THE USE OF ADENOSINE TRIPHOSPHATE (ATP) ASSAYS IN DESCRIBING THE LIMNOLOGY OF MOSS RESERVOIR, TEXAS

DISSERTATION

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By

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Limnological study of Moss Reservoir from May, 1975 through August, 1976 was conducted to evaluate the use of ATP assay in describing planktonic changes within the water column. Assay of ATP was used to quantify fractional components of the planktonic material, providing sensitive insight into fractional changes, especially in the nannoplankton (<10 μm). Plankton was initially collected with a tripartite closing net, especially designed and built to facilitate accurate sampling. Extraction of ATP from these samples was inefficient, although samples were representative of the planktonic material in the reservoir. The amount of plankton and detritic material collected by the net was too large for proper extraction. Experimentation revealed that ATP concentrations were linear from 0.1 to 1 ℓ of reservoir water. Net sampling was abandoned and 1 ℓ samples for ATP extraction were collected with a Van Dorn water bottle. An improved hot plate-water bath technique was tested and utilized for all ATP extractions. Total ATP varied seasonally and with depth. Fraction A (>165 μm), predominately zooplankton showed spring and late summer peaks of ATP. Late summer increases in the D fraction (<10 μm, nannoplankton) from bottom samples suggested a transition from aerobic to anaerobic bacteria.

Fractions B (64-165 μm) and C (10-64 μm) contained the most consistent ATP levels during the sampling period. Correlation of calculated planktonic biomass to ATP was best in the B and C fractions.

Texas reservoirs have been typically categorized as eutrophic, however low total phosphorus 10-30 μg ℓ^{-1} found in Moss Reservoir may represent a potentially limiting level. The reservoir tended to act as a phosphorus sink during this study. Nitrogen concentrations were never low enough to be considered limiting. Limestone formations in the impoundment basin and surrounding watershed provided constantly buffered reservoir water. Moss Reservoir was determined to be a warm, monomictic impoundment. Summer stratification created an anoxic hypolimnion zone below 8 m lasting from June through September. Annual heat content for the 1975-1976 sampling period was 12898 g cal cm^{-2}.

All physical and chemical parameters were combined in an effort to explain variation in ATP values. Multiple regression analysis of surface and bottom total ATP with physical and chemical parameters provided no significant multiple correlation. Simple correlation of surface total ATP and temperature was significant at $P < 0.001$.

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CHAPTER I

INTRODUCTION

Review of Literature on ATP Assay

Limnologists, naturalists and aquatic ecologists have, for years, been concerned with adequate data collection of the living components in lentic areas of aquatic systems. Biomass is classically defined as the quantity of living organisms in a community. Differentation between living biomass and microscopically calculated biomass is most important in quantitative plankton analysis. Accurate assessment of planktonic assemblages is essential to understanding the trophic relationships and production dynamics of standing waters (Odum 1971). For judging the trophic status of waterbodies, estimation of the living planktonic biomass has the potential to be a most useful tool.

Biomass of phytoplankton can be estimated by direct microscopic observation and pigment extraction (Eppley 1968). Carbon assimilation rates are often confused with biomass estimation but may be only indirectly related to biomass. Zooplankton biomass can practically be measured only by direct microscopic determination of numbers and volume (Beers and Stewart 1967). Microscopic examination is tedious, time-consuming, and in preserved samples does not allow discrimination between those organisms alive or dead at the time of capture. Organic carbon analysis is simple but unfortunately

carbon is dissolved or found in triptonic particulates and not associated with living organisms.

Classical biomass estimators, such as microscopic enumeration, pigment extraction, carbon analysis, and gravimetric determination, in fact, do not accurately measure planktonic biomass. It is impossible to completely differentiate living from dead cells in microscopic analyses of heterogenous plankton samples. Chlorophyll and other cell constituents may remain relatively unchanged long after a cell dies. Recently, use of various vital stains in fresh samples has permitted some differentiation between living and dead cells, but specific variability in stain uptake has been encountered in heterogenous plankton communities. To accurately determine total biomass in plankton communities one must measure some component present in all living cells, but not associated with dissolved organics or tripton. Also, such a cellular constituent would have to be present in some constant proportion to a primary cell constituent (i.e. carbon), in order that it be comparable to some other parameter as a check of accuracy.

Adenosine triphosphate (ATP) within cells is found in relatively constant proportion to cellular organic carbon (Holm-Hansen and Booth 1966). Furthermore, ATP is present only in living cells; it is almost instantaneously hydrolyzed upon death of cells (Chappelle and Levin 1968). Biomass quantification via ATP assay is based on the firefly luciferin-luciferase reaction described by Strehler and McElroy (1968). Simply

stated, one photon of light is released as each molecule of ATP is hydrolized when all reagents are in excess. The reaction can be summarized in two steps (Plant et al. 1968):

$$E + LH_2 + ATP \overset{MG^{++}}{\rightleftarrows} E - LH_2 - AMP + PP \quad (1)$$

$$E - LH_2 - AMP + O_2 \rightarrow E + AMP + CO_2 + H + T \quad (2)$$

where

E = Firefly Luciferase

LH_2 = Reduced Luciferin

AMP = Adenosine Monophosphate

PP = Pyrophosphate

T = Thiazolinone (dehydroluciferin)

H = Light (550 nm)

The firefly reaction is unique in that no other high energy phosphate compound except ATP will oxidize reduced luciferin. Hence, if ATP is extracted from living cells in a plankton sample, it may be quantified by its light-producing reaction with luciferin. The emitted light may be measured by any of a number of sensitive photomultiplier tubes. Not only is the light produced proportional to ATP concentration, but ATP concentration is proportioned to the number of cells. Bacterial cell concentration versus light units was linear over a functional range (Chappelle and Levin 1968).

Oceanographer Osmund Holm-Hansen pioneered ATP research in the hydrosphere at Scripps Institute of Oceanography (Holm-Hansen and Booth 1966). Holm-Hansen and his student, Rocky Booth, reported on their research measuring ATP

concentrations at various depths in the ocean. They designed and built a light detection device for the quantitative assay of ATP in sea water. The authors showed by direct observation and utilizing carefully monitored laboratory cultures as controls that the ATP assay was 50-2000 times more accurate than plate counts in estimating bacteria from deep ocean samples. Using the ATP assay, biomass profiles were made in a pelagic area off the California coast to depths of 1025 m. Water samples of 2 ℓ or less were collected and aliquots immediately filtered onto 0.45 μm membrane filters. Filters and the particulate (i.e. plankton and tripton material) trapped on and in them were plunged into test tubes containing 0.02 M Tris buffer boiling in a water bath. The test tubes were then removed from the water bath and extracts were brought to a standard volume, sealed, and frozen for later assay on shore. ATP concentration decreased rapidly below the euphotic zone but 4.3×10^6 and 0.33×10^6 bacterial cells ℓ^{-1} were estimated to be present at 500 and 1,025 m respectively (Holm-Hansen and Booth 1966).

Apparently Holm-Hansen had further thoughts about these bacterial estimates. Hamilton and Holm-Hansen (1967) analyzed the ATP content in a number of marine bacteria and found that the ratio of ATP to cellular organic carbon varied from 0.3 to 1.1%. Of the seven strains of bacteria examined, the average ratio was 0.7%. Thus the ratio of C to ATP was about 250:1. Using this average ratio the authors calculated that

the estimates of bacterial numbers made by Holm-Hansen and Booth (1966) were accurate. Holm-Hansen (1969) made additional deep ocean profiles off the southern California coast. He found that biomass estimates based on chlorophyll and ATP concentrations at 50, 100, and 200 m were correlated with those calculated from microscopic observations. Below 200 m, chlorophyll values were insignificant but ATP values and direct microscopic examination gave similar estimates. Analyses of natural plankton populations often require exposure of photosynthetic organisms to varying light conditions during collection, subsampling, and filtration. It is important that ATP levels remain relatively constant during changes in light. Holm-Hansen (1973) showed that when phytoplankton were subjected to light/dark changes or vice versa, initial ATP values shifted radically. However, following a period of 1 to 3 min. after a light/dark change, ATP levels returned to values approximately equal to those of the original conditions.

In the marine work described above, zooplankton were selectively removed prior to filtration; only marine bacteria and phytoplankton were assayed. However, there was no reason to believe that the ATP assay could not be used to quantify zooplankton biomass; Holm-Hansen simply eliminated the zooplankton to prevent higher ATP values which would interfere with correlation to chlorophyll.

Balch (1972) measured ATP content in the copepod Calanus finmarchicus. The ratio of ATP to total organic carbon (ca.

450:1) was very consistent between individuals. Balch noted that copepods maintain large fat globules as reserve energy stores. He thought that the amount of lipid might effect the carbon-ATP ratio because the fat storage bodies contain no ATP. Starvation of copepods for 0, 8, 14 and 23 days showed significant changes in the carbon-ATP ratio. However, the work by Balch suggested that zooplankton biomass, as measured by ATP, could be assayed with consistent results over a wide range of physiological conditions. It should be noted that all ATP work cited thus far has been marine.

While use of ATP assay expanded into diverse areas of plant science and medicine, its use in fresh water research also was initiated. Limnologists have been slow in evaluating the usefulness of ATP in fresh water research, as few published studies have resulted. Rudd and Hamilton (1973) reported biomass profiles from two experimental lakes in the Canadian shield. The authors found large seasonal changes in planktonic ATP throughout the water column. Significant differences in ATP concentrations were observed between three size fractions (0.22-250 μm, 10-250 μm, and 56-250 μm) of plankton (excluding zooplankton). Highest summer ATP levels (95% of total ATP) were found in the <10 μm size fraction, while the same fraction represented only 50% of the total ATP in winter. This finding alludes to the importance of nannoplankton and bacterial components in the water column.

Holm-Hansen and Booth's (1966) ratio of carbon to ATP (ca. 250:1) was originally formulated on laboratory algal culture analyses because of the realization that a low percentage (25-50) of the microscopically observable algal cells from natural populations were viable. Paerl (1975) re-examined the 250:1 ratio of carbon:ATP using samples collected from seven U. S. and Canadian lakes. Autoradiography was used to establish the number of viable cells present in direct microscopic counts and carbon values were calculated only from the viable cell estimates. Carbon:ATP ratios varied from 205:1 to 323:1 depending upon the primary phytoplankton component. Blue green algae characteristically possessing mucilagenous sheaths which contain carbon but no ATP, were numerically dominant in some lakes; samples from these lakes contained higher (i.e. 287 to 323:1) carbon:ATP ratios. Paerl also noted that diatoms, which characteristically have proportionally large amounts of silica in ponderous cell walls, were very abundant in some of the lakes. Lower carbon:ATP ratios (i.e. 205 to 236:1) were recorded from these lakes.

Cavari (1976) however, recently found C:ATP ratios which were considerably different from the 250:1 ratio. During early spring, while working on Lake Kinneret in Israel, a rather extensive bloom of the dinoflagellate Peridinuim cinctum occurred. Carbon:ATP ratios ranged from 918 to 2683:1. Laboratory investigation with the same organism revealed that high C:ATP ratios (highest 2374:1) were correlated with phosphorus

deficiency in the growth medium. When phosphorus was added the C:ATP ratio decreased to 297:1.

Less successful attempts to monitor viable biomass dynamics were made by Robinson and Kidd in New Mexico. ATP assays were used in combination with ^{14}C production estimates to access the trophic status of four reservoirs (Robinson 1975). The author concluded that short-term, summer analyses of ^{14}C production and ATP levels did not give a true indication of trophic status. Primary production and ATP levels did not necessarily correlate because individual species of phytoplankton have different rates of cell division (i.e. turnover times). Yearly average ATP levels were believed by the author to be useful for the ranking of trophic status of the reservoirs studied. Robinson felt that the utilization of ATP assay combined with carbon analysis was potentially very useful, as those measurements were routine and far less expensive than ^{14}C production experiments.

As the scope of the new technique was expanded into slightly new fields, problems were identified. Since the early work by Holm-Hansen and Booth (1966) and Hamilton and Holm-Hansen (1967) several researchers have documented problems with the ATP extraction process. Lee et al. (1971) attempted the extraction of ATP from soils with boiling Tris buffer. Boiling Tris extraction of soil samples, the procedure used by Holm-Hansen, yielded very poor results. Experimentation with a number of extractants revealed that dilute sulfuric acid

extraction provided the most quantitative ATP results. Karl and LaRock (1975) further studied problems of sediment and soil extraction in boiling Tris buffer. They theorized that "boundary areas" of cool fluid developed on the surface of soil particles in the boiling extracts. Heat transfer at the solid liquid boundary via conduction occurred at a very slow rate compared to heat convection at the liquid-cell interfaces (Knudson and Katz 1954). Organisms seemed to adhere to the solid surfaces of soil particles, where heating was slow, causing poor ATP extraction. Plankton samples often contain clumps of seston that are composed of aggregates of viable bacteria and plankton cells adhering to inert particulate (triptonic) materials. Many of the organisms to be assayed lie in boundary layers identified by Karl and LaRock and are not efficiently extracted. The result is a low temperature death (<90 C) and the subsequent hydrolysis of ATP yeilding inaccurately low, but possibly precise results (Picciolo et al. 1975). Similar problems, although not explained, were reported by Holm-Hansen (1975) for eutrophic coastal waters. Regressions of ATP over increasing sample volume (i.e. increasing biomass filtered) were not linear; at higher volumes the regressions tailed downward. The large quantities of dinoflagellates per unit of water volume that Holm-Hansen reported in these samples may have been phosphorus - limited, as has been suggested by Cavari (1976) or the mass of seston on the filters may have been so great as to have effectively created boundary layer

problems like those found associated with soil and sediment extraction in Tris buffer (Karl and LaRock 1975). Low temperature extraction may have also plagued Robinson and Kidd in the study of New Mexico reservoirs (Robinson 1975). Robinson points out that some of the reservoirs were at a high enough altitude to reduce the boiling point of water to about 95 C. The subsequent ATP extraction of planktonic material at this, or most probably a slightly lower temperature, may have produced precise but very inaccurate results.

Use of the ATP assay in plankton biomass analysis shows considerable promise. However, the exact relationship between ATP and viable organic carbon remains tenuous in heterogeneous plankton communities. It is clearly evident that there is a definite correlation between the two, provided that extraction is efficient. The ATP assay has been used in only a few marine and aquatic studies. Considering the tremendous diversity of ecological conditions in fresh water habitats alone, much research is needed before the assay will be established as a routine limnological tool.

Adenosine triphosphate analysis has been successfully applied in numerous other areas of research. Ching (1975) used ATP to estimate seed viability. Studies on the mechanisms of herbicide action by St. John (1975) employed ATP assay. Utilization of ATP assay has been extensive in biomedicine. Beutler and Baluda (1964) reported using ATP extractions in blood analyses, while Wolf (1975) showed specific ATP differences

between normal and sickle red blood cells. Gutekunst (1975) developed a procedure for detecting bacteria in urine using the luciferin-luciferase ATP assay technique. Differentiation of diseased from normal brain tissue with ATP assay was reported by Ebadi, Weiss, and Costa (1971).

Because the ATP assay has such wide-ranging application and potential in scientific research, enzymes for the light-producing reaction and photometers for light measurement are now commercially available. Crude and refined enzymes are available from biochemical supply houses; costs are variable depending upon purity. Holm-Hansen and Booth's (1966) original photometer has been re-engineered and is now commercially available. Two other ATP photometers are also being marketed. Picciolo (1975) compared the efficiency of all commercial ATP photometers.

Introduction to Problem

The limnology of reservoirs, particularly of those in the southwestern United States, is very poorly documented; fisheries studies are numerous however. Silvey and Harris (1947) began a 10-year study of the fish production in an east Texas reservoir in 1937. Previous to this study the then Texas Game, Fish, and Oyster Commission had sporadically monitored fish populations in public reservoirs in the state. Harris and Silvey (1940) stated:

> It is generally conceded by limnologists, biologists in general, sportsmen and laymen, that any artificial reservoir built

> by impounding a fresh water stream will within three to five years develop a maximum biological productivity. This maximum condition ordinarily remains during the following three to five years, at the end of which time the biological balance appears to be lost and productivity is poor.

Time has not altered this statement, according to Bennett (1970), and the sport fishery is most affected by this overall decline in productivity. Short-term or seasonal problems with tastes and odors may also arise in the multipurpose southwestern reservoirs. Extensive accumulation of sediments in reservoir basins, creating expansive shallow areas, tends to accompany the aging process. Nuisance blooms of certain species of phyto- and zooplankton may produce noxious tastes and odors which conventional water treatment cannot remove. Decreases in reservoir volume and the subsequent increase in submergent and emergent plant growth combine to produce the seemingly universal problem of eutrophication. All of these problems are in some way related to the complex dynamics of plankton-nutrient relationships. Dependence on impounded water for municipal use alone provides the impetus for continued research in an attempt to understand all aspects of reservoir limnology.

Research on the limnology and plankton-nutrient interactions in Texas reservoirs has been undertaken by J. K. G. Silvey (limnologist, and former chairman of the biology department at North Texas State University) and many of his students. Unfortunately most of these research efforts were never published. Extensive study has been made of nutrient relationships and production dynamics

in Lake Waco by Owen Lind and his students (Lind 1971; Kimmel and Lind 1972). However, it seems that few other studies of this nature exist for southwestern impoundments. This research was undertaken to further the knowledge of plankton dynamics in relation to nutrients and physical limnology in southwestern reservoirs.

By bringing current research techniques (ATP analysis)from marine origins to the fresh water sphere, I have attempted to further the holistic approach in lentic study. The primary objective of this research was to estimate planktonic biomass and controlling parameters (i.e. nutrients and hydrodynamics) within the water column of H. H. Moss Reservoir (hereafter MR). Distribution and type of organisms vertically within the water column was also of interest for correlation to ATP values. Holm-Hansen (1969) reported ATP profiles from deep ocean samples well below the euphotic zone. Few photosynthetic organisms were present, with bacteria contributing most of the viable biomass. In a laboratory experiment no significant differences were found in the ATP levels of algae maintained in light or darkness (Holm-Hansen 1973). Similarly, I was interested in vertical biomass differences, particularly between the euphotic and the aphotic zone in a reservoir. In winter complete mixing of the reservoir causes photosynthetic organisms to be transported into the aphotic zone. Summer thermal stratification tends to preclude living photosynthetic organisms from the aphotic zone. Comparison of ATP estimates of biomass to calculated microscopic

biomass, especially in the aphotic zone, was of interest to evaluate seasonal composition changes.

Importance of size-specific groups of viable plankton was hinted at by Rudd and Hamilton (1973). I wished to analyze specific sized groups of plankton to determine what contribution a particular size fraction had on the overall planktonic biomass within the reservoir. Of particular interest was the temporal contribution made by the nannoplankton to the total planktonic biomass.

I attempted to develop a combined net sampling and size fractionation technique to facilitate study of specific groups. Comparison of ATP assay results with microscopic volume calculations of plankton was made to determine how closely the two biomass estimation techniques compared. It should be pointed out that the purpose of this research was to correlate viability measurements, as documented by temporal changes in ATP concentration, with nutrient and hydrodynamic parameters. I did not attempt to investigate viable organic carbon to ATP relationships in the phytoplankton. Thus all inferences were based on measurements of ATP content and not viable organic carbon. Calculation of the annual heat storage capacity of MR was prompted by the apparent lack of such data for reservoirs in general and specifically for southwestern reservoirs.

CHAPTER II

STUDY AREA

H. H. Moss Reservoir is located in Cooke County, Texas, 16.09 Km (10 mi.) northwest of Gainesville. The reservoir was constructed in 1966 by damming Fish Creek, creating a 455-ha (1,125 a) impoundment (Table 1). The 168-km^2 (65 sq. mi.) drainage basin, supplying the 2.86 x 10^7 m^3 (23,210 ac-ft) reservoir, lies in the Red River drainage (Texas Water Development Board 1974). Construction was undertaken by the city of Gainesville in order to supply municipal and industrial water needs; however, no water has as yet been used for these purposes. North and South Fish Creeks form two large arms which comprise the reservoir (see Fig. 1). The North Fish Creek arm is largely wooded and much smaller than the other. South Fish Creek is broad and open except in the upper end, where a small expanse of woods remains. Runoff from shoreline areas is limited, as the basin has steep sides.

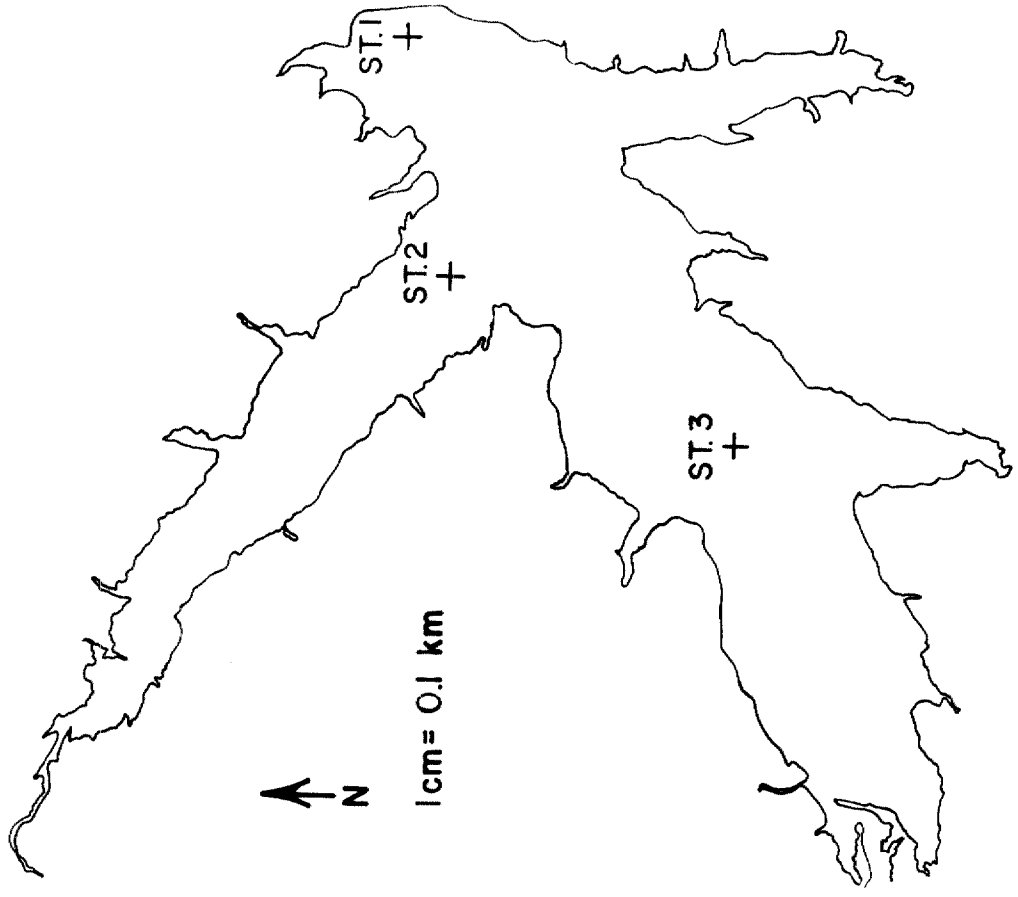
Shoreline area is predominately privately owned, with only two public access points to the reservoir. Development of the shoreline was slow until the 1970's. Today, the southern shore is crowded with camps and permanent homes while the northern shore remains relatively undeveloped.

Moss Reservoir is used extensively for recreation. A public beach with picnic area is available. Water skiing and fishing

Table 1. Morphometric characteristics of H. H. Moss Reservoir at normal pool level.

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
Annual Evaporation (May, 1975-April, 1976)	4.13(10^6)m^3
Annual Discharge (May, 1975-April, 1976)	2.64(10^6)m^3

Figure 1. H. H. Moss Reservoir, Red River Drainage, Cooke County, Texas.


ST.1
ST.2
ST.3
N
1cm= 0.1 km

seem to be the main boating interests, although sailboats are occasionally seen. During the winter little water-related activity occurs. Duck hunting is confined to the upper reaches of North and South Fish Creeks and fishermen are often out on pleasant days.

Selection of MR as a study site was based on many features. The drainage basin is relatively unpolluted in comparison to other local reservoirs. Runoff is from pasture and woodlands, with little tilled land, limiting the possibility of high turbidity in the reservoir and of extreme fluctuations in water level. The relatively small size of the reservoir allowed me to attain a generalized picture of the plankton. Moss Reservoir is small enough to be workable in all weather, yet large enough to be representative of southwestern reservoirs. Finally, there is no published data on this reservoir, which will eventually serve the city of Gainesville as a primary water supply and currently provides recreation for many North Texas water-sports enthusiasts.

CHAPTER III

MATERIALS AND METHODS

Field Biomass Studies

Sampling at three sites (see Fig. 1) on MR was initiated in May, 1975. Bi-weekly or monthly samples were collected above and below the euphotic zone at each site through August, 1976. All collections from Station 1 were made during darkness to reduce net avoidance by zooplankton. Duplicate samples for ATP extraction of planktonic organisms were collected, using a closing net and a Van Dorn water bottle. Initially, only a closing, tripartite, plankton net (Fig. 2) was used. Water bottle samples (1 ℓ) were used after problems were noted with net samples. This net was constructed of three nested nylon bags zippered to a canvas cylinder. Stainless steel plankton buckets of similar mesh were attached to the cod end of each net (inner 165 μm, middle 64 μm, outer 10 μm mesh). The three-stage closing net was lowered to the desired depth, towed vertically for 1 m, closed via messenger, and brought to the surface. Plankton was selectively filtered from approximately 70 ℓ of water during each net haul. Upon completion of a tow, each bag was detached and plankton clinging to the bags was rinsed into the attached buckets. The plankton buckets were removed and their contents rinsed into polyethelene bottles. Three sample fractions were obtained in this manner (A, >165 μm;

Figure 2. Tripartite, closing plankton sampler.

B, 64-165 μm; C, 10-64 μm). In addition, water for a fourth fraction (D <10 μm) and for 1-ℓ samples was collected with a Van Dorn water bottle. All samples for ATP extraction were placed in ice chests to maintain approximate field temperature for transport to a mobile laboratory on shore. The mobile laboratory provided a stable lakeside base with controlled environment for expedient sample extraction. Had I returned to the University to process samples, an additional 2 hours would have been necessary for raw water transportation. The additional time would have most probably caused death to many fragile plankton cells contained in the samples. On being returned to the mobile laboratory, whole water samples (1 ℓ) were filtered successively through plankton buckets which corresponded to the same fractions from the net. Therefore, due to mere differences in amount of water filtered, water bottle samples contained considerably less biomass than samples obtained using the net. The D(<10 μm) fraction, not collected by the net, consisted of all plankton and tripton in 0.5 ℓ of water which passed through the 10 μm filter. Material thus concentrated and retained in each size class from net or water bottle samples was filtered onto 0.45-μm membrane filters using 0.5 ATM pressure.

To determine the reliability of using a 1-ℓ sample for extraction, volumes of 0.1, 0.25, 0.5 and 1 ℓ were separately filtered through the A, B and C plankton buckets. Duplicate subsamples were run for comparison. All other conditions were maintained as previously described for sample collection, transport, and storage.

Sample extractions were performed in boiling Tris (hydroxymethyl) aminomethane (Tris) buffer solution at 0.02 M 7.75 pH, as described by Holm-Hansen and Booth (1966). Their procedure was modified somewhat, as I preferred to place the membrane filter organism-side down in a beaker containing 5-7 ml of boiling Tris buffer. All extraction beakers were boiling on a hot plate to insure the maintenance of maximum temperatures. Extraction on the hot plate proceeded for 1-2 min.; then the beakers were moved to a boiling water bath for an additional 8-10 min. Upon completion of extraction, material on the filters was discarded. The extract was brought to a final volume of 5 ml by adding Tris, transferred to test tubes, sealed, and frozen for later assay on a special photometer in the limnology laboratory at North Texas State University.

To assess boiling water bath effectiveness in plankton extraction, an experiment using the blue-green alga Microcystis arugenosa Kuetz was designed. Increasing duplicate volumes of a log-phase culture of the alga were extracted in boiling Tris buffer on a hot plate for 1 to 2 min. then transferred to a boiling water bath for an additional 8-10 min. Similar duplicate volumes from the same culture were extracted in boiling Tris buffer in a water bath only. The results led to the modification of Holm-Hansen and Booth's original procedure.

Plankton samples (1 ℓ for identification and enumeration) were taken with a Van Dorn bottle at Station 1 corresponding to ATP samples. These samples were preserved immediately after

collection with alcohol-formalin-acetic acid (1-2-1) fixative to a final 10% preservative solution. In order to complete sample collection and ATP extraction, which included night samples from Station 1, the sampling period often encompassed 24 h. Sample collection at Stations 2 and 3 initially were to include all parameters. However, nutrient (i.e. P and N) data were found to be similar at all three stations and these collections were abandoned. Time was a consideration and plankton samples from all stations could not have been adequately analyzed. Complete data collections(i.e. plankton, chemical and physical samples) were maintained at Station 1 because of my interest in the extensive hypolimnion zone developed at this site during summer. Stations 2 and 3, each only about 8 m deep, had no hypolimnion development.

Field Physical and Chemical Parameters

Collection of samples for determination of carbon (C), nitrogen (N), phosphorus (P), alkalinity, conductivity, and pH was made with the Van Dorn water bottle. Separate, duplicate samples were collected for each of the C, N, and P analyses and duplicates of combined samples were taken for measurement of alkalinity, conductivity, and pH. Samples for nitrogen determination were acidified with H_2SO_4 for preservation. Samples were stored in high density polyethylene bottles, refrigerated and/or frozen depending upon the length of time before samples were completed. Dissolved oxygen and temperature were determined in situ with a Weston and Stack Dissolved Oxygen

Analyzer. Morphological data were supplied by the city of Gainesville and hydrological data were obtained from U. S. Department of the Interior, Water Resources Division, in Fort Worth, Texas.

Laboratory Biomass Studies

Having completed collection and extraction of plankton samples, I carried out ATP analyses after returning to the limnology research laboratory at North Texas State University. Adenosine triphosphate stock solution was made from di-sodium ATP crystals (12.3 $mg\ell^{-1}$) and frozen in 1.0-ml portions in sealed glass vials. For each sample set a glass vial containing 1 ml of stock ATP was diluted to 100 ml with Tris buffer, producing a standard ATP solution of 100 $m\mu g\ ml^{-1}$) (0.2 ml = 20 mμg ATP). Stock solution stability, when frozen in the above manner, was excellent. No detectable deterioration occurred during this research. Crude, lyophylized luciferin-luciferase enzyme (Sigma Chemical Co.) was hydrolyzed with Tris buffer (pH 7.75, 0.02 M) approximately 20 h. before use. After standing for 3 to 4 h. at room temperature (22-24 C), the enzyme suspension was centrifuged 20 min. (3600 rpm), and the supernate was decanted and allowed to stand at room temperature for the remaining 16 h.

Analysis of extracted ATP samples and standards was carried out in a Model 2000 ATP Photometer (Fig. 3). The 60-second integration mode of the instrument was used for all data collection (SAI 1974). Photometric determination of ATP in samples

Figure 3. The Model 2000 ATP Photometer, SAI Technology.


LAB-LINE ATP PHOTOMETER
NO. 9140
INTEGRAL
INTERVAL
60
SENSITIVITY
ZERO
ASSAY
RESET
INTERVAL
MODE
POWER
LAB-LINE INSTRUMENTS, Inc.
Designers and Manufacturers
MELROSE PARK, ILLINOIS Pat. Pending

and standards was done in glass scintillation vials. An aliquot (200 ml) of extractant (i.e. of sample or standard) was placed in a scintillation vial. As the enzyme solution (200 ml) was added to the liquid in the scintillation vial the ATP photometer was activated, initiating a fifteen-second delay. During this delay the contents in the vial were thoroughly mixed, the bottom of the vial was wiped clean and the vial inserted into the counting chamber. The instrument integrated a 1 min. portion of the decay curve and a digital (LED) readout was produced (SAI 1974). These data were recorded. Sample ATP values were calculated from least squares regression of ATP concentration in standards. Such standard curves were prepared with each batch of enzyme. Crude enzyme (Sigma Chemical Co.) was of variable activity, necessitating the standardization of each quantity hydrated. Blanks (200 ml Tris + 200 ml enzyme) were run every sixteenth assay to determine the background enzyme count which was caused by traces of ATP in the crude enzyme. These background counts were subtracted from the corresponding 16 assays to attain a corrected count for calculating ATP concentration. Quality control was maintained by analysis of spiked, previously assayed samples.

Biomass estimates from plankton samples were calculated from direct microscopic observation. Duplicate, preserved 1-ℓ plankton samples were concentrated to 25 ml by centrifugation at 3600 rpm. A suction-siphon was used after centrifuging to remove the fluid above the pellet of seston in the bottom of

the centrifuge tube. Microscopic examination of the liquid which had been removed revealed the presence of few or no phytoplankton but numerous zooplankton. Fat storage bodies found in zooplankton caused these organisms to float, thus being removed in the supernate. Cell counts and measurements of the resuspended pellet were made in a Palmer-Maloney chamber with the aid of a Whipple disc inserted in the ocular. A phytoplankton count on one plankton sample consisted of enumerating 5 Whipple disc fields each from four Palmer-Maloney cell aliquots. Enumeration and measurement of phytoplankton was made at 200X, while identifications were made at up to 1000X. Due to sampling and concentration problems, zooplankton were enumerated, identified, and measured from the A (>164 μm) fraction ATP samples. Since these samples had already been used for ATP assay, remaining volumes were highly variable. To simplify this problem, all zooplankton in each sample duplicate were enumerated and some were measured. A Palmer-Maloney cell with Whipple disc in the ocular was used for this purpose. Microscopic magnification was 40X. Microscopic biomass estimates of plankton were calculated from volume formulas which most closely described the geometric shape of the organism. A formula assigned to describe an organism remained unchanged through the study, although volume estimates often varied in relation to specific, measured dimensions. In this way, seasonal size variability was accounted for.

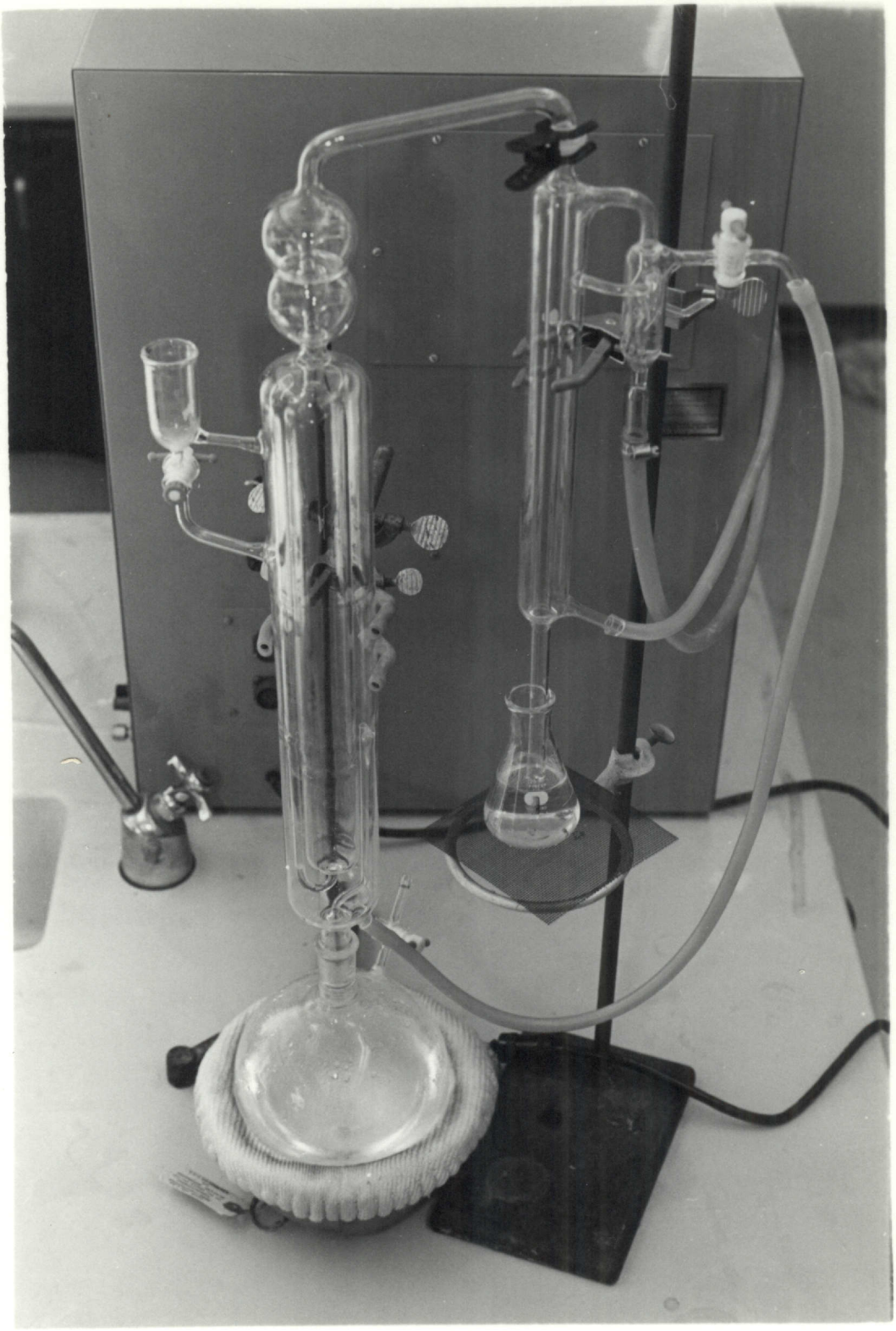
Laboratory Chemical Analyses

Carbon content was determined by direct injection of samples into a Beckman 915 Total Organic Carbon Analyzer. Particulate organic carbon (POC) was determined indirectly by sample filtration through 0.4 μm polyethelene membrane filters, then injection of the filtrate. Subtraction of the values obtained in this manner from total organic carbon (TOC) values yielded POC data.

Phosphorus and nitrogen determinations were made by the personnel in the Analytical Water Quality Laboratory, North Texas State University. Standard quality control procedures were followed for all analyses (U. S. EPA 1972). Known quantities of phosphorus or nitrogen were added to previously determined samples to assess recovery and accuracy of each sample run.

Total Kjeldahl nitrogen (TKN) was determined according to Standard Methods (1974). The procedure was modified so that the sample (250 ml) was refluxed in a specially designed and constructed steam distillation apparatus (Fig. 4). After distillation the sample volume was concentrated by evaporation, nesslerized, and an aliquot placed in a spectrophotometer. Absorbance values were measured at 425 nm using a 1-cm cell. A standard curve was generated by least squares regression from organic nitrogen standards processed identically to the samples. Sample TKN values were determined from the standard line.

Figure 4. Stream reflux apparatus for Kjeldahl nitrogen reduction.



Combined total nitrate-nitrite nitrogen (TN-NN) levels were measured, using the cadmium reduction method (U. S. Environmental Protection Agency 1974). A filtered sample was allowed to percolate through a copper-cadmium column to reduce nitrate to nitrite. The original nitrite plus the nitrate reduced to nitrite was determined in a spectrophotometer (Beckman Model 25) after azo dye formation with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride. Readings were made at 540 nm with a 1-cm light path. Standards were prepared and analyzed exactly as samples. Standard curves and sample values were obtained as with TKN.

Samples for total phosphorus (TP) were digested using a modification of the procedure in the fourteenth edition of Standard Methods (1974). A sample (200 ml) was placed in round-bottomed flask equipped with a teflon-lined screw cap. Digestion acid, 4 ml, and 1 gm of ammonium persulfate were added to the sample. The mixture was autoclaved for 1 h. at 20 psi. Phosphorus was determined colorimetrically according to a modified extraction procedure outlined by Stephens (1963). The cooled sample was placed in a 250 ml separatory funnel with 20 ml of mixed reagent. Color was allowed to develop for 10 min.; then the mixture was extracted with 29 ml of iso-butyl alcohol. The sample was shaken 60 sec., allowed to settle for 5 min., shaken for an additional 30 sec., and allowed to settle for an additional 5 min. The alcohol extract was removed, placed in a 25-ml volumetric flask and brought to 25 ml volume. Absorbance

values were measured in a spectrophotometer (Coleman 124), using a 10-cm cell at 690 nm. A standard curve was prepared (least squares regression) from phosphorus standards which had been digested and extracted following the same procedure. Sample phosphorus values were determined from the standard line.

Measurements of alkalinity were made according to Standard Methods (1974). Specific conductance was measured with a conductivity meter (YSI Model 31). Hydrogen ion concentration was measured with a pH meter (Beckman Expandomatic).

Since complete thermal and morphometric data were available, thermal energy content of the reservoir was calculated. Data of this nature appear to be absent for reservoirs in general and for southwest impoundments in particular. Precise volume estimates were obtained from daily gauge height averages (24 h.) supplied by the U. S. Geological Survey, Water Resources Division. Heat content calculations for the year August, 1975, through August, 1976, were made according to Rawson (1936).

Statistical Techniques

Multiple regression analysis of surface and bottom ATP concentrations at Station 1 on corresponding calculated biomass, TP, TN-NN, TKN, dissolved oxygen, pH, temperature, conductivity, and alkalinity was performed in an attempt to partition variability over the sampling period. One-way ANOVA was used to compare selected specific fraction means between sample sites on a particular date. The field extraction experiment was analyzed

by least squares regression for linearity of increasing sample volume to ATP concentration. Comparison of hot plate-water bath and water bath-only extraction of the Microcystis arugenosa culture consisted of the generation of two regression lines. The slopes of the two lines were statistically compared with a Student's t test.

CHAPTER IV

RESULTS

Biomass

Net samples were initially used, but when comparisons between samples obtained with 1-ℓ Van Dorn water bottles were made, extreme differences in concentration of ATP ℓ^{-1} were revealed (Fig. 5). Estimates of ATP ℓ^{-1} in each fraction (A, B, and C) during December and January were 5-8 times greater from 1 ℓ of reservoir water than those calculated from net samples (70 ℓ). An experiment was designed to determine if concentration problems, like those suggested by Holm-Hansen (1975), existed in plankton samples from MR. Smaller volumes of reservoir water were examined to investigate the propriety of using 1-ℓ samples. The ATP content of three fractions (A, B and C) was regressed on increasing volumes of sample (0.1, 0.25, 0.5, and 1 ℓ) (Fig. 6). The result was a very good ($R>0.98$) linear relationship between sample volume and ATP extracted in each size fraction. This indicated that no extraction problems were being encountered. Slope differences and apparent deviations from zero were attributed to differences in the ordinates and to changes in the background of the enzyme preparation at different instrument sensitivities.

Laboratory investigation of a common alga, Microcystis arugenosa revealed significant differences ($P>0.1$) in ATP levels

Figure 5. Fractional comparison of 1 ℓ (left bars) water bottle and 70 ℓ (right bars) net samples.

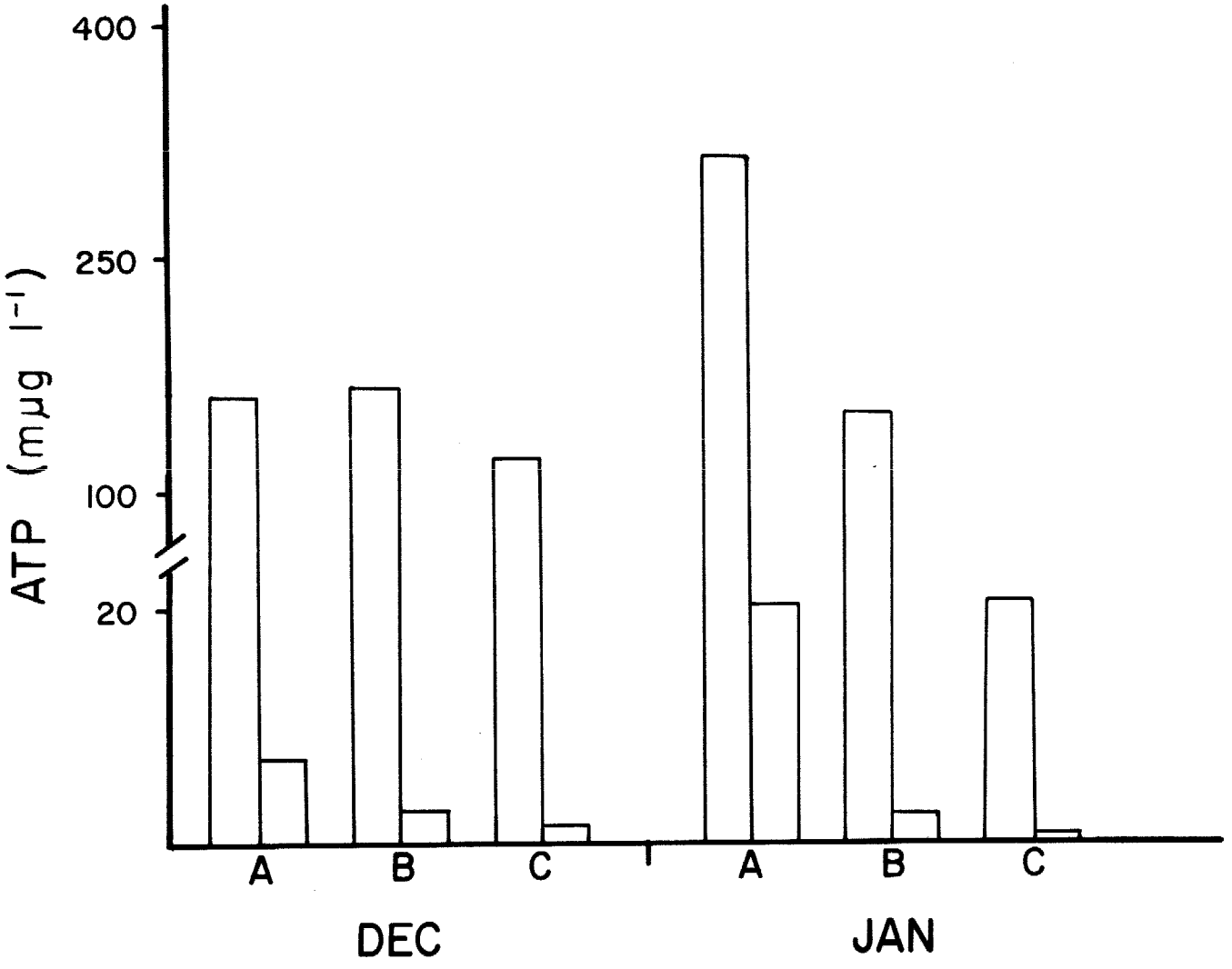
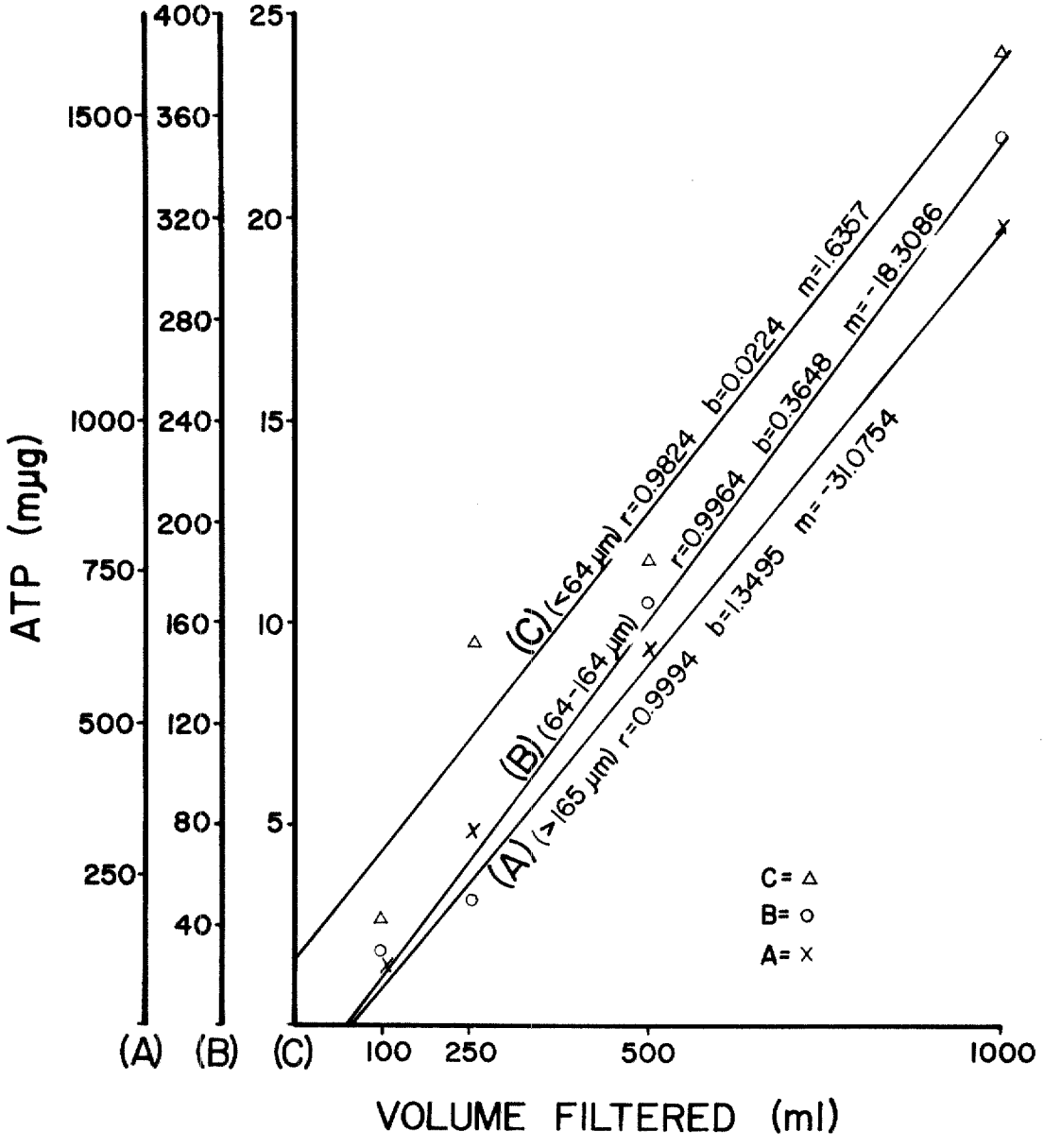

400
250
100
20
ATP (mμg l⁻¹)
A
B
C
A
B
C
DEC
JAN

Figure 6. Regression of ATP ℓ^{-1} change in three size fractions of plankton with increasing volume of water filtered (ml).


ATP (mμg)
400
360
320
280
240
200
160
120
80
40
25
20
15
10
5
1500
1000
750
500
250
(A)
(B)
(C)
100
250
500
1000
VOLUME FILTERED (ml)
(C) (<64 μm) r=0.9824 b=0.0224 m=1.6357
(B) (64-164 μm) r=0.9964 b=0.3648 m=-18.3086
(A) (>165 μm) r=0.9994 b=1.3495 m=-31.0754
C= △
B= ○
A= ×

between samples extracted in boiling Tris buffer on a hot plate and samples extracted in a boiling water bath (Fig. 7). All reservoir ATP samples were extracted with the combined hot plate-boiling water bath technique.

Mean surface (2.5 m) estimates of total ATP ℓ^{-1} showed generally similar trends at the three stations. Levels of ATP at Station 2 were much higher in March than at either of the other stations. Peaks of ATP occurred in May and August (Fig. 8). Mean ATP ℓ^{-1} values from bottom samples (Station 1 = 11.5 m, Station 2 and 3 = 7 m) revealed similarities to the surface values (Fig. 9). However, only Stations 1 and 2 had pronounced peaks of ATP in the August samples. Total ATP values were classified by fractional representation of the plankton.

Fraction A (>165 μm) consisted predominantly of zooplankton. During blooms of filamentous algae or diatoms large quantities of these phytoplankton occurred in the A fraction. The 65-165 μm component (fraction B) contained material characterized by Welch (1948) as "net plankton". In MR the net plankton component consisted of filamentous algae fragments, large green algae (Pediastrum sp.), dinoflagellates (Ceratium sp.), and flagellates (Euglena sp.). Nauplii and rotifers were observed in the B fraction also. The C fraction (10-64 μm) contained predominantly diatoms, unicellular green and blue green algae, and fragments of algal synobia, filaments and colonies. Microscopically observable material in the D fraction (<10 μm) was extremely limited. Unidentifiable detritus and fragments of cells were

Figure 7. Linear regression of samples extracted by combined hot plate-water bath extraction (x) and water bath (y) extraction alone.

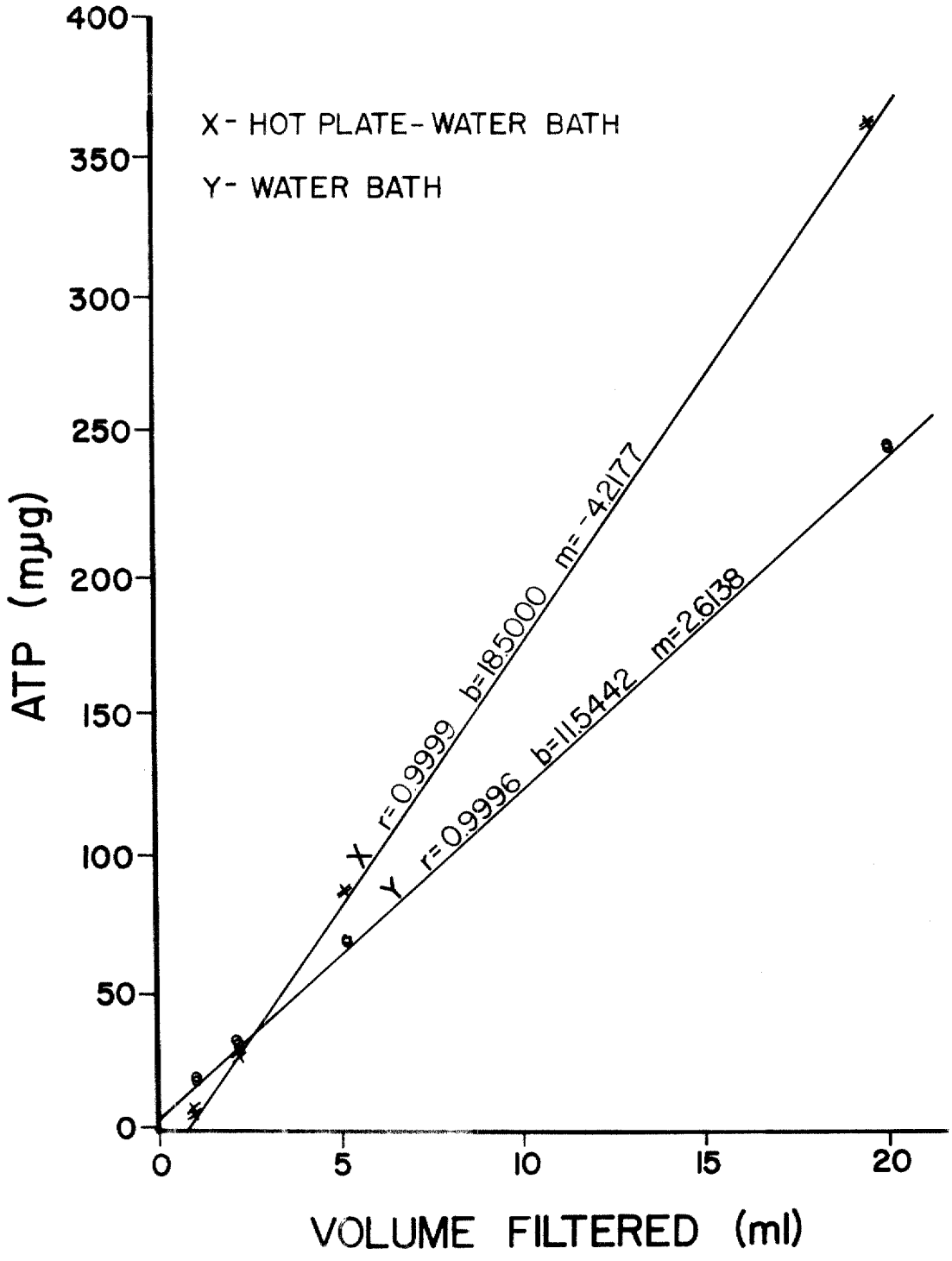

X- HOT PLATE- WATER BATH
Y- WATER BATH
X r=0.9999 b=18.5000 m=-4.2177
Y r=0.9996 b=11.5442 m=2.6138
ATP (mμg)
VOLUME FILTERED (ml)
400
350
300
250
200
150
100
50
0
0
5
10
15
20

Figure 8. Mean total surface ATP from three stations on H. H. Moss Reservoir.

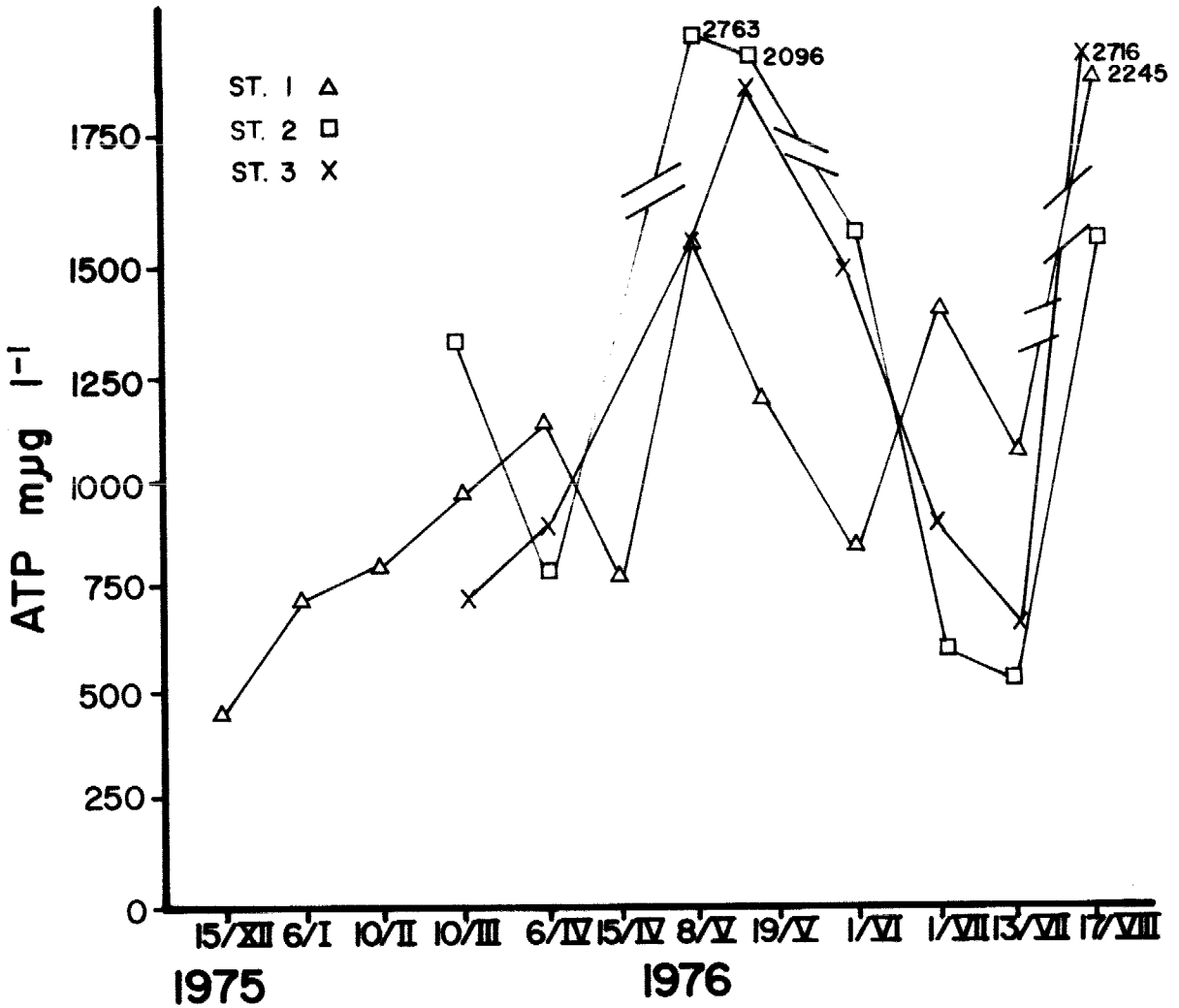
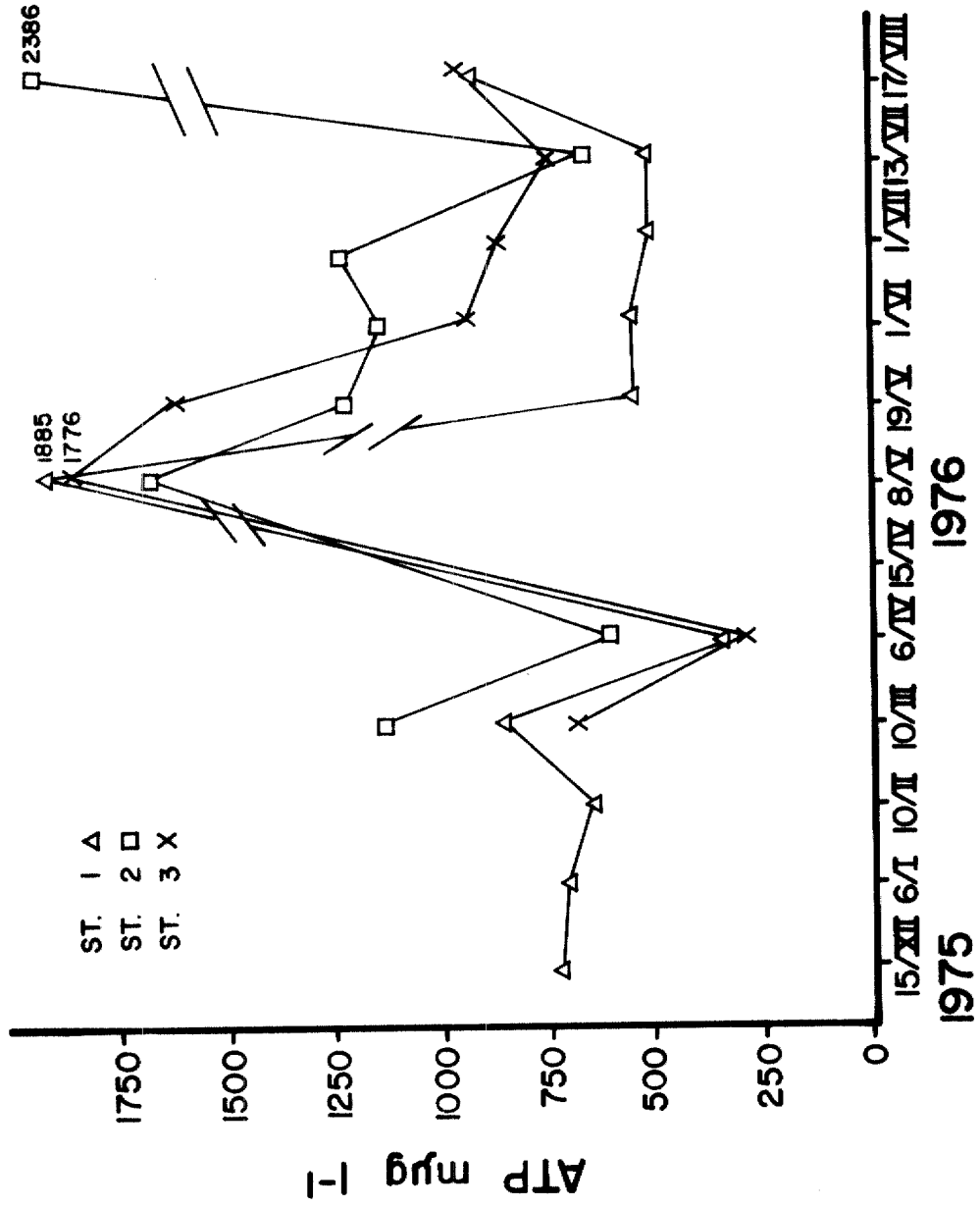

ST. 1 △
ST. 2 □
ST. 3 X
2763
2096
2716
2245
ATP mμg l⁻¹
1750
1500
1250
1000
750
500
250
0
15/XII 6/I 10/II 10/III 6/IV 15/IV 8/V 19/V 1/VI 1/VII 13/VII 17/VIII
1975
1976

Figure 9. Mean total bottom ATP from three stations on H. H. Moss Reservoir.


ATP mμg l-1
1750
1500
1250
1000
750
500
250
0
ST. 1 Δ
ST. 2 □
ST. 3 X
1885
1776
2386
15/III 1/9 1/10 II/10 III/10 9/III 15/III 8/X 19/X 1/XII 13/XII 17/XII
1975
1976

most commonly seen. Small diatoms, which predominated in the reservoir during winter, occurred in the D fraction at that time. Organisms found in reservoir plankton samples and their fractional distribution are shown in Table 2.

Mean ATP ℓ^{-1} values for the four surface fractions (A, >165 μm; B, 64-165 μm; C, 10-64 μm; D, <10 μm) from three stations are shown in Figures 10, 11 and 12. For each station comparison the A fraction contained the highest peak ATP concentrations during the sampling regime. Similar high ATP levels in the A fraction were observed at all stations in May and from Station 1 only in August. During spring (March through May) fractions C and D contained the lowest ATP levels. Late summer values showed considerable increases in all fractions at Station 1. Stations 2 and 3 had increases in B, C, and D components by August. Note the relative stability of the B fraction from March through June.

Concentrations of ATP in the four size fractions from samples taken below the euphotic zone at each station produced component ATP relationships similar to those seen in the surface samples (Fig. 13, 14 and 15). Particularly interesting was the dramatic increase of the ATP in the D fraction at Station 1 in August. At all three stations during August the D fraction contained the largest amount of ATP. Fractions A, B and C contained very low ATP levels during July and August at Station 1.

Table 2. Plankton found in H. H. Moss Reservoir with relative abundance† and size class distribution††.

PLANKTON	RELATIVE ABUNDANCE	SIZE CLASS
Chlorophyta		
Pandorina	* S, F	100% B
Tetraedon	*** F, W, Sp, S	100% C
Ankistrodesmus	** Sp, S	20% B, 80% C
Closteriopsis	** Sp, S	100% B
Franceia	** Sp, S	90% C, 10% D
Kirchneriella	** Sp, S	100% C
Oocystis	** Sp	100% B
Golenkinia	** Sp, S	90% C, 10% D
Lagerheimia	* Sp	90% C, 10% D
Dictyosphaerium	** S	90% B, 10% C
Westella	* F	100% C
Coelastrum	** S	70% B, 30% C
Crucigenia	*** W, Sp, S	90% C, 10% C
Scenedesmus	** Sp, S	100% C
Pediastrum	** Sp, S, F	20% A, 80% B
Arthrodesmus	* S, F	100% B
Cosmarium	* S, F	100% B
Micrasterias	* S, F	100% B
Staurastrum	*** Sp, S, F	70% B, 30% C
Euglenophyta		
Euglena	*** F	20% A, 80% B
Lepocinclis	*** F	100% B
Phacus	** S, F	100% B
Trachelomonas	*** F, W	100% C
Pyrrhophyta		
Glenodinium	*** F	100% C
Peridinium	*** S, F	100% C
Ceratium	*** F, W, Sp	100% A
Chrysophyta		
Dinobryon	**** Sp, S	20% A, 80% B
Mallomonas	** F	20% B, 80% C
Melosira	**** Sp	60% A, 40% B
Cyclotella	* Sp	40% B, 60% C
Rhizosolenia	* W	100% B
Asterionella	**** S	20% A, 80% B
Fragilaria	** F	100% B
Synedra	**** F, W	10% A, 20% B, 70% C
Cymbella	* S	100% A
Nitzschia	** F	100% C

Table 2. Continued

PLANKTON	RELATIVE ABUNDANCE		SIZE CLASS
Cyanophyta			
Chroococcus	*	F	30% B, 70% C
Gloeocapsa	**	S, F	100% B
Merismopedium	***	S, F	10% B, 90% C
Microsystis	***	S, F	20% B, 80% C
Phormidium	*	F	90% A, 10% B
Spirulina	**	S	20% A, 80% B
Anabaena	****	S, F	90% A, 10% B
Protozoa			
Centropyxis	**	F	80% A, 20% B
Difflugia	**	S, F	70% A, 30% B
Rotifera			
Polyarthra	**	W, Sp	100% A
Filinia	*	W	100% A
Keratella	***	W, Sp	90% A, 10% B
Asplanchna	**	Sp	100% A
Cladocera			
Diaphanosoma	**	Sp	100% A
Daphnia	***	Sp	100% A
Ceriodaphnia	***	Sp	100% A
Bosmina	*	Sp, S	90% A, 10% B
Bosminopsis	****	Sp, S	90% A, 10% B
Ostracoda			
Unknown	*	S	100% A
Copepoda			
Diaptomous	**	Sp	100% A
Cyclopoid	***	Sp, S, F	100% A
Nauplii	**	Sp, S	80% A, 20% B

† * Rare
** Occasional
*** Common
**** Numerous (Blooms)

W Winter
Sp Spring
S Summer
F Fall

††Fraction A >164 µm
Fraction B 64-164 µm
Fraction C 10-64 µm
Fraction D <10 µm

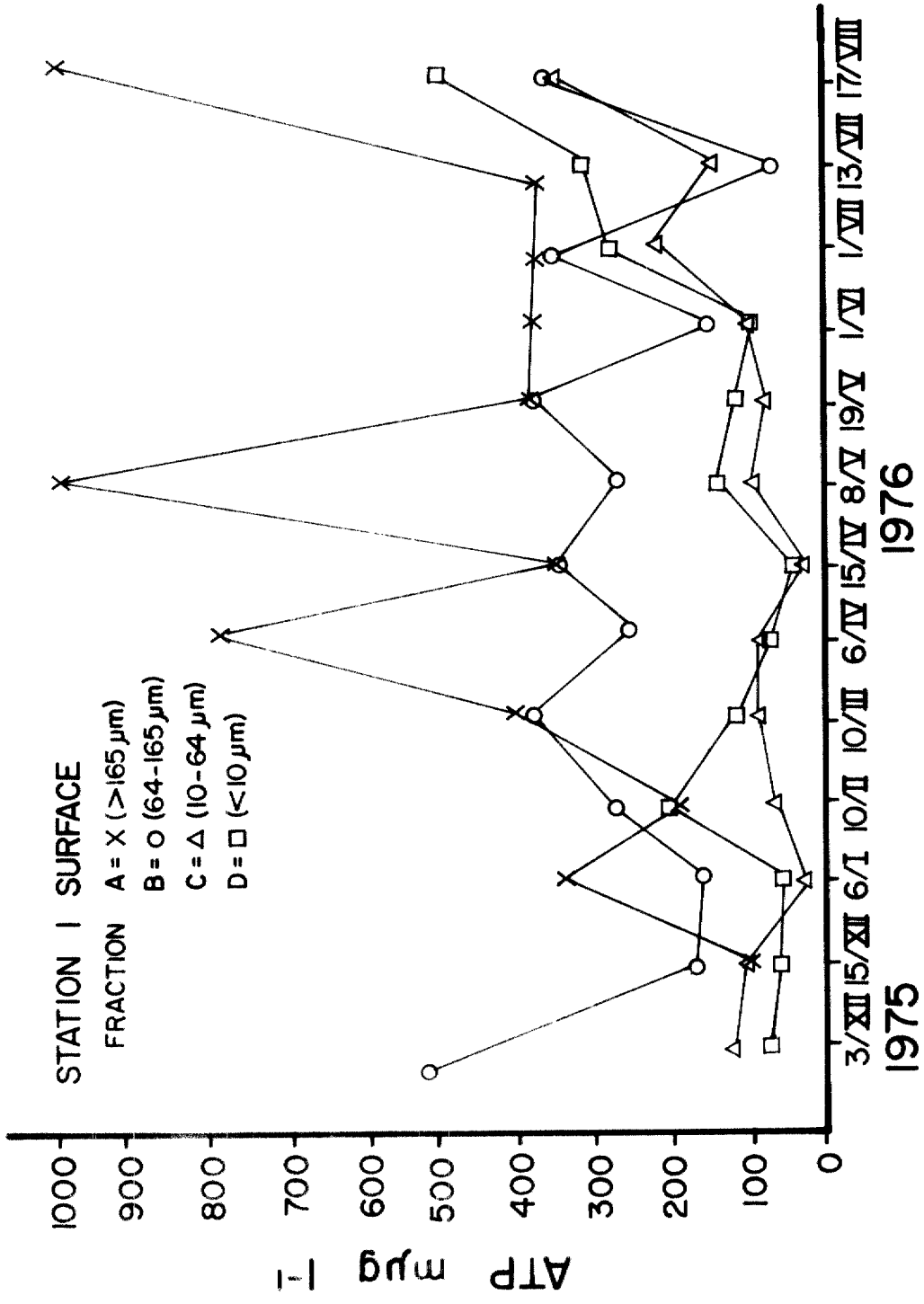

STATION 1 SURFACE
FRACTION A = X (>165 µm)
B = O (64-165 µm)
C = Δ (10-64 µm)
D = □ (<10 µm)
ATP mµg l⁻¹
1000
900
800
700
600
500
400
300
200
100
0
1975
1976
3/III 15/III 1/VI 10/VI 10/VII 9/IX 15/IX 8/X 19/X 1/XI 13/XII 17/XII

Figure 11. Fractional distribution of surface ATP at Station 2.

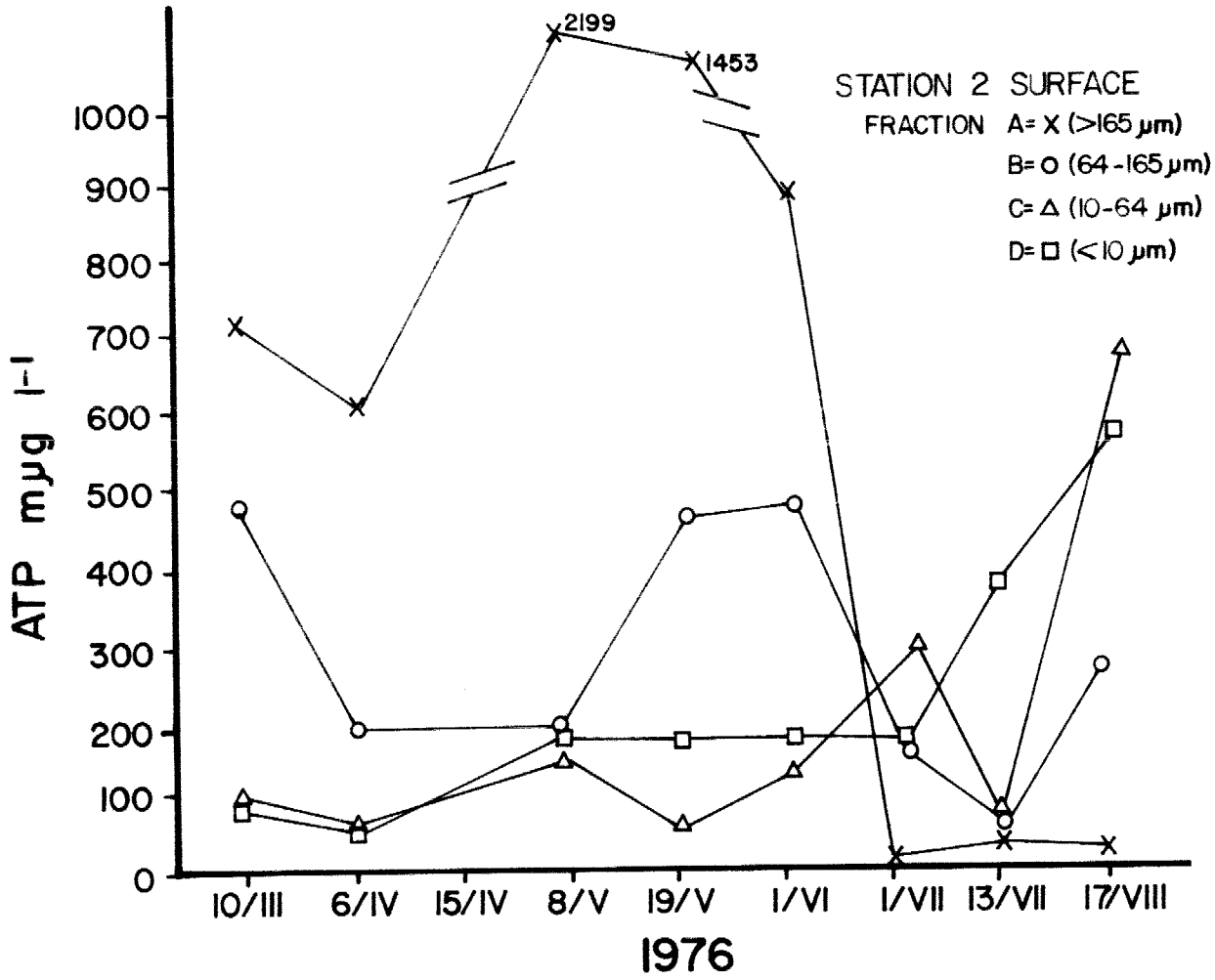

2199
1453
STATION 2 SURFACE
FRACTION A= X (>165 µm)
B= O (64-165 µm)
C= Δ (10-64 µm)
D= □ (<10 µm)
ATP mµg l-1
1000
900
800
700
600
500
400
300
200
100
0
10/III
6/IV
15/IV
8/V
19/V
1/VI
1/VII
13/VII
17/VIII
1976

Figure 12. Fractional distribution of surface ATP at Station 3.

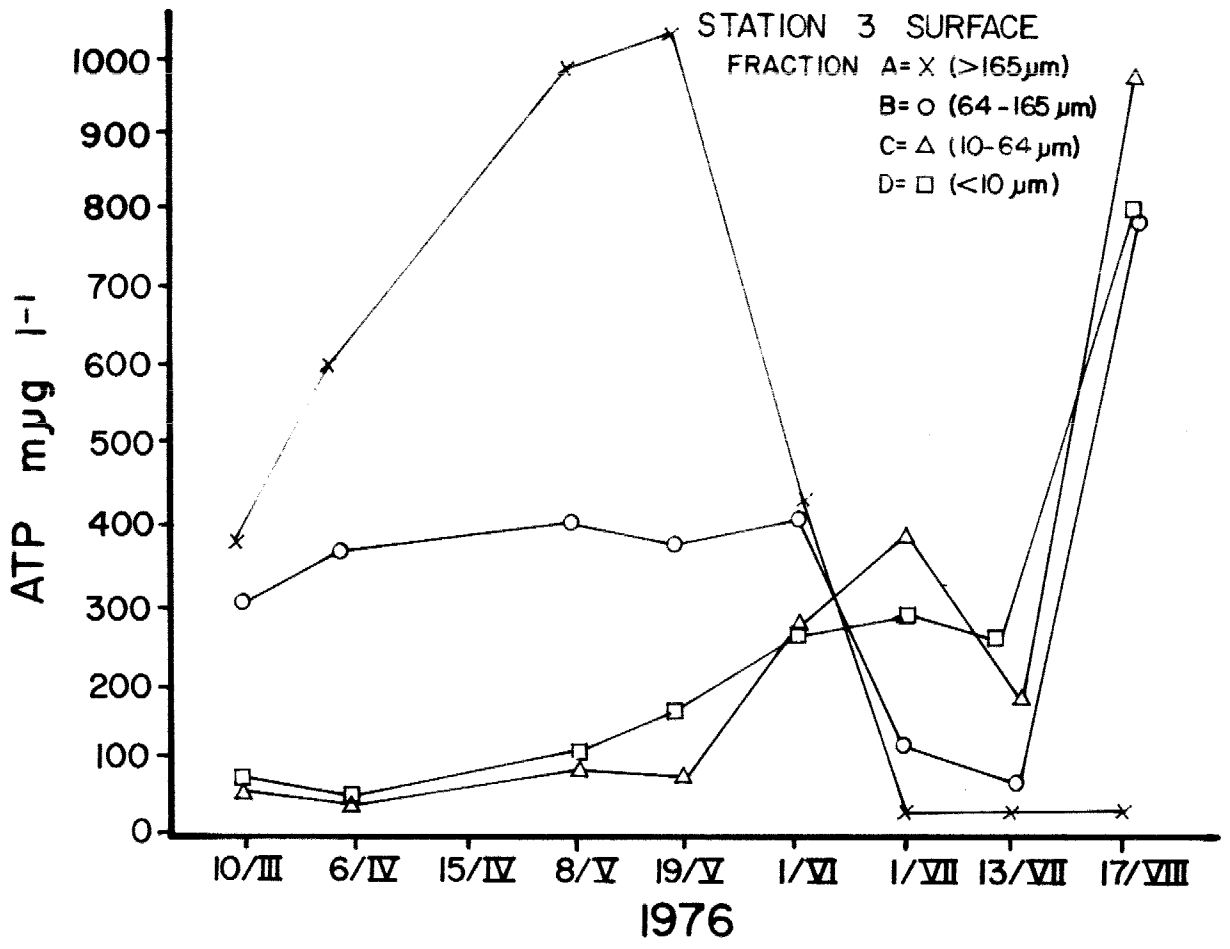

STATION 3 SURFACE
FRACTION A= X (>165µm)
B= O (64 - 165 µm)
C= Δ (10-64 µm)
D= □ (<10 µm)
ATP mµg l-1
1000
900
800
700
600
500
400
300
200
100
0
10/III
6/IV
15/IV
8/V
19/V
1/VI
1/VII
13/VII
17/VIII
1976

Figure 13. Fractional distribution of bottom ATP from Station 1.

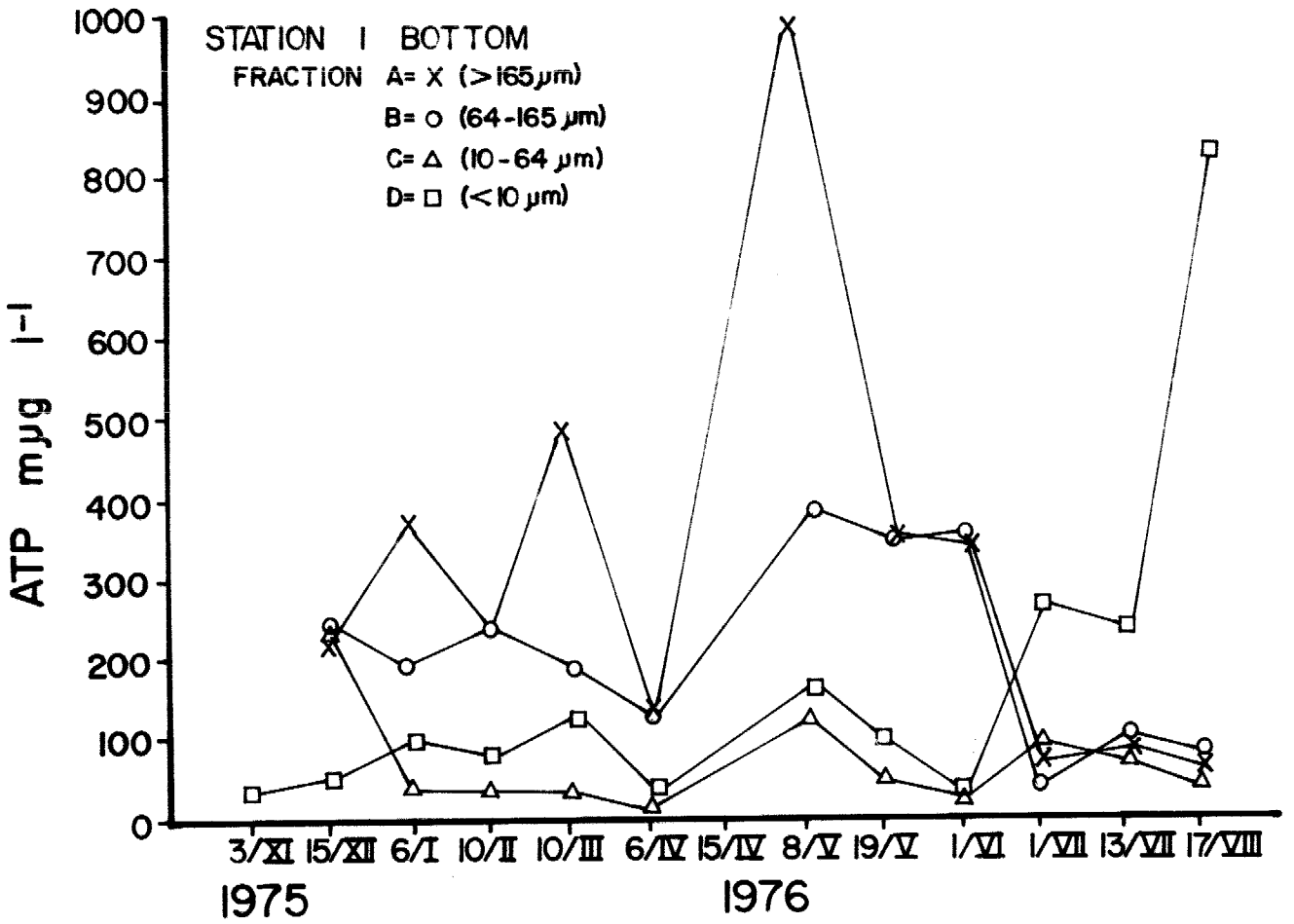

STATION I BOTTOM
FRACTION A= X (>165µm)
B= O (64-165 µm)
C= Δ (10-64 µm)
D= □ (<10 µm)
ATP mµg l-1
1000
900
800
700
600
500
400
300
200
100
0
3/XI 15/XII 6/I 10/II 10/III 6/IV 15/IV 8/V 19/V 1/VI 1/VII 13/VII 17/VIII
1975
1976

Figure 14. Fractional distribution of bottom ATP from Station 2.

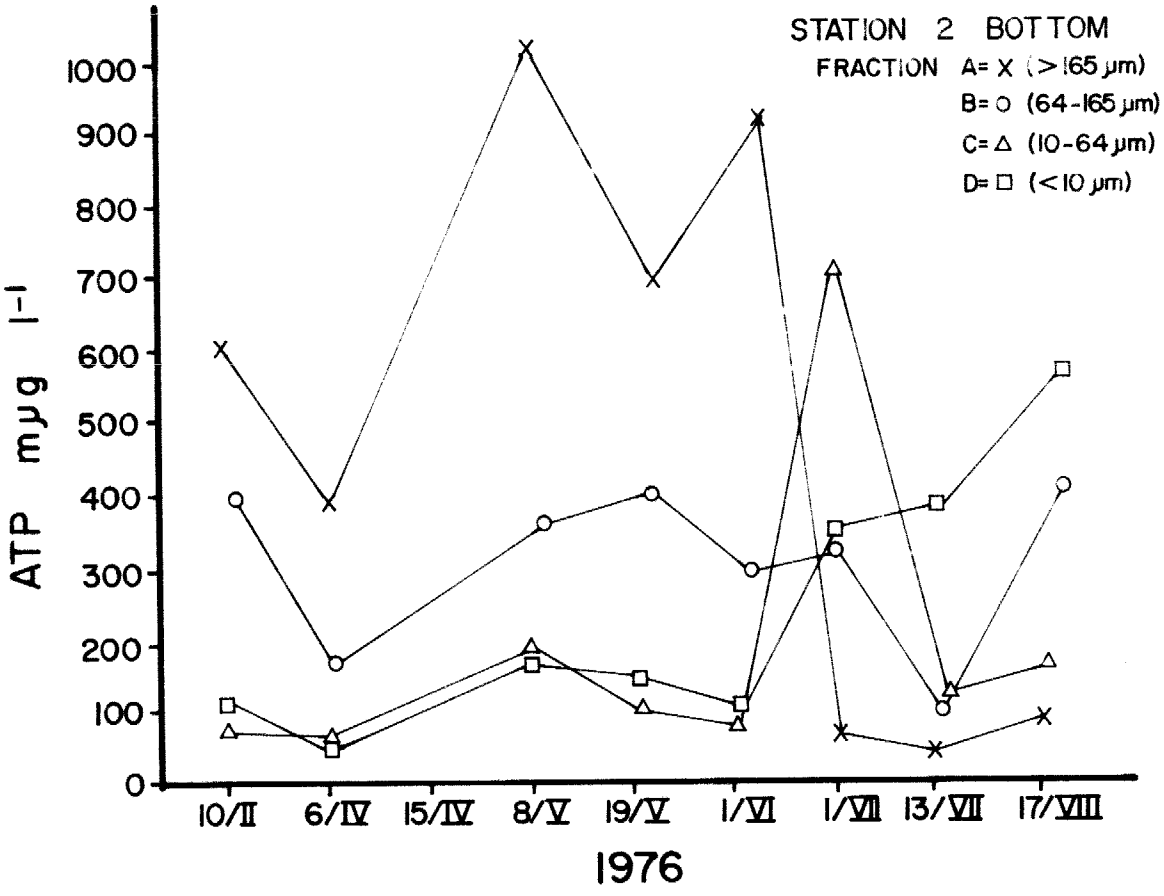
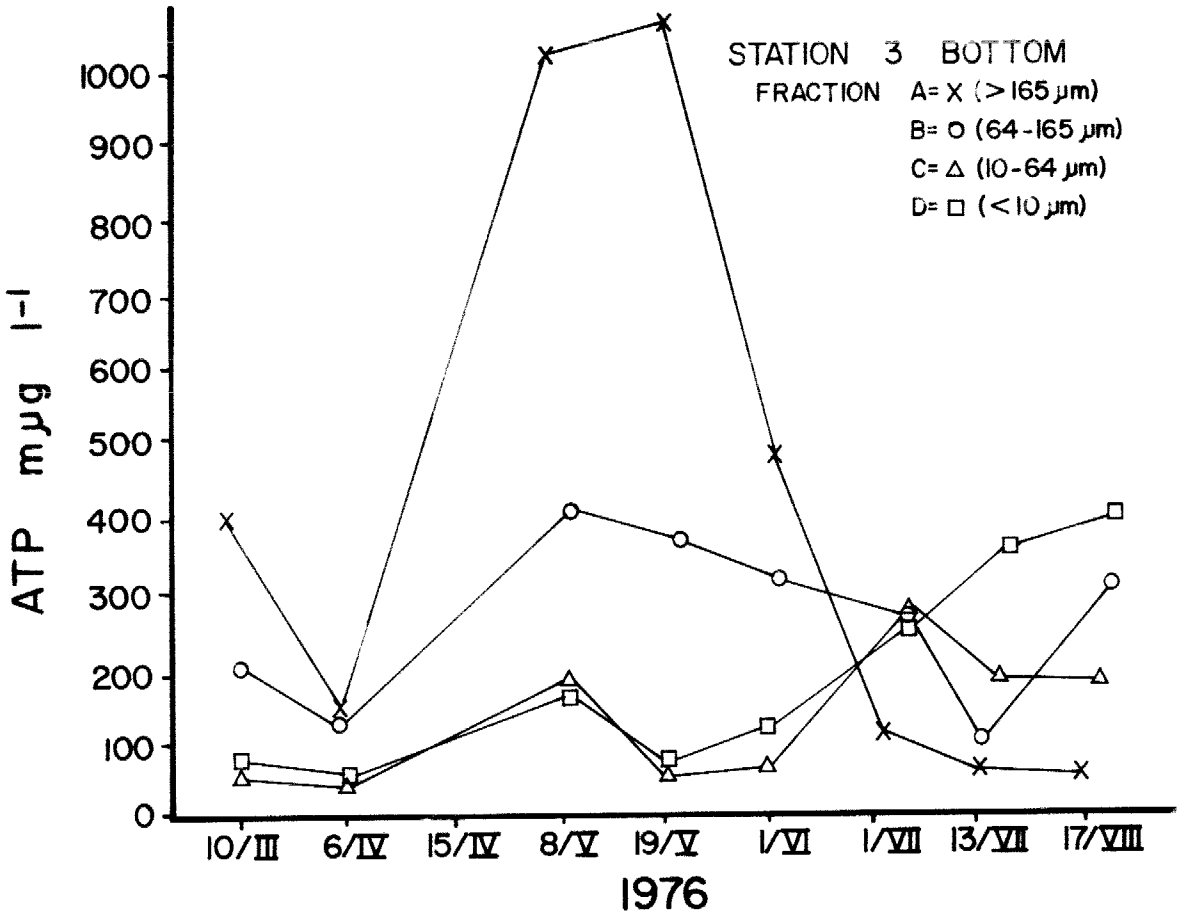

STATION 2 BOTTOM
FRACTION A= X (>165 µm)
B= O (64-165 µm)
C= Δ (10-64 µm)
D= □ (<10 µm)
ATP mµg l-1
1000
900
800
700
600
500
400
300
200
100
0
10/II
6/IV
15/IV
8/V
19/V
1/VI
1/VII
13/VII
17/VIII
1976

Figure 15. Fractional distribution of bottom ATP from Station 3.


STATION 3 BOTTOM
FRACTION A= X (>165 µm)
B= O (64-165 µm)
C= Δ (10-64 µm)
D= □ (<10 µm)
ATP mµg l-1
1000
900
800
700
600
500
400
300
200
100
0
10/III
6/IV
15/IV
8/V
19/V
1/VI
1/VII
13/VII
17/VIII
1976

Simple mean and variance calculations of a particular fraction suggested considerable differences between stations on any particular date. Further comparisons of individual fraction means, selected for apparent similarity, were made by one-way ANOVA (Table 3). Of the means compared, only one pairing proved not significant ($P > .001$).

When total ATP ℓ^{-1} concentrations at Station 1 were compared to total plankton biomass (calculated from separate plankton samples), considerable differences were noted (Fig. 16 and 17). Peak bottom biomass ($mm^3 \ell^{-1}$), calculated microscopically, occurred March 10 (Fig. 17), while the surface biomass peak did not occur until the subsequent sampling date (Fig. 15). High levels of ATP ℓ^{-1} were found during calculated biomass peaks but higher ATP ℓ^{-1} levels were recorded one or two samples after the calculated biomass peaks in both surface and bottom samples. A large rise in total ATP ℓ^{-1} was observed from bottom samples in August, neither following nor accompanied by a calculated biomass increase (Fig. 17). Note that separation of the three larger fractions (A, B and C) from the total ATP ℓ^{-1} value revealed that the dramatic ATP ℓ^{-1} increase was in the D fraction alone (Fig. 17). Concomitant surface increases occurred in the August samples (Fig. 16).

Breakdown of the total ATP ℓ^{-1} values by size fractions produced interesting comparisons between calculated biomass and the ATP ℓ^{-1} in a particular fraction. Calculated zooplankton biomass from the organisms actually present and remaining

Table 3. ANOVA of between-station comparisons of selected means.

Ho-$\bar{X}_1=\bar{X}_2=\bar{X}_3$	$P<0.001$ $F_{(1, 21)}$	
Date	Sample	F Value
10/III/76	Bottom Fraction A	
	$\bar{X}_1$ vs. $\bar{X}_2$	15.9059**
	$\bar{X}_1$ vs. $\bar{X}_3$	14.7630**
	$\bar{X}_2$ vs. $\bar{X}_3$	61.3166**
8/V/76	Surface Fraction B	
	$\bar{X}_1$ vs. $\bar{X}_2$	125.9611**
	$\bar{X}_1$ vs. $\bar{X}_3$	248.5320**
	$\bar{X}_2$ vs. $\bar{X}_3$	728.3596**
1/VII/76	Surface Fraction C	
	$\bar{X}_1$ vs. $\bar{X}_2$	2.9821 n.s.
	$\bar{X}_1$ vs. $\bar{X}_3$	33.1721**
	$\bar{X}_2$ vs. $\bar{X}_3$	16.2621**
17/VII/76	Bottom Fraction D	
	$\bar{X}_1$ vs. $\bar{X}_2$	139.6554**
	$\bar{X}_1$ vs. $\bar{X}_3$	95.9073**
	$\bar{X}_2$ vs. $\bar{X}_3$	65.5684**

**Highly Significant

Figure 16. Surface total ATP and total calculated biomass comparisons at Station 1.

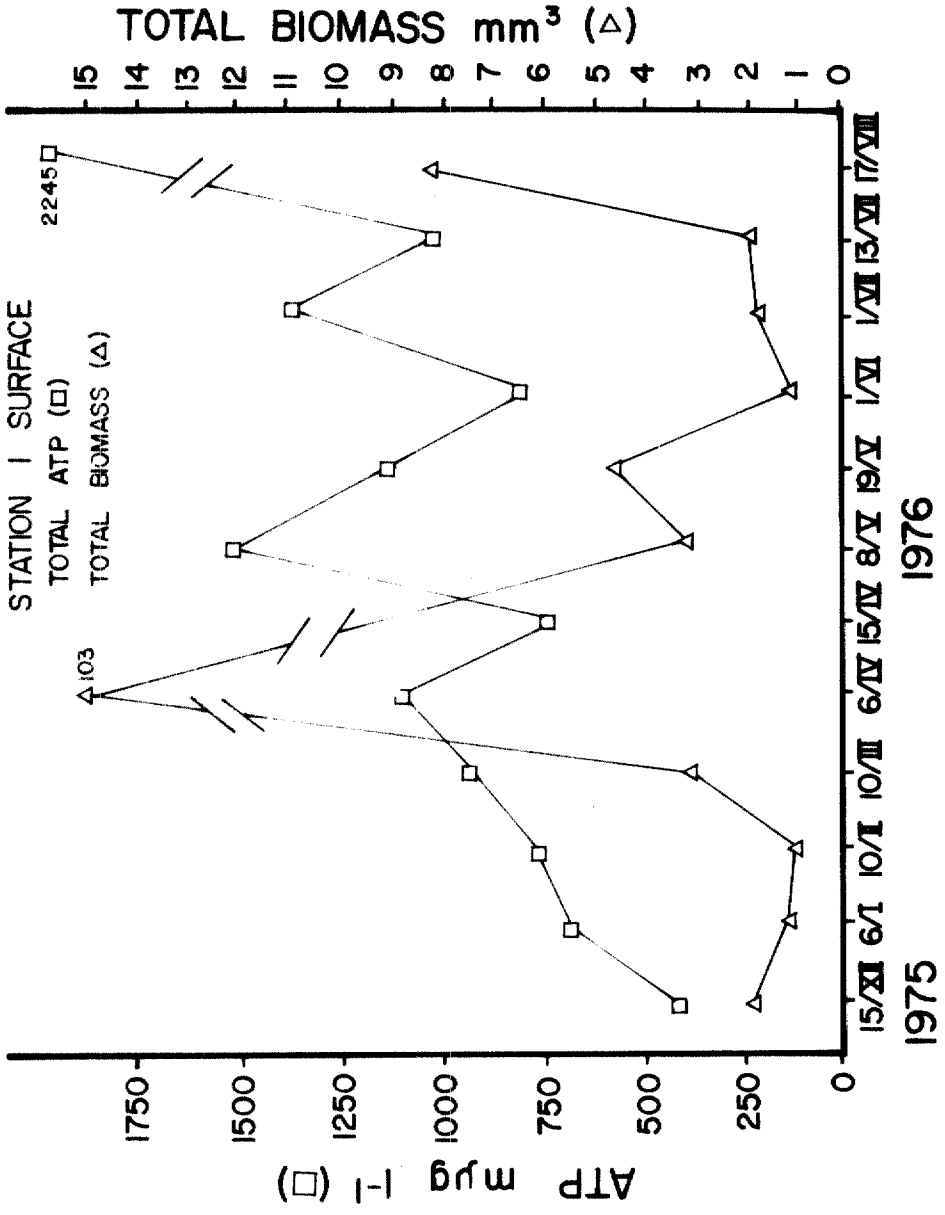
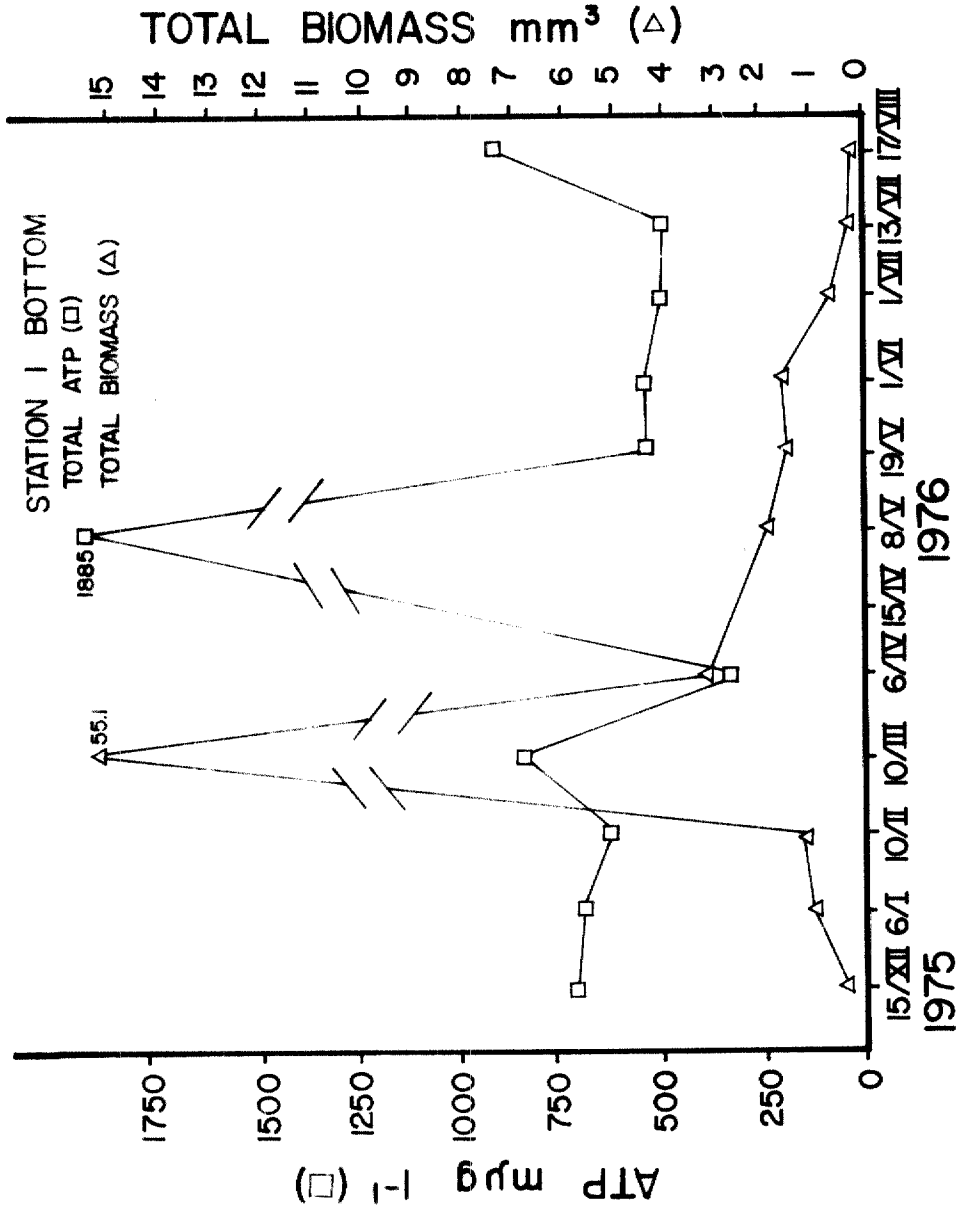

TOTAL BIOMASS mm³ (△)
STATION 1 SURFACE
TOTAL ATP (□)
TOTAL BIOMASS (△)
2245
103
ATP mμg l⁻¹ (□)
1975
1976
15/XII 6/I 10/I 10/III 6/IV 15/IV 8/V 9/V 1/VI 1/VII 13/VII 17/VII

Figure 17. Bottom total ATP and total calculated biomass comparisons at Station 1.


TOTAL BIOMASS mm³ (△)
STATION 1 BOTTOM
TOTAL ATP (□)
TOTAL BIOMASS (△)
1885
55.1
ATP mμg l⁻¹ (□)
1975
1976

after ATP extraction of bottom samples showed considerable deviation from the assessed ATP ℓ^{-1} (Fig. 18). When biomass (calculated from total plankton in the A fraction) was compared with ATP ℓ^{-1}, a more co-varying comparison resulted. Surface comparison of these two biomass estimations and ATP ℓ^{-1} show trends similar to those found for bottom samples (Fig. 19). Again, calculated biomass of zooplankton alone did not co-vary with ATP ℓ^{-1} as well as the biomass of all fraction A organisms calculated from separate plankton samples.

Erratic changes in ATP ℓ^{-1} from the surface B fraction were not represented in the calculated biomass data (Fig. 20). Divergence of the two parameters in the bottom samples was even more extreme, with agreement only between downward trends of each constituent (Fig. 21). Increases in ATP ℓ^{-1} were not shown as increased calculated biomass.

The C fraction (10-64 μm) ATP ℓ^{-1} comparison to calculated biomass provided definite concomitant increases or decreases of each component. Surface relationships between ATP ℓ^{-1} and calculated biomass were considerably divergent from May 19 through August (Fig. 22). Similar responses in ATP ℓ^{-1} and calculated volumes were observed in the bottom samples throughout the study (Fig. 23). Note that bottom calculated biomass values were relatively low compared to those at the surface. Calculated biomass comparisons to ATP values in particular and fractional considerations in general must be evaluated with the knowledge that separation of specific sized material is not 100 percent.

Figure 18. Bottom fraction A ATP relative to biomass calculated from plankton samples and from zooplankton retained in the A fraction ATP samples at Station 1.

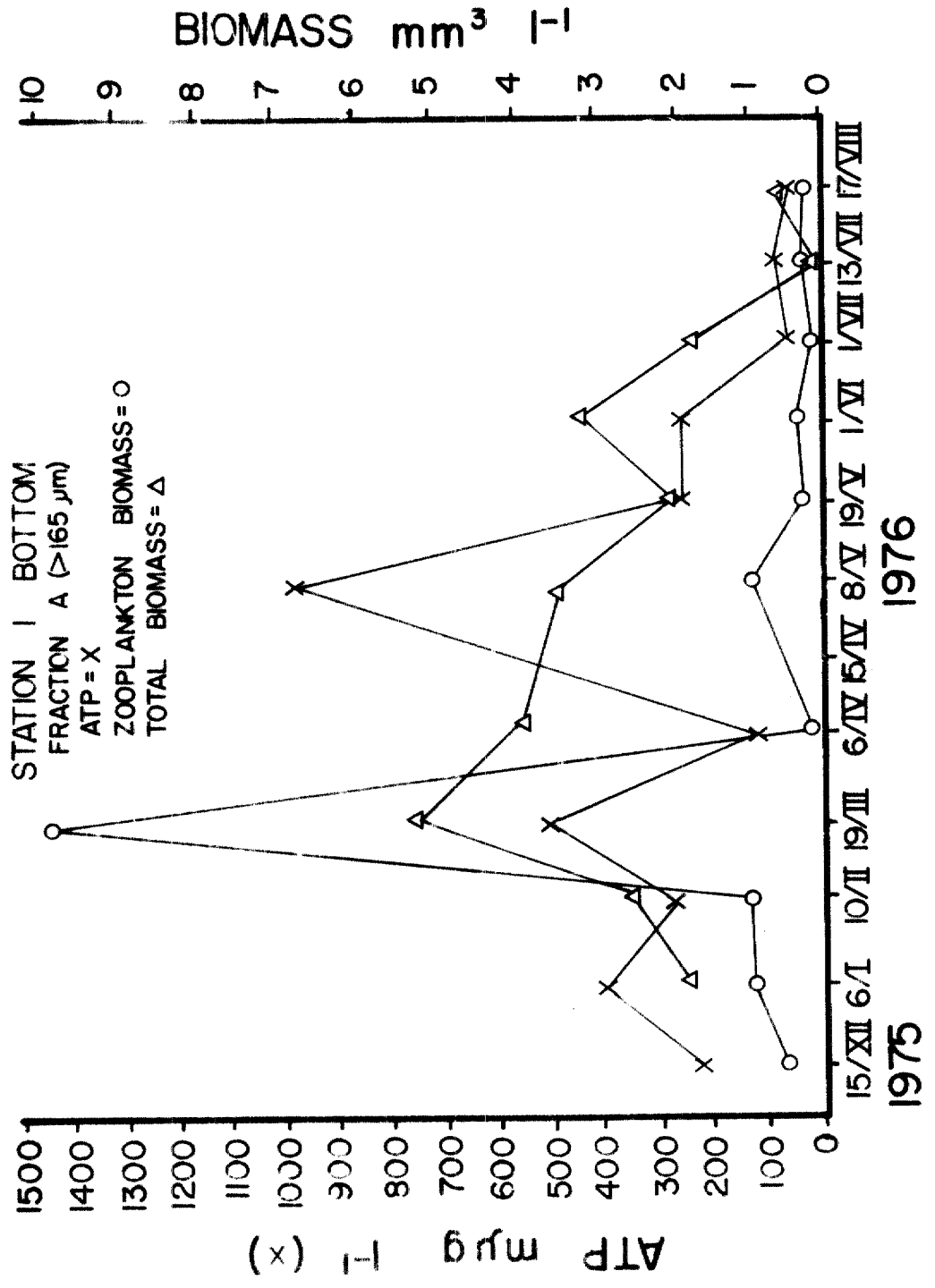

BIOMASS mm³ l⁻¹
ATP mμg l⁻¹ (x)
STATION I BOTTOM
FRACTION A (>165 μm)
ATP = X
ZOOPLANKTON BIOMASS = O
TOTAL BIOMASS = Δ
1975
1976
15/VIII/75
7/9
1/0
10/II
19/III
6/IV
8/V
19/V
1/VI
13/VI
17/VII
15/VIII

Figure 19. Surface fraction A ATP relative to biomass calculated from plankton samples and from zooplankton retained in the A fraction ATP samples at Station 1.

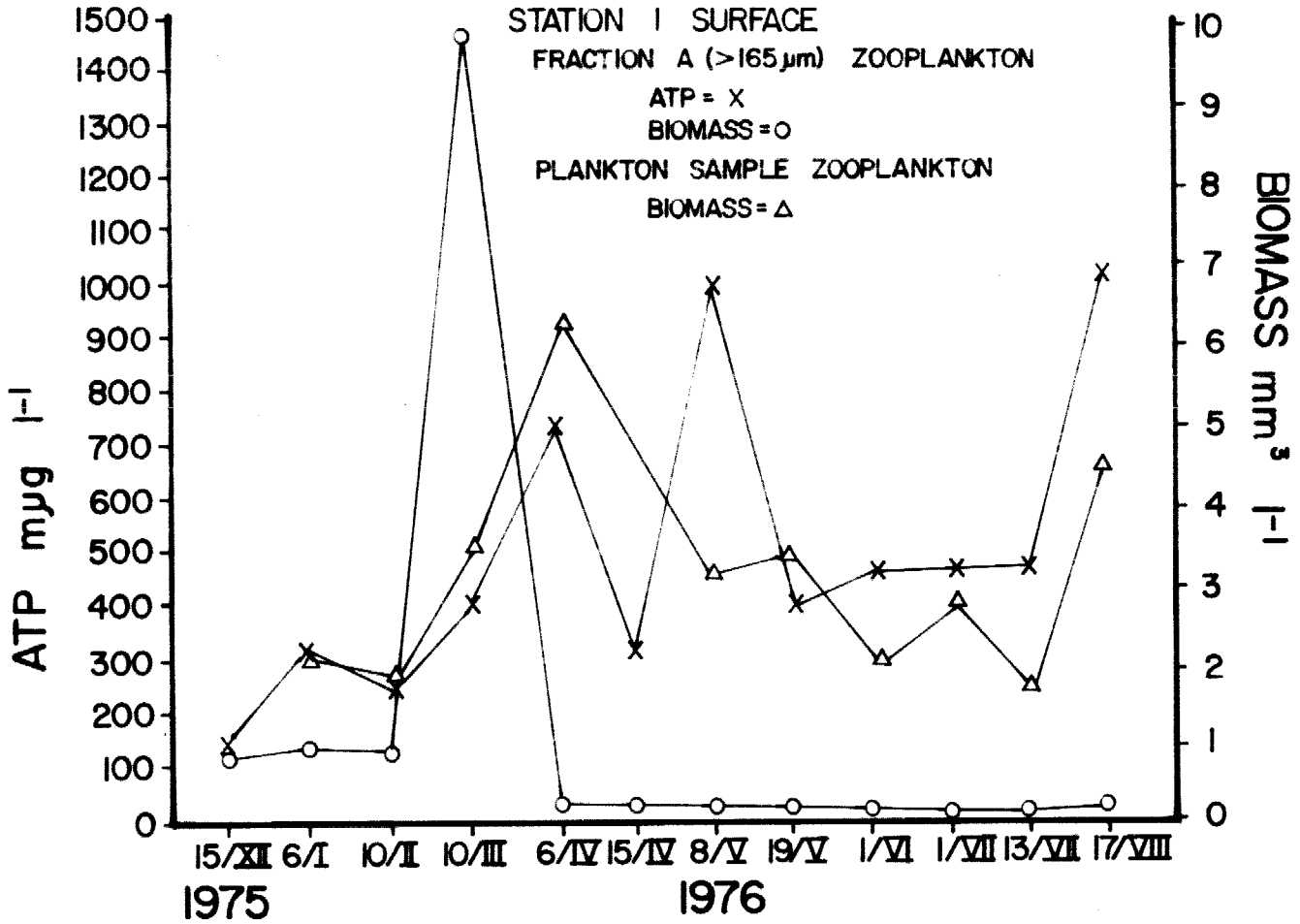

STATION I SURFACE
FRACTION A (>165 µm) ZOOPLANKTON
ATP = X
BIOMASS = O
PLANKTON SAMPLE ZOOPLANKTON
BIOMASS = Δ
ATP mµg l-1
BIOMASS mm³ l-1
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
10
9
8
7
6
5
4
3
2
1
0
15/XII
6/I
10/II
10/III
6/IV
15/IV
8/V
19/V
1/VI
1/VII
13/VII
17/VIII
1975
1976

Figure 20. Comparison of surface fraction B ATP to calculated volume at Station 1.

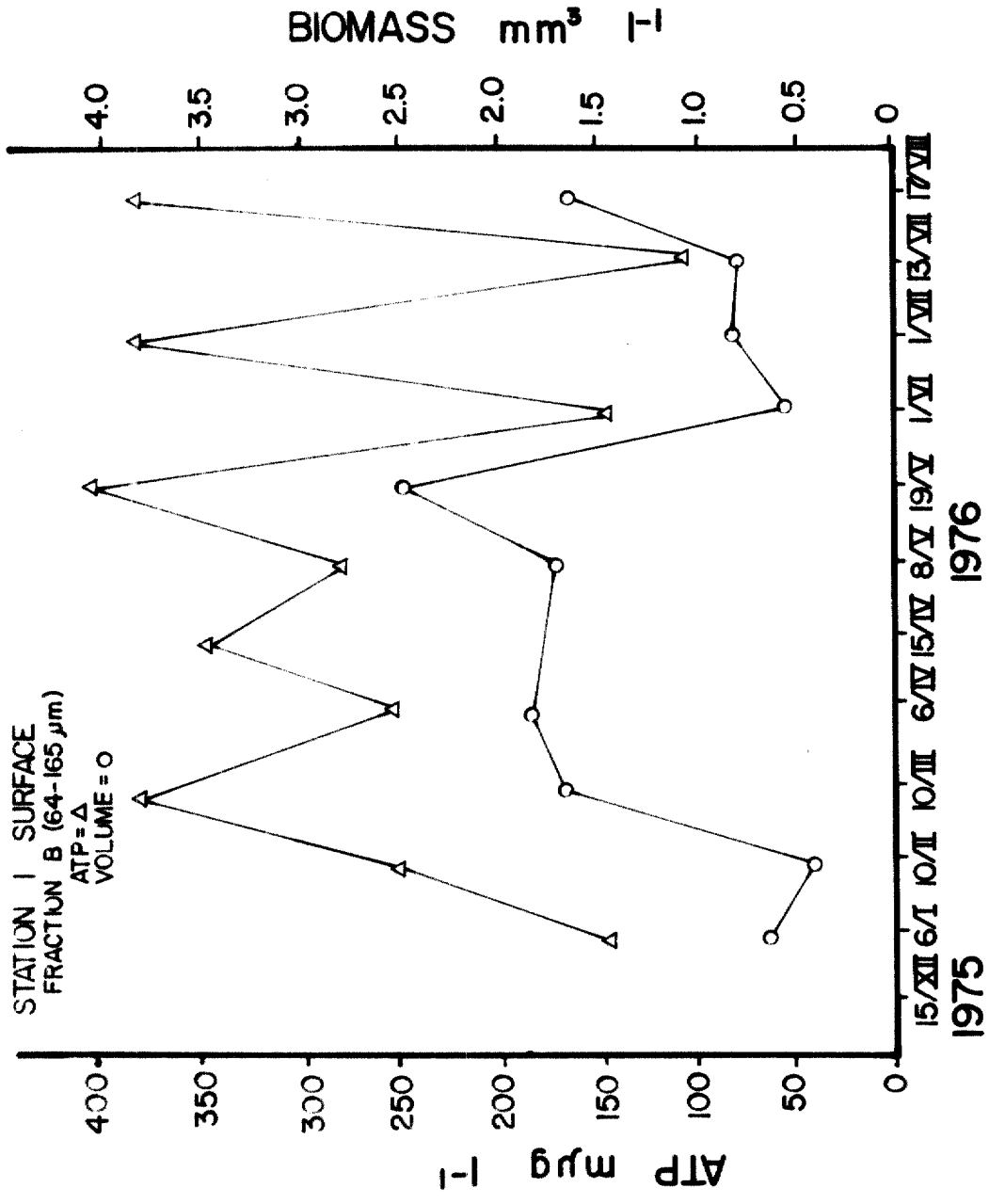

BIOMASS mm³ l⁻¹
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
ATP mμg l⁻¹
400
350
300
250
200
150
100
50
0
STATION 1 SURFACE
FRACTION B (64-165 μm)
ATP = Δ
VOLUME = o
15/XII 1/I 17/I 3/II 19/II 5/III 21/III 7/IV 23/IV 9/V 25/V 11/VI 27/VI 13/VII 29/VII
1975
1976

Figure 21. Comparison of bottom fraction B ATP to calculated volume at Station 1.

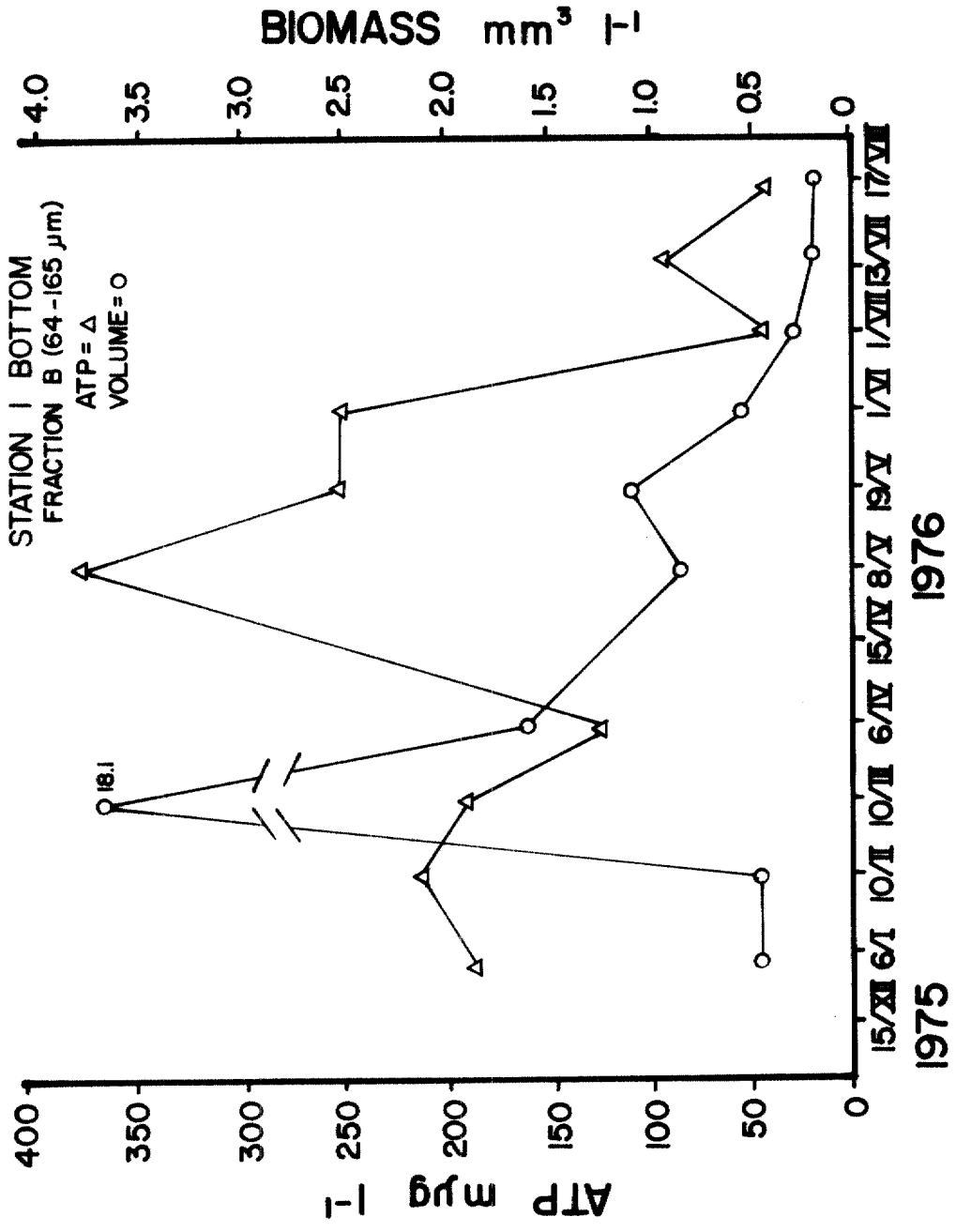

BIOMASS mm³ l⁻¹
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
STATION 1 BOTTOM
FRACTION B (64-165 µm)
ATP = Δ
VOLUME = o
18.1
ATP mµg l⁻¹
400
350
300
250
200
150
100
50
0
15/XII 1/I 10/II 6/III 15/III 8/IV 19/IV 1/VI 13/VI 17/VI
1975
1976

Figure 22. Comparison of surface fraction C ATP to calculated volume at Station 1.

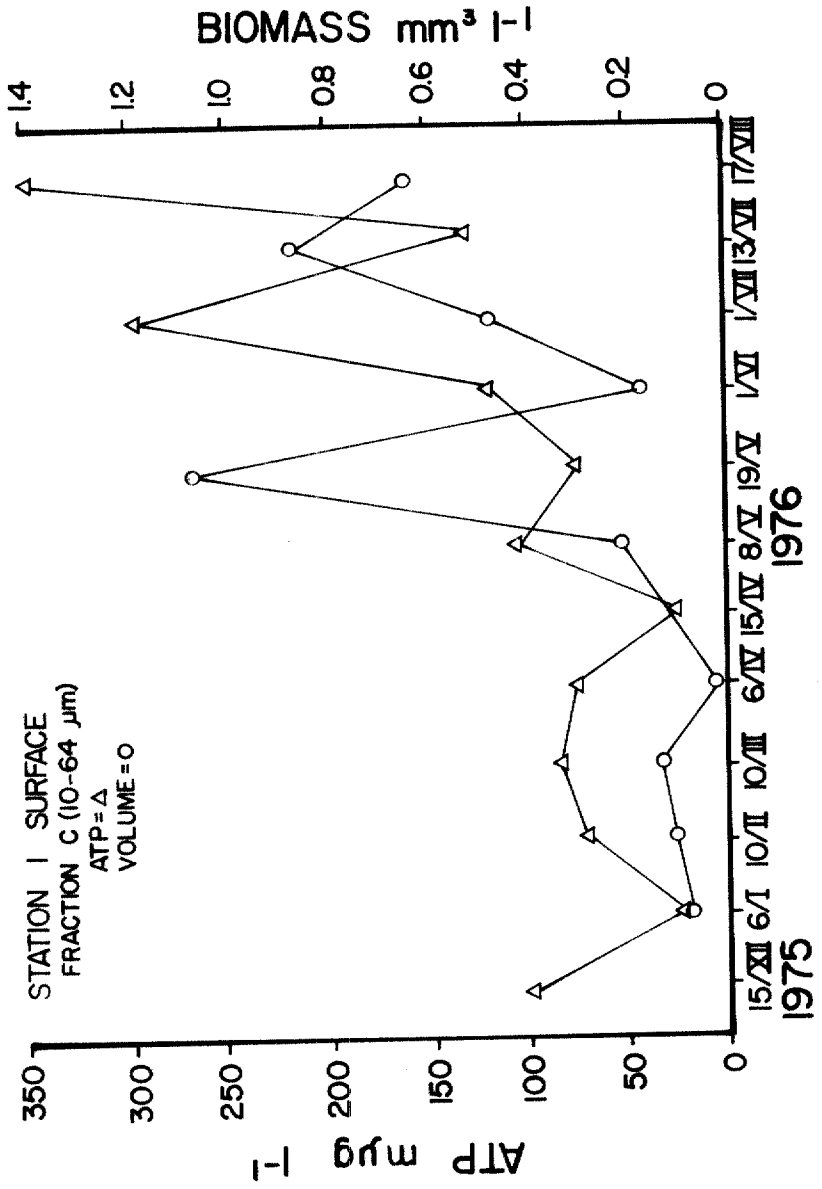
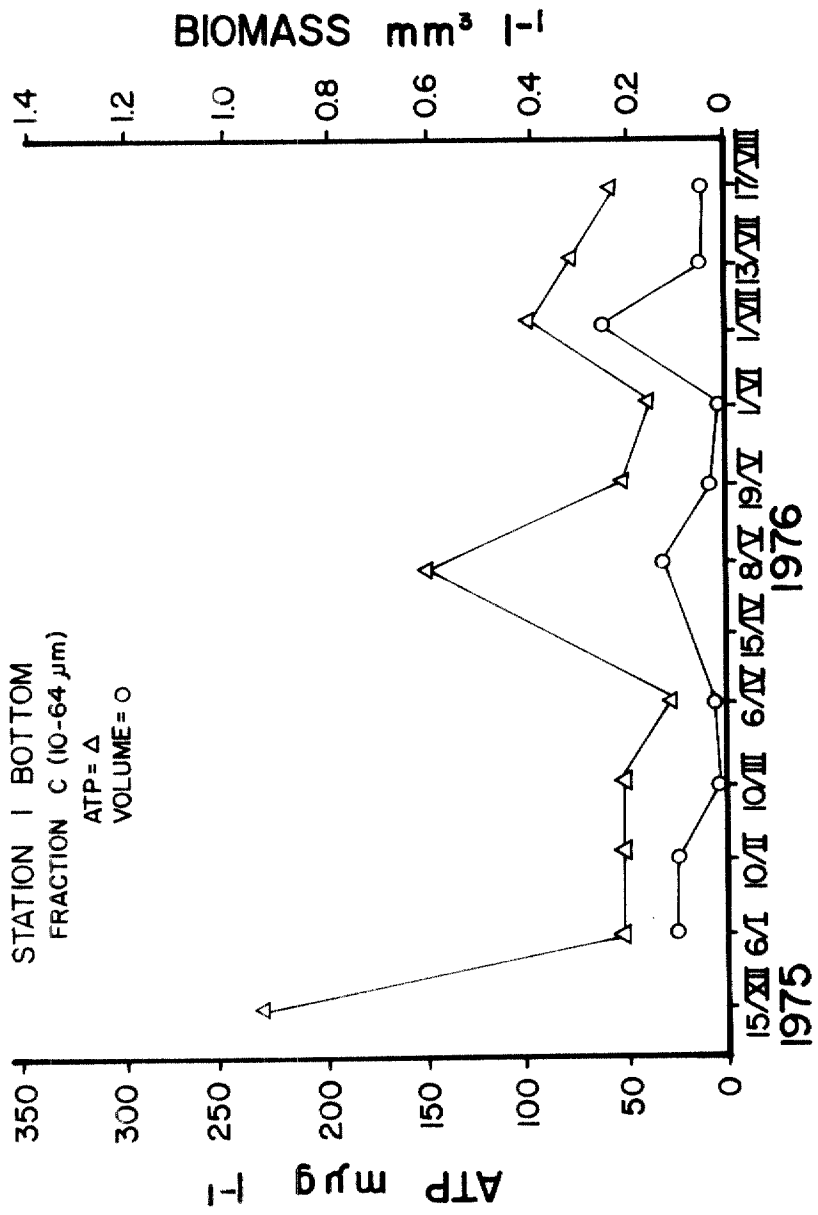

BIOMASS mm³ l-1
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
ATP mμg l-1
350
300
250
200
150
100
50
0
STATION 1 SURFACE
FRACTION C (10-64 μm)
ATP = Δ
VOLUME = 0
15/III 6/IV 10/II 10/III 9/IV 15/IV 8/V 19/V 1/VI 13/VI 17/VII
1975
1976

Figure 23. Comparison of bottom fraction C ATP to calculated volume at Station 1.


BIOMASS mm³ l⁻¹
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
ATP mμg l⁻¹
350
300
250
200
150
100
50
0
STATION 1 BOTTOM
FRACTION C (10-64 μm)
ATP= Δ
VOLUME= O
15/XII 1/I 10/II 10/III 9/IV 15/IV 8/V 19/V 1/VI 13/VI 17/VII
1975
1976

Physical-Chemical

Nitrogen analysis revealed high total Kjeldahl nitrogen (TKN) during late spring through summer into fall, with combined total nitrite-nitrate nitrogen (TN-NN) highest in winter (Fig. 24). Generally only small differences existed between surface and bottom values for either nitrogen type. Notable exceptions occurred for Kjeldahl nitrogen in early fall, 1975 (values were very erratic), and in mid-winter for TN-NN.

Total phosphorus (TP) data revealed very low levels and erratic, sometimes drastic changes (Fig. 25). Late summer and fall, 1975, bottom (11.5 m) sample values were considerably higher than those from the surface (2.5 m). Surface and bottom phosphorus levels were relatively similar during winter, spring, and summer. A notable exception was observed on July 1, 1976, when the bottom value was the highest recorded during the sampling period.

Alkalinity and pH were relatively constant for all three stations throughout the study. Means of duplicate bottom and surface samples from Station 1 were representative of the collection sites on the reservoir (Fig. 26). Yearly means of surface pH and alkalinity were 8.1 and 149 mg ℓ^{-1} respectively, while yearly bottom means were 7.4 and 146 mg ℓ^{-1} respectively. Conductivity, not as stable as pH and alkalinity, showed a general tendency to increase during this research (Fig. 27). Surface and bottom conductivity means were 234 and 251 μmhos respectively.

Figure 24. Mean total Kjeldahl and nitrite-nitrate nitrogen (N) from surface and bottom samples at Station 1.

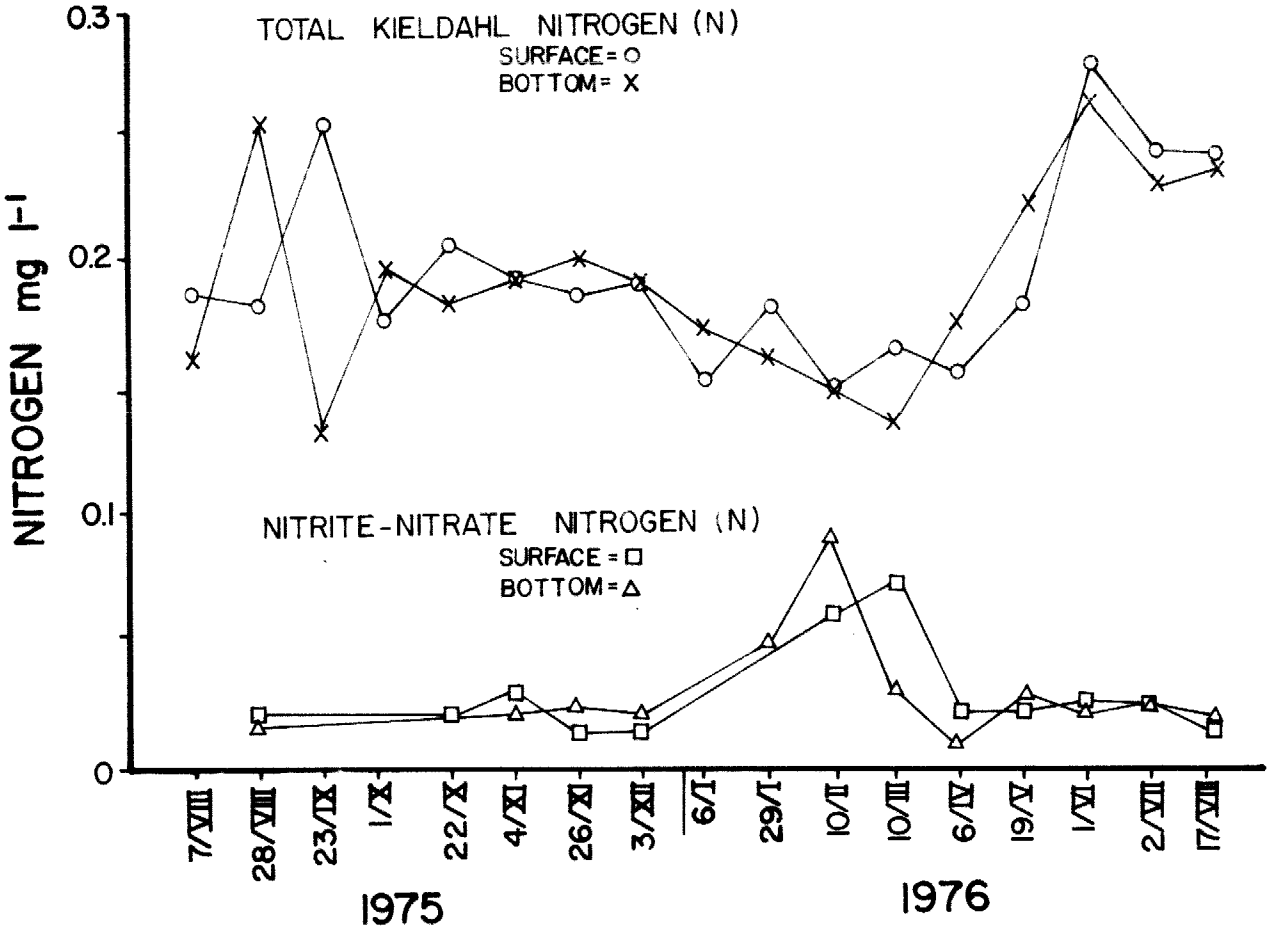

TOTAL KIELDAHL NITROGEN (N)
SURFACE = O
BOTTOM = X
NITRITE-NITRATE NITROGEN (N)
SURFACE = □
BOTTOM = △
NITROGEN mg l-1
0.3
0.2
0.1
0
7/VIII
28/VIII
23/IX
1/X
22/X
4/XI
26/XI
3/XII
6/I
29/I
10/II
10/III
6/IV
19/V
1/VI
2/VII
17/VII
1975
1976

Figure 25. Mean total phosphorus (P) from surface and bottom samples at Station 1.

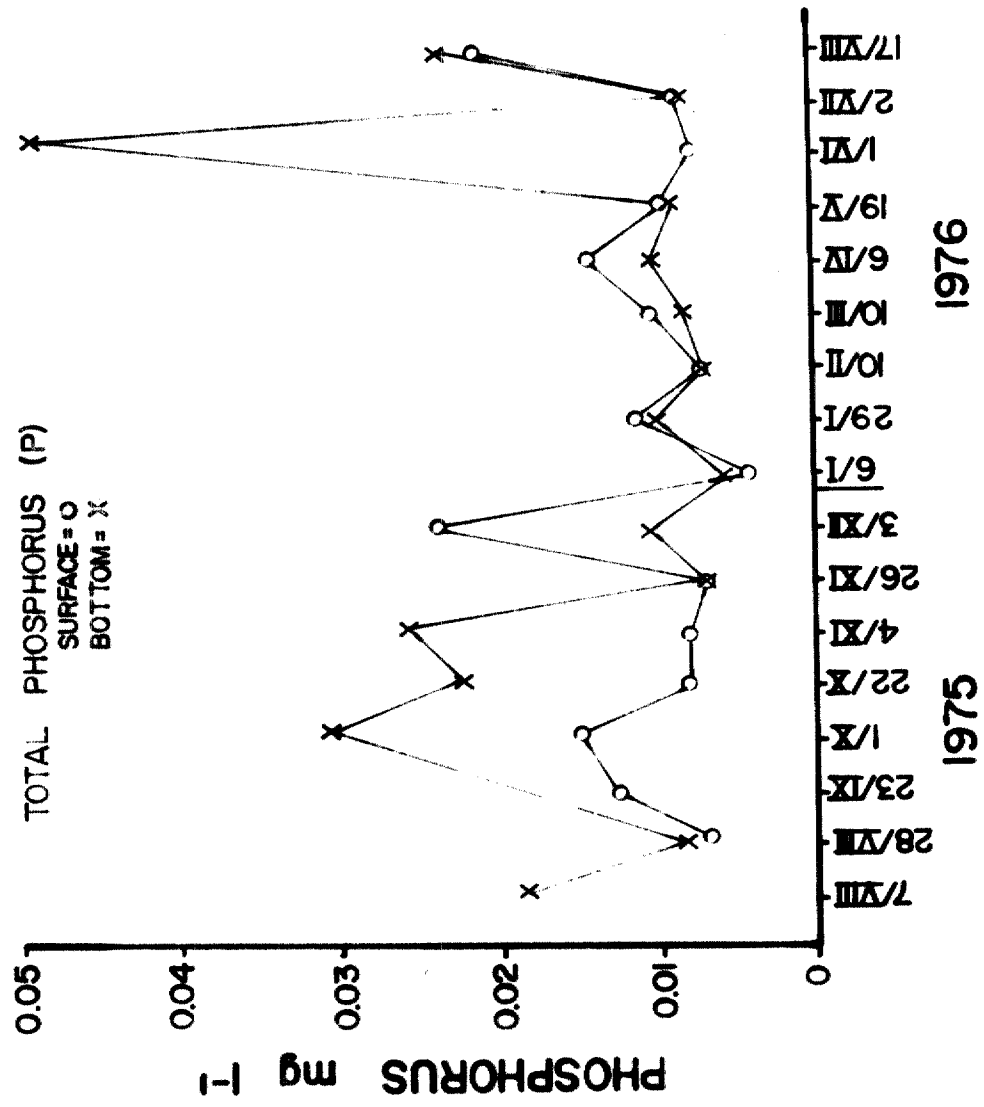

TOTAL PHOSPHORUS (P)
SURFACE = O
BOTTOM = X
PHOSPHORUS mg l-1
0.05
0.04
0.03
0.02
0.01
0
7/VIII
28/VII
23/IX
1/X
22/X
4/XI
26/XI
3/XII
6/I
29/I
10/II
10/III
6/IV
19/V
1/VI
2/VII
17/VIII
1975
1976

Figure 26. Mean alkalinity and pH from surface and bottom samples at Station 1.

ALKALINITY mg l-1 (CaCO3)
300
250
200
150
100
50
0
pH
9
8
7
6
5
4
3
2
1
0
pH
SURFACE = Δ
BOTTOM = □
ALKALINITY
SURFACE = O
BOTTOM = X
22/V
7/VI
23/VI
25/VII
7/VIII
28/VIII
19/IX
1/X
22/X
4/XI
3/XII
15/XII
6/I
29/I
10/II
10/III
6/IV
15/IV
8/V
19/V
1/VI
1/VII
13/VIII
17/VIII
1975
1976

Figure 27. Surface and bottom mean conductivity from Station 1.

µmhos
350
300
250
200
150
100
50
0
CONDUCTIVITY
SURFACE = O
BOTTOM = X
22/V
7/VI
25/VI
25/VII
7/VIII
28/VIII
19/IX
1/X
22/X
4/XI
3/XII
15/XII
6/I
29/I
10/II
10/III
6/IV
15/IV
8/V
19/V
1/VI
1/VII
15/VII
17/VIII
1975
1976

A dissolved oxygen (D.O.) profile from Station 1 (deepest station) showed that at no time was oxygen limited in the upper 7 m of the water column (Fig. 28). An essentially anaerobic zone, established in late June, 1974, and lasting through September, 1975 (June, 1976-August, 1976), extended downward from below the thermocline (8 m) to the bottom. The thermal profile at Station 1 revealed temperature maxima of 30 C and minima of 5.5 C (Fig. 29). Thermocline was established in early June, 1976, through the termination of sampling in August. Annual heat budget for the reservoir was calculated (Table 4). Heat absorbed by sediments was not accounted for; however, corrections were made for volume changes during the study. The annual heat content was 12,898 g cal cm^{-2}, calculated from August, 1975, and January, 1976, values.

Statistical

The relationship between ATP biomass, as dependent variable, and phosphorus, total Kjeldahl nitrogen (TKN), total nitrite-nitrate nitrogen (TN-NN), temperature, pH, alkalinity, conductivity, and dissolved oxygen as independent parameters was investigated, using multiple regression analysis. No multiple linear regressions were significant; however, simple correlation was best between temperature and total ATP ℓ^{-1} ($r = 0.6638$) in surface comparisons. No such correlation occurred in bottom parameter comparisons.

Figure 28. Vertical profile of dissolved oxygen in H. H. Moss Reservoir at Station 1.

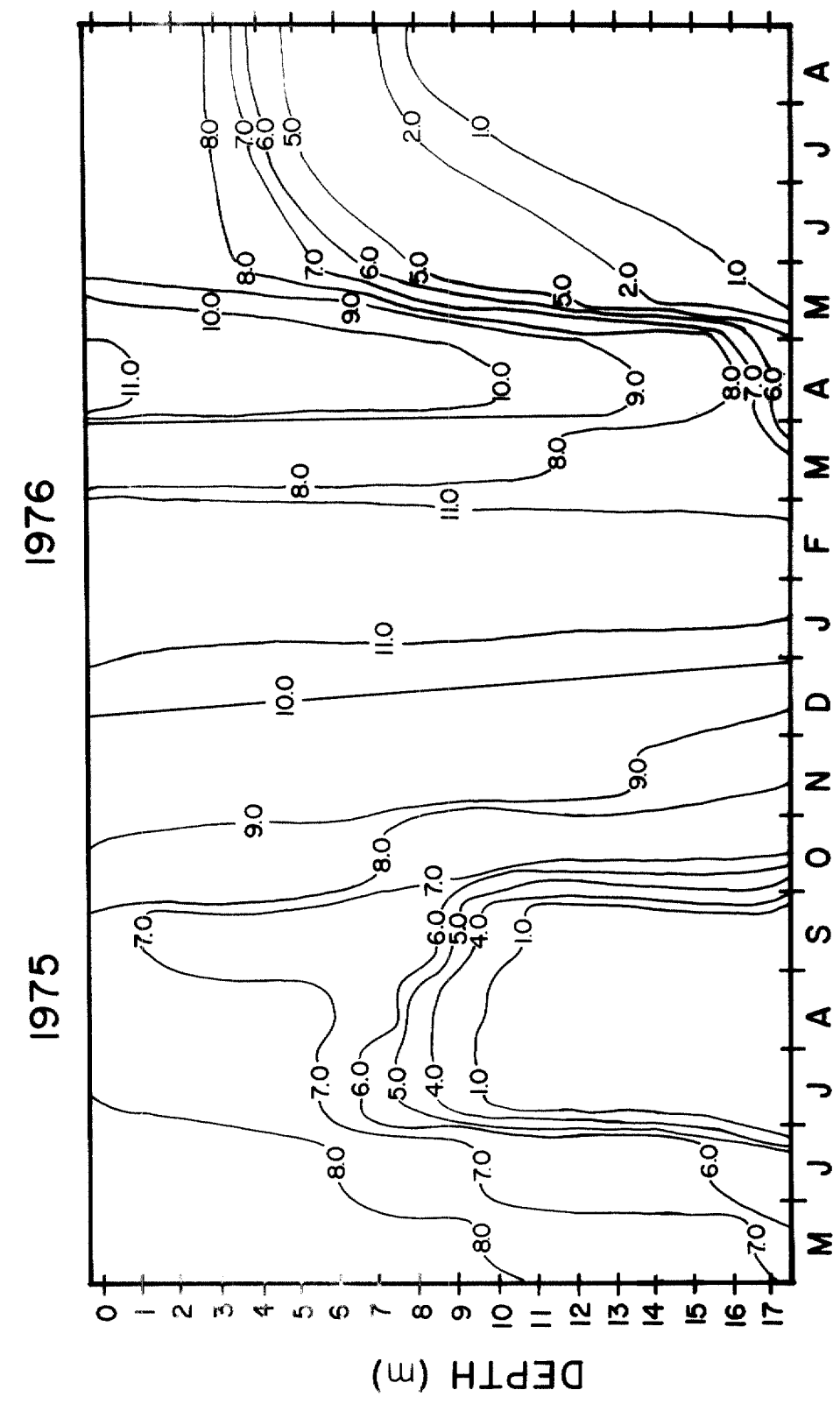

1975
1976
DEPTH (m)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
M J J A S O N D J F M A M J J A
1.0
2.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0

Figure 29. Vertical profile of temperature in H. H. Moss Reservoir at Station 1.

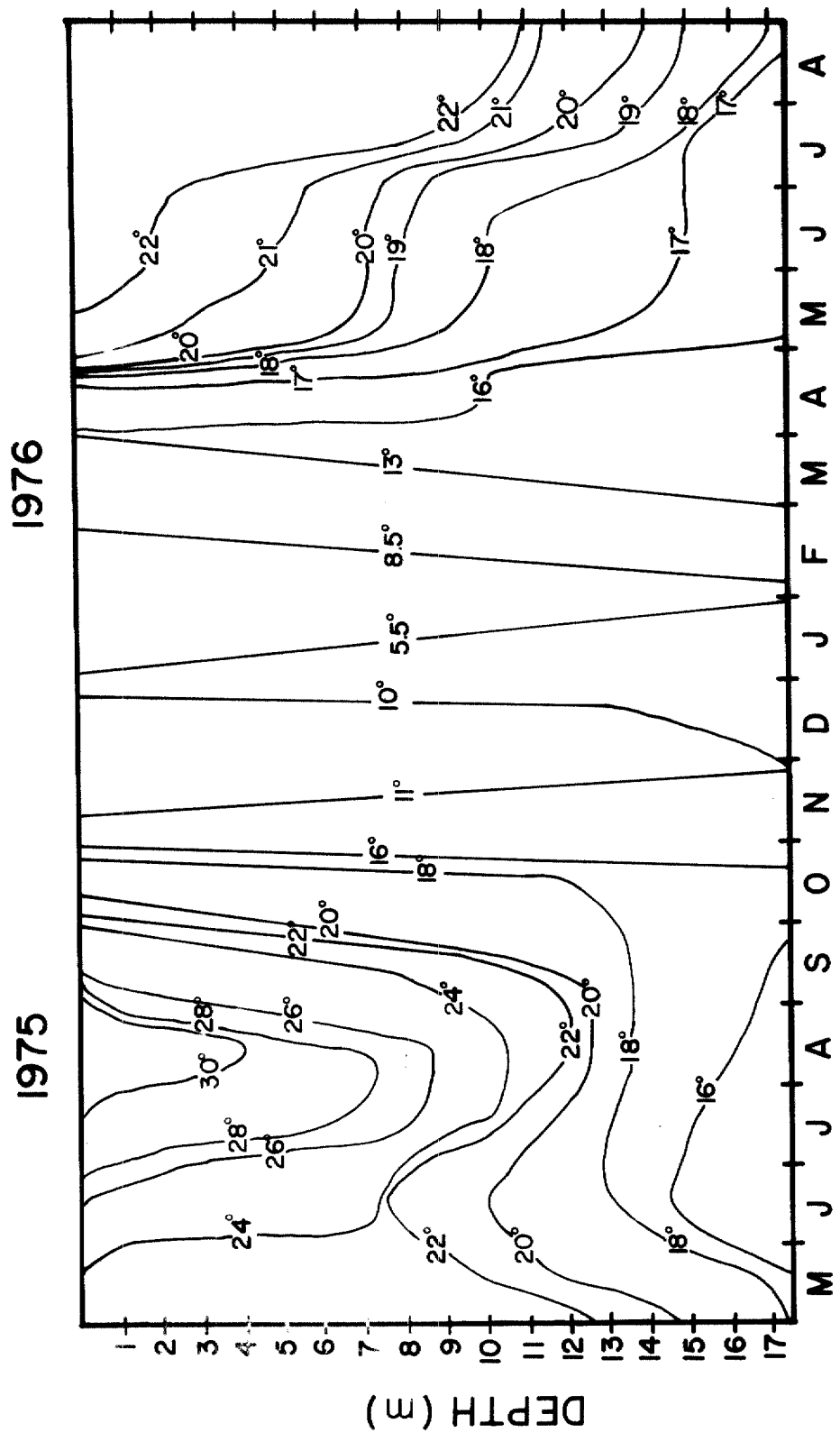

1975
1976
DEPTH (m)
M J J A S O N D J F M A M J J A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
30° 28° 26° 24° 22° 20° 18° 16° 13° 11° 10° 8.5° 5.5° 21°

Table 4. H. H. Moss Reservoir annual heat budget.

Depth (m)	Volume ($m^3 X 10^6$)	August Temps.	K Calories	January Temps.	K. Calories
0-2	4.48	30	134.4	5.5	24.64
2-5	7.64	30	229.2	5.5	42.02
5-8	5.41	28	151.5	5.5	29.75
8-11	4.54	26	118.1	5.5	24.97
11-14	3.20	20	64.0	5.5	17.60
14-17	2.05	18	36.9	5.5	11.27
17-19	0.29	16	4.6	5.5	1.59
			738.7		151.84
Area 4.55 km^2					

Heat Content $738.7 \times 10^6 - 151.84 \times 10^6 = 586.86 \times 10^6$ Kcal

1×10^6 g cal $Kcal^{-1} = 586.86 \times 10^{12}$ g cal

$$\frac{586.86 \times 10^{12} \text{ g cal}}{4.55 \times 10^6 (m^2) \times 10^4} = \frac{128.9802 \times 10^6}{10^4} = 128.9802 \text{ g cal cm}^{-2}$$

Maximum to minimum $\theta_{bs} - \theta_{bw} = 12{,}898$ g cal cm^{-2}

Monthly = 11,357 g cal cm^{-2}

CHAPTER V

DISCUSSION

Holm-Hansen (1975) encountered apparent extraction problems when conducting ATP assays in very eutrophic waters which contained high concentrations of suspended algae. He cited a particular "red tide" situation when the dinoflagellate Gymnodinium sp. was very abundant along the California coast. The amount of ATP measured in successively larger sample volumes resulted in a non-linear relationship; the response curve lagged inaccurately at its upper end. Although Rudd and Hamilton (1973) collected ATP data from a relatively eutrophic Canadian lake, no extraction problems were encountered. The authors used small sample sizes (250 ml) and removed zooplankton prior to extraction. Such differences in gross ATP ℓ^{-1} estimates which I found between 1-ℓ samples and the 70-ℓ net samples implied three problem sources. First, if the closing net did not actually filter 70 ℓ of water due to backwashing or other problems, ATP ℓ^{-1} estimation would be grossly incorrect; second, if the amount of planktonic material was too great, improper extraction, due to inefficient heating as suggested by Karl (1975) may have resulted in ATP loss; and finally, if large quantities of plankton were extracted properly, the ATP could have been adsorbed as reported by Lee et al. (1971).

It is unlikely that the net failed to filter 70 ℓ of reservoir water. The outer 10-μm mesh net filtered water slowly, and when lowered into the water column, the sides collapsed inwardly so that the net did not fill with water. The length (2 m) and diameter (30 cm) allowed for a capacity of much more than 70 ℓ. When towed vertically only one meter prior to closing, the net hardly filled to capacity; so there was little or no backwashing. These conclusions were verified by observations of net hauls using SCUBA.

Adsorption problems were reported by Lee et al. (1971) when attempts were made to extract ATP from sediments. To determine relative adsorption net sample fractions were spiked with di-sodium ATP stock solution during and after extraction. Spike recoveries ranged from 80 to 94%, providing evidence that, though some adsorption or loss might have occurred, the small amount observed could not account for what appeared to be gross ATP underestimation.

The third alternative was simply related to problems with extraction. Karl (1975) described boundary layer and differential heating problems when extracting ATP from sediments with boiling Tris buffer. The author showed that lowered temperatures in the extracted mass killed cells slowly and was ineffective in properly extracting ATP. Fluid dynamics and heat transfer problems of this type were elucidated by Knudson and Katz (1954). They believed that solid particles in liquid were heated by conduction, which occurred at a slower rate

than convection. A boundary layer was established, creating a sharp thermal gradient between the solid and surrounding liquid. The lower temperature of the sediment materials caused ATP loss, most probably becuase of improper denaturation of hydrolyzing enzymes. Inefficient extraction of dense net samples was encountered in the present study. The mass of plankton and tripton collected in the net samples apparently was improperly heated and incompletely extracted when immersed in boiling Tris buffer.

Since comparison of data from 1-ℓ (i.e. water bottle) samples to net samples (70 ℓ) revealed such large differences, the accuracy of ATP estimates from 1-ℓ samples in the present study was also questioned. Were 1-ℓ samples small enough to produce accurate ATP volumes and yet large enough to be representative of reservoir water? Holm-Hansen (1969) used 1- and 2-ℓ samples when extracting ATP from deep ocean waters and suggested using similar or smaller samples in eutrophic waters. Specific extraction of three different MR water volumes up to 1 ℓ produced linear ATP estimates. Since the D fraction (<10 μm) filtered volume, always 0.5 ℓ, had yielded consistent results and 92% recovery of spikes it was assumed to yield accurate information. From these data it is apparent that liter samples presented no filtration or extraction problems.

Critical temperatures of + 95 C must be maintained during extraction for proper enzyme denaturation and ATP release

(Holm-Hansen and Booth 1966). The use of hot plate-water bath combined extraction (i.e. instead of water bath alone) was established when extractions of varying volumes of laboratory algal cultures revealed significant differences. I suspected that problems with water bath use alone were related to initial cooling of the Tris solution when the wet, cool filter was added. The hot plate surface temperature (400 C) was much hotter than water bath (i.e. max. 100 C), thus reheating the slightly cooled extraction solution more rapidly. The result was that I abandoned use of net samples in favor of 1-ℓ water bottle samples. In my estimation net sampling might yield excellent results in less eutrophic waters. Analysis of sub-samples from net collections were not attempted. However, this technique might yield reproducible and accurate ATP results. Certainly net hauls are better estimates of actual biomass in the water column due to collection of larger sample sizes. Increased size of samples would prove most useful in analyzing the patchy plankton distribution which is often observed in lentic systems (Edmondson 1974).

I modified the procedure for enzyme hydration outlined by Strickland and Parsons (1972) for two reasons. Hydration with Tris (7.75 pH, 0.02 M) buffer was done to increase buffer capacity of the solution. The deionized water which was available to me had a low pH (5.5-6.0). If the enzyme had been prepared with the deionized water there might have been a chance that the resulting low pH would have interfered with the light

emission reaction. The preparation also differed from the suggested procedure in that the enzyme was allowed to stand for approximately 17 hours after centrifugation. This "aged" the solution and reduced the background counts for better reproducibility. No adverse effects were noted from these procedural changes.

Maximum total surface ATP values reported by Rudd and Hamilton (1973) for Lake 227 exceeded the maximum values I found in MR. Maximum surface total ATP values in MR never reached 3 $\mu g\ \ell^{-1}$, while values in Lake 227 (Canada) exceed 6 $\mu g\ \ell^{-1}$ in summer. Cavari (1976) reported maximum ATP ℓ^{-1} values of about 2.9 μg for Lake Kinneret, Israel, approximately the maximum levels observed in MR. Robinson (1975) reported peak ATP ℓ^{-1} for four New Mexican reservoirs. The highest values from MR (2.7 $\mu g\ \ell^{-1}$) exceeded the maximum ATP ℓ^{-1} (1.7 μg) from all four New Mexican impoundments. Radical monthly fluctuations in ATP levels were observed in each of these works as well as the present study. Two definite ATP peaks from MR were noted in spring and late summer. The spring peak was primarily due to zooplankton population increases, while the late summer peak was caused by a combination of filamentous blue green algae (Anabaena sp.) and undetermined material less than 10 μ in size.

The comparability of bottom samples from Station 1 (11.5 m) to those from Station 2 and 3 (7 m) is questionable). A thermocline existed during summer at about 7 m; consequently bottom

samples from Stations 2 and 3 were taken in the thermocline, while those from Station 1 were taken well below it. Bottom ATP estimates at all stations approached surface values for the same sampling dates prior to thermocline formation. Stations 1 and 3 showed similar, low ATP values after thermocline formation. At Station 2, bottom samples yielded much higher ATP levels in August. Maximum bottom total ATP found in MR was similar to that found in Lake 227 (Rudd and Hamilton 1973).

I had hoped to explain variability and changes in ATP by dynamics of specific physical and chemical parameters (Station 1), using multiple regression analysis. However, only simple correlation of temperature to total surface ATP was significant ($r = 0.66$). Small sample size (1 ℓ) and the Poisson distribution of plankton combined to produce variable numbers of organisms, and therefore, different ATP estimates with each replicate sample. The subsequent variability led to no significant multiple correlations. Holm-Hansen (1969) found that chlorophyll and ATP estimates were in excellent agreement with direct biomass estimation. Neither Rudd and Hamilton (1973) nor I found correlations between total ATP and direct microscopic biomass estimation. Robinson (1975) found variable correlations between total ATP and calculated phytoplankton and zooplankton biomasses in four New Mexican reservoirs. Although it is unclear exactly how Cavari (1976) arrived at calculated biomass values, there appeared to be little correlation between these and ATP content in Lake Kinneret.

However, calculated biomass (mm^3 ℓ^{-1}) did tract ATP dynamics in certain size fractions from MR. Bottom samples from fraction A (i.e. all organisms >165 μm; predominantly zooplankton) and fraction C showed similar increases and decreases throughout the sampling period. Less direct tracking occurred in the surface samples of fractions A and B. Some fractions at specific depths showed more correlation than others. It appears, with slight exception, that biomass calculated from direct microscopic examination bears considerably less correlation with ATP values than one would hope. The problem is twofold; first it is most difficult to separate, in preserved samples, those plankton which were living from those which were dead at the time of sample collection. Second, bacteria, epiphytic protozoa, and other easily destroyed or removed attached organisms cannot be seen in light microscope examination of preserved samples. Paerl (1975) addressed these problems with some success. He used autoradiography (radioactive ^{14}C uptake by phytoplankton; subsequent exposure of the filtered material to silver halide emulsion) to determine the percentage of living photosynthetic organisms in plankton samples. Comparisons of this estimated living planktonic biomass correlated well to ATP values. In an effort to estimate bacteria and protozoan non-photosynthetic organisms, Paerl used scanning electron microscopy to examine samples. Paerl suggested that, based on the autoradiography technique, only between 20 and 50% of the microscopically visible phytoplankton are viable, and

that, based on the scanning electron micrographs, normally less than 10% of the biomass is composed of bacteria.

The <10-μm size fraction in MR held more than 10% of the ATP, especially during summer, at all of the sampling stations. All material found in the <10-μm size fraction was not bacteria. In fact, during winter, small diatoms and in late spring, broken Scenedesmus sp. synobia were microscopically observed in the <10-μm fraction. Note that the D fractions from all stations in August were higher than any other fraction. Microscopically, these D samples contained less than 10% of the planktonic material found in the corresponding C fractions. The high D fraction ATP value in August from the 11.5 m (bottom) sample at Station 1 probably represented large concentrations of anaerobic bacteria. No oxygen and reduced iron, as evidenced by iron oxidation and settling upon filtration, were present at 11.5 m during this time period. The changes in bottom D fraction ATP levels, from winter to summer accompanied by thermocline formation and hypolimnion deoxygenation imply a change from aerobic bacteria to anaerobic bacteria. Rudd and Hamilton (1973) found that up to 95% of the ATP in summer samples from Lake 227 was present in the <10μm size fraction. The large amounts of ATP in the <10-μm size class from MR during summer indicate the major role that organisms from this fraction play. Nannoplankton of the C (10-64 μm) and D fractions contributed much of the living biomass found in the impoundment at any given time.

Fractions B, C, and of course D, may be compared between stations. Recall that all samples at Station 1 were collected at night. Because of day time zooplankton avoidance the A fractions are not comparable either in ATP ℓ^{-1} or calculated biomass (mm^3 ℓ^{-1}). Peaks of fraction A (observed as zooplankton) occurred in late spring at all three stations (surface and bottom) as water temperature rose and apparently in connection with no other planktonic fraction. The late summer surface A fraction peak at Station 1 was revealed only because night samples were collected. It was suspected, because of previous concomitant peaks between stations, that zooplankton were similarly abundant at the other stations but simply avoided the water bottle. Perhaps this could be avoided by use of a closing plankton net. August bottom (11.5 m) fraction A ATP was low at Station 1 presumably because of the anaerobic conditions prevailing below 8 m. The low A fraction ATP levels from the bottom (7 m) samples at Stations 2 and 3 may have been caused in part by low levels of dissolved oxygen and by zooplankton avoidance.

Fluctuations in fractions B and C (i.e. 64-165 and 10-64 μm respectively) were generally less than those observed in the A fraction. Although not constant, winter and early spring samples (B and C) were similar from both surface (2.5 m) and bottom (11.5 or 7 m). Viable photosynthetic organisms (observation) were apparently swept downward and kept in suspension by wind during complete reservoir mixing. The average euphotic

zone depth was 3.7 m (i.e. 1% light penetration). Light penetration at 2.5 m averaged 8.1% and never fell below 2%. Both the 7-and 11.5-m depths were continuously in the aphotic zone, with light penetration averaging 0.35 and 0.2% respectively. Organisms from the bottom samples could not maintain photosynthesis and their presence as viable organisms was most probably a result of wind mixing.

Dinobryon spp. dominated in B fractions during May and June. Rudd and Hamilton (1973) found that ATP contained in the 10-56-μm group was similar in amount to that in the 56-250-μm fraction. Although the B and C fractions of this study were not the same as those compared by Rudd and Hamilton, ATP in the smaller size fraction (C) rarely equaled or exceeded that in the larger (B) size fraction. Relative increases of both B and C fractions in August were caused by increased blue-green algae populations (Anabaena spp. and Microcystis spp.)

Comparison of a particular fraction between stations was complicated by large variances between sample replicates. Of the four sets of three means each (12 comparisons), tested by one-way ANOVA, 11 of the comparisons proved significantly different. Differences between these selected means were real as indicated by the low "within" sum of squares. Differences were attributed to actual organism differences between samples, not to assay or extraction variability of duplicate samples.

In addition to the ATP data, I began the study analyzing particulate organic carbon (POC). The carbon analyzer had a

number of mechanical problems, causing loss of some samples. Also, since samples were directly injected (40 μℓ), reproducibility between sample replicates was so poor as to be determined unusable for many samples. In effect, carbon data were not sufficient and were too unreliable (as measured by the techniques I had available) to be included.

Low total phosphorus (TP) values found in MR are atypical for southwestern impoundments (Smith 1973). Sams (1976) found similar low TP values in North Lake, an off-channel impoundment used as a cooling reservoir for an electric power generation facility. Total phosphorus levels were higher after turnovers in both bottom and surface samples. Bottom (11.5 m) TP values fluctuated more radically than those from surface samples. Surface TP values oscillated around an approximate mean of 10 $\mu g\ \ell^{-1}$. Since TP values were generally higher from bottom samples, I believe that during the study period MR was acting as a nutrient sink and sediment trap. Rainfall during the study was below the yearly average (71.12 cm compared to 81.28 cm) and as a consequence discharge was low. Autochthonous "seston rain" of complex organic phosphorus compounds and probably adsorbed inorganic phosphorus was concentrated in the aphotic zone. During summer stratification anaerobic conditions reduced some of the phosphorus to the inorganic free state. A portion of the hypolimnion phosphorus (inorganic or organic) most probably was adsorbed onto the montmorillinite clays present on the bottom and entering from the surrounding watershed and initially

lost. There is ample evidence for such phenomenon in the literature (see Golterman 1974 for summary). At thermocline breakup and remixing of hypolimnion waters with those of the epilimnion, considerable phosphorus might have been made available to photosynthetic organisms. The data showed that some phosphorus was carried to surface waters. However, in view of the presence of reduced iron in the hypolimnion (observed but not actually measured), a significant amount of the inorganic phosphorus would combine with iron in the presence of the added O_2, precipitate to the bottom, and be lost.

Fluctuations in nitrogen levels (TKN and TN-NN) generally coinside with development (TKN) and decomposition (TN-NN) of plankton communities in MR. Low winter TKN levels accompanied by high TN-NN values relate to low ATP and calculated biomass data for this period. There are simply too few organisms present to use the available inorganic nitrogen. In other words, plankton crops were limited by a paucity of some other nutrient, likely phosphorus, as indicated by the low observed concentrations. This was not substantiated by algal assay. Nitrogen was apparently not limiting during winter. Due to the large concentrations of TKN, combined with rapid turnover times for phytoplankton in spring and summer, limitation of nitrogen, in a readily utilizable form, most probably did not occur.

Moss Reservoir is surrounded and underlain by limestone formations. Alkalinity was maintained at relatively constant levels throughout the study. The excellent buffering capacity

of the reservoir allowed only moderate pH changes even though H_2S was noted in the hypolimnion during summer stratification. Hydrogen ion concentration and alkalinity were most constant during winter, when low temperatures and a relative sparse plankton community existed. The general increase in conductivity was due to overall concentration of dissolved materials, as little rainfall dilution occurred. High values for bottom samples were probably the result of increased ionic constituents caused by the reductive conditions during summer stratification.

The thermal and dissolved oxygen regimes observed in MR were typical of southwestern reservoirs with relatively steep walls (protection from wind) and moderate depths (>15 m). Larger Texas reservoirs such as Canyon, Possum Kingdom, and Travis exhibit regimes similar to Moss and typical of summer monomictic, temperate water bodies (personal observation; Fruh and Clay 1973). Heat content of MR (12,898 g cal cm^{-2}) is probably lower than many southwestern reservoirs. Many southwestern reservoirs, though as deep as MR, are severely wind-whipped and do not undergo summer stratification. Lind (1971) reported that Lake Waco (a reservoir) did not stratify in summer. It was his opinion that many Texas reservoirs do not stratify on a yearly basis.

Moss Reservoir appeared to be typical of a summer-stratified, southwestern impoundment. Accurate phosphorus determinations indicated the paucity of this nutrient in the aging reservoir, but did not explain biomass variability. ATP assay was useful

in monitoring seasonal changes in the planktonic biomass, particularly during stratification. Careful attention to methodology yielded precise and accurate data for ATP and phosphorus.

Further studies on MR should include the evaluation of the ratio of particulate organic carbon (POC) to ATP. A more complete evaluation of ATP ℓ^{-1} changes with subtle depth changes is warranted, as is the estimation of ATP at the mud water interface. The feasibility of using ATP assay to measure short term production should be compared to ^{14}C assimilation. Nutrient concentrations should continue to be monitored to determine if reduced allochthonous input resulting from low rainfall produced abnormally low phosphorus levels.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Usefulness of the ATP assay technique was shown to be dependent upon extracted volume and proper heating. Excess quantities of plankton and tripton decreased the efficiency of Tris buffer extraction of ATP. Net sampling of planktonic and triptonic materials was not useful in MR due to the large quantities of material collected. Modification of the ATP extraction procedure yielded more efficient removal of ATP from plankton.

Adenosine triphosphate concentrations differed between surface and bottom samples and between stations. These differences were due to relative abundance and not taxonomic changes. Fractional differences in ATP were observed seasonally and between sampling sites. Fractions B and C were most consistent during the sampling period. Winter bottom ATP values from B and C fractions represent some viable photosynthetic organisms swept downward during complete reservoir mixing. The average euphotic zone depth was 3.7 m, light never being limited at 2.5 m but always limited at 7 and 11.5 m. During summer, stratification and deoxygenation of the hypolimnion coincided with extreme increases in the ATP levels of the D fraction. This drastic increase of ATP in the anaerobic bottom waters suggested a transition from aerobic to anaerobic bacteria.

Comparison of total ATP to total calculated biomass produced poor results. Individual fraction ATP and calculated biomass comparisons revealed variable results. Fraction A (zooplankton) ATP and calculated biomass differences were partly explained by the retention of quantities of filamentous algae in filtered samples. Fractions B and C provided the best comparisons between ATP and calculated biomass.

Analysis of macronutrients (N, P and C) provided interesting insight into the probable mechanism of phytoplankton regulation. The carbon data which were collected were so erratic that they were not used. Nitrogen data were accurate and precise. Inorganic levels of nitrogen were highest when organic nitrogen was lowest. At no time did inorganic nitrogen reach levels low enough to be limiting. Analyses revealed such small quantities of TP that this macronutrient was potentially a limiting factor. Total phosphorus was consistently in the 10-30 $\mu g\ \ell^{-1}$ range with higher values recorded after reservoir turnover in late October. According to Wetzel (1975) MR, with 10-30 $\mu g\ \ell^{-1}$ TP should be considered a meso-eutrophic lake. The reservoir may tend to act as a nutrient sink and sediment trap. Other physical and chemical parameters revealed similar regimes during the two summers of sample collection.

Multiple regression analysis of surface and bottom total ATP with other data provided no significant multiple correlations. Temperature and TKN explained 66 and 32% respectively of the simple variance in surface ATP values. The temperature

correlation was significant at $P < 0.001$. No simple correlations were observed for bottom samples.

Future researchers using ATP for aquatic biomass estimation should consider a number of the findings from this study. Initial evaluation of maximum and minimum sample size for precise and accurate ATP extraction is essential. Use of the modified hot plate-water bath combined technique would be wise in waters containing large concentrations of planktonic and/or triptonic material. Size fractionation of plankton is only a partial answer; ultimately, combined techniques of autoradiography and scanning electron-microscopy need to be used to further the delineation between autotrophic and heterotrophic organisms. My data from ATP analyses suggested that this biomass estimation technique is equal to, or better than conventional means. Classification of the trophic status of lakes and reservoirs by the assay of planktonic ATP has more potential promise than ranking water bodies according to nutrient concentrations or carbon assimilation rates. ATP concentration in plankton cells has not been shown to be as dependent upon metabolic activity or physiological state as has ^{14}C uptake; nor does ATP change in relation to allochthonous input as nutrient levels might.

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