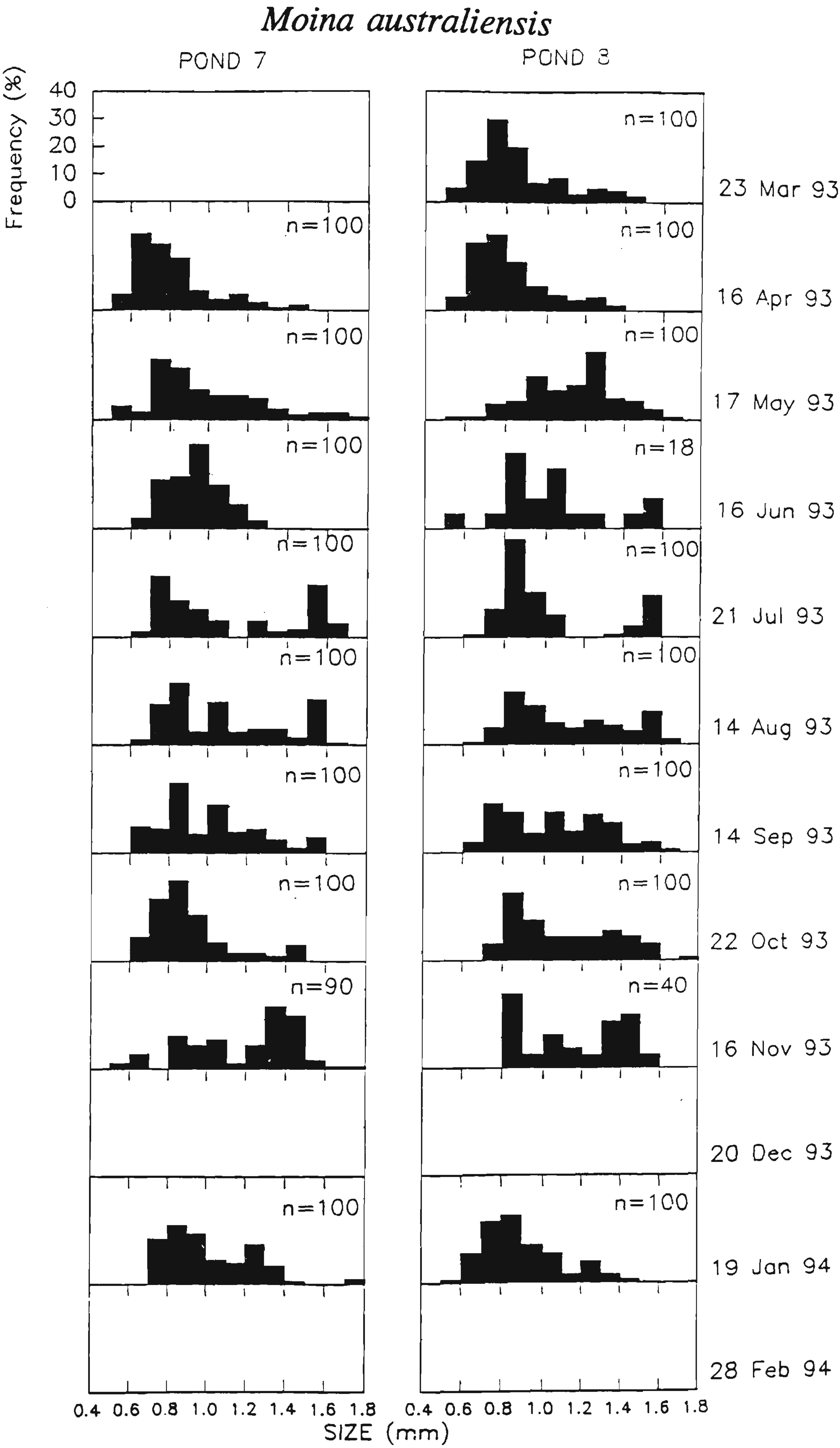
For *Moina australiensis*, active recruitments occurred in April, May and November in Pond 7, while most juveniles were released between March and June in pond 8 (Fig. 4.2). Large mature adults were abundant between July and November in pond 7, and between May and November in pond 8. Juveniles were well represented in the populations throughout the year, with larger individuals appearing before and after the peaks in population densities (cf. Fig. 3.2).

Fig. 4.1 Size-frequency histograms of *Daphnia carinata* in pond 7 and pond 8 over the period March 1993 to February 1994. Empty graphs indicate the months when *D. carinata* was absent or sample size was too small for analysis.



Daphnia carinata
POND 7
POND 8
Frequency (%)
60
40
20
0
n=45
23 Mar 93
n=100
16 Apr 93
n=100
n=100
17 May 93
n=100
n=100
16 Jun 93
n=100
n=100
21 Jul 93
n=100
n=100
14 Aug 93
n=100
n=100
14 Sep 93
n=100
n=100
22 Oct 93
n=100
n=100
16 Nov 93
20 Dec 93
n=100
n=22
19 Jan 94
28 Feb 94
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0
SIZE (mm)

Fig. 4.2 Size-frequency histograms of *Moina australiensis* in pond 7 pond 8 over the period March 1993 to February 1994. Empty graphs indicate the months when *M. australiensis* was absent or sample size was too small for analysis.



Moina australiensis
POND 7
POND 8
Frequency (%)
40
30
20
10
0
n=100
23 Mar 93
n=100
n=100
16 Apr 93
n=100
n=100
17 May 93
n=100
n=18
16 Jun 93
n=100
n=100
21 Jul 93
n=100
n=100
14 Aug 93
n=100
n=100
14 Sep 93
n=100
n=100
22 Oct 93
n=90
n=40
16 Nov 93
20 Dec 93
n=100
n=100
19 Jan 94
28 Feb 94
0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8
0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8
SIZE (mm)

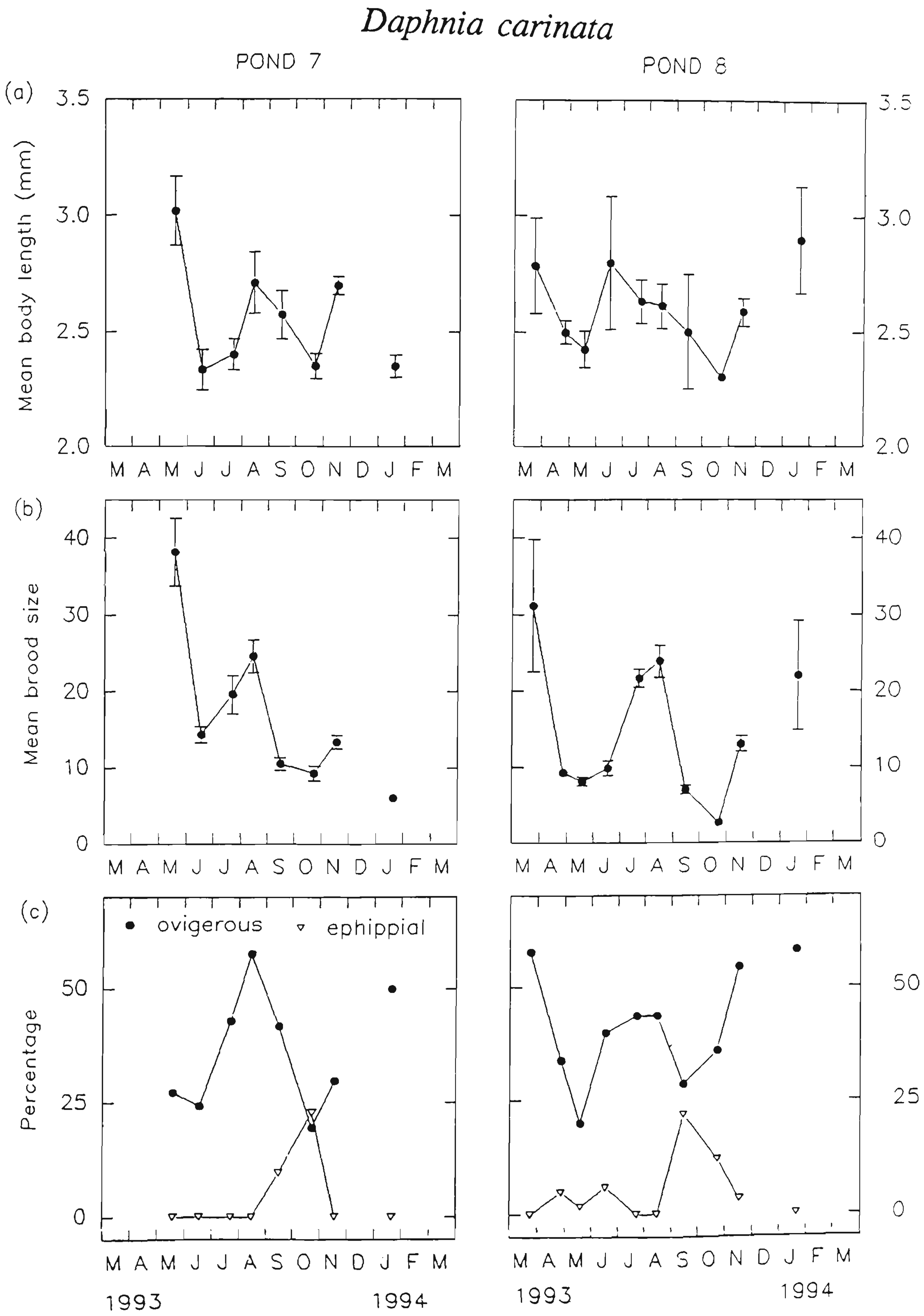
Seasonal variations of the mean size of mature females of *Daphnia carinata* and *Moina australiensis* are shown in Figs 4.3 and 4.4 respectively. The mean sizes of mature females of *D. carinata* and *Moina australiensis* were generally large at the onset of the populations and became smaller in the autumn. During winter months when chlorophyll *a* contents were high (Fig. 3.1), mean size of mature females reached a maximum and then decreased gradually until the following spring.

4.3.2 Dynamics of brood size, ovigerous and ephippial females

Seasonal variations of the mean brood size (i.e. the number of eggs/embryos per reproductive female) and the percentage of ovigerous and ephippial females (expressed as a percentage of the total number of mature females) of *Daphnia carinata* and *Moina australiensis* are summarized in Figs 4.3 and 4.4 respectively. The percentage of ovigerous females and mean brood size of *Daphnia carinata* were generally high at the onset of the population. Similar to body size, the percentage of ovigerous females and mean brood size reached a maximum in the winter, coinciding with a peak in chlorophyll *a* content, and then dropped to a minimum in the following spring when the population densities were highest (see Chapter 3). During that time, the percentage of ephippial females increased significantly. Similar patterns were observed in both ponds except that in pond 8 the percentage of ovigerous females was generally lower and the occurrence of ephippial females was more frequent as compared with pond 7. Male *D. carinata* appeared between May and November, accounting for 3 to 6% of the total population.

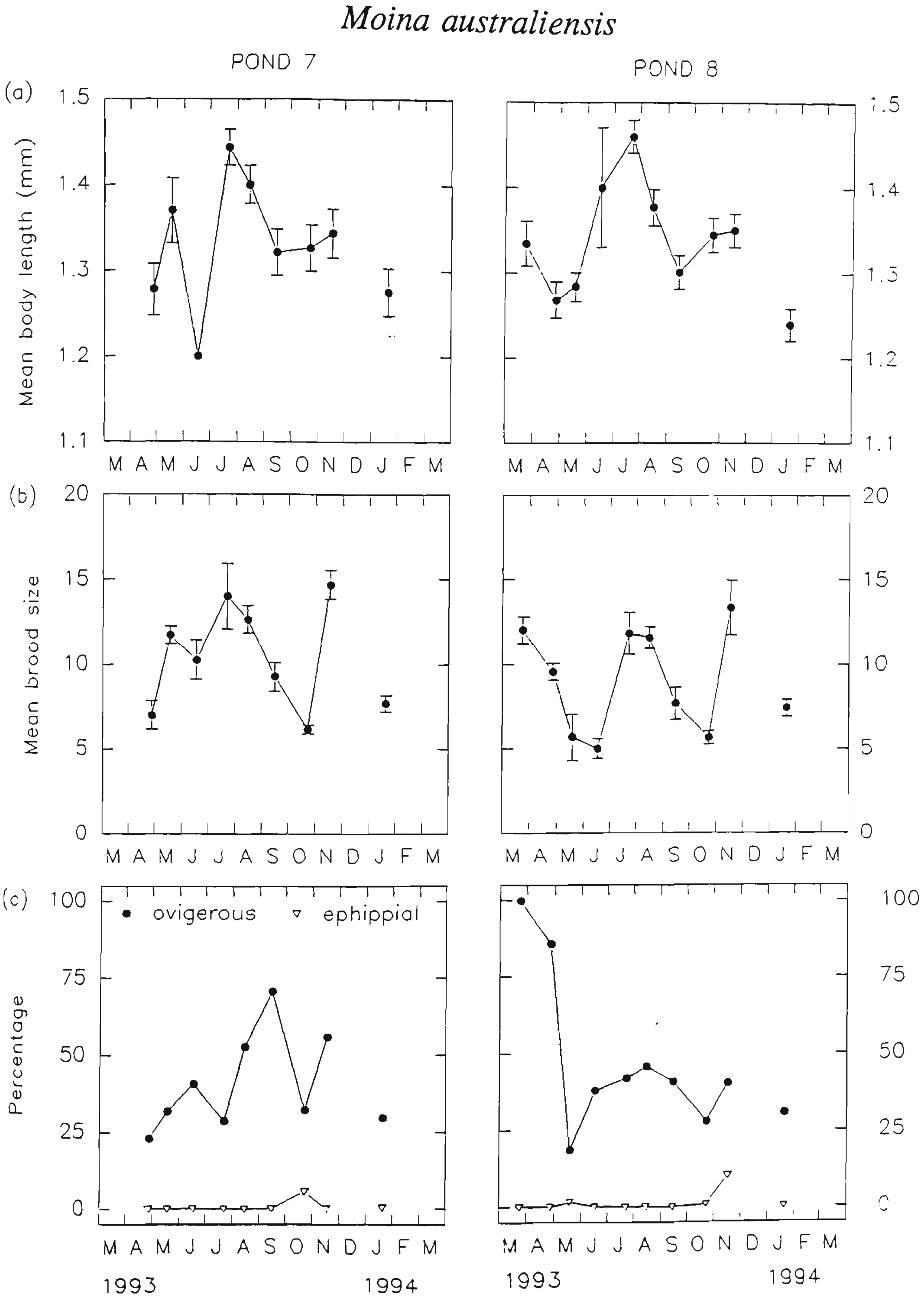
A similar pattern was also observed for *Moina australiensis* in pond 7 and pond 8

Fig. 4.3 Seasonal variations of (a) mean body length, (b) mean brood size and (c) percentage of ovigerous and ephippial females (expressed as a percentage of the total number of mature females) of *Daphnia carinata* in pond 7 and pond 8 over the period March 1993 to February 1994. Vertical lines are 1 S.E.. *D. carinata* was absent from the December 1993 and February 1994 samples.



Daphnia carinata
POND 7
POND 8
(a)
(b)
(c)
Mean body length (mm)
Mean brood size
Percentage
ovigerous
ephippial
M A M J J A S O N D J F M
1993
1994

Fig. 4.4 Seasonal variations of (a) mean body length, (b) mean brood size and (c) percentage of ovigerous and ephippial females (expressed as a percentage of the total number of mature females) of *Moina australiensis* in pond 7 and pond 8 over the period March 1993 to February 1994. Vertical lines are 1 S.E.. *M. australiensis* was absent from the December 1993 and February 1994 samples.



Moina australiensis
POND 7
POND 8
(a)
Mean body length (mm)
1.5
1.4
1.3
1.2
1.1
M A M J J A S O N D J F M
(b)
Mean brood size
20
15
10
5
0
(c)
Percentage
100
75
50
25
0
ovigerous
ephippial
1993
1994

where the mean brood size peaked in the winter and dropped to a minimum in October when the population density peaked. Significantly, strong correlations between the mean brood size of *Moina australienses* and that of *D. carinata* were observed in pond 7 ($r = 0.86$, $n = 5$, $P < 0.05$) and pond 8 ($r = 0.90$, $n = 6$, $P < 0.01$). In pond 7, the percentage of ovigerous females of *Moina australiensis* was highest in winter when the chlorophyll *a* contents peaked and started to drop in October. In contrast, the percentage of the ovigerous females in pond 8 remained low after the onset of the population. The ephippial females in pond 7 and pond 8 started to emerge in October and November respectively when food density was low. Male *Moina australiensis* was recorded in high numbers (9% of the total population) in Pond 7 in October when the population density peaked. They were observed again in both ponds in January when there was a sudden increase in population size.

Seasonal variations of the percentage of gravid females of *Mesocyclops australiensis* are shown in Fig. 4.5. These copepods had the highest percentage of reproductive females in the summer when rotifers were abundant.

Table 4.1 summarizes the life-history characteristics of *D. carinata* and *Moina australiensis*. *D. carinata* has a much larger adult size and brood size than *Moina australiensis* while the body sizes of their juveniles are similar. Moreover, *Moina australiensis* attained maturity at a smaller size. Males are smaller than females in both species.

Fig. 4.5 Seasonal variations of the percentage of the gravid females of *Mesocyclops australiensis* over the period March 1993 to February 1994. Vertical lines are 1 S.E..

Mesocyclops australiensis

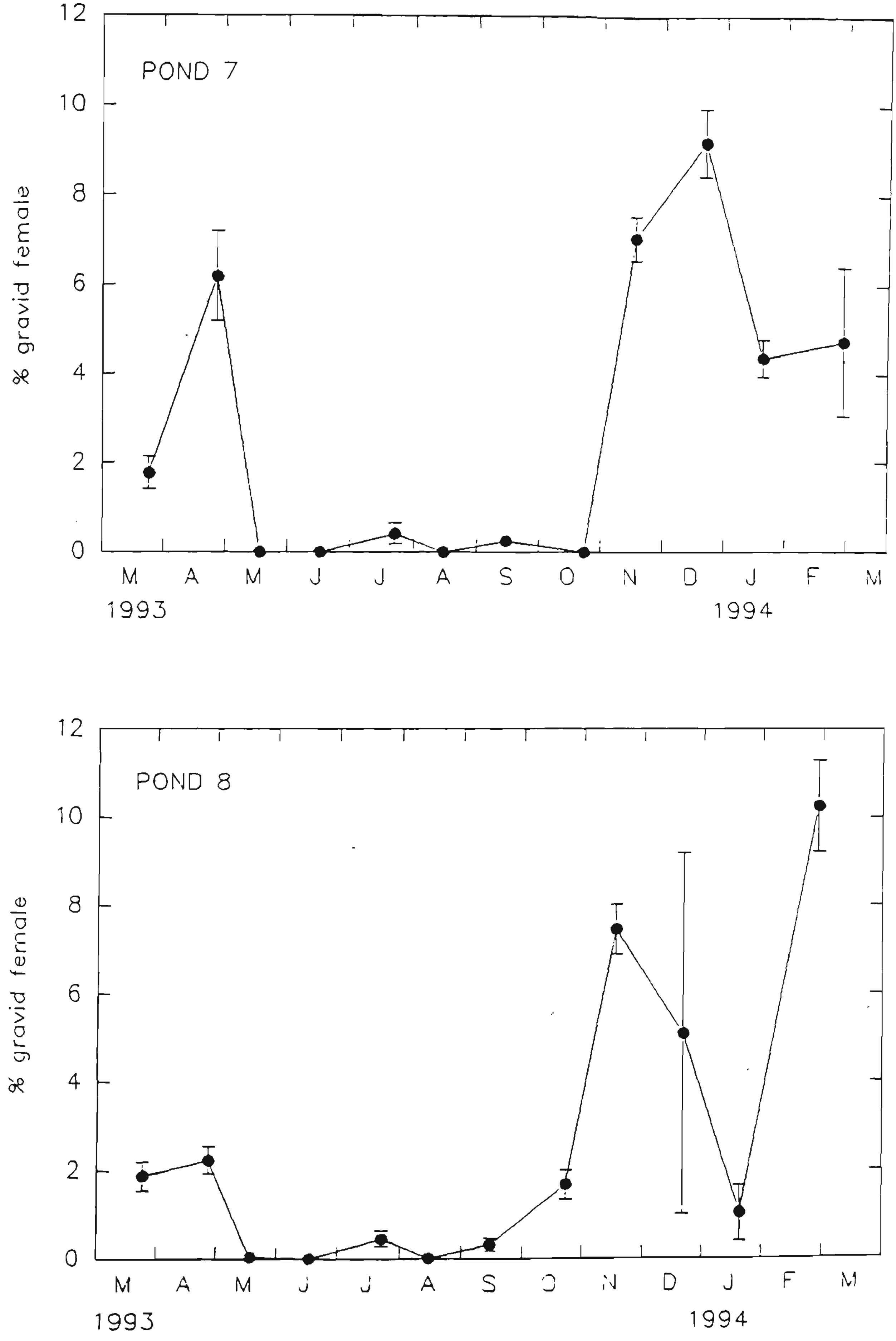


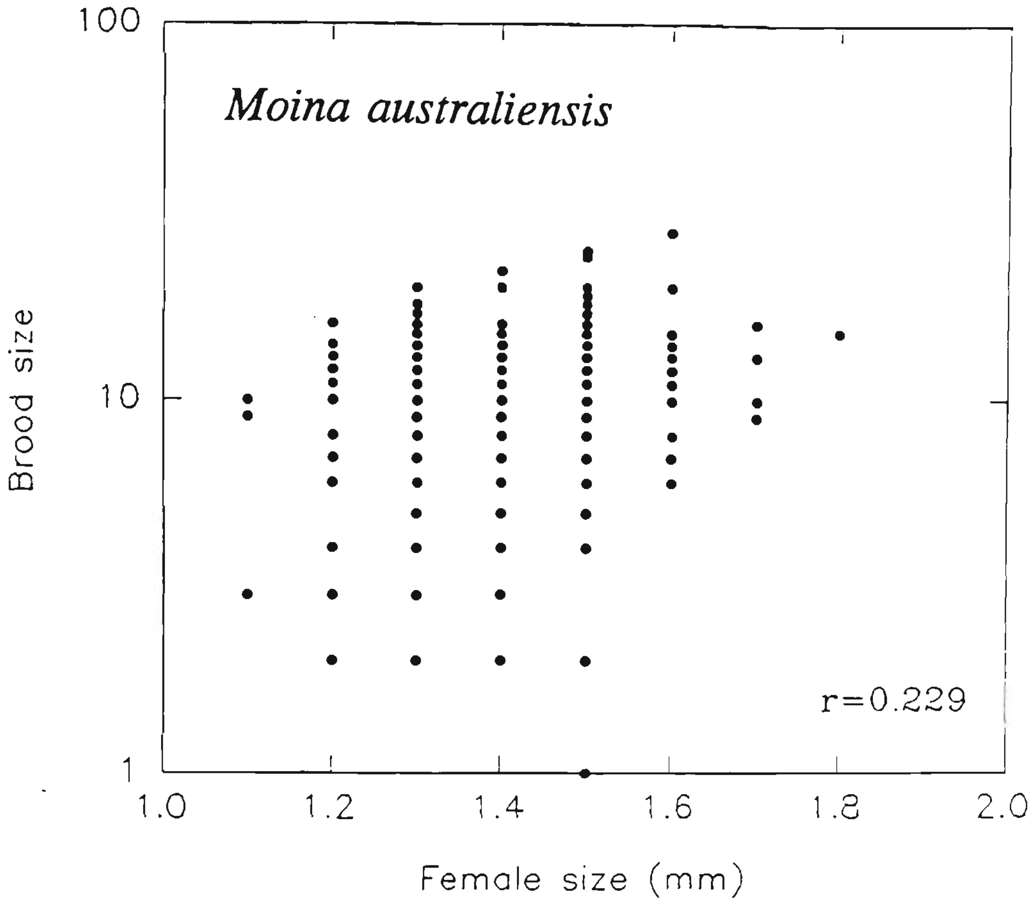
Table 4.1. Life-history characteristics of *Daphnia carinata* and *Moina australiensis* in the waste stabilization ponds at Werribee. (Note: Mature females are defined as individuals larger than the smallest reproductive female observed in the population.)

	D. carinata	*M. australiensis*
Size at birth (mm)	0.7	0.5
Maximum female size (mm)	4.2	1.8
Maximum male size (mm)	2.0	0.9
Minimum reproductive female size (mm)	2.1	1.1
Mean mature female size (± S.E.) (mm)	2.6 ± 0.049	1.3 ± 0.015
Maximum brood size	68	20
Average brood size (± S.E.)	15.8 ± 2.2	9.6 ± 0.70
Number of eggs in ephippium	2	2

4.3.3 Relationship between female size and brood size

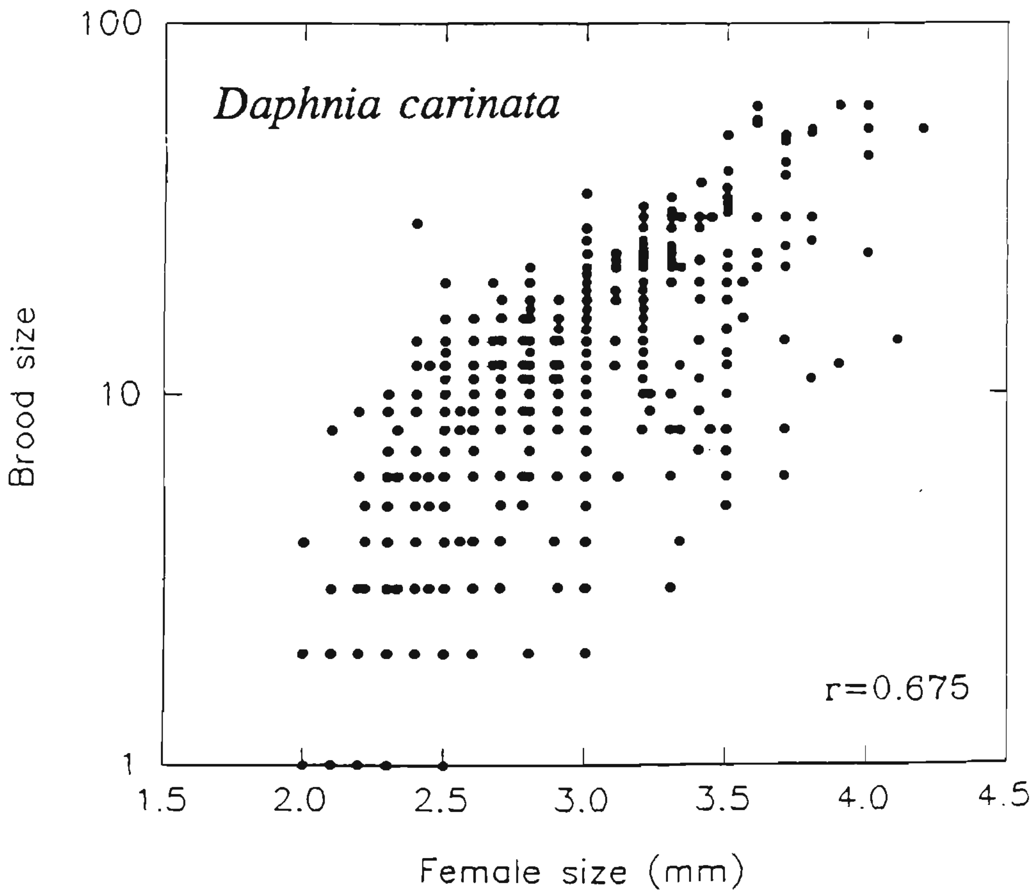
The relationships between brood size and body size of *D. carinata* and *Moina australiensis* based on pooled samples from both ponds over the entire study period are given in Fig. 4.6. The correlation coefficient (r) between body size and brood size of *Moina australiensis* was 0.229 ($n = 412$), indicating that changes in body size only account for 5% of the variations in brood size. Similar analyses based on monthly data revealed no significant correlations. By contrast, there is a much stronger correlation between brood size and body size of *D. carinata* ($r = 0.68$, $n = 533$, $P < 0.001$). Further analyses using monthly data from pond 8 revealed that the correlations between

Fig. 4.6 Relationships between the brood size (logarithmic scale) and the female body size of (a) *Moina australiensis* and (b) *Daphnia carinata*.



Moina australiensis
r=0.229
Brood size
100
10
1
1.0
1.2
1.4
1.6
1.8
2.0
Female size (mm)

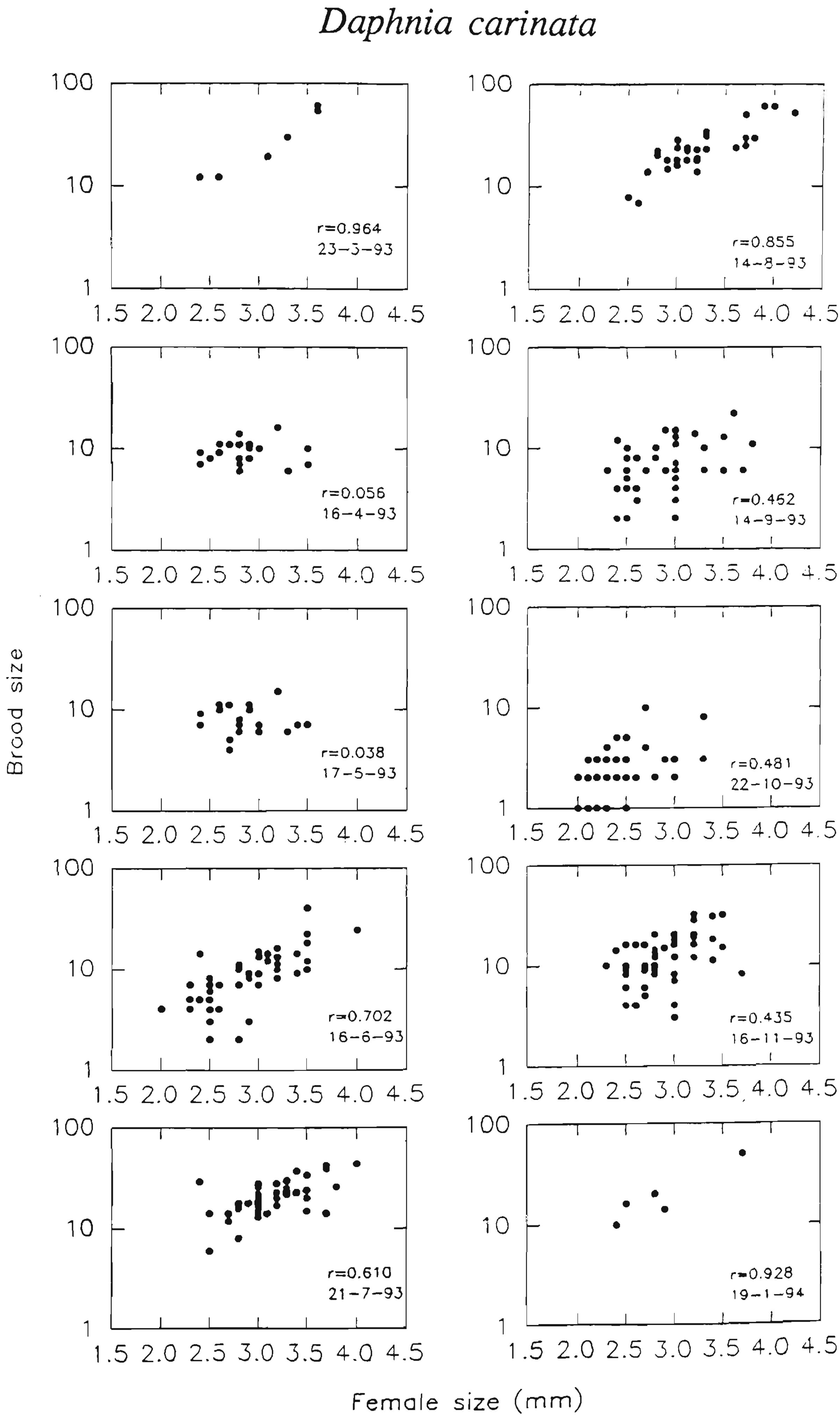
(b)



Daphnia carinata
r=0.675
Brood size
100
10
1
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Female size (mm)

brood size and body size were higher at lower population densities e.g. January and March, but lower at higher population densities e.g. September and October (Fig. 4.7), indicating that in a dense population where food availability is relatively scarce, body size does not have such a strong influence, if at all, on brood size. The seasonal variations of the mean brood size of a standard-sized female *D. carinata* (body length = 2.5 mm) were also examined by regressing brood size against body size using data collected each month (Fig. 4.8). It is apparent that the variations of brood size were not solely attributable to changes in body size. Instead, the peak of the brood size of standard-sized female coincided with that of the chlorophyll *a* content indicating the strong influence of food availability on the brood size of the female.

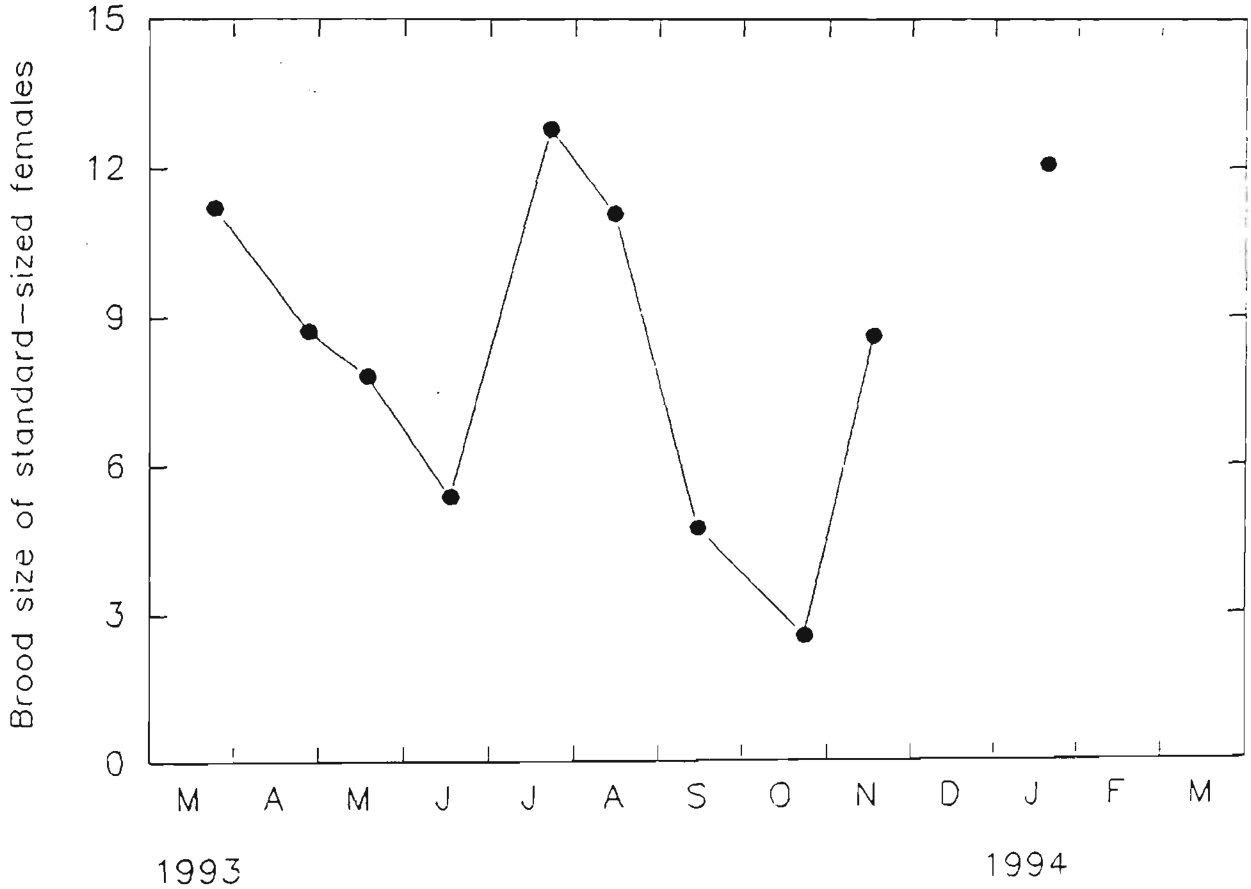
Fig. 4.7 Seasonal variations of the relationships between the brood size (logarithmic scale) and female body size of *Daphnia carinata* in pond 8 over the period March 1993 to February 1994. *D. carinata* was absent from the December 1993 and February 1994 samples.



Daphnia carinata
Brood size
Female size (mm)
r=0.964
23-3-93
r=0.855
14-8-93
r=0.056
16-4-93
r=0.462
14-9-93
r=0.038
17-5-93
r=0.481
22-10-93
r=0.702
16-6-93
r=0.435
16-11-93
r=0.610
21-7-93
r=0.928
19-1-94

Fig. 4.8 Seasonal variations of the brood size of standard-sized females of *D. carinata* over the period March 1993 to February 1994. *D. carinata* was absent from the December 1993 and February 1994 samples.

Daphnia carinata



4.4 DISCUSSION

Daphnia carinata in the Werribee ponds followed a similar population cycle as the Holarctic species summarized by Hebert (1978). The mean brood size and the percentage of breeding females were high at the onset of the population, but decreased as the population density increased. This cycle was repeated later in the year. The fundamental difference between *D. carinata* and the Holarctic species is that *D. carinata* is a cool-water species characterized by a winter-spring peak in its population density (Hebert, 1977a).

Previous studies have shown that the brood size of *Daphnia* depends crucially on food intake (Slobodkin, 1954; Green, 1954, 1956; Hall, 1964; Hebert, 1978). When the energy intake is in excess of maintenance requirements, energy is devoted to the production of parenthogenetic eggs. It has been demonstrated for *Daphnia pulex* that as high as 95% of the energy in excess of maintenance requirements could be expended on egg production (Richman, 1958). For *D. carinata* in both pond 7 and pond 8, the peaks in the mean brood size as well as the percentage of the ovigerous females coincided with periods of high chlorophyll *a* contents, indicating that excess energy might have been used in egg production under conditions of high food abundance. Significantly, the percentage of ovigerous females of *D. carinata* in pond 8 was lower than that in pond 7, reflecting the higher food availability in the latter.

Active breeding in August resulted in the density peaks in September and October. During this time, the brood size and the percentage of ovigerous females as well as the chlorophyll *a* content dropped to a minimum. The small brood size as well as the low percentage of reproductive females observed may suggest that the populations had reached the carrying capacity of the ponds, and that there was intense competition for the limited food resources. This is supported by the occurrence of smaller adults in October when growth conditions might be food-limiting. Although *D. carinata* could, in theory, utilize the sedimented detritus at the bottom of the ponds at low phytoplankton densities (see Horton *et al.*, 1979), the bottom surface area might at times be insufficient for each individual to forage effectively at such a high population density (Mitchell & Williams, 1982a).

In *Daphnia* populations, temperature may affect the size of the individual through an increase in longevity at low temperatures or increase in growth rate and reduction in instar duration at high temperatures (Green, 1956; Hebert, 1978). In this study, the clear relationship between the chlorophyll *a* concentration and the body size indicates that food availability may be a more important factor affecting the growth of *D. carinata* than temperature (cf. Mitchell & Williams, 1982a). Indeed, previous studies on *Daphnia* have shown that when food is abundant, individual growth is almost continuous, resulting in mature females with larger body size. Conversely, growth would stop upon maturity when food supply is low (George & Edwards, 1974; Hebert, 1978).

It has been reported for *Daphnia* that the brood size is positively correlated to the body size of the female (Green, 1954, 1956; Hebert, 1978). This is intuitive as a bigger individual would be expected to be able to produce and carry more eggs. However, this relationship only holds when there is plenty of surplus energy so that the production of eggs is limited by the holding capacity of the female (i.e. size of the brood chamber) rather than the energy available for egg production. In this study, the seasonal variation of the brood size of *D. carinata* cannot be completely explained by the variation of body size of the mature females although the seasonal patterns of these two attributes were very similar. As better food conditions which favour growth would also promote egg production, body sizes and brood sizes of mature females would be expected to fluctuate in parallel with food availability. Significantly, in a dense population where food resources are limited, increase in body size may not be accompanied by a 'proportionate' increase in brood size as would be observed during periods of high food abundance. Indeed, the importance of food availability in influencing brood size of *D.carinata* is clearly evident from an examination of the effect of varying food abundance on the brood size of standard-sized females.

In pond 7, the rapid reduction in food availability due to the increased cladoceran density in October probably induced the production of ephippial eggs (Slobodkin, 1954), while the more frequent occurrence of ephippial eggs in pond 8 reflected the general scarcity of food as confirmed by the inter-pond difference in chlorophyll *a* contents. The appearance of male individuals of *D. carinata* at that time further indicates that food availability is a stronger stimulus than physico-chemical factors in inducing sexual reproduction in these animals (cf. Daborn *et al.*, 1978).

The population density and the mean brood size of *D. carinata* in the stabilization ponds at Werribee were higher than those of other *Daphnia* species recorded in natural water bodies (e.g. Wright, 1965; George & Edwards, 1974; Huang & Hu, 1984), but were comparable to values recorded in other waste treatment facilities (e.g. Daborn *et al.*, 1978; Mitchell & Williams, 1982a). The larger brood size and higher population densities in the waste stabilization ponds are attributed to the lack of predation pressure by fish and the high nutrient inputs which enhance the primary productivity and thus the food availability for the zooplankton. The other co-existing cladoceran, *Moina australiensis*, exhibited similar seasonal patterns in mean body size, brood size and percentage of ovigerous females as those of *D. carinata*. Again, better food conditions resulted in bigger mean body size, larger mean brood size and higher percentage of ovigerous females. Males and ephippial females appeared when population density peaked.

Interestingly, despite the difference in the definitions of body size, the minimum reproductive size of female *D. carinata* recorded at Werribee was significantly smaller than that recorded in South Australia where the chlorophyll *a* contents were much lower (Mitchell & Williams, 1982a). This is counterintuitive as smaller reproductive females would be expected to occur under conditions of lower food abundance (Hall, 1964). It is possible that the smaller reproductive size of female *D. carinata* in the Werribee ponds could be a result of the microevolutionary adjustment by *D. carinata* in response to the fast-breeding tactics of *Moina australiensis* (see below).

Hebert (1977b) argued that if co-existing species practise resource partitioning, then food intakes should vary independently and there should be a lack of concordance in the egg production values for the two species. On the other hand, if there is substantial resource overlap, food intake and egg production rates should be similar provided that the two species do not differ in their physiological responses to an alteration in food abundance. In the present study, the strong correlation between the temporal trends in the egg production pattern of *D. carinata* and *Moina australiensis*, coupled with the fluctuations in the chlorophyll *a* contents clearly showed that there was no clear resource partitioning unless the different resources used by the two species underwent parallel variation in abundance. It was also unlikely that the two species had different physiological responses to variations in food availability. Moreover, the observation of smaller mean brood size for both species during periods of high population densities does not contradict my suggestion that there may be significant niche overlap between the two cladoceran species.

When the two species co-existed, the larger *D. carinata* (maximum size: 4.2 mm) should have competitive advantages over the smaller *Moina australiensis* (maximum size: 1.8 mm) due to the higher filtering efficiency (Brooks & Dodson 1965) and the ability to utilize sedimented food (Mitchell & Williams, 1982b). This is supported by the observation that *D. carinata* was always more abundant than *Moina australiensis* when they co-existed. Assuming that there is significant interspecific competition between *D. carinata* and *Moina australiensis*, an interesting question here is why *Moina australiensis* is able to co-occur with *D. carinata* in such an environment. There are a number of possible reasons. Firstly, conditions in the waste stabilization ponds may provide enough food for both species to co-occur, particularly during periods of high food abundance. The food concentration is higher than that concentration at which the maximum filtering rate occurs, and that the maximum filtering rate is limited by the structure of the food-gathering apparatus rather than the level of food supply (Porter *et al.*, 1982). Secondly, *Moina australiensis* could tolerate higher temperatures and flourish in both ponds in January when *D. carinata* was absent. Indeed, a change in temperature might reverse the competitive ability between the two species before competitive exclusion occurred (Hutchinson, 1961). Thirdly, the minimum reproductive size of female *Moina australiensis* (1.1 mm) was much smaller than that of *D. carinata* (2.1 mm) while their juveniles were similar in sizes. This may mean that *Moina australiensis* can reach sexual maturity in a shorter time period and start breeding at an earlier age as compared with *D. carinata*. Indeed, *Moina australiensis* is opportunistic, with higher intrinsic rate of increase (r_{max}), thus allowing its population size to increase rapidly within a short time period (Romanovsky, 1985). Such an interspecific difference in life-history strategies may have also contributed to the successful coexistence of the two species.

In summary, the two sympatric cladoceran species co-existed and exhibited similar patterns of reproductive characteristics and responses to biotic factors. It is apparent that food availability is a more important factor regulating the growth and the reproductive dynamics of the cladocerans as compared with temperature. When food conditions are favourable, females of both species produce bigger broods and have larger body sizes. When the population density is high and food availability low, body size and brood size are usually smaller. Female body size appeared to have a much stronger influence on brood size when food availability is high. Indeed, in the Werribee ponds where food is usually abundant and the weather relatively mild as compared with other temperate water bodies, effects of temperature might be less important than food availability in determining the reproductive characteristics of the cladoceran populations.

5. Seasonal dynamics and habitat utilization of waterbirds in the waste stabilization ponds (145W) at the Werribee Treatment Complex

5.1 INTRODUCTION

The Werribee Treatment Complex is internationally famous for the diversity and abundance of birdlife which are attributed to the high levels of nutrient input, the wide range of habitats created by different treatment processes as well as the lack of human disturbance. Composition of avifauna and their habitats in the Complex are described in chapter 1. This chapter will concentrate on the study of waterbirds in one series of waste stabilization ponds in the Complex. Waterbirds here are defined as the birds which spend most of their time in freshwater or brackish water including those in families Podicipedidae (Grebes), Ardeidae (Herons, egrets and bitterns), Ciconiidae (Storks), Plataleidae (Ibises and spoonbills), Anatidae (Geese, swans and ducks), Rallidae (Rails, Crakes, swamphens and coots) and Gruidae (Cranes).

Stabilization ponds in Werribee Treatment Complex occupy an area of 16.54 km^2 (Bisett & Pace, 1993) and offer an excellent habitat for waterbirds, particularly during drought and hunting seasons. At times, it supports over 32,000 wildfowl which represent almost 8% of the total number of wildfowl in Victoria (Lane & Peake, 1990). Indeed, the large numbers of Australian Shelduck (*Tadorna tadornoides*), Pacific Black Duck (*Anas superciliosa*), Grey Teal (*Anas gibberifrons*), Chestnut Teal (*Anas castanea*) and Pink-eared Duck (*Malachorhynchus membranaceus*) in the Complex are of state significance (Lane & Peake, 1990).

It has been suggested that the newly designed stabilization ponds constructed at Werribee might have detrimental effects on the waterbirds (Lane & Peake. 1990). In particular, the increased depth of the ponds may reduce the primary productivity at the bottom of the ponds due to reduced light penetration. This may adversely affect the diving ducks and the herbivorous species which feed on the bottom macrophytes. Moreover, the filtering ducks may also have a problem in collecting their food as diurnal migration of zooplankton may prevail in the deeper ponds. Furthermore, the rectangular shape of the new ponds results in a larger proportion of the ponds exposed to the disturbance from travelling vehicles (Lane & Peake, 1990). Although the primary aim of the Complex is to increase the efficiency of the stabilization ponds in the treatment process, major modifications of the old ponds should not be implemented unless possible impacts on the native widlife have been clearly assessed. The study outlined in this chapter will provide some background information on the waterbirds inhabiting the old-style ponds.

Preliminary studies show that waterbirds are restricted to the aerobic section of the stabilization ponds, however, no detailed information can be obtained from previous publications. Moreover, the trophic relationships of the waterbirds in the ponds have not been clearly addressed (D. Williams, personal communication). As the morphometry of the ponds may affect the distribution of aquatic birds (DesGranges & Darveau, 1985), the 145W stabilization pond series, consisting of ponds of similar size and shape, provided an ideal opportunity to study the habitat utilization of the waterbirds at different stages of the pond treatment process. In this chapter, I examine the distributions of waterbirds along the study pond series and explore the possible factors that may regulate the habitat utilization patterns of the waterbirds in the waste stabilization ponds at Werribee Treatment Complex.

5.2 MATERIALS AND METHODS

The study was undertaken from pond 1 to pond 8 of 145W stabilization pond series at Werribee Treatment Complex (Refer to chapter 2 for the detailed location and description of the study site). Field observations of waterbirds were conducted monthly from March 93 to January 94. At each field visit, waterbirds in pond 1 to pond 8 were identified following Simpson and Day (1989) and counted with a 10 X 50, 6.0° binocular (Nikon Sporting II). All data were gathered between 1000 and 1400.

In order to study the habitat utilization by waterbirds at different stages of the pond treatment process, the study pond series was divided into three different sections as in Table 5.1. (Refer to chapter 2 for a detailed description of the water characteristics in different ponds.)

Table 5.1 The characteristics of the three sections of the study pond series

Section	Pond	Area (Ha)	Characteristics
Anaerobic	1,2	9.02	High B.O.D., suspended solids and ammonia level.
			Low dissolved oxygen, pH level and phytoplankton abundance.
Facultative	3,4,5	17.37	Moderate B.O.D., suspended solids and ammonia level.
			High phytoplankton abundance.
			Large variation in Dissolved oxygen and pH (depend on algal abundance).
Maturation	6,7,8	16.45	Low B.O.D., suspended solids, ammonia level and phytoplankton abundance.
			High Dissolve oxygen, pH level and zooplankton abundance

Moreover, the monthly data are grouped into different seasons as follows:

Autumn - March to May

Winter - June to August

Spring - September to November

Summer - December to February

Bottom sediment samples were collected in pond 7 and pond 8 where the waterbirds were most abundant. Four locations were chosen randomly in each pond and samples were collected with a mud grab (LaMotte Code-1061) on board a rubber dingy. The sediment samples were sorted with a 250μm sieve and materials retained in the sieve were identified under a stereomicroscope.

5.3 RESULTS

The dominant waterbird species observed in the study area are summarized in Table 5.2. Eurasian Coot (*Fulica atra*), Musk Duck (*Biziura lobata*) and Scared Ibis (*Threskiornis aethiopica*) were also observed on a few occasions.

Table 5.2 Dominant waterbirds recorded in stabilization ponds (145W Group B) at Werribee Treatment Complex during March 93 and January 94.

Anatidae

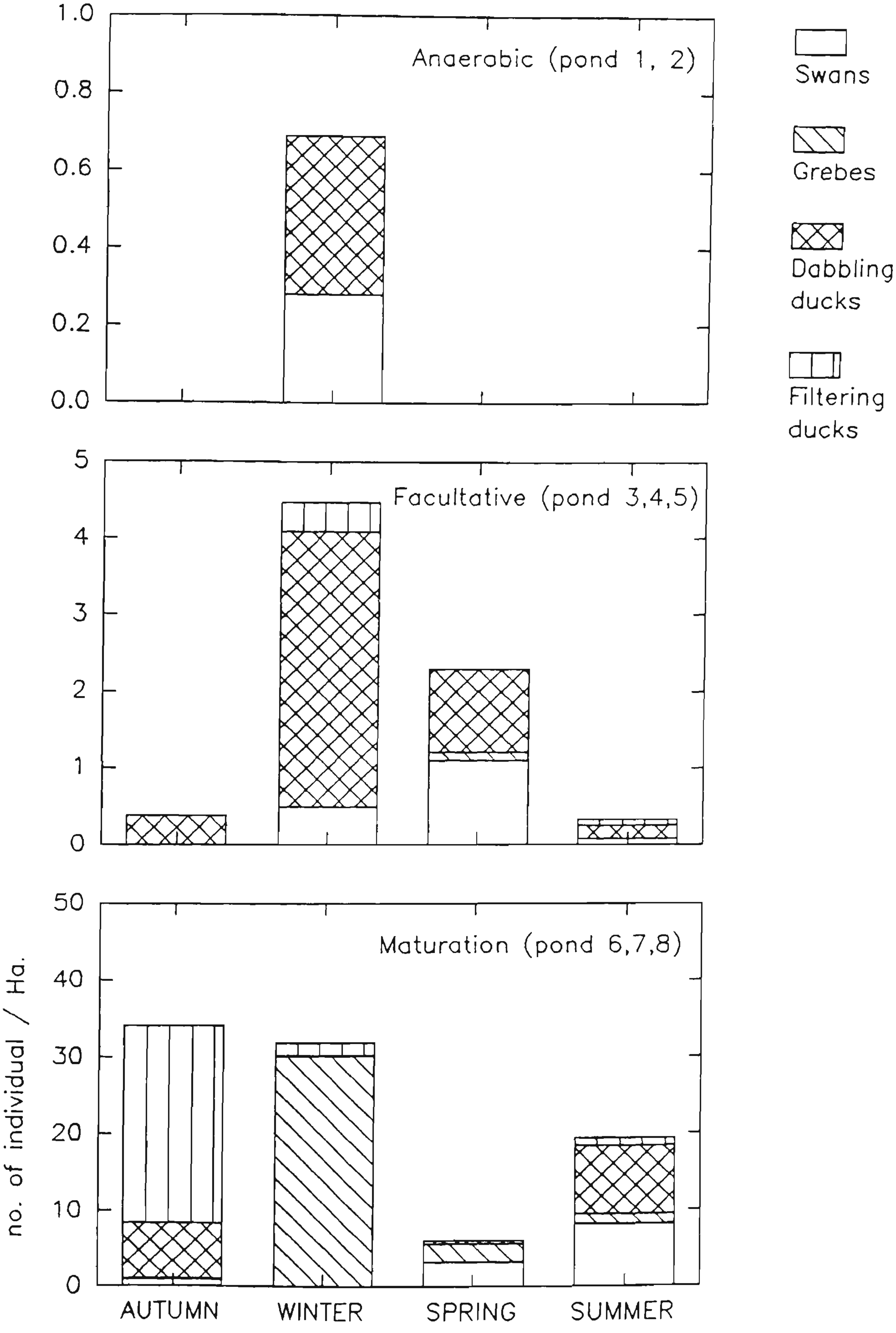
- Black Swans (*Cygnus atratus*)
- Pacific Black Duck (*Anas superciliosa*)
- Australia Shelduck (*Tadorna tadornoides*)
- Chestnut Teal (*Anas castanea*)
- Grey Teal (*Anas gibberifrons*)
- Pink-eared Duck (*Malachorhynchus membranaceus*)
- Australasian Shoveler (*Anas rhynchotis*)

Podicipedidae

- Hoary-headed Grebe (*Poliocephalus poliocephalus*)
- Australasian Grebe (*Tachybaptus novaehollandiae*)

Seasonal distribution and abundance of dominant waterbirds in different sections of the pond series are shown in Fig. 5.1. Different bird species are grouped into grebes, swans, dabbling ducks and filtering ducks. Dabbling ducks refer to those which collect food by dredging the mud in shallow water, stripping seeds from plants growing at the water's edge. They often leave the water and feed on the banks. The dabbling ducks

Fig. 5.1 Seasonal variations of the abundance of swans, grebes, dabbling ducks and filtering ducks at the anaerobic, facultative and maturation sections of the 145W stabilization pond series over the period March 93 to February 94.



Anaerobic (pond 1, 2)
Facultative (pond 3,4,5)
Maturation (pond 6,7,8)
Swans
Grebes
Dabbling ducks
Filtering ducks
no. of individual / Ha.
AUTUMN
WINTER
SPRING
SUMMER

observed in this study include the Grey Teal, Chestnut Teal, Australia Shelduck and Pacific Black Duck. Filtering ducks include Pink-eared Duck and Australasian Shoveler which filter their food from the water with the hairlike lamellae in their bills (Frith. 1977). Statistical analysis showed that there are significant differences between the observed and expected distributions of the total number of waterbirds in different sections of the series in all seasons ($\chi^2 > 36.45$, d.f. = 2, $P < 0.001$). Although flocks of Silver Gulls (*Larus novaehollandiae*) were often observed scavenging garbage in the first ponds, waterbirds were almost completely excluded from the anaerobic and facultative sections except in the winter when the detention time was longest (cf. Fig. 2.2). Highest abundance of filtering ducks (mainly Pink-eared Ducks), grebes and Black Swans was observed in the autumn, winter and summer respectively while dabbling ducks appeared in most of the year.

Dabbling ducks and swans often stood on the banks of the ponds and fed on the vegetation while filtering ducks and grebes usually stayed in the water. Once the ducks were disturbed, they usually took off and landed in the other ponds. On the other hand, the Black Swans seldom took off, instead, they tended to swim to the middle of the pond and stayed away from the banks if distributed. During the summer, the Black Swans often up-ended in the maturation ponds and could reach to the bottom with their long necks. Signs of swallowing were observed when they pulled their heads out of the water. This behaviour has not been observed in the Black Swans that occurred in the earlier ponds of the series. The Hoary-headed Grebes and Australasian Grebes were found often in mixed flocks during the winter. Although they are very similar in appearance, the latter can be easily distinguished by their diving behaviour when disturbed.

Interestingly, no benthic macrophyte was found in all the sediment samples. However, larval forms of midges (*Chironomus* spp.) and *Tubifex* spp. were abundant in all seasons. Significantly, all sediment samples had a high proportion of ephippial eggs of *Daphnia carinata*. Although sediment samples were not collected from the eariler ponds, the presence of the ephippial eggs should be restricted to the later section as preliminary study showed that the cladocerans were only present in the last few ponds.

5.4 DISCUSSION

Despite the frequent usage of waste stabilization ponds all over the world, habitat utilization and conservation of the avifauna in these systems have seldom been reported. In the Werribee Treatment Complex, it is clearly demonstrated that in addition to waste water treatment, waste stabilization ponds can provide excellent habitats for waterbirds.

High abundance of waterbirds has been observed in the study ponds throughout the year. On one occasion, there were as many as one thousand Pink-eared Ducks in a single pond. The occurrence of the waterbirds was generally restricted to the last few ponds in the series where the water quality was better in terms of higher dissolved oxygen and lower biochemical oxygen demand. Significantly, in the winter when the detention time was longest, the waterbirds extended their distribution to all the ponds in the series. This was mainly attributable to the improved water quality due to the prolonged detention time. During that period, ammonia concentration dropped to a minimum level while phytoplankton abundance and dissolved oxygen level were highest. This suggests that the distribution of the waterbirds along the pond series may be mainly controlled by the water quality in the ponds. It is therefore conceivable that the installation of mechanical aerators in the anaerobic ponds would extend the aerobic section of the pond series, hence increase the area of potential habitats for the waterbirds (Lane & Peake, 1990). However, from the observations in this study, waterbirds were usually absent from the ponds on windy days when the pond surface were rough and wavy. Therefore, the turbulence created by the aerators may create unfavourable conditions for the waterbirds.

Filtering ducks mainly composed of Pink-eared Ducks, appeared in large numbers in the Autumn which coincided with the re-emergence of the dominant zooplankton, *Daphnia carinata*, from the ephippial eggs. At times, there were more than 1000 Pink-eared Ducks in one pond, cruising slowly with their bills immersed in the water, filtering the microscopic organisms for food. They are carnivorous animals which feed mainly on insects, cladocerans and copepods by the filtering mechaism of their bills (Frith, 1977). Significant amount of zooplankton could have been removed from the ponds by the filtering ducks which might be crucial in the controlling of the population sizes of the zooplankton thus reducing the grazing pressure on the phytoplankton. This helps to maintain the abundance of phytoplankton at a level which is important for the proper functioning of the stabilization ponds, and facilitates the removal of nutrients as well as organic matter form the system.

Hoary-headed Grebes were highly abundant in the study ponds during the winter. Fjeldsa (1983) reported that the diet of Hoary-headed Grebe consisted mainly of nymphs of aquatic insects, midges and their larvae. Indeed, in this study, sediment samples revealed high densities of *Chironomus* larvae which might be the food source for the grebes. Kimerle and Anderson (1971) found that these midge larvae were important in the decomposition of organic matter in waste stabilization ponds. Notwithstanding, the nuisance caused by the emergence of the adult forms could be a problem to the nearby traffic and residential area. The Hoary-headed Grebes could be an effective biological control agent on the midges in waste stabilization ponds.

Swans are basically herbivorous animals, feed mainly in water, graze on the underwater vegetation with its long neck at up-end position (Owen & Black, 1990).

Moreover, they also dabble on the surface to collect duckweed and other floating plants (Frith, 1977). Indeed, field observations at the Werribee ponds revealed that the Black Swans fed actively at the bottom with the up-end position. It is interesting to note that in the stabilization ponds, no benthic marcophyte was found in the sediment samples. Instead, the sediment samples contained a large number of ephippial eggs of *Daphnia carinata*. I suspect that the swans may have been consuming the ephippial eggs which have a relatively high protein content. Although the ephippial eggs possess a protective layer that can withstand the digestive action of fish, the herbivorous swans may be able to utilize the eggs. The up-end feeding behaviour was only observed in the last few ponds during the summer. This observation does not contradict with the above speculation as *Daphnia* only appeared in the last few ponds and the ephippial eggs should be most abundant when the population was absent during the summer (see chapters 3 and 4). Nevertheless, this speculation cannot be confirmed until a gut content analysis has been conducted.

Notwithstanding the significant number of waterbirds in the waste stabilization ponds, their roles in the waste water treatment process are still uncertain (Mitchell, 1980). Being at the top trophic level of the food web, they can contribute to the removal of nutrients and organic materials from the system. However, the faecal materials from the waterbirds could increase the nutrient levels as well as the faecal coliform bacteria in the effluent. Whatever the role of the waterbirds in the treatment process is, as the coastal wetlands decline in area due to the increase in human population, stabilization ponds at Werribee can be identified as an important refuge for the conservation of waterbirds. In particular, the depth of the ponds can be a key factor affecting the feeding behaviour of the swans and the filtering ducks.

6. Concluding discussion

Werribee Treatment Complex has been regarded as a waste treatment facility which is both environmentally friendly and economically sustainable. In the treatment processes, recycling of resources is maximized while energy input is minimized. Moreover, through livestock production in the land and grass filtration areas, as much as 20% of the total operation cost of the complex is recovered. However, in terms of the volume of waste water treated per unit area, grass and land filtration are considered to be less efficient than stabilization ponds (see Table 1.1). It has been proposed that those areas currently used for land and grass filtration should be converted to stabilization ponds in order to increase the overall efficiency of the complex to meet the augmented sewage loading of the increasing population in Melbourne (Lane and Peake, 1990). Clearly, more information on the operational principles and treatment efficiency of the waste stabilization ponds at the Werribee Treatment Complex is needed to allow an informed decision to be made.

This study examined a waste stabilization pond series (145W) at the Werribee Treatment Complex. The nutrient dynamics and the prevailing ecological processes in the ponds were investigated. The findings showed that the quality of the effluent was largely controlled by the detention time of the system (cf. Meron *et al.*, 1965). In particular, prolonged detention would result in a significant increase in phytoplankton abundance which is a key-component in the treatment process. The algae not only stabilize the nutrients through direct uptake, they also produce oxygen via photosynthesis, facilitating aerobic degradation and nitrification by aerobic and nitrifying bacteria respectively. In the pond series at Werribee, ammonia in the raw sewage was completely removed through nitrification as well as algal cell uptake during periods of longest detention time. On the other hand, removal of phosphorus was insignificant throughout the study period. According to Gakstatter *et al.* (1978), water bodies receiving effluent from waste water treatment facility such as Port Phillip Bay are usually nitrogen-limited for algal growth. Therefore, the control the nitrogen content in the effluent should be of greater concern than phosphorus.

In the operation of the stabilization ponds, appropriate detention periods for the waste water in the system should be used in order to meet different treatment objectives (Meron, 1965). For instance, removal of biochemical oxygen demand usually requires a relatively short detention time, while a longer detention time is needed to achieve a significant increase in phytoplankton abundance and a low level of nitrogen in the effluent. Fitzgerald & Rohlich (1964) suggested that algal growth could be promoted by adding carbon dioxide to stabilization ponds, and this would improve the efficiency in nitrogen removal. However, this method is economically infeasible. One of the possible solutions is to feed the waste water into the stabilization pond system with intermittent flow, thus minimizing the loss of phytoplankton due to flushing. It should be noted that although waste water is being produced and distributed to the complex continuously, it can be fed to different pond series in rotation.

Higher phytoplankton abundance during the period of longest detention time increased the food density for the cladocerans in the stabilization ponds. As a result, the brood size of the cladocerans increased significantly. Without the predation of fish on the zooplankton, the population density of the cladocerans peaked in the following months. The increase in grazing pressure caused a drop in the phytoplankton number and consequently the population density of cladocerans decreased due to the reduced food supply. These cyclic changes in the phytoplankton and zooplankton populations clearly conformed to the classic model of predator and prey relationship. Indeed, my results showed that the most important factor controlling the abundance of the two dominant zooplankton, namely *Daphnia carinata* and *Moina australiensis*, was the density of phytoplankton which was the major food source for the two cladocerans. The impact of the physical and chemical factors on the cladocerans was less important.

The potential use of waste water as a resource has been a major issue around the world in recent years (Storm, 1979). Under proper management, the nutrients in the waste water can be recycled for agricultural as well as aquacultural uses (e.g. Noble, 1975; Lai & Lam, in press). Moreover, the treated waste water can also be re-used, and this would help to alleviate the shortage of water which is one of the most critical problems in Australia. In recent years, more attention has been paid to the recovering of biomass from waste water by harvesting zooplankton from stabilization ponds (e.g. Kawasaki *et al.*, 1982; Tarifeño-Silva *et al.*, 1982). The zooplankton have a high nutritive value and can be used as a potential feed for aquacultural purposes (Proulx & Noüe, 1985). Indeed, the results of this study have provided some insights into possible ways to maximize the efficiency of nitrogen removal in the system, and at the same time, optimize the yield of zooplankton harvesting. Phytoplankton production is firstly enhanced by prolonging the detention time of the pond systems, the increase in phytoplankton abundance not only results in the increase in the reproductive output of the zooplankton, but also produces an effluent with lower nitrogen level. The hatching of the parthenogenetic eggs and subsequent growth of the juveniles will boost the zooplankton populations which can then be harvested. Intensive grazing on the phytoplankton by zooplankton will produce an effluent with reduced levels of suspended solids.

It can be clearly demonstrated that waste stabilization ponds have a high potential for development. If the system is properly designed with appropriate detention time, high quality effluent with low levels of nutrients, suspended solids as well as biochemical oxygen demand can be produced. Economic benefits from zooplankton production can also help to recover part of the operational cost. Significantly, the productivity of the plankton in a stabilization pond, which is a three-dimensional system, should in theory be higher than that of the land and grass filtration areas used for livestock production. Lastly, the conservation value of stabilization ponds for waterbirds is particularly valuable in the present world where most of the wetlands have been destroyed, and more are threatened, due to urban development.

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8. Appendix

APPENDIX 1: A list of common bird species in the Werribee Treatment Complex. (Modified from Cropper, 1987)

SEABIRDS	
Cormorant, Great	*Phalacrocorax carbo*
Cormorant, Little Black	*Phalacrocorax sulcirostris*
Cormorant, Little Pied	*Phalacrocorax melanoleucos*
Jaeger, Arctic	*Stercorarius parasiticus*
Pelican, Australian	*Pelicanus conspicillatus*
Penguin, Little	*Eudyptula minor*
HERONS, ERGETS, BITTERNS, IBIS AND SPOONBILLS	
Bittern, Australasian	*Botaurus poiciloptilus*
Egret, Cattle	*Ardeola ibis*
Egret, Great	*Egretta alba*
Egret, Little	*Egretta garzetta*
Heron, Pacific	*Ardea pacifica*
Heron, White-faced	*Ardea novaehollandiae*
Ibis, Sacred	*Threskiornis aethiopica*
Ibis, straw-necked	*Threskiornis spinicollis*
Spoonbill, Royal	*Platalea regia*
Spoonbill, Yellow-billed	*Platalea flavipes*
WATERFOWL	
Duck, Blue-billed	*Oxyura australis*
Duck, Musk	*Biziura lobata*
Duck, Pacific Black	*Anas superciliosa*
Duck, Pink-eared	*Malachorhynchus membranaceus*
Hardhead	*Aythya australis*
Shelduck, Australian	*Tadorna tadornoides*
Shoveler, Australasian	*Anas rhynchotis*
Swan, Black	*Cygnus atratus*
Teal, Chestnut	*Anas castanea*
Teal, Grey	*Anas gibberifrons*

RAPTORS

Falcon, Brown	*Falco berigora*
Goshawk, Brown	*Accipiter fasciatus*
Harrier, Marsh	*Circus aeruginosus*
Kestril, Australian	*Falco cenchroides*
Kite, Black-shouldered	*Elanus notatus*
Kite, Whistling	*Haliastur sphenurus*

QUAILS

Quail, Brown	*Caturnix australis*
Quail, Stubble	*Caturnix novaezelandiae*

WADERS

Avocet, Red-necked	*Recurvirostra novaehollandiae*
Curlew, Eastern	*Numenius madagascariensis*
Dotterel, Red-kneed	*Erythrogonys cinctus*
Godwit, Bar-tailed	*Limosa lapponica*
Greenshank	*Tringa nebularia*
Knot, Red	*Calidris canutus*
Lapwing, Banded	*Vanellus tricolor*
Lapwing, Masked	*Vanellus miles*
oystercatcher, Pied	*Haematopus longirostris*
Plover, Black-fronted	*Charadrius melanops*
Plover, Double-banded	*Charadrius bicinctus*
Plover, Lesser Golden	*Pluvialis dominica*
Plover, Monogolian	*Charadrius mongolus*
Plover, Red-capped	*Charadrius ruficapillus*
Sandpiper, Curlew	*Calidris ferruginea*
Sandpiper, Pectoral	*Calidris malanotus*
Sandpiper, Sharp-tailed	*Calidris acuminata*
Snipe, Latham's	*Gallinago hardwickii*
Stint, Black-winged	*Himantopus himantopus*
Stint, Red-necked	*Calidris ruficollis*
Tattler, Grey-tailed	*Tringa brevipes*
Turnstone, Ruddy	*Arenatia interpres*

GULLS AND TERNS

Gull, Pacific	*Larus pacificus*
Gull, Silver	*Larus novaehollandiae*
Tern, Caspian	*Hydroprogne caspia*
Tern, Crested	*Sterna bergii*
Tern, Gairy	*Sterna nereis*
Tern, Little	*Sterna albifrons*
Tern, Whiskered	*Childonias hydrida*
Tern, White-winged	*Childonias leucoptera*

OTHER WATERBIRDS

Coot, Eursian	*Fulica atra*
Crake, Australian	*Porzana fluminea*
Crake, Spotless	*Porzana tabuensis*
Grebe, Great Crested	*Podiceps cristatus*
Grebe, Hoary-headed	*Poliocephalus poliocephalus*
Moorhen, Dusky	*Gallinula tenebrosa*
Rail, Buff-banded	*Rallus philippensis*
Swamphen, Purple	*Porphyrio porphyrio*

COCKATOOS, LORIKEETS AND PARROT

Galah	*Cacutua roseicapilla*
Parrot, Blue-winged	*Neophema chrysostoma*
Parrot, Orange-bellied	*Neophema chrysogaster*
Parrot, Red-rumped	*Psephotus haematonotus*
Rosella, Eastern	*Platycercus eximius*

PIGEONS AND DOVES

Pigeon, Feral	*Columba livia*
Turtle-dove, Spotted	*Streptopelia chinensis*

CUCKOOS, OWLS AND FROGMOUTH

Boobook, Southern	*Ninoxx novaeseelandiae*
Bronze-cuckoo, Horsefield's	*Chrysococcyx basalis*
Cuckoo, Pallid	*Cuculus pallidus*
Owl, Barn	*Tyto alba*

KINGFISHERS

Kookabura, Laughing	*Dacelo novaeguineae*

BUSH BIRDS

Blackbird	*Turdus merula*
Calamanthus	*Sericornis fuliginosus*
Chat, White-fronted	*Ephthianura albifrons*
Cisticola, Golden-headed	*Cisticola exilis*
Cuckoo-shrike, Black-faced	*Coracina novaehollandiae*
Fantail, Grey	*Rhipidura fuliginosa*
Fantail, Rufus	*Rhipidura rufifrons*
Fairy-wren, Superb	*Malurus cyaneus*
Goldfinch, European	*Carduelis carduelis*
Grassbird, Little	*Megalurus gramineus*
Greenfinch, European	*Carduelis chloris*
Honeyeater, White-naped	*Melithreptus lunatus*
Honeyeater, White-plumed	*Lichenostomus penicillatus*
Honeyeater, Yellow-faced	*Lichenostomus chrysops*
Magpie, Australian	*Gymnorhina tibicen*
Magpie-lark, Australian	*Grallina cyanoleuca*
Martin, Fairy	*Cecropis ariel*
Martin,Tree	*Cecropis nigricans*
Mynah, Common	*Acridotheres tristis*
Pipit, Richard's	*Anthus novaeseelandiae*
Raven, Little	*Corvus mellori*
Reed-warbler, Clamorous	*Acrocephalus stnetoreus*
Robin, Flame	*Petroica phoenicea*
Scrubwren, White-browed	*Sericornis frontalis*
Shrike-thrush, Grey	*Colluricincla harmonica*
Shrike-tit, Crested	*Falcunculus frontalis*
Silvereye	*Zosterops lateralis*
Skylark	*Alauda arvensis*
Songlark, Brown	*Cinclorhamphus cruralis*
Sparrow, House	*Passer domesticus*
Starling, Common	*Sturnus vulgaris*
Swallow, Welcome	*Hirundo neoxena*

Thornbill, Brown	*Acanthiza pusilla*
Thornbill, Yellow-rumped	*Acanthiza chrysorrhoa*
Wagtail, Willie	*Rhipidura leucophrys*
Wattlebird, Little	*Anthochaera chrysoptera*
Wattlebird, Red	*Anthochaera carunculata*