5 Ecological changes to phyto- and zooplankton communities as a consequence of different WSP effluent upgrade methodologies: results from pilot-scale investigations

5.1 Introduction

Waste stabilisation ponds are shallow flow-through open reactors in which complex interactions between the natural populations of bacteria, algae, protists and metazoans contribute toward significant reductions in the levels of suspended solids, organic matter and pathogens (Kryutčhkova, 1968; Andronikova, 1978; Hussainy, 1979; Mitchell, 1980; Uhlmann, 1980; Cauchie *et al.*, 2000b). These biotic communities have long been recognised as primary facilitators of the overall 'waste stabilisation' process; incorporating unstable organic materials into stable living tissue (Loedolff, 1965; Ehrlich, 1966; Kryutčhkova, 1968). The rich micro- and macrobiotic diversity also has a significant role to play in the overall treatment process, with complex and highly productive trophic interactions leading to accelerated biological stabilisation of the inflowing wastewater. Although high densities of metazoan zooplankton (rotifers, copepods, anomopods, ostracods) are often observed in these hypertrophic water bodies (e.g. Tschörtner, 1968; Daborn *et al.*, 1978; Hussainy, 1979; Mitchell and Williams, 1982a; 1982b; Dor *et al.*, 1987; Cauchie *et al.*, 1995; Roche, 1995), the mechanisms affecting both their temporal and spatial distribution, as well as factors dictating the extent of trophic interactions and subsequent biological productivity, remain poorly understood.

It is well known that aquatic invertebrates are responsible for a large part of the secondary production that takes place in shallow freshwater ecosystems, with invertebrate communities serving an integral role in both the detrital- and phytoplankton-grazing food chains. Zooplankton in particular play an essential part in the recycling of algal primary production within shallow freshwater environments, of which WSPs are no exception. Crustacean zooplankton have been recognised as playing a dominant role—in terms of that contributed by the total aquatic microfauna biomass (Downing and Rigler, 1984)—in the stabilisation of organic matter within WSPs, by feeding on phytoplankton and detrital seston and promoting the flocculation and sedimentation of particulate BOD_5 and SS within their faecal pellets (Loedolff, 1965; Uhlmann, 1967; Tschörtner, 1968; Dinges, 1973, cited in Maynard *et al.*, 1999; Hussainy, 1979; Uhlmann, 1980; Tanner *et al.*, 2005). Rotifers have also been cited as playing an important role in shaping the populations of certain lower forms of WSP organisms (e.g. bacteria, small phytoplankton and protozoa)—influencing the temporal succession of predominating planktonic species in such environments (deNoyelles Jr., 1967; USEPA, 1983; Roche, 1995).

In addition to ecological interactions, it has also been suggested that zooplankton populations can in some instances play a physical role in contributing to effective WSP performance. In cases of extreme secondary productivity within WSPs for example, *Daphnia* swarms may reach high enough densities (>1000 L^{-1}) to increase the turbulent dispersion of the water column to the extent necessary for maintenance of aerobic conditions via normal surface exchange and re-aeration processes (Uhlmann, 1980). Additionally, zooplankton (particularly *Daphnia* species) swarms can also reach a large enough density to cause micro-agitation and turbulence to such an extent that they can counteract the development of undesirable thermal stratification and even promote plug-flow conditions (Uhlmann, 1979). Despite the early recognition of the importance of zooplankton in overall pond function (e.g. Kryutčhkova, 1968; Tschörtner, 1968), and in spite of their more recently identified potential as a saleable commodity (e.g. as a proteinaceous aquacultural feedstuff or commercial source of chitin) (Proulx and de la Noüe, 1985a, 1985b; Cauchie *et al.*, 1995; Roche, 1998; Cauchie *et al.*, 2002), zooplankton dynamics in WSPs have remained largely uninvestigated. Little is also known about the seasonal dynamics of zooplankton in these extreme environments, and very few attempts have been made to quantify the *in situ* temporal ecology and biomass dynamics of these communities (e.g. Mitchell and Williams, 1982b; Cauchie *et al.*, 1995; Roche, 1995).

Due to the heightened trophic (hypereutrophic) state within WSPs, and as a result of their ever-changing nutritional inputs, very shallow hydraulic depth and often steep temperature, oxygen and redox potential gradients, they are notoriously unstable systems in an ecological context (Barica and Mur, 1980; Uhlmann, 1980). Unlike nutrient-poor 'oligotrophic' waterways, species richness is inherently reduced within these hypertrophic environments, such that continuous shifts in community dominance mean that there is rarely a chance for ecological 'stable-state' conditions to develop (Romanuk *et al.*, 2006). In addition to this, stochastic short-term variations in WSP hydrodynamic conditions—from both variable hydraulic inputs and also owing to diel variability in local weather patterns and its subsequent influence on pond mixing—often lead to a largely variable effluent water quality over relatively short timescales (Uhlmann, 1980). The combined result of these factors is a permanently 'transient mode' of ecological operation, whereby no biological equilibrium or 'steady-state' (in the strict sense of the word) with regard to abundances, biomass and oxygen budget can be maintained for any significant duration (Barica and Mur, 1980; Uhlmann, 1980).

As introduced (Section 1.3.1.1), the local Bolivar WSP network has undergone significant trophic and subsequent ecological state-changes in recent times as a result of the up-stream commissioning of an activated sludge plant (in February of 2001). The activated-sludge plant has been so efficient at sequestering nutrients up-stream, that the Bolivar WSPs are now at times thought to be nitrogen-limited (Cromar *et al.*, 2005). As a result of this treatment train upgrade, the Bolivar WSPs have gone from a more traditional nutrient-rich hypereutrophic state, to a situation where they are now commonly situated at the lower bounds of 'eutrophy' according to the trophic state index of Carlson (1977). Since this decline in nutritional and trophic status, algal population ecology within the WSPs has become increasingly volatile. In other shallow freshwater environments for example, a reduction in nutrient availability and corresponding trophic state is commonly accompanied by an increase in plankton species richness and diversity (Watson *et al.*, 1997; Olding *et al.*, 2000; Romanuk *et al.*, 2006) and so it is likely that this treatment plant upgrade has directly contributed to the heightened variability in algal ecology.

In addition to the increased variability in algal communities, the ecology of grazing zooplankton populations has also been unstable and largely variable, such that both of these groups have created significant follow-on problems for down-stream DAF/F plant treatment efficiency (see Sections 1.3.1–1.3.1.1 for more information). Despite the recognised 'problematic' nature of some zooplankton taxa (i.e. copepods and cladocerans such as *Daphnia*)—in terms of their adverse impact on DAF/F plant process efficiency (see Section 1.3.1)—there has been no prior assessment of zooplankton population ecology within the Bolivar WSPs. This is despite Martyn *et al*. (2004) highlighting the importance of zooplankton grazing in terms of the negative pressure they exert upon algal populations in the Bolivar stabilisation ponds. Currently, the reasons behind such periodic and unpredictable 'boom and bust' population dynamics remain unclear. Overall, it is possible that increased populations of zooplankton have been able to develop within the Bolivar WSPs as a result of the relatively recent (2001) up-stream activated sludge plant operations having reduced the levels of potentially toxic NH_4^+-N in the ponds by roughly 40% (Cromar *et al*., 2005; Sweeney *et al*., 2005a). Whilst it has been suggested that algal population crashes may be the result of increased grazing pressure from herbivorous zooplankton, and although there has been anecdotal evidence to suggest that high numbers of these organisms do at times exist (Herdianto, 2003), quantitative data is currently lacking.

The previous two chapters have investigated the treatment performance of several advanced in-pond upgrade methodologies with respect to a number of traditional water quality parameters. Initially, it was thought that treatment within such WSP upgrade systems may have a direct and manipulative influence on the out-flowing phyto- and/or zooplankton community structure (because these systems all involved a period of dark-exposure in addition to frequently reduced levels of dissolved oxygen). Since dark-survival capacity in phytoplankton is known to be highly species-specific (refer to Section 6.3.2), and since some zooplankton taxa (e.g. rotifers) are inherently less tolerant to low levels of DO (see Section 4.3.3), it was considered likely that different plankton species will most likely display varying degrees of survivorship during and following these advanced in-pond treatments. Through manipulation of the physical environment (e.g. via duckweed coverage or addition of rock media), it could therefore be possible to indirectly bring about a change the community structure of resident plankton populations—a sort of indirect 'biomanipulation'. As has already been introduced (Section 1.3.1.2), 'biomanipulating' plankton communities in this way could then have potentially significant follow-on implications for down-stream DAF/F plant efficiency; where plankton community structure is known to be capable of influencing process performance. It should be emphasized at this point that it was not the specific aim of this research to attempt to control plankton dynamics directly via manipulation of the aqueous environment; instead, ecological monitoring was conducted on each pilot upgrade system in addition to the primary assessment of their physical treatment performance.

Both phyto- and zooplankton population dynamics across each pilot plant upgrade system were therefore monitored as a means of assessing the likely ecological consequences of advanced WSP treatment within a duckweed, rock filter or attached-growth media upgrade. Although Pace and Orcutt (1981) concluded that protozoa can also make “a significant contribution to rates of grazing, nutrient regeneration, and secondary productivity” in freshwater environments (specifically lakes), they were not enumerated during the current research. Although there has been a considerable combined volume of research duckweed ponds, rock filtration and attached-growth media for the upgrading of final WSP effluents, there have—to the authors knowledge—been no prior quantitative assessments of either phytoplankton or zooplankton community dynamics within these upgrade environments. Finally, and based on these plankton community investigations, potential outcomes with respect to Bolivar DAF/F plant process efficiency will be discussed.

5.2 Methods

A detailed description of plankton sampling, counting and identification protocols is provided in Chapter 2. The reader is also redirected to Appendix E for information on individual organism biomass calculations. As an additional note concerning the results of statistical analyses presented during this Chapter, and because of the staggered operational schedule for the four experimental treatments (see Table 2.1), all statistical analyses for comparing the pilot plant influent with any of the treatment series were performed using only the data from the relevant time periods. In other words, only the *Period 1* 2005 influent data was compared with results from the DW Pond series, as was only the *Period 2* 2006 influent data used when assessing the performance of the AGM treatment series. Since both the RF and OP series were operated over the entire 2005–2006, results from these upgrade treatments were compared with the combined 2005–2006 influent data set.

5.3 Results and discussion

5.3.1 Comparative phytoplankton ecology of the pilot plant influent and the four advanced in-pond upgrades

Due to the relative complexity of performing detailed taxonomic identifications, phytoplankton were identified only to Genus. Moreover, taxonomic classification to the species level is often viewed as being redundant in an ecological context, with Warwick (1988) arguing that hierarchical aggregation of species data at the family level—even higher in some instances—commonly results in no loss of underlying ecological information. Additionally, the inclusion of frequently very low or zero species counts in biological data sets creates analytical problems for some commonly adopted multivariate statistical procedures. Therefore, and even where taxonomic classifications to species level were performed (i.e. for zooplankton populations), phyto- and zooplankton data sets are presented and discussed at the Genus level or higher.

A total of 42 phytoplankton genera were recorded during the approximate 12 month monitoring period from July 2005–August 2006 (Table 5.1). The pilot plant influent (Bolivar WSP effluent) algal community was most strongly represented by green algae (Chlorophyta), diatoms (Bacillariophyta; Bacillariophyceae), flagellate cryptophytes (Cryptophyta; Cryptophyceae) and also by a transient bloom of cyanobacteria (Cyanobacteria; Chroococcales). Although there was frequently in the order of 8–15 genera present at a given sampling interval, typically around 3–4 were numerically dominant at any one time (Figure 5.1). The relatively high level of taxonomic biodiversity in terms of the number of phytoplankton genera observed, was similar to other reports of algal population ecology in WSP environments (Raschke, 1970; Hussainy, 1979; Mitchell, 1980; Tharavathi and Hosetti, 2003) and was likely to have been linked to the reduced trophic status within a maturation WSP environment compared with other more highly polluted wastewater environments such as a high-rate algal pond (e.g. Canovas *et al.*, 1996).

Table 5.1. List of all phytoplankton taxa encountered in the pilot plant influent during monitoring from July 2005–August 2006.

Cyanobacteria (blue-green algae)
- Nostocales
    - Nostocaceae
        - *Anabaena*[†]
- Oscillatoriales
    - Oscillatoriaceae
        - *Lyngbya*[†]
    - Phormidiaceae
        - *Phormidium*[†]
        - *Planktothrix*[†]
    - Pseudanabaenaceae
        - *Pseudanabaena*[†]
- Chroococcales
    - Microcystaceae
        - *Microcystis flos-aquae*
    - Merismopediaceae
        - *Synechocystis*[†]

Bacillariophyta (diatoms)
- Bacillariophyceae
    - Bacillariales
        - *Nitzschia*
    - Cymbellales
        - *Gomphonema*
    - Naviculales
        - *Navicula*
    - Surirellales
        - *Surirella*[†]

Chlorophyta (green algae)
- Chlorophyceae
    - Chaetophorales
        - *Stigeoclonium*[†]
    - Chlorococcales
        - *Ankistrodesmus*
        - *Dictyosphaerium*[†]
        - *Hydrodictyon*[†]
        - *Oocystis*
        - *Pediastrum*
        - *Scenedesmus*
        - *Schroederia*
    - Tetrasporales
        - *Asterococcus*[†]
        - *Sphaerocystis*[†]
    - Volvocales
        - *Chlamydomonas*
        - *Volvox*[†]
- Trebouxiophyceae
        - *Actinastrum*[†]
    - Chlorellales
        - *Chlorella*
- Ulvophyceae
    - Cladophorales
        - *Cladophora*[†]
    - Ulotrichales
        - *Ulothrix*[†]
- Zygnematophyceae
    - Zygnematales
        - *Closterium*[†]
        - *Cosmarium*[†]
        - *Staurastrum*[†]

Cryptophyta
- Cryptophyceae
    - Pyrenomonadales
        - *Chroomonas*
        - *Cryptomonas*

Dinophyta (dinoflagellates)
- Dinophyceae
    - Peridiniales
        - *Ceratium*[†]
        - *Peridinium*[†]

Euglenozoa (Euglenoids)
- Euglenida
    - Euglenales
        - *Euglena*
        - *Trachelomonas*[†]

Ochrophyta
- Coscinodiscophyceae
    - Aulacoseirales
        - *Aulacoseira*[†]
    - Meloseirales
        - *Melosira*
    - Fragilariales
        - *Fragilaria*[†]
        - *Synedra*[†]
    - Thalassiosirales
        - *Cyclotella*
- Chrysophyceae
    - Ochromonadales
        - *Mallomonas*[†]

[†] Denotes rare or transient species observed relatively infrequently and/or in low numbers

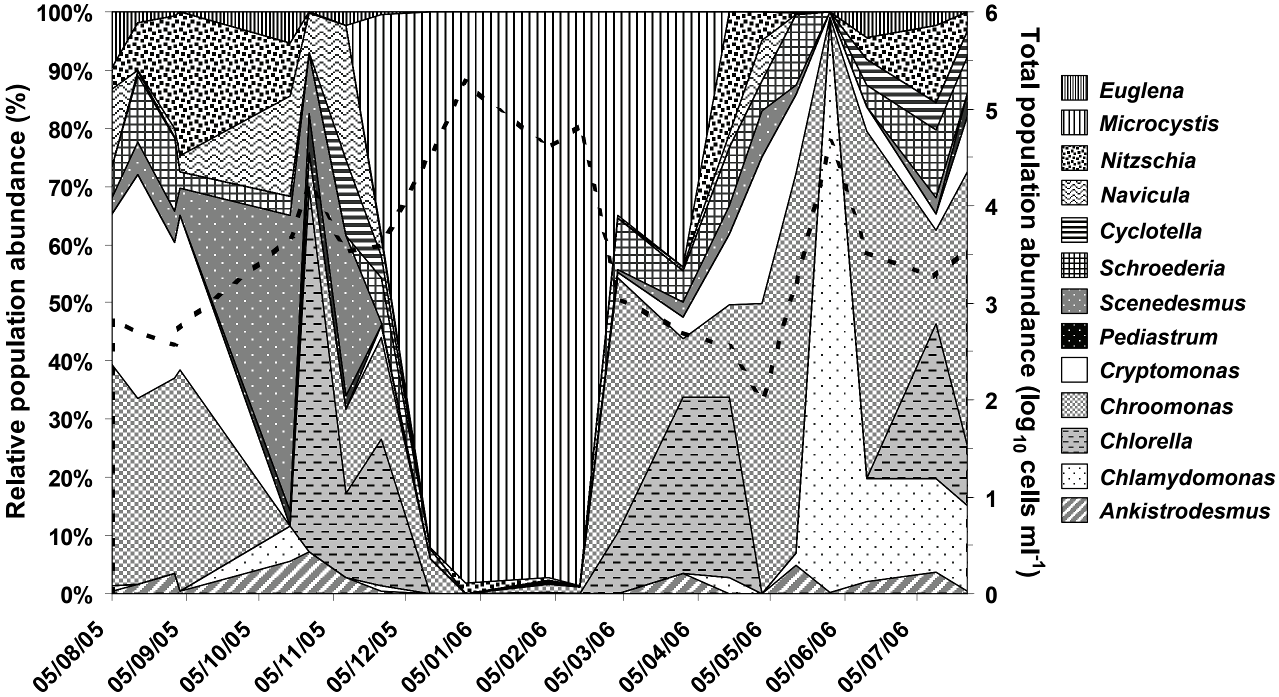

Figure 5.1. Pilot plant influent phytoplankton population dynamics during both monitoring *Period 1* of 2005 and *Period 2* of 2006 showing relative temporal abundance of the dominant genera (left *y*-axis) as well as total abundance ($\log_{10}$ cells ml^{-1}; broken line; right *y*-axis).

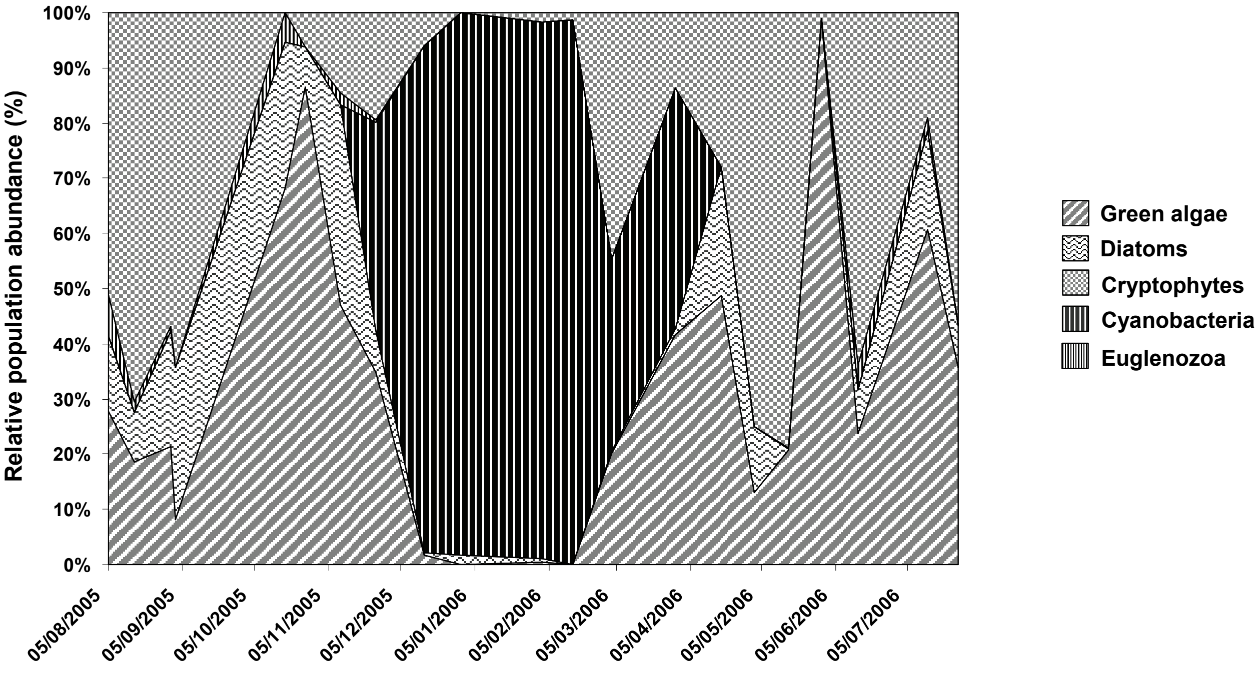

Figure 5.2. Pilot plant influent phytoplankton population dynamics during both monitoring *Period 1* of 2005 and *Period 2* of 2006 showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.

Average algal cell density in the influent wastewater during the combined 2005–2006 monitoring period was generally relatively low, with a mean of 2×10^4 and a median of 2×10^3 cells ml^{-1}. Average algal cell removals were greatest for the RF and AGM treatments, with approximate 1-log_{10} unit removals by the third Pond in both series (Figure 5.3). Algal cell removals were of a lower order in both the OP and DW series, where on average <0.5-log_{10} unit removals were achieved by the third Pond in both treatment trains. Not surprisingly, these algal cell removal trends reflected the previously reported trends for relative chlorophyll *a* removal efficiency across the four pilot upgrade systems (refer Figures 3.29 and 4.27).

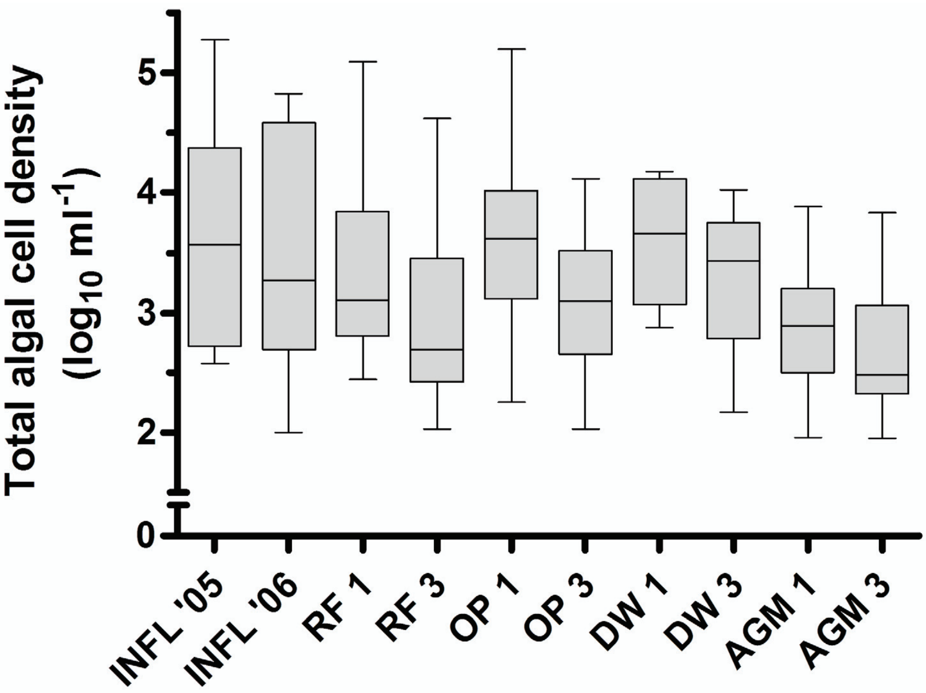

Figure 5.3. Box-plot showing total phytoplankton abundance for: the 2005 (INFL ‘05) and 2006 (INFL ’06) pilot plant Influent; Rock Filters 1 and 3 (RF-1; RF-3); Open Ponds 1 and 3 (OP-1; OP-3); Duckweed Ponds 1 and 3 (DW-1; DW-3); and Attached-Growth Media Reactors 1 and 3 (AGM-1; AGM-3). Abundance data sourced from the entire monitoring duration from July 2005–August 2006. The shaded ‘box’ represents the interquartile data range, the horizontal bar shows the median value, and the ‘whiskers’ show the absolute data range.

Overall, and as shown in Figure 5.1, the pilot plant influent phytoplankton community was largely represented by green algae such as species of *Chlorella*, *Chlamydomonas*, *Scenedesmus* and *Schroederia*, cryptophyte algae like *Chroomonas* and *Cryptomonas* species, as well as diatoms of the genus *Navicula* and *Nitzschia*. On average, green algae comprised approximately 32% of total influent population cell counts during the 2005–

2006 monitoring period, but relative contributions to daily totals ranged from 0.1–99% day-to-day depending on the corresponding density of cryptophytes, diatoms and cyanobacteria (Figure 5.2). As can be seen in the above Figures, there was also a transient but large-scale bloom of the colonial cyanobacterium *Microcystis flos-aquae* during the summer period from December of 2005 until February of the following year, during which time *Microcystis* represented on average 85% of the total population count, with an average density of 6×10^4 and a peak of 2×10^5 cells ml^{-1}. Burns and Xu (1990) commented on how during cyanobacterial blooms, the phytoplankton community frequently approximates a monoculture; something reiterated more recently by Simis *et al*. (2005). A similar trend was observed in the Bolivar WSPs, where the total community abundance was almost exclusively represented by *Microcystis* species (91–99%) from mid December of 2005 until mid February of 2006. Summer blooms of cyanobacteria in hypertrophic freshwater environments are a relatively common phenomenon (e.g. Nandini, 1999; Lund and Davis, 2000; Oufdou *et al*., 2000) and the occurrence of a *Microcystis* bloom in the Bolivar WSP was in line with the general ecology of this particular genus (Reynolds, 2006).

Martyn *et al*. (2004), following a 12 week case study of algal ecology in the Bolivar WSPs, commented on the increased prevalence of colonial cyanobacteria such as *Microcystis* species within the pond network. Since *Microcystis* species are not capable of nitrogen fixation (Reynolds, 2006), it seems unlikely that the large bloom of 2005–2006 was as a result of an ability to synthesize essential nutrients in the increasingly nitrogen-limited pond environment (see Cromar *et al*., 2005 for initial proposal of WSP nitrogen limitation at Bolivar). More likely perhaps, was that *Microcystis* species became dominant as a combined result of: the increasingly warmer late spring temperatures (>22ºC); their colonial morphology and resistance to grazing pressure; and also as a result of the capacity for gas vacuole production and buoyancy regulation (Reynolds, 2006) during the extended duration of highly turbid conditions prior to the onset of bloom formation. During the period from the first of October to mid November 2005 for example, the average WSP effluent turbidity was 62 NTU; peaking at 96 and 147 NTU following two extreme wind-induced resuspension events during October. Under these atypical and highly turbid conditions, the ability of *Microcystis* to regulate its buoyancy would have conveyed a significant competitive advantage in terms of the alga being able to undergo surface flotation, thereby maximizing its sunlight exposure and photosynthetic productivity. The competitive advantage possessed by *Microcystis* during turbid conditions may have also been aided further by the development of thermally stratified *in situ* conditions; given that thermal stratification has been shown to occur in the shallow Bolivar pond network under elevated temperatures (Sweeney *et al.*, 2005; Sweeney *et al.*, 2007).

Whilst there is a significant quantity of literature reporting on cyanobacterial blooms and the conditions conducive to their establishment, relatively little information exists relating to the environmental factors surrounding cyanobacterial bloom decay (Simis *et al.*, 2005). Amongst others, factors such as temperature, negative pressure from grazing activity, sedimentation and viral 'cyanophage' attack (Boon *et al.*, 1994; Gons *et al.*, 2002; Work and Havens, 2003; Brussaard, 2004; Simis *et al.*, 2005) have been proposed as major contributors to the termination of cyanobacterial blooms. Whilst reasons behind wholesale 'boom and bust' of cyanobacteria in the context of the current thesis was of secondary importance, it was likely that declining Autumn temperatures toward the end of March 2006 contributed significantly to the cessation of the *Microcystis* bloom. Additionally, some other factors—relating specifically to the zooplankton community structure surrounding both the establishment and decline of the cyanobacterial bloom in the Bolivar WSP—are discussed in more detail within the following Section 5.3.2.

Numerous studies have shown that WSP phytoplankton communities are most commonly dominated by species of green algae and to a lesser extent by diatoms (Raschke, 1970; Shillinglaw and Pieterse, 1977; Hussainy, 1979; Mitchell, 1980; Banat *et al.*, 1990; Tharavathi and Hosetti, 2003). With respect to the Bolivar WSP effluent, Figure 5.2 shows that the algal community was strongly represented by both green algae and diatoms, but that there was also a significant population of cryptophyte algae (*Chroomonas* and *Cryptomonas*) present for the majority of the 2005–2006 monitoring programme. Cryptophyte algae were also reported to have occurred locally in another WSP system at Gumeracha, South Australia (Mitchell, 1980). Numerically, the influent algal community was dominated by *Chlamydomonas*, *Chlorella*, *Chroomonas*, *Cryptomonas*, *Microcystis*, *Navicula*, *Nitzschia*, *Scenedesmus* and *Schroederia*, with these 9 genera constituting on average 94% of the total phytoplankton population abundance.

The phytoplankton community composition reported here was generally similar to that reported earlier by Martyn *et al*. (2004) for the Bolivar WSP system, although there were some notable trends for increases in the relative dominance of cryptophyte algae (*Chroomonas* and *Cryptomonas*) and also some larger and/or colonial green algae (*Pediastrum* and *Schroederia* species). Martyn and co-workers also noted a similar trend for reduced numbers of the classically abundant algal genera (such as *Ankistrodesmus* and *Euglena*) and increasing numbers of cryptophyte algae within the Bolivar WSPs; suggesting that it was a reflection of the reduced nutrient load in the pond system (since the up-stream commissioning of an activated sludge plant in 2001) in conjunction with the possible competitive successfulness of these new species under the "depleted nutrient" conditions. In addition to their possible physiological competitiveness under such conditions, it is also possible that the increased dominance of the abovementioned algal species within the Bolivar WSPs could be a consequence of the increased resistance of these algae to grazing pressure from zooplankton communities; something supported by the physical morphology of the larger (≈50μm), spiny and relatively unpalatable genera *Pediastrum* and *Schroederia* in particular. The work of Kobayashi (1991) also suggests that these larger algae would be unlikely to be eaten by the dominant zooplankton within the Bolivar WSPs (i.e. *Daphnia* and calanoid copepods); given that the mean maximum food particle sizes Kobayashi found in the guts of *Daphnia carinata* and *Boeckella triarticulata* were 24 and 33μm respectively.

Data from the monitoring of phytoplankton populations within Ponds 1 and 3 of each of the four pilot treatment systems is presented in Figures 5.4–5.19.

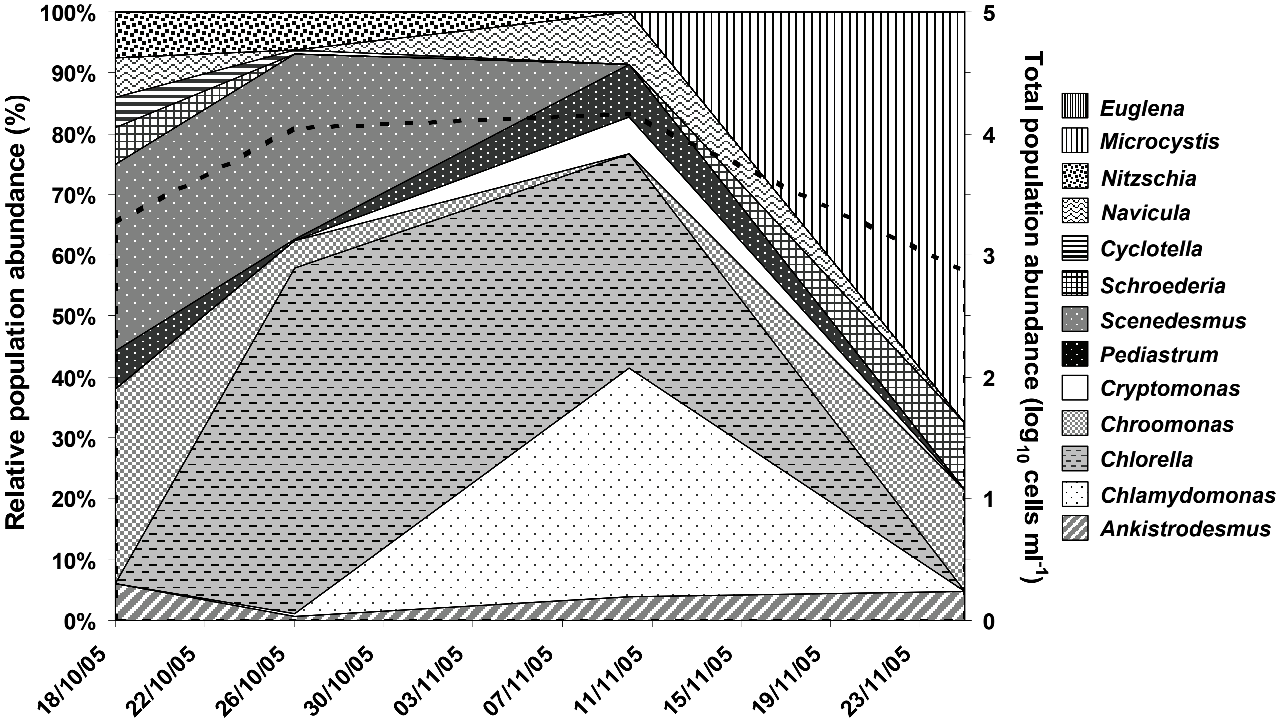

Figure 5.4. Duckweed Pond 1 phytoplankton population dynamics for a limited duration during the 2005 *Period 1* showing relative temporal abundance of the dominant genera (left *y*-axis) as well as total phytoplankton abundance (log$_{10}$ cells ml^{-1}; broken line; right *y*-axis).

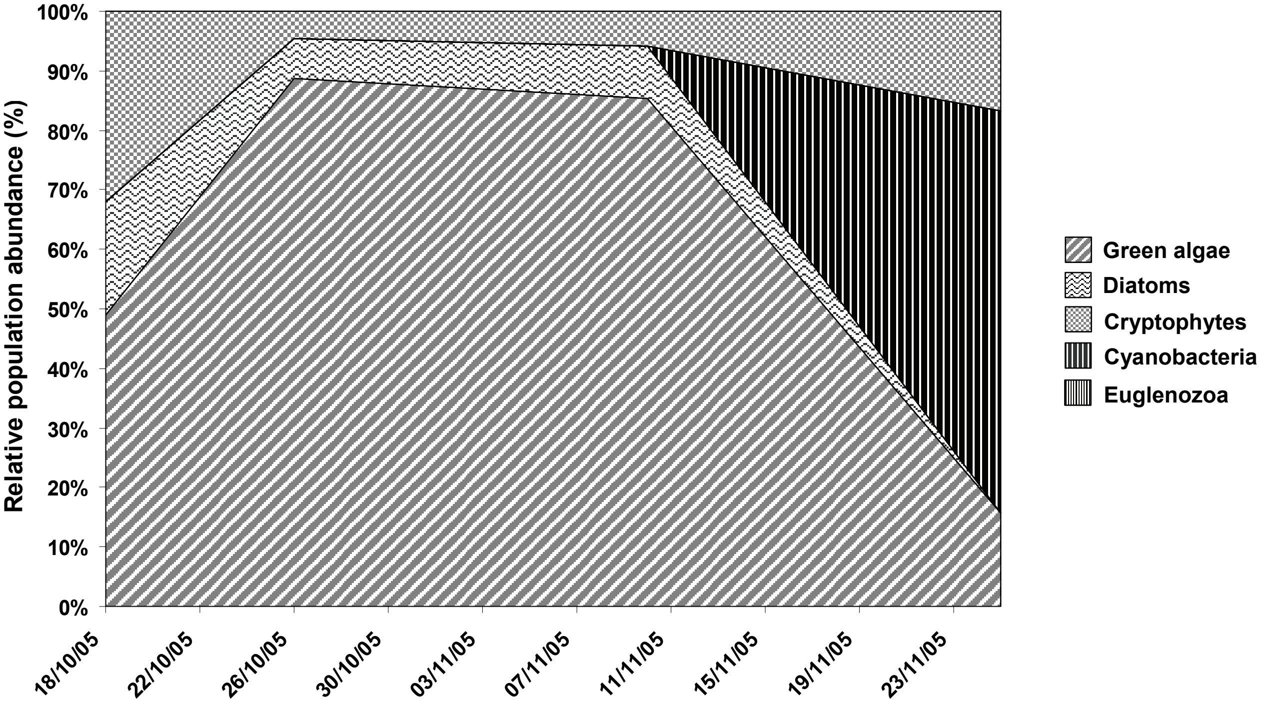

Figure 5.5. Duckweed Pond 1 phytoplankton population dynamics during the 2005 *Period 1* showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.

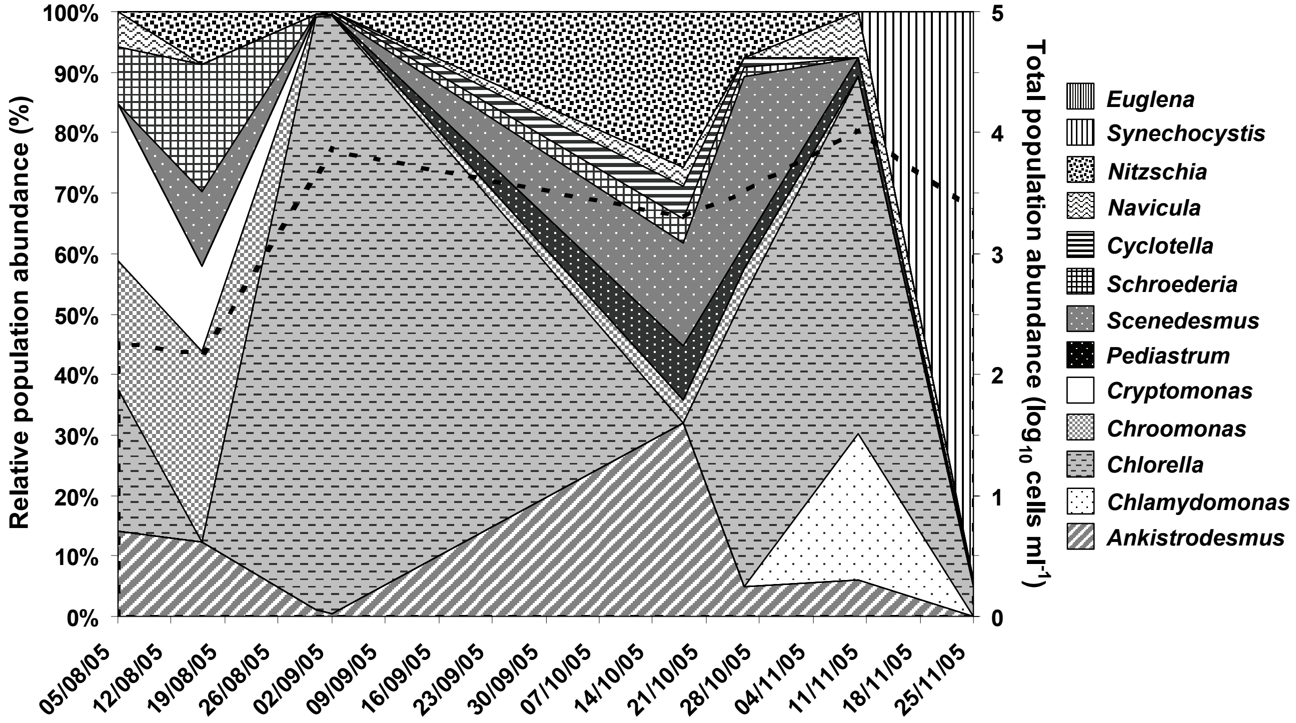

Figure 5.6. Duckweed Pond 3 phytoplankton population dynamics during the 2005 *Period 1* showing relative percentage temporal abundance of the dominant genera (left y-axis) as well as total phytoplankton abundance ($\log_{10}$ cells ml^{-1}; broken line; right y-axis).

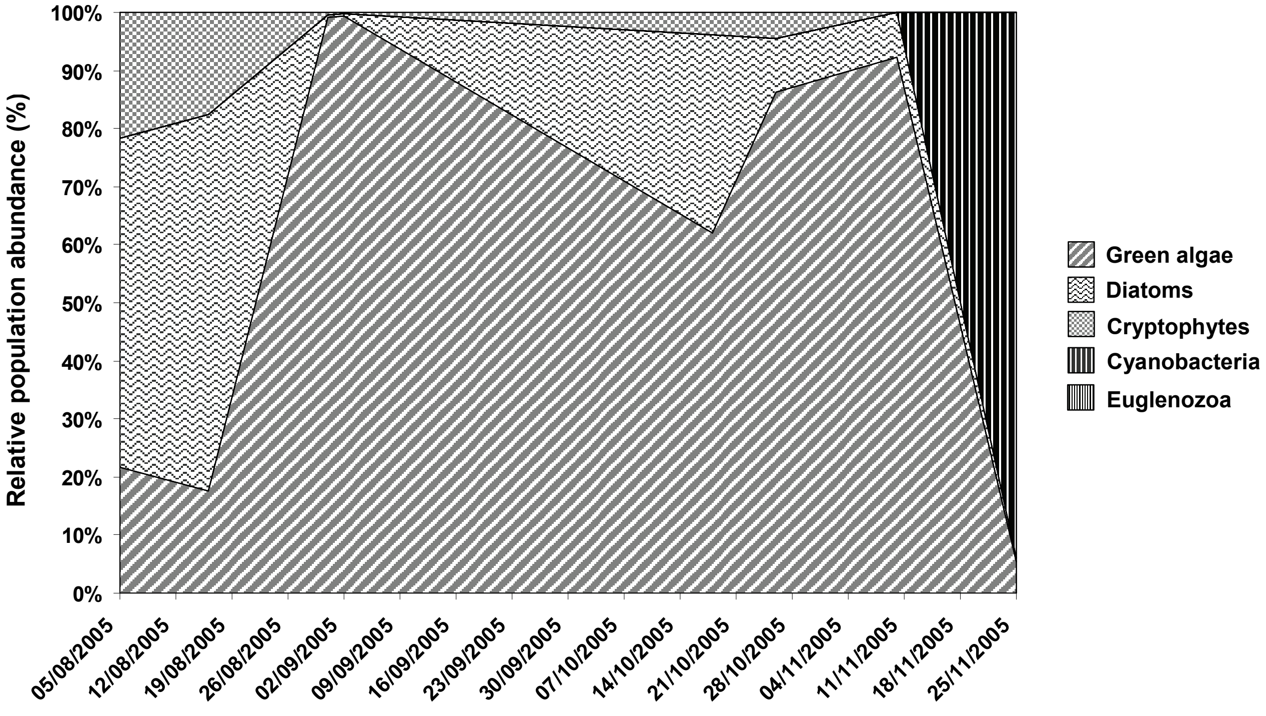

Figure 5.7. Duckweed Pond 3 phytoplankton population dynamics during the 2005 *Period 1* showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.

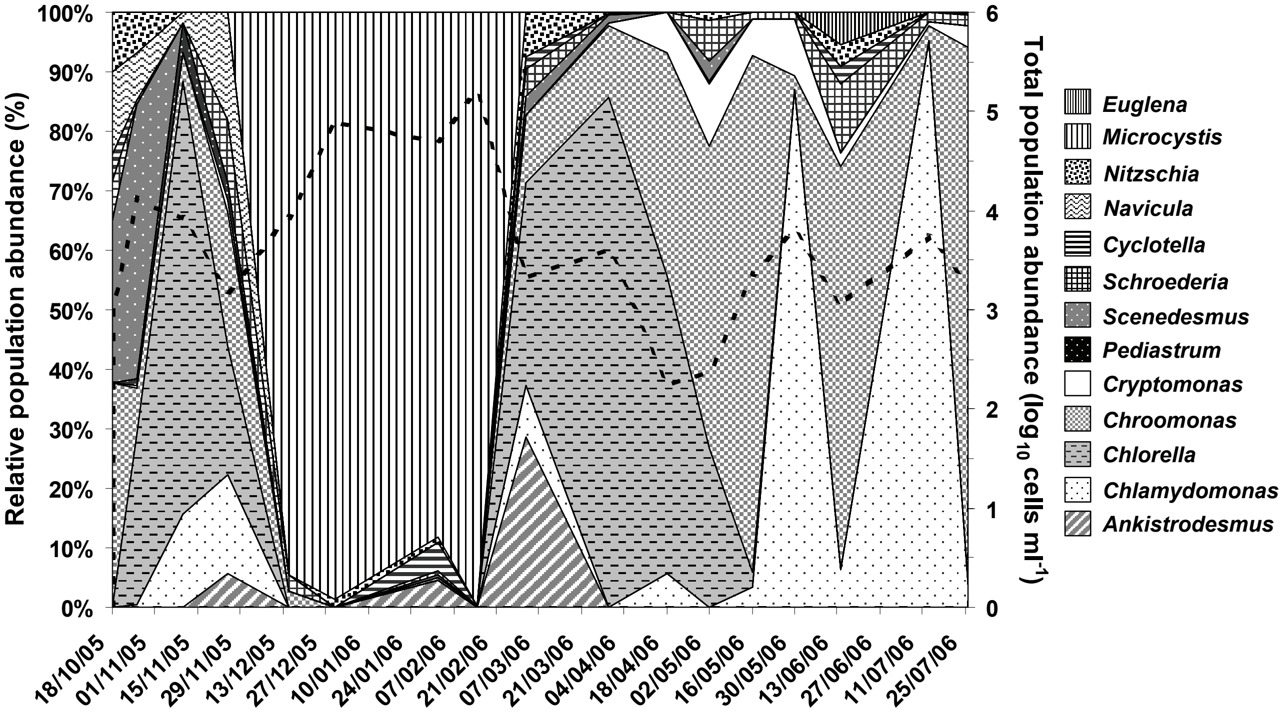

Figure 5.8. Open Pond 1 phytoplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total phytoplankton abundance ($\log_{10}$ cells ml^{-1}; broken line; right *y*-axis).

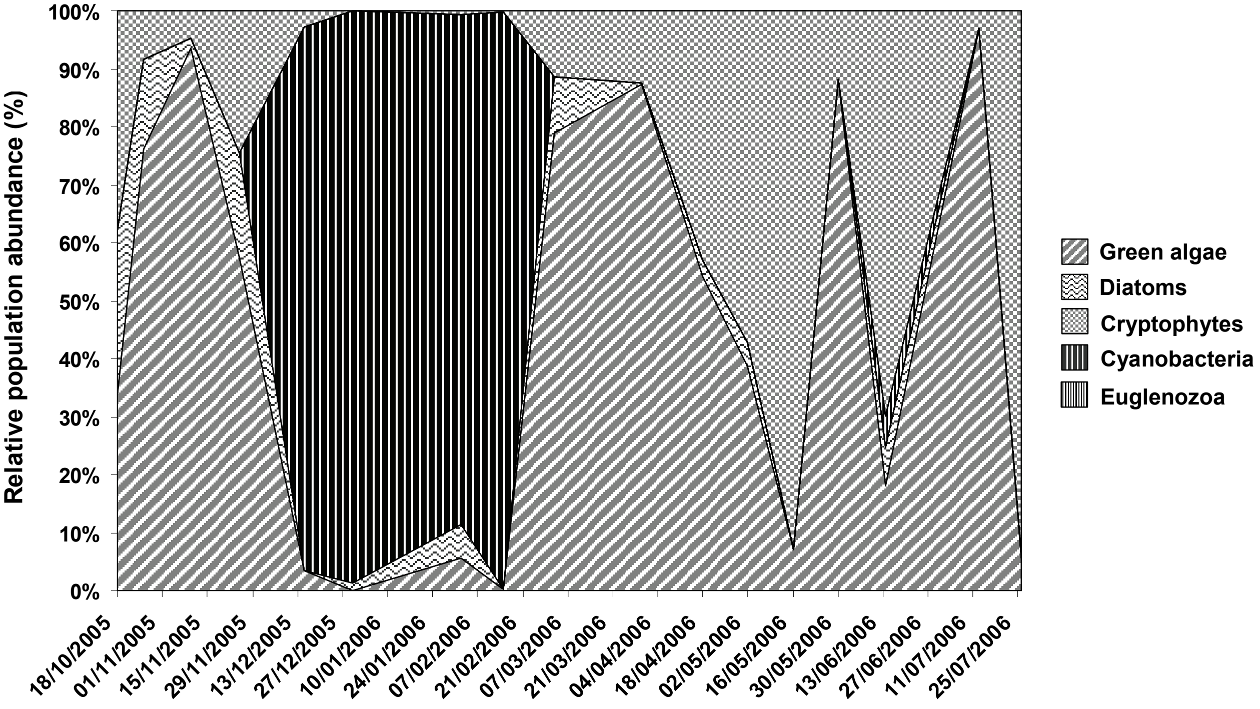

Figure 5.9. Open Pond 1 phytoplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.

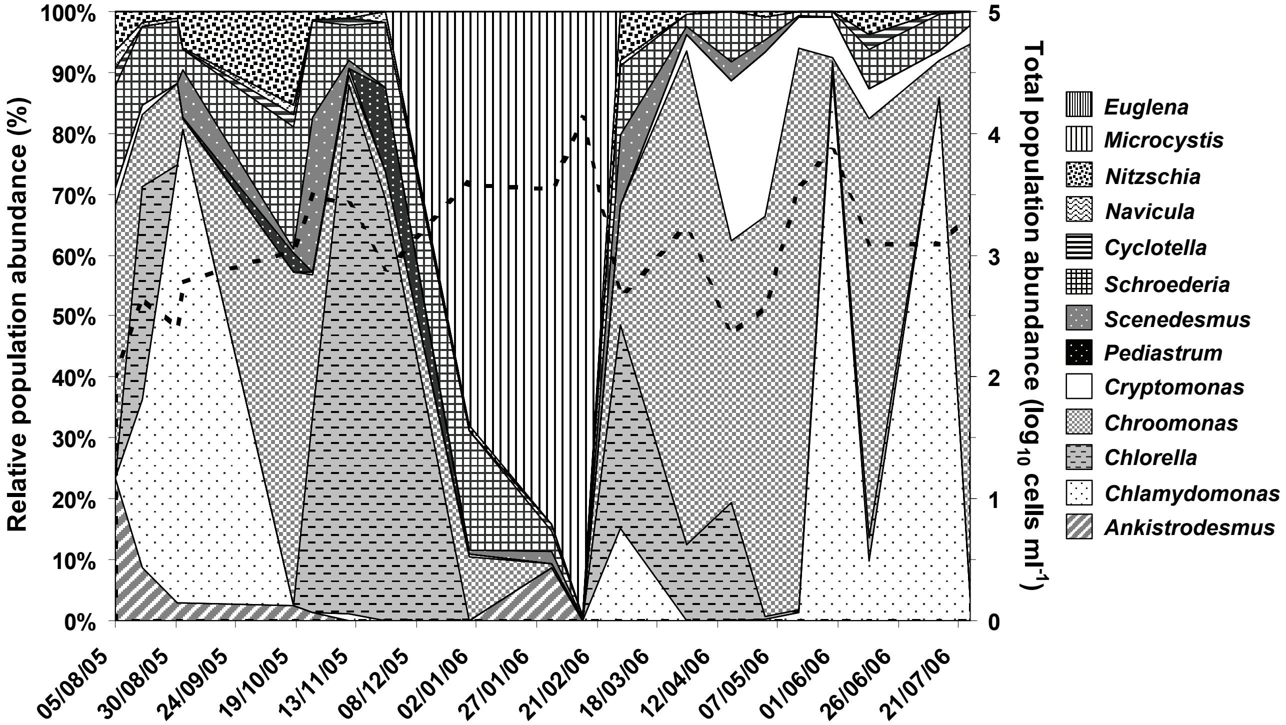

Figure 5.10. Open Pond 3 phytoplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total phytoplankton abundance ($\log_{10}$ cells ml^{-1}; broken line; right *y*-axis).

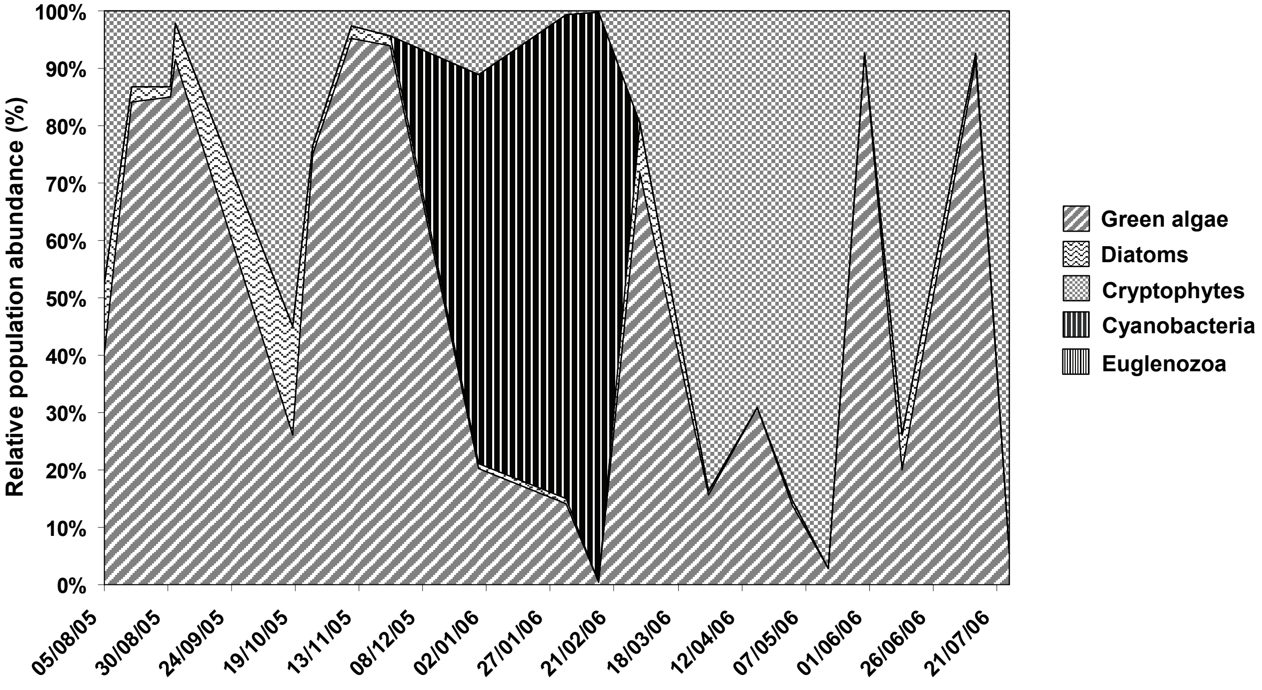

Figure 5.11. Open Pond 3 phytoplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.

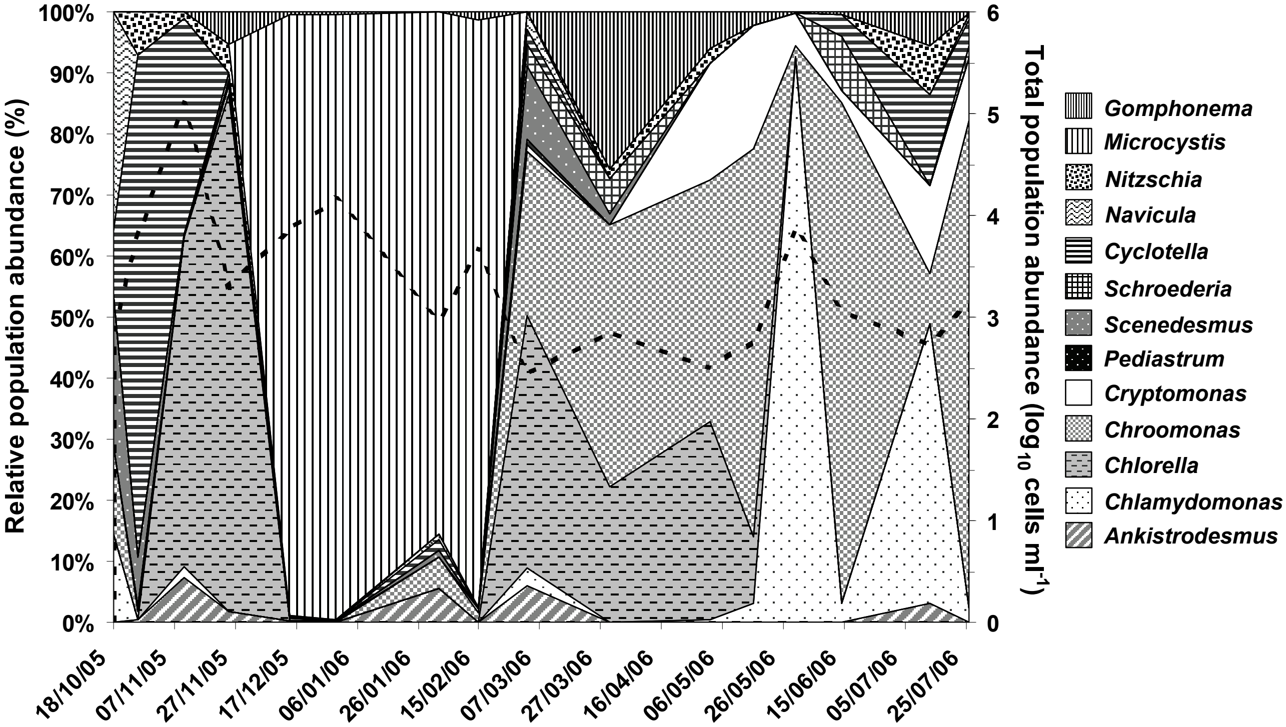

Figure 5.12. Rock Filter 1 phytoplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total phytoplankton abundance ($\log_{10}$ cells ml^{-1}; broken line; right *y*-axis).

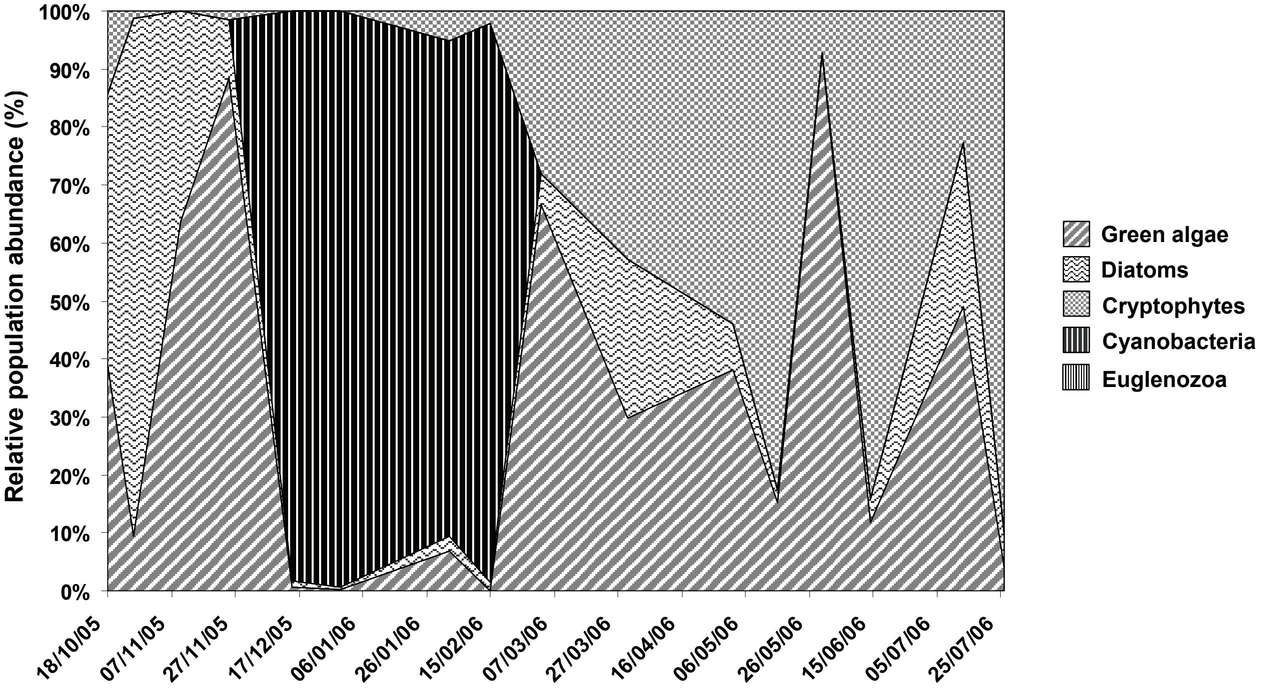

Figure 5.13. Rock Filter 1 phytoplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.

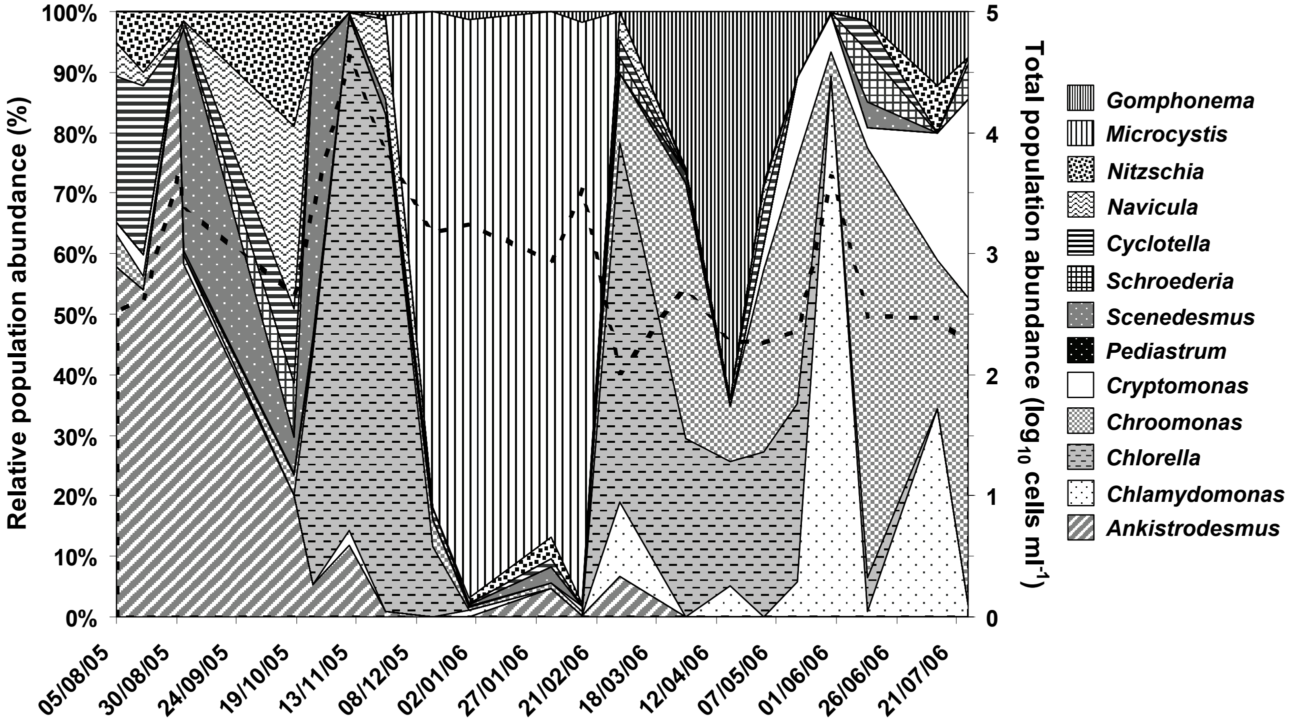

Figure 5.14. Rock Filter 3 phytoplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total phytoplankton abundance ($\log_{10}$ cells ml^{-1}; broken line; right *y*-axis).

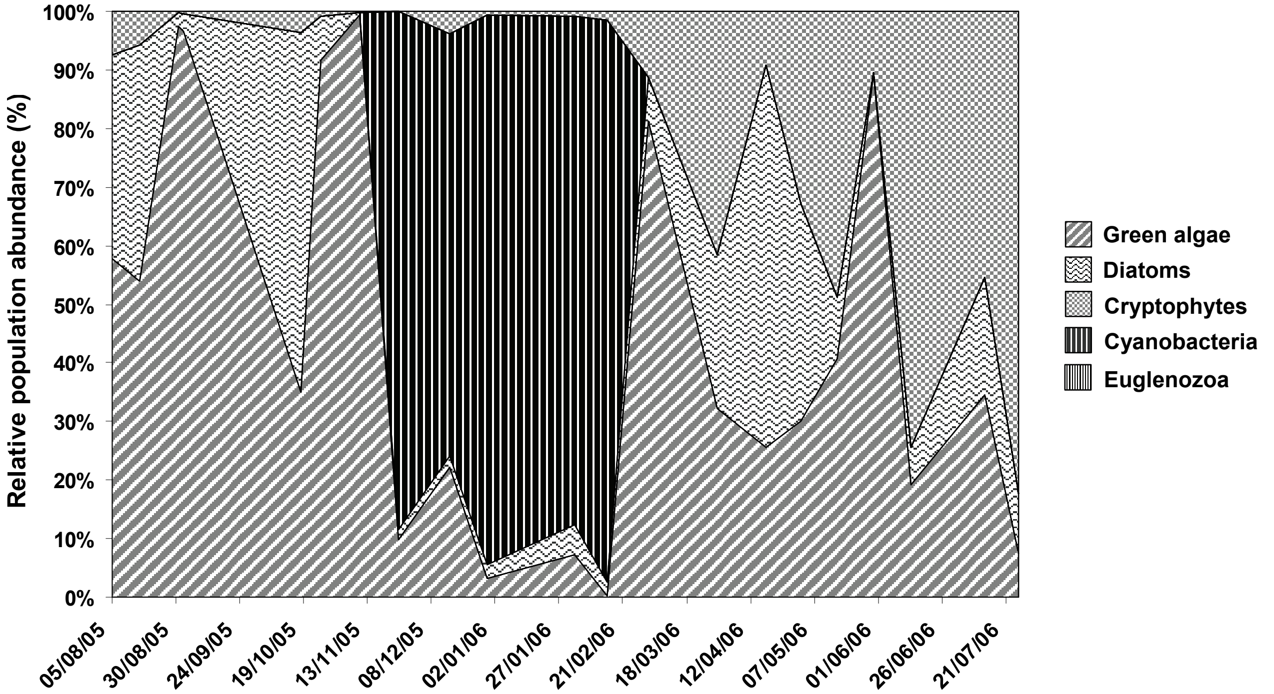

Figure 5.15. Rock Filter 3 phytoplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.

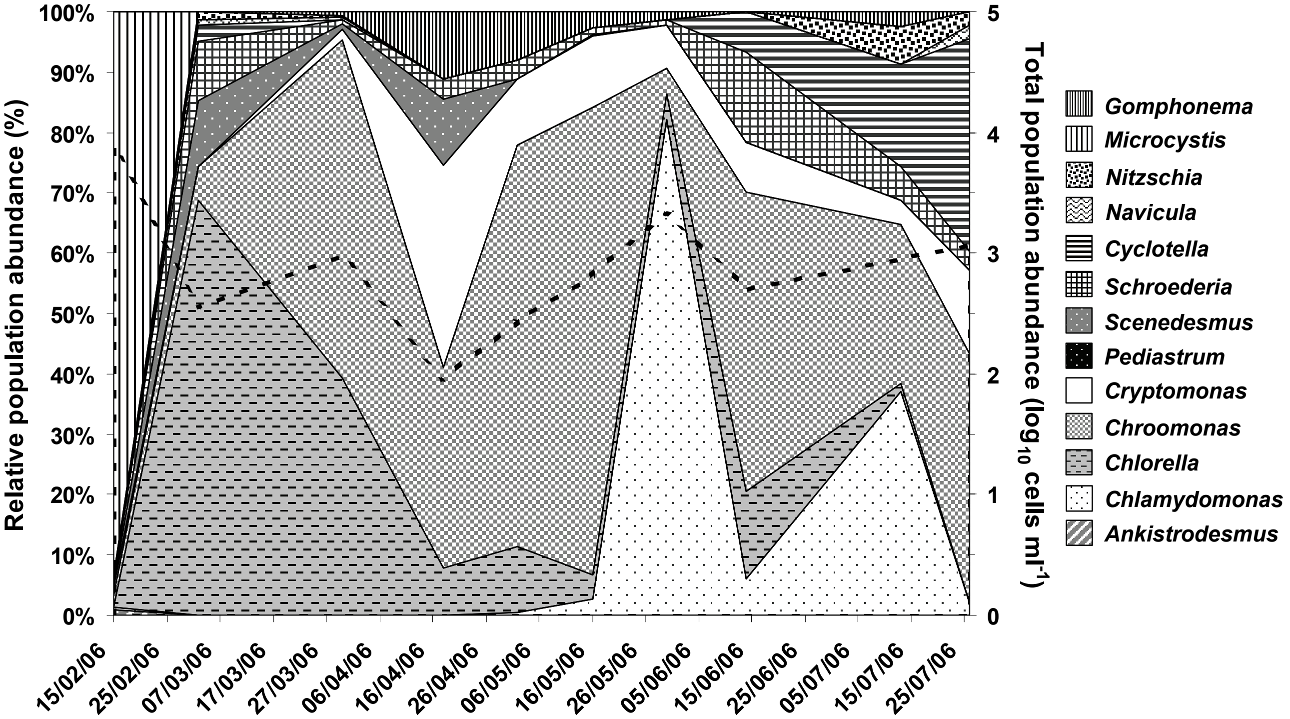

Figure 5.16. Attached-Growth Media Reactor 1 phytoplankton population dynamics during the 2006 *Period 2* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total phytoplankton abundance ($\log_{10}$ cells ml^{-1}; broken line; right *y*-axis).

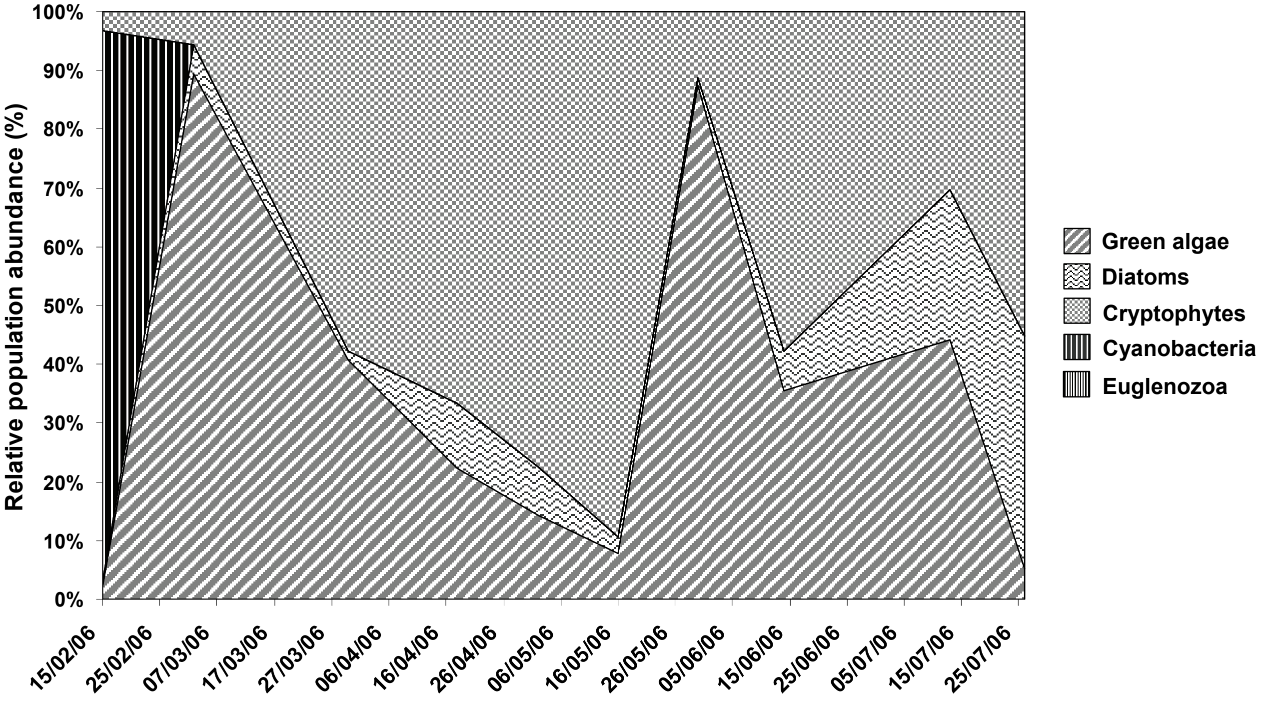

Figure 5.17. Attached-growth media Reactor 1 phytoplankton population dynamics during the 2006 *Period 2* showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.

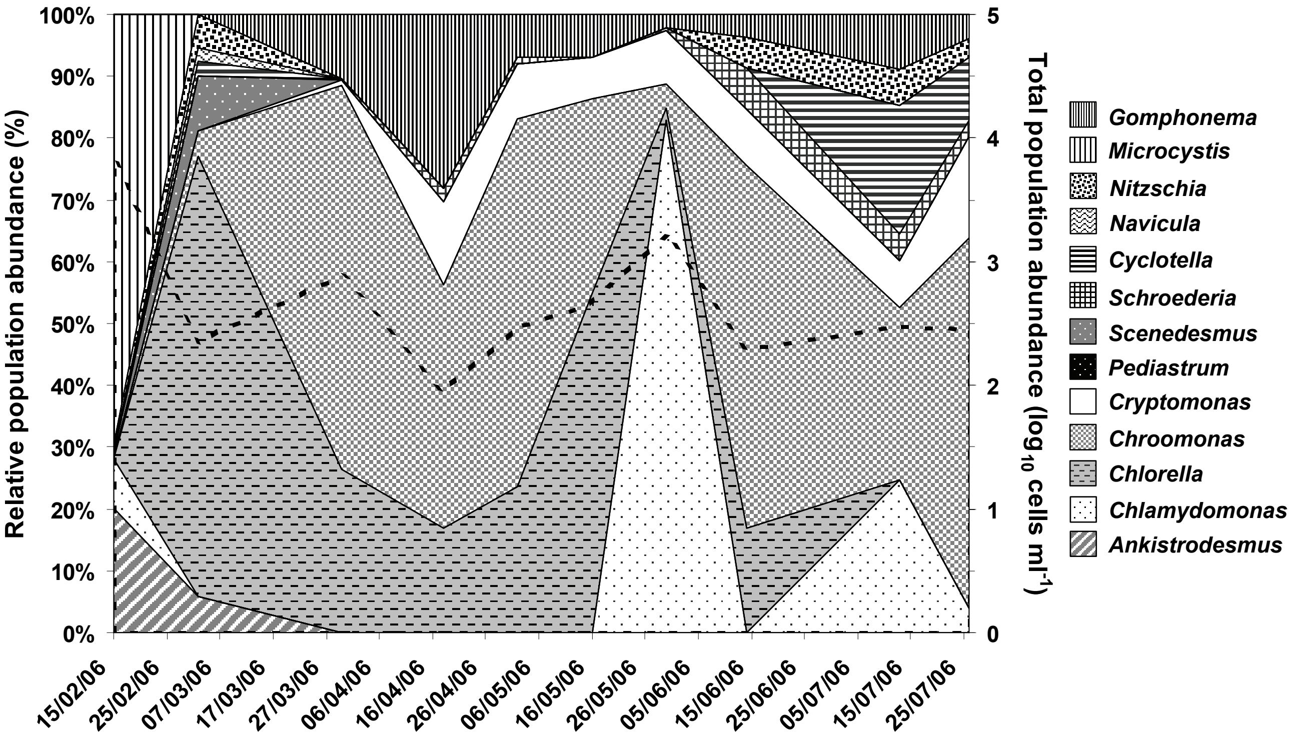

Figure 5.18. Attached-growth media Reactor 3 phytoplankton population dynamics during the 2006 *Period 2* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total phytoplankton abundance ($\log_{10}$ cells ml^{-1}; broken line; right *y*-axis).

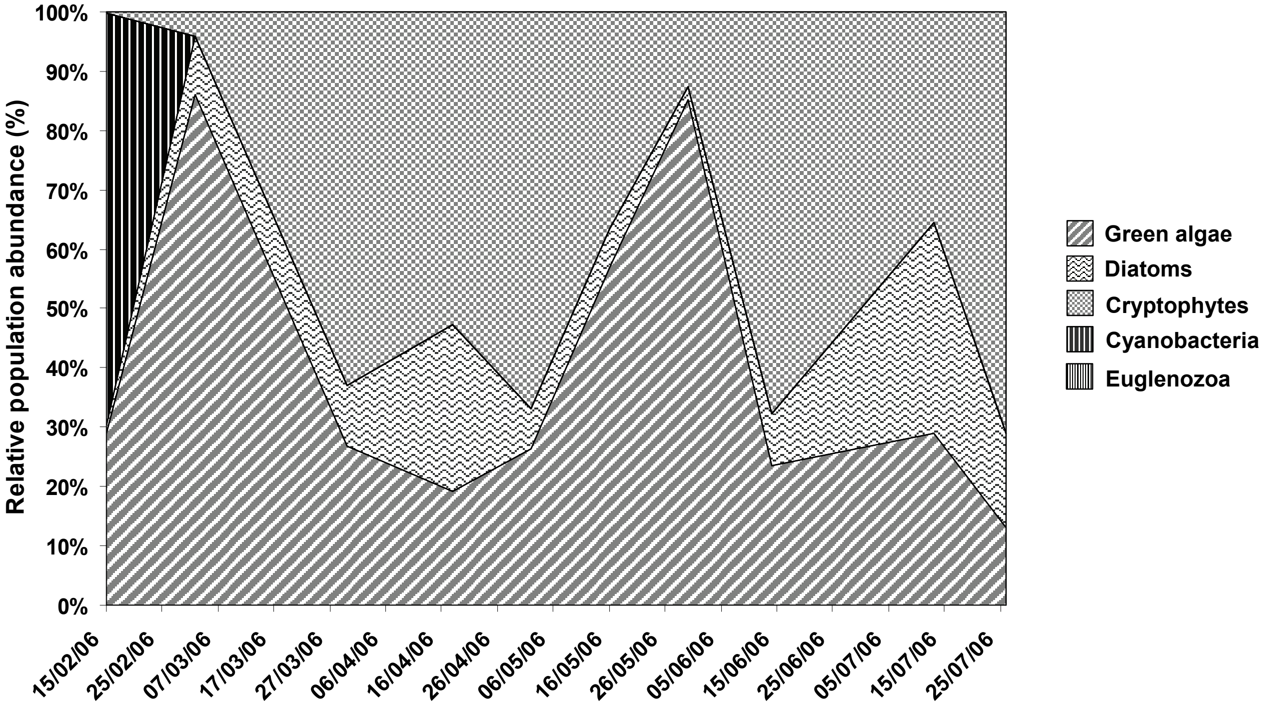

Figure 5.19. Attached-growth media Reactor 3 phytoplankton population dynamics during the 2006 *Period 2* showing relative percentage temporal abundance of: green algae; diatoms; cryptophytes; cyanobacteria; and Euglenozoa.

To the author's knowledge, there is very limited information available on the temporal ecology of phytoplankton communities under a duckweed surface cover. This is especially true in a wastewater environmental setting, where there have been few published reports on algal–duckweed interactions *per se* (e.g. Goldsborough and Robinson, 1985; Goldsborough, 1993; Hammouda *et al*., 1995; Szabó *et al*., 1998; Szabó *et al*., 1999; Özbay, 2002). Within these studies, some work has focused specifically on epiphytic non-suspended phytoplankton populations (i.e. Goldsborough and Robinson, 1985; Goldsborough, 1993), while others have provided little or no quantitative data (i.e. Hammouda *et al*., 1995; Özbay, 2002); such that these works are of limited relevance in the current setting. Based on the data of Duckweed treatment Ponds 1 and 3 (Figures 5.4–5.7), the relative phytoplankton population distributions within the DW Pond series remained essentially unchanged compared with the corresponding 2005 influent data (Figures 5.1 and 5.2). Numerically, the algal community within the DW ponds was dominated by *Ankistrodesmus*, *Chlamydomonas*, *Chlorella*, *Chroomonas*, *Microcystis*, *Nitzschia*, *Pediastrum*, *Scenedesmus* and *Schroederia*, with these 9 genera constituting—on average—94% of the total phytoplankton population.

The appearance of *Synedra* species within DW treatment series Pond 3 was the most visually apparent change in the phytoplankton community relative to influent community structure, although absolute cell numbers were always very low (maximum of 100 cells ml^{-1}). The increased dominance of *Ankistrodesmus* and *Pediastrum* species was also notable within the DW Pond series, however, absolute cell numbers were once again relatively low (maximum of 600 for *Ankistrodesmus* and 1×10^3 cells ml^{-1} for *Pediastrum*). Qualitatively, there appeared to be an increase in the fraction of green algae within the DW Pond series relative to 2005 influent levels (see also Figure 5.20(a)), although this trend could not be supported quantitatively (1-way ANOVA; $F_{(3,52)} = 1.179$; $p = 0.327$); with the absolute numbers of chlorophyte algal cells in the influent remaining effectively unchanged following passage through the three-pond Duckweed treatment series (both means during 2005 were in the range of $2–3\times10^3$ cells ml^{-1}). Interestingly, Hammouda *et al*. (1995) reported that *Lemna* coverage of wastewater was selective for diatom species, with observational results from their pilot-scale investigations suggesting that species of *Synedra*, *Navicula*, *Nitzschia*, *Melosira* and *Cyclotella* were the only phytoplankton able to withstand such conditions. This was in contrast to the results of the current work, which instead demonstrated an increase in the dominance of green algae; although there was qualitative evidence to suggest a slight increase in the influent-relative abundance of algal genera noted by Hammouda *et al*. (1995) within the *Lemna*-covered DW ponds reported here.

The presence of a dense surface cover of duckweed was shown to restrict the level of incident light penetration to the underlying water by a factor in excess of 99% (refer Section 3.3.3; Figure 3.4). In addition to presenting a physical barrier against light penetration, aquatic plants have long been suspected of suppressing phytoplankton growth through the excretion of inhibitory allelopathic chemical substances as a possible antagonistic strategy against other photosynthetic organisms competing for light and nutrients (Hutchinson, 1975; van Donk and van de Bund, 2002). Indeed, Gopal and Goel (1993) stated that it is a common observation that a dense growth of aquatic macrophytes often suppresses the growth of phytoplankton as well as filamentous algae; something supported more recently by the work of Parr *et al*. (2002). At the same time, there is evidence to suggest that some phytoplankton are capable of out-competing duckweed (*Lemna*) under conditions of a low density plant surface mat (Leng *et al*., 1995; Szabó *et al*., 1998). Additionally, some cyanobacteria have even been suggested as being capable of inhibiting the growth of duckweeds through the production of certain allelochemicals (Entzeroth *et al.*, 1985; Gleason and Case, 1986; Chauhan *et al.*, 1992).

As shown in the previous Figure 5.3, total phytoplankton density remained practically unchanged down the Duckweed Pond series relative to 2005 influent levels; suggesting no significant population growth or decline under the cover of duckweed. It was considered likely that the hydraulic retention time within the DW Pond series was too short (≈4 days) and that the plant mat surface area to volume ratio too small to promote large-scale algal removals as a result of competitive interactions between duckweed and phytoplankton. Given that the current duckweed surface mat was very thick (2–3cm) and structurally robust (see Section 3.3.3), it was perhaps unsurprising that there were no apparent adverse consequences for the duckweed community as a result of competitive interactions from the underlying phytoplankton communities; with the *Lemna* mat retaining its structural integrity and maintaining a complete surface coverage at all times.

Phytoplankton community structure within the Open Pond treatment series was very similar to that of the influent wastewater (compare Figures 5.1–5.2 and 5.8–5.11). Like the influent wastewater, the algal community in the OP series was again numerically dominated by *Chlamydomonas*, *Chlorella*, *Chroomonas*, *Cryptomonas*, *Microcystis*, *Nitzschia*, *Scenedesmus*, *Schroederia* as well as *Ankistrodesmus*, with these 9 genera constituting—on average—96% of the total phytoplankton abundance. On average, green algae constituted 46%, cryptophytes 32%, cyanobacteria 15% and diatoms 4% of the total population abundance during the entire 2005–2006 monitoring duration (Figure 5.20(a)). As shown in Figure 5.3, and similar to the results of the DW series above, only small-scale reductions in the total numbers of phytoplankton occurred down the Open Pond series relative to influent levels, with average cell densities remaining in the order of 10^3 cells ml^{-1} within OP-3. This was in spite of the Open Ponds containing, on average, approximately 70% greater total zooplankton biomass density compared with the DW ponds (see Figure 5.58) and presumably therefore being subjected to significantly elevated grazing pressure as a result; particularly by the larger and more efficient *Daphnia* species.

To the author's knowledge, there exists no published information relating to the plankton ecology of WSP effluent following rock filtration. Whilst a limited number of authors have reported on bulk measurements of algal biomass dynamics via chlorophyll analyses (see Section 3.3.6), so far no attempts have been made to directly assess the algal community structure of a rock filter effluent. The current work therefore represents the first reported attempt at monitoring the algal population ecology of a WSP effluent following rock filtration; the results of which are presented in Figures 5.12–5.15. Overall, the relative phytoplankton community distribution of the RF series effluent remained similar to that of the combined 2005–2006 influent wastewater, consisting predominantly of green algae (38%), cryptophytes (23%), cyanobacteria (22%) and diatoms (17%; see also Figure 5.20(a)). More specifically, the phytoplankton community during the entire 2005–2006 period had a similar ecological distribution to that of the pilot plant influent, being largely dominated by species of *Chlamydomonas*, *Chlorella*, *Chroomonas*, *Cryptomonas*, *Microcystis* and *Scenedesmus*. In addition to these common algal genera, there were some apparent differences as well; with species of *Ankistrodesmus*, *Cyclotella*, *Gomphonema* and *Oocystis* increasing in relative community abundance following Rock Filter passage. On average, these 10 genera constituted approximately 94% of the total phytoplankton population cell density at any given time.

As for the Duckweed Pond series above, it was thought that the relatively short retention time (1.5–2 days for the three filter series) within the Rock Filters may have resulted in insufficient exposure time to the modified *in situ* conditions to enable full realization of the likely selective pressures exerted by the arguably non-ideal Rock Filter environment (i.e. enhanced sedimentation potential, darkness and low DO concentration). Species of *Chlamydomonas* and *Chlorella* remained well represented in the RF treatment effluent (Chapter 9 discusses in greater detail the abilities of these algae to withstand simultaneous darkness and hypoxia), but it remains unclear if other phytoplankton genera would be as resilient to such conditions within a full-scale rock filter for example, where they would possibly be subjected to even lower oxygen concentrations and probably over a longer detention period. It should be emphasized at this point that the physical presence of cell populations in the above ecological monitoring data does not provide any insights into physiological activity or viability status at the time of sampling, and so whilst cells were indeed present in the final effluent, no attempts were made to determine whether they were alive or dead.

As will be described in Chapter 6 (Section 6.3.2), the variable capacity of phytoplankton for dark-survival is recognized as being a deterministic factor in the shaping of algal community composition; in the sense that a it can directly influence the successfulness of interspecies competition *during* as well as dictate the relative rates of cell death and subsequent species abundance and diversity *post*-darkness (Lee and Rhee, 1997; Agustí *et al*., 2006; Franklin *et al*., 2006). Prior to undertaking this research, it was initially considered possible that the *in situ* conditions existing within an in-pond upgrade system (such as a rock filter or duckweed-covered pond) may exert a negative selective pressure upon ‘less resilient’ members of the phytoplankton community. Based on the relevant published information, it appears likely that diatoms like *Navicula* and *Nitzschia*, cryptophytes like *Cryptomonas* and *Chroomonas* and other algae like *Cyclotella* and *Scenedesmus* are all well equipped to survive extended periods of continuous darkness (refer to Section 6.3 for more information) and so were more than likely biologically viable in the final RF effluent; although their capacity for *in situ* persistence under simultaneous dark–hypoxic conditions remains largely uninvestigated.

With respect to ecological monitoring of algal populations in attached-growth media systems, there is again very little published information available. Zhao and Wang (1998), following qualitative observations of their pilot-scale AGM system, found that species of, *Chlorella*, *Euglena*, *Nitzschia* and *Oscillatoria* were the dominant phytoplankton. Similarly, Peishi *et al*. (1993) commented on the prevalence of diatoms (Bacillariophyceae), cyanobacteria (*Oscillatoria*), Euglenophyceae and *Chlamydomonas* surrounding their 'fibrous carrier' AGM; however, neither of these studies offered quantitative information. As for the Rock Filter data above, the current work again represents the first reported attempt at probing the algal population ecology of a WSP effluent following treatment with an attached-growth media upgrade system. Results of these ecological investigations are shown in Figures 5.16–5.19 for AGM Reactors 1 and 3 respectively.

Overall, the relative phytoplankton community distribution of the AGM series effluent remained similar to that of the influent wastewater during the 2006 period, consisting almost entirely of green algae, diatoms, cryptophytes and cyanobacteria (see also Figure 5.20(a)). The phytoplankton community structure was largely dominated by similar genera such as *Chlamydomonas*, *Chlorella*, *Chroomonas*, *Cryptomonas*, *Microcystis* and *Schroederia*. In addition to these common algal genera, there were some apparent differences; with species of *Cyclotella* and *Gomphonema* increasing in relative community abundance following attached-growth media upgrade treatment. On average, these 8 genera constituted in excess of 94% of the total phytoplankton population cell density at any given time. On average, green algae constituted 37%, cryptophytes 43%, diatoms 11% and cyanobacteria 8% of the total population abundance during the 2006 period (Figure 5.20(a)). In terms of absolute cell numbers, the AGM series effectively yielded 1-$\log_{10}$ reductions in total algal population cell density by the last reactor in series (Figure 5.3), with individual removals for each algal genus most commonly of a similar order of magnitude. This magnitude removal was similar to that of the RF treatment and greater than the <0.5-$\log_{10}$ reductions achieved by the OP and DW series.

As a final point of note, it should be reemphasized that the RF, DW and AGM treatment series never experienced the same dense filamentous algal blooms (*Cladophora* and *Hydrodictyon*) that occurred periodically within the parallel OP series (see Section 3.3.7 for initial discussion). This ability to prevent the development of such filamentous algal populations would be expected to be considered advantageous in the context of the current work.

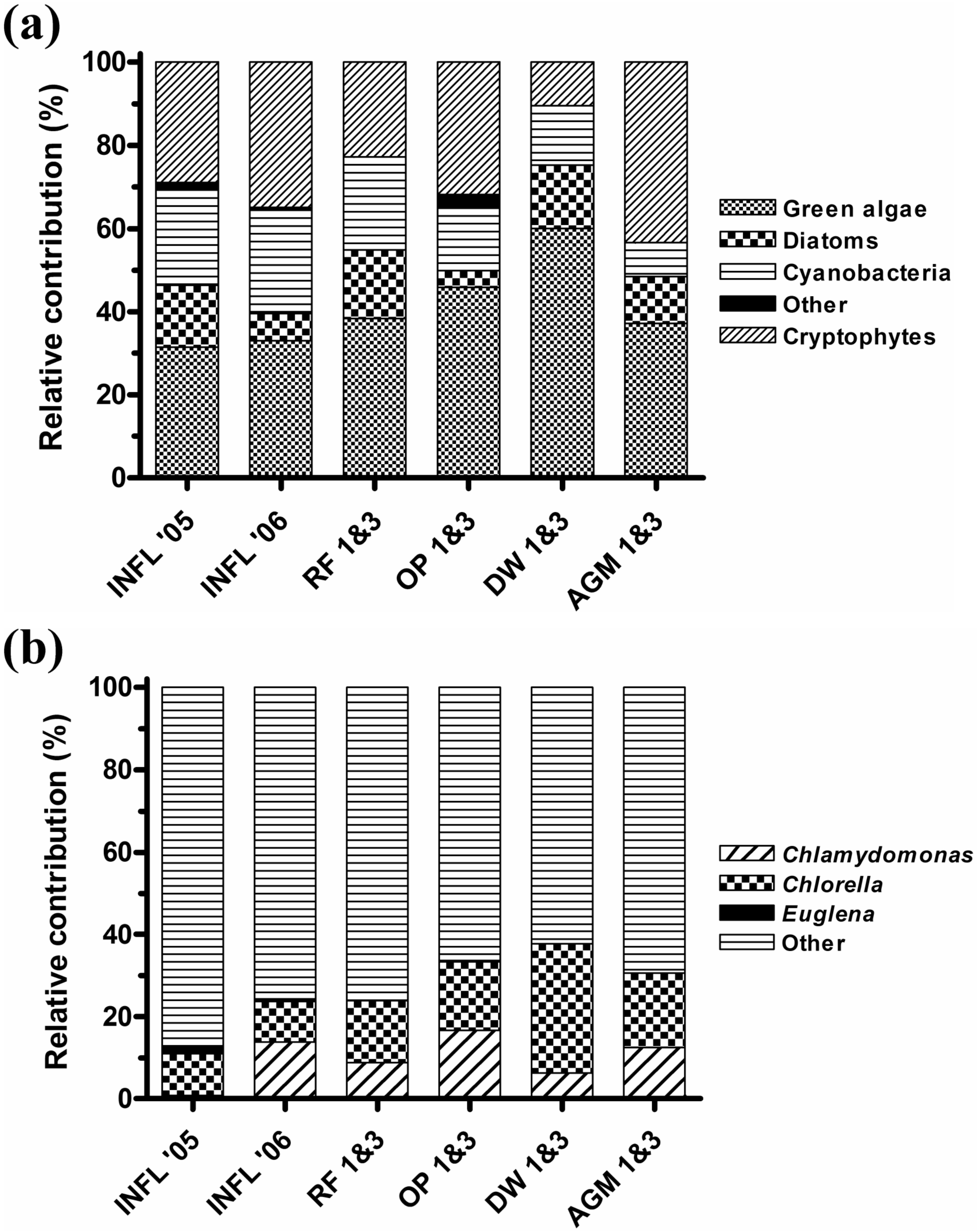

Figure 5.20. Mean percentage contributions of the four major phytoplankton groups (greens, diatoms, cyanobacteria and cryptophytes; **(a)**) and the three problem phytoplankton genera (*Chlamydomonas*, *Chlorella* and *Euglena*; **(b)**) to the total algal population. Data shown for: 2005 (INFL '05) and 2006 (INFL '06) pilot plant Influent; Rock Filters 1 and 3 (RF 1&3), Open Ponds 1 and 3 (OP 1&3), Duckweed Ponds 1 and 3 (DW 1&3) and Attached-Growth Media Reactors 1 and 3 (AGM 1&3). Average values for the RF and OP treatments were calculated from the combined Pond 1 and 3 data of the 2005–2006 monitoring duration.

As described previously (see Section 1.3.1), several phytoplankton genera have been identified as being "problematic" to DAF/F process efficiency. Buisine and Oemcke (2003) found that large, single-celled and motile phytoplankton (namely *Chlamydomonas* and *Euglena*) as well as small non-motile *Chlorella* species were especially difficult for the Bolivar DAF/F plant to remove. The authors, in referencing the work of Lucas (2000), suggested that the motility of *Euglena* and *Chlamydomonas* could possibly explain their low removals during the DAF/F process; however, it remained unclear why *Chlorella* was also consistently bypassing the treatment process. Despite there appearing to be some overall shifts within the four pilot upgrade systems relative to influent phytoplankton community structure, statistical analysis of the raw data from Figure 5.20(a) revealed that there were no significant changes in the relative fractions of the dominant phytoplankton groups between the pilot plant influent and any of the four treatment series (1-way ANOVA; $F_{(4,122)} \leq 4.196$; $p > 0.05$). Similarly, critical analysis of the data from Figure 5.20(b) showed that there were also no apparent differences in the relative abundance of total problematic phytoplankton (i.e. *Chlorella* + *Chlamydomonas* + *Euglena*) between the influent wastewater and any of the pilot treatment pond effluents (1-way ANOVA; $F_{(8,118)} = 0.530$; $p = 0.832$). Qualitative visual analysis of the above data suggested that the Rock Filters were likely to have produced an effluent with the lowest densities of so-called problem algae in comparison to all other treatments and the Duckweed Ponds the highest proportion of problem species. With respect to the performance of the remaining two pilot treatments, the combined levels of *Chlorella*, *Chlamydomonas* and *Euglena* in the final effluents appeared similar.

Szabó *et al*. (1998) suggested that from a total of 9 algal species tested, one *Chlamydomonas* and one *Chlorella* species were the two most capable of growth and competition under a 100% complete duckweed surface cover. Although there was qualitative evidence to suggest an increase in the relative dominance of *Chlorella* species within the DW Pond series compared with the other treatments and the influent wastewater, there was no statistical basis for this (1-way ANOVA; $F_{(3,33)} = 0.984$; $p = 0.412$). In actuality, the absolute numbers of *Chlorella* cells remained essentially unchanged following Duckweed Pond passage ($1–2\times10^3$ cells ml^{-1}) and it was only through changes in the relative numbers of other algal genera that *Chlorella* was shown to increase in relative abundance.

It was postulated prior to the commencement of the current research project (see Section 1.3.1.2 for initial discussion), that passage thorough an advanced upgrade process—like a rock filter or duckweed pond for example—may actively select for or against particular algal species that may be more resilient to the modified conditions within the confines of that particular upgrade (e.g. darkness, enhanced quiescence, biofilm entrapment, predation and/or biological attack); the question then was whether this *in situ* selectivity would be beneficial or antagonistic to DAF/F plant efficiency. Based on the above information, it does not appear likely that any of the WSP upgrade systems would be particularly selective (either of for against) with respect to altering the relative proportion of identified problem algae in their final effluents. Following this, it would not be expected that any beneficial manipulation of algal population ecology away from these problem organisms would occur as a result of WSP upgrading with: a rock filter; a duckweed cover; or attached-growth media addition. It should be cautioned, however, that the number of sample replicates was less than ideal for allowing definitive conclusions to be drawn regarding the ability of each upgrade system to attenuate these problem algal species (*n* = 18 for RF; *n* = 18 for OP; *n* = 6 for DW; *n* = 10 for AGM treatment). Nevertheless, these data do serve to provide some quantitative insights into the likely ecological shifts within each chosen upgrade system, and also constitute the only available information in the case of rock filters and attached-growth media systems.

Importantly, and regarding the specific applied focus of this research, despite all pilot upgrade treatments appearing to have produced an effluent with proportionally more *Chlorella*, *Chlamydomonas* and *Euglena* than was present in the influent, all treatments were in fact previously shown to be capable of significantly reducing the levels of chlorophyll *a* down the pond series (by somewhere in the order of 60–70%; see Sections 3.3.6 and 4.3.5). Furthermore, the RF and AGM treatment series in particular have already been shown to be capable of reducing the numbers of algal cells in the infiltrating wastewater by ≈1-$\log_{10}$ units (Figure 5.3). This means that even though there appeared to be small-scale (5–20%) qualitative increases in the fraction of problem algal species within the effluent of each pilot upgrade system, the *absolute* numbers of problem algal cells would be expected to be invariably reduced compared with influent numbers—especially for a rock filter or attached-growth media WSP upgrade. So in effect, and using the RF and AGM systems as examples, while these in-pond effluent upgrade systems would be expected to significantly reduce the total algal biomass load entering the Bolivar DAF/F plant, they would not be expected to do this *selectively* in terms of skewing the WSP effluent phytoplankton community structure either away from or toward these so-called ‘problem species’.

5.3.2 Comparative zooplankton ecology of the pilot plant influent and the four advanced in-pond upgrades

A total of 16 zooplankton taxa were recorded and identified (in addition to several unidentified rotifer species) during the approximate 12 month monitoring duration from July 2005 to August 2006 (Table 5.2). The reader is referred at this point to Appendix D for a photographic catalogue of the most commonly encountered zooplankton during the 2005–2006 monitoring duration. The zooplankton community within the pilot plant influent (Bolivar WSP effluent) was predominated by large-bodied cladocerans, copepods, copepod nauplii, rotifers and ostracods (Figure 5.21–5.24). Generally speaking, this zooplankton community structure was similar to the findings of Hussainy (1979) during observations of WSPs in Werribee, Victoria, Australia, and was also similar to that reported by Mitchell and Williams (1982b) following ecological monitoring of a WSP system in Gumeracha, South Australia. It was also similar in nature to the generic zooplankton community structure of freshwater environments in general (Downing and Rigler, 1984).

Table 5.1. Listing of the most commonly encountered zooplankton taxa, in addition to some miscellaneous invertebrates observed during pilot plant operational monitoring from July 2005–August 2006 (NB. some extremely rare organisms were not recorded).

Arthropoda (copepods)
- Calanoida
  - Centropagidae
    - *Boeckella triarticulata* Thompson, 1883
    - Unidentified nauplii and copepodites
- Cyclopoida
  - Cyclopidae
    - *Mesocyclops nothius* Kiefer, 1981
    - Unidentified nauplii and copepodites

Arthropoda (cladocerans)
- Diplostraca
  - Daphniidae
    - *Daphnia carinata* King, 1853 s.l.
    - *Simocephalus sp.*
  - Chydoridae
    - *Plexorus* sp.
  - Moinidae
    - *Moina micrura* Kurz, 1874

Arthropoda (ostracods)
- Podocopida
  - Cyprididae
    - *Bennelongia* cf. *barangaroo* (De Dekker, 1981)

Rotifera (rotifers)
- Ploima
  - Brachionidae
    - *Brachionus novaezealandia* (Morris) 1912
    - *Brachionus quadridentatus* Hermann, 1783[†]
    - *Keratella australis* (Berzins) 1963
    - *Keratella cochlearis* (Gosse) 1851[†]
    - *Keratella procurva* (Thorpe) 1891
    - *Keratella slacki* (Berzins) 1963
  - Lecanidae
    - *Lecane bulla* (Gosse) 1851
    - *Lecane ludwigii* (Eckstein) 1883
- Bdelloidea
  - Philodinidae
    - *Philodina* sp.[†]
  - Unidentified bdelloid rotifers (Adinetidae or Philodinidae)

Miscellaneous invertebrates:

- Arachnida: Hydrachnidae – Water mite[†]
- Insecta: Diptera: Chironomidae – Midge fly larvae
- Insecta: Hemiptera: Corixidae – Water boatman
- Insecta: Odonata: Anisoptera – Dragonfly larvae[†]
- Mollusca: Gastropoda – Snails
- Nematodes: Coelenterate – Hydra[†]
- Protozoa: Ciliophora: Ciliatea

[†] Denotes rare or transient species observed relatively infrequently and often in low numbers

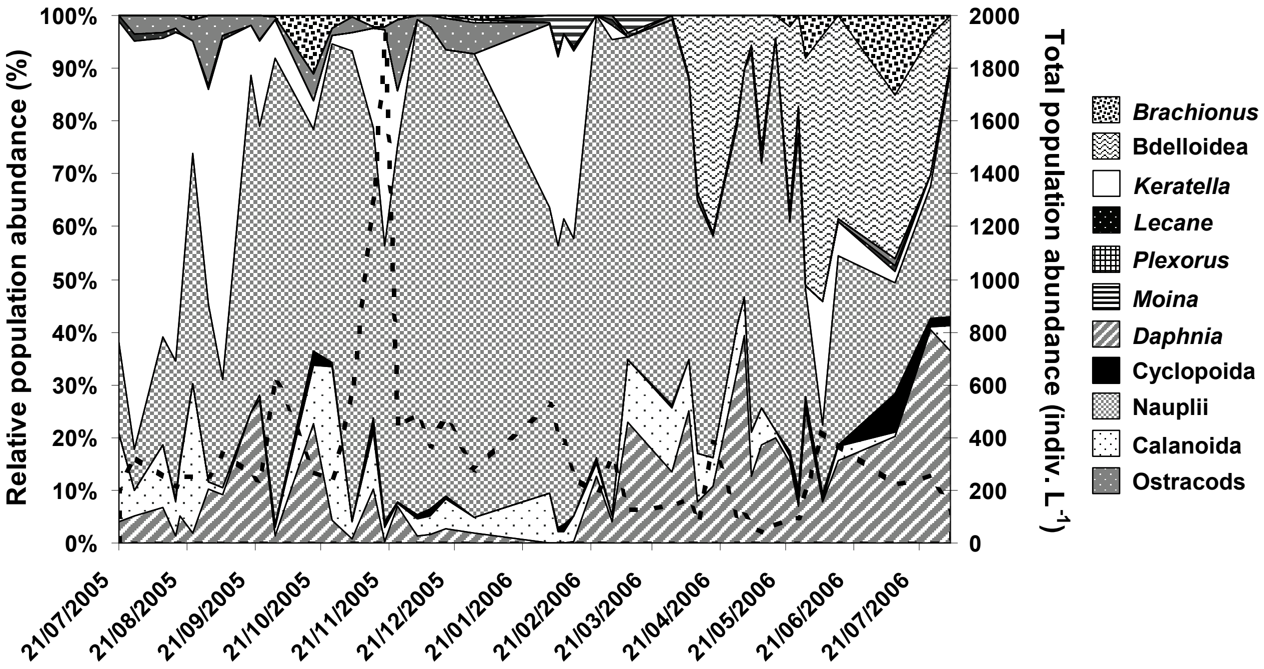

Figure 5.21. Pilot plant influent zooplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total zooplankton abundance (individuals L^{-1}; broken line; right *y*-axis).

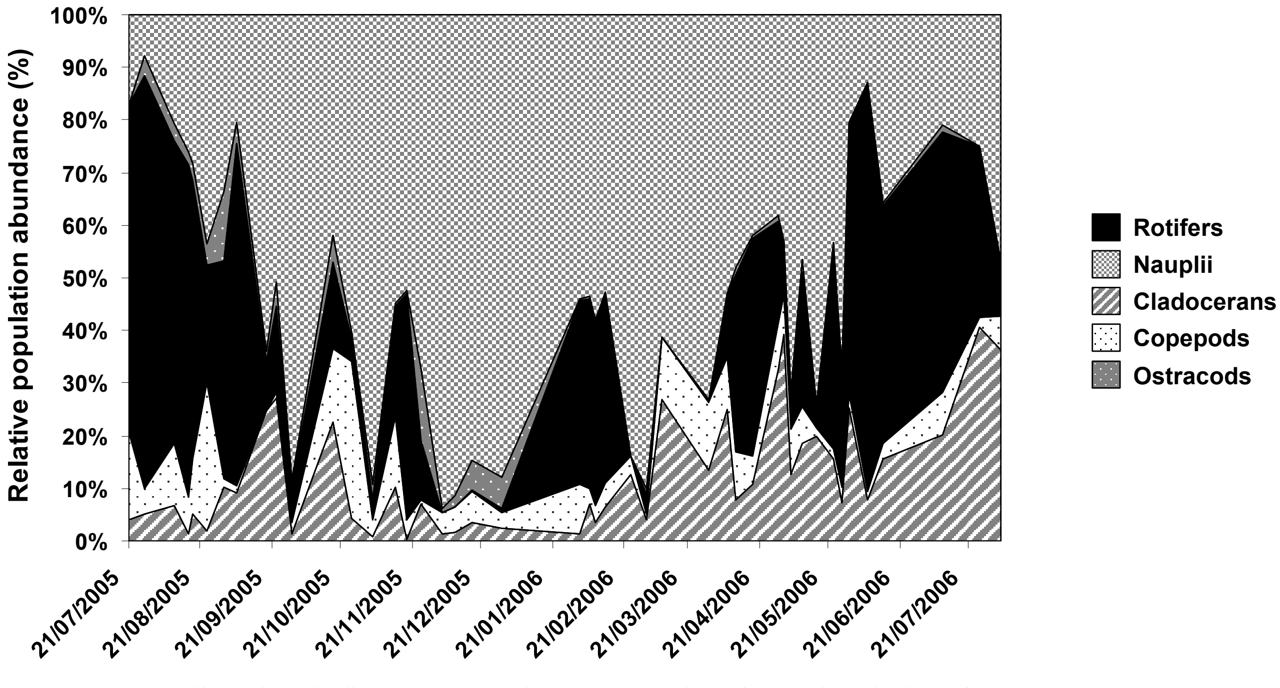

Figure 5.22. Pilot plant influent zooplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative temporal abundance of the dominant zooplankton groups.

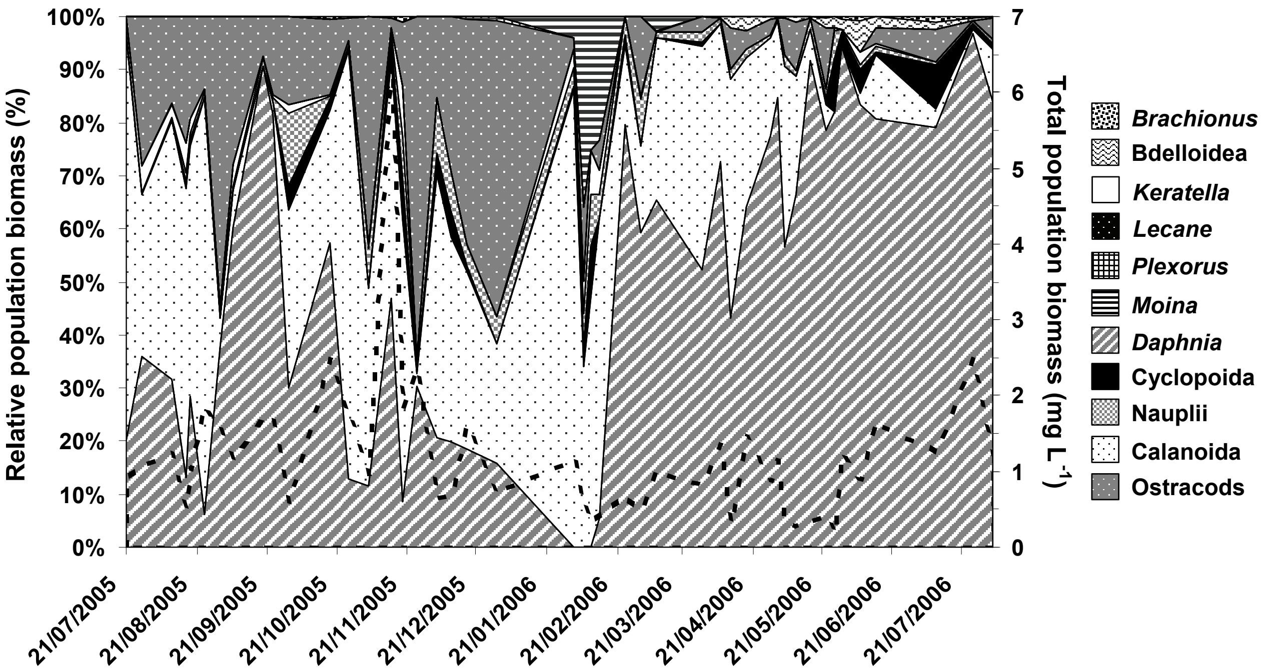

Figure 5.23. Pilot plant influent zooplankton biomass dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal biomass of the dominant genera (left *y*-axis) as well as total zooplankton biomass (mg L^{-1}; broken line; right *y*-axis).

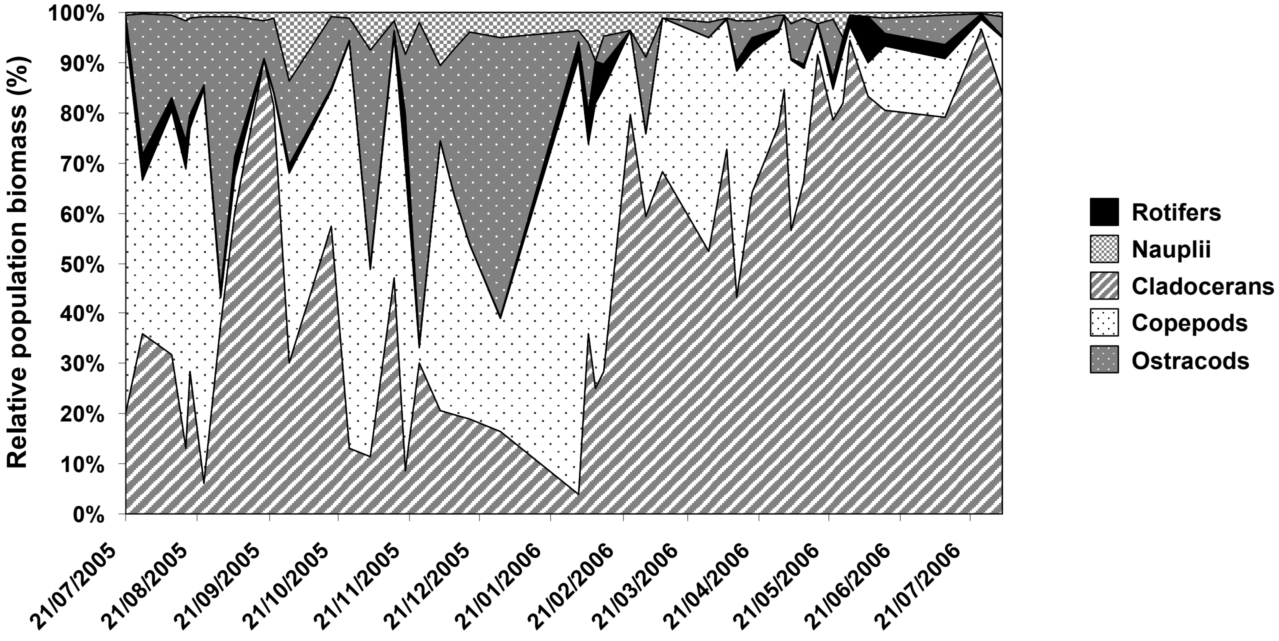

Figure 5.24. Pilot plant influent zooplankton biomass dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal biomass of the dominant zooplankton groups.

Average zooplankton density within the influent wastewater during the 2005–2006 monitoring period was moderate, with a mean of approximately 330 and a median of 250 organisms L^{-1} (Figure 5.21). Total zooplankton densities of this order were considered to be relatively low given the hypertrophic environmental status and given that counts also included copepod nauplii, although they were of a similar magnitude to those reported by Mitchell (1980) for another South Australian WSP with similar organic strength wastewater. Zooplankton populations were most abundant during the spring of 2005 (September–November), during which time the median population density was 390 individuals L^{-1} and several blooms occurred resulting in zooplankton densities in excess of 500 and up to 1920 individuals L^{-1} (Figure 5.21). Conversely, zooplankton were least abundant during autumn of 2006 (March–May), when median population density was just 103 individuals L^{-1}.

Copepod nauplii were both highly abundant (maximum of 1010 individuals L^{-1}) and numerically dominant within the influent wastewater, representing on average 52% of total zooplankton numbers during the 12 month monitoring duration. This was a similar trend to that noted by Nandini (1999), who also reported very high densities (500–2000L^{-1}) of copepod nauplii (*Mesocyclops* species) in the terminal WSP of the four-pond system (Delhi, India) on several occasions during the 10 month monitoring period. Rotifers were the second most abundant zooplankton group, representing on average 27% of the total population density. Interestingly, this general trend for 'nauplii–rotifer' dominance—in terms of relative population abundance—was similar to that noted by Shiel *et al*. (1982) following ecological monitoring of plankton communities in the lower River Murray, South Australia. *Daphnia carinata* was the dominant cladoceran species, making up 96% of total cladoceran density in the influent wastewater and 12% of the total zooplankton counts. Ostracods were the least abundant zooplankton group, constituting just 2% of the total population abundance on average.

As discussed earlier, *Daphnia* species are renowned for their competitive superiority under the notoriously variable and relatively unfavourable conditions synonymous with WSP environments (Cauchie *et al.*, 2000a). Further to this, and in fishless WSPs in particular, the larger grazers such as *Daphnia* species are much less susceptible to predation by invertebrate planktivores (e.g. predatory cladocerans and copepods, water mites and water boatmen) than are other smaller organisms (e.g. rotifers; Williamson, 1983; MacIsaac and Gilbert, 1989; Plaßmann *et al.*, 1997). In this sense *Daphnia* species often become the dominant member of the zooplankton community simply through sheer size and relative 'unpalatability' (Burns, 1998) as well as from their efficient grazing capabilities (Vanni, 1986) and the almost non-existent inter-specific competition resulting from an inherently restricted aquatic biodiversity within the relatively extreme and unfavourable pond environment (Mitchell and Williams, 1982c; Cauchie *et al.*, 2000a).

It should be noted at this point that due to the coarse pre-screening of the influent wastewater (2mm; refer Section 2.1), it was likely that some of the larger zooplankton (i.e. large-bodied cladocerans such as *Daphnia*) would have been under-sampled during pilot plant monitoring, possibly leading to an underestimation of the true *in situ* zooplankton population density within the Bolivar WSP network. Similarly, and as highlighted previously in Section 2.2.1, it was also likely that the 64μm aperture mesh used during on-site zooplankton sampling procedures could have led to under-sampling of some smaller zooplankton such as rotifers; resulting again in underestimation of the true *in situ* zooplankton density within the Bolivar ponds and pilot plant influent. At the same time, it was also likely that ostracod densities reported here would represent conservative estimates of the true population numbers due to the tendency of these organisms to substrate graze either on the pond bottom or on the pond walls (see Plate 3.4–3.5) rather than be pelagic or free-swimming in the water column. Regardless of this, sampling protocols and subsequent organismal counts did provide an accurate reflection of the number of zooplankton actually entering the pilot plant itself, and also effectively represented the vast majority of zooplankton populations (in terms of daily biomass) within both the influent and each of the pilot treatment series.

As was the case for the previous phytoplankton monitoring data, and although there were frequently in the order of 7–10 different species present in the influent wastewater at any given time, the community was most commonly dominated by just 3–4 organisms in terms of relative daily abundance and biomass (Figures 5.21 and 5.23). According to Burns (1998), the two largest crustacean zooplankton in Australasia, and potentially also the two most effective grazers of phytoplankton, are *Daphnia carinata* and calanoid copepods such as *Boeckella* species. These organisms were almost always present at all sampling intervals within both the influent and also the pilot treatment ponds and were frequently the dominant zooplankton in terms of their relative contributions to the total standing biomass. Ecological analysis of the pilot plant influent zooplankton community has shown a general temporal trend of oscillating *Daphnia* and copepod dominance in terms of their relative biomass contributions. This general trend has been observed by others for eutrophic freshwater environments (Pedrós-Alió and Brock, 1983; Adrian, 1997) and most likely relates to the general superiority of these zooplankton as well as competitive interactions between the two groups. Superficially, this trend for *Daphnia*–copepod dominance was also apparent in the work of Herdianto (2003) following short-term and infrequent monitoring of zooplankton populations within the Bolivar WSP network during 2001–2002; such that this is most likely a consistent ecological trend for this pond system.

Compared with other freshwater environments (i.e. oligotrophic/mesotrophic water bodies), hypertrophic wastewater environments are notorious for being associated with relatively low metazoan diversity (Dinges, 1973; Hussainy, 1979; Canovas *et al.*, 1996; Cauchie *et al.*, 2000a). For example Shiel *et al.* (1982)—following three year monitoring of the plankton diversity in the lower River Murray, South Australia—recorded a total of 133 zooplankton taxa in the relatively nutrient-poor waterway. It has been suggested by Mitchell and Williams (1982b) and later by Cauchie *et al.* (2000a) that such low-level biotic diversity in WSPs is a result of both a physiological intolerance by many aquatic organisms to the peculiar combination of abiotic conditions found within WSPs (namely the highly variable pH and DO conditions), and also from the competitive superiority of *Daphnia* species in these environments (e.g. Kring and O'Brein, 1976a; DeMott and Kerfoot, 1982; Vanni, 1986). *Daphnia* species were indeed competitively superior in the Bolivar WSPs also, and despite representing only 12% of the population in terms of absolute numbers, they constituted on average ≈50% of the total population biomass (refer Figure 5.23).

Notably, Figures 5.21 and 5.23 show an extensive and sometimes complete summer disappearance of *Daphnia* populations from December of 2005 until the beginning of March 2006, during which time the mean *Daphnia* density was just 6 individuals L^{-1};

with frequent zero counts recorded during February of 2006. This marked suppression of *Daphnia* populations coincided with the increased dominance of calanoid copepods (*Boeckella triarticulata*) and ostracods (*Bennelongia barangaroo*) as well as the first noted appearance of the smaller cladoceran zooplankton *Moina micrura* and *Plexorus* species; all of which appeared to conspicuously coincide with a large-scale cyanobacterial bloom (*Microcystis*) during the summer of 2005. Interestingly, Vanni and Temte (1990) reported that the summer disappearance of *Daphnia* in eutrophic freshwater environments generally coincides with periods of increased dominance by smaller cladocerans and copepods—a strikingly similar trend to that reported here for the Bolivar WSP system. Statistically, there was a significant negative association between the $\log_{10}$ abundance of *Microcystis* and *Daphnia* species (Spearman $r_s = -0.644$; $n = 20$; $p = 0.003$), a significant positive relationship between the $\log_{10}$ abundance of *Moina* and *Microcystis* species ($r_s = 0.544$; $n = 20$; $p = 0.013$) and a negative correlation between $\log_{10}$ population density of *Daphnia* and *Moina* species in the influent wastewater ($r_s = -0.426$; $n = 45$; $p = 0.004$). Although there was no statistically significant relationship between ostracod density and *Microcystis* in the influent wastewater ($p = 0.391$), it was apparent that ostracods did increase in relative biomass during the early stages of the cyanobacterial bloom. There are some reports of ostracods (*Cyprinotus*) grazing on cyanobacteria (*Nostoc*) (Grant *et al.*, 1983), but whether the abovementioned increase in ostracod biomass was directly related to the *Microcystis* bloom is unclear; since almost no information is available on the ecology of *Bennelongia* species. The current data does suggest, however, that *Bennelongia* species may be able to graze on—or in the very least are not inhibited by—*Microcystis*, and/or that the reduced competition from *Daphnia* in some way promoted ostracod development.

According to Mitchell and Williams (1982b) and also Cauchie *et al*. (2000a), water temperature is the most important factor influencing *Daphnia* productivity and population density in WSPs—an observation made somewhat earlier by Loedolff (1965) for zooplankton populations in WSP environments. Generally speaking, *Daphnia* density has been observed to be greatest during the Australian autumn–winter seasons and declines during the summer months (Hussainy, 1979; Mitchell and Williams, 1982b; Lund and Davis, 2000). While there were indeed a number of significant relationships between temperature and the occurrence of many zooplankton in the influent wastewater (i.e. *Daphnia*, *Moina*, *Plexorus*, *Lecane* and bdelloid rotifers; $p = 0.05–0.0001$) and although temperature has been suggested as the primary factor responsible for regulating zooplankton community structure in WSPs, it is likely that temperature in itself is not always the *direct* causal factor for such changes.

Whilst both Mitchell and Williams (1982b) and Cauchie *et al*. (2000a) cited temperature as the major factor behind the productivity and development of *Daphnia* populations in WSPs, it is likely that temperature also has an indirect but equally manipulative effect on zooplankton communities through its impact on resident phytoplankton populations. For example, summer blooms of unpalatable or inedible algae such as *Microcystis* or filamentous *Cladophora* species have elsewhere been reported to coincide with periods of low *Daphnia* density and increased copepod abundance (Mitchell and Williams, 1982b; Lund and Davis, 2000). Several additional authors have also linked the summer decline of large-bodied cladocerans such as *Daphnia* in hypertrophic environments to an increase in the relative abundance of grazing-resistant cyanobacteria (O'Brien and deNoyelles Jr., 1972; Jarvis, 1986; Vanni and Temte, 1990; Moss *et al*., 1991; Ghadouani *et al*., 1998; Lund and Davis, 2000); something that has in turn been linked to the mechanical interference of the feeding apparatus in *Daphnia* by unpalatable filamentous cyanobacteria (Dawidowicz, 1990; Gliwicz, 1990; DeMott *et al.*, 2001) as well as toxic inhibition by some cyanobacteria including *Microcystis* species (Lampert, 1982; Lampert, 1987; DeMott *et al.*, 1991; Forsyth *et al.*, 1992).

This trend for a reduction in *Daphnia* populations during *Microcystis* bloom conditions was also noted during the current work, and although there was also a significant negative relationship between *Daphnia* abundance and temperature in the influent wastewater ($r_s = -0.317$; $n = 44$; $p = 0.036$), this does not necessarily mean that temperature was the direct causal factor. It is considered more likely that temperature had an indirect but equally important role in shaping zooplankton communities by inducing seasonal changes in phytoplankton community ecology and therefore food resource availability. As another example, there was also a significant positive association between the numbers of *Moina* and temperature in the Bolivar WSP effluent ($r_s = 0.404$; $n = 44$; $p = 0.007$), however, it is unclear as to whether temperature alone was directly responsible for sudden occurrence of *Moina* species. Perhaps a more likely scenario was that temperature drove the late spring–summer cyanobacterial bloom of *Microcystis*, which in turn led to a reduction in the numbers of highly competitive *Daphnia* which then allowed populations of the smaller *Moina* (and to a lesser extent *Plexorus*) species to develop and persist for the duration of the cyanobacterial bloom.

Although there has been some evidence of effective cyanobacterial grazing by *Daphnia* species (e.g. Reynolds *et al*., 1982; Schoenberg and Carlson, 1984; Work and Havens, 2003) including *D. carinata* (e.g. Kobayashi, 1993; Matveev *et al*., 1994), the general trend from this significant body of work is that *Daphnia*—because of their non-selective feeding behaviour—are relatively ineffective cyanobacterial grazers and that grazing on cyanobacteria generally results in poor *Daphnia* fitness (Arnold, 1971; Jarvis, 1986; Gliwicz, 1990; DeMott *et al.*, 1991). At the same time, some authors have actually reported that increased grazing pressure by large-bodied zooplankton like *Daphnia* and calanoid copepods can itself be the cause a shift in the phytoplankton community structure toward one that is dominated by larger—sometimes colonial—algal species (Bergquist *et al.*, 1985). Large numbers of both *Daphnia* (135 individuals L^{-1}) and *Boeckella* (143 individuals L^{-1}) were indeed recorded just prior to the onset of the *Microcystis* bloom in late November, but whether this was actually a causal factor in the establishment of the cyanobacterial bloom remains unclear.

Considering all of the above information, the summer disappearance of *Daphnia* was considered most likely to have been a consequence of the largely unpalatable food resources at the time and also probably resulted from the competitive superiority of copepods (see below) under these conditions (e.g. Lampert, 1981; Thompson *et al*., 1982; Richman and Dodson, 1983; Gliwicz, 1990; Gliwicz and Lampert, 1990; DeMott *et al*., 2001). Thompson *et al*. (1982) for example noted a suppression of *Daphnia* filtration and feeding rate during times of colonial *Microcystis* dominance, and so it was most likely that the observed depression of *Daphnia* populations resulted from a similar and direct interference of grazing activity. It should also be stated at this point, that—to the best knowledge of the author—the particular species of cyanobacteria (*Microcystis flos-aquae*) responsible for the wholesale bloom within the Bolivar WSP network has not yet been reported as being capable of producing the toxin ‘microcystin’; such that the suppression of *Daphnia* populations as a result of cyanobacterial intoxication was deemed unlikely.

In terms of total population biomass, the calanoid copepod *B. triarticulata* was the dominant zooplankter during the peak of the *Microcystis* bloom from mid December 2005 to mid February 2006, with the combined adult and naupliar populations (assuming a similar calanoid:cyclopoid naupliar ratio as for adults at that time) representing on average >50% of the total zooplankton biomass during this two month period. Burns and Xu (1990) reported that *Boeckella* species (including *B. triarticulata*) are suitably equipped (in terms of their food handling and ingestion capability) to be able to graze effectively on a number of cyanobacterial species. Similarly, others have reported that copepods in general are more selective feeders than are cladocerans and can therefore reject unpalatable or poor quality food—namely cyanobacteria—on the basis of taste before it is ingested (DeMott, 1986; DeMott and Moxter, 1991; DeMott and Watson, 1991); giving them a competitive advantage over the more indiscriminate *Daphnia* during times of low food quality (DeMott, 1982; Richman and Dodson, 1983). This information could therefore go toward explaining the increased dominance of *B. triarticulata* during the 2005–2006 cyanobacterial bloom in the Bolivar ponds.

Interestingly, the dominance of calanoid copepods (*Boeckella*) over cyclopoids (*Mesocyclops*) in the Bolivar WSPs was unlike the trend reported by Mitchell and Williams (1982b) following monitoring of another WSP system in Gumeracha, South Australia. Mitchell and Williams reported only relatively low-density transient populations of the calanoid *B. triarticulata* in relation to the more dominant and widely represented cyclopoid *Mesocyclops*. Similarly, Hussainy (1979) observed no calanoid copepods in another Australian WSP from the state of Victoria, reporting only the presence of *Mesocyclops* in that system. Elsewhere, the ratio of calanoid to cyclopoid copepods has been seen as an indicator of trophic status, with a low ratio indicating more eutrophic conditions (Patalas, 1972 cited in Patil and Gouder, 1985; McNaught, 1975; Gannon and Stemberger, 1978; Adrian, 1997). It is possible then that the more refined nature of the tertiary maturation WSP effluent at Bolivar allowed for the increased prevalence of calanoid copepods over cyclopoids in comparison to the Gumeracha WSPs of Mitchell and Williams above. The data of Mitchell and Williams

(1982b; 1982c) showed an apparent trend for increasing prevalence of *B. triarticulata* with decreasing wastewater BOD_5 (from 17 to 7mg BOD_5 L^{-1}). Likewise, Cauchie *et al*. (2000a) reported no instances of calanoid copepod occurrence in their WSP under conditions of further elevated BOD_5 (mean concentration >50mg BOD_5 L^{-1}). This trophic status hypothesis remained qualitative, however, and no attempts were made to further correlate the calanoid:cyclopoid ratio with water quality parameters such as PO_4^{3-}-P, chlorophyll *a*, organic carbon or BOD_5.

Similarly to *B. triarticulata* above, and during the peak of the cyanobacterial bloom, the ostracod *Bennelongia barangaroo* was also strongly represented; constituting on average 21% of the total zooplankton biomass during the *Microcystis* bloom period of 2005–2006. Compared with cladoceran and copepod zooplankton, very little work on the ecology of freshwater ostracod species exists; although the work of Grant *et al*. (1983) does suggest that ostracods (*Cyprinotus* species) able to graze effectively on cyanobacteria. The particular ostracod Genus reported here (*Bennelongia*) was first classified in 1981 (De Deckker, 1981b; De Deckker, 1981a) and so information on its ecology in general is scarce. Furthermore, the current research represents the third known reporting of ostracods *per se* in Australian WSPs (alongside Hussainy, 1979 and Mitchell and Williams, 1982a), and the first known reporting of *Bennelongia* species in a wastewater environment. It can only be hypothesized then, that *B. barangaroo* was either able to effectively utilize *Microcystis* species as a food resource during the cyanobacterial bloom, and/or that it was able to predate upon the large numbers of copepod nauplii present during this time (presumably a consequence of the increased dominance of *B. triarticulata* as above). This latter theory of copepod predation is supported by the reporting of (De Deckker, 1983) and also by the significant correlation between $\log_{10}$ ostracod and naupliar abundance within the influent wastewater ($r_s = 0.423$; $n = 45$; $p = 0.004$).

With respect to some other zooplankton taxa, there were some additional and notable trends evident in the influent wastewater following the 2005–2006 monitoring programme. Figure 5.21 in particular showed a general trend of co-occurrence for populations of the cyclopoid copepod *Mesocyclops nothius* and the rotifer *Brachionus novaezealandia*, with the appearance of *B. novaezealandia* during October–November of

2005 and also during May–July of 2006 appearing to coincide with increased numbers of *Mesocyclops*. This trend was also supported statistically, with a highly significant positive relationship between the $\log_{10}$ abundance of *Mesocyclops* and *Brachionus* species in the influent wastewater during the entire 2005–2006 period ($r_s = 0.532$; $n = 45$; $p < 0.0002$). Countless reports of the predatory capabilities of cyclopoid copepods have been published since it was intensively investigated in the 1950s (Fryer, 1957). Adult cyclopoid species are well recognized as being carnivorous and are known to prey on small cladocerans, rotifers, as well as the smaller naupliar life stages of other copepods; with *Mesocyclops* in particular reportedly able to prey on rotifers including *Brachionus* species (e.g. Williamson, 1981; Williamson, 1983). Given that *Mesocyclops* densities were very low during periods where *B. novaezealandia* was absent (<1 individual L^{-1}), it was highly likely that the occurrence of *Mesocyclops* species in the Bolivar WSP system was strongly influenced by the availability of suitable populations of rotifer prey. Given the relatively small contribution of rotifers in general to the total zooplankton biomass (≈2% on average), no further attempts were made to delineate the causal relationships behind the temporal distribution of rotifer populations in the Bolivar WSPs.

Interestingly, the almost year-round presence of rotifer populations in the Bolivar WSP effluent provided additional support to the earlier suggestions (e.g. Sections 3.3.5; 3.3.6; 3.3.8) of the Bolivar effluent being highly refined and polished in nature. The universally small size of rotifers allows them to respire without the aid of conventional respiratory organs; respiring instead via their whole body surface. For this reason they are generally unable to persist in anaerobic environments, making them one of the more sensitive macrobiotic indicators of the level of organic pollution and subsequent aerobic status of a given waterway. Elsewhere, rotifers have been suggested as being good indicators of the trophic status or the level of organic pollution in aquatic environments (Gannon and Stemberger, 1978; Sládeček, 1983); with increasing rotifer abundance under conditions of improved water quality. The work of Nandini (1999) supported this concept in a wastewater context, reporting that the densities of rotifers were consistently higher in the terminal WSPs of a multi-pond series. The observation of almost perennial inhabitance by rotifers within the Bolivar WSP system therefore suggests that the

Bolivar effluent is of a sufficiently refined nature to allow for largely aerobic operation; something supported by the two month 24 hour online DO data of Figure 4.7.

Quantitative analyses of rotifer populations in WSPs world-wide are both rare and also commonly limited to reports of *Brachionus* species (e.g. Roche, 1995; Nandini, 1999; Cauchie *et al*., 2000a). Cauchie *et al.* (2000a) for example reported only sporadic populations of rotifers (commonly *Brachionus* species) in a Belgian WSP. It is possible that in the case of Cauchie and co-workers above, the higher organic strength of their wastewater (commonly >50mg BOD_5 L^{-1}) compared with that of the Bolivar ponds (annual mean of just 6mg BOD_5 L^{-1}) contributed to the observation of fewer rotifer populations, since these organisms are known to be less tolerant to environments with higher-level organic pollution (Gannon and Stemberger, 1978; Sládeček, 1983). At the same time, however, the likelihood of rotifer suppression by larger and more competitive *Daphnia* species (Gilbert, 1985; Gilbert, 1988; MacIsaac and Gilbert, 1989) should not be overlooked.

Martyn *et al*. (2004, p. 6), following a 12 week case study specifically investigating phytoplankton communities in the Bolivar WSPs, commented that rotifers had been observed “less frequently and in lower numbers” within the pond system since the up-stream installation of an activated sludge plant in 2001. Rotifer numbers during the 12 month monitoring programme reported here were generally considered to be moderate to low, with a mean density of 93 individuals L^{-1}. Rotifers were at times quite abundant in the Bolivar WSP effluent (maximum density of 830 individuals L^{-1}); however, since there has been no prior quantitative assessment of zooplankton populations in the Bolivar WSPs, no definitive assessments of the long-term dynamics of any zooplankton group can be made. In general, the ecological monitoring data presented above suggests that, in contrast to the classical view of inherent ecological instability in hypertrophic environments (Barica and Mur, 1980; Uhlmann, 1980), the Bolivar WSPs were ecologically quite stable and well balanced systems. In finishing, and as suggested somewhat earlier by Sládeček (1983), it is thought that the ecological monitoring of zooplankton populations in WSP effluents could perhaps be incorporated into more traditional water quality monitoring programmes as a relatively convenient, rapid and responsive measure of general pond treatment function and process efficiency.

As a final point of reference, it should be stated that ephippial female *Daphnia* (females containing resting eggs)—a result of sexual reproduction brought about by unfavourable conditions—were noted both very infrequently and in low densities during the total 12 month monitoring duration. This observation was similar to that made by Mitchell and Williams (1982b) during ecological monitoring of a WSP system in Gumeracha, South Australia, and suggested that the overall conditions were favourable for the year-round persistence of *Daphnia* populations in both the Bolivar WSP effluent and all four experimental pilot treatments. Similarly, *Daphnia* cyclomorphosis (distinct morphological changes) was not observed during the 2005–2006 monitoring period. Cyclomorphosis in *Daphnia* species has been reported elsewhere to be a defense mechanism against predation (Zaret, 1972) or an adaptation to seasonal temperature changes (Egloff, 1968). Instances of cyclomorphosis (such as dorsal carapace distension or helmet development) have been previously observed for *D. carinata* in another fishless WSPs from South Australia (Mitchell, 1978), however, they were not observed during the 12 month monitoring of *D. carinata* populations in the Bolivar WSPs. Although there were several different morphological forms of *D. carinata* observed during the 12 month experimental monitoring period, no instances of such extreme cyclomorphism were recorded during the current work. It was thought that the absence of discrete cyclomorphosis may have been related to the lack of significant predator populations (e.g. fish or invertebrate predators such as Notonectidae or cyclopoid copepods); however, no significant attempts were made to delineate the factors involved in *Daphnia* cyclomorphosis within the context of the current work.

The temporal distribution of zooplankton populations and their relative biomass within Ponds 1 and 3 of each of the four pilot treatment series is shown below in Figures 5.25–5.56.

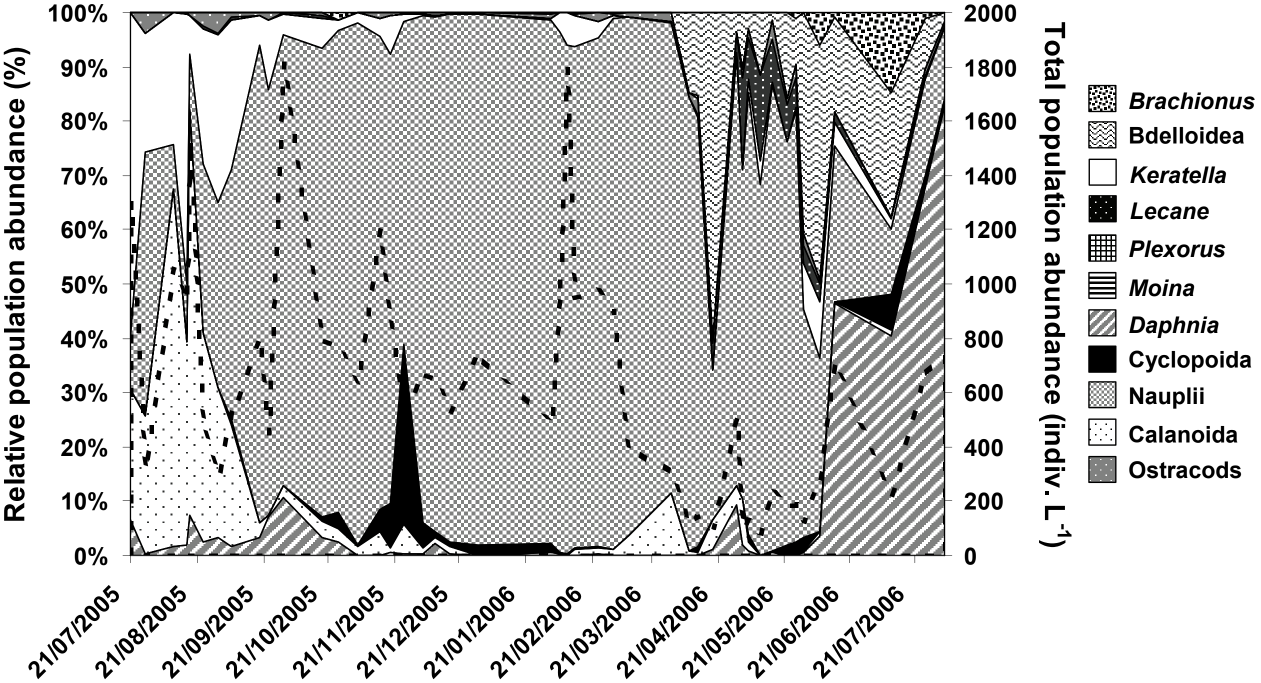

Figure 5.25. Rock Filter 1 zooplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total zooplankton abundance (individuals L^{-1}; broken line; right *y*-axis).

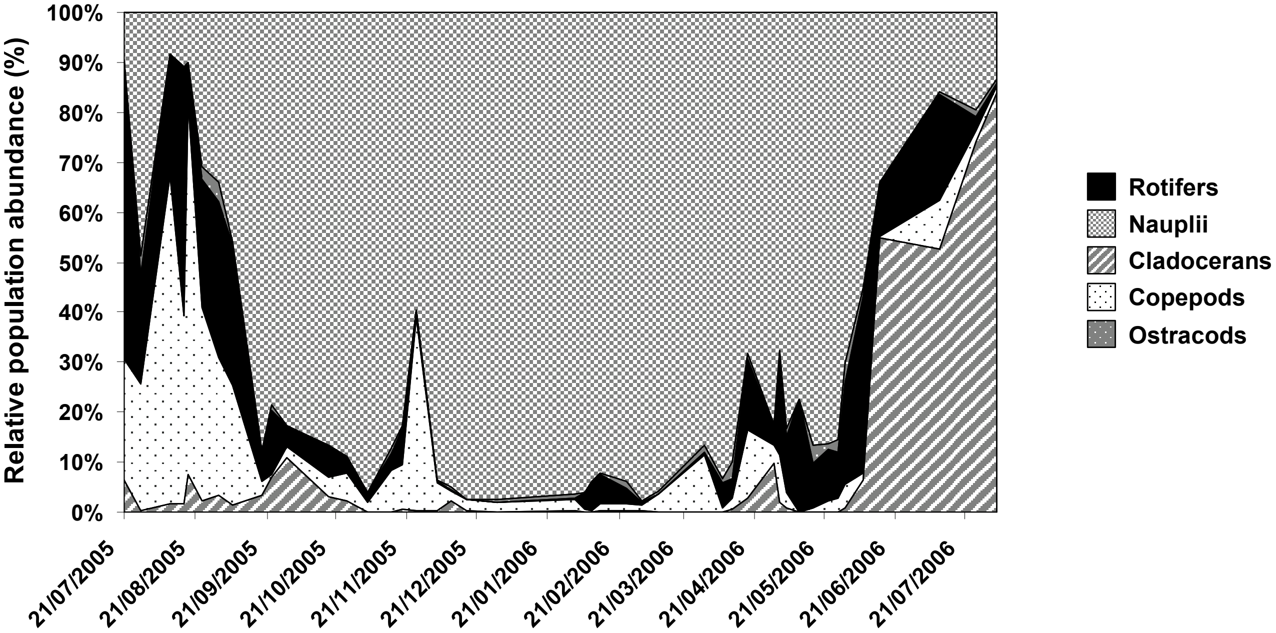

Figure 5.26. Rock Filter 1 zooplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of the dominant zooplankton groups.

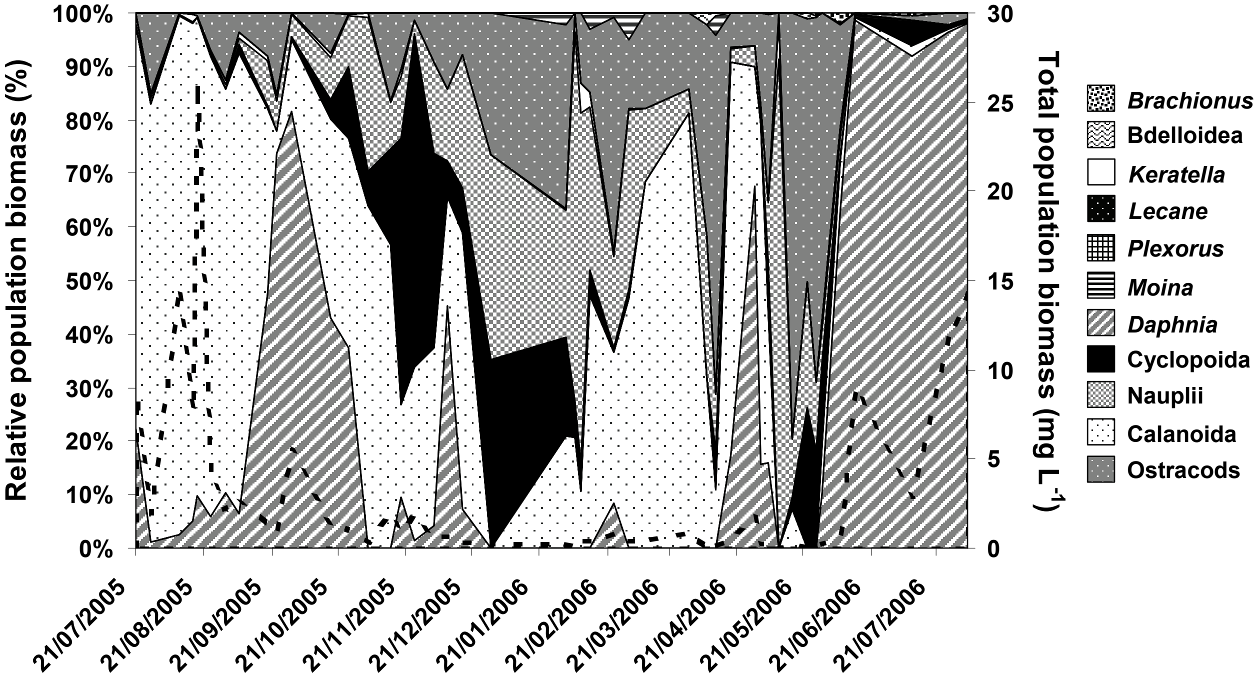

Figure 5.27. Rock Filter 1 zooplankton biomass dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal biomass of the dominant genera (left *y*-axis) as well as total zooplankton biomass (mg L^{-1}; broken line; right *y*-axis).

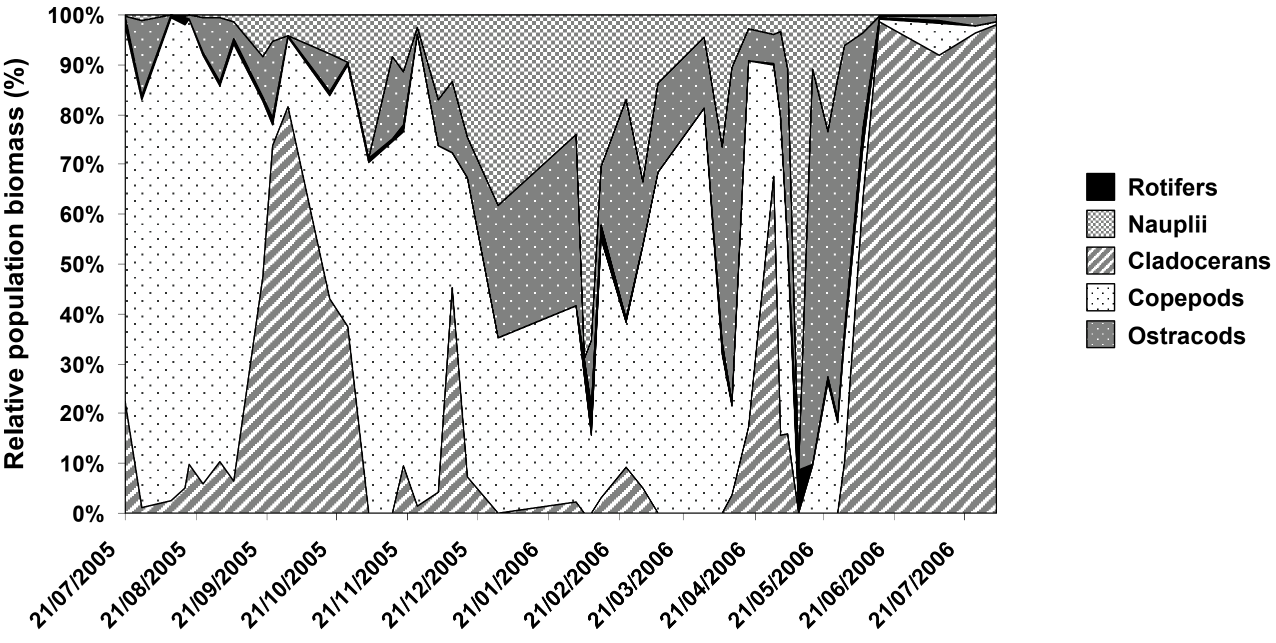

Figure 5.28. Rock Filter 1 zooplankton biomass dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal biomass of the dominant zooplankton groups.

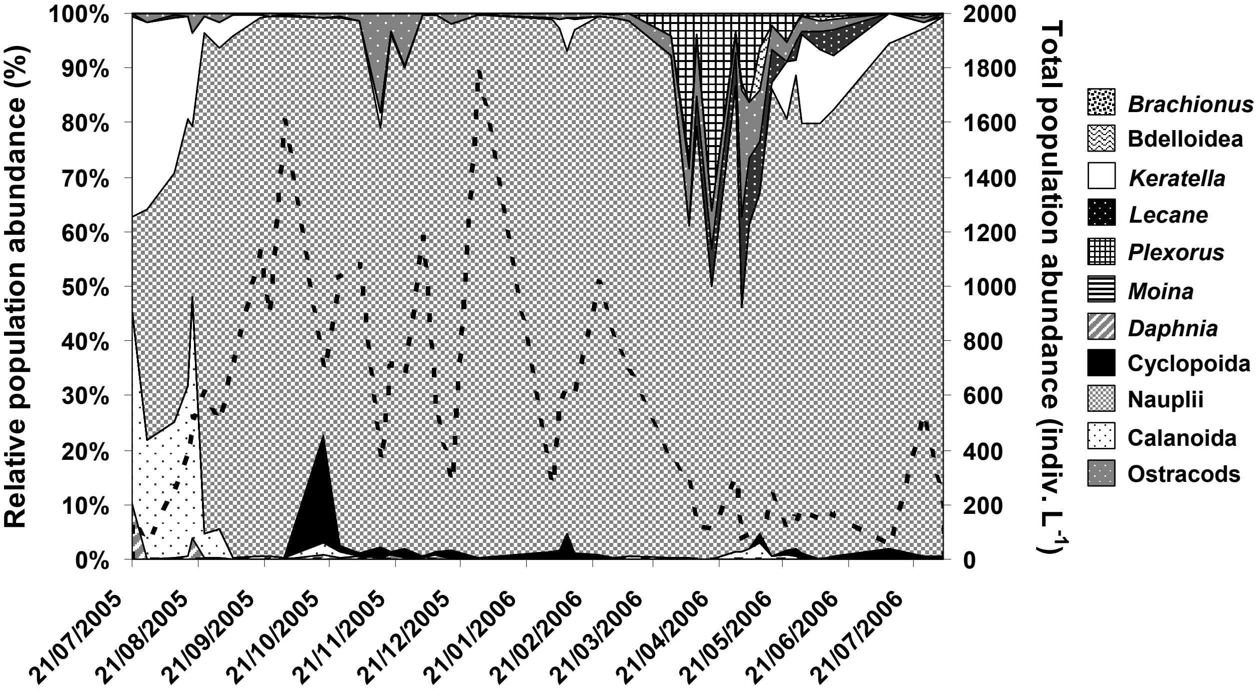

Figure 5.29. Rock Filter 3 zooplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total zooplankton abundance (individuals L^{-1}; broken line; right *y*-axis).

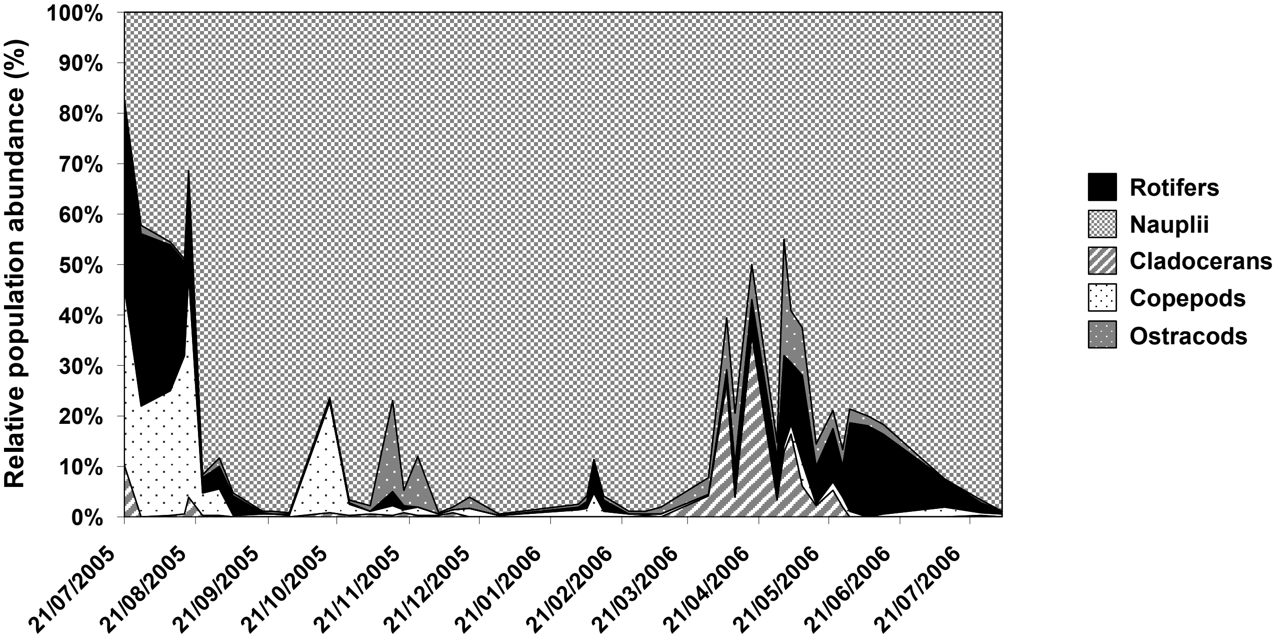

Figure 5.30. Rock Filter 3 zooplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of the dominant zooplankton groups.

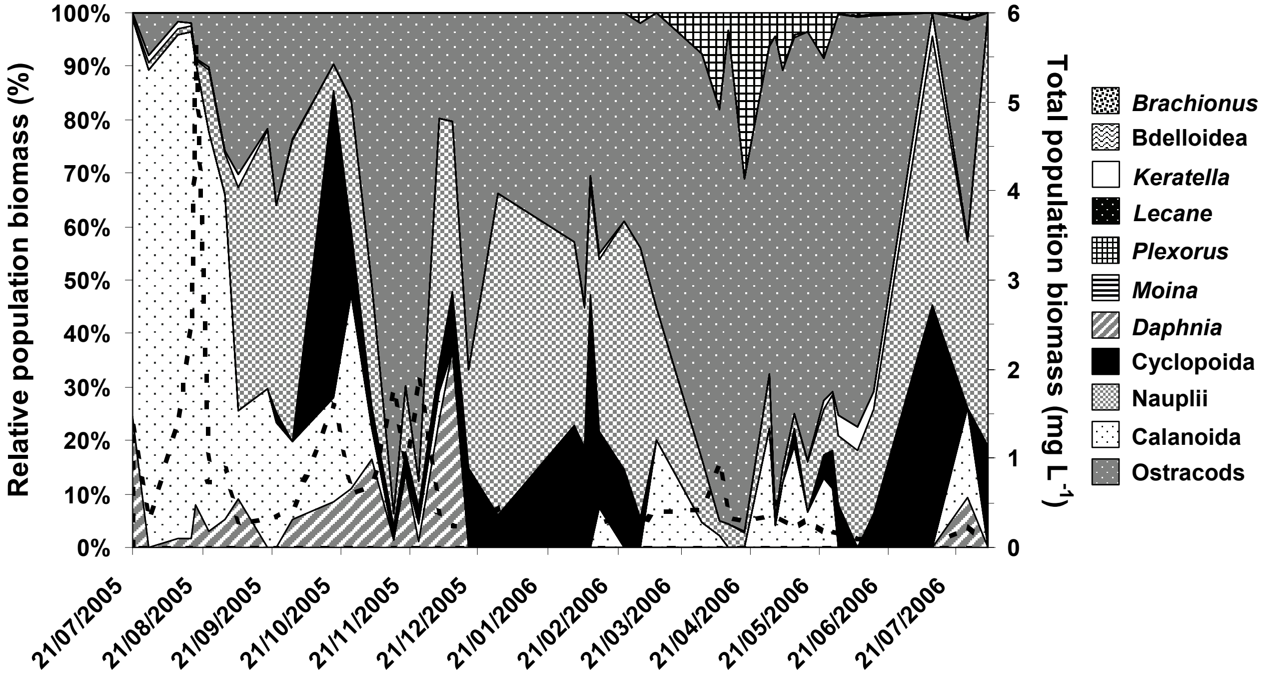

Figure 5.31. Rock Filter 3 zooplankton biomass dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal biomass of the dominant genera (left *y*-axis) as well as total zooplankton biomass (mg L^{-1}; broken line; right *y*-axis).

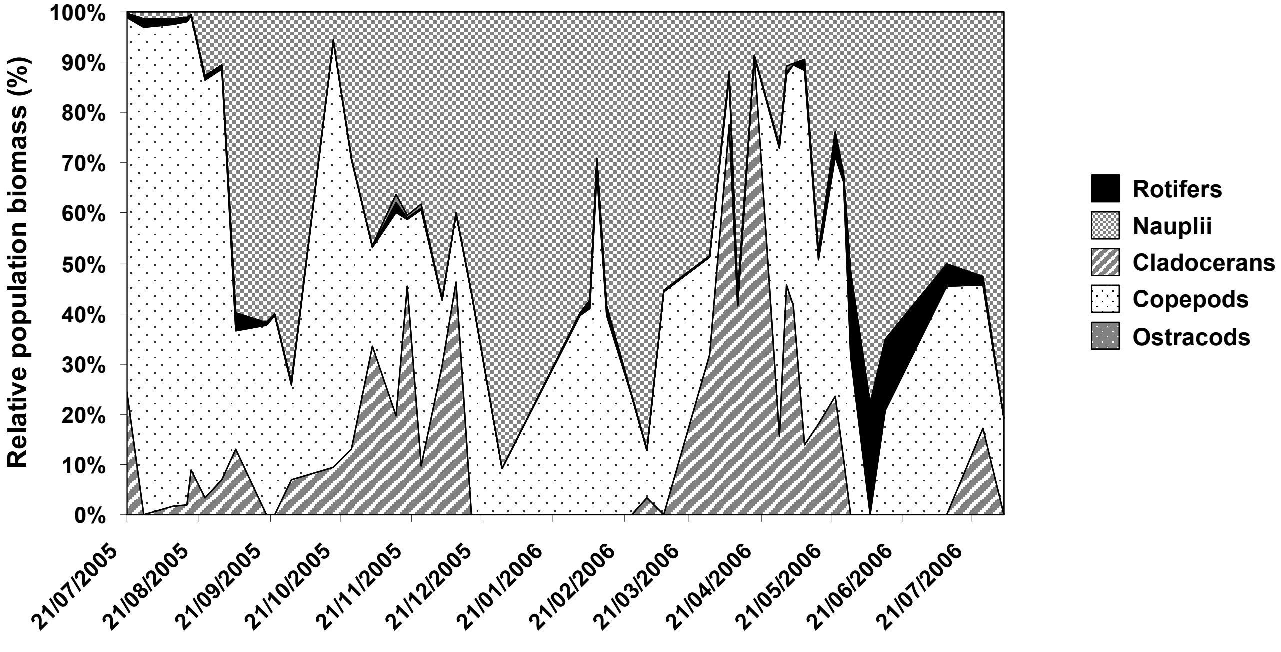

Figure 5.32. Rock Filter 3 zooplankton biomass dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal biomass of the dominant zooplankton groups.

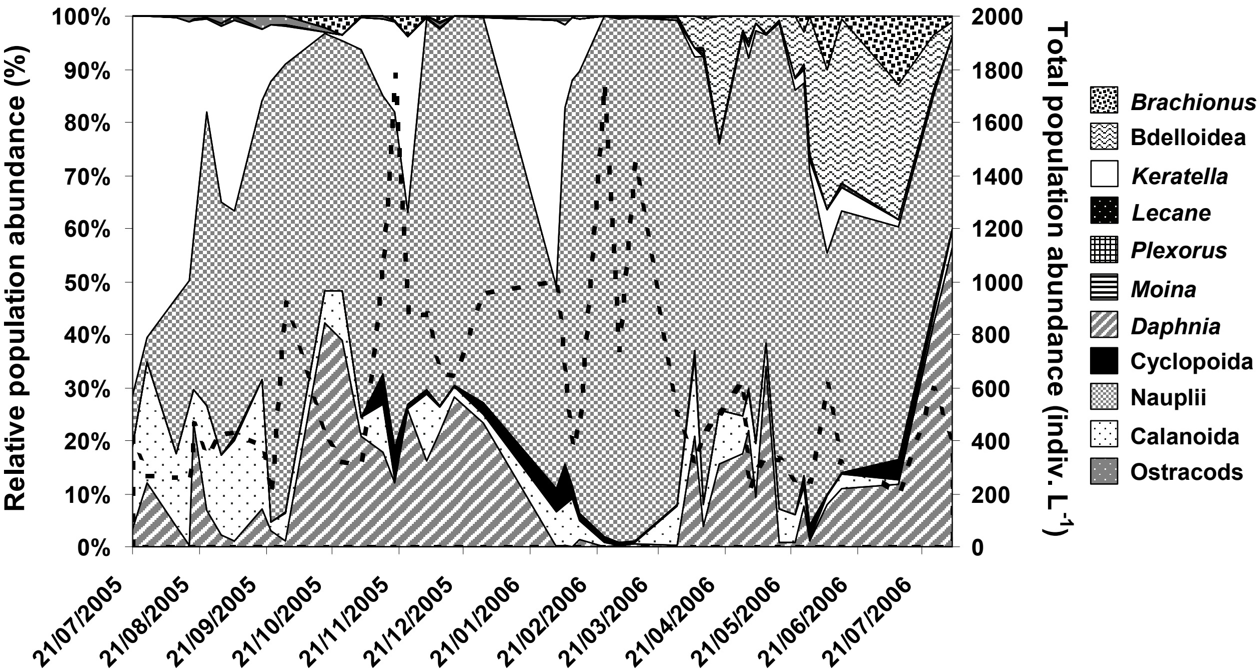

Figure 5.33. Open Pond 1 zooplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total zooplankton abundance (individuals L^{-1}; broken line; right *y*-axis).

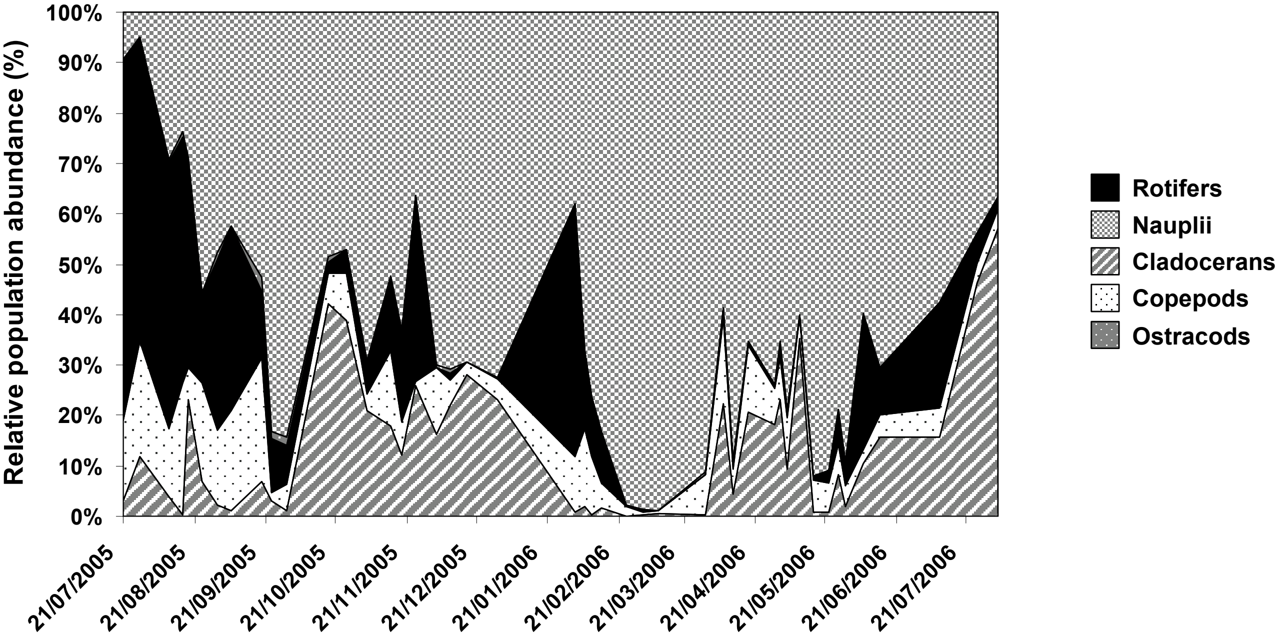

Figure 5.34. Open Pond 1 zooplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of the dominant zooplankton groups.

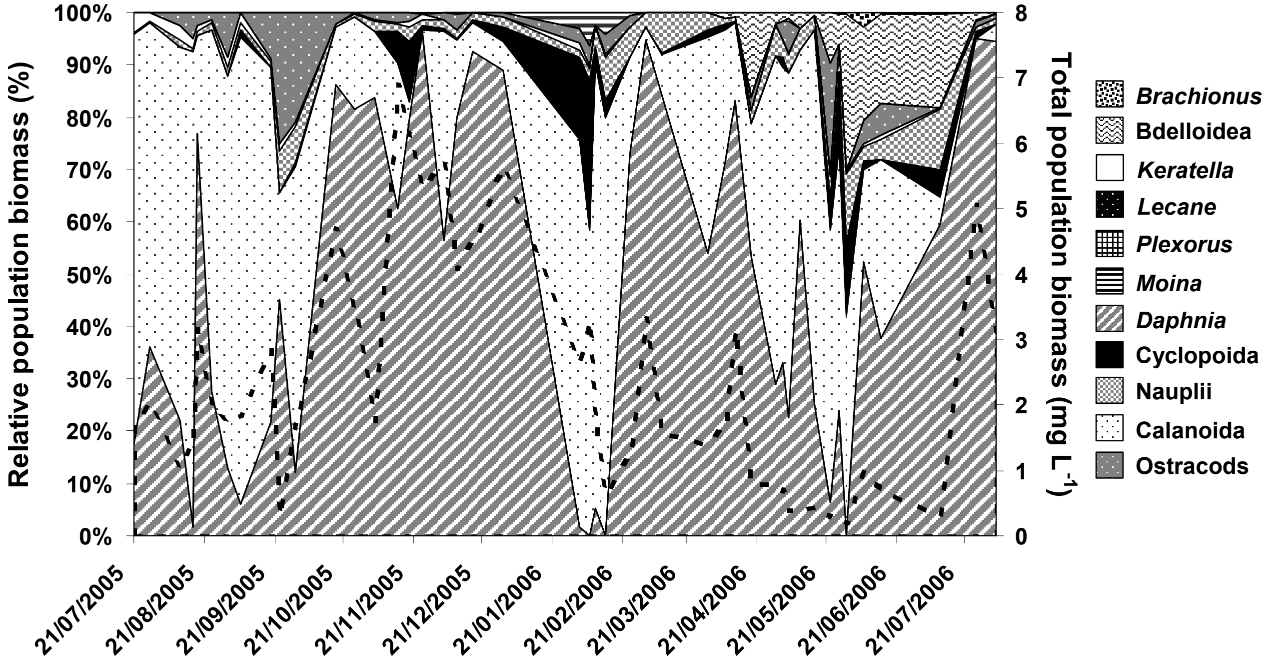

Figure 5.35. Open Pond 1 zooplankton biomass dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal biomass of the dominant genera (left *y*-axis) as well as total zooplankton biomass (mg L^{-1}; broken line; right *y*-axis).

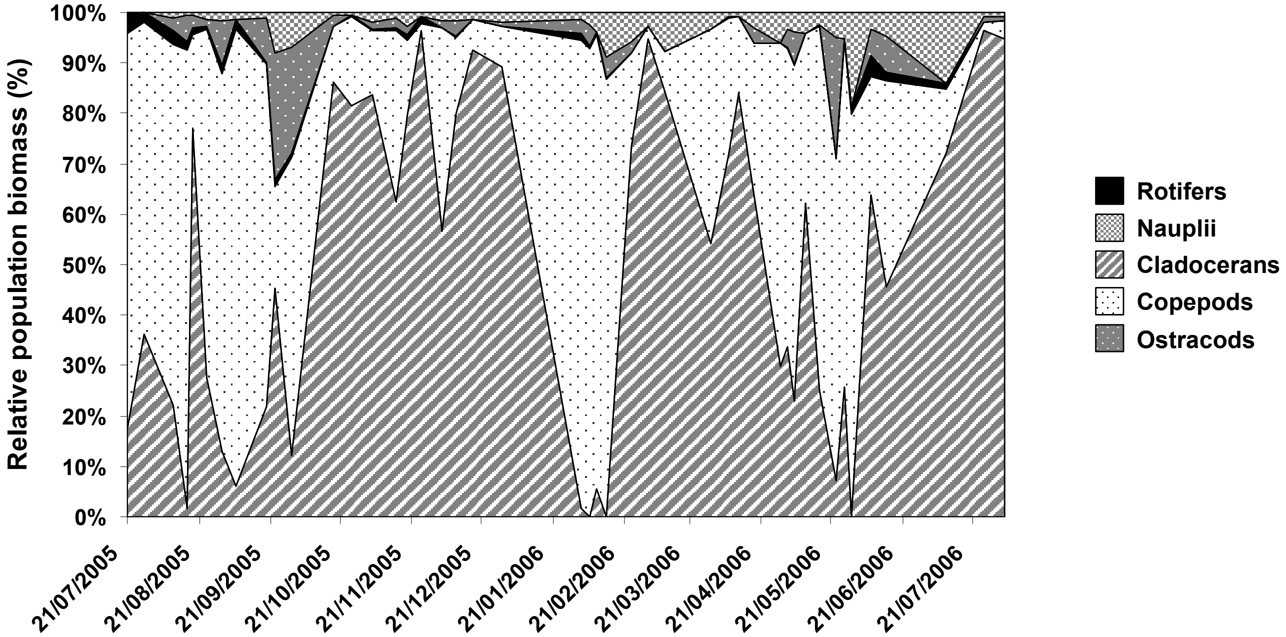

Figure 5.36. Open Pond 1 zooplankton biomass dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal biomass of the dominant zooplankton groups.

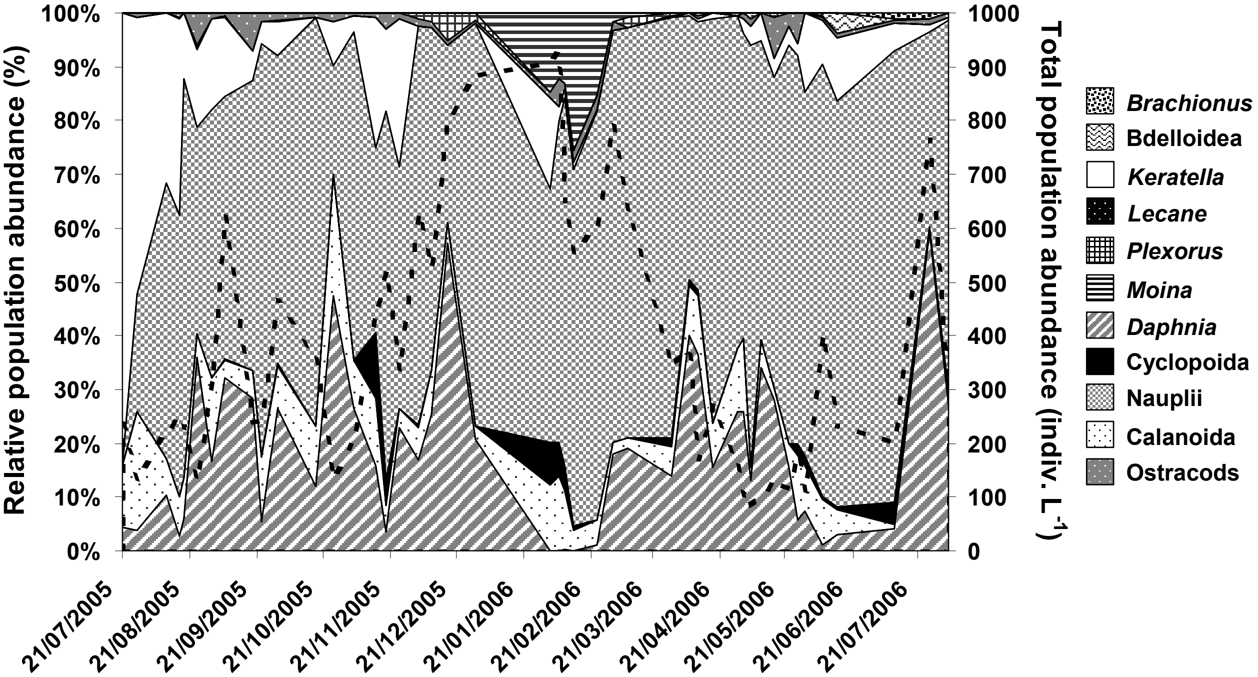

Figure 5.37. Open Pond 3 zooplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total zooplankton abundance (individuals L^{-1}; broken line; right *y*-axis).

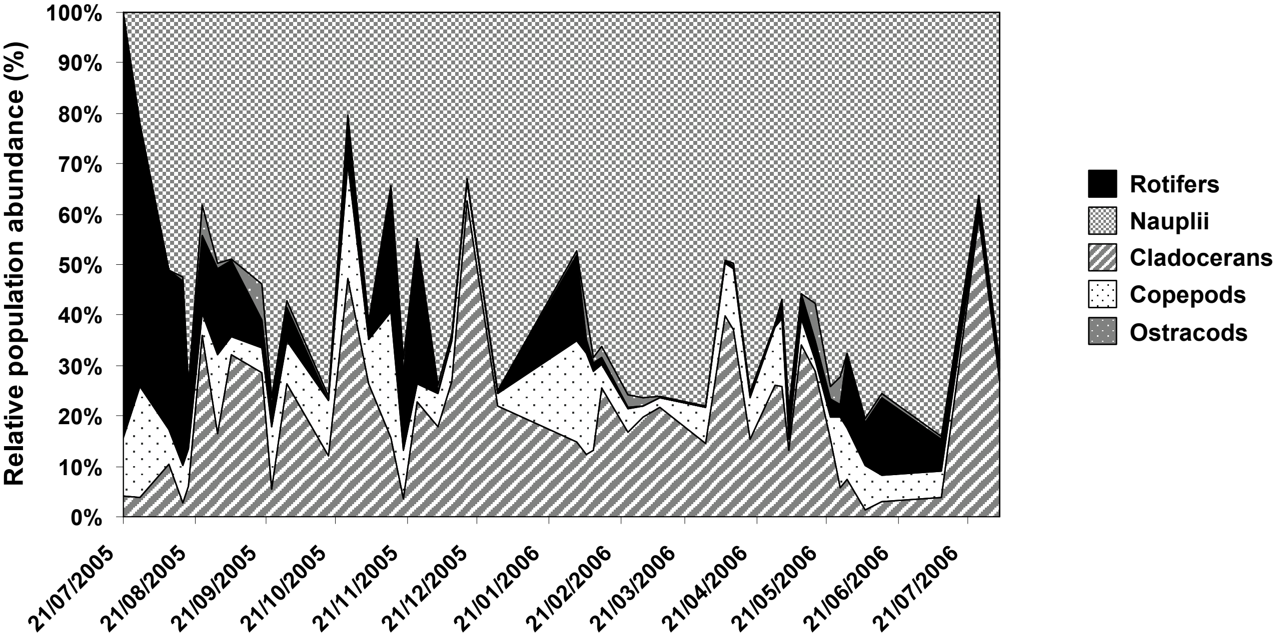

Figure 5.38. Open Pond 3 zooplankton population dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal abundance of the dominant zooplankton groups.

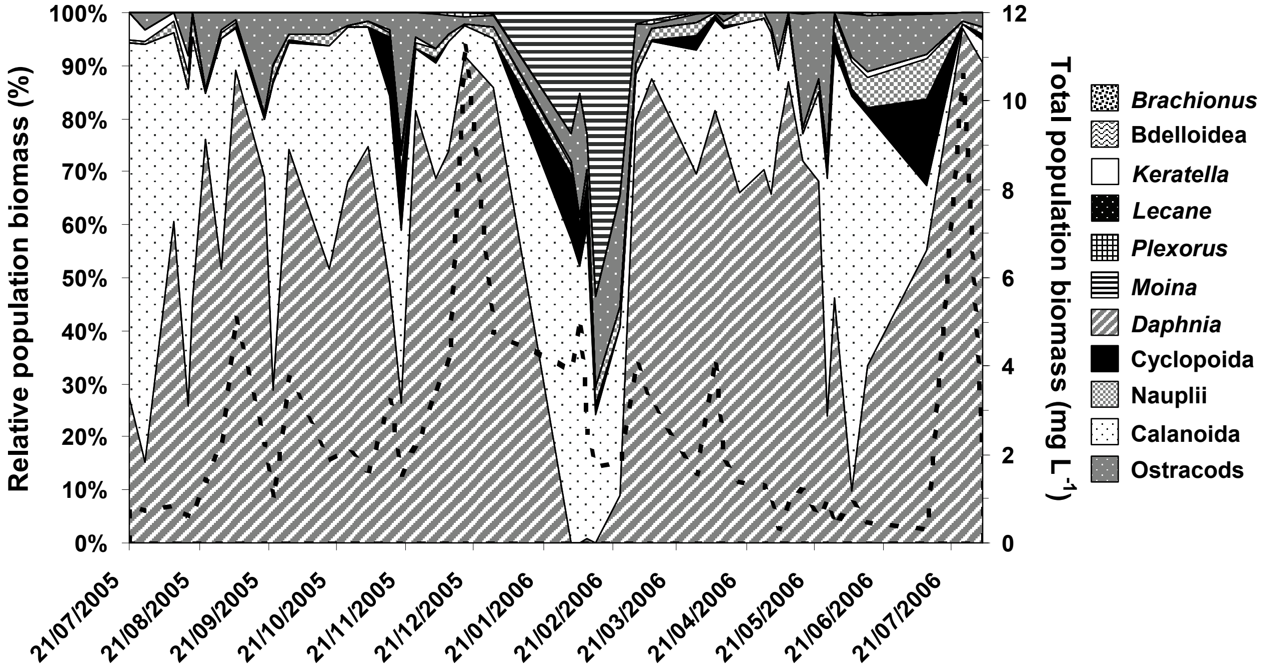

Figure 5.39. Open Pond 3 zooplankton biomass dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal biomass of the dominant genera (left *y*-axis) as well as total zooplankton biomass (mg L^{-1}; broken line; right *y*-axis).

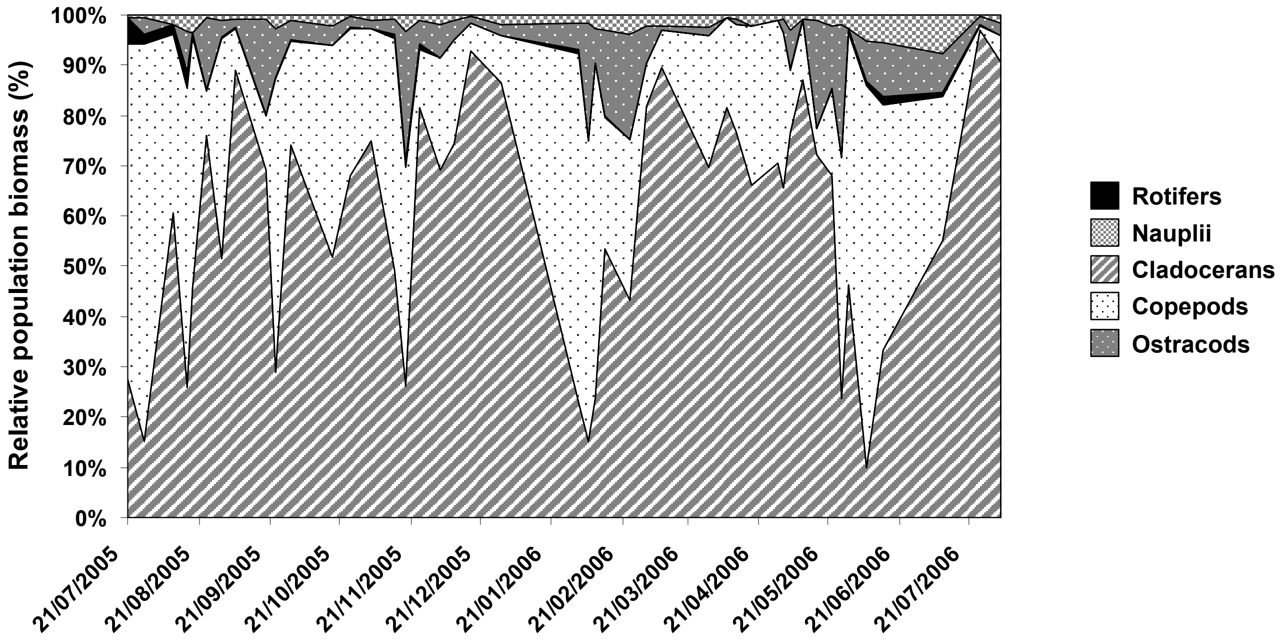

Figure 5.40. Open Pond 3 zooplankton biomass dynamics during the 2005 *Period 1* and 2006 *Period 2* showing relative percentage temporal biomass of the dominant zooplankton groups.

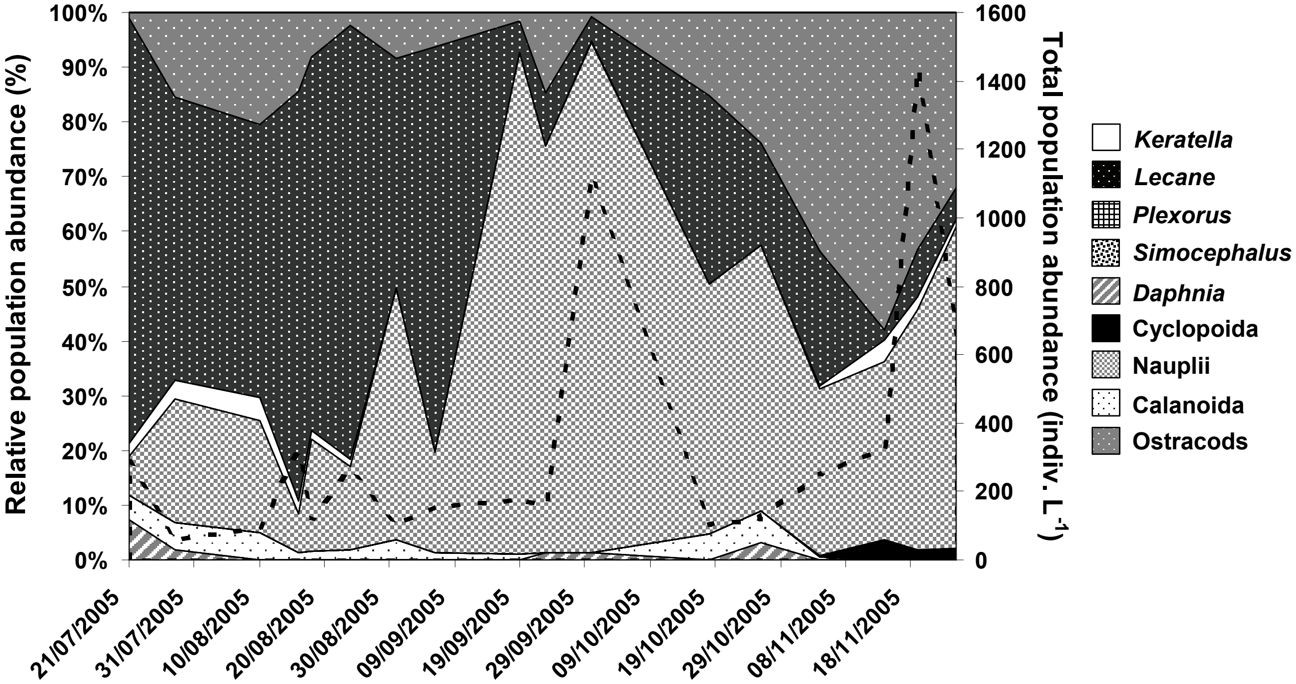

Figure 5.41. Duckweed Pond 1 zooplankton population dynamics during the 2005 monitoring *Period 1* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total zooplankton abundance (individuals L^{-1}; broken line; right *y*-axis).

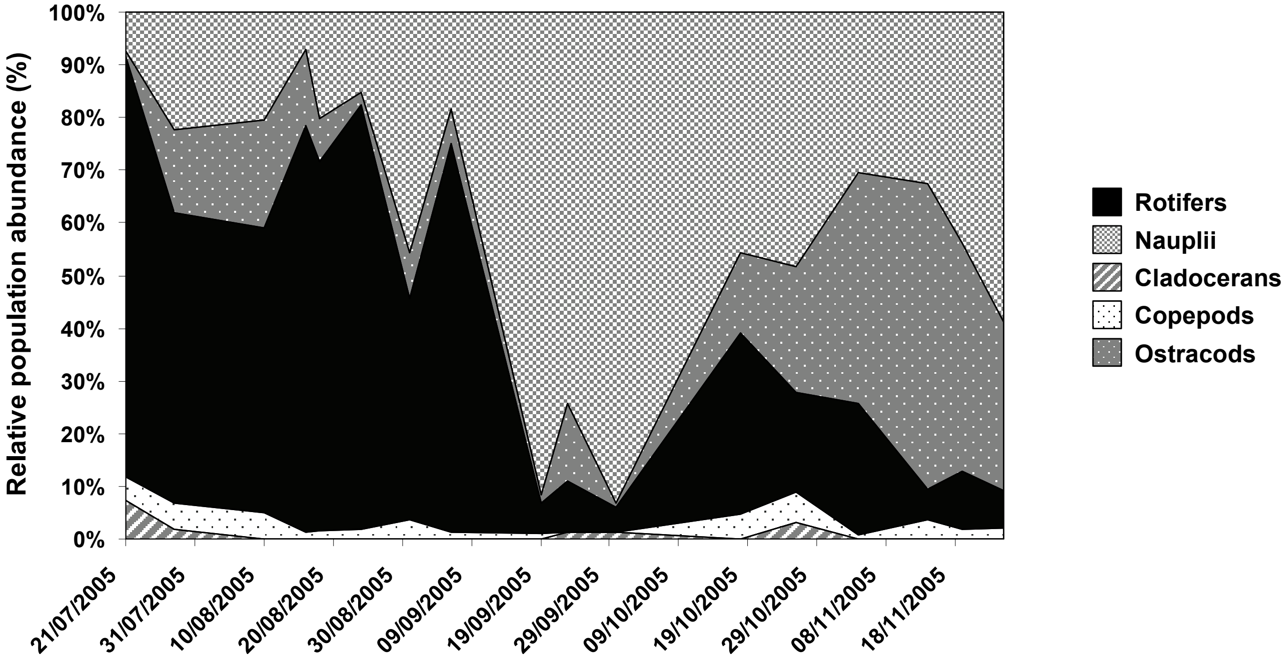

Figure 5.42. Duckweed Pond 1 zooplankton population dynamics during the 2005 monitoring *Period 1* showing relative percentage temporal abundance of the dominant zooplankton groups.

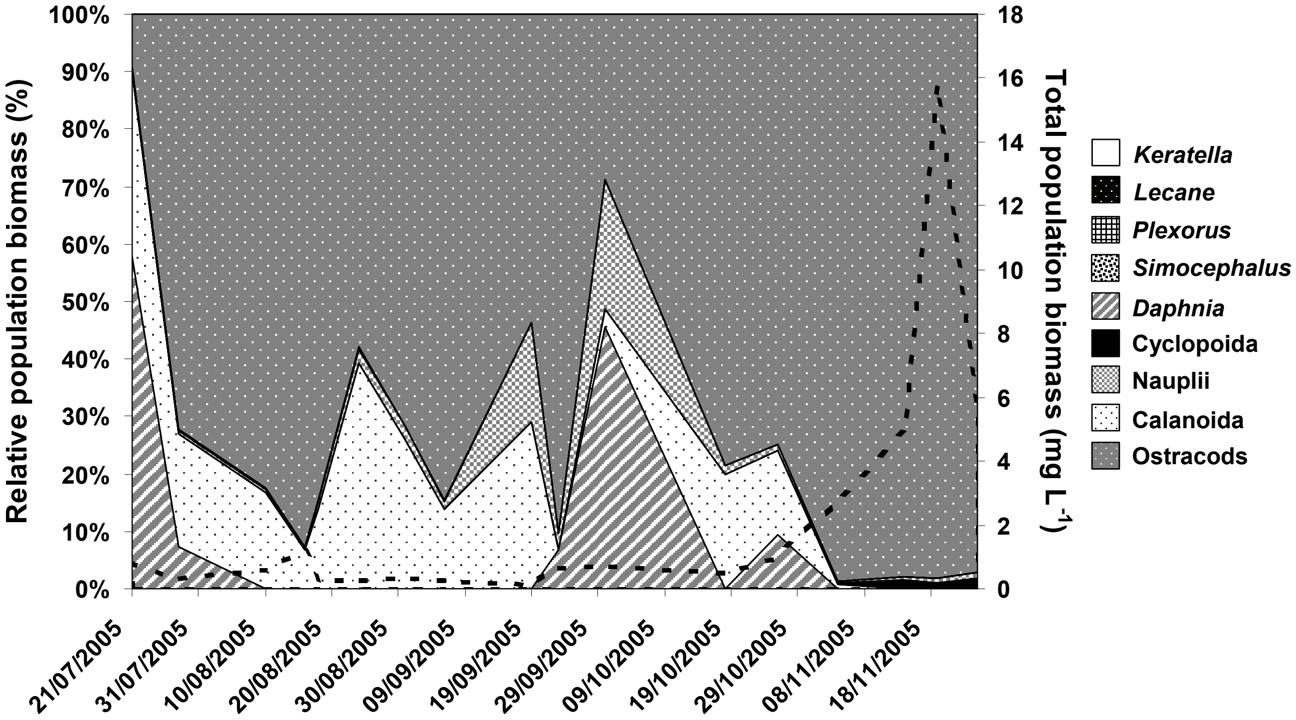

Figure 5.43. Duckweed Pond 1 zooplankton biomass dynamics during the 2005 monitoring *Period 1* showing relative percentage temporal biomass of the dominant genera (left *y*-axis) as well as total zooplankton biomass (mg L^{-1}; broken line; right *y*-axis).

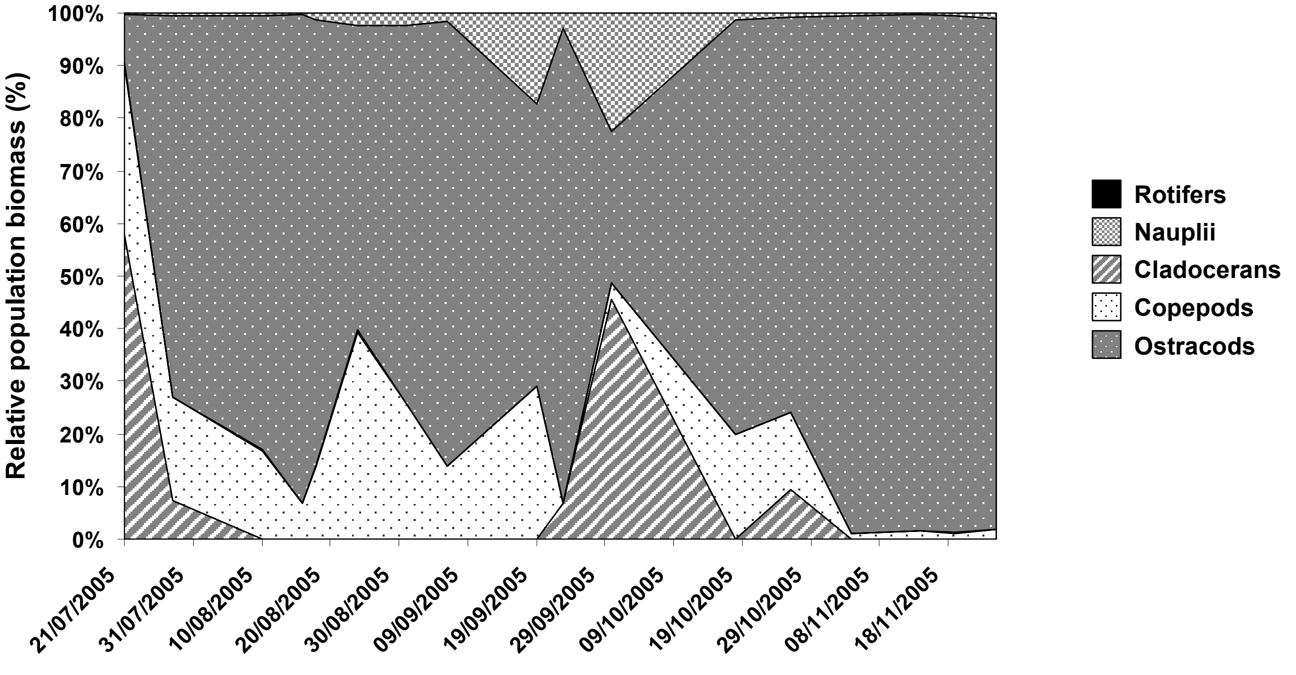

Figure 5.44. Duckweed Pond 1 zooplankton biomass dynamics during the 2005 monitoring *Period 1* showing relative percentage temporal biomass of the dominant zooplankton groups.

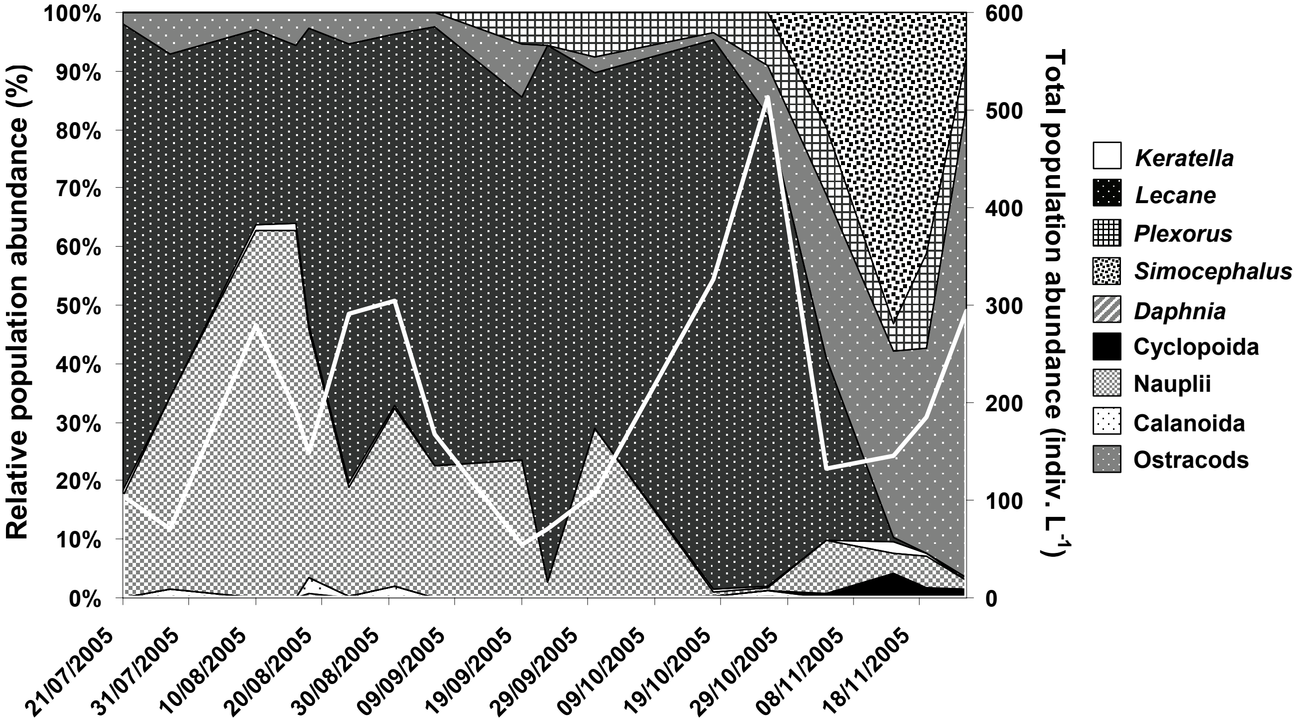

Figure 5.45. Duckweed Pond 3 zooplankton population dynamics during the 2005 monitoring *Period 1* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total zooplankton abundance (individuals L^{-1}; solid white line; right *y*-axis).

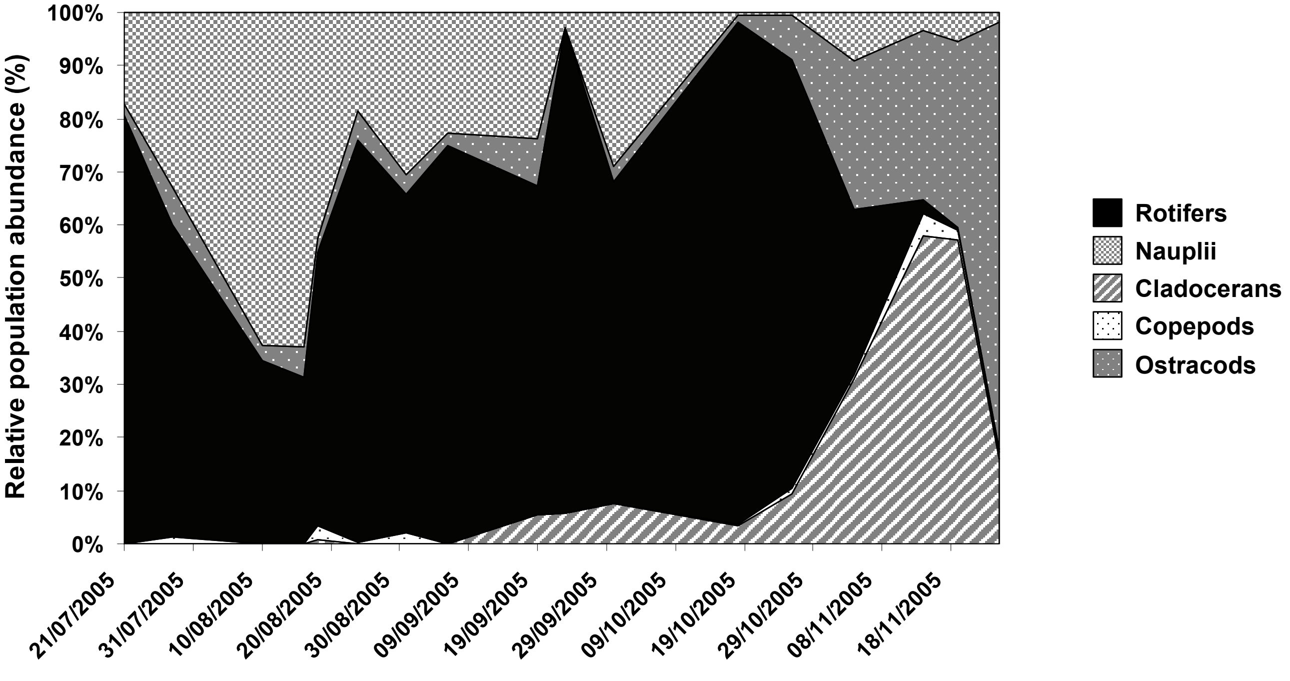

Figure 5.46. Duckweed Pond 3 zooplankton population dynamics during the 2005 monitoring *Period 1* showing relative percentage temporal abundance of the dominant zooplankton groups.

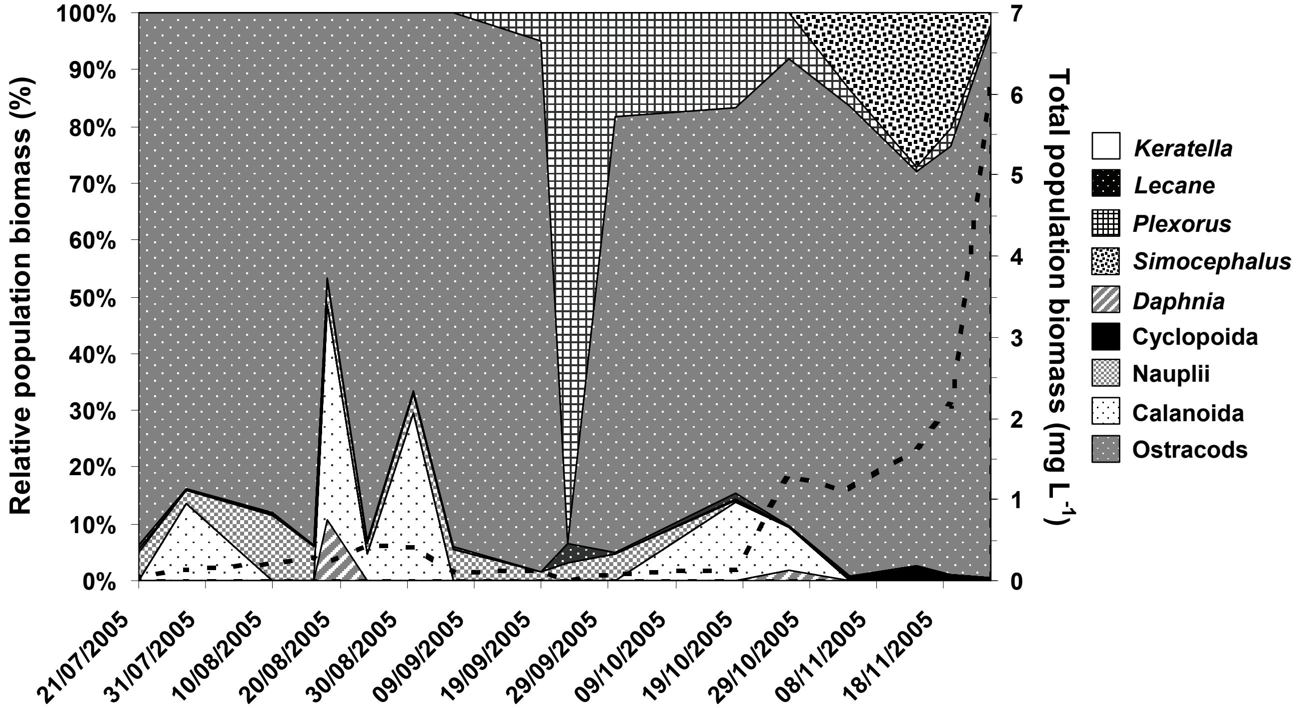

Figure 5.47. Duckweed Pond 3 zooplankton biomass dynamics during the 2005 monitoring *Period 1* showing relative percentage temporal biomass of the dominant genera (left *y*-axis) as well as total zooplankton biomass (mg L^{-1}; broken line; right *y*-axis).

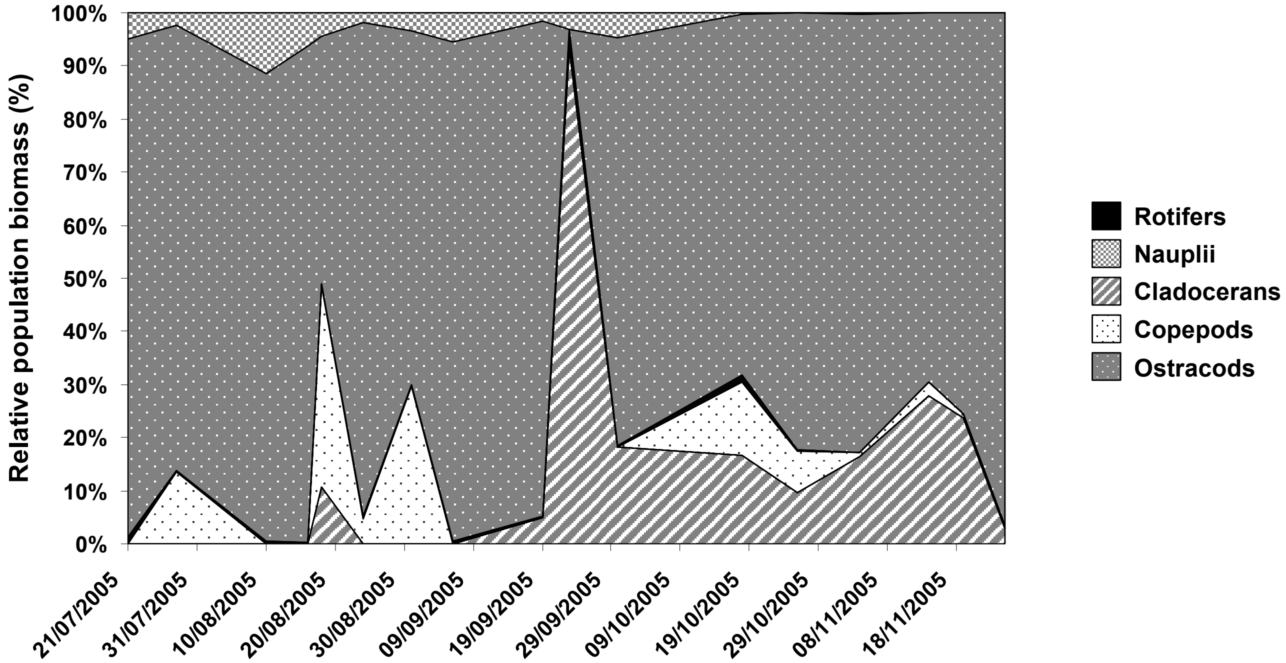

Figure 5.48. Duckweed Pond 3 zooplankton biomass dynamics during the 2005 monitoring *Period 1* showing relative percentage temporal biomass of the dominant zooplankton groups.

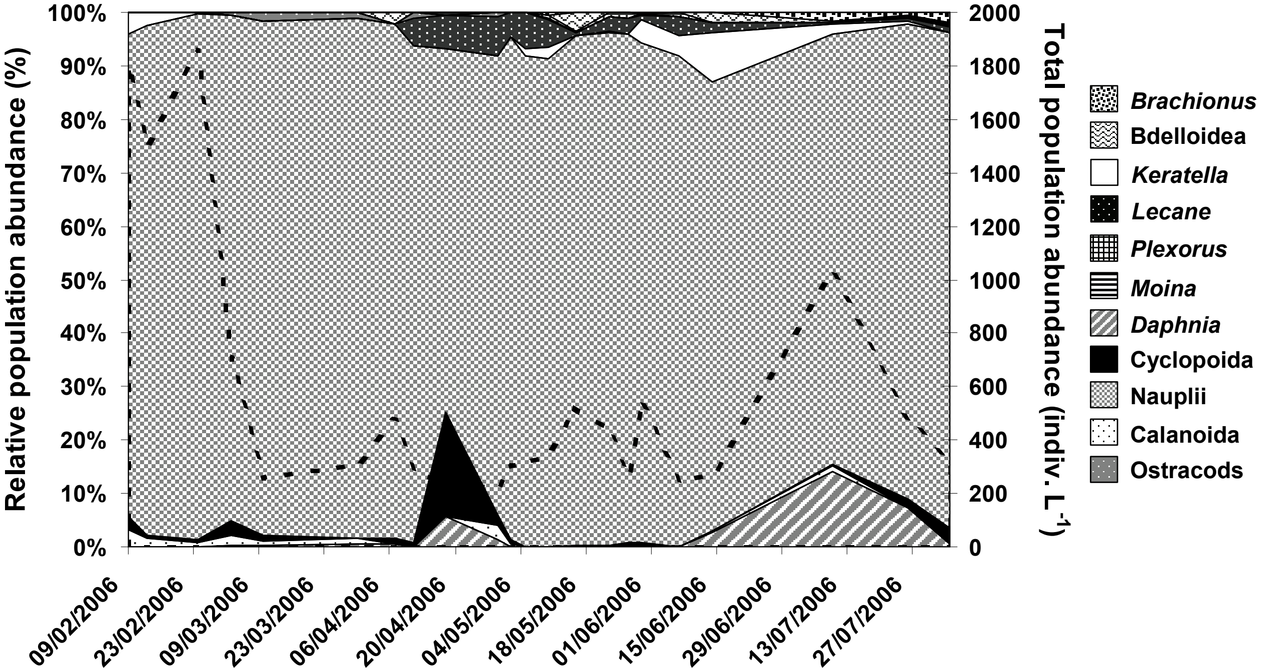

Figure 5.49. Attached-growth media Reactor 1 zooplankton population dynamics during the 2006 monitoring *Period 2* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total zooplankton abundance (individuals L^{-1}; broken line; right *y*-axis).

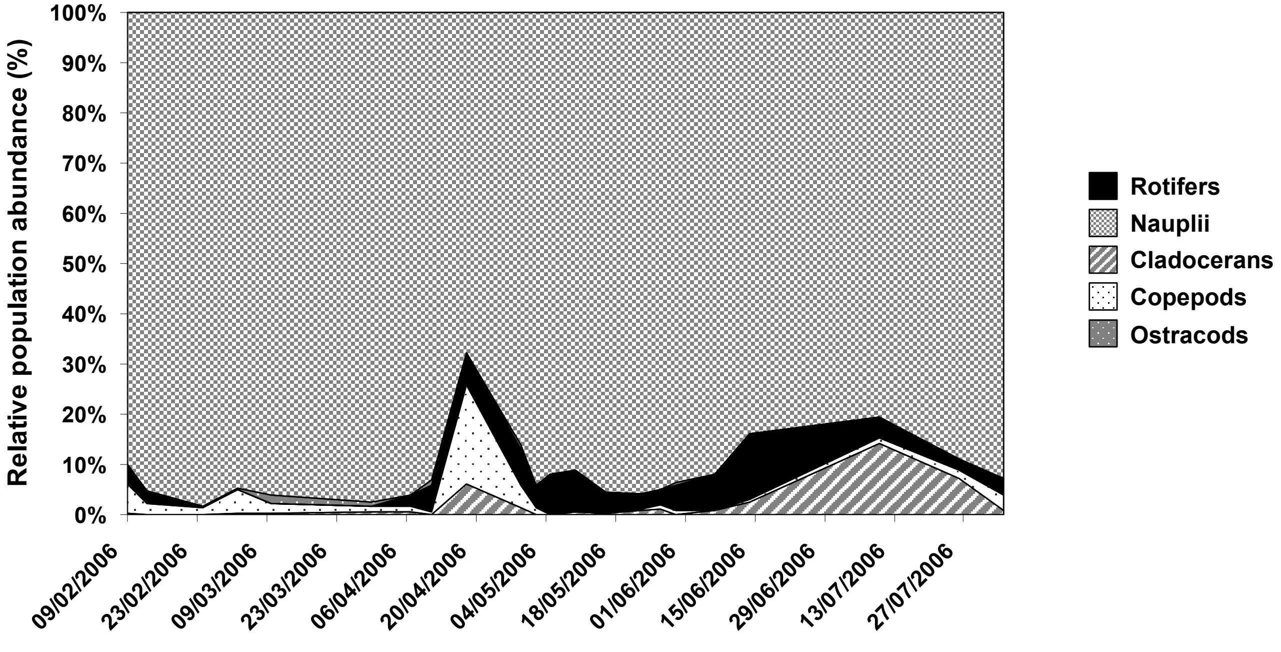

Figure 5.50. Attached-growth media Reactor 1 zooplankton population dynamics during the 2006 *Period 2* showing relative percentage temporal abundance of the dominant zooplankton groups.

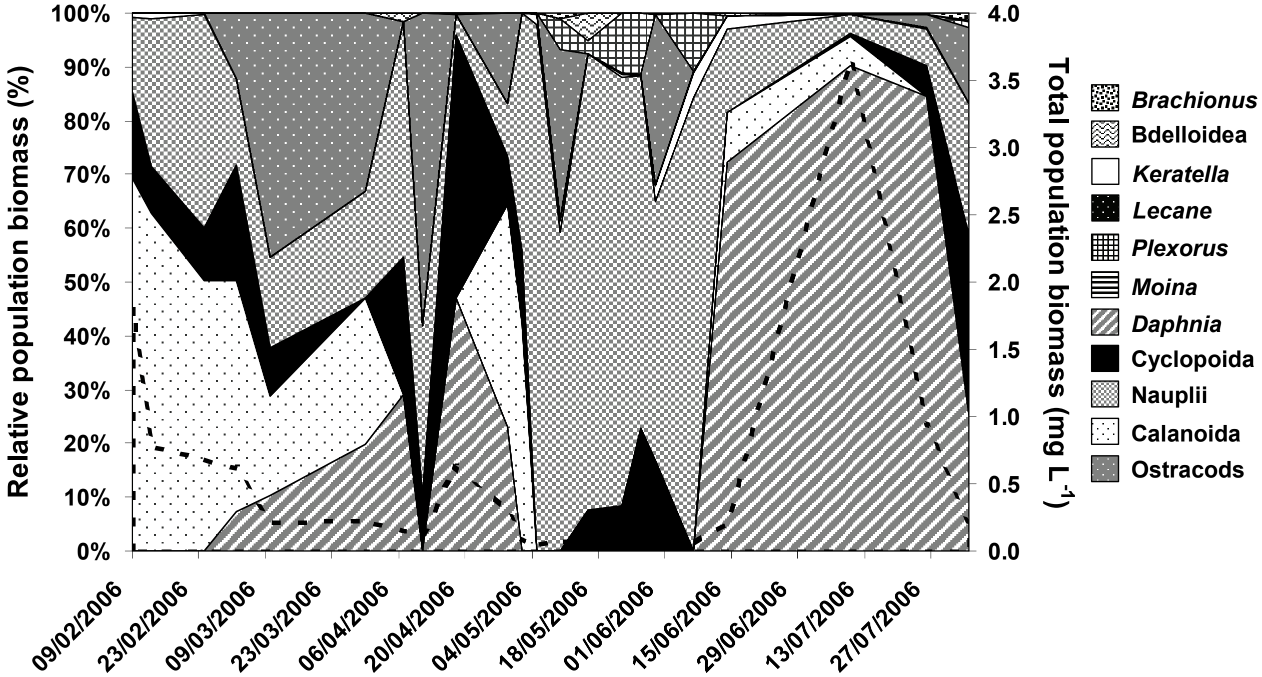

Figure 5.51. Attached-growth media Reactor 1 zooplankton biomass dynamics during the 2006 monitoring *Period 2* showing relative percentage temporal biomass of the dominant genera (left *y*-axis) as well as total zooplankton biomass (mg L^{-1}; broken line; right *y*-axis).

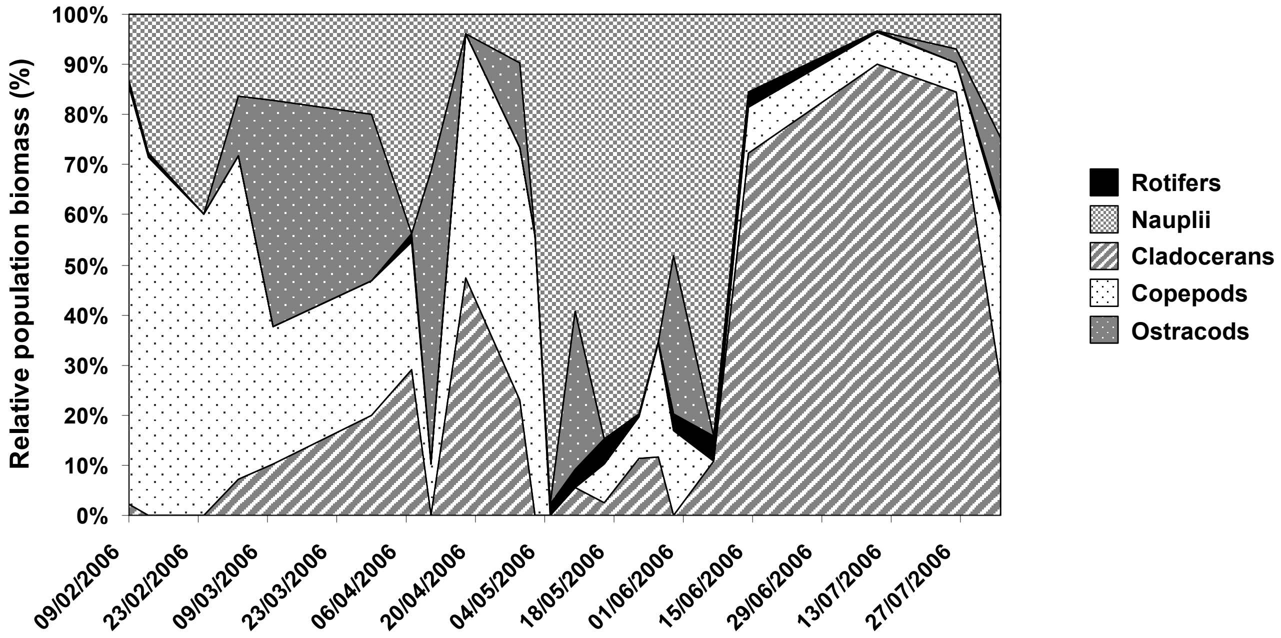

Figure 5.52. Attached-growth media Reactor 1 zooplankton biomass dynamics during the 2006 monitoring *Period 2* showing relative percentage temporal biomass of the dominant zooplankton groups.

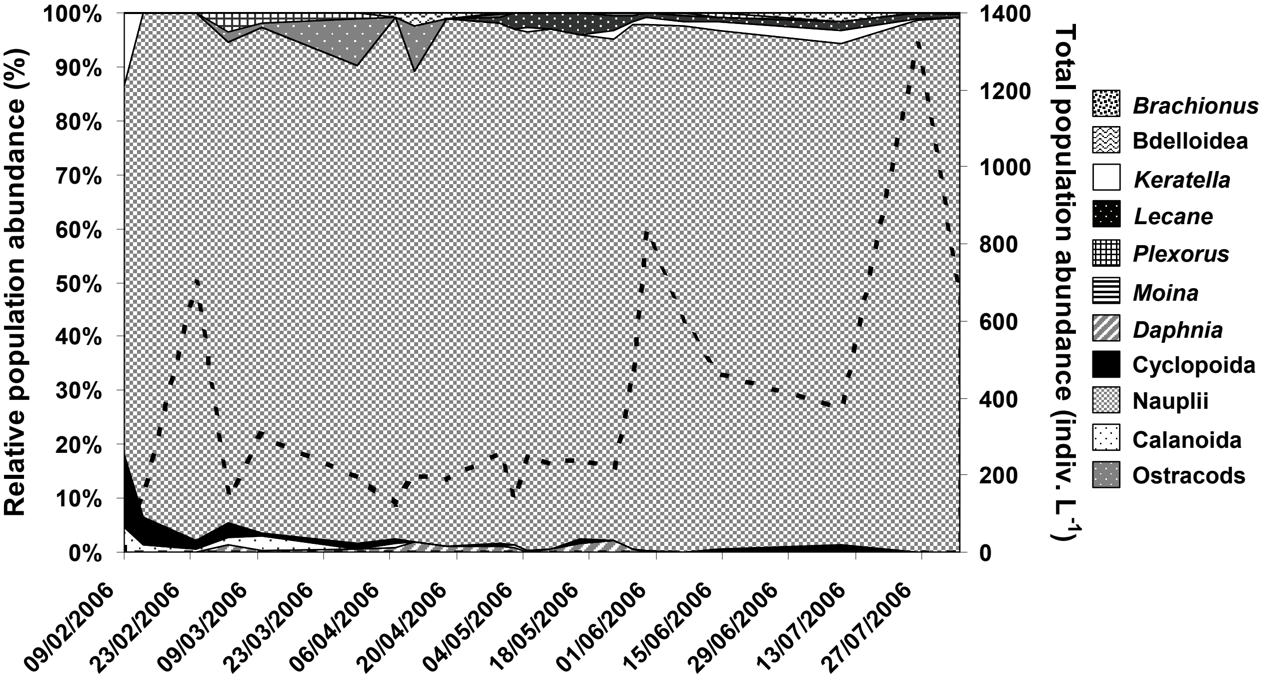

Figure 5.53. Attached-growth media Reactor 3 zooplankton population dynamics during the 2006 monitoring *Period 2* showing relative percentage temporal abundance of the dominant genera (left *y*-axis) as well as total zooplankton abundance (individuals L^{-1}; broken line; right *y*-axis)

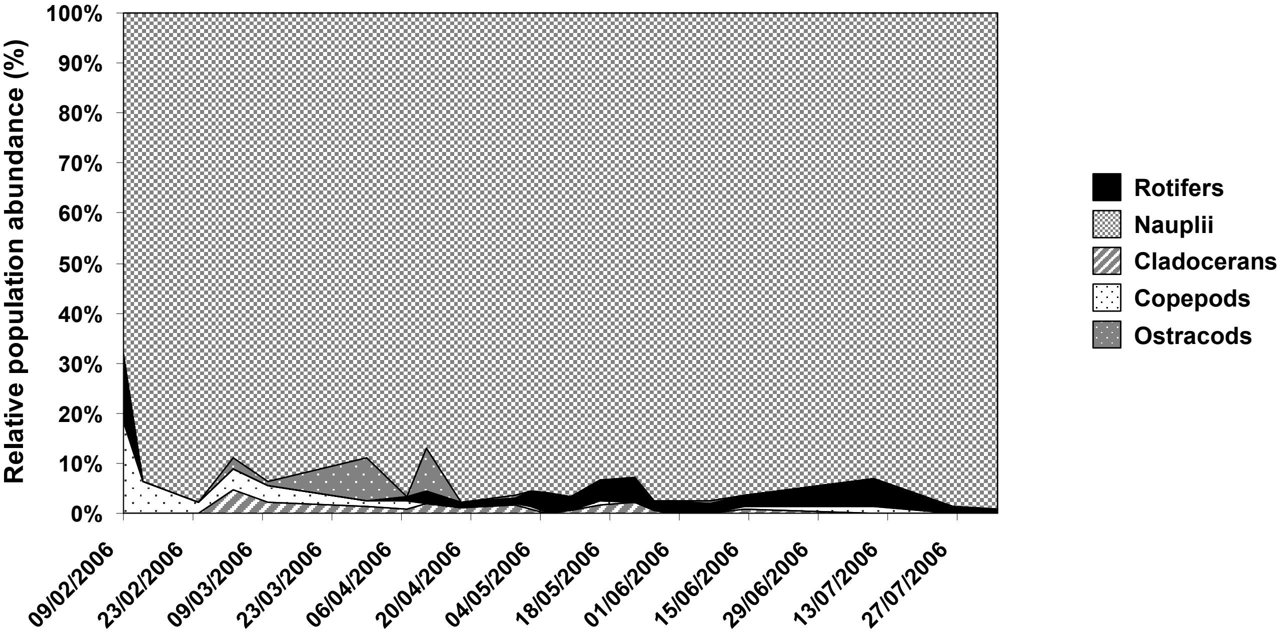

Figure 5.54. Attached-growth media Reactor 3 zooplankton population dynamics during the 2006 *Period 2* showing relative percentage temporal abundance of the dominant zooplankton groups.

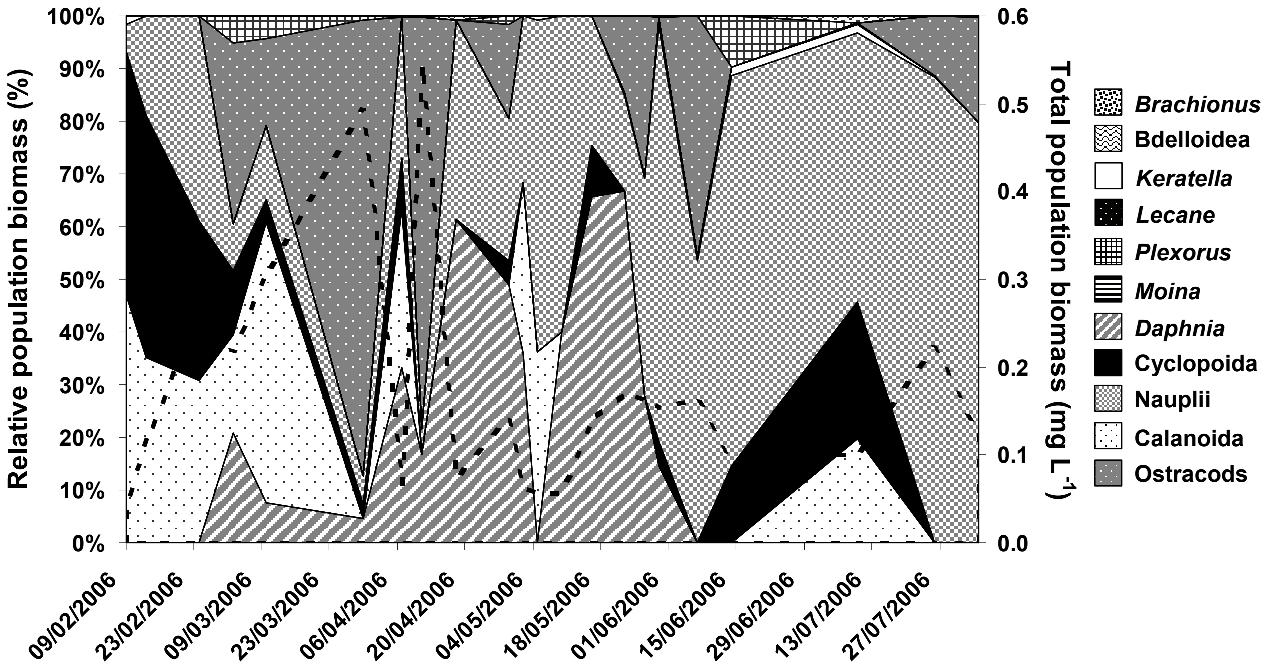

Figure 5.55. Attached-growth media Reactor 3 zooplankton biomass dynamics during the 2006 monitoring *Period 2* showing relative percentage temporal biomass of the dominant genera (left *y*-axis) as well as total zooplankton biomass (mg L^{-1}; broken line; right *y*-axis).

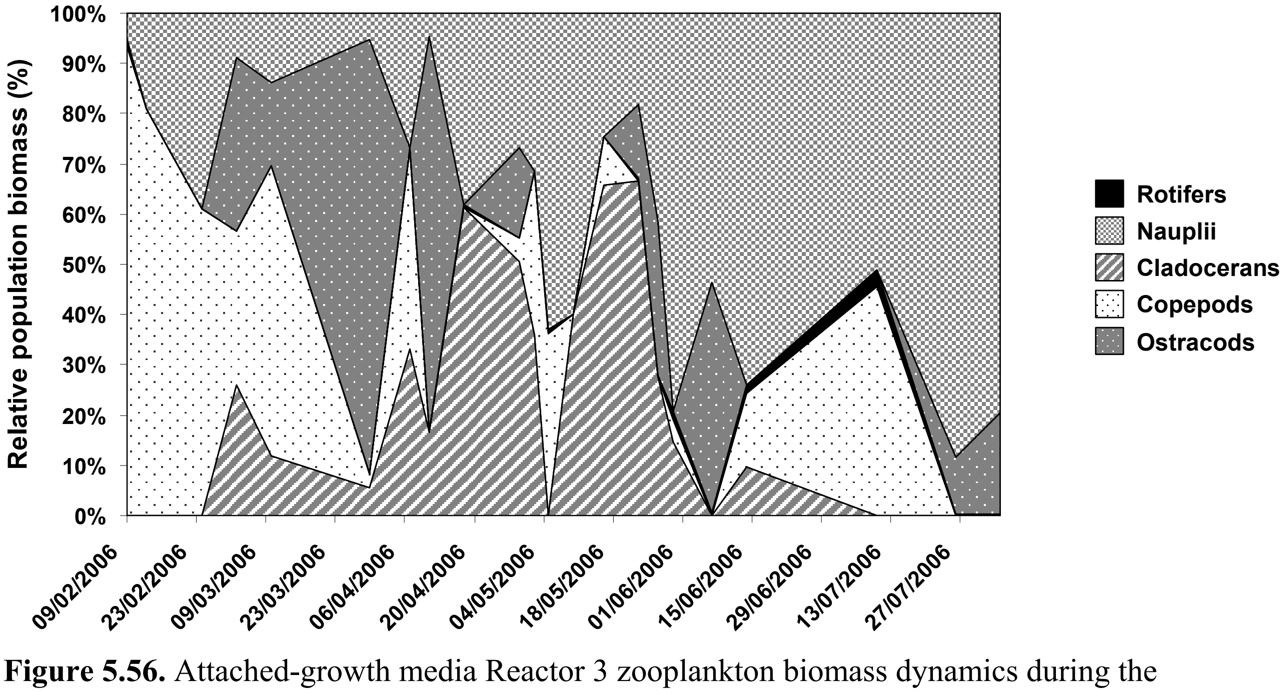

Figure 5.56. Attached-growth media Reactor 3 zooplankton biomass dynamics during the 2006 monitoring *Period 2* showing relative percentage temporal biomass of the four zooplankton groups.

Similar to the phytoplankton data of Section 5.3.1 above, there has so far been no prior research effort into the zooplankton ecology of WSP effluent following rock filtration. To the author's knowledge, there have been just two references to zooplankton *per se* in publications also reporting on rock filtration for the upgrading of WSP effluent (Hirsekorn, 1974; Tanner *et al.*, 2005); although in both cases the reports of zooplankton populations were strictly observational and taxonomically nonspecific. The current work therefore represents the first reported attempt to quantitatively assess the zooplankton population ecology of a WSP effluent following rock filtration. Average zooplankton density within the Rock Filters during 2005–2006 monitoring was increased slightly compared with the combined 2005–2006 influent wastewater of the same period (Figure 5.57); although only significantly for RF-1 (1-way ANOVA; $F_{(8,296)} = 6.252$; $p < 0.001$). Respective mean and median zooplankton densities were approximately 625 and 530 organisms L^{-1} for RF-1 and 510 and 380 organisms L^{-1} for RF-3.

Despite total zooplankton population abundance increasing somewhat within the Rock Filters compared with influent numbers, total zooplankton biomass (mg L^{-1}) actually decreased within the RF series compared with influent biomass (Figure 5.58); although this time only significantly for RF-3 (1-way ANOVA; $F_{(8,296)} = 11.244$; $p = 0.006$). The Rock Filters also produced a final effluent with significantly lower levels of zooplankton biomass than the parallel Open Ponds, with RF-3 effluent containing on average significantly less biomass that OP-3 ($p < 0.001$). Respective mean and median zooplankton biomass levels during 2005–2006 were in the order of 2.89 and 0.78mg L^{-1} for RF-1 and 0.66 and 0.35mg L^{-1} for RF-3. This increase in total abundance and decline in total biomass was a result of RF populations being predominated by smaller zooplankton—namely copepod nauplii and rotifers—which represented on average ≈85% of total population abundance and just 18% of the total zooplankton biomass for Rock Filters 1 and 3 (see Figures 5.25, 5.27 and 5.29, 5.31).

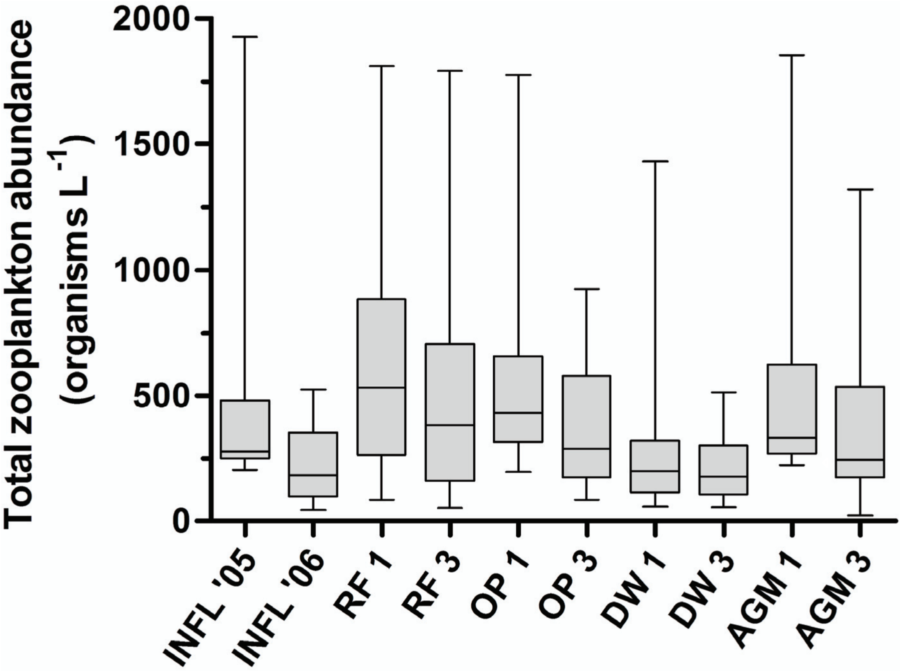

Figure 5.57. Box-plot showing zooplankton abundance data for: 2005 (INFL '05) and 2006 (INFL '06) pilot plant Influent; Rock Filters 1 and 3 (RF-1; RF-3); Open Ponds 1 and 3 (OP-1; OP-3); Duckweed Ponds 1 and 3 (DW-1; DW-3); and Attached-Growth Media Reactors 1 and 3 (AGM-1; AGM-3). Data sourced from the entire pilot plant monitoring period from July 2005–August 2006.

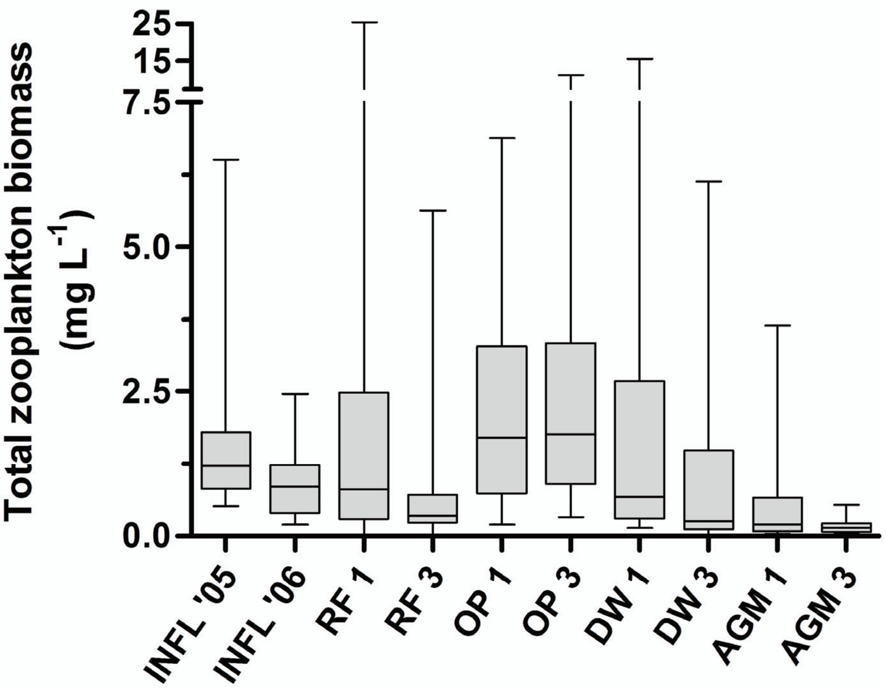

Figure 5.58. Box-plot showing zooplankton biomass data for: 2005 (INFL '05) and 2006 (INFL '06) pilot plant Influent; Rock Filters 1 and 3 (RF-1; RF-3); Open Ponds 1 and 3 (OP-1; OP-3); Duckweed Ponds 1 and 3 (DW-1; DW-3); and Attached-Growth Media Reactors 1 and 3 (AGM-1; AGM-3). Data sourced from the entire pilot plant monitoring period from July 2005–August 2006.

Overall, the zooplankton population within the RF series was represented most strongly—in terms of community biomass—by large-bodied zooplankton including cladoceran *Daphnia*, calanoid (*Boeckella*) and cyclopoid (*Mesocyclops*) copepods and ostracods (*Bennelongia* species); with these four genera comprising on average 84 and 76% of the total population biomass in Rock Filters 1 and 3 respectively. Rotifers, although highly abundant at times (maximum of 780 individuals L^{-1} in RF-1 during August 2005), were poorly represented in terms of their relative biomass contributions, with combined average rotifer biomass for Rock Filters 1 and 3 less than 1% of the total daily value. Relative to influent levels, there appeared to be an increase in the biomass of copepods and a decline in the dominance of *Daphnia* species within the RF series; with *Daphnia* and copepods constituting 48 and 34% of the respective influent biomass and 13 and 53% of the respective RF series biomass. This increase shift in community dominance from *Daphnia* to copepods has elsewhere been linked to decreased DO concentration (Hrbáček *et al.*, 1994) and so it is possible that reduced DO levels also contributed to the same observation during the current work.

There were several population peaks in zooplankton abundance within the RF series during 2005–2006, the most notable of which occurred during August of 2005 when the calanoid copepod *B. triarticulata* reached very high densities in RF-1 (>1000 individuals L^{-1}), corresponding to equally high RF-1 zooplankton biomass values in excess of 23mg L^{-1}. It should be noted that such high levels of total SS were not recorded from RF-1 effluent samples during the same period, and whilst there were indeed very high densities of zooplankton within the Rock Filter effluent mixing chambers themselves (see Plate 2.8), such high numbers of zooplankton were never observed in the corresponding daily effluent manifold grab samples. This was predominantly due to the rapid swimming velocities of larger zooplankton like copepods and *Daphnia* and their active avoidance of effluent manifold outflow streams (see Section 3.3.6 for initial discussion).

As described in Section 2.2.1, periodic full-depth water column samples could not be taken for regular *in situ* water quality analyses due to physical obstruction of the standard 40mm diameter column sampler by a network of PVC supports within the mixing chambers themselves. Water column samples were, however, collected for zooplankton analyses only using a separate narrow bore (20mm) column sampler following the recognition of the abovementioned problem and also following the frequent observation of greater zooplankton densities close to the water surface (< 20cm deep). This so-called ‘patchiness’ in zooplankton populations is a commonly reported phenomenon, whereby the spatial distribution of zooplankton within a given environment is not always uniform across a given vertical and/or horizontal plane. Some zooplankton, such as *Daphnia* for example, are known to commonly existing in aggregated swarms rather than as dispersed individuals (Uhlmann, 1980; Young *et al*., 1994); with swarming behaviour previously reported for *Daphnia* in WSP environments (Mitchell and Williams, 1982a; Cauchie *et al.*, 2002). As described in Section 2.2.1, steps were taken to minimize the confounding effects of patchy zooplankton distribution within the other three pilot treatment series; however, problems associated with non-uniform distribution of zooplankton populations within the Rock Filter mixing chambers in particular were difficult to control and so remained unconsolidated.

It should also be stated—with respect to the above issue—that the design of the current Rock Filter units was far more conducive to zooplankton proliferation than an unbroken ‘continuous-bed’ rock filter would be expected to be *in situ*. The uncovered mixing chambers that allowed significant populations of zooplankton to develop during the course of this research would not exist within a full-scale installation; such that zooplankton abundance and biomass would be expected to be significantly reduced compared with the values reported here. Whilst the presence of these open-air rock-free mixing chambers were not in line with the classical rock filter design, results from this work have shown that they can promote the development of sometimes dense zooplankton populations; populations that could potentially contribute to overall treatment efficiency as introduced earlier (see Section 5.1). It is suggested then, that future work could investigate the use of rock filters in both the traditional ‘continuous-bed’ and also the non-continuous ‘broken-bed’ style arrangements (as reported here) in order to assess the potential treatment benefits of the two configurations.

There was another particularly notable trend for declining dominance of both *Daphnia* and copepods—relative to the influent wastewater and also down the RF series (compare Figures 5.23, 5.27 and 5.31)—in place of an increased community representation by ostracods; with *Bennelongia* species constituting 45% of the average zooplankton biomass in RF-3 effluent. Not only did the relative community dominance of ostracods increase following Rock Filter treatment, but the absolute ostracod biomass also increased relative to influent levels; with this figure increasing from a mean of 164μg L^{-1} in the influent, to 236μg L^{-1} in RF-3. This showed that ostracods were not only surviving Rock Filter passage, but that they were also actively proliferating within the seemingly adverse conditions. The increased dominance of ostracods within the Rock Filter train was considered to have been a reflection of both the reduced algal abundance down the RF series (see Figure 5.3) and also the recognition that ostracods are omnivorous substrate feeders. Pennak (1953) for example reported that ostracods are generally omnivorous scavengers, with diets consisting mostly of bacteria, molds, algae and fine detritus. Therefore, it was likely that the reduced availability of traditional algal-based food resources within the Rock Filters could have favoured omnivorous ostracod species.

Furthermore, and during dark conditions or in the absence of suspended algae (conditions very similar to those within the Rock Filters), ostracods commonly undertake deposit feeding in sediments or browse on benthic biofilms or epiphytic growths (Grant *et al.*, 1983); with Grant *et al* (1983) reporting that dark vs. light feeding rates of the freshwater ostracod *Cyprinotus carolinensis* were not significantly different. A similar trend was noted by De Deckker (1983) for Australian ostracods in particular, with some genera most commonly found swimming near the bottom of water bodies. In addition to their capacity for omnivorous substrate grazing, some freshwater ostracods have also been shown to be very tolerant to conditions of hypoxia, with the oxygen concentration tolerance limit for the freshwater ostracod *Cytherissa lacustris* found to be less than 1mg O_2 L^{-1} at 10ºC over a period of 20 hours exposure (Newrkla, 1985). Therefore, the ability of ostracods to scavenge from substrate surfaces combined with their likely capacity for survival under hypoxic conditions were thought to have contributed to the observed increase in community dominance of *Bennelongia* species within the Rock Filter treatment series.

In passing, it should also be noted here that there were other freshwater invertebrates present that would have also contributed to enhanced treatment activity within the Rock

Filters. Freshwater snails for example are known to contribute significantly to the grazing and recycling of benthic materials and substrate-attached periphyton (Sigee, 2005), with their diet consisting of 50–90% detritus and <25% algae (Brönmark, 1989). Small snails (≈5mm) were omnipresent across all treatments during the entire 12 month pilot plant operational duration, and were observed to graze heavily on the walls of the polyethylene ponds—especially in the more exposed OP series. Partial Rock Filter disassembly also revealed high-density (estimated to be in the order of hundreds per m^2) snail populations within the filter body (Plate 5.1) and it is though that these macro-invertebrates would have contributed to overall RF (and probably also AGM) treatment performance and the internal recycling of accumulated particulate BOD_5 and SS.

In addition to their direct role in the processing of settled organics, benthic invertebrates such as snails and ostracods could have also contributed to the general maintenance of aerobic conditions within the upper layers of accumulated sediment. As discussed by Naméche *et al*. (1997), DO penetration into the sediment of WSPs is commonly restricted to the upper 1–2mm, with conditions below this upper aerobic zone markedly more reducing. Naméche and co workers also discussed how benthic invertebrates can assist in maintaining better mixing and oxygen availability in upper sediment layers through their physical activities; an effect collectively known as 'bioturbation'. Bioturbation activity also effectively increases the surface area of the sediment–water interface for normal exchange processes, as well as greatly enhancing the mobilization of sediment nutrients and organics throughout the sediment stratum—thereby shortening the diffusive transport distances for these pollutants (Svensson *et al.*, 2001).

It is possible then that invertebrate communities may have further contributed to the overall treatment performance of both the RF and AGM upgrade systems through physical bioturbation of the settled materials. Whilst no attempts were made to quantitatively assess the dynamics and/or activities of higher invertebrate communities within any of the pilot upgrade systems, it is suggested that their role in the overall treatment performance of each system (particularly the high-surface-area RF and AGM reactors) remains of potential significance. Interestingly, and although the treatment mechanisms in rock filters have been identified as overwhelmingly 'physical' in nature (see Section 1.2.8.6.1), results presented in both the current Chapter as well as Chapter 3

(Section 3.3.7.1) suggest that biological treatment activities might, in some instances, play a more active role in effective rock filter operation than previously credited.

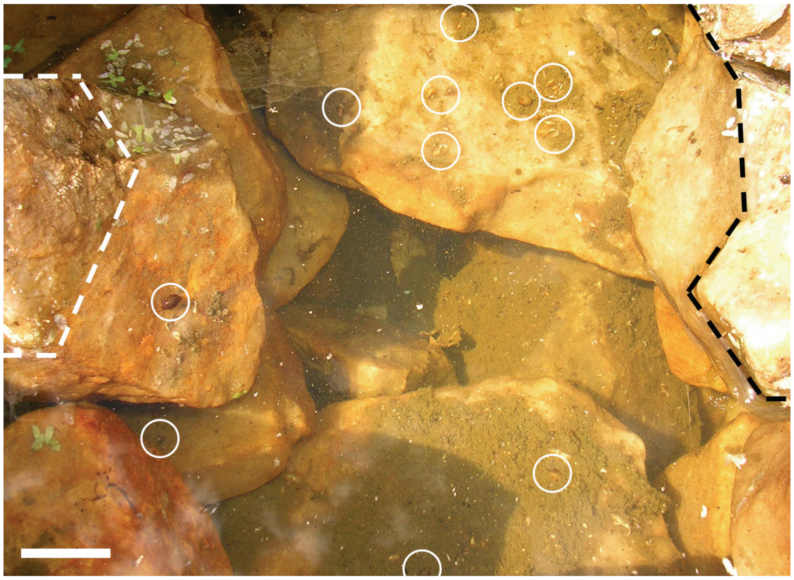

Plate 5.1. Detail of the internal rock media surfaces of RF-1 showing accumulations of flocculated detrital materials and a number of resident snails (circles). Broken lines indicate the water surface level. Scale bar (bottom–left) approximately 2cm in length.

In addition to a significant reduction in total population biomass, the relative biodiversity of zooplankton communities within the RF series was also significantly reduced compared with that of the influent wastewater (Figure 5.59); with the Shannon diversity indices (H') for RF-1 and RF-3 significantly lower than for the combined 2005–2006 pilot plant influent (Kruskal–Wallis test; $\chi^2_{0.05,7} = 74.76$; $p < 0.001$). This significant reduction in the magnitude of corresponding H' for Rock Filters 1 and 3 was also supported by the knowledge that just three zooplankton genera (*Boeckella*, *Mesocyclops* and *Bennelongia*) represented >93% of the total zooplankton biomass in RF-3 effluent; something that in turn was thought to have reflected the highly modified and largely unfavourable *in situ* Rock Filter conditions. To illustrate this point, darkened freshwater environments are generally recognized as being far less productive and biologically diverse that their illuminated counterparts (Janse and Van Puijenbroek, 1998; Daniel *et al.*, 2005). This inherent suppression of biological productivity and diversity is again reduced even further by de-oxygenation, such that 'dark–anoxic' environments are invariably less productive and less diverse than 'photic–oxic' ones (Detmer *et al.*, 1993; Wilk-Woźniak and Żurek, 2006). Following on from this and in conclusion, it would therefore be expected that rock filter effluents, in addition to having lower phytoplankton densities, would also have reduced levels of zooplankton biomass spread across fewer taxa than would a conventional untreated WSP effluent.

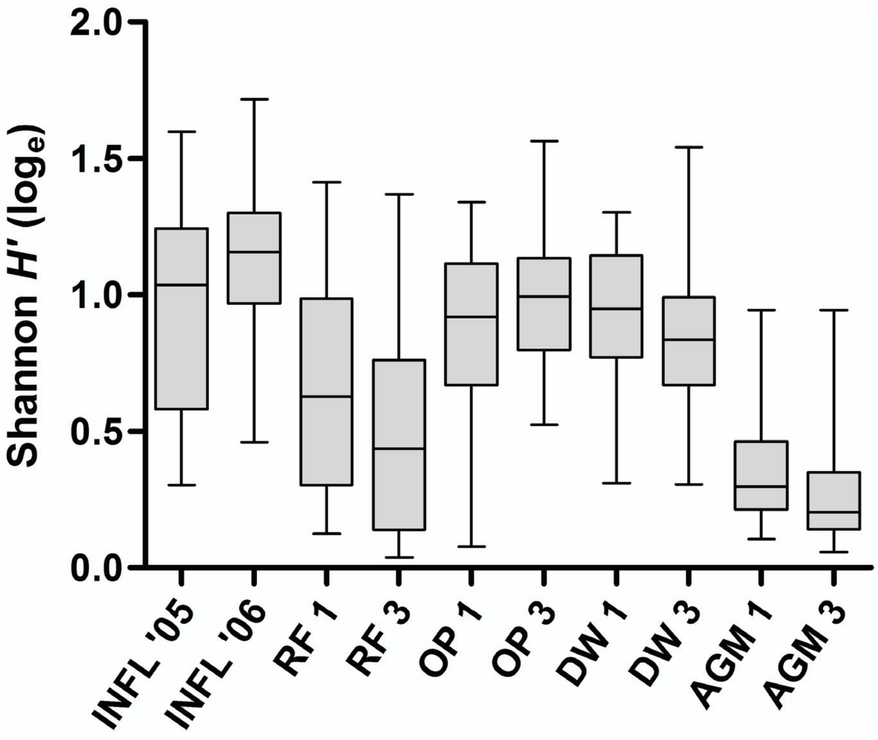

Figure 5.59. Box-plot of zooplankton community Shannon diversity indices (*H*′) for: 2005 (INFL '05) and 2006 (INFL '06) pilot plant Influent; Rock Filters 1 and 3 (RF-1; RF-3); Open Ponds 1 and 3 (OP-1; OP-3); Duckweed Ponds 1 and 3 (DW-1; DW-3); and Attached-Growth Media Reactors 1 and 3 (AGM-1; AGM-3). Data sourced from the entire pilot plant monitoring period from July 2005–August 2006.

Overall, the zooplankton community of the OP series was numerically dominated by large-bodied zooplankton, such as *Daphnia* and *Boeckella* species, as well as smaller zooplankton like rotifers (*Keratella* species) and copepod nauplii (Figures 5.33 and 5.37), with these four groups comprising on average 97 and 95% of the total population abundance in OP-1 and OP-3 respectively. Average zooplankton density within the Open Ponds during the 2005–2006 monitoring duration, as was the case for the RF treatment above, increased slightly compared with the influent wastewater during the same period (Figure 5.57); although once again only significantly for OP-1 (1-way ANOVA; $F_{(8,296)} = 6.252$; $p < 0.001$). Respective mean and median zooplankton densities during 2005–2006 were approximately 558 and 429 organisms L^{-1} for OP-1 and 377 and 286 organisms L^{-1} for OP-3. Unlike the trend for the Rock Filters above, this time the total zooplankton biomass was seen to have increased significantly within both OP-1 (1-way ANOVA; $F_{(8,296)} = 11.244$; $p = 0.033$) and OP-3 ($p = 0.013$) compared with 2005–2006 influent biomass levels (Figure 5.58); with respective mean and median zooplankton biomass values of 2.28 and 1.70mg L^{-1} for OP-1 and 2.39 and 1.76mg L^{-1} for OP-3. Like the influent wastewater, zooplankton communities in the OP series remained numerically dominated by copepod nauplii and rotifers; with these two groups constituting on average 73% of the total population abundance for the OP series. The significant increase in total zooplankton biomass within the OP series, on the other hand, was primarily a result of increased numbers of large-bodied zooplankton such as cladoceran *Daphnia* and also the calanoid copepod *B. triarticulata*; with these two zooplankton representing on average >87% of the total OP series population biomass (see Figures 5.35 and 5.39).

Overall, the zooplankton community structure in the OP treatment series was very similar to that of the influent wastewater; remaining both highly diverse and predominated (in terms of biomass) by large-bodied zooplankton species. As shown in Figure 5.59, relative biodiversity of zooplankton communities in the OP series remained statistically similar to that of the influent wastewater (Kruskal–Wallis test; $\chi^2_{0.05,7} = 74.76$; $p > 0.05$); again reflecting the ecological similarities between the two effluents. This apparent mirroring of the influent zooplankton community structure within the OP series was somewhat anticipated and was presumably a reflection of the similar and un-modified physical nature of their *in situ* environments. Unlike the RF series above, the transient appearance of *Moina* species was carried through from the influent wastewater down the OP series—being most apparent in OP-3 (see Figure 5.39). Also similar to the pilot plant influent, was the reduced dominance of ostracods (*Bennelongia* species) within the Open Ponds; with this group representing on average just over 5% of the total zooplankton biomass in the OP series. Notably, this was unlike the trend noted for the RF series above, where ostracods actually increased in total biomass to the point where they represented approximately 45% of the total population biomass by the final Rock Filter in the treatment series.

As discussed above, *Daphnia* and *Boeckella* species were seen to have dominated the zooplankton community within the pilot Open Ponds. Not only did their relative population abundance increase, but the absolute biomass of these two genera was also observed to have increased somewhat within the OP series relative to influent levels; with the combined biomass of these two zooplankton increasing from a mean of 949μg L^{-1} in the influent wastewater, to an average of 2089μg L^{-1} in both OP-1 and OP-3. It was considered likely that the increased prevalence of *Daphnia* and calanoid copepods within the OP series compared to the RF series was a result of the generally higher availability of algal food resources in the Open Ponds (see Figure 5.3) combined with the general competitive superiority of these zooplankton under such conditions. It was also possible that the open-air nature of the Open Ponds themselves enabled normal daily *in situ* fluctuations in light intensity to stimulate diurnal feeding and/or vertical migration rhythms these particular zooplankton (Starkweather, 1983; Stearns, 1986; Price, 1988)—facilitating their productivity in this way. Additionally, the increased dominance of *Daphnia* and *Boeckella* species in the OP series over ostracods for example (the dominant zooplankton group in the RF series) could have been due to the much higher levels of DO and corresponding pH in the Open Ponds compared with the Rock Filters (see Figures 3.10, 3.11 and 4.6, 4.9), as well as being a possible consequence of the 10-fold reduction in available surface area for substrate-grazing. Whilst the ultimate cause of these apparent differences between the zooplankton community structure of the RF and OP treatments remain uncertain, it goes beyond the scope of this thesis to elaborate any further as to the likely origins of such contrasting ecologies.

The operation of a parallel ‘non-interventional’ Open Pond treatment series has shown that in the absence of any distinct modifications to the physical environment, it would not be expected that the zooplankton community structure would be significantly modified from that of a conventional maturation WSP effluent. Results presented above have shown that the OP series zooplankton community remained similar in nature to the influent wastewater, and, instead of declining in total biomass as was the case following Rock Filter passage, zooplankton populations actually increased significantly in mass relative to influent levels.

Similar to the Rock Filter data presented above, there has once again been no significant prior research effort into the zooplankton ecology of a WSP effluent following passage under a duckweed surface cover. Whilst there have indeed been several observational references made to zooplankton in the context of wastewater-based research also incorporating duckweed (e.g. Cillie, 1962; Ehrlich, 1966; Tanner *et al*., 2005), there has so far been no direct quantitative assessments of zooplankton population ecology in wastewater with and without the presence of duckweed. Following this, the current work therefore represents—to the author's knowledge—the first reported attempt to quantitatively assess the zooplankton population ecology of a WSP effluent following upgrading with a duckweed surface cover. As can be seen in Figures (5.41 and 5.45), the zooplankton community of the DW treatment series was numerically dominated by copepod nauplii, rotifers (*Lecane* species) and ostracods (*Bennelongia* species), with these three zooplankton groups representing on average 91% of the total population abundance for the DW Pond series. There was also a transient population of smaller cladoceran zooplankton (*Plexorus* and *Simocephalus* species) from September–November of 2005, during which time they constituted anywhere from 4–58% of the total abundance figure.

Average zooplankton density within the Duckweed Ponds during 2005 monitoring *Period 1* remained relatively stable compared with the average numbers in the influent wastewater (see Figure 5.57). Statistically, there were no apparent differences between the average numbers of total zooplankton in the 2005 pilot plant influent and DW Pond 1 (1-way ANOVA; $F_{(6,134)} = 12.063$; $p = 0.22$), however, average zooplankton density was significantly reduced by the third DW Pond in series ($p = 0.008$). Respective mean and median zooplankton densities were approximately 332 and 198 organisms L^{-1} for DW-1 and 206 and 177 organisms L^{-1} for DW Pond 3. With respect to zooplankton biomass, and despite a significant decline in total population abundance in DW-3, zooplankton biomass levels remained statistically similar to 2005 influent biomass levels in both Ponds 1 and 3 of the DW series (1-way ANOVA; $F_{(6,134)} = 5.437$; $p \geq 0.38$); although total biomass appeared to be somewhat reduced in DW Pond 3 compared with the corresponding 2005 influent levels (Figure 5.58). When compared to the biomass levels in the parallel Open Ponds, however, there were significant differences between the two treatment trains; with DW Pond 3 producing lower overall zooplankton biomass levels than OP-3 ($p = 0.024$). Respective mean and median zooplankton biomass levels during 2005 were in the order of 2.14 and 0.68mg L^{-1} for DW-1 and 1.10 and 0.26mg L^{-1} for DW Pond 3.

This decline in total numbers and maintenance of population biomass down the DW Pond series was a reflection of the simultaneous decline in the numbers of larger *Daphnia* and *Boeckella* and the increased prevalence of ostracods within the Duckweed treatment Ponds compared with the 2005 influent wastewater (see compare Figure 5.21 Figures 5.43 and 5.47). *Daphnia* and *Boeckella* species in the 2005 influent constituted 70% of the total population biomass and ostracods 24%, but in DW-3 for example, *Daphnia* and *Boeckella* species represented just 6.7% of the total biomass and ostracods 78%. The same trend was also apparent in DW Pond 1, where ostracods comprised 76% of the total biomass pool on average. The reasons behind the comprehensive disappearance of *Daphnia* and *Boeckella* species in the DW treatment series—particularly by Pond 3—remain unclear. It is possible that it was related in some way to the highly shaded nature of the DW Ponds (see Figure 3.4) and the absence of significant quantities of incident sunlight to drive the necessary diel feeding rhythms in *Daphnia* and *Boeckella* species (as discussed for the OP series above); however, this hypothesis remains unconfirmed. It is apparent, however, that ostracods do not seem to require high levels of incident light in order to be successful in wastewater environments. This could in some way be linked to the recognition that aquatic chemistry in particular has been shown to be an important factor controlling the occurrence of freshwater ostracod species (Holmes, 1992); such that the moderating effect of darkness (within both duckweed-covered ponds and rock filters) on photosynthetically-driven fluctuations in pH and DO levels may result in a more favourable habitat for ostracod development.

There were several additional and notable ecological distinctions between the 2005 influent wastewater and population structure of the DW Pond series. First of these was the late 2005 increases in small cladoceran zooplankton such as *Simocephalus* and *Plexorus* species in DW Pond 3 (Figures 5.45 and 5.47). Curiously, these two zooplankton genera were either present in very low numbers (< 5 individuals L^{-1} for *Simocephalus*) or not recorded at all (*Plexorus*) in the 2005 influent wastewater, yet they were recorded in relatively high numbers (>75 individuals L^{-1}) in the DW Pond series—

particularly DW-3. Interestingly, the appearance of these two small-bodied cladocerans seemed to coincide with the onset of the November 2005 bloom of *Microcystis* and the decline in *Daphnia* dominance within the influent wastewater. As described earlier, reduced *Daphnia* dominance during warmer seasons has been observed to correspond with increased numbers of smaller cladoceran zooplankton (e.g. Vanni and Temte, 1990); all of which has also been linked to increased levels of cyanobacteria. It is therefore possible that the increased prevalence of the smaller cladoceran species of *Plexorus* and *Simocephalus* within the DW Pond series was linked to the *Microcystis* bloom in the influent wastewater around the same time.

Another notable ecological difference between the 2005 influent and zooplankton communities within the DW Pond series was the wholesale proliferation of small rotifers of the Genus *Lecane* (compare Figure 5.21 with Figures 5.41 and 5.45). Interestingly, *Lecane* species were all but absent in the 2005 influent wastewater, with a mean density of < 1 individual L^{-1}. At the same time, however, *Lecane* species appeared to thrive in the duckweed-covered Pond series, reaching densities of up to 230 and 514 individuals L^{-1} in DW-1 and DW-3 respectively and representing on average 42% of the DW series total population abundance (although mean biomass contributions remained < 1% on average due to their small size). This marked increase in *Lecane* numbers was thought to have been a result of the reduced numbers of *Daphnia* in the DW Pond series. Generally speaking, large cladoceran zooplankton such as *Daphnia* are known to be more effective grazers than rotifers and are therefore able to efficiently out-compete them for commonly shared food resources (Vanni, 1986; Gilbert, 1988; Lampert and Rothhaupt, 1991). In addition to this, small rotifers are often physically damaged by *Daphnia* as a result of being swept into their branchial chambers during non-selective feeding, often resulting in high rotifer mortality rates (Gilbert and Stemberger, 1985; MacIsaac and Gilbert, 1989). It should be noted here that other rotifers such as *Keratella procurva* were seen to have successfully coexisted with *Daphnia* in both the influent wastewater and also within the OP series, with both organisms simultaneously present in large numbers on numerous occasions. Unlike the smaller *Lecane* species, this *Daphnia*–*Keratella* coexistence was most likely able to occur because the much larger size of *K. procurva* (200–300μm; see Appendix D) restricted its rate of ingestion and subsequent physical damage by *Daphnia* during feeding (Gilbert and Stemberger, 1985;

Kobayashi, 1991) as well as the presence of large spines potentially limiting its palatability larger zooplankton (e.g. Marinone and Zagarese, 1991; Hamilton *et al*., 2005).

The above hypothesis of physical interference was supported statistically by the existence of a highly significant negative correlation between *Lecane* and *Daphnia* density within the 2005 influent wastewater and the combined data from DW Ponds 1 and 3 ($r_s = -0.615$; $n = 55$; $p < 0.00001$). At the same time, the marked proliferation of *Lecane* species within the DW Pond series may have also been related to the reduced abundance of copepods (namely *Boeckella* and *Mesocyclops* species) which may have normally preyed upon these small rotifers in the influent wastewater, thereby suppressing their numbers. In the absence of significant numbers of predatory copepods in the DW ponds, however, *Lecane* species were able to multiply to relatively high densities. This theory was again supported by significant negative correlations between *Lecane* density and the numbers of both *Boeckella* ($r_s = -0.429$; $n = 55$; $p = 0.001$) and *Mesocyclops* ($r_s = -0.495$; $n = 55$; $p = 0.0001$) in the 2005 influent wastewater and the combined data from DW Ponds 1 and 3. Following this, it was likely then that the greater abundance of *Lecane* species within the DW Pond series was a combined result of the reduced numbers of larger *Daphnia* and copepod species; although the relative influence of each of these species remains unclear.

Interestingly, small *Lecane* rotifers were seen to have successfully coexisted with the much larger ostracod *Bennelongia* in the DW Pond series during the 2005 monitoring period. The successful co-inhabitance of *Lecane* and *Bennelongia* species within the DW ponds most likely reflects the differing ecological and spatial niches occupied two zooplankton genera; with *Lecane* a planktonic suspension feeder and *Bennelongia* predominantly a substrate-grazing omnivore. The likely physical segregation of these zooplankton *in situ* probably enabled the much smaller planktonic *Lecane* species to avoid ingestion and possible maceration by the large numbers of ostracods also present at the same time within the duckweed-covered ponds; with this unique pattern of coexistence not apparent in either the influent wastewater or any of the other pilot treatment series.

With respect to the zooplankton biodiversity, and as shown in Figure 5.59, the relative Shannon diversity indices for zooplankton communities in Ponds 1 and 3 of the DW series remained similar to that of the 2005 influent wastewater (1-way ANOVA; $F_{(9,191)} = 10.695$; $p \geq 0.99$). This suggested that in spite of the strikingly different ecological compositions for the influent wastewater and DW series effluent, and despite obvious shifts in species dominance, there was nevertheless an apparent similarity between the two wastewaters in terms of the relative biodiversity of their zooplankton communities. It was considered likely that the apparent maintenance of influent community diversity within the DW Pond series, when compared with the RF series for example, was related to the less severe modification to the physical environment (i.e. the absence of rock media) and the maintenance of higher levels of DO within the duckweed-covered ponds compared with the Rock Filters (see Figure 3.10). In passing, this notion was actually raised somewhat earlier in Chapter 3 (Section 3.3.4), whereby it was proposed that that the reduced levels of DO—within the RF treatment series in particular—might have an influential role on the resident zooplankton community structure. As discussed above, reduced levels of oxygen in aquatic environments has been implicated as a causal factor in the reduction of biodiversity (e.g. Detmer *et al.*, 1993; Janse and Van Puijenbroek, 1998). Increased oxygen concentrations could therefore go toward explaining the relatively higher zooplankton community diversity in the DW series compared with Rock Filters, as well as the slightly lower level of biodiversity in comparison to the more oxygenated OP series; although no attempts were made to quantitatively correlate DO concentration with the magnitude of respective Shannon diversity indices during the current work.

In addition to the apparent maintenance of community diversity in the DW Pond series, there was also a trend of increased temporal stability of zooplankton populations within the duckweed-covered ponds compared with the 2005 influent wastewater. This general trend for reduced variability in zooplankton community structure within the DW series can be seen in both the corresponding box-plots of Figure 5.59 and also by comparing the corresponding community biomass area plots for the 2005 influent (Figure 5.23) and DW Ponds 1 and 3 (Figures 5.43 and 5.47). Elsewhere, macrophyte coverage has been suggested as having a stabilizing effect on the underlying zooplankton (*Daphnia*) populations by moderating the normal ‘boom and bust’ population fluctuations (Lau and

Lane, 2002). Similarly, Tanner *et al*. (2005) also noted that the high density zooplankton population booms observed in their pilot-scale open maturation pond system were not evident in the parallel wetland ponds containing both emergent macrophytes and duckweed; something the authors attributed to the altered physical environment and/or low oxygen levels within the wetland system.

The apparent ‘stabilizing effect’ of a duckweed surface cover has been discussed previously with respect to pH (see Section 3.3.4) and it is possible that this physicochemical stability had a positive effect toward maintaining more stable zooplankton populations within the pilot DW ponds. This also follows the reporting of O’Brien and deNoyelles Jr. (1972), who observed that vigorous daytime photosynthetic activity can result in large-scale fluctuations in pH and the promotion of highly alkaline conditions (pH 10.5–11) which can lead to increased mortality in crustacean zooplankton. In this sense, a complete duckweed surface cover could—by inhibiting algal photosynthesis—effectively moderate the extent of diel fluctuations in aqueous physicochemistry (e.g. pH, DO and alkalinity), thereby protecting the underlying zooplankton populations from such large-scale environmental variability and promoting a less volatile community structure. This concept can be visualized by comparing the respective zooplankton population biomass data of the OP series and the DW series (Figures 5.35 and 5.39 for the OP series and 5.43 and 5.47 for the DW series). As shown in these Figures, the temporal variability in zooplankton community structure and biomass dynamics is much greater for the un-covered Open Ponds during 2005, whereas the ‘boom and bust’ cycles for zooplankton populations in both ponds of the DW series are far less apparent during the same period. There was, however, one notable boom of ostracod biomass in the DW ponds during late November, but this was most likely related to the *Microcystis* bloom that occurred around the same time.

Within the relevant literature, there is evidence to suggest that a potentially beneficial role is played by aquatic macrophytes in the enhancement of zooplankton populations, with the plants in some instances serving as suitable zooplankton ‘refuges’. Aquatic macrophytes play host to a large variety of invertebrates such that the densities of zooplankton and other invertebrates are generally higher among the macrophyte communities than in the open water (Pennak, 1966). Very early work showed that

Lemna cover can provide favourable conditions for the growth of herbivorous metazoan zooplankton, such as *Daphnia* and rotifers, which can contribute to the removal of suspended algal and microbial biomass (Ehrlich, 1966). Others have also highlighted the potential beneficial role of aquatic macrophytes for the promotion of zooplankton populations and the subsequent enhancement of phytoplankton grazing and removal (van Donk and van de Bund, 2002). *Lemna* communities can not only serve as a suitable habitat for microinvertebrate zooplankton but also for macroinvertebrates such as various species of insect larvae (Gopal and Goel, 1993; Harper and Bolen, 1996) which can then contribute to the overall species richness and ecological function of the macrophyte treatment system. Data from monitoring of zooplankton populations in the DW Pond series reported here suggests that rather than serving as a suitable zooplankton refuge for enhanced secondary productivity, duckweed surface coverage actually suppresses the development of large zooplankton species in particular and resulting in significantly reduced total biomass levels compared with parallel un-covered ponds. It is unlikely then, than effective algal removal in duckweed pond systems would be as a result of increased grazing pressure from resident zooplankton communities; suggesting again that enhanced quiescent settling is the primary factor involved in effective solids removal in duckweed ponds.

The operation of a duckweed-covered Pond series has shown that zooplankton populations are moderated considerably by the presence of duckweed, in terms of both temporal variations in species and also total biomass, compared with the population structure of a maturation WSP effluent. Results have also demonstrated that in pilot-scale reactors, a duckweed surface cover can produce more stable zooplankton populations than those in a parallel un-covered Open Pond, as well as producing a population with a significantly lower overall biomass. Analysis of the zooplankton communities within these duckweed-cover ponds revealed a shift in community structure from one of dominance by large-bodied *Daphnia* and copepods to one dominated by smaller zooplankton such as ostracods, small cladocerans and rotifers. The implications of this apparent shift in ecological composition for DAF/F process efficiency will be discussed below.

As was the case for phytoplankton data of Section 5.3.1 above, published information regarding the zooplankton ecology of attached-growth media systems is virtually non-existent. The only work available appears to be that of Zhao and Wang (1998), who, following qualitative observations of their pilot-scale AGM system, commented on their observations of high numbers of rotifers, roundworms and *Daphnia* in their AGM system; although they offered no quantitative data in support of this. Results provided below therefore represent the first known attempt at quantitatively assessing the zooplankton ecology of a WSP effluent following upgrading via an attached-growth media system. As shown in Figures 5.49 and 5.53, the zooplankton community within the AGM treatment series was numerically dominated almost entirely by copepod nauplii and to a lesser extent by rotifers (*Lecane* and *Keratella* species), with copepod nauplii representing on average ≈93% of the total population abundance for the AGM series. Mean naupliar density in AGM Reactors 1 and 3 was in the order of 440 individuals L^{-1}, although nauplii were at times present in very high densities of up to 1800 individuals L^{-1} (AGM-1). Rotifers were far less abundant in the AGM series, with mean rotifer density for Reactors 1 and 3 during 2006 being <15 individuals L^{-1}.

Average zooplankton density within the AGM series during 2006 monitoring *Period 2* increased slightly compared with the average numbers in the influent wastewater during the same period (see Figure 5.57). Statistically, however, there were no apparent differences between the average numbers of total zooplankton in the 2006 pilot plant influent and either AGM Reactors 1 or 3 (1-way ANOVA; $F_{(6,142)} = 5.589$; $p > 0.76$). Respective mean and median zooplankton densities were approximately 578 and 330 organisms L^{-1} for AGM-1 and 368 and 244 organisms L^{-1} for AGM-3. With respect to zooplankton biomass, and despite a small but non-significant increase in total zooplankton abundance in the AGM series, zooplankton biomass levels decreased significantly in both Reactors 1 and 3 compared with 2006 influent biomass levels (1-way ANOVA; $F_{(6,142)} = 18.843$; $p < 0.001$); declining from an influent median of 0.85 down to a median of 0.15mg L^{-1} by AGM-3 (Figure 5.58). Furthermore, when compared to the total zooplankton biomass levels in the parallel Open Ponds, there were again significant differences between the two; with effluents from both AGM-1 and AGM-3 containing on average some 10-fold lower biomass levels than the corresponding Open Ponds ($p < 0.0001$). Respective mean and median zooplankton biomass level during

2006 were in the order of 0.51 and 0.20mg L^{-1} for AGM-1 and 0.17 and 0.14mg L^{-1} for AGM Reactor 3. Interestingly, average zooplankton biomass levels were also lower in the AGM effluent than they were in the parallel RF effluent, with mean AGM-1 and AGM-3 biomass values significantly lower compared to the respective pilot Rock Filters ($p < 0.01$).

The above increase in total numbers and dramatic decline in population biomass down the AGM series was a consequence of the apparent increase in the numbers of smaller copepod nauplii, combined with the almost complete disappearance of large-bodied zooplankton (*Daphnia*, *Boeckella* and *Bennelongia*) that were present in the influent wastewater. The reasons behind the large-scale disappearance of *Daphnia*, *Boeckella* and *Bennelongia* species in the AGM treatment series—as for the DW series above—remain unclear. It is possible that it was again related in some way to the shaded nature of the AGM reactors (as discussed for the OP series above) or perhaps the reduced oxygen concentration (see Figures 4.6 and 4.8); however, these theories remain unverified. Interestingly, the work of Chaston (1969) showed that copepod (*Cyclops* species) nauplii were much less tolerant to hypoxic conditions than were larger adults; implying that the reduced DO concentrations in the AGM reactors would not have favoured copepod nauplii. There have been reported instances of copepod nauplii competing with larger cladoceran zooplankton for food resources, as well as naupliar predation on small rotifers (Lang, 1997 cited in Maise, 2001); however, the likelihood of such interactions contributing to naupliar dominance in the AGM series appears small. It should be noted here, that increased numbers of these early naupliar life stages of copepods did not coincide with large numbers of adults. This notable absence of adults suggests that copepods were not actively reproducing within the AGM reactors; rather, their nauplii were somehow accumulating within the AGM series. Whatever the cause was for this apparent increase in naupliar abundance, the daily contribution of copepod nauplii to the total zooplankton biomass within the AGM series remained very low ($<70\mu g\ L^{-1}$ on average), such that nauplii were considered relatively unimportant on a population-scale.

Biodiversity of zooplankton communities in both Reactors 1 and 3 of the AGM series was reduced significantly compared with the corresponding 2006 influent wastewater

(Kruskal–Wallis test; $\chi^2_{0.05,7} = 74.77$; $p < 0.001$). This loss of empirical diversity no doubt reflected the wholesale disappearance of a number of the zooplankton species present in the influent wastewater; especially by the third AGM reactor in series. The reasons for the apparent decline in zooplankton community diversity within the AGM series are most likely similar to those already discussed for the RF series above, and were considered to have related specifically to the reduced oxygen levels and large-scale exclusion of incident light. As discussed previously, 'dark–anoxic' environments are inherently less biologically productive and diverse that 'light–oxic' ones. In this sense, it appears then that the remedial action of both the Rock Filters and AGM upgrade systems is two-fold; in that in addition to being highly competent at physically removing infiltrating volatile and non-volatile solids and BOD_5, they are also zones of reduced biological productivity and diversity such that there is very little 'new biomass' to exert a negative influence on the effluent water quality. As outlined in Section 5.1, WSPs are renowned for their ecological instability and highly variable effluent quality. Because both the Rock Filters and Attached-Growth Media upgrade systems have been shown to be biologically less productive and less diverse systems than a classical Open Pond environment, this could potentially serve as an additional 'ecological explanation' for the more stable and higher quality final effluents seen in these systems throughout Chapters 3 and 4.

Following analysis of both the AGM and RF data, there was no evidence to suggest that the addition of physical substrate (either artificial or natural rock) to a maturation WSP effluent served as a suitable 'refuge' for increased zooplankton activities; as has been suggested by others (e.g. Timms and Moss, 1984; van Donk and van de Bund, 2002). It was likely that in the case of the current work, the absence of predatory fish populations in the pilot reactors would have diminished any potentially beneficial effects that may have come from the presence of substrate refuges; although Mara *et al*. (2001) cited predation by higher invertebrates as being problematic for the maintenance of high-density zooplankton (*Daphnia*) communities. Conversely to enhancing zooplankton productivity, the large increases in the area of physical substrate within both the AGM and RF upgrade systems actually resulted in significantly enhanced removals of suspended particulates and algal biomass (see Sections 3.3.6 and 4.3.5) which in turn resulted in greatly reduced quantities of suspended 'food' in the water phase. This restriction in food availability for the majority of resident (planktonic) zooplankton grazers would have then led to reduced levels of secondary productivity and ultimately limited the amount of total zooplankton biomass present in the final effluents of these two upgrade systems. This could therefore go toward satisfying the earlier work of Mara *et al*. (2001, p. 24) who, following their investigations into the use of microcrustacean (*Daphnia*) WSPs, suggested that further work should focus on determining "*strategies to prevent (zooplankton) predation, such as the provision of underwater refuges.*" Results from the current work suggest that the provision of increased amounts of physical substrate (at least when oriented predominantly on a horizontal plane) is unlikely to enhance the secondary productivity of resident zooplankton populations.

Results from the operation of a pilot-scale horizontal-flow attached-growth media upgrade system have shown that this sort of in-pond upgrade has the potential to produce a final effluent with consistently lower levels of zooplankton biomass than is achievable in an un-covered open pond environment. Analysis of the zooplankton communities within the pilot AGM reactors revealed a marked shift in population structure away from a highly productive WSP community dominated by large-bodied *Daphnia* and copepods to one of very low secondary productivity. The implications of this notable change in ecological composition for DAF/F process efficiency are discussed in the following Section. In Chapter 3 (see Section 3.3.6) it was hypothesized that grazing interactions from resident zooplankton populations may have played a definable role in chlorophyll *a* (algal biomass) and SS removals within the pilot upgrade systems. Following ecological monitoring of zooplankton biomass within each of the pilot upgrade systems, however, it was considered likely that the higher-level algal biomass removals achieved by both the RF and AGM systems were predominantly a result of physical process interactions (i.e. sedimentation); although there was some evidence to suggest that invertebrate grazing could be important for the additional processing and stabilisation of accumulated algal solids (e.g. large zooplankton biomass levels seen in RF-1 as well as substrate-grazing snails and ostracods being omnipresent in the RF series). Given the generally low zooplankton biomass levels in the DW ponds, similar conclusions can probably be drawn regarding the significant chlorophyll *a* removals in the DW series, whereby enhanced quiescence and physical sedimentation were the most likely factors governing algal removal performance. This is in line with the earlier views of Zirschky and Reed (1988, p. 1254) who commented that "*mat formation is probably the most significant contribution that the duckweed plant makes to wastewater treatment*."

5.3.2.1 Incidence of problem zooplankton species: implications of the effluent upgrade systems for DAF/F process efficiency

As described previously (see Section 1.3.1), and similarly to phytoplankton above, several zooplankton have also been identified as being particularly "problematic" to DAF/F process efficiency. Buisine and Oemcke (2003) reported that both copepods and *Daphnia* are relatively unaffected by chemical coagulation and flocculation during DAF/F treatment; posing a threat to the filtration component of the DAF/F process by generating accelerated and dramatic headloss accumulation and necessitating frequent filter-bed backwashing. The authors did note, however, that jar testing results suggested that micro-bubbles (from the dissolved-air flotation step) can sometimes attach to these organisms and force them to float against their will. It is highly likely that the strong swimming capabilities of these particular zooplankton (see Section 3.3.6 for initial discussion) facilitate their resilience to flotation during DAF/F treatment. *Daphnia* species for example are capable of swimming at velocities in the range of 20–30m h^{-1} (Dodson *et al*., 1997) and copepods of similar size to the *Boeckella* species encountered during the current work are reportedly capably of sustained swimming (>1 hr) in an upward direction at speeds in the order of 30–90m h^{-1} (Enright, 1977).

It was initially hypothesized (see Section 1.3.1.2 for initial discussion) that passage through one or all of the investigated effluent upgrade systems might have a 'biomanipulating' effect on the resident zooplankton communities, and that this may then have follow-on implications in terms of reducing (or increasing for that matter) the burden on the Bolivar DAF/F plant. The following Section, therefore, seeks to provide information relating directly to the relative incidence problem zooplankton (i.e. cladoceran *Daphnia* and copepods) within each of the pilot upgrade systems, as well as outlining the potential down-stream implications of these findings for DAF/F plant process efficiency. Data showing the relative abundance and biomass of problem zooplankton in both the 2005 and 2006 influent wastewater as well as for the four pilot upgrade series is provided in Figures 5.60 and 5.61 respectively. It should be emphasized that since no distinction was made by Buisine and Oemcke (2003) regarding the problematic nature of calanoid and cyclopoid copepods, the two groups were assumed to be equally undesirable and as such have been grouped together for the purposes of this section.

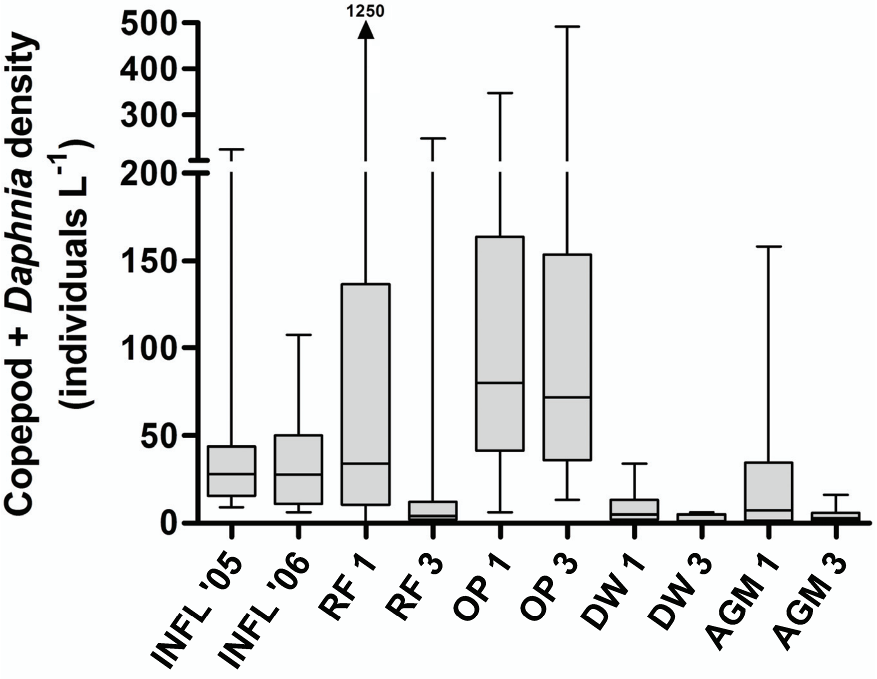

Figure 5.60. Box-plot showing the relative population densities of problem zooplankton for: 2005 (INFL '05) and 2006 (INFL '06) pilot plant Influent; Rock Filters 1 and 3 (RF-1; RF-3); Open Ponds 1 and 3 (OP-1; OP-3); Duckweed Ponds 1 and 3 (DW-1; DW-3); and Attached-Growth Media Reactors 1 and 3 (AGM-1; AGM-3). Data from the entire pilot plant monitoring period of July 2005–August 2006.

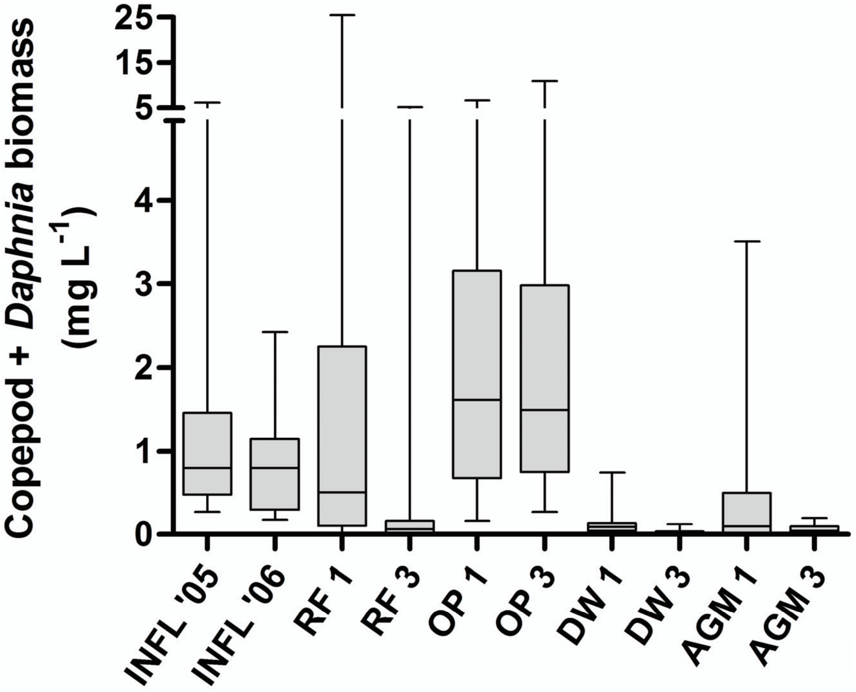

Figure 5.61. Box-plot showing the relative biomass densities of problem zooplankton for: 2005 (INFL '05) and 2006 (INFL '06) pilot plant Influent; Rock Filters 1 and 3 (RF-1; RF-3); Open Ponds 1 and 3 (OP-1; OP-3); Duckweed Ponds 1 and 3 (DW-1; DW-3); and Attached-Growth Media Reactors 1 and 3 (AGM-1; AGM-3). Data from the entire pilot plant monitoring period of July 2005–August 2006.

As shown in Figure 5.60 above, the Rock Filter series was able to drastically reduce the numbers of problem zooplankton compared with the influent wastewater, achieving a 10-fold reduction in the combined numbers of copepods and *Daphnia* by RF-3. Statistically, there were significant reductions in the numbers of problem zooplankton for RF-3 relative to combined 2005–2006 influent levels (1-way ANOVA; $F_{(4,220)} = 29.796$; $p < 0.0001$) but not RF-1 ($p = 0.979$). Similarly, the DW Pond series also yielded significant 10-fold reductions in the numbers of problem zooplankton, except this time it did so for both Ponds 1 and 3 of the treatment series relative to 2005 influent levels (1-way ANOVA; $F_{(6,131)} = 43.284$; $p < 0.001$). Like the Rock Filters above, the AGM upgrade treatment again achieved significant 10-fold reductions in the numbers of problem copepods and *Daphnia* for AGM-3 ($p < 0.001$) relative to 2006 influent levels (1-way ANOVA; $F_{(6,142)} = 23.623$) but not for AGM-1 ($p = 0.09$). Finally, and unlike the other treatment series, the Open Ponds actually yielded significant increases in the numbers of problem zooplankton for both Ponds 1 and 3 relative to combined 2005–2006 influent levels (1-way ANOVA; $F_{(4,220)} = 29.796$; $p < 0.001$); with median numbers of problem zooplankton increasing from 28 in the influent up to 70–80 in OPs 1 and 3. Performance data for the biomass dynamics of problem zooplankton (Figure 5.61) showed practically identical trends to those of abundance data discussed above, with virtually identical levels of statistical significance across all treatments.

In addition to the identified problem *Daphnia* species, it is likely that other large-bodied and highly motile cladoceran zooplankton (such as *Moina* or *Simocephalus* species for example) could also be problematic to DAF/F process performance. Given the incidence of these organisms was generally transient, however, and because they contributed only small biomasses across all treatments except for the OP series (up to 0.95mg L^{-1} for *Moina*), they were not considered to pose as significant a threat to DAF/F plant operational efficiency as the larger and more prevalent copepod and *Daphnia* species. Unlike *Moina* and *Simocephalus* species above, ostracods were consistently observed throughout the course of the current research and sometimes in high numbers. Furthermore, ostracod species were also observed to be highly motile within the pilot ponds and are thought to be at least as strong swimming as are *Daphnia* species. This observation of high-level motility in *Bennelongia* species is supported by the reporting of De Deckker (1983), where it was stated that a large number of Australian ostracod species are “effective swimmers”. Whilst actual ostracod swimming velocity remained unverified, based on visual observations it is estimated that *Bennelongia* species would be easily capable of swimming at speeds in the order of 10–20m hr^{-1}. Whether the similar size and motility of ostracods would then confer similar problems as for *Daphnia* and copepods—in terms of poor removal during the DAF/F process—remains unknown, although it is suggested that the topic does warrant future investigation. Should ostracods also be identified as problematic to DAF/F process efficiency, this could have consequences for the relative effectiveness of a duckweed surface cover to reduce the incidence of problem species; since this upgrade system produced—on occasion—high numbers of ostracods in the final effluent.

The above results suggest that a rock filter, a duckweed surface cover and an attached-growth media upgrade system all have the potential to significantly reduce the numbers of problem zooplankton in the final Bolivar WSP effluent. Whilst the experimental duration was not of sufficient length to guarantee the year-round effluent quality of particularly the DW and AGM upgrade systems, it is assumed that the above three effluent upgrade systems would be capable of providing a better quality influent for the Bolivar DAF/F plant than would a system consisting of essentially ‘no intervention’. Ecologically, and looking beyond the problem organisms, there were noticeable differences between the zooplankton communities of each pilot upgrade system; however, whether or not these additional ecological changes would further translate to better overall DAF/F plant performance remains to be seen. It is suggested that there is a need for additional investigations, such as flocculation jar-testing, in order to quantitatively determine the outcomes of these differential changes in zooplankton ecology for DAF/F process performance.

5.4 Conclusions

Analysis of phytoplankton populations from the final Bolivar WSP effluent revealed a highly diverse community spanning seven taxonomic phyla. Overall the WSP effluent was dominated by chlorophyte greens, cryptophytes, diatoms and cyanobacteria. The relatively high-level taxonomic diversity and the increased dominance of cryptophyte species was thought to have reflected the reduced trophic and nutritional status of the WSP network—a consequence of the 2001 activated sludge plant installation. Despite the reduced nitrogen availability within the pond system, the non-nitrogen-fixing cyanobacterium *Microcystis flos-aquae* was still able to undergo wholesale bloom formation and dominate the WSP community for a number of months from late spring to summer. Analysis of phytoplankton performance data showed that both the RF and AGM upgrade systems were able to yield approximate 1-$\log_{10}$ removals of total algal cells by the last reactor in series, in comparison to <0.5-$\log_{10}$ removals for the DW and OP treatments. Regarding the removal of so-called problem algae, performance data showed no significant trends for preferential removal of problem species in any of the four pilot treatment systems; however, results from Chapter 3 verified that the total quantity of problem algal biomass was invariably reduced following passage through all experimental upgrade series.

Results from ecological monitoring of zooplankton populations in the Bolivar WSP effluent have shown that smaller ‘microzooplankton’ (nauplii and rotifers) were frequently more abundant than were 'macrozooplankton' (cladocerans, copepods and ostracods), while macrozooplankton most commonly made a much greater average contribution to the total zooplankton biomass within the influent wastewater as well as each of the pilot treatment systems. Performance monitoring of the experimental pilot plant also revealed the presence of some organisms previously unreported for WSP environments. The presence of an ostracod species belonging to the Genus *Bennelongia* as well as rotifers of the Genus *Lecane* for example represented the first known reporting of these taxa in a wastewater setting. Performance monitoring data also provided the first detailed investigation into the temporal ecology of the three major freshwater zooplankton groups (rotifers, cladocerans and copepods) in a WSP environment, as well as offering the first quantitative insights into the zooplankton ecology of a rock filter, a duckweed-covered pond, or an attached-growth media system. Work presented here also constitutes the first known cataloguing of the seasonal distribution of rotifer and ostracod populations in an Australian WSP system; with Hussainy (1979) reporting only on the presence of rotifers and ostracods in another Australian WSP.

Results from this chapter demonstrated that the DW, RF and AGM treatment series all produced final effluents with significantly lower levels of total zooplankton biomass than the parallel OP series. Additionally, it was it was shown that a rock filter, a duckweed surface cover and an attached-growth media upgrade system all have the potential to provide a better quality influent for the Bolivar DAF/F plant by significantly reducing the numbers of so-called problem zooplankton in the Bolivar WSP effluent. The practical implications of these research findings and how they relate to process efficiency at the Bolivar WWTP will be discussed further in the general discussion of Chapter 10.

As outlined in Chapter 1 (Section 1.3.1.1) and again at the start of this Chapter (Section 5.1), the reasons behind the periodic and unpredictable 'boom and bust' dynamics of algal (and zooplankton) populations in the Bolivar WSPs remain unclear. During the current chapter, temperature was identified as an influential factor behind the temporal dynamics of both phyto- and zooplankton communities in WSP environments. In addition to its role in shaping the phytoplankton community structure (i.e. food resource availability), it was likely that temperature also had an indirect effect on particularly the zooplankton community structure through its interconnection with the rates of algal productivity and photosynthesis and the subsequent effects this has DO concentration and also pH; although the precise effects of these factors on zooplankton dynamics were not quantified. Whilst temperature obviously has an underlying role in seasonal variability, the complex nature of interconnections between multiple parameters during ecological analyses often make it difficult to ascertain the direct causal factors behind plankton population dynamics. Furthermore, and in most instances, it is unlikely that there is one single factor responsible for particular ecological shifts in population structure; rather, such changes arise from unique, complex and multidimensional changes in both physicochemical and biological parameters (Dor et al., 1987). Therefore, whilst temperature had an obvious underlying influence on both algal and zooplankton dynamics, it is likely that other factors such as competition and grazing interactions almost certainly have a significant role to play with respect to the unpredictable nature of plankton communities in the Bolivar WSPs. More work is needed in these areas before the seasonal dynamics of phyto- and zooplankton populations can be fully understood.