The ecology of sewage treatment gradients in relation to their use by waterbirds

Andrew J. Hamilton*, Wayne Robinson, Iain R. Taylor & Ben P. Wilson

Johnstone Centre, School of Environmental and Information Sciences, Charles Sturt University, P.O. Box 789, Albury, NSW 2640, Australia

(*Author for correspondence: Current address: Department of Primary Industries, Primary Industries Research Victoria – Knoxfield campus, Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia; E-mail: Andrew.Hamilton@dpi.vic.gov.au)

Received 27 August 2003; in revised form 28 May 2004; accepted 15 July 2004

Key words: sewage, waterbirds, waterfowl, zooplankton

Abstract

The distribution and abundance of waterbirds along sewage treatment gradients at the Western Treatment Plant (Victoria, Australia) were studied in late summer/early autumn 2000. In general, the highest densities and diversity of waterbirds, and of zooplankton, were found in the ponds towards the end of a treatment series. Filter-feeding waterfowl (Anatidae) probably used these ponds because of the availability of zooplankton as a food-source. Dissolved oxygen concentration generally increased along the treatment gradient and un-ionised sulphide concentration decreased, and it is possible that either one, or both, of these played a key role in determining the distribution of zooplankton.

Introduction

In most traditional sewage farms sewage flows through a series of several waste-stabilisation ponds (WSPs), and each pond has different limnological characteristics that are largely dependent on the stage of sewage treatment it represents. The ponds at the start of the series tend to be anaerobic, those in the middle, facultative, and those at the end, aerobic (Hawkes, 1983; Paul, 1995; Hodgson & Paspaliaris, 1996). The use of WSPs by waterbirds has been well documented (Uhler, 1964; Braithwaite & Stewart, 1975; Campbell & Milne 1977; Fuller & Glue, 1978, 1980, 1981; Maxson, 1981; Piest & Sowls, 1985; de Moor, 1993), but there have been no published studies that attempt to determine if birds exhibit a preference for ponds of a particular stage of treatment, and if so, why. Some studies have suggested that high invertebrate abundance in WSPs is the major factor attracting waterfowl, but such studies have generally looked at small systems with only one (Swanson, 1977; Piest & Sowls, 1985) or a few (Maxson, 1981) WSPs. There may be substantial variation in the abundance of invertebrates along a sewage treatment gradient.

Unlike some natural water-bodies, where nutrients might be limiting, it is likely that food webs in WSPs are not nutrient limited; most WSPs usually belong to either the eutrophic or hypertrophic categories of the OECD-Vollenweider trophic status classification scheme (OECD, 1982). Thus, factors determining invertebrate abundance in WSPs may be quite different to those in many natural systems. For example, ammonia and sulphide are produced through microbial processes in sewage and may reach levels that are toxic to planktonic crustacean invertebrates such as Daphnia magna Straus (Uhlmann 1980; Gersich & Hopkins, 1986). Also, low dissolved oxygen in WSPs, resulting from a very high biochemical oxygen demand, may limit invertebrate productivity (Mitchell & Williams, 1982a; Hathaway & Stefan, 1995).

Such direct effects are likely to provide only partial explanations for variations in invertebrate productivity along sewage treatment gradients; complex food web dynamics may also need to be considered (e.g. Abeliovich & Azov, 1976; Konig et al., 1987; Wrigley & Toerien, 1990). It is possible that non-food source related factors, such as the presence of oil, detergents or cyanobacterial toxins, may have an influence on the selection of WSPs by birds (Nero, 1964; Matsuanga et al., 1999).

The objective of this study was to develop an understanding of some aspects of the functioning of the planktonic food web at various stages of sewage treatment, and to relate these to waterbird use of a pond system at the Western Treatment Plant. The Australasian shoveler, Anas rhynchotis (Latham) was chosen as the 'target species' because it had been observed to use the site regularly, and in large numbers, during the previous summer/autumn. Also, it is a species that feeds exclusively at the water's surface (preliminary observations by A.J.H. at the site revealed no upending or dipping), and thus the distribution of this species in relation to potential planktonic invertebrate prey items could be studied. It is intended that certain physical and/or biological parameters could be used to monitor the status of ponds with respect to their function as waterbird habitat.

Methods

Site description

The Western Treatment Plant occupies 10 851 ha and is situated 35 km west of Melbourne (Victoria, Australia) on the shores of Port Phillip Bay (38° 00' S, 144° 34' E). It is recognised as a Wetland of International Importance for the numbers and diversity of waterbirds it supports (Ramsar Convention Bureau, 1984). A group of WSPs known as 145 West was chosen as the study site for two reasons. Firstly, it was known to support large numbers of waterfowl and other waterbirds (Lane & Peake, 1990; Hamilton 2002). Secondly, the WSPs within 145 West were of similar dimension and surface area (Fig. 1). The sewage entering the system is mostly raw, although it does undergo primary sedimentation of gross solids. Sewage flows from one pond to the next via drains (Fig. 1), and the hydraulic retention time of each pond is about 9 days. Within the 145 West system, we studied five independent series of seven WSPs (i.e. 35 WSPs). The mean surface area of these WSPs was 5.07 ha (s.e. = 0.12 ha). The only vegetation surrounding the ponds is short grass.

Current data on pond depths were not available, although the ponds are all roughly 80–90 cm deep. The only possible exceptions are the first two in each series, where sludge accumulates and is periodically dredged.

Study design

The study was restricted to 35 WSPs within the 145 West lagoon - five series consisting of seven ponds each (Fig. 1). Sewage enters the first pond in each series via a common open channel. There was no flow of sewage between ponds in adjacent series (i.e. north to south flow only) for the first seven ponds in each series; thus the series were independent of each other. But this was not the case for some of the ponds at the end of each series (Fig. 1); consequently, these ponds were not included in the study. The position each pond occupied in the series will henceforth be referred to as a particular stage of sewage treatment. Thus, the first pond, which received the raw sewage, was 'Stage 1', and so on through to 'Stage 7', the last pond.

Sampling was conducted on three dates: 19 February 2000, 26 February 2000 and 4 March 2000. This time of year was chosen in an attempt to coincide with expected peak abundances of Australasian shoveler, which usually occur in early autumn (Hamilton & Taylor, in press).

Waterbird surveys

Waterbird sampling started 30 min prior to sunrise and finished about 30 min after sunrise. All surveys were conducted from a car with the aid of a Leica[®] Televid 77 telescope $(20-70 \times zoom$ magnification). For each species, the number of individuals on the water was counted. It was not logistically feasible to fully randomise the order in



145 West supply carrier

Figure 1. Map of the 145 West sewage treatment lagoon at the Western Treatment Plant, Australia ($38^{\circ}00'$ S, $144^{\circ}34'$ E), showing the five series of seven ponds used in this study (i.e. ponds with numbers and marked with solid lines). Arrows denote drains between ponds. Boundaries around ponds are single-lane dirt vehicular tracks.

which the ponds were surveyed. Instead, the ponds were surveyed a series at a time by driving along the north – south tracks (Fig. 1). Once the end of one series was reached, sampling then proceeded along the adjacent series. One of the four 'corner' ponds (i.e. ponds 32, 26, 62 or 68 - Fig. 1) was chosen at random as the starting point. All ponds were observed from the track to their immediate

east. The total number of individuals of each species was recorded for each pond. Birds on pond embankments were not considered because the primary focus of this study was to relate waterbird numbers and distribution to water quality parameters.

Water sampling

Water samples were collected immediately following the waterbird surveys. Ponds were sampled in the same order as they were for the bird survey. A rapid sampling technique was adopted; this involved using a 21 plastic container attached to the end of a 3 m stick. All ponds were sampled from approximately halfway along their eastern margin, and two samples were collected from each. The first sample (600 ml in a plastic bottle) was used for chlorophyll a, sulphide and ammonia analyses. In order to prevent degradation of chlorophyll a, these samples were immediately stored in the dark. The second sample (1 l in a plastic bottle) was used to preserve invertebrates, and buffered formaldehyde $(20\% \text{ w/v sodium tetra-borate, Na}_2B_2O_4,$ 37% v/v formaldehyde) was added to each bottle prior to sample collection so that a final concentration of 5% v/v formaldehyde was attained upon addition of the sample (APHA et al., 1998). For the first and last pond in each series, an additional 1 l sample for nutrient analyses was collected.

An approximation of water temperature was required for the calculation of the dissociation constants for sulphide and ammonia (see below). The temperature of the middle pond in the middle series (Pond 47, Fig. 1) was recorded on each sampling date.

Storage and preservation of samples for nutrient analyses were in accordance with the Standard Methods outlined by APHA et al. (1998). From the 600 ml samples, a portion of each was vacuum filtered through a glass fibre filter (Whatman[®] GF/F, 0.7 μ m), and the filter was stored frozen at -70 °C. These filters were later used for chlorophyll a analysis. A further portion of about 80 ml was taken and stored frozen (-20 °C). These subsamples were analysed for ammonia. The remaining sample was preserved for subsequent sulphide analysis by adding 2 N Zinc acetate. $Zn(C_2H_3O_2)_2 \cdot H_2O$, (4 drops/100 ml of sample), and evacuating air from the bottle.

Water chemistry

Chlorophyll *a* concentration was used as an indicator of total phytoplankton biomass. It was extracted in 90% v/v acetone and analysed spectrophotometrically. The extraction, sample preparation and analysis protocols were the same as the APHA et al. (1998) method '10200 Parts 1 and 2', with the exception that a vortex was used in place of a tissue grinder. All nutrient and toxicant concentrations were determined using the Hach[®] DR/2000 spectrophotometric analysis system (Hach Co., Loveland, Colorado, USA).

The equilibrium of ammonia species was calculated according to Emerson et al. (1975), and sulphide speciation was calculated according to APHA et al. (1998) method 4500 H.

Due to limited availability of equipment, it was not possible to collect dissolved oxygen data on the days that invertebrate samples were taken. However, from 1 March 2000 to 3 March 2000 data were collected from the last pond of the middle series of seven ponds (Pond 50, Fig. 1). It was important to record fluctuations in dissolved oxygen over the diurnal period; instantaneous measurements at a particular time of day do not provide any indication of the exposure history an invertebrate would be likely to experience. LC82[®] Digital Oxygen Meters and ED500[®] Clarke membrane electrodes (both from TPS, Springwood, QLD, Australia) were used. In addition, instantaneous measurements of each pond in the middle series (Fig. 1) were made at sunrise on July 7 2000 using a Hach Sension[®] 156 Portable Multiparameter meter.

Enumeration of zooplankton

Rotifers were counted using a 1 ml Sedgwick Rafter counting cell and a compound microscope. Larger zooplankton were concentrated by passing 1 l of sample through a 158 μ m sieve. The organisms were then rinsed off the sieve with 70% v/v ethanol and counted directly in a Petri dish using a dissecting microscope.

Cyclopoid copepod nauplii were considered as a separate functional species. They cannot readily be identified beyond order (Hawking & Smith, 1997), and hence it was not possible to confidently link them to an adult form. More importantly though, their distinct form, by comparison to the copepodite life stages, warranted this separate classification. Two morphologically distinct ecotypes of Brachionus calciflorus (Pallas) were identified, one with spines, the other without. Spines are induced in the presence of predators (Shiel, R., 2001, pers. comm.). Therefore, from a functional plankton food-web perspective, it was considered reasonable to treat these two ecotypes separately as well. Thus, for the community analyses the two ecotypes were treated independently, but for the correlations with waterfowl abundance they were considered as one species (see description of analysis below). This approach was taken because the spines are likely to affect predation by other zooplankters but not waterfowl (both ecotypes are the same size).

Physical parameters

Upon return to the laboratory (about 2 h after collection) the pH (Hanna[®] HI 9321), conductivity (Hach Sension[®] 156) and turbidity (Hach 2100P) of all the 600 ml samples were recorded. pH data were required for determining sulphide and ammonia speciation. Similarly, conductivity data were needed purely to calculate the ionic strength for the sulphide speciation equations. Conductivity varied minimally between ponds (mean = 1949, se = 16 μ S cm⁻¹), and was considered unlikely to have any significant biological relevance, thus conductivity was not analysed in this context. In particular, direct effects on waterfowl resulting from such variation would be highly unlikely to occur (Goodsell, 1990). Turbidity data were used to make broad inferences about the possibility of light availability limiting phytoplankton growth.

Data analysis

For all analyses, waterbird data were corrected for the size of the pond; the first two ponds in each series were typically slightly smaller in area than the others. This was done by calculating the mean surface area of all 35 ponds and adjusting abundances for each pond accordingly.

All multivariate analyses were performed using PATN (Belbin, 1990). In order to improve clarity, taxa that did not occur on at least five pond-dates (see below) were not included in the analysis (Marchant, 1990). Waterbird and invertebrate density data were \log_{10} transformed and separate similarity matrices calculated using the Bray–Curtis dissimilarity measure (Bray & Curtis, 1957). The correlation between the waterbird and invertebrate similarity matrices was tested for significance using Mantel's *r* equivalent (Mantel, 1967) against 1000 randomisations of the matrices.

The 105 pond-dates - i.e. 35 ponds \times three dates – were reduced to fewer clusters with similar species composition using the agglomerative nonhierarchical clustering algorithm, ALOC. A maximum allocation radius of 0.6 defined 12 groups of pond-dates based on waterbird community composition and 14 groups based on invertebrate communities. These pond-date clusters were then classified in a hierarchical manner using flexible unweighted pair groups with arithmetic averaging (UPGMA: Sneath & Sokal, 1973). Groups of species having similar densities across pond-dates were classified using the Two-Step similarity measure and flexible UPGMA. After the classifications, groups of species and pond-date clusters were formed by visual inspection of the dendrograms and the resulting classification trees presented in two-way tables.

A limitation of the invertebrate data set was that different techniques were used to count rotifers and the larger zooplankton (because of size and magnification restrictions). Consequently, the per litre abundances of rotifers would not be expected to be as precise or accurate as those for the other zooplankton.

The effect of stage of sewage treatment on several variables was analysed using a repeatedmeasures analysis of variance (performed on SAS), with series being modelled as a blocking factor. The analysis made the assumption that there was no significant block (series) by treatment (stage) interaction; it was not possible to test for this interaction because of lack of replication within each series. However, in Figures 2, 4 and 5 data are presented independently for each series so that some appreciation of variation between series can be gained. The date by treatment interaction was tested for, and where it was found to be significant (p < 0.05) statistical inference about the treatment effect was not made, as there was a large number of factor levels due to the interaction. Most data did not require transformation, the exceptions being chlorophyll *a*, un-ionised ammonia (both square root transformed) and turbidity ($\log_{10} (x + 1)$ transformed). Unless otherwise stated, 'significance' for the treatment (stage) effect refers to the 0.05 probability level. Where significant differences were detected between sewage treatment stages, multiple comparisons using Tukey's Honestly Significance Difference (HSD) at p=0.05 were used to determine which stages of sewage treatment were different from others.

The relationship between selected species of waterfowl and potential invertebrate prey items was analysed using Spearman's rank correlation, r_s , (performed on SigmaStat[®], Version 1.0). Because of the large number of comparisons there was an increased likelihood of making a type one error. This was addressed by presenting significance at the p = 0.01 and 0.001 levels in addition to p = 0.05.

The nature of the design of the sewage treatment system meant that in each series the early stages of treatment were furthest away from the ocean, and the final stages were closest to it (Fig. 1). It is possible that waterbirds may have preferentially used the ponds at the end of the system because of their proximity to the coastal zone (assuming these birds also used the coast). However, this is considered unlikely because the ponds at the start of each series are still only about 2 km from the coast, and it is presumed that this distance would be considered minimal to a bird searching for a favourable feeding site. Most of the waterfowl species at the WTP are more numerous on the sewage ponds than the coastal zone (Loyn et al., 2002).

Results

Stage of sewage treatment and waterbird community structure

Five main groups of pond-date clusters were identified (Table 1, using a fusion distance of 0.8 as arbitrary cut-off). Group A was composed of the ponds at the early stages of treatment, and was characterised by a complete absence of birds. Stage 1 ponds occurred in this group on all occasions except one, and Stage 2 on all except three. Stages 3 and 4 were also represented in this group. Groups B and E comprised single observations of

Table 1. Two-way table showing 'clusters' and species groups of waterbirds based on abundance of birds across the 105 pond-dates (35 ponds \times 3 dates) at the 145 West series at the Western Treatment Plant



Each cluster represents a group of pond-dates, formed using the agglomerative clustering technique. The number of ponds from each 'stage' of sewage treatment (1 = raw sewage at start of series through to 7 = treated effluent at end of series) within each cluster are represented. Values within the table represent the mean number of birds within each species for that cluster. BBD = blue-billed duck; musk = musk duck; Hh = hardhead; BS = black swan; SD = Australian shelduck; coot = Eurasian coot; Shov = Australasian shoveler; grebe = hoary-headed grebe; CT = chestnut Teal; GT = grey teal; PeD = pink-eared duck; PBD = Pacific black duck. Data collected on 19 February 2000, 26 February 2000 and 4 March 2000.

a Eurasian coot (Fulica atra Linnaeus) and a chestnut teal (Anas castanea Eyton) on ponds at Stages 1 and 3, respectively. Group C comprised ponds from Stages 2–4, and was characterised by low abundances of black swan (Cygnus atratus Latham), Eurasian coot, grey teal (Anas gracilis Müller) and Pacific black duck (Anas superciliosa Gmelin) and no other species. All species were found in greatest abundance in Group D, and many species were only found in this group. This group was composed entirely of ponds from Stages 4 to 7, and it divided into two Sub-groups at a fusion distance of about 0.64. One of these, Subgroup D1, mainly comprised ponds from Stages 4 and 5, and the other, Sub-group D2, was dominated by ponds from Stages 6 and 7, although Stage 5 was also represented. Two of the diving duck species, musk duck (Biziura lobata Shaw) and hardhead (Aythya australis Eyton), were only found in Sub-group D2, and the other, blue-billed duck (Oxyura australis Gould), was more common in Sub-group D2 than D1. Also, black swan, Australian shelduck (Tadorna tadornoides Jardine & Selby), Eurasian coot, Australasian shoveler and hoary-headed grebe (Poliocephalus poliocephalus Jardine & Selby) were all more abundant in Sub-group D2 than D1.

Overall, there was a low level of ecological dissimilarity between waterfowl species. Nevertheless, the community did break into two separate groups at a fusion distance of 0.40 (Table 1). The three diving duck species comprised one of these groups, and the remaining species the other. Two defining characteristics tended to separate the diving duck group from the other. Firstly, it was generally composed of lower numbers of birds. Secondly, they were mainly found in Subgroup D2, which only included Stages 5–7, as opposed to the other waterbirds which were generally found in both Sub-group D1 (includes Stages 4–6) and D2 (although typically more numerous in D2).

Stage of sewage treatment and planktonic invertebrate community structure

As with the waterbirds, there was a low level of ecological dissimilarity among the invertebrate species, suggesting that they generally constituted one basic type of invertebrate community – one

that generally occurred most frequently in the ponds in the last few stages of treatment. Two main pond-date groups were identified: one consisted of only the first three stages (mainly the first two), and the other mainly comprised Stages 4–7, although the first three stages were also represented (Table 2).

The first of these groups (Clusters 1, 6, 8 and 4) was characterised by a large number of pond-dates that had either a complete absence (Cluster 4) or very low abundances of planktonic microfauna. The only exceptions to this were Cluster 6, and to a lesser extent Cluster 8, which had large numbers of an unidentified free-living nematode species. *Chironomus* sp. larvae were also more common in this cluster than any others, although abundance was still generally low (the life history of chironomids dictates that the vast majority of larvae would be expected to be found in the sediment).

The second group (Clusters 2, 3, 7, 5 and 9), composed mainly of Stages 4–7, and clearly contained more invertebrate species than the first. Nearly all species were more abundant in this group. All rotifer species, copepod nauplii, *Daphnia carinata* (King), *Pleuroxus inermis* (Sars) and an unidentified corixid species were entirely absent from Group A. The only exceptions to this trend were the unidentified nematode and chironomid species, which were both more dominant in the first group.

The invertebrate community could be broken down into two sub-communities, which separated at a fusion distance of 0.49 (Table 2). One contained all the rotifer species plus the dominant micro-crustacean, Mesocyclops sp., and a copepod nauplius. Presumably, the nauplius is that of Mesocyclops sp.; these two formed a distinct subgroup within this group, and Mesocyclops sp. was the only copepod found. Nevertheless, it was more practical to treat them as separate functional species because of their different form. The other subcommunity did not contain any rotifer species. D. carinata was the dominant micro-crustacean, the others being P. inermis and an unidentified ostracod. The unidentified species of nematode and corixids, and *Chironomus* sp. were also present in this community. It should be noted that the high abundance of nematodes (identified as a free living species by inspection of mouth parts) in Cluster 6 was the result of a large number (723) being re-





ang = Brachionus angularis; B. buda = Brachionus budapestinensis; B. caly. A and B = Brachionus calyciflorus ecotypes A and B respectively; B sp. A and B = unidentified Brachionus species A and B, respectively; Chiro. = Chironomus sp. larva. The ostracod and nematode were single species. Data collected on 19 February 2000, 26 February The number of ponds from each 'stage' of sewage treatment (1 = raw sewage at start of series through to 7 = treated effluent at end of series) within each cluster are represented. Values within the table represent mean densities (individuals 1^{-1}) within each species for that cluster. Nauplius = copepod nauplius; Meso = Mesocyclops sp.; B. 2000 and 4 March 2000. corded on one pond-date (Stage 1); they more frequently occurred in lower numbers in ponds further down the system, which is why they did not emerge as an out-lying group in the classification.

Correlation between waterbird and planktonic invertebrate communities

From the above analyses it is clear that most invertebrates and waterbirds were generally found in greatest numbers in the last 3 or 4 stages of sewage treatment. There was a significant correlation between the waterbird and planktonic invertebrate similarity matrices (Mantel's r equivalent = 0.28, n = 1000 randomisations, p < 0.001). Whilst many of the species making up the waterbird community are not likely to feed directly on the plankton, this overall relationship suggests that the planktonic invertebrate communities characterise the type of local pond ecosystem likely to support particular waterfowl communities. Benthic samples would be required to fully test this hypothesis. However, an hypothesis that can be tested with the existing data set is that the distribution of filter-feeding waterfowl species, which were only ever seen feeding on the water's surface at the 145 West ponds, is correlated with the distribution of potential invertebrate prey items. This is considered below.

Concentrations of potential toxicants along treatment gradients

Total sulphide and un-ionised sulphide concentrations clearly decreased along the sewage treatment gradients (Fig. 2a and b, respectively), although the effect depended on the date being sampled, and thus statistical inference about the stage effect was not made. There was a significant effect of stage of treatment on total ammonia concentration; concentrations in Stage 1 were significantly higher than for all the others, and concentrations in Stage 3 were significantly higher than for Stages 5 and 7 (Fig. 2c). There was no clear trend in the concentration of un-ionised ammonia along the sewage treatment gradient (Fig. 2d), and the effect of stage of treatment was not tested due to a significant date by stage interaction. pH tended to be higher in the ponds towards the end of the treatment series (Fig. 2e),

although statistical inference about the stage effect was not made because it was dependent on sampling date.

In Pond 50, the aerobic pond for which oxygen concentration was logged, there was a clear diel pattern in dissolved oxygen levels (Fig. 3). Levels generally increased after sunrise and then decreased after sunset.

Dissolved oxygen concentrations down the middle series of the 145 West ponds from the Stage 1 pond through to the Stage 7 pond were 0.04, 0.08, 0.01, 0.03, 0.03, 2.89, 5.88 mg 1^{-1} .

Correlations between sulphide and ammonia concentrations and the abundance of planktonic invertebrates

With the exception of B. calyciflorus, the abundance of all the dominant invertebrates was significantly negatively correlated with total sulphide concentration (Table 3). These correlations were stronger for the two microcrustacean species, Mesocyclops sp. and D. carinata, than for the rotifers (Table 3). None of the invertebrate species were significantly correlated with the concentration of un-ionised ammonia. The highest recorded concentration of total sulphide-S was 11.1 mg l^{-1} . The highest concentrations of un-ionised sulphide-S and un-ionised ammonia-N were 11.3 and 3.8 mg l^{-1} , respectively. There was also a significant negative correlation between un-ionised sulphide and chlorophyll a concentration ($r_s = -0.23$, p < 0.05); the correlation was checked only for this relationship as the un-ionised species is known to be toxic to algae but speciation does not influence toxicity to invertebrates. There was not a significant correlation between chlorophyll a and un-ionised ammonia (p > 0.05).

Potential food sources for filter-feeding waterfowl

The distribution and abundance of Australasian shoveler was significantly and strongly positively correlated with that of *Mesocyclops* sp. and *D. carinata* (Table 4), and weakly positively (and significantly) correlated with the distribution of *Brachionus angularis* (Gosse), *B. budapestinensis*



Figure 2. Concentrations of potential toxicants, and pH, along five series of WSPs at the 145 West lagoon at the Western Treatment Plant. Stage of sewage treatment refers to the position along the treatment series, with stage 1 representing the. The first ponds in the series (i.e. receiving raw sewage) are represented 'Stage 1', and so forth through to 'Stage 7' at the end of the series. Data were not collected for Stages 2, 4 and 6 for (c) and (d). Data collected on 19 February 2000, 26 February 2000 and 4 March 2000.

(Daday), *Filinia* sp. and total rotifers. A significant positive correlation was also observed between pink-eared duck (*Malacorhynchus membranaceus* Latham) abundance and *Mesocyclops* sp. density,

although the correlation was not as strong as that for Australasian shoveler. The abundance of pinkeared duck was not significantly correlated with *D. carinata*, and the only rotifer with which it was

Table 3. Correlation coefficients (r_s) for Spearman's rank correlation between dominant invertebrates/chlorophyll a and potential chemical toxicants at the 145 West series at the Western Treatment Plant

	Meso.	D. cari.	B. ang.	B. caly.	B. buda.	Filinia	Chl a	
T sul-S	-0.81^{***}	-0.70^{***}	-0.39***	0.01	-0.33***	-0.38***	NA	
NH ₃ -N	-0.08	0.16	-0.05	-0.03	-0.07	0.02	0.22	

T sul-S = total sulphide-S; NH₃-N = un-ionised ammonia-N; NA = not applicable; *Meso.* = *Mesocyclops* sp.; *D. cari.* = *D. carinata; B. ang.* = *B. angularis; B. caly.* = *B. calyciflorus; B. buda.* = *B. budapestinensis; Filinia* = *Filinia* sp.; Chl. *a* = chlorophyll *a.* *p < 0.05, **p < 0.01, ***p < 0.001. Data collected on 19 February 2000, 26 February 2000 and 4 March 2000.

Table 4. Correlation coefficients (r_s) for Spearman's rank correlation between density/concentration of dominant invertebrates/ chlorophyll *a* and abundance of filter-feeding waterfowl at the 145 West series at the Western Treatment Plant

	Meso.	D. cari.	B. ang.	B. caly.	B. buda.	Filinia	tot. rotif.	Chl. a
Shoveler	0.74*	0.63*	0.31**	-0.18	0.31**	0.20***	0.25**	0.04
Pink-eared duck	0.33*	0.02	0.26**	0.03	0.14	0.19	0.17	0.23*

Meso. = *Mesocyclops* sp.; *D. cari.* = *D. carinata*; *B. ang.* = *B. angularis*; *B. caly.* = *B. calyciflorus*; *B. buda.* = *B. budapestinensis*; *Filinia* = *Filinia* sp.; 'tot. rotif.' = total rotifers; Chl. a = chlorophyll a. ***p < 0.05, **p < 0.01, *p < 0.001. Data collected on 19 February 2000, 26 February 2000 and 4 March 2000.

significantly correlated was *B. angularis*. There was no significant relationship between chlorophyll *a* concentration (an indicator of total algal biomass) and Australasian shoveler abundance, however, a significant, but weak, correlation was observed with pink-eared duck.

Further to these correlations, it should be noted that both Australasian shoveler and pinkeared duck were only observed on ponds where *Mesocyclops* sp. was found (64 of the 105 ponddates). In contrast, both species were observed, sometimes in large numbers, in ponds where *D. carinata* was not found. Similarly, flocks of over 100 Australasian shoveler were observed feeding on two occasions on ponds where no rotifers were recorded. It should also be noted that no plant seeds, which could be a potential food source, were found in any of the plankton samples.

Other possible factors limiting productivity in ponds

Total phosphorus and dissolved reactive phosphorus concentrations were similar at both ends of the treatment system (Fig. 4a and b, respectively). Nitrate levels were lower at the end of the system, whereas nitrite levels were higher (Fig. 4c and d, respectively). Dissolved forms of nitrogen and phosphorus were detected in all sampled ponds. Cyanobacterial blooms were not observed on any of the ponds.

Turbidity generally decreased down the series (Fig. 5a), but statistical inferences about differences between stages of treatment were not made because of a significant date by stage interaction. Similarly, there was a significant date by stage interaction for chlorophyll *a* (Fig. 5b). Chlorophyll *a* concentration was not significantly correlated with turbidity ($r_s = -0.09$, p = 0.36).



Figure 3. Diurnal variation in dissolved oxygen concentration in an aerobic pond (Pond 50) in the 145 West series at the Western Treatment Plant from 2051 h on 1 March 2000 to 1200 h on 3 March 2000. Arrows denote sunrise (SR) and sunset (SS).



Figure 4. Concentrations of nutrients at the start (Stage 1, i.e. ponds receiving raw sewage) and end (Stage 7, i.e. ponds representing the most treated effluent) of each sewage treatment series. Each bar represents the mean for the three dates (+1 s.e.). Data collected on 19 February 2000, 26 February 2000 and 4 March 2000. *N.B.* The other important nutrient, ammonia, is presented in Figure 2.

Discussion

General trends in waterbird and planktonic invertebrate communities along sewage treatment gradients

In this study, the general ecology of WSPs was found to change markedly along sewage treatment gradients. Both planktonic invertebrate and waterbird numbers were generally highest in the last few ponds of each series, and the waterbird community was positively correlated with the invertebrate community. Whilst many waterbirds, such as bottom-feeding species, would be unlikely to feed directly on plankton, the correlation does imply that a diverse/abundant planktonic invertebrate community generally represents the type of pond ecosystem likely to support a high abundance and diversity of waterbirds.

In general, a particular WSP either supported an invertebrate and waterfowl community or it did not; there were not many different types of communities. From the perspective of managing WSPs for waterbird conservation purposes, this general finding suggests that a pond could be managed to simultaneously meet the needs of many different



Figure 5. Trends in turbidity and chlorophyll *a* concentration along five series of WSPs at the 145 West lagoon. Presentation and analysis is the same as that for Figure 2. Data collected on 19 February 2000, 26 February 2000 and 4 March 2000.

waterbird species. But the data from this study only represent a particular time of year, late summer, and so it was only possible to rigorously assess the distribution of some species. Australasian shoveler and hoary-headed grebe abundance did tend to change along a continuum of stage of sewage treatment. These were the two most abundant species in the study, and it is possible that similar trends may have emerged for other species had sampling been undertaken when they were more abundant. Pacific black duck was the only species that appeared to use the ponds at the earlier stages of treatment, as well as the later stages, although informal observations suggest that they rarely fed on these early treatment ponds.

Relationship between filter-feeding waterfowl and potential prey species

The findings of this study suggest that for Australasian shoveler and pink-eared duck the availability of invertebrate prey items in the plankton probably influenced the use of WSPs by these birds. Most correlative studies of waterbird and food abundance have been conducted on herbivorous and/or benthic feeding species that would gain little, if any, food from the plankton (Hanson & Butler, 1994; Lillie & Evrard, 1994; Nummi et al., 1994; Rehfisch, 1994). However, the young of some species often feed on plankton. The abundance of young of American black duck (Anas rubripes Brewster) for example were found to be significantly, and positively, correlated with both planktonic and benthic invertebrate biomass across 32 freshwater lakes in western Nova Scotia (Staicer et al., 1994).

According to the filtering efficiency studies of Crome (1985), organisms the size of *Mesocyclops* sp. and *D. carinata* would be extracted readily by both Australasian shovelers and pink-eared ducks. Australasian shoveler abundance was strongly positively correlated with the density of both these invertebrates, and pink-eared duck was correlated with *Mesocyclops* sp. but not *D. carinata*. The fact that pink-eared duck abundance was not correlated with *D. carinata*, and Australasian shoveler was, might reflect the much smaller numbers of pink-eared duck present during the study rather than any ecological difference.

Australasian shoveler and pink-eared duck abundances were also positively correlated with the abundance of some of the rotifer species. Two possible explanations for these correlations are that rotifers may be important prey items for these ducks, or conditions that favour rotifers may also favour other zooplankton that are prey for ducks. Rotifers have not been reported in the natural diets of Australasian shovelers (Frith, 1959; Marchant & Higgins, 2000), nor have they been reported as part of the diet of other shoveler species. It is possible, however, that they have been ignored because of their small size. Rotifers have not been reported in most dietary studies of pink-eared ducks (Marchant & Higgins, 2000). However, Frith (1959) noted that in a gizzard content survey of 138 birds in western New South Wales rotifers numerically accounted for 7.3% of the diet. But this would have accounted for a negligibly small proportion of the total biomass consumed, considering that the other prey items were substantially larger (mostly molluscs, crustaceans, insects, and plant seeds). Experiments by Crome (1985) demonstrated that extraction of organisms as small as rotifers from the water would be a highly

inefficient process for both Australasian shovelers and pink-eared ducks. It may be, however, that the very high densities of rotifers observed in some of the ponds may compensate for this.

Potential toxicity of sulphide, ammonia and low dissolved oxygen to planktonic invertebrates

Determining what influences the density of invertebrates, or any organism, along sewage treatment gradients is difficult because there are typically many confounding factors; many parameters change along a series of ponds. Thus, the effects of particular determinants cannot be separated by correlation (Ip et al., 1982). The approach taken in the present study was to identify correlations between potential toxicants and invertebrate numbers, and then test the likelihood of causation based on published bio-assay studies. Many bioassay studies have been conducted on Daphnia, as it is commonly used as a bio-indicator species (APHA et al., 1998). In contrast, there is little information available on the effects of toxicants on cyclopoid copepods such as Mesocyclops, a dominant zooplankter found in this study. Also, there

Table 5. Theoretical toxicities of sulphide, ammonia and low dissolved oxygen to D. carinata along the 145 West system at the Western Treatment Plant

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7
Sulphide	53.5 (11.3)	25.0 (6.3)	5.2 (1.7)	1.7 (0.2)	1.2 (0.0)	1.1 (0.0)	1.1 (0.0)
Ammonia	7.2 (6.6)	ND	0.6 (0.1)	ND	0.5 (0.1)	ND	0.8 (0.3)
Low DO ₂	85.8	64.6	93.7	89.1	89.1	0.0	0.0

Based on toxicity model of Hathaway and Stefan (1995) using data collected from the 145 West series on 19 February 2000, 26 February 2000 and 4 March 2000. Values represent daily percent mortality for each stage of treatment. Values for ammonia and sulphide represent the mean (s.e.) of 15 measurements (5 ponds \times 3 dates). Values for dissolved oxygen are based on measurements along the middle series of ponds in winter (7 July 2000). ND = no data collected for this stage.

appear to be no studies on the effects of sulphide, ammonia or low dissolved oxygen on rotifers. Therefore, the following discussion concentrates on the larger zooplankton species.

A model for estimating the toxicity of sulphide, ammonia and low dissolved oxygen to Daphnia (not species specific) in WSPs was developed by Hathaway & Stefan (1995), based on previously published bio-assay studies. When the data from the present study were run through this model, dissolved oxygen emerged as the most important toxicant to D. carinata, and sulphide also appeared to reach toxic levels in many of the ponds in the early stages of treatment (Table 5). Furthermore, these data were collected in winter, when dissolved oxygen levels in Australian WSPs would be expected to be at their lowest (Mitchell & Williams, 1982b). Also, dissolved oxygen concentration in a pond may vary substantially over a day. This is most likely the situation in aerobic ponds where oxygen concentration will gradually increase throughout the day as a result of oxygenic photosynthesis by algae before decreasing again at night as a result of microbial respiration, and this phenomenon can be seen clearly in Figure 3. It should also be noted that the values in Table 5 represent mortality per day, they do not take into account reproduction and population dynamics. Nevertheless, these values do give an indication of the relative significance of various toxicants. The apparent relatively high toxicity of ammonia in the first stage of treatment, 7.2%, is somewhat misleading, as it is an average of recordings including one pond-date that had a toxicity of 99.7%: the toxicity levels of all other pond-dates was less than 0.05%. This pond-date with high potential Daphnia mortality was characterised by a higher concentration of total ammonia; the pH was not higher than most other ponds.

Although ammonia toxicity to *D. carinata* was generally negligible according to the daily mortality model, it must be recognised that the parameters in this model were based on published acute toxicity tests (see references in Hathaway & Stefan, 1995), over 48 h, to a given population. Chronic toxicity work by Gersich & Hopkins (1986) demonstrated that after 21 days exposure to 0.87, 1.88 and 3.65 mg l^{-1} un-ionised ammonia-N, mortality of D. magna was 15, 65 and 100%, respectively. Furthermore, of those surviving the 0.87 and 1.88 mg l^{-1} treatments, sub-lethal reproductive effects were reported. The mean total young per individual decreased by 26 and 58%, respectively. Levels of un-ionised ammonia in the 145 West system were often in excess of 0.87 mg l^{-1} (Fig. 2d). However, D. carinata density was not correlated with un-ionised ammonia concentration, and D. carinata were generally found in greatest densities in the last two ponds of a series, whereas un-ionised ammonia levels were roughly consistent throughout the series (if not slightly higher towards the end, Fig. 2d). These results tend to suggest that even though ammonia toxicity may affect D. carinata productivity in most of the ponds throughout the 145 West system, at roughly equivalent levels for each stage of treatment, it is not likely to be a key factor in determining the relative distribution of the crustacean along the treatment series.

The fact that the density of *D. carinata* was significantly negatively correlated with sulphide concentration may have been due to direct toxicity. However, it is also possible that it was a spurious correlation, reflecting the fact that sulphide

concentration would be expected to be negatively correlated with dissolved oxygen concentration, as sulphide is produced under anaerobic conditions. It is also possible that in some ponds sulphide and anoxia had additive, or even possibly synergistic, toxic effects.

The effects of these toxicants on *Mesocyclops* sp., or any other copepods, are not well known. Mitchell & Williams (1982b) reported that *Mesocyclops* sp. grew in Australian WSPs at dissolved oxygen concentrations above 2 mg l^{-1} , with highest concentrations occurring above 8 mg l^{-1} .

Possible factors limiting pond productivity

Daphnia, Mesocyclops and rotifers are known to feed on single-celled green algae (e.g. Lubzens, 1987; Schlüter et al., 1987), and Daphnia has also been reported to feed on cyanobacteria (de Mott 1998; Thostrup & Christofferson, 1999). But food web structure in a WSP is likely to be complex; most rotifers for example also feed directly on fungi and bacteria (Lubzens, 1987), Daphnia and Mesocyclops feed on rotifers and protozoans (Gilbert & Williamson, 1978; Gilbert, 1988; Boyall, 1995), and Daphnia can even feed directly on bacteria, albeit inefficiently (McMahon & Rigler, 1965; Lampert, 1974; Peterson et al., 1978). Nevertheless, phytoplankton is likely to be an important food source for crustacean zooplankton, which in turn is probably the most important prey for filter-feeding waterfowl. Moreover, the phytoplankton is responsible for oxygenating the aerobic ponds, and thus may affect invertebrates through this route as well. Therefore, it is important to make an attempt to discern what factors influence phytoplankton biomass in WSPs to more fully understand the distribution of invertebrates, and ultimately waterfowl, along sewage treatment gradients.

Ammonia toxicity has been suggested to be a factor limiting algal production in WSPs, primarily through photosynthetic inhibition (Abeliovich & Azov, 1976). It is the un-ionised form that is toxic to algae (Warren, 1962). In the present study though, chlorophyll *a* concentration was not significantly correlated with un-ionised ammonia.

Chlorophyll *a* concentration was, however, significantly negatively correlated with un-ionised sulphide concentration. In contrast to sulphide

toxicity to invertebrates, toxicity to algae is strongly dependent on the form of sulphide, and it is the un-ionised species that is toxic, which predominates at low pH levels. Pearson et al. (1987) conducted *in vitro* experiments of the toxicity of both ammonia and sulphide in sewage to photosynthesis by four species of algae, and found that sulphide was more toxic than ammonia to all species.

Pearson et al. (1987) found that sensitivity to both sulphide and ammonia varied between species of algae found in WSPs. They estimated that a 50%reduction in photosynthesis resulted from exposure to 53.8, 13.7, 22.7 and 12.33 mg l^{-1} un-ionised ammonia-N for Chlorella, Euglena, Scenedesumus and Chlamydomonas respectively. In the present study, only one measurement above 3 mg l^{-1} was reported (5.3 mg l^{-1}). Pearson et al. (1987) also reported 50% inhibition of photosynthesis by unionised sulphide-S at 0.34, 0.71, 0.97 and 1.44 mg l^{-1} for *Chlorella*, *Euglena*, *Scenedesumus* and Chlamydomonas, respectively. In the first stage of treatment in the 145 West ponds un-ionised sulphide often reached levels in excess of 1 mg l^{-1} $(\text{maximum} = 3.8 \text{ mg } l^{-1}, \text{ mean} = 1.3 \text{ mg } l^{-1}), \text{ and}$ chlorophyll a concentration was generally very low. Based on these values, and the correlations described above, it would seem unlikely that ammonia toxicity greatly inhibits phytoplankton production (particularly algal) in the 145W system, whereas un-ionised sulphide may have a substantial influence on production in some ponds.

Cyanobacteria, which also use chlorophyll a as their photosynthetic pigment, are also an important constituent of the phytoplankton, particularly in WSPs. Unlike algae, cyanobacteria can carry out anoxygenic photosynthesis in addition to oxygenic and can actually thrive in the presence of sulphide. However, sulphide can be toxic to oxygenic photosynthesis in cyanobacteria. A study by Howsley & Pearson (1979) revealed that oxygenic photosynthesis was inhibited by 50% at concentrations of about 0.04, 0.37 and 0.21 mg l^{-1} un-ionised sulphide-S for Anabaena, Synechocystis and Oscillatoria, respectively. Whilst these species were not found in the 145 West system (A.J.H. unpublished data), the levels of un-ionised sulphide in the first few ponds in most series (Fig. 2b) would in theory be highly toxic to oxygenic photosynthesis by them. This may partly explain why dissolved oxygen only increased in the last couple of ponds in the series even though chlorophyll *a* concentration was relatively high in the preceding ponds; presumably cyanobacteria were undertaking anoxygenic photosynthesis in the ponds further up the series.

Another factor that may have influenced phytoplankton production is turbidity, which clearly decreased down the treatment gradient. However, chlorophyll *a* concentration was not significantly correlated with turbidity ($r_s = -0.09$, p = 0.36). The effects of turbidity are difficult to ascertain, since it may not only increase with increasing concentrations of suspended solids, but also with increased phytoplankton density itself.

The fact that ammonia, dissolved reactive phosphorus, nitrate, and nitrite were detected in all the ponds where they were measured suggests that nutrients were not limiting to primary production in this system. If a particular nutrient is available in the dissolved form (i.e. DRP, nitrate, nitrite and ammonia), it is considered to be in excess, and thus not limiting (Hecky & Kilman, 1988).

Conclusion

Waterbirds and zooplankton were generally found highest numbers in the ponds towards the end of the treatment series. Filter-feeding waterfowl probably used these ponds because of the availability of zooplankton as a food-source. Un-ionised sulphide and low dissolved oxygen stress may be important determinants of zooplankton community structure and size.

Acknowledgements

We wish to thank Russell Shiel for assistance with identifying zooplankton, Kylie Lewin for identifying the chironomid, and Lila Nambiar for determining the trophic class of the nematode. David Rogers, James Rogers and Steve Whitmore helped with the fieldwork. We also acknowledge the Johnstone Centre, Charles Sturt University, for covering the cost of reagents and filters. The Institute for Horticultural Development kindly let us use their laboratory equipment. The study was conducted under a Melbourne Water study permit (SP01/98). We are also grateful to Pam Rogers for graphical assistance.

References

- Abeliovich, A. & Y. Azov, 1976. Toxicity of ammonia to algae in sewage oxidation ponds. Applied and Environmental Microbiology 31: 801–806.
- APHA, AWWA & WEF, 1998. Standard Methods for the Examination of Water and Wastewater. 20th edn. American Public Heath Association, New York.
- Belbin, L., 1990. PATN: Technical Reference Manual. CSIRO, Canberra.
- Boyall, J., 1995. The role of protozoa in the shallow lagoon sewage treatment system at the Western Treatment Plant, Werribee. Department of Zoology, La Trobe University, Bundoora.
- Braithwaite, L. W. & D. A. Stewart, 1975. Dynamics of water bird populations on the Alice Springs Sewage Farm, N.T. Australian Wildlife Research 2: 85–90.
- Bray, J. R. & C. T. Curtis, 1957. An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs 27: 325–349.
- Campbell, L. H. & H. Milne, 1977. Goldeneye feeding close to sewer outfalls in winter. Wildfowl 28: 81–85.
- Crome, F. H. J., 1985. An experimental investigation of filterfeeding on zooplankton by some specialized waterfowl. Australian Journal of Zoology 33: 849–862.
- de Moor, P., 1993. Species changes at Sea Cow Lake over 13 years. Albatross 313: 3–4.
- de Mott, W. R., 1998. Utilization of a cyanobacterium and a phosphorus-deficient green alga as complementary resources by daphnids. Ecology 79: 2463–2481.
- Emerson, K., R. C. Russo, R. E. Lund, & R. V. Thurston, 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. Journal of the Fisheries Research Board Canada 32: 2379–2383.
- Frith, H. J., 1959, The ecology of wild ducks in inland N.S.W.: III food habits. CSIRO Wildlife Research 4: 131–155.
- Fuller, R. J. & D. E. Glue, 1978. Seasonal activity of birds at a sewage-works. British Birds 71: 235–244.
- Fuller, R. J. & D. E. Glue, 1980. Sewage works as bird habitat in Britain. Biological Conservation 17: 161–181.
- Fuller, R. J. & D. E. Glue, 1981. The impact on bird communities of the modernisation of sewage treatment works. Effluent Water Treatment Journal 21: 27–31.
- Gersich, F. M. & D. L. Hopkins, 1986. Site-specific acute and chronic toxicity of ammonia to *Daphnia magna* Straus. Environmental Toxicology and Chemistry 5: 443– 447.
- Gilbert, J. J., 1988. Suppression of rotifer populations by *Daphnia*: a review of the evidence, the mechanisms, and the effects on zooplankton community structure. Limnology and Oceanography 33: 1286–1303.
- Gilbert, J. J. & C. E. Williamson, 1978. Predator-prey behavior and its effect on rotifer survival in associations of *Mesocyclops edax*, *Asplancha girodi*, *Polyarthra vulgaris*, and *Keratella cochlearis*. Oecologia 37: 13–22.

- Goodsell, J. T., 1990. Distribution of waterbird broods relative to wetland salinity and pH in South-western Australia. Australian Wildlife Research 17: 219–229.
- Hamilton, A. J., 2002. The ecology of waterbirds at the Western Treatment Plant (Victoria), with particular reference to waterfowl, PhD Thesis, Charles Sturt University, Wagga Wagga.
- Hamilton, A. J. & I. R. Taylor, in press. Seasonal patterns in abundance of waterfowl (Anatidae) at a waste-stabilisation pond. Corella.
- Hanson, M. A. & M. G. Butler, 1994. Responses to food web manipulation in a shallow waterfowl lake. Hydrobiologia 279/280: 457–466.
- Hathaway, C. J. & H. G. Stefan, 1995. Model of *Daphnia* populations for wastewater stabilization ponds. Water Research 29: 195–208.
- Hawkes, H. A., 1983. Stabilization ponds. In Curds, C. R. & H. A. Hawkes (eds), Ecological Aspects of Used-water Treatment. Academic Press, London: 163–217.
- Hawking, J. H. & F. J. Smith, 1997. Colour guide to invertebrates of Australian inland waters. Co-operative Research Centre for Freshwater Ecology, Albury.
- Hecky, R. E. & P. Kilman, 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. Limnology and Oceanography 33: 796–822.
- Hodgson, B. & P. Paspaliaris, 1996. Melbourne Water's wastewater treatment lagoons: design modifications to reduce odours and enhance nutrient removal. Water Science and Technology 33: 157–164.
- Howsley, R. & H. W. Pearson, 1979. pH dependent sulphide toxicity to oxygenic photosynthesis in cyanobacteria. FEMS Microbiology Letters 6: 287–292.
- Ip, S. Y., J. S. Bridger, C. T. Chin, W. R. B. Martin & W. G. C. Raper, 1982. Algal growth in primary settled sewage: the effects of five key variables. Water Research 16: 621–632.
- Konig, A., H. W. Pearson & S. A. Silva, 1987. Ammonia toxicity to algal growth in waste stabilisation ponds. In Mara, D. D. & M. H. Mareos de Morte (eds), Water Science and Technology: 115–123.
- Lampert, W., 1974. A method for determining food selection by zooplankton. Limnology and Oceanography 19: 995–998.
- Lane, B. & P. Peake, 1990. Nature conservation at the Werribee Treatment Complex. Rep. No. 91/008. Melbourne and Metropolitan Board of Works, Melbourne.
- Loyn, R. H., E. S. G. Schreiber, R. J. Swindley, K. Saunders & B. A. Lane. Use of Sewage Treatment Lagoons by Waterfowl at the Western Treatment Plant – an Overview. Department of Natural Resources and Environment, Brett Lane and associates Pty Ltd, WaterECOscience. Heidelberg.
- Lillie, R. A. & J. O. Evrard, 1994. Influence of macroinvertebrates and macrophytes on waterfowl utilization of wetlands in the Prairie Pothole Region of northwestern Wisconsin. Hydrobiologia 279/280: 235–246.
- Lubzens, E., 1987. Raising rotifers for use in aquaculture. Hydrobiologia 147: 245–255.
- Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27: 209– 220.

- Marchant, R., 1990. Robustness of classification and ordination techniques applied to macroinvertebrate communities from the La Trobe River, Victoria, Australia. Australian Journal of Marine and Freshwater Research 41: 493– 504.
- Marchant, S. & P. J. Higgins, 1990. Handbook of Australian, New Zealand and Antarctic birds. Vol. 1, Ratitites to Ducks. Oxford University Press, Melbourne.
- Matsuanga, H., K. I. Harada, M. Senma, Y. Ito, N. Yasuda, S. Ushida & Y. Kimura, 1999. Possible cause of unnatural mass death of wild birds in a pond in Nishinomiya, Japan: sudden appearance of toxic cyanobacteria. Natural Toxins 7: 81– 84.
- Maxson, G. A., 1981. Waterfowl use of a municipal sewage lagoon. The Prairie Naturalist 13: 1–12.
- McMahon, J. W. & F. H. Rigler, 1965. Feeding rate of *Daphnia magna* Straus in different foods labelled with radioactive phosphorus. Limnology and Oceanography 10: 105–113.
- Mitchell, B. D. & W. D. Williams, 1982a. Factors influencing the seasonal occurrence and abundance of the zooplankton in two waste stabilization ponds. Australian Journal of Marine and Freshwater Research 33: 989–997.
- Mitchell, B. D. & W. D. Williams, 1982b. The performance of tertiary treatment ponds and the role of algae, macrophytes and zooplankton in the waste treatment process. Australian Water Resources Council Occasional Papers Series No. 2: 1– 90.
- Nero, R. W., 1964. Detergents deadly hazard to water birds. Audubon Magazine 66: 26–27.
- Nummi, P., H. Poysa, J. Elmberg & K. Sjoberg, 1994. Habitat distribution of the mallard in relation to vegetation structure, food and population density. Hydrobiologia 279/280: 247–252.
- OECD, 1982. Eutrophication of Waters. Monitoring, Assessment and Control. OECD, Paris.
- Paul, W., 1995. Lagoon technology at Melbourne Water's Western Treatment Plant. In Kolarik, L. O. & A. J. Priestley (eds), Modern Techniques in Water and Wastewater Treatment. CSIRO Publishing, East Melbourne: 141–148.
- Pearson, H. W., 1987. Algae associated with sewage treatment. In Da Silva, E. J. (ed.), Microbial Technology in the Developing World, Oxford University Press, Oxford: 260– 288.
- Peterson, B. J., J. E. Hobbie & J. F. Haney, 1978. *Daphnia* grazing on natural bacteria. Limnology and Oceanography 23: 1039–1044.
- Piest, L. A. & L. K. Sowls, 1985. Breeding duck use of a sewage marsh in Arizona. Journal of Wildlife Management 49: 580– 585.
- Ramsar Convention Bureau, 1984. Proceedings of the Second Conference of the Parties; Groningen, Netherlands, 7 to 12 May 1984. Convention on Wetlands of International Importance especially as Waterfowl Habitat. International Union for Conservation of Nature and Natural Resources, Gland.
- Rehfisch, M. M., 1994. Man-made lagoons and how their attractiveness to waders might be increased by manipulating the biomass of an insect benthos. Journal of Applied Ecology 31: 383–401.

- Schlüter, M., J. Groeneweg & C. J. Soeder, 1987. Impact of rotifer grazing on population dynamics of green microalgae in high-rate ponds. Water Research 21: 1293–1297.
- Sneath, P. H. A. & R. R. Sokal, 1973. Numerical Taxonomy. W.H. Freeman, San Fransisco.
- Staicer, C., B. Freedman, D. Strivastava, N. Dowd, J. Kilgar, J. Hayden, F. Payne, F. & T. Pollock, 1994. Use of lakes by duck broods in relation to biological, chemical, and physical features. Hydrobiologia 279/280: 185–199.
- Swanson, G. A., 1977. Diel food selection by Anatidae on a waste-stabilization system. Journal of Wildlife Management 41: 226–231.
- Thostrup, L. & K. Christoffersen, 1999. Accumulation of microcystin in *Daphnia magna* feeding on toxic *Microcystis*. Archives für Hydrobiologie 145: 447–467.
- Uhler, F. M., 1964. Bonus from waste places. In Linduska, J. P. (ed.), Waterfowl Tomorrow. Government Printing Office, Washington DC: 643–653.
- Uhlmann, D., 1980. Limnology and performance of waste treatment lagoons. Hydrobiologia 72: 21–30.
- Warren, K. S., 1962. Ammonia toxicity and pH. Nature 195: 47–49.
- Wrigley, T. J. & D. F. Toerien, 1990. Limnological aspects of small sewage ponds. Water Research 24: 83–90.