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Authors

Resh, Vincent H
Lamberti, Gary A
McElravy, Eric P
et al.

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THE SEPARATION OF THERMAL AND CHEMICAL EFFECTS IN EVALUATING GEOTHERMAL INFLUENCES ON AQUATIC BIOTA

by

VINCENT H. RESH
Principal Investigator

and

GARY A. LAMBERTI • ERIC P. McELRAVY • JOHN R. WOOD

Division of Entomology and Parasitology
University of California
Berkeley, California 94720

Office of the Director
CALIFORNIA WATER RESOURCES CENTER
University of California Davis, California 95616

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ABSTRACT

In this study a field based, stream microcosm system to experimentally separate the effects of the thermal and chemical components of geothermal fluids on the benthic community was developed. Using this system the separate and combined influences of these components on the numerical abundance of bacteria, the standing crop and productivity of algae, and the density, standing stock, and community structure of macroinvertebrates were evaluated. This microcosm study was designed on the basis of the results from an in-stream study, which was a natural comparison of benthic communities in geothermally influenced and uninfluenced stream segments.

Additionally, a new type of field experiment to determine the upper lethal temperature thresholds for three insect species found in a geothermally influenced stream was developed. This heat shock experiment simulated acute thermal effects on organisms that drift into the lethal temperature zones that seasonally occur in geothermal streams. It also mimicked the effects of heated effluents on aquatic organisms that are drawn into the cooling water intake of a power plant, or entrained in the discharge plume.

Although the in-stream study demonstrated that a moderate addition of geothermal fluids into a non-geothermal stream could substantially alter the structure of the benthic community, the analysis did not establish the cause of the alteration. The stream microcosm study demonstrated that the thermal component of those geothermal fluids had greater influence than the chemical component in determining the structure of the benthic community.

INTRODUCTION

Geothermally influenced habitats occur worldwide (Waring 1965), but the development of geothermal resources for energy production is limited to relatively few areas (Rinehart 1980). In North America, most of the geothermal resources are found in the western regions (Tillman 1980). The world's largest geothermal energy development for electric power production is located at The Geysers in northern California, U.S.A., and provides about 5% (1000 Mw) of that state's electricity. However, concerns regarding possible deterioration in environmental quality have accompanied geothermal energy development at The Geysers and other areas (e.g. Suter 1978, Katz 1981), and particular interest has focused on the influence of such development on aquatic biota (Resh et al. 1981). Aquatic habitats may be severely affected by effluents from geothermal operations, in particular the uncontrolled releases of geothermal fluids due to well blowouts, pipeline ruptures, and sump overflows.

The effects of geothermal development-related disruptions on aquatic habitats can be predicted by using natural hot springs as model ecosystems (Brock 1970), since these springs frequently add substantial amounts of heat energy, chemical substances, and water to non-geothermal aquatic habitats (Rinehart 1980). Consequently, such habitats simulate the important features of anthropogenic releases of geothermal effluents (Jones 1966). In North America, the ecology of natural geothermal habitats has been most intensively studied in Yellowstone National Park, U.S.A. (see Brock 1975, 1978 and many references therein). These

studies, along with those conducted in other areas (see review by Brock 1970), have shown that the water of geothermal habitats has two important components, high temperature and unique geochemistry, which limit the variety and types of organisms that can live in those habitats.

Unfortunately, nearly all studies of the influences of geothermal fluids on aquatic biota have failed to distinguish between the individual effects of the thermal and the chemical components. For example, Armitage (1958) reported that the biomass of aquatic insects greatly increased in the Firehole River (Yellowstone Park) below geothermal effluents. Although he attributed this increase to chemical enrichment (i.e. higher alkalinity due to bicarbonate input), which stimulated the growth of the insects' food (algae and other microorganisms), he *a priori* dismissed the increase in temperature as being of only secondary importance. In the nearby Gibbon River, Vincent (1967) also implied that chemical inputs were responsible for slightly higher standing crops and densities of insects downstream of geothermal additions, even though water temperature concurrently increased.

Boylen and Brock (1973) reported that thermal enrichment was primarily responsible for an increase in algal standing crop below geothermal additions to the Firehole River, although higher concentrations of algal nutrients were also found downstream of such inputs. In contrast, Castenholz (1976), working in geothermal habitats of New Zealand and Iceland, reported that sulfide concentration determined the species composition of

thermophilic algae. The small difference in the growth rates of bacteria in geothermal and non-geothermal segments of the Firehole River led Zeikus and Brock (1972) to conclude that bacterial species were optimally adapted to the temperature regimes in which they lived, and that geothermal influences were of minor importance. Based on this conflicting information concerning both macroinvertebrates and microorganisms, Brock (1978) emphasized that a major research goal for future biological studies of geothermal habitats should be to distinguish between chemical and thermal influences on aquatic biota.

Experimental analysis of the effects of thermal and chemical components in geothermally influenced habitats is needed to explain patterns of distribution, abundance, and diversity of biota in such systems, and to establish tolerance levels of specific taxa to geothermally generated heat and chemicals. In addition, the ecologically sound development of geothermal resources should be based on using that information to predict the effects of effluent components on aquatic biota so that mitigation efforts can be concentrated on the component that has the most pronounced impacts.

For these reasons, we developed a field-based, stream microcosm system to experimentally separate the effects of the thermal and chemical components of geothermal fluids on the benthic community. Using this system, we evaluated the separate and combined influences of these components on the numerical abundance of bacteria, the standing crop and productivity of

algae, and the density, standing stock, and community structure of macroinvertebrates. This microcosm study was designed on the basis of the results from an in-stream study, which was a natural comparison of benthic communities in geothermally influenced and uninfluenced stream segments.

Lethal thermal thresholds for aquatic organisms can be defined in two categories: thermal tolerance limits (chronic exposure) and thermal shock limits (acute exposure). These thermal limits are not necessarily the same for a given organism, and both may vary seasonally with acclimation temperature and life stage (Schubel et al. 1978). The effect of chronic exposure to heated discharges is usually determined by the standard bioassay of 24-, 48-, and 96-h exposure times. Although the effect of acute exposure to instantaneous thermal shocks has recieved much less study than chronic exposure (Sherberger et al. 1977), it is the most appropriate for determining the impact of entrainment or exposure to geothermal fluids.

We designed a new type of field experiment to determine the upper lethal temperature thresholds for three insect species found in a geothermally influenced stream. This heat shock experiment simulated acute thermal effects on organisms that drift into the lethal temperature zones that seasonally occur in geothermal streams. It also mimiced the effects of heated effluents on aquatic oganisms that are drawn into the cooling water intake of a power plant, or entrained in the discharge plume (Schubel et al. 1978).

STUDY AREA

This research was conducted in Big Sulphur Creek, Sonoma Co., California, U.S.A. (38° 47' N, 122° 47' W, elev. 670 m), a third-order stream that flows northwesterly through the The Geysers Known Geothermal Resources Area (Figure 1A,B). The drainage basin of Big Sulphur Creek is formed by a steeply sided valley in the Mayacmas mountain range of northern California. Vegetation is that of a mixed-species evergreen forest, dominated by Douglas fir (Pseudotsuga menziesii (Mirb.) Franco) and oak (Quercus spp.). Precipitation occurs primarily as rain ($\bar{x}$ = 137 cm/yr), 95% of which falls between November and April.

Our principle study site was at the confluence of Big Sulphur Creek and its tributary, Little Geysers Creek (Figure 1C). Big Sulphur Creek is a cold (i.e. non-geothermal) stream above that point; Little Geysers Creek is a heated (i.e. geothermal) stream formed by a number of small hot springs. Although the discharge volume of Little Geysers Creek is only about one-third that of Big Sulphur Creek (visual estimate), the thermal input of Little Geysers Creek raises water temperature in Big Sulphur Creek $\bar{x}$ = 7.5°C at 50 m downstream of the confluence dring spring and summer (e.g. from 19 - 26.5°C during 1 June - 18 July 1979). Likewise, the chemical input from Little Geysers Creek raises the concentrations of several substances in Big Sulphur Creek, most notably B, NO_3-N, and PO_4-P (Figure 2). The influence of these specific inputs is apparent for 250 m below the confluence. Smaller hot springs empty into Big Sulphur Creek below this point, which result in additional, but more localized, geothermal

influences throughout the stream's length.

MATERIALS AND METHODS

Experimental Design

An in-stream study was conducted to compare benthic communities in Big Sulphur Creek (BSC) at 100 m upstream and 50 m downstream of the geothermal input from Little Geysers Creek (LGC). These two sites were selected because they were the closest areas to the confluence that had similar physical characteristics, such as in water depth, current velocity, substrate composition, and illumination. Benthic macroinvertebrates and microorganisms were sampled using unglazed, red clay tiles (15.2 X 7.6 cm) that were placed directly on the substrate. At each site, 36 tiles were arranged in a 6 X 6 grid on the stream bottom in a shallow riffle that was moderately shaded by streamside vegetation. Five tiles were collected weekly at random from each site for seven consecutive weeks (1 June - 18 July 1979); the abundances of benthic bacteria, algae, macroinvertebrates, and total organic matter were determined on each tile.

An experimental study using stream microcosms was conducted near the confluence of BSC and LGC (Figure 3A) to assess the independent effects of the thermal and chemical components of those geothermal fluids from LGC on benthic biota. In this system, four microcosms were used (Figure 3B): 1) control: no thermal or chemical influence; source of water was BSC upstream of LGC; 2) thermal plus chemical (T+C): both thermal and

chemical influences; source of water was LGC; 3) thermal: thermal influence only; water from BSC upstream of LGC was heated using hot water from LGC in a heat-exchange system; 4) chemical: chemical influence only; water from LGC was cooled using cold water from BSC in a cold-water bath.

Water was transported from the two sources, BSC and LSC, to the microcosms using a gravity-feed design (Figure 3B). Over 300 m of 7.6 cm-diameter flexible, styrene pipe were necessary to generate sufficient pressure to maintain adequate flow rates to the microcosms. Each microcosm was a plexiglass trough (0.9 X 0.3 X 0.13 m deep) with an open top (Figure 3A); all fittings between the pipes and the microcosms were made of PVC. Water flowed through the microcosm only once and was then released into BSC.

All four microcosms were identical, except that the thermal microcosm was fitted with a specialized compartment at the input end, which contained the heat-exchange system that produced the thermal treatment (Figure 3). This closed compartment contained 100 thin-walled aluminum tubes (30 cm long; 0.6 cm diameter) through which hot water from LGC continually flowed. Cold water from BSC passed over the tubes and was thereby warmed via heat exchange, but without chemical addition, before it entered the thermal microcosm.

To produce the chemical treatment, six aluminum coils, each consisting of 18 tubes (60 cm long, 1.0 cm diameter) connected by vinyl tubing, were submerged in the cold section of BSC. Hot water from LGC circulated through the coils and was

thereby cooled while still retaining its chemical constituents, before being delivered to the chemical microcosm. No chemical precipitates were ever observed in the water of the chemical microcosm. Therefore, we infer that cooling did not reduce chemical concentrations.

Most of the material used to construct the microcosms (e.g. styrene, PVC, plexiglass) were relatively inert with regard to possible chemical contamination of the treatment water. However, we were concerned about potential contamination from the aluminum tubes used the heat-exchage units of the thermal and chemical microcosms. Aluminum concentration was measured in each of the four microcosms at the end of the 34-d study period and was found to be undetectable (i.e. <0.1 mg/l) in each case.

Water flow was maintained at the same constant levels in each of the microcosms (discharge = 0.1 l/sec; surface current velocity = 4 cm/sec; depth = 6 cm). Natural substrate was provided to each of the microcosms in a sterilized mixture of BSC stream gravel consisting of 1 l (volumetric displacement) each of three substrate size categories: 2-4 mm, 4-8 mm, and 8-16 mm (depth of substrate = 3 cm). Fourteen tiles (7.6 X 7.6 cm) were placed on top of the substrate in each microcosm; three tiles were randomly collected (and replaced by new tiles) from each microcosm after exposures of 3, 6, 11, 21, and 34 d (9 June - 13 July 1981) and sampled for benthic bacteria, algae, and total organic matter. The entire substrate from each microcosm (0.22 m^2) was collected and preserved after 34 d to inventory the benthic macroinvertebrate community.

Sample Collection and Measurement

Tiles were leached in stream-collected water for 14 d prior to use, and then scrubbed and autoclaved. After the appropriate exposure period, the tile was lifted off the substrate into a 125 um mesh bag to collect fleeing macroinvertebrates. Three separate 4-cm^2 scrapes of periphyton were taken from the upper surface of each tile using either a sterile scalpel and template for closely adherent algae, or a corkborer for more loosely attached algae. One scrape was appropriately preserved for each of three microbial analyses: number of bacteria (bacterial biomass), chlorophyll *a* and phaeophytin (algal standing crop), and ash-free dry weight (total organic content). The tile and net contents were then placed in 10% formalin for sorting, identification, and enumeration of macroinvertebrates in the laboratory. In BSC, biota collected from these tiles closely represented the zoo- and phyto-benthic communities occurring on natural stream rocks (Lamberti, unpub. data).

Samples for determination of bacterial biomass were preserved in a 0.5% glutaraldehyde solution that was buffered with a 0.067 M cacodylate solution. In the laboratory, bacteria were dispersed by homogenization, filtered through 0.2 μm Nucleopore membranes, stained with acridine orange, and viewed at 1500X using epifuorescence microscopy. Bacteria that fluoresced orange or green were directly counted (Barton and Lock 1979); ten fields per membrane were ennumerated and $\bar{x}$ counts were converted into density estimates.

Samples for chlorophyll *a* and phaeophytin analyses were filtered through glass fiber filters (Whatman GF/C), fixed with $MgCO_3$, and frozen in the field. In the laboratory, the filters were homogenized and photosynthetic pigments were extracted in 90% acetone for 24 h at 0°C. Spectrophotometric absorbances of the extracts were measured and pigment concentrations were calculated for both chlorophyll *a* and its degradation product, phaeophytin (Moss 1967a,b).

Samples for measurement of the total organic content of attached material were also filtered through glass fiber filters and frozen in the field. In the laboratory, the filters were dried (105°C) for 24 h in a vacuum oven, and then ashed (500°C) for 1 h in a muffle furnace. The difference between the ash and dry weights represents the organic content (i.e. the ash-free dry weight).

All macroinvertebrates (>125 μm in size) from each tile or natural substrate sample were identified and enumerated. Dry weight and ash-free dry weight of formalin-preserved macroinvertebrates were obtained using the procedure outlined above for total organic content.

Design of Heat Shock Experiment

Field experiments were conducted on 17 July 1982 (warm-acclimation test) and 14 January 1983 (cold-acclimation test) in BSC to determine acute thermal effects on three species of aquatic insects, the mayfly *Centroptilum* (probably *convexum* Ide) and the caddisflies *Helicopsyche borealis* (Hagen) and *Gumaga*

nigricula (McLachlan), which are common at The Geysers and/or have been important in previous studies (e.g. Resh et al. 1981). The experimental protocol was the same for all three species; the only exception was Centroptilum, which was not available for the cold-acclimation test in January. For each species, specimens were collected near the confluence of BSC and LGC (Fig. 1C) and ten animals were placed into each of 126 5-cm-diameter PVC cells, which were held in a floating rack (Fig. 9) anchored in BSC. Animals were held <24 h prior to testing. Forty-two cells were used in each of three replicates of a 6 X 7 time-temperature matrix.

Apparatus for the heat shock experiment consisted of seven ice chests (20 X 50 X 20 cm deep) used as static water baths. Temperature (+/- 0.5°C), dissolved oxygen, and water movement were maintained in the ice chests with a combination of aquarium heaters, air stones, and hand additions of warm water. The seven temperature baths ranged from 33 to 45°C, in 2-3°C increments. The control bath was maintained at ambient stream temperature for the test date (i.e. ≤30.5°C on 17 July 1982 and ≤9°C on 14 January 1983). The six exposure times ranged from 5 to 60 minutes for each temperature. To administer the heat shock, the cells were quickly transferred from the holding raft to one of the temperature baths on shore. After the appropriate time interval, cells were returned to the holding raft in the stream and the percent mortality was determined after 24 and 96 h. Mortality was indicated when the insect failed to respond to repeated probing.

RESULTS

In-Stream Study

This initial, descriptive study documented the development of the microbial and macroinvertebrate communities on tiles placed in Big Sulphur Creek (BSC) 100 m upstream and 50 m downstream of its geothermal tributary, Little Geysers Creek (LGC) (Figure 1C), during the period 1 June - 18 July 1979. Mean midday water temperatures during the study period were 36.5°C in LGC, 19°C in BSC 100 m upstream of LGC, and 26.5°C in BSC 50 m downstream of LGC. Thus, the 7.5°C difference between BSC-upstream and BSC-downstream is the amount that the geothermal LGC water heated BSC water. In contrast, the 10°C difference between LGC and BSC-downstream can be considered to be the amount that LGC water was cooled by mixing with BSC water.

Abundances of Microorganisms. The density of benthic bacteria in BSC increased after one week's growth to about 10^6 cells/cm^2 downstream of LGC as compared with 10^5 cells/cm^2 upstream (Figure 4A); densities then converged and remained similar at the two sites throughout the middle of the study period (i.e. weeks 2-5). However, after six weeks densities diverged again, and after seven weeks, there were significantly higher numbers of bacteria at the geothermally influenced downstream site (about 10^7 cells/cm^2) than at the uninfluenced upstream site (about 10^6 cells/cm^2) ($p<0.01$).

Chlorophyll *a* remained low at the upstream site (<0.2 μg/cm^2) throughout the study period (Figure 4B). At the

downstream site, chlorophyll *a* increased rapidly and peaked at 0.8 $\mu g/cm^2$ after two weeks, but then declined to <0.2 $\mu g/cm^2$ after five weeks. Similar to the bacterial pattern, chlorophyll *a* increased again, and after six weeks, resumed its previous high level. This fluctuation in downstream abundance was probably due to grazing by insect herbivores (see below). After seven weeks, chlorophyll *a* was significantly higher downstream of the geothermal input ($p<0.01$).

Phaeophytin, the chlorophyll *a* degradation product, ranged from 20-43% of the total pigment at the upstream site and 15-33% of the pigment at the downstream site, and exhibited dynamics that were similar to those of chlorophyll *a* (Figure 4C cf. 4B). Generally, higher amounts of phaeophytin were recorded at the downstream site and, after seven weeks' growth, phaeophytin was more abundant at the downstream site than at the upstream site ($p=0.06$).

The total organic content is a measure of microbial biomass plus associated non-living organic material, such as fine detritus that is trapped in the microbial matrix. The total amount of benthic organic matter (i.e. ash-free dry weight) was higher at the downstream site throughout the study period (Figure 4D), although the relative difference was smaller than with either chlorophyll *a* or phaeophytin. After seven weeks, the $\bar{x}$ organic content of the benthic material was 0.76 mg/cm^2 upstream compared with 1.26 mg/cm^2 downstream of the geothermal input ($p=0.07$). Similar to the chlorophyll *a* and phaeophytin patterns, the total downstream organic matter displayed an

initial rapid increase, which was followed by a decline, and then a second increase.

Abundances of Macroinvertebrates. The density of macroinvertebrates (Figure 5A) increased rapidly at the downstream site, peaked after three weeks at 325 individuals/0.012 m^2 tile (27,083/m^2), and then declined and stabilized at around 200 individuals/0.012 m^2 tile (16,667/m^2). At the upstream site, macroinvertebrate density both peaked and stabilized after five weeks at about 150 individuals/0.012 m^2 (12,500/m^2). After seven weeks, the density was significantly greater at the downstream site ($p<0.01$). At both sites, equilibrium densities were reached by five weeks, a period of time consistent with the results of many other artificial substrate colonization studies (see review by Rosenberg and Resh 1982).

The standing stock and species richness of macroinvertebrates were determined by combining the organisms collected from all five tiles at each sampling date into a single measurement. The standing stock of macroinvertebrates (Figure 5B) increased at the downstream site, peaking at 85 mg/0.06 m^2 (1.42 g/m^2) after six weeks, but remained low at the upstream site throughout the study period (<11 mg/0.06 m^2; 0.18 g/m^2). The inter-site difference in standing stock (Figure 5B) was much more striking than the difference in the density of macroinvertebrates (Figure 5A), and may have occurred for one or more reasons: 1) individuals grew at a faster rate at the downstream site; 2) downstream substrates were colonized by

progressively larger individuals over time; or 3) there were significant differences in macroinvertebrate community structure at the two sites.

The total number of macroinvertebrate species at each date was always higher at the downstream site (Figure 5C), although species richness converged at the two sites near the end of the study period. After seven weeks, 31 species were collected at the upstream site and 34 species were found downstream. A combined total of 53 species were found at the two sites during the seven-week study; of these, 33 species (62%) occurred at both sites. Thus, although most species were found at both sites, there were some differences in the macroinvertebrate communities at the two sites. This was especially true for the insect component of the macroinvertebrate fauna. For example, six species of Odonata were found at the downstream site, but only two species occurred upstream. No stoneflies (Plecoptera) were collected upstream, but three species occurred downstream. Although other aquatic insect orders showed similar species richnesses at the two sites, they differed in population densities. For example, we collected nine species of Trichoptera at the upstream site and ten species downstream; one caddisfly, Helicopsyche borealis (Hagen), which is a highly thermo-tolerant species frequently found in thermally enriched habitats (Wiggins 1977, Mackay and Wiggins 1979), occurred in much higher densities at the geothermally influenced downstream site (Figure 5D). H. borealis larvae graze algae in BSC and probably accounted for the oscillations in chlorophyll a

observed at the downstream site (Lamberti and Resh 1983). Brock (1967) suggested that other grazers may play a similar role in thermal streams of Yellowstone Park at temperatures <50°C.

Stream Microcosm Study

The thermal and chemical components of the geothermal fluids were analyzed both separately and together using the stream microcosm system described above. The duration of the experiment, about five weeks, was empircally determined from the results of the in-stream study, in which the density of macroinvertebrates stabilized after four to five weeks (Figure 5A).

Maroinvertebrate and microbial colonization of the four microcosms occurred primarily by the drift of organisms in water carried through the system's pipes. Although the tops of the microcosms were left open (to ensure normal light regimes), since only one egg mass (Trichoptera) was observed in the microcosms, oviposition was apparently a minor component of the colonization process when compared to drift.

Because of the different sources of colonists, the valid comparisons in this experiment were: 1) between the control and the thermal microcosms, whose common source of colonists was drift from the non-geothermal stream, BSC (Figure 3b); and 2) between the T+C and chemical microcosms, whose common source of colonists was drift from the geothermal stream, LGC.

The design of the heat-exchange elements of the microcosms resulted in effective heating of non-geothermal water (Figure 6A), and effective cooling of geothermal water (Figure 6B). To

determine the $\bar{x}$ temperature difference between the components of each experimental pair, the midpoint of each daily maximum-minimum reading was calculated, and the mean difference between midpoints was determined for the entire 34-d experimental period. Thus, cool BSC (i.e. control) water was heated $\bar{x}$ = 8.5°C in the thermal microcosm, an increase very close to the natural heating (7.5°C) of BSC by the inflow of LGC that was observed in the in-stream study. Hot LGC (i.e. T+C) water was cooled $\bar{x}$ = 14.5°C before it entered the chemical microcosm, a reduction somewhat larger than the natural cooling (10°C) of LGC by mixing with BSC. However, this further cooling of LGC water was advantageous because it allowed greater isolation of chemical from temperature effects. All four microcosms displayed a warming trend during days 10-15 (Figure 6), corresponding to a steady increase in air temperature during that period.

The two microcosms that had a chemical component (i.e. the T+C and chemical) contained concentrations of several substances that were considerably higher than those in BSC water (Figure 2). For example, B increased by >10X in BSC downstream of the natural geothermal input of LGC (from <0.1 to 1.2 mg/l). Likewise, NO_3-N increased almost 4X (from 0.03 to 0.11 mg/l), and PO_4-P increased 2X (from 0.01 to 0.02 mg/l). It is also important to note that since LGC water was diluted by BSC water about 3:1 before these measurements were taken, these increases in BSC indicate that those chemicals were carried in even higher concentrations in LGC, which was the source of water for the T+C and chemical microcosms.

The dissolved oxygen concentration, pH, and conductivity were measured in each microcosm at 3-4 day intervals (Table 1). The dissolved oxygen concentration was similar in both microcosms of each experimental pair, ranging from 81-84% saturation in the control and thermal microcosms, and from 54-64% in the chemical and T+C microcosms. Saturation levels were lowest in the cooled water of the chemical microcosm (54%) probably because reoxygenation of the cooled water did not occur before it flowed into the microcosm. Conductivity is positively influenced by temperature and thus was slightly higher in the warmer microcosm of each experimental pair. The pH was always near neutral in all four microcosms.

Responses of Microorganisms. Bacteria grew rapidly in all four microcosms during the period 0-20 d (Figure 7A,B); bacterial density stabilized in the control microcosm after 20 d, but continued to increase in the other microcosms. After 34 d, the density of benthic bacteria was significantly higher in the thermal microcosm than in the control (Figure 7A; $p<0.01$). However, there was no significant difference in bacterial density in the chemical and T+C microcosms (Figure 7B; $p=0.45$). The elevated temperature in the thermal microcosm apparently encouraged bacterial growth, but removal of the thermal component from the T+C water (i.e. chemical microcosm) had little effect on density.

The species composition and standing crop of algae both showed striking differeces among the microcosms. Algae in the control microcosm consisted principally of a thin layer of

diatoms (Chrysophyta: Bacillariophyceae), but also included small amounts of the the filamentous green alga Spirogyra (Chlorophyta: Zygnemataceae); in the thermal microcosm, Spirogyra predominated and grew into a thick mat that covered the substrate. Under the extreme temperature and chemical conditions in the T+C microcosm, thermophilic blue-green algae (Cyanophyta) predominated, with Oscillatoria, a filamentous blue-green, and Aphanocapsa, a colonial unicellular form, being the numerically dominant taxa. In the chemical microcosm, mixed green and blue-green algae were found, with Spirogyra and Oscillatoria being numerically dominant.

Algal standing crop (as indicated by chlorophyll a and phaeophytin) increased slowly from 0-10 d in all four microcosms (Figure 7C-F). In the thermal and chemical microcosms, algal growth accelerated from 10-20 d, but then stabilized after 20 d. Algal biomass remained low in the control microcosm throughout the study period, but increased steadily in the T+C microcosm. Both chlorophyll a and phaeophytin were significantly higher in the thermal microcosm than in the control after 34 d (Figure 7C, $p<0.01$; Figure 7E, $p<0.01$). Chlorophyll a and phaeophytin were lower in the T+C microcosm than in the chemical after 34 d (Figure 7D, $p=0.06$; Figure 7F, $p<0.01$). The amount of chlorophyll a degraded into phaeophytin provides some indication of the physiological state of the algae (Vollenweider 1969). The $\bar{x}$ degradation proportions after 34 d were similar in the thermal (29%) and control (24%) microcosms; thus, algae were of comparable "health" in these two microcosms.

In contrast, phaeophytin occurred in higher proportions in the chemical (34%) than in the T+C (14%) microcosm after 34 d. Thus, although chlorophyll a and phaeophytin were higher in the chemical microcosm than in the T+C, the algae in the T+C microcosm were in a better physiological condition.

These differences in proportions may be related to the relative productivity (i.e. rate of tissue elaboration) of algae in the T+C and chemical microcosms. Blue-green algae grew in a loosely attached mat over the substrate of the T+C microcosm, a growth form frequently seen in other thermal habitats (e.g. Brock 1978). We observed that portions of this mat periodically lifted off the substrate, tore free from the mat, and drifted out of the microcosm; this bare area was then rapidly covered by new growth. Thus, this sloughed algae represented primary production that was not expressed in the standing crop figures. The loss of algae/unit time is referred to as the "washout rate" by Brock (1970). We measured this loss (as dry weight) from each microcosms during five separate 24-h periods by collecting the sloughed algae in 1.0 mm mesh nets placed directly under the outflows (Table 2). There was no measurable loss of algae from the control microcosm due to sloughing. From the thermal and the chemical microcosms, there were similar absolute losses of algae (i.e. about 0.15 g 0.22 m^{-2} day^{-1}). When compared with the standing crops of microorganisms (also as dry weights), these represented daily losses of about 1% of the total standing crop from the thermal microcosm and 0.5% from the chemical microcosm. However, in the T+C microcosm, the loss was much

higher (0.81 g·0.22 m^{-2}·day^{-1}), which indicated that >2% of the total microbial biomass was sloughed each day.

Although the standing crop of algae was slightly higher in the chemical microcosm than in the T+C (Figure 4D), algal production was actually higher in the T+C microcosm since its standing crop of blue-green algae was periodically reduced by sloughing. The low proportion of algal phaeophytin (14%) in the T+C microcosm further substantiates the observed pattern of sloughing and vigorous regrowth that distinguished those thermophilic algae from those of the other three microcosms. The calculated rates of sloughing probably represent a slight underestimate of the actual losses, since the catchment nets did not retain algal clumps <1.0 mm in diameter. However, because most algae were sloughed in relatively large (>1.0 mm diameter) patches, this underestimate was probably not very great.

The total amount of organic matter was generally higher in the thermal microcosm than in the control (Figure 7G; $p<0.01$), as was also indicated by the other measurements of microbial biomass (e.g. bacteria, chlorophyll a). However, unlike the measurements of algae alone, total organic matter was slightly higher in the T+C microcosm than in the chemical microcosm (Figure 7H; $p=0.03$).

Responses of Macroinvertebrates. Since the entire macroinvertebrate fauna of each microcosm was inventoried after 34 d, all of the differences shown by these inventories are real (Figure 8). The number of macroinvertebrates in the thermal

microcosm declined by over 1,000 individuals when compared to the control (Figure 8A). However, the standing stock of macroinvertebrates increased slightly in the thermal microcosm (Figure 8B). As in the in-stream study, this increase may have been due to accelerated growth rates in this thermally enriched microcosm.

Macroinvertebrate species richness was similar in the control and thermal microcosms (45 cf. 44 species; Figure 8C) as was species composition. For example, a combined total of 54 species found in the two microcosms, of which 35 species were shared (65%); these totals are very similar to those found in the in-stream study. Apparently, most species colonizing the control microcosm from the cool section of Big Sulfur Creek were also able to tolerate the 8.5°C increase in water temperature in the thermal microcosm. However, some differences did exist. For example, the caddisflies Rhyacophila and Lepidostoma, which are taxa that normally occur in cool, well-oxygenated streams (Wiggins 1977), were found in the control but not in the thermal microcosm.

The distribution of those 54 species among the insect orders showed broad taxonomic diversity. There were 18 species of Diptera (33% of total), nine species of Coleoptera (17%), eight species of Trichoptera (15%), seven species of Ephemeroptera (13%), three species of Plecoptera (6%), and one species each of Odonata, Megaloptera, and Hemiptera. Non-insect taxa (e.g. crustaceans and oligochaetes) included six species (11%).

Macroinvertebrates were virtually absent from the T+C

microcosm (Figure 8A,B), where temperatures were extreme (e.g. to 47°C; Figure 6B). Only 85 individuals and three species were found in this microcosm, including 66 larvae (78% of total) of the chironomid midge Tanytarsus. The remaining macroinvertebrates were an oligochaete species (11%) and the ceratopogonid fly larva, Palpomyia (11%). Accordingly, the standing crop of macroinvertebrates was low in the T+C microcosm (Figure 8C).

Macroinvertebrates reached the highest density (5,678 individuals/0.22 m^2) in the chemical microcosm (Figure 8A), where water temperature was reduced from that found in the extreme T+C treatment. Standing stock in the chemical microcosm was also much greater than in the T+C microcosm (Figure 8B). However, as in the T+C microcosm, species richness in the chemical microcosm was low (i.e. 7 species; Figure 8C). Although diversity was inherently restricted in both microcosms because colonists came from the geothermal LGC, those species that colonized the chemical microcosm, when released from the extreme thermal stress, reached high density. In the chemical microcosm, as in the T+C, Tanytarsus was the most abundant macroinvertebrate (52% of total). Other abundant species in the chemical microcosm were the chironomid midge larva Polypedilum (14%) and an oligochaete species (26%). The results of both the stream microcosm experiment and the in-stream study are summarized in Table 3.

Heat Shock Field Experiment

This experiment was designed to measure the lethal thermal thresholds (LTT) of aquatic insects that were acclimated to warm (i.e. summer) or cold (i.e. winter) temperatures. H. borealis displayed the greatest resistance to thermal shock, whereas G. nigricula and Centroptilum sp. had lower LTTs.

Helicopsyche borealis. This caddisfly is able to withstand a thermal shock of 36°C for 1 h in both summer and winter with no increase in mortality over controls (Tables 4,5). In summer, thermal shocks of 39°C for 1 h or 41°C for 10 min produced >50% mortality. In winter, resistance to thermal shock was slightly lower; both 39°C for 20 min and 41°C for 5 min resulted in about 50% mortality.

Gumaga nigricula. This species can withstand 36°C for 1 h in summer with no mortality (Tables 4,5). However, thermal shocks of 39°C for 60 min or 41°C for five min produced >50% mortality. In winter, thermal shock resistance was substantially lower at 36° , 39°, and 41°C; all exposures at 41°C produced 100% mortality, 10 min at 39°C produced 50% mortality, and 1 h at 36°C resulted in about 50% mortality.

Centroptilum. This mayfly can withstand thermal shocks of 1 h at 33°C with no mortality and 1 h at 36°C with <10% mortality. However, at 39°C, >50% mortality occurs in 5 min.

Relevance to Previous Work. The LTT can vary with respect to acclimation temperature in any of three ways (Salmela and Anderson 1978). First, the LTT may be unaffected by acclimation temperature. Second, the LTT may vary directly with acclimation

temperature, increasing and decreasing with the seasonal fluctuation of temperatures; this is the most commonly observed relationship in aquatic organisms. Third, the LTT may be inversely related to the acclimation temperature. Our heat shock experiments indicate that the LTTs of H. borealis and G. nigricula vary directly with acclimation temperature, and are thus lower in the winter than in the summer. These results contrast with those found for two other caddisflies, Hydropsyche sp. (Sherberger et al. 1977) and Brachycentrus americanus (Salmela and Anderson 1978), which were unaffected by acclimation temperature. These differences may be due, in part, to the respective experimental protocols, especially the reduced number of time-temperature test combinations used in the other studies. In addition, we conducted our experiments in the field, which reduced stress from handling and pre-shock holding time. A further advantage of a field-based experiment is that pre- and post-shock holding conditions can be allowed to fluctuate with ambient field conditions.

In 1982 the maximum temperature at the study site in BSC was 30.5°C. In summer, the aquatic insects in this portion of the stream are living near their LTTs, and a relatively small increase in stream temperature (e.g. ΔT of 6-9°C) would exceed the LTT determined for all three species in this study. Power plants must generally have discharge waters with a $\Delta T > 7.7$°C to be economically practical (Schubel et al. 1978). This requirement would make summer discharges into geothermally heated waters, such as BSC, deleterious to aquatic life. In winter, there is a

small reduction in the LTTs for the two caddisflies. However, since ambient water temperatures are low, both caddisflies can withstand a ΔT of 25°C and would thus be less susceptible to the effects of thermal discharges. We conclude, as did Sherberger et al. (1977), that the magnitude of the thermal shock is not consequential in precipitating mortality unless it approaches the LTT, as influenced by the acclimation temperature.

DISCUSSION

Geothermal Influences: Synthesis

The stream microcosm study was not designed to simultaneously compare a control to three treatments (i.e. thermal, chemical, and T+C), but rather two separate controlled experiments were conducted. In the first, i.e. control cf. thermal, a thermal component was added to non-geothermal water. In the second, i.e. T+C cf. chemical, the thermal component was removed from geothermal water. In this latter comparison, water from LGC provided the T+C condition, which served as the control.

Thermal enrichment of non-geothermal water had dramatic effects on the benthic microbial community. In the stream microcosm study, the standing crop of algae was increased 40X by an increase of +8.5°C (thermal microcosm). Likewise, algal productivity, as determined by the rate of sloughing, was markedly accelerated, and the density of benthic bacteria increased two-fold. These patterns of relative increase are strikingly similar to those observed in the in-stream study, in

which algal standing crop was also about 40X higher at the downstream (i.e. thermally (+7.5°C) and chemically enriched) site after seven weeks, and bacteria also moderately increase. Apparently, thermal enrichment of BSC water alone (thermal microcosm) enhanced microbial growth in nearly the same proportions as thermal and chemical enrichment combined (BSC-downstream). The generally lower absolute amounts of algae and bacteria found in the in-stream study as compared with the microcosms (e.g. Figure 4B cf. 7C) were probably related to the grazing effects of herbivorous insects in BSC. For example, H. borealis larvae display a very low tendency to drift (Lamberti and Resh 1983) and thus failed to colonize the microcosms.

Less dramatic effects were observed in the responses of benthic macroinvertebrates to thermal enrichment; density declined in the thermal microcosm by about one-fourth, but standing stock and species richness were nearly the same as in the control microcosm. These results appear to contradict the findings of the in-stream study, in which there was a marked increase in macroinvertebrate density and biomass at the geothermally influenced site. However, since the colonists of BSC-downstream came from either the geothermal LGC and were adapted to geothermal conditions, or from the non-geothermal upstream section of BSC (Figure 1C), these colonists represented a broad range of thermal tolerances. Thus, the increase in the number of macroinvertebrates that occurred would be expected. However, macroinvertebrates that colonized the thermal microcosm came from only the non-geothermal BSC; since a narrow

range of thermal tolerances was probably represented, a density increase would not be expected and, in fact, macroinvertebrate density was lower in the thermal than in the control microcosm. However, the high species richness in both the control and thermal microcosms suggests that most of the cold-adapted macroinvertebrate species could tolerate a moderate increase in water temperature.

The removal of the thermal component from geothermal water resulted in an algal community with higher standing crop but lower productivity. This treatment also increased the density, standing crop, and species richness of macroinvertebrates. Although the extreme temperature and chemical conditions of geothermal water (T+C) could not be tolerated by most macroinvertebrates, following removal of the thermal component, the chemical conditions of geothermal water could be tolerated by many species.

Brock (1975) has indicated that thermal enrichment should affect aquatic habitats whose temperatures are <25°C more severely than those with higher temperatures. This is because few microorganisms have temperature/growth optima at <25°C. Consequently, establishment of certain microorganisms that grow at their temperature optimum should result when water is warmed above that point, thereby leading to increases in production, and possibly changes in community structure. In the stream microcosm study, this pattern was demonstrated by Spirogyra, which had a much lower standing crop in the control microcosm (t_{max}=23°C) than in the thermal microcosm (t_{max}=34°C). Since habitats

>25°C (before heating) should have some species growing optimally, further temperature increases should primarily result in community shifts rather than biomass differences, because the resident flora is replaced by other optimally growing species. In our study, mixed green and blue-green algae in the chemical microcosm (t_{max}=32°C) were replaced by an assemblage of blue-green algae in the T+C microcosm (t_{max}=47°C), but without significant increases in standing crop. Wilde and Tilly (1981) recorded similar shifts to blue-green dominance in thermally enriched (t_{max}>30°C) microcosms.

In most cases, generalized thermal discharges have been shown to have negative effects on macroinvertebrate communities, in particular on species richness (e.g. Logan and Maurer 1975, Dusoge and Wisniewski 1976, Ferguson and Fox 1978). In contrast, studies of moderate geothermal inputs into natural systems have shown little reduction in species richness, although there may be shifts in species composition (Armitage 1958, Vincent 1967), which is similar to the results of our in-stream study.

Certain taxonomic groups appear to respond favorably, in both density and production, to thermal additions, in particular the Oligochaeta (e.g. Nichols 1981 and references therein) and the Chironomidae (e.g. Vincent 1967, Ferguson and Fox 1978). At the extreme geothermal conditions in our stream microcosm study, the macroinvertebrate community was similarly dominated by chironomids and oligochaetes. In many cases, thermal enrichment also results in increased growth rates of aquatic invertebrates, as documented in groups as diverse as the Ephemeroptera (Humpesch

1978, Mattice and Dye 1978), the Oligochaeta (Wiśniewski 1976), the Nematoda (Laybourn 1979), and the Copepoda (Geiling and Campbell 1972). In both our in-stream and microcosm studies, increased growth rates may have been responsible for the increased mean weight of individuals in thermally enriched habitats, since the species composition remained similar.

Value of In Situ Bioassays

The development of *in situ* (i.e. field-based) bioassay procedures can greatly extend our knowledge of the effects of chemicals and temperature on aquatic biota (Alabaster 1978, Little and Maciorowski 1979). Such experimental approaches may be particularly effective for predicting the primary effects of environmental disturbances, once the main impacting factors have been identified (Rosenberg et al. 1981). Laboratory bioassay tests of chemical (LC_{50}) toxicity or thermal (LT_{50}) tolerance frequently have limited relevance to conditions in natural systems (Brock 1975), especially if conducted as short-term (acute) shock tests (Lehmkuhl 1979). Specific criticisms of laboratory bioassay studies have addressed: 1) chemical concentrations studied; 2) test duration; 3) trophic level and source of test organisms; 4) use of a single chemical as opposed to naturally-occurring mixtures; 5) selection of temperature; 6) lack of environmental variation imposed by field conditions; and 7) failure to assess broad taxonomic groups (Gray and Ventilla 1973).

In contrast, a well designed *in situ* bioassay can vary

important physico-chemical features while allowing natural environmental variation to exert some influence over the bioassay. For example, our stream microcosm study accomplished this goal and answered the above criticisms by: 1) exposing benthic communities to chemical concentrations typically found in geothermal habitats; 2) allowing sufficient time for biological response to occur; 3) assaying the wide range of trophic levels (autotrophs, microheterotrophs, macroheterotrophs) found in the natural community rather than using a stock culture; 4) using mixtures of geochemicals that occur naturally; 5) using natural thermal regimes; 6) incorporating environmental variation encountered in the natural system; 7) assessing the responses of many taxonomic groups, from bacteria to metazoans.

Buikema and Benfield (1979) have further suggested that toxicity tests should incorporate the synergistic effects of chemicals and temperature. For example, recent studies have demonstrated that invertebrates display increasing sensitivity to chemicals with increasing temperature (e.g. Cairns et al. 1978, Braginskiy and Scherban 1978). However, most *in situ* studies of combined thermal and chemical stresses have not been experimental, but rather have related existing thermal and chemical features to the observed structure of benthic communities (e.g. Dusage and Wiśniewski 1976, Letterman and Mitsch 1978).

Although the in-stream study conducted at The Geysers demonstrated that a moderate addition of geothermal fluids (i.e. from LGC) into a non-geothermal stream (i.e. BSC) could

substantially alter the structure of the benthic community, the analysis did not establish the cause of the alteration. The stream microcosm study demonstrated that the thermal component of those geothermal fluids had greater influence than the chemical component in determining the structure of the benthic community in Big Sulfur Creek. Thus, in future studies designed to evaluate the environmental effects of geothermal energy development, and as other sources of environmental disturbance as well, the development of field-based bioassay techniques may allow cause-and-effect relationships to be better elucidated and, consequently, enable appropriate mitigation procedures to be developed.

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TABLE 1. Dissolved oxygen concentration, conductivity, and pH in each stream microcosm ($\bar{x} \pm SD$).

	CONTROL	THERMAL	CHEMICAL	THERMAL + CHEMICAL
O_2 (mg/l)	7.6+0.6 (n=11)	6.8+0.6 (n=11)	4.6+0.2 (n=11)	4.2+0.2 (n=11)
$\bar{x}$ % saturation	81	84	54	64
µmhos/cm	340+65 (n=11)	400+70 (n=11)	370+25 (n=11)	560+30 (n=11)
pH	7.2+0.4 (n=8)	7.2+0.3 (n=8)	6.6+0.2 (n=8)	6.7+0.2 (n=8)

TABLE 2. Loss of periphyton due to sloughing in each stream microcosm. Measurements are dry weight $\bar{x}$'s for five separate 24-h periods.

	CONTROL	THERMAL	CHEMICAL	THERMAL + CHEMICAL
Slough (g/day)	0	0.16	0.15	0.81
Standing Crop ($g/0.22m^2$)	10.33	16.51	27.98	37.26
Slough/Day (% standing crop)	0	0.97	0.54	2.17

TABLE 3. Results of statistical tests (Student's t-test) comparing treatment pairs of the in-stream study and the stream microcosm study at the end of the respective study periods. Treatment pairs for the in-stream comparison: "+ Thermal and Chemical" represents BSC upstream cf. BSC downstream. Stream microcosm comparison: "+ Thermal" represents control cf. thermal; "- Thermal" represents thermal plus chemical cf. chemical. Comparisons without reported statistical significance indicate that quantities were inventories or pooled samples that are inappropriate for significance testing. Legend: I = increase; D = decrease; NC = no change; superscripts refer to p-values: $^{a} = p < 0.01$; $^{b} = p < 0.05$; $^{c} = p < 0.10$.

	IN-STREAM STUDY	STREAM MICROCOSMS	
	+ Thermal and Chemical	+ Thermal	- Thermal
Microorganisms			
Bacteria	I^{a}	I^{a}	NC
Chlorophyll a	I^{a}	I^{a}	I^{c}
Phaeophytin	I^{c}	I^{a}	I^{a}
1 Productivity		I	D
Ash-Free Dry Wt.	I^{c}	I^{a}	D^{b}
Macroinvertebrates			
Density	I^{a}	D	I
Standing Stock	I	NC	I
Species Richness	I	NC	I

TABLE 4. Mean % mortality after 24 h caused by acute thermal shock in warm-acclimated aquatic insects in BSC, 17 July 1982; control = 28°C; treatments = 33-45°C.

TEMP °C	EXPOSURE (min) 5	10	15	20	30	60
Helicopsyche borealis						
28	3	0	0	0	0	3
33	0	0	0	0	0	0
36	0	0	0	0	0	0
39	0	3	0	6	0	57
41	3	77	83	100	100	100
43	100	100	100	100	100	100
45	100	100	100	100	100	100
Gumaga nigricula						
28	0	0	3	0	0	0
33	0	0	0	0	3	3
36	0	0	0	3	0	0
39	3	6	3	13	10	90
41	53	93	97	100	100	100
43	100	100	100	100	100	100
45	100	100	100	100	100	100
Centroptilum sp.						
28	3	0	0	0	3	3
33	3	0	3	0	0	6
36	0	3	10	6	6	10
39	53	73	90	97	100	100
41	100	100	100	100	100	100
43	100	100	100	100	100	100
45	100	100	100	100	100	100

TABLE 5. Mean % mortality after 24 h caused by acute thermal shock in cold-acclimated aquatic insects in BSC, 14 January 1983; control = 6°C; treatments = 33-45°C.

TEMP °C	EXPOSURE (min) 5	10	15	20	30	60
Helicopsyche borealis						
6	0	3	0	0	0	0
33	0	0	0	0	0	0
36	0	3	0	0	0	3
39	0	20	10	47	97	100
41	50	77	100	100	100	100
43	100	100	100	100	100	100
45	100	100	100	100	100	100
Gumaga nigricula						
6	0	0	0	0	0	0
33	0	0	0	0	0	0
36	0	0	0	10	17	43
39	7	50	97	100	100	100
41	100	100	100	100	100	100
43	100	100	100	100	100	100
45	100	100	100	100	100	100

FIGURE LEGENDS

FIGURE 1. Maps showing detail of the study area. (A) The Geysers Known Geothermal Resources Area (K.G.R.A.) in relation to San Francisco Bay, California, U.S.A.; (B) major aquatic habitats in the principal development area at The Geysers; (C) study area and sampling sites at the confluence of Big Sulphur Creek (BSC) and Little Geysers Creek (LGC); U = BSC upstream of LGC, D = BSC downstream of LGC, M = location of stream microcosms.

FIGURE 2. Comparison of chemical concentrations in BSC at 100 m upstream and 50 m downstream of LGC on 6 May 1981. Points along broken line indicate equal concentrations upstream and downstream of LGC; points above the line indicate chemical inputs to BSC from the geothermally influenced LGC; points below the line indicate lower values in LGC than in BSC.

FIGURE 3. Design of stream microcosm study conducted at The Geysers. (A) Photograph showing <u>control</u> microcosm and three treatment microcosms; the heat-exchange unit of the <u>thermal</u> microcosm is at the upper left; (B) Schematic diagram showing sources of water, pattern of water movement, and location of heat-exchange units. Water was delivered to the microcosms solely by a gravity-feed system. See text for further details.

FIGURE 4. Dynamics of benthic microorganisms during the seven weeks of the in-stream study (1 June - 18 July 1979) designed to compare communities in BSC upstream and downstream of its

geothermal tributary, LGC. (A) Numerical abundance of bacteria; (B) Chlorophyll *a*; (C) Phaeophytin; (D) Total organic matter (dots and bars represent $\bar{x} \pm SE$; n=5).

FIGURE 5. Dynamics of benthic macroinvertebrates during the seven weeks of the in-stream study (1 June - 18 July 1979) designed to compare communities in BSC upstream and downstream of its geothermal tributary, LGC. (A) Density of all macroinvertebrates; (B) Standing stock (n=5 samples pooled); (C) Species richness (n=5 samples pooled); (D) Density of *Helicopsyche borealis* (Hagen).

FIGURE 6. Daily maximum-minimum temperature ranges in the four stream microcosms during the 34-d study period. (A) *Control* cf. *thermal*; (B) *T+C* cf. *chemical*.

FIGURE 7. Growth curves of benthic microorganisms in the four stream microcosms during the 34-d study period (9 June - 13 July 1981). (A,B) Numerical abundance of bacteria; (C,D) Chlorophyll *a*; (E.F) Phaeophytin; (G,H) Total organic matter. ($\bar{x} \pm$ 95% CL; n=3).

FIGURE 8. Inventory of benthic macroinvertebrates in the four stream microcosms at the end of the 34-d study period (13 July 1981). (A) Density; (B) Species richness; (C) Standing stock (dry weight).

FIGURE 9. Floating racks used to hold heat shock test cells in BSC; each rack contains three replicates of the 6 X 7 cell time-temperature matrix.

Figure 1

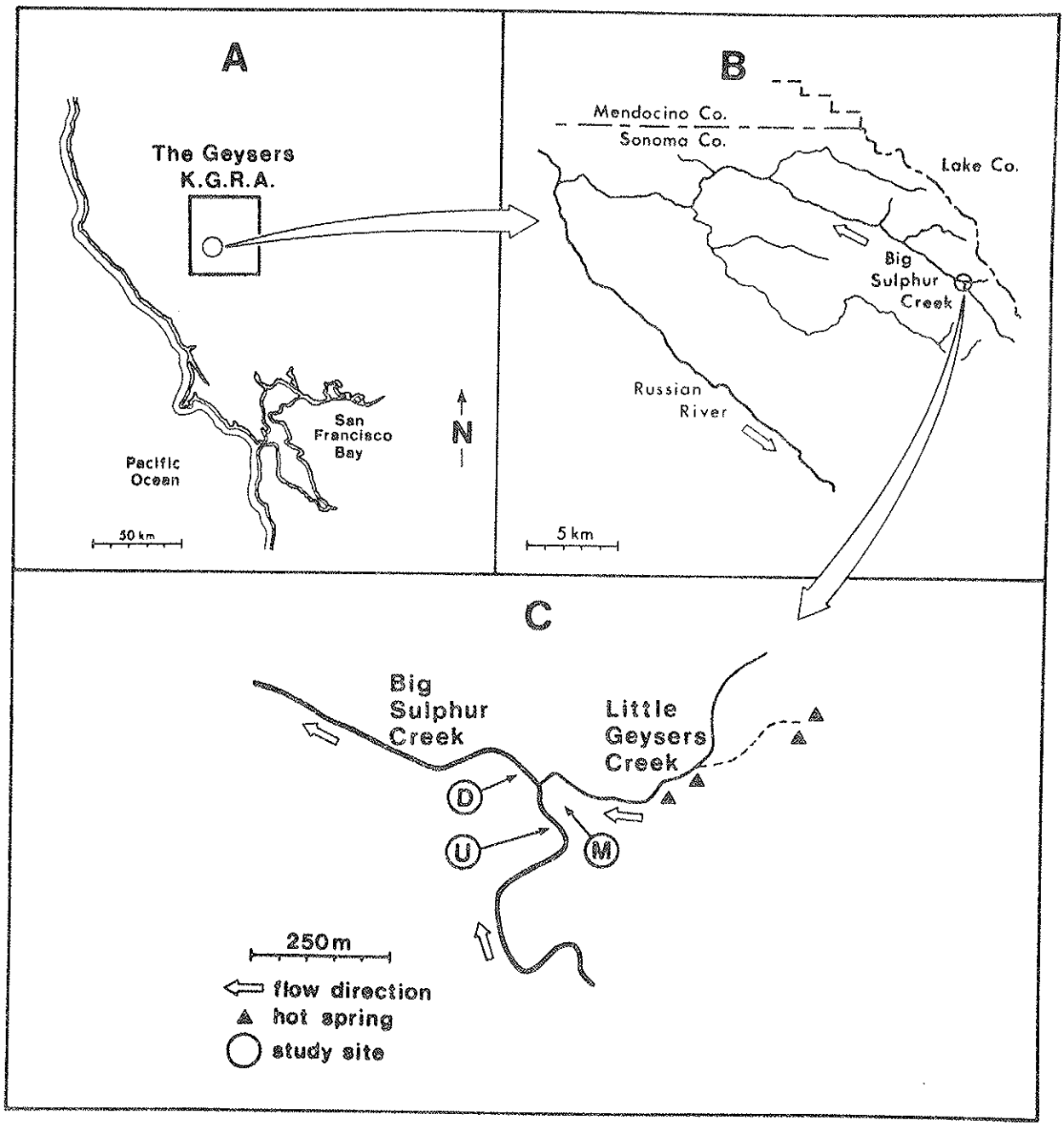

A
The Geysers
K.G.R.A.
San
Francisco
Bay
Pacific
Ocean
N
30 km
B
Mendocino Co.
Sonoma Co.
Lake Co.
Big
Sulphur
Creek
Russian
River
5 km
C
Big
Sulphur
Creek
Little
Geysers
Creek
D
U
M
250 m
flow direction
hot spring
study site

Figure 2

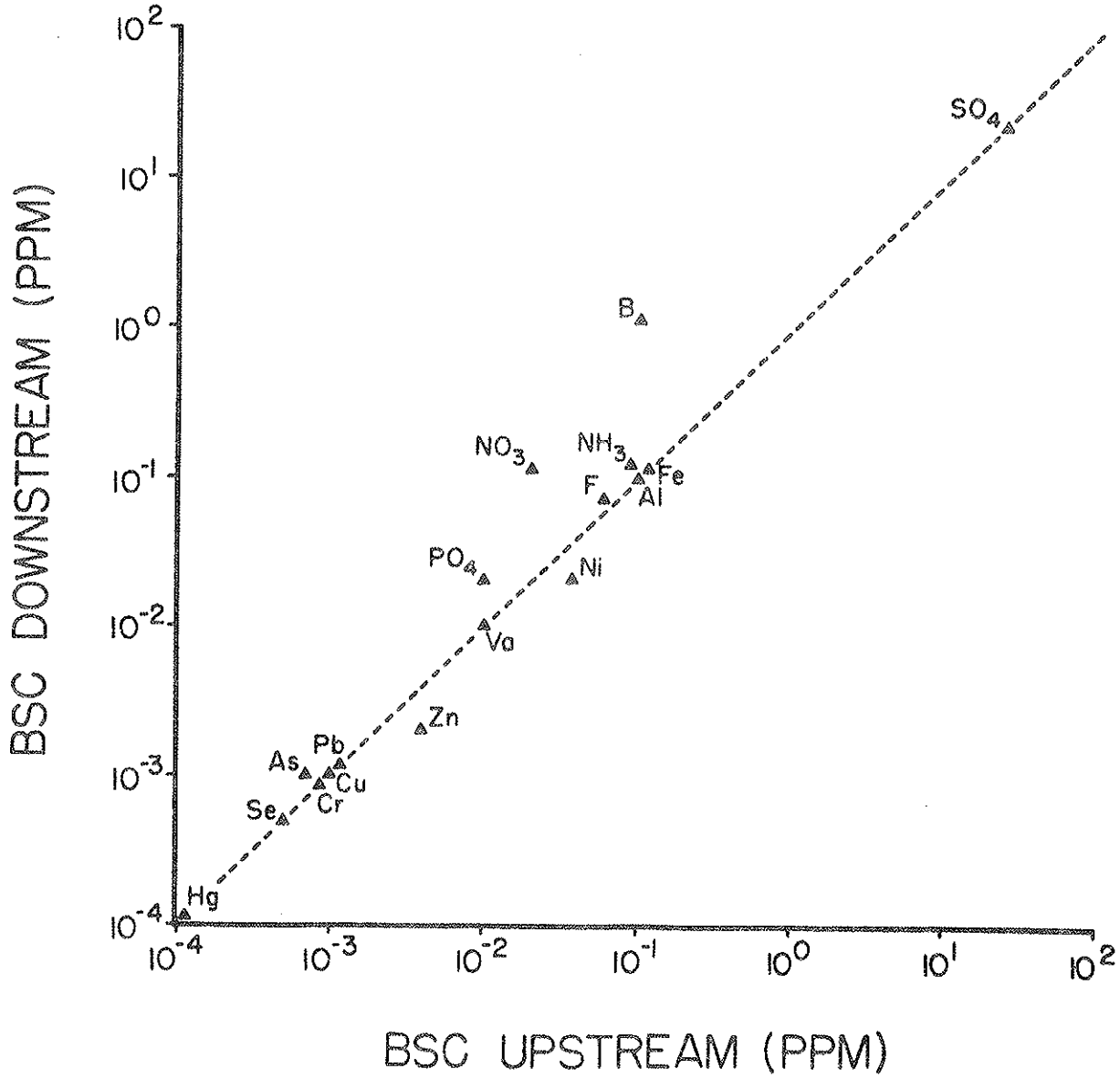
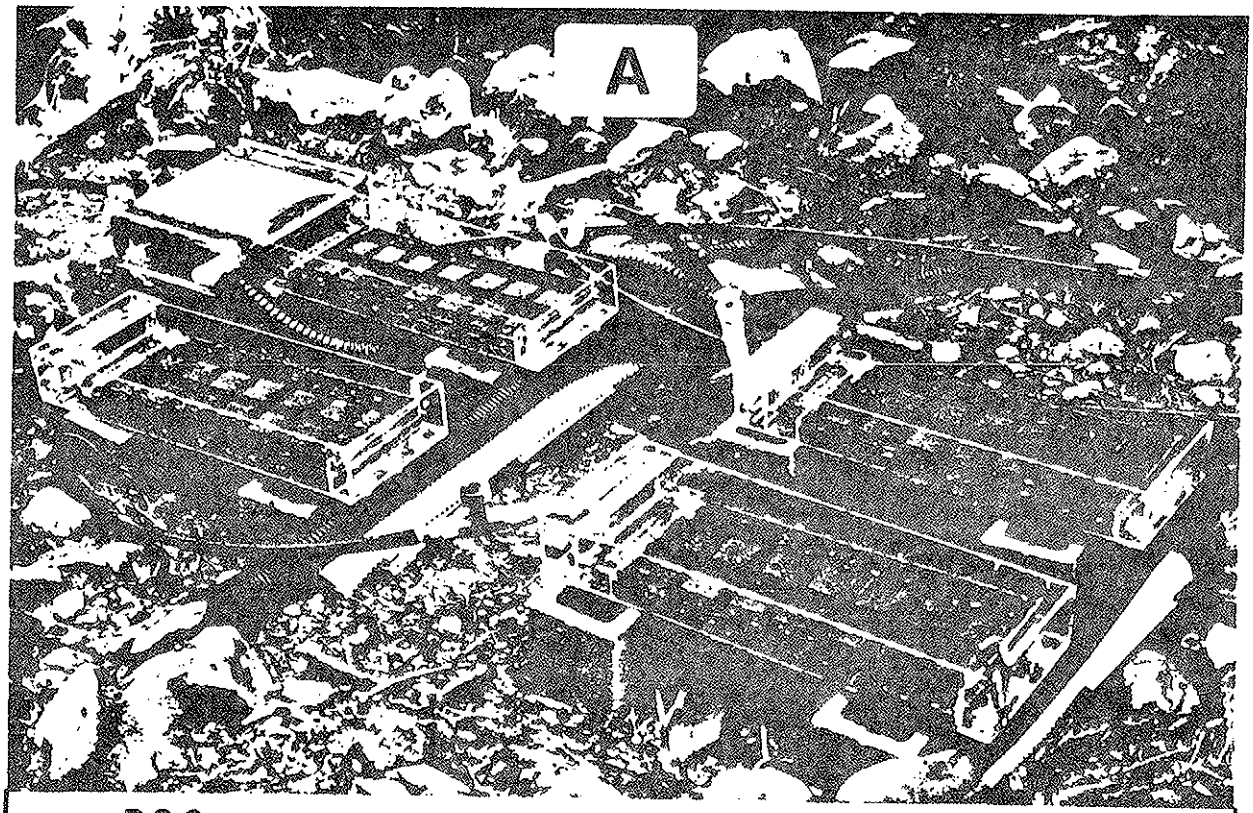
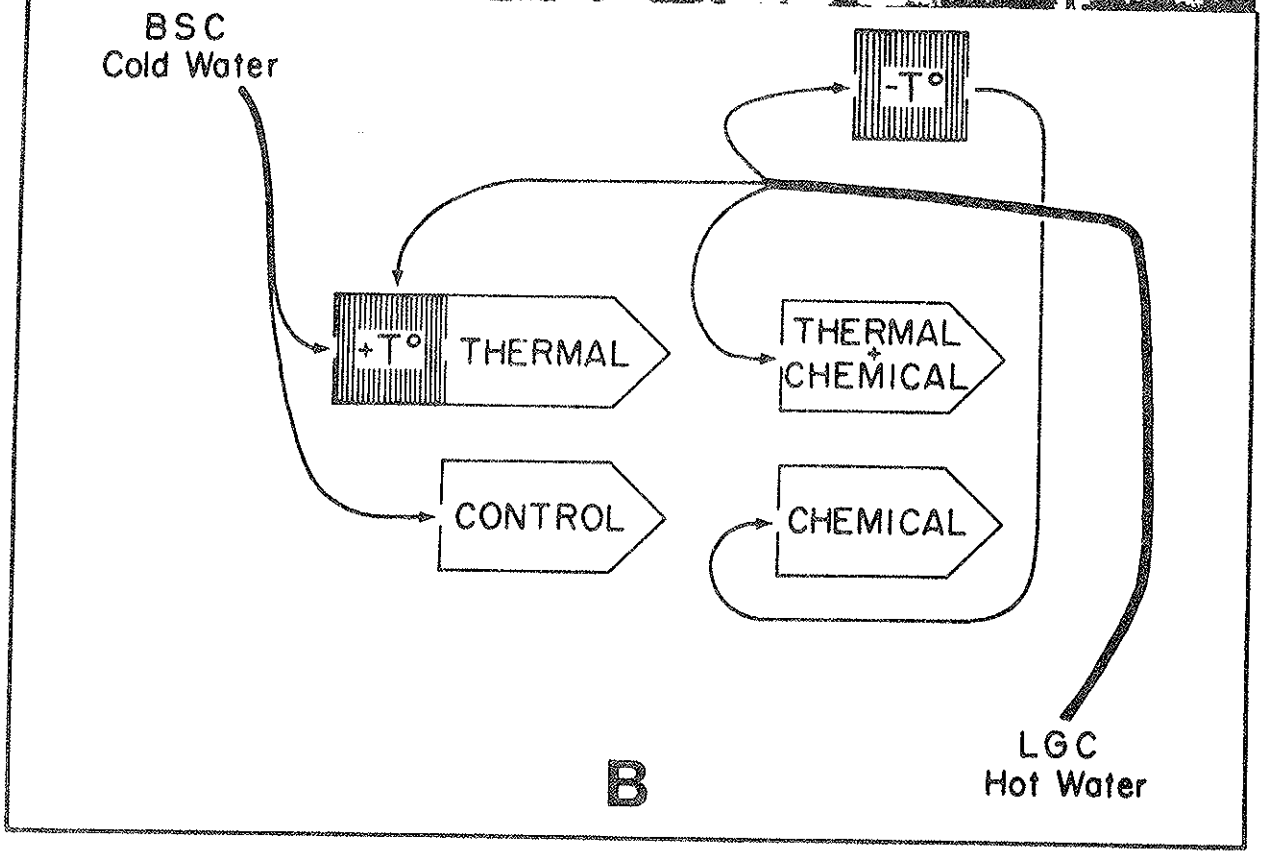

BSC DOWNSTREAM (PPM)
BSC UPSTREAM (PPM)
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
SO_4
B
NO_3
NH_3
Fe
F
Al
PO_4
Ni
Va
Zn
As
Pb
Cu
Cr
Se
Hg

Figure 3


A


BSC
Cold Water
-T°
+T°
THERMAL
THERMAL
+
CHEMICAL
CONTROL
CHEMICAL
LGC
Hot Water
B

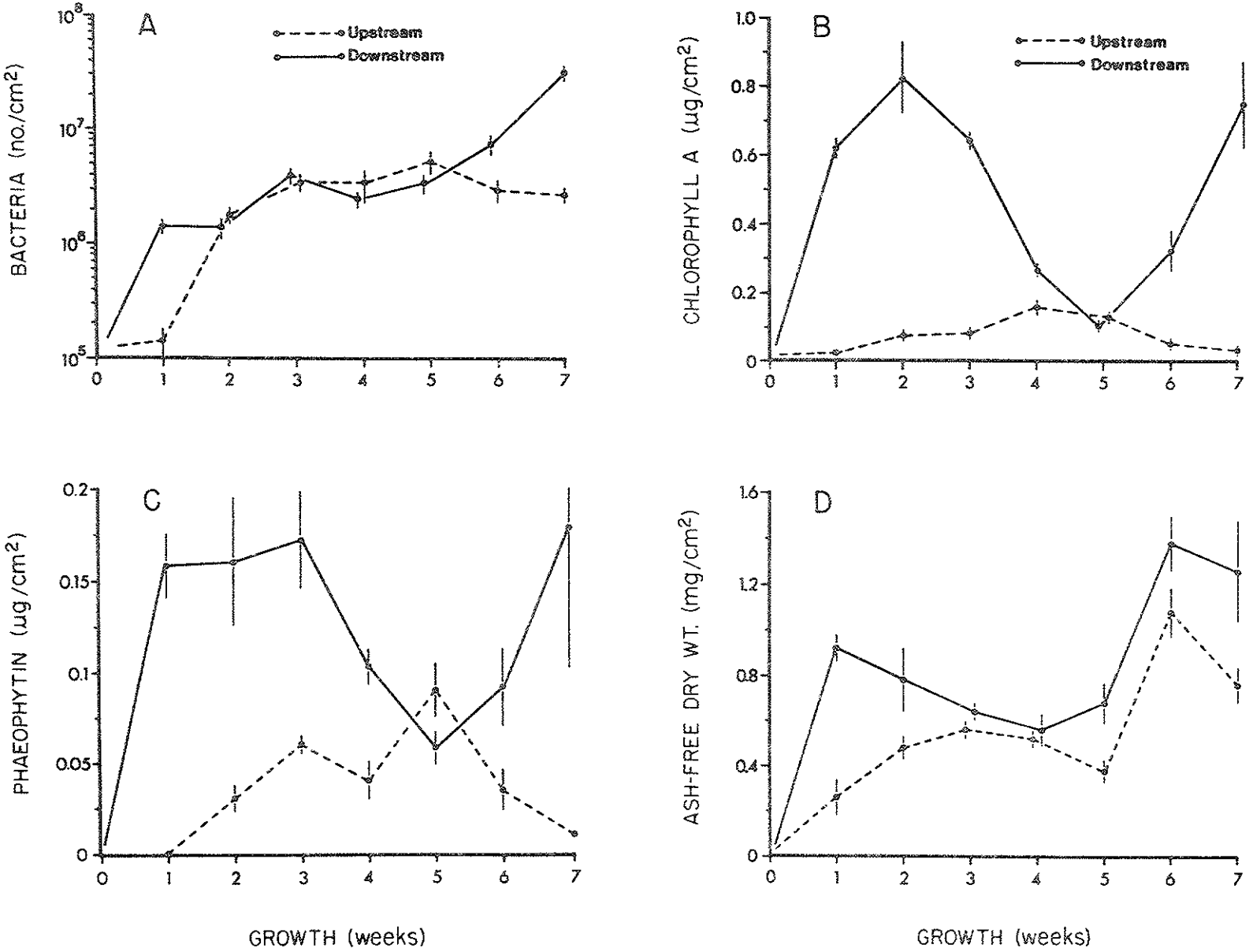

A
Upstream
Downstream
BACTERIA (no./cm²)
B
CHLOROPHYLL A (µg/cm²)
C
PHAEOPHYTIN (µg/cm²)
D
ASH-FREE DRY WT. (mg/cm²)
GROWTH (weeks)
GROWTH (weeks)

Figure 4

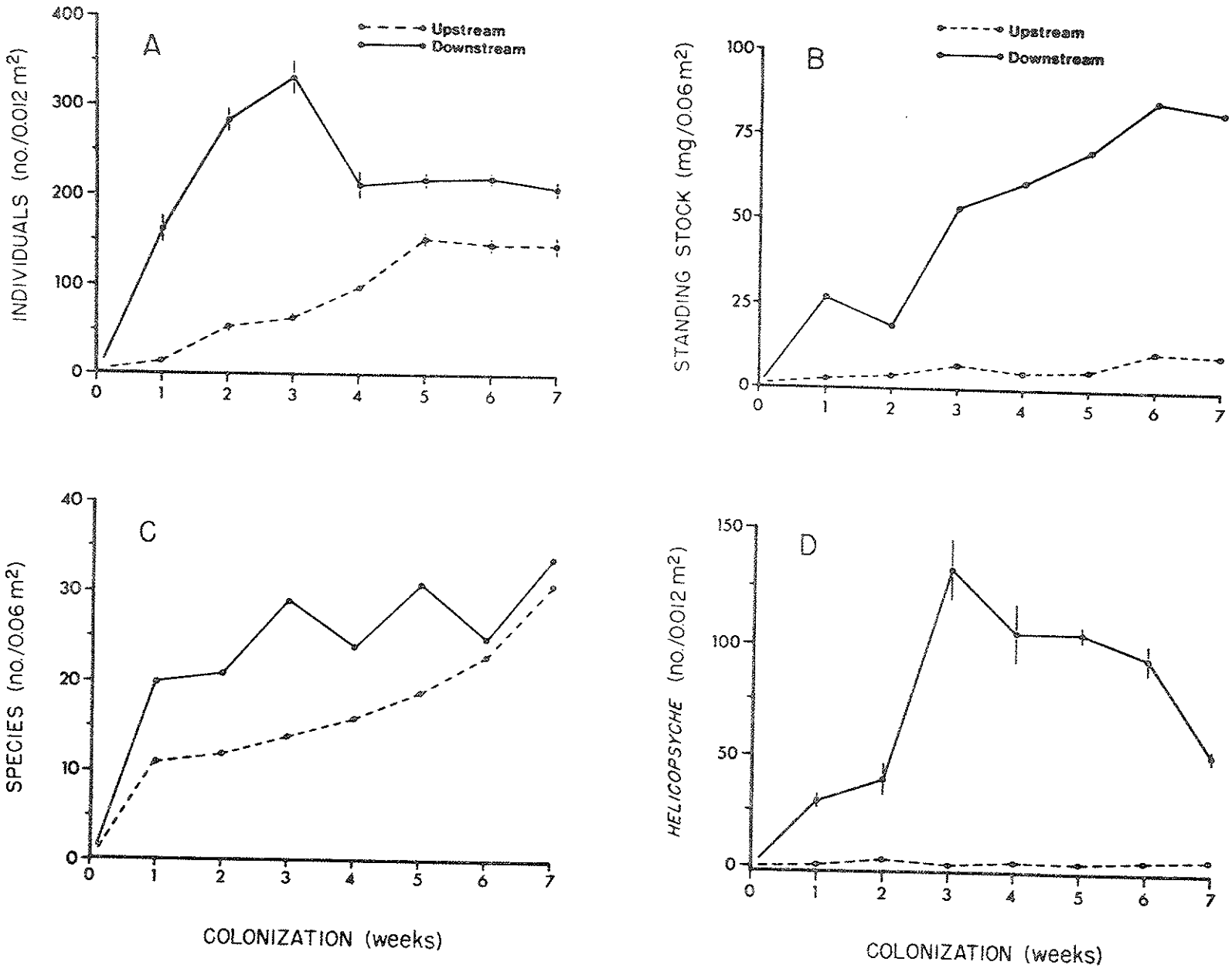

A
Upstream
Downstream
INDIVIDUALS (no./0.012 m²)
400
300
200
100
0
0 1 2 3 4 5 6 7
B
Upstream
Downstream
STANDING STOCK (mg/0.06 m²)
100
75
50
25
0
0 1 2 3 4 5 6 7
C
SPECIES (no./0.06 m²)
40
30
20
10
0
0 1 2 3 4 5 6 7
COLONIZATION (weeks)
D
HELICOPSYCHE (no./0.012 m²)
150
100
50
0
0 1 2 3 4 5 6 7
COLONIZATION (weeks)

Figure 5

Figure 6

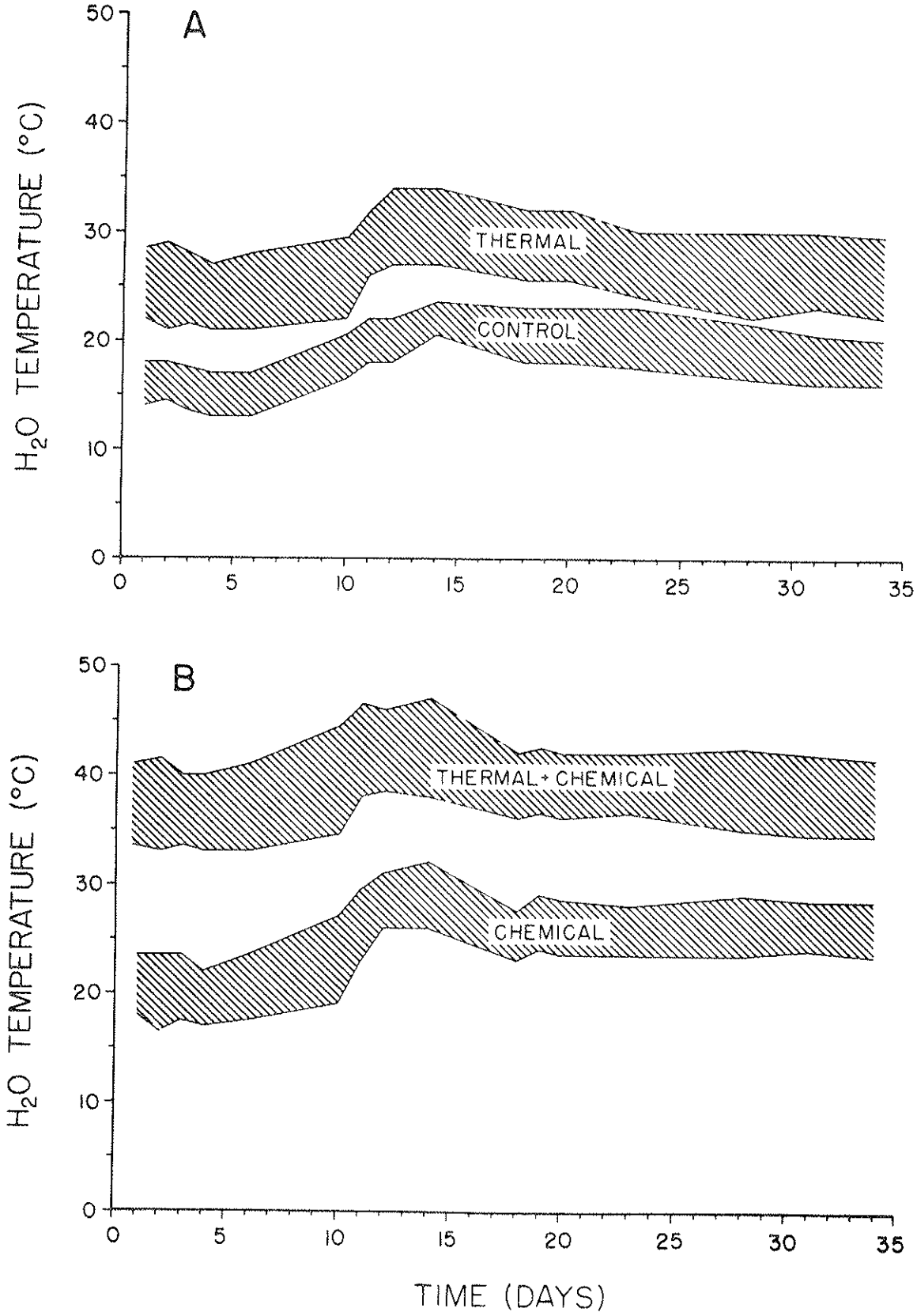

A
B
THERMAL
CONTROL
THERMAL + CHEMICAL
CHEMICAL
H_2O TEMPERATURE (°C)
TIME (DAYS)
0
10
20
30
40
50
0
5
10
15
20
25
30
35

Figure 7

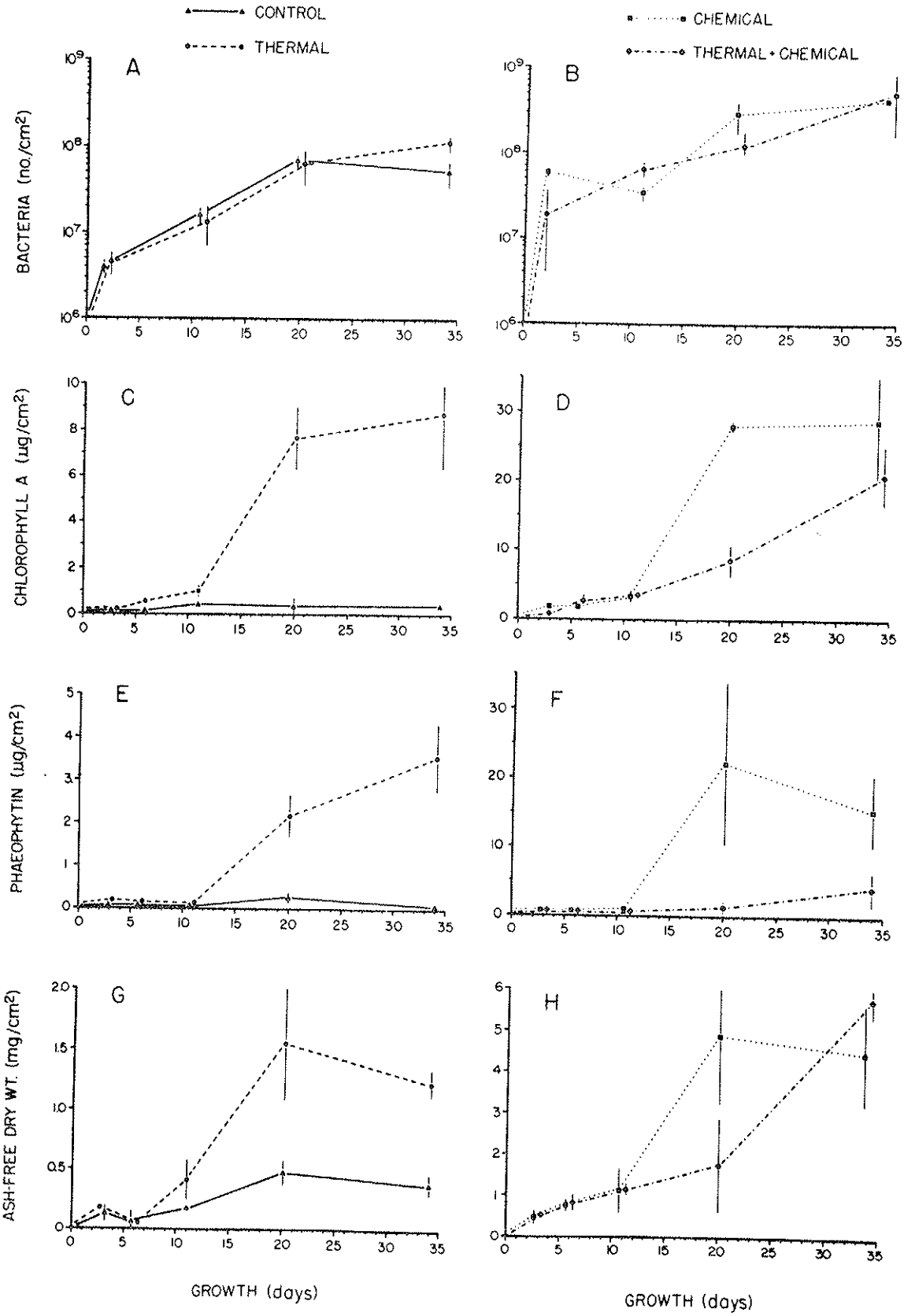
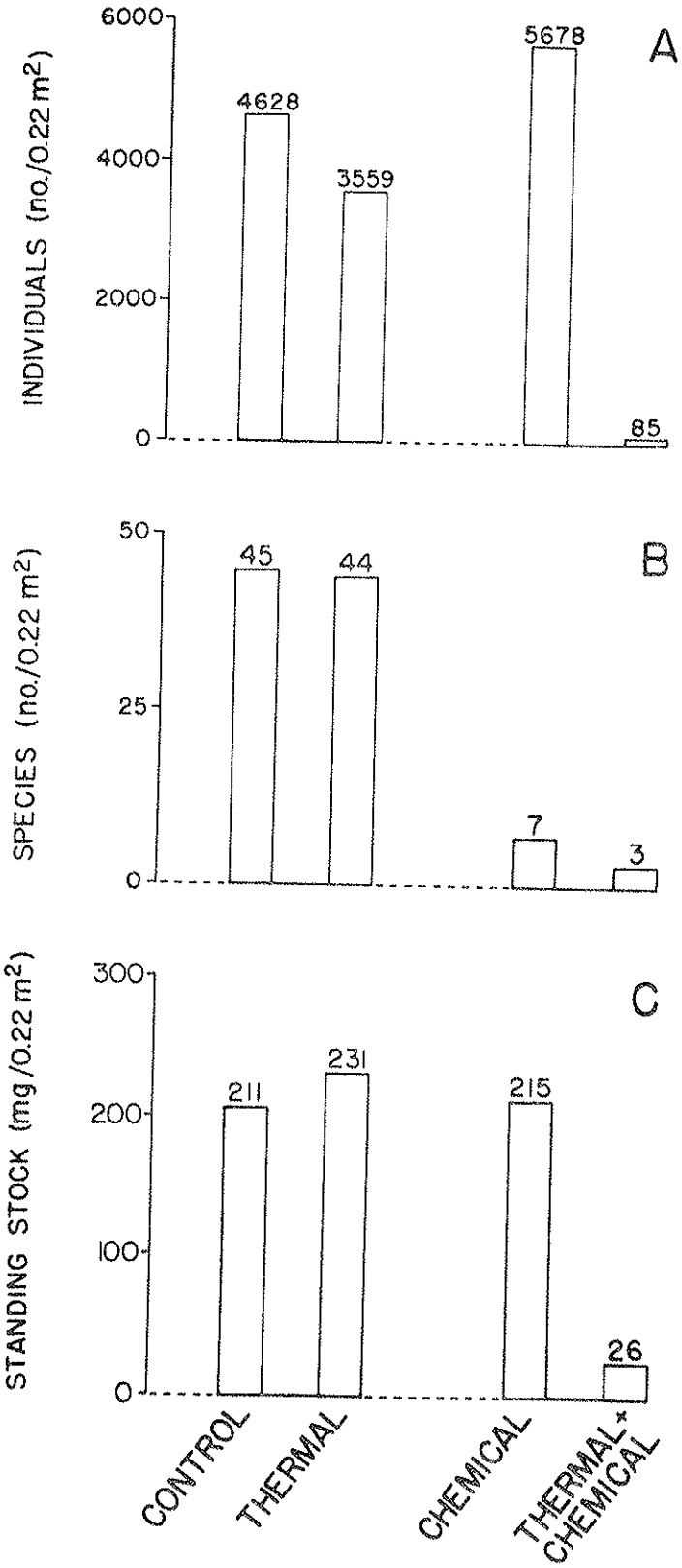

CONTROL
THERMAL
CHEMICAL
THERMAL + CHEMICAL
A
B
C
D
E
F
G
H
BACTERIA (no./cm²)
CHLOROPHYLL A (µg/cm²)
PHAEOPHYTIN (µg/cm²)
ASH-FREE DRY WT. (mg/cm²)
GROWTH (days)
GROWTH (days)

Figure 8


A
INDIVIDUALS (no./0.22 m²)
6000
4000
2000
0
4628
3559
5678
85
B
SPECIES (no./0.22 m²)
50
25
0
45
44
7
3
C
STANDING STOCK (mg/0.22 m²)
300
200
100
0
211
231
215
26
CONTROL
THERMAL
CHEMICAL
THERMAL* CHEMICAL