PRODUCTION AND NUTRIENT REMOVAL BY MACROPHYTE COMMUNITIES IN ARTIFICIAL STREAMS

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ABSTRACT

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by Glenn A. Duddles

Production and nutrient removal relationships were studied for three macrophyte communities in two continuous flow artificial streams. The streams were constructed for outdoor study to simulate natural growing conditions.

The artificial streams were established with water from the Red Cedar River which was stored in a large holding tank. Controlled enrichment in the form of phosphate and nitrate was added to stream A during two separate study periods. Daily upstream-downstream measurements were made to record nutrient removal within the system.

The macrophytes selected were *Ceratophyllum demersum* (L.), *Vallisneria americana* (Michx.) and *Elodea canadensis* (Michx.). These were gathered from local stream areas and planted in the streams on an artificial wire mesh substrate.

Community production measured by increase in dry weight was doubled in the enriched environment. There was not a noticeable difference in production between 4 mg/l and 2 mg/l enrichment levels. The *Ceratophyllum* community had the greatest cropped production (3.7 g dry wt/m^2/day) and the *Elodea* community the greatest cropped plus uncropped production (5.4 g dry wt/m^2/day).

The phosphate level was reduced by an average of 18.7% and the nitrate level by 19.5% in the artificial streams. A greater percentage of organic phosphorus and nitrogen was found in the macrophyte communities of the enriched stream. The average protein value of these communities was 19% including a maximum of 25% The N/P quotient for all the communities was relatively constant at 4.5.

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By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

Eutrophication is defined by Ruttner (1953) and Hutchinson (1957) as the natural process of lake maturation. This process proceeds at an infinitely slow rate under natural conditions. However, in recent years there has been increased alarm over an accelerated aging of many of our lakes and a degradation of our streams. This change is a direct result of pollution connected with civilization.

The first evidence of these problems occurs with the appearance of nuisance growths in the form of algae and higher aquatic plants. Such growths inevitably interfere with the municipal and recreational uses of the water.

A major source of the problem is excessive enrichment of natural waters with nutrients essential for plant growth, mainly nitrogen and phosphorus. In any nutrient problem, careful consideration must be given to the major sources which contribute the nutrients. A partial list of these would include nutrients from sewage, sewage effluents, industrial wastes, land drainage, applied fertilizers, precipitation, urban runoff, and release from bottom sediments.

The greatest contributor of nutrients from this list would be that from sewage and sewage effluents. For many years it was not uncommon for untreated sewage to be regularly discharged into rivers and lakes. The ramifications of this practice involve more than merely enrichment problems, and as a result of public interest, this type of pollution has been largely eliminated. Most sewage is now treated or at least partially treated before discharge into receiving waters.

However, in terms of enrichment, sewage treatment does not solve the problem involving eutrophication. The mechanical and biological sewage purification procedures used today remove only a small part of the effective nutritive material for plant growth. Organic phosphorus in the sewage plus simple and complex phosphates from synthetic detergents result in phosphorus concentrations far above the requirements for plant growth. Treatment or organic wastes results in an abundance of nitrogen in all forms. Ammonia and nitrite are transient forms and generally are oxidized to nitrates upon decomposition. Mackenthun (1964) reports secondarily treated sewage effluent from the Madison, Wisconsin, metropolitan area of 85 square miles with a population of 135,000 to annually contribute 8.5 pounds of nitrogen and 3.5 pounds of soluble phosphorus per capita.

This extreme enrichment has a tremendous effect on the growth of aquatic vegetation. Mackenthun (1965) indicates that submerged aquatic plants could be expected to produce at least 7 tons per acre containing 32 pounds of nitrogen and 3.2 pounds of phosphorus per acre.

This represents a significant removal of nutrients from the water by aquatic plants. William Beck (1962) removed 30 to 90% of the total nitrogen in a treated sewage effluent at Orlando, Florida, using beds of algae.

Oxidation ponds utilizing plants and algae for treatment have become widely used in small community operations. Bartsch and Allum (1957) studied the variability in design and loading for this method of treatment in the northern plains area. Although this treatment works sufficiently for stabilization of raw wastes, it does not eliminate the enrichment problem. The increased mass of algae and plants within the pond die and in time become a source of nutrient release within the ecosystem itself. Loehr and Stephenson (1965) discovered that the nitrogen and phosphorus content of a treated effluent was generally increased after time in an oxidation pond used as tertiary treatment.

The purpose of this study was to measure the effect of nitrogen and phosphorus enrichment on three higher aquatic plants and, conversely, the effect of the plants on the nutrient concentration in terms of nutrient uptake. This was done utilizing two separate 14 day growth periods during August 1967 at East Lansing, Michigan.

The three plants selected were *Ceratophyllum demersum*, *Vallisneria americana*, and *Elodea canadensis*. These were chosen on the basis of their availability and variety of growing nature. All three are vascular, submerged aquatic macrophytes found frequently in enriched environments. The *Ceratophyllum*, often referred to as coontail, was included because experiments by Schulze (1966) indicated that it had the greatest active production in a similarly enriched situation.

MATERIALS AND METHODS

Artificial Streams

A number of studies have been made concerning production by higher aquatic plants in natural and laboratory conditions, but none have incorporated the use of an artificial stream. Odum and Hoskin (1957) studied the production of microorganisms in an artificial laboratory system. Stokes (1960) and Kevern (1962) measured primary production by periphyton in a completely recirculating artificial stream.

The classical method for measuring growth by higher aquatics such as that used by Rickett (1922), Schuette (1928), or Penfound (1956) entails random cropping from a given area in a natural situation. A certain degree of error is inherent in growth measurements of this nature. Wetzel (1964) points out the importance of the rooting portions of macrophytes in determining production. Natural situations make accurate collection of entire rooted macrophytes extremely difficult. To minimize these problems, an artificial stream was constructed for this outdoor study to enable better control over the substrate area and certain growing conditions.

The channel dimensions were 104 feet in length, 2 feet in width and depth. The channels were constructed from 3/4 inch exterior grade plywood sheets attached in 8 foot lengths with wood screws. All joints were reinforced on the inside with 1×2 pine stripping and additional screws. Further strengthening was attained with a liberal application of Weldwood Plastic Glue to all fitted joints. This water insoluble glue also aided in making the channels watertight.

The eight foot sections were placed together upon the flat concrete edges of two large parallel reservoir tanks. These parallel units were then connected by two additional sections supported on a wood framework making a U-shaped channel out of 13 eight foot sections (Figure 1). All of the sections were firmly joined together using 1×6 pine boards with wood screws and sealed with glue. Crossbars were placed across the open top of the channels at four foot intervals to keep the sides from spreading.

A wall was constructed in the midpoint of the base, separating the structure into streams A and B. Throughout the study, stream A was used for experimentation while stream B was maintained as a control.

The interior of the streams was coated with two applications of Glidden Nu Pon Coat Epoxy Resin Paint which yielded a durable waterproof finish. All exterior surfaces were coated with a high quality exterior oil base paint.

Figure 1. Photograph of the entire artificial stream.

Flow Design

The streams were constructed so that water pumped in at each side of the separating midpoint would flow to the ends of each stream in a continuous non-recycling pattern. The water was discharged after overflowing the desired 18 inch depth at the end of streams A and B. The rate of discharge was in this way directly controlled by the incoming flow.

The water supply for this study was pumped from the Red Cedar River into the large storage tank alongside stream A. This was done with a large irrigation pump and 5 inch aluminum pipe. The storage tank was replenished as was necessary during the study period.

A Little Giant Submersible Pump, model #12NR was used to pump water from the storage tank into both streams. A 3/4 inch rubber hose attached to the pump was divided beyond an overflow shunt into feed lines for each stream. The overflow shunt and both feed lines contained compression valves to control the incoming flow (Figure 2).

Throughout the study, the incoming flow was maintained at 1.2 gpm which established a 16.5 hour retention time within streams. This flow was regularly checked with a volumetric container and a timer.

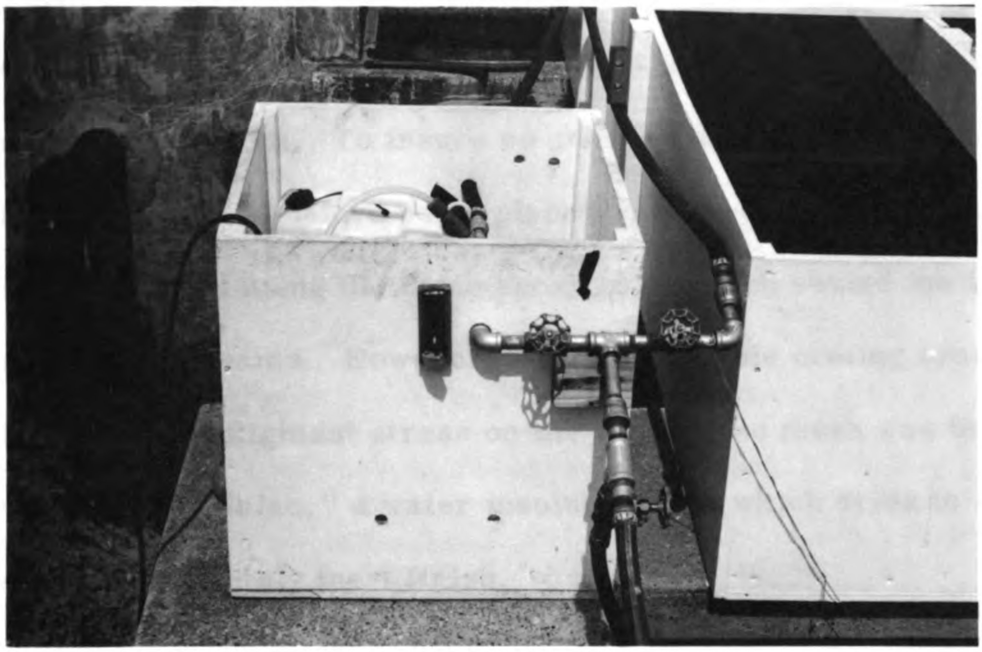


Figure 2. Photograph of the feed line assembly.

Substrate

In order to minimize the sorption, storage and release of phosphorus and nitrogen by substrate particles, an artificial substrate of 1/2 inch galvanized wire mesh was used in the streams. The mesh was formed into eight foot lengths and placed into each stream four inches from the bottom. To insure no contamination, the wire mesh was thoroughly coated before being placed into the channels. This was first attempted using the same epoxy paint which sealed the inner surfaces of the streams. However, this non-flexible coating cracked and chipped at the slightest stress on the wire. The mesh was then covered with "Flexiblac," a water insoluble paint which dries to a flexible, yet completely inert finish.

Aquatic Macrophytes

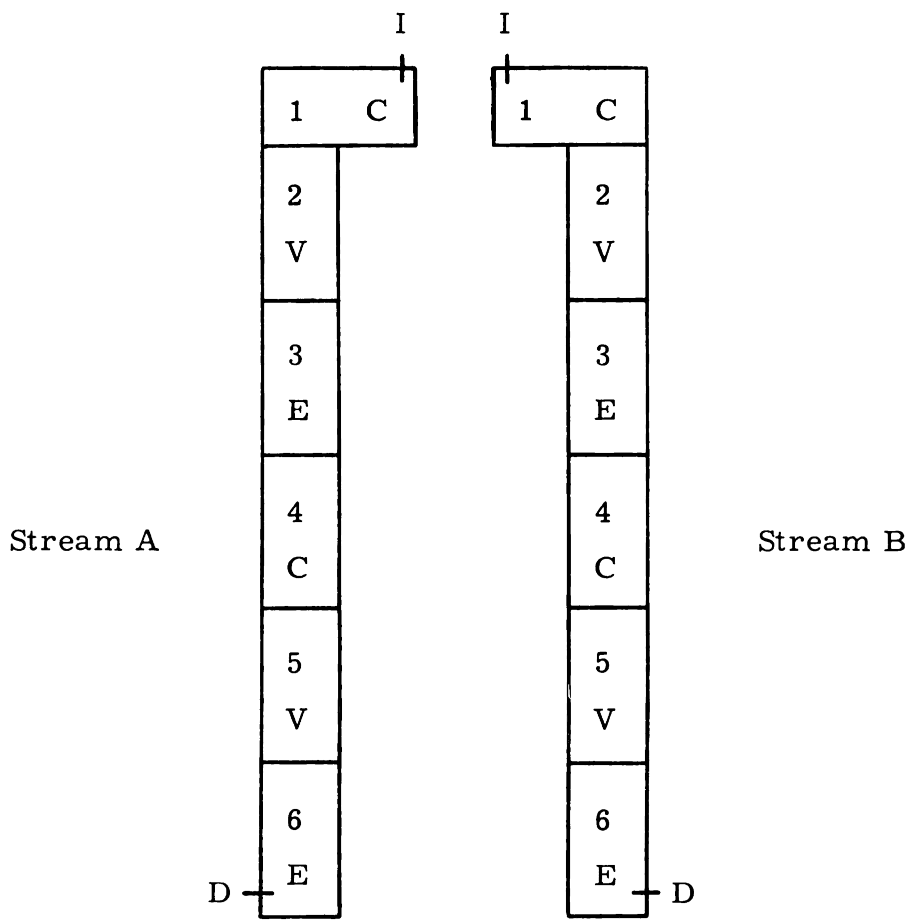
The plants used in this study were *Elodea canadensis*, *Vallisneria americana* and *Ceratophyllum demersum*. All were gathered from local streams and stored until planted in the artificial streams. At that time, each plant was hand picked to eliminate any damaged portions and wet weights were obtained for the plants placed in each section of the streams.

The plants were separated into the six equal eight foot sections within streams A and B. These sections were numbered 1 to 6 beginning at the head of each stream. Both streams thus contained two separate sections of each type of plant (Figure 3).

All of the Vallisneria was collected from the Grand River below the Waverly Road Bridge south and west of Lansing. These plants grew in abundance there scattered among others and were in excellent condition. Each plant was placed individually through the wire mesh with its root stock downward. This procedure, although tediously slow, had satisfactory results with minimum loss after planting.

The Elodea came from the Red Cedar River near the Zimmer Road Bridge west of Williamston, Michigan. It was planted in small clumps with the rooted portions pushed through the mesh 3 or 4 inches. Within a short time the clumps spread laterally, forming dense beds.

The Ceratophyllum also came from the Red Cedar River with half of it collected below the Zimmer Road Bridge and half at Ferguson Park in Okemos, Michigan. This plant had to be threaded down through the mesh and back out with both ends then extending upward. This procedure was necessary because of the non-rooting nature of this plant and was best accomplished with the wire out of the water and the plants placed in spaced rows.



I = Incoming Flow

D = Discharge

C = <u>Ceratophyllum</u>

V = <u>Vallisneria</u>

E = <u>Elodea</u>

Figure 3. Diagram of the sections of plants within streams A and B.

Nutrient Addition

The line of incoming flow into stream A passed through a small pump house at which point a concentrated nutrient feed solution was added. This was done with an electronic impulse regulated diaphragm feed pump from The Precision Chemical Pump Corporation, model #1201-1. This unit allowed accurate nutrient addition in amounts as small as 5 ml/min (Figure 4).

The nutrients added in this manner consisted of phosphate as the dibasic salt, KH_2PO_4 and nitrate nitrogen as potassium nitrate. The phosphate and nitrate concentrations were maintained at 4 ppm during the first growth period from August 1 to the 15th and at 2 ppm for the second period from August 18 to the 31st.

Community Production

Community production was measured by the increase in dry weight during the two week growth periods. The plants in all sections were cropped at a uniform height on the morning beginning a growth period. This was done utilizing a jig designed to rest on the sides of the channel with extensions down into the water and an adjustable horizontal edge. This adjustable edge was set at a level 6 inches above the wire mesh substrate and slowly moved down the stream while all plant material in contact with the edge was clipped

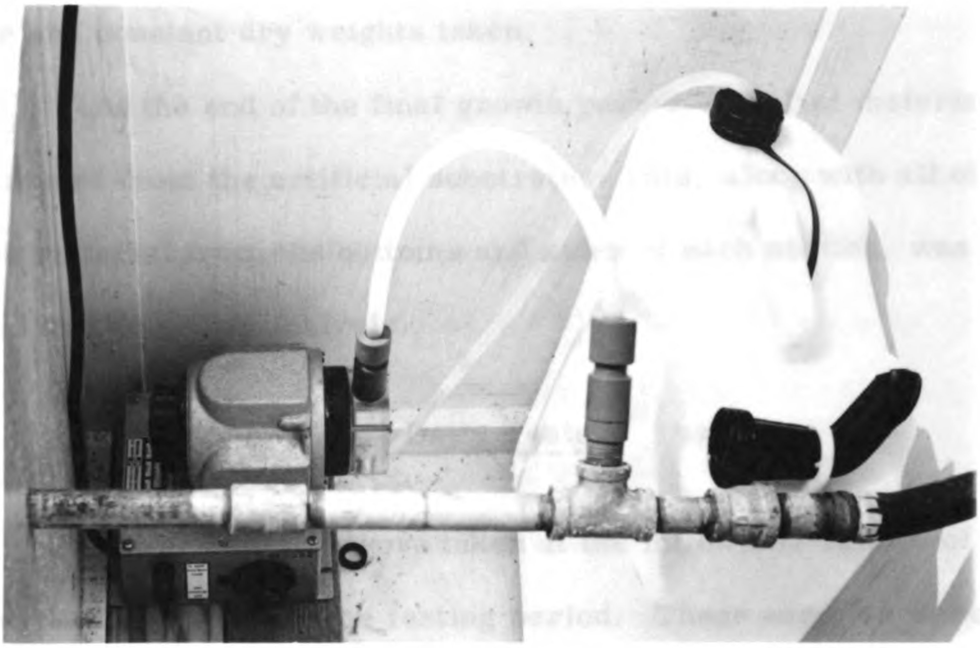


Figure 4. Photograph of the chemical feed pump assembly.

with hand grass cutters. This procedure was repeated at the end of the growth period and all cut material in each section was collected and wet weights obtained. The croppings were then dried in an oven at 105°C for at least 24 hours, after which they were cooled in a dessicator and constant dry weights taken.

At the end of the final growth period, all plant material was stripped from the artificial substrate. This, along with all other organic material from the bottoms and sides of each section, was weighed and dried for analysis.

Daily Tests

Water samples were taken at the inflow and outflow of each stream every day of the testing period. These samples were analyzed and the differences recorded daily.

Phosphate

Ortho-phosphate was measured daily using the ammonium molybdate-amino napthol sulfonic acid method as described in "Standard Methods for Examination of Water and Waste Water," APHA · AWWA · WPCF · , 1965. Total organic phosphate determinations were made on the cropped plant material and the final bottom scrapings following the appropriate test outlined in Standard Methods. The dried plant material was ashed in the furnace and uniformly mixed before testing for total phosphate.

Nitrogen

The Brucine Test was used to measure nitrate nitrogen while ammonia nitrogen was tested by Nesslerization. These procedures were followed as outlined in Standard Methods. Nitrite nitrogen was periodically checked using prepared powders developed by the Hach Chemical Company. The Bausch and Lomb Spectronic 20 was used to measure color in each case. Total Kjeldahl nitrogen determinations were made with a semi-micro method applying an Aminco system and a Sargent Spectro-Electro Titrator. Conversion to raw protein was made using a standard factor of 6.25.

Light, Temperature, and pH

Each time samples were taken, the air temperature and the water temperature were recorded. General observations of weather conditions and amount of light reaching the plants were noted daily. A Beckman Zeromatic II pH instrument was used to obtain pH readings.

RESULTS AND DISCUSSION

Study Conditions

Several distinct stages of plant condition were evident during the study period. Soon after the initial planting into the channels, most of the plants supported a dense growth of periphyton. This was a mixture of several species of algae which covered a major portion of the plants and channel walls. This first bloom seemed to reach its peak several days after the introduction of the river water supply and remained a factor throughout the study. These algae are generally associated with enriched habitats. (Prescott, 1962).

Near the end of the first growth period, a second bloom of *Oedogonium sp.* appeared in both streams and became the dominant algae. There was a continuous flow of fluffy clumps of this plant to the surface and out of the system.

The third algae of any significance was the filamentous *Cladophora glomerata* which began in the first three sections of stream A half way through the second growth period. Prescott (*op. cit.*) reports this algae is normally associated with cement walls. It probably came into the artificial stream from the large storage tank (Table 1).

Table 1. Identified algae from the artificial streams.*

I Mixed Periphyton	II	III
Scenedesmus alternans	*Oedogonium sp.*	*Cladophora glomerata*
S. bijuga		
S. dimorphus		
S. quadricauda		
Pediastrum boryanum		
P. duplex		
Palmodictyon viride		
Hormidium sp.		
Stigeoclonium sp.		
Mougeotia sp.		
Coelastrum microporum		
Misc. Diatoms		
Fragilaria crotonensis		
Melosira granulata		
Cocconeis sp.		
Synedra sp.		

*Algae identification by G. W. Prescott

The Elodea was planted in spaced clumps which soon spread laterally to form dense beds. An initial die back within these beds was followed by evident new growth. Long roots appeared all along the plant and particularly in the area beneath the wire mesh. Because of this attachment to the artificial substrate, very little Elodea floated away due to the failure of the plants to attach to the substrate.

During the first growth period, the bloom of mixed algae was particularly evident as periphyton on lower portions of the Elodea. New shoots of growth remained relatively free from the periphyton and the attached algae was less prominent along the edges of the channel where shading from the sides occurred. This was due mostly to the fact that the Elodea grew better in the shaded side areas than in the open center. Blackburn (1961) reported that high light intensities have an inhibitory effect on the growth of Elodea canadensis and that this effect follows a brief period of initial active growth and results in eventual death of the plants.

It was evident that some Elodea was gradually dying as the study progressed. Since both streams were completely unshaded, this may have been due to high light intensity. In one case, section 6 of stream B, it was necessary to replant fresh material before starting the second growth period.

Since Vallisneria freely roots to varied substrates, this plant was expected to thrive in the artificial streams. However, this is the one plant which failed to show significant growth during the entire study. At the time of planting, all of the large exterior leaves which were damaged or browned were stripped off so that only fresh live material was placed in the streams. Throughout the entire study, the large leaves deteriorated and continually broke loose and floated to the surface.

The Vallisneria did not attach to the artificial substrate as expected and periodically whole plants floated to the surface. These were returned to the mesh or recorded as lost material from the respective sections.

While making the final stripping of plants from the streams, it was discovered that the Vallisneria did not die entirely. The meristematic portion of each plant had been very active and a network of lateral stolons and new growth was evident beneath and just above the mesh. This growth was not measured in the cropped material for a growth period but was included in the total production at the end of the study.

The Ceratophyllum was by far the most successful plant in this experiment in terms of growth and plant condition. It grew rapidly and remained relatively free from periphyton. Wilkinson (1963) reported the optimum conditions for Ceratophyllum growth to be high light intensities at 30° C temperatures. This is substantiated by the success of the coontail in the similar conditions of the artificial streams.

Due to the non-rooting nature of Cerotophyllum, there was some difficulty in keeping this plant attached to the substrate. The slightest stress on the plants caused the slender stems around the wire to break. When the bloom of Oedogonium sp. reached its peak near the end of the first growth period, the mass of the algae caused much of the coontail to break loose from the artificial substrate. It was for this reason that sections A-4, B-1, and B-4 were stripped and replanted with fresh material before starting the second growth period. No further loss of Ceratophyllum was experienced and all sections were in good condition at the termination of the study.

The overall environmental conditions may have had an effect on the differences between growth period I and II. Period I from August 1 to the 14th was generally clear sunshine with very warm temperatures. On the other hand, period II from August 18 to the 31st was characterized by numerous overcast days and considerably cooler temperatures.

Merkle and Fertig (1963) describe an average pH difference of 0.88 between a rainy, overcast day and a bright sunlight day. This is largely a result of a shift in the HCO_3-CO_3 equilibrium caused by photosynthetic processes. This is also evident in the pH gradient established as the water passes over the plant material from head to discharge within the artificial streams. The difference in stream A was an average of 0.27 for the first period and 0.31 for the overcast second period. A maximum change of 0.5 occurred in both periods. The pH fluctuation in this study was apparently limited by the buffering capacity of the supply water (Appendices I and II).

Community Production

The term macrophyte community production was used in this study since no attempt was made to separate the higher aquatic plants from attached periphyton. Production was measured by the increase in material during a specified time interval. Naturally the actual amount of increase was directly related to the amount of original plant material placed in each section. To validly compare the growth between sections and streams, some method must be used to relate the growth back to the original stock. This was done by using a weight to weight percentage. Since the only measurement possible on original stock plant material was wet weights, all forms of growth are related by weight percentage back to the original wet weight planted.

Due to the fact that not all the growth was recorded in the cropping procedure and some plant stock was continually being lost, determining a set value for original starting stock presented several problems. This was further complicated by the fact that some sections were restocked with fresh material between the two study periods.

A series of formulas was designed to establish this starting value, taking into account the increases and decreases throughout the entire study. These formulas were used to determine all percentages expressed concerning growth and production (Appendix III).

Individual Sections

The results in Table 2 show that the production in the treatment stream A was consistently greater than in the control stream B. The range of production expressed in terms of percentage of increase (wet weight) from original starting mass was 78.5% maximum and 3.2% minimum for stream A compared to 39.2% maximum and 1.6% minimum for stream B. The only instance where a section in the control had more growth than the corresponding treated section involves section 6 in the second growth period. Here the non-treated _Elodea_ had more production than the treated. This may be attributed to several reasons. Section B-6 was the only _Elodea_ section which was restocked with fresh material before beginning the second period. As Blackburn (_op. cit._) pointed out, _Elodea canadensis_ responds under these conditions with an initial spurt of growth and then slowly dies off due to high light intensity. While this was going on in B-6, the _Elodea_ in A-6 continued its already downward progression.

It is noteworthy that the growth during the second period was consistently greater than that of the first even though the

Table 2. Macrophyte community production by individual sections for period I and II.

Section	Growth Period I					Growth Period II					Totals	
	WW C-1	% O.	DW C-1	% O.	% DW/WW	WW C-2	% O.	DW C-2	% O.	% DW/WW	TDWC	% O.
Stream A												
1	621	78.5	47.3	5.4	7.6	793	76.0	47.6	4.6	6.0	94.9	9.9
2	37	4.6	4.1	0.5	11.0	27	3.2	2.9	0.4	10.5	7.0	0.9
3	231	7.8	32.5	1.2	14.0	229	10.9	27.6	1.3	12.1	60.1	2.5
4	232	25.5	20.4	2.0	8.7	687	83.0	52.5	6.3	7.6	72.9	7.9
5	122	5.6	13.1	0.7	10.7	57	3.5	4.1	0.3	7.1	17.2	1.0
6	226	14.5	35.0	1.9	15.5	241	10.9	29.0	1.3	12.0	64.0	3.2
Total	1479	16.2	152.4	1.7	10.3	2034	23.4	163.7	1.9	8.1	316.1	3.7

Table 2. -- Continued

Stream B												
1	136	27.5	11.9	1.7	8.7	770	39.2	38.7	2.0	5.1	50.6	3.8
2	64	2.9	6.8	0.4	10.6	34	2.5	2.8	0.2	8.4	9.6	0.6
3	153	7.4	26.9	1.3	17.5	121	5.9	20.2	0.9	16.6	47.1	2.3
4	118	10.3	14.2	1.6	12.0	450	35.6	50.5	4.0	1.1	64.7	5.9
5	55	2.4	4.7	0.2	8.7	31	1.6	2.6	0.1	8.5	7.3	0.4
6	65	2.7	4.1	0.4	14.0	147	12.9	20.4	1.8	13.9	29.5	1.8
Total	591	6.0	73.6	0.7	12.5	1553	16.0	135.2	1.4	8.7	208.8	1.9

WW = Grams Wet Weight

C-1 = First Cropping

% O. = Percent of Original Starting Mass

DW = Grams Dry Weight

% DW/WW = Percent Dry Weight from Wet Weight

TDWC = Total Dry Weight Cropped Grams

concentration of nutrients was less. This indicates that it may be possible to reach a level of enrichment beyond which production is no longer increased. It is more likely that in the absence of minimum nitrogen and phosphorus concentrations, the effect of temperature, light and other environmental conditions becomes more prominent in influencing production.

Total Macrophyte Communities

In terms of cropped production, Ceratophyllum was the most productive of the three plants in the study (Table 3). This is most evident in the cropped plant material because the coontail had less unharvested growth. In all cases, the treated plants substantially out-produced the non-treated. The greatest difference in this respect was in the Elodea when considering total cropped and uncropped production. This indicates that the Elodea exhibited the greatest lateral growth in the lower portions. Merkle and Fertig (1963) showed that Elodea canadensis grew more rapidly near the bottom of a 2 foot deep pool exposed to high light intensities. The difference was from 0.89 cm/day increase in length at the bottom and 0.18 cm/day at the top. In view of previous discussion, this was probably due to the differences in light intensities at these levels. Even though the Elodea had more lateral uncropped growth than the others, when the results

Table 3. Total community production for each macrophyte community over the 30 day study.

	g Crop Dr. Wt.	% DW/WW	% of Origin	$\frac{g\ DW}{M^2}$ / day	Total Crop + Uncropped Dr. Wt.	% of Origin	$\frac{g\ DW}{M^2}$ / day	$\frac{ton\ DW}{acre}$ / year
Stream A								
Ceratophyllum	167.8	7.97	8.9	3.7	219.8	9.0	4.9	7.9
Vallisneria	24.2	9.80	0.9	0.5	31.5	1.2	0.7	1.1
Elodea	124.1	13.40	2.8	2.8	241.9	6.2	5.4	8.7
Stream B								
Ceratophyllum	115.3	6.70	4.8	2.6	176.4	4.0	3.9	6.2
Vallisneria	16.8	9.10	0.5	0.4	-----	---	0.4	0.6
Elodea	77.0	15.40	2.0	1.7	-----	---	1.7	2.7

are interpreted on the basis of increase from original starting mass, Ceratophyllum was the greatest producer.

The differences in the condition of the plants should be noted again. The Elodea grew at first but then tapered off continuously throughout the study while the Ceratophyllum remained in good condition. Hasler and Jones (1949) demonstrated that the more active higher aquatic plants had less attached algae. Some unknown mechanism inhibited phytoplankton production. It is probable that this is a result of light shading by the growth of the macrophyte. As a result of the plant condition, Elodea was covered with more periphyton than the active coontail. This may have been a significant contributing factor in the greater portion of uncropped growth from these sections.

Coontail also had the lowest percentage dry weight from wet weight plant material (7.3%). This results from higher water adherence to the coontail plants causing the wet weight value to be greater and the corresponding percentage to be lower.

The values obtained for cropped production are directly dependent on the procedures used in cropping. Due to the high variability inherent in such procedures, the significance of the values relate only to this particular study. The values expressed as total cropped plus uncropped production are valid for comparison in any situation of similar conditions.

When considering total production under enriched environments, the _Elodea_ community produced at a rate of 8.7 tons dry wt/acre/year. This was 3.2 times as much overall production when compared to the control stream B.

Bottom Deposits

At the beginning of the study period, the bottoms of the artificial streams were completely free from any deposited material. At the conclusion of the study, the detritus on the bottom of each section was collected and weighed (Table 4). This detritus appeared to be mainly decomposing plant material. In all cases there was more detritus deposited in stream A than stream B. The _Elodea_ was the greatest contributor of bottom deposits, followed by _Vallisneria_ and _Ceratophyllum_ respectively. The _Elodea_ community deposited bottom material in the enriched stream A at a rate of 18.1 tons dry wt/acre/year compared to 11.8 tons dry wt/acre/year in stream B.

Nutrient Observations

Phosphate

During the first growth period, the phosphate concentration was maintained at an average concentration of 4.07 mg/l in stream A. This concentration of soluble phosphate entering the system was reduced by an average of 0.73 mg/l before flowing out of the channel. The highest reduction during this period was 1.6 mg/l and the lowest 0.3 mg/l (Appendix IV).

Table 4. Total deposited bottom material for each macrophyte community over the 30 day study.

	Total g Wet Wt. Planted	g Dry Wt. Bottom Deposits	$\frac{\text{g DW D}}{\text{g WW P}}$	$\frac{\text{g DW D} / \text{M}^2}{\text{day}}$	$\frac{\text{Ton DW} / \text{acre}}{\text{year}}$
		Research Stream A			
<u>Ceratophyllum</u>	1746	302	0.17	3.4	5.4
<u>Vallisneria</u>	2728	677	0.25	7.5	12.0
<u>Elodea</u>	4355	1014	0.23	11.3	18.1
		Control Stream B			
<u>Ceratophyllum</u>	2887	202	0.07	2.2	3.5
<u>Vallisneria</u>	3882	502	0.13	5.5	8.8
<u>Elodea</u>	3659	669	0.18	7.4	11.8

DW = Dry Weight DW D = Dry Weight Deposited

WW = Wet Weight WW P = Wet Weight Deposited

In contrast, the control stream B remained at an incoming phosphate concentration of 0.5 mg/l throughout both testing periods. The reduction of phosphate concentration within the control was on the average 0.1 mg/l.

The incoming concentration of ortho-phosphate was lowered to an average of 2.5 mg/l in stream A for the second growth period. This brought about a corresponding lowering of the average phosphate concentration reduction within the system to 0.42 mg/l. The maximum reduction was 1.0 mg/l and the minimum 0.3 mg/l during this test period (Table 5).

Table 5. Average nutrient level and percent removal during period I and II.

	PO_4 ppm	Reduct. ppm	% Removal	NO_3 ppm	Reduct. ppm	% Removal
			Growth Period I			
Stream A	4.1	0.7	17.9	3.2	0.8	25.3
Stream B	0.5	0.1	20.0	0.4	0.1	25.0
			Growth Period II			
Stream A	2.5	0.4	16.8	2.1	0.5	21.0
Stream B	0.5	0.1	20.0	0.4	0.1	25.0

Nitrogen

The ammonium concentration remained at a level below 0.5 mg/l in both streams throughout the entire study. Likewise the nitrite concentration remained at an insignificant low level during the study. These concentrations were constant within the artificial stream situation. These transient forms of nitrogen are readily converted to the more stable nitrate in natural waters in the presence of oxygen, which was the case with the Red Cedar River and the large storage tank.

The nitrate concentration incoming to the control stream B was constant at near 0.4 mg/l during both growth periods. There was an average reduction of this concentration of about 0.1 mg/l for both periods (Table 5).

During the first growth period, the nitrate level was held at an average concentration of 3.16 mg/l for stream A. This level was lowered in the system by an average of 0.8 mg/l for the first 2 week period. The greatest reduction was 1.3 mg/l while the lowest was 0.1 mg/l.

The second growth period had an average incoming nitrate level of 2.1 mg/l with an average reduction of 0.44 mg/l. This included a maximum reduction of 0.7 mg/l and a minimum of 0.2 mg/l.

The soluble ortho-phosphate concentration coming into the enriched stream A was reduced by an average of 17.5% in passing through the channel. Similarly the nitrate concentration was lowered by 23.1% within the stream. These reductions, although measurable, are not as great as was expected for this study. Certainly the greatest factor limiting the removal of nutrients in this study was the condition of the plants. The photosynthetic activity of the plants was not great enough to counteract the buffering effect inherent in the water supply used. As a result, the pH was never raised to the point where active phosphate precipitation took place. Also a continual decomposition of plant material occurred, phosphorus was probably released within the system.

The retention time within the stream directly influenced the percentage of nutrients removed. If the retention time had been increased, it is likely that more nutrients would have been removed. At the waste treatment plant in Orlando, Florida, William Beck (op. cit.) showed that there was a direct relationship between retention time and the percent removal of nitrogen and phosphate in a tertiary treatment pond. He indicates the maximum removal to occur at retention times from 2 to 2.5 days, which is significantly longer than used in this study. His results show nitrogen removal up to 90% under controlled conditions. At retention times of less than one day, his nitrogen percentage removed was in the range of 20 to 30%.

Loehr and Stephensen (op. cit.) show that over long periods of time, the nitrogen and phosphorus concentrations may increase within tertiary treatment ponds. This is largely due to the release of nutrients from decomposition of material within the system. If a means of regularly harvesting the production was applied to biological tertiary processes, it is likely that significant removal of nutrients could be accomplished.

However, Bogan (1960) points out that there has been considerable difficulty in harvesting unicellular algae. Numerous attempts at harvesting algae incorporating screening, settling, centrifuging, and chemical coagulation have failed in developing an efficient, economical method. In comparison, the nature of higher aquatic macrophytes enables more efficient cropping. A number of mechanical harvesters have been developed to aid in the control of aquatic plant growths.

Organic Nutrient Levels

All of the macrophyte communities in stream A had higher percentages of organic phosphorus and nitrogen than those of stream B. (Tables 6 and 7). This agrees with Wetzel (*op. cit.*) in citing Gessnar and Kaukal (1952) about the uptake of phosphorus by *Elodea* being proportional to the concentrations present in the water.

The cropped material from the first section of *Ceratophyllum* in stream A consistently had higher percentages of nutrients than the succeeding sections. This difference is evident between the two separate sections of coontail and reflects again the better growth exhibited by this plant.

Table 6. Percent organic phosphorus and nitrogen in cropped material from period I.

Section	First Crop g Dry Wt.	% N	% P	N/P Quotient	% Protein
			Stream A		
1	47.3	2.67	0.61	4.4	16.7
2	4.1	1.80	0.43	4.2	11.2
3	32.5	1.25	0.39	3.2	7.8
4	20.4	2.06	0.75	2.8	12.9
5	13.1	1.76	0.38	4.6	11.0
6	35.0	1.50	0.46	3.3	9.4
			Stream B		
1	11.9	1.2	0.3	4.0	7.5
2	6.8	1.3	0.3	4.5	8.3
3	26.9	0.6	0.2	4.5	4.0
4	14.2	0.9	0.2	4.2	5.6
5	4.7	1.4	0.3	4.5	8.5
6	9.1	1.0	0.2	5.2	6.2

The total mg of nutrients per section is, of course, directly related to the production from that section. As a result, in some cases a section with a lower percentage of nutrients far exceeds another in actual mg of nutrients. This was evident several times in the case of Vallisneria.

Table 7. Percent organic phosphorus and nitrogen in cropped material from period II.

Section	2nd Crop g Dry Wt.	% N	% P	N/P Quotient	% Protein
			Stream A		
1	47.6	3.98	0.80	5.0	25.0
2	2.9	1.80	0.40	4.4	11.2
3	27.6	1.80	0.39	4.7	11.4
4	52.5	3.30	0.66	5.0	20.6
5	4.1	2.30	0.39	5.8	14.0
6	29.0	1.70	0.37	4.6	10.5
			Stream B		
1	38.7	3.0	0.4	7.6	19.0
2	2.8	1.0	0.3	3.5	6.3
3	20.2	0.8	0.2	4.1	4.9
4	50.5	1.3	0.3	4.1	8.0
5	2.6	1.5	0.3	5.8	4.5
6	20.4	0.9	0.2	4.3	5.4

At the end of the first growth period, some of the plants were in poor condition. This was more pronounced in control stream B and, as previously mentioned, several of these sections were replanted. In an effort to get the control plants in better condition, nitrate and phosphate enrichment (2 mg/l) was added to stream B for 50 hours. This treatment succeeded in aiding the plants of the control. A result of this interim exposure to nutrients shows up in the total nitrogen and phosphorus content of the control plants in period II. These values are higher in contrast to corresponding values from period I and the differences in relation to the treated stream A are not as great.

The N/P quotients remain fairly uniform for any one growth period in both stream A and B. These quotients which reflect the ratio of phosphorus and nitrogen range slightly higher during the second period than the first. This coincides with the general increases in production between the two growth periods and could be directly related to the interim treatment of stream B.

In terms of nutrient uptake per gram of plant material, there was an obvious gradient established between the three plants (Table 8). This could be due to the plants at the head of the channel being exposed to higher concentrations of incoming nutrients as a result of a nutrient gradient within each stream. The differences between treated and non-treated plants were correspondingly greatest between the upper sections. Here again it must be noted that *Ceratophyllum* had more production than the other plants.

A sharp gradient in protein content was also noted between the different plants. This was a result of the differences in nitrogen uptake by the plants. In the case of coontail, the results show that the protein content can be almost doubled by enrichment at the levels used in this study. The fact that the *Ceratophyllum* in stream A was 19% protein indicates a potential use for cropped macrophytes as a source of protein.

Table 8. Average organic nutrients and percent protein for each macrophyte community.

	g Dry Wt.	mg P	$\frac{mg\ P}{g\ DW}$	mg N	$\frac{mg\ N}{g\ DW}$	% Protein
			Stream A			
Ceratophyllum	167.8	1176.0	7.1	4172	24.8	18.8
Vallisneria	24.2	96.7	4.1	440	18.5	11.8
Elodea	124.6	505.6	4.0	1923	15.4	9.8
			Stream B			
Ceratophyllum	115.3	343.0	2.9	2091	18.1	10.0
Vallisneria	16.8	48.0	2.8	221	13.1	8.2
Elodea	77.0	136.0	1.8	595	7.7	5.1

The total amount of phosphorus and nitrogen remaining in the system over the 30 day study period was calculated using the incoming flow rate, the concentration of incoming nutrients and the average nutrient reduction within the system. The resulting values of 33 g of phosphorus and 119 g of nitrogen were compared with the organic nutrient totals from analysis of cropped material, uncropped material, and bottom sediments.

Only 29.6% of the phosphorus and 32.6% of the nitrogen theoretically remaining in the system was accounted for in the totals of analyses. There are a number of explanations for such a large discrepancy. Undoubtedly there was some nutrient adsorption to the sides, bottom, and substrate of the channels and also some loss of nutrients by evaporation. However, the most likely source of such a large discrepancy in balance lies in shortcoming of applied procedures. Tests for total phosphorus and total nitrogen were not run on the daily discharge from the enriched stream. As an unfortunate consequence, no measurement was obtained for that portion of nutrients taken up by planktonic forms which washed out of the system. This could account for a large portion of the disparity in nutrient balance. Another area for which no tests were taken concerns the release of nutrients during the night periods of the study. Any nutrients released at night would have washed out of the system without being measured.

SUMMARY

1. Macrophyte community production was measured in two artificial outdoor streams. The upstream-downstream nutrient uptake and removal relationships were studied in these streams.

2. Ceratophyllum demersum maintained the best condition over the study period and had the least attached periphyton. Elodea canadensis and Vallisneria americana supported dense growths of mixed periphyton and did not grow as well.

3. Production measured by increase in dry weight material noticeably increased with phosphate and nitrate enrichment. There was little difference in production between the 4 mg/l and 2 mg/l nutrient levels.

4. The Elodea canadensis community produced 5.4 g dry wt/m^2/day followed by the Ceratophyllum demersum community at 4.9 g dry wt/m^2/day and Vallisneria americana community at 0.7 g dry wt/m^2/day. A large portion of the production by the Elodea community took place at the lower depths and was not recorded in cropping procedures.

5. The phosphate level was reduced by an average of 18.7% and the nitrate level reduced by 19.5% in the artificial streams.

It is likely that these values could be increased using longer retention periods.

6. There was a definite increase in the organic phosphorus and nitrogen content of the cropped plant material in stream A. The N/P quotient for the plants was an average 4.5 and remained relatively constant for all the plants.

7. The Ceratophyllum demersum contained the highest percentage of organic phosphorus and nitrate and the highest average protein content of 19%.

8. The amount of detritus accumulated over a 30 day period was considerably higher in the enriched stream. The Elodea community deposited the greatest amount of detritus at a rate of 11.3 g dry wt/m^2/day.

LITERATURE CITED

APHA, AWWA, WPCF, 1965. Standard Methods for the Examination of Water and Waste Water. 12th Edition. Boyd Printing Co., Inc., Albany, N.Y. 769pp.

Bartsch, A. G., and M. O. Allum. 1957. An Oxidation Pond as a Tertiary Treatment Device. ASCE Sanitary Engineering Division. Vol. 91 SA 3:31-44.

Beck, W. M. 1962. The Biological Removal of Nitrogenous Compounds from Sewage Treatment Plant Effluents. Seminar on Biological Problems in Water Pollution. Public Health Service Publication No. 999-WP-25:306-308.

Bogan, R. H. 1960. The Use of Algae in Removing Nutrients from Domestic Sewage. Algae and Metropolitan Wastes, R. A. Taft Sanitary Engineering Center Technical Report W 61-3.

Blackburn, R. D., J. M. Lawrence, and D. L. Davis. 1961. Effects of Light Intensity and Quality on the growth of Elodea densa. Weeds 9:251-257.

Hasler, Arthur D., and Elizabeth Jones. 1949. Demonstration of the Antagonistic Action of Large Aquatic Plants on Algae and Rotifers. Ecol. 30:359-364.

Hutchinson, G. E. 1957. A Treatise on Limnology. John Wiley and Sons, Inc. Vol. 1:1015pp.

Kevern, Niles R. 1962. Primary Productivity and Energy Relationships in Artificial Streams. Thesis Ph.D. Michigan State University.

Loehr, R. C., and R. L. Stephenson. 1965. An Oxidation Pond as a Tertiary Treatment Device. ASCE Sanitary Engineering Division. Vol. 91 SA 3:31-44.

Mackenthun, K. M., W. M. Ingram, and R. Porges. 1964. Limnological Aspects of Recreational Lakes. U. S. Public Health Service Publication No. 1167. 176pp.

Mackenthun, K. M. 1965. Nitrogen and Phosphorus in Water. Public Health Service Publication 1305. 112pp.

Merkle, M. G., and S. M. Fertig. 1963. Some Effects of Plant Growth on the Aquatic Environment. NEWCC Proceedings 17:432-438.

Merkle, M. G., and S. N. Fertig. 1963. Problems and Techniques in Growing Certain Aquatic Species Under Greenhouse Conditions. NEWCC Proceedings 17:475-479.

Odum, H. T. 1956. Primary Production in Flowing Waters. Limnology and Oceanography. 1:102-117.

Penfound, W. T. 1956. Primary Production of Vascular Aquatic Plants. Limno. and Oceanogr. 1:92-101.

Prescott, G. W. 1962. Algae of the Western Great Lakes Area. Wm. C. Brown Co., Inc., Dubuque, Iowa. 946pp.

Rickett, H. W. 1922. A Quantitative Study of the Larger Aquatic Plants of Lake Mendota. Trans. Wisconsin Academy of Science. Vol. 20:501-527.

Rickett, H. W. 1924. A Quantitative Study of the Larger Aquatic Plants of Green Lake, Wisconsin. Trans. Wis. Acad. Sci. Vol. 21:381-414.

Ruttner, F. 1953. Fundamentals of Limnology. University of Toronto Press, Canada. 242pp.

Sawyer, C. N. 1960. Chemistry for Sanitary Engineers. McGraw-Hill Book Co., Ind., New York. 367pp.

Schuette, H. A., and H. Alder. 1928. Notes on the Chemical Composition of Some of the Larger Aquatic Plants of Lake Mendota. II *Vallisneria* and *Potomogeton*. Trans. Wis. Acad. Sci. Arts, Letters. Vol. 23:249-254.

Schulze, K. L. 1966. Biological Recovery of Waste Water. Jour. Water Pollution Control 38:1944.

Stokes, R. M. 1960. The Effects of Limiting Concentrations of Nitrogen on Primary Production in an Artificial Stream. Thesis M. S. Michigan State University.

Thomas, E. A. 1962. The Eutrophication of Lakes and Rivers, Cause and Prevention. Seminar on Biological Problems in Water Pollution. Public Health Service Publication No. 999-WP-25:306-308.

Welch, P. S. 1948. Limnological Methods. McGraw-Hill Book Co., Inc., New York. 367pp.

Wetzel, R. G. 1964. A Comparative Study of the Primary Productivity of Higher Aquatic Plants, Periphyton, and Phytoplankton in a Large, Shallow Lake. Int. Revue Ges. Hydrobiol. 49:1:1-61.

Wilkinson, R. E. 1963. Effects of Light Intensity and Temperature on the Growth of Waterstargrass, Coontail, and Duckweed. Weeds 11:287-290.

Appendix I. Daily pH, temperatures, and environmental conditions for period I.

Day August	Temp. Air	F° H_2O	E. C.	pH Stream A in	pH Stream A out	pH Stream A change	pH Stream B in	pH Stream B out	pH Stream B change
1	93	89	CS	8.6	8.7	0.1	8.6	8.7	0.1
2	88	86	S	8.6	8.7	0.1	8.6	8.6	0.0
3	90	90	CS	8.9	9.3	0.4	8.3	8.8	0.5
4	78	76	CS	8.5	8.9	0.4	8.5	8.3	-0.2
5	92	92	S	8.7	8.9	0.2	8.5	8.7	0.2
6	80	76	OC	8.5	9.0	0.5	8.6	8.7	0.1
7	85	83	S	8.7	9.1	0.4	8.6	8.6	0.0
8	80	78	OC	8.5	9.0	0.5	8.6	9.0	0.3
9	78	75	S	8.6	8.4	-0.2	8.5	8.6	0.1
10	82	80	S	7.8	8.1	0.3	7.6	8.0	0.4
11	81	78	OC	8.5	9.0	0.5	8.5	8.6	0.1
12	84	80	S	8.4	8.7	0.3	8.5	8.8	0.3
13	80	78	CS	8.4	8.5	0.1	8.5	8.5	0.0

E. C. = Environmental Conditions S = Sunny

CS = Cloudy Sun OC = Overcast

Appendix II. Daily pH, temperatures, and environmental conditions for period II.

Day August	Temp. Air	F° H_2O	E. C.	pH Stream A			pH Stream B		
				in	out	change	in	out	change
18	85	82	OC	8.3	8.5	0.2	8.4	8.5	0.1
19	68	65	OC	8.3	8.5	0.2	8.3	8.4	0.1
20	78	70	CS	8.2	8.5	0.3	8.2	8.5	0.3
21	77	70	CS	8.3	8.8	0.5	8.3	8.5	0.2
22	76	70	CS	8.2	8.7	0.5	8.3	8.6	0.3
23	72	65	CS	8.2	8.6	0.4	8.2	8.4	0.2
24	74	66	S	8.3	8.5	0.2	8.3	8.6	0.3
25	78	74	S	8.1	8.4	0.3	8.2	8.4	0.2
26				8.2	8.6	0.4	8.3	8.5	0.2
27				8.1	8.5	0.4	8.2	8.4	0.2
28	78	72	OC	8.2	8.4	0.2	8.1	8.4	0.3
29	75	70	OC	8.1	8.4	0.3	8.2	8.4	0.2
30	68	60	OC	7.9	8.1	0.2	8.0	8.0	0.0
31	70	65	CS	8.1	8.4	0.3	8.2	8.4	0.2

E. C. = Environmental Conditions   CS = Cloudy Sun

OC = Overcast   S = Sunny

Appendix III. Original starting plant mass and formulas for developing uniform origin.

A = Original Planted Material

B = Material Replanted Before Second Period

C = Positive Uncropped Growth or Negative Lost Material

Formula Original for First Cropping

$$F.O. = A \pm 1/4\ C$$

Formula Original for Second Cropping

Case 1. No Replant

$$F.O. = A \pm 3/4\ C$$

Case 2. Replant

$$F.O. = B \pm 1/4\ C$$

Formula Original for Total Cropped Material

$$F.O. = \frac{F.O.\ \text{1st crop} + F.O.\ \text{2nd crop}}{2}$$

Formula Original for Cropped Plus Uncropped Growth

Case 1. No Replant and No Lost Material

$$F.O. = A$$

Case 2. No Replant and Lost Material

$$F.O. = A - 1/2\ C$$

Case 3. Replant and No Lost Material

$$F.O. = A + B$$

Case 4. Replant and Lost Material

$$F.O. = A + B - 1/2\ C$$

Appendix III. -- Continued.

Section	O.P.	F.O.	C-1	S.O.	R.P.	C-2	F.O.	F.S.	U.G.	L.M.	T.C.	F.O.	T.G.	F.O.
Stream A														
1	790	873	621			793	1039	1123	333		1414	956	1747	790
2	796	815	37			27	853	873	77		64	834	141	796
3	2946	2657	231			229	2099	1790		1156	460	2378	460	2368
4	918	1014	232	695	733	687	830	1343	387		919	922	1306	1651
5	2166	1982	122	234		57	1614	1196		736	179	1798	179	1798
6	1559	1779	226	150		241	2219	2290	881		467	1999	1348	1559

Appendix III. -- Continued.

	Stream B													
1	493	690	136	1600	1759	770	1956	1440	788		906	1323	1694	2252
2	2144	1883	64	267		34	1361	830		1047	98	1622	98	1621
3	2064	2052	153	1204		121	2028	810		50	274	2040	274	2039
4	1140	895	118	414	1509	450	1264	1252		983	568	1079	568	2158
5	2315	2197	55	310		31	1961	1530		475	86	2079	86	2078
6	2451	2192	65	1050	1398	147	1139	1760		1039	212	1665	212	3335

O.P. = Original Planting
F.O. = Formula Original Mass used for Percent Increase
C-1 = First Cropping
S.O. = Strip Out
R.P. = Replant
C-2 = Second Crop

F.S. = Final Stripping
U.G. = Uncropped Growth
L.M. = Lost Material
T.C. = Total Cropped Growth
T.G. = Total Cropped plus Uncropped Growth

Appendix IV. Daily nutrient levels and percent removal for period I and II.

Day	Growth Period I						Growth Period II					
	Phosphate Level ppm			Nitrate Level ppm			Phosphate Level ppm			Nitrate Level ppm		
	in	out	Red.	in	out	Red.	in	out	Red.	in	out	Red.
1	2.6	2.2	0.4	0.5	0.4	0.1	2.2	1.2	1.0	2.1	1.5	0.6
2	3.2	2.5	0.7	0.6	0.5	0.1	1.9	2.2		0.9	2.0	
3	4.5	3.5	1.0	0.8	0.5	0.3	4.0	3.2	0.8	1.9	1.2	0.7
4	5.0	3.6	1.4	5.0	4.0	1.0	1.8	1.5	0.3	1.6	1.1	0.5
5	5.0	3.5	1.5	4.8	4.0	0.8	2.6	2.3	0.3	2.0	1.6	0.4
6	5.1	3.5	1.6	5.0	3.8	1.2	2.6	2.1	0.5	2.1	1.6	0.5
7	3.5	3.2	0.3	3.0	1.7	1.3	2.2	1.8	0.4	2.0	1.4	0.6
8	4.0	3.2	0.8	3.8	2.5	1.3	2.6	2.2	0.4	2.4	1.9	0.5
9	4.0	3.5	0.5	3.5	1.7	1.8	2.6	2.3	0.3	2.4	1.9	0.5
10	4.1	3.7	0.4	3.7	2.6	1.1	2.7	2.4	0.3	2.6	2.2	0.4
11	4.3	3.6	0.7	3.4	2.4	1.0	2.7	2.3	0.4	2.3	1.9	0.4
12	4.1	3.7	0.4	3.2	2.6	0.6	2.5	2.1	0.4	2.4	2.0	0.4
13	4.5	4.5	0.0	4.1	3.9	0.2	2.7	2.4	0.3	2.5	2.3	0.2
14	3.1	2.6	0.5	2.8	2.4	0.4	2.6	2.1	0.5	2.5	2.1	0.4
Ave.	4.07		0.73	3.16		0.8	2.5		0.42	2.1		0.44