

Chapter 6 ~ Discussion of the Results

6.1 Research synopsis

This research has sought to provide evidence for some of the nitrogen removal mechanisms, and pathways, operating seasonally within PFP WSP's in the UK. Pond performance was monitored in order to assess removal efficiencies for various physio-chemical parameters, and was found to be satisfactory. In-pond operating conditions showed that the pond fluctuated between aerobic and anoxic periods during summer and winter phases. The volatilization of ammonia contributed very little to overall permanent nitrogen removal from the system. Hydraulic tracer studies showed that there did appear to be much of a difference in pond hydraulic performance between summer and winter seasons. The use of ^{15}N as a stable isotope tracking technique helped to define where nitrogen was being removed from the system, and pinpoint some of the transformation pathways pertinent to the PFP's. The results from the molecular microbiological analysis indicated the possibility of previously unexplored biological participatory activity within the PFP nitrogen cycle.

6.2 PFP performance and removal efficiencies

Removal efficiencies for both the Green and Blue PFP's compared favourably, when considered with those reported from other pond systems presented in the literature. BOD_5 removal was excellent in all three experimental runs (measuring 80%, 79%, and 86% for the respective runs). In the Mèze WSP system in southern France, a total BOD_5 removal of 94% was achieved over a thirteen month period for the whole system (Picot *et al.*, 2009). The PFP's used in this research are coupled to maturation ponds and rock filters, ensuring that additional removal of all parameters from the PFP effluent is achieved. In the same study conducted by Picot *et al.* (2009), the mean influent total nitrogen concentration measured 51 mg/l, with an overall removal for the whole of the Mèze system equalling 65%, with the final concentration on the final effluent measuring 16 mg TKN/l. If the dilution factor of the raw wastewater into the Bradford PFP's is ignored, the mean TKN concentration of TKN ranged between 53.8 mg/l and 62.0 mg/l, which was slightly higher than the Mèze influent TKN concentration (51

mg/l). Overall, both the Green and Blue PFP's achieved good removals of total nitrogen in both winter, and summer. TKN removal efficiencies averaged 47% in the winter, and 52% in the summer, with the winter TKN concentrations measuring 11.5 mg/l and 14.9 mg/l in winter (2006 and 2007, respectively), and 10.7 mg/l in summer 2006, whereas the mean effluent TKN concentration in the Mèze ponds final effluent was 16 mg/l, with an overall removal of 68% (Picot *et al.*, 2009). Ammonia volatilization removals were found to be much higher in the south of France (accounting for 18% of total nitrogen removal) than in Bradford (a maximum of 0.1% in the summer, and 0.2% in the winter, of the total nitrogen removed from the PFP's). However, the removal of SS in the Bradford ponds (a maximum removal of 53% in winter 2006) compared poorly with the whole of the Mèze system (where the mean SS removal was 79%).

6.3 The insignificance of ammonia volatilization

As presented in section 5.5, the contribution of ammonia volatilization to overall nitrogen removal processes from the PFP's was negligible. Evidence from the winter and summer studies clearly suggests that the volatilization of ammonia does not constitute a significant nitrogen removal pathway for PFP within the UK.

This well corroborates the data already presented and discussed by Epworth (2004 – in section 3.4), who previously found that ammonia volatilization over a 13-week period in spring-time (from the same pond as used in this research), was responsible for a net loss of 0.019 g/d, equating to 0.19% of total N removed from the PFP daily. The experimental data tabulated in Appendix E reveal that ammonia volatilization appeared to be higher in the winter (25 g/ha d) than in the summer (11 g/ha d). This seasonal difference should in no way be taken as factual; these studies have limitations, providing more qualitative data than quantitative data, and it follows that, in order to make an adequate assessment of the effects of seasonality, rigorous longitudinal studies need to be conducted to enable the necessary statistical analyses to be validated.

It is widely perceived in the WSP community that ammonia volatilization must occur to a greater degree in summer because an increase in algal populations, associated pH increase, coupled with generally warmer temperatures, provide

ideal conditions for this mechanism to take place. From the science associated with the chemical dissociation of the ammonium ion, and the laws governing the ammonium and ammonia equilibrium, it is sensible to deduce that ammonia volatilization should theoretically occur at much higher rates in summer than in winter. If Emerson *et al.*'s (1975) table (Table 2, presented in Emerson *et al.* (1975)) is used in conjunction with the data presented in this thesis, it is possible to calculate a feasible quantity of ammonia volatilized from the PFP/d. The mean pH obtained from the pond effluent point during the winter study was 7.81, and the mean surface water temperature was 4.4 °C; according to these values the percentage of NH₃ in the PFP would lie around 1.14% (Emerson *et al.*, 1975). The mean ammoniacal nitrogen concentration in the water column throughout this study period (obtained from the weekly grab samples) was 7.6 mg NH₄⁺/l; 1.14% of this value equals ~0.09 mg NH₃/l. For the summer study, with the average water surface pH 8.4, an average water surface temperature of 14.1 °C, and a mean column concentration of 5.4 mg NH₄⁺/l, the percentage of NH₃ in the water would equate to ~7.43%, thus providing an available free ammonia concentration of 0.4 mg NH₃/l.

The higher quantity of ammonia volatilized from the winter study (shown in Figure 5.13, and Table E.2) may have occurred for several reasons. The most strikingly obvious of these was that the ammoniacal nitrogen concentration in the PFP water column was higher in the winter than in the summer, and therefore potentially more ammonia was available to be driven off as gas (although this does contradict the preceding paragraph and Emerson *et al.*'s (1975) calculations of the equilibrium between free and ionised ammonia). The mean column chlorophyll *a* concentration measured in the winter study was 315 µg/l, whereas the mean summer water column chlorophyll *a* concentration was 288 µg/l. Although this does not translate into a huge difference between summer and winter values, the slight elevation of winter chlorophyll *a* concentrations may have induced a higher photosynthetic output from the algae, so possibly boosting in-pond pH, of which the maximum value recorded during the winter 2006 experiment was 9.68. This is a postulate, and the actualities of the PFP diurnal cycle were difficult to ascertain, as only a very limited view could be obtained by the parameters measured from grab samples. It is highly probable that

photosynthetic reactions and algal activity were significantly higher in the summer than in the winter study. Although chlorophyll *a* concentrations were slightly less, increased algal activity would simultaneously have increased ammonia uptake by the collective biomass, so reducing free ammonia concentrations and subsequent volatilization; the algae at the PFP's surface may have caused a barrier to the escaping ammonia. Conversely, during the winter period algal reaction rates may have been lower because of the mean cooler temperature, and therefore the available NH₃ would have been freer to volatilize to atmosphere.

The effects of isotopic fractionation, as comprehensively discussed by Faure and Mensing (2005), may have come into play as a source of volatilization at the surface of pond (the heavier ¹⁵N being retained in the pond water), or in the diffusive extraction of nitrogen in the laboratory. However, the probability of this influencing the results gained from mass spectrometric analysis is likely to be small. The quantity of ¹⁵N in the winter samples was greater than in the summer samples, which confirmed that ammonia volatilization occurred to a higher degree in the winter study than in the summer study. The results presented in Table E.1 for the summer study show no increase in δ¹⁵N in the weeks following the stable isotope tracer addition. There may have been an elevated level of ¹⁵N in the first few weeks of the winter study but, due to complications in sample recovery, discussed in the previous chapter, (where no samples were analysed for the first three weeks of the experiment) it was not possible to ascertain this.

Rockne and Brezonik (2006) demonstrated the importance of ammonia volatilization in cold climates, as a valuable mechanism for permanent nitrogen removal from their systems. However, other prominent studies detailed in Chapter 2 (Shilton, 1996; Zimmo *et al.*, 2003; and Caicedo Bejarano, 2005) found the volatilization of ammonia to be insignificant with respect to total nitrogen removal. The findings from the two studies presented in this research, reveal that the contribution volatilization makes as a mechanism for nitrogen loss from PFP's within the UK, is not really very important, and any loss from the system via this mechanism must be taken as a bonus, rather than any significant N-removal phenomenon. Certainly, some degree of volatilization will always occur, purely

because of partial-pressure gas laws, temperature and pH gradients operating within a pond, but, as Shilton (1996) deduced, this study too provides evidence that it can be discounted as a major participatory mechanism (as was also found by Camargo Valero, 2008, for maturation ponds).

It is important to note that gaseous nitrogen removal from a WSP is not solely facilitated by the volatilization of ammonia, and in hindsight it would have been prudent to have measured NO_x and N_2 , among other gases, and their ^{15}N fractions, to determine the contribution of these to overall nitrogen removal from the system. The capture of $^{15}\text{N}_2$ would certainly have helped to elucidate the importance of denitrification within a PFP environment as a permanent nitrogen removal mechanism. Further study might have enabled the isolation of nitrifiers and denitrifiers to establish nitrification and denitrification rates, and map their spatial distribution within a PFP.

6.4 Pond hydraulic performance

As discussed in Chapter 5, the shape of the summer and winter RWT RTD curves were noticeably different. In both instances concerning the winter experiments, peak washout concentrations of the dye tracer were observed very shortly after spike introduction – an indication of severe hydraulic short-circuiting (Shilton and Harrison, 2002). Conversely, it took ~15 days for the peak concentration to appear in the summer spike.

The dispersion numbers obtained, were similar to those reported in the literature describing other hydraulic tracer studies undertaken on facultative WSP's; for example, Dorego and Leduc (1996) produced dispersion numbers of 0.392, 0.452 and 0.283 for three facultative ponds in Quebec, Canada, although it must be noted that these ponds were aerated facultative ponds, and undoubtedly the flow within the system, and thus the results, would have been influenced by the aeration processes. Marecos do Monte and Mara (1987), using Sulpho-rhodamine B as a dye tracer, found dispersion numbers of 0.595, 0.371, 0.574 and 0.523 from studies conducted on two facultative WSP in Portugal. Tracer studies within this research were conducted in parallel: once on each pond in the winter, and once on each pond in the summer. Dispersion numbers obtained in the winter months

were slightly larger than those calculated for the summer months. Marecos do Monte and Mara (1987) attributed these differences to the possibility of a greater degree of wind-induced mixing occurring in the winter months, and also to regular pond water turnover, which transported large sludge flocs from the base to the surface of the pond. The Esholt PFP's, similar to the two ponds studied in Portugal, were also heavily laden with solids flocs rising from the anaerobic sludge layer, although the site observations recorded in Table 5.7 show also that a degree of sludge feedback was occurring in the summer experiment as well.

The cumulative data obtained by these studies, and this research, strongly indicate that a high degree of dispersion occurs in facultative ponds, and that they operate somewhere in the region between the two ideal flow regimes. Torres *et al.* (1998), in their study of the internal hydrodynamics of three full-scale facultative WSP in Murcia, southern Spain, found a good graphical agreement of their experimental data with perfectly mixed reactor data, and therefore deduced that the ponds behaved as perfect mixed tank reactors. The shape of their RDT curves are similar to the winter 2007 RDT curves shown in Figures 5.41 and 5.42 of this research; however, their study does not provide dispersion numbers to validate their proposition that the ponds they studied operated as a completely mixed flow regime.

The relatively low recovery factor of RWT in each of the three runs presented in this thesis suggests a possible source of error in the experiments, from which it is inferred that the results obtained may not be representative of the actual mixing regimes of the Esholt PFP's. Hydraulic tracer studies are notoriously difficult to replicate in the natural environment, as many different influences and indeterminate environmental variables affect the outcome. Vassel (2009) commented, that a recovery factor of RWT below 50%, was poor in a hydraulic tracer study, and that ideally recovery should be higher, at around, and preferably above, 90% (in all three experimental runs presented here, all of the recovery factors measured less than 12%).

Although the total flow entering the system was rigorously controlled, Tables A.3, A.4 and A.5 show some variability in the weekly influent flows; furthermore,

flow variability at the effluent point of the ponds also differed from that at the influent, as these were corrected for net rainfall and evaporation. Shilton and Sweeney (2005) noted that fluctuating flows within the pond environment can result in erratic results, which inevitably introduce error in the calculation of the dispersion number. Indeed, if the dye slug had been introduced under different circumstances and the recovery factor were greater, it cannot be assumed that the mixing characteristics would have replicated those obtained therein.

Although RWT is recommended as a suitable dye tracer for use in a multitude of environments, yet Smart and Laidlaw (1977), and Vasudevan *et al.* (2001) have discussed a number of different environments where the transport of RWT throughout a system is comprehensively analysed. Indeed, their study reveals that RWT has some sorption capabilities on soil and organic matter as a function of time, pH and aqueous RWT concentration. Rates of adsorption and the fate of RWT have not been investigated within this research, but it is highly probable that the tracer studies were adversely impacted by: (a) the daily fluctuations in pH, (b) temperature, and (c) the abundance of many different types of organic particles and colloids within the PFP system.

The flow rate through the pond was very low, and Shilton and Harrison (2002) point out that at reasonable flow rates, the larger dye molecules will stay suspended in the smaller water molecule matrix; however, if a highly concentrated dye is introduced into slow flowing waters, the dye might sink. This action was certainly evident when the sludge buckets were collected at the end of each experiment: the sludge was coloured deep purple. Rhodamine WT is only marginally denser than water, but with hindsight, the small volume of liquid solution used for spike injection (~100 ml) containing a high concentration of RWT, and the isotope ammonium salt, may have been much denser than the pond water, so causing the RWT to sink upon introduction.

To overcome some of these problems, Vassel (2009) proposed that it was acceptable to dilute the RWT tracer solution, and introduce the entire amount as a pulse, at a sustained flow rate in combination with the influent wastewater, over a time period equal to ~2% of the theoretical hydraulic retention time. Shilton and

Harrison (2002) also note that RWT should be diluted in 10–12 litres of pond water prior to addition, to minimise density and temperature differences between the liquid dye and the pond water. For the experimental PFP's used herein with their set θ_0 , this would equate to 14.4 hours, just over half a day.

The results obtained in section 5.6 strongly suggest that the stable isotope tracer follows the same trajectory of the RWT through each PFP system. As has been mentioned, it is highly likely that the $^{15}\text{NH}_4\text{Cl}$ salt affected the passage of RWT in the ponds; it follows therefore that the RWT may have affected the passage of the $^{15}\text{NH}_4\text{Cl}$ (Vasel, 2009). If the ponds had been spiked exclusively using $^{15}\text{NH}_4\text{Cl}$, the results might have been different from those gained in the present studies. It would be prudent in future experiments, to spike the ponds independently, first with RWT, and second with the nitrogen stable isotope, to avoid conflict between the two tracers.

6.5 Stable isotope tracer mass balances

6.5.1 Winter 2006

The graphical data presented in Figure 5.45, showing $\delta^{15}\text{N}$ values for the initial experimental run of winter 2006, reveal that the largest ^{15}N fraction to leave the pond was the unchanged ^{15}N -ammonium. The next largest $\delta^{15}\text{N}$ peak was from ^{15}N -nitrate, and the suspended and soluble organic nitrogen fractions portrayed similar curves. In this initial experimental run, the shape of the normalised ^{15}N -ammonia curve in Figure 5.36 did not follow the normalised RTD curve of the RWT tracer. However, the sharp increase of ^{15}N -ammonia passing out of the effluent point within the first 24-hour period, indicates that a very high degree of hydraulic short-circuiting took place. Therefore a large fraction of ammonia was not treated at all within the system: this ammonium nitrogen was not removed but rather lost from the system as it exited untreated. This has implications for the engineering design of these systems, when with inlet and effluent points optimally positioned, and the aid of baffles or wind-induced mixers, the internal hydrodynamics of the pond could be altered to achieve higher removal efficiencies.

At first glance, the $\delta^{15}\text{N}$ data from the first experimental run shows that the rapid oxidation of ammonia to nitrate, is an important nitrogen transformation process which occurs in colder winter months. The peak nitrate $\delta^{15}\text{N}$ value occurred very rapidly, within the first 24-hour period after spike injection. As already discussed in section 5.7, when the $\delta^{15}\text{N}$ data are converted into actual concentration data, the concentrations are comparatively low when contrasted with the large curve presented in Figure 5.45. It is very difficult to pinpoint the exact nitrification mechanisms which were operating within the PFP during the winter 2006 experiment, as no molecular microbiological experimental work was undertaken on these samples. The shape of the nitrate peak is similar to the ammonium peak, and the rapid decrease in pond nitrate at the effluent point is largely attributed to washout from the pond. In part, this decrease may be attributed to denitrification processes, and, as observed in the molecular microbiological analysis conducted in the second and third experiments, denitrifiers were found to be present in both summer and winter pond environments.

The decrease in ^{15}N -ammonium happens, as an increase in ^{15}N -suspended organic nitrogen occurs, and this coincides loosely with a gradual increase in algal biomass noted by the rising chlorophyll *a* concentrations shown in Figure 5.11. It is, therefore, hard to distinguish if this increase is attributable to a delayed assimilation rate of algal uptake of the inorganic nitrogen fractions (where algal metabolism is appreciably slower in the winter than in the summer), or is a function of increased algal growth, and therefore assimilation, within the system.

The transformation of inorganic ^{15}N into the soluble organic fraction is interesting, as this rate is much faster than ^{15}N uptake into suspended organic forms. It is highly probable that soluble organic nitrogen is more readily detectable in the pond effluent, initially because of a combination of the degradation of dead nitrifiers, and dead algae, which would recycle a soluble form of organic nitrogen to the system.

6.5.2 Summer 2006 and winter 2007

The results shown in Figures 5.50 and 5.51 reveal the marked changes that the labelled ammonium underwent in summer and winter seasons. The passage of

ammonium leaving the system at the effluent point as an unchanged ^{15}N fraction from the $^{15}\text{NH}_4\text{Cl}$, corroborates the RWT RTD curves shown in Figures 5.40 and 5.43 for summer and winter, respectively.

The maximum peak concentrations of $\delta^{15}\text{N}$ (‰), the actual ^{15}N concentration within each sample, and the time elapsed in which these peaks occurred, are given in Table 6.1 for the summer 2006 and winter 2007 experimental runs only. In both these runs, the peak of ammonium leaving the system occurred exactly two days after the RWT peak passed out (summer 2006: RWT peak at 15 days, $^{15}\text{NH}_4\text{-N}$ peak at 17 days; and winter 2007: RWT peak within first 5 hours, $^{15}\text{NH}_4\text{-N}$ peak at 2 days).

When the $\delta^{15}\text{N}$ ammonium values were converted into actual ^{15}N concentrations (Figure 5.54), the peak concentration measured 34.8 $\mu\text{g/l}$ in summer 2007 and 135.6 $\mu\text{g/l}$ in winter 2007 – i.e., over four times as much leaving the pond unchanged in winter compared to summer. Although the PFP was fully facultative before the beginning of the winter spike, the presence of *Daphnia* rapidly consumed the pond algae, and chlorophyll *a* concentrations were almost identical to those observed in the first winter experiment. Under true facultative operating conditions, the passage of ammonium throughout the system may exhibit the same pattern as the two winter ammonium curves presented in this research; however, the notable absence of algae removes the fundamental assimilatory pathway that would, under facultative conditions, be responsible for the uptake of ammonium from the system.

The summer 2006 and winter 2007 experiments are compared and contrasted as they were conducted within the same PFP, using the same concentration of $^{15}\text{NH}_4\text{Cl}$.

Interestingly, the suspended organic nitrogen $\delta^{15}\text{N}$ values (shown in Figures 5.50 and 5.51) for summer and winter show similar trends. The $\delta^{15}\text{N}$ peak value for summer 2006 occurred at 17 days, and measured 1709.6 ‰. The ^{15}N peak value for winter 2007 was not much lower, measuring 1443.8 ‰, but occurred at 11 days. However, when the actual ^{15}N concentrations for these peaks are compared

(Figure 5.52), the ^{15}N incorporated into the maximum summer 2006 value measured 25.98 $\mu\text{g/l}$, compared with a maximum concentration of 9.54 $\mu\text{g/l}$ for winter 2007 – more than double.

Passages of the soluble organic nitrogen fraction between summer and winter data sets, also showed broadly similar patterns. The $\delta^{15}\text{N}$ peak height of summer 2006 (910.8 ‰) is questionable: it is possibly an anomaly from the main data set. The second highest value is 749.8 ‰, which occurred 24 days after spike injection. The maximum $\delta^{15}\text{N}$ value for winter 2007 was 644.1 ‰, and this occurred 19 days after spike injection. The trends for summer and winter soluble organic nitrogen fractions also correlate well, the bulk of the peak area occurring in 0 – 10₀, with very similar peak values. However, once $\delta^{15}\text{N}$ values are converted into actual ^{15}N concentrations (shown in Figure 5.53), it can be observed that over twice as much ^{15}N was incorporated into soluble organic nitrogen in the summer than in the winter (6.49 $\mu\text{g/l}$ and 2.59 $\mu\text{g/l}$ in summer and winter respectively).

Table 6.1: Summary of the maximum $\delta^{15}\text{N}$ values, corresponding ^{15}N concentrations per nitrogen fraction, and the elapsed time at which these peaks occurred in summer and winter.

Nitrogen fraction	Summer 2006			Winter 2007		
	$\delta^{15}\text{N}$ (‰)	^{15}N ($\mu\text{g/l}$)	Elapsed time of peak height (d)	$\delta^{15}\text{N}$ (‰)	^{15}N ($\mu\text{g/l}$)	Elapsed time of peak height (d)
Suspended organic nitrogen	1709.6	25.98	17	1443.8	9.54	11
Soluble organic nitrogen	910.8	6.49	29	644.1	2.59	19
Ammonium nitrogen	2415.3	34.76	17	5454.4	135.60	2
Nitrate nitrogen	740.2	0.31	13	4555.4	3.87	2

6.5.3 Comparison between this research and the literature

There is little published work which has attempted to differentiate between nitrogen removal mechanisms and pathways in WSP's, according to seasonal variations in temperate climates, and this makes comparisons difficult. However, comparative conclusions can be drawn between this research and the findings presented by authors conducting nitrogen research in WSP's in other parts of the world.

To date, Reddy (1983) provided the only other research which has used the stable nitrogen isotope method to trace the passage of, and transformation in $^{15}\text{NH}_4^+$ throughout a WSP. In the macrophyte ponds studied, the majority of the $^{15}\text{NH}_4^+$ was taken up by the plants, but in a control pond containing no plants, 4.6% of the added $^{15}\text{NH}_4^+$ was assimilated by the algae. A value only slightly higher than this was obtained for the winter 2007 experiment, where 8.13% of the total nitrogen spike was recovered as suspended organic ^{15}N , and this value was lower still for the winter 2006 experiment where the recovery factor of the same nitrogen fraction was only 1.91%.

In a mass balance approach undertaken by Zimmo *et al.* (2004), results for an algae-based WSP in Palestine, operating in cool temperatures similar to those in the UK, found just over 40% (the largest fraction) of the influent nitrogen fed to an algae-based WSP was washed out of the system in the pond effluent. In all three experimental runs conducted here, ammonium was also found to be the largest fraction leaving the PFP's, in both summer and winter seasons. The second largest removal from the system (just under 40%) was sedimented as pond benthos, and the third largest loss (~15%) was deemed to be through denitrification. Denitrification was not quantified within this research, but the sedimented nitrogen fraction, in all three experimental runs, was found to be sizeable, when the actual mass balances are analysed. Conversely, the ^{15}N mass balances reveal that the incorporation of the labelled ammonium into the sludge layer – by whichever mechanism or pathway – was not as great (although the winter 2006 contribution of ^{15}N to the sludge was not quantified). The summer 2006 experiment shows that only 1.4% of the ^{15}N spike was incorporated within

the pond benthos. Picot *et al.* (2009) found that sedimentation rates of nitrogen were higher in cooler periods of the year in the Mèze WSP in the south of France (before 31st May, where a mean value of 7.5 kg N/d was established) than in the warmer summer period (the mean average was given as 4.7 kg N/d). The seasonal differences in sedimentation cannot accurately be validated in this work, because of the limitations of the size of the data set.

In both winter and summer studies, ammonia volatilization was negligible, supporting the findings of Ferrara and Avci (1982), Shilton (1996), and Zimmo *et al.* (2004) who also concluded that ammonia volatilization accounted for a very small fraction of total nitrogen removed from their respective WSP's.

6.5.4 Summary of nitrogen mechanisms and pathways within the Esholt PFP's

This research has shown that ammonium nitrogen is rapidly taken up by the pond biomass (mainly algae) and assimilated into cell material, thus undergoing transformation from inorganic nitrogen to an organic fraction. This is subsequently released, either as soluble organic nitrogen, a by-product of cell metabolism, or by the degradation of cells, the result of algal mortality. More than double the amount of ¹⁵N was taken up in the suspended organic nitrogen fraction in summer than in winter, and just under five times as much ¹⁵N appeared as soluble organic nitrogen in summer. As temperatures and insolation are higher in summer than winter, and thus cell synthesis and metabolic functions occur faster, it is natural that a high proportion of the influent ¹⁵NH₄Cl should be absorbed very quickly by the components of the pond biomass that use inorganic substrates as their nutrient source. This is reflected by both the higher $\delta^{15}\text{N}$ and ¹⁵N concentration values for suspended organic nitrogen apparent in the summer data set. Preliminary findings for the summer experiment show therefore that a large proportion of the ¹⁵NH₄Cl left the pond unchanged, but that pond biomass does play a very important role in the uptake of influent ammonium nitrogen within the system.

In summary for each of the experiments, the following mechanisms and pathways played the most prominent roles in nitrogen removal/transformation within the Esholt PFP's:

Winter 2006:

- 1) Washout of ammonium measured at the pond effluent;
- 2) Biological uptake into suspended organic-N; and
- 3) Biological uptake into soluble organic-N Sedimentation of organic-N

Summer 2006:

- 1) Sedimentation of organic-N (according to the chemical mass balance);
- 2) Washout of ammonium measured at the pond effluent; and
- 3) Biological uptake into suspended organic-N

Winter 2007:

- 1) Washout of ammonium measured at the pond effluent;
- 2) Biological uptake into suspended organic-N; and
- 3) Biological uptake into soluble organic-N

6.6 Nitrification vs. algal assimilation in winter and summer

The data provided by the ^{15}N tracer studies in this research, indicated that the oxidation of nitrogen occurs to a significantly larger extent in the winter months. If Figures 5.45 and 5.51 are studied (which show graphical data for the winter 2006 and winter 2007 experiments, respectively) very rapid conversion of the $^{15}\text{NH}_4^+$ to $^{15}\text{NO}_3^-$ occurs within the first 24-hour period. The peak nitrate concentration occurred within the first 24-hour composite sample analysed in the winter 2006 experiment, and within the first 48-hour period in the winter 2007 experiment. If this is contrasted with the summer 2006 experiment (the data for which are shown in Figure 5.50), the difference between seasons is stark. The oxidation of $^{15}\text{NH}_4^+$ does happen in summer too; it also occurs instantaneously, yet to a much lesser extent. In all of the three experiments run, it must be noted that the total quantity of $^{15}\text{NO}_3^-$ recovered from the PFP's was very low, measuring only a fraction of a percent (0.3% recovered in winter 2006, 0.24%

recovered in summer 2006, and 0.99% recovered in winter 2007). Recovery was quantified as amount of ^{15}N contained within the nitrate fraction, measured in the composite samples collected from the pond effluent point. This clear evidence that nitrification processes predominate in winter months in the Esholt PFP's, goes against the findings of much of the other nitrification research published on WSP.

Studies have found evidence that nitrification does occur within WSP. Reddy (1983), in his ^{15}N tracer work found that significant quantities of $^{15}\text{NH}_4^+$ spike were converted to $^{15}\text{NO}_3^-$ within his pilot-scale WSP, especially in a pond supporting Water Hyacinths, another pond supporting Cattails and Elodea growth, and a control pond. This provides evidence of active nitrification occurring within these systems. These studies were conducted within WSP in Florida, but unfortunately no indication is given as to the time of year, and no temperature data supports the study.

Hodgson and Paspaliaris (1996), Hurse and Connor (1999), Picot *et al.* (2005) and Picot *et al.* (2009) observed the effect that seasonality has on ammonium removal within WSP: in all instances, ammonium removal was higher in the warmer summer months than the winter months. Picot *et al.* (2005) found that nitrification, coupled to denitrification, played a major role in removing nitrogen within maturation WSP in the south of France in warmer periods, at one point culminating in a peak concentration of 5 mg/l in October 2003 (an even higher peak concentration was recorded in June 2004), but decreasing as the temperature dropped, moving into the month of November. This research found that anaerobic ponds and secondary facultative ponds removed only a very little amount of nitrogen from their respective systems. The explanation provided for nitrification within the maturation ponds was that in October, as temperatures decreased, denitrification became limited and therefore nitrate concentrations increased; however, nitrate subsequently decreased in November which was coupled with an increase in ammonium. Subsequently, Picot *et al.* (2007) provided evidence for denitrification occurring in the same maturation ponds, with N_2O biogas being released from these. These patterns were not observed in the Esholt PFP's. Admittedly, with respect to organic nitrogen and ammonium concentrations, the

quantity of nitrate contained within the ponds was minimal, yet some interesting seasonal trends appear to have occurred.

The pattern of seasonal nitrification within the Esholt PFP's is interesting. Looking only at the $\delta^{15}\text{N}$ data plots, in both instances, the oxidation of the labelled ammonium nitrogen was instantaneous, producing the second largest peak $\delta^{15}\text{N}$ values behind the largest of ammonium. These values were recorded at 292.2‰ for winter 2006, and 4555.4‰ for winter 2007. When these values were converted into concentration data (Figures 5.49 and 5.55), the concentrations of ^{15}N were quite low – this is possibly because of the dilution effect upon the ^{15}N throughout the system. The very high peak $\delta^{15}\text{N}$ values, occurring within the first 48 hours, disappeared almost as quickly as it had occurred: this is indicative that some other transformation process happened to the nitrate within the system almost as soon as the nitrogen fraction was oxidised from $^{15}\text{NH}_4^+$. None of these patterns in the data is observed within the summer 2006 data set.

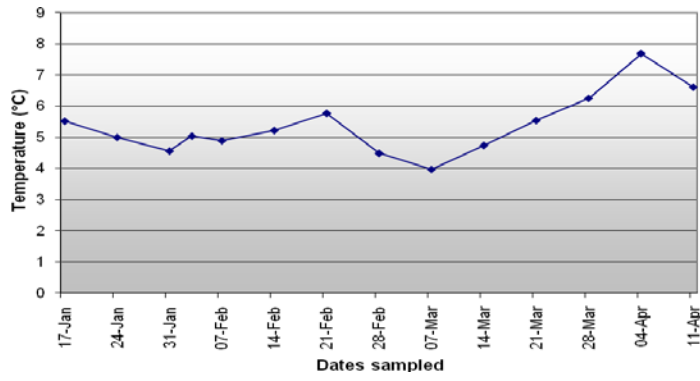
The following observations provide evidence to suggest that nitrification, and then denitrification, was occurring within the PFP's during the winter months:

1. The $\delta^{15}\text{N}$ signal of nitrate in the pond effluent was initially very high, but dropped rapidly, and there was no accumulation of nitrate within the pond effluent.
2. There was no accumulation of nitrate concentrations within the pond water column (Figures 6.1 (c) and 6.2 (c)), but the labelled ^{15}N studies confirmed that active nitrification pathways were operating.
3. Figures 5.8 and 5.10 show that there were, initially, very low concentrations of dissolved oxygen within the water columns, but these could have supported ammonia oxidising archaea and ammonia oxidising bacterial communities.
4. Only very low quantities of ammonia volatilization occurred within the ponds in winter time (and even less in summer time), and there is no ^{15}N enrichment of the gas samples stripped from the pond surface, strongly suggesting therefore that ammonia oxidisers utilised the ammonium within the system faster than it could be volatilized, and the volatilization mechanism is out-stripped.

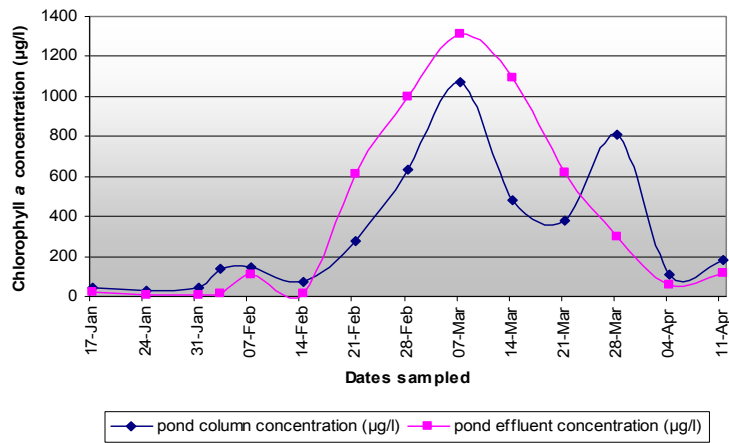
Figure 6.1a shows the mid-depth pond temperature taken at the time the corresponding grab samples were collected. The data presented in Figures 6.1a and 6.1b showing in-pond chlorophyll *a* and nitrate data are very interesting indeed. Algae have been shown readily to be the prime removers of ammonium within a WSP, and it is sensible to theorise that as algal activity within a WSP increases, they would out perform any bacterial (or other) community to sequester available ammonium for cell assimilation and metabolic processes. Figures 6.1b and 6.1c portray different findings. When the two data sets are compared and contrasted, it is clear that the production of nitrate follows exactly the same trend as the in-pond and pond effluent chlorophyll *a* concentrations, with two peak concentrations of nitrate and chlorophyll *a* occurring at exactly the same times in the second half of the experiment. These occurrences are not influenced apparently, by variations in pond temperatures.

Figures 6.2 (a), (b), and (c) show mid-pond depth temperature data, chlorophyll *a* concentrations, and in-pond nitrate concentrations, respectively, for the second winter experiment. Record of readings suggest that there is a somewhat different trend here when contrasted with the data of winter 2006. The in-pond production of nitrate within the PFP (concentrations of which were very low in comparison to ammonia data, for example) appears to be independent of both temperature and chlorophyll *a* concentrations, which indicates that a different nitrification mechanism was then active within this system. Chlorophyll *a* concentrations throughout the entirety of this experiment were extremely low, and therefore competition for available ammonium from algal biomass, would be significantly frugal, possibly leaving an available opportunity for another species to utilise the abundant supply. Three main peaks occur within the data observable in Figure 6.2 (c): one, two weeks after spike injection, a second, much smaller peak approximately half way through the experiment, and the third – the largest peak, one week before the end of the experiment.

(a)



(b)



(c)

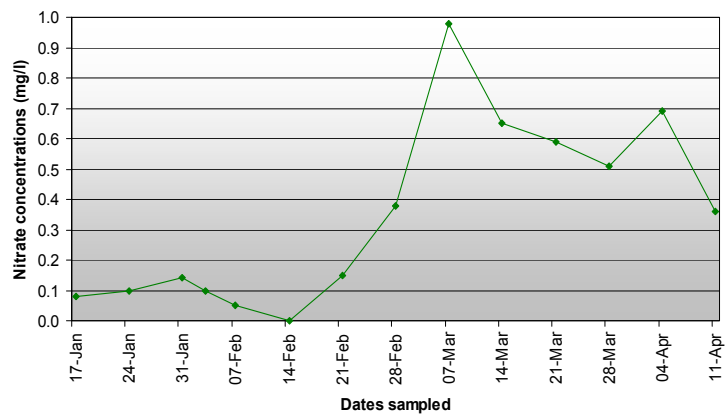
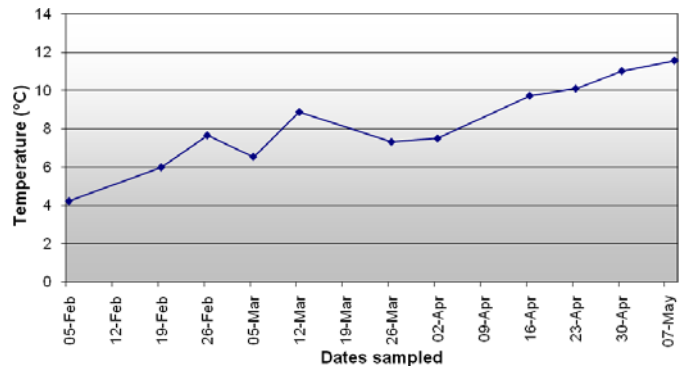


Figure 6.1: (a) winter 2006 pond mid-depth (75 cm) temperatures; (b) chlorophyll *a* column and effluent grab sample concentrations; (c) column grab sample nitrate concentrations.

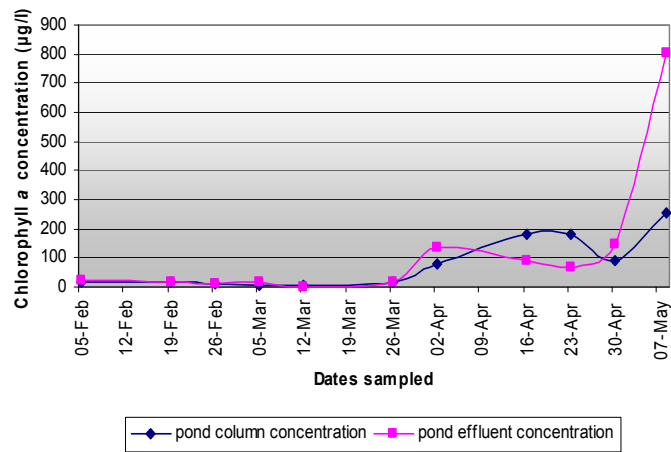
The corresponding ORP and dissolved oxygen data, discussed within sections 5.3.3 and 5.3.4, show that largely anoxic in-pond conditions prevailed for the majority of the winter experimental runs, with a recovery towards facultative conditions restored only towards the end of both experiments. Unfortunately, as already mentioned, no molecular microbiological work was undertaken on the winter 2006 samples, however the data to support nitrification and denitrification, can be found within the results presented for various nitrogen oxidising and nitrogen reducing communities, described in the results section 5.8 for the winter 2007 experiment. The PCR data shows that there were both AOA and AOB present, in the samples taken and analysed in the winter 2007 experiment (see Table 5.18), which demonstrates that aerobic nitrification could be taking place within the pond. The presence of anammox within the raw sewage influent and pond sludges, implies that these strict anaerobes may have been utilising some influent ammonium, as well as recycling some of the ammonia produced by the anaerobic sludges. The presence of denitrifiers (observed in the PCR analyses), widespread within the system, provides evidence that denitrification could be taking place throughout the whole pond environment. Indeed, when anoxic conditions prevailed within the ponds, every opportunity existed for the vertical migration of denitrifying communities expanding into the upper strata of pond water (they were strongly detected throughout the water column), thus increasing their territory, and possibly their activity within the pond.

A little harder to explain, is the rapid oxidation of ammonium to nitrate, within anoxic conditions. AOB and AOA both provide pathways for the aerobic oxidation of ammonia. Recently, Schmidt *et al.* (2002) have commented that, in low oxygen and anoxic environments, AOB have the ability to denitrify. It is possible therefore, that AOB communities residing at, or very near, the pond's surface, oxidise a portion of the influent ammonia, while at deeper layers, AOB communities present there revert to denitrification.

(a)



(b)



(c)

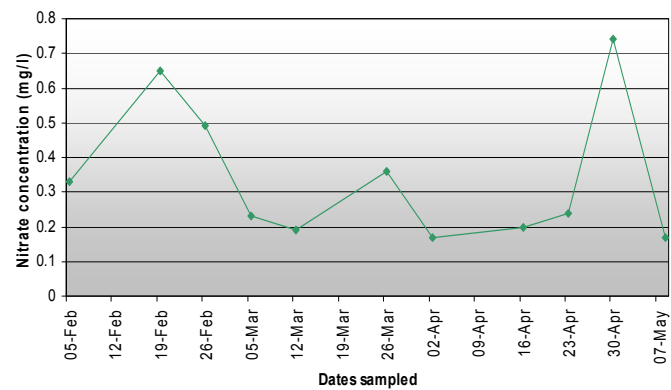


Figure 6.2: (a) winter 2007 pond mid-depth (80-cm) temperatures; (b) chlorophyll *a* column and effluent grab sample concentrations; (c) column grab sample nitrate concentrations.

It is possibly no surprise that anammox were present in the PFP and were found to be confined to the anaerobic depths of the sludge layers. Knowledge of their established presence within the pond ecosystem is useful, but actually conjures up more questions than answers. That they are present, does not necessarily mean that they are active. If they are active, questions arise about their rate of metabolic function and how quickly they can oxidise ammonium, their reaction rate coefficients, and species numeration, so that a net loss of ammonium from the system can be factored into the complete mass balance calculations. The present difficulties apparent in culturing the anammox group in a laboratory (Kuenen and Jetten, 2001) rule out the possibility of obtaining relevant data for this organism.

Anammox species were shown to be confined solely to sludge environments within the pond, and the level of their activity within the system could not be gauged. It is highly unlikely, that, given their spatial distribution, anammox species were solely responsible for the oxidation of ammonium within the PFP environment, especially given the pond hydraulic regime and mixing characteristics. In the last few years, a new concept in sedimentary geochemistry has challenged traditional views of ammonium oxidation within certain environments. The process of anoxic nitrification executed by species different from those which are involved with anammox processes, provides a new explanation for the disappearance of ammonium within anoxic environments. This is certainly a credible explanation for the oxidation of ammonia during highly anoxic in-pond episodes. Aller *et al.* (1998), Hulth *et al.* (1999), Anschutz *et al.* (2000) and Mortimer *et al.* (2004) are among the researchers who have found that anoxic nitrification occurs in solid phase marine sediments. Here ammonia is oxidised to nitrate, and a microbiological group reduces manganese oxides using the oxygen as a terminal electron acceptor (Bartlett, 2005). Research into anoxic nitrification comprises a relatively new field, and very little is known about the microorganisms responsible for this reaction. Just as anammox bacteria have been found in a multitude of freshwater and marine environments (Dalsgaard and Thamdrup, 2002; Dalsgaard *et al.*, 2003), it may be that other ammonia oxidisers pertinent to anoxic environments, and not measured in this study, have the ability to utilise ammonium within a PFP environment.