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THE RELATION BETWEEN CARBON ASSIMILATION AND BIOMASS
DYNAMICS IN A PHYTOPLANKTON COMMUNITY

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Douglas P. Wilcox, B. S.

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Production dynamics in the phytoplankton community of a mesotrophic Texas reservoir were measured weekly over a four month period using ^{14}C incubation and ATP assay methodologies. Assimilation values of ^{14}C ranged from 0.2 to 45 $\text{ug C l}^{-1}\text{hr}^{-1}$. Significant positive and occasionally negative changes in biomass (i.e. viable organic carbon) were observed in short term (4 hr) in situ incubations juxtapositioned with the ^{14}C experiments; viable organic carbon production, estimated with the ATP assay, ranged from -25 to +50 $\text{ug C l}^{-1}\text{hr}^{-1}$. Carbon assimilation and biomass changes did not correlate in either short term (4-5 hr.) or over the study period (6 months). However, weekly biomass trends were predicted by relative positive or negative biomass changes in the short term incubations. Biomass measurements gave a more sensitive insight into production dynamics in the phytoplankton community than did carbon assimilation measurements.

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CHAPTER I

INTRODUCTION

Accurate measurement of phytoplankton biomass dynamics is a basic problem in limnology, since production of planktonic and periphytic algae is the primary link in aquatic food chains. Numerous methods have been devised to quantify standing crops (i.e. instantaneous biomass) and primary production (i.e. biomass produced during some definite time period) of phytoplankton. Some of the more common methods used to estimate standing crops involve microscopic counts of cells, extraction of algal pigments such as chlorophyll a, and various gravimetric measurements, usually dry weight of cellular material (see Vollenweider 1974).

None of these methods accurately estimates biomass. Chlorophyll a is known to persist in significant amounts in the water column well after the demise of algal blooms (Talling and Driver 1963; Eppley 1968). It is very difficult to visually determine if algal cells to be included in cell counts are viable; and serious errors in biomass estimates determined in this manner may occur (Paerl 1976; Paerl and Williams 1976). The only really reliable information that may be generated from a temporal series of cell counts relates to relative specific and community succession. Dry weight measurements unavoidably measure detrital material as well as the viable

components of the seston in the water column. The implication here is that these methods do not provide the sensitivity needed to measure biomass, which Wetzel (1975) defines as the weight of all living and viable material at a given time in a given area.

Various researchers (Holm-Hansen and Booth 1966; Chappelle and Picciolo 1975; Holm-Hansen 1973; Paerl and Williams 1976; Rudd and Hamilton 1973; Holm-Hansen and Paerl 1972) have recently suggested that planktonic biomass may more accurately be estimated by quantification of some cellular biochemical component present only in living cells. Holm-Hansen suggested quantification of adenosine triphosphate (ATP) because: 1) it is present in all living organisms, 2) it is degraded very quickly upon death of the cell so its extracellular retention time is very short (i.e. minutes), 3) the steady state of ATP in algal cells is similar in light or in dark (see Holm-Hansen 1973), 4) adsorption of ATP on detrital material is insignificant except in sediments (Lee et al. 1971), and perhaps most importantly, 5) the ratio of cellular organic carbon to ATP is fairly uniform with ratios of 250-286:1 having been demonstrated (Hamilton and Holm-Hansen 1967; Holm-Hansen 1970). Cellular components such as NADPH and DNA have also been suggested as biomass estimators by Chappelle and Picciolo (1975) and Holm-Hansen et al. (1968). However, assays of these constituents apparently yield overestimates of viable carbon due to their significant association with non-living particulate material (Sutcliffe et al. 1970).

The ATP assay is by no means without problems. Perhaps the most controversial is the relationship between ATP measurements and viable organic carbon (VOC). Holm-Hansen (see Holm-Hansen 1973 and Holm-Hansen and Paerl 1972) has insisted that the 250:1 relationship holds in most plankton communities. Cavari (1975) demonstrated VOC:ATP ratios of 360-5000:1 in Israel's Lake Kinneret. The extremely high ratios in these data were due to a very dense bloom of Peridinium, a large phytoplankter which contains a greater percentage of cellular organic carbon than most other algae, and thereby yields higher C:ATP ratios. Since most aquatic systems are often more homogeneous with respect to their phytoplankton composition, very high C:ATP ratios may be exceptions rather than the rule. Further support of the 250-286 C:ATP ratio is given by Paerl and Williams (1976) in their investigations of different aquatic systems throughout the United States. Perry (1977) suggests that the C:ATP ratio will vary according to the amount and species of algae, especially blue-green forms, extant in the plankton community but that an overall ratio range of 250-306:1 is generally correct based on laboratory and field studies. It is clear that the ATP assay method of estimating planktonic biomass is very sensitive and may be quite useful in monitoring short term changes in microbial communities in lentic systems (see Stanford et al. 1977).

Another approach to understanding biomass dynamics involves monitoring photosynthetic processes (i.e. autotrophic activity). Yet this is difficult in aquatic systems where heterotrophic populations may represent a significant portion of the total biomass. It is important to point out here that natural plankton communities contain autotrophic and heterotrophic microbial biomasses. Both of the biotic components are primary contributors to higher trophic events, but there is a succinct difference between primary production and microbial production in plankton. The former refers to the amount of CO_2 assimilation or carbon fixation by phytoplankton per unit time and per unit water volume or surface area (Wetzel 1975); perhaps the latter should be thought of as the quantitative change in microbial biomass in the plankton, which would include phytoplankton and heteroplankton (i.e. all heterotrophic microbial life including many bacteria, protozoa, and fungi, but excluding by convention the zooplankton).

Quantification of primary production in plankton communities is now a routine limnological method. Since photosynthetic processes in most plants, including phytoplankton, involves quantitative assimilation of carbon dioxide, radioactively labeled CO_2 has been used as a tracer to yield estimates of uptake rates (see Steeman Nielsen 1952; Vollenweider 1974; Hall 1976). This methodology is so much more sensitive than estimates of CO_2 fixation from oxygen

flux measurements, that accurate studies of primary productivity in aquatic systems usually involve its use.

The original ^{14}C -primary production technique was very simple and straight forward. Water samples were placed in some type of transparent container, inoculated with a known amount of $\text{NaH}^{14}\text{CO}_3$, incubated for a short time period, fixed, filtered, and counted (using the Gieger-Muller counting system). The radioactive particulate material remaining on the filter represented the portion of the original inoculum that was autotrophically assimilated. Recently this method was modified by Schindler (1972) after he noted that a significant proportion of labeled carbon was passing through the filter in the form of labile organic carbon. To correct this, a portion of the incubated sample was bubbled with an inert gas to drive off excess or "unfixed" labeled carbon as CO_2 . A small aliquot was then taken and counted in a scintillation counter. Theodorsson and Bjarnason (1975) suggested a modification of the bubbling scheme in which sample aliquots were bubbled in scintillation vials rather than allihn filter tubes, thus reducing the number of aliquot transfers and errors associated with those transfers. An additional modification to the field procedure was introduced by Stuart (1977) who portended that an acid rather than a formalin fixative would better suit the technique since an acidified sample would not cause the foaming problem associated with a formaldehyde fixed sample. By alleviating this foaming process a

reduction in labile carbon loss could be realized. Although sufficient modifications have been made to increase the original sensitivity of the method, there are disadvantages that warrant special consideration. Briefly, these are the high costs initially involved (such as scintillation machinery, nuclear materials, and cocktails), special licensing and disposal required for radioactive tracer use, and the strict precautions needed to ensure that the ^{14}C stock solutions retain their specific activity and remain contaminant free.

As previously mentioned, the distinction between heterotrophic and autotrophic production in plankton is very difficult since the two are interrelated and both utilize inorganic carbon (as CO_2) for cell biosynthesis. The relationship between phytoplankton and heteroplankton is based on their extracellular products, for it is common knowledge that bacteria readily assimilate low weight molecular compounds as do phytoplankton, but their uptake rate is much lower than that of bacteria. A great percentage of these compounds come directly from extracellular metabolites produced by phytoplankton. In return, inorganic carbon (CO_2) is liberated during heterotrophic degradation of these compounds, which contributes to the carbon pool available to autotrophs (i.e. phytoplankton). This relationship is enhanced, positively or negatively, by the trophic status of the system. When estimating primary production these two constituents must be effectively separated. This is approached by use of opaque

and transparent incubation chambers for generating ^{14}C assimilation estimates. Assimilation measured in the dark bottle is assumed to be heteroplankton and is subtracted from light bottle values to yield an estimate of only autotrophic production (i.e. carbon assimilation).

Limnologically, it seems imperative to measure the primary production and microbial biomass of an aquatic system. Primary production measurements yield a quantitative insight into the amount of potential energy available to the "bio-activity" of the system. This bioactivity is considered to be the systems capacity for the formation of potential energy and the subsequent reconversion of this to kinetic energy on a unit volume of area basis (Goldman 1968). Concomittantly, biomass measurements and the taxonomic study of the phytoplankton yield a quantitative look at the total viability of the microbial community along with what organisms are present and responsible for the primary production of that system. These two parameters also provide an insight into the trophic status of the system from which certain preliminary assumptions regarding water quality, nutrient abundance, etc. may be ascertained.

Several recent studies have attempted to relate a quantitative relationship between biomass, as estimated by conventional techniques (i.e. cell counts, chlorophyll a, etc.), and primary production in several different aquatic systems (Schindler and Holmgren 1970; Hobbie et al. 1972; Tunzi and

Porcella 1974; Stadelman et al. 1974; and Vollenweider et al. 1974). These investigators suggest that chlorophyll a tends to correlate most reliably with primary production trends in the photic zone of the water column. Yet the relation is not particularly accurate because of the chlorophyll persistence previously mentioned and the fact that chlorophyll a content varies greatly according to light (intensity, quality, and duration), temperature, etc. The ATP assay seems to hold promise for better analyses of biomass dynamics and primary production in lentic systems because instantaneous measurements of viable organic carbon may be made in size fractionated plankton samples (see Boswell 1977 and Stanford et al. 1977). The relationship between phytoplankton biomass dynamics and primary production has not been researched from this standpoint. Although it may be inferred from documentation of rapid turnover of nutrients (i.e. C, N, and P) by planktonic microbes (see Wetzel 1975), there exists no data that demonstrates short term (i.e. hourly biomass changes in plankton communities. Such studies would yield useful interpretive knowledge of the intricacies of lentic carbon dynamics (see Stanford and Silvey 1977).

Short term biomass dynamics and the applicability of ATP assays to phytoplankton production studies were investigated over a 6 month period in a North Texas reservoir. Fluctuations in VOC were measured along with primary productivity in the laboratory algal culture and in the reservoir. Planktonic

biomass changes were recorded in incubation chambers over four hour time periods; these studies were conducted weekly and interpreted in conjunction with primary production data. Since Parker et al. (1974) have shown that selection of size and construction of incubation chambers can significantly affect the outcome of primary production studies, I also attempted to delineate if container effects were a factor in estimating biomass changes in the incubation chambers. The major objective of this work was to compare phytoplankton biomass changes with primary production estimates in time series samplings and to assess the proposition that biomass changes quantified with ATP assays might be more informative than ^{14}C tracer methods for estimating primary production in lentic systems.

Two basic investigations were undertaken in this study design: 1) the feasibility and precision of algal production estimates generated from short term biomass changes initially were investigated with favorable results in carefully controlled laboratory experiments and therefore, 2) biomass, primary production, and some physiochemical dynamics were studied in the reservoir over the period from July to December, 1976.

CHAPTER II

DESCRIPTION OF STUDY AREA

H. H. Moss Reservoir, selected as a study area, is located in Cooke County, Texas, 16.09 km (10 miles) northwest of Gainesville. The impoundment lies in the Red River drainage basin and receives runoff from an area of 168 km² (65 sq. miles) via North and South Fish Creeks (see Figure 1). The ratio of surface area to volume is high due to the shallow basin in Moss Reservoir (Figure 1: Table I), a feature very typical of reservoirs in the southwestern U.S. Much of the runoff is a result of fairly infrequent spates; there is little sustained discharge from either South or North Fish Creeks.

Moss Reservoir was built in 1966 to supply future potable water needs for Gainesville and surrounding areas. To date it has been used only for recreational purposes. Thus, the water volume of the reservoir has remained stable at 2.86 X 10 m². Most of the southern shore is crowded with private homes and fishing campus, but the northern shore is mostly undeveloped pasture land above the steep shoreline.

The reservoir lies in the lower Cretacious Comanche limestone formation and hence its waters are well buffered where pH valued trend around 8.0 - 8.3, except during summer

Fig. 1--Map of H. H. Moss Reservoir, Cooke County,
Texas indicating sampling site and tributaries.

TABLE I
MORPHOMETRIC FEATURES OF H.H. MOSS RESERVOIR
COOKE COUNTY, TEXAS

Elevation (MSL)	220 m
Surface Area	4.55 km ²
Volume	2.86 (10 ⁷)m ³
Mean Depth	6.03 m
Maximum Depth	20 m
Watershed Area	168 km ²

stratification when hypolimnion pH readings fall to the 6.5 - 6.8 range. The reservoir is susceptible to occasional blue-green algal blooms and macrophytic vegetation (e.g. Typha, Potamogeton, Chara, etc.) abounds throughout most of the year. Boswell (1977) has classified Moss Reservoir as mesotrophic based on C, N, and P measurements.

Moss Reservoir has an abundance of several game fish (see Table II) and is fished rather heavily 8-9 months of the year. Although fishing pressure is heavy, the reservoir is still in its prime with regard to the quantity and quality of available fish habitat.

TABLE II
FISH OF MOSS RESERVOIR*

Species Name	Common Name
<u>Lepomis macrochirus</u>	Bluegill
<u>Lepomis magalotus</u>	Longear Sunfish
<u>Lepomis cyaneus</u>	Green Sunfish
<u>Lepomis microlophus</u>	Red-ear Sunfish
<u>Merone chrysops</u>	White Bass
<u>Micropterus salmoides</u>	Largemouth Bass
<u>Pomoxis annularis</u>	White Crappie
<u>Dorosoma cepedianum</u>	Gizzard Shad
<u>Moxostoma erythrurum</u>	Redhorse Sucker
<u>Cyprinus carpio</u>	Carp
<u>Ictalurus natalis</u>	Yellow Bullhead
<u>Ictalurus melas</u>	Black Bullhead
<u>Ictalurus punctatus</u>	Channel Catfish
<u>Ictalurus furcatus</u>	Blue Catfish
<u>Ichthyobus bubalus</u>	Smallmouth Buffalo
<u>Aplodinotus grunniens</u>	Freshwater Drum
<u>Lepisosteus oculatus</u>	Spotted Gar
<u>Lepisosteus osseus</u>	Longnose Gar
<u>Chaenobryttus gulosus</u>	Warmouth

*Listed according to population density from data obtained in concurrent studies on Moss Reservoir (Beitinger 1977).

CHAPTER III

MATERIALS AND METHODS

Laboratory Investigations

Biomass, cell numbers, and primary production dynamics of Scenedesmus quadricauda in unialgal batch culture were monitored weekly under strictly controlled environmental conditions in which light intensity (i.e. 165 ft. candles) and temperature (i.e. 23-25° C) were maintained at ranges in which nutrient depletion (due to excessive growth) would least likely occur. Primary production estimates were generated (^{14}C radioisotope tracer technique, see Stadelman 1974 and Stuart 1977) at the same time intervals as biomass and cell counts. An initial biomass determination was made on the culture using the ATP assay procedure and at the same time aliquots (e.g. 50 ml) of culture were placed in light and dark containers, inoculated with ^{14}C , and incubated in the same environmental chamber as the original culture. Another culture sample was assayed at the end of the five hour ^{14}C incubation period. The usually positive difference between the initial and final measurements yielded quantitative biomass changes per hour (i.e. Δ -biomass hr^{-1}). Similarly, primary productivity rates were calculated in mgC hr^{-1} from carbon assimilation data. Cell counts were also made at the beginning and end of each incubation period.

The final aspect of the design was to statistically compare the three parameters.

The *S. quadricauda* culture was reared in a 19 liter autoclaved pyrex carboy which was fitted with a filtered air inlet and a resealable outlet tube. Air was bubbled through an isopropyl alcohol bath, filtered through an activated charcoal column, and then wetted with distilled water before entering the culture flask. Turbulence from bubbled air kept the algal cells in suspension and provided a partial inorganic carbon source (CO_2) for the alga. Fifteen liters of Bristol's algal medium was used as a growth solution and was prepared according to procedures outlined by Stein (1973). The culture was in a heavy growth phase throughout most of the study. After preparation, the culture was placed in an environmental chamber where light banks were set to provide 165 ft candles (measured using a G.E. Light Meter Type 213) of cool white fluorescent light and the temperature was set to maintain a range of 23-25° C. Also in the same environmental chamber a rotary shaker was installed to provide agitation (100 rpm) for ^{14}C samples while they were being incubated.

ATP assays were performed essentially according to Holm-Hansen and Booth (1966), Parsons and Strickland (1968), and Hamilton and Rudd (1973). Boswell (1977) recommended a modification to the extraction process whereby a hot plate was used instead of a boiling water bath during the initial

steps of the extraction. He found that extraction efficiencies were much greater since no significant temperature changes occurred in the critical first 1-3 minutes of the extraction process (e.g. the temperature of the boiling Tris remained constant upon introduction of the sample when a hot plate was used).

Since the culture was in growth phase and cell number ranged from $58,000 \text{ ml}^{-1}$ to $188,000 \text{ ml}^{-1}$ over the five week experimental period, only 10 ml of culture was needed for each ATP assay. Each of 5-10 ml samples was filtered on Gelman membrane filters with a pore size of $1.2 \mu\text{m}$. This particular pore size was selected in an attempt to separate bacteria from the alga cells during filtration of culture aliquots. It was assumed that due to the size of *S. quadricauda* only bacteria, if there were any in the culture, would pass through the filter.

After filtration, the filter and included cells were quickly emersed in boiling Tris buffer (.02 M; pH = 7.75) contained in beakers on a hot plate. After heating for approximately three minutes at 100°C , the samples, in beakers, were placed in a hot water bath, also at 100°C , for an additional two minutes for the purpose of handling more filters on the hot plate. The filters were then washed with hot Tris and discarded and extracts were again placed in the hot water bath for another three to four minutes to finalize extractions and reduce sample volume to approximately 4 ml. Hot extracts

were poured into test tubes, brought to constant volumes of 5 ml with hot Tris, and allowed to cool. Samples were then stored at -20°C (Holm-Hansen and Booth 1966).

The frozen samples were assayed for ATP content in the following manner. A standard ATP solution of 100 mg ml^{-1} ($.2\text{ ml} = 20\text{ mg ATP}$) was made by diluting di-sodium ATP crystals (12.3 mg l^{-1}). This solution was divided into 1 ml fractions which were frozen until use. Crude lyophilized luciferin-luciferase enzyme (from Sigma Chemical Company) was hydrolyzed with Tris buffer ($.02\text{ M}$; $\text{pH} = 7.75$) approximately 20 hours before use. After aging three to four hours at room temperature ($22-24^{\circ}\text{C}$) the enzyme suspension was centrifuged 20 minutes (3200 rpm) at room temperature, the supernate decanted, and allowed to stand at room temperature for the remaining 16 hours.

Analyses of the extracts and standards were made using a Model 2000 ATP Photometer, JRB Company, (see Figure 2). A 0.20 ml aliquot of extract (i.e. of sample or standard) was placed in a scintillation vial. As an equal aliquot of enzyme was added to the liquid in the vial, the photometer was activated. Following a 15 second delay, the vial was placed into the counting chamber. The instrument integrated a one minute portion of the decay curve and a digital (LED) readout was produced (see Holm-Hansen 1973 and Chappelle and Picciolo 1975). Concentrations of ATP in extracts were calculated from least squares linear regression of data

Fig. 2--Model 2000 ATP Photometer (JRB Co.)

generated from assay of ATP standards. Since a crude enzyme of variable activity was utilized, a fresh set of standards was prepared prior to analysis of each set of extracts. Background assays of the enzyme and occasional sample spikes were performed on the samples to maintain quality control. Values obtained from standard curve interpretations were extrapolated to cellular organic carbon by multiplying $\mu\text{g ATP l}^{-1}$ by a factor of 276 (Holm-Hansen and Hamilton 1967; Holm-Hansen 1973).

Cell counts were determined in cultures using a Model ZBI Coulter Counter (Coulter Electronics, Inc.). Aliquots (i.e. 1/2 ml) of samples were taken before and after the incubation time period and diluted with a known amount of counting fluid (Isoton). One ml was then counted automatically by the machine. The actual figures for cell numbers per unit volume were derived from dilution factors and the change in cell number was found by subtracting initial from final concentrations.

Primary production estimates were determined using the ^{14}C tracer technique originally proposed by Steeman Nielson (1952) with analysis modifications (see below) recommended by Schindler et al. (1972). A stock solution of radioactively labeled sodium bicarbonate ($\text{NaH}^{14}\text{CO}_3$; activity of 5 mCi), obtained from New England Nuclear (Boston, Mass.) was made by diluting the 5 mCi reagent with 500 ml of buffered (pH = 10.2) and sterilized distilled water; resulting in a

final activity of 10 mCi ml^{-1} . This final solution was divided into 80 ml aliquonts, each of which were placed in presterilized heavy glass tubes fitted with serum vial stoppers. Containers of this type provided eash transport of stock solution to the field and minimum contaminant problems when removing aliquonts for inoculation. Samples of 50 ml each were obtained from the culture and placed in 50 ml opaque or transparent Erlenmyer flasks. Four transparent and three opaque flasks were used, permitting sample replication. Each bottle was inoculated with 50 ml (i.e. $0.5 \mu\text{Ci}$) of ^{14}C stock solution and incubated for four hours on a small rotary shaker in the same environmental chamber that contained the parent culture. At the end of four hours, the samples were "fixed" (i.e. the cells killed) using 0.25 ml of concentrated HCl. The HCl was used in place of the normally employed formaldehyde fixative to alleviate foaming during sample processing (Stuart 1977). An aliquont (5 ml) of fixed sample was placed in a scintillation vial (Theodorsson and Bjarnason 1975) and bubbled at a rate of 150 ml min^{-1} (see Figure 5) with Argon (any inert gas may be used) for 30 minutes (Schindler et al. 1972), after which time 15 ml of a xylene based scintillation cocktail (Aquasol) was added to the vial and shaken to homogenize both the cocktail and sample. Each sample was then counted in a Beckman LS-250 scintillation counter for ten minutes each using the ^{14}C and H^3 mode. Background and stock activity counts were

made each time sample sets were analyzed. Data obtained from each method were converted from decays per minute (dpm) to $\text{mgC l}^{-1}\text{hr}^{-1}$ using the following formula derived by Steeman Nielsen (1952) as modified by Lind (1974).

$$\text{mgC l}^{-1} \text{ unit of time}^{-1} = \frac{r}{R} (v) (c) (f)$$

Where:

r = uptake of radioactive carbon in counts per minute =
 counts min^{-1} for filtered volume $\times \frac{\text{volume of bottle}}{\text{volume filtered}}$

R = ^{14}C inoculated in cpm

v = dilution volume

c = inorganic carbon (C^{12}) available in ppm

f = correction of slower uptake of ^{14}C as compared with
 $\text{C}^{12} = 1.06$.

Field Investigations

Light, dissolved oxygen, turbidity, and inorganic carbon profiles were measured in situ each week during field investigations. Other pertinent data were available from concurrent studies on the reservoir (Perry 1977). Taxonomic identifications and cell volumes were determined using microscopic analyses. Biomass changes (i.e. Δ -Biomass hr^{-1}) were measured in special incubation chambers as described in following paragraphs. Rates of primary productivity were delineated by both dissolved oxygen and ^{14}C methodologies throughout the field study; a four week study of heterotrophic assimilation of ^{14}C labeled glucose was also undertaken.

Weekly dissolved oxygen and temperature profiles were obtained in the water column of the reservoir using a YSI Model 31 combination D.O./Temperature meter. Light penetration data, also determined in situ, was expressed as percent of surface irradiation using a Kahlsco Model 268 WA 370 submarine photometer equipped with a surface (i.e. deck) photocell. Turbidity was monitored with a Hach Turbidimeter and inorganic carbon in the system was measured using an Oceanography International Total Carbon System Model 303.

Samples (1 liter) to be used for taxonomic and cell volume determinations were collected with a 4 liter Van Dorn bottle at depths of 1, 3, and 6 meters, which essentially were top, middle, and bottom of the photic zone respectively. These samples were passed through a 164 μ mesh stainless steel net to remove zooplankton. The filtrate was concentrated (1000:10) by centrifugation and preserved in a solution of 6 parts water - 3 parts ethanol - 1 part formalin (Prescott 1970). Aliquots (.1 mm³) of the preserved sample were placed in a Palmer-Maloney counting chamber, sized and enumerated using a whipple disc and stage micrometer. Phytoplankton samples were enumerated by counting 6 whipple disc fields each, from 3 Palmer-Maloney cell aliquots. Enumeration and measurements were made at 200X, whereas identifications were made from 200X to 1000X (Stein 1973).

One liter water samples for biomass determinations were obtained with the 4 liter Van Dorn bottle at depths of 1, 3,

and 6 meters. When using 3 liter plexiglas containers (see Figure 3), one Van Dorn bottle was used to fill each of the 12 containers. However, when using the glass BOD bottles (see Figure 4), one dark bottle and one light bottle were filled from the same Van Dorn sample; a second Van Dorn sample was immediately obtained, at the same depth, and the remaining light and dark bottles in the set were filled. This sampling regime was done to provide replicate samples for statistical purposes in checking heterogeneity in plankton distribution in the lake. In all cases, samples were incubated for four hours at the same depths from which they were taken. Immediately after incubation chambers were set in situ, two additional one liter samples were obtained from each of the three depths (i.e. 1, 3, and 6 meters). These were returned to a mobile field laboratory located on shore for processing. Each of these one liter samples (i.e. for initial biomass measurement) were passed through a 164 μ mesh stainless steel net to remove zooplankton. Four aliquots, 250 ml in volume, were then each filtered on a Gelman membrane (1.2 μ m). This pore size was selected to attempt to segregate the majority of phytoplankton from heterotrophic bacteria by allowing the latter to pass through with the filtrate. Extraction of ATP from plankters retained on filters was performed exactly as in laboratory studies described above. At the end of the four hour incubation period, this same procedure was used to process samples from the chambers (i.e. either 3 liter

Fig. 3--Plexiglas incubation chambers used in Δ -biomass studies.

Fig. 4--Glass BOD bottle apparatus used in both Δ -biomass and primary production studies.

Fig. 5--Non-filtration bubbling apparatus used in carbon assimilation determinations.

plexiglas or 300 ml glass BOD bottles). Differences between initial and final or incubated biomass measurements were analyzed for significance using a two sample T-test. Significant positive or negative changes in biomass were assumed to quantitatively represent phytoplankton growth activity in the photic zone of the reservoir.

Autotrophic production was monitored using the assimilation of labeled inorganic carbon ($\text{NaH}^{14}\text{CO}_3$; prepared as described above for laboratory studies). Light and dark bottle sets (i.e. light and dark 300 ml glass BOD bottles) were shaded, carefully filled with water from the selected depths via a Van Dorn bottle, and inoculated with 1 ml of $\text{NaH}^{14}\text{CO}_3$ using an autosyringe system capable of rapidly dispensing a constant volume of inoculum. Two light and dark bottle sets were incubated for four hours at 1, 3, and 6 meters in the water column. Assimilation rates were analyzed and primary productivity calculated as described above for laboratory procedures. For comparative purposes, pre and post incubation analyses of dissolved oxygen concentration were also determined in these same light and dark bottle sets to yield primary productivity estimates calculated from D.O. changes. A YSI O_2 meter fitted with a special BOD probe was used in these analyses. Changes in D.O. were expressed as $\text{mg O}_2 \text{ m}^{-3} \text{ hr}^{-1}$ and converted to carbon synthesis rates using a correction factor of 0.375 (Lind 1974).

Heterotrophic production analyses were facilitated by use of radioactive ^{14}C - glucose (New England Nuclear). Preparation of the stock solution was made by diluting the concentrated form of glucose (activity = .15 mCi) to a volume of 25 ml with a final activity of $6\ \mu\text{Ci ml}^{-1}$. Since glucose, labeled or unlabeled, is readily assimilated by most heterotrophic organisms, extreme precautions were taken to avoid contamination of the stock solution. Water to be incubated was taken with a plexiglas Van Dorn bottle that was prewashed with alcohol before each sample was obtained. The inoculum was prepared in sterilized stoppered serum vial and delivered to each BOD bottle with a sterile syringe and needle. After an incubation time of four hours, the samples were raised and immediately fixed using 2 ml of concentrated hydrochloric acid. The samples were then counted using the same technique as described for ^{14}C primary production analyses.

Statistical Techniques

Initial and final ATP measurements, in laboratory culture and field samples, were demonstrated to be significantly different using the students - t statistic. Linearity of light penetration and ATP standards were described by least squares regression analysis. A two level nested ANOVA (Sokal and Rohlf 1969) was used to delineate container effects (or lack of) between plexiglas and glass incubation chambers.

CHAPTER IV

RESULTS

Laboratory Study

Production of Scenedesmus quadricauda in batch culture was accurately quantified by weekly measurements of particulate organic carbon (POC) and viable organic carbon (VOC), as estimated from ATP assays. Measurements of POC were less sensitive than either VOC or cell counts (Figure 6). Viable organic carbon (VOC) appeared to be more valuable in monitoring the decline in culture growth than did the other parameters since numerous dead cells were not included in the biomass estimate; and hence the curve tailed off. Cell counts and POC unavoidably included nonviable cells. Production in the culture was readily evident from both biomass and ^{14}C primary productivity measurements (Figure 7). Since the culture contained fewer bacteria than would be found in most aquatic systems, the biomass changes (i.e. Δ -biomass) were considered to estimate production rates of S. quadricauda. As was expected, the two productivity curves (i.e. ^{14}C primary productivity and biomass) were closely correlated. Δ -biomass measurements contained greater amplitude (i.e. sensitivity) over the five week growth period than did ^{14}C primary productivity estimates.

Fig. 6--Weekly trends of standing crop (i.e. initial biomass) (Δ — Δ — Δ), cell number ($\circ$ — $\circ$ — $\circ$) and particulate organic carbon (POC) ($\bullet$ — $\bullet$ — $\bullet$) of *S. quadricauda* in laboratory culture.

Fig. 7--Primary productivity (●—●) in comparison with Δ -biomass (Δ — Δ) of *S. quadricauda* in laboratory culture.

Field Studies

Summer stratification occurred during July-September and was terminated by turnover or circulation in October when air temperatures cooled. Anoxic conditions prevailed in a well defined hypolimnion during August and September, but disappeared as fall turnover ensued (see Figures 8, 9, 10). Light penetration curves were similar throughout the study period; the photic zone varied little from a depth of zero to six meters.

Blue-green algae, particularly Microcystis sp. (Anacystis) were abundant in the phytoplankton community throughout the study period. Blooms of Cyanophyta were noted in late August, early September, and mid December. Diatoms were occasionally present in large numbers and temporally contributed about 30 percent of the total seston composition. Increasing amounts of the green alga, Gleocystis, were noted in the later part of the study (Figure 11).

No differences in Δ -biomass estimates could be detected due to incubation containers. As might be expected, Δ -biomass measurements were very different at the three depths in the photic zone (Table III).

Neither carbon synthesis (i.e. primary productivity) nor Δ -biomass estimations, as determined from dissolved oxygen data, compared well to the same measurements using ^{14}C and ATP respectively.

Fig. 8--Monthly dissolved oxygen and temperature profile for July and August.

Fig. 9--Monthly dissolved oxygen and temperature profile for September and October.

Fig. 10--Monthly dissolved oxygen and temperature profile for November and December.

Fig. 11--The percentage of the total standing crop that each algal phylum occupied on each sampling date in Moss Reservoir from July through December, 1976.

TABLE III

TWO LEVEL NESTED ANALYSIS OF VARIANCE TEST FOR DIFFERENCES BETWEEN
 PLEXIGLAS AND GLASS INCUBATION CHAMBERS, AS WELL AS
 DIFFERENCES BETWEEN DEPTHS AND Δ -BIOMASS

Date	Source	ss	df	F	P
9-22-76	Chambers	1,870.0	1	.0535	ns
	Depths	139,800.0	4	27.36***	0.001
	Within Subgroups	23,000.0	18		
	Total	162,800.0	23		
11-10-76	Chambers	176.0	1	.0021	ns
	Depths	34,000.0	4	154.55***	0.001
	Within Subgroups	10,000.0	18		
	Total	350,000.0	23		

***Indicates a highly significant difference between depths with regard to Δ -biomass.

Standing crop dynamics as estimated by VOC measurements (i.e. instantaneous biomass) showed that a greater percentage of phytoplankton was viable during December than in August or September, when cell counts indicated the greatest concentration of phytoplankton cells were present in the photic zone (see Figures 12, 13, 14).

Perhaps the most stimulating information revealed during the study was that relative positive or negative biomass estimates, derived from weekly four hour incubations in situ, very confidently predicted 92 percent of the weekly trends in instantaneous biomass (i.e. standing crops) at all three sampling depths (see Figures 15, 16, 17).

Periods of greatest primary productivity, as determined by the ^{14}C method, existed during August, September, and October (see Figure 18). These rates contributed significantly to the high mean production rate observed throughout the study (see Table IV). Significant correlations between temporal carbon assimilations and Δ -biomass dynamics were not evident at all depths (see Figures 19, 20, 21). As might be expected, no consistent relationship existed between VOC (i.e. instantaneous biomass or standing crop) and primary productivity, although on a few dates high carbon assimilation rates seemed to correspond to high standing crop levels (see Figures 12, 13, 14).

Six meter primary productivity values were not used in the estimation of activity coefficients since primary

Fig. 12--Temporal comparisons of standing crop (i.e. initial biomass) (○—○—○) and primary productivity (△—△—△) at 1 meter in Moss Reservoir, July through December, 1976.

Fig. 13--Temporal comparisons of standing crop (i.e. initial biomass) (●—●—●) and primary productivity (Δ—Δ—Δ) at 3 meters in Moss Reservoir, July through December, 1976.

Fig. 14--Temporal comparisons of standing crop (i.e. initial biomass) (○—○—○) and primary productivity (△—△—△) at 6 meters in Moss Reservoir, July through December, 1976.

Fig. 15--Temporal trends in standing crop (●—●—●)
at 1 meter in Moss Reservoir July-December, 1976.
 Δ -biomass is expressed as + or - changes on each date.
Numerals above each + or - represent the actual Δ -biomass
as hourly VOC changes (i.e. $\text{mgC m}^{-3} \text{ hr}^{-1}$).

Fig. 16--Temporal trends in standing crop (●—●—●) at 3 meters in Moss Reservoir July-December, 1976. Δ -biomass is expressed as + or - changes on each date. Numerals above each + or - represent the actual Δ -biomass as hourly VOC changes (i.e. $\text{mgC m}^{-3} \text{ hr}^{-1}$).

Fig. 17--Temporal trends in standing crop (●—●—●) at 6 meters in Moss Reservoir July-December, 1976. Δ -biomass is expressed as + or - changes on each date. Numerals above each + or - represent the actual Δ -biomass as hourly VOC changes (i.e. $\text{mgC m}^{-3} \text{ hr}^{-1}$).

Fig. 18--Comparisons of primary productivity at
1 (●—●), 3 (●---●), and 6 (●-----●) meters in Moss
Reservoir from July-December, 1976.

TABLE IV

WEEKLY DATA COMPARISONS BETWEEN MEANS OF STANDING CROP
 Δ -BIOMASS, AND PRIMARY PRODUCTIVITY FOR THE
 PERIOD JULY-DECEMBER, 1976

Date	Initial Biomass mgC m ⁻²	Biomass Change mgC m ⁻² day ⁻¹	Primary Productivity mgC m ⁻² day ⁻¹	Carbon Turnover Time* (days)
July 24	951	865	1425	.42
31	887	-442	2062	.33
August 14	956	.	1769	.39
18	1228	-208	1571	.56
31	1220	-127	737	1.24
September 8	927	635	1409	.46
14	995	-385	2276	.38
22	1296	-1495	1399	.69
29	1369	-1915	1709	.59

TABLE IV--- Continued

Date	Initial Biomass mgC m ⁻²	Biomass Change mgC m ⁻² day ⁻¹	Primary Productivity mgC m ⁻² day ⁻¹	Carbon Turnover Time* (days)
October				
6	1085	-451	1216	.79
13	962	1138	2290	.40
20	848	2904	1006	1.42
26	. . .	. . .	279	. . .
November				
10	1083	598	867	1.64
December				
1	1696	3225	942	1.72
7	2006	-1404	834	2.32
15	1932	-82	657	2.37
22	2004	2148	968	2.52
Means	1262	+313	1302	1.07

*These values obtained using the means of 1 and 3 meters (i.e. 6 meter values were not included).

Fig. 19--Temporal comparison of Δ -biomass (●---●---●) and primary production (Δ — Δ — Δ) rates measured at 1 meter in Moss Reservoir, July through December, 1976.

Fig. 20--Temporal comparison of Δ -biomass ($\bullet\text{---}\bullet\text{---}\bullet$) and primary production ($\Delta\text{---}\Delta\text{---}\Delta$) rates measured at 3 meters in Moss Reservoir, July through December, 1976.

Fig. 21--Temporal comparison of Δ -biomass ($\bullet\text{---}\bullet\text{---}\bullet$) and primary production ($\Delta\text{---}\Delta\text{---}\Delta$) rates measured at 6 meters in Moss Reservoir, July through December, 1976.

productivity was low because of subdued light at that depth. These coefficients in the form of Biomass/Production (B/P) ratios indicated that VOC turnover rates varied inversely with phytoplankton densities (i.e. when one parameter is high, the other is low) 71 percent of the time. As expected, these ratios also revealed that carbon turnover times were greatest on an areal basis (m^2) during July through October, whereas the opposite was true during November through December (see Table IV). A B/P ratio for the photic zone was estimated for the study period (see Table IV). However, if the six meter biomass and primary production values are included in determination of carbon turnover, then they would increase by an order of magnitude.

The short term experiment involving ^{14}C labeled glucose assimilation during December indicated that trends in glucose uptake followed similar trends in labeled sodium bicarbonate assimilation and biomass changes. The large amount of glucose assimilated suggests that a large bacterial population was present at six meters (see Table V). Further support indicating a substantial heterotrophic population was demonstrated by the quantification of light and dark bottles during primary productivity experiments (see Table VI).

TABLE V
 ^{14}C GLUCOSE ASSIMILATION AS COMPARED
 TO PLANKTON STANDING CROP AND
 PRIMARY PRODUCTIVITY

Date	Depth in Meters	^{14}C *Glucose Uptake $\text{mgC m}^{-3} \text{ hr}^{-1}$	Primary Productivity $\text{mgC m}^{-3} \text{ hr}^{-1}$	Initial Biomass mgC m^{-3}
Dec. 1, 1976	6	2.30	.25	270
Dec. 7, 1976	6	4.84	.73	305
Dec. 15, 1976	6	2.88	.36	296
Dec. 22, 1976	6	5.23	1.94	382

*Same dpm (1.1×10^7) as $\text{NaH}^{14}\text{CO}_3$ used in primary production estimates.

TABLE VI
COMPARISON OF LIGHT AND DARK BOTTLE ^{14}C ASSIMILATION
AT 1, 3, AND 6 METERS OF THE PHOTIC ZONE IN MOSS
RESERVOIR DURING NOVEMBER AND DECEMBER, 1976

Date	Depth (Meters)	Light Bottle	Dark Bottle
Nov. 10	1	36.87	8.47
	3	15.07	7.90
	6	8.00	7.44
Dec. 1	1	45.25	15.25
	3	22.50	13.70
	6	14.35	13.98
Dec. 7	1	37.75	11.75
	3	16.70	9.55
	6	9.15	8.95
Dec. 15	1	26.30	9.17
	3	17.05	8.32
	6	10.05	8.55
Dec. 22	1	29.78	9.12
	3	14.88	8.12
	6	8.22	8.32

*Values are represented in $\text{mgC m}^{-3} \text{ hr}^{-1}$.

CHAPTER V

DISCUSSION

Primary productivity and Δ -biomass methodology as used for rate measurements in this study really quantify different entities. Primary productivity is by definition the rate of assimilation or fixation of inorganic carbon by autotrophs. Organic compounds resulting from this synthesis are mostly in the form of new cellular material and thus ^{14}C primary production estimates include rates of algal production or the change in cellular material per unit of time. However, during periods of growth in phytoplankton populations, newly synthesized organic compounds may be released from cells via excretion, secretion, and respiration. These compounds are primarily produced by autotrophs and are primarily consumed by heterotrophic microbes (Wetzel 1975). In some cases, low molecular weight metabolites of autotrophic growth are reassimilated by autotrophs as a nutrient source, although this is a matter of considerable debate among algal physiologists (Brock 1967). In either case, it is now generally agreed that estimates of primary productivity should include consideration of ^{14}C in dissolved organic compounds because serious underestimates of ^{14}C fixation may otherwise occur (Stuart 1977). Hence, the non-filtration technique was developed.

Measurements of Δ -biomass do not account for extracellular losses of organic carbon but rather only reflect population dynamics. Thus, Δ -biomass measurements should not necessarily correlate with primary production in identical algal cultures. The fact that they did in my laboratory study of *S. quadricauda* indicates that during its growth the incorporation of inorganic carbon into cell constituents far exceeded extracellular release and essentially the two methods were measuring rates of change in algal biomass.

The lack of correlation between primary productivity and Δ -biomass in the field studies was not surprising; since the two parameters are actually not comparable in field situations. Unlike laboratory cultures, plankton in natural systems are composed of heterogenous populations which respond to a variety of environmental gradients. Calculations of Δ -biomass (using the ATP assay) are quite dependent upon C:ATP ratios. The selection of 250:1 used here was by virtue of literature recommendations. However, Perry (1977) has recently suggested that presence of high concentrations of blue-green algae may alter this ratio upward because the mucilaginous sheaths of blue-green cells are probably responsible for higher C:ATP ratios. Since blue-greens were occasionally abundant in the phytoplankton of Moss Reservoir, biomass calculations may have been low resulting in poor correlation to ^{14}C derived productivity values. Furthermore, biomass measurements in the field likely included a variable amount of heteroplankton (i.e.

bacteria and fungi), especially bacteria, in spite of the attempt to prevent this by size selective filtration. Heteroplankton have been demonstrated to be a significant (20-50 percent) portion of the seston biomass (Hobbie et al. 1972; Sorokin and Kadota 1972). Obviously, if a substantial population of heteroplankton is present when ATP assays for standing crop of Δ -biomass are made, their presence will be reflected. Boswell (1977) indicated this when he fractionated the plankton standing crop into various size classes. He concluded that approximately 30 percent of the total microbial standing crop was composed of heterotrophic organisms. This may be a significant proportion of the Δ -biomass measurement since I tried separation of phytoplankton and bacteria using a 1.2 μ m filter which may, in fact, have retained larger amounts of bacteria than originally thought.

Changes in standing crop (i.e. Δ -biomass) do not measure excretion and secretion that takes place when phytoplankton metabolize inorganic carbon. It has been well documented (Fogg 1971; Nalwewajko and Lean 1972; and Wright 1975) that phytoplankton do excrete labile organic compounds such as glycollate, lactone, mesotartrate, polysacchrides, organic acids, etc. as products of photorespiration and dark reactions of photosynthesis. At times, this extracellular release may represent a substantial proportion (20-30 percent) of the assimilated carbon depending on physiological and environmental conditions. This is particularly exemplified in my

study at 6 meters in the water column where light levels were low. Consequently, carbon assimilation (i.e. primary productivity) was low but standing crop and Δ -biomass were very high. As a matter of fact, approximately 50 percent of the total seston biomass and 71 percent of the Δ -biomass were found at this depth. Thus, a large seston standing crop does not necessarily mean that primary production rates will follow.

The prediction of relative weekly changes in standing crops by 4-5 hour Δ -biomass measurements is not easily explained. In view of recent studies (Eppley and Sharp 1975) in which phytoplankton regeneration times were discussed as varying from several hours to weeks, it was very difficult for me to attribute a major proportion of the Δ -biomass to phytoplankton turnover during short time periods (4-5 hr.). However, studies by Brock (1967) indicate that turnover times for bacteria may range from 1.5 to 11 hours. It is also perhaps pertinent that an unavoidable and often criticized consequence of enclosing lake plankton in non-circulating incubation chambers is the settling of plankters and detritus to the bottom of the chambers. This phenomenon may enhance bacterial growth in the chambers during incubation. The settled particulate material may serve as a nutrient rich substrate for bacterial growth due to the increased concentration of extracellular organic compounds (i.e. excretion products) and to the abundance of organic compounds adsorbed to the detritus (Shah and Fogg 1973). Thus, a large percentage

(50-75 percent) of Δ -biomass may, in fact, be due to changes in heteroplankton carbon. Further support to this effect was demonstrated in recent studies (Stanford et al. 1977) when no significant change in total standing crop was observed over a three day period. However, short term changes occurred within size classes, especially in the

10 μ m group (i.e. mostly bacteria). The fact that a substantial heterotrophic population did exist in the water column was established using ^{14}C - glucose assimilation and quantification of light and dark bottles in organic carbon assimilation experiments (Romanenko 1964). Measurements of Δ -biomass probably reflect the activity of the smaller size classes within the total plankton community. In other words, the total standing crop may change very slowly, but the internal cycling of biomass (i.e. the smaller size classes) is very fast (Stanford et al. 1977). Therefore, this internal activity apparently expresses a propensity (or lack of) for growth in the total plankton community and reflects whether the standing crop is increasing or decreasing in size in response to environmental or physiological conditions. The few instances where Δ -biomass measurements failed to estimate the standing crop during the following week may possibly be explained by temporal population growth and decline of patterns in the plankton community. Measurement of Δ -biomass during late growth phase of the plankton community would yield a positive prediction while the population would actually

decline the following week. Conversely, for negative

Δ -biomass measurements made just prior to phytoplankton blooms, an increase would actually be observed. This phenomenon occurred very rarely (i.e. approximately 8 percent of the time) in my study indicating that perhaps prediction in excess of one week might be feasible. However, when monitoring biomass dynamics, the sampling schedule undertaken should clearly be no more than 1-1.5 weeks because the chances of observing the above phenomenon would increase significantly.

The sensitivity of the ATP assay in describing very small changes in microbial biomass has clearly been established. Although the Δ -biomass cannot be used to estimate primary production, it does describe changes in VOC which may be properly interpreted as an estimate of microbial production. In describing primary production only the cycle or flow of inorganic carbon (i.e. into and out of the cell) is described, whereas in Δ -biomass studies, the result of carbon assimilation and subsequent functions is realized. The mere fact that 92 percent of the weekly trends in standing crop (i.e. biomass) were predicted from Δ -biomass estimations lends support to the importance of developing this assay as a production estimator (i.e. biomass monitor). However, until some method is discovered that will successfully separate autotrophs and heterotrophs, their independent biomass dynamics and the effects of those dynamics on their surrounding environments cannot correctly be assessed.

CHAPTER VI

CONCLUSION

It was confirmed that short term (4-5 hr.) biomass changes (Δ -biomass) could be measured using the ATP assay. Primary productivity (i.e. carbon assimilation) and

Δ -biomass did not correlate due to the differences in cellular functions measured. The expected correlation between standing crop and Δ -biomass was indeed positive. In addition,

Δ -biomass changes predicted relative standing crop changes because of the ability of the ATP assay to measure small succinct biomass changes which reflect growth trends in the plankton community. A real understanding of plankton ecology must be based on quantification of heterotrophic and autotrophic events because both are vitally fundamental to higher trophic events.

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