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SENSITIVITY OF MARINE CILIATES (PROTOZOA, CILIOPHORA) TO HIGH THERMAL STRESS

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by

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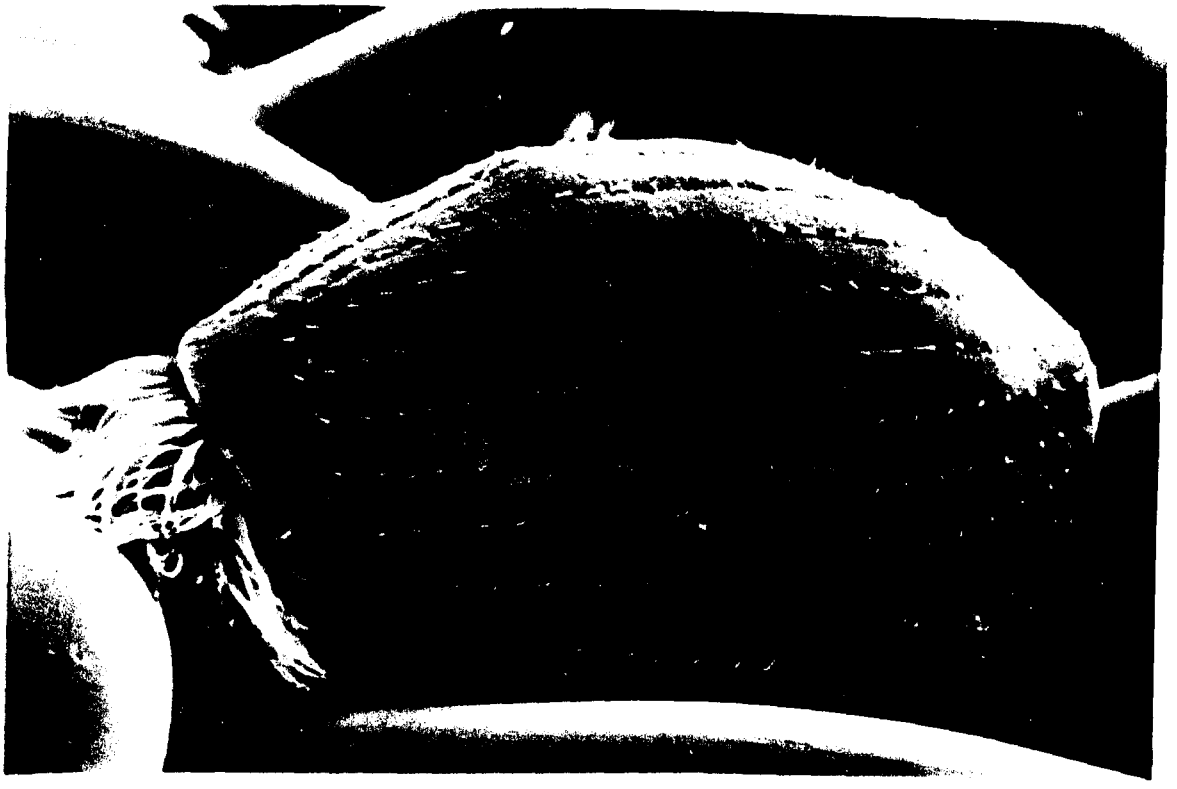

Euplotes harpa

(Magnification: 950X)

This thesis has been examined and approved.

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TO MY DAD

IN MEMORY OF HIS LOVE

AND DEVOTION

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ABSTRACT

SENSITIVITY OF MARINE CILIATES (PROTOZOA, CILIOPHORA) TO HIGH THERMAL STRESS

by

EDWIN A. MARTINEZ

The range of sensitivity of marine ciliates to temperatures near lethal maxima was determined under a variety of biotic and abiotic factors. Twelve species representing different taxa, sizes and modes of feeding and locomotion were examined. A simple heating apparatus consisting of a heated microscope stage was employed to subject the organisms to acute heat stress.

Ciliates are sensitive to elevated temperatures, especially when near their lethal limits. Heat resistance was influenced by cultivation temperature, salinity and nutrition. Temperatures of LD_{50} ranged from 31.5°C for Uronema marinum to 39.3°C for Dexiotricha sp. When cultivated at low temperatures (10°C), Euplotes crassus was more resistant to heat shock at higher salinities (35 o/oo) than at low salinities (15 o/oo). Thermal sensitivity depended also on acclimation temperature. Further, when cultured on a single species of bacteria, E. crassus was more sensitive to heat stress than when cultivated on an assortment of bacteria. The type of bacteria offered as food also determined its sensitivity to heat shock.

Aside from accelerating the final outcome, a temperature increase from 15° to 28°C had little influence on the competitive interaction between E. crassus and Strombidium sulcatum at the food levels tested (0.001% and 0.015% proteose peptone and yeast extract). At 30°C, a temperature above the optimum for E. crassus growth, the competitive effects of E. crassus on S. sulcatum were further reduced. The data suggest that E. crassus may have a competitive advantage over E. harpa at 15°, but not at 28°C. It appears that the magnitude of a population is affected by the presence of other ciliates only when temperatures are below the optimum values of the interacting species. If temperature increases beyond these maxima, species interaction becomes negligible.

INTRODUCTION

Over the past decade the increasing problem of thermal addition to aquatic environments resulting from power generation provided impetus for many studies concerned with the effects of chronic and acute heat stress on the physiology and ecology of many organisms. Among these are investigations not only on primary producers, but also on vertebrates, and many invertebrates. Major reviews of studies of heat stress include Coutant (1969), Fry (1967), Kennedy & Mihursky (1967), Krenkel & Parker (1969) and McWhinnie (1967); also the Proceedings of the 2nd Thermal Workshop of the U.S. International Biological Program (1969) and Gibbons & Sharitz (1974).

Estimates indicate that by 1980 one-third of the new and expanded power-generating installations requiring large volumes of water for their cooling systems will be located in estuarine and coastal areas (Anonymous, 1970; Clark & Brownell, 1973; and Picton, 1960). For reasons outlined below, this study focuses on the effects of heat on marine Ciliophora.

Ciliated protozoa are shown to be important constituents of benthic communities of estuaries and of tidal marshes in particular (Borror, 1965, 1968, 1972, 1975; Brown, 1973; Fenchel, 1967, 1968a, b, 1969; Johannes, 1965; Kirby, 1934; and Webb, 1956), yet the literature reveals that they have received very little attention with regards to heat stress

as an environmental factor.

Presently, little information is available on the exact trophic relationships of ciliates as prey for animals; however, their role as bacterivores and consumers of protophytes is well established. Several researchers (e.g., Burkholder, 1959; Fenchel, 1970; and Odum, 1970) have stated that ciliates comprise a definite link in the decomposer food chain system, while others believe that their major role lies in other equally important ecological functions. For example, according to Johannes (1965) ciliates maintain the bacterial concentration in a state of "prolonged physiological youth" by grazing and thus promote normal succession and recycling of nutrients (see also Bick, 1973 and references therein). Their numbers alone suggest that they exert a significant influence on the community. In fine sands and areas rich in bacterial growth, such as tidal marshes, ciliates range from 10^6 to $4x10^7$ individuals per m^2 and are 10-100 times more numerous than the total number of metazoa (Fenchel, 1967). Their biomass (0.03-2.3g wet wt./m^2) is of the same order and often larger than the biomass of micrometazoa ($\sim$ 1.5g/m^2). Populations of ciliates inhabiting salt marshes are as rich and diverse as populations inhabiting sandy habitats of more open coasts (Borror, 1968). Further, ciliates may account for up to 25% or more of the total animal respiration of sublittoral sands (Fenchel, 1969); comparable estimates were established by Wieser & Kanwisher (1961) in Spartina flats. Thus, they make a considerable contribution to the total

energetics of these communities, and therefore information regarding their physiological tolerances is of concern to the complete understanding and protection of these "areas of critical environmental concern" (Clark & Brownell, 1973).

The majority of previous research on thermal effects on ciliated protozoa is physiological or biochemical in scope. For example, the influence of temperature on nutrition and metabolism has received substantial emphasis (see reviews by Hall, 1967; Holz, 1964; and Hutner, 1961; also Reid et al., 1969; and Rosenbaum et al., 1966); the effects of certain salts and chemicals have been examined, and problems associated with physiological adaptation and mating types have attracted considerable attention (Crippa-Franceschi et al., 1973; Crippa-Franceschi & Genermont, 1973; Hairston & Kellerman, 1965; Hipke & Hanson, 1974; Holz et al., 1959; Hutchison, 1915; Kitching, 1948; Phelps, 1949; Poljansky, 1973; Poljansky & Irlina, 1973; Poljansky & Sukhanova, 1967; Propper, 1965; and Vogel, 1966). Other studies have shown that high temperatures can induce morphological alterations. These changes are manifested in heat impairment during stomatogenesis (e.g., Frankel, 1964) and on the overall size and general morphology of cells (James & Read, 1957; Kiesselbach, 1935; Rosenbaum et al., 1966; and Thormar, 1962a).

Generally, ciliates are cosmopolitan and temperature apparently plays a minor role in their ecology. However, several researchers have found that in some instances temperature is indeed significant in controlling the geographical

distribution and occurrence of ciliates. For example, the freshwater ciliate Neobursaridium gigas occurs only in tropical regions and reproduces only at temperatures between 22° and 26°C (Dragesco, 1968). Euplotes antarcticus, recently described from Antarctic sea ice by Lee & Fenchel (1972), survives only in temperatures below 17°C and reproduces only between -2° and 10°C, the optimum being approximately 5°C. Dingfelder (1962) also showed that, among other Protozoa, certain ciliates can tolerate high temperatures of temporary shallow freshwater puddles. For the most part, however, the research cited above was conducted mainly on freshwater species of Paramecium, Tetrahymena or Colpidium; only the studies of Fenchel (1968b), Kiesselbach (1935), Lee & Fenchel (1972) and Vogel (1966) dealt with marine ciliates. Of the investigations concerned with effects of high temperatures resulting from or related to man induced environmental changes, only those of Lee et al. (1971) and Saks et al. (1974) consider, among other salt marsh organisms, the impact of high temperatures on a marine ciliate (Euplotes vannus). Cairns (1969a, b) demonstrated changes in species diversity and in stability of freshwater protozoan communities as a consequence of high thermal shock. Hence, for both basic and practical reasons, a major thrust of the present study was to survey the ranges of sensitivity to high temperatures on selected representatives of marine Ciliophora.

In comparative studies the median tolerance limit (LD_{50}) or median tolerance temperature has been used to define the physiological tolerance of organisms under stressful

experimental situations (Kinne, 1963, 1964, 1967; and Vernberg & Vernberg, 1970). One objective of this study, therefore, was to determine the LD_{50} of several species of ciliates. To achieve this, a simple heating apparatus was designed and constructed so that organisms could be subjected to abrupt increases in temperature while simultaneously allowing observation of the cells (see Materials and Methods). Salinity and temperature behave synergistically to influence the response of organisms to thermal changes (Kinne, 1964; McLeese, 1956; and Vernberg & Vernberg, 1974). Since both of these ecological parameters are important in tidal marshes, another aim was to examine the effects of acclimation temperature and salinity on ciliate responses to high thermal stress. Nutrition, a factor that may influence resistance to heat stress, also was investigated.

Since so many variables are involved, optimum conditions are difficult to define even in the laboratory; indeed, in the field optimum conditions may never be realized. Nevertheless, the laboratory situation enables us to control variables and thus treat experimental organisms in such a manner whereby a standard optimum state may be achieved. Optima are especially useful as points of reference; i.e., they not only enable one to make comparisons between species, but also allow better comprehension of a particular organism's tolerance limits. Therefore, another goal was to determine the temperature of optimum growth for each ciliate.

According to Clark & Brownell (1973) Atomic Energy Commission calculations show that if all power plants under

construction or in operation in the Hudson estuary were cooled with once-through systems, the temperature of a 35-mile stretch of the estuary would be raised by 5-6°C. Such increases above ambient are even more crucial during summer when estuarine organisms may already be living near their upper thermal limits. Therefore, while exposure to abrupt increases in temperature may certainly be lethal, prolonged exposure to sublethal temperatures may be even more significant. Thus, experiments were conducted to evaluate the upper thermal limits for significant growth and survival.

Under natural conditions, increasing temperatures may modify competitive interaction. This may be displayed in the exclusion of the more sensitive species, or conversely in the enhancement of particular species at the expense of others as they are favored by elevated temperatures. Freshwater algae, for instance, have been shown to exhibit this phenomenon as have other components of marine aufwuchs communities (Cairns, 1956; Saks et al., 1974). To test the extent or occurrence of this principle, I chose Strombidium sulcatum, a planktonic form, and Euplotes crassus, a benthic ciliate for competitive interaction experiments at different temperatures. Both are ubiquitous marine ciliates with similar growth rates at extreme temperatures (see Results). Sublethal increases in temperature may favor one ciliate at the expense of another, even if environmental temperatures do not reach lethal levels. This may be significant in terms of ecosystem succession and fouling phenomena. Competitive interaction is usually more severe among

closely related species and when a common resource such as food is exploited by similar means. For these reasons I have studied the interaction between E. crassus and E. harpa at extreme temperatures; both are benthic and feed by grazing along the substrate.

In summary, this research documents the ranges of sensitivity to high temperatures on selected representatives of marine ciliates. Temperature optima, upper thermal limits of significant growth and survival, and temperatures of LD_{50} were determined for twelve species. The effects of nutrition and the influence of temperature and salinity on ciliate responses to acute heat stress were also tested. Finally, the influence of increasing temperature and food concentration on competitive interaction was examined.

MATERIALS AND METHODS

Twelve species of marine ciliates were isolated from mass and raw cultures of collections made in the spring of 1973 and 1974, and the summer and fall of 1974. They were collected from New Hampshire tidal marshes at Odiorne Point, Rye (43° 2' 22" N; 70° 42' 57" W) and Adams Point, Durham (43° 5' 22" N; 70° 52' 15" W). One species, Euplotes alatus, was collected at mid tide level of a sandy habitat at Rye Beach, New Hampshire (43° 00' 40" N; 70° 44' 15" W). Several taxonomic and morphologic groups are represented as well as different modes of feeding and locomotion; i.e., benthic and planktonic, and include the largest and smallest forms (Table 1). To show the basic morphological characters of the ciliates and to demonstrate their relative proportions, illustrations drawn to scale are presented in Figures 1-23. Identifications were made with living ciliates and with ciliates prepared for cortical staining according to a modification of the NMF (Nigrosin-$HgCl_2$-Formalin) method (Borror, personal communication), the Chatton-Lwoff silver impregnation technique as modified by Corliss (1953), Feulgen nucleal reaction, and iron hematoxylin. The smaller species were extracted from salt marsh sediments, algae, or detritus by filtration through a series of filters fitted with nylon gauze of mesh sizes ranging from 1050μm to 64μm and from marine sand by the seawater-ice method (Uhlig, (1964).

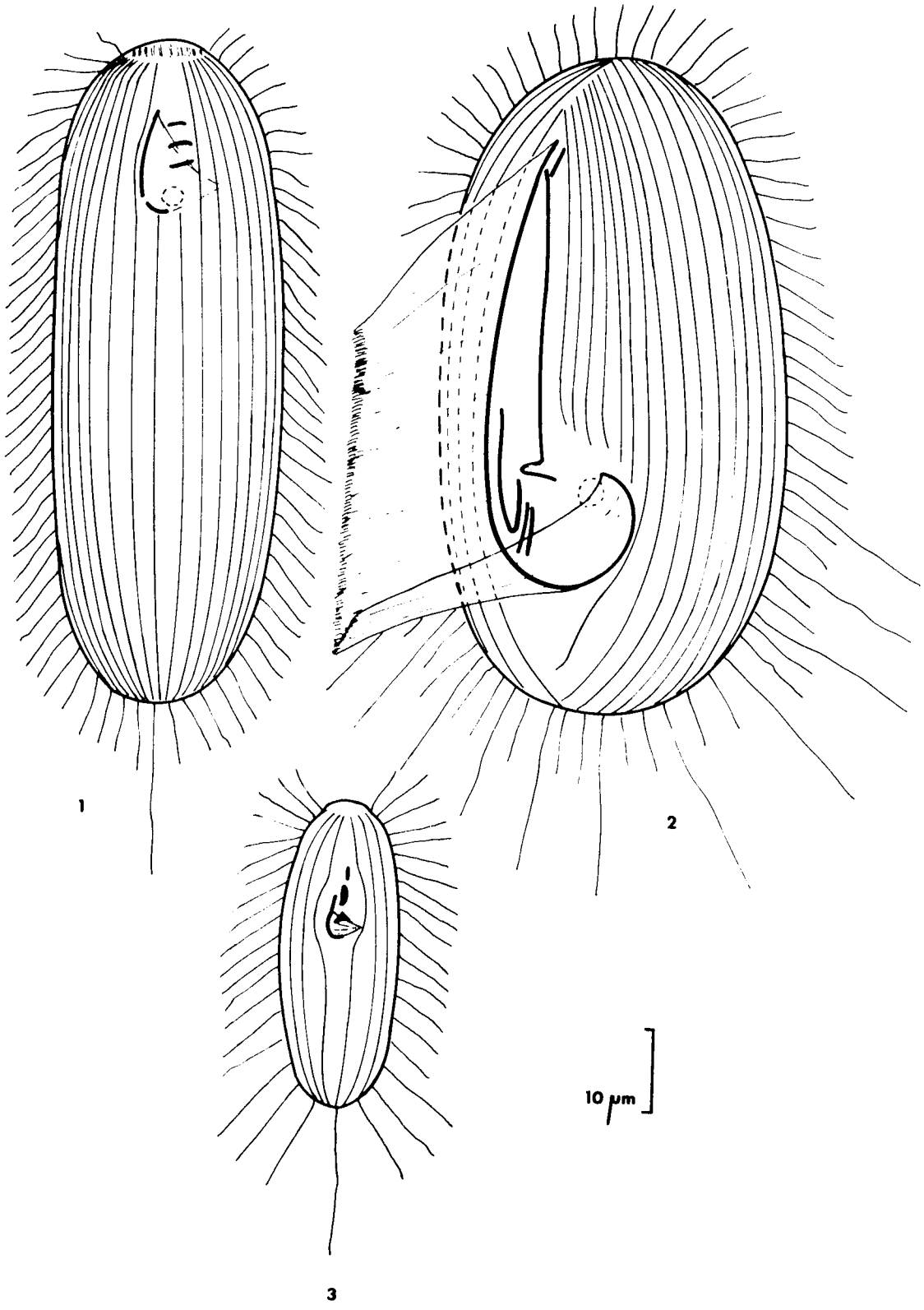
Table 1. TAXONOMIC POSITION AND CELL DIMENSIONS OF TEST CILIATES

Taxa	Species	Mean Length (μm)	Mean Width [a]
Phylum Ciliophora			
Class Oligohymenophora			
Subclass Hymenostomata			
Order Scuticociliatida			
Family Loxocephalidae	*Dexiotricha* sp.	78.4	25.1
Family Pleuronematidae	*Pleuronema coronatum*	78.9	43.8
Family Uronematidae	*Uronema marinum*	30.8	13.4
Class Polyhymenophora			
Subclass Spirotricha			
Order Heterotrichida			
Family Condylostomatidae	*Condylostoma arenarium*	353.3	96.1
Order Oligotrichida			
Family Strombidiidae	*Strombidium sulcatum*	40.6	30.2
Order Hypotrichida			
Family Oxytrichidae	*Tachysoma saltans*	48.0	25.3
	Trachelostyla pediculiformis	143.9	26.8
Family Euplotidae	*Euplotes alatus*	41.0	26.2
	E. crassus	91.4	57.8
	E. harpa	145.8	86.9
	E. raikovi	45.2	30.2
	E. trisulcatus	55.2	36.2

[a]Cell dimensions of 10 individuals at 20°C and 25 o/oo S

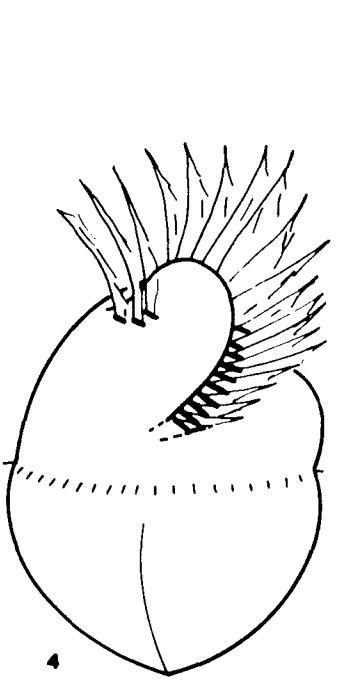
Figures 1-3.

1. Dexiotricha sp.
2. Pleuronema coronatum Kent, 1881
3. Uronema marinum Dujardin, 1841


1
2
3
10 µm

Figures 4-6.

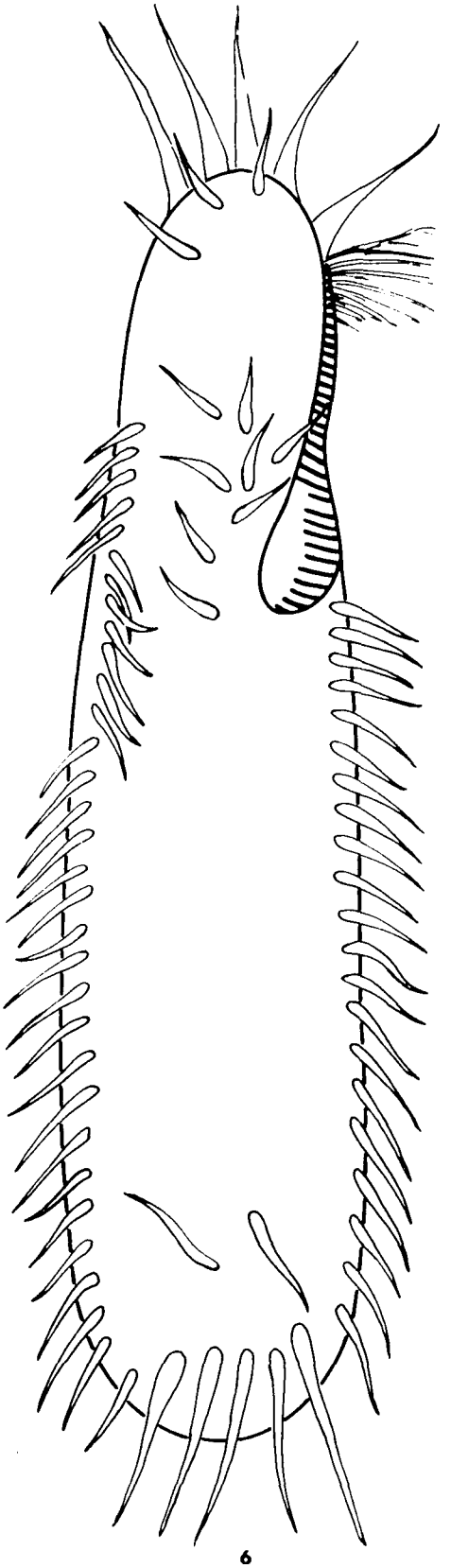
4. Strombidium sulcatum Claparède & Lachmann, 1859
5. Tachysoma saltans (Cohn, 1866)
6. Trachelostyla pediduliformis (Cohn, 1866)



4

10 µm

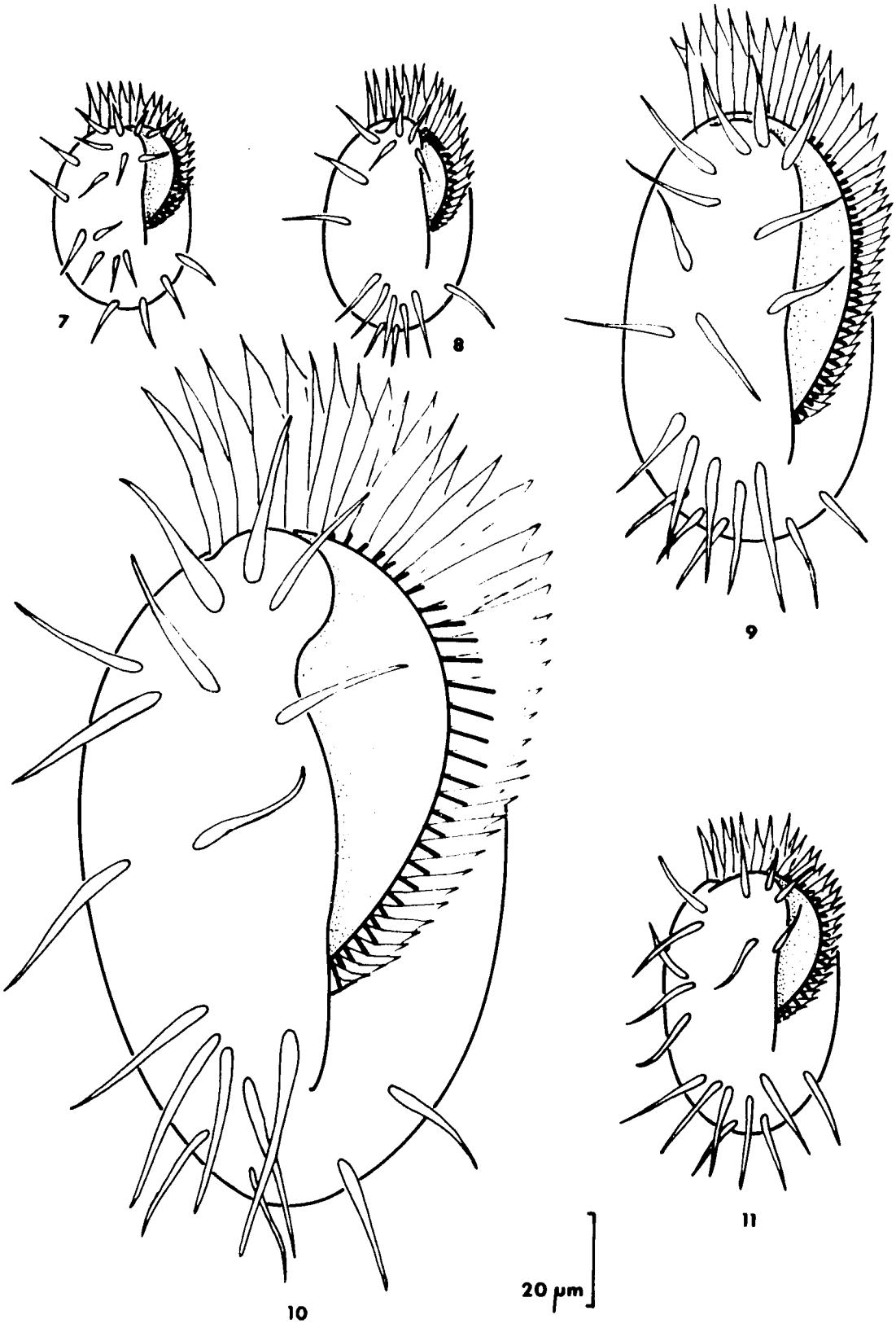
5



6

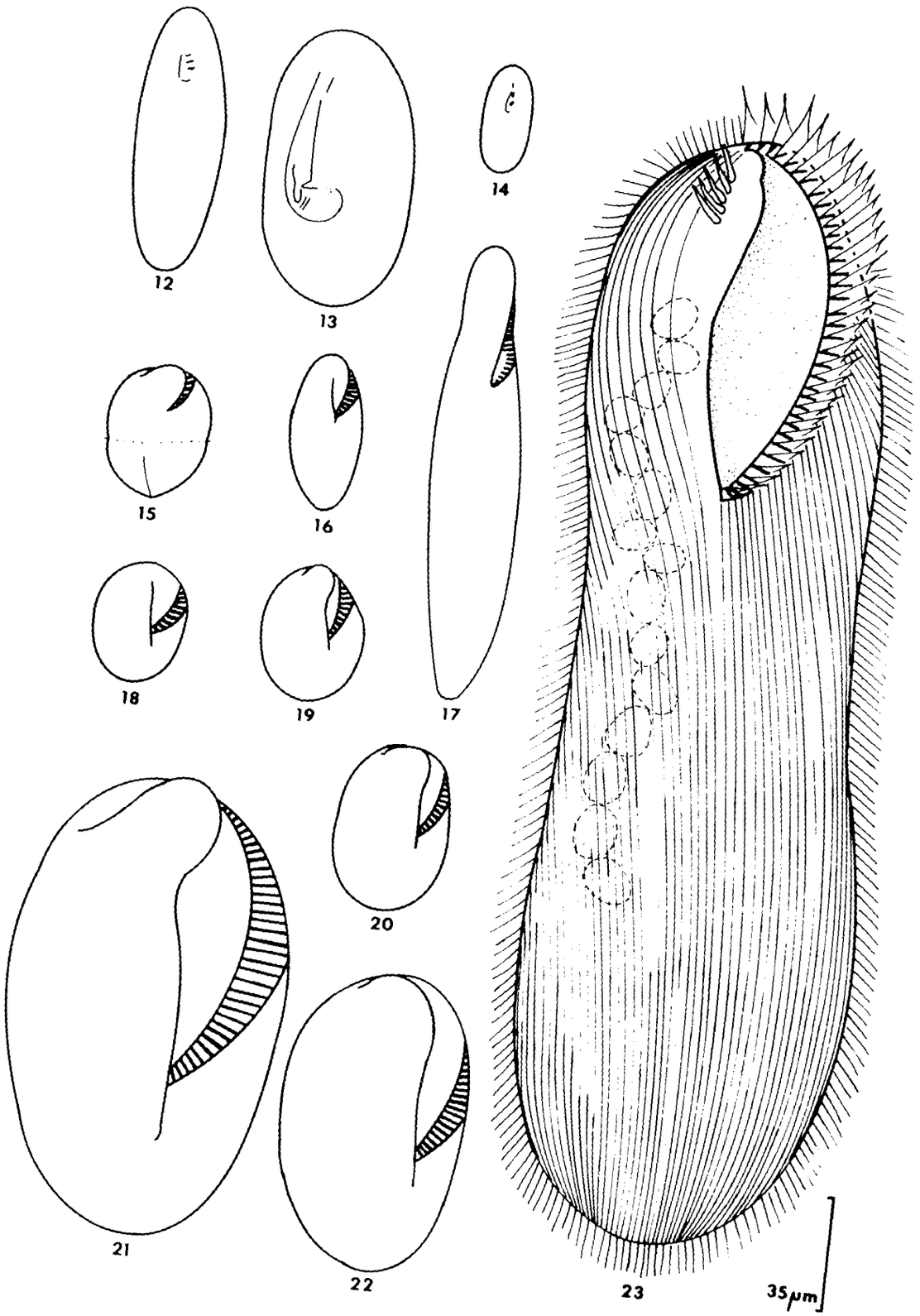
Figures 7-11.

7. Euplotes alatus Kahl, 1932
8. E. raikovi Agamaliev, 1966
9. E. crassus (Dujardin, 1841)
10. E. harpa Stein, 1859
11. E. trisulcatus Kahl, 1932


7
8
9
10
11
20 µm

Figures 12-23.

12. Dexiotricha sp.
13. Pleuronema coronatum
14. Uronema marinum
15. Strombidium sulcatum
16. Tachysoma saltans
17. Trachelostyla pediculiformis
18. Euplotes alatus
19. E. raikovi
20. E. trisulcatus
21. E. harpa
22. E. crassus
23. Condylostoma arenarium Spiegel, 1926


12
13
14
15
16
17
18
19
20
21
22
23
35µm

Clone cultures of isolated ciliates were maintained at 20°C in 100 ml seawater (25 o/oo S) contained in 250 ml capacity Erlenmeyer flasks. As a nutrient, either rice grains or a 4:1 mixture of proteose peptone and yeast extract (PPY) was added to seawater. Depending upon experimental requirements, concentrations of PPY ranged from 0.001-0.015%. Salinity and temperature were varied according to experimental design, and pH typically was 7.8-7.9.

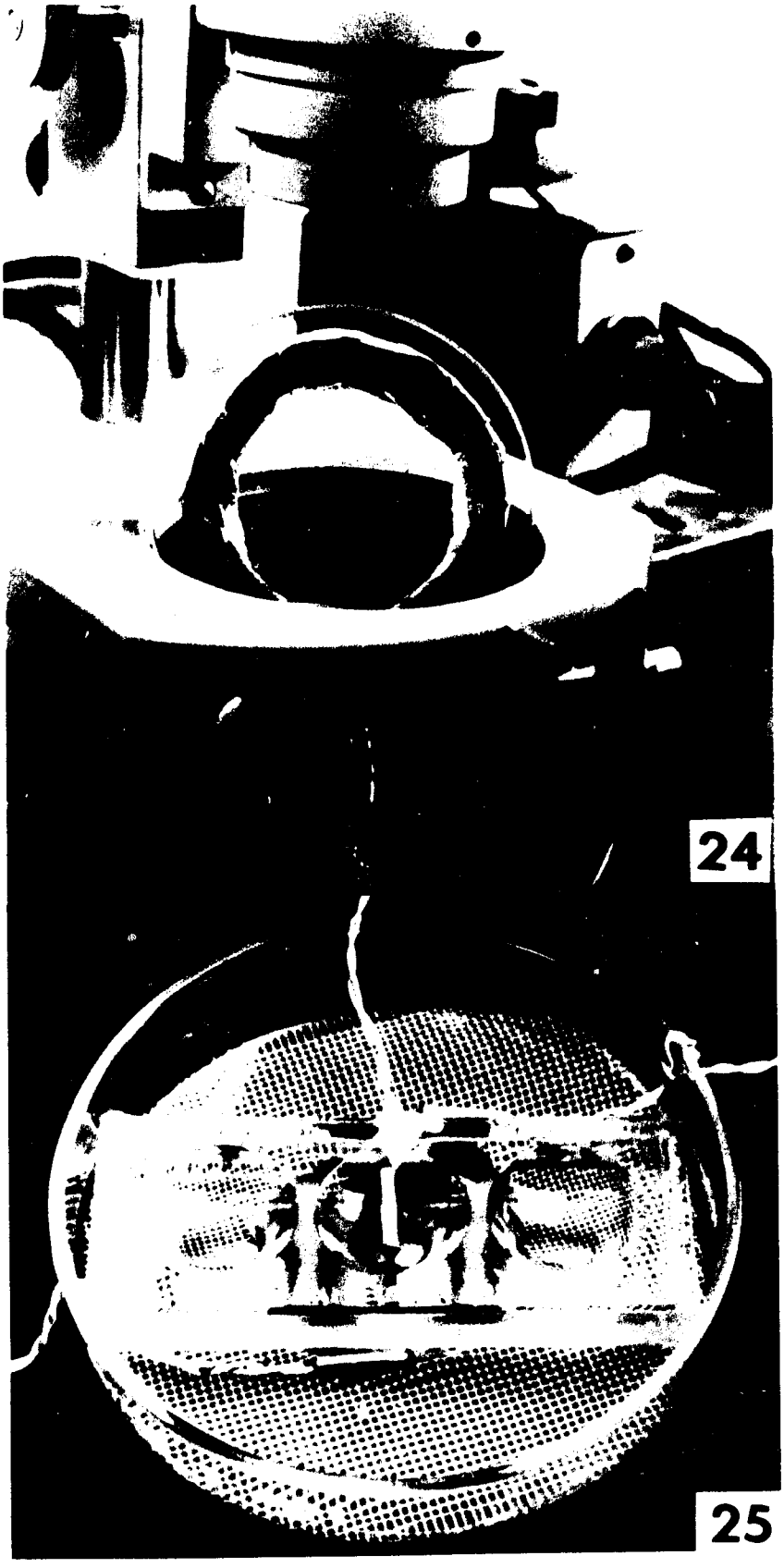
HEATING APPARATUS. A resistance heater epoxyed to the bottom of a glass stage of a dissecting stereomicroscope allowed administration of acute heat stress (Fig. 24). The flow of current through the heating element was regulated with a transformer and a Variac rheostat.

The test chamber was the center well of 3-hole spot plates made of clear glass, and of 1 ml capacity (Fig. 25). Two thermistor probes, one on either side of the test chamber, monitored the temperature of the water bath. To determine heating rates of the test solutions, I fitted another thermistor probe into one of the test chambers by filing a groove on the surface of the spot plate thus accommodating the wire leads of the thermistor probe (2 x 13 mm). The wire leads were then epoxyed into the groove, flush with the surface of the chamber which was then closed with a cover slip during heating rate determinations. All thermistor probes were connected through a switch box to a temperature indicator (Atkins Technical, Inc., Model 3HOI-C10).

Figure 26 illustrates the heating characteristics of

Figure 24. Heating apparatus attached to stereomicroscope.

Figure 25. Test chamber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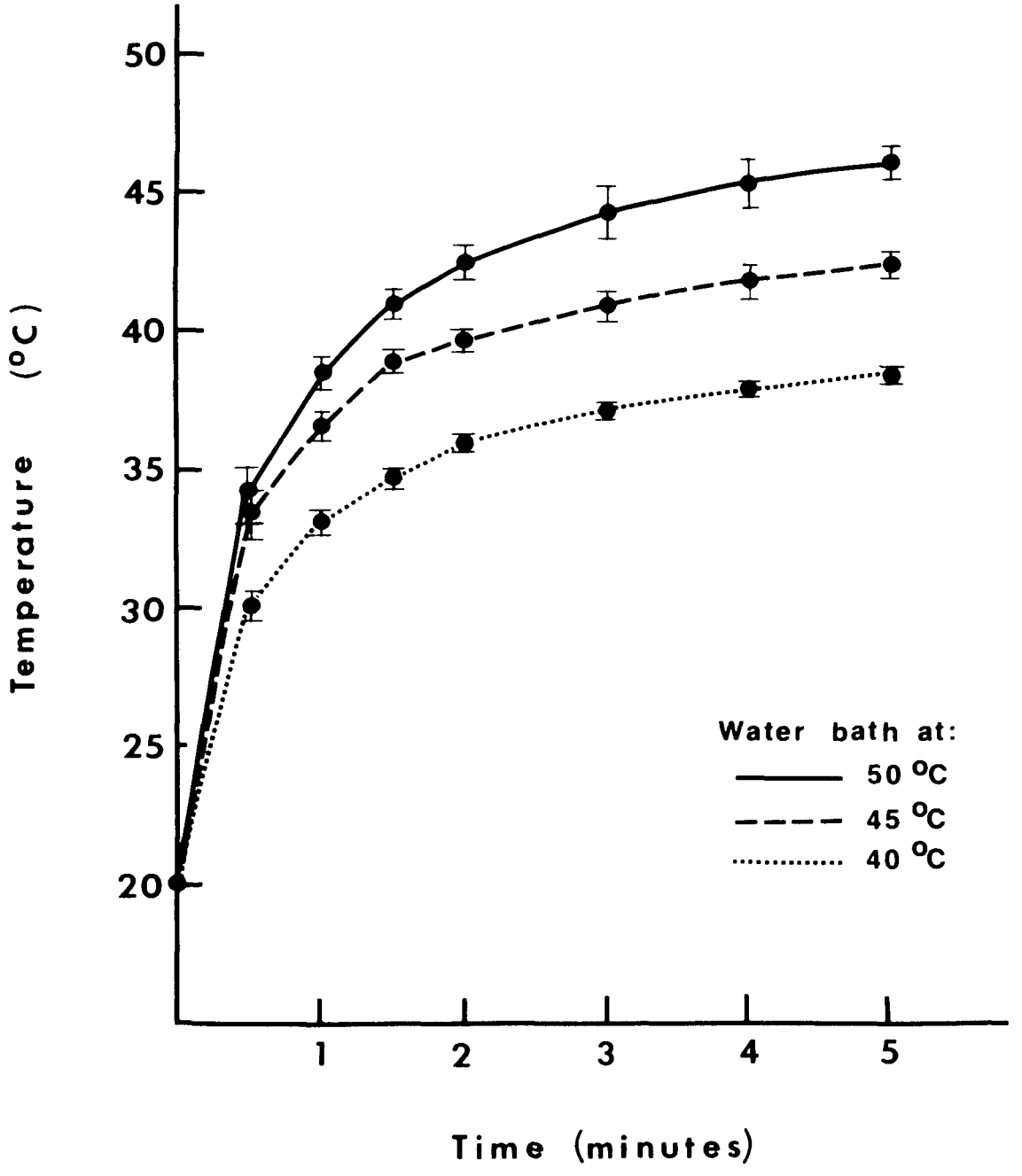
25

Figure 26. Heating rates of test solution when water bath is at 40°, 45° and 50°C. Each point represents the mean of 10 replicates. Vertical lines about the means are standard deviations.


50
45
40
35
30
25
20
Temperature (°C)
1
2
3
4
5
Time (minutes)
Water bath at:
50 °C
45 °C
40 °C

the test chambers when the water bath temperature was held constant at 40°, 45° and 50°C. These water bath temperatures permitted a selection of test temperatures that could be administered rapidly with minimum time variation. Variability between replicates was sufficiently small such that at any instant the temperature of the test solution could be predicted accurately so long as the temperature of the water bath was maintained constant. With establishment of the heating characteristics of the test chamber, many spot plates of similar design and dimensions could be used successively during actual determinations of lethal thermal limits.

MEDIAN TOLERANCE TEMPERATURES. Since one objective was to determine the median tolerance temperature (LD_{50}) of each species, ciliates were subjected to acute heat stress with the apparatus described above. Heating rates were selected so that all LD_{50}'s were attained within approximately 60 seconds. This minimized variability of the time factor between species while subjecting them to an abrupt increase in temperature as rapidly as possible. In a typical experiment, a known number of ciliates (average: 30-40) contained in 1 ml of fresh culture fluid (seawater of salinity comparable tc that of original culture) was introduced into a preheated test chamber and sealed with a cover slip. After heating to the desired temperature for a predetermined period (e.g., 45, 60 or 75 seconds), the test chamber was removed from the hot water bath and immediately transferred (within 3 seconds) to a cold one (5-8°C) in a refrigerator. This

effectively stopped further heating of the organisms. For example, when heated to 34.5° and to 39.9°C, the temperature of the test solution begins to drop after 10 seconds in a cold water bath of 8°C. By the end of three minutes the temperature drops to 25-26°C and to 26.6°C, respectively. Test cells were harvested from cultures in log growth, a condition determined from previous observations on growth rates, vigor and general condition of the cells and of the culture as a whole. At least three, but usually five to six replicates were conducted at each temperature. After heat treatment and subsequent cooling, the ciliates were maintained at room temperature (20-23°C) for 30-60 minutes prior to determination of mortality rates to allow for recovery from temperature shock. With the exception of actual heating, controls received similar treatment. Death was defined as the point at which the ciliates ceased motility. Additional comments and observations on mortality will be discussed later.

GROWTH RATES AND SURVIVAL LIMITS. To determine upper thermal limits of significant growth (generation time within 72 hours) and temperature of optimum growth (maximum growth rate) for each species, cultures were initiated with 2-10 individuals in 1 ml of 25 o/oo S seawater at temperatures between 20° and 38°C (2-5 replicates at each temperature). Growth rates were estimated after 3-10 days by total cell counts; these provide reasonable estimates of the mean generation time (Fenchel, 1968b). The ciliates were counted under a

dissecting microscope by removing them with a pipette from an evaporating dish. Rates of growth for Uronema marinum were obtained with 0.05 ml cultures initiated with 1-2 cells, and were checked with greater frequency. Upper thermal limits of survival were obtained when cultures either exhibited just slight growth or when at least 75% of the original number of cells remained after exposure to experimental temperatures for up to 72 hours. To examine the influence of salinity-temperature interaction on reproductive rates, Dexiotricha sp. was grown at 15° and 30°C, Euplotes crassus and Strombidium sulcatum at 10°, 20° and 30°C; and all three at salinities of 15, 25 and 35 o/oo. These three species represent not only different taxa, planktonic and benthic forms, but also exhibit wide ranges of thermal tolerance thus making them appropriate candidates for this experiment.

NUTRITION EFFECTS. To determine the effects of nutrition on the heat sensitivities of ciliates, I cultivated Euplotes crassus on monobacterial cultures of Arthrobacter marinus and Pseudomonas cuprodurans, and on bacteria present in unsterilized seawater. Pure strains of A. marinus and P. cuprodurans were maintained on nutrient agar slants (medium 2216E, ZoBell, 1941) at 4°C. Millipore filtered (0.22μm) seawater was enriched with sterile PPY (0.01%) and aliquots, each of 100 ml contained in 250 ml capacity Erlenmeyer flasks, were inoculated with either A. marinus or P. cuprodurans and incubated at 20°C for 24 hours. The inocula were transferred directly from the agar slants with an inoculating loop. The purity of these monobacterial cultures

was confirmed by inoculating 0.01 ml samples on nutrient agar plates. Unfiltered seawater enriched with PPY was also incubated at 20°C for 24 hours. Although no attempt was made at identification of the bacteria present in the unfiltered seawater, this assortment provided a suitable substrate for culturing E. crassus.

Before introducing E. crassus into the bacterized seawater, the ciliates were serially washed in two baths of sterile seawater for 15-20 minutes each, then transferred through three baths of an antibiotic mix (Table 2, modified from Lee et al., 1970) for 15, 30 and 60 minute durations. A 15 minute wash in sterile seawater concluded the series. Except for the unfiltered seawater cultures, all techniques were aseptic.

Cells were harvested during log growth and tested for resistance to heat by introducing them with a small volume (~ 0.5 ml) of culture fluid into 37-38°C seawater. After 0.5, 1, 2, 3, 4, 5 and 6 minutes the dish containing the ciliates was removed from the heat source (Precision oven) to a cold water bath, and then to room temperature before determination of survival rates. At least four replicates were conducted for each period of heat treatment.

COMPETITIVE INTERACTION. To ascertain whether or not the outcome of competitive interaction is affected by temperature, I cultured Euplotes crassus and Strombidium sulcatum first as isolates then in mixed cultures at 15°, 28° and 30°C. The similarity in their temperature growth

Table 2. ANTIBIOTIC MIXTURE

Constituents[a]	µg/100 ml
Albamycin	200
Chloromycetin	250
Streptomycin sulfate	2000
Fungizone	50

[a]Albamycin (Sodium novobiocin), Mix-O-Vial, The Upjohn Co.; Chloromycetin (Chloramphenicol), Parke, Davis & Co.; Streptomycin sulfate, Eli Lilly & Co.; Fungizone (Amphotericin B), E. R. Squibb & Sons, Inc.

requirements makes them suitable candidates for this experiment. All cultures were inoculated with 10 individuals of each species and a ratio of 1:1 was retained in the inocula of the paired species, thus eliminating any initial numerical advantage. Since competitive interaction may be enhanced not only by temperature changes but also by availability of food, two levels of food concentration were offered at the 15° and 28°C experiments. Seawater (25 o/oo S) enriched with 0.001% PPY constituted a low food supply and seawater with 0.015% PPY was arbitrarily established as the higher food level. The experiment was repeated with two closely related species, Euplotes crassus and E. Harpa, at 15° and 28°C.

Cultures were 1 ml in volume and contained in sealable vials (Wheaton snap cap bottles, 4 ml capacity; Carolina Biological Supply Co.). The mean number of ciliates of triplicate cultures was determined by direct cell counts following the technique described above (see pp. 24-25). Statistical methods are according to Sokal & Rohlf (1969) and Young & Veldman (1972).

RESULTS

HEATING APPARATUS. Table 3 provides a comparison of the response of ciliates to acute heat stress administered with the heating apparatus. In all but three cases, namely Dexiotricha sp., Strombidium sulcatum and Euplotes harpa cultured at 20°C and 25 o/oo S, variability in mortality rates decreased with increasing test temperatures. The coefficient of variation (CV) for Condylostoma arenarium, for example, diminished from 93% to 0% as these ciliates were subjected to temperatures ranging from 37° to 40.9°C. Greater uniformity in response was therefore obtained as shock temperatures were increased. Although care was taken to minimize variability in the manner in which replicates were conducted both within a particular species and between different species, it is possible that along with the natural variation inherent in the response of biological systems to a stimulus, small thermal gradients in the test chamber may also have contributed to the total variability in mortality rates. However, the heating apparatus provides reasonably good estimates of median tolerance temperatures, especially since these are so close to the LD_{100} limits, often less than 1°C (see Fig. 27).

GROWTH RATES AND SURVIVAL LIMITS. In addition to temperatures of LD_{50}, Table 4 also shows temperatures of optimum growth, and upper thermal limits of significant growth and survival for all the ciliates studied. Temperatures

Table 3. COMPARISON OF VARIABILITY IN RESPONSE TO ACUTE HEAT STRESS

Organism (Culture conditions)	Test temp. (°C)	N	# cells per N	% Mortality Mean ± S.E.	CV[a]
Condylostoma arenarium (20°, 25 o/oo)	37.0	4	26-30	10.7 ± 5.00	93.0
	38.4	8	24-34	38.8 ± 7.25	52.0
	39.9	5	27-32	69.5 ± 5.11	16.7
	40.9	3	15-28	100.0 ± 0.00	0.0
Dexiotricha sp. (20°, 25 o/oo)	36.5	3	34-36	9.2 ± 6.75	60.8
	38.4	4	35-40	36.5 ± 1.18	64.6
	39.9	4	40	60.0 ± 10.02	33.8
	40.9	5	20-40	100.0 ± 0.00	0.0
Pleuronema coronatum (20°, 25 o/oo)	32.0	5	25-33	17.8 ± 3.39	42.7
	34.6	12	26-38	22.0 ± 1.64	25.6
	36.5	3	40	40.0 ± 2.40	10.5
	37.0	4	30-40	100.0 ± 0.00	0.0
Strombidium sulcatum (20°, 25 o/oo)	34.6	5	44-62	33.8 ± 2.72	18.0
	36.5	5	52-60	51.0 ± 5.82	25.5
	37.0	5	45-65	98.5 ± 1.61	3.7
Euplotes crassus (10°, 15 o/oo)	30.1	8	25-33	17.5 ± 5.04	81.1
	32.0	8	25-32	68.3 ± 4.92	20.4
	34.5	6	24-35	83.8 ± 6.78	19.8
	37.0	3	30-31	100.0 ± 0.00	0.0
E. crassus (10°, 25 o/oo)	32.0	8	24-29	8.3 ± 2.20	74.6
	34.5	8	24-30	79.0 ± 8.20	29.2
	37.0	3	29-35	100.0 ± 0.00	0.0

Table 3. Cont'd.

Organism (Culture conditions)	Test temp. (°C)	N	# cells per N	% Mortality Mean ± S.E.	CV
E. crassus	34.5	4	26-36	3.8 ± 2.20	115.0
(10°, 35 o/oo)	36.5	5	23-38	20.0 ± 1.88	21.0
	37.0	4	25-28	100.0 ± 0.00	0.0
E. crassus	32.0	5	18-30	33.9 ± 6.39	42.2
(20°, 15 o/oo)	34.5	8	38-42	42.0 ± 5.00	34.0
	36.5	5	28-38	73.7 ± 4.96	15.0
	37.0	5	40-44	100.0 ± 0.00	0.0
E. crassus	34.5	5	25-40	21.9 ± 1.24	124.0
(20°, 25 o/oo)	36.5	5	23-30	65.8 ± 9.00	27.4
	37.0	4	27-40	100.0 ± 0.00	0.0
E. crassus	34.5	10	34-40	10.0 ± 2.78	88.0
(20°, 35 o/oo)	36.5	7	26-39	40.4 ± 7.66	50.0
	37.0	3	29-30	100.0 ± 0.00	0.0
E. crassus	34.5	3	20-25	12.5 ± 7.76	107.0
(30°, 15 o/oo)	36.5	5	28-40	12.0 ± 3.27	60.8
	37.0	3	25-30	100.0 ± 0.00	0.0
E. crassus	34.5	4	40-50	8.2 ± 3.40	82.8
(30°, 25 o/oo)	35.5	6	35-56	26.3 ± 3.90	36.4
	37.0	3	38-57	100.0 ± 0.00	0.0
E. crassus	34.5	3	38-40	7.7 ± 3.64	90.0
(30°, 35 o/oo)	36.5	6	31-41	7.2 ± 2.08	70.8
	37.0	3	25-45	97.8 ± 2.00	4.1

Table 3. Cont'd.

Organism (Culture conditions)	Test temp. (°C)	N	# cells per N	% Mortality Mean ± S.E.	CV
E. harpa	32.0	5	26-35	3.7 ± 2.48	143.0
(20°, 15 o/oo)	34.5	5	28-37	21.9 ± 6.43	65.3
	37.0	5	28-37	100.0 ± 0.00	0.0
E. harpa	34.5	5	21-30	7.6 ± 1.56	46.0
(20°, 25 o/oo)	36.5	5	29-40	15.0 ± 3.00	49.0
	37.0	6	26-35	97.9 ± 1.30	3.3
E. harpa	34.5	6	26-34	7.4 ± 3.26	108.1
(20°, 35 o/oo)	36.5	6	29-36	10.0 ± 3.10	76.0
	37.0	3	23-29	100.0 ± 0.00	0.0
E. raikovi	34.5	6	32-40	10.6 ± 0.57	14.0
(20°, 25 o/oo)	36.5	4	31-40	28.0 ± 1.00	5.0
	37.0	3	36-57	100.0 ± 0.00	0.0
E. trisulcatus	37.0	5	24-35	17.7 ± 2.72	34.5
(20°, 25 o/oo)	38.4	5	26-42	39.0 ± 3.39	19.5
	40.9	3	32-37	100.0 ± 0.00	0.0
Tachysoma saltans	36.5	5	25-50	44.7 ± 7.65	38.3
(20°, 25 o/oo)	37.0	5	80-250	69.7 ± 10.03	34.1

[a]CV: Coefficient of variation

Figure 27. Ranges of tolerance to acute heat stress.

1. Uronema marinum
2. Pleuronem coronatum
3. Strombidium sulcatum
4. Euplotes crassus
5. E. raikovi
6. E. harpa
7. E. trisulcatus
8. Condylostoma arenarium
9. Dexiotricha sp.

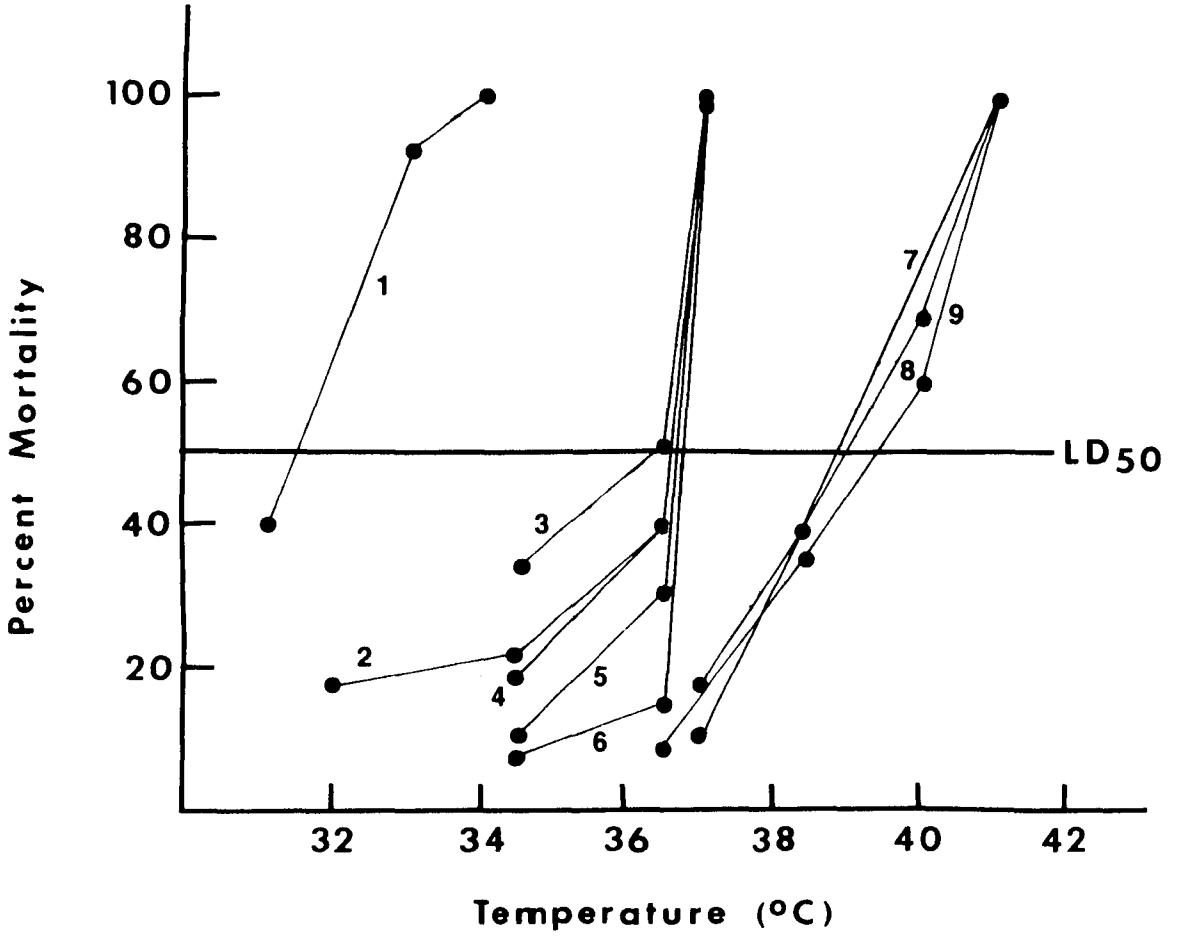

100
80
60
40
20
Percent Mortality
LD50
1
2
3
4
5
6
7
8
9
32
34
36
38
40
42
Temperature (°C)

Table 4. TEMPERATURES OF OPTIMUM GROWTH, UPPER THERMAL LIMITS OF SIGNIFICANT GROWTH AND SURVIVAL, AND TOLERANCE TO ACUTE HEAT STRESS (LD_{50}) FOR 12 SPECIES OF CILIATES

Organism	Temperature (°C)			
	Temp. of optimum growth	Upper lim. of signif. growth	Upper lim. of surv.	Temp. of LD_{50}
Dexiotricha sp.	34.0	37.0	38.0	39.3
Pleuronema coronatum	31.0	32.0	33.0	36.5
Uronema marinum	25.0	27.0	29.0	31.5
Condylostoma arenarium	29.0	35.0	36.5	39.0
Strombidium sulcatum	31.0	33.0	33.0	36.5
Euplotes alatus	27.0	31.0	32.0	35.5
E. crassus	29.0	32.0	33.0	36.6
E. harpa	30.0	31.0	33.0	36.8
E. raikovi	30.0	32.0	33.0	36.7
E. trisulcatus	33.0	35.0	35.0	38.7
Tachysoma saltans	34.0	35.0	35.0	36.8
Trachelostyla pediculiformis	31.0	34.0	35.0	37.0

of optimum growth ranged considerably. For instance, Uronema marinum reproduced most rapidly at 25°C, whereas both Dexiotricha sp. and Tachysoma saltans attained maximum growth rates at 34°C.

The upper thermal limits of significant growth lie within two degrees of the upper thermal limits of survival. Dexiotricha sp., for example, reproduces at 37°C, and although it can tolerate 38°C, it cannot divide at this temperature. Uronema marinum divides at 27°C, but can only manage to survive at 29°C. For several species, the temperatures recorded for upper thermal limits of significant growth and for survival were identical.

NUTRITION EFFECTS. When cultivated on single species of bacteria, Euplotes crassus was more sensitive to high temperatures than when grown on an assortment of bacteria (Fig. 28). Further, resistance to heat apparently is dependent on the particular species of bacteria on which the ciliates are cultivated. With the exception of the 4 minute heat exposure for E. crassus grown on P. cuprodurans vs. E. crassus grown on an assortment of bacteria, the differences between means are statistically significant both at the 95 and 99% confidence limits.

SALINITY EFFECTS. Dexiotricha sp., S. sulcatum and E. crassus reproduced at nearly all temperature-salinity combinations examined (Table 5). Without exception, generation time was inversely proportional to culture temperature, regardless of the salinity. Furthermore, the ranges of variation in

Figure 28. Rate of survival of Euplotes crassus at 37-38°C: Effect of nutrition. Ciliates cultivated at 20°C on Arthrobacter marinus (.....), Pseudomonas cuprodurans (-----) and on an assortment of bacteria (_____). Vertical lines about the means are standard deviations.

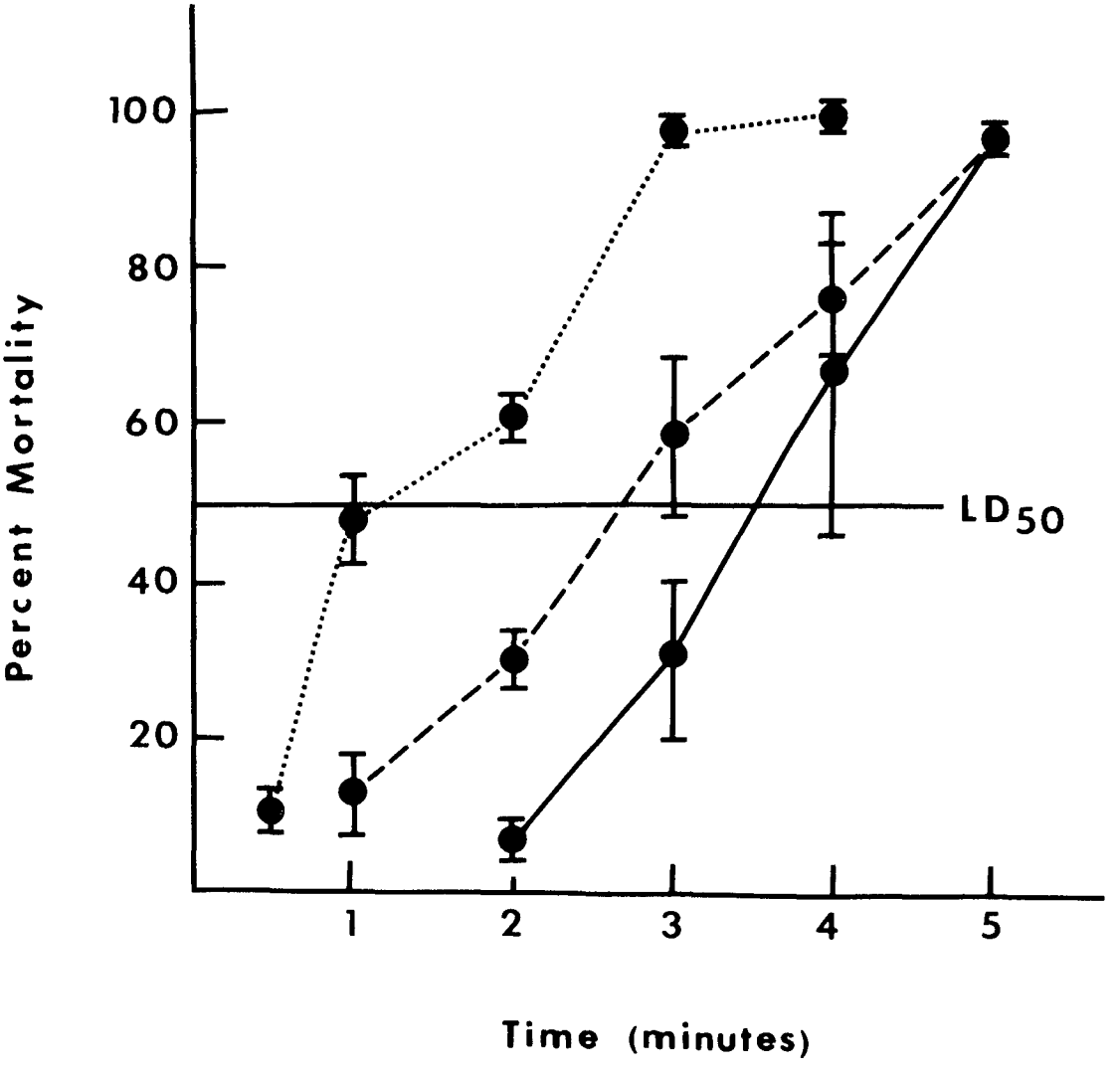

100
80
60
40
20
Percent Mortality
LD50
1
2
3
4
5
Time (minutes)

Table 5. EFFECT OF TEMPERATURE AND SALINITY ON THE GENERATION TIME (IN HOURS) OF THREE SPECIES OF MARINE CILIATES

Organism	Temp. (°C)	Salinity (o/oo) 15	25	35
Dexiotricha sp.	15	65.8 ± 11.6[a]	65.1 ± 1.2	76.5 (15.0)[b]
	30	9.3 ± 0.5	8.6 ± 0.0	8.7 ± 0.9
Strombidium sulcatum	15	60.4 ± 12.1	54.1 ± 16.5	42.6 ± 1.5
	20	28.5 ± 2.3	16.8 ± 3.5	19.5 ± 0.2
	30	12.9 ± 1.0	9.9 ± 0.8	7.9 ± 0.9
Euplotes crassus	10	53.3 (0.0)	59.4 (0.0)	62.4 (5.9)
	20	15.2 (0.5)	15.4 (0.3)	15.4 (0.5)
	30	--------	10.1 ± 0.9	9.5 ± 1.0

[a]Mean ± S.D.

[b]Values in parentheses = Range

generation time among cultures at any particular salinity were non-overlapping (compare values in columns in Table 5). There also appears to be a decrease in variability of generation time with culture temperature at nearly all salinities tested. In marked contrast, however, there was a higher degree of variability in generation time among cultures at any particular temperature; that is, with few exceptions most pairs of values at any particular temperature were within one standard deviation of each other. In addition, there appeared to be no consistent trend in variability as a function of salinity. An obvious exception is the apparent relationship between increased salinity and decreased generation time of S. sulcatum at 30°C.

At 30°C, low salinity inhibited E. crassus reproduction; i.e., fewer than 25% of the attempts at culturing E. crassus at 30°C and 15 o/oo were successful. Strombidium sulcatum appears to be inhibited almost as much by that combination of salinity and temperature (30°C and 15 o/oo); the values in Table 5 were obtained only after several attempts. Dexiotricha sp. showed no indication of reproduction being restricted at 30°C and 15 o/oo; all cultures developed without difficulty and with minimum variability. However, at certain temperatures and salinities, a positive correlation exists between culture salinity and heat shock resistance. For example, when E. crassus is cultured at 10° and 15°C, the differences in mortality rates between organisms grown at salinities of 15, 25 and 35 o/oo are significant ($P = 0.05$) at shock temperature

of 30° and 32°C. At heat shocks of 34.5° and 36.5° only ciliates cultivated at 35 o/oo were significantly more resistant (Figs. 29, 30). The influence of salinity on heat resistance diminished as E. crassus was cultivated at 20° and 30°C (Figs. 31, 32). At heat shocks of 37°C neither salinity nor culture temperature regimes influenced the resistance of E. crassus to heat stress. Figure 33 summarizes the effects of salinity-temperature interaction on the heat resistance of E. crassus.

In Figure 34 the average generation time of seven species of ciliates is plotted as a function of incubation temperatures. Generally, mean generation time decreased as ciliates were cultivated at successively higher temperatures until their individual growth optima were reached. Beyond their temperatures of optimum growth they either stopped reproducing (such as E. harpa where only a few individuals divided at temperatures above 30°C) or divided at much slower rates.

INFLUENCE OF TEMPERATURE ON TWO-SPECIES INTERACTIONS. At the low food level (0.001% PPY), isolated populations of Strombidium sulcatum achieved nearly identical maximum numbers (approximately 400/ml) at rates that were dependent upon culture temperature (see Figs. 35-37); population growth phase occurring in nine days at 15°C, five days at 28°C, and in only four days at 30°C. In mixed cultures with Euplotes crassus, however, S. sulcatum reached different population maxima at different temperatures (approximately

Figure 29. Effect of salinity on the heat resistance of Euplotes crassus cultivated at 10°C (..... 15 o/oo, ----- 25 o/oo, ——— 35 o/oo).

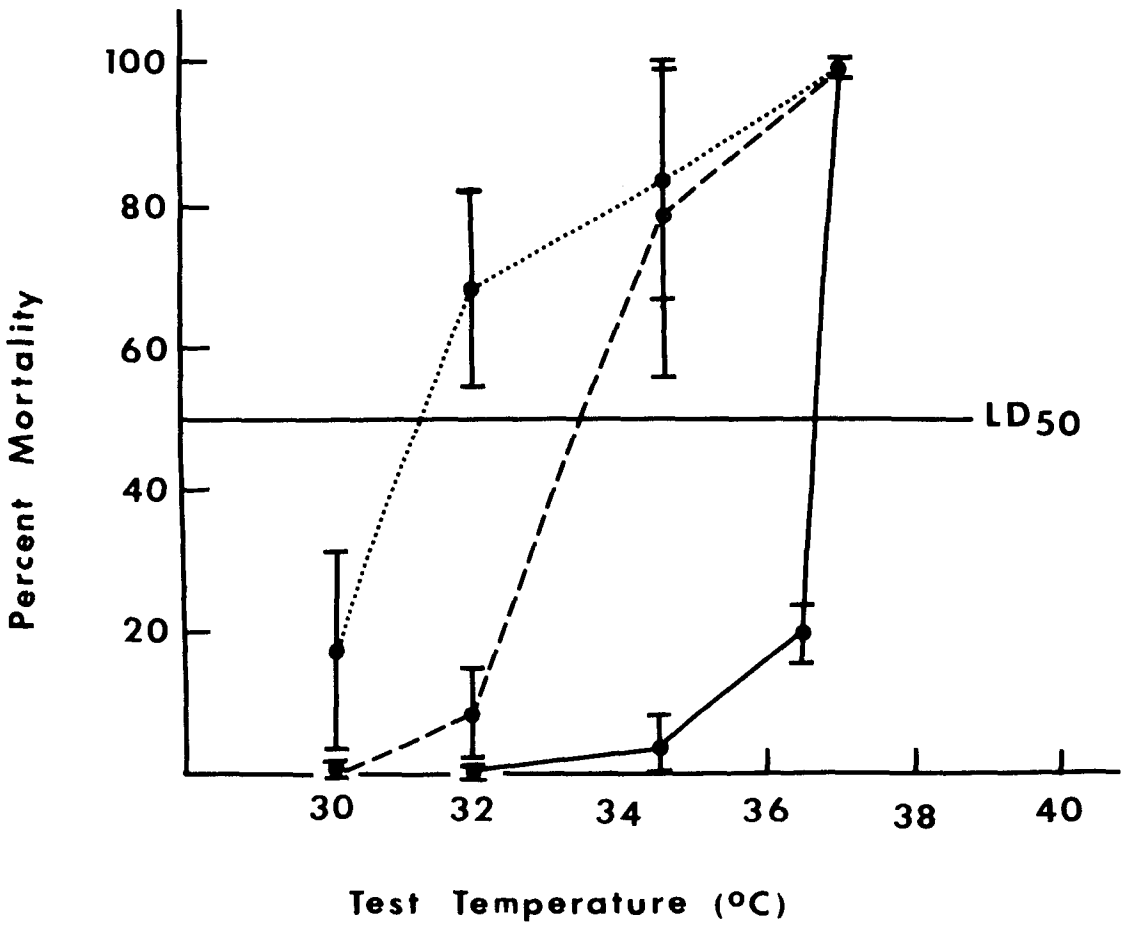

100
80
60
40
20
Percent Mortality
LD50
30
32
34
36
38
40
Test Temperature (°C)

Figure 30. Effect of salinity on the heat resistance of Euplotes crassus cultivated at 15°C (..... 15 o/oo, ----- 25 o/oo, ——— 35 o/oo).

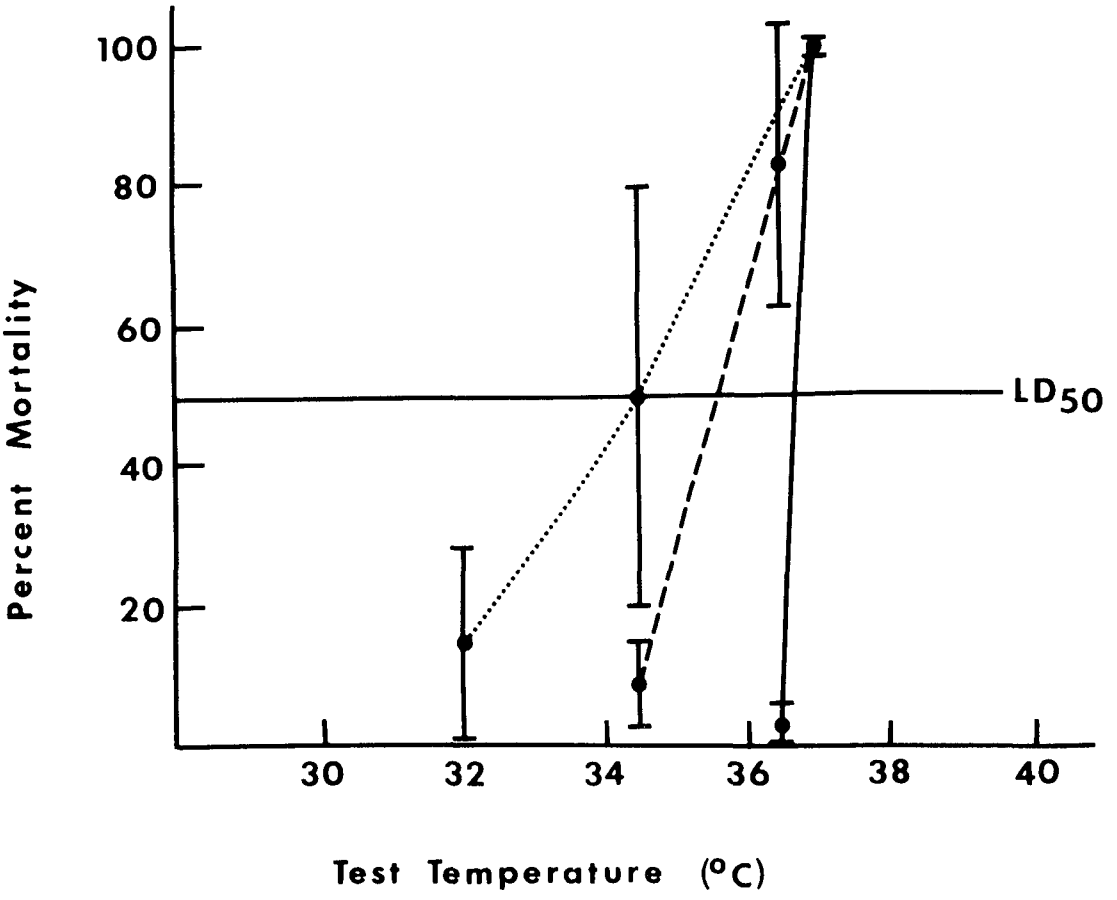

100
80
60
40
20
Percent Mortality
LD50
30
32
34
36
38
40
Test Temperature (°C)

Figure 31. Effect of salinity on the heat resistance of Euplotes crassus cultivated at 20°C (..... 15 o/oo, ----- 25 o/oo, ——— 35 o/oo).

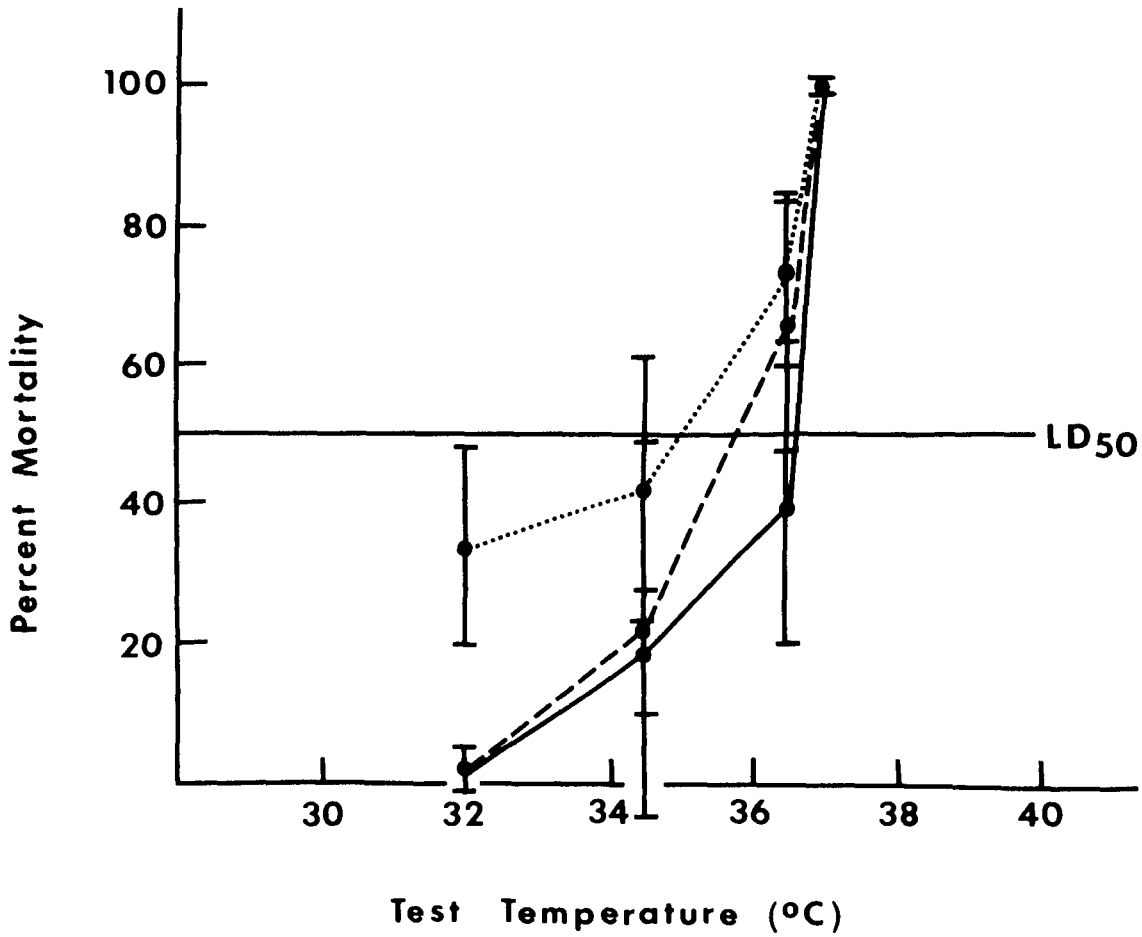

100
80
60
40
20
Percent Mortality
LD50
30
32
34
36
38
40
Test Temperature (°C)

Figure 32. Effect of salinity on the heat resistance of Euplotes crassus cultivated at 30°C (..... 15 o/oo, ----- 25 o/oo, ——— 35 o/oo).

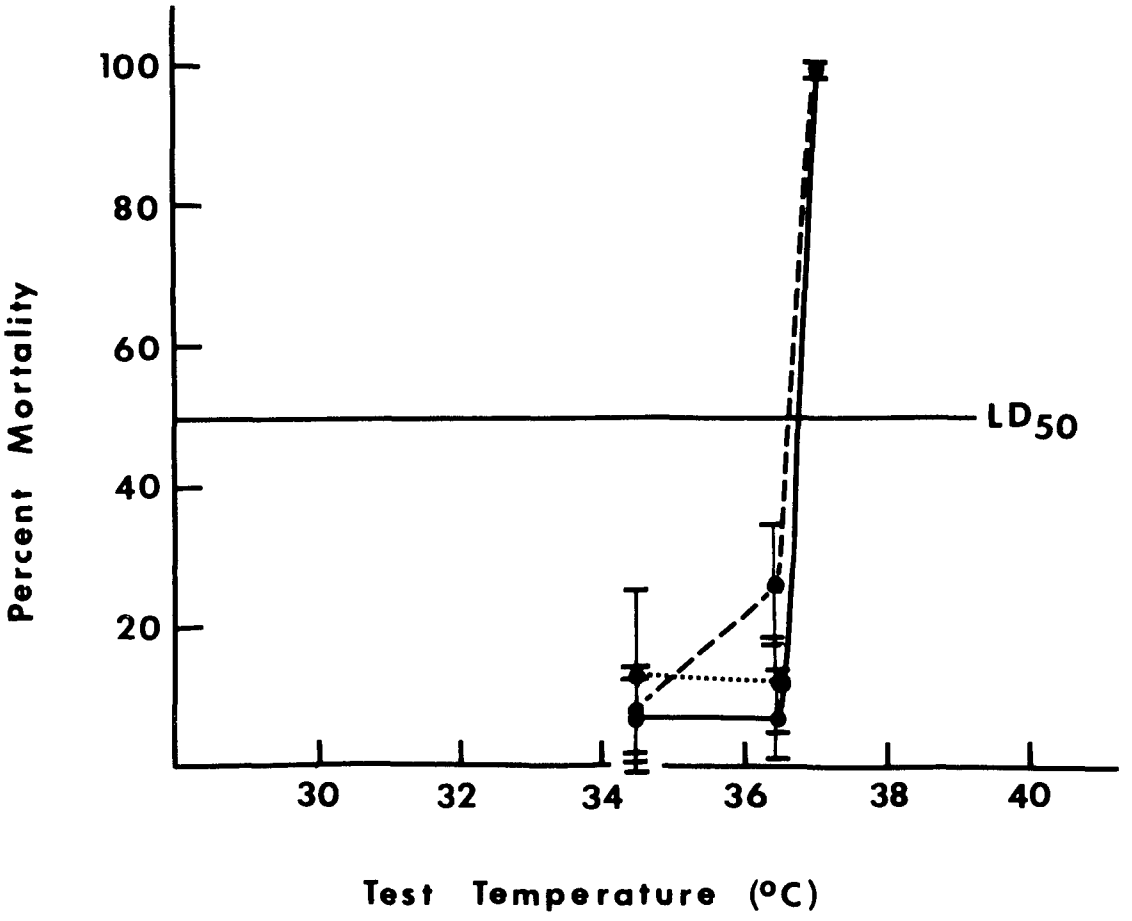

100
80
60
40
20
Percent Mortality
LD50
30
32
34
36
38
40
Test Temperature (°C)

Figure 33. Influence of temperature and salinity on the heat resistance of Euplotes crassus.

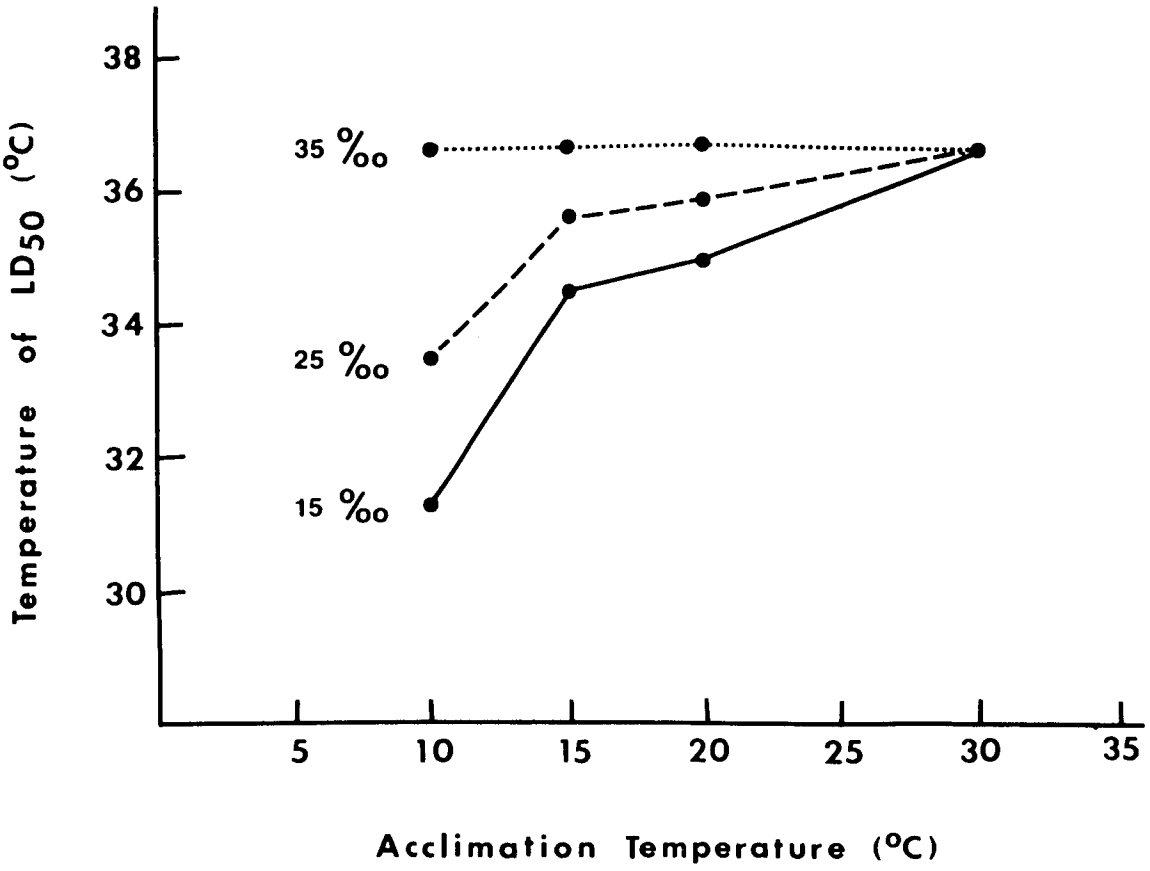

38
36
34
32
30
35 ‰
25 ‰
15 ‰
Temperature of LD_{50} (°C)
5
10
15
20
25
30
35
Acclimation Temperature (°C)

Figure 34. Generation time of seven species of ciliates at different temperatures.

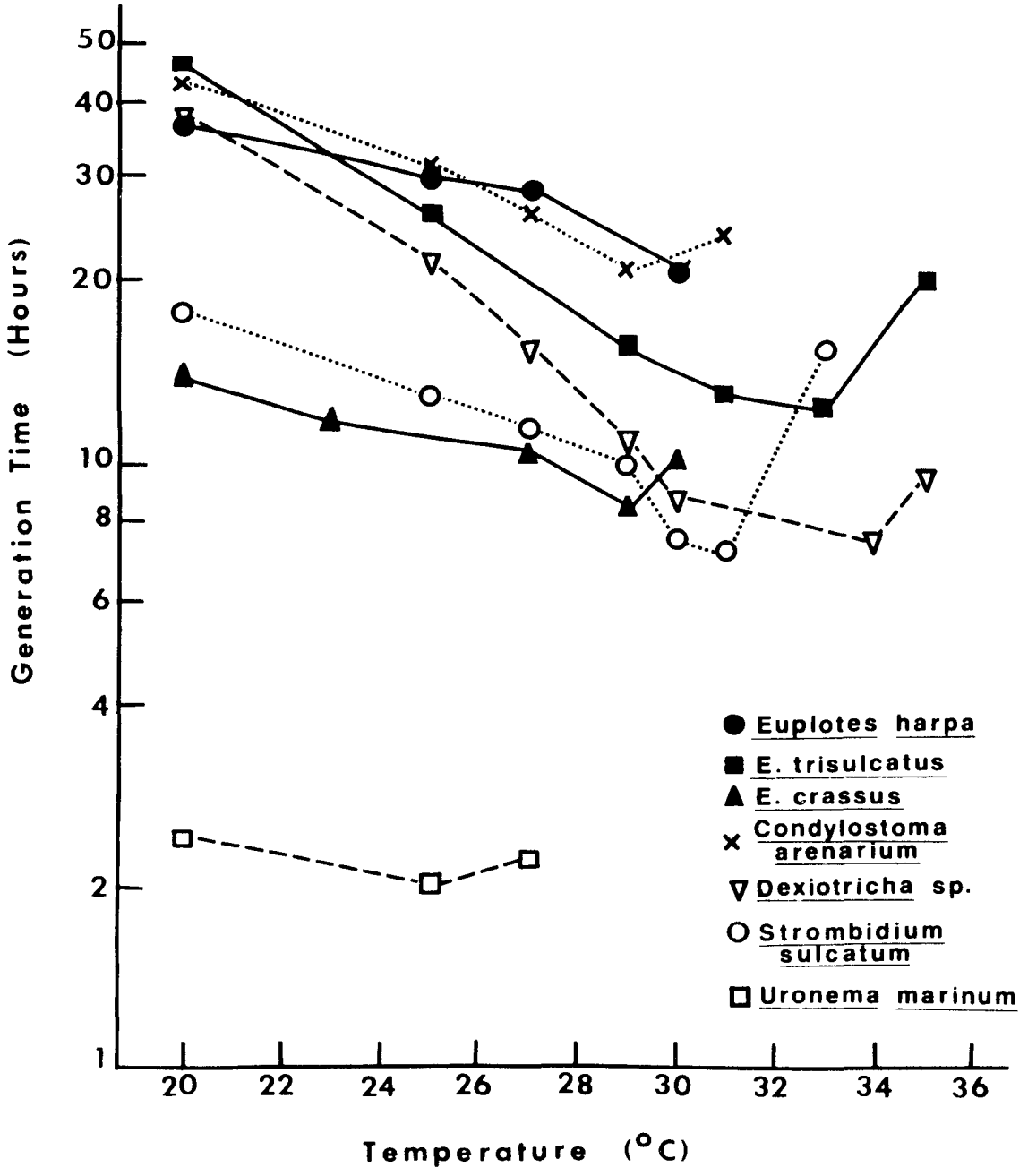

Generation Time (Hours)
50
40
30
20
10
8
6
4
2
1
20
22
24
26
28
30
32
34
36
Temperature (°C)
Euplotes harpa
E. trisulcatus
E. crassus
Condylostoma arenarium
Dexiotricha sp.
Strombidium sulcatum
Uronema marinum

Figure 35. Interaction between Euplotes crassus and Strombidium sulcatum at 15°C and 0.001% proteose peptone and yeast extract.

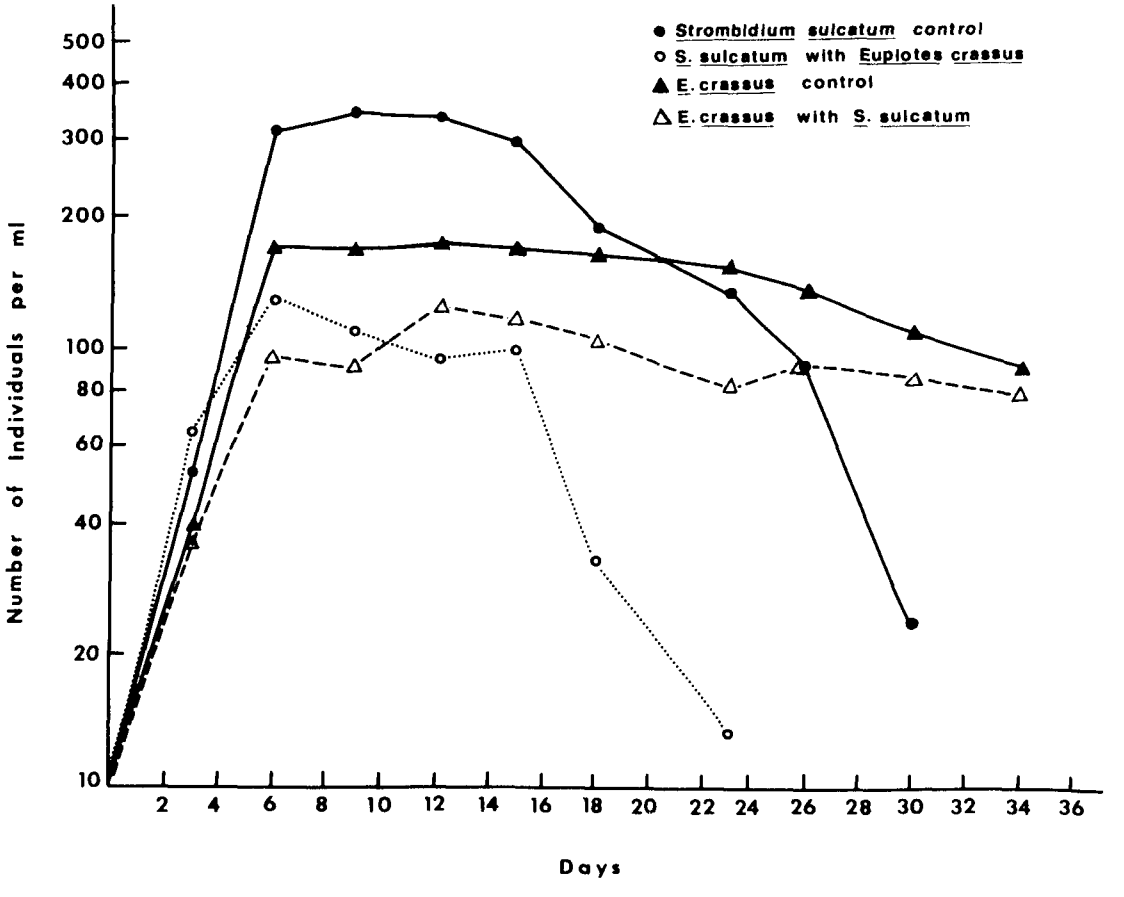

● Strombidium sulcatum control
○ S. sulcatum with Euplotes crassus
▲ E. crassus control
△ E. crassus with S. sulcatum
Number of Individuals per ml
500
400
300
200
100
80
60
40
20
10
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
Days

Figure 36. Interaction between Euplotes crassus and Strombidium sulcatum at 28°C and 0.001% proteose peptone and yeast extract.

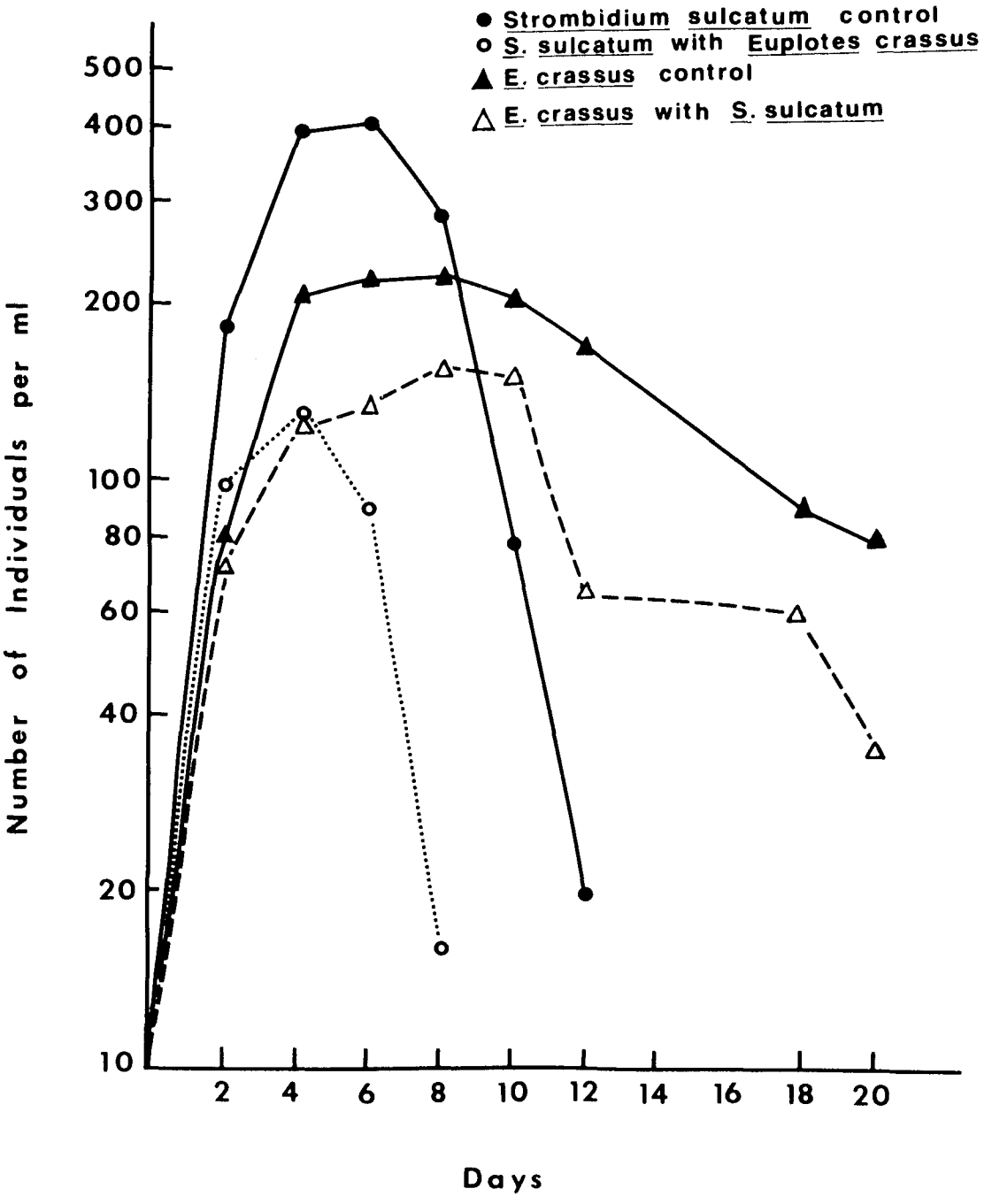
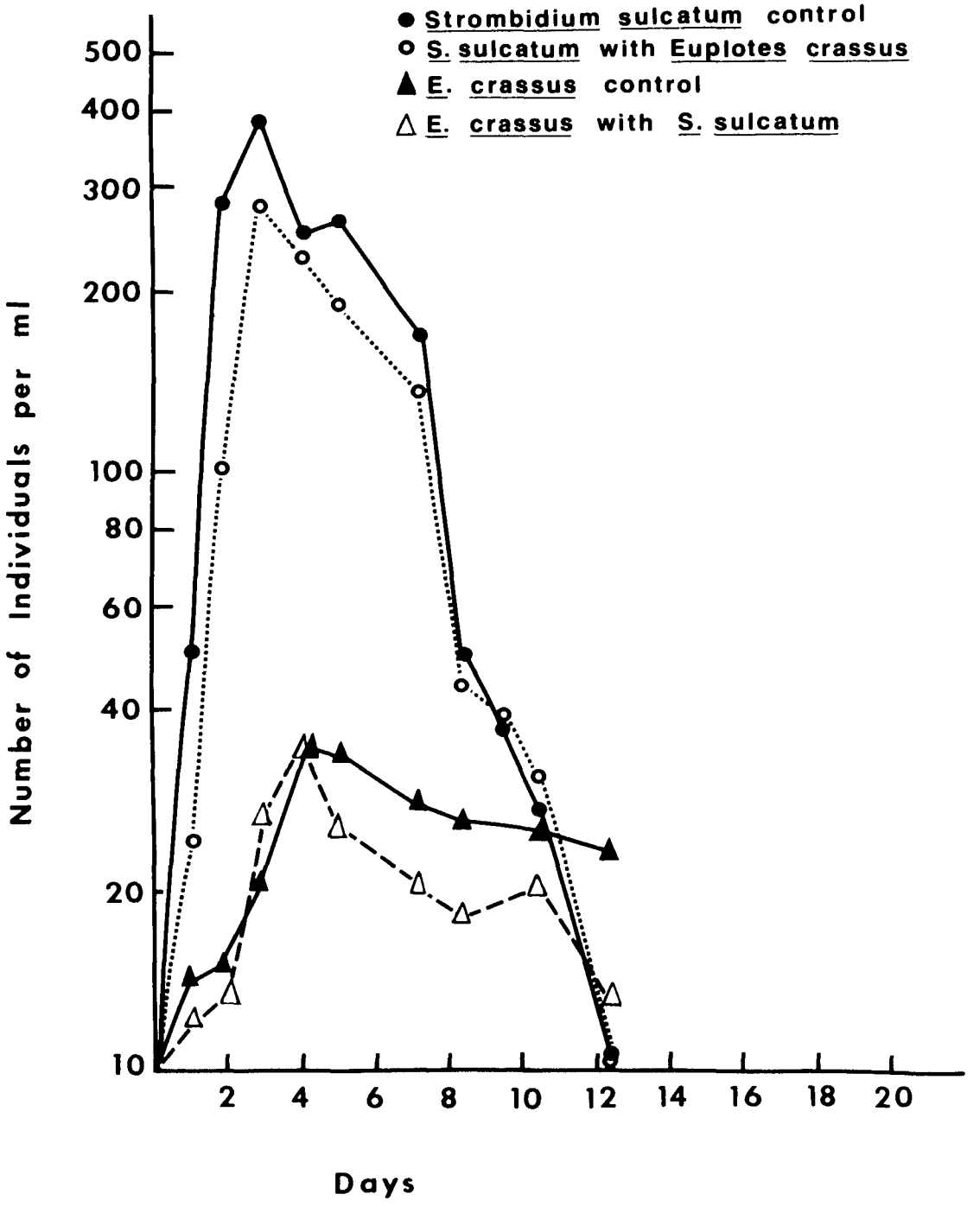

● Strombidium sulcatum control
o S. sulcatum with Euplotes crassus
▲ E. crassus control
△ E. crassus with S. sulcatum
Number of Individuals per ml
500
400
300
200
100
80
60
40
20
10
2
4
6
8
10
12
14
16
18
20
Days

Figure 37. Interaction between Euplotes crassus and Strombidium sulcatum at 30°C and 0.001% proteose peptone and yeast extract.


● Strombidium sulcatum control
o S. sulcatum with Euplotes crassus
▲ E. crassus control
△ E. crassus with S. sulcatum
500
400
300
200
100
80
60
40
20
10
Number of Individuals per ml
2
4
6
8
10
12
14
16
18
20
Days

130/ml when cultivated at 15° or 28°C, but reaching 280/ml at 30°C, nearly as high as when grown in isolation). These data suggest temperature-related differences in the effect of E. crassus upon S. sulcatum at 28° and 30°C.

At low food level, isolated populations of E. crassus achieved nearly identical maximum numbers (approximately 175-200/ml) at culture temperatures of 15° and 28°C, but attained only a population size of 35/ml when grown at 30°C. In mixed cultures with S. sulcatum, population growth curves were similar to those of isolated E. crassus cultures, but generally with slightly lower population numbers. These data suggest a relatively limited effect of S. sulcatum upon E. crassus growth, compared with temperature effects at this food level.

At the higher food level (0.015% PPY), isolated populations of S. sulcatum reached significantly higher population densities faster at 28°C than at 15°C (Figs. 38, 39). In mixed cultures with E. crassus, similar results occurred, but population densities were only about half that of isolated cultures. These data suggest no obvious temperature related difference in the effect of E. crassus upon S. sulcatum at these two test temperatures.

Population densities achieved by culture of E. crassus at this food level were nearly identical, regardless of temperature and the presence or absence of S. sulcatum. These data suggest a relatively limited effect of S. sulcatum upon E. crassus growth.

Since there appeared to be a lesser effect of

Figure 38. Interaction between Euplotes crassus and Strombidium sulcatum at 15°C and 0.015% proteose peptone and yeast extract.

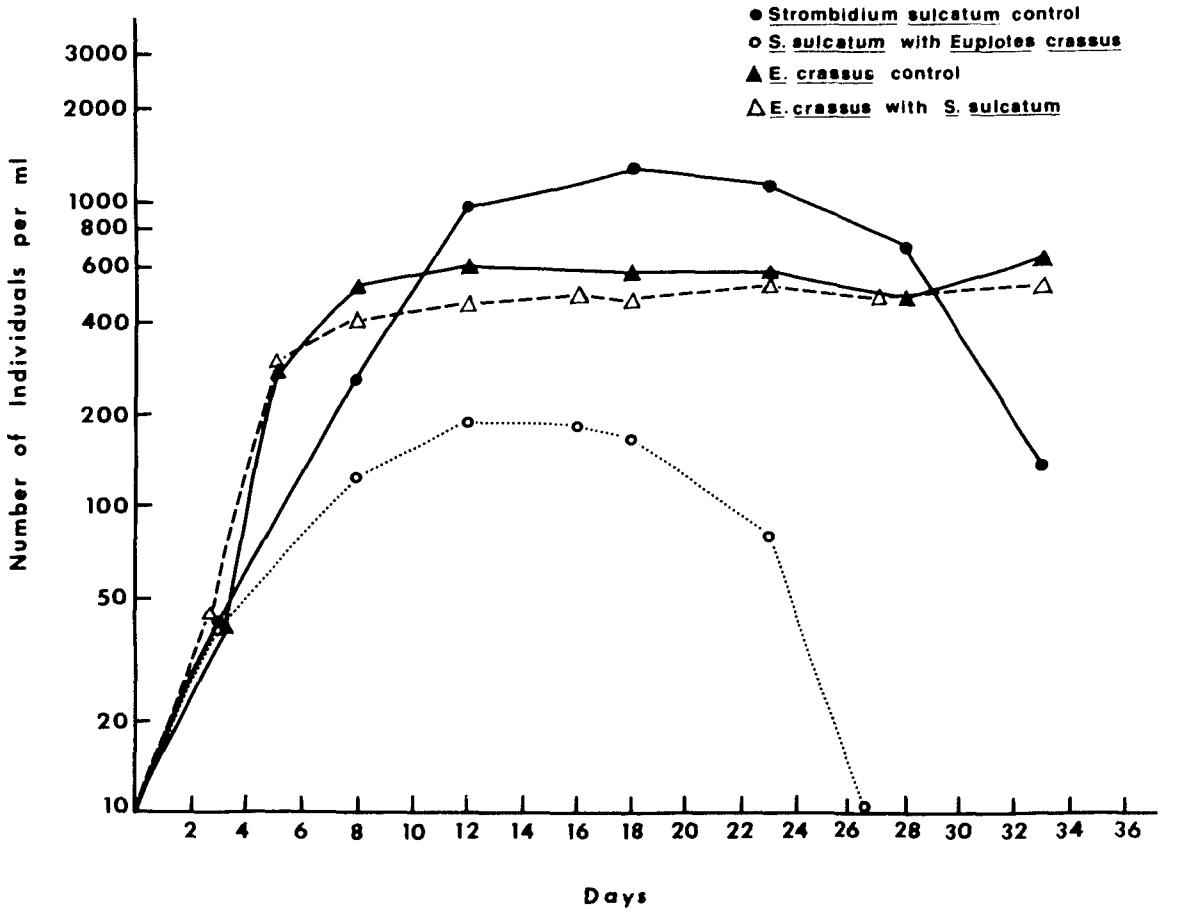
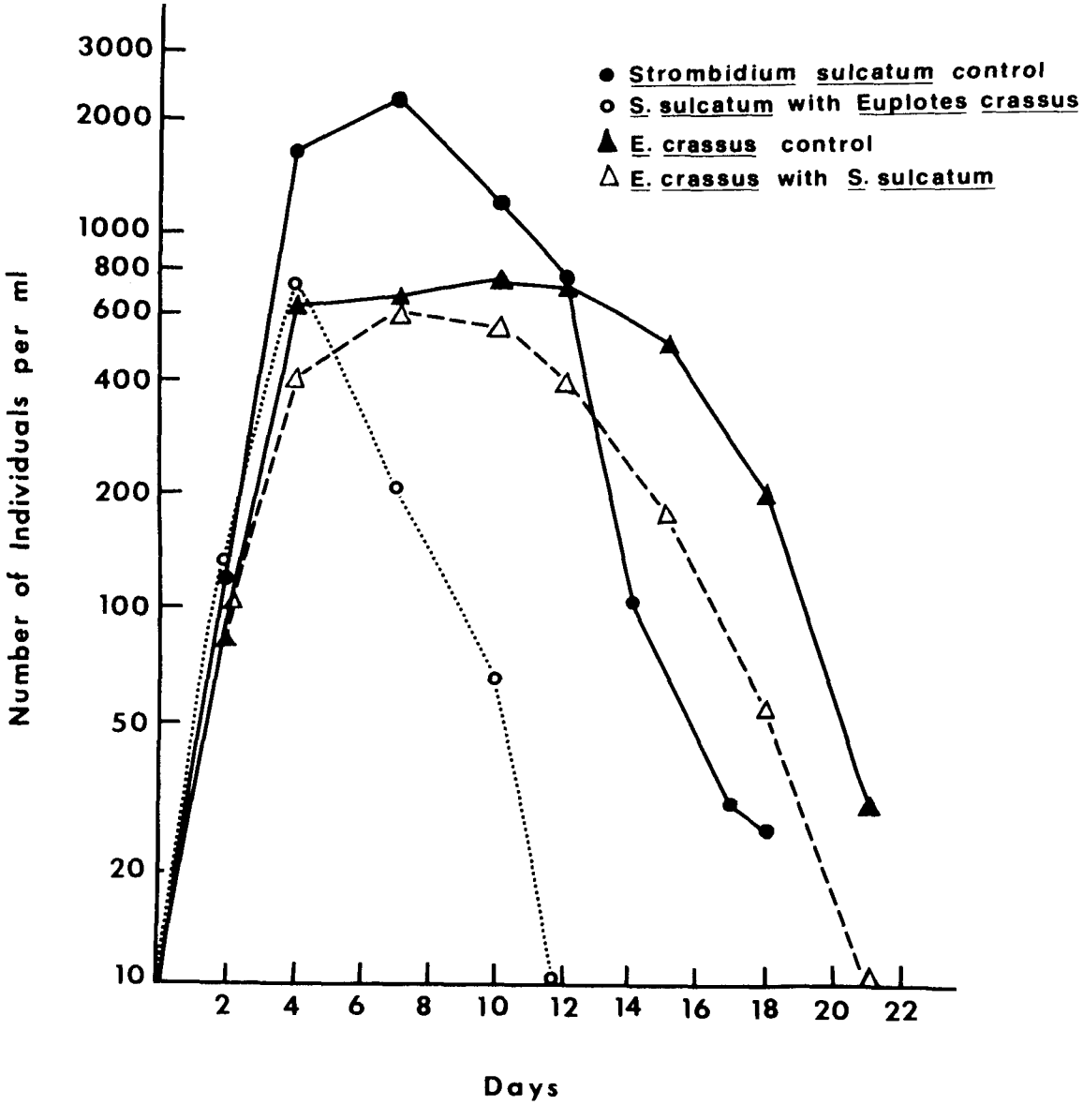

● Strombidium sulcatum control
○ S. sulcatum with Euplotes crassus
▲ E. crassus control
△ E. crassus with S. sulcatum
Number of Individuals per ml
3000
2000
1000
800
600
400
200
100
50
20
10
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
Days

Figure 39. Interaction between Euplotes crassus and Strombidium sulcatum at 28°C and 0.015% proteose peptone and yeast extract.


3000
2000
1000
800
600
400
200
100
50
20
10
Number of Individuals per ml
2
4
6
8
10
12
14
16
18
20
22
Days
● Strombidium sulcatum control
o S. sulcatum with Euplotes crassus
▲ E. crassus control
△ E. crassus with S. sulcatum

S. sulcatum upon E. crassus at these temperatures at a food level of 0.015% compared with 0.001%, no two-species interactions were observed at 30°C at high food level. That is, at the higher food level, E. crassus populations showed no indications of being influenced by the presence of S. sulcatum, hence there appeared no need to determine the temperature effects upon their population growth rate at 30°C.

Similar observations of isolated and mixed populations of two congeners, Euplotes crassus and E. harpa, yielded strikingly different results (Figs. 40 and 41). At the food concentration employed (0.001% PPY), and at both culture temperatures (15° and 28°C), population growth curves for isolated and mixed populations were not consistent; neither species affected the growth and survival of the other.

In summary, temperatures of optimum growth ranged widely among the different species of ciliates examined. Both temperature optima and upper thermal limits of growth are close to thermal limits of survival. Heat sensitivity depended not only upon the variety, but also on the quality or type of nutrients available to E. crassus. When cultivated at low temperatures, heat resistance in E. crassus increased with increasing salinity. Acclimation to high temperatures similarly increased its resistance to sudden heat stress.

The ability of Dexiotricha sp., S. sulcatum and E. crassus to reproduce over a wide range of temperatures and salinities demonstrates the capacity of these organisms to tolerate and to adjust to extreme changes in environmental

Figure 40. Interaction between Euplotes crassus and E. harpa at 15°C and 0.001% proteose peptone and yeast extract.

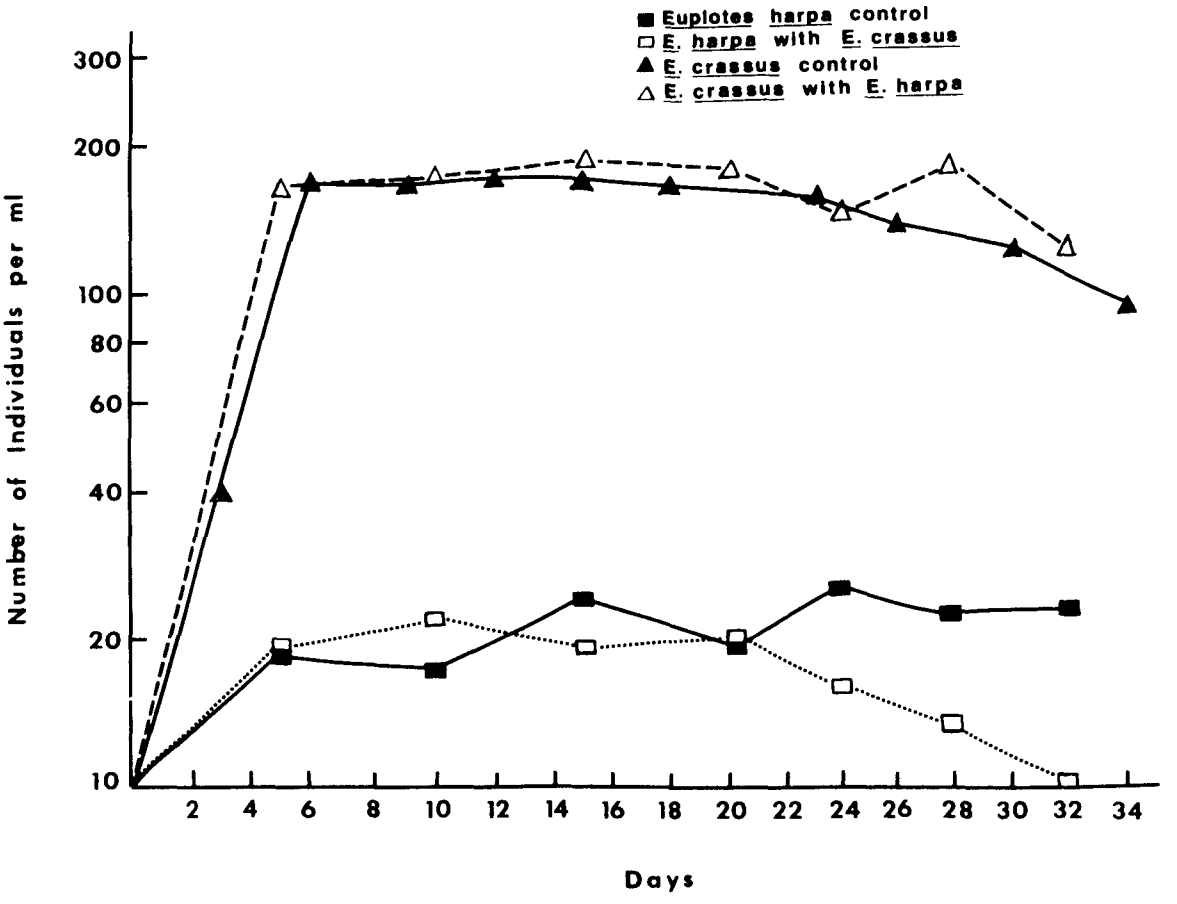
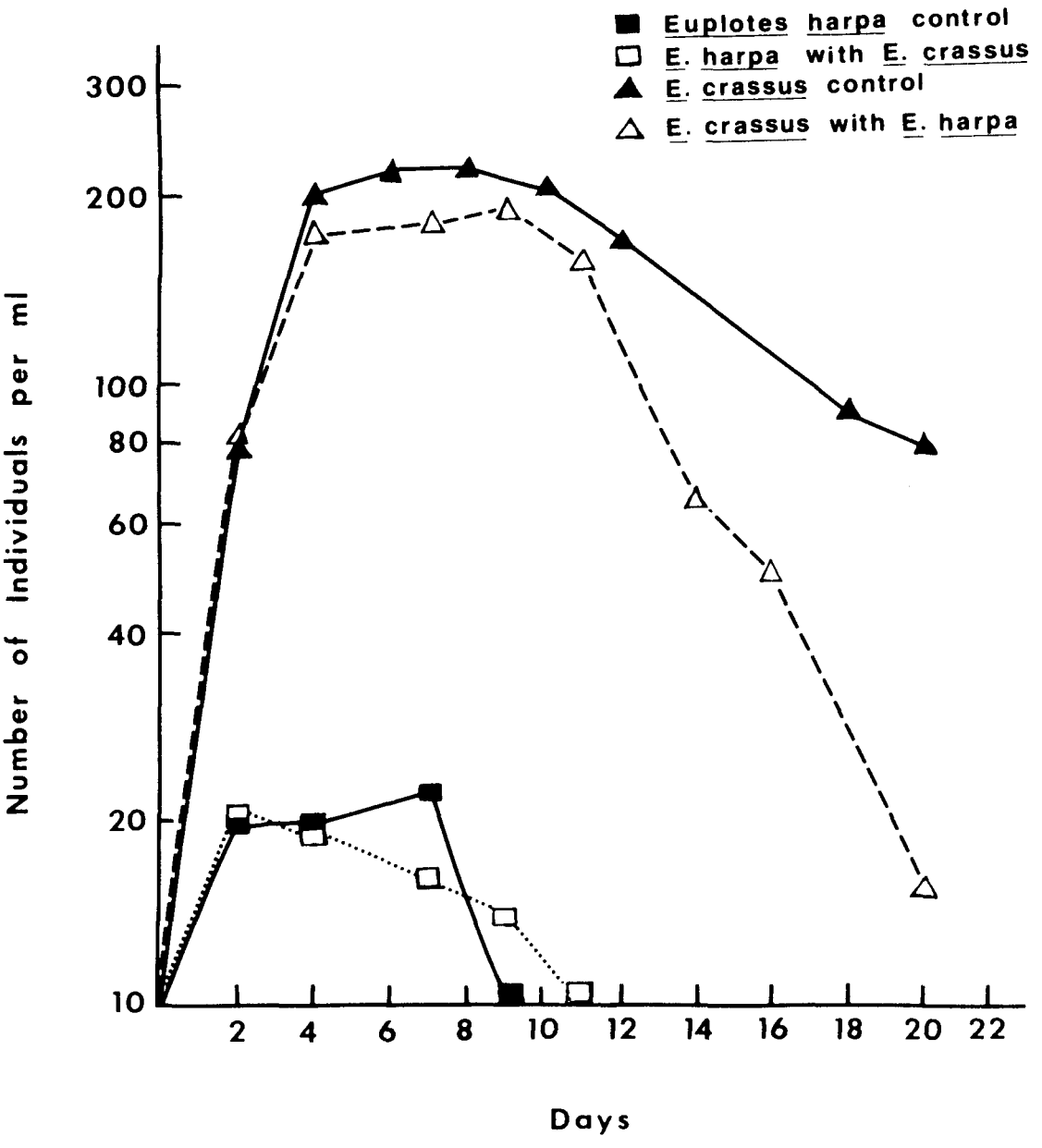

■ Euplotes harpa control
□ E. harpa with E. crassus
▲ E. crassus control
△ E. crassus with E. harpa
Number of Individuals per ml
300
200
100
80
60
40
20
10
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
Days

Figure 41. Interaction between Euplotes crassus and E. harpa at 28°C and 0.001% proteose peptone and yeast extract.


Euplotes harpa control
E. harpa with E. crassus
E. crassus control
E. crassus with E. harpa
300
200
100
80
60
40
20
10
Number of Individuals per ml
2
4
6
8
10
12
14
16
18
20
22
Days

conditions. However, as shown with E. crassus and to a lesser extent with S. sulcatum, temperatures above optimal limits in conjunction with low salinities inhibited reproduction.

Thermal changes had little influence on the competitive interaction between E. crassus and S. sulcatum; i.e., no competitive advantage was given to either species as a result of increasing temperatures. Further, temperature elevations beyond the growth optimum of E. crassus actually reduced any competitive effects of this species over S. sulcatum. Changing the food concentration only affected the total number of organisms produced; higher densities of ciliates were attained at the higher food level than at the lower, again without influencing competitive interaction. Temperature effects on the interaction between two species of Euplotes are inconclusive.

DISCUSSION

I have demonstrated that ciliates are particularly sensitive to sudden elevations in temperature, especially near their lethal limits. Additionally, thermal requirements for growth and survival are influenced by both biotic and abiotic factors. Before discussing the effects of these factors on the heat sensitivity of ciliates, a few comments concerning my observations on the behavioral responses during heat tolerance determinations may be appropriate.

The pattern of behavior elicited by heat shock was similar among the various species of ciliates studied. During the initial 30-40 seconds of heat treatment, the rate of locomotion accelerated considerably, eventually becoming erratic; i.e., uncoordinated or lacking normal direction. Similar observations of accelerated locomotion were made by Tawada & Miyamoto (1973) and Tawada & Oosawa (1972) on the response of Paramecium caudatum to thermal changes. This behavioral response is considered by these and other investigators to be a mechanism manifested in the dynamic properties of the cell membrane as a receptor for detecting optimal temperature conditions and as an avoidance mechanism from lethal conditions (see Jahn & Bovee, 1967).

Sleigh (1956) observed that increasing temperatures increased ciliary beat frequency in Stentor polymorphus, and that this was accompanied by reduction in metachronal wave length and increased wave velocity. The frequency and wave

velocity increased steadily with temperature up to 25-28°C, above which rates of increase declined. Observations by Machemer (1972) further show that reduction of temperature causes a counterclockwise shift in beat direction in Paramecium multimicronucleatum. Such temperature-related alterations of ciliary activity have been associated with membrane-regulated changes of intracellular calcium concentration (Dryl, 1970; Eckert, 1972; Murakami & Eckert, 1972 and Satir, 1975). External stimuli, whether chemical, mechanical, electrical, thermal, or alteration in viscosity of culture medium, induce changes in membrane potential which in turn regulate the rate of calcium uptake by the cell. Murakami & Eckert (1972) demonstrated that increased influx of intracellular Ca^{2+} due to mechanical stimulation of the cell membrane induced higher frequency of ciliary beating. These and other researchers hypothesize that the role of calcium in ciliary activity is analogous to that played in the activity of muscle ATP-ase --- ATP system (see also Dryl, 1970 and Sleigh, 1970).

In ciliates where coagulation of cytoplasmic proteins did not occur such as Strombidium sulcatum, Trachelostyla pediculiformis and Condylostoma arenarium, cytolysis, vesiculation or complete fragmentation of the cell was prominent. Coagulation at death was typical among Euplotes. Contortions of the organism, cytoplasmic bulges or extrusions were most evident in C. arenarium. Just prior to death or during highly stressful sublethal temperatures, impaired

locomotion was characterized by spinning or tight circling movements as in Euplotes or by jerky back and forth movements as in T. pediculiformis.

Although ciliary activity was in some cases observed in immobile ciliates, lack of mobility of the organism as a whole was chosen as the criterion for determining death for several reasons. First, it was easier and quicker to determine cell immobility rather than to ascertain whether or not membranelles or cilia were active, particularly in the smaller organisms such as Uronema marinum and Tachysoma saltans. Secondly, ciliary activity persisted even in ciliates that had undergone cytolysis or fragmentation, and therefore I felt was not as reliable a criterion as loss of mobility. Lastly, in studies where many ciliates were subjected to stress of one form or another (e.g., Giese, 1938 and Nyberg, 1974), lack of mobility was relied upon as a criterion for death.

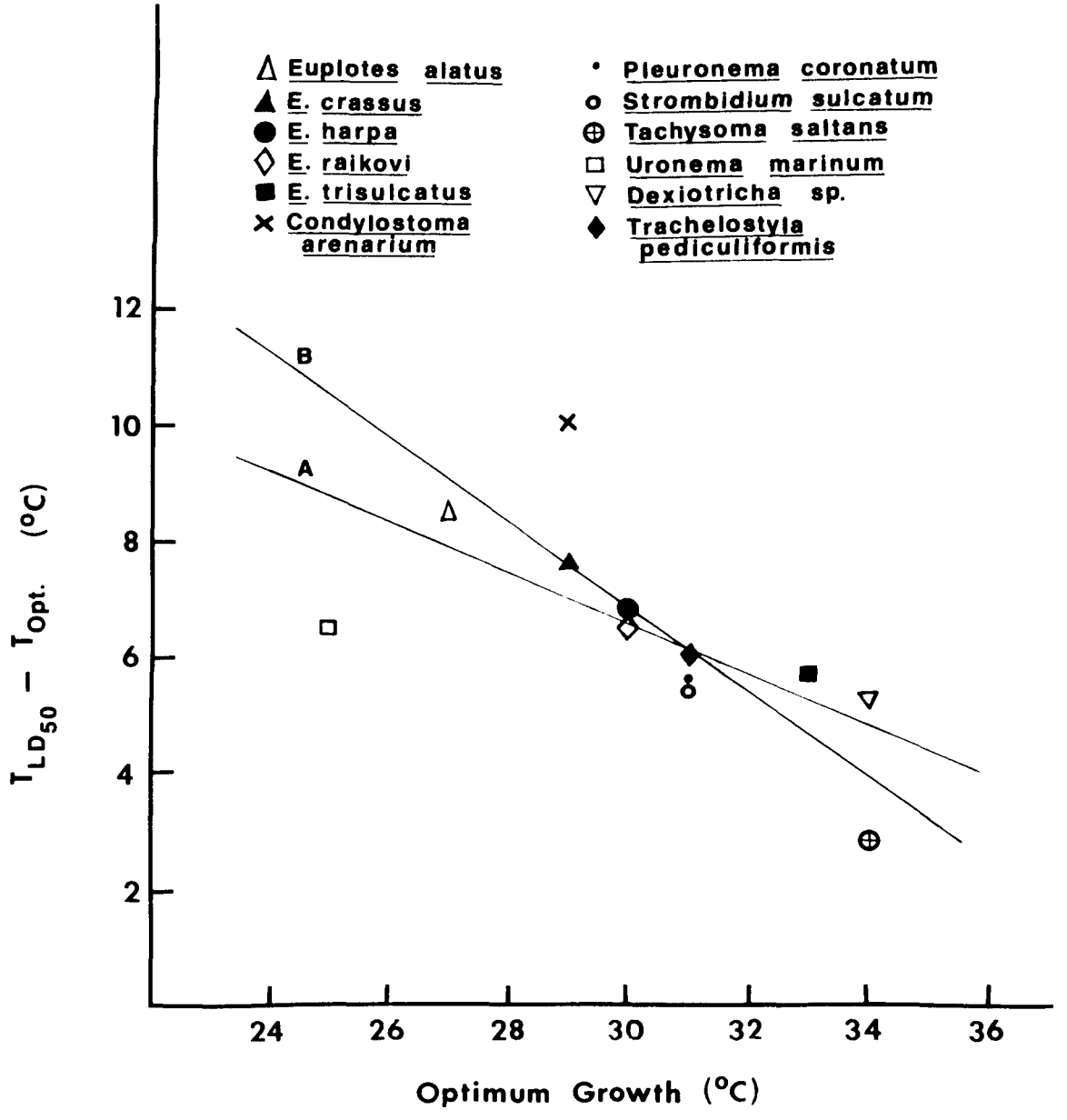
The high sensitivity of ciliates to elevated temperatures is suggested by the small differences between temperatures of LD_{50} and LD_{100}. In several replicates, during the course of heat shock experiments, an increase of 1-2°C meant the difference between 0% and 100% mortality. Similar sensitivities to high thermal stress were observed for several species of estuarine bivalves (Kennedy & Mihursky, 1971). Hedgpeth & Gonor (1969) point out that the distribution and abundance of many invertebrates, especially those that occur in large populations, are affected by small

fluctuations in environmental temperatures. According to them, such knowledge is necessary for the understanding of any adverse affects of artificial thermal changes on populations of organisms.

However, ciliates exhibit wide ranges of sensitivity in response to acute heat stress. Uronema marinum was the most sensitive; its LD_{50} was recorded at 31.5°C (Table 4, Fig. 27). Dexiotricha sp. and C. arenarium had the highest resistance to heat shock; their LD_{50}'s registered at 39.3° and 39°C, respectively. These temperatures are lower than those reported by Cairns (1972) for freshwater ciliates, where the mean for Spirostomum ambiguum was 46.8°C, that for Colpidium colpoda was 43.4°C, for Stentor coeruleus it was 48.2°C, for Tetrahymena pyriformis, 45.9°C, and for Paramecium multimicronucleatum, 42.9°C. These discrepancies may be attributed to the fact that Cairns acclimated his organisms to 25°C, whereas I cultured my ciliates at 20°C. Also, his ciliates were obtained from a commercial source where the culture media provided may be richer in nutrients than that contained in the simple seawater-rice medium employed in the present investigation.

When the difference betwen the temperature of optimum growth and the temperature of LD_{50} ($T_{LD_{50}} - T_{opt.}$) is plotted against the temperature of optimum growth, an inverse linear relationship is evident (Fig. 42). A low correlation ($r = 0.210$) was obtained when U. marinum data were included in the computation of regression analysis (regression line A, Fig. 42). When

Figure 42. Relationship between temperature of optimum growth and temperature of LD_{50}. (Regression lines fitted by the method of least squares, y = a + bX. Line A includes Uronema marinum data, a = 19.78, b = -0.44, N = 12. Line B excludes Uronema marinum data, a = 28.5, b = -0.72, N = 11).


Euplotes alatus
E. crassus
E. harpa
E. raikovi
E. trisulcatus
Condylostoma arenarium
Pleuronema coronatum
Strombidium sulcatum
Tachysoma saltans
Uronema marinum
Dexiotricha sp.
Trachelostyla pediculiformis
B
A
12
10
8
6
4
2
24
26
28
30
32
34
36
$T_{LD_{50}} - T_{Opt.}$ (°C)
Optimum Growth (°C)

U. marinum was not considered (regression line B), the correlation became significant (P = 0.05). This suggested that U. marinum was indeed significantly different from other ciliates in its sensitivity to heat stress. However, it was further noted that the correlation of regression analysis was statistically significant also when C. arenarium, for example, is not considered in the analysis. This analysis, therefore, does not really provide a statistical measure to support the observed differences in heat sensitivity. Nevertheless, the recorded differences in LD_{50} (Fig. 27) and generation time as a function of temperature (Fig. 34) provide sufficient evidence that U. marinum is strikingly different from other ciliates in its thermal sensitivity. Further, Strombidium sulcatum, Euplotes trisulcatus and Tachysoma saltans are of comparable dimensions, and yet are considerably more resistant to acute heat stress than U. marinum. Therefore, differences in response to temperature changes do not appear to be a function of either cell size, cell shape, nucleo-cytoplasm ratio, nuclear configuration, or proximity of nucleus to cell membrane.

As noted by Fenchel (1968b), maximal reproductive rates are generally inversely correlated with cell size. Uronema marinum, the smallest ciliate tested, had the shortest division time (Fig. 34). Condylostoma arenarium and E. harpa, both among the largest ciliates studied, took longer to divide than the other ciliates. The correlation between cell size and generation time does not hold, however, for S. sulcatum, E. trisulcatus and E. crassus. The former two species are

much smaller than E. crassus (see Table 1) and yet have longer generation times; the difference in generation time between E. trisulcatus and E. crassus being considerable. Therefore, factors other than cell size or cell shape must be considered in order to explain both the differences and/or similarities in generation time and sensitivities to high temperatures. Evidence from other studies indicate that such factors are physiologically related. For example, the nature and properties of enzyme systems and proteins play a significant role in the temperature requirements and adaptations of psychrophilic, mesophilic and thermophilic bacteria (Christophersen, 1967, 1973; Farrel & Rose, 1967).

Nutritional requirements of protozoa can be modified by increases in temperature. In the flagellate Ochromonas malhamensis, for example, a shift of culture temperature from 34° to 38°C increased the requirement for thamine 1000 times, and increased the B_{12} requirement by approximately 3000 times (Hall, 1965, 1967). Chemically-defined culture medium supplemented with nucleic acid derivatives enhanced Tetrahymena pyriformis growth at 37°C and further addition of phospholipids permitted equivalent growth at 39° and 1/3 of that at 40°C (Rosenbaum et al., 1966). Cytochemical investigations on Blepharisma intermedium have shown that conditions are optimal for cell division when acclimated to 28°C (Kasturi et al., 1969). At that temperature, required quantities of glycogen, basic proteins, lipids, enzymes and phosphatases are present. However, at 30°C, metabolic patterns were altered. Glycogen

reserves, proteins and lipids were diminished, enzyme activity decreased and changes in the free amino acids were observed with the simultaneous blockage of cell division.

The thermal resistance of E. crassus was shown to vary according to the quantity and type of bacteria on which it was cultivated (see Results). Undoubtedly, the assortment of nutrients provided by the rich bacterial growth in the unfiltered seawater confers greater heat resistance to E. crassus than the limited diet provided by single species of bacteria. Perhaps for similar reasons, E. crassus demonstrates more resistance to heat when cultivated on Pseudomonas cuprodurans than when cultivated on Arthrobacter marinus. That is, certain micronutrients that may be lacking in A. marinus but not in P. cuprodurans could explain the difference in sensitivity to heat stress when E. crassus is cultured on these bacteria. The need for trace metals (e.g., iron, magnesium, manganese, zinc and copper by Protozoa) have also been shown to increase sharply with temperature elevations (Hall, 1965). Possibly, in addition to biochemical differences (e.g., utilization of different amino acids, presence or absence of particular vitamins), P. cuprodurans differs from A. marinus in elemental composition and these differences are reflected in the thermostability of E. crassus.

Acclimation experiments provide still further evidence for a physiological basis to heat resistance. For example, E. crassus exhibited increased resistance to heat shock when it was cultivated at successively higher temperatures (see

Fig. 33). Changes in the thermoresistance of Paramecium caudatum accomplished either through acclimation to relatively high temperatures or through "heat hardening" (a short-term shock to high temperatures) were accomplished by changes in resistance to the action of other agents (Irlina, 1967; Poljansky, 1973; and Poljansky & Sukhanova, 1967). Along with an increase in thermostability, an increase in resistance to ethanol and a decrease in resistance to potassium cyanide was noted. Cold adapted Paramecium exhibited greater resistance to cyanide, but were most sensitive to malonate. Along with resistance to potassium cyanide at low temperatures, they also exhibited high succinic dehydorgenase activity. Thus, changes in thermostability accompanied by changes in sensitivity to inhibitors of respiration and glycolysis when ciliates are acclimated to different temperature regimes indicate that basic metabolic alterations, specifically enzymatic alterations, are occurring simultaneously.

In addition to a physiological basis for resistance to thermal stress, genetic and caryonidal differentiation hypotheses have been put forward to account for changes in resistance. In 1887 Dallinger increased the resistance of Tetramitus rostratus and other flagellates to heat by gradually submitting cultures to elevated temperatures up to 70°C. It is likely that selection played a major role in those experiments (Fauré-Fremiet, 1967; Noland & Gojdics, 1967). More recently, Poljansky & Irlina (1973) have shown that different clones within P. caudatum display hereditary

changes in thermostability and that significant interclone differences are seen in the shape of their thermostability curves. It has been suggested that temperature adaptation and resistance to calcium chloride may be examples of differential gene action induced by environmental changes as described by the Jacob & Monod hypothesis (Preer, 1967). Essentially, this hypothesis asserts that gene action alterations are a result of environmental changes affecting the action of certain repressor substances acting on the genes.

Whatever arguments are set forth to explain differences or similarities in tolerances to heat stress among ciliates, the fact remains that thermostability depends upon the physiological state of these organisms, and in turn the physiological condition is under the direct influence of the interaction between the biotic and abiotic factors of the environment.

As stated earlier, environmental impact and the ecological significance of thermal addition have been the bases for much research dealing with resistance adaptation in response to heat stress. Alteration of environmental temperatures, however, may also lead to modifications in competitive interaction. For example, freshwater blue-green algae replace diatoms and other green algae when temperatures are increased from 20° to 40°C (Cairns, 1956). In the presence of other salt marsh organisms (e.g., the nematode, Chromadorina germanica or the foraminiferan, Allogromia laticollaris), Euplotes vannus demonstrates dramatic changes

in its growth patterns in comparison with its control cultures (Saks et al., 1974). To help explain natural field distribution of ciliates, Hairston & Kellerman (1965) demonstrated competitive exclusion between two species of Paramecium under a variety of conditions. Paramecium biaurelia (formerly P. aurelia Variety 2; see Sonneborn, 1975) consistently won over P. triaurelia (formerly P. aurelia Variety 3) at low temperatures and low food levels. In the present study, growth rates of ciliates suggest that changes in environmental temperatures could result in changes in the relative dominance of one species at the expense of others (see Fig. 34).

Strombidium sulcatum, Euplotes crassus and E. harpa were selected for an experiment designed to test the effects of culture temperature on growth and survival of 1- and 2-species cultures. As long as culture temperatures were below those of optimum growth, temperature did not appear to have much influence on competitive interaction between species of ciliates. For example, although inhibited in the presence of one another, due perhaps to the limiting conditions of food and space, a shift in temperature from 15° to 28°C provided no significant advantage to either S. sulcatum or E. crassus; it only accelerated the final outcome of the experiments (Figs. 35, 36). However, at 30°C the influence of temperature becomes evident (Fig. 37). This two-degree increment places E. crassus beyond its limits of efficient growth and it dies out independent of any interaction with S. sulcatum. At this temperature the inhibitory influence

of E. crassus on S. sulcatum is negligible in comparison to 15° and 28°C experiments (i.e., further elevation in temperature reduces competitive effects of E. crassus on S. sulcatum), while the effect of S. sulcatum on E. crassus is even less noticeable; i.e., both the control and experimental E. crassus cultures attain similar population densities. At each temperature a noticeable difference in rates of decline between E. crassus and S. sulcatum control cultures is observed; S. sulcatum cultures decline at a faster rate. This suggests that S. sulcatum is more sensitive to changing culture conditions than E. crassus. Therefore, this is another factor influencing population densities of ciliates operating independently of either temperature or the presence or absence of other ciliates. Although the 30°C experiment was not conducted at the higher food level (see Materials and Methods), the differences between the results of the high and low food level experiments at 15° and 28°C are mainly one of total number of organisms produced. There again, the final outcome of the experiment appears to be little influenced by the two temperatures employed (Figs. 38, 39).

The results of the competition experiments between E. crassus and E. harpa are not as dramatic as between E. crassus and S. sulcatum. These two species of Euplotes have considerably different generation times throughout their temperature ranges (see Fig. 34) and therefore interaction between them would seem to be reduced. The population growth of one species was unaffected by the presence of the other at

either 15° or 28°C (Figs. 40, 41). However, although E. harpa did not attain high densities to begin with, an obvious decline in its experimental cultures (i.e., in the presence of E. crassus) was evident at 15°C. Similarly, at 28°C E. crassus began to show indications of inhibition even after E. harpa cultures had expired. One might interpret these observations as a response of the cultures to autotoxins. This is particularly suggestive of E. crassus at 28°C. However, by this same reasoning control cultures should have declined at similar rates; this was not the case especially with the 15°C E. harpa cultures. It is more likely that the decline of E. harpa at 15°C and E. crassus at 28°C is a result of competition for food in mixed cultures, in spite of low numbers and early decline of E. harpa at 28°C. Although the data may be insufficient (Fig. 40) or strong enough (Fig. 41) to draw a final conclusion, it appears that E. crassus may have a competitive edge over E. harpa at 15°C, but not (or at least less) at 28°C. The failure of E. harpa to develop at the higher food level may be related to its inability to keep up with too rapid a build up of bacteria and metabolic products under the limited space provided.

In summary, the duration of population growth is a function of temperature and food supply; high temperatures stimulate fast growth and high food concentrations produce greater number of organisms. This is in agreement with previous studies (e.g., Fenchel, 1968b). The magnitude of

a population is affected by the presence of other ciliates only when temperatures are below optimum values of the interacting species. Such species interaction appears to be one of inhibition due to competition for limited food and space. Beyond temperatures of optimum growth, species interaction becomes negligible. Finally, the rate of decline is related to the physiology of the individual species as well as temperature.

With the exception of Uronema marinum, the ciliates in general adapted readily to temperatures up to 30°C. Studies with freshwater ciliates show that temperatures for optimum growth range between 25° and 32°C (Phelps, 1946; Prescott, 1957; Propper, 1965; and Thormar, 1962b). This was shown to be true also for the marine ciliate, Diophrys scutum (Fenchel, 1968b). In the present investigation this was observed for the majority of the ciliates studied. Three species, Dexiotricha sp., E. trisulcatus and Tachysoma saltans exhibited slightly higher temperature optima, 33-34°C (Table 4). Upper thermal limits of significant growth and survival generally were 2-4°C higher than temperatures of optimum growth. In shallow water habitats such as New Hampshire tidal marsh pannes, the mean surface and bottom temperatures during the summer months reach up to 28-29°C and 27-29°C, respectively (Kelts, personal communication). The total summer temperature range is approximately 14°C. During a warm August day (air temperature, 32°C) in a salt marsh panne 20 cm in depth at Adams Point, New Hampshire, temperatures were as follows. The surface

registered at 34.5°C. Immediately below a surface mat of green algae (Cladophora), the temperature was 32-33°C; the water column was 28-29°C, and the bottom was 25°C. Ciliates are therefore well adapted to the usual temperature conditions of these environments.

A sudden increase in ambient water temperature such as occurs at power plant discharge sites, would undoubtedly eliminate the vast majority if not all the ciliates living in the immediate vicinity. Farther from the point of discharge, elevations in temperature may be such as to either eliminate the more sensitive species (e.g., Uronema marinum), thus disrupting normal community succession patterns of the ecosystem, or alter community composition by the imposition of physiological stress on ciliates with lower tolerance limits.

There is no doubt that many factors are involved in the heat sensitivity not only of marine ciliates, but of many other organisms. All act in concert to influence an organism's response to thermal changes. Those examined in the present study are some of the more obvious and perhaps the most significant ecologically. The results reported demonstrate that alteration of these factors (particularly temperature), whether gradual or sudden, may have a significant affect on the growth and survival of marine ciliates. The information presented should be of interest both from the purely ecological point of view as well as from the more practical applications of environmental quality.

With the ever increasing demand for energy in the form of electrical power, it is reasonable to assume that increasing numbers of power-generating facilities will be located in coastal areas. Present trends indicate this to be the case. It is hoped that the results of this research, coupled with previous investigations, will further illustrate the need for careful evaluations of both siting and design of such facilities.

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