THE ENERGETICS OF *AMOEBA PROTEUS* LEIDY

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by

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Abstract.

The study dealt with the energetics of the large naked sarcodine, Amoeba proteus, when fed a range of Tetrahymena pyriformis concentrations (125 - 4000 cells $500\mu l^{-1}$) at $10^{o}C$, $15^{o}C$ and $20^{o}C$.

Part 1 of the thesis was concerned with measuring the individual parameters of the energy budget equation, namely consumption, production and respiration. The dried weights of the predator Amoeba and the prey species Tetrahymena were $0.147pg\ \mu m^{-3}$ and $0.162pg\ \mu m^{-3}$ respectively, regardless of temperature. The calorific content of A. proteus was found to be $17.51J\ mg^{-1}$ and was unaffected by temperature; the energy content of T. pyriformis was higher - $19.80J\ mg^{-1}$ at $20^{o}C$ and $15^{o}C$, and $18.28J\ mg^{-1}$ at $10^{o}C$. Energy yields were determined by combustion of freeze-dried pellets in a Phillipson micro-bomb calorimeter.

The effect of the environmental parameters, temperature and food concentration, on the generation times of A. proteus were investigated. Doubling times ranged from 44 to 84 hours, 71 to 112 hours and 372 to 2,926 hours at $20^{o}C$, $15^{o}C$ and $10^{o}C$ respectively.

The rate of consumption increased with increasing temperature, attained a peak and decreased thereafter. The food level promoting maximum consumption decreased with decreasing temperature. As a consequence of the extended generation times with decreased temperature, consumption per generation was greatest at $10^{o}C$.

Maxima energy intakes of 92,931μJ, 17,294μJ and 8,127μJ were calculated for 20°C, 15°C and 10°C respectively.

The volume of protoplasm produced over the cell cycle was the parameter used to measure production. The cell volume doubled over a generation from the daughter cell to the point before fission. The rate of production was influenced by temperature and food concentration and was found to be linear throughout the cell cycle. Increasing temperature increased the rate of production, while increased food supply initially increased the production up to a threshold level, after which the rate decreased. Maximum production was attained at a food concentration of 2000 cells $500\mu l^{-1}$ for 20°C, 1500 cells $500\mu l^{-1}$ for 15°C and 500 cells $500\mu l^{-1}$ for 10°C. Further, decreasing the temperature increased the size of the Amoeba cells; a function of the long generation times at the lower temperatures.

Respiration was measured by Cartesian diver microrespirometry. The rate of oxygen consumption per unit volume (μm^3) was dependent upon temperature, $5.40 \times 10^{-10}\mu l\ O_2\ h^{-1}$, $2.61 \times 10^{-10}\mu l\ O_2\ h^{-1}$ and $2.34 \times 10^{-10}\mu l\ O_2\ h^{-1}$ at 20°C, 15°C and 10°C respectively.

Part 2 of the thesis was concerned with the compilation of a series of both generation and instantaneous energy budgets for individual Amoeba spanning the range of food concentrations and temperatures investigated. The biological efficiencies, linking the parameters of the budget equation, were compared with the relevant published data. Assimilation efficiencies for A. proteus ranged from 22% to 59% regardless of temperature. Net

production efficiencies were high - 65% to 82% - at 15°C and 20°C but low at 10°C (11% to 49%). Gross production efficiencies were also higher at 15°C and 20°C (16% to 47%) than at 10°C (4% to 29%).

In Part 3, the distribution of A. proteus and related species in the field was discussed, with particular reference to a Sphagnum bog-pool. A tentative annual production estimate, based upon both the field and laboratory experiments, of 49.74kJ m^{-2} yr^{-1} (to a depth of 10cm) was calculated.

The thesis was concluded with a General discussion in Part 4.

CONTENTS

Figures.

Plates.

Tables.

General Introduction.

(a) History.

In 1675 Leeuwenhoek, on observing a body of standing rain water, described a species of Vorticella (ref. cited in Viswanath and Pillia, 1968) becoming the first to report on the previously unknown microscopic life form, the protozoa.

Since that time protozoa have been found to occupy an amazing array of habitats from the marine environment to the leaves of plants. As Noland and Gojdics (1967) pointed out, their distribution is restricted by excessive salinity, pH and predation. Further, a utilizable food source and liquid water between O-52^{o}C is also required with some degree of dissolved oxygen. These flexible requirements explain the cosmopolitan occurrence of protozoa which are to be found at practically all levels of the aquatic environment.

Amoeba proteus is a large, free-living naked sarcodine, commonly mononucleate and polypodial. Such forms have been known for over 200 years although the earliest reports on A. proteus were confused by the endless name changing that has plagued this species. Illustrating this confusion, Leidy (1879, ref. cited in Mast and Johnson, 1931) published the following list of synonyms for A. proteus Leidy:-

Der kleine Proteus	Rösell 1755
Volvox Chaos	Linnaeus 1760
Volvox Proteus	Pallas 1766
Chaos Protheus	Linnaeus 1767
Proteus diffluens	Muller 1786
Vibrio Proteus	Gmeling 1788
Amiba Roesili	Bory 1824
Amibi divergens	Bory 1822
Amibi Mülleri	Bory 1824
Amoeba princeps	Ehrenberg 1831
Amiba princeps	Dujardin 1841
Amoeba ramosa	Fementrol
Amoeba communis	Duncan 1877
Amoeba chaos	Leidy 1878
Amoeba proteus	Leidy 1879

Later research papers attempted to document the life-history of this species, notably those publications of Sister Monica Taylor and her associates at Glasgow (1918, 1920, 1921, 1924). Taylor described fragmentation of large A. proteus into a multitude of tiny spores, an observation which is questionable in view of the fact that subsequent researchers have failed to obtain such spores. Other accounts of the life-history are equally debatable, such as the process of conjugation between two cells reported by Prandtl (1907).

With the development of improved culturing techniques for A. proteus, initially due to Taylor (1918, 1920, 1924) and Taylor and Hayes (1921), this organism became popular with experimentors who produced papers largely based on observations of the cells' behaviour in processes such as feeding, locomotion, stimulation and starvation (Beers, 1924; Mast, 1925, 1939; Mast and Root, 1916; Mast and Hahert, 1935; and others).

Recently, research interest in Amoeba has centred around the many micro-manipulations perfected by the cell biologist such as nuclear transplantations, enucleation studies and even the micropuncture of contractile vacuoles (Lorch and Danielli, 1953; Jean, 1970; Schmidt-Nielsen and Schrauger, 1963; and others). Together with echinoderm eggs, amoebae have been among the most important cells used to elucidate the properties of cell protoplasm (Lorch, 1973), a consequence of their large size and ease of culture.

For such a well documented species as A. proteus, which is known to all biologists from school age upwards, it is perhaps surprising that studies relating to its energetics are virtually non-existant.

(b) Protozoan Ecology.

Reviews on the ecology of protozoa are few in the literature and are largely biased towards the ciliated protozoa (Noland and Gojdics, 1967; Faure-Fremiet, 1967; Fenchel, 1969; Bick and Kunze, 1971; Finlay, 1977).

Protozoa have been shown, however, to be numerically important in aquatic ecosystems. Grabacka (1971), Goulder (1971) and Finlay (1977) have all reported concentrations of ciliates of several thousand cm^{-2} from freshwater benthic habitats. Fenchel (1975) found the small benthic zooflagellates of an Arctic Tundra pond to be the most important single group of microfauna utilizing the bacteria. Further, he suggested that the zooflagellates are of probable importance in other aquatic systems.

In a study on the microfauna of Canadian mosses, Fantham and Porter (1945) found the Sarcodina, notably the Testacea, to be the dominant component out of all the microfauna. The importance of this group in Sphagnum bogs has been noted by other researchers including Paulson (1952), de Graff (1956, 1957) and Heal (1961).

The importance of the sarcodines in the benthic environment has never been considered, although Fenchel (1967) reported that naked amoebae appeared to play a small quantitative role in the marine sediments examined. The author pointed out that many individuals may have been overlooked as a result of the inefficiency of the extraction technique, and the difficulties in the detection of amoebae within sediment samples.

Sherman (1915, 1916) documented the protozoan populations of fertile soils and concluded that the flagellates dominated at

approximately 10,000 cells g^{-1}, although the ciliates and amoeboid forms were numerically important at approximately 1,000 cells g^{-1}.

In view of the quantitative importance of protozoa, in conjunction with their short generation times, it is surprising that studies aimed at assessing their role and impact in the aquatic ecosystem are lacking.

Elucidation of the energy transformations and flow through the saprovore food chain have been neglected, even although species of free-living protozoa, particularly the ciliates, are now recognised as forms of importance in polluted environments, extreme examples of which are to be found in the activated-sludge treatment of sewage plants. Curds, Cockburn and Vandyke (1968) showed that the introduction of ciliates into activated-sludge significantly improved the quality of the effluent, apparently by removing large numbers of suspended bacteria.

With increasing bacterial populations developing in the water systems as a consequence of human settlement, it is clearly of importance to gain knowledge about the relations of the protozoa within such systems. In addition, available publications suggest that a major proportion of the energy flow of aquatic environments is channelled through the decomposer food chains rather than the grazer chains. Teal (1962), Heald (1969) and Mann (1972) have indicated that less than 10% of the productivity of marine waters is utilised directly by herbivores. Much of the plant biomass enters the aquatic system as particulate matter where it becomes available as an energy source for the micro-organisms, the benthic macrofauna

and ultimately the higher trophic levels. A similar point, illustrating the importance of those organisms utilizing the considerable energy reserves within the detritus sink, was made by Efford (1969) who discussed the energy transfer through a freshwater lake.

With reference to non-polluted aquatic ecosystems, Legner (1973) has suggested that microphagous ciliates may release metabolites to the environment which stimulate the utilization of substrates by bacteria, thereby aiding the degradation process. Similarly, the grazing activity of protozoa is known to stimulate bacterial growth and increase the rates of saprophytic decay and mineralization (Fenchel and Harrison, 1976).

The importance of ciliates in the food chain has highlighted the need for gathering information on the other protozoan groups within the saprovore web. Reluctance to investigate the sarcodines and flagellates is probably due to the diffuculties associated with identifying these groups. As Bovee (1953c) stated, "one of the knottiest problems in zoology is the specific identification of naked, free-living amoebas of the order Amoebida". Keys are beginning to appear, for example, the recent publication by Page (1976) on the identification of freshwater amoebae. Further, the small size of protozoa presents problems for the investigator, necessitating the development of carefully controlled laboratory experiments to complement the results of field studies. Recently, improved culture methods and new microtechniques have improved matters considerably, although the would-be experimentor must still be prepared to develop and modify existing techniques.

(c) Ecological Energetics.

The term ecological energetics covers any bioenergetic approach which contributes to the understanding of energy transformations between ecological units (be they individuals or trophic levels). Energetics studies are one branch of ecology which, when conducted under conditions approximating the natural environment, significantly contribute to the understanding of how an ecosystem functions.

Since Lindeman's now classic concept on the community dynamics of a cedar bog lake (Lindeman, 1942) there has been much interest in measuring the rate of energy transfer in aquatic systems. Slobodkin (1959, 1962), Phillipson (1966), Kleiber (1961), MacFadyen (1964) and others, have all contributed to this trend in ecology. Further, Winberg (1962, 1964, 1965, 1967, 1968) produced a series of papers on the application of bioenergetics to hydrobiology.

The first investigation on the subject of energy transformations in an organism was undertaken by Hiratsuka (1920) who published information concerning the amount of food converted by silkworms into body biomass. However, it was not until Ivlev (1939a, 1945), that the theories on energy transformation within an organism were developed and summarised in the following equation:-

$$Q = Q' + Q_R + Q_T + Q_V + Q_W$$

where:- Q = quantity of energy consumed by the organism.
Q' = the energy accumulated in growth.
Q_R = the egested energy.
Q_T = the energy of primary heat.
Q_V = the energy of external work.
Q_W = the energy of internal work.

Ricker (1946) pointed out that Q_T, Q_V and Q_W could be combined under the term "Respiration". In other words, the total energy cost incurred for the animals bodily functions.

The equation was thus simplified to:-

Input = Growth + Respiration + Egestion

or, after Heal (1967b) to:-

Consumption (C) = Production (P) + Respiration (R) + Egestion (E)

where the total energy assimilated equals the sum of Production and Respiration.

From an ecological viewpoint, the energy of an individual comprises the sum of energy gains and losses of that individual over a period of time. These energy exchanges are governed by the same thermodynamic laws that describe purely physical energy transfers and transformations. Phillipson (1966), in the context of ecological energetics, has defined the first law: "energy may be transformed from one form into another but is neither created nor destroyed", and the second law: "processes involving energy transformations will not occur spontaneously unless there is a degradation of energy from a non-random to a random form".

The parameters of the budget equation must be expressed in comparable energy units. Traditionally the calorie, defined as the amount of heat required to raise the temperature of one gram of water through one degree centigrade, has been used in energetic studies. In accordance with the Standard International Practice the joule (4.187J = 1 calorie) has replaced the calorie as the unit of energy and is used in present day studies.

An energy equation of the described form (page 7) details the requirements of the animal under study in terms of the total energy consumption and the resulting distribution of that energy within the animal in relation to some period of time. However, perhaps the main functions of an energy budget study are best summed up in the colloquial quotation of H.S. Jennings, 1920:

> "To become personally intimate with particular amoebae or infusoria; to control their goings out and comings in; their diet and personal habits; to interfere with their social and domestic relations; to feed them and mate them; to make them do and live as we want them to live - this is what we have to do if we are to really understand their lives, their behaviour, their growth, their matings, their heredity, their evolution".

As Jenning's statement infers, to fully understand the requirements of protozoa, detailed studies on individual species must be undertaken.

Recently, the importance of intense laboratory investigations on the energy balances of important species under controlled environmental conditions was stressed by Kajak (1970). By combining such information with field population studies, the role of the major protozoan groups in the energy flow through the food web can be estimated.

Detailed studies on Consumption, Production and Respiration are not practical for all of the species in an ecosystem, especially in view of the diversity of species within the protozoan community. Noland (1925) published the first important study on the distribution of freshwater ciliates, although it was Picken (1937) who elaborated on the complex structure of protozoan communities in

general. He paralleled the successive hierarchies from the bacterial and detritus feeding species through to the carnivorous forms with the population pyramidal structure to which Elton (1927) had drawn attention for other animal communities.

Recent field studies by Bryant and Laybourn (1972/73) and Finlay (1977) have illustrated this species diversity at least with regard to the ciliated protozoa. The earlier study reported a total of 59 species of benthic ciliated protozoa from Loch Leven while Finlay found 91 species of ciliates from three shallow freshwater benthic sites.

As Phillipson (1975) believes, the functioning of an ecosystem can only be understood by subdividing it into a large number of relatively simple units, for example species populations. This approach of dissecting the complex system down to representative species is the only means for a full understanding of protozoan dynamics. By studying individual species, selected on the basis of being "typical" of that group, it is hoped that future incorporation of such results will allow the compilation of a complex, but meaningful, model.

Studies relating to the energetics of protozoan species are often only concerned with partial budget equations. Consumption and Production studies for the ciliated protozoa are most prevalent in the literature. Coleman (1964), Proper and Garver (1966), Curds and Cockburn (1968, 1971), Laybourn (1976c) and Laybourn and Stewart (1975) have all published such results from which the gross production efficiencies, indicating the efficiency with which an animal converts energy, were determined.

Energy studies, concerned with the parameter of Respiration under normal environmental conditions, have also been published recently for ciliates. The energy losses of Stentor coeruleus, Podophrya fixa and Didinium nasutum have all been determined by Laybourn (1975b, 1976b, 1977) over varying conditions of temperature and food concentration. Further, Laybourn and Finlay (1976), investigated the respiratory losses in relation to ciliate cell weight.

Overall protozoan energy budgets are few, and predominately for the ciliated protozoa. Laybourn (1973, 1976a) determined a series of budget equations for Colpidium campylum and Stentor coeruleus, while Stachurska (ref. cited in Klekowski and Fischer, 1975) compiled energy budgets for Dipleptus cygnus when fed a range of Colpidium colpoda concentrations.

The only published energy budget equation for the sarcodines was by Heal (1967a) for the small naked amoeba, Acanthamoeba, when cultured on yeast at 25^{o}C.

The aims of the present study.

The aim of the present investigation was the construction of a detailed series of energy budgets for the microbivore sarcodine, Amoeba proteus. The components of the energy budget equation were determined under laboratory conditions, where the effects of temperature and food availability were related to the energy uptake and utilization by the cell. A series of temperatures and food concentrations representative of field conditions was considered. A maximum temperature of 20°C was used as water temperatures in the field for middle and high latitudes rarely exceed this level. A lower limit of 10°C was imposed by the limitations of the laboratory. Bryant and Laybourn (1972/73) reported ciliate concentrations as low as $2cm^{-2}$ while Finlay (1977) found concentrations up to 83,000 cm^{-2}. A wide range of Tetrahymena food concentrations, spanning the extremes of 125 - 4,000 cells $500\mu l^{-1}$ was therefore investigated.

A subsidiary part of the project was concerned with the distribution of A. proteus and related species in the natural environment, with particular reference to a Sphagnum bog pool.

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PART 1.

Energetic - Physiological Investigation in the laboratory.

Chapter 1.

1.1. Mass culturing of the experimental organisms.

1.1.1. Introduction.

Taylor (1918) and Taylor and Hayes (1921) had early success with amoebae cultures containing decaying wheat grains and an abundance of mixed ciliates and flagellates. Similarly, Wilson (1900) obtained a flourishing bacterial and protozoan growth suitable for sustaining amoebae by using a tub with pond mud containing Nitella, two or three opened mussels, and a crayfish cut into several pieces. It is perhaps not surprising that it was the wheat, hay or rice infusion which was favoured by subsequent workers interested in maintaining a constant supply of amoebae for research or teaching purposes.

The preparation of these infusions has been described long past by numerous workers, Dawson (1928), Hahnert (1932), Halsey (1936), and others. Later, Mast (1939) and Williamson (1944) attempted to achieve more uniform populations of amoebae by feeding their cultures with separately grown ciliates and flagellates, but it was not until Prescott and James (1955) developed a system based on an inorganic amoebae medium combined with axenically grown Tetrahymena as food that homogeneous mass cultures of Amoeba were possible. The initial salts medium was later modified by Prescott and Carrier (1964) to cover the basic ion requirements of the amoebae while keeping the total ion concentration low.

1.1.2. Methods of mass culture.

1.1.2.1. Tetrahymena pyriformis.

T. pyriformis (strain G.L.) from the Culture Collection, Cambridge, was grown axenically in 250ml conical flasks containing 150ml of proteose-peptone medium (Appendix 1). This medium is commonly used for the culture of Tetrahymena, although many researchers prefer a 2% proteose-peptone solution, Løvlie (1963), Rasmussen, Buhse and Groh, (1975) and Orias and Pollock (1975). The only obvious effect of the dilute medium (0.5%) was a slower growth rate which had the advantage of maintaining the cultures in exponential growth for longer periods of time.

Sterile technique was applied at all times, and the stock was maintained by subculturing with 5ml of inoculum every four or five days. The Tetrahymena were incubated in the dark at 20°C, 15°C and 10°C depending upon the experimental temperature required. Tetrahymena were maintained throughout the whole study period by this method.

1.1.2.2. Amoeba proteus.

A. proteus (Carlsberg "A" strain), reportedly free of cytoplasmic bacteria (Cult. Collection catalogue) was obtained from the Culture Collection, Cambridge. At least 500 amoebae were placed in 9cm diameter petri dishes containing inorganic medium (after Prescott and Carrier, 1964) to a depth of 5 - 10mm (Appendix 1). The medium was non-nutritive and did not promote bacterial growth. This gave the potential for producing amoebae cultures in which the bacterial population remained at an insignificant level (Prescott 1956).

The food organisms, T. pyriformis, were washed twice by centrifugation in conical bottom centrifuge tubes at 300g for 2 - 4 minutes. The proteose-peptone was immediately decanted and the Tetrahymena were resuspended in inorganic medium. This procedure was repeated twice to ensure that traces of proteose-peptone were removed.

A Nephelometer head (Evans Electroselenium Ltd.) in conjunction with an EEL galvanometer was calibrated (as detailed in Appendix 2) and used to estimate the concentrations of washed Tetrahymena cells. This standardised the feeding procedure ensuring that the cultures of Amoeba were not overfed. 10ml of dense Tetrahymena suspension (approximately 8.0×10^4 cells ml^{-1}) were added every 2 or 3 days to the amoebae cultures which were adjusted to maintain a population of around 3.0×10^4 amoebae per petri dish. When the bottom of a culture vessel became contaminated, the amoebae were suspended in fresh inorganic media and emptied into sterile petri dishes. The amoebae cultures were kept in the dark at $20^{\circ}C$, $15^{\circ}C$ and $10^{\circ}C$, depending upon the required experimental temperature.

Covering the bottom of the petri dishes with a layer of non-nutrient agar, as suggested by Prescott (1956), was found to be unnecessary. Rather than making the cultures more stable, the agar tended to encourage bacterial contamination.

Reserve stock cultures of A. proteus were maintained using a modified method based on that of Chalkley (1930) and Sheib (1935). Three polished rice grains were placed in a small crystallising dish containing glass distilled water to a depth of not more than 4cm. The dishes were left for 24 hours to allow bacterial growth to

develop after which they were seeded with various ciliated protozoa and Amoeba from healthy mass cultures. The culture vessels were covered to minimise evaporation and stored in the dark at 20°C. Mixed cultures of this type were easily maintained, often lasting several months without subculturing, but produced relatively few amoebae and considerable debris.

1.2. Culturing technique for A. proteus under experimental conditions.

1.2.1. Introduction.

Having successfully managed to maintain clean healthy stock cultures of both Amoeba and Tetrahymena, a well defined medium suitable for both organisms had to be found for the subsequent experimental work.

It would have been desirable to have excluded bacteria from the cultures, thus simplyfying the system, especially with regard to the Consumption and Production studies. To date, all attempts to obtain axenic or monoxenic growth of A. proteus have been unsuccessful emphasising that the nutritional requirements of Amoeba are not fully understood and that carnivorous amoebae are adapted to prey on specialised motile organisms in a dilute medium (Griffin, 1973). Nardone (1959) succeeded, however, in maintaining A. proteus in dixenic culture using Tetrahymena in conjunction with one of several bacterial species.

A medium had therefore to be found which permitted the growth of amoebae but suppressed the reproduction of both the Tetrahymena and bacterial populations. It was also desirable to

use a dilute medium which approximated that found in the field situation.

Prescott's inorganic medium, as used in the mass culture systems, was unsatisfactory as the Tetrahymena became inactive and their overall numbers decreased markedly with time (Figure 1). These phenomena were unimportant in the mass cultures where the surviving numbers and behaviour of the Tetrahymena cells was unimportant as they were rapidly consumed. For the subsequent experiments, where well defined ratios of prey to predator were important, a random dispersion of active ciliates was required.

Several other inorganic media were tried, notably Chalkley's (1930) and Chapman-Andresen's (1962) modified Pringsheims solution, but all were found to have the same drawbacks as Prescott's with regard to the survival of the Tetrahymena cells.

1.2.2. Soil Extract Media.

A well chosen soil and water medium provide not only a supply of essential mineral nutrients and trace elements, but the latter are naturally chelated, pH is buffered, and toxic products are rendered innocuous. This form of medium is often highly variable depending upon the initial soil type, however, by using a garden loam soil readily available in the University Grounds, a soil extract medium (hereafter termed S.E.M.) was produced which proved to be suitable for sustaining both Tetrahymena and Amoeba in an active condition.

1.2.3. Preparation of S.E.M.

A 1cm deep layer of soil was placed in a 2.0l conical flask. After the addition of 1.5l glass distilled water the vessel was

Figure 1.

The percentage decrease of the Tetrahymena population with time after addition to sterile Prescott's Inorganic medium.

The protozoan suspension contained in three 500 ml conical flasks (A, B and C) was sampled periodically and counted electronically (Coulter Counter).

The addition of a dense suspension of the bacterium Aerobacter aerogenes had no effect on the ciliate population decrease (C).

Figure 2.

The percentage increase of the Tetrahymena population with time after addition to S.E.M. (A and B).

The addition of A. aerogenes had a marked effect on increasing the ciliate population (C).

% decrease in cell number

% increase in cell number

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a marked

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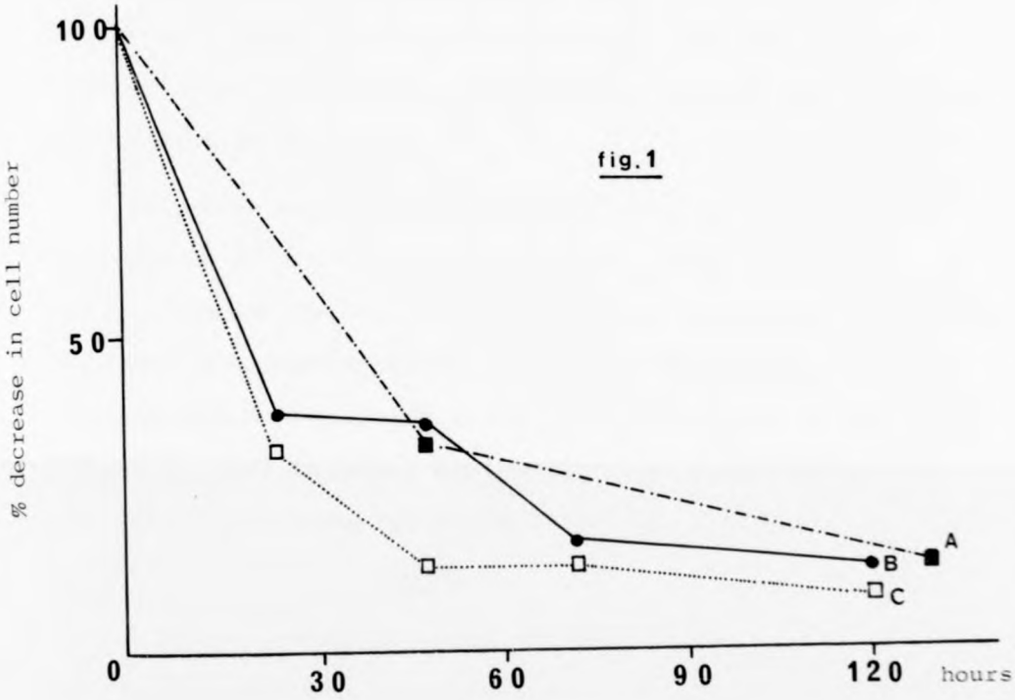


fig.1

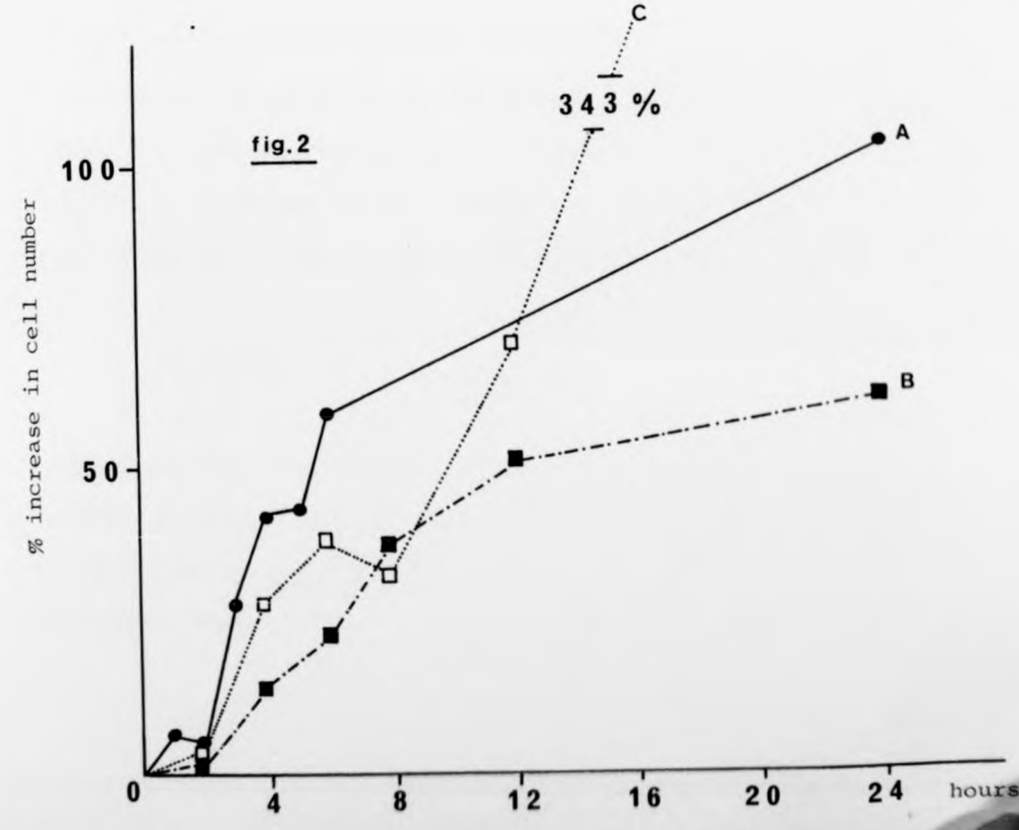


fig.2

autoclaved and left to settle for at least two weeks. The liquid medium was siphoned off and filtered using grade "C" glass fibre filters. The filtering process was repeated until no residue was apparent on the paper.

The medium was diluted with distilled water to its weakest form capable of maintaining the protozoa, thereby minimizing bacterial growth and indirectly Tetrahymena replication. Figure 2 indicates the dramatic rise in the ciliate population when S.E.M. was innoculated with a relatively high concentration of Aerobacter aerogenes. This bacterial species was used because of the work undertaken by Laybourn and Finlay (1976) who successfully cultured T. pyriformis on A. aerogenes.

In order to standardise the diluting of the S.E.M. a galvanometer with attached Nephelometer head (EEL) was used to adjust the batches of media to a reference turbidity level. The pH of the S.E.M. was always close to 6.0.

Having established an easily prepared and reproducible medium which, if care was taken, was not subject to excessive bacterial growth, it remained to run a control to ensure that the basic medium was not contributing to the nutrition of the amoebae.

1.2.4. The "Death Rate" of A. proteus.

To ensure that both the S.E.M. and the low bacterial background population were not being directly utilised by the amoebae for growth, a control was devised which investigated the time taken for a population of Amoeba to die when grown in the absence of a protozoan food.

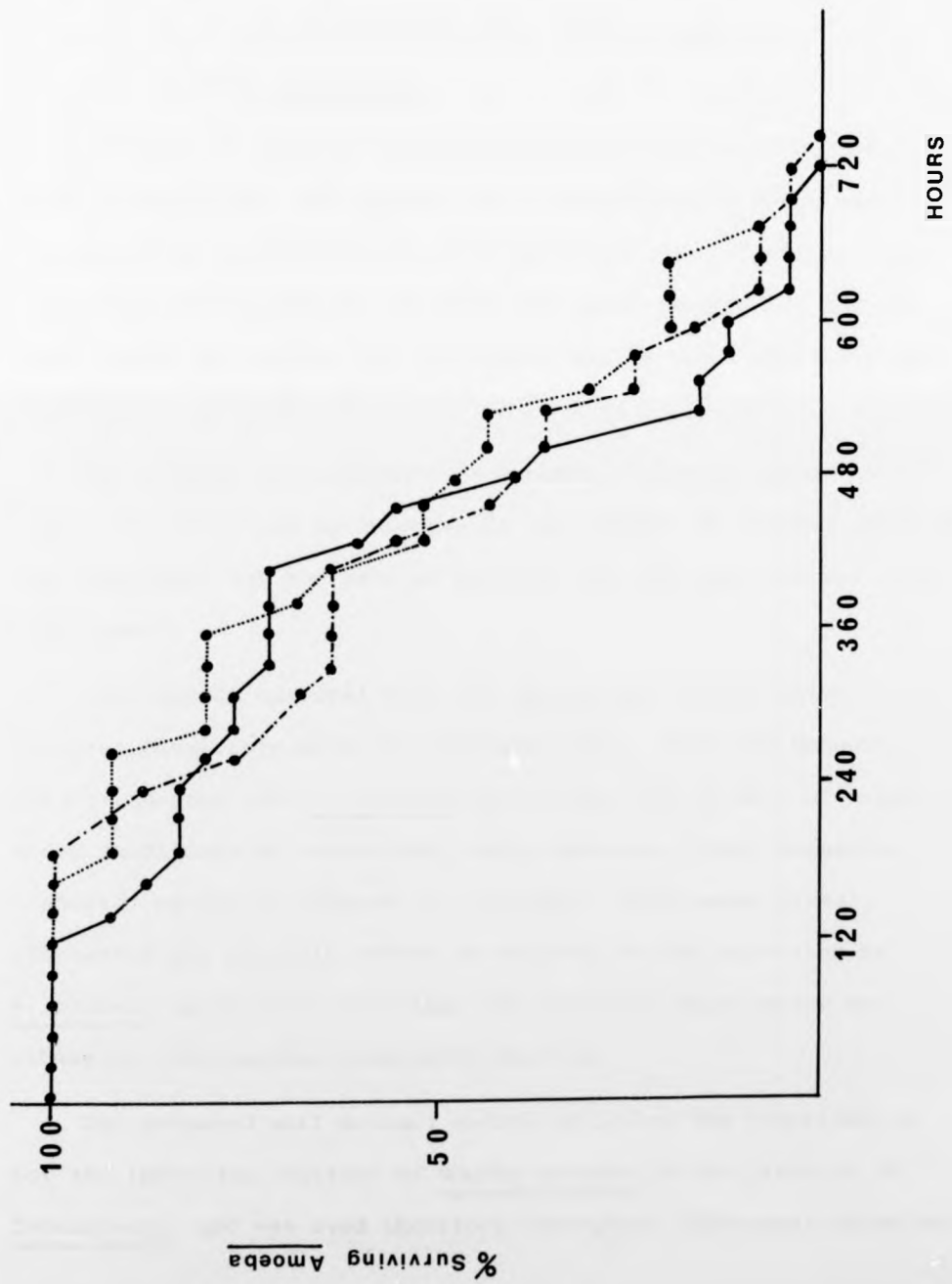
Figure 3.

The "Death Rate" of A. proteus.

Sterile Prescott's medium.

Sterile S.E.M.

S.E.M. containing A. aerogenes.


% Surviving Amoeba
100
50
120
240
360
480
600
720
HOURS

Three different conditions were examined:-

1. Sterile Prescotts Inorganic medium.
2. Sterile S.E.M.
3. S.E.M. inoculated with a dense suspension of A. aerogenes.

Solid watch glasses containing 1ml of media and 5 washed amoebae were used. The amoebae were transferred to fresh watch glasses every 24 hours to minimise bacterial contamination. Five replicates under each set of conditions were carried out and the total number of amoebae was recorded every 24 hours until all the populations had died out.

The results are tabulated in Appendix 3 and presented in Figure 3. There was no increase in the numbers of amoebae throughout the experiment and the rate of decrease was the same for all three conditions.

The longest survival time for Amoeba was 30 days which compares favourably with the published data. Mast and Hahnert (1935) reported that A. proteus can survive for 20 days or longer under conditions of starvation, while Andresen (1946) suggested a shorter period of between 10 - 20 days. Williamson (1944) discounted any possible effect of bacteria in the nutrition of A. proteus as he found that when the protozoan food source was exhausted, the amoebae invariably starved.

The proposed soil extract medium satisfied the requirements for the individual culture of Amoeba proteus in the presence of Tetrahymena, and was used therefore throughout subsequent experiments.

1.2.5. Experimental Vessel.

Preliminary studies on the growth of individual protozoa in Butt Cavity slides, depression slides and small petri dishes, highlighted the need for a vessel which had a volume large enough to make any evaporation irrelevant, and small enough for examination on a microscope stage. Covered solid watch glasses containing 1ml of sterile S.E.M. satisfied these requirements and were used throughout the entire research programme.

The watch glasses were washed with concentrated sulphuric acid (36N) after use and rinsed in distilled water. This careful cleaning procedure was found to be essential if a build up of contaminants on the glassware was to be avoided. Various workers agree that this unknown contamination is a general problem which can lead to the sudden death and lysing of the protozoan culture (pers. comm., Salt 1976).

1.2.6. "Watch glass culture" procedure.

Individual amoebae were placed in solid watch glasses containing 1ml of sterile S.E.M. and a known concentration of washed T. pyriformis. The washing procedure consisted of 3 gentle centrifugations (300g) using sterile S.E.M. A small number of bacteria was inevitably transferred on the surface of the amoebae. This provided the small background population of bacteria necessary for the growth of the Amoeba. The Tetrahymena cell counts were made using a Coulter Counter, model Z.B. (Plate 1). The use of the Coulter Counter for counting ciliated protozoa is not unique to this study. Laybourn (1973), Ricketts and Rappitt (1974) and Curds (pers. comm. 1976) have all used the instrument to count ciliates.

Plate 1.

The Coulter Counter.

1. Electronic circuit/control box.
2. Aperture viewing microscope.
3. Orifice tube.
4. Mercury manometer.
5. Vacuum tube.

ntrol box.

oscope.


COULTER COUNTER
MONITOR
1
5

1.2.7. Coulter Counter.

The number of particles (cells) suspended in an electrically conductive liquid (0.9% NaCl solution) was counted by drawing the sample through a small, 140μ, aperture (Plate 2) by means of a vacuum pump. A constant sample volume, 500μl, was measured each time by the passing of a column of mercury in a manometer over start and stop probes which activated the electronic counter. As a cell passed through the orifice it changed the resistance between two electrodes, one inside and one outside the aperture tube. To ensure that only Tetrahymena cells were being counted, the upper and lower threshold switches, which act as electronic gates above and below which there are no counts, and the aperture current and amplification switches, which alter the sensitivity of the instrument, were all set to predetermined levels (see calibration procedure, Appendix 4).

It was found that after the addition of electrolyte, the number of Tetrahymena suspended in the sample rapidly increased in number before decreasing as the cells lysed (Figure 4). Care was taken therefore to ensure that the counts were made immediately after the addition of the NaCl electrolyte.

As previously stated, S.E.M. also promoted cell division and although the initial concentration increased, the characteristic decrease associated with the other inorganic media, was not found and the protozoa remained in an active condition. It would have been desirable to have the food organism maintained at a constant concentration throughout the experiments but this was not possible. It was necessary therefore to document the change in Tetrahymena cell number and volume over the experimental period.

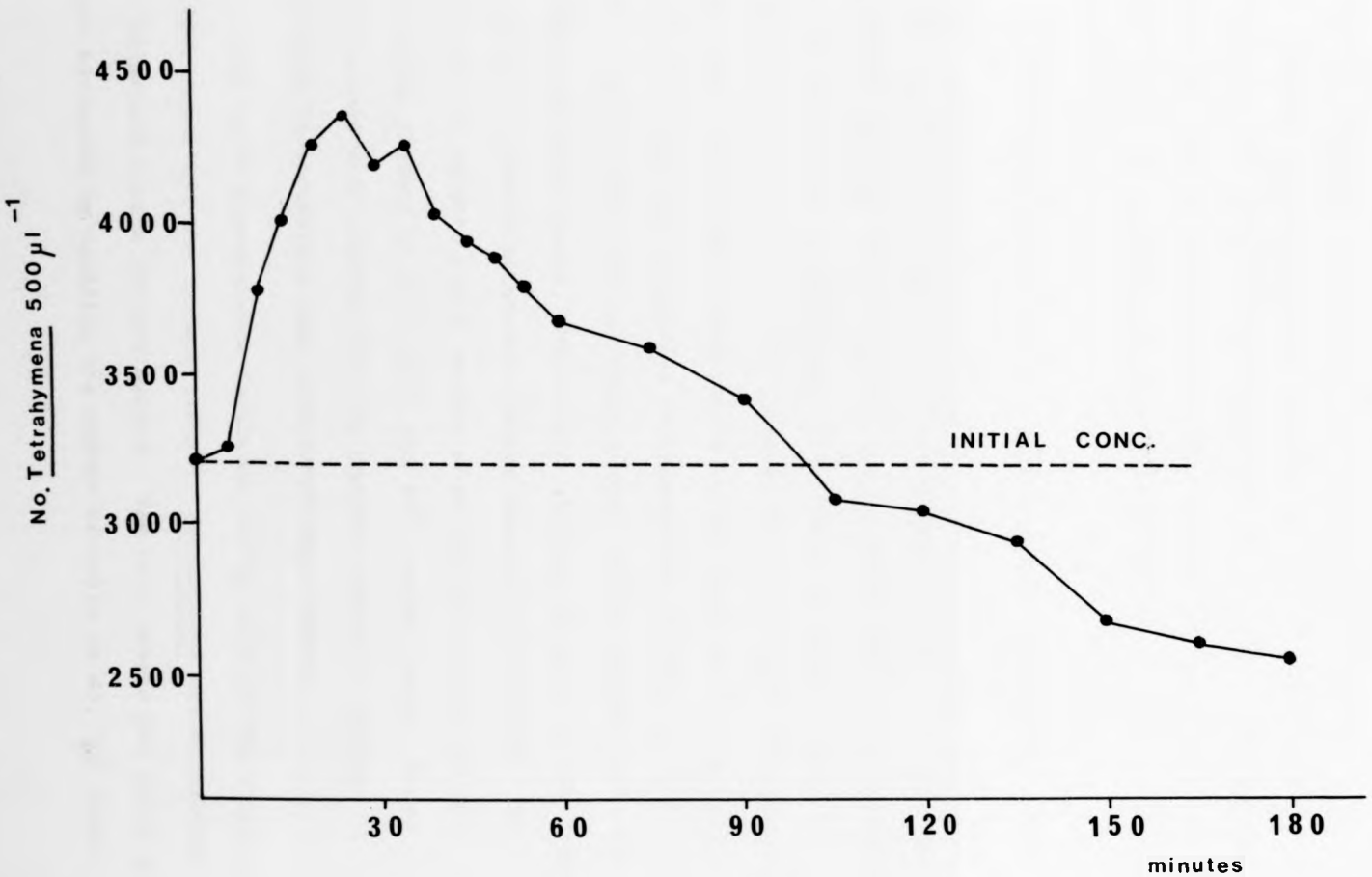
Plate 2.

140μ glass orifice tube.

Figure 4.

The effect of 0.9% NaCl electrolyte on the Tetrahymena cell count.

(for each point, n = 4).


4500
4000
3500
3000
2500
No. Tetrahymena 500µl^{-1}
INITIAL CONC.
30
60
90
120
150
180
minutes

1.2.8. Change in the number of *Tetrahymena* with time after addition to S.E.M.

1.2.8.1. Methods.

Washed *Tetrahymena* were counted and 1ml of the required suspension was pipetted into a sterile watch glass. Due to the rapid mobility of the cells, a pipette with a wide orifice was used to obtain accurate cell concentrations (pers. comm. Ricketts, 1976).

Three concentrations, 125, 1000 and 4000 *Tetrahymena* cells $500\mu l^{-1}$ were prepared for the three temperatures $10^{o}C$, $15^{o}C$ and $20^{o}C$. A similar set of vessels was prepared, which included the predator *Amoeba* , to investigate the possible impact of consumption on the numbers of *Tetrahymena*. The number of amoebae added to each watch glass varied with temperature and ciliate concentration employed. Preliminary experiments on the culture of *A. proteus* indicated that the population increased most rapidly at $20^{o}C$, over the range of temperatures investigated. It was assumed that for subsequent experiments, those watch glasses cultured at the higher temperatures would accumulate larger amoebae populations. 30 amoebae, 10 amoebae and 6 amoebae were therefore pipetted into the watch glasses at $20^{o}C$, $15^{o}C$ and $10^{o}C$ respectively. These populations were greater than the maximum number of *Amoeba* expected to accumulate over subsequent experiments.

The watch glasses were incubated in the dark at the required temperature, and the concentration of *Tetrahymena* was determined at intervals over a 30 hour period. The cell counts per watch glass were estimated by counting the number of cells in 40, 5µl drops

automatically dispensed from an Eppendorf pipette. After the watch glass was sampled, the vessel was discarded. Sufficient replicates had to be set up initially to enable a series of counts to be made at a series of intervals over the 30 hour experimental period.

1.2.8.2. Results.

In all cases the basic pattern was the same. The numbers of Tetrahymena increased over the 30 hour experimental period (Figures 5 - 7). The cells were not undergoing normal reproduction but were reacting to the stimulatory effect of the new culture conditions. At 10°C, mortality of the Tetrahymena cells was greater than at the higher temperatures. This was due to the fact that 10°C is close to the lower tolerance limit for this laboratory strain, shown by the very slow growth rate of cultures at this temperature.

The effect of including the predator A. proteus had no significant effect on the numbers of Tetrahymena, as is shown by the degree of overlap of the 95% confidence limits in the data (Appendix 5).

After 24 hours, the population of Tetrahymena showed a sharp increase. The cells at this point commenced normal cell division and growth, presumably due to the build up of a detectable bacterial flora which was utilized by the Tetrahymena as a food source. For subsequent experiments, amoebae were transferred to fresh culture conditions every 24 hours. Within this period the concentration of Tetrahymena was found to vary between the initial

Figure 5.

The change in Tetrahymena cell number and volume with time after addition to S.E.M.

Initial concentration, 125 Tetrahymena $500\mu l^{-1}$.

- ● $20^{\circ}C$
- ■ $15^{\circ}C$
- o $10^{\circ}C$
- —— cell volume (μm^3).
- ..... cell number (with Amoeba).
- -·-·- cell number (without Amoeba).

Number Tetrahymena $500\mu l^{-1}$

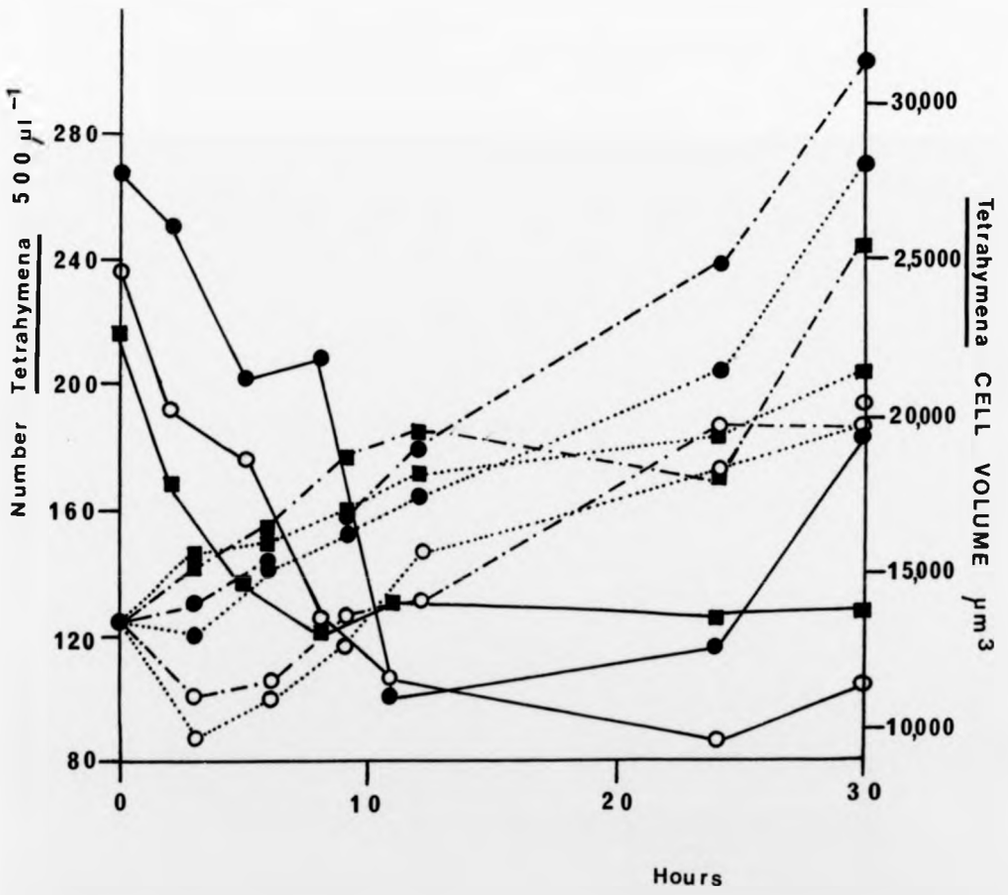

Number Tetrahymena 500 µl⁻¹
280
240
200
160
120
80
0
10
20
30
Hours
30,000
25,000
20,000
15,000
10,000
Tetrahymena CELL VOLUME µm3

Figure 6.

The change in Tetrahymena cell number and volume with time after addition to S.E.M.

Initial concentration, 1000 Tetrahymena $500\mu l^{-1}$.

● $20^{o}C$

■ $15^{o}C$

o $10^{o}C$

—— cell volume (μm^{3}).

..... cell number (with Amoeba).

-·-·- cell number (without Amoeba).

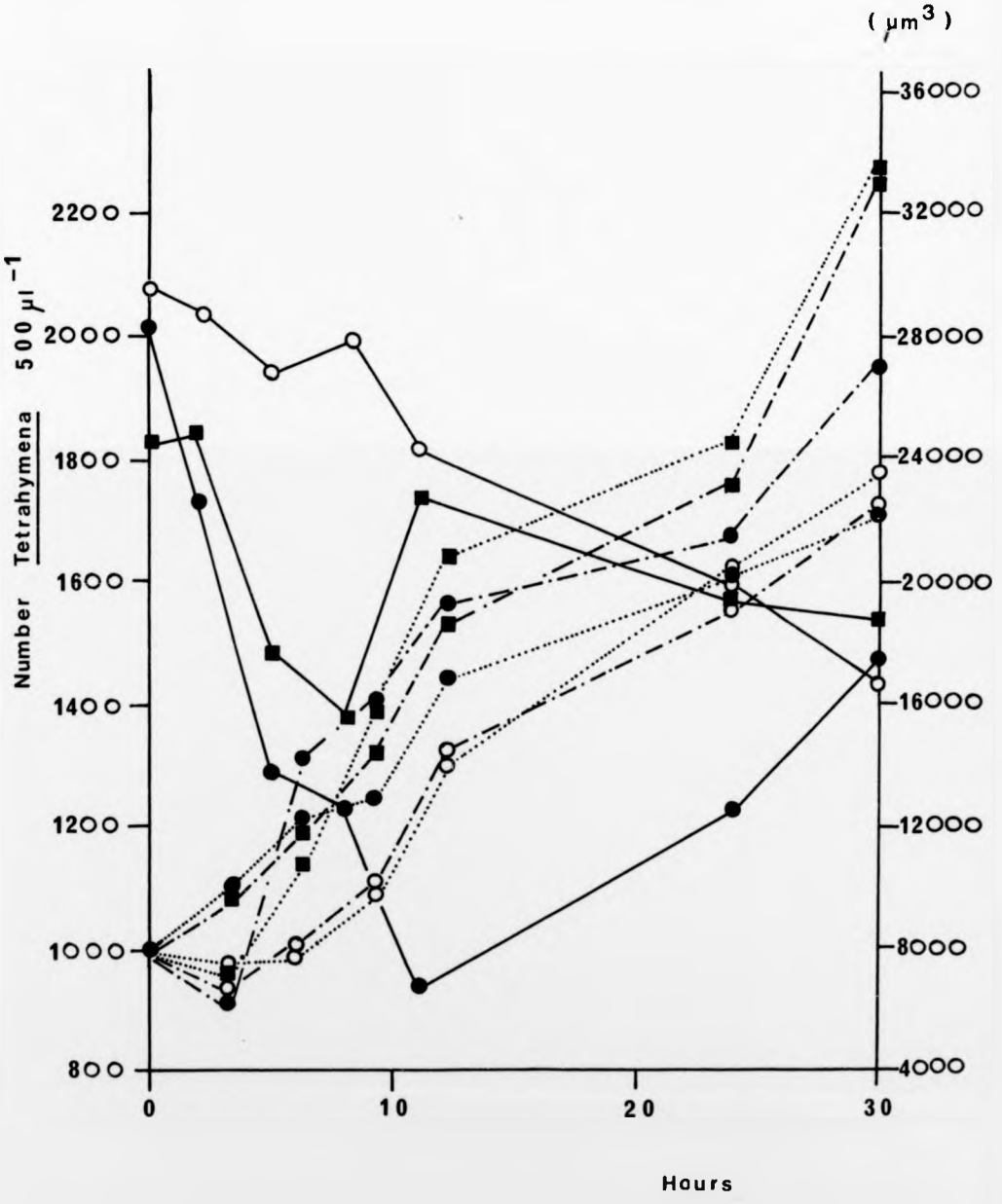

(μm³)
36000
32000
28000
24000
20000
16000
12000
8000
4000
2200
2000
1800
1600
1400
1200
1000
800
Number Tetrahymena 500 μl⁻¹
0
10
20
30
Hours

Figure 7.

The change in Tetrahymena cell number and volume with time after addition to S.E.M.

Initial concentration, 4000 Tetrahymena $500\mu l^{-1}$.

● $20^{o}C$

■ $15^{o}C$

o $10^{o}C$

—— cell volume (μm^{3}).

..... cell number (with Amoeba).

-·-·- cell number (without Amoeba).

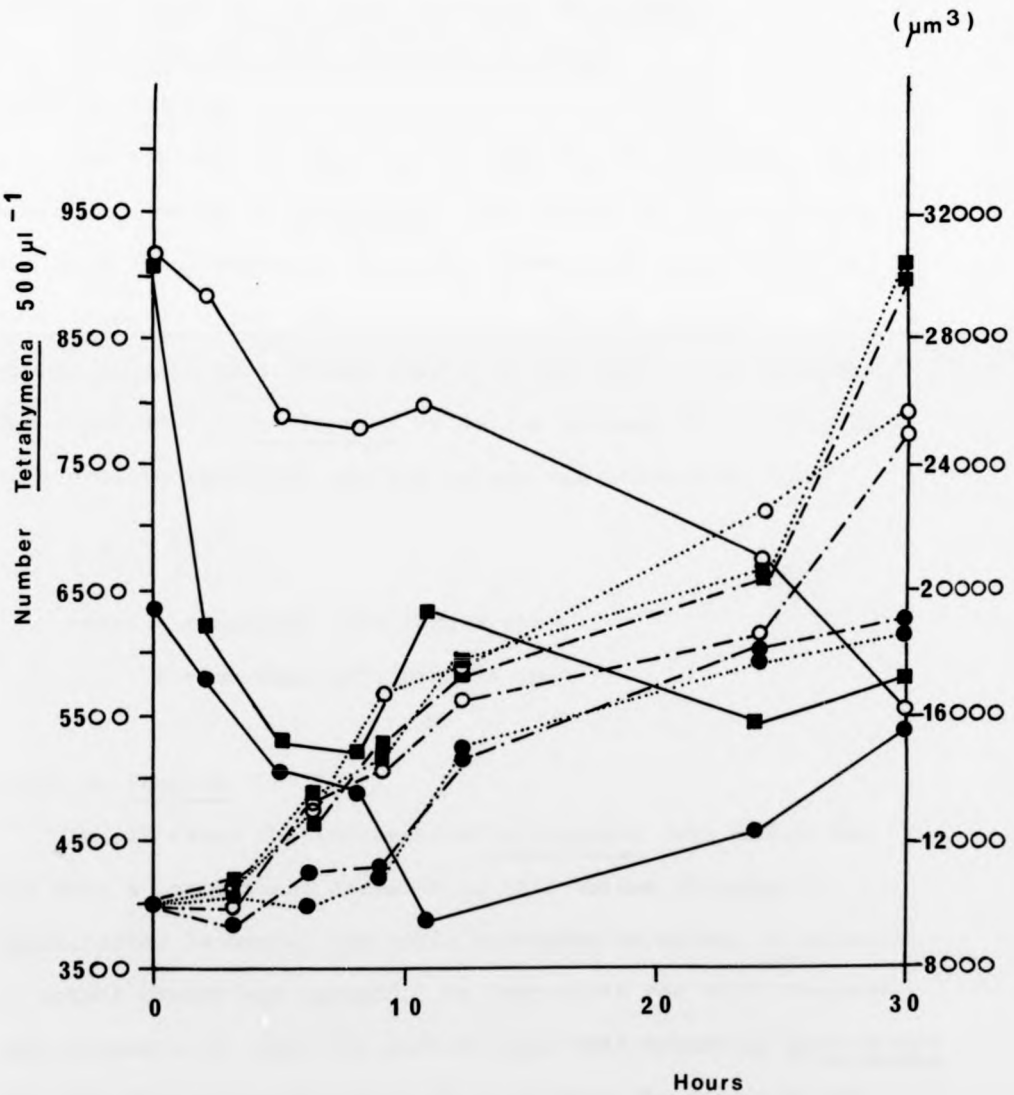
nd

M.

ymena 500µl^{-1}.

eba).

Amoeba).



cell concentration and an average increase of 69% at 20°C, 64% at 15°C and 51% at 10°C.

1.2.9. Change in the cell volume of Tetrahymena with time after addition to S.E.M.

1.2.9.1. Methods.

The initial procedure was as detailed in Section 1.2.8.1. where the change in Tetrahymena cell number was investigated. For each time interval, 50 cells, fixed in 4% Glutaraldehyde, were measured using a microscope and eyepiece graticule. The change in mean cell volume over a 30 hour period was recorded. The shape of the Tetrahymena cells was assumed to be equivalent to a prolate spheroid, and the volume was calculated from:

$$\frac{4}{3}\pi \cdot \frac{a}{2}\cdot\left(\frac{b}{2}\right)^2$$

where a = maximum cell length (μm)

b = maximum cell breadth (μm)

1.2.9.2. Results.

In all cases the increase in Tetrahymena cell number was met with a concomitant decrease in cell volume (Figures 5 - 7). Again, after 24 hours, the cells increased in volume as a return to normal growth was resumed. To compensate for this changing cell volume with time, the overall mean cell volume of Tetrahymena over the 24 hour period was calculated from the measured cell volumes at each time interval. Neither temperature nor the initial Tetrahymena cell concentration significantly affected the average cell volume. The data is tabulated in Appendix 6 where the

respective standard deviations about the means are included.

An overall cell volume of 19,500 μm^3 was calculated for Tetrahymena pyriformis, regardless of temperature, over the 24 hour experimental period. This estimate was used throughout the subsequent feeding experiments. An initial cell volume of 26,481 μm^3, representing the mean cell volume of Tetrahymena when cultured in 0.5% proteose-peptone, was also obtained and was used throughout the calorimetry and dry weight investigations.

Curds and Cockburn (1971) reported a considerable range in the volume of Tetrahymena grown in continuous culture. Cells were found to vary between 5.4×10^3 and 49.5×10^3 μm^3, a range which encompasses the results of the present study.

1.2.10. Conclusions.

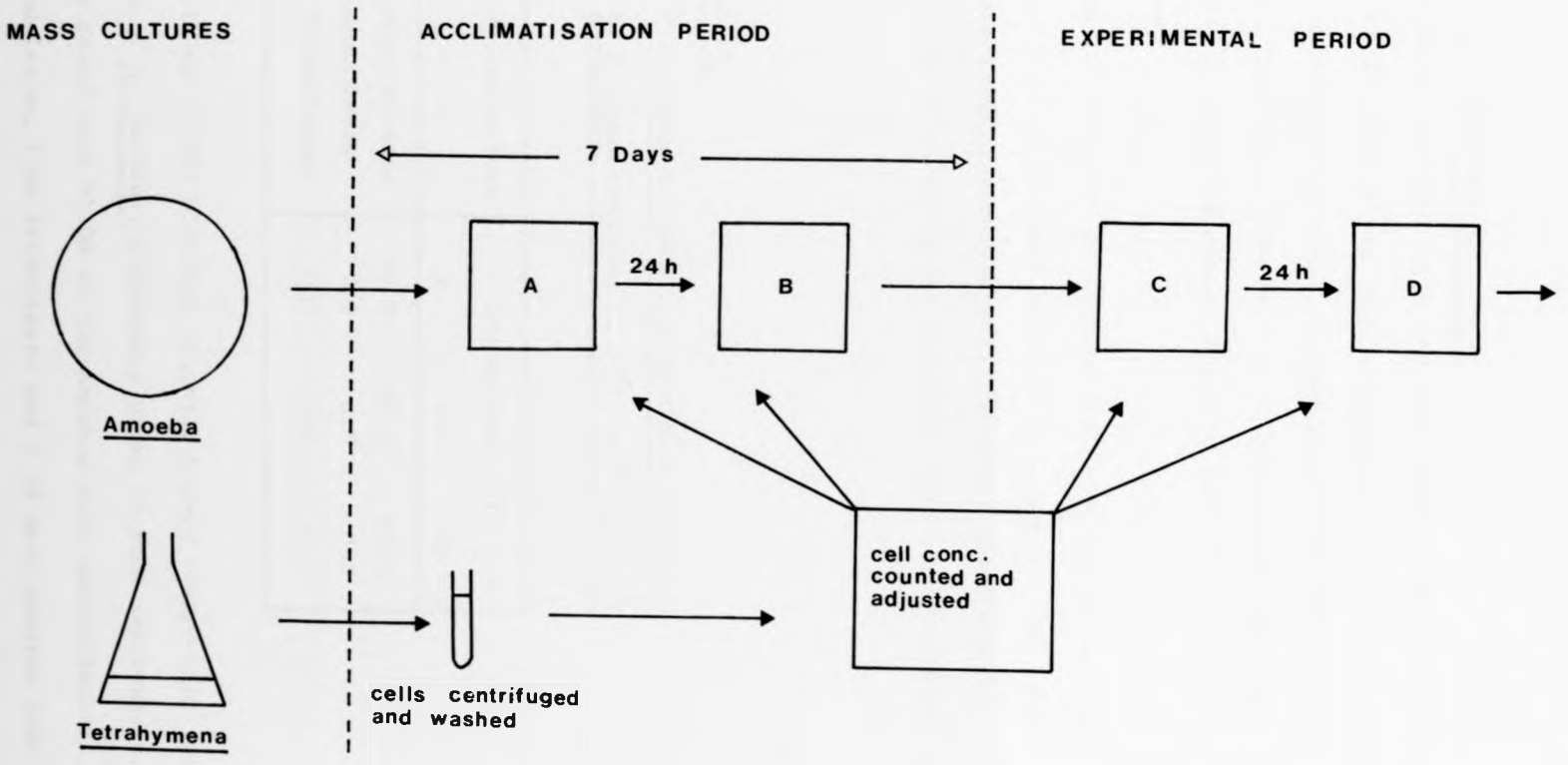
Since the changes regarding the cell volume and number of Tetrahymena pyriformis over a 24 hour period could be predicted and quantified, all specimens of amoebae for subsequent experiments were acclimatised to the relevant food concentration and temperature by growing them for at least 7 days in watch glasses. Amoeba were transferred every 24 hours to fresh vessels containing the correct initial Tetrahymena concentration in S.E.M.

This "watch glass" procedure was used throughout the entire study period and is summarised diagrammatically in Figure 8.

Figure 8.

Diagrammatic representation of the "watch glass culture" method.

A, B, C and D are watch glasses. Individual *Amoeba* were transferred every 24 hours to fresh culture conditions.


MASS CULTURES
ACCLIMATISATION PERIOD
EXPERIMENTAL PERIOD
7 Days
A
24 h
B
C
24 h
D
Amoeba
Tetrahymena
cell conc.
counted and
adjusted
cells centrifuged
and washed

1.2.11. Multinucleate A. proteus.

As early as 1906, Stolc and later Levy (1924), observed as many as six nuclei per single Amoeba cell. The occurrence of such multinucleate cells in subsequent experiments would produce incomparable results. An investigation into the distribution of multinucleate forms in the cultures was undertaken.

Table 1 lists the percentage frequency of the various nucleate forms of randomly selected A. proteus. 200 cells were taken from mass cultures at the three temperatures, stained with 1% methyl green in acetic acid, and examined microscopically.

Table 1.

Percentage frequency of nucleate forms in A. proteus cultured at 20°C, 15°C and 10°C.

Nucleate Form	Temperature (°C)		
	20	15	10
Mononucleate	98.0	97.5	96.0
Binucleate	2.0	2.5	3.5
Trinucleate	0.0	0.0	0.5

Chalkley (1931) obtained slightly higher frequencies for cultures of A. proteus, presumably grown at room temperature. Chalkley found that 91.8% of the amoebae were mononucleate, 5.7% binucleate, 1.4% trinucleate and 1.1% were greater than

trinucleate. The occurrence of the low numbers of multinucleate forms found for the present study was probably a function of the improved culture conditions employed.

It can be concluded that although there is a trend towards a greater proportion of multinucleate forms in cultures with decreasing temperatures, the majority of forms were mononucleate. Results obtained from multinucleate amoebae throughout subsequent experiments were disregarded.

Chapter 2.

2.1. Dried Weight and Calorimetry determinations.

2.1.1. Introduction.

In ecological energetics, which is the study of energy transformations from one form to another within ecosystems, there is a need for a common unit of energy, traditionally the calorie, but more recently the joule. The calorific content of the organisms in any energetics study is an important variable, and so the energy values of both A. proteus and T. pyriformis were determined. Because of the difficulties in obtaining monospecific samples of sufficient size for direct calorimetry, few studies measure the energy content of both species. With the exception of Laybourn (1973) who compiled a series of energy budgets for Colpidium campylum when fed on the bacterium Moraxella (sp), the present study is rare in that the energy content of both protozoan species was considered. Heal (1967a), who has published the only other energy budget for a sarcodine, (Acanthamoeba, cultured on the yeast Saccharomyces cerevisiae) used dry weight estimates to describe the budget.

The heats of combustion can be measured directly using a micro-bomb calorimeter as described by Phillipson (1964). This technique is suitable where the material readily burns, as was the case for the protozoan pellets. An alternative approach to

calorimetry, in cases where combustion is incomplete, is the use of an indirect chemical method. Two such approaches are described by Crisp (1971). The first measures the total oxidisable matter in the sample and applies an appropriate oxy-calorific coefficient to convert the oxygen demand to energy units. The second approach analyses the biochemical components of a sample and multiplies them by an appropriate calorific content conversion.

Before the energy values of Amoeba and Tetrahymena were determined, it was necessary to investigate the relationships between protozoan cell volume and the cell dry weight. In addition, dry weight measurements are important in ecological studies as they allow the expression of abundance as a single, readily comparable value.

Curds and Cockburn (1971) estimated the dry weight of Tetrahymena using an interference microscope. The methods more commonly adopted, however, rely on counting, centrifuging, drying and weighing procedures, (Heal, 1967a; Ormsbree, 1942; Laybourn, 1973; and others).

Determination of the Dried Weights of Tetrahymena and Amoeba.

2.1.2. Materials and Methods: Tetrahymena pyriformis.

Cultures were grown in 0.5% proteose-peptone as described previously (Page **13**). A suspension of Tetrahymena, in the logarithmic phase of growth, was washed twice in sterile distilled water by centrifugation (Page **14**) and counted electronically using the Coulter Counter (Page **23**). The washed suspension of cells was further centrifuged until a pellet was formed. The supernatant fluid was retained for counts of the protozoa. The pellet containing a known number of Tetrahymena cells was carefully collected and vacuum freeze-dried for 24 hours, the time required to reach a constant weight. The dried pellet was weighed using a microbalance and the average weight of an individual Tetrahymena cell calculated. 10 such replicate experiments were carried out for each of the three temperatures, 10°C, 15°C and 20°C. The weights obtained were converted to unit protoplasm terms using the mean cell volume derived for Tetrahymena cultured in proteose-peptone, namely 26,481μm^3, as detailed on Page 32.

2.1.3. Materials and Methods: Amoeba proteus.

Mass cultures of Amoeba were grown as described previously (Section 1.1.2.). Amoebae were washed in sterile distilled water to remove adhering bacteria. This was done by gently rotating the culture dishes until the amoebae had been swept into the centre of the vessel. Amoebae were then pipetted onto a fresh petri dish containing sterile distilled water. This rotation and washing procedure was repeated at least 3 times.

The number of amoebae in a washed suspension was estimated by counting 30, 5µl drops, sampled with an Eppendorf pipette. The amoebae were harvested by centrifugation and the number of cells left in the supernatant was accounted for. The pellet obtained was freeze-dried until constant weight and weighed on a micro-balance.

The weight of an individual Amoeba was calculated, with 10 replicates being carried out at 20°C, 6 replicates at 15°C and 6 replicates at 10°C. The number of replicates was dependent upon the availability of cellular material, with less at the lower temperatures where the growth of cultures was slow.

The mean cell volume of A. proteus grown in mass culture at 10°C, 15°C and 20°C was determined to enable the volume to dry weight conversions to be calculated. 50 amoebae were randomly selected from the mass cultures at each of the three temperatures investigated. The volumes of the cells were ascertained by the compression technique as described in Section 5.1.2., and the mean cell volume for each temperature calculated as 859 ± 191 (S.D.) x $10^3 \mu m^3$, 1077 ± 232 (S.D.) x $10^3 \mu m^3$ and 2022 ± 585 (S.D.) x $10^3 \mu m^3$ at 20°C, 15°C and 10°C respectively.

2.1.4. Results.

The dried weights of Tetrahymena and Amoeba per unit protoplasm when cultured at 10°C, 15°C and 20°C are given in Tables 2 and 3 respectively.

In both cases, regardless of temperature, the variation between weights was not great allowing a mean conversion factor to be calculated for each of the two protozoan species. The overall

Table 2.

Dried weights of T. pyriformis (pg μm^{-3}) for 20°C, 15°C and 10°C.

Temperature	Sample	Dried weight pg μm^{-3}
20°C	1	0.133
	2	0.147
	3	0.168
	4	0.123
	5	0.197
	6	0.202
	7	0.118
	8	0.143
	9	0.139
	10	0.137
	Mean 0.150 ± 0.029 (S.D.)	
15°C	1	0.146
	2	0.170
	3	0.157
	4	0.149
	5	0.152
	6	0.147
	7	0.188
	8	0.166
	9	0.128
	10	0.223
	Mean 0.163 ± 0.027 (S.D.)	
10°C	1	0.236
	2	0.186
	3	0.168
	4	0.170
	5	0.177
	6	0.145
	7	0.107
	8	0.215
	9	0.155
	10	0.168
	Mean 0.173 ± 0.036 (S.D.)	

Overall mean 0.162 ± 0.031 (S.D.)

Table 3.

Dried weights of A. proteus (pg μm^{-3}) for 20°C, 15°C and 10°C.

Temperature	Sample	Dried weight pg μm^{-3}
20°C	1	0.123
	2	0.123
	3	0.148
	4	0.114
	5	0.110
	6	0.095
	7	0.129
	8	0.114
	9	0.124
	10	0.117
	Mean 0.120 ± 0.014 (S.D.)	
15°C	1	0.171
	2	0.148
	3	0.123
	4	0.171
	5	0.141
	6	0.161
	Mean 0.152 ± 0.019 (S.D.)	
10°C	1	0.181
	2	0.148
	3	0.195
	4	0.195
	5	0.139
	6	0.157
	Mean 0.169 ± 0.020 (S.D.)	

Overall mean 0.147 ± 0.025 (S.D.)

conversion for converting the volume of Tetrahymena protoplasm (μm^3) to dry weight terms (pg) was found to be 0.162pg μm^{-3} of cell protoplasm. The value for Amoeba was less at 0.147pg μm^{-3} of cell protoplasm.

2.1.5. The Determination of the energy content of A. proteus and T. pyriformis: Materials and Methods.

Dried protozoan biomass for both species, Tetrahymena and Amoeba, was collected as a result of the dried weight determinations. As before, the number of replicates was a function of the quantity of cells harvested. Material was compressed to form pellets within the weight range of 1 - 6mg. The calorific content of the respective pellets was determined by burning the material in a micro-bomb calorimeter as designed by Phillipson (1964) and manufactured by Gentry-Weigert of Aiken, Carolina.

The apparatus consisted of a stainless steel bomb which contained the sample pellet and oxygen under a pressure of 30 atmospheres. The pellet was ignited by passing an electric charge of 35V through a fine platinum wire attached to the sample. The heat liberated by the oxidation of the pellet increased the temperature of the bomb, producing a voltage potential in the copper ring and thermocouple junctions on which the bomb was seated. The voltage was directly proportional to the temperature change and was measured on a potentiometric Telsec Chart Recorder.

The potentiometer reading was calibrated according to Phillipson (1964) and Prus (1968a). Weighed pellets of Benzoic acid, C_6H_5COOH, yielding 26.455 Jmg^{-1} as determined by the National

Figure 9.

The Benzoic Acid Calibration.

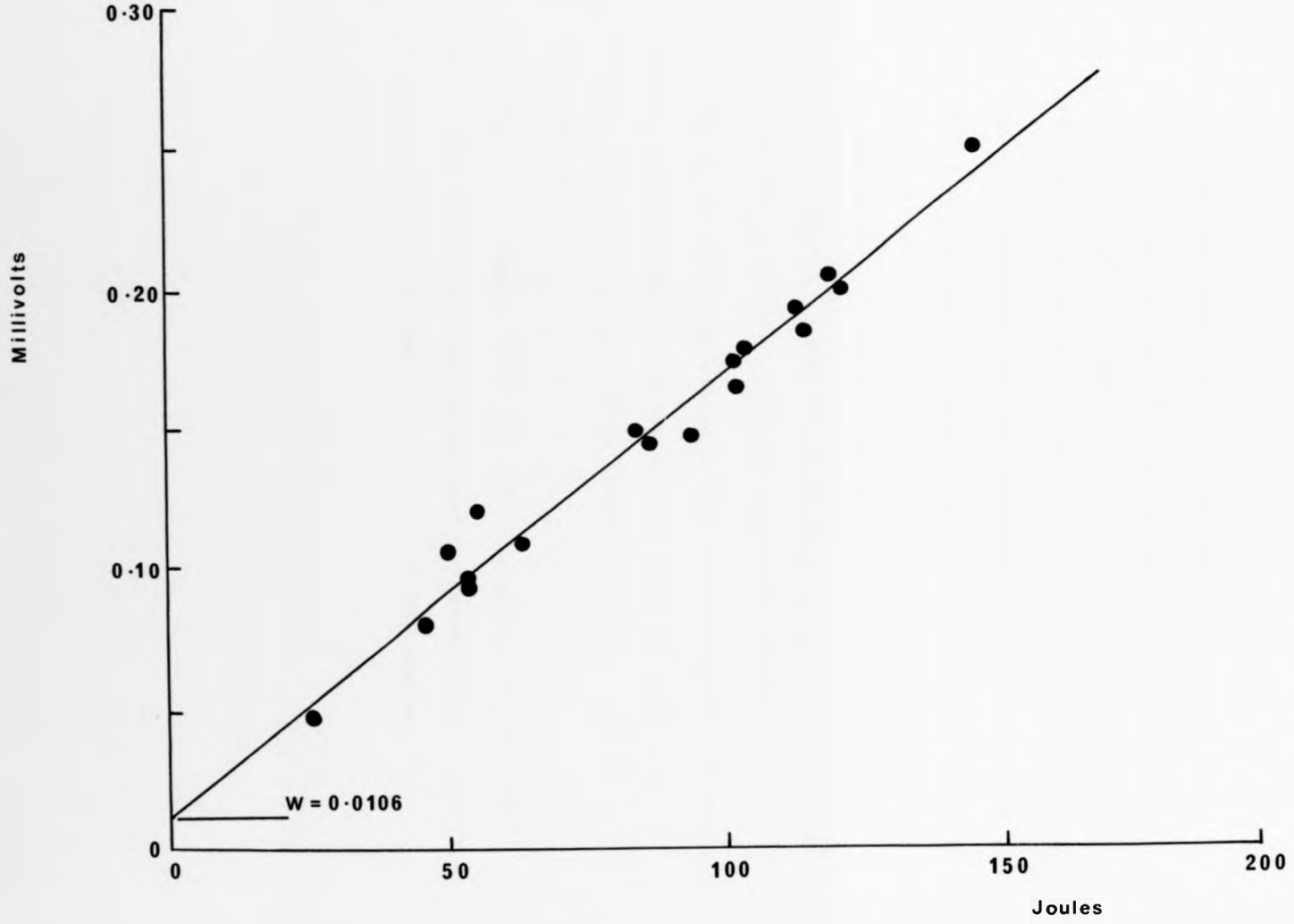
$y = 0.0016\ x + 0.0106$

$r = 0.9889$

$n = 18$

$p = 0.001$ (highly significant)

$W = 0.0106$


0·30
0·20
0·10
0
Millivolts
W = 0·0106
0
50
100
150
200
Joules

Physical Laboratories, were used for the calibration. 18 acid pellets within the range 1.01 - 5.42mg were combusted. Over the range examined the relationship between the energy content of Benzoic acid and the potentiometer recording in millivolts was found to be linear (Figure 9).

The value between O and the intercept, W, represents the heat output of the platinum wire, a value of 0.0106mV. Corrections were also applied for prefiring and postfiring changes in potential, as outlined by Crisp (1971). The nitric acid correction of Golley (1961) was not employed as the heat generated by the acid production was considered negligible by Paine (1964).

Due to the vigorous nature of the reaction within the bomb, accurate measurements of the ash content were not possible. An estimate was obtained from those samples leaving a residue on the platinum pan.

The potentiometer trace was used in conjunction with the calibration factor and the corrections mentioned to determine the calorific content, in Jmg^{-1} dry weight, of the samples.

2.1.6. Calorimetry: Results

The Benzoic acid calibration was linear and highly significant ($p = 0.001$) over the range of pellet weights combusted as shown in Figure 9. The wire correction W, which compensates for the heat of combustion of the platinum ignition wire, was found to be 6.10J. The results are given in Appendix 7.

The relationships between the pellet weight and energy yield for T. pyriformis and A. proteus are shown in Figures 10 - 12. Again, in all cases, the relationships were linear and highly significant ($p = 0.001$) over the range of pellets combusted. For all linear relationships, the regression lines were calculated with the method of least squares.

The calorific contents of Tetrahymena, joules mg^{-1}, are presented in Table 4. A t-test indicated no significant difference ($p = 0.001$) between the energy content of Tetrahymena cells cultured at 20°C and 15°C. When the results for those temperatures were compared with the values obtained for 10°C, a highly significant difference (t-test; $p = 0.001$) was found. In other words, the mean energy content of Tetrahymena cells cultured at 20°C and 15°C was greater at 19.80 $\pm$ 0.72 (S.D.) joules mg^{-1} compared with those cells cultured at 10°C, where a lower mean energy value of 18.28 $\pm$ 0.41 (S.D.) joules mg^{-1} was obtained.

Temperature was found to have no significant effect on the energy content of A. proteus, an overall mean value spanning the three temperatures was therefore adopted (Table 5). This value shows that the mean calorific content of A. proteus was in all cases lower than that of the food organism, T. pyriformis.

Figure 10.

The relationship between pellet weight and energy yield of Tetrahymena at 15°C and 20°C.

$y = 0.0471 x + 0.4157$

$r = 0.9964$

$n = 16$

$p = 0.001$ (highly significant)

Figure 11.

The relationship between pellet weight and energy yield of Tetrahymena at 10°C.

$y = 0.0518 x + 0.3569$

$r = 0.9912$

$n = 8$

$p = 0.001$ (highly significant)

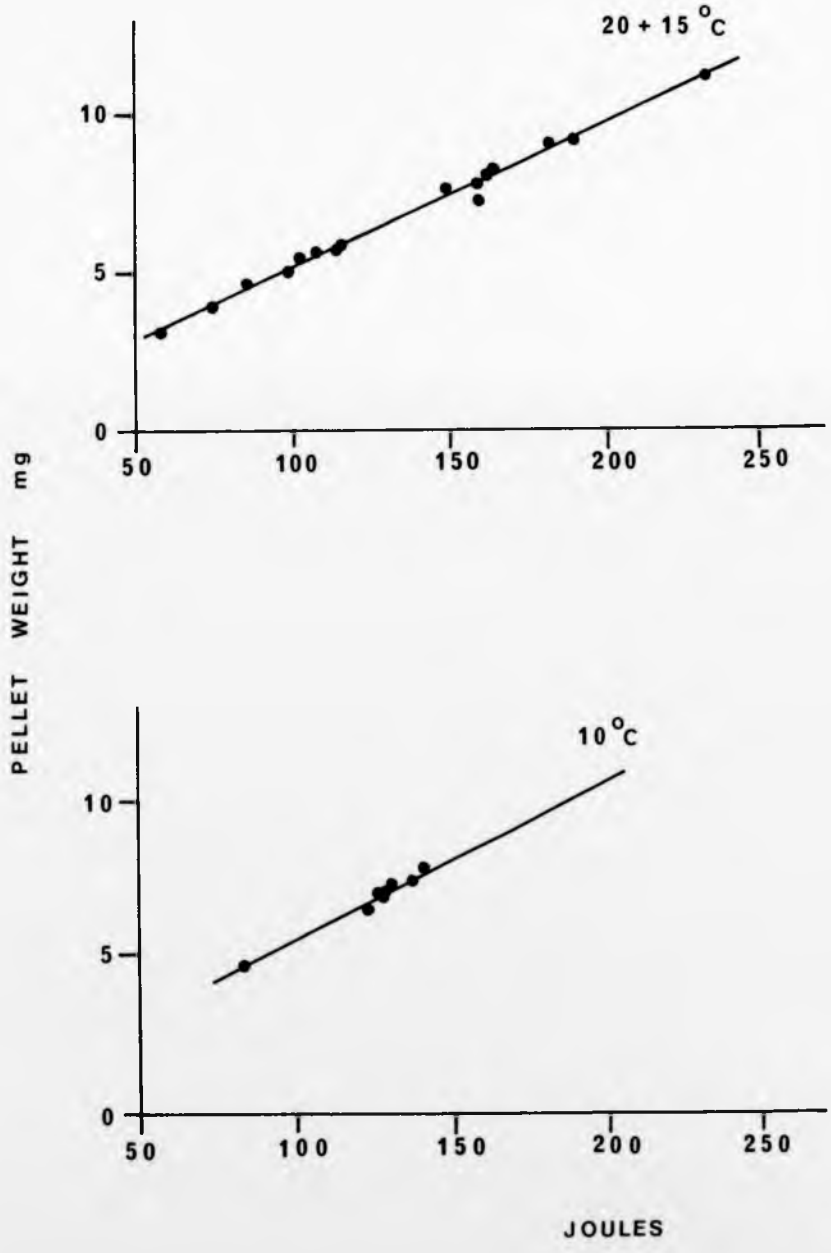

20 + 15 °C
10
5
0
50
100
150
200
250
PELLET WEIGHT mg
10 °C
10
5
0
50
100
150
200
250
JOULES

Figure 12.

The Relationship between pellet weight and energy yield of Amoeba.

$y = 0.0539 x + 0.1359$

$r = 0.9907$

$n = 20$

$p = 0.001$ (highly significant)

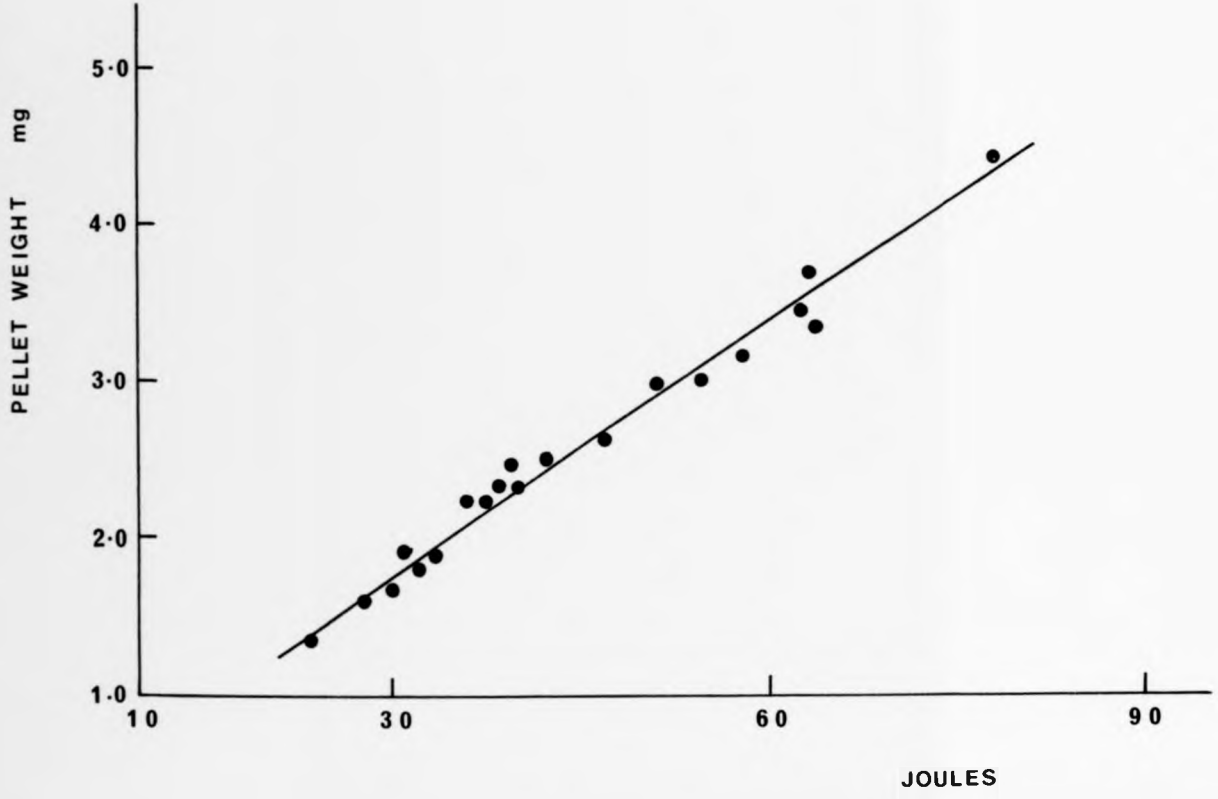

PELLET WEIGHT mg
5·0
4·0
3·0
2·0
1·0
10
30
60
90
JOULES

Table 4.

Calorific determinations of Tetrahymena pyriformis.

Temperature	Sample	Joules mg^{-1} dry weight
20°C	1	21.64
	2	19.73
	3	19.34
	4	19.74
	5	20.33
	6	18.71
	7	19.72
	8	19.10
15°C	9	19.30
	10	19.85
	11	20.38
	12	19.93
	13	20.55
	14	19.78
	15	18.75
	16	19.91
Combined mean 19.80 ± 0.72 (S.D.)		
10°C	17	18.32
	18	18.97
	19	18.46
	20	18.12
	21	17.61
	22	18.58
	23	18.09
	24	18.07
Mean 18.28 ± 0.41 (S.D.)		

The estimated ash contents for Tetrahymena and Amoeba were 2.0% and 4.2% respectively. It was not deemed necessary to correct the calorific values to ash-free terms as the percentage ash content for both species was low.

Table 5.

Calorific determinations of Amoeba proteus.

oteus.

Temperature	Sample	Joules mg^{-1}
20°C	1	17.97
	2	16.32
	3	18.29
	4	19.05
	5	17.54
	6	17.28
	7	17.10
	8	18.21
15°C	9	16.37
	10	16.06
	11	16.24
	12	18.42
	13	18.33
	14	17.88
	15	17.34
	16	17.21
10°C	17	17.40
	18	16.69
	19	18.19
	20	17.27
Overall mean 17.51 ± 0.77 (S.D.)		

2.1.7. Calorimetry and Dried Weight determinations: Discussion.

Since Slobodkin and Richman's paper of 1961, much information on the calorific value of animals has been published. Typical publications on this topic include those by Golley (1961), Paine (1964), Cummins and Wuycheck (1967) and Thayer et al (1973).

According to Paine (1971), the calorific values of animals should fall between a lower limit of 3.74kcal g^{-1} (equivalent to 15.65J mg^{-1}) set by glucose, and an upper limited of 9.37kcal g^{-1} (equivalent to 39.20J mg^{-1}) as determined by the value for oils and fatty acids. Because of the chemical constitution of an organism, representatives of a major group will have intermediate values between these extremes, probably biased towards the lower end of the range. This supports the results obtained for the protozoan species investigated in the present study.

Much of the literature, with regard to the calorific content of aquatic animals, has been summarised by Prus (1970). The range of values, covering 63 species, extended from 4.2 to 6.8 kcal g^{-1} ash free dry weight (17.57 - 28.45J mg^{-1} dry weight) although the majority of the species, 37 in all, were grouped by Prus within the range 5.2 - 6.0kcal g^{-1} (21.76 - 25.10J mg^{-1} dry weight).

Cummins and Wuycheck (1967) compiled an extensive list of comparative calorific data to aid ecological energeticists. It indicated significant differences in energy value, on an ash free basis, between various ecological categories. The primary producers had a mean calorific rating equivalent to 19.58J mg^{-1}, the detritus consumers a mean of 20.44J mg^{-1} and the macroconsumers

had a mean value of 24.35J mg^{-1}. When the latter categorie was subdivided into aquatic and terrestrial consumers, the energy content of aquatic macroconsumers was lower at 22.86J mg^{-1} as opposed to 25.52J mg^{-1} for their terrestrial counterparts.

Although the results for Amoeba and Tetrahymena were, in general, slightly lower than the calorific values presented by Cummins and Wuycheck (1967) and Prus (1970), they were close enough to the reported values to be accepted with confidence, especially in view of the fact that most of the comparable data relates to multicellular invertibrates.

Published calorific values for protozoan species are few. Slobodkin and Richman (1961) found the energy content of Tetrahymena to be equivalent to 24.84J mg^{-1}, by bomb calorimetry, while Klekowski and Shuskina (1966) found Paramecium caudatum to have a much lower value equivalent to 16.01J mg^{-1}, by analysis of the chemical composition of the cell. 20.15J mg^{-1} was the value reported by Laybourn (1973) for the ciliate Colpidium campylum obtained by micro-bomb calorimetry.

The results of the present study certainly tend to agree with those of Laybourn (1973) and place the protozoa at the lower limit of the invertebrate scale with the values obtained for Tetrahymena pyriformis being 19.80J mg^{-1} (20°C and 15°C) and 18.28J mg^{-1} (10°C) while the energy content of A. proteus averaged an overall lower value of 17.51J mg^{-1}, regardless of the culture temperature.

It is of interest to note that temperature was found to have a significant effect on the calorific content of T. pyriformis, with a lower value being obtained for cells cultured at 10°C.

Laybourn (1973) measured the energy content of Colpidium campylum at 10°C, 15°C and 20°C but found no difference attributable to temperature. Similarly, temperature had no effect on the energy content of A. proteus in the present study.

It would appear that Tetrahymena has a different body composition at 10°C, possibly as a result of the inability of some metabolic pathways to operate at lower temperatures. 10°C has already been shown to present problems for the laboratory culture of this organism, suggesting that this temperature is close to its lowest thermal limit (Page 27).

The lower calorific content of Amoeba, as compared to Tetrahymena, is also important as it represents an interspecific difference between the two protozoan groups. These differences are sufficient to prevent total confidence in the application of a general calorific value for all protozoan ecological studies. However, as more data becomes available, overall conversions may become appropriate for the major protozoan groups. The findings of Laybourn (1973) for Colpidium were close to those reported for Tetrahymena at 15°C and 20°C in the present study. It is probable that the energy content of ciliated protozoa is close to 20.00J mg^{-1} dry weight when under conditions of normal growth.

The calculated dry weight values, 0.0043µg per cell for T. pyriformis regardless of temperature, and 0.13, 0.16 and 0.30µg per cell for A. proteus at 20°C, 15°C and 10°C respectively, are useful for comparative and conversion purposes. Biomass values often indicate the bulk of material in terms of wet weight, but

these are mainly dependent upon the cell water content, which is variable. Dry weight values estimate the quantity of organic and non-organic matter and are therefore a better measure of the amount of material present which could be metabolically useful to a predator species.

The dry weight estimates, in conjunction with the calculated cell volumes, permitted the determination of a conversion factor linking the cell volume and cell weight. It was found for A. proteus that the weight of a unit of protoplasm (μm^3) was 0.147pg, while the same for T. pyriformis was 0.162pg, irrespective of temperature.

The higher value for Tetrahymena may have been due to the different culture techniques employed between the ciliates and amoebae, Tetrahymena having been grown under the artificial condition of proteose-peptone solution. The result obtained, 0.162pg μm^{-3} protoplasm, however does compare favourably with the value of 0.170pg μm^{-3} reported by Laybourn (1973) for Colpidium campylum cultured on bacteria. This suggests that a standard conversion for ciliate protoplasm to dry weight units may be applicable, regardless of species.

The overall cell weight of Tetrahymena, 4290pg per cell (assuming a cell volume of 26481 μm^3, Page **32**) agrees closely with the published data. Cameron (1973), in a review on the cell growth cycle of Tetrahymena reported a range of dry cell weights of between 2400 - 4250pg per cell. Similarly, Scherbaum and Rasch (1957) gave the approximate dry weight of a Tetrahymena cell as 5000pg.

With reference to A. proteus, the dry cell weight was found to be 0.13µg at 20°C, a value close to the published one of 0.10µg per cell for A. proteus, presumably cultured at room temperature, by Kawai, Maki, Akaboshi and Shimizu (1976), who used amoebae as an experimental organism to clarify the lethal action of thermal neutrons. Comparative data for the conversion factor, 0.147pg μm^{-3} protoplasm, is not available for A. proteus. However, the value of 0.116pg μm^{-3} can be calculated from the dry weight estimate of Kawai et al (1976) and the volume determinations from the present study. In addition, Heal (1967a) estimated the dry weight of Acanthamoeba as 0.3738mg per 10^6 amoebae when cultured on yeast at 25°C. Byers et al (1969) calculated the cell volume of the same amoebae to be 3365 μm^3, which gives a conversion value of 0.111pg μm^{-3}.

The value of 0.147pg μm^{-3} found for A. proteus in the present study, and the calculated values of 0.116pg μm^{-3} for Amoeba and 0.111pg μm^{-3} for Acanthamoeba indicate an overall value of 0.12pg μm^{-3} for the naked amoebae. It may be that the sarcodines, in general, have a lower unit dry weight value than the ciliated protozoa.

Chapter 3

3.1. Reproduction of A. proteus.

3.1.1. Introduction.

The reproductive rates, under the various food concentrations and temperatures, must in themselves be regarded as separate variables in an energy budget study as the amounts of protoplasm available for subsequent trophic levels ultimately depend upon the rates and quantities of protoplasm produced over the life cycle.

Reproduction in Amoeba is by binary fission and the study of its energetics is simplified by the fact that there is no distinction between growth of the individual and the production of reproductive material over the generation. Asexual reproduction is therefore a direct product of growth which is constant over the cell cycle (Section 5.1.4.). Zeuthen (1951) observed that for Tetrahymena the rate of metabolism changed during the actual fission process. The same is likely to be true for A. proteus, however, the time taken for the division process is small in relation to the overall generation time. Changes in the energy expended over this time can therefore be disregarded.

The rate of reproduction has often been used as a parameter in protozoan growth studies, as was the case for Acanthamoeba (Heal 1967a), where the number of generations with time was

considered as opposed to direct volume measurements. However, such studies fail to make allowance for the mean cell volume variation within populations subjected to different environmental conditions. In other words, conditions promoting high rates of reproduction do not necessarily result in a large increase in cell protoplasm.

Early reproduction studies, with reference to A. proteus, were largely concerned with describing and attempting to clarify the contradictory life history of the cell. Calkins (1905) maintained that the sexual cycle of A. proteus began with the encystment of the adult, while Stolc (1906) and Prandtl (1907) were reporting conjugation between two amoebae cells. In recent publications, emphasis has been placed on inheritance studies, where multiplication rates have been indicative of the variations between strains (Hawkins and Danielli, 1963; Sophina, 1975).

Studies on the effects of food concentration and temperature on the rate of reproduction have largely been ignored in the sarcodines, with the exception of Heal's (1967) Acanthamoeba study. As previously stated, the majority of investigations relating to the reproduction of the naked amoebae had no direct bearing on their ecology. Data regarding the effects of environmental parameters, such as temperature and food condition, are secondary.

Comparable data can be found for the ciliated protozoa with the effect of food concentration on the rate of reproduction being noted as early as 1924 by Cutler and Crump. The effect of temperature has also been well documented in the ciliates since the time of Woodruff and Baitsell (1911 a,b). With the exception

of Laybourn and Stewart (1974), who investigated the effect of temperature and food consumption on the reproduction of Colpidium campylum, there have been few attempts to combine both variables in protozoan studies.

As the length of time needed to complete a generation varies depending upon the prevailing environmental conditions (Berkeley and Campbell, 1971) an energy budget is most meaningful over the complete cell cycle. The aim of this section, therefore, was to examine the effects of both food concentration and temperature on the generation times of A. proteus, with a view to compiling generation energy budgets and forming comparisons with such information as is available in the literature.

3.1.2. Materials and Methods.

40 replicate watch glasses, each containing 1ml of the appropriate Tetrahymena concentration over the range 125 - 4000 cells 500 μl^{-1} at the required temperature (10°C, 15°C and 20°C) were set up as detailed in Sections 1.2.5. - 1.2.10. Initially, a single Amoeba was added to each watch glass, after which the number of amoebae cells were counted every 12 hours for cultures at 15°C and 20°C, and every 24 hours for cultures at 10°C, where reproductive rates were lower. Amoebae were transferred to fresh culture conditions every 24 hours to prevent bacterial contamination and to adjust the changing Tetrahymena concentration (Page 27). The length of each experimental period was at least 264 hours for 20°C and 15°C, and 408 hours for 10°C.

The number of amoebae (logarithmic) from the sum of 40 replicates was plotted against time and linear regressions were computed

for the exponential phase of growth. The generation times for A. proteus were calculated from these regressions.

Experiments were conducted over a two year period.

Figure 13.

The effect of temperature on the multiplication of A. proteus (125 Tetrahymena 500µl^{-1}).

20°C : $y = 0.0036 \pm sx + 1.6804$

$n = 18$

$r = 0.9925$

$p = 0.001$

$s = 0.0347$

15°C : $y = 0.0027 \pm sx + 1.5010$

$n = 18$

$r = 0.9939$

$p = 0.001$

$s = 0.0231$

10°C : $y = 0.0005 \pm sx + 1.6148$

$n = 18$

$r = 0.9365$

$p = 0.001$

$s = 0.0233$

plication

).

LOG. NO. CELLS
3·5
3·0
2·5
2·0
1·5
1·0
0
50
100
150
200
250
300
350
400
450
TIME h
20°C
15°C
10°C

Figure 14.

The effect of temperature on the multiplication of A. proteus (250 Tetrahymena 500μl^{-1}).

$20^{o}C$: $y = 0.0042 \pm sx + 1.5894$

$n = 19$

$r = 0.9982$

$p = 0.001$

$s = 0.0200$

$15^{o}C$: $y = 0.0037 \pm sx + 1.5404$

$n = 19$

$r = 0.9971$

$p = 0.001$

$s = 0.0228$

$10^{o}C$: $y = 0.0008 \pm sx + 1.5907$

$n = 16$

$r = 0.9894$

$p = 0.001$

$s = 0.0137$

iplication

1
).

Figure 15.

The effect of temperature on the multiplication of *A. proteus* (500 *Tetrahymena* 500μl^{-1}).

$20^{\circ}C$: $y = 0.0051 \pm sx + 1.4331$

$n = 17$

$r = 0.9991$

$p = 0.001$

$s = 0.0163$

$15^{\circ}C$: $y = 0.0035 \pm sx + 1.5483$

$n = 18$

$r = 0.9947$

$p = 0.001$

$s = 0.0281$

$10^{\circ}C$: $y = 0.0007 \pm sx + 1.6042$

$n = 16$

$r = 0.9808$

$p = 0.001$

$s = 0.0171$

plication

).

20°C
15°C
10°C
LOG. NO. CELLS
3·5
3·0
2·5
2·0
1·5
1·0
0
50
100
150
200
250
300
350
400
TIME h

Figure 16.

The effect of temperature on the multiplication of A. proteus (1000 Tetrahymena 500μl^{-1}).

$20^{o}C$: $y = 0.0065 \pm sx + 1.3059$

$n = 15$

$r = 0.9982$

$p = 0.001$

$s = 0.0259$

$15^{o}C$: $y = 0.0043 \pm sx + 1.3013$

$n = 14$

$r = 0.9934$

$p = 0.001$

$s = 0.0307$

$10^{o}C$: $y = 0.0004 \pm sx + 1.6322$

$n = 17$

$r = 0.9535$

$p = 0.001$

$s = 0.0164$

plication

1).

20 °C
15 °C
10 °C
LOG NO CELLS
3·5
3·0
2·5
2·0
1·5
1·0
0
50
100
150
200
250
300
350
400
TIME h

Figure 17.

The effect of temperature on the multiplication of A. proteus (2000 Tetrahymena 500μl^{-1}).

$20^{\circ}C$: $y = 0.0062 \pm sx + 1.4609$

$n = 18$

$r = 0.9991$

$p = 0.001$

$s = 0.0202$

$15^{\circ}C$: $y = 0.0044 \pm sx + 0.9263$

$n = 9$

$r = 0.9974$

$p = 0.001$

$s = 0.0098$

$10^{\circ}C$: $y = 0.0003 \pm sx + 1.6180$

$n = 17$

$r = 0.9587$

$p = 0.001$

$s = 0.0114$

tiplication

μl^{-1}).

Figure 18.

The effect of temperature on the multiplication of A. proteus (4000 Tetrahymena 500μl^{-1}).

20°C : $y = 0.0073 \pm sx + 1.1317$

$n = 14$

$r = 0.9991$

$p = 0.001$

$s = 0.0187$

15°C : $y = 0.0028 \pm sx + 1.2945$

$n = 12$

$r = 0.9911$

$p = 0.001$

$s = 0.0177$

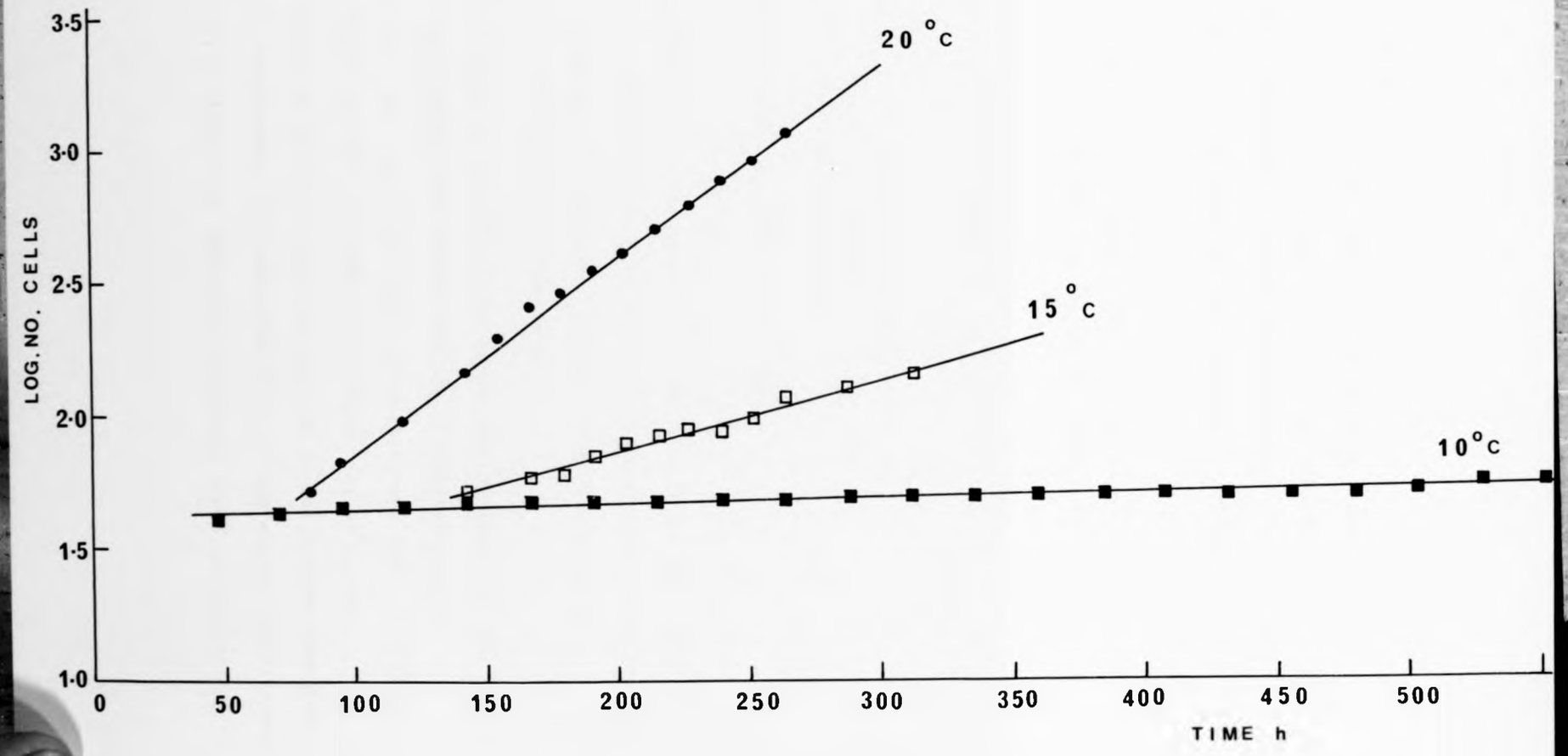
10°C : $y = 0.0001 \pm sx + 1.6482$

$n = 22$

$r = 0.8487$

$p = 0.001$

$s = 0.0135$


3·5
3·0
2·5
2·0
1·5
1·0
LOG. NO. CELLS
20 °C
15 °C
10 °C
0
50
100
150
200
250
300
350
400
450
500
TIME h

3.1.3. Results.

The effect of temperature on the multiplication rate of A. proteus is shown in Figures 13 - 20, the raw data being presented in Appendix 8. In all cases of food condition the generation times increased as the temperature was lowered from 20°C to 10°C.

The computed regressions were all highly significant (P = 0.001) when the equation below was employed:-

$$F = \frac{r^2}{(1-r^2)} \times n-2$$

where r = correlation coefficient.
degrees of freedom = 1 and n-2.
F = variance ratio.

(after Bannister, 1978, pers. comm.).

The increase in the generation time with increasing temperature was significantly different (P = 0.001) for each food concentration. Table 6 compares the regression coefficients (b values) by employing a modification of the t-test.

The length of the generation times, as influenced by the various Tetrahymena food concentrations, is illustrated in Figures 21 - 23. Additional food concentrations, 6000 and 8000 cells $500\mu l^{-1}$ at 20°C and 3000 cells $500\mu l^{-1}$ at 15°C, were investigated to obtain further information on the pattern emerging for each temperature. By comparing pairs of regression coefficients (Table 6), a significant downward and upward trend was indicated

The change in number of A proteus with time.

Figure 19.

The effect of temperature on the multiplication of A. proteus (6000 Tetrahymena 500μl^{-1}).

$20^{o}C$: $y = 0.0047\ x + 1.5323$

$n = 15$

$r = 0.9949$

$p = 0.001$

proteus with time.

tiplication

h^{-1}).

Figure 20. The change in number of A. proteus with time.

The effect of temperature on the multiplication of A. proteus (3000 Tetrahymena $500\mu l^{-1}$).

$15^{o}C$: $y = 0.0028 x + 1.5009$

$n = 17$

$r = 0.9945$

$p = 0.001$

roteus with time.

iplication

$^{-1}$).

Table 6.

Comparison of the regression coefficients (b_1,b_2) from the linear relationships describing the multiplication of A. proteus at 10°C, 15°C and 20°C over a range of food concentrations.

Modification of the t-test (after Bailey, 1959).

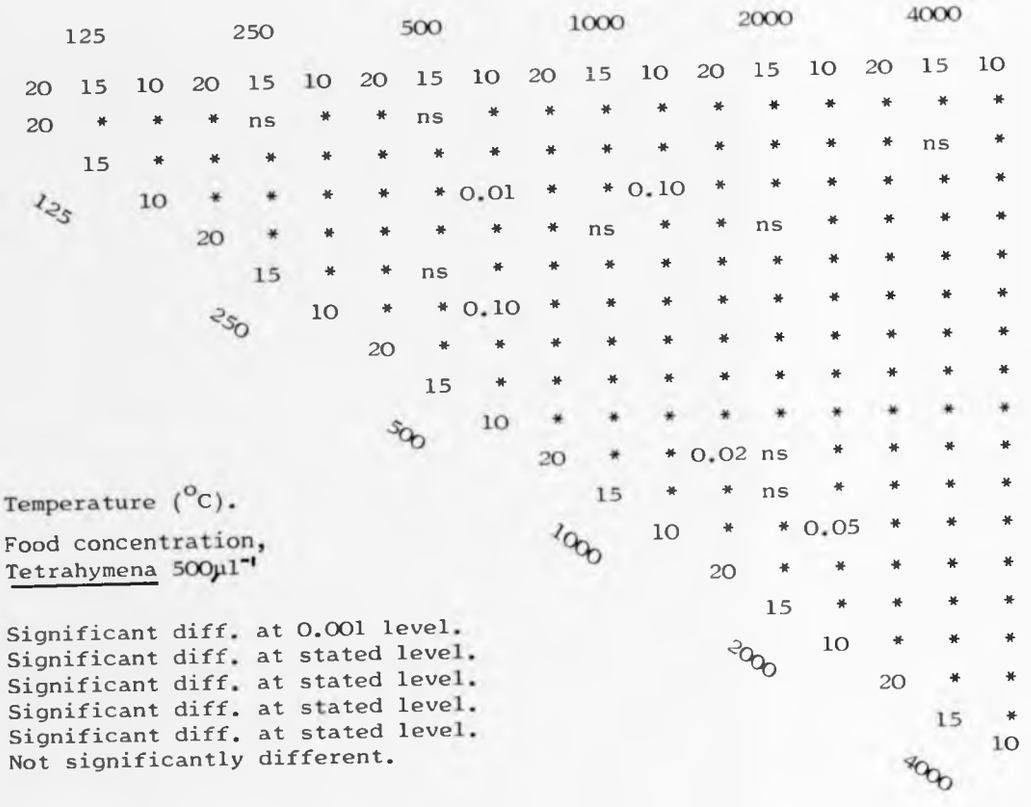
$$t = \frac{b_1 - b_2}{s\sqrt{\frac{1}{\sum_1 (x - \bar{x}_1)^2} + \frac{1}{\sum_2 (x - \bar{x}_2)^2}}}$$

where:

$$s^2 = \frac{(n_1 - 2)s_1^2 + (n_2 - 2)s_2^2}{n_1 - n_2 - 4}$$

s^2 = variance

degrees of freedom = $n_1 + n_2 - 4$



10, 15 and 20 = Temperature (^{o}C).

125 - 4000 = Food concentration, Tetrahymena 500μl^{-1}

* = Significant diff. at 0.001 level.
0.01 = Significant diff. at stated level.
0.02 = Significant diff. at stated level.
0.05 = Significant diff. at stated level.
0.10 = Significant diff. at stated level.
ns = Not significantly different.

Table 7.

The generation times of A. proteus.

Temp. ^{o}C	Initial food conc. 500ul^{-1}	Calculated gen.T. (h)	Extrapolated gen.T. (h)
20	125	83	84
	250	71	70
	500	59	58
	1000	47	49
	2000	48	44
	4000	46	50
	*6000	64	62
	*8000	0	0
15	125	111	107
	250	81	91
	500	86	77
	1000	70	71
	2000	68	78
	*3000	107	93
	4000	107	112
10	125	600	615
	250	375	431
	500	428	372
	1000	750	561
	2000	1000	1247
	4000	3000	2926

* Additional replicates.

for the condition of increasing food concentration. The best fitting curves, based on a modified parable equation, were computed where:

$$y = a + b\left(\frac{x}{1000}\right) + c \log x$$

a, b and c were constants.

(after Bannister, 1978, pers. comm.).

The equations were very significant for 20°C and 10°C ($p = 0.01$), but just outside the 5% significance level ($p < 0.1 > 0.05$) for 15°C.

The extrapolated generation times, from Figures 21 - 23, together with the calculated generation values are compared in Table 7 for each respective food concentration and temperature. The extrapolated generation times were used throughout subsequent calculations.

The range of generation times varied markedly with both temperature and food concentration. At 20°C the range extended over 44 to 84 hours, with a 100% mortality of cells within 180 hours, (Appendix 8), at the high food concentration of 8000 Tetrahymena $500\mu l^{-1}$. Although this concentration was outside that experienced in the field situation, it did substantiate the parabolic shape of the graph. Figure 21 indicates this pattern for Amoeba cultured at 20°C, where an optimum generation time was found for the relatively high food level of 2000 cells $500\mu l^{-1}$.

Extrapolated gen.T. (h)
84
70
58
49
44
50
62
0
107
91
77
71
78
93
112
615
431
372
561
1247
2926

Figure 21.

The effect of food concentration on the generation time (h) of A. proteus at 20°C.

$$y = 184.04 + 10.20 \left(\frac{x}{1000}\right) - 48.47 \log x.$$

$p = 0.01$ (very significant).

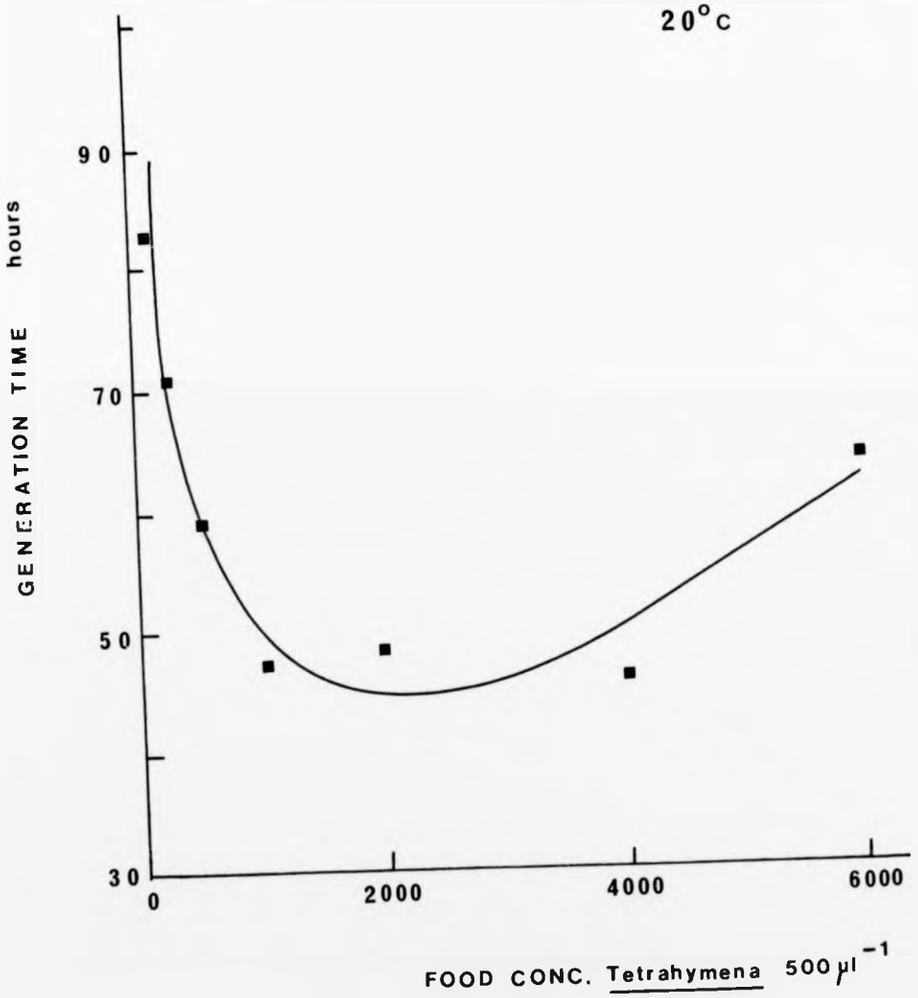

20°C
GENERATION TIME hours
90
70
50
30
0
2000
4000
6000
FOOD CONC. Tetrahymena 500 µl⁻¹

Figure 22.

The effect of food concentration on the generation time (h) of A. proteus at 15°C.

$$y = 244.99 + 27.44 \left(\frac{x}{1000}\right) - 67.24 \log x.$$

$p < 0.1 > 0.05$ (not significant).

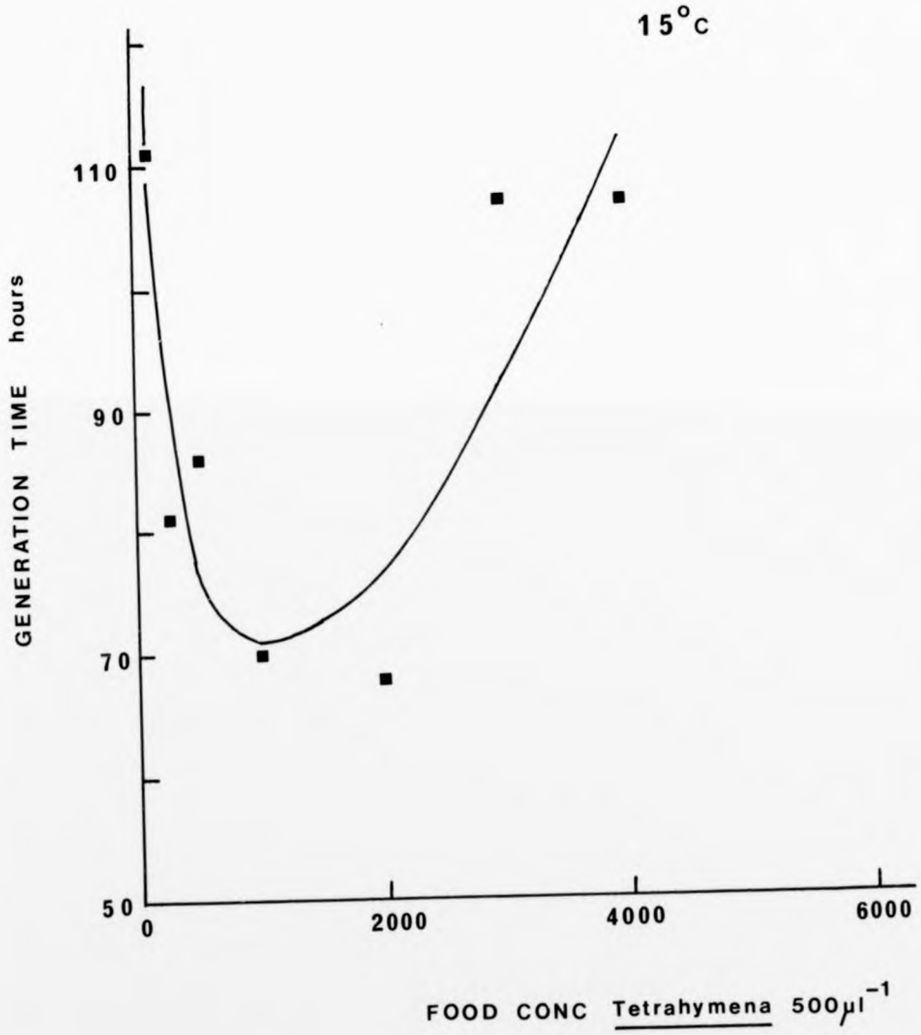
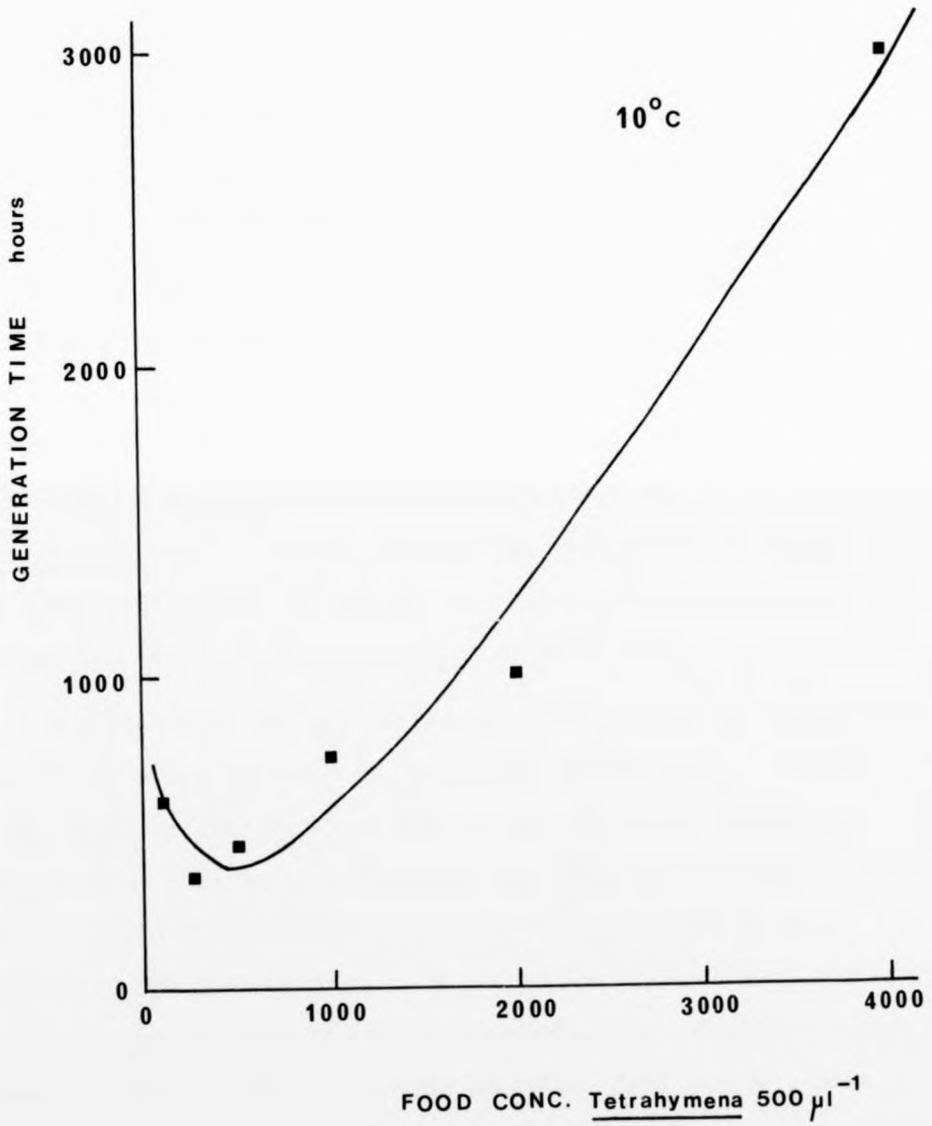

15°C
GENERATION TIME hours
110
90
70
50
0
2000
4000
6000
FOOD CONC Tetrahymena 500µl⁻¹

Figure 23.

The effect of food concentration on the generation time (h) of A. proteus at 10°C.

$$y = 2633.03 + 993.48 \left(\frac{x}{1000}\right) - 1021.74 \log x.$$

$p = 0.01$ (very significant).


10°C
GENERATION TIME hours
3000
2000
1000
0
0
1000
2000
3000
4000
FOOD CONC. Tetrahymena 500 µl⁻¹

At 15°C, (Figure 22), the shortest generation time was obtained at a lower food level, approximately 1000 Tetrahymena cells $500\mu l^{-1}$. A higher range of generation times, 71 - 112 hours, was found for Amoeba cultured over the range of food concentrations investigated.

The longest generation times were obtained at 10°C (Figure 23) where the range was 372 - 2,926 hours. Increasing the food concentration had a particularly marked effect on the length of the cell cycle at this temperature. The long generation times, found for cells cultured at the higher Tetrahymena concentrations, reflect the intolerance of Amoeba to such conditions; the optimum generation time being found at the low level of 500 Tetrahymena $500\mu l^{-1}$.

The length of the lag period before the onset of exponential growth was normally about 36 hours, but occasionally, a longer delay of up to 150 hours was found. The length of the phases are shown in Figures 13 - 20, where the time between 0 and start of the computed regressions is indicative of the lag period. Sophina (1975) found delays in the division of different strains of A. proteus of between 3 to 10 days after the transfer of cells from cultures at 17°C to cultures at 10°C. Heal (1967a), however, found that Acanthamoeba readily adapted to low temperatures within 24 hours of transfer from cultures at 25°C.

As care was taken to avoid heat shocks and sudden changes throughout the entire experimental programme, variations in the length of the lag phases cannot be satisfactorily explained. It is relevant that after the onset of cell division, normal

Figure 24.

Comparison of the generation times for A. proteus when cultured at different temperatures.

▲ - results of present study.

Δ - published results from the references below.

Prescott (1955)

Hishfield et al (1960)

James (1959)

*Stolc (1905)

*Schaeffer (1916)

*Levy (1924)

*Hartmann (1926)

Hawkins and Danielli (1963)

*Chalkley (1931)

Salt (1968)

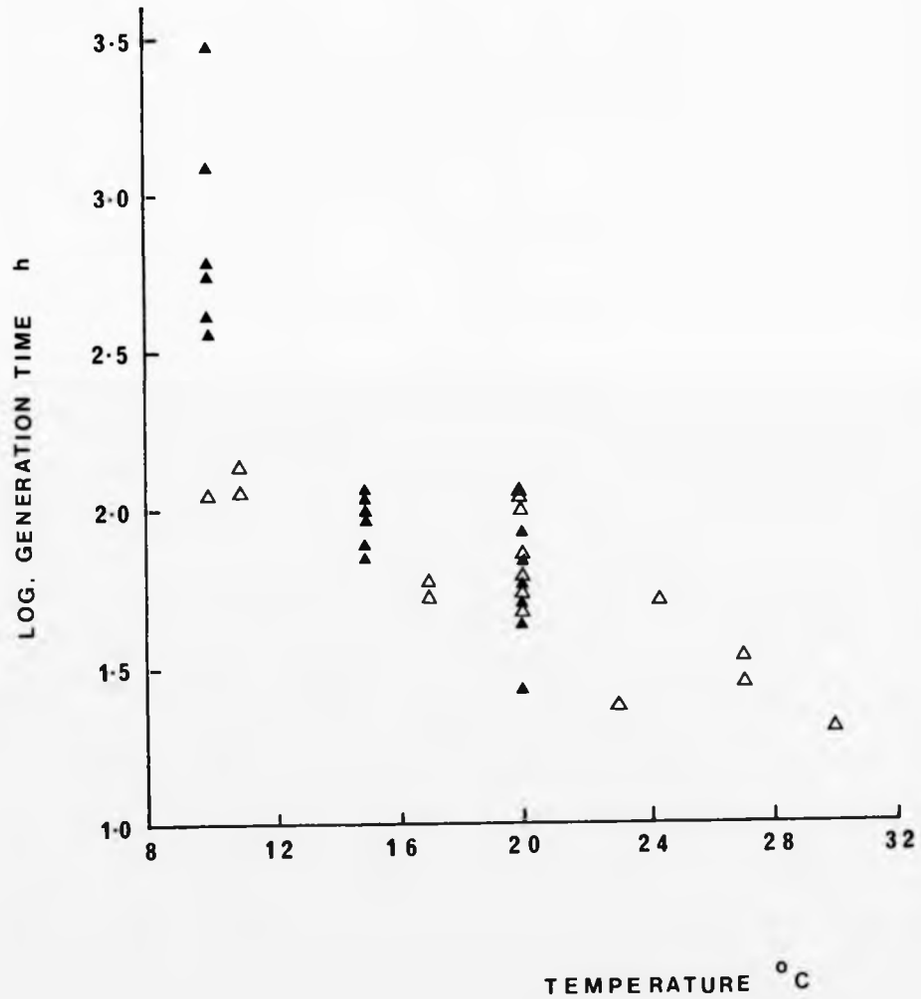
*Williamson (1944)

*The temperature used by these authors was not quoted. It was assumed that cultures were grown at room temperature (20°C).

nces below.

was not quoted.

n at room



exponential growth proceeded in all cases.

The results of past publications relating to the effect of temperature on the generation time of A. proteus have been plotted together with the results of the present study (Figure 24). Again, it is apparent that temperature is an important factor in determining the length of the cell cycle.

The rates of reproduction for Amoeba under the various experimental conditions examined, are presented in Figure 25. Increasing temperature over the range 10°C to 20°C increased the rate of reproduction while the food concentration determined the optimum condition for the multiplication of Amoeba within each temperature regime.

A widely adopted method for comparing the magnitude of the increase in a rate process with temperature is Van't Hoff's Q_{10} approximation, which is the factor by which the velocity of a rate process is increased for a 10°C rise in temperature.

$$Q_{10} = \left[\frac{V_2}{V_1}\right]^{\frac{10}{t_2 - t_1}}$$

where: V_1 and V_2 are the velocities and t_1 and t_2 are temperatures (°C).

The Q_{10} values (Table 8) were found to be between 1.30 - 5.42 for A. proteus over 15 - 20°C. Between the temperatures 10 - 15°C, values were very high, 21.36 - 801.06, indicating that Amoeba

Figure 25.

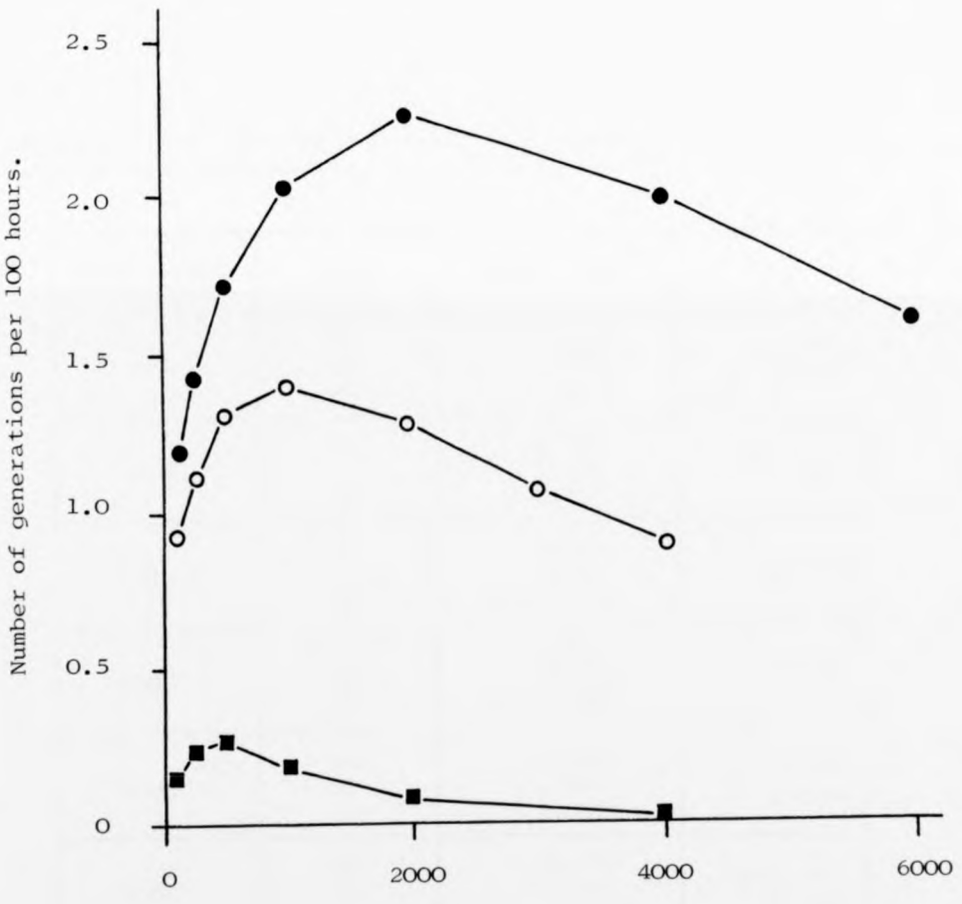
Rate of reproduction in A. proteus with regard to food concentration and temperature.

●—● 20°C

○—○ 15°C

■—■ 10°C

rature.


2.5
2.0
1.5
1.0
0.5
0
0
2000
4000
6000
Number of generations per 100 hours.
Initial food concentration ,
Tetrahymena 500ul^{-1}.

at 10°C was approaching the lower limit of its thermal range.

The effect of food concentration and temperature on the generation time of A. proteus is summarised in a three dimensional diagram (Figure 26).

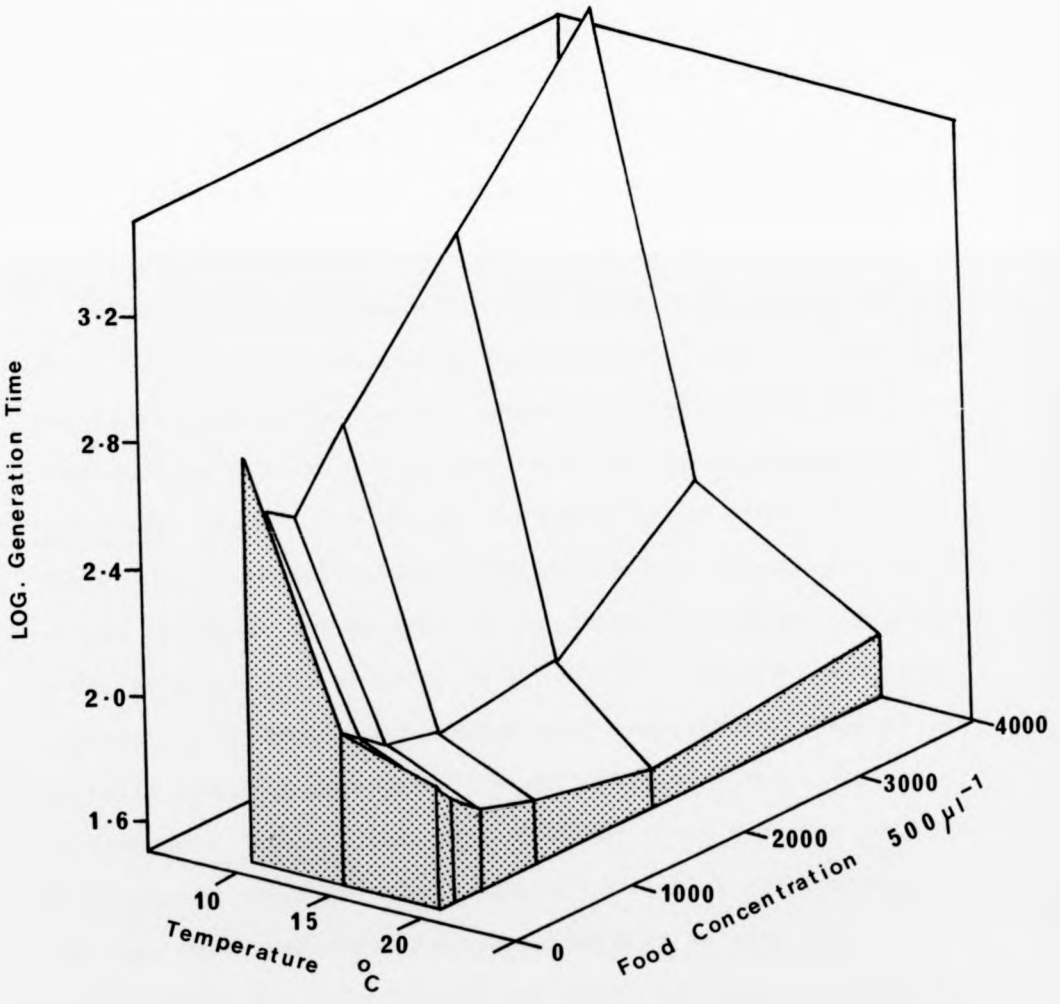
Table 8.

Amoeba proteus reproductive rates: Q_{10} values.

Tetrahymena conc.	Temp.	Rate $100h^{-1}$	Q_{10}'s.
125	10 15 20	0.167 0.901 1.205	29.108 1.789
250	10 15 20	0.267 1.234 1.408	21.360 1.302
500	10 15 20	0.234 1.163 1.695	24.702 2.124
1000	10 15 20	0.133 1.428 2.128	115.280 1.490
2000	10 15 20	0.100 1.470 2.083	216.090 2.008
4000	10 15 20	0.033 0.934 2.174	801.061 5.418

Figure 26.

Three dimensional diagram summarising the effect of food concentration and temperature on the generation time of A. proteus.


LOG. Generation Time
3·2
2·8
2·4
2·0
1·6
10
15
20
Temperature °C
0
1000
2000
3000
4000
Food Concentration 500 µl⁻¹

3.1.4. Discussion.

Cell division in Amoeba proteus is possible between 4°C and 35°C (Daniel and Chalkley, 1932) although at temperatures approaching these extremes it is not normal. At the lower temperature only 15% of the cells divided and the frequency of multinucleate forms increased, whereas at 35°C, mortality was high at 56%. Mitotic division was found to be normal only within the range 11°C to 27°C (Daniel and Chalkley, 1932; James, 1959). The temperatures used in this study were close enough to this range to assume normal division.

The decrease in generation times with increasing temperature obtained for the present study was expected. Heal (1967a) found an almost linear relationship between temperature and the reproductive rate of Acanthamoeba when fed on Saccharomyces cerevisae. Sopina (1975) used b-coefficients, which characterise the slope of the multiplication curve, to investigate the role of the cytoplasm and nucleus in the inheritance of multiplication rates of A. proteus at 25°C, 17°C and 10°C. In all cases, the regression coefficients decreased with temperature. Hawkins and Danielli (1963) investigated the multiplication rates in two strains of A. proteus over 11 - 27°C and found a marked increase in generation time with temperature. A similar relationship has been found for other Protozoa, the most recent study being by Finlay (1977) who investigated the effect of temperature on the generation times of 10 ciliate species. Again, an increase in the length of the cell cycle was found as the temperature was lowered.

Figure 24 illustrates the variation which exists in the generation times of A. proteus at different temperatures. As has been shown for this study, part of the variation can be explained by the influence of food concentration. The species of food organism (Salt, 1968) and the culture medium used can also be expected to influence the length of the cell cycle. In addition, different strains of amoebae show different generation times, as has been demonstrated by Hawkins and Danielli (1963), Hawkins and Cole (1965) and Sopina (1975).

Optimum generation times were found at a Tetrahymena concentration less than the maximum level investigated for 20°C, 15°C and 10°C. It is likely that at excessive concentrations the amoebae became subject to mechanical interference from colliding Tetrahymena. Further, it is probable that Amoeba has a limit on the rate of enzyme production above which it fails to cope efficiently with the digestion of captured cells. As the Amoeba were found to have high ingestion rates at the highest Tetrahymena concentrations investigated (Chapter 4), the culture of cells at such food levels prolonged the generation time.

Stachurska (1970) found an optimum food concentration for the generation time of the predatory ciliate Dileptus cygnus fed on Colpidium colpoda at 22°C. The population was most dynamic at a certain food concentration, above and below which, the generation time increased. The same was true for the results of the present study (Figures 21 - 23). This characteristic curve has also been found by Rudzinska (1951) for the suctorian ciliate Tokophyra infusorium. Reproduction in this group involves budding and an optimum temperature was obtained for this process, above

and below which, embryo formation decreased.

The rate of multiplication is dependent upon such temperature dependent factors as the rate of digestion and the rate of synthesis of new materials necessary for growth (Danielli, 1959). Further, the rate of phagocytosis is important being determined again by temperature and also by the abundance of food (Chapter 4).

Pace (1933) investigated the relation of inorganic salts on the growth of A. proteus and reported no correlation between the numbers of Chilomonas and the numbers of amoebae. Comparable data are not available for A. proteus, however, Pace's report is the exception for Protozoa in general. Cutler and Crump (1927), found a relationship for the soil amoebae Hartmanella hyalina whereby an increased bacterial food supply resulted in a gradual increase in the rate of reproduction. The effect of food concentration on the reproduction of ciliates has been well documented. Cutler and Crump (1924) obtained evidence that bacterial concentration was important in determining the number of divisions of Colpidium colpoda. As the bacterial concentration increased, the number of ciliate divisions likewise increased.

Phelps (1936) grew Colpidium in axenic culture and obtained a levelling off in the reproductive rate at high nutrient concentrations. Harding (1937) also obtained a levelling effect at higher food concentrations in monoxenic cultures of Glaucoma pyriformis (syn. T. pyriformis). A similar pattern was found by Laybourn and Stewart (1974) again for Colpidium, when cultured at 10°C, 15°C and 20°C.

The relationship between food concentration and the multiplication rate for A. proteus showed a peak followed by a decrease at all temperatures. In the studies previously cited, it is probable that had the range of bacterial concentrations been extended, the characteristic decrease in the rate of reproduction would have been found.

The shift in optimum food concentration with temperature found for Amoeba proteus is ecologically significant as the greatest reproductive rate and hence the shortest generation time was attained at lower food levels as the temperature was decreased. As the available food source can be expected to decrease with temperature (Chapter 8) in the field situation, amoebae appear to be adapted to exploit these changing variables by having the ability to adjust their reproductive pattern accordingly.

Chapter 4.

4.1. Consumption.

4.1.1. Introduction.

Consumption studies are of fundamental importance in considering the transfer of energy through a biotic community. The process is therefore at the heartof most ecological relationships. By considering the relationship between the predator A. proteus and the prey species Tetrahymena, an estimate of the impact that Amoeba is exerting on the ciliate population can be found. The simplification of considering only two "typical" species is most useful if the classification is adopted whereby organisms of a community are divided into trophic levels. The performance of a key species, in this case the microcarnivore A. proteus, becomes useful in estimating the impact of all the predators of the protoxoan community at this level.

With regard to predator-prey relationships, the effect of increasing predator numbers is reflected in the number and composition of the prey population (Salt, 1967, 1968, 1974). Griffiths and Holling (1969) argue, however, that although a well defined predator density control may be demonstrated in the laboratory, the effect only becomes important at a predator density above that expected under normal field conditions. They

conclude that for practical purposes, the effect can largely be ignored. This is certainly the case for A. proteus and large naked amoebae in general, as the numbers in the field appear to be at a constantly low level (Chapter 8).

Earliest reports on the feeding of A. proteus were concerned with describing the process of ingestion and the food preferences of the species, and include studies by Kepner (1913), Mast and Root (1916), Shaeffer (1916a) and Beers (1924). Some years later, Williamson (1944) followed these authors with a more detailed study on the nutrition of A. proteus by comparing the suitability of various protozoan species as food items for the predator Amoeba.

Quantitative estimates of the food consumption of protozoa, with regard to temperature and food concentration, are fragmentary in the literature. The most comprehensive studies for the sarcodines have been by Salt (1961, 1968) where he described some aspects of the feeding behaviour of A. proteus. An earlier attempt at quantifying the number of organisms consumed by Amoeba was undertaken by Mast and Fennell (1938) who investigated the relation between food abundance and frequency of ingestion when Amoeba was cultured with Chilomonas. An indication of the number of Chilomonas consumed per day was also given by Schaeffer (1916, 1917) and by Mast and Hahnert (1935).

It must be noted that studies on food uptake by pinocytosis in amoebae have been made by Lewis (1931), Chapman-Andresen (1962), Chapman-Andresen and Holter (1955), and others. The possibility of pinocytosis contributing to the overall nutrition has not been

discounted, and has been commented on in the discussion.

This present study was therefore the first detailed investigation to examine the effect of both food concentration and temperature on the rate of food uptake in A. proteus.

4.1.2. Selection of the experimental method for the determination of the rate of consumption in Amoeba.

There have been many nutritional studies on soil amoebae grown in axenic culture; Reich (1955) on Mayorella palestinensis, Neff (1958) on Acanthamoeba, and others. Even if the problems associated with the axenic culture of A. proteus could be overcome (Page **15**), such studies are so artificial that they are of limited use for extrapolation to field conditions.

The use of radioactive labelling techniques has gained momentum for nutritional studies on the invertebrates. The ingestion rates of an isopod were obtained from radiotracer experiments by Hubbell (1965). The feeding of Foraminefera in the laboratory has also been studied with tracers by Lee et al (1966), while the use of ^{14}C in the study of invertebrate nutrition, in general, has been discussed by Sorokin (1966). The use of tracers with regard the protozoa, however, are not well developed and so their use in this present study were rejected.

Heal (1967b) described a method involving a non-nutrient medium system in which the number of predators and prey were counted at the beginning and end of a specified time. Due to the build up of metabolites, the length of the experiment was kept short. However, the method relied on the food organisms not

dividing throughout the experimental period, which was not the case for Tetrahymena on addition to soil extract media (Page 27). Similarly, the method of Curds and Cockburn (1968) was rejected as it relies on the culturing of both organisms in a nutrient medium system.

Salt (1964) developed a method of photo-recording for counting the numbers of protozoa in culture. By using time-lapse photography, the change in the cell numbers with time was followed. Although this technique was tried several times in preliminary studies, negatives with discernable counts were not obtained.

The method finally adopted was one of direct observation, using 100x magnification, where the number of protozoa captured per hour was recorded. Two considerations had to be made in the design of the experiments. The first of these was that the number of prey organisms changed over the 24 - hour experimental period. The available food ratio therefore increased relative to the amoebae with time. Secondly, it was essential to distinguish between short-term variations in the rate of food capture and the long-term mean rate of capture.

This problem was brought to light by Mast and Fennell (1938) who studied the frequency of ingestion of Chilomonas by A. proteus. The number consumed was greatest in the first 30 minute period of the experiment as opposed to a more settled rate after 60 minutes. In addition, Salt (1961) reported a 24 hour cyclic behaviour whereby amoebae ate voraciously for the first few hours and then little for the remainder of the cycle. The cells then

became active and resumed searching for food.

Both these observations may have been a function of the sudden feeding of the Amoeba, especially in the case of Salt whose experiments were conducted using starved amoebae. Throughout this present study, amoebae were never subject to sudden change, however, the possibility of long periods of inactivity was considered.

4.1.3. Consumption: Materials and Methods.

Amoebae were grown in solid watch glasses (Sections 1.2.5. - 1.2.10.) under the appropriate set of food conditions and temperature.

Randomly selected watch glasses containing amoebae were microscopically observed over a one - hour period, and the number of Tetrahymena captured was recorded. Direct observation had the additional advantage of providing information on the behaviour of the animals. As detailed on page 26, the number of amoebae per watch glass was kept at a low level. This was important as Salt (1967) maintained that there are few predators which are totally unresponsive to their own density. A high density of Amoeba was found to decrease the rate of consumption of Paramecium (Salt 1968).

Experiments were conducted in growth room at 10°C, 15°C and 20°C (± 1°C). The microscope used had a direct light source, but no increase in temperature within the watch glass was measured. Harrington and Leaming (1900) found red light to least inhibit protoplasmic flow. In addition, Mast (1910) concluded that red light was only slightly active in causing reactions in A. proteus.

Table 9.

Average number of Tetrahymena consumed per hour by A. proteus individual.

Temp. °C		Initial food concentration 500µl^{-1}. 125	250	500	1000	2000	4000	8000*
20	Period 1 (0-10h)	0.60	0.50	1.15	1.10	2.10	3.20	0.90
	Period 2 (23-24h)	1.10	1.65	1.20	2.05	2.20	2.00	0.30
	Average no. of cells consumed h^{-}	0.85	1.07	1.17	1.57	2.14	2.60	0.60
15	Period 1 (0-10h)	0.55	1.10	0.95	1.65	2.80	2.50	-
	Period 2 (23-24h)	0.85	0.65	1.00	1.20	1.80	2.45	-
	Average no. of cells consumed h^{-}	0.70	0.87	0.92	1.42	2.30	2.47	-
10	Period 1 (0-10h)	0.75	0.65	0.60	1.25	0.90	0.60	-
	Period 2 (23-24h)	0.30	0.70	0.60	0.65	1.00	0.50	-
	Average no. of cells consumed h^{-}	0.52	0.67	0.60	0.95	0.95	0.55	-

* = additional food concentration.

Raw data in Appendix 9.

ation 500µl^{-1}.		
00	4000	8000*
10	3.20	0.90
20	2.00	0.30
14	2.60	0.60
80	2.50	-
80	2.45	-
30	2.47	-
90	0.60	-
00	0.50	-
95	0.55	-

ntration.

A red filter was therefore used throughout the experiments to avoid adverse effects on the behaviour of the cell.

To ensure an accurate estimate of the long-term mean rate of capture, 40, one - hour replicates were made for each set of conditions. By observing such a large number, amoebae from all stages in the cell cycle were considered. To compensate for the changing food concentrations, the replicates were carried out over two time intervals. 50% of the one - hour long observations were made between 0 - 10 hours, when the available food concentration was lowest (Page 27). The remainder were made at 23 - 24 hours when the food concentration was greatest. Time zero represented the point at which the watch glasses were set up with the initial Tetrahymena concentrations.

4.1.4. Results.

A total of 720 hours were spent observing the feeding of Amoeba over the 3 temperatures and 6 food concentrations investigated. The influence of these variables on the mean rates of food consumption are given in Table 9. In all cases, the total number of Tetrahymena captured over the generation of Amoeba was found to increase with a decrease in temperature, regardless of food concentration (Figure 27). This was a function of the increasing generation times found for A. proteus when the temperature was decreased (Page 70).

No apparent difference was found for the average rate of consumption over the first experimental period, 0 - 10 hours, when compared with the second period, 23 - 24 hours. In other

Figure 27.

The effect of temperature and food concentration on the consumption per generation of Tetrahymena by A. proteus.


- 20°C


- 15°C

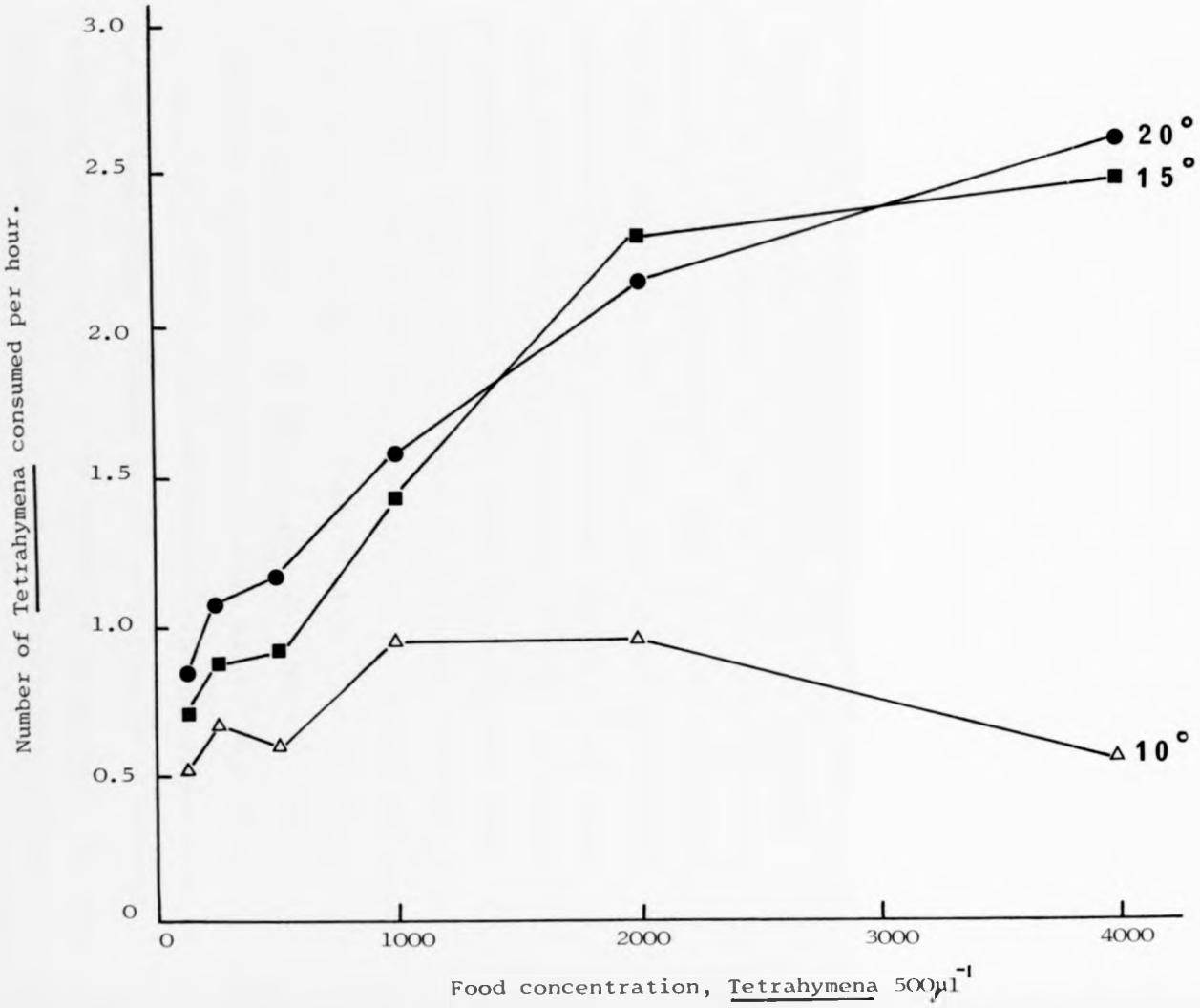

- 10°C

entration

trahymena

Figure 28.

The effect of temperature and food concentration on the rate of consumption of Tetrahymena by A. proteus.


3.0
2.5
2.0
1.5
1.0
0.5
0
0
1000
2000
3000
4000
20°
15°
10°
Number of Tetrahymena consumed per hour.
Food concentration, Tetrahymena 500µl⁻¹

words, a change in Tetrahymena concentration representing an increase of approximately 70% (Page 31), was not great enough to affect the rate of food uptake over the 24 hour experimental period. The Amoeba consumption rates (h^{-1}) are graphically presented in Figure 28. The rates of consumption at 20°C and 15°C were similar although, in general, the rate of food capture was slightly higher at 20°C. Consumption at 10°C was low by comparison. It was assumed that all cells captured were digested.

Increasing food concentration increased the rate of consumption by Amoeba over the range 125 - 4000 Tetrahymena 500μl^{-1} at 15°C and 20°C. The additional food concentration investigated, 8000 cells 500μl^{-1} (Table 9), confirmed that the rate decreased at high food levels. The optimum Tetrahymena concentration for maximum consumption in Amoeba at 20°C was of the order 6000 cells 500μl^{-1}. Similarly, at 15°C, the peak consumption rate was not reached within the range 125 - 4000 Tetrahymena 500μl^{-1} although Figure 28 indicates that 4000 cells 500μl^{-1} was close to the food concentration for optimum consumption by A. proteus. The maximum consumption at 10°C was found at a food level of approximately 1500 Tetrahymena cells 500μl^{-1}. The discrepancy in the 10°C curve at 500 cells 500μl^{-1} was attributable to the very low and variable rates of capture observed for this temperature. Amoebae at 10°C were sluggish in movement, resulting in many failures in the capture of Tetrahymena. Further, cells at this temperature often displayed a reluctance to feed, whereas at the higher temperatures the stimulatory response to prey was high.

The three-dimensional diagram (Figure 29) summarises the effects of both variables on the rates of Tetrahymena consumption per hour for A. proteus. Ecologically, it was unimportant that the maximum consumption rates were not found for 20°C and 15°C as biomass values in excess of 4000 Tetrahymena cells $500\mu l^{-1}$ are outwith those expected in the field situation.

The results of the consumption section were converted to energy units (joules). The mean volume of a Tetrahymena cell was taken as $19,500\mu m^3$ (Page **32**) while the conversions for volume to dry weight and dry weight to energy units, as determined in Chapter 2, were employed. The energy levels, representing the numbers of Tetrahymena consumed per hour and per generation by A. proteus are presented in Table 10. These values were used in the compilation of the series of energy budgets for A. proteus (Chapter 7).

Figure 29.

A three-dimensional representation of the effects of temperature and food concentration on the rate of consumption of Tetrahymena by A. proteus.

the

tration

ena by

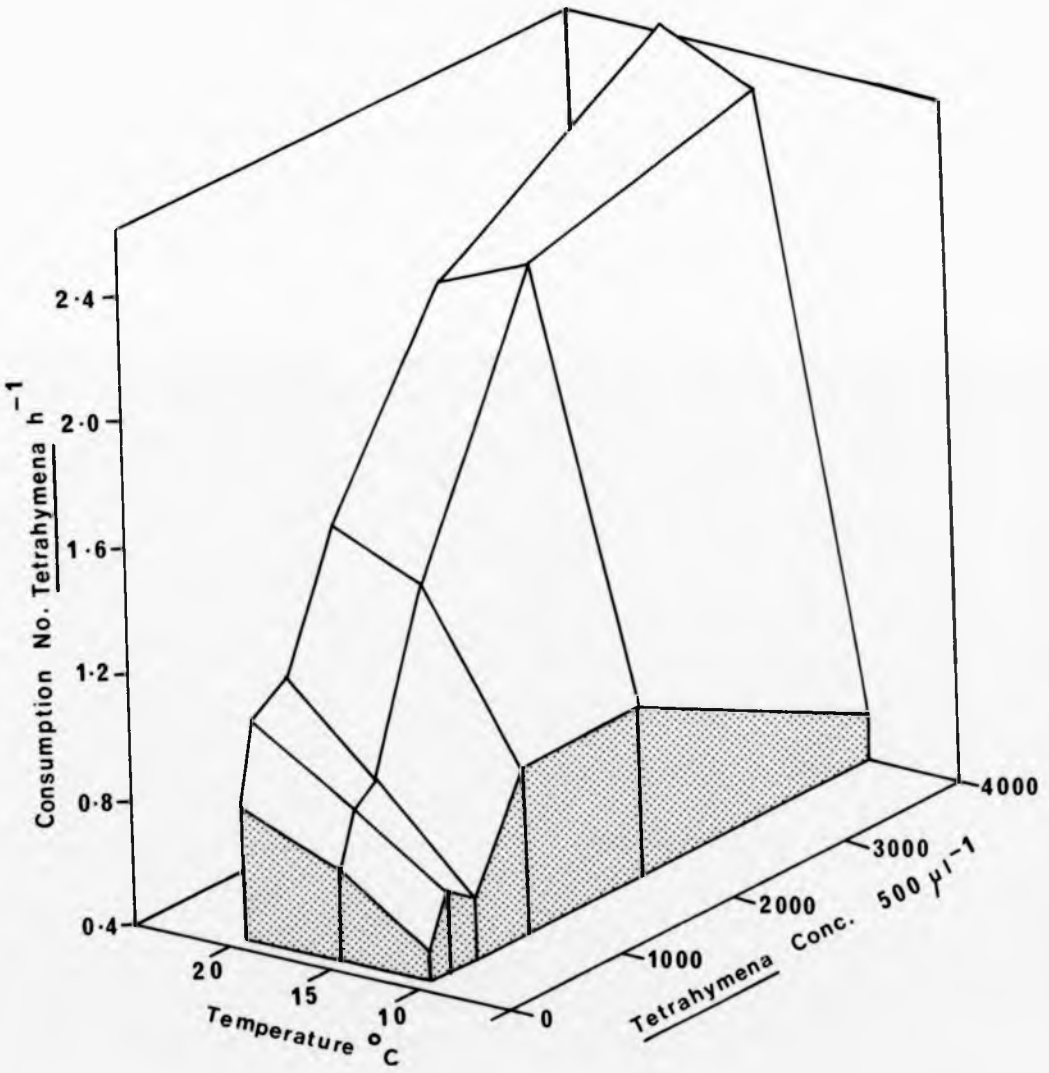


Table 10.

The energy consumption of A. proteus as influenced by temperature and Tetrahymena concentration.

Temp. °C	Initial food Concentration (cells 500µl^{-})	µJ consumed per hour	µJ consumed per generation
20	125	53.14	4463.68
	250	66.89	4682.49
	500	73.14	4242.38
	1000	98.15	4809.40
	2000	133.78	5886.56
	4000	162.54	8127.16
15	125	43.76	4682.49
	250	54.39	4949.44
	500	57.51	4428.68
	1000	88.77	6302.92
	2000	143.79	11215.48
	4000	154.41	17294.59
10	125	30.03	18467.34
	250	38.69	16675.46
	500	34.65	12889.02
	1000	54.86	30776.00
	2000	54.86	68409.41
	4000	31.76	92931.47

4.1.5. Discussion.

There are numerous problems associated with selecting a "typical" food organism for a nutritional study of A. proteus. The fact that amoebae, in general, utilise such a diverse array of food items is perhaps the greatest problem. The food preferences of amoebae are not sufficiently documented to warrant the selection of a "typical" individual food item with any degree of certainty. The diversity of food types utilised by amoebae was illustrated by Old (1977) who recently described a giant soil amoeba (probably of the genus Vampyrella, pers. comm., 1977) which can penetrate and digest the contents of conidia from the fungus Cohliobolus sativus. Admittedly, the feeding behaviour of this large amoeba is exceptional, but it is possible that the preferences of A. proteus in the field are diverse. According to Schaeffer (1916a),"a hungry amoeba eats any organism it can get hold of". Kepner (1913) found amoeboid forms to react to motile organisms, i.e. flagellates, ciliates and rotifers, and "non-motile" forms such as desmids, Oscillatoria and encysted Chlamydomonas. In addition, Mast and Root (1916) observed Amoeba feeding on rotifers.

Laboratory food preference experiments on A. proteus are contradictory, with Schaeffer (1916a) stating that they favoured small flagellates, while Mast and Hahnert (1935) reported A. proteus as having no preference between the large ciliate Colpidium and the small flagellate Chilomonas. Perhaps the problem was best resolved by Prescott and James (1955) when they stated that A. proteus grew adequately on T. pyriformis, but consumed other protozoa as well.

As previously stated in Section 1.2. on the problems associated with the culture of Amoeba, Tetrahymena alone is not nutritionally adequate for the growth of A. proteus; bacteria are also required. The direct contribution of these bacteria on the energy uptake of A. proteus however has been considered negligible (Page 20).

The possibility of pinocytosis supplementing the nutrition of Amoeba was also considered. The majority of authors who have studied this process have used artificially high concentrations of sugars and proteins to induce the process. It is thought unlikely that pinocytosis contributed to the energy uptake of Amoeba in the dilute soil extract cultures. As pointed out by Chapman-Andresen and Holter (1955), pinocytosis is a useful tool for physiologists interested in introducing specific substances into a cell. In this study, pinocytosis was never observed and hence its possible influence on the nutrition of A. proteus was discounted.

The total number of Tetrahymena consumed over a generation invariably increased with decreasing temperature; a function of the increased volume attained and the long generation times found for the lower temperatures. The impact of predation on the ciliate population was greatest at the higher temperatures, as was shown by a comparison of the feeding rates (Figure 28). A. proteus at 20°C consumed only marginally more food per hour than those cells at 15°C, but considerably more than amoebae cultured at 10°C. This reduction in feeding was observed to be a function of the rate of locomotion which was visibly less at the lower temperature. In addition, the rate of food vacuole

digestion was slow at 10°C. Although no experiments were conducted to measure these rates, the nature of the experiments, involving long periods of observation, revealed that digestion proceeded faster at higher temperatures. At 15°C and 20°C the amoebae had a relatively constant capture and digestion rate of Tetrahymena, and although periods of satiation occurred, these were of short duration.

Kepner (1913) reported that the relative number of food vacuoles did not make any marked difference to the uptake of food by Amoeba, but these experiments were presumably at room temperature (20°C) where digestion was rapid. In a study on the feeding behaviour of Woodruffia on Paramecium, Salt (1967) found restraints on the capture rate after the ingestion of only 3 ciliates, possibly due to the sheer volume of prey mass consumed. A. proteus did not have these strict constraints, being of a more elastic structure, and was observed to ingest as many as seven Tetrahymena within one hour at 20°C. At the lowest temperature studied, 10°C, digestion was slow and after the ingestion of one or two Tetrahymena, the feeding response was stopped, resulting in the reduced rate of capture reported.

The cyclic feeding behaviour of A. proteus, as reported by Salt (1961), was not observed in the present study with the amoebae showing no recognisable pattern in their rate of capture over the 24 hour experimental period. The study on the growth of Amoeba by Prescott (1955) substantiates this absence of a feeding cycle. Prescott obtained gradually increasing weight values over the cell cycle, an unlikely

condition had the feeding behaviour been cyclic.

Mast and Fennell (1938) studied the relation between temperature and the frequency of ingestion of chilomonads by A. proteus. Increasing consumption was found between 5 - 26°C, thereafter the rate decreased to zero at 40°C. The results of the present study suggest that the peak consumption rate for A. proteus may have been found at about 22°C, had the range of temperatures been extended. Metalinikow (1912) maintained that low temperatures retarded the frequency of ingestion in paramecia whereas high temperatures accelerated the capture rates. With reference to the ciliated protozoa, Laybourn (1975) found that the consumption of bacteria by Colpidium was not greatly affected by temperature, although the rate was slightly higher at 20°C compared with 15°C, and lower at 10°C.

Food availability was found to be an important variable in determinating the rate of consumption for A. proteus. The frequency of ingestion increased with increasing food concentration to an optimum rate and thereafter decreased as the cell became saturated with food vacuoles. Mast and Fennell (1938) observed that the concentration of chilomonads did not appreciably affect the rate of ingestion by amoebae. Unfortunately, they only investigated two food concentrations and not a complete range as was the case for the present study. When wider food ranges are considered, the predator is generally found to respond to changes in the level of food concentration. Heal (1967a) found consumption to be linearly related to food availability when Acanthamoeba was

cultured with different yeast concentrations. Harding (1937) obtained a similar pattern for the feeding behaviour of Glaucoma pyriformis. Increasing the food concentration resulted in an increase in the number of food vacuoles formed. Laybourn (1976) examined the rate of consumption in Podophrya when fed on Colpidium campylum. The number of prey captured increased with prey concentration and then decreased at the highest food concentration. An optimum consumption rate was also found by Rigler (1961) for Daphnia magna. Below 10^5 yeast cells ml^{-1} the feeding rate was proportional to the concentration of food, while above this level of prey, there was little effect on the feeding rate. Galkowskaya (1961) found the rate of consumption of the rotifer Brachionus calyciflorus to increase with increasing food availability up to a maximum after which the rate levelled off. King (1967) published a similar result for Euchlaris dilatata when fed on Chlamydomonas, although no levelling period in the feeding rate at high food concentrations was obtained.

It was significant that the maximum consumption rates for Amoeba occurred at lower food levels as the temperature was reduced from 20°C to 10°C. As the numbers of food items, notably the ciliates and flagellates, decrease in the field with decreasing temperature (Chapter 8), it is probable that this shift in threshold level is ecologically advantageous to the amoebae, enabling them to excercise optimum feeding rates, regardless of temperature.

It is difficult to compare the rates of consumption found for A. proteus in the present study with previous publications which have been carried out over a variety of conditions. Salt (1961)

found previously starved Amoeba to consume between 1.7 to 2.3 Tetrahymena per hour while unstarved amoebae captured 1.2 cells per hour, presumably at room temperature. This compares favourably with the range of between 0.85 to 2.60 obtained in the present study for 20°C. Mast and Fennell (1938) reported a consumption rate for A. proteus of between 19 - 30 Chilomonas $hour^{-1}$ at 20°C presumably when cultured under conditions of ample food. Assuming Chilomonas has a cell volume corresponding to an ellipsoid (Appendix 10), the volume was calculated to be 2,453μm^3. This estimated figure suggests that an individual Tetrahymena of mean cell volume 19,500μm^3 (Page 32) was equivalent in volume to 7.9 Chilomonas. The biomass of Tetrahymena protoplasm consumed at 20°C for conditions of abundant food (2,000 and 4,000 Tetrahymena 500μl^{-1}) in this study therefore corresponded to 17 - 21 Chilomonas per hour, a range within the same order of magnitude as that found by Mast and Fennell (1938).

It is tentatively suggested that the volume of protoplasm consumed per amoebae may be relatively constant regardless of prey species. Obviously further comparisons are required, but if this were the case, laboratory energy studies, incorporating only one prey species, may be more meaningful for extrapolation to the field situation than is often argued.

Chapter 5.

5.1. Production.

5.1.1. Introduction.

There is a noticeable lack of information in the literature concerning growth of sarcodines in general. Direct comparisons on the effect of varying the food availability and temperature have never been carried out for A. proteus or related species.

Recent energetics studies, with the exception of Heal's (1967a) investigation on the small sarcodine Acanthamoeba, have centred around the ciliated protozoa. Curds and Cockburn (1968a, 1971) studied the growth in batch culture of T. pyriformis, while Laybourn has documented the energy expenditure for production in the ciliates, C. campylum (1973), P. fixa (1976c) and S. coeruleus (1976a).

Physiologists investigating the growth rate of cells are often interested in the increase in total dry mass, which makes allowance for the synthesis of new cellular material. Energeticists, however, require only a measure of the increase in protoplasm volume over the cell cycle to permit an estimate of production.

The determination of production often presents a problem for those researchers investigating higher animals. The various stages in growth and reproduction, in conjunction with the loss of products such as hair and exudates, represent difference levels of energy expenditure and energy loss. In protozoa, the task is simplified, as production is equivalent to growth which equals reproduction; the total amount of protoplasm produced in one life cycle being passed on to subsequent generations. Because asexual reproduction is a direct product of growth, the rate of reproduction has been used by workers as a means of estimating production. One such study was that of Heal's (1967a) for the soil amoeba Acanthamoeba. This method of estimating growth was rejected for the present study as a result of the findings of Kimball, Caspersson, Svensson and Carlson (1959). They concluded that the rate of growth and the rate of reproduction in Paramecium may be capable of independent variation. In addition, Laybourn (1973) stated that the two rates, production and reproduction, should be considered separately as the reproduction of a dense population is not necessarily indicative of a large increase in protoplasm production.

The most widely adopted method for determining the cell volumes of protozoa is that of direct microscopic measurement of the length and breadth of the cell. The nearest geometric shape is then determined and the appropriate formula applied. Earlier in this present study, the volume of T. pyriformis was obtained by assuming that the shape of the cell approximated to a prolate spheroid (Page 31).

More recently, the Coulter Counter in conjunction with a size analyzer has been used for volume determinations, notably by Rickets (1974) and Laybourn (1973), for the ciliated protozoa. In addition, Weik and John (1977) sized the small amoeba Naegleria using a Coulter Counter in conjunction with a pulse height analyzer. These methods were found unsuitable for A. proteus as the considerable volume and variable shape of the cell repeatedly fouled the available aperture sizes.

Chalkley (1931) estimated the volume of Amoeba by repeatedly drawing the cell into and ejecting the cell from a capillary pipette, thus stimulating the organism to assume a spherical shape. The diameter was then measured and the volume calculated. Earlier, Chalkley (1929) developed a sophisticated apparatus, designated the "optical apparatus" for measuring the cell volume of amoebae. This method relied on estimating the diameter an amoeba would have if spherical in shape. This diameter was calculated from a series of axial measurements which, when plotted graphically, provided an estimate of the diameter of a "spherical" amoeba. It was assumed that the organism was at all times comparable in form to an ellipsoid of rotation. This somewhat complicated method did not stand the test of time, possibly as it was time consuming and involved the construction of special optical apparatus for the measurement of the axes, and was seldom used by subsequent workers excepting Belda (1942) and Pace and Frost (1952).

An obvious method for measuring the volume of an amoeboid cell relies on introducing the cell into a calibrated cylindrical capillary and measuring the length. Petrerfi (1937) used this

technique on Amoeba sphaeronucleata as did Mast and Fowler (1935) and Ord (1968a) for A. proteus.

The colorimetric method of Holter (1945) consisted of sucking an Amoeba into a wide capillary with a small quantity of dye solution. The volume of the dye and Amoeba phase was known from measuring the length within the capillary and the volume of dye was estimated by micro-colorimetry. By subtraction, the volume of the cell was found. This method, however, was developed specifically for the large amoeba Chaos chaos, and problems associated with the evaporation of the dye solution have been reported by Lumsden and Robinson (1953).

Volume measurements can also be calculated from the results of reduced weight and density measurements. The former are obtained using Zeuthen's Cartesian diver balance (1948) while the density estimates are derived from the starch density gradient technique, as described by Løvtrup (1950).

The final option open for the determination of the cell volume of naked amoebae relies on compression techniques. These range from the sophisticated "Roto-compressor" developed by American protozoologists, and reported on by Hahnert (1972/73), and the compression chamber of Lumsden and Robinson (1953), through to the flattening of amoebae cells to a known depth between glass slides (Scholander, Claff and Sveinssen, 1952a). Prescott (1955) in his studies on A. proteus made a chamber by etching a depression on a glass slide with Hydrofluoric acid. The amoebae were then flattened to a depth of less than 10μ. The method used for this study was a compression technique, chosen because of its speed and accuracy in determining the volume of

Amoeba cells.

The aim of this section was to obtain production estimates for A. proteus when grown at the various food concentrations and temperatures, which could be incorporated into the series of energy budgets being compiled. Additionally, the various hourly production rates were examined and discussed in terms of the consumption levels and temperatures employed.

5.1.2. Compression technique: Materials and Methods.

Amoebae for experiments were cultured in solid watch glasses under the appropriate conditions as detailed in Sections 1.2.5. - 1.2.10.

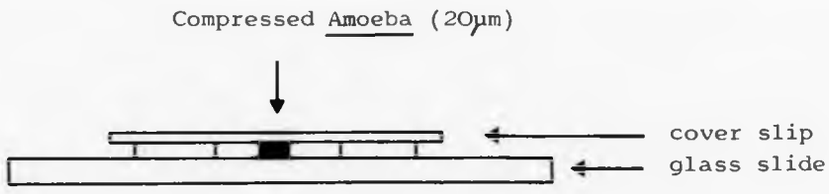
The technique of measuring the cells in a calibrated capillary was investigated, however, two serious difficulties were encountered. As A. proteus is polypodial the organism did not fully fill the capillary, resulting in an overestimated cell volume. When the capillary diameter was reduced to overcome this problem, a high mortality, as a result of cells rupturing, was found. The reduced-weight density method was also tried in preliminary experiments, but it soon became apparent that the method was time consuming and not suited for large numbers of volume determinations.

The method finally adopted for measuring the cell volume of A. proteus consisted of flattening individual cells to a uniform depth of 20µm in a Hawksley Standard Bacterial Counting Chamber. As this process ruptured live specimens, cells were fixed in Carnoy's flud (90% ethylalcohol, 10% Acetic acid). A drop of

fixative was pipetted onto the cover slip and placed on top of the counting slide, which contained the amoebae in a minimum of culture solution. In this manner the cells were fixed and compressed in the same movement. The counting chamber with cover slip is shown in cross section below (Figure 30).

Figure 30.

"Compression Chamber".



Uniformity in the method was achieved by rounding the amoebae before fixation by repeated ejection from a micropipette. This ensured complete withdrawal of the pseudopodia prior to the flattening process. Compressed amoebae were projected with a Camera-Lucida microscope and the image traced with a planimeter. The volume was calculated from the product of the area and the depth.

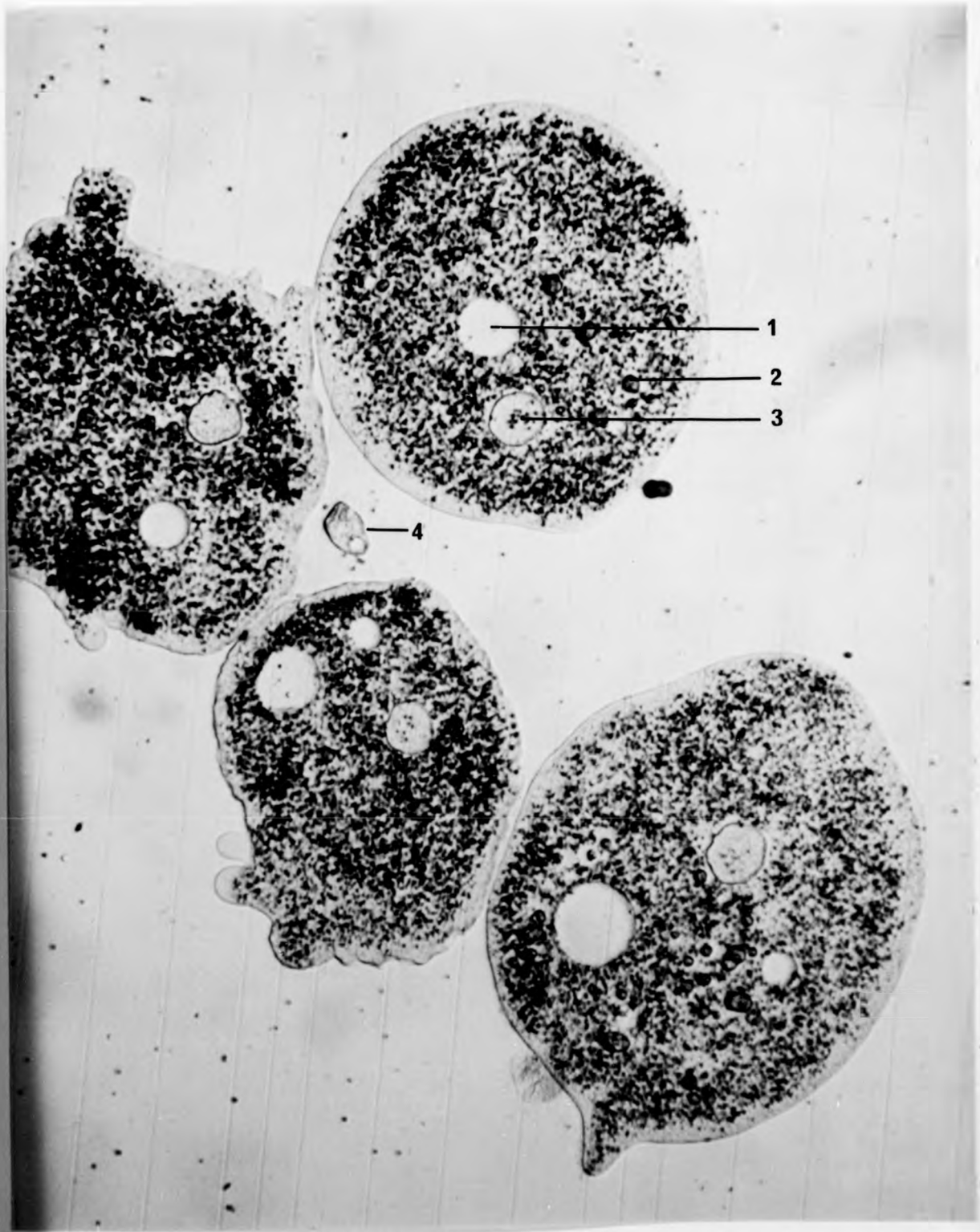
Lumsden and Robinson (1953) proposed a correction factor to compensate for the rounded edge along the perimeter of the compressed cell. This was necessary in their study as the depth of amoebae was considerable at 77µm. No correction was employed in the present method as the flattening process was severe (Plate 3) and the error at the perimeter therefore negligible.

Plate 3.

4 compressed *Amoeba* cells (20µm depth).

1. Contractile vacuole.
2. Food vacuole.
3. Nucleus.
4. *Tetrahymena* cell.

th).



5.1.3. The pattern of production of A. proteus over a life cycle: Materials and Methods.

In order to compile instantaneous and generation energy budgets (Chapter 7), it was necessary to ascertain the type of growth pattern followed over the cell cycle, and by what factor the cell volume of a newly divided Amoeba increased over a generation. If the rate of cell production was not constant over the cycle, the calculation of the generation budgets would have to make allowance for this fact.

Amoebae were cultured under two differing food concentrations, 125 and 4000 Tetrahymena cells $500\mu l^{-1}$ at $20^{o}C$ and $15^{o}C$ in solid watch glasses (Page 33). Due to the difficulty in selecting newly divided amoebae from cultures at $10^{o}C$, where generation times ranged from 372 - 2,926 hours, volume determinations were not carried out at this temperature for the initial experiments. Similarly, only 2 food concentrations were investigated for $20^{o}C$ and $15^{o}C$ at this stage. It was assumed that the pattern of Amoeba cell growth was the same for $10^{o}C$ and for other Tetrahymena levels.

Cultures were periodically examined and newly divided amoebae were pipetted out and transferred to fresh watch glasses containing the appropriate food concentrations. Amoeba form a characteristic rounded shape at the onset of cell division, and with practice, dividing cells can be selected. Care was taken not to expose the amoebae to strong light as this resulted in unequal cell division. As Prescott (1956) reported, only amoebae kept in weak light or complete darkness divided to yield two daughter cells of equal volume. Individual amoebae of a known age were

fixed and flattened to determine their cell volume. The change in cell size with time over the cycle was thus determined.

5.1.4. Results.

Growth was found to be generally linear over the cell cycle (Figures 31 - 34) although a tendency towards decreasing growth and a period of levelling before cell division could also be argued, especially with regard to Figure 31. This discrepancy in interpretation of the results arises from the high degree of scatter in the graphs, but this can be accounted for. It is accepted that there were inaccuracies inherent in the methods employed, particularly with regard to inconsistencies in the flattening and tracing techniques. These were slight, however, as close agreement was found between the volumes of the daughter cells of a newly divided cell (Appendix 11). The degree of scatter was almost certainly due to the size variation between amoebae of the same age, i.e. growth stage. Variations in the feeding rates, digestion rates and in the initial daughter cell volumes of amoebae from like cultures, all gave rise to inconsistency in the volumes of equally aged cells.

It can be concluded that the Amoeba cells doubled in volume over the period between the newly divided daughter cell and the point before the onset of binary fission. In addition, the rate of production (growth) of protoplasm over the cell cycle was linear.

Figure 31.

Growth of A. proteus over the cell cycle when cultured at the food concentration of 125 Tetrahymena $500\mu l^{-1}$ at $20^{o}C$.

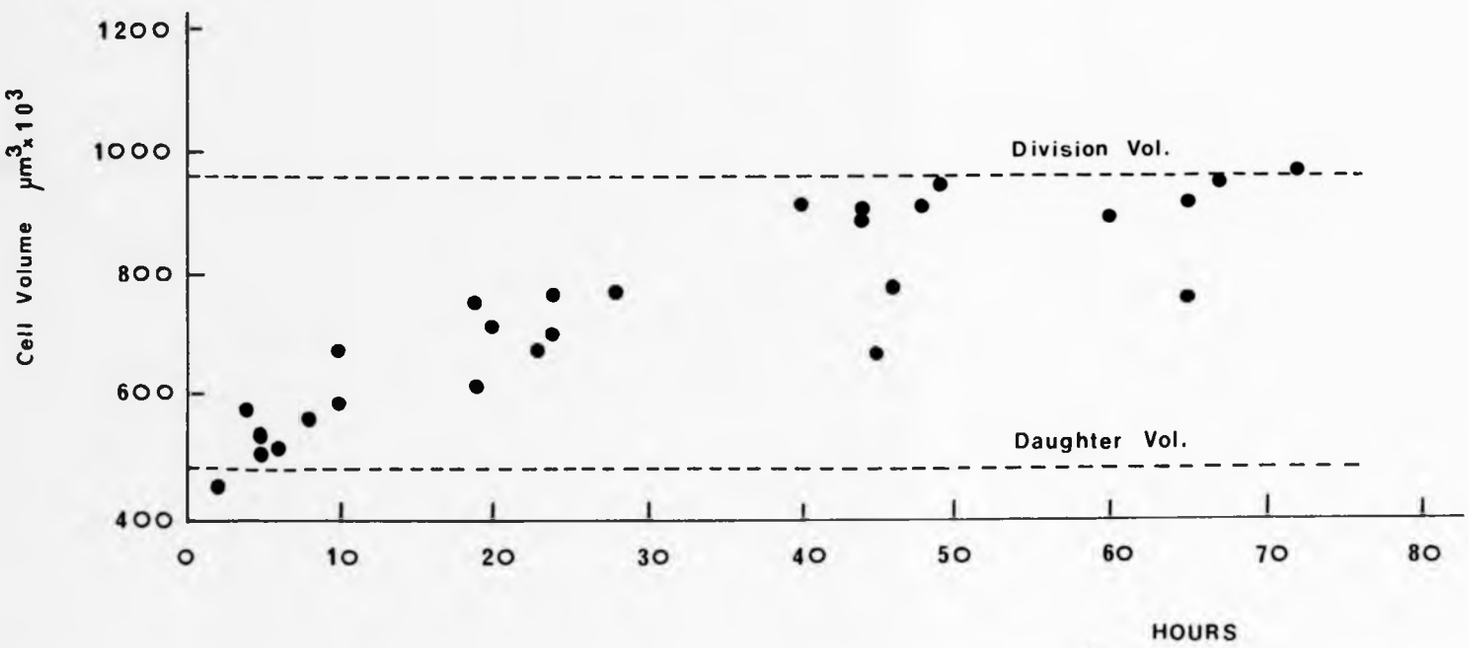

1200
1000
800
600
400
Cell Volume $\mu m^3 \times 10^3$
Division Vol.
Daughter Vol.
0
10
20
30
40
50
60
70
80
HOURS

Figure 32.

Growth of A. proteus over the cell cycle when cultured at the food concentration of 4000 Tetrahymena 500μl^{-1} at 20^{o}C.

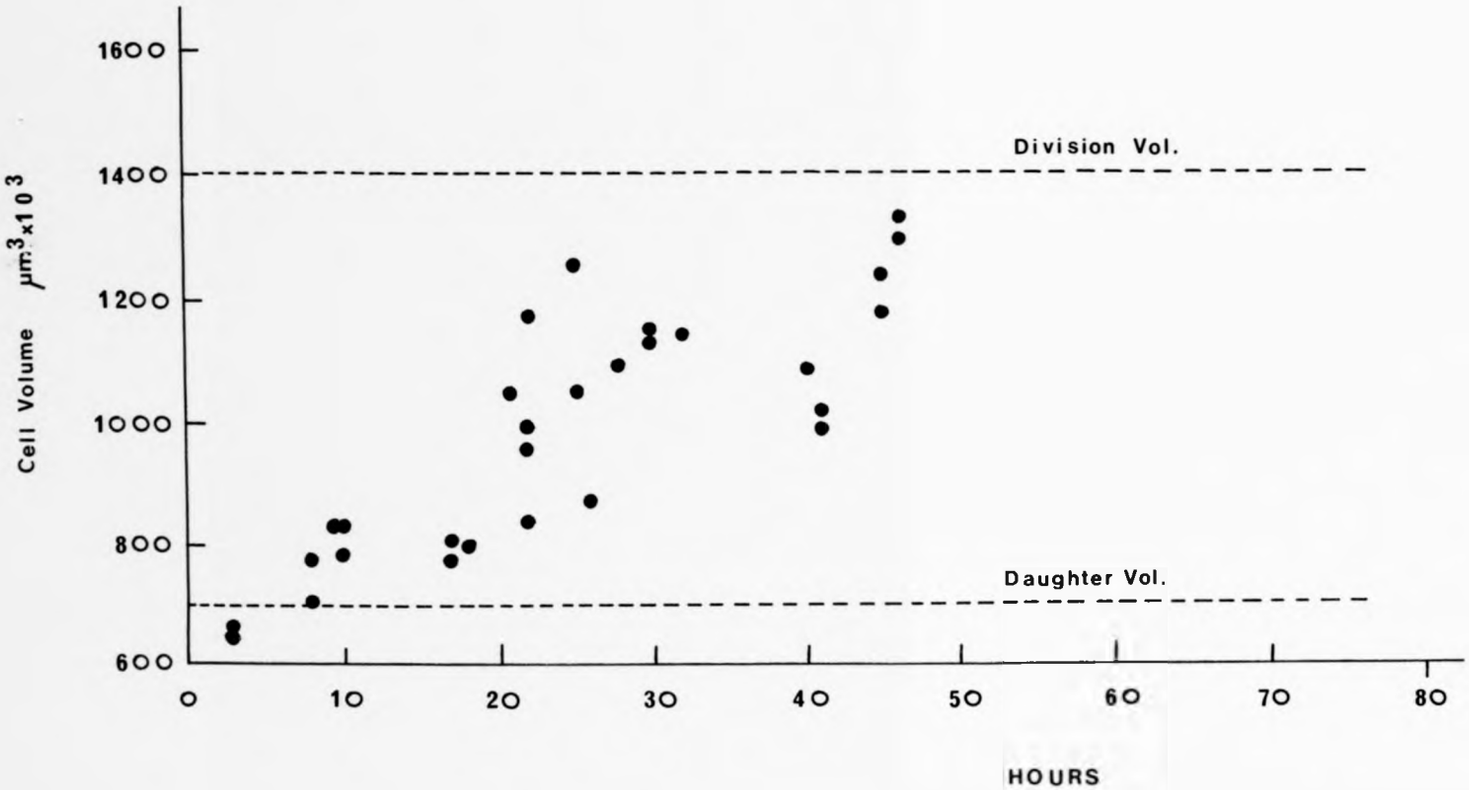

1600
1400
1200
1000
800
600
Cell Volume $\mu m^3 \times 10^3$
Division Vol.
Daughter Vol.
0
10
20
30
40
50
60
70
80
HOURS

Figure 33.

Growth of A. proteus over the cell cycle when cultured at the food concentration of 125 Tetrahymena $500\mu l^{-1}$ at $15^{o}C$.

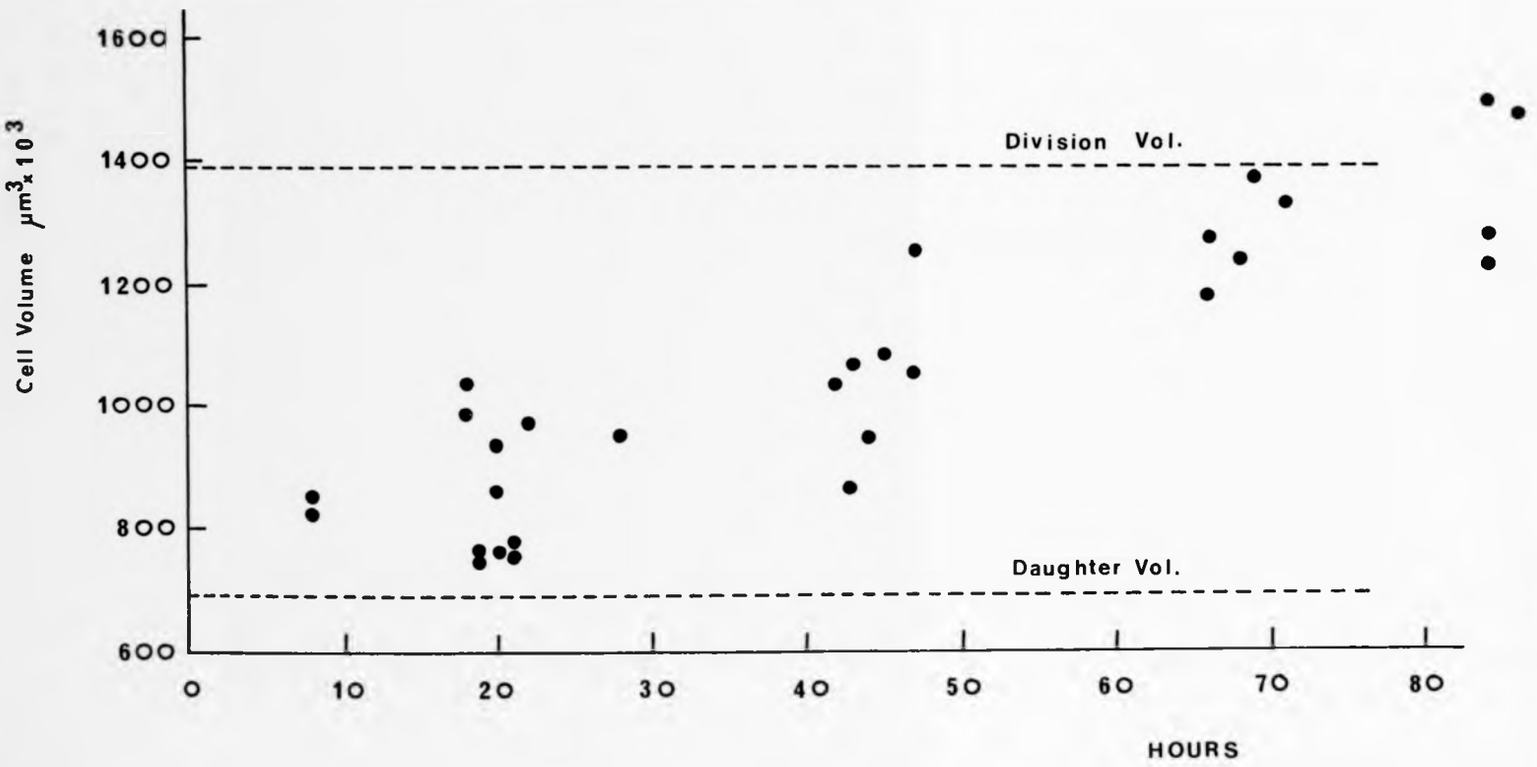
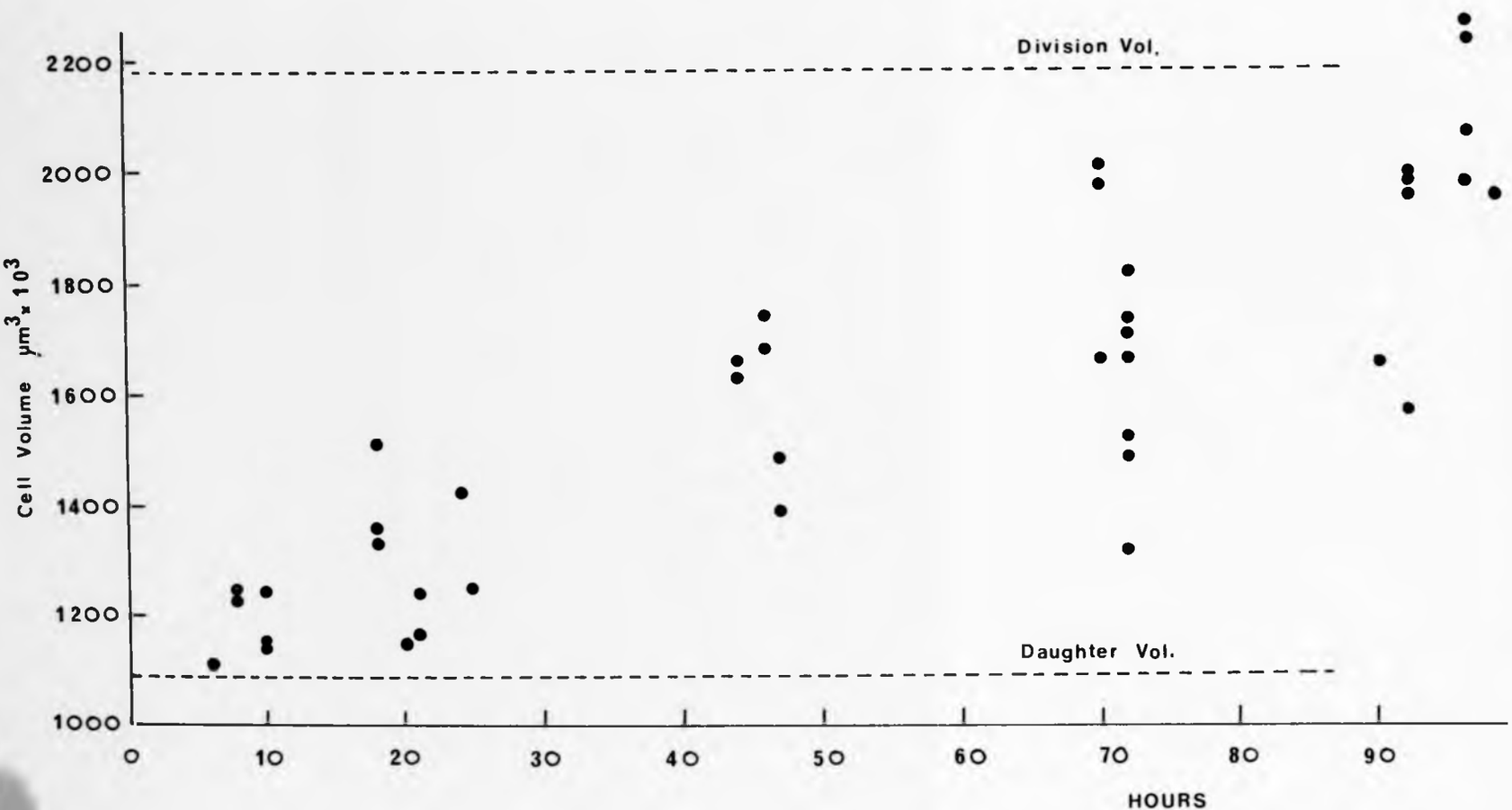

1600
1400
1200
1000
800
600
Cell Volume $\mu m^3 \times 10^3$
Division Vol.
Daughter Vol.
0
10
20
30
40
50
60
70
80
HOURS

Figure 34.

Growth of A. proteus over the cell cycle when cultured at the food concentration of 4000 Tetrahymena $500\mu l^{-1}$ at $15^{o}C$.


Division Vol.
Daughter Vol.
Cell Volume $\mu m^3 \times 10^3$
2200
2000
1800
1600
1400
1200
1000
0
10
20
30
40
50
60
70
80
90
HOURS

5.1.5. The determination of the overall (Total) production per generation of individual A. proteus cells: Materials and Methods.

As the cell volume was shown to double over a generation, the production of Amoeba was estimated by selecting newly divided cells, the volumes of which were determined by the compression technique described. In other words, the volume of protoplasm produced over a generation was equal to the volume of a newly divided cell.

The volumes of twenty such cells were determined for all the food concentrations (125 - 4000 Tetrahymena $500\mu l^{-1}$) and temperatures ($10^{o}C$, $15^{o}C$ and $20^{o}C$) investigated. The mean volume of twenty daughter cells was calculated and used as an estimate of the cell production. The mean growth rates per hour were calculated by dividing the overall Amoeba production by the appropriate generation time (Chapter 3).

For subsequent calculations, all volumes were converted to dry weight using the conversion values of 0.162 and 0.147pg μ^{-3} for Tetrahymena and Amoeba respectively. The dry weight estimates were converted to joules using the values 19.80J mg^{-1} ($15^{o}C$ and $20^{o}C$) and 18.28J mg^{-1} ($10^{o}C$) for T. pyriformis, and 17.51J mg^{-1} for A. proteus (Chapter 2).

Experiments were conducted throughout a two year period of the study.

5.1.6. Results.

Temperature was found to exhibit a pronounced effect on the total production of amoebae cells over the cell cycle. The largest cells were all obtained at the lowest temperature 10°C, regardless of the initial food concentration of the cultures. The total production per generation decreased with increasing temperature over the range investigated, being least at 20°C where the overall cell production was less than half that at 10°C. The mean production values for Amoeba are given in Table 11 and the relationship is presented in Figure 35, where the height of each bar is a measure of the total cell production for the various conditions employed. The complete list of volume determinations for A. proteus is given in Appendix 11.

Table 11.

The effect of temperature and food concentration on the production μm^3 ($\times 10^3$) of A. proteus per generation.

For each value n = 20; the 95% confidence limits are included.

Food conc. of Tetrahymena $500\mu l^{-1}$.	Temperature (°C)		
	20	15	10
125	480 ± 19	695 ± 40	1016 ± 63
250	575 ± 25	751 ± 32	1421 ± 13
500	659 ± 27	815 ± 47	1455 ± 82
1000	693 ± 36	808 ± 29	1648 ± 85
2000	683 ± 39	986 ± 56	1557 ± 73
4000	701 ± 33	1092 ± 51	1461 ± 86

Figure 35.

The effect of temperature on the total cell production per generation of A. proteus.

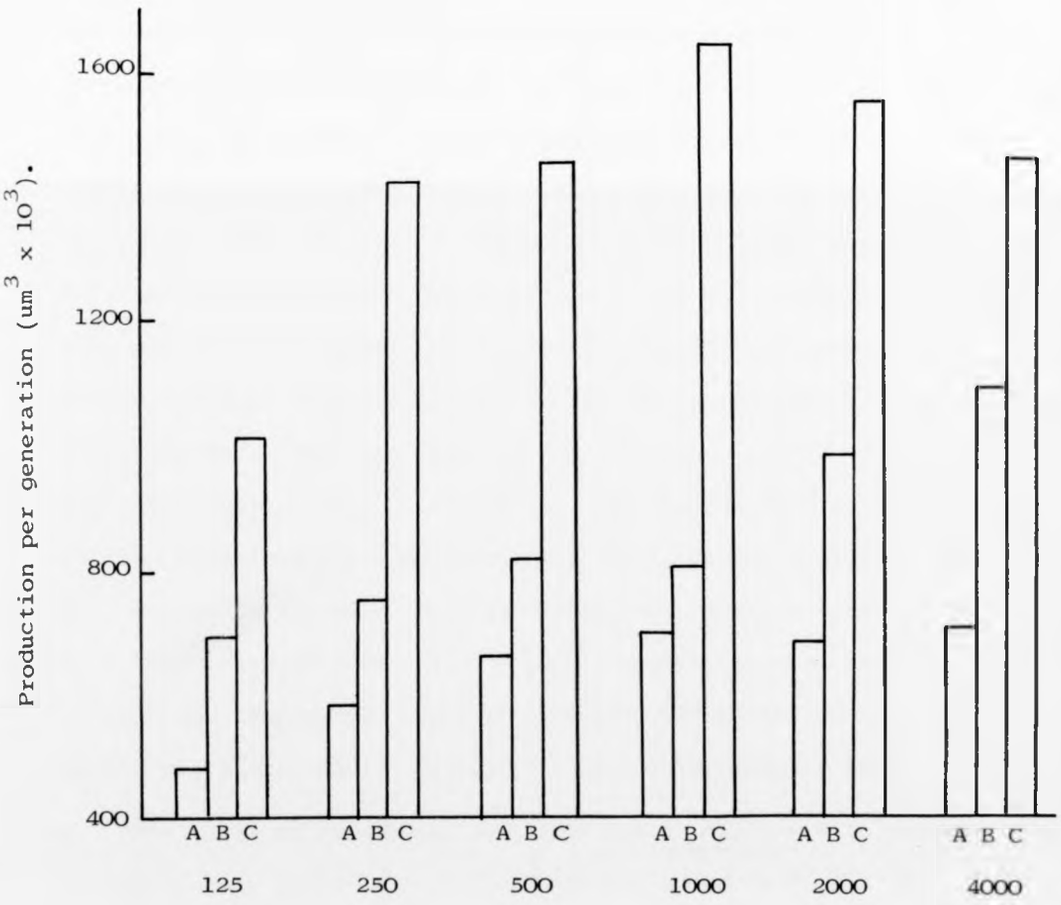
A - 20°C

B - 15°C

C - 10°C

total

A. proteus.



In addition, cell production per generation was influenced by the food concentration of Tetrahymena under which the amoebae were cultured. Figures 36 and 37 show an initial increase in the amount of production over the cell cycle as the level of food availability was increased over the range 125 - 4000 Tetrahymena cells $500\mu l^{-1}$. The tendency thereafter was to form a production peak at higher food levels. At $20^{o}C$ (Figure 36) the maximum production per generation was obtained on the food concentration of 1000 Tetrahymena $500\mu l^{-1}$. Increasing the food availability further up to 4000 cells $500\mu l^{-1}$ had no effect on the overall production, suggesting that amoebae at this temperature could tolerate these high food concentrations. At $15^{o}C$ (Figure 36), although the peak production per cell cycle was not reached within the food range examined, the decreasing pattern of the curve indicates that the peak was close to the food level of 4000 Tetrahymena $500\mu l^{-1}$. Again, a tolerance to high numbers of food organisms was indicated. For $10^{o}C$, however, (Figure 37) the overall cell production decreased after reaching a maximum at a food level of 1000 cells $500\mu l^{-1}$, suggesting that amoebae at this low temperature had a narrow food concentration range within which they could efficiently cope with ingested food.

The rates of production per hour for A. proteus with regard to temperature and food concentration are shown in Figure 38. Although amoebae cells cultured at $10^{o}C$ were the largest in volume their rates of production were lowest increasing with temperature up to $20^{o}C$. The maximum rate of production for $20^{o}C$ was approximately $15,500\mu m^{3}\ h^{-1}$ and was found to decrease with

Figure 36.

The effect of food concentration on the total production per generation of A. proteus at 20°C and 15°C (± 95% confidence limits).

curves fitted by eye.

Figure 36.

The effect of food concentration on the total production per generation of A. proteus at 20°C and 15°C (± 95% confidence limits).

curves fitted by eye.

total

at

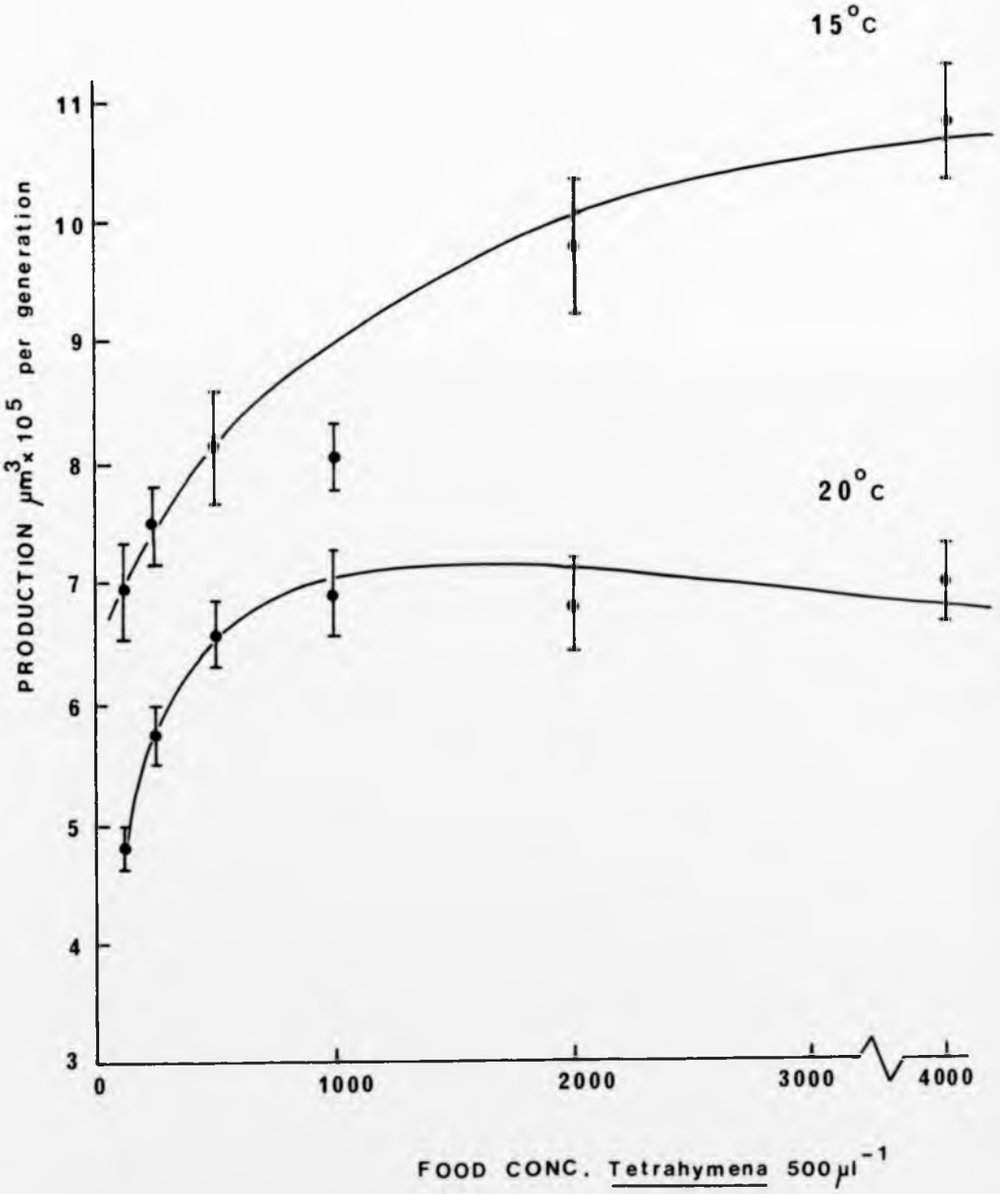

15°C
20°C
PRODUCTION $\mu m^3 \times 10^5$ per generation
11
10
9
8
7
6
5
4
3
0
1000
2000
3000
4000
FOOD CONC. Tetrahymena 500 μl^{-1}

Figure 37.

The effect of food concentration on the total production per generation of *A. proteus* at 10°C (± confidence limits).

curves fitted by eye.

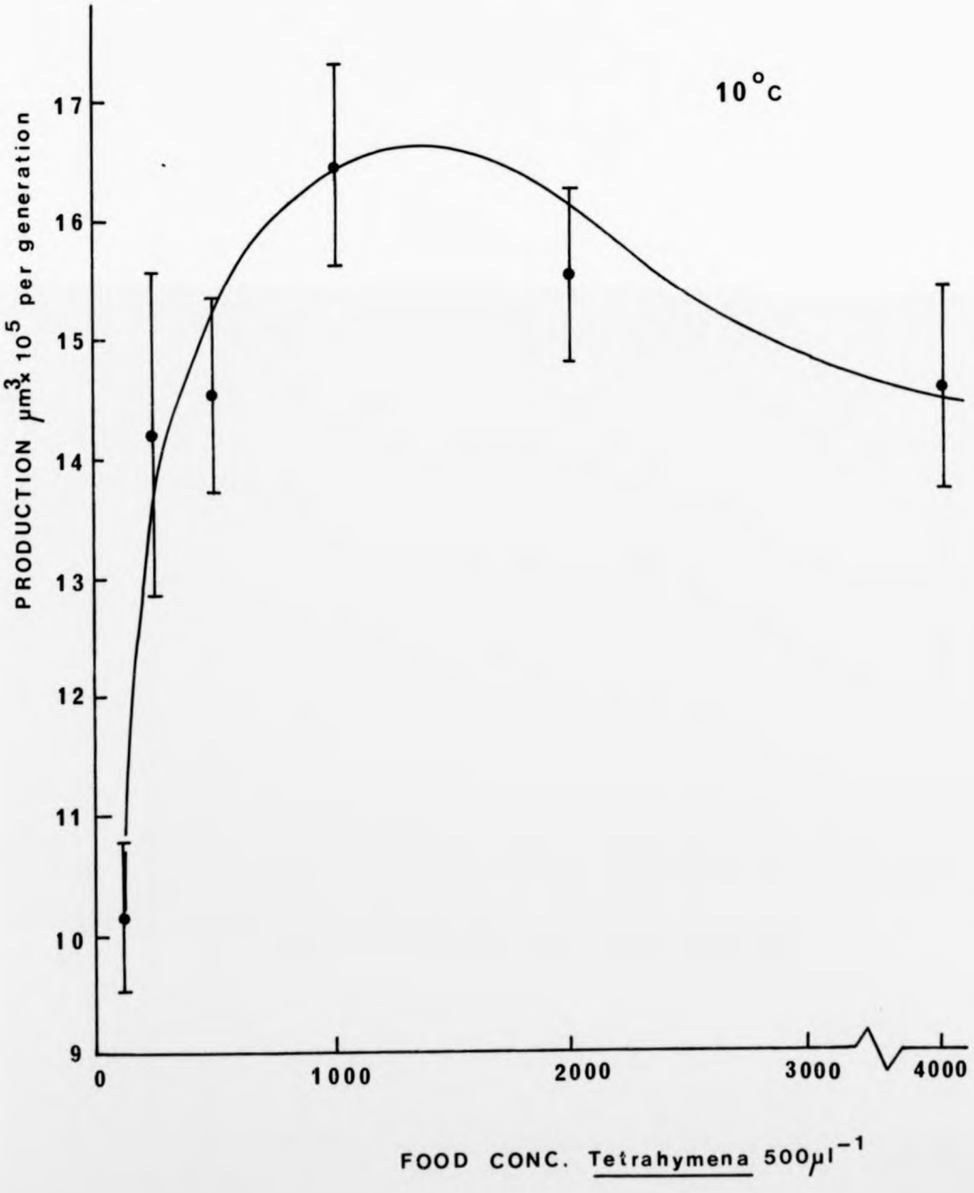

10°C
PRODUCTION μm³ x 10⁵ per generation
17
16
15
14
13
12
11
10
9
0
1000
2000
3000
4000
FOOD CONC. Tetrahymena 500μl⁻¹

Figure 38.

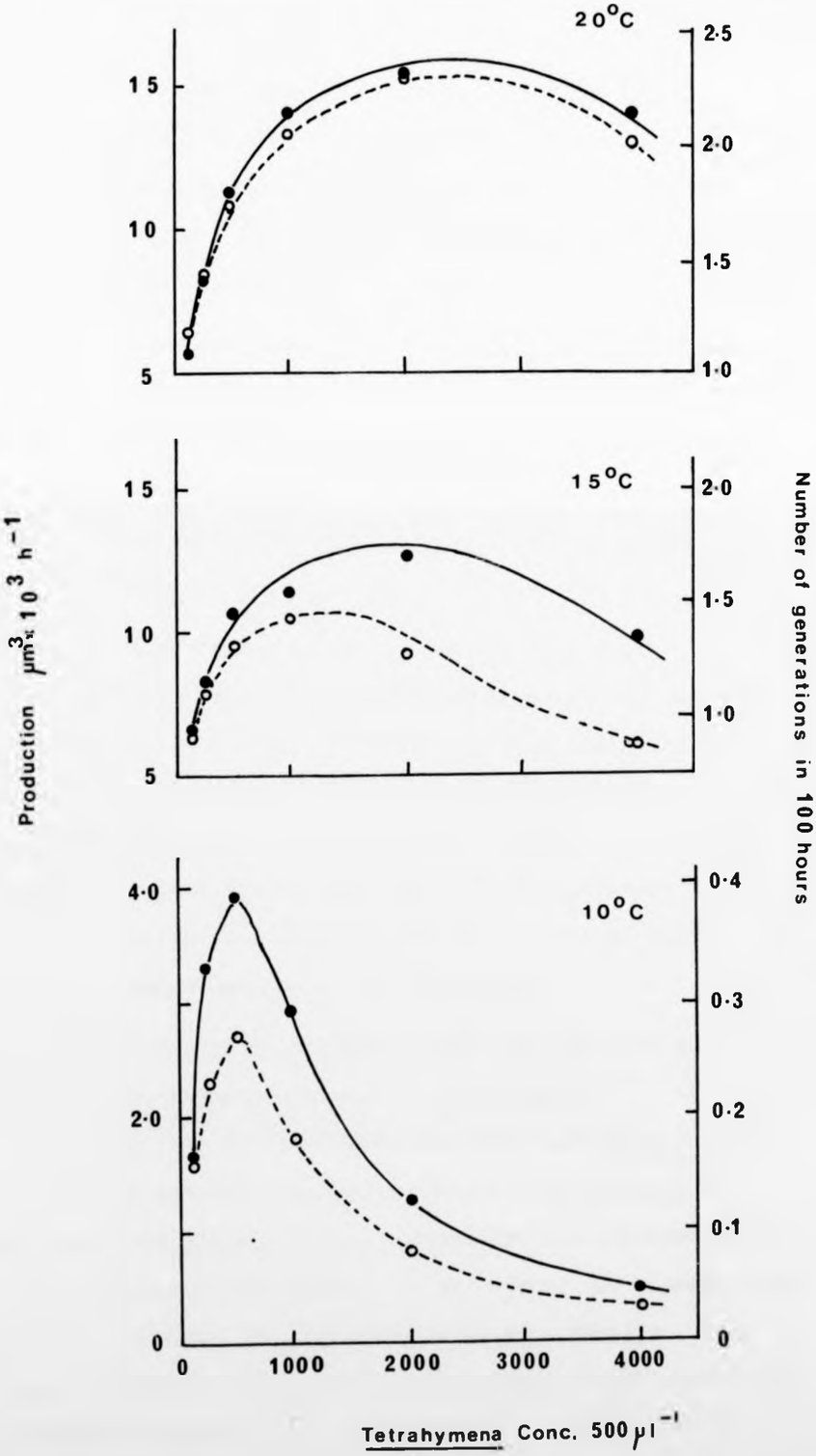
The effect of food concentration and temperature on the rate of production (h^{-1}) of A. proteus.

●—●

The effect of food concentration and temperature on the rate of reproduction ($100h^{-1}$) of A. proteus.

o····o

Curves fitted by eye.


20°C
15°C
10°C
Production $\mu m^3 \times 10^3\ h^{-1}$
Number of generations in 100 hours
Tetrahymena Conc. 500 μl^{-1}
0
1000
2000
3000
4000

temperature, the maxima being approximately 12,500μm^3 h^{-1} at 15°C and 3,900μm^3 h^{-1} at 10°C. The rates of reproduction, a parameter often used to indirectly measure growth are also given in Figure 38, and were found to closely follow the pattern obtained for production.

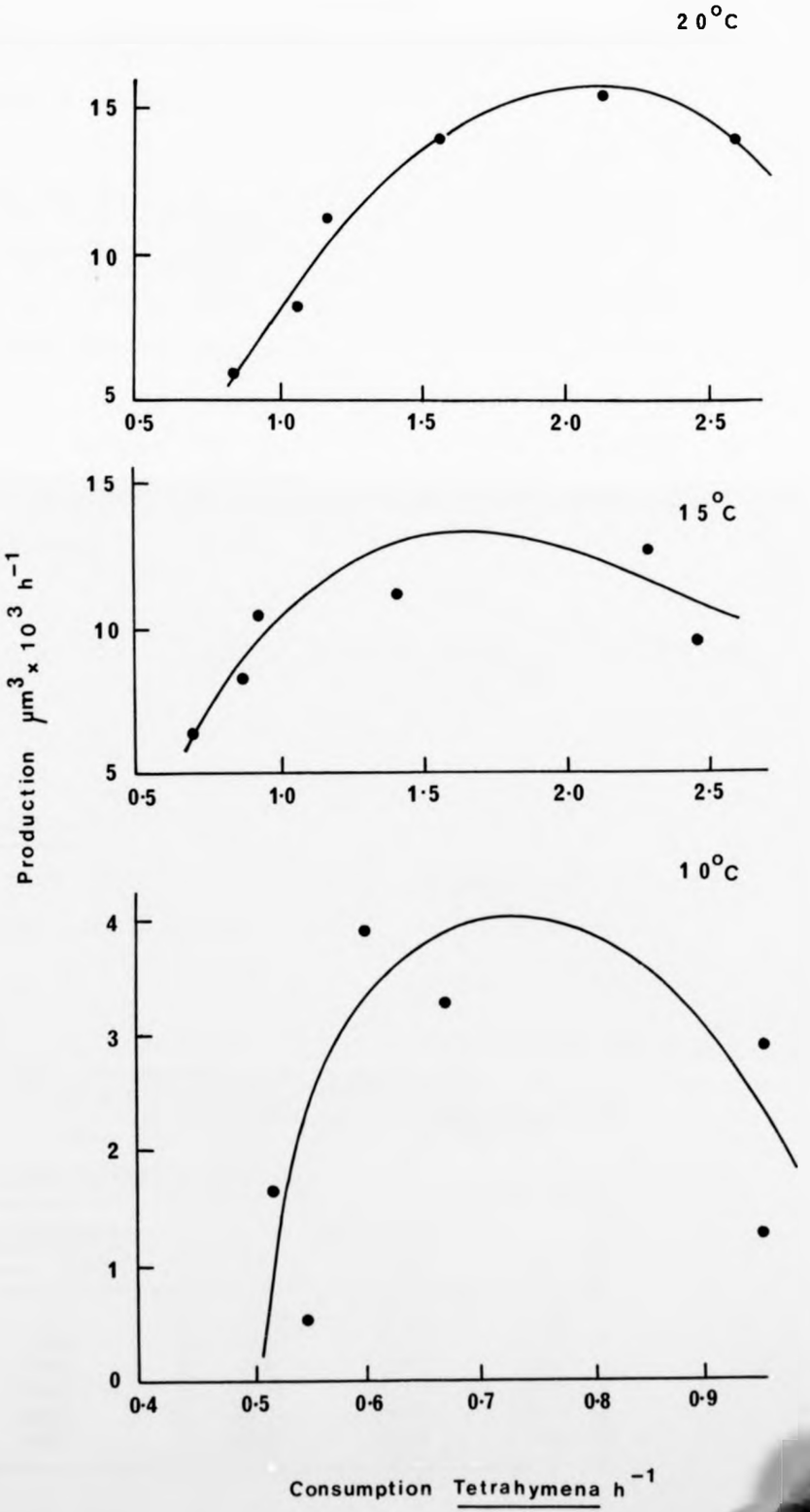
An increase of 10°C within the temperature range studied was sufficient to raise the amount of protoplasm produced per hour by approximately 300%. The increase in the magnitude of the production rate was greatest for the first 5°C rise over 10°C - 15°C, where an average Q_{10} value of 10.27 was calculated. Between 15°C - 20°C the Q_{10} value was lower at 1.54.

Figure 39 illustrates the effects of consumption, in terms of the numbers of Tetrahymena ingested per hour, on the rates of Amoeba production. The graph is similar to that depicting the rate of production against food concentration (Figure 38) as food consumption was essentially a factor of food availability. For all temperatures investigated, the rate of production increased with increasing consumption until a level at which the production peaked and thereafter decreased.

The maximum rate of production at 20°C was attained at a consumption level of approximately 2.2 Tetrahymena h^{-1}. At 15°C, the maximum rate of production was less, 1.7 Tetrahymena h^{-1}, while at 10°C the optimum rate was on only 0.7 Tetrahymena h^{-1}. In other words, the maximum rate of production was achieved on less food as the temperature decreased. In all cases, the highest level of consumption resulted in a decreased rate of production, i.e. production attained a maximum at a peak ingestion level, less than that of maximum consumption.

Figure 39.

The effect of consumption on the rate of production (h^{-1}) of *A. proteus* at 20°C, 15°C and 10°C.


20°C
15
10
5
0·5
1·0
1·5
2·0
2·5
15°C
10°C
4
3
2
1
0
0·4
0·6
0·7
0·8
0·9
Production µm³ x 10³ h⁻¹
Consumption Tetrahymena h⁻¹

For later consideration, the volume determinations were converted to energy units (Table 13).

5.1.7. The determination of the mean cell volume of A. proteus: Materials and Methods.

The mean cell volumes of amoebae when cultured in watch glasses under the various conditions of food and temperature were required for the respiration study (Chapter 6.). As the daughter cell volumes were known and the division volume was twice that of a newly divided cell, the mean cell volumes were calculated from:

$$M.C.V. = \frac{\text{Daughter volume + Theoretical division vol.}}{2}$$

5.1.8. Results.

The mean cell volumes ($\mu m^3 \times 10^3$) of Amoeba under the experimental conditions are tabulated below in Table 12.

Table 12.

The mean cell volumes of A. proteus when cultured at various conditions of temperature and food concentration. ($\mu m^3 \times 10^3$).

Tetrahymena conc. $500\mu l^{-1}$	Temperature (^oC)		
	20	15	10
125	720	1042	1524
250	862	1126	2131
500	988	1222	2182
1000	1039	1212	2472
2000	1024	1479	2335
4000	1051	1638	2191

The effect of temperature and food concentration on the mean cell volume of A. proteus is summarised in a three-dimensional diagram (Figure 40). Again temperature exerted a marked effect on the size of the amoebae cells, with the largest individuals from cultures at 10°C and the smallest cells from cultures at 20°C. The fact that the peaks in cell volume were not found for the range of food concentrations investigated at 15°C and 20°C was ecologically unimportant as 4000 cells $500\mu l^{-1}$ was outwith that concentration expected in the natural situation.

Figure 40.

A three-dimensional diagram illustrating the effect of temperature and food concentration on the M.C.V. of A. proteus.

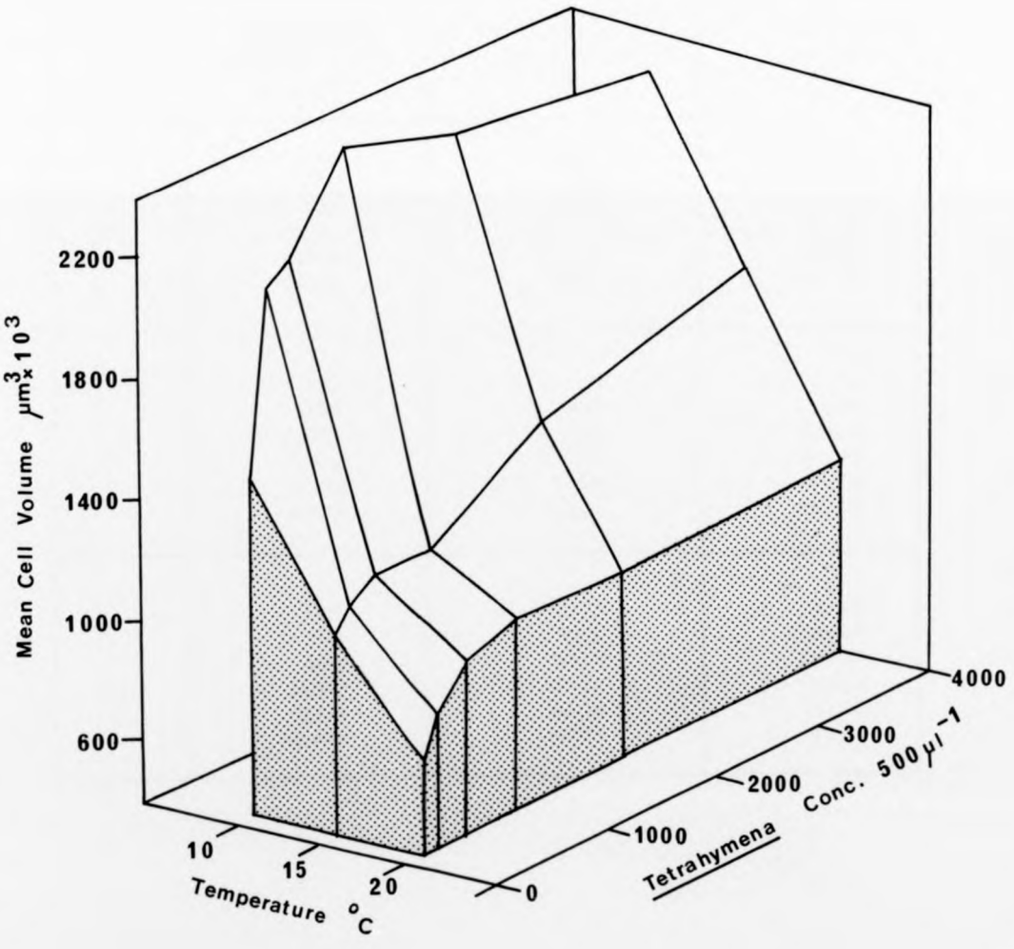

Mean Cell Volume $\mu m^3 \times 10^3$
2200
1800
1400
1000
600
10
15
20
Temperature °C
0
1000
2000
3000
4000
Tetrahymena Conc. 500 μl^{-1}

Table 13.

Production of A. proteus as influenced by food concentration and temperature.

Temp. ^{o}C	Tetrahymena concentration $500\mu l^{-1}$	Production per generation (μJ)	Production per hour (μJ)
20	125	1235	14.70
	250	1480	21.14
	500	1696	29.24
	1000	1784	36.41
	2000	1758	39.95
	4000	1804	36.08
15	125	1789	16.72
	250	1933	21.24
	500	2098	27.25
	1000	2080	29.29
	2000	2538	32.54
	4000	2811	25.10
10	125	2615	4.25
	250	3658	8.49
	500	3745	10.07
	1000	4242	7.56
	2000	4008	3.21
	4000	3760	1.28

5.1.9. Discussion.

Energetics studies of the protozoa have been neglected in the past, especially with reference to the sarcodines, although reviewing the available literature does allow some comparisons to be drawn concerning the present production study, as growth has often been used as a measure of the intensity of a treatment.

The range of volumes obtained for A. proteus in this study were, by comparison, within the expected order of magnitude. Chalkley (1931) published a range of estimates for the volume of amoebae cells prior to cleavage of between 1,823 x 10^3 to 2,842 x 10^3 μm^3, by measuring the cells in a calibrated pipette. This represents a production range per generation of 911 x 10^3 - 1,421 x 10^3 μm^3 for Amoeba presumably cultured at room temperature (20°C). The values from the present study were less, a consequence of the different culture methods employed. In addition, the capillary technique, as already stated, overestimates the cell volume measurements. Prescott (1955), using a compression technique, found the cell volume of newly divided A. proteus to be 497 x 10^3 μm^3 at the higher culture temperature of 23°C, a value which agrees with the trend of decreasing cell volume with increasing temperature, found in the present study. Ord (1970), again using the capillary technique, estimated the volume of A. proteus division spheres to lie between 2,400 x 10^3 to 3,800 x 10^3 μm^3 at 18 - 19°C. Again these high values, representing production values of 1,200 x 10^3 - 1,900 x 10^3 μm^3 may be explained in terms of the measuring technique and culture methods employed.

A general rule for predicting the effect of temperature on the cell size of protozoa can not be applied, as the contradictory

published data show. Laybourn and Finlay (1976) measured the cell volume of 5 ciliates when cultured at 20°C, 15°C and 8.5°C. The cell size decreased with increasing temperature for T. pyriformis whereas with Spirostomum teres, cells grown at 20°C were approximately 40% larger than those cultured at 15°C and 8.5°C. For the remaining three species, Frontonia, Paramecium and Vorticella, the optimum temperature for increased cell size was found to be 15°C.

Summers (1963) found no variation in the cell size of Tetrahymena between 10°C - 20°C but at 25°C, where growth was at its optimum, smaller cells were obtained. Increasing the temperature further increased the volume of the Tetrahymena. Laybourn (1975) found temperature to have no marked effect on the mean cell volume of Colpidium campylum when cultured between 10°C - 20°C, as was the case for the axenically grown T. pyriformis described in Chapter 1.

In the present study, temperature had an important effect on the cell size and production of A. proteus. Cell volume increased markedly with decreasing temperature over the temperature range investigated, with the overall cell production per generation at 10°C more than twice that found for 20°C.

Changing the available food concentration also influenced cell production. At 20°C, maximum production per generation was reached when the amoebae were grown at food levels greater than 1000 Tetrahymena $500\mu l^{-1}$. At 15°C, the relationship was less well defined, although at food concentrations greater than 2000 cells $500\mu l^{-1}$ the increase in cell production was small. For the

lowest temperature investigated, 10°C, the production per generation peaked at 1000 Tetrahymena 500µl^{-1} thereafter, increasing the food concentration decreased the cell size. The overall pattern was therefore one in which the cell production (growth per generation) increased with increasing food concentration (food consumption) to a maximum level which was maintained at 20°C and 15°C, but decreased at 10°C within the food range investigated. The decrease in cell size at 10°C for 2000 - 4000 Tetrahymena 500µl^{-1} was attributable to the low tolerance of amoebae to high food conditions at this temperature.

As discussed in Chapter 4, amoebae at 10°C had an optimum feeding rate at approximately 1500 Tetrahymena 500µl^{-1}, after which the rate decreased. At 15°C and 20°C, the rate of ingestion increased to approximately 2000 cells 500µl^{-1} after which the high level of consumption was maintained over the cell concentrations examined. The pattern for cell production with changing food conditions was therefore similar to that found for consumption. Both studies indicated an intolerence in Amoeba to high food levels at 10°C, where a limitation was imposed on the rate of ingestion and presumably also on the rate of metabolism of ingested cells.

The apparent inefficiency whereby amoebae are unable to utilize high food concentrations at low temperatures is unimportant for Amoeba in the field as the available food source invariably tends to decrease with temperature. Further discussion regarding conditions in the wild is given in Chapter 8.

Comparable data for the effects of food concentration on cell size and cell production are available for the ciliated protozoa. Harding (1937) first demonstrated that the cell size attained could be controlled by the concentration of food (bacteria) available. The cell volume of Glaucoma pyriformis (syn. T. pyriformis) was found to increase with increasing bacterial supply in a batch culture system. A similar pattern was found for Tetrahymena by Curds and Cockburn (1971). In addition Laybourn (1975) found that increasing the bacterial concentration (and therefore consumption) resulted in an increase in the cell volume of Colpidium at 10°C, 15°C and 20°C. A threshold consumption level was found above which the cell volume remained constant, a result similar to that found for A. proteus when cultured at 15°C and 20°C.

As the cell cycle progressed from that of the daughter cell volume, the rate of growth of A. proteus was linear. Prescott (1955) measured the reduced weight (weight in water) of individually growing Amoeba cells at 23°C using Cartesian divers. In addition, he followed the change in cell volume throughout the cycle by a compression technique. Similar curves for both the volume and reduced weight determinations with time were found; cells increased in mass after division until the growth rate gradually slowed to a plateau 4 hours before cleavage. Conversely, a constant rate of increase in the cell volume over the period between divisions was found by Chalkley (1931), again for A. proteus. These conflicting results, in the construction of individual cell growth curves, are in part due to the inaccuracies inherent in the different techniques employed.

With reference to the present study, linear growth was assumed although it is accepted that in some cases, notably Figure 31, a period of reduced growth before cleavage could be interpretated. This was of such short duration, when compared with the length of the cell cycle, that it can be disregarded. Prescott (1959) suggested that the depression in growth before division was due to a decrease in the activity of the cell resulting in the termination of feeding. Throughout the consumption studies (Chapter 4), cells were observed to divide within 30 minutes of ingesting a Tetrahymena, which suggests that feeding was continuous throughout the entire cell cycle and that growth was therefore linear throught as well.

Decreasing temperature was found to decrease the rate of production in A. proteus between 20°C and 10°C, regardless of the food condition. Although there are no comparable data for the effect of temperature on the rate of production in sarcodines, it is expected that this pattern will be universal throughout the group, as temperature determines the rates of physiological processes within the cell. Lower growth rates were generally found for Stentor cultured at 15°C as compared with 20°C (Laybourn 1976). In other words, the physiological processes were accelerated as a function of increased temperature.

When the effect of food concentration on the rate of production was investigated for A. proteus, a characteristic graph was found in which the rate of growth increased to a maximum and thereafter declined. It is of interest to note that when the rate of reproduction was used as a measure of growth, a

similar set of graphs was obtained, indicating the suitability of both methods in production studies (Figure 38).

It was noted that the rate of production attained a maximum at a peak less than the maximum consumption level (Figure 39). The decrease in the rate of production at high levels of Tetrahymena ingestion was due to the slow rate of digestion and consequently the high egestion rates for these food concentrations. As previously stated, this apparent inefficiency is unlikely to be of significance in the field, as such high food levels are rarely encountered.

A more significant result from an ecological viewpoint was the shift in the peak production rate, relative to consumption, with temperature. As the temperature was lowered, the optimum production rate occurred at a decreasing food level. At 20°C, the peak production was found at approximately 2,200 Tetrahymena cells $500\mu l^{-1}$ representing a consumption rate of 2.2 Tetrahymena h^{-1}. This decreased to a level of approximately 1,800 Tetrahymena or 1.7 cells h^{-1} at 15°C and to approximately 500 Tetrahymena $500\mu l^{-1}$ or 0.7 cells h^{-1} at 10°C. This shift in the optimum food level for Amoeba with temperature appears to be an adaptation whereby amoebae in the natural situation can best utilise the available food source, which fluctuates with temperature. In other words, this ensures that amoebae in the field exploit the available food source without sacrificing efficiency, obtaining a greater production on less food as the temperature is lowered.

Heal (1967a) found an optimum consumption level for Acanthamoeba when cultured on yeast, below which growth, as measured by the rate of reproduction increased linearly and above which the rate of production

the rate was constant. Further comparisons are available in the data pertaining to the ciliated protozoa. As early as 1924, Culter and Crump reported the division rate of Colpidium to gradually increase with increasing food supply, until a level at which it became independent of the food condition. Curds and Cockburn (1968) also found the yield of Tetrahymena to be initially linear as the food concentration was increased until a food level was reached which inhibited the rate of production. Similar patterns in the rate of cell production, for increasing food availability, have been reported for Colpoda steinii by Proper and Garver (1966) and for the carnivorous ciliate Podophrya fixa (Laybourn 1976c).

In conclusion, it was established that both temperature and food concentration affected the production of A. proteus. Comparing both variables, temperature displayed the most marked effect with an increase in the rate of protoplasm production of approximately 300% over a temperature difference of only $10^{o}C$. The responses to varying the food concentration over the extremes 125 to 4000 Tetrahymena $500\mu l^{-1}$ were also high with increases in cell production of 68%, 64% and 62% for $20^{o}C$, $15^{o}C$ and $10^{o}C$ respectively.

Until a method for determining the production of protozoa in the wild can be successfully developed, the results of laboratory studies must be accepted. The present study has highlighted the importance of considering, in detail, both temperature and food concentration in such studies.

Chapter 6.

6.1. Respiration.

6.1.1. Introduction.

The list of publications detailing the oxygent requirements of the protozoa is extremely long, the first being Vernon's (1895) study of a radiolarian. Although numerous species have been investigated over the years, there has been an emphasis towards the ciliates, in particular, species of the genus Paramecium (Barrett, 1905; Lund, 1918 a,b,c; Necheles, 1924; Leichsenring, 1925; Kalmus, 1928; Howland and Bernstein, 1931; Pace and Kimura, 1944; Pringle and Stewart, 1961; Stewart, 1966). Recent publications include those of Sarojini and Nagabhushanam (1967) who presented a comparative study of the respiration of 13 free-living ciliates, while Laybourn (1973, 1975b, 1976, 1977) and Laybourn and Finlay (1976) investigated the respiratory energy losses of 5 ciliate species.

Literature pertaining to the respiration of the Rhizopoda is less extensive. Emerson (1929), using a Warburg "macrorespirometer", measured the oxygen consumption of a culture of Amoeba cells. Similarly, Pace and Kimura (1946), Pace and Belda (1944) and Pace and Frost (1952) measured the oxygen uptake of the large amoeba, Pelomyxa carolinensis (syn. Chaos chaos). Further, the

Warburg apparatus was used by Reich (1948) for his studies on the soil amoeba Mayorella palestinensis and by Neff et al (1958) for Acanthamoeba.

Cook (1966) developed oxygen diffusion probes for a study on the adaptations to temperature in Euglena gracilis. Weik and John (1977) adapted the technique to measure the rate of oxygen uptake in Naegleria gruberi as did Byers et al (1969) in a study on Acanthamoeba castellanii.

Howland and Bernstein (1931) measured the oxygen consumption of isolated cells of the Heliozoan Actinosphaerium eichhornii by employing a "microrespirometer", a modification of that developed by Kalmus (1927) for Paramecium. Subsequently, the development of the Cartesian diver microrepirometer and similar flotation methods, allowed the accurate measurement of the oxygen uptake of single cells with sensitivities as low as $0.1\mu\mu l\ O_2\ h^{-1}$ (S.I. equivalent, picolitres). Using such techniques Zeuthen (1943) measured the respiration of Difflugia, Holter and Zeuthen (1948) and Zeuthen (1953) investigated C. chaos, while recently, Hamburger (1975) measured the rate of oxygen uptake of the soil amoeba, Acanthamoeba.

6.1.2. Cartesian diver microrespirometry.

Since the first use of Cartesian divers by Linderstrøm-Lang (1937), physiologists and bioenergeticists have had an instrument for the accurate determination of oxygen consumption at the cellular level. Numerous variations on the basic device have since been developed, most aimed at improving the sensitivity of the apparatus.

Miniaturisation by Zeuthen (1943, 1955) increased the usefulness of the technique, while the Ampulla diver (Zethen, 1953) was an additional varient on the diver model, sensitive enough to measure the oxidative activity of a single nerve cell (Hyden, Løvtrup and Pigon, 1958). Offshoots like the diver balance of Zeuthen (1948) provided a method for the accurate determination of the reduced weight (weight in water) of individual cells.

Attempts have also been made to remove the tediousness of the measurements associated with Cartesian microrespirometry by developing an automatically-recording diver method. Løvlie and Zeuthen (1962) placed an Ampulla diver in a density gradient where it moved to find its own density. Changes in the gas phase resulted in the diver being displaced. The movements of the diver were recorded by photography. A second approach was that of Larsoson and Løvtrup (1966) who used a magnetic force to float a diver in aqueous medium. The changing force required to float the diver was indicative of the changing gas phase, and was recorded on a chart recorder.

Plate 4.

Cartesian Diver Apparatus.

1. Manometer.
2. Regulation screw.
3. Horizontal microscope.
4. Water bath.
5. Cooling coil.
6. Stirrer unit.
7. Heater unit.
8. Tube containing flotation medium (O.1N NaOH) and diver.

edium (O.1N NaOH)

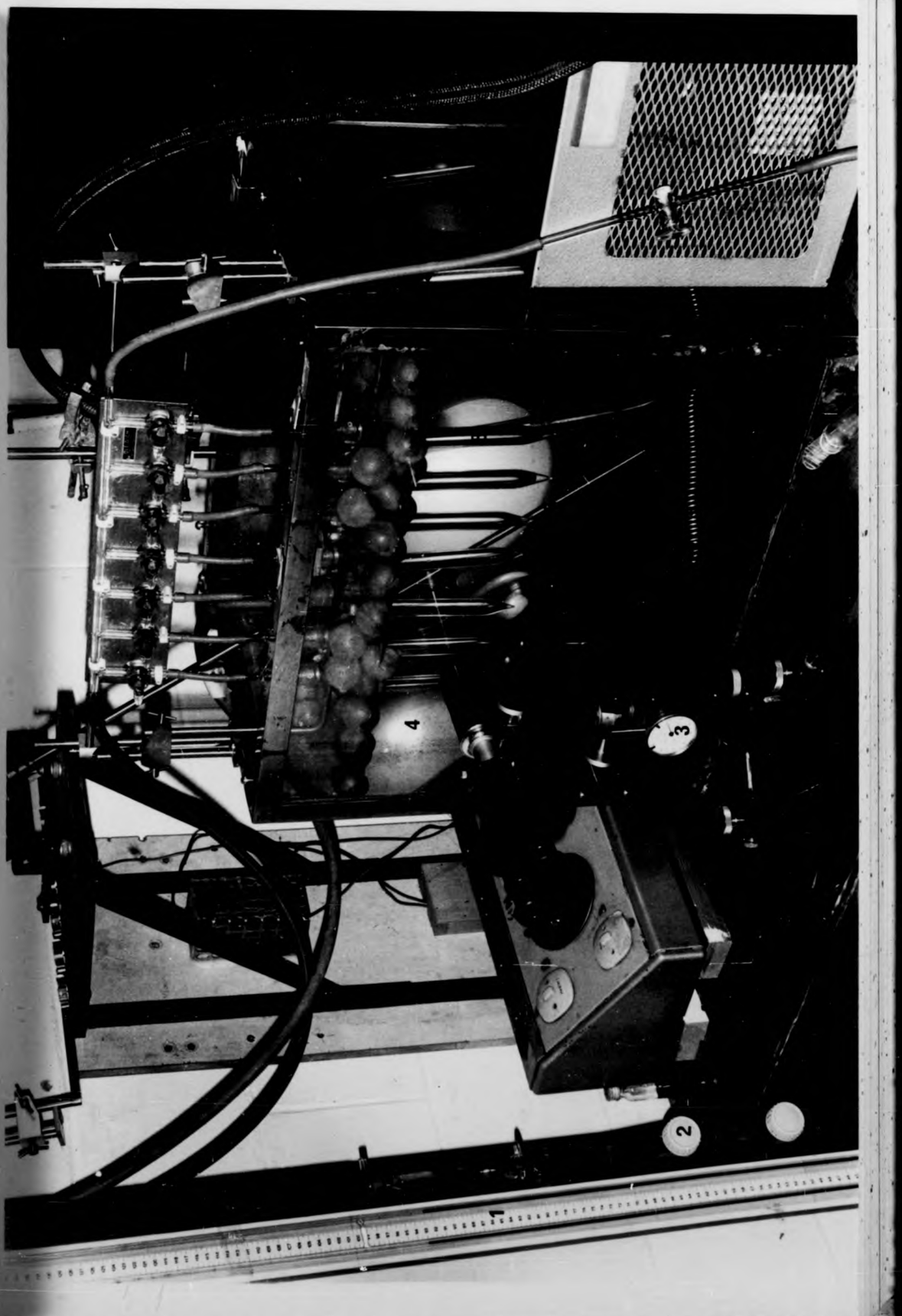


Figure 41.

Diagrammatic representation of the Cartesian apparatus.

Po - Normal pressure for system.

ΔP - Equilibrium pressure change.

1 - Pressure of system crudely adjusted by syringe.

2 - Flotation medium.

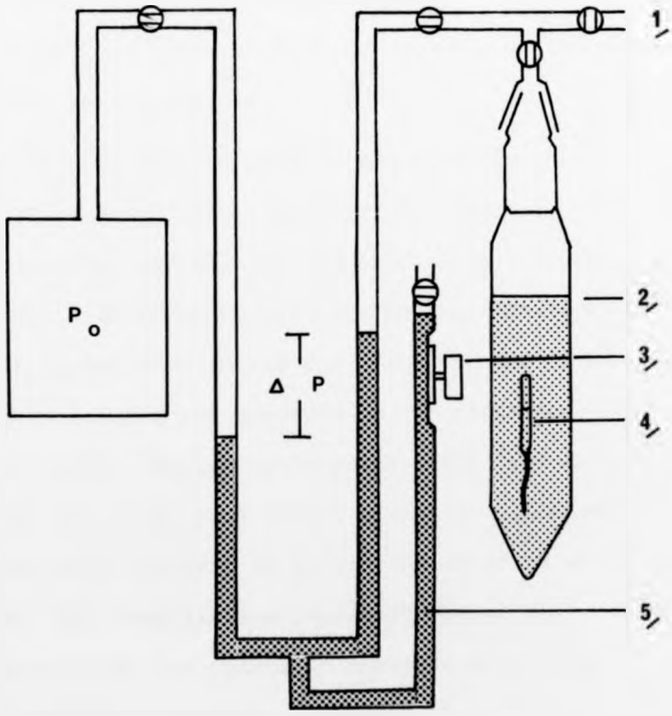
3 - Fine pressure regulation screw.

4 - Cartesian diver.

5 - Manometer containing Brodies fluid.

ted by syringe.

id.



6.1.3. Materials and Methods.

The methods used in this study were similar to those employed and developed by Holter (1943) and Linderstrøm-Lang (1943). The diver system used incorporated the stoppered diver of Zeuthen (1950a), the technique having been most recently outlined by Klekowski (1971).

The apparatus, as shown in Plate4 and diagrammatically in Figure 41, was a constant volume, changing pressure system. A stirrer, cooler unit, and thermostatically controlled heater all combined to regulate the system to with $\pm$ 0.1^{o}C of the experimental temperature. Where greater temperature variation occurred the results were discarded.

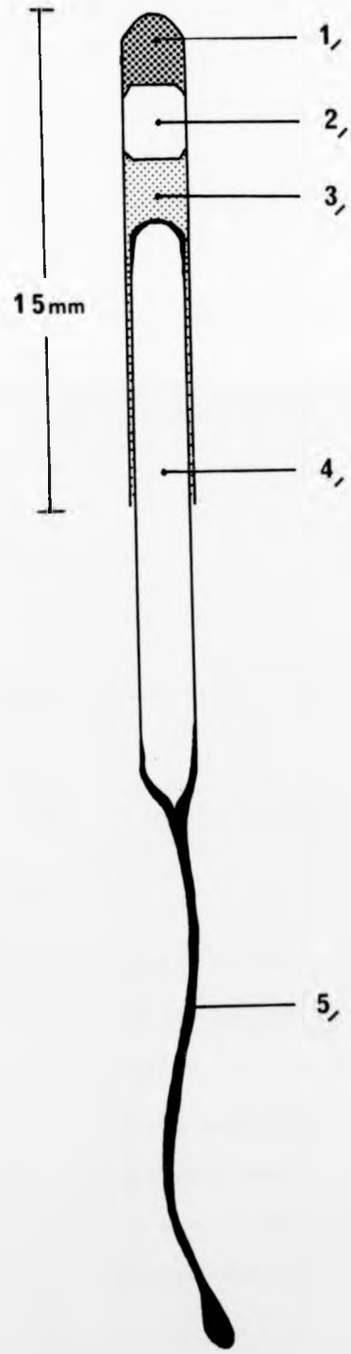
The method relied on the buoyancy of the diver in the flotation medium (0.1N NaOH) being measured. Changes of pressure on the flotation medium were transferred to the gas space in the diver. A decrease in pressure on the flotation medium resulted in an increase in the gas space and the diver rose. Conversly, increasing the pressure on the flotation medium lowered the diver. Oxygen consumption within the diver chamber changed the gas phase such that a series of different pressures with time were required to return the diver to an equilibrium level. The "equilibrium pressure" change was controlled manometrically, the change in pressure, ΔP, being proportional to the rate of oxygen consumption.

Small stoppered glass divers, of the type presented diagrammatically in Figure 42, were made from thin walled capillary tubing, drawn out in a microflame. The diver consisted of a diver

Figure 42.

Zeuthen's stoppered Cartesian diver.

1. S.E.M. containing amoebae cells.
2. Vg, Gas phase.
3. CO_2 Absorbant NaOH.
4. Hollow glass diver stopper.
5. Diver tail.

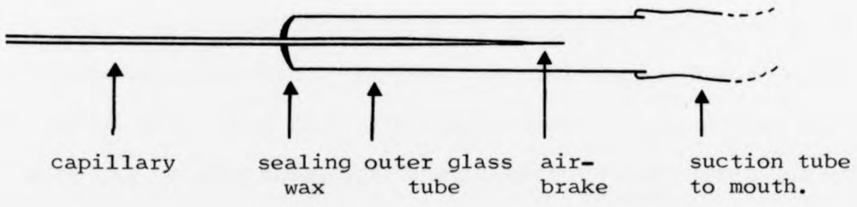

1
2
3
15mm
4
5

chamber containing the amoebae cells within a drop of S.E.M., a gas phase and CO_2 absorbing O.1N NaOH. A hollow glass stopper gave the diver buoyancy and was inserted into the neck of the diver. The divers were calibrated such that a known gas volume of between O.66 - 1.5Oμl floated them in the NaOH flotation medium. Adjustments were made as necessary to the divers by adding or removing glass from the stopper tail.

Manipulations of inserting the amoebae and the respective gas and liquid phases were carried out using a braking pipette (Holter 1943), diagrammatically shown below in Figure 43.

Figure 43.

Braking pipette.



The inner diameter of the capillary was constant and known. The volume of gas phase introduced into the pipette was therefore determined by measuring the length of the phase in the capillary.

Individual divers were filled with between 2 to 6 amoebae, subjectively grouped into one of three size classes, small, medium or large cells. It was of importance to consider differently sized animals from over the life cycle, as the relation between

metabolic rate and body size is one of the classical topics of physiology. Calculation of the respiratory component of an energy budget must therefore take the increasing volume of the cell over the generation into consideration. The subjective selection of the respective amoebae was considered to be accurate enough for the purpose of calculating an average oxygen consumption value, as the distinction between newly divided, mid-cycle and post-cycle amoebae was obvious after a year of observing and maintaining amoebae cultures.

To free the amoebae of bacteria and other contaminants, the cells were transferred through six changes of sterile soil extract media. A sample of the washings from the last transfer was used as a control on each diver run to determine whether any bacterial respiration was present.

The S.E.M. phase containing the Amoeba cells and gas phase were pipetted into the diver head simultaneously. The NaOH stage was added last. The diver filling procedure is summarised in Figure 44. Divers were left to equilibrate for at least 1.5 hours before the commencement of the manometer readings. The pressure (P) of the system was adjusted crudely by the use of a syringe and finely by the regulation screw (Plate 4) to return the diver to a reference level, determined by viewing with a horizontal microscope. The manometer levels (mm) were recorded every 30 minutes, and the pressure change with time was noted.

Being a "closed vessel" respirometer, the length of the experiment was a compromise between the time required to obtain a series of recordings reflecting a constant rate of pressure change, and the time before the onset of oxygen depletion and the increase

Figure 44.

Procedure for filling
the Cartesian diver.

1. The diver cap was filled with soil extract media.
2. The appropriate diver gas phase and S.E.M. containing the amoebae were sucked into the capillary of the Braking pipette.
3. The gas phase and S.E.M. were introduced into the diver cap.
4. The S.E.M. to the rear of the gas phase was flushed out and replaced with NaOH (O.1N).
5. The diver tail was firmly inserted into the cap.

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S.E.M.

nto the

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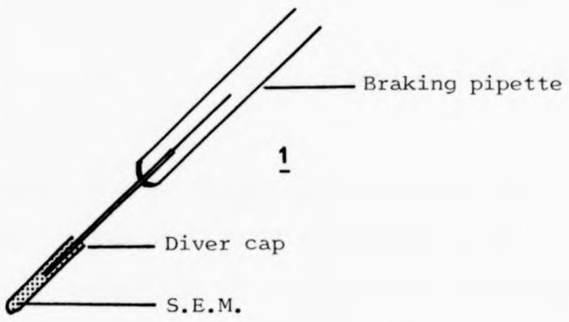
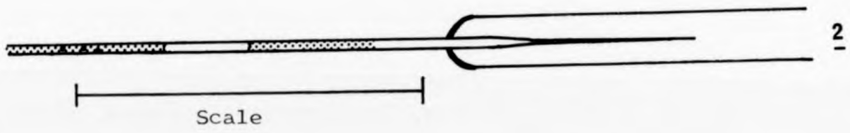
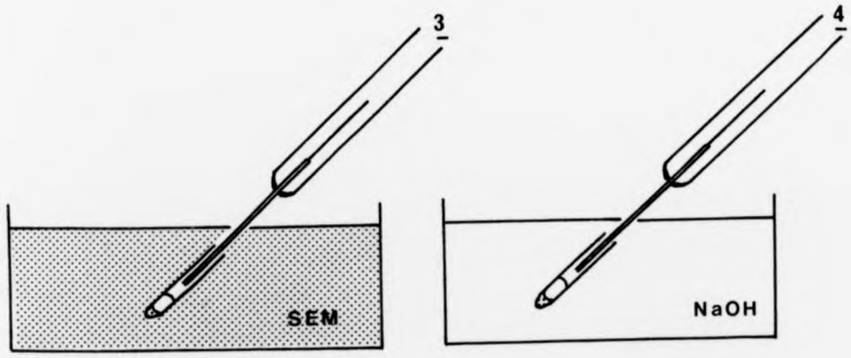
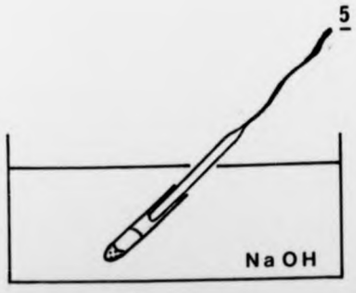
O.1N).

nto the cap.









of metabolites. Experiments over a 4 - 5 hour period satisfied these requirements.

The respiratory rates of Amoeba cells spanning the three size classes, small medium and large, were measured for cells cultured at the food conditions of 125, 1000 and 4000 Tetrahymena cells $500\mu l^{-1}$ at $10^{o}C$, $15^{o}C$ and $20^{o}C$. Cells for experimentation were prepared by growing the amoebae in solid watch glasses under the appropriate conditions, as outlined in Sections 1.2.5. - 1.2.10. Four replicate divers were used for each particular size class, food concentration and temperature employed. In addition, by loading several amoebae into each diver (between 2 - 6 cells) part of the variation in the behaviour of individual amoebae throughout the experiment was accounted for.

6.1.4. Results.

A specimen Table of results obtained for one diver run is presented below (Table 14), while the complete series of repiration data is given in Appendix 12.

Table 14.

Specimen of diver results.

Conditions	Manometer Readings			Time (minutes)
	Left	Right	P	
1.2µl diver containing 3 large amoebae at 20°C after culture at a food conc. of 125 Tetrahymena $500u^{-1}$.	748	518	230	0
	673	434	239	30
	733	488	245	60
	695	444	251	90
	705	445	260	120
	746	483	263	150
	753	484	269	180
	806	534	272	200

Results where cell division in the amoebae occurred during a run were discarded.

A graph of the equilibrium pressure (mm) against time was plotted for each set of results. One such graph corresponding to the results above, is shown in Figure 45. The change in equilibrium pressure, Δ P, per hour was obtained from the slope of the graph, and was proportional to the rate of oxygen consumption.

Regressions were fitted for each set of results using the method of least squares. All the regressions were highly significant ($p = 0.001$) excepting two cases where $p = 0.01$.

Figure 45.

Typical Cartesian plot depicting the change in equilibrium pressure with time (●).

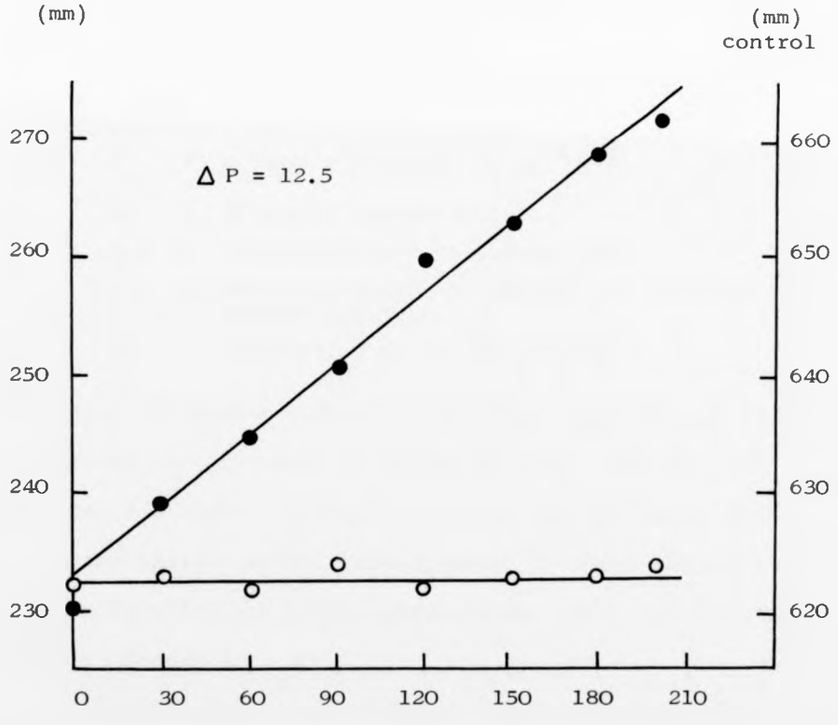
$y = 0.2074 x + 232.1072$

$n = 8$

$r = 0.9945$

$p = 0.001$ (highly significant)

The results of the accompanying control diver are also given (O).



125 Tetrahymena $500\mu l^{-1}$
$20^{o}C$.

The control divers showed no significant change in P with time, indicating that oxygen consumption was attributable to the Amoeba alone.

The rate of respiration per hour was calculated from the formula:

$$V_{O_2} = \frac{Vg \cdot \Delta P}{Po} \quad \frac{273}{T}$$

where, V_{O_2} = volume of consumed O_2 μl^{-1} h^{-1}.

Vg = volume of gas phase (μl).

Δ P = Manometer pressure change (mm).

Po = Normal pressure for system i.e. 10,000mm Brodies solution.

T = Temperature of system (Kelvin).

The oxygen consumption rates per individual amoebae, for each set of conditions are presented in Tables 15 - 17. The rate of respiration per individual Amoeba was greatest in the large cells and least in the smaller cells, regardless of the food concentration or temperature at which the cells were cultured. This relationship is presented in Figure 46.

Average values for the rate of oxygen consumption per unit protoplasm, spanning the small, medium and large replicates, were calculated for 20°C, 15°C and 10°C (Table 18). The overall rate of respiration per individual was divided by the appropriate Amoeba mean cell volume (Page 126). It should be noted that the standard deviations were in all cases high as a consequence of the variable behaviour of amoebae in different divers.

Tables, 15, 16 and 17.

The oxygen uptake of A. proteus (μl x 10^{-4} h^{-1} per individual) at 20°C, 15°C and 10°C.

Each value was obtained from the results of 1 diver run.

per individual)

diver run.

Table 15. 20°C.

Food conc. $500\mu l^{-1}$.	Small amoebae	Mean	Medium amoebae	Mean	Large amoebae	Mean	Overall mean
125	4.47 1.90 1.42 2.98	2.69 ± (S.D.) 1.35	7.69 2.80 1.85 2.57	3.73 ± (S.D.) 2.67	4.66 5.59 7.48 4.07	5.45 ± (S.D.) 1.49	3.96 ± (S.D.) 1.39
1000	3.13 3.41 1.97 4.11	3.15 ± (S.D.) 0.89	5.02 6.51 5.10 4.01	5.16 ± (S.D.) 1.03	9.96 4.84 8.29 7.74	7.71 ± (S.D.) 2.13	5.34 ± (S.D.) 2.28
4000	2.24 4.18 3.73 3.62	3.44 ± (S.D.) 0.84	7.31 5.13 6.46 6.86	6.44 ± (S.D.) 0.94	1.18 6.84 4.98 7.09	7.68 ± (S.D.) 2.90	5.85 ± (S.D.) 2.18

Table 16. 15°C.

Food conc. $500\mu l^{-1}$.	Small amoebae	Mean	Medium amoebae	Mean	Large amoebae	Mean	Overall mean
125	2.43 2.60 1.29 1.30	1.90 ± (S.D.) 0.71	1.20 2.08 2.37 3.05	2.17 ± (S.D.) 0.77	3.51 2.43 4.11 4.03	3.52 ± (S.D.) 0.77	2.53 ± (S.D.) 0.87
1000	1.92 3.07 3.87 3.92	3.19 ± (S.D.) 0.93	3.47 3.11 2.18 4.84	3.40 ± (S.D.) 1.10	5.54 4.82 5.22 7.61	5.80 ± (S.D.) 1.24	4.13 ± (S.D.) 1.45
4000	2.41 1.50 1.56 2.11	1.89 ± (S.D.) 0.44	2.95 3.50 3.54 3.46	3.36 ± (S.D.) 0.28	5.68 4.61 3.31 4.69	4.57 ± (S.D.) 0.97	3.27 ± (S.D.) 1.34

Table 17. 10°C.

Food conc. $500\mu l^{-1}$.	Small amoebae	Mean	Medium amoebae	Mean	Large amoebae	Mean	Overall mean
125	4.12 4.60 3.52 3.85	4.02 ± (S.D.) 0.46	4.73 3.78 4.69 4.54	4.43 ± (S.D.) 0.44	5.19 5.12 6.04 8.92	6.32 ± (S.D.) 1.78	4.92 ± (S.D.) 1.23
1000	3.62 4.39 3.00 3.33	3.58 ± (S.D.) 0.59	4.57 3.67 2.60 4.73	3.89 ± (S.D.) 0.98	6.59 7.56 9.52 4.93	7.15 ± (S.D.) 1.92	4.87 ± (S.D.) 1.98
4000	3.63 3.19 4.45 2.12	3.35 ± (S.D.) 0.97	3.14 3.09 3.04 3.13	3.10 ± (S.D.) 0.04	4.56 8.41 3.66 5.37	5.50 ± (S.D.) 2.06	3.98 ± (S.D.) 1.32

Figure 46.

Oxygen uptake of subjectively selected small, medium and large A. proteus cells.

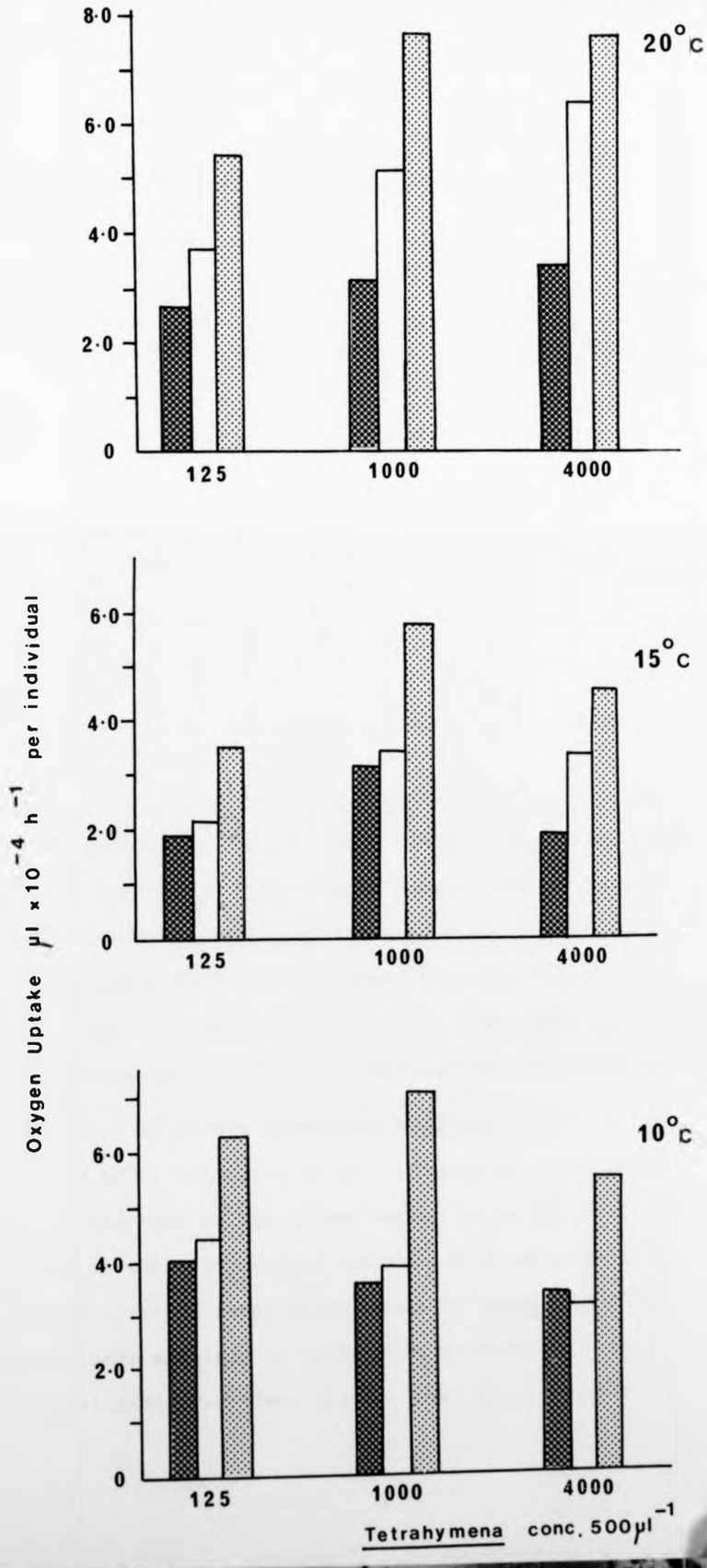

8·0
6·0
4·0
2·0
0
20°C
125
1000
4000
6·0
4·0
2·0
0
15°C
125
1000
4000
Oxygen Uptake µl x10⁻⁴ h⁻¹ per individual
6·0
4·0
2·0
0
10°C
125
1000
4000
Tetrahymena conc. 500µl⁻¹

Table 18.

The O_2 consumption of A. proteus $\mu l\ h^{-1}$ per unit protoplasm at 20°C, 15°C and 10°C.

M.C.V. $\mu m^3 \times 10^3$	Food conc. $500\mu l^{-1}$	Temp. °C.	O_2 cons. $\mu l \times 10^{-10}$ $h^{-1}\ \mu m^{-3}$	S.D.	Overall mean O_2 cons. $\mu l \times 10^{-10}$ $h^{-1}\ \mu m^{-3}$
720	125		5.50	1.93	
1039	1000	20	5.14	2.19	5.40
1051	4000		5.57	2.07	
1042	125		2.43	0.83	
1212	1000	15	3.41	1.20	2.61
1638	4000		2.00	0.82	
1524	125		3.23	0.81	
2472	1000	10	1.97	0.80	2.34
2191	4000		1.82	0.60	

The conversions, 5.40×10^{-10}, 2.61×10^{-10} and $2,34 \times 10^{-10}$ ul $O_2\ h^{-1}$ per unit protoplasm for the three temperatures investigated were used to obtain estimates of the oxygen consumption of Amoeba for all the conditions of culture used throughout the present study, namely Tetrahymena food concentrations over the range 125 - 4000 cells $500\mu l^{-1}$ at 10°C, 15°C and 20°C. The Amoeba mean cell volume determinations (Page 126) were multiplied accordingly.

The overall rates of oxygen uptake per cell were found to be similar at 20°C and 10°C, but lower at 15°C (Figure 47). At 20°C, this was in part a function of the higher degree of activity observed for cells at this temperature compared with those at 15°C and 10°C. The high rates of respiration found for Amoeba at 10°C were due to the volumes attained by cells when cultured at this temperature, approximately 2.3 times greater than those at 20°C

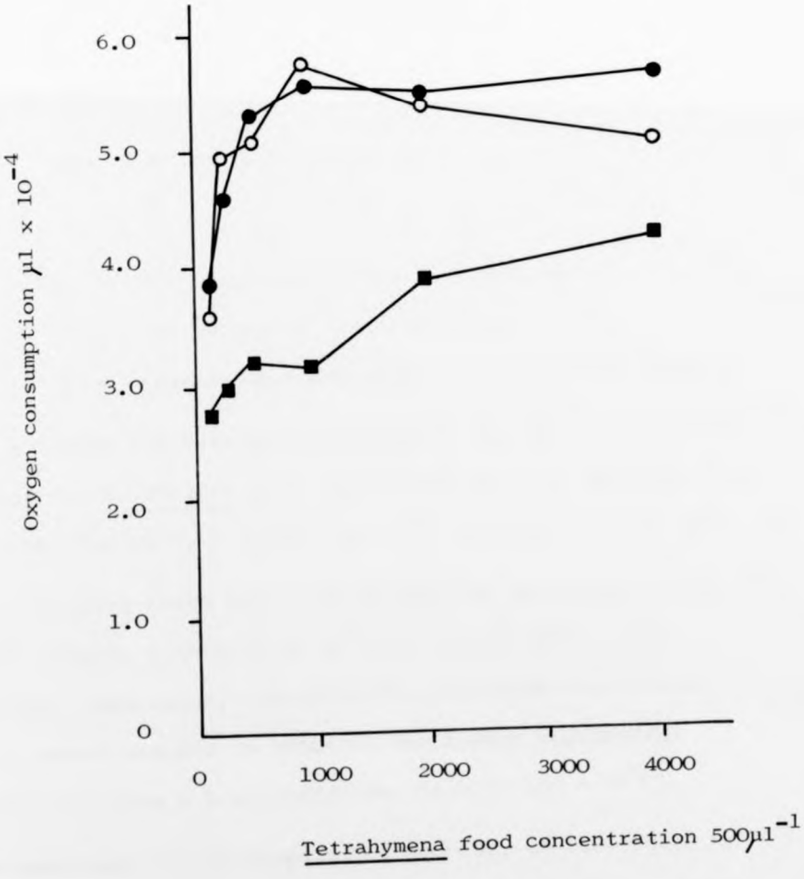
Figure 47.

The oxygen consumption of A. proteus cells as influenced by temperature (10°C, 15°C and 20°C).

● 20°C

■ 15°C

○ 10°C


6.0
5.0
4.0
3.0
2.0
1.0
0
Oxygen consumption µl x 10^{-4}
0
1000
2000
3000
4000
Tetrahymena food concentration 500µl^{-1}

and 1.9 times greater than those cultured at 15°C (Page **126**).

Oxygen consumption as a function of cell size (small, medium or large) is shown in Figure 48 for the data obtained in the present study and in Figure 49, where the results are compared with the available published data for the naked amoebae. Individual respiratory rates for an organism are related to body weight, a relation described by the exponential equation:

$$R = a.w^{b}$$

or logarithmically:

$$\text{Log. } R = \text{Log. } a + b.\ \text{Log. } W$$

where:

R = the respiratory rate per individual.

W = the weight of the individual.

b = regression coefficient describing the slope.

The b values (regression coefficients) for the intraspecific regressions for A. proteus were significant for all temperatures, with p = 0.01 for 20°C, p = 0.02 for 15°C and p = 0.02 for 10°C.

An increasing trend with temperature for the coefficients was indicated, ranging from 0.74 at 10°C to 1.16 at 20°C. The interspecific regression, comparing the published respiration values for naked amoebae in general, was highly significant (p = 0.001) and gave a b - coefficient of 0.75 (20 - 30°C).

The magnitude of the respiratory process, as influenced by temperature, was compared by calculating the Q_{10} values. These were highest for an increase of temperature between 15°C and 20°C where the range was 1.76 - 3.15. Between 10 - 15°C the values were all less than 1, (Table 19).

Figure 48.

Intraspecific size dependence of Amoeba on the rate of oxygen uptake ($\mu l\ h^{-1}$) at 20°C, 15°C and 10°C.

20°C

y = 1.1634x - 3.8605
n = 9
r = 0.8876
p = 0.01 (very significant)

15°C

y = 0.9847x - 3.9612
n = 9
r = 0.7705
p = 0.02 (significant)

10°C

y = 0.7425x - 3.6965
n = 9
r = 0.7671
p = 0.02 (significant)

Culture conditions:

● = 125 Tetrahymena $500\mu l^{-1}$
o = 1000 Tetrahymena $500\mu l^{-1}$
■ = 4000 Tetrahymena $500\mu l^{-1}$

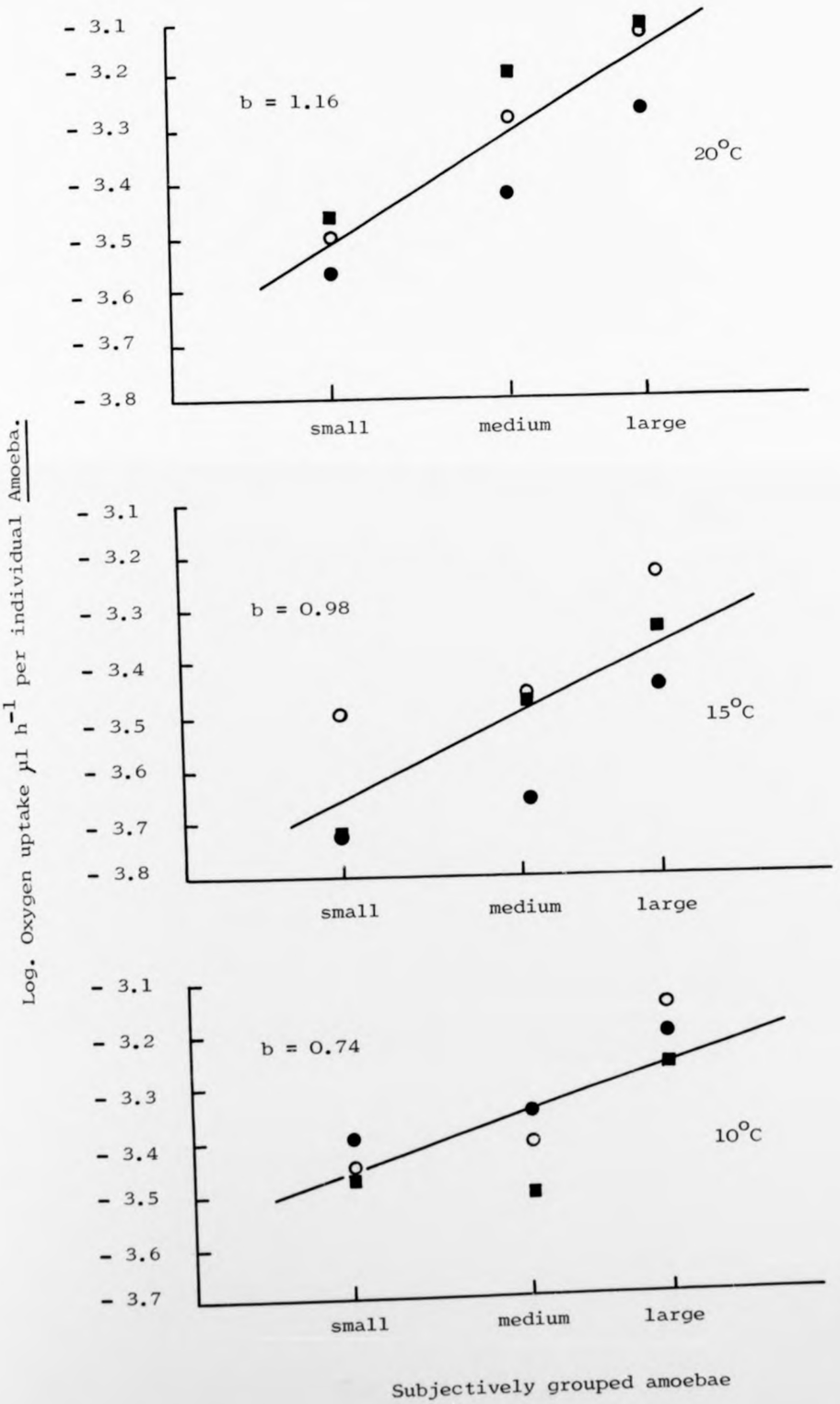

- 3.1
- 3.2
- 3.3
- 3.4
- 3.5
- 3.6
- 3.7
- 3.8
b = 1.16
20°C
small
medium
large
b = 0.98
15°C
b = 0.74
10°C
Log. Oxygen uptake µl h⁻¹ per individual Amoeba.
Subjectively grouped amoebae

Figure 49.

Interspecific size dependence of amoebae on the rate of oxygen uptake ($\mu l\ h^{-1}$) of cells cultured between 20°C - 30°C.

$y = 0.7503x - 7.0349$

$n = 38$

$r = 0.9547$

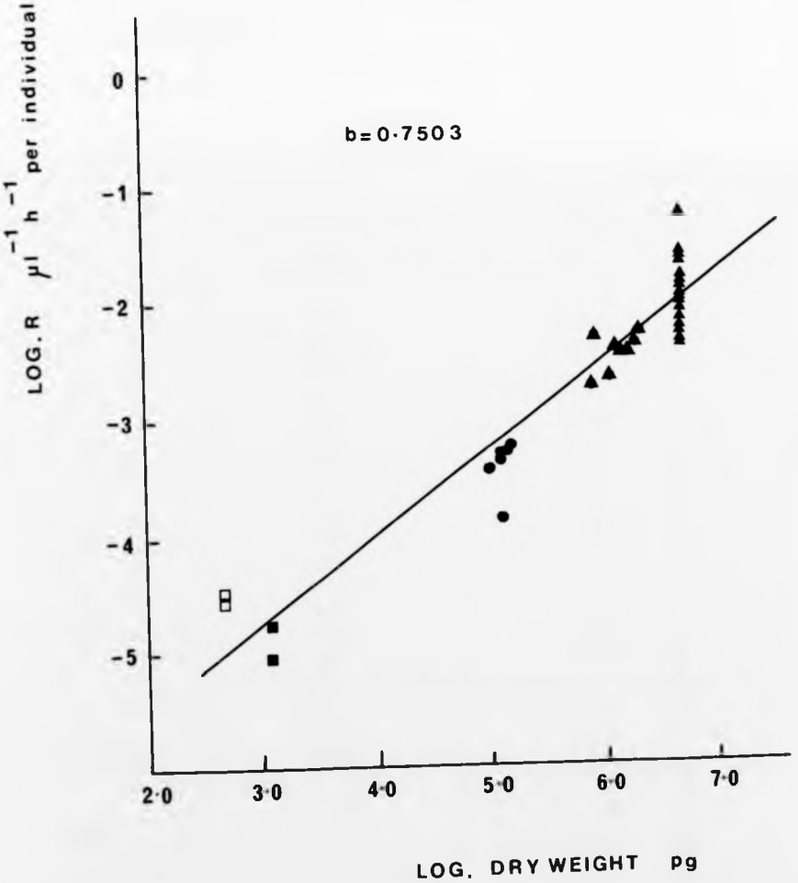
$p = 0.001$

● = A. proteus, present study.

▲ = Chaos (sp.).

□ = Acanthamoeba.

■ = Mayorella.


b = 0·7503
LOG. R $\mu l^{-1} h^{-1}$ per individual
0
-1
-2
-3
-4
-5
2·0
3·0
4·0
5·0
6·0
7·0
LOG. DRY WEIGHT pg

By comparing the Q_{10}'s for the overall rates of respiration per unit volume, the additional values of 4.28 ($15^{o}C$ - $20^{o}C$) and 1.24 ($10^{o}C$ - $15^{o}C$) were obtained.

Table 19.

The Q_{10} values describing the rates of oxygen consumption by A. proteus.

A) per individual cell.

Initial food concentration	Temperature ^{o}C.	Q_{10}
125	10	
	15	0.58
	20	2.04
250	10	
	15	0.35
	20	2.50
500	10	
	15	0.39
	20	2.79
1000	10	
	15	0.30
	20	3.15
2000	10	
	15	0.50
	20	2.05
4000	10	
	15	0.69
	20	1.76

B) per unit volume (μm^3).

	20	
	15	4.28
	10	1.24

The suitability of the oxygen consumption values per unit protoplasm calculated for A. proteus at 20°C, 15°C and 10°C were compared with the available published data for the naked amoebae in general (Figure 50). The assumptions and data used in the calculations required for the compilation of this graph are presented in Appendix 13. As a consequence of the various techniques employed by individual researchers, and the assumptions made regarding the cell volumes, a high degree of scatter in the graph was found. Those values for the larger species showed a gradual increase in the rate of oxygen consumption per unit volume with increasing temperature. The values obtained for A. proteus in the present study lie within this general pattern. The smaller amoebae species, Mayorella and Acanthamoeba were found to have much higher respiratory demands per unit volume (μm^3). The energy requirements of such naked amoebae species must therefore be regarded as outwith the present findings for A. proteus.

An oxycalorific coefficient of 4.85 kcal l^{-1} O_2 (Winberg 1971) was used to convert values of oxygen consumed into energy units (Table 20).

Figure 50.

The effect of temperature on the rate of oxygen uptake per unit volume (μm^3) of the naked amoebae in general.

- ■ A. proteus, present study.
- A. proteus.
- Chaos (sp.).
- ○ Acanthamoeba (sp.).
- □ Mayorella (sp.).

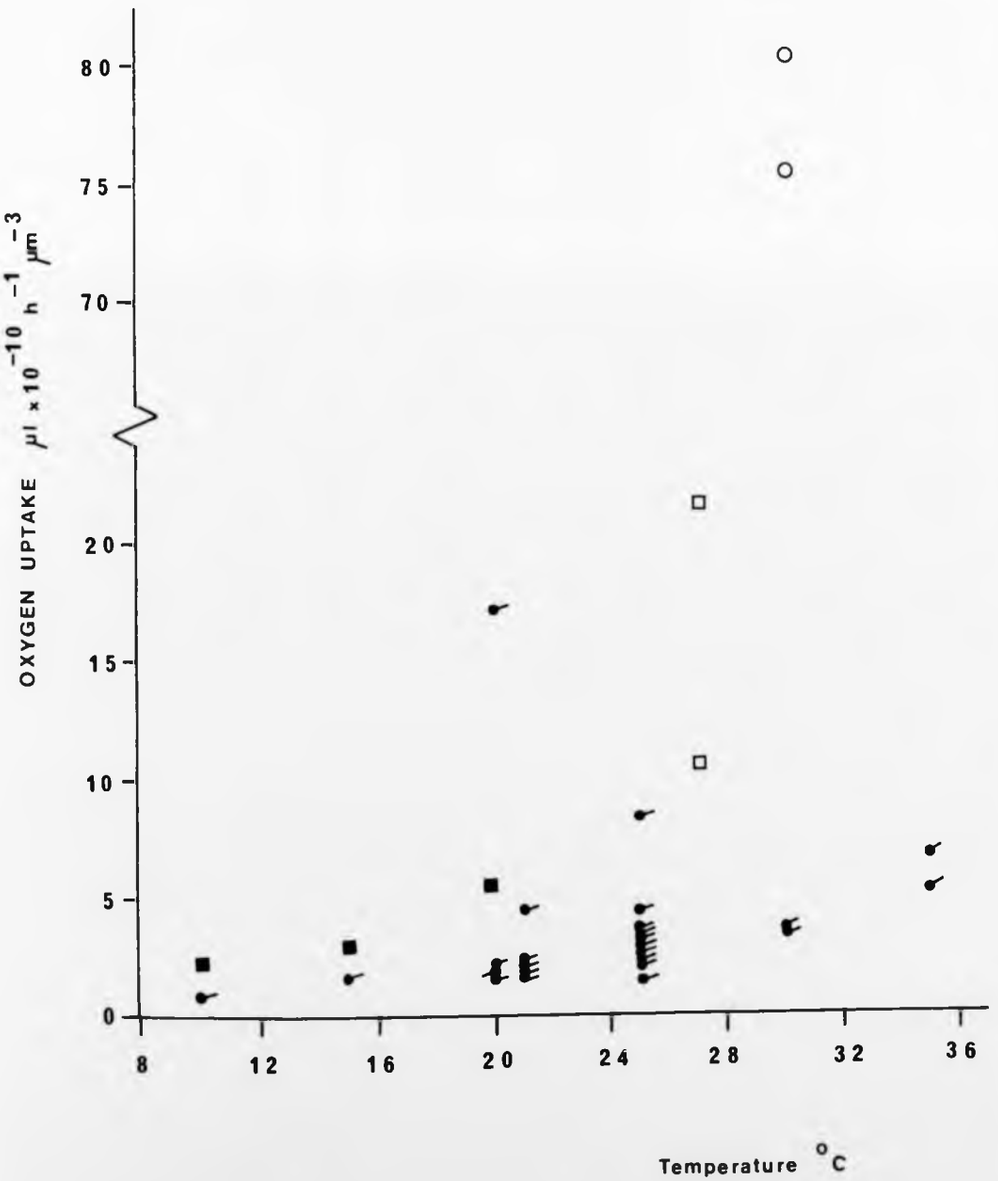

OXYGEN UPTAKE µl x 10⁻¹⁰ h⁻¹ µm⁻³
80
75
70
20
15
10
5
0
8
12
16
20
24
28
32
36
Temperature °C

Table 20.

The respiratory energy losses of A. proteus as influenced by temperature and the culture food concentration.

Food conc. $500\mu l^{-1}$.	Temp.	M.C.V. ($\mu m^3 \times 10^3$)	O_2 consumed $\mu l \times 10^{-4} h^{-1}$ per individual	Resp. energy losses h^{-1} (μJ)	Resp. energy losses per generation (μJ)
125		720	3.89	7.89	663.10
250		862	4.65	9.44	660.52
500		988	5.33	10.81	626.98
1000	20	1039	5.61	11.38	557.62
2000	^oC	1024	5.53	11.22	493.68
4000		1051	5.67	11.50	575.00
125		1042	2.72	5.52	590.53
250		1126	2.94	5.97	542.91
500		1222	3.19	6.47	498.42
1000	15	1212	3.16	6.41	455.25
2000	^oC	1479	3.86	7.83	610.97
4000		1638	4.27	8.66	970.48
125		1524	3.57	7.24	4455.06
250		2131	4.99	10.12	4361.72
500		2182	5.10	10.35	3850.02
1000	10	2472	5.78	11.73	6580.53
2000	^oC	2335	5.46	11.08	13816.76
4000		2191	5.13	10.41	30459.66

6.1.5. Discussion.

Cartesian diver microgasometric technique, whether it incorporates the standard Cartesian diver, Ampulla diver, Gradient diver or the Stoppered diver as was used in the present study, is undoubtedly the most accurate method available for the laboratory bioenergeticist interested in determining the rate of respiration of micro-organisms.

As each diver was loaded with a small number of cells, permitting observation throughout the experimental period, assessment of the animals condition and behaviour was possible. In addition, the activity of the amoebae within the diver was not restricted and the active metabolic rate, as opposed to the normal or resting metabolic rate as is so often the case in macrorespirometers, was measured.

The effect of culturing amoebae at different food concentrations had an indirect bearing on the respiration of individual amoebae. Increasing the food concentration, increased the size of the amoebae cells (Chapter 5), and thereby increased the overall oxygen consumption per individual. The influence of cell volume on the overall oxygen uptake of the cell is shown in Figures 46 and 47 where the amount of oxygen consumed at 10°C was comparable to that consumed at 20°C, even although the mean rate of respiration per unit protoplasm showed a marked decrease with temperature (Table 18).

The effect of temperature on the rate of oxygen uptake has been well documented with regard the ciliated protozoa. Paramecium aurelia and P. caudatum showed a continual rise in the rate of respiration between 15°C - 35°C (Pace and Kimura, 1944), suggesting

that the optimum temperature for this species was high. 25°C has been found to be the optimum temperature for respiration in many ciliates, for example, Spirostomum ambiguum (Sarojini and Nagabhushanam, 1966), Tetrahymena geli (Pace and Lyman, 1947) and well fed Podophyra fixa (Laybourn, 1976b), all have their maximum rate of oxygen uptake at this temperature.

With reference to the naked amoebae, Pace and Belda (1944) studied the effects of temperature on the rate of respiration of Pelomyxa carolinensis (syn. Chaos chaos) by means of a Barcroft-Warburg respirometer over the temperature range 15°C - 40°C. An increase in respiratory rate was found up to 35°C, with temperatures above this proving lethal to the cells.

The effect of temperature on the rate of oxygen uptake by A. proteus in the present study was complicated by the increasing cell volumes found as the temperature was decreased. Comparisons of the rate of respiration were made on the oxygen uptake per unit volume of the naked amoebae species in general (Figure 50). Studies on the large amoeba Chaos chaos by Scholander et al (1952), Pace and Belda (1944), Pace and Frost (1952), Holter and Zeuthen (1948), Pace and Kimura (1946) and Claff and Tahmisian (1949), and on A. proteus by Emerson (1929) showed a trend of increasing oxygen consumption per unit volume with increasing temperature. The small naked amoebae (Reich, 1948; Byers et al, 1969), indicated much higher rates of oxygen consumption, suggesting that such species are not comparable to the large amoebae. Chaos chaos and A. proteus are essentially sedentary cells, relying on the capture of passing food, whereas the smaller amoeboid forms are more active, especially with regard to their feeding behaviour, and

consequently expend much greater respiratory energy losses per unit volume.

The interpretation of the results comparing the rates of respiration in amoebae must be treated with care as the methods employed by individual researchers varied greatly with regard to the respiration and volume measurements. In some case, the temperatures employed were not stated and a room temperature of 20°C was assumed. In others, the volume measurements were omitted and values were taken from the results of other workers using the same species. The relationship did show, however, that the results obtained for A. proteus were within the expected order of magnitude. The trend of decreasing metabolic rate with decreasing temperature was important as it suggests that amoebae have the ability to conserve energy when the temperature and consequently the feeding rates (Chapter 4) are low.

The b-values (regression coefficients) gave an indication of the relation between body size and rate of metabolism and increased with temperature from 0.74 at 10°C to 1.16 at 20°C for A. proteus. The subject has a long history ever since Sarrus and Rameau (1839) found that the metabolic weight did not increase directly with weight, thereby forming the basis of the surface law of metabolism or law of Rubner (1883). By comparing b-values, it is possible to determine whether the rate of respiration is dependent upon weight, b = 1.01, or on surface area, b = 0.67 , (Bertalanffy, 1957). Numerous authors have reviewed this topic, notably Brody (1945), Zeuthen (1953), Hemmingsen (1960)and Kleiber (1961), resulting in a more realistic value of 0.75, when corrected to 20°C, being adopted. Hemmingsen (1960) proposed

a "unicellular line" of 0.76 based on the results of various unicellular organisms. A linear relationship ($p = 0.001$) between cell size and respiration on an interspecific basis for the naked amoebae ($20^{o}C$ - $30^{o}C$) gave a similar b-coefficient of 0.75 (Figure ~~53~~ 49).

Published b-values for protozoa vary considerably, examples of which are 0.27 at $8.5^{o}C$, 0.44 at $15^{o}C$ and 0.42 at $20^{o}C$ found for a range of ciliates by Laybourn and Finlay (1976). Scholander et al (1952) obtained a b-value of 0.55 at $25^{o}C$ covering 3 protozoan species; 2 ciliates and 1 sarcodine. Laybourn (1975) investigated the respiratory energy losses in Stentor coeruleus and reported b-coefficients of 0.60 at $15^{o}C$ and 0.67 at $20^{o}C$. Higher values for Didinium of 0.96 - 1.00 over the temperature range $10^{o}C$ - $20^{o}C$ were published by Laybourn (1977). Further, a value of 0.74, corrected to $28^{o}C$, was reported by Vernberg and Coull (1974) for the interstitial ciliate Tracheloraphis. Verberg et al (1970) compared some published values for aquatic invertebrates obtained under different conditions and found a range extending from 0.42 to 1.05. Where the significance of such differences was tested, the variability of the respiratory measurements was such that differences were not significant (Schiemer et al, 1974).

The b-coefficients for A. proteus were 1.16 ($20^{o}C$), 0.98 ($15^{o}C$) and 0.74 ($10^{o}C$). Two sources of innaccuracy in the determination of these b-values can be considered. The variable behaviour of different amoebae within the diver chamber must in itself constitute a source of variation, while the subjective selecting of small, medium and large amoebae was only an approximation of cell volume.

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The errors were most compounded at 10°C where some amoebae were observed to spend long periods of inactivity and where the volume range was greatest. Accepting that the b-coefficients were merely estimates, an increasing trend was found with increasing temperature, a fact which was also reported by Laybourn and Finlay (1976) with reference to a range of ciliated protozoa.

Q_{10} values, indicating the magnitude of the change in respiratory metabolism with temperature, were between 1.76 - 3.15 for 15°C - 20°C, but less than 1.0 for an increase in temperature over 10°C - 15°C. More meaingful results were obtained for the rates of respiration per unit protoplasm (μm^3), where an increase in temperature between 10°C - 15°C gave a Q_{10} value of 1.24 while between 15°C - 20°C a higher value of 4.28 was found.

Comparable data for the rate of oxygen consumption by A. proteus are not available with the exception of Emerson (1929), however, by comparing the data for amoebae species in general (Figure 49) the rate of respiration of A. proteus was found to be intermediate between the smaller naked amoebae, such as Mayorella (Reich 1948) and the larger species C. chaos (Scholander et at, 1952 and others).

Sarojini and Nagabhushanam (1967), made a comparative study of the respiration of 13 free living ciliates using a Warburg respirometer at 24 - 25°C, a technique ill-suited for the accurate determination of the oxygen demand of micro-organisms. They concluded that there were no trends in the rate of respiration in relation to different cell volumes, and that respiration was a function of activity, rather than volume. Conversly, Laybourn and Finlay (1976) reported a linear relationship between cell

size and oxygen uptake when they compared seven species of ciliates using the more accurate Cartesian microrespirometer. The results of the present study for the naked amoebae agree with those of Laybourn and Finlay (1976) supporting the relationship between cell volume and respiration.

In conclusion, the aim of this section was to obtain conversions which could be used in conjunction with volume measurements to estimate the respiration of A. proteus or related species in the laboratory. Interpretation of the available data has suggested that a distinction must be made between small ($<200\mu$ long) and large ($>200\mu$ long) naked amoebae. The results of the present study on A. proteus, 5.40, 2.61 and 2.34×10^{-10} $\mu l\ h^{-1}$ for $20^{o}C$, $15^{o}C$ and $10^{o}C$ respectively, are probably representative of large naked carnivorous amoebae species in general.

PART 2.

Energy Budgets and Biological Efficiencies.

Chapter 7.

7.1. Energy budgets.

7.1.1. Introduction.

Energy budgets give information on the intensity with which an individual or population acts on its environment. In other words, how much energy in the form of food is consumed, the amount of energy assimilated, and that energy lost from the organism through egestion and excretion. When used in conjunction with field data, the parameters of an energy budget can be used to place an animal in the food web and assess its contribution to energy flow through the ecosystem.

The key assumption, is of course, that conditions in the laboratory approximate those in the natural situation. It is unfortunate that in compiling the budgets, it is necessary and unavoidable to use simplifications such as a single food source and a restricted range of environmental variables.

For evaluation of such budgets in the field, a second assumption concerning the choice of experimental organism is made. Due to the species diversity of the protozoan community, consideration of the energy balance of all the links in the food web is impossible, and so representative species from the various trophic levels have to be considered. It is assumed that the

energy requirements of related species within the group are similar. For the present study, Amoeba proteus was selected as a "typical" large naked sarcodine for an investigation on the energetics of carnivorous amoebae.

Comparable protozoan budgets are not common in the literature. The only published energy budget for a sarcodine is that of Heal (1967a) for the small amoebae Acanthamoeba fed on yeast cells at 25°C. The remaining protozoan budgets all relate to the ciliated protozoa (Laybourn 1973, 1976 and Stachuska, see Klekowski and Fischer, 1975).

The preceding chapters have described how the various components of the budget equations were derived. The results were discussed largely in non-energetic units, as it was felt that to do otherwise would have resulted in the loss of valuable data on the physiology of the feeding, growth and respiration processes.

This section therefore deals with the energy consumed by A. proteus and the subsequent fate of that energy. In other words the proportion used for production, that lost as heat as a consequence of the cells activity and metabolism, and the energy lost through egestion and excretion. From these components, the efficiency of converting the ingested energy into new protoplasm (the gross production efficiency), the assimilated energy into new protoplasm (the net production efficiency) as well as the efficiency of assimilating the consumed energy was determined for all conditions of food availability and temperature investigated.

7.1.2. Methods.

The series of energy budgets for A. proteus were calculated from the budget equation suggested by Heal (1967b):

$$C = P + R + E$$

$$A = P + R$$

C is equal to the amount of ingested food, P equals the amount of protozoan protoplasm produced, R is the energy lost due to respiration, and E is the energy excreted and egested. A is the proportion of the ingested energy which is assimilated.

The components, C, P and R were measured directly, while E, representing that proportion of ingested energy not assimilated, was calculated from the difference between the ingested and assimilated energy values. The methodology associated with these parameters has been fully described in the previous chapters.

For studies in ecological energetics, the data must be presented in units of heat energy, and so in accordance with the Système Internationale, the joule was used throughout the present study. The conversions employed for the production and consumption values were determined by microbomb calorimetry in Chapter 2 where it was found that the calorific value of A. proteus was 17.51 joules per mg, regardless of temperature, while the energy content of T. pyriformis was higher at 19.80 joules per mg for 20°C and 15°C, and 18.28 joules per mg at 10°C. The respiration data was converted to energy equivalents using the oxycalorific coefficient of 4.85 calories cm^{-3} O_2 (Winberg, 1971).

It is theoretically possible to measure, simultaneously, all the parametersof the budget equation over an experimental period of time. The budget thus derived corresponds to the "Instantaneous budget" of Klekowski (1970a). The length of time on which the equation is calculated depends on the species under investigation, and variables such as the length of any feeding and growth cycles. For A. proteus, where cyclic patterns were not observed, the length of the period was purely arbitrary. A series of instantaneous energy budgets, spanning a one-hour period of the cell cycle were therefore compiled.

Although such instantaneous budgets are useful for comparing the effects of different environmental conditions on the energy balance of a species, they do not describe the action of that species in the natural environment. The variable of generation time must be considered in this context and so a second series of budgets, spanning the generation times, were calculated for Amoeba cultured under each set of food concentrations and temperature investigated.

Throughout the text, the various parameters namely consumption, production and repiration have been tabulated in energy units (Tables 10, 13 and 20). Assimilation was found by summing the components P + R, while egestion was calculated as the proportion of ingested food not assimilated.

7.1.3. Results: Instantaneous energy budgets.

The various energy parameters, including the calculated energy losses due to egestion are presented in Table 21.

Interpretation of these equations for an individual *Amoeba* cultured at 20°C with a *Tetrahymena* concentration of 125 cells $500\mu l^{-1}$ is as follows; the *Amoeba* cell consumed 53.14 μJ per hour of which 22.59 μJ was assimilated and 30.55 μJ egested. Of the assimilated energy 14.70 μJ was incorporated as cell biomass every hour while 7.89 μJ was respired as the cost of maintenance.

The effect of varying the food concentration on the components C, P and R of the energy equation has been discussed fully in preceding chapters. The general pattern for consumption and production was that as the food supply increased, the energy intake and growth of the amoebae cells also increased to a maximum at which the consumption rate levelled (15°C and 20°C) or decreased (10°C), while the growth rate peaked and then fell for all temperatures. The tolerance of amoebae to high food conditions was found to decrease with temperature.

The energy losses due to respiration were found to increase gradually with increasing food availability at all the temperatures investigated, although at 10°C, a decrease was found at the highest food concentration. The magnitude of the respiratory energy losses for *Amoeba* was a function of the cell size, with the largest cells being obtained when amoebae were cultured at the higher *Tetrahymena* concentrations.

Table 21.

Instantaneous (1 hour) energy budgets for individual *A. proteus* at different temperatures and food concentrations.

Food conc. $500\mu l^{-1}$.	C	P	R	E	A	
125	53.14	14.70	7.89	30.55	22.59	
250	66.89	21.14	9.44	36.31	30.58	
500	73.14	29.24	10.81	33.09	40.05	20°C
1000	98.15	36.41	11.38	50.36	47.79	
2000	133.78	39.95	11.22	82.61	51.17	
4000	162.54	36.08	11.50	114.96	47.58	
125	43.76	16.72	5.52	21.52	22.24	
250	54.39	21.24	5.97	27.18	27.21	
500	57.51	27.25	6.47	23.79	33.72	15°C
1000	88.77	29.29	6.41	53.07	35.70	
2000	143.79	32.54	7.83	103.42	40.37	
4000	154.41	25.10	8.66	120.65	33.76	
125	30.03	4.25	7.24	18.54	11.49	
250	38.69	8.49	10.12	20.08	18.61	
500	34.65	10.07	10.35	14.23	20.42	10°C
1000	54.86	7.56	11.73	35.57	19.29	
2000	54.86	3.21	11.08	40.57	14.29	
4000	31.76	1.28	10.41	20.07	11.69	

Figure 51.

The rate of egestion as a factor of energy consumption in A. proteus.

$y = 0.7670x - 11.5637$

$n = 18$

$r = 0.9764$

$p = 0.001$

● = 20°C

■ = 15°C

o = 10°C

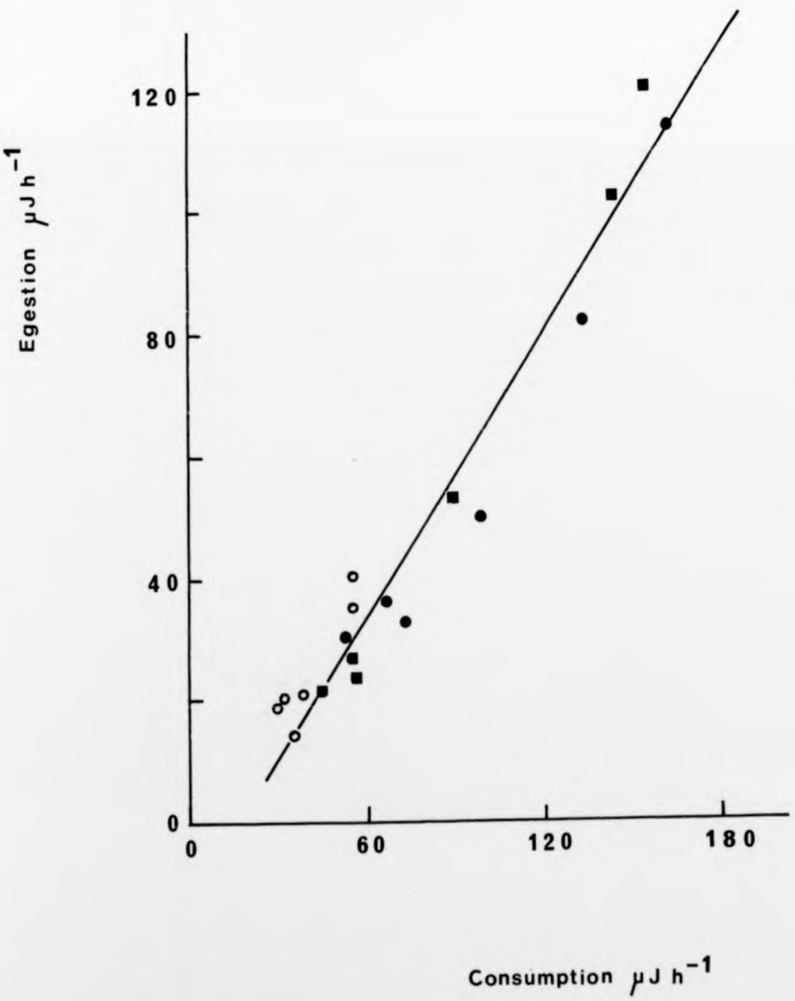

Egestion μJ h⁻¹
120
80
40
0
0
60
120
180
Consumption μJ h⁻¹

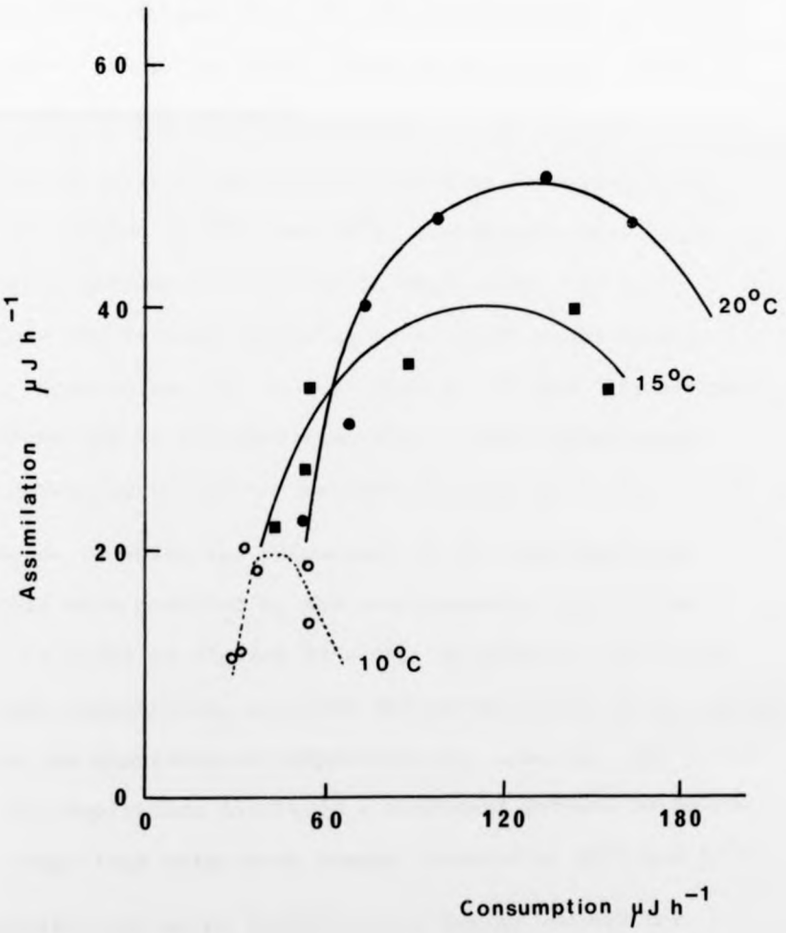
Figure 52.

The effect of consumption on the rate of assimilation in A. proteus at 20°C, 15°C and 10°C.

● 20°C

■ 15°C

o 10°C


60
40
20
0
0
60
120
180
Assimilation μJ h⁻¹
Consumption μJ h⁻¹
20°C
15°C
10°C

The rate of egestion and excretion ($\mu J\ h^{-1}$) as influenced by consumption ($\mu J\ h^{-1}$) is given in Figure 51. A linear relationship was found whereby increased food concentration, and hence consumption, resulted in an increased rate of egestion, regardless of temperature. The relationship between the rate of consumption and egestion was such that when consumption was low at, for example, 40 μJ per hour, 45% of the ingested energy was egested. When consumption was high at, for example, 160 μJ per hour a greater proportion, 70% of the consumed energy was egested.

The rate of assimilation (P + R) per hour increased with increased consumption at 15°C and 20°C, the maximum rate being attained before maximum consumption in both cases. At 10°C, however, where the consumption rates were in all cases lower, the pattern emerging was not so well defined (Figure 52), although a similar trend can be interpretated with a peak assimilation rate again occurring before the maximum consumption level.

The degree to which the parameters of the Instantaneous energy budgets were modified by the environmental variable of temperature is shown in Figures 53 - 56. In general, the rates of production, consumption, egestion and assimilation for A. proteus decreased as the experimental temperature was lowered. The energy losses due to respiration displayed a different pattern being low at 15°C in comparison with those losses incurred at 20°C and 10°C.

The complete series of Instantaneous energy budgets for A. proteus when cultured at 10°C, 15°C and 20°C over the Tetrahymena food range 125 - 4000 cells $500\mu l^{-1}$, are presented in diagrammatic

Figure 53.

The effect of temperature (10°C, 15°C and 20°C) on the rate of egestion in A. proteus.

6 different food concentrations were investigated. (125 - 4000 Tetrahymena 500μl^{-1}).

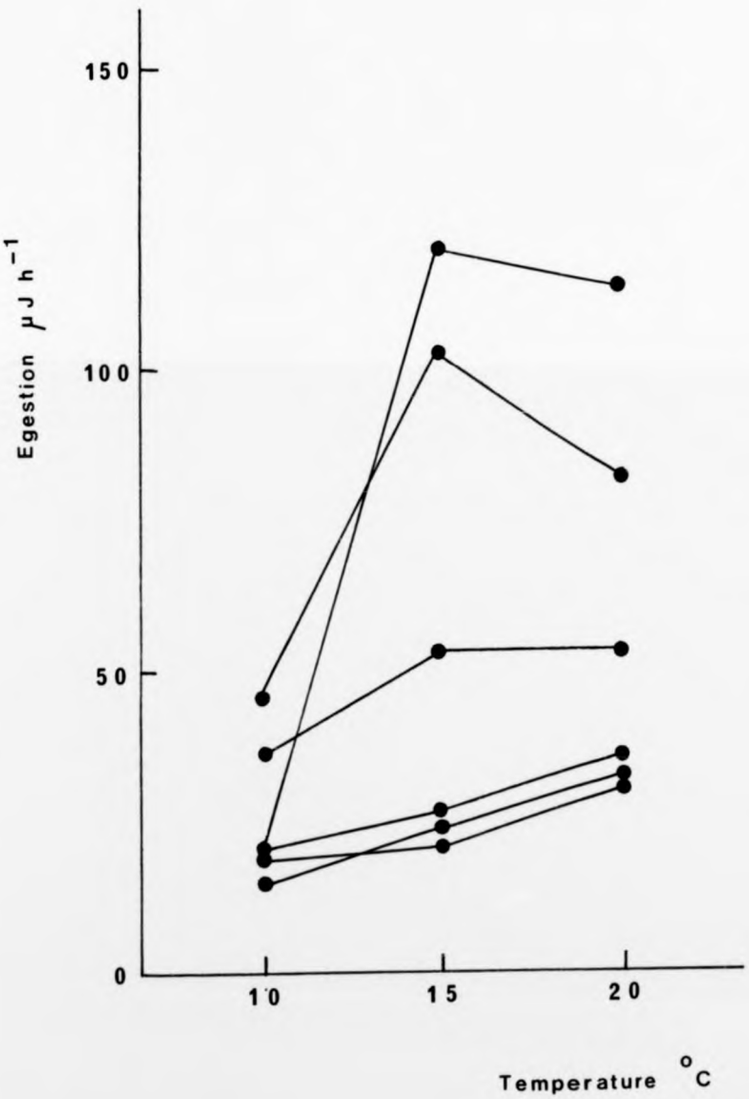

150
100
50
0
Egestion μJ h⁻¹
10
15
20
Temperature °C

Figure 54.

The effect of temperature ($10^{o}C$, $15^{o}C$ and $20^{o}C$) on the rate of consumption in A. proteus.

6 different food concentrations were investigated. (125 - 4000 Tetrahymena $500\mu l^{-1}$).

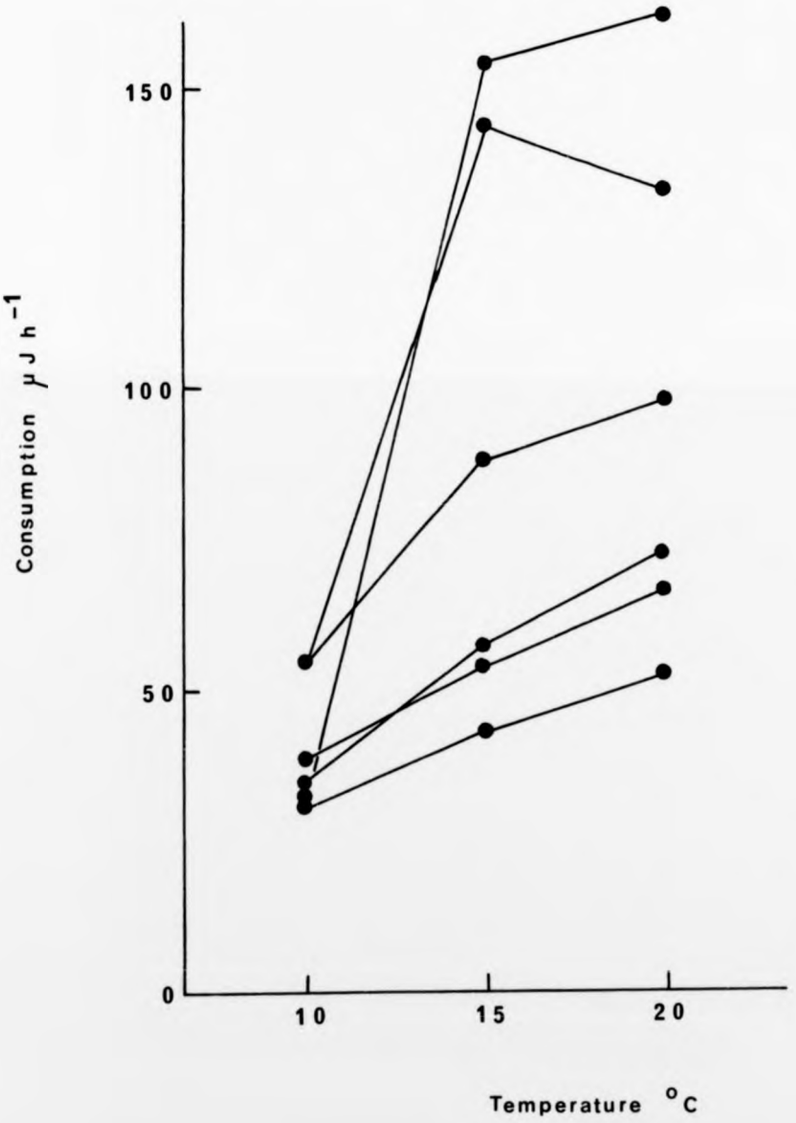

150
100
50
0
Consumption μJ h⁻¹
10
15
20
Temperature °C

Figure 55.

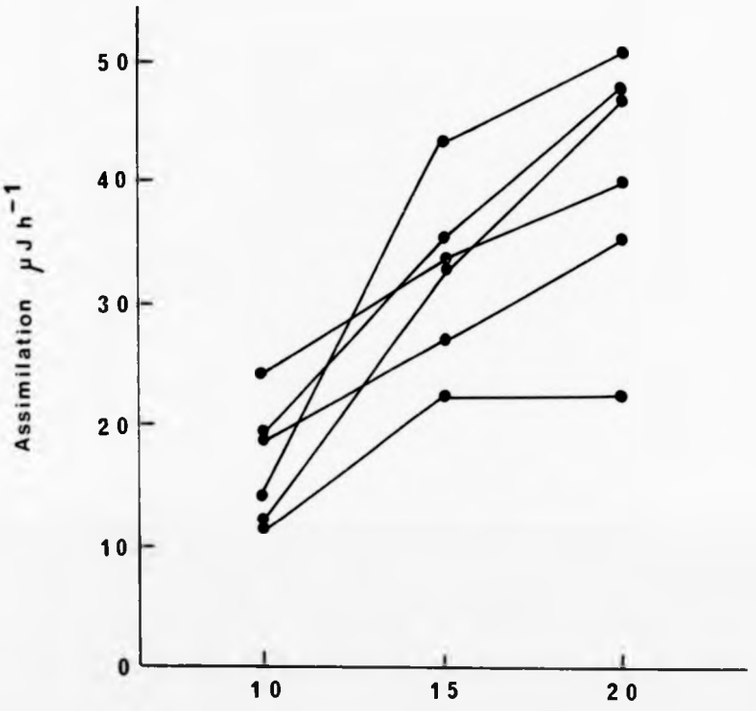
The effect of temperature ($10^{o}C$, $15^{o}C$ and $20^{o}C$) on the rate of assimilation in A. proteus.

Figure 56.

The effect of temperature ($10^{o}C$, $15^{o}C$ and $20^{o}C$) on the rate of production (o–o) and respiration (●–●) in A. proteus.

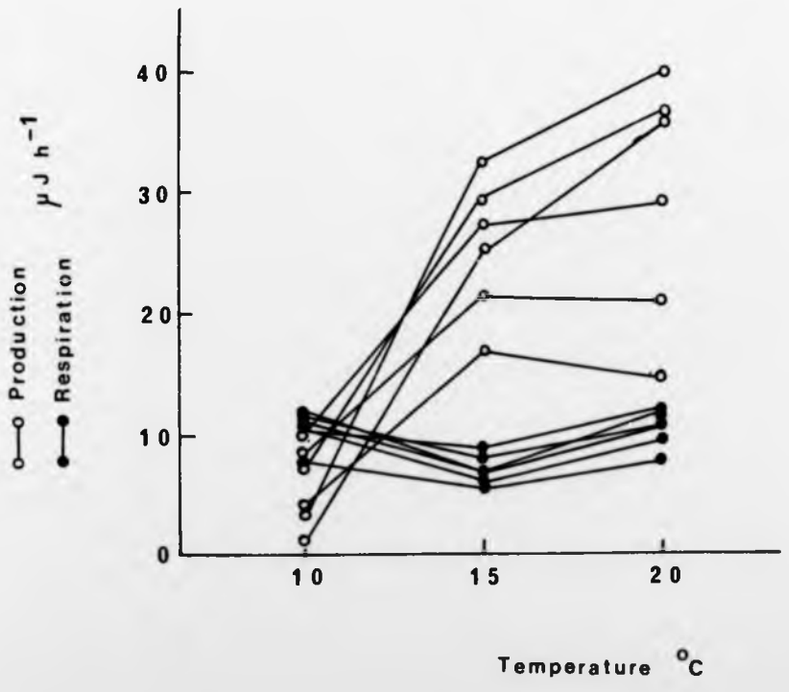
6 different food concentrations were investigated.
(125 - 4000 Tetrahymena $500\mu l^{-1}$).

Figure 55.



teus.

Figure 56.



Figures 57 - 62.

Diagrammatic presentation of the instantaneous energy budgets (per hour) of *A. proteus* in relation to temperature ($20^{o}C$, $15^{o}C$, $10^{o}C$) and food concentration (125 - 4000 *Tetrahymena* $500\mu l^{-1}$).

Figure 57.

125 Tetrahymena 500µl^{-1}.

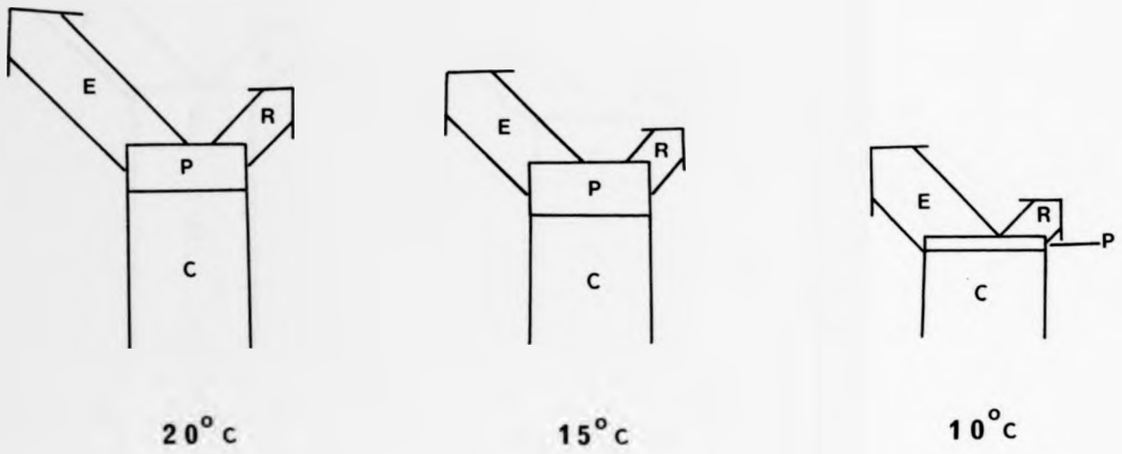


Figure 58.

250 Tetrahymena 500μl^{-1}.

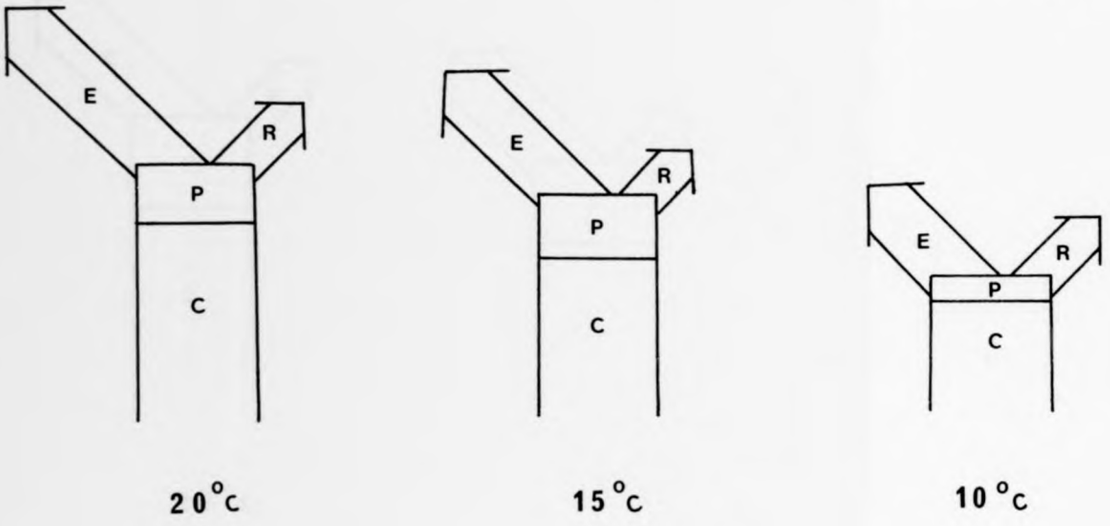


Figure 59.

500 Tetrahymena 500μl^{-1}.

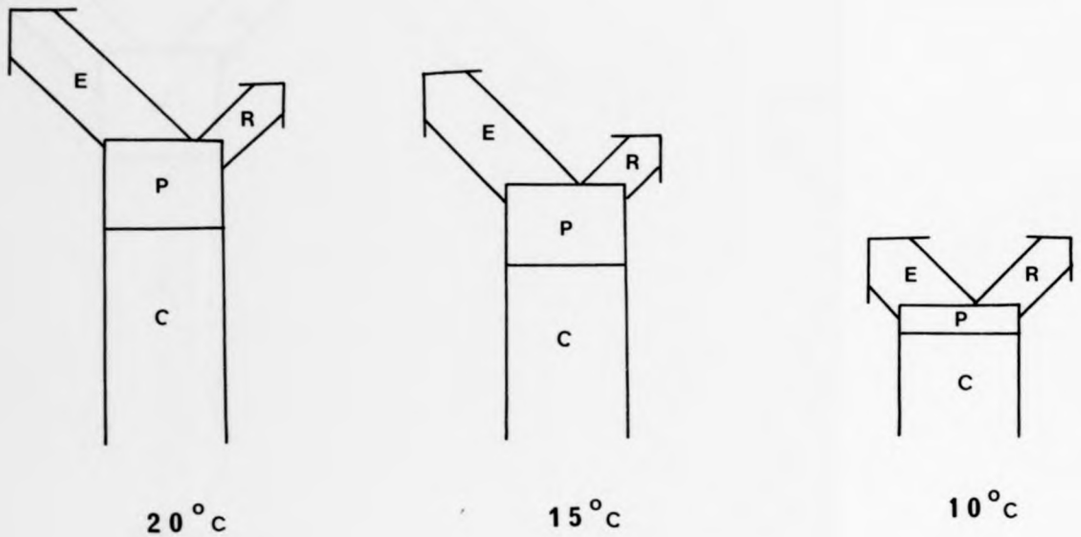
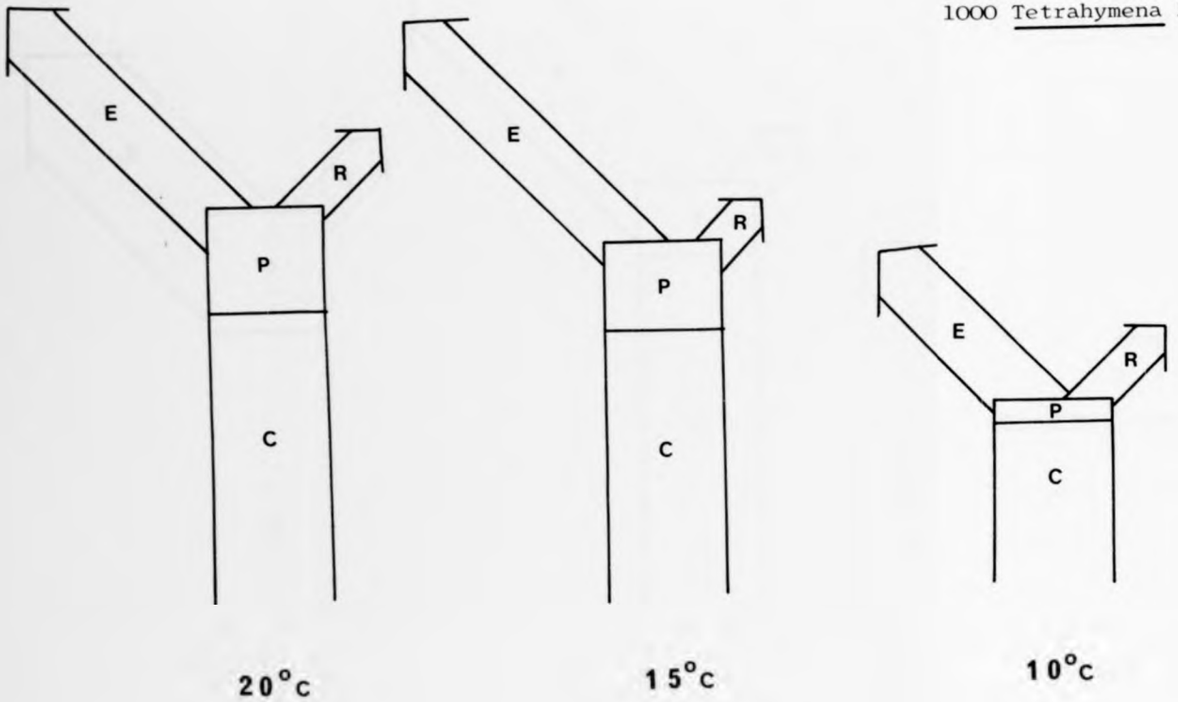


Figure 60.

1000 Tetrahymena 500µl^{-1}.



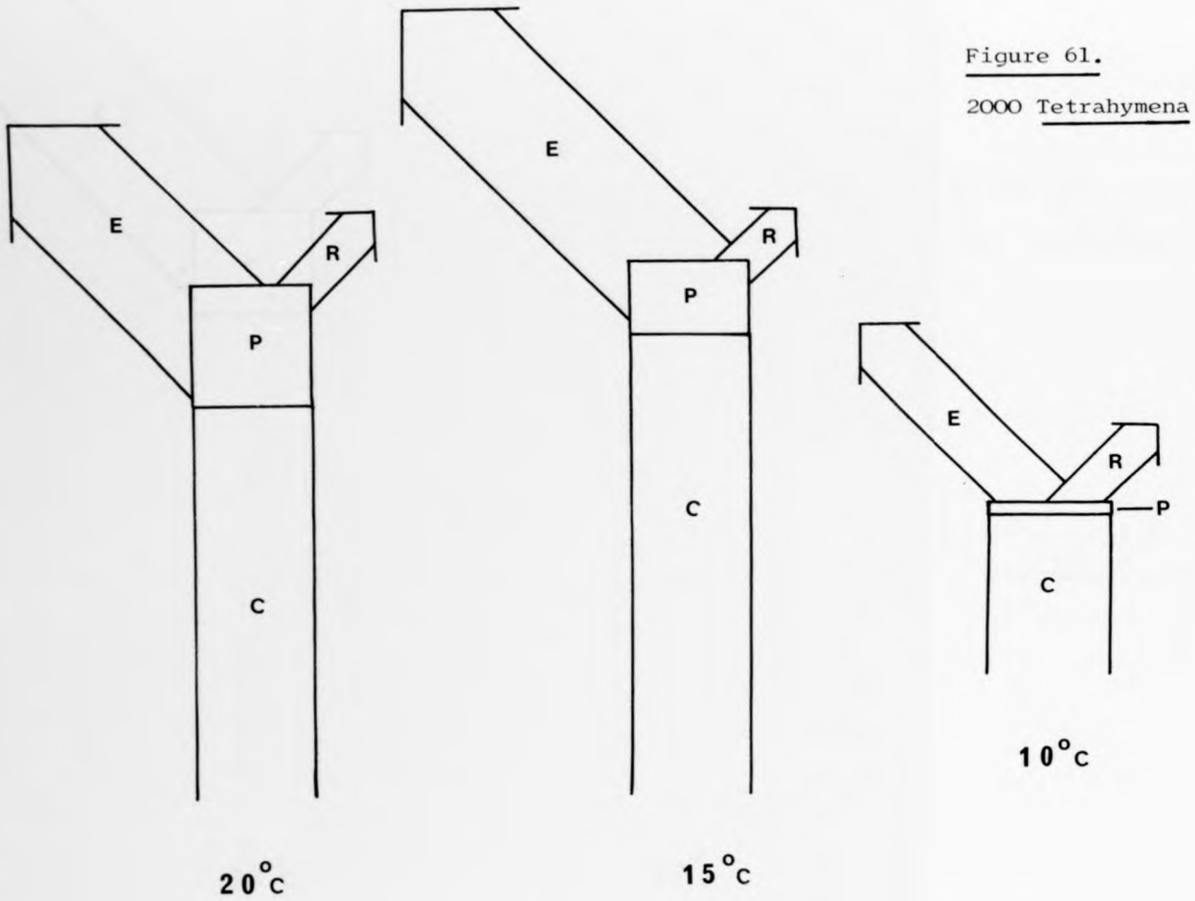
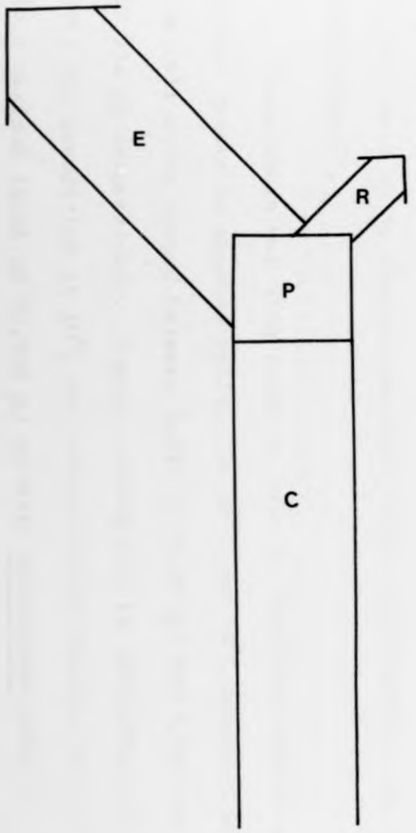
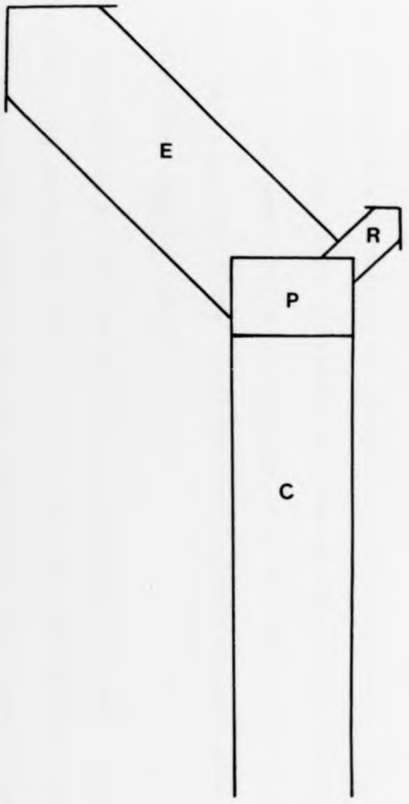


Figure 61.

2000 Tetrahymena $500\mu l^{-1}$.



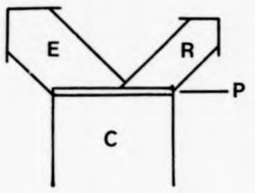
20°C



15°C

Figure 62.

4000 Tetrahymena $500\mu l^{-1}$.



10°C

form in Figures 57 - 62. The figures are drawn to scale and represent the energy consumed (μJ) and the subsequent fate of that energy, i.e. the proportion that is utilized as production, and that part lost through respiration and egestion.

The points which are immediately apparent from the set of diagrams is that the rate of consumption, for all cases of food concentration, decreased with temperature. The rate of production was also considerably less at the lowest temperature, 10°C, while the respiratory cost of that production was high.

7.1.4. Results: Generation energy budgets.

When the variable of generation time was considered, a series of budgets covering the complete life cycle of A. proteus were compiled, as shown in Table 22. Again these equations are presented diagrammatically in Figures 63 - 65, where the height of each bar represents the total energy consumption per generation for individual Amoeba cells.

Consumption at low levels of food availability was relatively constant. At concentrations greater than 500 Tetrahymena cells $500\mu l^{-1}$ the energy uptake over the generation increased markedly in all cases up to the maximum food level investigated, i.e. 4000 cells $500\mu l^{-1}$.

Temperature was also found to have an important effect on the energy uptake by amoebae cells. At 20°C and 15°C consumption over the cell cycle ranged between 4,242 to 8,127 μJ and 4,429 to 17,294 μJ respectively. Energy consumption by individual cells over the generation at 10°C was substantially higher, increasing to a maximum level of 92,931 μJ at 4000 Tetrahymena $500\mu l^{-1}$. This

Food conc. $500\mu l^{-1}$	C	P	R	E	A	% N.P.E.	% G.P.E.	% Ass. Eff.	Temp. (^{o}C)
125	4463.68	1235.00	663.10	2565.58	1898.10	65.06	27.67	42.52	
250	4682.49	1480.00	660.52	2541.97	2140.52	69.14	31.61	45.71	
500	4242.38	1696.00	626.98	1919.40	2322.98	73.01	39.98	54.76	
1000	4809.40	1784.00	557.62	2467.78	2341.62	76.19	37.)9	48.69	20
2000	5886.56	1758.00	493.68	3634.88	2251.68	78.07	29.86	38.25	
4000	8127.16	1804.00	575.00	5748.16	2379.00	75.83	22.20	29.27	
125	4682.49	1789.00	590.53	2302.96	2379.53	75.18	38.21	50.82	
250	4949.44	1933.00	542.91	2473.53	2475.91	78.07	39.05	50.02	
500	4428.68	2098.00	498.42	1832.26	2596.42	80.80	47.37	58.63	
1000	6302.92	2080.00	455.25	3767.67	2535.25	82.04	33.00	40.22	15
2000	11215.48	2538.00	610.97	8066.51	3148.97	80.60	22.63	28.08	
4000	17294.59	2811.00	970.48	13513.11	3781.48	74.33	16.25	21.86	
125	18467.34	2615.00	4455.06	11397.28	7070.06	36.99	14.16	38.28	
250	16675.46	3658.00	4361.72	8655.74	8019.72	45.61	21.94	48.09	
500	12889.02	3745.00	3850.02	5294.00	7595.02	49.31	29.05	58.93	
1000	30776.00	4242.00	6580.53	19953.47	10822.53	39.20	13.78	35.16	10
2000	68409.41	4008.00	13816.76	50584.65	17824.76	22.48	5.86	26.06	
4000	92931.47	3760.00	30459.66	58711.81	34219.66	10.99	4.04	36.82	

Table 22.

Energy budgets (μJ) for one generation of *A. proteus* at different temperatures and food concentrations.

Figure 63.

Diagrammatic presentation of the generation energy budgets in relation to food concentration at 20°C.

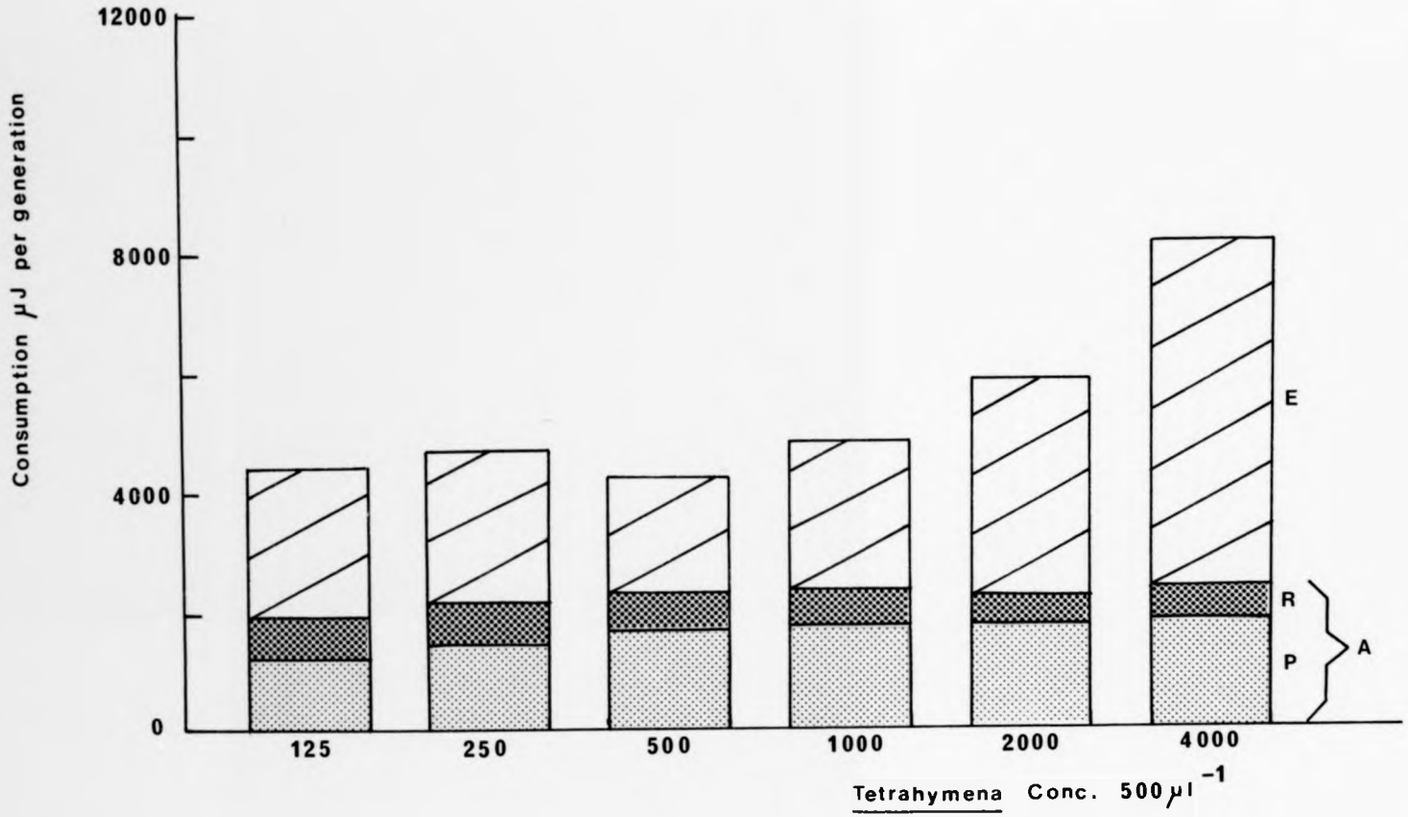

12000
8000
4000
0
Consumption μJ per generation
E
R
P
A
125
250
500
1000
2000
4000
Tetrahymena Conc. 500μl⁻¹

Figure 64.

Diagrammatic presentation of the generation energy budgets in relation to food concentration at 15°C.

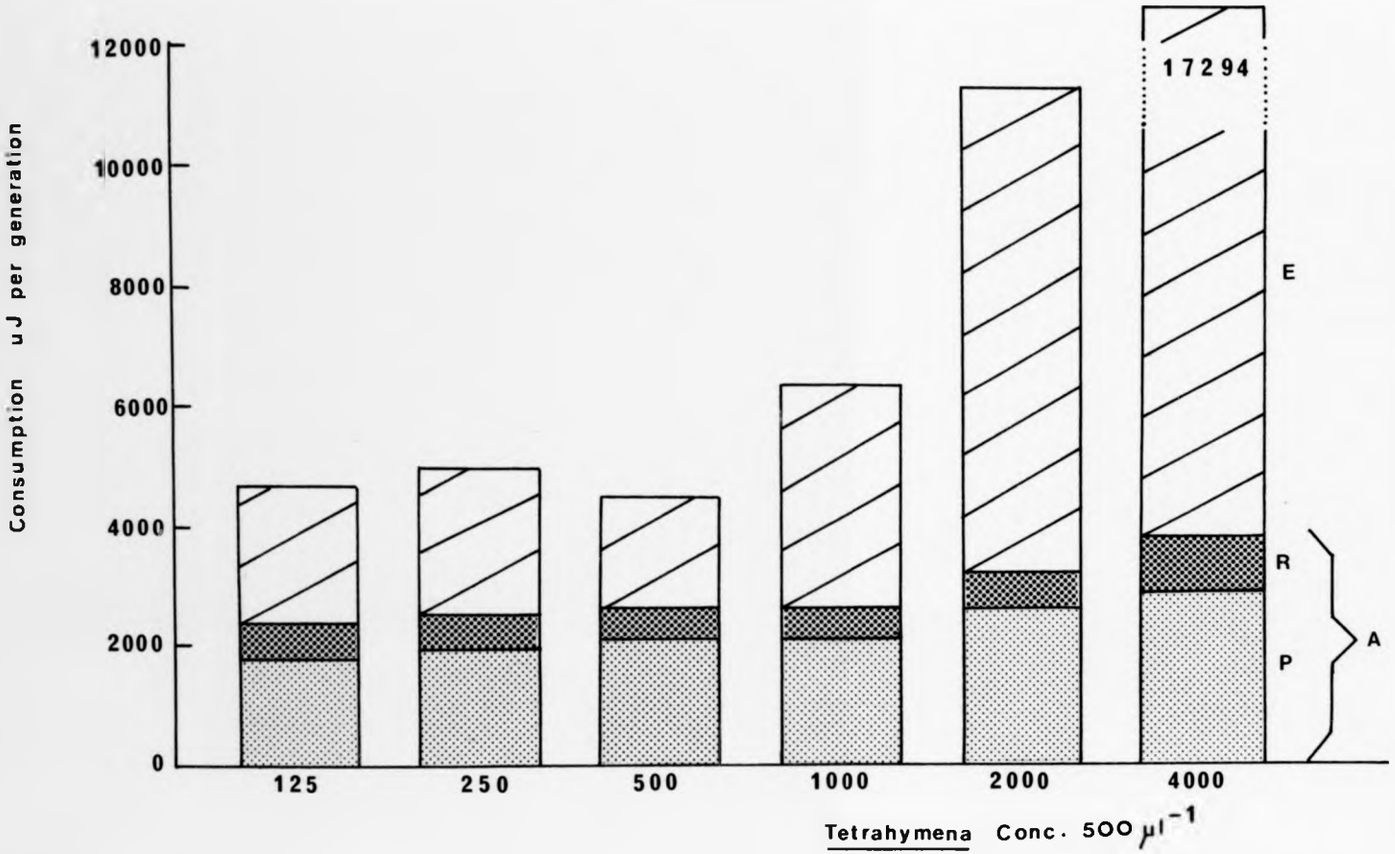
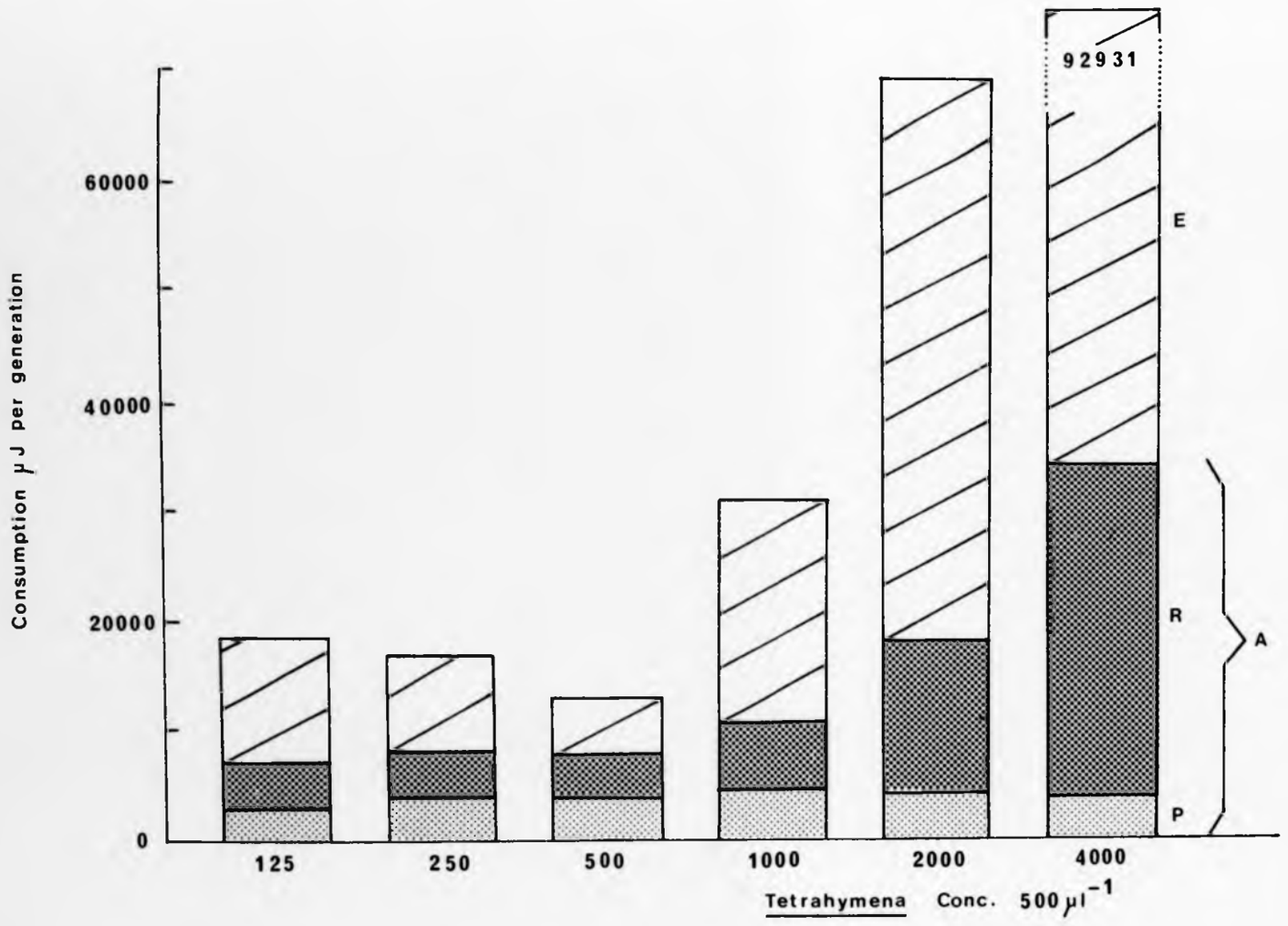

Consumption uJ per generation
12000
10000
8000
6000
4000
2000
0
17294
E
R
P
A
125
250
500
1000
2000
4000
Tetrahymena Conc. 500 µl⁻¹

Figure 65.

Diagrammatic presentation of the generation energy budgets in relation to food concentration at 10°C.


92931
60000
40000
20000
0
Consumption μJ per generation
E
R
A
P
125
250
500
1000
2000
4000
Tetrahymena Conc. 500 μl⁻¹

high energy uptake per generation at 10°C was a consequence of the long generation times of amoebae when cultured at this temperature (Chapter 3).

Relative to this high consumption, production per generation at 10°C was low, the maximum being 4,242 μJ as compared to the maxima of 1,804 μJ and 2,811 μJ per generation for cells cultured at 20°C and 15°C respectively. Again it is apparent from Figures 63 to 65 that the cost of this production, in terms of the respiratory energy losses, was high for the lower temperature, 10°C.

Although the more obvious trends are apparent from the energy budget equations and diagrams presented, the effects of the environmental variables investigated namely, temperature and food concentration, cannot be fully understood until the various efficiencies, which link the parameters of the budget equation, are examined.

The aim of the following section was therefore to detail these biological efficiencies.

7.1.5. Biological efficiencies.

The basic parameters of an energy budget C, P and R can be related to each other in the form of non-dimensional ratios or as percentages (efficiencies). The concept of biological efficiency was first introduced by Terroine (1922) for micro-organisms. The efficiencies U^{-1}, K_1 and K_2, corresponding to the assimilation, gross production and net production efficiencies, were later theoretically developed by Ivlev (1939a,b, 1945 and 1966) and applied to hydrobiological research by Winberg (1962, 64, 65, 67 and 68) and others.

The parameters of the budget for A. proteus increased linearly throughout the cell cycle, it was not therefore necessary to distinguish between the "Instantaneous" budget efficiencies and the generation budget efficiencies, as they were the same in both cases.

7.1.6. The assimilation efficiency.

(Prus, 1972, after Ivlev).

The assimilation efficiency of an organism describes the proportion of energy consumed by the animal which is not egested:

$$\% \text{ Assimilation efficiency} = \frac{A}{C} \times 100$$

where:

A = amount of energy assimilated.

C = amount of energy consumed.

The relevant biological efficiencies found for A. proteus are presented in Table 22.

The assimilation efficiencies for Amoeba initially increased with increasing food concentration, reached a peak efficiency, and thereafter decreased markedly. Figures 66 to 68 show the effect of increasing consumption (a factor of the food concentration) on the assimilation efficiency at 20°C, 15°C and 10°C. These efficiencies were similar for Amoeba cultured at 20°C and 15°C, although for cells grown at the lower levels of food availability, the efficiency of assimilation was slightly higher at 15°C. The maximum assimilation efficiency at 20°C was 54.76% at a consumption level of approximately 74μJ h^{-1}, while the lowest value obtained at this temperature was found at the highest food concentration investigated, a value of 29.27% when the cell ingested 160μJ h^{-1}. For 15°C the peak assimilation efficiency was higher at 58.63% and occurred at a lower level of consumption, 58μJ h^{-1}. As was the case for amoebae cultured at 20°C, the highest ingestion level produced the lowest efficiency, 21.86% for 15°C.

The maximum assimilation efficiencies at 10°C were again found at the lower food levels, with an increase in consumption beyond the peak assimilation efficiency (58.93%) resulting in a sharp drop in efficiency. For this temperature, the maximum assimilation efficiency was obtained at an even lower level of consumption, approximately 35μJ h^{-1}. The lowest recorded efficiency for 10°C was 26.06%, obtained when the cells energy consumption was high at 60μJ h^{-1}.

Figure 66.

The percentage assimilation efficiency of A. proteus at various levels of energy consumption for 20°C.

A = percentage energy consumed that is used for growth and respiration.

E = percentage energy egested.

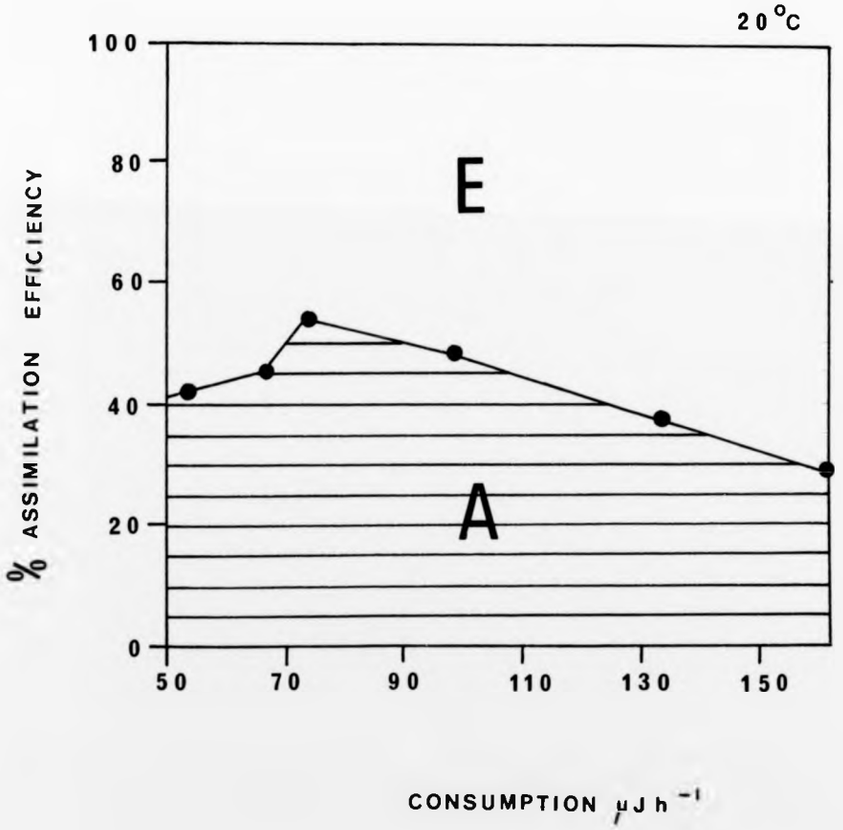

20 °C
100
80
60
40
20
0
E
A
% ASSIMILATION EFFICIENCY
50
70
90
110
130
150
CONSUMPTION µJ h⁻¹

Figure 67.

The percentage assimilation efficiency of A. proteus at various levels of energy consumption for 15°C.

A = percentage energy consumed that is used for growth and respiration.

B = percentage energy egested.

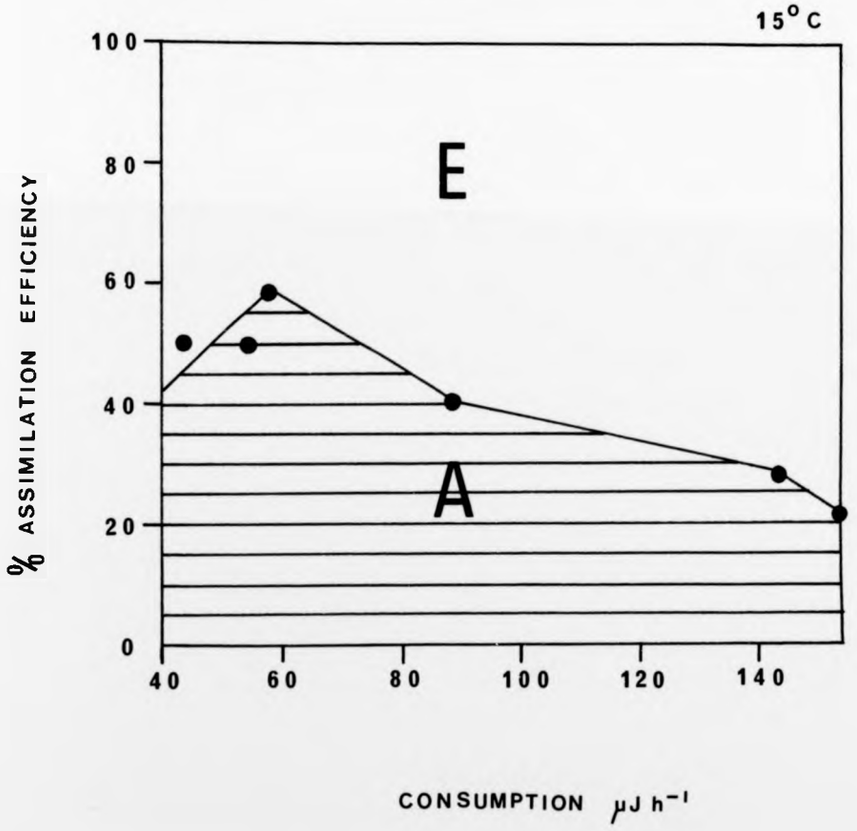
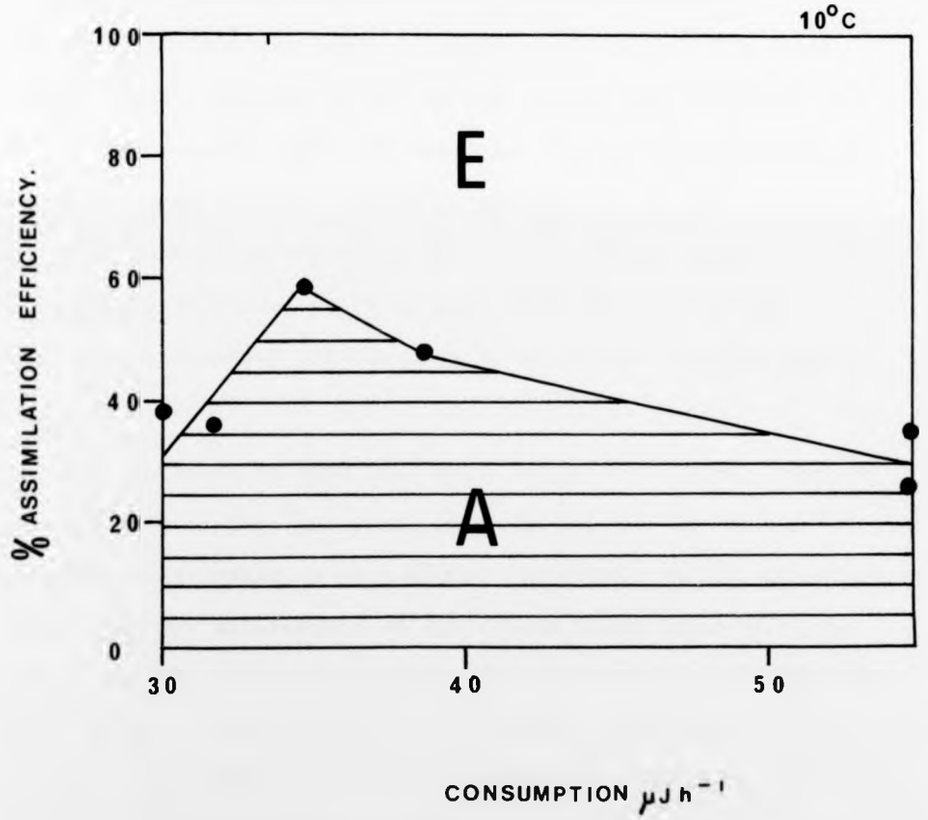

15° C
100
80
60
40
20
0
E
A
% ASSIMILATION EFFICIENCY
40
60
80
100
120
140
CONSUMPTION μJ h⁻¹

Figure 68.

The percentage assimilation efficiency of A. proteus at various levels of energy consumption for 10°C.

A = percentage energy consumed that is used for growth and respiration.

B = percentage energy egested.


10°C
100
80
60
40
20
0
E
A
% ASSIMILATION EFFICIENCY.
30
40
50
CONSUMPTION μJ h⁻¹

7.1.7. Discussion.

Assimilation efficiencies have been shown to increase from low to high temperatures for insect larvae (Raulerson,1970). However, for the present study the magnitude of the efficiencies did not show an apparent dependence upon temperature. Although the highest overall efficiency for A. proteus was found at 10°C (58.93%), the maximum efficiencies at 20°C and 15°C were of the same order of magnitude at 54.76% and 58.63% respectively. The results of the present study indicated that the optimum assimilation efficiency occurred at lower levels of consumption as the temperature was lowered. Animals in the field therefore could be expected to be operating at, or close to, their peak efficiency by having compensatory assimilation efficiencies to match the changing food supply.

It is of interest to note that even though the assimilation efficiencies were of the same order of magnitude for the three temperatures investigated, a much higher proportion of the assimilated energy was used for maintenance at the lowest temperature 10°C, as is shown by the high range of $\frac{R}{P}$ ratios, 1.03 to 8.13. By comparison, the ranges at 20°C and 15°C were low at 0.28 to 0.54 and 0.22 to 0.34 respectively. The combination of high assimilation efficiencies and low metabolic requirements in part explains the rapid growth rates found for these higher temperatures compared with the slow rates for amoebae at 10°C.

According to Duncan and Klekowski (1975) the published assimilation efficiencies for invertebrates are usually between 10 - 70%. Wiegert (1964) reported a high assimilation efficiency of

66% for the fluid-feeding invertebrate Philaenus. The food of such organisms is in a readily digestible form and they may therefore represent one of the ultimate invertebrate groups with regard to assimilation efficiency. In addition, McNeill (1970) suggested that the assimilation efficiency of the mirid Leptopterna dolabrata (28 - 36%), which feeds largely on cell contents, may be similar to that of animals feeding on naked cells, which would presumably include the carnivorous protozoa.

Published assimilation efficiencies for protozoa are variable. Heal (1967a) obtained an assimilation efficiency value of 58% for the small naked amoeba, Acanthamoeba at 25°C, a result which compares favourably with the range found for A. proteus. Other published protozoan assimilation efficiencies refer to the ciliated protozoa. The carnivorous ciliate Podophyra fixa had assimilation efficiencies within the range 50 - 66% (Laybourn,1976b,c) while Stachurska (ref. cited in Klekowski and Fischer, 1975) obtained a wider range of values of between 26 - 71% for the carnivorous ciliate Dipletus cygnus.

Curds and Cockburn (1968) investigated the growth and feeding of Tetrahymena pyriformis in axenic culture and reported an assimilation efficiency of 23% which was close to the value of 27% for the bacterial feeding ciliate Spirostomum ambiguum (Walczak, ref. cited in Klekowski and Fischer, 1975). Laybourn (1976a) reported a higher range of values for the bacterial consumer Stentor coeruleus ranging between 65 - 83%, while the lowest reported range of protozoan assimilation efficiencies was 3 - 16% for the ciliate Colpidium campylum when fed on A. aerogenes between 10°C and 20°C (Laybourn, 1973).

Differences in the assimilation efficiencies of animals are related to the feeding types and to the food items themselves. The efficiency with which Asellus aquaticus assimilated food components was found to vary by Prus (1976a). 72.3% of the total carbohydrate content of the food was assimilated, compared with 54.1% of the total lipid content, while only 32.4% of the protein content was assimilated.

The food concentration under which the Amoeba were cultured was also found to have a marked effect on the assimilation efficiency. Under conditions of excess food, and therefore high consumption, the assimilation efficiencies were lower as a greater proportion of the ingested food was only partially digested before egestion. A similar result was found by Laybourn (1973) for the ciliate Colpidium campylumunder conditions of ample food.

From the results presented and from the comparable published data, the carnivorous protozoa feeding on high energy foods, tend to have higher assimilation efficiencies than the bacterial grazers. Similarly, with reference to the macro-invertebrates, detritus-deposit feeding species tend to have the lowest efficiencies. A. aquaticus (Prus, 1972) when fed on decaying leaves was found to have a low assimilation efficiency of 26 - 35% while herbivores such as Onychiurus (Healy, 1967) were higher at 37 - 40%. The carnivores displayed the greatest efficiencies with, for example, the assimilation of Macrocyclops albidus (Klekowski and Shushkina, 1966) being greater than 50% and the carnivorous opisthobranch Navanax inermis averaging 62% (Paine, 1965).

It is unfortunate that further comparable data is, to date, unavailable for the sarcodines, but it would appear that the maximum assimilation efficiency of the naked amoebae may well be close to the value of 58%, as reported by Heal (1967a) for Acanthamoeba and for A. proteus in the present investigation.

7.1.8. The net production efficiency.

(Prus, 1972, after Ivlev).

The net production efficiency of an organism is the efficiency of utilisation of assimilated energy for growth.

$$\% \text{ Net production efficiency} = \frac{P}{A} \times 100$$

where:

P = amount of protoplasm produced in energy units.

A = amount of energy assimilated.

The N.P.E. values obtained in the present study for A. proteus are given in Table 22.

Throughout the range of food concentrations examined at 20°C, 15°C and 10°C, the net production efficiency values were slightly higher at 15°C than at 20°C, while those values obtained at 10°C were low by comparison. The values at 20°C ranged between 65.06% and 78.07%, at 15°C between 75.18% and 82.04% while at 10°C the net production efficiencies were less at 10.99% to 49.31%. The effect of food consumption on the net production efficiency of Amoeba is presented in Figures 69 - 71, for the three temperatures investigated. At 20°C and 15°C, although peaks are discernable at approximately 130μJ h^{-1} and 100μJ h^{-1} respectively, these were not so well defined as the maxima for the assimilation efficiencies. For 15°C and 20°C, after an initial rise in the net production efficiency values, an increase in the energy consumption only marginally affected the production efficiencies, and it was not until the highest levels of

consumption were reached, at approximately 140μJ h^{-1}, that a decrease in efficiency was apparent. At 10°C, a more distinct peak in the net production efficiency was found which decreased with increased consumption beyond 35μJ h^{-1}.

The lightly shaded areas in Figures 69 - 71 correspond to the proportion of energy being lost through respiration. It is apparent that respiratory energy losses were considerably greater at 10°C than at 15°C and 20°C.

Figure 69.

The percentage net production efficiencies of A. proteus at various levels of energy consumption for 20°C.

P = percentage of assimilated energy for growth.

R = percentage of assimilated energy for respiration.

cwth.

epiration.

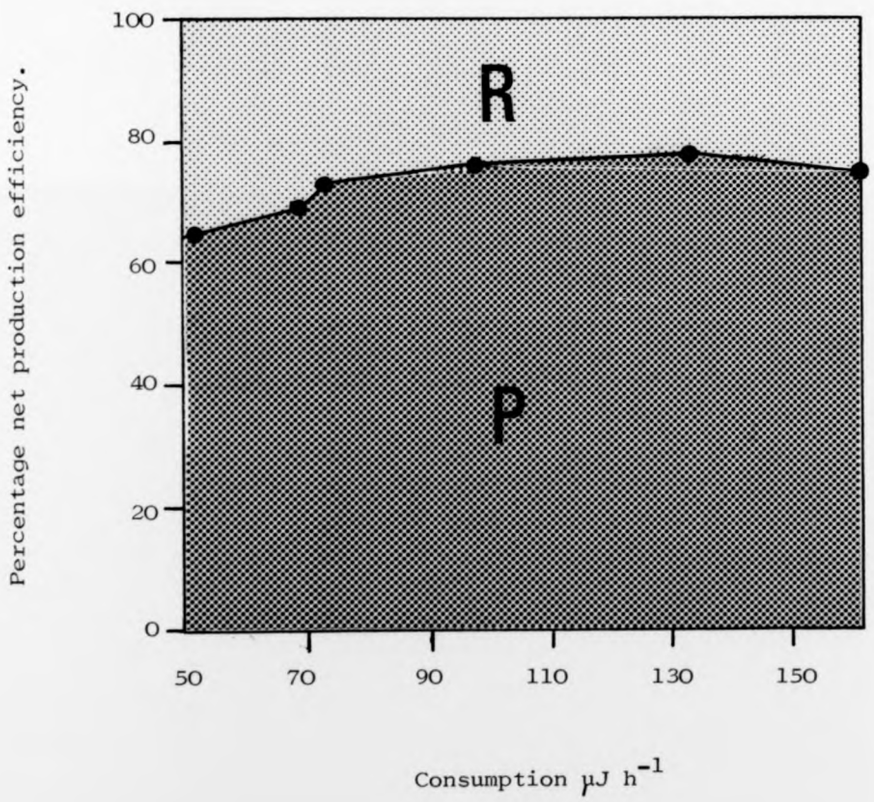


Figure 70.

The percentage net production efficiencies of A. proteus at various levels of energy consumption for 15°C.

P = percentage of assimilated energy for growth.

R = percentage of assimilated energy for respiration.

wth.

piration.

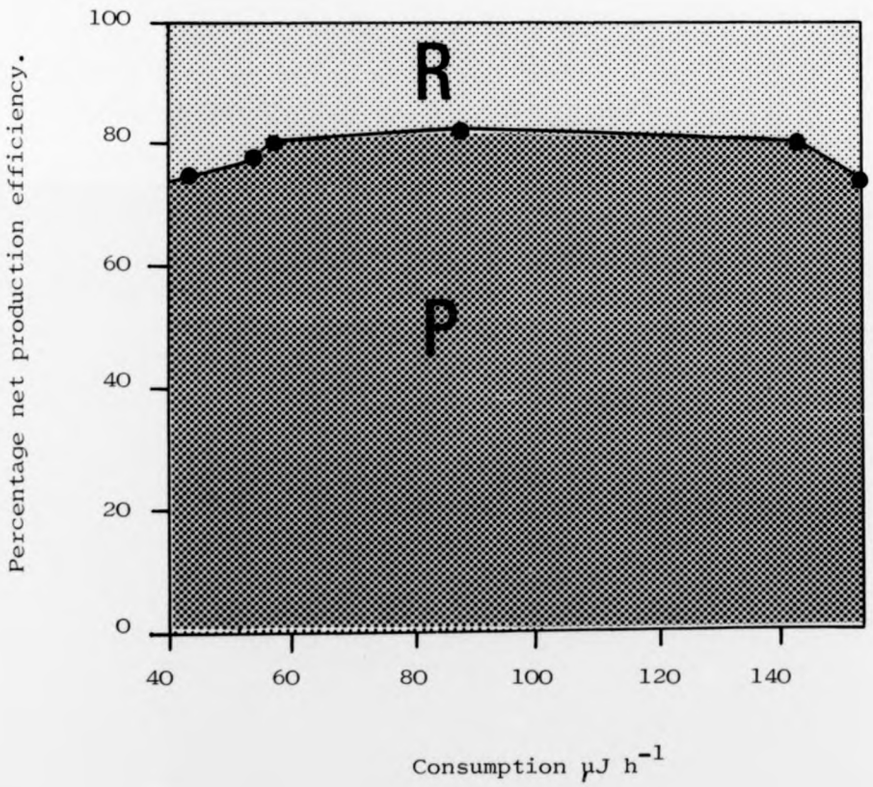


Figure 71.

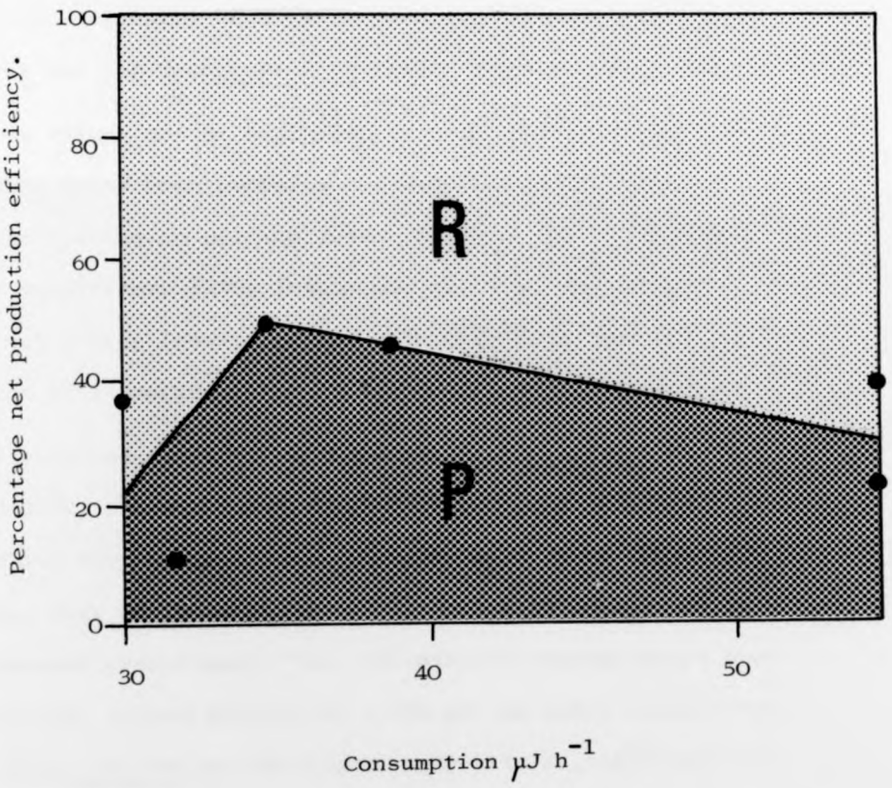
The percentage net production efficiencies of A. proteus at various levels of energy consumption for 10°C.

P = percentage of assimilated energy for growth.

R = percentage of assimilated energy for respiration.

wth.

piration.



7.1.9. Discussion.

The net production efficiency of protozoa can be expected to be high since continuous growth occurs throughout the life cycle. A high proportion of the assimilated energy is therefore channelled into production. At 15°C and 20°C, high values up to 82% were obtained for A. proteus, while Laybourn (1976a,c) reported exceptionally high values for the ciliates Stentor coereuleus and for Podophyra fixa within the range 96 - 99%.

These values can be explained in terms of the organisms life-style, where both protozoa are essentially sedentary carnivores feeding on passing prey. Further, both organisms have low respiratory rates (Laybourn, 1976 a,b) and hence respiratory energy losses are low with resulting high net production efficiencies.

Efficiencies ranging from 65 - 82% for A. proteus at 15°C and 20°C, and 63% for Acanthamoeba (Heal, 1967a) are intermediate for the protozoa. Although amoebae are basically sedentary, they do incur relatively high energy losses due to their constant cytoplasmic flow. Respiratory energy losses over the generation ranged between 18 - 35% of the total assimilated energy for A. proteus at the higher temperatures, 15°C and 20°C, resulting in the intermediate production efficiencies reported. The higher efficiency values found for amoebae at 15°C were in part due to the lower energy losses at this temperature (Figures 69 - 71), the cells being more active at 20°C compared with those at 15°C. Heal's (1967a) study on Acanthamoeba has indicated a possible tendency to lower production efficiency values in smaller

amoebae (63%). This can be accounted for in terms of the greater energy losses incurred through respiration for such amoebae (Page160).

Active protozoa, in general, have lower net production efficiencies with the carnivorous ciliate Dipleptus cygnus reported as having a net production efficiency of 25 - 52% (Stachurska, ref. cited in Klekowski and Fischer, 1975), and the bacteriovorous ciliates Tetrahymena pyriformis and Spirostomum ambiguum efficiencies of 37% and 55% respectively (Curds and Cockburn, 1968; Wakzak, ref. cited in Klekowski and Fischer, 1975).

The net production efficiencies of A. proteus at 10°C were low at 11 to 49%, a range attributable to the high respiratory cost of the large cells found for this temperature. The energy losses due to respiration were 51 to 89% of the assimilated energy, a considerably higher range than that for 15°C and 20°C (18.35%).

An exception to the trend suggested by the published and reported net production efficiencies was the study of Laybourn (1973). High values ranging between 60 - 84% for Colpidium cells cultured over the temperature range 10°C to 20°C were obtained. It is possible that the respiration values in her study were underestimated as a consequence of using the insensitive Warburg respirometer which was used to determine the rates of oxygen consumption. Alternatively, as more protozoan species are examined, such anomalous results may become the rule thereby discounting any suggested trend between different protozoan types.

Net production efficiency values for macro-invertebrates are variable. Again the mode of life for the animal largely determines the energy losses and hence the net production of the organism. Duncan, Schiemer and Klekowski (1975) obtained a high value of 82% for the nematode Plectus palustris, an animal which incurs an almost insignificant energy loss when feeding. Conversely, radiotracer studies on an algal feeding ephemeropteran nymph by Trama (1957) estimated the net production efficiency of this group to be low at 28%, while an intermediate value of 49% was found for the carnivorous opisthobranch Navanax inermis (Paine, 1965).

The relationship between the net production and assimilation efficiencies of a variety of aquatic consumers has been investigated by Welch (1968). As the former increased the latter decreased. In other words, as the amount of food being assimilated by the animal decreased, a greater proportion of that energy was channelled towards growth. Certainly for A. proteus at 20°C and 15°C this relationship can be applied. As the assimilation efficiency fell, the net production efficiency remained high, until the assimilation efficiency was at such a level, less than 30%, that the proportion of energy being used for growth had to decrease in order to satisfy the cells metabolic energy requirements. For 10°C, the relationship between the net production and assimilation efficiencies, as suggested by Welch (1968), did not hold. The issue was confused at this temperature by the high respiratory energy losses of the amoebae cells, a function of their large volume. Although the assimilation

efficiencies were relatively high for 10°C, up to 89% of that energy was used for respiration, resulting in the low net production efficiencies.

Welch (1968) has suggested that as the assimilation efficiency falls there is a compensatory increase in the net production efficiency. Finlay (1978) has pointed out that this compensatory effect would be to the animals advantage in cases where the animal had difficulties in digesting the available food sources. In such cases, the assimilation efficiency would be low, however the animal could compensate by channelling a high proportion of that assimilated energy into production. Carefoots (1967) data for *Aplysia* when fed on algae of varying digestibility demonstrates this point where the net production efficiency was found to increase with decreasing digestibility. Certainly, for *Amoeba* at the highest food concentration, digestion of food vacuoles was incomplete (Chapter 4) resulting in a lower rate of assimilation. A compensatory increase in the net production efficiency could therefore be argued in this case for 20°C and 15°C.

Welch (1968) proposed an alternative explanation whereby the assimilation and net production efficiencies were governed by consumption. For *A. proteus* at 15°C and 20°C the peak efficiencies were at food levels of approximately 58 $\mu J\ h^{-1}$ and 74 $\mu J\ h^{-1}$ respectively. Below these consumption levels the assimilation efficiencies decreased with a concomitant lowering of the two components of assimilation, namely production and respiration. As production can decrease to a greater extent relative to respiration,

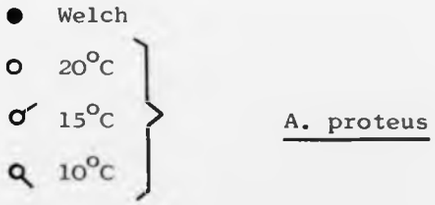
and can in fact become negative, a fall in the net production efficiency was found. As consumption was increased beyond the peak levels of 58 $\mu J\ h^{-1}$ and 74 $\mu J\ h^{-1}$ there was a decrease in the assimilation efficiency, but an increase in the components of assimilation. Again the component of respiration remained relatively constant, while production increased. The overall effect was a gradual increase in the net production efficiency up to a food level where the amoebae had difficulty in digesting the high level of energy consumption and where the rate of assimilation was very low.

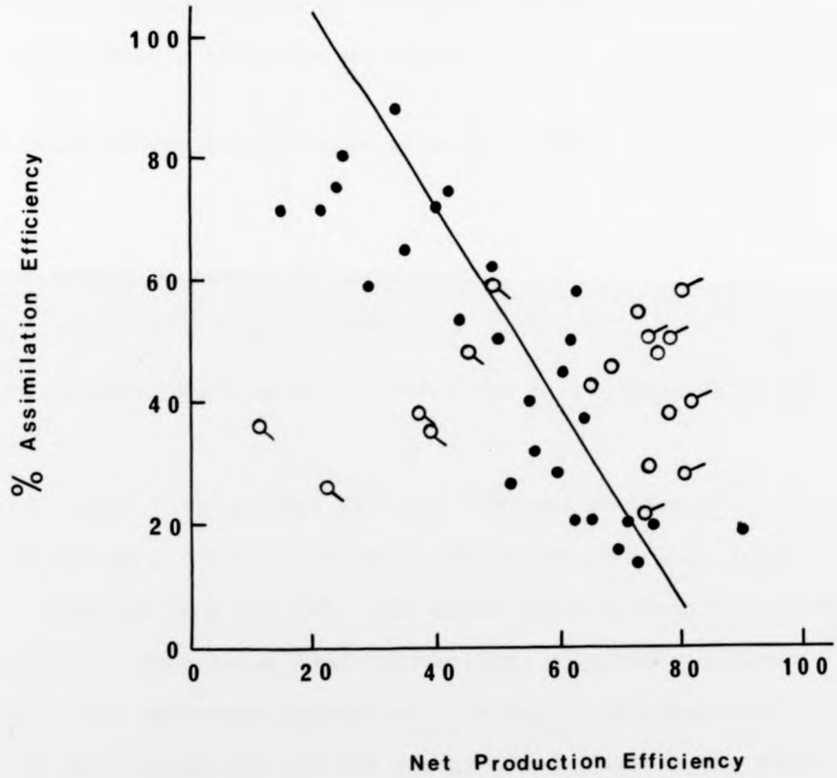
Richman (1958) and Klekowski and Sushkina (1966) working with crustacea, and Laybourn (1973) and Stachurska (ref. cited in Klekowski and Fischer, 1975) with ciliates, all found a compensatory increase in the assimilation efficiency when consumption was decreased. Certainly for A. proteus at the higher temperatures, a relationship as suggested by Welch (1968) between the assimilation and net production efficiencies was found. It is probable that under field conditions, where amoebae are utilising several food items over constantly changing concentrations, both interpretations of Welch may be relevant.

Welch demonstrated the relationship between net production efficiency and assimilation efficiency as a possible aid to researchers in the field of invertebrate aquatic ecology. The present study has shown the importance of considering both food concentration and temperature when estimating biological efficiencies, as much of the data, especially at the lower temperature 10°C, does not correspond to the linear relationship proposed by Welch (1968), (Figure 72).

Figure 72.

A comparison of the results found for A. proteus with the linear relationship proposed by Welch (1968).




% Assimilation Efficiency
100
80
60
40
20
0
0
20
40
60
80
100
Net Production Efficiency

7.1.10 The gross production efficiency.

(Prus 1972, after Ivlev).

The gross production efficiency of an animal is the percentage of ingested material that is eventually converted to consumer biomass, giving an indication of the efficiency of an organism as a convertor of energy.

$$\% \text{ Gross production efficiency} = \frac{P}{C} \times 100$$

where:

P = amount of energy for production.

C = amount of energy consumed.

The gross production efficiencies for A. proteus are given in Table 22.

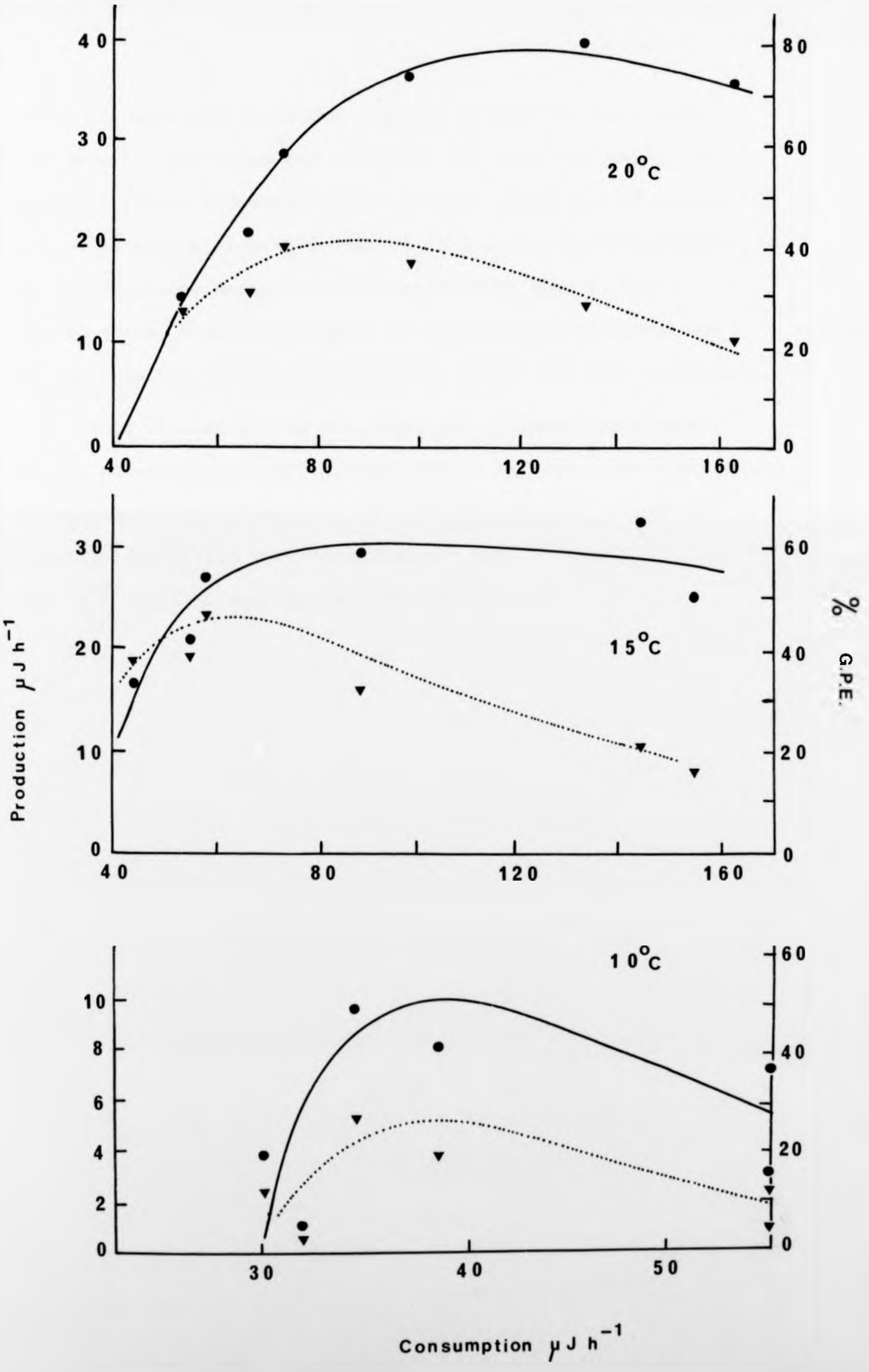
The range of values for 20°C and 15°C was similar at 22 - 40% and 16 - 47% respectively. Those for 10°C were lower, however, ranging from 4 - 29%. The gross production efficiencies increased with increasing food consumption, attained a maximum conversion and decreased thereafter. Figure 73 compares the effect of increasing the rate of energy consumption on the rate of production (growth) and on the gross production efficiency of A. proteus. The efficiency of Amoeba as a convertor of energy was greatest at consumption levels approximating 70μJ h^{-1}, 60μJ h^{-1} and 35μJ h^{-1} for the temperatures 20°C, 15°C and 10°C respectively. Increased consumption beyond the peak production rate, decreased the gross production efficiency, while the rate of production levelled and ultimately decreased at the highest consumption levels. In other words, the highest conversions occurred at the point where growth attained a maximum. The gross production efficiency was

Figure 73.

The rate of production and gross production efficiency in A. proteus as influenced by consumption.

● Production

▼ % Gross production efficiency


20°C
15°C
10°C
Production μJ h⁻¹
% G.P.E.
Consumption μJ h⁻¹

therefore dependent upon the quantity of food consumed which was in turn based upon the available food concentration. The amount of food necessary for the maximum conversion of ingested energy to Amoeba biomass decreased with decreasing temperature, thereby ensuring maximum production on less food at lower temperatures, a situation which is ecologically advantageous to cells in the natural situation for middle and high latitudes.

It is of interest to note that the minimum consumption levels necessary for maintaining amoebae at zero production were extrapolated from Figure 73. The levels required to prevent negative production were approximately 40μJ h^{-1} for 20°C, 35μJ h^{-1} for 15°C and 30μJ h^{-1} for 10°C.

7.1.11. Discussion.

Knowledge of the gross production efficiency of an animal is of importance as it gives an indication of the efficiency of an organism as a convertor of consumed energy for growth. This production is then potentially available for utilisation by subsequent trophic levels.

Laybourn (1976c) has suggested that carnivorous protozoa tend to have higher gross production efficiencies than most bacterial and fungal feeding species. The predaceous ciliated protozoa, Stentor coeruleus and Podophyra fixa, showed high gross production efficiencies ranging between 64 - 82% (15°C and 20°C) and 50 - 66% (15°C) respectively (Laybourn, 1976 a,c). Stachurska (ref. cited in Klekowski and Fischer, 1975) reported, however, a lower range of values, 7 - 32%, for the carnivorous ciliate Dipleptus cygnus when cultured over a range of food concentrations.

In comparison to the predaceous ciliates, the bacteriophagous protozoa display on the whole lower efficiencies. Spirostomum ambiguum (Wakzak, ref. cited in Klekowski and Fischer, 1975) gave a low yield (G.P.E. = 15%) as did Colpidium campylum (Laybourn, 1973) with maximum production efficiencies of 11% at 20°C, 9% at 15°C and 3% at 10°C. Fischer (ref. cited in Klekowski and Fischer, 1975) reported that the gross production efficiency of Tetrahymena when fed Azotobacter was 34% while Curds and Cockburn (1968) reported a slightly higher value of 50% for T. pyriformis when cultured on Klebsiella aerogenes. An exceptionally high conversion value of 78% was found by Proper and Garver (1966) for

Colpoda steinii when fed the bacterium Escherichia coli. This high figure for a bacteriophagous protozoan was almost certainly due to the high culture temperature of 30°C used. As has been shown for A. proteus in the present investigation and for Colpidium (Laybourn, 1973), the highest growth efficiencies were obtained at the higher temperatures of 20°C and 15°C.

The only published production efficiency values for the sarcodines were by Heal (1967a) for Acanthamoeba where the gross production efficiency was 37% at 25°C, and tentative estimates for the conversion of Tetrahymena dry mass to Amoeba dry mass by Griffin (1960) of approximately 50% and 35% for A. proteus and C. chaos. These figures correspond well with the values for A. proteus at 20°C and 15°C where gross production efficiencies ranged between 16 -47%.

As Engelmann (1961) pointed out, slow growing and non-reproducing organisms tend to convert less food to protoplasm than fast growing animals. As protozoa have no reproductive stages, but are in a state of continuous growth over a relatively short cell cycle, their gross production efficiencies are generally higher than those of other aquatic invertebrates. The benthic nematode Plectus palustris (Duncan et al, 1974) when cultured in conditions of abundant bacterial food supply displayed a very low gross production efficiency of 10%. Similarly, low efficiencies were reported for Tribolium (13 - 23%) and for Asellus aquaticus (5%) by Prus (1968b and 1972 respectively). McNeill (1971) found the growth efficiencies of the heteropterean Leptopterna dolabrata to range from 15.6 - 16.8%.

Further, the food type consumed is important in determining the gross production efficiency of the animal. The herbivorous and detritus feeding macro-invertebrates display low conversion efficiencies with Gere (1956) reporting values as low as 0.7% for litter feeding Diplopoda and Isopoda. Carnivorous macro-invertebrates are in general higher with, for example, Paine (1965) reporting a gross production efficiency of 30% for the opisthobranch Navanax inermis. As already shown, a similar trend regarding food types can be shown for the protozoa, although efficiencies tend to be higher throughout as a consequence of the mode of growth.

Excepting the present investigation for A. proteus and that of Laybourn (1973) for Colpidium, studies on the gross production of protozoa at temperatures at or approaching 10°C have not been undertaken. It is apparent that the efficiency of utilisation of consumed food for growth decreases markedly with temperature; as low as 4% for A. proteus and 3% for C. campylum at 10°C. This suggests that the gross production efficiency of protozoa in the field, where temperatures are generally lower than 15°C for temperate regions, may be less than the results of laboratory studies, largely conducted at temperatures greater than 15°C, indicate. The present study has supported existing publications in showing that both food concentration and temperature are important variables which must be considered in determining the gross production efficiency of protozoa.

PART 3.

Field studies on amoebae.

Chapter 8.

8.1. The Distribution of *Amoeba proteus* and related species.

8.1.1. Introduction.

Protozoa occur wherever moisture is present, in the sea, in all types of freshwater habitat, and in the soil. Their cosmopolitan distribution is aided by their ease of dispersion, a function of their size, and by the ability of many species to form resting stages, commonly cysts, which can be wind dispersed. Puschkarev (1913) estimated that there are approximately two protozoan cysts per $1m^3$ of air. Although a seemingly unimportant number their rapid reproductive cycle enables such a small quantity of cysts to become readily established in favourable environments.

The factors which are thought to influence their distribution include temperature, the dissolved oxygen content, the chemical composition and the pH of the water, in addition to their food types and the adaptability of individual species to adjust to environmental changes. Although protozoa are relatively unprotected and hence in intimate relationship with their environment, they are safeguarded by having a high degree of tolerance to the constantly changing environment.

Several species of Ciliata and Mastigophora in addition to a single amoeboid species have been reported from a thermal spring in Virginia, where the water temperature was 34 - 36°C (Glaser and Coria, 1935). Gojdics (1967) has pointed out that protozoa can be found in water between the extremes of 0 - 52°C, while a laboratory investigation by Chambers and Hale (1932) showed that if ice was prevented from forming, there was no visible damage to amoebae cells cultured at -5°C.

Protozoa are in general tolerant to changes in pH, with studies by Singh (1948) on the protomyxan Leptomyxa reticulata showing the tolerance of laboratory cultures over the pH range 4.2 - 8.7. Wang (1928), however, has shown that the temporal distribution of ciliates can be influenced by the hydrogen ion concentration. Further, studies on the Testacea have revealed the importance of both water-content and pH on the distribution of these protozoan forms (Bartos, 1940, 1946; de Graaf, 1956, 1957; Heal, 1961).

Oxygen availability has been shown by Moore (1939) and Webb (1961) to be of importance in determining the distribution of protozoa, again with regard to the ciliated protozoa. Further, food concentration can also be expected to fluctuate in the field, particularly as a consequence to temperature changes. Grabacka (1971) and Goulder (1974) have recently shown that both the quality and quantity of food can affect the distribution of ciliates. The extensive publication of Sandon (1932), however, serves to illustrate the array of micro-organisms which have been reported as being acceptable food items for the protozoa.

The distribution of and numbers of ciliated protozoan species from the aquatic environment, is well documented, (Noland and Gojdics, 1967; Faure-Fremiet, 1967; Fenchel, 1969; Bick and Kunze, 1971; Bryant and Laybourn, 1972/3; Finlay, 1977), although publications on the quantitative occurrence of the naked amoebae are lacking in the literature. Statements such as that of Kepner and Taliaferro (1913) "in material taken from a pond southwest of the University, we found great numbers of A. proteus" have failed to be supported throughout subsequent publications. Those papers dealing with microfaunal populations, rarely consider the naked amoebae as a commonly accurring group (West, 1901; Brown, 1911; Hausman, 1917; Graff, 1927; Hempstead and Jahn, 1936; Lackey, 1938; Fantham and Porter, 1945; Cole, 1955; Bamforth, 1958). Further, Finlay (pers. comm., 1976) recorded only one finding of a large naked amoebae species throughout an intensive two-year sampling programme of the benthos of Airthrey loch. Admittedly, Finlay was sampling for the ciliated protozoa, and may have overlooked some amoeboid species but amoebae could certainly not be considered as a common species for this environment.

Bovee (1965a) maintained that A. proteus usually frequents the shallow, shaded, clear, slow moving waters of lakes, ponds and streams, while Vickerman and Cox (1967) suggested that A. proteus is found in large permanent bodies of water, rarely in abundance and never in temporary ponds. Sarcodines are certainly abundant in marsh areas, notably Sphagnum bogs where high populations of testate amoebae have been reported by Fantham and Porter (1945), de Graaf (1956, 1957) and Heal (1961, 1962).

In view of the fact that little is known about the function of naked amoebae within the complex protozoan community structure, highlighted by Picken (1937), Faure-Fremiet (1950) and Webb (1956), and the fact that the present study was the first investigation aimed at detailing the energy requirements of a large naked amoeba (A. proteus) some quantitative information on the distribution of this species and comparable species was sought.

It must be stressed that the intention of this final section was merely to give an approximation as to the numbers and possible impact of A. proteus and related species in the field. To fully document the ecology of naked amoebae in the wild was outwith the scope of this thesis, and would have constituted a full time study in itself.

8.1.2. Preliminary qualitative survey: Materials and Methods.

The literature (Bovee, 1965a; Vickerman and Cox, 1967) suggested that A. proteus were commonest in shallow clear bodies of water of a permanent nature, frequently on the undersides of the leaves of aquatic plants (MacKinnon and Hawes, 1961).

A preliminary qualitative survey, spanning such habitats, was undertaken in November, 1976. Samples of the vegetation (approximately 10g wet weight) were collected from the five areas listed below. The samples were washed in the laboratory within two hours of collection and the washings were microscopically searched for large naked amoebae species (>200µ in length).

Site 1.

Airthrey loch: A small eutrophic loch on the University of Stirling campus. The deptch of this body of water never exceeds 5m. Samples of both decaying and fresh vegetation from around the seven emerging macrophytes, characteristic of this loch, were collected. 4 surface sediment samples were also sampled, diluted and examined.

Site 2.

The Forth and Clyde Canal: This now dissused canal offered a permanent slow moving body of water. 6 samples were collected from around the emerging macrophytes which were abundant at the edge of the canal.

Site 3.

The Sheriffmuir reservoir: Large areas at the margin of this moorland reservoir were sheltered and shallow (10cm) with abundant plant growth. 6 samples were taken from amongst the benthic vegetation and decaying matter.

Site 4.

Inlet stream: The vegetation and decaying leaf litter of this shallow (20cm), slow moving, inlet stream serving the Sheriffmuir reservoir was sampled at 6 sites.

Site 5.

In view of the abundance of Rhizopoda reported from fern and bog areas, 4 moss samples were collected from the moorland surrounding the Sheriffmuir reservoir.

8.1.3. Preliminary qualitative survey: Results.

A total of 33 samples were searched for large ($>200\mu m$) naked amoebae species, and with the exception of the 4 moss samples, no amoebae of this type were found.

It was therefore decided to select and concentrate on a wet-bog area for future study into the distribution of the naked amoebae.

8.1.4. The distribution of the Sarcodina of a Sphagnum bog with emphasis on the large naked amoebae: Site sampled.

The series of raised bogs, collectively known as Flanders Moss, in the upper part of the Forth Valley, Scotland, is the most extensive area of continuous raised bog (about $10Km^2$) in Britain. The vegetation of the bog surface is characterised by small scale rather than large scale pattern. A site was therefore sought in January, 1977 which consisted of a uniform stand of wet moss with overlying water, suggesting that the chosen area would remain moist throughout the year.

A bog pool ($2m^2$) containing submerged Sphagnum palustre and surrounded by overhanging Myrica gale, Calluna vulgaris and small Betula (sp.) was selected for study.

8.1.5. Materials and Methods.

Heinis (1945) studies the vertical distribution of Testacea in Sphagnum stands and concluded that most species were to be found in the top 10cm. For the present quantitative investigation, two separate 10cm long strands of Sphagnum moss, A and B, were randomly chosen from the submerged Sphagnum mass.

Within 1 hour of collection, the samples were rinsed thoroughly with filtered (0.45µm membrane) overlying water from the bog pool site. The washings were placed in an 8.5 diameter petri dish and the total number of sarcodine species contained within a $4cm^2$ area on the dish were counted and identified. By multiplying by the factor of 14.19, the values for samples A and B were converted to numbers per strand of Sphagnum.

On each sampling occasion, 1 per month for the year 1977, a $1000cm^3$ quadrat was pushed into the Sphagnum mass to be depth of 10cm, and the moss contained was collected and dried in an oven at 80^oC until standard weight. As the variation between quadrat weights over the year was not great, a mean dry weight of 10.30 ± 2.60 (S.D.)g was calculated. By similarly drying the individual washed Sphagnum strands, conversions were calculated which were used to convert the numbers of individuals per strand to numbers per $1000cm^3$. Time alone dictated the restricted number of samples which were examined throughout the year, however, it was hoped that some notes could be concluded about the distribution of amoebae in the field.

Although the food preferences of carnivorous amoebae in the wild are not fully understood (see discussion Chapter 8), the numbers of possible food items in the Sphagnum bog were estimated in an attempt to gain information on the biomass available for consumption by the large naked amoebae component of the bog.

A. Ciliates and flagellates.

A 250ml sample of water surrounding the Sphagnum was collected by filling a submerged bottle. 5µl samples of this suspension were dispensed automatically from an Eppendorff pipette and were inspected before evaporation significantly reduced their volume. 50 such samples were examined for each monthly collection, and the number of ciliates and flagellates within 3 size classes, less than 50µm, 50 - 100µm and greater than 100µm were recorded.

B. Rotifers and nematodes.

Although it is unlikely that the naked amoebae were utilising such large invertebrates as the rotifers and nematodes within the Sphagnum, Mast and Root (1916) did report A. proteus feeding frequently on rotifers and on one occasion a nematode.

The numbers of rotifers and nematodes were estimated in conjunction with the sarcodine counts, by recording the number of individuals on an area of $4cm^2$ of a petri dish. These values were multiplied by the appropriate conversion and expressed as numbers per $1000cm^3$.

C. Diatoms and desmids.

The algal forms represented by the diatoms and desmids were abundant, and although not normally a constituent in the diet of a truly carnivorous amoeba, their common occurrence in the Sphagnum samples, warranted their mention.

The numbers of diatoms and desmids were estimated from counts of the washings of the collected Sphagnum strands. In this case, however, a much smaller area of the petri dish was examined, $0.1cm^2$ as opposed to $4cm^2$.

Appropriate conversions were calculated and employed as previously described.

The physical parameters of temperature and pH were recorded for each sampling occasion. Mid-morning temperatures of the water surrounding the Sphagnum mass were read directly using a mercury thermometer. A sample of the water overlying the Sphagnum mass was collected and the pH measured within 1 hour using a Corning-EEL model 7 pH meter and an Activion electrode.

The following publications were used in the identification of the Sarcodina.

Testacea: West (1901), Deflandre (1918, 1928, 1929), Hoogenraad (1935), Hoogenraad and Groot (1934/35), Paulson (1952/53), de Graaf (1956).

Actinopoda: Deflandre (1918).

Naked amoebae: Deflandre (1918), Page (1976).

8.1.6. Results.

For each monthly set of results, the mean number of Sarcodine species, from the two strands A and B, were calculated. The results are presented in Table 23.

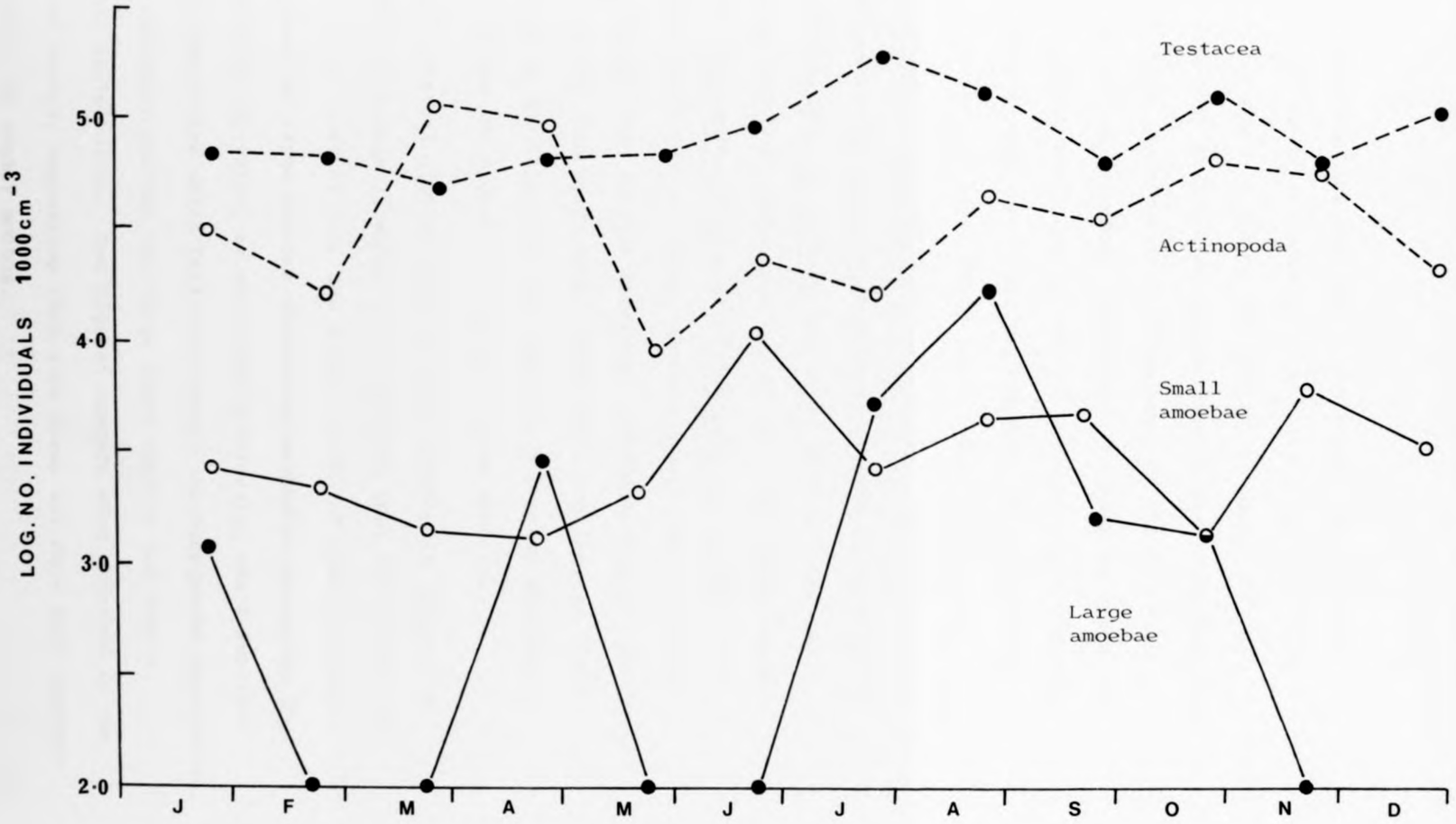
The Testacea constituted the dominant component of the Sarcodina with concentrations as high as 18.7 million m^{-2} (to a depth of 10cm) being recorded. The population remained high throughout the year with peak numbers being obtained in the summer months between June and August (Figure 74). The bulk of the individuals were represented by the genera, Arcella, Centropyxis, Difflugia, Euglypha and Nebela (Appendix 14).

Similarly the Actinopoda, represented predominately by the species Actinophrys sol, was abundant throughout the year reaching a maximum of 11.3 million m^{-2}, although no seasonal variation was apparent (Figure 74).

The numbers of naked amoebae were consistently lower (Table 23), especially with regard to those species greater than 200µm in length. The numbers of small amoebae (<200µm) ranged between 0.1 - 1.1 million cells m^{-2} (to a depth of 10cm) being found on

Figure 74. } The number of Sarcodina (per 1000cm^3) from a
Table 23. } Sphagnum bog over a 1 year sampling programme.

	Actinopoda	Small naked amoebae (<200µm)	Large naked amoebae (>200µm)	Testacea
J.	30573	2720	1198	67674
F.	17867	2184	0	64067
M.	112660	1624	0	47196
A.	88109	1260	3076	62107
M.	9456	2357	0	69069
J.	22708	10853	0	88943
J.	16069	2396	5040	187450
A.	45322	4157	15575	126829
S.	32648	4229	1522	58360
O.	61855	1305	1305	117217
N.	59706	5825	0	60460
D.	23337	3177	0	97401


LOG. NO. INDIVIDUALS 1000cm^{-3}
5·0
4·0
3·0
2·0
J
F
M
A
M
J
J
A
S
O
N
D
Testacea
Actinopoda
Small amoebae
Large amoebae

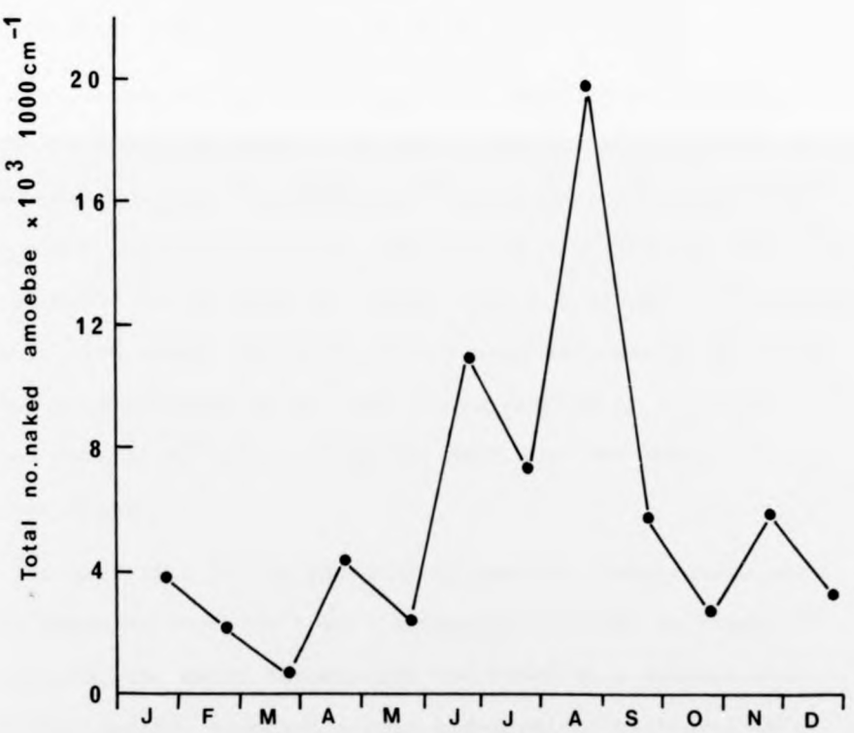
every monthly sampling occasion. The large naked amoebae (>200um) were fewer, between 0 - 1.5 million cells m^{-2} and were only recorded in 50% of the monthly samples. It must be stressed that on those occasions when no large amoebae were recorded, this was probably a consequence of the limited number of samples examined. Therefore, although no large amoebae species were found on half of the sampling occasions, it is probable that such cells were present at the site but at a low background level which was not detected in the restricted sampling programme.

No particular naked amoebae species could be considered as being dominant. A. proteus was found on three separate occasions, which was more than any of the other large amoebae species. Thecamoeba striata and Valkampfia (sp.) were the commonest small amoebae found, although there were also many small unidentified species recorded. Identification, particularly of the smaller amoeboid forms, presented a problem, as often knowledge of the life cycle is required, a process obviously requiring the individual culture of sampled species.

The seasonal distribution of naked amoebae in general is presented in Figure 75 where it is apparent that the numbers of amoebae were greatest over the summer months of June to August. The numbers of large amoebae fluctuated markedly throughout the year (Figure 74) which, as mentioned previously, was a function of the sample size which failed to detect the background population. It is not clear whether the large naked amoebae did show a seasonal distribution. The highest numbers were obtained in the month of August, suggesting that such forms may have been commoner throughout the summer months.

Figure 75.

The change in the total naked amoebae population of a Sphagnum bog over a 1-year sampling programme.


Total no. naked amoebae x 10^3 1000cm^-1
20
16
12
8
4
0
J
F
M
A
M
J
J
A
S
O
N
D

The complete species list of sarcodines found throughout the 1 year sampling programme is given in Appendix 14. Figure 76 illustrates how the number of ciliates and flagellates fluctuated throughout the year. The peak values were found in the summer months with the smallest cells, those less than 50µm, being prevalent throughout the year. The absolute numbers of cells recorded for each month sampled are given in Appendix 15.

The numbers of rotifers, nematodes, diatoms and desmids was considerable throughout the year (Appendix 16). The rotifers ranged between 0.9 - 7.4 million m^{-2} (to a depth of 10cm) with no apparent seasonal pattern. The numbers of nematodes were also high at 0.5 - 7.2 million m^{-2}, again with the numbers fluctuating randomly each month. However, the diatoms and desmids were by far the most numerous of all the groups sampled at 13,400 - 702,000 million m^{-2} with a distinct peak over the months of July and August.

The variation in the physical parameters, temperature and pH, as measured over the 1 year programme is given in Figure 77. As expected, the water temperature increased to a maximum over the summer months, reaching a peak mid-morning level of 14.6°C in July. The lowest temperature recorded was for over the months of January and February, when the temperature dropped to 2°C. The pH values of the water overlying the Sphagnum were relatively constant throughout the year, varying between the close confines of 3.5 and 3.8.

Figure 76.

The numbers of flagellates and ciliates in a Sphagnum bog-pool throughout a 1 year sampling programme.

●—● Flagellates < 50μm.

●-● Flagellates 50 - 100μm.

o—o Ciliates <50μm.

o-o Ciliates 50 -100μm.

o—o Ciliates >100μm.

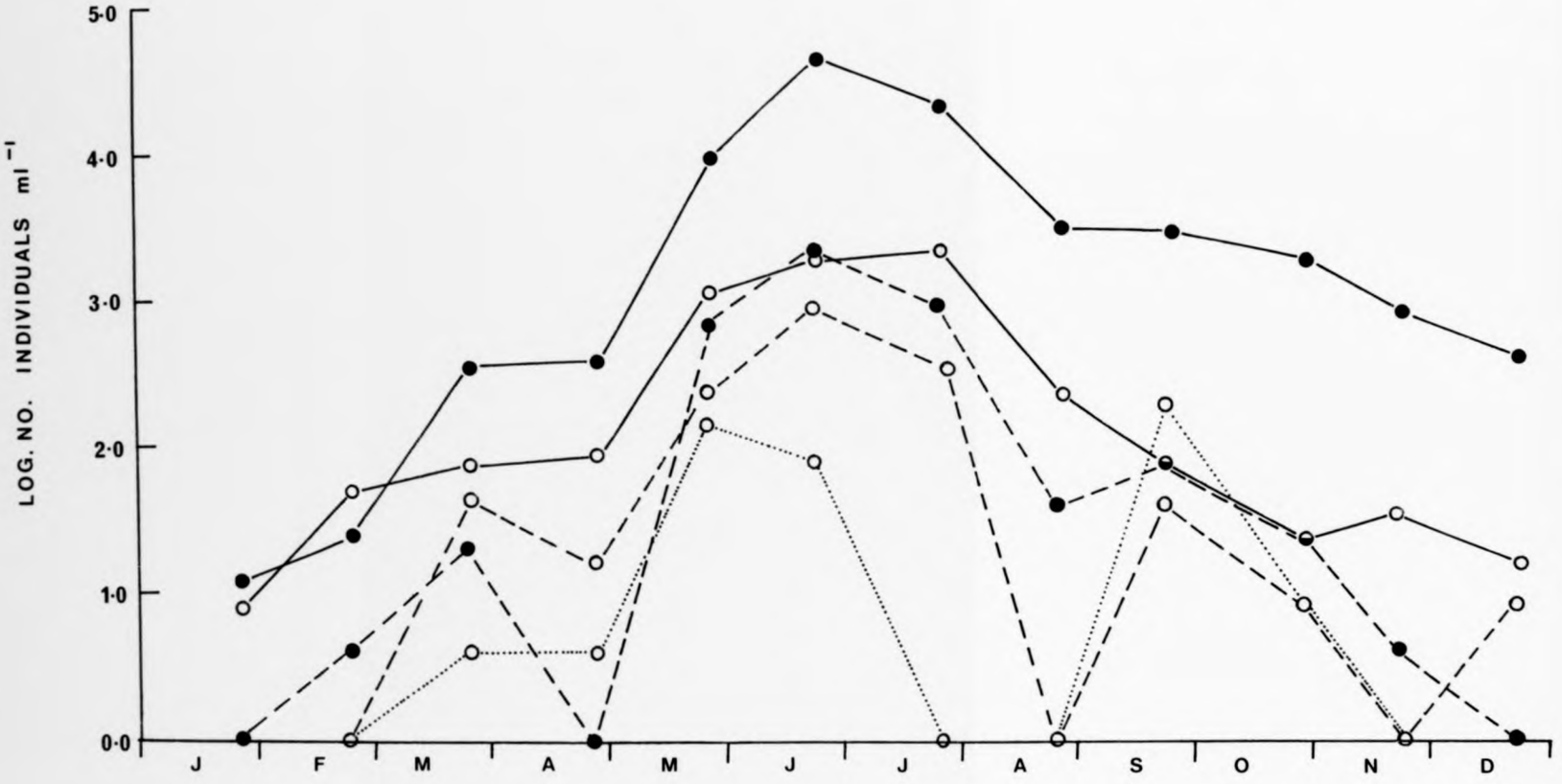
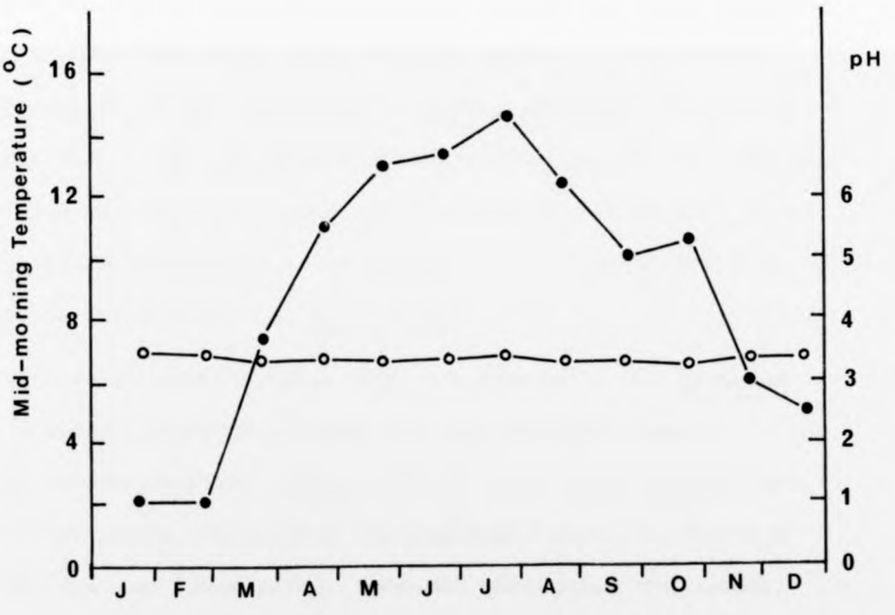

LOG. NO. INDIVIDUALS ml⁻¹
5·0
4·0
3·0
2·0
1·0
0·0
J
F
M
A
M
J
J
A
S
O
N
D

Figure 77.

The temperature and pH of the water overlying the *Sphagnum* mass throughout the 1 year sampling programme.

● Temperature (^{o}C)

o pH


Mid-morning Temperature (°C)
16
12
8
4
0
pH
6
5
4
3
2
1
0
J
F
M
A
M
J
J
A
S
O
N
D

8.1.7. Discussion.

Quantitative samples provide information about the absolute numbers in the field which are essential for a clear understanding of changes in population composition. The present investigation has indicated trends in the sarcodine population of a Sphagnum swarm, although at best, these suggestions on seasonal distribution are tentative.

While the Testacea and Actinopoda were the major constituents, the present investigation must centre around the naked amoebae, in particular the large naked amoebae species, whose energy requirements may be comparable to Amoeba proteus. In view of the differing respiratory requirements (Chapter 6) and mode of nutrition of the small amoeboid forms, less than 200µm, it is too great an assumption to encompass these forms within the energetic requirements of A. proteus.

The large naked amoebae were not common in the Sphagnum mass sampled, although a trend towards increased numbers in the summer months could be interpretated. This peak corresponded to the increased temperature and available food, in the form of ciliates and flagellates, recorded throughout the summer. Increased ciliate biomass over the summer months has recently been reported by Finlay (1977) for the benthic protozoa of a freshwater loch. Similar seasonal fluctuations, again for the ciliated protozoa, have been reported by Wang (1928), Moore (1939) Fenchel (1967) and Goulder (1974).

For the amoebae species collected in the course of the sampling programme, the food vacuoles were in all cases small

showing no discernible remains. It is likely therefore that the amoebae were ingesting the ciliated and flagellated protozoa, which were in general small (<50μ), however this is speculation.

Published reports detailing the feeding habits of A. proteus are varied and in some cases open to question. It is generally accepted that the ciliated protozoa, particularly Tetrahymena (Prescott and James, 1955) and Paramecia (Taylor, 1924) notably Paramecium bursaria (Williamson, 1944), in addition to the small flagellate Chilomonas paramecium (Williamson, 1944) are suitable food items for the culture of A. proteus. Further, Beers (1924) has fully described the ingestion of the large ciliate Frontonia. Less likely food items have been published bt Mast and Root (1916) who have reported A. proteus feeding on rotifers, on occasion taking up to two days to ingest the small metazoan. Similarly, these authors observed Amoeba on one occasion feeding on a small nematode, while Czerny (1868) reported A. proteus feeding on amphibian eggs.

If rotifers were being consumed in the field, then amoebae would be at all times under conditions of excess food supply, as the numbers of rotifers was high throughout the year. However, with the rotifers from Flanders moss ranging up to 1000μm in length, it is thought more likely that the amoebae were utilising the ciliated and flagellated protozoa. The seasonal fluctuations of these protozoa would therefore have an effect on the distribution of the amoeboid species.

The question of whether Amoeba proteus encysts or forms resting stages has plagued early publications. Hausmann (1920) reported the liberation of amoeboid spores from a large, unencysted

Amoeba, with these spores developing directly into adult A. proteus. Hulpieu and Hopkins (1927) also published a process involving the liberation of spores from an amoeba, although these spores passed through stages resembling intermediate amoebae species before adopting the morphology of A. proteus. In addition, Taylor (1924) and Jones (1928) have reported the liberation of small cysts from degenerating Amoeba proteus cells, produced as a result of fragmentation of the nucleus within the "mother" amoeba. Such processes are though unlikely as Halsey (1936), in a study designed to elucidate the life-cycle of A. proteus, found no signs of encystment, sporulation or fragmentation in over 400 cells which were allowed to degenerate in isolation.

With regard to the life cycle of A. proteus in the field under unfavourable conditions, such as those of low temperature and food concentration, one must again resort to speculation. Certainly, for the present investigation, within the temperature range investigated, decreasing temperature markedly increased the cell size (Page 126), with a concomitant tendency to form multinucleate amoebae (Page 34). Daniel and Chalkley (1932) reported that cell division in A. proteus is possible between 4°C and 35°C, with only 15% of the amoebae dividing at the lowest temperature. In addition, 25% of those cells cultured at less than 11°C showed nuclear division without cytoplasmic division. In view of the low growth and feeding rates found for A. proteus at 10°C, (Pages 123 and 90) and the very high generation times (Page 70), it is suggested that amoebae in the field may overwinter by reducing their energy consumption to a minimum, i.e. that required to cover metabolic losses.

In addition, cells may suppress cell division, opting for nuclear division when the cell attains a size such that it is outwith the control of a single nucleus. On the return to favourable conditions, the cells would be expected to divide to mononucleate forms. Although on occasion Amoeba (presumably multinucleate forms) were found to divide into three or four individuals throughout the generation time experiments at 10°C, (Appendix 8), the limited field data does not bear out this theory. No sudden increase in amoebae numbers was found at the onset of more favourable temperature in the summer months nor were there any cells of particularly large volume found for those months when temperatures were less than 10°C. However, amoebae cells do appear to be present throughout the year, as A. proteus was recorded in January when the mid-morning temperature was low at 2°C, a temperature outside that possible for cell division. There is obviously a need for a more detailed sampling programme to be undertaken if the fluctuations and life cycles of amoeboid populations are to be fully understood.

Papers dealing with the distribution of the microfauna of various habitats have highlighted the cosmopolitan occurrence of the large naked amoebae species, and the fact that they are never found in abundance. Cole (1955) studies the microbenthic fauna of the bottom deposits of two Minnesota lakes and found Amoeba and Pelomyxa palustris to be present in small numbers in the top sediments. Lackey (1938) sampled the open water of several freshwater lakes and reported A. proteus from

only 5% of the samples collected, while Bragg (1960), in a study of the protozoa of a freshwater lake in Oklahoma, recorded A. bigemma (syn. Mayorella) on one occasion, A. proteus 8 times and A. discoides 16 times over a 1-year period.

Ponds have often been considered as an optimum site for collected Amoeba proteus, however, Dickinson (1948) in an investigation of the microfauna of the ponds and ditches of Northern Florida, reported the only sarcodine species to be the testate amoeba Arcella and Difflugia. Taylor (1947), however, found the new species Amoeba kerri from the freshwater pools on the shore at Millport, Scotland. In addition, Hausman (1917) reported the presence of A. proteus in low numbers in clear small pools containing decomposing organic sediment. Amoeba proteus was found to be commonest in ponds and along stream margins by Lackey (1938) where it was present in 36% of the samples collected.

Amoeba taylorae, a species up to 500µm in length, was reported to be present in small numbers from around the water plants of a Scottish freshwater loch by Hayes (1955). Similarly, Graff (1927) while sampling the Rhizopoda from an area around a Montana Lake, found A. proteus to be rare with only a few individuals present amongst the pond weeds. Lastly, Fantham and Porter (1945) studies the microfauna of 93 samples of Canadian mosses, and although 108 species of Sarcodina were reported, 93 of these were testate amoebae. They concluded that the vast majority of Canadian mosses sheltered relatively few Amoebina.

It is apparent that Amoeba proteus and related naked amoebae species are never abundant in the field, but appear to form a characteristic low background level in many of the stable freshwater environments such as the sediments and vegetation of some freshwater lakes and pools in addition to Sphagnum bog areas. Those publications concerned with recording the numbers of protozoa in the natural situation have probably underestimated the naked amoebae as a consequence of the problems associated with detecting amoebae and extracting them from collected material.

8.1.8. Production estimate for the large naked carnivorous amoebae of a Sphagnum mass.

Only a tentative estimate of production for the large naked amoebae can be made in view of the small numbers of sampling sites investigated which have accentuated the variation between monthly samples.

Population production was calculated according to the formula of Galkorskaja as reported in Kajak (1967) and Heal (1971).

$$P = \frac{N_o + N_1}{2} \cdot \frac{1}{D} \cdot t$$

where:

P equals production, N_o and N_1 are the initial and final biomass values with regard to time t, the length of the period in days, while D is the period of doubling of an individual (generation time) in days. All the steps involved in the calculation have been included in Appendices 17 - 21.

On those sampling occasions where no naked amoebae were found, zero production for that month was recorded. Had a more detailed sampling programme been undertaken, amoebae would probably have been found for these periods, (previous discussion). It is suggested, however, that the production would have been negligible in any case for those months when the temperature was low, as the laboratory study for A. proteus has shown that net production efficiencies were substantially lower at 10°C compared with those at 15°C and 20°C. In other words, as the temperature was decreased a smaller proportion of the assimilated energy was channelled into growth. Further, the lower limit for reproduction in A. proteus was close to 8°C for the present study (Appendix 18).

The average standing crop of the ciliated and flagellated protozoa for each monthly sampling occasion was 0.14J ml^{-1} (Appendix 20) which approximately corresponds to the laboratory food concentration of 1000 Tetrahymena cells 500μl^{-1} (i.e. 0.12J ml^{-1}, Appendix 21). The mean temperature throughout the year was 8.9°C which is close to 10°C, the lowest temperature investigated for the laboratory situation. From the present study on A. proteus, the gross production efficiency was 13.78% for 10°C when cultured at 1000 Tetrahymena cells 500μl^{-1}. As the total annual production for the large naked amoebae was estimated at 1.93 x $10^{11}\mu m^3$ per $1000cm^3$ (Appendix 17) equivalent to 49.74kJ m^{-2} yr^{-1} (to a depth of 10cm), an annual consumption of 360.96kJ m^{-2} yr^{-1} was calculated. This represented a total consumption of 1.22 x $10^{14}\mu m^3$ of protoplasm or 5.96 x 10^{10} small protozoan cells ($<50\mu m$ in length).

The assimilation efficiency for A. proteus under the laboratory culture conditions of 1000 Tetrahymena cells $500\mu l^{-1}$ at $10^{o}C$ was 35.16%. Again, assuming that this value approximates to that for all the large naked amoebae, the total amount of material assimilated by the amoebae in the Sphagnum mass was 126.91kJ m^{-2} yr^{-1}.

An annual percentage energy budget covering the total large naked amoebae component of a Sphagnum stand can be proposed, where it is assumed that the average field conditions approximate the laboratory study undertaken for A. proteus at $10^{o}C$ when cultured at 1000 Tetrahymena cells per $500\mu l$.

C	=	P	+	R	+	E
100%		14%		21%		65%
		A	=	35%		

Comparative production estimates for the protozoa are few in the literature, making comparisons difficult. Heal (1967a) speculated that the annual production of the small soil amoebae, Acanthamoeba, was about 200g m^{-2} (equivalent to 3502kJ m^{-2} yr^{-1}, assuming 17.51J mg^{-1} dry protoplasm, Chapter 2). Schönborn (1977) estimated the production of the Testacea, obtaining a low value equivalent to 3.5kJ m^{-2}. In the same study, Schönborn also estimated the production of four species of loricate ciliates reporting a value of 0.03kJ m^{-2} yr^{-1}. Recently, Finlay (1978) published data for the total ciliate production from the benthos of a shallow (<5m) freshwater loch, where he estimated the production of three sites, of varying depths, to be 3450, 1490 and 403kJ m^{-2} yr^{-1}. Further, the production of the total benthic protozoa of a mesotrophic reservoir was estimated to be 20.9kJ m^{-2} by Sorokin (1972) for a six months period over the summer.

Publications detailing the annual production for the zoobenthos are equally variable. Mason (1977) reported the production of the invertebrate macrofauna of two small eutrophic lakes to range between 99 - 276kJ m^{-2} yr^{-1}, while the maximum production of the zoobenthos from nine Soviet lakes was 712kJ m^{-2} yr^{-1} (Winberg, 1972). A higher value of 2500kJ m^{-2} yr^{-1} was suggested by Morgan and McLuskey (1974) for the total zoobenthos of Loch Leven.

Certainly, the tentative annual production estimate of 49.74kJ proposed for the large naked amoebae, inhabiting a Sphagnum mass was at the lower end of the range for invertebrate production. It is however of sufficient magnitude to warrant consideration of the role of amoebae in the energy flow through aquatic ecosystems, especially in view of the fact that the annual consumption by amoebae in a Sphagnum bog is within the order of 360kJ m^{-2} yr^{-1}.

PART 4

General discussion.

General discussion.

As Phillipson (1966) pointed out, without unlimited numbers of research workers, a thermodynamic study of any living system must, of necessity, be restricted. The diversity of protozoa in the field (Picken, 1937; Faure-Fremiet, 1950: Webb, 1956) complicates the selection of a typical species to represent a trophic level, although in practice the choice is simplified by the fact that there are only certain species which can be successfully cultured in the laboratory. A technique for the controlled culture of the carnivorous amoeba, A. proteus, in soil extract media, when fed on the ciliate T. pyriformis was developed (Chapter 1), allowing a thorough research programme to be undertaken on such factors as dry weight, calorific value, rate of growth, consumption, reproduction and intensity of metabolism. The understanding of these functions clarified the energy flow through this species, and possibly large carnivorous naked amoebae in general. Andresen (1956) pointed out that the large freshwater amoeba C. chaos was very similar cytologically to A. proteus, although herbivorous species such as Pelomyxa palustris were quite different. Similarly, the testate amoebae must be considered separately as the production of a shell may constitute a considerable energy expenditure as is the case in some macro-invertebrates. Hagvar (1975) has shown that the formation of the pupal exuvium of the Coleoptera accounts for a substantial proportion of the net production. In addition, the respiratory requirements of the small naked amoebae have been shown to be quite different from those of A. proteus (Page 160), suggesting that the energetics of such species should also be considered separately.

The effects of both temperature and food concentration were found to influence all the parameters of the energy budget equations compiled for A. proteus, and it is likely that these variables are of importance in the natural environment. Wood (1956) stated that temperature was probably the most important variable affecting living cells, and that in general the rate of biological activity increases with increasing temperature within certain limits. Further, ciliate numbers are undoubtedly variable in the wild with concentrations as low as $2cm^{-2}$ (Bryant and Laybourn, 1972/73) and as high as $83,000cm^{-2}$ (Finlay, 1977) being reported for freshwater environments, a condition which must affect the energetics of predator species.

The most apparent difference between laboratory and natural situation is the variable nature of conditions in the field. Temperature fluctuates constantly thereby influencing the abundance of the food organism. In addition, the prey of most predators are not scattered randomly throughout the environment (Taylor, 1961) as is the case in laboratory culture systems. By culturing with only one food source, the predator is further removed from the field condition. Recently, Rubin and Lee (1976) suggested that food information can be processed by a predator (ciliate) to yield energetic advantages by selecting those species of food organism which advance the cell cycle fastest by 'recognising' the molecules it requires, thereby avoiding the synthesis of new products. Such a condition is impossible when only one prey species is available. Generalisations are inevitable in the laboratory construction of energy budgets, however, and are partly overcome by the detailed investigation of as many

environmental variables as is feasible. A. proteus, in the present study, was subjected to a wide range of food concentrations (125 - 4000 Tetrahymena $500\mu l^{-1}$) at 20°C, 15°C and 10°C.

A variable which may influence the energy budget for a given species is the food source selected. For the present study, Tetrahymena was used in view of the acceptable nature of this species, especially for the development of mass culture systems of Amoeba (Prescott, 1956). The food preferences of A. proteus have been reported as diverse and summarised by Sandon (1932). However, the results of the present consumption studies, in conjunction with the published data, tentatively suggest that the volume of protoplasm consumed per Amoeba over a generation may be relatively constant, regardless of prey species (Page[103]). In addition, the fact that the calorimetry results for Tetrahymena (Chapter 2) are close to those reported by Laybourn (1973) for Colpidium, make it probable that the energy content of the ciliated protozoa as a group is close to 20.00J mg^{-1} dry weight. These factors combine to make laboratory studies with a single prey species more meaningful for extrapolation to the field situation.

The rate of consumption increased markedly with increasing temperature over the range 10°C - 20°C in A. proteus (Figure 28), a consequence of the increased rate of digestion and locomotion at the higher temperatures. Conversly, digestion at the lowest temperature, 10°C, was observed to be slow and the capture rate reduced as the level of activity, notably with regard to the cells locomotion, was reduced. A similar pattern of increasing

food consumption with increasing temperature was reported by Mast and Fennell (1938) for the ingestion of chilomonads by A. proteus. Food concentration also influenced theenergy intake of Amoeba with the frequency of ingestion increasing with increasing food availability until a level at which the cell failed to cope with the volume of captured food. At the highest food levels the rate of consumption decreased, largely as a result of the physical contact of colliding Tetrahymena. Mast and Fennell (1938) pointed out that A. proteus does not feed when unattached, a condition which arose at the highest food levels in the present study.

There was a direct relationship between the energy consumed and the energy egested (Figure 51). Ryther (1954b) pointed out that Daphnia pulex fed on Chlamydomonas cells defecated green masses of undigested algae at high algal concentrations and suggested that the cells passed through so rapidly that the efficiency of digestion and subsequent assimilation was greatly reduced. A similar condition was found for A. proteus, as the maximum rate of growth was attained on a food concentration less than that promoting maximum consumption. The optimum Tetrahymena concentrations for consumption by Amoeba were approximately 6000, 4000 and 1500 cells $500\mu l^{-1}$ at $20^{o}C$, $15^{o}C$ and $10^{o}C$ respectively, while the maximum growth rates were attained at levels of approximately 2000, 1500 and 500 Tetrahymena $500\mu l^{-1}$, again at $20^{o}C$, $15^{o}C$ and $10^{o}C$. In addition, the rate of assimilation decreased markedly at the highest consumption levels investigated (Figure 52). In other words, for Amoeba cultured at the highest food concentrations, growth decreased as a greater proportion of the ingested energy was egested.

For the consumption, production and reproduction studies, the optimum rates were found on less food as the temperature was decreased from 20°C to 10°C. This situation is ecologically advantageous to Amoeba in the field where the number of food items decrease with decreasing temperature (Section 8.1.6.) The cell adjusted its consumption level to match the changing environmental conditions, and as a consquence, maintained its rate of production and reproduction at an optimum level for each temperature. These changes were reflected in the gross production efficiencies for A. proteus, where maxima were attained at consumption levels of 70µJ h^{-1}, 60µJ h^{-1} and 35µJ h^{-1} (Figure 73) at 20°C, 15°C and 10°C respectively, thus ensuring maximum production on less food at lower temperatures. Further, Amoeba has been shown to be able to withstandlower food levels with decreasing temperatures while still maintaining positive production. The minimum energy intake per hour at 20°C was 40µJ decreasing to 35µJ at 15°C and 30µJ at 10°C (Figure 73).

Respiration was measured by Cartesian diver microrespirometry. The techniques employed in past publications for the measurement of the oxygen uptake of protozoa are variable (Section 6.1.1.), although macrorespirometers of the Warburg type feature prominently in the literature. As many cells are required for this form of respirometer, the washing of individual protozoa is impossible, resulting in the inclusion of surface bacteria. The importance of removing adhering bacteria is due to their exceptionally high rates of respiration. Doetsch and Cook (1973) stated that active bacteria can consume as much as 500µl O_2 h^{-1} per mg dry weight. The equivalent weight of A. proteus cells would consume only 3.7µl O_2 h^{-1}. It is apparent that the

inclusion of bacteria in the respirometer would result in erroneous results. Cartesian diver microrespirometry had the advantage of measuring the oxygen uptake of a small number of cells (up to 6 Amoeba per diver), allowing the individual washing of cells (Page145) and thereby eliminating bacterial respiration.

The effect of culturing Amoeba under different food concentrations indirectly affected the rates of respiration by increasing the size of those cells cultured under high levels of Tetrahymena. Temperature also influenced the cell volume (a decrease from 20°C to 10°C increased the volume by 100%), and as such the total respiration per individual, with the overall oxygen consumption of cells at 10°C being comparable to those cultured at 20°C (Figure 47). The rates of oxygen consumption per unit volume decreased with decreasing temperature. At 20°C a value of 5.40×10^{-10} µl h^{-1} was obtained which decreased to 2.61×10^{-10} µl h^{-1} and $2,34 \times 10^{-10}$ µl h^{-1} for 15°C and 20°C. The high rate of respiration for 20°C was due to the degree of locomotion, resulting in a high degree of energy expenditure. A linear relationship between cell volume and oxygen uptake, as demonstrated by Brody (1945), Zeuthen (1953), Kleiber (1961) and others, was found for A. proteus with the b-regression coefficients increasing with temperature from 0.74 at 10°C to 1.16 at 20°C (Figure 48). An interspecific comparison of naked amoebae (Figure 49) gave a b-coefficient of 0.75 (20°C - 30°C), the same as the 'unicellular' line proposed by Hemmingsen (1960) when corrected to 20°C.

The importance of considering a range of temperatures applicable to field conditions in a laboratory study has been highlighted throughout the present study. In particular, temperatures at or approaching 10°C have been, in the past, neglected for protozoan energetics studies, excepting Laybourn (1973, 1976b) and Laybourn and Finlay (1976). Decreasing temperature was found to markedly reduce the rates of reproduction, production , consumption, assimilation and egestion, in addition to extending the generation times for A. proteus at 10°C, to a maximum of 2926 hours as opposed to 84 hours at 20°C (Page 70). As a result, the total consumption and respiration values per generation were high for 10°C, 12,889 to 92,931µJ respectively. By comparison, the maximum energy consumption for 20°C and 15°C was 17,294µJ while the maximum respiratory energy loss was only 970µJ (Table 22). Because of the exceptional respiratory losses incurred by Amoeba at 10°C, a consequence of their large volume (up to 2,472 x 10^3µm), the cost of production was high. A major proportion of the assimilated energy (up to 89%) was respired resulting in the low net production efficiencies found for Amoeba (11 - 49%) at 10°C, compared with the higher range of 65 - 82% at 20°C and 15°C.

The gross production efficiencies of A. proteus indicating the percentage of ingested material converted to consumer biomass, were again low for 10°C (4 - 29%), a consequence of the fact that up to 33% of the ingested energy was respired while 63% of the ingested energy was lost through egestion for amoebae cultured with 4000 Tetrahymena 500µl^{-1} (Table 22).

In ecology today there is a need for reference regressions as an aid to ecologists. Welch (1968) investigated the relationship between net production and assimilation efficiencies for a variety of aquatic invertebrates. The most obvious aspect of the relationship was that as the former increased the latter decreased. In general, the relationship held for A. proteus at 15°C and 20°C, but was not applicable for the data at 10°C (Figure 72) as the issue was confused by the high respiratory losses and resulting low net production efficiencies for this temperature. The present investigation has highlighted the need for more detailed studies to be undertaken on the energetics of protozoan species before relationships such as that of Welch, can be accepted with confidence.

Finlay (1978) has recently found evidence suggesting that the computed linear regressions relating production and respiration in homiotherms, poikilotherms and short lived poikilotherms (McNeil and Lawton, 1970) for various groups of metazoans, are not applicable to the ciliated protozoa. A new regression, with a slope of approximately 1.0 and an elevation considerably below those of the regressions for poikilotherms, was indicated. Such results further outline the need for additional ecological studies on the protozoa.

Detailed field studies on the naked amoebae have not been undertaken, probably due to the problems associated with their extraction and indentification. The limited sampling programme conducted for the sarcodines as part of the present study has indicated that although large carnivorous amoebae are seldom common, they do constitute a characteristic background level in

many stable freshwater environments. A tentative estimate of their annual production in a Sphagnum bog-pool, 49.74kJ m^{-2} yr^{-1} (to a depth of 10cm), was calculated from the results of both the laboratory and field data. Further, it was suggested that the impact of amoebae on the ciliate and flagellate population may be of the order of 370kJ m^{-2} yr^{-1}, while the energy flow (assimilation) through the population was 127kJ $m^{-2}yr^{-1}$.

The effective running of ecological systems depends upon the transfer of energy from organism to organism. The present investigation has combined the results of detailed laboratory experiments with a sampling programme and has served to elucidate the energetics of a previously neglected trophic level. The complexity of biotic relations within an ecosystem are such that interference of any one component is likely to upset the pattern of energy flow throughout the system. The value of species such as A. proteus, which are to be found only from stable environments, has recently been recognised by Mills (1976) who investigated the suitability of Amoeba as an indicator of water quality. It is hoped that the energetics study reported here may contribute to a fuller understanding of the natural aquatic environment and help to guard against any permanent imbalance which may arise through man's intervention.

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APPENDICES

Appendix 1.

0.5% Proteose-peptone for the mass culture of Tetrahymena pyriformis.

10g Proteose-peptone.

2.5g Yeast extract.

1 litre Glass distilled water.

1 litre Prescott's inorganic media.

Inorganic culture medium (after Prescott and Carrier 1964) for the mass culture of Amoeba proteus.

Stock A. - 10g $CaCl_2$

6g KCl

2g $MgSO_4.7H_2O$

10g NaCl

1 litre Glass distilled water

Stock B. - 2g $CaHPO_4$ (anhydrous, finely granular)

1 litre Glass distilled water

Stock B was evenly suspended before sampling. 1 ml of each stock solution was added to 1 litre of Glass distilled water. The pH of the final solution was adjusted to between 6.4 - 6.6 with KOH.

Appendix 2.

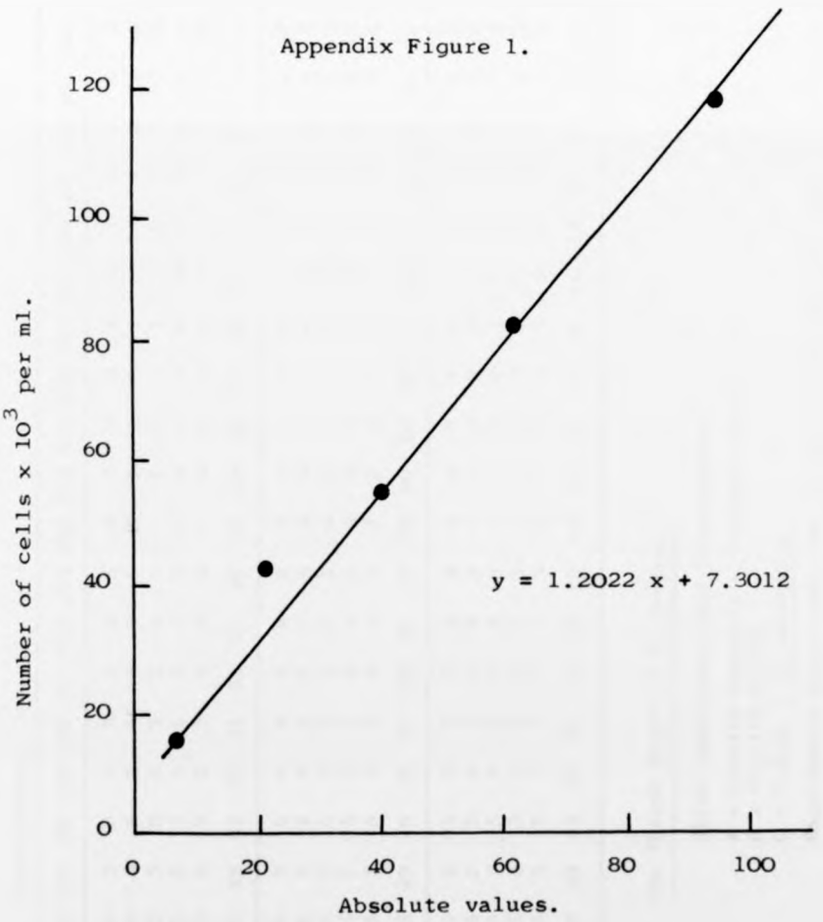
Calibration of Nephelometer Head.

To determine the concentration of Tetrahymena suspended in Prescott's inorganic media an EEL Nephelometer head with attached Galvanometer was calibrated using the inorganic medium as a blank in conjunction with a blue filter.

Protozoan counts were made with a Sedgewick-Rafter counting cell. For each point, n = 50.

The calibration graph is given below in Appendix Figure 1.

Appendix Figure 1.



Appendix 3.

Time (hours). ——→

	0	24	48	72	96	120	144	168	192	216	240	264	288	312	336	360	384	408	432	456	480	504	528	552	576	600	624	648	672	696	720
	5	5	5	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4	3	3	3	3	2	2	2	2	1	1	1	1	0
	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	4	2	2	0	0	0	0	0	0	0	0	0
A	5	5	5	5	5	5	3	2	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4	4	4	3	2	2	1	1	0	0	0	0
	5	5	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4	4	3	2	1	1	0	0	0	0	0	0	0	0	0
Total	25	25	25	25	25	25	23	22	21	21	21	20	19	19	18	18	18	18	15	14	10	9	4	4	3	3	1	1	1	1	0
	5	5	5	5	5	5	5	5	4	4	4	4	3	3	3	3	3	3	3	3	2	2	2	1	0	0	0	0	0	0	0
	5	5	5	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4	4	4	4	4	4	3	3	3	3	3	1	1	0
B	5	5	5	5	5	5	5	5	5	5	5	5	4	4	4	4	3	2	2	2	2	1	1	0	0	0	0	0	0	0	0
	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4	4	4	2	2	2	2	2	2	2	2	2	2	2	1	0	0
	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2	2	2	2	1	1	0	0	0	0	0	0
Total	25	25	25	25	25	25	25	25	23	23	23	23	20	20	20	20	17	16	13	13	12	11	11	7	6	5	5	5	2	1	0
	5	5	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4	4	3	3	3	3	3	2	2	1	0	0	0	0	0
	5	5	5	5	5	5	5	5	5	3	2	2	2	2	2	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
C	5	5	5	5	5	5	5	5	5	5	5	3	3	2	2	2	2	2	2	2	2	2	2	2	2	1	0	0	0	0	0
	5	5	5	5	5	5	5	5	5	5	5	5	5	4	3	3	3	3	3	3	3	3	2	2	0	0	0	0	0	0	0
	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	3	2	2	2	2	2	2	2	2	2	1	1
Total	25	25	25	25	25	25	25	25	25	23	22	19	19	17	16	16	16	16	14	11	10	9	9	6	6	4	2	2	2	1	1

The "Death Rate" of A. proteus.

Three conditions were investigated;

A - Sterile Prescott's inorganic media
B - Sterile S.E.M.
C - S.E.M. + A. aerogenes

Experiments were conducted in solid watch glasses initially containing 1 ml of media and 5 amoebae. Amoeba were transferred to fresh culture dishes every 24 hours to prevent bacterial contamination.

Appendix 4.

The Calibration of the Coulter Counter.

The procedure was as described by Strickland and Parsons (1972).

Latex spheres (12.45μ diameter) were suspended in electrolyte (0.9% NaOH solution). The initial count was adjusted so that the count was less than the number causing a 5% coincidence when the threshold mode switch was set on separate, the lower threshold at 5 and the upper threshold at 100. The amplification and aperture current settings were adjusted such that the pulses were 1.5 to 2.0 cm in height on the oscilloscope screen.

The threshold mode switch was locked and the upper threshold set at 50, with the lower threshold at 5. The lower threshold was increased by intervals of 5 units and the count recorded after each increase.

The mean counts were plotted as shown in Appendix Figure 2.

The volume equivalent was obtained from:

Volume of latex spheres = $25.2\mu m^3$

Threshold value for mode (t_L)

at sensitivity = 1
(i.e. amplification x aperture current)

The calibration factor x^1 at sensitivity y^1

$$= x^1 = \frac{y^1}{y} \cdot x$$

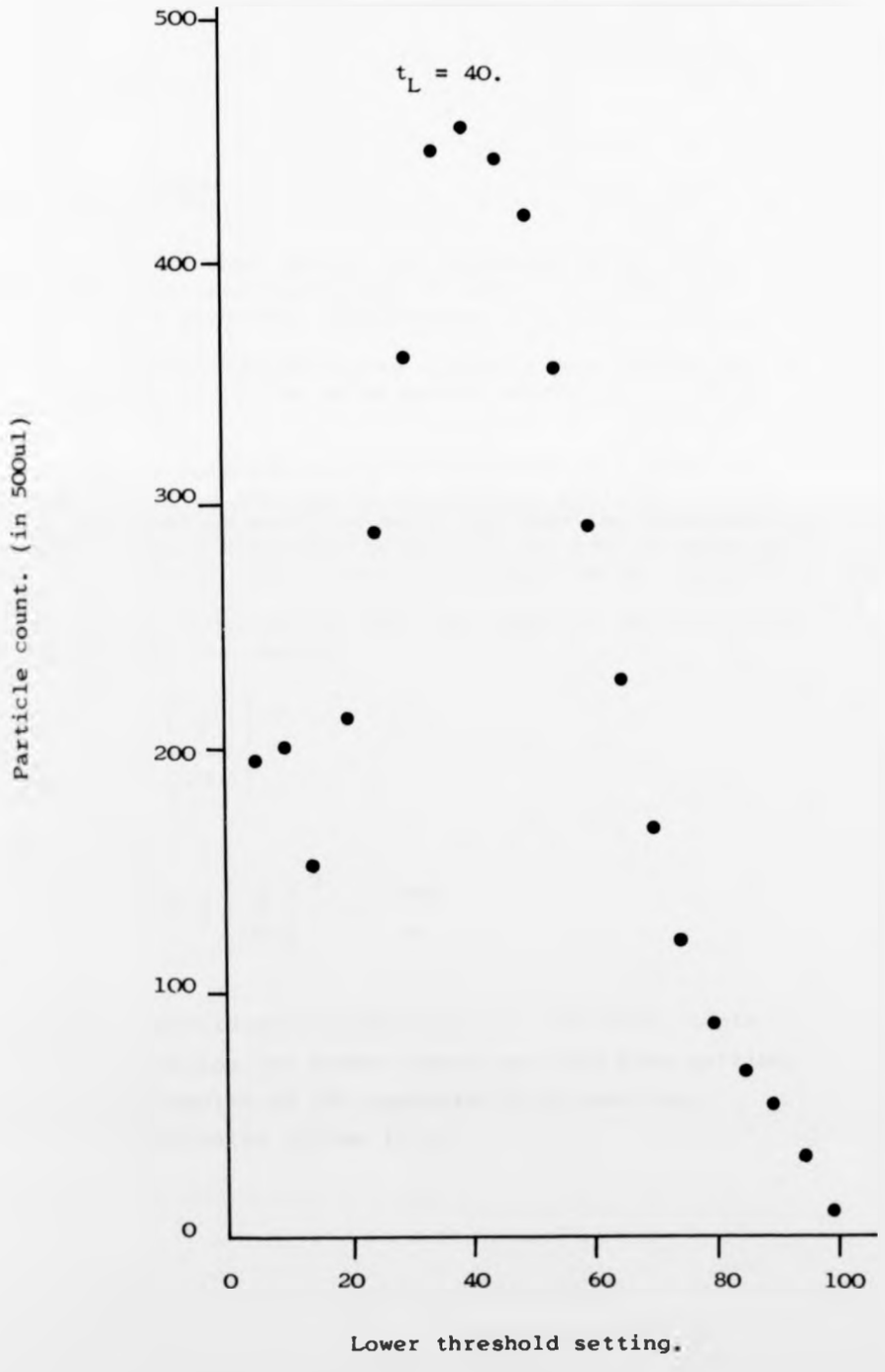
where:

x = the volume equivalent of 1 threshold division
y = sensitivity, 1

The settings used to count the Tetrahymena cells were:

sensitivity 16, lower threshold 2, upper threshold 100.

Appendix Figure 2.



Appendix 4 (contd.)

Coincidence Correction

The possibility that two or more particles were in the sensing zone at the same time leads to what is arbitrarily called primary and secondary coincidence.

1. Primary coincidence is the loss of counts resulting from only one pulse being generated for two or more particles.

2. Secondary coincidence is the counting of a particle whose size is the sum of two or more particle volumes which normally would be below the counting threshold. This is more difficult to correct for but is normally negligible if the concentration limit is not exceeded.

In all cases a correction (n") was added to the raw count to give the correct one where:

$$n'' = p\left[\frac{\bar{n}^1}{1000}\right]^2$$

and:

$$p = 2.5 \times \left[\frac{d}{100}\right]^3 \times \frac{500}{V}$$

n" = the coincidence correction, i.e the lost counts.

$\bar{n}^1$ = the average raw number count for that size setting.

d = the diameter of the aperture in micrometres.

V = the manometer volume in µl.

Appendix 5.

Change in Tetrahymena cell number after addition to S.E.M. (125 cells $500\mu l^{-1}$, $20^{o}C$.). Values = cell count in 5µl. Means ± 95% confidence limits.

watch glasses without Amoeba						watch glasses with 10 Amoeba					
Time (hours)						Time (hours)					
3h	6h	9h	12h	24h	30h	3h	6h	9h	12h	24h	30h
2	5	2	2	4	5	2	1	4	2	3	6
0	0	3	1	6	4	1	3	1	1	0	3
0	1	2	2	1	6	2	2	3	3	3	2
0	1	3	3	0	3	1	3	0	1	1	1
1	0	1	0	2	0	0	4	1	1	0	4
4	1	3	0	4	4	1	3	1	2	1	1
1	0	1	4	4	2	1	2	1	2	3	2
0	4	2	3	1	3	2	0	3	3	1	0
1	2	0	2	4	3	2	2	3	0	2	4
0	1	0	1	2	1	1	3	2	1	1	1
1	1	2	2	2	5	1	1	2	1	2	1
2	0	1	2	1	1	1	1	2	2	1	1
1	3	2	0	0	3	2	0	1	2	2	4
2	1	0	0	1	3	2	2	1	2	3	5
0	2	3	4	5	4	2	0	0	3	0	5
0	0	3	2	2	2	1	1	2	2	2	2
1	1	2	2	3	3	2	1	0	2	3	5
0	2	2	1	3	2	1	1	1	2	5	1
0	1	0	2	2	4	2	0	1	1	1	3
1	2	3	1	1	6	2	0	1	1	4	4
0	3	1	1	2	4	3	3	2	3	3	6
0	2	1	3	1	2	3	1	3	1	4	2
2	0	3	4	2	5	0	1	2	1	2	4
3	2	2	2	2	4	0	3	1	1	2	3
2	3	1	3	5	3	1	0	1	1	3	0
2	3	2	2	2	3	1	0	2	2	2	1
1	3	1	2	3	3	1	2	2	1	2	5
0	1	2	1	3	2	0	2	1	3	1	5
4	0	0	2	4	2	2	2	3	1	0	3
2	0	0	1	5	3	0	0	1	0	1	1
3	1	0	2	0	3	1	3	3	2	2	2
0	2	2	3	4	4	0	0	1	1	2	4
1	6	1	3	1	3	0	2	0	2	2	1
1	1	2	2	1	3	1	2	1	1	3	3
2	1	2	1	2	2	0	1	1	1	0	4
4	2	3	2	3	1	2	1	3	3	1	3
2	2	1	1	5	2	2	0	2	2	2	2
2	1	1	1	0	2	0	1	1	3	5	2
3	1	2	0	1	3	1	2	1	1	4	1
1	2	2	2	2	4	1	0	1	2	3	2
130 ± 37	145 ± 37	160 ± 31	180 ± 33	240 ± 49	305 ± 41	120 ± 25	143 ± 35	155 ± 29	165 ± 25	205 ± 41	272 ± 51

Appendix 5 (contd.)

Change in Tetrahymena cell number after addition to S.E.M. (1000 cells 500μl^{-1}, 20°C.). Values = cell count in 5μl. Means ± 95% confidence limits.

watch glasses without Amoeba						watch glasses with 25 Amoeba					
Time (hours)						Time (hours)					
3h	6h	9h	12h	24h	30h	3h	6h	9h	12h	24h	30h
11	9	19	10	17	17	10	18	16	21	12	16
7	14	14	14	19	20	14	11	13	19	14	18
10	9	17	16	13	31	13	13	14	11	18	14
8	20	17	13	26	17	12	13	14	21	13	14
13	14	15	18	13	20	8	13	11	9	14	19
7	20	10	21	16	28	17	9	13	11	15	15
9	11	10	19	10	17	16	9	10	9	13	16
6	12	9	24	18	27	11	12	12	10	16	15
8	13	13	18	16	25	15	12	9	13	14	14
10	16	11	19	19	26	9	10	13	15	12	21
6	14	10	13	15	22	8	15	12	13	19	15
9	16	10	11	10	21	8	12	16	13	16	18
9	13	17	21	10	25	10	11	12	10	14	22
8	14	16	11	24	13	9	15	11	9	20	16
10	19	15	16	24	17	11	11	12	14	13	17
8	16	12	18	12	21	8	13	16	20	12	17
10	11	13	19	21	10	6	14	14	15	14	15
9	9	16	18	16	11	18	15	14	13	20	19
12	6	15	15	29	23	9	14	11	13	19	14
8	8	17	15	19	16	13	11	16	19	12	20
11	10	13	15	11	20	14	12	13	15	20	22
10	11	10	13	19	25	13	15	12	12	22	15
10	15	18	11	23	11	8	11	14	13	21	16
8	14	9	10	14	18	10	13	14	17	15	15
9	10	16	16	22	30	10	17	11	16	15	20
5	17	13	14	14	18	12	11	9	20	19	21
11	13	12	21	20	20	10	8	10	19	17	15
10	9	8	17	20	12	12	10	11	18	16	21
6	9	16	14	15	15	10	15	12	16	18	17
9	11	13	12	12	14	13	16	12	19	14	21
9	19	17	13	13	25	9	10	13	10	13	17
10	14	18	11	19	17	10	8	11	11	16	19
8	13	10	20	24	11	9	9	11	17	18	15
11	13	12	19	10	24	6	11	10	16	20	16
12	10	13	15	11	18	11	11	12	10	15	14
11	12	14	14	19	19	18	12	14	11	13	17
10	15	18	16	13	20	9	10	13	22	16	20
11	15	16	18	16	15	10	13	12	15	18	19
11	19	20	13	16	19	11	12	11	15	17	17
7	13	15	15	10	22	11	10	12	9	19	17
917 ± 57	1315 ± 108	1392 ± 98	1565 ± 106	1670 ± 153	1950 ± 165	1102 ± 92	1212 ± 74	1240 ± 57	1447 ± 199	1605 ± 88	1722 ± 76

Appendix 5 (contd.)

Change in Tetrahymena cell number after addition to S.E.M.

(4000 cells $500\mu l^{-1}$, $20^{o}C$). Values = cell count in 5µl.
Means ± 95% confidence limits.
* diluted 1 : 4 before counts.

watch glasses without Amoeba						watch glasses with 30 Amoeba					
Time (hours)						Time (hours)					
3h	6h	9h*	12h*	24h*	30h*	3h	6h	9h*	12h*	24h*	30h*
38	46	40	52	64	76	39	34	56	58	44	84
33	43	64	40	68	76	35	54	48	72	88	92
35	48	48	44	64	84	45	36	40	44	76	68
44	48	44	64	56	92	46	42	56	72	92	100
37	57	36	76	60	68	31	50	72	64	64	60
41	47	52	68	56	64	41	50	56	64	48	84
52	43	48	60	68	68	49	38	40	52	52	92
29	58	44	56	64	84	44	52	56	52	60	80
43	49	60	64	64	72	40	56	64	64	56	72
46	51	64	60	60	88	38	58	72	56	72	64
31	38	52	56	60	88	46	38	48	56	76	64
38	45	52	64	56	80	42	40	60	64	72	68
44	39	48	68	48	84	50	44	36	48	96	76
51	43	44	60	68	64	43	52	64	48	64	92
36	62	64	48	60	76	48	55	52	44	88	72
32	46	44	48	68	84	38	54	56	64	68	60
37	51	48	44	72	88	40	51	64	56	68	88
44	56	44	52	60	92	34	48	44	56	56	76
30	63	56	56	56	88	32	44	64	60	48	68
51	38	68	56	56	80	46	57	58	56	60	92
44	52	52	60	60	80	46	50	45	56	76	76
47	39	56	56	52	72	48	41	72	68	80	72
40	41	64	52	60	64	41	51	56	44	80	60
38	54	60	60	68	64	39	42	48	72	84	76
41	41	56	48	64	68	51	39	60	68	76	100
42	51	56	48	56	68	43	45	40	72	76	92
38	50	52	56	52	64	35	44	44	56	60	80
37	42	48	60	60	76	38	51	72	56	68	72
29	47	48	52	64	84	34	56	68	52	88	76
38	48	40	60	64	80	49	50	60	64	80	104
46	54	48	64	68	76	46	50	40	68	72	72
44	55	56	60	60	88	40	48	52	68	80	96
48	40	48	48	60	88	41	49	68	56	52	76
36	60	60	60	64	84	38	45	68	52	80	88
46	52	40	56	64	72	44	47	64	64	72	72
43	47	52	56	64	76	50	46	56	56	84	72
29	50	48	56	56	92	39	50	64	48	88	96
41	44	44	56	68	80	38	49	64	56	64	88
45	41	40	52	60	68	44	54	60	48	80	76
33	46	56	64	72	76	41	52	64	60	72	80
3992	4797	5110	5650	6160	7790	4180	4780	5677	5835	7152	7940
± 196	± 202	± 245	± 225	± 170	± 274	± 161	± 188	± 315	± 253	± 406	± 366

Appendix 5 (contd.)

Change in Tetrahymena cell number after addition to S.E.M.

(125 cells 500μl^{-1}, 15°C). Values = cell count in 5μl.
Means ± 95% confidence limits.

watch glasses without Amoeba						watch glasses with 4 Amoeba					
Time (hours)						Time (hours)					
3h	6h	9h	12h	24h	30h	3h	6h	9h	12h	24h	30h
1	4	3	2	4	1	1	0	2	1	4	2
1	2	0	3	0	3	2	1	0	2	4	1
3	3	2	0	1	3	3	1	1	2	1	4
0	1	2	2	2	2	3	3	0	0	1	3
2	1	2	1	1	5	1	3	0	2	3	1
3	2	1	3	4	4	2	1	3	2	2	1
0	3	2	0	2	2	2	1	1	0	2	2
2	1	0	2	0	2	2	0	1	1	2	0
0	1	1	2	4	5	1	2	0	2	2	1
1	0	1	2	1	2	1	4	4	1	3	2
2	0	0	2	4	3	0	2	0	3	2	0
0	1	1	0	1	0	1	2	3	5	0	3
2	0	3	3	0	1	0	3	4	1	1	1
2	2	2	1	0	3	3	1	3	1	2	1
4	4	1	2	3	2	3	1	3	2	1	1
3	2	3	5	0	3	1	3	3	1	0	4
2	1	1	1	1	1	2	1	3	3	1	1
2	4	4	3	3	1	2	3	0	2	2	4
0	4	3	1	1	1	2	3	1	2	1	3
2	2	2	2	1	6	1	1	1	3	4	2
1	2	2	1	2	4	1	3	4	1	4	2
0	1	2	3	0	4	0	1	2	2	3	4
0	0	3	2	1	2	2	0	3	2	3	0
1	0	2	2	1	2	2	2	2	3	3	1
1	0	3	3	1	3	1	2	1	4	3	4
2	0	2	3	3	3	2	2	1	1	1	1
1	2	0	2	1	3	1	1	1	1	0	2
1	0	1	1	1	3	0	1	2	2	1	2
1	1	3	2	4	4	1	1	3	1	1	4
0	2	4	0	3	4	4	0	1	2	0	4
2	2	3	3	2	2	1	0	1	2	3	1
2	1	1	2	1	2	2	1	0	2	2	3
2	1	1	2	1	1	2	2	2	1	1	4
2	2	0	2	2	0	1	1	1	0	1	2
2	2	1	1	1	2	0	1	1	1	1	0
3	3	1	2	3	1	1	2	0	1	4	3
0	1	2	2	2	2	0	0	1	2	0	0
1	1	1	1	3	3	1	2	3	2	2	4
2	1	2	2	3	0	1	2	2	2	2	1
1	2	3	1	1	3	2	1	0	1	1	3
142 ± 32	155 ± 37	177 ± 34	185 ± 32	172 ± 40	245 ± 43	145 ± 30	151 ± 32	160 ± 40	172 ± 31	185 ± 38	205 ± 43

Appendix 5 (contd.)

Change in Tetrahymena cell number after addition to S.E.M.

(1000 cells 500μl^{-1}, 15°C). Values = cell count in 5μl.
Means ± 95% confidence limits.

watch glasses without Amoeba						watch glasses with 8 Amoeba					
Time (hours)						Time (hours)					
3h	6h	9h	12h	24h	30h	3h	6h	9h	12h	24h	30h
10	13	13	14	22	28	6	8	16	13	18	29
14	8	13	14	19	26	8	11	16	11	19	27
8	9	15	18	25	26	14	16	15	14	24	28
9	10	10	17	21	25	6	14	11	23	20	25
9	11	12	16	17	28	13	15	15	20	19	16
8	10	17	16	22	23	7	13	12	15	15	23
14	15	14	17	20	16	8	12	12	16	19	23
9	14	11	13	16	17	9	7	10	18	17	29
8	15	10	18	16	25	6	14	10	12	19	14
10	18	10	14	19	16	9	8	14	18	19	21
7	11	11	12	17	28	9	14	17	19	18	26
13	17	13	13	23	26	13	11	13	18	13	27
9	11	10	16	18	23	9	12	13	16	21	19
14	17	10	19	11	24	13	14	13	17	10	16
11	15	14	21	12	29	14	13	10	15	18	23
15	12	14	18	15	23	9	13	9	16	23	27
7	12	12	11	14	14	10	15	15	14	22	20
8	11	16	16	18	24	15	11	19	11	19	24
8	13	19	20	18	18	7	11	15	23	18	29
9	12	13	15	11	28	9	10	18	17	19	18
15	9	17	18	15	19	8	9	11	16	22	27
15	9	13	18	22	21	10	9	17	18	18	28
7	12	17	19	12	20	8	9	9	12	17	27
13	12	9	11	13	24	7	8	13	15	16	26
8	13	14	10	24	28	9	10	19	15	20	15
11	11	13	13	19	26	14	7	16	22	22	23
14	13	13	15	17	15	14	11	13	16	21	26
12	8	10	17	19	23	13	10	14	14	18	21
11	14	9	16	20	18	6	15	11	22	19	16
10	10	11	17	15	24	13	17	16	18	14	15
11	9	11	18	14	23	7	8	19	23	18	25
8	11	16	14	18	28	11	9	9	22	13	14
14	17	16	13	16	16	10	12	11	19	18	29
9	11	19	19	16	17	8	13	17	17	19	19
7	11	13	21	15	23	8	13	13	14	22	28
15	13	13	14	18	24	11	10	15	12	21	25
11	11	19	15	20	28	11	14	18	18	19	23
10	9	10	17	22	19	10	12	13	15	15	25
7	8	12	18	19	20	9	7	15	12	11	20
9	12	17	14	16	26	10	11	12	13	20	19
1042 ± 83	1192 ± 80	1322 ± 88	1533 ± 105	1760 ± 109	2272 ± 134	977 ± 81	1140 ± 82	1385 ± 90	1647 ± 106	1832 ± 96	2287 ± 147

Appendix 5 (contd.)

Change in Tetrahymena cell number after addition to S.E.M.

(4000 cells 500μl^{-1}, 15°C). Values = cell count in 5µl.
Means ± 95% confidence limits.
* diluted 1 : 4 before counts.

watch glasses without Amoeba						watch glasses with 10 Amoeba					
Time (hours)						Time (hours)					
3h	6h*	9h*	12h*	24h*	30h*	3h*	6h*	9h*	12h*	24h*	30h*
48	40	40	44	56	88	48	52	44	60	84	112
52	44	60	60	52	108	52	56	64	44	80	84
52	52	56	68	92	100	52	36	56	60	76	80
36	32	84	64	88	104	40	44	44	72	64	88
44	36	80	52	96	92	28	52	40	56	40	92
32	40	52	64	56	84	44	56	36	44	64	100
40	40	48	68	60	108	48	32	48	40	64	72
32	48	36	48	64	96	28	36	36	64	84	76
40	72	40	72	52	108	44	32	32	72	64	100
48	60	44	44	56	56	28	40	52	76	76	104
40	52	36	40	72	92	32	56	48	56	48	96
52	32	68	64	68	88	52	68	48	48	56	68
44	32	68	44	64	84	48	56	48	56	88	104
28	36	48	80	56	92	28	48	52	72	64	88
28	48	72	72	80	68	40	44	36	52	68	76
28	72	36	40	84	64	32	52	40	56	80	88
36	32	48	68	60	84	48	60	76	60	72	104
40	32	64	60	64	92	56	32	64	48	72	104
40	40	60	64	96	84	48	52	64	80	84	100
28	40	56	44	52	100	32	56	48	48	68	112
52	40	32	68	68	100	32	40	40	60	80	92
48	56	64	44	76	108	36	48	76	44	72	96
52	40	64	56	64	92	32	44	48	76	76	68
40	64	36	76	68	92	32	72	68	56	64	72
48	36	48	60	72	88	28	72	48	72	56	96
48	68	60	44	52	76	36	40	64	60	76	72
36	56	56	72	44	108	36	48	64	80	40	104
52	68	40	44	76	104	44	44	44	72	44	96
24	44	68	68	72	76	36	48	68	64	72	100
44	40	56	72	88	72	52	52	60	56	52	96
24	44	60	48	64	88	52	48	40	48	72	92
40	52	36	44	76	76	48	68	52	48	72	84
40	44	64	68	48	92	48	56	44	76	76	64
44	44	40	76	80	108	52	60	48	56	52	112
48	40	64	36	60	92	32	36	64	48	68	84
52	48	64	68	56	100	32	36	48	48	64	116
36	52	68	64	72	64	36	52	44	60	84	100
52	56	44	44	52	112	52	36	64	64	68	100
48	44	40	52	68	104	48	44	52	60	76	96
32	48	36	56	60	84	44	48	76	60	44	84
4140 ± 259	4660 ± 348	5323 ± 425	5800 ± 384	6710 ± 416	9070 ± 432	4090 ± 277	4880 ± 331	5220 ± 369	5930 ± 339	6760 ± 396	9180 ± 417

Appendix 5 (contd.)

Change in Tetrahymena cell number after addition to S.E.M. (125 cells 500µl^{-1}, 10°C). Values = cell count in 5µl. Means ± 95% confidence limits.

watch glasses without Amoeba						watch glasses with 2 Amoeba					
Time (hours)						Time (hours)					
3h	6h	9h	12h	24h	30h	3h	6h	9h	12h	24h	30h
2	1	2	0	2	2	1	0	0	1	1	1
1	1	2	0	3	5	1	0	0	2	2	1
0	0	1	1	0	3	0	2	2	1	2	1
1	0	1	2	2	1	0	3	1	0	0	2
3	1	1	1	1	2	1	0	2	2	2	3
0	2	1	0	3	3	3	0	2	3	2	2
0	1	0	0	0	1	0	1	2	2	0	2
0	1	1	0	2	0	0	1	0	2	1	1
3	0	3	2	2	1	1	1	5	2	2	1
0	1	0	2	2	1	1	2	1	0	1	1
1	0	1	3	2	2	2	3	1	0	3	3
2	0	1	3	0	2	1	1	0	1	5	2
1	3	1	1	3	2	1	0	3	3	1	2
1	2	1	2	1	1	0	1	2	1	1	2
1	0	2	1	2	3	3	2	0	1	2	3
0	1	1	0	5	2	2	0	1	2	1	2
1	2	0	4	1	2	2	0	2	0	3	2
1	1	0	0	3	2	1	1	0	3	2	2
1	2	0	0	1	3	1	1	0	3	2	3
1	0	2	2	2	3	0	0	2	2	3	2
1	2	2	0	1	1	0	2	1	1	1	1
3	1	2	0	3	2	0	0	1	0	2	0
1	0	0	2	2	2	1	1	0	0	2	1
1	2	2	3	2	2	1	1	2	4	3	1
0	1	0	2	3	3	1	0	2	0	4	2
1	1	2	1	3	1	1	2	2	3	1	2
1	0	0	2	2	1	2	1	1	0	1	2
1	2	1	0	1	0	1	1	1	0	2	2
0	1	1	1	2	1	1	1	1	3	1	3
1	2	2	3	0	3	1	1	1	3	2	2
0	1	1	0	3	3	0	1	0	1	2	2
2	1	2	3	2	3	1	0	0	1	2	1
1	1	3	2	2	3	1	1	0	0	1	3
1	1	2	0	2	2	0	2	1	2	0	1
0	1	2	1	1	2	0	0	2	2	1	2
0	1	1	0	2	2	0	0	0	1	1	1
3	3	1	2	2	1	1	2	1	2	2	3
2	1	1	3	1	2	0	2	1	1	2	2
1	0	2	0	2	1	1	1	3	3	2	3
1	1	3	4	1	2	1	2	1	1	1	2
102 ± 27	105 ± 25	127 ± 27	132 ± 39	185 ± 32	195 ± 30	87 ± 24	100 ± 27	117 ± 33	147 ± 36	172 ± 31	185 ± 24

Appendix 5 (contd.)

Change in Tetrahymena cell number after addition to S.E.M.

(1000 cells 500µl^{-1}, 10°C). Values = cell count in 5µl.
Means ± 95% confidence limits.

watch glasses without Amoeba Time (hours)						watch glasses with 4 Amoeba Time (hours)					
3h	6h	9h	12h	24h	30h	3h	6h	9h	12h	24h	30h
13	9	12	15	11	28	10	11	13	15	21	16
6	8	9	13	21	16	11	8	11	11	12	18
11	10	13	9	12	14	7	5	12	16	14	25
10	10	11	12	14	15	12	11	8	12	16	22
6	8	17	12	17	16	9	8	12	9	16	14
8	11	12	14	23	16	7	13	8	16	13	20
14	12	11	14	20	18	7	8	9	11	14	10
9	7	9	16	16	25	7	13	8	14	19	20
10	11	7	10	16	15	9	12	12	13	20	15
7	10	12	9	13	13	10	11	12	10	15	17
11	9	13	16	13	11	12	10	9	9	16	25
10	11	12	11	14	24	13	10	14	10	14	27
9	10	13	15	14	10	10	8	11	15	13	18
7	9	8	12	16	22	12	8	14	17	17	13
7	13	9	15	15	24	6	10	12	16	14	10
8	7	12	10	14	16	11	7	9	13	13	22
9	12	10	10	16	22	8	11	12	15	15	19
11	12	8	13	17	14	13	12	13	16	17	18
7	10	12	14	18	20	9	10	14	9	16	20
12	9	12	17	18	22	6	9	12	13	17	10
10	9	11	16	18	10	5	11	8	10	18	14
11	7	11	15	19	20	11	10	12	18	18	12
6	11	7	9	15	17	11	9	13	15	19	24
7	13	9	12	20	19	10	11	9	17	15	11
10	14	14	16	15	17	13	13	7	11	16	13
7	9	13	13	15	15	7	10	8	10	20	25
7	7	12	15	16	28	9	8	13	15	11	16
13	6	14	14	19	16	11	7	12	15	21	16
10	11	12	15	11	17	13	8	8	13	17	14
11	10	13	11	15	18	8	11	10	12	14	27
12	10	9	10	14	12	12	8	12	9	22	18
9	12	8	18	15	10	13	10	12	16	13	17
11	13	11	17	15	21	14	10	11	10	19	22
7	8	12	14	13	19	12	11	14	13	18	21
8	9	10	16	17	15	9	9	13	14	16	19
15	12	13	15	19	18	10	10	11	17	16	15
8	13	14	11	13	20	7	11	7	11	13	18
10	10	9	13	14	10	6	13	13	14	16	17
10	7	7	15	16	14	10	8	9	12	15	19
11	12	10	9	17	12	9	11	12	11	17	17
945 ± 70	1002 ± 63	1102 ± 70	1327 ± 79	1585 ± 83	1722 ± 147	972 ± 74	985 ± 58	1097 ± 66	1307 ± 82	1615 ± 82	1785 ± 142

Appendix 5 (contd.)

Change in Tetrahymena cell number after addition to S.E.M. (4000 cells $500\mu l^{-1}$, $10^{o}C$). Values = cell count in 5µl. Means ± 95% confidence limits. * diluted 1 : 4 before counts.

watch glasses without Amoeba						watch glasses with 6 Amoeba					
3h*	6h*	9h*	12h*	24h*	30h*	3h*	6h*	9h*	12h*	24h*	30h*
56	40	28	56	52	40	56	40	36	52	92	60
52	32	36	64	56	56	48	24	40	44	60	104
52	76	28	40	76	68	36	36	58	56	64	56
32	48	36	52	72	76	56	48	24	56	76	40
24	32	32	48	52	44	52	40	52	56	56	68
48	52	32	60	56	84	32	36	40	48	52	48
36	48	48	48	56	88	28	44	44	56	60	52
32	36	44	48	72	40	36	28	40	48	56	52
36	52	32	56	56	96	36	32	48	52	52	88
52	36	40	40	76	68	32	44	44	52	52	36
32	28	36	52	68	52	20	68	48	56	56	44
20	32	52	32	44	60	40	36	36	64	76	76
20	60	48	36	60	64	42	28	52	56	72	40
28	36	60	60	60	60	32	52	40	40	56	104
36	28	32	48	76	80	56	24	28	48	56	72
52	46	48	52	52	48	28	36	28	60	64	84
36	32	52	52	60	52	40	36	52	52	60	48
36	48	48	64	56	56	48	40	36	48	76	72
36	44	32	64	52	48	48	40	40	48	72	52
36	40	48	60	52	60	24	44	32	52	52	96
28	32	52	60	44	72	44	48	28	36	44	68
32	32	32	52	60	76	24	48	48	32	48	44
56	52	36	56	88	72	36	48	52	52	76	68
32	48	52	44	80	44	44	36	60	60	68	64
32	40	44	48	64	76	52	28	60	40	60	60
44	52	36	52	52	52	48	36	56	52	60	80
40	36	28	40	44	56	32	52	44	64	56	64
32	32	36	48	56	92	40	40	52	60	76	48
40	48	44	60	68	96	36	32	56	64	52	48
40	60	40	48	56	44	52	56	28	48	44	40
40	48	48	52	56	68	52	32	32	60	52	68
36	24	40	64	76	48	24	44	36	64	60	60
44	52	52	64	60	52	32	40	36	44	60	64
36	24	60	56	44	60	40	44	44	48	60	52
52	36	32	52	52	80	36	28	36	52	52	56
36	52	48	60	56	68	36	52	28	64	56	36
44	36	40	36	52	60	56	40	40	56	48	80
40	52	48	56	64	48	48	36	44	44	44	76
36	52	60	44	60	72	40	52	36	60	56	40
44	44	40	68	72	68	48	28	40	52	60	60
3840 ± 282	4245 ± 344	4200 ± 285	5230 ± 273	6020 ± 332	6360 ± 477	4025 ± 310	3990 ± 294	4185 ± 302	5240 ± 243	5980 ± 326	6170 ± 553

Appendix 6.

Tetrahymena cell volumes after addition to S.E.M.

Cell conc. 500µl⁻	Temp. °C.	Time (h).	M.C.V. µm³ n = 50	standard deviation	overall M.C.V. ± S.D. (0 - 24h)
125	20	0	27826	8749	20163 ± 6890
		2	26125	8228	
		5	21372	7016	
		8	21905	7159	
		11	11074	2515	
		24	12679	4756	
		30	19575	6493	
1000	20	0	28302	7853	16085 ± 7843
		2	22472	7495	
		5	13863	4448	
		8	12593	3855	
		11	6697	2236	
		24	12585	4256	
		30	17549	9239	
4000	20	0	19438	6263	14322 ± 3523
		2	17077	5672	
		5	14041	5611	
		8	13566	4029	
		11	9437	4074	
		24	12373	4054	
		30	15570	6032	
Overall mean (20°C) = 16857 ± 2996					
125	15	0	22732	6401	16051 ± 3694
		2	17965	6456	
		5	14771	6025	
		8	13094	4664	
		11	14039	4377	
		24	13703	5087	
		30	13855	4862	
1000	15	0	24579	6739	20868 ± 3849
		2	24999	6367	
		5	17633	4466	
		8	15611	4358	
		11	22764	7630	
		24	19624	4722	
		30	18863	5003	
4000	15	0	30312	7576	19091 ± 5826
		2	18991	4488	
		5	15161	4398	
		8	14815	4078	
		11	19343	6464	
		24	15926	5168	
		30	17189	5177	
Overall mean (15°C) = 18670 ± 2436					

Appendix 6 (contd.)

Tetraymena cell volumes after addition to S.E.M.

Cell conc. 500µl⁻	Temp. °C.	Time (h).	M.C.V. μm^3 n = 50	standard deviation	overall M.C.V. ± S.D. (0 - 24h).
125	10	o	24669	6806	16403 ± 5728
		2	20252	5111	
		5	18624	5109	
		8	13557	3605	
		11	11621	3636	
		24	9694	3080	
		30	11513	2937	
1000	10	0	29753	8520	26219 ± 3613
		2	28780	8628	
		5	26870	5782	
		8	27735	8186	
		11	24249	5451	
		24	19925	5408	
		30	16791	4454	
4000	10	0	30721	8722	26301 ± 3443
		2	29416	8197	
		5	25553	7085	
		8	25197	7144	
		11	25919	6590	
		24	21000	5615	
		30	16169	4723	
Overall mean 10°C = 22974 ± 5691					

Mean cell volume at time Oh (10°C, 15°C and 20°C) = 26481 ± 3857μm^3

Mean cell volume over 24 hour experimental period = 19500 ± 4385μm^3 (10°C, 15°C and 20°C).

Appendix 7.

Calibration of the Micro-Bomb Calorimeter with Benzoic acid pellets (1mg = 26.455J).

Wire correction (W):

from the calibration, 1 division = 2.88J (equivalent to 0.005mV).

(W) from Figure 9 = 0.0106mV.

∴ Wire correction in joules = 6.10J.

Sample Number	Wt. Benzoic acid pellet (mg)	calories liberated (J)	Millivolts recorded on Potentiometer after correcting for cooling.	Potentiometer scale reading, no. divisions.	Calorific equiv. of 1 division (J)
1	3.89	102.91	0.178	35.52	2.90
2	1.91	50.53	0.106	21.16	2.39
3	2.11	55.82	0.120	23.95	2.33
4	4.47	118.25	0.205	41.10	2.88
5	4.26	112.70	0.194	38.73	2.91
6	3.86	102.12	0.165	32.95	3.10
7	1.01	26.72	0.047	9.40	2.84
8	4.30	113.76	0.185	37.10	3.07
9	2.03	53.70	0.095	18.98	2.83
10	3.55	93.91	0.147	29.45	3.19
11	3.85	101.85	0.173	34.70	2.94
12	3.15	83.33	0.148	29.58	2.82
13	1.76	46.56	0.081	16.12	2.89
14	5.42	143.39	0.249	49.74	2.88
15	2.40	63.49	0.108	21.52	2.95
16	4.55	120.37	0.200	39.95	3.01
17	3.28	86.77	0.144	28.80	3.01
18	2.04	53.97	0.093	18.65	2.89
					Mean = 2.88
					S.D. = 0.21

Appendix 8.

Raw data for the calculation of the generation times of *A. proteus*.

The increasing number of cells with time (h) in each of 40 replicate watch glasses at different levels of *Tetrahymena* food concentration and temperature.

Appendix 8 (contd.)

Food concentration 125.500µl^{-1} (20°C).

Time(h). →	0	12	24	36	48	60	72	84	96	108	120	144	168	180	192	204	216	228	240	252	264
1	1	1	1	1	2	3	3	2	3	3	3	3	3	3	3	3	4	4	4	4	4
2	1	1	1	2	2	2	2	3	3	3	3	3	5	7	9	9	9	12	12	12	14
3	1	1	1	1	2	2	2	4	4	4	4	5	6	5	5	5	5	5	4	4	5
4	1	1	1	2	2	2	2	3	4	4	2	2	3	3	3	3	4	4	4	4	4
5	1	1	1	2	2	2	2	3	3	3	4	5	5	6	6	6	7	8	11	12	12
6	1	1	1	1	2	2	2	4	3	3	4	4	6	7	11	11	11	11	10	11	11
7	1	1	1	1	2	2	2	2	3	3	3	3	4	4	6	6	6	6	8	9	9
8	1	1	1	1	2	2	2	2	2	2	3	3	4	5	5	5	6	6	8	9	11
9	1	1	1	2	2	2	2	2	3	4	5	5	5	6	9	9	8	8	10	11	13
10	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3
11	1	1	1	1	2	2	2	3	3	3	4	5	6	7	7	7	7	7	10	11	11
12	1	1	1	2	2	2	1	1	1	1	2	2	2	4	4	4	5	5	7	7	9
13	1	1	1	2	2	2	2	4	4	6	8	9	13	13	14	14	15	15	16	16	16
14	1	1	1	2	3	3	3	2	3	3	3	4	4	6	6	6	6	9	9	10	11
15	1	1	1	1	2	2	2	4	3	4	6	6	8	9	10	12	13	15	15	17	18
16	1	1	1	1	2	2	2	3	3	3	3	4	4	4	3	4	4	4	6	7	7
17	1	1	1	1	2	2	2	2	2	2	4	3	3	4	5	5	5	8	14	14	14
18	1	1	1	1	1	1	1	1	2	2	2	4	5	5	5	5	6	6	7	8	9
19	1	1	1	1	2	2	3	3	3	3	3	4	5	6	7	7	7	7	10	10	10
20	1	1	1	1	2	2	2	4	4	4	5	6	7	7	9	8	8	5	5	6	8
21	1	1	1	2	2	2	2	2	2	2	4	5	7	7	10	11	11	12	13	13	14
22	1	1	1	1	2	2	2	2	2	3	4	4	5	6	6	6	6	8	11	13	15
23	1	1	1	1	2	2	2	2	2	2	4	6	7	8	10	10	10	10	10	11	11
24	1	1	1	1	2	2	3	4	4	4	4	4	4	5	5	6	7	7	8	10	11
25	1	1	1	2	2	2	2	1	1	1	2	2	3	3	4	4	4	3	1	0	0
26	1	1	1	2	2	2	2	2	3	4	4	5	8	10	11	12	12	12	14	15	15
27	1	1	1	1	2	2	2	3	2	2	2	3	4	4	5	5	5	5	7	8	10
28	1	1	1	1	2	2	2	3	4	4	5	5	5	6	6	7	8	8	8	9	9
29	1	1	1	1	2	2	2	2	4	4	5	6	9	9	11	11	11	10	12	12	12
30	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	4	6	6	6	8	10
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	4	4	4	4
32	1	1	1	1	1	1	1	2	2	2	3	4	5	6	6	6	7	7	8	9	10
33	1	1	1	1	2	2	2	2	3	3	3	4	4	5	6	6	7	10	12	13	15
34	1	1	1	2	2	2	2	2	3	3	4	3	3	3	5	6	6	6	7	7	9
35	1	1	1	1	2	2	2	3	4	5	7	7	7	8	7	7	7	7	8	8	8
36	1	1	1	1	2	2	2	2	3	3	3	4	6	8	9	9	9	9	11	12	13
37	1	1	1	1	2	2	2	3	4	4	8	9	11	11	14	14	14	15	15	17	17
38	1	1	1	1	2	2	2	1	1	1	1	1	2	4	4	4	4	4	4	5	5
39	1	1	1	1	2	1	2	1	1	1	1	1	1	2	2	2	2	2	3	2	2
40	1	1	1	1	2	2	2	2	4	4	4	6	8	7	7	7	7	8	11	11	12
Tot.	40	40	40	52	78	78	79	96	110	117	144	158	203	229	262	270	283	300	346	372	401

Appendix 8 (contd.)

Food concentration 250.500μl^{-1} (20°C).

Time (h). →	0	12	24	36	48	60	72	84	96	108	120	144	168	180	192	204	216	228	240	252	264
1	1	1	1	1	2	2	2	2	3	3	3	3	3	3	5	5	5	6	11	12	12
2	1	1	1	1	2	2	2	2	2	2	2	4	4	4	4	7	7	7	8	8	8
3	1	1	2	2	2	3	4	4	4	6	6	7	7	9	10	14	14	13	15	16	19
4	1	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2
6	1	1	1	1	1	1	1	1	1	1	1	2	2	4	4	4	6	8	8	9	9
7	1	1	1	1	1	2	2	2	3	3	3	4	5	6	8	8	9	9	12	14	15
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	2	2	2	2	3	4	6	8	8	10	14	14	16	17
10	1	2	2	2	2	4	4	4	4	7	7	10	12	15	16	21	24	27	28	33	37
11	1	1	1	2	3	3	5	6	9	11	11	12	14	15	17	19	24	28	28	31	33
12	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3	3
13	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	3	3	3	3	3
14	1	1	2	2	2	2	2	2	3	3	3	5	5	5	6	7	8	8	8	10	10
15	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	1	1	1	1	2	2	2	2	4	4	4	4	6	7	8	10	10	8	13	15	15
17	1	1	2	2	2	2	3	3	3	4	4	5	8	11	13	18	18	23	25	28	33
18	1	1	2	2	2	2	2	2	2	2	3	3	3	2	2	3	3	4	4	6	6
19	1	1	1	1	1	1	1	1	4	4	4	5	6	7	7	10	10	12	12	13	15
20	1	1	1	1	1	2	2	2	2	2	2	3	3	3	4	5	5	5	5	6	8
21	1	1	1	1	1	2	1	1	1	1	1	1	2	2	2	4	4	7	9	10	11
22	1	1	2	2	2	2	4	4	4	5	6	7	7	8	13	12	15	16	20	23	24
23	1	1	1	1	1	1	2	2	2	2	2	5	6	7	9	10	10	12	12	13	13
24	1	1	1	1	1	1	2	2	2	3	3	3	4	4	4	4	4	7	7	7	9
25	1	1	1	2	2	2	2	2	3	3	3	3	3	3	4	5	5	5	7	8	10
26	1	1	1	1	2	2	2	2	2	1	1	0	0	0	0	0	0	0	0	0	0
27	1	1	1	1	1	1	1	1	1	2	2	3	4	4	4	5	6	6	8	8	9
28	1	1	1	2	2	2	2	2	2	2	2	3	3	3	4	4	5	6	6	9	10
29	1	1	2	2	2	2	3	4	4	4	4	4	4	4	4	7	9	6	9	10	12
30	1	1	1	1	1	1	1	1	1	1	2	2	2	4	4	4	4	4	4	4	4
31	1	1	1	1	1	2	2	3	4	4	5	9	9	16	16	15	15	19	21	21	23
32	1	1	1	1	1	1	2	2	3	3	4	4	4	5	5	8	9	11	12	14	14
33	1	1	1	1	2	2	2	3	3	3	5	6	6	7	8	8	8	11	12	13	15
34	1	1	1	1	2	2	2	2	4	3	3	5	5	6	8	8	10	11	13	14	14
35	1	1	1	1	1	1	1	2	2	2	2	2	1	1	2	2	2	2	2	2	2
36	1	1	1	1	2	2	2	2	2	2	2	2	2	2	4	4	5	5	7	8	8
37	1	1	2	2	3	3	5	5	5	5	6	7	8	9	10	10	15	17	20	22	22
38	1	1	2	2	2	2	2	2	2	2	1	1	1	1	2	2	2	2	4	4	4
39	1	1	1	1	2	2	4	4	4	6	6	7	8	10	12	11	14	14	15	17	18
40	1	1	1	1	2	2	3	3	3	3	3	7	9	9	13	18	18	18	24	25	26
Tot.	40	41	49	52	63	68	82	89	106	117	124	157	176	208	246	287	320	359	412	458	494

Appendix 8 (contd.)

Food concentration 500.500 μl^{-1} (20°C).

Time (h). ⟶	0	12	24	36	48	60	72	84	96	108	120	144	168	180	192	204	216	228	240	252	264
1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	3	6	5	5	6	7	8
2	1	1	1	1	1	1	1	1	1	1	2	2	3	4	4	6	6	7	8	8	9
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	2	2	2	2	2	3	4	4	5	8	9	9	11	13	16	16	21	23
5	1	1	1	1	1	2	2	2	2	2	2	3	5	6	6	6	6	7	12	12	12
6	1	1	1	1	2	2	2	2	3	4	4	5	5	8	8	9	10	13	14	14	14
7	1	1	1	2	1	1	1	1	1	4	4	4	4	4	7	8	8	11	15	16	16
8	1	1	1	1	1	1	1	2	2	2	4	4	6	6	6	6	9	9	10	11	11
9	1	1	1	1	1	1	2	2	2	2	4	7	8	9	9	11	15	15	18	25	26
10	1	1	1	1	1	1	1	2	2	2	3	3	4	5	6	6	6	6	8	9	10
11	1	1	1	1	1	1	2	2	2	3	4	4	4	4	6	6	9	11	14	16	16
12	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	6	8	8	8
13	1	1	1	1	1	1	1	1	2	2	2	3	4	4	5	7	8	9	12	15	17
14	1	1	1	1	1	1	2	2	2	2	3	3	4	6	7	7	7	14	14	14	14
15	1	1	1	2	2	2	2	4	4	4	4	4	5	9	13	14	16	18	21	24	24
16	1	1	1	1	1	1	1	2	2	2	2	4	4	4	4	8	8	8	10	14	15
17	1	1	1	1	1	1	1	2	2	2	3	4	5	6	6	8	8	9	11	14	14
18	1	1	1	1	1	1	1	1	1	3	3	5	7	7	8	12	12	12	14	20	20
19	1	1	1	1	1	1	2	2	2	2	3	4	4	4	5	6	7	7	10	10	10
20	1	1	1	1	1	1	1	1	1	2	2	2	3	4	4	5	7	7	8	8	9
21	1	1	2	2	2	2	2	3	4	4	4	4	5	7	7	8	11	15	17	17	17
22	1	2	2	2	2	2	4	4	4	4	5	6	7	7	9	10	12	14	14	18	20
23	1	1	1	1	1	1	1	2	2	2	3	4	4	7	8	8	8	9	12	12	14
24	1	1	1	1	1	1	1	1	1	1	1	2	4	5	7	9	11	12	13	13	16
25	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	1	1	2	2	2	2	2	4	4	4	4	4	6	6	8	8	7	7	12	14	14
27	1	1	1	1	1	1	1	2	2	2	3	4	5	8	8	10	10	11	13	14	14
28	1	1	1	1	1	2	2	2	3	4	4	6	8	8	9	13	15	17	17	19	20
29	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	6	9	9	9	11
30	1	1	1	1	2	2	2	2	2	4	4	6	8	9	9	9	12	14	15	17	20
31	1	1	1	2	2	2	2	2	2	2	3	3	7	7	8	9	10	14	16	20	23
32	1	1	1	1	1	2	2	2	3	3	3	6	8	8	8	9	10	14	14	17	17
33	1	1	1	1	1	1	2	2	2	2	2	2	3	3	4	4	4	4	5	6	8
34	1	1	1	2	2	2	2	4	4	4	4	8	9	9	11	13	13	14	18	22	22
35	1	1	1	1	1	1	1	1	2	2	2	2	4	4	4	8	8	9	14	14	14
36	1	1	1	1	1	1	2	2	2	3	4	4	4	9	9	9	9	11	13	16	18
37	1	1	1	1	1	1	2	2	2	3	4	4	4	8	8	8	11	13	14	15	15
38	1	1	1	1	2	2	2	2	2	3	4	5	6	8	9	9	9	9	11	11	11
39	1	1	1	1	1	1	2	2	2	2	3	4	5	6	7	8	10	10	10	10	10
40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Tot.	40	41	43	48	50	52	62	76	85	97	118	148	189	229	259	304	342	398	468	532	562

Appendix 8 (contd.)

Food concentration 1000.500μl^{-1} (20°C).

	Time (h). → 0	12	24	36	48	60	72	84	96	108	120	144	168	180	192	204	216	228	240	252	264
1	1	1	1	1	1	1	1	2	2	2	4	4	6	7	8	11	13	12	14	21	21
2	1	1	1	1	1	1	1	1	1	2	2	2	4	5	8	8	11	14	14	15	15
3	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	1	1	1	1	1	1	2	2	2	2	4	4	8	9	9	16	17	17	28	28	31
5	1	1	1	1	1	1	2	2	2	2	2	3	4	5	8	9	13	17	17	19	30
6	1	1	1	1	1	1	2	2	2	4	4	4	6	7	10	12	12	16	21	21	22
7	1	1	1	1	1	2	2	2	2	2	4	4	5	6	7	8	16	16	16	22	31
8	1	1	1	1	1	1	1	1	1	1	1	2	3	3	6	7	11	10	13	16	20
9	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	4	4	10	10	10
11	1	1	1	1	1	1	1	2	2	2	2	2	4	5	6	8	8	14	16	11	11
12	1	1	1	1	1	1	1	3	3	3	3	3	4	4	4	4	4	5	5	5	9
13	1	1	1	1	1	1	1	2	2	2	4	5	6	6	10	12	13	18	20	27	27
14	1	1	1	1	1	1	2	2	2	2	4	8	8	8	9	14	15	15	25	30	30
15	1	1	1	1	1	2	2	2	2	2	4	4	8	8	11	14	16	16	28	31	33
16	1	1	1	1	1	1	1	1	1	2	2	2	4	4	5	7	8	13	15	16	24
17	1	1	1	1	1	1	1	1	1	2	2	3	4	4	8	8	8	14	15	16	16
18	1	1	1	1	1	1	1	1	2	2	2	3	5	5	5	8	8	9	14	15	15
19	1	1	1	1	1	1	1	1	2	3	3	4	4	8	8	9	13	15	15	22	27
20	1	1	1	1	1	1	2	3	4	4	4	4	8	8	8	8	8	8	10	13	15
21	1	1	1	1	1	1	1	1	3	4	4	9	11	13	17	18	18	22	26	28	34
22	1	1	1	1	1	2	2	2	1	1	1	1	1	2	2	2	4	4	4	7	9
23	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	5	5	6	6	8
24	1	1	1	1	1	1	1	2	2	3	4	5	6	7	9	10	13	13	17	16	16
25	1	1	1	2	2	2	2	3	4	4	4	7	7	10	14	14	23	29	28	34	51
26	1	1	1	2	2	2	3	3	3	3	6	6	6	8	12	12	16	23	23	26	32
27	1	1	1	1	1	1	2	2	2	2	2	4	5	7	8	10	14	14	19	24	29
28	1	1	1	1	1	1	1	2	2	2	3	6	7	8	9	14	16	16	29	32	32
29	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	4	5	5
30	1	1	1	2	2	2	2	4	4	4	7	10	13	14	15	24	29	28	44	47	58
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	6	7	7
32	1	1	1	1	2	2	4	4	4	5	6	8	10	15	16	20	23	27	38	44	55
33	1	1	1	2	2	2	2	3	4	4	6	9	12	15	19	20	30	31	43	49	59
34	1	1	1	1	1	1	1	1	2	2	4	4	4	5	6	6	9	11	14	14	14
35	1	1	1	1	1	1	1	2	2	2	5	5	9	13	19	20	23	36	37	41	52
36	1	2	2	2	2	2	3	3	3	3	5	7	8	8	8	13	13	13	15	17	17
37	1	1	1	1	1	1	1	1	2	2	2	2	4	5	8	10	13	15	20	22	27
38	1	1	1	1	2	3	3	3	3	3	3	4	7	12	14	19	24	25	36	40	46
39	1	2	2	2	2	2	4	4	5	7	7	11	16	20	32	33	34	56	63	64	85
40	1	1	1	1	1	1	1	1	1	1	1	2	4	6	7	10	12	15	23	24	29
Tot.	40	42	42	46	48	51	64	77	85	96	126	164	228	278	354	430	521	620	791	885	1052

Appendix 8 (contd.)

Food concentration 2000.500μl^{-1} (20°C).

	Time (h). →																				
	0	12	24	36	48	60	72	84	96	108	120	144	168	180	192	204	216	228	240	252	264
1	1	1	1	1	2	2	4	4	6	6	7	10	12	13	15	15	21	24	32	39	45
2	1	1	2	2	2	3	3	3	5	5	7	9	11	14	14	16	19	22	24	29	38
3	1	1	1	1	1	1	1	2	2	2	2	4	4	5	8	10	16	19	24	29	36
4	1	1	1	1	1	1	1	2	2	2	4	5	8	10	15	17	18	21	29	35	43
5	1	1	1	1	1	2	2	2	4	4	4	4	4	6	7	8	10	10	11	12	17
6	1	1	1	2	2	2	2	3	4	4	4	4	8	10	12	15	18	21	25	33	40
7	1	2	2	2	2	4	4	4	4	5	8	8	12	14	16	18	23	25	25	29	37
8	1	1	1	1	1	2	2	2	2	4	4	6	8	8	8	10	13	15	17	19	30
9	1	1	1	1	1	1	2	2	2	2	3	4	5	6	8	9	10	13	16	23	25
10	1	1	1	1	1	1	1	1	1	1	2	2	4	4	4	8	8	9	15	17	19
11	1	1	1	1	1	1	1	2	2	2	3	3	6	8	11	11	15	20	25	29	43
12	1	1	1	2	2	2	3	3	3	4	5	6	10	12	13	16	20	21	26	32	42
13	1	1	1	1	1	1	2	2	2	3	4	4	4	5	8	8	11	13	16	21	25
14	1	1	1	1	1	1	1	1	2	2	4	5	8	9	14	15	17	20	25	29	41
15	1	1	1	1	2	2	2	3	3	3	6	7	9	11	12	14	15	25	32	39	47
16	1	1	1	1	2	2	2	2	2	3	4	6	10	11	16	16	18	24	29	29	36
17	1	1	1	1	2	2	2	4	4	4	5	6	8	12	16	24	24	33	38	45	51
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	4	4	4
19	1	1	2	2	2	3	3	3	3	3	3	4	6	7	9	10	12	15	18	22	28
20	1	1	1	1	2	2	2	4	4	5	6	6	7	10	12	17	20	23	28	34	39
21	1	1	1	1	1	1	1	1	1	1	1	2	4	4	8	8	8	13	15	21	23
22	1	1	1	1	1	1	1	2	2	2	4	5	5	5	7	8	9	12	15	17	19
23	1	1	1	1	1	2	2	2	3	3	3	4	6	6	10	14	15	20	25	28	33
24	1	1	1	1	1	1	1	2	2	2	4	6	6	7	10	11	13	15	19	21	30
25	1	1	1	1	1	2	2	2	2	4	6	7	9	11	13	15	18	20	24	35	37
26	1	1	1	1	1	2	2	2	2	3	4	5	7	7	8	14	14	14	14	15	20
27	1	1	1	2	2	2	3	4	4	5	8	9	10	11	14	18	21	24	33	37	47
28	1	1	1	1	1	2	2	2	2	4	4	6	7	8	11	11	14	20	32	26	36
29	1	1	1	1	2	2	2	3	4	5	6	6	8	12	12	23	24	29	34	36	48
30	1	1	1	1	2	2	2	3	3	3	3	4	7	8	11	14	15	17	26	29	33
31	1	1	1	1	1	1	1	1	2	2	2	2	3	4	6	6	7	10	13	14	16
32	1	1	1	1	2	2	2	4	4	4	6	8	11	13	14	18	18	23	33	41	44
33	1	2	2	2	2	2	2	2	4	4	8	10	14	18	24	30	31	34	40	45	52
34	1	1	1	1	1	1	1	2	3	4	4	6	9	10	10	12	13	15	19	19	27
35	1	1	1	1	2	2	2	3	4	4	6	7	8	10	14	15	17	17	17	18	26
36	1	1	1	1	1	2	2	2	4	4	4	5	7	8	8	11	14	17	22	24	31
37	1	1	1	2	2	2	3	4	4	5	7	7	9	9	9	12	12	15	20	23	29
38	1	1	1	2	2	2	3	4	4	4	6	6	7	10	10	14	17	19	26	31	39
39	1	1	1	2	1	2	2	2	2	3	4	4	7	7	7	11	14	14	15	20	23
40	1	1	1	1	1	1	1	1	2	2	2	2	4	6	8	10	13	14	14	18	23
Tot.	40	42	44	50	59	70	80	98	116	133	178	215	293	350	433	534	617	737	905	1067	1322

Appendix 8 (contd.).

Food concentration 4000.500μl^{-1} (20°C).

	Time (h). ⟶																					
	0	12	24	36	48	60	72	84	96	120	144	156	168	180	192	204	216	228	240	252	264
1	1	1	1	1	1	1	1	2	2	3	4	6	6	7	8	9	10	12	16	21	28
2	1	1	1	1	1	1	1	1	2	4	4	5	7	8	9	9	11	12	16	20	28
3	1	1	1	1	1	1	1	1	1	1	3	4	4	4	6	8	12	13	15	19	19
4	1	1	1	1	1	1	1	1	2	2	2	2	2	2	4	6	8	10	14	16	21
5	1	1	1	1	2	2	2	2	4	4	6	9	13	13	16	17	20	24	30	34	37
6	1	1	1	1	1	1	1	1	2	4	4	7	7	7	7	8	9	10	15	20	29
7	1	1	1	2	2	2	2	2	3	6	8	12	15	16	17	17	20	25	33	36	42
8	1	1	1	1	1	1	1	1	1	1	2	3	4	4	7	10	13	13	13	18	28
9	1	1	1	1	1	1	1	1	1	1	2	4	4	4	4	4	4	5	6	8	11
10	1	1	1	1	1	1	1	2	2	2	4	7	8	9	11	15	16	23	32	37	43
11	1	1	1	1	2	2	2	2	4	5	6	9	10	13	16	19	28	28	30	38	48
12	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	8	9	10
14	1	1	1	1	1	1	1	1	1	3	4	4	4	6	8	11	15	17	24	26	39
15	1	1	1	1	1	1	1	1	1	4	4	5	6	6	9	10	13	16	21	22	28
16	1	1	1	1	1	1	1	1	1	2	4	4	7	7	8	13	16	22	27	30	35
17	1	1	1	1	1	1	1	1	1	2	4	4	8	9	10	13	14	17	20	24	30
18	1	1	2	2	2	2	2	2	2	5	8	8	12	14	16	17	21	25	30	36	47
19	1	1	1	1	1	1	1	1	2	2	2	2	3	4	5	5	5	8	10	12	19
20	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	4
21	1	1	1	1	2	1	1	1	1	1	2	2	2	2	4	4	4	5	8	10	14
22	1	1	1	1	1	2	2	2	2	4	7	8	10	11	15	19	21	28	31	37	49
23	1	1	1	1	2	2	2	2	4	6	8	9	13	15	17	18	21	25	31	36	44
24	1	1	1	1	1	1	1	2	2	2	4	6	7	8	9	11	14	17	23	24	29
25	1	1	1	1	1	1	2	2	2	2	3	4	7	11	16	17	19	27	31	32	39
26	1	1	1	1	1	1	1	1	1	3	4	4	6	7	8	12	15	17	20	24	28
27	1	1	1	1	1	1	1	1	1	2	4	5	8	9	11	11	13	16	21	24	29
28	1	1	1	1	1	1	1	1	1	2	4	4	5	6	8	10	15	16	16	24	28
29	1	1	1	1	1	1	1	1	2	2	4	8	8	10	12	13	17	21	29	31	39
30	1	1	1	1	1	1	1	1	1	2	3	3	6	7	10	11	12	15	16	24	31
31	1	1	1	1	1	1	2	2	2	2	5	7	8	13	14	16	19	29	31	38	48
32	1	1	1	1	1	1	1	1	1	2	3	3	6	6	8	10	11	13	18	26	29
33	1	1	1	1	1	1	1	1	1	2	4	4	6	7	7	8	8	9	14	22	29
34	1	1	1	1	1	1	1	1	1	1	2	4	4	5	8	8	11	13	16	20	25
35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	3	4	6	7	10
36	1	1	1	1	1	1	1	2	2	2	4	8	8	8	8	11	14	20	25	30	37
37	1	1	1	1	1	1	1	1	2	3	4	6	8	8	9	15	16	17	22	26	31
38	1	1	1	1	2	2	2	2	2	3	3	3	6	6	6	7	9	10	10	14	19
39	1	1	1	1	1	1	1	1	2	2	4	7	8	9	10	10	15	19	25	29	36
40	1	1	1	1	1	1	1	1	1	1	2	2	2	3	3	3	3	4	5	8	10
Tot	40	40	41	42	47	46	48	52	67	99	149	197	252	289	350	412	501	611	760	914	1150

Appendix 8 (contd.).

Food concentration 6000.500μl^{-1} (20°C).

	Time (h). ⟶																	
	0	12	24	36	48	60	72	96	120	132	144	156	168	180	192	204	216
1	1	1	1	1	1	1	2	2	2	2	2	3	3	3	3	4	5
2	1	1	1	1	1	1	1	2	2	2	2	2	3	3	3	4	5
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
5	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
6	1	1	1	1	1	2	2	2	2	2	3	3	4	4	4	6	8
7	1	1	2	2	2	2	4	4	6	8	10	12	13	14	18	20	24
8	1	1	1	2	2	2	2	2	2	2	3	4	6	7	11	11	12
9	1	1	1	1	1	1	2	2	2	3	4	5	6	7	7	9	11
10	1	1	1	2	2	2	2	4	4	5	7	8	12	13	16	19	20
11	1	1	1	1	2	2	2	2	4	4	5	5	6	6	6	9	10
12	1	1	1	2	2	2	2	2	4	4	8	8	8	10	15	15	16
13	1	1	1	1	2	2	2	2	3	4	5	6	7	8	10	11	11
14	1	1	1	1	2	2	2	2	3	3	3	3	4	4	4	4	5
15	1	1	2	2	2	2	2	4	4	4	5	5	7	7	7	8	10
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	2	2	2	2	3	3	5	6	7	8	12	14	15
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	2	2	2	2	4	4	4	8	9	12	13	14	17	21
20	1	1	2	2	2	2	3	4	4	6	7	7	7	7	8	8	12
21	1	1	1	2	2	2	2	2	2	3	3	3	6	7	10	11	11
22	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	3
23	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	8
24	1	1	1	1	1	1	2	2	2	2	2	2	3	4	5	5	7
25	1	1	2	2	2	2	2	2	3	4	5	5	5	8	10	10	10
26	1	2	2	2	2	3	4	4	6	6	7	8	9	11	15	16	19
27	1	1	1	2	2	2	2	2	4	4	5	5	4	4	4	4	4
28	1	1	1	1	2	2	2	2	4	4	5	5	6	6	7	7	8
29	1	2	2	2	2	4	4	4	6	6	7	8	10	11	14	15	16
30	1	1	1	1	2	2	2	2	3	3	3	3	5	6	8	8	9
31	1	1	1	1	1	2	2	2	2	3	3	4	4	4	4	6	7
32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
33	1	1	1	1	2	2	2	2	2	4	4	4	8	9	11	14	16
34	1	1	1	1	2	2	2	2	4	4	5	7	9	12	14	15	19
35	1	1	2	2	2	2	3	4	4	6	8	7	7	8	9	11	11
36	1	1	1	2	2	2	2	2	3	4	5	5	6	7	7	9	12
37	1	1	1	1	1	2	2	2	2	4	4	5	5	6	7	7	8
38	1	1	1	2	2	2	2	2	4	4	5	5	5	5	7	9	10
39	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	1	1	1	1	2	2	2	2	3	4	4	4	4	8	8	9	11
Tot.	40	42	46	54	62	68	76	85	111	130	161	174	209	238	288	326	378

Appendix 8 (contd.).

Food concentration 8000 . 500μl^{-1} (20°C).

Time (h). ⟶	0	12	24	36	48	60	72	84	96	108	120	144	168	180	192
1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
2	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
3	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
4	1	1	1	1	2	2	2	2	2	2	2	1	0	0	0
5	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
6	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
8	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
9	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
10	1	1	1	1	1	1	2	2	2	1	0	0	0	0	0
11	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
12	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
13	1	1	1	1	1	1	2	2	2	0	0	0	0	0	0
14	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
15	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
16	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
17	1	1	1	1	1	1	2	2	2	0	0	0	0	0	0
18	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
19	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
20	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
21	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
22	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
23	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
24	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
25	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
26	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
27	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
28	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
29	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
30	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
31	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
32	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
33	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
34	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
35	1	1	1	1	1	2	2	2	2	2	2	1	1	1	0
36	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
37	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
39	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
40	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Tot.	40	40	39	38	38	39	41	41	39	31	25	10	6	3	0

Appendix 8 (contd.)

Food concentration 125.500μl^{-1} (15^{o}C).

Time (h) →	0	12	24	36	48	60	72	84	96	108	120	144	168	180	192	204	216	228	240	252	264
1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4
2	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2
3	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4	4	4
4	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	3	4	4	4
5	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	5	5	7	9
6	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4
7	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	4	4	4	4	4
8	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	3	4	4	4
9	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4
10	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	4	5	5	5	6	6
11	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	4	4	4	4
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
13	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3	4	4	4	4
14	1	1	1	1	1	1	1	1	1	2	2	2	4	4	4	4	4	5	5	5	5
15	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	4	4	4	4	4	4
16	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	5
19	1	1	1	2	2	2	2	2	2	2	3	3	3	4	5	5	5	6	7	7	7
20	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	3	3	4	4	4	4
21	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
22	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3
23	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	4	5	5
24	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4
25	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
26	1	1	1	1	1	1	1	1	1	1	1	2	2	2	3	3	3	4	4	4	4
27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
28	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	6	6	7	7
29	1	1	1	2	2	2	2	2	2	2	2	2	4	4	4	4	4	4	4	4	5
30	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
32	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	3	3	3	4
33	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	4	4	4
34	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	5	6	6	7
35	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	4	4
36	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4	5	6
37	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4	4	4
38	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
39	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	4	4	4
40	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	4	4
Tot.	40	40	40	42	46	46	50	53	54	64	68	76	81	88	105	113	127	141	146	154	161

Appendix 8 (contd.)

Food concentration 125.500μl^{-1} (15°C).

Time (h) →	0	12	24	36	48	60	72	84	96	108	120	144	168	180	192	204	216	228	240	252	264
1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4
2	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2
3	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4	4	4
4	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	3	4	4	4
5	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	5	5	7	9
6	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4
7	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	4	4	4	4	4
8	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	3	4	4	4
9	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4
10	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	4	5	5	5	6	6
11	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	4	4	4	4
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
13	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3	4	4	4	4
14	1	1	1	1	1	1	1	1	1	2	2	2	4	4	4	4	4	5	5	5	5
15	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	4	4	4	4	4	4
16	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	5
19	1	1	1	2	2	2	2	2	2	2	3	3	3	4	5	5	5	6	7	7	7
20	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	3	3	4	4	4	4
21	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
22	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3
23	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	4	5	5
24	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4
25	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
26	1	1	1	1	1	1	1	1	1	1	1	2	2	2	3	3	3	4	4	4	4
27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
28	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	6	6	7	7
29	1	1	1	2	2	2	2	2	2	2	2	2	4	4	4	4	4	4	4	4	5
30	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
32	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	3	3	3	4
33	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	4	4	4
34	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	5	6	6	7
35	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	4	4
36	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4	5	6
37	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4	4	4
38	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
39	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	4	4	4
40	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	4	4
Tot.	40	40	40	42	46	46	50	53	54	64	68	76	81	88	105	113	127	141	146	154	161

Appendix 8 (contd.)

Food concentration 250.500μl^{-1} (15^{o}C).

	Time(h) → 0	12	24	36	48	60	72	84	96	108	120	144	168	180	192	204	216	228	240	252	264
1	1	1	1	1	1	2	3	3	3	3	3	3	4	4	5	5	6	7	10	11	13
2	1	1	1	1	1	1	1	1	2	2	2	2	4	5	5	6	7	8	8	8	10
3	1	1	1	1	1	1	2	2	2	2	3	3	4	4	5	6	8	8	8	8	8
4	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	3	4	4	4	6	8
5	1	1	1	1	2	2	2	1	1	1	2	3	4	4	4	4	4	4	4	4	3
6	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	3	3	3	3	3	4
7	1	1	1	1	1	1	2	2	2	2	3	3	4	6	7	7	7	8	10	11	12
8	1	1	2	2	2	2	2	2	3	3	3	3	3	4	6	6	6	7	8	8	9
9	1	1	1	1	2	2	2	2	2	3	4	4	7	8	8	8	9	11	13	13	13
10	1	1	1	1	1	1	2	2	2	2	2	2	3	3	3	3	3	4	5	5	5
11	1	1	1	1	1	1	2	2	2	2	2	2	3	3	3	4	6	6	6	6	6
12	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4	4	4	3	3
13	1	1	1	1	2	2	2	2	3	3	4	4	4	4	8	8	8	8	8	10	14
14	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	4	4	5	6	7
15	1	1	1	1	1	1	2	2	2	2	2	2	4	5	7	7	7	7	7	8	10
16	1	1	1	1	1	1	1	2	2	2	2	2	3	3	3	3	3	3	3	3	3
17	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
18	1	1	1	1	1	1	2	2	2	2	2	2	4	4	5	5	6	7	8	8	8
19	1	1	1	1	1	1	1	1	2	2	2	2	4	4	4	5	6	6	8	9	9
20	1	1	1	1	1	1	2	1	1	1	1	1	2	2	2	2	2	2	2	2	2
21	1	1	1	1	1	1	1	1	2	2	2	2	3	3	4	4	4	6	7	7	7
22	1	1	1	1	1	1	1	2	2	2	2	2	3	3	3	5	6	6	7	7	7
23	1	1	1	1	1	1	2	2	2	2	4	4	4	4	5	5	5	6	7	7	7
24	1	1	1	1	1	1	2	2	2	2	2	2	3	3	3	4	4	4	6	6	7
25	1	1	1	1	2	1	1	1	2	2	2	2	3	3	4	4	4	5	6	6	6
26	1	1	1	1	1	1	2	2	2	2	2	2	4	4	5	6	8	8	8	8	8
27	1	1	1	1	1	1	1	2	2	2	2	2	3	3	4	4	4	4	4	4	4
28	1	1	1	1	1	1	2	2	2	2	4	4	4	5	6	6	8	8	8	9	12
29	1	1	1	1	1	1	2	2	2	2	2	2	4	4	4	5	8	8	9	10	13
30	1	1	1	1	1	1	2	2	2	2	3	3	3	4	6	6	6	8	9	9	13
31	1	1	1	1	1	1	2	1	1	2	3	3	4	4	5	5	5	5	5	6	6
32	1	1	2	2	2	2	2	2	3	3	4	4	7	7	8	8	8	10	13	13	13
33	1	1	1	1	2	2	2	2	2	2	4	4	7	7	7	7	7	7	7	7	7
34	1	1	2	2	2	2	2	2	2	3	4	4	7	8	8	8	9	10	15	15	15
35	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	5	5	6	6	6	6
36	1	1	2	2	2	2	2	3	4	4	4	4	7	7	6	7	8	8	11	12	12
37	1	1	1	1	1	1	2	2	2	2	4	4	3	3	4	4	4	5	6	6	7
38	1	1	1	1	1	2	2	2	2	2	3	2	2	3	3	3	3	3	3	3	3
39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
40	1	1	1	1	2	2	2	2	4	4	4	6	7	8	8	8	9	9	11	11	11
Tot.	40	40	45	51	52	70	72	81	85	105	107	151	160	186	199	220	240	275	287	314	

Appendix 8 (contd.)

Food concentration 500.500μl^{-1} (15^{o}C).

Time (h). ⟶	0	12	24	36	48	60	72	84	96	108	120	144	168	180	192	204	216	228	240	252	264
1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	5	6	7	7	7	8	12
2	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3
3	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	5	5	6
4	1	1	1	1	1	2	2	2	2	2	3	4	4	4	7	7	7	7	7	11	13
5	1	1	1	1	1	1	1	1	1	2	2	2	2	3	4	4	4	5	7	7	7
6	1	1	1	1	2	2	3	3	3	3	4	4	4	4	6	7	8	8	8	10	12
7	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	5	6	7	8	8	8
8	1	1	1	1	1	1	2	2	2	3	3	3	3	3	3	3	4	4	4	4	4
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	2	3	3	3	2	2	2	2	2	2	2	3	3	3	3	3	3	5
11	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	5	7	7	7	7	7
12	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	4	4	4	4	4	4
14	1	1	1	1	2	2	2	2	2	2	4	4	4	4	4	4	5	6	7	9	9
15	1	1	1	1	1	1	2	2	2	1	2	2	3	4	4	4	4	4	5	5	5
16	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	3	4	5	5
17	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	3	5	5	4	4
18	1	1	1	1	1	1	1	2	2	2	3	4	4	4	8	8	8	9	9	10	10
19	1	1	1	1	2	2	2	2	2	2	2	3	3	3	3	4	5	6	7	7	7
20	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	4	4	4	4	7	8
21	1	1	1	1	2	2	1	1	1	2	2	2	2	2	3	4	4	5	7	8	8
22	1	1	1	2	2	1	1	1	1	2	2	2	2	3	4	4	4	4	4	4	4
23	1	1	1	1	2	2	2	1	1	1	2	2	2	2	2	2	4	4	5	5	6
24	1	1	1	1	2	2	3	2	2	2	3	4	4	4	5	6	8	8	8	9	14
25	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	5	6
26	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	4	4	5	6	8
27	1	1	1	1	2	2	2	2	2	2	4	4	4	4	8	8	8	8	8	8	8
28	1	1	1	1	1	1	1	2	2	3	3	4	4	5	6	8	8	8	9	9	10
29	1	1	1	1	1	2	3	2	3	2	3	4	5	5	6	6	7	8	7	7	10
30	1	1	1	1	1	2	2	2	2	2	4	4	4	4	7	7	7	7	7	7	7
31	1	1	1	1	1	1	1	1	2	2	2	2	3	4	4	4	5	6	8	8	8
32	1	1	1	1	1	1	2	2	2	2	3	3	4	4	4	5	8	8	8	9	9
33	1	1	1	1	1	1	1	2	2	2	2	2	3	3	3	3	4	5	6	6	6
34	1	1	1	2	2	2	2	2	2	4	4	4	4	6	8	8	8	8	9	10	10
35	1	1	1	1	2	2	2	2	2	4	5	5	5	7	8	8	8	9	10	15	15
36	1	1	1	2	2	2	2	2	2	3	3	4	4	4	8	8	8	8	9	12	12
37	1	1	1	1	1	1	2	2	2	2	2	4	4	5	5	7	8	8	8	8	9
38	1	1	1	1	1	2	2	2	2	4	4	4	3	3	6	6	6	6	6	7	9
39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	3	3	3	3
40	1	1	1	2	2	2	2	2	2	2	4	4	4	4	5	6	7	7	7	9	10
Tot.	40	39	39	44	55	60	67	67	71	83	100	114	123	132	175	190	212	224	242	273	302

Appendix 8 (contd.)

Food concentration 1000.500μl^{-1} (15°C).

Time (h). ⟶	0	12	24	36	48	60	72	84	96	108	120	144	168	180	192	204	216	228	240	252	264
1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	5	6	8
2	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	5	7	7	9
3	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	6	6	6
4	1	1	1	1	1	1	1	1	1	2	2	2	2	4	4	4	4	4	5	6	8
5	1	1	1	1	2	2	2	2	2	2	2	2	3	4	4	4	4	8	8	8	8
6	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	3
7	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	3	3	3	4	5	6
8	1	1	1	1	1	1	1	1	2	2	2	2	4	4	4	6	6	6	7	8	8
9	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	4	5	6
10	1	1	1	1	1	1	1	1	1	1	1	2	2	2	3	3	4	4	7	7	7
11	1	1	1	1	2	2	2	2	2	2	2	3	4	4	5	5	5	5	5	7	8
12	1	1	1	1	1	1	1	1	1	1	2	2	2	3	4	4	4	4	6	7	7
13	1	1	2	2	2	2	2	2	2	2	2	3	4	4	5	6	8	8	8	10	12
14	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	4	4	4	5	8
15	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	6	6
16	1	1	1	1	1	2	2	2	2	1	1	1	1	1	2	2	3	3	3	3	4
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	3	3
18	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	3	3	3	5	5	5
19	1	1	1	1	1	1	1	1	1	1	1	1	4	4	4	6	8	8	8	8	8
20	1	1	1	1	1	1	1	1	1	1	1	2	2	2	3	4	4	4	4	5	7
21	1	1	1	2	2	2	2	2	2	2	2	2	3	3	4	5	6	7	8	8	8
22	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	4
23	1	1	1	1	1	1	1	1	1	2	2	2	2	4	4	4	4	6	7	7	7
24	1	1	2	2	2	2	2	2	2	2	4	4	4	6	7	8	8	9	13	13	14
25	1	1	2	2	2	2	2	2	2	2	2	2	2	4	4	5	6	6	7	8	8
26	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	6	8	8	9
27	1	1	1	1	1	1	1	1	1	2	2	2	2	4	4	4	5	6	8	8	8
28	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	4	4	7	8	8
29	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	5	5
30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	3	3	3	3	3
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
32	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	4	4	4	6	8
33	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	7	8
34	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	4	4	4	5	6	8
35	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	4	4	4	5	8
36	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	4	4	6	6	7	7
37	1	1	1	1	1	1	1	1	2	2	2	2	4	4	5	6	8	8	8	8	8
38	1	1	1	1	2	2	2	2	2	2	2	3	4	4	5	7	8	8	8	10	12
39	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	5
40	1	1	1	1	1	1	1	1	1	2	2	2	2	3	3	4	4	4	5	7	8
Tot.	40	40	43	44	47	48	49	50	53	62	70	78	92	106	134	150	174	189	222	253	285

Appendix 8 (contd.)

Food concentration 2000.500μl^{-1} (15^{o}C).

Time (h). ⟶	0	12	24	36	48	60	72	84	96	108	120	144	168	180	192	204	216	228	240	252	264
1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
2	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	4
3	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
7	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	4
8	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	3	3	4	4
9	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	4	4
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2
13	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	3	4	5
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2
17	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	4	5
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	3	3
22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4
24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	4	4
27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	4
28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
29	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	4
30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	3
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4
32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3
33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2
34	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4
35	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	4	4	4	5	5
36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2
37	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4
38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
39	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	4
40	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	4
Tot.	40	40	40	40	40	40	40	40	40	40	42	44	46	53	61	67	78	81	93	110	122

Appendix 8 contd.

Food concentration 3000. 500µl^{-1} (15^{o}C).

Time (h) ⟶	0	12	24	36	48	60	72	84	96	108	120	144	168	180	192	204	216	228	240	252	264
1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	4
2	1	1	1	1	2	2	2	2	2	2	2	3	3	4	4	4	4	4	4	8	8
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	4	4	6	7	8
5	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	4	4	4	4	4	4
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4
7	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	4
8	1	1	1	1	1	1	2	2	2	2	2	2	2	2	4	4	4	4	4	4	5
9	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	4
10	1	1	1	1	2	2	2	2	2	2	2	2	3	3	4	4	4	4	4	4	4
11	1	1	1	1	1	1	1	1	1	4	4	4	4	4	4	5	6	7	8	8	8
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4
14	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4
16	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	4	4	4	4	4
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3
18	1	1	1	2	2	2	2	2	2	2	2	3	4	4	4	4	5	6	7	7	8
19	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	4	4	4	4	4
20	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	3	3	4
21	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	4	4	4	7
22	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	4	4
23	1	1	1	2	2	2	2	2	2	2	3	3	4	4	4	4	4	4	4	6	8
24	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	4	4	4	4	4
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2
26	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4
27	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	4	4	6	8
28	1	1	1	1	2	2	2	2	2	2	4	4	4	4	4	4	7	8	8	8	8
29	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4
30	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	4	4
31	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	4	4	4
32	1	1	1	1	1	2	2	2	2	2	2	2	3	3	4	4	4	4	4	4	6
33	1	1	1	1	2	2	2	2	2	2	2	2	2	4	4	4	4	4	4	7	7
34	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	4	4	4
35	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	4	4	4	4	7	8
36	1	1	1	1	1	1	2	2	2	2	2	2	2	2	4	4	4	4	4	4	6
37	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	4	4
38	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	4	4	5	5	6
39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
40	1	1	1	1	1	1	1	2	2	2	2	2	2	4	4	4	4	4	4	7	8

Tot.	40	40	39	41	45	48	52	55	58	65	71	75	84	95	107	114	131	138	146	170	193

Appendix 8 (contd.)

Food concentration 4000. 500μl^{-1} (15°C).

Time (h). →	0	12	24	36	48	60	72	84	96	108	120	144	168	180	192	204	216	228	240	252	264	288	312
1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4	4	4	4
2	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4	4	4
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3
5	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3
6	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	4	4	4
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	4	4	4	5
10	1	1	2	2	2	2	2	2	2	2	2	3	3	4	4	4	4	4	5	5	7	8	8
11	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	3	3	3	4	4	4
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	4
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	1	4	4	4
14	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	3	4	4	4
19	1	1	1	1	1	1	1	1	2	2	2	2	4	4	4	4	4	5	5	6	8	8	8
20	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	4	4	4	4	4	4
21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	4	4	4	4	4	5	6	8
24	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	4	4	4	4	4	4	6	8
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
26	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	4	4	4	4	4	4	5	6
27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2
28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2
29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3
32	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	4	4	4	4	5	6	7	8
33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4
34	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	4	4	4
35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
37	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4
38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2
40	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	4	5	6	8
Tot.	40	40	42	42	42	42	43	42	44	44	47	50	58	59	69	78	83	87	88	97	115	127	141

Appendix 8. (contd.)

Food concentration 125 500μl^{-1} (10°C).

	Time (h) → 0	24	48	72	96	120	144	168	192	216	240	264	288	312	336	360	384	408	432	456	480
1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	4	4
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
5	1	2	2	2	2	2	2	2	2	4	4	4	4	4	4	4	4	4	4	4	4
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3
8	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
9	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2
10	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
11	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3	3
12	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3	3
13	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	3
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	4	4	4	4	4
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2
18	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3	4
21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
22	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	3	4
23	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3
24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
27	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3	3
28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
33	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	4
34	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
37	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2
38	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
39	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3
40	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3
Tot.	40	41	41	43	43	45	51	55	55	57	56	58	59	60	59	61	62	62	64	67	74

Appendix 8 (contd.)

Food concentration 250.500μl^{-1} (10°C).

Time (h) →	0	24	48	72	96	120	144	168	192	216	240	264	288	312	336	360	384	408
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
4	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	2	2	2	2	2	2	2	4	4	5	6	6
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
10	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	3	5
11	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
12	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
13	1	1	2	2	2	2	2	2	3	3	3	3	3	3	3	5	5	5
14	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	4	4	4
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	3	3
16	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
20	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3
21	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3
22	1	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2
27	1	1	1	1	1	1	1	1	3	3	3	3	3	3	3	4	4	4
28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2
30	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3
31	1	1	1	2	2	2	2	2	2	3	4	4	4	4	4	5	5	5
32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
34	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
35	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3
36	1	1	1	1	1	1	2	2	2	2	3	3	3	4	4	4	4	4
37	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	4	4
38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
40	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3
Tot.	40	42	44	46	46	49	52	54	57	58	60	60	62	68	71	78	82	85

Appendix 8 (contd.)

Food concentration 500 500µl^{-1} (10°C).

	Time (h) ⟶																	
	0	24	48	72	96	120	144	168	192	216	240	264	288	312	336	360	384	408
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2
3	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
4	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	4	4	4
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	2	2	2	2	2	2	2	2	2	2	2	4	4	4	4
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
15	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	4	4	4
22	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24	1	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
26	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	1	1	1	2	2	2	2	2	2	3	3	4	4	4	5	6	6	6
28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2
31	1	1	2	2	2	2	2	2	2	2	2	3	4	4	4	4	4	4
32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
34	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
36	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
37	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3
38	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3
39	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	4	4	4
40	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3
Tot.	40	41	41	47	47	49	51	52	56	57	58	60	62	62	71	78	77	78

Appendix 8 (contd.)

Food concentration 1000.500μl^{-1} ($10^{0}C$).

Time (h) ⟶	0	24	48	72	96	120	144	168	192	216	240	264	288	312	336	360	384	408	432
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
3	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	4	4	4
11	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
18	1	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4
19	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
20	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2
23	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3
24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
27	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
28	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
33	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2
34	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2
35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2
37	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2
39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Tot.	40	42	43	44	46	49	52	53	54	54	53	53	54	56	57	60	61	64	64

Appendix 8 (contd.)

Food concentration 2000.500μl^{-1} (10°C).

Time (h) →	0	24	48	72	96	120	144	168	192	216	240	264	288	312	336	360	384	408	432
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	2	2	2	2	2	2	2	2	3	4	4	4	4	4	4
4	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2
14	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
17	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3
18	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
32	1	1	1	1	2	2	2	2	2	2	2	2	2	2	4	4	4	4	4
33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
34	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2
35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
37	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
38	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Tot.	40	40	43	44	46	47	47	46	46	47	48	49	49	50	54	55	55	56	56

Appendix 8 (contd.)

Food concentration 4000.500μl^{-1} ($10^{o}C$).

Time (h) →	0	24	48	72	96	120	144	168	192	216	240	264	288	312	336	360	384	408	432	456	480	504	528	552
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
4	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1
11	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
12	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	4	4
13	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3
21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3
26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
34	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3
35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
37	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
38	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
39	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3
40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Tot.	40	38	41	43	46	46	47	47	47	47	48	48	48	48	49	49	49	49	48	48	48	50	54	54

Appendix 9.

Number of Tetrahymena consumed per hour by A.proteus . (20°C).

Food conc. per 500µl	8000	4000	2000	1000	500	250	125
Cells consumed (0 _ 10 h)	0	4	0	2	2	1	0
	0	6	1	2	2	1	2
	0	3	3	0	0	0	0
	2	2	0	0	3	1	1
	0	5	1	0	0	0	0
	0	2	0	2	3	0	1
	0	3	4	2	1	1	0
	1	0	3	0	0	0	0
	4	2	1	0	1	2	1
	5	6	3	2	2	0	0
	0	1	2	0	0	0	0
	0	1	4	1	1	0	1
	1	3	2	3	1	1	0
	0	4	2	0	1	1	2
	0	4	2	1	0	0	0
	3	0	4	0	2	0	1
	0	3	2	3	0	1	0
	0	2	3	0	0	1	2
	2	2	2	3	3	0	1
	0	5	3	1	1	1	0
Mean	0.90	3.20	2.10	1.10	1.15	0.50	0.60
Cells consumed (0 - 10 h)	1	2	3	4	0	1	0
	0	1	3	3	2	1	0
	0	1	2	0	1	2	0
	1	3	3	1	2	4	1
	0	2	1	0	1	2	0
	0	5	2	1	0	0	1
	0	0	2	0	1	2	3
	0	2	4	1	3	0	2
	0	4	2	4	2	1	0
	0	1	2	5	1	1	2
	1	1	0	0	1	3	1
	0	3	4	3	2	1	0
	1	2	3	4	3	2	1
	0	1	1	5	0	4	0
	0	3	2	1	1	0	3
	1	0	2	2	0	2	5
	0	2	3	4	0	1	0
	1	4	3	1	0	1	0
	0	1	1	0	3	3	2
	0	2	1	2	1	2	1
Mean	0.30	2.00	2.20	2.05	1.20	1.65	1.10
Overall mean	0.60	2.60	2.14	1.57	1.17	1.07	0.85

Appendix 9 (contd.).

Number of Tetrahymena consumed per hour by A.proteus (15°C).

Food conc. per 500µl	4000	2000	1000	500	250	125
Cells consumed (0 - 10 h)	5	4	2	1	0	1
	3	4	4	2	2	0
	2	3	2	0	0	0
	2	0	2	2	1	2
	2	1	2	0	0	1
	5	2	2	1	1	0
	2	1	2	1	3	0
	4	2	0	2	2	0
	0	2	2	0	2	1
	1	3	1	1	2	1
	0	4	0	2	3	0
	3	5	3	1	1	0
	6	4	2	0	1	1
	4	4	1	1	0	1
	3	4	2	1	1	1
	3	3	2	1	0	0
	0	2	1	1	0	0
	2	4	0	0	3	0
	0	1	3	2	0	1
	3	3	0	0	0	1
Mean	2.50	2.80	1.65	0.95	1.10	0.55
Cells consumed (0 - 10 h)	2	2	1	0	3	1
	2	4	3	0	1	0
	0	0	1	2	1	0
	6	1	0	2	1	0
	3	4	1	2	0	4
	2	1	0	0	2	1
	1	1	1	2	0	0
	1	0	1	2	1	0
	4	0	2	1	1	3
	7	4	0	0	0	2
	1	1	1	0	0	1
	2	2	2	1	0	1
	2	0	2	2	1	0
	3	0	1	1	0	1
	2	3	3	1	0	0
	1	3	0	0	1	0
	2	2	1	2	0	1
	0	2	1	0	0	0
	5	3	2	2	0	1
	3	3	1	0	1	1
Mean	2.45	1.80	1.20	1.00	0.65	0.85
Overall mean	2.47	2.30	1.42	0.92	0.87	0.70

Appendix 9 (contd.).

Number of Tetrahymena consumed per hour by A.proteus (10°C).

Food conc. per 500µl	4000	2000	1000	500	250	125
Cells consumed (0-10 h)	0	0	3	2	0	3
	0	0	2	0	0	2
	1	0	0	1	0	0
	1	0	2	0	2	0
	0	2	3	0	0	1
	1	1	1	0	2	1
	1	1	0	2	3	0
	1	1	1	1	1	0
	0	3	1	1	0	0
	0	1	1	0	0	0
	0	0	1	0	0	2
	2	1	2	1	0	1
	1	1	1	0	1	0
	0	1	2	1	0	0
	1	0	2	1	1	1
	0	3	0	2	2	1
	0	0	1	0	0	0
	1	2	1	0	0	2
	2	0	0	0	1	0
	0	1	1	0	0	1
Mean	0.60	0.90	1.25	0.60	0.65	0.75
Cells consumed (0-10 h)	1	0	2	0	0	1
	1	0	0	0	0	0
	0	0	0	2	1	0
	1	0	0	1	0	1
	0	1	0	0	1	0
	0	2	0	1	0	0
	1	1	1	0	3	0
	2	0	0	1	1	0
	0	1	0	0	0	0
	0	1	2	0	0	0
	1	1	1	0	1	1
	0	1	2	0	2	0
	1	5	1	2	0	1
	0	0	0	1	1	0
	1	2	0	1	0	0
	0	1	2	0	0	1
	0	1	0	1	1	0
	1	0	1	0	1	1
	0	2	0	1	0	0
	0	1	1	1	2	0
Mean	0 .50	1.00	0.65	0.60	0.70	0.30
Overall mean	0 .55	0.95	0.95	0.60	0.67	0.52

Appendix 10.

Estimation of the cell volume of Chilomonas.

Assumptions:

1. ellipsoid in shape
2. length 25μm
3. breadth 12.5μm

$$\text{volume} = \pi \frac{d^2 \times l}{6}$$

d = diameter

l = length

Appendix 11

Production data.

Appendix 11 (contd.)

Increase in the cell volume of A.proteus with time.

125 Tetrahymena.500µl^{-1} (20°C)			4000 Tetrahymena.500µl^{-1} (20°C)		
	Age of cell (h)	Cell vol. ($\mu m^3 \times 10^3$)		Age of cell (h)	Cell vol. ($\mu m^3 \times 10^3$)
1	2	454	1	3	669
2	4	570	2	3	661
3	5	528	3	8	776
4	5	504	4	8	702
5	6	512	5	10	784
6	8	561	6	10	826
7	10	669	7	17	801
8	10	586	8	17	776
9	19	751	9	18	793
10	19	611	10	21	1040
11	20	710	11	22	983
12	23	669	12	22	1164
13	24	760	13	22	950
14	24	694	14	22	834
15	28	768	15	25	1247
16	40	908	16	25	1040
17	44	884	17	26	867
18	44	900	18	28	1090
19	45	669	19	30	1148
20	46	776	20	30	1123
21	48	908	21	32	1140
22	49	941	22	40	1082
23	60	884	23	41	983
24	65	917	24	41	1016
25	65	760	25	45	1230
26	67	941	26	45	1173
27	72	966	27	46	1288
			28	46	1321

Appendix 11 (contd.)

Increase in the cell volume of A.proteus with time.

125 Tetrahymena 500µl^{-1} (15°C)			4000 Tetrahymena 500µl^{-1} (15°C)		
	Age of cell (h)	Cell vol. ($\mu m^3 \times 10^3$)		Age of cell (h)	Cell vol. ($\mu m^3 \times 10^3$)
1	8	826	1	8	1247
2	8	842	2	8	1222
3	18	983	3	8	1107
4	18	1032	4	10	1148
5	19	743	5	10	1239
6	19	751	6	10	1140
7	20	760	7	18	1503
8	20	933	8	18	1330
9	20	859	9	18	1354
10	21	776	10	20	1148
11	21	751	11	21	1445
12	22	974	12	21	1239
13	28	950	13	21	1164
14	42	1032	14	24	1420
15	43	1065	15	25	1247
16	43	867	16	44	1660
17	44	950	17	44	1627
18	45	1082	18	46	1685
19	47	1156	19	46	1742
20	47	1255	20	47	1486
21	66	1181	21	47	1387
22	66	1272	22	70	1660
23	68	1239	23	70	2007
24	69	1371	24	70	1974
25	71	1330	25	72	1486
26	84	1495	26	72	1313
27	84	1280	27	72	1519
28	84	1230	28	72	1668
29	86	1478	29	72	1734
			30	72	1709
			31	72	1817
			32	90	1652
			33	92	1982
			34	92	1569
			35	92	1990
			36	92	1957
			37	96	1974
			38	96	2065
			39	96	2230
			40	96	2263
			41	98	1957

Appendix 11 (contd.) 20°C.

Division and daughter cell volumes ($\mu m^3 \times 10^3$). A = division volume. B = daughter volume.

Food conc.	125		250		500		1000		2000		4000	
	A	B	A	B	A	B	A	B	A	B	A	B
	865	420 445	1121	536 585	1349	650 699	1339	655 684	1313	661 652	1602	710 892
	931	495 436	1140	570 570	1341	683 658	1471	731 740	1552	829 723	1501	791 710
	982	512 470	1146	488 658	1365	813 552	1346	751 795	1528	821 707	1391	684 707
	956	453 503	1153	575 578	1381	723 658	1351	671 680	1333	585 748	1338	691 647
	949	453 495	1191	587 604	1357	677 680	1325	671 654	1123	537 586	1222	619 603
	1023	487 536	1098	520 588	1297	578 619	1387	801 586	1421	694 727	1330	661 669
	1098	586 512	1292	634 658	1235	601 634	1478	908 570	1544	793 751	1499	760 739
	940	448 492	979	469 510	1330	603 727	1467	719 748	1292	723 569	1457	755 702
	890	460 430	1255	652 603	1346	677 669	1297	628 669	1311	628 683	1373	779 694
	964	468 496	1114	553 561	1288	666 622	1404	669 735	1247	603 644	1305	661 644
Mean A ± (S.D.)	960 ± 66		1149 ± 86		1329 ± 44		1386 ± 66		1366 ± 142		1402 ± 113	
Mean B ±)S.D.)	480 ± 40		575 ± 53		659 ± 58		693 ± 78		683 ± 84		701 ± 72	

Appendix 11 (contd.). 15°C.

Division and daughter cell volumes ($\mu m^3 \times 10^3$). A = division volume. B = daughter volume.

Food conc.	125		250		500		1000		2000		4000	
	A	B	A	B	A	B	A	B	A	B	A	B
	1469	751 718	1498	758 740	1676	900 776	1478	760 718	2023	1115 908	2321	1082 1239
	1478	743 735	1750	842 908	1495	826 669	1651	900 751	2461	1280 1181	2255	1181 1074
	1236	635 601	1635	844 791	1497	754 743	1610	900 710	2172	1173 999	2221	1247 974
	1139	586 553	1421	727 694	1808	900 908	1660	809 851	1784	908 876	2147	1206 941
	1544	710 834	1561	791 770	1565	769 796	1701	854 847	1999	925 1074	2238	1115 1123
	1577	817 760	1379	776 603	1544	727 817	1560	784 776	1883	983 900	2205	1148 1057
	1441	707 734	1330	678 652	1792	892 900	1693	842 851	1709	866 843	2213	1331 882
	1399	690 704	1521	761 760	1932	900 1032	1511	685 826	1891	900 991	2139	1074 1065
	1287	621 666	1483	698 785	1635	933 702	1791	817 874	1965	1024 941	2023	1057 966
	1333	811 522	1457	732 725	1362	685 677	1612	841 771	1841	907 934	2073	1024 1049
Mean A ± (S.D.)	1390 ± 140		1503 ± 123		1631 ± 173		1627 ± 94		1973 ± 216		2183 ± 89	
Mean B ± (S.D.)	695 ± 86		751 ± 69		815 ± 101		808 ± 62		986 ± 120		1092 ± 109	

Appendix 11 (contd.). 10°C.

Division and daughter cell volumes ($\mu m^3 \times 10^3$). A = division volume. B = daughter volume.

Food conc.	125		250		500		1000		2000		4000	
	A	B	A	B	A	B	A	B	A	B	A	B
	2130	1098 1032	3444	1751 1693	3229	1669 1560	3177	1584 1593	2967	1467 1500	3001	1553 1448
	2065	1114 951	3477	1412 2065	2275	1123 1152	3551	1736 1815	2953	1561 1392	3512	1719 1793
	1916	985 931	2717	1396 1321	2820	1364 1456	3482	1748 1734	3441	1770 1671	3007	1608 1399
	2412	1201 1211	3271	1718 1553	3011	1619 1392	3004	1550 1454	3299	1669 1630	2777	1534 1243
	2378	1256 1122	2898	1420 1478	2991	1557 1434	2975	1571 1404	3617	1888 1729	2566	1443 1123
	1751	921 830	2131	991 1140	3315	1718 1597	3441	1779 1662	2841	1531 1310	2271	1132 1139
	1911	1010 901	3237	1742 1495	2561	1234 1327	3031	1481 1550	3219	1656 1563	2961	1448 1513
	1995	956 1039	2569	1330 1239	2881	1478 1403	2877	1433 1444	2765	1504 1261	3114	1578 1536
	2131	983 1148	2824	1437 1387	3246	1669 1577	3342	1657 1685	3119	1593 1526	2914	1497 1417
	1623	891 732	1855	1001 854	2781	1543 1238	4081	2022 2059	2915	1563 1352	3107	1636 1471
Mean A ± (S.D.)	2031 ± 249		2842 ± 545		2911 ± 324		3296 ± 363		3117 ± 277		2923 ± 334	
Mean B ± (S.D.)	1016 ± 135		1421 ± 137		1455 ± 175		1648 ± 181		1557 ± 156		1461 ± 184	

Appendix 11 (contd.)

Raw data for the calculation of the mean cell volume of Amoeba cells from the mass cultures. ($\times 10^{-3} \mu m^3$).

Temp. °C.	Cell volumes					Mean cell volumes
20	1082	991	578	718	983	859 ± 191 (S.D.)
	974	900	817	661	950	
	669	958	941	900	1082	
	611	495	570	983	851	
	702	751	611	892	957	
	867	1222	1082	834	918	
	628	487	966	570	1074	
	760	1115	950	1007	1115	
	677	586	941	751	1197	
	826	900	727	991	1131	
15	966	842	1082	1222	892	1077 ± 232 (S.D.)
	784	1387	1032	983	1123	
	1164	950	1396	1181	1363	
	991	900	1181	1016	875	
	1222	1057	1197	970	1148	
	743	1230	1296	1007	1057	
	809	1404	1115	1181	826	
	1156	1660	809	1065	1668	
	727	1057	727	1330	1494	
	652	1049	1057	950	834	
10	2420	1189	2237	2370	3006	2022 ± 585 (S.D.)
	1404	1883	3039	2106	2147	
	1652	1880	1990	2238	1470	
	1536	1412	1825	1858	2056	
	1478	2312	2751	1181	2048	
	3171	2593	1470	1255	1825	
	2981	1660	2923	1561	1189	
	2593	1495	1742	2428	1553	
	1693	1916	2880	1231	3130	
	1486	2131	2081	2544	2155	

Appendix 12.

Cartesian respiration data.

The terms, left and right, refer to

the manometer readings (mm.)

Appendix 12 (contd.)

Food concentration 125.500μl^{-1} (20°C).

1. 1.2µl diver containing 3 large amoebae.
2. 1.5µl diver containing 2 large amoebae.
3. 1.5µl diver containing 2 large amoebae.
4. 1.2µl diver - control.

Time (min.)	0	30	60	90	120	150	180	200
left	748	673	733	695	705	746	753	806
1. right	518	434	488	444	445	483	484	534
diff.	230	239	245	251	260	263	269	272
left	600	683	720	684	686	701	733	787
2. right	381	476	495	460	457	467	496	545
diff.	219	297	225	224	229	234	237	242
left	538	593	622	608	604	616	665	710
3. right	469	528	557	537	530	535	580	619
diff.	69	65	65	71	74	81	85	91
left	876	928	926	945	956	959	992	1058
4. right	254	305	304	321	334	336	369	434
diff.	622	623	622	624	622	623	623	624

Food concentration 125.500μl^{-1} (20°C).

1. 1.2µl diver containing 4 small amoebae.
2. 0.9µl diver containing 4 small amoebae.
3. 1.5µl diver containing 4 medium amoebae.
4. 1.8µl diver containing 3 large amoebae.
5. 1.5µl diver - control.

Time (min.)	0	30	60	90	120	150	180
left	902	861	799	773	810	915	837
1. right	456	415	350	323	353	457	378
diff.	446	446	449	450	457	458	459
left	1997	911	865	829	728	976	930
2. right	398	303	250	206	97	338	287
diff.	609	608	615	623	631	638	643
left	897	803	809	756	934	772	876
3. right	464	368	372	351	485	323	422
diff.	433	435	437	441	449	449	454
left	626	552	551	481	689	469	666
4. right	632	555	552	478	679	458	651
diff.	- 6	- 3	- 1	3	10	11	15
left	659	586	570	573	731	634	709
5. right	615	542	530	530	687	590	665
diff.	44	44	40	43	44	44	44

Appendix 12 (contd.)

Food concentration 125.500μl^{-1} (20^{o}C).

1. 1.5μl diver containing 3 small amoebae.
2. 1.2μl diver containing 2 small amoebae.
3. 0.9μl diver - control.

	Time (min.)	0	30	60	90	120	150	175
1.	left	735	720	734	707	686	668	579
	right	633	615	618	590	563	542	449
	diff.	102	105	116	117	123	126	130
2.	left	1045	1052	1003	1035	989	965	984
	right	419	424	376	407	359	333	351
	diff.	626	628	627	628	630	632	633
3.	left	986	1013	949	942	952	848	927
	right	418	446	383	376	386	283	359
	diff.	568	567	566	566	566	565	566

Food concentration 125.500μl^{-1} (20^{o}C).

1. 1.5μl diver containing 2 medium amoebae.
2. 1.8μl diver containing 6 medium amoebae.
3. 1.5μl diver containing 4 medium amoebae.
4. 1.2μl diver - control.

	Time (min.)	0	30	60	90	120	150	180	210
1.	left	722	728	782	765	749	726	732	774
	right	505	510	560	536	515	487	486	524
	diff.	217	218	222	229	234	239	246	250
2.	left.	588	609	659	624	597	580	595	635
	right	619	636	676	636	604	584	597	631
	diff.	-31	-27	-17	-12	- 7	- 4	- 2	4
3.	left	784	803	839	780	791	778	791	815
	right	491	509	542	482	486	471	483	506
	diff.	293	294	297	298	305	307	308	309
4.	left	851	902	907	870	852	847	865	864
	right	419	470	476	440	420	418	434	432
	diff.	432	432	431	430	432	429	431	430

Appendix 12 (contd.)

Food concentration 1000.500μl^{-1} (20^{o}C).

1. 1.5µl diver containing 4 small amoebae.
2. 1.5µl diver containing 5 small amoebae.
3. 1.5µl diver containing 4 small amoebae.
4. 1.5µl diver - control.

	Time (min.) 0	30	60	90	120	150	180
	left	1210	1129	1111	1138	1090	1119	1115
1.	right	361	283	260	286	230	255	247
	diff.	849	846	851	852	860	864	868
	left	1157	1063	1030	1042	1014	1053	1060
2.	right	425	328	290	298	259	291	294
	diff.	732	735	740	744	755	762	766
	left	832	743	712	713	702	729	740
3.	right	613	523	490	488	473	497	505
	diff.	219	220	222	225	229	232	235
	left	1067	1048	1008	1008	1011	1046	1054
4.	right	324	305	265	264	266	301	309
	diff.	743	743	743	744	745	745	745

Food concentration 1000.500μl^{-1} (20^{o}C).

1. 1.5µl diver containing 5 large amoebae.
2. 1.5µl diver containing 4 large amoebae.
3. 1.2µl diver - control.

	Time (min.) 0	30	60	90	120	150	180
	left	503	476	478	482	508	555	570
1.	right	678	625	613	599	607	638	634
	diff.	-175	-149	-135	-117	-101	- 83	- 64
	left.	836	784	787	791	814	848	856
2.	right	475	420	414	410	429	456	453
	diff.	361	364	373	381	385	392	403
	left	815	778	772	776	804	818	820
3.	right	459	423	416	421	447	461	463
	diff.	356	355	356	355	357	357	357

Appendix 12 (contd.)

Food concentration 1000.500μl^{-1} ($20^{o}C$).

1. 1.5µl diver - control.
2. 1.2µl diver containing 4 small amoebae.
3. 1.5µl diver containing 5 large amoebae.
4. 1.5µl diver containing 3 large amoebae.

	Time (min.)	0	60	90	120	150	180
	left	659	647	669	655	640	656
1.	right	539	527	549	535	520	536
	diff.	120	120	120	120	120	120
	left	705	710	726	700	735	725
2.	right	515	506	514	481	507	492
	diff.	190	204	212	219	228	233
	left	916	935	954	943	976	970
3.	right	371	365	369	341	359	337
	diff.	545	570	585	602	617	633
	left	667	689	686	672	707	704
4.	right	525	534	522	501	524	513
	diff.	142	155	164	171	183	191

Food concentration 1000.500μl^{-1} ($20^{o}C$).

1. 1.5µl diver containing 6 medium amoebae.
2. 1.5µl diver containing 5 medium amoebae.
3. 1.5µl diver containing 3 medium amoebae.
4. 1.2µl diver - control.
5. 1.5µl diver containing 3 medium amoebae.

	Time (min.)	0	6o	90	120	150	180
	left	1198	1187	1192	1210	1221	1247
1.	right	189	159	154	163	156	175
	diff.	1009	1028	1038	1047	1065	1072
	left	519	531	526	533	555	582
2.	right	599	592	574	568	522	591
	diff.	- 80	- 61	- 48	- 35	- 27	- 9
	left	667	675	681	678	701	728
3.	right	489	486	486	479	497	516
	diff.	178	189	195	199	204	212
	left	794	792	795	799	805	833
4.	right	415	413	417	421	427	453
	diff.	379	379	378	378	378	380
	left	802	808	822	820	841	853
5.	right	409	406	417	410	425	435
	diff.	393	402	405	410	416	418

Appendix 12 (contd.)

Food concentration 4000.500µl^{-1} (20°C).

1. 1.8µl diver containing 3 large amoebae.
2. 1.5µl diver containing 6 medium amoebae.
3. 1.2µl diver containing 4 small amoebae.
4. 1.5µl diver - control.

	Time (min.)	0	30	60	90	120	150	180
	left	884	910	914	920	935	930	949
1.	right	668	682	678	674	678	663	669
	diff.	216	228	236	246	257	267	280
	left	792	834	825	831	852	844	854
2.	right	738	771	746	741	748	712	708
	diff.	54	63	79	90	104	132	146
	left	1039	1068	1056	1072	1073	1065	1059
3.	right	578	604	590	603	597	586	574
	diff.	461	464	466	469	476	479	485
	left	831	856	839	851	866	848	856
4.	right	720	745	728	739	756	737	745
	diff.	111	111	111	112	110	111	111

Food concentration 4000.500µl^{-1} (20°C).

1. 1.5µl diver containing 5 small amoebae.
2. 1.2µl diver containing 3 small amoebae.
3. 1.5µl diver containing 4 small amoebae.
4. 1.8µl diver - control

	Time (min.)	0	30	60	90	120	150	180
	left	611	673	743	771	827	847	957
1.	right	611	674	737	759	802	818	922
	diff.	0	- 1	6	12	25	29	35
	left	992	1055	1119	1146	1184	1200	1142
2.	right	384	445	502	524	556	569	505
	diff.	608	610	617	622	628	631	637
	left	579	659	705	747	779	824	751
3.	right	662	734	773	812	840	878	802
	diff.	-83	-75	-68	-65	-61	-54	-51
	left	740	808	842	877	910	950	891
4.	right	580	648	682	717	749	789	730
	diff.	160	160	160	160	161	161	161

Appendix 12 (contd.)

Food concentration 4000.500μl^{-1} (20^{o}C).

1. 1.8µl diver containing 6 medium amoebae.
2. 1.5µl diver containing 4 medium amoebae.
3. 1.2µl diver containing 3 medium amoebae.
4. 1.5µl diver - control.

	Time (min.)	0	30	60	90	120	150	180
	left	766	788	774	755	801	805	805
1.	right	689	701	680	654	689	681	673
	diff.	77	87	94	101	112	124	132
	left	576	600	601	582	631	615	626
2.	right	801	817	804	777	817	792	797
	diff.	-225	-217	-203	-195	-186	-177	-171
	left	998	1012	1014	989	1048	1024	1057
3.	right	524	540	534	504	547	517	540
	diff.	474	472	480	485	501	507	517
	left	819	817	804	783	835	807	851
4.	right	671	669	656	635	688	657	702
	diff.	148	148	148	148	147	150	149

Food concentration 4000.500μl^{-1} (20^{o}C).

1. 1.5µl diver containing 4 large amoebae.
2. 1.2µl diver containing 3 large amoebae.
3. 1.5µl diver containing 4 large amoebae.
4. 1.5µl diver - control.

	Time (min.)	0	30	60	90	120	150	180
	left	752	749	733	704	670	710	719
1.	right	575	564	536	500	453	485	484
	diff.	177	185	197	204	217	225	235
	left	1004	990	968	925	917	948	950
2.	right	430	409	380	331	316	340	336
	diff.	574	581	588	594	601	608	614
	left	1077	1043	1039	999	994	1028	1028
3.	right	396	364	341	290	279	301	290
	diff.	681	679	698	709	715	727	738
4.	left	832	795	775	727	724	751	741
	right	556	519	499	451	449	473	463
	diff.	276	276	276	276	275	278	278

Appendix 12 (contd.)

Food concentration 4000.500μl^{-1} (20^{o}C).

1. 1.8µl diver containing 6 medium amoebae.
2. 1.5µl diver containing 4 medium amoebae.
3. 1.2µl diver containing 3 medium amoebae.
4. 1.5µl diver - control.

	Time (min.)	0	30	60	90	120	150	180
	left	766	788	774	755	801	805	805
1.	right	689	701	680	654	689	681	673
	diff.	77	87	94	101	112	124	132
	left	576	600	601	582	631	615	626
2.	right	801	817	804	777	817	792	797
	diff.	-225	-217	-203	-195	-186	-177	-171
	left	998	1012	1014	989	1048	1024	1057
3.	right	524	540	534	504	547	517	540
	diff.	474	472	480	485	501	507	517
	left	819	817	804	783	835	807	851
4.	right	671	669	656	635	688	657	702
	diff.	148	148	148	148	147	150	149

Food concentration 4000.500μl^{-1} (20^{o}C).

1. 1.5µl diver containing 4 large amoebae.
2. 1.2µl diver containing 3 large amoebae.
3. 1.5µl diver containing 4 large amoebae.
4. 1.5µl diver - control.

	Time (min.)	0	30	60	90	120	150	180
	left	752	749	733	704	670	710	719
1.	right	575	564	536	500	453	485	484
	diff.	177	185	197	204	217	225	235
	left	1004	990	968	925	917	948	950
2.	right	430	409	380	331	316	340	336
	diff.	574	581	588	594	601	608	614
	left	1077	1043	1039	999	994	1028	1028
3.	right	396	364	341	290	279	301	290
	diff.	681	679	698	709	715	727	738
4.	left	832	795	775	727	724	751	741
	right	556	519	499	451	449	473	463
	diff.	276	276	276	276	275	278	278

Appendix 12 (contd.)

Food concentration 125.500μl^{-1} (15°C).

1. 1.2µl diver containing 4 large amoebae.
2. 1.2µl diver containing 2 large amoebae.
3. 1.2µl diver containing 3 large amoebae.
4. 1.2µl diver - control.

	Time (min.)	0	30	60	90	120	150	180
	left	643	632	706	692	682	702	700
1.	right	575	559	634	609	589	604	597
	diff.	68	73	72	83	93	98	103
	left	954	909	1019	997	981	993	1010
2.	right	401	355	464	440	421	432	443
	diff.	553	554	555	557	560	561	567
	left	389	352	463	436	449	442	452
3.	right	693	657	761	731	736	725	729
	diff.	-304	-305	-298	-295	-287	-283	-277
	left	833	797	893	873	888	871	871
4.	right	453	417	514	494	507	490	492
	diff.	380	380	379	379	381	381	379

Food concentration 125.500μl^{-1} (15°C).

1. 1.2µl diver containing 2 large amoebae.
2. 1.2µl diver containing 4 medium amoebae.
3. 1.26µl diver containing 4 small amoebae.
4. 1.2µl diver -control.

	Time (min.)	0	30	60	90	120	150	180
	left	918	940	947	942	932	928	910
1.	right	606	627	627	620	603	599	578
	diff.	312	313	320	322	329	329	332
	left	630	658	667	649	649	643	663
2.	right	754	783	785	762	758	747	759
	diff.	-124	-125	-118	-113	-109	-104	- 96
	left	1057	1085	1080	1067	1055	1057	1074
3.	right	513	540	532	517	502	503	517
	diff.	544	545	548	550	553	554	557
	left	684	708	706	691	679	678	711
4.	right	718	743	739	722	711	712	746
	diff.	- 35	- 35	- 33	- 31	- 32	- 34	- 35

Appendix 12 (contd.)

Food concentration 125.500μl^{-1} (15^{o}C).

1. 1.2µl diver containing 3 small amoebae.
2. 1.2µl diver containing 4 small amoebae.
3. 1.2µl diver containing 4 small amoebae.
4. 1.26µl diver - control.

	Time (min.)	0	30	60	90	120	150	180
	left	-	630	723	743	775	783	770
1.	right	-	628	715	733	762	768	750
	diff.	-	2	8	10	13	15	20
	left	441	446	545	566	590	626	586
2.	right	716	715	809	824	847	877	832
	diff.	-275	-269	-264	-258	-257	-251	-246
	left	991	984	1078	1098	1114	1145	1109
3.	right	425	419	510	529	544	572	531
	diff.	566	565	568	569	570	573	578
	left	963	955	1057	1079	1078	1106	1068
4.	right	431	422	525	547	546	575	536
	diff.	532	533	532	532	532	531	532

Food concentration 125.500μl^{-1} (15^{o}C).

1. 1.26µl diver containing 4 medium amoebae.
2. 1.2µl diver containing 4 medium amoebae.
3. 1.2µl diver containing 3 medium amoebae.
4. 1.2µl diver - control.

	Time (min.)	0	30	60	90	120	150	180
	left	1174	1169	1124	1121	1099	1064	1237
1.	right	424	418	368	365	338	301	459
	diff.	750	751	756	756	761	763	778
	left	873	868	820	818	799	765	943
2.	right	572	568	518	515	494	458	615
	diff.	299	300	302	303	305	307	328
	left	787	766	759	730	706	688	838
3.	right	620	598	590	558	531	511	654
	diff.	167	168	169	172	175	177	184
	left	749	702	699	672	647	629	804
4.	right	655	606	605	578	553	535	690
	diff.	94	96	94	94	94	94	94

Appendix 12 (contd.)

Food concentration 1000.500µl^{-1} (15^{o}C)

1. 1.26µl diver containing 3 small amoebae.
2. 1.2µl diver containing 2 small amoebae.
3. 1.2µl diver containing 4 small amoebae.
4. 1.2µl diver - control.

	Time (min.)	0	30	60	90	120	150	180
	left	987	973	954	947	949	908	947
1.	right	383	373	352	340	340	298	335
	diff.	604	600	602	607	609	610	612
	left	952	941	920	908	912	885	918
2.	right	398	388	365	347	350	320	352
	diff.	554	553	555	561	562	565	566
	left	544	536	520	528	510	510	526
3.	right	641	616	596	596	572	564	572
	diff.	-97	-80	-76	-68	-62	-54	-46
	left	707	685	670	677	646	665	660
4.	right	541	519	503	512	480	498	494
	diff.	166	166	167	165	166	167	166

Food concentration 1000.500µl^{-1} (15^{o}C).

1. 1.2µl diver control.
2. 1.2µl diver containing 4 medium amoebae.
3. 1.2µl diver containing 3 medium amoebae.
4. 1.26µl diver containing 4 medium amoebae.

	Time (min.)	0	3o	60	90	120	150	180
	left	601	590	595	574	568	540	511
1.	right	659	652	656	636	629	601	572
	diff.	-58	-62	-61	-62	-61	-61	-61
	left	599	597	592	581	574	546	523
2.	right	669	664	653	636	622	588	556
	diff.	-70	-67	-61	-55	-48	-42	-33
	left	962	970	945	922	927	904	870
3.	right	454	464	438	407	408	383	344
	diff.	508	506	507	515	519	521	526
	left	1056	1063	1042	1021	1018	1002	959
4.	right	396	400	373	348	341	324	277
	diff.	660	663	669	673	677	678	682

Appendix 12 (contd.)

Food concentration 1000.500µl^{-1} (15°C).

1. 1.2µl diver containing 4 large amoebae.
2. 1.2µl diver containing 2 large amoebae.
3. 1.2µl diver containing 3 large amoebae.
4. 1.26µl diver - control.

	Time (min.)	0	60	90	120	150	180	210
	left	650	617	698	615	615	594	586
1.	right	604	546	619	528	517	485	470
	diff.	46	71	79	87	98	109	116
	left	909	886	945	872	-	845	836
2.	right	421	396	453	376	-	334	320
	diff.	488	490	492	496	-	511	516
	left	731	730	715	699	671	675	665
3.	right	711	697	674	653	618	614	595
	diff.	20	33	41	46	53	61	70
	left	871	861	865	856	841	850	833
4.	right	438	428	429	423	407	416	399
	diff.	433	433	432	433	434	434	434

Food concentration 1000.500µl^{-1} (15°C).

1. 1.2µl diver containing 4 small amoebae.
2. 1.2µl diver containing 3 large amoebae.
3. 1.26µl diver containing 4 medium amoebae.
4. 1.2µl diver - control.

	Time (min.)	0	30	60	90	120	150	210
	left	968	961	924	889	846	854	919
1.	right	509	498	457	416	366	354	416
	diff.	459	463	467	473	480	500	503
	left	657	658	635	585	554	560	652
2.	right	652	650	618	564	524	504	579
	diff.	5	8	17	21	30	56	73
	left	952	952	927	872	845	858	943
3.	right	476	473	444	385	351	337	414
	diff.	476	479	483	487	494	521	529
	left	617	602	565	510	484	494	567
4.	right	670	657	617	563	536	547	620
	diff.	- 53	- 55	- 52	- 53	- 52	- 53	- 53

Appendix 12 (contd.)

Food concentration 1000.500μl^{-1} (15°C).

1. 1.2µl diver containing 4 large amoebae.
2. 1.2µl diver containing 2 large amoebae.
3. 1.2µl diver containing 3 large amoebae.
4. 1.26µl diver - control.

	Time (min.)	0	60	90	120	150	180	210
	left	650	617	698	615	615	594	586
1.	right	604	546	619	528	517	485	470
	diff.	46	71	79	87	98	109	116
	left	909	886	945	872	-	845	836
2.	right	421	396	453	376	-	334	320
	diff.	488	490	492	496	-	511	516
	left	731	730	715	699	671	675	665
3.	right	711	697	674	653	618	614	595
	diff.	20	33	41	46	53	61	70
	left	871	861	865	856	841	850	833
4.	right	438	428	429	423	407	416	399
	diff.	433	433	432	433	434	434	434

Food concentration 1000.500μl^{-1} (15°C).

1. 1.2µl diver containing 4 small amoebae.
2. 1.2µl diver containing 3 large amoebae.
3. 1.26µl diver containing 4 medium amoebae.
4. 1.2µl diver - control.

	Time (min.)	0	30	60	90	120	150	210
	left	968	961	924	889	846	854	919
1.	right	509	498	457	416	366	354	416
	diff.	459	463	467	473	480	500	503
	left	657	658	635	585	554	560	652
2.	right	652	650	618	564	524	504	579
	diff.	5	8	17	21	30	56	73
	left	952	952	927	872	845	858	943
3.	right	476	473	444	385	351	337	414
	diff.	476	479	483	487	494	521	529
	left	617	602	565	510	484	494	567
4.	right	670	657	617	563	536	547	620
	diff.	- 53	- 55	- 52	- 53	- 52	- 53	- 53

Appendix 12 (contd.)

Food concentration 4000μl^{-1} (15°C).

1. 1.26µl diver containing 3 small amoebae.
2. 1.2µl diver containing 5 small amoebae.
3. 1.2µl diver containing 4 small amoebae.
4. 1.5µl diver- control.

	Time (min.)	0	30	60	90	120	150
	left	869	900	813	808	806	799
1.	right	463	493	402	396	392	384
	diff.	406	407	411	412	414	415
	left	855	886	805	798	793	787
2.	right	475	502	414	405	399	389
	diff.	380	384	391	393	394	398
	left	518	545	450	451	443	439
3.	right	680	705	607	605	590	583
	diff.	-162	-160	-157	-154	-147	-144
	left	869	801	834	835	821	818
4.	right	294	226	258	261	247	243
	diff.	575	575	575	574	574	575

Food concentration 4000μl^{-1} (15°C).

1. 1.2µl diver containing 5 medium amoebae.
2. 1.2µl diver containing 5 medium amoebae.
3. 1.26 diver containing 5 medium amoebae.
4. 1.5µl diver containing control.

	Time (min).	0	30	60	90	120	150	180
	left	610	596	574	559	672	656	637
1.	right	467	451	420	397	504	481	457
	diff.	143	145	154	162	168	175	180
	left	792	783	746	744	856	831	830
2.	right	365	346	301	295	395	363	356
	diff.	427	437	445	449	461	468	474
	left	864	852	824	824	926	904	900
3.	right	308	291	250	251	341	309	300
	diff.	556	561	574	573	585	595	600
	left	747	702	647	633	694	690	681
4.	right	376	331	277	262	324	320	310
	diff.	371	371	370	371	370	370	371

Appendix 12 (contd.)

Food concentration 4000μl^{-1} (15°C).

1. 1.26µl diver containing 3 large amoebae.
2. 1.2µl diver containing 3 large amoebae.
3. 1.2µl diver containing 4 large amoebae.
4. 1.5µl diver - control.

	Time (min.)	0	30	60	90	120	150	180
	left	1208	1289	1172	1222	1255	1259	1297
1.	right	203	278	154	198	223	218	249
	diff.	1005	1011	1018	1024	1032	1041	1048
	left	903	984	867	926	956	976	994
2.	right	385	460	339	389	413	427	440
	diff.	518	524	528	537	543	549	554
	left	521	500	515	514	521	498	474
3.	right	197	174	184	174	177	148	115
	diff.	324	326	331	340	344	350	359
	left	635	631	618	621	559	611	614
4.	right	224	219	205	211	148	200	204
	diff.	411	412	413	410	411	411	410

Food concentration 4000μl^{-1} (15°C).

1. 1.26µl diver - control.
2. 1.2µl diver containing 4 large amoebae.
3. 1.2µl diver containing 5 small amoebae.
4. 1.2µl diver containing 3 medium amoebae.

	Time (min.)	0	30	60	90	120	150	180
	left	1049	1025	1030	1020	1014	1005	983
1.	right	229	205	212	201	196	185	163
	diff.	820	820	818	819	818	820	820
	left	801	788	801	798	792	790	775
2.	right	359	345	349	340	323	310	285
	diff.	442	443	452	458	469	480	490
	left	608	599	610	609	603	594	575
3.	right	460	450	456	448	438	423	395
	diff.	148	149	154	161	165	171	180
	left	544	532	558	549	543	527	514
4.	right	487	473	495	478	468	448	431
	diff.	57	59	63	71	75	79	83

Appendix 12 (contd.)

Food concentration 125.500μl^{-1} ($10^{o}C$).

1. 1.2μl diver containing 3 large amoebae.
2. 1.5μl diver containing 3 large amoebae.
3. 1.2μl diver - control.
4. 1.2μl diver containing 3 large amoebae.

	Time (min.)	0	30	60	90	120	150	180
	left	635	623	647	656	656	646	660
1.	right	588	570	586	587	583	563	572
	diff.	47	53	61	69	73	83	88
	left	645	667	673	680	680	675	684
2.	right	559	571	572	572	571	560	562
	diff.	86	96	101	108	109	115	122
	left	820	840	838	839	840	835	835
3.	right	451	469	468	469	469	465	463
	diff.	369	371	370	370	371	370	372
	left	330	352	364	370	373	365	386
4.	right	227	243	246	242	236	221	237
	diff.	103	109	118	128	137	144	149

Food concentration 125.500μl^{-1} ($10^{o}C$).

1. 1.5μl diver containing 3 large amoebae.
2. 1.2μl diver containing 3 medium amoebae.
3. 1.5μl diver containing 4 small amoebae.
4. 1.26μl diver - control.

	Time (min.)	0	30	60	90	120	150
	left	850	843	827	820	817	817
1.	right	508	488	460	447	434	426
	diff.	342	355	367	373	383	391
	left	700	684	676	667	665	672
2.	right	597	574	561	546	536	539
	diff.	103	110	115	121	129	133
	left	777	777	761	754	765	757
3.	right	535	521	504	490	497	485
	diff.	242	256	257	264	268	272
	left	1083	1077	1057	1050	1050	1043
4.	right	326	320	298	292	292	285
	diff.	757	757	759	758	758	758

Appendix 12 (contd.)

Food concentration 125.500ul^{-1} (10^{o}C).

1. 1.2µl diver containing 4 small amoebae.
2. 1.2µl diver containing 2 small amoebae.
3. 1.5µl diver containing 5 small amoebae.
4. 1.26µl diver - control.

	Time (min.)	0	30	60	90	120	150
	left	732	710	683	696	662	630
1.	right	484	455	419	426	383	346
	diff.	248	255	264	270	279	284
	left	695	670	655	657	611	588
2.	right	488	462	441	436	389	362
	diff.	207	208	214	221	222	226
	left	684	642	654	633	600	594
3.	right	498	448	450	426	387	376
	diff.	186	194	204	207	213	218
	left	1092	1053	1052	1022	994	992
4.	right	228	190	189	159	129	123
	diff.	864	863	863	863	865	864

Food concentration 125.500µl^{-1} (10^{o}C).

1. 1.26µl diver - control.
2. 1.5µl diver containing 3 medium amoebae.
3. 1.2µl diver containing 3 medium amoebae.
4. 1.5µl diver containing 2 medium amoebae.

	Time (min.)	0	30	60	90	120	150	180
	left	1068	1029	1012	994	972	975	1042
1.	right	304	266	248	231	209	211	278
	diff.	764	763	764	763	763	764	764
	left	549	513	501	473	474	465	545
2.	right	625	582	568	533	528	513	591
	diff.	- 76	- 69	- 67	- 60	- 54	- 48	- 46
	left	696	662	653	623	627	619	707
3.	right	521	480	466	432	430	419	500
	diff.	175	182	187	191	197	200	207
	left	1017	968	973	943	965	944	1035
4.	right	310	260	261	225	245	220	310
	diff.	707	708	712	718	720	724	725

Appendix 12 (contd.)

Food concentration 1000.500μl^{-1} (10°C).

1. 0.9µl diver containing 4 large amoebae.
2. 1.2µl diver containing 5 large amoebae.
3. 1.5µl diver - control.
4. 1.2µl diver containing 4 large amoebae.

	Time (min.)	0	30	60	90	120	150	180
	left	651	688	639	637	649	668	580
1.	right	595	614	548	530	525	523	414
	diff.	56	74	91	107	124	145	166
	left	1254	1249	1229	1270	1274	1255	1228
2.	right	229	201	159	177	163	120	76
	diff.	1025	1048	1070	1093	1111	1135	1152
	left	1195	1159	1153	1153	1154	1105	1100
3.	right	270	235	227	227	229	180	175
	diff.	925	924	926	926	925	925	925
	left	1065	1020	1026	1027	1035	967	989
4.	right	368	312	309	305	303	221	240
	diff.	697	708	717	722	732	746	749

Food concentration 1000.500μl^{-1} (10°C).

1. 1.2µl diver - control.
2. 1.5µl diver containg 4 small amoebae.
3. 1.5µl diver containing 3 small amoebae.

	Time (min.)	0	30	60	90	120	180	210
	left	768	806	805	808	790	778	817
1.	right	332	369	368	371	353	341	380
	diff.	436	437	437	437	437	437	437
	left	787	819	822	827	807	818	840
2.	right	328	358	354	356	333	325	352
	diff.	459	461	468	471	474	485	488
	left	794	824	825	826	820	840	833
3.	right	329	354	351	347	340	354	341
	diff.	465	470	474	479	480	486	492

Appendix 12 (contd.)

Food concentration 1000.500µl^{-1} (10°C).

1. 1.5µl diver - control.
2. 1.5µl diver containing 4 large amoebae.
3. 1.5µl diver containing 4 small amoebae.
4. 1.2µl diver containing 3 small amoebae.

	Time (min.)	0	30	60	90	120	150	180
	left	880	952	968	1004	1022	1030	1025
1.	right	323	394	410	447	465	472	467
	diff.	557	558	558	557	557	558	558
	left	779	880	895	913	950	940	937
2.	right	412	507	512	525	544	526	517
	diff.	367	373	383	388	406	414	420
	left	701	708	686	712	729	733	751
3.	right	278	280	251	273	285	285	296
	diff.	423	428	435	439	444	448	455
	left	960	985	1019	1033	1066	1054	1040
4.	right	396	412	436	448	477	457	439
	diff.	564	573	583	585	589	597	601

Food concentration 1000.500µl^{-1} (10°C).

1. 1.5µl diver - control.
2. 1.5µl diver containing 5 medium amoebae.
3. 1.2µl diver containing 5 medium amoebae.
4. 0.9µl diver containing 3 medium amoebae.
5. 0.66µl diver containing 2 medium amoebae.

	Time (min.)	0	30	60	90	120	150
	left	878	924	934	937	912	938
1.	right	254	301	309	312	287	313
	diff.	624	623	625	625	625	625
	left	840	850	863	873	841	894
2.	right	339	340	344	345	307	352
	diff.	501	510	519	528	534	542
	left	1233	1239	1257	1252	1221	1277
3.	right	119	122	130	118	74	125
	diff.	1114	1117	1127	1134	1147	1152
	left	702	715	724	711	670	722
4.	right	438	449	449	431	388	436
	diff.	264	266	275	280	282	286
	left	1136	1141	1157	1135	1073	1189
5.	right	201	194	206	174	108	213
	diff.	935	947	951	961	965	976

Appendix 12 (contd.)

Food concentration 4000.500μl^{-1} (10°C).

1. 1.5µl diver containing 4 small amoebae.
2. 1.2µl diver containing 4 small amoebae.
3. 1.5µl diver containing 5 small amoebae.
4. 1.2µl diver - control.

	Time (min.)	0	60	90	120	150	180	210
	left	518	511	512	499	477	479	630
1.	right	574	554	552	531	507	504	648
	diff.	- 56	- 43	- 40	- 32	- 30	- 25	- 18
	left	924	928	934	927	908	901	1050
2.	right	296	284	282	270	253	236	379
	diff.	628	644	652	657	655	665	671
	left	383	405	390	387	380	358	516
3.	right	633	633	613	601	587	559	708
	diff.	-250	-228	-223	-214	-207	-201	-192
	left	403	411	395	377	372	353	385
4.	right	619	628	611	594	588	569	601
	diff.	-216	-217	-216	-217	-216	-216	-216

Food concentration 4000.500μl^{-1} (10°C).

1. 1.5µl diver containing 5 medium amoebae.
2. 1.2µl diver containing 3 medium amoebae.
3. 1.2µl diver containing 3 medium amoebae.
4. 1.5µl diver - control.

	Time (min.)	0	60	90	120	150	180	210
	left	881	888	863	857	906	911	914
1.	right	691	683	653	643	685	684	685
	diff.	190	205	210	214	221	227	229
	left	1239	1228	1227	1200	1264	1255	1262
2.	right	469	451	444	415	473	461	463
	diff.	770	777	783	785	791	794	799
	left	682	678	690	649	721	715	729
3.	right	811	798	806	761	829	818	830
	diff.	-129	-120	-116	-112	-108	-103	-101
	left	438	396	402	377	434	393	380
4.	right	997	955	960	936	993	951	939
	diff.	-559	-559	-558	-559	-559	-558	-559

Appendix 12 (contd.)

Food concentration 4000.500μl^{-1} (10°C).

1. 1.2µl diver containing 3 large amoebae.
2. 1.5µl diver containing 3 large amoebae.
3. 1.2µl diver containing 4 large amoebae.
4. 1.5µl diver - control.

	Time (min.)	0	60	90	120	150	180	240
	left	641	585	565	554	543	526	485
1.	right	623	548	523	510	494	468	415
	diff.	18	37	42	44	49	58	70
	left	731	675	668	664	659	628	589
2.	right	545	473	459	447	433	389	331
	diff.	186	202	209	217	226	239	258
	left	980	936	918	912	905	872	823
3.	right	369	313	290	277	266	224	161
	diff.	611	623	628	635	649	648	662
	left	843	811	803	786	763	733	679
4.	right	411	380	371	354	331	301	248
	diff.	432	431	432	432	432	432	431

Food concentration 4000.500μl^{-1} (10°C).

1. 1.2µl diver containing 5 small amoebae.
2. 1.2µl diver containing 5 medium amoebae.
3. 1.5µl diver containing 4 large amoebae.
4. 1.2µl diver - control.

	Time (min.)	0	30	60	90	120	150	180
	left	762	782	784	784	803	815	837
1.	right	757	773	773	767	780	786	805
	diff.	5	9	11	17	23	29	32
	left	1033	1056	1058	1045	1086	1099	1108
2.	right	581	598	593	575	609	612	614
	diff.	452	458	465	470	477	487	494
	left	568	559	570	569	600	615	617
3.	right	920	900	909	898	924	928	921
	diff.	-352	-341	-339	-329	-324	-313	-304
	left	688	673	695	681	669	682	639
4.	right	574	559	580	566	555	567	524
	diff.	114	114	115	115	114	115	115

Appendix 13.

1. A comparison of the published respiration data for the naked amoebae.

2. The volume estimates used to convert the oxygen consumption values per individual to unit protoplasm (μm^3) terms.

A. proteus (Emerson, 1929). The cell volume at 20°C was equal to 859,000μm^3 (present study).

Chaos chaos. An average cell volume of 35,145,000μm^3 was reported by Pace and Belda (1944).

This value was used for;

Pace and Frost (1952)
Pace and Kimura (1944)
Claff and Tahmisian (1949)

Chaos chaos. (Holter and Zeuthen, 1948).

A range of cell volumes was reported;

50,000,000 - 98,000,000μm^3.

Chaos chaos. (Scholander et al, 1952).

A range of values was extrapolated from the graph presented by these authors;

5,500,000 - 15,000,000μm^3.

Acanthamoeba. An average value of 3,365μm^3 was used.

(Byers et al, 1969).

The same value was also used for the data of Hamburger, 1975.

Mayorella palestinensis. A value of 7,891μm^3 was calculated.

The dry weight of 10 million amoebae was equal to 11.6mg (Reich, 1948). Using the conversion 0.147pg.μm^{-3}, the volume estimate was derived.

Appendix 13.

Species	Author	Oxygen cons. $\mu l\ h^{-1}\ ind^{-1}$	Oxygen cons. $\mu l\ h^{-1}\ \mu m^{-3}$	T. °C.
A. proteus	Emerson (1929)	1.37×10^{-4}	1.60×10^{-10}	20
A. proteus	Present study	5.05×10^{-4}	5.40×10^{-10}	20
		3.31×10^{-4}	2.61×10^{-10}	15
		4.59×10^{-4}	2.34×10^{-10}	10
C. chaos	Pace and Belda. (1944)	5.04×10^{-3}	1.44×10^{-10}	15
		7.05×10^{-3}	1.91×10^{-10}	20
		9.01×10^{-3}	2.45×10^{-10}	25
		13.24×10^{-3}	3.72×10^{-10}	30
		17.75×10^{-3}	5.08×10^{-10}	35
C. chaos	Scholander et al. (1952)	1.80×10^{-3}	3.27×10^{-10}	
		2.30×10^{-3}	2.87×10^{-10}	
		3.50×10^{-3}	3.68×10^{-10}	
		3.60×10^{-3}	3.56×10^{-10}	25
		4.00×10^{-3}	4.44×10^{-10}	
		5.00×10^{-3}	8.33×10^{-10}	
		4.50×10^{-3}	3.46×10^{-10}	
		5.50×10^{-3}	3.67×10^{-10}	
C. chaos	Holter and Zeuthen. (1948)	2.30×10^{-2}	2.35×10^{-10}	
		1.10×10^{-2}	2.04×10^{-10}	
		1.30×10^{-2}	1.67×10^{-10}	21
		1.40×10^{-2}	1.69×10^{-10}	
		2.20×10^{-2}	4.40×10^{-10}	
C. chaos	Claff and Tahmisian (1949)	6.00×10^{-2}	1.71×10^{-9}	20
P. carolinensis = C. chaos	Pace and Kimura (1944)	2.44×10^{-3}	6.95×10^{-11}	10
		5.59×10^{-3}	1.59×10^{-10}	20
		1.17×10^{-2}	3.32×10^{-10}	30
		2.69×10^{-2}	7.67×10^{-10}	35

Appendix 13 (contd.)

Species	Author	Oxygen cons. $\mu l\ h^{-1}\ ind^{-1}$	Oxygen cons. $\mu l\ h^{-1}\ \mu m^{-3}$	T.°C.
P. carolinensis = C. chaos	Pace and Frost. (1952)	9.80×10^{-3} 7.20×10^{-3} 1.07×10^{-2} 5.00×10^{-3}	2.79×10^{-10} 2.05×10^{-10} 3.04×10^{-10} 1.42×10^{-10}	25
Acanthamoeba	Byers et al (1969)	2.54×10^{-5}	7.55×10^{-9}	30
Acanthamoeba	Hamburger (1975)	2.70×10^{-5}	8.02×10^{-9}	30/31
Mayorella paelstinensis	Reich (1948)	8.30×10^{-6} 1.70×10^{-5}	1.05×10^{-9} 2.15×10^{-9}	27

Appendix 14.

Sarcodina - Flanders moss (Sphagnum bog-pool).

Species and monthly occurrence.

numbers represent species per $1000cm^3$. at sites A and B.

Testacea species.	J A	J B	F A	F B	M A	M B	A A	A B	M A	M B	J A	J B	J A	J B	A A	A B	S A	S B	O A	O B	N A	N B	D A	D B
Antarcella pseudarcella (Penard)			1719	1271			1329																	
Arcella catinus (Penard)					1827																			
A. dentata (Ehrenberg)		2396																						
A. discoides (Ehrenberg)	30450	45524	41269	**34313**	17863	34712	40320	17274	6154	17476	8273	40474	43128	60480	59961	41106	48719	15224	88739	22967	10293	34654	35279	12709
A. mitrata (Leidy)	7188	1719					5040		1589	2758														
A. polypora (Penard)										1379	4497	2396							13050					
A. vulgaris (Ehrenberg)								1329													1507			
Assulina seminulum (Ehrenberg)	3045																							
Bullinularia indica (Penard)							2520																	
Centropyxis aerophila v. sphagnicola (Deflandre)	3045				3654		2520	1329		7943	4136	4497		9584	2520									

Testacea species.	J A	J B	F A	F B	M A	M B	A A	A B	M A	M B	J A	J B	J A	J B	A A	A B	S A	S B	O A	O B	N A	N B	D A	D B
C. arcelloides (Penard)	3045						5040						2396								2058			
Centropyxis (sp.) (Stein)							5040		9231	17476	4136	2248			2520	7495	9135		2610		13560			
Cryptodifflugia compressa (Penard)													4792											
Cucurbitella mesiliformis (Penard)		2396									2248				3747				5220					
Difflugia bacillifera (Penard)					1624				1538				2396											
D. oblonga (Ehrenberg)	3045		1271						1538	1589	1379	2248	9584	5040		4567	2707		2988		1507			
D. oblonga v. longicollis (Gassowsky)					1624																			
D. rubescens (Penard)			1719	1271			7560	2657	3077	12710	28956	13491	35940	25200	26233	4567	2707		7830				12709	
D. tuberculata (Wallich)						1827																		
Difflugia (sp.) (Leclerc)	6090			1624			2657	1538			11031	4497			11243									
Difflugiella oviformis (Penard)			1271						1538															
Diplochlamys fragilis (Penard)	1271																							

Testacea species.	J		F		M		A		M		J		J		A		S		O		N		D	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Diplochlamys (sp.) (Greeff)																							5040	
Euglypha ciliata (Ehrenberg)			1719		2542		1827				3077				2248		9584		10080		7495		18269	
E. compressa (Carter)	6090	4792	6878		2542	3248	1827		2520		1329		3077	6355	2758		2248	4792	5040		3747		18269	18946
E. laevis (Ehrenberg)			1719																					
Euglypha (sp.) (Dujardin)																								
Heleopera (sp.) (Leidy)											1329													
Hyalosphenia cuneata (Stein)																								
H. elegans (Leidy)		2396			1271	1624											2397	5040						
H. papilio (Leidy)			343	9	1271																			
Hyalosphenia (sp.) (Stein)			1719						2520						2248	4792							5040	
Lesquereusia modesta (Rhumbler)	2396						1827																	
Microcorycia flavia (Greef)												1538												

Testacea species.	J A	J B	F A	F B	M A	M B	A A	A B	M A	M B	J A	J B	J A	J B	A A	A B	S A	S B	O A	O B	N A	N B	D A	D B
Nebela collaris (Ehrenberg)					1827				1379				2397	2520			8120	1522						
N. dentistoma (Penard)			3439	1271						1589														
N. flabellulum (Leidy)			3439		1827	5040	1329	4615	12710		2758	2248	64691	45360	13702		5413	1522	4176		6176	4520		6355
N. militaris (Penard)											4497						2707	1522	2088		4117		5040	6355
N. minor (Penard)																								
Nebella (sp.) (Leidy)					3248																			
Parmulina cyanthus (Penard)						3654																		
Penardochlamys arcelloides (Deflandre)									1589															
Phryganella hemisphaerica (Penard)					1827																			
Placocista spinosa (Leidy)															3747		1522							
Plagiopyxis callida (Penard)																						1507		
Pontigulasia spectabilis (Penard)											2248		2397											

Testacea species.	J A.B	F A B	M A B	A A B	M A B	J A B	J A B	A A B	S A B	O A B	N A B	D A B
Pontigulasia (sp.) (Rhumbler)				1329							2058	
Pyxidicula operculata (Ehrenberg)	3045	1719										
Sexangularia polyedra (Deflandre)			2542 1624				2397	4567				
Sexangularia (sp.) (Awerintzew)	3045			2520 1329								5040
Trigonopyxis arcula (Leidy)			1827									
Trinema lineare (Penard)											2058	
Wailesella (sp.) (Deflandre)							2520					6355
Unidentified testate amoebae												
<100µm	12179	1719 5083 1624		2520 1329 6154 6355 6894 13491			2397 2520	7495 4567	1522		8234	
>100µm	3045		1827	2520 3986 1538 4766			2520				4117 1507	

Actinopoda species.	J A	J B	F A	F B	M A	M B	A A	A B	M A	M B	J A	J B	J A	J B	A A	A B	S A	S B	O A	O B	N A	N B	D A	D B
Actinophrys sol (Ehrenberg)	39583	19168	10317	25417	139656	84040	126000	43849	4615	14298	20682	24734	11979	20160	44971	45673	37892	27404	44369	79341	55581	61774	40319	6355
Actinosphaerium eichhornii (Ehrenberg)					1624																2058			
Acanthocystis chaetophora (Schrank)		2396					5040	1329																

Naked amoebae species >200µm	J A	J B	F A	F B	M A	M B	A A	A B	M A	M B	J A	J B	J A	J B	A A	A B	S A	S B	O A	O B	N A	N B	D A	D B
Amoeba laureata (Penard)															3747									
Amoeba proteus (Leidy)		2396											7560		4567									
Chaos carolinense (Wilson)							1538						2520											
Chaos (sp.) (Linnaeus)															22837									
Polychaos dubium (Schaeffer)							4615																	
Trichamoeba (sp.) (Fromentel)																			2610					
Unidentified (sp.)																								

Naked amoebae species < 200um	J A	J B	F A	F B	M A	M B	A A	A B	M A	M B	J A	J B	J A	J B	A A	A B	S A	S B	O A	O B	N A	N B	D A	D B
Acanthamoeba (sp.) (Vovkonsky)																	3945							
Cochliopodium (sp.) (Hertwig and Lesser)											4136													
Gocevia fonbrunei (Pussard)							2520																	
Hartmannella (sp.) (Alexeieff)				1827 1624																				
Mayorella (sp.) (Schaeffer)	3045		1271								2248										6027			
Rugipes bilzi (Schaeffer)																								
Thecamoeba striata (Penard)			1271								4136	2248					5413		2610		4117			
Thecamoeba (sp.) (Fromentel)											5040													
Valkampfia (sp.) (Chatton-Lalung-Bonnaire)	2396		1624								2520	4792												
Unidentified (sp.)									1538	3177 1379					3747	4567					1507		6355	

Appendix 15.

Numbers of flagellates and ciliates ml^{-1}

from a Sphagnum bog-pool.

Sampling date	Mid-morn. temp. °C.	pH	Numbers represent the mean of 50, 5µl drops expressed in terms of per ml. Flagellates in 3 size classes (μm^3) 50	50–100	100	Ciliates in 3 size classes (μm^3) 50	50–100	100
25.1.77	2.0	3.8	12	0	0	8	0	0
24.2.77	2.0	3.8	24	4	0	48	0	0
24.3.77	7.5	3.6	360	20	0	72	44	4
28.4.77	11.0	3.6	384	0	0	84	16	4
26.5.77	13.0	3.6	9840	680	0	1120	240	160
21.6.77	13.5	3.7	41360	2320	0	2000	880	80
22.7.77	14.6	3.8	21520	880	0	2320	320	0
25.8.77	12.3	3.6	2920	40	0	240	0	0
23.9.77	10.0	3.6	2840	80	0	80	40	200
29.10.77	10.5	3.5	1800	24	0	24	8	8
24.11.77	6.0	3.7	832	4	0	36	0	0
22.12.77	5.0	3.7	412	0	0	16	8	0

Appendix 16.

The mean number of rotifers, nematodes and diatom/desmids in a Sphagnum mass. (numbers per 1000cm^3).

Months	Rotifers	Nematodes	Diatoms/Desmids (x 10^8)
J	69957	5765	1.34
F	17718	9009	2.14
M	9439	2639	6.18
A	17319	27399	3.92
M	47702	5510	4.93
J	45703	10627	7.02
J	56658	17144	12.70
A	45146	72199	19.50
S	74177	16155	2.06
O	36278	20067	1.86
N	29275	31184	1.76
D	50508	40538	2.36

Appendix 17.

Dates	Days	$T^{O}C$	D	N_0 (x 10^9)	N_1 (x 10^9)	P (x 10^9)	P diurnal (x 10^9)
25.1.77 - 24.2.77	34	2	-	3.602	0	0	0
24.2.77 - 24.3.77	35	5	-	0	0	0	0
24.3.77 - 28.4.77	37	9	62.50	0	6.684	1.978	0.053
28.4.77 - 26.5.77	33	12	7.33	6.684	0	15.046	0.456
26.5.77 - 21.6.77	35	13	5.67	0	0	0	0
21.6.77 - 22.7.77	31	14	4.62	0	7.948	26.666	0.860
22.7.77 - 25.8.77	34	13	5.67	7.948	26.415	103.029	3.030
25.8.77 - 23.9.77	29	11	10.42	26.415	2.943	40.854	1.409
23.9.77 - 29.10.77	36	10	17.84	2.943	2.679	5.673	0.157
29.10.77 - 24.11.77	26	8	-	2.679	0	0	0
24.11.77 - 22.12.77	28	5	-	0	0	0	0

Notes to Appendix 17.

1. $T^{o}C$ = The mean temperature in degress centigrade, spanning the period concerned.

2. D = The generation time in days. Appendix Figure 3 was used to estimate the generation time for each temperature.

3. N_o = The biomass on the first date of the period concenred. Values are in volume units (μm^3 per $1000cm^3$).

   Decreased temperature was found to increase the cell volume. The required volumes for individual amoebae at the various temperatures were read directly from Appendix Figure 4.

   N_1 = The biomass (μm^3 per 1000 cm^3) on the second date of the period concerned.

4. P = The total production of large naked amoebae for each respective period.

5. Production quantities (μm^3) were converted to energy terms by adopting the conversions O.147pg μm^3, where the volumes were transformed to dry weight units, and 17.51 Jmg^{-1}, which converted the weights to energy units.

Appendix 17:

Data used for the calculation of the *A. proteus* production estimiate.

24.11.77 - 22.12.77	26	28	8	5	-	-	2.679	0	0	0	0	0	0	0

Appendix 18.

The relationship between temperature and the b-coefficients (slope) describing the increase of amoebae with time.

For estimating the generation times of the large naked amoebae species at specific temperatures, a plot of the b-values against temperature was used. The results for Amoeba proteus, cultured at 10°C, 15°C and 20°C, over a range of food concentrations, provided the necessary b-coefficients (Chapter 3).

A mean intercept value (a) was used throughout, as the variation between temperatures was not great; $a = 1.4678 \pm 0.2032$ (S.D.).

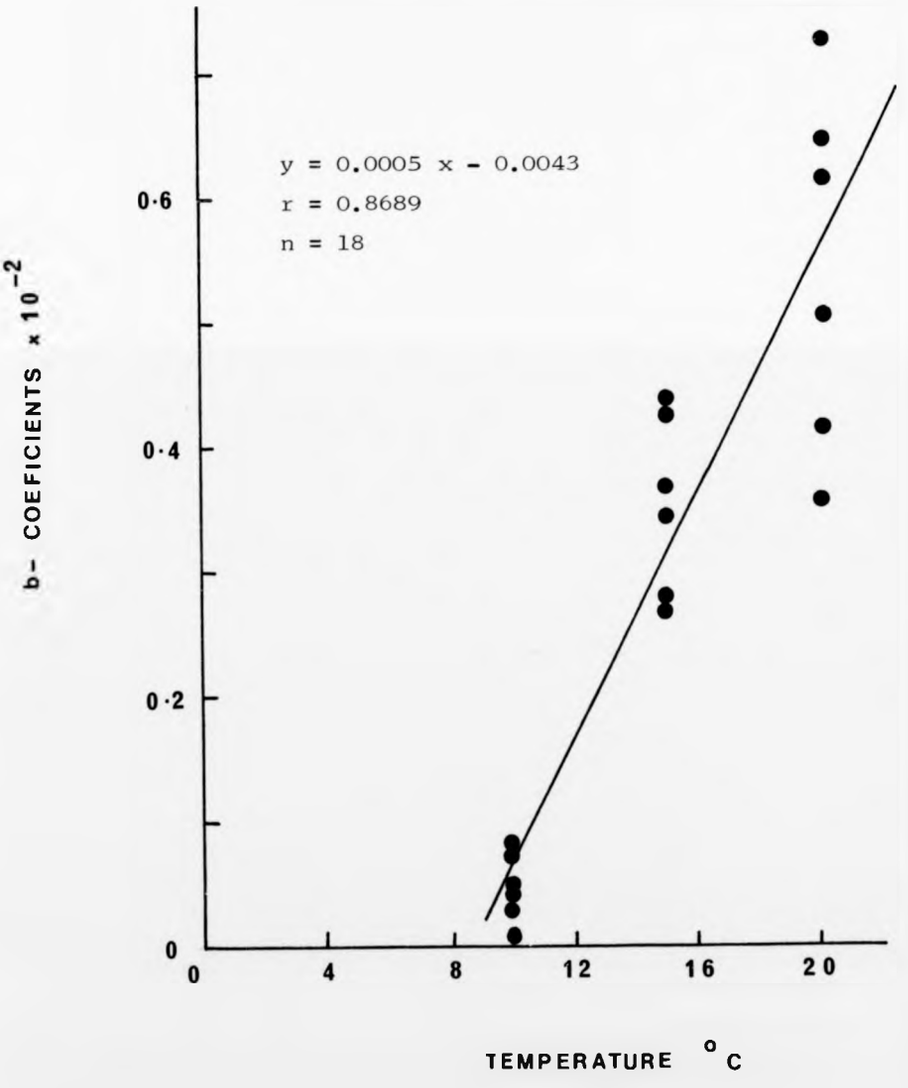
The required generation times were obtained by extrapolating the b-coefficients from Appendix Figure 3.

$$\log. \; n = a + bt_1 \qquad (1)$$

$$\log. \; 2n = a + bt_2 \qquad (2)$$

generation time (hours) = $t_1 - t_2$ when $n > 1$.

Appendix Figure 3.

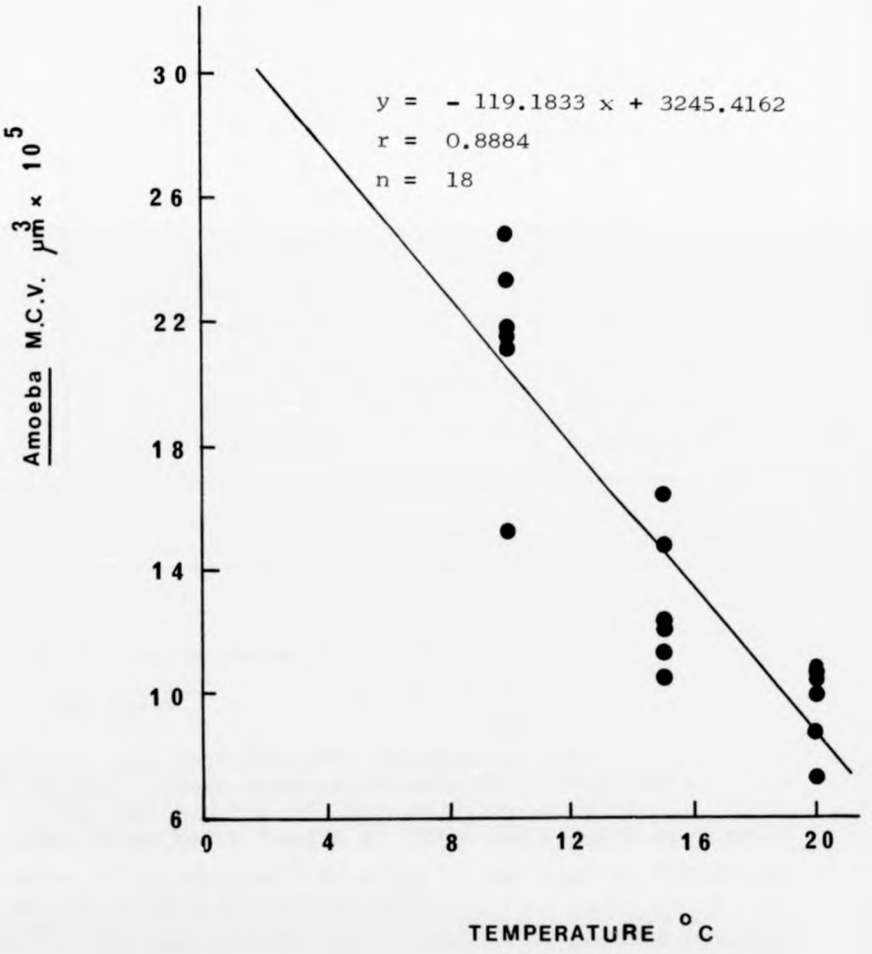


Appendix 19.

The relationship between temperature and the mean cell volume of *Amoeba proteus* cells.

The data for *A. proteus* (Chapter 5) at 10°C, 15°C and 20°C was used to plot the graph. The regression covers all levels of food concentration, thereby providing the "best estimate" for the variable food conditions in the field.

Appendix Figure 4.



Appendix 20.

The total ciliate and flagellate biomass approximations for the sampled Sphagnum bog-pool, Flanders Moss.

Months	Volume (μm^3 protoplasm ml^{-1})	$J.ml^{-1}$
J	4.09×10^4	1.26×10^{-4}
F	3.62×10^5	1.12×10^{-3}
M	4.85×10^6	1.49×10^{-2}
A	2.34×10^6	7.22×10^{-3}
M	9.28×10^7	0.29
J	2.71×10^8	0.84
J	1.13×10^8	0.35
A	8.62×10^6	2.65×10^{-2}
S	3.86×10^7	0.12
O	6.50×10^6	2.00×10^{-2}
N	1.99×10^6	6.14×10^{-3}
D	1.30×10^6	4.03×10^{-3}
	Mean =	0.14 ± 0.25 (S.D.)

Assumptions:

1. All the species approximated to an ellipsoid:

$$v = \frac{d^2 \times l}{6}$$ where, d = depth, l = length

2. Those species less than 50μm had a length of 25μm and a depth of 12.5μm. Those species between 50 - 100μm had a length of 75μm and a depth of 37μm while those cells greater than 100μm had a length of 100μm and a depth of 50μm.
3. A conversion of 0.162pg μm^{-3} (Chapter 2) was used to transform the volume units into dry weight units.
   19.04 Jmg^{-1}, the mean of the conversions as determined for 10^oC, 15^oC and 20^oC (Chapter 2), was used to convert the dry weight biomass values to energy units (joules).

Appendix 21.

Laboratory food concentrations (Tetrahymena) expressed in equivalent energy units (joules).

Concentration of Tetrahymena (ml^{-1})	Equivalent volume of Tetrahymena protoplasm (μm^3)	$J.ml^{-1}$
250	$4,875 \times 10^3$	1.50×10^{-2}
500	$9,750 \times 10^3$	3.01×10^{-2}
1000	$1,950 \times 10^4$	6.01×10^{-2}
2000	$3,900 \times 10^4$	0.12
4000	$7,800 \times 10^4$	0.24
8000	$1,560 \times 10^5$	0.48

Assumptions:

1. The mean cell volume of T. pyriformis cells was $19,500\mu m^3$ (Section 1.2.9.2.).
2. The conversions, $0.162pg.\mu m^{-3}$ and $19.04J\ mg^{-1}$ (Chapter 2) were applicable.