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# THE EFFECTS OF ENVIRONMENTAL STRESS ON THE COMMUNITY STRUCTURE AND PRODUCTIVITY OF SALT MARSH EPIPHYTIC COMMUNITIES

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Progress Report

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## I. COMPLETED PROJECTS

A brief synopsis of each of the full papers, all appended as part of this report, follows:

1. Howard A. Rubin and John J. Lee. 1976. Informational energy flow as an aspect of the ecological efficiency of marine ciliates. J. Theoretical Biology, 62:69-91.

The role of information processing in food web energetic transformations was experimentally tested using 2 marine ciliates from the same habitat, Euplotes vannus and Uronema marinum, as experimental animals. In these experiments, caloric value of food was normalized so that information transfer could be measured. The informational quality of food was experimentally demonstrated by 3 independent means: 1) decay of synchrony in optimally reproducing cultures; 2) switching time lags; 3) comparison of generating times on calorically normalized diets. Information utilization was measured in terms of cell cycle advancement. Reproduction of the cilitates was often delayed when they were transferred from a diet of one algal species to a diet of another species. These induction time lags ($\gtrsim$ 2.5 hr) were interpreted as a consequence of adaptive enzyme formation, an information recognition response. Simple food web experiments tested whether or not the ciliates selected food to maximize their biotic potential.

Uronema was the ecologically more efficient of the 2 species tested. When logarithmically growing and fed certain strains of chlorophytes, its ecological efficiency approached 20%, while on diets of other chlorophyte species it dropped to only 6%. Ecological efficiency for Euplotes on various diets ranged from 2-12%.

The data clearly show that nutritional quality factors (molecular information) is part of energy flow in at least some food webs since the reproduction of the ciliates studied was neither a strict function of feeding rate nor strictly coupled with the calorific values of the food

organisms themselves. A theoretical construct was designed to quantitatively evaluate the informational gain in food-consumer relationships. The difference between the energetic cost of growth for the consumer on the diet most informationally impoverished and other foods was chosen as a base to which all other foods could be compared. The difference between the energetic cost of growth on the base food and other food species can therefore be expressed in temrs of energy saved per food organism processed per consumer generation. A new unit of information gain in food web transformations, the Cyberon, can be translated to other energy units through fundamental respiration relations. The theoretical energetic implications for resource distribution and utilization at the nanno, micro and meio levels of marine communities are discussed.

2. Norman M. Saks, Robert J. Stone and John J. Lee 1976. Autotrophic and heterotrophic nutritional budget of salt marsh epiphytic algae. J. Phycol. 12:443-448.

In this study we attempted to estimate the relative importance of primary production to heterotrophy in algae from the same assemblage. The algae were quite heterogeneous in their growth responses to nutrients added in enrichment culture. At the levels tested inhibition of growth by the addition of amino acids was as common as growth enhancement. Most of the sugars tested, with the exception of glucose, were non-stimulatory or inhibitory at a 10 mM concentration. Glucose stimulated the growth of 6 species and inhibited 3 others. Fructose almost totally inhibited the growth of 6 species but tripled the growth of another. A complex vitamin mixture stimulated 82% of the species tested. Some natural products and mixtures of metabolites were quite stimulating; some species were stimulated 10-15 times the levels of controls.

In 8 of the 11 species tested, photoassimilation of glucose and acetate were noted. Except for 2 species, uptake of radionuclide labeled organic substrates was only a fraction of the total carbon being photosynthetically fixed by the algae. In several cases, photosynthesis was

enhanced in the presence of glucose. Acetate, on the other hand, depressed photosynthetic rates in 6 of the 11 species. With only one exception all of the algae were obligate phototrophs under the test conditions.

The data suggest to us that the benthic and epiphytic algal assemblages of littoral and shoal marine communities are well adapted to play mixed trophic roles at lower steps in the detrital food web.

3. John J. Lee, John H. Tietjen and J. R. Garrison. 1976. Seasonal switching in the nutritional requirements of Nitocra typica Boeck, a harpacticoid copepod from salt marsh aufwuchs communities. Trans. Amer. Micros. Soc., 95:628-637.

Microcrustacea are extremely important members of most marine food webs. Pioneering studies by L. Provasoli and his associates showed that the species that they studied had dietary requirements which were satisfied by very few species of algae. Our studies on nematodes and foraminifera suggest that the phenomenon is more widespread than initially believed. The base of knowledge on this important aspect of food web dynamics is extremely limited and must be expanded in order to develop a meaningful overview of secondary production. Our choice of an experimental animal was based on several factors but most important, we needed an animal whose distribution in the community over time was fairly broad. We reasoned that an organism found in the community throughout the year was more likely to be a feeding generalist.

The aim of the current study was to measure in model microcosms the trophic relationships of a microherbivore at the beginning of the growing season (late May-early June, 15° C); and later the same summer (July-August, 25° C). Both temperature and food quality affected the growth rate and fecundity of Nitocra typica Boeck, the chosen herbivore. On adequate diets, generation time was 31-41 days at 15° C, while it was

13-24 days at 25° C. Species and even strains of the algal species varied in their nutritional value for the copepods. Taken as a group, diatoms were better food sources than the chlorophytes tested. Size of the algal food did not seem to be an important factor in the feeding of Nitocra typica. Very surprisingly, the animal is really a nutritional specialist and has very complex and graded nutritional requirements which seem to flexibly adapt it to feed on different species and strains of algae which are likely to occur in the community at different seasons.

4. M. E. McEnery and John J. Lee. 1976. Allogromia laticollaris - a foraminiferan with an unusual apogamic metagenic life cycle. J. Protozool. 23:94-108.

Allogromia laticollaris is an unusual meiofaunal species because it has a life cycle which is extremely plastic and seemingly responsive to environmental changes. Before we began this study it was known that the species was capable of reproducing by at least six different means (sexual, endogenous budding, exogenous budding, binary fission, cytotomy and schizogony). Under particular environmental or dietary conditions, some types of reproduction are favored over others.

In this study we described the life cycle of 3 strains of the species grown under defined conditions on a uniform diet. Almost bizarrely, each of the 3 strains had a different, nonclassical, and basically apogamic life cycle. The strain isolated at the lab in Cold Spring Harbor has a regular 26 day life cycle that alternated between 2 cytologically different schizonts. By comparison, the life cycles of the strains isolated from Towd Point, Southampton, L. I. and Sippewissett Marsh near Woods Hole, Mass. were abbreviated. The details of the life cycles are quite fascinating and present some very challenging problems for the cell biologist.

5. William A. Muller. 1975. Competition for food and other niche-related studies of 3 species of salt marsh foraminifera. Mar. Biol., 31-339:351.

Several hundred species of foraminifera grow in various salt marshes throughout the world. Very little is known of the differences in their niches which allow some species to grow while others in the same marsh do not. Tracer feeding and microcosm experiments provided some of the data used for testing a graphic representation of the Hutchinsonian niche. A polygon was constructed using 6 factors: temperature, salinity, pH, feeding rate, interspecific competition, and intraspecific competition as niche determinants. The effects of these biotic and abiotic factors on the generation time of 4 species of foraminifera were tested. Biotic factors were more difficult to evaluate than the abiotic ones. An equation, based on competitive feeding experiments, was developed to reduce data to a single coefficient. One species, Spiroloculina hyalina, did not compete with the other species for food, but Allogromia laticollaris and Rosalina leei were competitive. Intraspecific competition (crowding) seems to be an important factor limiting the exploitation of available community space by Allogromia laticollaris but it was not a limiting factor for the other 2 species. A. laticollaris was characterized as a rare species which may become locally abundant when dominant species are missing. S. hyalina is also a rare species with a high biotic potential but with very specialized niche requirements. It will bloom in physically unstressed environments provided that moderate numbers of appropriate species of bacteria are present. R. leei is stable, a conspicuous species whose moderate numbers are relatively unaffected by physical stress and competition.

6. John J. Lee, John H. Tietjen, Norman M. Saks, George G. Ross, Howard Rubin, William A. Muller. 1975. Educing and modeling the functional relationships within sublittoral salt marsh aufwuchs communities - inside one of the black boxes. Estuarine Res., Vol. I. 710-734.

The gist of our paper is that at the present time, information on the small organism population structure of at least one community, and by inference other communities, is too fragmentary to be used in modeling and predicting their community structure and function. Studies of salt marsh aufwuchs community foraminifera, nematodes and microalgae grown in gnotobiotic culture suggest it is unreasonable to assume that measurements made on one species will be good estimators for similar parameters of other species or the assemblage as a whole. Nutritional experiments suggest many meiofauna are selective feeders and gain different nutritional values from the food they assimilate. Many problems relating to the population structure and dynamics of aufwuchs microbial and meiofaunal food webs were illustrated and discussed.

7. John H. Tietjen and John J. Lee. 1975. Axenic culture and uptake of dissolved organic substances by the marine nematode Rhabditis marina Bastian. Cahiers de Biologie Marine 16:685-693.

The purpose of this study was to gain insight into the nature of quality factors inherent in food web transformations. Despite the widespread occurrence and abundance of free-living marine nematodes, successful continuous culture of these organisms in the laboratory has been limited to but a few species. Rhabditis marina is capable of digesting only a small number of species of bacteria in the environment because it will grow in continuous monoxenic culture with very few. Thus our task was very tedious even in the preliminary stages. In our

search for a substitute for the living bacteria diet, we found that many aseptic washings and gentle centrifugations of the nematodes with high concentrations of antibiotics followed by transfer to numerous replicates of experimental media were required before we found one with sufficient growth factors. R. marina grew axenically on Graces Insect Media and a marine salt base. The addition of casamino acids, hemoglobin and crude natural materials rich in lipid stimulated growth and reproduction. Lipid rich materials as TEM (triethylene amine) and TWEEN 80 were stimulatory when supplemented with whole egg powder. Combinations of fatty acids were able to replace the undefined lipid mixtures. It is suspected that R. marina has fewer fatty acid synthetic abilities than other free living nematodes. Unlike many types of marine invertebrates, the worm was unable to take up dissolved organic substances unless it was feeding.

8. John J. Lee, Marie E. McEnery, Eileen M. Kennedy and Howard Rubin. 1975. A nutritional analysis of a sublittoral epiphytic diatom assemblage from a Long Island salt marsh. J. Phycol. 11:14-49.

This paper is the product of one of the most time consuming and difficult projects we have ever attacked in the laboratory. At the onset we hoped that a study of the auxotrophic profile of the microbial assemblage would give us insight into some of the subtle variables which regulate population succession and information within the assemblage. Several important technological gaps were bridged on the way to reaching our goals. We designed a series of differential media which are effective as tools for the isolation and nutritional characterization of the algae and bacteria from the community. Many diatom species can be recognized by their colony type or growth pattern on solidified differential media. A dichotomous key and illustrated plates have been developed as an aid to their identification. The changes in population structure of the community were also reflected by changes in the nutritional patterns in the community as judged by qualitative and quantitative differences in the growth of diatoms and bacteria on the differential media tested. The comparative

abilities of individual species isolated from the community to grow on the wide range of test media was evaluated. A measure, the Colony Band-Width Index (CBI) was developed as a comparative aid. A high CBI indicates that a species has a wide range of organic tolerance, possibly no macro- or micronutrient requirements, or is nutritional quite versatile. The nutritional requirements and selectivity of media for individual species is also discussed. A rank order listing of media for the growth of individual species is given.

The distribution of many species in the community was seasonal. Distribution patterns were tested statistically. Many species showed high correlation with others in abundance or in their appearance in the community indicating possible common abiotic and biotic dependencies. These correlations are tabulated.

Perhaps the most significant advance is the development of new tools which we can use to isolate and study ecologically significant organisms in the laboratory. The new media and methods give promise that the functional interrelationships between diatoms from the same community assemblage may someday be accessible for detailed analysis. They may also be useful for the culture of many new species of protozoa and micrometazoan herbivores. The auxotrophic profile of the diatom assemblage suggests that some measure of stability within this important component of the community is achieved through heterotrophic diversity. The same might be suggested as a key factor in community succession.

9. John J. Lee and William A. Muller. 1975. Culture of salt marsh microorganisms and micrometazoa. In: Culture of Marine Invertebrate Animals (ed. W. L. Smith and M. H. Chaney). Plenum Press. pp. 87-107.

The purpose of the conference was to bring together experts on the culture of microorganisms and micrometazoa of northeastern U. S. estuarine and coastal waters. In our paper we discuss the biotic and

gnotobiotic cultures of salt marsh microorganisms and micrometazoa. The paper is sufficiently detailed so that a beginning graduate student could use it as a laboratory manual for the study of near shore bacteria, algae, protozoa and micrometazoa. We also elaborate on our experience in this field and give helpful hints on pitfalls to be avoided.

10. John J. Lee, Lawrence J. Crockett, Johnny Hagen and Robert J. Stone. 1974. The taxonomic identity and physiological ecology of Chlamydomonas hedleyi (the algal flagellate symbiont from the foraminifer Archaias angulatus). Br. Phycol. J. 9:407-422.

Symbiosis between algae and invertebrates is a significant phenomenon in the marine environment in which it occurs. Although symbiotic associations between foraminifera and various species of algae have been known for over 65 years, the taxonomic identity of any of the symbionts or their metabolic contributions to their hosts has never been clarified. In this paper we discuss the fine structure, taxonomic identity, and physiological ecology of a symbiotic alga from a foraminifera which we recently isolated into axenic culture. The symbionts has no vitamin or organic requirements but growth is 3 x in the presence of thiamin, and 2 x in the presence of 1 μM glutamic acid, histidine and methionine. Urea was the best nitrogen source. The symbiont fixed $\sim 7 \times 10^{-7}$ M carbon $hr^{-1} gm^{-1}$, 57% of which was released into the medium as a chromatographically homogeneous organic molecule provisionally identified as mannitol.

11. Norman M. Saks, John J. Lee, William A. Muller and John H. Tietjen. 1974. The growth of salt marsh microcosms subjected to thermal stress. Presented at the Thermal Ecology symposium. In: Thermal Ecology, AEC Symposium Series, pp. 391-398.

In this paper we discussed some of our preliminary experiments on the effects of temperature and interspecific competition between pairs of organisms, a marine benthic feeding ciliate, *Euplotes vannus*, and either *Allogromia laticollaris*, a foraminifera, or *Chromadorina germanica*, a benthic nematode. At 15° C, neither *Allogromia* nor *Chromadorina* reproduce in the presence of *Euplotes* but the small numbers in the inoculum reduce *Euplotes* populations to $\leqslant$ 1/2 that of controls. At 25° C each of the species was affected by the presence of another species. This was reflected by changes in both amplitude and phase of populations. The effect of temperature on the growth and survival of their food was also studied.

12. John J. Lee. 1974. Towards understanding the niche of foraminifera. In: Foraminifera: (R. H. Hedley & G. Adams, eds). pp. 207-260. Academic Press, London & New York.

In this chapter, many facets of the niches of salt marsh foraminifera are discussed. The point of the review is to give a microbiologist's microscale perspective of the functional roles of a few littoral benthic foraminifera in their communities and the methods which can be used to gain insight into this aspect of their biology. Both field and laboratory approaches are stressed in the review. Patchiness, substrate preferences, seasonality, culture methods, nutrition and growth factors, interspecific and intraspecific competition, ecological efficiency and symbiosis in foraminifera are discussed in detail.

13. G. F. Coccetti and John J. Lee. 1977. The potential effects of energy related activities on the seasonal trajectories of benthic marine diatoms. Accepted for presentation at the Savannah River Ecology Symposium on: Energy and Environmental Stress in Aquatic Systems, Nov. 2-4, 1977. Augusta, Ga.

One of the most difficult problems in studying the cause and effects of changes in natural communities is to separate various types of community influencing factors without doing violence to the community itself. Manipulation of diatom assemblages in situ offered us an attractive opportunity to study equilibrium trajectories of different natural populations under the same environmental conditions and the replicate populations under chronic stress. The conditions of the experiment excluded the possible regulatory effects of spatial heterogeneity, recruitment and selective consumption.

In one set of experiments the effects of water quality were tested on the development of inocula from grossly similar but fairly distant locations. In another set, the native inoculum was incubated in the continual presence of Fe, Cu, Cr, Pb, Zn and crude oil. Seasonal succession took place in all of the control and experimental communities. During the course of succession the trajectories of assemblages under some conditions converged while others diverged. Since the random fluctuations in the environment were constant for all assemblages, the abundances of individual species were due to niche packing and interspecific restraints on the biotic potential of selected diatom species as well as the change in water quality. The most diverse community was an experimentally mixed one. The high diversity of this community was probably due to niche packing, a phenomenon which restricted the realized niches of many species and hindered their blooming. Paradoxically twice as many species (40%) had their greatest niche breadth in the mixed community as did species in the tranplanted (~ 20%) or native controls (17%). Enrichment of the incubation medium with heavy metals

and oil altered the community structure and diversity of most experimental vessels. Growth of some species was depressed while that of others was enhanced. The trajectories of the communities enriched with oil and Pb were quite similar throughout the summer. The Cu and Cr assemblages initially followed the trajectory of oil and Pb but later diverged. Greatest species diversity was consistently found in the community incubated in the presence of Cu. The Fe enriched community generally diverged from all the others. Recurrent group analysis identified distinctive groups of species for each of the experimental assemblages as well as controls.

The amount of work in making each biological assessment is a serious limiting factor. There may be many possible trajectories for each diatom assemblage. We must increase the number of replicates in different experimental contexts to give greater insight into the stochastic and historical aspects of assemblage trajectories. If we knew the ecological consequences of driving the trajectories of the diatom communities toward favoring the abundances of the particular groups of species we have identified through recurrent analysis we could attach significance to this aspect of our results. In final analysis the ecological significance of individual species can only be measured in assemblage and community contexts over wide ranges of environmental conditions and community histories.

The <u>in situ</u> semi-holistic experimental approach which we have used here seems to hold promise of eventually gaining the insights we are looking for. Connell (1976) in his recent review endorses this type of controlled field experiments for revealing the extent of biological interactions. The trick, as he emphasizes, is to make sure that all the environmental and biological factors, except the ones being tested, vary to the same degree in the experimental and control sites. Although there are many variables which have to be properly evaluated, including interactions with consumers, recruitment, and spatial heterogeneity, there are only finite possible combinations of organisms in functioning communities. Even if it were possible to throw together a large mixed assortment of algae and small animals only certain combinations

of organisms would emerge in time and function together. These contextual relationships are the substance which form realized niches.

Though the pathways toward developing realistic synthetic conceptualizations of the structural-functional relationships in communities of small organisms seem very long at this time, the ways are at least approachable, and progress can be made.

14. John J. Lee and Michael Grofik. 1977. A note on the effect of mechanical shear on meiofaunal viability. Ms accepted for presentation at the Savannah River Ecology Symposium on: Energy and Environmental Stress in Aquatic Systems. Nov. 2-4, 1977. Augusta, Ga.

During the course of procedures involving the gnotobiotic isolation and aseptic transfer of meiofauna we have often had the opportunity to observe the animals under the microscope. The manipulation of many meiofaunal species by conventional aseptic techniques often results in reduced meiofaunal viability. This is a very important consideration since many of our experiments are quantitative and one must be aware that techniques which work so well with other organisms must be modified for meiofauna. Since turbulence and shear are fairly common natural phenomena in the littoral and sublittoral zones from which our organisms are isolated, we felt that there was need for more formal examination of this phenomenon.

Three of the 5 species of meiofauna tested were extremely sensitive to the shear stress to which they were exposed in the experimental apparatus. The most sensitive organism was Chromadorina germanica (50% of the animals were killed by shear stress as low as 13 mdynes/mm$^2$). It took 16, 24 and 49 mdynes/mm$^2$ to respectively kill half of the Nitocra, Leptocaris and Diplolaimella tested. In terms of velocity 50% of the 3 most sensitive species were killed with exposure to velocities between 40-70 mm/sec. We realize that our experiments have modeled only a very simplified dissipative process compared with the turbulent mixing layers

likely to be encountered in natural situations. However, we feel that we have demonstrated that in spite of evidence of physiological robustness many meiofaunal species are mechanically quite fragile. This sensitivity to shear stress undoubtedly is a factor which affects the distribution of organisms in the field. It also follows that in the design of engineering projects, where large numbers of meiofauna might be affected, some provision for the lessening of turbulent mixing might diminish the impact on this important group of animals.

15. W. A. Muller and J. J. Lee. 197-. The effects of stress on meiofauna. Ms accepted for presentation at the Savannah River Ecology Symposium on Energy and Environmental Stress in Aquatic Systems, Nov. 2-4, Augusta, Ga.

The effects of potential power plant effluent related chronic or acute stresses upon small marine animals (two species of littoral benthic foraminifera, Allogromia laticollaris and Rosalina leei; a marine ciliate Euplotes vannus, and a nematode, Chromadorina germanica) were studied. Exposure to 30° C was lethal for juveniles of A. laticollaris and R. leei; some of the juvenile nematodes survived; and ~ 75% of the ciliate cultures grew almost normally. At 30 ° C fecundity of three of the animals was also reduced significantly (1.11 to 11.03% of controls). R. leei was most inhibited by heavy metals (0-77% of control at 1 to 10 ppm) and E. vannus least affected (20.48 to 102.39% of control at 1 to 10 ppm). A. laticollaris and R. leei cultures are most significantly inhibited by crowding while this stress has little or no effect upon E. vannus and C. germanica. Evidence suggests that wetlands management strategies can be developed to minimize the negative effects of power plant related stresses upon salt marsh community productivity and structure.

16. William A. Muller and John J. Lee. 197-. Biological interactions and the realized niche of Euplotes vannus from the salt marsh aufwuchs. Ms. accepted for publication in J. Protozool.

Euplotes vannus, a hypotrich ciliate, grows well over broad ranges of temperature and salinity. E. vannus requires higher densities of food (>1 x $10^4$ cells/ml) for rapid reproduction than do the other herbivores, the foraminiferan Allogromia laticollaris (>1 x $10^2$ cells/ml) and the nematode Chromadorina germanica (> 1 x $10^3$ cells/ml), to which it was compared. If food levels were initially very high (~ 1 x $10^8$ cells/ml) the ciliates reproduced rapidly and consumed the algae faster than it could reproduce. Some balance between the algae and the ciliates was achieved at initial algal concentrations of ~ 1 x $10^5$ cells/ml. In microcosm experiments at 25° C with equal numbers of C. germanica and A. laticollaris, E. vannus proved to be a very poor competitor; reaching only 20% of control levels when grown with C. germanica and only 13% when cultured with A. laticollaris. It was a better competitor in 2-species microcosms, at lower temperatures, and when its ratio to the other species was initially higher.

The experimental evidence suggests that E. vannus is best adapted to being a migrating initial colonizer of fresh algal blooms.

17. Howard Rubin and John J. Lee. 197-. A view of operon at the ecosystem level. Ms submitted, J. Theoret. Biol.

Within the last several decades, molecular and cellular biologists have drawn attention to adaptive mechanisms whereby cells retain the ability to produce metabolic enzymes without the energetic costs of consistently manufacturing them. The energy thus saved is utilized for other functions. This paper is an extension and generalized conceptualization of the microcosm experiments with 2 species of marine ciliates in which we made parallel measurements of information and calorific

transfers (in this progress report summarized earlier). The model ciliates are automata which perform many steps concurrently. Viewed as a whole the digestive and intermedia ry metabolic processes of the model ciliate appear to be a composite of many functioning operons.

In simulations in which a single ciliate species was placed in an environment containing a food of high informational value in patches and another food, randomly distributed, low informational content, the model ciliates fed selectively on the better food. When the reverse food distribution was simulated, results were similar. When both of the foods were available in the environment in a random distribution, the ciliate population was less productive than it was in the patchy environment. When two species of ciliates with different information processing abilities were placed in patchy environment of two foods, each of high value to only one ciliate, only one ciliate was able to achieve full biotic potential. Competition for a single food of high informational value distributed in patches resulted in population distributions and population sizes that were a function of inoculum size, generation time, and a random variable. Competition for a single randomly distributed food of high informational value to both ciliates resulted in a rapid extinction of both populations.

A natural environment for the two organisms was simulated by having five food species of different informational value present; three of the foods were close to optimal for one ciliate, one optimal for the other, and one of high value to both. This supported populations of both organisms at all times, but the combined population was less than that which could be supported when only a single species was growing on the patch. When the same foods were randomly distributed the specialist was not able to exploit its food source because of the interference in switching caused by coincidence with other foods.

Secondary productivity in our model was directly linked to temporal and spatial distribution of various food organisms. Simulated environments with patchy food distribution were as much as 60 times more productive than environments in which the algae were randomly distributed. Since patchiness is a quality of many aquatic communities one wonders

if it is not a mechanism for lowering competition and increasing diversity of the habitat to provide more niches for highly specialized and very productive organisms. This is logical if optimization principles govern ecosystem development. This is not meant to deemphasize the importance of the opposite situation which has some of the same optimal characteristics. Uniform, almost pure, monocultures of some plants and their consumers are as highly productive as patchy ones and for the same reason.

In our simulations, interpatch distance directly affected secondary productivity through switching and inter- and intraspecific competition. When patches of different species of food are too close, switching can become important. Because of recent advances in the molecular biology of ciliates and bacteria, it is easy to understand some of the functional bases for switching in protists; whether switching can be an important factor in metazoa is still in question.

## II. WORK IN PROGRESS

In addition to the completed or nearly completed projects briefly summarized in the previous section and appended to this report, many subprojects are in progress. For the sake of continuity, progress to data on all incomplete subprojects will be summarized here.

A. Comparative quantitative and qualitative studies on the role of diverse meiofaunal species in the detritus food chains of marine marshes including:

a) mineral cycling, energy flow, biological half-life, trophic efficiency estimations;
b) food web complexion studies and "special growth factors";
c) estimations of biotic potential and realized productivity

The aim of the subproject is to gain comparative insight into the functional complexities of micro- and meiofaunal food webs and thereby obtain some reasonable bases of interpreting some of the changes which take place, or could take place, in stressed communities. During the past year, a number of separate, but related, studies were directed toward these goals.

Although there is great need for comparative microherbivore niche studies, and for more than 1 year we have now maintained 6 unstudied species of newly cultured harpacticoid copepods (*Paramphiascoides* spp, *Tachidius discipes*, *Mesochra* cf *lilljeborge*, *Heterolaephonte stromi* and *Harpacticus* sp), we chose to follow up on some interesting issues raised by our paper on *Nitocra typica*. We found (Lee *et al*. 1976) that, although this animal is a nutritional specialist, temperature affects the nutritional requirements of various life cycle stages so that it is flexibly adapted to feed on different species and strains of algae which are likely to occur in the community at different seasons. New studies completed this year utilized a greater variety of potential food

organisms adapted for living in aufwuchs and benthic communities at different seasons of the year. The results of these studies indicate that the animal has an interesting blend of "r" and "k" characteristics. (See Tables 1 and 2 and Figs. 1-28).

At 5° C, characteristic of mid-winter *in situ* temperatures, adult *Nitocra* maintained themselves on certain algal diets but did not reproduce. When gravid females were transferred to 5° C their egg sacs detached but the eggs did not hatch. If the females were re-transferred to higher temperatures they produced new egg sacs which hatched. Occasionally egg sacs, detached from females at 5° C, hatched when placed in higher temperature incubators. The females, and frequently the egg sacs also, remained viable for up to 4 months. The lowest temperature at which a life cycle could be completed was 10° C. At this minimum temperature the intrinsic rates of increase of copepods grown on diets of winter strains of algae were no greater than on diets of summer strains. On the other hand, reproduction was generally better at higher temperatures on diets of summer isolates than winter ones (Tables 1 and 2). Rates of population increase roughly doubles as temperature was increased 10° C.

Table 1 . Intrinsic rate of increase (r)[1] of Nitocra typica on different diets at 10, 15, 20, and 25C .

| Species | 10C | 15C | 20C | 25C |
|---|---|---|---|---|
| Cylindrotheca closterium (9) | 0.043 | 0.086 | 0.119 | 0.175 |
| Cylindrotheca fusiformis (B1-27) | 0.048 | 0.094 | 0.102 | 0.164 |
| Nitzschia sp. (Pb-7) | 0.049 | 0.101 | 0.103 | 0.133 |
| Cylindrotheca sp. (8) | 0.055 | 0.080 | 0.112 | 0.161 |
| Phaeodactylum tricornutum (39) | 0.015 | 0.061 | 0.030 | 0.129 |
| Cylindrotheca closterium (Amp.) | 0.040 | 0.087 | 0.110 | 0.126 |
| Achnanthes sp. (110) | 0.051 | 0.088 | 0.114 | 0.119 |
| Cylindrotheca closterium (709) | 0.046 | 0.060 | 0.172 | 0.173 |
| Dunaliella parva (14) | 0.051 | 0.078 | 0.091 | 0.147 |
| Chlorococcum sp. (38) | 0.029 | -- | 0.054 | 0.112 |
| Nannochloris sp. (41) | 0.030 | -- | 0.079 | 0.149 |
| Dunaliella sp. (55) | -- | 0.054 | 0.059 | 0.016 |
| Dunaliella sp. (98) | 0.014 | 0.020 | -- | -- |
| Unidentified chlorophyte (105) | -- | -- | -- | -- |
| Chlamydomonas sp. (BL-1) | 0.052 | 0.095 | 0.113 | 0.172 |
| Chlamydomonas hedleyi (Arch) | 0.044 | 0.063 | 0.094 | 0.121 |
| Unidentified chlorophyte (50) | 0.048 | 0.079 | 0.092 | 0.094 |
| Navicula salinarum (W503) | -- | 0.082 | -- | 0.114 |
| Amphora coffeaeformis (W504) | 0.009 | 0.063 | 0.082 | 0.148 |
| Navicula sp. (W505) | 0.032 | 0.065 | 0.076 | 0.095 |
| Navicula salinarum (W506) | 0.018 | 0.063 | 0.082 | 0.140 |
| Cylindrotheca fusiformis (W507) | 0.009 | 0.089 | -- | 0.076 |
| Amphora sp. (W508) | -- | 0.050 | | 0.139 |
| Stauroneis salina (W509) | 0.049 | 0.064 | 0.074 | 0.157 |
| Nitzschia sp. (W510) | 0.038 | -- | 0.046 | 0.091 |
| Nitzschia frustulum (W511) | -- | 0.053 | 0.054 | 0.106 |
| Navicula cincta (W512) | 0.046 | -- | -- | 0.112 |

[1] $r = (1/t)\ln(N_t/N_o)$

r = intinsic rate of increase

T = total # days (10C - 78 days; 15C - 46 days; 20C - 37 days; 25C - 26 days)

$N_o$ = # organisms at beginning ($N_o$=2)

$N_t$ = # organisms at termination

Table 2. Intrinsic rate of increase (r) of *Nitocra typica* on mixed diets at 20° C.

| Species | r |
|---|---|
| *Nitzschia* sp (Pb-7) + *Chlamydomonas* sp (BL-1) | 0.195 |
| *Chlamydomonas hedleyi* (Arch) + *Cylindrotheca fusiformis* (BL-27) | 0.179 |
| *Cylindrotheca* sp (8) + *Chlorococcum* sp (38) | 0.173 |
| *Nitzschia* sp ((Pb-7) + *Achnanthes* sp (110) | 0.198 |

**Cylindrotheca sp.**
**(8)**

Fig. 1

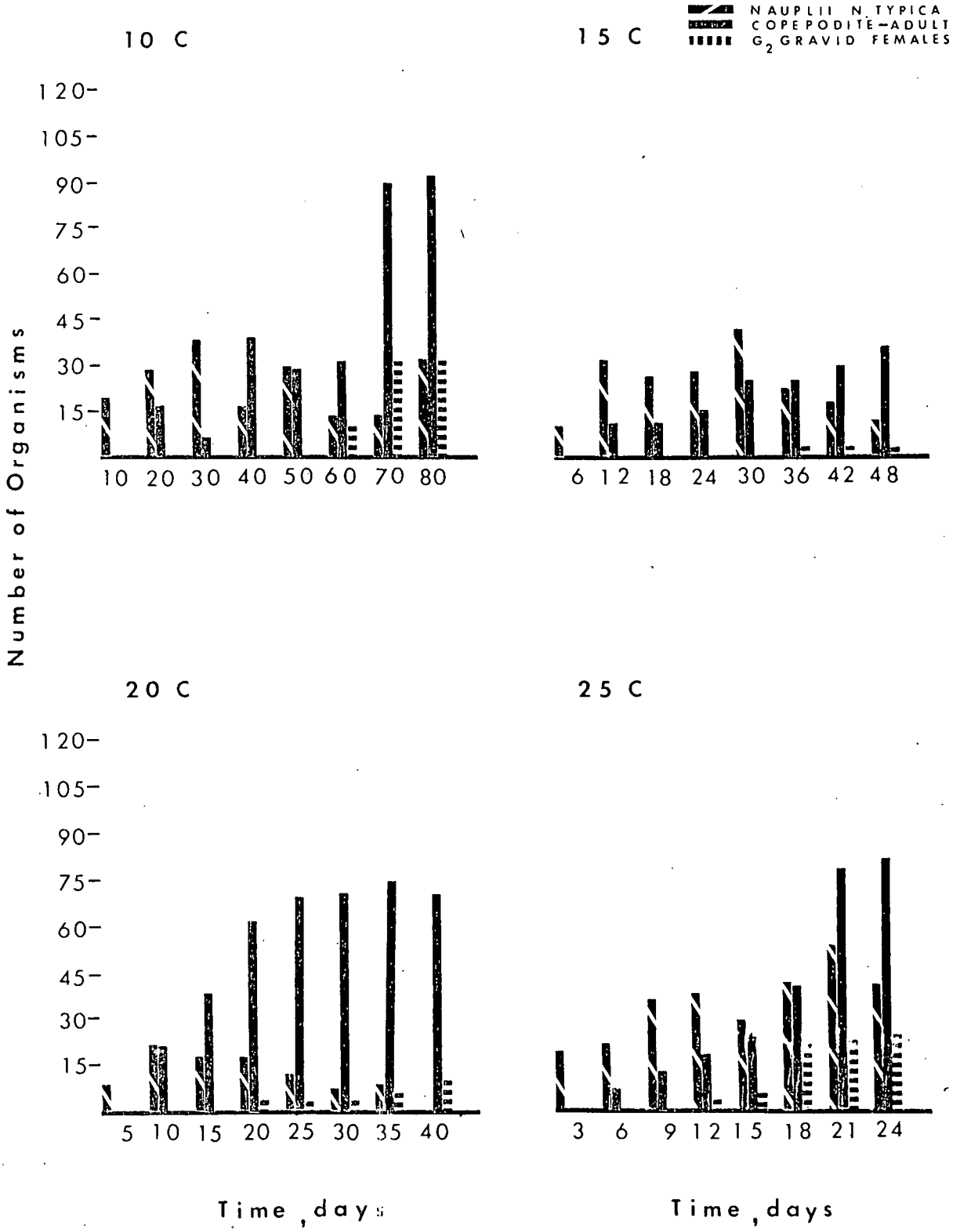


Fig. 2

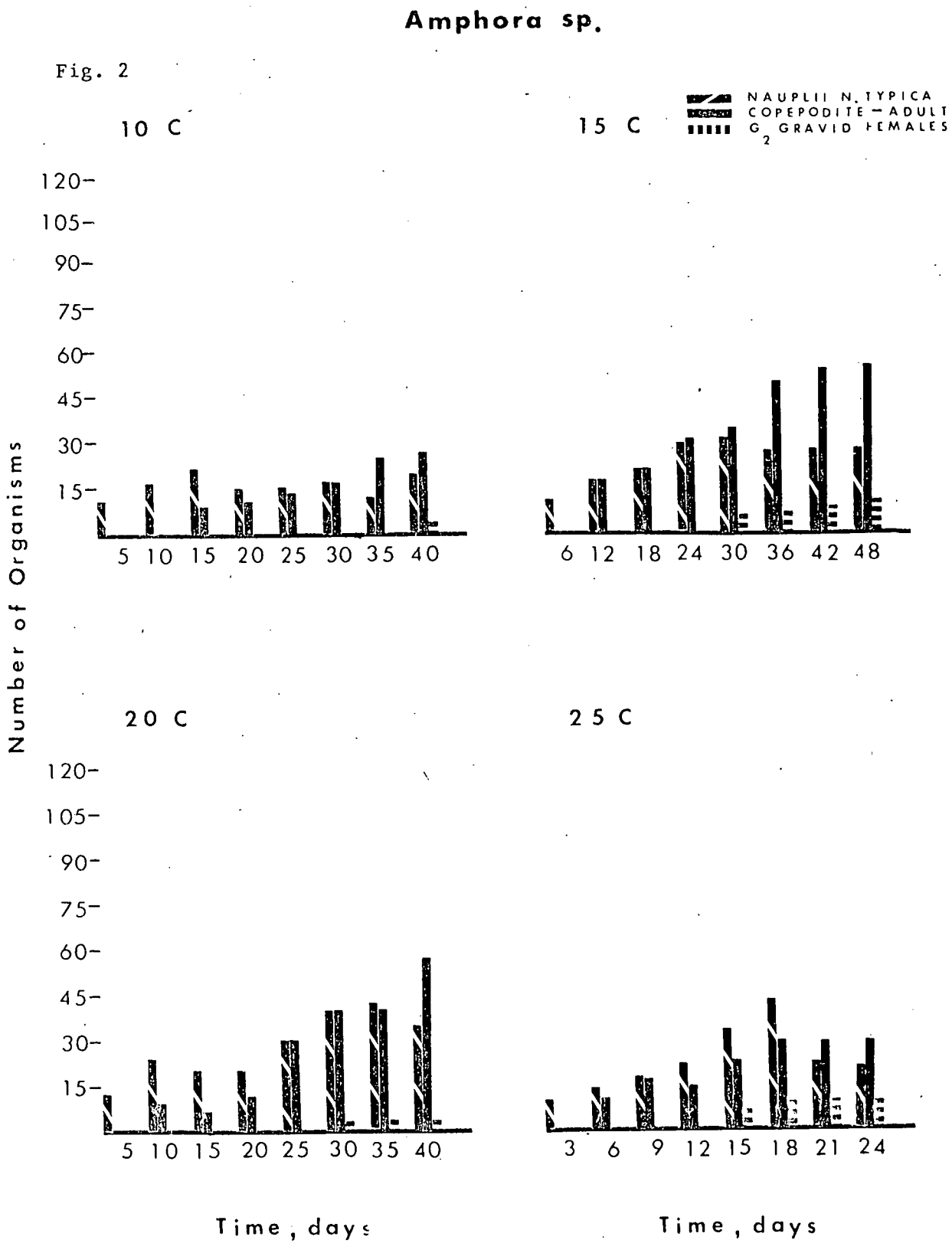

Amphora sp.
NAUPLII N. TYPICA
COPEPODITE – ADULT
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120
105
90
75
60
45
30
15
5 10 15 20 25 30 35 40
6 12 18 24 30 36 42 48
3 6 9 12 15 18 21 24
Time, days
Time, days

Fig. 3

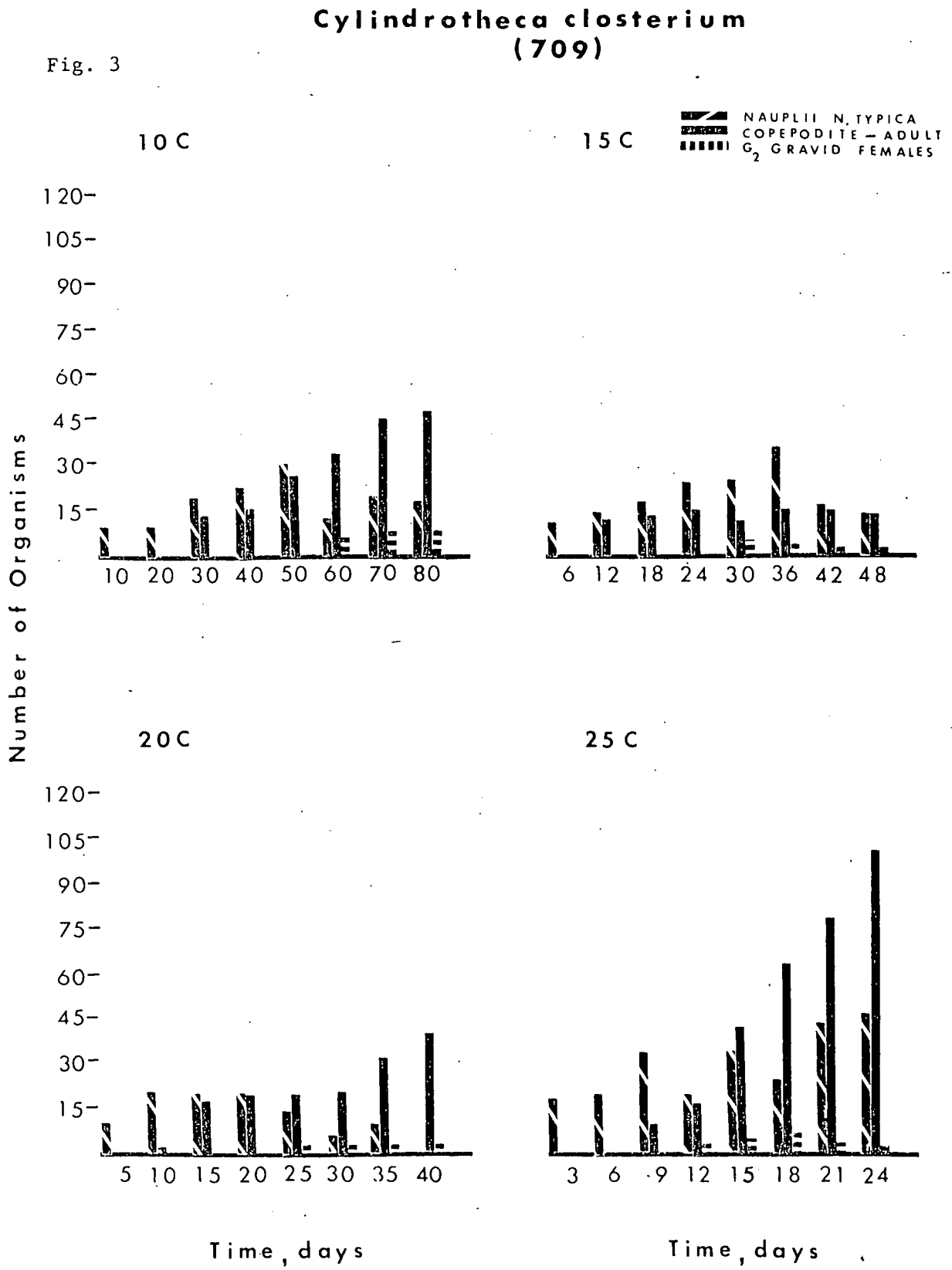

Cylindrotheca closterium
(709)
NAUPLII N. TYPICA
COPEPODITE – ADULT
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
5 10 15 20 25 30 35 40
3 6 9 12 15 18 21 24
Time, days
Time, days

Fig. 4

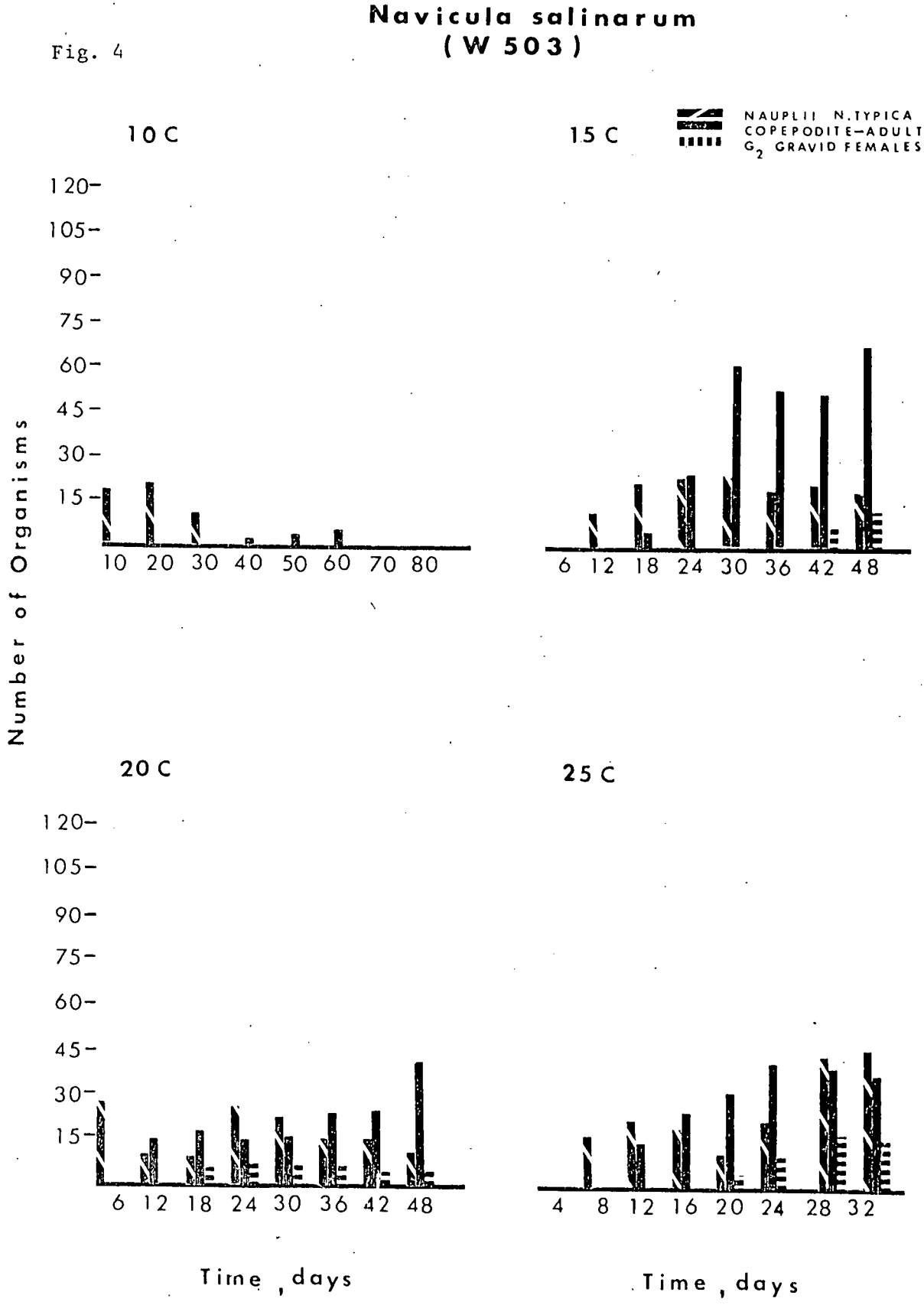

Navicula salinarum
(W 503)
NAUPLII N.TYPICA
COPEPODITE–ADULT
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
Time, days
Time, days

Fig. 5

**Amphora coffeaeformis**
**( W 504 )**

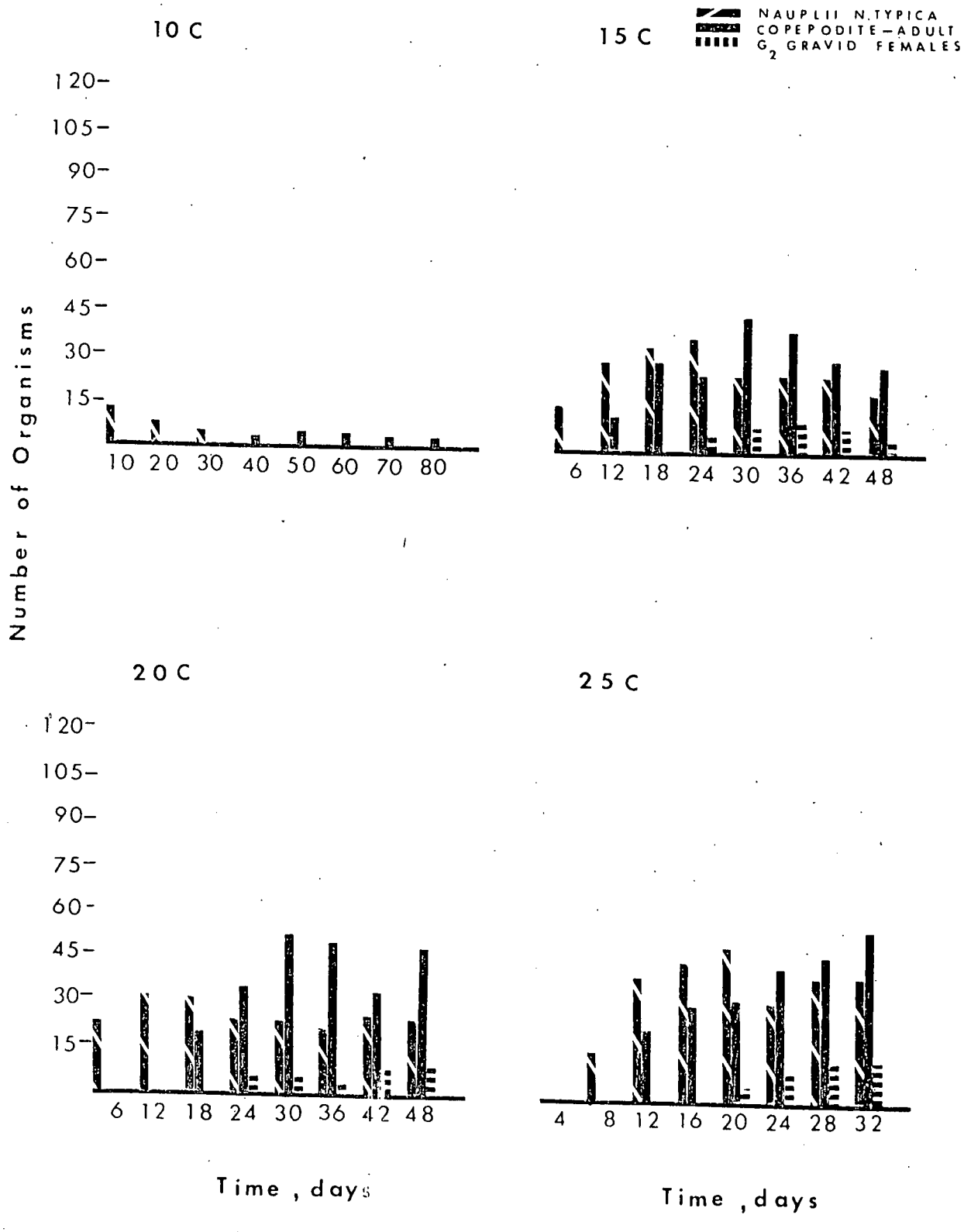


Fig. 6

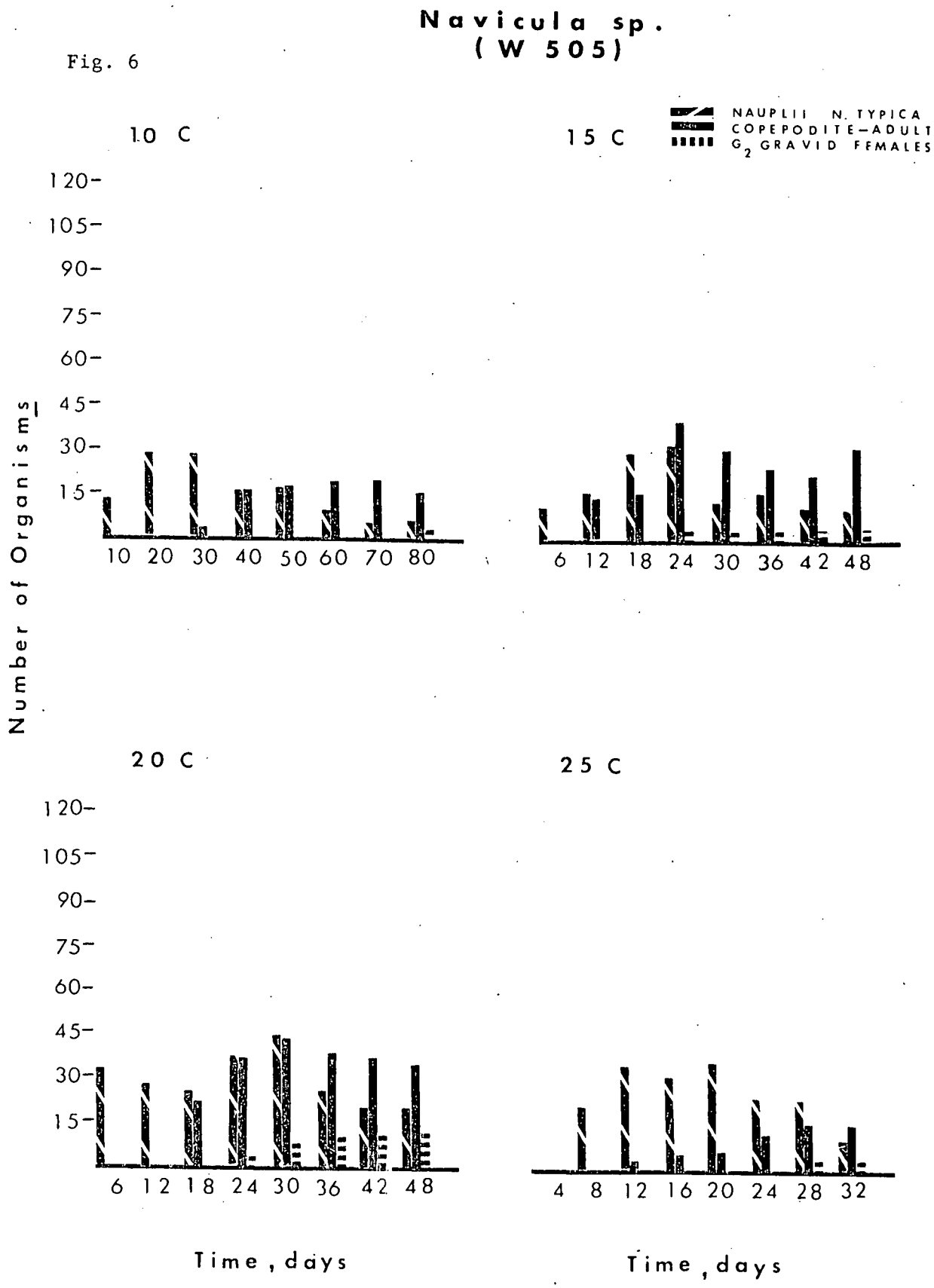

Navicula sp.
(W 505)
NAUPLII N. TYPICA
COPEPODITE–ADULT
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
Time, days
Time, days

Fig. 7

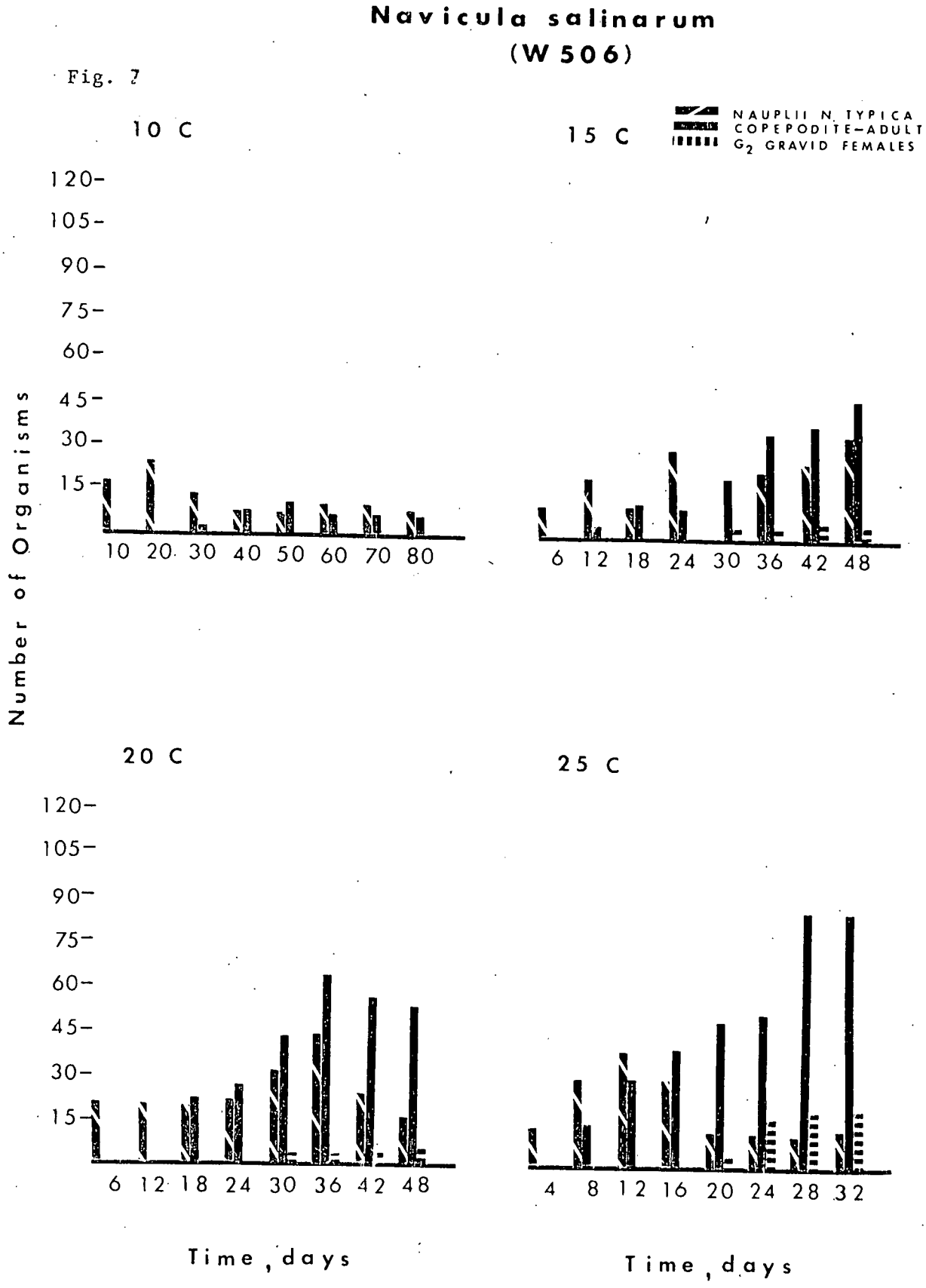

Navicula salinarum
(W 506)
NAUPLII N. TYPICA
COPEPODITE-ADULT
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
Time, days
Time, days

Fig. 8

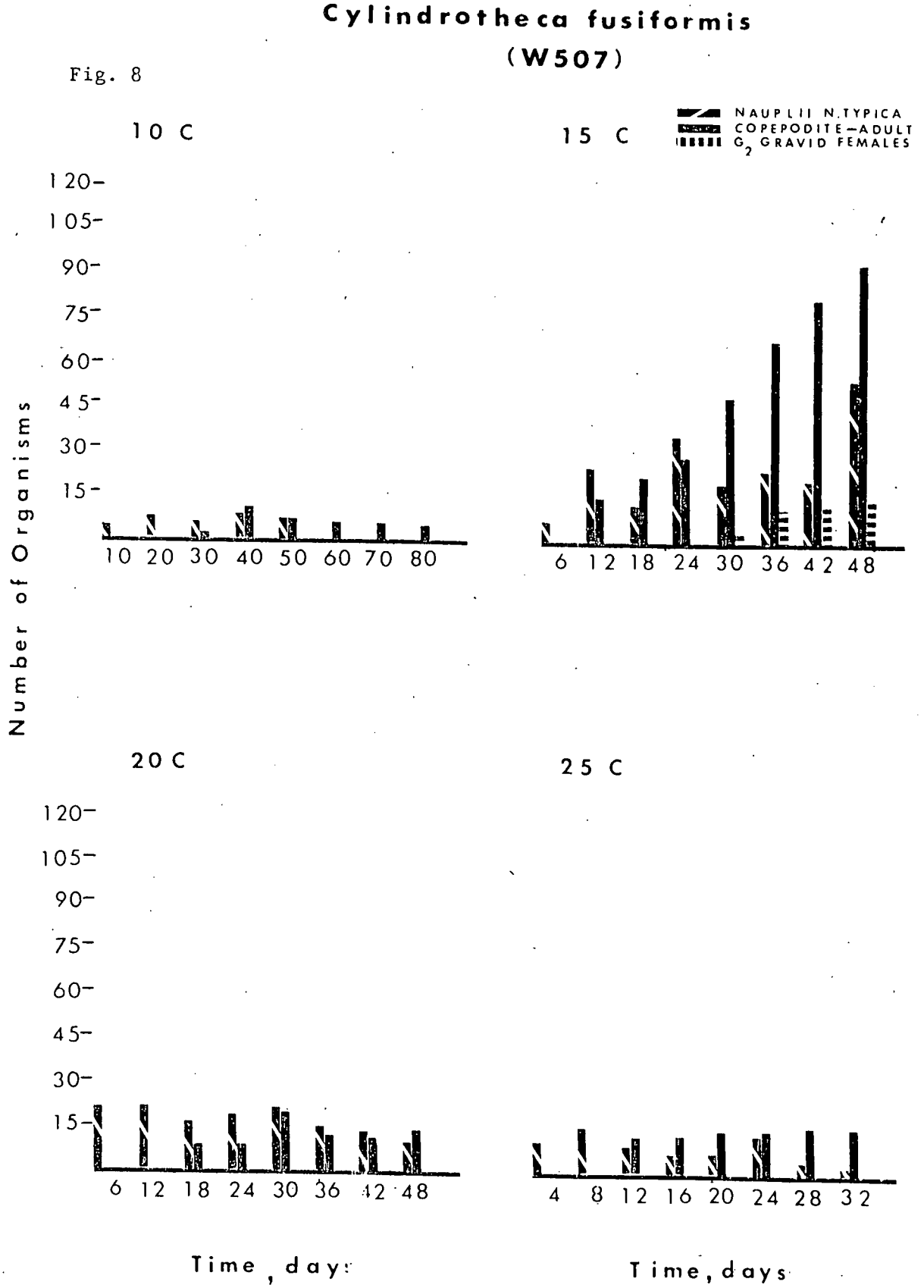

Cylindrotheca fusiformis
(W507)
NAUPLII N.TYPICA
COPEPODITE–ADULT
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
Time, days
Time, days

Fig. 9

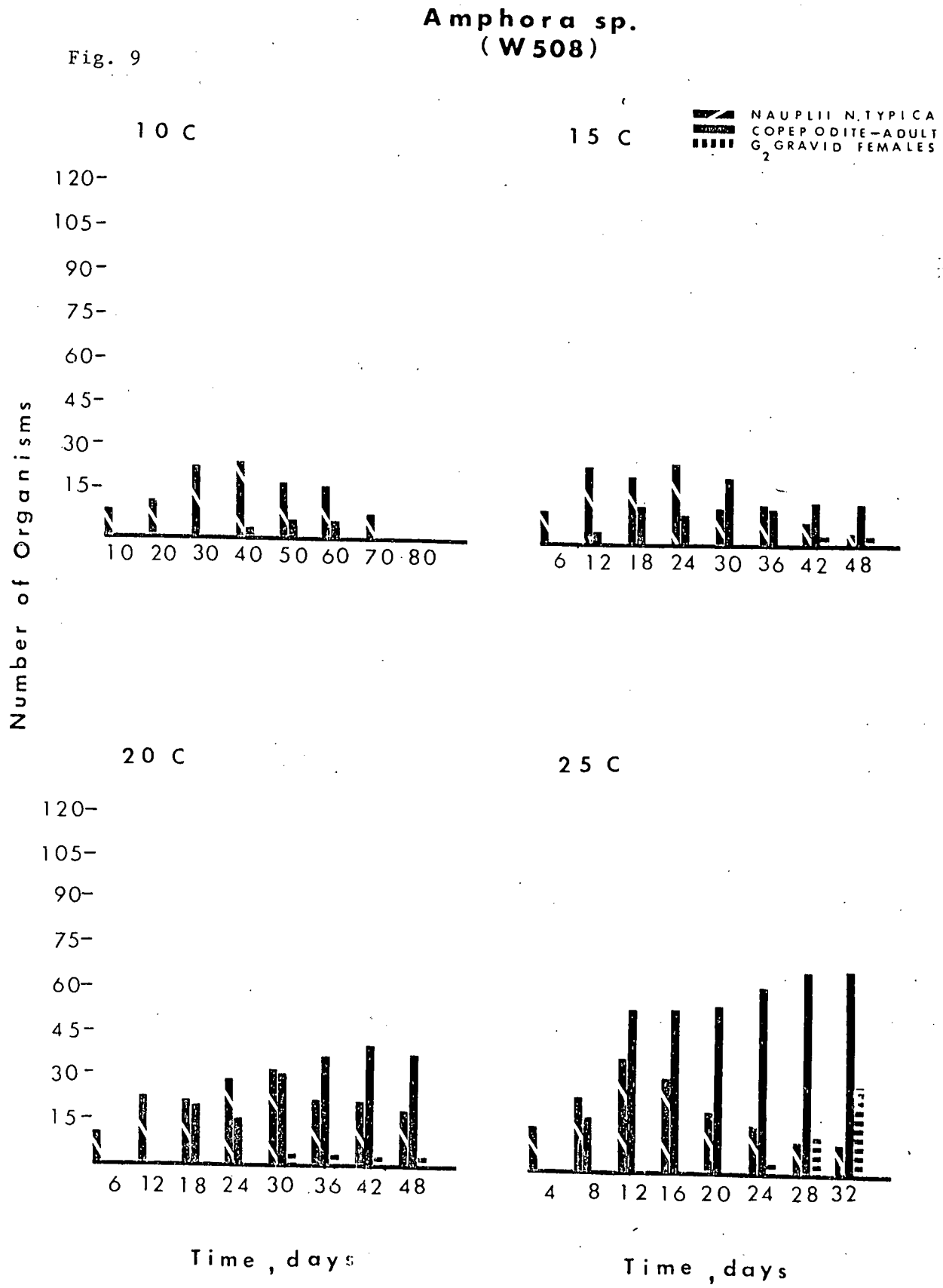
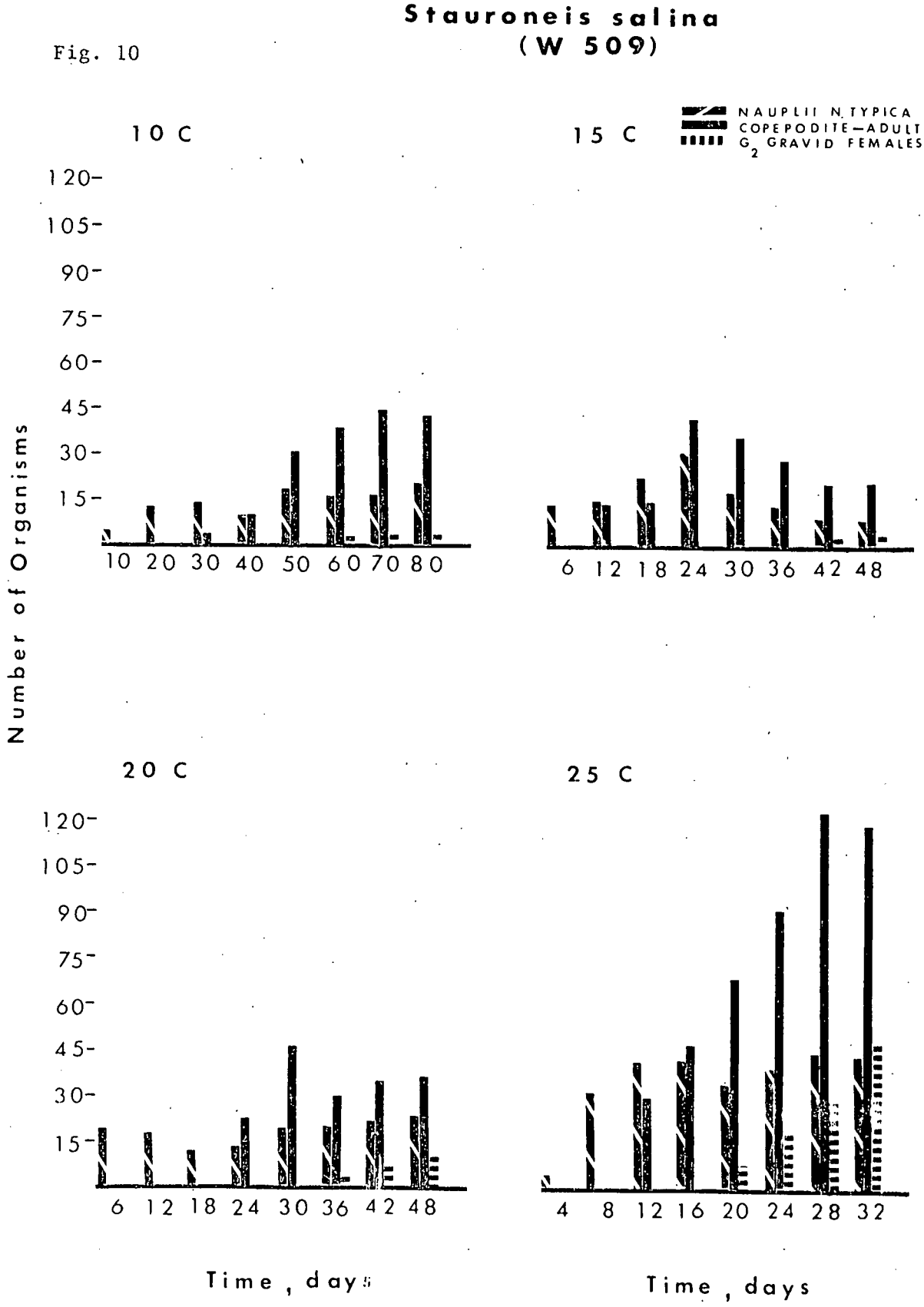

Amphora sp.
(W508)
NAUPLII N.TYPICA
COPEPODITE-ADULT
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120-
105-
90-
75-
60-
45-
30-
15-
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
Time, days
Time, days

Fig. 10


Stauroneis salina
(W 509)
NAUPLII N. TYPICA
COPEPODITE—ADULT
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
Time, days
Time, days

**Nitzschia communis var.**
**(W 510)**

Fig. 11

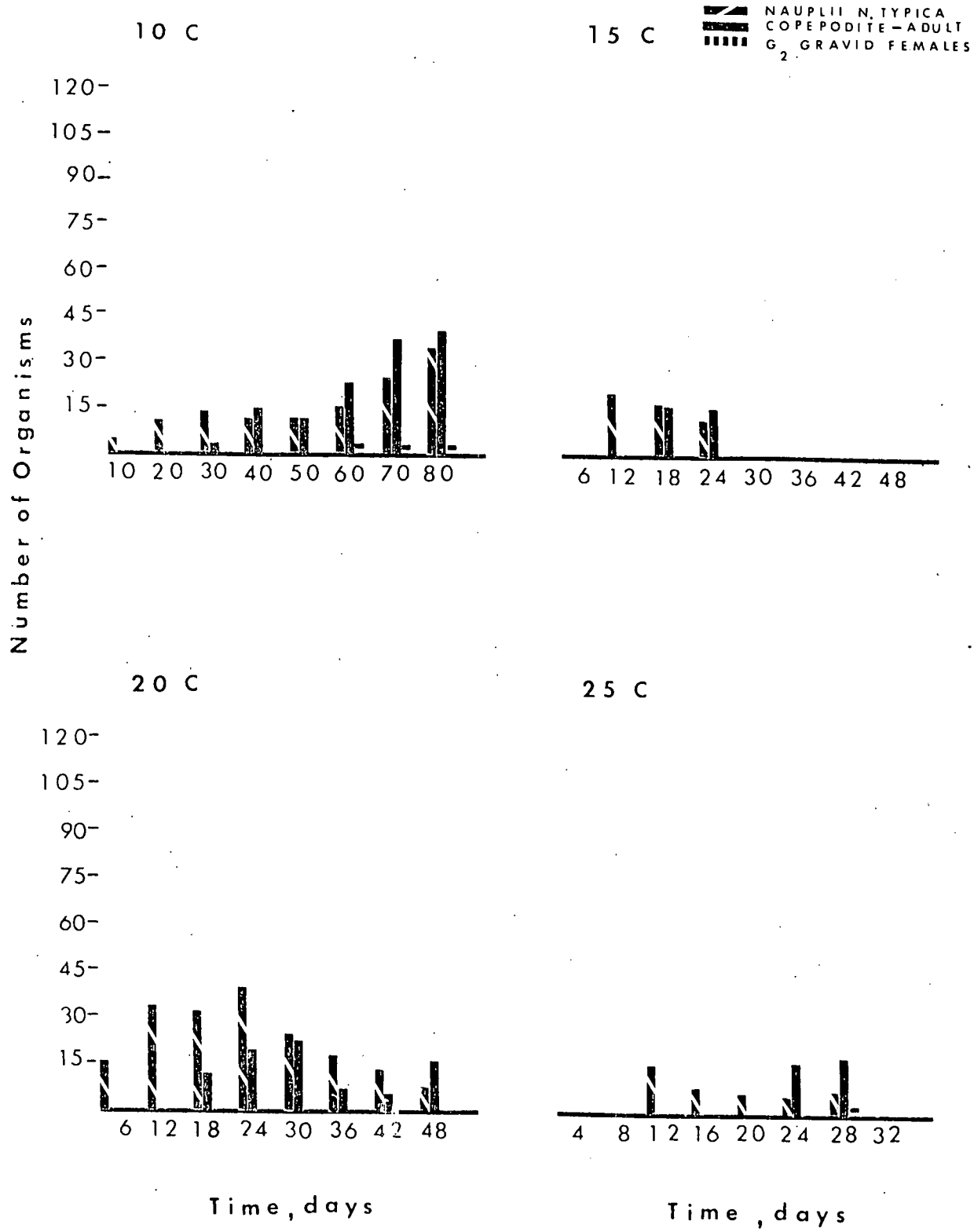


Fig. 12

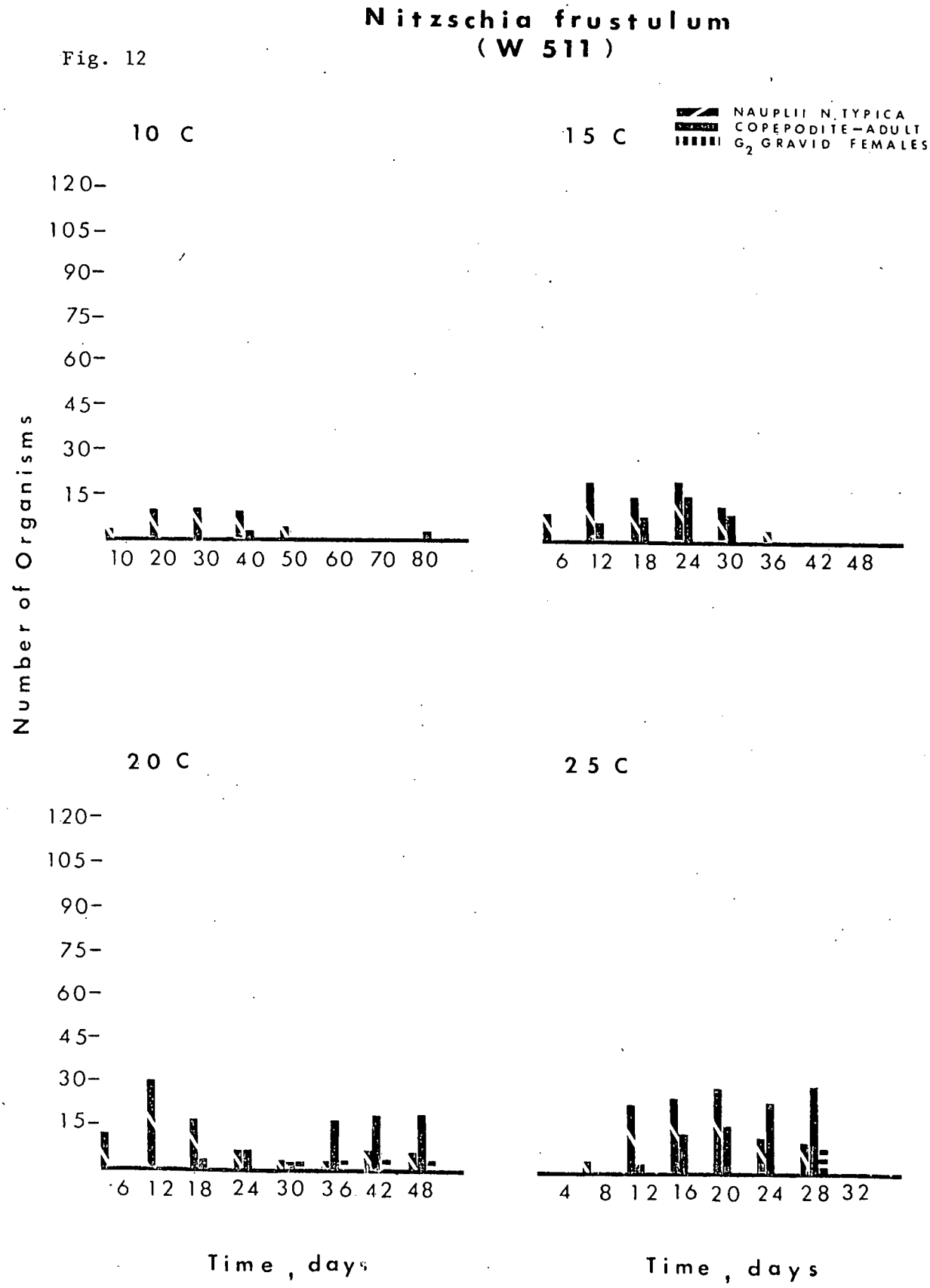

Nitzschia frustulum
(W 511)
NAUPLII N. TYPICA
COPEPODITE–ADULT
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
Time, days
Time, days

Fig. 13

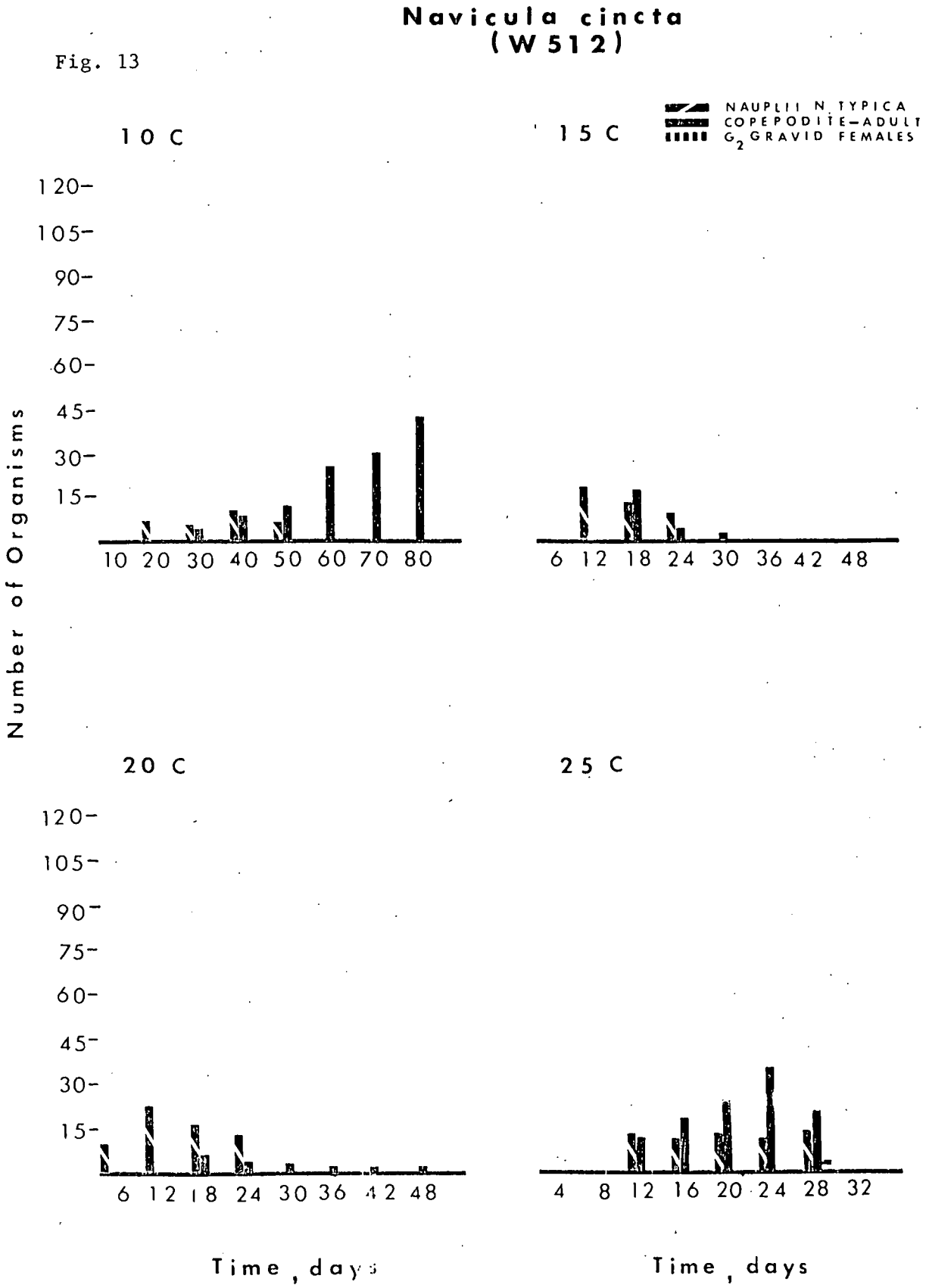

Navicula cincta
(W 512)
NAUPLII N. TYPICA
COPEPODITE–ADULT
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
Time, days
Time, days

Fig. 14

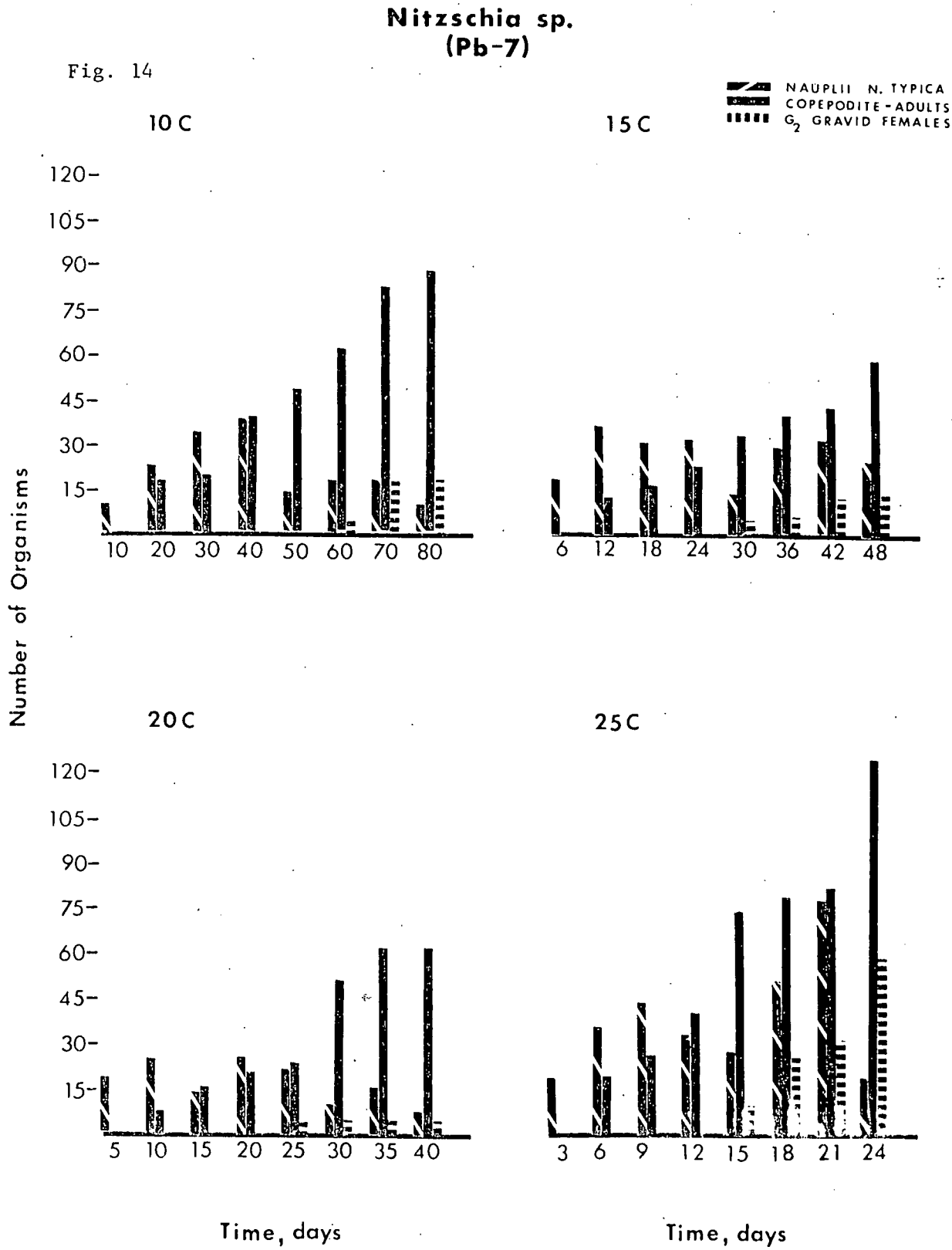
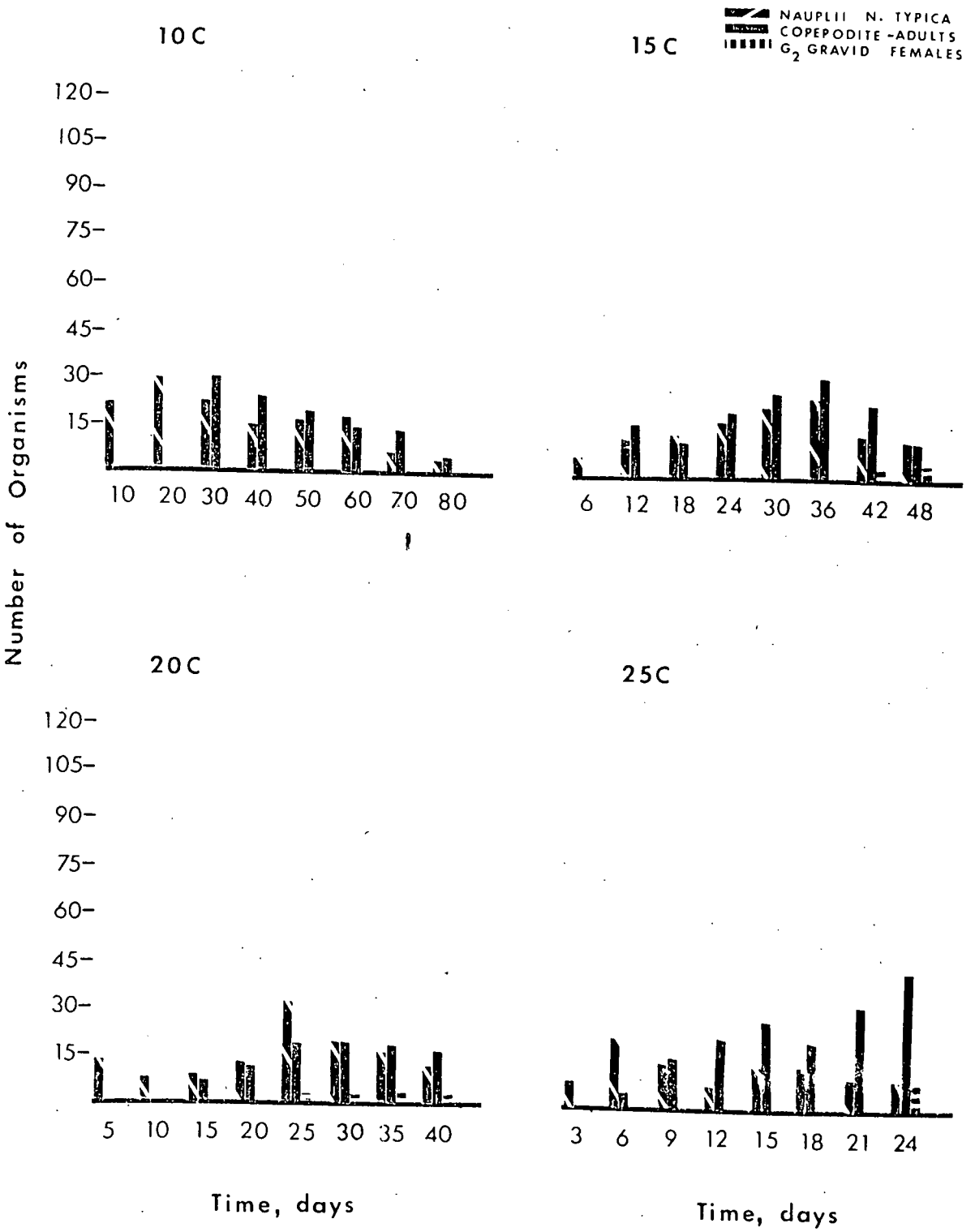

Nitzschia sp.
(Pb-7)
NAUPLII N. TYPICA
COPEPODITE - ADULTS
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120-
105-
90-
75-
60-
45-
30-
15-
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
5 10 15 20 25 30 35 40
3 6 9 12 15 18 21 24
Time, days
Time, days

Fig. 15

**Phaeodactylum tricornutum**
**(39)**



**Cylindrotheca closterium**
**(9)**

Fig. 16

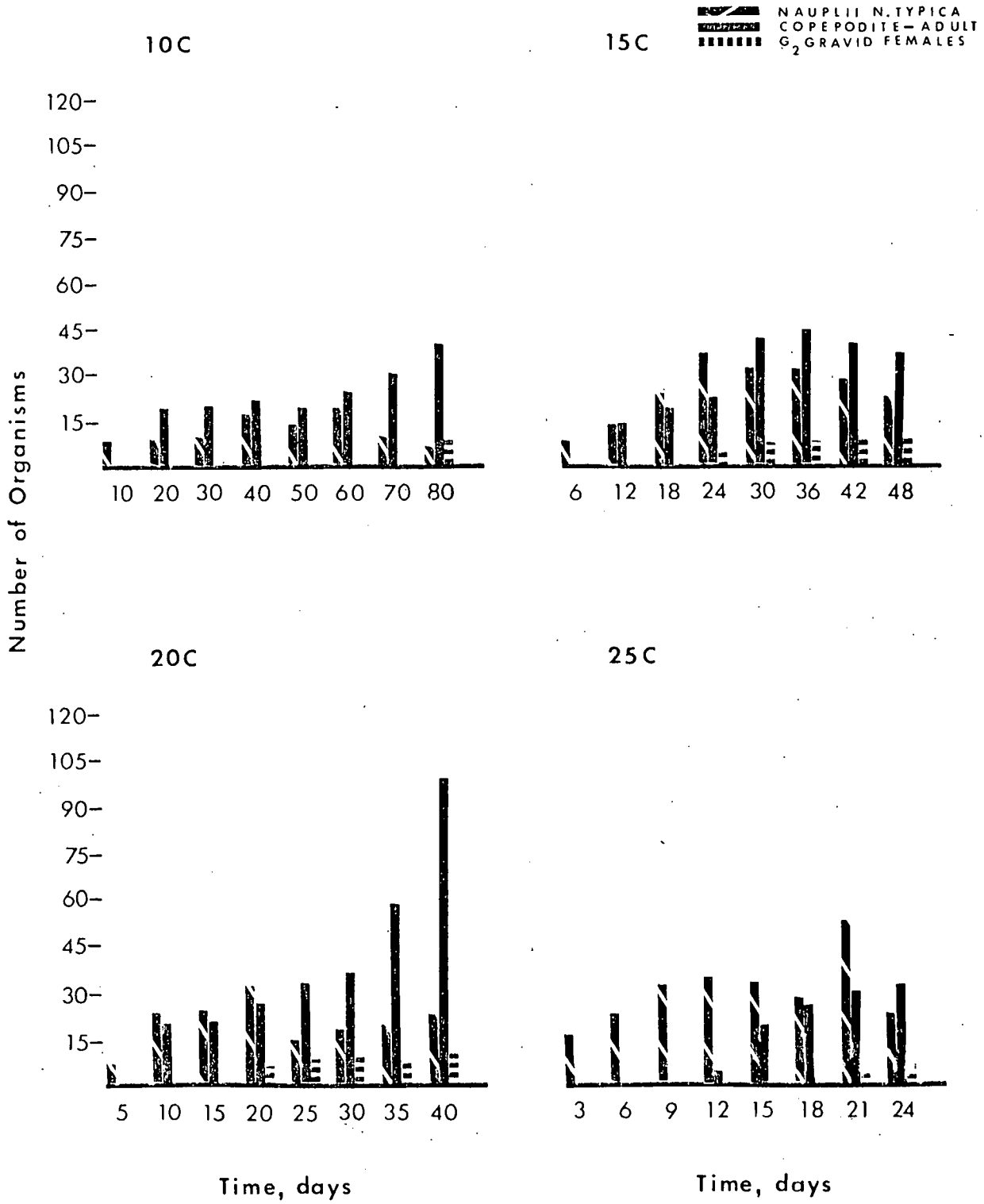


Fig. 17

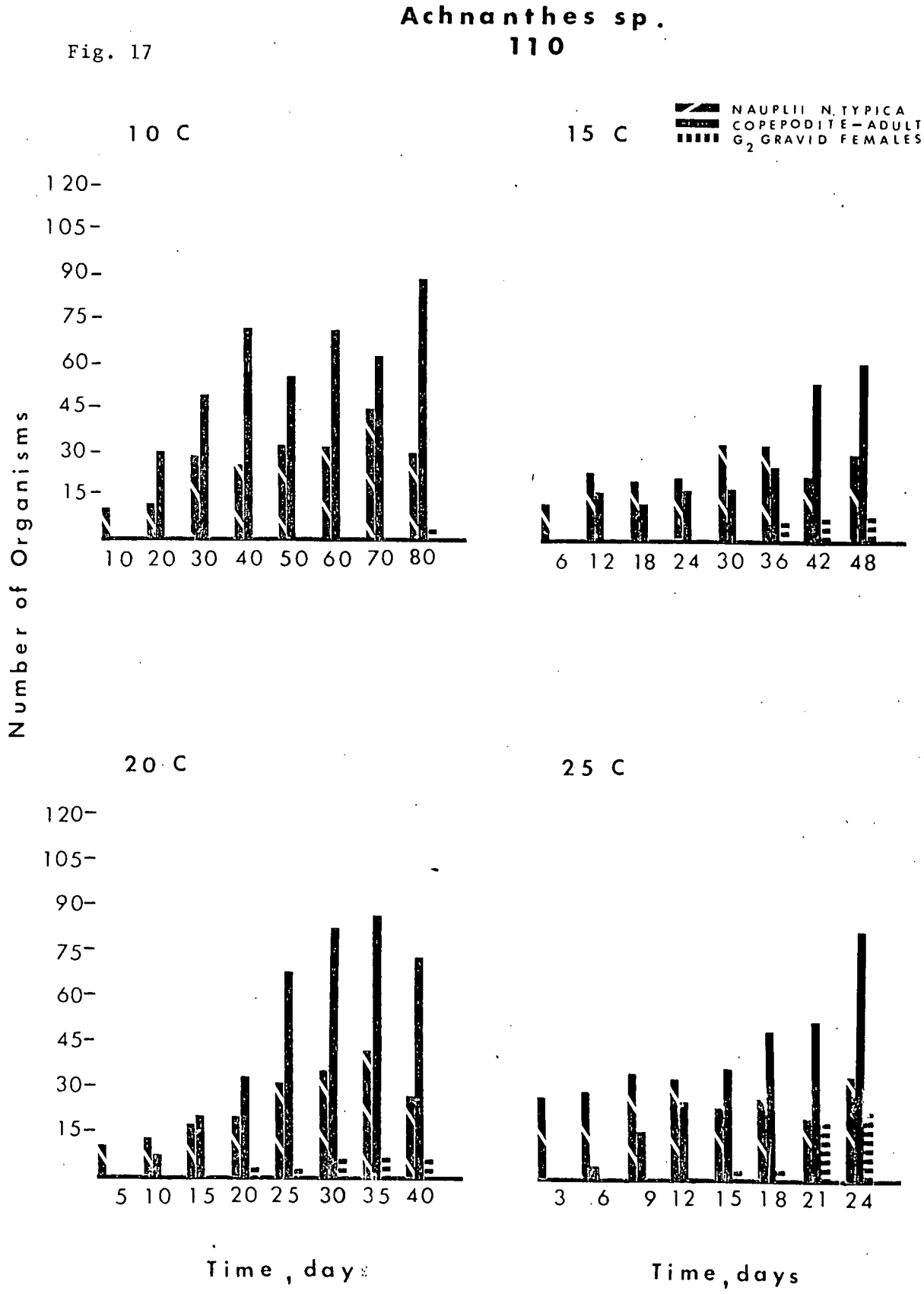

Achnanthes sp.
110
NAUPLII N. TYPICA
COPEPODITE–ADULT
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
5 10 15 20 25 30 35 40
3 6 9 12 15 18 21 24
Time, days
Time, days

Fig. 18

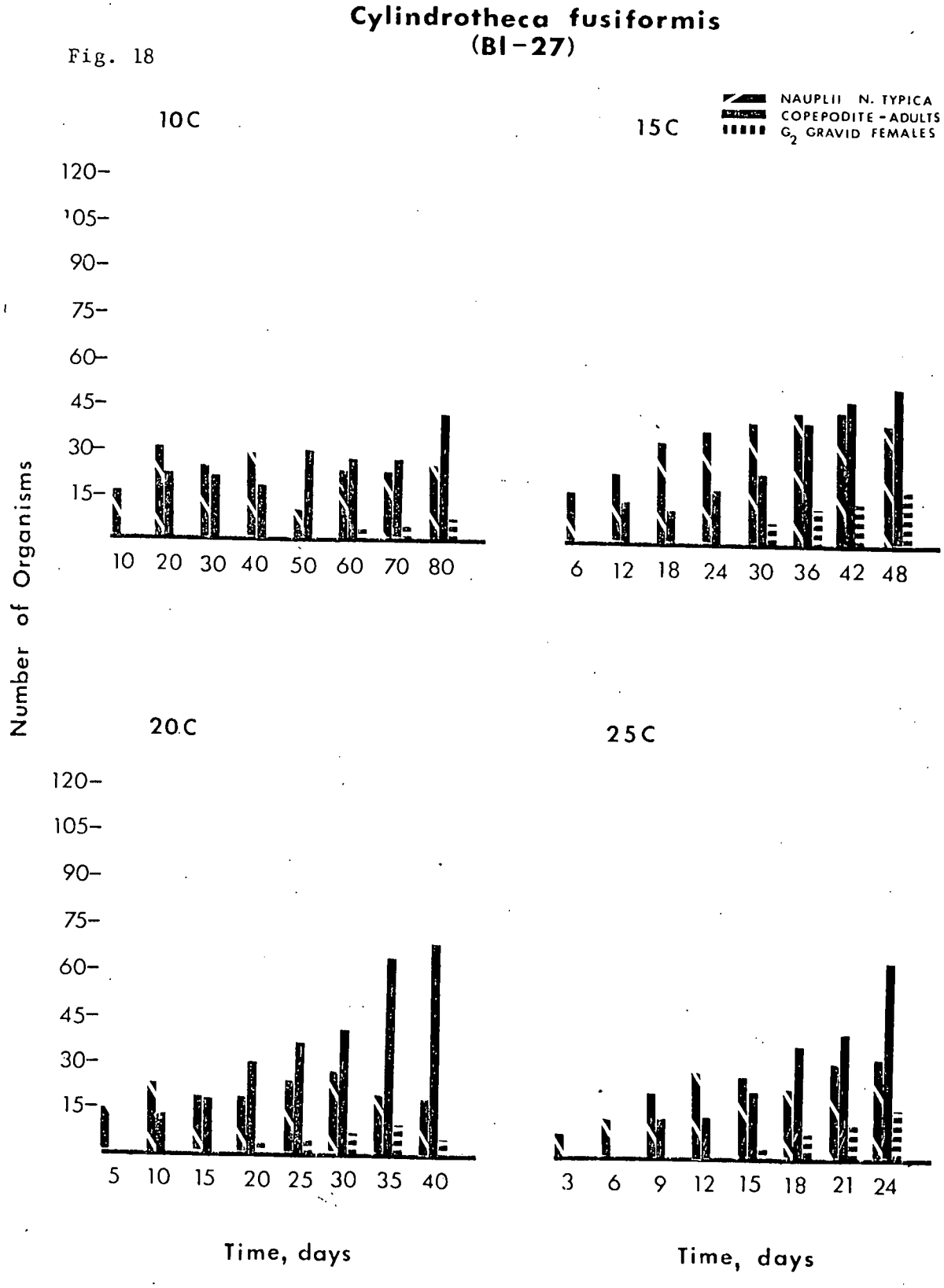

Cylindrotheca fusiformis
(BI-27)
NAUPLII N. TYPICA
COPEPODITE - ADULTS
G2 GRAVID FEMALES
10C
15C
20C
25C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
5 10 15 20 25 30 35 40
3 6 9 12 15 18 21 24
Time, days
Time, days

Fig. 19

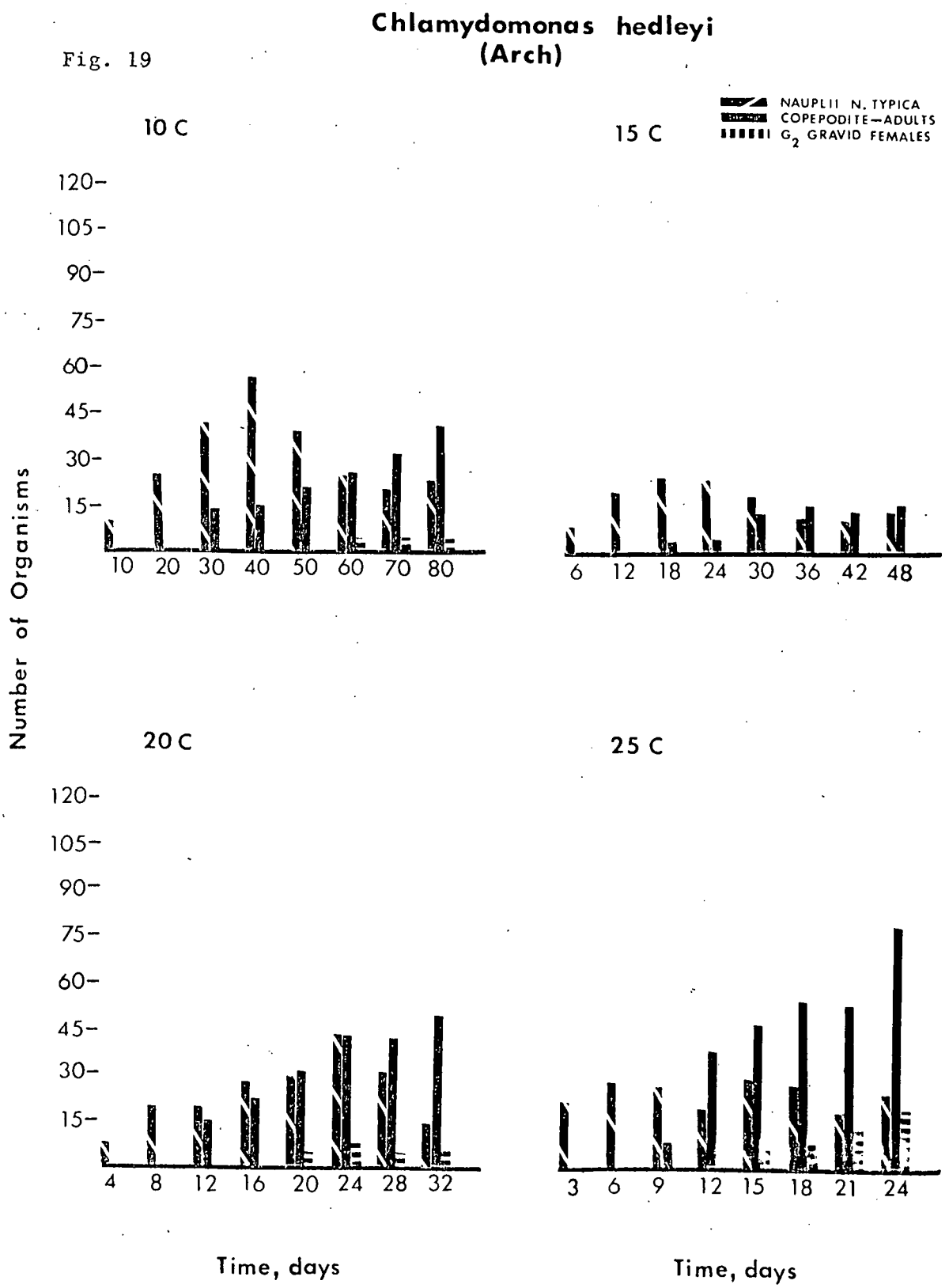

Chlamydomonas hedleyi
(Arch)
NAUPLII N. TYPICA
COPEPODITE—ADULTS
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
3 6 9 12 15 18 21 24
Time, days
Time, days

Fig. 20

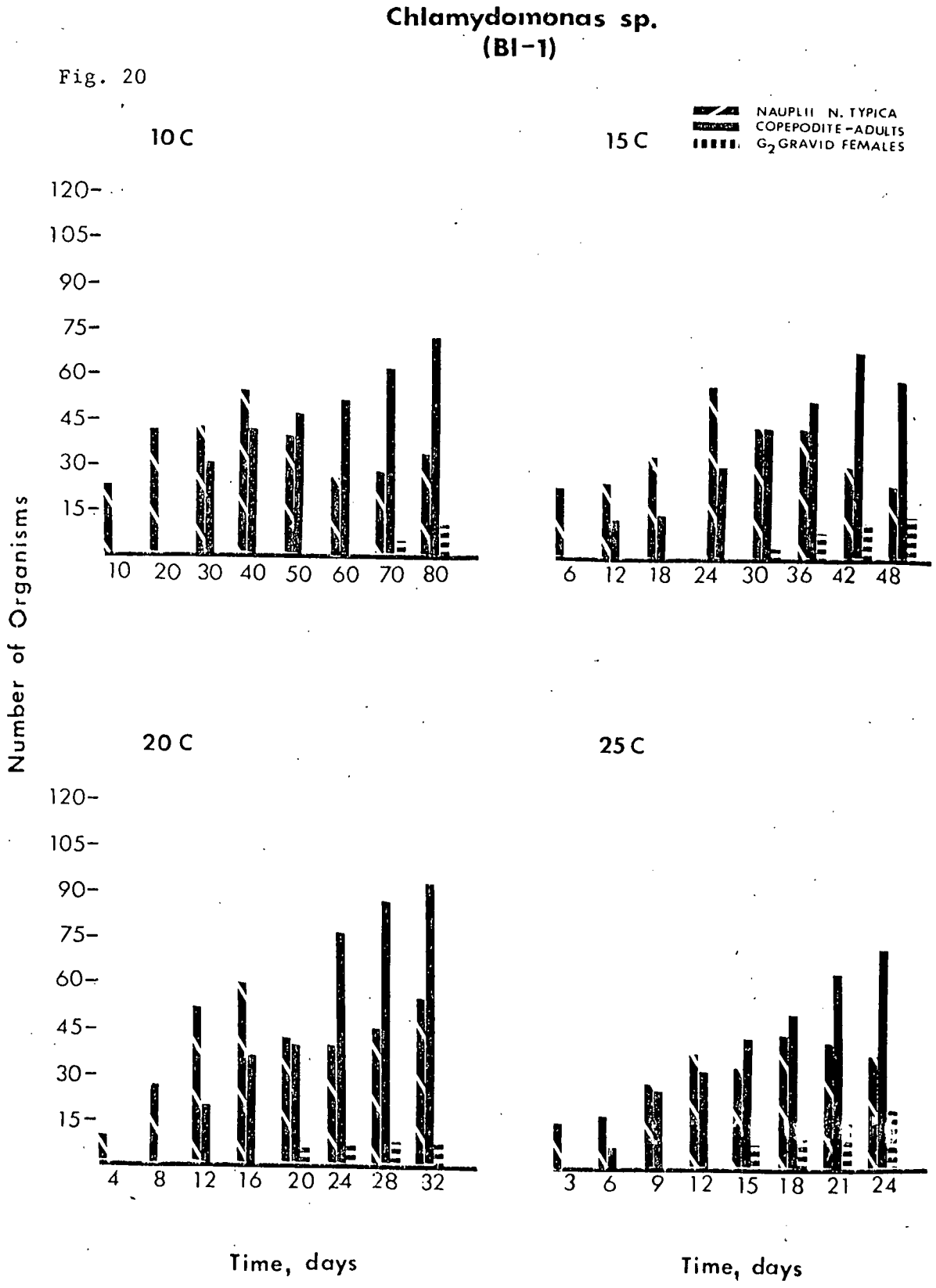

Chlamydomonas sp.
(BI-1)
NAUPLII N. TYPICA
COPEPODITE-ADULTS
G2 GRAVID FEMALES
10 C
15 C
20 C
25 C
Number of Organisms
120-
105-
90-
75-
60-
45-
30-
15-
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
3 6 9 12 15 18 21 24
Time, days
Time, days

Fig. 21

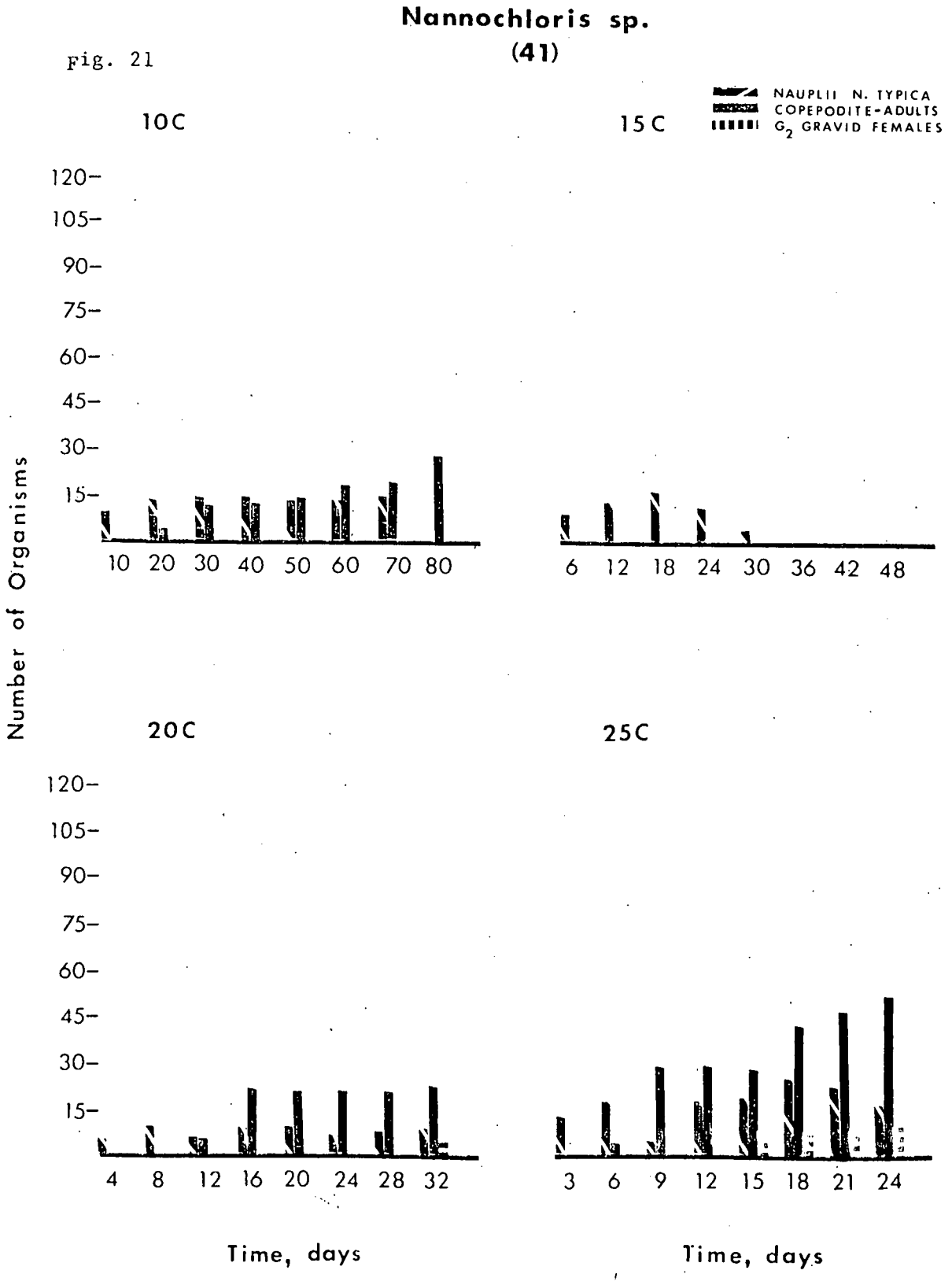

Nannochloris sp.
(41)
NAUPLII N. TYPICA
COPEPODITE-ADULTS
G2 GRAVID FEMALES
10C
15C
20C
25C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
3 6 9 12 15 18 21 24
Time, days
Time, days

Fig. 22

**Dunaliella sp.**
**(98)**

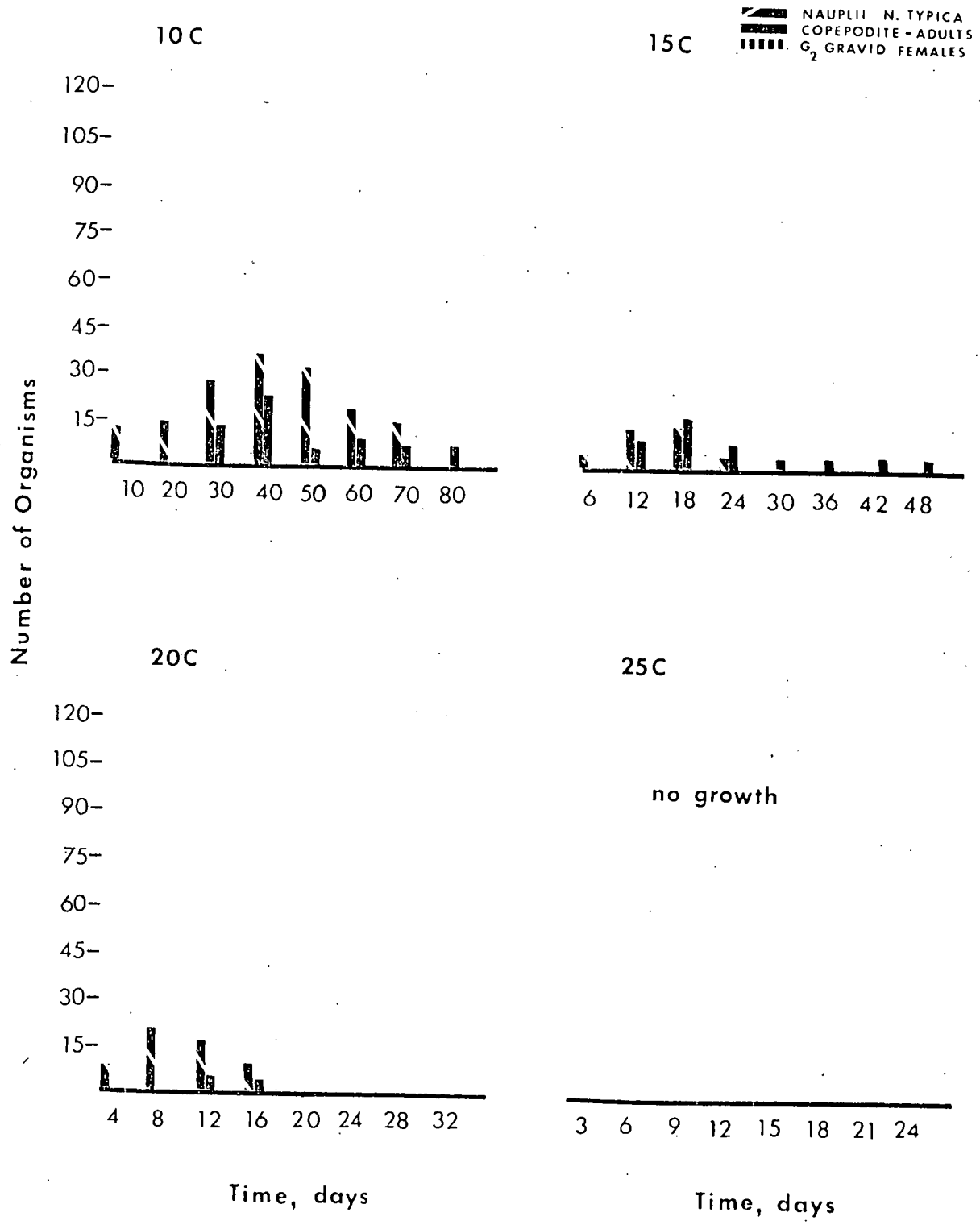
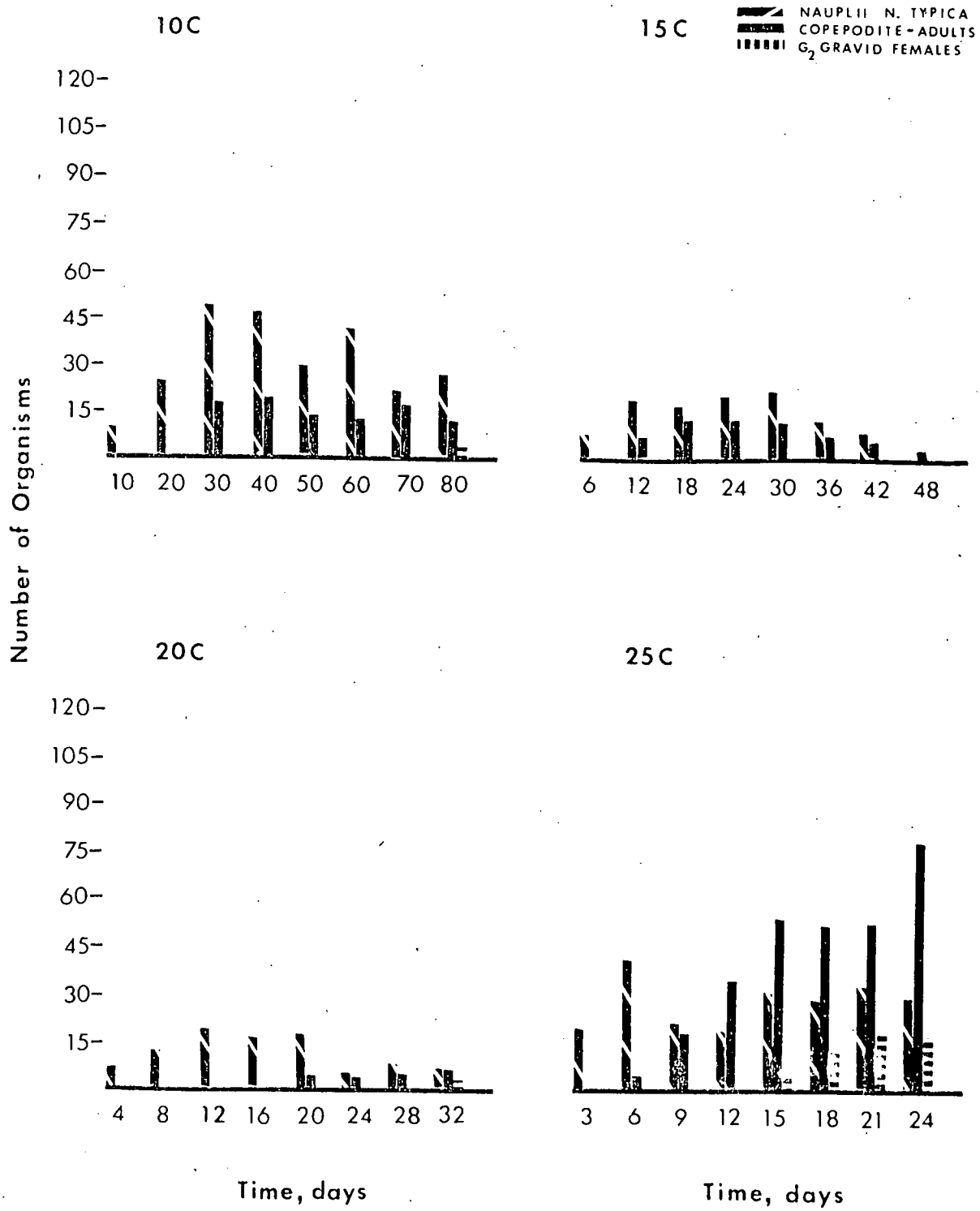


Fig. 23

**Chlorococcum sp.**
**(38)**



## Unidentified chlorophyte (50)

Fig. 24

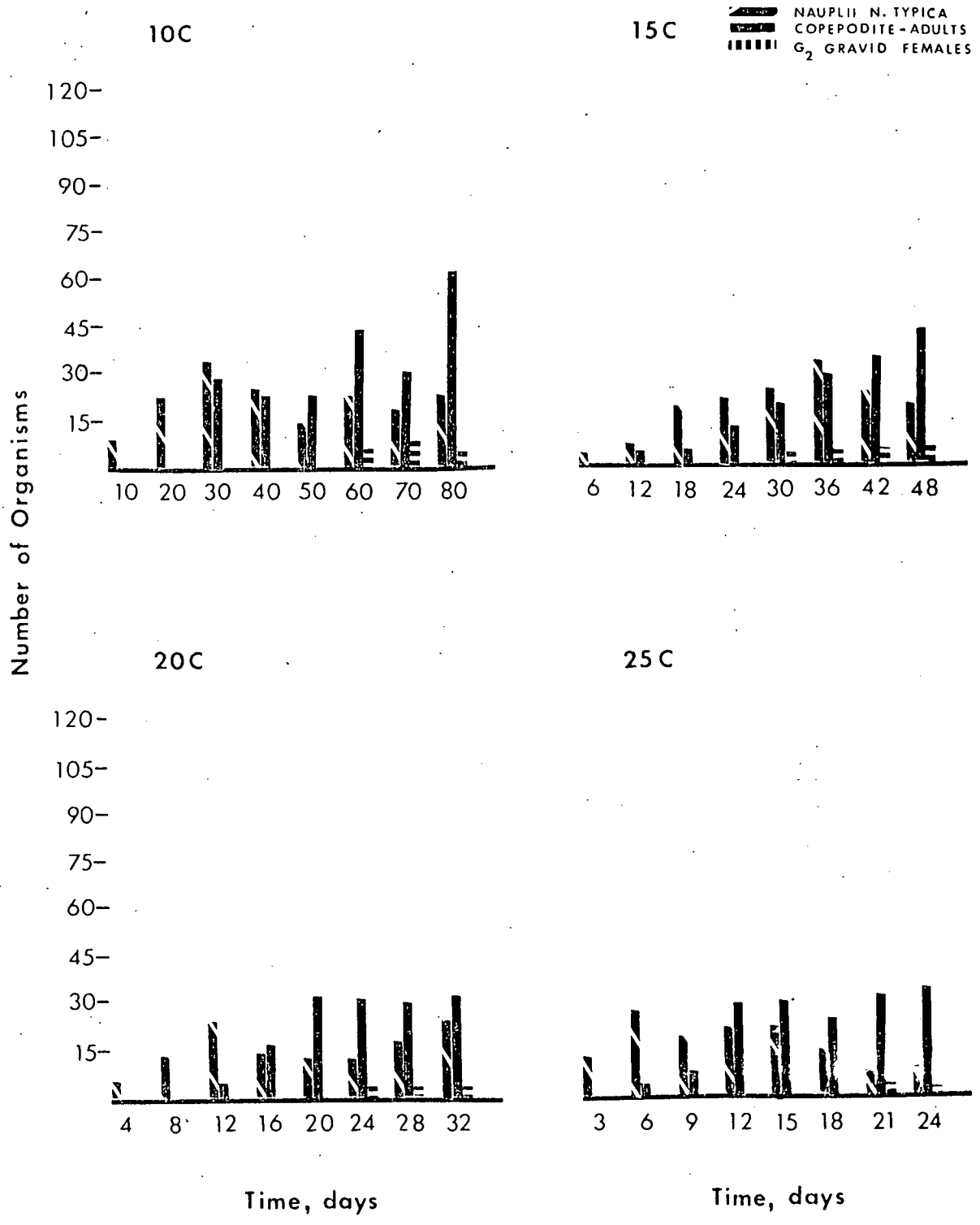


Fig. 25

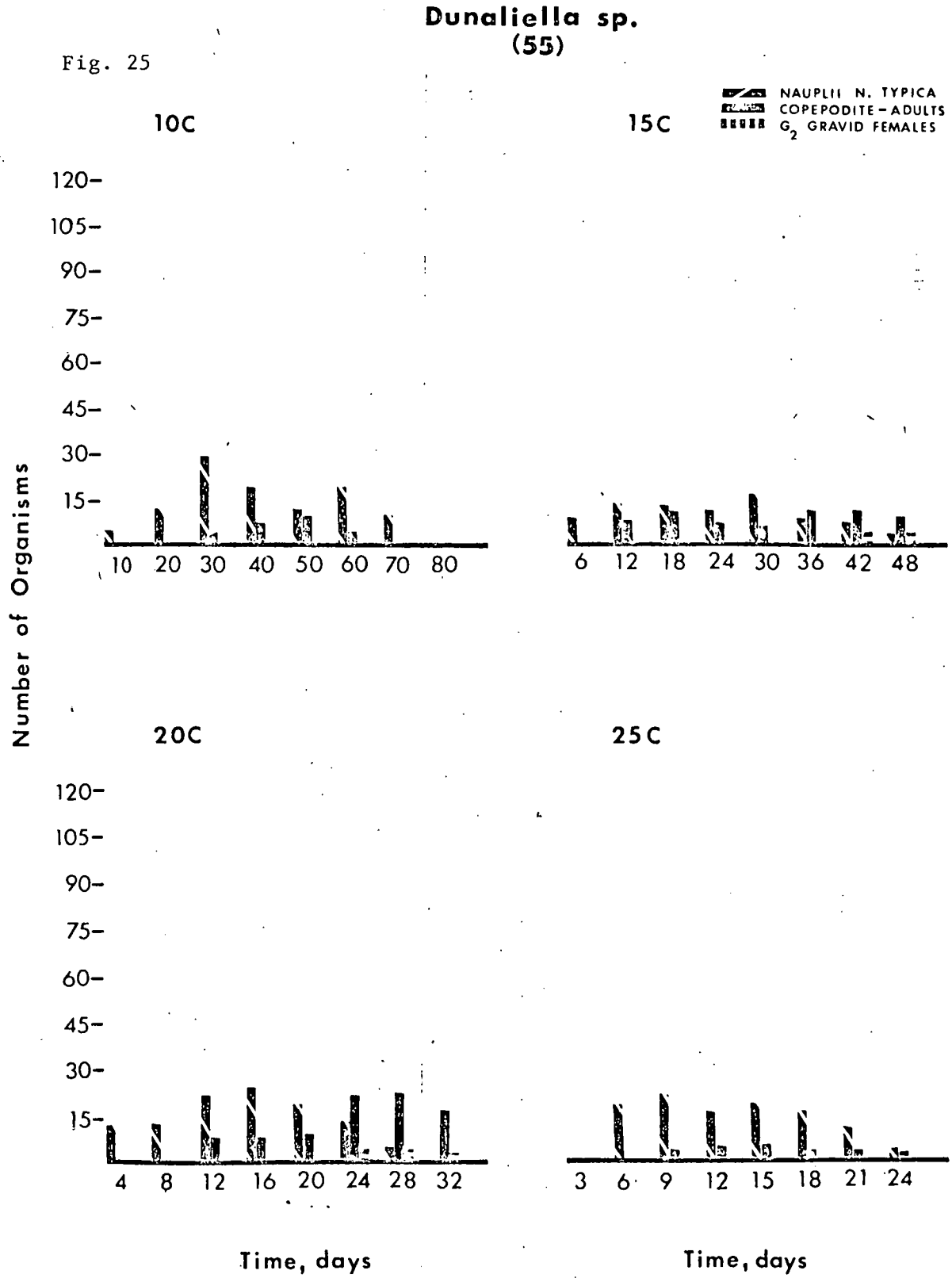

Dunaliella sp.
(55)
NAUPLII N. TYPICA
COPEPODITE - ADULTS
G2 GRAVID FEMALES
10C
15C
20C
25C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
3 6 9 12 15 18 21 24
Time, days
Time, days

Fig. 26

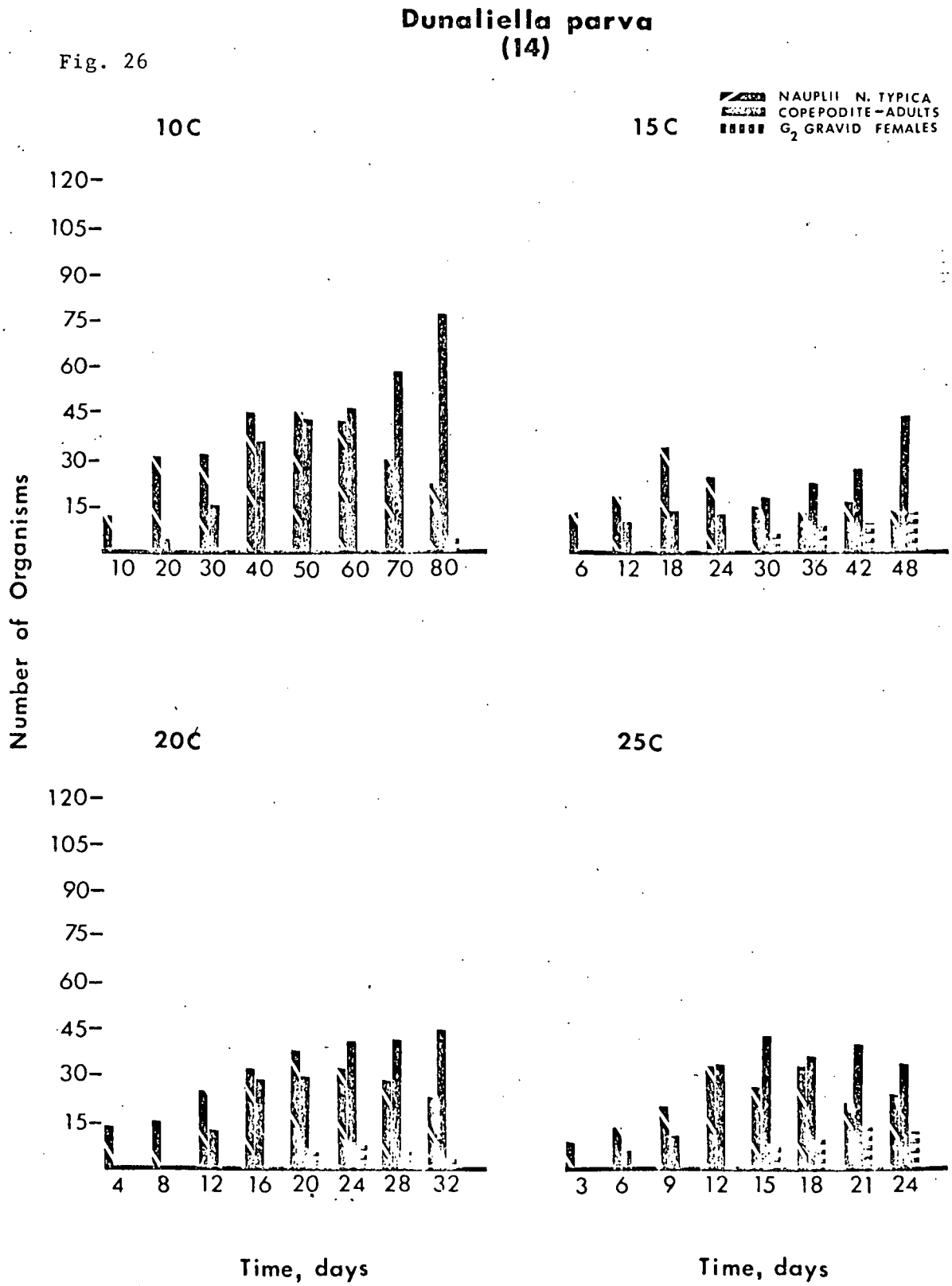

Dunaliella parva
(14)
NAUPLII N. TYPICA
COPEPODITE-ADULTS
G2 GRAVID FEMALES
10C
15C
20C
25C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
3 6 9 12 15 18 21 24
Time, days
Time, days

Fig. 27

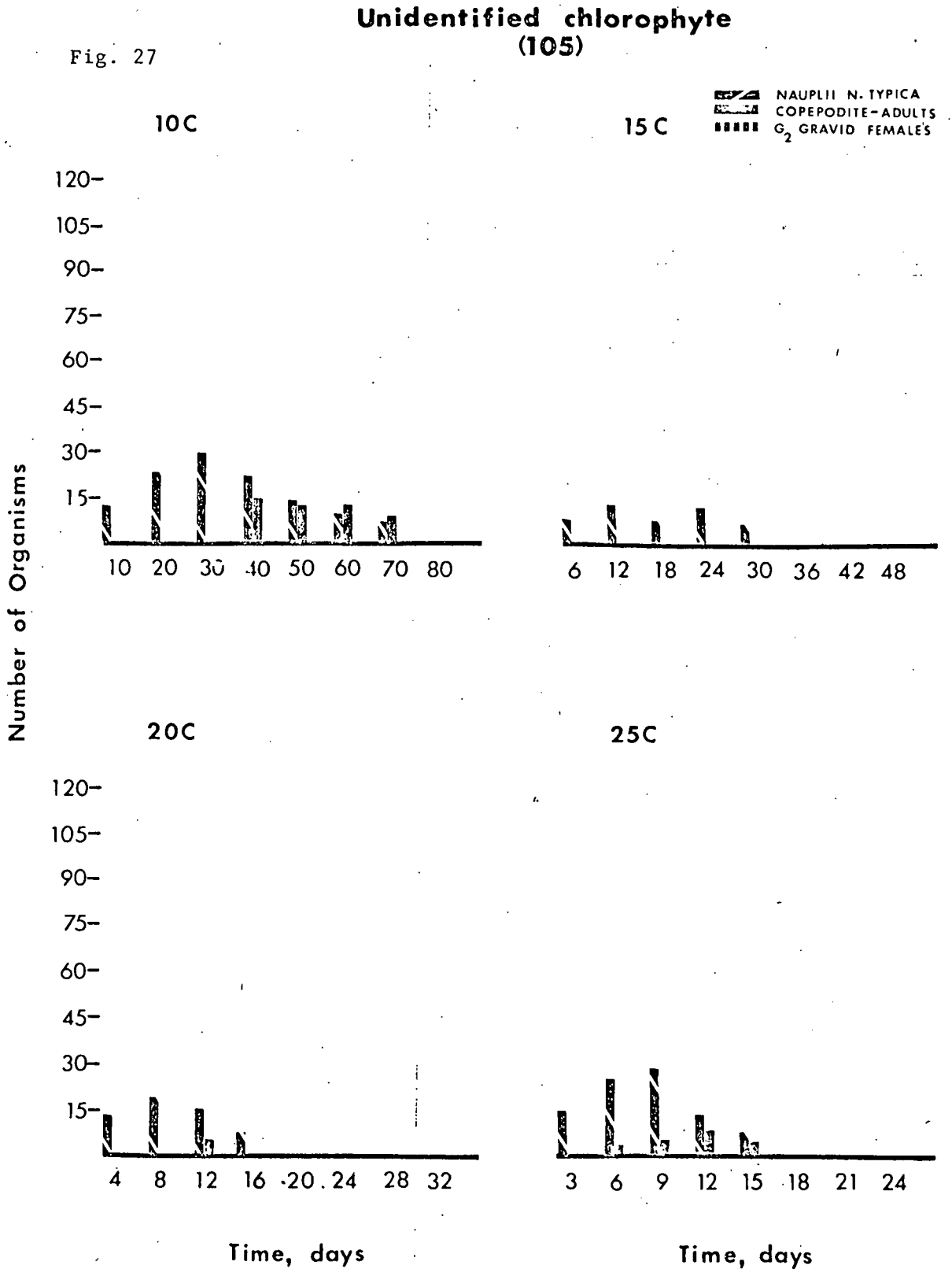
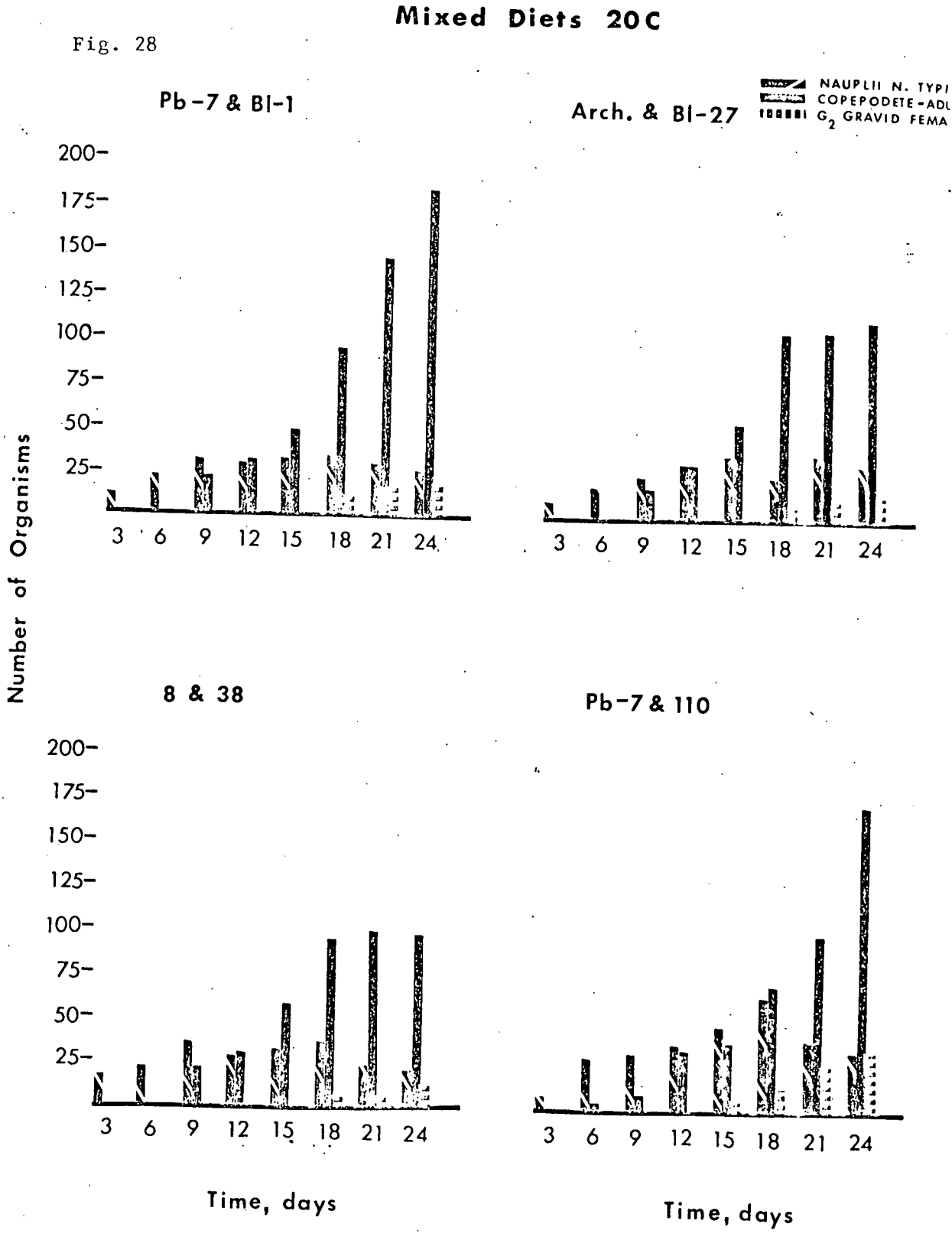

Unidentified chlorophyte
(105)
NAUPLII N. TYPICA
COPEPODITE-ADULTS
G2 GRAVID FEMALES
10C
15C
20C
25C
Number of Organisms
120
105
90
75
60
45
30
15
10 20 30 40 50 60 70 80
6 12 18 24 30 36 42 48
4 8 12 16 20 24 28 32
3 6 9 12 15 18 21 24
Time, days
Time, days

Fig. 28


Mixed Diets 20C
NAUPLII N. TYPI
COPEPODETE-ADU
G2 GRAVID FEMA
Pb-7 & Bl-1
Arch. & Bl-27
8 & 38
Pb-7 & 110
Number of Organisms
200
175
150
125
100
75
50
25
3 6 9 12 15 18 21 24
Time, days

An important aspect of decomposition community dynamics are the rates of mineral cycling by the small animals. This past year we completed studies of the following meiofaunal species: Allogromia laticollaris (foraminifera); Rosalina leei (foraminifera; Quinqueloculina lata (foraminifera); Rhabditis marina (nematode); Nitocra typica (copepod). In these experiments 5 μCi/ml of labeled ($^{14}C$, $^{35}S$, $^{32}P$) were inoculated into a solidified nematode growth medium with a shallow liquid overlay. Large populations of each of the various stages of the animals were transferred onto the labeled media and were allowed to grow 1-2 weeks depending on the life cycle of the animals. After growing in the labeled medium, the animals were transferred onto "cold" (unlabeled) media and 10 adults, intermediate stages and juveniles were picked, washed 3 X and placed in scintillation vials. An aliquot of the final wash also was placed in a vial as a control, reflecting relative carry-over as a check on procedure. Animals were picked successively every day and at the end of the 2 week period all vials were counted on a Beckman liquid scintillation counter. The results were then plotted in CPM/μgm nematode to determine biological half-life.

The results of these experiments are summarized in the accompanying figures (29-40). The biological half-life of $^{14}C$ in Monhystera differs in each age class. The C $T_b$ for adults was ~ 2 d; intermediate stages ~ 5 d; and newly hatched juveniles 2.3 d. The $T_b$ for S was: 2 d for adults; 1 d for intermediate stages; and 3 d for juveniles. The $T_b$ for P was: 9.6 d for adults; 5 d for intermediates; and 1 d for juveniles. The $T_b$ for S in Rhabditis marina adults was ~ 1.5 d while for intermediate stages it was ~ 7 d; for juveniles 10 d. The $T_b$ for adult A. laticollaris for S was 2 d and for P it was 3 d. Turnover was much faster in juveniles; it was 6 hr for S and 1.5d for P. Turnover rates for Quinqueloculina lata were similar to adult A. laticollaris while those of Rosalina leei were closer to juvenile A. laticollaris. The mineral turnover rates of Nitocra typica were also very rapid: C $T_b$ = 4.4 d; P $T_b$ = 1.6 d; and S $T_b$ = 4.5 d.

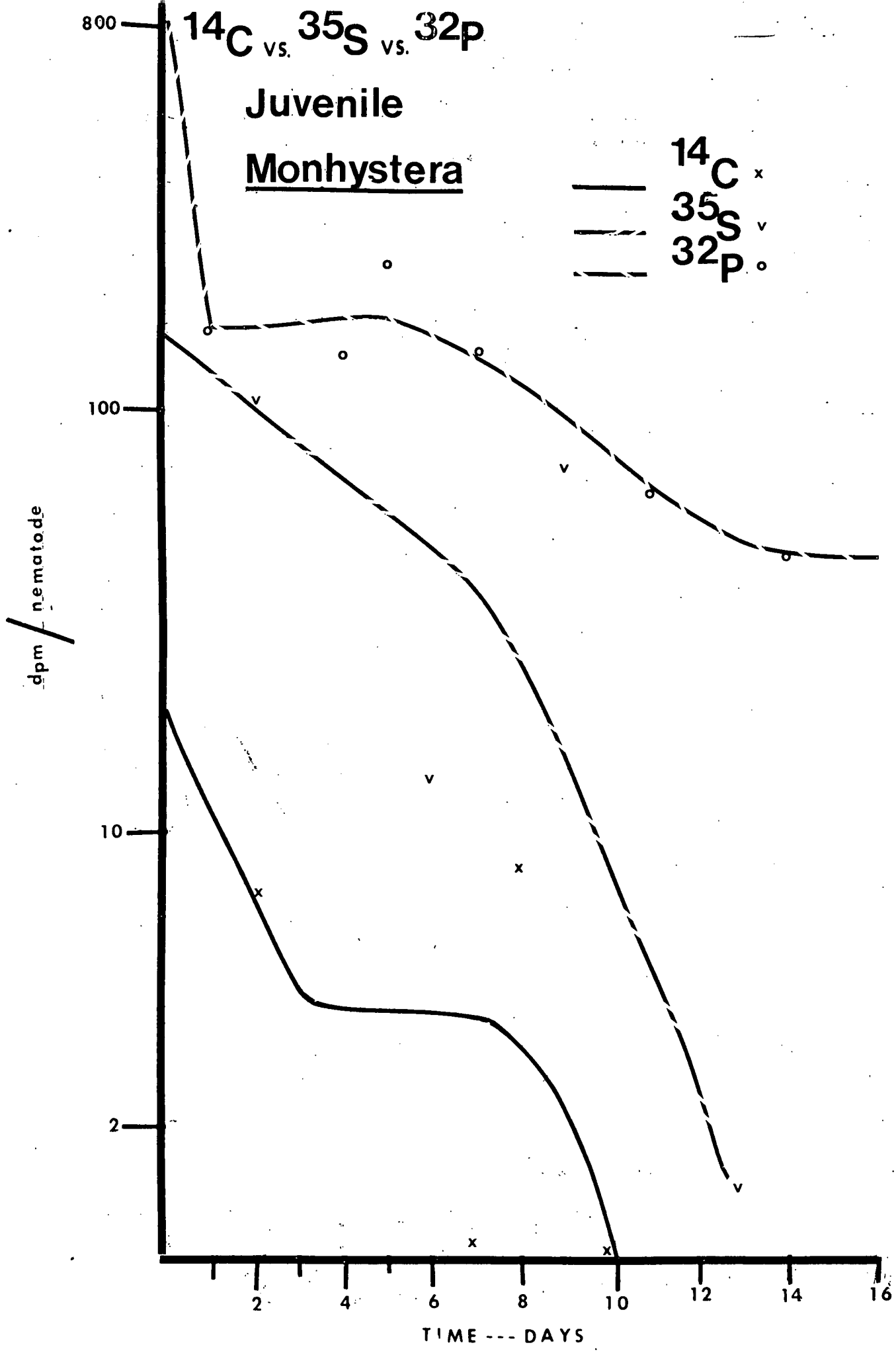

14C vs. 35S vs. 32P
Juvenile
Monhystera
14C x
35S v
32P o
800
100
10
2
dpm / nematode
2
4
6
8
10
12
14
16
TIME --- DAYS

Fig. 30

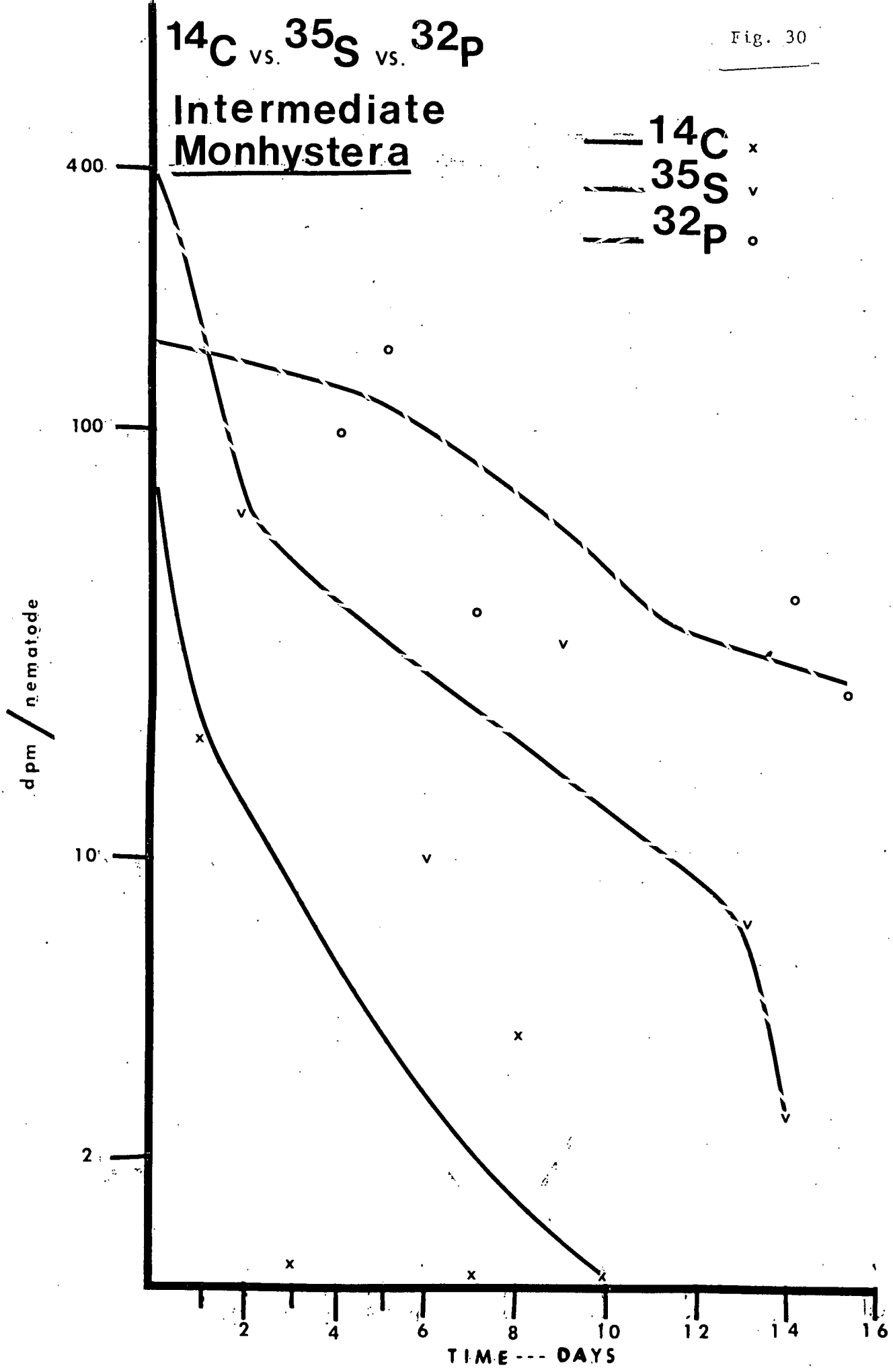
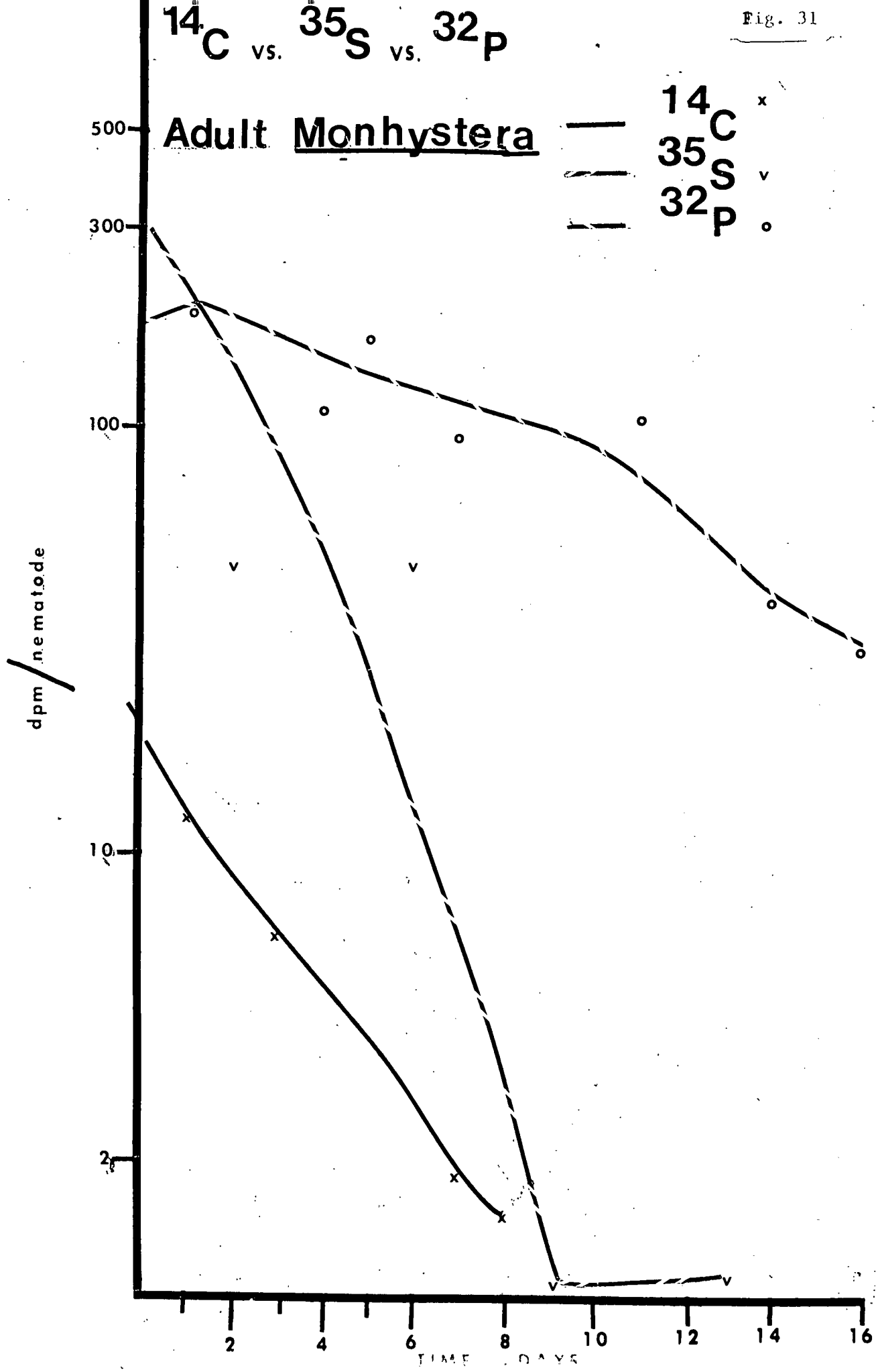

14C vs. 35S vs. 32P
Intermediate
Monhystera
14C x
35S v
32P o
400
100
10
2
dpm / nematode
2
4
6
8
10
12
14
16
TIME --- DAYS

Fig. 31


14C vs. 35S vs. 32P
Adult Monhystera
14C x
35S v
32P o
500
300
100
10
2
dpm/nematode
2
4
6
8
10
12
14
16
TIME - DAYS

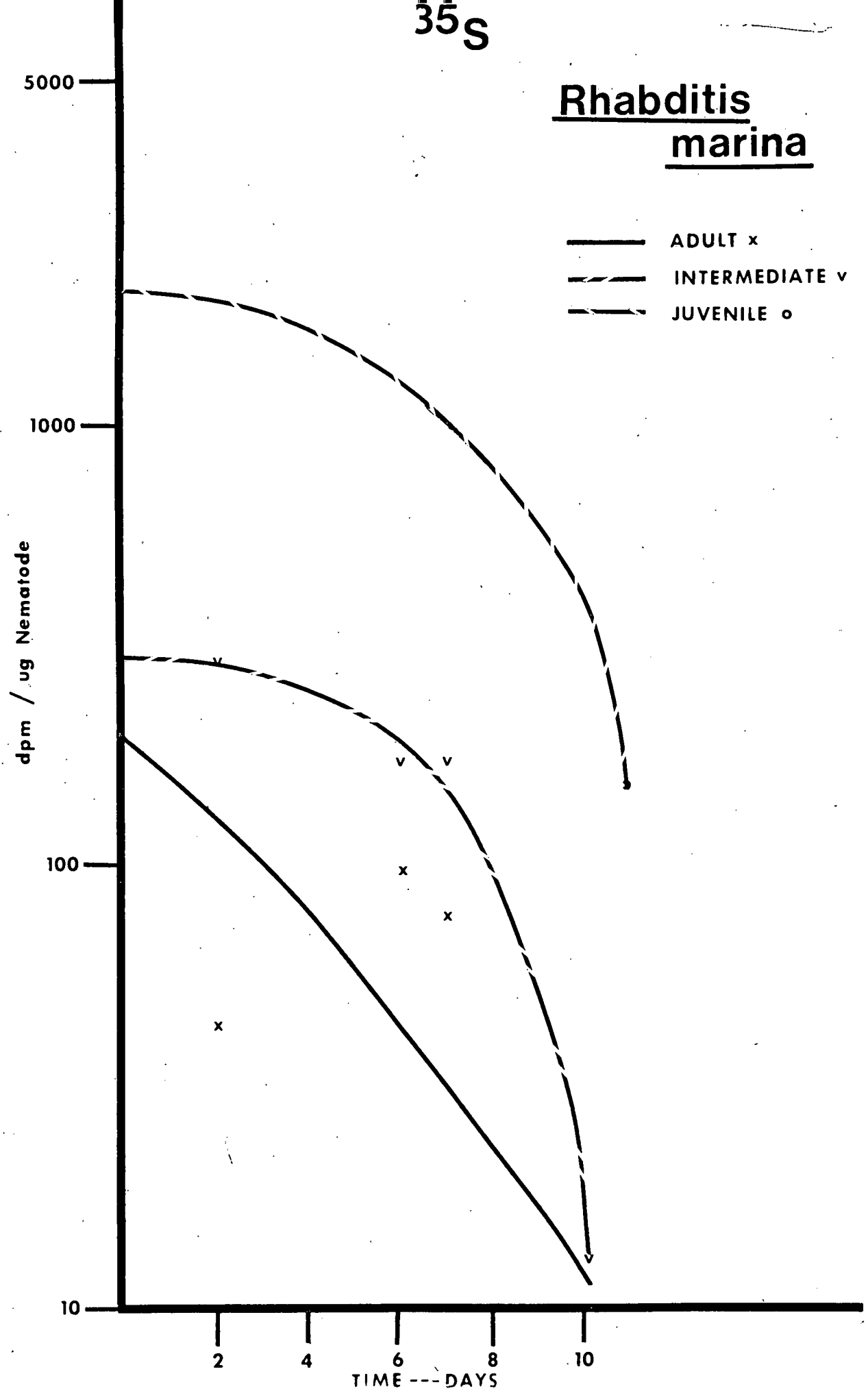

35S
Rhabditis marina
ADULT x
INTERMEDIATE v
JUVENILE o
5000
1000
100
10
dpm / ug Nematode
2
4
6
8
10
TIME --- DAYS

Fig. 33

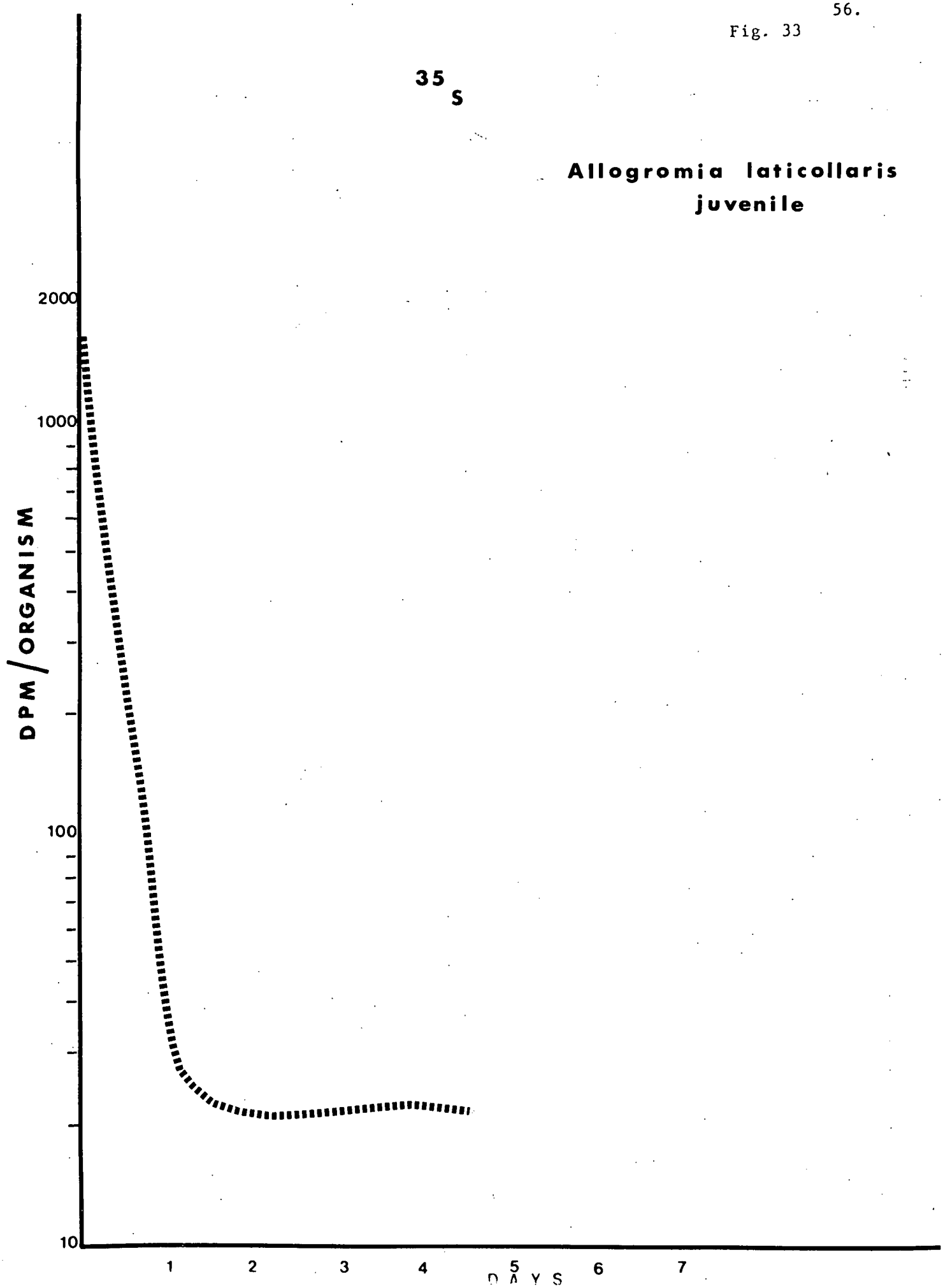

35 S
Allogromia laticollaris
juvenile
2000
1000
100
10
DPM/ORGANISM
1
2
3
4
5
6
7
DAYS

Fig. 34

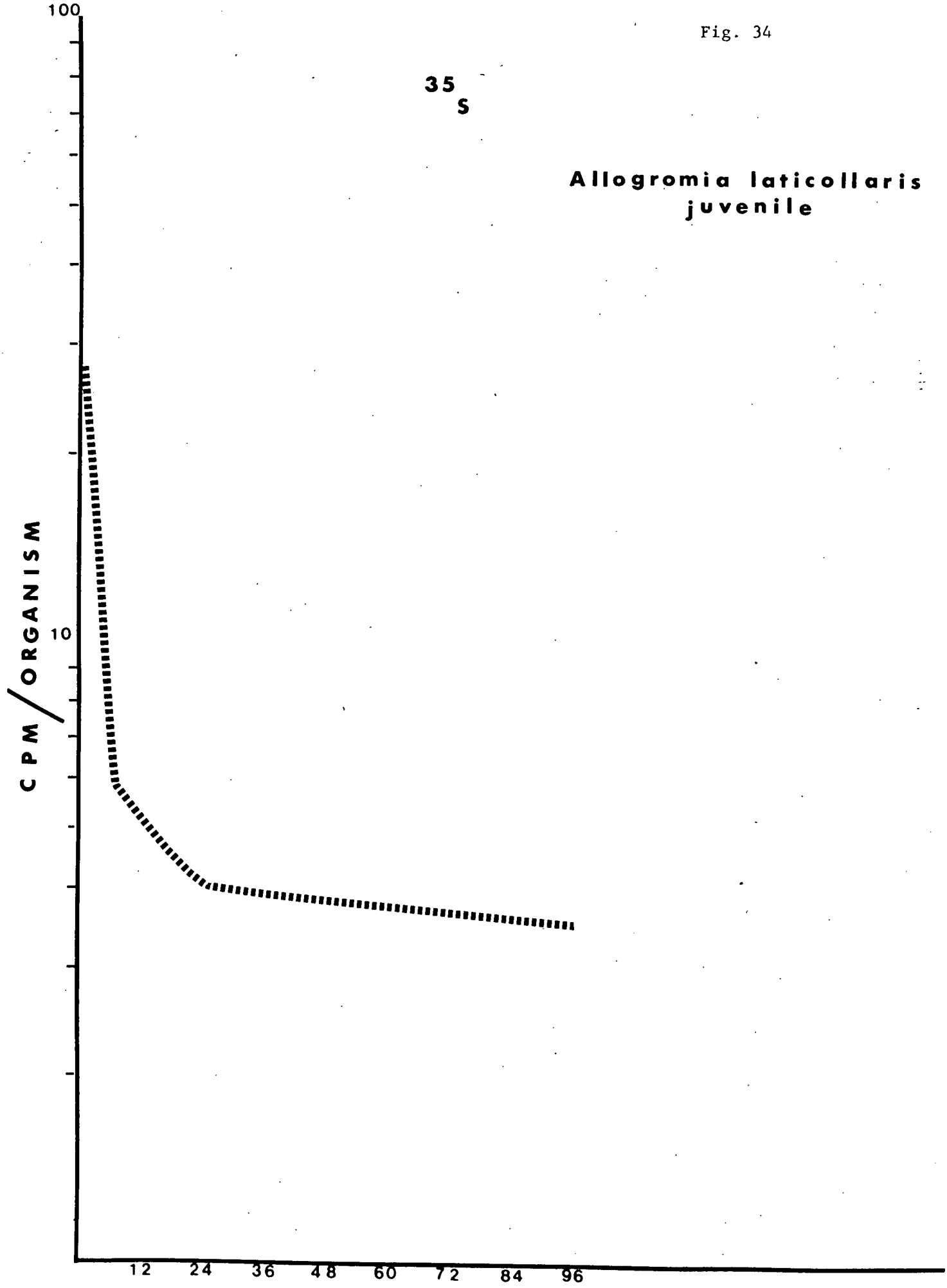

35 S
Allogromia laticollaris
juvenile
CPM/ORGANISM
100
10
12
24
36
48
60
72
84
96

Fig.35

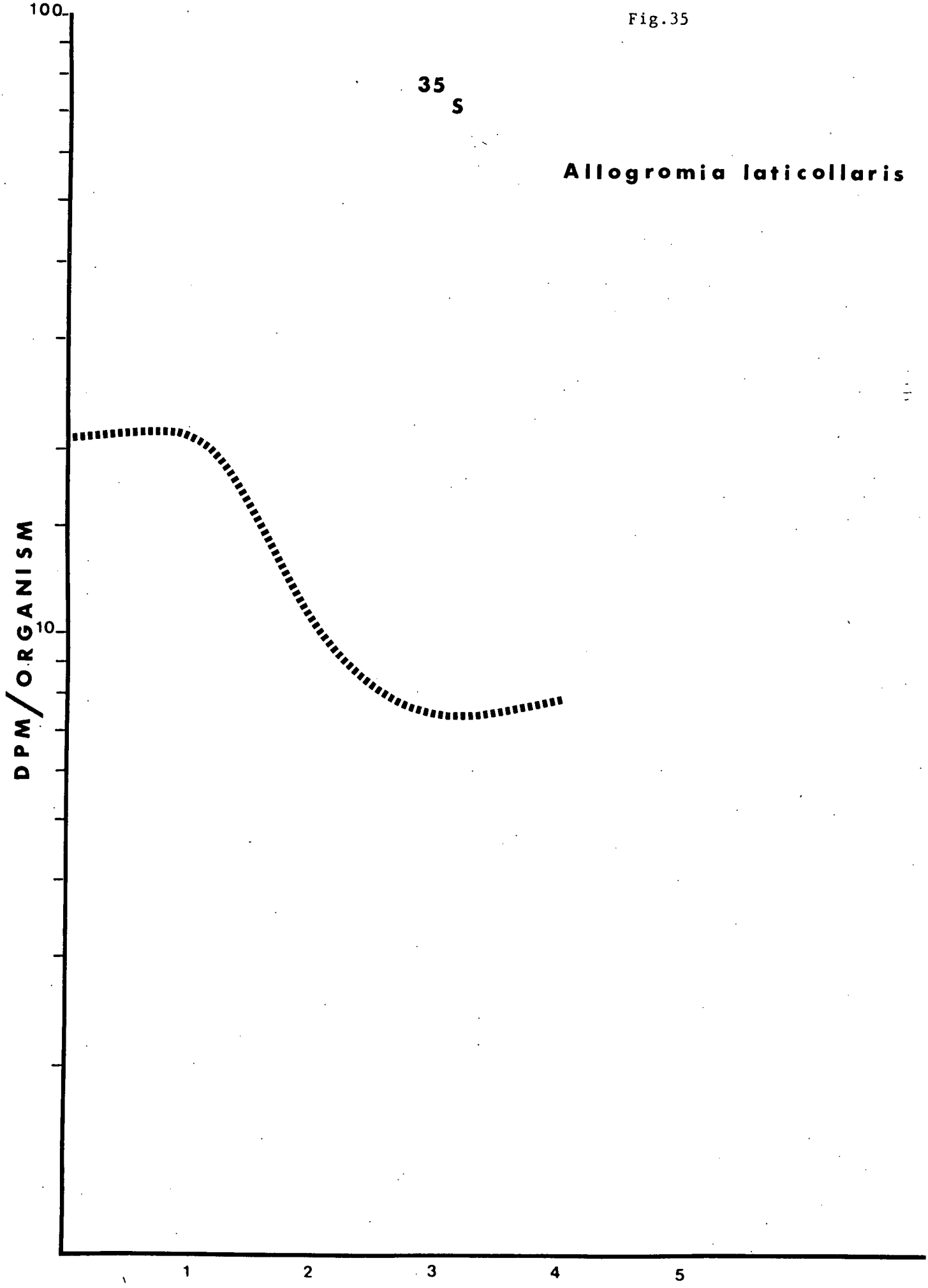


Fig. 36

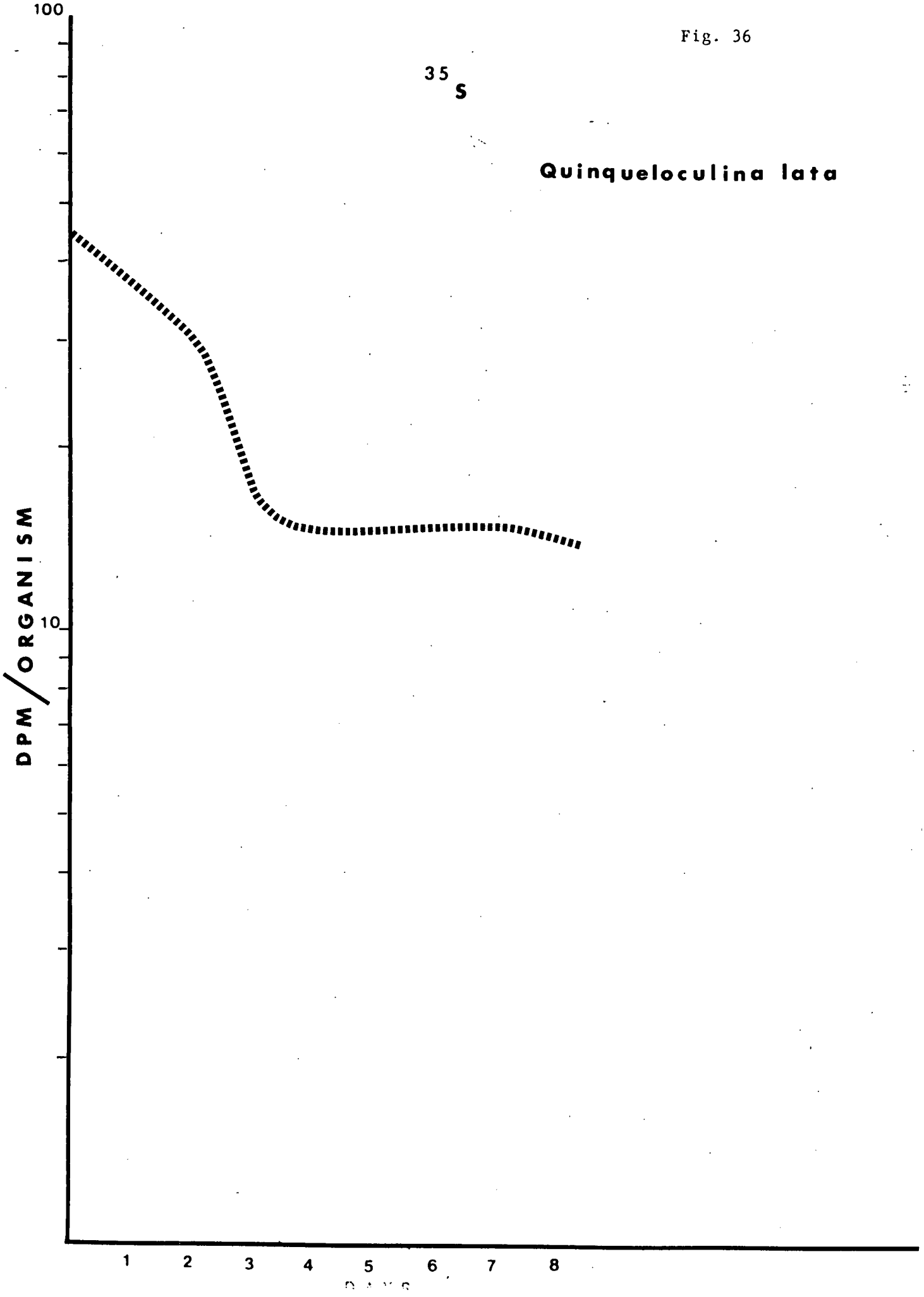


Fig. 37

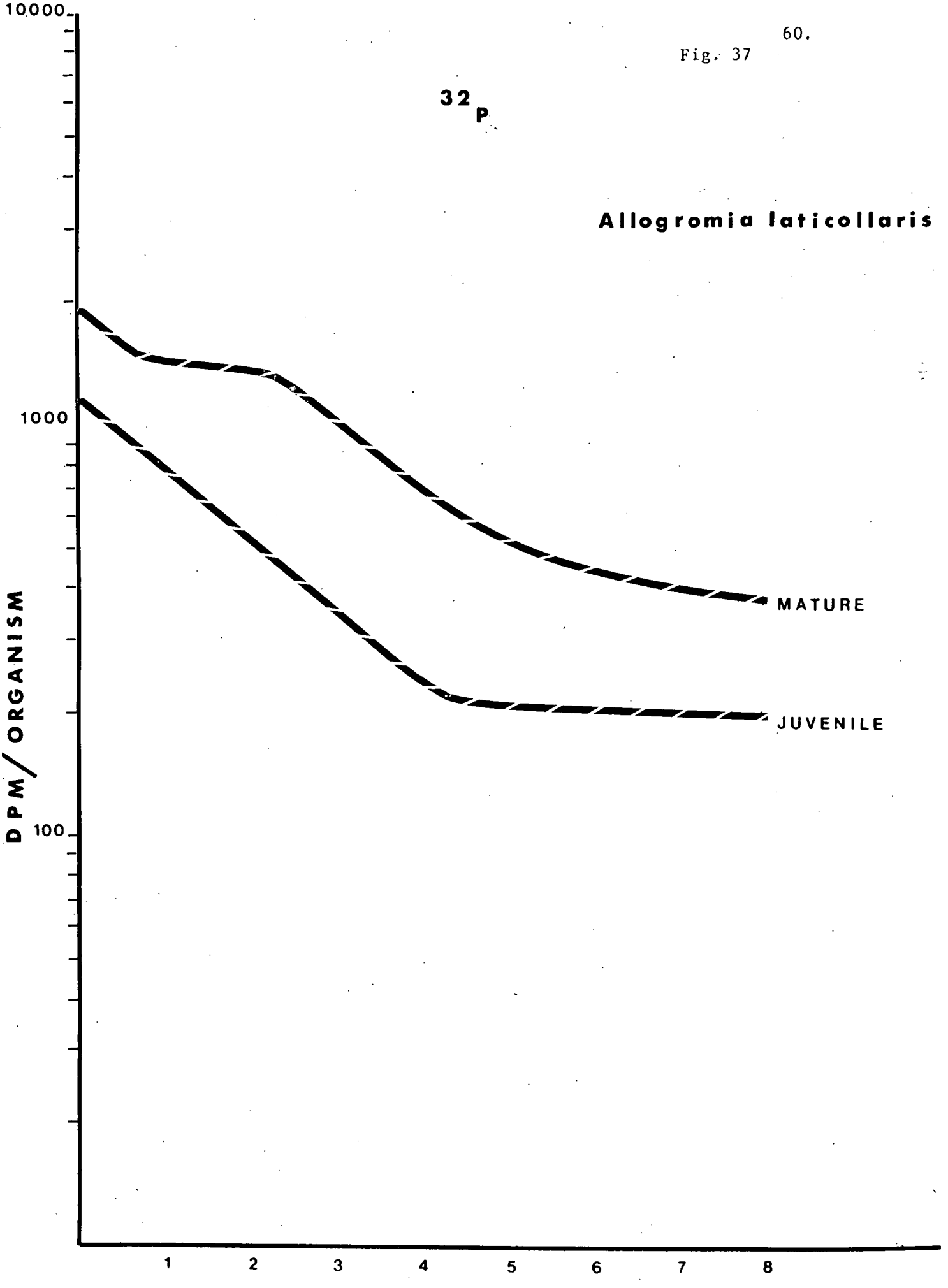

32P
Allogromia laticollaris
10000
1000
100
DPM/ORGANISM
MATURE
JUVENILE
1
2
3
4
5
6
7
8

Fig. 38

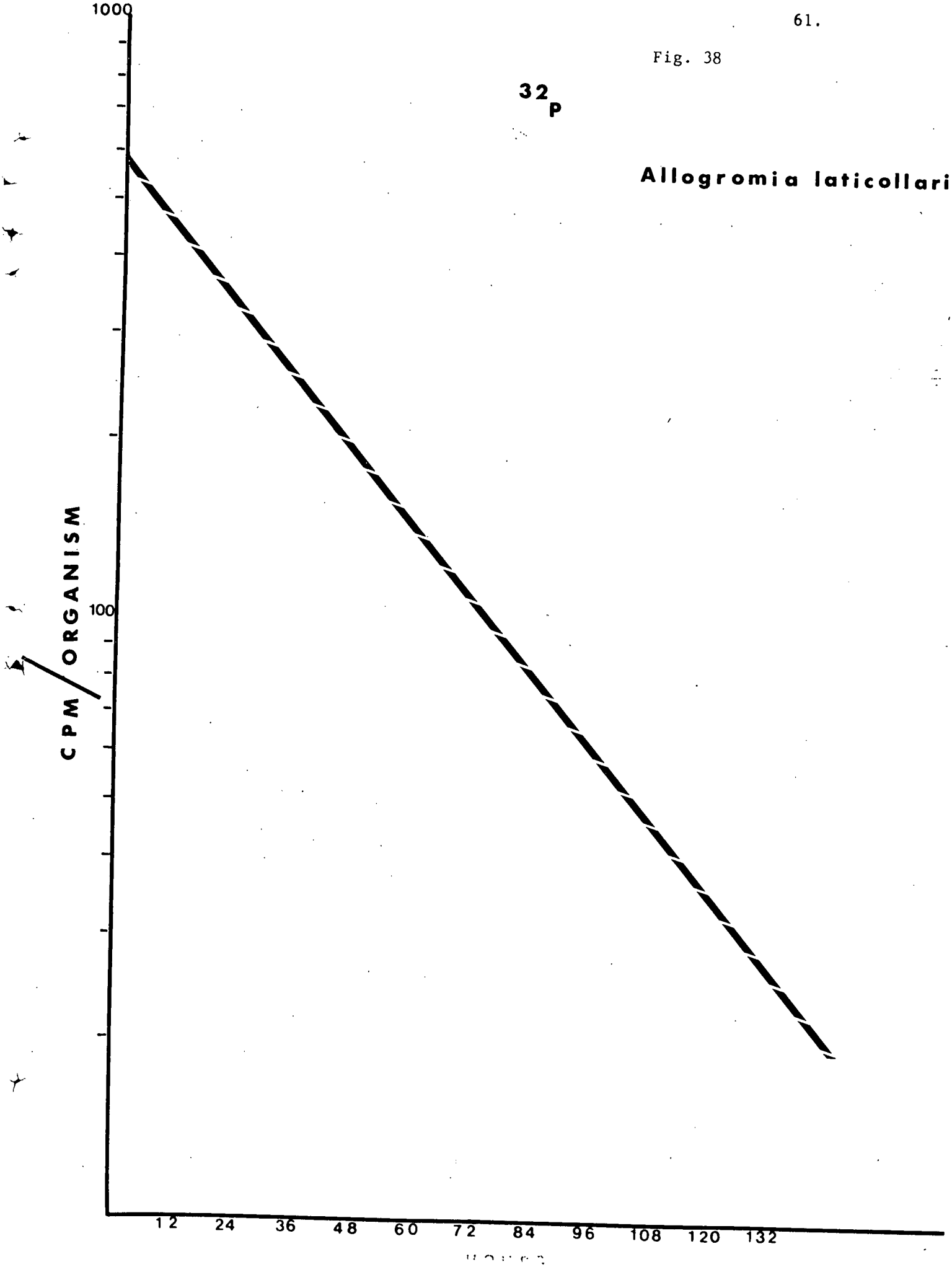

32P
Allogromia laticollaris
1000
100
CPM/ORGANISM
12
24
36
48
60
72
84
96
108
120
132

Fig. 39

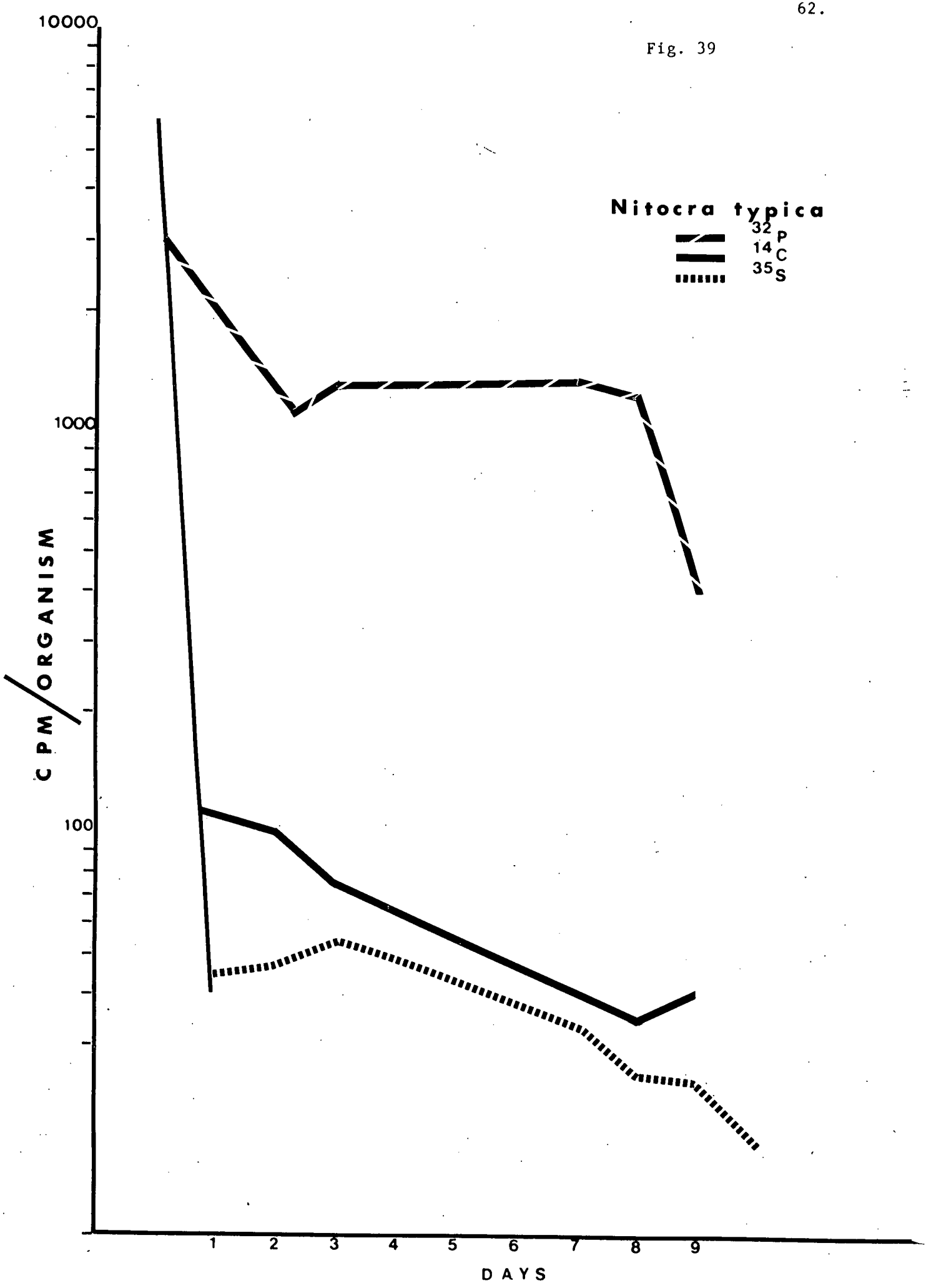
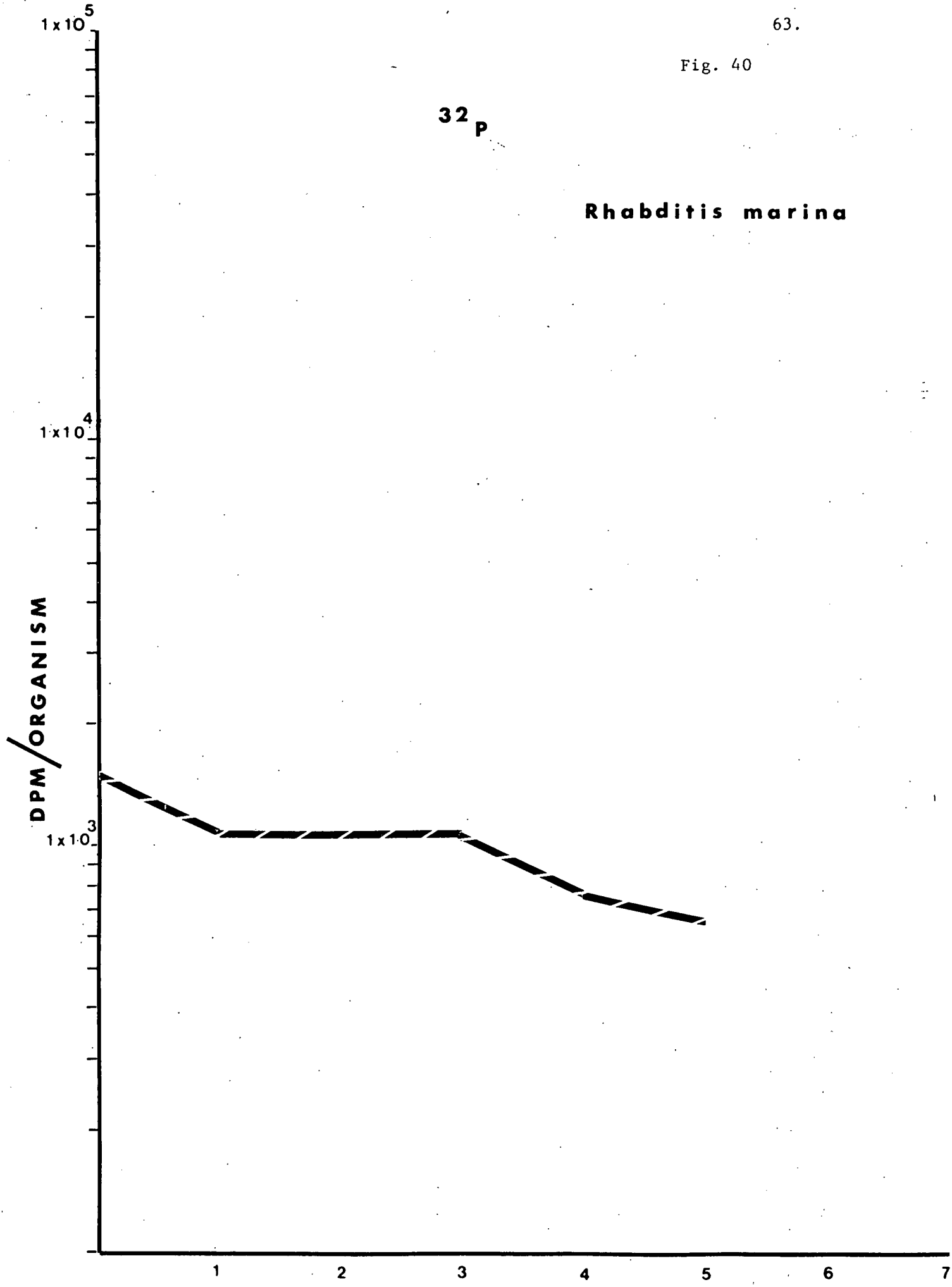

Nitocra typica
32 P
14 C
35 S
10000
1000
100
CPM/ORGANISM
1
2
3
4
5
6
7
8
9
DAYS

Fig. 40


32P
Rhabditis marina
1x10^5
1x10^4
1x10^3
DPM/ORGANISM
1
2
3
4
5
6
7

The tracer feeding experiments were done in 3 stages. Bacteria or algae were inoculated with 10 μCi/ml $^{32}P$; bacteria grew for 24 hrs and algae 48-72 hrs. The bacteria were spun down 3 x 10,000 rpm at 5° C in a Sorvall refrigerated centrifuge; the algae washed 3 x at 5,000 rpm for 10 min. in a Sorvall GLC-1 centrifuge. The remaining pellets were resuspended in 0.3 ml of sea water; 0.1 ml was placed in a scintillation vial; 0.1 ml was counted using a hemocytometer or a B & L spectronic 20 spectrophotometer; the final 0.1 aliquot was added to tubes of 50 nematodes.

The nematodes were allowed to feed for 24 hrs, after which time they were picked, washed, and placed on unlabeled media for 2-8 hrs in order to allow any indigested foods remaining in their gut to be voided. The worms were then washed and counted on a Beckman liquid scintillation counter.

In tracer feeding experiments with Monhystera denticulata, Chromadorina germanica and Rhabditis marina, we found a wide range in rates of consumption of different species of algae and bacteria. Data was presented at the Estuarine Conference. Not all the potential food ingested was digested. Rates of assimilation ranged from a low of 22% to 100%. There was, for instance, 4 orders of magnitude difference in rates of consumption of 2 different but closely related species of algae by one worm. Rhabditis marina digested $1 \times 10^{-4}$ mg of Dunaliella parva/day/worm and digested 88% of what it ate, while it digested 31% or $6 \times 10^{-8}$ mg/worm/day of Chlamydomonas hedleyi, a symbiotic alga isolated from a foraminifera. Rhabditis also ate fairly large amounts ($2 \times 10^{-5}$) of two other species of algae, Nitzschia acicularis and Phaeodactylum tricornutum, and 5 species of bacteria, A 5-3, A 1-1, A 5-6, D 1-1 and D 5-2. Chromadorina consumed very low numbers of bacteria and generally consumed large quantities of most species of algae tested. The feeding patterns of Monhystera on algae were similar to those of Rhabditis. One hundred per cent of one species of bacterium (B1-1)

fed to the worm was eaten while only 4% of another species (C1-4) was eaten.

We have also begun to test trophic efficiency and mineral cycling in another series of experiments. Into the vessels of a Gilson respirometer, worms and bacteria or bacteria alone were inoculated into media containing various $^{14}C$ labeled substrates. $^{14}CO_2$ was trapped in 40% KOH moistened filter paper placed in the center well of the flask. At the end of each experiment (3 hrs) the substrate turnover was measured. Overall respiration was measured by means of the Gilson apparatus. Initial results with Rhabditis marina and Monhystera sp indicate that substrate turnover is ~ 125-150% of the rate of the flasks with bacteria alone. Preliminary estimates of nematode ecological efficiencies and associated trophic dynamics are tabulated (Table 3).

We have also studied the ecological efficiency ($E_e$%) of populations of one worm, Rhabditis marina, as a function of time. For these experiments the worms were inoculated into large numbers of flasks with 4 species of bacteria in a medium which would promote the slow steady growth of the bacteria. Bacteria alone were inoculated into control flasks. Measurements were made weekly. At the beginning of each experiment a known quantity of labeled bacteria was added to each flask. Each week a census of the worm population was made and their rates of growth, respiration and ingestion were measured. The data (Table 4; Fig. 41) suggest that Rhabditis, at least grown this way, has a very low ecological efficiency. Additional experiments are in progress.

One of the key issues in defining the biotic variables which regulate community structure is whether many of the meiofauna are so highly specialized for resource partitioning that they are stenotrophic (restricted to only a few species in their diet). If many species are so specialized, then food webs and community structure must be evaluated in very complex ways here-to-fore only theoretically considered by ecosystem cybernetics. (For an extensive discussion see COO 3254-16 and COO 3254-22 appended to this report.) This year we began experiments on the effect of food density on the diet and stability of meiofaunal

TABLE 3.

Preliminary estimates of marine nematode efficiencies

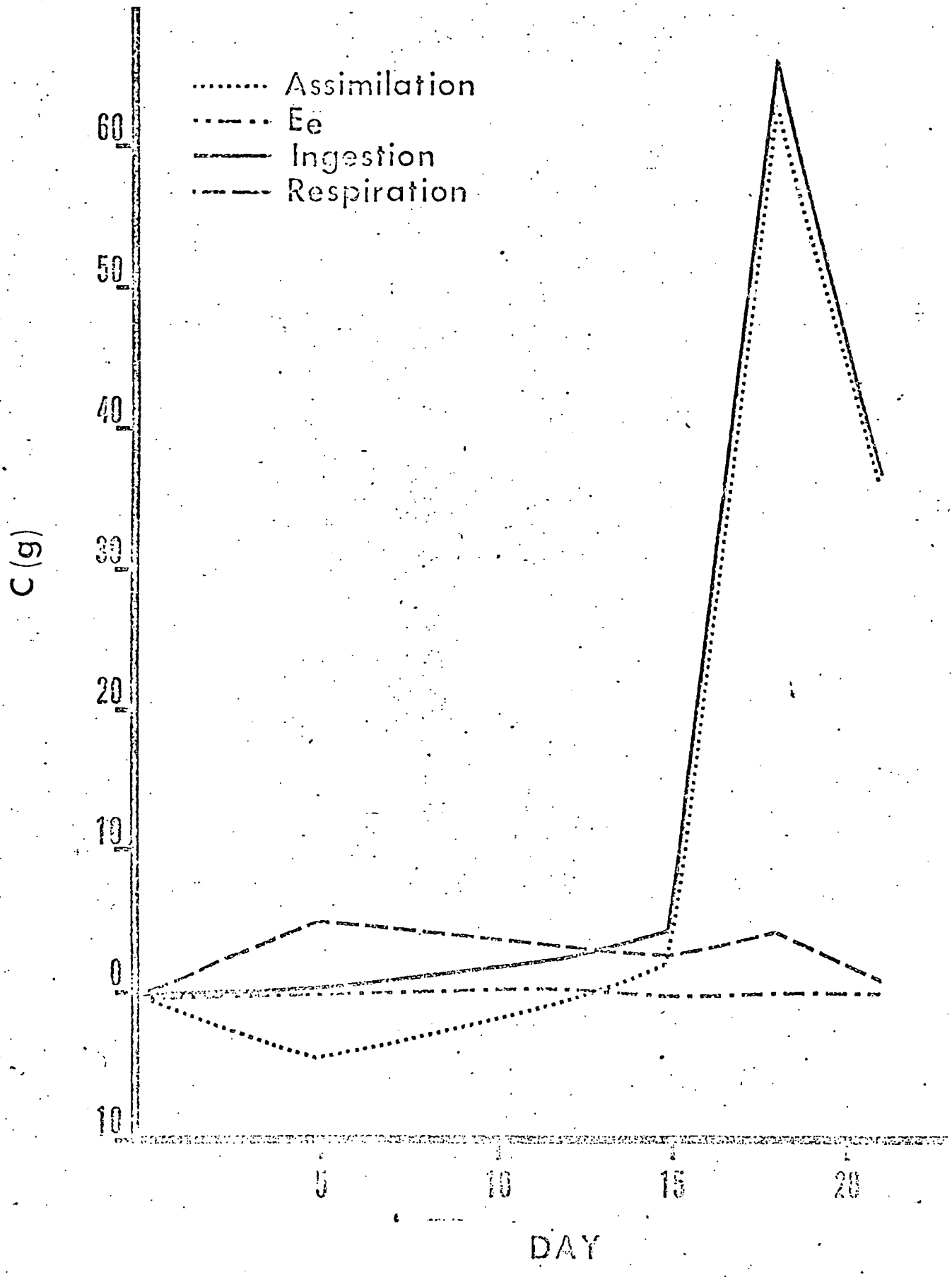
| | Ingestion µgC/pop/gen | Respiration µgC/pop/gen | | Assimilation µgC/pop/gen | Production | % Ea=A/I | % Et=P/A | % Ee=P/I |
|---|---|---|---|---|---|---|---|---|
| *Monhystera* sp | 980 µg | $C^{14}$ | 1082µg | -102 | 50 µg | - | - | 5.10 |
| | | GILSON | 321 µg | -121 | | - | - | |
| *Rhabditis marina* | 12 grams | $C^{14}$ | 23.35µg | ~12 | 1584 µg | ~100 | 0.013 | 0.013 |
| | | GILSON | 11.26µg | ~12 | | ~100 | 0.013 | |

TABLE 4

Energetic consideration of the growth of Rhabditis marina fed a mixture of 4 species of bacteria (unidentified species of Pseudomonas strains R 1, R 2, R 4 and R 6) at 25 C.

| Week | Population | Ingestion µgC/worm/hr | Respiration µl $O_2$/hr/g | Production µg C/worm | $E_e$=P/I |
|---|---|---|---|---|---|
| 1(10/31) | 38 | $4\times10^{-8}$ | 1452 | $1.6\times10^{-4}$ | 0.0% |
| 2(11/ 6) | 1477 | $0.88\times10^{-8}$ | 375 | $1\times10^{-5}$ | 0.31% |
| 3(11/10) | 2886 | $1.02\times10^{-8}$ | 474 | $3.6\times10^{-5}$ | -0.099% |
| 4(11/13) | 2713 | $12.3\times10^{-8}$ | 758 | $3.7\times10^{-6}$ | -0.0002% |
| 5(11/16) | 2602 | $6.21\times10^{-8}$ | 142 | $4.6\times10^{-6}$ | +0.004% |

Fig. 41


Assimilation
Ee
Ingestion
Respiration
C (g)
60
50
40
30
20
10
0
10
5
10
15
20
DAY

populations. A priori one might expect that if Lotka-Volterra kinetics dominate feeding relationships that the animals will feed upon the most abundant prey species. When a particular prey species declines numerically, the consumer would be expected to switch to another, more abundant prey. If this is not true and switching does not occur, then any large deviations from a strictly abundant relationship may be taken as evidence of dietary specialization and used as a measure of food web stability and informational complexion.

Allogromia laticollaris was chosen as the initial experimental animal for this subproject because its diet has received some prior attention in our laboratory and because it is very hardy and easily manipulated in dietary experiments. Allogromia used in these experiments were uniform in size and at a mid life cycle, a very actively feeding life cycle stage. Except for controls, the total concentration of both species of algae was kept at a constant level, $1 \times 10^6$ or $10^7$/ml. This concentration is at a level fairly close to the saturation limits for feeding by Allogromia. The concentration of each of the food species was varied in proportion to each other from 1:4-4:1 of the reference species. Controls contained only a single species and were varied only in concentration at levels which paralleled those in experimental vessels. The results of our initial experiments are presented graphically (Figs. 42-46). As can be seen, the presence of a second potential food species variously effected the predator's feeding patterns. Phaeodactylum tricornutum was not eaten in as great a quantity in the presence of Dunaliella parva as it was alone (Fig. 42). On the other hand, when Phaeodactylum was present in small concentration (<2:1) feeding on Dunaliella was enhanced (Fig. 43); switching occured after 1:1 prey concentration.

Feeding of Allogromia on mixed diets of Nanochloris sp (strain 41), Chlamydomonas subehrenberghii (strain 93) and Dunaliella parva seemed to follow Lotka-Volterra kinetics even though there was at least an order of magnitude difference in the consumption of Nanochloris in the presence of Dunaliella parva and the closely related Chlamydomonas subehrenberghii (Fig. 43). In the presence of large amounts of each other, neither Nitzschia acicularis nor Cylindrotheca closterium were eaten at control levels (Fig. 44). Switching was not evident. Nanochloris were eaten in relatively constant quantities in the presence of Dunaliella salina, but in the presence of Cylindrotheca closterium

switching was evident at a ratio of 2:1 or greater (Fig. 45). The reverse (*Cylindrotheca* eaten in the presence of *Nanochloris*) was also true. Switching was also seen in mixtures of *Dunaliella parva* and *Cylindrotheca closterium* (Fig. 46); feeding on *Dunaliella* was negligible below the 2:1 ratio of *D. parva*:*C. closterium*.

Fig. 42. The effect of varying concentration of 2 species of prey on consumption by *Allogromia laticollaris*.

1. *Phaeodactylum tricornutum* (39) control.
2. *Dunaliella parva* (14) in the presence of *Phaeodactylum tricornutum*.
3. *Phaeodactylum tricornutum* in the presence of *Dunaliella parva*.
4. *Dunaliella parva* control.

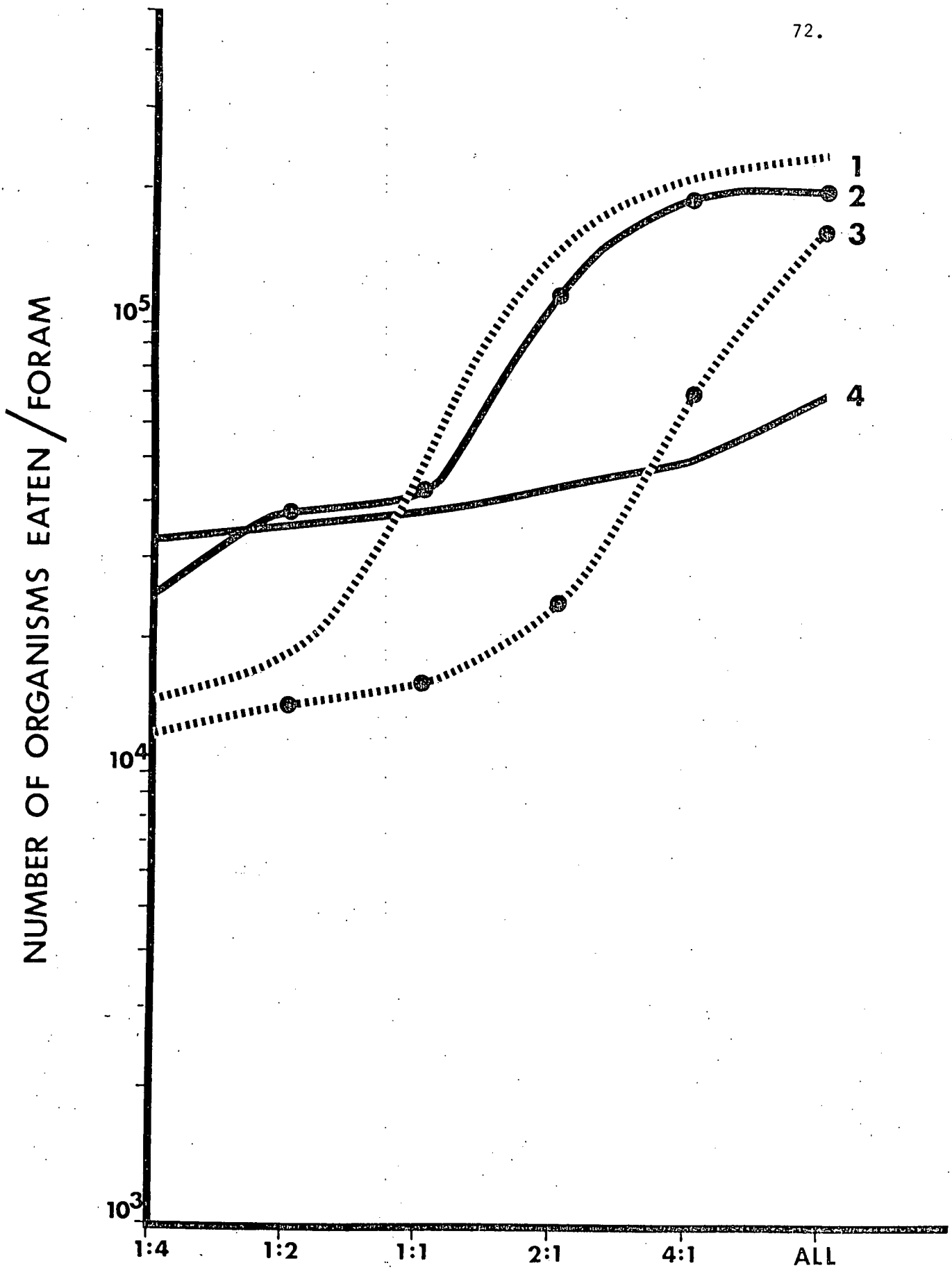

NUMBER OF ORGANISMS EATEN / FORAM
$10^5$
$10^4$
$10^3$
1:4
1:2
1:1
2:1
4:1
ALL
1
2
3
4

Fig. 43. The effect of varying concentration of 3 species of prey on consumption by *Allogromia laticollaris*.

1. *Nanochloris* spp (41) in the presence of *Dunaliella parva* (14).
2. *Chlamydomonas subehrenberghii* (93) in the presence of *Nanochloris* spp (41).
3. *Dunaliella parva* (14) in the presence of *Nanochloris* spp (41).
4. *Nanochloris* sp (41) in the presence of *Chlamyodmonas subehrenberghii* (93).

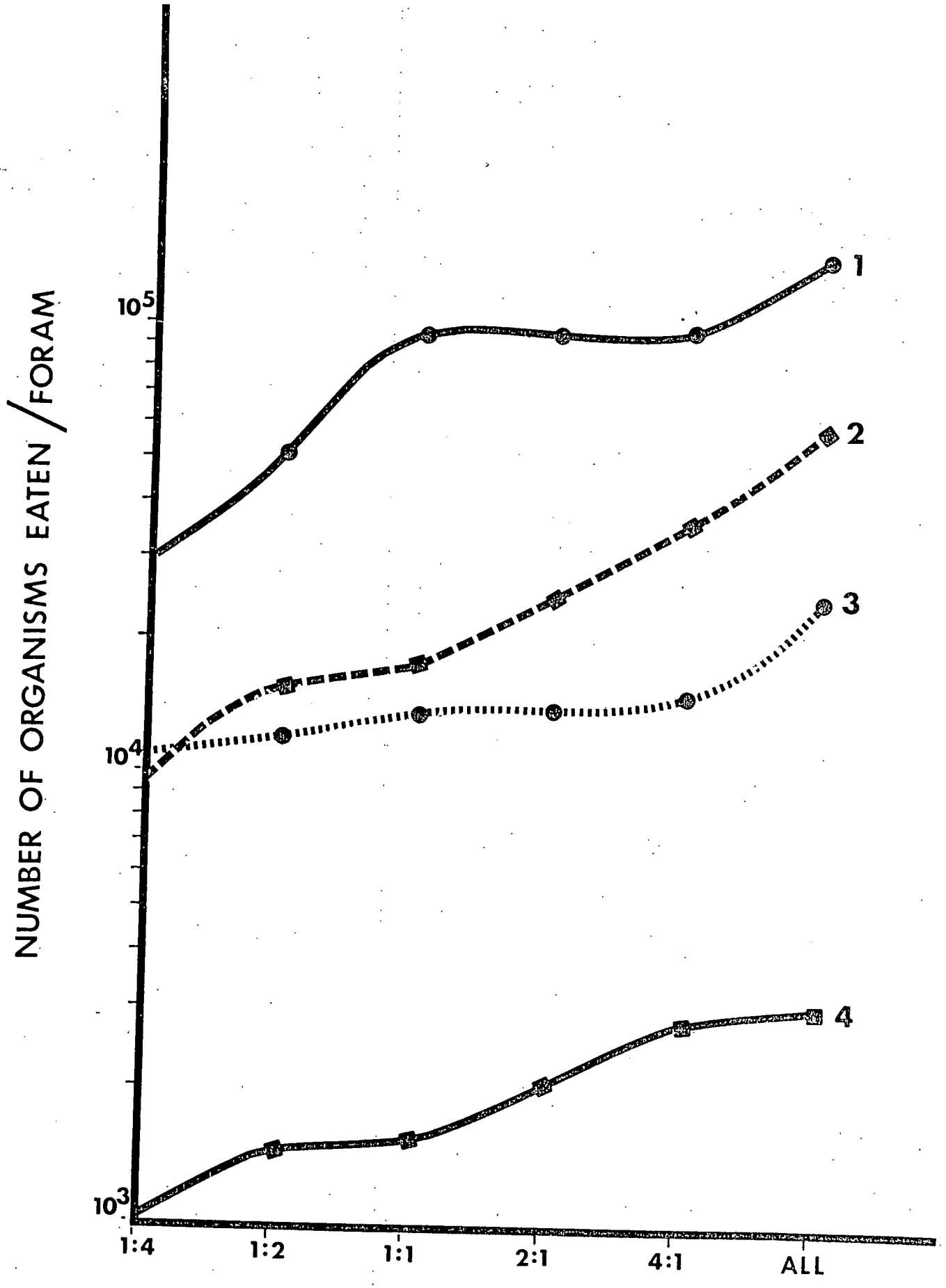

NUMBER OF ORGANISMS EATEN /FORAM
$10^5$
$10^4$
$10^3$
1
2
3
4
1:4
1:2
1:1
2:1
4:1
ALL

Fig. 44. The effect of varying concentration of 2 species of prey on consumption by *Allogromia laticollaris*.

1. *Nitzschia acicularis* (8) in the presence of *Cylindrotheca closterium* (9).
2. *Nitzschia acicularis* (8) control.
3. *Cylindrotheca closterium* (9) control.
4. *Cylindrotheca closterium* (9) in the presence of *Nitzschia acicularis* (8).

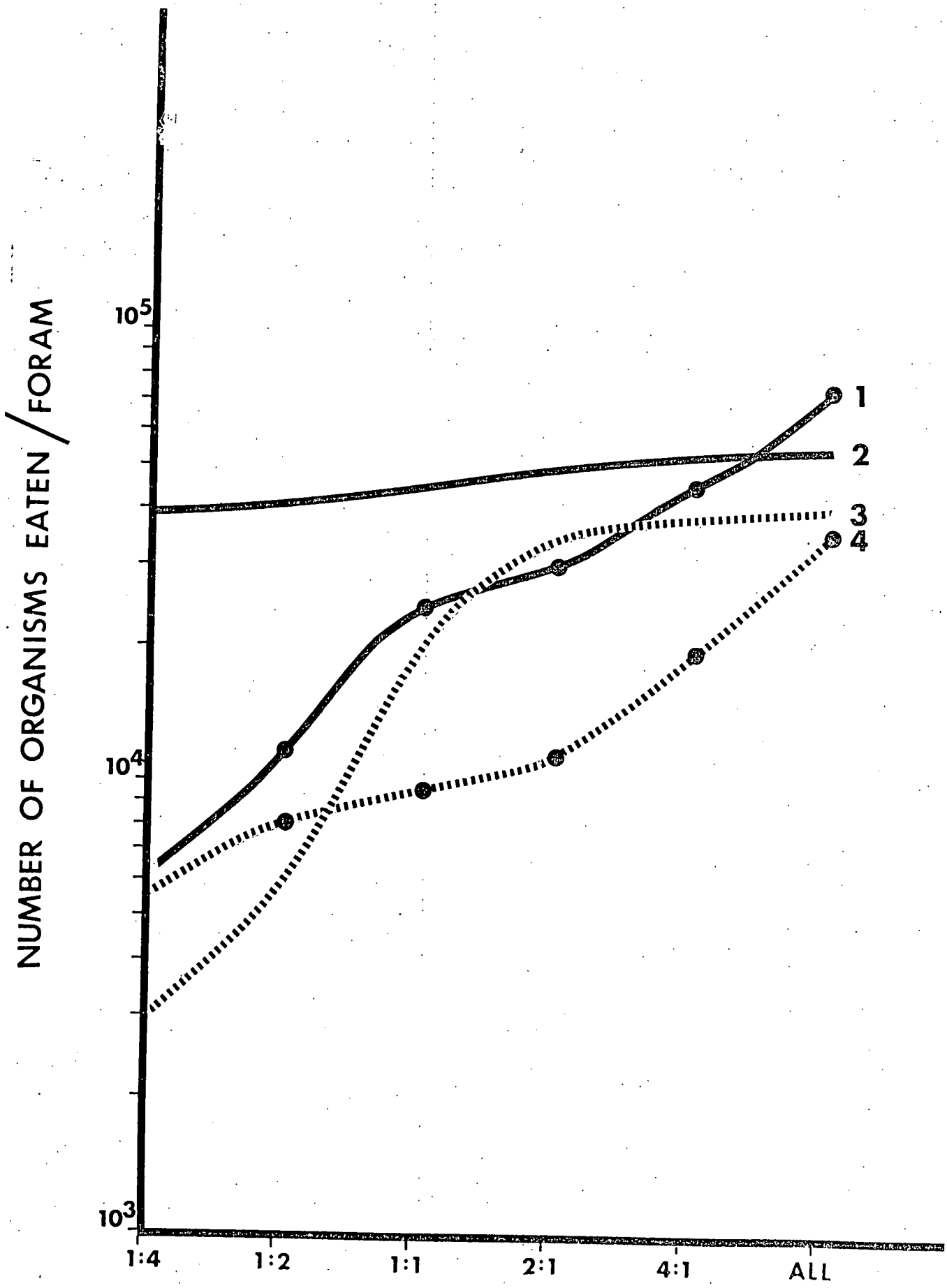

NUMBER OF ORGANISMS EATEN / FORAM
$10^5$
$10^4$
$10^3$
1
2
3
4
1:4
1:2
1:1
2:1
4:1
ALL

Fig. 45. The effect of varying concentration of 3 species of prey on consumption by *Allogromia laticollaris*.

1. *Cylindrotheca closterium* (9) in the presence of *Nanochloris* sp (41).
2. *Dunaliella salina* (13) in the presence of *Nanochloris* sp (41).
3. *Nanochloris* sp (41) in the presence of *Dunaliella salina* (13).
4. *Nanochloris* sp (41) in the presence of *Cylindrotheca closterium* (9).

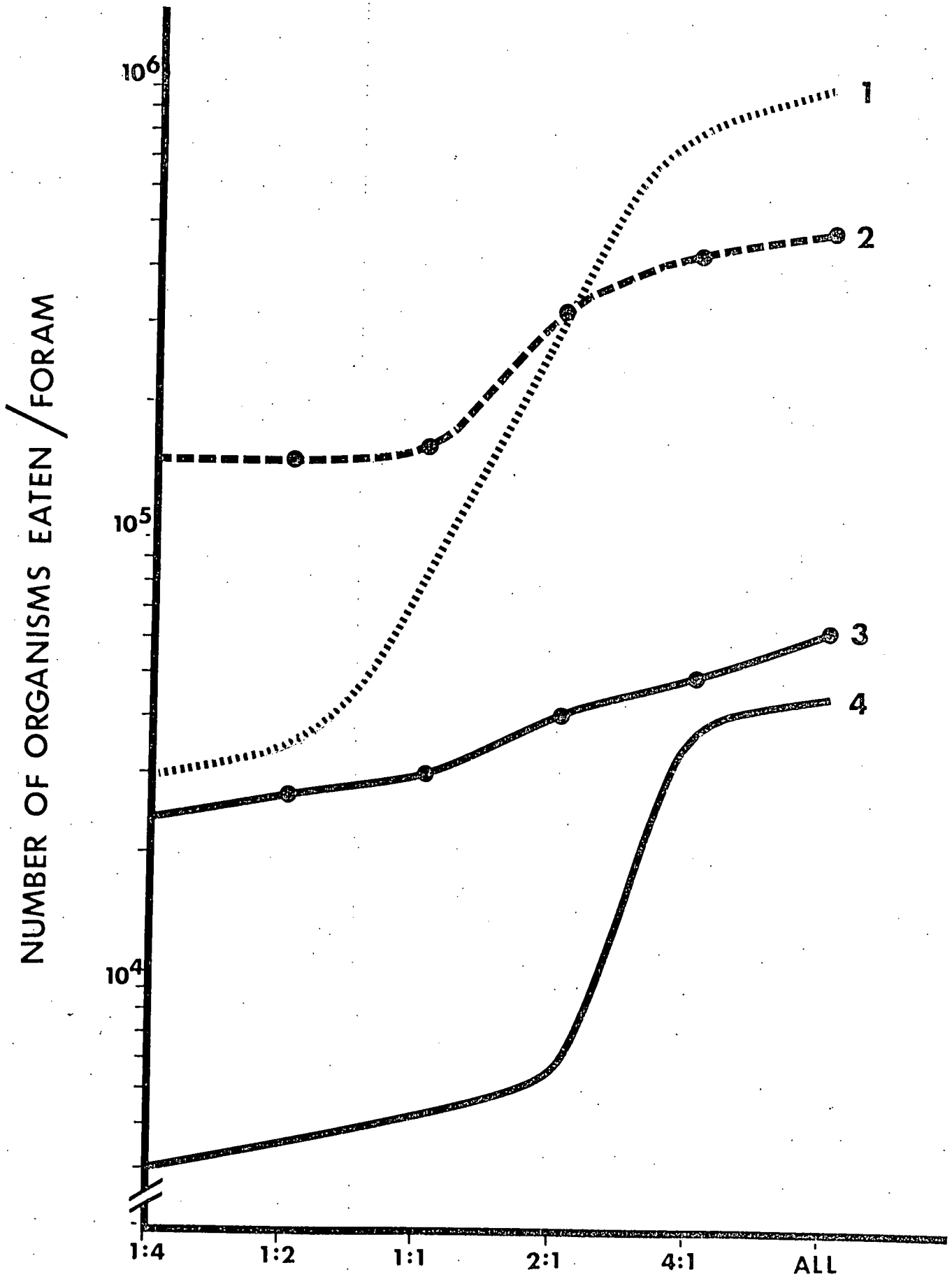
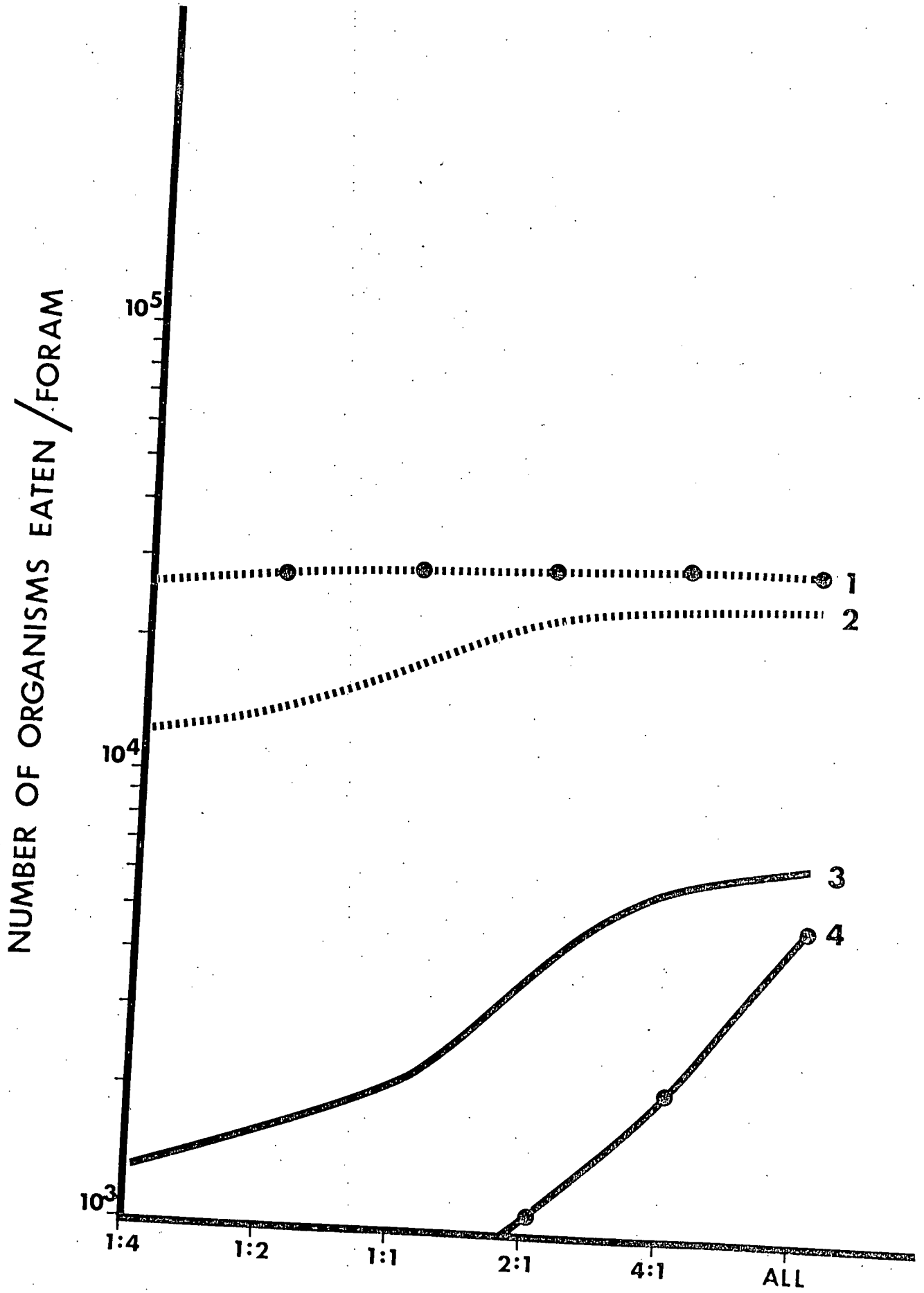

NUMBER OF ORGANISMS EATEN /FORAM
$10^6$
$10^5$
$10^4$
1
2
3
4
1:4
1:2
1:1
2:1
4:1
ALL

Fig. 46. The effect of varying concentration of 2 species of prey on consumption by *Allogromia laticollaris*.

1. *Cylindrotheca closterium* in the presence of *Dunaliella parva* (14).
2. *Cylindrotheca closterium* control.
3. *Dunaliella parva* control.
4. *Dunaliella parva* in the presence of *Cylindrotheca closterium*.


NUMBER OF ORGANISMS EATEN /FORAM
$10^5$
$10^4$
$10^3$
1
2
3
4
1:4
1:2
1:1
2:1
4:1
ALL

B. Stress and field experiments on transplanted and local natural communities to test the changes in community structure due to local water quality, heavy metals, etc.

Experiments on the effects of heavy metal ions on the growth of epiphytic communities begun last year were continued. Choice of concentrations to be tested was based on Dr. Grant Gross' analyses of the levels of metalic ions present in the N. Y. C. Ambrose light tower sludge dumping area. Thus far we have measured $LD_{50}$ and MLD for 3 species of worms and their food when grown under optimal conditions. For both the algae and the worms the toxicity of the various ions was generally: Ag>Hg>Cu>Pb (Tables 5,6). Tolerance was generally lower for exposure to metals for long periods. The data for 30 days exposure are also tabulated (Table 7). In general, metallic ions were more lethal to the foraminifera and nematodes than they were to the algae. With few exceptions they and 2 species of algae were killed by the lowest levels of metallic ions tested in the experiment (Table 5). The MLD for most algae was $\sim 5 \times 10^{-4}$ g/L (0.5 $^{o}/ooo$) of lead, chromium and mercury. Chlorophyte Bl-1 was a bit more resistant to lead. All the organisms were more sensitive to silver; MLD for this ion was $\leqslant 1 \times 10^{-6}$ g/L (0.005 $^{o}/ooo$).

TABLE 5

The toxicity of selected heavy metals to various salt marsh aufwuchs algae. *

| Alga Tested | Ag | | Cr | | Cu | | Hg | | Pb | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $LD_{50}$ | MLD | $LD_{50}$ | MLD | $LD_{50}$ | MLD | $LD_{50}$ | MLD | $LD_{50}$ | MLD |
| BL-1 | 7 | 10 (1)** | 40 | 80 (4) | 22 | 60 (2) | 40 | 60 (3) | 90 | >100 (5) |
| SH-1 | 5 | 9 (1) | 40 | 60 (4) | 30 | 60 (3) | 40-50 | 50 (2) | 80 | >100 (5) |
| 8 | 3 | 5 (1) | 70 | 80 (4) | 9 | 60 (3) | 20 | 30 (2) | 100 | >100 (5) |
| RF-8 | 2 | 5 (1) | -- | -- | -- | 50 (4) | 1 | 6 (2) | 20 | 30 (3) |
| 9 | 3 | 5 (1) | 70 | 80 (4) | 9 | -- (2) | 10-20 | 40 (3) | 100 | >100 (5) |
| BL15a | 3 | 5 (1) | -- | -- | 20 | 50 (3) | 3 | 5 (1) | 10 | 30 (2) |
| BL-17 | 4 | 6 (2) | -- | -- | 20 | 40 (4) | 2 | 5 (1) | 20 | 30 (3) |
| 38 | 7 | 10 (1) | 20 | 90 (4) | 40 | 60 (2) | 40-60 | 60 (3) | >100 | >100 (5) |
| 50 | 10-20 | -- (2) | 40 | 80 (4) | 30 | 80 (3) | 4 | 8 (1) | >100 | >100 (5) |
| 55 | 0.8 | 1-10 (1) | 80 | 80 (4) | 69 | 100 (3) | 4 | 10 (2) | >100 | >100 (5) |

* all values in ppm

** values in parenthesis represent relative toxicity on a scale of 1-5, where 1 = most toxic, 5 = least toxic.

TABLE 6

The acute toxicity of selected heavy metals to 3 species of salt marsh aufwuchs nematodes.*

| | Chromadorina | | | Monhystera ** | | Rhabditis ** | |
|---|---|---|---|---|---|---|---|
| Metal | $LD_{50}$ | MLD | | $LD_{50}$ | MLD | $LD_{50}$ | MLD |
| Ag | 0.25 | 0.5 | 24 hr | 0.5-1 | > 1 | 1 | > 1 |
| | | 0.4 | 3 d | | | | |
| | | 0.3 | 6 d | | | | |
| Hg | 0.25 | ~ 1 | 24 hr | 0.1 | > 1 | > 1 | > 1 |
| | | 0.8 | 3 d | | | | |
| | | 0.75 | 6 d | | | | |
| Pb | ~5-10 | > 100 | | > 100 | > 100 | > 1 | > 1 |
| Fe | >100 | >100 | 24 hr | > 40 | > 40 | > 1 | > 1 |
| Cr | >100 | >100 | 24 hr | ~ 75 | ≈ 100 | > 1 | > 1 |
| Cu | 2-3 | > 75 | 24 hr | 3 | > 74 | > 1 | > 1 |
| | | 75 | 3 d | | | | |

* all values in PPM

** data for 24 hr

TABLE 7

Preliminary study of the effects of heavy metal ions on the growth of selected salt marsh microorganisms and micrometazoa. *

| Organism | Lead | | Chromium | | Mercury | | Silver | |
|---|---|---|---|---|---|---|---|---|
| | LD 50/30 | MLD | LD 50/30 | MLD | LD 50/30 | MLD | LD 50/30 | MLD |
| Algae | | | | | | | | |
| BL-1 | $6x10^{-4}$ | $1x10^{-3}$ | $1x10^{-4}$ | $3x10^{-4}$ | $2x10^{-4}$ | $5x10^{-4}$ | $1.5x10^{-6}$ | $7x10^{-6}$ |
| RF-1 | $4x10^{-4}$ | $5.5x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-6}$ | $<1x10^{-6}$ |
| SH-1 | $5x10^{-4}$ | $9x10^{-4}$ | $1x10^{-4}$ | $3x10^{-4}$ | $1x10^{-4}$ | $4x10^{-4}$ | $2.5x10^{-6}$ | $5x10^{-6}$ |
| RF-8 | $2x10^{-4}$ | $6x10^{-4}$ | $2x10^{-4}$ | $3x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-6}$ | $<1x10^{-6}$ |
| BL-15A | $2x10^{-4}$ | $5x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-6}$ | $<1x10^{-6}$ |
| 8 | $5.5x10^{-4}$ | $9x10^{-4}$ | $4x10^{-4}$ | $5x10^{-4}$ | $3x10^{-4}$ | $4x10^{-4}$ | $<1x10^{-6}$ | $<1x10^{-6}$ |
| 9 | $6x10^{-4}$ | $6x10^{-4}$ | $2x10^{-4}$ | $3x10^{-4}$ | $4x10^{-4}$ | $5x10^{-4}$ | $<1x10^{-6}$ | $<1x10^{-6}$ |
| 38 | $4x10^{-4}$ | $7x10^{-4}$ | $2x10^{-4}$ | $3x10^{-4}$ | $2x10^{-4}$ | $3x10^{-4}$ | $2x10^{-6}$ | $3x10^{-6}$ |
| 39 | $8x10^{-4}$ | $1x10^{-3}$ | $2x10^{-4}$ | $4x10^{-4}$ | $1x10^{-4}$ | $1x10^{-4}$ | $1x10^{-6}$ | $4x10^{-6}$ |
| 50 | $5x10^{-4}$ | $7x10^{-4}$ | $2x10^{-4}$ | $3x10^{-4}$ | $2x10^{-4}$ | $3x10^{-4}$ | $2x10^{-6}$ | $3x10^{-6}$ |
| 55 | $5x10^{-4}$ | $9x10^{-4}$ | $3x10^{-4}$ | $4x10^{-4}$ | $1x10^{-4}$ | $2x10^{-4}$ | $2x10^{-6}$ | $<1x10^{-6}$ |
| Nematodes | | | | | | | | |
| *Chromadorina* | $<1x10^{-4}$ | $2x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-6}$ | $<1x10^{-6}$ |
| *Rhabditis* | $2.5x10^{-4}$ | $4x10^{-4}$ | $1.5x10^{-4}$ | $3x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $2x10^{-6}$ | $4x10^{-6}$ |
| *Monhystera* | $2.5x10^{-4}$ | $7x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $1.5x10^{-6}$ | $6x10^{-6}$ |
| Foraminifera | | | | | | | | |
| *Spiroloculina hyalina* | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-6}$ | $<1x10^{-6}$ |
| *Rosalina leei* | $5x10^{-4}$ | $9x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-6}$ | $<1x10^{-6}$ |
| *Allogromia laticollaris* | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $<1x10^{-4}$ | $1.5x10^{-6}$ | $3x10^{-6}$ |

* MLD and LD 50/30 given in g/l.

Fig. 47. The effect of acute pH shock on the growth of adult *Allogromia laticollaris*.

| | |
|---|---|
| Curve 1 | control |
| Curve 2 | pH 3 exposure 30 min. |
| Curve 3 | pH 3 exposure 1-4 hr |
| Curve 4 | pH 6 exposure 0.5-5 hr |

**Allogromia laticollaris**

Fig. 47

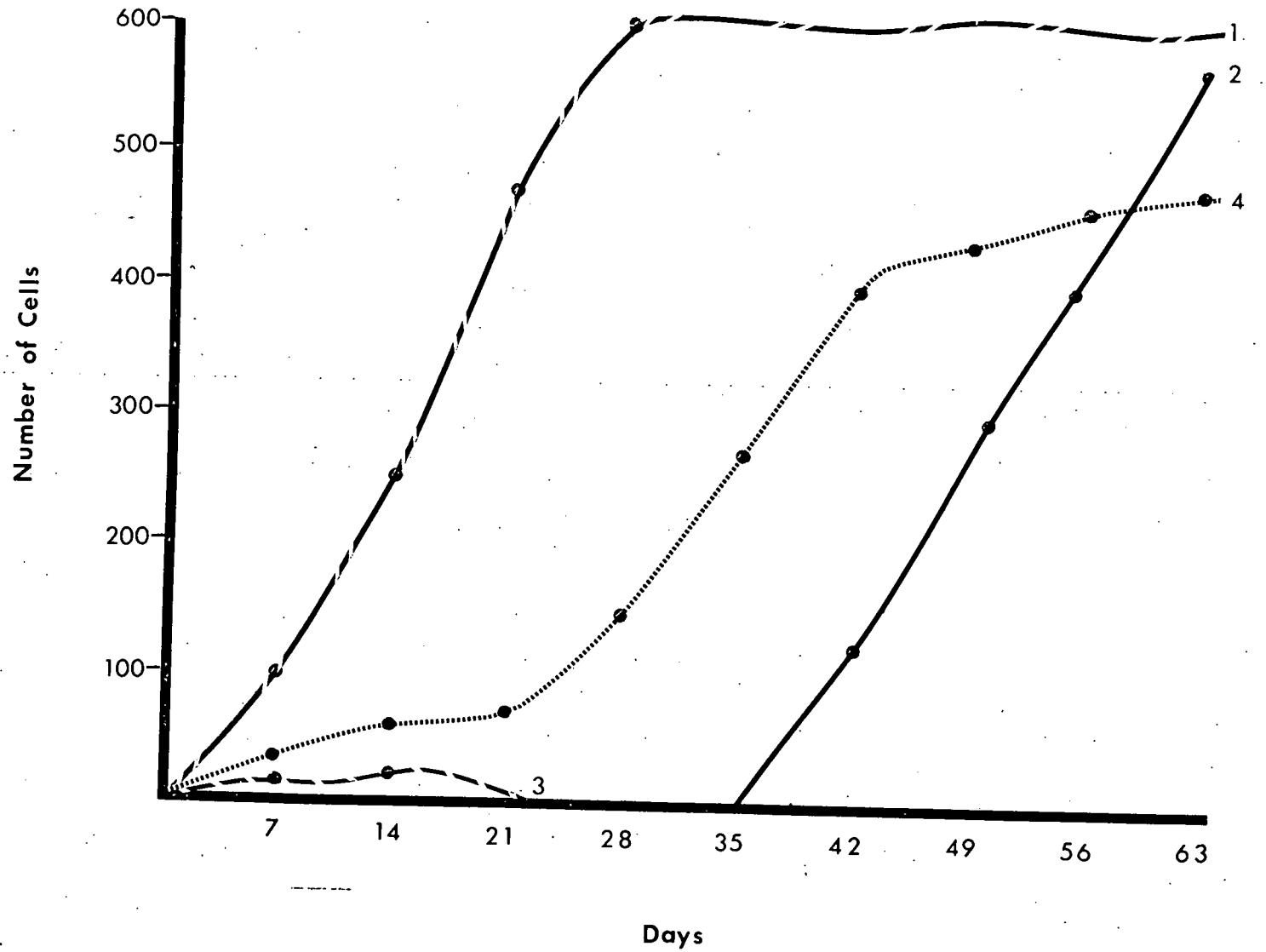


Fig. 48 The effect of acute pH shock on the growth and reproduction of *Euplotes vannus*

Curve 1 control

Curve 2 pH 3

Curve 3 pH 6

NB: Results were the same for exposure times from 0.5-4hr.

**Euplotes vannus**

Fig. 48

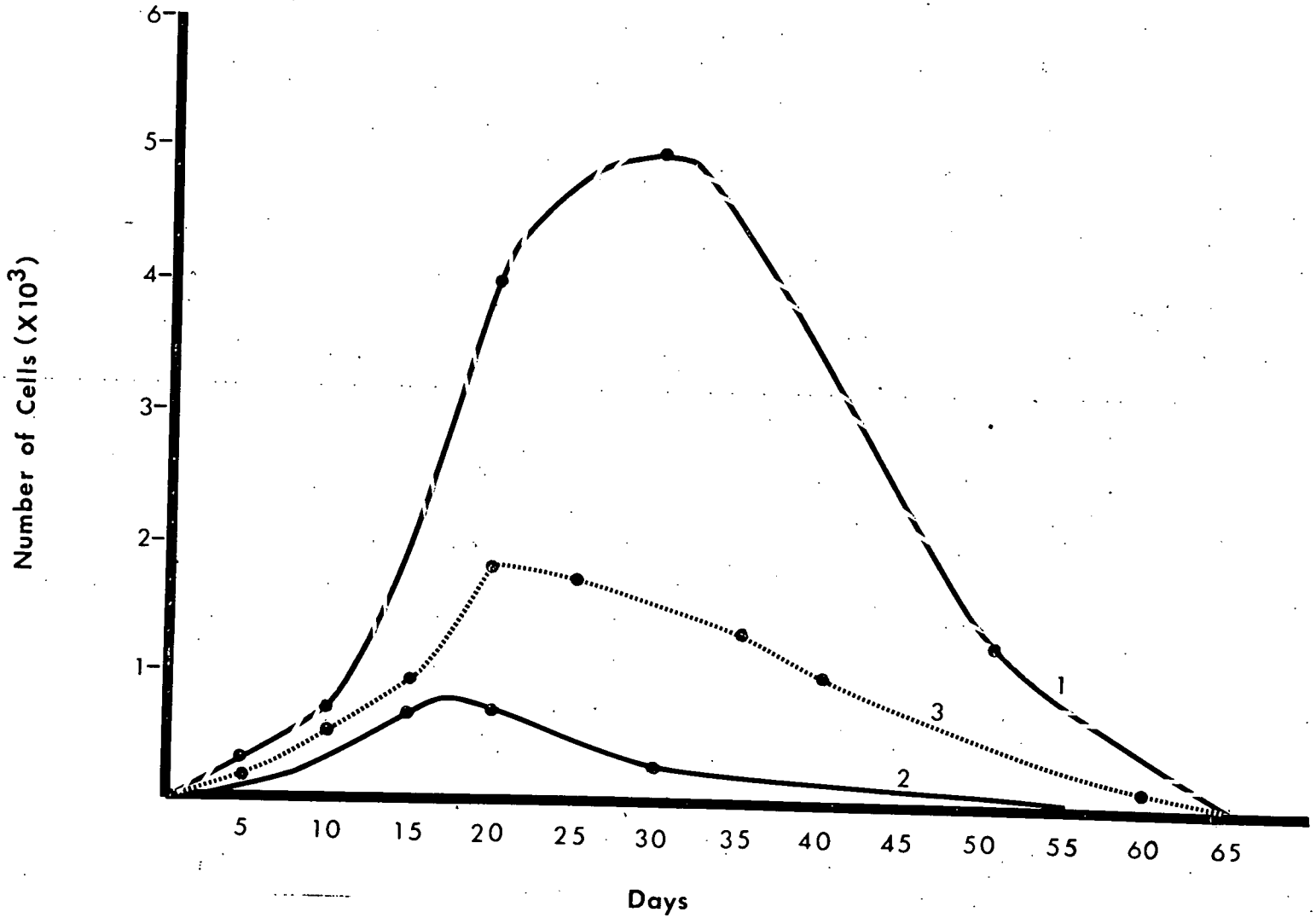


Fig. 49 The effect of chronic exposure of Euplotes vannus to Pb, Zn, Hg, Co, Mn or Fe

Curve 1 $\leq$ 5 ppm

Curve 2 10 ppm

**Euplotes vannus**

Fig. 49

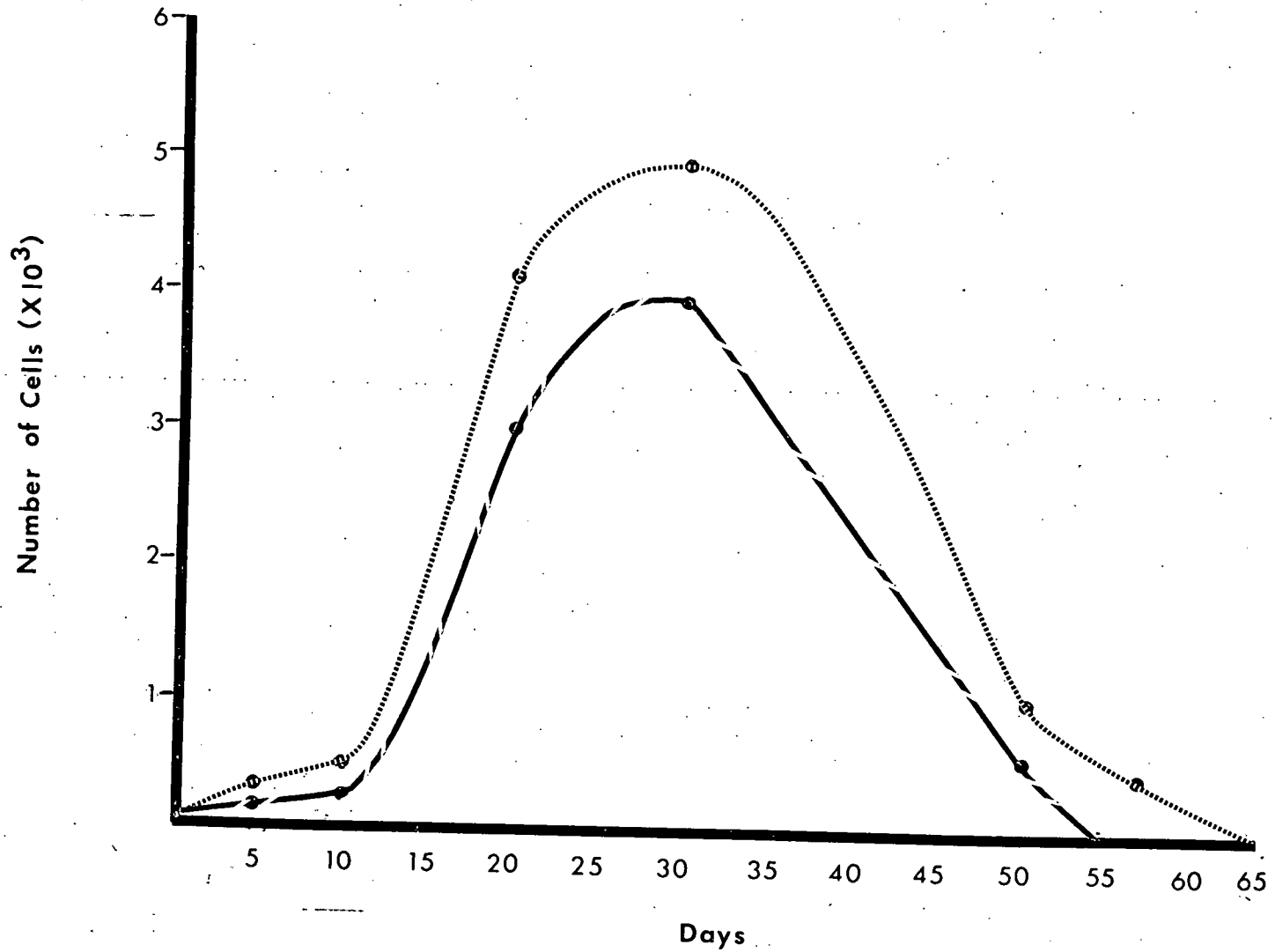


Fig. 50 The effect of chronic exposure of juvenile *Allogromia laticollaris* to Pb, Zn, Hg, CO, Mn or Fe.

Curve 1 control

Curve 2 Pb, Hg, Fe or Cu > 1 ppm

Curve 3 Zn or Cu > 1 ppm

## Allogromia laticollaris

Fig. 50

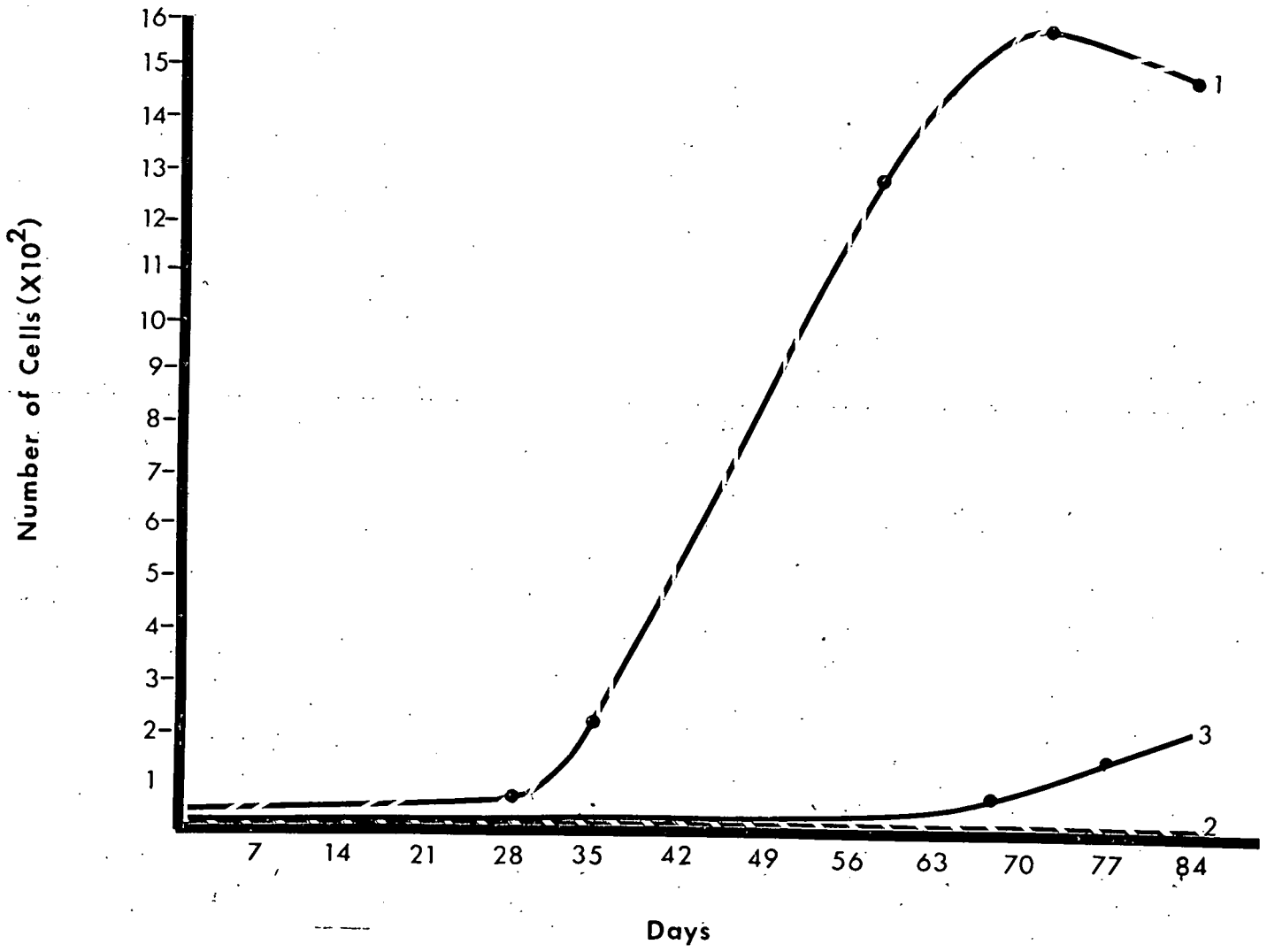


Fig. 51 The effect of chronic exposure of adult *Allogromia laticollaris* and *Rosalina leei* to Pb, Zn, Hg, Co, Mn or Fe

Curve 1 *Allogromia* control and exposure to ≤0.1 ppm Pb, Zn, Hg, Cu or Co.

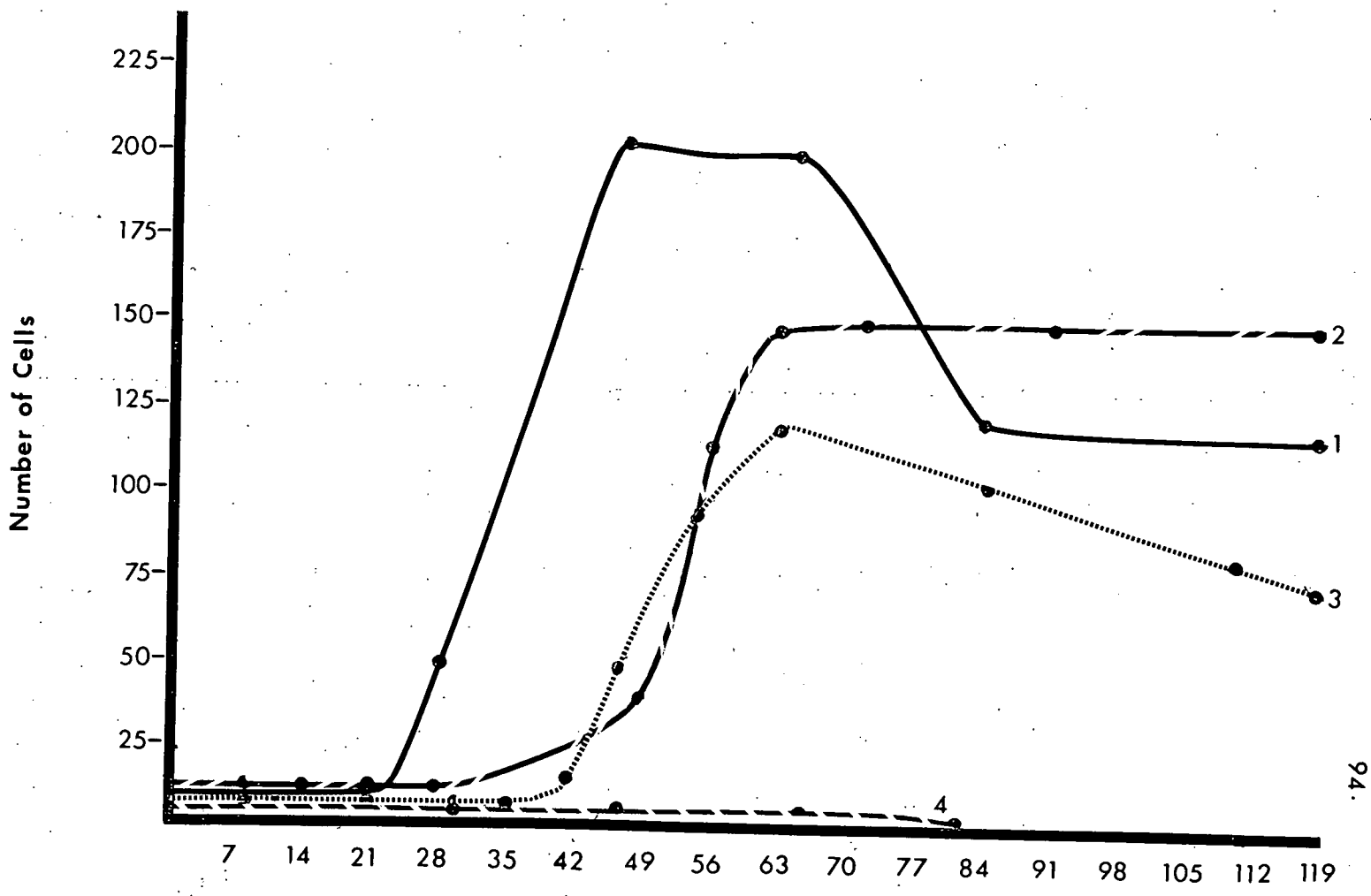
Curve 2 *Rosalina* control

Curve 3 *Allogromia* exposure to 1-5 ppm Pb, Hg, Zn, Co or Cu; 0.1 ppm Fe

Curve 4 Exposure of *Allogromia laticollaris* to any of the metallic ions ≥ 10 ppm; or *Rosalina leei* to metallic ions ≥ 1 ppm.

**Allogromia laticollaris**
**Rosalina leei**

Fig. 51



C. Field and laboratory studies aimed at characterizing the changes in the bacterial and unicellular algal communities over a summer growing season.

For the last few years we have been broadening and extending our knowledge of the natural community. The study of the diatom assemblage was finally completed this year and is included in this progress report. The bacterial population structure of the community is still being studied by one of our graduate students, Miss Eileen Kennedy. Her methods for collection and enumeration of the bacterial colonies were detailed in the progress report 4 years ago (NYO 3995-20; pp 14-15).

About 18 hrs after incubation, all plates were examined and any bacterial growth recorded. They were then incubated at 20° C in a 12 L/12 D environmental chamber and rechecked for growth after 4 and 10 days.

Bacterial colony types were described on the basis of relative size, pigmentation, whole colony appearance, edge and surface. The frequency of each was noted. Each different type was isolated by restreaking on a fresh plate of its initial isolation medium. Pure cultures were originally maintained on slants of the same medium but as a matter of practicality were transferred to a medium containing the following: yeast extract (Difco), 0.5%; Bactotryptone (Difco), 0.5%; Base B without $B_{12}$ (Lee et al. 1966) 100 ml. pH was adjusted to ~ 7.5.

The results were quite dramatic. The natural sea water based media, which are the standard media for this type of work, were quite inferior to the artificial sea water in both the isolation of bacteria and algae. The algal component of the microbial community was simpler to evaluate than the bacterial, since we were able to recognize easily the growth of the same species on different media. The total number of bacteria per gram (dry weight) of the community was estimated variously by individual media. Only after we identify each species and see the range of media on which each

species grew well will we be able to estimate accurately the bacterial component. Three species grew very well on unenriched sea water alone (Fig. 52). The number of bacteria growing on sea water agar fluctuated from $< 1 \times 10^6 - 2 \times 10^6$/g in early summer to a peak of $\sim 6 \times 10^6$/g on field trip #9 in early August (Fig. 54). Only 1-4 species were isolated on the sea water agar plates. Two species were consistently isolated on the sea water agar plates. Two species were consistently isolated on erdschreiber throughout the summer (Fig. 53). Although there were slight fluctuations in the populations, both species were quite abundant ($\sim 3 \times 10^6$/g; Fig. 54). The number of different species isolated on this medium gradually increased over the summer from 1 in early June to 7 in mid-August. Among the natural sea water based media, marine nutrient agar (Difco, Bacto Nutrient Agar 23 g/l, distilled sea water 0.5 l, sea water 0.5/l) and marine trypticase soy agar (B.B.L. trypticase soy agar 40 g/l distilled water 0.5/l, sea water 0.5/l) best showed fluctuations in the natural community. Two peaks in the total number of bacteria/g were observed (Fig. 55), a peak in late June (field trip #3) and another peak in early August (Field trips 8,9). These peaks coincide in time with the 2 annual summer blooms of foraminifera (see Matera and Lee, in last year's progress report). The 2 peaks were observable in all of the sea water based media except erdschreiber [sea water $KNO_3$ 0.01% (w/v), $NaH_2PO_4 \cdot H_2O$ 0.01% (w/v), and soil extract 5% (w/v)] and medium d. Growth on erdschreiber was depressed during the first peak. The second summer peak on other media was preceded by a late July peak on these latter media (Fig. 55). The growth of species b2, b3, c4, c5, d1, e3, and e4 were possibly correlated with both peaks. Species a2, d3, e1, e3 and e4 were depressed in numbers during the first peak. The greatest total numbers of species were isolated on marine trypticase agar (13 colony types; Fig. 56); on marine nutrient agar 10 types were isolated. Five colonial types were isolated on unenriched sea water agar and 7 types were isolated on erdschreiber. Medium d (sea water

enriched with $KNO_3$ 10 mg% and $NaH_2PO_4 \cdot H_2O$ 10 mg%) was more selective, since it promoted the growth of 2 species of bacteria and only a few other species grew on the medium all summer.

The seasonal changes in the community reflected by growth on the series A media are quite interesting. Many species which grew on this medium were agar digesters. On medium A 1, unenriched artificial sea water, there was a steady increase in total number of bacteria/g throughout the summer (Fig. 57). Of the 3 species which grew on the medium only 2, A 1-1 and A 1-2, grew in the early part of the summer (Fig. 56). The first isolation of species A 1-3 was in mid-August (Fig. 57). The pattern of colony growth on medium A 2 (enriched with $NaNO_3$) and A 3 (enriched with $NH_4Cl$) was similar. The same progressive general increase in number of bacteria/g was observed although the peak population was not as great as in medium A 1 (Fig. 57). The number of species growing on the media gradually increased throughout the summer (Fig. 56). A total of 7 species grew on both media (Figs. 59, 60). We found nitrite inhibited bacterial growth. Only on August 19 did we grow a small number of colonies of a single species on medium A 4 (Figs. 54, 56). The growth on medium A 5, which is enriched with $NH_4NO_3$ and $NaH_2PO_4$, was very interesting since it paralleled the growth pattern observed in medium D the sea water media also enriched with $NO_3^-$ and $H_2PO_4^-$. Growth on medium A 5 was minimal during the 2 seasonal peaks on other media (Fig. 54). Two peaks, one in week 7 and one in week 9, immediately preceding and immediately following those observed on other media, were noted. Species A 5-1 was present in both peaks and greatly depressed in the collections on August 5. Species A 5-3 was present in large numbers only during weeks 6 and 7 (Fig. 61).

Throughout the season, 2 species of bacteria were consistently isolated on medium B 1 (Fig. 62). Though there were minor fluctuations, populations of species B 1-1 numbered $\sim 2.5 \times 10^6$/g of the epiphytic community while species B 1-2 numbered $\sim 2 \times 10^5$/g. Small numbers of 6 other species were collected in late summer. In terms

of total numbers of bacteria, the peptone enriched medium B 2, was the best. On it 4 species grew well (> 1 x $10^6$). Peaks in the population were observed on field trips 3, 7, and 10 (Figs. 63, 64). Only 1 species, B 3-1, grew well on medium B 3, the medium enriched with urea (Fig. 65). Substantial growth was noted on this medium from the 6th field collection (July 23) until a decline which began on the 11th collection (Fig. 63). From mid-July on, there was a progressive increase in the number of species collected on this medium (Fig. 66).

The same trend of progressive increase in the number of species isolated from the community on successive collections which we observed on media A-5 and B 3 was observed on all the series C media (Fig. 66). Ten species grew on media enriched with $B_{12}$ (C 1) and 11 species grew on the media enriched with thiamin, biotin and $B_{12}$ (C 2) (Figs. 67-70). Organisms with these requirements were not isolated from the community until the 6th or 5th field collections respectively. Though the population trends on both media at first seem quite similar (Fig. 71) closer examination of the growth patterns of individual species on both media shows that different species were isolated on each of the media. Peaks in the portion of the community isolated on these media were observed on either the 6th and 9th collections in medium C 1 or the 5th and 8th weeks on medium C 2. Although medium C 3 could be described as an artificially based erdschreiber, the growth on both media (Figs. 55, 56, 66, 71) were quite dissimilar in respect to the seasonal trends in both the total numbers and types of bacteria isolated. The growth of one organism, C 3-1, which was one of the most abundant species at the beginning of the season, gradually declined over the course of the summer (Fig. 72). Another species, C 3-4, grew well from mid-July to the end of August. One species, C 4-1, grew well from the 4th to the 11th field collection on medium C 4 (Fig. 73). During this period, the fluctuations in the number of organisms isolated on this vitamin enriched medium were minimal.

Though there were exceptions, there was a general trend of seasonal succession and increasing numbers of species which grew on the series D media (Figs. 66, 74, 75, 76). On medium D 1 which contained acetate, lactate and glucose as substrates, only 1 species, D 1-1, was found in quantity throughout the summer (Fig. 76). Species D 1-2 was abundant in early summer and gradually diminished in numbers. Species D 1-4 was found from weeks 3-8, species D 1-5 from 7-8, species D 1-6 from 8-10, and species D 1-7 was found only in weeks 11 and 12. The increase in the total numbers of bacteria isolated on the glutamate enriched medium (D 2) as the summer progressed was due both to the increase in the populations of species D 2-1 and D 2-3 and to the increase in the total number of species which grew on this medium (Fig. 78). Peak growth on medium D 2 was observed on collections from weeks 7, 9, 10. We observed peak growth on medium D 4 during weeks 3, 5-7 and 10. Though the species isolated were different, the population trends observed on medium D 2 were also observed on media 4-8. Mannitol, glycerol and extracts of Enteromorpha each stimulated the growth of 7 species while only 5 species grew on the acrylic acid enriched plates. The pattern of growth observed on medium D 7 (autoclaved Enteromorpha community) was similar to the growth observed on medium C 4 (vitamins 8A; Figs. 71, 74). It seems probable that autoclaved Enteromorpha stimulated the bacteria.

The 127 bacterial isolates have been subjected to 54 morphological and physiological tests. The results (Table 7 ) describe 117 "unit characters" of each organism and these will be scored for analysis by computer. The Jaccard coefficient, $S_j$, will be used to determine the similarities between each of the isolates. The strains will be clustered by a single linkage technique using the GTP-2 program supplied to us by Dr. Rita Colwell of the University of Maryland. We have just finished entering the program into our computer and are presently coding our data in the required form.

Using a program obtained from Dr. Rita Colwell, the bacterial isolates have been subjected to computer analysis. For adansonian classification similarity indicies S, which does not include negative matches, and M which does include such patches, were calculated for each pair of organisms and the organisms were grouped into clusters on the basis of the S values. Four groups were created at the highest S value of 95% and all strains were clustered at an S value of 48%. A similarity matrix is presently being developed to summarize and graphically represent the results obtained.

Fig. 52.

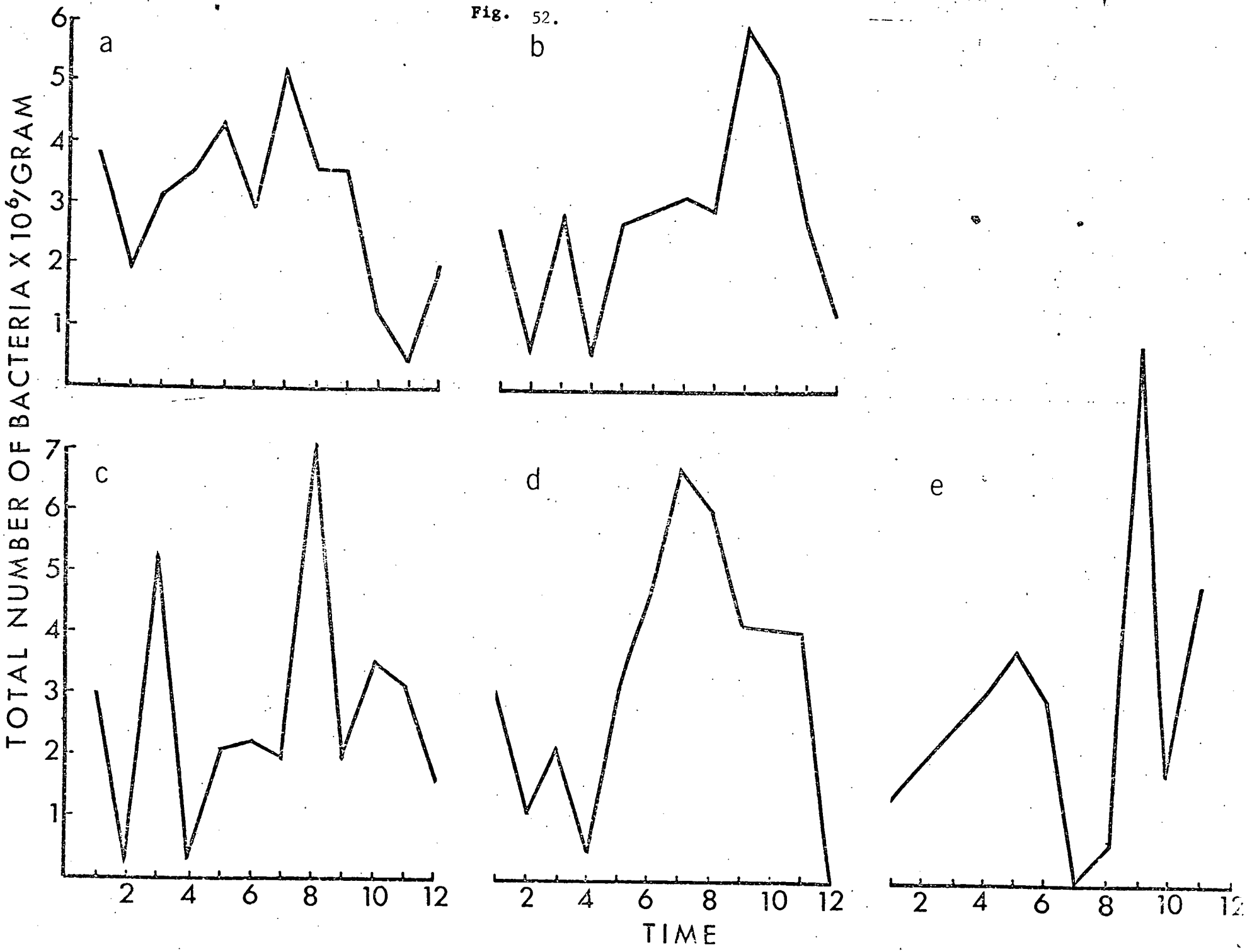

a
b
c
d
e
TOTAL NUMBER OF BACTERIA X 10⁶/GRAM
TIME
1
2
3
4
5
6
7
2
4
6
8
10
12

Fig. 53

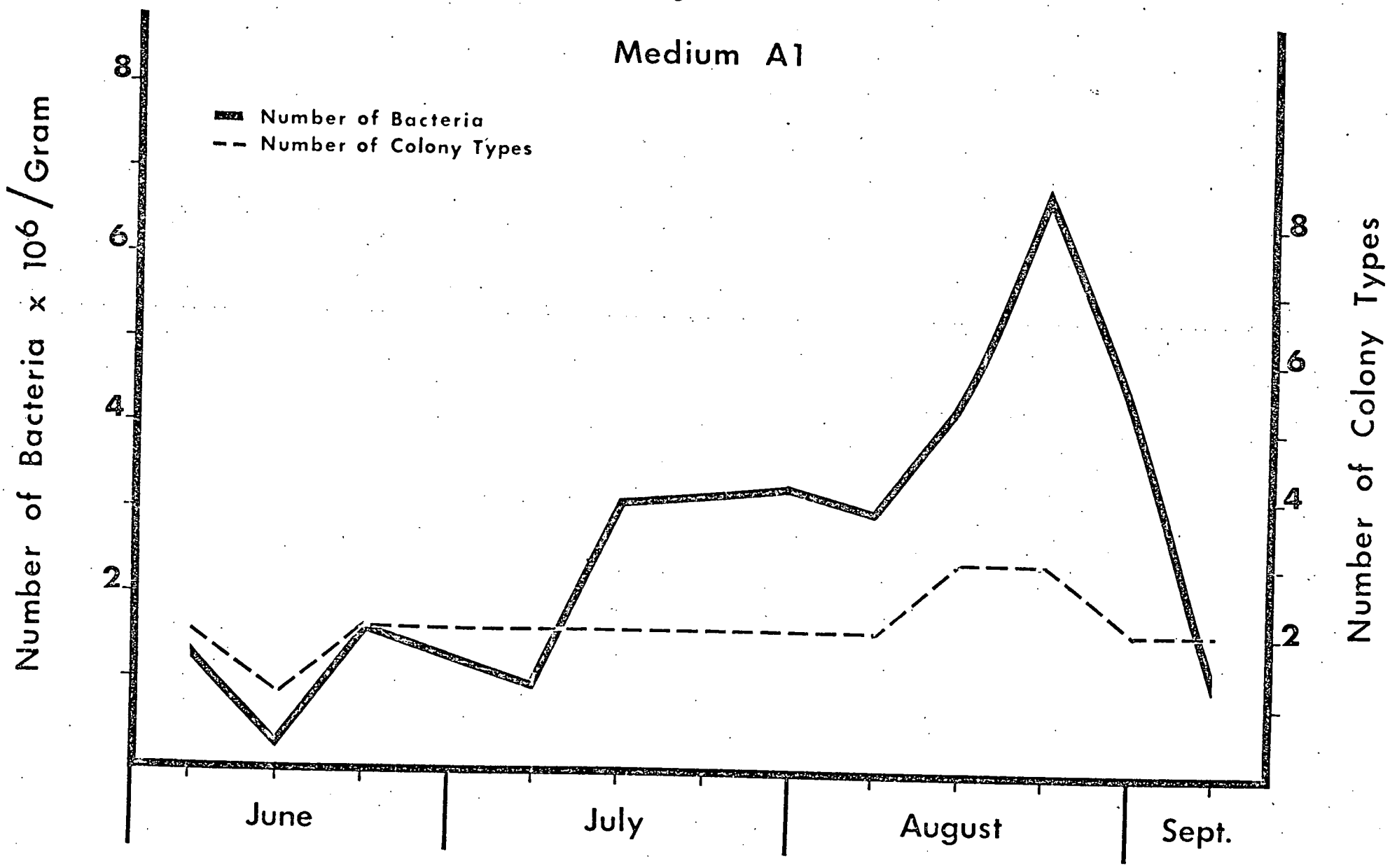

Medium A1
Number of Bacteria
Number of Colony Types
Number of Bacteria x 10^6 /Gram
Number of Colony Types
8
6
4
2
June
July
August
Sept.

Fig. 54

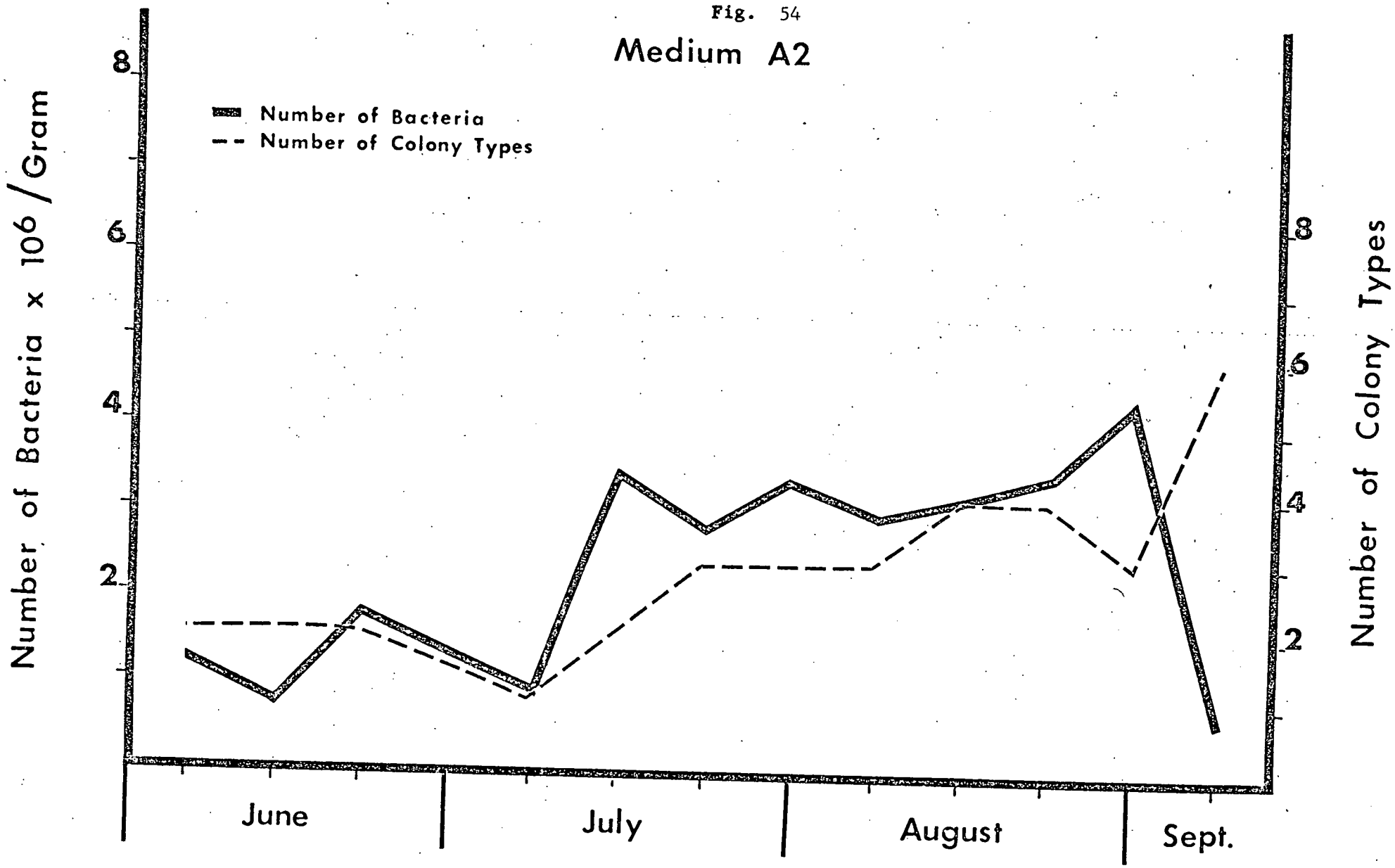

Medium A2
Number of Bacteria
Number of Colony Types
Number of Bacteria x 10^6 /Gram
Number of Colony Types
8
6
4
2
8
6
4
2
June
July
August
Sept.

Fig. 55

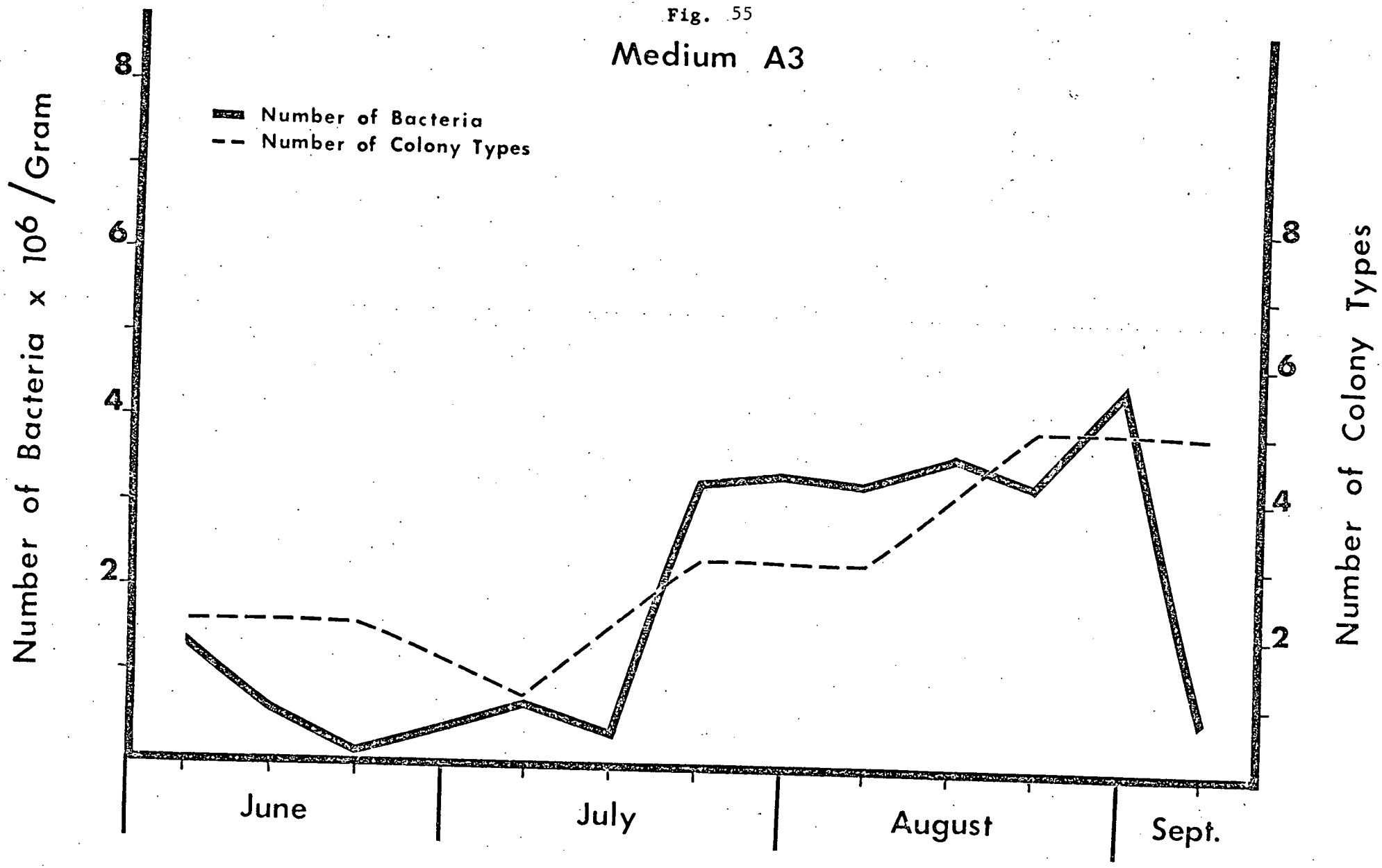

Medium A3
Number of Bacteria
Number of Colony Types
Number of Bacteria x 10^6 /Gram
Number of Colony Types
8
6
4
2
June
July
August
Sept.

Fig. 57

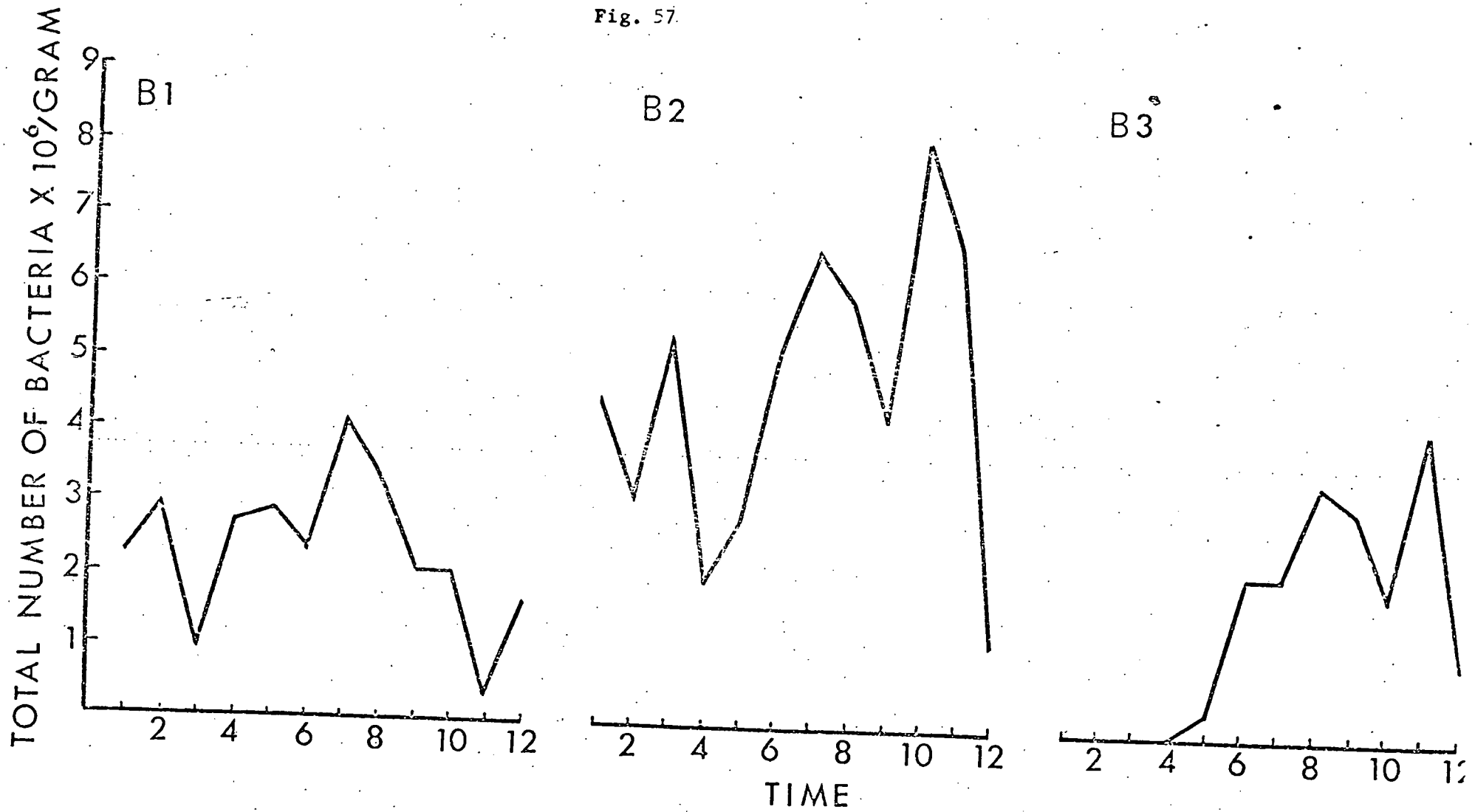

TOTAL NUMBER OF BACTERIA X 10^6/GRAM
9
8
7
6
5
4
3
2
1
B1
B2
B3
2
4
6
8
10
12
TIME

Fig. 56

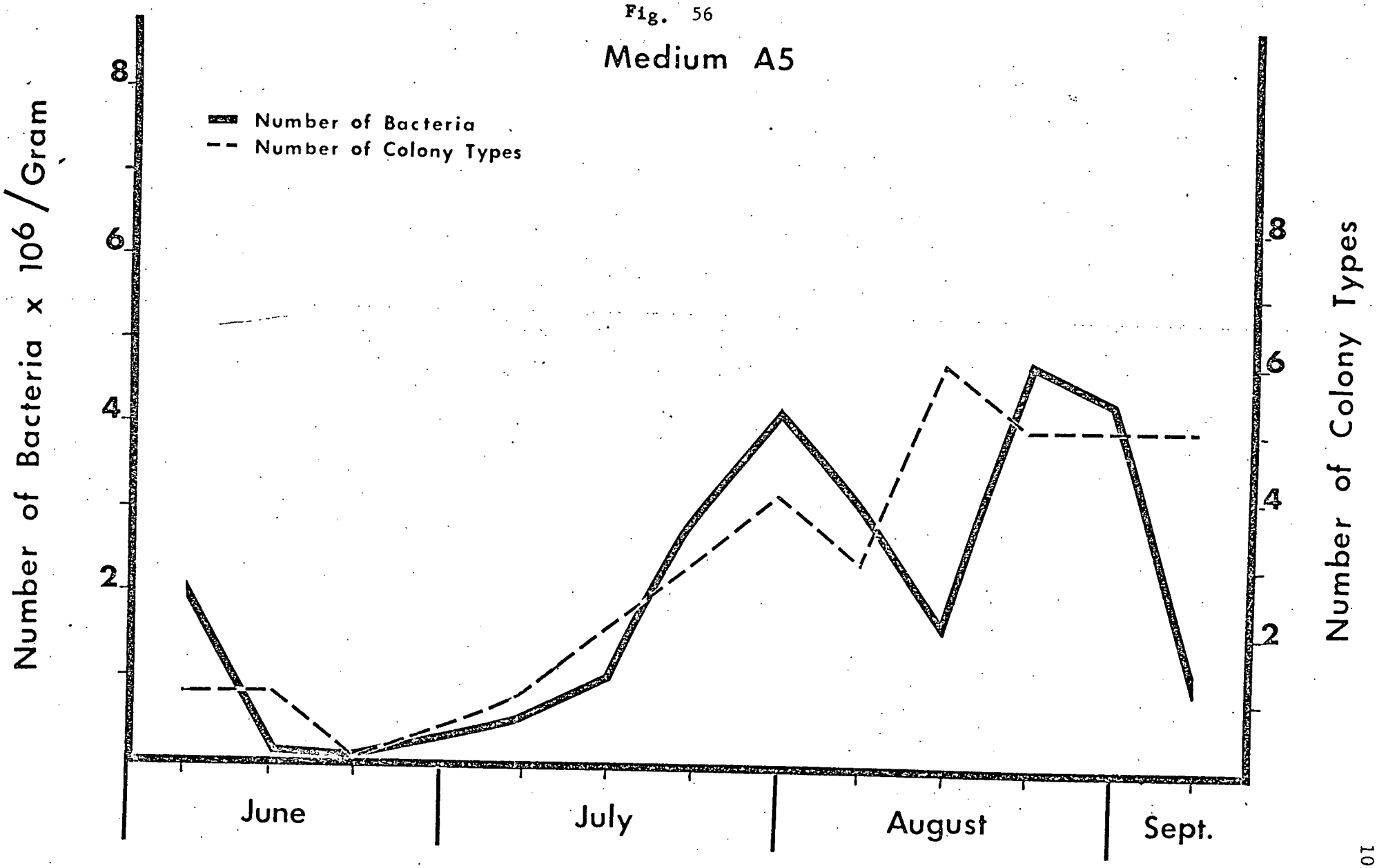

Medium A5
Number of Bacteria
Number of Colony Types
Number of Bacteria x 10^6 /Gram
Number of Colony Types
8
6
4
2
June
July
August
Sept.

Fig. 57

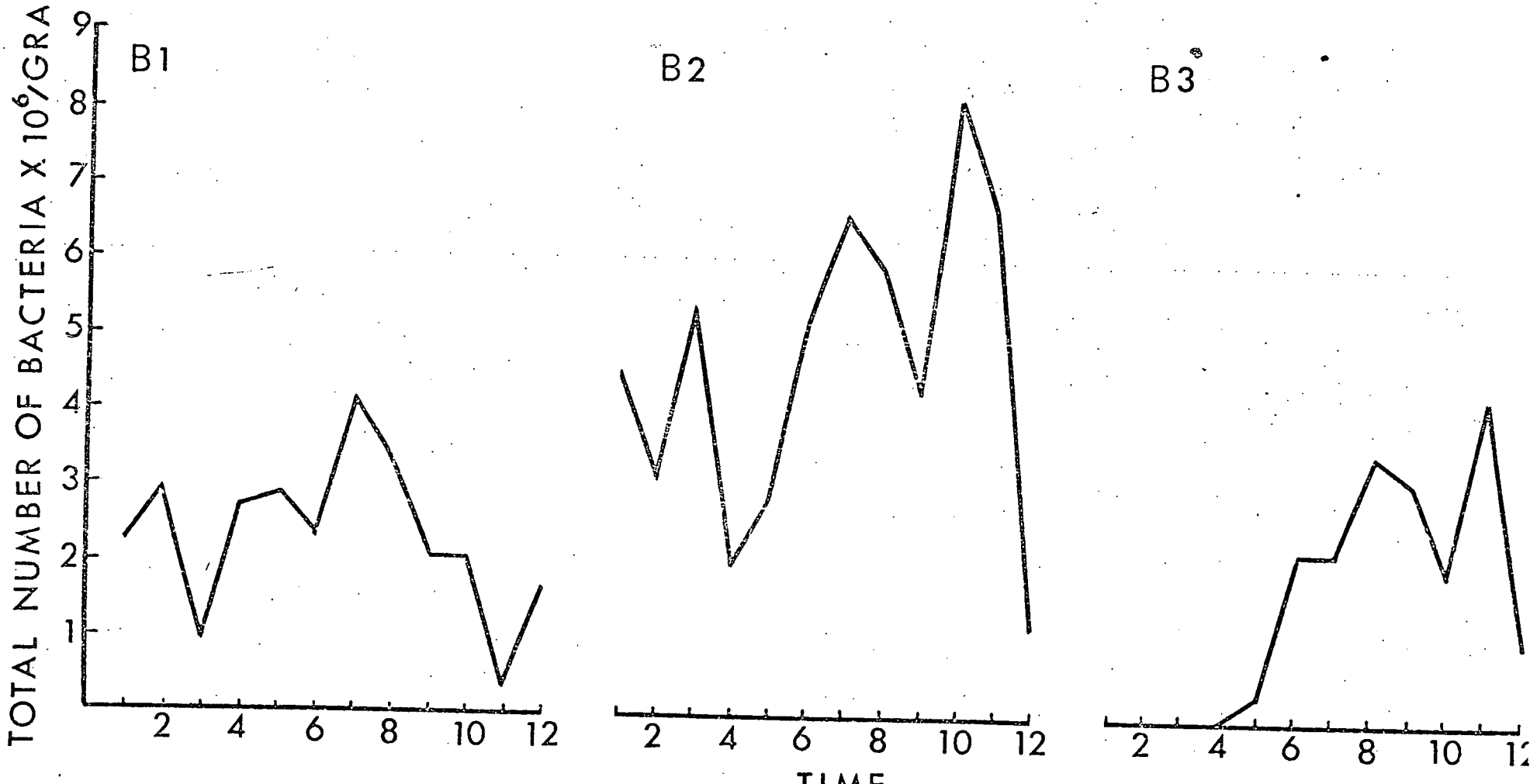

TOTAL NUMBER OF BACTERIA X 10⁶/GRAM
9
8
7
6
5
4
3
2
1
B1
B2
B3
2
4
6
8
10
12
TIME

Fig. 58

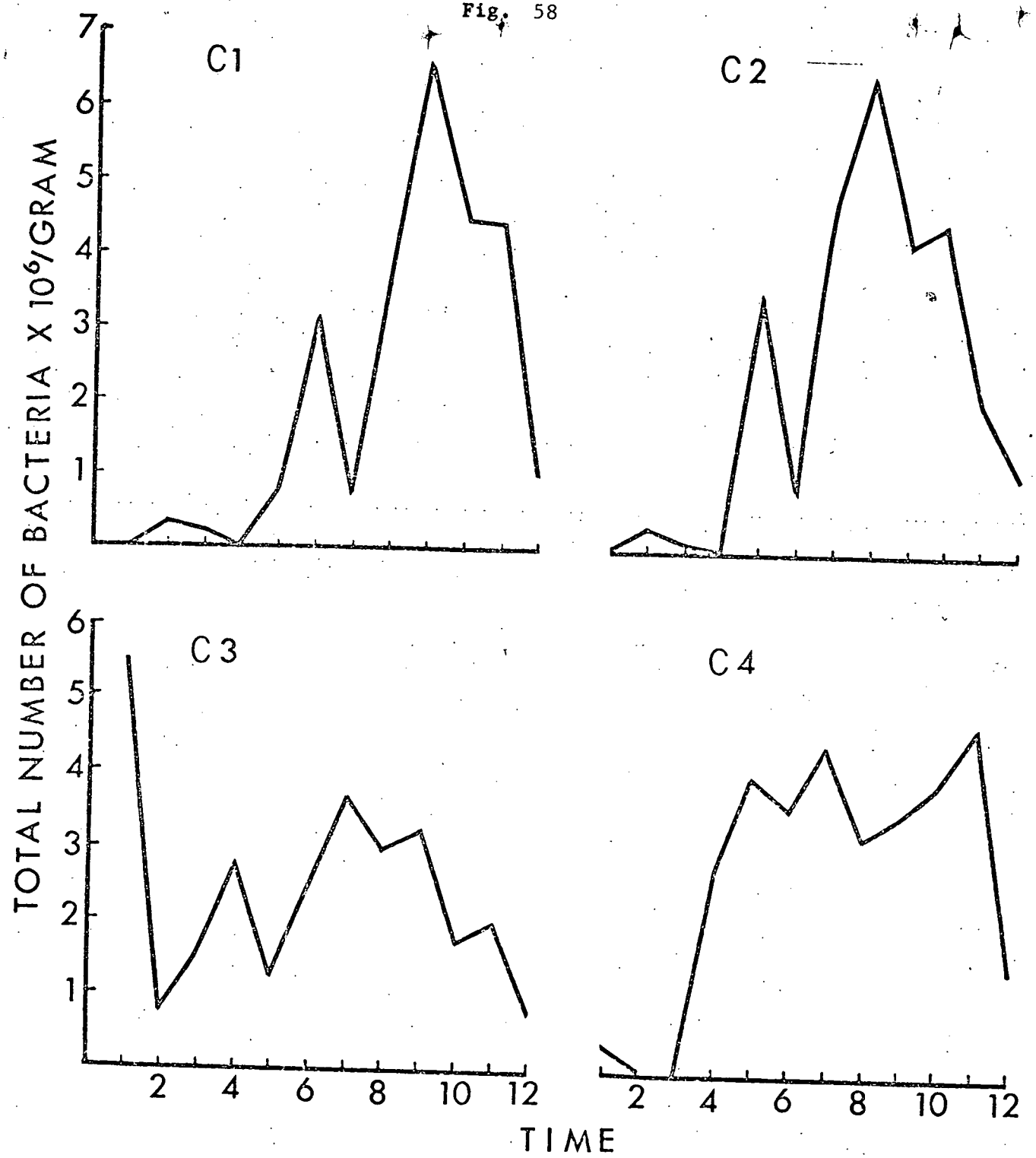

C1
C2
C3
C4
TOTAL NUMBER OF BACTERIA X 10⁶/GRAM
TIME
1
2
3
4
5
6
7
2
4
6
8
10
12

Fig. 59

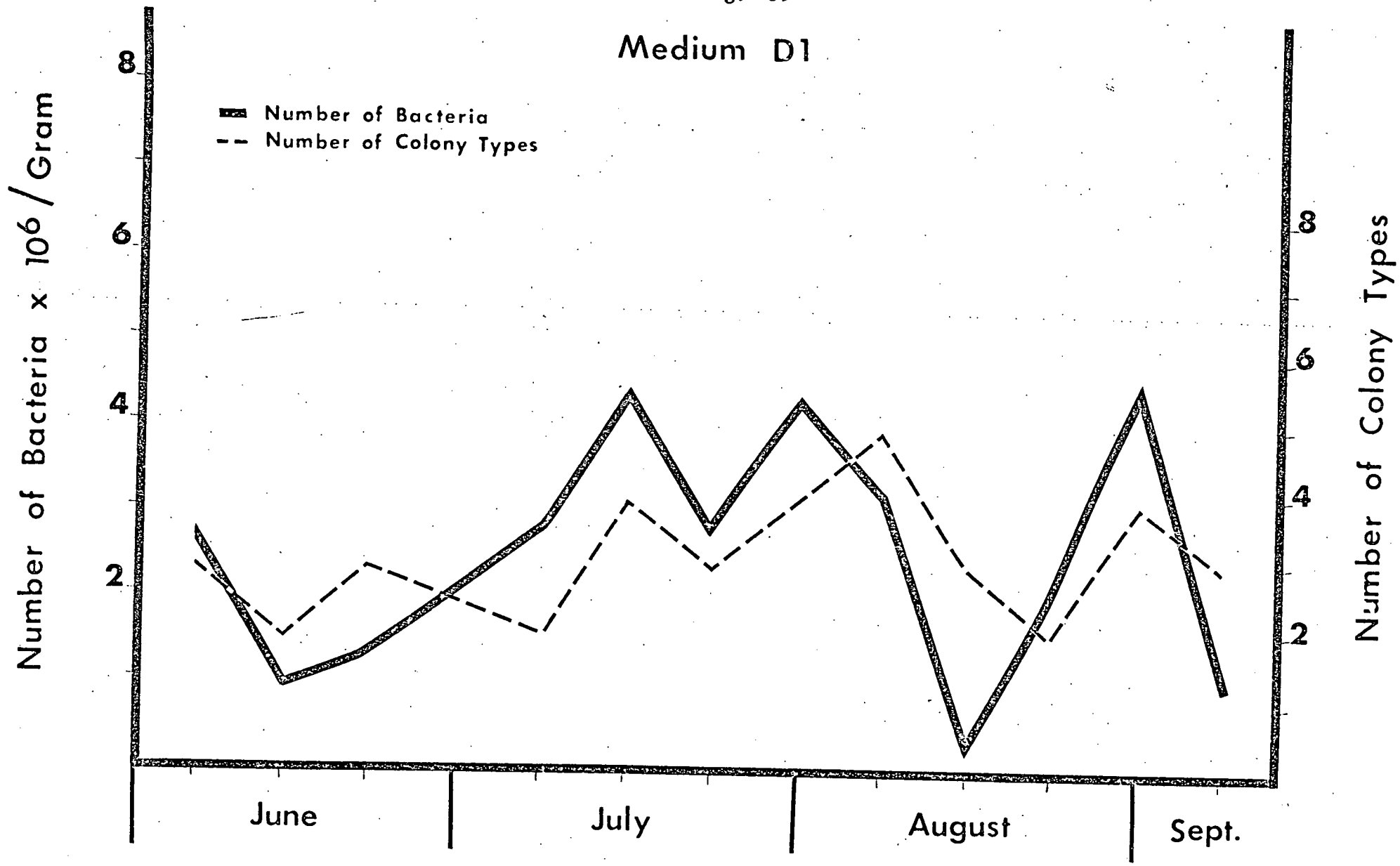

Medium D1
Number of Bacteria
Number of Colony Types
Number of Bacteria x 10^6 / Gram
Number of Colony Types
8
6
4
2
8
6
4
2
June
July
August
Sept.

Fig. 60

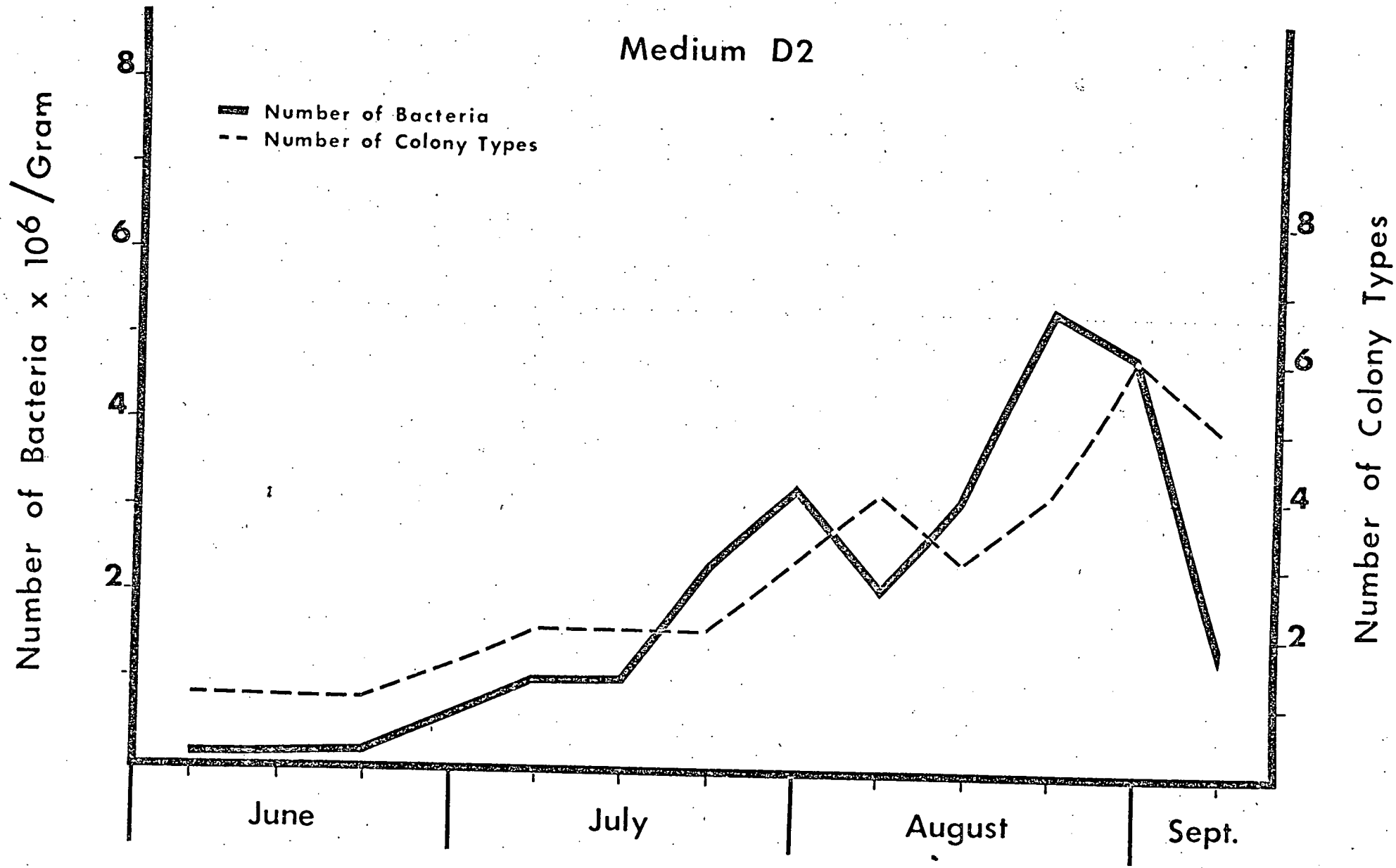

Medium D2
Number of Bacteria
Number of Colony Types
Number of Bacteria x 10^6 /Gram
Number of Colony Types
8
6
4
2
8
6
4
2
June
July
August
Sept.

Fig. 61

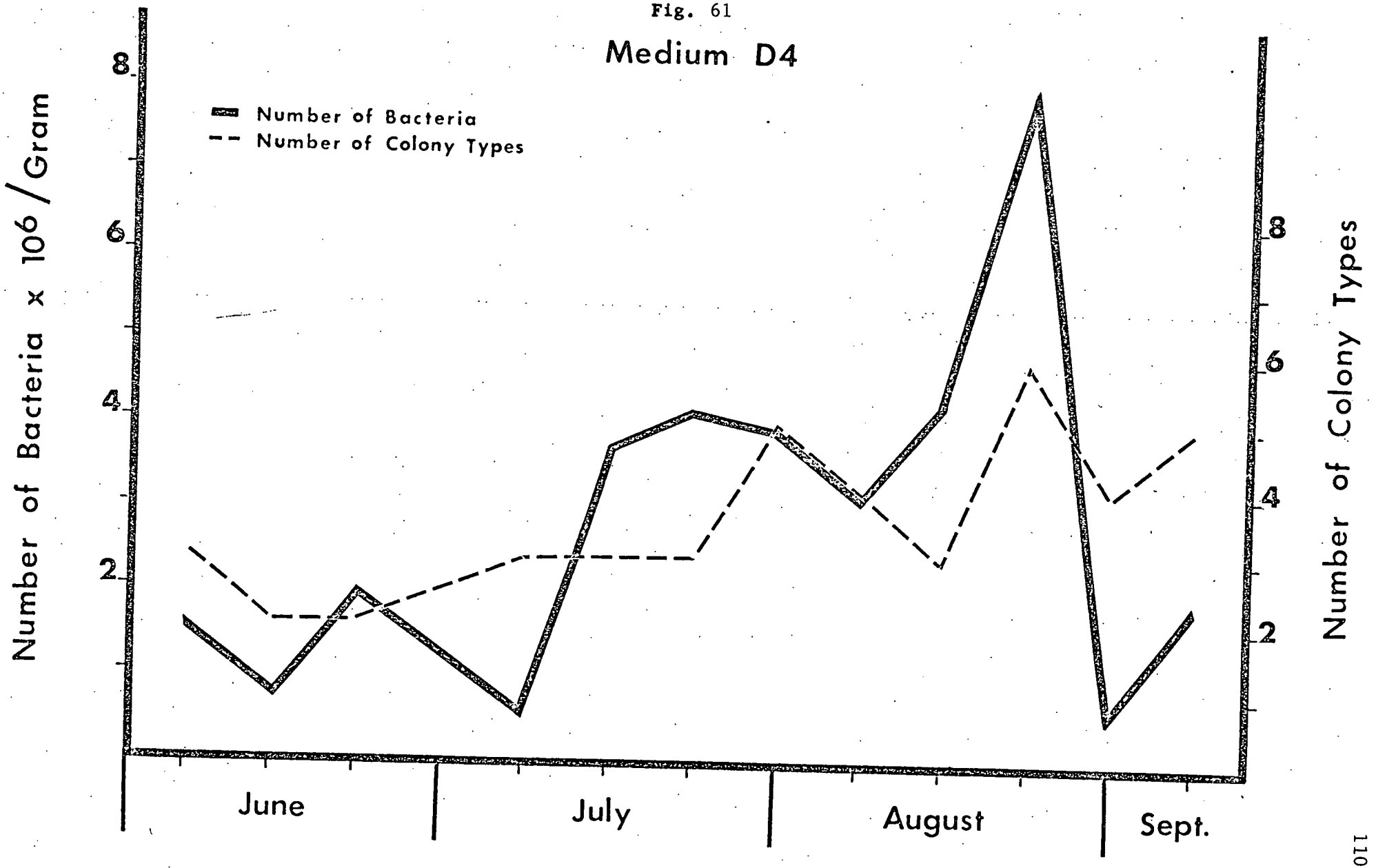

Medium D4
Number of Bacteria
Number of Colony Types
Number of Bacteria x 10^6 /Gram
Number of Colony Types
8
6
4
2
June
July
August
Sept.

Fig. 62

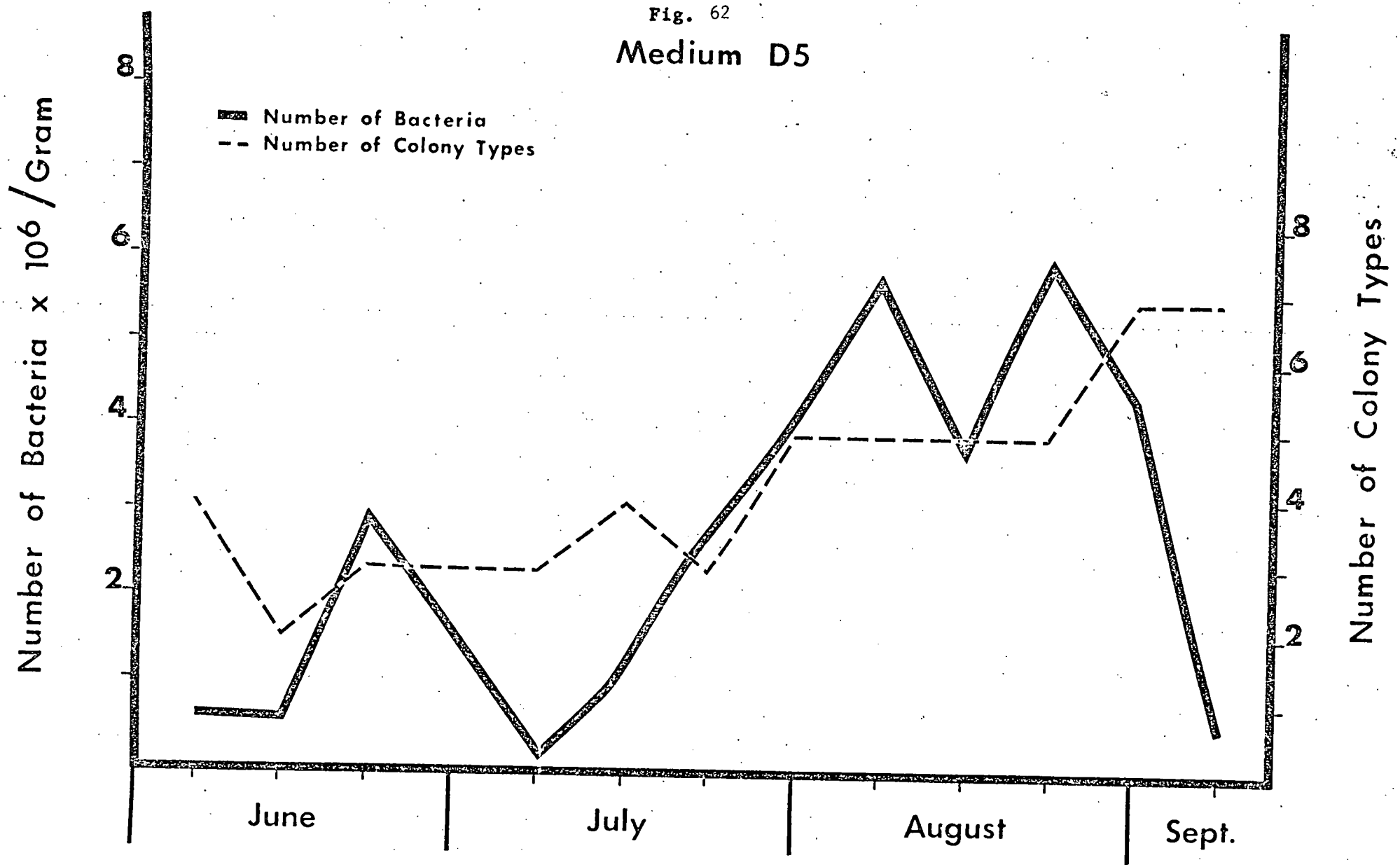

Medium D5
Number of Bacteria
Number of Colony Types
Number of Bacteria x 10^6 /Gram
Number of Colony Types
8
6
4
2
June
July
August
Sept.

Fig. 63

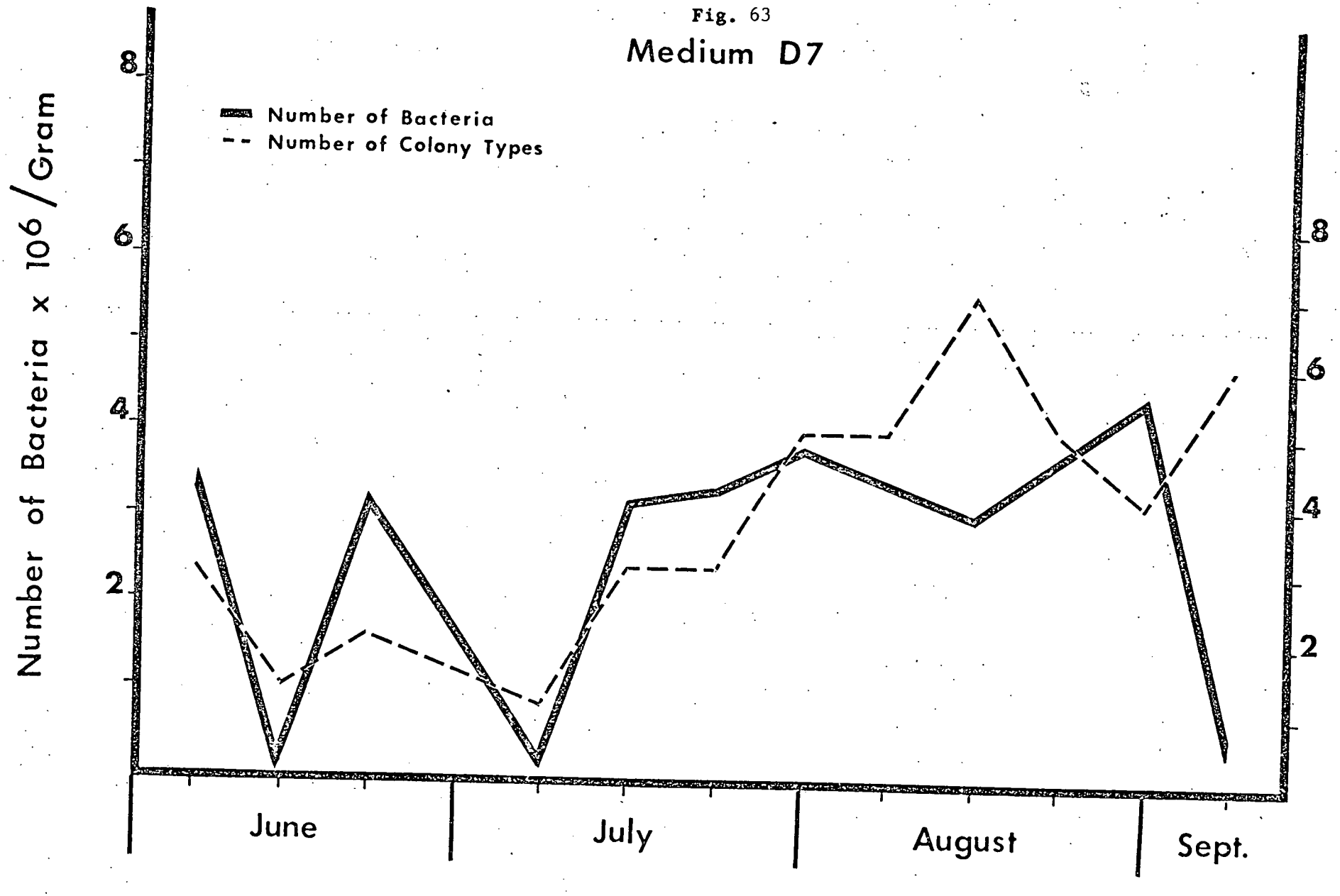

Medium D7
Number of Bacteria
Number of Colony Types
Number of Bacteria x 10^6 /Gram
Number of Colony Types
8
6
4
2
June
July
August
Sept.

Fig. 64

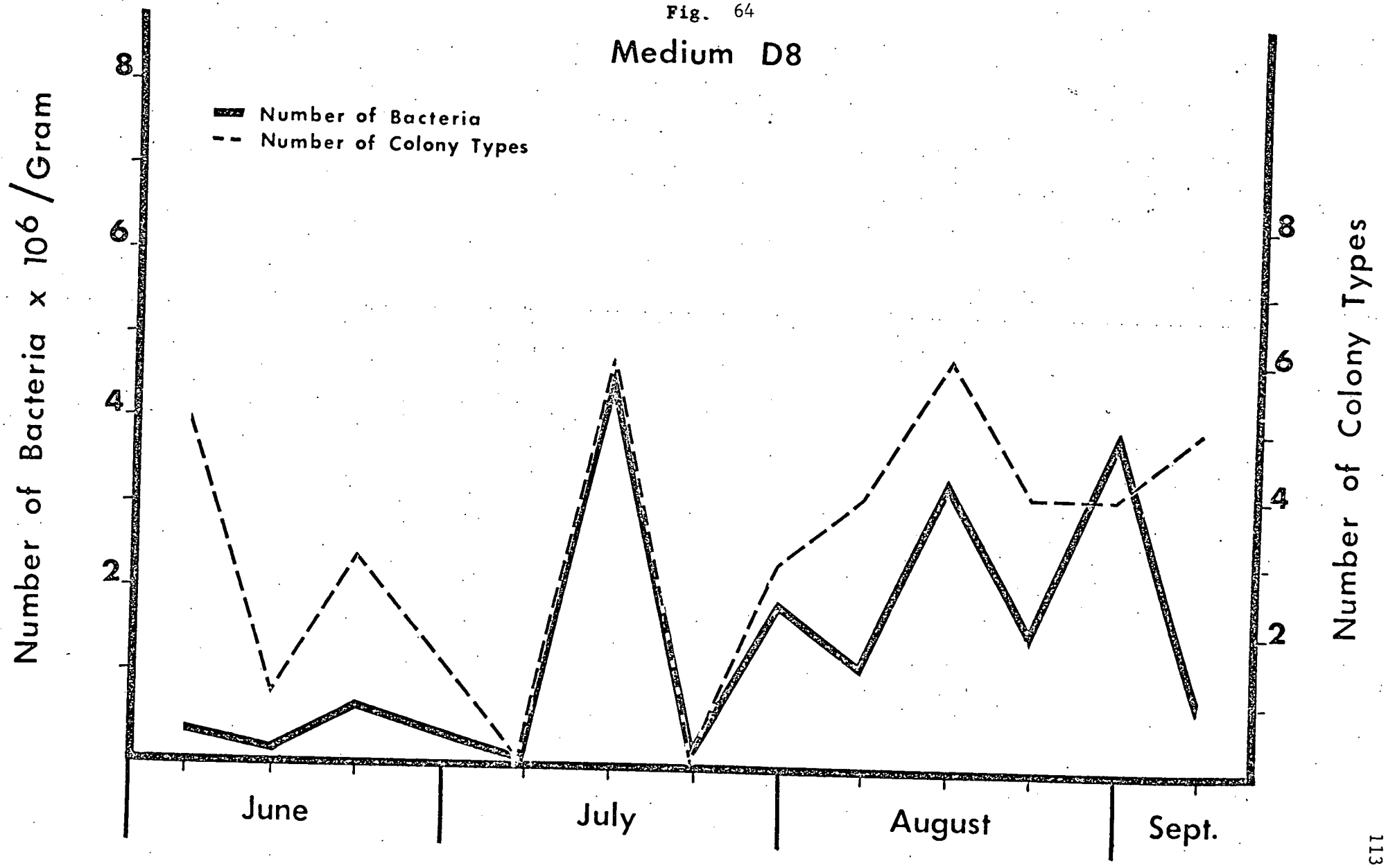

Medium D8
Number of Bacteria
Number of Colony Types
Number of Bacteria x 10^6 /Gram
Number of Colony Types
8
6
4
2
June
July
August
Sept.

Fig. 65

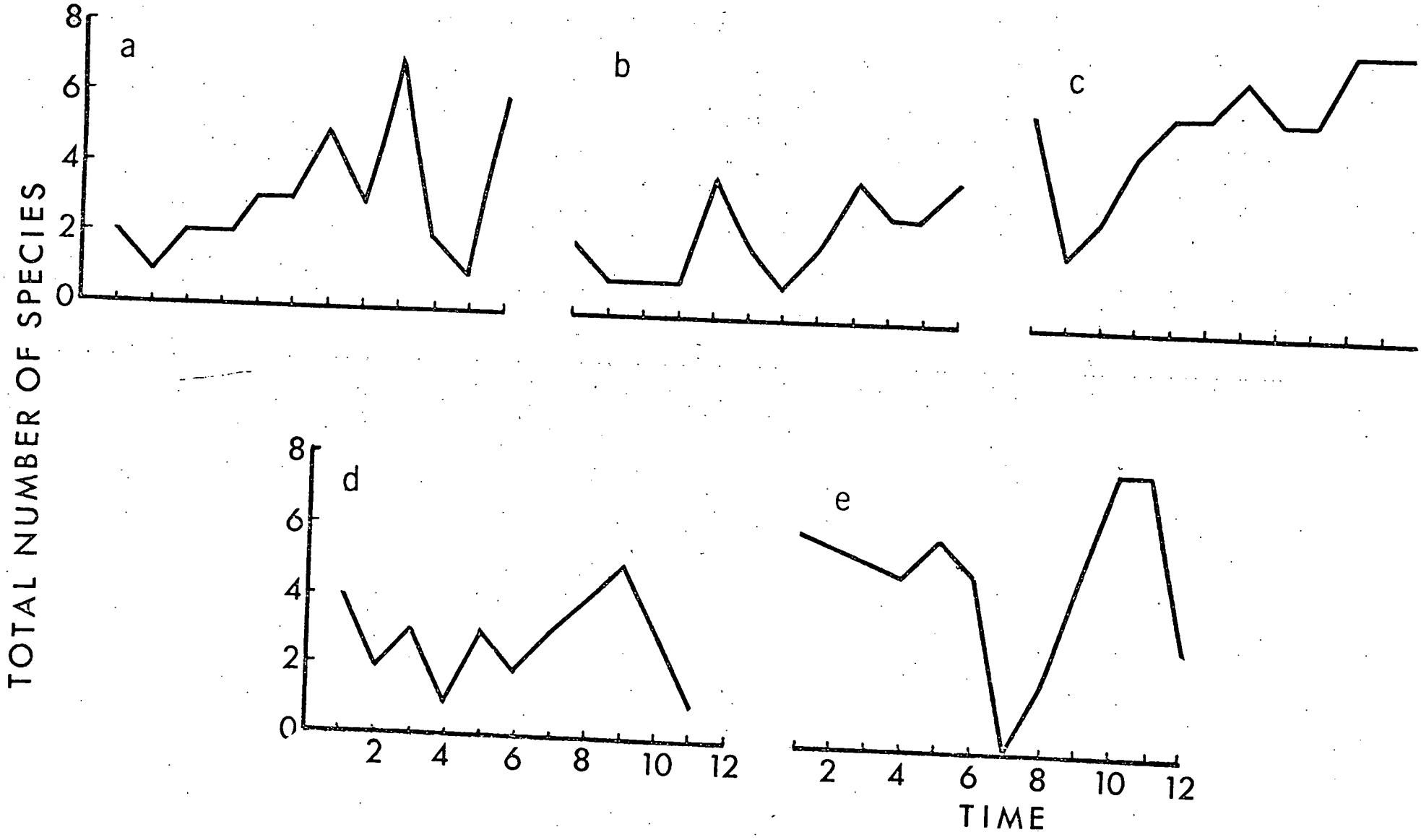


Fig. 66

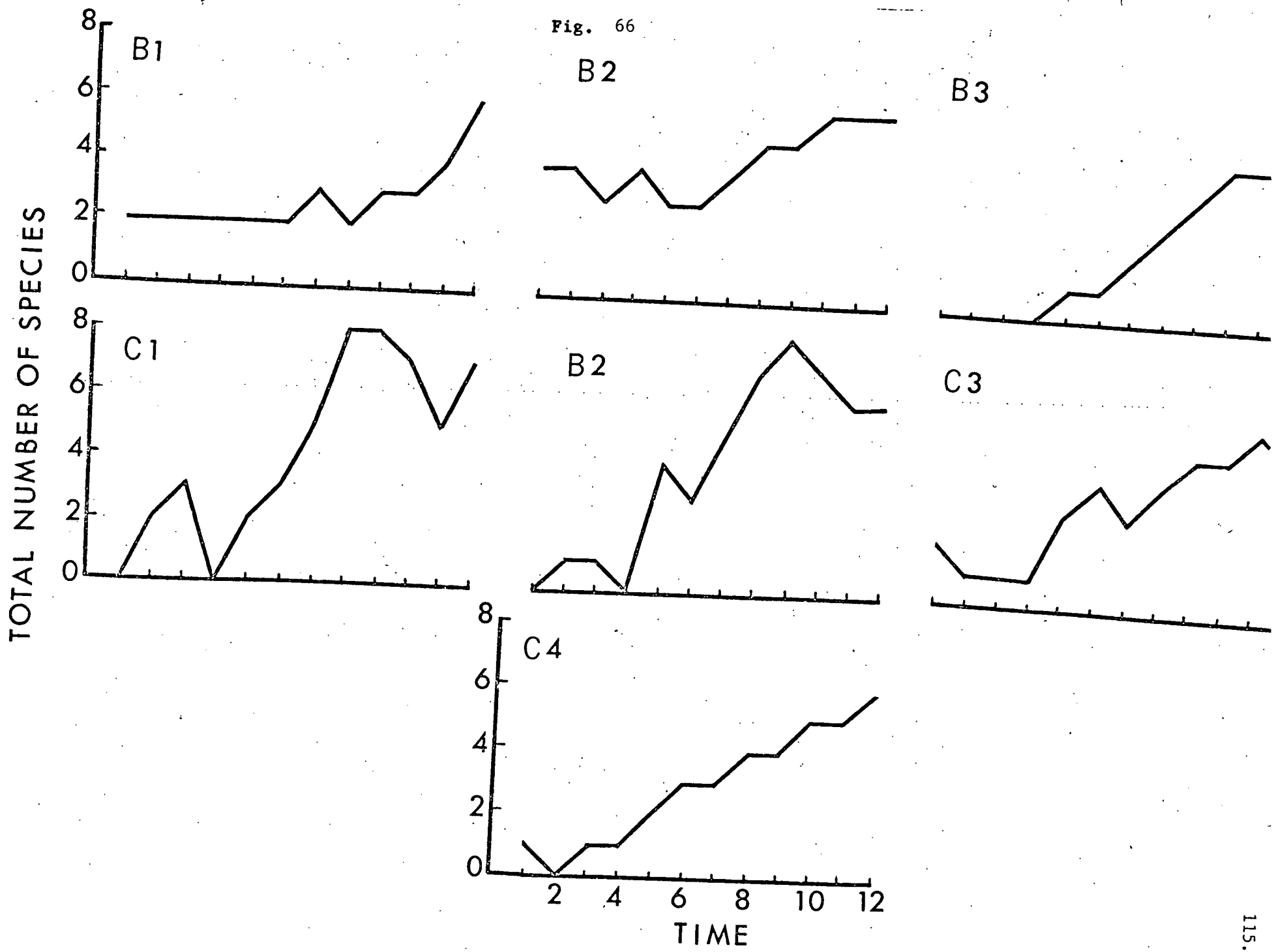


Fig. 67

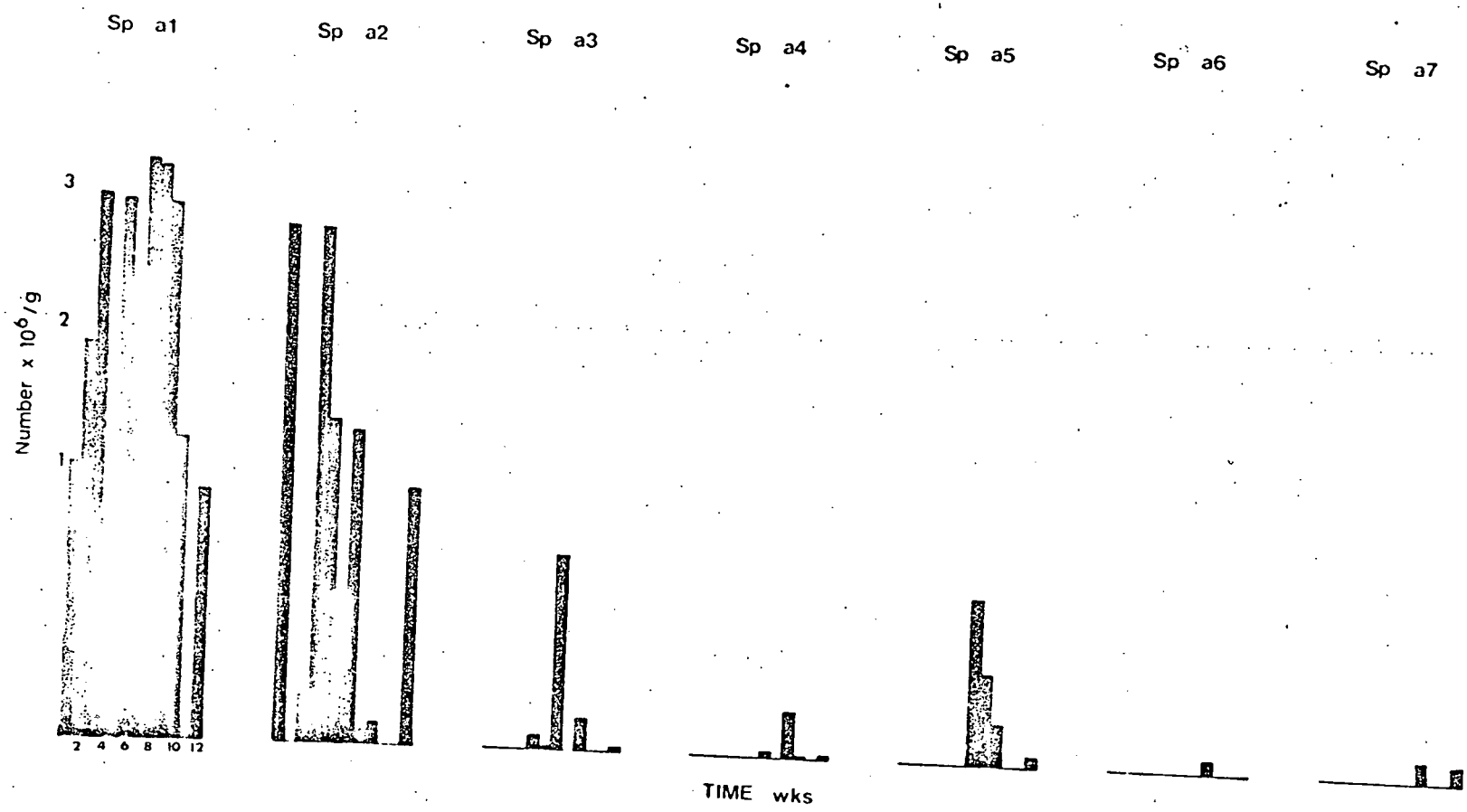

Sp a1
Sp a2
Sp a3
Sp a4
Sp a5
Sp a6
Sp a7
Number x 10^6/g
3
2
1
2 4 6 8 10 12
TIME wks

Fig. 68

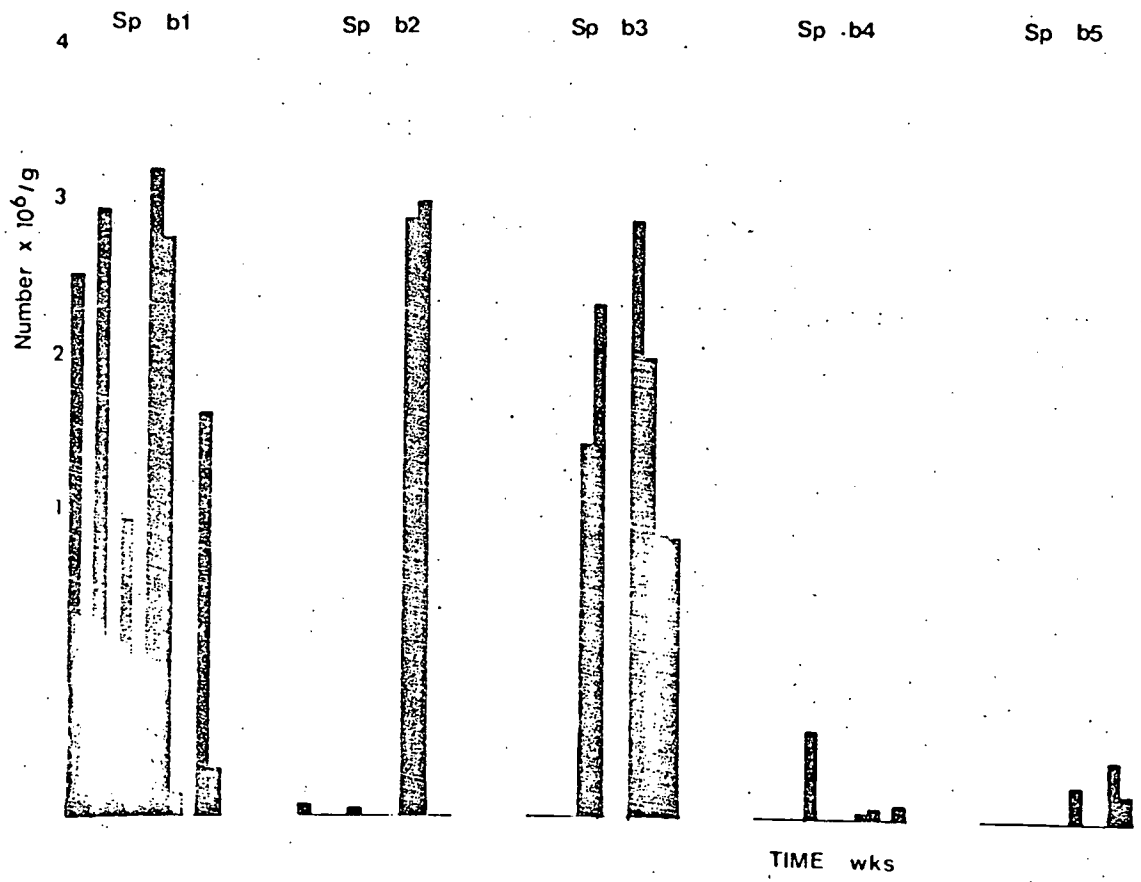


Fig. 69

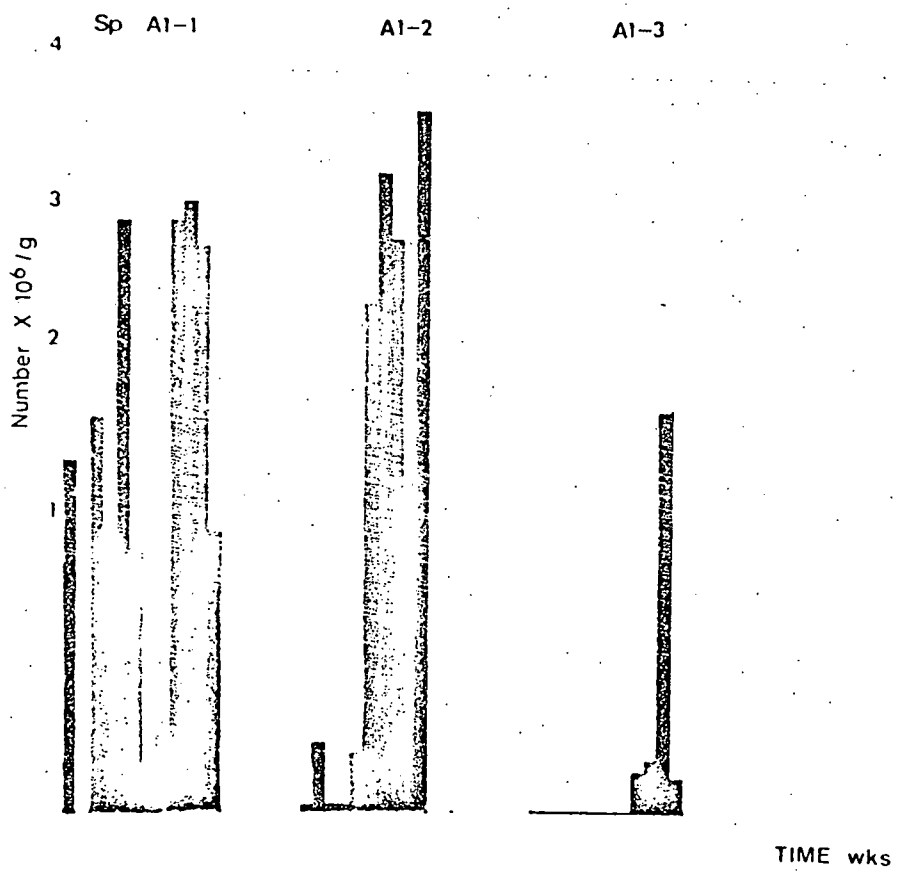


Fig. 70

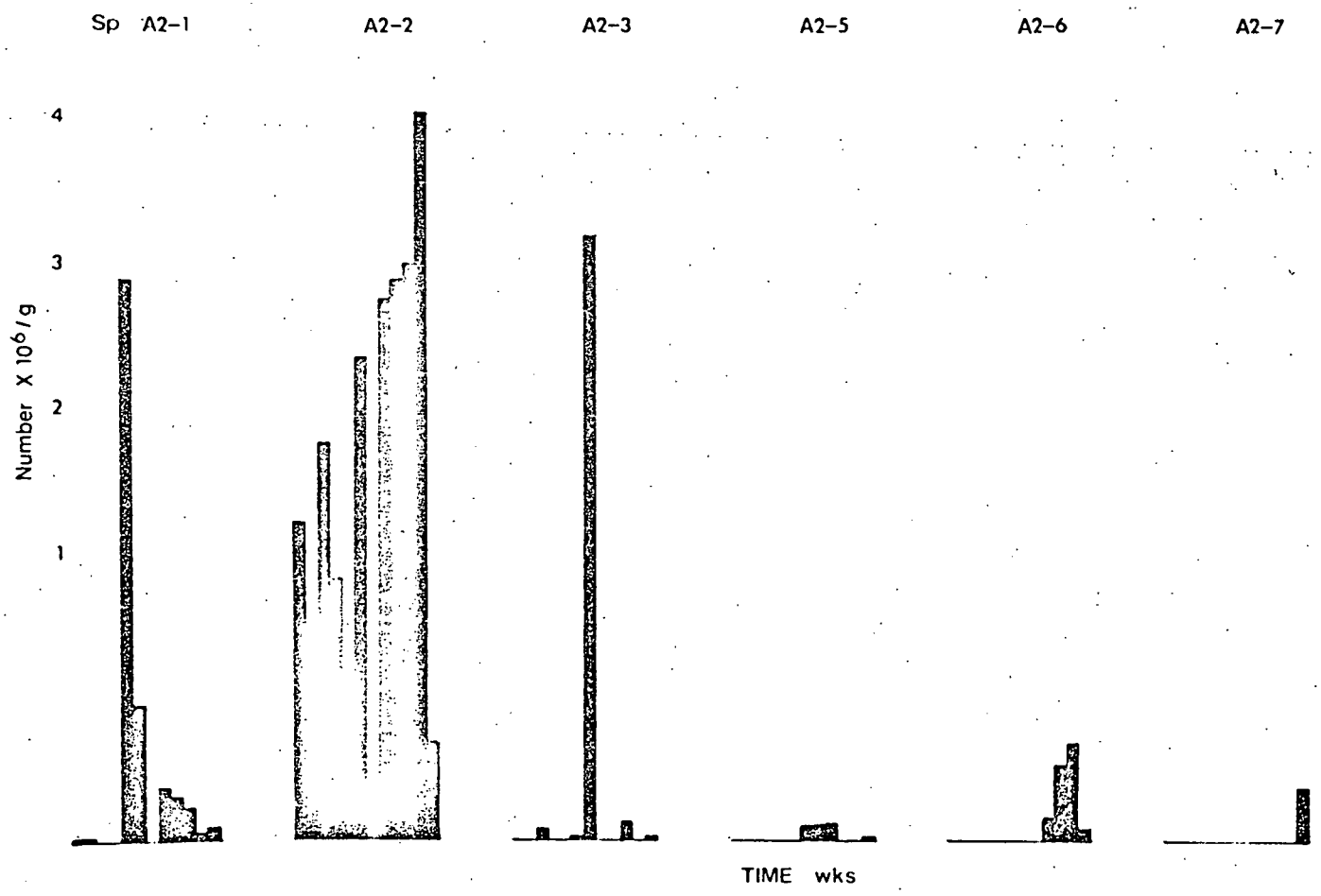


Fig. 71

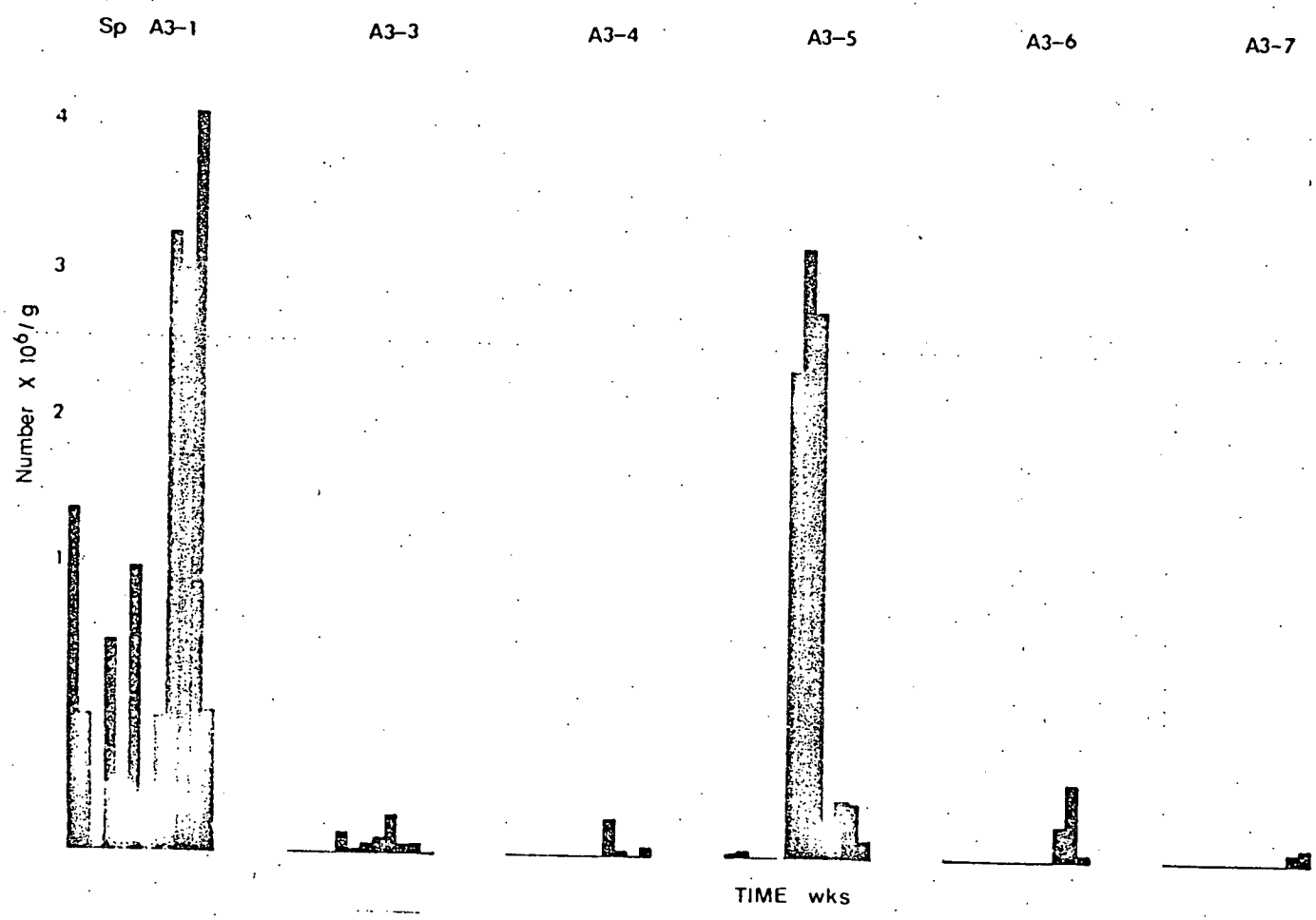


Fig. 72

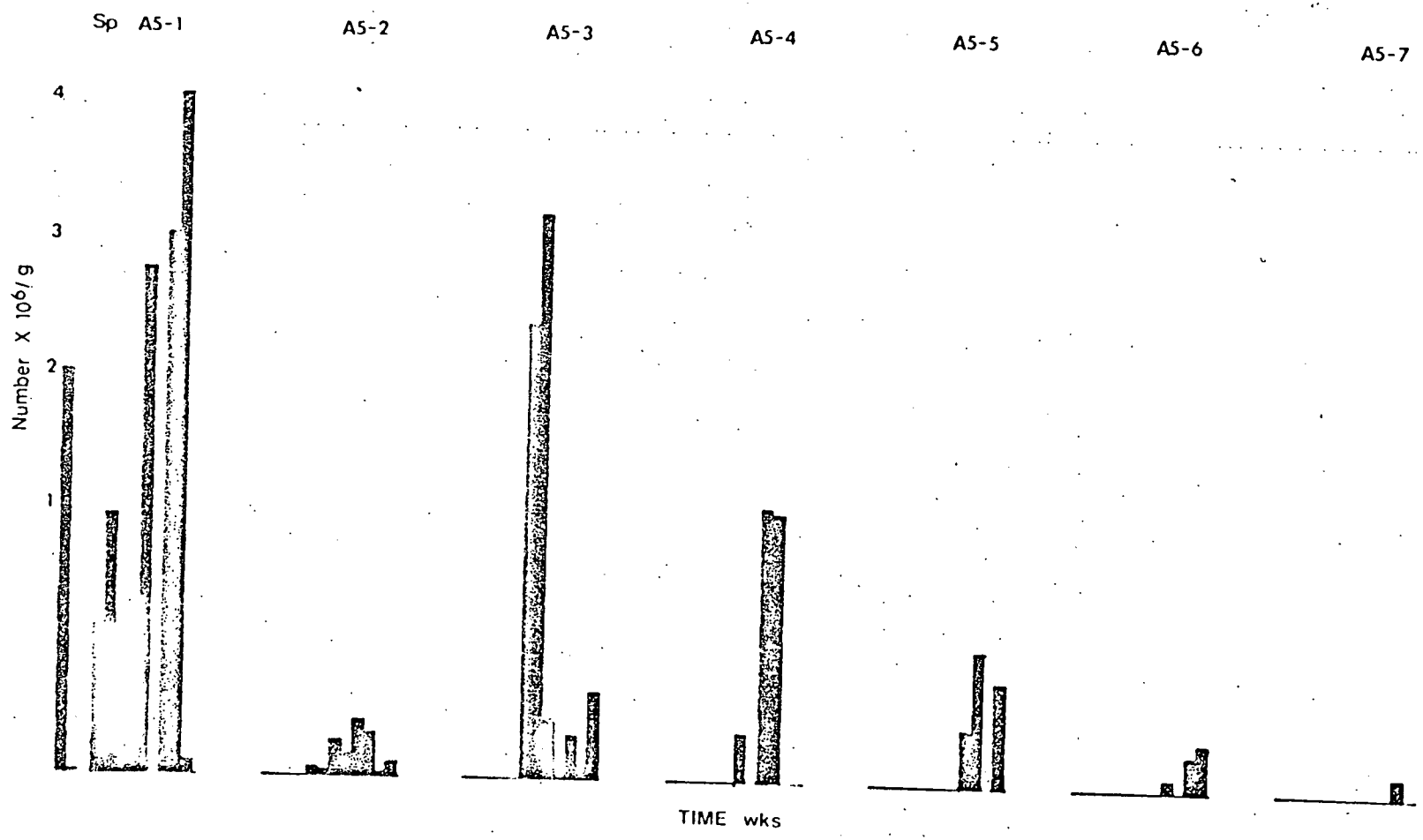


Fig. 73

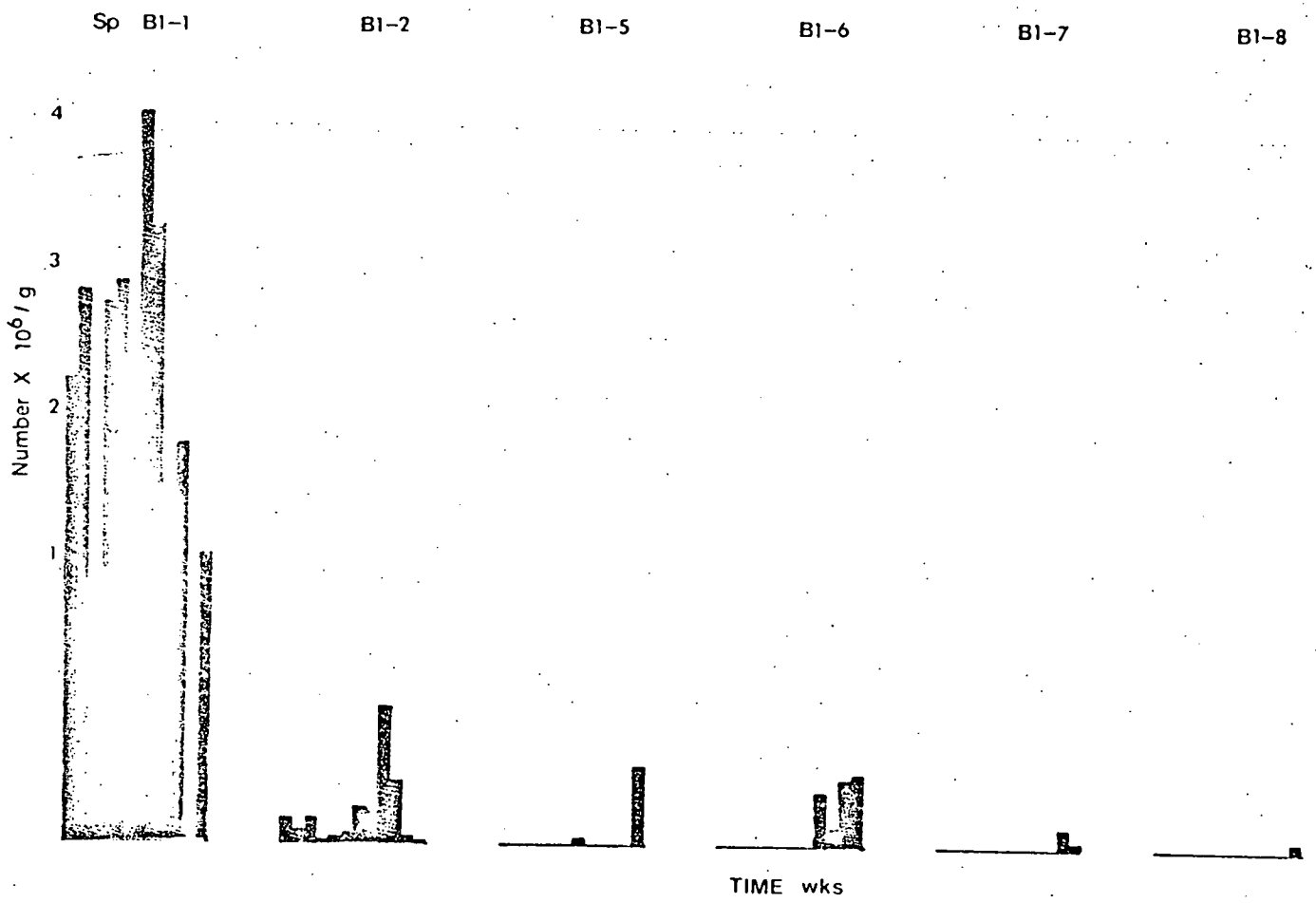


Fig. 74

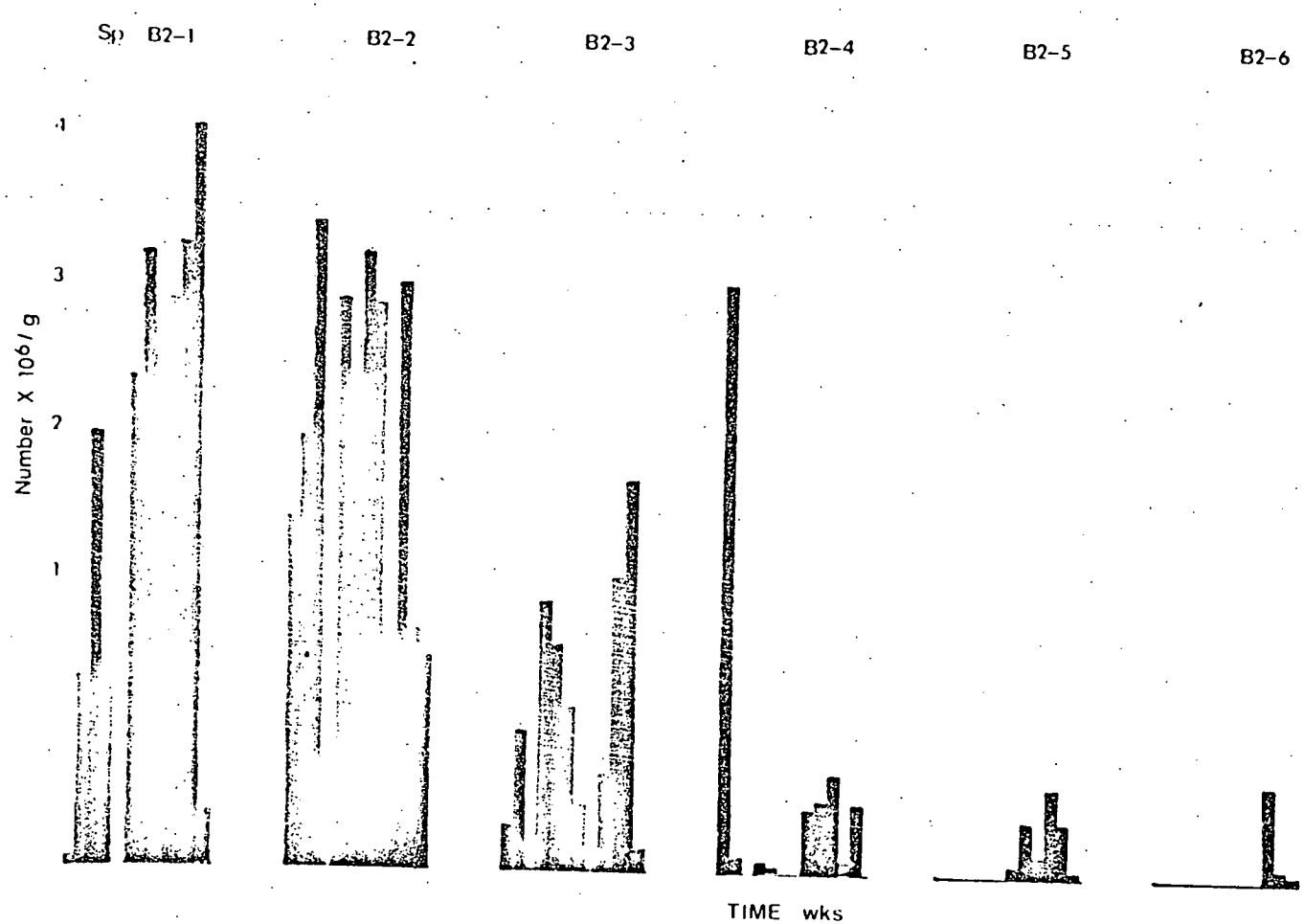


Fig. 75

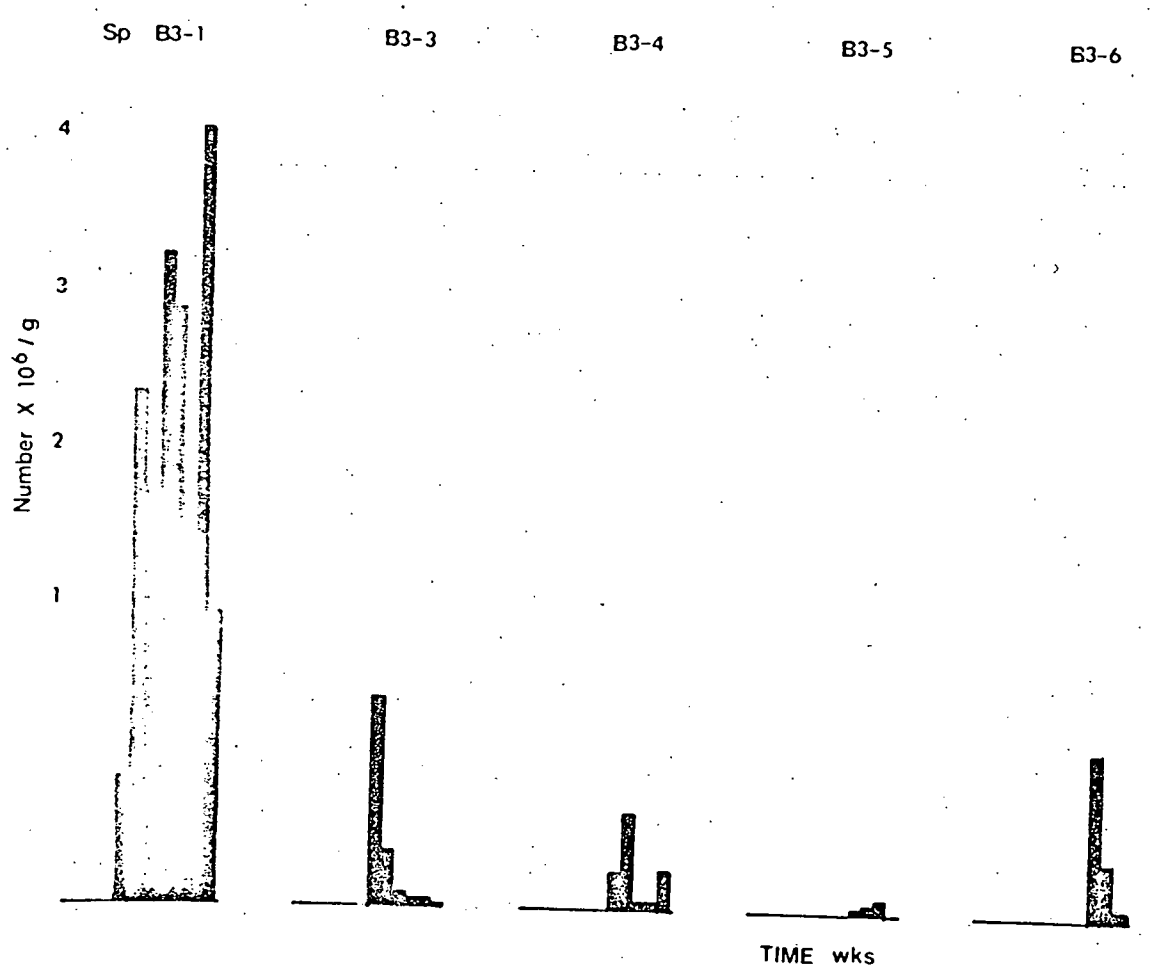


Fig. 76

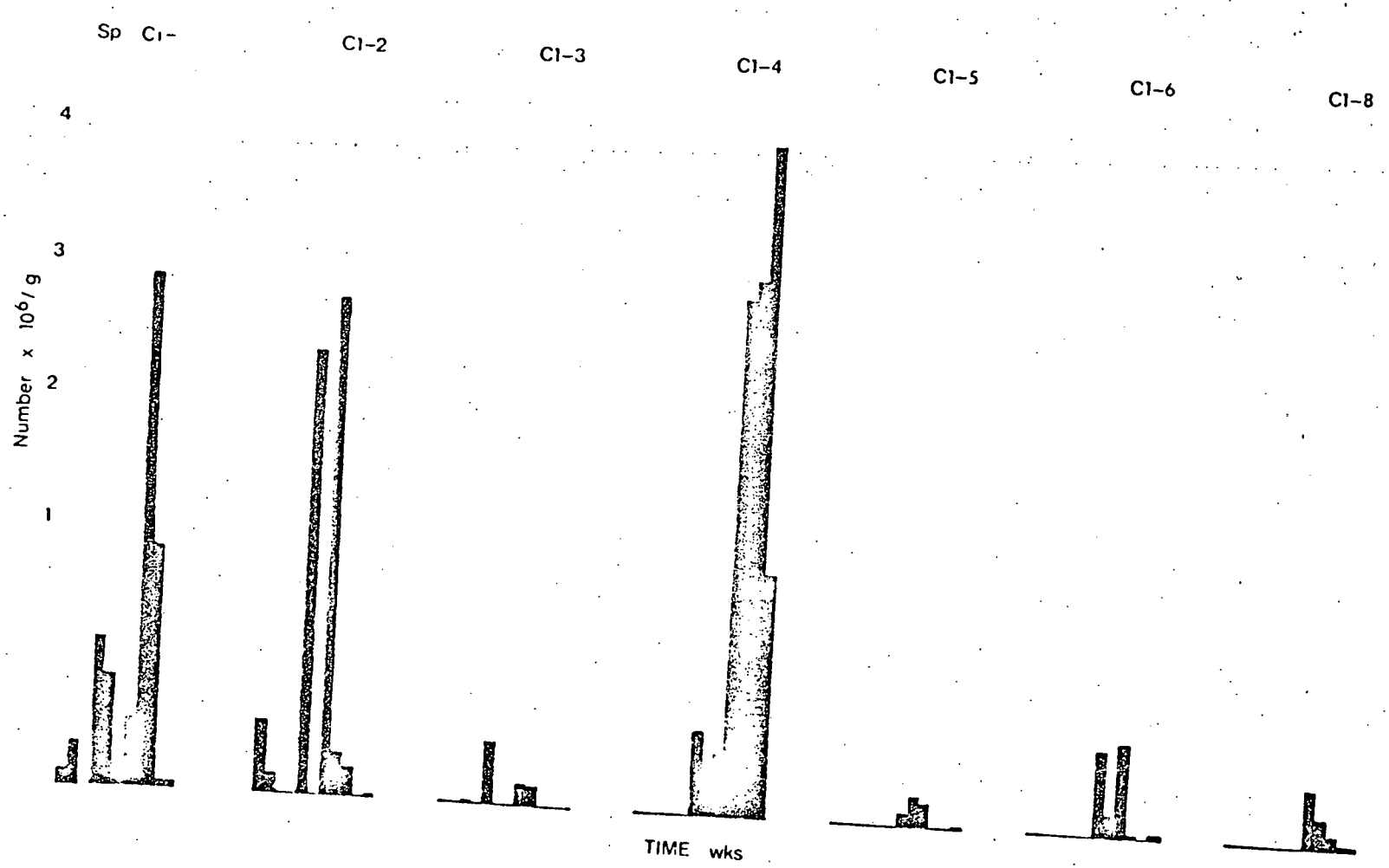


Fig. 77

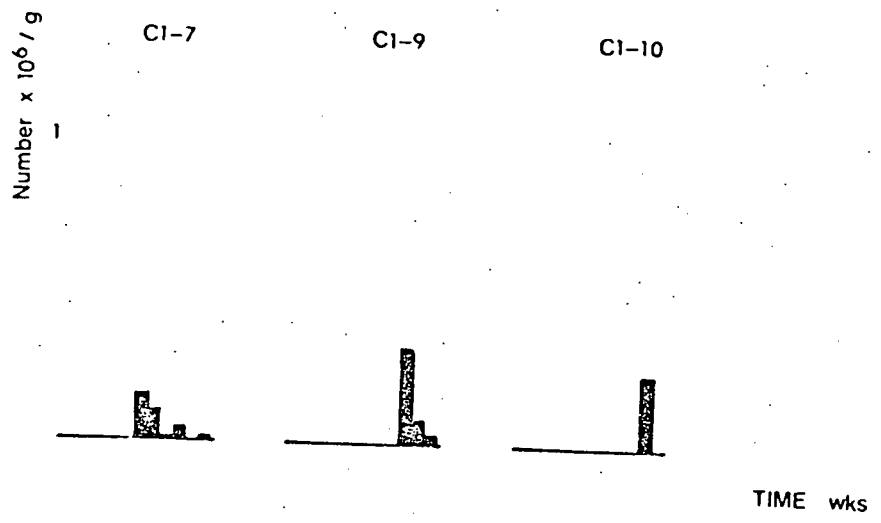


Fig. 78

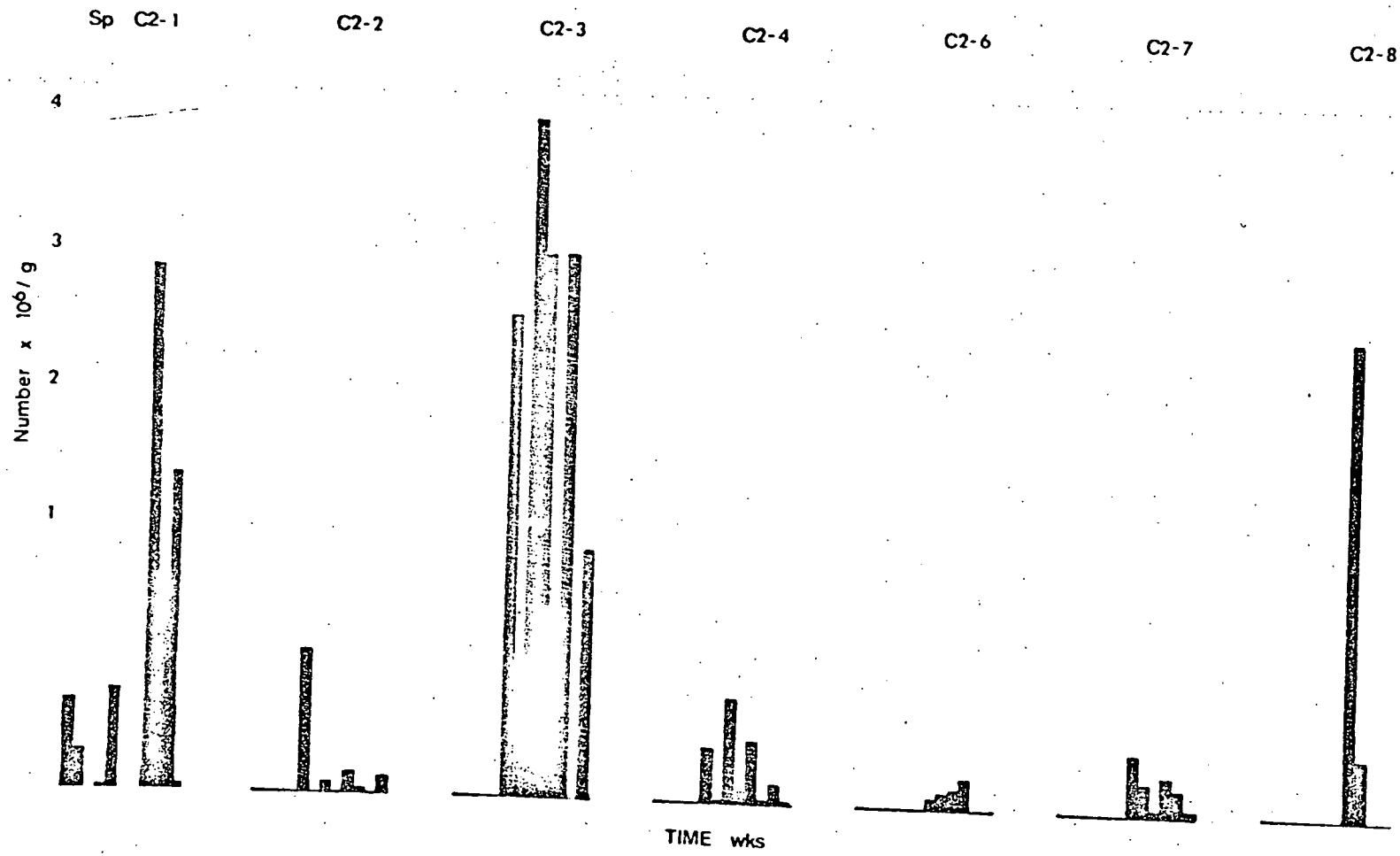


Fig. 79

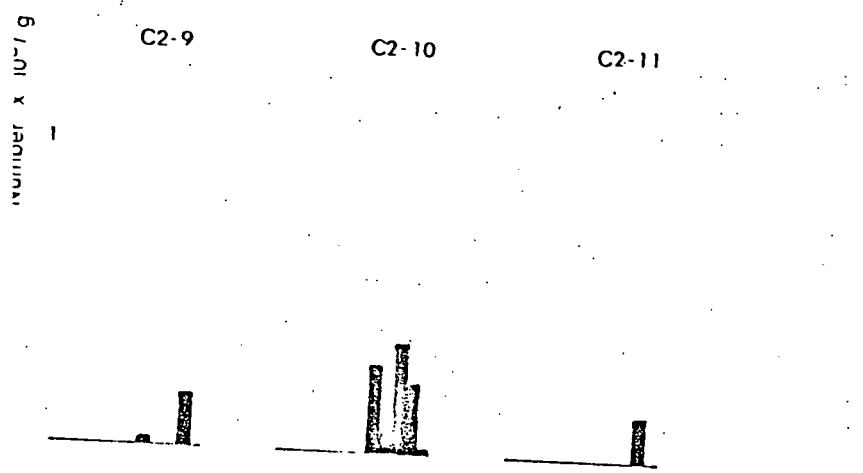


Fig. 80

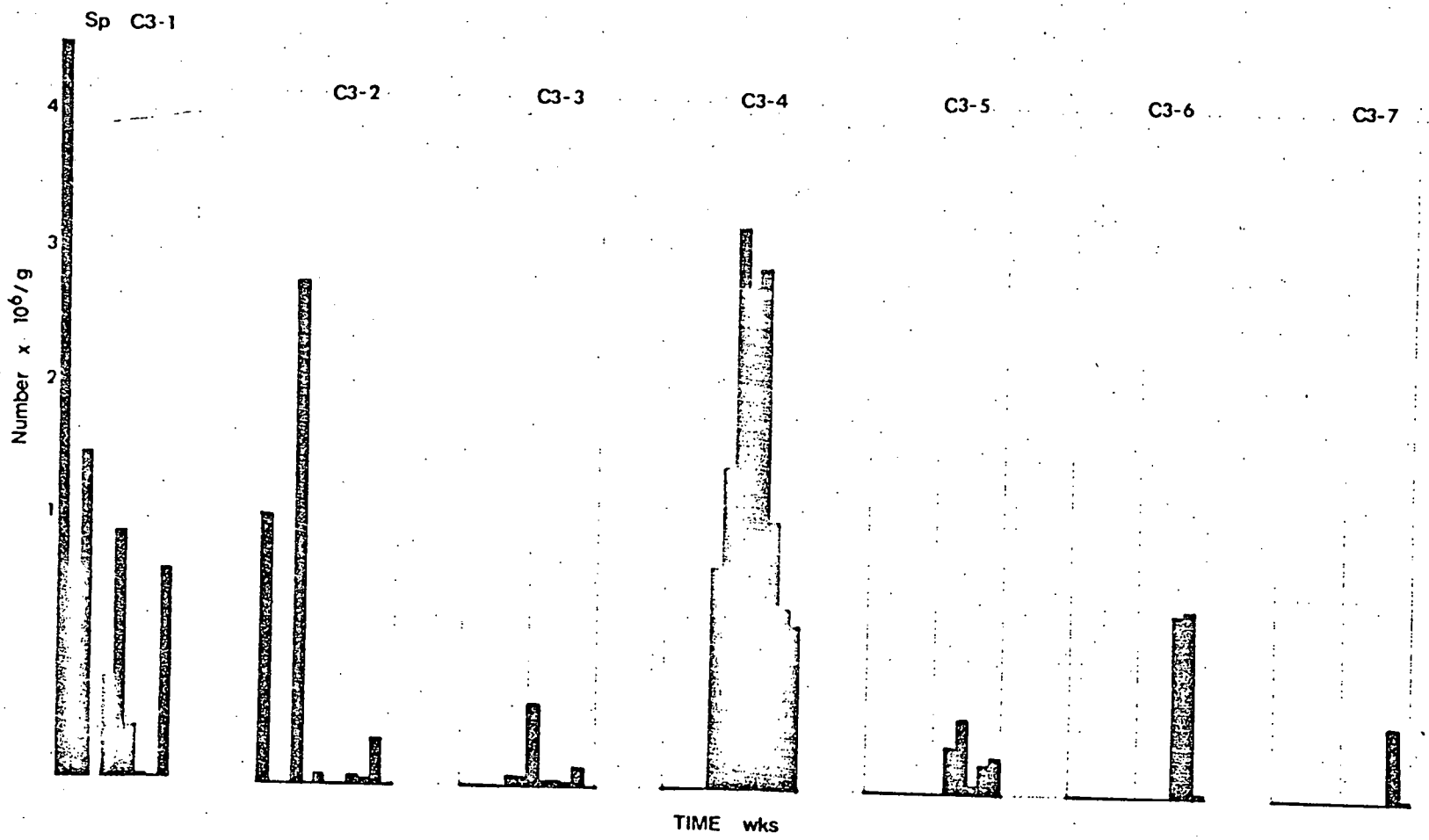


Fig. 81

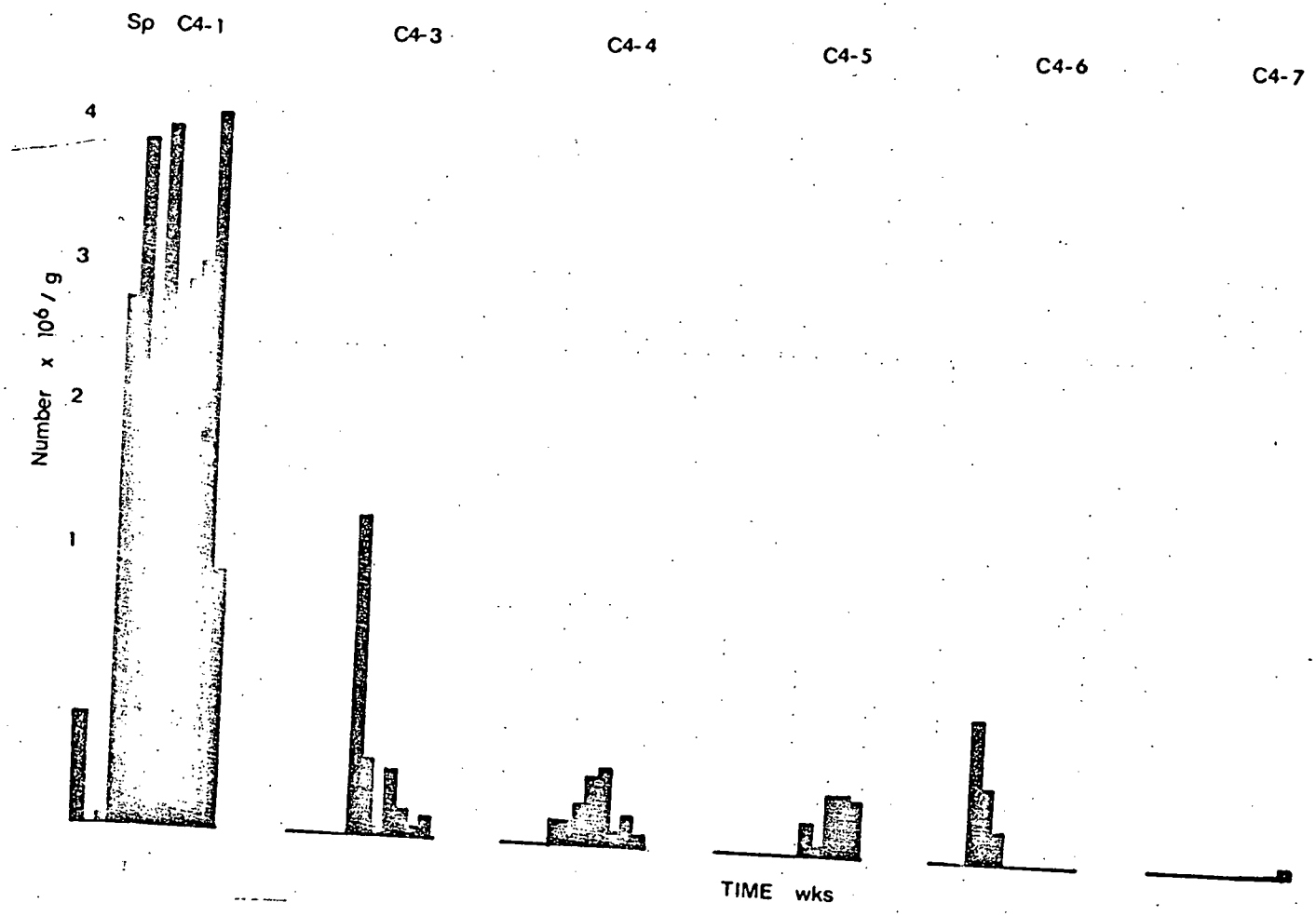


Fig. 82

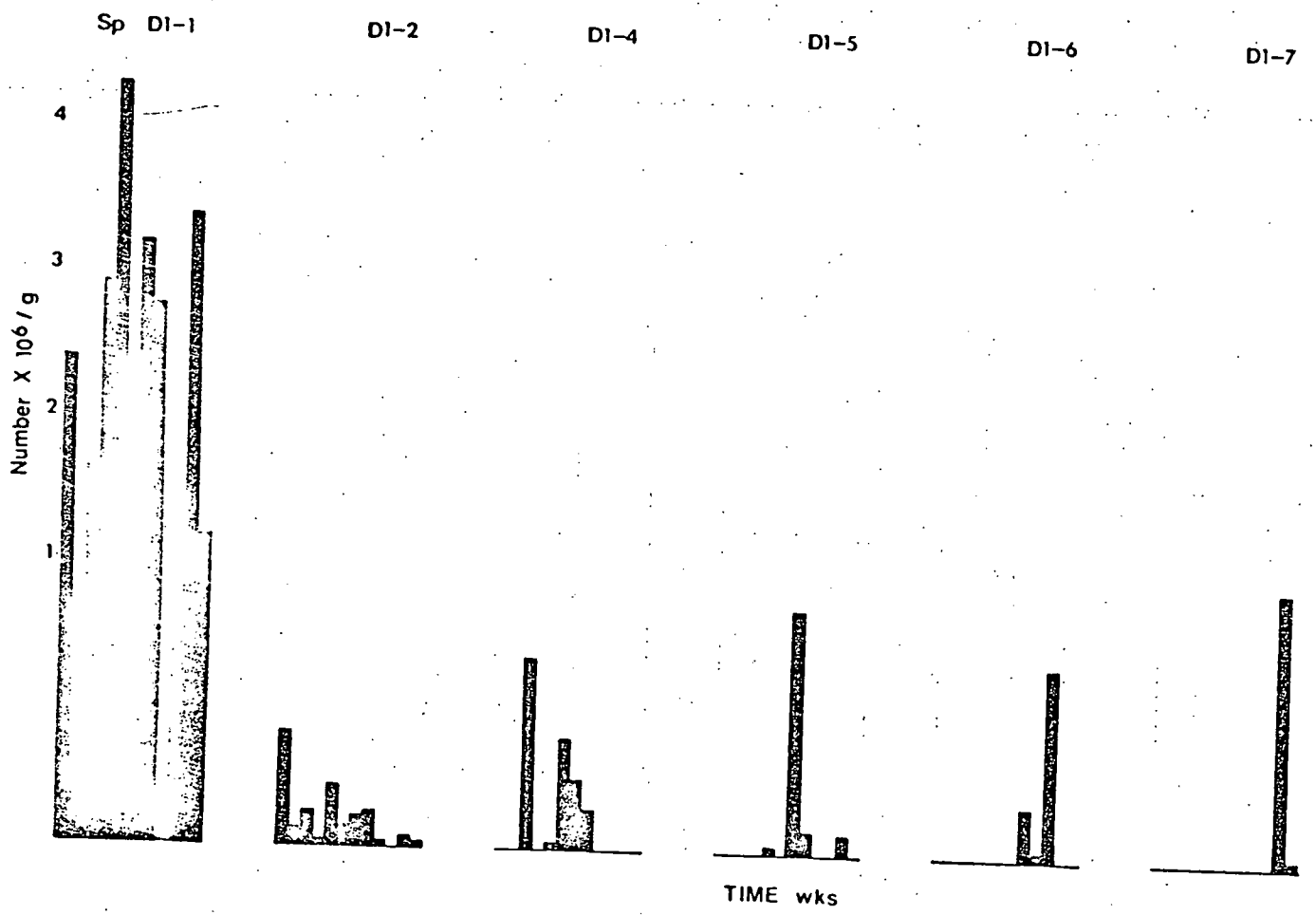
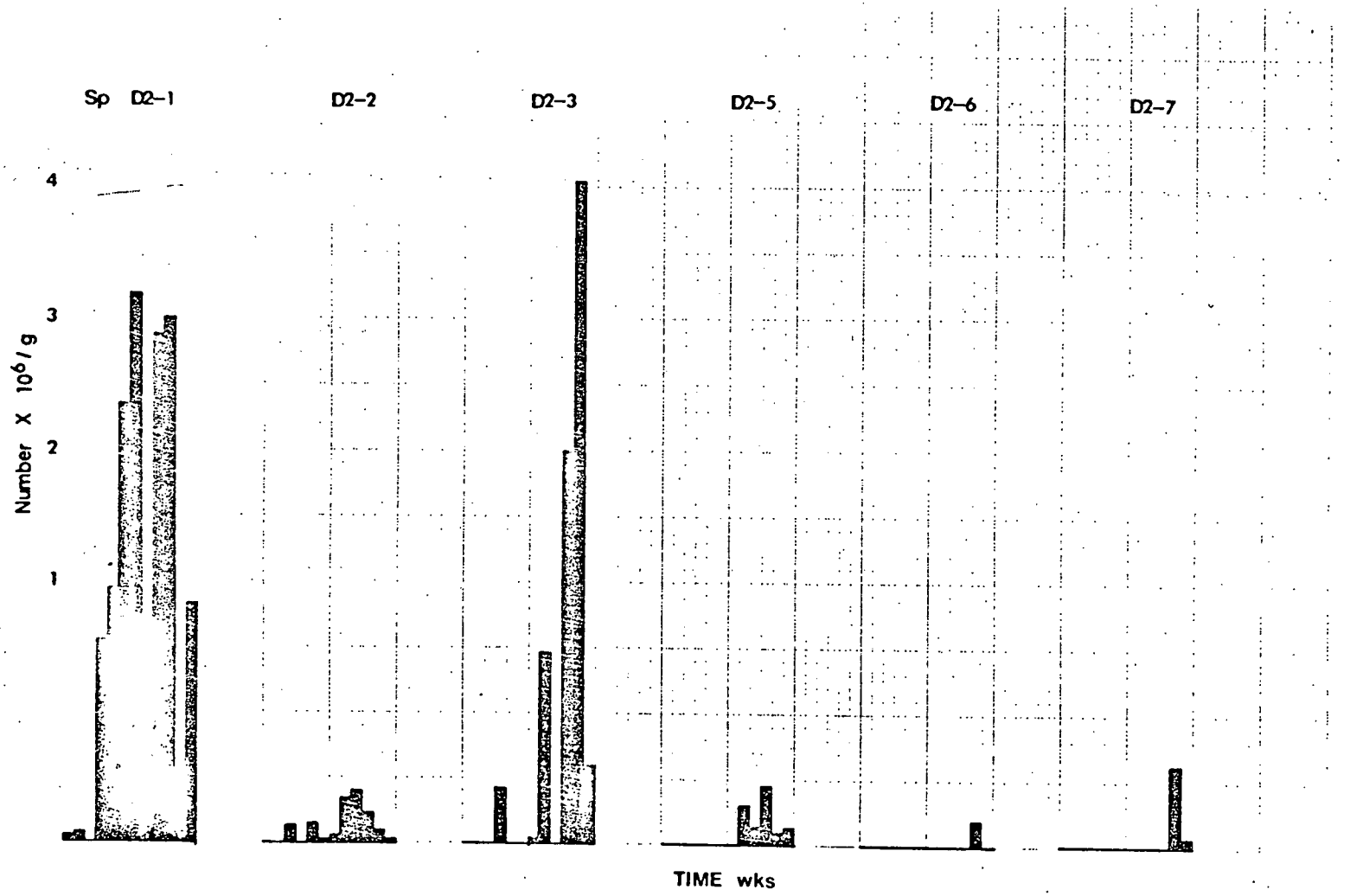


Fig. 83



Results are tabulated (Tables 9,10 ). As expected, and as can be seen by inspection of Table 8, most of the isolated organisms are gram negative rods which demonstrate diverse physical characteristics. Close inspection of the data strongly indicates that organisms isolated on our series of differential media demonstrate varied ability to attack glucose, sucrose and lactose (Table 8). Such results begin to point out the utility and importance of diverse complex and synthetic media in the isolation and enumeration of the bacterial inhabitants of the salt marsh community. By employing such media, we have obtained a more comprehensive picture of the metabolic capabilities of salt marsh bacteria. We hope in the next year to complete this project as a companion to the diatom assemblage paper (COO 3254-9) included in this progress report. Together they should give us a cohesive picture of auxotrophic pathways of carbon cycling in the microbial assembly of the marsh.

Table 8 Characteristics of the bacteria isolated from epiphytic communities of *Enteromorpha intestinalis*.

| Organism | Shape | | | Ends | | Length (μm) | | | | L:W | | | Diameter (μm) | | Occurrence | | | | Gram | Motility |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Straight Rod | Curved Rod | Coccus | Tapered | Square | ≤ 0.5 | 0.6 - 1.2 | 1.3 - 3.0 | ≥ 3.0 | 3 : 1 | 2 : 3 : 1 | 1 : 1 | ≤ 0.5 | ≥ 0.6 | Singles | Pairs | Short Chains | Long Chains | | |
| a3 | + | | | | + | | | + | | | + | | | | + | | | | − | + |
| a4 | | | + | | + | | | | | | | | | + | + | | | | − | |
| a6 | + | | | | + | | + | | | | + | | | | + | + | | | − | + |
| a6* | + | | | | + | | + | | | | + | | | | + | | | | − | + |
| a7 | + | | | | + | | | + | | + | | | | | + | | | | − | + |
| c2 | + | | | | + | | | + | | | + | | | | + | | | + | − | + |
| c4 | + | | | | + | | | + | | | | + | | | + | | | | − | + |
| c6 | + | | | | + | | | + | | | | + | | | + | | + | | − | + |
| c7 | + | | | | + | | | + | | | | + | | | + | | + | | − | + |
| c8 | + | | | | + | | | + | | | | + | | | + | | | | − | + |
| c9 | + | | | | + | | | + | | | | + | | | + | + | | | − | |
| c711 | | | + | | + | | | | | | | | + | | + | + | | | − | |
| c712 | + | | | | + | | | + | | | + | | | | + | + | | | − | |
| c715 | + | | | | + | | | + | | + | | | | | + | + | | | − | |
| d1 | + | | | | + | | + | | | | + | | | | + | + | | | − | |

| Organism | Glucose, Aer. Acid | Glucose, Anaer. Acid | Glucose, Aer. Alk. | Glucose, Anaer. Alk. | Glucose, Gas | Lactose, Aer. Acid | Lactose, Anaer. Acid | Lactose, Aer. Alk. | Lactose, Anaer. Alk. | Lactose, Gas | Sucrose, Aer. Acid | Sucrose, Anaer. Acid | Sucrose, Aer. Alk. | Sucrose, Anaer. Alk. | Sucrose, Gas | Mannitol, Aer. Acid | Mannitol, Anaer. Acid | Mannitol, Aer. Alk. | Mannitol, Anaer. Alk. | Mannitol, Gas | Mannose, Aer. Acid | Mannose, Anaer. Acid | Mannose, Aer. Alk. | Mannose, Anaer. Alk. | Mannose, Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a3 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| a4 | + | + | | | | | | + | + | | + | + | | | | + | + | | | | + | + | | | |
| a6 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| a6* | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| a7 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| c2 | + | + | | | | | | + | + | | + | + | | | | + | + | | | | + | + | | | |
| c4 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| c6 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| c7 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| c8 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | + | + | | | |
| c9 | + | + | | | | | | + | + | | + | + | | | | + | + | | | | + | + | | | |
| c711 | + | + | | | | | | + | + | | + | + | | | | + | + | | | | + | + | | | |
| c712 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| c715 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| d1 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | + | + | | | |

| Organism | Galactose, Aer. Acid | Galactose, Anaer. Acid | Galactose, Aer. Alk. | Galactose, Anaer. Alk. | Galactose, Gas | Maltose, Aer. Acid | Maltose, Anaer. Acid | Maltose, Aer. Alk. | Maltose, Anaer. Alk. | Maltose, Gas | Arabinose, Aer. Acid | Arabinose, Anaer. Acid | Arabinose, Aer. Alk. | Arabinose, Anaer. Alk. | Arabinose, Gas | Sorbitol, Aer. Acid | Sorbitol, Anaer. Acid | Sorbitol, Aer. Alk. | Sorbitol, Anaer. Alk. | Sorbitol, Gas | Xylose, Aer. Acid | Xylose, Anaer. Acid | Xylose, Aer. Alk. | Xylose, Anaer. Alk. | Xylose, Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a3 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| a4 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| a6 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | + |
| a6* | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| a7 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| c2 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| c4 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| c6 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| c7 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| c8 | | | + | + | | | | + | + | | | | + | + | | + | + | | | | | | + | + | |
| c9 | + | + | | | | + | + | | | | | | + | + | | + | | | | | | | + | + | |
| c711 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| c712 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| c711 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| d1 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |

| Organism | Inositol, Aer. Acid | Inositol, Anaer. Acid | Inositol, Aer. Alk. | Inositol, Anaer. Alk. | Inositol, Gas | Methyl Red Reaction | Voges-Proskauer Test | Oxidase (Kovacs) | Starch Hydrolysis | Gelatin Hydrolysis, ≤8 da | Gelatin Hydrolysis, ≥9 da | Indole | Lysine Decarboxylase | Ornithine Decarboxylase | Arginine Decarboxylase | Ammonification | Nitrate Reduction → $NO_2$ | Nitrate Reduction → Gas | Catalase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a3 | + | + | | | | | | + | | | | | | | | | | | |
| a4 | + | + | | | | + | | + | | | + | + | | | | + | + | + | + |
| a6 | + | + | | | | | | + | | | | | | | | + | + | | + |
| a6* | + | + | | | | | | + | | | | | | | + | + | + | | + |
| a7 | + | + | | | | | | + | | | | | | | | + | + | | + |
| c2 | + | + | | | | + | | + | + | | | | | | | + | + | + | + |
| c4 | + | + | | | | | | + | | | + | + | | | | + | + | | + |
| c6 | + | + | | | | | | + | | + | | | | + | | + | + | | + |
| c7 | + | + | | | | | | | + | | | | | + | | + | + | | + |
| c8 | | | + | + | | | | + | + | | | | | + | | + | + | | + |
| c9 | | | + | + | | | | + | | | | | | + | | + | + | + | + |
| c711 | + | + | | | | + | | + | | + | | | | + | + | + | + | | + |
| c712 | + | + | | | | + | | + | | + | | + | | + | + | + | + | | + |
| c715 | + | + | | | | | | + | | + | | | | | | + | + | | + |
| d1 | | | + | + | | | | + | + | + | | | | | | + | + | | + |
| | | | | | | | | + | + | + | | | + | + | | + | + | | + |

| Organism | Medium a | Medium b | Medium c | Medium d | Medium e | Medium A1 | Medium A2 | Medium A3 | Medium A4 | Medium A5 | Medium B1 | Medium B2 | Medium B3 | Medium C1 | Medium C2 | Medium C3 | Medium C4 | Medium D1 | Medium D2 | Medium D4 | Medium D5 | Medium D7 | Medium D8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a3 | | | + | | + | | | | | | | + | | | | | | + | + | + | + | | |
| a4 | | | + | | + | | | | | | | + | | | | | | | | | + | | |
| a6 | | | + | | + | | | | | | | + | | | | | | + | + | + | | | |
| a6* | | | + | | + | | | | | | | + | | | | | | + | + | | | | |
| a7 | | | + | | + | | | | | | | + | | | | | | + | + | + | + | | |
| c2 | | | + | | + | | | | | | | + | | | | | | + | + | + | + | | |
| c4 | | | + | | + | | | | | | + | + | | | | | | | | | | | |
| c6 | | | + | | + | | | | | | | + | | | | | | + | + | + | | | |
| c7 | | | + | | + | | | | | | | + | | | | | | + | + | + | + | | |
| c8 | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| c9 | | | + | | + | | | | | | | + | | | | | | | | + | + | | |
| c711 | | | + | | + | | | | | | | + | | | | | | + | + | + | + | | |
| c712 | | | + | | + | | | | | | + | + | | | | | | | | | | | |
| c715 | | | + | | + | | | | | | | + | | | | | | | | + | + | | |
| d1 | | | + | | + | | | | | | | + | | | | | | + | + | + | + | | |

| Organism | Shape | | | Ends | | Length (μm) | | | | L:W | | | Diameter (μm) | | Occurrence | | | | Gram | Motility |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Straight Rod | Curved Rod | Coccus | Tapered | Square | ≤ 0.5 | 0.6 - 1.2 | 1.3 - 3.0 | ≥ 3.0 | 3 : 1 | 2 -3:1 | 1 : 1 | ≤ 0.5 | ≥ 0.6 | Singles | Pairs | Short Chains | Long Chains | | |
| e2 | + | | | | + | | | | + | + | | | | | + | | | | − | + |
| e5 | + | | | | + | | + | | | | + | | | | + | | + | | − | + |
| e7 | + | | | | + | | | | + | + | | | | | + | | | | − | + |
| e8 | + | | | | + | | | + | | + | | | | | + | | + | | − | + |
| A1-1 | + | | | | + | | | + | | + | | | | | + | | + | | − | + |
| A1-2 | + | | | | + | | | + | | + | | | | | + | | + | | − | |
| A1-712 | + | | | | + | | | + | | | + | | | | + | + | | | − | |
| A1-714 | + | | | | + | | | + | | | + | | | | + | + | | | − | |
| A1-714* | | + | | | + | | | | + | + | | | | | + | | + | | − | |
| A2-1 | + | | | | + | | | + | | + | | | | | + | | + | | − | + |
| A2-2 | + | | | | + | | | + | | + | | | | | + | | | | − | |
| A2-3 | + | | | | + | | | + | | + | | | | | + | | | | − | + |
| A2-3* | + | | | | + | | | + | | + | | | | | + | | + | | − | + |
| A2-4 | | + | | | + | | | + | | + | | | | | + | | | | − | + |
| A2-714* | | + | | | + | | | | + | + | | | | | + | | | | − | + |

| Organism | 14* | * | 3 | 2 | 1 | 714* | 714 | 712 | 2 | 1 | 8 | 7 | 5 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Glucose, Aer. Acid | | + | + | + | | + | | | | + | + | + | + | + |
| Glucose, Anaer. Acid | | + | | + | | + | | | | + | + | + | + | + |
| Glucose, Aer. Alk. | + | | | | + | + | | + | + | + | | | | |
| Glucose, Anaer. Alk. | + | | + | | + | + | | + | + | + | | | | |
| Glucose, Gas | | | | | | | | | | | | | | |
| Lactose, Aer. Acid | | | | | | | | | | | | | | |
| Lactose, Anaer. Acid | | | | | | | | | | | | | | |
| Lactose, Aer. Alk. | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lactose, Anaer. Alk. | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lactose, Gas | | | | | | | | | | | | | | |
| Sucrose, Aer. Acid | | | | | | | + | | | + | + | + | + | + |
| Sucrose, Anaer. Acid | | | | | | | + | | | + | + | + | + | + |
| Sucrose, Aer. Alk. | + | + | + | + | + | + | | + | + | + | | | | |
| Sucrose, Anaer. Alk. | + | + | + | + | + | + | | + | + | + | | | | |
| Sucrose, Gas | | | | | | | | | | | | | | |
| Mannitol, Aer. Acid | + | | | | | + | + | + | + | + | + | + | + | + |
| Mannitol, Anaer. Acid | + | | | | | + | + | | | + | + | + | + | + |
| Mannitol, Aer. Alk. | | + | + | + | + | | | | | | | | | |
| Mannitol, Anaer. Alk. | | + | + | + | + | | | + | + | | | | | |
| Mannitol, Gas | | | | | | | | | | | | | | |
| Mannose, Aer. Acid | + | | | | | | + | + | + | + | + | | + | + | + |
| Mannose, Anaer. Acid | + | | | | | | + | + | | + | + | | + | + | + |
| Mannose, Aer. Alk. | | + | + | + | + | + | | | | | | + | | |
| Mannose, Anaer. Alk. | | + | + | + | + | + | | | + | | | + | | |
| Mannose, Gas | | | | | | | | | | | | | | |

| ganism | Galactose, Aer. Acid | Galactose, Anaer. Acid | Galactose, Aer. Alk. | Galactose, Anaer. Alk. | Galactose, Gas | Maltose, Aer. Acid | Maltose, Anaer. Acid | Maltose, Aer. Alk. | Maltose, Anaer. Alk. | Maltose, Gas | Arabinose, Aer. Acid | Arabinose, Anaer. Acid | Arabinose, Aer. Alk. | Arabinose, Anaer. Alk. | Arabinose, Gas | Sorbitol, Aer. Acid | Sorbitol, Anaer. Acid | Sorbitol, Aer. Alk. | Sorbitol, Anaer. Alk. | Sorbitol, Gas | Xylose, Aer. Acid | Xylose, Anaer. Acid | Xylose, Aer. Alk. | Xylose, Anaer. Alk. | Xylose, Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| e2 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| e5 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| e7 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | | | + | + | |
| e8 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| 1-1 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| 1-2 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| 1-712 | | | + | + | | | | + | + | | + | + | | | | + | + | | | | + | + | | | |
| 1-714 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| 1-714* | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| 2-1 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | + | + | | | |
| 2-2 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | + | + | | | |
| 2-3 | + | + | | | | + | + | | | | | | + | + | | | | + | + | | | | + | + | |
| 2-3* | + | | | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| 2-4 | + | | | + | | + | + | | | | + | + | | | | | | + | + | | | | + | + | |
| 2-714* | + | + | | | | + | + | | | | | | + | + | | | | + | + | | + | + | | | |

| genism | Inositol, Aer. Acid | Inositol, Anaer. Acid | Inositol, Aer. Alk. | Inositol, Anaer. Alk | Inositol, Gas | Methyl Red Reaction | Voges-Proskauer Test | Oxidase (Kovacs) | Starch Hydrolysis | Gelatin Hydrolysis, ≤8 d | Gelatin Hydrolysis, ≥9 d | Indole | Lysine Decarboxylase | Ornithine Decarboxylase | Arginine Decarboxylase | Ammonification | Nitrate Reduction → $NO_2$ | Nitrate Reduction → Gas | Catalase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| e2 | + | + | | | | | + | + | + | + | | + | + | + | | + | + | | + |
| e5 | + | + | | | | | | | + | + | | | + | + | | + | + | | + |
| e7 | + | + | | | | | + | | + | + | | | + | + | | + | + | | + |
| e3 | + | + | | | | | + | | + | + | | | + | + | | + | + | + | + |
| -1 | + | + | | | | | | + | + | + | | + | + | + | | + | + | | + |
| -2 | + | + | | | | | | | | | | | + | + | | + | + | + | + |
| -712 | + | | | + | | | | + | | | | | + | + | | + | + | | + |
| -714 | | | + | + | | + | | | | | | | + | + | | + | + | | + |
| -714* | + | + | | | | | + | | + | + | | + | + | + | | + | + | | + |
| -1 | | | + | + | | | | + | + | + | | | + | + | | + | + | | + |
| 2 | | | + | + | | | | + | + | + | | | + | + | | + | + | | + |
| 3 | | | + | + | | | | + | + | + | | | + | + | + | + | + | | + |
| 3* | | | + | + | | | | + | + | | + | | + | + | | + | + | | + |
| 4 | | | + | + | | | | + | + | + | | | + | | | + | + | | + |
| 714* | | | + | + | | | | + | + | + | | | + | + | | + | + | | + |

| Organism | Medium a | Medium b | Medium c | Medium d | Medium e | Medium A1 | Medium A2 | Medium A3 | Medium A4 | Medium A5 | Medium B1 | Medium B2 | Medium B3 | Medium C1 | Medium C2 | Medium C3 | Medium C4 | Medium D1 | Medium D2 | Medium D4 | Medium D5 | Medium D7 | Medium D8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| e2 | | | + | | + | | | | | | | + | | | | | | + | | | | | |
| e5 | | | + | | + | | | | | | | + | | | | | | + | | | | | |
| e7 | | | + | | + | | | | | | | + | | | | | | + | | | | | |
| e8 | | | + | | + | | | | | | | + | | | | | | + | | | | | |
| A1-1 | | | + | | + | | | | | | + | + | | | | | | + | + | | + | | |
| A1-2 | | | + | | + | | | | | | + | + | | | | | | | | | + | | |
| A1-712 | | | + | | + | | | | | | + | + | | | | | | + | | | + | | |
| A1-714 | | | + | | + | + | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| A1-714* | | | + | | + | | | | | | | + | + | | | | | + | + | + | + | | |
| A2-1 | | | + | | + | | | | | | + | + | | | | | | | | | + | | |
| A2-2 | | | + | | + | | | | | | | + | | | | | | + | | | | | |
| A2-3 | | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + |
| A2-3* | | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| A2-4 | | | + | | + | | | | | | + | + | | | | | | | | | | | |
| A2-714* | | | + | | + | | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

| Organism | Shape | | | Ends | | Length (μm) | | | | L:W | | | Diameter (μm) | | Occurrence | | | | Gram | Motility |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Straight Rod | Curved Rod | Coccus | Tapered | Square | ≤ 0.5 | 0.6 - 1.2 | 1.3 - 3.0 | ≥ 3.0 | 3 : 1 | 2 -3:1 | 1 : 1 | ≤ 0.5 | ≥ 0.6 | Singles | Pairs | Short Chains | Long Chains | | |
| A3-1 | + | | | | + | | | + | | + | | | | | + | | | | − | + |
| A5-1 | + | | | | + | | | + | | | + | | | | + | + | | | − | + |
| A5-1* | + | | | | + | | | + | | + | | | | | + | | + | | − | + |
| A5-1' | + | | | | + | | | + | | + | | | | | + | | | | − | + |
| A5-3 | + | | | | + | | | + | | + | | | | | + | | + | | − | + |
| A5-4 | + | | | | + | | + | | | | + | | | | + | | + | | − | + |
| A5-5 | + | | | | + | | | + | | + | | | | | + | | + | | − | + |
| A5-5* | + | | | | + | | | + | | + | | | | | + | | + | | − | + |
| A5-6 | | + | | | + | | | + | | + | | | | | + | | | | − | + |
| A5-7 | + | | | | + | | | + | | + | | | | | + | | | | − | + |
| B1-2 | + | | | | + | | | + | | | + | | | | + | + | | | − | + |
| B1-6 | + | | | | + | | | + | | + | | | | | + | + | | | − | + |
| B1-7 | + | | | | + | | | + | | + | | | | | + | | | | − | + |
| B2-2 | + | | | | + | | | + | | | + | | | | + | + | | | − | + |
| B2-4 | + | | | | + | | | | + | + | | | | | + | | + | | − | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | |

| rganism | Galactose, Aer. Acid | Galactose, Anaer. Acid | Galactose, Aer. Alk. | Galactose, Anaer. Alk. | Galactose, Gas | Maltose, Aer. Acid | Maltose, Anaer. Acid | Maltose, Aer. Alk. | Maltose, Anaer. Alk. | Maltose, Gas | Arabinose, Aer. Acid | Arabinose, Anaer. Acid | Arabinose, Aer. Alk. | Arabinose, Anaer. Alk. | Arabinose, Gas | Sorbitol, Aer. Acid | Sorbitol, Anaer. Acid | Sorbitol, Aer. Alk. | Sorbitol, Anaer. Alk. | Sorbitol, Gas | Xylose, Aer. Acid | Xylose, Anaer. Acid | Xylose, Aer. Alk. | Xylose, Anaer. Alk. | Xylose, Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A3-1 | + | | | + | | + | | | + | | | | + | + | | | | + | + | | | | + | + | |
| A5-1 | | | + | + | | + | + | | | | | | + | + | | | | + | + | | + | + | | | |
| A5-1* | | | + | + | | + | + | | | | | | + | + | | | | + | + | | + | + | | | |
| A5-1' | + | + | | | | + | + | | | | | | + | + | | | | + | + | | + | + | | | |
| A5-3 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| A5-4 | + | + | | | | + | + | | | | | | + | + | | | | + | + | | + | + | | | |
| A5-5 | + | + | | | | + | + | | | | | | + | + | | + | + | | | | + | + | | | |
| A5-5* | + | + | | | | + | + | | | | | | + | + | | + | + | | | | + | + | | | |
| A5-6 | | | + | + | | + | + | | | | | | + | + | | + | + | | | | + | + | | | |
| A5-7 | | | + | + | | + | | | + | | | | + | + | | + | | | + | | + | | | + | |
| B1-2 | + | + | | | | + | + | | | | | | + | + | | | | + | + | | | | + | + | |
| B1-6 | + | + | | | | + | + | | | | | | + | + | | + | + | | | | + | + | | | |
| B1-7 | + | + | | | | + | + | | | | | | + | + | | + | + | | | | + | + | | | |
| B2-2 | + | | | + | | + | + | | | | | | + | + | | + | + | | | | + | + | | | |
| B2-4 | | | + | + | | + | + | | | | | | + | + | | + | + | | | | + | + | | | |

| rganism | Inositol, Aer. Acid | Inositol, Anaer. Acid | Inositol, Aer. Alk. | Inositol, Anaer. Alk. | Inositol, Gas | Methyl Red Reaction | Voges-Proskauer Test | Oxidase (Kovacs) | Starch Hydrolysis | Gelatin Hydrolysis, ≤8 d | Gelatin Hydrolysis, ≥9 d | Indole | Lysine Decarboxylase | Ornithine Decarboxylase | Arginine Decarboxylase | Ammonification | Nitrate Reduction → $NO_2$ | Nitrate Reduction → Gas | Catalase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A3-1 | | | + | + | | | | + | + | | + | | | | | + | + | | + |
| A5-1 | | | + | + | | | | + | + | + | | | | | | + | + | | + |
| A5-1* | | | + | + | | | | + | + | + | | | | | | + | + | | + |
| A5-1' | | | + | + | | | | + | + | + | | | | | | + | + | | + |
| A5-3 | | | + | + | | | | + | + | + | | | | | | | + | | + |
| A5-4 | | | + | + | | | | + | | | | | | | | | + | | + |
| A5-5 | | | + | + | | + | | + | + | + | | + | + | + | | + | | | + |
| A5-5* | | | + | + | | + | | + | + | + | | + | + | + | | + | | | + |
| A5-6 | | | + | + | | + | | + | + | + | | + | + | + | | + | + | | + |
| A5-7 | | | + | + | | | | + | | | | + | + | + | | + | + | + | + |
| 01-2 | | | + | + | | | | + | + | | + | + | + | + | | | + | | + |
| 01-6 | | | + | + | | + | | + | | | + | + | | | + | + | + | | + |
| 1-7 | | | + | + | | | | | + | + | | | + | + | + | + | + | | + |
| 2-2 | | | + | + | | | | + | + | | + | | + | + | | + | + | | + |
| 2-4 | | | + | + | | + | | + | | | | | + | + | + | + | + | | + |

| Organism | Medium a | Medium b | Medium c | Medium d | Medium e | Medium A1 | Medium A2 | Medium A3 | Medium A4 | Medium A5 | Medium B1 | Medium B2 | Medium B3 | Medium C1 | Medium C2 | Medium C3 | Medium C4 | Medium D1 | Medium D2 | Medium D4 | Medium D5 | Medium D7 | Medium D8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A3-1 | | | + | | + | | | | | | + | + | | | | | | | | | + | | |
| A5-1 | | | + | | + | | | | | | + | + | | | | | | + | | | | | |
| A5-1* | | | + | | + | | | | | | + | + | | | | | | + | | | | | |
| A5-1' | | | + | | + | | | | | | + | + | | | | | | + | | | | | |
| A5-3 | + | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | + | + | + |
| A5-4 | | | + | | + | | | | | | | + | | | | | | | | | | | |
| A5-5 | | | + | | + | | | | | | + | + | | | | | | + | + | | + | | |
| A5-5* | | | + | | + | | | | | | + | + | | | | | | + | + | | + | | |
| A5-6 | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| A5-7 | | | + | | + | | | | | | + | | | | | | | + | + | + | + | | |
| B1-2 | | | + | | + | | | | | | | | | | | | | + | + | + | + | | |
| B1-6 | | | + | | + | | | | | | + | | | | | | | + | | + | + | | |
| B1-7 | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| B2-2 | | | | | + | | | | | | + | + | | | | | | + | | | | | |
| B2-4 | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |

| Organism | Shape | | | Ends | | Length (μm) | | | | L:W | | | Diameter (μm) | | Occurrence | | | | Gram | Motility |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Straight Rod | Curved Rod | Coccus | Tapered | Square | ≤ 0.5 | 0.6 - 1.2 | 1.3 - 3.0 | ≥3.0 | 3 : 1 | 2 -3:1 | 1 : 1 | ≤ 0.5 | ≥ 0.6 | Singles | Pairs | Short Chains | Long Chains | | |
| B2-Rh | + | | | | + | | | + | | + | | | | | + | | + | | − | + |
| B2-Rh* | + | | | | + | | | + | | + | | | | | + | | | | − | + |
| B3-1 | | + | | | + | | | + | | | + | | | | + | | | | − | + |
| B3-5 | + | | | | + | | | | + | + | | | | | + | | + | | − | + |
| B3-711 | + | | | | + | | | + | | | + | | | | + | | + | | − | + |
| B3-712 | + | | | | + | | | + | | | + | | | | + | + | | | − | + |
| B3-Ps | + | | | | + | | | + | | + | | | | | + | | | | − | + |
| B3-Rh | + | | | | + | | | + | | + | | | | | + | | | | − | + |
| C1-1 | + | | | | + | | + | | | | | + | | | + | + | | | − | + |
| C1-1* | + | | | | + | | | + | | + | | | | | + | | | | − | |
| C1-1' | + | | | | + | | + | | | | | + | | | + | | | | − | + |
| C1-2 | + | | | | + | | | | + | + | | | | | + | | | | − | + |
| C1-2* | + | | | | + | | | + | | | | + | | | + | | | | − | + |
| C1-3* | + | | | | + | | | | + | + | | | | | + | | | | − | + |
| C1-4 | + | | | | + | | | + | | | + | | | | + | | | | − | + |

| Organism | Glucose, Aer. Acid | Glucose, Anaer. Acid | Glucose, Aer. Alk. | Glucose, Anaer. Alk. | Glucose, Gas | Lactose, Aer. Acid | Lactose, Anaer. Acid | Lactose, Aer. Alk. | Lactose, Anaer. Alk. | Lactose, Gas | Sucrose, Aer. Acid | Sucrose, Anaer. Acid | Sucrose, Aer. Alk. | Sucrose, Anaer. Alk. | Sucrose, Gas | Mannitol, Aer. Acid | Mannitol, Anaer. Acid | Mannitol, Aer. Alk. | Mannitol, Anaer. Alk. | Mannitol, Gas | Mannose, Aer. Acid | Mannose, Anaer. Acid | Mannose, Aer. Alk. | Mannose, Anaer. Alk. | Mannose, Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B2-Rh | + | + | | | | | | + | + | | | | + | + | | + | + | | | | + | + | | | |
| B2-Rh* | + | + | | | | | | + | + | | + | + | | | | + | + | | | | + | + | | | |
| B3-1 | + | + | | | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| B3-5 | + | + | | | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| B3-711 | + | + | | | | | | + | + | | + | + | | | | | | + | + | | | | + | + | |
| B3-712 | + | + | | | | | | + | + | | | | + | + | | | | + | + | | + | + | | | |
| B3-Ps | + | | | + | | | | + | + | | | | + | + | | | | + | + | | + | | | + | |
| B3-Rh | + | + | | | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| C1-1 | + | + | | | | | | + | + | | + | + | | | + | | | + | + | | | | + | + | |
| C1-1* | + | + | | | | | | + | + | | + | | | + | | | | + | + | | + | + | | | |
| C1-1' | + | + | | | | + | + | | | | + | + | | | | | | + | + | | | | + | + | |
| C1-2 | + | + | | | | + | + | | | | + | + | | | | | | + | + | | | | + | + | |
| C1-2* | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| C1-3* | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| C1-4 | + | + | | | | + | + | | | | + | + | | | | | | + | + | | | | | + | + |

| Ganism | Galactose, Aer. Acid | Galactose, Anaer. Acid | Galactose, Aer. Alk. | Galactose, Anaer. Alk. | Galactose, Gas | Maltose, Aer. Acid | Maltose, Anaer. Acid | Maltose, Aer. Alk. | Maltose, Anaer. Alk. | Maltose, Gas | Arabinose, Aer. Acid | Arabinose, Anaer. Acid | Arabinose, Aer. Alk. | Arabinose, Anaer. Alk. | Arabinose, Gas | Sorbitol, Aer. Acid | Sorbitol, Anaer. Acid | Sorbitol, Aer. Alk. | Sorbitol, Anaer. Alk. | Sorbitol, Gas | Xylose, Aer. Acid | Xylose, Anaer. Acid | Xylose, Aer. Alk. | Xylose, Anaer. Alk. | Xylose, Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B2-Rh | + | + | | | | + | + | | | | | | + | + | | + | | | + | | | | + | + | |
| B2-Rh* | | | + | + | | + | | | + | | + | + | | | | | | + | + | | + | | | + | |
| B3-1 | | | + | + | | | | + | + | | + | + | | | | | | + | + | | | | + | + | |
| B3-5 | | | + | + | | + | + | | | | + | + | | | | | | + | + | | | | + | + | |
| B3-711 | | | + | + | | + | + | | | | + | + | | | | | | + | + | | | | + | + | |
| B3-712 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | | | + | + | |
| B3-Ps | | | + | + | | + | | | + | | + | | | + | | | | + | + | | | | + | + | |
| B3-Rh | | | + | + | | + | + | | | | + | + | | | | | | + | + | | | | + | + | |
| C1-1 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| C1-1* | + | + | | | | + | + | | | | | | + | + | | + | + | | | | + | + | | | |
| C1-1' | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| C1-2 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| C1-2* | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| C1-3* | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| C1-4 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |

| Organism | Inositol, Aer. Acid | Inositol, Anaer. Acid | Inositol, Aer. Alk. | Inositol, Anaer. Alk. | Inositol, Gas | Methyl Red Reaction | Voges-Proskauer Test | Oxidase (Kovacs) | Starch Hydrolysis | Gelatin Hydrolysis, ≤8 da | Gelatin Hydrolysis, ≥9 da | Indole | Lysine Decarboxylase | Ornithine Decarboxylase | Arginine Decarboxylase | Ammonification | Nitrate Reduction → $NH_3$ | Nitrate Reduction → Gas | Catalase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B2-Rh | | | + | + | | | | | | | | | + | + | + | + | + | | + |
| B2-Rh* | | | + | + | | | | | | | | | + | + | + | + | + | + | + |
| B3-1 | + | + | | | | | | + | | | | | + | + | | + | + | | + |
| B3-5 | + | + | | | | | | + | | | + | | + | + | + | + | + | | + |
| B3-711 | + | + | | | | + | | + | + | + | | | + | + | | + | | | + |
| B3-712 | + | + | | | | | | + | + | + | | | + | + | + | + | + | | + |
| B3-Ps | + | | | + | | | | + | + | | + | | + | + | + | + | + | | + |
| B3-Rh | + | + | | | | | | + | + | + | | | + | + | + | + | + | | + |
| C1-1 | + | + | | | | + | | | + | | + | | | | | + | + | | + |
| C1-1* | + | + | | | | | | | + | + | | | | | + | + | + | | + |
| C1-1' | + | + | | | | | | | | | + | | | | | + | + | | |
| C1-2 | + | + | | | | | | | + | | + | | | | | + | + | | + |
| C1-2* | + | + | | | + | + | | + | + | + | | + | | | + | + | + | | + |
| C1-3* | + | + | | | | + | | + | | | + | | | | + | + | + | | + |
| C1-4 | + | + | | | + | | | + | + | + | | | | | + | + | + | | + |

| Organism | Medium a | Medium b | Medium c | Medium d | Medium e | Medium A1 | Medium A2 | Medium A3 | Medium A4 | Medium A5 | Medium B1 | Medium B2 | Medium B3 | Medium C1 | Medium C2 | Medium C3 | Medium C4 | Medium D1 | Medium D2 | Medium D4 | Medium D5 | Medium D7 | Medium D8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B2-Rh | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| B2-Rh* | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| B3-1 | | | + | | + | | | | | | + | + | | | | | | + | | | | | |
| B3-5 | | | + | | + | | | | | | | | | | | | | | | | | | |
| B3-711 | | | + | | + | | | | | | | + | | | | | | + | | + | + | | |
| B3-712 | + | | + | | + | + | + | | | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| B3-Ps | | | + | | + | | | | | | + | + | | | | | | | | | | | |
| B3-Rh | | | + | | + | | | | | | | | | | | | | | | | | | |
| C1-1 | | | + | | + | | | | | | | + | | | | | | + | + | + | + | | |
| C1-1* | | | + | | + | | | | | | + | + | | | | | | | | | | | |
| C1-1' | | | + | | + | | | | | | + | + | | | | | | | | + | | | |
| C1-2 | | | + | | + | | | | | | + | + | | | | | | | | | | | |
| C1-2* | | | + | | + | | | | | | + | + | | | | | | | | | | | |
| C1-3* | | | + | | + | | | | | | | + | | | | | | + | | | + | | |
| C1-4 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

| Organism | Shape | | | Ends | | Length (μm) | | | | L:W | | | Diameter (μm) | | Occurrence | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Straight Rod | Curved Rod | Coccus | Tapered | Square | ≤ 0.5 | 0.6 - 1.2 | 1.3 - 3.0 | ≥3.0 | 3 : 1 | 2 -3:1 | 1 : 1 | ≤ 0.5 | ≥ 0.6 | Singles | Pairs | Short Chains | Long Chains | Gram | Motility |
| C1-4* | + | | | | + | | | + | | + | | | | | + | + | | | - | + |
| C1-5 | + | | | | + | | | | + | + | | | | | + | | + | | - | + |
| C1-8 | + | | | | + | | | + | | + | | | | | + | | | | - | + |
| C1-10 | + | | | | + | | | + | | | + | | | | + | + | | | - | + |
| C2-2 | + | | | | + | | | | + | + | | | | | + | | + | | - | + |
| C2-3 | + | | | | + | | | + | | | + | | | | + | | | | - | + |
| C2-4 | + | | | | + | | + | | | | | + | | | + | | | | - | + |
| C3-1 | + | | | | + | | | + | | + | | | | | + | + | | | - | + |
| C3-2 | + | | | | + | | | + | | + | | | | | + | | | | - | + |
| C3-4 | + | | | | + | | | + | | + | | | | | + | | | | - | + |
| C3-4* | + | | | | + | | | + | | + | | | | | + | | | | - | + |
| C3-5 | + | | | | + | | | + | | + | | | | | + | | | | - | + |
| C3-7* | + | | | | + | | | + | | | + | | | | + | + | | | - | + |
| C3-711 | + | | | + | | | | + | | + | | | | | + | | | | - | + |
| C3-712 | + | | | | + | | | + | | + | | | | | + | | | | - | + |

| ganism | Glucose, Aer. Acid | Glucose, Anaer. Acid | Glucose, Aer. Alk. | Glucose, Anaer. Alk. | Glucose, Gas | Lactose, Aer. Acid | Lactose, Anaer. Acid | Lactose, Aer. Alk. | Lactose, Anaer. Alk. | Lactose, Gas | Sucrose, Aer. Acid | Sucrose, Anaer. Acid | Sucrose, Aer. Alk. | Sucrose, Anaer. Alk. | Sucrose, Gas | Mannitol, Aer. Acid | Mannitol, Anaer. Acid | Mannitol, Aer. Alk. | Mannitol, Anaer. Alk. | Mannitol, Gas | Mannose, Aer. Acid | Mannose, Anaer. Acid | Mannose, Aer. Alk. | Mannose, Anaer. Alk. | Mannose, Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-4* | + | | | + | | | | + | + | | + | | | + | | + | | | + | | + | | | + | |
| 1-5 | + | | | + | | + | | | + | | + | | | + | | + | | | + | | + | | | + | |
| 1-8 | | | + | + | | + | + | | | | | | + | + | | | | + | + | | + | | | + | |
| -10 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| -2 | + | | | + | | + | | | + | | + | | | + | | + | | | + | | + | | | + | |
| -3 | + | | | + | | | | + | + | | + | | | + | | + | | | + | | | | + | + | |
| -4 | | | + | + | | + | + | | | | | | + | + | | | | + | + | | + | + | | | |
| -1 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| -2 | | | + | + | | + | + | | | | | | + | + | | + | + | | | | | + | + | | |
| -4 | + | | | + | | | | + | + | | | | + | + | | + | | | + | | + | | | + | |
| -4* | | | + | + | | + | + | | | | | | + | + | | | | + | + | | + | + | | | |
| -5 | | | + | + | | + | + | | | | | | + | + | | | | + | + | | + | + | | | |
| -7* | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| -711 | + | | | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| -712 | + | | | + | | + | | | + | | + | | | + | | + | | | + | | + | | | + | |

| | 12 | 11 | * | | * | | 2 | 1 | 4 | 3 | 2 | 10 | 8 | 5 | 4* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Galactose, Aer. Acid | + | + | + | + | + | | + | + | + | | + | | | + | |
| Galactose, Anaer. Acid | | | + | + | + | | + | + | + | | | | | | |
| Galactose, Aer. Alk. | | | | | | + | | | | + | | | + | | + |
| Galactose, Anaer. Alk. | + | + | | | | + | | | | + | + | | + | + | + |
| Galactose, Gas | | | | | | | | | | | | | | | |
| Maltose, Aer. Acid | + | + | + | + | | | + | + | + | | + | | + | + | + |
| Maltose, Anaer. Acid | | | + | + | | | + | + | + | | | | + | | |
| Maltose, Aer. Alk. | | | | | + | + | | | | + | | | | | |
| Maltose, Anaer. Alk. | + | + | | | + | + | | | | + | + | | | + | + |
| Maltose, Gas | | | | | | | | | | | | | | | |
| Arabinose, Aer. Acid | + | + | + | + | + | + | + | + | + | + | + | | + | + | + |
| Arabinose, Anaer. Acid | | | + | + | + | | + | + | + | | | | + | | |
| Arabinose, Aer. Alk. | | | | | | | | | | | | | | | |
| Arabinose, Anaer. Alk. | + | + | | | | + | | | | + | + | | | + | + |
| Arabinose, Gas | | | | | | | | | | | | | | | |
| Sorbitol, Aer. Acid | + | + | + | + | + | | + | + | + | | + | | + | + | |
| Sorbitol, Anaer. Acid | | | + | + | + | | + | + | + | | | | + | | |
| Sorbitol, Aer. Alk. | | | | | | + | | | | + | | | | | |
| Sorbitol, Anaer. Alk. | + | + | | | | + | | | | + | + | | | + | + |
| Sorbitol, Gas | | | | | | | | | | | | | | | + |
| Xylose, Aer. Acid | + | + | + | + | + | | + | + | + | | + | | + | + | |
| Xylose, Anaer. Acid | | + | + | + | | | + | + | + | | | | + | | |
| Xylose, Aer. Alk. | | | | | + | | | | | + | | | | | + |
| Xylose, Anaer. Alk. | + | + | | | + | | | | | + | + | | | + | + |
| Xylose, Gas | | | | | | | | | | | | | | | |

| Organism | Inositol, Aer. Acid | Inositol, Anaer. Acid | Inositol, Aer. Alk. | Inositol, Anaer. Alk. | Inositol, Gas | Methyl Red Reaction | Voges-Proskauer Test | Oxidase (Kovacs) | Starch Hydrolysis | Gelatin Hydrolysis, ≤8 day | Gelatin Hydrolysis, ≥9 day | Indole | Lysine Decarboxylase | Ornithine Decarboxylase | Arginine Decarboxylase | Ammonification | Nitrate Reduction → $NO_2$ | Nitrate Reduction → Gas | Catalase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C1-4* | | | + | + | | | | + | + | + | | | | | + | + | + | | + |
| C1-5 | + | + | | | | | | + | + | | + | | | | + | + | + | | + |
| C1-8 | + | + | | | | | | + | + | + | | | | | | + | + | | + |
| C1-10 | + | + | | | | + | | + | + | + | | + | + | + | | + | + | | + |
| C2-2 | | | + | + | | | | | | | + | | | | | + | + | | + |
| C2-3 | | | + | + | | | | + | | | | | | | | + | | | + |
| C2-4 | + | + | | | | | | + | | | + | | | | | + | | | + |
| C3-1 | + | + | | | | | | + | + | + | | + | + | + | | + | + | | + |
| C3-2 | + | + | | | | | | + | + | + | | | + | + | | + | + | | + |
| C3-4 | | | + | + | | | | + | | | | | + | + | | | + | | + |
| C3-4* | + | + | | | | | | | | | | | + | + | | + | + | | + |
| C3-5 | + | + | | | | | | + | + | + | | | + | + | | + | + | | + |
| C3-7* | + | + | | | | | | + | + | | + | + | + | + | | + | + | | + |
| C3-711 | + | | | + | | | | + | + | | + | | + | + | | + | + | + | + |
| C3-712 | | | + | + | | | | + | + | | + | | + | + | | + | + | | + |

| Organism | Medium a | Medium b | Medium c | Medium d | Medium e | Medium A1 | Medium A2 | Medium A3 | Medium A4 | Medium A5 | Medium B1 | Medium B2 | Medium B3 | Medium C1 | Medium C2 | Medium C3 | Medium C4 | Medium D1 | Medium D2 | Medium D4 | Medium D5 | Medium D7 | Medium D8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C1-4* | | | + | | + | | | | | | + | + | | | | | | + | + | | | | |
| C1-5 | + | | + | + | + | | + | + | | + | | + | + | + | + | + | + | + | | + | + | + | + |
| C1-8 | | | + | | + | | | | | | + | + | | | | | | + | | | | | |
| C1-10 | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| C2-2 | + | | + | + | + | + | | + | | | | + | | + | + | + | + | + | | + | + | | + |
| C2-3 | | | + | | + | | | | | | | | | | | | | + | | + | + | | |
| C2-4 | + | + | + | + | + | + | + | + | | | + | + | | + | + | + | + | + | + | + | + | + | + |
| C3-1 | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| C3-2 | | | + | | + | | | | | | + | + | | | | | | | + | | | | |
| C3-4 | | | + | | + | | | | | | | | | | | | | | + | | | | |
| C3-4* | | | + | | + | | | | | | | | | | | | | | | | | | |
| C3-5 | | | + | | + | | | | | | | + | + | + | | | | + | + | + | + | | |
| C3-7* | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| C3-711 | | | + | | + | | | | | | + | + | | | | | | + | | + | + | | |
| C3-712 | + | + | + | + | + | + | + | + | | + | | + | + | + | + | + | + | + | + | + | + | + | + |

| Organism | Shape | | | Ends | | Length (μm) | | | | L:W | | | Diameter (μm) | | Occurrence | | | | Gram | Motility |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Straight Rod | Curved Rod | Coccus | Tapered | Square | ≤ 0.5 | 0.6 - 1.2 | 1.3 - 3.0 | ≥3.0 | 3 : 1 | 2 -3:1 | 1 : 1 | ≤ 0.5 | ≥ 0.6 | Singles | Pairs | Short Chains | Long Chains | | |
| C3-712* | | + | | | + | | + | | | | + | | | | + | | | | - | + |
| D1-1 | + | | | | + | | | + | | + | | | | | + | + | | | - | + |
| D1-1* | + | | | | + | | | + | | | + | | | | + | + | | | - | + |
| D1-2 | + | | | | + | | | | + | + | | | | | + | | + | | - | + |
| D1-3 | + | | | | + | | | + | | | + | | | | + | + | | | - | + |
| D1-4 | + | | | | + | | | + | | + | | | | | + | + | | | - | + |
| D1-5 | + | | | | + | | | | + | + | | | | | + | | | | - | + |
| D1-7 | + | | | | + | | | + | | + | | | | | + | + | | | - | + |
| D1-7* | + | | | | + | | | + | | | | + | | | + | | | | - | + |
| D2-1 | + | | | | + | | + | | | | | + | | | + | | | | - | + |
| D2-2 | + | | | | + | | | | + | + | | | | | + | | + | | - | + |
| D2-3 | + | | | | + | | | + | | + | | | | | + | | + | | - | + |
| D2-3* | + | | | | + | | | + | | + | | | | | + | + | + | | - | + |
| D2-5 | + | | | | + | | | + | | + | | | | | + | + | | | - | + |
| D2-5* | + | | | | + | | | + | | | + | | | | + | | | | - | + |

| ganism | Glucose, Aer. Acid | Glucose, Anaer. Acid | Glucose, Aer. Alk. | Glucose, Anaer. Alk. | Glucose, Gas | Lactose, Aer. Acid | Lactose, Anaer. Acid | Lactose, Aer. Alk. | Lactose, Anaer. Alk. | Lactose, Gas | Sucrose, Aer. Acid | Sucrose, Anaer. Acid | Sucrose, Aer. Alk. | Sucrose, Anaer. Alk. | Sucrose, Gas | Mannitol, Aer. Acid | Mannitol, Anaer. Acid | Mannitol, Aer. Alk. | Mannitol, Anaer. Alk. | Mannitol, Gas | Mannose, Aer. Acid | Mannose, Anaer. Acid | Mannose, Aer. Alk. | Mannose, Anaer. Alk. | Mannose, Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ~~C3-712A~~ | + | + | | | | + | + | | | | | | + | + | | | | + | + | | + | + | | | |
| D1-1 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | + | + | | | |
| D1-1* | | | + | + | | | | + | + | | | | + | + | | | | + | + | | + | | | + | |
| D1-2 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| D1-3 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| D1-4 | + | + | | | | + | + | | | | + | + | | | | | | + | + | | | | + | + | |
| D1-5 | + | | | + | | + | | | + | | + | | | + | | + | | | + | | | | + | + | |
| D1-7 | + | + | | | | + | | | + | | + | | | + | | | | + | + | | | | + | + | |
| D1-7* | + | + | | | | | | + | + | | | | + | + | | + | + | | | | | | + | + | |
| D2-1 | + | + | | | | | | + | + | | + | + | | | | + | + | | | | + | + | | | |
| D2-2 | + | + | | | | + | + | | | | + | + | | | | | | + | + | | + | + | | | |
| D2-3 | + | | | + | | | | + | + | | + | | | + | | | | + | + | | | | + | + | |
| D2-3* | + | + | | | | + | | | + | | + | + | | | | + | + | | | | + | + | | | |
| D2-5 | + | | | + | | | | + | + | | + | | | + | | | | + | + | | + | | | + | |
| D2-5* | + | | | + | | | | + | + | | + | | | + | | | | + | + | | + | | | + | |

| Organism | Galactose, Aer. Acid | Galactose, Anaer. Acid | Galactose, Aer. Alk. | Galactose, Anaer. Alk. | Galactose, Gas | Maltose, Aer. Acid | Maltose, Anaer. Acid | Maltose, Aer. Alk. | Maltose, Anaer. Alk. | Maltose, Gas | Arabinose, Aer. Acid | Arabinose, Anaer. Acid | Arabinose, Aer. Alk. | Arabinose, Anaer. Alk. | Arabinose, Gas | Sorbitol, Aer. Acid | Sorbitol, Anaer. Acid | Sorbitol, Aer. Alk. | Sorbitol, Anaer. Alk. | Sorbitol, Gas | Xylose, Aer. Acid | Xylose, Anaer. Acid | Xylose, Acr. Alk. | Xylose, Anaer. Alk. | Xylose, Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C3-712* | + | | | + | | + | | | + | | + | | | + | | + | | | + | | + | | | + | |
| D1-1 | | | + | + | | + | | | + | | | | + | + | | | | + | + | | | | + | + | |
| D1-1* | + | | | + | | + | | | + | | | | + | + | | | | + | + | | | | + | + | |
| D1-2 | + | | | + | | + | | | + | | + | | | + | | | | + | + | | | | + | + | |
| D1-3 | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| D1-4 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| D1-5 | + | | | + | | + | | | + | | | | + | + | | | | + | + | | + | | | + | |
| D1-7 | + | + | | | | + | + | | | | + | + | | | | | | + | + | | + | + | | | |
| D1-7* | + | + | | | | + | + | | | | + | + | | | | + | + | | | | | | + | + | |
| D2-1 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | | | + | + | |
| D2-2 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| D2-3 | + | | | + | | + | | | + | | | | + | + | | + | | | + | | | | + | + | |
| D2-3* | + | + | | | | + | + | | | | + | + | | | | + | + | | | | | | + | + | |
| D2-5 | | | + | + | | + | | | + | | + | | | + | | | | + | + | | | | + | + | |
| D2-5* | | | + | + | | + | | | + | | + | | | + | | | | + | + | | | | | + | |

| Organism | Inositol, Aer. Acid | Inositol, Anaer. Acid | Inositol, Aer. Alk. | Inositol, Anaer. Alk. | Inositol, Gas | Methyl Red Reaction | Voges-Proskauer Test | Oxidase (Kovacs) | Starch Hydrolysis | Gelatin Hydrolysis, ≤8 day | Gelatin Hydrolysis, ≥9 day | Indole | Lysine Decarboxylase | Ornithine Decarboxylase | Arginine Decarboxylase | Ammonification | Nitrate Reduction → $NO_2$ | Nitrate Reduction → Gas | Catalase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C3-712* | + | + | | | | | | + | | | + | | + | + | | + | | | + |
| D1-1 | | | + | + | | | | + | + | + | | | | | | + | + | | + |
| D1-1* | | | + | + | | | | + | + | + | | | | | | + | + | | + |
| D1-2 | | | + | + | | | | + | | | + | | | | | + | + | | + |
| D1-3 | | | + | + | | | | + | | | + | + | | | | + | + | | + |
| D1-4 | | | + | + | | | | + | + | | + | + | | | + | + | + | | + |
| D1-5 | | | + | + | | | | + | + | + | | | | | | + | + | | + |
| D1-7 | + | + | | | | | | + | + | | + | + | | | | + | + | | + |
| D1-7* | + | + | | | | + | | + | + | | + | | | | + | + | + | | + |
| D2-1 | | | + | + | | + | | + | + | + | | + | | | + | + | + | | + |
| D2-2 | | | + | + | | + | | + | + | | | | | | + | + | + | | + |
| D2-3 | | | + | + | | | | + | + | + | | | | | + | + | + | | + |
| D2-5 | | | + | + | | | | + | + | + | | | + | + | | + | + | | + |
| D2-5* | | | + | + | | | | + | + | + | | | + | + | | + | + | | + |

| Organism | Medium a | Medium b | Medium c | Medium d | Medium e | Medium A1 | Medium A2 | Medium A3 | Medium A4 | Medium A5 | Medium B1 | Medium B2 | Medium B3 | Medium C1 | Medium C2 | Medium C3 | Medium C4 | Medium D1 | Medium D2 | Medium D4 | Medium D5 | Medium D7 | Medium D8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C3-712* | | | + | | + | | | | | | | + | | | | | | | | | | | |
| D1-1 | | | + | | + | | | | | | + | + | | | | | | + | | | + | | |
| D1-1* | | | + | | + | | | | | | + | + | | | | | | + | | | + | | |
| D1-2 | | | + | | + | | | | | | | | | | | | | + | | | | | |
| D1-3 | | | + | | + | | | | | | + | + | | | | | | + | + | | | | |
| D1-4 | | | + | | + | | | | | | | | | | | | | | | | | | |
| D1-5 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| D1-7 | + | + | + | + | + | | | | | | + | + | | + | + | + | + | + | + | + | + | + | + |
| D1-7* | | | + | | + | | | | | | | + | | | | | | + | + | + | + | | |
| D2-1 | | | + | | + | | | | | | | + | | | | | | + | + | + | + | | |
| D2-2 | + | | + | + | + | + | + | + | | + | | + | + | + | + | + | + | + | + | + | + | + | + |
| D2-3 | | | + | | + | | | | | | | + | | | | | | + | | | | | |
| D2-3* | | | + | | + | | | | | | + | + | | | | | | + | + | | + | | |
| D2-5 | | | + | | + | | | | | | + | + | | | | | | + | | | | | |
| D2-5* | | | + | | + | | | | | | + | + | | | | | | + | | | | | |

| Organism | Shape | | | Ends | | Length (μm) | | | | L:W | | | Diameter (μm) | | Occurrence | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Straight Rod | Curved Rod | Coccus | Tapered | Square | ≤ 0.5 | 0.6 - 1.2 | 1.3 - 3.0 | ≥ 3.0 | 3 : 1 | 2 -3:1 | 1 : 1 | ≤ 0.5 | ≥ 0.6 | Singles | Pairs | Short Chains | Long Chains | Gram | Motility |
| D2-6 | + | | | | + | | | + | | + | | | | | + | + | | | - | + |
| D2-7 | + | | | | + | | | + | | + | | | | | + | + | | | - | + |
| D2-711 | + | | | | + | | | + | | | + | | | | + | | | | - | + |
| D2-712 | + | | | | + | | | | + | + | | | | | + | | | | - | + |
| D4-1 | + | | | | + | | | + | | + | | | | | + | | + | | - | + |
| D4-4 | + | | | | + | | | + | | | + | | | | + | | | | - | + |
| D4-4* | + | | | | + | | | + | | + | | | | | + | | | | - | |
| D4-6 | + | | | | + | | + | | | | + | | | | + | | | | - | + |
| D4-7 | + | | | | + | | | | + | + | | | | | + | | | | - | + |
| D5-1 | + | | | | + | | | | + | + | | | | | + | + | | | - | + |
| D5-1* | + | | | | + | | | + | | + | | | | | + | | | | - | + |
| D5-2 | + | | | | + | | | + | | + | | | | | + | | | | - | + |
| D5-2* | + | | | | + | | | + | | + | | | | | + | | + | | - | + |
| D5-4 | + | | | | + | | | + | | + | | | | | + | | | | - | + |
| D5-5 | + | | | | + | | | + | | + | | | | | + | | + | | - | + |

| Organism | Glucose, Aer. Acid | Glucose, Anaer. Acid | Glucose, Aer. Alk. | Glucose, Anaer. Alk. | Glucose, Gas | Lactose, Aer. Acid | Lactose, Anaer. Acid | Lactose, Aer. Alk. | Lactose, Anaer. Alk. | Lactose, Gas | Sucrose, Aer. Acid | Sucrose, Anaer. Acid | Sucrose, Aer. Alk. | Sucrose, Anaer. Alk. | Sucrose, Gas | Mannitol, Aer. Acid | Mannitol, Anaer. Acid | Mannitol, Aer. Alk. | Mannitol, Anaer. Alk. | Mannitol, Gas | Mannose, Aer. Acid | Mannose, Anaer. Acid | Mannose, Aer. Alk. | Mannose, Anaer. Alk. | Mannose, Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D2-6 | + | + | | | | | | + | + | | + | + | | | | + | + | | | | + | + | | | |
| D2-7 | + | | | + | | | | + | + | | + | | | + | | | | + | + | | + | | | + | |
| D2-711 | + | + | | | + | | | + | + | | + | + | | | | + | + | | | | + | + | | | |
| D2-712 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| D4-1 | + | | | + | | + | | | + | | + | | | + | | + | | | | | + | | | | |
| D4-4 | + | + | | | | | | + | + | | + | + | | | | + | + | | | | + | + | | | |
| D4-4* | + | | | + | | | | + | + | | | | + | + | | | | + | + | | + | | | + | |
| D4-6 | + | | | + | | + | | | + | | + | | | + | | + | | | + | | + | | | + | |
| D4-7 | + | | | + | | + | | | + | | + | | | + | | + | | | + | | + | | | + | |
| D5-1 | + | + | | | | | | + | + | | + | + | | | | + | + | | | + | + | + | | | |
| D5-1* | + | + | | | + | | | + | + | | + | + | | | + | + | + | | | + | + | + | | | |
| D5-2 | + | + | | | | | | + | + | | + | + | | | | + | + | | | | + | + | | | |
| D5-2* | | | + | + | | | | + | + | | | | + | + | | | | + | + | | + | + | | | |
| D5-4 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| D5-5 | + | + | | | | + | + | | | | + | + | | | + | + | + | | | | + | + | | | |

| Organism | Galactose, Aer. Acid | Galactose, Anaer. Acid | Galactose, Aer. Alk. | Galactose, Anaer. Alk. | Galactose, Gas | Maltose, Aer. Acid | Maltose, Anaer. Acid | Maltose, Aer. Alk. | Maltose, Anaer. Alk. | Maltose, Gas | Arabinose, Aer. Acid | Arabinose, Anaer. Acid | Arabinose, Aer. Alk. | Arabinose, Anaer. Alk. | Arabinose, Gas | Sorbitol, Aer. Acid | Sorbitol, Anaer. Acid | Sorbitol, Aer. Alk. | Sorbitol, Anaer. Alk. | Sorbitol, Gas | Xylose, Aer. Acid | Xylose, Anaer. Acid | Xylose, Aer. Alk. | Xylose, Anaer. Alk. | Xylose, Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D2-6 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | | | + | + | |
| D2-7 | + | | | + | | + | | | + | | | | + | + | | | | + | + | | + | | | + | |
| D2-711 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | | | + | + | |
| D2-712 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | | | + | + | |
| D4-1 | + | | | + | | + | | | + | | + | | | + | | + | | | + | | | | + | + | |
| D4-4 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| D4-4* | + | | | + | | + | | | + | | | | + | + | | | | + | + | | | | + | + | |
| D4-6 | + | | | + | | + | | | + | + | | | + | + | | | | + | + | | + | | | + | |
| D4-7 | + | | | + | | + | | | + | | + | | | + | | | | + | + | | + | | | + | |
| D5-1 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| D5-1* | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| D5-2 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| D5-2* | | | + | + | | + | + | | | | + | + | | | | + | + | | | | + | + | | | |
| D5-4 | + | + | | | | + | + | | | | + | + | | | | | | + | + | | + | + | | | |
| D5-5 | + | + | | | | + | + | | | | | | + | + | | + | + | | | | + | + | | | |

| Organism | Inositol, Aer. Acid | Inositol, Anaer. Acid | Inositol, Aer. Alk. | Inositol, Anaer. Alk. | Inositol, Gas | Methyl Red Reaction | Voges-Proskauer Test | Oxidase (Kovacs) | Starch Hydrolysis | Gelatin Hydrolysis, ≤8 day | Gelatin Hydrolysis, ≥9 day | Indole | Lysine Decarboxylase | Ornithine Decarboxylase | Arginine Decarboxylase | Ammonification | Nitrate Reduction → $NO_2$ | Nitrate Reduction → Gas | Catalase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D2-6 | | | + | + | | + | | + | + | + | | + | | + | + | + | + | | + |
| D2-7 | + | | | + | | | | + | + | + | | | + | + | + | + | + | | + |
| D2-711 | + | + | | | | | | + | + | + | | + | + | + | | + | + | | + |
| D2-712 | | | + | + | | | | + | + | | + | + | + | + | | + | + | | + |
| D4-1 | | | + | + | | | | + | + | | | | + | + | | + | + | | + |
| D4-4 | + | + | | | | | + | + | + | + | | + | + | + | | + | + | | + |
| D4-4* | | | + | + | | | | + | + | | + | | + | + | | + | + | | + |
| D4-6 | | | + | + | | | | + | + | | + | | + | + | | + | + | | + |
| D4-7 | | | + | + | | | | + | + | | + | | + | + | | + | + | | + |
| D5-1 | + | + | | | | | + | + | + | + | | + | + | + | | + | + | | + |
| D5-1* | + | + | | | | + | | + | + | + | | + | + | + | | + | + | | + |
| D5-2 | + | + | | | | | + | + | + | + | | + | + | + | | + | + | | + |
| D5-2* | + | + | | | | | | + | + | + | | | + | + | | + | + | | + |
| D5-4 | | | + | + | | | | + | | | | | + | + | | + | + | | + |
| D5-5 | | | + | + | | + | | + | | + | | + | + | + | + | + | + | | + |

| Organism | Medium a | Medium b | Medium c | Medium d | Medium e | Medium A1 | Medium A2 | Medium A3 | Medium A4 | Medium A5 | Medium B1 | Medium B2 | Medium B3 | Medium C1 | Medium C2 | Medium C3 | Medium C4 | Medium D1 | Medium D2 | Medium D4 | Medium D5 | Medium D7 | Medium D8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D2-6 | | | + | | + | | | | | | + | + | | | | | | + | | + | + | | |
| D2-7 | + | | + | + | + | | | | | | + | + | | | + | | + | + | + | + | | + | + |
| D2-711 | | | + | | + | | | | | | | + | | | | | | + | + | + | + | | |
| D2-712 | | | + | | + | | | | | | | + | | | | | | | | | | | |
| D4-1 | | | + | | + | | | | | | | | | | | | | | | | | | |
| D4-4 | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| D4-4* | | | + | | + | | | | | | | | | | | | | | | | | | |
| D4-6 | | | + | | + | | | | | | + | + | | + | | | | + | + | + | + | + | |
| D4-7 | | | + | | + | | | | | | | | | | | | | + | | | + | | |
| D5-1 | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| D5-1* | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| D5-2 | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| D5-2* | | | + | | + | | | | | | + | + | | | | | | + | | + | + | | |
| D5-4 | | | + | | + | | | | | | | + | | | | | | + | | + | + | | |
| D5-5 | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |

| Organism | Shape | | | Ends | | Length (μm) | | | | L:W | | | Diameter (μm) | | Occurrence | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Straight Rod | Curved Rod | Coccus | Tapered | Square | ≤ 0.5 | 0.6 - 1.2 | 1.3 - 3.0 | ≥3.0 | 3 : 1 | 2 -3:1 | 1 : 1 | ≤ 0.5 | ≥ 0.6 | Singles | Pairs | Short Chains | Long Chains | Gram | Motility |
| D5-5* | + | | | | + | | | + | | + | | | | | + | | | | - | + |
| D5-8 | + | | | | + | | | + | | + | | | | | + | | + | | - | + |
| D5-711 | + | | | | + | | | + | | + | | | | | + | | | | - | + |
| D7-1 | + | | | | + | | | + | | + | | | | | + | | + | | - | + |
| D7-1* | + | | | | + | | | | + | + | | | | | + | | + | | - | + |
| D7-2 | + | | | | + | | | + | | | + | | | | + | | | | - | + |
| D7-3 | + | | | | + | | | + | | + | | | | | + | | | | - | + |
| D7-6 | + | | | | + | | | + | | + | | | | | + | | | | - | + |
| D7-6* | + | | | | + | | | | + | + | | | | | + | | + | | - | + |
| D7-7 | + | | | | + | | | | + | + | | | | | + | | + | | - | + |
| D7-7* | + | | | | + | | | + | | + | | | | | + | | + | | - | + |
| D7-7' | + | | | | + | | | + | | + | | | | | + | | + | | - | + |
| D7-711 | + | | | | + | | | + | | | + | | | | + | | | | - | + |
| D7-Ps | + | | | | + | | | + | | | + | | | | + | | | | - | + |
| D8-1 | + | | | | + | | | + | | | | + | | | + | + | | | - | + |

| Organism | Glucose, Aer. Acid | Glucose, Anaer. Acid | Glucose, Aer. Alk. | Glucose, Anaer. Alk. | Glucose, Gas | Lactose, Aer. Acid | Lactose, Anaer. Acid | Lactose, Aer. Alk. | Lactose, Anaer. Alk. | Lactose, Gas | Sucrose, Aer. Acid | Sucrose, Anaer. Acid | Sucrose, Aer. Alk. | Sucrose, Anaer. Alk. | Sucrose, Gas | Mannitol, Aer. Acid | Mannitol, Anaer. Acid | Mannitol, Aer. Alk. | Mannitol, Anaer. Alk. | Mannitol, Gas | Mannose, Aer. Acid | Mannose, Anaer. Acid | Mannose, Aer. Alk. | Mannose, Anaer. Alk. | Mannose, Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D5-5* | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| D5-8 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | | | |
| D5-711 | + | | | + | | + | | | + | | + | | | + | | | | + | + | | + | + | | | |
| D7-1 | + | + | | | | + | + | | | | + | + | | | | | | + | + | | + | | | + | |
| D7-1* | | | + | + | | | | + | + | | + | + | | | | | | + | + | | + | + | | | |
| D7-2 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | + | + | | | |
| D7-3 | + | | | + | | | | + | + | | | | + | + | | | | + | + | | + | | | + | |
| D7-6 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| D7-6* | + | + | | | | | | + | + | | + | | + | + | | + | + | | | | + | + | | | |
| D7-7 | + | + | | | | | | + | + | | + | | | + | | + | + | | | | + | + | | | |
| D7-7* | + | + | | | | | | + | + | | + | + | | + | | | | + | + | | | | + | + | |
| D7-7' | + | + | | | | | | + | + | | + | + | | | | + | + | | | | + | + | | | |
| D7-711 | | | + | + | | | | + | + | | | | + | + | | + | + | | | | + | + | | | |
| D7-Ps | + | | | + | | | | + | + | | | | + | + | | + | + | | | | + | + | | | |
| | | | | | | | | | | | | | | | | | | + | + | | + | | | + | |
| 08-1 | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | | | + | |

| ganism | Galactose, Aer. Acid | Galactose, Anaer. Acid | Galactose, Aer. Alk. | Galactose, Anaer. Alk. | Galactose, Gas | Maltose, Aer. Acid | Maltose, Anaer. Acid | Maltose, Aer. Alk. | Maltose, Anaer. Alk. | Maltose, Gas | Arabinose, Aer. Acid | Arabinose, Anaer. Acid | Arabinose, Aer. Alk. | Arabinose, Anaer. Alk. | Arabinose, Gas | Sorbitol, Aer. Acid | Sorbitol, Anaer. Acid | Sorbitol, Aer. Alk. | Sorbitol, Anaer. Alk. | Sorbitol, Gas | Xylose, Aer. Acid | Xylose, Anaer. Acid | Xylose, Aer. Alk. | Xylose, Anaer. Alk. | Xylose, Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D5-5* | + | | | + | | + | | | + | | | | + | + | | | | + | + | | + | | | + | |
| D5-8 | + | + | | | | + | + | | | | | | + | + | | + | + | | | | + | + | | | |
| D5-711 | + | | | + | | + | | | + | | | | + | + | | | | + | + | | + | | | + | |
| D7-1 | + | + | | | | | | + | + | | + | + | | | | | | + | + | | | | + | + | |
| D7-1* | | | + | + | | | | + | + | | + | + | | | | | | + | + | | + | + | | | |
| D7-2 | + | | | + | | + | | | + | | | | + | + | | | | + | + | | | | + | + | |
| D7-3 | | | + | + | | | | + | + | | | | + | + | | | | + | + | | | | + | + | |
| D7-6 | + | + | | | | + | + | | | | + | + | | | | | | + | + | | + | + | | | |
| D7-6* | + | + | | | | + | + | | | | + | + | | | | | | + | + | | | | + | + | |
| D7-7 | | | + | + | | + | + | | | | | | + | + | | | | + | + | | | | + | + | |
| D7-7* | + | + | | | | + | + | | | | + | + | | | | | | + | + | | | | + | + | |
| D7-7' | + | + | | | | + | + | | | | | | + | + | | | | + | + | | + | + | | | |
| D7-711 | + | + | | | | + | + | | | | | | + | + | | | | + | + | | + | + | | | |
| D7-Ps | | | + | + | | + | | | + | | | | + | + | | | | + | + | | | | + | + | |
| D8-1 | + | | | + | | + | | | + | | + | + | | | | + | + | | | | + | + | | | |

| Organism | Inositol, Aer. Acid | Inositol, Anaer. Acid | Inositol, Aer. Alk. | Inositol, Anaer. Alk. | Inositol, Gas | Methyl Red Reaction | Voges-Proskauer Test | Oxidase (Kovacs) | Starch Hydrolysis | Gelatin Hydrolysis, ≤8 day | Gelatin Hydrolysis, ≥9 day | Indole | Lysine Decarboxylase | Ornithine Decarboxylase | Arginine Decarboxylase | Ammonification | Nitrate Reduction → $NO_2$ | Nitrate Reduction → Gas | Catalase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D5-5* | | | + | + | | | | + | + | + | | | + | + | | + | + | | + |
| D5-8 | | | + | + | | | | + | | | + | | + | + | | + | + | | + |
| D5-711 | | | + | + | | | | + | + | | + | | + | + | | + | + | | + |
| D7-1 | | | + | + | | + | | | + | + | | | + | + | | + | + | | + |
| D7-1* | | | + | + | | + | | | + | | + | | + | + | | + | + | | + |
| D7-2 | | | + | + | | | | + | + | | + | | + | | + | + | + | | + |
| D7-3 | | | + | + | | | | + | + | | | | + | + | | + | + | | + |
| D7-6 | | | + | + | | | | + | + | + | | | + | | | + | + | | + |
| D7-6* | | | + | + | | | | + | + | + | | | + | + | | + | + | | + |
| D7-7 | | | + | + | | + | | | + | | + | + | | | | + | + | | + |
| D7-7* | | | + | + | | | + | + | + | + | | + | + | + | | + | + | | + |
| D7-7' | | | + | + | | | + | + | + | + | | + | + | + | | + | + | | + |
| D7-711 | | | + | + | | | | + | | | | | + | + | | + | + | + | + |
| D7-Ps | | | + | + | | | | + | + | | + | | + | + | | + | + | | + |
| D8-1 | + | + | | | | + | | | | | | | | | | + | + | | + |

| Organism | Medium a | Medium b | Medium c | Medium d | Medium e | Medium A1 | Medium A2 | Medium A3 | Medium A4 | Medium A5 | Medium B1 | Medium B2 | Medium B3 | Medium C1 | Medium C2 | Medium C3 | Medium C4 | Medium D1 | Medium D2 | Medium D4 | Medium D5 | Medium D7 | Medium D8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D5-5* | + | | + | + | + | + | + | + | + | + | + | + | | | | | | + | + | + | + | + | + |
| D5-8 | | | + | | + | | | | | | | + | | + | | | + | + | | | | | |
| D5-711 | | | + | | + | | | | | | + | + | | + | + | + | + | + | | + | + | | + |
| D7-1 | | | + | | + | | | | | | | + | | | | | | | | | | | |
| D7-1* | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| D7-2 | | | + | | + | | | + | | | + | + | | + | + | + | + | + | + | + | + | | + |
| D7-3 | | | + | | | | | | | | | + | | | | | | | | | + | | |
| D7-6 | + | + | + | + | + | | + | + | | | + | + | | + | + | + | + | + | | + | + | + | + |
| D7-6* | | | + | | + | | | | | | + | + | | | | | | + | | | + | | |
| D7-7 | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| D7-7* | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| D7-7' | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| D7-711 | | | | | + | | | | | | + | + | | | | | | + | | | + | | |
| D7-Ps | | | + | | + | | | | | | + | + | | | | | | | | | | | |
| D8-1 | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |

| Organism | Shape | | | Ends | | Length (μm) | | | | L:W | | | Diameter (μm) | | Occurrence | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Straight Rod | Curved Rod | Coccus | Tapered | Square | ≤ 0.5 | 0.6 - 1.2 | 1.3 - 3.0 | ≥ 3.0 | 3 : 1 | 2 -3:1 | 1 : 1 | ≤ 0.5 | ≥ 0.6 | Singles | Pairs | Short Chains | Long Chains | Gram | Motility |
| D8-2 | + | | | | + | | | + | | + | | | | | + | | + | | - | |
| D8-2* | | + | | | + | | | + | | + | | | | | + | | | | - | + |
| D8-3 | + | | | | + | | | + | | + | | | | | + | | + | | - | + |
| D8-6 | + | | | | + | | | + | | | + | | | | + | | + | | - | + |
| D8-7 | + | | | | + | | | + | | + | | | | | + | | + | | - | + |
| D8-711* | + | | | | + | | | + | | + | | | | | + | | | | - | + |
| D8-712 | + | | | | + | | | + | | + | | | | | + | | + | | - | + |

| ganism | D8-2 | D8-2* | D8-3 | D8-6 | D8-7 | D8-711* | D8-712 |
|---|---|---|---|---|---|---|---|
| Glucose, Aer. Acid | + | + | + | + | | + | + |
| Glucose, Anaer. Acid | + | + | + | + | | + | + |
| Glucose, Aer. Alk. | | | | | + | | |
| Glucose, Anaer. Alk. | | | | | + | | |
| Glucose, Gas | | | | | | | |
| Lactose, Aer. Acid | + | | + | | | + | |
| Lactose, Anaer. Acid | + | | + | | | + | |
| Lactose, Aer. Alk. | | + | | + | + | | + |
| Lactose, Anaer. Alk. | | + | | + | + | | + |
| Lactose, Gas | | | | | | | |
| Sucrose, Aer. Acid | + | + | | | | + | + |
| Sucrose, Anaer. Acid | + | + | | | | + | + |
| Sucrose, Aer. Alk. | | | + | + | + | | |
| Sucrose, Anaer. Alk. | | | + | + | + | | |
| Sucrose, Gas | | | | | | | |
| Mannitol, Aer. Acid | + | | | | + | | + |
| Mannitol, Anaer. Acid | + | | | | + | | + |
| Mannitol, Aer. Alk. | | + | + | + | | + | |
| Mannitol, Anaer. Alk. | | + | + | + | | + | |
| Mannitol, Gas | | | | | | | |
| Mannose, Aer. Acid | + | + | + | + | + | + | + |
| Mannose, Anaer. Acid | | + | | | + | | + |
| Mannose, Aer. Alk. | | | | | | | |
| Mannose, Anaer. Alk. | + | | + | + | | + | |
| Mannose, Gas | | | | | | | |

| rganism | D8-2 | D8-2* | D8-3 | D8-6 | D8-7 | D8-711* | D8-712 |
|---|---|---|---|---|---|---|---|
| Galactose, Aer. Acid | + | | + | + | + | + | + |
| Galactose, Anaer. Acid | | | | | | | + |
| Galactose, Aer. Alk. | | + | | | | | |
| Galactose, Anaer. Alk. | + | + | + | + | + | + | |
| Galactose, Gas | | | | | | | |
| Maltose, Aer. Acid | + | + | | | + | | + |
| Maltose, Anaer. Acid | | + | | | | | + |
| Maltose, Aer. Alk. | | | + | + | | + | |
| Maltose, Anaer. Alk. | + | | + | + | + | + | |
| Maltose, Gas | | | | | | | |
| Arabinose, Aer. Acid | + | + | + | + | + | + | + |
| Arabinose, Anaer. Acid | + | + | + | + | + | + | + |
| Arabinose, Aer. Alk. | | | | | | | |
| Arabinose, Anaer. Alk. | | | | | | | |
| Arabinose, Gas | | | | | | | |
| Sorbitol, Aer. Acid | + | + | | | + | | |
| Sorbitol, Anaer. Acid | + | + | | | + | | |
| Sorbitol, Aer. Alk. | | | + | + | | + | + |
| Sorbitol, Anaer. Alk. | | | + | + | | + | + |
| Sorbitol, Gas | | | | | | | |
| Xylose, Aer. Acid | + | | + | + | | + | + |
| Xylose, Anaer. Acid | + | | + | + | | + | + |
| Xylose, Aer. Alk. | | + | | | + | | |
| Xylose, Anaer. Alk. | | + | | | + | | |
| Xylose, Gas | | | | | | | |

| Organism | Inositol, Aer. Acid | Inositol, Anaer. Acid | Inositol, Aer. Alk. | Inositol, Anaer. Alk. | Inositol, Gas | Methyl Red Reaction | Voges-Proskauer Test | Oxidase (Kovacs) | Starch Hydrolysis | Gelatin Hydrolysis, ≤8 days | Gelatin Hydrolysis, ≥9 days | Indole | Lysine Decarboxylase | Ornithine Decarboxylase | Arginine Decarboxylase | Ammonification | Nitrate Reduction → $NO_2$ | Nitrate Reduction → Gas | Catalase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D8-2 | | | + | + | | | + | | + | | + | | | | + | + | + | | + |
| D8-2* | | | + | + | | + | | + | + | + | | | | | + | + | + | | + |
| D8-3 | | | + | + | | | | + | | | | | | | + | + | + | | + |
| D8-6 | | | + | + | | | | + | | | | | | + | + | + | + | | + |
| D8-7 | + | + | | | | | | + | | | | | + | | + | + | + | | + |
| D8-711* | | | + | + | | | | + | + | + | | | + | | | + | + | | + |
| D8-712 | | | + | + | | | | + | + | + | | + | + | + | | + | + | | + |

| Organism | Medium a | Medium b | Medium c | Medium d | Medium e | Medium A1 | Medium A2 | Medium A3 | Medium A4 | Medium A5 | Medium B1 | Medium B2 | Medium B3 | Medium C1 | Medium C2 | Medium C3 | Medium C4 | Medium D1 | Medium D2 | Medium D4 | Medium D5 | Medium D7 | Medium D8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D8-2 | | + | | + | + | + | + | + | + | + | | + | + | + | + | + | + | + | + | + | + | + | + |
| D8-2* | | | + | | + | | | | | | | + | | | | | | | + | | | | |
| D8-3 | | | + | | + | | | | | | + | + | | | | | | + | + | | | | |
| D8-6 | | | + | | + | | | | | | + | + | | | | | | + | + | | | | |
| D8-7 | | | + | | + | | | | | | + | + | | | | | | + | + | + | + | | |
| D8-711* | | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| D8-712 | | | + | | + | | | | | | | + | | | | | | + | + | + | + | | |

TABLE 9

Synopsis of data obtained thus far on the characteristics of bacteria isolated from epiphytic communities living on *Enteromorpha intestinalis*.

Total number of isolates: 128

| Characteristic (s) | # of organisms | % of organisms tested |
|---|---|---|
| rod | 126 | 98 |
| coccus | 2 | 2 |
| gram negative | 127 | 99 |
| gram positive | 1 | 1 |
| motile | 111 | 87 |
| non-motile | 17 | 13 |
| gram negative, motile rod | 111 | 87 |
| gram negative, non-motile rod | 14 | 11 |
| gram negative, non-notile coccus | 2 | 2 |
| ferment glucose | 67 | 54 |
| oxidize glucose | 27 | 22 |
| (-) glucose | 29 | 24 |
| ferment sucrose | 45 | 42 |
| oxidize sucrose | 24 | 23 |
| (-) sucrose | 37 | 35 |
| ferment lactose | 21 | 20 |
| oxidize lactose | 17 | 17 |
| (-) lactose | 65 | 63 |
| ferment mannose | 27 | |
| oxidize mannose | 17 | |
| (-) mannose | 15 | |
| ferment galactose | 16 | 35 |
| oxidize galactose | 6 | 13 |
| (-) galactose | 24 | 52 |
| ferment mannitol | 30 | 39 |
| oxidize mannitol | 16 | 20 |
| (-) mannitol | 33 | 41 |
| nitrate reduction to $NO_2$ | 80 | 93 |
| nitrate reduction to $NH_4$ | 86 | 100 |
| nitrate reduction to gas | 2 | 2 |

TABLE 10

Synopsis of characteristics of isolates from individual media.

| | GLUCOSE | | | | SUCROSE | | | | LACTOSE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Medium | # tested | # fermenting | # oxidizing | # negative | # tested | # fermenting | # oxidizing | # negative | # tested | # fermenting | # oxidizing | # negative |
| a | 5 | 1 | 0 | 4 | 5 | 1 | 3 | 1 | 5 | 0 | 0 | 5 |
| c | 9 | 3 | 0 | 6 | 9 | 3 | 2 | 4 | 8 | 0 | 1 | 7 |
| d | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| e | 4 | 4 | 0 | 0 | 4 | 4 | 0 | 0 | 4 | 0 | 0 | 4 |
| A 1 | 5 | 2 | 0 | 3 | 5 | 2 | 0 | 3 | 5 | 0 | 0 | 5 |
| A 2 | 6 | 2 | 2 | 2 | 6 | 0 | 0 | 6 | 6 | 0 | 0 | 6 |
| A 3 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| A 5 | 9 | 5 | 1 | 3 | 8 | 4 | 0 | 4 | 8 | 0 | 2 | 6 |
| B 1 | 3 | 3 | 0 | 0 | 2 | 2 | 0 | 0 | 2 | 0 | 0 | 2 |
| B 2 | 3 | 3 | 0 | 0 | 3 | 3 | 0 | 0 | 3 | 1 | 1 | 1 |
| B 3 | 4 | 4 | 0 | 0 | 5 | 0 | 4 | 1 | 5 | 0 | 1 | 4 |
| C 1 | 10 | 7 | 2 | 1 | 8 | 4 | 3 | 1 | 6 | 4 | 0 | 2 |
| C 2 | 3 | 0 | 2 | 1 | 3 | 0 | 2 | 1 | | | | |
| C 3 | 9 | 3 | 3 | 3 | 10 | 3 | 1 | 6 | | | | |
| D 1 | 8 | 3 | 4 | 1 | 8 | 1 | 5 | 2 | 8 | 1 | 5 | 2 |
| D 2 | 10 | 6 | 4 | 0 | | | | | 10 | 2 | 2 | 6 |
| D 4 | 5 | 1 | 4 | 0 | | | | | 5 | 0 | 3 | 2 |
| D 5 | 9 | 7 | 1 | 1 | 9 | 9 | 0 | 0 | 9 | 3 | 2 | 4 |
| D 7 | 11 | 6 | 3 | 2 | 10 | 4 | 4 | 2 | 10 | 2 | 2 | 6 |
| D 8 | 8 | 7 | 0 | 1 | 8 | 5 | 0 | 3 | 6 | 4 | 0 | 2 |

D. Mixed culture studies on the synergistic or antagonistic effects of algae and bacteria from natural associations including:

i) the effects of epiphytes on the growth and differentiation of Enteromorpha

ii) the effects of Enteromorpha on its epiphytes

iii) the isolation and identification of extra cellular, possibly community regulatory, substances released into the media.

1) Nitrogen budget of the aufwuchs community (Dissertation project of Ms Patricia Sullivan).

Preliminary experiments in the summer of 1973, using the acetylene reduction technique (Hardy et al. 1968), have suggested that $N_2$ fixation does occur among the epiphytes of Enteromorpha. Published data indicate that the blue green algae are the most important nitrogen fixers in the sea (Stewart, 1966; Brooks et al. 1971). Therefore, attempts are currently being made to isolate the blue-green epiphytes of Enteromorpha. The technique being followed is basically that of Van Baalen (1961) who succeeded in isolating a number of blue-greens occurring in coastal marine environments, many from the Long Island area. A small piece of Enteromorpha is transferred into an enrichment media (a modification of Provasoli's 1957, ASP-2 media) and incubated in a light bank with airation. Later, plates are inoculated with 0.1 ml, sealed and incubated in the light for up to 3 months. After growth, algal colonies were picked and transferred.

Temperature has been used as a selective factor in isolating blue-greens from water and soil (Allen and Stainer, 1967). Incubation at 35 C suppressed eukaryotic algae development. This technique was not successful with the salt marsh Enteromorpha epiphytes, due to the ability of these organisms to tolerate high temperatures (Saks and Lee 1973).

The addition of cyclohexamide (actidione) to media is now being used to inhibit eukaryotic algae (McCurdy and Hodgson 1973). Pure cultures of blue-greens are difficult to obtain because bacteria live in gelatinous sheaths. Vibration of algal samples with glass beads in a Bradson Sonifier followed by washing with sterile modified ASP-2 media and low pressure (2 1/2 $lbs/in^2$) filtration through 0.8 $\mu$ filters also have been tried in an attempt to obtain axenic cultures.

Field studies using the acetylene reduction technique have shown that nitrogen fixation occurs among the epiphytes of Enteromorpha. The nitrogen fixing members of the Enteromorpha epiphytic community, 10 bacteria and 4 blue green algae, have been isolated in laboratory culture. The bacteria and a number of the blue green algal species are geing maintained axenically. These organisms are being studied in order to determine the physiological factors limiting nitrogen fixation. Experiments have shown that temperature and salinity are important parameters influencing the amount of nitrogen that can be fixed by bacteria (see figs. 84 and 85). Preliminary studies indicate that blue green algae fix nitrogen at a comparatively much lower rate ($10^1$ vs $10^{-4}$ g at N/g. Thus it would appear that bacteria play the major role in nitrogen fixation in this community.

2. Enteromorpha culture

Cultures of Enteromorpha intestinalis presently being maintained have been clones from one culture which demonstrated good growth, slight differentiation and zoospore production. Attempts at removing the bacterial contaminant by washing, dragging and using antibiotics have been unsuccessful. Cultures which may appear to be bacteria-free within 103 weeks of transfer (i.e., no visible turbidity in the liquid overlay or around the germlings) have been found to contain a fine film of bacteria on the solid substrate. In cultures where this film is extensive, the transferred germlings, as well as those resulting from zoospore production seem to be able to successfully attach to the substrate and survive but do not differentiate. In cultures where attachment does not occur, the algae become surrounded by a slight cloud of

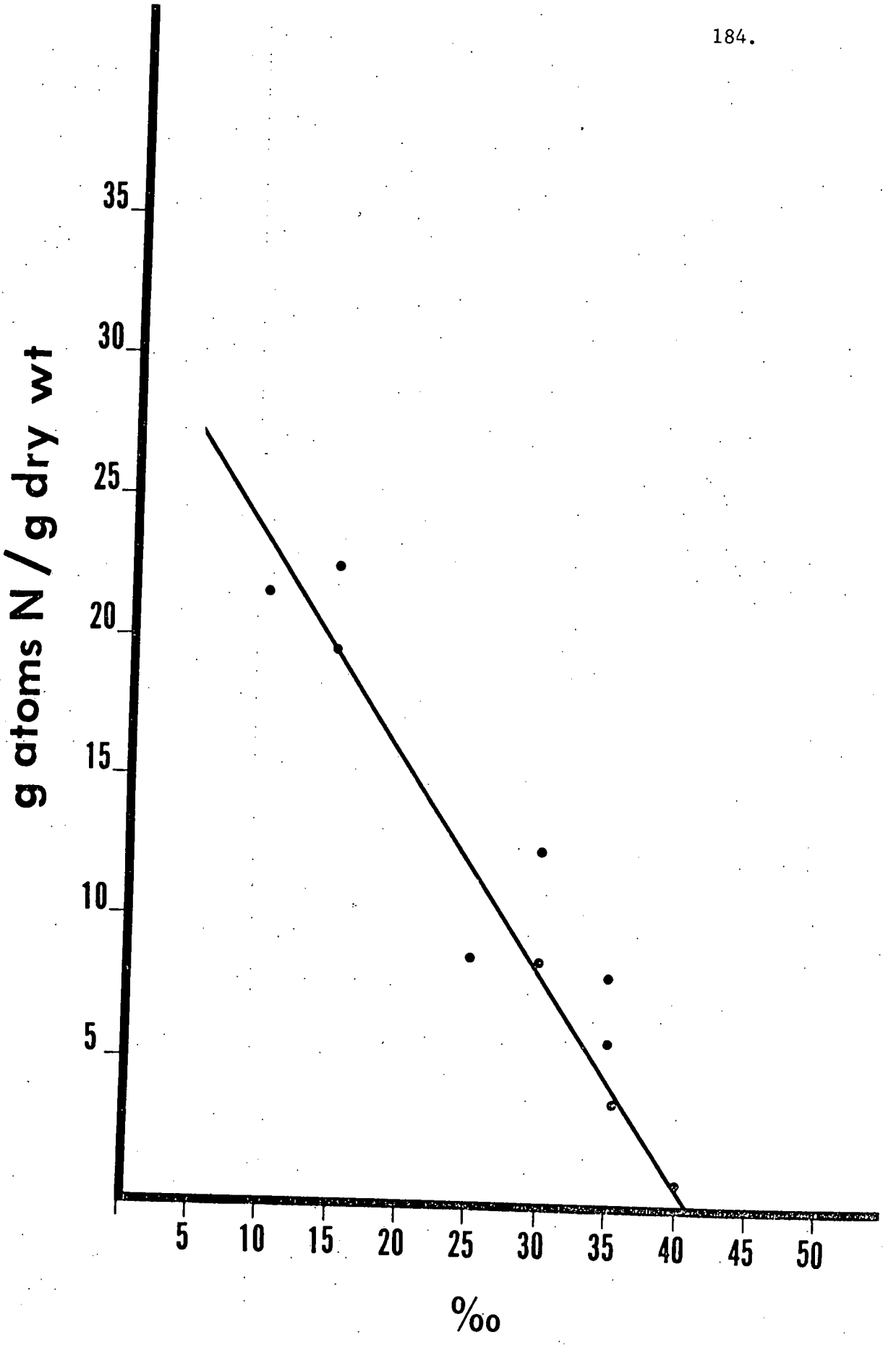



Fig. 84

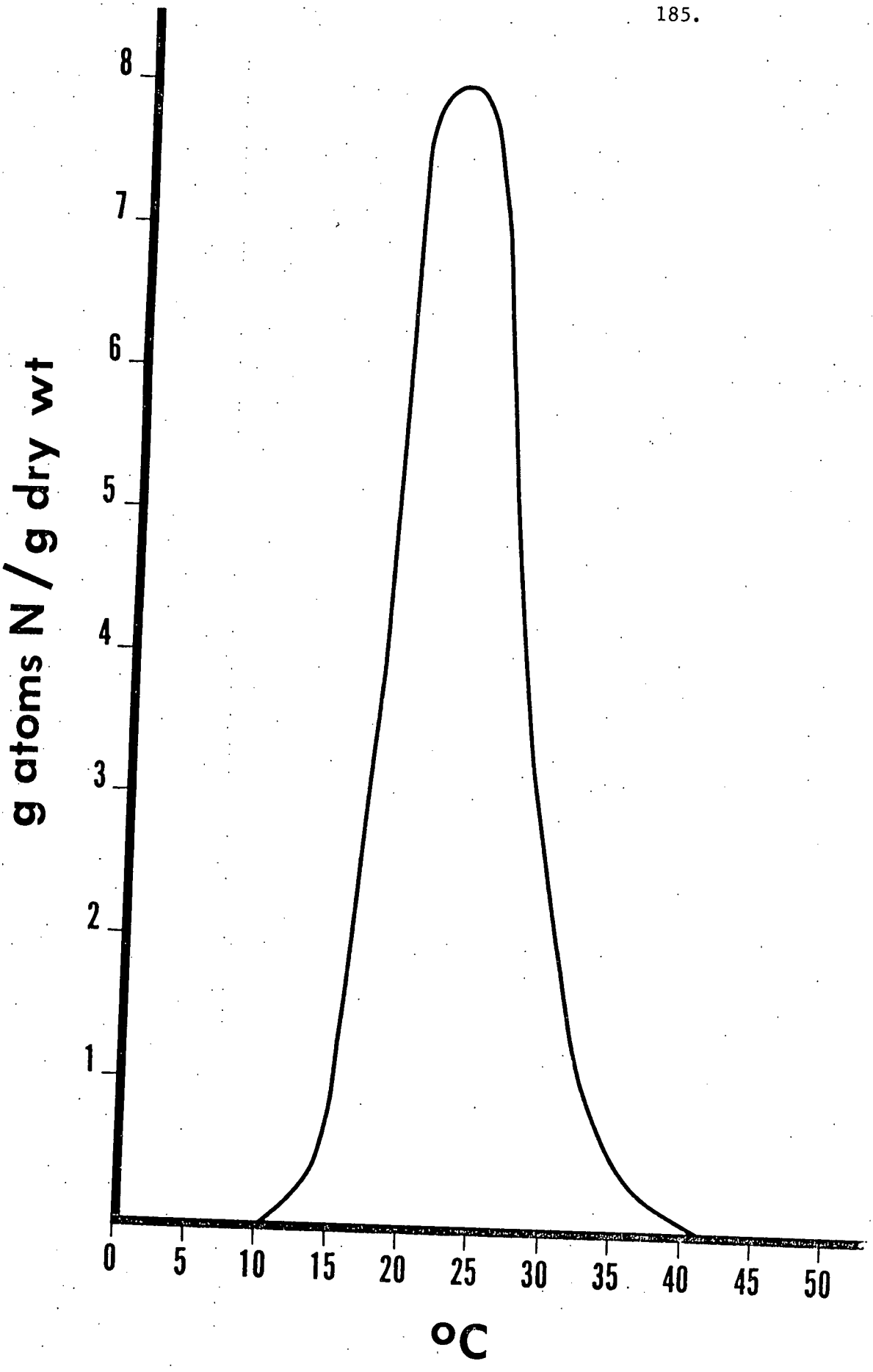



Fig. 85.

bacteria, bleach and die.

Routine maintenance of Enteromorpha has been bothered by an increasing proportion of unsuccessful transfers. Within the last few months, various changes have been introduced into the culturing program. Stock medium S is being used in place of Base B for the Enteromorpha medium and 150 ml Ehrlenmeyer flasks with some solid substrate and 25 ml liquid overlay have replaced tubes in a portion of the transfers. Both changes have been successful. We have obtained hundreds of germlings from the flask cultures alone.

Experiments were designed to determine any changes in the morphological and/or reproductive characteristics of Enteromorpha when it is grown parabiotically with each of the 128 bacterial strains.

The initial or pilot experiments were set up using specially designed long, flat, rubber-stoppered glass tissue culture tubes (Bellco Glass Co.). Slants consisting of 3 ml of IMS agar (Isolation Solution Medium S, without vitamins, Lee et al. 1970), were overlain with 5 ml of liquid IMS. Cloned axenic germlings of Enteromorpha were washed twice in a sterile basal salt medium (BSM) (Table 11) and one or two of these were transferred aseptically into each of the experimental tubes. Each of the bacterial strains were grown overnight in 5 ml of liquid bacterial stock medium and washed twice and resuspended in 5 ml of the BSM. 0.1 ml aliquots of each strain were inoculated into duplicate experimental tubes containing the germlings. The mixtures were incubated in a 12 L;12 D 20° C environmental chamber (Sherer CEL 4-4). Observations were recorded once a week for six weeks.

Subsequent parabiotic experiments were identical to the pilot set-up with regard to media, inoculation procedures, and length of incubation. The only innovation was the use of experimental tubes containing only liquid media with no agar base. In each of the experiments, controls tubes with Enteromorpha alone were maintained.

Many of the bacteria are incapable of stimulating any morphological changes in the algal equivalent to or beyond the levels of control cultures (Table 12). Forty seven of the 128 bacteria (37%) have

no visible effect on the germling used as an inoculum. The feathery growth characteristic of the controls was attained in 42 (~ 33%) of the parabiotic mixtures. In the remaining 39 mixtures, some elongation of the thalli of the germling occured. For the majority of these (31), elongation was temporary or transitory with algal growth ultimately reverting to mold-like colonies. Normal tubular growth of the thalli was maintained throughout the duration of the experiment in only 8 of the mixtures.

Reproduction of the macroalga did not take place in the control cultures but did occur in 38 (30%) of the parabiotic mixtures. New germlings appeared after varying periods of incubation and most of these did not undergo thallal elongation. In only 10 instances, morphological changes in the newly produced germlings were observed.

The growth of many of the bacteria appears to be influenced by the alga. Turbidity was markedly increased in most of the slant mixtures although such changes were not observed in liquid cultures or in control cultures of bacteria alone.

TABLE 11

Basal Salts Medium

| | |
|---|---|
| $Na_2SiO_3 \cdot 9H_2O$ | 7.00 mg % |
| NaCl | 2.50 % |
| $MgSO_4 \cdot 7\ H_2O$ | 0.90 % |
| KCl | 0.07 % |
| Tris | 0.10 % |
| NTA | 7.00 mg % |
| $NaHCO_3$ | 10.00 mg % |
| P II metals | 3.0 ml/100 ml |

TABLE 12

The effects of bacteria on the growth, differentiation and reproduction of Enteromorpha.

| | |
|---|---|
| Group I: | Organisms having no effect on Enteromorpha |
| Group II: | Organisms producing feathery growth of Enteromorpha but without effect on its reproduction |
| Group III: | Organisms producing abnormal, mold-like colonies of Enteromorpha but without effect on its reproduction |
| Group IV: | Organisms producing normal, elongated growth of Enteromorpha, but without effect on its reproduction |
| Group V: | Organisms having no effect on the growth of Enteromorpha but with which zoospore production alone takes place |
| Group VI: | Organisms having no effect on the growth of Enteromorpha but with which zoospore production |
| Group VII: | Organisms producing feathery growth of Enteromorpha and with which unelongated zoospores are produced. |
| Group VIII: | Organisms producing abnormal, mold-like colonies of Enteromorpha and with which unelongated zoospores are produced. |
| Group IX: | Organisms producing normal, elongated growth of Enteromorpha and with which unelongated zoospores are produced. |
| Group X: | Organisms producing feathery growth of Enteromorpha and with which zoospores and new elongated germling are produced. |
| Group XI: | Organisms producing abnormal, mold-like colonies of Enteromorpha and with which zoospores and new elongated germlings are produced. |
| Group XII: | Organisms producing normal, elongated growth of Enteromorpha and with which zoospores and new elongated germlings are produced. |

## GROUP I

| | | | |
|---|---|---|---|
| C702 | C3-711 | D2-707 | D5-705* |
| C704 | D1-701 | D2-711 | D5-708 |
| C708 | D1-704 | D2-712 | D5-711 |
| A5-704 | D1-706 | D4-701 | D7-701* |
| A5-705 | D1-707 | D4-704 | D7-702 |
| A5-705* | D1-707* | D4-707 | D7-703 |
| B3-711 | D2-701 | D5-701* | D7-707 |
| C1-705 | D2-702 | D5-702 | D7-707* |
| C1-708 | D2-703 | D5-702* | D8-703 |
| C3-705 | D2-703* | D5-705 | D8-706 |

## GROUP II

| | | | | |
|---|---|---|---|---|
| A704 | A2-703 | B3-Ps | C3-701 | D2-706 |
| C706 | A2-714* | C1-702' | C3-702 | D4-704* |
| C709 | A5-701 | C1-703* | C3-704 | D7-706 |
| D702 | A5-701* | C1-704* | D1-701 | D7-707* |
| C705 | A5-703 | C1-7010 | D1-702 | D7-Ps |
| C707 | A5-706 | C2-702 | D1-703 | |
| A2-701 | B2-Rh | C2-704 | D1-705 | |

## GROUP III

| | | |
|---|---|---|
| C708 | C1-701 | D4-706 |
| A1-701 | C1-701* | D8-712 |
| A1-714* | C1-701* | |
| B2-704 | C3-712 | |
| B3-705 | | |

## GROUP IV

A3-701
B1-706
B2-702
D7-701

## GROUP V

C1-704
C3-704*
D1-707
D1-707*

## GROUP VI

A2-703'
D8-702'

## GROUP VII

| | |
|---|---|
| A707 | A5'-701 |
| C711 | B2-Rh* |
| E702 | B3-Rh |
| A2-704 | |

## GROUP VIII

| | | |
|---|---|---|
| A706 | B1-702 | D7-706* |
| A706* | B3-712 | D8-702 |
| A1-702 | C3-712 | D8-707 |
| A2-702 | D5-704 | |

## GROUP IX

D701
A1-712
A5-707
B1-707

## GROUP X

C715
A1-714

## GROUP XI

| | |
|---|---|
| A703 | D7-711 |
| B3-701 | D8-701 |
| C2-703 | D8-711 |
| C3-707* | |

## GROUP XII

C707

Ten bacterial strains, representing those with a range of activity regarding their effect on algal growth, were chosen for further study. Each of these strains was tested for its ability to take up a variety of organic substrates. Twenty four hour cultures were washed twice and resuspended in 5 ml sterile BSM. 0.1 ml aliquots were used to inoculate duplicate experimental tubes containing 5 ml IMS supplemented with each of the following substrates: 0.1 M, 10 mM, and 1 mM glucose; 0.1 m, 10 mM, and 1 mM mannitol; 0.1 m, 10 mM and 1 mM Na glycerophosphate; 0.1 m, 10 mM and 1 mM Na acetate; 0.1 m, 10 mM and 1 mM lactic acid; 0.1 m, 10 mM and 1 mM succinic acid; 10 mM and 1 mM glutamic acid. Controls consisted of IMS alone. The cultures were incubated at 20° C for 36 hrs. The optical density at 600 nM of each was determined with a Gilford Micro Sample Spectrophotometer. Results can be found in Table 13.

Tracer studies using $^{14}C$ mannitol as a substrate for the bacteria and $H^{14}CO_3$ for the Enteromorpha are planned to determine those substances which may be involved in any cross-feeding between the organisms.

TABLE 13

The effects of low molecular metabolites on the growth of bacteria isolated as epiphytes on Enteromorpha.

| SUBSTRATE | ORGANISM a3 | a4 | d 1 | e 7 | A1-712 | A5-1 | A5-7 | B1-2 | D1-1 | D2-5 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.1 m glucose | 0.26 | 0.07 | 0.19 | 0.17 | 0.40 | 0.30 | 0.27 | 0.29 | 0.22 | 0.26 |
| 10 mM glucose | 0.59 | 0.12 | 0.26 | 0.24 | 0.68 | 0.69 | 0.37 | 0.34 | 0.41 | 0.31 |
| 1 mM glucose | 0.23 | 0.23 | 0.18 | 0.27 | 0.36 | 0.31 | 0.36 | 0.28 | 0.29 | 0.27 |
| 0.1 m mannitol | 0.40 | 0.14 | 0.40 | 0.90 | 0.62 | 1.05 | 0.58 | 0.49 | 0.51 | 0.38 |
| 10 mM mannitol | 0.53 | 0.12 | 0.55 | 0.97 | 0.54 | 0.89 | 0.69 | 0.63 | 0.49 | 0.28 |
| 1 mM mannitol | 0.15 | 0.14 | 0.15 | 0.14 | 0.16 | 0.23 | 0.20 | 0.16 | 0.16 | 0.17 |
| 0.1 m Na gly $PO_4$ | 0.23 | 0.04 | 0.13 | 0.19 | 0.06 | 0.07 | 0.07 | 0.01 | 0.00 | 0.01 |
| 10 mM Na gly $PO_4$ | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1 mM Na gly $PO_4$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.1 m Na acetate | 0.58 | 0.53 | 0.04 | 0.84 | 0.29 | 0.81 | 0.46 | 0.44 | 0.38 | 0.54 |
| 10 mM Na acetate | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 | 0.00 | 0.00 |
| 1 mM Na acetate | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.1 m lactic acid | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 10 mM lactic acid | 0.06 | 0.00 | 0.01 | 0.00 | 0.00 | 0.05 | 0.01 | 0.01 | 0.00 | 0.00 |
| 1 mM lactic acid | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.1 m succinic acid | 0.02 | 0.09 | 0.02 | 0.23 | 0.12 | 0.03 | 0.08 | 0.01 | 0.03 | 0.27 |
| 10 mM succinic acid | 0.02 | 0.09 | 0.02 | 0.25 | 0.11 | 0.02 | 0.09 | 0.03 | 0.02 | 0.34 |
| 1 mM succinic acid | 0.02 | 0.10 | 0.02 | 0.09 | 0.09 | 0.02 | 0.09 | 0.03 | 0.02 | 0.18 |
| 10 mM glutamic acid | 0.01 | 0.00 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.03 | 0.04 |
| 1 mM glutamic acid | 0.07 | 0.07 | 0.05 | 0.05 | 0.11 | 0.08 | 0.09 | 0.02 | 0.04 | 0.04 |

Some of our efforts have been directed toward in situ studies of the effects of the Millstone Power Plant effluents on the organisms and the functioning of decomposition communities and the lower steps of the detrital food web. It is alleged by some plant critics that the pile-up and rafting of undecomposed or slowly decomposing sea grasses in the bay adjacent to the effluent is due to effluent effects at low tide and under certain wind current conditions. By agreement with the plant management, their environmental consultants, and ourselves we have undertaken the study of this possible concern. The Millstone plant is located on Long Island Sound in close proximity to the mouth of the Niantic River in Connecticut. Because vandalism of untended experimental apparatus is a problem, a small cove, the Millstone Pools, near the cooling uptake system in Niantic Bay, was chosen for a control site (Fig. 86). There is restricted land access to it. The effluent from the plant is released into a former granite quarry (~ 1000 ft long and 125 ft deep). Flow rate through the quarry is ~ 4-5 ft/sec.

Several different types of experiments were incubated in the control and experimental sites. The in situ incubators (Fig. 87) employed in our previous studies in Jamaica Bay, N. Y., Greater Sippewissett Marsh, Mass, and Towd Point, N. Y. (Coccetti and Lee ms in this report) were also used in these studies. The transparent lucite apparatus holds 10 tissue culture flasks covered on one side with 1 μm nylon filters bonded to the plastic. The flasks retain most microorganisms and all meiofauna and allow free exchange of sea water between the vessels and the surrounding waters. Vessels contained natural decomposition communities from the experimental or control sites (Millstone Pool, Greater Sippewissett Marsh, and Towd Point). Community composition, rates of microbial growth (estimated by the ATP method of Holm-Hansen), nitrogen fixation, and rates of Spartina decomposition were studied in the Millstone Pool and in the quarry. The vessels were sampled biweekly.

Many problems, some beyond our control, have caused us difficulty in successfully completing this subproject. Our first problem was caused by larval Littorina littorea which settled on the apparatus in the effluent quarry, grew rapidly, and rasped holes in the nylon filters. We solved

this problem by redesigning the apparatus and protecting the filters with an outer layer of fiberglass screening. Hurricane Belle destroyed our apparatus last summer. Dismantling of the underwater naval research porject in the quarry and plant shut down have prevented us from obtaining well controlled and continuous observations. The results of various fragmentary experiments indicate that the rate of detrital decomposition is accelerated in the effluent quarry. On various dates it was more than twice the rate found in the Millstone Pool (significant at greater than the 98% confidence level). The microbial biomass of cultures incubated in the quarry was generally double those incubated in the Millstone Pool. The community structure studies are still in progress (Fig. 88).

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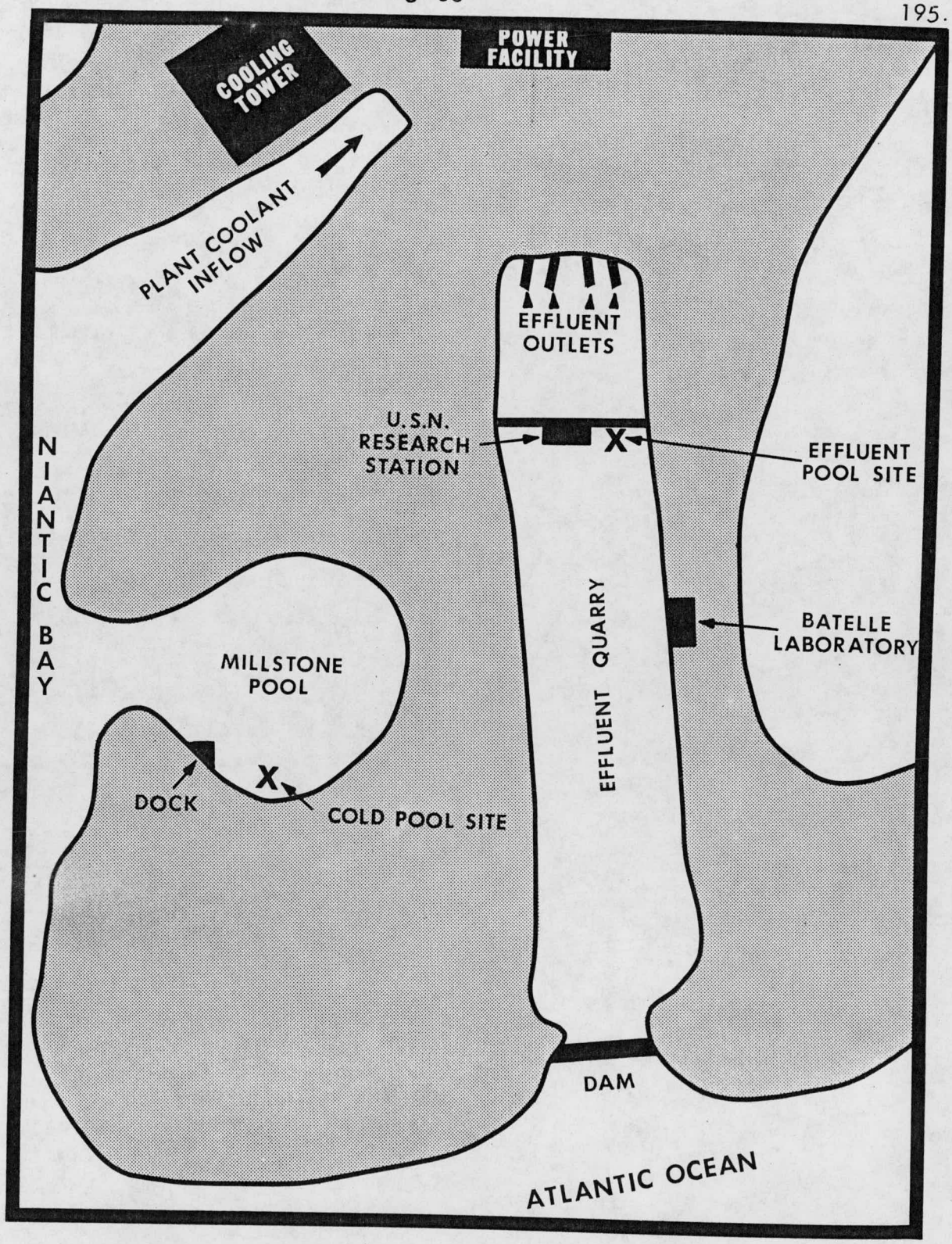
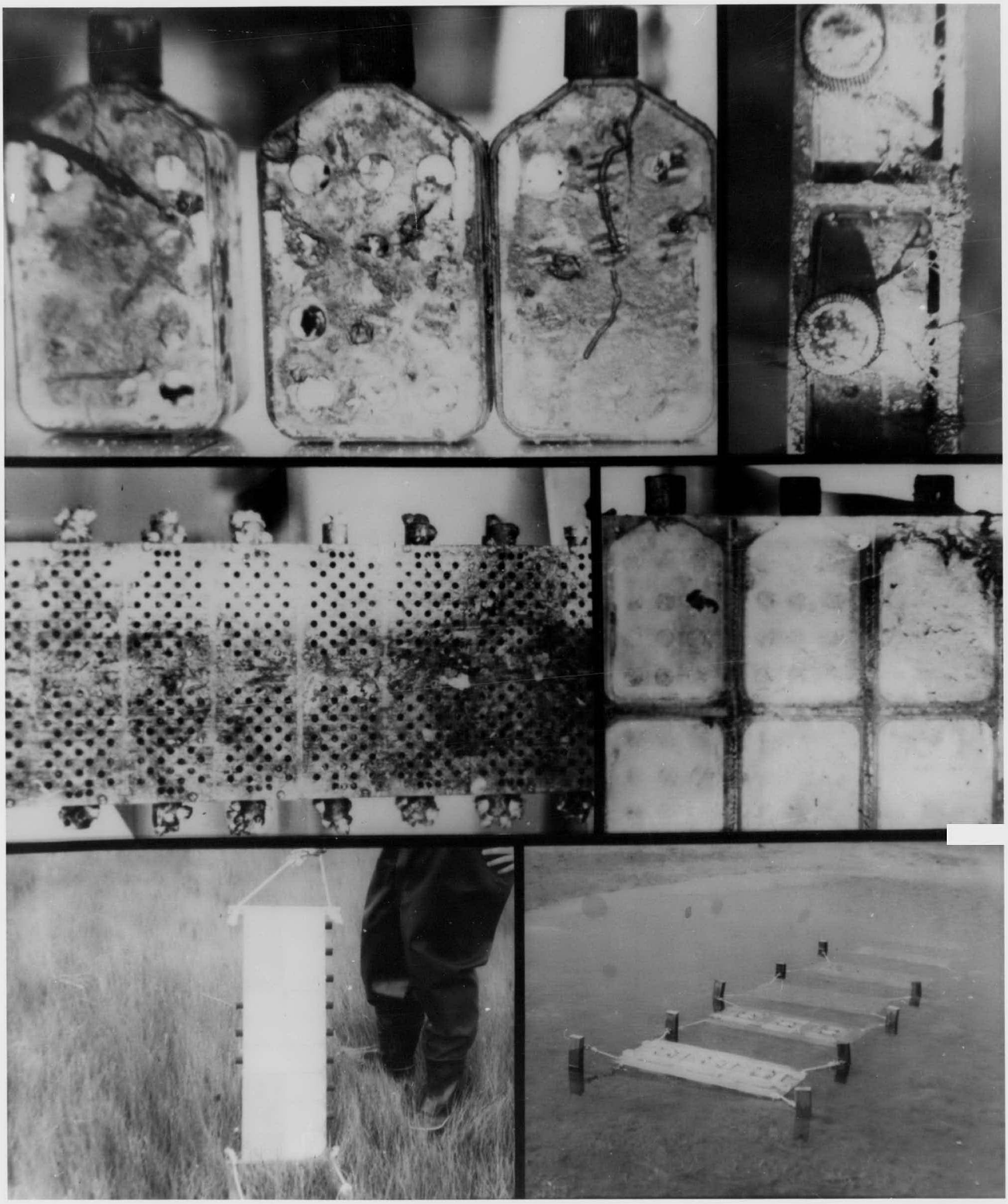
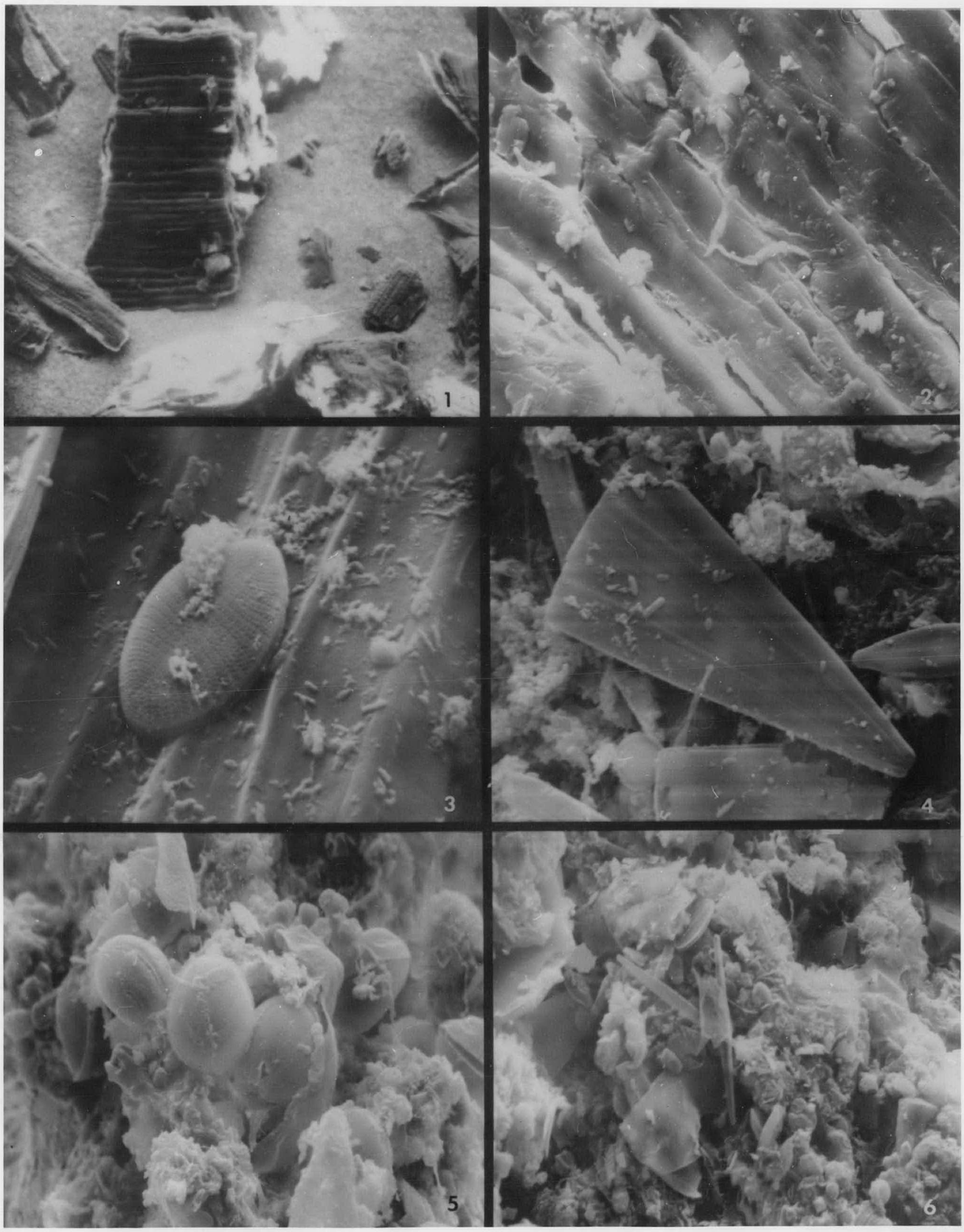
Fig. 86




POWER FACILITY
COOLING TOWER
PLANT COOLANT INFLOW
EFFLUENT OUTLETS
U.S.N. RESEARCH STATION
X
EFFLUENT POOL SITE
NIANTIC BAY
EFFLUENT QUARRY
BATELLE LABORATORY
MILLSTONE POOL
DOCK
X
COLD POOL SITE
DAM
ATLANTIC OCEAN

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