Chapter 5 ~ Results

5.1 Introduction

This chapter presents the results obtained from the Esholt ponds, over the three-year research period. The main focus of the results is on the stable isotope tracer studies, and their supporting chemical data, which consumed the bulk of the research time both on site and in the laboratory. The results are set within the context of overall pond performance, and the physiochemical conditions operating within the pond during each experimental run. Sludge accumulation rates are presented, and their relationship with pond performance discussed. Ammonia volatilization is also quantified in terms of a viable mechanism for ammonia-nitrogen removal from the pond system, and volatilization rates are calculated. The hydraulic characteristics and performance of the ponds are also reported, and normalised residence time distribution (RTD) curves are contrasted with the normalised concentrations of ammonium-nitrogen from the stable isotope work. Finally, findings from the molecular microbiological analysis are presented, and correlated with the chemical data, to portray the bacterial microbiological make-up of the PFP's.

5.2 Routine sampling and pond performance

The operation of the Green and Blue PFP's during the research time can be divided into five main periods, which are presented in Table 5.1.

Both the Green and Blue PFP's were operating as healthy facultative ponds when they were taken over in November 2004. The Green PFP was operating with a load of 120 kg/ha d and a theoretical HRT of 60 days, and the Blue PFP was operating at the UK optimum loading of 80 kg/ha d with a theoretical HRT of 60 days. At the end of April 2005 the Green PFP loading was adjusted to 80 kg/ha d and the theoretical HRT reduced to 30 days in preparation for the first spiked winter experimental run. The Blue PFP was monitored until the first week in February 2006, and on Tuesday 14th February it was desludged. In the weeks running up to this date, the pond was alternating between facultative and completely anoxic conditions, indicating pond failure. It was these prevailing conditions which resulted in the decision to desludge the pond; this eliminated the physical possibility of anaerobism, brought about by the sludge layer, in subsequent experimental runs. Site observations recorded the week before, noted that the pond was almost black, with large sludge mats floating on the surface.

During the winter of 2005/2006, the Green pond did not fare much better. In the run up to the winter 2006 spike, large accumulations of *Daphnia* were recorded, the pond was murky brown in colour, and again there were significant numbers of sludge mats floating on the surface. However, due to the constraints of experimental commitments downstream, within other units comprising the pilot-scale WSP and rock filter system, (also being used to study stable isotope nitrogen transformations), the Green PFP was the only pond at this time which feasibly could be used, even though it was operating at sub-optimal conditions. This first run was therefore used as a pilot test study, to ensure that the stable nitrogen isotope would be detectable in the pond effluent, and also to observe how nitrogen was removed from a failing PFP.

The Blue PFP was refilled with water shortly after desludging, and the correct wastewater/freshwater flows set on Thursday 4th May, providing adequate time for at least three theoretical HRT's to pass, before the scheduled summer experimental run.

The first experimental run was conducted in the winter of 2006, from Friday 20th January until Monday 17th April in the anoxic Green PFP. The second and third experimental runs took place in the facultative Blue pond: the experimental run of summer 2006 was started on Monday 14th August, and continued until Thursday 12th November; the third experimental run conducted during the winter of 2007 was started on Monday 5th February and continued until Sunday 6th May.

Table 5.1: A summary of the operational periods and characteristics of both PFP's during the research period.

Date/Period	Green PFP	Blue PFP
Period 1: October 2004 to April 2005	λ_s = 120 kg/ha d θ_0 = 60 d Ponds monitored continuously	λ_s = 80 kg/ha d θ_0 = 60 d Ponds monitored continuously
Period 2: End April 2005 to December 2005	Pond adjusted to λ_s = 80 kg/ha d and θ_0 = 30 d. Pond monitored continuously	Pond run and continuously monitored, and loaded as above
Period 3: January 2006 to April 2006	1st pilot study spike conducted on this pond, λ_s = 80 kg/ha d and θ_0 = 30 d. After end of experiment, pond monitored until end July 2006	Pond emptied and desludged mid February. Pond refilled during March and April, and restarted properly beginning of May and allowed to re-stabilize
Period 4: August 2006 to November 2006	Pond decommissioned due to anaerobic conditions prevailing; desludging was deemed necessary	Summer spike conducted, λ_s = 80 kg/ha d and θ_0 = 30 d.
Period 5: February 2007 to May 2007	–	Winter spike conducted, λ_s = 80 kg/ha/d and θ_0 = 30 d.

5.2.1 Influent and effluent wastewater characteristics

Figures 5.1, 5.2, and 5.3 present the different fractions of the average blended wastewater and freshwater influent, and BOD_5 and COD components for each experimental run. The influent wastewater and PFP effluent physicochemical characteristics for the weekly sampling regime, collected throughout each of the three experimental runs, are presented in Tables 5.2, 5.3, and 5.4 for winter 2006, summer 2006 and winter 2007, respectively.

From Figure 5.1 it is possible to see that 44% of the influent BOD_5 and 38% of the influent COD were almost instantaneously removed from the wastewater stream, and theoretically settled out via the mechanism of sedimentation to the base of the pond. Of the remaining freely available BOD_5 (56%), and COD (62%), over half of these fractions (56% for BOD_5, and 53% of COD) were in the soluble form, enabling immediate uptake by pond bacteria.

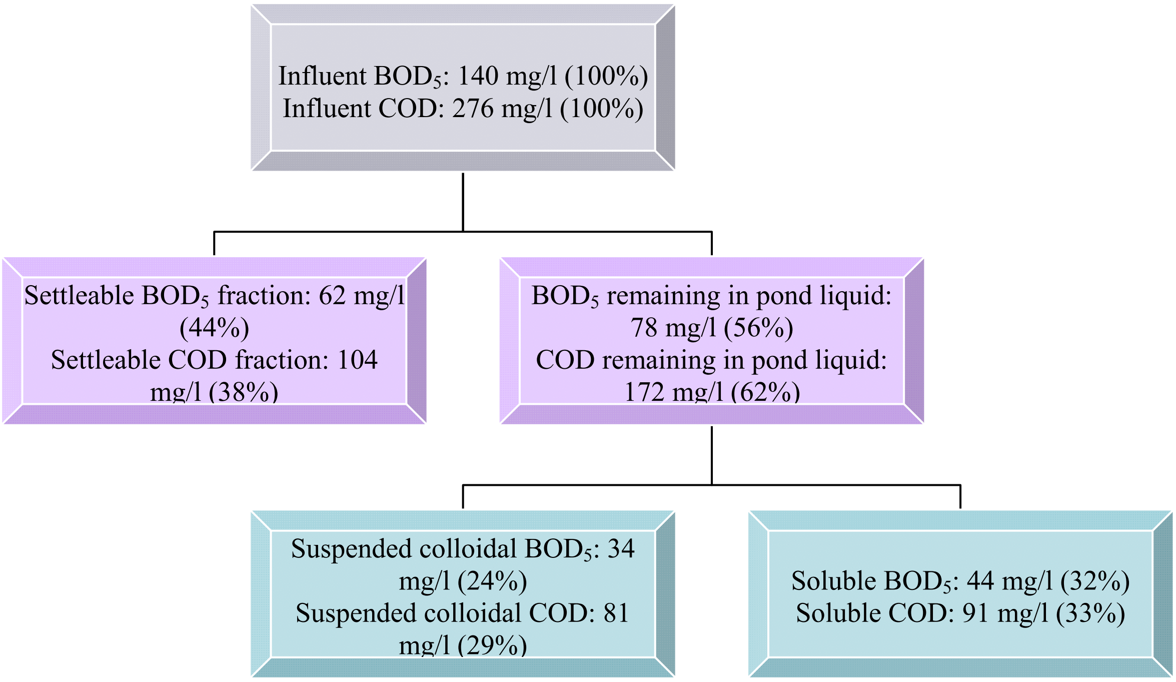


Figure 5.1: The different fractions of influent BOD_5 and COD for the winter 2006 experimental run (conducted in the Green PFP).

Table 5.2: A summary of the influent and effluent wastewater characteristics for the Green PFP during the winter 2006 experiment.

Parameter	Influent	Effluent	Removal
Suspended solids (mg/l)	78 ± 8.7*	37 ± 3.8	53%
Total BOD_5 (mg/l)	140 ± 19.2	28 ± 2.7	80%
Soluble BOD_5 (mg/l)	44 ± 2.8	13 ± 2.3	70%
Total COD (mg/l)	276 ± 37.4	74 ± 10.2	73%
Soluble COD (mg/l)	91 ± 9.6	35 ± 10.2	62%
Ammonium (mg/l)	12.0 ± 1.6	7.3 ± 1.1	39%
Total TKN (mg/l)	21.1 ± 2.2	11.5 ± 0.8	45%
Soluble TKN (mg/l)	15.7 ± 1.7	8.6 ± 0.9	45%
E. coli (cfu/100 ml)	2.78E+06	9.89E+04	2 $\log_{10}$

*parameters are presented as the mean value of all parameters throughout the experimental time, ± the standard error of the mean.

Despite the Green PFP operating anoxically for the first half of the winter 2006 run (see section 5.3), mean removal efficiencies for SS, total BOD_5, COD, and total TKN were high. Other parameters presented in Table 5.2 also show very high removal efficiencies, all of which indicated good pond performance. All effluent concentrations fall within the maximum permissible limits stipulated in the UWWTD, which are shown in Table 1.1 (total SS = 37 mg/l, UWWTD ≤150 mg/l; soluble BOD_5 = 13 mg/l, UWWTD ≤25; total COD = 74 mg/l, UWWTD ≤125; total TKN = 11.5 mg/l, UWWTD ≤15). However, the effluent concentrations obtained for these parameters well exceed the maximum permissible limits set for discharge into salmonid or cyprinid receiving water bodies, outlined in the Fisheries Directive (presented in Table 1.2). The mean influent settleable solids content of the raw wastewater measured 12 ml ± a standard error of the mean of 4.6.

Influent BOD_5 and COD concentrations, presented in Figure 5.2, for the summer 2006 experimental run, are both lower than those measured during the winter 2006 run. The settleable BOD_5 fraction (45%) is similar to the winter 2006 value (44%), but the settleable COD fraction (45%) is higher than for winter 2006 (38%). The available soluble BOD_5 and COD fractions are very similar to those observed in the influent wastewater of winter 2006 (32% and 33% respectively); the summer 2006 soluble fractions of both parameters measuring 32% and 31% respectively.

As with winter 2006 run, all effluent concentrations for the parameters shown in Table 5.3 fall within the maximum permissible limits stipulated in the UWWTD (soluble BOD_5 = 9 mg/l; total COD = 107 mg/l; total TKN = 10.7 mg/l). Again, all effluent concentrations exceeded the maximum permissible limits outlined in the Fisheries Directive, and would not be at all suitable for discharge into these specified waters. The mean settleable solids content of the raw wastewater entering the pond throughout this period, was 18.4 ml ± a standard error of the mean of 1.4.

Figure 5.3 and Table 5.4 present influent and effluent wastewater characteristics for the experimental run of winter 2007. The mean BOD_5 concentration entering the pond, was higher in this experiment, than the mean concentrations observed in both the winter and summer of 2006. The mean settleable BOD_5 and COD fractions were also the highest in this data set.

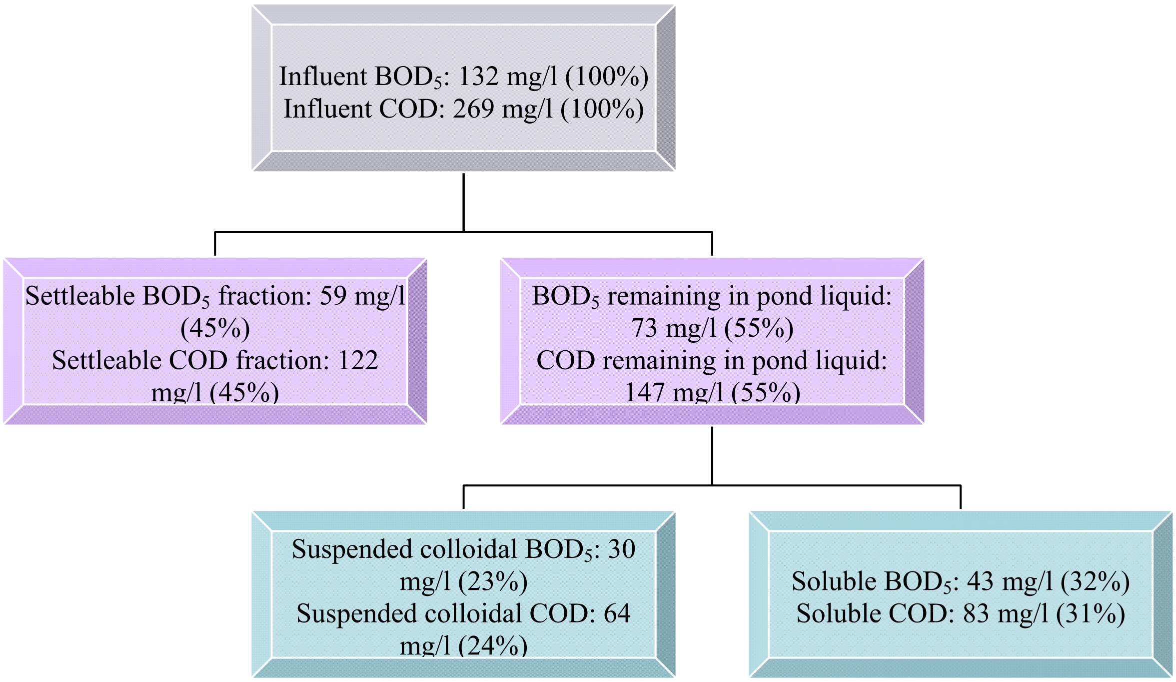


Figure 5.2: The different fractions of influent BOD_5 and COD for the summer 2006 experimental run (conducted in the Blue PFP).

Table 5.3: A summary of the influent and effluent wastewater characteristics for the Blue PFP during the summer 2006 experiment.

Parameter	Influent	Effluent	Removal
Suspended solids (mg/l)	74 ± 12.9*	62 ± 6.5	16%
Total BOD_5 (mg/l)	132 ± 12.3	28 ± 3.2	79%
Soluble BOD_5 (mg/l)	43 ± 4.6	9 ± 3.1	79%
Total COD (mg/l)	269 ± 35.1	107 ± 8.1	60%
Soluble COD (mg/l)	83 ± 10.1	55 ± 9.8	34%
Ammonium (mg/l)	12.5 ± 1.3	4.6 ± 0.8	63%
Total TKN (mg/l)	22.1 ± 1.8	10.7 ± 0.9	52%
Soluble TKN (mg/l)	17.2 ± 1.5	6.4 ± 0.8	63%
E. coli (cfu/100 ml)	1.40E+07	8.66E+04	3 $\log_{10}$

*parameters are presented as the mean value of all parameters throughout the experimental time ± the standard error of the mean.

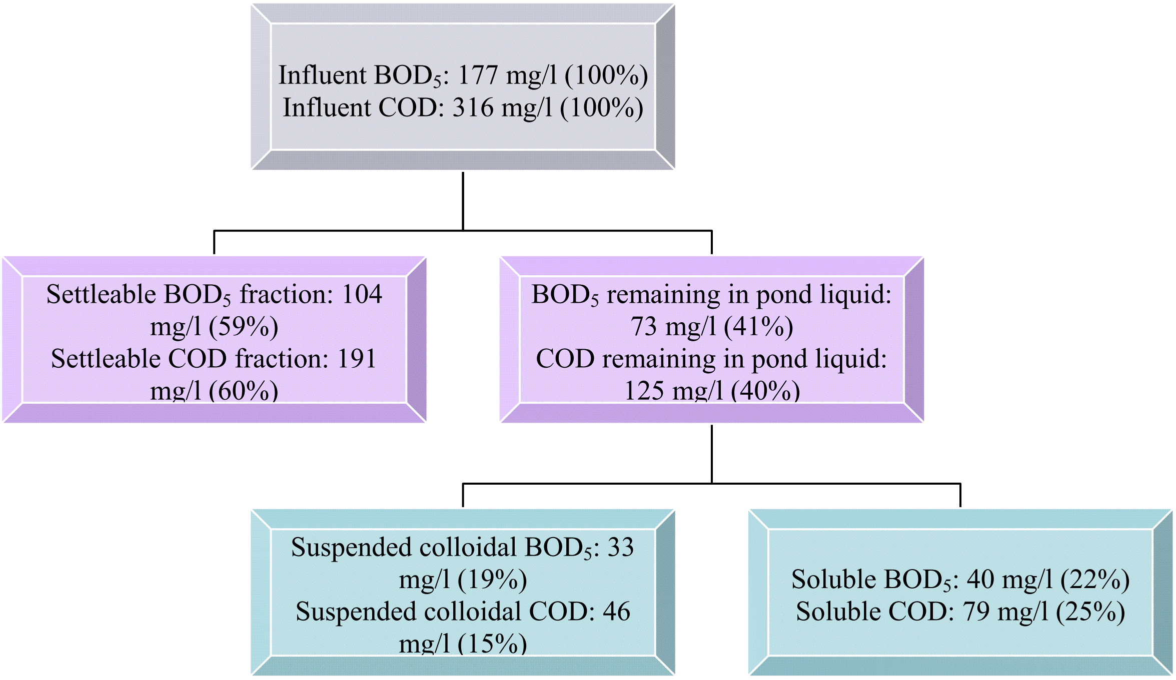


Figure 5.3: The different fractions of influent BOD_5 and COD for the winter 2007 experimental run (conducted in the Blue PFP).

Table 5.4: A summary of the influent and effluent wastewater characteristics for the Blue PFP during the winter 2007 experiment.

Parameter	Influent	Effluent	Removal
Suspended solids (mg/l)	42 ± 6.3*	21 ± 4.7	50%
Total BOD_5 (mg/l)	177 ± 16.0	24 ± 4.5	86%
Soluble BOD_5 (mg/l)	40 ± 4.8	14 ± 2.1	65%
Total COD (mg/l)	316 ± 34.5	83 ± 10.5	74%
Soluble COD (mg/l)	79 ± 14.0	36 ± 6.1	54%
Ammonium (mg/l)	17.1 ± 3.8	7.0 ± 0.6	59%
Total TKN (mg/l)	29.1 ± 2.2	14.9 ± 1.6	49%
Soluble TKN (mg/l)	22.3 ± 2.3	10.6 ± 2.2	52%
E. coli (cfu/100 ml)	1.23E+07	6.46E+04	3 $\log_{10}$

*parameters are presented as the mean value of all parameters throughout the experimental time, ± the standard error of the mean.

This experimental run also revealed good pond performance of removal efficiencies. The effluent concentrations for the total SS (21 mg/l), soluble BOD_5 (14 mg/l), and total COD (83 mg/l) fall well within the prescribed consent limits of the UWWTD. The mean total TKN concentration of 14.9 mg/l provides the only exception in all three data sets. This value is, for practical purposes, equal to the 15 mg/l consent advocated in the Directive. As for the winter 2006 and summer 2006 data sets, all effluent concentrations exceeded the maximum permissible limits outlined in the Fisheries Directive. The mean settleable solids content of the influent wastewater was similar to that measured for the summer 2006 run. The value measured 18.5 ml, with a standard error of the mean of ±1.8.

Figure 5.4 presents a histogram of the mean removal efficiencies for various parameters, measured for each of the experimental runs. Overall, it can be seen that there is little variation between summer and winter data sets for most of the parameters presented. Both total and soluble COD removal is shown to occur to a lesser degree in the summer, as is suspended solids removal. Suspended solids removal in the summer faired poorly against both winter sets, a mean SS removal of 16% recorded for the whole data set. Under healthy operating PFP conditions, this may be explained by a typical increase in algal concentrations throughout the summer months, however, as can be seen in Figure 5.12, Chlorophyll *a* concentrations were not particularly high. The site observations recorded in Table 5.7 reveal that there was a considerable amount of sludge feedback throughout the summer period, this being the most likely cause of increased SS within the pond effluent, therefore impacting the removal efficiency of this parameter. As expected, removal efficiencies for the parameters of ammonium, total, and soluble TKN are higher in the summer period, than in the two winter periods.

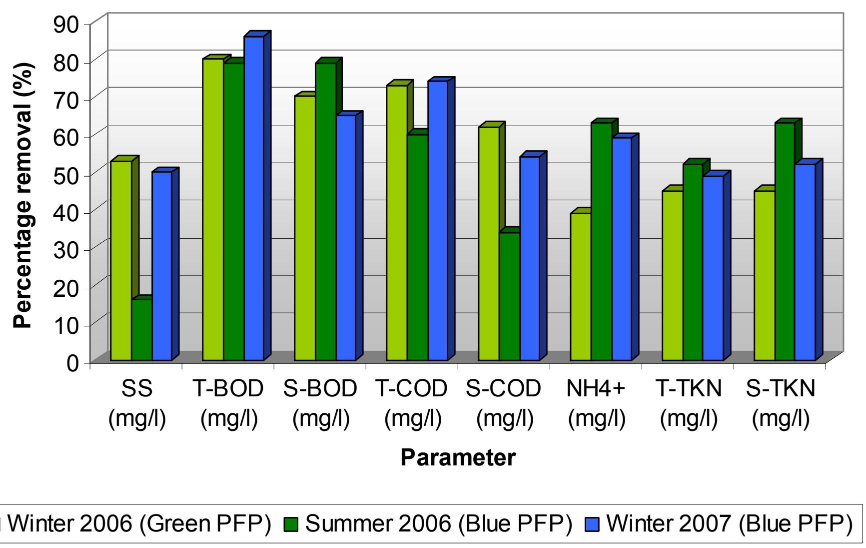


Figure 5.4: Removal efficiencies for various pond performance parameters.

5.3 In-pond studies

The physiochemical environments within the Green and Blue PFP's, during each experimental run, are discussed within this section.

5.3.1 Weather and temperature data

Net rainfall and evaporation, obtained on site at Esholt wastewater treatment works are given in Appendix A, and were used to adjust effluent flow data. The weather data presented in Table 5.5 provides mean monthly air temperatures, among other meteorological parameters. The data were obtained from the Bradford Meteorological Office (Met Office, 2009), which is located 53.7981°N, and 1.7509°W , not too far from the Esholt Wastewater Treatment Works, located 53.5105°N, and 1.4313°W (Abis, 2002).

The data presented in Table 5.5 show that the winter of 2006 was, on average, cooler than the winter of 2007. Thirteen more days of air frost are recorded in the winter of 2006 (using data from February to April, and excluding data from January 2006 and May 2007), and the hours of total sunshine are fewer than those recorded for winter 2007.

5.3.2 Site observations

The visual inspection of a WSP can very often provide an immediate assessment of its operating conditions. Site observations were logged each week and accompany the grab samples collected. These observations are recorded in Tables 5.6, 5.7, and 5.8 for winter 2006, summer 2006, and winter 2007 respectively.

Prior to the commencement of the winter 2006 experiment, the Green PFP smelled very strongly of H_2S, was almost black in colour, and had large deposits of sludge on its surface. These factors suggested a very anoxic, or possibly anaerobic, pond. On the day of spike injection, there was no improvement in pond condition. One week after spike injection (on Tuesday 31st January), no odour was present, but the pond remained apparently anoxic, at least in appearance until the Tuesday 21st February, when facultative conditions were rapidly recovered. The pond remained green and apparently facultative for the remainder of the experiment, apart from an episode recorded on Tuesday 4th of April, when the pond appeared again to be anoxic.

Table 5.5: Monthly weather characteristics for the City of Bradford (Met Office, 2008).

Experimental Run 1: Winter 2006						
Month	**Mean max. air temp. (°C)**	**Mean min air temp. (°C)**	**Mean air temp. (°C)**	**Days of air frost**	**Total rainfall (mm)**	**Total sunshine duration (hrs)**
January	6.3	1.9	4.1	7	16.3	29.0
February	6.5	1.5	4	9	40.4	71.3
March	7.4	1.1	4.3	12	113.2	8.72
April	11.5	4.6	8.1	2	52.5	160.9
Experimental Run 2: Summer 2006						
Month	**Mean max. air temp. (°C)**	**Mean min air temp. (°C)**	**Mean air temp. (°C)**	**Days of air frost**	**Total rainfall (mm)**	**Total sunshine duration (hrs)**
August	19.1	12.3	15.7	0	92.7	121.4
September	19.6	12.3	15.9	0	82.7	131.6
October	15.4	9.3	12.4	0	89.6	89.8
November	11.0	4.8	7.9	2	78.6	85.7
Experimental Run 3: Winter 2007						
Month	**Mean max. air temp. (°C)**	**Mean min air temp. (°C)**	**Mean air temp. (°C)**	**Days of air frost**	**Total rainfall (mm)**	**Total sunshine duration (hrs)**
February	8.6	2.9	5.8	8	103.4	80.3
March	10.3	3.5	6.9	2	49.4	125.9
April	15.1	6.5	10.8	0	4.2	194.8
May	15.3	7.8	11.6	0	68.8	130.5

Table 5.6: PFP operating conditions observed for the Green pond during the winter 2006 experimental run.

Observation date	Green PFP observations
17th January	Pond almost black; strong odour; slight green colouration in places.
31st January	Pond dark brown; sludge mats present on pond surface.
7th February	Pond murky brown; lots of sludge present on pond surface.
14th February	Pond still murky brown; sludge continuing to feedback.
21st February	Pond very green; sludge continuing to feedback.
28th February	Pond very green; no sludge mats visible
7th March	Pond very green; half ice covered.
14th March	Pond very green.
21st March	Pond murky green; surface scum present.
28th March	Pond clear green; no sludge feedback.
4th April	Pond is brown.
11th April	Pond very green; sludge mats present on pond surface.

The Blue PFP, prior to the summer 2006 experimental run, was the characteristic green of a well operating and healthy PFP. On the day of spike injection, the Rhodamine WT (RWT) spread rapidly throughout the pond, colouring the water – after a few hours, completely red. This made it very difficult to assess pond conditions, as, for the first few weeks up until Tuesday 12th September, the pond was purple, which, without the addition of RWT, would indicate the predominance of purple sulphur bacteria and anoxic/anaerobic conditions. From observations recorded between Tuesday 19th September and Tuesday 24th October, a bout of *Daphnia* infestation appeared to cause a significant reduction in the algal population, and the pond rapidly turned brown – the characteristic colour of the onset of anoxia. However, the brown colour was quite possibly the result of the action of the RWT visible from subsurface layers, because algal depletion, from the algal band in the surface waters, increased pond water transparency. The growth of duckweed, which occurred between the Tuesday 3rd and Tuesday 10th of October, could also explain the brown colour of the pond. Duckweed cover is a barrier to insolation, which automatically limits algal photosynthesis, and the ability of the pond to re-oxygenate from natural diffusion of O_2 in the atmospheric boundary layer. By the end of the experimental run, the pond had completely recovered, and the rich green algal layer was restored.

Table 5.7: PFP operating conditions observed for the Blue pond during the summer 2006 experimental run.

Observation date	Blue PFP observations
14th August	Pond green; sludge feedback occurring.
22nd August	Pond brown/green; no sludge mats.
29th August	Pond red/brown in colour; sludge mats on pond surface.
4th September	Pond red/brown; scum on pond surface.
12th September	Pond bright green; algal foam on pond surface.
19th September	Pond brown; large *Daphnia* population present; sludge mats on pond surface.
3rd October	Pond purple/brown; lots of duckweed growth; *Daphnia* present.
10th October	Pond is purple; completely covered in duckweed.
17th October	Pond red/brown in colour.
24th October	Pond red/brown in colour.
7th November	Pond very green.

Unlike the winter 2006 experimental run, the Blue PFP, prior to the winter 2007 run, was operating facultatively, with a notable rich green algal band. On the day of spike injection, Monday 5th of February, *Daphnia* were noticeable at the pond's surface, although in small numbers. For the majority of the experimental timeframe – over two months until mid-April, excessive *Daphnia* populations caused very real problems in terms of algal biomass, depleting it rapidly. The grazing effects of *Daphnia* bring about anoxia in ponds, as a huge impact is made upon the algal mechanism for oxygenating pond waters, through photosynthesis, and oxygenation rates lower purely because of reduced alga. The pond was very pink, and mostly transparent throughout this time, a change most likely caused by the high content of RWT. The transparency of the pond waters indicated very low biomass yields, while *Daphnia* populations remained high. On Monday 12th of March, sewage fungus was present on the pond's surface – characteristically indicative of anoxic conditions. No odour was detectable during this time, and in-pond sulphide concentrations all measured zero, thus discounting sulphide toxicity as an active inhibitory process. The pond remained purple/brown until Monday 2nd of April, when it appeared that facultative conditions had been restored.

Table 5.8: PFP operating conditions observed for the Blue pond during the winter 2007 experimental run.

Observation date	Blue PFP observations
5th February	Pond very green and facultative looking; *Daphnia* just starting to colonise.
12th February	Pond pink (RWT) but very clear; *Daphnia* still present, sludge deposits on the surface.
19th February	Pons very pink (RWT); large amounts of *Daphnia* still present; sewage fungus on pond surface.
26th February	Pond very clear and brown; no green colouration at all; devoid of algae.
5th March	Pond red/brown; very high *Daphnia* population; pond clear and devoid of algae.
12th March	Pond is red/brown; extremely high content of *Daphnia*; sewage fungus on pond surface.
19th March	Pond mucky brown but clear; *Daphnia* levels significantly decreased
26th March	Pond murky brown and scummy; *Daphnia* still present, but populations decreased further and appear to be dying out.
2nd April	Pond very green; no *Daphnia*; algal film on surface.
16th April	Pond very green, lots of algal foam on ponds surface.
23rd April	Pond very green; algal film and lots of foam on pond surface.
30th April	Pond very green; oil-like algal film on surface; lots of sludge feeding back.
8th May	Pond very green, sludge feedback continuing.

5.3.3 ORP data

In-pond oxidation/reduction potential (ORP) profiles for each experimental run are presented in Figures 5.5, 5.6, and 5.7. The data presented in Figure 5.5, together with the site observations in Table 5.6 reveal that according to the definitions advocated by Charpentier *et al.* (1987), the pond was completely anoxic. In fact, from Tuesday 17th January to Friday 3rd of February 2006, practically no ORP stratification was observed − even at the pond surface, which would indicate very severe anoxic conditions, approaching anaerobism. From the Tuesday 21st February onwards, ORP values at the pond surface were recorded at just below 50 mV, and reached a maximum of 100 mV one week before the end of the experiment on Tuesday 4th April. The profiles obtained from Tuesday 21st of February onwards are characteristic of a well functioning PFP, where positive ORP values extending to a depth of ~50 cm, represent oxic conditions where the highly concentrated algal band resides. At a depth of around 50 cm, a sharp decrease in ORP values marks the facultative zone within the pond, the transitory period between oxic and anoxic conditions. Between depths of 50 and 75 cm, all ORP values are negative, as expected for this anaerobic zone of the pond.

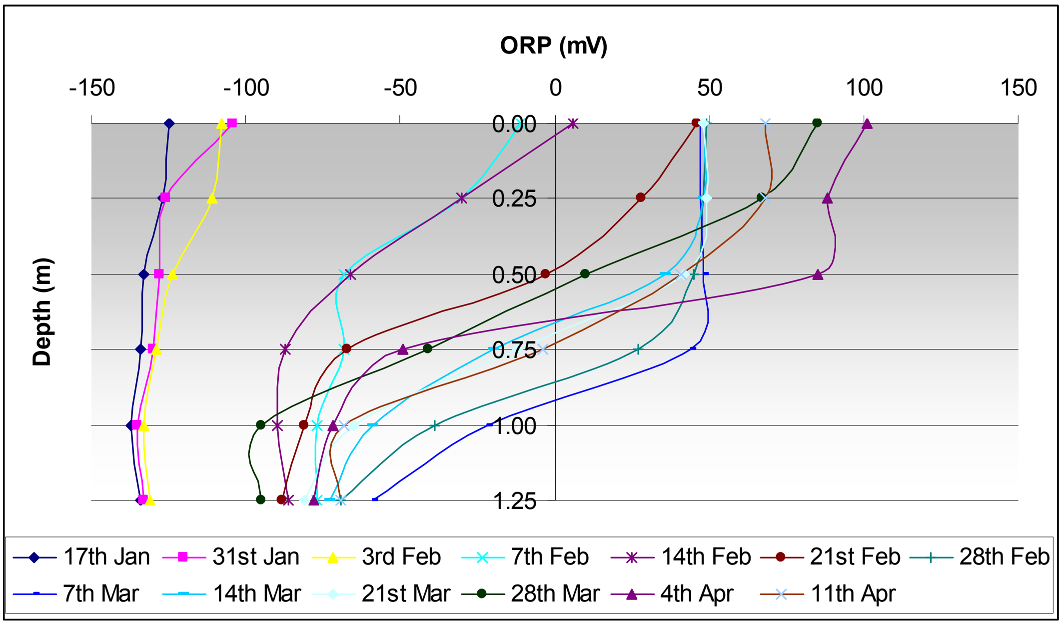


Figure 5.5: ORP profile data from within the Green PFP, winter 2006.

The summer 2006 ORP profiles for the Blue PFP, reveal that it was working facultatively from the start of the experiment for a period of five weeks, until Tuesday 19th September, when ORP values become negative at the surface of the pond, and continued to decrease with depth. This occurrence directly coincided with the presence of *Daphnia* within the pond, as shown from the site observations presented in Table 5.8. Fully anaerobic conditions were reached around Tuesday 31st October, where the ORP at the pond's surface measures −150 mV, and quickly decreases towards −300 mV. All profiles measured from Tuesday 19th September onwards, still represent some degree of in-pond stratification, where, although anoxic, ORP values were still higher in the surface layers than in layers closer to the bottom of the pond. The pond did not recover facultative conditions for the remainder of the experiment from Tuesday 19th September onwards – showing that two full nominal HRT's experienced total anoxia. An anomalous value was observed on Tuesday 3rd October at a depth of 1.2 m, where the ORP rose sharply from ~ −230 mV at 1 m to ~ −30 mV. This was attributable possibly to the fouling of the probe by solid material at that depth.

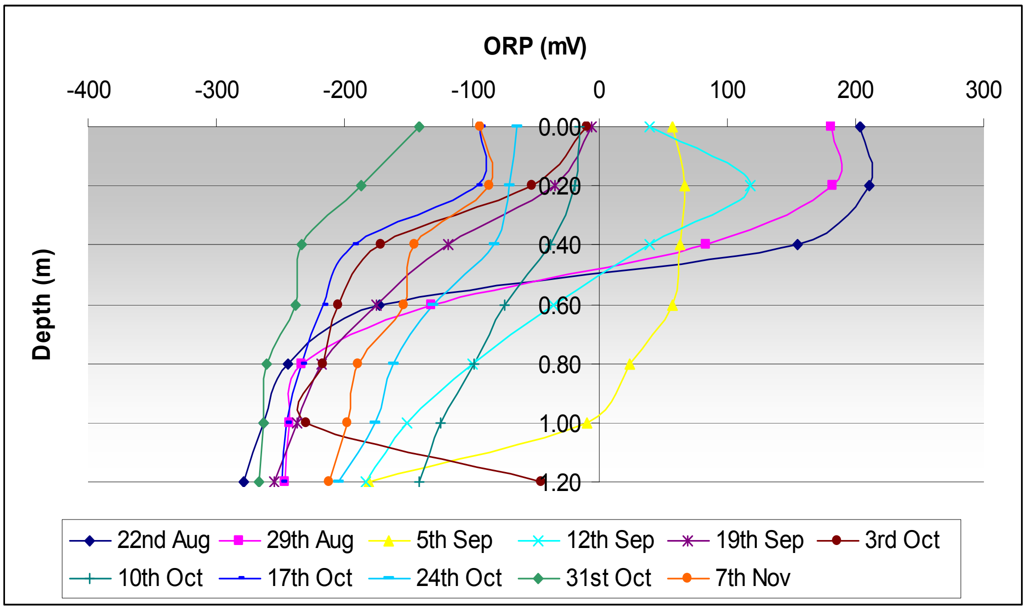


Figure 5.6: ORP profile data from within the Blue PFP, summer 2006.

The ORP data from the start of the winter 2007 experiment on Monday 5th February until Monday 12th March, are highly likely to be incorrect, and do suggest that the probe was contaminated, or simply not working properly. Highly reductive conditions are always associated with facultative depths below the facultative zone, principally because of the anaerobic conditions of the sludge; these profiles appear to be anomalous, especially as this period was observed during the height of winter (with low temperature and sunlight intensity), and the pond had frozen on a few occasions. However, inspection of the DO data (Figure 5.9) reveals that the Blue PFP did actually contain uniformly distributed DO concentrations throughout the depth profile from Monday 5th February until Monday 12th of March, high enough to maintain positive ORP values longitudinally within the profile. Indeed, when a depth of 1 m is reached, the profiles for these five weeks show a rapid decline in ORP values towards anoxia. Despite the adverse effects of predatory *Daphnia* populations grazing the algae from the onset of the first week of the experiment, aerobic conditions are maintained throughout the pond, and appear not to be stratified. From Monday 26th March until the end of the experiment (excluding the data recorded on Monday 2nd April), facultative conditions were restored to the pond, where the noticeable transition between positive surficial ORP values, and negative ORP values below the facultative zone of the pond, are observed.

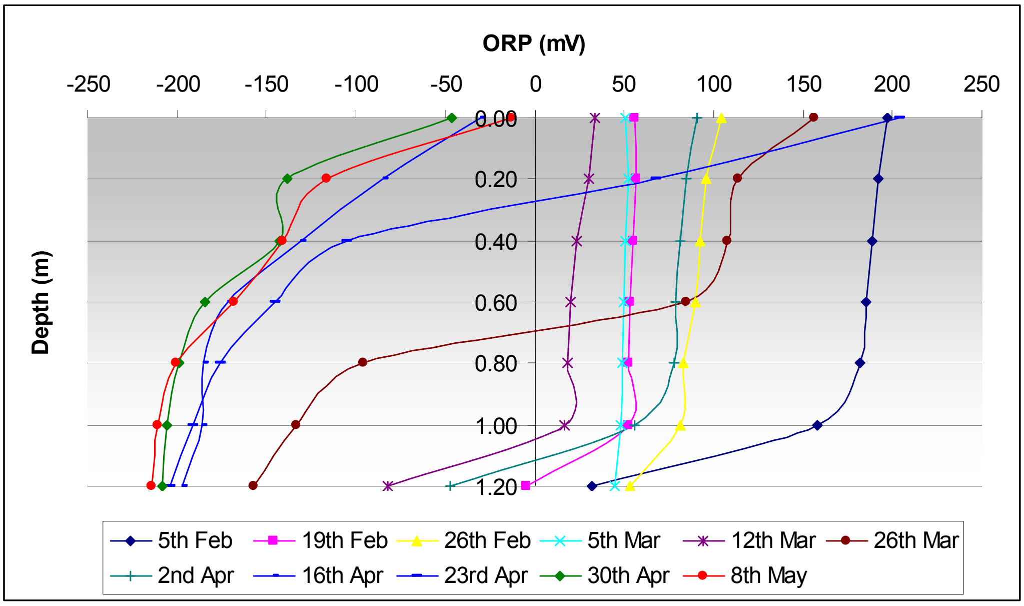


Figure 5.7: ORP profile data from within the Blue PFP, winter 2007.

5.3.4 Dissolved oxygen data

The DO profile for the winter 2006 experimental run is presented in Figure 5.7. The DO concentrations within the pond from Tuesday 17th January until Tuesday 7th February are very near zero throughout the pond depth – apart from a very slight increase at the pond surface. However, the maximum value at the surface for this period was only 0.59 mg DO/l on the 17th of January. Data from Tuesday 14th February reveal that the DO concentration was unstratified through out the pond depth and measured ~2 mg DO/l. This occurred at around the time facultative conditions were being restored. From Tuesday 21st February until the end of the experiment, classic DO profiles, characteristic of a well functioning facultative pond are observed, with saturated oxygen-rich concentrations in the upper zone of the pond correlating with the presence of the algal band. Note that facultative conditions had been restored, as detailed in Table 5.6 and Figure 5.5 with lower concentrations occurring below the oxypause.

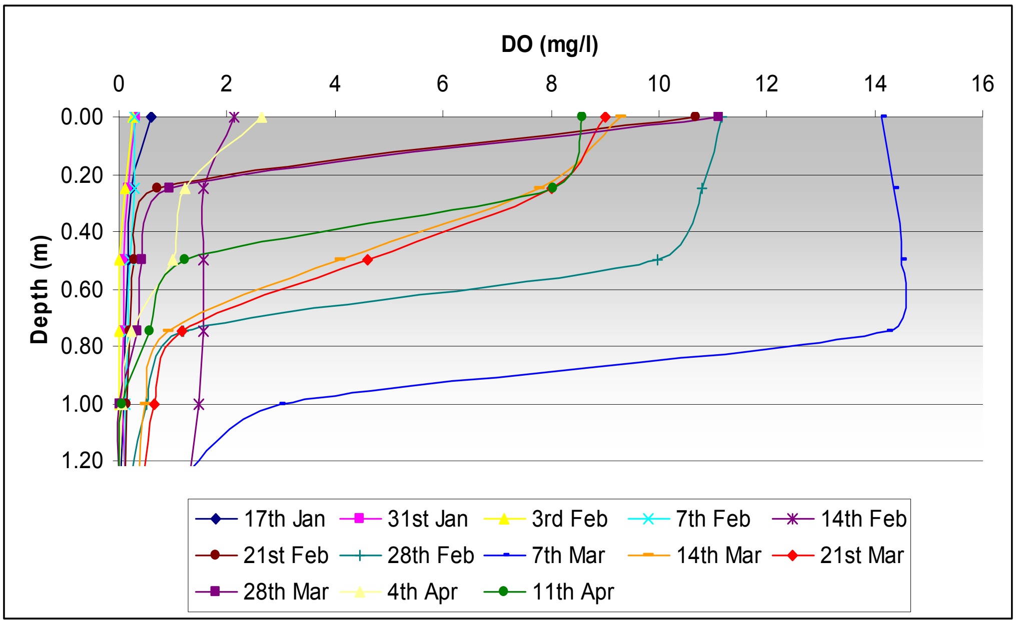


Figure 5.8: DO profile data from within the Green PFP, winter 2006.

Apart from two dates, DO profiles were taken during the summer 2006 experiment. DO concentrations for the Blue pond were surprisingly low, even at the surface, despite it's being green and facultative before spike injection. When analysed in conjunction with the summer 2006 ORP profile, these results are contextualised and are not as anomalous as they seem; the corresponding ORP profile values indicate total anoxia. For all dates apart from Tuesday 17th October (where the profile shows virtually zero DO within the whole profile), DO concentrations were higher from the surface of the pond. They show a gradual decrease, until the oxypause is reached at a depth of ~ 40 cm; below this, they approach zero DO, and thus stratification may be observed. On Saturday 12th September and Tuesday 7th November a considerably elevated DO concentration at the surface of the pond is evident. This, when cross-referenced with Table 5.7, the dense green of the pond indicated high algal concentrations.

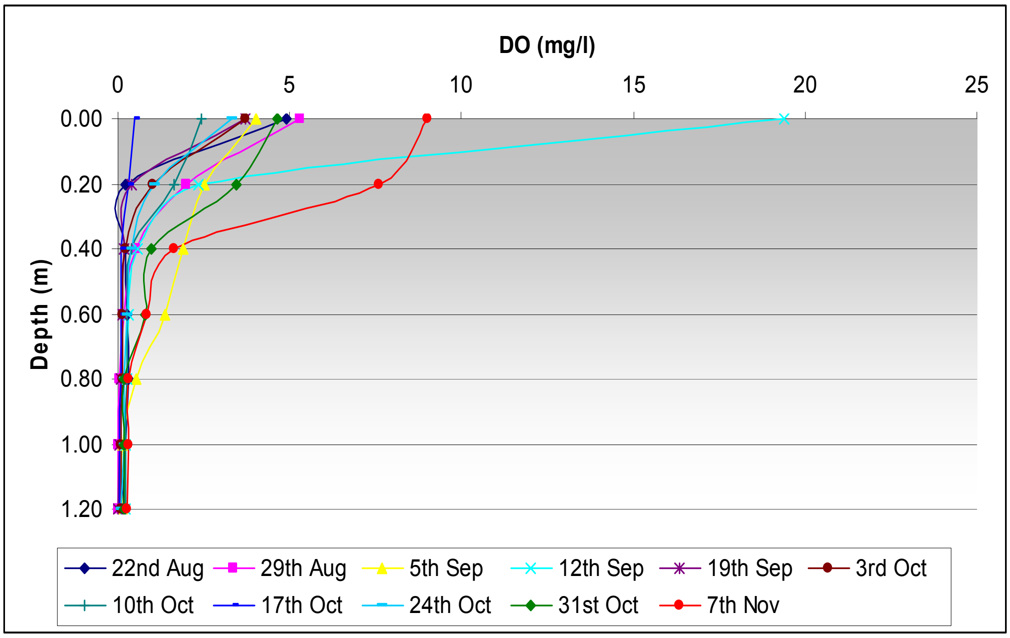


Figure 5.9: DO profile data from within the Blue PFP, summer 2006.

The DO data presented in Figure 5.10 for the winter 2007 experimental run, show that for eight consecutive weeks out of the eleven weeks sampled, there was no DO stratification throughout the pond. The ORP profile data corroborates, and shows that six out of the eleven weeks had unstratified conditions (certainly until a depth of 120 cm, where values dropped rapidly towards anoxia). The data for the first four weeks of the experiment show relatively no change in stratification – which is somewhat surprising; although WSP's are typically unstratified in cold and winter climates, this usually pertains only to temperature depth profiles, whereas DO concentrations usually show a gradient from the surface of the pond to the base of the pond. It is unlikely that the DO probe was malfunctioning, as it was cleaned and re-calibrated every time before use (responding accordingly), and the electrodes showed no sign of fouling throughout the time on site. The very high levels of *Daphnia* present throughout this period, contributed to heavy algal grazing, thus removing the mechanism of algal oxygenation to surface layers. Reductions in algal concentrations result typically in the anoxic conditions which occur in facultative WSP's. Cooler winter temperatures and reduced sunlight are additional factors which result in pond anoxia. The profiles taken during the middle portion of the experimental time (for the weeks beginning Monday 12th March, Monday 26th March, Monday 16th April, and Monday 23rd April) show, that apart from slight elevated DO concentrations at surface of the pond, concentrations were all <1 mg DO/l.

The profile data from three weeks towards the end of the experiment (Monday 2nd April, Monday 30th April, and Tuesday 8th May), show more classic DO profiles from PFP's, which coincide with elevated algal concentrations as observed on site, in Table 5.8.

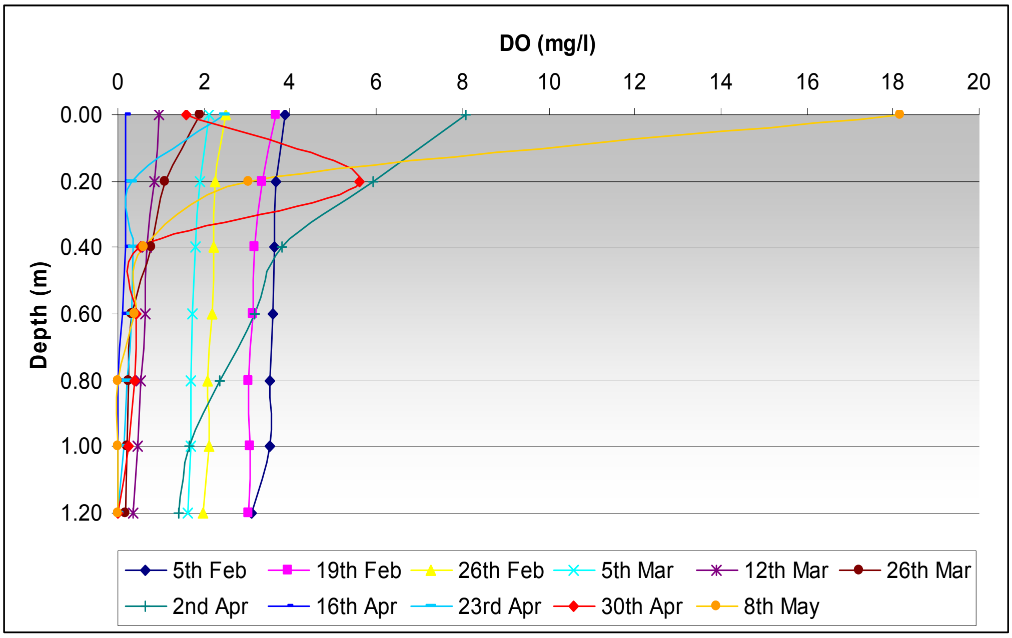


Figure 5.10: DO profile data from within the Blue PFP, winter 2007.

5.3.5 Chlorophyll *a* data and profiles

Chlorophyll *a* column and effluent concentrations for the winter 2006 experimental run, are shown in Figure 5.11. From Tuesday 17th January until Tuesday 14th February, in-pond column concentrations are well below the minimum concentration of 300 µg/l specified by Pearson and Mara (1987b) for the maintenance of facultative conditions. As already discussed, the ORP and DO data show that the pond was anoxic during this time; the low chlorophyll *a* concentrations presented in Figure 5.10, for these dates, compound this assertion further. From Tuesday 21st February, chlorophyll *a* concentrations show a marked increase, culminating in a peak value of 1072 µg/l observed on Tuesday 7th March. After this, column concentrations began steadily to decrease, then increased again, producing a secondary (but smaller) peak of 806 µg/l on Tuesday 28th of March. By Tuesday 4th of April, they had fallen below the 300 µg/l minimum threshold. Effluent chlorophyll *a* concentrations tend to map the column chlorophyll *a* concentrations, although from Tuesday 21st February until Tuesday 21st of March, effluent chlorophyll *a* concentrations were higher than the in-pond column concentration; the most plausible explanation is that the position of the algal band was level with the pond effluent point when the grab samples were taken.

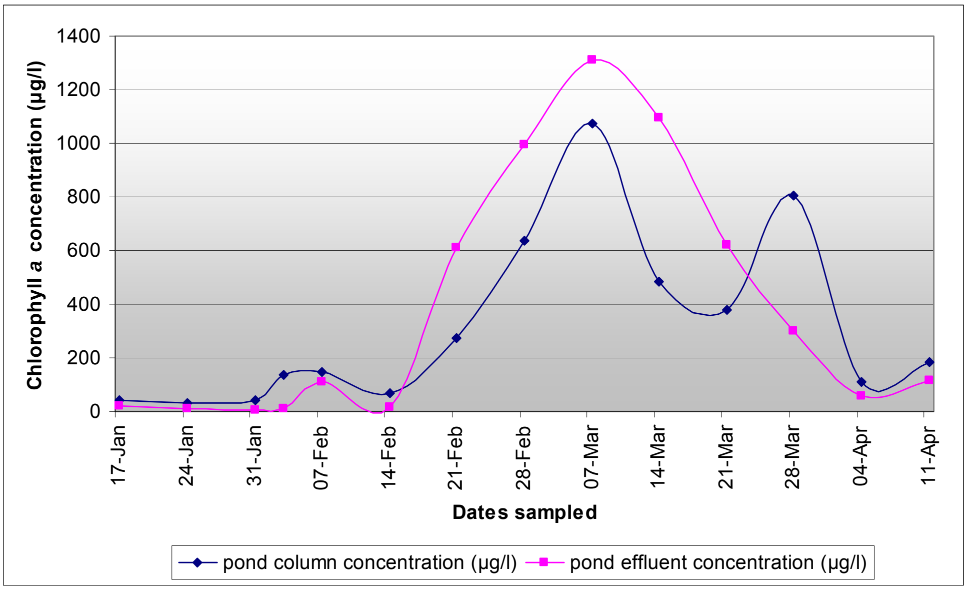


Figure 5.11: Chlorophyll *a* pond column and effluent concentrations for the Green PFP, winter 2006.

The Blue pond, at the start of the summer 2006 experiment, was visibly green and operating facultatively. However, two days after the spike injection on Wednesday 16th August, the chlorophyll *a* column concentration measured 279 µg/l. The next two consecutive dates sampled (on Tuesday 22nd August and then on Tuesday 12th September) revealed that chlorophyll *a* concentrations slumped well below the minimum 300 µg/l threshold necessary for the maintenance of facultative conditions. Concentrations improved on Tuesday 19th September, and then dipped again in the run up to October. Chlorophyll *a* levels stayed relatively low until the beginning of November; this was brought about by *Daphnia* grazing and a bout of duckweed growth. By the end of the experiment, on Tuesday 7th November, the pond had recovered fully, and facultative conditions were restored, which resulted in a high column concentration of 671 µg chlorophyll *a*/l.

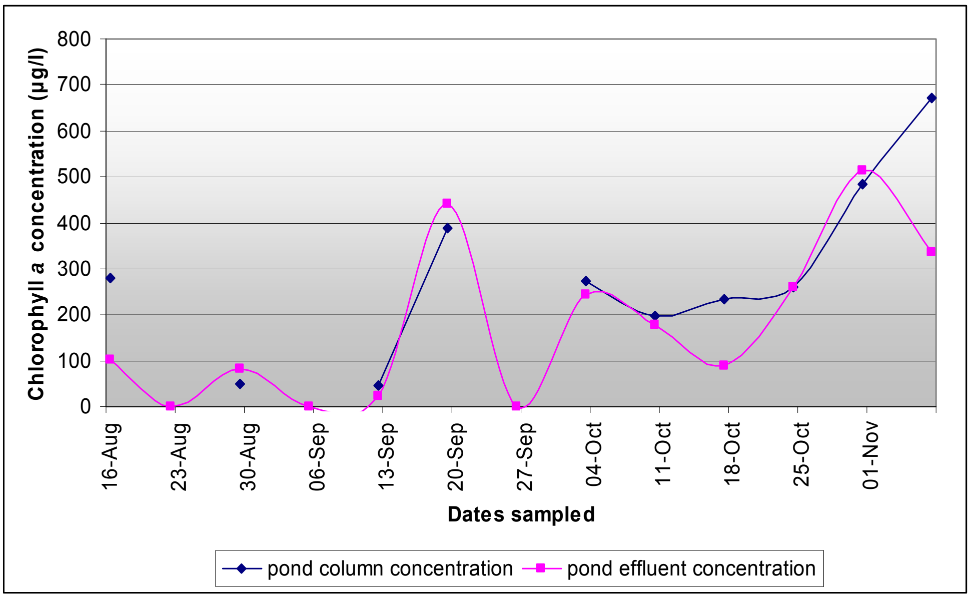
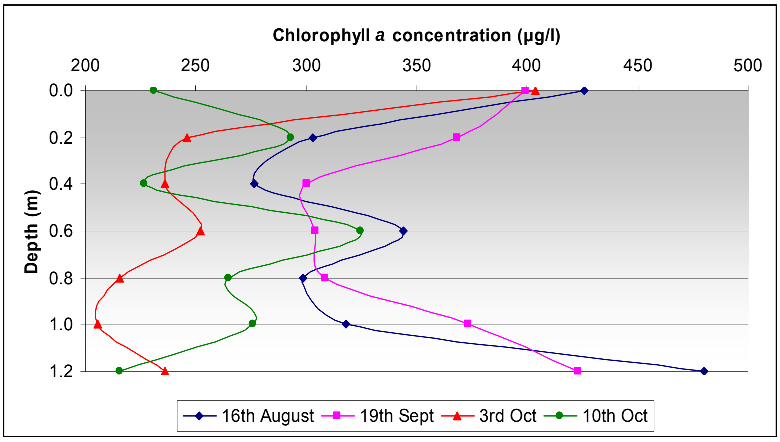


Figure 5.12: Chlorophyll *a* pond column and effluent concentrations for the Blue PFP, summer 2006.

Figure 5.13 shows a more detailed analysis of chlorophyll *a* stratification within the Blue PFP throughout the experimental period. The four dates sampled from the start of the experiment, until Tuesday 10th October, reveal somewhat haphazard stratification profiles. The profile on Wednesday 16th August, two days after spike injection, shows that the chlorophyll *a* concentration at the pond's surface is 426 µg/l, which is relatively low for a facultative WSP at the height of summer. Concentrations decline sharply at a depth of 40 cm, increase at a depth of 60 cm, then decrease again at depth of 80 cm, after which they increase dramatically to 480 µg/l, a higher concentration than that measured at the pond's surface. Profile data for Tuesday 19th September and Tuesday 3rd of October also show a pattern similar to the data collected on Wednesday 16th August, although the chlorophyll *a* concentration at 120 cm on Tuesday 3rd October is less than the pond surface concentration. Elevated concentrations of chlorophyll *a* at these depths may be explained by the predominance of non-motile algal species which had fallen to the bottom of the pond. Another likely explanation is provided by the deposition of dead algae gradually settling out at these depths, which would also contribute chlorophyll *a* to appropriate grab samples.



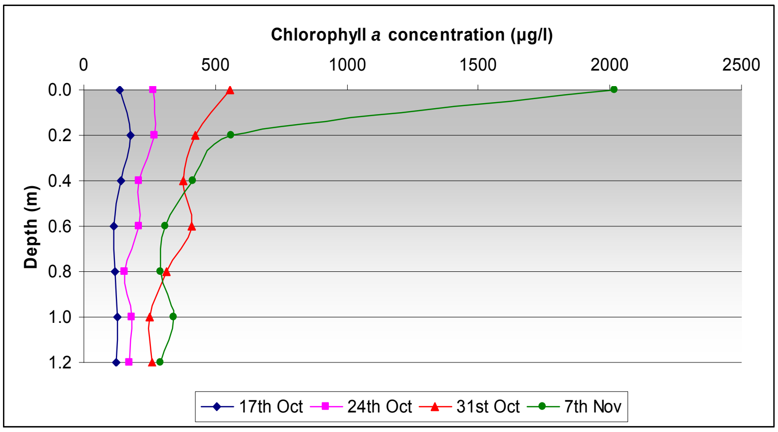


Figure 5.13: Chlorophyll *a* in-pond stratification data for the Blue PFP, summer 2006.

The profile obtained on Tuesday 10th October presents a different trend altogether. In the upper pond strata, a higher chlorophyll *a* concentration was obtained at a depth of 20 cm depth, this was probably the zone containing the dense algal band. Concentrations then fluctuated, until the maximum measured depth of 120 cm. The onset of anoxia occurred on Tuesday 10th October, and by Tuesday 17th October the pond was fully anoxic (see Table 5.7). This coincided with chlorophyll *a* profiles from Tuesday 17th October till Tuesday 31st October showing relative longitudinal uniformity. The last set of grab samples collected within this experimental run (on Tuesday 7th November), also showed little fluctuation within the pond's depth, excluding the sample collected from the surface.

The winter column and effluent chlorophyll *a* data, presented in Figure 5.14 show that, for the entire winter 2007 experimental run, chlorophyll *a* concentrations were very low, and initially, for almost two thirds of the experiment, did not rise to above 20 μg/l. From Monday 26th March, in-pond concentrations increased a little, but gradually fell again over a period of one month until Monday 23rd April. The sharp increase in chlorophyll *a* concentrations in the last week of the experiment coincided with complete *Daphnia* die-off and the restoration of facultative conditions.

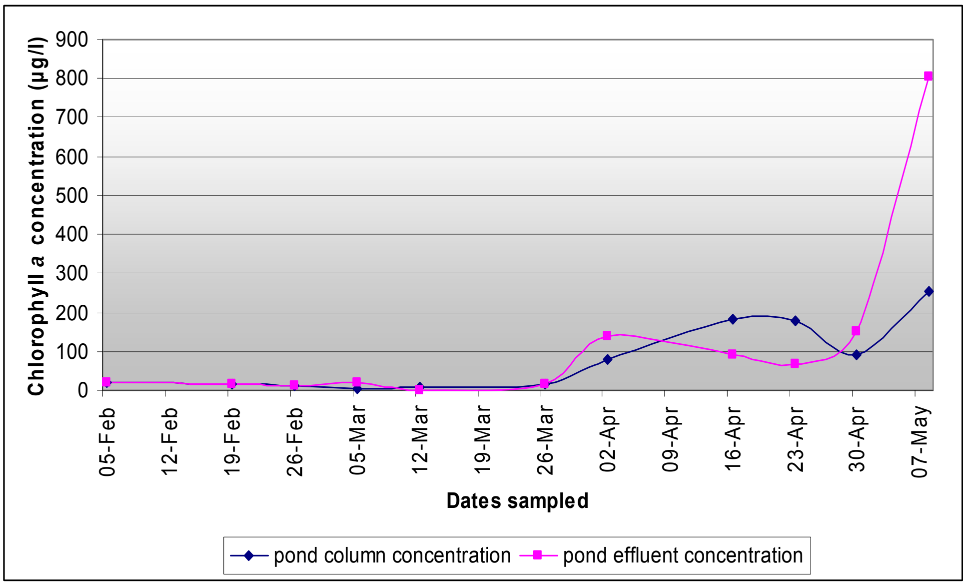


Figure 5.14: Chlorophyll *a* pond column and effluent concentrations for the Blue PFP, winter 2007.

Figure 5.15 shows profile chlorophyll *a* data for the winter 2007 experimental run. The top graph presented in Figure 5.15 shows profiles for the first half of the experiment. There appears to be no general trend within the data captured and presented in this graph, apart from an initial increase in chlorophyll *a* concentrations from the pond surface to depths of 40 cm. The overall concentrations of chlorophyll *a*, were so minimal within this data set, that the degrees of stratification shown in Figure 5.15 were likely to have made no tangible difference to biochemical processes within each tier of the pond.

Site observations presented in Table 5.8 show that from Monday 2nd April the Blue PFP was slowly beginning to recover after the interminable presence of *Daphnia* populations. This is reflected by the increasing chlorophyll *a* concentrations in the upper layers of pond water, with the highest concentration measuring >2,000 μg chlorophyll *a*/l at the pond surface, on Monday 16th of April 2007.

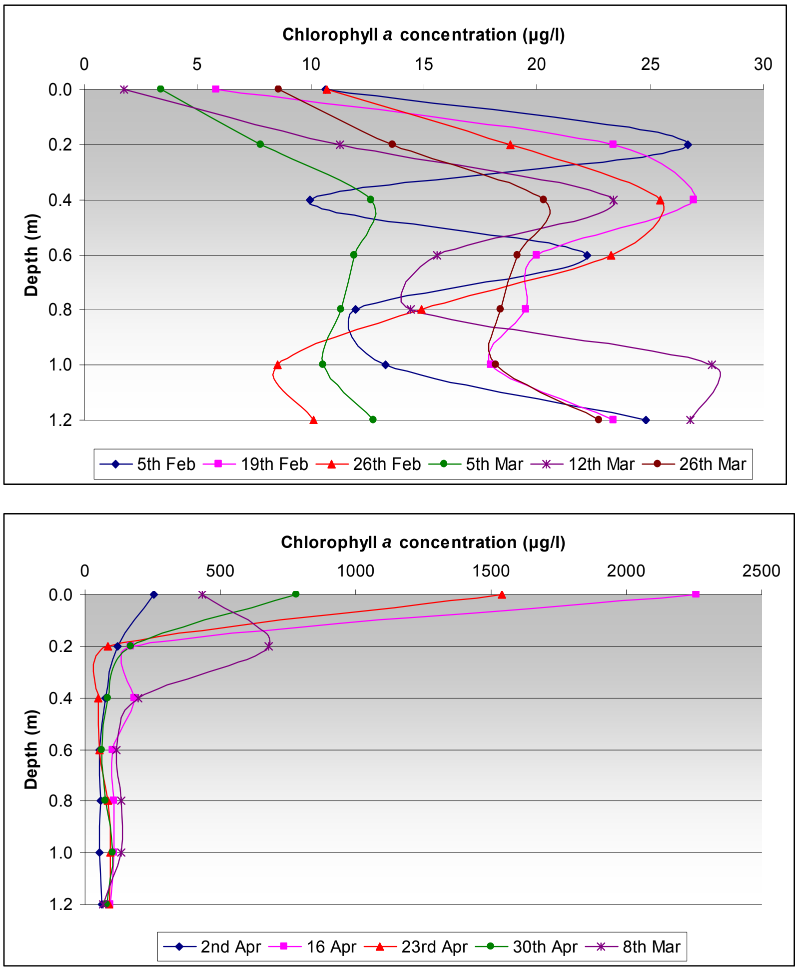


Figure 5.15: Chlorophyll *a* in-pond stratification data for the Blue PFP, winter 2007.

The chlorophyll *a* profiles presented in the lower graph in Figure 5.15 show no stratification below depths of 40 cm. As site observations reveal that algal populations were observed to regenerate in the upper tier of the pond form Monday 2nd April, a concomitant increase in chlorophyll *a* concentrations in surface waters was also measured.

5.3.6 Nitrate profiles

Nitrate concentrations for column grab samples, collected from the Green pond during the winter 2006 experiment, are presented in Figure 5.16. During the anoxic period, which lasted for the whole of the first month, nitrate concentrations were very low and, apart from the result obtained on Tuesday 31st January, all measured below 0.1 mg/l. Over the following two weeks, nitrate concentrations in the pond column increased, culminating with a peak concentration of 1 mg/l on

Tuesday 7th of March. This peak concentration occurred in conjunction with peak concentrations observed for DO and chlorophyll *a*. For the remainder of the experiment, when the pond was operating facultatively, nitrate concentrations stayed in the range 0.5−0.7 mg/l, apart from a decrease to below 0.4 mg/l on Tuesday 11th April, the last week grab samples were collected and analysed.

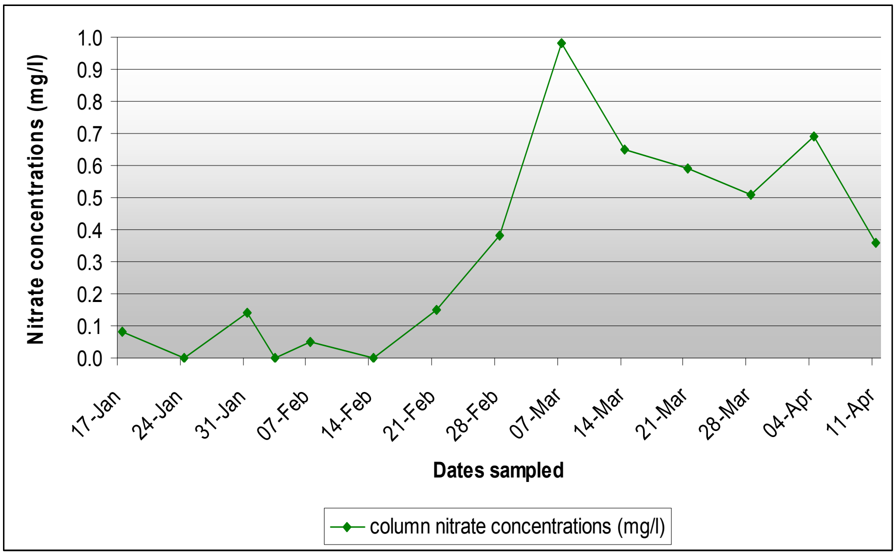


Figure 5.15: Pond column nitrate data for the Green PFP, winter 2006.

Nitrate depth profiles were obtained for some of the weeks sampled in the summer 2006 experimental run. Figure 5.17 presents the data for six of the weeks sampled over the twelve-week experimental period. There appears to be no trend in the data, with nitrate concentrations showing high variability between the different depths measured. The data for Tuesday 22nd August show that a maximum nitrate concentration was found at a depth of 20 cm; this probably coincided with increased chlorophyll *a* concentrations (profiles of which were not measured on this day). Both profiles for Tuesday 3rd October and Tuesday 12th October show high nitrification occurring at the surface layer of the pond, with concentrations falling to zero at a depth of 20 cm. The profile on Tuesday 3rd October shows peaks of increased nitrate concentration at a depth of 60 cm, which drop again by 80 cm, to reach a maximum concentration of only 1.4 mg/l at a depth of 100 cm.

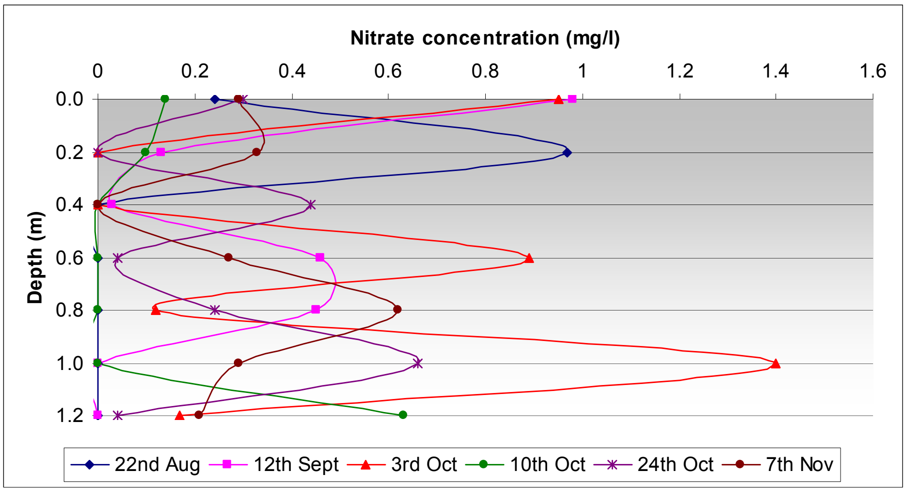
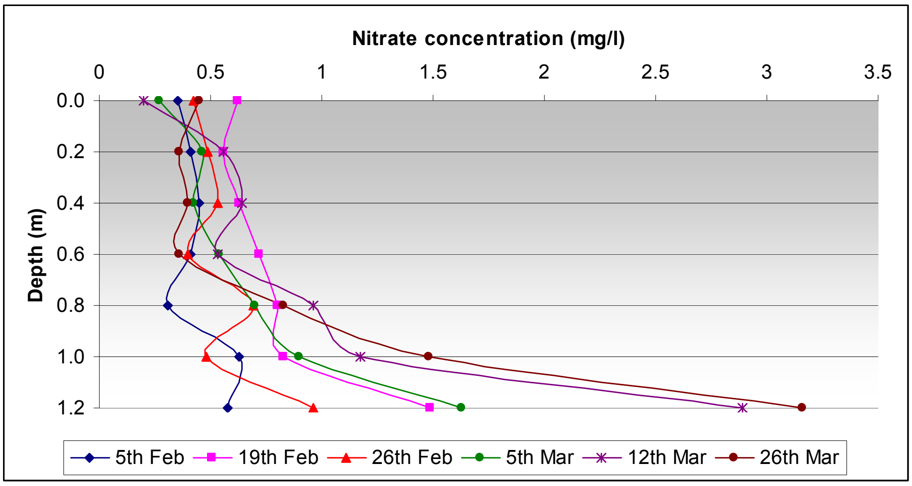


Figure 5.17: Nitrate depth profile concentrations for some of the weeks sampled in summer 2006.

Figure 5.18 shows data for nitrate profiles collected throughout the winter 2007 experimental run. The data show a different trend from that collected for the summer 2006 experiment. Data collected on Monday 5th and Monday 26th February exhibit no tangible stratification of concentrations. Data for the profiles on Monday 19th February, and those on Monday 5th, Monday 12th and Monday 26th March reveal that there is a correlation between increasing nitrate concentrations with increasing depth, and, in all cases, a sharp increase in nitrate concentration at a depth of 120 cm. Data from Monday 2nd April and Tuesday 8th May, also show a similar trend, although concentrations throughout the pond profile to a depth of 100 cm fluctuated between depths. Data for Monday 23rd Monday 30th April do not follow any particular trend, and nitrate concentrations remain fairly low (< 0.2 mg/l) throughout the profile, apart from a large peak concentration of 1.98 mg/l at a depth of 40 cm. A peak nitrate concentration of 1.13 mg/l occurred within the profile for the 23rd of April at a depth of 80 cm, otherwise nitrate concentrations were low.



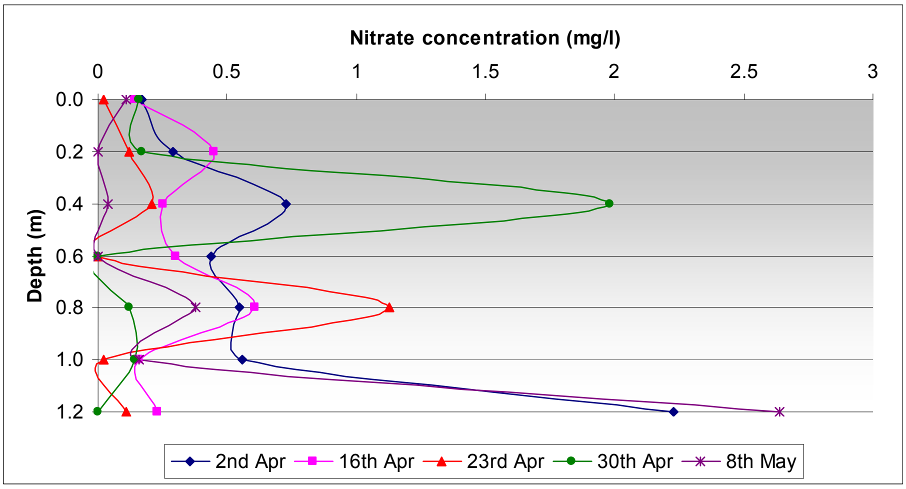


Figure 5.18: Nitrate depth profile concentrations for some of the weeks sampled in winter 2007.

5.3.7 Ammonium profiles

Figure 5.19 shows the pond column nitrate concentration data for the winter 2006 experimental run. Ammonium concentrations were highest during the anoxic period for the first half of the experiment and peaked at 15.7 mg/l on Friday 3rd February. As the transition between anoxic and facultative conditions occurred, and algal populations started to regenerate, column ammonium concentrations started to decrease too, and for the last third of the experiment, were consistently around 4 mg NH_4^+/l. The mean column ammonium concentration for the whole of the winter 2006 period measured 7.7 mg NH_4^+/l with a standard error of ± 0.98.

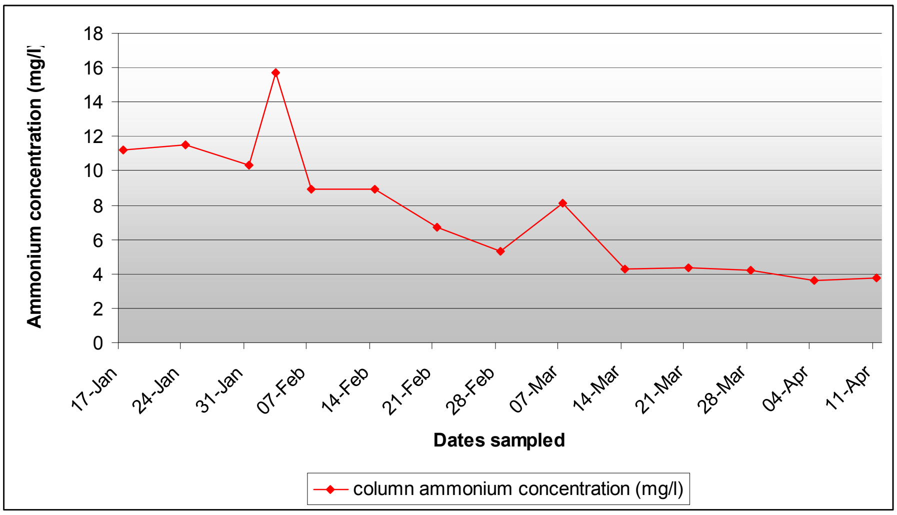


Figure 5.19: Pond column ammonium data for the Green PFP, winter 2006.

Ammonium concentration profiles are shown in Figure 5.20 for nine out of the thirteen weeks sampled during the summer 2006 experimental run. The mean ammonium column concentration for the summer 2006 period was 5.4 mg $NH4^+$/l, with a standard error of ± 0.59 and, as is usual with ponds operating in summer time periods, measured the lowest of all three experiments. The profile taken one week after spike injection (on Tuesday 22nd August) shows that the ammonium concentration was zero at the pond surface, and remained below 0.5 mg NH_4^+/l to a depth of 40 cm. Below this depth, the concentration rapidly but consistently increased, to a depth of 100 cm, then increased by 9 mg NH_4^+/l between 100 and 120 cm, which could be caused by the mineralisation of sludge releasing ammonium to the water column. This trend is not matched by the profile data obtained on Tuesday 12th September, where very little stratification in ammonium occurred throughout the water column. Data obtained on Tuesday 19th September show a trend similar to that on Tuesday 22nd August. The sample data from the other weeks show little to no ammonium stratification occurring at any depth. Data on Tuesday 3rd October and on Tuesday 24th October show no stratification, apart from increases in concentration at a depth of 60 cm which occurred around the facultative boundary. But below this depth the concentration dropped again, revealing that, along with the other unstratified data on several dates, the release of ammonium from the mineralisation of sludge did not appear to have occurred.

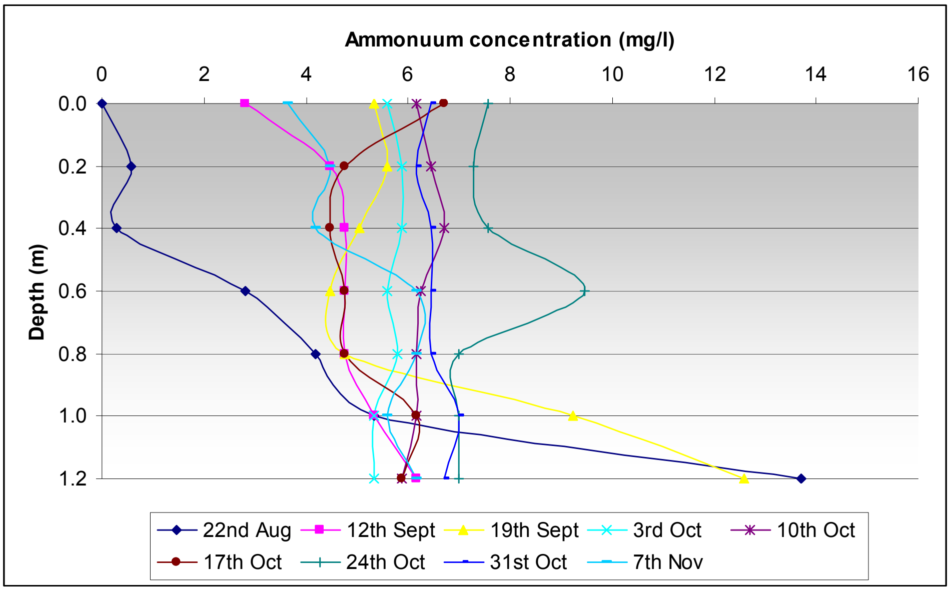


Figure 5.20: Ammonium depth profile concentrations for some of the weeks sampled in summer 2006.

The ammonium concentration profiles collected for nine out of the eleven weeks sampled during the winter 2007 experimental run, are shown in Figure 5.21. The mean column concentration measured 8.5 mg NH_4^+/l with a standard error of ± 0.62 (higher than the previous winter experiment conducted on the Green pond). All of the data presented show very high ammonium stratification throughout the pond depth profiles, and all data, without exception, follow the same trend. Ammonium concentrations measured at the Blue PFP's surface (Figure 5.20) ranged between 5.6 and 8.1 mg NH_4^+/l (with the exception of Monday 23rd April which measured 3.1 mg NH_4^+/l). At a depth of just 20 cm, all ammonium concentrations had dropped close to zero. This was followed by a concomitant increase in concentrations between depths of 40 and 60 cm, to those approximately measured at the surface. Another decrease in ammonium concentrations almost to zero, occurred between depths of 80 and 100 cm in all the grab samples analysed. This was followed by another sharp increase between depths of 100 and 120 cm, where ammonium concentrations measured approximately the same as those found in surface pond water samples and samples collected at depths of 40 and 60 cm. The increase in ammonium concentration at a depth of 120 cm could be explained by the mineralisation of sludge, and subsequent recycling of ammonium to the overlying water column. The identical longitudinal stratification pattern of all the data, does not appear to be affected at all by the change of oxic to anoxic conditions, recorded mid-way through this experiment, indicated in Figure 5.10. The stratification of ammonium appears also to be independent of chlorophyll *a* concentrations; as shown in Figure 5.15, during the first half of this experiment the chlorophyll *a* concentration profiles, for individual dates sampled do show stratification, although none of these profiles followed the same trend. During the second half of the experiment, chlorophyll *a* profiles (apart from those monitored on Tuesday 8th May) show elevated concentrations at the surface of the pond, and from a depth of 40 cm downwards, no stratification occurred.

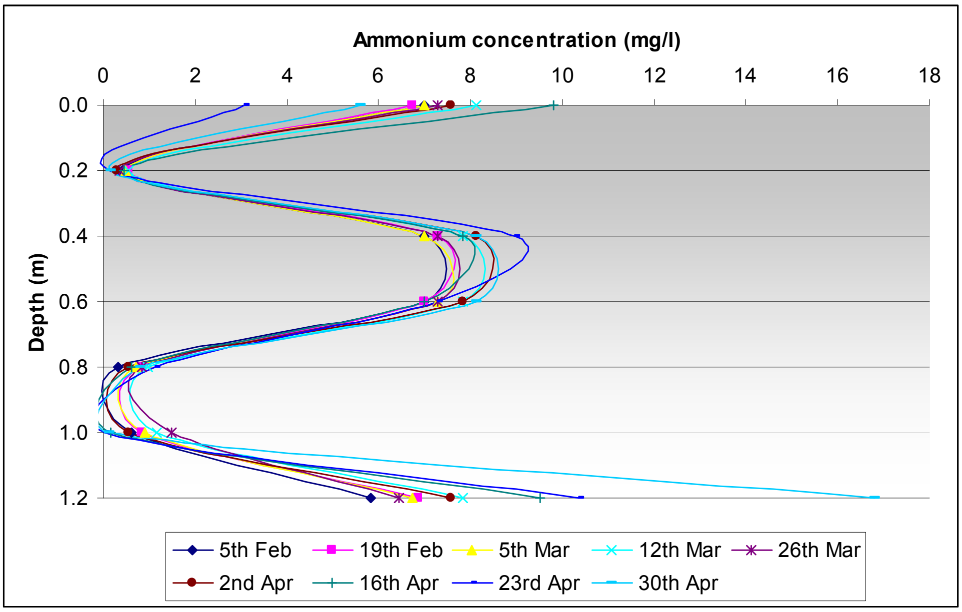


Figure 5.20: Ammonium depth profile concentrations for most of the weeks sampled in winter 2007.

5.4 Sludge accumulation

The Green and Blue PFP's were inherited after four years and five months of operation. In November 2004, the sludge depth profiles of both ponds were taken (according to the method described in section 4.8.2) to assess the accumulation of sludge within this time. A summary of sludge accumulation rates with respect to reduced PFP volume, is presented in Table 5.9. Figures 5.22 and 5.23 show the sludge depth profiles for the Green and Blue PFP's, respectively.

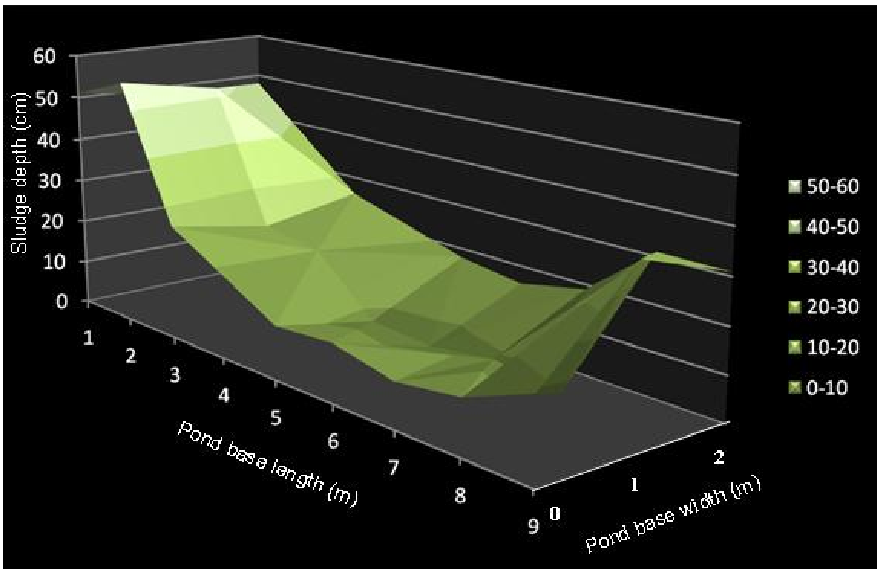


Figure 5.22: Sludge depth profile for the Green PFP after four years and five months of operation.

The total integrated sludge volume calculated for the Green PFP was approximately 3.63 m^3, leaving an effective pond volume of 47.67 m^3. The total integrated sludge volume for the Blue PFP equalled 3.1 m^3, leaving an effective pond volume of 55.7 m^3. In both Figures 5.22 and 5.23, the highest area of sludge accumulation fans out as a conical portion surrounding the pond inlet structure. The Green PFP profile shows slightly more sludge accumulation round the pond inlet than the Blue PFP profile; this is attributed to the different loading regimes that were applied to each of the ponds during their first four years of operation. Site records for the time note that both ponds were operating facultatively.

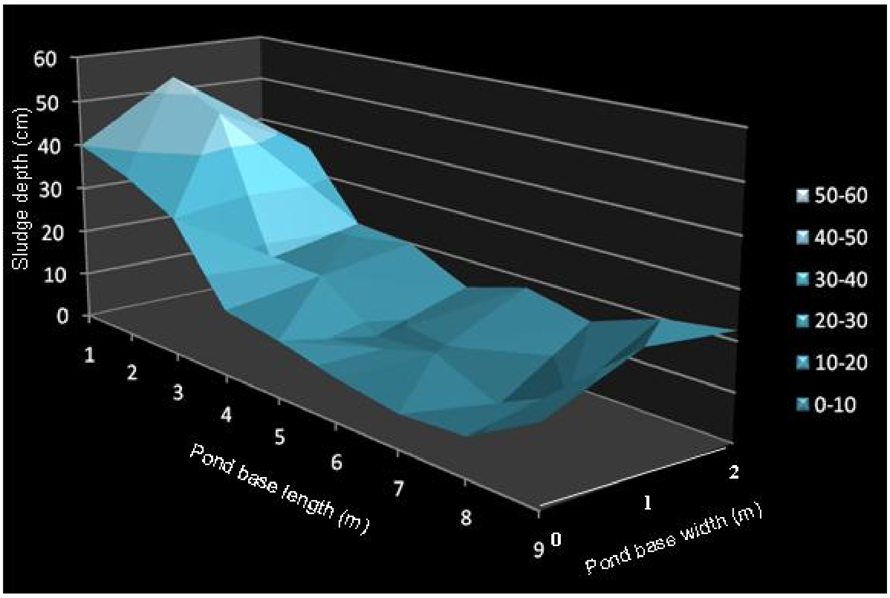


Figure 5.23: Sludge depth profile for the Blue PFP after four years and five months of operation.

The second sludge depth profiles were taken on Monday 10th October 2005, after ponds had been operating for five years and four months. The data for the Green PFP are shown in Figure 5.24, and for the Blue PFP and in Figure 5.25. Figure 5.24 shows that, in the eleven-month period after the first profiles were taken, a marked increase in sludge build-up (contributing ~30 cm) around the inlet area had occurred. Sludge build-up had also occurred at the effluent point, but only by ~5 cm. The total sludge accumulation observed from pond profiles alone equalled 0.9 m^3, which produced a net sedimentation rate of 0.31 cm^3/d. This reduced the effective pond volume from 47.7 m^3 to 46.8 m^3, equalling a 2% increase in the amount of sludge stored within the system. The increase in sludge height at the pond inlet was deep enough to envelope the influent plastidrain pipe, and may have altered the pattern of the flow entering the pond. This quite possibly limited the proper mixing of influent settleable and suspended solids, causing clogging of the influent pipe and resulting in a localised zone of anoxic conditions through the strata of the pond.

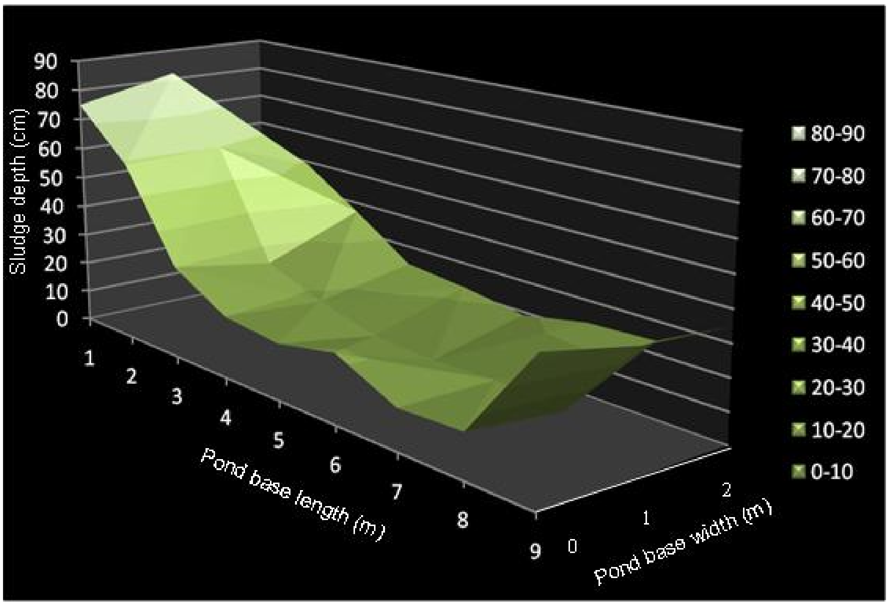


Figure 5.24: Sludge depth profile for the Green PFP after five years and four months of operation.

The profile taken after the same period of time, for the Blue PFP, is shown in Figure 5.25. A net increase of ~10 cm is observed at both the pond influent and effluent points. The net accumulation of sludge over this eleven-month period measured slightly more than that in the Green PFP, equalling 1 m^3, and contributing a net sedimentation rate of 0.29 cm^3/d. The effective PFP volume (effective volume = volume of PFP − net sludge volume) was reduced by 2% over this period, from 95% to 93%. Although the Blue PFP contained marginally less sludge than the Green PFP, poor pond performance over the winter months led to the decision to desludge the pond in early February 2006.

The sludge depth profile for the Green PFP was taken approximately one month after the end of the winter 2006 stable isotope and RWT dye tracer study, and is shown in Figure 5.26 (This was five years and eleven months from the initial pond start-up). Interestingly, the volume of sludge around the pond inlet appears to have reduced over this seven-month period, but increased slightly beneath the pond effluent point. The effective pond volume was reduced by 1% from 91% to 90%, with the net sludge volume equalling 5.2 m^3. The net sedimentation rate for this period measured 0.7 m^3, equating to a net sedimentation rate of 0.31 cm^3/d.

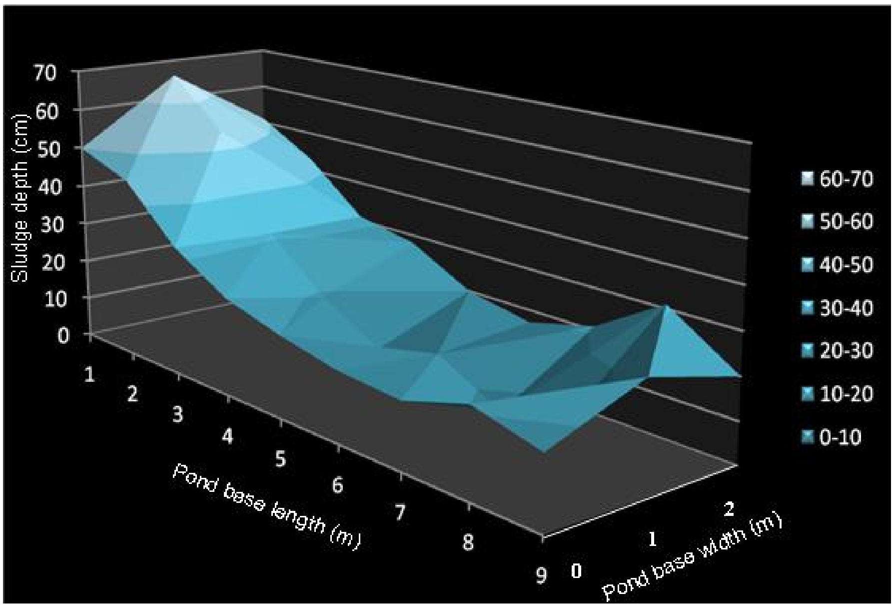


Figure 5.25: Sludge depth profile for the Green PFP after five years and four months of operation.

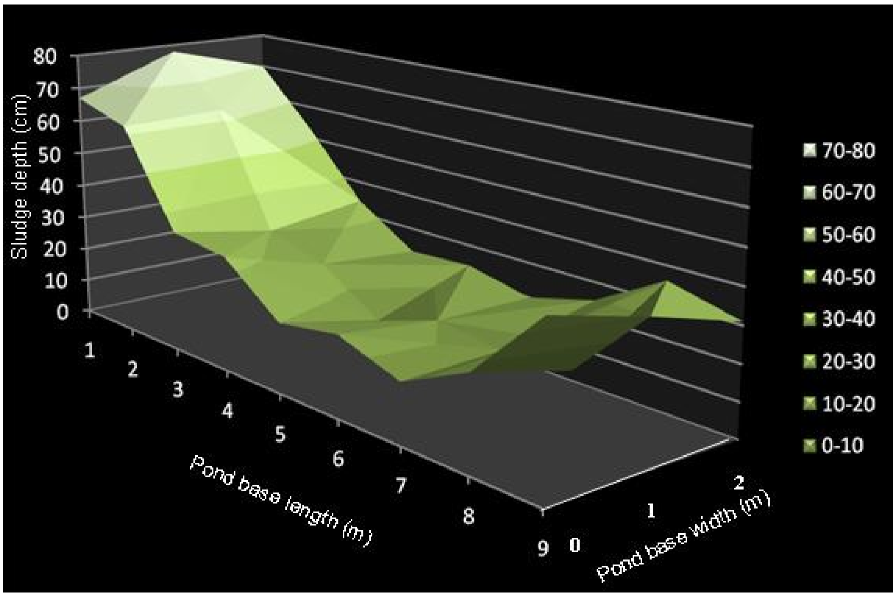


Figure 5.26: Sludge depth profile for the Green PFP after five years and eleven months of operation.

Desludging the Blue PFP did not ensure the absolute removal of the heavily thickened sludge on the PFP floor. Every effort was made to remove as much as possible, but the base of the pond was not level, and small isolated pockets of sludge remained over a few areas of the pond floor. A gully ran down the length of the PFP to the left of the pond influent, which also contained a small fraction of sludge. The total net integrated sludge volume was surprisingly high for such a short operational period, i.e. 2.4 m^3, attributable to the relative freshness of the sludge, which had possibly not undergone the same degree of compaction as the older sludges (as can be seen in Figure 5.26). The shape of the profile is skewed from the right side of the figure to the left. This marks the location of the gully, showing that there was old sludge contained within this section of the base of the pond.

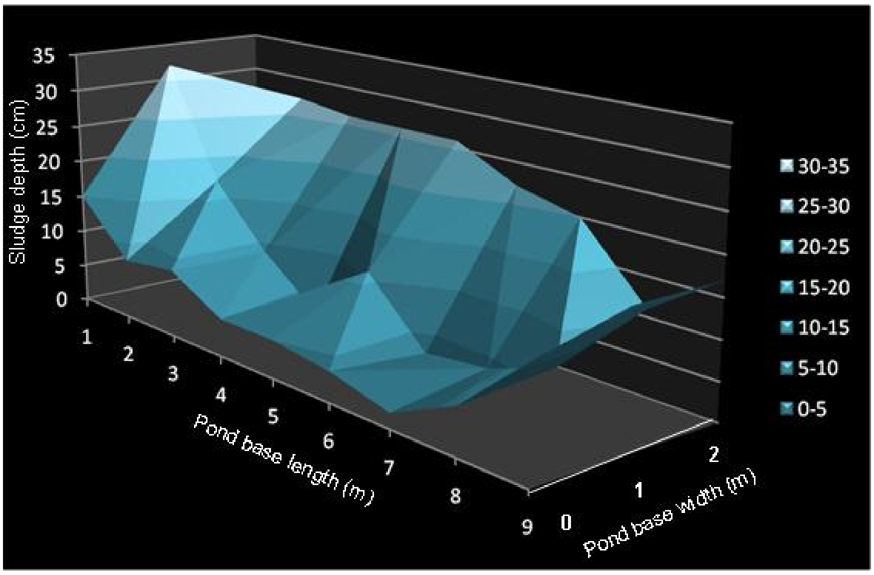


Figure 5.27: Sludge depth profile for the Blue PFP after seven months of operation.

Figure 5.27 shows the final sludge depth profile for the Blue PFP, taken one month after the end of the winter 2007 tracer studies, and after thirteen months of operation. The sludge volume had increased by 2% to 6%, only 1% less than the sludge volume measured in the same pond before it was desludged. The total amount of net sedimented sludge over the six month period was 1 m^3, which equalled a net sedimentation rate of 0.53 cm^3/d. The sludge profile had become more uniform over the base of the pond too, the sludge contained within the gully having been redistributed over the pond floor.

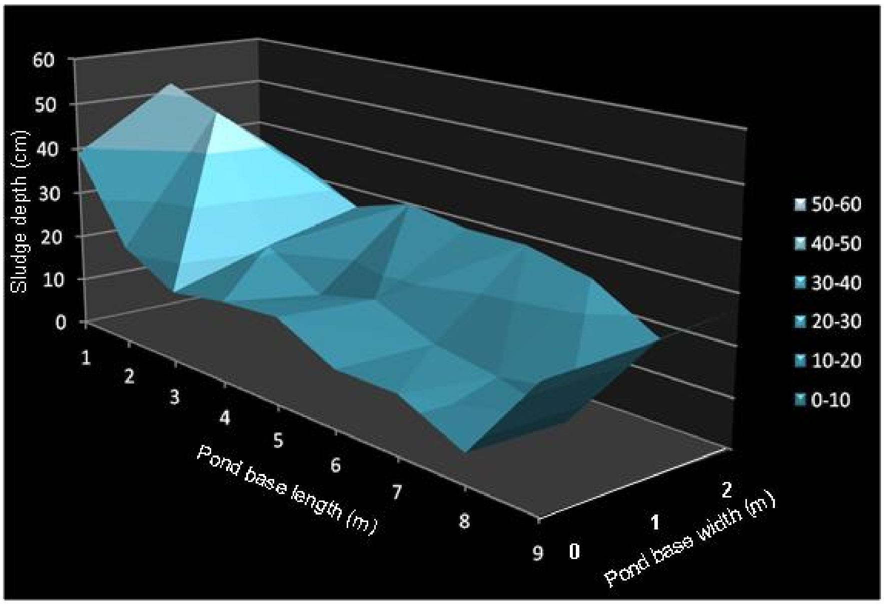


Figure 5.28: Sludge depth profile for the Blue PFP after seven months of operation.

Table 5.9: Net sludge accumulation rates measured by the white towel test over the research period.

Dates profiles measured	**Green PFP**			**Blue PFP**		
	Integrated sludge volume	Effective pond volume	Percentage pond volume reduced	Integrated sludge volume	Effective pond volume	Percentage pond volume reduced
04/11/2004	3.6 m^3	47.7 m^3	~ 7%	3.1 m^3	55.7 m^3	~ 5%
10/10/2005	4.5 m^3	46.8 m^3	~ 9%	4.1 m^3	54.7 m^3	~ 7%
23/05/2006	5.2 m^3	46.1 m^3	~ 10%	***Desludged***		
06/12/2006	***Experimental work finished on the Green PFP***			2.4 m^3	56.4 m^3	~ 4 %
12/06/2007				3.4 m^3	55.4 m^3	~ 6%

5.5 Ammonia volatilization

Two assessments of ammonia volatilization were made, using a new and improved ammonia volatilization capture chamber and absorbtion system, as described in Camargo Valero and Mara (2007). The first was conducted over a nominal HRT of $3\theta_0$ ($1\theta_0$ = 30 d) during the winter of 2006; the second was also conducted for a $3\theta_0$ period, later on in the same year in the summer of 2006.

5.5.1 Winter 2006

The mean air temperature recorded by the Bradford Met Office from January until March 2006, measured 4.1°C. (Including data for April, this mean value was 5.1°C). This corresponds closely with the mean surface water temperature measured from the Green PFP, which was 4.4°C. The maximum temperature measured at the pond surface throughout the $3\theta_0$ time was 15°C, and the minimum was −0.5°C. Figure 5.29 shows temperature profile data for the majority of weeks sampled throughout the study.

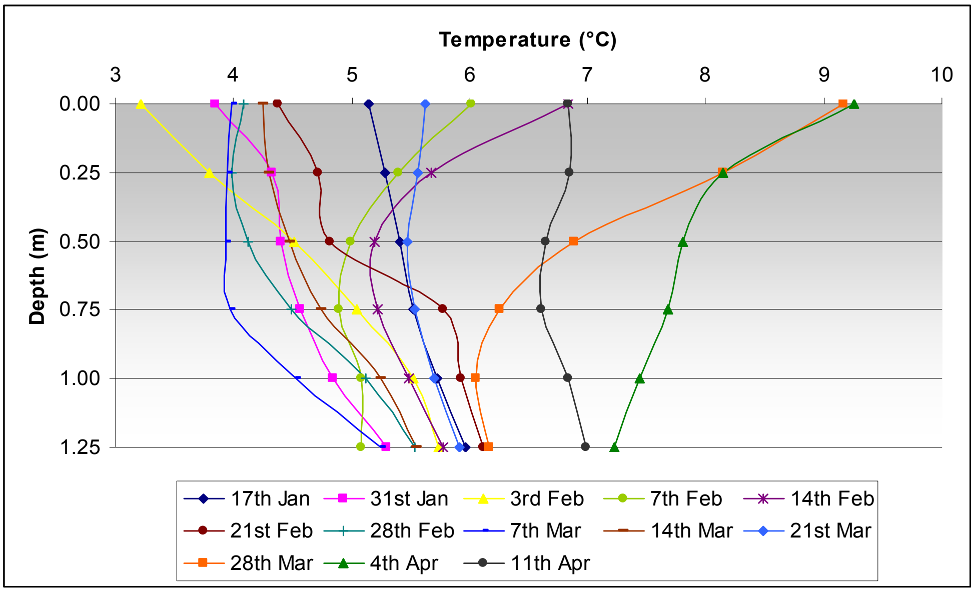


Figure 5.29: Temperature stratification data in the Green PFP, winter 2006.

The mean pH recorded at the pond effluent throughout the study period, was 7.81, with a minimum value of 7.34, and a maximum value of 9.68. Profile data for individual dates sampled are shown in Figure 5.30. pH values at the pond surface, as shown in Figure 5.30, ranged between a minimum of 7.38 on the first date sampled, and a maximum of 8.81 on Tuesday 28th of March. In all data sets, a stratification of pH occurs, where decreases in pH correlate with increasing depth throughout the water column. Stratification became more pronounced on dates sampled nearer to the end of the experiment, where facultative conditions were being restored and algal populations regenerating. A small range of pH is observed at a depth of 125 cm, where the average pH was neutral at 7.03, with a minimum value of 6.92, and a maximum of 7.19.

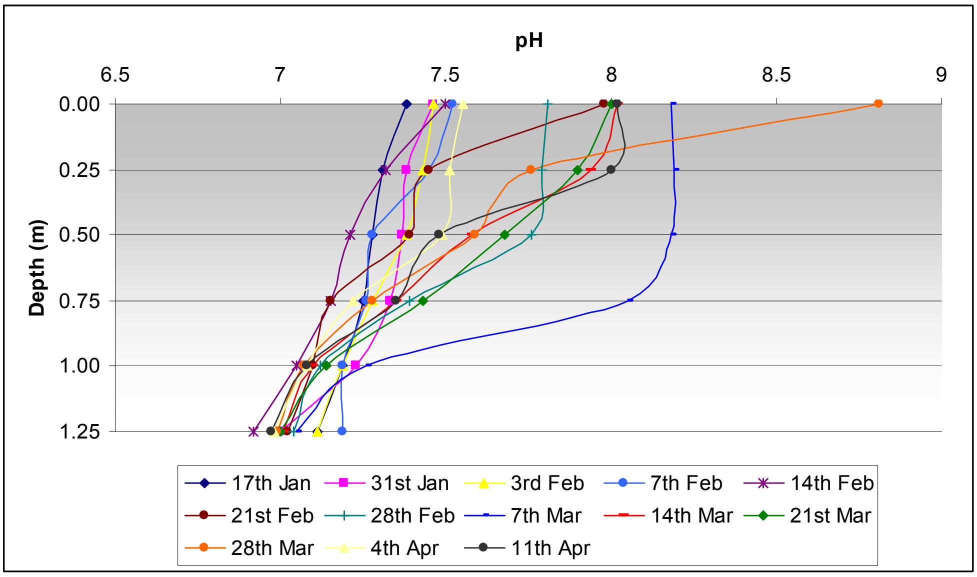


Figure 5.30: pH stratification in the Green PFP, winter 2006.

Figure 5.31 presents the total weekly ammonia volatilization recorded for 8 weeks over the winter 2006 period; the results are expressed in terms of grams of ammonia volatilized per hectare per day.

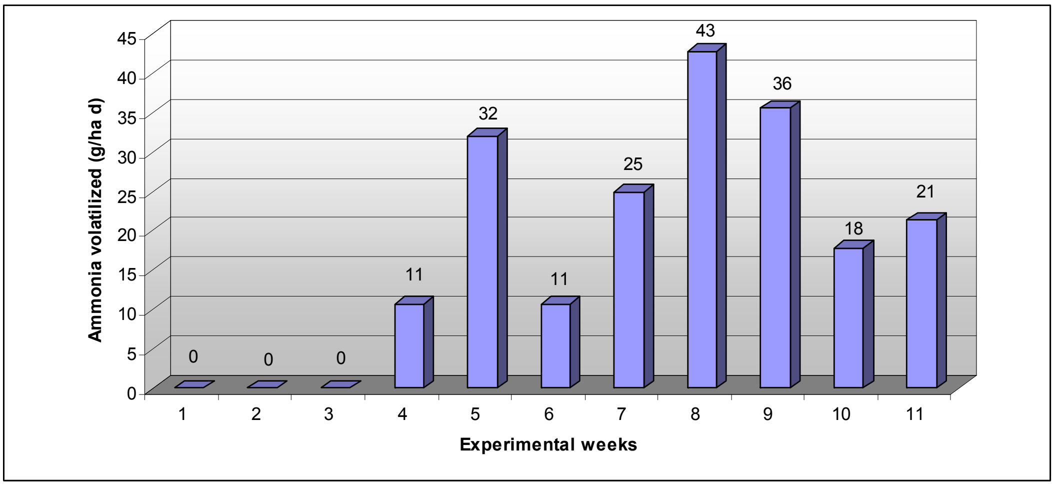


Figure 5.31: Total weekly ammonia volatilization recorded for individual weeks, winter 2006. Week 1 marks the total ammonia volatilized from the start of the experiment (from the 20th of January until the 31st of January), where consecutive weeks mark the volatilized ammonia collected between the weekly sampling schedule (until the end of the experiment), during which time boric acid from the absorbance unit was collected, and new boric acid added to the absorbance system.

The data for each ammonia volatilization experiment are presented in full in Appendix E. Unfortunately, the samples collected during the first three weeks were not measured. On one occasion, the bungs in the absorbance column, and in the three conical flasks, had completely blown out, and on two other occasions the glass bottles used to collect the samples broke in the refrigerator while pending analysis. The highest quantity of ammonia volatilized occurred in the experimental week ending Friday 24th of March, 2006 (week 8). The second highest quantity was recorded for the following week, which ended on Friday 31st of March. The third highest quantity was recorded during the week ending on Friday 3rd of March (week 5). If these data are compared with the pond column chlorophyll *a* data presented in Figure 5.10, the patterns are roughly the same: where chlorophyll *a* concentrations were highest, so was the rate of ammonia volatilization. Interestingly, from this limited data set, it can be observed that the highest rates of ammonia volatilized occurred in conjunction with periods when the pond was operating at its most facultative. Pond column ammonium concentrations were also lower for the second half of the experiment (around 5 mg NH_4^+/l); this is shown in Figure 5.19. The average quantity of ammonia volatilized over the eight week period was 2.45 mg NH_3/m^2 d, which equates to 24.5 g NH_3/ha d. This quantity, applied to the experimental period of 90 d, produced a calculated value of 2.21 kg NH_3 volatilized for the whole experiment.

5.5.2 Summer 2006

The summer assessment of ammonia volatilization was also conducted for a $3\theta_o$ period, from Monday 14th August, until Sunday 12th November. The average air temperature recorded by the Bradford Met Office for the summer months of August to October 2006 was 14.7°C (which is lowered to 13°C if data for November is included). This compared favourably with the mean water temperature of the Blue PFP's surface throughout this time, which measured 14°C. The mean maximum air temperature observed for this period measured 18°C (the maximum temperature recorded at the pond's surface being 20.8°C), and the minimum mean air temperature observed was 11.3°C. (The actual minimum temperature recorded at the pond surface was 6.12°C). Temperature profiles recorded for the Blue PFP, shown in Figure 5.32, indicate that the pond was fairly well mixed thermally throughout the $3\theta_0$ period. In fact, no degree of thermal stratification was recorded at all for the dates of Tuesday 10th, Tuesday 17th, and Tuesday 31st October. With the exception of these three data sets, in all the data presented the temperature profiles were uniform from depths below 20 to 40 cm deep, where slight increases in temperature were observed close to the surface of the pond. These increases however, did not exceed a 1 °C rise. The profile recorded for Tuesday 12th September presents the only really thermally stratified data set, with approximately a 5°C decrease in temperature between the surface of the pond, and a depth of 60 cm. The highest mean water column temperatures were observed on Tuesday 5th September, and Tuesday 12th of September, and measured 16°C. The coolest mean water column temperature was measured on Tuesday 7th of November at 7.1°C.

pH profile data are presented in Figure 5.33. Eight of the eleven weeks sampled show that practically no stratification of pH occurred, and that for these eight profiles the mean range was relatively small, approximately between 7.6 and 8.2. Only three dates observed produced stratified pH profiles; these were in the first, second and fourth weeks into the study (Tuesday 22nd August, and Tuesday 29th of August, and Tuesday 12th September, respectively).

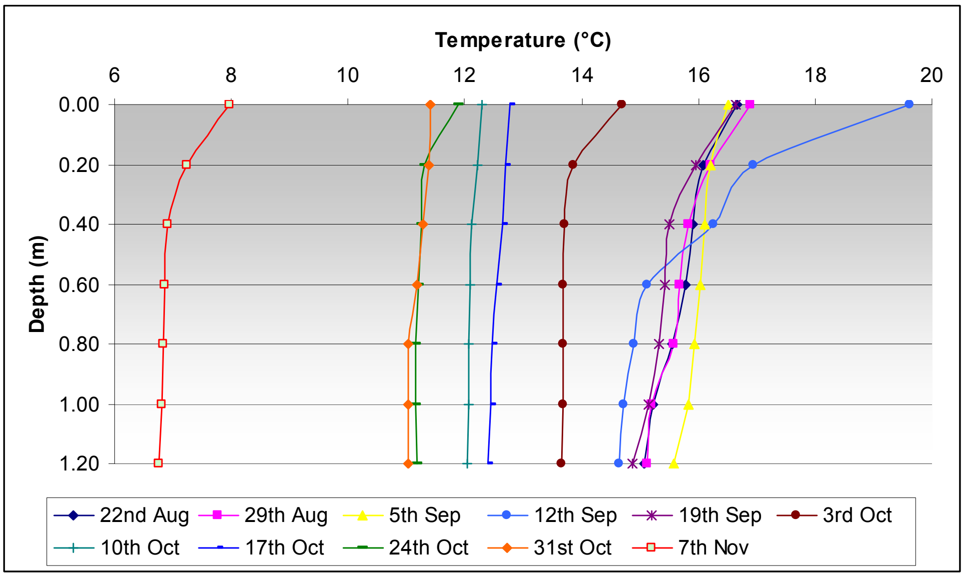


Figure 5.32: Temperature stratification data in the Blue PFP, summer 2006.

The higher surface pH values observed in these profiles fit roughly with the surface chlorophyll *a* concentrations profile as shown in Figure 5.12. This is interesting because the mean chlorophyll *a* concentrations observed within the first month of the experiment were <300 µg chlorophyll *a*/l, with maximum values of approximately 400 µg chlorophyll *a*/l; surface pH values were highest and the most stratified at this time. However, during the total timeframe, in-pond algal concentrations did not rise above 400 µg chlorophyll *a*/l until the last two weeks of the experiment, when theoretically, photosynthetic rates may have been higher, thus increasing alkaline conditions at the pond surface and elevating the pH.

Figure 5.34 presents the total quantity of ammonia volatilized during the thirteen weeks throughout the 2006 summer experimental period. The highest amount of ammonia volatilized was measured in the week ending Tuesday 17th October, viz. 29 g/ha d; the second highest quantity measured (25 g/ha d) was obtained during the first week, which ended Tuesday 22nd of August. Neither of these weeks contained especially high chlorophyll *a* concentrations; in fact, pond column concentrations revealed <300 μg chlorophyll *a*/l, below the recommended minimum level for sustaining facultative conditions. During weeks five and twelve, a recurring problem caused the bungs sealing the absorbance apparatus to blow out. The pump flow rate required was kept constant at the required flow rate of 3 l/min, and the pipe network was checked for blockages, yet the problem was nit eradicated. The boric acid, collected from the absorbance system for this week, revealed no detectable ammonia volatilized from the system, although it is highly likely that ammonia was volatilizing from the pond surface continually throughout the summer period.

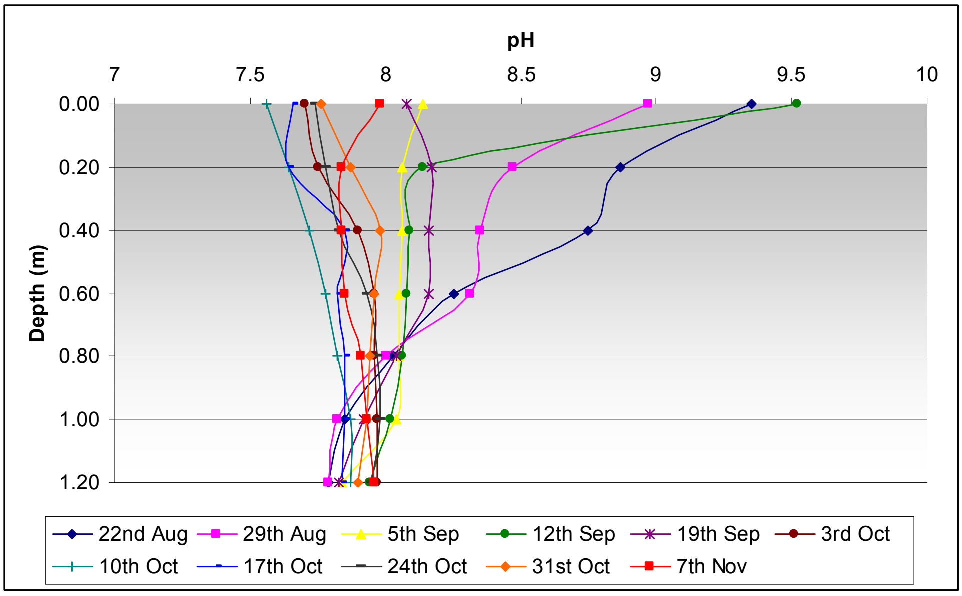


Figure 5.33: pH stratification in the Blue PFP, summer 2006.

The analysis of the ammonia volatilization data is presented in full in Appendix E. A calculation of the average volatilization of ammonia for the total 3θ_0 period revealed that 1.07 mg NH_3/m^2 d which equates to 10.7 g/ha d, was being volatilized from the Blue PFP. The total ammonia volatilized for the $3\theta_0$ period was 0.96 kg. Very surprisingly, the quantity of ammonia volatilized in the summer period was just under two and a half times more than the quantity measured for the winter period of the same year. The assessments of the physiochemical parameters of temperature (Figure 5.32), and pH (Figure 5.33), reveal that the conditions for the volatilization of ammonia were more favourable in the summer months than in the winter months. The mean monthly sunshine hours (presented in Table 5.5) were fewer in the winter months too (36.3 hours per month using data for the months of January to March); an increase, more than three-fold, in sunshine hours was observed for the summer months (114.2 hours per month using data from the months of August to October).

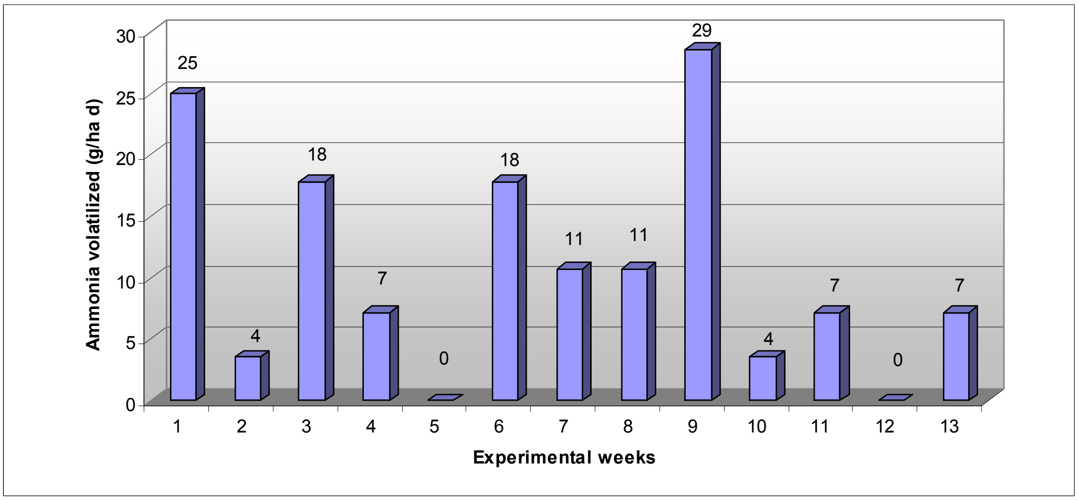


Figure 5.34: Total weekly ammonia volatilization recorded for individual weeks, summer 2006. Week 1 marks the total ammonia volatilized from the start of the experiment (from the 14th of August until the 22nd of August), where consecutive weeks mark the volatilized ammonia collected between the weekly sampling schedule (until the end of the experiment), during which time boric acid from the absorbance unit was collected, and new boric acid added to the absorbance system.

5.6 Pond hydraulic performance

The results of each of the Rhodamine WT (RWT) tracer studies are presented in Figures 5.35 and 5.36, (winter 2006), 5.38 and 5.39 (summer 2006), and 5.41 and 5.42 (winter 2007). All the important findings revealed from the tracer study experiments are summarised in Table 5.10.

Prior to the running of each experiment, the sonde was left to log hourly and record background data from the pond effluent point, for a period of one week.

Analysis of this data found that the fluorometer was picking up background concentrations of a substance, which may have been RWT, or another compound or organic substance capable of fluorescing under the probe's optical sensor. The average background concentrations of this pre-spike hourly data were taken; they were 2.1 μg/l, 9.4 μg/l, and 2.2 μg/l for winter 2006, summer 2006, and winter 2007, respectively. Interestingly, the summer 2006 background concentration was over four times higher than the winter 2006 and 2007 concentrations, indicating that elevated algal concentrations (or other pond biomass) may have interfered with the absorbance measurements collected by the fluorometer. In the calculation of the dispersion numbers and RWT recovery for each data set, the background concentration ($C_{background}$) was subtracted from the observed RWT concentration (C_{actual}), to give the spike concentration of RWT ($C_{actual} - C_{background} = C_t$). Therefore, in each data set, the RWT concentration at the start of each experiment (i.e., at t = 0), was set to zero. In the event, towards the end of the nominal $3\theta_0$ time, where the majority of RWT had been washed out of the PFP's and negative C_t concentrations were obtained, these values were reset to zero, as, theoretically at least, the effluent at that time contained no RWT from the experimental spike.

5.6.1 Winter 2006

The data presented in section 5.3 show that the Green pond used in the winter 2006 study was anoxic/anaerobic for at least half of the experimental run time. As observable in Table 5.10, this experimental run recorded the lowest RWT peak ($C_{t\ max}$ = 34.8 μg/l) when compared with those of summer 2006 and winter 2007 ($C_{t\ max}$ summer 2006 = 45.8 μg/l; and $C_{t\ max}$ winter 2007 = 109.6 μg/l). It must be noted, however, that the maximum peak obtained for winter 2006 was recorded as an isolated value, two hours after spike injection. RWT concentrations for the third and fourth hour after spike injection, measured 21.4 and 21.7 μg/l respectively, but, from the fifth hour onwards, the concentration steadily decreased from 18.6 μg/l, until around day twenty-five of the experiment. Not surprisingly, as a result of the early peak concentration of RWT, measured at the pond effluent, the calculated index of hydraulic short circuiting for this experiment was the largest of all three runs (α_s = 1.0).

The actual RWT concentration, and RTD graphs, presented in Figure 5.35 and 5.36 respectively, show a sustained gradual flush-out of RWT from the pond over the $3\theta_0$ hydraulic retention time. Apart from the elevated RWT concentrations measured over the first few hours, the curves in Figures 5.35 and 5.36, for the first thirty day period, show a steady downward lag of decreasing RWT concentration. The curve plateaus for much of the second hydraulic retention time (30 ~ 55 d), and then concentrations decrease further to form another plateau from day fifty-five onwards, until gradually fading just before the end of the actual ninety-day experimental run.

The total RWT recovery from this experiment (recovery was calculated as the total quantity of RWT which passed out of the PFP effluent point) was very low, measuring 0.99 g, just 3.09% of the total concentration of the spike injected. This relatively low recovery, and (apart from initial hydraulic short circuiting events) the relatively gradual release rates of RWT from the pond system, can well be explained by the large conical volume of sludge (comprised mostly of thick settleable solids) surrounding the pond inlet works, recorded in Figure 5.24. The portion of this sludge was deep enough to cover influent plastidrain pipe; therefore, it is highly likely that the sludge acted as a physical barrier to influent flow, and thus the RWT too. It is probable that the bulk of the RWT did not pass out from this portion of sludge, but remained in the interstitial sludge water between the sludge colloidal solids, therefore inhibiting active transport and mixing of the dye. Indeed, when the sludge collection receptacles were removed from the PFP's, in all cases, especially in the two receptacles nearest the pond inlet, the sludge was a rich pink/purple colour – the colour of Rhodamine WT.

The sludge barrier also provides part of the explanation for the average hydraulic retention time of RWT within the PFP, where $\bar{t}$ = 28.21 days, the highest average retention time measured from each of the three runs. This tracer study also exhibited the largest calculated dispersion number (D/uL = 0.4566) of the three runs. This value represents non-ideal arbitrary flow, characteristic of a dispersed flow hydraulic regime, neither satisfying ideal flow characteristics for plug flow (D/uL = 0), or completely mixed ($D/uL = \infty$).

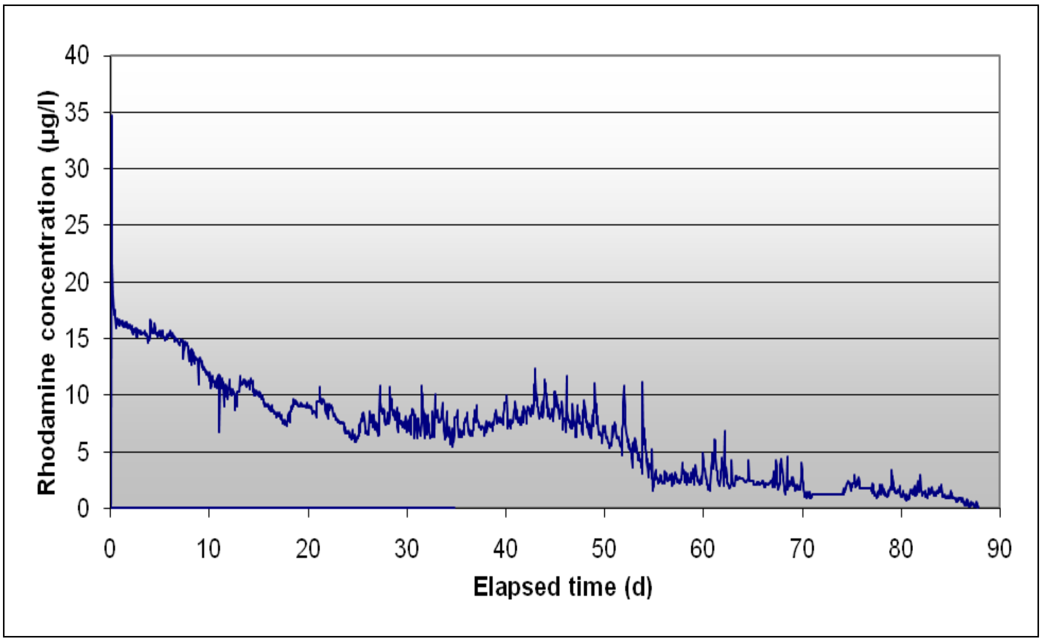


Figure 5.35: RWT concentration measured at Green pond effluent, over the experimental theoretical hydraulic retention time of $3\theta_0$ (90 d), winter 2006.

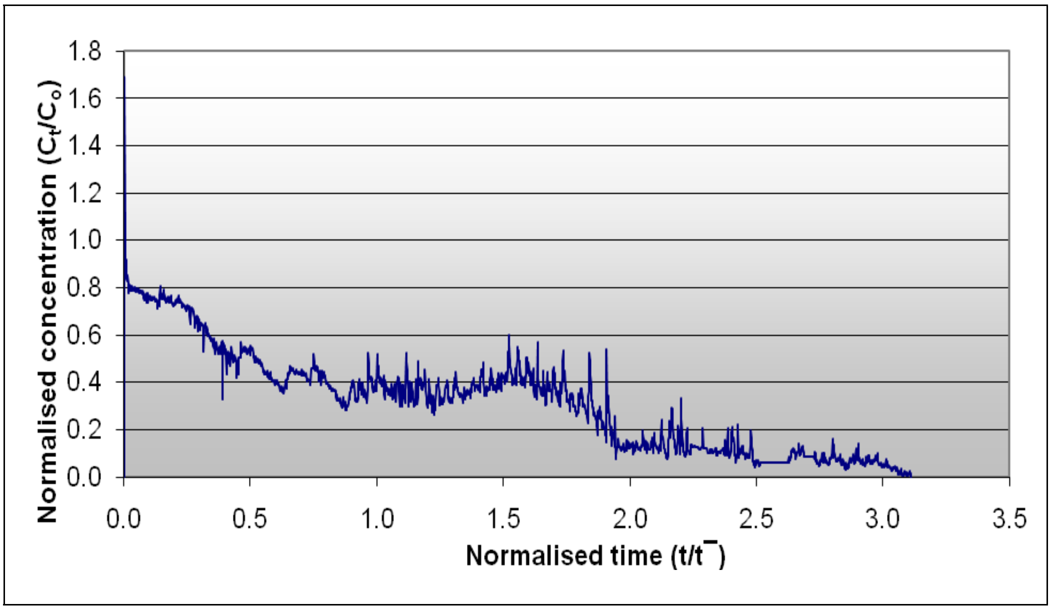


Figure 5.36: Normalised RTD curve for the first experimental run conducted on the Green pond, winter 2006.

The concentration of ^{15}N exiting the pond as $^{15}N{-}NH_4^+$ was calculated (Appendix D), and the data normalised to produce the normalised concentration curve as recorded in Figure 5.37. The shape of the data does not fit with the normalised RDT curve presented in Figure 5.36. The normalised concentration of $^{15}N{-}NH_4^+$ has a maximum peak value of just over 0.16, and occurs marginally later than the maximum peak height of 0.055, observable in Figure 5.36. After a normalised time period of t/t¯ = 0.3, the normalised $^{15}N-NH_4^+$ concentration shows an overall steady decrease although there are seven pronounced peaks and troughs occurring from t/t¯ = 0.3 to t/t¯ = 2.0. From Figure 5.37 it is evident that, at normalised time of t/t¯ = 2.1, the main portion of the $^{15}N-NH_4^+$ has been washed from the system, leaving a trace tail where gradual dissipation from the PFP occurs.

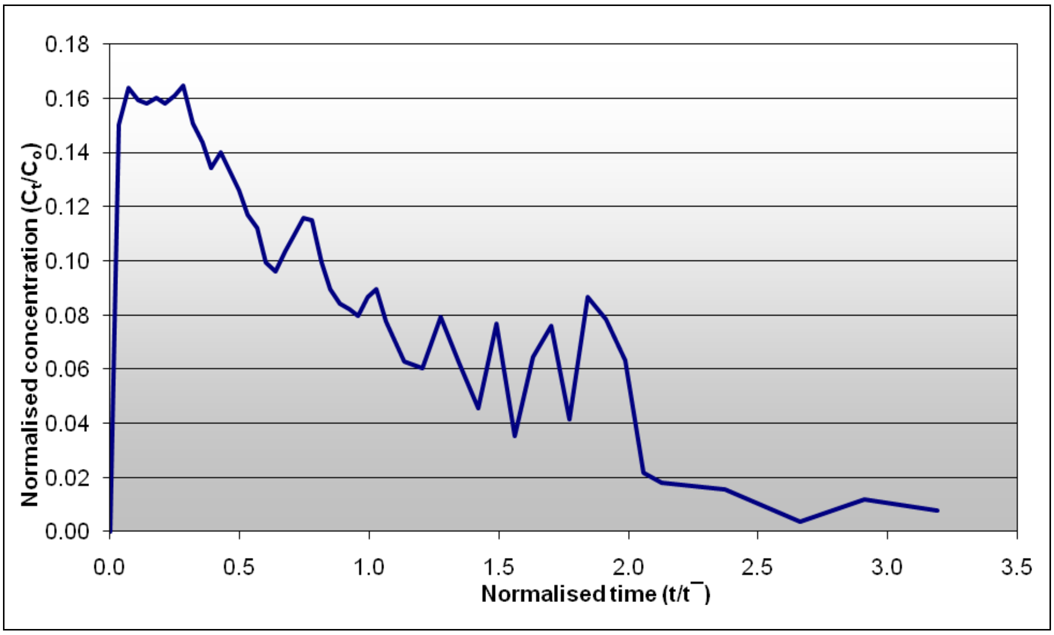


Figure 5.37: Normalised concentration curve for ^{15}N stable isotope tracer, for the first experimental run conducted on the Green pond, winter 2006.

5.6.2 Summer 2006

The RWT concentration and RTD curves produced for the experimental run of summer 2006 (Figures 5.38 and 5.39, respectively) are very different from those of the first experimental run. A degree of hydraulic short circuiting is observed ten hours after spike injection (0.42 d), with a preliminary peak of 31.5 µg RWT/l. Figure 5.38 shows an overall steady increase of RWT passing out of the pond from t = 0 until t = 15 d. The maximum peak concentration (C_t = 45.8 µg/l) is reached 15.8 d after spike injection. Following that, if the noise in the data set is ignored, there is a steady decline in RWT washout form the pond until the end of the $3\theta_0$ (90 d) experimental run. On the thirty-first day the concentration plateaus for two days, then on day thirty-three, the concentration of RWT leaving the pond plummets, and does not rise again until the thirty-sixth day. This corresponds with a large rainfall event (personal site observations), which is the most plausible explanation for the disturbance in the data trend.

The average hydraulic retention time ($\bar{t}$ = 26.69 d) for this experimental run was 1.5 days shorter than for winter 2006, only 2.2 days shorter than the actual HRT, and 3.3 days shorter then the nominal HRT. The index of short-circuiting (α_s = 0.41) is less than half that observed for the first experimental run of winter 2006, indicating that there is a better degree of mixing occurring within the pond. The dispersion number calculated (D/uL = 0.2358) is half that obtained for the winter 2006 experimental run, again, exhibiting arbitrary flow, but approximating more of a plug flow regime than the winter 2006 spike. This value still represents a large degree of dispersion within any reactor.

RWT recovery at the pond effluent point was higher in this experiment (measuring 7.66%) than in the winter 2006 run. However, the amount of RWT was much lower than expected. The pond had been completely desludged four months prior to the start of the experimental run, and the sludge depth profile (Figure 5.26) revealed that the conical portion, beneath the pond influent plastidrain pipe, was not deep enough to cover the bottom of the pipe, as was the case for the Green PFP in winter 2006. The low RWT recovery from the pond effluent is most likely attributable to the density of RWT (RWT is slightly denser than water), and the dye settling out at the base of the pond over the length of the experiment.

The trend of the normalised data plotted in Figure 5.50 for $^{15}N{-}NH_4^+$ shows a remarkably good fit with both the RWT concentration, and normalised concentration curves, presented in Figures 5.38 and 5.39. In all of the Figures, three smaller main peaks are noticeable (occurring at ~2 d, ~7 d, and ~13 d) before the maximum peak concentration is reached, 15.8 d after spike injection. After the first HRT, the $^{15}N{-}NH_4^+$ normalised concentration curve shows a rapid, but sustained decrease in concentration, which is congruent with the curves recorded in Figures 5.38 and 5.39. From the evidence presented in Figure 5.50, the $^{15}N{-}NH_4^+$ concentration is not affected by the rainfall event occurring at day 33 of the experiment; this is marked by the relatively smooth path of the curve during this time.

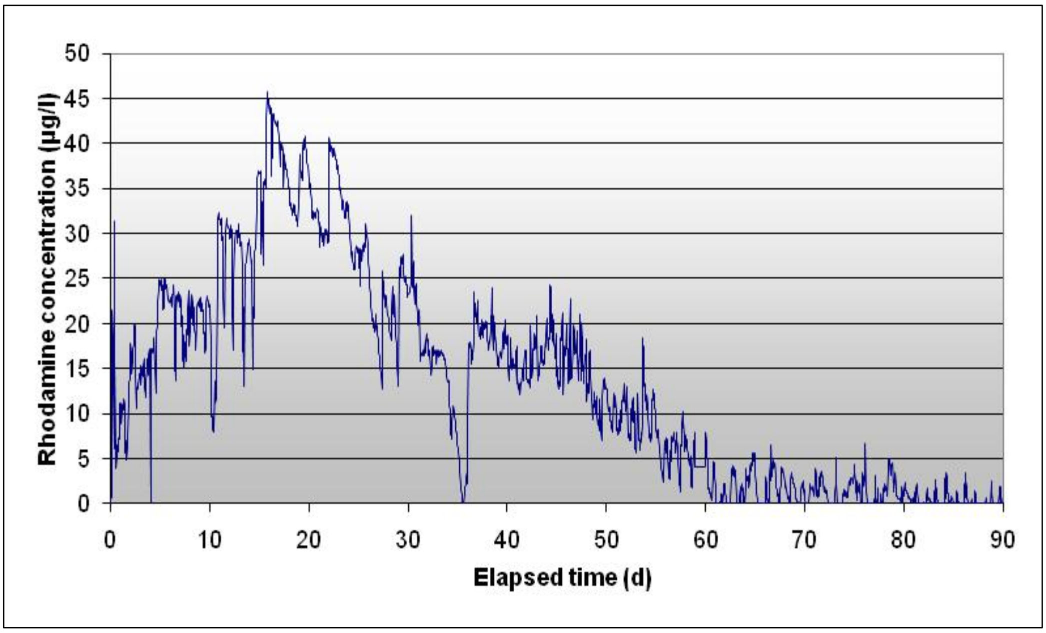


Figure 5.38: RWT concentration measured at Blue pond effluent over the experimental theoretical hydraulic retention time of $3\theta_0$ (90 d), summer 2006.

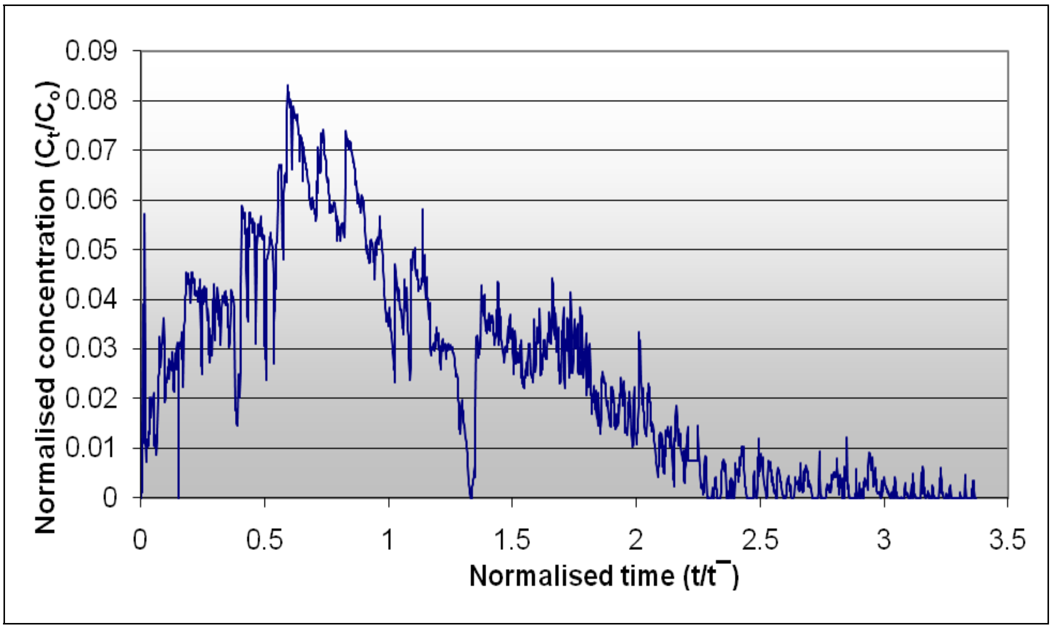


Figure 5.39: Normalised RTD curve for the second experimental run conducted on the Blue pond, summer 2006.

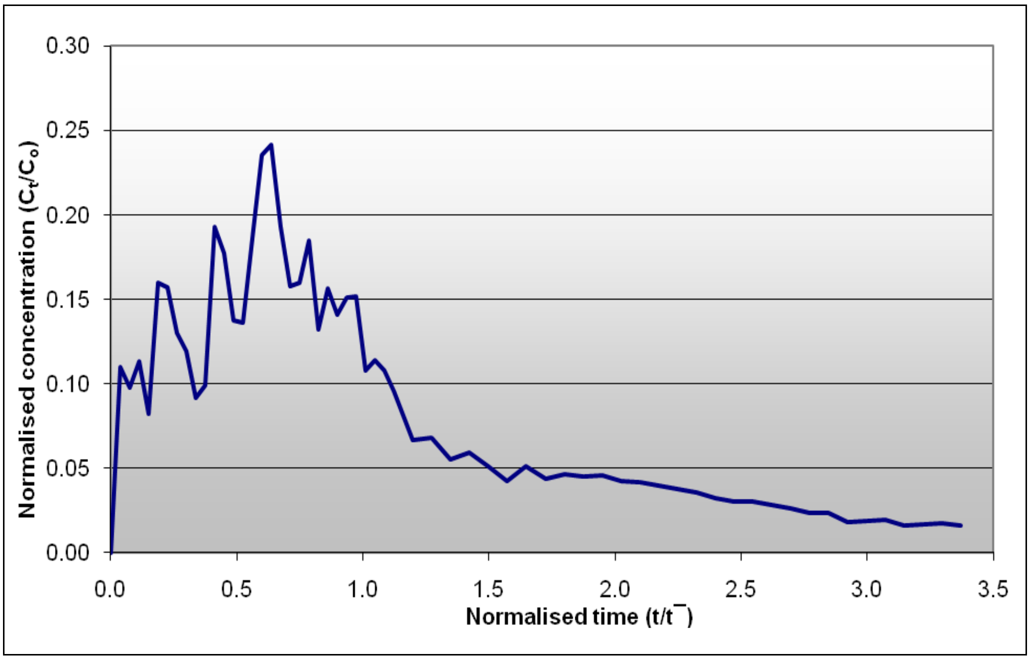


Figure 5.40: Normalised concentration curve for [15]N stable isotope tracer, for the second experimental run conducted on the Blue pond, summer 2006.

5.6.3 Winter 2007

The data obtained from the tracer study conducted in winter 2007 have their own unique characteristics. However, comparisons can be drawn between both the two winter runs.

The maximum peak RWT concentration ($C_{t\ max}$) was reached 0.21 d (5 h) after spike injection, and measured 109.6 µg/l. This is interesting, because there was a similar occurrence in the winter 2006 run. As described in section 5.3, the Blue PFP, at the start of the spike, was operating facultatively and had a rich green surface layer of algae, characteristic of a well operating facultative pond, whereas the Green pond in winter 2006 was operating anoxically. As already mentioned, the concentration of RWT used in each spike was based on calculations which assumed a completely mixed hydraulic regime for each PFP, and a spike concentration of 110 µg RWT/l pond volume prepared. The maximum spike concentration reached after 0.21 d, was practically identical to the calculated theoretical in-pond concentration, assuming a completely mixed model. Indeed, the curves of the graphs in both Figures 5.41 and 5.42, at first sight, appear to mimic a completely mixed flow regime, but the dispersion number produced (0.3421) is characteristic of an intermediate or arbitrary flow regime.

The very short time taken for the maximum spike concentration to reach the effluent point (demonstrated in Figures 5.41 and 5.42) shows a very large degree of hydraulic short- circuiting, which produced an index of short-circuiting of 0.99 − the same value as was calculated for the winter 2006 spike. Evidence of hydraulic short-circuiting is clearly shown in Figure 5.44 for the winter experimental run of 2007, where a red plume of RWT can be seen reaching the PFP effluent point, just two hours after it was introduced into the pond. The furthest end of the plume is visible in the top right hand corner of the pond, directly adjacent to, and only around 50 cm away from, the effluent point. Three noticeable troughs appear in the degree of RWT washout from the Blue PFP during this run. They occur approximately seven days, thirteen days, and twenty days after spike injection. These troughs could result from rainfall and freezing events, which would inevitably introduce pulses of additional water to the pond, thus creating a flow surcharge at the effluent point.

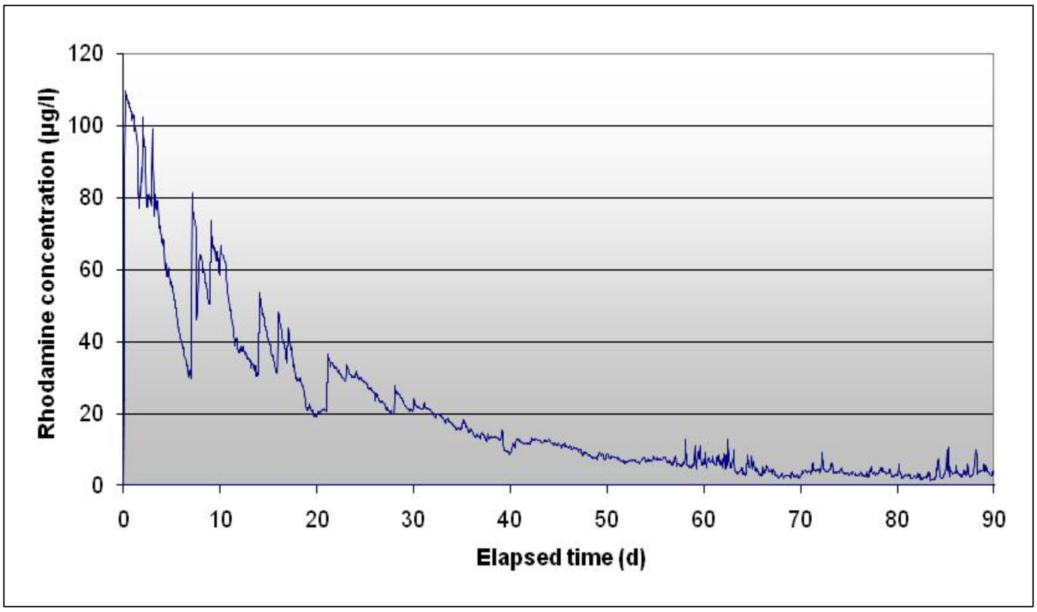


Figure 5.41: RWT concentration measured at Blue pond effluent over the experimental theoretical hydraulic retention time of $3\theta_0$ (90 d), winter 2007.

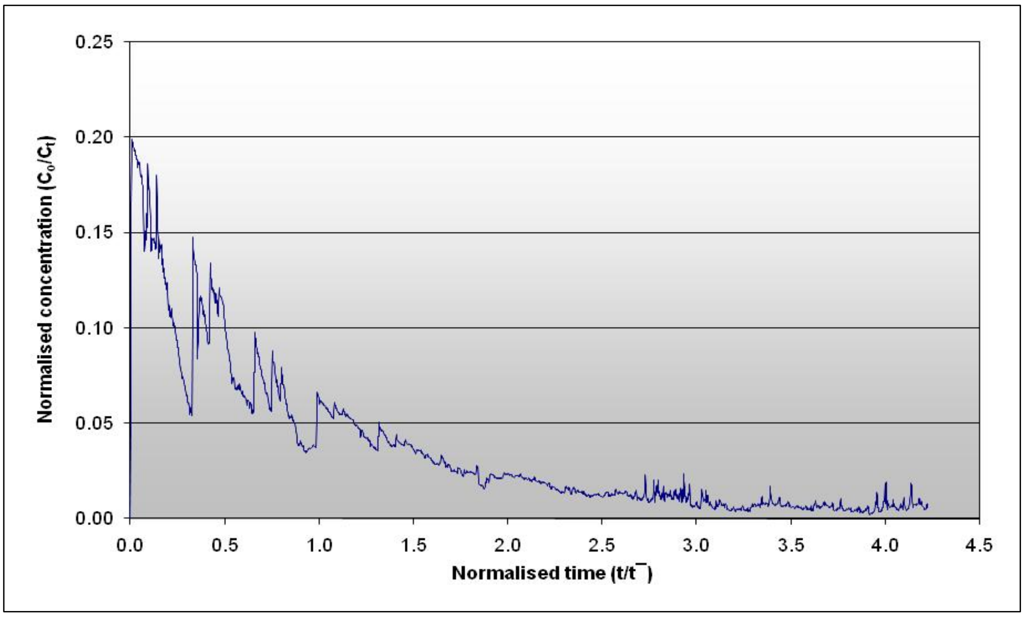


Figure 5.42: Normalised RTD curve for the third experimental run conducted on the Blue pond, winter 2007.

Where the peak concentration in the winter 2006 spike appeared to be an isolated reading, the general curve of the graph in Figure 5.35 shows a rapid but consistent decrease in RWT concentration over time. The overall trend of the graph shows a decrease in RWT concentration until around 50 d after spike injection, when the concentration of RWT stabilises, with an average value for the remaining forty days of 4.7 µg/l. The average retention time of the tracer within the PFP, for this study, was the lowest of all three runs ($\bar{t}$ = 21.3 d); this is almost a third of the nominal and actual HRT's, even though the actual HRT was identical to the summer run (θ = 28.9 d). The RWT recovery from this spike was the highest of all three runs: over a tenth (11.47%) of the initial spike concentration was recovered from the outflow at the pond effluent. As with the winter and summer 2006 experiments, the sludge retrieved after $3\theta_0$ was bright purple.

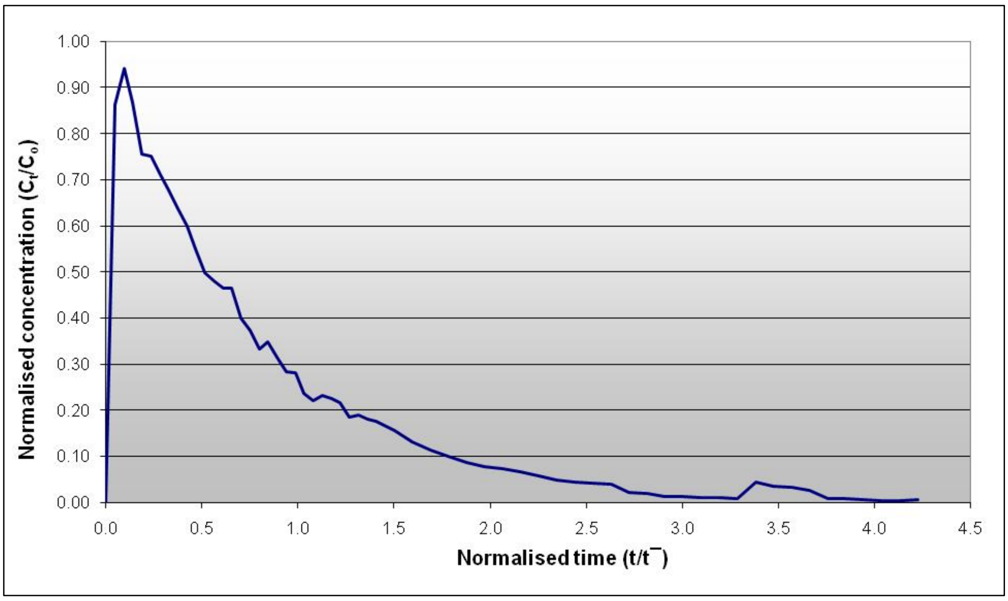


Figure 5.43: Normalised concentration curve for ^{15}N stable isotope tracer, for the third experimental run conducted on the Blue pond, winter 2007.

The normalised $^{15}N{-}NH_4^+$ concentration graph presented in Figure 5.43 shows a very agreeable fit with the RWT RTD curve in Figure 5.42. As with the summer 2006 run, the passage of $^{15}N{-}NH_4^+$ exiting the system appears to follow the path of the RWT dye tracer. As with the summer 2006 experiment, the data set presented in Figure 5.51 for $^{15}N{-}NH_4^+$, not only correlates well with the RWT spike, but are also apparently not influenced by rainfall or other events which induced the interference with the RWT spike; this is represented by the degree of noise in all of the RWT graphs.

Figure 5.44: Evidence of hydraulic short circuiting exhibited in the Blue PFP, two hours after spike injection, winter 2007.

Table 5.10: Data summary table for the three hydraulic tracer studies.

	Winter 2006	Summer 2006	Winter 2007
Nominal hydraulic retention time (θ_0)*	30 d	30 d	30 d
Actual hydraulic retention time (θ)†	29.0 d	28.9 d	28.9 d
Average retention time ($t/\bar{t}$)‡	28.2 d	26.7 d	21.3 d
Dispersion number $\left(\frac{D}{uL}\right)$	0.4566	0.2358	0.3421
Maximum RWT concentration ($C_{t\ max}$)	34.8 µg/l	45.8 µg/l	109.6 µg/l
Time taken to reach peak/maximum concentration (t_p)	0.08 d (2 hrs)	15.8 d (381 hrs)	0.21 d (5 hrs)
Index of short circuiting (α_s)	1.00	0.41	0.99
RWT recovery	0.99 g (3.09%)	2.48 g (7.66%)	3.71 g (11.47%)

* The nominal HRT represents the days specified for one theoretical HRT (chosen to be 30 d), where three theoretical HRT were needed in order to ensure successful, and near complete, washout of RWT from the PFP's.

† The actual hydraulic retention time is based on the mean effluent flow leaving the PFP's over the duration of the $3\theta_0$ period. Full calculations are presented in Appendix A.

‡ The average retention time of RWT in each pond was calculated by equation 2.44.

5.7 Stable isotope tracer studies and nitrogen mass balances

This section presents all the results from the stable isotope tracer studies. Mass balances are calculated for each experimental run, from chemical data, and are contrasted with mass balances prepared from the ^{15}N content of the $^{15}NH_4Cl$ stable isotope tracer spike.

5.7.1 Winter 2006

The Green PFP was spiked with 2.4 g of ^{15}N on Friday 20th January 2006 for a period of $3\theta_0$ (90 d). As already discussed, in-pond operating conditions were not ideal at the time, with the pond being anoxic for approximately two thirds of the experiment. Figure 5.45 shows the mass spectrometry data of daily composite samples for organic and inorganic ^{15}N nitrogen fractions, exiting the pond at the effluent point.

The ^{15}N data gathered from the mass spectrometer does not provide a concentration of ^{15}N for a particular sample; the results obtained are expressed as $\delta^{15}N$ in parts per thousand. This is actually the ratio of ^{15}N:^{14}N with respect to the standard – air – which has a known isotopic ^{15}N fraction.

The mass spectrometry data were converted into actual concentration data (μg/l) using a novel calculation procedure developed by Camargo Valero (2008), which is presented in equation D.1 in Appendix D.

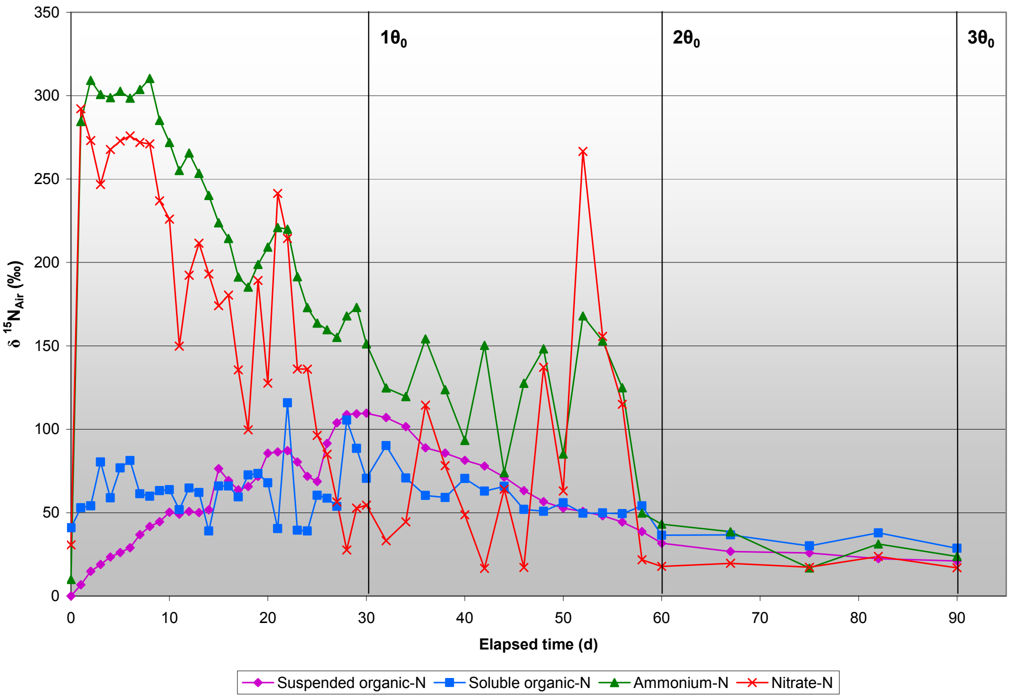


Figure 5.45: Mass spectrometry ^{15}N data for nitrogen fractions measured at the pond effluent point, winter 2006.

Figure 5.46 shows the concentration data for the ^{15}N-enriched suspended organic nitrogen fraction, exiting the Green PFP over a nominal 3θ HRT. It can be seen that incorporation into the suspended organic nitrogen fraction is low; a value of just over 0.6 μg/l is the peak value achieved for this dataset. The assimilation of ^{15}N into pond biomass occurs within the first twenty-four hours and increases consistently for the first eleven-day period of the study. Two small peaks are observed at approximately fifteen days and twenty-one days after spike injection, before the main peak occurs after thirty days. A gradual and sustained decrease in ^{15}N expulsion occurs for much of the second HRT, where the tail end of the curve (from $2\theta_0$ to $3\theta_0$) shows a gradual fade out of the plateau. The suspended organic-^{15}N nitrogen fraction does not return to its background level after the $3\theta_0$ period, but it is postulated that gradual washout would return this concentration to near zero.

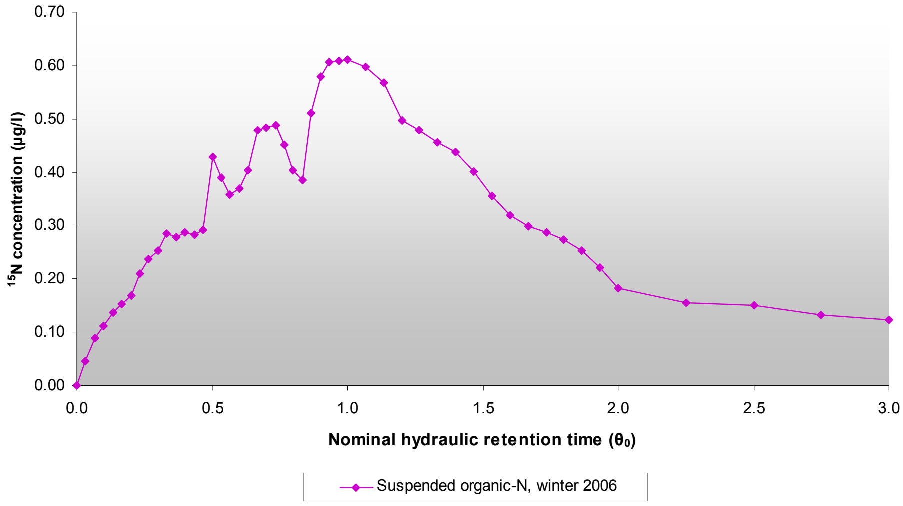


Figure 5.46: ^{15}N enriched suspended organic-nitrogen, winter 2006.

The ^{15}N discharge of soluble organic-nitrogen from the PFP shows a distinct random pattern (Figure 5.47), and does not follow, in any way, the pattern of the suspended organic nitrogen fraction. The first two data points show that the ^{15}N, within the first twenty-four hours, is transformed into this organic nitrogen fraction, but, after a seventy-two hour period, fluxes in the ^{15}N concentration leaving the pond are observed bi-daily (these are shown by the pronounced peaks and troughs occurring in the graphical data). The two peak concentrations evident in Figure 5.46 coincide with the peaks displayed in the soluble organic ^{15}N data. After the third nominal HRT, weekly composite samples were prepared; a soluble organic-N fraction was not observed in the tail end of the experiment, possibly because of dilution causes by the combining of samples to make the composite.

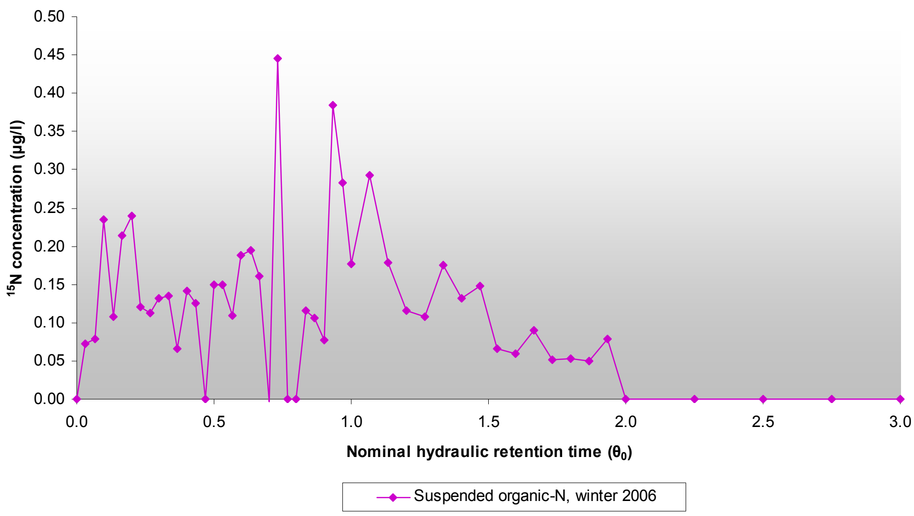


Figure 5.47: ^{15}N enriched soluble organic-nitrogen, winter 2006.

The peak concentration of ^{15}N-ammonium leaving the pond effluent (Figure 5.48) appears to be instantaneous, showing a very high degree of hydraulic short circuiting within the system. A primary pulse of ^{15}N passes out of the system over the first eight day period, but six other peaks are observed over the $3\theta_0$ experimental time. A peak ^{15}N concentration of 7.7 µg ^{15}N/l occurs on day eight. Although successive peaks do occur throughout the data set, the overall trend shows a steady decline over a $2\theta_0$ time period.

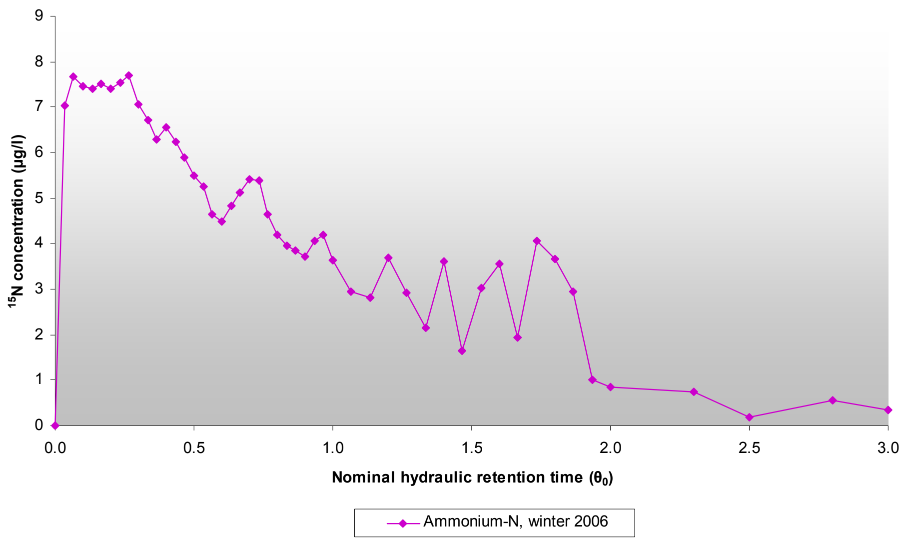


Figure 5.48: ^{15}N enriched ammonium-nitrogen, winter 2006.

The transformation of $^{15}NH_4^+$ occurs most rapidly in the oxidation of ammonium to nitrite, then nitrate (Figure 5.49). Within the first 24 hour period after spike injection, a peak maximum concentration of 0.18 µg ^{15}N/l had been reached. The anomalous data point observed at around day 50 could genuinely be attributed to an isolated event occurring within the PFP, or may have resulted in sample carry over, or memory effect from the mass spectrometry reagent columns.

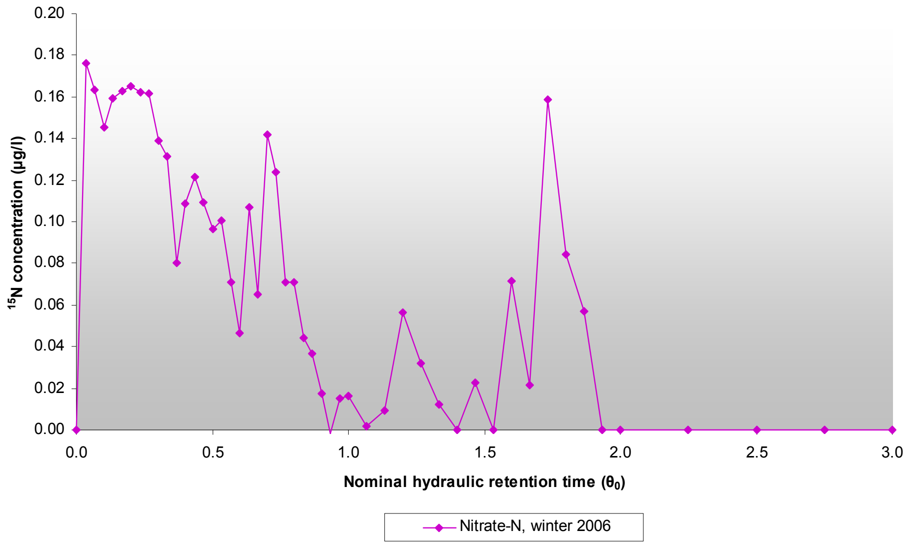


Figure 5.49: ^{15}N enriched nitrate-nitrogen, winter 2006.

A nitrogen mass balance was prepared for the winter 2006 experiment, and the data is presented in Table 5.11. The Green PFP was loaded with a mean daily load of 10.8 kg N/ha d throughout the $3\theta_0$ period. Data for the four nitrogen fractions measured in the pond effluent, ammonia volatilization, and nitrogen contributed to the pond sludge are presented.

Table 5.11: A nitrogen mass balance for the Green PFP, winter 2006.

Nitrogen fraction	Mean amount of N/d (kg N/ha d)	Percentage of total N recovered
(Total TKN in influent)	**(10.80)**	**(100%)**
Effluent suspended organic-N	1.53	14.2%
Effluent soluble organic-N	0.68	6.3%
Effluent NH_4^+-N	3.85	35.6%
Effluent NO_3^--N	0.29	2.7%
Volatilized ammonium fraction	0.025	0.2%
Sedimented N fraction	2.53	23.4%
Total accounted for	**8.91**	**82.4%**

Table 5.12 presents the recovery factors for each of the four ^{15}N fractions leaving the pond in the pond effluent. Unfortunately, the sludge data were not correctly recorded, therefore the amount of ^{15} nitrogen contributed to the sludge has had to be omitted for this experiment. A total of 22.4% of the ^{15}N spike was recovered

from the pond effluent. The largest fraction leaving the pond was in the unchanged ammonium form. From the data recorded, it is possible to see that very little of the total ^{15}N was incorporated into the matrix of pond biomass, and subsequently re-released as a suspended organic nitrogen fraction. The transformation of ^{15}N into oxidised forms of nitrogen was also very low, which implies that nitrification processes did not predominate, or certainly did not play an important role in nitrogen removal from the system. The data presented in Appendix E, reveals that some ^{15}N was measurable in the boric acid samples which stripped volatilized ammonia from the pond. However, when compared to the background concentration of ammonia volatilized, there was no increase in ^{15}N from these samples; the mechanism of ammonia volatilization is therefore discounted as an active participatory mechanism for nitrogen removal, in anoxic PFP's during winter.

Table 5.12: Recovery of ^{15}N from organic and inorganic fractions within the Green PFP effluent, winter 2006.

Nitrogen fraction	^{15}N recovered (µg)	Recovery
(Total ^{15}N in influent spike)	**(2,400 000)**	**(100%)**
Effluent ^{15}N suspended organic-N	45,771	1.9%
Effluent ^{15}N soluble organic-N	13,310	0.6%
Effluent ^{15}N ammonium-N	471,057	19.6%
Effluent ^{15}N nitrate-N	7,193	0.3%
Volatilized ^{15}N fraction	0	0%
Sedimented ^{15}N fraction	–	–
Total ^{15}N recovered	**537,331**	**22.4%**

5.7.2 Summer 2006 and winter 2007

Datasets for summer 2006 and winter 2007 are compared and contrasted, helping to elucidate the variables in nitrogen removal mechanisms and pathways between summer and winter. The Blue PFP was spiked with the same quantity of ^{15}N as $^{15}NH_4Cl$ (8.47 g) on both summer and winter occasions, to better enable direct comparisons to be drawn between the two datasets. Figure 5.50 shows the combined mass spectrometry data found for each ^{15}N nitrogen fraction measured, exiting the pond effluent for the summer 2006 study. Figure 5.51 demonstrates similar data found for the subsequent winter.

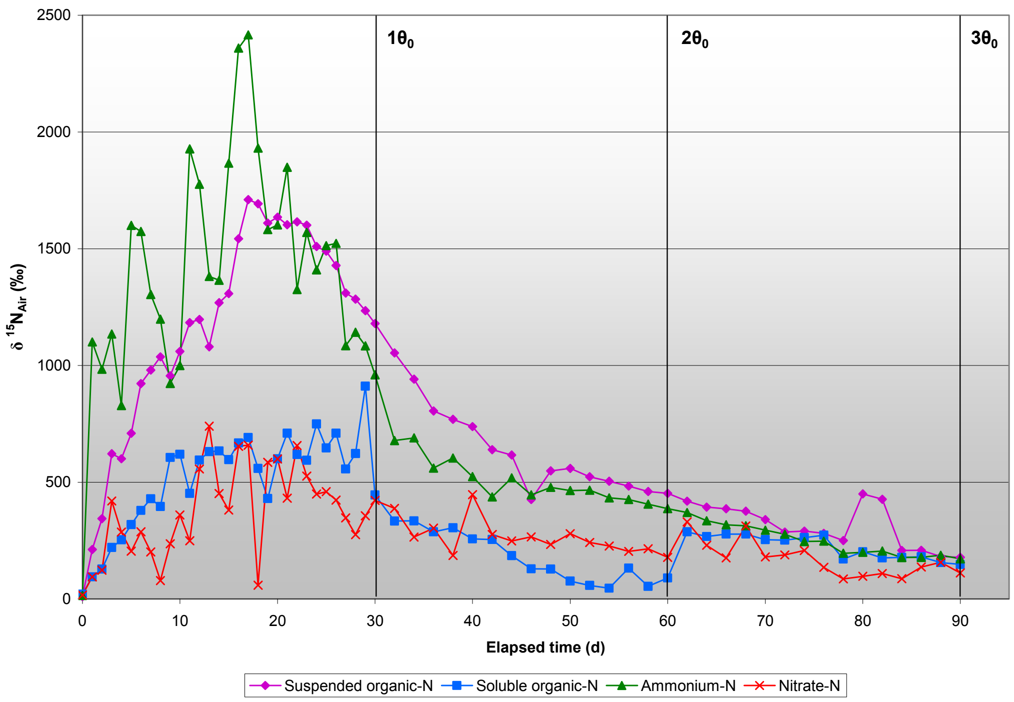


Figure 5.50: Mass spectrometry ^{15}N data for nitrogen fractions measured at the pond effluent point, summer 2006.

Both Figures 5.50 and 5.51 show that unchanged ammonium is the predominant nitrogen fraction leaving the pond, irrespective of summer or winter conditions. This was also found to be true for the winter 2006 ^{15}N data, presented in Figure 5.45.

There are a number of indelibly stark contrasts between the summer and winter data-sets. First, every curve of the graphs seen in Figure 5.50 and 5.51, is different. Apart from the static observed in the data-set, the peaks of all nitrogen fractions in the summer data-set occur simultaneously. The transformation of the ^{15}N into the three fractions of suspended, and soluble, organic nitrogen and nitrate nitrogen, occur much more gradually in the summer than in the winter. The role of algae in assimilating the ^{15}N into cellular biomass represents the second largest $\delta^{15}N$ peak leaving the pond. This is in direct contrast with the winter 2007 data-set, where nitrate nitrogen provides the second largest peak after ammonium. As already discussed in section 5.6, the amount of ammonium leaving the pond in the treated wastewater stream for the summer and winter data-set, follows the almost identical route of the RWT dye tracer. As with these studies, a very high degree of ammonium short-circuiting occurs, and this is more radically observed in the winter data-set. The hydraulic tracer studies revealed large degrees of dispersion from the two extremes of ideal flow, and when these are coupled with the ^{15}N tracer studies, it can be seen that insufficient mixing occurs within these unbaffled PFP's, and this leads to relatively large quantities of ammonium leaving the pond unchanged.

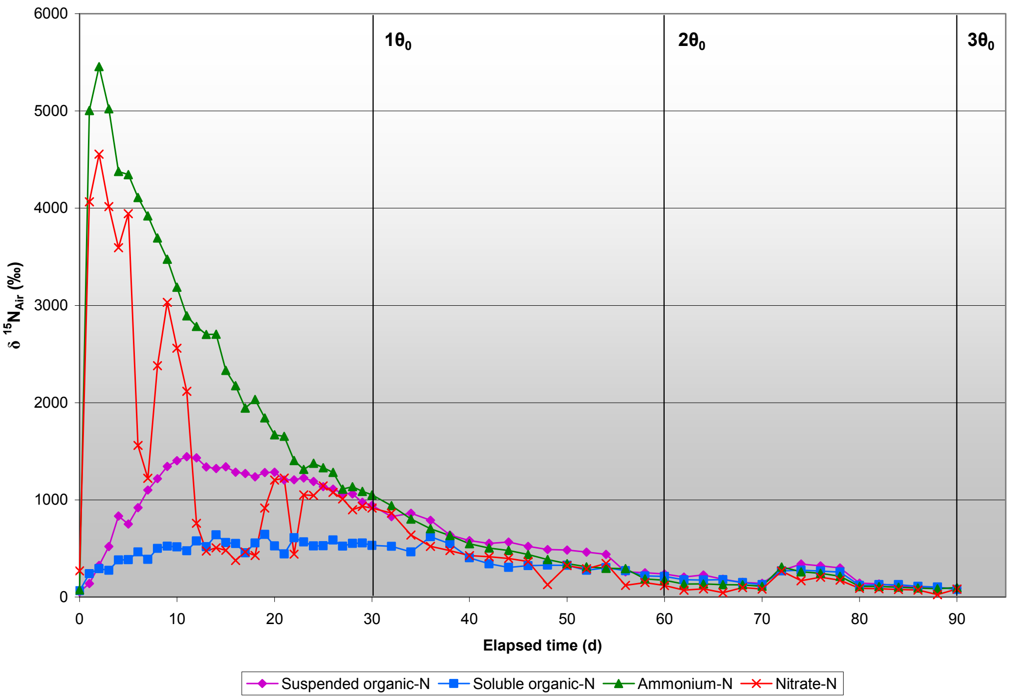


Figure 5.51: Mass spectrometry ^{15}N data for nitrogen fractions measured at the pond effluent point, winter 2007.

The raw mass spectrometry data were converted into actual ^{15}N concentrations within the samples analysed. It is demonstrably evident in Figure 5.52, that the incorporation of ^{15}N into algal biomass was much larger in the summer than in the winter. The peak concentration of ^{15}N suspended organic-nitrogen in the summer study, measured just over 25 µg $^{15}N/l$, and occurred around 17 days after spike injection. The winter data-set reveals a peak concentration for this nitrogen fraction of 10 µg $^{15}N/l$, but occurring much sooner than the summer peak concentration (after an elapse of ten days). A more rapid increase in ^{15}N assimilation is presented by the pre-peak data but, once the peak concentrations have been reached, a gradual downward lag ensues, indicating that there is a sustained washout from the system during this time.

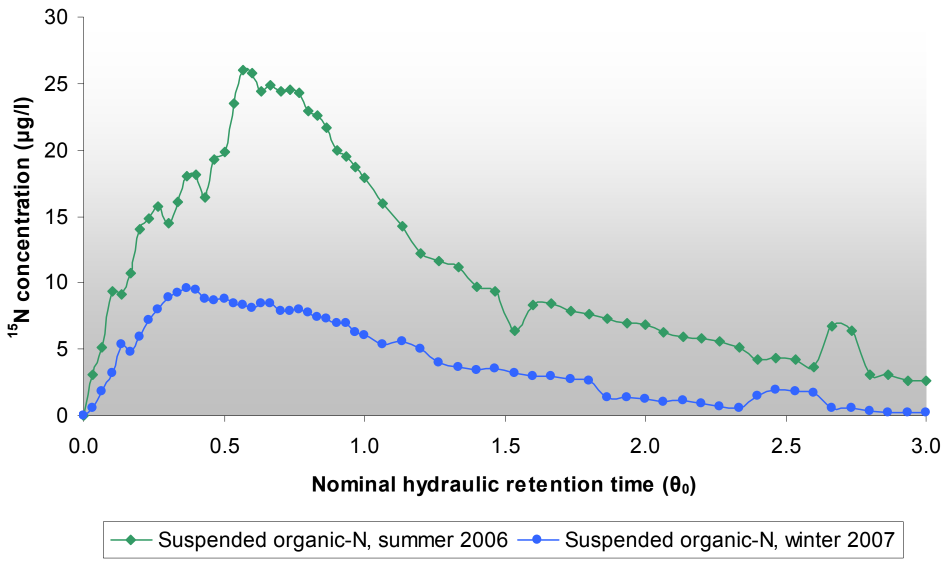


Figure 5.52: ^{15}N enriched suspended organic-nitrogen, for summer 2006 and winter 2007.

The ^{15}N soluble organic-nitrogen data are seen in Figure 5.53. The peak concentrations of this fraction are smaller than the soluble organic nitrogen fractions, but relatively the same pattern is apparent. Around twice as much ^{15}N is incorporated into suspended organic nitrogen in the summer than in the winter. The peak height area occurs between seven and thirty days, and is much broader than the suspended organic nitrogen peak observed in Figure 5.52. The winter curve shows that ^{15}N concentrations do not fluctuate as much as those observed in the summer data-set. Both data-sets indicate in this instance that there is an increase in this nitrogen fraction in the last HRT, which again is more pronounced in the summer data-set. A likely interpretation of this evidence is the degradation from the suspended organic fraction appearing as a soluble organic fraction.

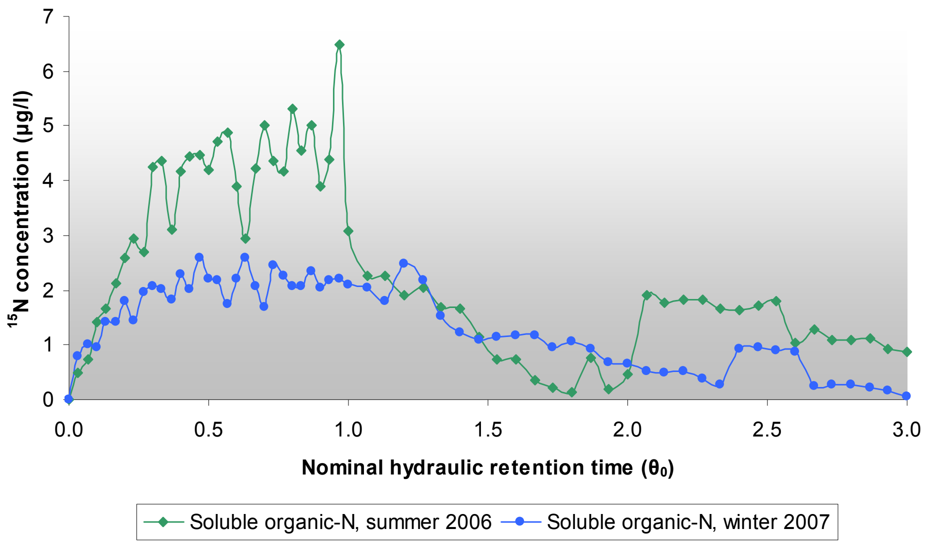


Figure 5.53: ^{15}N enriched soluble organic-nitrogen, for summer 2006 and winter 2007.

The quantity of ^{15}N leaving the pond as unchanged ammonium is clearly seen in Figure 5.54. When the isotopic $\delta^{15}N$ data are observed in Figure 5.50, the ammonia peak appears quite high next to the other nitrogen fractions. When the ratio data are however converted into concentration data and compared, the summer peak is dwarfed by the winter peak. The peak concentration of ammonium ^{15}N leaving the pond effluent in winter 2007 occurs just forty-eight hours after spike injection, with a concentration of over 130 µg ^{15}N/l. Ammonium washout from the summer study is observed to be more gradual than in the winter study. A peak concentration of <40 µg ^{15}N/l occurs seventeen days after spike injection. The Blue PFP in the summer contained a slightly higher concentration of chlorophyll *a* than the winter pond when it was spiked, but, bearing in mind that the same quantity of ^{15}N was used in the spike for both seasons, the data clearly show, as in the case for the suspended organic-nitrogen fraction, that more ammonium nitrogen is utilised in the summer than in the winter.

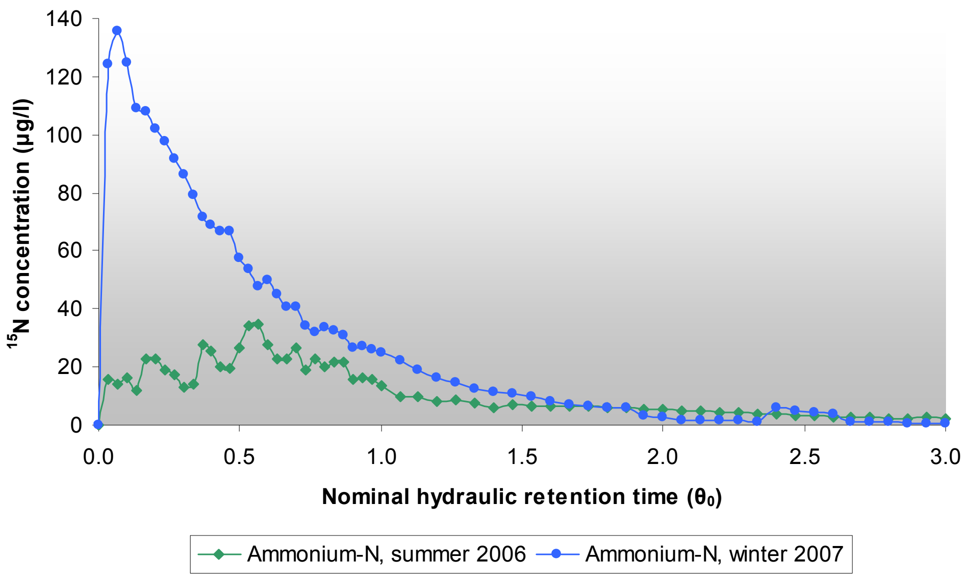


Figure 5.54: ^{15}N enriched ammonium-nitrogen, for summer 2006 and winter 2007.

Although Figure 5.51 shows that nitrate-nitrogen produced the second biggest peak value in the winter 2007 $\delta^{15}N$ data, when these data were transformed into concentration data, the ^{15}N nitrate concentrations were not too high. An identical pattern was discovered in the winter 2006 experiment: ammonium-nitrogen was rapidly oxidised upon entering the pond during the winter months. Figure 5.55 shows the concentration data for the ^{15}N-nitrate summer 2006 and winter 2007 curves. After ten days the nitrate concentration rapidly declined, and fast approached the natural background concentrations observed in the PFP pre-spike. In contrast, the summer dataset shows a negligible transfer of ammonium-nitrogen into an oxidised fraction. Although comparisons can only be drawn between three studies, and not validated by further experimental runs, there is a possibility that nitrification is a predominant pathway for ammonia nitrogen removal in PFP's, in the UK, during winter scrutiny.

The recovery factors for individual ^{15}N fractions observed from the pond effluent are illustrated in Tables 5.14 and 5.16 for summer and winter respectively. The mass balances prepared for the two experimental runs, are presented in Tables 5.13, and 5.15. As for the winter 2006 experiment, no change in the background ^{15}N concentration was observed in the summer 2006 ammonia volatilization study.

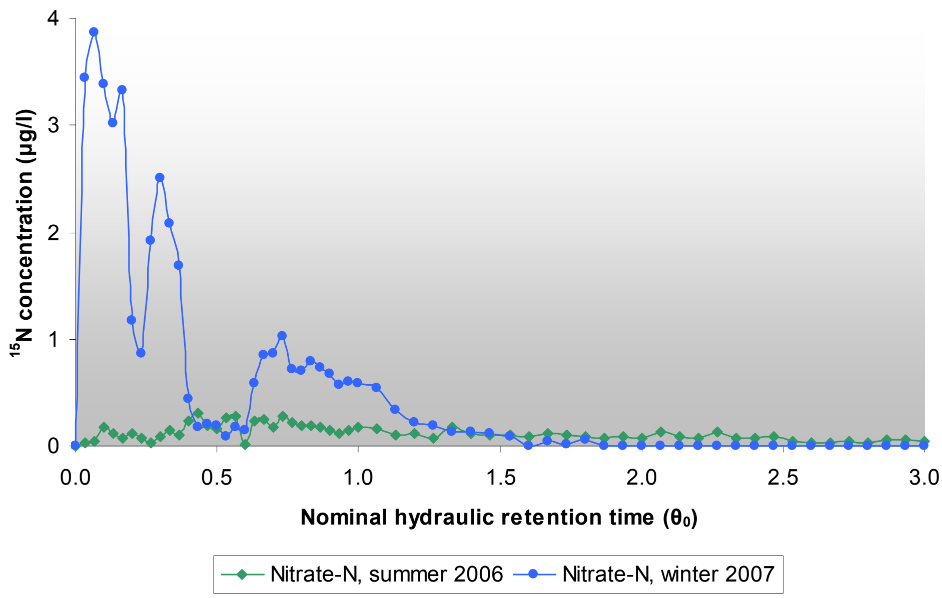


Figure 5.55: ^{15}N enriched nitrate-nitrogen, for summer 2006 and winter 2007.

The ^{15}N recovery tables reveal that more ^{15}N was recovered from the winter 2007 spike than from the summer 2006 spike. In the summer 2006 experiment, the largest portion of ^{15}N was recovered as suspended organic-nitrogen, and although the ammonium peak was larger than the other nitrogen fractions recorded in Figure 5.50, the actual concentration recovered was secondary to the ^{15}N in the suspended organic-nitrogen fraction. In the winter, the largest portion of ^{15}N was recovered as the unchanged ammonium-nitrogen fraction. The recovery of effluent soluble organic-nitrogen in the summer was almost double the winter recovery factor. Nitrate ^{15}N recovery was higher in the winter study than the summer study.

Table 5.13: A nitrogen mass balance for the Blue PFP, summer 2006.

Nitrogen fraction	Mean amount of N/d (kg N/ha d)	Percentage of total N recovered
(Total TKN in influent)	**(10.78)**	**(100%)**
Effluent suspended organic-N	2.16	20.0%
Effluent soluble organic-N	0.90	8.3%
Effluent NH_4^+-N	2.31	21.4%
Effluent NO_3^--N	0.12	1.1%
Volatilized ammonia fraction	0.01	0.1%
Sedimented N fraction	3.09	28.7%
Total accounted for	**8.59**	**79.6%**

Table 5.14: Recovery of ^{15}N from organic and inorganic nitrogen fractions within the Blue PFP effluent, summer 2006.

Nitrogen fraction	^{15}N recovered (µg)	Recovery
(Total ^{15}N in influent spike)	**(8,470 000)**	**(100%)**
Effluent ^{15}N suspended organic-N	1,984 865	23.4%
Effluent ^{15}N soluble organic-N	380,750	4.5%
Effluent ^{15}N ammonium-N	1,907 141	22.5%
Effluent ^{15}N nitrate-N	20,089	0.24%
Volatilized ^{15}N fraction	0	0%
Sedimented ^{15}N fraction	119,000	1.4%
Total ^{15}N recovered	**4,411 845**	**52.0%**

Table 5.15: A nitrogen mass balance for the Blue PFP, winter 2007.

Nitrogen fraction	Mean amount of N/d (kg N/ha d)	Percentage of total N recovered
(Total TKN in influent)	**(14.26)**	**(100%)**
Effluent suspended organic-N	2.15	15.1%
Effluent soluble organic-N	1.8	12.6%
Effluent NH_4^+-N	3.5	24.5%
Effluent NO_3^--N	0.25	1.8%
Volatilized ammonia fraction	–	–
Sedimented N fraction	1.8	12.6%
Total accounted for	**9.5**	**66.6%**

Table 5.16: Recovery of ^{15}N from organic and inorganic fractions within the Blue PFP effluent, winter 2007.

Nitrogen fraction	^{15}N recovered (µg)	Recovery
(Total ^{15}N in influent spike)	**(8,470 000)**	**(100%)**
Effluent ^{15}N suspended organic-N	688,356	8.1%
Effluent ^{15}N soluble organic-N	228,011	2.7%
Effluent ^{15}N ammonium-N	4,691 857	55.4%
Effluent ^{15}N nitrate-N	83,631	0.99%
Volatilized ^{15}N fraction	–	–
Sedimented ^{15}N fraction	40,296	0.5%
Total ^{15}N recovered	**5,732 151**	**67.7%**

5.8 Molecular microbiology

During the summer 2006 and winter 2007 experimental runs samples were taken from different areas of the Blue PFP for molecular microbiological analysis (described in section 4.7). The primary objective of the analysis was to confirm the presence, or absence, of specific groups of nitrogen-using microorganisms. The groups targeted in the analysis were ammonia-oxidising bacteria (AOB), ammonia-oxidising archaea (AOA), ANNAMOX, methanotrophs and denitrifiers.

A summary of the main findings from samples taken during the summer of 2006 is represented in Table 5.17. The PCR amplification process, using nested CTO, revealed that there were AOB present in all of the samples analysed. Only three bands from the DGGE gel were excised and sequenced, all being samples taken from beneath the sludge inlet pipe. Sequence identification in a BLAST (Basic Local Alignment Search Tool) search of public library databases, revealed that there was a 98% identity with three types of bacteria: uncultured *Nitrosomonas* sp. AF527015 (Rowan *et al*., 2003), an 82% match with *Nitrosospira* sp. AY635573 (Hornek, unpublished), and an 85% match with uncultured *Nitrosospira* sp. Isolate AY773203 (Nyberg and Schnurer, unpublished). Five bacteria classified as unidentified bacterium amoa17 ammonia monooxygenase gene AF272507, with percentage identities in the range 97% to 99% were also found in the inlet sludges, as identified by Purkhold *et al*., (2000).

Four samples were found to contain anaerobic ammonium oxidisers (ANAMMOX). The bacterium gene AB240351 (uncultured) was found (Nakamura, 2005) in a water column sample which was taken in early August, 2006, as were two uncultured *Desulfobulbus* sp., DQ831537 and DQ831533 (Silva and Purdy, unpublished). The former species was also found in a sludge sample taken from the middle of the pond in mid-August. Two other bacterial species were found in a sludge sample taken from a similar location in early August. These were *Sphingopyxis alaskensis* complete genome CP000356 (Copeland and Lucas, unpublished), and an uncultured Planctomycete clone DQ534744 with a 100% identity match (Hamersley, unpublished).

A methanotroph identified as *Methylomonas* sp. pmoA gene AF150801 (Costello and Lidstrom, 1999) was found in sludge samples taken from the middle of the pond, and also within a pond column and effluent sample.

Table 5.17: Presence and absence results for various nitrogen utilising bacterial groups found in samples taken during the summer of 2006.

Sample location	**Ammonia-oxidisers**		**Anammox**	**Methanotrophs**		**Denitrifiers**	
	AOB	AOA	Pla46-Amx368	pmo682	MB661	nirS	nirK
Water column	+	−	+	+	−	+	+
Inlet sludge	+	±±	±	−	−	+	+
Mid-pond sludge	+	−	+	+	+	+	+
Effluent sludge	+	−	+	+	+	+	+
Water column 60 cm	+	−	−	+	−	+	+
Influent	+	−	−	−	−	+	+

Key: − = no band present
± = weak band present
±± = very weak band
+ = band present

In all samples analysed from every location within the pond (including the pond influent wastewater), denitrifying bacteria were present.

The presence or absence results for the samples taken during the winter of 2007, are presented in Table 5.18. AOB were found to have just as strong a presence in the winter months as in the summer months. All samples were identified as having an affirmative AOB presence when a nested CTO was undertaken in the PCR stage. All species sequenced, were found to contain the ammonia monooxygenase gene, and were found to be quite similar to those found in nitrifying wastewater treatment plants, as identified by Purkhold *et al*., (2000).

AOA were present in all of the winter 2007 samples analysed (apart from one effluent sample), whereas only one faint band was detected in the DGGE gel, for an inlet sludge sample, analysed from the summer sample set.

ANAMMOX bacteria were observed in six samples from the winter sample set. These were all contained within sludge samples, except for one influent wastewater sample, which was collected in the first half of February 2007. None of the bands observed in the DGGE gels was excised or sequenced.

In most of the samples analysed for methanotrophic bacteria, very weak positive, weak positive or positive bands were, identified in a wide variety of samples when PCR products were run in a DGGE gel. These samples were not sequenced, however, and species identification was unconfirmed.

As for all of the samples analysed in the summer data set, denitrifiers were found in abundance in every sample analysed in the winter sample set.

Table 5.18: Presence and absence results for various nitrogen utilising bacterial groups found in samples taken during the winter of 2007.

Sample location	**Ammonia-oxidisers**		**Anammox**	**Methanotrophs**		**Denitrifiers**	
	AOB	AOA	Pla46-Amx368	pmo682	MB661	nirS	nirK
Water column	+	±/±±	−			+	+
Inlet sludge	+	±	+	+	+	+	+
Mid-pond sludge	+	+/±±	+	±	+	+	+
Effluent sludge	+	±±	±	+	+	+	+
Water column 60 cm	+	±±	−	±±	−	+	+
Influent	+	+/±	+	±±	±±	+	+

Key: − = no band present

± = weak band present

±± = very weak band

\+ = band present