Investigation of Some Characteristics of the Nitella Population in Mountain Lake, Giles County, Virginia

by

Charles Kelly Jervis

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APPROVED:

Dr. Bruce C. Parker, Chairman

Dr. Ernest F. Benfield

Dr. Robert A. Paterson

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(ABSTRACT)

The Nitella which grows in Mountain Lake, Giles County, Virginia, was investigated and found to be Nitella megacarpa (T.F.A.) em. R.D.W. after N. furcata subsp. megacarpa and not N. flexilis (L.) Ag. as previously reported.

Internodal cell length was found to increase with depth, pigment content changed with depth, biomass distribution had decreased since an earlier study, primary productivity was low and was associated with the depth of maximum biomass and pigment content, and the furcations of the branchlets increased in shallower water.

ACKNOWLEDGEMENTS

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Additionally, several fellow students have been very helpful in many aspects of this research. Marolyn Parson, Laura King, and Karen Gearhart helped in collections and helped work out the countless fine details of running the instruments and making measurements. Instrumental in collecting the samples were the SCUBA divers Kent Carlson, Kate Breeden, and Jennifer Rothrock. John Randall and Llyn Sharp assisted in the photography of the macroscopic samples and Dr. Rob Soring gave valuable advice in the chromosome staining technique.

Finally, I want to express may deepest thanks to my parents, James and Carol, and to my wife, Jody and her parents, Benjamin and Claire Matthias, without whom none of this would have been possible and with whom all of this has been enjoyable.

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SECTION 1

INTRODUCTION AND LITERATURE REVIEW

History of Mountain Lake: Mountain Lake, an oligotrophic lake found in Giles County, Virginia, is an extensively studied site. It is the only natural lake in the unglaciated Appalachians and only one of two natural lakes in Virginia. The history of Mountain lake has been discussed by Sharp (1936) and Parker et al. (1975). The lake lies at an elevation of 1200 meters at the head of the south fork of Little Stony Creek and is fed by subsurface streams and runoff. Mountain Lake has been the subject of numerous studies (Table 1). In several of the studies (discussed below), charophytic algae have been mentioned. The only species mentioned are Nitella flexilis (L.) Ag., Chara schweinitzii A. Br., and Chara braunii Gm..

Charophyte Background: Charophytes are an ancient group of algae. Fossils from the late Silurian onward clearly identifiable as charophytic oogonia (Flugel 1977) have been placed into at least 10 extinct genera by Johnson (1969). The evolutionary line of which the charophytes are members is postulated to have led to higher plants (Pickett-Heaps and Marchant 1972). This relationship is based mainly upon three pieces of evidence:

1. Phragmoplastic mode of cell division resembling that found in higher plants;

2. Sterile jacket cells associated with reproductive structures;

3. Sperm flagella placements which resemble those of Bryophytes.

It is because of these distinctions from other green algae that the charophytes are placed in their own division, Charophyta.

In addition to the possible evolutionary significance of charophytes, they are economically important in several ways. As reviewed by Wood and Imahori (1965), they have been used in fish culture, as fertilizers, polishes, and in insect control. They also are economically important in water transport systems because they can clog irrigation ditches and filter systems. They also are indicators of chemical changes in aquatic habitats. They are very sensitive to phosphorus concentrations (Andrews 1987, Forsberg 1965) and thus may be used as indicator plants for pollution analyses. Some studies indicate that they concentrate heavy metals and may be of value in purification systems. An extensive review of their life histories can be found in Groves and Bullock-Webster (1920), Fritsch (1956), and Wood and Imahori (1965).

Charophytes in Mountain Lake: There are six extant genera of characeous algae: Chara, Lamprothamnium, Nitellopsis, Lychnothomus, Nitella and Tolypella. Only Nitella and Tolypella have oogonia with two tiers of five coronal cells (Bold 1978). Both of these genera occur in North America (Tindall, et al. 1965). Nitella flexilis (L.) Ag. and the single tiered forms, Chara braunii Gm. (Dubay 1976) and Chara schweinitzii A.

Br. (Whitford 1964; Obeng-Asamoa 1971), have been reported in Mountain Lake.

N. flexilis is described by Whitford and Shumacher (1973) and Edmondson (1963) as being uncorticated, green pigmented and filamentous in appearance and having many branches. The branches are in whorls of five to six (Wood and Imahori 1964, 1965). Current authorities recognize N. flexilis as monoecious (Sawa 1965; Proctor 1971), though Wood and Imahori (1964, 1965) placed N. flexilis as conspecific with the dioecious Nitella opaca. Vernon Proctor and F.K. Daily (pers. comm.) have pointed out problems caused by Wood and Imahori (1964, 1965) and both have placed the two species as not conspecific. F.K. Daily commented that the protandry (advanced antheridial development) reported by Hutchinson (1975 b) and others may be one cause for confusion in identification. Chromosome number distinctions have been made between the two by Tindall and Sawa (1964) and Guerlesquin (1984) with N. flexilis having n = 12 where N. opaca has n = 6. Dubay and Simmons (1981) spent some time in discussing differences in an attempt to resolve the taxonomy of the dominant macrophyte in Mountain Lake.

N. flexilis was first reported in Mountain Lake by Forest (1954). Subsequent investigators have either mentioned N. flexilis specifically or have noted the presence of Nitella beds. Dubay (1976) and Dubay and Simmons (1979, 1981) focused mainly on the species of Nitella and attempted to determine whether N. flexilis or C. braunii made up the dominant macrophyte in the lake. They concluded that N. flexilis was the

dominant macrophyte with small amounts of other macrophytes occasionally present. The only other mention of a charophyte was by Whitford (1964) and Obeng-Asamoa (1971) who reported C. schweinitzii as a very minor constituent of the macrophyte community. Table 1 presents a summary of the emphases of previous studies.

The purposes of the current study were:

1. To verify the presence of Nitella flexilis (L.) Ag. in Mountain Lake;

2. To determine variations of morphological characteristics associated with depth through analysis of internodal cell length, chlorophyll and carotenoid content, and branching patterns;

3. To measure current biomass distribution associated with depth and in relation to the previous study (Dubay 1976);

4. To estimate primary productivity of Nitella and analyze changes in productivity as depth increases;

5. To formulate an overall picture of the ecological role of the species of Nitella in Mountain Lake.

The following hypotheses or predictions were made:

Table 1. Summary of work of previous investigators of Mountain Lake.

Investigator(s)	Year	Emphasis
Hutchinson and Pickford	1932	limnology
Coker and Hayes	1940	phytoplankton
Forest	1954	algae list
Roth and Neff	1964	limnology
Whitford	1964	phytoplankton
Marland	1967	paleontology
Obeng-Asamoa	1971	phytoplankton
Obeng-Asamoa and Parker	1972	phytoplankton
Simmons and Neff	1973	phytoplankton productivity
Parker, Wolfe and Howard	1975	geology
Parker	1976	trophic state
Dubay	1976	macrophytes
Dubay and Simmons	1979,1981	macrophytes

1. Based upon preliminary observations of samples taken in 1986 and upon a review of the literature, Nitella megacarpa (T. F. A.) em. R.D.W. and not Nitella flexilis occurs in Mountain Lake. The prediction is that the identification of N. flexilis is incorrect.

2. There will be a difference in internodal cell length, pigment content, and overall branching pattern as depth increases of N. megacarpa due to differences in light intensity or temperature.

3. Since Mountain Lake has changed slightly during the ten years since Dubay's (1976) biomass study (Parson pers. comm.), the prediction is that there has been a change in the biomass distribution since Dubay's study.

4. Primary productivity will vary with depth.

5. Primary productivity will be at a maximum at the depth associated with maximum biomass due to changes in temperature and light.

SECTION 2

SPECIES IDENTIFICATION

Introduction: Wood and Imahori (1965) briefly discuss their reasons for the major revision of Charophyte classification. Since this group is an extensively studied one, and since the revision was taxonomically reductionist, many species were lumped. The result has been a confusion of nomenclature caused by the proliferation of polynominal names. Classification and identification are still in an active state of flux because of the earlier revision and the more recent collections of newer taxonometric ultrastructural data and cytological information.

Materials and Methods: Samples were collected from two to ten meters using both an Ekman dredge (1986 collections) and divers in SCUBA (1987 collections). Initial collections did not conform to the descriptions of N. flexilis. Thus, additional samples were taken for morphological analysis. In 1986 samples were preserved in FAA and sent to Dr. F. K. Daily at the Cryptogamic Herbarium of Butler University for taxonomic verification.

Morphological data were collected on samples during 1986 and 1987 and included observations of oogonial dimensions, antheridial diameter, internodal cell width, furcations, dactyls and chromosome number. Measurements were made using an Aus Jena Nomarski filter equipped microscope. Photographs of chromosomes were taken using a Nikon camera attachment.

Chromosome staining on antheridial filaments followed the aceto-carmine method given in Clark (1981). Chromosomal interpretative drawings were made using an 8 x 10 inch enlargement of a 35 mm transparency photomicrograph. Average values for the measurements of the Mountain Lake samples were compared with each other and with other reported ranges or values where available from Daily (1953), Allen (1954), Wood and Imahori (1964, 1965) and Tindall and Sawa (1964).

Discussion: All sexually reproductive Nitella specimens taken from Mountain Lake were monoecious although frequently antheridial development preceded the oogonial development. This is protandry as reported by Robinson (1906), Groves and Bullock-Webster (1920), and Hutchinson (1975 b). All specimens were typical of N. megacarpa (T. F. A.) em. R. D. W. after N. furcata subsp. megacarpa.

Morphological data presented in Tables 2 and 3 indicate an overlap of ranges for several characteristics. The chromosome number observed was n = 18 (Figures 1 and 2). This is consistent with that reported previously for N. megacarpa, but not for N. flexilis (Tindall and Sawa 1964; Bhatnagar and Bhatnagar 1978; Guerlesquin 1984; Khan and Sarma 1984; Wood and Imahori 1965). Nitella collected from six meters and below lacked sex organs but resembled sterile N. megacarpa but not N. flexilis in dactyl furcations. This reflects morphological variations with depth reported by Kashimura (1960) and Forsberg (1965) for related species.

The large chromosome number reported by Wood and Imahori (1965) for N. megacarpa, n= ca. 30, may indicate that they examined a polyploid sample or that they did not observe complete separation of replicated chromosomes. Such a high number for this species has not been reported elsewhere.

Voucher samples collected by Dubay (1976) and deposited in the herbarium of the University of Virginia's Mountain Lake Biological Research Station were located, but lacked detectable sex organs. Vegetatively, these specimens resembled my collections of N. megacarpa. Voucher samples from my collections, prepared according to Dewees and Hooper (1975), are on file with the herbarium of Virginia Polytechnic Institute and State University in Blacksburg.

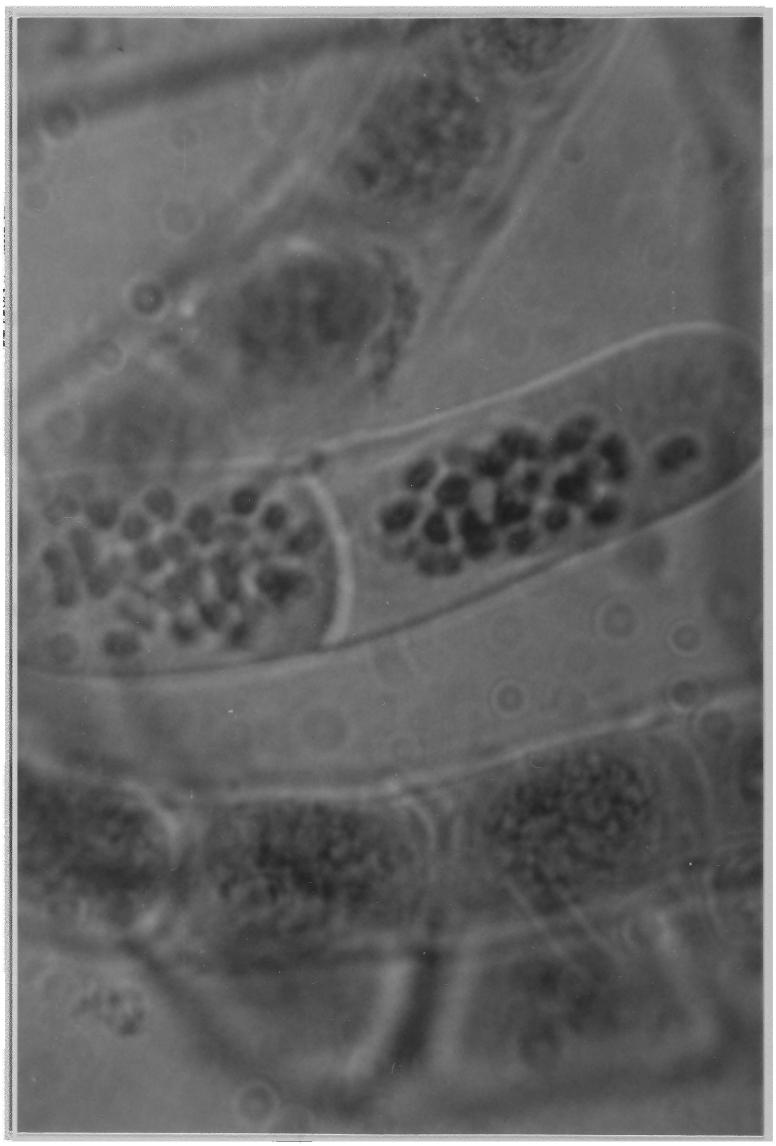

Figure 1. Photomicrograph of antheridial filaments stained to show chromosomes. Bar = 1 micrometer.

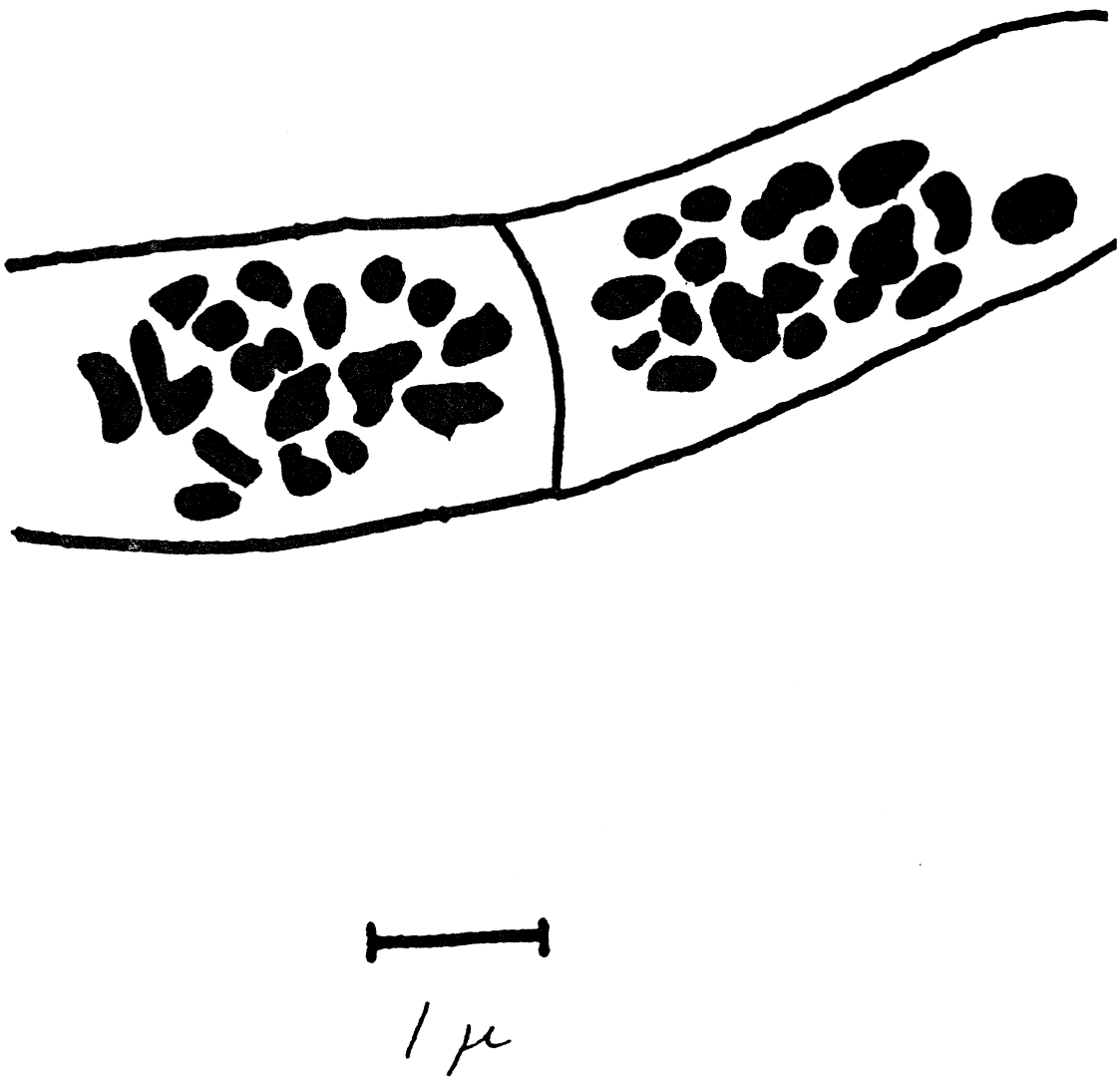

Figure 2. Interpretative drawing of Figure 1 showing chromosome number n = 18.

Table 2. Means ± 1 standard error for morphological characteristics measured on Nitella megacarpa from Mountain Lake for two sampling years. All measurements are in micrometers.

Characteristic	1986	n	1987	n
Oogonium width	----		387 ± 13	20
Oogonium length	432 ± 8.2	45	503 ± 16	20
Internodal length	445 ± 7.8	60	397 ± 7.1	100
Antheridium dia.	288 ± 2.7	60	314 ± 3.2	84
Oospore length	529 ± 7.3	53	469 ± 3.0	45
Oospore width	505 ± 7.7	53	377 ± 4.0	45

Table 3. Comparison between the mean values of 1986 and 1987 samples and values reported from the literature. All dimensions in micrometers. (L) = length, (W) = width. Numbers in () are for N. flexilis, all others are for N. megacarpa.

	Allen	Daily	Tindall & Sawa	Wood & Imahori	1986	1987
Int. cell w.				to 1700 (500-1000)	445 ± 7.8	397 ± 7.1
Ant. dia.	400	265 (504-630)	(540-610)	240-285 (555-580)	288 ± 2.7	314 ± 3.2
Oosp. l.	370-450 (525)	370-400 (420-580)	(380-440)	375-405 (445-525)	529 ± 7.3	469 ± 3.0
Oosp. w.	(450)	318-368 (400-445)	(350-430)	345-360 (420-435)	505 ± 7.7	337 ± 4.0
Oogo. l.				480-510 (695-780)	432 ± 8.2	503 ± 16
Oogo. w.				360-435 (585-630)		387 ± 13
n			18(12)	ca.30 (12)		18

SECTION 3

MORPHOLOGICAL VARIATIONS WITH DEPTH

Introduction: Charophytes, like many other plants, exhibit phenotypic plasticity. Variations in morphology as a result of variations in light intensity or spectral quality, temperature, or nutrient concentrations and pH for charophytes have been observed by others. In my study, I investigated internodal cell length, a characteristic frequently reported to change with depth (Forsberg 1965; Kashimura 1960), although not previously for N. megacarpa.

Materials and Methods: Samples were collected using an Ekman dredge (1986) and divers in SCUBA (1987). These samples were rinsed in lake water at the site, placed in plastic bags, and transported back to the laboratory on ice in the dark. At the laboratory observations of presence or absence of antheridia and oogonia or oospores, furcation frequency, and internodal cell length were made. Internodal cell length was measured to the nearest whole millimeter using a hand held ruler. Photographs were made using a Yashica FX-2 35 mm camera and either Ektachrome 400 or 160 ASA or Kodachrome 60 ASA transparency film and Hoya Macro-lens attachments.

Discussion: It was noted earlier that sexually mature Nitella megacarpa was not found below six meters. This observation is consistent with those of Groves and Bullock-Webster (1920) and Karling (1924) who noted that

Nitella spp. do not fruit in deep waters. One objective of this study was to determine if other characteristics of Nitella megacarpa in Mountain Lake varied with depth.

Choudhary and Wood (1973), Starling et al. (1974), and Hutchinson (1975 b) noted that the form of a species is highly variable in vegetative parts. Within one aquatic system, one can find diffuse forms or compact forms, depending upon the depth at which they are growing. Figures 3 and 4 illustrate two forms of Nitella found growing in Mountain Lake. The shallow water form is consistently more furcate than the deeper water form.

Forsberg (1965) investigated the influence of red and blue light on the growth of internodal cells in Chara spp. from Swedish lakes. Kashimura (1956, 1960) investigated the influence of light on internodal cell elongation of N. flexilis from Japanese lakes. Stross (1979) reported variations in internodal cell length in N. flexilis with depth in Lake George, New York. All concluded that in deeper depths, in dimmer light, and in the case of Forsberg (1965) in red light, the internodal cells were longer. Forsberg's (1965) observations concerning red light were made on sprigs allowed to grow under filters of blue and red. He admitted that his red filters allowed an excess of far red light to enter the growth chamber, and thus may have caused spurious results.

In Mountain Lake, internodal cell lengths increased with depth in all cases except May 1987, when there was little difference with depth (Table

4). Sediments collected in May contained large numbers of newly germinating oospores suggesting that Nitella was just beginning to develop. Nitella sufficiently mature to have measurable internodal cells probably overwintered in a vegetative form. Dubay (1976) noted greatly reduced biomass in winter months. Hutchinson (1975 b) reported that Nitella spp. starts growth and active reproduction in May. My observations are consistent with these.

Although attempts to culture N. megacarpa from more than 100 oospores following procedures of Shen (1971) and Sokol and Stross (1986) were unsuccessful, maintenance of plants in aquaria in Mountain Lake water and sediment was successful. In culture, plants from lower depths, once exposed to higher light intensities and temperatures, showed development of variations in morphology. Newly formed internodal cells were shorter and accompanied by numerous stubby branchlets. This reduction in the length of the internodal cells corresponds with my observations of shallow water forms in Mountain Lake as well as with observations reported by Kashimura (1960) who reported experimental induction of side shoot development at higher light intensities. Figure 5 illustrates this growth habit of plants maintained in an aquarium.

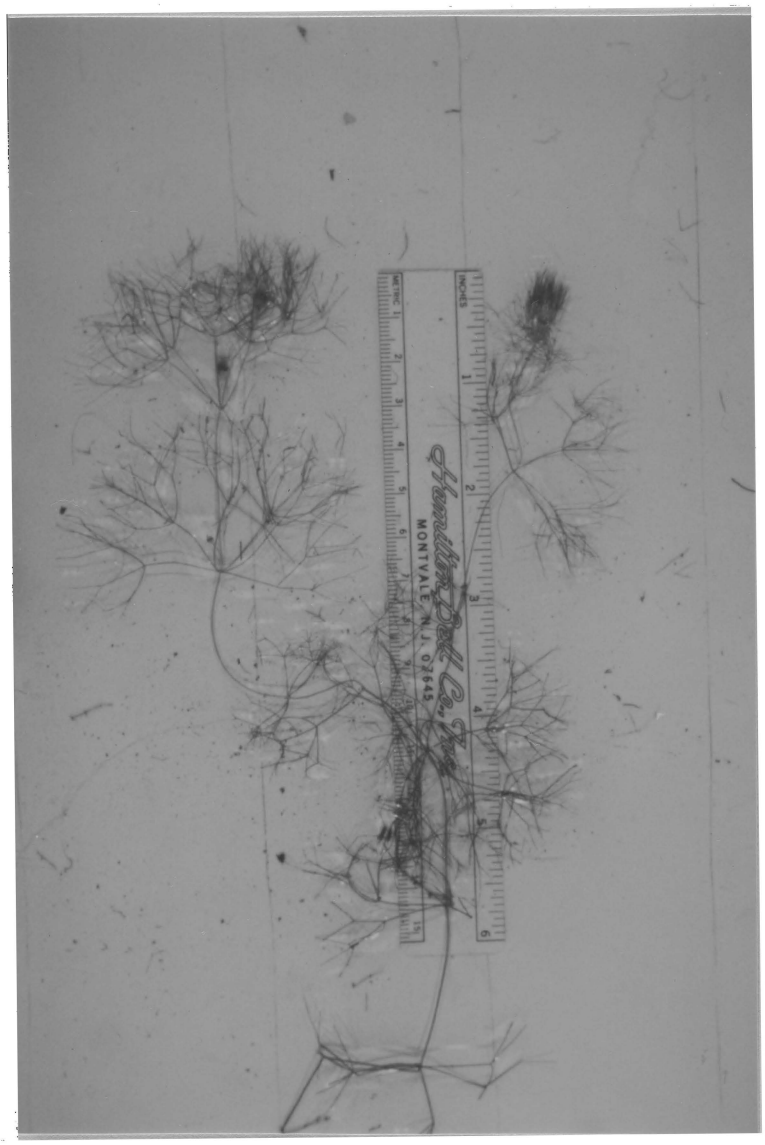

Figure 3. Shallow water growth form of N. megacarpa showing high degree of furcation and branching characteristic of high light intensities.

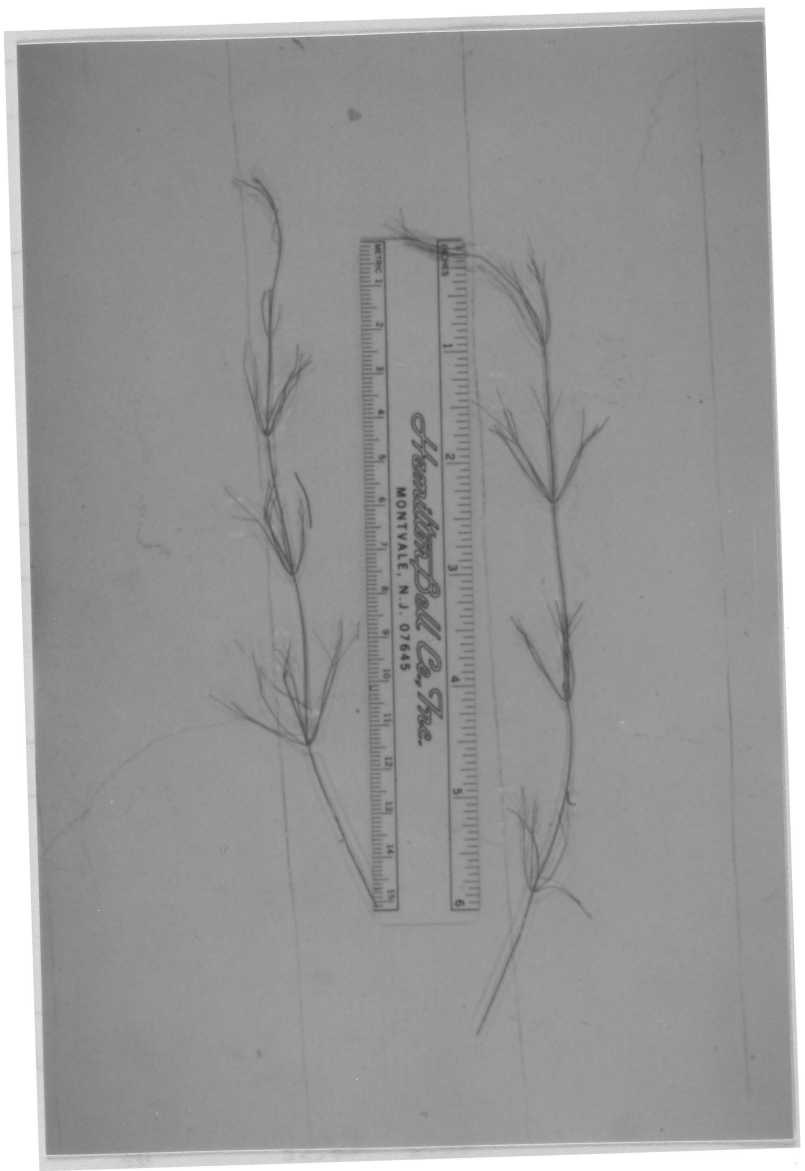

Figure 4. Deep water growth form of N. megacarpa showing reduced frequency of furcation and branching and longer internodal cells characteristic of dim light.

Table 4. Internodal cell lengths of Nitella megacarpa in Mountain Lake. Measurements are in mm. Means ± 1 s.e. Depth is in meters.

DATE	AUG 86	
DEPTH	n	MEANS
2.5	18	21.89 ± 1.43
3.5	20	29.35 1.50
4.5	20	30.70 0.97
5.5	20	40.83 2.30

DATE	SEPT. 86	
DEPTH	n	MEANS
2.5	30	20.50 ± 1.61
3.5	50	31.62 1.06
4.5	50	35.06 0.86
5.5	50	41.18 1.21
6.5	50	41.96 2.75
7.5	50	36.80 1.81
8.5	50	46.36 2.75

DATE	MAY 87	
DEPTH	n	MEANS
3.5	20	33.55 ± 2.42
4.5	40	32.85 1.33
6.5	80	33.92 0.87
7.5	100	32.52 0.67
8.5	80	38.36 0.94

DATE	JULY 87	
DEPTH	n	MEANS
2.5	20	16.15 ± 0.61
4.5	20	25.00 1.88
5.5	20	45.00 1.86
6.5	20	42.65 1.44
7.5	20	43.75 2.55
8.5	20	46.70 1.64
9.5	20	58.40 1.93

DATE	SEPT. 87	
DEPTH	n	MEANS
2.5	50	12.98 ± 0.58
3.5	50	28.36 0.67
4.5	30	29.67 0.99
5.5	40	52.67 2.08
6.5	50	39.92 1.15
8.5	50	59.12 1.64

Figure 5. Growth form of a sample from 5 meters depth held in an aquarium in bright light and at room temperature. Note short internodal cells and stubby branches, which developed after transplanting to the laboratory.

SECTION 4

PIGMENT VARIATIONS

Introduction: Variation in pigment content in a plant can be environmentally related. Light and temperature are especially important in influencing variation. Pigment content is often used as an indicator of biomass and photosynthetic potential. Since charophytes respond to changes in light and temperature by changes in morphology, I was interested in possible changes in pigment content as depth, and therefore light and temperature, changed.

Materials and Methods: Plants were collected using an Ekman dredge (1986) and divers in SCUBA (1987) and were placed in plastic bags, sealed, and placed on ice until return to the laboratory. At the laboratory, subsamples of 2 to 3 centimeters of apical tips were taken and processed according to Vollenweider (1969) and Littler and Littler (1985), i.e. subsamples were blotted dry, weighed, and then pigment was extracted with basic acetone following Standard Methods (1985). Calculations for chlorophylls followed Standard Methods (1985). Carotenoid calculations followed Andrews *et al.* (1984). Remaining sample material was used for internodal cell measurements, voucher sample preparation, or morphological measurements. Carotenoids were calculated from: C=(4 x A x V)/M where C=total carotenoid pigments in milli specific units per gram fresh weight which approximates milligrams per gram fresh weight, A= absorbance at 480 nm, V=volume of 90 % acetone used in extraction, and

M=mass fresh weight in grams. Chlorophyll calculation formulas were those of Standard Methods (1985).

Discussion: Factors influencing the chromatic adaptation of algae have been reviewed for marine algae by Dring (1981) and for freshwater Charophyta by Ramus et al. (1976) and Andrews et al. (1984). Their general conclusions are that amounts of chlorophyll a and b, the two present in Nitella according to Bold (1978), may change in these ways:

1. Total pigment content increases with depth.

2. Ratio of pigments changes (a/b decreases) with depth.

Pigment content changed with depth changes (Table 5). Chlorophyll a and b both increased slightly as depth increased down to a depth corresponding to maximum biomass development. Below the depth of maximum biomass concentration, pigment content decreased. The ratio of a/b increased along the same pattern as the pigments. The clear exception is the month of July 1987. This month the chlorophyll a follows the expected pattern, chlorophyll b drops consistently, and the ratio rises consistently.

The patterns observed in Mountain Lake are contrary to those seen elsewhere. In Mountain Lake, there is no consistent increase in chlorophyll b content; therefore, the ratio of a/b does not consistently decrease with depth as observed elsewhere.

Alternative reasons for this pattern of chromatic adaptation may be those suggested by Andrews et al. (1987) and Barko and Filbin (1983):

1. Since lateral branchlets are the main sites of photosynthesis, they will be more abundant in plants in areas with maximum photosynthetic potential (Andrews et al. 1987).

2. Chlorophyll a + b content is closely associated with light, but is also influenced by temperature gradients which may promote patterns of adaptation opposite those expected from light alone (Barko and Filbin 1983).

Temperature data collected by Marolyn Parson (1987 in preparation) in this laboratory, suggest a coincidence between maximum Nitella chlorophyll and biomass and the metalimnion in Mountain Lake. Additionally, observations of branching and furcations, mentioned earlier, also indicates that surface area is larger in plants growing in shallow water. These two factors may account for the pattern in chromatic adaptation with depth observed in Nitella.

Two other factors which may be adaptations to decreased temperature and light mentioned by Andrews et al. are a reduced rate of dark respiration and an increase in the amount of accessory carotenoid pigments. No investigation of respiration was made in the present study. Carotenoids were only measured in the September 1987 samples (Table 6), but show a pattern

of change associated with depth, i.e. decrease with depth to approximately maximum biomass depth, then increase.

Table 5. Chlorophyll concentrations of *Nitella megacarpa* in Mountain Lake by depth. Values are in mg/g fresh weight ± 1 standard error.

JUNE 1987 DEPTH		Means		
	n	A	B	RATIO A/B
3.5	9	.327 ± .005	.070 ± .002	4.75 ± .159
4.5	3	.511 .012	.121 .004	4.22 .039
5.5	3	.481 .006	.123 .001	3.92 .039
6.5	3	.288 .009	.053 .002	5.47 .049
7.5	3	.507 .008	.116 .004	4.35 .079
8.5	3	.440 .015	.090 .004	4.93 .058

JULY 1987 DEPTH		Means		
	n	A	B	RATIO A/B
3.5	9	.367 ± .011	.096 ± .003	3.83 ± .054
4.5	3	.487 .005	.144 .003	3.38 .027
5.5	3	.322 .007	.123 .002	2.62 .014
6.5	3	.378 .008	.092 .002	4.13 .018
7.5	3	.450 .008	.120 .002	3.75 .032
8.5	3	.477 .023	.115 .007	3.90 .042

AUGUST 1987 DEPTH		Means		
	n	A	B	RATIO A/B
2.5	3	.551 ± .079	.171 ± .026	3.22 ± .038
3.5	3	.484 .016	.147 .004	3.28 .034
4.5	3	.682 .036	.187 .010	3.65 .028
5.5	3	.377 .102	.112 .032	3.37 .052

JULY 1987 DEPTH		Means		
	n	A	B	RATIO A/B
2.5	4	.815 ± .092	.231 ± .025	3.52 ± .066
4.5	4	.849 .061	.216 .009	3.92 .201
6.5	6	.854 .087	.218 .022	3.93 .070
8.5	2	.704 .066	.173 .015	4.07 .035
10.5	2	.655 .066	.169 .027	3.91 .236

SEPTEMBER 1987 DEPTH		Means		
	n	A	B	RATIO A/B
2.5	3	.636 ± .102	.208 ± .042	3.09 ± .120
3.5	3	.611 .059	.181 .014	3.37 .088
4.5	3	.357 .040	.106 .013	3.36 .089
5.5	3	.591 .062	.159 .023	3.80 .044
6.5	3	.644 .017	.187 .003	3.44 .103
7.5	3	.641 .044	.175 .017	3.71 .333

Table 6. Mean carotenoid pigment content of Nitella megacarpa in Mountain Lake in milli specific pigment units per gram fresh weight ± 1 standard error. Measured and calculated after Andrews et al. (1984).

SEPTEMBER 1987 DEPTH	n	CAROTENOIDS MILLI SPECIFIC PIGMENT UNITS PER GRAM FRESH WEIGHT Means
2.5	3	197 ± 32.3
3.5	3	147 ± 11.5
4.5	3	92 ± 9.8
5.5	3	146 ± 16.2
6.5	3	162 ± 8.08
7.5	3	172 ± 13.3

SECTION 5

BIOMASS DISTRIBUTION

Introduction: Nitella spp. have been shown to respond to changes in nutrient content of sediments or water. The plants are especially sensitive to changes in phosphorus concentrations (Starling et al. 1974, Andrews 1987). They respond by slowing growth rate, by producing abnormally formed cells, or by dying. Parson (pers. comm.) has indicated that her study suggests that the nutrient quality of Mountain Lake has changed since 1971. I was interested in seeing if the Nitella biomass has changed since Dubay's (1976) study.

Materials and Methods: Dubay (1976) first reported on the biomass distribution of macrophytes in Mountain Lake and identified the dominant species as Nitella flexilis (L.) Ag.. His survey sampled along transects shown in Figure 6. In the summer of 1986, preliminary surveys along his transects indicated that transects 1 and 5 contained little, if any, Nitella. Dubay's (1976) transects 2, 3, and 4 were sampled on every collection date in my study. Transects 1 and 5 were sampled on three occasions, but yielded no Nitella. Consequently, my study concentrated on the transects which contained the greatest biomass from Dubay's (1976) study (i.e. transects 2, 3, and 4).

The procedures used here are essentially those described by Westlake (1969). Samples were collected in 1986 with an Ekman dredge and in 1987

by divers in SCUBA. For diver collected samples a 20 x 20 cm grid was placed on the bottom at the appropriate depth and the area within the grid was cleared by hand. Samples were rinsed in the field, placed in plastic bags and stored on ice until return to the laboratory. In the laboratory, each bag's contents were placed in a No. 40 mesh Hubbard sieve and rinsed clean with runnng tap water. Other debris was removed by hand at this time.

Samples were either placed directly into tared beakers or, if fresh weights were to be determined for a dry weight conversion factor as shown in Appendix D, blotted dry and placed into tared beakers. Samples were dried at 100 Celcius for 24 hours and weighed. Subsamples were ashed at 500 Celcius temperature in a muffle furnace for 5 hours and ash weight was determined. For blotted samples, wet weights were determined prior to drying and ashing (Appendix D).

Ash free dry weights (AFDW) were calculated by substracting ash weight from dry weight. Values for all samples collected at given depths were averaged to get the mean AFDW / square meter at each depth. All transects were pooled, since I was only concerned with the depth relationships of the biomass distribution and not the transect distribution.

Discussion: Maximum biomass is found along transects 2, 3, and 4. The positions of the transects used in my study are shown in Figure 6. No Nitella was recovered from the area of transect 1 and only one small piece was recovered from transect 5.

My data indicate that there has been a reduction in biomass of Nitella since 1976 (Table 7). The maximum biomass is less than 30 % of the maximum value recorded by Dubay (1976). Additionally, the depth of maximum concentration is less predictable and usually shallower than the six meters reported by Dubay (1976) and Dubay and Simmons (1979, 1981). Maximum biomass development is now between four and six meters for most of the study period. No Nitella was recovered from deeper than 10 meters. Dubay and Simmons (1981) reported growth down to 11 meters.

Dubay (1976) reported a maximum of 356.2 grams AFDW / square meter Nitella biomass in Mountain Lake. My values parallel those from Stross (1979) for Lake George, New York (175 g dry wt. / square meter) or by Likens (1985) for Mirror Lake, New Hampshire, (60 g dry wt. / square meter). Changes in biomass over long time periods have also been reported for an English lake by Macan (1977) and for Lake George, New York by Stross (1979).

Furthermore, although not quantitatively assessed, the SCUBA collections disclosed a major increase in the biomass of Ceratophyllum sp. in Mountain Lake. This genus was found previously in only one Dubay and Simmons (1981) collection in a very small amount.

Figure 6. Transects on Mountain Lake map indicating sites of my collections.

Table 7. Depth distribution of Nitella megacarpa as mean ±1 standard error biomass in Mountain Lake summer 1986-1987. Values are means of samples collected from 3 transects on the south, east, and west of the lake basin.

September 1987 DEPTH	n	AFDW/m^2
2.5	4	12.89 ± 4.32
3.5	9	10.10 ± 3.10
4.5	18	14.70 ± 2.28
5.5	8	16.33 ± 4.36
6.5	7	17.61 ± 6.09
7.5	2	1.72 ± 1.11
8.5	4	5.16 ± 2.21

May 1987 DEPTH	n	AFDW/m^2
3.5	1	6.34 ± 0
4.5	2	21.40 ± 5.12
6.5	4	14.32 ± 7.11
7.5	4	10.35 ± 3.39
8.5	1	12.92 ± 0
9.5	4	4.70 ± 2.16

July 1987 DEPTH	n	AFDW/m^2
2.5	1	3.94 ± 0
3.5	2	2.48 ± 1.10
4.5	2	30.34 ± 26.7
5.5	2	30.91 ± .28
6.5	2	32.31 ± 8.12
7.5	2	79.51 ± 45.1
8.5	2	20.74 ± 7.52
9.5	2	7.30 ± 5.55

September 1987 DEPTH	n	AFDW/m^2
2.5	3	10.59 ± 2.37
3.5	4	41.86 ± 25.5
4.5	4	42.20 ± 10.5
5.5	4	29.05 ± 13.2
6.5	3	100.11 ± 88.3
7.5	5	25.51 ± 5.59
8.5	1	9.47 ± 0
9.5	1	5.89 ± 0

SECTION 6

PRIMARY PRODUCTIVITY

Introduction: Stross (1979) reported measuements of 5 mgC fixed / gram dry weight / hour for N. flexilis under laboratory conditions. No reports of primary productivity of N. megacarpa have been made prior to my study, and no reports of in situ measurements on any charophyte have been made. Since net carbon fixation rates determine biomass and are related to chlorophyll content, I assessed primary productivity at different depths in Mountain Lake.

Materials and Methods: I measured in situ primary productivity levels of Nitella to determine whether there was variation with depth. Littler and Littler (1985) reviewed methods for measurements with macrophytic algae and it is their calculation technique which is used:

$$P = (S \times C \times V \times 1.05)/(W \times B \times H)$$

where P=primary production in mgC / g dry weight/ hour, S=corrected sample activity in disintegrations per minute, C=available carbon dioxide in mg/L determined from pH and total alkalinity, V=volume of incubation vessel in L, 1.05 is the isotopic discrimination constant, W=activity

added in disintegrations per minute, B=mass dry weight in grams incubated material, and H=time of incubation in hours before fixing samples.

Steeman-Nielsen (1952) introduced methods for measuring primary productivity by uptake and fixation of radioactive carbon (C-14). The basic method is recognized to contain numerous sources of errors. Modifications of the basic method to lessen the effect of various errors have been made (Parker and Samsel 1974; Parker and Parson 1987). The method used here is a modification of one developed by Parker and Parson (1987). A modified diagram of their procedure is given below.

Measurements were made in May 1987 to determine if uptake was linear over time for apical portions removed from Mountain Lake. Since damage occurs when the sprigs are removed from the thallus, the possibility exists that the plant may have an immediate decrease in uptake or an abrupt shut down due to stress. Samples from four meters were collected and processed, except they were suspended at four meters and fixed after 2, 4, and 6 hours.

Measurements were made in July and September 1987. Samples were removed by divers from 3.5, 5.5, and 7.5 meters in July and from 3.5, 5.5, 6.5, and 7.5 meters in September. In both cases they were incubated for four hours at depths of collection. Results are summarized in Table 8.

Discussion: Measurements from May indicate a linear relationship ($r = .94$ with $p < .05$) for uptake over time. This suggests that minimal aber-

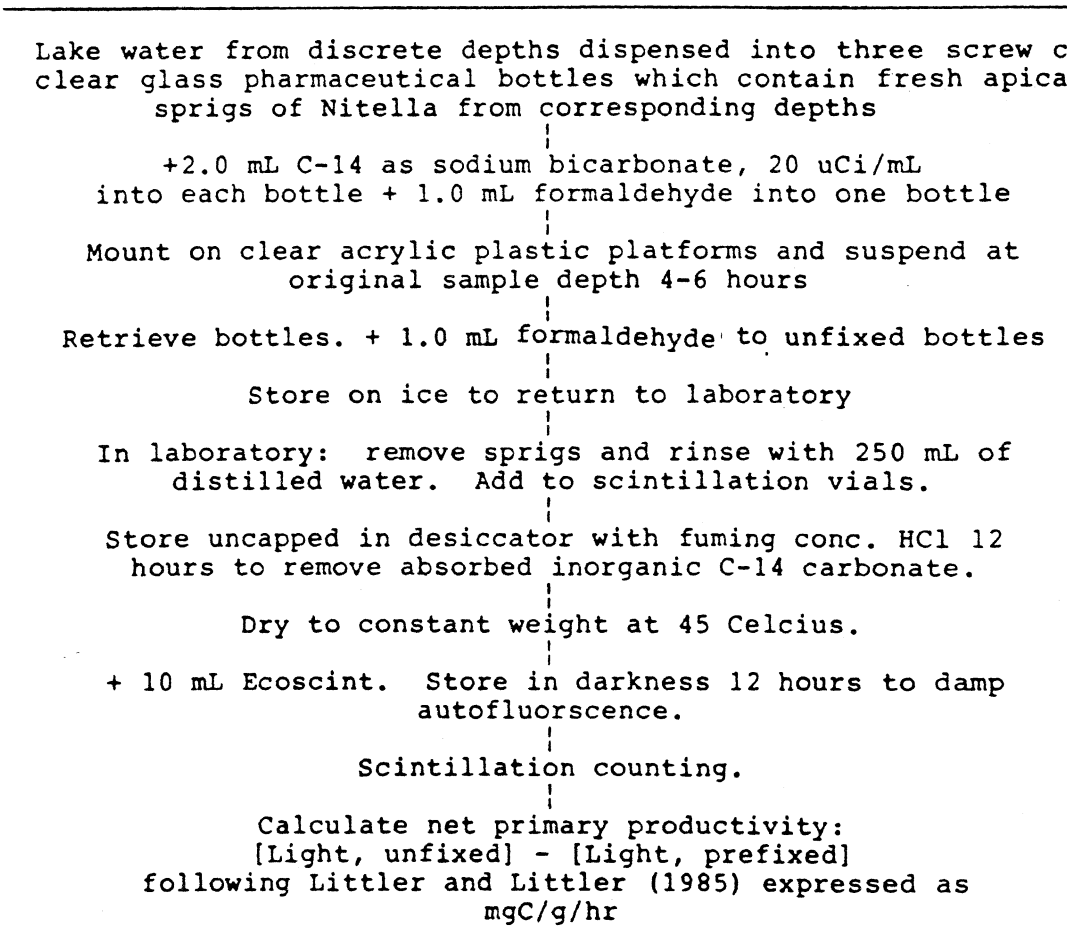

Figure 7. Flowchart procedure for primary productivity modified after Parker and Parson (1987).

Table 8. Summary results of primary productivity measurements of Nitella megacarpa in Mountain Lake.

May 1987 Depth = 4 meters, surface light average 752 µE m^{-2} sec^{-1}	
Time	mgC/g/hr
2	.062 ± .01
4	.072 .02
6	.086 .005

July 1987 Time = 4 hours, surface light average 143 µE m^{-2} sec^{-1}	
Depth	mgC/g/hr
3.5	.008 ± .008
5.5	.053 .07
7.5	.004 .0004

September 1987 Time = 4 hours, surface light average 111 µE m^{-2} sec^{-1}	
Depth	mgC/g/hr
3.5	.001 ± .0004
5.5	.002 .0002
6.5	.004 .000012
7.5	.001 .00020

rations occur over time for the time period studied. The median time of four hours was chosen for future incubations.

July and September measurements indicate a correspondence between depth of maximum uptake (photosynthetic activity) and biomass. Although light levels, measured with a LiCor 185B photometer, were quite low at the surface because of the cloud cover, and thus at of incubation also, the pattern of production is obvious. However, the values are quite low. This may have been a stress related depression in uptake, which, though not causing aberration over the time observed, may have caused an abnormally low rate after removal from the substrate. Alternatively, the apical sprigs which are actively growing may also be actively recycling fixed carbon-14 through respiration.

Note that the highest productivity rates were at 5.5 and 6.5 meters during the July and September experiments. These depths approximate those at which Nitella was at maximum biomass.

Stross (1979), in his laboratory measurements, used larger sections of N. flexilis, under light intensities of 7500 lux and 18 Celcius, with constant rotation, complete combustion of plant materials and recovery of carbon dioxide gas for counting. He reported rates as high as 5 mg C fixed / gram dry weight / hour. He explained that he used incubation conditions which maximized productivity. Thus, comparisons between his laboratory resluts and my field results may not be valid.

SECTION 7

CONCLUSIONS

1. The species of Nitella present in Mountain Lake is Nitella megacarpa (T. F. A.) em. R. D. W. after N. furcata subsp. megacarpa. The dactyls and furcation pattern, the overlap of morphometric data, and especially the chromosome number suggest this conclusion. It is probable, considering the confusion created by Wood and Imahori (1964, 1965) as discussed by Proctor and Daily (pers. comm.) that the earlier identifications by Forest (1954), Dubay (1976) and others were incorrect. In fact, there is no evidence that these earlier investigations examined sex organs or attempted to ascertain whether a monoecious species other than N. flexilis existed in Mountain Lake.

2. Morphological characteristics vary with depth. A Model I Analysis of Variance for differences in mean internodal cell lengths as depth increased showed significant variation ($p < .05$). The variation in branching patterns, obvious from Figures 3 and 4, indicate other variations with depth. These seem likely to be in response to differing light intensities and /or spectral distributions. The change in internodal cell length and the furcation formation in plants held in an aquarium (Figure 5) indicate that future investigators may be able to determine experimentally what relationships exist between light intensity and internodal cell length. It is possible that this pattern of change in internodal cell length and furcation frequency

may indicate the presence of two populations of Nitella megacarpa in Mountain Lake. One shallow water population, sexually reproductive and seasonal, disappears in fall and begins to reestablish in May. Between 2 to 4 m, germinating oospores were commonly observed. This shallow population is adapted to warm temperatures in the stratified lake epilimnion each summer, but the population may be intolerant of cold temperatures which develop by November overturn. The other population is a long celled, vegetatively reproducing deep water form found below six meters depth. This deep population may be perennial and adaptated to constant low light intensity and temperatures ranging between 4 and 12 Celcius.

3. The pigment content varies with depth, although not consistently. A Model I Analysis of Variance for differences between the means of the pigment content as depth increased showed a significant difference ($p < .05$) evident in June and July 1986 for chlorophyll a and b as well as the ratio a/b in August 1986 and in chlorophyll a and total carotenoids for September 1987. On all other sampling dates, there was no significant difference ($p > .05$) in pigment measurements. There was a change, though not statistically significant, in chlorophyll a and b content and the ratio a/b on other sampling dates. This is likely in response to the effects of both temperature and light intensity. Spectral distributions in Mountain Lake need to be measured in order to determine the possible relationships between these factors. The erratic nature of these pigment changes reflects those reported for C. hispida reported by Andrews et al. (1984).

4. The biomass of Nitella in Mountain Lake is much lower than previously reported by Dubay (1976). However, there is not a statistically significant difference associated with depth as measured with a Model I Analysis of Variance. Long term changes in macrophyte denisities and distributions have been previously reported by Macan (1977) for other lakes. These changes may be in reponse to changes in the nutrient content of the water (currently under investigation by Marolyn Parson) or the substrate (see Appendix B).

5. The estimates of primary productivity for Nitella megacarpa are low. The depth of maximum carbon fixation is between 4.5 and 6.5 meters. These correspond with depths of maximum biomass and with peaks in the concentrations of chlorophyll a and b. Production is quite low. This suggests three possibilities:

   a. The phytoplankton may contribute more significantly to the oxygen production than Nitella;

   b. The rate of production of Nitella measured here is artifically low because of stress to the plants when the apices were removed causing partial shut down of photosynthesis; such would have to be uniform, because experiments over time show no adverse changes in primary productivity rates;

   c. The rate of Nitella primary productivity is correct.

6. Observations of the oxygen content of the metalimnion (see Appendix A), from data collected by Marolyn Parson, indicate that there is still supersaturation of the metalimnion. Dubay (1976) sought to link Nitella biomass distribution to this metalimnetic oxygen maximum (MOM). Nitella megacarpa has decreased in biomass, yet this supersaturation remained at the same basically low level as reported by Dubay and Simmons (1979) (i.e. between 102 and 115 % saturation) and even went as high as 124 % on one date, August 1987. This persistence of the supersaturation in 1986 and 1987 at the same time that a decrease in the biomass is observed suggests that the hypothesis that the two are linked is not correct. Indeed, Hutchinson (1975 a) discussed physical factors which also influence MOM. Cold water with a high percentage saturation of oxygen, may, if warmed gradually, retain the original oxygen content. However, at the now higher temperature, the previously unsaturated or saturated water will be supersaturated. The gradual warming of Mountain Lake may be responsible for physical supersaturation of the MOM. Since the change in biomass of Nitella is drastic (< 30 % of what was previously reported is now present), and since the MOM still persists, one should look at factors other than the contribution of the macrophytes as a source of the supersaturation.

7. It is possible, as Dubay and Simmons (1979) mention, that attached diatoms can account for some of the oxygen production. Qualitative observations of samples, made while gathering the morphometric data, indicate that the Nitella is a heavily colonized substrate for diatoms

and protozoa (e. g. Gomphonema and Vorticella spp.) as well as a place of attachment for invertebrate eggs. Quite likely the diatom contribution has lessened since there has been a reduction in the biomass of Nitella for colonization. This diatom colonization pattern may also be seasonal and this seasonality may contribute to the MOM seasonal pattern. Since my primary productivity measurements were made on actively growing Nitella tips, these may not reflect the events on heavily epiphytized older stalks. However, the persistence of the MOM even with reduced macrophyte biomass may indicate a stronger physical factor influence than perviously accepted or may be an indicator of physical, phytoplanktonic, or epiphytic factors not previously considered.

It is also possible that Nitella maximum biomass is located in the MOM because it grows best there. Phragmoplastic algae have glycolate oxidase photorespiration. This mode of respiration enables plants to live more efficiently in high oxygen environments by enabling them to convert the oxygen to carbon dioxide (Tolbert 1974). This ability to utilize the higher oxygen of the supersaturated metaliminion gives Nitella an advantage over phycoplastic algae. It may thus be that the MOM is the cause of the higher biomass of Nitella rather than Nitella being the cause of the MOM.

8. During this study, the Chambers-Kalff (1985) equation to estimate maximum depth of charophyte colonization was applied using average

Secchi depths from 1985 - 1987 gathered by Marolyn Parson. The average depth was 6.1 meters. When substituted into the equation:

$$\log (Z) = .87 \log (D) + .31$$

where Z = maximum depth of colonization and D = average Secchi depth, the prediction is that the depth of maximum colonization will be 9.8 meters. The depths of the current collection are within the range predicted by this equation, not exceeding 10 meters.

Overall, one can conclude that there has been a decrease in the biomass of Nitella in Mountain Lake since Dubay's (1976) study. At the same time, there is a pronounced MOM, as observed during Dubay's study. This suggests that the two may not be linked and that MOM is caused by other influences. The Nitella population(s) exhibit morphological and probably physiological variations associated with depth. These may be caused by changes in light intensity and quality, substrate or water nutrient variations (Appendix B), and temperature changes as depth increases. The beds are not as widespread as they once were, evidenced by the paucity of collection materials obtained by divers. It seems that the abundance is more patchy today. The plants which are there have a low productivity as measured here. However, they do produce some oxygen, provide a source of food for grazers and provide a substrate for diatoms and other attached algae (e.g. Bulbochaete sp.) as well as invertebrate eggs.

Mountain Lake, the only natural lake in the unglaciated Appalachians, is changing. All oligotrophic lakes age. It is possible that the changes in Nitella biomass are natural results of the long term changes in the lake such as observed for other lakes by Macan (1977) and Stross (1979). It is also possible that the changes may be the result of activities by the hotel around the lake. Periodic perturbations in phosphate concentrations in the lake have been noted by Dr. Parker (pers. comm.) as a result of leakage from the hotel laundry. Construction at the hotel may have resulted in increased silt deposition on the bottom of the lake. Not only is phosphate essential in growth of charophytes (Andrews 1987), but it has a rather narrow range for growth stimulation, above which it becomes inhibitory or toxic. According to Daily (1953), silt is another factor inhibiting charophytes.

If the changes in Nitella biomass and factors associated with the biomass (i.e. oxygen production, food availability, breeding substrates, etc.) are the result of natural aging of the lake, the observations may be valuable in a longitudinal study of natural lake aging. If the changes are the result of the activities of man, the Nitella may be an indicator of the environmental impact of such activities on the lake. At this point, my study doesn't allow specific interpretations of the cause of the decrease in biomass and allows only speculation concerning the impact of this decrease. It is easy to speculate that decreased biomass means that those organisms which use Nitella for food or breeding will be harmed by the decrease. It is also easy to speculate that the oxygen contribution, however small, made by Nitella will have an impact on all life in the

lake. Since Nitella, as the dominant macrophyte in Mountain Lake, plays a variety of roles in the lake, the impacts of its decrease will be many. Obviously, further study of the use of Nitella by organisms in the lake and of the causes of its decrease need to be done.

SECTION 8

SUMMARY

A. The Nitella in Mountain Lake is Nitella megacarpa (T. F. A.) em. R. D. W. after N. furcata subsp. megacarpa.

B. The Nitella biomass has decreased since Dubay's (1976) study and is now less than 10 % of what he reported.

C. The Nitella morphology varies with depth, i.e. internodal cell length increases with depth and furcations decrease.

D. The Nitella is located where a MOM develops.

E. The Nitella pigment content varies with depth, although not predictably so from a statistical point of view.

F. The Chambers-Kalff (1985) equation to predict the maximum depth of colonization for charophytes is valid for this lake.

G. Based upon the only study of N. megacarpa primary productivity in Mountain Lake, primary productivity is too low to account for MOM.

H. Morphological variations may indicate the existence of two populations of Nitella in Mountain Lake.

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APPENDIX A

OXYGEN SUPERSATURATION

Dubay (1976) and Dubay and Simmons (1979) investigated the relationship between the position of Nitella megacarpa in Mountain Lake and the metalimnetic oxygen maximum (MOM). They and others investigated the hypolimnetic oxygen deficit (Obeng-Asamoa 1971; Roth 1963). As part of her study on the phytoplankton and the trophic state of Mountain Lake, Marolyn Parson gathered oxygen concentration data. The data for 1987 are summarized in Table 9.

These data indicate that, even though the biomass of Nitella megacarpa in Mountain Lake has decreased, the supersaturation of the MOM still persists in the summer months. One would then think that the macrophyte contribution is not important as Dubay (1976) and Dubay and Simmons (1979) concluded.

It is also possible that the change in biomass is associated with a change in the trophic state of Mountain Lake. Thus, in addition to the supersaturation observations, calculations of the hypolimnetic oxygen deficit were performed following methods used by Roth (1963) and Lind (1985) using morphological data from Roth (1963) in Table 10. The result was a value of .009 mg / sq. cm / day. This value is within the range for oligotrophic lakes described by Hutchinson (1975 a). This indicates that the change

in biomass of Nitella is not associated with a change in trophic state as measured by this trophic state indicator.

Table 9. Oxygen concentrations, temperatures, and per cent saturation at selec+ed depths for summer months, 1987.

Depth	May			June		
	t	0	%	t	0	%
.1	18.3	7.55	92	21	7.15	94
1	18.3	7.50	92	21	6.95	91
6	7	10.75	100	9.2	10	102
10	5	9.55	88	6	10.2	95
14	5	8.25	75	5.8	7.7	71.5
18	5	7.40	71	5.8	7.3	53
22	5	6.85	62	6.0	5.5	52
	July			August		
	t	0	%	t	0	%
.1	23	7.85	104	21	7.7	101
1	23	7.85	104	21	7.7	101
6	12	9.2	98	15.5	10.65	124
10	6.3	9.45	89	7	10.4	102
14	5.3	8.1	74	5.5	6.8	63
18	5.3	6.7	61	5.2	6.05	55
22	5.3	4.6	41	5.9	3.65	34
	September					
	t	0	%			
.1	18	8.55	104			
1	18	8.4	104			
6	18	8.1	100			
10	8	10.2	102			
14	5.8	3.45	32			
18	5.2	3.6	33			
22	5.0	2.25	20.5			

Table 10. Morphometric data from Roth (1963).

Maximum Length	870 m
Maximum Effective Length	845 m
Maximum Width	266 m
Maximum Effective Width	344 m
Mean Width	218 m
Maximum Depth	31.5 m
Mean Depth	9.75 m
Mean Depth/Maximum Depth Relation	.310
Maximum Depth/Surface Area Relation	.073
Direction of Major Axis	NE-SW
Area	189,000 m^2
Length of Shoreline	2,160 m
Shoreline Development	1.37
Volume	1,850,000 m^3
Volume Development	.93
Mean Slope of Basin	15.0 %
Elevation	1,180 m
Location	37° 21'56''N, 80° 31'39''W
Area of Drainage Basin	0.95 km^2
Area of Drainage Basin/Area of Lake relation	5.02

Depth	Area m^2	Volume m^3
0	189300	------
2	164500	353800
4	137200	310400
6	11500	247400
8	93300	208000
10	75800	168732
12	67800	143400
14	60000	134400
16	50650	110566
18	40600	91100
20	18000	56932
22	2400	27466
24	1200	3532
26	493	1640
28	164	628

APPENDIX B

SUBSTRATE ELEMENTAL COMPOSITION

Obeng-Asamoa (1971) reported selected elemental and ionic concentrations of the sediments in Mountain Lake. During the summer of 1987 samples were collected using an Ekman dredge along transect 3. Three samples were collected, pooled, and analyzed (Table 11).

Not much can be said from these data. It seems that there is some phosphorus accumulation in the substrate betwen July and September. Further study needs to be done in order to investigate possible relationships between substrate nutrients and Nitella distribution.

Table 11. Selected elemental concentrations in parts per million and pH of sediments from selected depths in Mountain Lake, July and September 1987. * - Sample too small to test.

Date	Depth (m)	pH	P	K	Ca	Mg	Nitrate
7-87	2	5.2	8	34	461	39	*
	3	5.0	11	37	498	46	*
	4	4.8	9	31	360	36	3
	5	5.1	12	33	552	57	*
9-87	2	5.4	7	34	432	37	5
	4	5.4	16	36	588	48	13
	6	4.9	10	40	468	48	13
	8	5.0	18	47	552	59	15
	10	5.2	18	39	612	56	13

APPENDIX C

INSTRUMENTATION

A summary of the instrumentation used in the analyses in this research is given in Table 12.

Table 12. Instrumentation for the procedures in this study.

Procedure	Instrument Used
Collections	Ekman dredge and SCUBA
Photography	35 mm SLR camera
Microscopy	Aus Jena Nomarski filter equipped microscope
Chlorophyll absorbances	Perkin Elmer 552 Spectrophotometer
Masses	Mettler H80 Balance
Drying	Blue M Oven
Ashing	Thermolyne Muffle Furnace Barber Coleman 293 C Controls
Screening	CBS GEO # 40 Hubbard Screen
Scintillation Counts	Beckman LS-3150 T Counter
Chlorophyll analysis	Waring Homogenizer
Temperature	YSI Model 33 S-C-T Meter
pH	Corning 610 A Meter
Light	LiCor 185B photometer

APPENDIX D

FRESH-DRY WEIGHT CONVERSIONS

Traditionally, weights are presented as dry weights for all data. For chlorophyll analysis dry weights are not used because of the degradation product formed when the pigments dry. Therefore, the following information is presented for utilization in the conversions of fresh weights to dry weights or ash weights.

Percent fresh weights which are water and corresponding dry weight percentages as well as the percentage of the dry weights which are ash were calculated for 71 samples collected over the two year period of this study. Samples included ones from all depths and all transects. The percentage water was 93.25 ± 2.67. The percentage of the dry which was ash was 23.33 ± 10.81. From these, conversions of fresh weight to dry weights can be made.

APPENDIX E

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