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Metabolism of a Laboratory Stream Microcosm

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Metabolism of a Laboratory Stream Microcosm[1]

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Introduction

THE MICROCOSM METHOD

Wherever the energy of the sun is being absorbed on earth, living organisms use it to develop complex associations capable of survival. In order to compete, such complexes must recirculate the chemical raw materials making use of whatever physical processes are at hand. Food must be passed from producer to consumer, and the chemicals passed from regenerating consumer back to primary producer. Sometimes there is only molecular diffusion; at other times there are available strong turbulent mechanisms for exchanging substances as effectively as in the blood circulatory systems within organisms. The term ecosystem has been used for the combination of communities of organisms and their circulation systems. Understanding the ecosystem is a main objective of ecology. Unfortunately, most ecosystems in nature are large consequently difficult to manipulate.

Smaller systems could be handled experimentally. A microcosm is by definition a miniature system. To quote Webster's dictionary it is a "small community, a little world." It seems a logical methodology to study experimental misrocosms as a means of understanding the larger macrocosms.

It is also conceivable that study of microcosm can lead to the invention of new ecosystems not present in nature. Perhaps such ecosystems can be developed to maintain themselves by internal ecological mechanisms to man's benefit. The microcosm therefore may be an approach to *community engineering*, one of the bright possibilities for man after his fossil power is gone.

Whereas most kinds of experiments involve isolating a part of a system for study, a microcosm experiment is designed to retain as much of the total complexity as possible, including especially the producer, consumer-regenerator, and circulating phases. This paper is a report on the behavior of a laboratory stream microcosm and the inferences that might be drawn from it about larger flowing systems in nature.

PROBLEMS IN STREAM ECOLOGY

In previously reported studies on natural flowing systems some perplexing issues and hypotheses have been proposed and discussed to account for the patterns of biota and metabolism known to occur in streams. Some of these questions are as follows:

1. *Are stream communities more productive than other aquatic communities* as suggested by data summarized previously (Odum, 1956a)? If true on the average, does the current modify production because of the circulation per se within the community as well as by bringing fresh media and requirements from outside of the community? Gessner (1937, 1955) has shown that single vascular water plants metabolize more rapidly in circulation. Fox, Simmonds, and Washburn (1935) have described the high metabolism of current adapted animals. Does a current also increase the metabolism of communities isolated in steady state? Do the metabolic rates of ecosystems, which have both photo-synthetic aufwuchs and respiratory slimes change with the current?

2. *Is the metabolism of an adapted steady state community proportional to current velocity at which it grows?* Does the metabolism of an aufwuchs-slime community in adjustment to one current velocity change if the current velocity is changed? These

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questions need solution to allow an understanding of metabolism in streams. Are the variations in flow in streams accompanied by variations in metabolism? Is the predominance of aufwuchs in rapids an indication that metabolism is greatest there?

3. *Can an aufwuchs-slime community concentrate and store inorganic nutrients* from passing water as plankton cultures do? Can aufwuchs-slime communities concentrate and store *organic nutrients* from dilute flowing waters for future metabolism as an energy source? Sargent and Austin (1949, 1954) reported some results which suggested that coral reefs might have the ability to extract organic stuff from dilute concentrations in tropical ocean water. Some perpelexing decreases in biochemical oxygen demand in Silver Springs (Odum, 1957) could be similarly interpreted.

Following Pütter's hypothesis, much work such as that of Bond (1933) showed that dilute natural concentrations of dissolved organic matter could not support larger consumer organisms directly under conditions of still water. Bacteria on the other hand were able to grow in dilute media or on surfaces adjacent to media with low organic concentrations (Zobell and Anderson, 1936). Presumably low nutrient media under flowing conditions can also support bacteria on surfaces. The possibility of larger consumers deriving energy from dilute organic matter concentrations flowing past the organisms has not been eliminated. If true for sessile consumers the possibility that it could be true for plankton organisms might be reconsidered for turbulent water. That dissolved organic matter concentrations are maintained at such low levels in the ocean suggests continual metabolic use by plants, animals, or bacteria.

4. *What is the course of metabolism and holistic community structure during succession and climax?* Butcher (1940, 1945, 1947, 1949) and others have described patterns of brief succession and climax in stream communities. The types and kinds of final states have seemed to be infinitely numerous under different hydrographic conditions. What measures of total community structure and function in common among these taxonomically diverse steady states can be observed in a microcosm?

5. *What is a design for a flowing respirometer for attached stream cultures?* What conditions are necessary to produce a typical periphyton (aufwuchs) in place of a plankton type community? Can such communities be cultured at will under controlled conditions? L. Whitford has recently cultured a single species of stream algae in an open circulating trough (personal communication).

Stream Microcosm Apparatus

After a number of trials and failures an apparatus was finally evolved which yielded aufwuchs communities readily and which permitted continual metabolic measurements. In Figure 1 is the microcosm as finally modified. As always, the authors regret that hindsights were not foresights.

A pump circulates water in a closed circuit through the inner tube of a condenser illuminated by four 150 watt R-40 General Electric photo-flood lights. All other tubing is covered with aluminum foil and is in the dark. The temperature is regulated by flow of tap water in the condenser jacket. The temperature is indicated by a thermistor and bridge. While the community is developing and while measurements are not being made, the water is passed through an aerator tank where a flow of air bubbles maintains a constant oxygen and carbon dioxide-bicarbonate condition. Thus all experiments

receive the same constant carbon dioxide pressure of the natural air. Because o
continual introduction from the outside, no shortage of carbon dioxide can dev
Carbon dioxide can control rates by its concentration but can never be limiting i
sense of becoming unavailable. A tube from a resevoir carbuoy permits renewal an
placement of medium which is removed for analysis or which evaporates in the aer
Measurements of the water properties are made by drawing samples from a tap
times a glass wool filter is used to catch pseudoplankton and make chances of plug
in the smaller tubes less likely.

When metabolic rates are measured, water is routed through a different route s

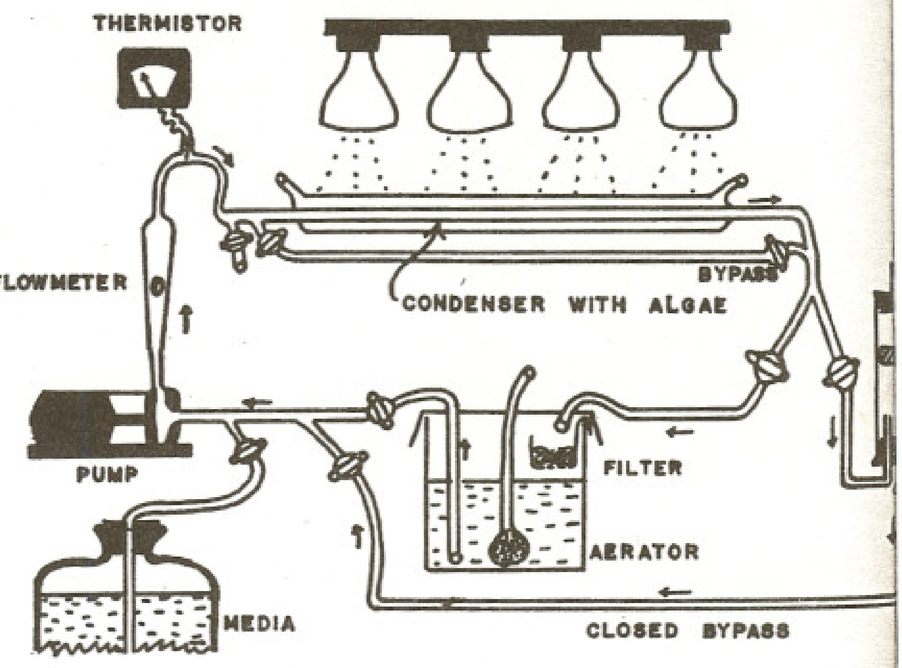

FIG. 1. Flowing microcosm apparatus and respirometer.

to bypass the aerator (See Figure 1). After ten minutes of circulation to permit m
ing, two 5 cc oxygen samples are drawn for Winkler analysis in a syringe. After a m
bolic period of an hour duplicate samples are again drawn for analysis. The aver
volume of the total metabolic system during this period (about 400 ml) is measu
from the height of a circular paraffin block in the water resevoir cylinder of the byp
(Figure 1). After these measurements are made, the system is again routed through
aerator where the oxygen concentrations are returned to normal in a few minutes.

A circuit is available to bypass the main photosynthetic community in the conden
also. Current velocities are controlled with a stopcock and indicated by the position
a glass bead in the cone-shaped tube, which after calibration serves as a flowmeter. T
pump, Model D–6 produced by Eastern Industries, is a stainless steel centrifugal t
powered by a 1/30 Hp electric induction motor, which ran continuously for a y
without major mishap.

The Winkler analysis in the syringe is rapid and accurate. One 0.05 cc drop each
manganous sulfate, potassium-hydroxide-potassium iodide solution, and sulfuric a

in usual concentrations are placed in cups of a depression slide. These drops are drawn into the syringe containing the 5 cc water sample in the usual sequence. The clear iodine-containing solution is titrated with a 0.00625N thio-sulfate solution in a bottle. With a fully developed aufwuchs-slime community the oxygen changes for the volume of the system of about 400 cc are as much as 5 mg/1/hr. These large changes in concentration far exceed the variation in values due to analytical error or due to heterogeneity of the waters sampled. No correction was made for oxygen added in reagents.

A considerable effort was made to adapt the gold-zinc electrode used by Ohle (1953) for continuous instantaneous readings of oxygen in the flowing system. These efforts were unsuccessful. It was not possible to obtain readings which could be repeated. Apparently, continuously changing electrode surfaces, attachments of slime, and other current-sensitive effects could not be reproduced. The attempts included washing of electrodes in acid, flushing electrodes with distilled water while not in use, and study of time curves of amperes following closure of electrical circuit. It was apparent during the two months of work with the zinc electrode in the system that only bacteria and fungi were being cultured in thick fuzzy mats. The first success with algae followed the removal of the zinc from the system.

Similar failure to obtain reproducible results in polarographic oxygen measurements in continuous flow systems were recently reported by Ambuhl (1955) and Lynn and Okun (1955).

Where phosphate and nitrate analysis were made to indicate nutrient levels, colorimetric methods were used with a Bausch and Lomb spectronic 20 instrument. The strychnidine method for nitrate (Zwicker and Robinson, 1944) and the ammonium molyddate method for phosphorus were used (Robinson and Thompson, 1948).

A number of the early experiments were terminated after 3 weeks by the slippage of the thick mats of filamentous and gelatinous aufwuchs from the side of the condenser so that the system became plugged. It was not until later experiments that this was prevented by placing a cylindrical layer of plastic window screen within the condenser for the algae to develop on. Thereafter no massive clumps broke loose.

The current velocities were fairly uniform in the condenser tube. Flow of water con-
aining particles showed the observer a fairly laminar flow. For example, when the flow
eter indicated an average flow of 0.1 m/sec there was a flow of this magnitude within
5 mm. of the wall or less. Although 0.08–0.2 m/sec flow in the free water of streams is
t large, a flow of this magnitude within the aufwuchs occurs in nature only when much
ater currents are occurring in the free water. Thus 0.08–0.2 m/sec is referred to as
gh current velocity subsequently in this discussion.

Sequence of Experiments

m November 1955 to November 1956 manipulations of the stream microcosm were
d. During the course of these experiments the metabolism of the community under
nd dark conditions was measured. Except during metabolism measurements the
ser portion of the community was in continuous light 24 hours a day. When
tion rates were measured, black covers were placed over the condenser. Thus
denser might be described as a flowing light and dark bottle. The oxygen de-
s for the system were recorded in mg/hr as respiration and represented by R. The
n changes during illumination were considered to be the net ...

community represented by P_n. The oxygen changes of respiration (R) were subtrac algebraically from the oxygen changes during photosynthesis (P_n) to obtain the gr primary production P (photosynthesis).

The sequence of measurements of photosynthesis and respiration through the s cessive experiments is recorded in the series of graphs in Figues 2–11. Extrapolat lines are dashed. Where photosynthesis of the system was in excess of the respirati of the whole system, the area between the graphs is shaded to indicate net storage organic matter. Nutrient conditions, the gross color of the community, and other prop ties of the experiments are described in chronological order.

At no time did any plankton communities develop. The system filtered and remov all pseudoplankton whether the glass wool filter was used or not. No algal blooms ev developed in the water—only on the tube surfaces.

A. SUCCESSION AND METABOLISM FOLLOWING INTRODUCTION OF A READY-FORMED COMMUNITY; HIGH CURRENT VELOCITY (FIGURE 2)

The first success in developing a green aufwuchs followed the introduction of blade of eel grass and water from Silver Springs, Florida enriched with a commerci inorganic fertilizer mixture (hyponex). For the chemical characteristics of Silve Springs water see Odum (1957). Previously, many introductions of algae, lake waters and soils had given opportunity for many species to become "seeded." The eel gras blade and its already existing aufwuchs demonstrated a high metabolism from the star which gradually diminished. It was immediately obvious that the initial community wa being replaced by succession. This succession passed through a period of rich diatom growth followed by a replacement with blue-green algae. The eel grass showed decom position indicating release of organic supplements. P and R were about equal but both decreased as the excess nutrient levels diminished (Figure 2). The stimulating effec of the addition of a dilute natural medium is indicated on February 18. Since durin January and February neither P nor R was much in excess, the total organic matte originally added had not diminished although the apparent decomposition of the ee grass blade indicated a reworking of the organic matter. The experiment ended when the tubes plugged. The current velocity during these experiments was high, 0.1–0.2 m/sec.

RADIOPHOSPHORUS UPTAKE (FIGURE 2)

On February 16, 1956 10 microcuries of p^{32} as phosphate were added to the aerator reservoir (1 liter). A survey meter showed the immediate localization of the radio-active phosphorus in the walls of the tubes, especially the tygon. After one hour and at intervals, five cc samples were evaporated in nickel planchettes and assayed for radio-activity in a proportional scaler. The community was in light for 6 hours and then put in the dark for 6 hours. The levels were not at any time significantly higher than back-ground. There had been an effective removal of the added phosphorus. The implication of the rapid uptake of the phosphorus by the aufwuchs is that there is a high turnover and flux of phosphorus although the concentrations in the water at any time are ex-

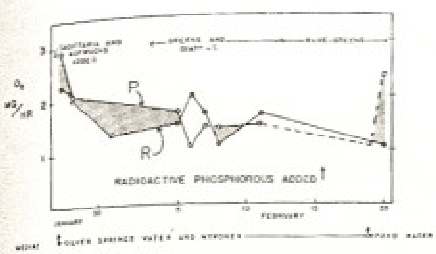

Fig. 2. Succession and metabolism in the microcosm at high current velocity following introduction of water and a ready-formed community.

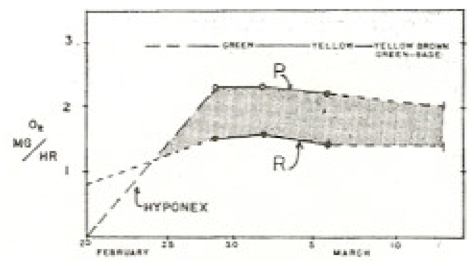

Fig. 3. Growth and succession in a fertilized system at high current velocity.

B. GROWTH AND SUCCESSION IN A FERTILIZED SYSTEM

(FIGURE 3)

On February 20 after the condenser was cleaned out except for small bits of aufwuchs firmly attached in crevices, and an extremely rich inorganic medium was introduced consisting of one gram of hyponex per liter. This system contained far higher concentrations of nutrients than in later experiments and might be compared to the condition initially in fertilized ponds. Within a week a brilliant green aufwuchs developed with a great excess of photosynthesis over respiration meaning that organic matter was being synthesized (Figure 2). No further nutrients were added and as conditions in the recirculating medium changed, the community passed through a succession of color to a brilliant yellow and then orange. There was some green at the upstream end of the community and on the inside of the thick gelatinous mats of algae. Examination of the aufwuchs showed a small blue-green alga almost of bacterial size as dominant. The orange color was due to the small blue greens. Many other blue greens were also present. With the increasing deposits of organic matter, the system finally plugged on March 14.

C. COMMUNITY SUCCESSION WITH LOW NUTRIENT WATER, NUTRIENT-SATURATED SUBSTRATES, AND HIGH CURRENT VELOCITY (FIGURE 4)

After the condenser communities were mostly removed, a low nutrient medium resembling types more often found in nature was introduced. This was a medium described by Rhode quoted in Brunel, Prescott, and Tiffany (1950). Although it was not realized until later, the previous history of the microcosm was important since the films of slime in the dark tubes of the system and in the plastic were holding organic and inorganic nutrients thus affecting the system's metabolism for many days subsequently. Thus this experiment began with a history of 3 months rich growths with ample opportunity for invisible storage of organic matter and nutrients in the walls of the system. Efforts to remove all visible accumulation of organic matter did not reduce this effect appreciably.

With the dilute Rhode medium a community developed less rapidly than with the hyponex but in the subsequent weeks did cover the system and pass through an evolution of color from green to yellow (Figure 4).

Perplexing at first was the fact that photosynthesis did not exceed the respiration of the system during the entire growth period. Here one had a net loss of organic matter of the ecosystem but a regular succession and growth of the visible community. Such metabolic regimes are likely to be common in nature where blooms are observed but where the total P/R is less than 1. Whether the photosynthetic part of the community was deriving heterotrophic energy supplements was not certain but there was ample opportunity for this to have occurred. The aufwuchs slipped and plugged on April 9. The slipping occurred as the older cells touching the glass turned brown.

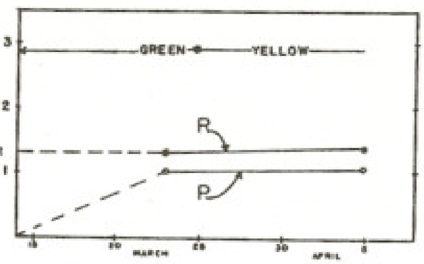

FIG. 4. Community succession with low nutrient water, nutrient-saturated substrates, and high current velocity.

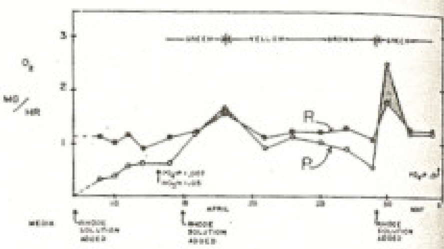

FIG. 5. Bloom pulses in a nutrient-starved steady state community adapted to high current velocities.

D. BLOOM PULSES IN A NUTRIENT-STARVED STEADY STATE COMMUNITY ADAPTED TO HIGH CURRENT VELOCITIES

(FIGURE 5)

After the deposits of the system were cleaned out and new media added, the succession and trends followed a new growth sequence similar to that previously described. As the condenser surfaces became covered with algae, the community became green and and photosynthesis increased indicating that there was net plant production and growth. However, in the total system, respiration (R) was generally in excess of photosynthesis (P, Figure 5). Although there was an obvious growth of the photosynthetic part of the community, the inorganic nutrient levels (nitrate and phosphate) were low resembling the conditions for growths that often occur in natural systems. The succession of color of aufwuchs from green to yellow was reversible. When additional new medium was added to replace that drawn off during measurements, a pulse in photosynthesis and respiration occurred with a return to the green color. As in previous sequences these was a tendency for the metabolic rate curves to level off with metabolism of both photosynthesis and respiration at about 1 mg/hr for the whole microcosm. A somewhat peculiar characteristic of the system was the tendency for low photosynthesis that occurred when the total volume of the system became low on April 13 and again on April 29. Although the nutrient levels in the water were insignificant compared to those found in the aufwuchs-slimes, the decrease of circulating volume affected the ratio of P and R. On May 4 the aufwuchs again slipped and plugged the system just after its bloom condition.

E. COMMUNITY SUCCESSION AND STEADY STATE ADJUSTMENT TO LOW CURRENT VELOCITIES (FIGURE 6)

Again the system was cleaned except for a few spots to act as a "seed" to provide the same possible populations. Whereas the four previous sequences had been studied under conditions of high current velocity (0.09–0.21 m/sec), the next sequence beginning on May 5 (Figure 6) was studied under conditions of low current velocity (0.02 m/sec) with the same dilute inorganic medium and with the same "seeding technique." It was reasoned that the effect of current velocity on steady state adjustments might be determined.

The sequence of events was remarkably similar with a rapid growth of the green aufwuchs over the surfaces of the condenser tube and a development of a large photosynthetic and respiratory metabolism. After the peak of the activity the community again turned yellow as the metabolism adjusted to lower steady state levels of P and R.

The principal difference between this sequence and the ones at high velocity was the lower initial respiratory rate prior to the algal bloom and lower metabolic rates after the steady state began to develop.

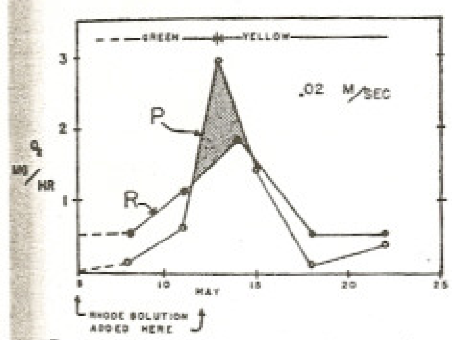

FIG. 6. Community succession and steady state adjustment to low current velocity.

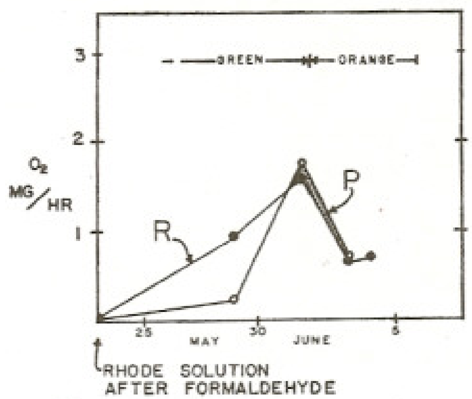

FIG. 7. Community succession and metabolism at high current velocity after sterilization.

F. COMMUNITY SUCCESSION AND METABOLISM AT HIGH CURRENT VELOCITY AFTER STERILIZATION (FIGURE 7)

While efforts were being made to determine how respiration could exceed photosynthesis for long periods without organic matter, a test was made to determine if there was some inherent oxygen loss somewhere such as in a cavitation action of the pump.

10% formaldehyde was circulated in the system for an hour and then flushed out with a continual flow of tap water for a day. Then the dilute nutrient plankton medium was introduced again with a pinch of aufwuchs saved from the former community. The growth sequence which developed (Figure 7) was similar in community composition, characteristics, and color with a green surface bloom followed by an orange color as the blue greens experienced a pigment change. The initial metabolism measurements following the formaldehyde treatment showed no oxygen increase or decrease thus indicating that there were no non-biological oxygen changes due to the circulation process. During the treatment, visible slime clumps had been removed that had previously been residual through the previous experiments. Thus the total bacterial concentrations and organic storage were diminished at the start of this particular sequence. The metabolism of bacterial slimes in the dark tubing increased more rapidly than that of the algae as shown on the measurements of May 29. The respiration rates were somewhat lower than in earlier experiments at similar high current velocity.

G. EFFECT OF GLUCOSE ON METABOLISM AND SUCCESSION

(FIGURE 8)

On June 5 after another community stoppage was removed, a growth sequence was repeated which followed the usual previous pattern with a green bloom turning toward a yellow steady state as the metabolism diminished. As indicated in Figure 8, 1 gm of glucose was added at the time of the initial algal aufwuchs bloom. Neither photosynthesis nor respiration was stimulated by this supplemental carbohydrate source. Instead the pattern of previous experiments prevailed, with a lowering of metabolic rates and yellowing of the community.

On the other hand when a supplemental inorganic nutrient source was added there was an immediate increase of photosynthesis and respiration. Unlike the stimulatory action shown in an addition of nutrients without organic matter present on April 29, the respiration this time exceeded the photosynthesis in a respiratory bloom.

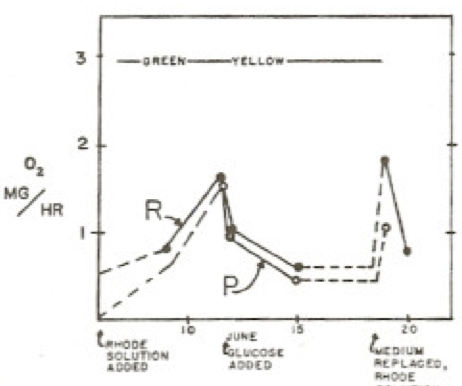

FIG. 8. Effect of glucose on succession and metabolism.

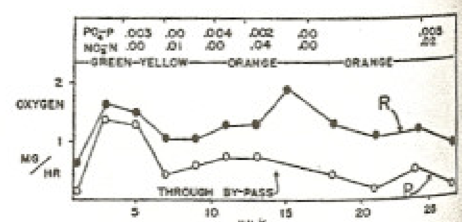

FIG. 9. Characteristics of a steady state based on organic reserves. (Inorganic nutrients self maintained at very low levels).

H. CHARACTERISTICS OF A STEADY STATE BASED ON ORGANIC RESERVES: P/R RATIO LESS THAN ONE

(FIGURE 9)

After cleaning and flushing the visible contents as usual, a new sequence was started with two innovations made on July 1. First, the plastic liner was introduced that permitted indefinite maintenance of the community without slippage and stoppage. Second, all water replacements were made with glass distilled water. The initial establishment of the community over the condenser surfaces followed the previous patterns with a green mat turning yellow after the initial burst of photosynthesis and respiration.

Unlike the two previous sequences the July patterns showed a great excess of respiration over photosynthesis with R values higher than those in the most recent sequences. The maintenance of this excess of R over P in steady state without addition of anything but distilled water indicated a steady organic nutrient supplement. Since the tubes had been flushed out, it was inferred that the residual slime communities in the dark tubes (tygon and plastic covered with aluminum) were holding and storing organic matter for energy sources from previous sequences when these materials were in excess. The glucose in the experiment that had preceded this sequence had apparently been carried over. Another possibility is that dark respiration exceeds light respiration.

On July 15 the circulation was routed through the condenser by-pass for an hour. This by-pass had been exposed to the concentrated solutions of the winter experiments and was known to have developed extensive bacterial slimes which were visibly pink and which had shown strong radio phosphorus uptake. After the water had been routed through the by-pass and returned to the main system, a temporary stimulation of respiratory rate was immediately measured.

From July 10 to August 14 the trend of the respiration rate curve was down. This pattern also suggested a diminishing organic matter source.

I. A STEADY STATE REGIME WITH A BALANCE OF P AND R

(FIGURE 10)

To test the hypothesis that the source of excess respiratory activity was in the dark tubes and that organic matter was stored either on the slimes or in the plastic, the tygon tubing was replaced with glass on August 13. Immediately, the excess respiration disappeared, and after the initial pulse, the photosynthesis became equal to or in excess of the respiratory rate.

On August 18 a mixed organic extract of some former community aufwuchs stored in the refrigerator was added to the system with a small stimulating effect. On September 3 after 10 days without disturbance, a balance of P and R was again in effect. To determine the effect of inorganic nutrients on the respiratory part of the community, inorganic medium was added and the community kept in darkness. After 3 hours, the respiration had increased tremendously but by the next day it was low again. At this time and while the community was still in darkness, glucose was added with an immediate stimulation of respiration which again had returned to normal by the next day.

J. STUDY OF THE VARIATION OF METABOLIC RATE WITH EXPERIMENTAL CHANGES OF CIRCULATION RATE

(FIGURE 12)

As illustrated in Figure 12, a number of series of measurements of photosynthesis and respiration were made of the microcosm under different current velocities and various circumstances over the several months of experiments (Figures 2–11). The communities studied had been growing at one velocity and had been adapted to this

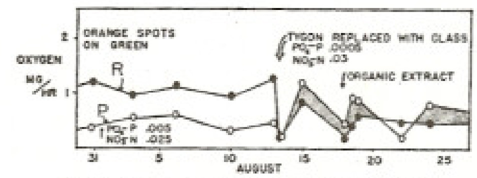

FIG. 10. Change from a steady state with excess of respiration to a balance of photosynthesis and respiration.

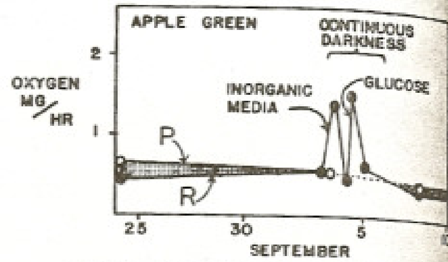

FIG. 11. Inorganic and organic stimulation of respiration under steady state conditions of balanced photosynthesis (P) and respiration (R).

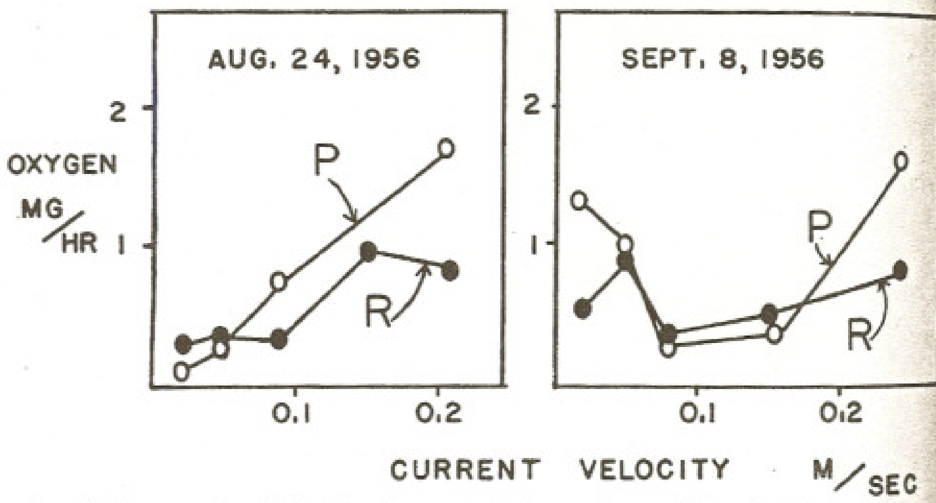

FIG. 12. Two examples of the effect of current velocity on the metabolism of a flowing microcosm in steady state.

velocity. It was found that any change in current velocity caused pronounced effects on the photosynthesis and respiration rates (Figure 12). In several series there was a clear correlation of current velocity with metabolic rate (Figure 12a). In other series there was a maximum or a minimum in the functional relationship of metabolic rate and current velocity (Figure 12b).

If more than one phase of the flow route of raw materials and energy from euphotic zone to regenerating zone and back is current sensitive, it is reasonable that the total metabolism should be a complex function of current velocity. Therefore it is not surprising that the ratio of P/R is modified by the rate of current circulation. Judging in a general way from the current experiments, the total community metabolism possible is greater in high current velocities since the metabolisms of individual plant and animals adapted to high current are greater.

Discussion

METABOLIC ZONATION IN THE MICROCOSM

The distribution of metabolism in the stream microcosm in 3 parts is similar in pattern to that in many streams, lakes, and marine situations. There is (1) a euphotic zone where photosynthesis is occurring, (2) a regeneration zone in the dark where respiratory processes predominate with a release of nutrients, and (3) a circulating medium. The slimes and porous plastics are like the oozes as reservoirs for organic and inorganic storage. As in classical ideas of the balanced aquarium, photosynthesis is dependent on the respiration and vice versa in metabolic symbiosis although separated into separate zones. The discussion by Steele (1956) of a shelf environment, the Fladen ground, or the lake with epilimnion and hypolimnion (Ohle, 1956) may be cited as two examples of macrosystems of similar structure where metabolic rates have been estimated.

THE COMPETITION BETWEEN P AND R FOR INORGANIC NUTRIENTS

When P is equal to R in steady state, the system fulfills the conditions for a closed self regulating cycle system described by Lotka (1925). As Redfield brought out in 1934 such coupling of regeneration and production will tend by a natural selection process to adjust the ratios of the chemical elements in the regenerating system to resemble those in the primary production part of the system.

The photosynthetic and respiratory systems are not, however, necessarily sequentially symbiotic but may exhibit competition also. Where organic matter is available in the community from imports or earlier storage, there may be an excess of respiration over photosynthesis. Under these conditions the bacterial heterotrophs as well as the autotrophs may be limited by the same inorganic nutrients. A competition results. The rapid uptake of radio-phosphorus (Experiment A) in both the condenser euphotic zone and in the regeneration zones of the microcosm is an indication of such competition for phosphorus.

Renn (1937) with experiments on stored sea water showed that the bacterial assimilation of inorganic phosphate was markedly increased if 10 to 60 mg./1 glucose were added. In spite of his own experimental demonstration that marine bacteria with an organic substrate could lower phosphate concentrations below detectable limits, Renn somehow concluded that bacteria do not complete with algal phytoplankton for nutrients. His discussion emphasized the role of bacteria in releasing phosphorus after short life span of a few days. He did not discuss steady state regimes or those where respiration was greater than photosynthesis.

THE EXTRACTION, STORAGE, AND METABOLISM OF ORGANIC MATTER BY AUFWUCHS-SLIME COMMUNITIES

In several of the experimental sequences, there were indications that organic matter could be extracted from the water and stored rapidly during periods of excess dissolved organic matter for use many days later. In experiments C, D, and H there were long periods of time when respiratory metabolism of the whole microcosm exceeded the photosynthetic metabolism (Figures 4, 5 and 9). The quantity of such stored organic matter metabolized during each sequence was determined from a balance sheet calculation using the following equation:

$$P + I = R + S$$

where P is the production, S is the storage, R is the respiration and I the organic matter saved from an earlier time as an import.

TABLE 1

Balance Sheet Calculations for Experimental Sequences

Community	P/R	Calculation[a]	Total During Sequence Gm	Chlorophyll Per Area Gm/m^2	Assimilat Numbe mg/mg/
Fertilized Hyponex, autotrophic March 14, 1956	1.3	P: 2.25 mg/hr for 20 days R: 1.50 mg/hr for 23 days S: final dry weight I: by difference	1.07 0.83 1.30 1.05	0.38	1.37
Dilute media, nutrient rich substrates, heterotrophic April 7, 1956	0.68	P: 1.00 mg/hr for 18 days R: 1.25 mg/hr for 21 days S: final dry weight I: by difference	0.43 0.63 0.45 0.65	0.12	1.8
Dilute media, heterotrophic May 5, 1956	0.74	P: 1.0 mg/hr for 25 days R: 1.20 mg/hr for 28 days S: final dry weight I: by difference	0.60 0.81 1.08 1.29	0.21	1.0
Dilute medium low current heterotrophic June 5, 1956	0.69	P: 0.75 mg/hr for 9 days R: 0.75 mg/hr for 13 days S: final dry weight I: by difference	0.16 0.23 0.23 0.30	0.095	1.43
Dilute medium low current May 24, 1956	0.84	P: 0.65 mg/hr for 17 days R: 0.82 mg/hr for 17 days S: final dry weight I: by difference	0.27 0.33 0.20 0.26	0.033	3.0

[a] P, gross production; R, community respiration; S, storage; I, organic matter (stored previously and metabolized as an impo

In Table 1 are given balance sheet calculations for 5 cases for which data are ava able. For these calculations the approximations were used that one gram of dry weig of aufwuchs was equivalent to one gram of oxygen metabolism and that the phot synthetic quotient was unity. P and R were obtained from the metabolic graphs. T period of time used for calculating R was greater than for calculating P since the fi few days of each sequence were without appreciable plant communities. The storage was determined as the dry weight of the community aufwuchs removed from the syste at the end of the sequence. I was calculated by difference. In most of the cases the magn tude of organic matter metabolized as an energy supplement was apparently as great the production, even though the growth of plants in the euphotic zone was the visib conspicuous process.

CHLOROPHYLL AND ASSIMILATION NUMBER

Especially since the studies of Willstatter and Stoll chlorophyll has been related t photosynthetic rates in physiological studies of individual plants. Even closer relatio ships have been found between chlorophyll of natural communities and their produc tion as in the papers of Manning and Juday (1941), Ichimura (1956), and Edmondso (1955). Gessner (1949) found similar quantities of chlorophyll per area in lakes, field and forests. It is of interest, therefore, to determine the relationship of chlorophyll i the microcosm to metabolism and area of the community. A useful function is the as similation number (ratio of mg carbon-dioxide consumed per hour to mg chlorophyll)

In Table 1 are given the chlorophyll values and assimilation numbers. The quan tities of chlorophyll per area are between 0.09 and 0.38 gm/m^2. These values are lowe by a factor of ten than those in terrestrial communities but similar in order of magnitud to those in many aquatic communities. The assimilation numbers between 1.0 and 1.9 mg/hr are similar to those in Silver Springs (0.72) (Odum, 1957) and the Eniwetok coral reef (Odum and Odum, 1955). They are vastly smaller than values of 20 found in algal cultures during successional states (Ryther, 1956).

These comparisons do indicate similarities between the flowing microcosm and the macrocosm. Chlorophyll is apparently as valid a measure of the photosynthesis in steady state adjustment in microcosm as in some similar natural ecosystems.

THE SPECIES DIVERSITY STRUCTURE OF THE COMMUNITIES

Following the procedure used by Yount (1956) on aufwuchs in Silver Springs, counts were made of the species in the aufwuchs of the microcosms at a number of stages during the year. The remarkable tendency for natural communities to have a logarithmic distribution of rarity was utilized to obtain an estimate of diversity. Without actually identifying most species, the cumulative appearance of new species was plotted as a function of the logarithm of the number of individuals examined. These counts were made on bits of aufwuchs placed on microscope slides. The plotted points are given in Figure 13. The lines are fairly straight. The slope of the line is the index of

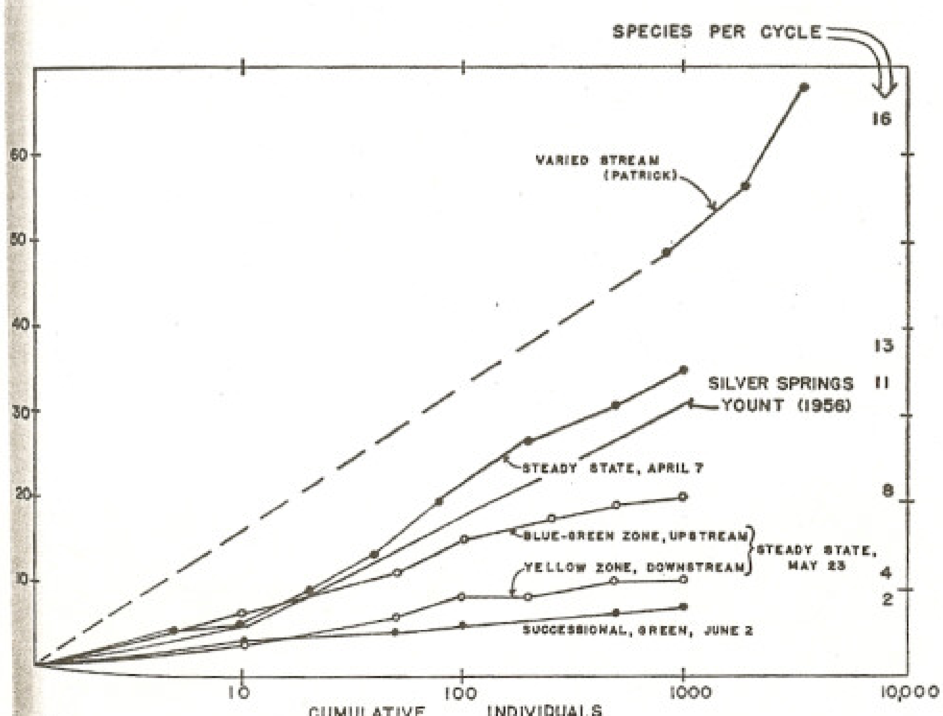

FIG. 13. Cumulative species as a function of logarithm of the number of individuals counted. The slope expressed as species/cycle is the index of diversity used to compare microcosm with macrocosm.

versity expressed as species/cycle. A cycle is defined as a 10 fold increase in individuals examined. The tendency for straight lines indicates that logarithmic patterns e found in the microcosm as in the Silver Springs System. That the species structure is similar in this respect to typical aufwuchs in nature is a justification for regarding the microcosms as real miniatures.

The species diversities resembled those in Silver Springs regarding relationship to succession. The species variety was less during successional bloom than at steady state.

Dr. Francis Drouet kindly identified the dominant algae of the microcosm as *Phormidium tenue* (Menegh.) Gom. Also present in lesser numbers was *Phormidium inundatum* Kütz, *Plectonema nostocorum* Born, *Anabaena oscillaroides* Bory, *Anabaena variabilis* Kütz, *Stigeoclonium tenus* (Ag.) Kütz, *Scenedesmus quadricauda* (Turp.) Bréb., *Scenedesmus bijuga* (Turp.) Lagerh., *Scenedesmus obliquus* (Turp.) Kütz, and *Calothrix parietina* (Nag.) Thur.

THE YELLOW PIGMENT CHANGE AND ANIMAL SUCCESSION FOLLOWING INITIAL SUCCESSIONAL BLOOM

Kingsbury (1956) found in a culture of a blue-green alga *Plectonema nostocorum* Bornet ex Gomont that with the disappearance of nutrients the aging and cessation of growth of the culture was accompanied by a change in overall pigment in the algae from green to yellow. He was able to restore the original color and stimulate further growth with added nitrate. Kingsbury determined that the yellow pigments were carotenoids. He believed that photosynthesis had stopped or been much reduced in such cells.

As described in the many sequences (Figures 2–11) a similar color change took place in the sessile blue-green algae dominating the microcosm although photosynthesis did not cease entirely. The absorption spectrum of the orange community is in Figure 14. The pattern resembles that given by Kingsbury (1956) with evidence of carotenoid

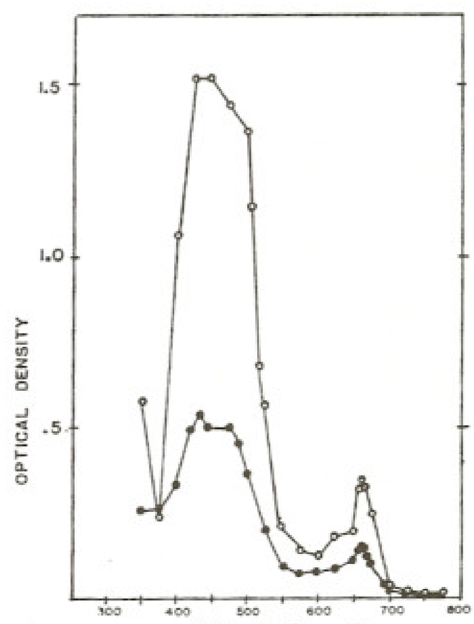

FIG. 14. Absorption spectra of acetone extracts of aliquots of the steady state community. Carotenoids and chlorophyll

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minance although chlorophyll is still present.

Throughout the studies the upstream end of the community for about two inches was een while the rest of the community was brilliant orange. In August the condenser ibe with community was disconnected and reversed. Within a week the green color had eformed in the upstream end of the community indicating a gradient with respect to he flow of the water. Presumably the green represented the zone where nutrients regenerated in the dark tubes were being removed. Although different in color, microscopical examination did not show differences in species composition or species variety.

Although the meaning of the color shift is not fully understood, it served as a most sensitive visible indicator of the existence of net plant production. Kingsbury postulated that chlorophyll was being used for its nitrogen content and that the plants were metabolically inactive.

In the long steady state series beginning on August 14, the orange color formed as usual but in time disappeared being replaced by a dull green. No nutrients had been added and no spurt in metabolism measured. Examination showed that a large population of herbivores and carnivores had developed in the aufwuchs just as in Silver Springs aufwuchs after the first month. *Arcella* and a flatworm were dominant. In the previous sequences, yellow cells followed the first two weeks algal bloom and did not have appreciable microzoa. It is conceivable that the later development of the animals in the older steady states serves in total function as a means of regenerating nutrients from filamentous algal cells thus preventing the nutrient condition that produces the yellow color.

EFFICIENCY

A rough measurement of incident light energy was made with the help of Dr. J. S. Kirby-Smith of Oak Ridge Laboratories. The galvanometer deflection produced by a standard lamp on an Epply Pyrheliometer was compared at the same distance with the deflection produced by one of the floodlamps. Then a Weston photronic cell was placed opposite the floodlamp so that the microammeter deflection of the photronic cell was found corresponding to a measured quantity of total energy received (1.82 watt/cm^2/microampere). Then the photronic cell was covered with a slit the width of the condenser community and was placed at the microcosm. From the microampere readings, the incident total energy was estimated for the entire condenser of 60 cm length. Rabinowitch (1951:839) figures that 8% of the total energy of incandescent lights of this size is of a wave length suitable for photosynthesis. Using this figure, it is found that the aufwuchs of the microcosm is receiving 0.27 KCal/hr useable light energy. This is of the order of magnitude of full daylight. The maximum gross production of 2.5 mg/hr oxygen figured at 3.5 Cal/gm is 8.7×10^{-3} KCal/hr. The production thus is about 3 percent of usable light energy reaching plant level. It is interesting that the efficiencies in the microcosm resemble those of naturally adapted communities in nature (1–8% of visible light energy absorbed).

SUMMARY

1. A flowing water laboratory stream microcosm was constructed in which it was possible to culture and study the growth, succession, and metabolism of whole aufwuchs-slime communities.

2. The communities developing in the microcosm during succession, resembled communities in nature with respect to P/R ratio (0.6–1.3), chlorophyll (0.03–0.38 gm/m²) assimilation number (1–3 mg/mg/hr), species diversity index (2–13 species/cycle) efficiency (3%), and the low concentrations of nitrate (0.05 ppm) and phosphate (0.005 ppm) maintained in the medium.

3. Under conditions of constant illumination steady state adjustments of whole aufwuchs communities are possible for considerable times with an excess of respiration over photosynthesis as well as with a balance of photosynthesis and respiration.

4. During community succession metabolism was maximal during succession rather than at climax.

5. The metabolism of successional and steady states of whole communities is a complex function of the current velocity. There is a greater metabolism at greater velocities in many cases but not all.

6. Aufwuchs communities show strong powers of taking up both dissolved inorganic and organic nutrients for future use both as raw materials and as an energy source.

7. Pigment changes of the blue-green algal mats of the aufwuchs from green to orange were a sensitive visible indicator of the metabolic condition of the community.

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