SODIUM CHLORIDE-INDUCED STRUCTURAL AND FUNCTIONAL CHANGES IN MONOALGAL LABORATORY CULTURES AND NATURAL PHYTOPLANKTON ASSEMBLAGES IN RENEWAL AND STATIC BIOASSAYS

By

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CHAPTER I

STATEMENT OF PROBLEM

Surface waters of Oklahoma and other states of the American South and West face water quality degradation due to an increased concentration of dissolved salts. As demand for high quality fresh water increases, more attention will be focused on the mineral pollution of surface waters. Information now exists on the effects of salinity for hypersaline lakes, brackish water communities, and marine systems. This is presently contrasted by an overall lack of information dealing with the salinization of freshwaters. Furthermore, most studies to date have dealt with water quality requirements for "out-of-stream" uses such as agricultural irrigation or varied municipal and industrial needs. The "in-stream" effects of mineral pollution, especially its impacts upon the ecology of the primary trophic level, have generally been neglected (Sorensen et al. 1977). Any alteration in the composition or metabolism of the primary producers, would affect changes in both higher trophic levels, and in the physical and chemical qualities of the impacted systems. This is especially important in large systems, where phytoplanktonic productivity often represents the major input of new organic matter (Wetzel 1983), as phytoplankton fix, by means of photosynthetic pigments, almost all energy used by the entire ecosystem (Sakshaug 1980).

The salinity problem is especially acute in the Colorado River

Basin, where it is estimated that ca. 52% of salt loading comes from irrigated agriculture (Law and Hornsby 1982). Oil shale leachates are also highly saline, containing large quantities of the sodium salts dawsonite [$NaAl(OH)_3$], nahcolite ($NaHCO_3$), and halite (NaCl) (Fransway and Wagenet 1981). The problem will be heightened as water withdrawal for energy development increases, leaving less water available for downstream salt dilution (Law and Hornsby 1982). In 1972, the seven-state Colorado River Basin Salinity Forum was created to consider the problem of salinity, and establish criteria for salinity in the basin (Lawrence and Saunders 1981).

In several Oklahoma reservoirs the salinity problem is also severe. Nine of the State's reservoirs, including Keystone and Texoma are considered to have poor water quality in terms of total dissolved solids (Oklahoma Water Resources Board 1980). Salinity in the Red River (Oklahoma-Texas border) and its major tributaries has nearly eliminated its use for agricultural irrigation and other beneficial uses (Laughlin 1981). Several large scale salinity control projects have been undertaken in Oklahoma (U.S. Army Corps of Engineers 1978).

The problem of excess salinity in surface waters is not restricted to the United States. In Wellington Reservoir in Western Australia, models have predicted that unless action is taken soon, a severe salinity problem will exist in the near future (Imberger 1981).

This study was undertaken to test the following hypotheses: 1) rapid elevation of media NaCl-salinity to concentrations typically found in salinized Oklahoma reservoirs alters primary productivity in unsalinized monoalgal cultures of Anabaena flos-aquae and Selenastrum capricornutum, and in natural phytoplanktonic associations, during

static batch bioassays; 2) NaCl-salinity concentration affects phytoplanktonic metabolism (primary production and pigment concentrations) and community structure in both the laboratory and in situ under continuous culture bioassay conditions; and 3) experimental systems which differed in complexity and in their degree of realism toward the natural environment could be used in an integrated approach to study phytoplankton-salinity interactions.

CHAPTER II

INTRODUCTION

The Salinity of Surface Waters

Salinity is a term by which we understand the degree of saltiness of a particular water (Lyman 1969). In oceanography, salinity refers to the total solids in the water after all carbonates have been converted to oxides and all bromides and iodide have been replaced by chloride, and all organic matter has been oxidized (American Public Health Association et al. 1981). It is usually expressed in parts per thousand (°/₀₀).

For inland waters, the total ionic salinity is for practical purposes the sum of the four major cations and anions respectively: Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}; HCO_3^{-}, CO_3^{2-}, SO_4^{2-}, and Cl^{-} (Wetzel 1983). Freshwater salinities are usually expressed in mg $liter^{-1}$ or milliequivalents $liter^{-1}$.

The salinity of all surface waters is controlled by rock dominance, atmospheric precipitation, and the overall evaporation-precipitation process (Gibbs 1970). In either marine or freshwater systems, salinity is considered an allogenic type environmental factor that limits the distribution and growth of biota, and over which organism have no direct control (Smayda 1980).

Marine Systems

The average total salinity of ocean water is ca. 35 °/oo. Although there is variability in total salinity, excess salinities are uncommon in the oceans (Pearse and Gunter 1957). Sodium chloride constitutes ca. 27 °/oo of the total dissolved solids (ca. 77%), with potassium, magnesium, and calcium salts comprising the remainder (Odum 1971). The solubility of NaCl (natural mineral halite) is 35.7 g per 100 ml water (Weast 1980). In most cases, the relative proportions of these salts are constant, and analysis of the chloride component is usually all that is required for total salinity determination (Odum 1971). Normal sea water has a monovalent:divalent cation ratio of ca. 6.5 (Provasoli et al. 1954), and an alkaline pH of ca. 8.2, due to the greater cation electrical dissociation force (Odum 1971).

Most authors agree that salinity is an important ecological variable in marine environments, especially in inshore areas (McLachlan 1961), although where fluctuations are moderate, salinity may not be a significant regulatory factor in biotic succession (Smayda 1980).

Many classification schemes have been devised to indicate salinity tolerance or preference for various components of the biota. Kolbe (1927) devised such a scheme for diatoms, which is amendable to other algal taxa. He divided diatom algae into four basic groups, as shown below:

Polyhalobiens - Species that can stand a salt concentration greater than normal seawater.

Euhalobiens - Develop best at a salinity of 30-40 °/oo (17-20 °/oo NaCl).

Mesohalobiens - Optimum salinity of 5-20 °/oo (NaCl).

Olgiohalobiens - Prefer very low salt concentration.

Species capable of growth over a wide salinity range (euryhaline) are considered by some authors to be relatively rare (Pearse and Gunter 1957), although empirical data has shown that phytoplankton species collectively can grow over a relatively wide salinity range (Braarud 1951; McLachlan 1961).

Estuarine Systems

Estuarine systems are semi-enclosed coastal water bodies with free connection to the open sea, and include coastal bays, tidal marshes, and river mouths (Odum 1971). In these ecotonal systems, salinity constitutes a striking example of an environmental factor which limits the population growth and distribution of organisms (Reid and Wood 1976). Most estuarine waters are classified as mixohaline, varying in salinity from 0.5 to 30 °/°°, and contain a unique association of marine, fresh-water, and some euryhaline organisms.

In highly stratified or "salt-wedge" type estuaries, more dense salt water forms a "wedge" below the freshwater overflow, with development of a halocline (Pritchard 1952; Odum 1971). Some hypersaline estuaries such as the Laguna Madre of Texas commonly have 50-80 °/°° salinity, and under certain conditions can reach 104 °/°° (Pearse and Gunter 1957). Conover (1964) reports that most higher plants remained sterile where summer salinities exceeded 65 °/°° in such habitats, and that only blue-green algae could grow epiphytically at such high salinities. Sanders (1979) reports similar results for algal mats growing on the North Carolina coast, where salinity stress effectively reduced species diversity and the blue-green alga Lyngbya dominated the intertidal zone.

In other estuarine research, Paasche (1975) determined that the distribution of brackish-water diatoms was largely governed by a physiological intolerance to high and low salt concentrations. Vosjan and Siezen (1968) report the marine alga Chlamydomonas uva-maris withstood great salinity variations (10-100 °/₀₀), whereas the fresh water green alga Scenedesmus obliquus showed optimum growth at the lowest possible salinity. Phytoplankton biomass variations in the Lower Hudson Estuary were primarily the result of material fluxes due to estuarine circulation (including salinity) and biological and particulate processes (Neale et al. 1981). More detailed information for estuarine systems is provided by Remane and Schlieper (1971) and Reid and Wood (1976).

Inland Saline Lakes

Inland saline lakes comprise 0.008% of the earth's water, as compared to 0.009% for freshwater lakes. Despite this near equality, few extensive studies have been made on saline lakes (Williams 1981). Saline lakes form when either evaporation exceeds inflow or by inflow being saline or both (Eugster and Hardie 1978).

The salt content and type in these lakes is an expression of the climatic and physiographic factors responsible for their formation (Rawson and Moore 1944), and presents on a worldwide basis a bewildering variety of compositions and concentration ranges (Eugster and Hardie 1978). However, most saline lakes occurring in arid locales are dominanted by Na^+ and Cl^-(Wetzel 1983). The chemical limnology of these systems deals especially with evaporation, concentration, precipitation of compounds, and the relative changes in ionic abundance (Cole 1968).

Eugster and Hardie (1983) provide an extensive review of the physical and chemical processes of saline lakes.

Although inland saline waters have been regarded by many as simply extensions of coastal brackish waters, their chemical and especially biological natures are more aligned with inland fresh waters (Beadle 1959; Williams 1972). Generally, the diversity of species is inversely correlated with salinity (Cole 1983), as a few kinds of tolerant organisms occur in large numbers (Bradbury 1971). Periphytic and planktonic diatom and blue-green algae (such as Chaetoceros, Spirulina, Nodularia, and Anabaena) dominate primary production, as macrophytes are almost entirely absent (Williams 1972). Exceptions are the branched green alga Ctenocladus circinnatus, and the green flagellate Dunaliella, the latter thriving in water saturated with NaCl (Cole 1983). Partially because of the lack of rooted littoral vegetation, and decreased habitat heterogeneity, saline lakes have reduced species diversity and simplified trophic structure (Williams 1972). However, despite the extreme physiologic stress on the primary producers of saline lakes, some extraordinarily high production rates have been recorded (Goldman et al. 1967; Edmondson 1969), and are much greater than peak productivities for eutrophic fresh waters (Westlake 1963).

Borax Lake, California, is a saline lake that has been studied in detail (Wetzel 1983). Because of its shallow depth, ca. 70% of the total organic carbon fixed in the lake was attributable to the periphyton (Wetzel 1964).

Rawson and Moore (1944) studied saline (total dissolved solids = 10,000-118,000 mg liter^{-1}:Na_2SO_4 dominated) eutrophic lakes in Saskatchewan, Canada. They report that in over 60 lakes, most

phytoplankton associations were dominated by euryhaline species of blue-green algae.

More detailed reviews of saline lakes are provided by Cole (1968), International Association of Limnology (1969), Walker (1973), and Williams (1981).

Freshwater Systems

Over large regions of the temperate zone, the general dominance relationships for the major ions that comprise the total ionic salinity of freshwater are:

$$\text{Cations: } Ca > Mg \geq Na > K$$

$$\text{Anions: } CO_3 > SO_4 > Cl$$

Ions are supplied to fresh waters by the natural weathering of soil and rock, and from the atmosphere. Important processes involved in the weathering of materials include solution, oxidation and reduction, hydrogen ion activity, and complex formation, whereas dry and wet precipitation dominate atmospheric inputs (Gorham 1961). An increasing percentage of ions are now delivered to fresh waters from pollution sources in the watershed and from atmospheric sources.

Wetzel (1983) has classified the major ions of freshwater into two groups. Conservative ions are those that undergo relatively small biotically-induced changes, and generally includes Mg^{2+}, Na^{+}, K^{+}, and Cl^{-}. Dynamic ions are those strongly influenced by metabolism, and includes Ca^{2+}, SO_4^{2-}, and the inorganic carbon species.

The specific salinization pathway followed for a given body of fresh water is site specific, being dependent upon many variables. However, Schoeller (1961) has generalized three different stages of

water salinization, as summarized below:

Stage	Anions	Cations
1	$HCO_3 > Cl > SO_4$	$Ca > Mg > Na$
	or $CO_3 > SO_4 > Cl$	
2	$Cl > SO_4 > HCO_3$	$Ca > Na > Mg$
	$SO_4 > Cl > HCO_3$	
3	$Cl > SO_4 > HCO_3$	$Na > Ca > Mg$
		and then $Na > Mg > Ca$

This succession is governed by the relative solubilities of the compounds, with the carbonates of alkaline earth metals precipitating first ($CaCO_3$, $MgCO_3$), followed by $CaSO_4$ and $MgSO_4$. This process leading to the development of sodium chloride salterns is fairly common in North America (Cole 1983).

In large river-reservoir systems that dominate the Western United States, salinity increases are resulting from two basic causes, as outlined by Blackman et al. (1973). Salt loading effects are caused by discharge of additional mineral salts into stream systems, and include contributions from both natural and anthropogenic sources. Natural sources encompass point discharges such as saline mineral springs or seeps, and diffuse discharges such as salt accretions from large drainage areas. Anthropogenic salt loading involves the discharge of brines or brackish waters brought to the surface by oil and gas operations [old oil fields may produce 100 times more salt water that oil: (U.S. Public Health Service 1964)], municipal and industrial discharge, leaching of spent oil shale and soil overburden (Cleave et al. 1980), and irrigation return flows.

The second major reason that rivers and reservoirs are becoming saltier is due to salt concentrating effects (Blackman et al. 1973). These occur as a result of consumptive loss of water from the stream system, and include municipal and industrial withdrawals, evaporation, and irrigation withdrawal when seepage does not return to the stream. Removal of water above a salinity source diminishes the amount of water available for dilution (Blackman et al. 1973).

Cleave et al. (1979, 1980) studied the effects of saline oil shale leachates on phytoplankton productivity and species composition in the Colorado River basin. Results from batch bioassays indicated that increases of salinity in the Colorado River could favor presently uncommon Cyanophytes, and stimulate the productivity of Scenedesmus species. Further toxicity testing showed that a Synedra species indigenous to Lake Powell (Colorado River reservoir in northern Arizona and southern Utah) was more tolerant to salinity addition than the standard test alga Selenastrum capricornutum (Cleave et al. 1981).

The relationship between the growth and composition of a phytoplankton association and dissolved solids content of water was first studied by Pearsall (1922, 1932) in English lakes. He found that desmid algae characterized waters with a high (ca. 3.2) monovalent: divalent cation ratio (Na + K:Ca + Mg), whereas diatoms occurred in waters having a lower ratio (ca. 1.1) and high silicon and nitrate-nitrogen concentrations. Several studies report that the monovalent:divalent cation ratio did not affect algal growth (Moss 1972; Batchelder 1981).

Sze and Kingsbury (1972) reported a marine trend in seasonal succession for saline Onodaga Lake, New York. Despite high salinity

(ca. 3000-6000 µmhos) caused by industrial discharge and extensive NaCl deposits, this lake did not show a limited phytoplankton flora, but rather a large number of species and a regular succession (Sze and Kingsbury 1974).

Not all salinity increases favor blue-green algal development. In the lower Chowan River in northeastern North Carolina, Paerl (1982) found that salinity increases (from ca. 0.2°/°° to 2.0°/°°) were a strong deterrent to blue-green algal bloom formation, even when other environmental conditions proved conducive to blooms.

Of 134 shallow prairie pothole lakes in southwestern Manitoba, Canada, those in the 300-3000 µmhos salinity range were the most productive, with ca. 37% developing heavy Aphanizomenon blooms (Barica 1978).

In Rajashan, India, Gupta (1972) found luxuriant populations of the blue-green algae Anabaenopsis and Arthrospira inhabiting the high salt-high pH waters of Sambhler Lake. Blinn et al. (1981) found phytoplankton species preference for various salinity regimes for a small creek system in Arizona, with low algal densities (mostly diatoms) attributed to low residence times, restricted light, and an ionic gradient.

In a field study on the effects of NaCl additions to a small stream in Ontario, Canada, Dickman and Gochnauer (1978) found decreased algal diversity on artificial substrate samplers after 28 days at a 1000 mg liter^{-1} station.

Salinity and Algal Physiology

Introduction. Osmoregulation in algae functions to maintain a different concentration of dissolved solids inside and outside the cell at physiological concentrations that allow for normal operation (Wetzel 1983). The dynamics of water movement into and out of algal cells is given by the equation:

$$\psi = \psi_p + \psi_\pi + \psi_m$$

[ψ = water potential, ψ_p = pressure potential or turgor pressure, ψ_π = osmotic potential, and ψ_m = matric potential (Fogg et al. 1973)]. Many freshwater algae have an osmotic pressure of ca. 0.5 megapascals (MPa), and marine forms have a pressure of ca. 1.0 MPa above the local seawater, which averages ca. 3.0 MPa (Blinks 1951). Dissolved solids decrease the osmotic potential of algal cell sap, thereby decreasing its water potential. As algae accumulate ions to concentrations higher than the surrounding medium, a water potential gradient develops, and causes a net increase of water in the cells via osmosis (Lee 1980). In some freshwater flagellates, this water imbalance is corrected by contractile vacuoles, which expel water and solutes to the outside of the cell. For algae inhabiting salty water, solute accumulation is an adaptive mechanism to increase their internal salt concentrations and prevent plasmolysis (Salisbury and Ross 1978). Algal cells that face a fluctuating salinity regime need a mechanism for both maintaining turgidity when the environmental solution has a greater osmotic pressure than the cell sap (Blinks 1951), and for preventing the cell from bursting when in either a hypotonic or isotonic environment (Raven 1976;

Weier et al. 1982). The salinity range occupied by species populations, and ultimately the structure and function of the algal association as a whole, depends on the efficiency of the physiological mechanisms by which individual cells are adapted to changes in environmental salinity (Wetzel 1983).

Most information concerning ion transport and hence osmoregulation in algae results from work done on the giant cells of coencytic algae such as Halicystis, Hydrodictyon, and certain Charaphytes, or work on unicellular, essentially non-vacuolate algae such as Euglena and Chlorella.

In all algae, organic and inorganic solutes are maintained within a specific range (Raven 1976). This implies control of the passage of materials across the differentially permeable membranes of the cell, the plasmalemma (cell membrane) and the tonoplast (vacuolar membrane). Materials can be actively taken across either membrane, exchanged, or diffuse passively into the cell. The partitioning of material fluxes into these components is exceedingly complex (Raven 1976).

Although both organic and inorganic solutes contribute to osmoregulation, only the inorganic ions will be considered here. Inorganic ions for this discussion are classified into those that are metabolically incorporated inside the algal cell, and those that function mainly in an osmoregulatory role. The first group includes the nutrient anions PO_4^{3-}, SO_4^{2-}, NO_3^-, and HCO_3^-, and the cations NH_4^+, and H^+. For the nutrient anions PO_4^{3-} and SO_4^{2-} supplied to cells at ecologically realistic concentrations, net influx is very large compared to efflux (Raven 1976). The second group is the primary interest of this discussion, and contains K^+, Na^+, Ca^{2+}, Mg^{2+}, and Cl^-. These

solutes represent the major osmoregulatory ions in algae, and are responsible for the higher osmotic pressures related to the maintenance of isotonicity (osmosis equilibrium), turgor generation, and the storage of nutrients or energy (Raven 1976).

Except for Mg^{2+}, which is in the chlorophyll molecule, these ions are not needed for metabolism per se (Raven 1976) (several other exceptions to this general rule will be discussed in more detail later). Since this study dealt with NaCl-salinity, I will briefly review the transport of Cl^- and the monovalent cations Na^+ and K^+.

Chloride Ion. Anions are in general actively transported into algal cells (Raven 1976). Chloride ion uptake is not an exception to this rule, as it appears to be actively taken up as an essential ion in all photosynthetic microorganisms (O'Kelley 1974; Lüttge and Higinbotham 1979), although exceptions have been reported (Raven 1976).

In most algae, the active nature of Cl^- transport at the plasmalemma and tonoplast in an inward direction causes a higher electrochemical potential for Cl^- in the cytoplasm and vacuole as opposed to the external environment (Raven 1976). Chloride fluxes are generally lower in freshwater species and higher in marine species. A "pump and leak" situation probably exists for Cl^-, with a net influx of Cl^- in the light, and a smaller net influx, or a net efflux, in the dark (Raven 1976). Kylin (1967) found Cl^- uptake in P-starved cells of Scenedesmus in the light to be inhibited by the re-addition of PO_4^{3-}. In Chlorella, energy for the Cl^- "pump" appears to be ATP from oxidative phosphorylation (in the dark) or cyclic photophosphorylation (in the light) (Raven 1976).

Monovalent cations. Most electrochemical data suggest active Na^+

extrusion and active K^+ influx occur at the plasmalemma, and that active Na^+ influx at the tonoplast may also be common (Raven 1976). This preferential accumulation of K^+ over Na^+ is particularly clear in marine algae, which live in a high Na^+ environment, but do not contain more Na^+ than freshwater species and higher plants (Lüttge and Higinbotham 1979). Sodium slowly but continuously diffuses into the cell (Salisbury and Ross 1978), and must be "pumped" out due to its dangerous effects in high concentrations on cytoplasmic enzymes and proteins (Lüttge and Higinbotham 1979). In some species, Na^+ efflux may be coupled to K^+ influx or H^+ efflux, and can be light-stimulated (Raven 1976).

The role the Na^+ "pump" plays during algal growth is not clear. There is some evidence to suggest that Na^+ efflux may be linked to the influx of phosphorus as PO_4^{3-} (Raven 1976), a known limiting nutrient in many aquatic systems.

The role of Na^+ as an essential nutrient for algal growth has received considerable attention. Allen (1952) reported that some blue-green algae required Na^+ for growth, while other species were indifferent to either Na^+ or K^+. This interchangable relationship between Na^+ and K^+ had been reported for Oscillatoria much earlier (Benecke 1898). Emerson and Lewis (1942) found little or no growth for the coccoid blue-green alga Chroococcus if Na^+ was omitted from the medium. Allen and Arnon (1955) showed a specific Na^+ requirement for Anabaena cylindrica, with 5 ppm required for optimal growth. Clear-cut Na^+ and K^+ requirements were also found for Anabaena variabilis and Anacystis nidulans (Kratz and Meyers 1955). Some members of the Chlorophyceae may also require Na^+ (Eyster 1962).

Sodium apparently plays a role in the nitrogen fixation process by blue-green algae, where Brownwell and Nicholas (1967) believe that it is required in the conversion process of molecular nitrogen to ammonia. Ward and Wetzel (1975) found decreased rates of acetylene reduction for Na^+ deficient cultures of Anabaena cylindrica Lemm. Based on such evidence, some researchers have concluded that Na^+ is a required nutrient for all members of the Cyanophyceae (Allen and Arnon 1955). Yet the relationship between Na^+ levels in excess of any supposed minimum required concentration and blue-green algal halotolerance at high or fluctuating salinities is not clear. Batterton and Van Baalan (1971) have reported that growth inhibition at high NaCl concentrations is apparently due more to ionic stress than osmotic stress. Wetzel (1983) notes that blue-green algae adapt readily to increasing salinity by means of genetic change and that this characteristic is shared by most freshwater bacteria. This evolutionary euryhalinity (Hutchinson 1967) may be due in part to the inherent capacity of the blue-green algal cells to extrude Na^+ (Batterton and Van Baalan 1971). It is also known that blue-green algae do not possess large sap vacuoles, and hence the vacuolar sap, which is often a major determinant of osmotic pressure, is absent. However, a considerable part of the osmotic pressure in blue-green algal cells is contributed by soluble products of photosynthesis (Fogg et al. 1973), which might also increase their ability to tolerate elevated or fluctuating external salinity.

Osmotic stress. In general, slow changes in osmotic pressure are tolerated much more readily than quick ones, although some algae do not show the usual plasmolytic response upon sudden environmental salinity change (Blinks 1951). Wetherell (1961) tested 13 species of freshwater

unicellular green algae for salinity tolerance and osmotic shock. After initial culture at seawater salinity (31 °/oo), cultures were transferred to media of both higher and lower salinity and growth was measured after 5-12 days. Wetherell found Chlamydomonas and Coelastrum species strongly inhibited at regular seawater salinity, some Chlorella species tolerated full strength sea water, and only Chlamydomonas eugamentos showed growth at the higher salinity. Wetherell (1961) concluded that adaptation to the physiological stress of high salinity is not readily induced by preliminary culture at sub-limiting salinity levels.

Brown (1982) found that osmotic shock in the marine unicellular alga Nannochloris bacillaris Naumann, caused by transferring cells from 200% to 7% artificial sea water, had little effect on growth. Equal shocks in an upward direction caused long lag phases in growth, totally inhibited photosynthesis, and often led to cell death.

Salinity and the Oklahoma River-Reservoir System

Oklahoma is a diverse state in terms of climate, water resources, and water quality. It is considered an ecotonal region between the humid east and the semi-arid west. Average rainfall decreases sharply from east to west, with 142 cm annually in the southeastern corner of the State, to 38 cm in the western Panhandle (Oklahoma Water Resources Board 1980). Long hot summers and short winters result in a mean annual temperature range of 14-18 °C, with annual lake evaporation averaging ca. 122 cm in the east and 165 cm in the southwest corner.

The Arkansas River in the north and the Red River in the south comprise the two major drainage systems in Oklahoma. Most of the 50 major reservoirs are located in the eastern one-half of the State, and

are designated multi-purpose projects (Oklahoma Water Resources Board 1980). The only natural water bodies in the State occur as playa lakes in the west and oxbow lakes in the southeast (Penfound 1953). Water quality in the southeastern portion of Oklahoma is generally good, with most waters low in mineralization (Oklahoma Water Resources Board 1980).

Both major river systems in Oklahoma have documented salinity problems (U.S. Public Health Service 1964; Oklahoma Water Resources Research Institute 1980; Oklahoma Water Resources Board 1980). Although impoundment has the effect of reducing dissolved mineral concentrations, relatively high concentrations can persist and flucuate in both time and space.

In northern Oklahoma, the Arkansas River receives highly saline water from its Cimmaron River and Salt Creek tributaries. The Cimmaron River is one of the most highly mineralized streams in the State, draining areas containing large amounts of soluble salts (NaCl, Na_2SO_4, $MgCl_2$, $CaCl_2$). Certain stretches of the Cimmaron River have a mean annual chloride concentrations of 34,000 mg $liter^{-1}$ (U.S. Army Corps of Engineers 1978).

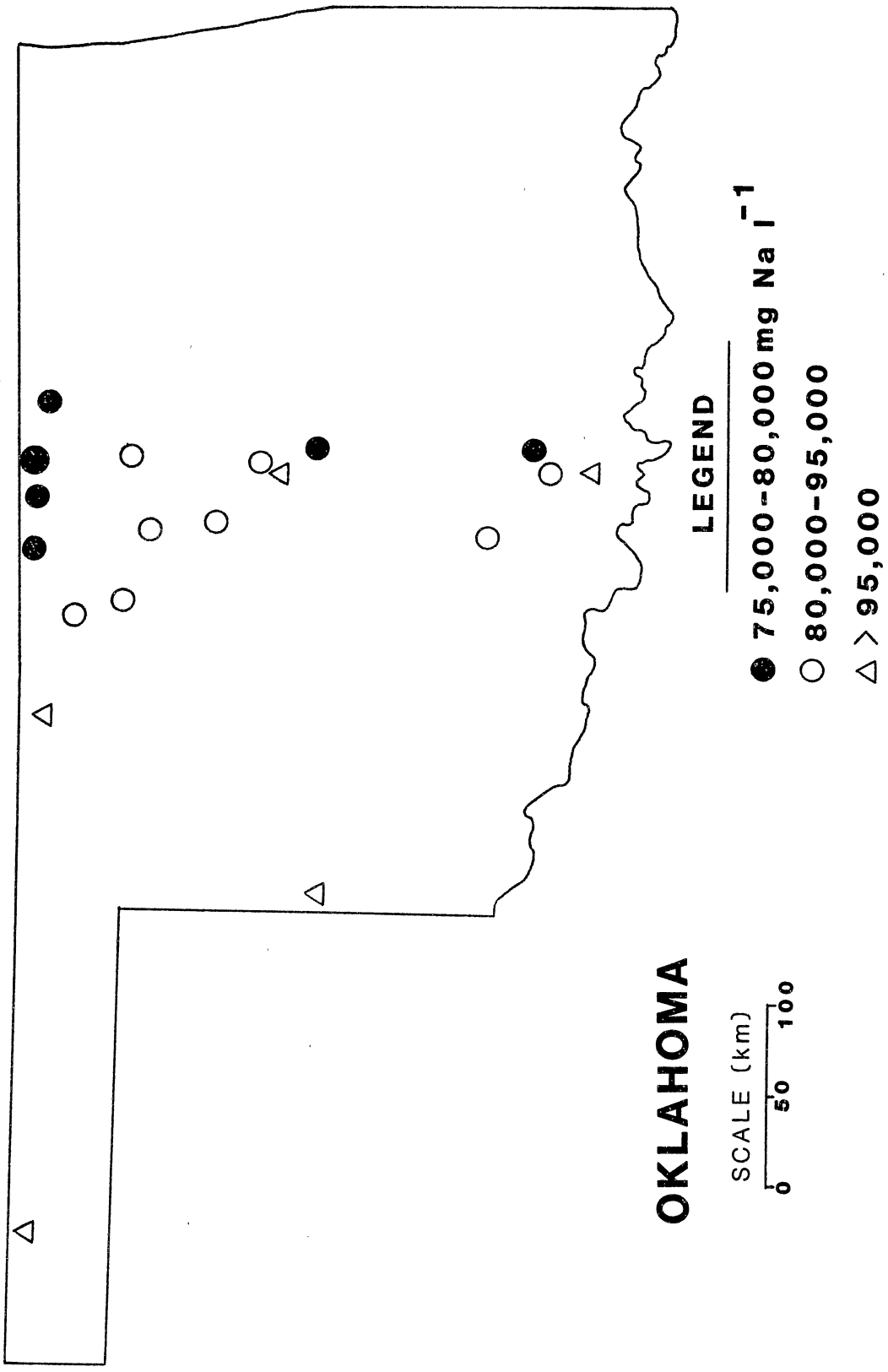
Keystone Reservoir, at the confluence of the Arkansas and Cimmaron Rivers receives ca. 10,800 metric tons of salt per day (Oklahoma Water Resources Research Institute 1980). Salinity in the surface waters of the Cimmaron Arm of Keystone Reservoir ranges from ca. 2000 to 4000 µmhos. During a period of epilimnetic withdrawal, a build-up of highly mineralized Cimmaron River water in the hypolimnion temporarily produced meromictic conditions in Keystone Reservoir, with a chemocline located at ca. 12 m, and an 8000 µmhos increase from the top to bottom of the

monimolimnion (Eley 1970).

The Red River (Oklahoma-Texas border) drains ca. 242,000 km^2, with ca. 90% of the drainage area being downstream from major salt sources (U.S. Army Corps of Engineers 1976). Texoma Reservoir, a large impoundment located in the middle of the drainage area, has an average of 3000 metric tons of chlorides enter it every day. In 1976, ca. one-third of the total chlorides were from controlable oil field activities, with the remaining two-thirds from natural sources (brine springs, seeps, and salt plains) in the Wichita and Red River drainages (U.S. Army Corps of Engineers 1976). The 1976 cost estimate for control of the natural sources was ca. 124 million dollars, with an annual cost of ca. 8 million. The salinity control projects are elaborate engineering schemes used to divert usable and uncontaminated water around identified salt flats and natural brine sources by means of dikes, dams, and retention reservoirs (Oklahoma Water Resources Board 1980).

Oklahoma groundwater can also be highly mineralized. Figure 1 shows the location of brines containing high concentrations of sodium. This is important during low stages when stream flow may be largely maintained by more concentrated groundwater inflow. For example, stream water sampled in Harmon County in southwestern Oklahoma during low flow periods averaged 119,100 mg $Cl^{-}liter^{-1}$, and 366,000 mg $liter^{-1}$ total dissolved solids (Pettyjohn et al. 1983). There is generally an inverse relationship between stream discharge and dissolved solids concentration, although exceptions can occur in large streams whose drainage covers several types of soils and rocks (Oklahoma Planning and Resources Board 1945).

Figure 1. Approximate geographic locations of brines containing high concentrations of sodium in Oklahoma. Adapted from Collins (1974).


OKLAHOMA
LEGEND
75,000-80,000 mg Na l-1
80,000-95,000
> 95,000
SCALE (km)
0
50
100

The biological consequences of elevated or fluctuating salinity have been inadequately addressed in Oklahoma. Of the available studies, few deal with effects on the primary trophic level. For example, Clemens and Finnell (1957) found conductivities of 4,800 to 42,100 μmhos in Salt Creek, a brine-polluted (from oil fields) tributary to the Little Washita River. They report an inverse relationship between the number of animals present and the stream chloride concentration. Jenkins (1976) used total dissolved solids as one of the characteristics of Oklahoma reservoirs to predict fish production. Koschsiek et al. (1971) reported a negative correlation between species diversity of net zooplankton in Keystone Reservoir and conductivity. In the Arkansas River below Keystone dam, a rich diversity of algae with a variety of salinity preferences were reported (U.S. Army Corps of Engineers 1977). On Texoma Reservoir, Hubbs et al. (1976) showed that annual stratification began with a salinity barrier restricted to the old river valley, and that the halocline was reinforced by the thermocline. Baglin (1972) reported higher diversity and increased numbers of surface phytoplankton correlated with areas of Texoma Reservoir highest in salinity. He found that dinoflagellate and euglenoid algae preferred areas highest in salinity and turbidity.

Madden and Morris (1978) found higher dissolved solids concentrations in the Washita River after discharge from a demineralization plant began in 1975. Rice (1974) related cation concentrations in an unmineralized Arkansas reservoir to phytoplankton succession. Other general sources of information on Oklahoma phytoplankton are the Environmental Protection Agency (1979) and Pfiester et al. (1980).

Sangre Isle Reservoir

The field study site for this project was Sangre Isle Reservoir (SIR), a small eutrophic impoundment 8 km southwest of Stillwater, Oklahoma in Payne County (SE1/4 Sec29 T19N R2E) (Figure 2). It was chosen because of its close proximity to the Oklahoma State University main campus, and because of its relatively low salinity. Soils in the region belong to the Zaneis-Stonebury-Renfrow association, being composed of mostly clay loams (U.S. Soil Conservation Service 1978). Most of the watershed is used for suburban residential housing.

Salinity of the SIR surface water ranges from ca. 150 to 300 μmhos (25 °C), with Ca^{2+} being the dominant cation, followed by Na^{+}, Mg^{2+}, and K^{+}. The monovalent:divalent cation ratio during the summer of 1983 was ca. 0.55 (Table 1). Francko (1983) presents data on the occurrence of dissolved regulatory molecules in SIR.

Sangre Isle Reservoir occupies the opposite end of the salinity spectrum for Oklahoma surface waters relative to the larger river-reservoir systems previously discussed (Keystone and Texoma Reservoirs). Therefore, the response of natural SIR phytoplanktonic assemblages to experimental manipulation is not directly applicable to the presently more salinized systems as a whole. However, since the salinity fluctuations caused by saline inflows and circulation patterns within larger systems can bring phytoplankton in low salinity waters in contact with higher and more variable salinity, a guarded analogy is realistic.

Figure 2. Morphometric map of Sangre Isle Reservoir, Payne County, Oklahoma. The depth contours are in meters, as adapted from the original map done in feet by Dr. T.C. Dorris, Department of Zoology, Oklahoma State University. (● = Station 1; ▲ = Chemostat test site).

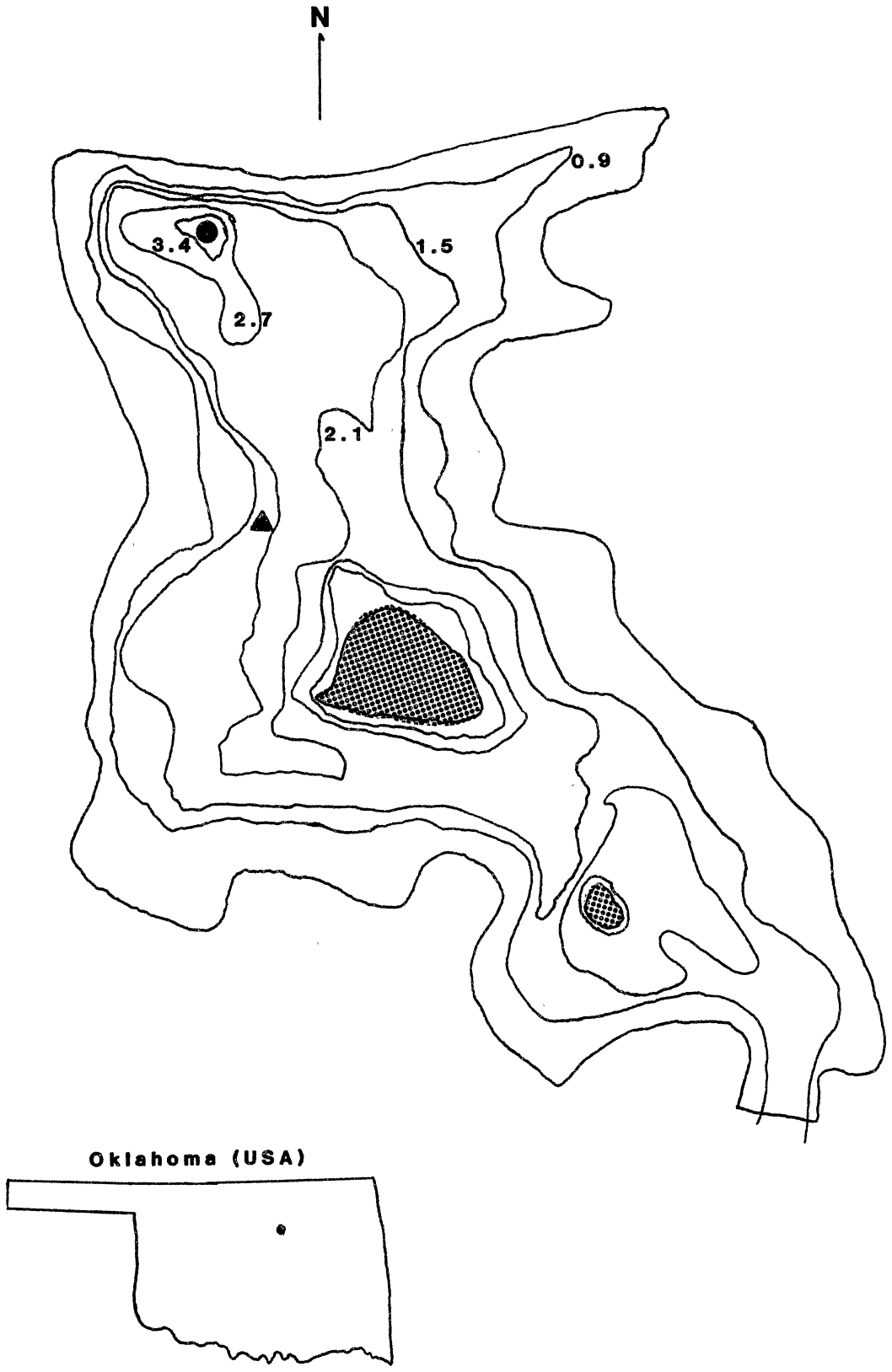

N
0.9
1.5
3.4
2.7
2.1
Oklahoma (USA)

Table 1. Major cation concentrations for the surface water of Sangre Isle Reservoir, Payne County, Oklahoma.

Date	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	$\frac{Na^{+} + K^{+a}}{Ca^{2+} + Mg^{2+}}$
6- 3-83	22.4	7.2	11.0	5.2	0.55
7-29-83	23.1	9.2	12.1	5.9	0.56

Analysis was done by the Oklahoma State University Water Quality Research Laboratory, using a Perkin Elmer 5000 Atomic Absorption Spectrophotometer.

All values are in mg $liter^{-1}$.

[a] Monovalent:divalent cation ratio.

CHAPTER III

STATIC BATCH BIOASSAYS

Introduction

A batch culture is a closed system containing a limited volume of medium with necessary nutrients added, and into which is inoculated a relatively small number of cells (Fogg 1975). Growth in such a system follows a characteristic pattern. A lag phase of variable duration is followed by an exponential growth phase. During the latter, cells generally show carbon metabolism similar to those in continuous culture (Sharp et al. 1980), and average cell properties during exponential phase remain relatively constant (Rhee 1980). At some point, a stationary phase is induced by either nutrient limitation and/or accumulation of inhibitory products (Rhee 1980). The final stage in the growth cycle is a death phase.

The basic advantage of this experimental approach is its simplicity. Static or non-renewal batch bioassays using numerous representative algal species (Anabaena, Anacystis, Chlorella, Selenastrum, etc.) have been employed extensively to research physiological function (Lewin 1962), nutrient limitation and eutrophication (Environmental Protection Agency 1971, 1978, 1979), and toxicity (Patrick 1964; Walsh and Merril 1982). General methods for the culture of algae have been given by Stein (1973) and Fogg (1975). Batchelder (1981) and Cleave et al. (1981) have employed this technique to study

the influence of numerous cations upon algal growth.

Short-term static bioassays have often been applied to natural planktonic associations in situ. One of the most common and useful techniques has been to use the light and dark bottle method (either O_2-evolution or ^{14}C-assimilation) to estimate phytoplanktonic photosynthesis (Vollenweider 1969). Phytoplankton are amendable to study in enclosures of various types because of their size and life histories. However, the conditions experienced by phytoplankton cells within an experimental vessel deviate from those unenclosed (Talling 1969), but negative effects can be minimized by short incubation periods of ca. 4-6 hours (Vollenweider 1969). In situ primary productivity incubation bioassays have been used to test for nitrogen and phosphorus limitation (Gerhart and Likens 1975; O'Brien and deNoyelles 1976), and to study the effects of Na^+ on blue-green algal growth (Ward and Wetzel 1975).

Methodological Approach

To test the response of single algal species under defined laboratory culture conditions, and of natural planktonic associations in situ to elevated NaCl-salinity, static batch bioassays were conducted. Additionally, these simpler studies were implemented to yield preliminary data for consideration in designing the continuous culture bioassays, and to provide a basis for comparison between the simpler and more complex methodologies employed in this study.

Materials and Methods

In situ Primary Productivity Bioassays

Primary productivity. Samples for productivity analyses were collected from Sangre Isle Reservoir (SIR) at Station 1 (Figure 2) in January and June through October of 1983 from a depth of 0.1 m using a 2-liter plastic Van Dorn water sampler. Bottles (125-ml Pyrex ground-glass stoppered) were filled and placed in a dark box, where solutions of 5N NaCl and $NaH^{14}CO_3$ (100 µl injection = ca. 1 µCi) were added. All bottles were then tightly sealed, mixed by repeated inversion, and incubated in situ at the 0.1-m depth using a productivity bottle rack (Korstad 1983). The top and neck of dark bottles were wrapped in aluminum foil to exclude light. Incubation periods ranged from 3.6 to 4.4 h and bracketed 1200 h. Bottles were retrieved and filtered (30 ml) in semi-darkness within 1 h through 0.45µm Millipore membrane filters at a vacuum not exceeding 200 mm Hg. Filters were placed in scintillation vials and frozen. The next day, 15 ml of Budget-Solve liquid scintillation cocktail was added to each vial, followed by radioassay on a Beckman LS-3100 Series liquid scintillation counter (Schindler and Holmgren 1971: Wetzel and Likens 1979). Each filter was counted for 10 minutes on at least 2 occasions, with the mean was used for calculations. Estimates represent a mean of 2-4 light bottles for all dates except 17 January and 14 July.

Physicochemical variables. Total alkalinity was determined by acid titration to a pH of ca. 4.6 using the method of Wetzel and Likens (1979). All pH measurements were made on an Orion Research Model 701A pH meter.

Salinity pertubations were accomplished by the addition of appropriate volumes of 5N NaCl to the treatment bottles. Salinity increases of ca. 200-10,000 µmhos simulated naturally-occurring salinity increases, and were determined following incubation using the electrical conductivity method (American Public Health Association et al. 1981). Two to 5 salinity treatment levels within the stated range were used. A Markson Electromark Analyzer was used for all conductivity measurements, and a stirred water bath maintained a constant temperature (25 °C) for all samples.

Dissolved oxygen was determined using the azide modification to the Winkler method as given by the American Public Health Association et al. (1981). Temperature of SIR surface water was measured with a Yellow Springs Instrument Co. Model 43TD Telethermometer. All turbidity measurements were taken in the laboratory on a Hach Model 16800 nephelometer as described by Nolen (1983) and Nolen et al. (1984). Secchi depth was measured according to Lind (1979).

Phytoplankton enumeration and analysis. Samples for phytoplankton enumeration were taken from the same water sample used for productivity and pigment determinations. Small volume (10 ml) samples were preserved with 2 drops of Lugol's solution (Wetzel and Likens 1979). Some concentrated samples were also prepared by settling 120 ml of sample water with 4 ml Lugol's solution in a graduated cylinder. After ca. 1 month, the supernatant was decanted and a concentration factor recorded. Identification was to the genus level using keys of Smith (1942), Whitford and Schumacher (1973), and Prescott (1978). The scientific names presented are those listed in Prescott (1978). Quantification was by direct counting of 30-40 randomly selected fields using a Palmer-

Maloney counting chamber and a Nikon Model S-kt binocular light microscope. For filamentous algae, the average length of a filament was determined for each sample (Wetzel and Likens 1979). This was necessary since individual cells of Oscillatoria species could not be differentiated using our techniques. Species diversity ($\bar{d}$) was calculated using the Shannon index (Shannon and Weaver 1963; Pielou 1975) based on the number of cells ml^{-1} for each genus encountered ($\bar{d}_n$).

Algal pigments. Chlorophyll a was determined as an estimate of algal biomass present in SIR, using the method of Wetzel and Likens (1979). Chlorophyll a was chosen because of its importance as a photosynthetic pigment, and its practicality as a biomass estimate for natural populations (see Senft 1978). Volumes (25-100 ml) of 0.1-m depth water were filtered onto Whatman 934-AH glass fiber filters and frozen. Three filters for each date were extracted in 90% (vol/vol) alkaline acetone and fluorescence was measured on a Turner Model 111 fluorometer. Sample fluorescence values were compared to a chlorophyll a standard curve, prepared by serial dilution of a 1000 µg $liter^{-1}$ stock solution. The stock solution was prepared with pure chlorophyll a extracted from Anacystis nidulans (Sigma Chemical Co., St. Louis, MO), and dissolved in 90% alkaline acetone. Wratten filters No. 47B and No. 25 were used as the activation and emission filter respectively. All estimates were corrected for phaeopigments by acidification with 2 drops of 4N HCl, with remeasurment of fluorescence after 2 minutes.

A methodological shortfall of the fluorometric procedure is that it does not distinguish chlorophyll a from chlorophyllide a (chlorophyll a minus phytol) (Jeffery 1974). This implies an overestimation of chlorophyll a and an underestimation of degradation products (Round

1981). Jeffery (1974) also warns that grossly exaggerated phaeopigment values will result when excessive chlorophyll b is present.

Statistical analysis. Statistical analysis of productivity data was accomplished using Statistical Analysis Systems, Inc. (SAS) computer programs. Analysis of variance tests were used to indicate if differences among sample means were large enough to imply that the corresponding population means differed. Duncan's multiple range test was used as a mean separation technique, with a significance level of 0.05.

Laboratory Batch Bioassays

Batch bioassays were conducted in the laboratory using a filamentous Cyanophyte (Anabaena flos-aquae; GR stain, originally isolated from Lake Erie by G-Yull Rhee) and a Chlorophyte (Selenastrum capricornutum Printz; UTEX 1948). A blue-green alga was chosen because of their importance in eutrophic waters (Wetzel 1983) and their reported halotolerance (Fogg et al. 1973). Selenastrum capricornutum Printz is a small unicellular alga that belongs to a group of ubiquitous algae with a wide tolerance to environmental conditions (Rodhe 1978). Selenastrum capricornutum has been a widely used test organism in static laboratory bioassays (Environmental Protection Agency 1978, 1979).

Methods common to both the Anabaena and Selenastrum bioassays include the use of 250 ml of autoclaved Moss media (Moss 1972) in 500-ml Pyrex Erlenmeyer flasks. Cell densities were estimated for both species by measuring optical density (OD) at 600 nm on a Spectronic 20, and relating sample OD to a OD - cell number plot prepared by serial dilution. Treatment cultures for both bioassays were amended with a

sterile 5N NaCl solution.

For the 112-h Anabaena bioassay, replicated (n=2) treatment levels of 701 (T1), 2337 (T2), and 4675 (T3) mg NaCl l^{-1} accompained an equal replication of unamended controls. Ten-ml of a non-axenic stationary-phase culture of Anabaena flos-aquae was used as inoculum for each culture. Cultures were maintained in an environmental growth chamber at 25 °C under a L:D 14:10 cycle that delivered ca. 40 μEin m^{-2} s^{-1} of cool white fluorescent light. Cultures were manually shaken and randomly rearranged at least twice daily. Chlorophyll a and relative carbon assimilation were determined in late exponential growth phase at 112 h using methods previously discussed. For the determination of relative carbon assimilation, two 125-ml Pyrex ground glass bottles were used for each culture, with an incubation period of 1.1 h.

A 14-day Selenastrum bioassay employed 3 replicated (n=4) treatment levels of 538 (T1), 2735 (T2), and 11,500 (T3) mg NaCl l^{-1} with an equal replication of unamended controls. An axenic stationary phase culture of Selenastrum capricornutum Printz was aseptically centrifuged, washed in sterile distilled water, recentrifuged, and cells resuspended in sterile Moss media. After complete mixing, 2.5 ml were distributed as inoculum to each flask. Cultures were randomly assigned to positions on a shaker table, with constant mixing and daily rearrangement throughout the bioassay. Four cool white fluorescent lights provided sufficient light on a 12:12 L:D cycle. A 25 °C constant temperature room was used for the bioassay. Samples for relative carbon assimilation analyses and cell counts were aseptically withdrawn at day 6 and 14 during the middle of the light period. Samples were enclosed for 1-h incubations in 7-ml stoppered culture tubes on day 6, and 24-ml screw-cap scintillation

vials on day 14.

Results

In situ Primary Productivity Bioassays

Physicochemical variables. The results of selected water quality analyses for the surface water of SIR are shown in Table 2. Alkalinity remained relatively constant (ca. 2.0 meq liter^{-1}) throughout the summer and fall of 1983. A low conductivity range of 160 to 300 μmhos indicates the naturally low salinity of SIR. Secchi depths remained less than 1.0 m during the sampling period, reaching a minimum of 0.15 m on 27 October. This minimum Secchi depth corresponded to the maximum turbidity of 94 nephelometric turbidity units (NTU) also on 27 October, a result of high silt and clay loading caused by heavy rains.

Phytoplankton. Table 3 presents the results of phytoplankton enumeration and pigment analyses. Sangre Isle Reservoir phytoplankton during the study period were dominated by members of the Chlorophyta and Cyanophyta. The filamentous blue-green alga Oscillatoria was especially dominant from 14 July to 18 October, when it comprised from 43 to 81% of the total cell number. During this time interval, Oscillatoria had a mean filament length of ca. 122 μm. Other major phytoplankton genera included the green algae Ankistrodesmus and Scenedesmus, the blue-green algae Chroococcus and Aphanocapsa, and the Cryptophyte Cryptomonas (see Table 3).

Species richness data indicate the variable presence of other "minor" genera, which can be important members of the total algal association as a result of their contribution to productivity or species diversity. Numeric species diversity ($\overline{d}_n$) tended to be in the higher 2.00 to 3.00 range when species richness was high. However, low $\overline{d}_n$

Table 2. Selected water quality parameters for Sangre Isle Reservoir during 1983.

Water quality parameter	17 Jan	16 Jun	23 Jun	1 Jul	14 Jul	29 Jul	15 Aug	30 Aug	15 Sep	18 Oct	27 Oct
Alkalinity (meq liter^{-1})	1.2	1.9	1.9	1.9	1.9	2.0	2.2	2.1	2.1	2.2	1.4
Conductivity (μmhos at 25 °C)	299	213	212	206	209	253	287	246	253	253	160
Dissolved oxygen (mg liter^{-1})	12.5	8.5	7.7	8.2	7.1	6.9	5.3	8.4	4.8	7.4	ND
Secchi depth (cm)	45	95	85	63	53	50	65	45	38	35	15
Temperature (°C at 0.1 m)	2.0	26.0	29.8	27.2	28.1	29.8	30.5	31.0	23.2	18.0	ND
Turbidity (NTU)[a]	ND	12	14	24	23	19	16	31	47	28	94

[a] Nephelometric turbidity units.

ND = No data.

Table 3. Algal association parameters for Sangre Isle Reservoir for selected dates in 1983.

Date	Sample type	Species richness	Species diversity ($\bar{d}_n$)	Total cell density (No. ml^{-1} x 10^4)	Chlorophyll a (μg $liter^{-1}$)	Phaeopigments[a] (μg $liter^{-1}$)	Numerically dominant phytoplankton[d]
1/17	ND	ND	ND	ND	2.0	3.8	ND
6/16	10[c]	5	2.19	1.1	4.1	4.4	1,4
6/23	C[b]	20	3.02	4.0	4.5	5.5	3,6
7/1	10	10	2.76	1.7	17.2	16.3	1,3
7/14	C	11	2.32	1.7	18.4	22.2	4,5
7/29	C	15	1.25	5.6	10.6	9.8	5
8/15	10	10	2.93	2.3	6.7	10.5	4,5
8/30	10	8	1.75	5.8	10.9	10.2	5
9/15	10	8	1.94	4.2	14.5	12.2	3,5
10/18	C	14	2.88	3.5	14.1	13.7	2,5
10/27	10	4	1.46	3.2	8.4	8.1	3,4

[a] Degraded chlorophyll. [b] C = 120-ml concentrated algal sample. [c] 10 = 10-ml sample.
[d] Numbers correspond to the following algal genera: 1 = Ankistrodesmus (Chlorophyta); 2 = Aphanocapsa (Cyanophyta); 3 = Chroococcus (Cyanophyta); 4 = Cryptomonas (Cryptophyta); 5 = Oscillatoria (Cyanophyta); 6 = Scenedesmus (Chlorophyta). ND = No data.

accompanied relatively high species richness in SIR on 29 July. This low diversity was caused by Oscillatoria comprising ca. 81% of the total cell number, and the presence of ca. 14 "minor" genera.

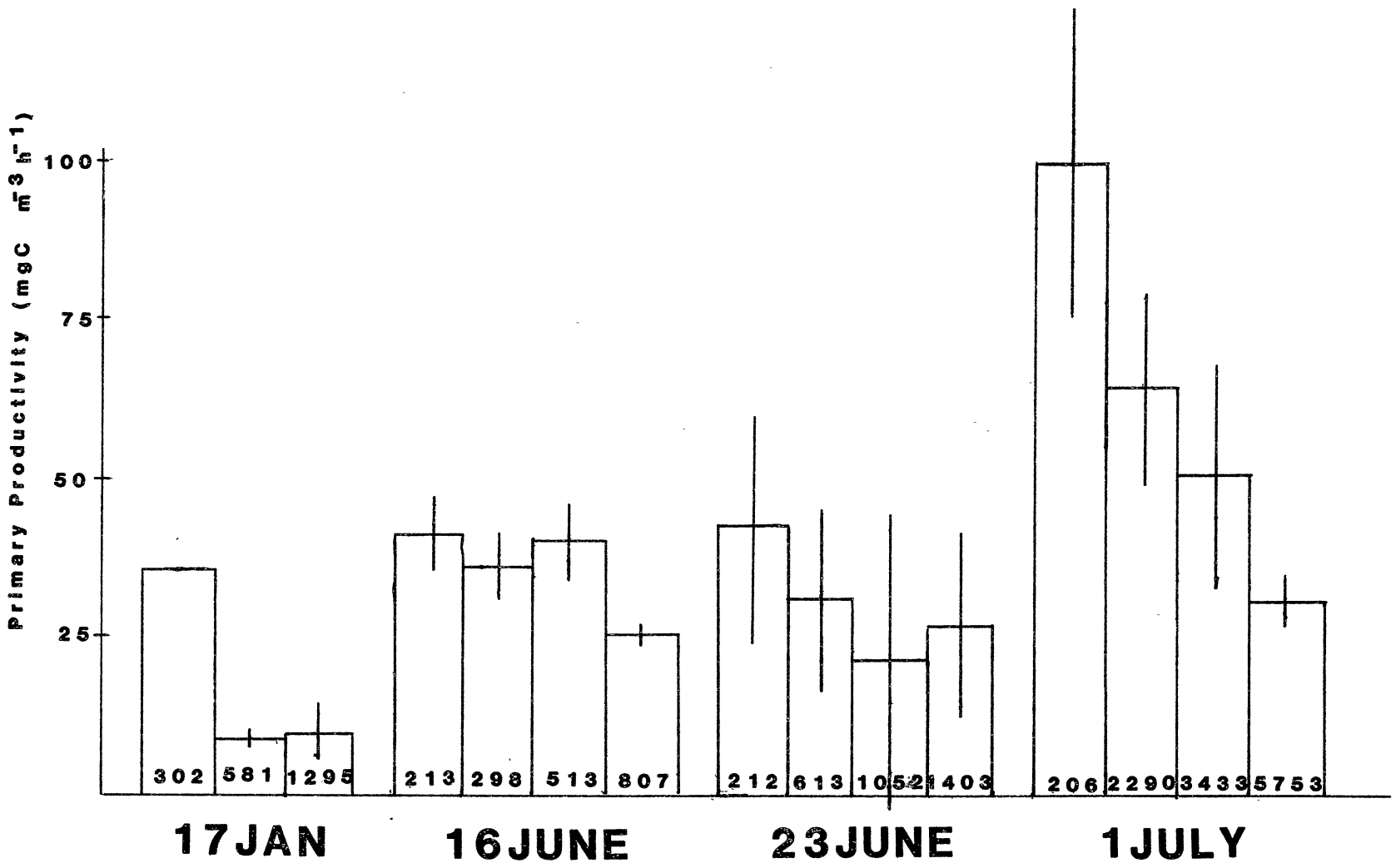
Total cell density reached a maximum level (ca. 5.7×10^4 cells ml^{-1}) in late July and again in late August. At both of these times, the blue-green alga Oscillatoria was the dominant phytoplankter.

Chlorophyll a reached a maximum value in mid-July, accompanied by a higher phaeopigment concentration. Chlorophyll a and phaeopigment concentrations were generally equivalent, although sampling dates in June, July, and August showed phaeopigments exceeding functional chlorophyll a.

Primary productivity. Figure 3 presents productivity data for January and June through October, 1983. When viewed as a general time series, there was a productivity peak in mid-July, followed by declining productivity through mid-August. Subsequently, a second increase began on 30 August, peaking at the maximum productivity for the experimental period on 18 October (197 mg C m^{-3} h^{-1} fixed by unperturbed phytoplankton). The drastic decline on 27 October was probably due to an increase in abiogenic turbitity that occurred on 20 October.

Algal responses to the numerous elevated salinity treatments varied. Statistical tests indicate no significant differences on 23 June and 14 July between control and treatment productivities, or among treatments. In contrast are data from 17 January, 16 June, 15 August, 18 October, and 27 October, where a salinity increase of ca. 500 µmhos reduced productivity significantly. On 29 July and 15 September, NaCl pertubation significantly reduced productivity only at the upper treatment levels of ca. 6000 and 9500 µmhos. On 30 August, productivity

Figure 3. Primary productivity for the in situ primary productivity incubation bioassays with natural Sangre Isle Reservoir phytoplankton associations. Dates for 1983 include: 17 Jan, 16 Jun, 23 Jun, 1 Jul, 14 Jul, 29 Jul, 15 Aug, 30 Aug, 15 Sept, 18 Oct, 27 Oct. Conductivity during the C-14 incubation period is shown at the base of each bar in μmhos at 25 ^{o}C.


Primary Productivity (mgC m-3 h-1)
100
75
50
25
302
581
1295
213
298
513
807
212
613
1052
1403
206
2290
3433
5753
17JAN
16JUNE
23JUNE
1JULY

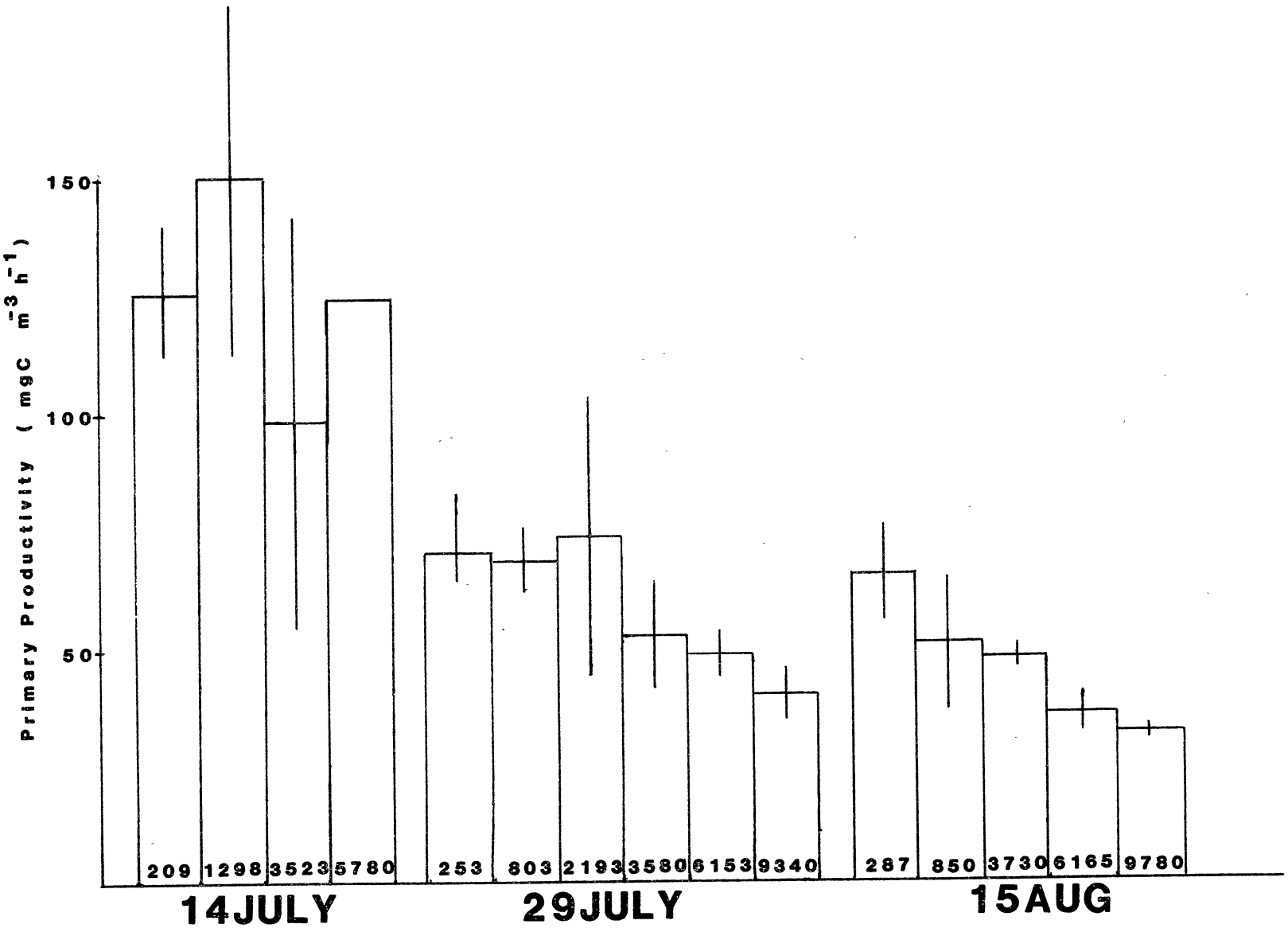


FIGURE 3 - continued

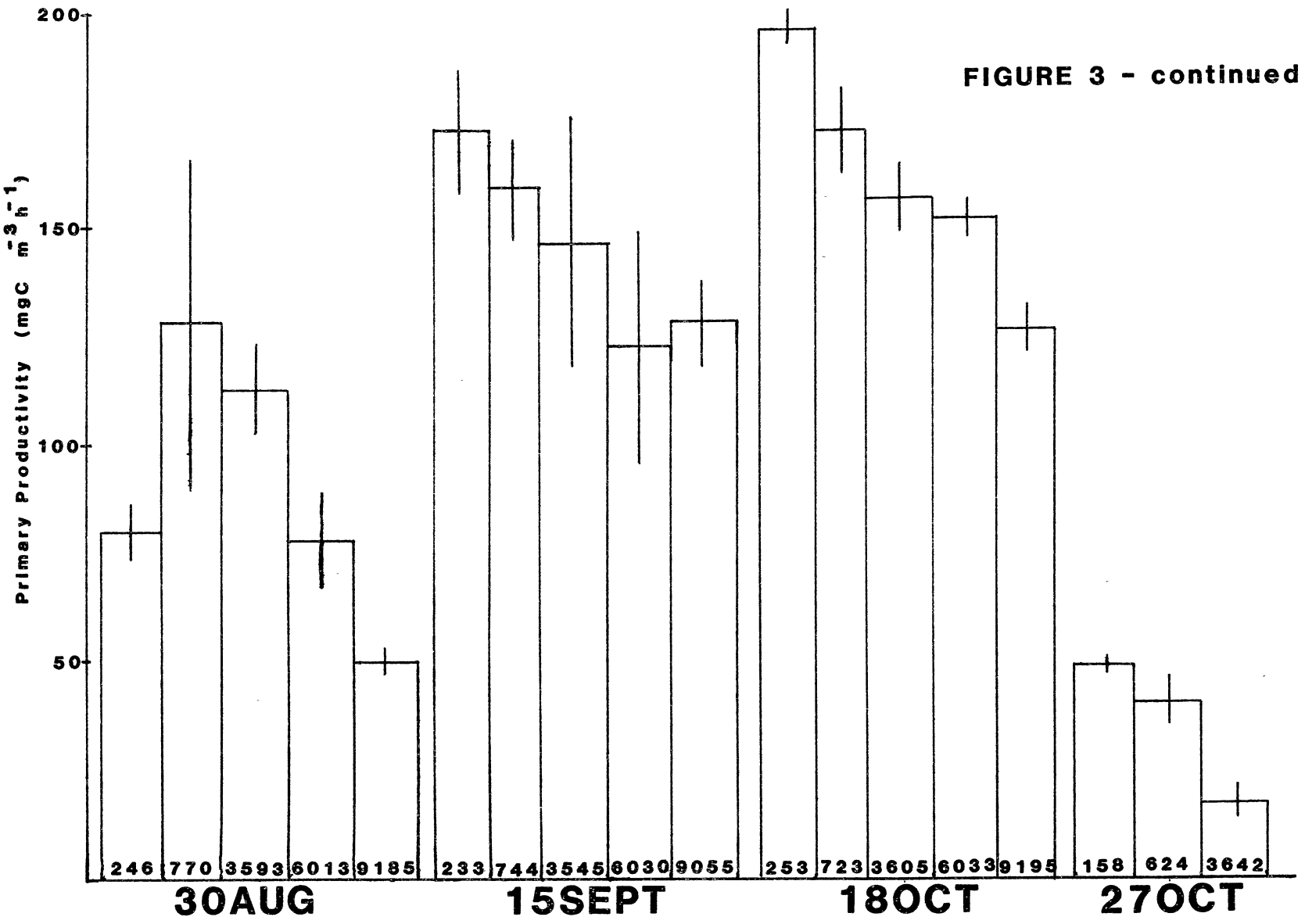


FIGURE 3 - continued

was maximum at 770 and 3593 μmhos, equal to the control at 6013 μmhos, and reduced from the control at 9185 μmhos (control = 296 μmhos).

Laboratory Batch Bioassays

Anabaena-bioassay. Results from the 112-h static Anabaena flos-aquae salinity bioassay are shown in Table 4. No significant differences in cell density were observed among the treatments and control. A significant inhibition of both relative carbon assimilation and chlorophyll a concentration occurred between 701 and 2337 mg NaCl l^{-1}. Further inhibition did not occur at the 4675 mg NaCl l^{-1} level.

Selenastrum bioassay. All cultures of Selenastrum capricornutum Printz showed some growth in cell number over the day 6 to day 14 interval (Table 5). A highly significant depression occurred at treatment level 3 (T3 = 11,500 mg NaCl l^{-1}) for both cell density and relative carbon assimilation. Inhibition of relative carbon assimilation during both the exponential phase and the late exponential-stationary phase was not caused by a decrease in cell numbers for T1 and T2. Although relative carbon assimilation was greatly reduced between day 6 and 14 in all cultures, the percent inhibition of ^{14}C-uptake for T1 remained constant. During late exponential to stationary phase, Selenastrum was more tolerant to salinity at the T2 salinity level.

Table 4. Treatment level data, relative carbon assimilation, and algal biomass for a 112-hour laboratory batch bioassay with Anabaena flos-aquae in late exponential phase of growth.

	Treatment mg NaCl l^{-1}	Conductivity (µmhos 25 °C)	Cell density (cells ml^{-1} x 10^6)	Relative carbon assimilation (CPM[b] ± 1SD x 10^4)	Chlorphyll a (µg Chl. a l^{-1} ± 1SD x 10^2)
C[a]	9	362	6.52	5.9 ± 0.6	14.2 + 0.5
T1	701	2180	8.75	5.4 ± 0.2	14.1 + 0.6
T2	2337	5280	10.40	4.5 ± 0.5	9.5 + 0.8
T3	4675	9710	7.39	4.7 ± 0.2	9.0 + 0.2

[a] C = Control.
T1 = Treatment level 1 (etc.).

[b] Counts per minute (quench corrected).

Table 5. Relative carbon assimilation and cell densities for day 6 and 14 of a 14-day laboratory batch bioassay with Selenastrum capricornutum Printz. The percent inhibition from the control is given in parentheses below each relative carbon assimilation value.

Day	Growth phase	Relative carbon assimilation (CPM[b] ± 1SD x 10^3)				Cell density (cells ml^{-1} x 10^6)			
		C[a]	T1	T2	T3	C	T1	T2	T3
6	E[c]	275 ± 2.8	221 ± 1.6 (20)	98 ± 0.8 (64)	0.6 ± 0.006 (99)	1.3	1.6	1.2	0.05
14	E-S[d]	19 ± 4.4	15 ± 3.9 (21)	15 ± 3.2 (21)	0.7 ± 0.2 (96)	3.8	4.2	3.4	0.11

[a] C = Control (NaCl = ca. 9 mg $liter^{-1}$; conductivity = ca. 360 µmhos).
T1 = Treatment 1 (NaCl = 538 mg $liter^{-1}$; conductivity = 1281 µmhos).
T2 = Treatment 2 (NaCl = 2735 mg $liter^{-1}$; conductivity = 6771 µmhos).
T3 = Treatment 3 (NaCl = 11,500 mg $liter^{-1}$; conductivity = 22,500 µmhos).

[b] Counts per minute (quench corrected).

[c] Exponential phase.

[d] Exponential to stationary phase.

Discussion

In situ Primary Productivity Bioassays

Three altered productivity responses to the rapid elevation of NaCl-salinity occurred during this series of in situ bioassays in addition to that of no response to salinization. Sodium chloride had no effect on C-fixation on 23 June when species diversity was high, and again on 14 July during a peak in algal pigment concentration per cell. Data for these dates agrees with Ward and Wetzel (1975), who report that the addition of 50, 100, and 200 mg Na^+ $liter^{-1}$ elicited neither a stimulatory nor an inhibitory response in photosynthetic C-fixation in natural associations in Michigan lakes.

A phyto-inhibitory effect, initiated at a conductivity ca. 2-4 times above the normal SIR level, occurred with the greatest frequency, taking place on 17 January, 16 June, 15 August, and 18 and 27 October. On the latter 3 dates, additional NaCl further reduced productivity, yielding an inverse relationship between productivity and salinity. The second response type was characterized by a significant phyto-inhibitory effect only above a minimum conductivity of ca. 6000 µmhos. The SIR phytoplankton associations on both 29 July and 15 September exhibited this behavior.

The final and perhaps most interesting response occurred on 30 August, when mean incubation conductivities of 770 and 3593 µmhos caused an apparent stimulation of productivity. Significant inhibition occurred only at the highest salinity level of 9185 µmhos, for an association dominated by the non-heterocystous blue-green alga Oscillatoria. This indicates a positive factor interaction between

increased salinity in the 770 to 3600 µmhos range, and C-fixation. Ward and Wetzel (1975) also found increased C-fixation in natural blue-green populations when small amounts of Na^+ (5 mg $liter^{-1}$) were added.

Further variation in response is noted among experimental dates with similar phytoplankton associations. On both 14 July and 15 August, phytoplankton associations were dominated by Cryptomonas and Oscillatoria. On the first date, no significant reduction in productivity occurred up to 5780 µmhos, while on 15 August, significant inhibition began at 850 µmhos. These data indicate a physiological change in the ability of the phytoplankton to adapt to the experimental pertubation. Some of this increased adaptability might be due to a more abundant supply of stored or external nutrients. Heath (1979) found that cadmium stress may be greatest in systems that are phosphorus limited. It is also known that species living under stress are usually more tolerant to additional stress of the same type than are species not initially perturbed (Jernelöv and Rosenberg 1976). However, the SIR phytoplankton are not naturally salinity-stressed. Therefore, some other limitations were probably acting in a synergistic manner with salinity to cause the observed shift in tolerance.

Adequate supplementary data (i.e., nutrients, light, etc.) to more completely explain the observed variability in the phytoplankton acute response to elevated salinity are not available. However, the existence of such variability on a seasonal basis is a significant finding. Because similar phytoplankton associations exhibited variable tolerance to salinity stress, other factors that interact and alter this tolerance were present, and should be given more consideration in future studies.

Laboratory Batch Bioassays

Anabaena Bioassay. The level at which significant inhibition of relative carbon assimilation began during the 112-h Anabaena flos-aquae bioassay approximates a range in absolute Na^+ concentrations of 270-900 mg $liter^{-1}$. This level is well above a minimum requirement of 5 mg Na^+ $liter^{-1}$ for Anabaena cylindrica (Allen and Arnon 1955). These results agree with Batchelder (1981), who found that Na^+ concentrations of 46 and 184 mg $liter^{-1}$ did not affect the dry weight yield of Anabaena subcylindrica in batch culture. Also in agreement is Ward (1974), who found no differences in ^{14}C-uptake and chlorophyll a among cultures of Anabaena cylindrica Lemm. grown in batch cultures with 50 mg Na^+ $liter^{-1}$.

The inhibition of ^{14}C-uptake between treatment levels T1 and T2 was due more in part to a reduction in functional chlorophyll a than to a depressed growth rate. Cell density actually increased slightly between T1 and T2 as chlorophyll a concentration was depressed. No further inhibition occurred between salinity levels T2 and T3, indicating a sharp decline in the rate of inhibition. The salinity level at which further reductions would occur is not shown by these data.

Selenastrum bioassay. An overriding depression of ^{14}C-uptake occurred in C1, T1, and T2 cultures of Selenastrum at the onset of stationary phase (between day 6 and 14). During this time, cell density increased 2-3 fold in C1, T1, and T2. In T2 cultures, an apparent partial acclimation occurred, as the percent inhibition in ^{14}C-uptake from the control was reduced by ca. 40% to an inhibition level consistent with T1 cultures. Significant inhibition began at the lowest treatment level (T1 = 538 mg NaCl $liter^{-1}$). However, it is likely that

inhibition began at a somewhat lower concentration, as Cleave et al. (1981) found growth of the same alga was initially depressed at 250 mg NaCl liter^{-1}. Cleave et al. (1981) also noted that growth was further depressed as concentration increased, a situation that also occurred during this bioassay.

A NaCl concentration of 11,500 mg liter^{-1} (22,500 µmhos) effectively reduced ^{14}C-uptake and cell division to insignificant levels, thereby preventing any form of exponential growth in T3 cultures. This response of Selenastrum was consistent with preliminary in situ primary productivity incubation bioassays conducted in 1982 with SIR phytoplankton associations. In those studies not presented here, I found productivity inhibition of > 90% when incubation conductivity was ca. 20,000 µmhos.

CHAPTER IV

LONGER-TERM BIOASSAYS

Continuous Culturing of Phytoplankton

Introduction

Recent work in experimental phytoplankton ecology has indicated a lack of knowledge concerning the applicability and extrapolation of static laboratory bioassay results to natural phytoplankton associations (Anon 1969; Barlow et al. 1973; Cain and Trainor 1973; Walsh and Merril 1982). Laboratory algal bioassays have been conducted under a variety of simplified and highly artificial systems. Most laboratory batch experiments consist of a single "test organism," in artificial media with concentrations of critical nutrients one or more orders of magnitude greater than observed in the field. In batch systems, no input or output of materials occurs; growth in these confined enclosures creates a constantly changing environment (Rhee 1980). Since growth limitations are a function of the rate of nutrient input, and not their levels at a given time (Rhee 1980), conclusions drawn from batch bioassays concerning growth phenomena are highly suspect. In a closed system, organisms can be expected to react to decreases in limiting factors, shifts from one limiting factor to another, and to the build up of metabolic products (Jannasch 1967a). The short-term nature of these studies adds to their lack of realism, thereby further confounding the

formulation of realistic ecological hypotheses (Kaleff and Knoechel 1978). Because of these obvious shortcomings, researchers have called for the development and application of flow-through techniques using algal species and associations (deNoyelles et al. 1980; Walsh and Merril 1982). To meet the need for greater experimental realism and greater extrapolation potential to natural aquatic communities, many continuous and semi-continuous culture techniques have been used.

Continuous culture of phytoplankton consists of maintaining a culture at some chosen point on its growth curve by the regulated addition of fresh medium (Fogg 1975). Extensive reviews on the theory and methodology are available (c.f. Herbert et al. 1956, 1965; Jannasch 1965; Fuhs 1966; Malek and Fencl 1966; Jannasch and Mateles 1974; Rhee 1980).

Behind all methods in continuous culture is the concept of steady state. Theoretically, a system in steady state is in a dynamic equilibrium, where inflows balance outflows of material and energy (Odum 1971). For algal continuous culture the steady state can also be defined as a time-independent state where growth is counter balanced by the removal of cells (Jannasch 1967b). A steady state is the basis for most kinetic investigations which seek to establish relations between growth and environmental factors (Odum 1971). The basic requirements of a steady state in continuous culture are: 1) inflow equal to outflow; 2) constant flow rate and volume; 3) complete mixing and dispersion of the microorganisms so that dilution proceeds in an indiscriminate fashion and at the same rate for all species present; and 4) maintenance of a uniform environment (Porcella 1969; Odum 1971). Rhee (1980) notes that a steady state will be achieved only if one nutrient is limiting

for growth, and a constant proportion of cells are viable. Judgement as to when a culture has reached a steady state has been based on different standards and measurements (c.f. Fuhs 1969; Goldman et al. 1974).

The turbidostat and the chemostat are two different continuous culture methods used to achieve a steady state. In a turbidostat, dilution is controlled by a photometric device to keep population density (culture turbidity) constant and thus balance growth (Fogg 1975). Due to its higher level of complexity and cost, this technique will not be discussed further (see Munson 1970). The second method, which I have attempted to use both in situ and in the laboratory, is the chemostat. A chemostat is a refined continuous culture system that depends on the addition of fresh medium to the culture at a constant rate (Porcella 1969; Fogg 1975). The population density is self-adjusting to a maximum rate which is determined by the rate of supply of the limiting nutrient (Fogg 1975). A continuous overflow of spent medium keeps the culture from accumulating excretory products (Rhee 1980). Advantages of the chemostat culture system include: 1) the use of low nutrient concentrations; 2) the achievement of a dynamic equilibrium between nutrient input and growth; 3) the laboratory chemostat theoretically provides a steady state culture under a constant physical environment which is nonvariant chemically and biologically (Porcella 1969); and 4) although difficult to operate in the field, the chemostat can allow the study of acute and chronic responses to experimental perturbation under natural conditions of light, nutrient concentrations, turbidity, etc. (deNoyelles and O'Brien 1974; deNoyelles et al. 1980; Knoechel and deNoyelles 1980).

In terms of environmental realism, the chemostat technique ranks

somewhere between experimental ponds and large bag experiments and batch cultures (Kaleff and Knoechel 1978). Cain and Trainor (1973) state that although continuous culture is potentially the most useful, it also can be the most demanding. But several studies have indicated that the chemostat can yield results comparable to those of the larger experimental systems, but with a smaller resource commitment (O'Brien and deNoyelles 1976; deNoyelles et al. 1980). Despite its advantages, the chemostat does not allow the reproduction of natural conditions (Odum 1971; Rhee 1980), since the steady state achieved is a function only of the constant rate of nutrient input. In natural populations, Rhee (1980) suggests that if a steady state ever existed, it would be the result of a combined effect of numerous environmental factors. Odum (1971) points out that most individual species are not in a steady state, but rather in transient states due to seasonal or other periodic environmental changes.

Laboratory Artificial-Media Chemostats

The chemostat method for continuous culturing of phytoplankton has been employed in the laboratory by many investigators. Most research to date has been with a representative "test organism," such as the green alga Selenastrum capricornutum Printz (EPA 1979). The U.S. Environmental Protection Agency (1971) suggested the chemostat as one method for the assay of algal growth-nutrient relationships in natural and enriched waters.

In one of the earlier continuous culture designs, Carpenter (1968) employed a simple laboratory chemostat to test nutrient limitation for axenic cultures of two planktonic marine diatoms and one freshwater

flagellate. Prance and Benson-Evans (1973) demonstrated that species of Ulothrix could be cultured with chemostat techniques at realistic nutrient levels. The uptake of radioactive ^{137}Cs by the blue-green alga Plectonema purpureum was shown in chemostat culture by Watts and Harvey (1963). Fuhs (1966, 1969) has done extensive experimental work with experimental seawater chemostats in culturing species of Cyclotella and Thalassiosira. Bahner et al. (1975) studied toxicant-environmental stress interactions using a flowing-water bioassay that allowed control of temperature and salinity.

A similar but less demanding system termed the semi-continuous flow culture is defined as a fixed volume culture from which a withdrawal of cell mass, medium, and metabolites occur at regular intervals, with fresh medium being added to replace that which is removed (Reynolds et al. 1976). The relationship between semi-continuous flow cultures and continuous flow cultures was investigated in experiments designed to model the effects of temperature on phenol toxicity to Selenastrum capricornutum. In these studies, Reynolds et al. (1976) found that results from the semi-continuous and continuous cultures were not in agreement. Cain and Trainor (1973), Klotz et al. (1975), and Cain et al. (1979), used small volume axenic cultures of Selenastrum capricornutum in semi-continuous culture to show changes in algal carrying capacity above and below a wastewater treatment plant outfall. Fay and Kulasooriya (1973) describe semi-continuous culture apparatus which they used to culture axenically several unicellular, colonial, and filamentous algae.

Laboratory Natural-Water Chemostats

Some researchers desiring to more meaningfully extrapolate culture responses to the natural community as a whole have chosen to enclose a sample of that indigenous community, instead of choosing a representative "test organism" (deNoyelles et al. 1980). This method is much more promising, since no single species can be expected to represent adequately the response of a natural association of some dozens of species (Barlow et al. 1973). Recent research has shown that standing stock and rate measurements may provide little evidence that a system has been impacted, while estimates of change in population structure and succession can more truly measure pollution stress (Menzel 1977).

Considering such studies on marine systems, Dunstan and Menzel (1971) used 15-liter seawater continuous cultures treated with secondary sewage treatment plant effluent. To study competitive interaction, Fisher et al. (1974) enclosed both natural marine phytoplankton communities, and species-defined communities, and measured species compositional changes caused by polychlorinated biphenyls.

In a freshwater study, Porcella (1969) used unfiltered water from Lake Tahoe containing its natural assemblage of algae in chemostat culture to test raw sewage and treatment plant effluents. Nutrient enrichment experiments using 12-liter culture flasks containing pond water algal communities were conducted by Barlow et al. (1973). They found good correlation between chemostat culture response and the response of experimental ponds treated in a similar way.

In comparative studies on algal bioassay techniques, O'Brien and deNoyelles (1976) performed simultaneous batch culture bioassays, primary productivity bioassays, and continuous culture chemostat bioassays.

These were then compared to a series of similarily treated (treatment was nutrient additions) experimental ponds. They report that both the batch and chemostat bioassays yielded results similar to the ponds. Results from the primary productivity bioassays were different. Gehart and Likens (1975) compared results of four different bioassay techniques, including: 1) short-term (4-30 h) ^{14}C- bioassays; 2) laboratory chemostat culture of natural phytoplankton; 3) large polyethylene tube enclosures; and 4) long-term ^{14}C-bioassays on subsamples of water from enriched chemostat cultures. In tests for N and P limitation, the last three methods showed agreement that N and P were limiting production. The short-term ^{14}C-bioassay again did not yield conclusions similar to the other bioassays.

In situ Chemostats

The most realistic form of continuous culture to date, as applied to phytoplankton ecology, has been the in situ chemostat. This unit was originally described by deNoyelles and O'Brien (1974), when it was used for nutrient enrichment studies. The development of this technique can be seen in the recent work by deNoyelles et al. (1980) and Knoechel and deNoyelles (1980).

Materials and Methods

In situ Chemostat Bioassays

Methodological Approach

To test the response of natural reservoir assemblages of phytoplankton to elevated NaCl-salinity, in situ chemostat bioassays

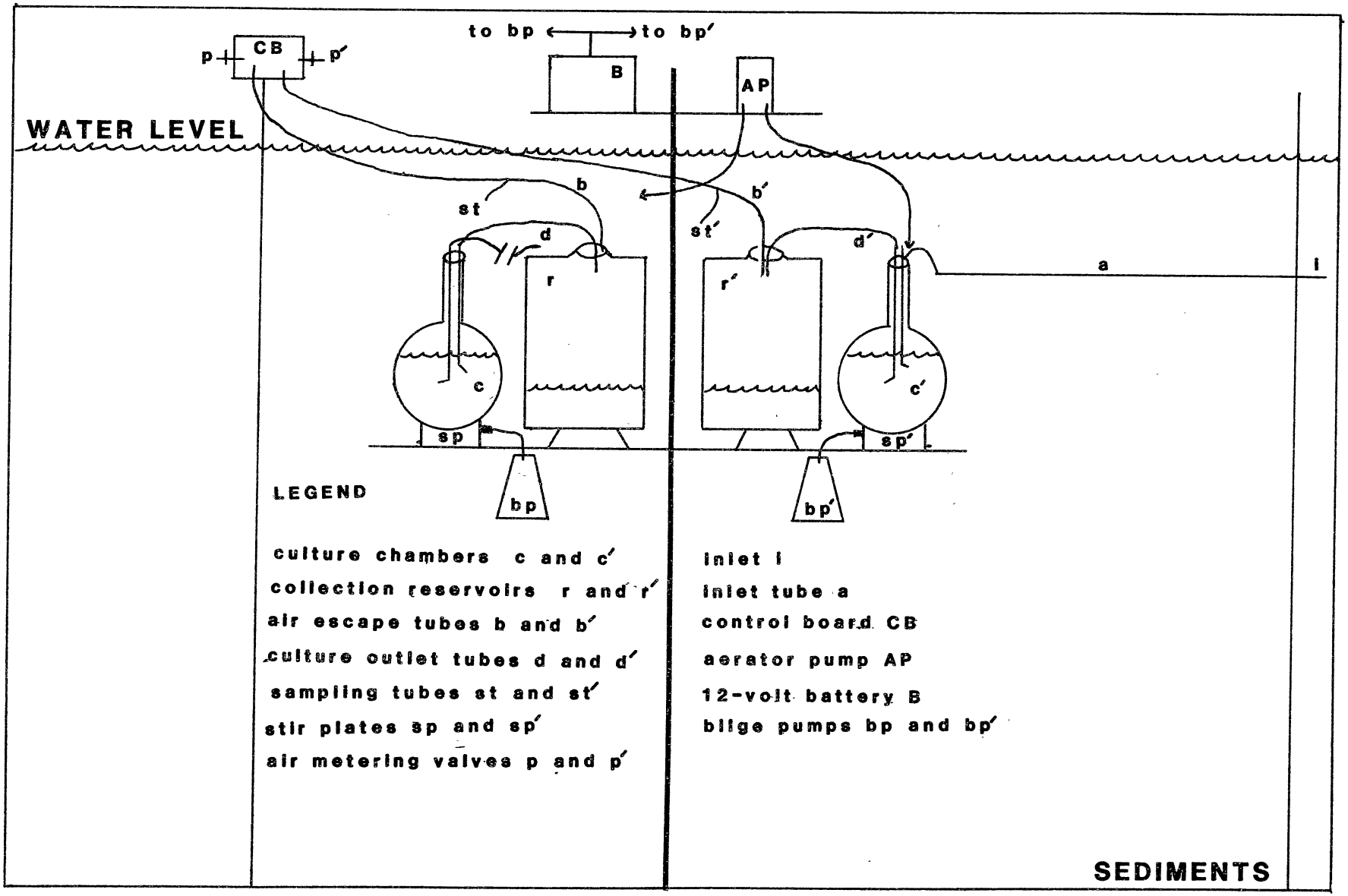
were conducted. Since questions concerning both short-term metabolic responses and longer-term successional changes caused by elevated NaCl-salinity were being addressed, I believed that the application of the chemostat technique under natural reservoir conditions would result in a more rigorous experimental design.

In situ Continuous-culturing System

An in situ chemostat was constructed following the basic design of deNoyelles and O'Brien (1974). A diagram of the unit is shown in Figure 4. It consisted of two 3-liter round-bottom boiling flasks which served as culture chambers, and two 11.35-liter opaque polyethylene carboys, which served as collection reservoirs. Culture chambers and collection reservoirs were paired, and connected to each other by a length of tubing. All tubing used in the unit was 6 mm OD, 3 mm ID Tygon (Trademark-Norton Speciality Plastics Division) tubing. All enclosures were mounted onto an angle-iron frame. To insure adequate mixing and random withdrawal of all culture chamber contents, cultures were stirred continuously with submerged water-driven magnetic stirrers (Hartell, Italy: from Fisher Scientific), which rotated a stirring bar inside each flask. Reservoir water was forced into the stir plates by two small bilge pumps, which operated off a 12-volt battery mounted on supports at the surface.

To operate the unit, water was collected from the 0.5-m stratum with a polyethylene Van Dorn sampler, and 2.5 liter was transferred into each culture flask (c) and (c'). To insure that no leakage occurred, a non-toxic aquarium sealant was applied around rubber stoppers and rubber-glass connections. The entire unit was then submerged and bolted

Figure 4. In situ continuous culturing system patterned after deNoyelles and O'Brien (1974).


to bp
to bp′
p
CB
p′
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b
b′
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a
i
r
r′
c
c′
sp
sp′
bp
bp′
LEGEND
culture chambers c and c′
collection reservoirs r and r′
air escape tubes b and b′
culture outlet tubes d and d′
sampling tubes st and st′
stir plates sp and sp′
air metering valves p and p′
inlet i
inlet tube a
control board CB
aerator pump AP
12-volt battery B
bilge pumps bp and bp′
SEDIMENTS

onto metal posts anchored in the sediments. The midpoint of the culture flasks was set at a depth of about 0.4-0.5 m below the water surface. To begin flow through the culture chambers (c) and (c'), metering valves (p) and (p') were opened at the above surface control board (CB). As air escaped into collection reservoirs (r) and (r') through lines (b) and (b'), raw lakewater was forced into inlet (i), located about 3.5 m from the center of the unit. Total reservoir depth at inlet was ca. 2.0 m.

The inlet was also placed at a depth of 0.4-0.5 m below the water surface by passing the tube end through a piece of drilled pipe, and attaching the pipe to a supporting rod driven into the sediments. Influent water was then split by a "Y" adaptor in inlet tube (i), and forced by water head pressure into both culture chambers. This pressure arises from the relative elevations of the inlet tube (i) and culture outlet tubes (d) and (d'). Ideally, a constant flow of water moves through the culture chambers, and into the collection reservoirs via tubes (d) and (d'), until all air in the reservoirs has been displaced by water.

The above water control board consisted of metering valves (p) and (p'). These were 24-ml scintillation vials filled with about 8 ml of water. The vial was fitted with a tight rubber stopper, and attached via tubing to a small pin valve. The outlet of lines (b) and (b') terminated in these small vials. Escaping air from (r) and (r'), was bubbled out, and the rate of escaping air, quantified visually as a bubbling rate, was directly proportional to the water flow through the culture chambers. Operation of the two culture chambers at equivalent flow rates was attempted by matching bubbling rates.

For experimentation, one culture flask served as a control and the other as a NaCl-treatment. Specific volumes of 1N NaCl were manually injected into the treatment flask.

Experimental salinity levels were determined using both my results from batch studies and published values for Oklahoma reservoirs. Impoundments such as Keystone Reservoir, which routinely has salinities in the 2000 to 4000 µmho range at some stations (Eley 1970; Kochsiek et al. 1971; Environmental Protection Agency - STORET), served as an upper salinity limit.

The unit was placed in Sangre Isle Reservoir (SIR) (see Figure 2). Water depth at this location was ca. 1.5-m. Location at this relatively shallow depth was necessary, because periodic raising of the unit for sampling and maintenance could not be accomplished by boat, and earlier attempts to attach the unit to floats at deeper sites had failed. The sampling procedure consisted of raising the unit to a depth of about 20 cm below the water surface, where the required operations could be completed more easily. Clip clamps were used to temporarily shut off lines (a), (b) and (b') from normal operation. A small battery-operated bait aerator was then used to pump air into each sealed culture chamber, forcing fresh culture water up the outlet tubes (b) and (b'), and through sampling tubes (st) and (st') into dark plastic bottles for separate analyses. The volume sampled never exceeded 20% of the total culture volume. Manual input of 0.5-m SIR water following sampling increased culture volume to 2.5 liter. No analyses were conducted on water in the collection reservoirs, since these enclosures had no means of mixing and were opaque.

Two experiments were conducted using the in situ chemostat.

Experiment 1 was a 92-h investigation that took place from 23 October 1983 to 27 October 1983. Experiment 2 followed experiment 1, taking place from 29 October to 30 October, and lasting 22 h. The control culture chamber, treatment culture chamber, and 0.5-m depth of SIR were sampled.

Analytical Methods

Flow rates. Average flow rates for a given time interval were estimated by recording water level increases in the collection reservoirs. These depth measurements were then correlated to changes in volume. It did not prove practical to rely on bubbling rates for flow calculations.

Primary productivity. For experiment 1, ^{14}C-primary productivity was determined at the midpoint of the experiment (41 h) prior to NaCl treatment, and at the end of the experiment (92 h). Modifications of the method presented previously include the use of 24-ml screw cap glass scintillation vials as incubation chambers. This was done to increase sample size and minimize the volume of culture water withdrawn. New vials were soaked in distilled water, washed in Contrad 70 (Decon Laboratories, Malvern, PA), and rinsed five times in distilled-deionized water. Normal 125-ml Pyrex ground-glass stoppered bottles were also used as a check against possible enclosure effects in the smaller bottles. Upon filling, small vials were fitted with parafilm to restrict the introduction of air bubbles. Incubation times were 4.25 h (1223-1638) and 4.35 h (1252-1713) for the 41-h and 92-h determinations respectively. The entire bottle volume (24 ml) was filtered from the small scintillation vials, while 25-30 ml was subsampled from the large

bottles.

In order to determine if further elevations of NaCl-salinity altered acute algal productivity differently in treatment and control chambers, additional volumes of 5N NaCl were injected into the bottles prior to incubation for the 92 h experiment. Two increased NaCl levels were used. Injections of 91 µl into culture samples in 24 ml bottles raised NaCl-salinity by 490 µmhos. Injections of 100 µl into 0.5-m depth SIR water in 125-ml bottles raised NaCl-salinity by 466 µmhos, while 750 µl resulted in ca. 3484 µmhos elevation. For SIR, this was considered an acute ^{14}C-primary productivity incubation bioassay as presented in Chapter III.

Salinity. Culture salinity was monitored daily using the electrical conductivity method as disscussed previously.

Algal pigments. Chlorophyll a was determined as an estimate of algal biomass present in the culture chambers and SIR. Volumes of culture and SIR water (50 and 75 ml, respectively) were filtered, with fluorometric determination as outlined previously. All SIR values represent a mean of 3-4 samples, and all chemostat culture values represent a mean of 2 samples, except for 41-h determination in experiment 1, which had only 1 sample.

Chlorophyll a activity, which is the weight of carbon assimilated per hour per weight of phytoplankton chlorophyll a was calculated as the ratio of primary productivity to chlorophyll a (Yentsch 1962). This ratio has also been termed assimilation number or photosynthetic index (Round 1981).

Light and visibility. A Li-cor Model LI-188B integrating quantum/radiometer/photometer was used to determine light transmission

through the water column near the chemostat. Measurements were taken on 18, 24, 25, and 30 October 1983. Light intensity was measured at 0.1-m, 0.5-m, and 1.0-m depths. These values were then compared to illumination directly above the water surface. Secchi disk visibility was measured on 18, 21, 24, 25, 29, and 30 October.

Turbidity. Reservoir turbidity (0.1-m depth) was monitored from 18 to 30 October 1983. Samples from the culture chambers were occasionally measured to determine if culture turbidity levels differed from SIR levels.

Temperature. Water temperature at the chemostat site and at Station 1 was monitored using a telethermometer. Temperature was measured at 1-m intervals in SIR.

Phytoplankton enumeration and analysis. Samples for phytoplankton enumeration were taken at 0 hours for the initial 0.5-m SIR water placed into the culture chambers, and at 41 h and 90 h from SIR, and culture chambers. Small volume samples (10 ml) were preserved with Lugol's solution (Wetzel and Likens 1979). Methods for identification and enumeration of the phytoplankton were the same as those presented in Chapter III.

To provide more accurate abundance estimates, total cell volumes were determined using the linear dimensions of at least 10 randomly selected individuals (Lind 1979), with calculation of a volumetric factor using equations provided by Wetzel (1984). Total cell volume for each genus was then calculated by multiplying cell density estimates (No. ml^{-1}) by the volumetric factor for that genus. Data are presented as total cell volume (μm^3 ml^{-1}), total numeric density (No. ml^{-1}), and and as percentages of both total estimates for the following dominant

genera: Ankistrodesmus, Chroococcus, Cryptomonas, Oscillatoria, and Synedra. A miscellaneous class includes all other minor algal genera encountered. To further delineate changes in species distribution, species diversity ($\bar{d}$) was calculated using both biovolume ($\bar{d}_v$) and number of cells per ml ($\bar{d}_n$) with the Shannon index. To evaluate the diversity component due to the distribution of individuals among species, equitability was calculated using a table of Lloyd and Ghelardi (1964) as published in Weber (1973). This dimensionless ratio is based upon MacArthur's (1957) broken stick model, as discussed in Pielou (1975).

Further comparisons were made using an index of community similarity (Psc) as given by Whittaker and Fairbanks (1958). Percentage community similarity (Psc) was used to compare the number of species and their relative abundance as biovolume of each from two groups.

Statistical analyses. Statistical analyses of algal pigment and productivity data were done with Statistical Analysis Systems, Inc. (SAS) computer programs. Analysis of variance and Duncan's multiple range tests were used. A significance level of 0.10 was used except where otherwise noted. To test for significant differences between total cell density and total cell volume, a confidence interval was set on each value ($P \geq 0.10$). Confidence limits were based on the expectation of a Poisson variable, as tabulated in Pearson and Hartley (1970). To determine significance, the simple procedure of Lund et al. (1958) was employed. Counts or volumes were judged significantly different if no overlap occurred between the confidence limits of the values being compared.

Modifications for the twenty-two hour bioassay. For experiment 2,

all chemostat operational methods and analytical techniques used were the same as those described for experiment 1. Primary productivity was determined only at the end of the experiment (22 h) on 30 October 1983 (1424-1920; 4.83-h incubation time). Injection of 7.5 ml 1N NaCl occurred initially at 0 h, and was followed by a 12.5 ml injection at 17 h. The additional incubation bottle injections of 5N NaCl just prior to incubation for samples at 22 h were done as in experiment 1. Only 24-ml incubation vials were used. Algal pigments were determined for the SIR water that was initially placed into the culture chambers, and again at 22 h. No intermediate determinations were made.

Laboratory Chemostat Bioassay

Methodological Approach

A laboratory continuous flow bioassay was conducted to test the response of natural reservoir assemblages of phytoplankton to elevated NaCl-salinity under a more controlled and replicable environment. I considered this technique to be both a semi-realistic and manageable compromise between the highly-simplified and artificial batch cultures, and the highly complex in situ continuous culturing method.

Laboratory Continuous Culturing System

A laboratory continuous culturing system for use with natural algal associations was constructed following the general methodology described in Barlow et al. (1973), Wetzel and Likens (1979), and deNoylles et al. (1980). Four 3.0-liter autoclaved boiling flasks served as culture chambers. Culture chambers were placed on a multiple magnetic stirring unit, which allowed for equal mixing and random withdrawal of contents.

A separate magnetic stirrer was used for the common influent water reservoir (11.35-liter opaque polyethylene carboy), which was located adjacent to and elevated above the cultures. Four glass tubes were suspended at the same depth from the influent reservoir carboy top down into the water. Fresh water was then dripped into the cultures by siphon action through Tygon tubing (ID = 1.6 mm; OD = 4.8 mm). Screw clamps were used to regulate the inflow rate to each culture.

Influent water was collected daily from Sangre Isle Reservoir (SIR) near station 1 (total depth ca. 1.5 m). A full carboy of water was collected from directly beneath the ice between 0830 and 1430 hours from 20 to 27 January 1984. During this period, SIR was covered with ice (ca. 0.1-m thickness) except for a small area near the northern island. It was anticipated that daily additions of fresh reservoir water would result in a higher similarity between the unperturbed cultures and the SIR algal association.

A Buchler 4-channel peristaltic pump was positioned between the culture chambers and four 1-liter Erlenmeyer flasks, which served to hold culture chamber effluents. Attempts were made to maintain a constant culture volume by matching the siphoned inflow rate to the pumped outflow rate. Neither inflow nor outflow were filtered.

Light to the cultures was provided on a 12:12 L:D (L = 0600-1800 h) cycle using 6 fluorescent lamps (40W, cool white) located 0.4 m above the culture midpoint. Total light intensity, measured at the culture midpoint between the four cultures, was about 10.7 μEin m^{-2} s^{-1}. It was assumed that this light was considerably greater than that occurring directly below the ice in SIR during this period. Water temperature for the cultures and common influent reservoir was allowed to equilibrate to

room temperature. No attempts were made to simulate the natural winter conditions of light and temperature of SIR in the laboratory.

For experimental purposes, two cultures were randomly chosen for NaCl treatment, with the others serving as controls. Treatment flasks received a sterilized solution of 1N NaCl and control cultures received an equivalent volume of sterile distilled-deionized water. Solutions were located above the light bank, and dripped into the cultures using screw clamps to regulate inflow volumes. An ecologically realistic salinity regime similar to that used for the in situ bioassays was again used.

Sampling of the cultures was accomplished by temporarily closing inflow and outflow lines. Complete mixing of the cultures was followed by siphoning of a sample into containers for analysis. The volume sampled did not exceed 10% of the total culture volume, and equal withdrawals were made from all cultures. Sampled volumes were replaced immediately with fresh water from the influent reservoir following the withdrawal of all culture water required for the various analyses.

The inner walls of the culture chambers were brushed vigorously at least once per day in order to discourage the growth of attached algae. A 170-hour laboratory bioassay was conducted between 20-27 January 1984.

Analytical Methods

Culture hydraulics. Outflow rates were calculated from culture water effluent volume determinations for 3-6 time intervals each day. Culture volume was monitored using culture "depth" measurements. These "depth" measurements were made by recording the level of water in the culture chambers with a plastic ruler taped to the outside surface of

each flask. These linear measurements were then converted to volumetric changes. In this manner, changes in culture volume could be easily determined without disturbing the culture. Inflow volumes were then calculated using outflow and culture volume data. It did not prove practical to rely upon drip rates or changes in the influent reservoir volume over time to calculate individual culture inflow rates. Outflow rates were averaged on a time-weighted basis for each major time period that preceeded sampling for primary productivity, pigments, and algal identification (i.e. longer time intervals received greater weight in the calculation).

Primary productivity. Primary productivity determinations were divided for analysis and interpretation purposes into five groups, as shown in Table 6. The basic monitoring of culture productivity (determination group No. 4) was accompanied by productivity determinations for the raw SIR surface water (No. 1), and for water that had been stored in the influent chamber reservoir for ca. 24 hours (No. 3). To test for an acute response to elevated NaCl-salinity, the productivity of freshly sampled SIR surface water with unaltered algal populations was tested at 3 salinity levels (No. 2). To test for the effect of the continuous flow bioassay on the acute response of cultured populations to elevated NaCl-salinity, the productivity of culture water samples amended with additional NaCl was determined for selected times (No. 5).

All determinations were made using 24-ml borosilicate glass scintillation vials. These screw-capped vials were then turned upside down, randomly assigned to a position in a test tube rack, and placed under the same light bank used for the cultures. Incubation periods

Table 6. Primary productivity determination groups for a 170-hour laboratory chemostat bioassay.

Group No.	Productivity determination	Experimental day							
		0	1	2	3	4[a]	5	6	7
1	Raw SIR Surface water	X	X	X	X		X	X	X
2	Raw SIR Surface water plus additional NaCl	X					X	X	X
3	Influent storage reservoir water		X	X	X				
4	Raw culture chamber water	X	X	X	X	X	X	X	X
5	Culture chamber water plus additional NaCl							X	X

[a] NaCl additions began to treatment cultures.

SIR = Sangre Isle Reservoir.

X = productivity determination.

ranged between 2.0 and 4.4 hours in the later one-half of the light period. Other specific details for the productivity determinations are the same as those for the small bottle in situ chemostat bioassays previously discussed.

Salinity. Culture salinity was measured using the electrical conductivity method at 25 °C. Sodium chloride additions began at 93.5 hours. Daily measurements prior to this time were increased in frequency (4-8 times/day) following the salinity increase in the treatment cultures. Attempts were made to maintain treatment culture salinity at ca. 1000 μmhos (ca. 5 times natural SIR conductivity), by frequent measurements and adjustment of the NaCl input.

For productivity determination group No. 2 (see Table 6), three acute salinity levels were used. Additions of 19 μl, 80 μl, and 144 μl of 5N NaCl increased conductivity in the incubation bottles ca. 460, 1800, and 3400 μmhos respectively.

For productivity determination group No. 5, control culture samples received 80 μl 5N NaCl, and treatment culture samples 45 μl prior to incubation. These additions raised conductivity in both control and treatment incubation bottles to a roughly equal conductivity of 2000 μmhos.

This procedure was used to test the hypothesis that treatment culture phytoplankton would exhibit a greater degree of tolerance to further acute NaCl-salinity increases after being cultured at a moderately elevated salinity, than control culture phytoplankton.

Temperature. Culture water temperature was measured at least twice daily using a mercury thermometer. Sangre Isle Reservoir temperature was also monitored at the collection site to determine the difference

between the natural conditions and the lab environment.

Algal pigments. Chlorophyll a and phaeopigments were determined using the procedure described previously. Sample volumes for raw SIR influent water (n=3) and cultures (n=2) were 150 ml and 50 ml, respectively. Culture samples were withdrawn daily between 1340 and 1623 hours. On day 4 at 0945 hours, an additional pigment determination was made just prior to the initial salinity increase to delimit any potential salinity-induced pigment changes.

Phytoplankton enumeration and analysis. Ten-ml culture samples for algal identification and quantification were taken at 24, 70, and 166 hours, at the same time samples were withdrawn for pigment and productivity analyses. For comparison purposes, day 0, 3, and 7 samples for influent SIR water were counted. Identification was to the generic level, with all other enumeration and computational details employed the same as those used for the in situ chemostat bioassays.

Statistical analyses. Statistical Analysis Systems, Inc. (SAS) computer programs were used for analysis of variance and multiple range tests. A significance level of 0.10 was used. Other statistical methods used have been presented previously.

Results

In situ Chemostat Bioassays

Ninety-two Hour Bioassay

Flow rates. Table 7 lists the average flow rate for each submerged culture chamber of the chemostat. Also tabulated are the resulting dilution rate and mean residence time. Poor correlation existed between

Table 7. Flow rate characteristics in control and treatment chambers for a 92-hour in situ chemostat bioassay.

Time interval No.	Time interval (hours)	ΔT (hours)	Control chamber			Treatment chamber		
			Average flow rate[a]	Dilution rate[b]	Mean residence time[c]	Average flow rate	Dilution rate	Mean residence time
1	0 - 19.5	19.5	0	0	ND	0	0	ND
2	19.5 - 40.8	21.3	71	0.03	36	198	0.08	13
3	40.8 - 65.0	24.2	0	0	ND	68	0.03	37
4	65.0 - 89.7	24.7	0	0	ND	206	0.08	12

[a] Average flow rate in milliliters per hour.

[b] Dilution rate ($hours^{-1}$) calculated by: average flow rate (ml h^{-1})/culture volume (ml).

[c] Mean residence time (hours) calculated by: 1/dilution rate.

ND = No data.

control chamber and treatment chamber flow rates. Extensive periods of no flow occurred for both culture chambers, and this problem was especially acute for the control chamber.

Salinity. Additions of NaCl solutions to the chemostat culture resulted in a "stepwise" increase in conductivity, while the control culture and Sangre Isle Reservoir (SIR) remained at normal levels (Figure 5). Each injection independently raised the NaCl concentration in the flask ca. 175 mg l^{-1} (3.0×10^{-3} M). The different rate of decline in conductivity during time intervals 3 and 4 was caused by different flow rates through the culture chamber during each interval. At 92 h, most of the high conductivity water had been displaced from the culture, as shown by a conductivity of 232 μmhos.

Primary productivity. Primary productivity estimates for both the 41-h and 92-h incubations are shown in Figure 6. At 41 h, enclosure effects caused by the 24-ml bottles significantly reduced productivity as compared to the 125-ml bottles. This reduction was not apparent at 92 h. Comparisons between the 24-ml bottle productivity estimates for treatment culture, control culture, and SIR (all at "normal" conductivities), showed no significant differences at either 41 or 92 hours.

Pigments and chlorophyll a activity. For comparison purposes, chlorophyll a and phaeopigment estimates for 16 and 18 October (pre-bioassay) are included in Figure 7 with all pigment data for the 92-h bioassay. In response to the three fold increase in turbidity that occurred on 20 October, both chlorophyll a and phaeopigment decreased from pre-bioassay concentrations. An apparent lag period was present between the rise in turbidity and the decrease in pigment

Figure 5. Treatment culture, control culture, and Sangre Isle Reservoir conductivity during a 92-h in situ bioassay. Time intervals 1-4 are delimited above the abscissa. (TC = treatment culture; CC = control culture; SI = Sangre Isle Reservoir)

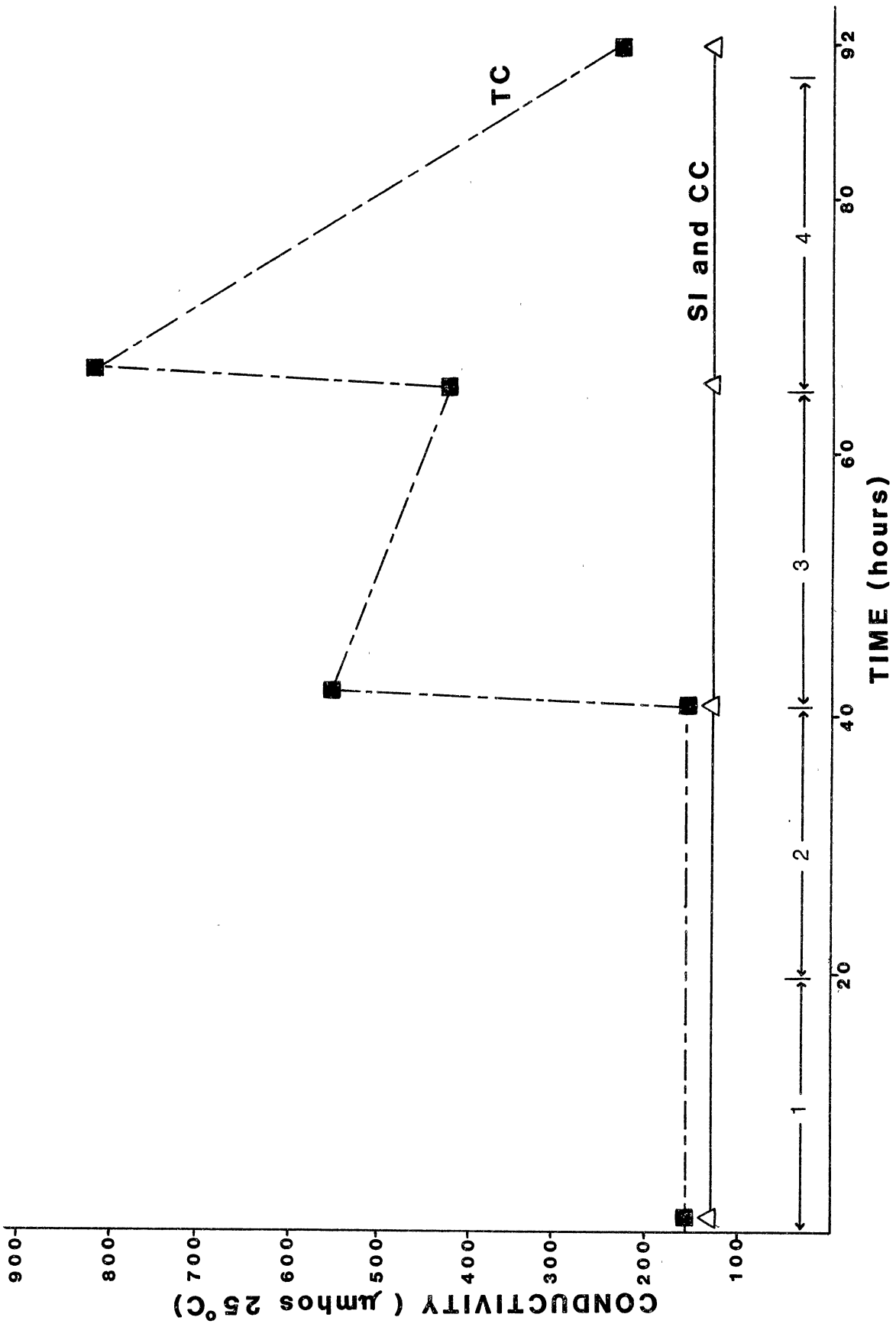

TC
SI and CC
TIME (hours)
1
2
3
4
20
40
60
80
92
CONDUCTIVITY (μmhos 25°C)
100
200
300
400
500
600
700
800
900

Figure 6. Primary productivity during a 92-h in situ bioassay for Sangre Isle Reservoir (SI), control culture (CC), and treatment culture (TC). The open bars represent productivity estimates derived from 24-ml bottles, and the stippled bars for 125-ml bottles. Error bars indicate $\pm$ 1SD. Conductivity during the C-14 incubation period is shown at the base of each bar in µmhos at 25 ^{o}C.

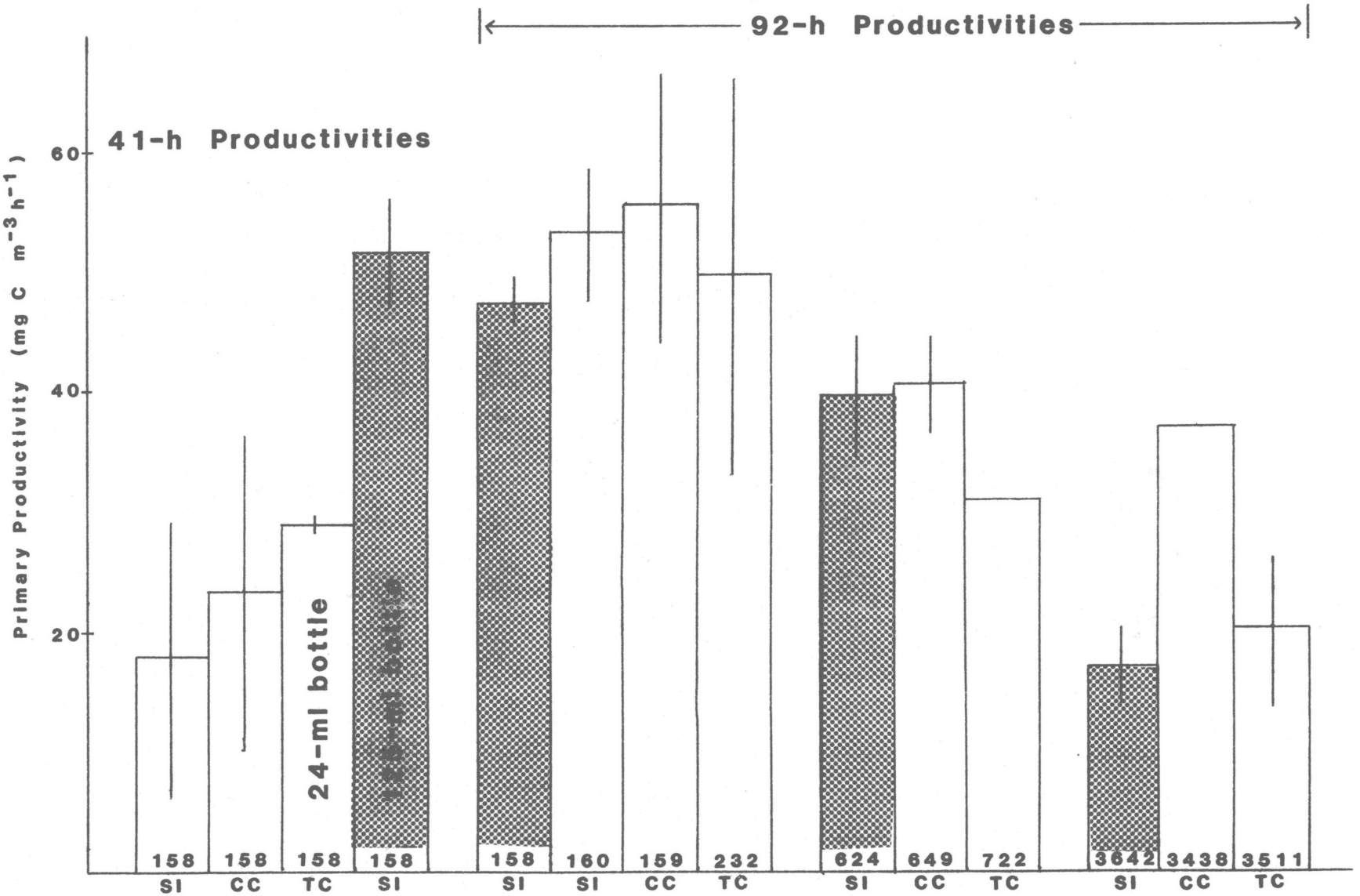
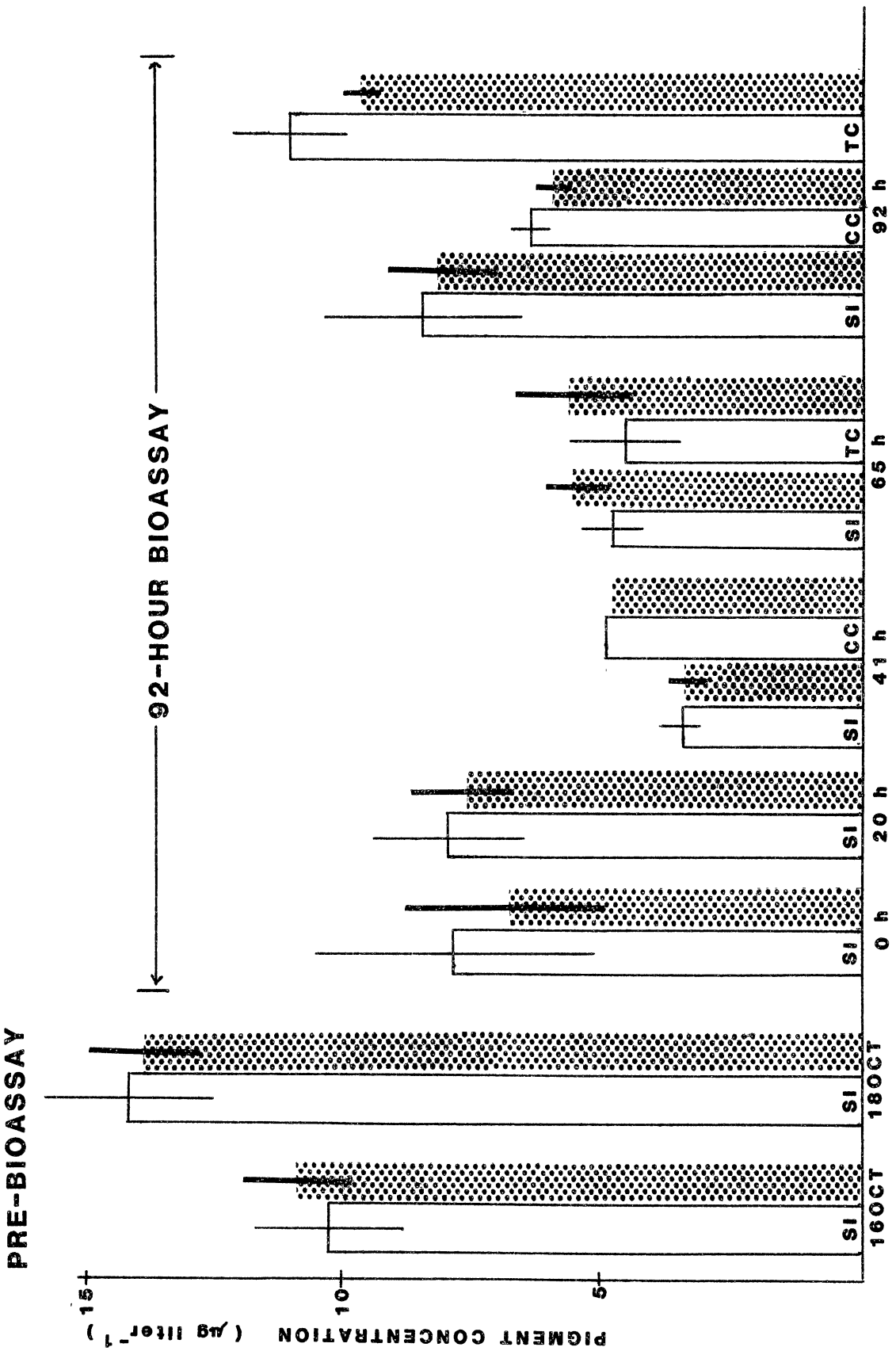

92-h Productivities
41-h Productivities
Primary Productivity (mg C m-3 h-1)
60
40
20
24-ml bottle
158
SI
158
CC
158
TC
158
SI
158
SI
160
SI
159
CC
232
TC
624
SI
649
CC
722
TC
3642
SI
3438
CC
3511
TC

Figure 7. Chlorophyll *a* (open bars) and phaeopigments (stippled bars) during a pre-bioassay period (16 and 18 Oct) and a 92-h in situ bioassay in Sangre Isle Reservoir. Error bars indicate ± 1SD for Sangre Isle Reservoir (SI), control culture (CC), and treatment culture (TC).


PRE-BIOASSAY
92-HOUR BIOASSAY
PIGMENT CONCENTRATION (µg liter⁻¹)
15
10
5
16OCT
18OCT
0 h
20 h
41 h
65 h
92 h
SI
CC
TC

concentrations. Excluding 16 October and the 65-h estimates on 26 October, all samples exhibit phaeopigment concentrations slightly less than chlorophyll *a*. The only statistically significant differences occurred at 92 h, when the treatment chemostat culture showed higher levels of both functional and non-functional chlorophyll *a*, when compared to the static control culture. The resultant salinity regime in the treatment culture did not alter the concentration of either pigment significantly from SIR levels.

Chlorophyll *a* activity decreased sharply from a high pre-bioassay level of 14.0 mg C h^{-1} mg Chl. a^{-1} on 18 October, to turbidity-depressed levels at both 41 and 92 hours (Figure 8). At 92 h, the static control culture ratio was ca. 28% greater than the ratio for SIR, and ca. 50% higher than the treatment culture ratio.

Physiochemical variables. Secchi disk transparency (Figure 9) and light transmission (Figure 10) both decreased in response to the 20 October turbidity increase (Figure 9). At the 0.4-0.5-m culture chamber depth, light levels remained above 1% of surface intensity for the entire experimental period. Turbidity for the treatment culture was ca. 21% above reservoir levels at 92 h (27 October), while the control chamber was ca. 26% below SIR turbidity at the same time.

Water temperature at Station 1 in SIR and at the chemostat test site remained essentially isothermal. Temperatures ranged from 15.9 to 18.8 °C (Figure 11).

Phytoplankton enumeration and analysis. Figures 12 and 13 show relative proportions of the top five algal genera as percents of total numbers per milliliter reservoir or culture water and total cell volume. Total numeric and volumetric densities are also shown. The initial

Figure 8. Chlorophyll *a* activity for 18 October (pre-bioassay), and for 92-h and 22-h in situ bioassays. Ratio estimates are shown for Sangre Isle Reservoir (SI), treatment culture (TC), and control culture (CC).

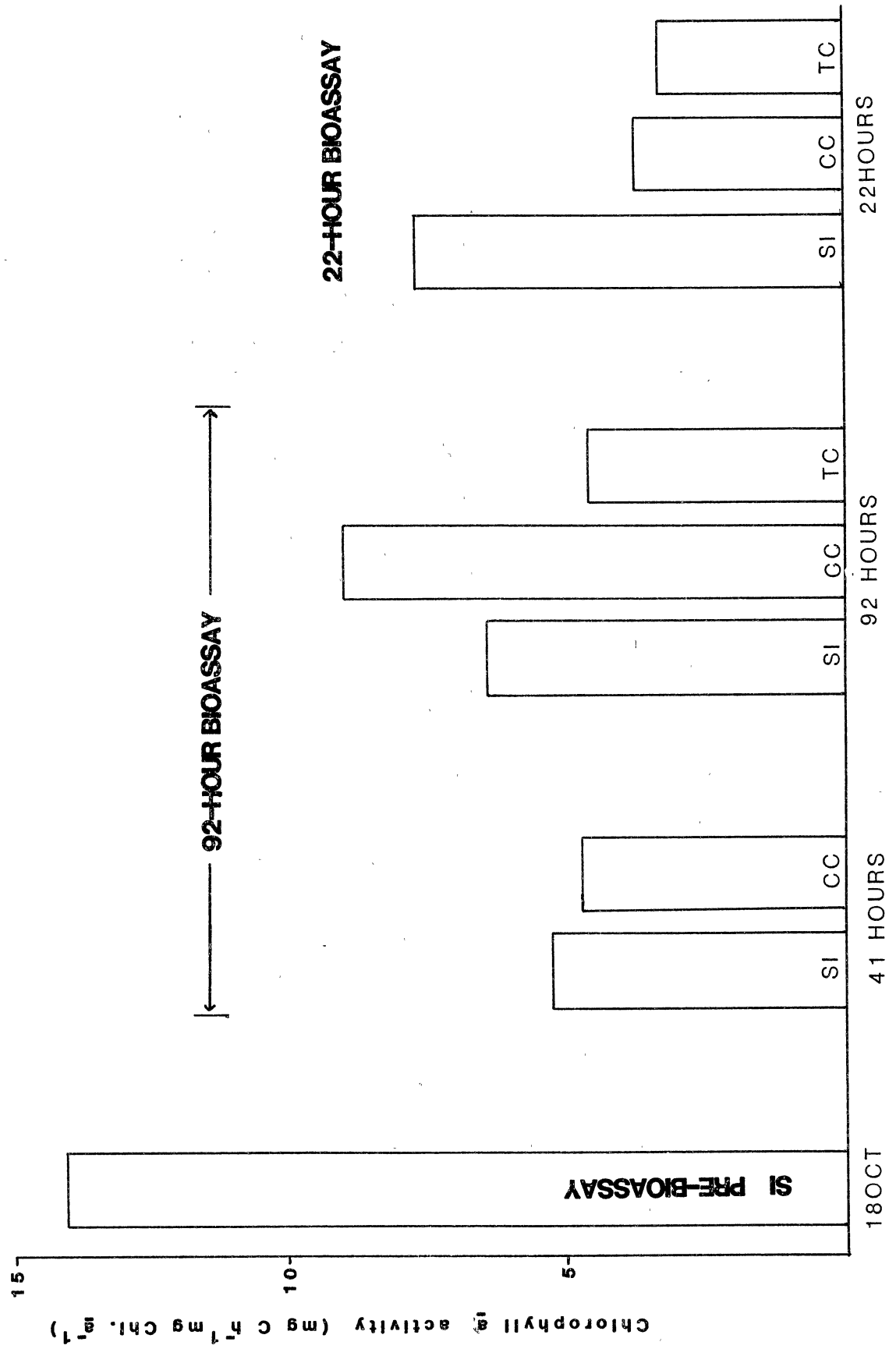

Chlorophyll a activity (mg C h⁻¹ mg Chl. g⁻¹)
15
10
5
SI PRE-BIOASSAY
18OCT
SI
CC
41 HOURS
92-HOUR BIOASSAY
SI
CC
TC
92 HOURS
22-HOUR BIOASSAY
SI
CC
TC
22HOURS

Figure 9. Turbidity (0.1-m depth) and Secchi depth (SD) for Sangre Isle Reservoir from 18 to 30 October 1983, with selected chemostat culture turbidities. Turbidity units are nephelometric turbidity units (NTU). (Control culture =⬡; Treatment culture = ▲)

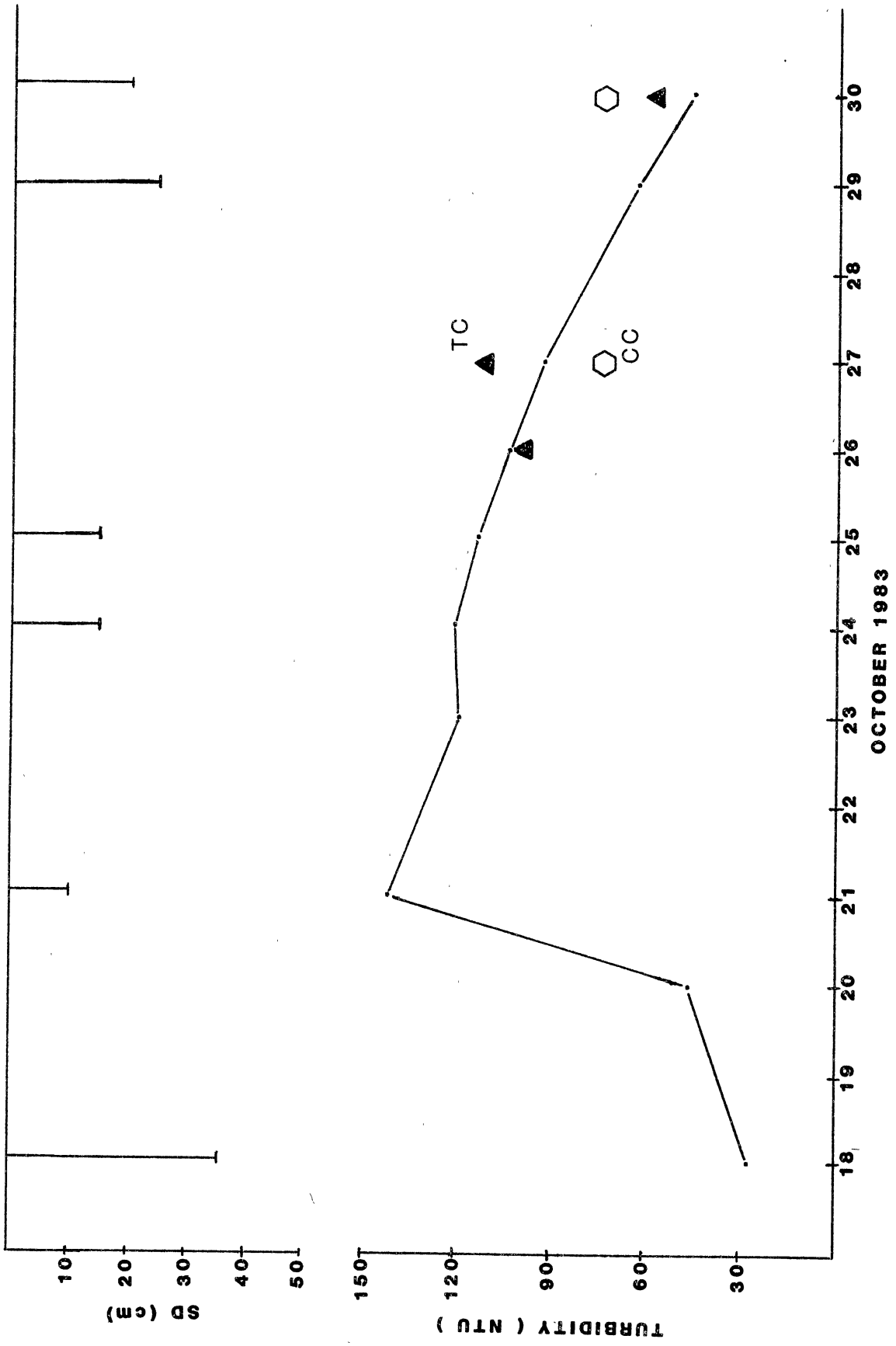

SD (cm)
10
20
30
40
50
TURBIDITY (NTU)
150
120
90
60
30
TC
CC
18
19
20
21
22
23
24
25
26
27
28
29
30
OCTOBER 1983

Figure 10. Light extinction in Sangre Isle Reservoir for 18, 24, 25, and 30 October 1983. All measurements were taken at the chemostat test site. (S = surface intensity)

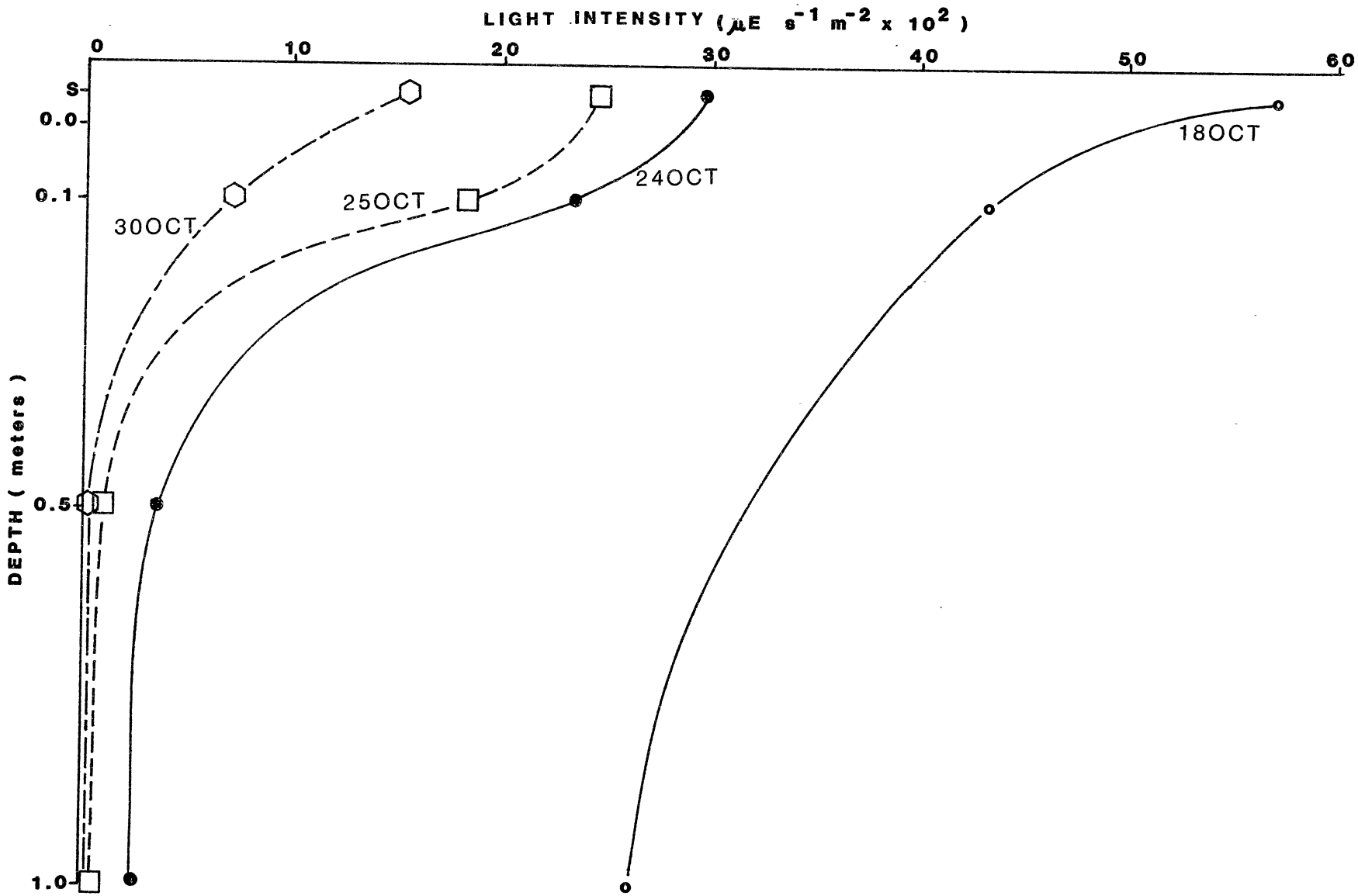

LIGHT INTENSITY (μE s⁻¹ m⁻² x 10²)
0
10
20
30
40
50
60
S
0.0
0.1
0.5
1.0
DEPTH (meters)
30OCT
25OCT
24OCT
18OCT

Figure 11. Temperature profiles for Sangre Isle Reservoir at Station 1 (30 October 1983) and at the chemostat test site (CTS) on 18, 24, and 30 October 1983.

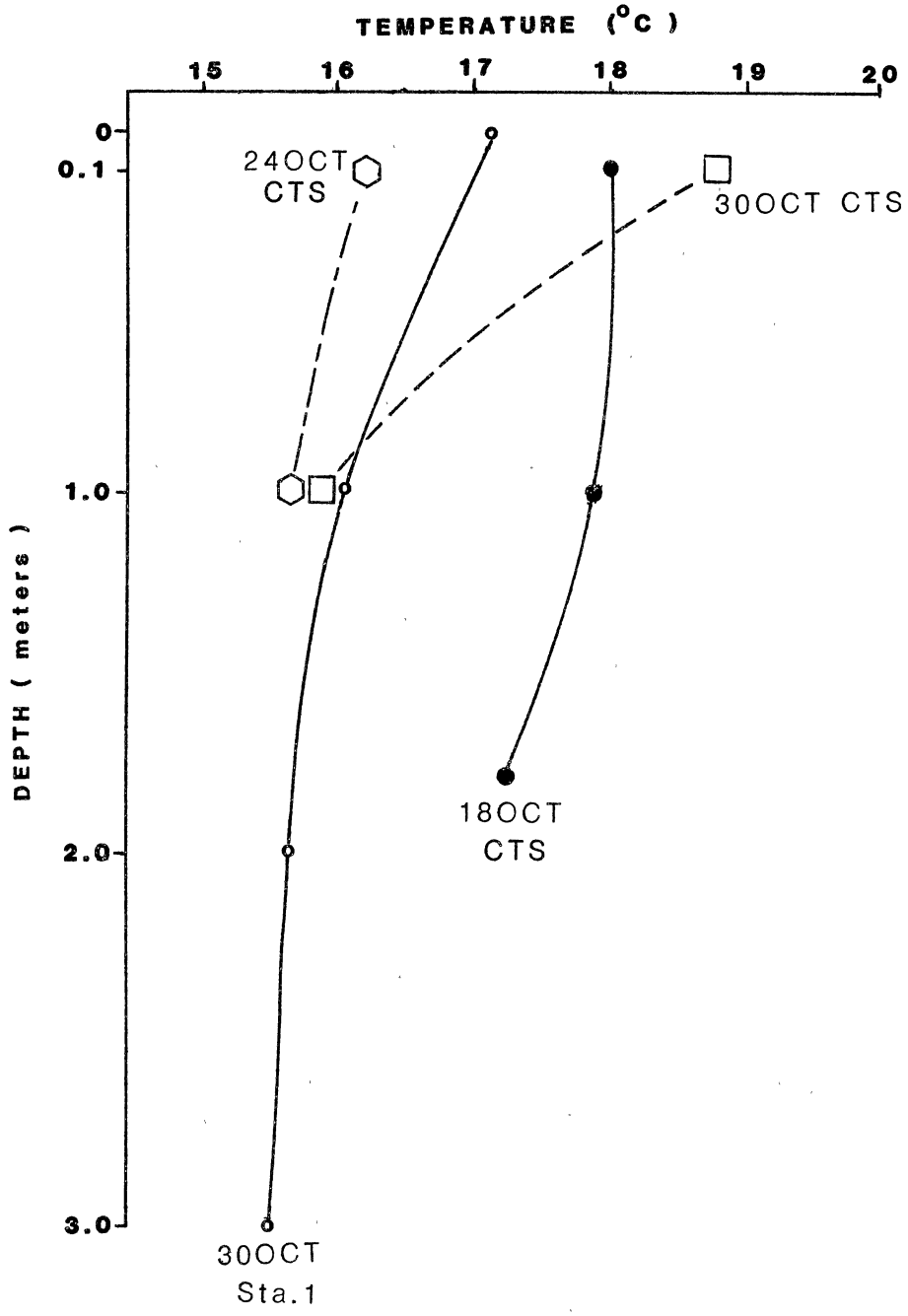

TEMPERATURE (°C)
15
16
17
18
19
20
0
0.1
1.0
2.0
3.0
DEPTH (meters)
24OCT
CTS
30OCT CTS
18OCT
CTS
30OCT
Sta.1

Figure 12. Algal density and species distribution for initial Sangre Isle Reservoir water placed into both the treatment and control cultures for a 92-h in situ bioassay. Open bars are percents of either total numeric or volumetric density. Horizontally-striped bars represent total algal density as cells/ml. Vertically striped bars represent total algal cell volume. Bar numbers correspond to the following algal genera:
1 = Ankistrodesmus; 2 = Chroococcus; 3 = Cryptomonas;
4 = Oscillatoria; 5 = Synedra; 6 = all other genera.

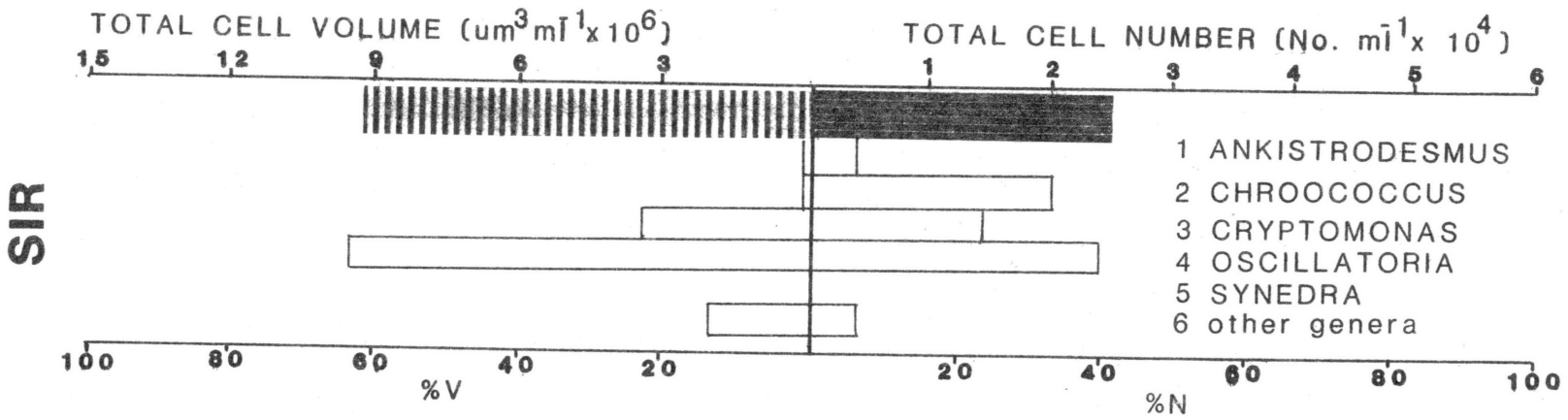
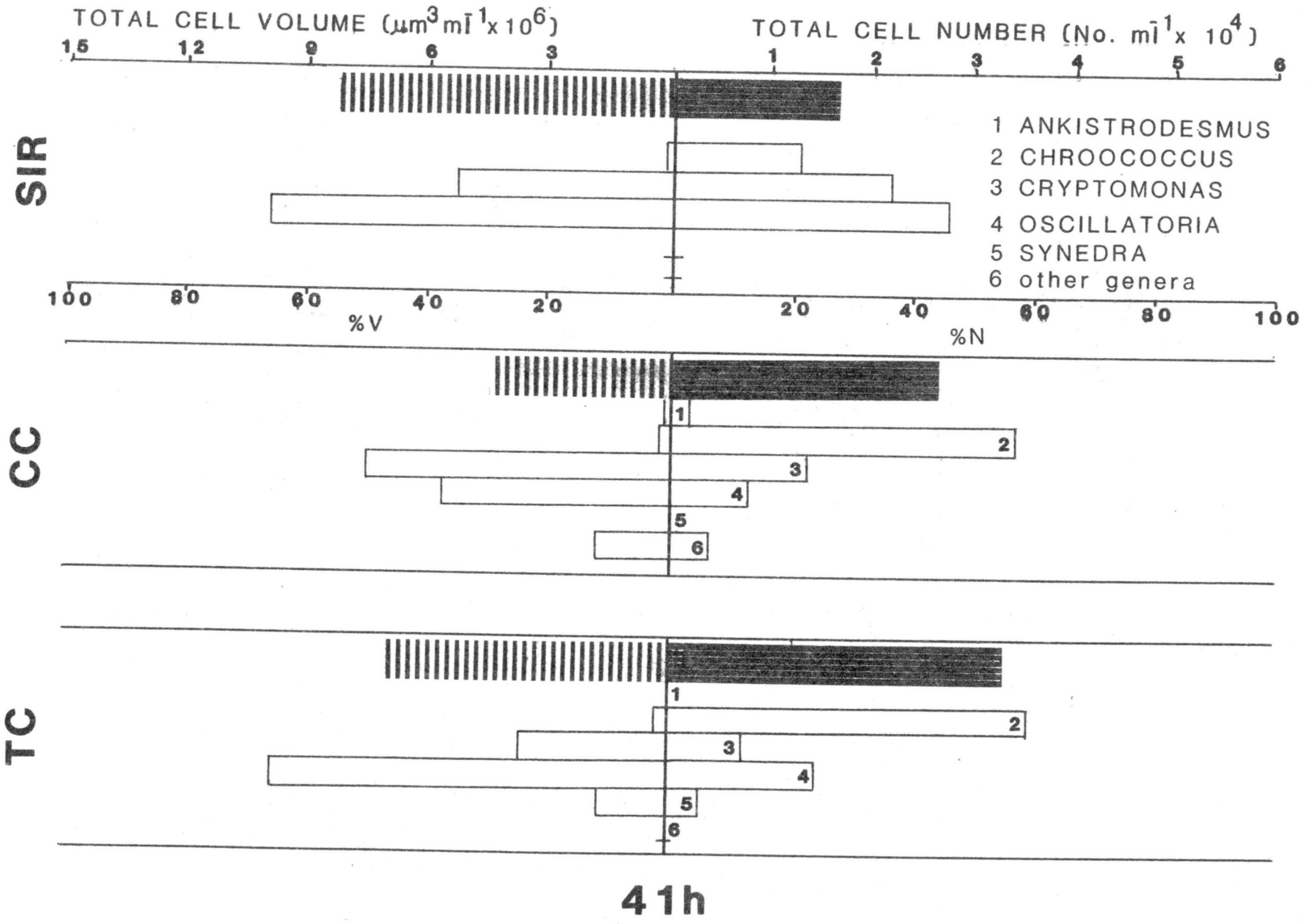

TOTAL CELL VOLUME (um3 ml-1 x 10^6)
TOTAL CELL NUMBER (No. ml-1 x 10^4)
1,5
1,2
9
6
3
1
2
3
4
5
6
SIR
1 ANKISTRODESMUS
2 CHROOCOCCUS
3 CRYPTOMONAS
4 OSCILLATORIA
5 SYNEDRA
6 other genera
100
80
60
40
20
%V
20
40
60
80
100
%N

Figure 13. Algal density and species distribution at 41 h and 92 h for a 92-h in situ bioassay. Estimates are presented for Sangre Isle Reservoir (SIR), control culture (CC), and treatment culture (TC). Open bars are percents of either total numeric or volumetric density. Horizontally-striped bars represent total algal cell volume. Bar numbers correspond to the following algal genera:
1 = Ankistrodesmus; 2 = Chroococcus; 3 = Cryptomonas;
4 = Oscillatoria; 5 = Synedra; 6 = all other genera.


TOTAL CELL VOLUME (µm³ml⁻¹x10⁶)
1.5
1.2
.9
.6
.3
TOTAL CELL NUMBER (No. ml⁻¹x 10⁴)
1
2
3
4
5
6
SIR
1 ANKISTRODESMUS
2 CHROOCOCCUS
3 CRYPTOMONAS
4 OSCILLATORIA
5 SYNEDRA
6 other genera
100
80
60
40
20
%V
20
40
60
80
100
%N
CC
TC
41h

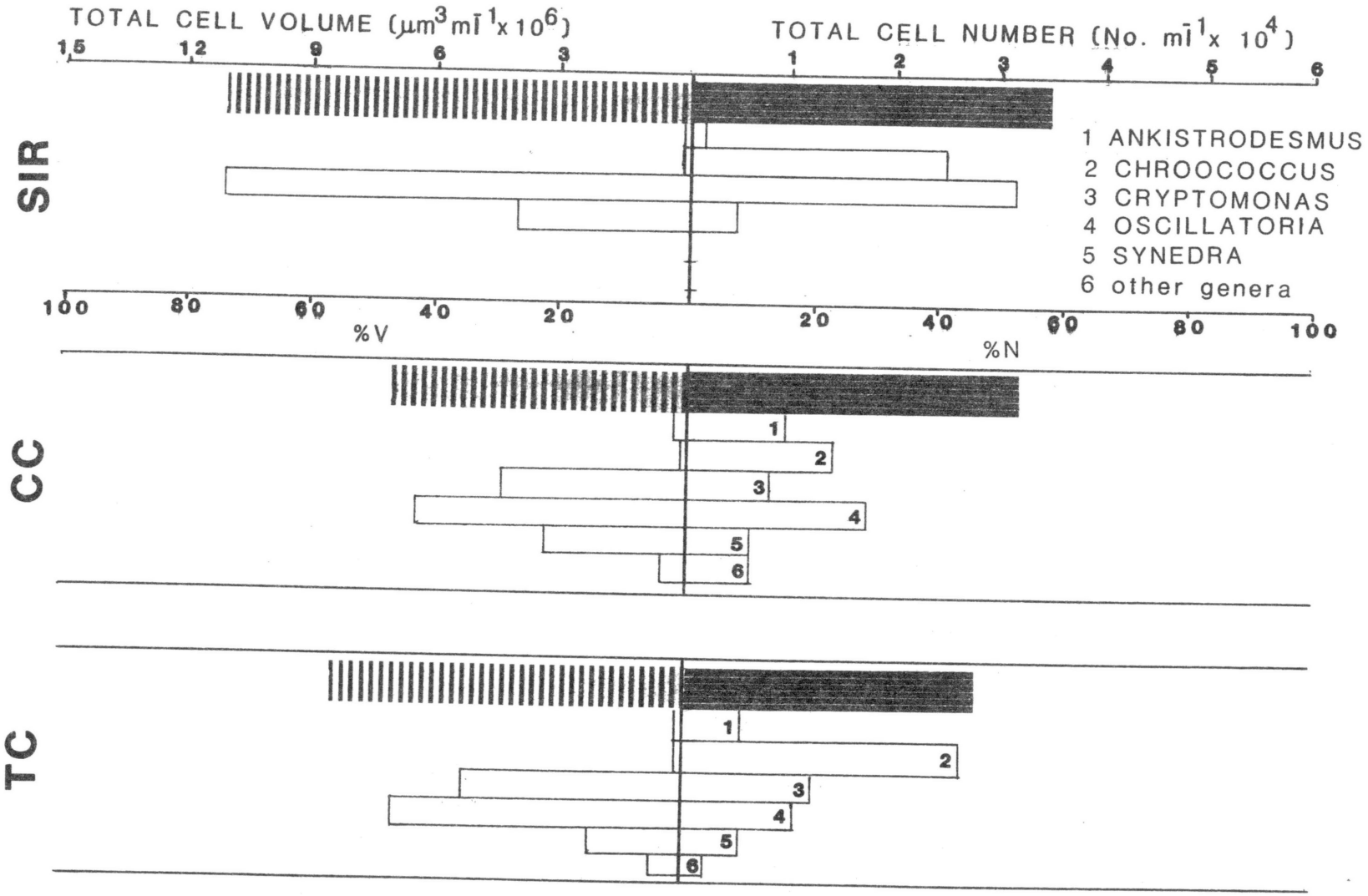


Figure 13 continued - 92h

influent to the cultures was dominated by the blue-green filamentous alga Oscillatoria, and by members of the genus Cryptomonas. For SIR, this codominance continued throughout the 92-h duration, although a shift in primary dominance from Oscillatoria to Cryptomonas occurred between 41 and 92 h. This shift did not occur to the same extent in the treatment or batch culture. At 41 h, species diversity ($\bar{d}$) calculated from biovolume data ($\bar{d}_V$) was higher for both cultures than for SIR (Table 8). This was apparently due to a higher species richness component of diversity in the cultures. At 92 h, $\bar{d}_V$ variation between cultures and SIR was due more in part to a lower equatability (E_V) for the SIR association than to increased species richness for the cultures.

Results from similarity index comparisons using the Percentage of community similarity (Psc) index appear in matrix format in Figure 14. Comparisons for both cultures versus SIR show decreases in similarity occurring between 41 and 92 h, while the control versus treatment culture comparison increased slightly. The significance of these differences is not known.

Twenty-two Hour Bioassay

Flow rates. Control chamber flow rates showed wide variation between time intervals 1 and 2 (Table 9). Treatment chamber flow rates exhibited more consistency throughout the 22-h experiment. All flow rates were higher than desired. The static conditions that dominated the control culture during the 92-h bioassay were avoided in both culture chambers during this bioassay.

Salinity. The 0-h injection of NaCl into the treatment culture

Table 8. Algal abundance and community structure parameters for a 92-hour in situ bioassay.

Algal association variable	0 h	41 h			92 h		
	SIR[a]	SIR	CC[b]	TC[c]	SIR	CC	TC
Species richness	5	3	7	6	5	8	6
Equitability (E_v)	0.65	0.77	0.64	0.57	0.49	0.61	0.75
Total cell density (No. milliliter^{-1} x 10^4)	2.5	1.6	2.6	3.3	3.5	3.2	2.8
Species diversity ($\bar{d}_n$)	2.00	1.51	1.98	1.77	1.71	2.75	2.18
Total cell volume (μm^3 milliliter^{-1} x 10^6)	9.2	8.2	4.5	7.1	11.1	7.0	8.5
Species diversity ($\bar{d}_v$)	1.39	0.96	1.80	1.44	1.04	1.89	1.79

[a] Sangre Isle Reservoir

[b] Control culture

[c] Treatment culture

Figure 14. Comparison of algal associations in Sangre Isle Reservoir (SI), control culture (CC), and treatment culture (TC) using the Percentage of community similarity (Psc) index based on biovolume at 41 and 92 h for a 92-h in situ bioassay. At 41 h for example, the SI vs. CC comparison shows 72% similarity between their respective algal associations, while the SI and TC associations were more similar at 87%.

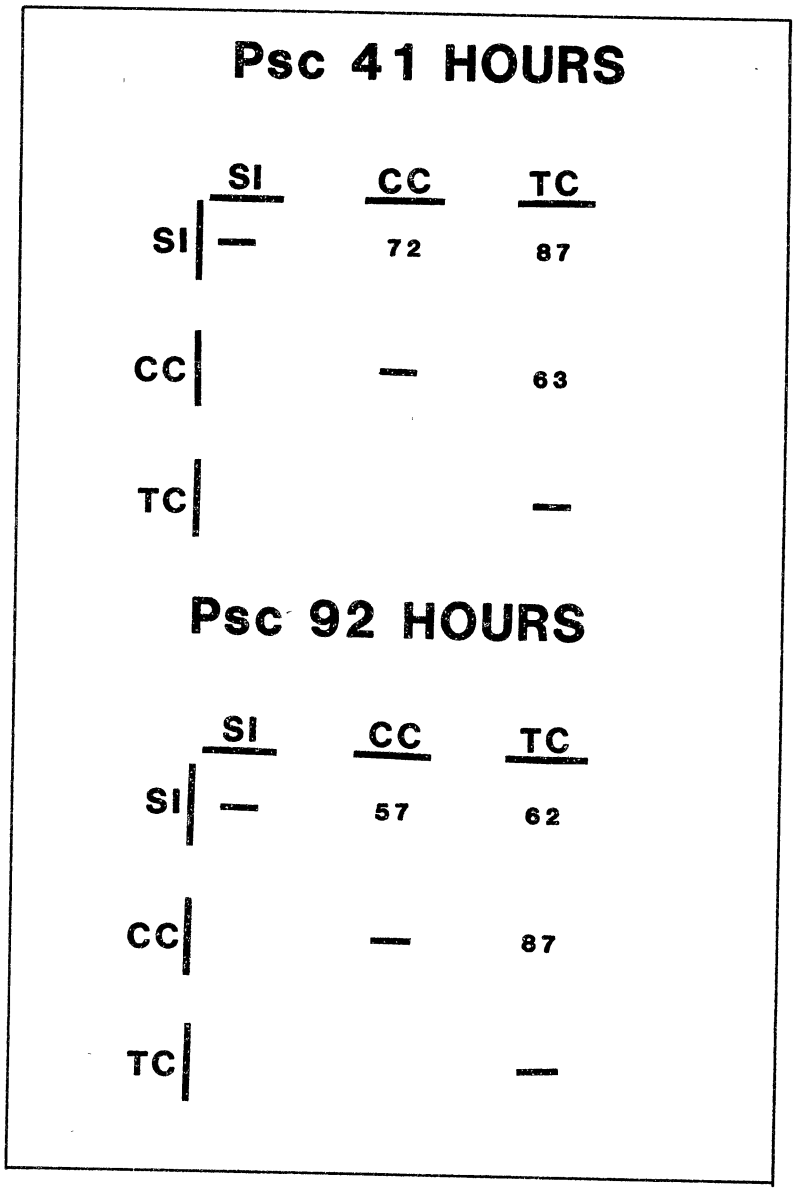

Psc 41 HOURS
SI
CC
TC
SI
—
72
87
CC
—
63
TC
—
Psc 92 HOURS
SI
CC
TC
SI
—
57
62
CC
—
87
TC
—

Table 9. Flow rate characteristics in control and treatment chambers for a 22-hour in situ chemostat bioassay.

Time interval No.	Time interval (hours)	ΔT (hours)	Control chamber			Treatment chamber		
			Average flow rate[a]	Dilution rate[b]	Mean residence time[c]	Average flow rate	Dilution rate	Mean residence time
1	0 - 17.0	17.0	129	0.05	20.0	668	0.27	3.7
2	17.0 - 22.0	5.0	1815	0.73	1.4	460	0.18	5.6

[a] Average flow rate in milliliters per hour.

[b] Dilution rate (hours^{-1}) calculated by: average flow rate (ml h^{-1})/culture volume (ml).

[c] Mean residence time (hours) calculated by: 1/dilution rate.

elevated conductivity ca. 3-fold from the control culture and SIR level of 160 µmhos. The 17-h injection took place just 5 h before pigment and productivity determinations, raising conductivity ca. 6-fold above ambient levels (Figure 15). At 22 h, treatment culture conductivity was 432 µmhos. Additional inputs of 19 µl and 144 µl of 5N NaCl raised conductivity in the incubation bottles an average of 490 µmhos and 3279 µmhos respectively.

Primary productivity. Estimates for chemostat and SIR primary productivities appear in Figure 16. Chemostat productivities were significantly depressed compared to SIR values. Control versus treatment productivities were not significantly different under all salinity regimes tested. Sangre Isle Reservoir phytoplankton significantly decreased productivity ca. 36% and 87% as an acute response to the incubation bottle increases in NaCl. Control culture productivity decreased at 632 µmhos and 3450 µmhos salinity. Treatment culture phytoplankton did not show an acute response to a doubling of conductivity above the previously elevated levels.

Pigments and chlorophyll a activity. The SIR water used for initial culture chamber influent for the experiment contained ca. 26 µg l^{-1} chlorophyll a and 15 µg l^{-1} phaeopigment (Figure 17). At 22 h, SIR chlorophyll a was significantly greater than chlorophyll a concentration in either culture. Treatment and control cultures were not significantly different from each other. Phaeopigment concentrations were not significantly different for SIR and the control culture, but both were greater than treatment culture phaeopigment. Overall phaeopigment variation for all sampled units was generally small compared to chlorophyll a variation.

Figure 15. Treatment culture, control culture, and Sangre Isle Reservoir conductivity during a 22-h in situ bioassay. Time intervals 1 and 2 are delimited above the abscissa. (TC = treatment culture; CC = control culture; SI = Sangre Isle Reservoir)

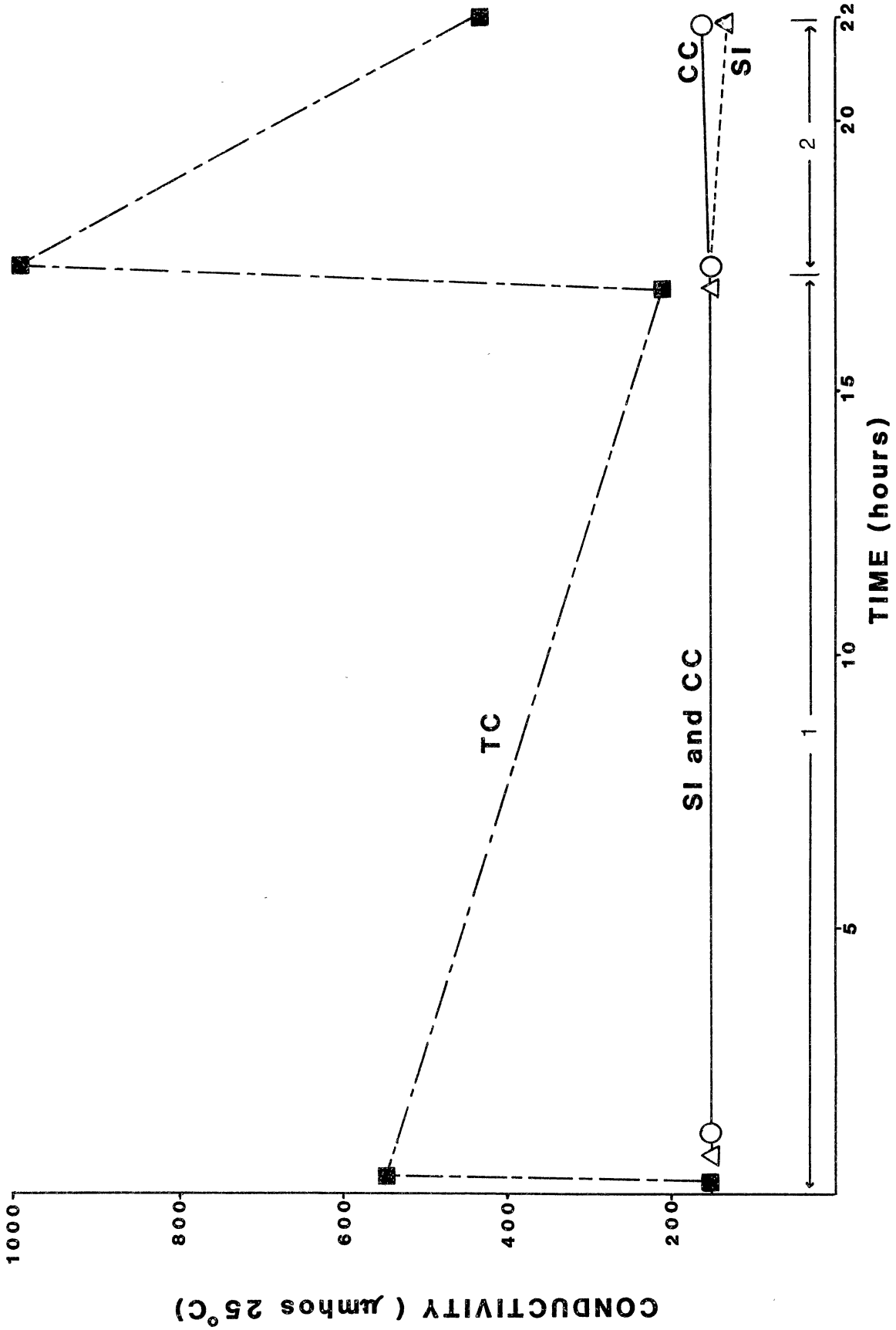

CONDUCTIVITY (μmhos 25°C)
1000
800
600
400
200
TIME (hours)
5
10
15
20
22
1
2
TC
SI and CC
CC
SI

Figure 16. Final primary productivities for a 22-h in situ bioassay. Error bars indicate ± 1SD for Sangre Isle Reservoir (SI), control culture (CC), and treatment culture (TC). Estimates were derived using 24-ml incubation bottles. Conductivity during the C-14 incubation period is shown at the base of each bar in µmhos at 25 °C. The bars are grouped as follows:
Group A = samples incubated at the initial 22-h conductivity;
Group B = samples incubated with an additional 19 µl 5N NaCl;
Group C = samples incubated with an additional 144 µl 5N NaCl.

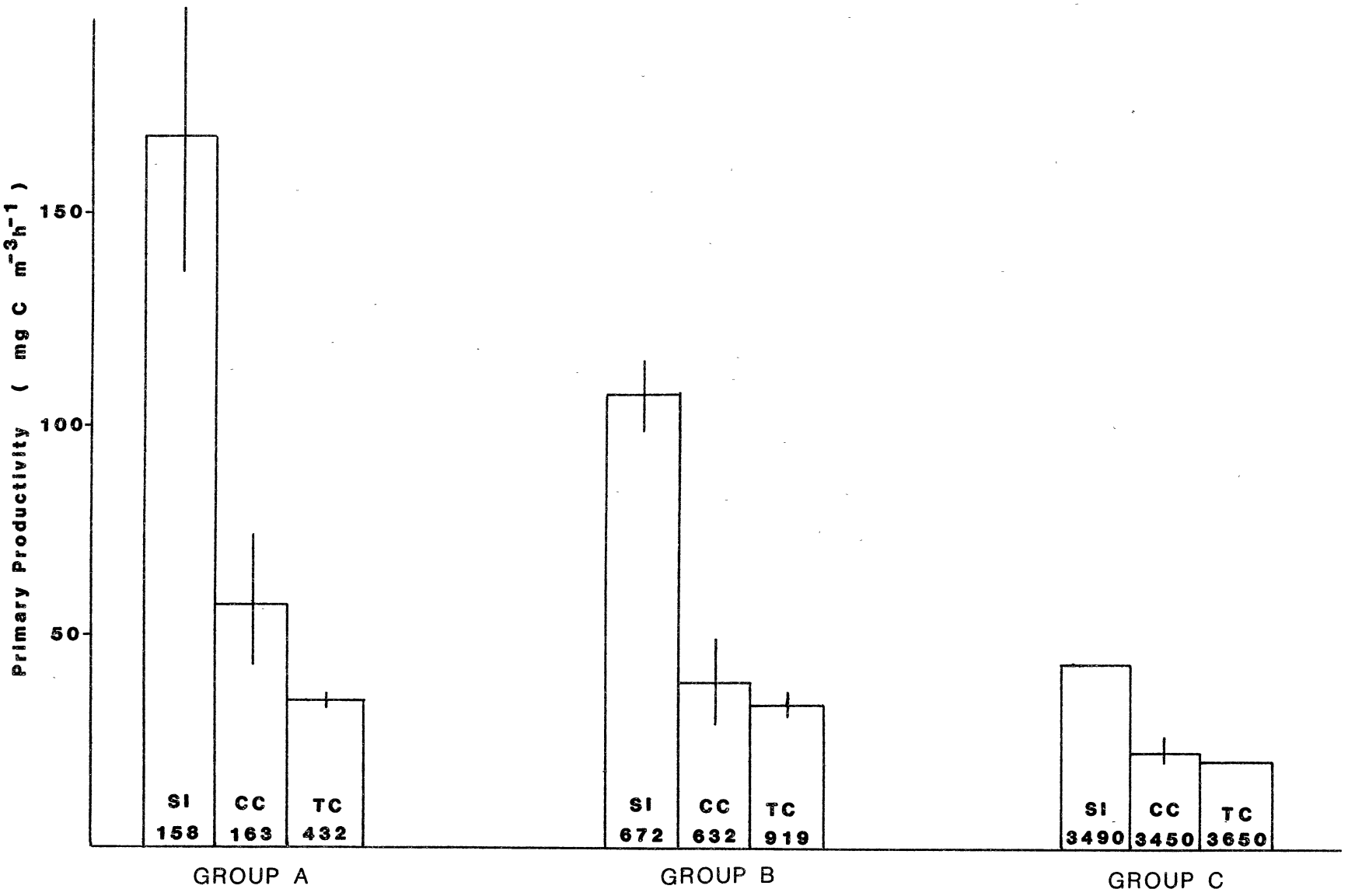

Primary Productivity (mg C $m^{-3}h^{-1}$)
150
100
50
SI
158
CC
163
TC
432
SI
672
CC
632
TC
919
SI
3490
CC
3450
TC
3650
GROUP A
GROUP B
GROUP C

Figure 17. Chlorophyll a (open bars) and phaeopigments (stippled bars) at 0 and 22 h for a 22-h in situ bioassay. Error bars indicate ± 1SD for Sangre Isle Reservoir (SI), control culture (CC), and treatment culture (TC).

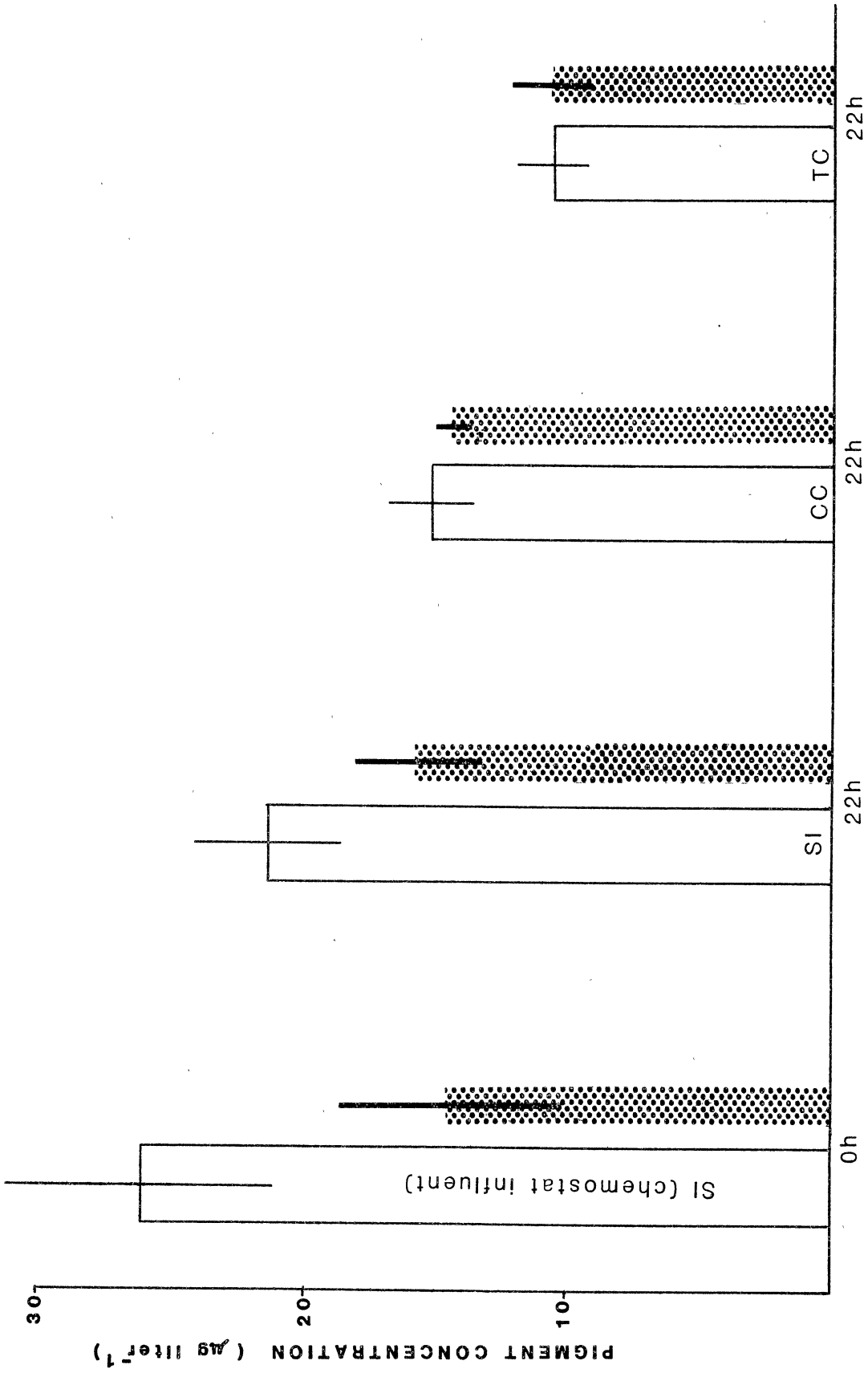

PIGMENT CONCENTRATION (μg liter⁻¹)
30
20
10
SI (chemostat influent)
0h
SI
22h
CC
22h
TC
22h

Figure 18 gives chlorophyll a on both a per cell and per cubic micrometer of cell volume basis. Both show a depressed trend for the treatment culture.

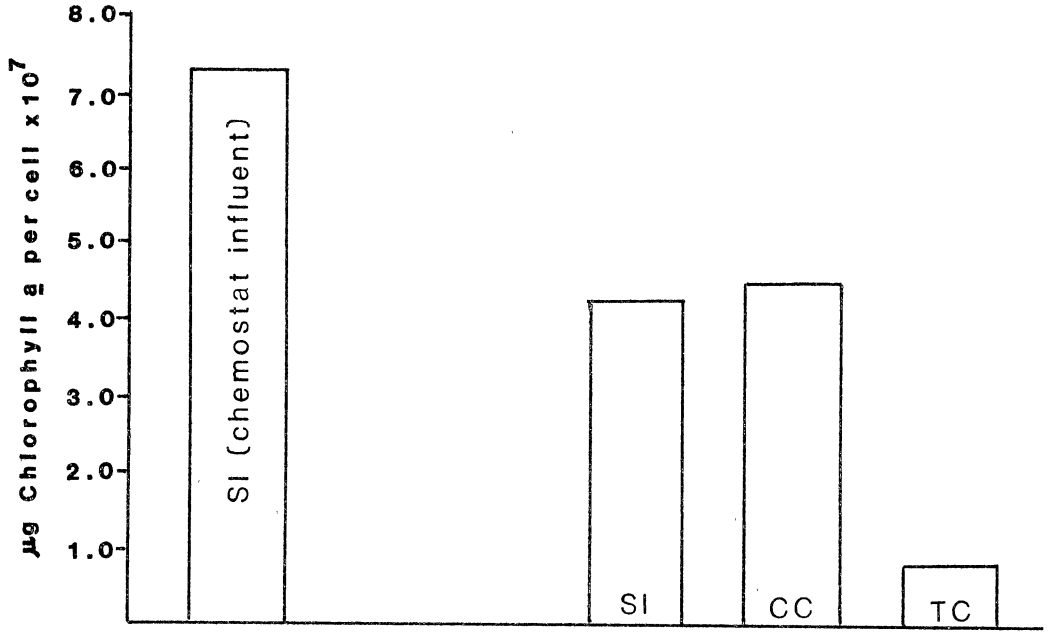
Physiochemical variables. Turbidity in SIR decreased slightly during the bioassay. Turbidity levels were ca. 50 nephelometric turbidity units (NTU) in SIR surface water during the experiment (Figure 9). The culture turbidity at 22 h was elevated above the SIR level for both treatment (38%) and control (79%) cultures, indicating that despite mixing, the enclosures acted as traps for particles causing turbidity.

Secchi depth transparency decreased slightly from 29 to 30 October (Figure 9) with subsurface light intensity for 30 October (Figure 10) less than that for days with significantly higher turbidity during the previous bioassay. Both of these apparent contradictions seem attributable to the ca. 70% cloud cover that was present at 1500 h when these readings were taken.

Reservoir temperature for 30 October at both Station 1 and at the chemostat test site are shown in Figure 11. A slight thermal stratification was present at the chemostat test site at 22 h. Station 1 values were basically isothermal.

Phytoplankton enumeration and analysis. Figures 19 and 20 give taxonomic and species distribution results. The algal taxonomic groupings used for the bar graphs are the same as those for the previous bioassay. Initial influent water to both cultures showed Cryptomonas and Oscillatoria as biovolume dominants. The small colonial blue-green alga Chroococcus was numerically important in the influent water, but due to its small size, was only a minor contributor to the total cell volume. Total cell volume remained constant from 0 to 22 h in SIR.

Figure 18. Chlorophyll a per cell and chlorophyll a per cubic micrometer total algal cell volume for a 22-h in situ bioassay. Ratio estimates are shown for Sangre Isle Reservoir water used for chemostat influent at 0 h, and for Sangre Isle Reservoir (SI), control culture (CC), and treatment culture (TC) at 22 h.


µg Chlorophyll a per cell x 10^7
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
SI (chemostat influent)
SI
CC
TC

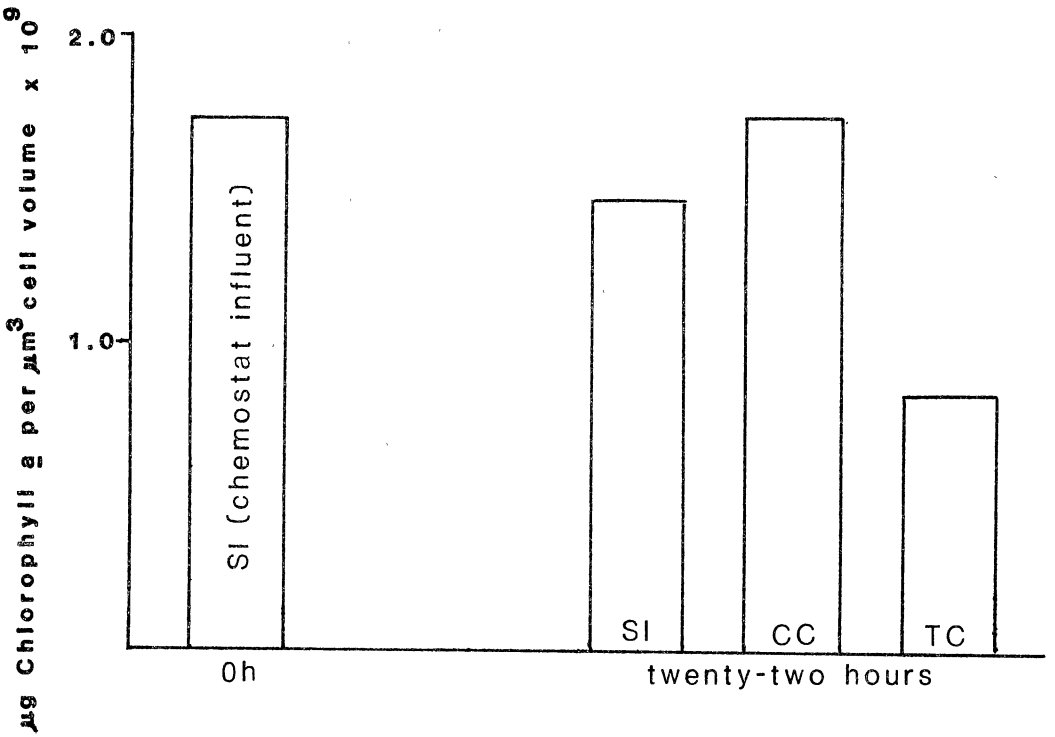

µg Chlorophyll a per µm^3 cell volume x 10^9
2.0
1.0
SI (chemostat influent)
SI
CC
TC
0h
twenty-two hours

Figure 19. Algal density and species distribution for initial Sangre Isle Reservoir water placed into both the treatment and control cultures for a 22-h in situ bioassay. Open bars are percents of either total numeric or volumetric density. Horizontally-striped bars represent total algal density as cells/ml. Vertically-striped bars represent total algal cell volume. Bar numbers correspond to the following algal genera:
1 = Ankistrodesmus; 2 = Chroococcus; 3 = Cryptomonas;
4 = Oscillatoria; 5 = Synedra; 6 = all other genera.

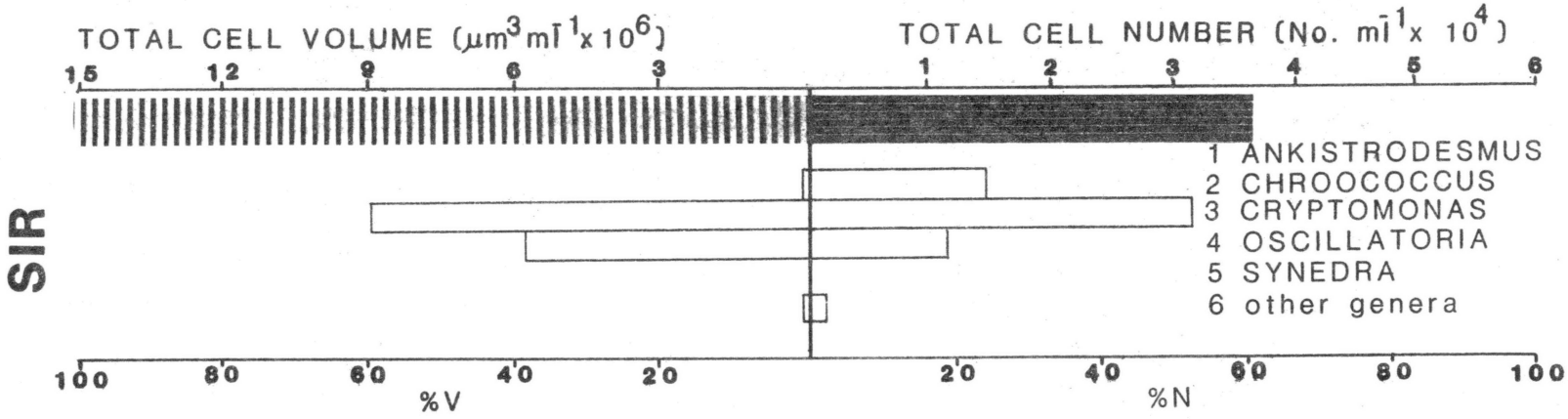
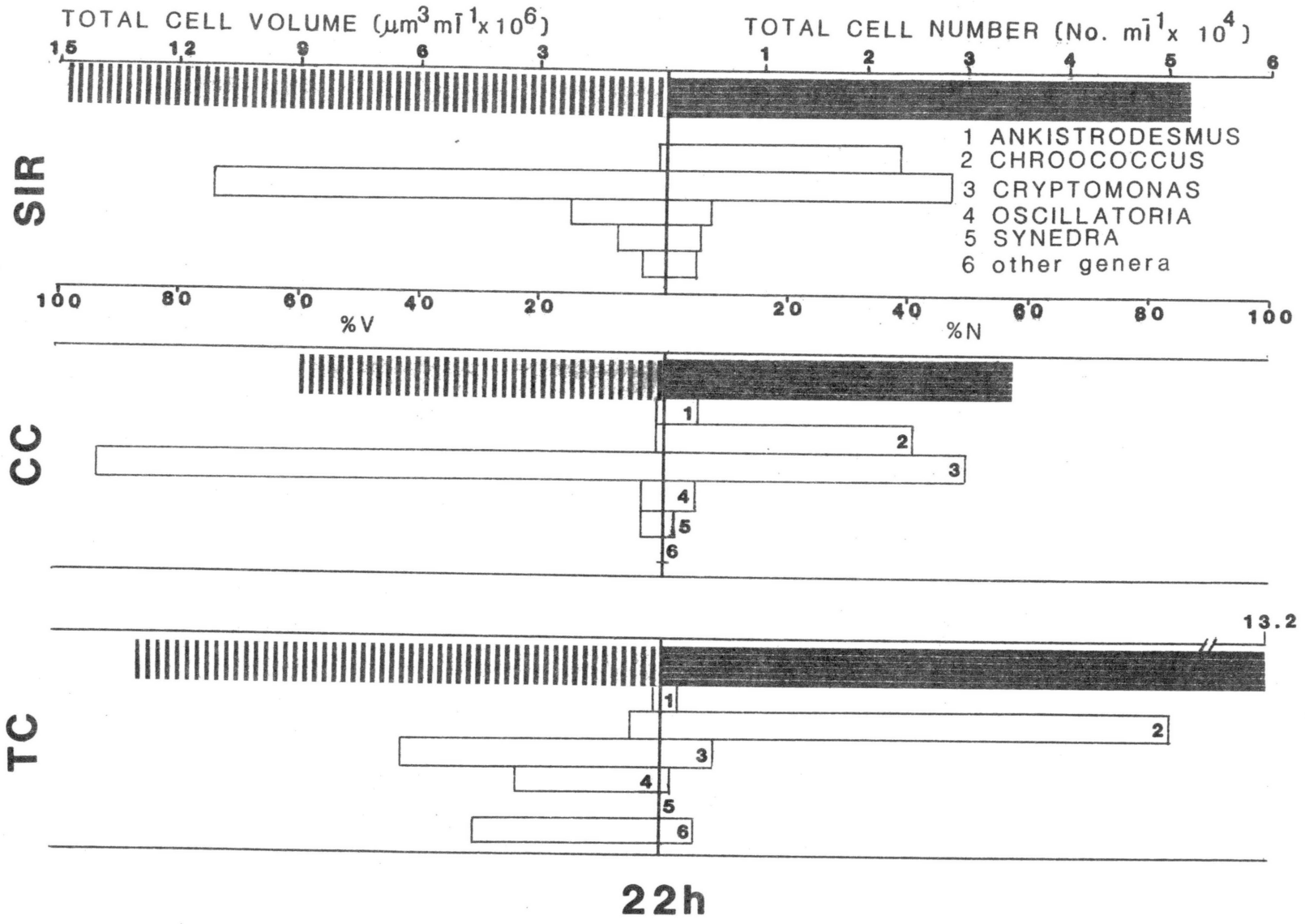

TOTAL CELL VOLUME ($\mu m^3 ml^{-1} \times 10^6$)
TOTAL CELL NUMBER (No. $ml^{-1} \times 10^4$)
15
12
9
6
3
1
2
3
4
5
6
SIR
1 ANKISTRODESMUS
2 CHROOCOCCUS
3 CRYPTOMONAS
4 OSCILLATORIA
5 SYNEDRA
6 other genera
100
80
60
40
20
20
40
60
80
100
%V
%N

Figure 20. Final algal density and species distribution for a 22-h in situ bioassay. Open bars are percents of either total numeric or volumetric density. Horizontally-striped bars represent total density as cells/ml. Vertically-striped bars represent total algal cell volume. Bar numbers correspond to the following algal genera:
1 = Ankistrodesmus; 2 = Chroococcus; 3 = Cryptomonas;
4 = Oscillatoria; 5 = Synedra; 6 = all other genera.


TOTAL CELL VOLUME ($\mu m^3 ml^{-1} \times 10^6$)
TOTAL CELL NUMBER (No. $ml^{-1} \times 10^4$)
1.5
1.2
9
6
3
1
2
3
4
5
6
SIR
1 ANKISTRODESMUS
2 CHROOCOCCUS
3 CRYPTOMONAS
4 OSCILLATORIA
5 SYNEDRA
6 other genera
100
80
60
40
20
%V
%N
CC
TC
13.2
22h

Species diversity based on biovolume ($\bar{d}_V$) remained relatively low in SIR, increased in the 22-h treatment culture, and decreased in the 22-h control culture (Table 10). A large increase in Chroococcus density occurred under NaCl treatment culture conditions. However, testing of both numeric and volumetric densities revealed no statistically significant differences.

Analysis of similarity percentages (Psc-biovolume) (Figure 21) indicate that the SIR algal assemblage at 22 h showed greater similarity to the control culture (81%) than to the treatment culture (60%). Treatment culture versus control culture comparison showed the least similarity (47%). Sangre Isle Reservoir at 0 h and SIR at 22 h were 77% similar. The significance of these differences is not known.

Laboratory Chemostat Bioassay

Influent Water Quality and Usage

Temperature, dissolved oxygen, pH, total alkalinity, and conductivity for the surface water of Sangre Isle Reservoir (SIR) all remained fairly constant throughout the 8-day sampling period (Table 11). The percent saturation of dissolved oxygen was ca. 65% at the constant temperature of 4 °C in SIR. Although not shown, pH means for all cultures during the bioassay ranged from 8.04 to 8.17, with a mean culture temperature of 24.0 °C.

Table 11 also lists the total time a given SIR water sample was used for influent to the four culture chambers, and the total volume used during that time period.

Table 10. Algal abundance and community structure parameters for a 22-hour in situ bioassay.

Algal association variable	0 h	22 h		
	SIR[a]	SIR	CC[b]	TC[c]
Species richness	6	7	6	8
Equitability (E_V)	0.44	0.47	0.32	0.88
Total cell density (No. milliliter^{-1} x 10^4)	3.6	5.2	3.5	13.2
Species diversity ($\bar{d}_n$)	1.88	2.02	1.79	1.12
Total cell volume (μm^3 milliliter^{-1} x 10^6)	15.1	14.8	8.8	12.9
Species diversity ($\bar{d}_V$)	1.11	1.40	0.73	2.31

[a] Sangre Isle Reservoir

[b] Control culture

[c] Treatment culture

Figure 21. Comparison of algal associations in Sangre Isle Reservoir (SI), control culture (CC), and treatment culture (TC) using the Percentage of community similarity (Psc) index based on biovolume for a 22-h in situ bioassay. Only final comparisons at 22 h are shown.

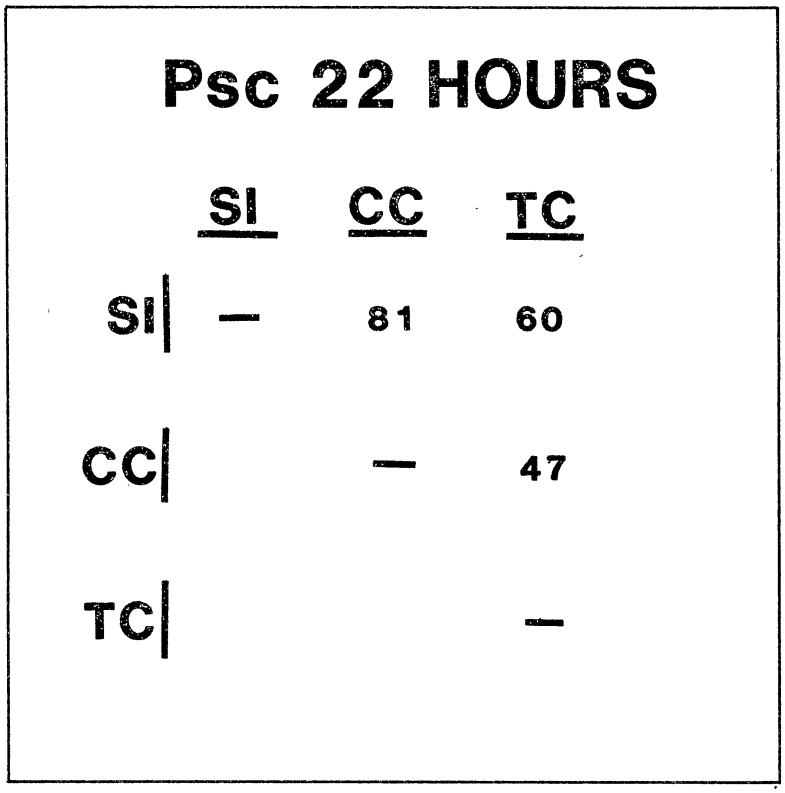

Psc 22 HOURS
SI
CC
TC
SI
—
81
60
CC
—
47
TC
—

Table 11. Selected water quality and chemostat usage parameters for influent Sangre Isle Reservoir (SIR) surface water used for a laboratory chemostat bioassay.

SIR sample date Jan 1984	Water quality					Usage parameters	
	Temperature (°C)	Dissolved oxygen (mg liter^{-1})	pH	Total alkalinity (mg l^{-1} $CaCO_3$)	Conductivity (μmhos 25 °C)	Time used (hours)	Volume used (liter)
20	4	9.19	7.64	90	190	25.3	ND
21	4	ND	ND	ND	185	26.8	3.6
22	ND	8.42	7.54	ND	189	19.0	3.6
23	ND	8.18	7.85	94	181	21.8	3.6
24	ND	ND	ND	ND	ND	24.0	4.9
25	4	8.57	7.66	90	192	23.8	5.1
26	ND	ND	7.80	ND	ND	24.6	ND
27	4	9.32	7.75	90	ND	4.9	ND

ND = No data.

Culture Hydraulics

Due to the use of the peristaltic pump for regulation of outflow from the cultures, outflow rates were more consistent than inflow rates during the bioassay. Because of the manual nature of inflow water regulation, fresh influent water was delivered to the four cultures at widely variable rates. Upon adjustment, inflow drip rates for the cultures were similar. Due to the very slow rates of addition of fresh SIR water, air bubbles developed in the Tygon tubing, cutting off the inflow. For any given time period, culture volume could either remain constant (inflow = outflow), increase (inflow > outflow), or decrease (inflow < outflow). As the time interval between inflow adjustments increased, culture volume tended to decrease. To compensate for this, relatively large inflow volumes were used over short time intervals to maintain culture volumes at ca. 3.0 liters.

Outflow rates are shown with estimates for total culture outflow and mean experimental outflow rate in Table 12. Variation in outflow rates among cultures was caused in part by the position of culture outflow tubing in the channels of the peristaltic pump. In an attempt to reduce this variation, relative channel positions were changed 3 times during the bioassay, and all outflow tubes were replaced with new tubing on day 4. A second cause of outflow variation among cultures was apparently due to their proximity to the peristaltic pump.

The coefficient of variation (CV) for outflow rates for all cultures within a given time interval was ca. 15-20% (Table 12). Lower variation in outflow rates was observed when comparisons were made between control culture 1 (C1) and treatment culture 1 (T1) (ca. 2-19%: mean = 12%), and between control culture 2 (C2) and treatment culture 2

Table 12. Average outflow rate for control and treatment cultures for seven major time intervals during a 170-hour laboratory chemostat bioassay.

Day	Time interval	ΔT (hours)	Average outflow rates (ml hour^{-1}) Control 1	Control 2	Treatment 1	Treatment 2	CV
1	1530 - 1546	24.3	31	46	36	36	16.9
2	1546 - 1547	24.0	30	30	39	29	14.7
3	1547 - 1223	20.6	26	25	34	24	16.8
4	1223 - 1540	27.3	25	22	31	22	17.0
5	1540 - 1629	24.8	48	34	43	33	18.3
6	1629 - 1607	23.6	48	34	43	33	18.3
7	1607 - 1052	18.7	42	31	41	31	16.8
Total outflow (ml)			6090	5411	6496	5082	
Mean outflow rate[a] (ml h^{-1})			37.6	33.4	40.2	31.4	
Mean residence time[b] (hours)			90	100	80	104	

[a] Mean outflow rate is a weighted average for each culture for the entire bioassay.

[b] Calculated using average outflow rates and an average culture volume of 3000 ml.

CV = Coefficient of variation.

(T2) (ca. 2-17%: mean = 4%). Additionally, both total outflow and mean outflow rates indicate a higher similarity between C1 and T1, and C2 and T2, than for other comparisons.

Inflow volumes to the cultures appear in Table 13. The highest inflow volumes correspond to the highest outflow rates for a given time interval, as a constant culture volume was sought. When the inflow volume data are standardized to a basic 24.0-hour time interval, the range in inflow volume is 710 to 1683 ml. Culture inflow variations for all time intervals ranged from ca. 12 to 23% (CV) for all cultures. However, inflow variability between C1 and T1 averaged ca. 12% (CV), while the C2 and T2 comparison was only ca. 5% (CV). Total and mean inflow data also show greater similarity for the above mentioned control-treatment pairs.

After reexamination of the experimental design, it was determined that an inflow-outflow gradient had existed during the bioassay, and was the major cause of the observed variation in flows. This gradient was apparently due to the arrangement of the culture chambers in relation to the influent reservoir carboy, and the peristaltic pump. Those cultures closer to the pump (C1 and T1) had higher outflow rates, and consequently higher inflow volumes. Therefore, because of the more similar flow characteristics between C1 and T1, and C2 and T2, greater emphasis will be placed on these comparisons.

Salinity

Following the NaCl addition at 93.5 hours, the salinity of T1 and T2 varied within a range of ca. 780-1100 μmhos conductivity, with most values in the 800 to 900 μmhos range (Figure 22). Minor conductivity

Table 13. Inflow volume of Sangre Isle Reservoir surface water for control and treatment cultures for seven major time intervals during a 170-hour laboratory chemostat bioassay.

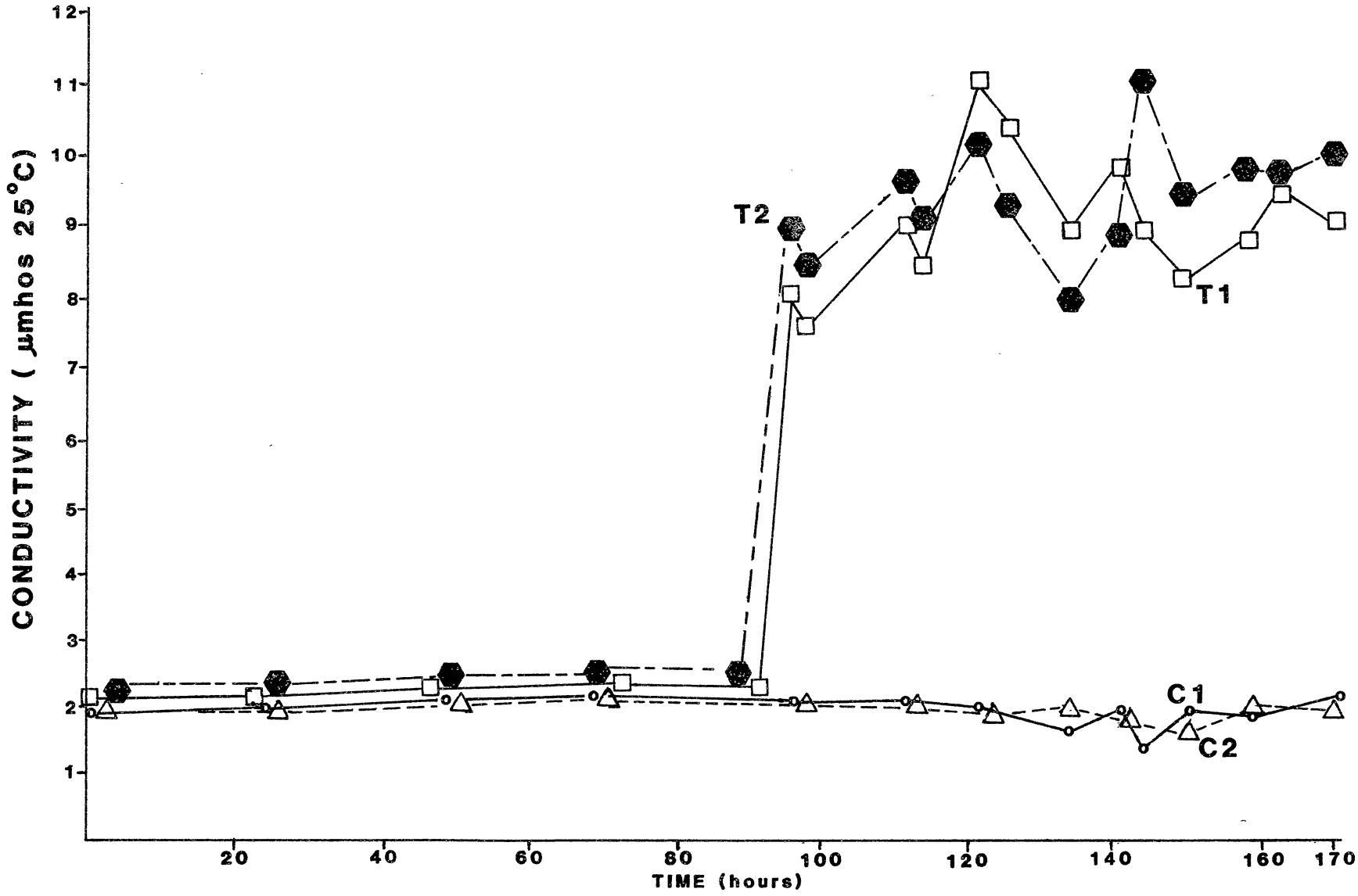
Day	Time interval[a]	ΔT (hours)	Inflow volume (ml)				CV
			Control		Treatment		
			1	2	1	2	
1	1530 - 1546	24.3	674	967	713	861	16.9
2	1546 - 1547	24.0	778	959	1071	795	15.5
3	1547 - 1223	20.6	848	700	1039	692	19.9
4	1223 - 1540	27.3	966	888	1174	903	13.4
5	1540 - 1629	24.8	1542	1164	1335	1157	14.0
6	1629 - 1607	23.6	1405	1121	1271	1089	11.9
7	1607 - 1052	18.7	1311	911	1141	785	22.6
Total inflow			7524	6710	7744	6282	
Mean inflow[b]			1075	959	1106	897	
SD			340	154	202	169	

[a] The first time listed is for experimental day "n-1." For example, control culture 1 had a total inflow volume of 674 ml during the time interval that began at 1530 h on day 0 and ended at 1546 h on day 1. Each time interval directly preceeded sampling for all cultures.

[b] Calculated as the average inflow volume for the average time interval duration of 23.3 hours.

CV = Coefficient of variation.
SD = Standard deviation of inflow volumes.

Figure 22. Temporal variations in conductivity for treatment and control cultures during a 170-h laboratory chemostat bioassay. (C1 = control 1; C2 = control 2; T1 = treatment 1; T2 = treatment 2)


CONDUCTIVITY (µmhos 25°C)
12
11
10
9
8
7
6
5
4
3
2
1
T2
T1
C1
C2
20
40
60
80
100
120
140
160
170
TIME (hours)

reductions resulted from distilled-deionized water inputs into the control cultures.

Pigments

Chlorophyll a. A general trend of decreasing chlorophyll a in all cultures (Figure 23) followed an influent water chlorophyll a concentration decrease (Figure 24) throughout the bioassay period. Chlorophyll a varied more in C1 than in the other three cultures. All cultures show an apparent "leveling off" trend in chlorphyll a after 144 h. Sodium chloride treatment did not affect chlorophyll a concentration significantly.

Phaeopigments. Degraded chlorophyll exhibited a greater degree of variability than functional chlorophyll a, both within and among cultures, although no general increase or decrease was apparent (Figure 25). Treatment culture phaeopigment means taken before and after NaCl additions were not significantly different from each other nor from the control means. Both treatment and control cultures showed a general increase in the phaeopigment:chlorophyll a ratio over the bioassay period, with T1 significantly greater than other cultures over the NaCl-treatment period (Figure 26).

Primary Productivity

Laboratory incubations of raw SIR water (Group No. 1, Table 6), with ^{14}C resulted in a wide range of productivity estimates (Figure 27). After peaking at 121 mg C m^{-3} h^{-1} on 23 January, a decreasing productivity trend continued through 27 January. The effect of storage on the productivity of the SIR water samples in the influent reservoir carboy (Group No. 3, Table 6) was variable.

Figure 23. Temporal variations in chlorophyll a for treatment and control cultures during a 170-h laboratory chemostat bioassay. (C1 = control 1; C2 = control 2; T1 = treatment 1; T2 = treatment 2)

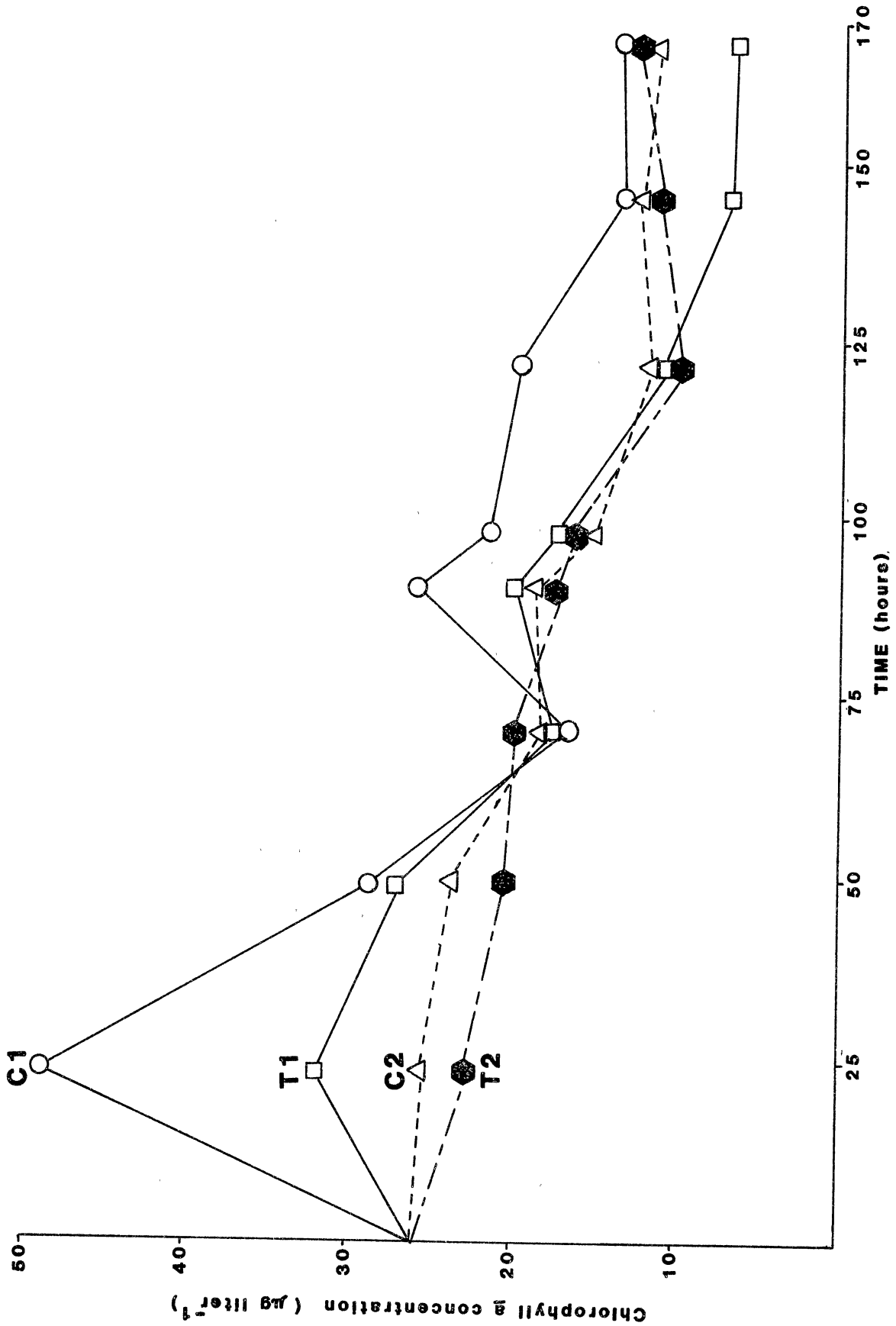

C1
T1
C2
T2
50
40
30
20
10
25
50
75
100
125
150
170
TIME (hours)
Chlorophyll a concentration (μg liter⁻¹)

Figure 24. Chlorophyll a and phaeopigment concentrations of Sangre Isle Reservoir water used as culture influent water during a 170-h laboratory chemostat bioassay. Error bars indicate ± 1SD for chlorophyll a (solid line) and phaeopigments (dashed line).

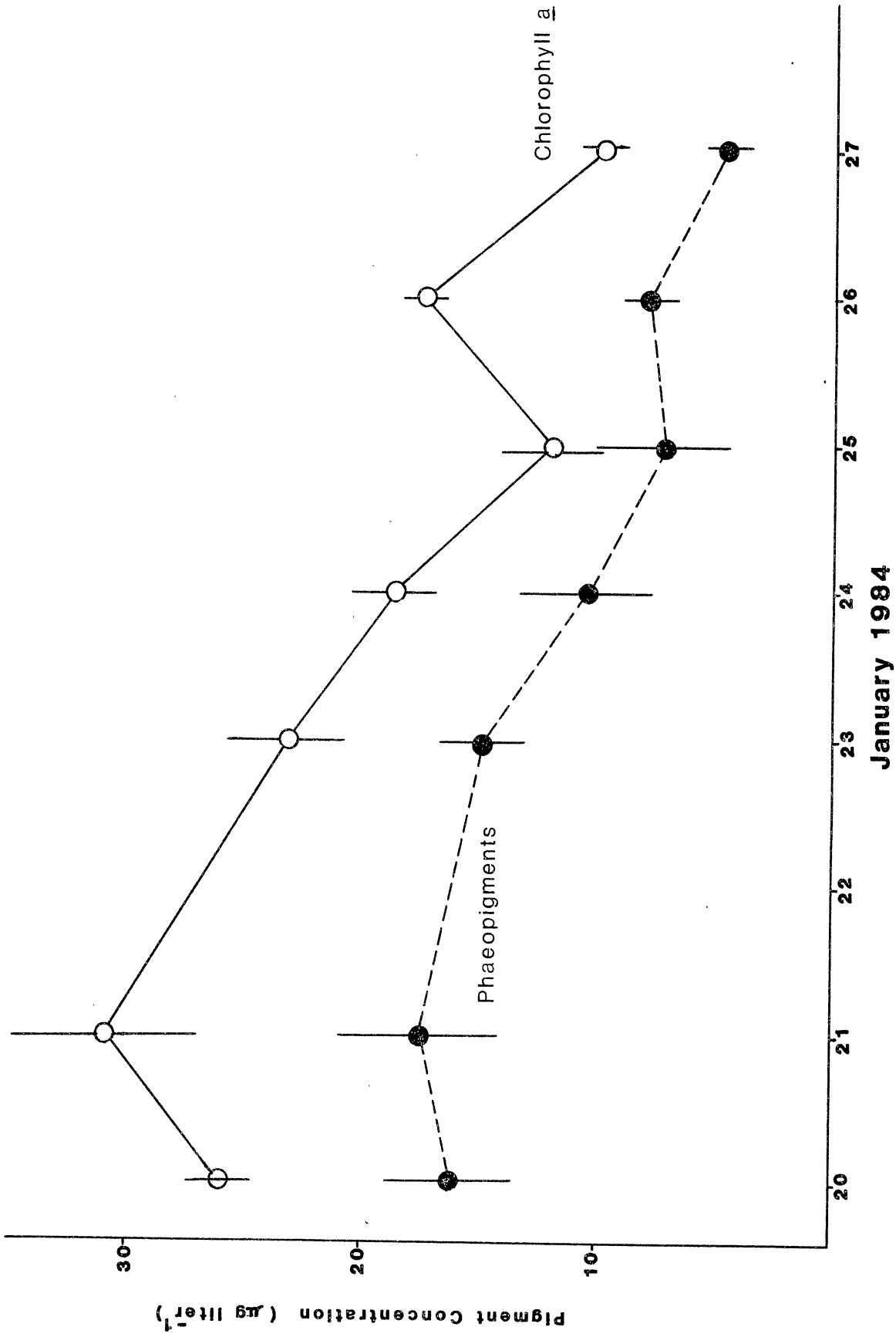
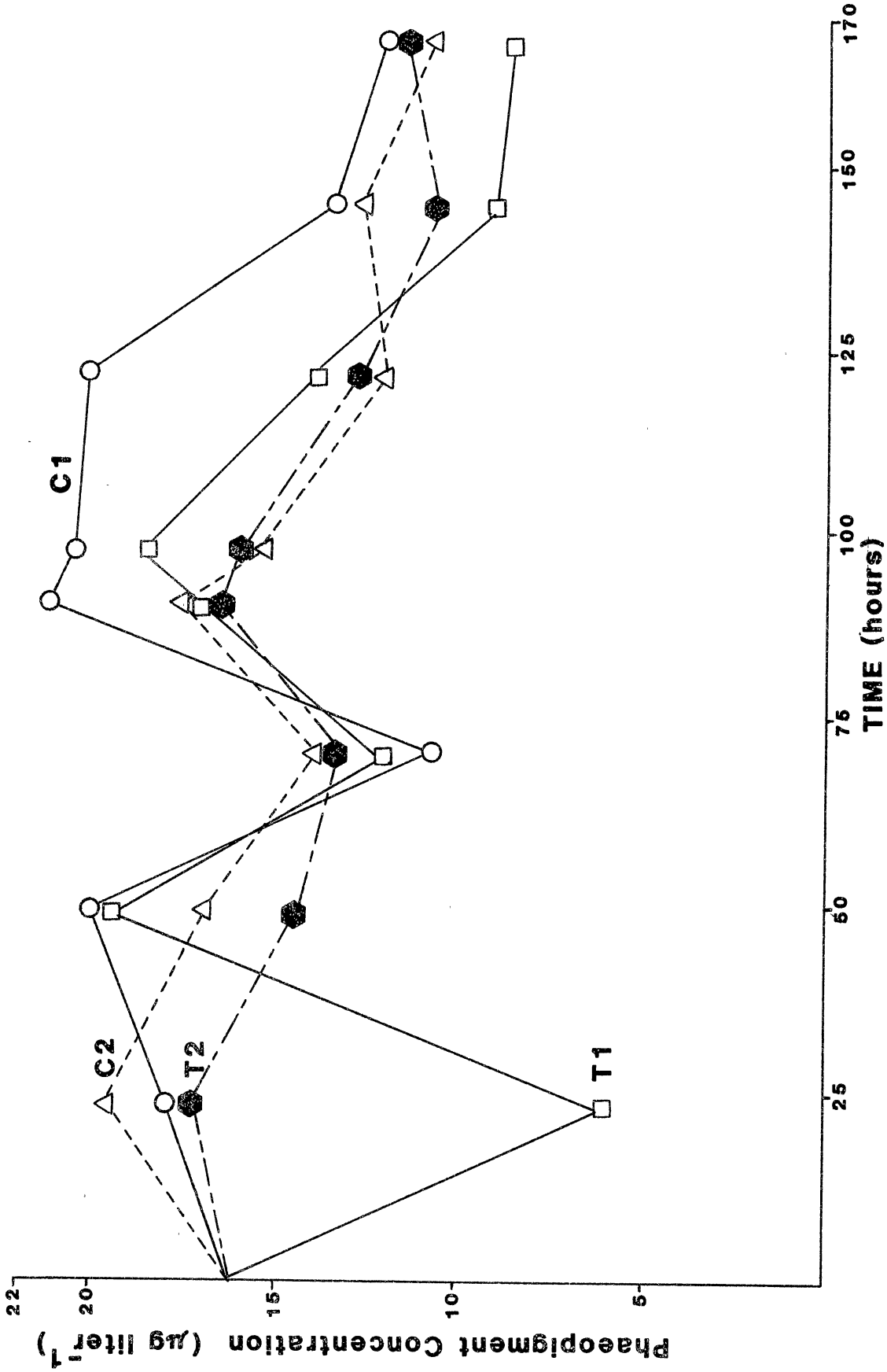
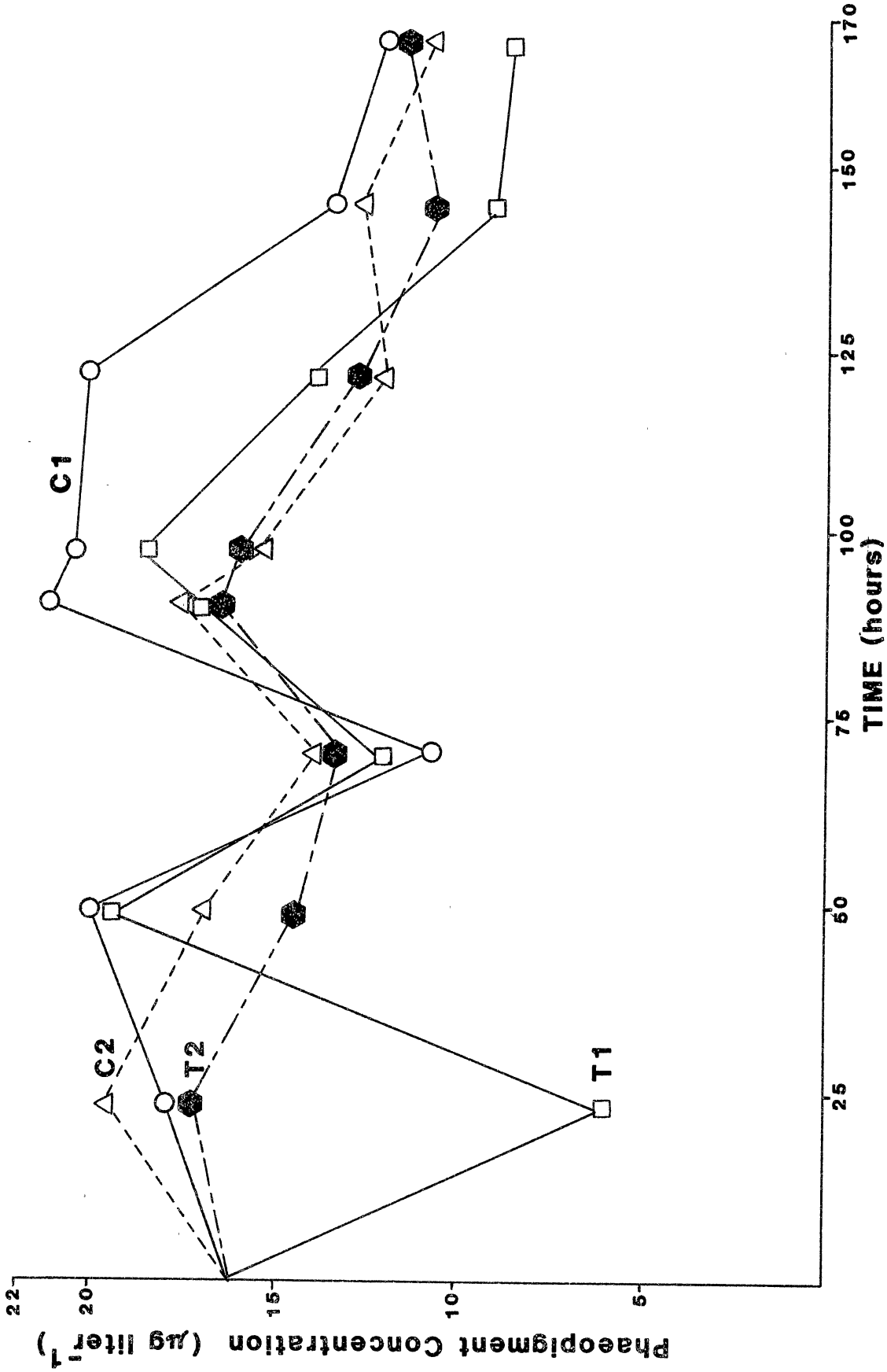

Chlorophyll a
Phaeopigments
Pigment Concentration (μg liter⁻¹)
30
20
10
20
21
22
23
24
25
26
27
January 1984

Figure 25. Temporal variation in phaeopigments (degraded chlorophyll) for treatment and control cultures during a 170-h laboratory chemostat bioassay. (C1 = control 1; C2 = control 2; T1 = treatment 1; T2 = treatment 2)


Phaeopigment Concentration (µg liter⁻¹)
22
20
15
10
5
25
50
75
100
125
150
170
TIME (hours)
C1
C2
T2
T1


Phaeopigment Concentration (µg liter⁻¹)
22
20
15
10
5
25
50
75
100
125
150
170
TIME (hours)
C1
C2
T2
T1

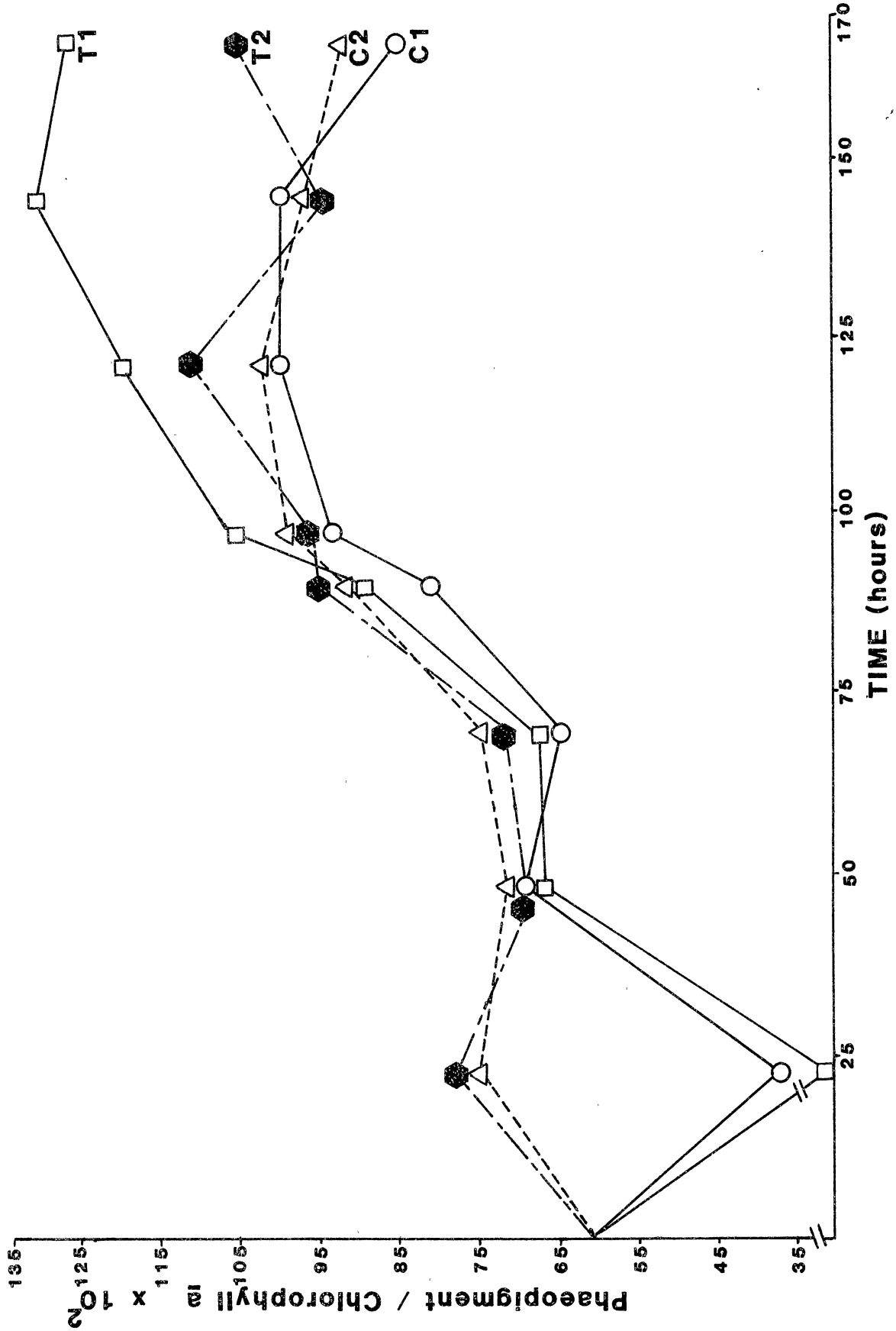
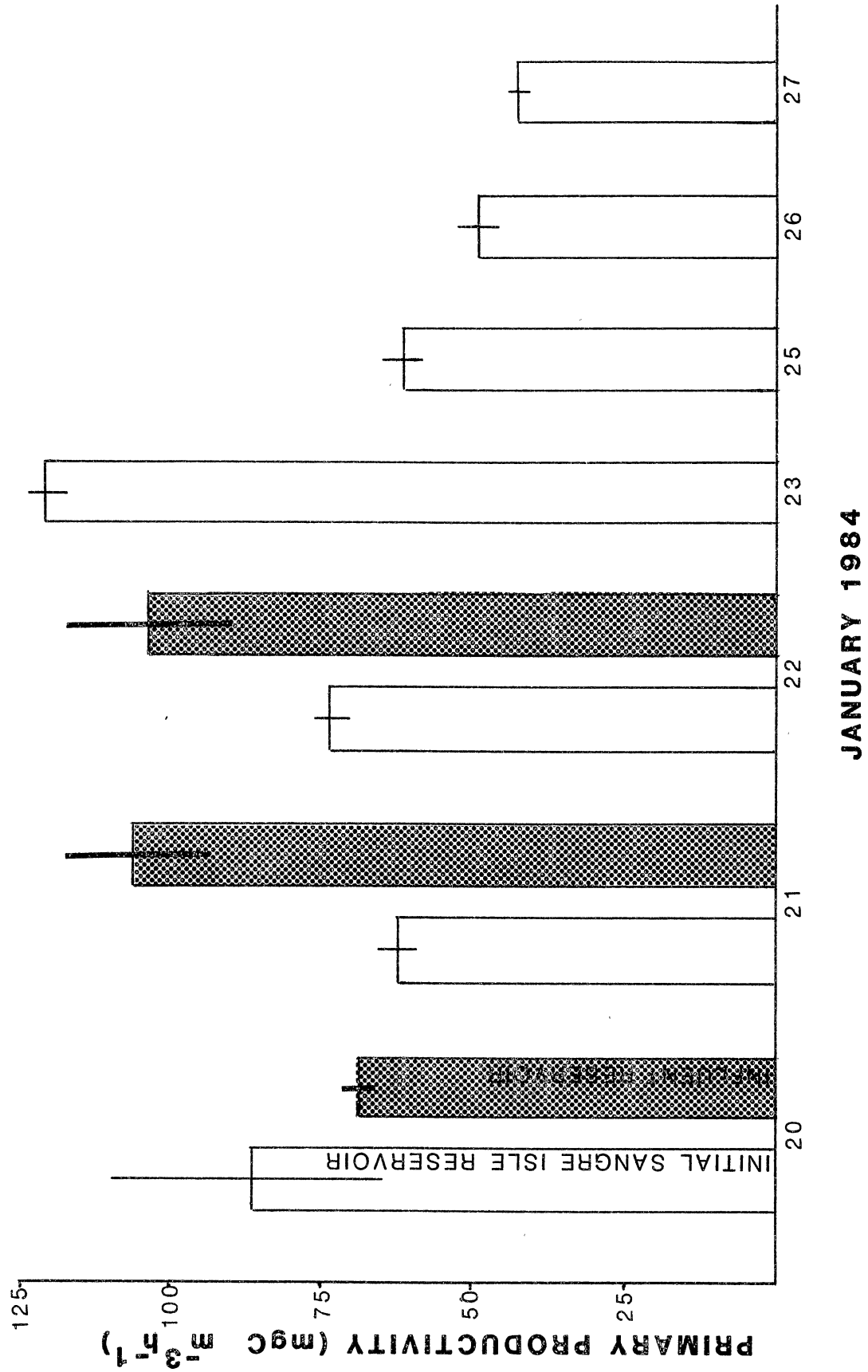

T1
T2
C2
C1
Phaeopigment / Chlorophyll a x 10²
135
125
115
105
95
85
75
65
55
45
35
25
50
75
100
125
150
170
TIME (hours)

Figure 27. Initial primary productivity of laboratory chemostat influent water collected from Sangre Isle Reservoir from 20 to 27 January 1984, with storage productivities on 20-22 January. (Open bars = initial Sangre Isle Reservoir estimates; stippled bars = influent reservoir productivity estimates for water sampled from Sangre Isle Reservoir on the day recorded on the abscissa, but determined on the following day after storage in the common influent reservoir that fed the 4 cultures. Storage times were: 20 Jan = 25.3 h; 21 Jan = 26.8 h; 22 Jan = 19.0 h)


PRIMARY PRODUCTIVITY (mgC m⁻³ h⁻¹)
125
100
75
50
25
INITIAL SANGRE ISLE RESERVOIR
20
21
22
23
25
26
27
JANUARY 1984

Culture chamber productivity estimates appear in Figure 28. All cultures except T2 reached maximum productivity at 48 hours, then steadily declined until the 121 to 144-h time period. All cultures increased in productivity at 167 h. Salinization did not affect treatment culture productivity immediately after introduction at 93.5 hours. Final (167 h) treatment culture productivities significantly differ from each other and from the controls, being both stimulated and inhibited relative to the more consistent control culture responses.

The acute response of the incoming SIR surface water algal associations to three elevated NaCl-salinity levels on 20, 25, 26, and 27 January is shown in Figure 29 (Group No. 2, Table 6). All dates show productivity inhibition at salinity levels 2 (total conductivity = ca. 2000 μmhos) and 3 (total conductivity = ca. 3600 μmhos), although the percent of inhibition among dates was not consistent. An increase in NaCl concentration of ca. 30 mM (level 3) decreased productivity by 72, 79, and 71 percent on 20, 26, and 27 January, respectively. However, on 25 January this same increase reduced productivity by only 32%.

Figure 30 shows the comparative productivity estimates at 145 and 167 h at normal culture salinities (normal control $\bar{x}$ = 176 μmhos, n = 4; normal treatment $\bar{x}$ = 997 μmhos, n = 4), and when the appropriate additional volumes of 5N NaCl had been added to the incubation bottles to raise both control and treatment samples to near 2000 μmhos ($\bar{x}$ = 1942 $\pm$ 106 μmhos, n = 8). In contrast to the general results of the productivity bioassays on fresh SIR surface water (Figure 27), both control cultures had statistically similar productivities at normal and elevated salinities at 145 h. Productivity for both treatment cultures was significantly stimulated by the higher incubation salinity

Figure 28. Temporal variations in productivity for treatment and control cultures during a 170-h laboratory chemostat bioassay. (C1 = control 1; C2 = control 2; T1 = treatment 1; T2 = treatment 2)

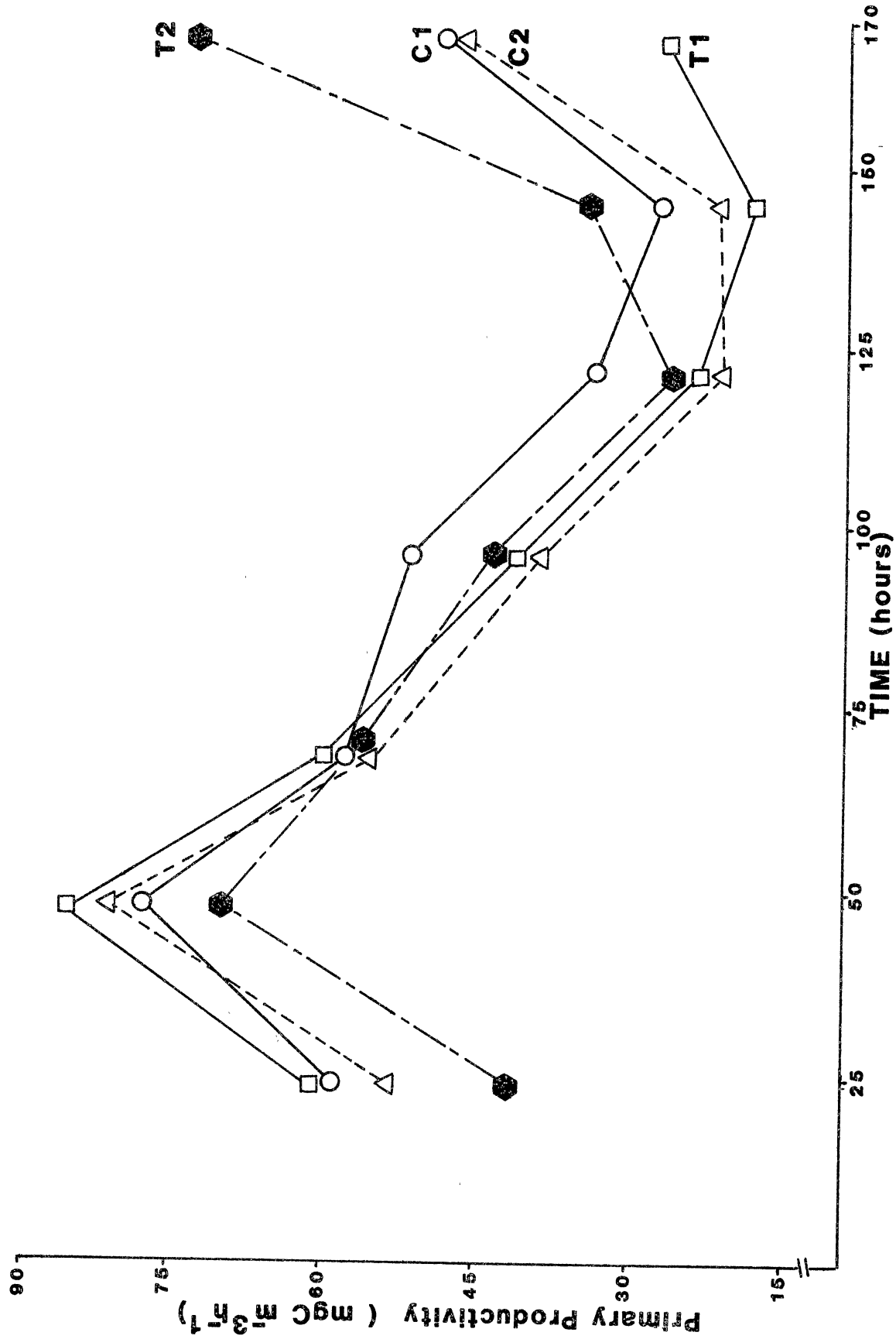
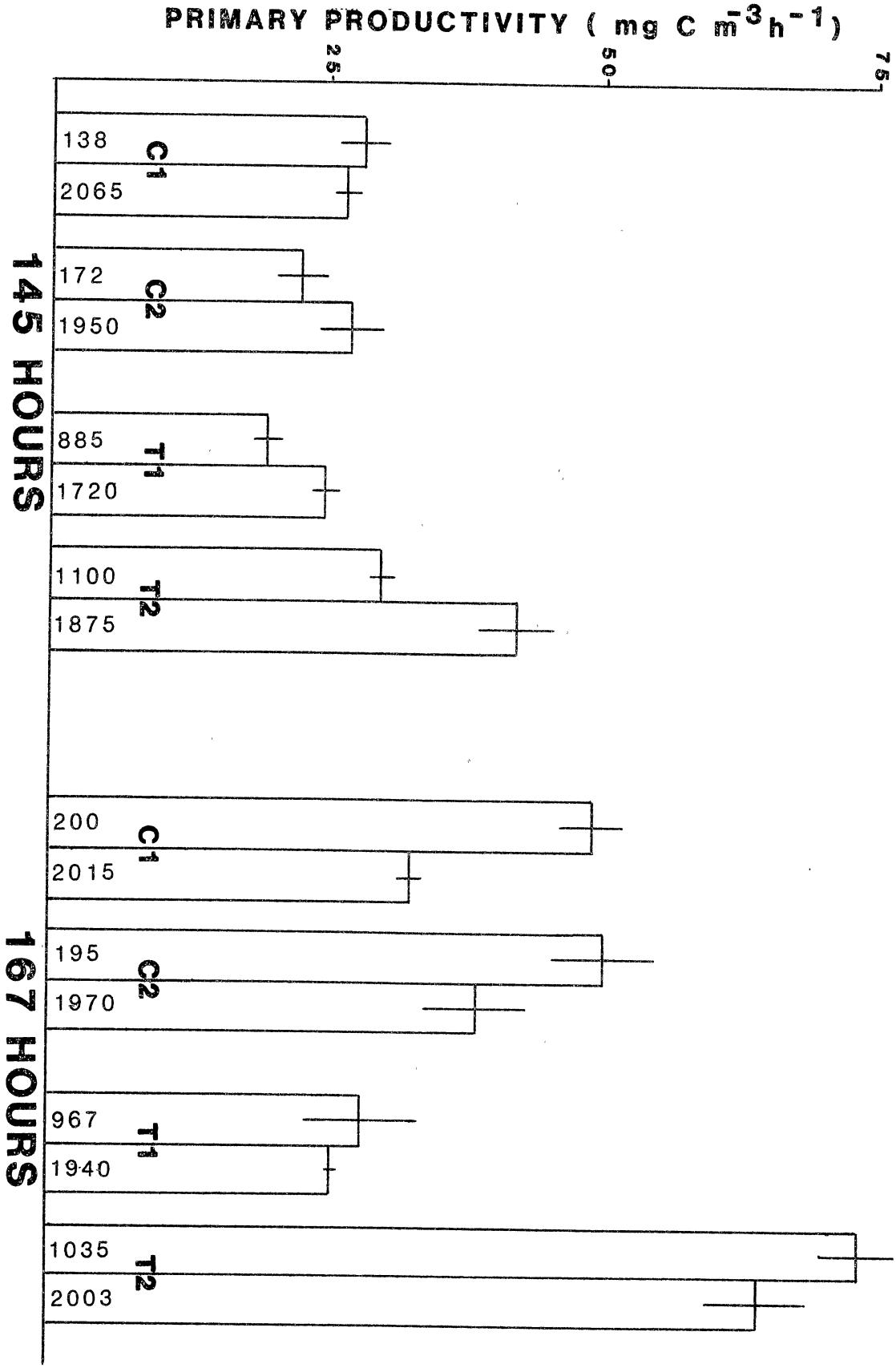

T2
C1
C2
T1
Primary Productivity (mgC m⁻³ h⁻¹)
90
75
60
45
30
15
25
50
75
100
125
150
170
TIME (hours)

Figure 29. Primary productivity of Sangre Isle Reservoir surface water used as laboratory chemostat culture influent. (Open bars = raw Sangre Isle Reservoir surface water; horizontally-striped bars = + ca. 460 µmhos conductivity; dotted bars = + ca. 1800 µmhos; vertically-striped bars = + ca. 3400 µmhos; # = no +460 µmho treatment level on 20 January)

PRIMARY PRODUCTIVITY (mg C m⁻³h⁻¹)
100
75
50
25
Raw Sangre Isle Reservoir surface water
+460 µmhos
+1800 µmhos
+3400 µmhos
#20 JAN
25 JAN
26 JAN
27 JAN

Figure 30. Primary productivity comparison at 145 and 167 h between laboratory chemostat culture samples at normal experimental salinity and at roughly 2000 µmhos incubation salinity. Culture samples were withdrawn and incubated with and without additional NaCl. For a given treatment or control culture, both determinations are shown together, with incubation conductivity at 25 °C inside each bar. Error bars indicate ± 1SD (n=4) for control 1 (C1), control 2 (C2), treatment 1 (T1), and treatment 2 (T2).


PRIMARY PRODUCTIVITY (mg C m⁻³h⁻¹)
25
50
75
145 HOURS
138
C1
2065
172
C2
1950
885
T1
1720
1100
T2
1875
167 HOURS
200
C1
2015
195
C2
1970
967
T1
1940
1035
T2
2003

at 145 h. At 167 h, all cultures except T1 showed significant reductions in productivity at the ca. 2000 µmhos level, with the highest level of inhibition (34%) occurring in C1.

Phytoplankton Enumeration and Analysis

The culture "start-up" water for the bioassay (SIR, 20 January 1984) was dominated by the members of the Cryptophyte genus Cryptomonas (Figure 31). Of the five genera present, Cryptomonas accounted for 89% of the total cell number, and 97% of the total cell volume. This overwhelming dominance resulted in low biovolume equitability (E_V = 0.25), and low species diversity ($\bar{d}_n$ = 0.67; $\bar{d}_V$ = 0.22) (Table 14). Both species diversity indices increased for 23 and 27 January SIR samplings, due mainly to population increases of the green alga Ankistrodesmus and the blue-green alga Chroococcus. However, due to the small size of these algae, both contributed little to the total cell volume, as E_V increased only slightly. During the 8-day SIR sampling period, a declining Cryptomonas population caused total cell volume to decrease.

In C1 and C2, Cryptomonas remained the biovolume and numeric dominant through at least the first 70 h of the bioassay (Figure 32). Between 70 and 166 h, both the diatom Synedra and the blue-green alga Chroococcus showed significant population increases. At 166 h, C1 was dominated numerically by Chroococcus (58%), while biovolume dominants were Cryptomonas (41%) and Synedra (93%). At the same time, C2 was dominated numerically by both Synedra (41%) and Chroococcus (34%), and volumetrically by Synedra (62%) and Cryptomonas (32%).

Figure 31. Algal density and species distribution for Sangre Isle Reservoir surface water on 20, 23, and 27 January 1983. All laboratory chemostat cultures started the bioassay with 3.0 liters of the 20 January sample. Daily changes of influent water are represented by the 23 and 27 January associations. Open bars are percents of either total numeric or volumetric density. Horizontally-striped bars represent total algal density as cells/ml. Vertically-striped bars represent total algal cell volume. Bar numbers correspond to the following algal genera:
1 = Ankistrodesmus; 2 = Chroococcus; 3 = Cryptomonas;
4 = Oscillatoria; 5 = Synedra; 6 = all other genera.

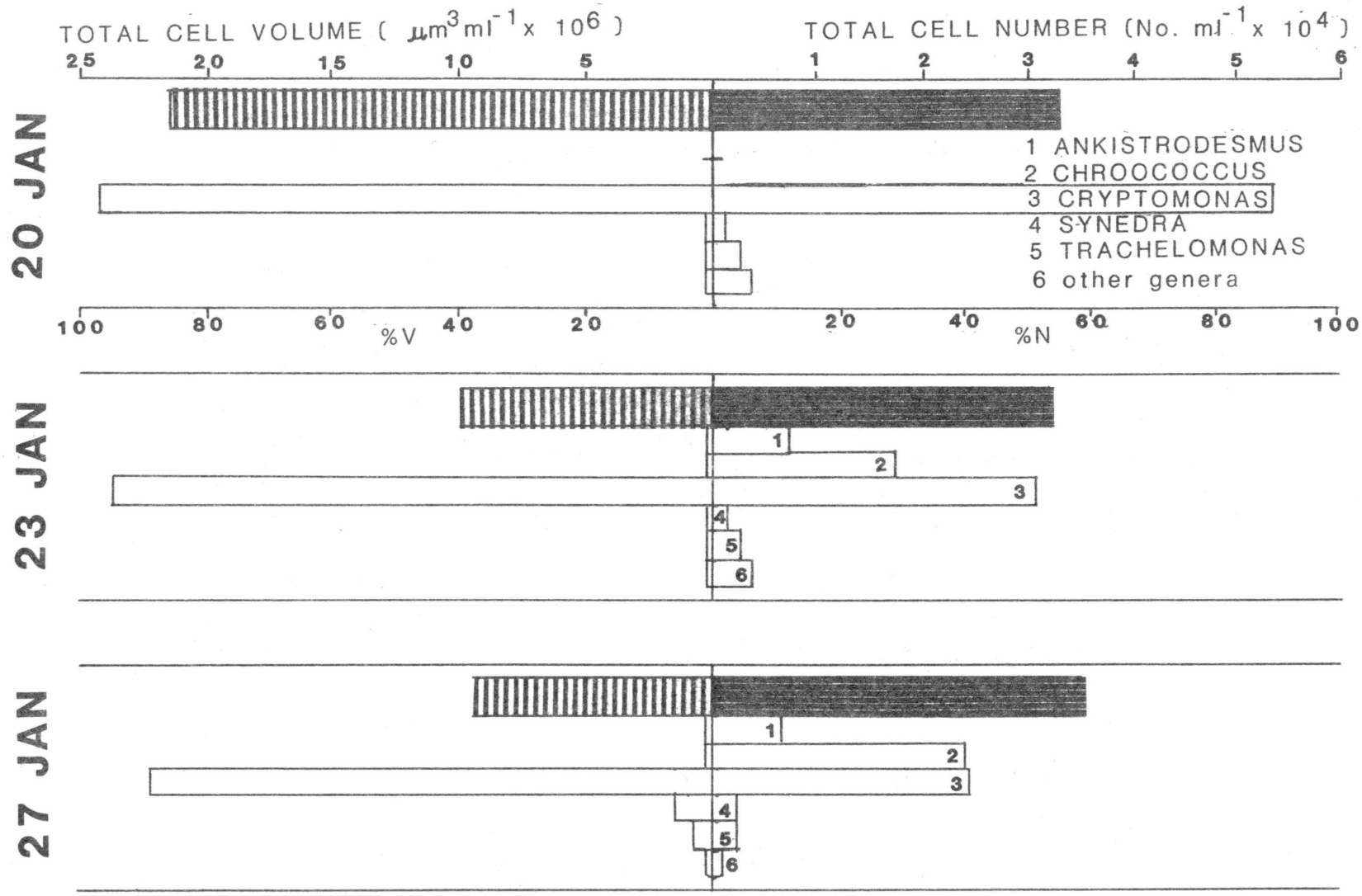

TOTAL CELL VOLUME (µm³ ml⁻¹ x 10⁶)
TOTAL CELL NUMBER (No. ml⁻¹ x 10⁴)
25 20 15 10 5
1 2 3 4 5 6
20 JAN
1 ANKISTRODESMUS
2 CHROOCOCCUS
3 CRYPTOMONAS
4 SYNEDRA
5 TRACHELOMONAS
6 other genera
100 80 60 %V 40 20
20 40 60 %N 80 100
23 JAN
1
2
3
4
5
6
27 JAN
1
2
3
4
5
6

Table 14. Algal abundance and community structure parameters for Sangre Isle Reservoir surface water, January 1984.

Algal association variable	Sampling date, January 1984		
	20	23	27
Species richness	5	7	7
Equitability (E_v)	0.25	0.29	0.34
Total cell density (No. milliliter^{-1} x 10^4)	3.3	3.2	3.6
Species diversity ($\bar{d}_n$)	0.67	2.20	2.15
Total cell volume (μm^3 milliliter^{-1} x 10^6)	21.6	10.1	9.5
Species diversity ($\bar{d}_v$)	0.22	0.84	1.01

Figure 32. Algal density and species distribution for control and treatment cultures at 24, 70, and 166 h during a 170-h laboratory chemostat bioassay. Open bars are percents of either total numeric or volumetric density. Horizontally-striped bars represent total algal density as cells/ml. Vertically-striped bars represent total algal cell volume. Bar numbers correspond to the following algal genera:
1 = Ankistrodesmus; 2 = Chroococcus; 3 = Cryptomonas;
4 = Oscillatoria; 5 = Synedra; 6 = all other genera.

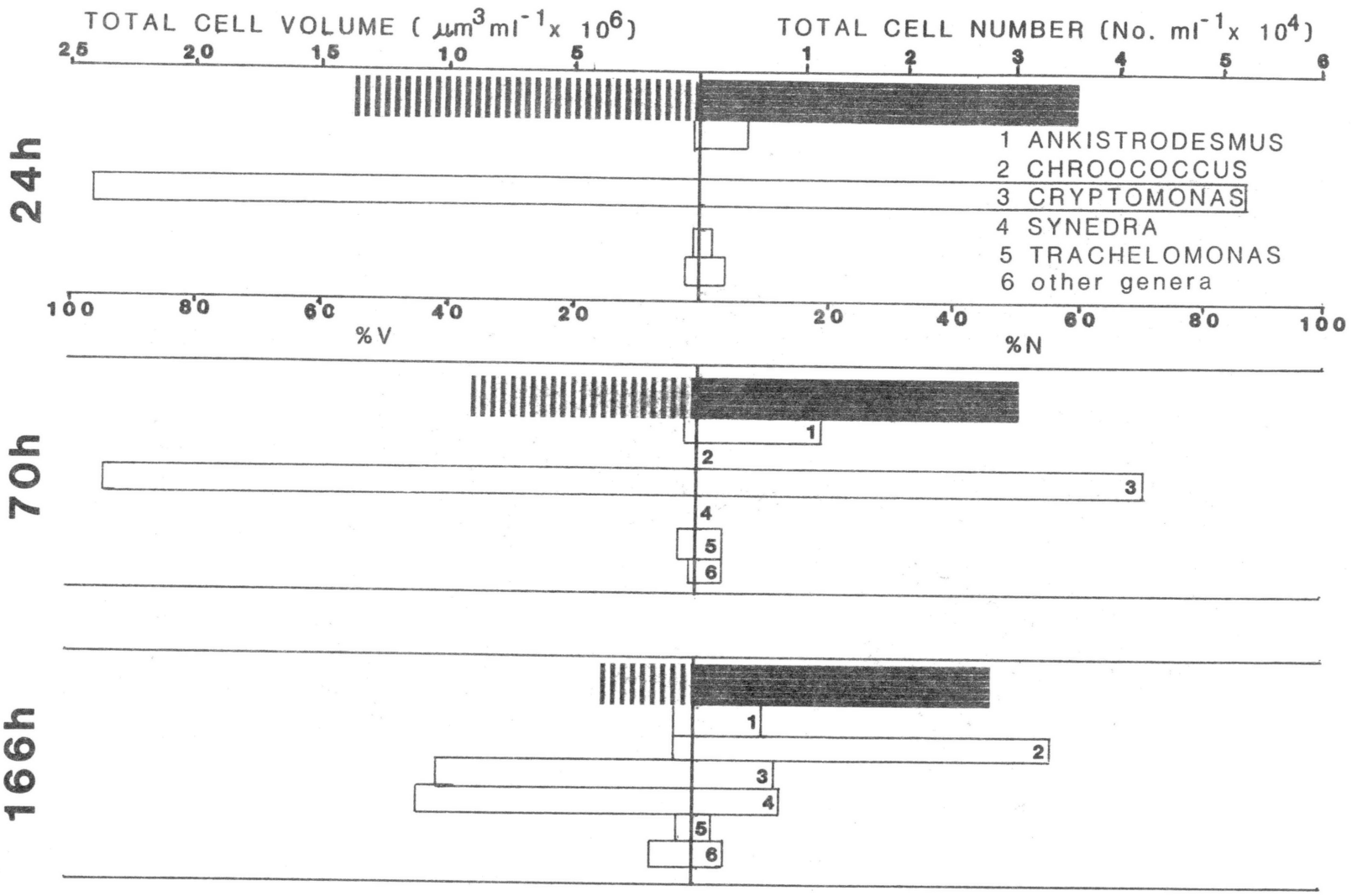


Figure 32 Control Culture 1

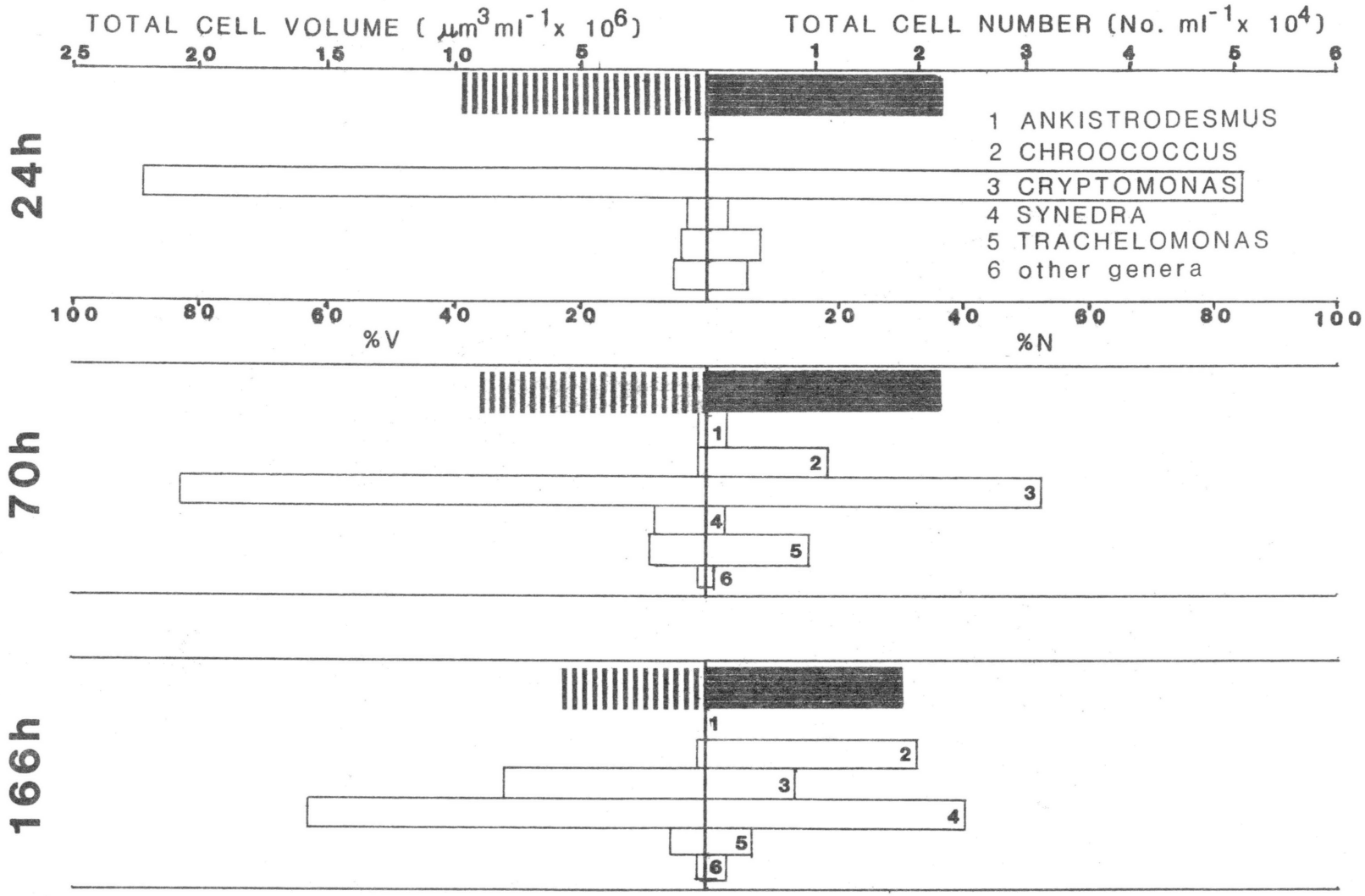


Figure 32 continued-Control Culture 2

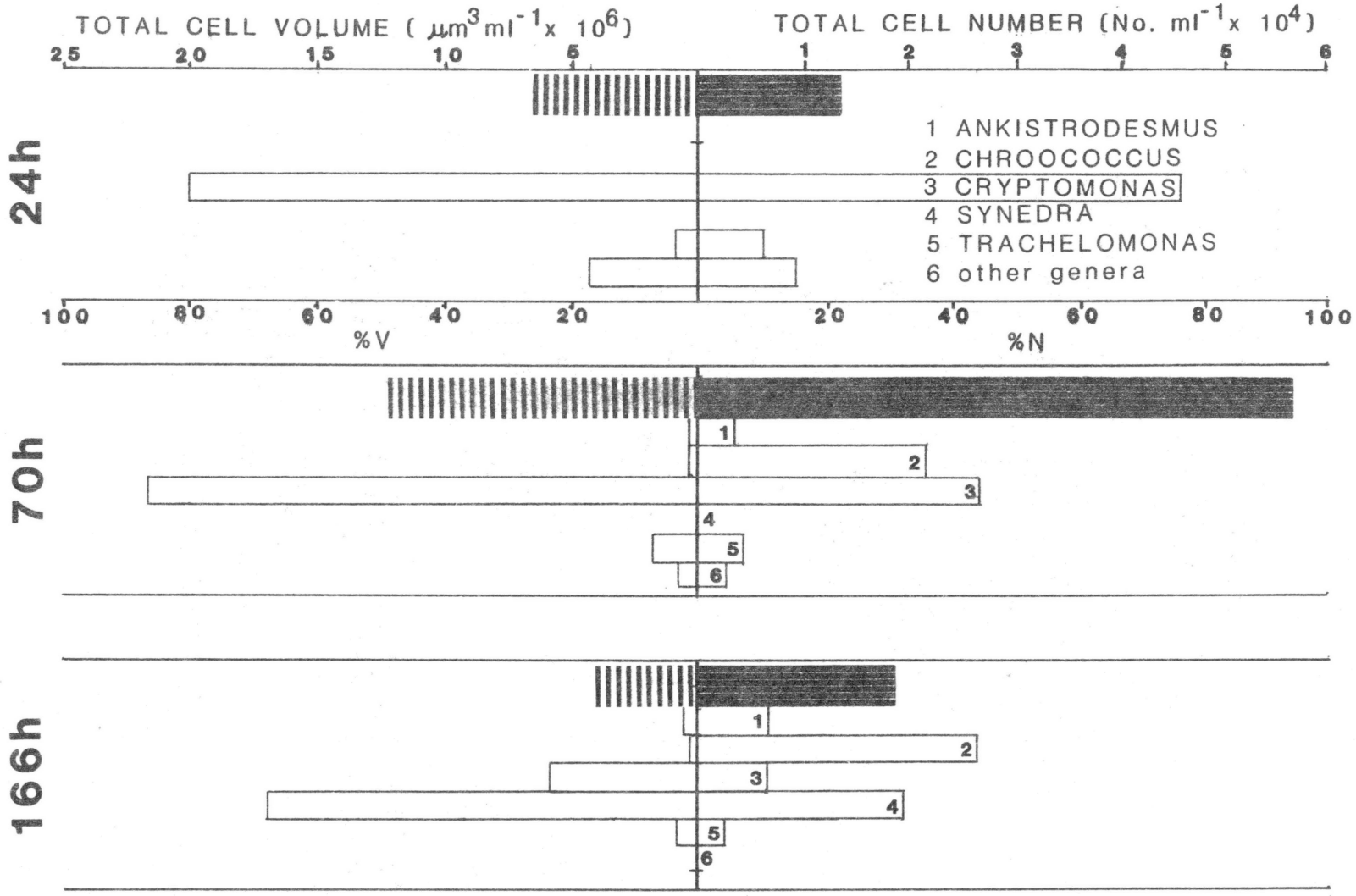


Figure 32 continued -Treatment Culture 1

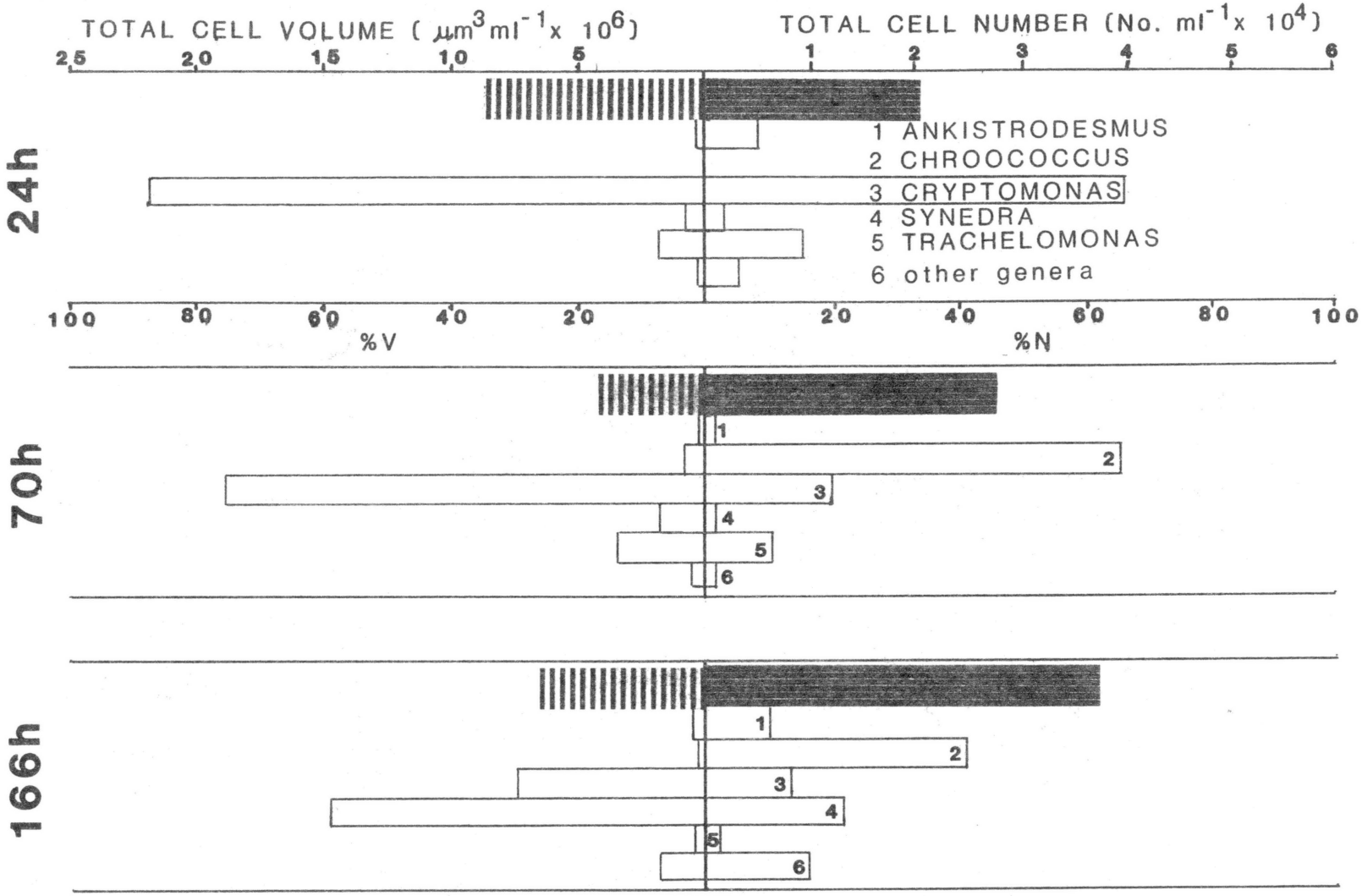


Figure 32 continued-Treatment Culture 2

Although the control cultures retained a species distribution similar to the initial 20 January SIR algal association through the first 24 hours, total cell volume was reduced up to 55% during this period. Total cell volume continued to decrease throughout the bioassay for both control cultures, although numeric density remained relatively constant (Figure 32).

Both species diversity indices increased in C1 as a result of increased equitability (E_V) and constant species richness (Table 15). Biovolume species diversity ($\overline{d}_V$) in C2 remained constant, and numeric species diversity ($\overline{d}_n$) was variable.

Treatment cultures followed a species distributional trend similar to that of the control cultures, as early domination by Cryptomonas was followed by increasing Synedra and Chroococcus populations (Figure 32). Prior to salinization in T1, large increases in Chroococcus and Cryptomonas populations caused total numeric density and total cell volume to increase by factors of 4.4 and 2.1, respectively. Similar increases did not occur in any of the other cultures.

In T2, Synedra was present at about 5% of the total cell volume at 24 and 70 hours, prior to increasing to 59% at 166 hours. In T1, Synedra was not detected until 166 hours, when it composed nearly 68% of the total cell volume.

For both treatment and control cultures, the total cell volume was on the average nearly two times more evenly distributed among the algal genera present, than the total cell volume in SIR influent samples (see equitability, E_V; Tables 14 and 15). This contributed in general to a higher biovolume species diversity in the cultures, as species richness in the cultures remained similar to the SIR influent associations.

Table 15. Algal abundance and community structure parameters for treatment and control cultures at 24, 70, and 166 hours during a 170-hour laboratory chemostat bioassay.

Algal association variable	Control						Treatment[a]					
	1			2			1			2		
	24	70	166	24	70	166	24	70	166	24	70	166
Species richness	6	5	8	6	7	5	5	8	6	6	7	9
Equitability (E_V)	0.41	0.57	0.70	0.52	0.39	0.59	0.75	0.47	0.52	0.46	0.56	0.54
Total cell density (No. milliliter^{-1} x 10^4)	3.6	3.0	2.9	2.2	2.3	1.8	1.3	5.7	1.7	2.0	2.8	3.8
Species diversity ($\bar{d}_n$)	1.64	1.89	2.32	1.78	2.32	1.88	1.91	2.28	2.01	2.12	1.71	2.51
Total cell volume (μm^3 milliliter^{-1} x 10^6)	14.1	9.0	4.0	9.7	8.9	5.6	6.0	12.6	3.8	8.4	3.9	6.4
Species diversity ($\bar{d}_V$)	1.03	1.23	2.08	1.34	1.15	1.27	1.57	1.55	1.34	1.18	1.62	1.90

[a] Sodium chloride treatment began at about 94 hours.

Comparisons of the algal associations in the four cultures 24 h before (70 h) and 72 h after (166 h) the onset of treatment culture salinization are shown in Figure 33. All Percentage of community similarity (Psc) values lie within a relatively narrow 75-90% similarity range. At 166 h, control versus treatment comparisons do not indicate any altered succession caused by NaCl. The lower variation in flow for the control and treatment culture pairs previously discussed (C1 and T1; C2 and T2) did not result in greater algal association similarity.

Discussion

In situ Chemostat Bioassays

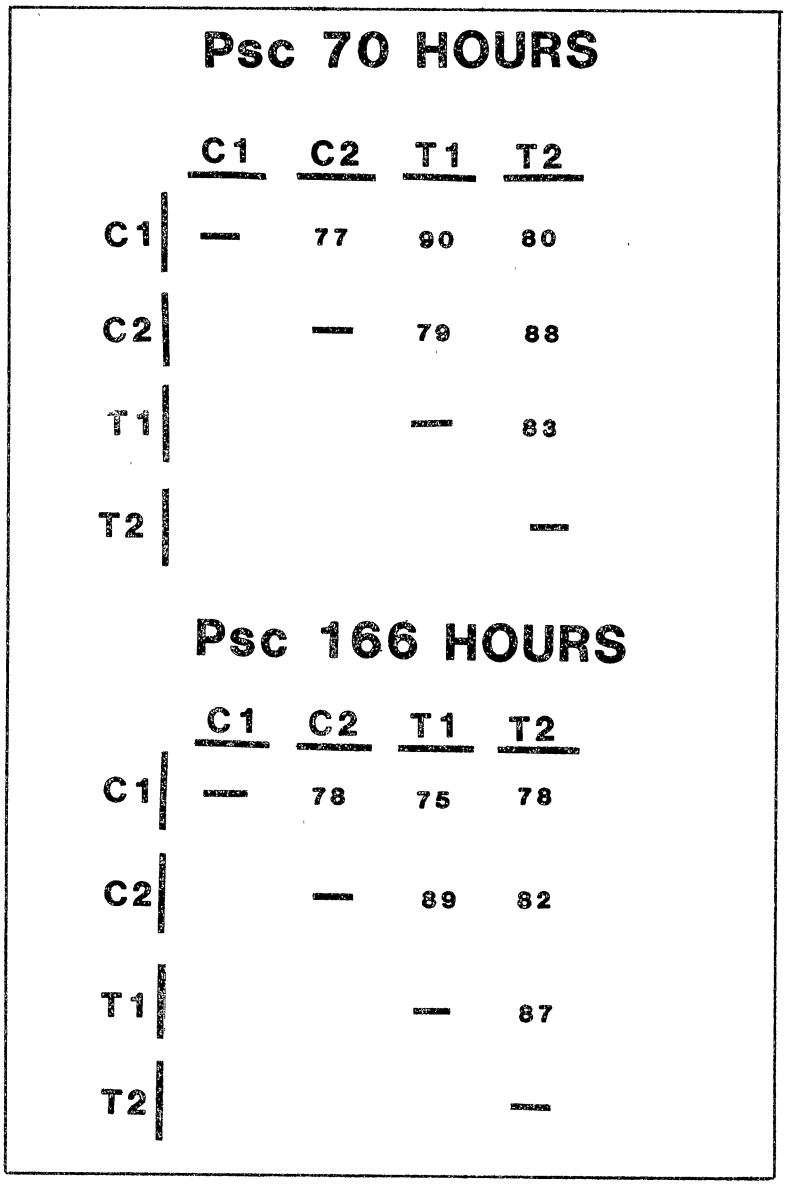
Ninety-two Hour Bioassay

Chemostat operation. Problems in maintaining equitable flow rates through the chemostat culture chambers introduced complications into the interpretation of experimental results. Therefore, an alternate interpretation is provided that will summarize these problems, discuss the implication of these errors on the experimental design, and state conclusions based on available data.

During time interval 1 (Table 7), no flow occurred through either culture chamber. This essentially made each culture a 2.5-liter batch system. Following time interval 2, during which both cultures had flow, the control culture remained static for the duration of the experiment, while flow through the treatment culture was widely variable. These variable flow rates caused dilution rates and mean residence times to vary ca. 200-300% between time intervals.

An explanation for the poor mechanical performance of the

Figure 33. Comparison of algal associations in control and treatment cultures before (70 h) and after (166 h) NaCl additions during a laboratory chemostat bioassay. Comparisons are based on biovolume estimates, using the Percentage of community similarity (Psc). Sodium chloride additions began at ca. 94 h to the treatment cultures. (C1 = control 1; C2 = control 2; T1 = treatment 1; T2 = treatment 2).


Psc 70 HOURS
C1 C2 T1 T2
C1 — 77 90 80
C2 — 79 88
T1 — 83
T2 —
Psc 166 HOURS
C1 C2 T1 T2
C1 — 78 75 78
C2 — 89 82
T1 — 87
T2 —

experimental system is difficult, since many attempts were made during the analytical period to identify and remedy mechanical problems. These attempts included (see Figure 4): 1) adjustment of the relative elevations of inlet (i) and the influent line (a) to lines (d) and (d'); 2) priming influent line (a); 3) extensive checking of all lines, glass tubing, and connections for blockage; and 4) frequent adjustment of air outflow regulation values (p) and (p').

Because of the noted failure to control flow rates, it is clear that the control culture can be viewed as nothing more than a static batch system, since it is neither an acceptable control, nor an acceptable continuous flow culture. The treatment culture will be considered a continuous culture that never reached equilibrium between reservoir water inflow and algal growth.

Primary productivity. At 41 h (prior to any NaCl additions), similar productivity estimates (24-ml bottles) indicate that sufficient flow through each culture chamber removed any potential inhibitory or stimulatory enclosure effects brought about by static conditions of time interval 1 (Figure 6).

Depressed values for productivity in the 24-ml bottles were attributed in part to high dark fixation, which ranged from 37 to 47% of the light bottle mean. In contrast, 125-ml bottles had dark fixation at ca. 8% of the light bottle mean. Since precautions were taken to eliminate all light from the dark bottles, the factors involved in this marked increased in dark fixation for the smaller bottles are not known. Depression of 24-ml light bottle counts may have been due to different glass quality of the small bottles (Ohle 1958).

Data from the primary productivity incubation bioassay of 0.5-m

Sangre Isle Reservoir (SIR) water at 92 h indicate significantly reduced primary productivity at 624 μmhos (125 ml bottle estimates, $P \geq 0.05$). However, subsamples taken from the slightly salinized treatment chemostat culture at 92 h (232 μmhos) did not differ significantly from SIR productivity estimates (24-ml bottle estimates). For this comparison, a static conductivity of 624 μmhos was present in the batch test, and a decreasing conductivity (ca. 817 μmhos at 65 h) in the chemostat. Rapid inflow rates to the treatment chemostat culture brought about two changes. First, conductivity decreased ca. 585 μmhos during a 25 h time interval. Second, new phytoplankton brought into the treatment culture flask came into contact with a progressively lower conductivity. The short mean residence time during time interval 4 (12 h) meant that no phytoplankton sampled at 92 h had been in contact with the 817 μmhos water initially present at 65 h. Therefore, sampling of the treatment culture at 92 h was in essence sampling a briefly enclosed subsample of SIR. Had productivity been analyzed on a sample of culture effluent water collected during time interval 4, a more legitimate comparison could have been drawn.

Other researchers that have applied the in situ continuous culturing technique to perturbational studies have operated with slower flow rates (ca. 50 ml h^{-1}), and consequently longer (ca. 90-110 h) mean residence times (deNoyelles et al. 1980). A longer mean residence time in the treatment culture would have theoretically allowed adequate time for a potentially altered metabolic response to develop into a successional change. Since unfiltered inflow water was used, any potential response to NaCl perturbation was diminished by incoming water. The phytoplankton association that had possibly been influenced

by the higher salinity early in time interval 4 had been removed prior to any sampling. This may confirm a warning (deNoyelles et al. 1980) that dilution of the species response will occur when flow continuously enters the experimental chamber. Ideally, flow rate should be controlled so as to minimize the unnatural conditions of enclosure, while still allowing the researcher to detect a change in species distribution with reasonable analytical effort. Barlow et al. (1973) believed that in perturbational studies that last for more than a few hours, species succession is most likely occurring. Venrick et al. (1977) noted successional changes in short-term ^{14}C primary productivity bioassays. During this bioassay, if substantial successional change occurred in the treatment culture, it was not detected by the enumeration procedures used.

The static control culture, which had remained in a "no flow" condition for over 40 h showed a similar production rate to SIR. This appears to be attributable in part to a smaller but more photosynthetically active algal association.

Statistical comparisons among those bottles incubated with additional 5N NaCl are not possible, due to unequal and inadequate replication. The data available (Figure 6) suggest that those phytoplankton assemblages under a moderately elevated (but decreasing) NaCl salinity regime (ca. 700 µmhos), and those under normal low salinities (ca. 150 µmhos), are equally inhibited by further increases in salinity up to ca. 3500 µmhos. These results agree with earlier work by Wetherell (1961) who concluded that adaptation to the physiological stress of elevated salinity was not readily induced by preliminary culture at sub-limiting salinity levels. Further investigations would

be required to determine if phytoplankton assemblages composed of various taxonomic groups continuously cultured at elevated salinities would exhibit a tolerance or suceptibility to additional salinity increases, and at what salinity level siginficantly altered primary productivity would occur. Information of this type might prove valuable, since saline intrusions into thermally stratified reservoirs could affect phytoplankton at different and variable salt concentrations.

For the entire experiment, replicate determinations in the 24-ml bottles had a coefficient of variation (CV) range from 2-64%, with a mean of 28%. For the 125-ml bottles, a lower CV range from 4-18% resulted, with a mean of 11%. Other researchers have found a wide range of variability in productivity determinations (Venrick et al. 1977). Because of the over two-fold greater variation among small bottle replicates, their use to reduce sampling volume is not recommended for future field research.

Pigments and chlorophyll a activity. The overall decrease and subsequent increase in pigment levels was clearly influenced to a major extent by the changes in abiogenic turbidity. Light availability in the reservoir decreased substantially from 18 October when turbidity was 28 NTU, to 24 and 25 October, when turbidity was 122 and 116 NTU, respectively. As light became more limiting in the reservoir, phytoplankton loss rates increased. However, cell integrity and pigment concentrations persisted for several days prior to declining to their lowest levels on 25 October, five days after the rapid turbidity increase. With the settling of some of the silts and clays, light levels improved, Secchi depth transparency increased, and pigment levels

increased. This type of response to high abiogenic turbidity was also recorded in a eutrophic Nebraska reservoir by Schwartzkopf and Hergenrader (1978), and in numerous Oklahoma reservoirs and ponds by Claffey (1955). Contrasting this, Hunter (1982) found significantly higher chlorophyll concentrations at stations in Keystone Reservoir (Tulsa, Oklahoma) that had highest turbidities. In Texas reservoirs, Harris and Silvey (1940) reported maximum phytoplanktonic production occurring both at times of maximum and minimum turbidity.

In addition to light limitation, both SIR and culture algae may have faced a more limited phosphorus supply, as adsorption to clay-sized particles and sedimentation may have occurred.

Phaeopigments are a degraded form of chlorophyll which are non-functional due to the loss of the magnesium atom of the tetrapyrole ring (Bogarad 1962). These "Mg-free" components are formed when a chlorophyll molecule comes in contact with acid, or by exposure to prolonged darkness (Lorenzen 1965; Yentsch 1965). During ths study, functional chlorophyll _a_ was almost always equal to degraded chlorophyll for all samples. This suggests that either the enclosures and salinity increases had no effect on factors controlling the chlorophyll degradation pathway in SIR, or that this rough equality might be a consequence of the analytical procedure.

Phaeopigments were slightly greater than chlorophyll _a_ at 65 h in both SIR and the treatment culture. Although not significant, this could have been caused by the delayed degradation of algal cells, the input of allocthonous plant detritus, or light restriction.

Prior to the 92-h sampling, rapid inflow of fresh SIR water diminished any potential change in pigment levels from developing in the

treatment culture, while static conditions decreased pigment concentrations in the control culture.

A chlorophyll *a* activity for 18 October (pre-bioassay) of 14.0 mg C h^{-1} mg Chl. *a*$^{-1}$ was higher than all values I found in the literature. For example, Ryther and Yentsch (1957) report a chlorophyll activity range of 1-11 mg C h^{-1} mg Chl.$^{-1}$, with most below a value of 3.7, for light-saturated natural marine phytoplankton associations. Manning and Juday (1941) found an average chlorophyll activity value of 2.7 for some Wisconsin lakes. Hickman (1973) reported a chlorophyll activity range of 0.5 to 8.5 for surface in situ incubations in an English pond. For Lake Kinneret, Israel, a range of 0.4 to 7.8 was found by Berman and Pollingher (1974). Considering these data, it is evident that the pre-bioassay algal assemblage inhabiting the surface waters of SIR was highly efficient at carbon fixation, and that this efficiency declined following the turbidity increase.

During the bioassay, chlorophyll *a* activity ranged from 4.6 to 9.0 mg C h^{-1} mg Chl. *a*$^{-1}$ in cultures and SIR. A higher 92-h chlorophyll *a* activity for the control culture indicates a smaller but more efficient phytoplankton association than in either the treatment culture or SIR. This 92-h static control culture algal association was characterized by a high numeric diversity ($\overline{d}_n$ = 2.75), a moderate biovolume diversity ($\overline{d}_v$ = 1.89), and the lowest total cell volume of all sampled units (Table 8 and Figure 13).

The treatment culture at 92 h had the lowest chlorophyll *a* activity, although it was not significantly different from SIR. Care should be exercised in comparing these ratios, since errors in both chlorophyll *a* and productivity are compounded by taking their quotient

(Ryther and Yentsch 1957).

Species distribution. Total cell volume for SIR varied slightly during the experiment, with a range of 8.2 to 11.1 x 10^6 μm^3 ml^{-1}. Total cell density for SIR ranged from 1.6 to 3.5 x 10^4 cells ml^{-1}, with all culture densities within the upper 50% of that range. Confidence intervals set on both abundance estimates for SIR and cultures showed no significant differences at 41 or 90 h for all comparisons. For the treatment culture versus SIR comparison, this was predictable considering the high flow rates, short mean residence times, and rapidly declining NaCl-salinity levels in the treatment culture. However, similar abundance parameters for the static batch culture would not have been predicted.

Despite relatively similar total abundance values for SIR and the cultures, changes in species distribution did occur. At 41 h, biovolume species diversity ($\bar{d}_v$) for SIR and the two cultures diverged (Table 8). Biovolume species diversity was increased in the cultures by a reduction in the dominance of Cryptomonas and Oscillatoria, and a more even distribution of individuals among species. This slight increase in species evenness is evidenced by higher biovolume equitability (E_v). Presumably by their physical nature (decreased turbulence, increased attachment sites, etc.), control and treatment cultures exhibited larger populations of opportunistic genera such as Anabaena, Schroederia, and Synedra. I have called these species opportunistic, since they increased in abundance in the artificial enclosures that represented a new area for colonization. These species were not detected in SIR samples.

This phenomenon of similar succession in both cultures is

quantitatively expressed as a higher Psc (Percentage community similarity) value for control culture versus treatment culture 92 h comparison, than for either culture versus the SIR association. These data suggest that enrichment of the cultures with the opportunistic species listed occurred independently of inflow of fresh reservoir water. This was especially true for the diatom Synedra, which at 92 h accounted for 22% and 14% of the total biovolume of the control and treatment cultures, respectively. The increase in the Synedra population may have been due to the predominately benthic habit of this genus (Patrick and Reimer 1966).

Therefore, as members of the genera Anabaena, Schroederia, and Synedra increased in significance in the culture algal associations, they caused an enhancement of species diversity indices due to increased species richness and equitability. At 41 h, the higher $\overline{d}_V$ in the cultures was due to a higher species richness component. At 90 h, the cultures had a higher equitability component to species diversity. Such changes in species diversity for algal associations under approximately 10 species, agrees with the work of Sager and Hasler (1969).

At 41 h, two additional species distributional changes occurred. First, a 70% decrease in Oscillatoria triggered an ca. 50% reduction in total cell volume for the control culture. The reason that the treatment culture did not follow this trend may be due to the higher treatment culture inflow rate during time interval 2. Second, members of the blue-green alga genus Chroococcus numerically dominated both cultures. Although their biovolume contribution was minor due to their small cell size, the higher metabolic rates of these small cells may have caused the slight increase in productivity at 41 h (Figure 6).

Some Chroococcus species are benthic in habit (Round 1981), and this may have also contributed to their culture increases.

Twenty-two Hour Bioassay

Chemostat operation. High flow rates through the treatment chamber (time intervals 1 and 2) and control chamber (time interval 2) resulted in high dilution rates and short mean residence times (Table 9). For interpretation, both cultures were considered to be continuous. However, due to the short duration of the bioassay and flow rate variation, steady state conditions were not achieved.

Primary productivity. A severe reduction of productivity in both continuous cultures indicates strong phyto-inhibitory effects by the chemostat apparatus on the enclosed algal populations. Although the treatment culture productivity was depressed significantly from the SIR level, only a small percentage of that appears attributable to NaCl-salinity. Because productivity reduction in the control culture was statistically equal to that of the treatment culture, some mutually negative effect of enclosure was responsible. Elevated culture turbidities may have been partially responsible for decreased productivity and chlorophyll a, presumably through reduction of available light.

Productivity of treatment culture algae showed no acute response when conductivity was raised to 919 µmhos for the productivity incubation period. This suggests that either the previous salinity increase had already inhibited that portion of algal productivity that was susceptable to a moderate (500-1000 µmhos) salinity increase, or that the chemostat itself caused the reductions. The remaining portion

represented productivity not affected by the moderate salinity increase. A treatment culture incubation conductivity of 3650 μmhos does appear to significantly reduce productivity, although lack of replication does not allow for statistical comparisons.

Due to the severe inhibition of productivity in the continuous flow control culture, it is difficult to ascertain any true NaCl-induced effects. This is equally true for both samples incubated directly from the culture, and those incubated with additional volumes of NaCl solution.

Pigments and chlorophyll a activity. Despite short mean residence times in both continuous cultures, chlorophyll a concentration and chlorophyll a activity of the culture algae were reduced from SIR levels (Figure 17). Sodium chloride treatment did not reduce chlorophyll a or chlorophyll a activity significantly below control levels.

Phaeopigments can be useful indicators of the past history of phytoplankton populations (Lorenzen 1967). Currie (1962) discovered that ingested chlorophyll a was rapidly broken down to pheophorbide in the guts of small marine animals. Lorenzen (1967) suggested that a primary source of phaeopigments in the ocean results from zooplankton grazing. Consequently, the phaeopigment:chlorophyll ratio has been used as a measure of grazing pressure, and as a measure of population viability (Lorenzen 1967). A high ratio indicates more zooplankton grazing, higher levels of detrital chlorophyll, and a less viable phytoplankton population. Although not statistically significant, both cultures show larger phaeopigment:chlorophyll a ratios, which suggests another negative influence on the enclosed populations, possibly due to increased grazing activity. Ratio differences are due in most part to

chlorophyll *a* reductions, since phaeopigment concentrations varied only slightly among the sampled units.

The increase in the Chroococcus population in the treatment culture caused chlorophyll *a* per cell to decrease ca. 5-fold below control culture and SIR levels. For chlorophyll *a* per cubic micrometer of algal cell volume, the treatment culture ratio was ca. 50% lower than the others. The smaller relative differences in this ratio (Chl. *a* μm^{-3} cell volume) between treatment culture and controls were due in part to the minor cell volume increase that accompanied the large numerical increase in Chroococcus in the treatment culture.

Species distribution. Of biological significance in terms of species distribution was the numeric increase of the coccoid blue-green alga Chroococcus in the treatment culture. Environmental conditions present in the treatment culture favored the growth of two members of this genus, decreasing numeric diversity ($\bar{d}_n$) to 1.12, as compared to 1.79 and 2.02 for the control culture and SIR, respectively.

Members of the Chroococcus genus are considered unicellular, although individuals often occur grouped in aggregates of 2 to 8 cells due to the failure of cellular division products to separate promptly (Bold and Wynne 1978). Although Prescott (1980) refers to members of this genus as usually free-floating, Round (1981) notes that they can also grow as a member of the phyto-benthos, attached to rocks or sediments. A colonial mucilage is present around the aggregates, although it does not intermingle with that of other colonies. The average volume of a single Chroococcus cell during this experiment was ca. 5 μm^3. According to the size classification scheme in Wetzel (1983), individual Chroococcus cells would be considered ultraplankton.

Because of numerous abiotic and biotic interactions that occurred within the treatment culture, many of which were not estimated, the Chroococcus increase cannot be solely attributed to increased NaCl-salinity. It is evident that Chroococcus growth and reproduction were stimulated by environmental conditions present in the treatment culture. However, all comparisons to the control culture, which did not support a similar succession, must be tempered by the fact that during time interval 2, the average flow rate through the control culture was nearly 4 times greater than the treatment culture rate. Because of this difficulty, culture chamber environmental factors affected by flow rate cannot be ruled out as influences upon Chroococcus and other species.

The interpretation of similarity comparisons must also be viewed with caution. The higher Percentage community similarity (Psc) between the control culture algal association and the SIR association was due to a roughly equivalent distribution of the total cell volume for each among similar species present. This high Psc value (81%) occurred despite the considerably larger total cell volume present in SIR. This similar distribution pattern in the control and SIR at 22 h is evidenced by the relatively close equitability values (E_V) of 0.32 and 0.47 respectively. The Psc index has been shown by Brock (1977) and Pinkham and Pearson (1976) to fail (i.e., dissimilar data yields similar Psc values) when the relative abundances of the taxa remain the same, but the overall abundances change, a situation that parallels the control culture versus SIR comparison noted above. Figure 20 shows the treatment culture association was more similar in total cell volume to the SIR association, however, the equitability was much greater for the treatment culture (E_V = 0.88), hence the lower Psc value for this

comparison. Consequently, the higher similarity between the algal associations in the control culture and SIR appears to be illusory.

In the treatment culture, as numeric species diversity ($\overline{d}_n$) decreased (1.88 at 0 h to 1.12 at 22 h) biovolume species diversity ($\overline{d}_v$) increased (1.11 at 0 h to 2.31 at 22 h). This $\overline{d}_v$ increase is due mostly to a decrease in Cryptomonas volume, and an increase in volume contributions of such relatively minor species as Chlamydomonas and Francia. A high equitability based on biovolume (E_v) value of 0.88 for the 22-h treatment culture expresses this more even distribution of total cell volume among the species present.

The control culture also showed a decrease in the absolute biovolume of Cryptomonas, although no concurrent increase in minor species was detected. The fact that Cryptomonas biovolume decreased in the control culture suggests that higher salinity alone did not cause Cryptomonas to decline in the treatment culture. This means that either the moderately elevated salinity concentrations were partially responsible for the observed $\overline{d}_v$ increase, or that the minor algal species that largely contributed to the higher treatment culture $\overline{d}_v$ were not detected in the control culture and SIR due to possible errors in enumeration.

The observed treatment culture changes in species distribution are perhaps a competitive release phenomenon, triggered by the combined effects of selective zooplankton grazing and salinity inhibition on the biomass dominant Cryptomonas. Porter (1977) has noted that Cryptomonads belong to a "reaction group" of algae that are known to decline in numbers when grazer density is experimentally increased-a situation that might have existed in the culture chambers. Porter

(1977) also states that high algal diversity occurs when there are no numerically dominant species, and that a diversity increase may be brought about by zooplankton grazing. My data suggest that as a dominant larger-volume phytoplankter such as Cryptomonas decreases in importance, and a smaller-volume opportunistic species such as Chroococcus becomes numerically dominant, $\overline{d}_v$ will increase, while $\overline{d}_n$ decreases. This increase in $\overline{d}_v$ does not indicate a shift to a more "stable" community, but rather denotes the onset of a period of transition and change (Porter 1977; Round 1981). The exact role NaCl-salinity played in bringing this about, and how it would affect succession beyond this point are unknown.

Numerous authors have suggested that blue-green algae can tolerate relatively high salt concentrations (Batterton and Van Baalan 1971; Fogg et al. 1973). Emerson and Lewis (1942) found that Chroococcus grew little if at all when Na^+ was omitted from the medium. As Chroococcus increased in the treatment culture, the filamentous blue-green alga Oscillatoria decreased in both absolute numbers and volume. This difference is perhaps due more in part to the small size and high metabolic activity of the Chroococcus species, than to the inhibition of Oscillatoria by elevated salinity. Since certain species of Oscillatoria have nitrogenase activity under microaerophilic conditions (Kenyon et al. 1972), it would be of practical interest to study the ecology of salinity in terms of nitrogen fixation, species succession, and eutrophication in Oklahoma impoundments.

Laboratory Chemostat Bioassay

Chemostat Operation

Since the flow regime of the cultures was dominated by a continuous and variable outflow, and semi-continuous and variable inflow, care must be exercised in the interpretation of results. Although this variation was greater than anticipated, it does not completely negate planned comparisons, and lower variation was exhibited by the control-treatment pairs noted (C1 and T1: C2 and T2).

Pigments, Productivity, and Species Distribution

The general decrease in chlorophyll *a* for all cultures during the bioassay was caused in part by the overall decline in chlorophyll *a* in the SIR influent water, and probably to photo-reduction of chlorophyll *a* under the artificially high light intensities used. This photo-reduction phenomenon has been reported for marine algae by Bieble (1952). The increased phaeopigment:chlorophyll *a* ratio that occurred for all cultures during the 70 to 96-h time interval continued to increase significantly for only T1 from 96 to 167 h. An increase in this pigment ratio means a less viable phytoplankton association (Lorenzen 1967). Consequently, the productivity depression for T1 at 167 h was due in part to the relative decrease in functional chlorophyll *a*.

Decreasing carbon fixation rates during the 50 to 120-h time interval followed the decline in culture chlorophyll *a*. As culture chlorophyll *a* began to level off (ca. 125-144 h), productivity subsequently increased significantly. This productivity increase in all

cultures occurred despite a decrease in the productivity of SIR influent water, and corresponded to higher Synedra and Chroococcus population densities in most cultures. This common increase in productivity per unit chlorophyll a (chlorophyll a activity) during the final 26 h for all cultures suggests that after an initial acclimation period characterized by a decreasing Cryptomonas population, smaller volume but more productive species became adapted to the artificial laboratory conditions, and began to increase in importance. The higher rate of increase in chlorophyll a activity exhibited by T2 and C2 during the last 26 hours appears to be related to the longer mean residence times occurring in those cultures.

Some interesting salinity-productivity relationships result from the disparity between the acute response of the algal associations from fresh SIR water (Figure 29), and culture water (Figure 30) to elevated NaCl-salinity at incubation. On four occasions, when fresh SIR water was incubated at ca. 2000 µmhos total conductivity, the average productivity reduction was ca. 54%. However, at 145 hours, both control cultures show statistically similar productivities at normal (ca. 150 µmhos) and elevated (ca. 2000 µmhos) salinity levels. This control culture anomaly was accompanied by an 18% stimulation for both treatment cultures when NaCl-salinity was approximately doubled from the previously elevated level (ca. 1000 µmhos) to ca. 2000 µmhos. These results indicate that the laboratory culturing system had either increased the culture algae's short-term physiological tolerance to NaCl, or that a change in species composition to a more NaCl tolerant algal assemblage had been induced. Although culture algal abundance and composition data are not available at 145 hours, it is clear that as

Cryptomonas declined in the cultures, Synedra and Chroococcus increased. However, despite decreasing Cryptomonas numbers in SIR, this genus remained the overwhelming biovolume dominant (> 80% to total cell volume) throughout the sampling period.

This anomaly at 145 hours is further delimited by 167-hour productivity results. At 167 hours, both treatment and control cultures were dominated by a Synedra, Cryptomonas, and Chroococcus association, and both show productivity inhibition at 2000 μmhos. Control culture productivity reductions were on the average ca. 2.5 times greater than the average treatment culture reduction.

These data tend to support two important points, one methodological, and the other biological. First, results obtained with an acute primary productivity bioassay may not agree with longer-term laboratory bioassays. This point has practical considerations, since most algal bioassays are short-term in nature. Similar conclusions have been made by Gerhart and Likens (1975) and O'Brien and deNoyelles (1976) in nutrient limitation studies. Secondly, a slightly greater tolerance to elevated NaCl-salinity accompanied the shift from Cryptomonas to Synedra and Chroococcus in the treatment cultures. This tolerance to the 2000 μmhos incubation salinity was at least temporarily and partially induced by preliminary culturing at ca. 1000 μmhos. These results differ from the in situ continuous culture bioassay results presented earlier, and from work by Wetherell (1961) on salinity tolerance adaptation for unicellular green algae. Wetzel (1966) and Provasoli (1969) have suggested that high Na^+ levels might favor blue-green algae, while Hutchinson (1967) considers the diatoms as a taxon possessing high euryhalinity. The relationship between these

published observations and the species distributional results presented here is unconfirmed.

In conclusion, there is little evidence to indicate that NaCl-salinity elevated 4-5 times above normal low conductivity significantly altered either the metabolism or the species composition of the natural winter associations of Oklahoma reservoir phytoplankton used in this bioassay. There does exist, however, some evidence to suggest that preliminary culturing at ca. 1000 μmhos can induce limited tolerance to secondary NaCl-salinity increases, and that this tolerance may be supported by a shift in species composition from a low diversity Cryptophyte dominated association, to one dominated by a diatom and a small blue-green alga.

In retrospect, a lengthened bioassay might have allowed for the separation of salinity-induced effects from those resulting from natural laboratory succession. Future bioassays of this type might include the use of multiple culture salinity levels under higher replication, the placement of an additional pump for more precise inflow regulation, the application of a more natural temperature and lighting regime, and the use of coordinated in situ productivity determinations.

CHAPTER V

CONCLUSIONS AND IMPLICATIONS

Static Bioassays

In situ Primary Productivity Bioassays

During the summer and fall of 1983 in the surface water of Sangre Isle Reservoir, elevations in NaCl-salinity most often caused acute productivity inhibition initiated at either a relatively low (ca. 500-600 μmhos) or high (ca. 6000 μmhos) conductivity for phytoplankton associations composed mainly of green and blue-green algae. On relatively fewer dates a similar salinity range had no effect, or caused stimulation of productivity. Variability in the NaCl-induced productivity response for similar phytoplankton assemblages on different dates indicated an unknown factor interaction.

Laboratory Batch Bioassays

Anabaena flos-aquae

Significant inhibition in relative carbon assimilation and chlorophyll *a* content began between ca. 700 and 2340 mg NaCl $liter^{-1}$ (2180-5280 μmhos) for *Anabaena flos-aquae* in late exponential growth phase. No further inhibition in either metabolic variable occurred at 4675 mg NaCl $liter^{-1}$ (9710 μmhos). Cell densities were not affected by NaCl treatments.

Selenastrum capricornutum

Relative carbon assimilation of Selenastrum capricornutum in exponential growth phase was significantly depressed (-20% from control) at 538 mg NaCl liter^{-1} (1280 µmhos), with further reductions as concentration increased. Inhibition of ^{14}C-uptake was reduced as cultures at 2735 mg NaCl liter^{-1} (6770 µmhos) began the stationary growth phase. Cell densities were not affected up to 2735 mg NaCl liter^{-1}. Sodium chloride at 11,500 mg liter^{-1} (22,500 µmhos) nearly totally inhibited ^{14}C-uptake and cell division.

Renewal Bioassays

In situ Chemostat Bioassays

Ninety-two Hour Bioassay

Extensive periods of zero flow through the control chamber and excessively high flows through the treatment chamber introduced extreme variability, and forced reexamination of the experimental design.

The high treatment culture flow rates caused relatively short mean residence times, and dilution of any possible NaCl-induced effect upon the structure or function of the Sangre Isle Reservoir algal association with the relatively low salinity levels (ca. 800 µmhos) used.

In situ culturing at a moderately elevated but decreasing NaCl-salinity (ca. 700 µmhos) did not confer greater tolerance (in terms of productivity) to a secondary salinity increase up to ca. 3500 µmhos.

After ca. 40 h in a "no flow" static condition, chlorophyll *a* concentration of the control culture was reduced, but algal abundance and productivity remained similar to Sangre Isle Reservoir levels.

Significant increases of Anabaena, Schroederia, and Synedra occurred in both control and treatment culture chambers, independent of a concurrent Sangre Isle Reservoir increase. This caused species diversity to increase in the cultures as compared to Sangre Isle Reservoir. This culture enriching effect upon species diversity was unrelated to culture flow characteristics.

The use of small volume bottles for enclosures during productivity determinations is not recommended due to excessive variation among replicates.

Twenty-two Hour Bioassay

High flow rates through both treatment and control cultures resulted in high dilution rates and short mean residence times. Sodium chloride additions to the treatment culture were rapidly diluted.

A strong phyto-inhibitory effect by the chemostat unit on the enclosed phytoplankton populations reduced productivity and chlorophyll a in both cultures as compared to Sangre Isle Reservoir. Sodium chloride additions to the treatment culture did not further reduce these variables.

Culture phaeopigment:chlorophyll a ratios were slightly higher than Sangre Isle Reservoir levels, suggestive of additional chemostat stress on the enclosed phytoplankton populations.

Species distribution in both culture chambers was altered from that present in Sangre Isle Reservoir. In both cultures, Cryptomonas declined in abundance. Both Chlamydomonas and Francia increased in abundance in the treatment culture, but not in the control culture, causing equitability and species diversity to increase in the treatment

culture.

A large numeric and biologically significant increase in the small-volume blue-green alga Chroococcus occurred only under treatment culture conditions. This phenomenon can not be attributed solely to NaCl additions, due to uncertainity caused by flow variation and other unestimated factors.

Laboratory Chemostat Bioassay

A re-examination of the experimental design used for the 170-hour laboratory bioassay revealed a flow gradient which caused variation in inflows and outflows among the cultures to be greater than expected. However, due to the random assignment of cultures to either treatment or control groups, planned control-treatment comparisons remained valid.

Sodium chloride-salinity elevated 4-5 times above a normal low conductivity (ca. 200 μmhos) did not significantly alter either the metabolism (primary production and chlorophyll a concentration) or species composition of a natural winter association of algae from Sangre Isle Reservoir.

A shift in the structure of the algal association from a low diversity assemblage dominated by Cryptomonas species, to a more diverse association dominated by Synedra and Chroococcus took place in all cultures. This compositional shift may have increased tolerance to a rapid secondary NaCl elevation.

Preliminary culturing of the natural Sangre Isle Reservoir algal associations at ca. 1000 μmhos NaCl-salinity at least temporarily induced tolerance to an acute doubling of NaCl-salinity upon productivity incubation.

Chlorophyll *a* showed a general decrease throughout the 170-h bioassay for all cultures, causing the phaeopigment:chlorophyll *a* ratio to increase. This indicates a trend toward a less viable phytoplankton association.

A common productivity increase during the final 26 h of the bioassay suggests an initial acclimation period is required (both taxonomically and physiologically) as natural phytoplankton populations adjust to a new laboratory environment.

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VITA 2

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