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Nutritive Value and Chemical Composition of Certain Fresh-water Plants of Minnesota

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Approximately ten per cent of the area of Minnesota is lake and stream; the lakes alone are said to number nearly 13,000. There is an abundance of vegetation in these lakes regardless of drouth conditions in adjacent areas. Thus drouth cycles should not present serious problems of providing forage for livestock in such regions if the aquaculture were known to be adequate from a nutrition standpoint and its harvest were practicable.

It is generally known and accepted that seaweeds contain large quantities of minerals and vitamins; with the exception of iodine, it seems reasonable that similar nutrients exist in plants grown in fresh-water. There is an abundance of such forage available, and if the figures given by Rickett (1921) for Lake Mendota, Wisconsin, are applied to the 5,600 square miles of lake surface in Minnesota (Anon., 1936), there would be a yield of over 860,445 tons of dry plant forage in one season. Since Gortner (1934) found that several important representatives of these plants contain an average of 14.39 per cent crude protein, this tonnage would give a possible yield of at least 123,818 tons of protein. There is also the possibility of profitably disposing of the tons of plant material which choke up beaches and boat channels, if the material can be used for fertilizer or animal food.

Before practical use may properly be made of fresh-water aquaculture, detailed knowledge as to the chemical and biochemical nature of the proteins, carbohydrates, and minerals of such plants would be highly desirable, and laboratory studies of their nutritive value should be made with test animals. Little knowledge of this kind is so far available and no detailed nutrition studies have been reported. It is not known whether the proteins in such plants are biologically important for animals, nor whether vitamins occur in sufficient quantity to make them potentially important food products.

The present bulletin is an extensive study of some of these problems applied to representative fresh-water plants of Minnesota. Part I presents the general biochemical, chemical, and nutritional studies; Part II presents detailed studies of the nitrogen-containing components of *Elodea;* Part III presents studies on the carbohydrates. One species, *Elodea canadensis,* was employed for all phases of the work. Two species, *Elodea canadensis* and *Myriophyllum spicatum,* were employed for the general studies and the carbohydrate studies.

I. Nutritive Value and General Chemical Composition of Species of *Elodea, Myriophyllum, Vallisneria,* and Other Aquatic Plants[1]

J. Wesley Nelson and Leroy S. Palmer

The nutritive value of the higher aquatic plants is of interest from several standpoints. Not only are they potentially valuable as forage for livestock, but they are used as food by a varied aquatic fauna including the insects among the invertebrates and fishes, birds, ducks, and geese and such mammals as the muskrat, beaver, and moose among the vertebrates.

The study of the nutritive value of certain aquatic plants presented in this section of the bulletin includes their proximate analyses, palatability, digestibility, the biological value of the proteins, and their mineral and vitamin contents.

REVIEW OF LITERATURE

A search through the literature shows that there has not been a great deal of nutritional work carried out on fresh-water plants. The proximate and ash analyses that have been reported for the plants studied in this bulletin are summarized in Tables 1, 2, and 3.

Ferle (1904) reported on the use of *Elodea canadensis* as a new fodder plant in Germany. The only feeding experiment on fresh-water plants found in the literature was reported by this worker, and it was not extensive. Two hogs were kept on the same kind and amount of food, and one hog received an additional 900 grams of *Elodea* daily for two months. The hog that had the extra supplement of *Elodea* gained 615 grams more than the control hog. It was stated that cattle relished *Elodea* and geese used it as food.

Some Holland workers (Anon., 1918; 1919) reported the proximate analyses of three aquatic plants, *Lemna triscula* (duckweed), *Elodea canadensis,* and *Azolla* sp. They stated that these plants could be utilized either fresh or as ensilage as food for cattle and pigs.

The University of Wisconsin has carried on a general investigation of the productivity of Wisconsin lakes (Juday, 1935). Rickett (1920, 1921, 1924) has collected samples and estimated the yields of the aquatic flora found in several Wisconsin lakes. Schuette and Hoffman (1921) and Schuette and Alder (1927; 1929 a, b) reported the chemical composition of six of the large aquatic plants found in Lake Mendota, namely,

[1] Part I of this bulletin submitted to the Faculty of the Graduate School of the University of Minnesota by J. Wesley Nelson in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, March 1938.

Table 1. Proportion of Components (Chiefly Dry Basis) in Elodea canadensis

	Hoffmeister (See Ferle, 1904)		Ferle (1904)	Holland workers (See Anon, 1918)	Rickett (1924)	Gortner (1934)	Harper and Daniel (1934)
Fresh basis							
				per cent			
Dry matter	12.00	.....	12.10	5.4	7.10	18.40	.....
Dry basis							
Ash	19.22	20.16	16.00	19.0	.....	27.86	.....
Crude protein	14.37	19.56	17.50	25.9	.....	12.13	10.47 15.19
Ether extract	2.32	2.26	2.96	1.8	.....	1.71	12.44 .....
Crude fiber	16.89	16.54	15.60	18.5	.....	16.20	.....
N-free extract	44.17	41.48	47.04	35.2	.....	42.10	.....
CO_2	13.82	14.57	.....	.....	.....	.....	.....
Sand	0.24	0.26	.....	.....	.....	.....	.....
SiO_2	2.48	2.63	.....	.....	.....	.....	.....
P	0.54	0.41	.....	.....	.....	.....	0.231 0.148
S	0.15	0.12	.....	.....	.....	.....	0.196 .....
Cl	0.20	0.81	.....	.....	.....	.....	.....
Na	1.06	1.15	.....	.....	.....	.....	.....
Mg	0.89	0.92	.....	.....	.....	.....	.....
K	3.62	3.44	.....	.....	.....	.....	.....
Ca	2.98	3.22	.....	.....	.....	.....	10.80 8.44
Fe	1.71	1.90	.....	.....	.....	.....	8.65

Table 2. Proportion of Components (Chiefly Dry Basis) in Myriophyllum spicatum

	Birge and Juday (1922)	Schuette and Hoffman (1921)	Gortner (1934)	Gortner (1934)	Gortner (1934)
Fresh basis					
		per cent			
Dry matter	.....	9.80*	13.60	.....	.....
Dry basis					
Ash	20.72	20.72	20.26	17.08	17.08
Crude protein	20.19	18.75	17.65	17.34	17.53
Ether extract	2.95	2.44	1.28	1.65	1.21
Crude fiber	15.58	15.01	11.15	13.19	14.93
N-free extract	40.56	35.88	49.66	50.74	49.25
SiO_2	1.96	1.96	.....	.....	.....
P	.....	0.55	.....	.....	.....
S	.....	1.36	.....	.....	.....
Cl	.....	1.62	.....	.....	.....
Mg	.....	0.81	.....	.....	.....
Ca	.....	3.06	.....	.....	.....
Al	.....	2.25	.....	.....	.....
Mn	.....	trace	.....	.....	.....
Fe	.....	0.06	.....	.....	.....

* This analysis by Rickett (1924).

Table 3. Proportion of Components (Chiefly Dry Basis) in Vallisneria spiralis

	Birge and Juday (1922)	Rickett (1924)	Schuette and Alder (1927)	Gortner (1934)
	Fresh basis			
		per cent		
Dry matter	.....	7.10	.....	.....
	Dry basis			
Ash	20.70	.....	25.18	28.63
Crude protein	17.50	.....	11.80	15.03
Ether extract	2.41	.....	0.73	1.29
Crude fiber	13.10	.....	14.00	18.26
N-free extract	46.29	.....	41.41	36.79
SiO_2	0.52	.....	5.45	.....
P	0.52	.....	0.23	.....
S	.....	.....	0.85	.....
Cl	.....	.....	1.32	.....
Na	.....	.....	0.60	.....
Mg	0.81	.....	1.13	.....
K	.....	.....	4.55	.....
Ca	3.06	.....	5.83	.....
Al	.....	.....	0.30	.....
Mn	.....	.....	0.37	.....
Fe	.....	.....	0.57	.....

Cladophora, Myriophyllum, Vallisneria, Potamogeton, Castalia odorata and *Najas flexilis,* and of *Chara* sp. from Green Lake, Wisconsin.

Harper and Daniel (1934) gave the N, P, and Ca content of 13 species of aquatic plants found in Oklahoma. *Elodea canadensis,* one of these species, ranked fourth, eighth, and first in per cent of N, P, and Ca, respectively.

Gortner (1934) reported the proximate analyses of 28 samples representing the dominant vegetation types found in lakes near St. Paul, Minnesota. He suggested that lake vegetation might be a good source of forage in years of drouth.

Mršić (1936) stated that the peasants in Yugoslavia used water plants as forage in districts subject to drouth in summer and where there was an abundance of vegetation in clear, cool waters. He also stated that cattle liked this fodder and digested it as easily as ordinary green fodder. However, vegetation from marshy and warm waters could not be used as cattle objected to its musty odor.

METHODS OF EXPERIMENTATION

The three species of aquatic plants used in this study were selected for the following reasons: (1) They are common to all types of lakes; (2) they apparently grow as well in mud as in sand; (3) they are the predominating species; (4) they are high in protein; (5) they are low in fiber, and (6) they have been studied by other investigators. These species are *Vallisneria spiralis* (eelgrass or water celery), *Myriophyllum spicatum* (water milfoil), and *Elodea canadensis* (ditch moss, water thyme, or water weed). *Elodea* is found abundantly in a lotic environ-

ment (running-water series), and the other two species predominate in a lentic environment (standing-water series).

These species are classified by Welch (1935) as higher aquatic plants which are rooted in the bottom, may occur as land forms but are normally submerged, and are characterized by a creeping axis bearing long, branching, and leafy shoots with no floating leaves. *Vallisneria* and *Myriophyllum* have emergent inflorescences but no aerial foliage. *Elodea* has the inflorescence entirely submerged, but the essential organs are raised to the surface. *Elodea* may also live unattached for considerable periods. The roots probably serve mainly for anchorage, as the absorption of nutrient materials is performed mostly by the body of the plant. They are found in the zone of submerged hydrophytes down to a depth of six meters.

Collection and Preparation of Samples

Vallisneria was obtained from a shallow channel that connects the two ends of Lake Owasso, by cutting the plants off under water with a scythe from the side of a boat and then picking up the plants as they drifted on the surface. *Myriophyllum* was obtained from deeper water at the south end of Lake Owasso, by simply pulling the plants into the boat with a rake or by hand. Both of these plants were in full bloom when collections were made in September 1934 and in August 1935. Lake Owasso is about five miles north of St. Paul, has a very muddy bottom, and at times is almost choked up with vegetation.

Elodea was obtained from Rice Creek in July 1935 and from Coon Creek in August 1935. The collectors picked the young green sprigs by hand while wading in the creek. These creeks are about 11 and 21 miles, respectively, north of St. Paul, in Anoka County; they have a sandy bottom and clear, cool water two to four feet deep.

All the samples were taken into the laboratory and the fresh, green parts of the plants, free of extraneous material, were selected. After thoroughly washing under tap water, the plants were spread out in thin layers and dried in the Division of Agronomy's drying tower in a current of air at 60° C. for five days. The material was then ground in a Wiley mill to pass through a medium-mesh screen and was stored in large cans at room temperature. For some analyses, special samples were taken as will be described later.

Methods of Chemical Analysis

The methods of analysis used were those of the Association of Official Agricultural Chemists (1935) except where specifically noted. All determinations were made in triplicate. CO_2 was determined by the method of Neal and Palmer (1931) with the modification of Phillips, Goss, and Browne (1933) of heating to only 70° C. to prevent the liberation of CO_2 from the uronic acids. Ca, P, and Mg were determined by the method of Morris, Nelson, and Palmer (1931). The different forms

Table 4. Composition of Rations in Per Cent

Ration No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Ingredient																								
Plant	.....	.....	.....	.....	.....	28	50	50	.....	.....	70.5	67	67	67	67	67	67	60	87.5	70	63.7	63.4	58	70.5
Commercial casein	.....	.....	.....	.....	.....	.....	.....	.....	27	.....	.....	.....	.....	.....	.....	.....	18	18	.....	.....	.....	.....	.....	.....
Purified casein	18	18	18	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	5	5	.....	.....	.....	.....	.....	.....	.....	5
Dried fat-free whole egg	.....	.....	.....	.....	.....	.....	.....	.....	.....	5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Gelatin	.....	.....	.....	15	13.5	15	15	15	.....	.....	.....	11	10.8	.....	.....	.....	.....	.....	.....	.....	11	.....	11	.....
Zein	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	11	.....	.....	.....	.....	.....	.....	.....	10	7	.....
Cystine	.....	.....	.....	.....	.....	0.3	0.3	0.3	.....	.....	.....	.....	0.2	.....	.....	0.3	.....	.....	0.5	0.5	0.3	.....	.....	0.5
Gluten	.....	.....	.....	.....	.....	.....	15	15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Gluten, acid washed	.....	.....	.....	15	13.5	15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Soft wheat, low P	.....	.....	.....	33	29.7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Corn, yellow	.....	.....	.....	33	29.7	9	9.1	8.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Dextrin	.....	.....	.....	.....	.....	20.4	.....	.....	35	66.2	.....	.....	.....	.....	6	5.7	.....	.....	.....	.....	.....	.....	.....	.....
Sucrose	63.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Cornstarch	.....	65.2	67.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Agar	.....	.....	.....	.....	.....	.....	.....	.....	.....	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Lard	12	.....	.....	.....	10	10	10	10	22	20	27.5	22	22	22	22	22	15	15	10	27.5	22	24.8	22	22
Butterfat	.....	9	9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Salt mixture Hawk-Oser (1931)	4.4	4.4	4.4	.....	.....	.....	.....	.....	4	4.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
NaCl	.....	.....	.....	1	0.9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
$CaCO_3$	.....	.....	.....	3	2.7	2.3	0.6	1.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3	.....	.....	.....
NaH_2PO_4	.....	.....	.....	.....	.....	.....	.....	0.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Dried brewers' yeast	.....	.....	.....	.....	.....	.....	.....	.....	10	.....	.....	.....	.....	.....	.....	.....	.....	5	.....	.....	.....	.....	.....	.....
Nopco vitamin B concentrate	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Betaxin	.....	.....	0.3*	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Vitamin G containing clay adsorbate	.....	2.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
U.S.P. cod-liver oil	2	1	1	.....	.....	.....	.....	.....	2	2	2	.....	.....	.....	.....	.....	.....	2	2	2	.....	1.8	2	2

* Milligram per cent.

of S were determined by the method of Woodman and Evans (1933). Smith and Ross' (1925) perchlorate method was used to determine Na and K. Mn was determined by the method of Bolin (1934). For Fe and Cu, special samples were taken with precautions to avoid contamination. These samples were ground by hand with an agate mortar and pestle. Total Fe was determined by the method of Elvehjem (1930) as modified by Farrar, Jr. (1935) and available Fe by Kohler, Elvehjem, and Hart's (1936) modification of Hill's (1930) dipyridyl method. Cu was determined by the carbamate method as outlined by Conn, Johnson, Trebler, and Karpenko (1935).

Ascorbic acid, total carotenoids, and β-carotene were determined on special samples to avoid any oxidative deterioration. The *Elodea* samples for these determinations were collected from Coon Creek on August 24, 1936. Only the young green sprigs were taken, and the leaves averaged two millimeters by seven millimeters in size. The *Vallisneria* samples were collected from Lake Owasso on August 28, 1936. These plants were in full bloom, but only the green leaves were collected. The *Myriophyllum* samples were also collected from Lake Owasso at the same time. Most of the plants were in full bloom, but only the young green tips about four inches long were selected.

The fresh green plants were taken from the water, placed in jars containing ice water, and taken into the laboratory. Each sample was then taken separately, washed in tap water, and dried between paper towels until the surface water was absorbed, taking care to remove the plants before plasmolysis of the cells occurred, as shown by examining the leaves under a low-power microscope. They were then placed in tared tubes, sealed, and weighed. This gave the fresh green weight. Similar samples were taken and the dry matter determined by heating in a vacuum oven at 100° C. for five hours.

The samples intended for carotenoid determinations were frozen in a dark cold room at —22° C. and held there until analyzed by a slight modification of the method described by Miller (1935).[2]

The samples for ascorbic acid were placed in tared tubes containing enough acetic acid to make an eight per cent solution, weighed, and held near 0° C. until analyzed. These samples were extracted and titrated with the 2,6-dichlorophenolindophenol reagent according to the method of Bessey and King (1933).

Methods of Biological Analysis

The same general technique in the care of the rats was used in all the biological assays. The rats were kept in individual metal cages on raised ½-inch-mesh screens. The cages were washed and sterilized weekly. The temperature of the room was kept practically constant at 78° F. the year round. They were fed *ad lib.* and given distilled water

[2] We wish to thank Dr. Elmer S. Miller for his assistance in the determination of the carotenoid pigments.

containing two drops of 10 per cent KI solution saturated with I_2 per gallon of water. The food cups used were of the McCollum type, and daily supplements were fed in Fischer "Franke" type feed cups. Weekly, and in some cases daily or biweekly, records were taken of the weights and food intakes. The composition of all the rations used is given in Table 4. The casein was prepared and purified according to the method of Palmer and Kennedy (1927). Commercial wheat gluten was dispersed in boiling water containing 26 milliliters concentrated HCl per liter of water, allowed to settle, filtered, and the treatment repeated. The wheat gluten was then washed once with water on the filter and dried. This decreased the P content from 0.3 to 0.087 per cent. Dextrin was prepared by heating tapioca in an autoclave with steam at 15 pounds pressure for four hours. The dextrin was then dried and ground. Butterfat was prepared by melting butter at 40° C. and carefully filtering through dry filter paper. The other constituents were commercial products.

Table 5. Results of Individual Chemical Analyses of Elodea canadensis Calculated on Air-dry, Dry, and Fresh Basis

	Air-dry basis				Dry basis	Fresh basis
	Individual analyses			Average	Average	Average
				per cent		
Dry matter	96.94	96.88	97.01	96.94	.....	.....
Ash	21.31	21.14	21.14	21.20	21.87	1.64
Crude protein	26.09	25.91	25.97	25.99	26.81	2.02
Ether extract	3.27	3.45	3.54	3.42	3.53	0.27
Crude fiber	15.37	14.69	14.69	14.92	15.39	1.16
N-free extract	.....	.....	.....	31.41	32.40	2.44
CO_2	1.38	1.47	1.47	1.44	1.49	0.112
Sand	2.23	2.26	2.12	2.20	2.27	0.17
SiO_2	6.09	6.16	6.02	6.09	6.28	0.47
P	0.55	0.54	0.55	0.55	0.57	0.043
S, total	0.25	0.26	0.26	0.26	0.27	0.020
S, SO_4	0.07	0.07	0.07	0.07	0.07	0.0053
S, organic	.....	.....	.....	0.19	0.20	0.015
Cl	0.59	0.52	0.57	0.56	0.58	0.044
Na	0.46	0.49	0.48	0.48	0.50	0.038
Mg	0.63	0.64	0.63	0.63	0.65	0.049
K	3.51	3.61	3.51	3.54	3.65	0.27
Ca	2.74	2.69	2.69	2.71	2.80	0.21
Mn	0.320	0.318	0.324	0.321	0.331	0.025
Fe, total	0.366	0.403	0.383	0.384	0.408	0.031
Fe, available	0.303	0.303	0.303	0.303	0.322	0.024
				milligram per cent		
Cu	0.79	0.76	0.82	0.79	0.84	0.063
			Fresh basis			
				per cent		
Dry matter	7.58	7.76	7.21	.....	.....	7.52
				milligrams per cent		
Carotenoids	6.07	5.28	5.61	.....	75.1	5.65
β-carotene	.....	1.81	1.52	.....	22.2	1.67
Ascorbic acid	0.80	0.79	0.78	.....	10.5	0.79

A very uniform and as nearly homogeneous stock of rats as could be obtained was supplied by the Nutrition Section of the Biochemistry Division from lines that had been inbred for over 30 generations. It was possible to use all males for the biological assays; the females were being used for breeding and vitamin E assay. They were put on experiment when they were 28±two days old and weighed 60±two grams. A system of random sampling was employed for allotting the rats for each assay to five groups representing positive and negative control animals and a group for the animals on each of the three plants. Each rat was given a number, and slips bearing the rat numbers were drawn at random from a box and allotted in order to the five groups. Approximately ten rats were used in each group for each assay.

The formulas used in the statistical treatment of the results are given by Treloar (1933).

Vitamin B (B_1), the antineuritic vitamin, was determined by the method of Chase and Sherman (1931) using 2.4 per cent of vitamin G

Table 6. Results of Individual Chemical Analyses of Myriophyllum spicatum Calculated on Air-dry, Dry, and Fresh Basis

	Air-dry basis				Dry basis	Fresh basis
	Individual analyses			Average	Average	Average
				per cent		
Dry matter	90.77	90.70	90.80	90.76	.....	.....
Ash	12.76	12.40	12.50	12.55	13.83	1.84
Crude protein	23.21	23.59	23.53	23.44	25.83	3.44
Ether extract	2.31	2.20	2.20	2.24	2.47	0.33
Crude fiber	12.75	12.85	12.85	12.82	14.13	1.88
N-free extract	.....	.....	.....	39.71	43.74	5.83
CO_2	1.29	1.34	1.33	1.32	1.47	0.194
Sand	0.71	0.70	0.70	0.70	0.77	0.10
SiO_2	2.65	2.69	2.68	2.67	2.94	0.39
P	0.38	0.37	0.38	0.38	0.42	0.056
S, total	0.39	0.39	0.38	0.39	0.43	0.057
S, SO_4	0.19	0.19	0.19	0.19	0.21	0.028
S, organic	.....	.....	.....	0.20	0.22	0.029
Cl	1.23	1.28	1.15	1.22	1.34	0.18
Na	0.69	0.69	0.66	0.68	0.75	0.10
Mg	0.64	0.66	0.72	0.67	0.74	0.099
K	1.61	1.74	1.76	1.70	1.87	0.25
Ca	2.45	2.50	2.57	2.51	2.77	0.37
Mn	0.466	0.470	0.463	0.466	0.513	0.068
				milligrams per cent		
Fe, total	67.0	68.0	58.0	64.0	66.0	8.8
Fe, available	33.0	32.0	32.0	32.0	33.0	4.4
Cu	0.82	0.87	0.88	0.86	0.89	0.12
				Fresh basis		
				per cent		
Dry matter	12.54	13.71	13.32	.....	.....	13.32
				milligrams per cent		
Carotenoids, total	13.42	15.46	15.21	.....	110.04	14.70
β-carotene	2.79	2.91	2.86	.....	21.4	2.85
Ascorbic acid	3.60	2.80	3.60	.....	24.8	3.30

containing clay adsorbate in the ration instead of autoclaved yeast. The positive controls received 0.15 gram daily of vitamin B (B_1) adsorbate, similar to International Standard vitamin B_1 adsorbate.

The vitamin G (B_2) assay method of Bourquin and Sherman (1931) was used to determine the d-riboflavin fraction. Betaxin was used at a 0.3 milligram per cent level to supply vitamin B (B_1) instead of alcoholic extract of wheat. The crystalline vitamin B (B_1) was dissolved in water and added to the purified casein which was then ground in a ball mill to give a homogeneous mixture. The positive controls received 0.25 gram daily of vitamin G containing clay adsorbate. The vitamin B and G adsorbates were supplied by the Vitab Products, Inc., New York, through the courtesy of Mr. C. L. Bowman, Vice President. The B adsorbate was made from rice-polish extract and the G adsorbate from milk whey. Betaxin was purchased from the Winthrop Chemical Co., Inc., New York.

Vitamin D was determined by the United States Pharmacopoeia

Table 7. Results of Individual Chemical Analyses of Vallisneria spiralis Calculated on Air-dry, Dry, and Fresh Basis

	Air-dry basis				Dry basis	Fresh basis
	Individual analyses			Average	Average	Average
				per cent		
Dry matter	95.80	95.77	95.68	95.75	.....	.....
Ash	14.94	15.00	14.95	14.96	15.64	0.81
Crude protein	14.40	14.69	14.45	14.51	15.15	0.78
Ether extract	3.93	4.07	4.31	4.10	4.28	0.22
Crude fiber	15.54	15.27	14.64	15.15	15.82	0.81
N-free extract	.....	.....	.....	47.03	49.11	2.53
CO_2	0.57	0.53	0.60	0.57	0.60	0.031
Sand	0.09	0.10	0.07	0.09	0.09	0.0046
SiO_2	1.04	1.04	0.99	1.02	1.07	0.055
P	0.19	0.20	0.20	0.20	0.21	0.011
S, total	0.36	0.35	0.36	0.36	0.38	0.020
S, SO_4	0.20	0.20	0.20	0.20	0.21	0.011
S, organic	.....	.....	.....	0.16	0.17	0.0088
Cl	2.00	1.78	1.97	1.92	2.01	0.10
Na	2.28	2.25	2.33	2.29	2.39	0.12
Mg	0.75	0.72	0.72	0.73	0.76	0.039
K	6.36	6.51	6.57	6.48	6.77	0.35
Ca	1.48	1.50	1.47	1.48	1.55	0.08
Mn	0.036	0.039	0.037	0.037	0.039	0.002
				milligrams per cent		
Fe, total	44.0	43.0	45.0	44.0	45.0	2.30
Fe, available	17.0	18.0	18.0	18.0	19.0	0.98
Cu	0.32	0.31	0.32	0.32	0.33	0.017
			Fresh basis			
				per cent		
Dry matter	5.14	5.11	5.20	.....	.....	5.15
				milligrams per cent		
Carotenoids	4.69	4.66	6.15	.....	100.4	5.17
β-carotene	0.89	0.85	0.97	.....	17.5	0.90
Ascorbic acid	4.1	3.9	3.6	.....	75.7	3.9

(1937) method. As the rachitogenic ration had to be modified to incorporate large amounts of the plants, the ration for the controls was correspondingly changed so that in all cases the percentages of Ca and P were constant.

Vitamin E was determined by the method described by Palmer (1937). However, it was necessary to feed as much of the plant as the animals would eat during the first 13 days of the gestation period in order to demonstrate the presence of any vitamin E.

The biological value of the protein was determined by the method of Mitchell (1923 and 1924) as modified by Mitchell and Carman (1926). The coefficients of apparent digestibility for ash, dry matter, and protein were determined in conjunction with the biological values.

EXPERIMENTAL RESULTS

Chemical Analyses

The results of the triplicate determinations for all the chemical analyses are reported in Tables 5, 6, and 7. The chemical analyses are averaged and calculated on the dry and fresh basis.

The general chemical composition of these three plants seems to be similar. They differ chiefly in dry matter. *Myriophyllum* has more than twice as much dry matter; therefore, on the fresh basis it has the highest percentage of all constituents except Fe, sand, SiO_2, N, K, and ascorbic acid. *Elodea* with 0.408 per cent Fe on the dry basis is one of the richest plant sources of Fe. More remarkable yet is the fact that 79 per cent of Fe in *Elodea* is available for biological use. *Elodea* also has the highest percentage of sand and SiO_2. *Vallisneria* has the highest percentage of Na, K, and ascorbic acid.

From the percentages of β-carotene and ascorbic acid it is possible to calculate the International Units of vitamins A and C. The conversion factors are 0.6 microgram β-carotene per International Unit of vitamin A and 0.05 milligram ascorbic acid per International Unit of vitamin C. Thus *Elodea, Myriophyllum,* and *Vallisneria* contain 27.83, 47.5, and 15.02 International Units of vitamin A, and 0.158, 0.66, and 0.78 International Units of vitamin C per gram of fresh plant, respectively.

Chlorophyll A and B were also determined along with the carotenoid pigments because of interest in the dark green color of most fresh-water plants. *Elodea, Myriophyllum,* and *Vallisneria* contained 39.88, 72.21, and 25.26 milligrams per cent of chlorophyll A and 16.26, 26.09, and 35.49 milligrams per cent of chlorophyll B, respectively, on the fresh basis.

There is a question as to the form in which the inorganic elements occur in the ash owing to the possibility of sulphates, phosphates, silicates, etc., but if they are calculated as the oxides, except for Na and K which are added as such, their sum is slightly more than the ash content. The calculated figures for *Elodea, Myriophyllum,* and *Vallisneria* are

Table 8. Comparison of the Mineral Composition of an Adequate Rat Ration with the Mineral Composition in Dry Matter of Elodea, Myriophyllum, and Vallisneria

Element	Adequate rat ration*	*Elodea*	Approximate ratio *Elodea* to adequate ration	*Myriophyllum*	Approximate ratio *Myriophyllum* to adequate ration	*Vallisneria*	Approximate ratio *Vallisneria* to adequate ration
				per cent			
P	0.51	0.57	1.0	0.42	1.0	0.21	0.5
Cl	0.53	0.58	1.0	1.34	2.5	2.01	4.0
Na	0.19	0.50	2.5	0.75	4.0	2.39	13.0
Mg	0.08	0.65	8.0	0.74	9.0	0.76	9.0
K	0.83	3.65	4.0	1.87	2.0	6.77	8.0
Ca	0.55	2.80	5.0	2.77	5.0	1.55	3.0

* Figures from Osborne and Mendel (1918).

22.75, 15.55, and 15.99, and the ash percentages are 21.87, 13.83, and 15.64, respectively. This indicates that practically all the mineral constituents have been accounted for.

It is interesting to compare the values given by Osborne and Mendel (1918) for the normal mineral composition of a rat's diet with the mineral composition of these plants. This is done in Table 8 from which it can be seen that 20 per cent of any of these plants in a ration would furnish almost the same amount of the mineral elements listed as found in this normal rat diet. The only constituent that will be critically low is P.

Table 9. Analyses of Samples of Lake-water Vegetation from Representative Minnesota Lakes

No.*	Plant*	Dry-basis analyses		
		Ca	P	Mg
		per cent	per cent	per cent
1.	*Myriophyllum spicatum*	3.39	0.233	0.858
2.	*Myriophyllum spicatum*	5.10	0.184	0.631
3.	*Myriophyllum spicatum*	5.71	0.260	0.792
4.	*Potamogeton amplifolius*	7.55	0.219	0.595
5.	*Potamogeton amplifolius*	13.66	0.142	0.844
6.	*Potamogeton Richardsonii*	13.41	0.148	0.711
7.	*Potamogeton pectinatus*	1.82	0.294	0.654
9.	*Najas flexilis*	5.70	0.266	0.961
12.	*Najas flexilis*	9.43	0.184	0.854
13.	*Najas flexilis*	7.57	0.169	0.795
15.	*Ceratophyllum demersum*	4.47	0.286	0.834
16.	*Ceratophyllum demersum*	4.27	0.193	1.490
18.	*Vallisneria spiralis*	1.79	0.186	0.803
19.	*Heteranthera dubia*	6.52	0.219	0.612
20.	*Nymphaea advena*	1.04	0.253	0.395
21.	*Chara* sp.	24.64	0.051	0.757
22.	*Ruppia occidentalis* and *Potamogeton pectinatus*	2.61	0.284	0.763
23.	Largely *Myriophyllum spicatum*	7.00	0.117	1.098

* These numbers and plant samples are the same as those given in the publication by Gortner (1934).

Chemical analyses for Ca, P, and Mg were made on various other aquatic plants available from the study by Gortner (1934). These results are given in Table 9. As pointed out by Gortner, some of the *Potamogeton* and *Chara* especially are marl-formers, and this is emphasized here by their high Ca content.

Biological Analyses

A preliminary experiment was carried out to determine if all growth factors of the vitamin "B complex" present in the B and G adsorbates were present in these plants. Ration 1, Table 4, was used for the vitamin "B complex" free ration. Two rats were used for each plant and two for negative controls and one for a positive control. Figure 1 shows that these plants probably contain all the growth factors of the "B complex" occurring in the adsorbates fed to the positive control. *Elodea* and *Myriophyllum* are both about three times as rich in these factors, or in the limiting one, as is *Vallisneria.*

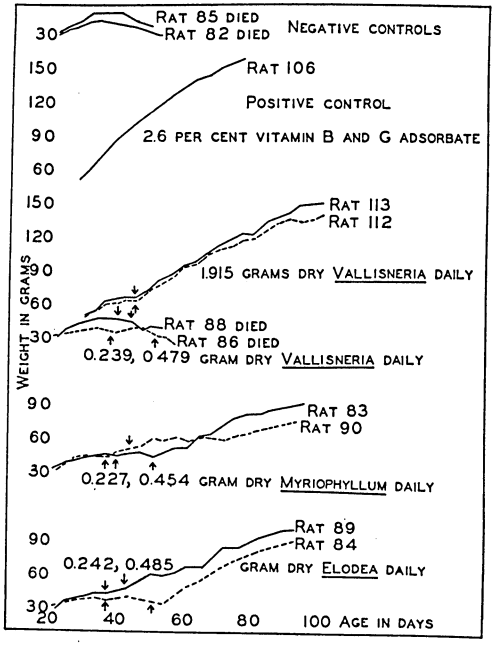

FIG. 1. GROWTH CURVES OF RATS ON RATION NO. 1 USED FOR THE BIOLOGICAL ASSAY OF THE VITAMIN "B COMPLEX"

Arrows indicate the beginning of supplement feeding.

Biological Assay for Vitamin B (B_1)

Ration 2, Table 4, was used for the determination of vitamin B (B_1). On this ration, the rats became depleted of their vitamin reserves and lost weight or remained stationary over a three-day period in an average of 16.14 days. At the time they were started on the four weeks assay, their average weight was 84.41 grams. The nine animals used as negative controls continued to lose weight and died in an average of 21.89 days, with an average loss in weight of 11.23 grams. Their food intake averaged only 2.75 grams a day. If a daily supplement of the vitamin G containing clay adsorbate was fed, the animals ate more and lived the full four weeks but still continued to lose weight.

The seven animals used as positive controls were given an average daily supplement of 0.14 gram of the vitamin B (B_1) adsorbate. They showed an average weekly gain of 14.06 grams.

Table 10. Biological Assay for Vitamin B (B_1), Using Ration 2

Plant	No. of rats	Food eaten, daily average		Gain in weight, weekly average	Vitamin B (B_1)		
		Total	Plant		Units	Standard deviation	Coefficient of variation
		grams	grams	grams	per gram		per cent
Elodea	10	6.42	0.7657	8.65	3.76±0.17	0.78±0.12	20.74
Myriophyllum	12	6.59	0.9050	7.25	2.61±0.14	0.74±0.10	28.45
Vallisneria	11	8.23	1.9940	11.20	1.83±0.12	0.57±0.08	31.08
Negative controls	9	2.75		−11.23±1.09		4.86±0.77	43.28
Positive controls	7	8.12		14.06±0.61		2.41±0.43	17.13
					Difference		
Elodea—Myriophyllum					1.15±0.17		
Elodea—Vallisneria					1.93±0.17		
Myriophyllum—Vallisneria					0.78±0.01		

At least ten rats were used in the assay of each plant. The rats were fed the dry plant in a separate Fischer "Franke" type feed cup daily over a four-week period. If they did not eat enough to maintain a steady gain in weight, the plant was added to the ration in place of a like amount of starch. The Chase and Sherman (1931) units of vitamin B (B_1) per gram of *Elodea, Myriophyllum,* and *Vallisneria* were found to be 3.76, 2.61, and 1.83, respectively. Thus *Elodea* contains twice as much vitamin B (B_1) as *Vallisneria,* the difference being 1.93±0.17 units. The difference between *Elodea* and *Myriophyllum* is 1.15±0.17, and between *Myriophyllum* and *Vallisneria* it is 0.78±0.01 units. All these differences are significant. The data for the vitamin B (B_1) assays are found in Table 10, and the average growth curves are plotted in Figure 2.

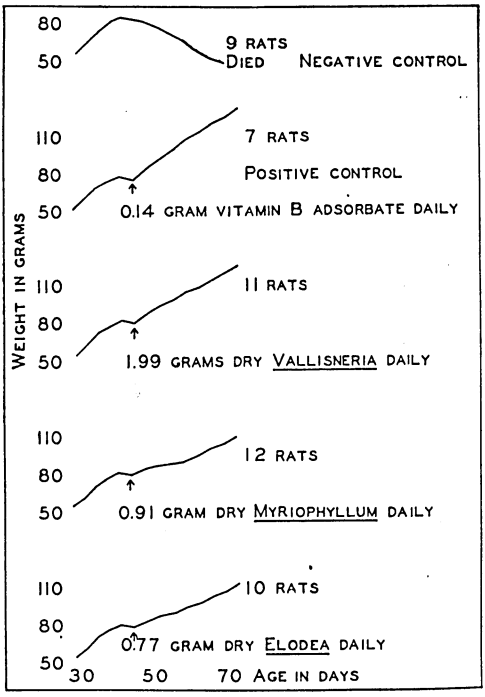

FIG. 2. AVERAGE GROWTH CURVES OF RATS ON RATION NO. 2 USED FOR THE BIOLOGICAL ASSAY OF VITAMIN B (B_1)

Arrows indicate the beginning of supplement feeding.

Biological Assay for Vitamin G (B_2)

Ration 3, Table 4, was used for the biological assay of the d-riboflavin fraction of vitamin G (B_2). On

this ration the rats showed a depletion time of 17.57 days at an average weight of 79 grams. The 10 animals that were continued on ration 3 for four weeks more as negative controls did not continue to lose weight as did the negative controls for vitamin B (B_1). Instead they showed an average gain of 1.53 grams per week for the four-week period on an average daily food intake of 5.43 grams. Bourquin and Sherman (1931) define a unit of vitamin G as "that amount which when fed as a daily allowance induces a gain of three grams per week in an experimental animal standardized as here described and fed a basal ration which is sufficiently freed from vitamin G to result in a loss of weight during the test period." The average loss of weight on the Bourquin-Sherman diet is 0.5 gram a week. As pointed out by Levine and Remington (1937), a correction should be made when the growth of the negative controls is appreciable. Their method is to divide the difference between the net gain of the experimental animals and the net gain of the negative controls by 3.5, which is the net gain per week equivalent to a Bourquin-Sherman unit. The units of vitamin G calculated both by the usual method and by the Levine-Remington method are given in Table 11, and the average growth curves are plotted in Figure 3.

For positive controls, six animals were given a daily supplement of 0.25 gram of the vitamin G containing clay adsorbate. Their average weekly gain was 13.96 grams.

Nine rats each were used for the vitamin G assay of *Elodea* and *Vallisneria,* and eight rats for *Myriophyllum.* The dry plant was fed in a separate feed cup daily over a four-week period. The Bourquin-Sherman (1931) units of vitamin G (B_2) for *Elodea, Myriophyllum,* and *Vallisneria* were 8.68, 7.60, and 5.73, respectively. The difference between *Elodea* and *Vallisneria* was 2.95± 0.87, which is just significant. The difference between *Elodea* and *Myriophyllum* was 1.08±0.94 and between *Myriophyllum* and

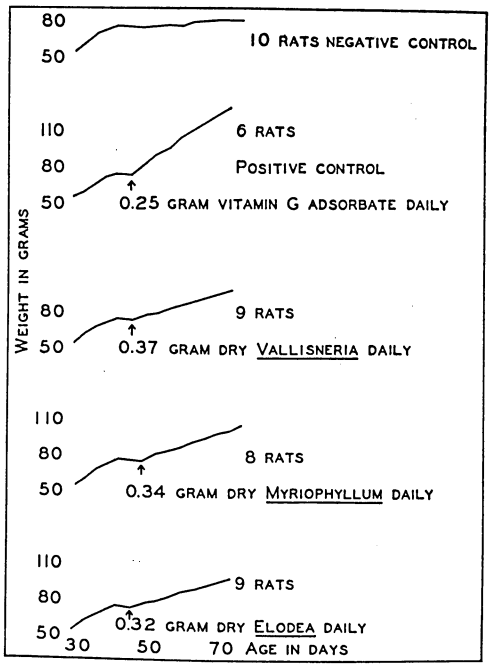

FIG. 3. AVERAGE GROWTH CURVES OF RATS ON RATION NO. 3 USED FOR THE BIOLOGICAL ASSAY OF VITAMIN G (B_2)

Arrows indicate the beginning of supplement feeding.

Table 11. Biological Assay for Vitamin G (B_2), Using Ration 3

Plant	No. of rats	Food eaten, daily average		Gain in weight, weekly average	Vitamin G (B_2)		
		Total	Plant		Units	Standard deviation	Coefficient of variation
		grams	grams	grams	per gram		per cent
Elodea	9	6.73	0.3247	8.19	8.68±0.73	3.26±0.52	37.52
Myriophyllum	8	6.34	0.3351	7.72	7.60±0.60	2.53±0.43	33.29
Vallisneria	9	6.01	0.3655	6.19	5.73±0.47	2.09±0.33	36.47
Negative controls	10	5.43		1.53±0.50		2.33±0.35	152.29
Positive controls	6	7.61		13.96±0.76		2.77±0.54	19.84
					Difference		
Elodea—Myriophyllum					1.08±0.94		
Elodea—Vallisneria					2.95±0.87		
Myriophyllum—Vallisneria					1.87±0.76		

Units of vitamin G (B_2) corrected for negative controls, Levine and Remington (1937)

Plant	Net gain in weight weekly average	Vitamin G (B_2)		
		Units	Standard deviation	Coefficient of variation
	grams	per gram		per cent
Elodea	6.68	6.09±0.56	2.49±0.40	40.89
Myriophyllum	6.19	5.18±0.53	2.23±0.38	43.05
Vallisneria	4.66	3.70±0.40	1.76±0.28	47.55
		Difference		
Elodea—Myriophyllum		0.91±0.77		
Elodea—Vallisneria		2.39±0.69		
Myriophyllum—Vallisneria		1.48±0.66		

Vallisneria, 1.87±0.76, neither of which is significant. The units corrected according to Levine and Remington (1937) were 6.09, 5.18, and 3.70 for the three plants in the same order. Here also, the only difference that was significant was the one between *Elodea* and *Vallisneria,* which was 2.39±0.69. The difference between *Elodea* and *Myriophyllum* was 0.91±0.77 and between *Myriophyllum* and *Vallisneria* was 1.48±0.66 of the corrected vitamin G (B_2) units.

From these results it appears that of these two factors vitamin B (B_1) is the limiting vitamin and *Vallisneria* is the poorest in both factors.

Biological Assay for Vitamin D

The method of the United States Pharmacopoeia (1937) for cod-liver oils was used to secure standardized rachitic rats up to the time the eight-day assay period begins. Then a modification of the official rachitogenic ration (No. 4 in Table 4) had to be made for each plant owing to the fact that assays of non-appetizing plants high in ash and low in vitamin D were being attempted. First it was found that feeding the plants separately or mixed with the official ration resulted either in very little plant being eaten, or in the animals going off feed and not eating enough even to maintain their weight. In the few cases in which the animals did maintain their weight, there was no evidence of any heal-

ing rickets. By adding 10 per cent lard and more protein to the plants a much more palatable food was obtained.

The rations finally selected were No. 6 for *Elodea,* No. 7 for *Myriophyllum,* and No. 8 for *Vallisneria* for the eight-day plant assay periods, and No. 5, which was the same as No. 4 plus ten per cent lard, for the positive and negative controls. These rations were all practically isodynamic and equal as to Ca and P. Ration 6 contained 1.24 per cent Ca and 0.235 per cent P for a ratio of 5.28. Ration 7 contained 1.13 per cent Ca and 0.237 per cent P for a ratio of 4.77. Ration 8 contained 1.13 per cent Ca and 0.245 per cent P for a ratio of 4.61. The rats ate well and gained much more on these diets containing the plants and the lard than they did on the standard rachitogenic ration. Ration 5 contained 1.08 per cent Ca and 0.238 per cent P for a ratio of 4.54. During the tenth and eleventh days of the assay period, all animals were on ration 4 which had 1.26 per cent Ca and 0.291 per cent P for a ratio of 4.33.

Table 12 shows the extent and degree of calcification of the rachitic metaphysis of the bones examined by the line test according to the procedure of Bills *et al.* (1931). Owing to the necessary modification of the rachitogenic diet, the results for vitamin D are not very clear. The negative controls that received 10 per cent lard added to the rachitogenic ration showed healing ranging from one plus to three plus, with an average value of two plus. It was not a characteristic healing along the epiphysial line but an irregular calcification in the metaphysis. This compared to the negative controls on the standard rachitogenic diet, which showed no healing at all, indicates that the lard changed the rachitic picture. The type and degree of healing rickets obtained is seen in Figure 4, which shows a typical bone from each group in the assay.

Table 12. Biological Assay for Vitamin D

Plant	Ration No.	No. of rats	Food eaten, daily average		Gain in weight, 10-day average	Degree of healing rickets
			Total (10 days)	Plant (8 days)		
			grams	grams	grams	
Elodea	6	10	8.24	2.285	22.80	+
Myriophyllum	7	6	8.45	1.920	19.00	0
Vallisneria	8	9	7.49	1.790	22.11	++
Negative controls	4	4	5.78	.....	8.00	0
Negative controls	5	5	5.60	.....	11.40	++
				milligrams*		
Positive controls	4	1	5.10	5.35	5.00	++
Positive controls	5	6	5.32	6.76	4.67	+++

* Reference cod-liver oil.

There is no definite evidence in the literature that lard contains vitamin D; Osborne, Mendel, and Park (1923) obtained marked rickets on diets containing up to 24 per cent lard. Karelitz and Shohl (1927) produced rickets when 10 per cent lard was added to the Steenbock-Black diet No. 2965. However, they state that the relationship between the

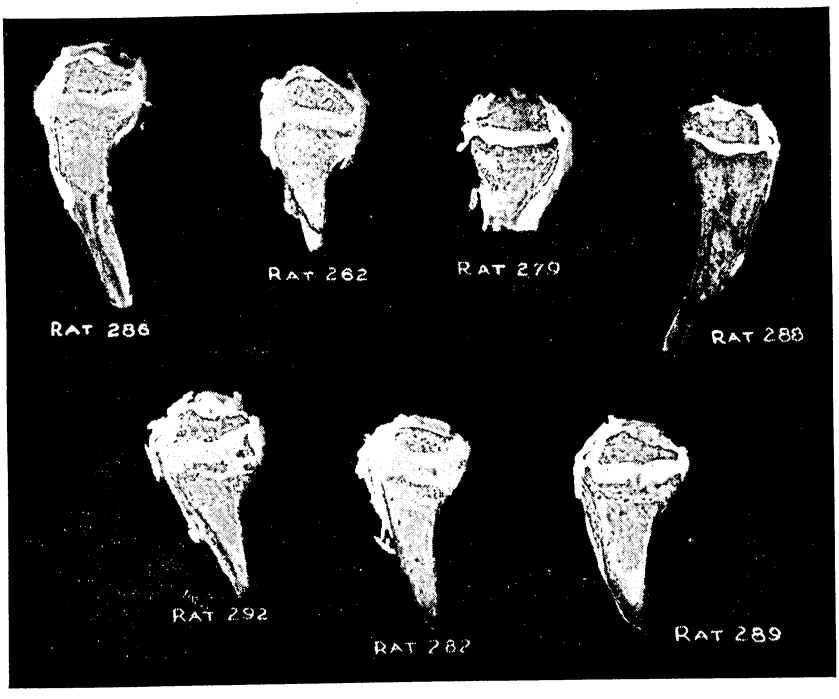

FIG. 4. PHOTOMICROGRAPHS OF TYPICAL SPECIMENS SHOWING TYPES AND DEGREES OF HEALING RICKETS

Rat 286
0 healing on ration No. 4

Rat 262
+ healing on ration No. 5

Rat 279
+ + healing on ration No. 4 plus 5.35 milligrams reference cod liver oil daily

Rat 288
+ + + + healing on ration No. 5 plus 5.35 milligrams reference cod liver oil daily

Rat 292
+ healing on ration No. 6 average 2.68 grams *Elodea* daily

Rat 282
0 healing on ration No. 7 average 1.79 grams *Myriophyllum* daily

Rat 289
+ + healing on ration No. 8 average 1.94 grams *Vallisneria* daily

fat and the mineral salts may alter the requirements of the latter. Beard (1926) found that in making up rachitogenic rations for mice no fat should be added to the food mixture, otherwise satisfactory rickets might not develop. Butterfat is usually eliminated from a rachitogenic ration as McCollum *et al.* (1926) found that five per cent of butterfat in a rachitogenic ration sometimes provided enough vitamin D to give normal bones.

In the tests of *Elodea* and *Myriophyllum* less healing was found in most cases than for the negative controls, which would indicate a complete lack of vitamin D in these plants. But in the case of *Vallisneria* the calcification in the metaphysis was at least as great as in the negative controls on lard and in most cases greater. Calculated as International Units, *Vallisneria* contained not over 0.36 International Units per gram of dry plant because 1.79 grams of the dry plant did not produce so much

healing as 6.76 milligrams of reference cod-liver oil containing 95 International Units per gram. However, if the negative control value of two plus is substracted from the value of two plus obtained for *Vallisneria,* it leaves a value that is definitely rachitic. Therefore, in the case of *Vallisneria,* there is either no depressing effect on the healing owing to the presence of lard or else there is some vitamin D present. If this is so, it can probably be accounted for by the fact that these plants were exposed to more sunshine than the other plants as they were cut under water and then allowed to float on the surface for a time until collected. However, in all cases the plants were covered with at least a thin film of water during the time they were exposed to sunshine.

The positive controls were likewise affected by the lard. The same amount of reference cod-liver oil (5.35 milligrams per day) that gave two plus healing on the standard rachitogenic ration gave a strong three plus healing on the rachitogenic ration containing 10 per cent added lard.

Biological Assay for Vitamin E

All of the rats used for the biological assay of vitamin E were given ration 9 from the time they were three weeks old and were carried through one resorption starting at 90 days of age before being used for assay. From the results given in Table 13 it must be concluded that there is no vitamin E in *Elodea* or *Myriophyllum.* At least, there is not enough present in the maximum amounts which the animals would eat during that part of the gestation period when they require vitamin E to allow the animals to give birth to any young. Assuming that the first five days of the gestation period is the most important time for administering vitamin E, the rats on *Elodea* consumed a maximum of 39.4 grams and those on *Myriophyllum* a maximum of 23 grams during this period. Up to the time the erythocyte sign was seen, the rats on *Elodea* had consumed a maximum of 73 grams and those on *Myriophyllum* had consumed a maximum of 54 grams. When only 18 grams of *Vallisneria* were eaten the first five days of gestation and only 37 grams from conception to the erythocyte sign, not enough vitamin E was furnished for

Table 13. Biological Assay for Vitamin E, Using Ration 9

Plant	Amount of plant eaten			No. of rats	Efficiency values			
	First 5 days gestation	First 13 days gestation	Total		T.L.E.*	T.I.E.†	L.L.E.‡	L.I.E.§
	grams	grams	grams		per cent	per cent	per cent	per cent
Elodea	9 to 39.4	17 to 73	17 to 118	7	0	0	0	0
Myriophyllum	3 to 23	3 to 54	3 to 103.3	8	0	0	0	0
Vallisneria	3.7 to 18	3.7 to 37	3.7 to 50	4	0	0	0	0
Vallisneria	13.7 to 26.8	47.9 to 67	47.9 to 105.3	4	100	87.18	100	76.92
Vallisneria	14.4 to 15.3	43.1	43.1	3	100	94.74	100	52.63

* Total litter efficiency, percentage of pregnancies resulting in live and still-born young.

† Total implant efficiency, percentage of placental implantations resulting in live and still-born young.

‡ Live litter efficiency, percentage of pregnancies resulting in litters containing living young.

§ Live implant efficiency, percentage of placental implantations resulting in live young.

Table 14. Coefficients of Apparent Digestibility with Rats on Ration 11

Plant	Rat No.	Ash	Dry matter	Crude protein
		per cent	per cent	per cent
Elodea	230	28.01	61.42	69.33
	232	25.19	60.10	67.85
	233	26.86	58.52	72.12
	234	23.32	60.00	69.18
	235	28.80	59.87	69.60
	236	17.12	57.84	71.67
	237	24.05	60.48	72.12
	238	25.50	60.58	72.69
	239	19.79	58.93	68.57
	240	23.41	59.86	69.68
Mean	n=10	24.21±0.77	59.76±0.23	70.28±0.37
Standard deviation	.....	3.60±0.54	1.06±0.16	1.75±0.26
Coefficient of variation	.....	14.86	1.77	2.49
Myriophyllum	230	23.32	45.39	25.25
	231	21.33	44.65	26.99
	232	27.57	48.49	28.58
	233	26.48	46.71	26.82
	234	27.58	47.77	27.37
	235	26.80	46.53	29.60
Mean	n=6	25.51±0.72	46.59±0.39	27.44±0.38
Standard deviation	.....	2.62±0.51	1.43±0.28	1.39±0.27
Coefficient of variation	.....	10.28	3.07	5.07
Vallisneria	236	64.77	61.45	33.39
	237	66.31	61.08	30.51
	238	66.73	63.00	28.98
	239	59.84	56.86	23.91
	240	67.17	61.98	32.37
	241	61.78	59.84	22.41
Mean	n=6	64.43±0.85	60.70±0.61	28.60±1.23
Standard deviation	.....	3.07±0.60	2.21±0.43	4.46±0.87
Coefficient of variation	.....	4.76	3.64	15.59

the production of any young. However, all cases in which the rats ate at least 13.7 grams of *Vallisneria* during the first days and at least 43.1 grams from conception to the erythocyte sign did give birth to living young in at least 50 per cent of the implantations.

As positive controls, four animals were given 0.75 milligram of wheat-germ oil on the day they were mated, in addition to large amounts of these plants immediately before and during the gestation period. This was the minimum level of vitamin E, as found by biological assay, that would allow living young to be born. This procedure should detect the presence of anything in these plants which was destroying the vitamin or preventing normal gestation. However, out of 43 implantations there were only eight resorptions and one dead young compared to 34 living young. Therefore, *Elodea* and *Myriophyllum* actually lacked vitamin E.

Biological Value of the Protein

A study of the biological value of the protein in these plants would appear to be a simple matter because of a high percentage of protein and an ample supply of the "B complex" vitamins, making it unnecessary to add any vitamin B supplements to complicate the protein assays. However, preliminary experiments indicated that considerable fat would have

to be added to these plants in order to make them more appetizing. This was probably needed to mask the high salt content. Rations were first made up which contained just sufficient amounts of these plants to furnish the rats nitrogen at the endogenous level. However, this amount of protein failed completely to satisfy the needs of the rats for maintenance. Therefore rations were made up to contain the maximum amount of plant that could be added along with the necessary fat. Table 4 gives the composition of ration 11, which was used to determine the biological value of the proteins for maintenance and the coefficients of apparent digestibilities, and ration 10, which was used during the low N periods.

The results of the biological-value experiments proved to be very disappointing. The values for *Myriophyllum* and *Vallisneria* varied greatly, both positive and negative values being obtained because the animals refused to eat sufficient food to maintain their weight. Consequently these results had to be discarded. The coefficient of apparent digestibility of the protein of these two plants, shown in Table 14, was only about 28 per cent. Therefore one must conclude that the proteins in both *Myriophyllum* and *Vallisneria* are of such poor quality that they will not maintain a rat in nitrogen balance.

Elodea, however, presented an entirely different picture. The rats actually gained weight on *Elodea* as the sole source of protein as shown in Table 15. The biological value averaged 42.71 ± 0.99 with a standard deviation of 4.63 ± 0.70 and a coefficient of variation of 10.85. This value should be considered a value for maintenance plus growth since the rats were not full grown and gained some weight. The coefficient of apparent digestibility of the protein, shown in Table 14, was 70.28 per cent. Therefore the protein in *Elodea* must be considered as being of fairly good quality as well as quantity.

Table 14 also gives the coefficients of apparent digestibility for dry matter, which were about the same for all three plants, and for ash which were the same for *Elodea* and *Myriophyllum* but 2.5 times as high for *Vallisneria.*

The effect of cystine as a supplement to these plants was tried by the paired feeding method and by comparisons with *ad libitum* feeding. The rations used are given in Table 4 and the results are given in Table 16. Nine young male rats were divided into three pairs and one trio to give equal starting weights. Four of the rats on ration 11 determined the food consumption of the other five rats, one determining the food intake of two other members of the trio and the other three of their pair mates. Each of these five rats received 20 milligrams of cystine daily in addition to the basal ration. On the same food intake, the animals receiving the cystine supplements showed a gain of 3.0, 5.0, 8.0, 2.0, and 4.0 grams over their controls. The average daily gain of the animals receiving cystine supplements was 1.55 grams, while the controls averaged 1.09 grams. Two additional animals that were fed the same ration *ad libitum* with 20 milligrams of cystine daily showed an average daily

Table 15. Biological Value of Proteins in Elodea Using Ration 11, 21.2 Per Cent Protein (Results on Daily Basis)

Rat No.	Body weights		Food intake	N intake	Total fecal N	Metabolic N in feces	Food N in feces	Absorbed N	N in urine	Endogenous N in urine	Food N in urine	N retained	Biological value
	Initial	Final											
	gm.	gm.	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	per cent
230	363	373	10.91	363.2	111.40	20.07	91.33	271.87	204.46	67.09	137.37	134.50	49.47
232	348	354	12.76	424.1	136.36	28.71	107.65	316.45	254.74	56.76	197.98	118.47	37.44
233	365	374	12.51	415.7	115.90	24.39	91.51	324.19	231.49	68.52	162.97	161.22	49.73
234	357	375	15.06	499.1	153.84	32.53	121.31	377.79	289.77	63.98	225.79	152.00	40.23
235	338	351	14.71	490.4	149.10	30.89	118.21	372.19	280.40	66.86	213.54	158.65	42.63
236	381	390	14.32	471.6	133.62	28.78	104.84	366.76	291.26	66.66	224.60	142.16	38.76
237	357	382	15.91	525.8	146.60	35.80	110.80	415.00	301.54	72.78	228.76	186.24	44.88
238	354	362	12.21	404.1	110.36	27.23	83.13	320.97	251.03	66.95	184.08	136.89	42.65
239	349	351	11.49	377.1	118.52	24.82	93.70	283.40	220.51	64.33	156.18	127.22	44.89
240	369	384	14.40	473.9	143.68	30.82	112.86	361.04	293.37	63.94	229.43	131.61	36.45
Average	358	370	13.43	444.5	131.94	28.40	103.53	340.97	261.86	65.79	196.07	144.90	42.71

gain of 2.97 grams. This indicates that limiting the food intake to that of the controls also limited the possible gains.

The beneficial effect of additions of cystine to the proteins in *Elodea* is also indicated by the supplementary effect of additions of 11 per cent zein, which is rich in cystine, while 11 per cent gelatin and 5 per cent casein, which contain less cystine, do not improve the diet unless cystine is also added. These results are shown in Table 16, also. Eighteen per cent casein, of course, completely satisfies the protein requirements in itself. In the light of the recent work of Womack, Kemmerer, and Rose (1937), this beneficial effect of cystine must be interpreted as showing that *Elodea* contains some methionine but not enough to allow for the synthesis of all the cystine that is needed by the animal. Although only one rat was used in each of the protein supplement experiments, they indicate clearly that the dietary essential amino acids must be pretty well supplied by *Elodea,* and only small supplements of methionine or cystine, or of proteins containing these amino acids, are needed to make *Elodea* complete as the sole source of protein in the diet.

The Van Slyke analysis of the water-soluble protein in *Elodea,* as

Table 16. Growth Experiments with Rats on Elodea With and Without Protein Supplements

Ration No.	Supplement cystine daily	Rat No.	Paired feeding: Period on experiment	Paired feeding: Food eaten, daily average: Total	Paired feeding: Food eaten, daily average: *Elodea*	Weight: Start	Weight: End	Gain in weight daily average
	mgm.		days	gm.	gm.	gm.	gm.	gm.
11	none	311	11	5.89	4.15	60	74	1.27
11	20	307	10	5.87	4.14	60	77	1.70
11	20	312	11	5.89	4.15	60	79	1.73
11	none	308	11	4.98	3.51	58	65	0.64
11	20	309	11	4.98	3.51	58	73	1.36
11	none	313	11	5.95	4.19	58	74	1.45
11	20	314	11	5.95	4.19	59	76	1.55
11	none	315	10	5.35	3.77	62	72	1.00
11	20	316	10	5.35	3.77	62	76	1.40
			Ad libitum feeding					
11	20	305	14	6.57	4.63	58	94	2.57
11	20	306	14	7.71	5.44	58	105	3.36
	% protein							
12	11 gelatine	50	21	3.10	2.08	63	48	−0.71
13	11 gelatine + 0.2 cystine	67	20	7.80	5.23	63	108	2.25
14	11 zein	70	21	6.80	4.56	64	98	1.62
15	5 casein	47	21	5.00	3.35	59	64	0.24
16	5 casein + 0.3 cystine	47	21	11.71	7.85	64	144	3.81
17	18 casein	19	40	10.98	7.36	53	153	2.50
17	18 casein	20	38	10.29	6.89	64	150	2.26
17	18 casein	21	38	11.68	7.83	56	165	2.87
18	18 casein + 5 yeast	13	11	7.60	4.56	53	74	2.45
18	18 casein + 5 yeast	14	17	8.60	5.16	59	99	2.35
18	18 casein + 5 yeast	15	9	7.10	4.26	66	83	1.89

Table 17. Growth Experiments with Rats on Myriophyllum With and Without Protein Supplements

Ration No.	Supplement amino acid or protein	Rat No.	Period on experiment	Food eaten, daily average		Weight		Gain in weight, daily average
				Total	*Myriophyllum*	Start	End	
	per cent		days	gm.	gm.	gm.	gm.	gm.
11	none	226	14	3.21	2.27	61	44	—1.21
11	none	227	14	3.16	2.24	60	42	—1.29
19	0.5 cystine	218	14	3.43	3.00	60	39	—1.50
19	0.5 cystine	219	9	2.56	2.24	60	40	—2.22
20	0.5 cystine	222	14	4.57	3.20	60	55	—0.36
20	0.5 cystine	223	14	4.64	3.25	58	54	—0.29
21	0.5 cystine+ 11 gelatine	243	8	6.88	4.38	85	87	0.25
21	0.5 cystine+ 11 gelatine	245	8	5.88	3.75	75	71	—0.50
22	10 zein	298	14	2.71	1.72	62	44	—1.29
17	18 casein	33	15	5.10	3.42	78	71	0.87
18	18 casein+ 5 yeast	32	14	6.20	3.73	62	82	1.43

Table 18. Growth Experiments with Rats on Vallisneria With and Without Protein Supplements

Ration No.	Supplement amino acid or protein	Rat No.	Period on experiment	Food eaten, daily average		Weight		Gain in weight, daily average
				Total	*Vallisneria*	Start	End	
	per cent		days	gm.	gm.	gm.	gm.	gm.
11	none	228	14	3.57	2.52	62	45	—1.21
11	none	229	14	3.21	2.27	62	46	—1.14
19	0.5 cystine	220	11	3.45	3.02	60	38	—2.00
19	0.5 cystine	221	10	3.10	2.71	62	39	—2.30
20	0.5 cystine	224	14	3.57	2.50	60	50	—0.71
20	0.5 cystine	225	14	3.54	2.48	61	49	—0.86
21	0.3 cystine+ 11 gelatine	252	8	4.36	2.78	70	61	—1.13
21	0.3 cystine+ 11 gelatine	255	8	6.13	3.90	79	75	—0.50
22	10 zein	299	19	4.21	2.67	59	54	—0.26
22	10 zein	300	19	4.26	2.70	59	54	—0.26
23	7 zein+ 11 gelatine	301	11	4.82	2.80	58	57	—0.09
23	7 zein+ 11 gelatin	302	10	4.30	2.49	61	59	—0.20
24	0.5 cystine+ 5 casein	303	19	5.37	3.79	61	70	0.47
24	0.5 cystine+ 5 casein	304	18	5.17	3.64	62	73	0.61
17	18 casein	35	28	6.00	4.02	58	80	0.79
18	5 yeast+ 18 casein	34	14	7.10	4.26	79	95	2.50

given in Part II of this bulletin, indicates a high percentage of tryptophane, tyrosine, histidine, and lysine while the arginine is only moderately high and the cystine is definitely low. Thus the biological analysis confirms the chemical analysis as to the fairly good quality of the protein in *Elodea*.

Similar *ad libitum* feeding growth experiments were carried out with *Myriophyllum* and *Vallisneria* and are shown in Tables 17 and 18, but in no case did supplements of cystine, gelatin, or zein improve the quality of the proteins sufficient to allow growth. Supplements of 5 per cent casein plus 0.5 per cent cystine allowed a slight amount of growth, but 18 per cent casein was required to give good growth. These experiments indicate a much greater deficiency in the dietary essential amino acids in *Myriophyllum* and *Vallisneria* than in *Elodea* and are further proof of their low biological value.

DISCUSSION

Table 19 gives a comparison of the data on the proximate and mineral analyses of the fresh-water plants in this bulletin with that of other workers on the same plants (see Tables 1, 2, and 3) and on alfalfa. There is

Table 19. Proximate and Mineral Analyses (Chiefly Dry Basis) of Fresh-water Plants Compared with Published Data on Same Plants and Alfalfa

	Elodea		*Myriophyllum*		*Vallisneria*		Alfalfa
	Data this paper	Ferle (1904)	Data this paper	Schuette and Hoffman(1921)	Data this paper	Schuette and Alder (1927)	Woodman, Evans and Norman (1933)
			Fresh basis				
				per cent			
Dry matter	7.52	12.00	13.32	9.80*	5.15	7.10*	29.35
			Dry basis				
				per cent			
Ash	21.87	19.69	13.83	20.72	15.64	25.18	7.72
Crude protein	26.81	16.97	25.83	18.75	15.15	11.80	17.29
Ether extract	3.53	2.29	2.47	2.44	4.28	0.73	1.88
Crude fiber	15.39	16.72	14.13	15.01	15.82	14.00	35.63
N-free extract	32.40	42.83	43.74	35.88	49.11	41.41	37.48
CO_2	1.49	14.19	1.47	.....	0.60	.....	.....
Sand	2.27	0.25	0.77	.....	0.09	.....	.....
SiO_2	6.28	2.56	2.94	1.96	1.07	5.45	0.10
P	0.57	0.48	0.42	0.55	0.21	0.23	0.15
S, total	0.27	.....	0.43	.....	0.38	.....	0.76
S, SO_4	0.07	0.14	0.21	1.36	0.21	0.85	0.09
S, organic	0.20	.....	0.22	.....	0.17	.....	0.67
Cl	0.58	0.51	1.34	1.62	2.01	1.32	0.61
Na	0.50	1.11	0.75	.....	2.39	0.60	0.04
Mg	0.65	0.91	0.74	0.81	0.76	1.13	0.25
K	3.65	3.53	1.87	.....	6.77	4.55	2.40
Ca	2.80	3.10	2.77	3.06	1.55	5.83	1.44
Mn	0.331	.....	0.513	trace	0.039	0.37	0.0047†
Fe, total	0.408	.....	0.066	0.06	0.045	0.57	0.0132‡
Fe, available	0.322	.....	0.033	.....	0.019	.....	0.0036‡

* This analysis by Rickett (1924).

† This analysis by Bolin (1934).

‡ This analysis by Sherman, Elvehjem and Hart (1934).

a close agreement on the whole, both among the three aquatic plants and in general with alfalfa. Considerable variability might occur owing to the maturity of the plant at the time of sampling, the subsequent treatment, and the nature of the soil and water in which the plant was growing. The outstanding differences will be pointed out here.

The method of determining the dry-matter content depends primarily upon getting the fresh green weight. The method described in this bulletin insures that result. Thus the dry-matter content found for *Elodea* is lower than reported by some workers, although it is similar to that reported by Rickett (1924). For *Myriophyllum* the dry-matter content is similar to that reported by Gortner (1934). These aquatic plants contain much less dry matter than alfalfa. However, Woodman *et al.* (1934) reported only 13.54 per cent dry matter in pre-bud alfalfa, and Peterson and Skinner (1931) reported only 6.2 per cent dry matter in lettuce. Therefore, the fact that these plants grow completely submerged in water does not mean that when they are freed of their surface water they will necessarily contain less dry matter than land plants.

The ash content found for *Myriophyllum* and *Vallisneria* was found to be lower than that reported by most workers. These plants contain considerably more mineral matter than alfalfa.

The crude-protein content of *Elodea* found in this study is higher than that reported by some workers but similar to that reported by the Holland workers (1918). The figure for protein in *Myriophyllum* is also higher, while for *Vallisneria* it is similar to that reported by Gortner (1934) and between that of Schuette and Alder (1927) and Birge and Juday (1922). These plants compare favorably in crude protein content with the best of the land forage plants as they are just as rich or richer than alfalfa which represents the legumes.

Crude fiber is uniformly low in these aquatic plants compared to alfalfa. The reason for this is that the buoyancy of the water holds the plants up so that they do not need so many cellulose strands.

There is only one report on the CO_2 content of these plants in the literature, and that is by Ferle (1904) for *Elodea*. His figure is about ten times that obtained in this study. The method he used is not stated. There is a possibility that he heated his material high enough to liberate CO_2 from the uronic acids and pentoses present, as his figure corresponds to the sum of these two, as given in Part III of this bulletin. Phillips *et al.* (1933) gives values for the CO_2 content of land plants from 0.9 to 4.43 per cent, which corresponds to the results found for the aquatic plants analyzed in this investigation. Evidently the large amount of minerals present in these plants exists in a more stable form, possibly as proteinates. This possibility is supported by the fact that it is practically impossible to separate and concentrate the proteins free of ash either by water, alcohol, salt, acid, or alkali extraction or by electrical dialysis. Preliminary experiments were done along these lines for the purpose of obtaining the crude proteins for biological studies.

A higher content of SiO_2 was obtained for *Elodea* than that reported by Ferle (1904). For *Vallisneria,* the content of SiO_2 was lower than that reported by Schuette and Alder (1927) but similar to that reported by Birge and Juday (1922).

The P content of these aquatic plants is higher than that reported by most workers for alfalfa; however, it is more like the figure given by Woodman and Eden (1935).

The S content found in this study is slightly less than that reported by other workers for these plants and relatively much less than for alfalfa. This is especially true for the organic S.

The Na content of *Vallisneria* found in this study is about four times that reported by Schuette and Alder (1927). These aquatic plants appear to contain from 12 to 60 times as much Na as does alfalfa.

The Mn values for *Elodea* and *Myriophyllum* are similar to the value Schuette and Alder (1927) reported for *Vallisneria,* while the value found in this study for *Vallisneria* is only a fraction of the value they reported. These plants may be 100 times richer in Mn than is alfalfa.

The total Fe content found for *Vallisneria* is less than the value reported in the literature. These plants are much richer in Fe than is alfalfa. Two of the richest sources of Fe listed by Sherman *et al.* (1934) are yeast and spinach with 0.43 and 0.53 per cent total Fe and 0.28 and 0.12 per cent available Fe, respectively. Thus *Elodea* appears to be one of the richest plant sources of available Fe, with 0.322 per cent.

Table 20. Fertilizer Units

Plant	N	P_2O_5	K_2O
	parts	parts	parts
Elodea canadensis	4.29	2.61	8.79
Myriophyllum spicatum	4.13	1.92	4.51
Vallisneria spiralis	2.42	0.92	16.30

The concentration of a few of these substances is expressed in fertilizer units in Table 20. Thus these plants could be used as complete fertilizers, with *Elodea* ranking as a 4-2-8 type, *Myriophyllum* as a 4-2-4 type, and *Vallisneria* as a 2-1-16 type. This would be one way of combating the natural forces of wind, stream, run-off water, and wave action which remove the richest of the surrounding land, the top soil, and bring it into the lakes and streams. To this very rich nutrient solution in the lake are added the remains of plant and animal life. If this ideal seedbed cannot be used to produce food for animals, owing apparently to its musty and fishy odor, it could be used to produce a concentrated fertilizer. This would be especially profitable around many of the resort lakes in Minnesota, where it is a common practice to cut the lake "weeds" with a motor-powered sickle attached to the front end of a flat-bottom boat. This is done to clean up bathing beaches and to keep boat lanes open. As the wind blows the plants in to shore, it is neces-

Table 21. Vitamin and Protein Values (Dry Basis) of Fresh-water Plants Compared with Each Other and with Published Data on Alfalfa

	Data this paper			
	Elodea	*Myriophyllum*	*Vallisneria*	Alfalfa
β-carotene—milligrams per cent	22.2	21.4	17.5	1.7*
Vitamin A—International Units per gram	370.1	356.6	291.7	7.5-60.0†
Vitamin B (B_1)—Chase-Sherman Units per gram	3.76	2.61	1.83	1.7-7.0‡
Ascorbic acid—milligrams per cent	10.5	24.8	75.7	.....
Vitamin C—International Units per gram	2.1	5.0	15.1	.....
Vitamin D—International Units per gram	0.0	0.0	trace	0.0.0.3†
Vitamin E	0.0	0.0	present§	present‖
Vitamin G (B_2) Bourguin-Sherman Units per gram	8.68	7.60	5.73	0.4-5.3‡
Biological value of protein—per cent	42.71	0.0	0.0	62.0¶
Coefficient of apparent digestibility of protein—per cent	70.28	27.44	28.60	42.0¶

* Smith (1932).
† Scheunert and Schieblich (1934).
‡ Douglass, Tobiska, and Vail (1933).
§ 43.1 grams gave a live implant efficiency of 52.63 per cent.
‖ 15 per cent in ration gave litters (Hathaway, Davis, and Graves, 1932).
¶ Nevens (1921).

sary to dispose of them. Owing to their lack of crude fiber, they would disintegrate rapidly and easily when applied to the land. Furthermore, when used as fertilizer they would not have to be washed as would be the case if used as animal food.

Table 21 gives a comparison of the vitamin and protein values of fresh-water plants and alfalfa. Of interest from the viewpoint of using these aquatic plants as a forage crop is the fact that they appear to contain all the vitamins in at least as high an amount as alfalfa. Of more significance is the fact that the biological value of the protein in *Elodea* is about 70 per cent that of alfalfa, and the digestibility is apparently better.

Cystine seems to be a limiting amino acid in *Elodea* as well as in alfalfa (Haag, 1931). The very low biological value and digestibility of the proteins in *Myriophyllum* and *Vallisneria* practically eliminates them as a potential animal food. This might be one explanation for their unpalatability, because if the right supplements of protein, fat, and vitamins are made, *Myriophyllum* and *Vallisneria* seem to be eaten readily and give good gains. Under such conditions, there is no evidence of any toxicity whatsoever connected with these plants, at least for rats. This suggests the possibility that some other plant or plants might be found that would be suitable to aquaculture and also highly desirable as a forage plant. *Elodea* from lakes might be as satisfactory as from cool, fresh-water streams.

CONCLUSIONS

Proximate analyses indicate that *Elodea, Myriophyllum,* and *Vallisneria* with their high protein, high carbohydrate, and low fiber should be highly nutritive.

These plants are sufficiently rich in minerals so that at a 20 per cent level in rations (dry basis) they would furnish all the minerals needed in nutrition, with the possible exception of P, Na, and Cl.

No evidence was obtained in this study that any of the other elements present in large excess are toxic (at least for rats).

Vitamins B (B_1), C, and G (B_2), and carotene as a source of vitamin A are adequately supplied by these plants, but vitamins D and E are lacking, especially in *Elodea* and *Myriophyllum.*

The greatest difference among these three plants from the nutritive standpoint is in the quality of their proteins. The proteins in *Myriophyllum* and *Vallisneria* have very low digestibility, and their biological value is too low to be accurately determined. The protein of *Elodea,* however, has a biological value of 42 per cent and a coefficient of apparent digestibility of 70 per cent.

Small supplements of cystine appear to supply the only dietary essential amino acid missing from *Elodea.*

Elodea may well serve as a forage crop for livestock under local conditions where there is cool, fresh water and plenty of hand labor.

These plants would have a high nutritive value for agriculture if used as a complete fertilizer to return to the soil the nutrients which have been washed away.

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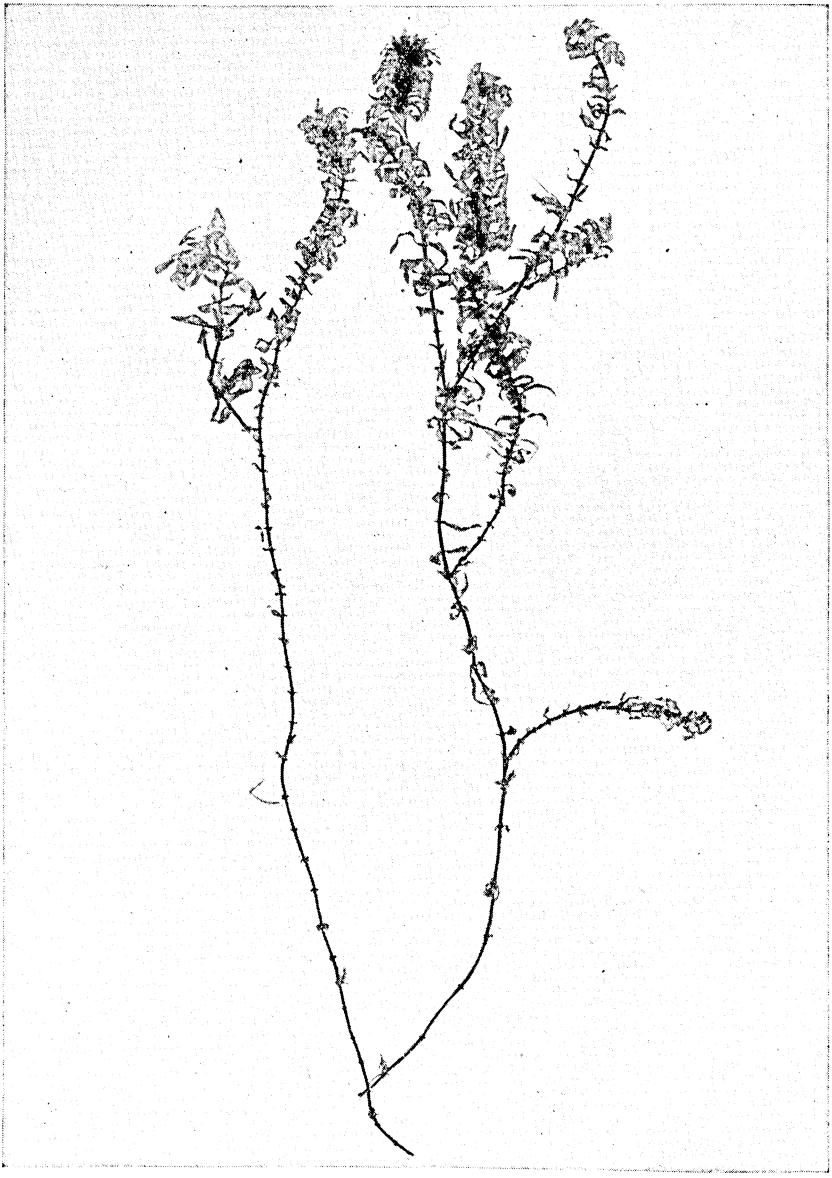
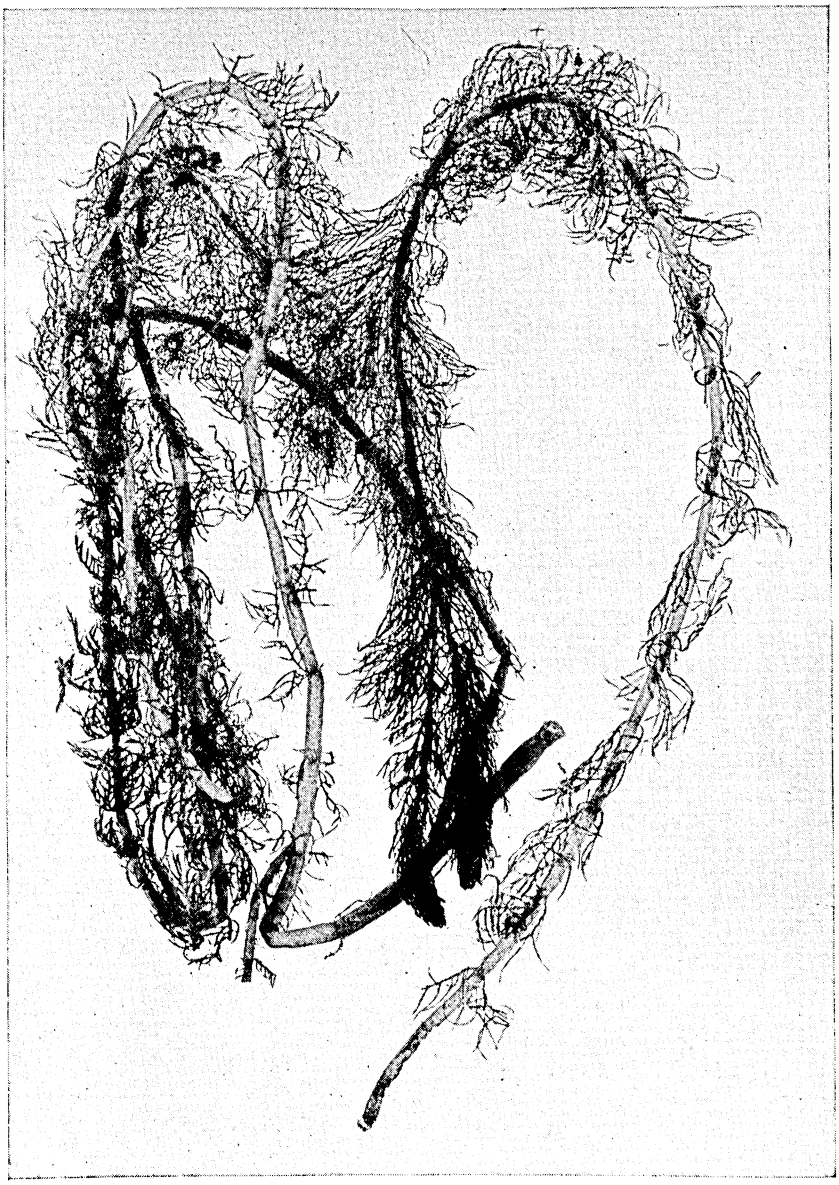


Elodea canadensis

Common names: Ditch moss, water thyme, water weed. (Natural size.)



Myriophyllum spicatum

Common name: Water milfoil. (Natural size.)

Vallisneria spiralis

Common names: Eelgrass, water celery. (Natural size.)

II. The Nitrogen Distribution of *Elodea canadensis*

ARNE N. WICK AND W. M. SANDSTROM

THE NITROGEN CONTENT of various fresh-water plants has been determined (Schuette, 1922-1929; Juday and Birge, 1922; Juday, 1935; Gortner, 1934; Harper and Daniels, 1934), but in no case has the nature of the nitrogen compounds been examined. On the other hand, rather complete studies have been reported on leafy land plants, such as alfalfa (Osborne *et al.*, 1921; Vickery, 1924-1925; Chibnall and co-workers, 1924, 1926) and spinach (Chibnall, 1923). These studies indicate that a considerable portion of the nitrogen is in the form of compounds of lower molecular weight than proteins. It seemed desirable to determine the nitrogen distribution of a typical Minnesota aquatic plant; *Elodea canadensis* was selected because it has been widely used for other physiological studies and because it is comparatively rich in nitrogen.

MATERIAL AND METHODS

The *Elodea canadensis* was obtained from Coon Creek near Fridley, Minnesota, at intervals during the summer of 1935. Only the green plants from a pure stand were used, and the material was carefully washed in the laboratory. Representative samples were taken for total nitrogen and for total solid determinations.

PRELIMINARY EXPERIMENTS

Water Extraction

The material was ground ten times with an excess of water in a food chopper using the finest adjustment. The resulting dark green material was so finely divided that no fibers could be detected. It was stored overnight at 0° C. The next day the water was filtered off through a cloth towel, after which additional water was expressed by hand. The residue was placed in a duck cloth and more water expressed in a hydraulic press. The liquids obtained above were united and labeled "the water extract." The residue was allowed freely to imbibe wash water which was again expressed. This was repeated until a negligible amount of nitrogen was obtained in the wash water. The water extract contained 23 per cent, and the successive washings 5.5, 1.8, and 0.4 per cent, respectively, of the total leaf nitrogen. The washings and the original water extracts were combined for further study.

The Water-Insoluble Residue

Attempts were now made to extract the remaining nitrogen in the water-insoluble residue by various solvents. The extractions were made on separate residues resulting from the water extract. Residues were permitted to stand in solvents for 12 hours at 0° C. before expressing the solvent. Table 1 shows results obtained with these solvents.

Table 1. Per Cent of Total Leaf Nitrogen Extracted by Solvents on Residue Resulting from Water Extract

1.	10 per cent sodium chloride	1.6 per cent
2.	2 per cent hydrochloric acid	1.43 per cent
3.	70 per cent alcohol	0.8 per cent
4.	0.3 per cent sodium hydroxide	5.7 per cent

Since the salt, acid, and alcohol showed negligible extraction power, it was decided to confine the extractions to the two solvents, water and alkali. Although most proteins are soluble in alkali, the amount of nitrogen extracted by the 0.3 per cent alkali in this case is very low. Osborne *et al.* (1921)', when working with alfalfa, found that 0.3 per cent alkali was not sufficient to extract any considerable amount of nitrogen. He then used 0.3 per cent alkali in 60 per cent alcohol and heated the ground residue together with solvent at 60° C. for three minutes before expressing the liquid; this procedure increased the nitrogen content of the extraction more than four times. The same procedure was used on *Elodea* and an increase of extracted nitrogen was obtained in about the same ratio.

Preliminary Attempts to Increase the Extractable Nitrogen

Attempts were made to increase the nitrogen extracted by the two solvents. For these experiments four lots of *Elodea* were gathered at different intervals during the summer. Each lot was subjected to a slightly different procedure for the extractions, including three washings.

Table 2. Nitrogen Extracted from Leaf Tissue

Lot number	1	2	3	4
Water-soluble nitrogen (per cent of total leaf nitrogen)	26.8	26.2	28.5	25.0
Alkali-soluble nitrogen (per cent of total leaf nitrogen)	31.7	15.8	19.6	27.7
Total nitrogen extracted	58.5	42.0	48.1	52.7

Lot One.—This lot was treated according to the previously mentioned methods. The water-soluble nitrogen was extracted after grinding. The alkali-soluble nitrogen was extracted using 0.3 per cent alkali in 60 per cent alcohol and heated at 60° C. for three minutes before squeezing.

Lot Two.—This lot was frozen in an icebox set at —18° C., and

ground in the frozen state. The nitrogen was extracted by the previously described method.

LOT THREE.—The *Elodea* comprising this lot was ground in a ball mill for 72 hours in a room at −18° C. In extracting the alkali fraction, the residue and the 0.3 per cent alkali (in 60 per cent alcohol) was heated at 60° C. for six minutes instead of three minutes.

LOT FOUR.—This lot was packed and frozen with carbon-dioxide ice for 24 hours. The extractions were performed in the usual manner.

ISOLATION OF THE PROTEINS

The Water-Soluble Protein

The water extract from lot 4, like all the others, was a dark green colloidal solution. It was centrifuged to remove all gritty material. Attempts were made to get rid of the chlorophyll by filtering through a thick wad of paper pulp as described by Chibnall and Grover (1926). However, the quantity of solution in this case made this impractical. The protein was then precipitated by acetic acid at pH of 4.5 as determined by the spot plate method. The protein was readily precipitated but carried the chlorophyll with it, leaving a clear brown solution. Heating or the addition of alcohol produced no additional coagulation. The protein was then separated from the mother liquor and transferred to distilled water. A little alkali was added to complete the peptization of the protein. The protein was then reprecipitated by bringing the solution to a pH of 4.5 and the protein again centrifuged off. The chlorophyll which was adsorbed on the protein was removed by extractions with acetone. The protein was then a grayish color and after drying was very easily pulverized. This protein without further treatment was found to contain 64.6 per cent ash.

Qualitative tests showed that the ash contained considerable calcium. It was hoped that the ash content of the protein could be reduced by precipitating the calcium before coagulating the protein. This was attempted on a new water-soluble fraction prepared for this purpose. The calcium was precipitated by potassium oxalate and allowed to stand overnight in an icebox. This, however, precipitated not only the calcium but also the protein, as only 1.9 per cent of the nitrogen remained in solution. The protein, containing 64.6 per cent ash, was then subjected to dialysis in a collodion bag for three days, but very little, if any, protein went into solution. After three days, the protein was separated by centrifuging and dried. A quantitative analysis of calcium, magnesium, and phosphorus was determined on this ash.

The Alkali-Soluble Protein

The alkali solution of lot 4 was centrifuged in the same manner as the water-soluble fraction to remove all foreign material. The protein was

Table 3. Ash Constituents of the Proteins from Lot 4*

	CaO	MgO	P_2O_5
	per cent	per cent	per cent
Water-soluble protein (6.8% ash)	24.3	1.7	32.0
Alkali-soluble protein (9% ash)	18.0	4.3	16.0

* Ash analyses were run by J. W. Nelson.

easily precipitated at pH 4.5, centrifuged, and dialyzed. After three days of dialysis, the protein was centrifuged without difficulty since it had not gone into solution. It was then dried and its ash content determined. A quantitative analysis for calcium, magnesium, and phosphorus was determined on the protein after ashing.

Although no complete extraction of the nitrogen or purification of the proteins was obtained in the preliminary experiments, it was deemed advisable to determine the distribution of the nitrogen that could be extracted.

FINAL EXPERIMENTS

The final lot (lot 5) was collected August 29, 1935, and taken to the laboratory and washed. Representative samples were taken for solid matter and total nitrogen determinations. The weighed material was ground with water and set aside at 0° C. until the next day. The water was then expressed as previously described. Three washings were added to the original extract. The water-insoluble residue was placed in 0.3 per cent alkali in 60 per cent alcohol and permitted to stand overnight at 0° C. The next day the alcohol-alkali solution and residue were heated at 60° C. for three minutes. The liquid was then expressed by hand and then by the hydraulic press. The residue was washed by permitting it to imbibe 0.3 per cent sodium hydroxide and the liquid removed in the press. Three such washings were made, after which the washings and the original extract were combined. The water-soluble protein and the alkali-soluble protein were precipitated, separated from the mother liquor, and dialyzed for one week.

Method of Analysis of Protein-Free Water Extract

This extract was analyzed for the following:

1. Ammonia Nitrogen

This was determined by the method of Vickery and co-workers (1935). It involves the distillation of a liquid sample containing ammonia nitrogen from a buffered alkaline reagent in a vacuum apparatus at 40° C. The ammonia is absorbed in an acid solution and the ammonia is determined by the Nessler method.

2. Amide Nitrogen

This was determined as described by Vickery *et al.* (1935). An aliquot of extract was made normal with respect to sulfuric acid and the mixture hydrolyzed for three hours on a water bath. The increase of ammonia was calculated as being due to amide nitrogen.

3. Other Amide Nitrogen

This represents the further increase of ammonia nitrogen after hydrolysis with 20 per cent hydrochloric acid for 16 hours.

4. Amino Nitrogen

Amino nitrogen was determined by the Van Slyke method. The ammonia was first removed as described in the ammonia determination.

5. Peptide Nitrogen

Peptide nitrogen represents the increase of amino nitrogen after hydrolysis with 20 per cent hydrochloric acid for 16 hours.

6. Nitrate Nitrogen

The nitrate nitrogen was estimated by the Gunning Kjeldahl method modified to include the nitrogen of the nitrates.

7. The Van Slyke nitrogen distribution was made by the traditional method (Morrow and Sandstrom, 1935). Cystine was determined on both the bases and the filtrate from the bases by the colorimetric method of Folin and Marenzi (1929). The value for cystine in the filtrate is not added into the total since it is already included in the amino nitrogen of the filtrate.

RESULTS

The analyses of nitrogen fractions on this lot of *Elodea* gave the values reported in Table 5. The Van Slyke nitrogen distribution of the two proteins is recorded in Table 6, which also contains, for purposes of comparison, the analysis obtained by Chibnall and Nolan (1924) on the water-soluble protoplasmic protein isolated from the leaves of the green alfalfa plant.

Table 4. Nitrogen Content of Dried Samples of Lot Five

Sample	1	2	3	4	5	6	Average
Dry matter in per cent of wet weight	7.27	8.31	8.20	8.10	7.81	6.68	7.72
Total nitrogen in per cent of dry weight	4.12	4.00	4.09	3.82	3.94	4.15	4.02

Weight of material on wet basis	2470.0	grams
Weight of material on dry basis (7.72% of 2470)	190.9	grams
Weight of nitrogen (4.02% of 190.0)	7.675	grams

Table 5. Distribution of Extractable Nitrogen in Lot Five

	Per cent of total leaf nitrogen	Per cent of water-extractable nitrogen
1. Water-soluble protein nitrogen	18.63	58.84
2. Ammonia nitrogen	0.22	0.71
3. Amide nitrogen	0.36	1.11
4. Other amide nitrogen	3.48	10.97
5. Amino nitrogen	5.48	17.25
6. Peptide nitrogen	1.02	3.40
7. Nitrate nitrogen	0.00	0.00
8. Alkali-soluble protein	21.60	.....
9. Nitrogen in residue	16.30	.....
Total	67.15	92.28

DISCUSSION

The high nitrogen content of *Elodea* is striking; it contains over 4 per cent of nitrogen in the oven-dried material as compared to the legume hays with 1.9 to 3.6 per cent (Morrison, 1936). Of this nitrogen, some 51 per cent has been accounted for as protein or as simpler nitrogen compounds.

The extraction of the nitrogen presented considerable difficulty. Similar results were obtained by Osborne, Wakeman, and Leavenworth (1921) who could extract only 57 per cent of the nitrogen in alfalfa. It is possible that untried techniques might yield better results; thus, the protein may be bound in a complex which might be broken if the proper technique were known. This is suggested by the experiments with alkalis. Aqueous 0.3 per cent alkali extracted approximately 6 per cent of the total nitrogen, while the same alkali in 60 per cent alcohol at 60° C. extracted nearly 20 per cent of the nitrogen.

The nitrogen, unaccounted for by extraction plus that in the residue, comprises nearly one third of the total. One possibility of a major source of discrepancy lies in the determination of the total solid material from which the calculation of total nitrogen is made. Thus, the material was drained by gravity, and six samples were taken for analysis with results ranging from 6.68 to 8.31 per cent of solids. An average figure was used for computations, which figure may not be representative. It is also recognized that the press cloths held some material.

The values for the fractions account for the major portion of the soluble nitrogen extracted and are somewhat similar to those found for leafy land plants, with the exception of the fraction labeled "other amide nitrogen." This was determined on an aliquot subjected to much more drastic treatment than that necessary to split the ammonia from asparagine and allontoin (Vickery, Pucher, and Leavenworth, 1935). Since the value for the "other amides" was rather high, the determination was repeated during the summer of 1937. At that time the "other amide nitrogen" was approximately one third of the regular amide nitrogen.

It is to be noted that the values reported in Table 5 were determined on a lot collected on August 29, whereas the smaller values were obtained on a lot collected a month earlier in the following season. Part of the volatile amines produced by the more drastic hydrolysis was not ammonia, as evidenced by the color, and the decomposition point of its chloroplatinate was a deep orange and decomposed at from 280° to 300° C., whereas the regular amide nitrogen gave exclusively crystals of the chloroplatinate of ammonia.

The two proteins were peculiar in resisting all efforts to obtain them ash free. The metals are obviously held tenaciously by the protein. In comparing the Van Slyke analysis of the two proteins of *Elodea* and that which Chibnall obtained from alfalfa, one can see several large differ-

Table 6. Nitrogen Distribution in Elodea (Per Cent of Nitrogen)

	Water-soluble protein			Alkali-soluble protein	Data of Chibnall and Nolan
	A	B	Average		
Ammonia nitrogen	5.61	5.10	5.35	3.28	5.51
Acid-insoluble humin	3.55	3.12	3.33	6.18	1.46
Acid-soluble humin	7.12	9.34	8.23	4.80	1.22
Basic nitrogen					
Arginine	7.30	7.46	7.38	9.70	15.32
Histidine	5.71	3.27	4.49	3.43	3.09
Cystine	0.12	0.18	0.15	0.11	0.84
Lysine	10.25	12.00	11.12	10.10	9.97
Filtrate nitrogen					
Amino	56.41	54.28	55.34	56.37	58.56
Non-amino	5.39	1.85	3.62	7.81	3.19
Cystine	0.37	0.32	0.35	0.22	.....
P.T.A. humin	0.50	0.88	0.69	0.95	0.04
Total	101.94	96.68	99.70	102.91	99.20
Per cent nitrogen	.....	.....	10.71	9.66	.....
Per cent ash	.....	.....	2.41	8.34	.....

ences. The humin nitrogen in both proteins of *Elodea* is much higher than that in alfalfa. This suggests that there is more trytophane in *Elodea.* However, the per cent arginine in alfalfa protein is nearly double that in the *Elodea* protein. The cystine in alfalfa is higher than it is in *Elodea.* This is to be expected as Chibnall probably used the total sulfur method, whereas in our studies a specific color test was employed.

SUMMARY

The nitrogen distribution of *Elodea canadensis* has been determined. Of the water-soluble nitrogen, over one half was isolated as a protein and one third was found to be distributed as simpler compounds such as ammonia, amides, amino acids, and polypetides. Like the land plants which have been studied, not all of the nitrogen compounds were extractable.

Two proteins, one water-soluble and the other alkali-soluble, were isolated and characterized by the Van Slyke nitrogen distribution. It was not possible to prepare these proteins ash-free in spite of several modifications.

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III. The Nature of the Carbohydrates of Species of *Elodea, Myriophyllum, Ceratophyllum, Ruppia,* and *Ranunculus*

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Fresh-water plants have been suggested as a source of cattle feed because they are fairly abundant in certain regions and because they have a high content of crude protein (Gortner, 1934) accompanied by a low content of crude fiber (Schuette and Alder, 1927; Schuette and Hoffman, 1921). It seemed desirable, therefore, to make a more nearly complete study of the various carbohydrate constituents of certain more common species of Minnesota aquatic plants.

MATERIAL AND METHODS

The plants chosen were *Ceratophyllum demersum, Myriophyllum spicatum, Ruppia occidentalis, Elodea canadensis,* and *Ranunculus aquatilis.* The *Elodea* was obtained at Rice Creek and the other four species at Lake Owasso, Minnesota, all on July 1, 1935. Typical fresh samples were selected, washed free from adhering dirt, and immediately pressed free of excessive water by placing between newsprint.

Representative samples were taken for dry-matter determinations, and two 50-gram samples of each were placed in alcohol containing a little powdered calcium carbonate for the determination of soluble sugars (Assoc Off. Agri. Chem., 1935). The remainder was divided into two groups. One part was frozen in order to determine, some time later, the effect of freezing on the simple sugar content of the material; the other fraction was placed in hot air chambers until thoroughly dry and then pulverized in a ball mill. The powder was kept in well-stoppered bottles until ready for polysaccharide analysis. The residue from the alcohol extraction was dried, weighed, and pulverized in a ball mill. This powder was kept in well-stoppered bottles until ready for starch determination. In all cases, the samples were re-dried immediately before analysis.

The determinations of soluble sugars were made on the alcohol extracts (Assoc. Off. Agri. Chem., 1935). The reducing sugars were determined by the Shaffer and Hartman method (1921) and calculated as glucose. Sucrose was determined by hydrolysis with invertase (Assoc. Off. Agri. Chem., 1935). Total soluble sugars were determined after hydrolysis with hydrochloric acid but gave the same values as those for sucrose by the invertase method; hence it was assumed that sucrose was the only disaccharide present.

Starch was determined by a taka diastase hydrolysis (Collins, 1927) on the residue from the alcohol extraction. The hydrolysis proceeded to the glucose stage, and the results were multiplied by 0.9 to obtain starch values.

The dried whole material was used for the following determinations: ash-free crude fiber (Assoc. Off. Agri. Chem., 1935, p. 340); pentose-yielding materials (Assoc. Off. Agri. Chem., 1935, p. 344); ash-free Cross and Bevan cellulose by Phillips' modification (1932); and the uronic-acid anhydrides (Dickson *et al.*, 1930; Phillips *et al.*, 1933).

Table 1. Proximate Analysis of Samples

Material	Dry matter in per cent	Ash in per cent of dry matter	Crude protein in per cent of dry matter
Ceratophyllum	10.21	15.7	12.9
Myriophyllum	16.14	13.4	14.8
Ruppia	17.47	15.9	16.5
Elodea	11.09	22.9	25.8
Ranunculus	15.18	17.8	8.4

The official phloroglucinol method is taken as a measure of pentosan content, but it is to be recognized that the process of converting pentosans to furfural will also convert hexuronic acids by decarboxylation to pentoses, and these ultimately to furfural. Consequently this fraction is labeled "pentose-yielding materials."

For the determination of uronic-acid anhydrides (Dickson *et al.*, 1930), the preliminary heating below 100° C. was continued for one hour in order to remove all carbon dioxide arising from carbonates, since these plants may contain as much as 20 per cent of calcium carbonate.

All analyses were made in duplicate and, in the case of the soluble sugars, on two lots from each plant carried through the alcohol extraction. The duplicate determinations agreed very well except in the analyses for uronic-acid anhydrides, where the greatest difference between duplicates was 1.71 per cent in the runs on *Ceratophyllum.* The greatest percentage deviation was 9.9 per cent in the case of *Elodea;* this

Table 2. Carbohydrate Distribution in Samples

Material	Reducing sugar	Sucrose	Starch	Crude fiber	Cellulose	Pentose-yielding material, as pentosan	Uronic-acid anhydride
Ceratophyllum	0.28	0.86	5.51	16.89	15.51	6.18	9.5
Myriophyllum	0.64	1.37	6.00	13.08	11.48	9.29	9.2
Ruppia	0.15	1.58	3.04	16.72	16.40	9.41	11.4
Elodea	0.45	0.85	1.28	12.30	11.85	6.84	7.3
Ranunculus	0.64	2.30	5.08	16.52	14.50	10.56	9.8

figure is large because of the low uronic-acid content of *Elodea*. From other work in the Division of Agricultural Biochemistry, it has been found that other carbohydrates are slowly oxidized to yield carbon dioxide; thus, 1 gm. carbohydrate gives 0.45 per cent of carbon dioxide, equivalent to 1.80 per cent of uronic-acid anhydrides (Guanzon and Sandstrom, 1937). Since these corrections are low, they are not applied to the uronic-acid anhydride values as determined.

Crude protein and ash determinations were also made for general interest. The former was calculated from Kjeldahl determinations using the factor 6.25. Table 1 reports these values calculated on the oven-dried basis.

Table 2 shows the carbohydrate distribution; all values are reported as percentages of the oven-dried material.

EFFECT OF FREEZING ON COMPOSITION OF SAMPLES

A portion of the fresh material from each species was immediately placed in a refrigerator at —17° C. for 30 days, after which the material was treated in the same manner as the unfrozen samples for sucrose and reducing-sugar analyses. Table 3 presents the analysis of comparable samples of the frozen and the unfrozen material. No very great changes or tendencies were detected.

Table 3. Sugars in Fresh and Frozen Material

	Fresh		Frozen	
	Reducing sugars	Sucrose	Reducing sugars	Sucrose
Ceratophyllum	0.28	0.86	0.41	1.10
Myriophyllum	0.64	1.37	0.71	2.09
Ruppia	0.15	1.58	0.36	1.60
Elodea	0.45	0.85	0.31	0.70
Ranunculus	0.60	2.30	0.62	2.06

DISCUSSION

The values for reducing sugars and for sucrose are low as compared with alfalfa and timothy hays (Morrison, 1936). However, the starch contents of the water plants studied are higher than those of the same two hays so that the totals for the sugars and starches in these aquatic plants (6.65, 8.01, 4.77, 2.18, and 8.02 per cent, respectively, for the plants analyzed) extend both above and below the values for alfalfa and timothy hays (4.85 and 5.70 per cent, respectively).

Crude-fiber values of from 12.30 to 16.89 per cent are from one-half to one-third those reported for hays (Morrison, 1936, pp. 954-966). Arber (1920) has summarized the structural differences encountered in aquatic plants and pointed out in particular that water plants need less fiber for structural purposes. The close agreements between crude-

fiber and cellulose contents suggest that the fiber consists mainly of cellulose and that little lignification has taken place. The cellulose here referred to is the "Cross and Bevan cellulose" (Phillips, 1932) and may contain small quantities of materials closely related to cellulose.

The values obtained by the official method for the estimation of pentosans are reported as "pentose-yielding materials." Since both uronic acids and pentosans are converted to furfural by boiling with 12 per cent hydrochloric acid, the "pentosan" values should include the five-carbon residue of the hexuronic acid. We have recalculated our values for uronic-acid anhydrides as the equivalent in pentosan (factor=0.75) and in Table 4 have compared these with the "pentosan" values obtained.

Table 4. Comparison of Pentosan and Uronic Acid Content

	Uronic-acid anhydride	Pentosan equivalent to uronic-acid anhydride	Pentosan found
Ceratophyllum	9.5	7.13	6.18
Myriophyllum	9.2	6.90	9.29
Ruppia	11.4	8.55	9.41
Elodea	7.3	5.48	6.84
Ranunculus	9.8	7.35	10.56

With the exception of *Ceratophyllum,* most of the "pentosan" material appears to exist in the form of uronic-acid anhydrides. In the case of *Ceratophyllum* the results are not easily explained, although it was pointed out earlier that the large amount of carbonates present required some modification of the method for determining uronic-acid anhydrides. It is felt that any errors in this determination tend to give too high values, owing to the slow evolution of carbonate-carbon dioxide.

The comparatively high uronic-acid contents, as compared with land plants (Guanzon and Sandstrom, 1937), accompanied by low pentosan values when corrected for the equivalent weight of uronic acids, are in keeping with the theory of Spoehr (1919). He postulated that pentoses, and hence pentosans, arose from the decarboxlyation of hexuronic acids by ultraviolet light. The amount of ultraviolet light that reaches the submerged plant is very low (Luckiesh, 1922) and hence little decarboxylation takes place, with the resultant higher content of uronic acids and little true pentosan.

CONCLUSIONS

1. Five species of aquatic plants abundant around Minnesota were analyzed for certain carbohydrate constituents. In all cases the sugars were lower than those in cultivated hays such as timothy and alfalfa, but the quantities of starch more or less offset this deficiency.

2. The crude fiber is lower than that of most hays and consists almost entirely of cellulose.

3. The content of "pentose-yielding materials" can largely be accounted for on the basis of the uronic acids present in these plants.

4. Storage at —17° C. appears to have slight effect on the soluble-carbohydrate content.

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