COO-3390-7

THE ROLE OF PLANKTONIC PROTOZOA IN THE MARINE FOOD CHAIN

Seasonal Changes, Relative Abundance, and Cell Size Distribution of Tintinnida

Progress Report

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Reporting Period: May 1, 1972 - April 30, 1973

PREPARED FOR THE U. S. ATOMIC ENERGY COMMISSION
UNDER CONTRACT NO. AT(11-1)-3390

DISCLAIMER

ABSTRACT

Tintinnida in local waters were identified on a seasonal basis to relate occurrences of species to water temperature. The winter-spring community comprised 4 species, which is considered to be low diversity by comparison with other published investigations.

Variability in size of a species was detectable at a glance, when lorica measurements were plotted according to their lengths and widths. The oral diameter of Tintinnopsis tubulosa was a conservative dimension--the most useful for taxonomic purposes. Length was quite variable, and possibly a useful guide to the physiological status of the population.

Size distribution of Tintinnopsis tubulosa in situ was compared with their dimensions in vitro. It was concluded that the length-frequency distribution may be a useful index to tintinnid growth rate in situ.

Two closely related eurythermal species of Tintinnopsis, isolated from the winter plankton, were grown in mixed agnotobiotic culture. Comparable growth responses were obtained for both of them at several concentrations of food, and at temperatures up to 15°C. Survival was poor at 20°C, however. Neither predation nor probiosis was detected.

INTRODUCTION

New research projects initiated during the previous contract period centered on the following:

(1) The principal species of Tintinnida in local waters were identified, and their relative abundances were established.

(2) The natural populations of Tintinnida were characterized according to their size distribution.

(3) Comparisons were made between the size spectrum of Tintinnida in nature, and the same species in culture. The in vitro studies were done at different temperatures and at several concentrations of food.

(4) The growth of 2 closely related species of Tintinnida in mixed culture was studied, to see (a) if food concentration acted selectively on either species, and (b) whether temperature was a selective factor.

(5) Predator-prey relationships were identified in which an omnivorous ciliate fed upon Tintinnida.

Several research projects were completed during the previous contract period. Completed research is summarized in the attached reprints and preprint. These papers describe (1) the methods used for continuous cultivation of Tintinnida, and some potential uses for the system in marine ecology; (2) nutritional factors that affect the abundance of nitrogen-containing particulate inclusions in marine dinoflagellates; (3) methods for preserving Tintinnida.

Removed

1. The objectives of this investigation were to identify the principal species of Tintinnida in the local plankton community, and to establish their relative abundances. Changes in the communities are now being observed as frequently as weather permits, usually several times weekly, so that changes in the tintinnid composition of the plankton can be related to water temperature.

During the first few months of this investigation, water temperature ranged from 3 to 9°C (winter-spring), and 4 species were generally found: Codonella acuta, Stenosemella oliva, Tintinnopsis beroidea, and T. tubulosa. The methodology employed, and the early observations are summarized in the following abstract submitted to the IV International Congress on Protozoology, Round Table on Ecology of Marine Protists.

Subsequent to writing this abstract, it has been found that T. tubulosa and Codonella are persistent representatives in the plankton, while Stenosemella and T. beroidea are found less frequently.

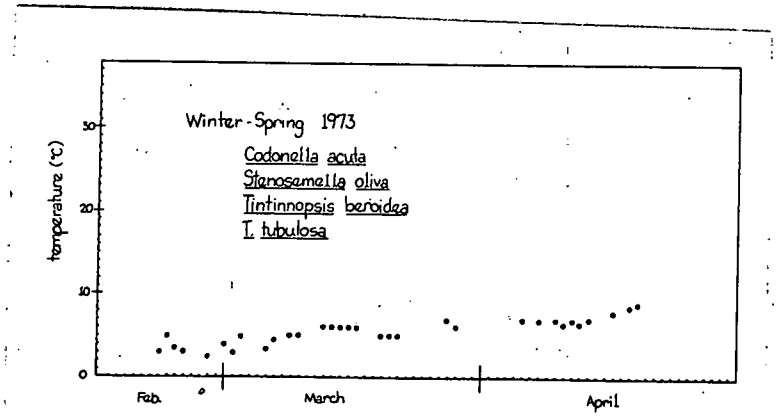


Fig. 1 Water temperature at Coney Island, N.Y. winter-spring, 1973.

Seasonal changes in tintinnid populations.
Changements selon la saison de la population Tintinnide.

GOLD, K. (Osborn Laboratories of Marine Sciences, New York Aquarium, New York Zoological Society, Brooklyn, N.Y. 11224, U.S.A.)

Tintinnida were collected from a local inshore station just beyond the surf zone, by hand-towing a 5" diameter, #25 plankton net (mesh size 64 µm) through waist-high water. During January-March 1973, when water temperature was low (3 to 5.5°C), there were 3 genera present. Species names tentatively assigned to the representatives, in order of their estimated relative abundance are: Codonella acuta, Stenosemella oliva, Tintinnopsis beroidea, and T. tubulosa. All are believed to be endemic to local waters and characteristic of the winter planktonic protozoan community.

Samples were brought to the laboratory within minutes of collection, examined immediately, or kept cold until processed, either for staining or for isolation of individuals into food-enriched culture medium (Gold, 1968). An ice-water or 5° C water bath sufficed for short holding periods up to several hours. Tintinnida generally remained highly motile, swam to the surface of the culture vessel, and stayed within their loricas when handled in this way. Where longer holding periods are desired, it is recommended that the larger zooplankton be filtered off, as they are believed to feed on tintinnids.

T. beroidea was isolated from the winter plankton and established in vitro for the third consecutive year, thus confirming its endemic nature. T. tubulosa was also established in vitro using methods similar to those described earlier (Gold, 1968). Codonella and Stenosemella, on the other hand, did not persist in culture when the same methods and foods were applied. Both Tintinnopsis species tolerate 10° C well (2-3x in situ temperature), and each of these species is recommended where a laboratory strain of Tintinnida is needed.

Preservation of samples was with Schaudinn's fixative added directly to the sea water. Identification of the species, and measurements were made on this preserved material, in settling chambers at an inverted microscope. As cells generally remained within the loricas on fixation, Schaudinn's solution is recommended as a preservative for Tintinnida in natural plankton samples where biological stains are to be employed or where permanent mounts are desired. A number of biological stains have been tested on both cultured and natural plankton samples -- to improve contrast and facilitate identifications -- and the results will be described.

Gold, K. 1968. J. Protozool. 15, 193-4.

Supported by a contract with the U.S. Atomic Energy Commission, reference number C00-3390-6.

2. Populations of Tintinnida were characterized according to their size distribution. While the length-width (l-w) diagrams shown here are not quantitative with respect to numbers of individuals, they do give an indication of the relative abundances of the protozoa in the plankton-net samples. Three distinctly different size groupings were distinguishable. Two of the groups are easily identifiable as T. tubulosa and Codonella, while the third, and smallest group, consists of Stenosemella or a composite of the latter plus T. beroidea. In these studies, size was considered to be more important than the species making up the clusters on the diagrams: the nutritional value of the protozoa to higher trophic levels is probably similar.

The data from early February is worthy of special attention (Fig. 2). Note that (1) Codonella (the widest group) was absent from the plankton on 2-1-73, (2) there was a small contingent of Codonella on 2-5, and (3) there was a conspicuously larger population of Codonella on 2-8.

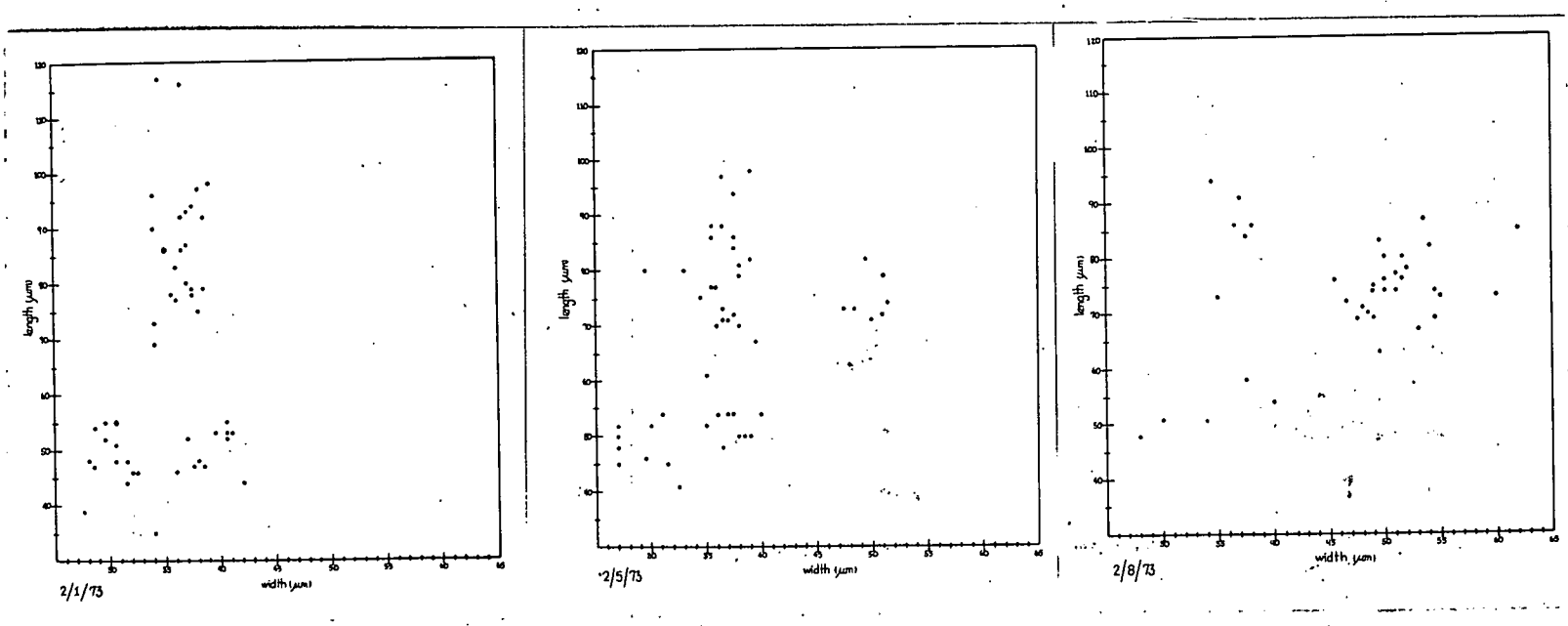


Fig. 2. Length-width distribution of Tintinnida, winter, 1973.

The sudden appearance of a species of tintinnid in the plankton in these waters does not necessarily mean that there has been a sudden "bloom" of that species. Coastal waters here are markedly influenced by tides, currents and wave action, and there is heavy grazing by invertebrates. All of these undoubtedly contributed to the observed changes in population densities. This method of following tintinnid population growth will be used in further studies in small ponds or embayments, however, where the influences of some of these physical factors are less severe.

Similar populations have been found on other occasions (Fig. 3-4); a difference may be seen, however. Codonella dimensions appeared to be much more variable in late April than they were in earlier plankton samples: loricas were also narrower. A possible reason for these anomalies is that look-alike species may be present that will be discernable only when sufficient numbers of measurements have been taken to yield distinct clusters.

Fig. 3-4. Length-width distribution of Tintinnida. Below, winter; right, spring.

Fig. 3

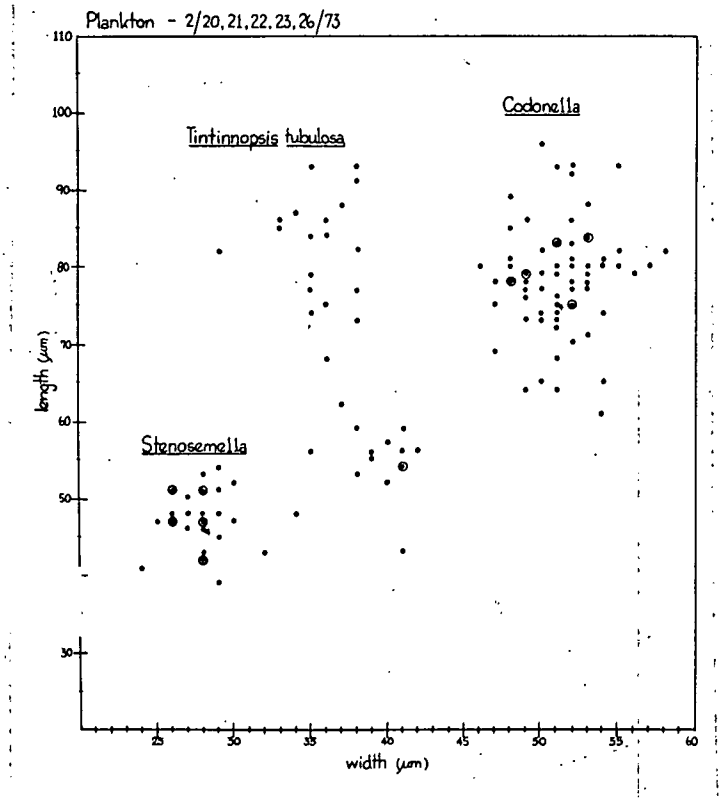
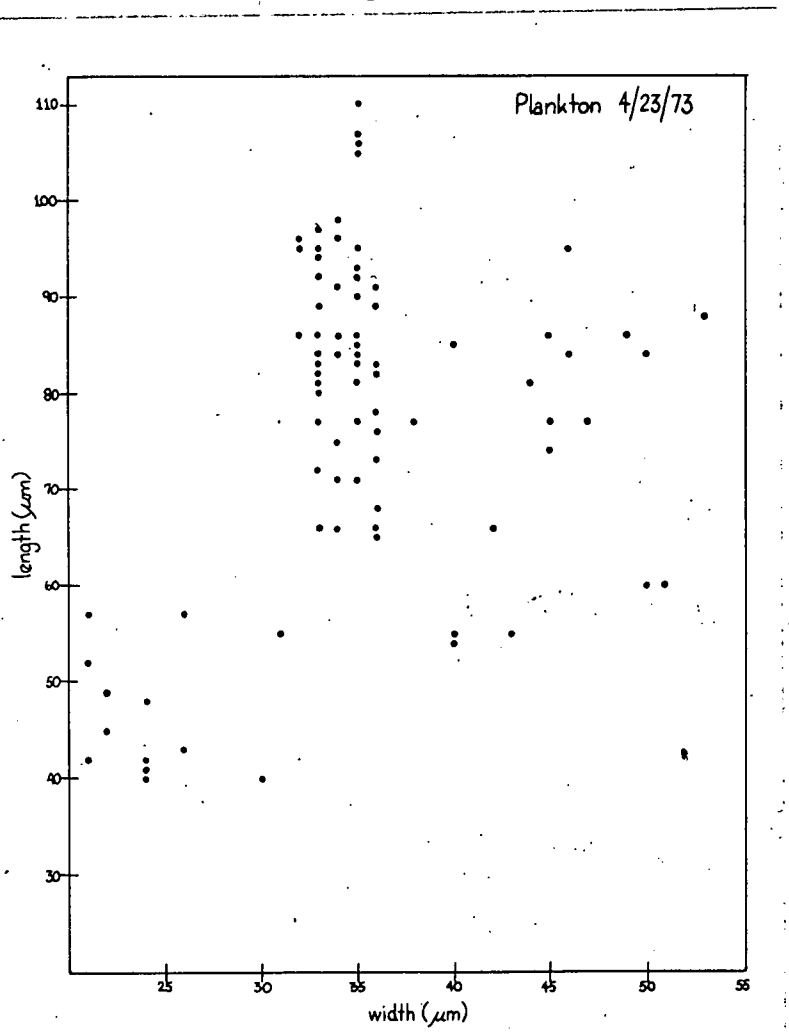


Fig. 4



Gold (1969) reported that, *in vitro*, lorica width was a far more stable feature of tintinnid morphology than the length. Hence, the width dimension was considered to be a better guide to speciation than either the length alone or the length:width ratio. That observation appears to hold for cells *in situ* as well.

The length of the lorica seems to be an excellent guide to the status of a population with respect to cell growth and division. Where there are sufficient numbers of Tintinnida available *in situ* for a study, the size (length) frequency will undoubtedly be a useful index to growth rate. The length range for *T. tubulosa* during the winter, for example, was considerable *in situ*, and this is suggestive of an actively growing population containing both juvenile and older contingents. The April population lacks a juvenile contingent. The status of the *Codonella* population is uncertain; it would be desirable to see the ranges of sizes of this species in culture.

3. The size distribution of T. tubulosa was investigated under a variety of cultivation conditions, e.g., at different concentrations of food, at elevated temperatures, and at different stages in the growth cycle.

Figure 5 shows the length-width distribution of a culture measured while it was in log growth with a doubling time of approximately 1.5 days. The similarities of this figure and the plankton samples shown above are obvious. It must be emphasized that these cells were grown in a defined sea water substitute under controlled experimental conditions (Gold, 1968 and later papers). Comparisons of Fig. 5 with the April plankton sample (Fig. 4) are especially pertinent, since the in situ temperature was close to the experimental temperature of 9°C. In the experimental culture, there was a conspicuous population of small T. tubulosa, whereas juveniles were absent from the plankton on 4-23-73. The absence of a contingent of small loricas, and the presence of considerably larger individuals in the April sample, suggest that this was a mature population, and that division was limited by as yet unknown environmental factors.

Fig. 6 shows the lorica dimensions of T. tubulosa at 15° C, which was approximately 5 x the in situ temperature at the time the protozoa were isolated, and 6° higher than the usual growth temperature. There was little if any effect on size distribution as a function of time of exposure to the elevated temperature. Measurements were also taken of empty loricas to see if juveniles and older cells were equally affected by temperature. The data suggest that the casting off of empty loricas is probably independent of the temperature, and independent of the age of the cells as well.

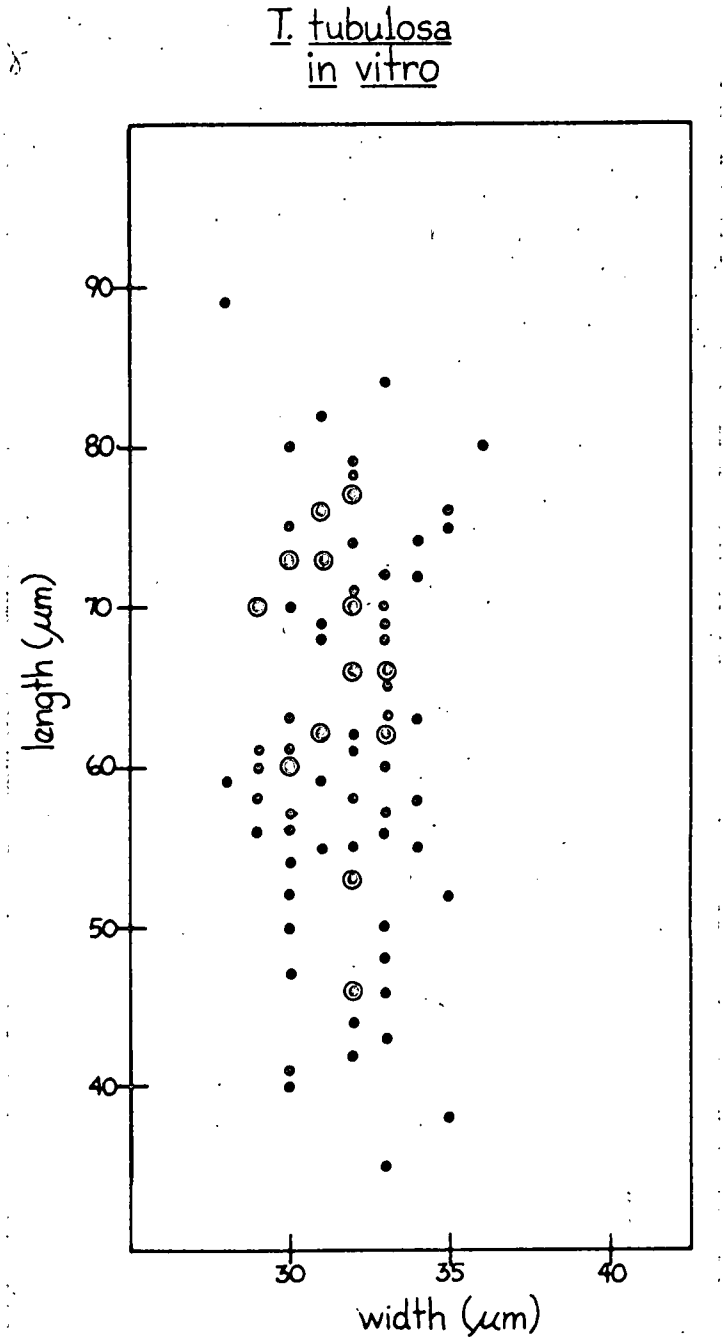


Fig. 5. Length-width diagram of T. tubulosa grown in vitro at 9° C. Log-growth culture.

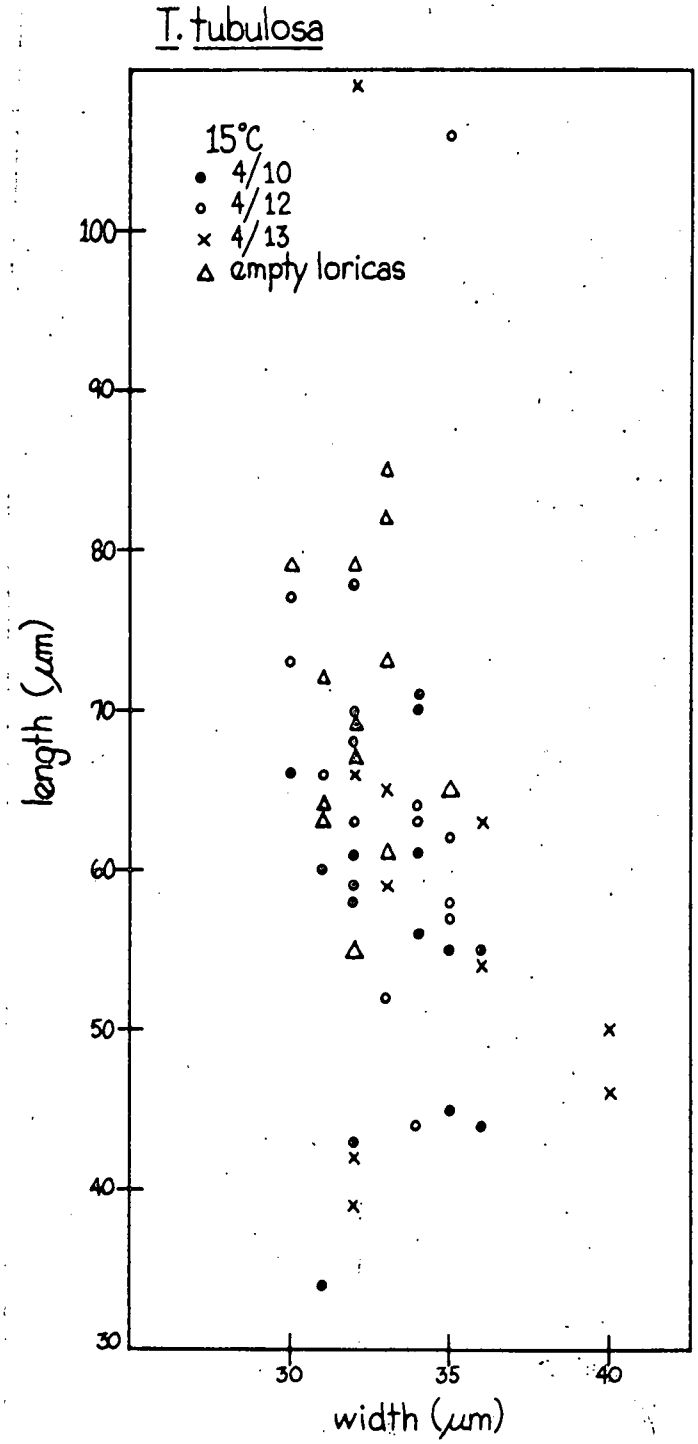


Fig. 6. Length-width diagram of T. tubulosa grown in vitro at 15° C.

4. Two closely related species of Tintinnida were grown in mixed culture to test the effects of (a) different concentrations of phytoflagellate foods, and (b) extremes in temperature conditions, on species dominance.

The size relationship of the 2 species of Tintinnopsis (beroidea and tubulosa) is shown in Fig. 7. The inherent difficulties in separating certain members of the two populations are apparent: small tubulosa are easily confused with wide beroidea. Therefore, additional characteristics, such as cell movement or their appearance (the contractile stalk of tubulosa was generally prominent), were taken into consideration when counts were made of the living cells.

Phytoflagellate foods known to be growth-promoting to Tintinnida were added to the culture vessels at different concentrations (Figs. 8a-f). Besides these additions, there was an abundant diatom flora present that persisted throughout the experiment. The diatoms, which were not inhibitory to the protozoa, included Detonula and Chaetoceros spp ., Nitzschia closterium, and other small pennate species. Despite the presence of the diatoms, cell yield for both species of tintinnid was proportional to the amount of phytoflagellate food added initially. Note, also, that the growth responses of both species were similar at all food concentrations.

Confirmation that the protozoa were sharing the same pool of food organisms may be seen in Fig. 9 in which the combined populations (T. tubulosa + T. beroidea) responded to the phytoflagellates in the same way as T. beroidea alone, grown in the absence of diatoms (cf Figs. 9-10).

Neither a probiotic (growth enhancing) nor a predatory effect was observed between the 2 protozoa.

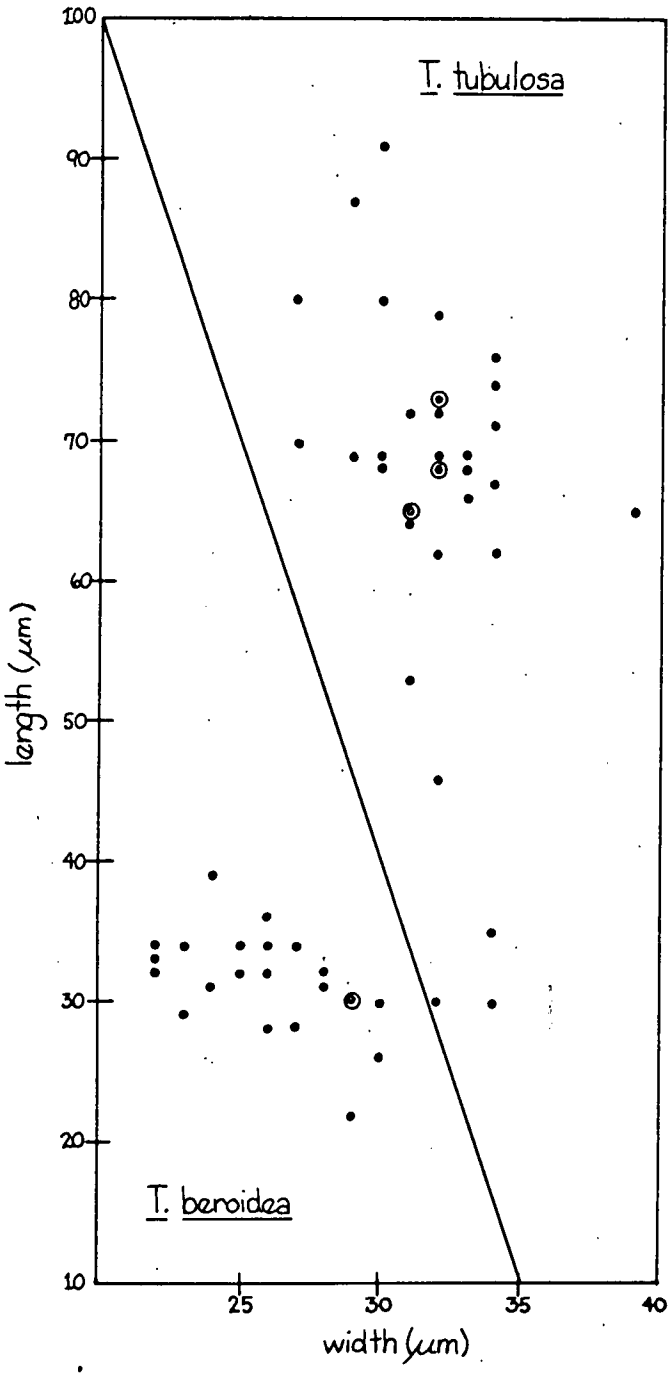


Fig. 7. Size relationship of T. beroidea and T. tubulosa grown in mixed culture.

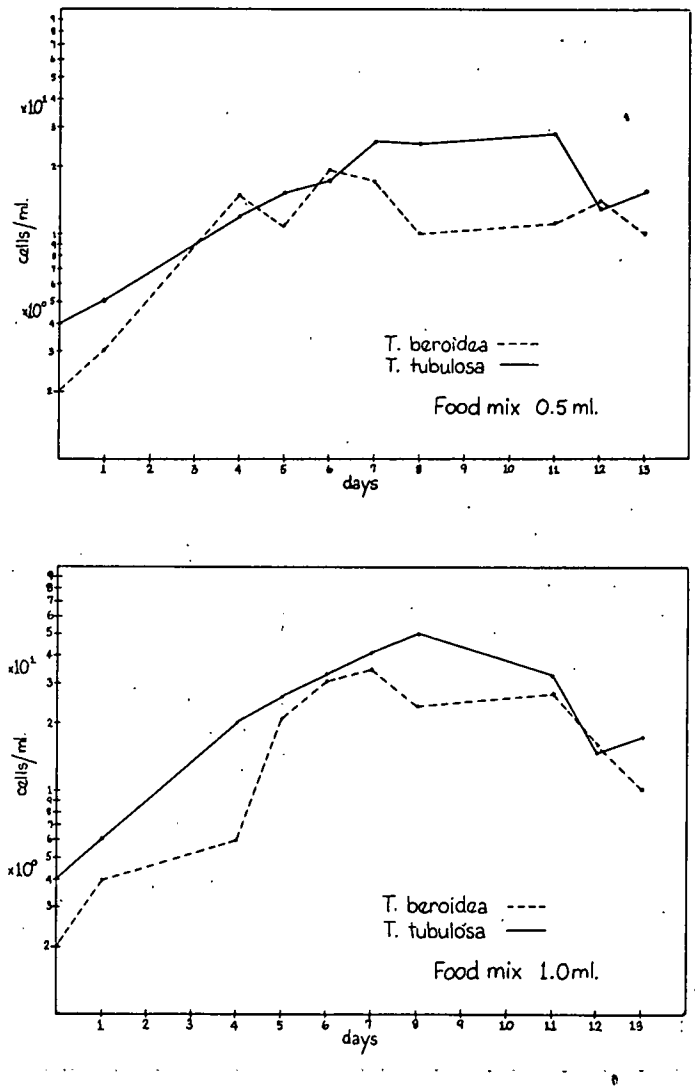


Fig. 8a-b. Growth responses of two species of Tintinnida in mixed culture. (Low food.)

Fig. 8c-f. Growth responses of two species of Tintinnopsis in mixed culture. (Intermediate and high food concentrations.)

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Fig. 9. A composite diagram of T. beroidea + T. tubulosa grown in mixed culture at different food concentrations. Agnotobiotic. Diatoms abundant.

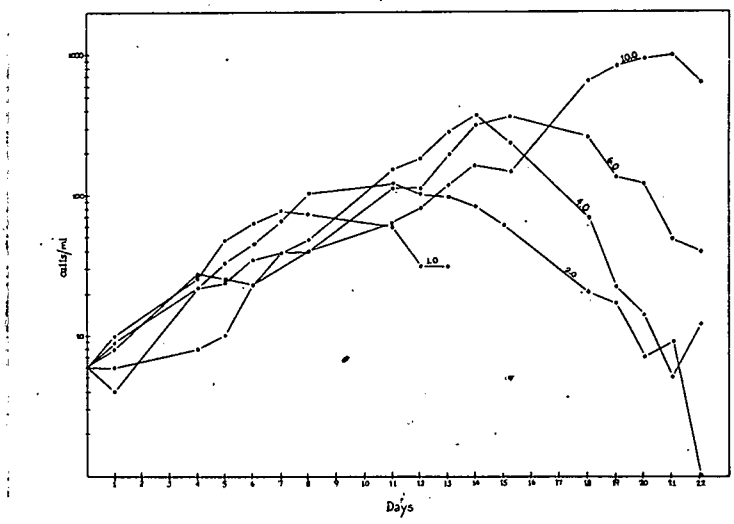
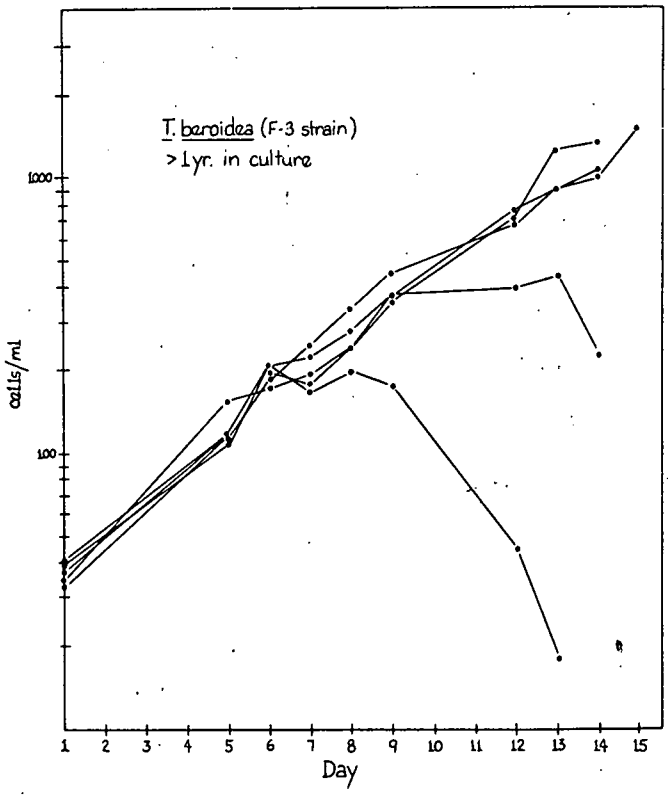
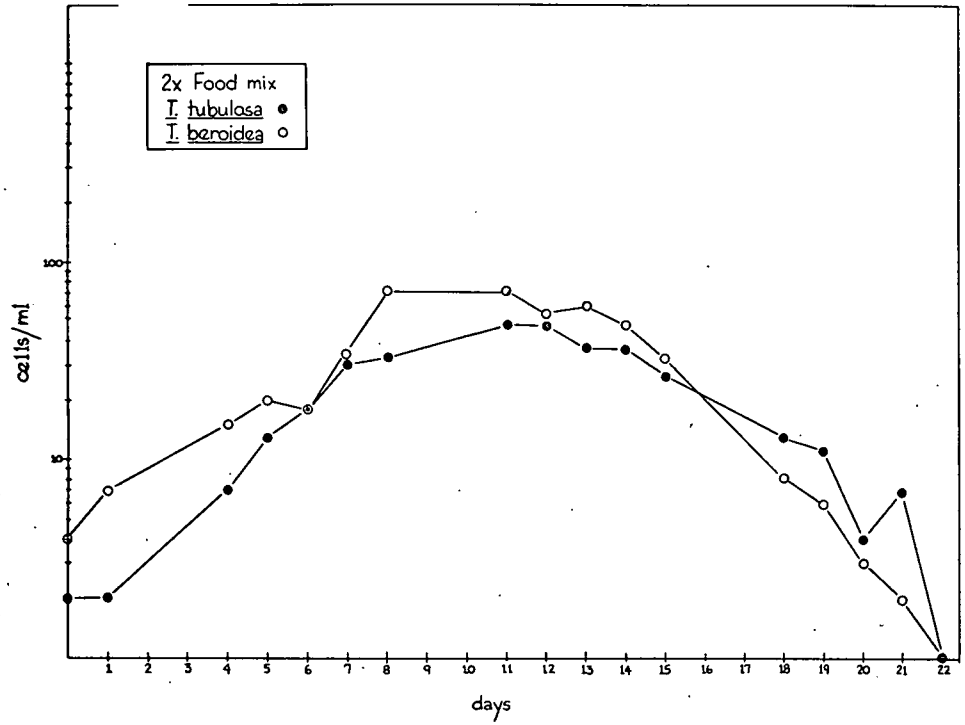
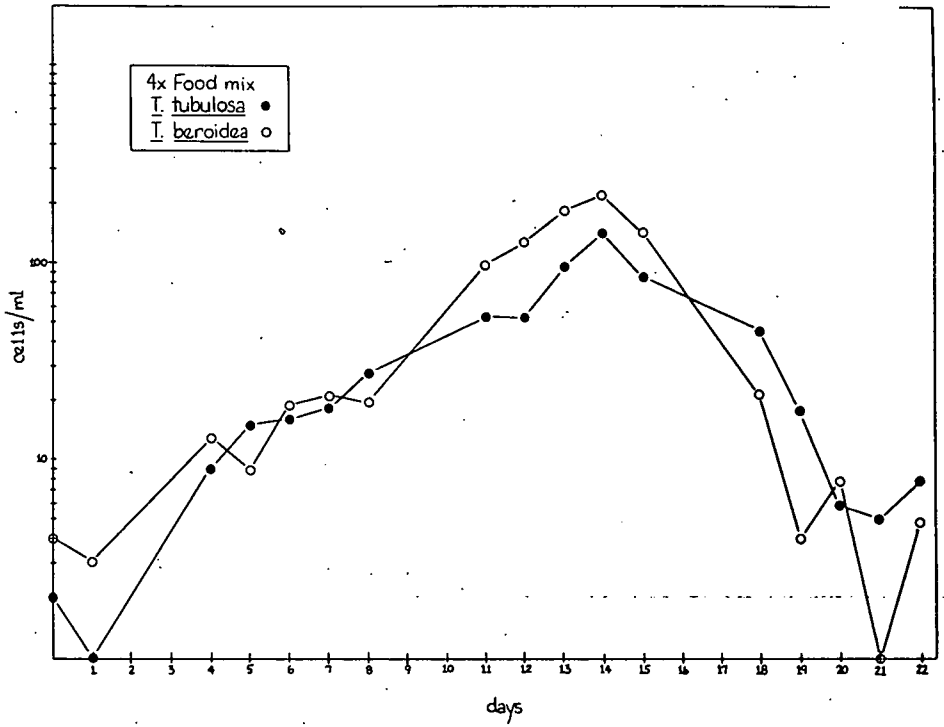
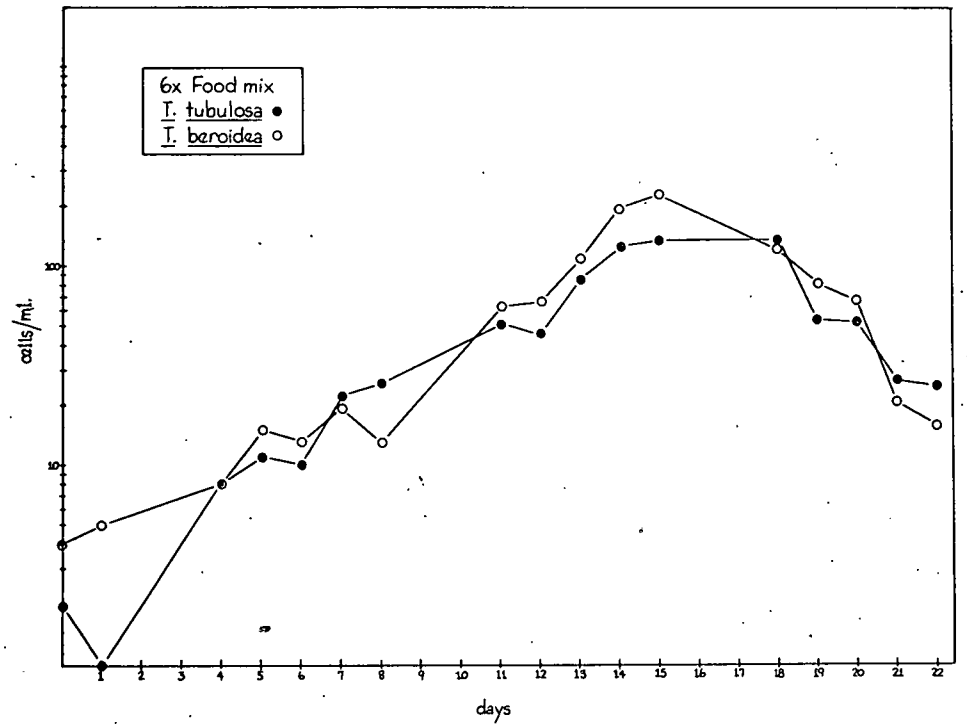


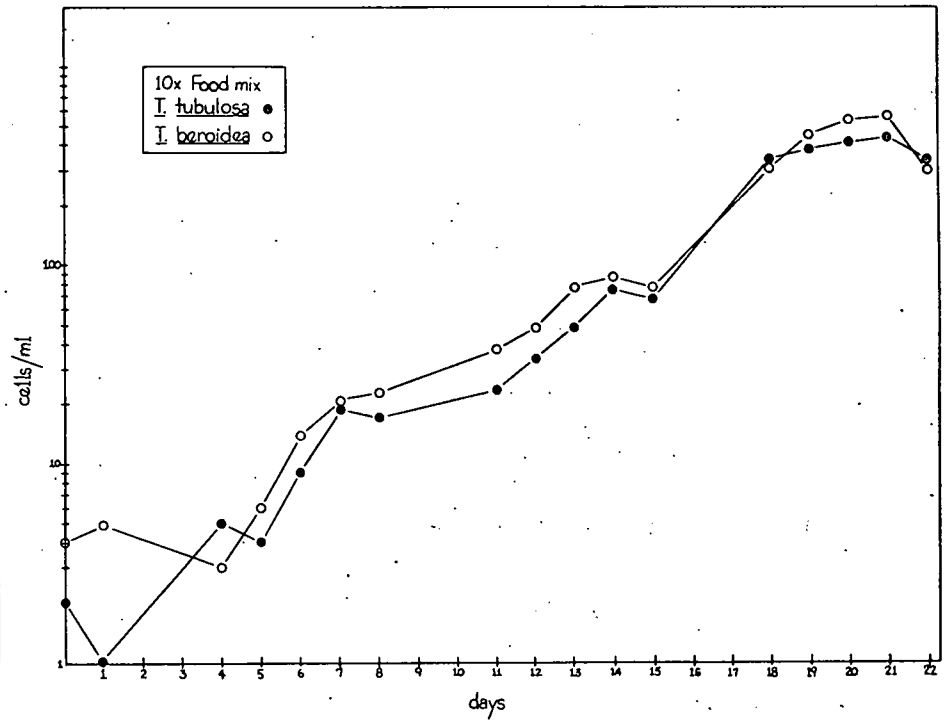
Fig. 10. Growth responses of T. beroidea at different concentrations of food. Agnotobiotic. Diatoms absent.




2x Food mix
T. tubulosa ●
T. beroidea ○
cells/ml
100
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
days


4x Food mix
T. tubulosa ●
T. beroidea ○
cells/ml
100
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
days


6x Food mix
T. tubulosa ●
T. beroidea ○
cells/ml.
100
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
days


10x Food mix
T. tubulosa ●
T. beroidea ○
cells/ml
100
10
1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
days

The preceeding figures (8a-f) show that the concentration of food was not a selective factor in determining species dominance. It was thought that perhaps thermal stress might be.

This hypothesis was tested when the cultures in Figs. 8a-b were reinoculated with food organisms, and placed at 9 and 15 C. Since both cultures resumed growth, indicating that the inoculum was a viable one, only the 15 C culture was followed in detail. (Fig. 11). Cell growth was excellent at the elevated temperature, provided that there was an adequate supply of phytoflagellate food present. Not unexpectedly, the protozoa seemed to have an increased food requirement. The responses of the 2 food-limited species, to a 5-fold increase in the food ration, were rapid and dramatic.

At a time when both Tintinnopsis were in a new log-growth phase, additional thermal stress was applied. Temperature was raised to 20 C, at which time the first indications of thermal stress were detected. (1) There was a marked decline in the cell yield. (2) T. tubulosa lost the ability to fabricate a lorica. (3) There was a change in the appearance of the Feulgen positive nuclei, i.e., T. tubulosa nuclei were highly fragmented. T. beroidea seemed to fare better than T. tubulosa at 20 C. Their loricas were well developed and the nuclei were not as fragmented. Summer temperatures in local waters exceed 20 C, and it will be interesting to see if T. tubulosa is excluded from the plankton at that time.

Several conclusions can be reached from these experiments. Tintinnopsis spp. are remarkably eurythermal, a feature that probably accounts for their success in neritic plankton communities where there is generally an abundance of food. The two species coexist quite well, and neither the food concentration nor thermal stress influence species dominance. The principal foods utilized by Tintinnopsis spp. were phytoflagellates, and even though they probably fed on diatoms somewhat, those species apparently were not nutritionally balanced, and would not support protozoan growth.

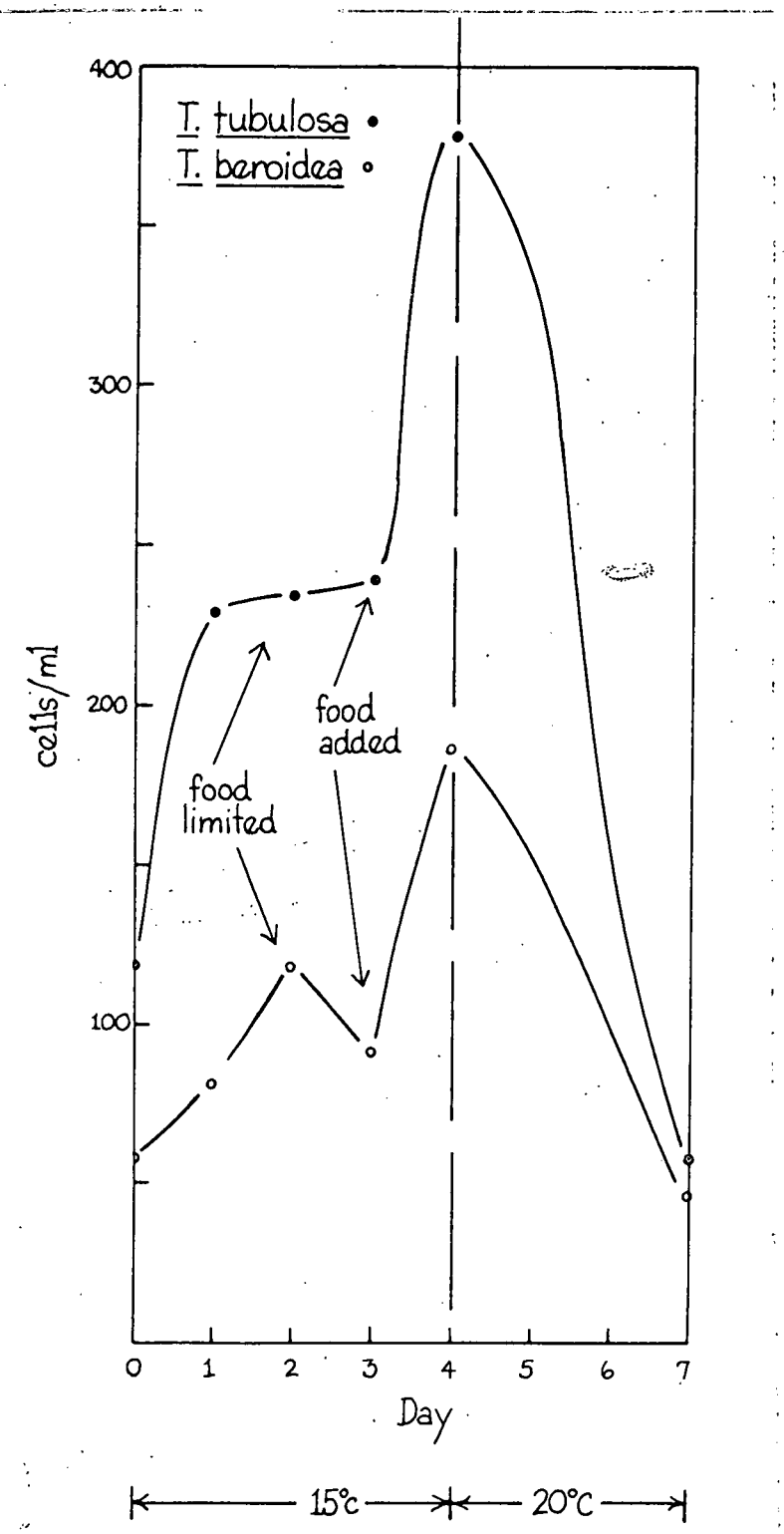


Fig. 11. Growth of T. tubulosa and T. beroidea at 15 C, and the inhibitory effect of 20 C.

5. An example of protozoan predation is shown in Figs. 12a-c. Strobilidium sp. is a large non-loricate ciliate, isolated from the local plankton. Identical culturing conditions were used to grow all of the ciliates, i.e. they were grown on an algal-food diet.

Note that T. tubulosa could not withstand the grazing pressure applied by Strobilidium, and the tintinnids declined steadily. The benefit to Strobilidium was not immediately obvious, although there appeared to be a small effect on the lag period and on cell yield.

In order to confirm that the tintinnid decline resulted from predation rather than inhibitory substances released by Strobilidium, the latter was filtered out of a culture, and a substantial portion of the supernatant was added to a T. tubulosa culture. There were no adverse effects on the tintinnid.

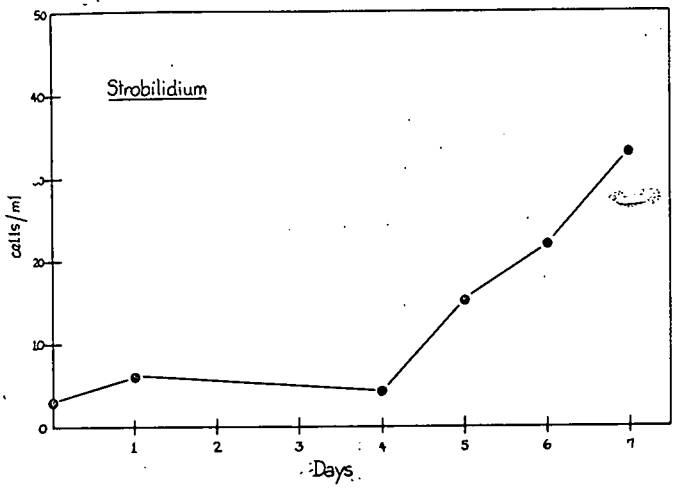


Fig. 12a. Growth of Strobilidium sp. on a mixed algal-food diet.

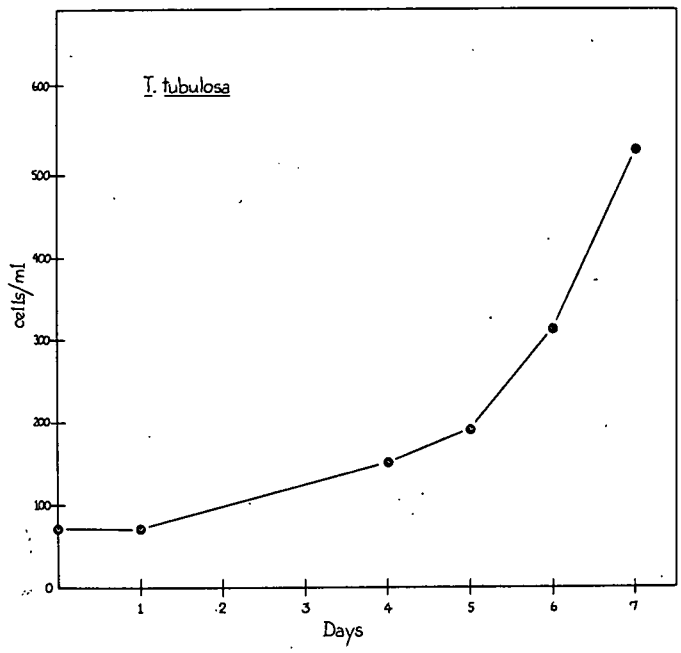


Fig. 12b. Growth of T. tubulosa on a mixed algal-food diet.

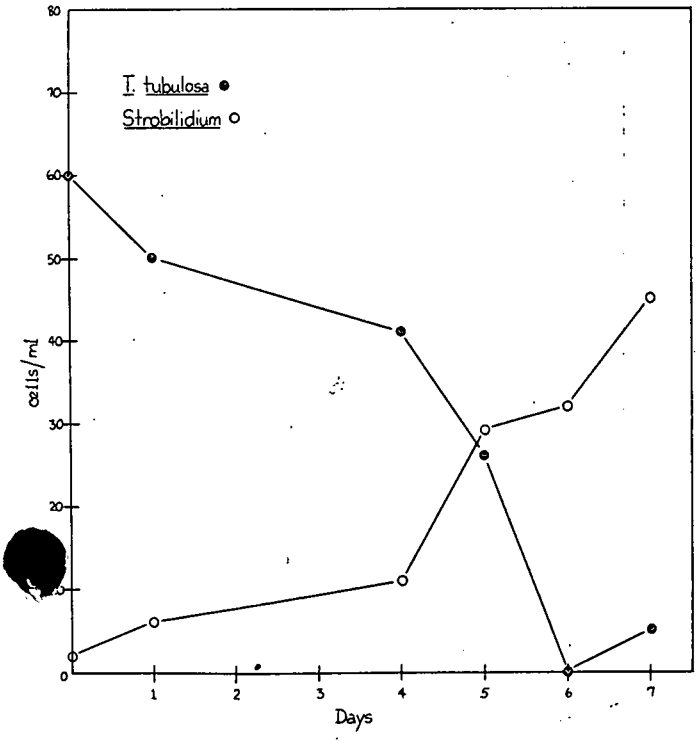


Fig. 12c. Predation by Strobilidium sp. on Tintinnopsis tubulosa.