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The purpose of this problem was to determine the succession of the varied forms of microscopic animal life, protozoan and metazoan, in different natural pond cultures.

From the standpoint of the teacher, it was thought desirable to make as wide an acquaintance as possible with a variety of microscopic forms. To learn what kinds of organisms might, with reasonable certainty, be expected in a given type of culture, and at what time in the life of the culture these forms would be most likely to occur were two major aims in the problem.

Fifteen cultures were made up from a variety of different infusion materials, including leaf mold, several kinds of algae, submerged grass and aquatic vegetation, and bottom silt and debris from different fresh-water habitats. Three concentrations, dilute, medium, and concentrated, were used in making the cultures, which were all maintained under ordinary laboratory conditions.

Observations on the surface of the cultures were made daily or every other day for the first week and once a week thereafter. Observations were continued over a period of eight weeks in the case of all cultures but one, which was kept under observation for twelve weeks. The cultures were made up at different time intervals, so that not all cultures were under observation at the same time.

The organisms were catalogued and their percentage abundance tabulated for each observation according to the following procedure. From each culture, 25 drop samples were taken from

the surface with a pipette, and the organisms occurring in each drop were listed. At the end of the 25 observations, the number of drops in which any organism had appeared was put down as its percentage abundance for that day.

The relative percentage distributions thus obtained were charted for the eight or twelve-week period of observation in a bar graph for a number of the more typical cultures. The graphs show what organisms appear in the early, middle, and late life of a culture, and at which period they are abundant or even dominate the culture.

A classified list of the identifiable organisms found in the course of the study was compiled, and a summarization table made of the periods in the life of a culture when certain organisms most commonly appeared. Only those organisms occurring with considerable regularity in large numbers were included in the summarization table.

Results of the study may be briefly summarized as follows:
Organisms tending to appear for a short time in the early life of a culture are: Synura, Volvox, Gonium, Pandorina, Eudorina, Trachelomonas, Peridinium, Holophrya, Monads, Chilomonas, Planaria, Gammarus, Copepoda, Ostracoda, Cladocera, and Nauplius

Organisms appearing in large numbers in the early life of a culture are: Colpoda, Paramecium, Hypotricha, Halteria, Urocentrum, Loxophyllum, Lionotus, Trachelius, Balanonema, and Lembadion.

Organisms appearing in greatest abundance in the late maturity of a culture are: Amoeba, Arcella, Blepharisma, Lachrymaria, Chilodon, Spirostomum, Stentor, and Paramecium.

In the old-age period of a culture only diatoms and algae flourish in abundance. Nematodes and Rotifera are usually common, and small numbers of Astasia, Peranema, Copepoda, Ostracoda, Cladocera, and Nauplius are found.

Paramecium is often found in great numbers in both the early and late maturity of a culture, but tends to be dominant in the early maturity period.

As to the kind of organisms appearing in a given type of culture, no definite statement can be made. The organisms listed in the summarization table in the last three periods of a culture cycle were found in practically all cultures brought in. However, greater numbers of Vorticella were found in cultures containing a large quantity of filamentous algae.

In the first two or three days of a culture, a greater variety of species is usually found than in the later periods, but the numbers of individuals of a species are not as abundant, except in the case of Chilomonas and monads, which usually occur in larger numbers than any other protozoan.

SUCCESSION OF ANIMAL LIFE IN DIFFERENT CULTURES

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SUCCESSION OF ANIMAL LIFE IN DIFFERENT CULTURES

INTRODUCTION

Nature of Problem

Shifts of plant and animal populations, effects of changing environment upon the fauna and flora in a given habitat, the struggle for existence, the interaction of organism upon organism, and numerous other biological phenomena can be made easily accessible to the student of biology in the study of the sequence of changes in a culture of microscopic organisms. Here, within the confines of a small battery jar one can watch the transformation from a small pioneer community of a few individuals to a climax community where life becomes very stable. Between these two extremes, progressive changes go on with startling rapidity. Teeming populations of organisms spring up, reach their maximum, and suddenly decline. Others take their place. Eventually, the culture reaches a stage where animal domination is replaced by a period of floral dominance of algae or diatoms. Various animal organisms continue to exist, but their numbers are limited.

What is the nature of this sequence of events? Are there only certain organisms which manifest this tendency to appear in vast numbers and so dominate the culture for

a time? Or, in different cultures and under different conditions, will any organism become the dominating form? Are different organisms to be expected at the beginning, middle, and end of such a culture? Can one observe any general tendency in the similarity of the types of organisms which appear and the time of appearance and disappearance of these organisms?

Historical Background of Problem

That some fairly regular sequence of forms occurs in various types of cultures had been pointed out by an anonymous observer as far back as the time of Leeuwenhoek. According to Woodruff (13), Dujardin had also noticed this phenomenon, but no exact experiments were undertaken to determine what the regular sequence, if any, might be, until Woodruff's own classical experiment in 1912. Prior to this time, Peters (8) had considered the problem mainly from the viewpoint of the chemico-biological relations involved. He attempted to correlate the chemical changes in liquid culture media with the succession of organisms occurring in a culture. Only a few type organisms were considered, and their presence and maximum numbers were noted with regard to the rising or falling acidity of the culture.

Woodruff at first contemplated a comprehensive tabulation of the entire plant and animal life in the

course of hay infusions, but found the task to be too large an undertaking. Instead, he confined himself to the protozoa, and paid particular attention to certain characteristic forms appearing in great numbers. From the large numbers of cultures kept under observation, he was able to establish a definite series for the dominant organisms in hay infusions. Woodruff's cultures were derived from five cc. of "seed material" from aquaria and standing laboratory cultures which were used to inoculate the sterile hay media.

Eddy (2) studied the protozoan succession in pond cultures which he kept under certain controlled conditions. By varying the temperature, oxygen content, light, presence or absence of soil, and the type of infusion material in his cultures, Eddy found that the hay infusion sequence described by Woodruff did not always occur. However, the general course of events was much the same.

Limitation of Problem

In none of the studies described above has there been made available to the student of microscopic animal life any idea of the wealth and variety of protozoan forms occurring in the succession. Neither has attention been called to the more common multicellular forms, exclusive of the Rotifera, beyond a general mention of crustaceans.

This thesis attempts to include a catalogue of all the more common forms encountered in a culture cycle, even though, in regard to a large number of the organisms, no general rule of succession could be formulated.

The purpose of this problem was to determine the succession of the varied forms of microscopic animal life, protozoan and metazoan, in different natural pond cultures kept under ordinary laboratory conditions. Both the quality and concentration of infusion material in the different cultures were varied in order to determine what effects these variations might have on the sequence.

From the standpoint of the teacher, a wide acquaintance with fresh-water forms is highly desirable. This problem has made the realization of this acquaintance possible. By making a study of the successions occurring in cultures from different habitats, the teacher may soon familiarize herself with the forms commonly occurring in a given locality.

MATERIALS AND METHODS

Sources and Treatment of Cultures

Natural pond material was used in all fifteen of the cultures set up for observation. The type of infusion material included leaf mold, several kinds of algae, submerged grass and aquatic vegetation, and bottom silt and debris from various fresh water habitats. Among the localities from which the infusion material was collected were temporary wayside ditches, cattail swamps, a permanent slough rich in organic material, a large clear lake, and several small but fairly permanent ponds.

According to Libbie Hyman's recommendations (3), the cultures were set up in three concentrations designated as "dilute", "medium", and "concentrated." Battery jars of approximately two-liter capacity were set up for all cultures. For the dilute cultures, a layer of infusion material was placed in the bottom of the jar with a few sprigs of aquatic vegetation and then filled with water. In the medium concentrations, the jar was half filled with infusion material and half with water. In the concentrated cultures, the jars were filled with infusion material and enough water added to cover it. Distilled water was added to the pond water brought in except in the case of culture G_1, where tap water was added. Glass

plates were placed over the cultures to prevent evaporation. All cultures were kept in the laboratory where they were subject to the same temperature and approximately the same light conditions.

Method of Observation

Observations were made daily, or every other day, for the first week and once a week thereafter. Samples were collected with a pipette, care being taken to secure samples from all parts of the culture surface. It was first intended to make careful observations on both bottom and surface of the cultures. However, when it was found that the bottom of the cultures generally presented a "diluted" top condition, it was decided to employ the limited time available to detailed observations on the surface only. The bottom of the cultures was kept under general observation, particular attention being paid to the bottom only when sudden changes occurred at the surface, or towards the end of observation of a particular culture.

Since the purpose of the problem was to determine the general sequence of events in the cultures under observation, some method of computing relative abundance of organisms was desired. At first, it was thought that the designations of "rare", "frequent", "common",

"abundant", and "very abundant" (indicating dominance) would be the most suitable method of tabulating this relative abundance. However, it was soon found that certain "local" distributions interfered with the general picture of the culture. One organism might be very abundant in one or two spots on the surface but not at all distributed over the rest of the surface. After the preliminary eight-week observation period on the four earliest cultures, the following method was decided upon.

Twenty-five drop samples were taken from each culture. The organisms occurring in each drop were listed. At the end of the twenty-five observations, the number of drops in which any given organism had appeared was put down as its percentage abundance for that day. Thus, if an organism had occurred in all twenty-five drops, it was listed as present 100%; if present in twenty drops, as 80%, etc. In this way, a picture of the relative percentage distribution of an organism in a culture was obtained. This relative distribution was deemed sufficient to indicate the dominant tendency of an organism in the surface culture. Organisms that were present in the largest percentage of the samples taken in a given culture at a given time were regarded as dominant in that culture at that time.

The data on the first four cultures, B, C, K, and L, which had been tabulated in the manner first mentioned, was transposed into this percentage tabulation. Approximate percentages of 20, 40, 60, 80, and 100 were substituted respectively for "rare", "frequent", "common", "abundant", and "very abundant". Since these first four cultures were of very dilute concentration and did not run the cycle typical of the more concentrated cultures, such transposition was considered sufficiently accurate to permit using the data in the general observations on results of the study.

Method of Determination

Of necessity, identification of organisms often had to be limited to a classification into some of the larger taxonomic groups. Among the protozoa, the larger and more easily identifiable forms were put into their genera. Other forms more difficult of identification were put into larger categories, such as hypotrichs and minute ciliates, the latter designation being a cover-all for several tiny holotrichs which were not identified and were never very abundant. Vorticella was used to include all vorticellid forms exclusive of Zoothamnion and Epistylis, which occurred more commonly and were easily identified. Woodruff's designation of "monads" was used to include all minute

colorless flagellates, exclusive of Chilomonas, and "phytomonads" was used to designate all minute chlorophyll-bearing flagellates exclusive of the Euglenidae.

Among the multicellular forms, the Rotifera were lumped together with the exception of Lepadella, Stephanops, Noteus, and Pterodina, which were listed separately. It was not found feasible to attempt a closer identification of the multicellular groups Nematoda, Gastrotricha, and Rhabdocoela, and they were listed as such. Crustaceans were classified into their orders, including Copepoda, Ostracoda, and Cladocera, while all forms of the nauplius stage of whatever order were classified simply as Nauplius. Exceptions to this classification of the Crustacea into orders were made in the case of Gammarus and Asellus, both of which were very common in the first days of some cultures. Annelids were classified down to their genera.

It is to be hoped that invertebrate specialists and protozoologists will not look askance upon the taxonomic efforts made. As Woodruff says, "one who has not attempted to follow in detail a series of cultures....has not, I think, an adequate realization of the wealth of forms which will develop." This "embarrassment of riches" makes the problem of taxonomy alone an impressive one.

GENERAL ACCOUNT OF CULTURE CYCLES

Biological Succession

Woodruff found that within 48 hours after his hay infusions had been made up and seeded, countless numbers of bacteria appeared. After bacterial action had put the hay into a form available for animal life, a great growth of protozoa followed. Monads, Colpoda, Hypotricha, Paramecium, Vorticella, and Amoeba appeared in the serial order given and attained their maximum growth or dominated the culture in the same order. There was a corresponding order in the disappearance of these organisms from the culture, except in the case of Amoeba, which had the shortest cycle in the protozoan sequence.

With the disappearance of the last dominant protozoans, Rotifera became numerous; diatoms, desmids, and filamentous algae flourished; and several species of crustaceans became more or less abundant. At this stage in the cultures, the "climax" had been reached. Under optimum conditions of light and temperature, green plants and animals adjusted themselves to a more or less balanced state of existence.

Peters had found a somewhat similar series of events in his study on the chemico-biological relations in liquid media. He defined four recognizable periods in the

life of a culture. The first period, one of high acidity, was characterized by an abundance of bacteria. Then, during a period of falling acidity, certain organisms with a limited time range appeared in great abundance, the organisms able to withstand a higher concentration of acidity appearing first. In this period came Colpoda, Amoeba, heterotrichs and hypotrichs. In the third period, designated as the "later culture history", great numbers of Stentor and Rotifera flourished, while Paramecium was also abundant, its wide pH range enabling it to be present in large numbers in both second and third periods. Finally, the fourth period, one of faunal sterility, corresponds to Woodruff's "self-supporting microcosm", where algae and crustaceans flourish, but where protozoa and bacteria never again occur in abundance.

Eddy found much the same general sequence as the two preceding workers. However, his sequence varied more than that of Woodruff, probably due, in part, to the greater variety of infusion material used.

The sequence of events in the culture cycles described above refers only to the surface of the cultures. Though both Woodruff and Eddy studied the bottoms of cultures also, they were not able to establish any definite succession of forms occurring on the bottom. Neither did they find organisms appearing in any degree of abundance

compared to that found at the surface. Amoeba appeared to be the only permanent bottom inhabitants, other forms coming down as stragglers or brought down by the sudden dropping of the surface zoogloea.

Factors Determining Biological Succession

Several factors have been regarded as important in determining the biological succession of protozoa.

Peters considered concentration of the acidity of the culture the most efficient factor in determining this succession. He found that the period of Colpoda dominance always coincided with a period of high acidity, while the period of Paramecium dominance corresponded with a lower acidity. An index to the adjustment of these two organisms to acidity was experimentally obtained by determining the minimum amount of HCl which would kill the organisms instantly when added to the normal culture media. It was found that Colpoda always required a higher concentration than Paramecium.

Food had always been regarded as a determining factor favoring growth and reproduction. Peters found, however, that even with an abundance of food present, Stentor did not flourish until the proper acidity conditions had been established.

In addition to the importance of food, Woodruff

suggested that potential of division might be an important factor in large infusions. In a series of experiments, Woodruff showed that the rate of division decreased successively from monads to Colpoda, from Colpoda to Hypotricha, and from Hypotricha to Paramecium.

Interaction of organism upon organism was regarded by Woodruff as an important factor in the decline of the various dominants. Thus Colpoda were found to ingest monads in great numbers, while hypotrichs ingested the Colpoda.

Other important factors considered as having important effects on protozoan populations were summarized by Johnson (4). These include the effects of metabolic products, the effects of the numbers of protozoans themselves, the effects of growth factor requirements as well as the basic nutrients, the effects of the oxygen and carbon dioxide tension of the medium, and the effects of temperature. Specific effects of these various factors on protozoan populations are still being investigated.

DATA

Description of Specific Cultures of Experiment

Culture A. Culture A was begun on December 22, and observations were continued for twelve weeks. Infusion material consisted of a mass of Vaucheria, with some Spirogyra and Zygnema, and silt from a slough rich in organic material. Infusion material was made up in medium concentration.

Culture B. This culture was begun on November 9 and continued for eight weeks. Infusion material, of dilute concentration, consisted of a small mass of Oedogonium and a few sprigs of Myriophyllum, together with a thin bottom layer of silt from a permanent pond.

Culture D_1 and D_2. Both cultures were begun on February 14 and run for eight weeks as were all following cultures. Infusion material was composed of silt, leaf mold, and Spirogyra from a permanent swamp. D_1 was made up in dilute concentration, and D_2, in medium concentration.

Culture E_1 and E_2. Infusion material was composed of small amounts of Zygnema, Oedogonium, Vaucheria, and silt from a wayside ditch. E_1, which was made up in nearly dilute concentration, did not decompose but presented a "balanced aquarium" aspect throughout. E_2 was made

up in approximately the same concentration, but went through a fairly rapid decomposition.

Culture F_1 and F_2. Infusion material was composed of leaf mold, Spirogyra, and Vaucheria from a permanent swamp. F_1 was made up in a concentrated culture, and F_2, in dilute concentration.

Culture G_1 and G_2. Infusion material consisted of submerged grass and algae (Tetraspora) from a permanent lake in North Albany. G_1 was made up in medium concentration with tap water, and G_2, medium concentration with distilled water.

Culture H_1 and H_2. Infusion material included silt and cattail vegetation from a cattail marsh. H_1 was made up into a concentrated culture, and H_2, in dilute concentration.

Cultures C, K, and L. These three dilute cultures were begun at the same time as Culture B and run for the same length of time. Due to their dilute concentration and the lack of decaying organic material, their cycle was not typical, and the data regarding them will be included only in some of the general observations. Infusion material for Culture C was made up of silt and Myriophyllum from a permanent pool. Infusion material for Culture K was made of Lemna, Ceratophyllum, and silt.

Infusion material for Culture L was composed only of Azolla.

Sequence of Cultures

Results for the cultures not given in Plates I-XIX are included here. (For sequence of events in cultures A, B, D_2, E_1, F_1, G_1, G_2, H_1, and H_2, see Plates I-XIX.)

Culture D_1. On the first and third days of observation Culture D_1 was similar in practically all respects to D_2, except that on the third day, both Colpoda and Paramecium were rising in abundance in the more concentrated culture (D_2). In the dilute culture (D_2) Colpoda had appeared but were not abundant, while Paramecium did not appear until the fifth day of observation, when Paramecium bursaria showed a sudden rise in the dilute culture.

On the fifth day, Colpoda reached its maximum of 92%; Paramecium bursaria was present in 80% of the samples; while the other three most prominent forms, Chilomonas, monads, and phytomonads were present as in D_2.

In the second week Colpoda declined to 44%, after which it disappeared. Paramecium bursaria maintained its maximum of 80%, and Paramecium sp. and Loxophyllum reached their maximum of 80%. Hypotrichs reached their low maximum of 72%. A small number of Phacus had appeared,

but Euglena were entirely lacking. The dominant organism was Arcella, which was present in 96% of the samples taken.

In the third week, both species of Paramecium and Loxophyllum had declined sharply to 8%, hypotrichs had disappeared, and Peranema had reached its maximum of 64%. Arcella was the dominant organism and reached its maximum of 100%.

In the fourth week, the dominant ciliate was Halteria. Arcella had declined sharply to 4%, and Paramecium and Loxophyllum had disappeared.

In the fifth and sixth weeks of observation the culture presented a dearth of all ciliates except Halteria, which persisted throughout the culture in small numbers. Chilomonas, which reached its maximum of 100% in the fourth and fifth weeks, disappeared in the sixth week, and monads disappeared at the same time.

The seventh and eighth weeks showed a dominance of diatoms and phytomonads with an occasional Halteria, Trachelomonas, Peranema, copepod, and Nauplius present.

Culture E_2. E_2 was identical with that of E_1 on the first and third days of observation except for the sudden appearance of a fairly large number of Spirostomum, (50%), and Vorticella, (60%), on the third day in this culture. On the fifth day, Aspidisca and other hypotrichs reached their maximum, Aspidisca appearing in 96% of the samples.

Chilomonas was only occasionally present, while in E_1 it was present in great abundance. Neither Vorticella nor phytomonads were present in abundance as in E_1.

In the third week, the abundance of monads was about equal in the two cultures, while phytomonads and Vorticella were the only other organisms present in any abundance in E_2.

In the fourth, fifth, and sixth weeks only monads and phytomonads were abundant. Other organisms occasionally present but never appearing in more than 30% of the samples were Coleps, Colpoda (did not attain a maximum in this culture), hypotrichs, Pandorina, Arcella, Lepadella, Nematoda, Nauplius, and Cladocera.

By the eighth week, only phytomonads were present in abundance, while Chilodon, Euglena, monads, Rotifera, copepods, and Nauplius were occasionally present. Instead of 100% diatoms as in E_1, filamentous algae flourished.

Culture F_2. This culture presented the same picture as F_1 (Plate IX and X) on the first day of observation, except for the abundance of hypotrichs.

On the third day, hypotrichs were on the decline, and Vorticella were present in small numbers, while in F_1 they were at a maximum.

On the fifth day, hypotrichs had practically disappeared, and Vorticella were present only in small

numbers, while, in F_1, these two organisms were dominant.

In the second week, Chilodon and Paramecium were absent, and hypotrichs were only occasionally present, while these three organisms were among the dominants in F_1.

In the third week, Chilodon, Colpoda, and Paramecium were absent, while all three were abundant in F_1. Hypotrichs, which began a secondary rise, had declined to 16% in F_1.

In the fourth week, Colpoda and Paramecium were the only dominant ciliates in F_1, while hypotrichs had a secondary rise and were the dominant ciliates in this culture.

In the fifth week, phytomonads, Chilomonas, and monads were present in abundance, while Paramecium was the dominant ciliate in F_1.

In the sixth week, only Chilomonas was present in abundance, while phytomonads and monads were abundant in F_1.

In the seventh week, there was another maximum rise of hypotrichs, and Chilodon and Rotifera had suddenly appeared in abundance, while, in F_1, Colpoda had a secondary rise and Arcella, Amoeba, and Chilomonas were abundant.

In the eighth week, hypotrichs declined, phytomonads and diatoms were the dominant organisms; in F_1, Chilomonas and diatoms were the most abundant, rhabdocoels and

nematodes had increased greatly in numbers, and there was a sudden rise of large numbers of Blepharisma.

DISCUSSION

Interpretation of Data

In interpreting the data from the cultures described above, Culture A, (Plates I and II) as one of the most typical cultures studied, will be interpreted in detail, and its findings compared with the general sequence described by Peters, Woodruff, and Eddy.

It will be seen from Plates I and II that the succession of organisms has divided the culture into three rather definite periods, coinciding fairly accurately with the first week (the first three observations), the period from the second to the seventh week, and the period from the eighth to the twelfth week.

During the first week there was a fairly wide distribution of ciliates and flagellates, Vorticella reaching a maximum and then suddenly disappearing at the end of the first week. Chilomonas shared the same fate as Vorticella. Practically all the metazoan population disappeared at the end of the first week, only the hardier Gastrotricha and Rotifera remaining until the end of the third week.

After the sudden disappearance of the varied population of the first week came a dominance of Colpoda, followed very quickly by a dominance of Paramecium. Then for a period

of four weeks Colpoda and Paramecium were the dominant organisms. In the sixth and seventh week, there was a rise of Vorticella, which reached a maximum in the eighth, ninth, and tenth weeks, together with the hypotrichs, which had their rise in the ninth week. Rotifers appeared in the ninth week, and Nematodes began competing with the Colpoda and Paramecium dominants in the fourth week, after which they persisted steadily until the eleventh week.

Comparing results of this culture with Woodruff's sequence, it will be seen that Chilomonas takes the same place as monads and then abruptly declines. Colpoda is next to reach its maximum, followed by a short decline, and then a secondary and long-continued maximum, Colpoda remaining even after Paramecium has disappeared. The Paramecium maximum comes after the first rise of Colpoda but coincides with Colpoda's second maximum. Vorticella had a maximum the first week, followed by a decline and a second maximum in the eighth week. Only the second maximum corresponds to Woodruff's picture. The first would be out of order. However, the amount of "seed" material brought in must be considered. Woodruff inoculated his cultures with five cc. of "seed" material, which did not contain a great abundance of any one form. Culture A, when brought into the laboratory, contained large numbers of Vorticella and Zoothamnion attached to the algal

filaments. Within a few days, the Vorticella had multiplied so abundantly that they presented a "frosty" appearance over the sides of the battery jar. As soon as decomposition set in, the Vorticella apparently could not thrive in the acid medium and suddenly disappeared. Their second maximum is more in accordance with their proper rank in the "old age" stage of a culture.

Hypotrichs appeared a little later than Vorticella, their later appearance putting them fourth in order of succession, instead of third as in Woodruff's sequence. Amoeba were the last to appear in any abundance and were still present when the final observations were made.

Among the metazoans, only the Nematoda showed up as an organism thriving in the "old age" of a culture.

Though a large number of Rotifera were "seeded" in the culture at the beginning, they did not survive long. Other species of these organisms appeared after the passing of the protozoan dominants.

Diatoms and phytomonads appeared in great abundance at the end of the culture. Diatoms had appeared in smaller numbers earlier in the culture but were not recorded, because their significance in the cycle was not then realized. Peranema appeared definitely to belong to a later stage of the culture.

Culture B. (Plates III and IV) This culture, it will

be remembered, was very dilute, and did not have the environmental conditions afforded by rapid decomposition of more concentrated cultures. There was no abundance of monads at the beginning, and Chilomonas occurred only sporadically. Colpoda dominance was absent entirely; this may have been due to the lack of monads. Paramecium held a sustained distribution from the third to the seventh week but was never abundant. Paramecium bursaria appeared first followed by Paramecium sp.

Other ciliates occurring in equal abundance during the period when Paramecium was present included Urocentrum, Spirostomum, and Stentor.

Hypotrichs appeared in fair abundance, one maximum period represented by Stylonychia, the second and later maximum being represented by Euplotes.

Amoeba appeared in orthodox fashion in the last two weeks of the culture. Arcella was also present in fairly large numbers since the fourth week.

It is rather significant that the crustaceans maintained themselves until the fifth week. That they did not reappear at the end of the culture may probably be due to the scarcity of diatoms and algae, which had not yet begun to establish themselves at the end of the eighth week.

Culture D_2. (Plates V and VI) The Colpoda, Paramecium, hypotrich, and rhizopod sequence followed approximately

the usual succession, if we substitute Arcella for Amoeba. The sudden rise of Amoeba in the second week and its sudden disappearance is not typical, and may be an early stage in the life cycle of some protozoan as suggested by Woodruff. Monads persisted throughout the culture to the eighth week.

The dominance of Euglena for the last five weeks, with large numbers of Phacus and Spondylomorum, made this culture significantly different, but did not interfere with the other dominants in the culture.

Nematodes again significantly reached their maximum towards the end of the culture along with the Rotifera.

Culture E_1. (Plates VII and VIII) This dilute culture failed to show the usual sequence. There was only a very short maximum of Colpoda, maintained for one week. Vorticella had two periods of maxima. Chilomonas and monads persisted almost throughout the life of the culture, and phytomonads were constant dominants. Lachrymaria had a period of dominance coinciding with that of Colpoda.

Culture F_1. (Plates IX and X) The order of dominance in this culture was Chilodon, hypotrichs and Vorticella, Paramecium and Colpoda, and Amoeba. Difflugia and Arcella were evidently introduced into the culture at the beginning in large numbers and continued throughout the life of the culture. Chilomonas persisted as a dominant throughout.

Rhabdocoela were also seeded in large numbers at the beginning and persisted throughout the culture. Nematodes reached their maximum in the last three weeks of the culture cycle.

Culture G_1. (Plates XI and XII) Halteria was the first dominant, reaching its maximum on the fifth day. Colpoda and Paramecium were again dominant simultaneously, from the second to the fifth week. Neither hypotrichs nor Vorticella attained a maximum, and Amoeba reached its low maximum in the third and fourth week. Monads were persistently dominant until the sixth week. Nematoda had their rise in the third and fourth week. The rise of a small number of Nauplius, Ostracoda, Copepoda, and Gammarus in the last week indicated a beginning of the "balanced state" that was to ensue. Rotifera and Rhabdocoela were also flourishing along with Euglena, Phacus, and phytomonads.

Culture G_2. (Plates XIII, XIV, and XV) The only striking difference between these two concentrated cultures is the length of the Colpoda-Paramecium dominance, which was still at its peak in G_2 and had subsided in the sixth week in G_1. Probably snails are to blame. In G_1, there were several snails which had escaped capture when the culture was brought in and which kept the surface comparatively "clean." G_1 had the usual surface scum and

decomposition products, which were not consumed by the scavengers present in G_1. Rotifera and Nematodes showed a characteristic late rise.

Culture H_1. (Plates XVI and XVII) This culture differs from H_2 chiefly in its lengthened cycle. Paramecium had a four-week period of dominance. Colpoda never reached a maximum and disappeared after the fifth week. Urocentrum was dominant in the third week with Paramecium and Vorticella. Amoeba reached its maximum in the third week, while Paramecium was dominant. Rhabdocoels and Nematoda rose in numbers in the last three weeks of the culture, rhabdocoels having persisted throughout the life of the culture.

Culture H_2. (Plates XVIII and XIX) This dilute culture of decomposing cattail vegetation went through its cycle very rapidly. Both Colpoda and Paramecium attained their low maximum in the second week, the hypotrichs attaining theirs already on the third day. Halteria had a maximum from the second to the fourth week, and Urocentrum in the third week. Vorticella attained its maximum in the second week. Arcella attained its maximum in the fourth week. Phytomonads and monads persisted throughout, and Chilomonas disappeared in the seventh week.

Classified List of Forms Studied

Phylum: Protozoa

Subphylum: Plasmodroma

Class: Mastigophora

Subclass: Phytomastigina

Order:	Chrysomadina	
	Synura*	At surface on light side.
Order:	Cryptomonadina	
	Chilomonas*	At surface.
Order:	Phytomonadina	
	Phytomonads	Persistent throughout.
	Volvox*	Occurs only first two days of culture.
	Gonium*	First few days.
	Spondylomorum	Sometimes persistent throughout.
	Pandorina*	First few days.
	Eudorina*	First few days.
Order:	Euglenoidina	
	Euglena	Persistent throughout. On light side.
	Phacus	
	Trachelomonas*	Abundant on first few days.
	Astasia***	Bottom and surface.
	Peranema***	
	Anisonema***	Bottom and surface.
	Entosiphon	
Order:	Dinoflagellata	
	Peridinium*	At surface on light side.

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* Organisms so marked occur in early life of culture.
** Occurring in early maturity of culture.
*** Occurring in late maturity of culture.
**** Occurring in old age of culture.
Organisms not marked did not exhibit enough regularity in time of appearance to warrant placing them in any particular age of the culture cycle.

Subclass: Zoomastigina

Order: Rhizomastigina

Mastigamoeba***	In great numbers in surface zoogloea.

Order: Protomonadina

Monads	
Dendromonas	

Class: Sarcodina

Subclass: Rhizopoda

Order: Amoebina

Amoeba***	In surface scum or at bottom.

Order: Testacea

Arcella***	In great numbers in surface scum or on bottom.
Difflugia	

Subclass: Actinopoda

Order: Heliozoa — None of helizoans ever found very abundantly. More often on bottom than on top.

Actinophrys	
Sphaerastrum	
Heterophrys	
Hedriocystis	

Subphylum: Ciliophrya

Class: Ciliate

Order: Holotricha

Cranotheridium	Rare.
Didinium**	
Coleps	
Holophrya	
Lachrymaria***	
Lionotus**	
Loxophyllum**	Sometimes common.
Trachelius**	
Dileptus**	
Chilodon***	Common.
Paramecium**	Next in abundance to Colpoda.
P. bursaria**	
Colpoda**	Most abundant ciliate to occur
Frontonia**	
Lembadion**	

	Glaucoma**	
	Balanonema**	
	Urocentrum**	Abundant in some cultures.
	Ophryoglena**	
Order:	Heterotricha	
	Bursaria**	
	Metopus**	At bottom; rarely at surface.
	Spirostomum***	Often at bottom.
	Stentor***	Usually attached to algal filaments.
	Halteria**	Often abundant.
	Strombidium**	
Order:	Hypotricha	
	Oxytricha**	
	Urostyla**	
	Stylonychia**	
	Euplotes**	
	Aspidisca**	
	Uroleptus**	
	Stichotricha**	
Order:	Peritricha	
	Epistylis	Attached to algal filaments.
	*Vorticella****	Most often attached to algae.
	Zoothamnion	
	Vaginicola*	Attached to algae and roots of Lemna.
	Cothurnia*	Often attached to crustaceans.
Class:	Suctoria	
	Podophrya	
Phylum:	Platyhelminthes	
Class:	Turbellaria	
Order:	Rhabdocoelida	
	Rhabdocoela***	Persistent. Most abundant during late maturity.
Order:	Tricladida	
	Planaria*	

*Vorticella may be abundant in both early and late life of culture.

Phylum: Nemertea
Nemerteans

Phylum: Nemathelminthes

Class: Nematoda
Nematodes*** Occur in great numbers in late maturity.

Phylum: Trochelminthes

Class: Rotatoria
Rotifera Several unidentifiable forms are included here.
Noteus
Stephanops
Pterodina
Rotifera vulgaris**** Most common rotifer in old age culture.
Notommata
Lepadella**** Also very common.
Philodina
Conochilius

Class: Gastrotricha
Gastrotricha (Mostly of the genus Chaetonotus)

Phylum: Annelida

Class: Oligochaeta
Nais
Dero
Stylaria
Pristina
Chaetogaster
Tubifex

Phylum: Arthropoda

Subclass: Entomostraca

Order: Branchiopoda

Suborder: *Cladocera****

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*Cladocera and other crustacea may appear in early and later life of cultures.

Order: *Copepoda****

Order: *Ostracoda****
*Nauplius**** (For all orders).

Subclass: Malacostraca

Order: Amphipoda
*Gammarus****

Order: Isopoda
Asellus*

Class: Arachnoidea

Subclass: Arachnida

Order: Acarina
Hydrachnidae (Hydracarina)*

Class: Hexapoda

Order: Chironomidae
Chironomus

Summarization of Age Periods of Organisms

Some organisms appear to have a very definite place in the periods of a culture, which might be termed "youth", "early maturity", "late maturity", and "old age." The organisms which appear more or less definitely to fall into one of these periods in the life of a culture are put into the following table. Organisms which occur more or less sporadically or persist throughout the culture in varying numbers are not included.

Youth	Early Maturity	Late Maturity	Old Age
Synura	Colpoda	Amoeba	Diatoms
Volvox	Paramecium	Arcella	Vorticella
Gonium	Hypotricha	Blepharisma	Astasia
Pandorina	Halteria	Lachrymaria	Peranema
Eudorina	Urocentrum	Chilodon	Nematodes
Trachelomonas	Loxophyllum	Spirostomum	Rotifers
Peridinium	Lionotus	Stentor	Copepoda
Holophrya	Trachelius		Ostracoda
Monads	Balanonema		Cladocera
Chilomonas	Lembadion		Nauplius
Planaria			
Gammarus			
Copepoda			
Ostracoda			
Cladocera			
Nauplius			

Suggestions for Culturing Microscopic Forms

Should the teacher see fit to provide her own cultures for classroom study, the following suggestions are the outcome of this problem.

Members of the Volvocidae group are most often found in clear ponds and must be studied the first or second day after the culture is brought in. They do not survive when decomposition sets in.

Other forms that must be studied the first few days, since they quickly disappear when the water begins to foul, are listed in the first column in the preceding table.

To obtain Colpoda, Paramecium, Stylonychia, and other ciliates in great abundance for classroom use, it is well to make up a medium concentration of infusion material. (See p.4) Colpoda usually appear within about four or five days, followed by Paramecium and hypotrichs within about a week. It is suggested that the teacher bring in infusion material from three or four different sources and that the cultures be started at different intervals. This procedure would insure a greater variety in the successions of the cultures and increase the chances of obtaining the desired protozoans.

If Euglena are desired, the cultures should be placed where at least one side of the culture jar is

exposed to the light. Euglena, which are present in practically every culture brought into the laboratory, will congregate at the surface film on the side exposed to the light. A green rim at this site is usually evidence of their presence. The presence of Euglena is more probable if care is taken to bring in a considerable amount of algae.

To obtain Vorticella, masses of filamentous algae should be brought in. Both Spirogyra and Vaucheria were found to contain vast numbers of Vorticella attached to them, but the Vorticella must be studied the first week, since they disappear when decomposition takes place. An old culture will also usually contain Vorticella in abundance, but they will generally not be found during the mature age of the culture when Colpoda, Paramecium, etc., are dominant.

If planarians, copepods, cladocerans, and other crustaceans are wanted for study, infusion material composed largely of filamentous algae will usually supply the conditions needed. The infusion material should be made up in dilute concentration. If the organisms are to be kept under observation for some time, it is best not to include too many of them in the culture. One or two snails introduced into such a culture will assist in keeping the culture fresh and prevent decomposition.

To obtain nematodes, rotifers, and diatoms in abundance, one need only prevent a culture from evaporating and let it age. Within eight or ten weeks, in dilute concentrations where organic decomposition has taken place (in infusions of leaf mold, silt, debris, etc.), an abundance of nematodes, rotifers, and diatoms will usually occur in the order given.

SUMMARY

Results from this study may be summarized as follows:

1. Any material that will furnish organic decomposition products is suitable to serve as infusion material for a culture of microscopic animal life in which to study biological successions.

2. Dilute concentrations, if they contain decomposing material, will run their cycles much more rapidly than a more concentrated culture. If a dilute concentration is used, care must be taken to see that sufficient organic material is present for the decomposition process. If only green algae or other vegetation are used in small quantities, a balanced aquarium may result, and a typical laboratory cycle will not be obtained.

3. Variations in the typical "biological succession" as established by Woodruff for hay infusions are obtained from different natural pond cultures, the sequence in all probability varying with the conditions established by the different infusion material.

4. In general, it may be said that a much larger amount of "seed" is introduced into the cultures when natural pond cultures are set up in the way described. This large initial seeding will cause considerable variations in the early stages of the culture, though once decomposition sets in, the cycle may, and often does, run

a biological succession, typical of laboratory cultures.

5. Usually a greater variety of forms appears in such cultures the first week, and these are succeeded by fewer species which dominate the culture in vast numbers.

6. Protozoans that appear to have a dominating tendency during the Colpoda-Paramecium period are Lachrymaria, Urocentrum, Halteria, Loxophyllum, and Chilodon. Further study would be needed to determine whether such dominants could be regarded in any regular sequence of pond cultures.

7. Organisms usually found only in the first few days of a culture are Synura, Volvox, Gonium, Pandorina, Eudorina, Trachelomonas, Peridinium, Holophrya, Monads, Chilomonas, and Planaria.

8. Organisms appearing in the early maturity of cultures are Colpoda, Paramecium, Hypotricha, Halteria, Urocentrum, Loxophyllum, Lionotus, Trachelius, Balanonema, and Lembadion.

9. Organisms occurring in greatest numbers in the late maturity of the cultures studied are Amoeba, Arcella, Blepharisma, Lachrymaria, Chilodon, Spirostomum, and Stentor.

10. Forms occurring in greatest numbers in the old age period of a culture are diatoms, Vorticella, Astasia, Peranema, and Nematoda.

11. Crustaceans occur during both the youth and old age periods of a culture.

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EXPLANATION OF PLATES

Plates I to IX are horizontal bar graphs representing the relative percentage distributions of the organisms appearing in the designated cultures. Only the more typical cultures are thus represented. The name of the organism is given in a vertical column to the left, while, to the right of each organism, is marked off on a bar the percentage of times that organism appeared in the culture on each successive period of observation.

Plate I, for culture A, shows a twelve-week period of observation. The following plates show an eight-week period of observation for each culture, after which time the observations were discontinued.

Each of the ten small squares in the vertical column for each day or week of observation represents 10%. If an organism appeared in all the samples taken in a culture on one day of observation, that organism would be marked 100% and is indicated in the graph by a complete horizontal bar for that day or week. The numerical percentage is also given for each organism below its horizontal bar on the graph.

Cultures

Culture A: Medium concentration. Vaucheria, Spirogyra, and silt from slough.

Culture B: Dilute concentration. *Oedogonium*, *Myriophyllum*, and silt from permanent pond.

Culture D_2: Medium concentration. Leaf mold, *Spirogyra*, silt from permanent pond.

Culture E_1: Dilute concentration. *Zygnema*, *Oedogonium*, *Vaucheria*, and silt from wayside ditch.

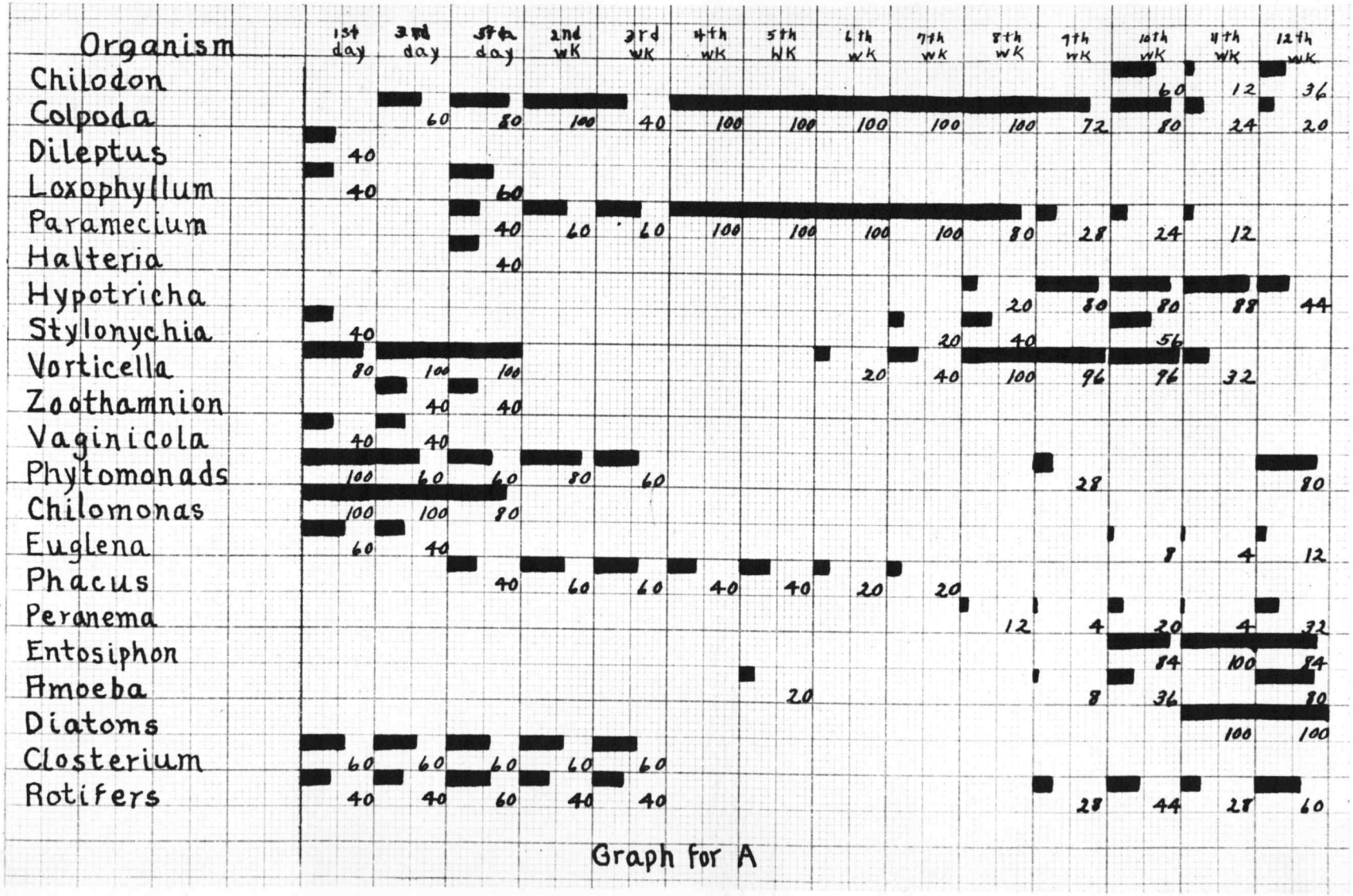
Culture F_1: Concentrated culture. Leaf mold, *Spirogyra*, and *Vaucheria*, and silt from wayside ditch.

Culture G_1: Medium concentration with tap water. Submerged grass and *Tetraspora* from permanent lake.

Culture G_2: Medium concentration with distilled water. Infusion material as above.

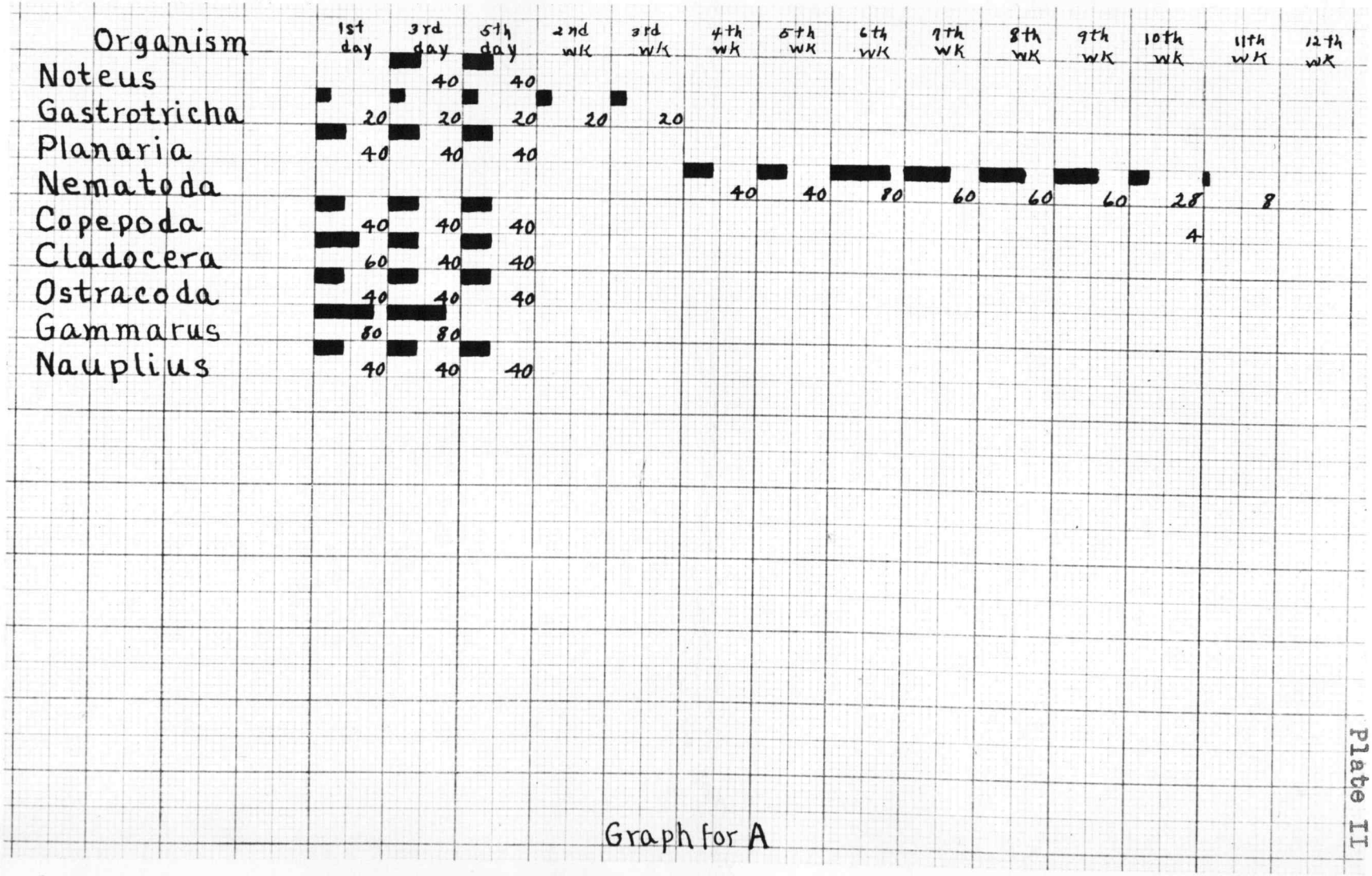
Culture H_1: Concentrated culture. Silt and cattail vegetation.

Culture H_2: Dilute concentration. Infusion material as above.



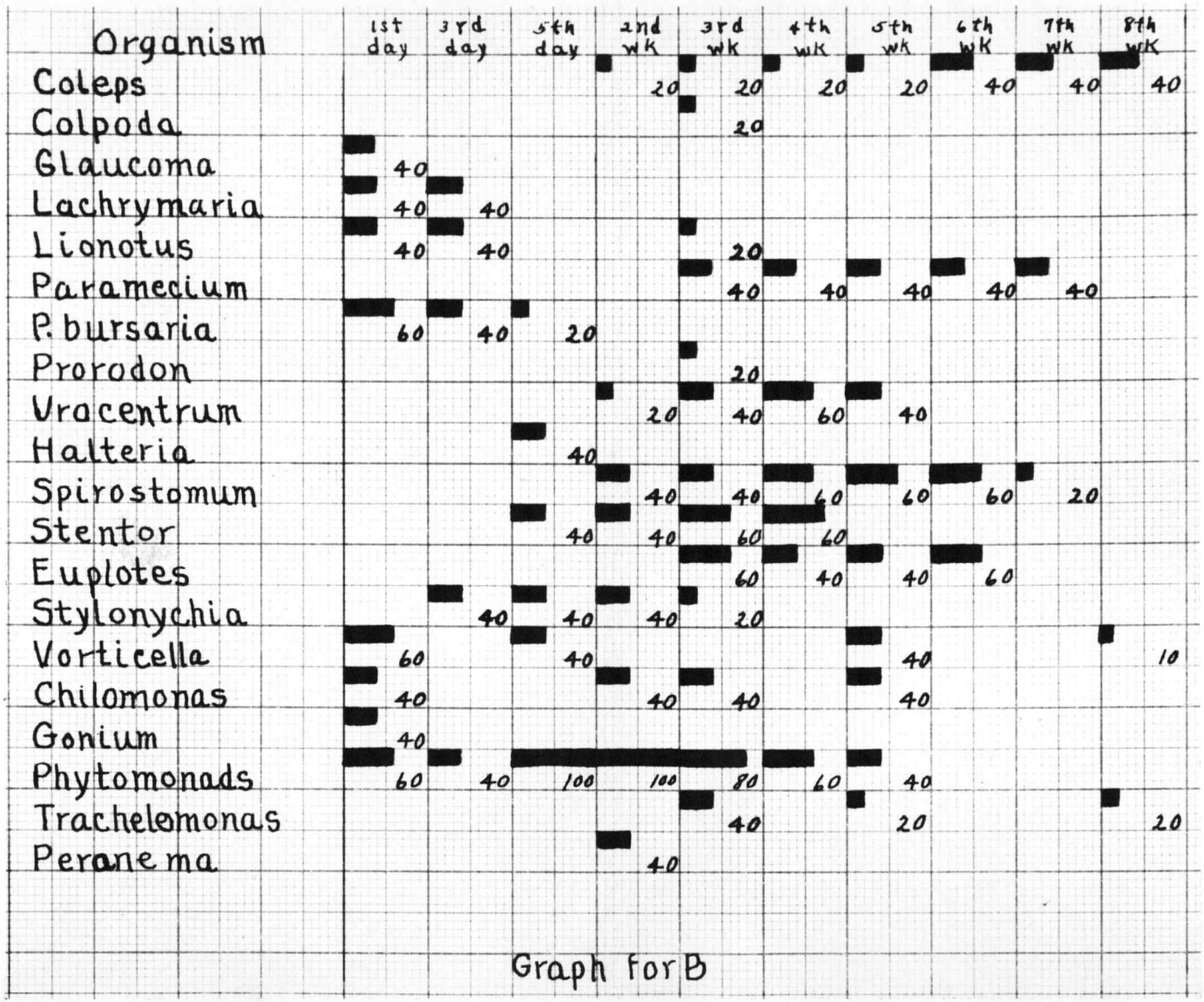
Graph for A

Plate I



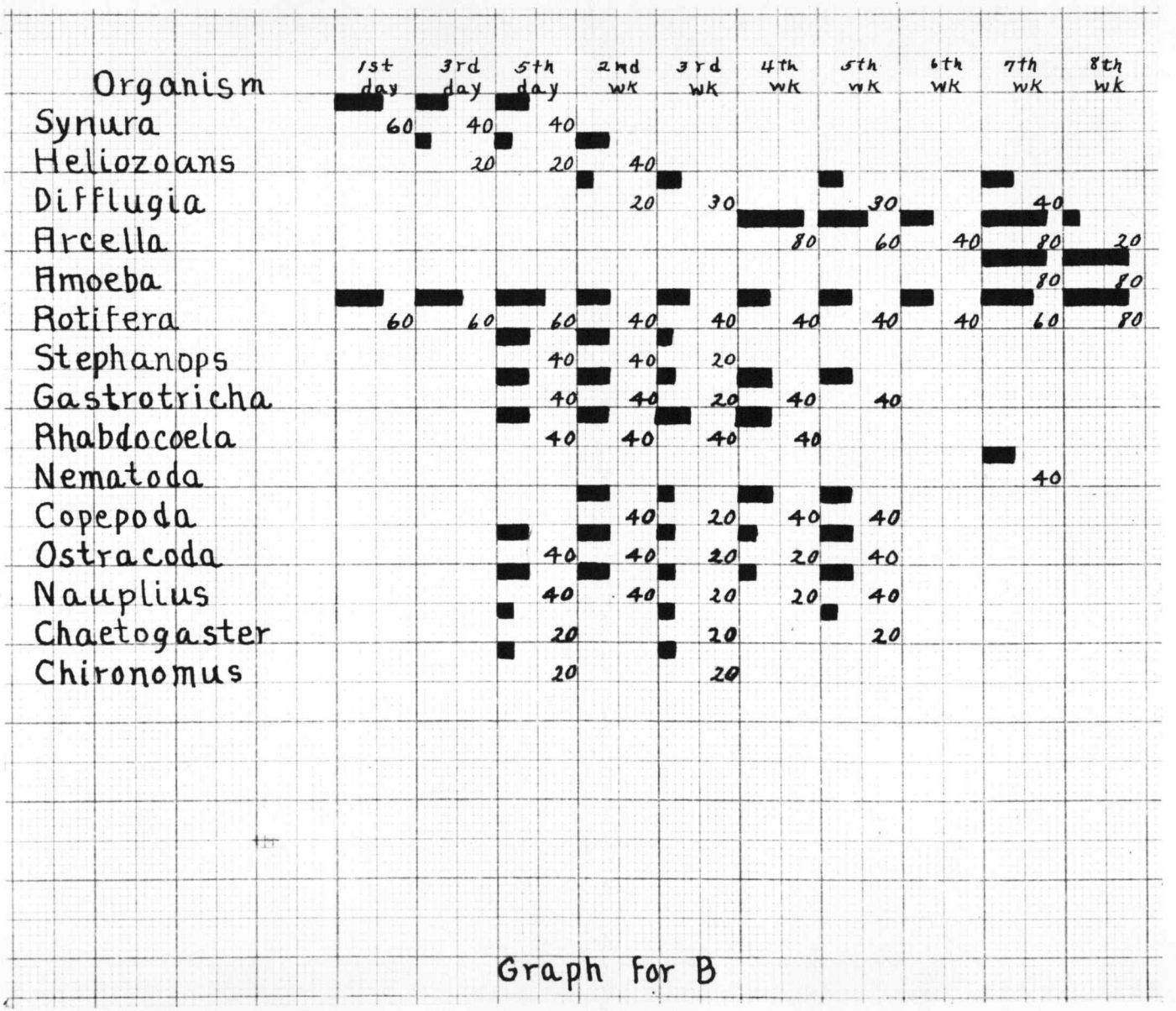
Graph for A

Plate II



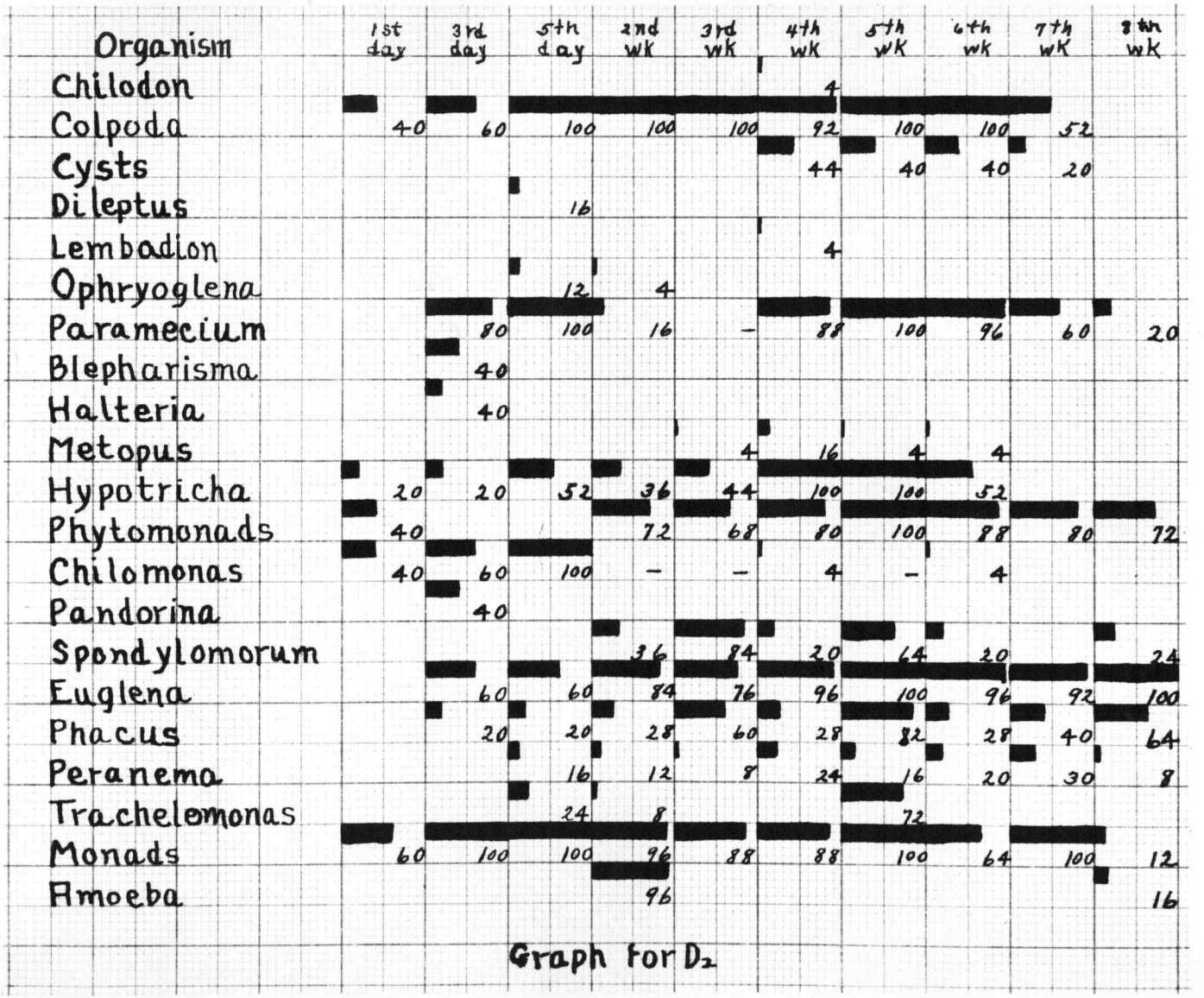
Graph for B

Plate III



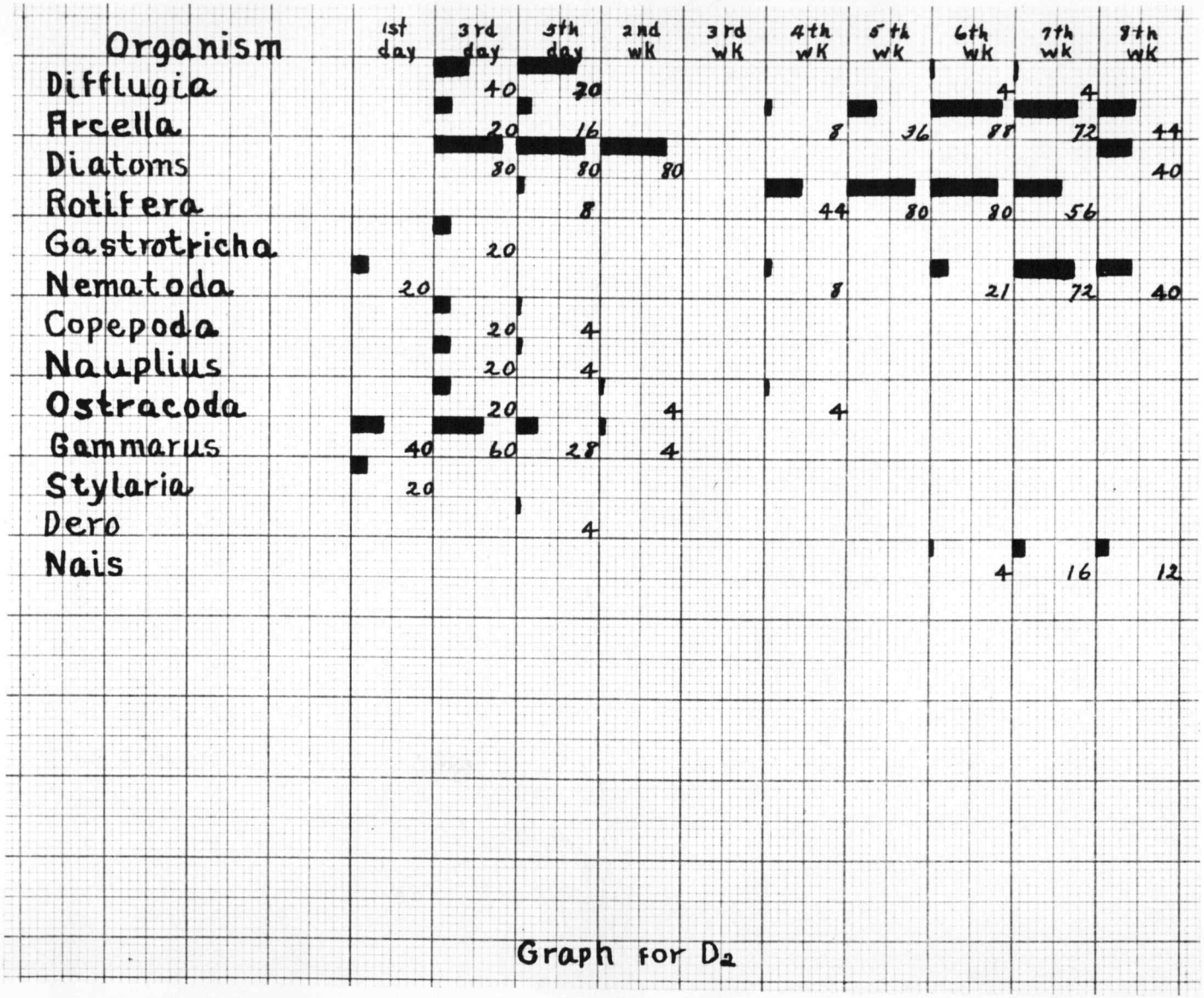
Graph for B

Plate IV



Graph for D_2

Plate V



Graph for D_2

Plate VI

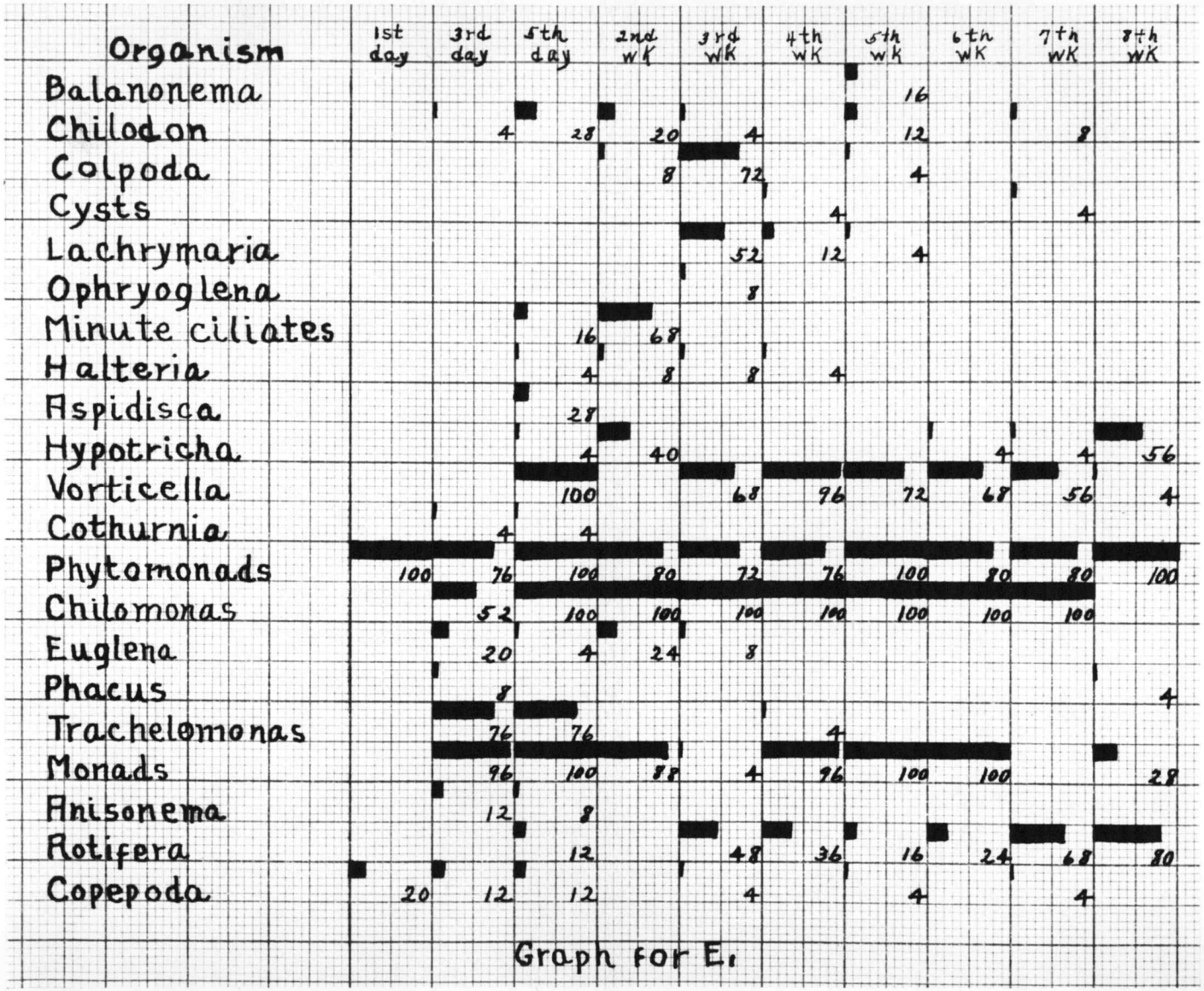
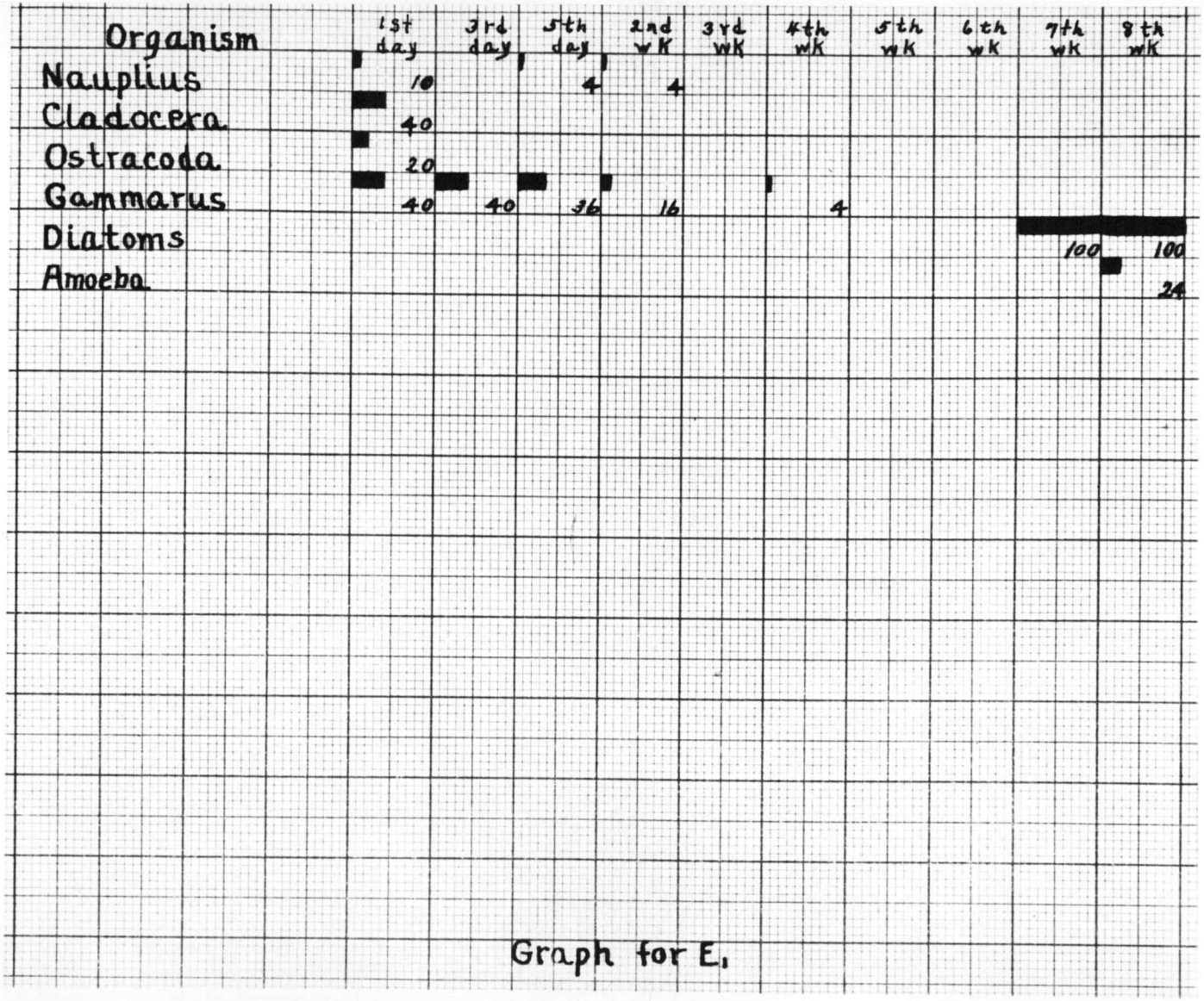
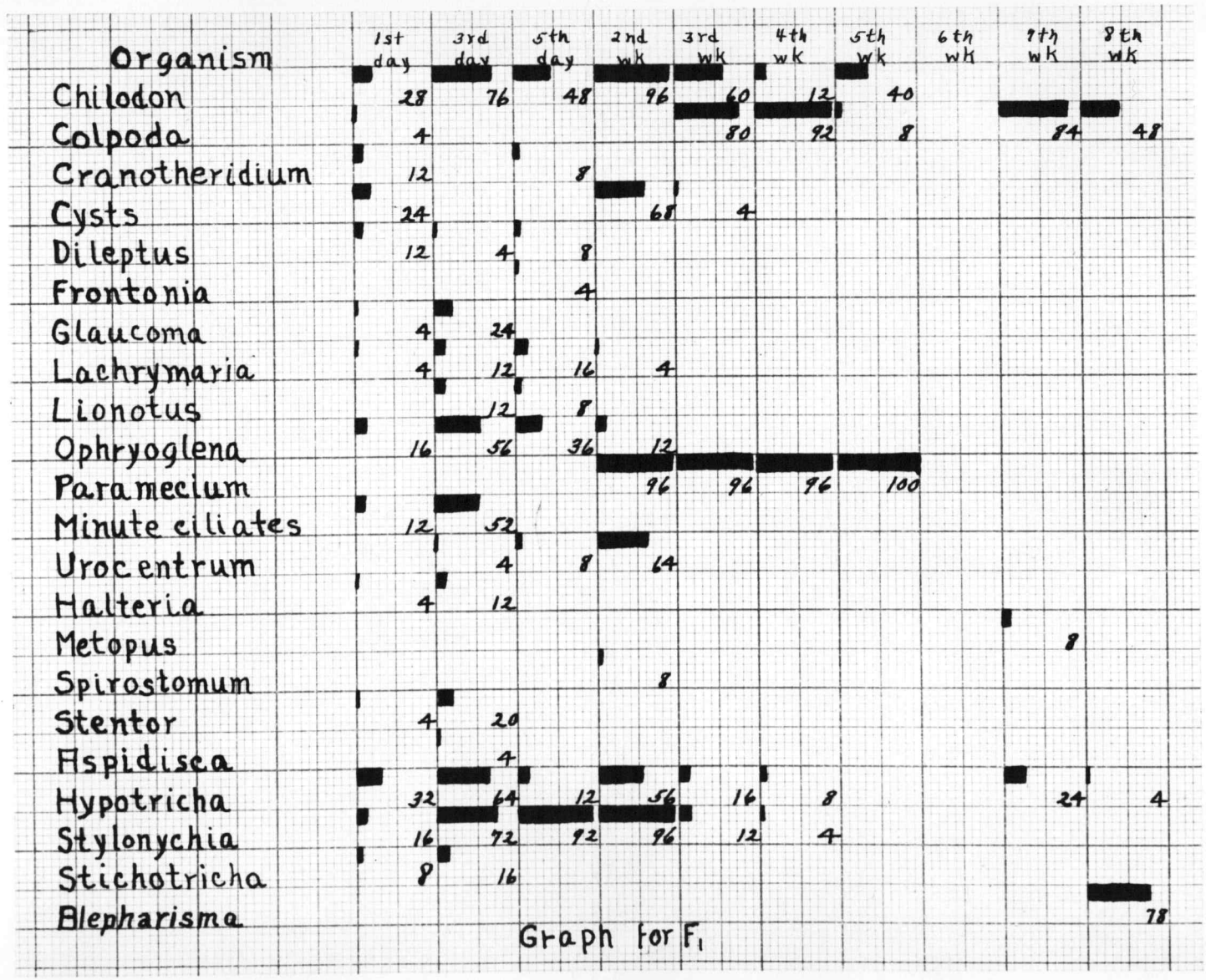

Organism
1st day
3rd day
5th day
2nd wk
3rd wk
4th wk
5th wk
6th wk
7th wk
8th wk
Balanonema
Chilodon
Colpoda
Cysts
Lachrymaria
Ophryoglena
Minute ciliates
Halteria
Aspidisca
Hypotricha
Vorticella
Cothurnia
Phytomonads
Chilomonas
Euglena
Phacus
Trachelomonas
Monads
Anisonema
Rotifera
Copepoda
Graph for E.

Plate VII



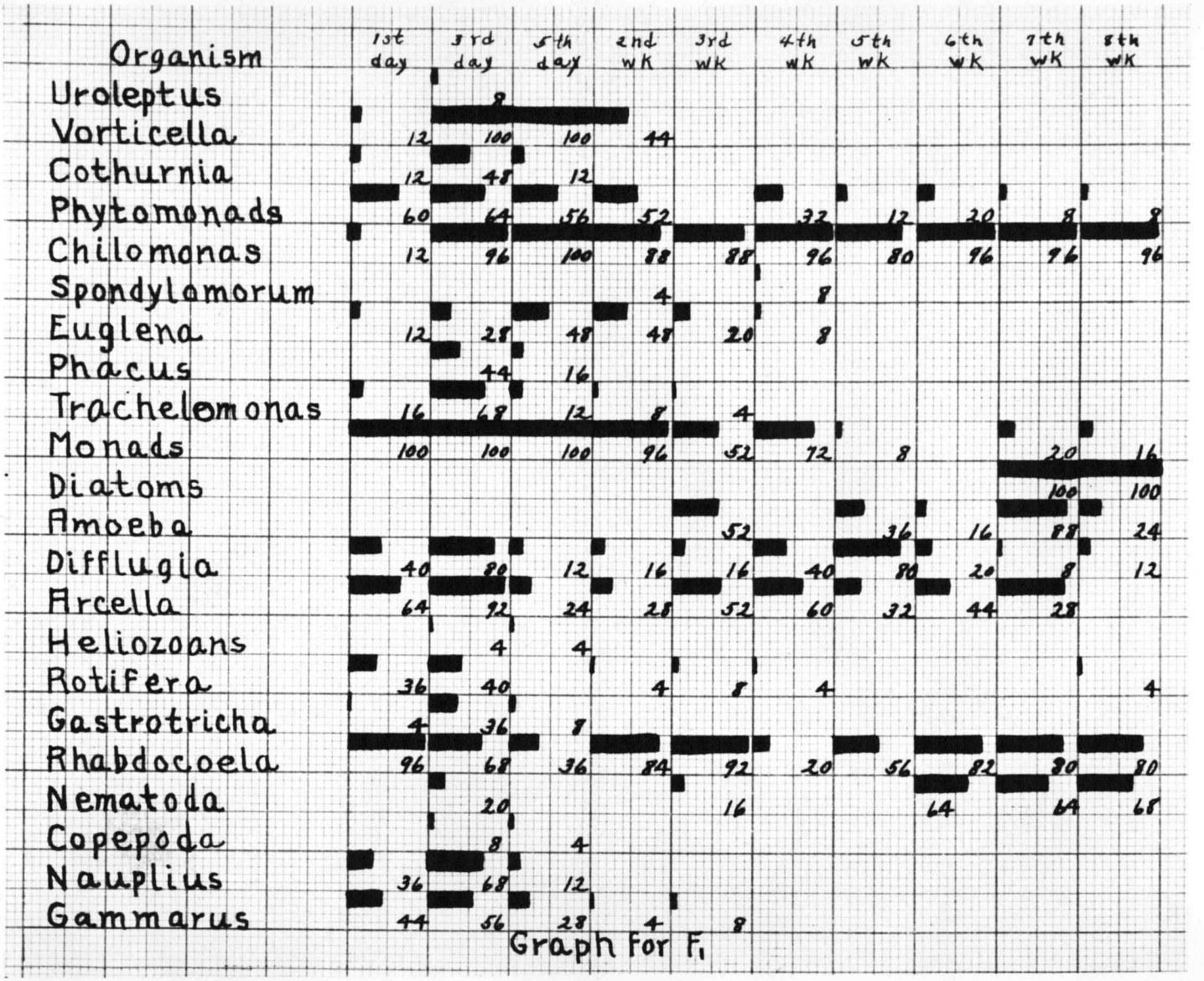
Graph for E.

Plate VIII



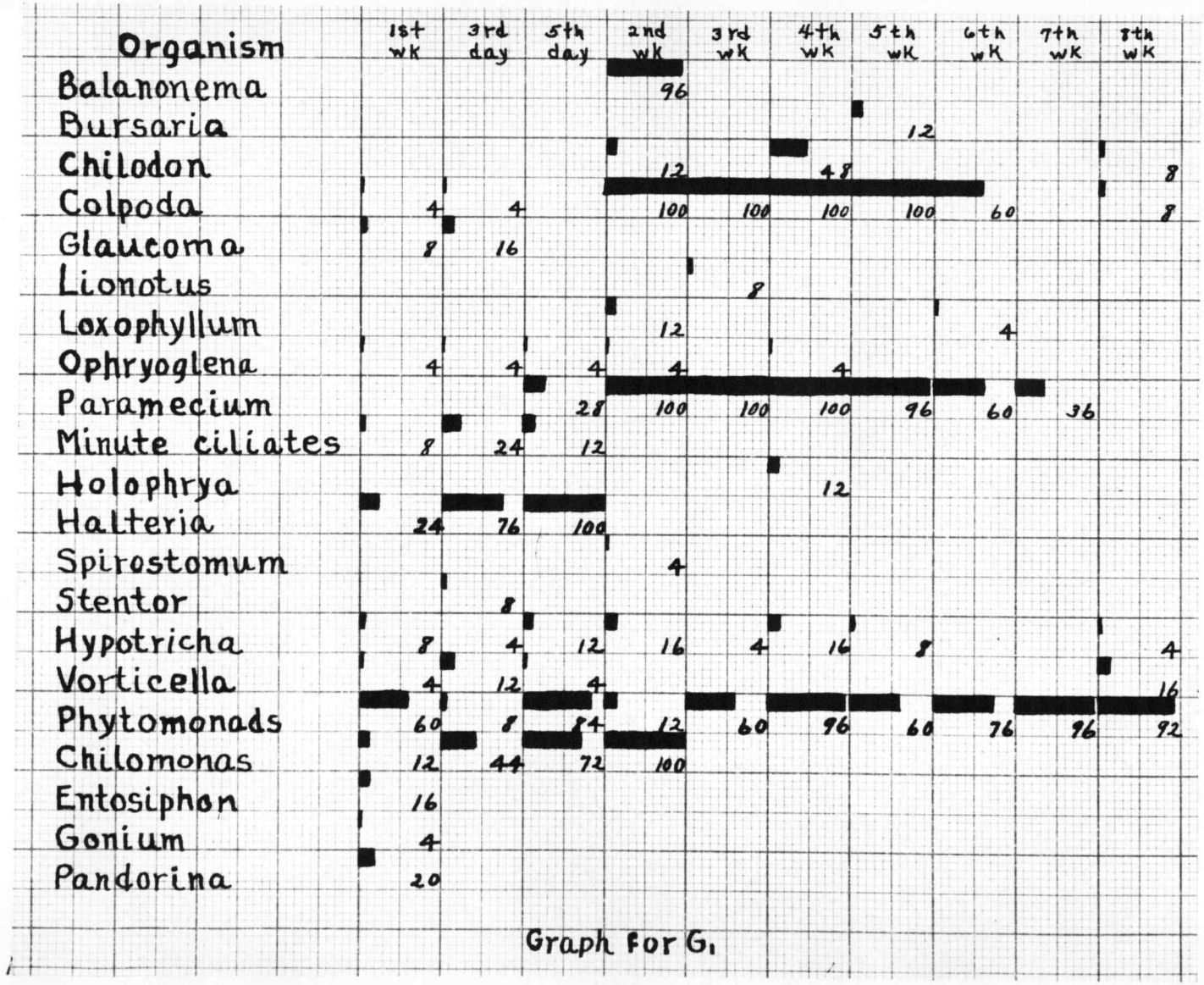
Graph for F_1

Plate IX



Graph For F_1

Plate X



Graph for G1

Plate XI

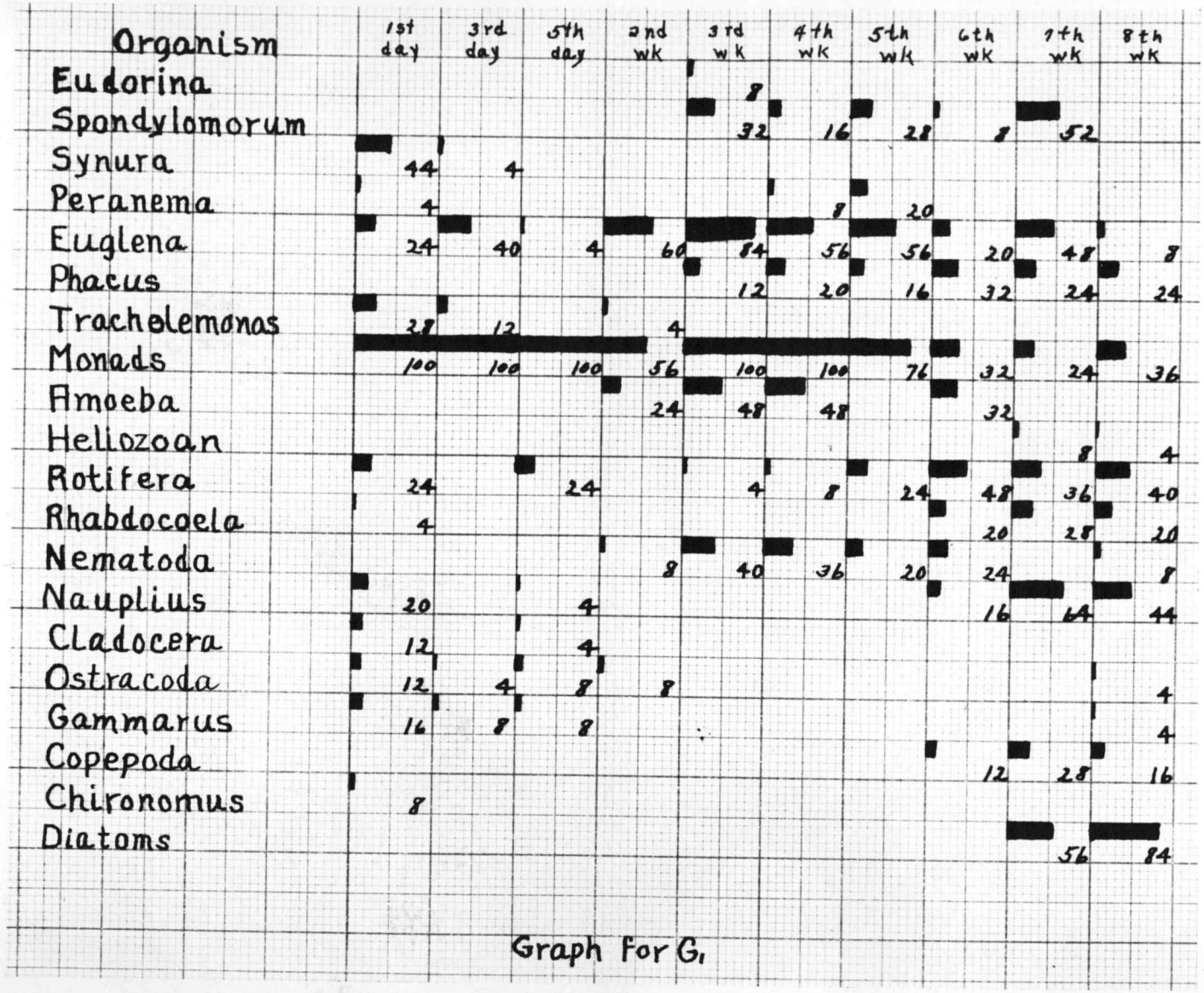
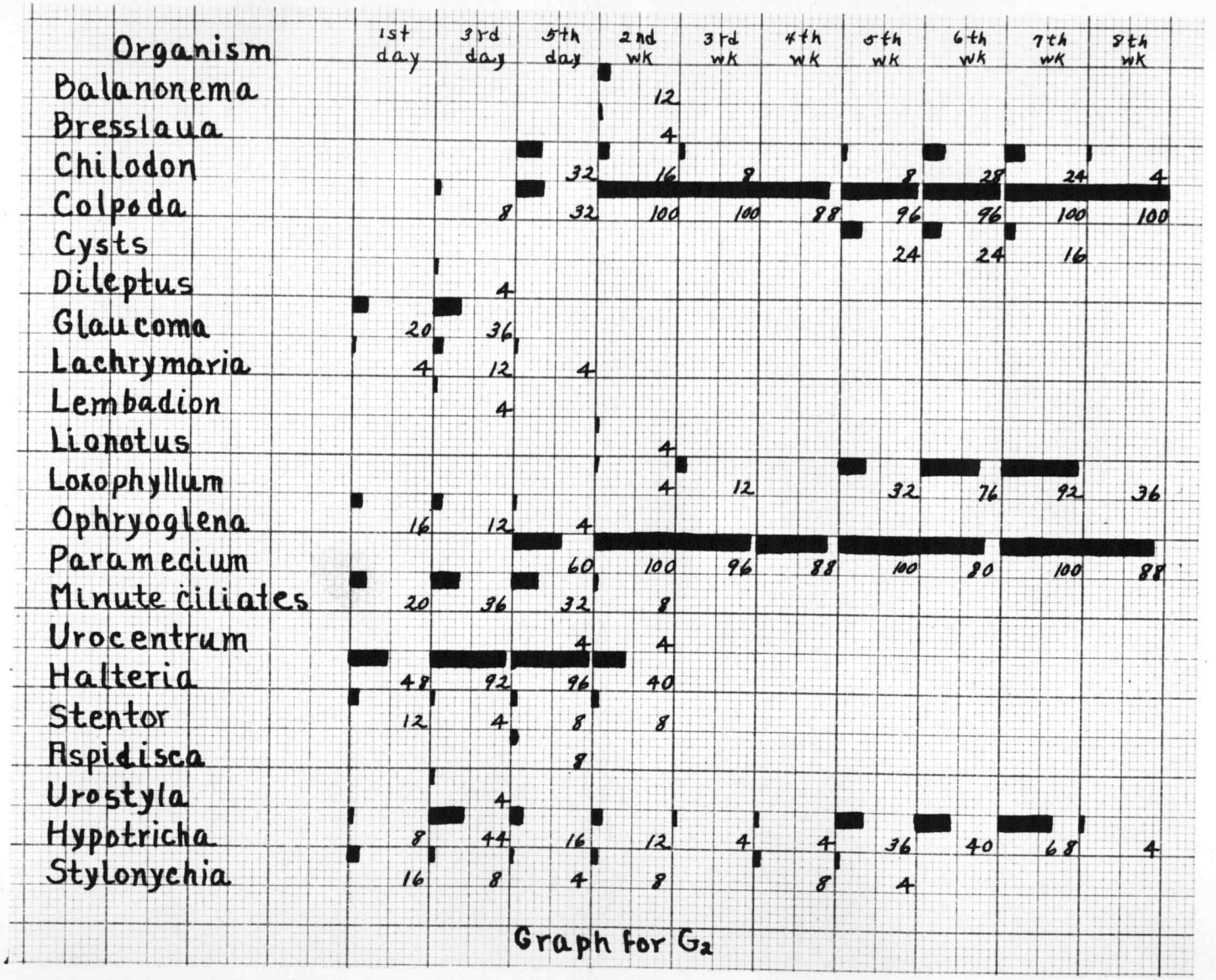
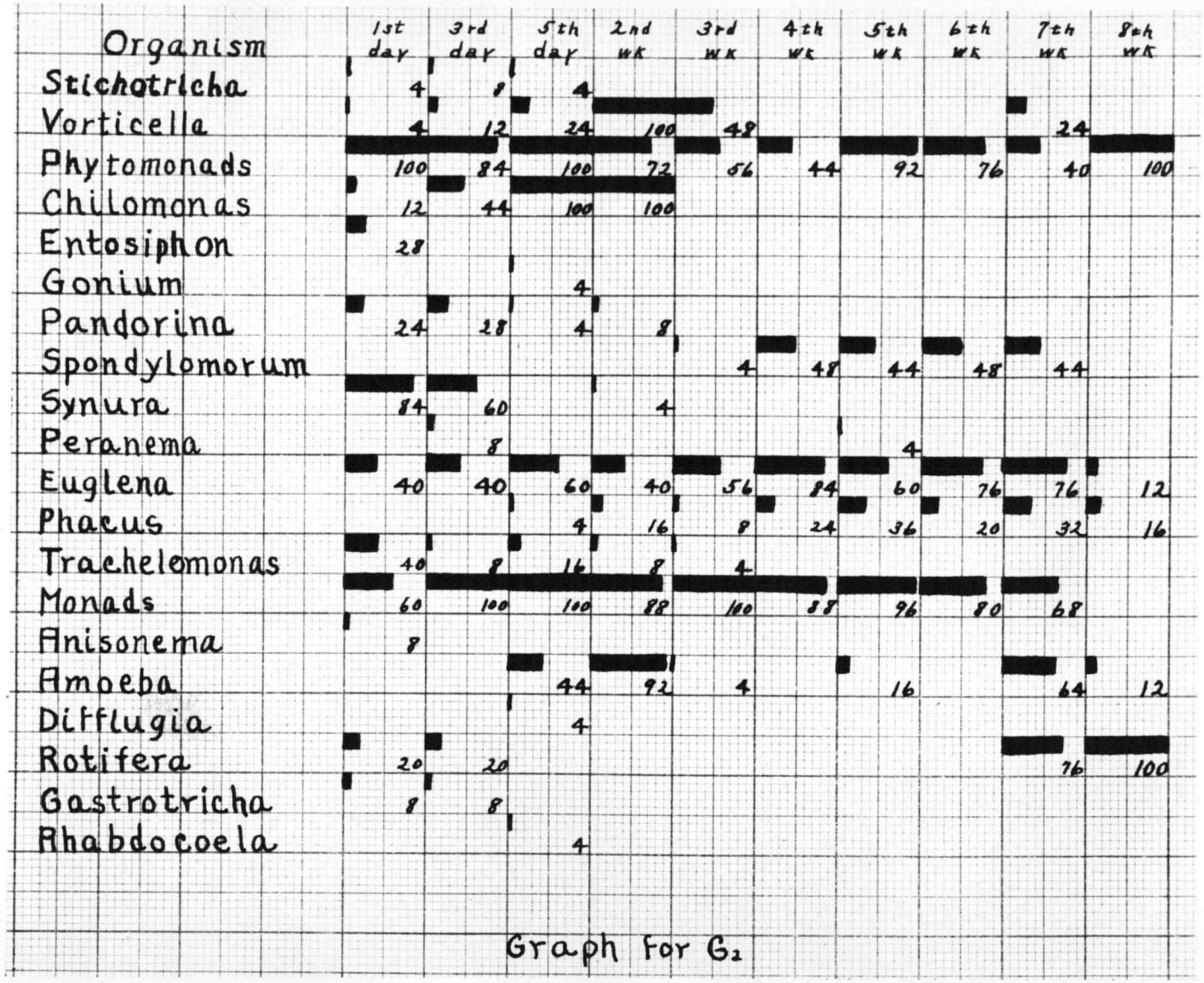

Organism
1st day
3rd day
5th day
2nd wk
3rd wk
4th wk
5th wk
6th wk
7th wk
8th wk
Eudorina
Spondylomorum
Synura
Peranema
Euglena
Phacus
Trachelemonas
Monads
Amoeba
Heliozoan
Rotifera
Rhabdocoela
Nematoda
Nauplius
Cladocera
Ostracoda
Gammarus
Copepoda
Chironomus
Diatoms
8
32 16 28 8 52
44 4
4
8 20
24 40 4 60 84 56 56 20 48 8
12 20 16 32 24 24
28 12 4
100 100 100 56 100 100 76 32 24 36
24 48 48 32
8 4
24 24 4 8 24 48 36 40
4 20 28 20
8 40 36 20 24 8
20 4 16 64 44
12 4
12 4 8 8 4
16 8 8 4
12 28 16
8
56 84
Graph For G1

Plate XII



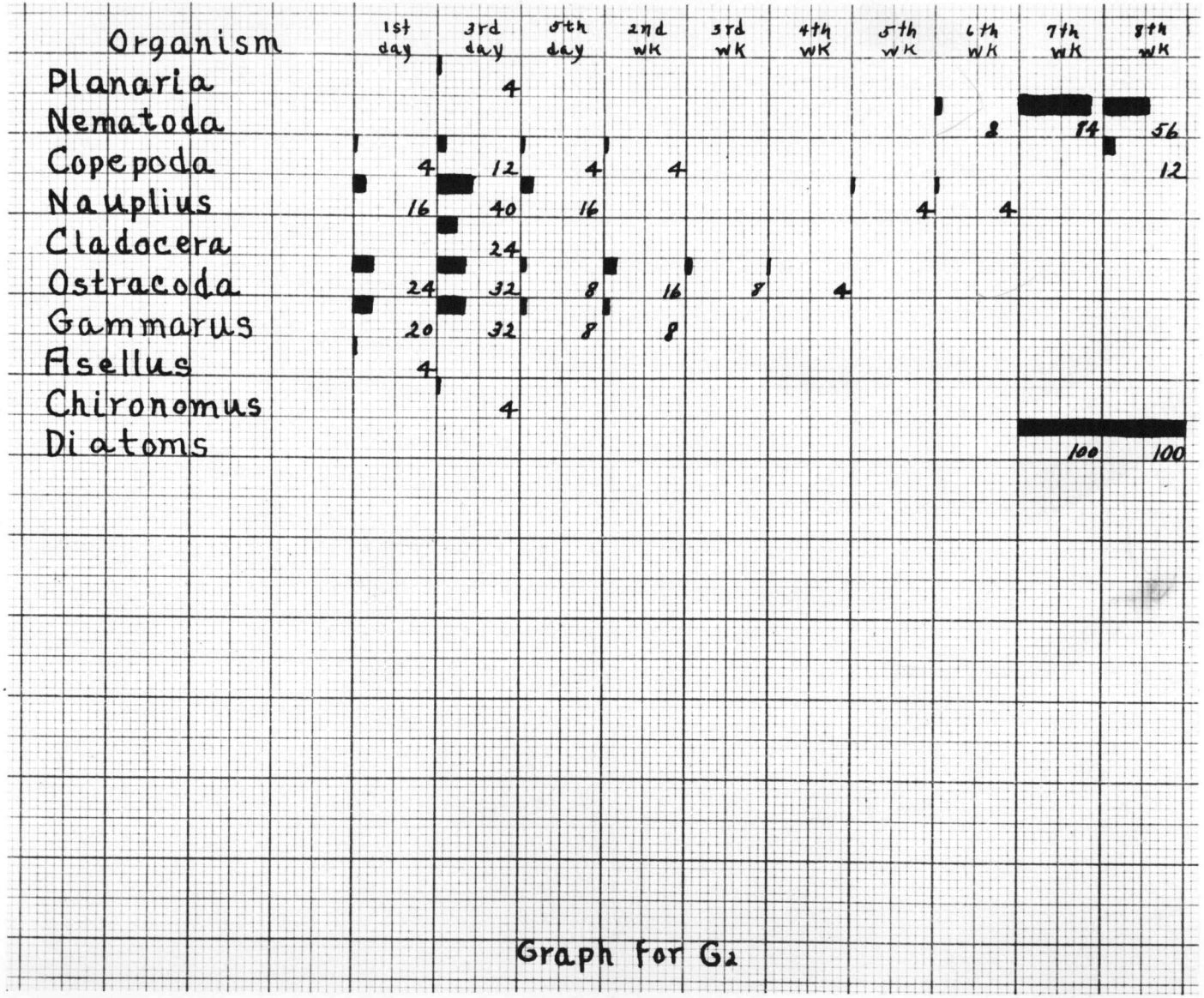
Graph for G_2

Plate XIII



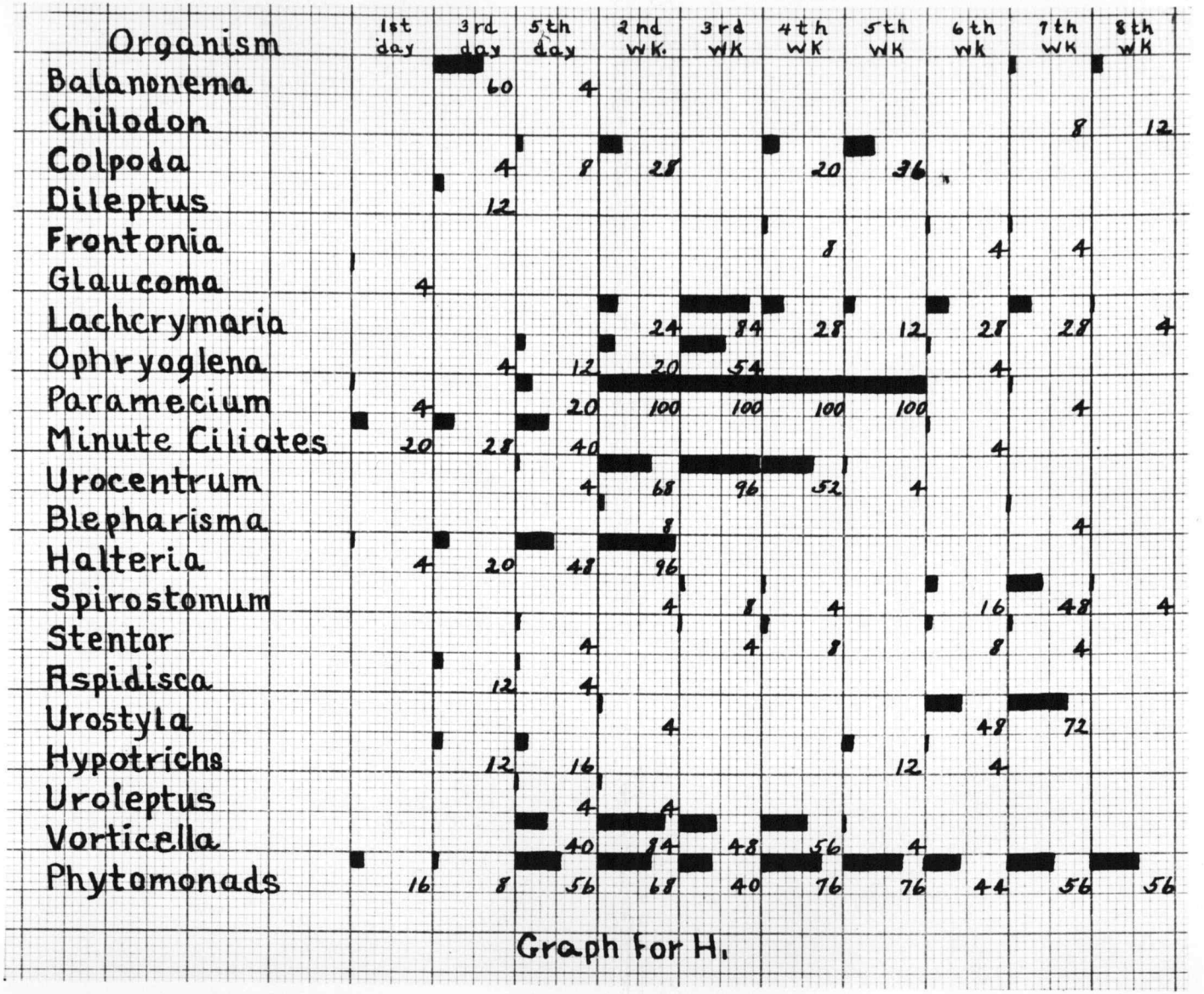
Graph for G_2

Plate XIV



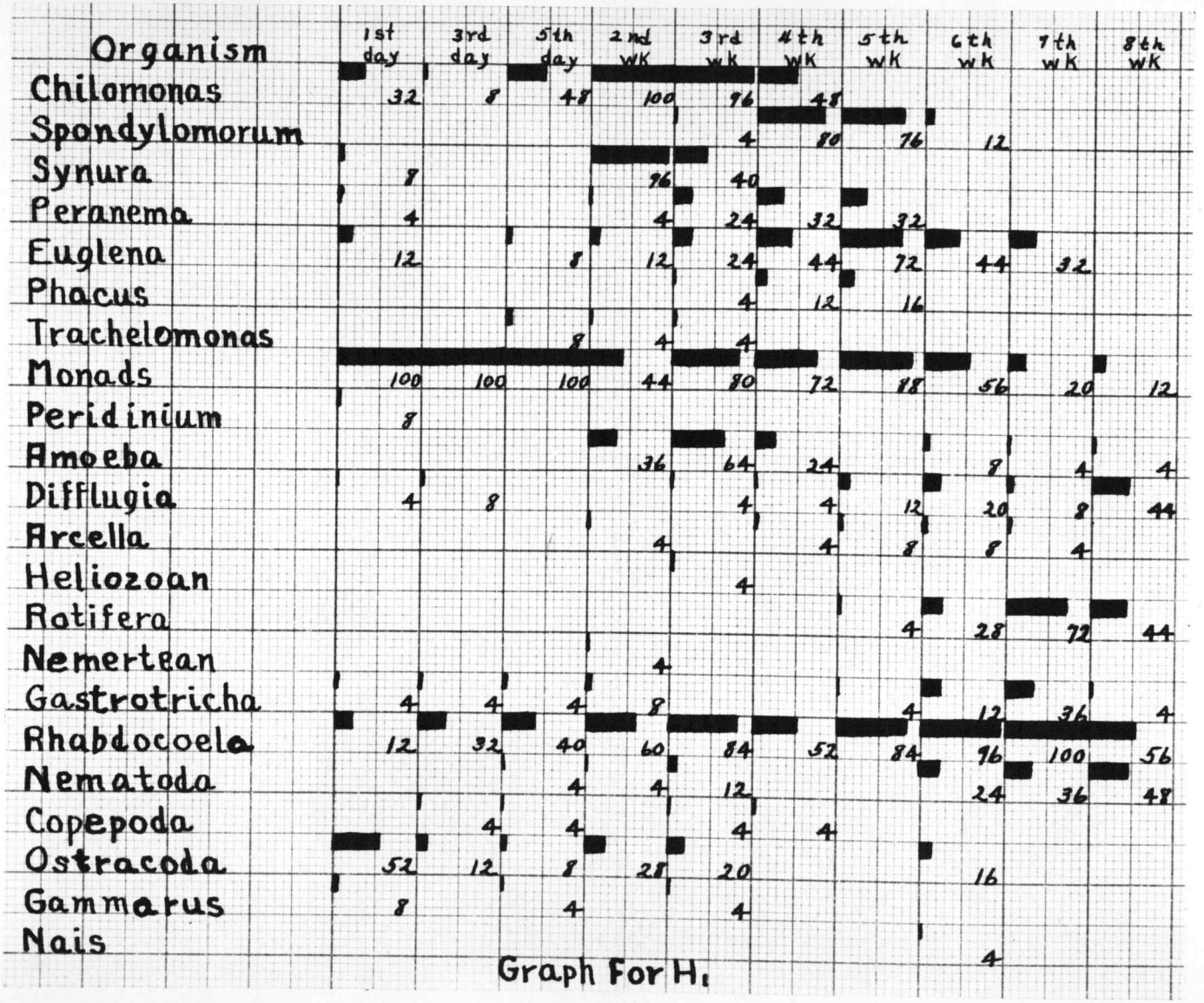
Graph For G_2

Plate XV



Graph for H.

Plate XVI



Graph For H.

Plate XVII

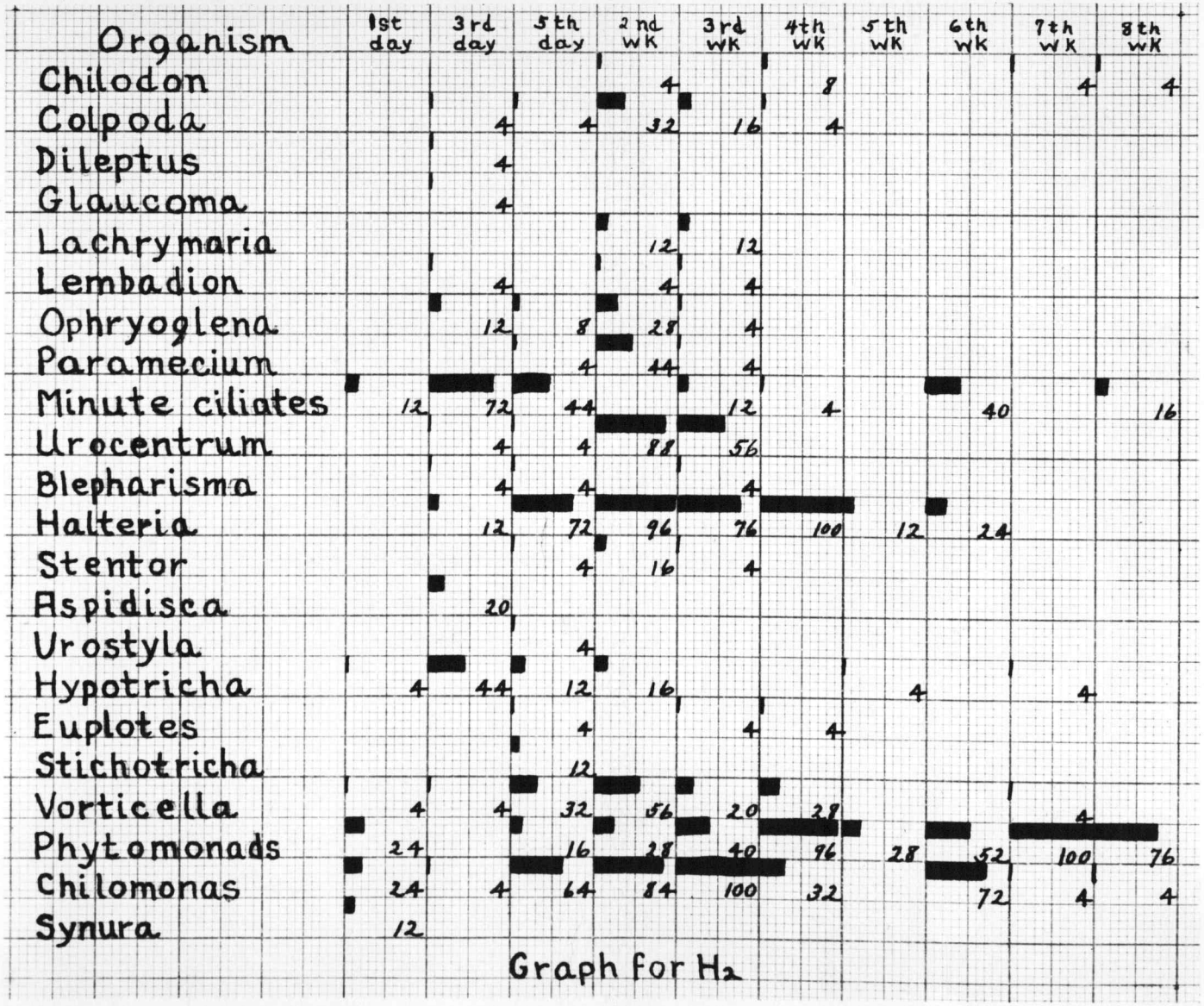
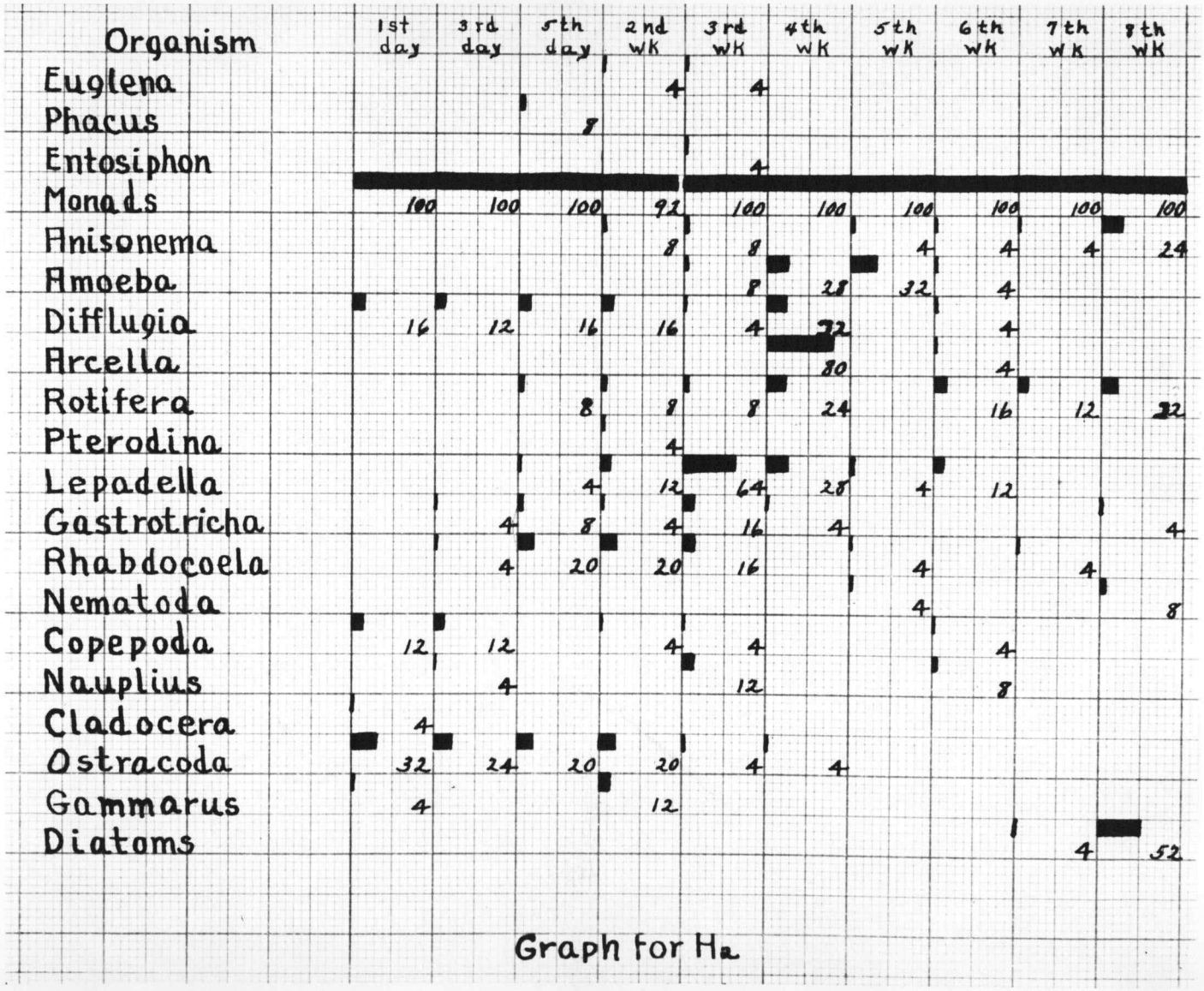

Organism
1st day
3rd day
5th day
2nd wk
3rd wk
4th wk
5th wk
6th wk
7th wk
8th wk
Chilodon
Colpoda
Dileptus
Glaucoma
Lachrymaria
Lembadion
Ophryoglena
Paramecium
Minute ciliates
Urocentrum
Blepharisma
Halteria
Stentor
Aspidisca
Urostyla
Hypotricha
Euplotes
Stichotricha
Vorticella
Phytomonads
Chilomonas
Synura
Graph for H2

Plate XVIII



Graph for Ha

Plates XIX