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PHYTOPLANKTON AND NUTRIENT ANALYSIS OF A NUCLEAR FUEL-STORAGE POND AT SELLAFIELD

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Summary

- The aim of the project was to identify and enumerate phytoplankton cells and analyse phosphate and nitrate concentrations in samples from a nuclear fuel-storage pond from Sellafield. The samples were taken in five different bays, at two depths and at two time points.

- All samples contained large numbers of phytoplankton cells.

- The dominant species in bays B1 to B4 was a filamentous cyanobacterium, *Oscillatoria limnetica*. Bay B5 was dominated by small single cells of the chlorophyte, *Stichococcus bacillaris*. Other species in significant numbers that were found in nearly all samples were *Haematococcus pluvialis, Chlamydomonas* sp. and *Chlorella* sp. (all Chlorophyceae) and the cyanobacterium *Synechococcus*. The numbers of other species that were identified were neglectable.

- There was no clear difference in species composition and numbers between the samples taken at different depths.

- There was no significant difference in the species composition between the samples taken in June and July 2007, even when the July samples showed a slightly higher diversity. The difference between the two sampling time points was more of a quantitative nature; the number of *O. limnetica* was reduced in July compared to June, but the number of small phytoplankton species, especially *S. bacillaris*, increased in July.

- The chemical analysis revealed only low concentrations of phosphate and nitrate that were mainly below the detection limit of our detection method. Only the July sample contained detectable amounts of nitrogen.

1. Outline of this study

The aim of this project was the analysis of samples from five bays of a nuclear fuel-storage pond at the Sellafield site to determine phytoplankton species composition and phosphate and nitrate concentrations. Water samples were taken at the end of June and the end of July 2007 from all five bays (two sampling stations at bay B5) and at two depths each (upper and lower part of the sampling station), and analysed at the Centre for Ecology & Hydrology (CEH) at Lancaster. Phytoplankton identification and enumeration was done by light microscopy on concentrated samples fixed with Lugol's solution. Phosphate and nitrate concentration were determined through colorimetric analysis.

2. Methods

2.1. Samples

Water samples were taken by Sellafield personnel at the end of June and at the end of July 2007, and were delivered to CEH on the 29/06/7 and 31/07/07. Those samples will be referred to in the report as **June** and **July samples**, respectively. Each bay of the fuel-storage pond (called **B1 – B5**) contained one sampling station with the exception of the largest bay B5 which contained two stations (called **B5AB & B5CD**). In addition, samples at each station were taken at two depths, at the upper level of the water column (**U**) and at the lower level (**L**).
Approximately 500 ml of each sample have been mixed with 2.5 ml of Lugol's Solution (95 g KI, 14 g I_2, 10 ml acetic acid, 100 ml deionised water) to preserve phytoplankton cells for identification and enumeration. The samples taken for chemical analysis were not treated.

2.2. Phytoplankton Identification & Enumeration

Phytoplankton identification and enumeration was done according to the method used in CEH's regular monitoring programme of the English Lake District.

Samples were carefully mixed before being poured into 3 x 100 ml labelled measuring cylinders and left to settle for seven days. The upper 90 ml of each cylinder were carefully removed, the remaining 10 ml sample from each of the three measuring cylinders were combined and left to settle for another seven days. Afterwards, the upper 25 ml of each sample were again removed, with exactly 5 ml of sample remaining. This led to a concentration factor of [x60] which is taken into account for the determination of cell numbers. The samples were stored in 10 ml glass vials and are stable for at least 20 years in good storage conditions.

Light microscopic analysis of phytoplankton was done at 100x and 400x magnification using bright field and phase contrast microscopy. The microscope was also equipped with a calibrated square eyepiece graticule. For each sample, cells in 100 random square fields were identified and counted under 100x and 400x magnification each, with the exception of highly abundant species for which fewer fields were analysed (minimum five fields and 200 cells).
Enumeration of an algal population is based on the number of individuals observed in the number of random fields within a chamber of known area and volume. The counting chamber that was used was designed for the evaluation of algal populations by Lund (Lund JWG,

1959. A simple counting chamber for nanoplankton. Limnol. & Oceanogr. *4*, 57-65.). Taken into account was also the concentration factor of the samples, which finally led to a conversion into cells ml^{-1} in the original sample.

2.3. Chemical Analysis

Samples for chemical analysis were filtered through glass microfibre filters (Whatman GF/C) after arrival at CEH and stored at 4°C in the dark until further processing.

The analysis determined the amount of soluble reactive phosphorous and nitrate-nitrogen in the water samples. Samples were analysed colorimetrically using a SEAL AQ2 discrete analzyer. Phosphate was determined using the molybdenum blue chemistry. Nitrate was reduced to nitrate using hydrazine. The detection limits for this methods were 0.005 mg l^{-1} for phosphate and 0.01 mg l^{-1} for nitrate.

3. Results

3.1. Phytoplankton Identification & Enumeration

In all samples the nanoplankton (detectable at 100x magnification) was dominated by a filamentous cyanobacterium, *Oscillatoria limnetica*. A second species that was always present in the nanoplankton in significant numbers was *Haematococcus pluvialis* (Chlorophyceae). Those species, especially *O. limnetica*, reached high cell numbers in the bays B1 to B4 at both depths. While the two species were also present in significant numbers in the samples from bay B5, the dominant species in these samples was the small green alga *Stichococcus bacillaris*. In addition to *S. bacillaris*, the picoplankton (detectable at 400x magnification) always contained moderate to high numbers of other small green algae (*Chlamydomonas*, *Chlorella*) and the cyanobacterium *Synechococcus*. Other species were found as well in both size classes, but not in significant numbers.

There was no major difference in the species composition between the June and July samples but the July samples contained on average a lower number of cells. In addition, the species composition and cell numbers were not generally different between the two depth's levels of the same sample.

While most samples contained some silt or detritus, the amount in some of the samples was quite high. This made counting of phytoplankton rather difficult as present cells might not have been detected because they were covered. The samples that were mostly affected by this were B5CDL (June), and B1U, B2U, B2L & B5CDL (all July).

The calculated number of cells (chains/colonies/filaments) per millilitre for all detected species are shown in Table 1 for the June samples and in Table 2 for the July samples.
Table 3 gives the phylogenetic classification of the species that were detected.

Table 1: Phytoplankton counts for samples taken in June 2007. Numbers are given as cells (or chains/colonies/filaments) ml^{-1}.

Species	Type of count	B1U	B1L	B2U	B2L	B3U	B3L	B4U	B4L	B5ABU	B5ABL	B5CDU	B5CDL [a]
Aulacoseira islandica	Chain (Cells)	0.53											
Botryococcus sp.	Colony						0.53						
Chlamydomonas sp.	Cell	143.06	126.23	193.55	126.23	252.45	67.32	33.66	50.49	437.58	42.08	252.45	42.08
Chlorella sp.	Cell	151.47	403.92	151.47	143.06	210.38	336.6	58.91	925.65	336.6	159.89	176.72	126.23
Chroomonas sp.	Cell			8.42	16.83								
Coenochloris fottii	Colony (Cells)						0.53 (2.1)		0.53 (2.1)				
Cryptomonas sp.	Cell	8.42	17.88	25.25			0.53	8.95			8.42		
Dictyosphaerium tetrachotomum	Colony (Cells)											0.53 (5.26)	
Fragillaria crotonensis	Chain (Cells)	1.05 (1.58)											
Gymnodinium sp.	Cell					1.05							
Haematococcus pluvialis	Cell	34.72	35.77	7.36	11.57	16.83	18.94	13.15	14.20	25.77	13.68	30.51	19.99
Lyngbya martensiana	Filament	0.53	1.05									0.53	23.67
Oocystis sp.	Colony (Cells)									0.53 (2.1)			
Oscillatoria limnetica	Filament	1846.26	3021.87	3855.58	4523.6	12781.8	10183.36	10656.76	8489.64	1248.37	540.73	675.91	573.34
Planktothrix agardhii	Filament												0.53
Pyramimonas sp.	Cell							16.83					
Small unident. flagellate	Cell								563.81	361.85	210.38	126.23	227.21

Table 1 (cont.): Phytoplankton counts for samples taken in June 2007. Numbers are given as cells (or chains/colonies/filaments) ml^{-1}.

Species	Type of count	B1U	B1L	B2U	B2L	B3U	B3L	B4U	B4L	B5ABU	B5ABL	B5CDU	B5CDL [a]
Staurastrum hirsutum	Cell	0.53											
Stichococcus bacillaris	Cell	117.81	84.15	84.15	84.15	176.72	143.06	302.94	2709.63	48637.74	28021.4	46534.03	31303.18
Synechococcus sp.	Cell	42.08	58.91	25.25	25.25	67.32	42.08	92.57	33.66	193.55	143.06	151.47	227.21

[a] large amount of silt & detritus present

Table 2: Phytoplankton counts for samples taken in July 2007. Numbers are given as cells (or chains/colonies/filaments) ml^{-1}.

Species	Type of count	B1U [b]	B1L	B2U [b]	B2L [b]	B3U	B3L	B4U	B4L	B5ABU	B5ABL	B5CDU	B5CDL [b]
Aulacoseira islandica	Chain (Cells)	0.53 (3.16)	0.53 (4.21)										
Chlamydomonas sp.	Cell	50.49	58.91	67.32	117.81	168.3	134.64			67.32	168.3	42.08	8.41
Chlorella sp.	Cell	58.91	252.45	1851.3	521.73	5091.08	3744.68	967.73	546.98	319.77	210.38	126.23	33.66
Chroomonas sp.	Cell	33.66	8.42				67.32				16.83		
Coenochloris fottii	Colony (Cells)		0.53 (2.11)										
Cosmarium variolatum	Cell	1.05											
Cryptomonas sp.	Cell	8.42		16.83	8.42								1.05
Dictyosphaerium pulcellum	Colony (Cells)										1.05 (4.21)		
Euglena mutabilis	Cell	3.68	0.53					0.53		0.53			
Geminella minor	Filament				14.73 [a]		2.63				1.05		
Gloeocystis vesiculosa	Colony (Cells)		0.53 (8.42)					2.1 (6.31)	2.1 (5.26)	2.1 (7.89)			
Gymnodinium sp.	Cell	0.53											
Haematococcus pluvialis	Cell	11.05	11.57	33.14	18.41	53.65	32.09	71.01	44.71	83.11	18.94	61.54	26.83
Lyngbya martensiana	Filament												0.53
Nitzschia/Synedra sp.	Cell	0.53		25.77	16.83								
Oocystis sp.	Colony (Cells)						8.42						
Oscillatoria limnetica	Filament	2977.16	3760.9	1898.86	1299.22	2224.98	1237.85	2099.95	1599.04	56.81	65.75	44.18	62.59

Table 2 (cont.): Phytoplankton counts for samples taken in July 2007. Numbers are given as cells (or chains/colonies/filaments) ml^{-1}.

Species	Type of count	B1U [b]	B1L	B2U [b]	B2L [b]	B3U	B3L	B4U	B4L	B5ABU	B5ABL	B5CDU	B5CDL [b]
Planktothrix agardhii	Filament									0.53	0.53		0.53
Pyramimonas sp.	Cell						8.42			0.53			
Small unident. Flagellate	Cell							185.13	84.15	168.3	2019.6	126.23	100.98
Spirogyra sp.	Filament										2.1		
Staurastrum proboscideum	Cell								0.53				
Staurastrum punctulatum	Cell	3.16		1.05			0.53					0.53	
Stichococcus bacillaris	Cell	58.91		286.11	33.66	1851.3	1043.46	656.37	816.26	124202.94	33827.63	112253.88	39633.87
Synechococcus sp.	Cell	92.57	50.49	168.3	126.23	58.91	42.08	33.66	75.74	168.3	294.53	286.11	235.62
Ulothrix sp.	Filament				0.53								

[a] larger bundles of G. minor present, single filaments uncountable; [b] large amount of silt & detritus present

Table 3: Phylogenetic classification of detected phytoplankton

Species	Classification
Aulacoseira islandica	Bacillariophyceae (Diatom), chain-forming
Botryococcus sp.	Chlorophyceae, colony-forming, single cells uncountable
Chlamydomonas sp.	Chlorophyceae, single cells, identification to species level not possible with available methods
Chlorella sp.	Chlorophyceae, single cells, identification to species level not possible with available methods
Chroomonas sp.	Chlorophyceae, single cells, identification to species level not possible with available methods
Coenochloris fottii	Chlorophyceae, colony-forming
Cosmarium variolatum	Chlorophyceae, single cells
Cryptomonas sp.	Cryptophyceae, single cells, identification to species level not possible with available methods
Dictyosphaerium pulcellum	Chlorophyceae, colony-forming
Dictyosphaerium tetrachotomum	Chlorophyceae, colony-forming
Euglena mutabilis	Euglenophyceae, single cells
Fragillaria crotonensis	Bacillariophyceae (Diatom), chain-forming
Geminella minor	Chlorophyceae, filamentous
Gloeocystis vesiculosa	Chlorophyceae, colony-forming
Gymnodinium sp.	Dinophyceae, single cells, identification to species level not possible with available methods
Haematococcus pluvialis	Chlorophyceae, single cells
Lyngbya martensiana	Cyanobacteria, filamentous
Nitzschia/Synedra sp.	Bacillariophyceae (Diatom), single cells, both species look very similar and further identification was not possible with available methods
Oocystis sp.	Chlorophyceae, colony-forming, identification to species level not possible with available methods
Oscillatoria limnetica	Cyanobacteria, filamentous
Planktothrix agardhii	Cyanobacteria, filamentous
Pyramimonas sp.	Prasinophyceae, single cells, identification to species level not possible with available methods
Small unident. Flagellate	Round cells (3-5 µm) with 1 or 2 flagella; further identification with available methods not possible.
Spirogyra sp.	Chlorophyceae, filamentous, identification to species level not possible with available methods
Staurastrum hirsutum	Chlorophyceae, single cells
Staurastrum proboscideum	Chlorophyceae, single cells
Staurastrum punctulatum	Chlorophyceae, single cells
Stichococcus bacillaris	Chlorophyceae, single cells or short filaments
Synechococcus sp.	Cyanobacteria, single cells, identification to species level not possible with available methods
Ulothrix sp.	Chlorophyceae, filamentous, identification to species level not possible with available methods

3.2. Chemical Analysis

Phosphate concentrations were very low in all samples and either below or slightly above the detection limit of our method (0.005 mg l^{-1} for phosphate, 0.01 mg l^{-1} for nitrate). The nitrogen concentrations of the June samples were below the detection limit as well, with the exception of sample B1L. In comparison, the July samples showed significantly higher concentrations (Table 4).

Table 4: Analysis of phosphate and nitrogen concentration

Sample	June 2007		July 2007	
	PO_4-P [mg/l]	TON [mg/l]	PO_4-P [mg/l]	TON [mg/l]
B1U	**0.009**	**<0.010**	**0.006**	**0.114**
B1L	**0.010**	**0.021**	**<0.005**	**0.093**
B2U	**0.005**	**<0.010**	**<0.005**	**0.080**
B2L	**<0.005**	**<0.010**	**<0.005**	**0.076**
B3U	**<0.005**	**<0.010**	**<0.005**	**0.090**
B3L	**<0.005**	**<0.010**	**<0.005**	**0.076**
B4U	**<0.005**	**<0.010**	**<0.005**	**0.074**
B4L	**<0.005**	**<0.010**	**<0.005**	**0.081**
B5ABU	**<0.005**	**<0.010**	**<0.005**	**0.094**
B5ABL	**0.006**	**<0.010**	**<0.005**	**0.126**
B5CDU	**<0.005**	**<0.010**	**<0.005**	**0.086**
B5CDL	**<0.005**	**<0.010**	**<0.005**	**0.120**