

ECOLOGICAL ENGINEERING
AND
THE CHARA PROCESS
APPLIED TO
THE RABBIT LAKE DRAINAGE BASIN

Final Report

for

CAMECO

By

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and

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1.0 INTRODUCTION

In March 1988, a "Proposal to Apply Ecological Engineering in the Rabbit Lake Watershed" was submitted to the Regulatory Agencies. This was followed by an investigation of the Rabbit Lake drainage basin in the same year.

It was found, as predicted earlier from the water quality data, that ^{226}Ra was removed below the Sedimentation Dam by an extensive population of *Nitella flexilis*, a species of the Characeae. After the first year of assessing the ecological, geochemical and hydrological conditions of the drainage basin, it appeared that the Chara Process with Ecological Engineering was a suitable decommissioning approach for the Rabbit Lake drainage basin.

Design criteria were developed for the implementation of this decommissioning approach during 1988. A report was issued in December 1988, entitled "Ecological Engineering and the Chara Process Applied to the Rabbit Lake Drainage Basin. Phase 1: Design Parameters for the Implementation in the Link Lake System".

As Ecological Engineering was a novel technology, the decommissioning was approached stepwise. First, the reduction of ^{226}Ra and uranium was to be achieved with a biological polishing system consisting of an underwater meadow of *Nitella*. Such a task was never before attempted. Therefore, on an experimental basis in June 1989, twelve tonnes of *Nitella flexilis* were collected from a natural population in Lower Link Lake and transplanted

to four areas in Upper Link Lake. After the transplant operation, a construction report, describing the procedure, was submitted in November 1989. In the last two years, the growth and distribution of the population was monitored in Upper Link lake. The transplanted *Nitella* population has now overwintered twice. Hence, conclusions can be drawn with respect to the feasibility of establishing the required biological polishing system.

Second, in order to proceed with the implementation of the decommissioning approach, the entire drainage basin has to be considered with respect to water quality and ecological stability. Ecological Engineering decommissioning measures will achieve a low maintenance or, in the long term, completely self-sustaining ecosystem. Therefore, within the work program, factors which affect the algal populations in the long term were addressed. These factors were addressed through the study of *Nitella* populations present in Lower Link Lake between the years 1988 to 1991. Information on the validity of the design criteria, determined at the onset of the program, were derived.

A third aspect which has to be addressed for the decommissioning of the drainage basin is the fate of the contaminants. The algal population dynamics provide a sink for the contaminants ^{226}Ra and uranium, namely the sediment. The long term stability of the contaminants in the sediment and the magnitude of the natural flux from the sediment to the water has to be determined. This aspect was addressed through the development and the testing of sediment interstitial solution samplers, instruments otherwise called pore water peepers, which are used to collect solutions with dissolved ion concentrations representative

of the sediment environment. It is assumed that the mobile fraction of ^{226}Ra and uranium would reflect sediment fluxes. Data on the sediment interstitial solutions' mobile ^{226}Ra and uranium content have now been collected covering an extensive period of time.

This report summarizes the conclusions on the experimental transplant operation and the application of Ecological Engineering to the entire drainage basin, with reference to the three important aspects of decommissioning.

1.1 1991 Objectives

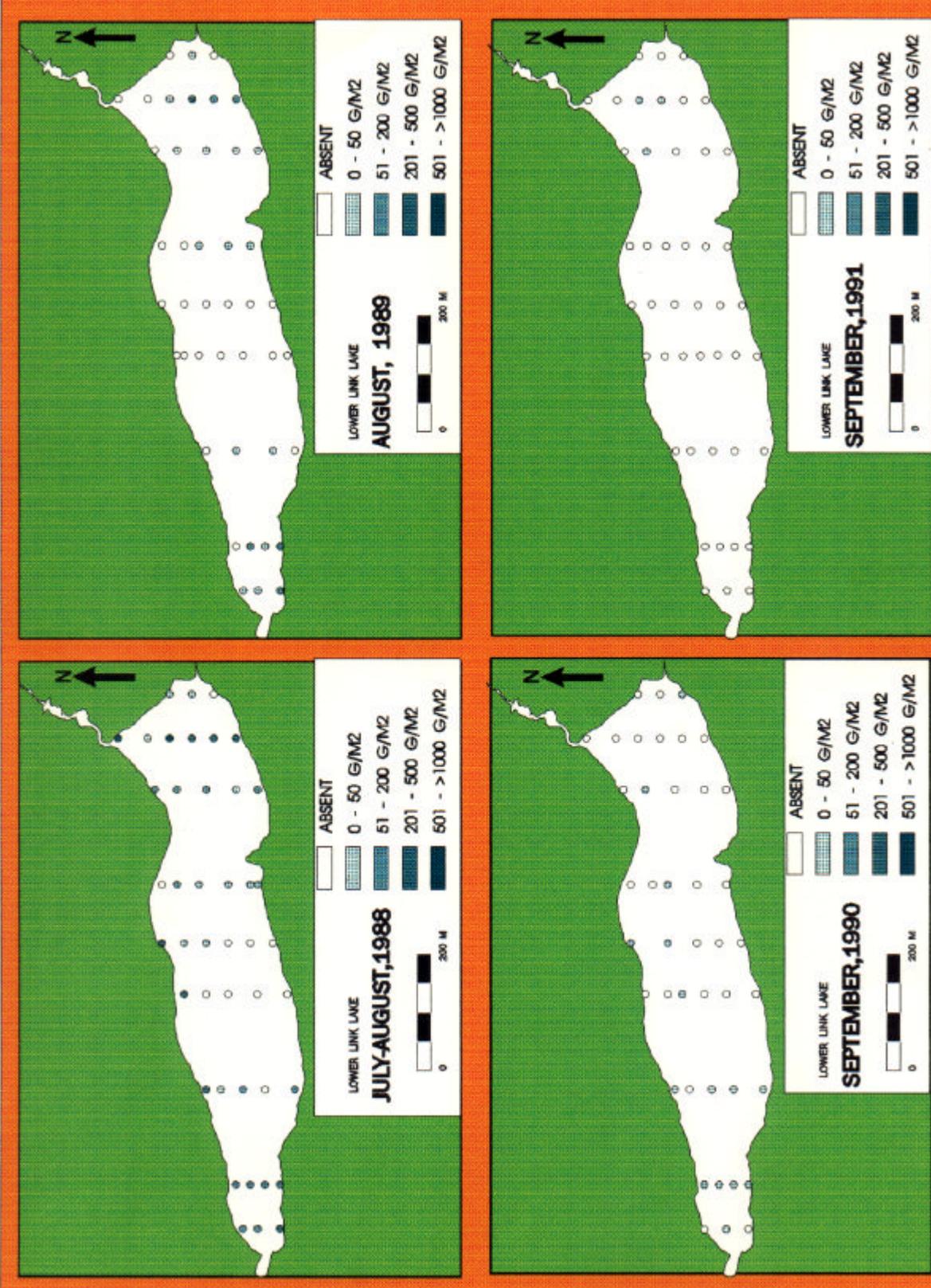
- 1) Confirm the growth controlling factor, water level, through continued standing biomass quantification in Lower Link Lake.
- 2) Quantify nutrient status of the drainage basin through on-site nutrient determination in conjunction with SRC determinations.
- 3) Define conceptual approach to control of phytoplankton blooms.
- 4) Determine turn-over rates of *Nitella* populations with new technique (Calcofluor staining).
- 5) Continue evaluation of ^{226}Ra flux from sediment (ULL Delta and BAD).
- 6) Continue monitoring the distribution of transplanted *Nitella* in Upper Link Lake.

2.0 CONFIRMATION OF GROWTH CONTROLLING FACTORS IN LOWER LINK LAKE

Surveys of the distribution of Characean populations in Lower Link lake, performed in 1988, indicated a strong relationship between water depth and *Nitella flexilis*'s distribution. Specifically, *Nitella* was not found at depths less than 0.6 m, or greater than 1.9 m. High density populations were confined to depths of 0.9 to 1.6 m. Strong relationships with other factors, such as substrate type, flow regime, or proximity to inflow or outflow regions were not observed. Water depth, due to its related light attenuation characteristics, was therefore the most important design criteria in the implementation of the Chara Process in Upper Link Lake.

In the years following the 1988 investigation, the *Nitella* populations, in terms of density and distribution, greatly declined. The first evidence of this decline was observed in June of 1989; *Nitella* biomass located near the inflow of Upper Link Lake was at densities similar to the fall of 1988, but was, for the most part, dead.

The August, 1989 survey revealed that the *Nitella* population was, by then, confined to a much smaller area, typically at depths greater than 1 m. Results of the September, 1990 survey indicated an even smaller and less dense *Nitella* population, while the September 1991 survey found negligible amount of *Nitella* at only three locations (Schematic 1).



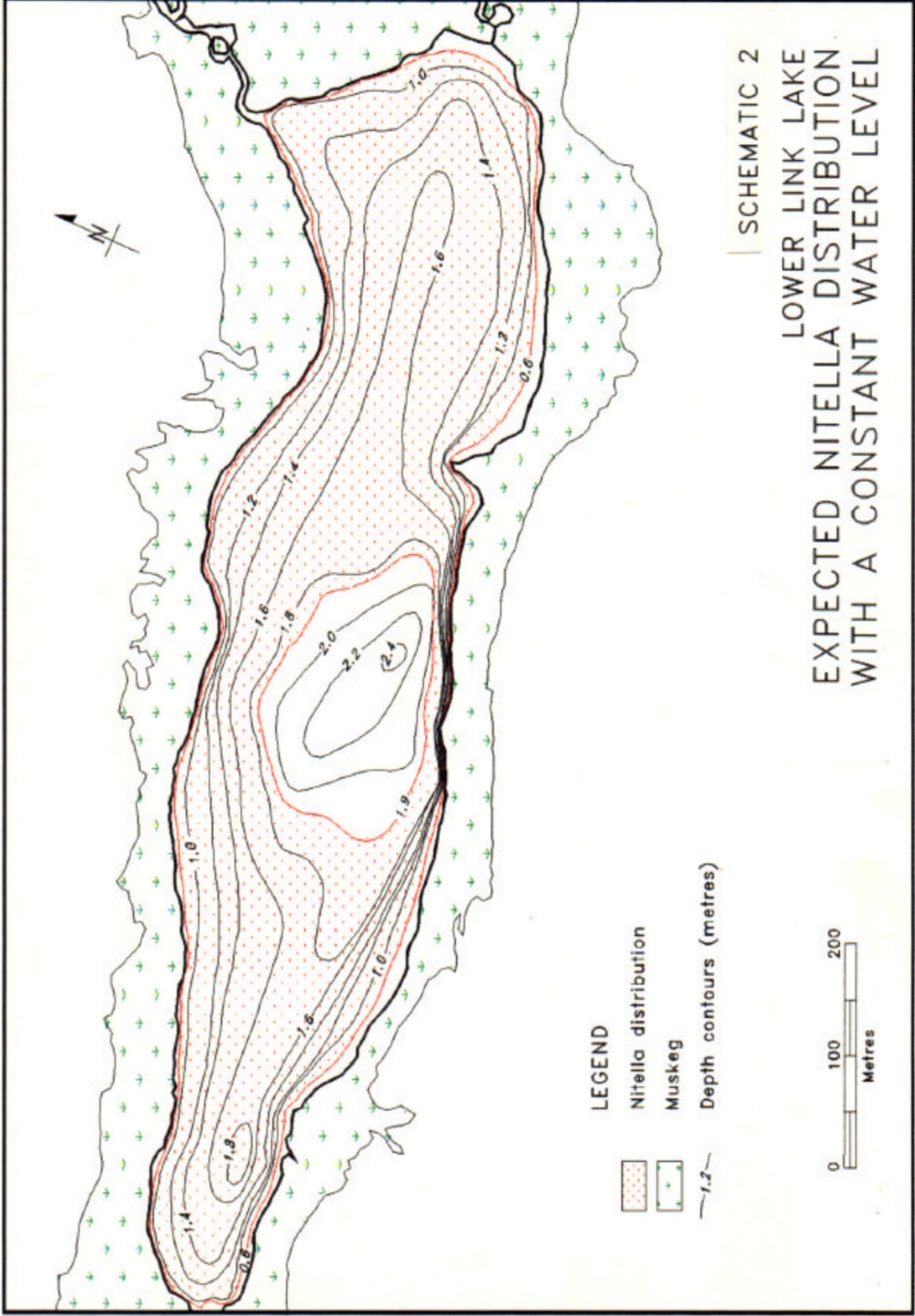
Schematic 1: Distribution and density of Characean populations in Lower Link Lake, 1988 - 1991

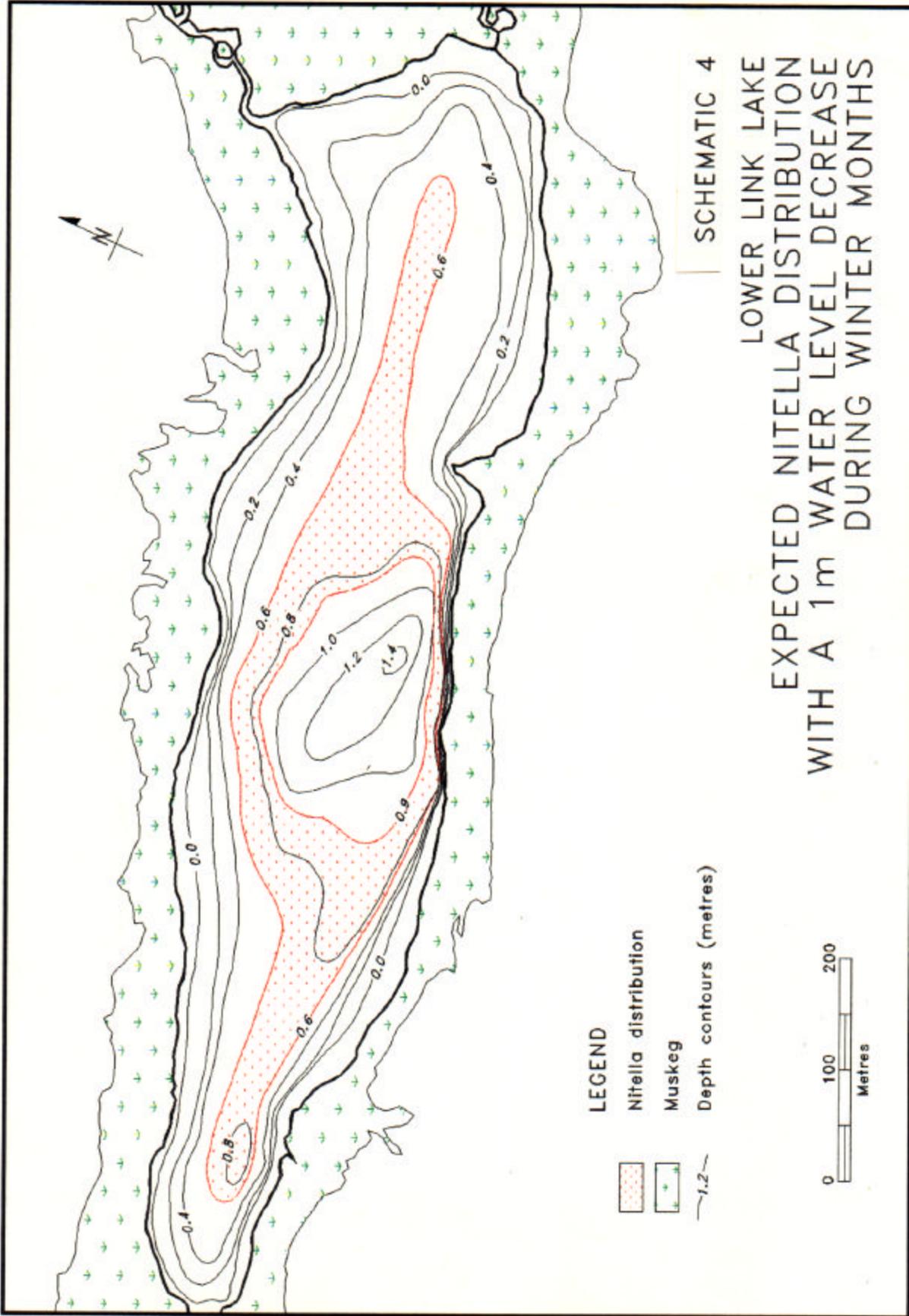
Overall, it appears that changes in Lower Link Lake's water levels during 1989, 1990 and 1991 are responsible for the decline of Lower Link Lake *Nitella* population, such that, in 1991, negligible amounts of *Nitella* remain in the lake (Schematic 1).

During the April, 1990 site visit, the water level in Lower Link lake was 0.55 m lower than levels measured in the growing seasons of 1988 and 1989, due to failure of the beaver dam at Lower Link Lake Outflow. Failure of the beaver dam again occurred in late August 1991, and Lower Link Lake water levels were as low as 1 m below normal levels during freeze-up this fall (R. Scott, personal communication).

With decreases in water level, the formation of bottom bound ice over much larger areas of Lower Link Lake is an obvious cause for the decline of the *Nitella* population. Schematic 2 outlines the expected *Nitella* population distribution if water levels remain constant year round, resembling that of the distribution of *Nitella* in 1988. Depths at Lower Link Lake's shoreline increase to 1 m with 2 to 3 m distance from the shore. Ice thicknesses, measured in April, 1990 were between 0.50 and 0.75 m. Therefore, bottom bound ice would form only around the perimeter of Lower Link Lake. The previous growing season's *Nitella* population distributed at depths greater than 0.75 m would remain unfrozen and survive until spring.

Schematic 3 presents the expected *Nitella* distribution if the water level decreased by 0.5 m in the winter, and raised to high water levels during the summer. The *Nitella* population distribution would be only marginally decreased; for instance, the portion of the population





SCHEMATIC 4

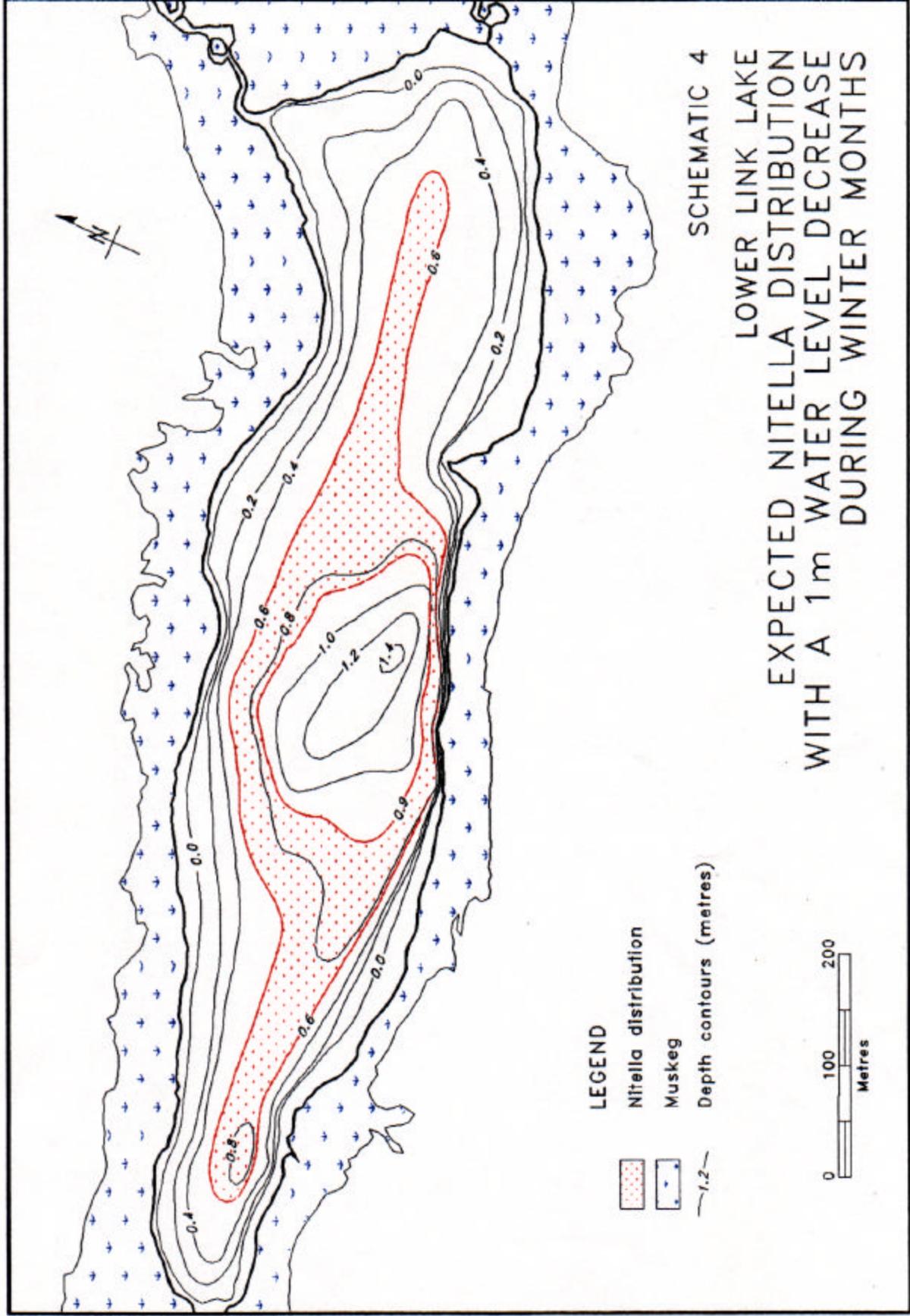
LOWER LINK LAKE
 EXPECTED NITELLA DISTRIBUTION
 WITH A 1m WATER LEVEL DECREASE
 DURING WINTER MONTHS

near the Lower Link Lake inflow would remain below the ice, and likely survive. A further reduction in the distribution could be due to the plant height, as the tips may be frozen in the overlying ice.

Schematic 4 outlines the expected *Nitella* population distribution if water levels decrease by 1 m, as may have been the case during the 1990-91 winter. The distribution would be more than halved, while the remaining population is that low density fraction grown at summer depths of 1.6 m or deeper. Again, with increasing plant height, even larger areas of the remaining population would be damaged upon spring break-up.

These considerations indicate that ice formation with water level decreases during winter can alone account for the decline of the Lower Link Lake *Nitella* population. Formation of exceptionally thick ice (up to 1.2 m) in some years would only exacerbate the situation. Other changes due to the water level fluctuations, such as the draining of the surrounding muskeg's water with a different chemistry may also play a role. It may, for instance, significantly increase light attenuation as humic acid concentrations increase.

The lower depth limit for the expected *Nitella* distribution of 1.9 m was based light attenuation measurements in Upper and Lower Link Lakes. Measurements in 1988 in Upper Link lake were conducted after the single 1988 phytoplankton bloom had abated. In 1991 measurements were repeated but during an algal bloom. Therefore greater light attenuation with depth was measured in 1991, compared to 1988 (Figure 1). This increase



SCHEMATIC 4
 LOWER LINK LAKE
 EXPECTED NITELLA DISTRIBUTION
 WITH A 1m WATER LEVEL DECREASE
 DURING WINTER MONTHS

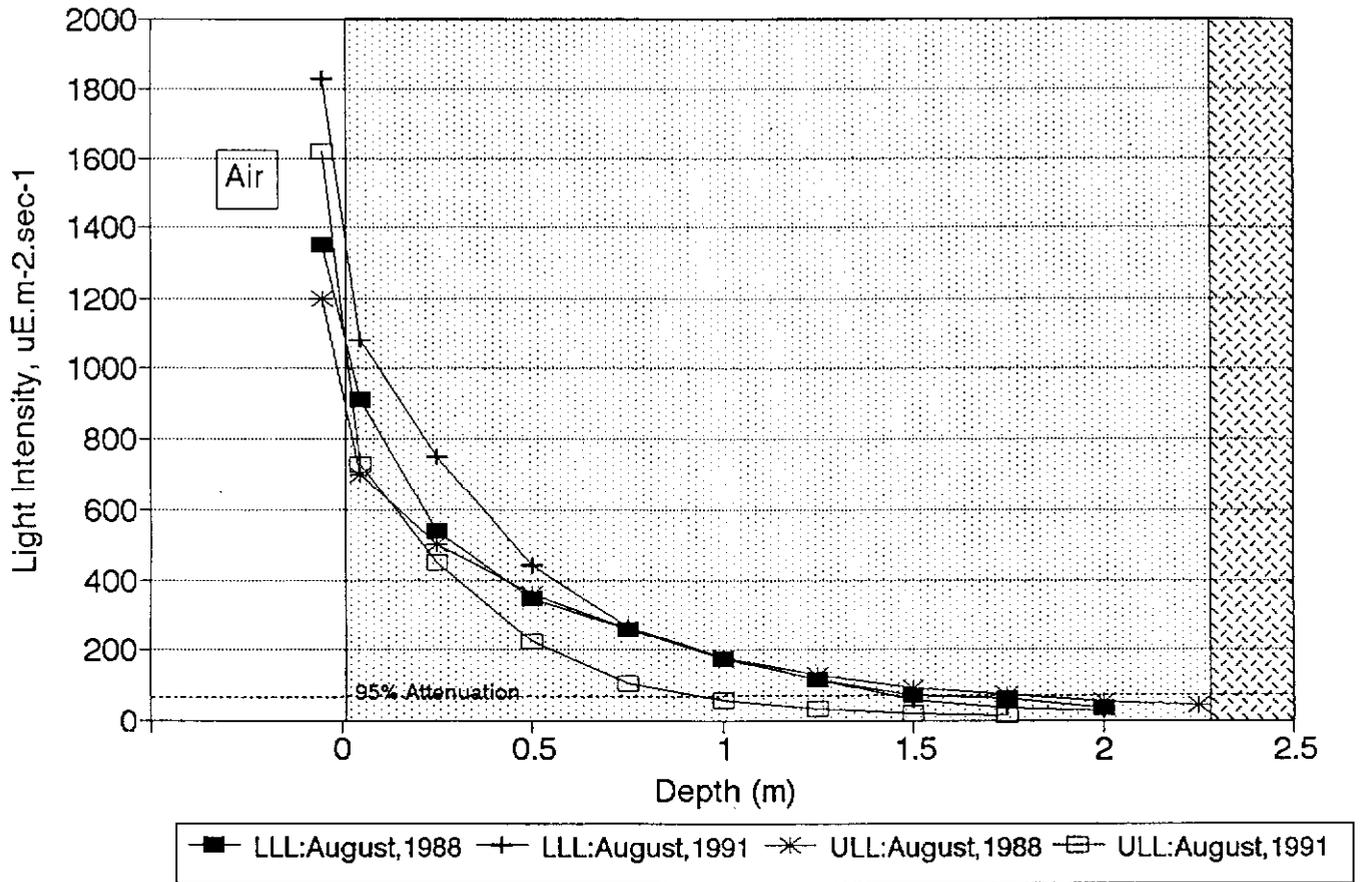


Figure 1: Light attenuation with depth: Upper and Lower Link Lakes.

in light attenuation, and the observations during 1990 and 1991, indicate that the algal bloom is continuous. These limitations on *Nitella* growth lead to the following conclusions.

The design criterion, water level, must be adjusted based upon the light attenuation data. Overall, water depth must be maintained year round such that the maximum sediment surface area is beneath water depths between 0.6 and 1.9 m, thereby providing the *Nitella* populations with appropriate light levels over the largest area. As this surface area will be reduced with further light attenuation by phytoplankton blooms, measures to eliminate algal blooms should be taken.

3.0 QUANTIFICATION OF THE RABBIT LAKE DRAINAGE BASIN NUTRIENT STATUS

Phosphate, nitrate and ammonia concentrations were measured in the field using Hach test kits in samples collected from throughout the Rabbit Lake Drainage basin in June, August and September, 1991. Samples from the same locations were also submitted to SRC. The primary goal behind collection of this database was the determination of the drainage basin's nutrient status. The nutrients are considered a key factor in controlling phytoplankton blooms in Upper Link Lake. At the same time, a validation of nutrient values determined with the Hach kit would provide a substantial cost savings (50 nitrate tests, \$81; 500 ammonia tests, \$18-; 50 phosphate tests, \$25-) over SRC analyses (\$33/sample).

In addition to the cost savings, accurate determination of potentially volatile or oxidizable compounds such as ammonia is difficult, if performed days after sample collection and shipment. Figures 2, 3 and 4 present pairs of Hach and SRC results for phosphate, nitrate and ammonia, respectively.

The two procedures yielded very similar results for phosphate at concentrations greater than 2 mg/l PO₄ (Figure 2). The detection limit for phosphate by SRC is 0.05 mg/l, while for the Hach tests, 0.1 mg/l. Biologically significant phosphate concentrations are in the order of 0.1 mg/l. Therefore the concentrations in the Link Lake system are such that, with the Hach kit, determination, for the most part, of appropriate data can be obtained for the Rabbit Lake Drainage basin. However, only with an improved Hach kit detection limit could data be collected of comparable precision as SRC.

Comparison of nitrate values determined by the two procedures indicates a much poorer correlation (Figure 3). As the detection limit of the Hach nitrate test is 0.88 mg/l, Hach concentration points at the 0.88 to the left of the 1:1 diagonal line can be ignored. However, SRC detected concentrations of nitrate of up to 4 mg/l when the Hach test results were less than detection limit. Biologically significant concentrations of nitrate are around 0.8 mg/L. It would appear that, again, the precision of the Hach kit, in terms of detection limit, is marginal. However, given the high nitrate concentrations prevalent in the Rabbit Lake drainage basin, the Hach kit should serve well, with periodic verification against SRC determinations.

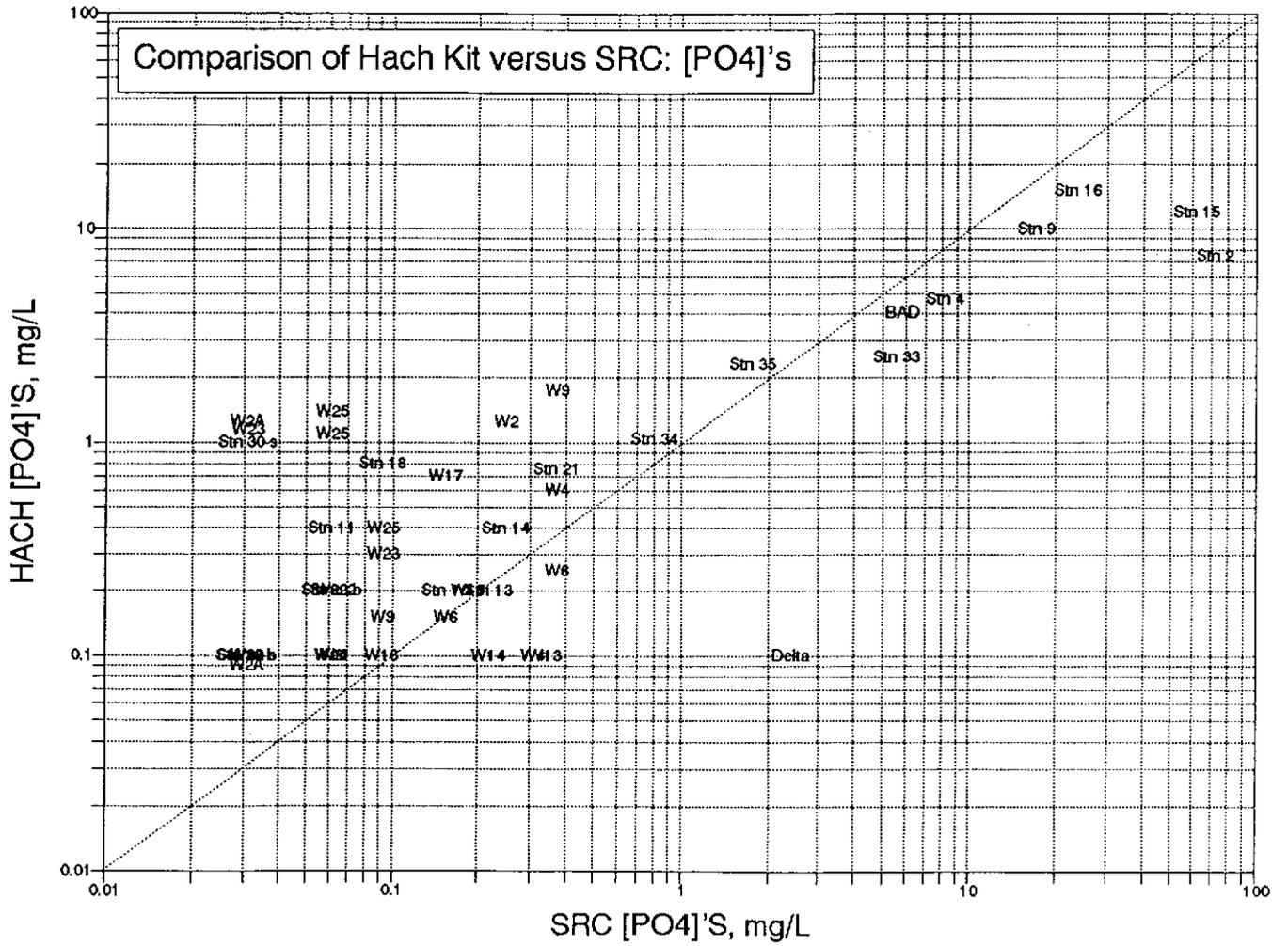


Figure 2: Comparison of Hach Kit versus SRC: Phosphate concentrations.

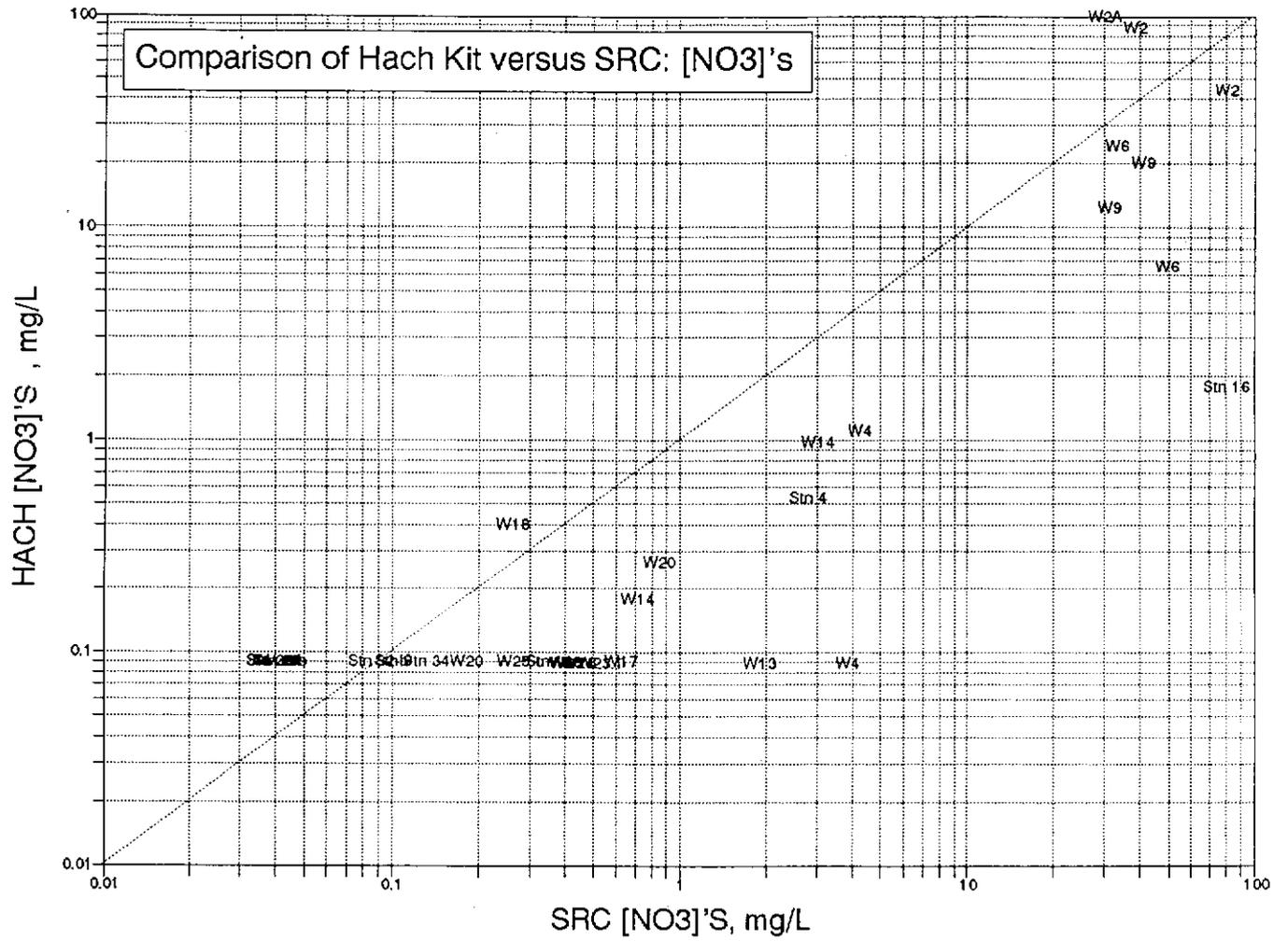


Figure 3: Comparison of Hach Kit versus SRC: Nitrate concentrations.

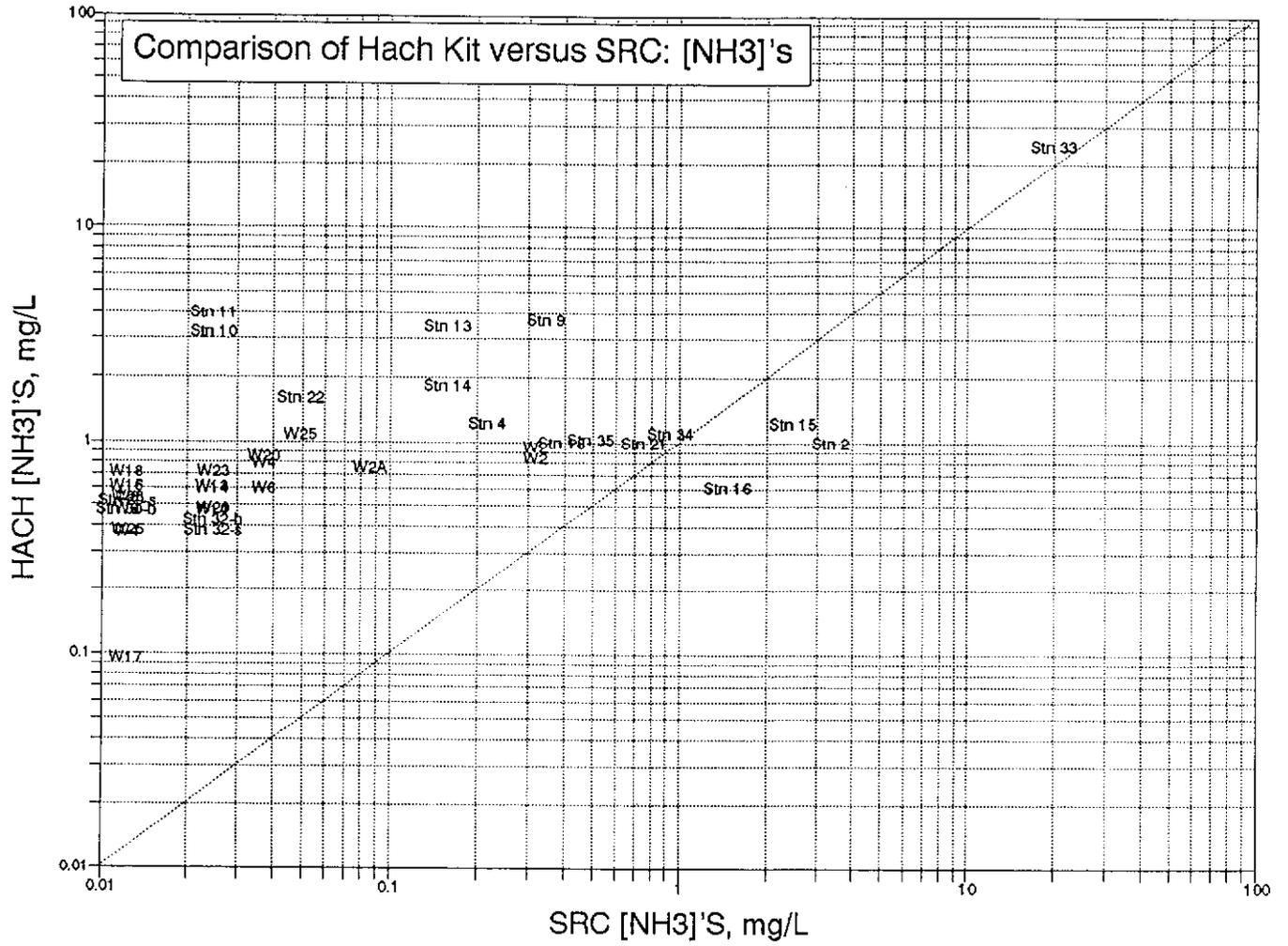


Figure 4: Comparison of Hach Kit versus SRC: Ammonia concentrations.

At higher concentrations, a closer correlation between Hach and SRC is noted. Hach results are typically lower than SRC results. This may indicate that following preservation with sulphuric acid, algae and bacteria present in the samples may have released some nitrate.

Contrary to nitrate results, the Hach kit tests yielded higher results for ammonia than did the SRC procedure. Although SRC determined values of less than 0.1 mg/l ammonia for a large number of samples, the Hach tests gave up to 3 mg/l. As less than 1% of the ammonia can be expected to be volatilized in these circumneutral waters, even at temperatures of up to 30° C (MOE, 1978), loss of ammonia may occur between collection and acidification.

Biologically significant concentrations of ammonia are in the range of 0.01 mg/l, which is below the detection limit of SRC. It is evident, however, that ammonia is plentiful in the Rabbit Lake drainage basin. For the evaluation of the nutrient status in this drainage basin, we have chosen to use Hach kit results, as a larger data base is available for the analysis.

3.1 The Nutrient Status of the Rabbit Lake Drainage Basin

Factors such as light, heat, carbon dioxide, oxygen and nutrients are essential for the growth of plants. Any one of these factors, if in short supply, can limit plant growth. In aquatic

systems, nutrients, particularly phosphorus, are consumed until their concentrations place a limitation on the growth of aquatic plants.

The three nutrients are plotted for three sampling times in Figures 5, 6 and 7. The data concerning the nutrient status of the Rabbit Lake system indicate that highly productive aquatic ecosystems can be expected. Drainage from the west waste rock pile contains 30 to 100 mg/l nitrate, concentrations characteristic of hypereutrophic ecosystems. Phosphate concentrations in the drainage basin, ranging between 0.1 and 1 mg/l, are characteristic of eutrophic, bordering on hypereutrophic, ecosystems.

Phosphate: Both Hach and SRC results indicate that low concentrations (0.03 to 0.3 mg/l) of phosphate are entering the west waste rock pond at W2A. However, samples taken in June 1991 indicated that up to 1 mg/l is found in the south drainage ditch (W4B). This may explain the higher concentrations of phosphate downstream at old airport road (W6). The phosphate is utilized in Upper Link lake and the BAD, indicated by concentration decreases. Over the length of Lower Link Lake the concentrations increase again. This increase in phosphate concentrations over Lower Link Lake noted in June was evident in August (Figure 6), but not as pronounced in September (Figure 7). Given that PWP phosphate concentrates are always quite high, flux of phosphate from sediments may account for these observed concentration increases.

Rabbit Lake Drainage Basin
June 9, 1991: [PO₄], [NO₃] and [NH₃]

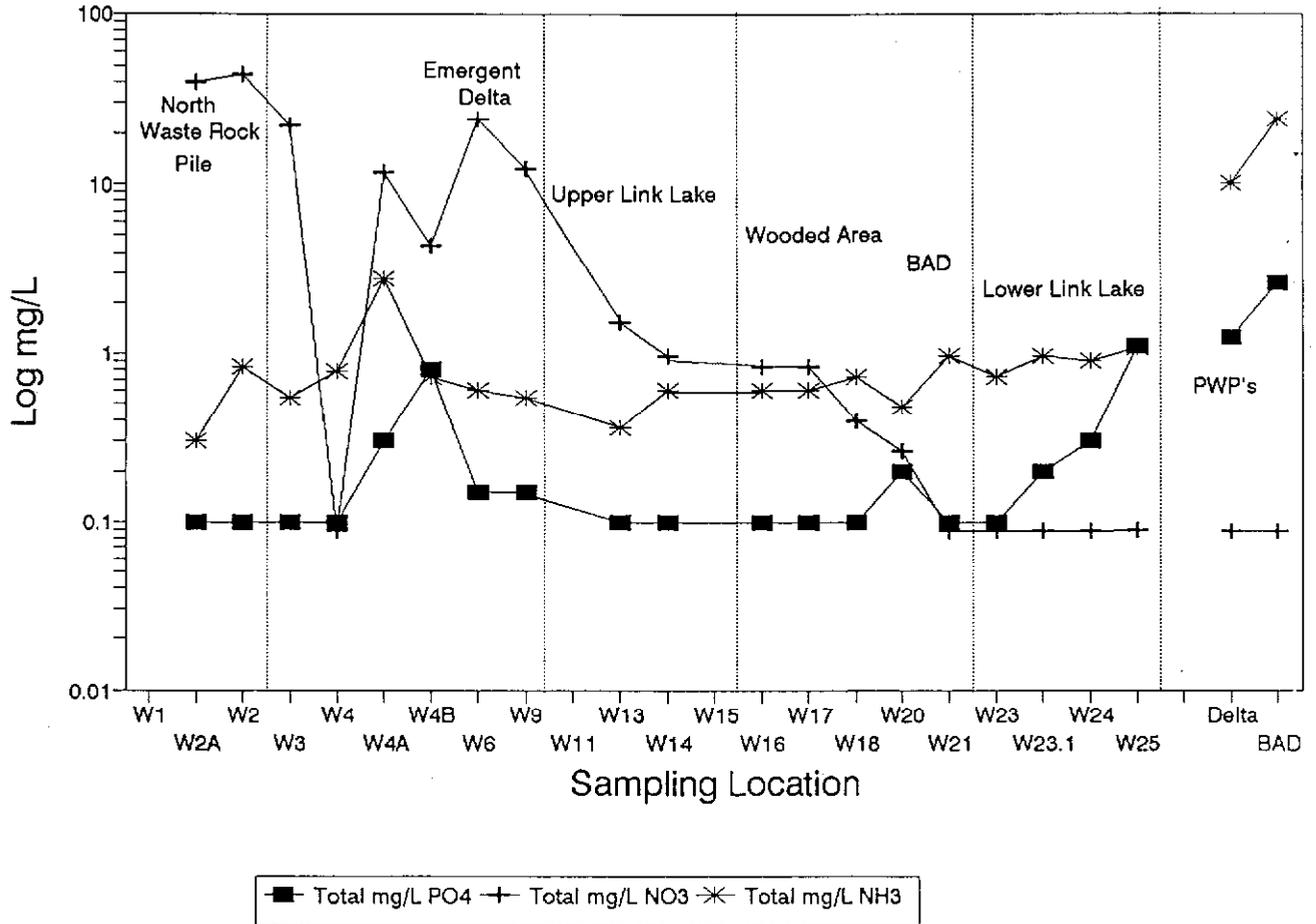


Figure 5: Rabbit Lake Drainage Basin, June 9, 1991
Phosphate, Nitrate and Ammonia

Rabbit Lake Drainage Basin
August 2, 1991: [PO4], [NO3] and [NH3]

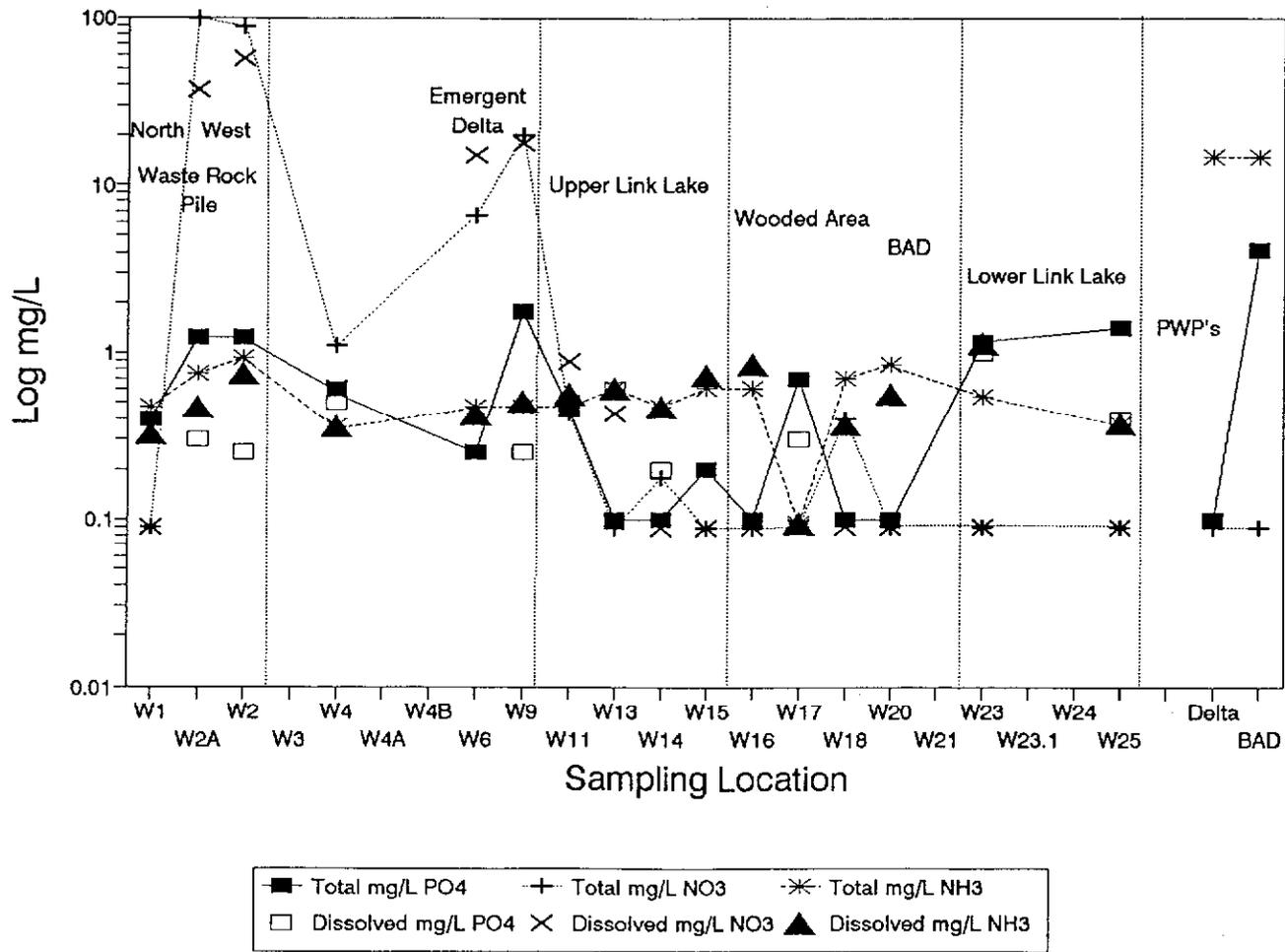


Figure 6: Rabbit Lake Drainage Basin, August 2, 1991
Phosphate, Nitrate and Ammonia

Rabbit Lake Drainage Basin September 23, 1991: [PO4], [NO3] and [NH3]

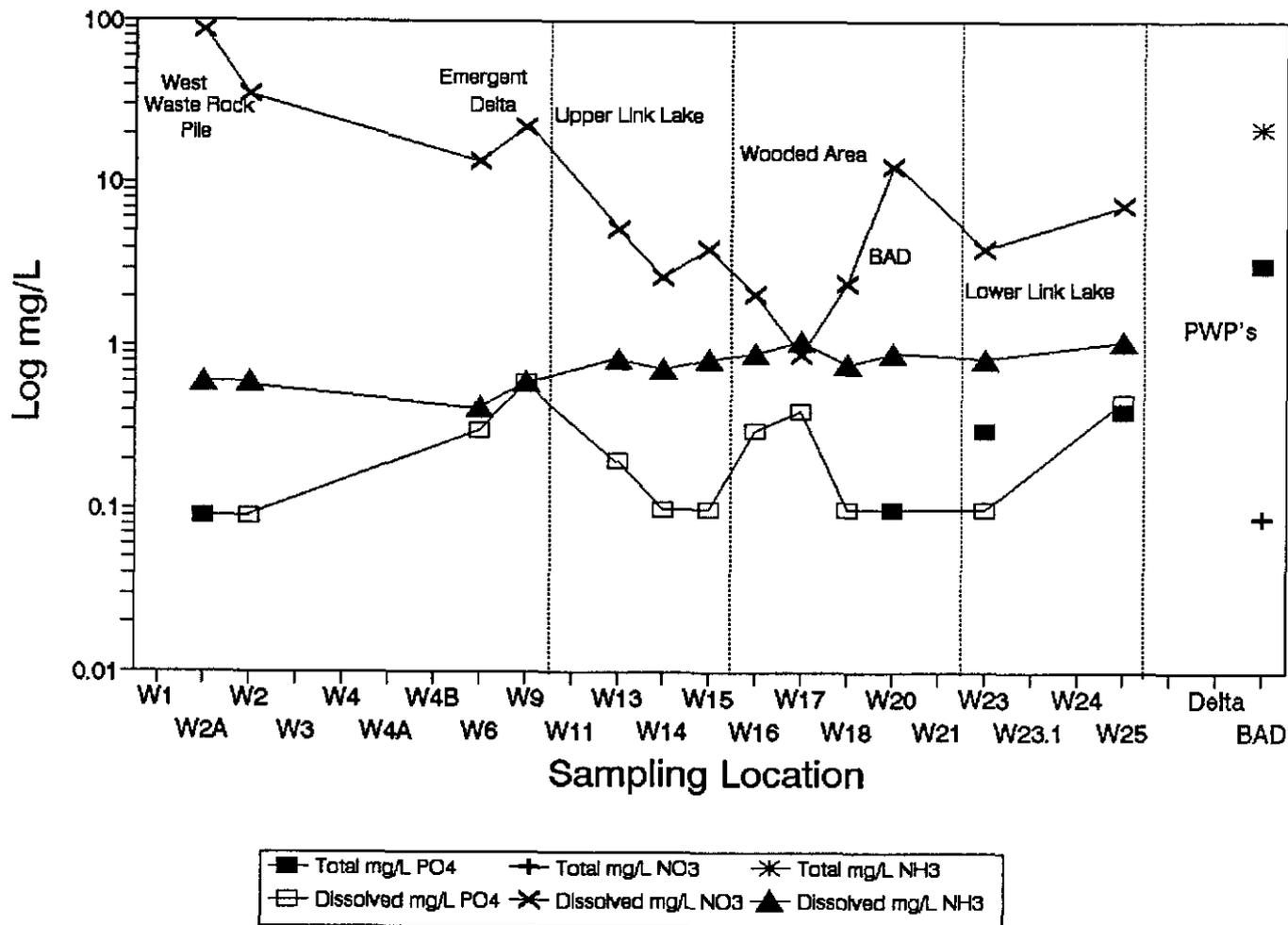


Figure 7: Rabbit Lake Drainage Basin, September 23, 1991
Phosphate, Nitrate and Ammonia

These three sets of data indicate that the west waste rock pond is a minor source of phosphate. The basin draining into the south ditch provides a significant supply of phosphate to Upper Link Lake. Concentrations of phosphate in Upper Link Lake remained relatively constant throughout the growing season. Lower Link Lake concentrations were higher than upstream. Unfortunately, this latter trend cannot be verified using SRC results, where pairs of Hach/SRC data are available.

Nitrate: Early in the growing season, nitrate concentrations decreased over the drainage basin from 40-50 mg/l in the west waste rock pond (W2) to less than 0.1 mg/l over the length of Lower Link Lake. It is interesting to note that nitrate concentrations decreased from 1 to 0.1 mg/l between Sedimentation Dam and the BBD, suggesting this area as a nitrate sink. In August 1991, despite continued input of 9 mg/l nitrate at the Delta, nitrate concentrations in Upper Link Lake remained below 0.5 mg/l, while in the BAD and Lower Link Lake, concentrations were only 0.1 mg/l.

In September 1991, the concentrations of nitrate throughout the system were significantly higher than in June or August; Upper Link Lake was discharging 2 mg/l nitrate, while the BAD and Lower Link Lake contained around 5 mg/l.

Overall, the Rabbit Lake Drainage Basin receives a very large loading of nitrate from the west waste rock pond, which is biologically captured or diluted by fresh waters to

concentrations at the most, less than 5 mg/l. Concentrations indicating eutrophic conditions are 0.8 mg/l and higher.

Ammonia: Concentration of ammonia in the Rabbit lake drainage basin are quite consistent over the drainage path, and relatively consistent over the growing season. In June 1991, concentrations ranged from 0.2 in the west waste rock pond feed (W2A), peaking at 1.2 mg/l in a waters draining to the south drainage ditch. In August, 1991, concentrations were usually 0.4 to 1 mg/l over the drainage basin, with the exception of the subsurface streams passing beneath the wooded area, where the concentration of ammonia was only 0.1 mg/l.

The virtually continuous cyanobacteria bloom observed over the 1991 growing season clearly suggests that the system is in fact very eutrophic. Given the very high loading of nitrate, moderate loadings of ammonia, but low, relative to nitrogen, concentrations of phosphate, phosphate concentrations may in fact be limiting growth. This suggestion, based on the nutrient concentrations in water passing through the drainage basin, should be reflected in biomass nutrient ratios.

3.2 Nitrogen:Phosphorus Ratios in Drainage Basin Water and Biota

In June, August and September of 1991, extensive nutrient surveys were conducted in the Link Lake drainage system. These nutrients were measured on site using Hach field kits, with some duplicates sent to SRC, an analytical laboratory.

For the analysis of nutrient ratios, Hach and SRC concentrations were averaged, and the ratio of N:P was determined. The ratio was then plotted on log scale for each of the three periods during the summer of 1991. These data are presented in Figure 8.

Large concentrations of N (in the form of nitrate) are found in the west waste rock pond. The water contains very little P, so the ratio of N:P is high (around 2000). As the water travels from the west waste rock pond to Upper Link Lake, and thence to Lower Link Lake, phosphorus levels slightly increase, while nitrate levels, on the other hand are being diluted or utilized by the biota, thereby overall decreasing the N:P ratio. With the exception of August 1991 samples, the ratio reaches 7.5:1 when the water reaches the BAD. In August, this optimal nutrient ratio was reached earlier in the drainage basin. The ratios calculated from the waters are reflected in the associated biota. Table 1 data, collected in 1989, summarizes the NPK concentrations in biota.

All plants require nitrogen and phosphorus to grow. Nitrogen is a basic component of proteins and nucleic acids, and phosphorus is found in energy transfer molecules and nucleic

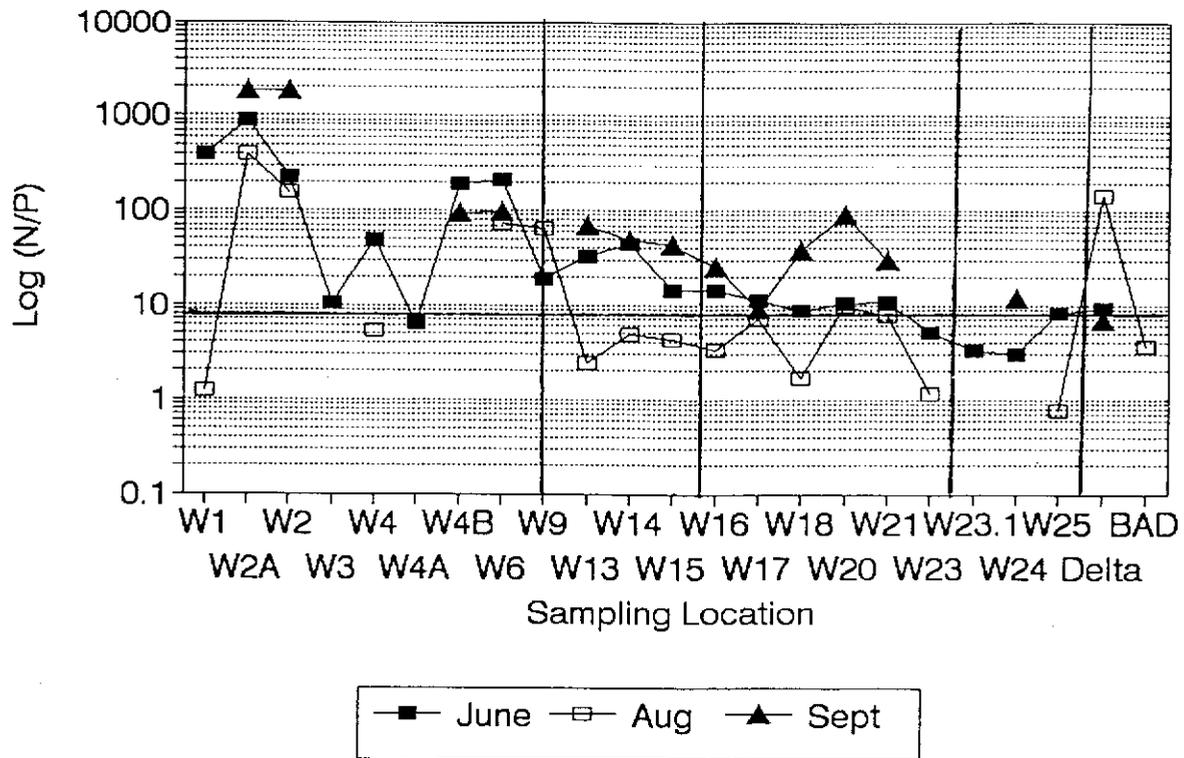


Figure 8: Rabbit Lake Drainage Basin: N:P ratios, summer, 1991

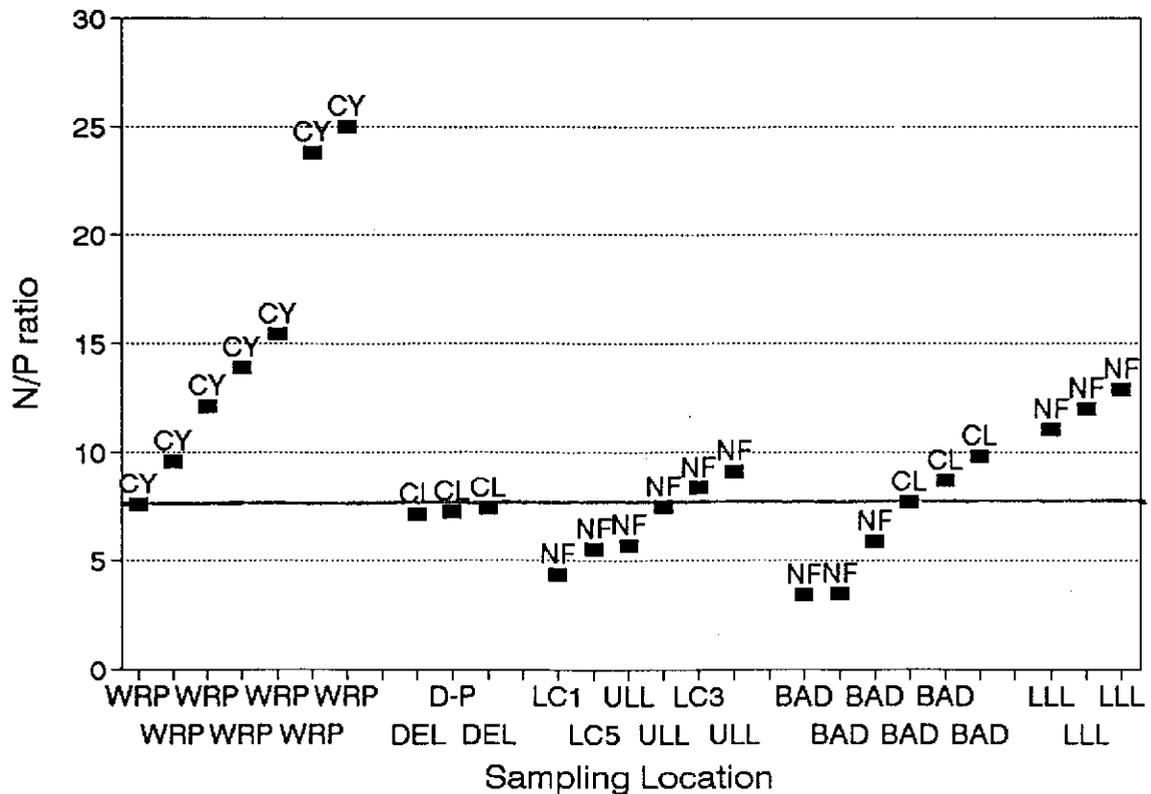


Figure 9: N:P ratios in algae growing in the Rabbit Lake Drainage Basin.

Table 1: Nutrient content in algae growing the Rabbit Lake Drainage Basin.

SAMPLE CODE	SAMPLE LOCATION	SAMPLE NAME	ALGAE	SAMPLE DATE	LOI 550 C	NUTRIENTS		TOTAL			
						NO2+NO UG/G	TKN %	N %	P %	K %	N/P
83	BAD	RECV ST#34	Nitella	10/7/89		1	2.9	2.9	0.84	2.6	3.45
84	BAD	RECV ST#35	Nitella	10/7/89		1	3	3	0.86	2.2	3.49
16	LC1	Q#1	Nitella	12/7/89		1	2.7	2.7	0.62	1.3	4.35
22	LC5	Q#1	Nitella	12/7/89		1	3.1	3.1	0.56	1.2	5.54
86	ULL	'88 UPTAKE	Nitella	21/6/89		8	3.2	3.2	0.56	2.9	5.71
73	BAD	RECV ST#23	Nitella	10/7/89		1	2.7	2.7	0.46	1.4	5.87
34	DELT-OUT	FIL L	Chlorophytes	11/7/89	66.6	1	2.5	2.5	0.35	0.6	7.14
14	D-POND	PERI-ANAL	Chlorophytes	22/6/89		1	1.3	1.3	0.18	0.82	7.22
35	DELT-OUT	FIL D	Chlorophytes	11/7/89	60.5	1	2.6	2.6	0.35	0.51	7.43
85	ULL	1988 T1	Nitella	21/6/89		1	3.3	3.3	0.44	1.8	7.50
49	WRP	M-LC3,26HR	Cyanophytes	11/7/89		10	0.6	0.6	0.079	0.68	7.59
26	BAD	FIL D	Chlorophytes	10/7/89	84.6	1	4.7	4.7	0.61	1.1	7.70
19	LC3	Q#1	Nitella	12/7/89		1	2.5	2.5	0.3	0.79	8.33
25	BAD	FIL L	Chlorophytes	10/7/89	86.7	1	4.8	4.8	0.55	0.9	8.73
89	ULL	'88 TZ.2	Nitella	5/7/89		1	3.1	3.1	0.34	1.2	9.12
38	WRP	MAT L	Cyanophytes	6/7/89	14.9	11	0.65	0.65	0.068	0.57	9.56
4	BAD	PERI-ANAL	Chlorophytes	22/6/89		1	4.8	4.8	0.49	1.2	9.80
60	LLL	RECV ST#5	Nitella	9/7/89		1	2.1	2.1	0.19	1.1	11.05
13	ND-SOURC	PERI-ANAL	Cyano/Chlorophytes	22/6/89		1	1.1	1.1	0.098	0.88	11.22
64	LLL	RECV ST#10	Nitella	9/7/89		1	3	3	0.25	2	12.00
47	WRP	M-LC1,26HR	Cyanophytes	11/7/89		9	0.86	0.86	0.071	0.67	12.11
63	LLL	RECV ST#9	Nitella	9/7/89		1	2.7	2.7	0.21	2.1	12.86
39	WRP	MAT D	Cyanophytes	6/7/89	18.4	12	0.97	0.97	0.07	0.43	13.86
37	WRP	FIL D	Cyanophytes	6/7/89	31.4	1	1.5	1.5	0.097	0.66	15.46
36	WRP	FIL L	Cyanophytes	6/7/89	39.4	1	2	2	0.084	0.84	23.81
48	WRP	M-LC2,26HR	Cyanophytes	11/7/89		8	0.19	1.9	0.076	0.55	25.00

acids. Nitrogen and phosphorus are, therefore, the basic building blocks of life. The ratio of these elements in plant tissues is thus quite constant. Plants which are sufficient in both N and P generally contain these elements in a ratio of 7.5:1 N/P (Raven, 1981). However, both N and P can be taken up in excess and stored in plant tissues. Both N and P can be in sub-optimal concentrations, which slows or stops growth. The result of starvation or "luxury uptake" is that the optimal 7.5:1 ratio can change. If analysis of plant tissues indicates that the ratio is lower than 7.5:1, either N is sub-optimal, or P is in superabundance. A ratio greater than 7.5:1 indicates that N is in abundance and/or P is deficient.

Ratios ranged from 3-6 for algae from ULL, BAD, and LLL (a value less than 7.5 indicates P overabundance). Those algae with a ratio between 7.1 and 7.7 were collected in areas between the WRP and the BAD, and those with a ratio in excess of 8, were found predominantly in the WRP, with some of the lower numbers belonging to algae from other locations (Figure 9).

The algae from the WRP (CY = cyanophytes, blue green algae) are shown to have ratios from about 7.5 to 25, indicating that most are P deficient (or contain a superabundance of N). Those chlorophytes (green algae) in the delta region (CL) were P sufficient. Those *Nitella flexilis* (NF) in Upper Link Lake also straddled the 7.5 line, representing the 'healthy' line. Those NF and CL (*Nitella* and Chlorophytes) in the BAD straddled the line, with the

NF being somewhat below and the chlorophytes somewhat above. In Lower Link Lake, all *Nitella* measured either contained a slight excess of N or a slight deficiency in P.

4.0 STATUS OF TRANSPLANTED *NITELLA* IN UPPER LINK LAKE

The distribution, density and overall population structure of *Nitella flexilis* biomass transplanted to Upper Link Lake in June 1990 has widely varied according to season, location and year observed. This suggests that other factors, in addition to the design criterion, water depth between 0.6 and 1.9 m, play a role.

Observations of the *Nitella* populations in Transplant Zones 1 through 4 were carried out in regions along the transects through each zone. However, qualitative observations on the establishment of the algae over areas other than along the transects were made in addition. The locations of the transects are given in Figure 10.

4.1 Frequency of Presence and Percent Cover by *Nitella*

Transplant Zone 1: In 1989, *Nitella* biomass was distributed over of the soft mine slime deposits of a 1.2 hectare area of the submerged Delta at depths between 0.6 and 1.2 m. The transplanted *Nitella* remained in situ over the 1989 growing season, but remained unattached

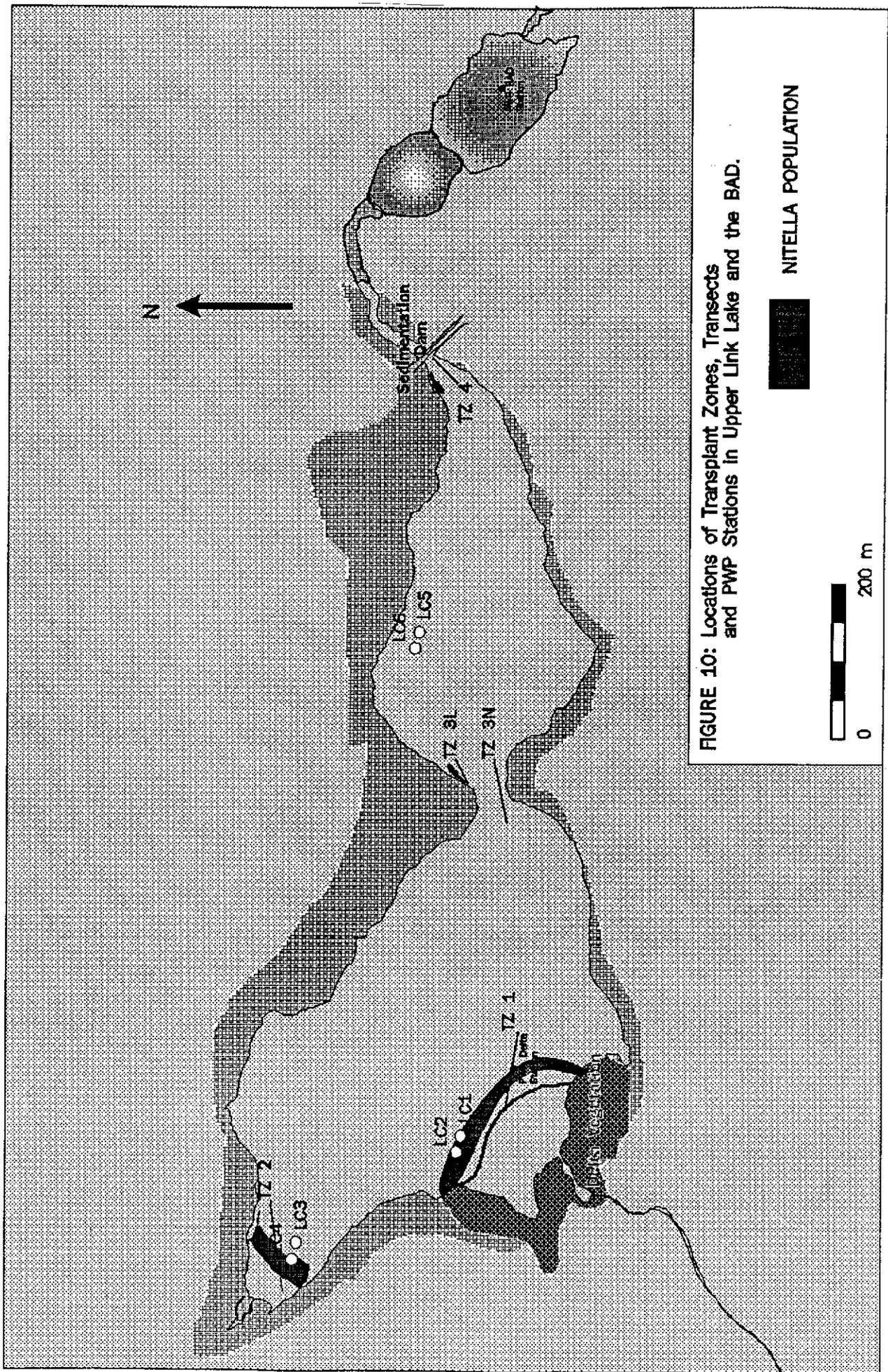


FIGURE 10: Locations of Transplant Zones, Transects and PWP Stations in Upper Link Lake and the BAD.

to, rather than anchored by rhizoids in, the sediment. This can now be explained by the nutrient status of the drainage basin.

In spring of 1990, very little *Nitella* was found over the TZ-1 transect and the remaining biomass consisted of short free shoots lying on the sediment. By September 1990, a higher frequency of anchored clumps of *Nitella* were observed over the transplant zone (Figure 11). As expected, by June 1991 the populations had not expanded since the previous September. However, by August 1991, both the frequency of presence of, and percent cover by *Nitella* had greatly increased. Significant amounts were found beyond the transplant zone at depths greater than 1.6 m. As well, most *Nitella* observed was anchored to the sediments, comparable to the population structure Lower Link Lake's *Nitella* population in 1988. This could be related to the nutrient ratio changes over the 1991 growing season (Figure 8).

In September 1991, the population structure had entirely changed from the anchored larger clumps of *Nitella* to a fragmented, unanchored distribution of short shoots spread over the entire transplant zone. Detachment of shoots from the sediment is likely related to the rate of basal decay. The violent storm on August 30, 1991 may also have contributed to damage of the already vulnerable plants.

Transplant Zone 2: Since the time of transplant over soft organic sediments of the 0.6 hectare area of TZ-2, *Nitella* biomass has remained, to date, at good densities. Similar frequencies of presence and percent cover were observed over 1989 and 1990 (Figure 12).

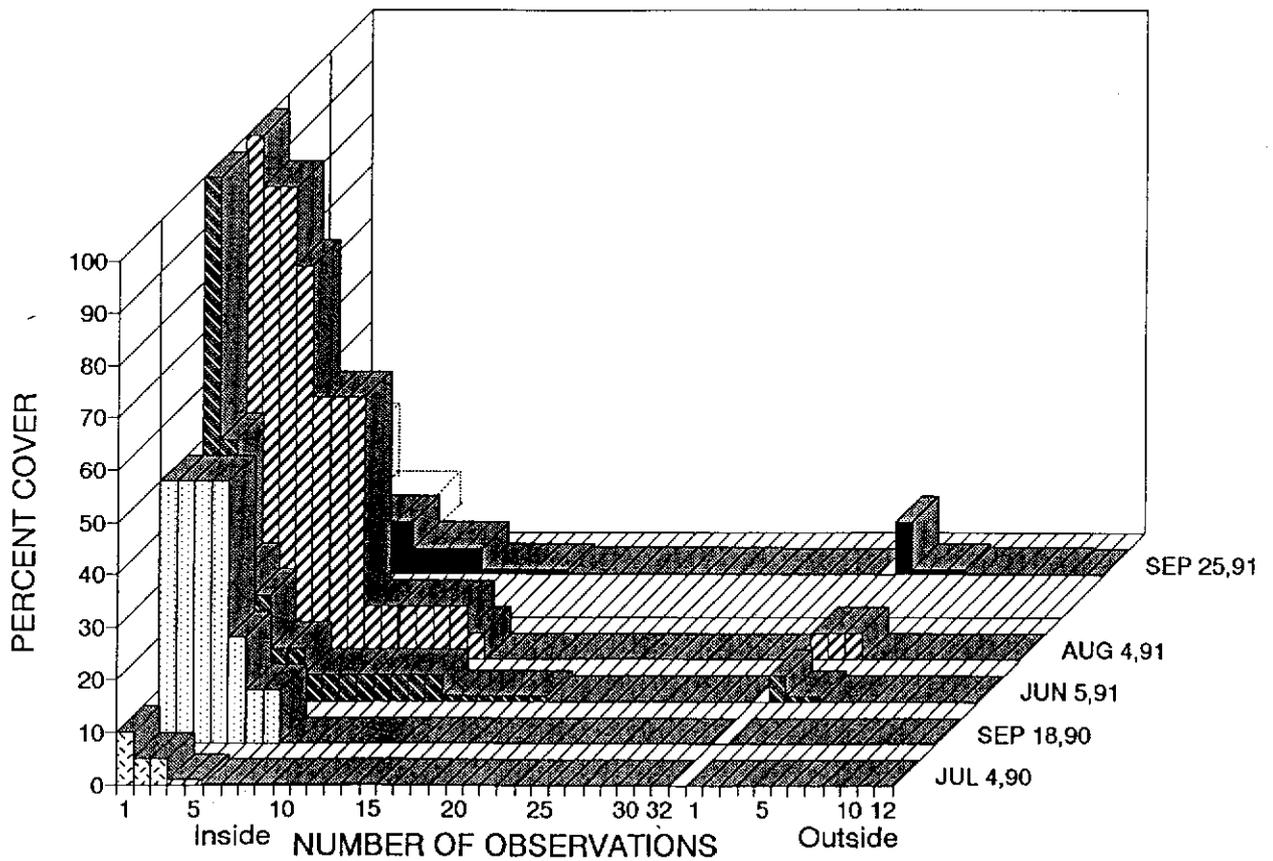


Figure 11: TZ-1: Frequency of occurrence and percent cover of *Nitella* inside and outside transplant zone.

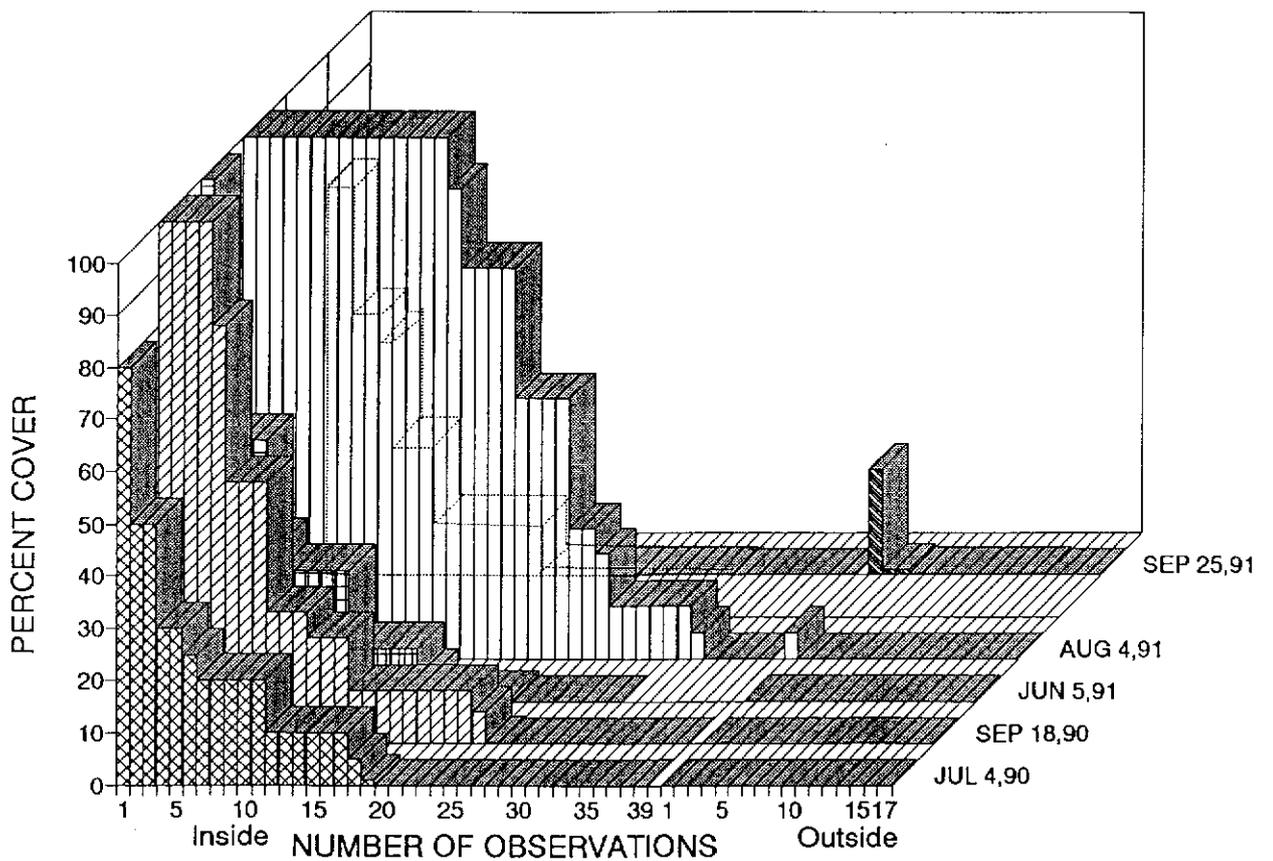


Figure 12: TZ-2: Frequency of occurrence and percent cover of *Nitella* inside and outside transplant zone.

However, between June and August 1991, the population increased its area both locally around existing nuclei and extended into both deeper and shallower areas beyond the original transplant zone.

During the August 1991 survey, the *Nitella* biomass was anchored to the sediments by rhizoids, such that the population appeared as did the 1988 Lower Link Lake population. However, by September, this population had detached from the sediment-bound portions of the shoots and fragmented plants were widely distributed over the area. The plant population remained attached to the sediment only in the area closest to the fresh water inflow in the north-west area of TZ-2.

Transplant Zone 3 Lateral: Transplanting *Nitella* biomass over a 0.35 ha area in the vicinity of the Narrows, or the Transplant Zone 4, was a long shot. Given the high rate of suspended solids settling to the bottom (4.3 to 10.5 g/m²/day, 1990 sedimentation trap results), the lack of organic sediments in this area is a clear indication that vigorous sweeping of the lake bottom occurs. This is probably the reason for the rocky bottom, scoured year round by currents, especially during winter when ice forms, increasing flow velocities at the narrow's bottom.

In only months following transplant, *Nitella* biomass was sparse along the TZ-3 narrows transect, while the TZ-3 lateral transect still supported moderate amounts of *Nitella* (Figure

13). However, between fall 1989 and spring 1990 virtually all *Nitella* biomass was scoured from the area along the TZ-3L transect.

In 1991, surveys of the TZ-3L transect were continued and in fact, *Nitella* had been moved into the transect area at one location. By August 1991 the area cover had expanded, but by September, *Nitella* was fragmented, as was observed along the TZ-1 and TZ-2 transects.

Transplant Zone 4: As in Transplant Zone 3, southern half of Transplant Zone 4's lake bottom is comprised of exposed rocks and boulders, with a thin matrix of sediment. The transect surveyed for this zone spans this southern half. The northern half has, overall, a larger area of the lake bottom covered with sediment.

All of the *Nitella* disappeared between 1989 and 1990 from the area along the TZ-4 transect (Figure 14). To date, as at the narrows, *Nitella* has not re-established along the TZ-4 transect. However, patches of *Nitella* remain along the northern half of the transplant zone.

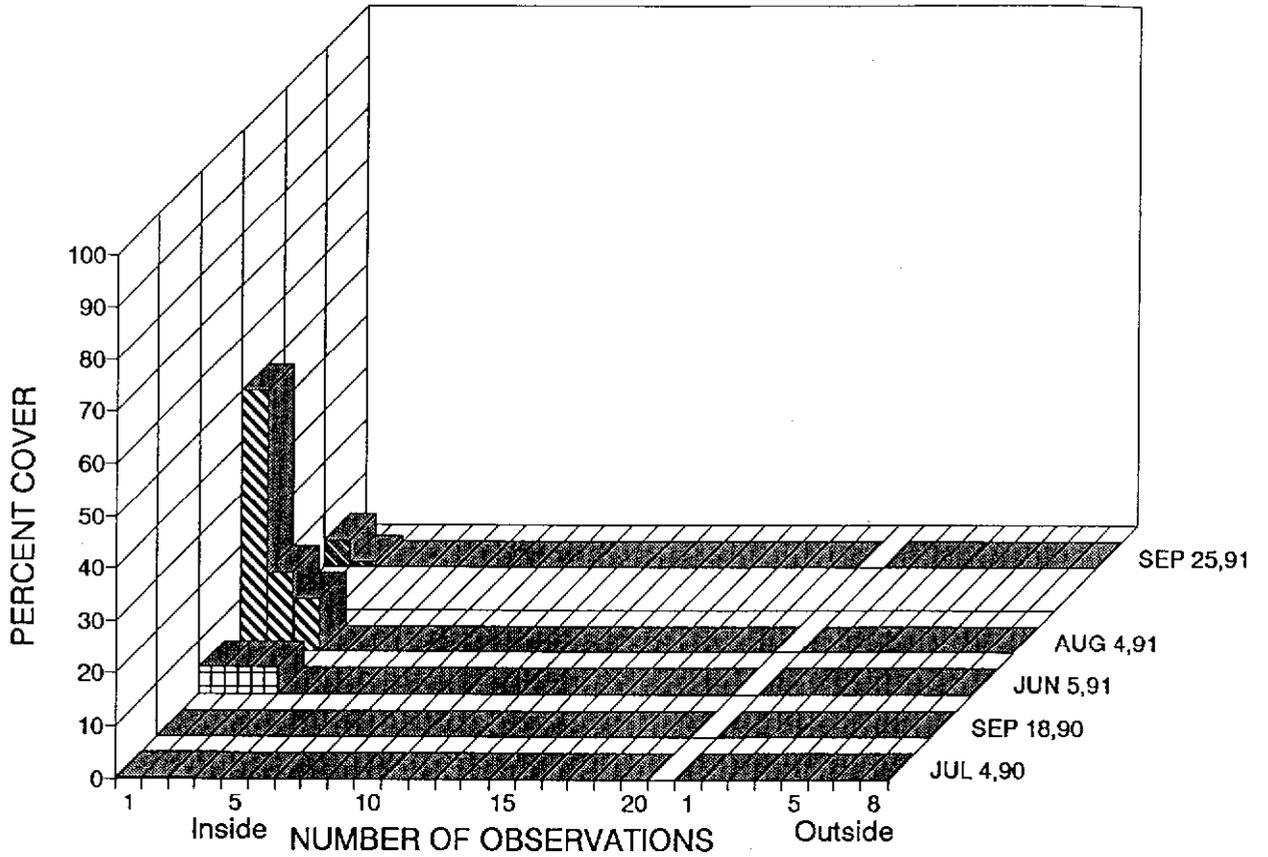


Figure 13: TZ-3L: Frequency of occurrence and percent cover of *Nitella* inside and outside transplant zone.

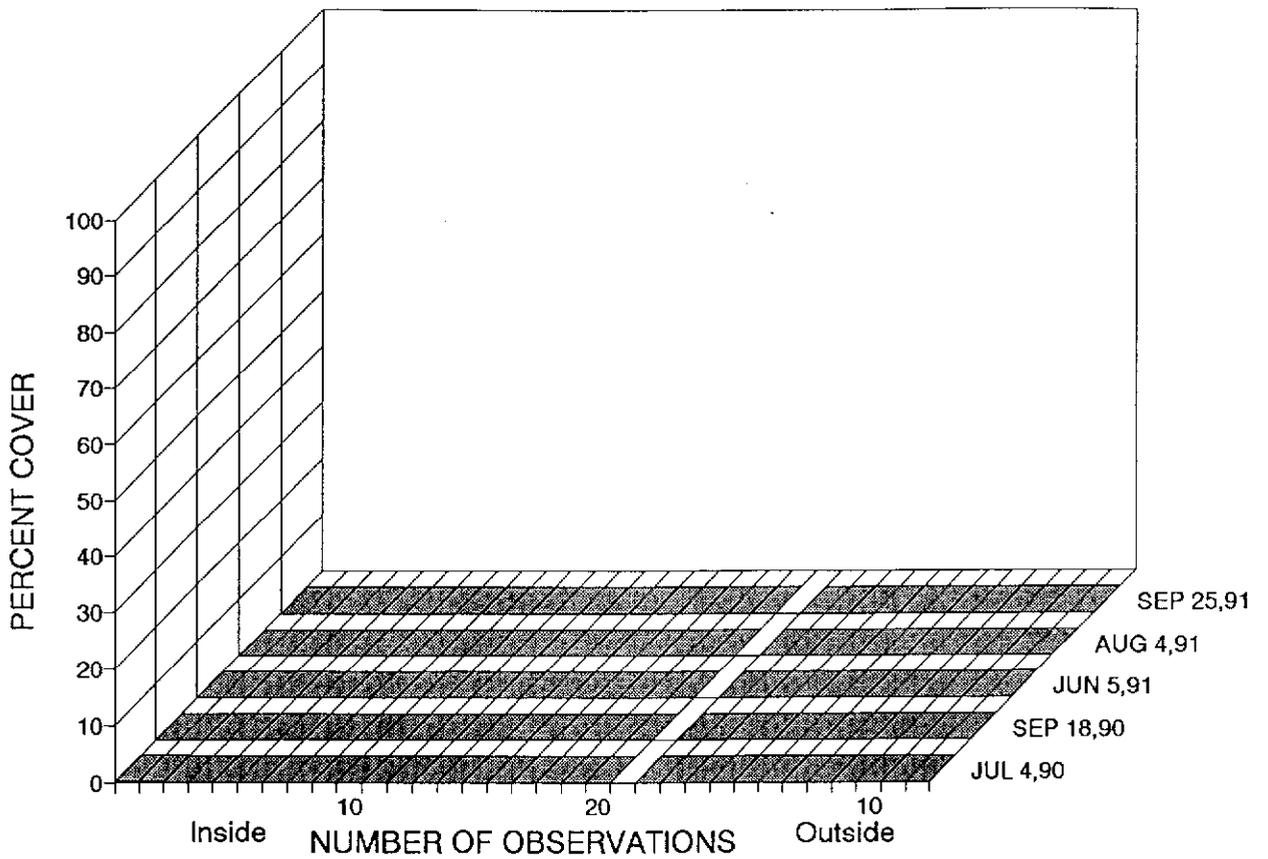


Figure 14: TZ-4: Frequency of occurrence and percent cover of *Nitella* inside and outside transplant zone.

4.2 Factors Determining *Nitella* Distribution in Upper Link Lake

In the 1988 Report (Kalin, Smith and Vandergaast, 1988), the water depths in Upper Link Lake at which *Nitella* could be expected to colonize were placed at 0.6 to 1.9 m, based on the *Nitella* distribution in Lower Link Lake, and the comparable light attenuation in the two lakes.

Lower Link Lake's *Nitella* population structure, and the nature of the underlying substrate, significantly differ from those of Upper Link Lake. The *Nitella* population over the expanse of Lower Link Lake was, at the very least, anchored by rhizoids to the loose sediment or sandy silt found over most of the lake, while commonly sub-sediment plant storage structures (bulbils) were observed underlying the green portion of the *Nitella* population. Furthermore, oospore, or seed, production by the *Nitella* population was commonly observed over Lower Link Lake. Prolific growth of *Nitella* was observed in areas of very high water velocity (relative to the lake at large), such as the BAD or the Lower Link Lake outflow channel, but in these areas, anchorage by rhizoids was seldom observed; the 'spaghetti-like' *Nitella* biomass, with interwoven shoots up to 1 m long, simply remained in position by its overall mass.

On the contrary, in Upper Link Lake, *Nitella* was transplanted to some areas where anchorage of the plants by rhizoids was not possible due to the rocky bottom, and these same regions clearly may periodically have strong currents such that anchorage by rhizoids

would have failed upon erosion of sediment. Even in areas where soft sediment underlies the lake, observations of *Nitella* anchoring itself by rhizoids are infrequent, with the exception of the August 1991 survey. Meanwhile, oospore development in Upper Link Lake has been observed on one occasion in 1990 only, among plants in a pond beside Sedimentation Dam which is isolated by muskeg from the lake at large. Overall, the higher water movements, indicated by rocky substrates in some areas, combined with poor anchorage by *Nitella*, indicates that establishment of a population is not controlled by depth alone.

Nitrate concentrations in the water and ammonia concentrations in the sediments of Upper Link lake are the factors which can now be identified as responsible for both the poor anchorage of *Nitella*, and the absence of oospore development in Upper Link Lake.

Several observations should be restated for discussion of this hypothesis. First, as described in Section 3.2 and 3.3, Upper Link Lake has, typically, higher concentrations of nitrate, and similarly high concentrations of ammonia, compared to water passing through the BAD or Lower Link Lake. Therefore, *Nitella* populations in Lower Link Lake may be more dependent on the sediment as a source of nitrogen and phosphorus, and hence produce rhizoids.

The failure of *Nitella* growth inside LC2 (Delta limnocorral underlain by mine slimes) and LC4 (north-west corner limnocorral underlain by organic sediment), but success of *Nitella*

in terms of growth and overwintering in LC6 (limnocorral below narrows overlying sandy sediment) may have been due to shifts in nutrient ratios due to the sediment enclosure. Increases in ammonia concentrations, through a) microbial reduction of nitrate to ammonia, b) decay of phytoplankton to ammonia, and c) flux of ammonia from the organic sediments to the water columns of LC2 and LC4 may have killed the *Nitella*. The enclosure of relatively inorganic sediments in LC6 may not have altered the nutrient ratios as drastically.

5.0 EVALUATION OF RADIUM²²⁶ IN SEDIMENTS IN THE RABBIT LAKE DRAINAGE BASIN

5.1 Sediment Interstitial Solution Chemistry

Uranium and ²²⁶Ra are governed by different chemical dynamics, uranium belonging to the actinides and ²²⁶Ra to the alkaline earth elements. Thus, the sorption processes which govern their removal from the water and the adherence to different components in the sediments are different. Data on mobility of ²²⁶Ra sediments are lacking although sorption and adsorption processes are well-studied under laboratory conditions (Benes and Majer (1980) in " Trace Chemistry in Aqueous Solutions").

Within the Ecological Engineering decommissioning program, the quantification of the flux of ^{226}Ra and uranium from the sediment overlaid by a Characean underwater meadow in the Rabbit Lake drainage basin was pursued.

The concentrations of ^{226}Ra and uranium in the sediments, based on dried material, indicates, as anticipated, that the sediments are high in both the elements. Exploration crews use the concentration of uranium in lake sediments to locate ore bodies, as was the case for Rabbit Lake. Mobilization of heavy metals from sediments is governed mainly by chemical changes in the water, such as changes in redox conditions, pH changes in the water, changes in the total salt concentrations, and changes in the concentrations of complexing agents. These factors will also govern the mobility of ^{226}Ra and uranium in the sediments. Interstitial water characteristics have therefore been determined together with the concentrations of the contaminants of concern.

Interstitial solution in the sediments was collected with pore water peepers, which were modified after Carignan (1984) to accommodate the retrieval of a larger sample volume required for radionuclide analysis. The goal of these measurements using the pore water peepers is to determine the concentrations of specific compounds in the interstitial water which are mobile, or free to diffuse along concentration gradients within the sediment. The instruments were equipped with $0.45\ \mu\text{m}$ membranes and filled with distilled water, which allows only "dissolved" elements to diffuse in or out of the instrument's chamber according to concentration gradients, while screening against entry of compounds bound to larger

colloidal or particulate components of the sediment. The rationale behind the selection of the membrane material and pore size is described by Salomons & Forster (1984).

In Figures 15 to 21, the concentrations of Na, K, Ca, Mg, Fe, ^{226}Ra and U are given for the interstitial solution over time in the sediment. Figure 22 provides an overview of sediment interstitial chemistry data between 1989 and 1991. The numbers 1 through 8 denote the PWP from the 1989 investigation and B and D, those from 1990/91. The drastic increase in the first sampling period after 14 days in the sediment is expected as the sampling chamber was initially filled with distilled water. Introduction of the distilled water behind the permeable membrane creates a steep concentration gradient from the sediment interstitial solution dropping across the membrane to the distilled water chamber.

The time required for equilibration is of special interest. Equilibration was evaluated from the change in concentrations of Na, K, Ca, Mg and Fe. Equilibration was achieved before 14 days, as no further increases in the pore water peeper concentrations were noted for elements in the samples taken during subsequent retrievals, representing longer periods for equilibration with the sediment. In fact, the dissolved concentrations in the sediment interstitial solution were strikingly constant over the period of examination (Figures 15, 16, 17 and 18) with the exception of K and Na.

Potassium was the only element where concentrations fluctuated after 14 days. The concentrations decreased after the initial range of 2.5 mg/L at 14 days, to a range of 0.5 to

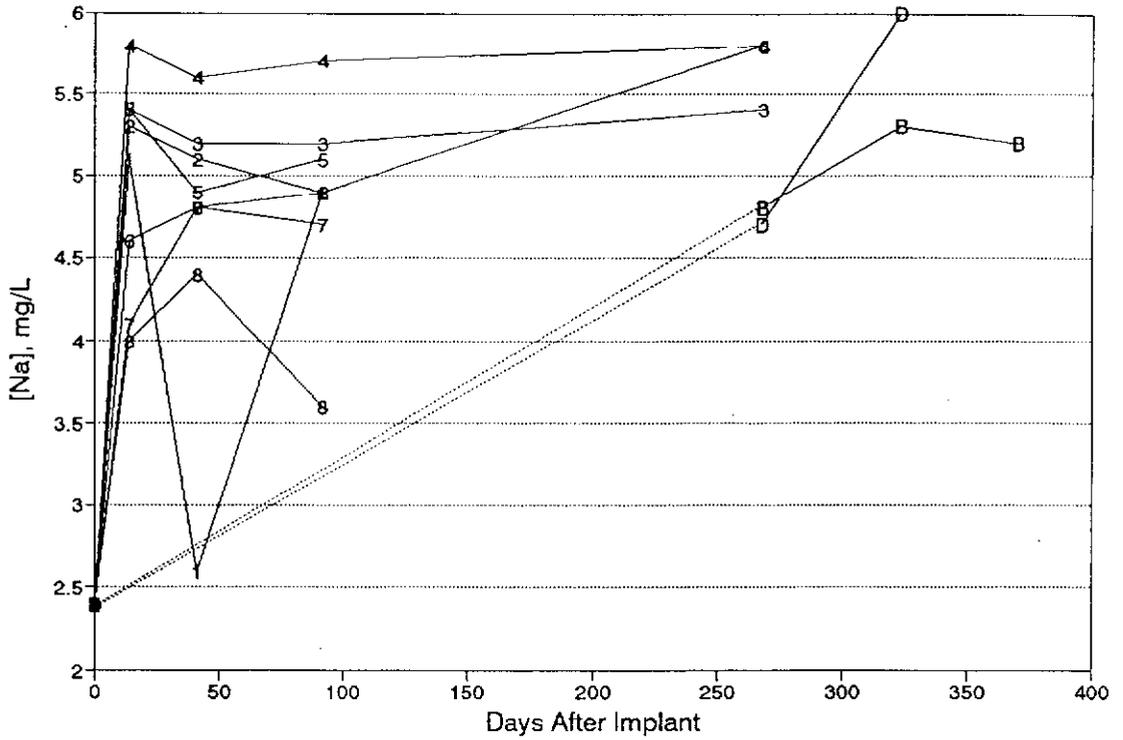


Figure 15: Sediment Interstitial solution concentration of Sodium. The values 1 through 8 are PWP's in limnocorrals, while B represents the BAD and D represents the Delta. Dashed lines are used when a large interpolation is made.

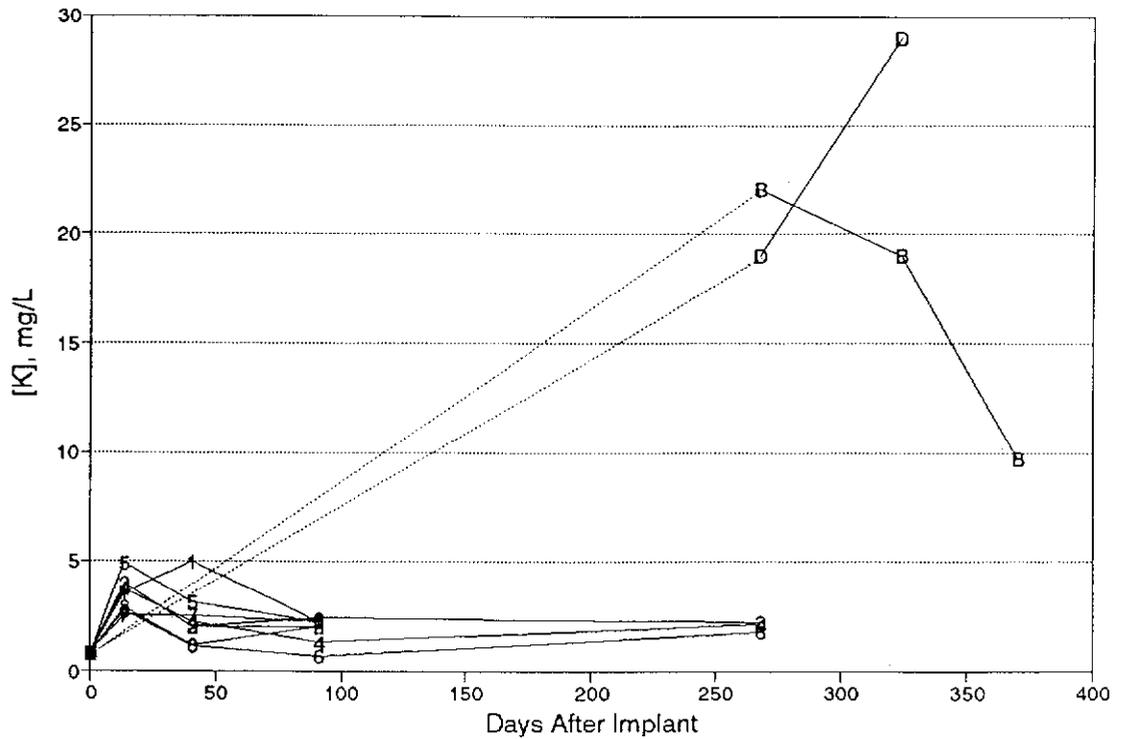


Figure 16: Sediment Interstitial solution concentration of Potassium. The values 1 through 8 are PWP's in limnocorrals, while B represents the BAD and D represents the Delta. Dashed lines are used when a large interpolation is made.

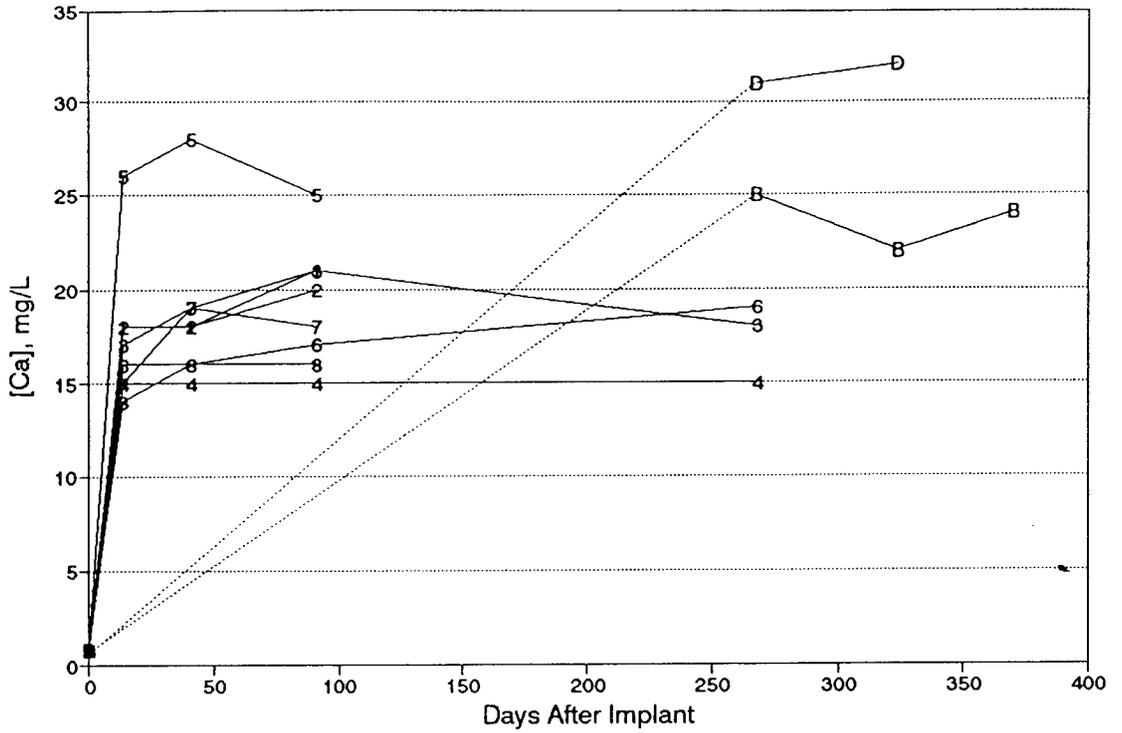


Figure 17: Sediment Interstitial solution concentration of Calcium. The values 1 through 8 are PWP's in limnocorrals, while B represents the BAD and D represents the Delta. Dashed lines are used when a large interpolation is made.

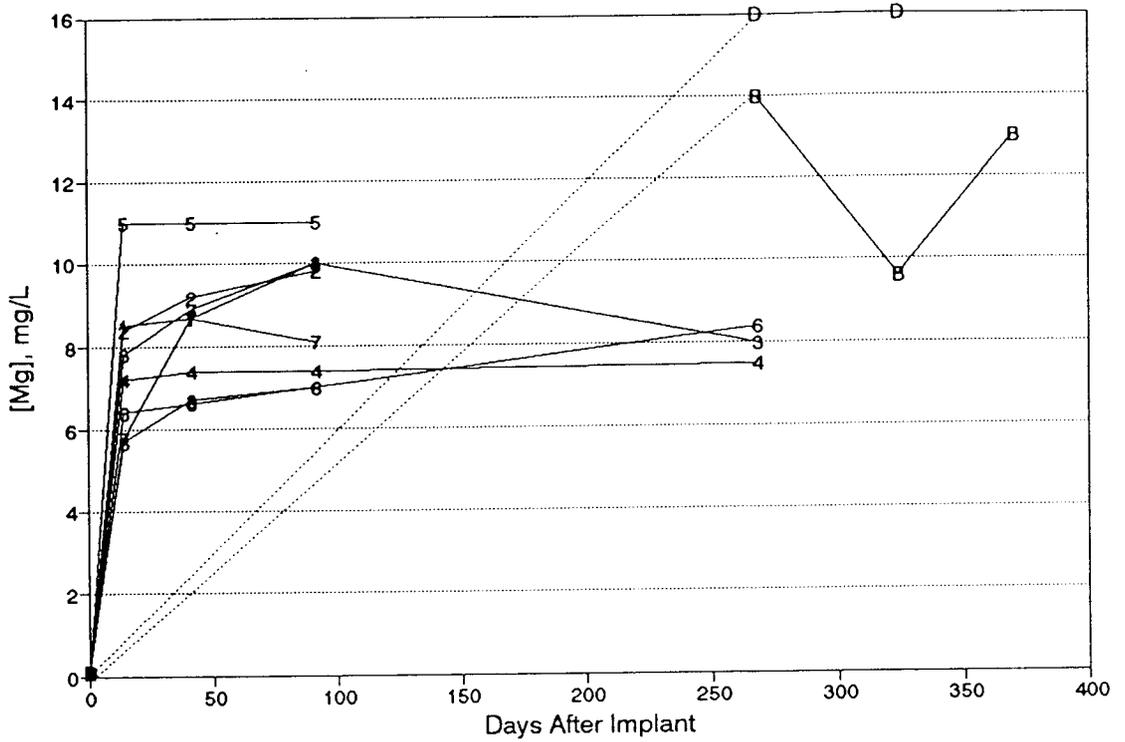


Figure 18: Sediment Interstitial solution concentration of Magnesium. The values 1 through 8 are PWP's in limnocorrals, while B represents the BAD and D represents the Delta. Dashed lines are used when a large interpolation is made.

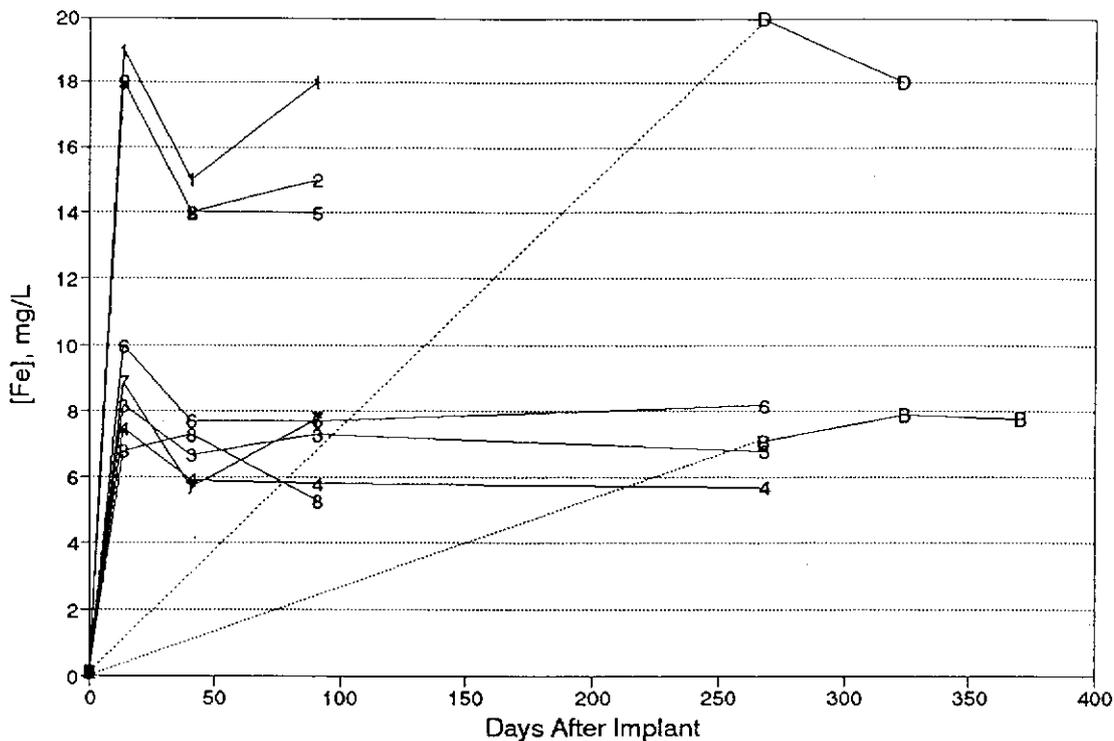


Figure 19: Sediment Interstitial solution concentration of Iron. The values 1 through 8 are PWP's in limnocorrals, while B represents the BAD and D represents the Delta. Dashed lines are used when a large interpolation is made.

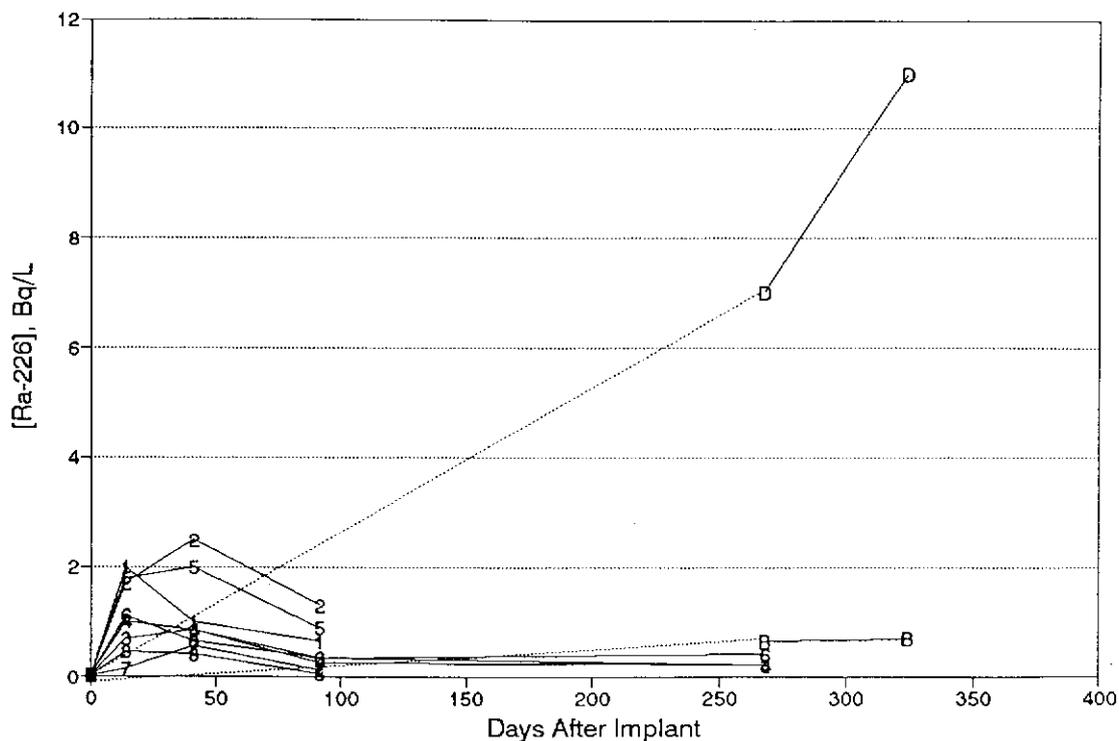


Figure 20: Sediment Interstitial solution concentration of Ra^{226} . The values 1 through 8 are PWP's in limnocorrals, while B represents the BAD and D represents the Delta. Dashed lines are used when a large interpolation is made.

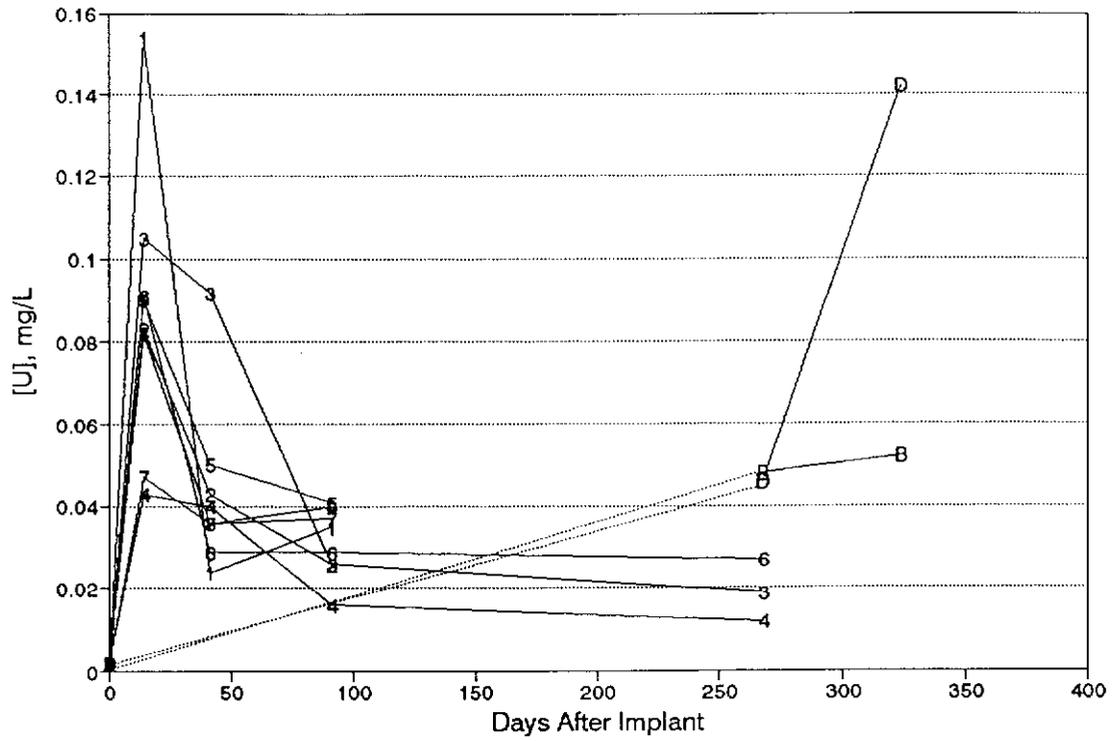


Figure 21: Sediment Interstitial solution concentration of Uranium. The values 1 through 8 are PWP's in limnocorrals, while B represents the BAD and D represents the Delta. Dashed lines are used when a large interpolation is made.

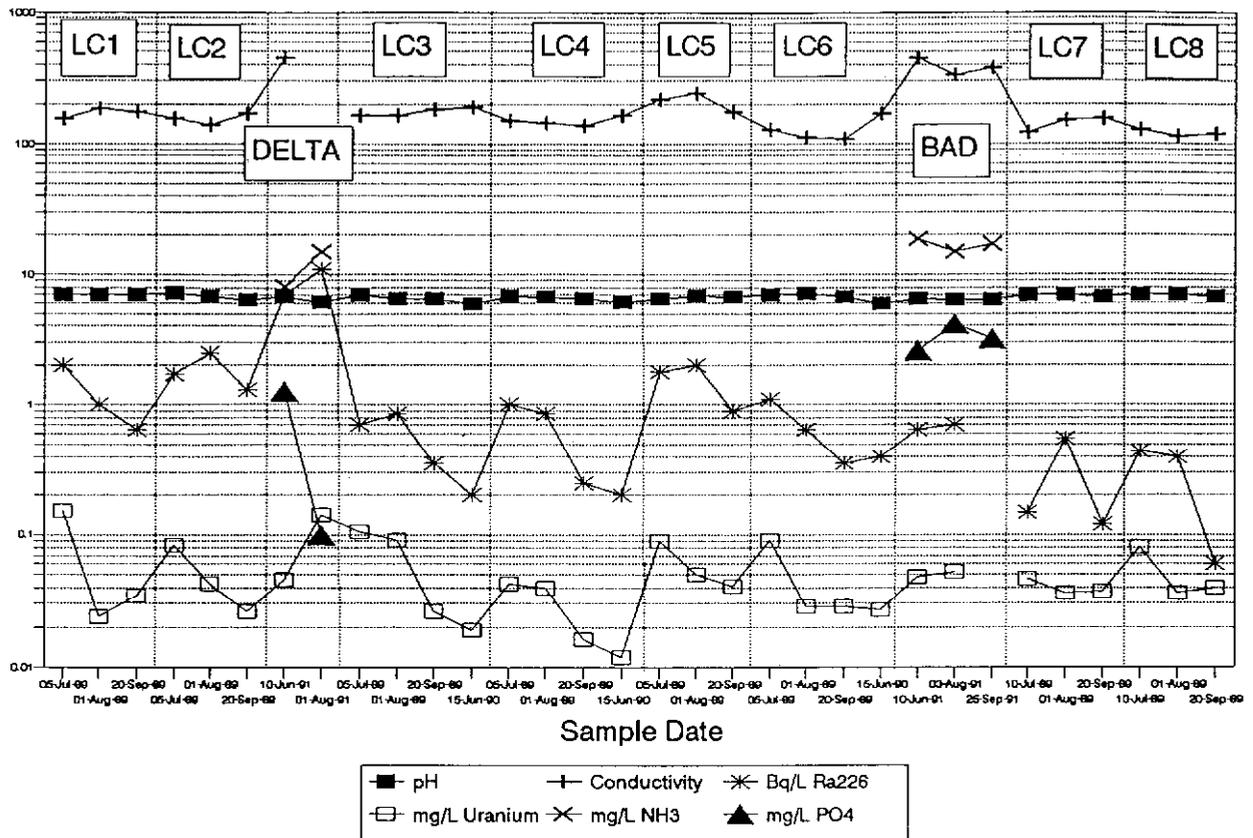


Figure 22: Overview of Sediment Interstitial Solution Chemistry: 1989-1991

2.5 mg/L after 90 days. ^{226}Ra concentrations determined from the pore water peeper solutions exhibited a similar trend as potassium in that, between 14 and 40 days in the sediment, the concentrations overall decreased (Figure 20). Where the initial concentrations (1 to 2.5 Bq/l at 18 days) were higher, the decline in ^{226}Ra concentration was more steep, compared to pore water peeper concentrations which were initially lower, below 1 Bq/l.

In June, 1990 pore water peepers, which had remained in the sediment for almost 1 year, thereby overwintering, were retrieved where possible. The concentrations of all elements remained in the same ranges as those ranges determined after 90 days incubation in the sediment. This constancy in concentrations over an entire year indicates, first, that the sediment interstitial solution chemistry is relatively constant. Furthermore, the pore water peeper technique effectively samples interstitial solutions.

The key questions, with respect to the interstitial concentrations, is whether the concentrations of mobile forms of ^{226}Ra in the sediment interstitial solutions are constant, in relation to the concentrations in the solids. Through detailed geochemical interpretation of the data, it may, in fact, be possible to quantitatively inter-relate the available data concerning interstitial solution and lake chemistry. That such an interpretation is required should be evident.

From the PWP concentrations, collected at different intervals for up to 268 days in the Limnocorrals between 1989/90 and those placed in the Delta sediments and the BAD

sampled after 263, 324 and 370 days indicate the following. The dissolved or mobile fraction in the sediment is related to the location. In the *Nitella* sediment in the BAD the longer exposure did not alter the fraction of dissolved and hence mobile fraction of Ra^{226} . This, however, was not the case for the PWP submerged in the Delta, where the mobile fraction increased from 2.5 Bq/l in Limnocorrals 1 and 2, to 11 Bq/l for the longer exposure.

The concentration of Ra^{226} in the *Nitella* sediments in the BAD were 6 Bq/g and ranged from 14 to 22 Bq/g dry weight in the Delta sediments in the limnocorrals. For the location on the Delta where the 1990/1991 PWP was incubated, no solid samples of the sediment were collected. The concentrations in the solid phase of the sediment do not appear to affect the mobile ^{226}Ra fraction as much as either location or sediment condition. The important fact is, that *Nitella* sediment did not behave as did Delta sediment, based on the long term incubations of the PWP's.

5.2 Sources and Loadings of ^{226}Ra

The annual loading of ^{226}Ra in Upper Link lake was estimated at 4.1×10^8 Bq/year (Kalin, Smith and Vandergaast, 1988). Between 1985 and 1987, the average flow at Airport Road was 22.6 l/s, while at Sedimentation Dam, 26.9 l/s. The ^{226}Ra loading estimate at Sedimentation Dam was based on an average concentration of 0.44 Bq/l.

^{226}Ra concentrations at Old Airport Road (W6), and especially at the end of the flow path through the vegetated Delta (W9), are usually lower (up to 50%) than at Sedimentation Dam (W15). Flux of ^{226}Ra from the Delta sediments is believed to be the source of ^{226}Ra loading in addition to the west waste rock pond (W2). If the water entering Upper Link lake at W9 is assumed to contain 25% less ^{226}Ra than that leaving Sedimentation Dam, then the additional loading of ^{226}Ra by the Delta is 1.96×10^8 Bq/year.

If this additional ^{226}Ra loading is arising from a 1 to 4 hectare submerged Delta area, then the average flux rate over the year is 13 Bq $^{226}\text{Ra}/\text{m}^2$ Delta/day (4 ha active area), 27 Bq $^{226}\text{Ra}/\text{m}^2$ Delta/day (2 ha active area) or 54 Bq $^{226}\text{Ra}/\text{m}^2$ Delta/day (1 ha active area).

Data obtained from the Pore Water Peeper experiments can be used to estimate the flux of ^{226}Ra from the submerged Delta from a different approach. ^{226}Ra concentrations reached as high as 11 Bq/L inside the pore water peepers after a 324 day incubation period in the Delta sediments (Figure 20). However, the 1989 pore water peepers extracted after only 14 days of incubation may provide an approximation of the upper limit of the flux rate, by using the shortest incubation period (rate obtained when $[^{226}\text{Ra}]_{\text{inside PWP}} < [^{226}\text{Ra}]_{\text{outside PWP}}$).

The pore water peepers contained <0.01 Bq/l ^{226}Ra at the time of implant (Day 0). After 14 days, the PWP's (125 ml volume) in LC1 and LC2 had, on average, 1.85 Bq/l. This means that 0.231 Bq of ^{226}Ra diffused from the surrounding sediment through the 18 cm^2

PWP membrane into the PWP chamber in 14 days. Therefore, the flux rate was 9.17 Bq $^{226}\text{Ra}/\text{m}^2$ PWP membrane/day.

The 1990-91 Delta pore water peepers contained <0.01 Bq/l ^{226}Ra at the time of implant (Day 0). After 324 days, The PWP's (125 ml volume) had 11 Bq/l. This means that 1.375 Bq of ^{226}Ra diffused from the surrounding sediment through the 18 cm^2 PWP membrane into the PWP chamber in 324 days. Therefore, the flux rate was 2.36 Bq $^{226}\text{Ra}/\text{m}^2$ PWP membrane/day.

Using these estimates, a one ha active Delta area would increase the loading by 0.9 to 3.3 $\times 10^7$ Bq/year (324 versus 14 day incubation data); over two ha, 1.7 to 6.7 Bq/year; and over four ha, 3.4 to 13.4 $\times 10^8$ Bq/year. Comparison of the ^{226}Ra loading estimate from water quality data with the Pore Water Peeper data suggests that a four ha active Delta area is releasing ^{226}Ra at rates comparable to estimates using 14 day PWP data.

5.3 The Delta *Nitella* Population: Annual ^{226}Ra Capture

Of the 4 ha submerged Delta area where the 1987 Machibroda study determined radioactivities of greater than 1000 cps, 75% (or 3 ha) of the area has a depth between 0.6 and 1.6 m, and is therefore suitable for *Nitella* growth. In 1988, transplant racks of *Nitella*

produced a net biomass of 0.5 kg/m^2 over the ice-free season (1989 and 1990 rack experiments yielded lower biomasses). Analysis of 1991 Delta *Nitella* indicates that the biomass contained, on average, $40 \text{ Bq/g } ^{226}\text{Ra}$. A homogenous, dense population of *Nitella* would therefore harbour up to $2 \times 10^4 \text{ Bq } ^{226}\text{Ra}$ per m^2 of Delta sediment. Overall, the 4 ha Delta could alone support a *Nitella* population containing $6 \times 10^7 \text{ Bq}$ of ^{226}Ra , or 31 % of the total ^{226}Ra annual loading generated within Upper Link Lake.

5.4 Determination of *Nitella* Populations' Biomass Turnover Time:

Development of the Calcofluor Technique

The rates at which new *Nitella* biomass, the 'filter', is generated by shoot apices growth, and at which the filter, loaded with ^{226}Ra , is relegated to the sediments upon decay of the plant bases, are essential for estimation of the biomass turnover time and the overall performance of the Chara Process. We have adopted the term, "apical growth and basal decay", or AG/BD from Andrews and coworkers (1984).

The observed standing biomass of a *Nitella* population seen at any particular time is the result of the dynamic equilibrium of these two opposing processes, growth and decay. For the build-up of standing biomass, apical growth must exceed basal decay. However, to

account for a relatively stable standing biomass of a mature population, the annual average rate of decay must match that of growth.

Determination of site-specific rates of *Nitella* biomass growth and decay must be obtained in the field, as both growth and decay rates are temperature dependent and therefore are be seasonally dependent, although not necessarily in phase. In 1988, a technique was used, whereby colour-coded acrylic rings were placed over individual shoots. This was done in the BAD and Lower Link Lake. The migration of the rings downwards with decay of the lower portions of the plants was followed, while the accumulation of new shoot biomass above the ring, indicating new growth, was quantified. These early results indicated that growth over 98 days was 200 to 300 g/m² and decay was 1500 to 400 g/m², yielding an overall turnover time of at least once per year. However, as the technique provided only crude estimates, clearly improvements to the technique were required.

In 1990, Calcofluor, or Fluorescent Brightener #28 (C₄₀H₄₂N₁₂Na₂O₁₀S₂) was identified as a chemical used to examine cell wall development. It is an effective stain of cellulose which fluoresces under ultraviolet light. Application of Calcofluor, instead of acrylic rings, was postulated as a more refined method to estimate apical growth and basal decay.

Preliminary tests in the lab indicated that Calcofluor concentrations of only 6 mg/l effectively stained *Chara vulgaris* shoots for several days. In June 1991, the technical concept (though not yet a technique) was taken to the field, where 125 l volume square Fabrine tents open

on bottom were placed over existing *Nitella* populations in TZ-1, TZ-2 and the BAD. Dissolved Calcofluor was injected into the tents at concentrations of 5, 50 and 500 mg/l, and the tents filled with the Calcofluor solution were left in situ for 24 hours. Upon tent removal samples were collected to verify that the *Nitella* biomass was in fact stained.

In August and September, 1991 shoots were recovered from the plots and examined under UV light. Overall, the results indicated that Calcofluor effectively stains the youngest cell walls of *Nitella* shoot cells when free of detritus, periphyton and invertebrate frustules (see Plate 1). Stirring of the solutions in the tents may well have increased the surface area of *Nitella* shoots stained. Second, although no toxicity data on Calcofluor exists, field results indicate that *Nitella* survived staining. Third, the BAD *Nitella* site's three month results indicate that Calcofluor is not readily biodegraded, as fluorescence was still visible after this period.

Although the Calcofluor technique appears to have excellent potential for determination of apical growth and basal decay rates of *Nitella*, specific rates cannot be derived from the 1991 data. Unfortunately, plots encompassing rooted *Nitella* biomass with good densities could not be exactly pinpointed during tent installation due to poor visibility, and on most occasions, little biomass was stained and available for sampling during the following site visits. Second, the shoots in TZ-1 and TZ-2 were not, for the most part, anchored in June and September, and exchange of stained for unstained biomass was suspected. Although the BAD *Nitella* provided excellent examples of staining after three months, shoots comprising

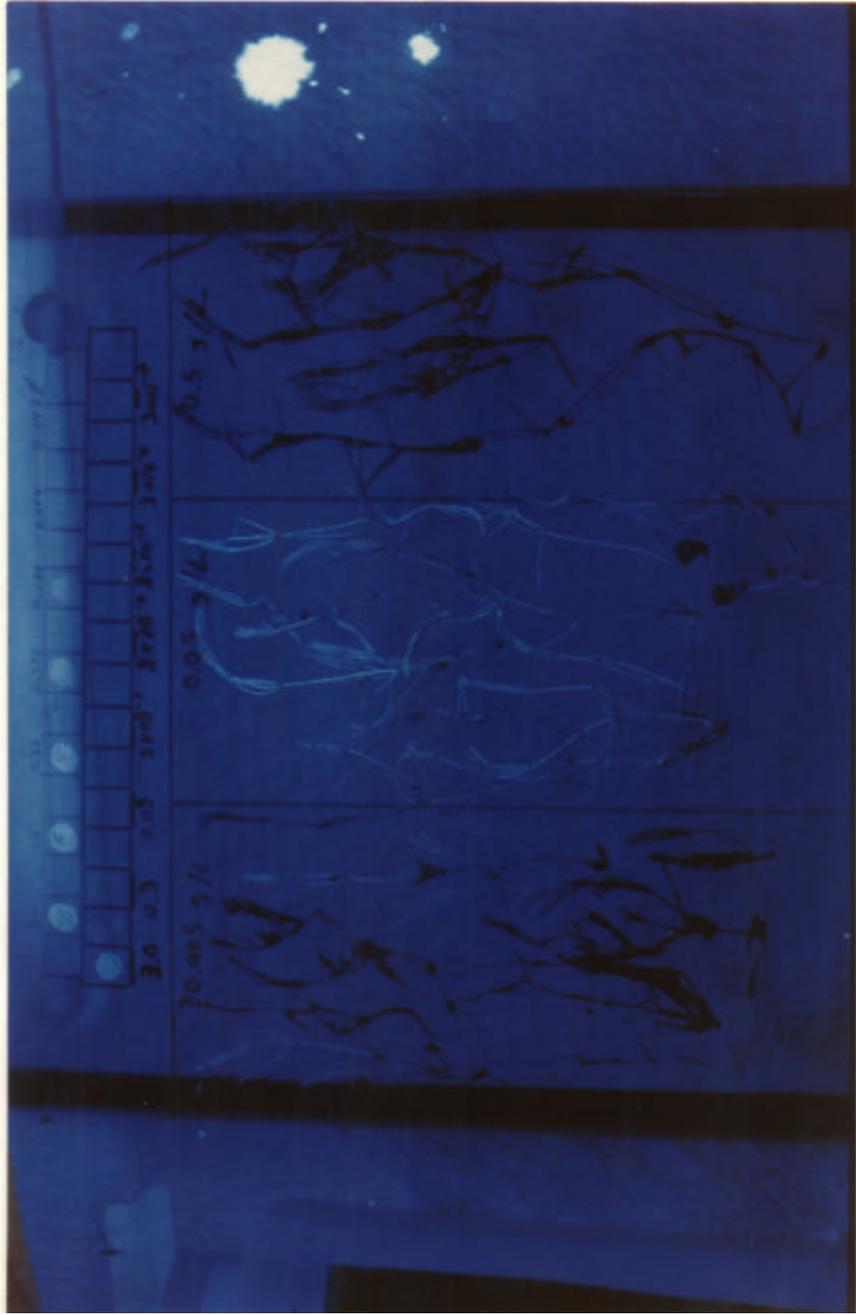


Plate 1: Example of UV fluorescence of Calcofluor-stained *Nitella* shoots.

this population's biomass are up to 1 m long and 'spaghetti-like', and tracing a shoot apex back to the basal node without breaking the shoot is difficult to say the least.

Although the target, determination of AG/BD rates, was not achieved, in 1991 the technique was developed to a workable state. By placement of a 2 x 2m netting fence around an representative area of the *Nitella* population, import and export of biomass should be prevented. Staining of the entire plot should provide sufficient biomass for even sampling, after overwintering, in the second year. Using the larger plot size, 25 x 25 cm blocks of biomass can be collected each month in order that calcofluor results can be tied in with specific data on standing biomass as g/m^2 . Concurrent placement and growing of additional *Nitella* biomass using the rack technique would provide newly grown biomass with minimal detritus over its surface, aiding Calcofluor staining.

6.0 CONCLUSIONS

The Rabbit Lake drainage basin is a eutrophic, i.e., nutrient rich water body. The nutrient ratios indicate that blooms of blue green algae can be expected to occur as long as the waste rock pile produces nitrate rich waters. In order to affect this nutrient ratio, and thereby the algal bloom, it is suggested that a wetland should be developed around the waste rock pile, or any suitable location before Upper Link Lake.

The need for this measure is reflected in the growth form of *Nitella* transplanted to Upper Link Lake. Although the plants establish and survive, towards the end of the growing season the plants fragment. This is attributed to the changes in nutrient ratios which occur after August. In August, the best growth was observed and at that time the nutrient ratio was optimal in Upper Link Lake.

The second reason for building a nitrate sink before the water enters Upper Link Lake is that continuous algal bloom increases light attenuation, which in turn reduces the area in which *Nitella* colonization can occur.

With the field program results of the 1988 feasibility study, it was determined that the values for key design parameters are as follows:

- | | | |
|---|-------------------|-----------------------------|
| 1) Water depth for growth of <i>Nitella</i> : | | 0.6 to 1.9 m |
| 2) Standing Biomass (SB) of <i>Nitella</i> : | | 100 to 400 g/m ² |
| 3) <i>Nitella</i> turnover rate: | | 1.0 SB/yr |
| 4) Contaminant Uptake: | ²²⁶ Ra | 10 to 30 Bq/g |
| | Uranium | 1 to 2 mg/g |

The first design parameter, water depth, was based on the depths at which *Nitella* was distributed in Lower Link Lake in 1988, and light attenuation. This parameter has to be redefined, taking into account the depths at which ice scouring occurs.

All other data collected since 1988 do not contradict the values of the remaining design criteria.

The long term exposure of the PWP's comparing the Delta sediments in the transplant zone on the Delta to that in the BAD, suggest that *Nitella* sediments have a significantly lower

mobile fraction of Ra ²²⁶ than the Delta sediments. The results also indicate that the mobile fraction differs from location to location, suggesting the existence of hot spots in the Delta.

The complexity of sediment chemistry and the factors controlling ²²⁶Ra mobility is evident. As at this stage a reliable data set is available on mobile fractions of the contaminant of concern, it is suggested that geochemical simulations are used to finalize the stability of ²²⁶Ra in *Nitella* sediments. The existing data can be used to confirm the results of the simulations.

The methods of quantifying the turnover rates of the *Nitella* populations are plagued with problems. The Calcofluor staining, however, confirms significant basal decay and accumulation of material on the *Nitella* underwater meadow. The experience gained with the fluorescent stain can be used to obtain reliable turn-over rates when the populations are re-established.

7.0 REFERENCES

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