EFFECTS OF SALINITY ON THE METABOLISM AND BIOLOGY
OF PHYTOPLANKTONIC COMMUNITIES

David A. Francko
Botany and Microbiology Department
Oklahoma State University

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WATER RESEARCH

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Principal Investigator: David A. Francko
                          Assistant Professor, Department of Botany and
                          Microbiology

Collaborator   : .Bill Thompson
                          M.S. Degree candidate, Department of Botany and
                          Microbiology

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1. Summary

Static and renewal algal bioassays were conducted in both the laboratory and
in situ to test for changes in algal metabolism and species composition under
elevated NaCl-salinity.

In situ primary productivity incubation bioassays conducted in a small non-
salinized (ca. 250 μmhos) eutrophic reservoir [Sangre Isle Reservoir (SIR),
Oklahoma] showed a variable acute phytoplanktonic response; including
inhibition (at either ca. 500 or 6000 μmhos), stimulation (ca. 800-3700 μmhos)
or no effect.

Static laboratory batch bioassays with Anabaena flos-aquae and Selenastrum
capricornutum showed significant inhibition of C-14 uptake above 2180 μmhos
and at 1280 μmhos respectively. For Selenastrum, 22,500 μmhos caused nearly
total inhibition of C-14 uptake and cell division.

Flow rate variability between treatment and control cultures for 92-h and
22-h in situ chemostat bioassays confounded results. Enclosure of natural
phytoplanktonic associations either strongly inhibited or had no effect on
productivity and chlorophyll a. Species diversity increased in enclosed
cultures compared to SIR. A Chroococcus population increase occurred under
salinized conditions during the 22-h bioassay. Preliminary elevation of
salinity from ca. 200 to 700 μmhos did not confer greater tolerance to a
second salinity increase to ca. 3500 μmhos.

Sodium chloride additions raising conductivity 4-5 times above normal levels
did not alter either the metabolism or species composition of a natural winter
algal association during a 170-h laboratory chemostat bioassay. Preliminary
culturing at ca. 1000 μmhos temporarily induced tolerance to a salinity
increase to 2000 μmhos. This tolerance may be attributable in part to a shift
in dominance from Cryptomonas to Synedra and Chroococcus. Stimulation of pro-
ductivity occurred in salinized cultures at 145-h upon acute doubling of salinity.

2. Statement of Problem

Many large river-reservoir systems that dominate the surface water resources of the American South and West face water quality degradation due to an increased concentration of dissolved salts. Increases in total salinity arise as salt loading from such natural sources as saline mineral springs, saline seeps, and discharges from salt accretions occurs. Anthropogenic sources that contribute to salt loading include oil and gas operations, municipal and industrial discharge, leeching of spent oil shale, and saline irrigation return flows (Blackman et al. 1973; Cleave et al. 1981). Additionally, as consumptive loss of water occurs via municipal, industrial, or agricultural withdrawals, and naturally by the process of evaporation, the quantity of water available for dilution is diminished.

This salinity problem is acute in the two major river systems in Oklahoma, with nine of the State's reservoirs considered to have poor water quality in terms of total dissolved solids (Oklahoma Water Resources Board 1980). For example, the Cimmaron River of the Arkansas River drainage basin is one of the most highly-salinized streams in the State, draining areas containing large amounts of soluble salts ($NaCl$, Na_2SO_4, $MgCl_2$, $CaCl_2$). 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), and salinity in the Cimmaron Arm of the impoundment ranges from ca. 2000–4000 micromhos (μmhos).

In the Red River drainage basin, ca. 90% of the drainage area is 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 chloride enter it every day. In 1976, one-third of the total chlorides were from controllable oil field activities, with the remaining two-thirds from natural sources (U.S. Army Corps of Engineers 1976). The 1976 cost estimate to control the natural sources alone, via an elaborate system of dikes, dams, and retention reservoirs, was ca. 124 million dollars (U.S. Army Corps of Engineers 1976).

The biological and ecological consequences of elevated or fluctuating salinity have been inadequately addressed in Oklahoma and in the entire American West. Of the available studies, few deal with effects on the primary trophic level. Any alteration in the composition or metabolism of the primary producers of Oklahoma reservoirs, 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 (such as many Oklahoma reservoirs), where phytoplanktonic productivity often represents the major input of new organic matter, as phytoplankton fix almost all energy used by the entire system (Sakshaug 1980; Wetzel 1983).

This study employed an integrated laboratory-field approach to experimentally 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; and 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.

3. Results and Discussion

Laboratory Batch Bioassays

Results from a 112-h static laboratory bioassay using monoalgal cultures of the filamentous blue-green alga Anabaena flos-aquae in late exponential growth phase are shown in Table 1. Algae of this type play an important role in the eutrophication of Oklahoma surface waters. Significant inhibition in relative carbon assimilation and chlorophyll a concentration occurred between 701 and 2337 mg NaCl liter^{-1}. Further inhibition did not occur as NaCl salinity was elevated to 4675 mg liter^{-1}. Cell density was not affected by salinization. These data indicate that reductions in the productivity of Anabaena flos-aquae may be predicted under moderate NaCl salinization.

In a similar type bioassay, the unicellular green alga Selenastrum capricornutum showed significant inhibition in relative carbon assimilation at 538 mg NaCl liter^{-1} (Table 2). For cells in exponential growth phase, relative carbon assimilation was reduced further as salinity was increased. A NaCl concentration of 11,500 mg liter^{-1} effectively reduced carbon assimilation and cell division to insignificant levels, a response consistent with results from 1982 in situ bioassays using natural phytoplankton associations. These data are important as a reference standard since Selenastrum is a widely-used test alga for pollution bioassays.

In situ Primary Productivity Bioassays

Primary productivity incubation bioassays were conducted in situ for January and June through October of 1983 in Sangre Isle Reservoir (SIR), a eutrophic low-salinity (ca. 200 μmhos) impoundment located 8 km southwest of Stillwater in Payne County. Figure 1 presents productivity data for these natural phytoplankton bioassays. 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 (23 June and 14 July), or caused a stimulation of productivity (30 August). Variability in response for similar phytoplankton assemblages on different dates indicated an unknown factor interaction.

In situ Continuous Flow Bioassays

Continuous flow bioassays were conducted in situ at SIR using a continuous culture system (chemostat) described by DeNoyelles and O'Brien (1974). Excessively high and variable flow rates occurred during 92-h and 22-h bioassays. Only selected results from the 22-h experiment are presented.

Injections of NaCl into the treatment culture of the chemostat raised conductivity ca. 6-fold above the natural SIR and control culture levels, although high flow rates caused rapid dilution. A strong phyto-inhibitory effect by the chemostat unit reduced productivity and chlorophyll a in both cultures compared to SIR (Figure 2 - Productivity data only). Additional injections of NaCl into the incubation bottles at 22 h were made, but control versus treatment productivities were not significantly different under all salinity regimes tested. Increased turbidity and/or increased grazing pressure in the cultures may have been responsible for the inhibition of productivity.

Figure 2 shows algal density and species distributional results at 22 h. Although Cryptomonas remained the biovolume dominant in cultures and SIR, a large numeric and biologically significant increase in the small-volume blue-green alga Chroococcus occurred under NaCl treatment culture conditions. Because of the Chroococcus population increase, treatment culture numeric species diversity $(\bar{d}_n)$ was reduced compared to the control culture and SIR (Table 3). Biovolume species diversity $(\bar{d}_v)$ for the treatment culture increased, as the distribution of individuals among species increased (E_v). These phenomena can not be attributed solely to NaCl additions, due to uncertainty caused by flow variation and other unestimated factors.

Laboratory Chemostat Bioassay

A 170-h laboratory chemostat bioassay was conducted using the general methodology described by Barlow et al. (1973) and deNoyelles et al. (1980). Unfiltered water was used in the bioassay, and was collected daily from SIR.

A flow gradient caused variation in inflows and outflows among cultures to be greater than expected. Figure 4 shows the elevated salinity regime for treatment cultures. A general trend of decreasing chlorophyll a in all cultures caused the phaeopigment:chlorophyll a ratio to increase, with treatment culture 1 significantly greater than the other cultures over the NaCl-treatment period (Figure 5).

Salinization did not affect treatment culture productivity immediately after introduction at 94 h. Final treatment culture productivities were significantly different from each other and from the controls, being both stimulated and inhibited relative to the more consistent control culture responses (Figure 6).

To test the hypothesis that treatment culture phytoplankton would exhibit a greater degree of tolerance to further acute NaCl salinity increases after initial culturing at a moderately elevated salinity (ca. 1000 μmhos) 145 and 167-h subsamples were incubated at both normal salinity and at ca. 2000 μmhos. Figure 7 shows that preliminary culturing at ca. 1000 μmhos temporarily induced tolerance to an acute doubling of NaCl salinity. An 18% stimulation of productivity occurred in 145-h treatment cultures.

A shift in the structure of the algal associations in all cultures from a low diversity assemblage dominated by Cryptomonas to a more diverse association dominated by Synedra and Chroococcus may have been partially responsible for the increased tolerance to a rapid secondary NaCl elevation (Figure 8). In general, sodium chloride salinity elevated 4-5 times above SIR levels did not alter a natural winter association of algae either metabolically or structurally.

4. Groups Benefited

Research results summarized here and treated more comprehensively by Thompson (1984) will be of benefit to the following groups: 1) water quality management agencies (e.g. Oklahoma Water Resources Board, Oklahoma Department of Health, Pollution Control Coordinating Board, U.S. Army Corps of Engineers, etc.); 2) fisheries management agencies (e.g. Oklahoma Department of Wildlife Conservation and U.S. Fish and Wildlife Service); and 3) academic ecologists.

5. New Contributions

Collectively, these data represent the most comprehensive study to date concerning phytoplankton-salinity interactions in Oklahoma reservoirs. To our knowledge, this research effort represents the first time that in situ continuous culturing methods have been employed to study the ecology of mineral pollution in Oklahoma. Field studies on the structural and functional changes that result from salinization of previously unimpacted natural associations provide a data base for comparison to future studies on salinized Oklahoma impoundments. Seasonal data on the acute response of natural phytoplankton assemblages to elevated NaCl salinity allows for the first time a preliminary basis for estimating impacts on higher trophic levels (i.e. zooplankton and fish). The integrated laboratory-field experimental approach used for this study allows for comparisons between methodologies that differ in complexity and potential for extrapolation to natural aquatic ecosystems.

6. Publications Supported by Regents Water Grant Funding

Francko, D.A. 1983. Dissolved cyclic adenosine 3':5'-monophosphate in a eutrophic reservoir lake. Proc. Ok. Acad. Sci. 63:9-11.

Francko, D.A. 1983. Size-fractionation of alkaline phosphatase activity in lakewater by membrane filtration. J. Fresh. Ecol. 2:305-309.

Francko, D.A. 1984. The significance of cyclic nucleotides in phytoplanktonic carbon metabolism: A current view. Verh. Internat. Verein. Limnol. 22: (in press).

Francko, D.A. 1984. Phytoplanktonic metabolism and cyclic nucleotides: I. Nucleotide-induced perturbations of carbon assimilation. Arch. Hydrobiol. 100:341-354.

Francko, D.A. 1984. Phytoplankton metabolism and cyclic nucleotides: II. Nucleotide-induced perturbations of alkaline phosphatase activity. Arch. Hydrobiol. 100:409-421.

Francko, D.A. Relationships between phosphorus functional classes and alkaline phosphatase activity in reservoir lakes. J. Fresh. Ecol. 3: (in press).

Francko, D.A. A tissue solubilizer method for the measurement of chlorophyll a and ^{14}C-uptake in algae. J. Plankton Res. (in review).

Francko, D.A. Axenic growth of Nelumbo lutea: Use as a model physiological system. J. Aquat. Plant Manage. (in review).

Thompson, W.C. 1984. Sodium chloride-induced structural and functional changes in monoalgal laboratory cultures and natural phytoplankton assemblages in renewal and static bioassays. M.S. Thesis, Oklahoma State University, 194 pp.

Thompson, W.C. and D.A. Francko. Laboratory and in situ renewal bioassays to test for structural and functional changes in natural phytoplanktonic associations. (in prep).

Thompson, W.C. and D.A. Francko. Primary productivity incubation bioassays to test for salinity stress in Oklahoma reservoir phytoplankton (in prep).

7. Papers Presented

Thompson, W.C. 1982. Effects of increasing sodium chloride salinity on
    relative carbon fixation in natural populations of pelagial phytoplankton.
    Oklahoma Academy of Sciences Annual Meeting, University of Science
    and Arts of Oklahoma, Chickasha.

Thompson, W.C. 1983. Use of an in situ chemostat to study the effects of
    NaCl salinity on reservoir phytoplankton. Oklahoma Academy of Sciences
    Annual Meeting, Oral Roberts University, Tulsa.

Francko, D.A. 1983. To quench our thirst: The present and future American
    water crises. Department of Forestry Seminar Series, Okla. State Uni-
    versity, September 1983.

Francko, D.A. 1983. What's in a lake? Green Country Teachers Workshop,
    Phillips Petroleum Co., Bartlesville, OK, October 1983.

Francko, D.A. 1983. Biomanipulation of plant productivity in lake ecosystems,
    OK. Conference on Science and Technology, Tulsa, OK, December 1983.

Francko, D.A. 1983. The significance of cyclic nucleotides in phytoplanktonic
    metabolism: A current view. XXII. Congress, International Association
    of Theoretical and Applied Limnology, Lyon, France, Aug. 1983.

Francko, D.A. 1983. Epilimnetic phosphorus cycling: Are there two major
    mechanisms? Annual Meeting, Great Plains Limnology Conference, Lake of
    the Ozarks, MO, October 1983.

Francko, D.A. 1983. Cyclic nucleotides as regulators of algal carbon assimi-
    lation, Annual Meeting, Okla. Acad. Sci., Tulsa, OK, November 1983.

Francko, D.A. 1984. Epilimnetic phosphorus cycling. How many mechanisms?
    Int. Sym. on Phosphorus-Plankton Dynamics, 26th Conference on Great Lakes
    Research, St. Catharines, Ontario, April 1984.

Francko, D.A. 1984. Nucleotide-induced perturbation of algal C-assimilation:
    A plausible model. 47th Annual Meeting. Amer. Soc. Limnol. Oceanogr.,
    Vancouver, B.C., Canada, June 1984.

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    1973. Mineral pollution in the Colorado River basin. J. Water Pollut.
    Contr. Fed. 45:1517-1557.

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Sakshaug, E. 1980. Problems in the methodology of studying phytoplankton. Pages 57-95 in I. Morris, ed. The physiological ecology of phytoplankton. University of California Press, Berkeley and Los Angeles. 625 p.

Thompson, W.C. 1984. Sodium chloride-induced structural and functional changes in monoalgal laboratory cultures and natural phytoplankton assemblages in renewal and static bioassays. M.S. Thesis. Oklahoma State University, Stillwater, Oklahoma. 194 p.

U.S. Army Corps of Engineers. 1976. Arkansas-Red River basin chloride control; Texas, Oklahoma, Kansas. (Red River Basin). Design memorandum No. 25, general design, phase I - plan formulation. Tulsa District.

Wetzel, R.G. 1983. Limnology. 2nd edition. Saunders College Publishing, Philadelphia. 767 p.

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

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

[b] Counts per minute (quench corrected).

Table 2. 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 $(CPM^b + 1SD \times 10^3)$				Cell density $(cells\ ml^{-1} \times 10^6)$			
		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.

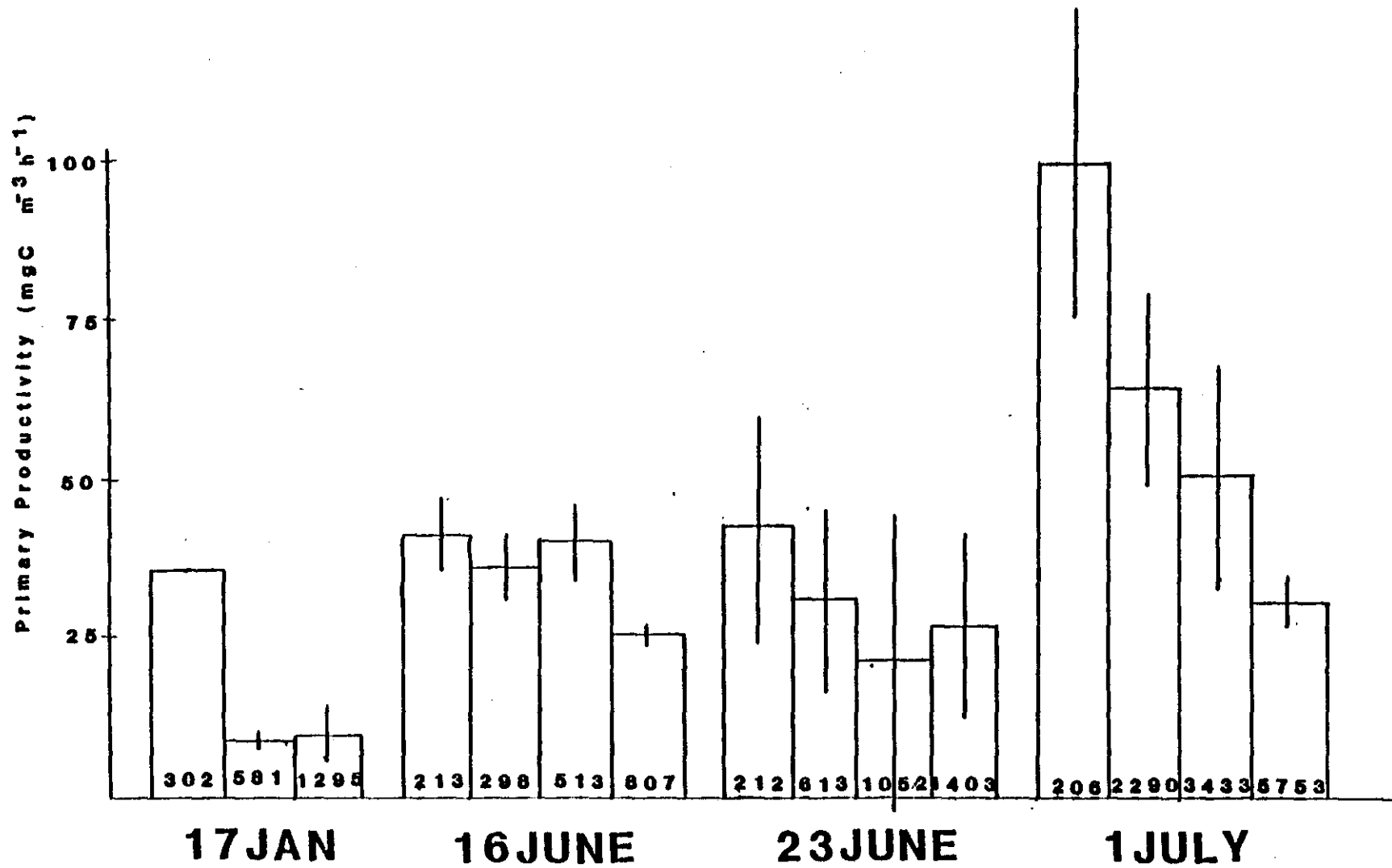Table 3. Algal abundance and community structure parameters for a 22-hour in situ bioassay.

Algal association variable	0 h SIR[a]	22 h		
		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 1. 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 $^{\circ}$C.
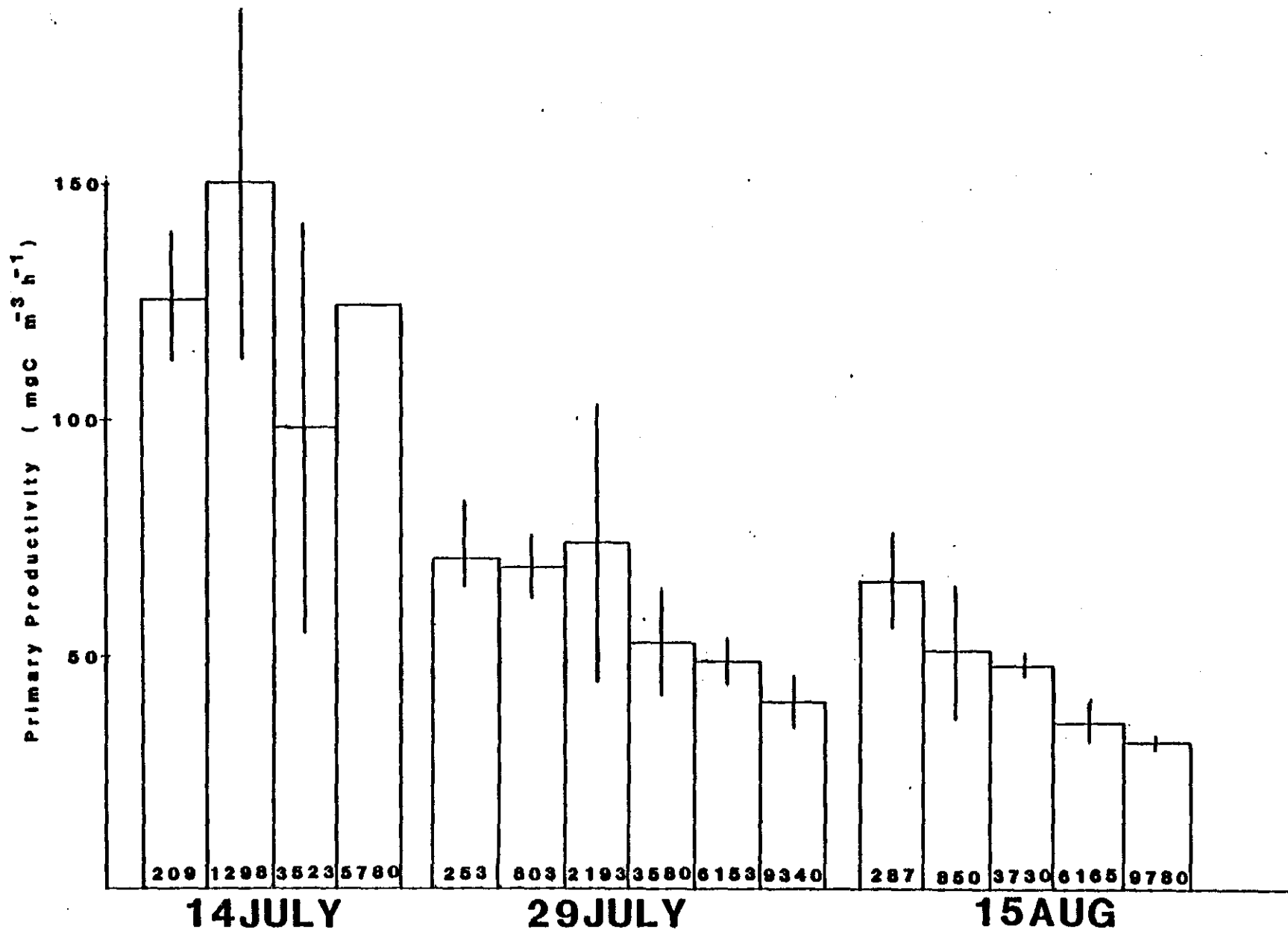

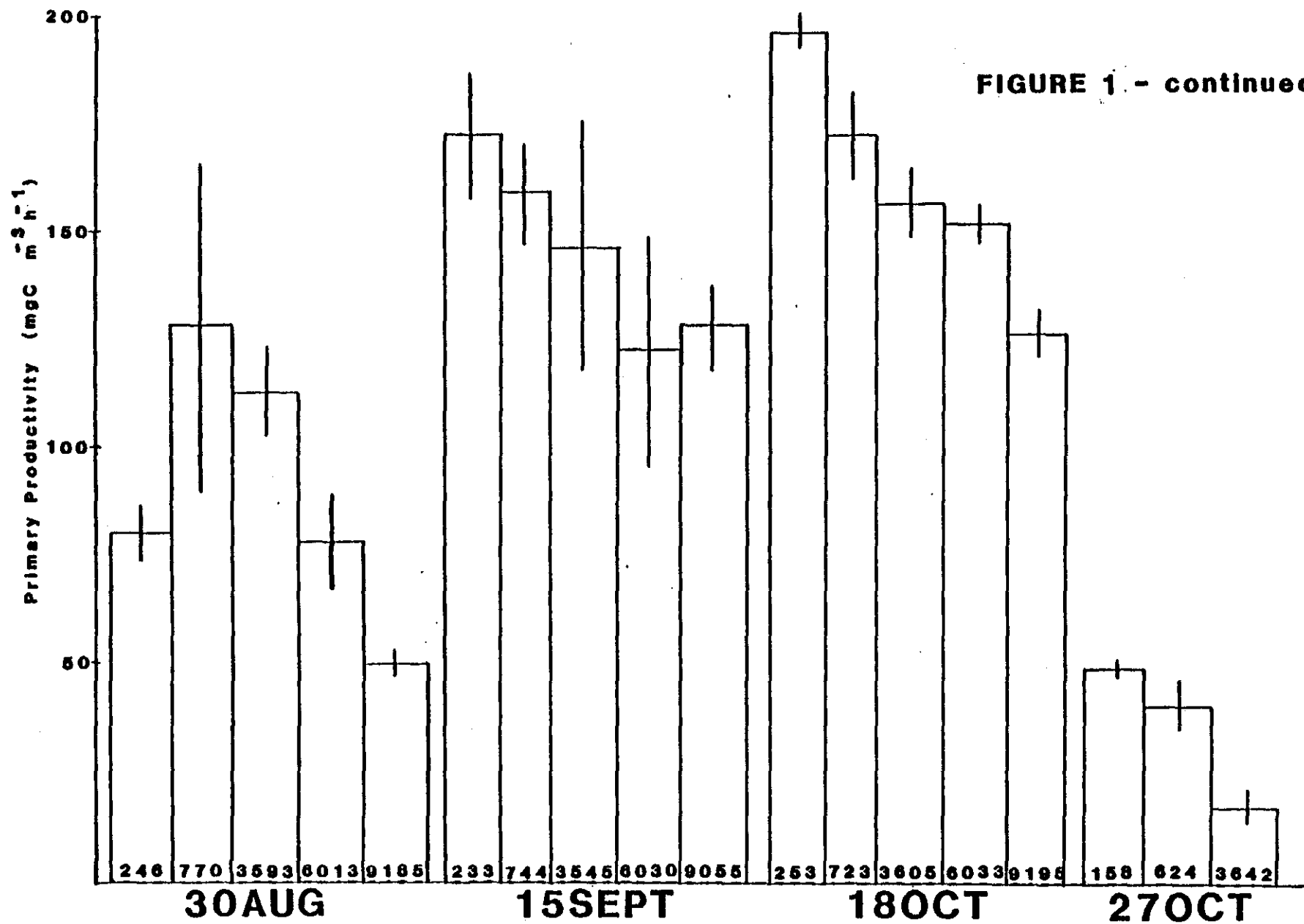Y-axis: Primary Productivity (mgC m^{-3} h^{-1})

100 — 75 — 50 — 25

Bar base values:

17 JAN: 302 | 581 | 1295

16 JUNE: 213 | 298 | 513 | 807

23 JUNE: 212 | 613 | 1052 | 1403

1 JULY: 206 | 2290 | 3433 | 5753

FIGURE 1 - continued

FIGURE 1 - continued

Figure 2. 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 $^{\circ}$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	CC	TC
158	163	432

GROUP A

SI	CC	TC
672	632	919

GROUP B

SI	CC	TC
3490	3450	3650

GROUP C
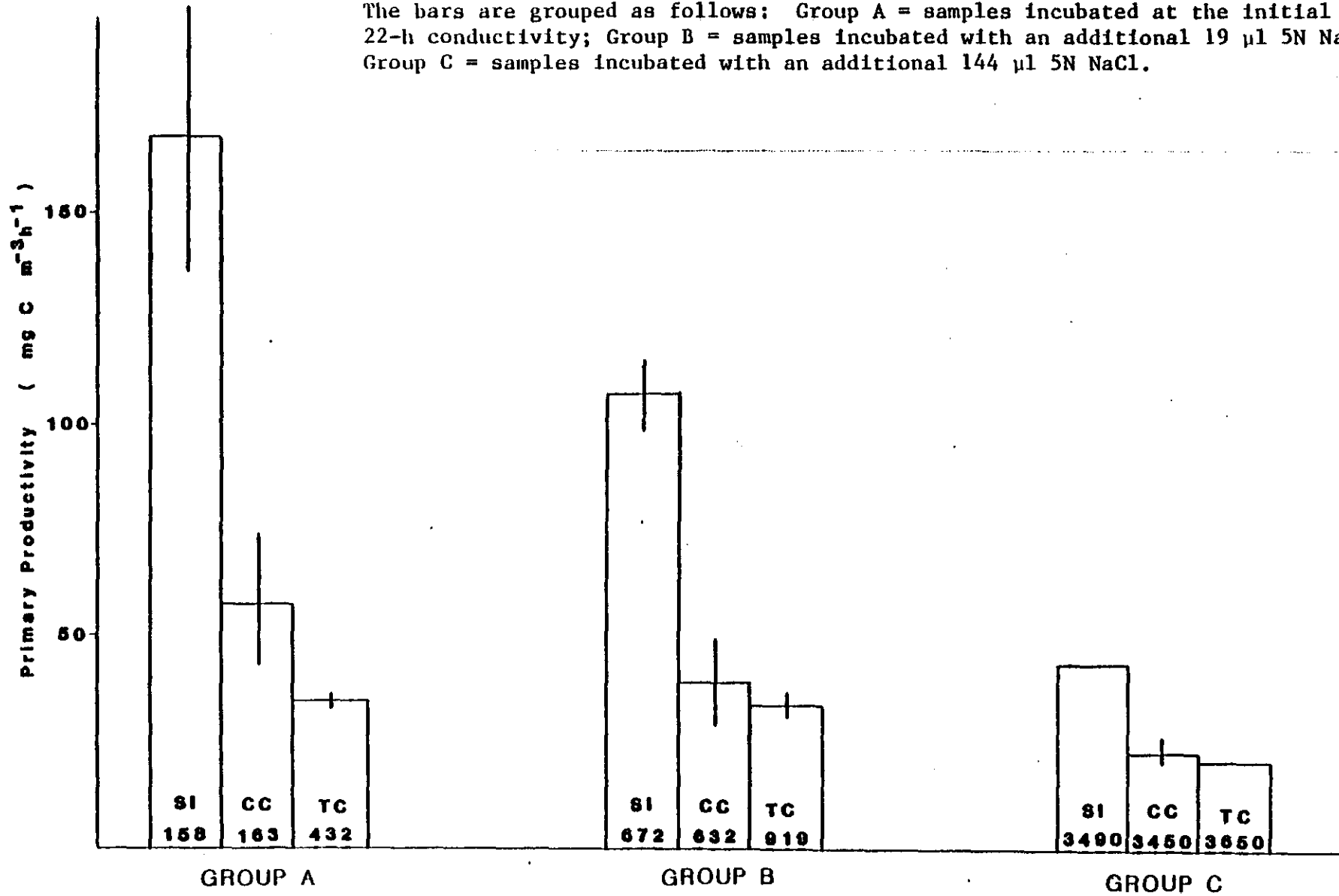
Figure 3. Final algal density and species distribution for a 22-h in situ bioassay. Open bars are percents of either total numeric or volumetric density. Horizontally-striped bars represent total density as cells/ml. Vertically-striped bars represent total algal cell volume.
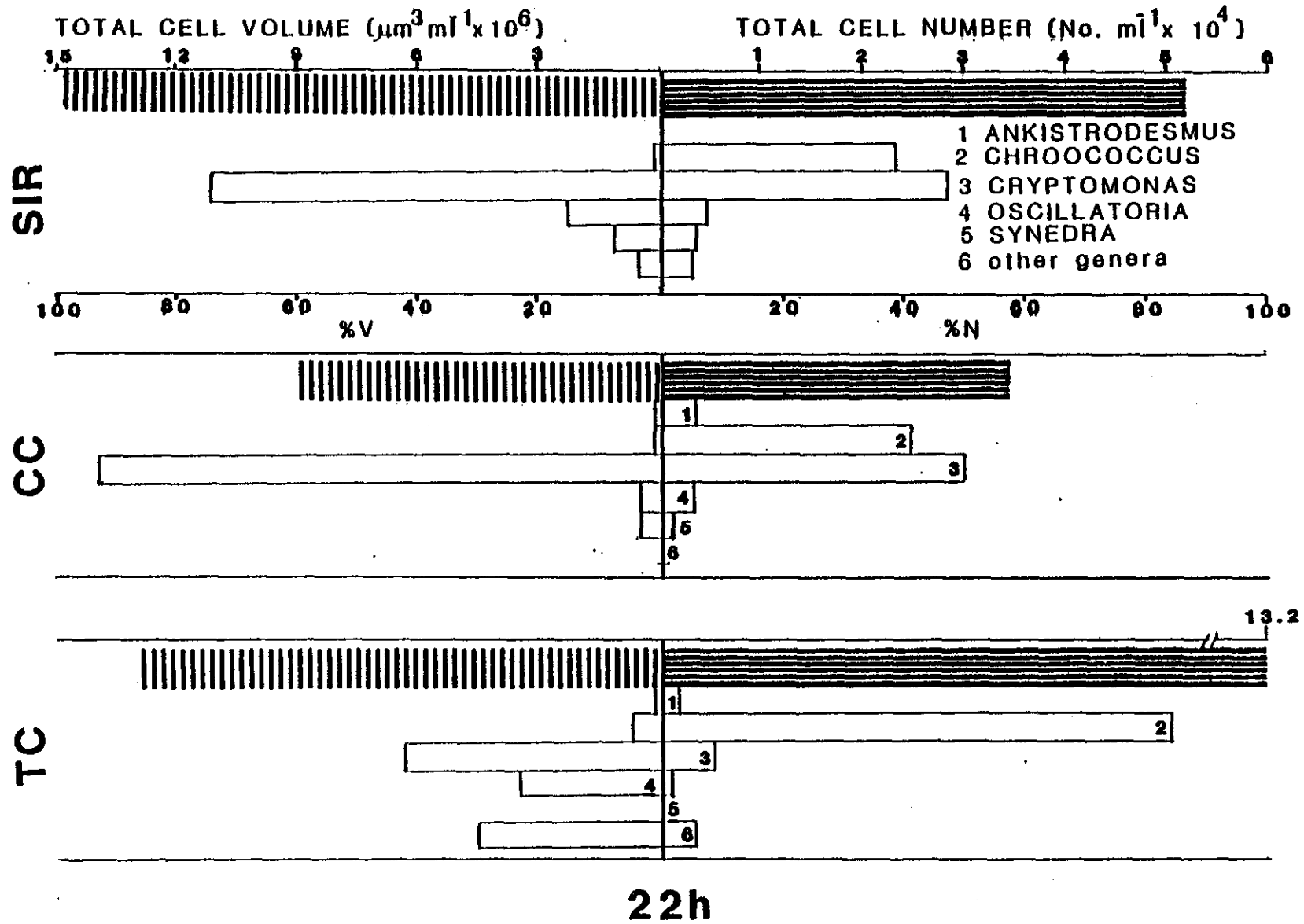

TOTAL CELL VOLUME ($\mu m^3 ml^{-1} x 10^6$)

TOTAL CELL NUMBER (No. $ml^{-1} x 10^4$)

1 ANKISTRODESMUS
2 CHROOCOCCUS
3 CRYPTOMONAS
4 OSCILLATORIA
5 SYNEDRA
6 other genera

SIR

CC

TC

%V   %N

13.2

22h

Figure 4. 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)
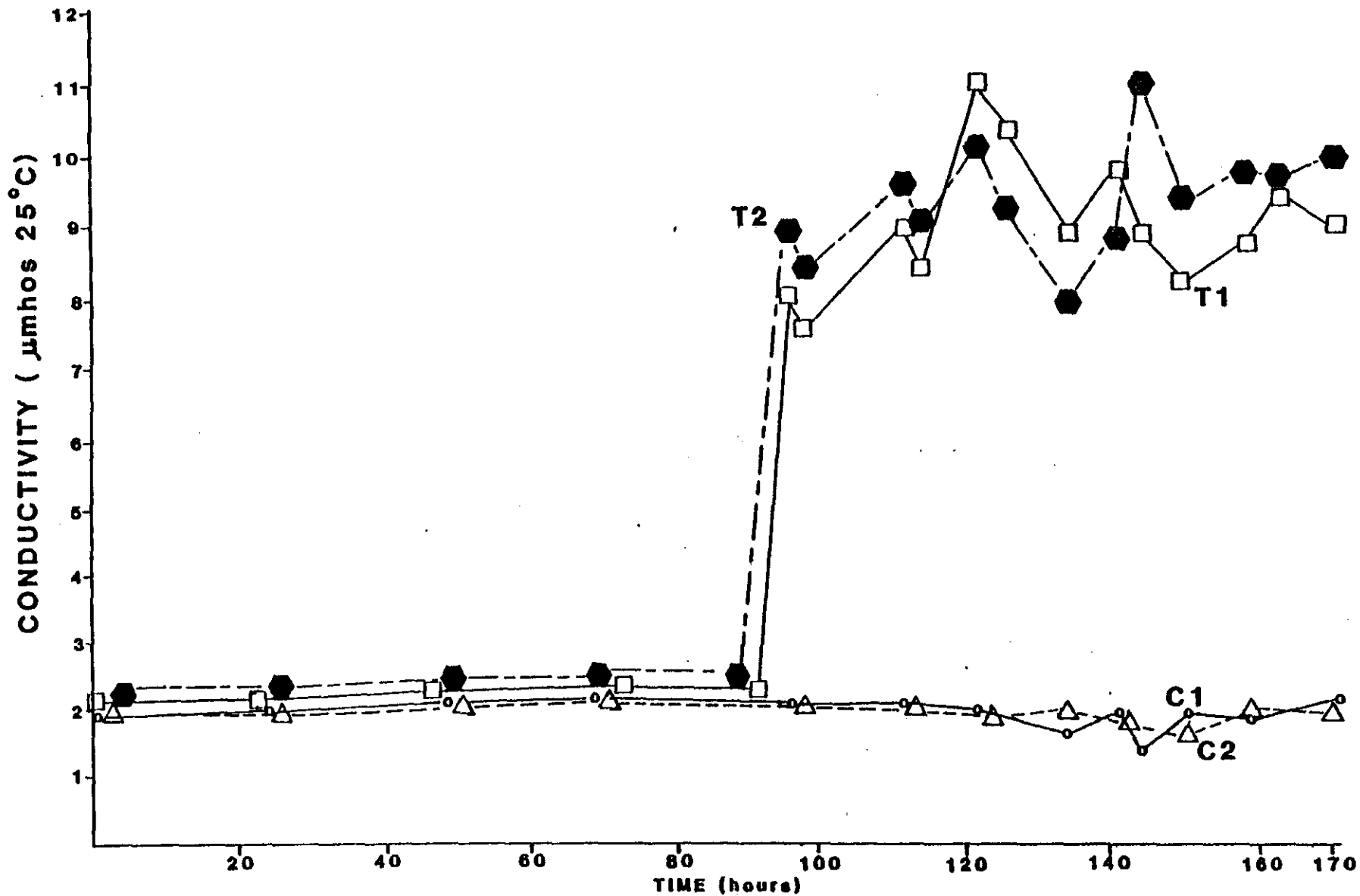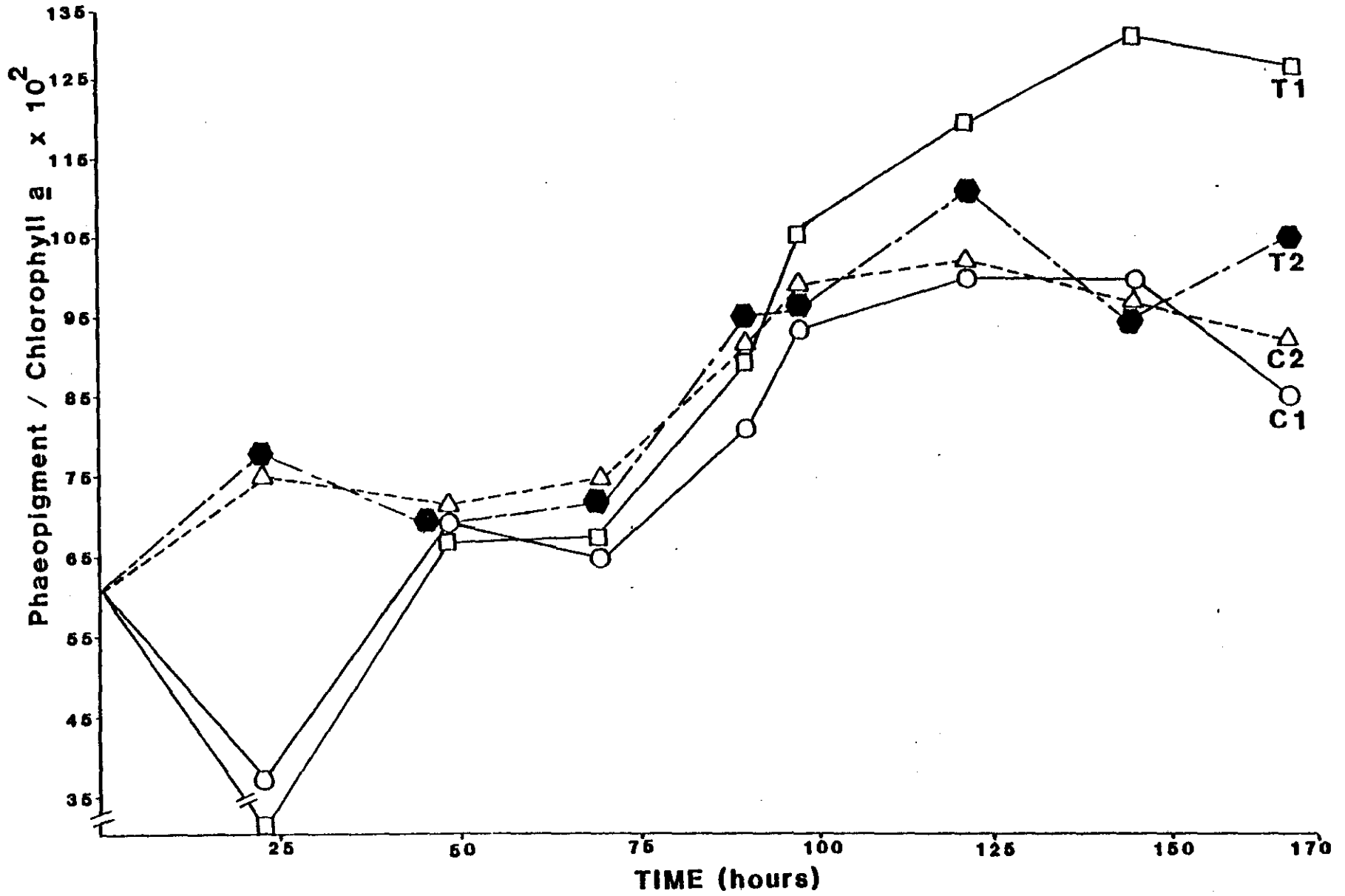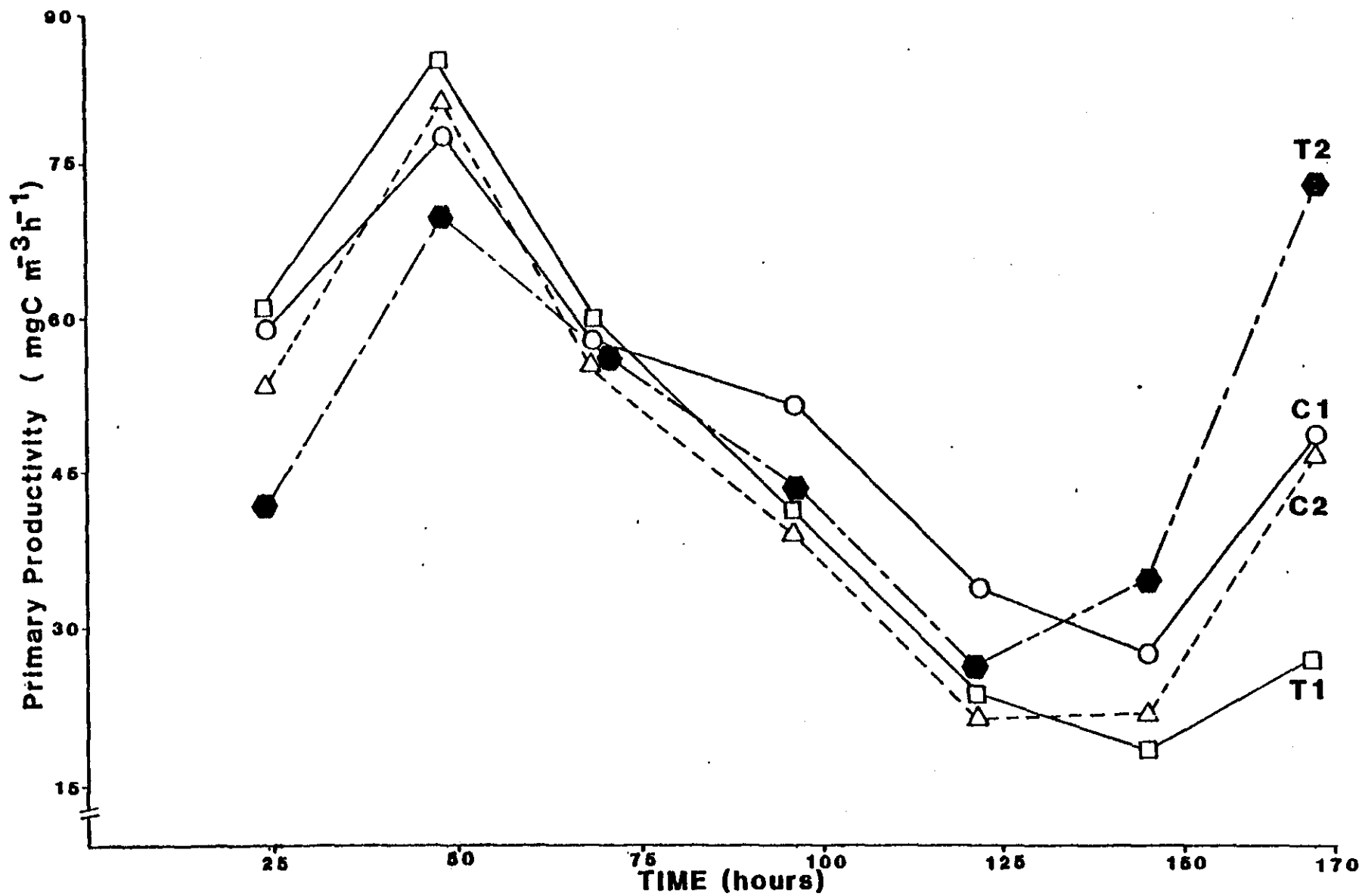
Figure 5. Phaeopigment:chlorophyll a ratio for treatment and control cultures during a 170-h laboratory chemostat bioassay. (C1 = control 1; C2 = control 2; T1 = treatment 1; T2 = treatment 2)

Figure 6. 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)

Figure 7. 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).
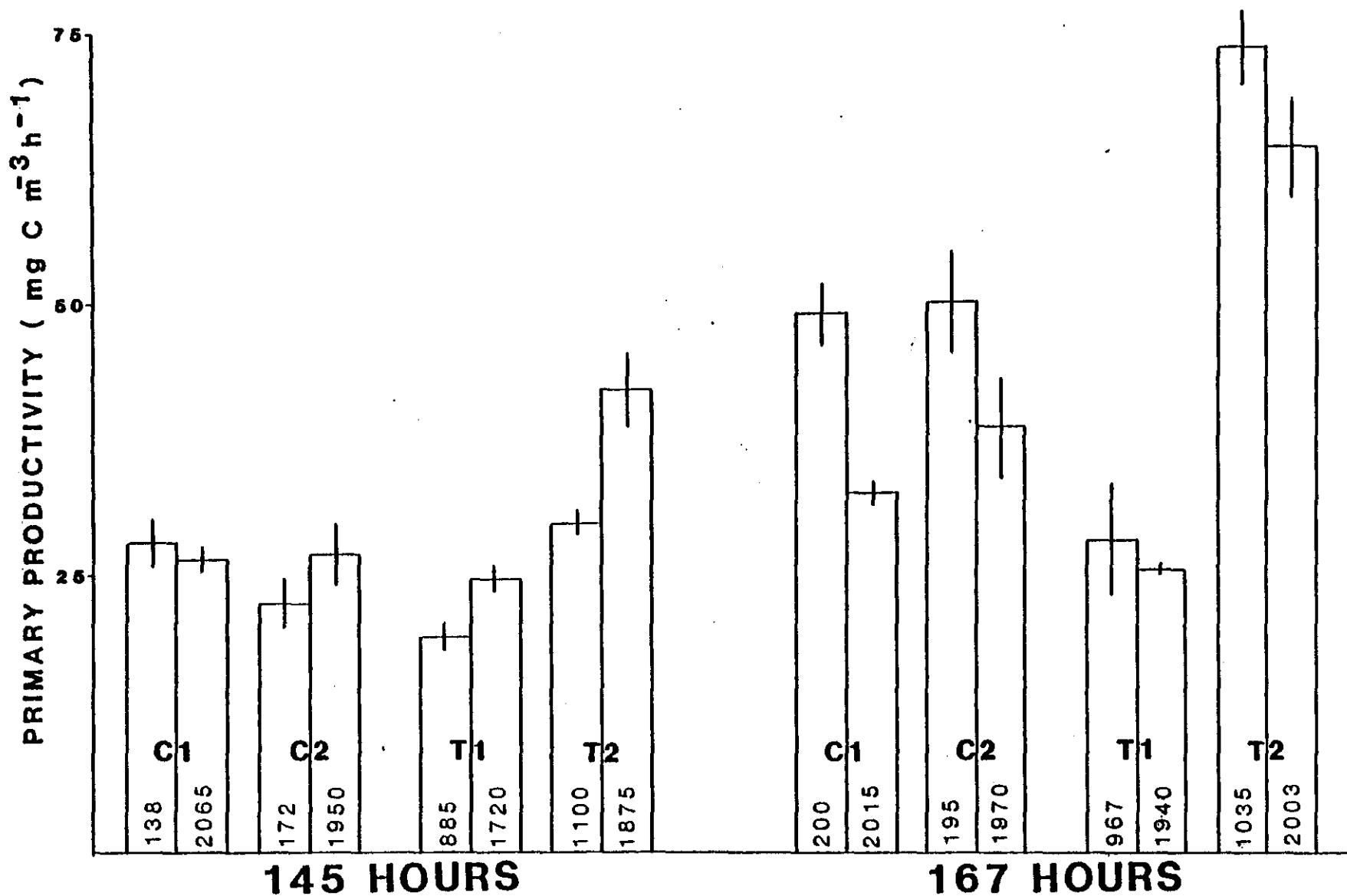
Figure 8. Algal density and species distribution for control and treatment cultures at 24, 70, and 166 h during a 170-h laboratory chemostat bioassay. Open bars are percents of either total numeric or volumetric density. Horizontally-striped bars represent total algal density as cells/ml. Vertically-striped bars represent total algal cell volume.
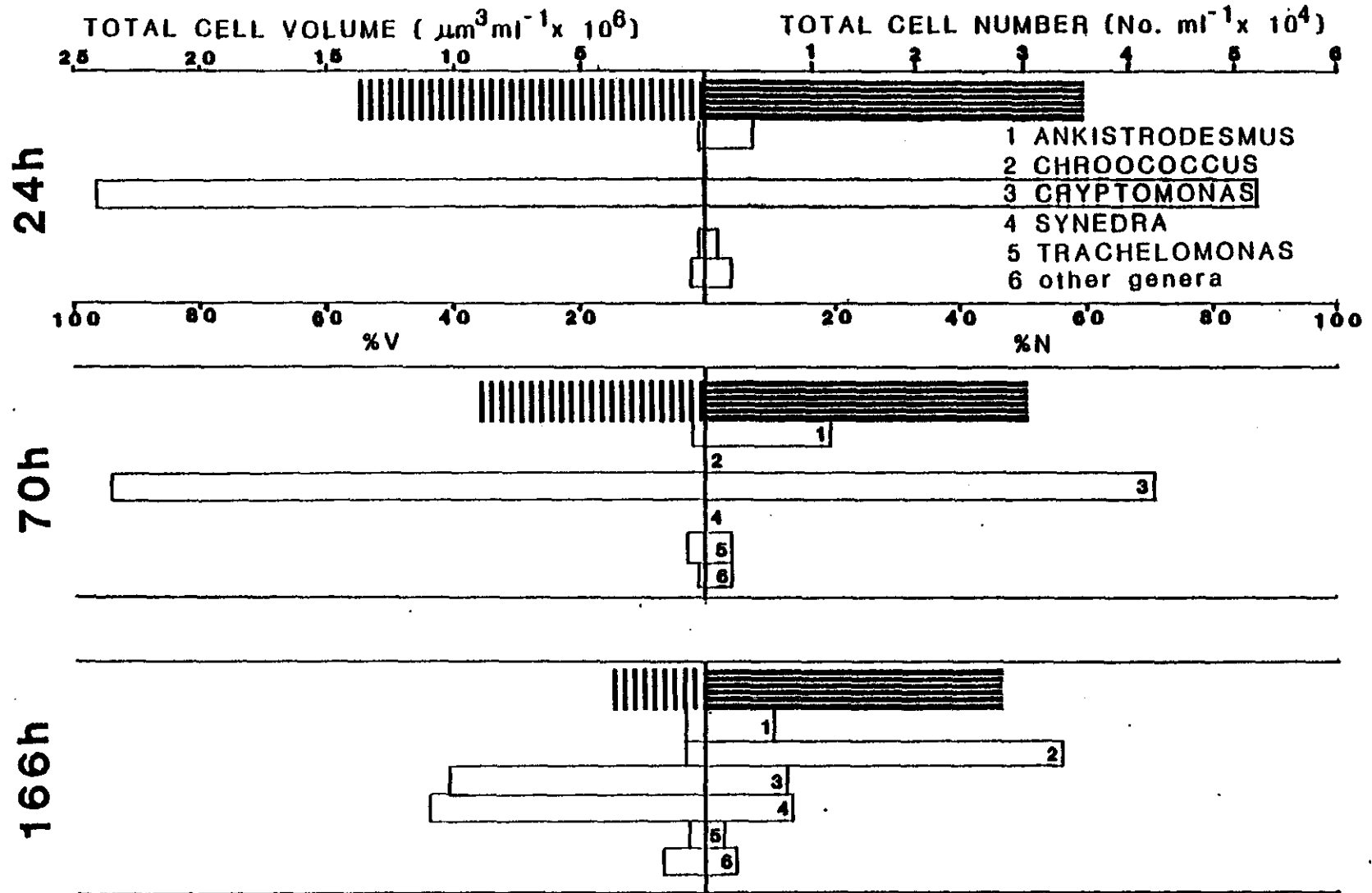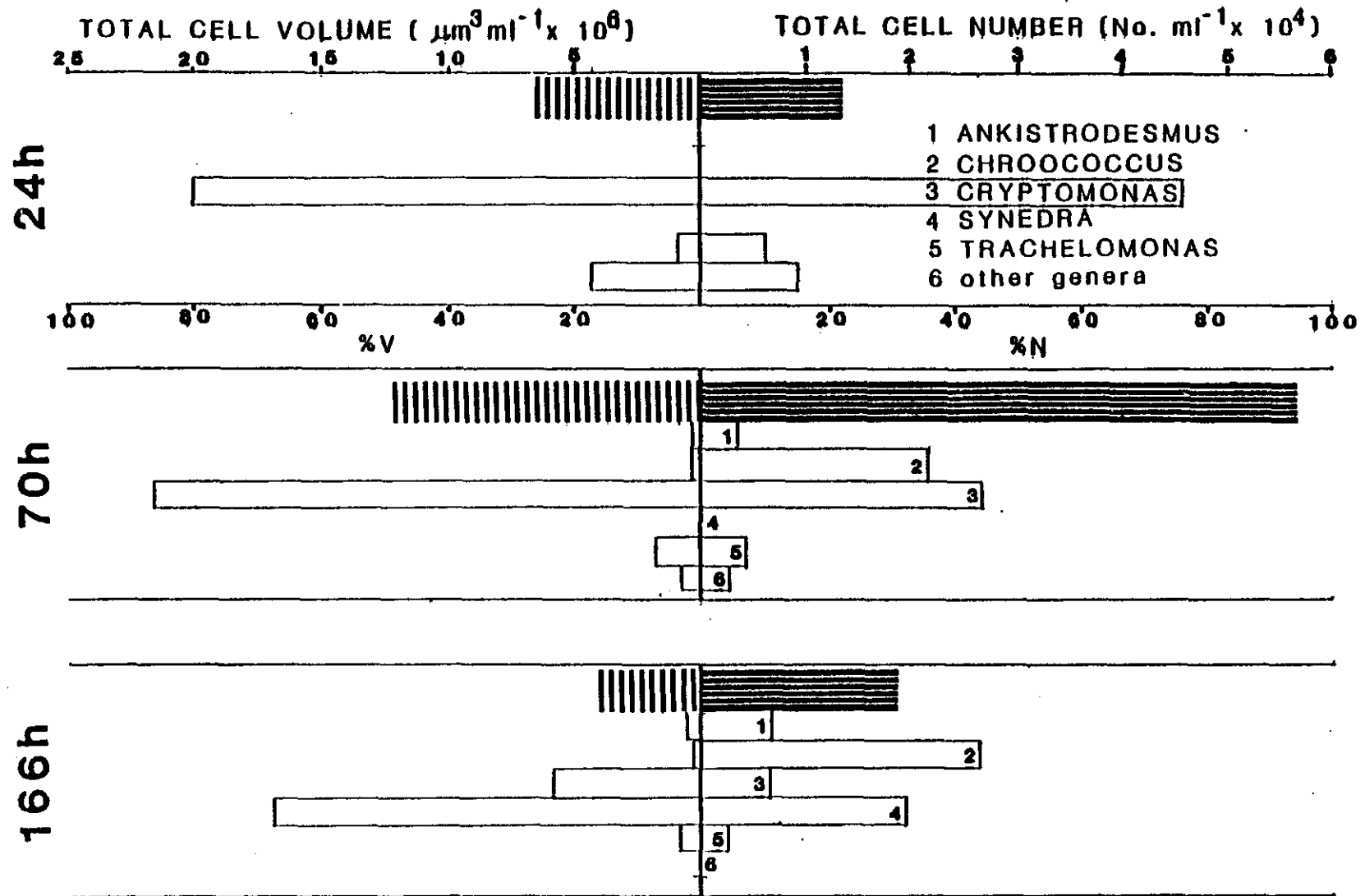

Figure 8 Control Culture 1

Figure 8 continued -Treatment Culture 1