OPHELIA, 5: 123-136 (May 1968)

THE ECOLOGY OF MARINE MICROBENTHOS III. THE REPRODUCTIVE POTENTIAL OF CILIATES

TOM FENCHEL
Marine Biological Laboratory, Helsingør, Denmark.

ABSTRACT

Growth rates of populations of 9 benthic marine ciliate species were measured in pure cultures at different temperatures. All species had a maximal growth rate above 20°C which is above the average temperature in their natural environment. Several species multiplied at 4°C and one species also at 0°C, and it is suggested that all species are able to multiply at temperatures between 0 and 4°C at very slow rates after long periods of adaptation.

At 20°C generation times varied between 2.4 hours (*Uronema marina*) and 46 hours (*Condylostoma patulum*) corresponding to intrinsic rates of natural increase (r, per day): 6.65-0.36 and finite rates of increase (λ, per day): ca. 1000-1.4.

The maximal reproductive rate of ciliates was found to be correlated with cell size. It was found that $T = k \times v^{0.44}$, where T is generation time and v is the average body volume.

The reproductive rates of ciliates are compared with values of r for small metazoans (based on the literature or estimated from published data on fecundity and generation times). It is recommended that r is evaluated for a greater number of species as a measure of reproductive potential since this renders comparisons between different animals possible.

INTRODUCTION

Knowledge on the reproductive potentials is of importance when the role played by different organisms in natural environments is to be evaluated. The purpose of the present paper is to report on the reproductive rates of some benthic ciliates and to compare them with the reproductive rates of other animal groups.

It is well known that when an animal population is allowed to increase unchecked, the increase of the population is at any moment proportional to the size of the population at that particular moment, thus:

$$dN/dt = rN$$

or in an integrated form:

$$N_t = N_o e^{rt} \qquad (1)$$

where N_o and N_t are the population sizes at time 0 and t respectively, e is the base of natural logarithms and r is a constant known as the "intrinsic rate of natural increase". If T is the generation time and R_o is the net reproductive rate per generation (viz. N_{t+T}/N_t) (1) gives

$$r = \frac{\log^e R_o}{T} \tag{2}$$

For protozoa and other animals which multiply by division into two daughter individuals T is the period of time between two successive divisions and $R_o = 2$, thus:

$$r = \frac{\log_e 2}{T} = \frac{0.693}{T} \tag{3}$$

The "finite rate of increase" λ is defined as $\lambda = e^r$ and is, as may be seen from (1), a measure of how many times a population will multiply per unit of time.

Several authors (Andrewartha & Birch, 1954; Macfadyen, 1963; Slobodkin, 1962; Smith, 1954) have stressed the great value of r in ecological work since it is a true measure of the reproductive potential which allows comparisons between very different kinds of animals. The fact that this measure has been used to a small extent only in practical ecological work is due to difficulties in determining r in the great majority of metazoan species which have overlapping generations and deposit their eggs over a longer period of time, and r has only been determined with accuracy in few cases (see above-mentioned literature and the discussion in the present paper for references and methods for determination of r in metazoan populations).

For any animal population r may assume several values, one for each set of environmental factors (availability and nature of food, salinity, temperature, pH, etc., etc.,). If $r = 0$ the population size is constant and if r is negative it is decreasing. Under optimal conditions r will assume a value characteristic for the species in question. This value will be a measure of the reproductive potential of the species. In practice absolute optimal conditions will probably never be observed in the field nor will they be reproduced in the laboratory, but close approximations are often easily obtained.

The value or r is of interest because this as mentioned above, makes it possible to compare reproductive rates of different kinds of animals and also because exponential growth of populations of small animal species is not uncommon in nature. Bick (1964) demonstrated exponential growth in protozoan successions under laboratory conditions. I have observed exponential growth phases of ciliate populations in succession experiments as well as in the field (unpublished observations). Similar blooms of planktonic organisms are well known.

The generation time (T) or the intrinsic rate of natural increase (r) of some protozoan species frequently used in physiological and biochemical work have often been determined (see for example Phelps (1946) and Prescott (1957) for the

ciliate *Tetrahymena* (since the rate of the logarithmic growth of these organisms is used as a measure of the effect of various factors (temperature, nutrition, drugs, etc.) but few of these works have an ecological scope and no comparisons have been made between the values of r for a larger number of species.

Bick (1964) determined r from the growth of a number of ciliate species and the coelenterate *Hydra* in mixed cultures and demonstrated the importance of r for the understanding of the course of microfaunal successions.

My sincere gratitude is due to Miss Harriet H. Hansen for maintaining and counting the ciliate cultures and to Dr. Grete Møller Christensen for making cultures of unicellular algae available for me. I also acknowledge the support rendered through contract no. F 6 1052 67 C 0089 between the United States' Office of Naval Research, Department of the Navy, and the Marine Biological Laboratory, Helsingør.

MATERIAL AND METHODS

Nine species were used in the present study. *Uronema marina* Dujardin, 1841, *Aspidisca angulata* Bock, 1952, and *Litonotus lamella* (Ehrenberg, 1838) were isolated from sand samples from the Helsingør Beach; *Euplotes vannus* (O.F.M., 1786), *Diophrys scutum* Dujardin, 1841, *Lacrymaria marina* Dragesco, 1963 and *Condylostoma patulum* Claparède & Lachmann, 1858 were isolated from sediment samples from the Nivå Bay; *Keronopsis rubra* (Ehrenberg, 1838) was isolated from the trays in the sea-water system of the laboratory and an undescribed philasterid was isolated from a culture of lamellibranch larvae (see Fenchel, 1968).

Animals which were used for initiating cultures were taken from cultures in their logarithmic phase with the exception of *Condylostoma* and *Lacrymaria* because the reproductive rate of these species always decreased somewhat after some generations in pure culture, probably due to some deficiency of micro-nutrients in the food offered. These species were taken directly from mixed cultures.

All cultures were initiated with 2 to 12 individuals. The small species (*Uronema* and the philasterid) were cultured in a drop of culture fluid between two coverglasses sealed with vaseline, the other species were kept in Boveri dishes. Aged seawater (19‰ S) with the food item added was used as culture fluid.

After the culture fluid and ciliates had been added to the culture vessels these were incubated at the desired temperature. At intervals of some hours or once a day – according to the expected reproductive rate of the species examined – the culture vessels were placed under the dissection microscope and the number of ciliates counted. An experiment was terminated when the logarithmic growth phase had ended or when the number of animals was too large for direct counting. In the latter case the animals were counted a last time by removing them one by one with a pipette or after killing and staining them with methyl green in acetic acid. Due to the method of direct countings the accuracy is considered to be absolute·

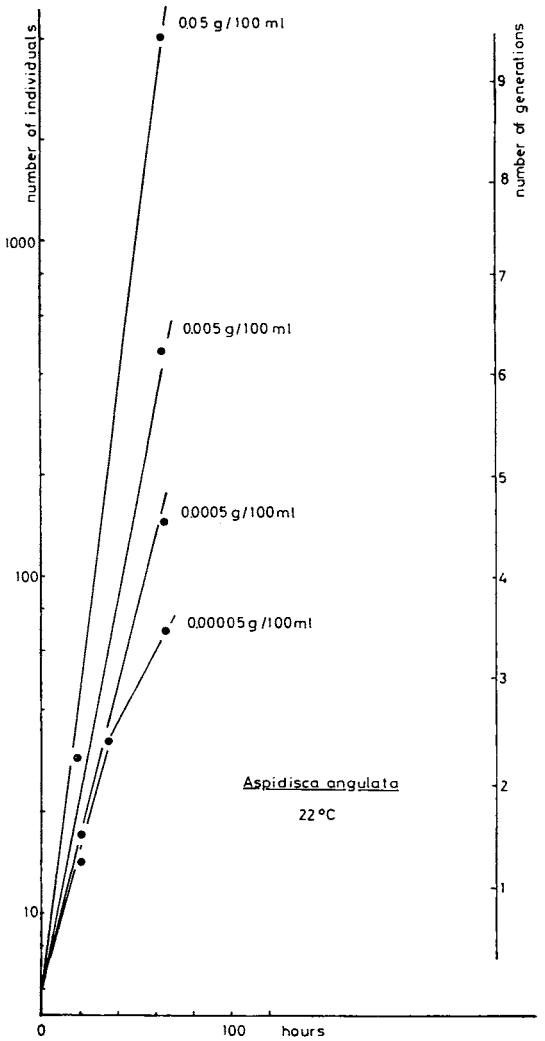

FIG. 1. Growth of cultures of *Aspidisca angulata* in different concentrations of peptone solutions.

Most species were tested at several temperatures between 0° and 22°C, *Diophrys* also at 27° and 32°C (see Fig. 5). All experiments were at least made in duplicates.

Bacteria grown in peptone solutions were used as food for *Uronema, Aspidisca* and *Euplotes* (yeast gave a slightly lower value of r in *Euplotes*); the diatom *Phaeodactylum* was used for *Keronopsis* and *Diophrys* (yeast, the flagellate *Dunaliella* and benthic diatoms gave a slightly slower growth, and *Isochrysis* gave no growth

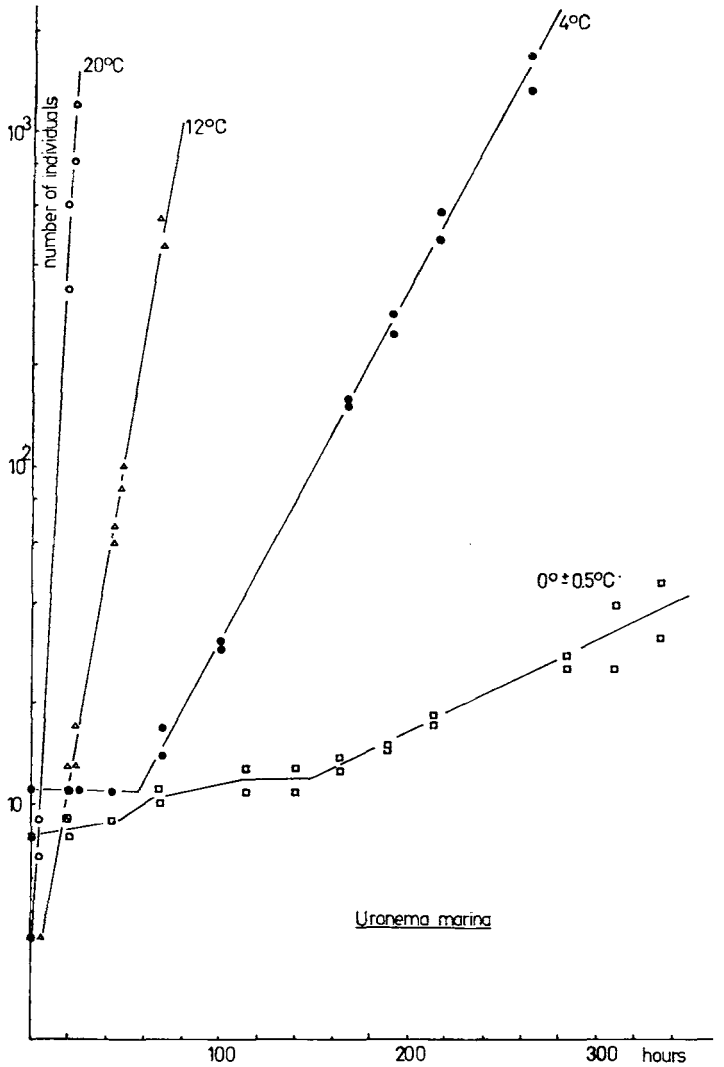

FIG. 2. Growth of cultures of *Uronema marina* at four temperatures.

at all in *Diophrys*). *Dunaliella* was used for *Condylostoma*. Bits of living mussel tissue were used for the histophagous philasterid. The carnivores *Litonotus and Lacrymaria* were fed with *Uronema marina* which were taken from peptone cultures and rinsed in sea-water before feeding.

The concentration of the food item influences the value of r as seen in Fig. 1, which shows the growth of *Aspidisca* in different concentrations of peptone in sea-water. An about 25 hour old solution of 25-50 mg peptone in 100 ml sea-

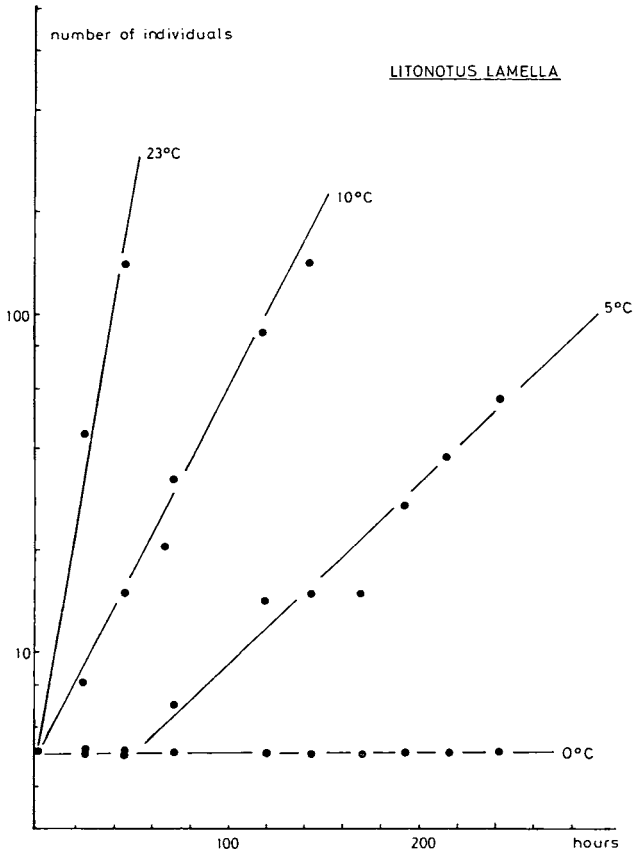

FIG. 3. Growth of cultures of *Litonotus lamella* at four different temperatures.

water was found to give best results for species which were fed with bacteria. Freshly made solutions gave rise to a comparatively long lag phase in the growth of the populations.

The optimal concentrations of other food items was not investigated, but since these items were considered harmless to the ciliates, even in very high concentrations, they were added in so large amounts that the reproductive rate of the ciliates could not be affected by lack of food. In most cases the experiments were reasonably reproducible even though two series of cultures of *Uronema* gave somewhat diverging results (Fig. 4).

Figs. 2 and 3 show the development of cultures of *Uronema* and *Litonotus* respectively at various temperatures.

T was estimated from growth curves describing the logarithmic phase and r was calculated from (3).

The volumes of ciliates were measured as described in Fenchel (1967).

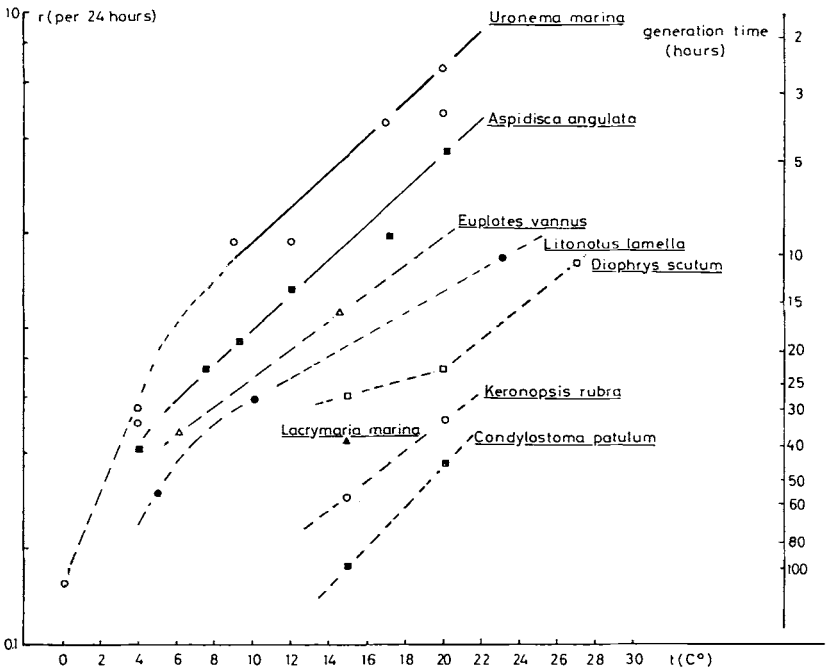

FIG. 4. Reproductive rates (r and T) at different temperatures of 8 species of ciliates. Curves fitted by eye.

In all experiments – with the exception of those on *Uronema, Litonotus* and *Diophrys* at temperatures above 10°C – a lag phase was apparent in the beginning of the experiments. The lag phase was longest at low temperatures (Figs. 2, 3).

A lag phase seems sometimes inevitable in protozoan cultures (see Hall, 1967) and the reason for this is not always clear. I my experiments the long lag phase at low temperatures is probably to some extent due to a period of temperature adaptation (stock cultures were kept at room temperature). The lag phase of the two species which were taken from mixed cultures may also be due to a period of adaptation, in this case to a new diet.

RESULTS

In Fig. 4 the reproductive rates of the investigated species (as r and as T) are plotted against temperature. As pointed out by Phelps (1946) Q_{10} may be expected to vary with temperature. The number of different temperatures used during the present study is therefore too small to give a detailed picture of the effect of temperature on the reproductive rate.

For *Tetrahymena* Phelps found three distinct values of Q_{10} with sharp breaks between them: Q_{10} was 9.7 at 7.8-12.3°C, 2.9 at 12.3-20.0°C and 1.5 at 20.0-28.6°C. Highest growth rate was found at 29°C, and at about 32°C no growth occurs. Neither did growth occur below 5-6°C.

The results of the present investigation indicate a constant Q_{10} in the interval from 4-8°C to 20°C for *Uronema* and *Aspidisca* which were tested at several temperatures. At lower temperatures Q_{10} increases in accordance with the findings of Phelps. The values of Q_{10} of the various species in the interval 8-20°C varied between 3.5 (*Uronema*) and 2.3 (*Litonotus*).

Only one species, *Diophrys scutum,* was cultured at temperatures above 23°C. The highest growth rate was found at 27°C while nearly no growth occurred at 31-32°C.

Uronema grew both at 4°C and at 0°C. *Aspidisca* grew at 4°C, and it was not incubated at lower temperatures. *Litonotus* multiplied at 5°C but not at 0°C during 10 days of incubation. *Diophrys* did not grow at 4°C and 0°C. All species survived for at least 2-3 weeks at 0°C and behaved normally even though no multiplication took place.

James & Read (1957) demonstrated that the cell size of *Tetrahymena* increased in incubations at low temperatures. This effect was observed in several of the species studied; it was especially evident in *Uronema.*

On Fig. 5 the reproductive rate at 20°C, as expressed by r, λ, and T, is plotted against the volume of the animals. A correlation between cell size and reproductive rate is evident. Calculation of the regression line gives the equation r (per day) $= 105.9 \times v^{-0.44}$ or T (hours) $= 0.0065 \times v^{0.44}$ when the volume v is given in μ^3. At 15° the calculation gave a somewhat different exponent (0.42) since Q_{10} is not quite identical in the different species.

The largest and the smallest species studied (*Condylostoma* and *Uronema* respectively) almost represent the extremes in size of ciliates, and their reproductive rates are therefore considered as nearly showing the limits in variation of reproductive rates in these animals.

At 20°C, r for *Uronema* has the value 6.65 per day, i.e. a generation time of 2.5 hours. At the same temperature *Condylostoma* has a generation time of about 46 hours corresponding to a value of $r = 0.36$ per day. The enormous difference in reproductive potential between these two species is made clear when λ is considered. A population of *Condylostoma* will under optimal conditions at 20°C increase by a factor 1.4 in 24 hours. During the same period a population of *Uronema* will increase by a factor of 1000.

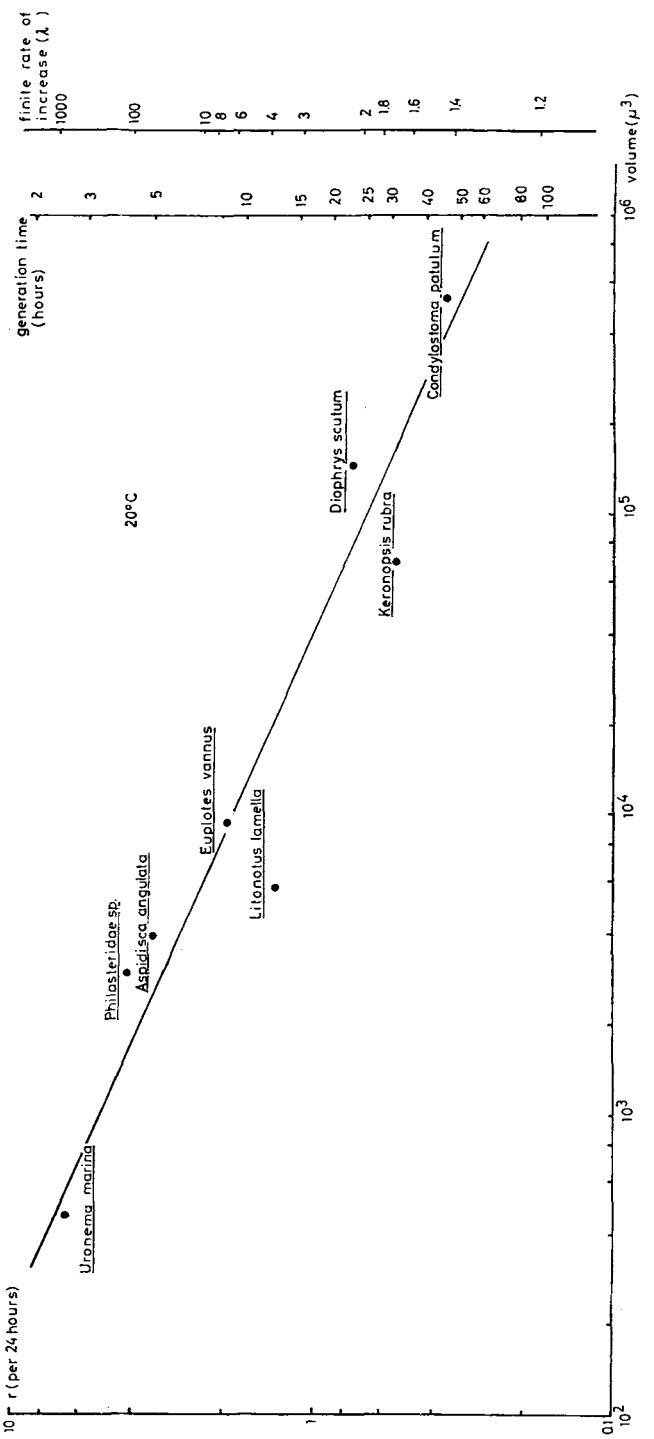

FIG. 5. Reproductive rates (r, λ and T) at 20°C plotted against body volume.

DISCUSSION

Previous studies have shown that several freshwater ciliates have an optimum temperature in the region 25-32°C (Phelps, 1946; Prescott, 1957, a.o.). During the present investigation this was also shown to be the case with *Diophrys scutum*, and all species studied – with the possible exception of *Lacrymaria* – have their temperature optimum above 20°C. Thus the optimum temperature is far above the average temperature of the sea. In temperate regions temperatures of 25-30°C can only be reached in very shallow water on hot summer days. However, the relations between temperature and reproductive rates of the well studied species indicate a better adaptation to low temperatures than found for the *Tetrahymena* studied by Phelps (1946), which would not divide below 5-6°C. The present findings indicate that reproduction will occur nearly troughout the year exept in very shallow water during periods of extreme cold.

Studies on field populations of sand ciliates indicated multiplication even at 0 to + 1°C (Fenchel, 1967, and unpublished observations). It is possible that the other species studied may divide at even lower temperatures than 4°C like *Uronema* which divided at 0°C. Efimoff (1924) found that *Paramecium* has a generation time of 13 days at 0°C. If a long lag period due to cold adaptation exists, my experiments have been too short to show these very slow rates.

It is obvious that the standing crop will not be a measure of the biological activity when comparisons at different temperatures are made. The population sizes need not decrease much with decreassing temperatures since the smaller rate of reproduction will be counteracted by a smaller rate of predation. Some mortality factors will of course remain constant and a small decrease in population size of ciliates may therefore be expected in winter though not at all comparable to the decrease in the reproductive rate which will be 10 to 20 times lower at 4°C than at 20°C. A small decrease in size of ciliate populations was actually found in field populations (Fenchel, 1967). In contrast, several groups of micro-metazoans do not reproduce at all in winter (Muus, 1967; Theisen, 1966) and field populations of species belonging to these groups show much more pronounced summer peaks and winter minima.

It is well known that when the total animal kingdom is considered the reproductive potential tends to decrease with increasing body size (see Smith, 1954).

A clear correlation between body size and reproductive potential within an animal group has not, however, been demonstrated before. This correlation allows one to estimate the generation time of a ciliate species when its body volume is known and the great variation in reproductive potential within the ciliates may be explained by the great variation in size of this group (more than three decades).

The reason why the exponent in the equation $r = k \times \text{volume}^{-0.44}$ just takes the value —0.44 is not understood. Before a theoretical discussion can be fruitful more data should be provided, also from other groups, and the intrinsic rates of

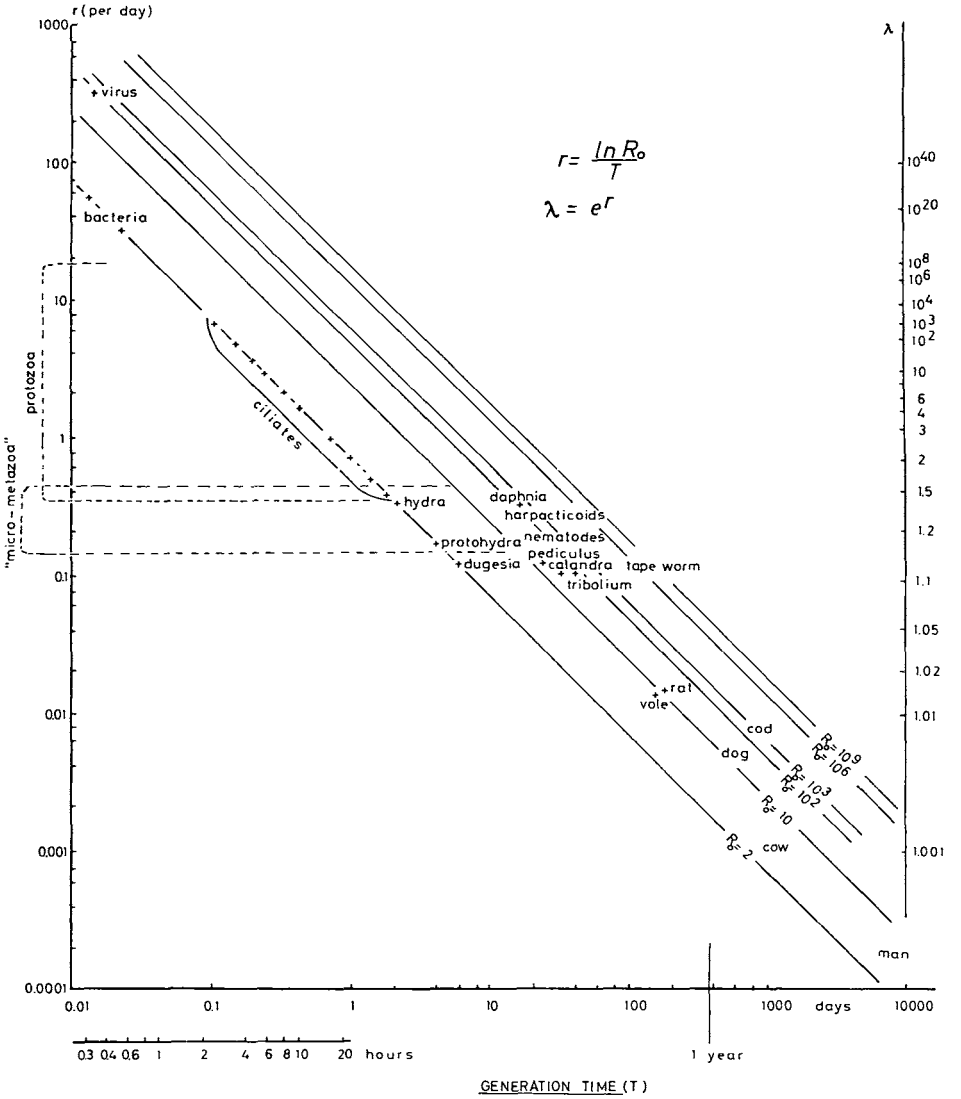

FIG. 6. Graph showing the interrelationships between r, λ, T and R_0 and the maximal reproductive rates of some animals. Redrawn and modified after Smith (1954). Data on reproductive rates have been taken from Smith when not otherwise stated in the text.

natural increase should be compared at the optimum temperature rather than at an arbitrarily chosen temperature.

Fig. 6 allows one to compare the reproductive potential (r and λ), generation time and the net reproductive rate per generation (R_0) for all types of organisms from virus to man.

Measurements of r of bacteria are numerous. The data on *Escherichia coli* represents populations grown on peptone broth and glucose respectively and show the order of magnitude of r for bacteria.

The data on ciliates derive from the present study (20°C values, thus somewhat below optimum rate in contrast to other data on the figure) except those on *Paramecium caudatum* and *Tetrahymena pyriformis* which fit into the present data according to their sizes (Data from Andrewartha & Birch, 1954 and Phelps, 1946).

Since several groups of protozoa – especially within the flagellates – contain species which are much smaller than the smallest ciliates it is probable that the range of the reproductive rates of protozoa in general should be extended somewhat in the direction of higher rates as indicated on Fig. 6.

Due to the difficulties in calculating the reproductive potential of metazoans not multiplying by division there are still very few data with which the present findings may be compared. Most ecologists have given egg numbers, development time and/or estimates of generation time as measures of reproductive rates, but these data are very difficult or impossible to evaluate. As may be seen from (2) and Fig. 6 and as pointed out by Smith (1954) and Slobodkin (1962) the effect of increasing R_0 (viz. the offspring per female per generation) is surprisingly small compared with the effect of decreasing T. Thus, for example, the tape worm does not have an especially high reproductive rate as is commonly believed, in spite of the enormous numbers of eggs produced (Fig. 6).

Andrewartha & Birch (1954) give methods available for calculating r in metazoan populations. This requires a life-table of the animal population in question and a table of age-specific fecundity. If l_x is the chance of survival at age x and m_x is the fecundity at age x then $R_0 = \Sigma l_x m_x$. For approximate calculation, T may be estimated and r calculated from (2). The real value of T may be difficult to evaluate and methods have been developed for evaluating r independent of T and the exact value of T is then calculated from r and R_0.

In the present investigation it is of interest to compare the reproductive potential of ciliates with that of micro-metazoans living in soft sediments.

Stiven (1962) gives r = 0.34 per day for *Hydra* growing under optimal conditions, and Cole (1960) found r to be 0.12 for the large flatworm *Dugesia*. K. Muus (1966) found a generation time for the small coelenterate *Protohydra*, which lives in estuarine sediments, of 4 days at 20-22°C. *Protohydra* reproduces by division. This gives a value of 0.17 per day for r which seems low for this small animal when compared with *Hydra*. Maybe conditions were not optimal under the culture conditions. Frank et al. (1957) found r of *Daphnia pulex* to be 0.30 per day.

From the studies of Muus (1967) rough estimates of r may be calculated for a number of benthic harpacticoids. R_0 seems to be of the magnitude 100 (estimated from the total number of eggs produced by each female in mixed cultures and the sex ratio) and generation times are 12 to 25 days. This gives values of r between 0.2 and 0.4. On the basis of the studies by Nielsen (1949) and Hopper & Meyers

(1966) it seems reasonable to estimate r of small nematodes to be somewhat smaller – 0.1 to 0.2 – since R_0 is of the same magnitude as that of harpacticoids but T is higher (from 20 days to more than a month). The relatively small variation in reproductive potentials within the micro-metazoa (the "meiofauna") could be expected since the "group" is per definition of a restricted size range. Very small metazoans (rotifers, chaetonotoid gastrotrichs, small turbellarians) may be expected to have somewhat higher values of r, but no data are available.

Thus r of the metazoan microfauna may be estimated to lie between 0.1 and 0.4 per day under optimal conditions at temperatures around 20° C. The corresponding λ-values render these values intelligible. Thus a population of nematodes can be expected to increase by a factor 1.1-1.2 per day and a population of harpacticoids can be expected to increase by a factor 1.2 to 1.5 per day under optimal conditions. For comparison populations of ciliates will, at 20° C, increase by a factor between 1.4 and about 1000 per day according to the species. The very large ciliates like *Condylostoma* play a relatively small quantitative role in most microfaunal communities in comparison to smaller species. The dominating ciliate species in fine sand are of the size range 5×10^3 to 5×10^4 μ^3, thus the reproductive rates of the dominating species will be 1.0-2.8 (r, per day) or 2.5-20 (λ, per day). The very small ciliates like *Uronema* dominate the first phases of successions on decaying organic material, but under other conditions they often play a relatively small quantitative role.

The relatively high values of r for *Hydra* and *Daphnia* when compared with the estimates of r for harpacticoids, nematodes and *Protohydra* are probably due to the fact that the former are well known laboratory animals especially cultured to find the maximal value of r while the latter have been kept in uncontrolled mixed cultures. It must also be stressed again that the values of r for the latter forms are only rough estimates based on partly inadequate data.

It is believed that exact evaluations of r for a larger number of metazoans inhabiting microfaunal communities would be a great advance for the understanding of these communities since this will render it possible to make relevant comparisons of the reproductive potentials between different species and groups of organisms. Such evaluations can probably now be made without great difficulties since culture methods are being developed for a large number of forms (Hopper & Meyers, 1966; Muus, 1967; Theisen, 1966; von Thun, 1966) provided that the relevant data from these cultures are collected.

REFERENCES

ANDREWARTHA, H. G. & L. C. BIRCH. 1954. The Distribution and Abundance of Animals. The University of Chicago Press, Chicago. 782 pp.

BICK, H. 1964. Die Sukzession der Organismen bei der Selbstreinigung von organisch verunreinigtem Wasser unter verschiedene Milieubedingungen. Min. ELF des Landes Nordrhein/ Westfalen. Düsselsorf. 139 pp.

COLE, L.C. 1960. A note on population parameters in cases of complex reproduction. Ecology, **41**: 372-375.

EFIMOFF, W.W. 1924. Über Ausfrieren und Überkältung der Protozoen. Arch. Protistenk., **49**: 431-446.

FENCHEL, T. 1967. The ecology of marine microbenthos. I. The quantitative importance of ciliates as compared with metazoans in various types of sediments. Ophelia, **4**: 121-137.

— 1968. The ecology of marine microbenthos. II. The food of marine benthic ciliates. Ibid., **5**: 73-121.

FRANK, P.W., C.D.BOLL & R.W.KELLY. 1957. Vital statistics of *Daphnia pulex* DeGeer as related to density. Physiol. Zool., **30**: 287-305.

HALL, R.P. 1967. Nutrition and growth of protozoa. *In* Tze-Tuan Chen (ed.): Research in Protozoology, vol. 1, pp. 337-404, Pergamon Press, Oxford.

HOPPER, B.E. & S.P.MEYERS. 1966. Aspects of the life cycle of marine nematodes. Helgoländer wiss. Meeresunters., **13**: 444-449.

JAMES, T.W. & C.P.READ. 1957. The effect of incubation temperature on the cell size of *Tetrahymena pyriformis*. Expl. Cell. Res., **13**: 510-516.

MACFADYEN, A. 1964. Animal Ecology. Aims and Methods. Sir Isaac Pitman & Sons Ltd. London. 344 pp.

MUUS, B. 1967. The fauna of Danish estuaries and lagoons. Meddr Danm. Fisk.- og Havunders., N.S., **5**: 1-316.

MUUS, K. 1966. Notes on the biology of *Protohydra leuckarti* Greef (Hydroidea, Protohydridae). Ophelia, **3**: 141-150.

NIELSEN, C.OVERGAARD. 1949. Studies on the soil microfauna. II. The soil inhabiting nematodes. Natura jutl., **2**: 1-131.

PHELPS, A. 1946. Growth of protozoa in pure culture. J. exp. Zool., **102**: 277-292.

PRESCOTT, D.M. 1957. Relation between multiplication rate and temperature in *Tetrahymena pyriformis*, strains HS and GL. J. Protozool., **4**: 252-256.

SLOBODKIN, L.B. 1962. Growth and Regulation of Animal Populations. Holt, Rinehart and Winston, New York. 184 pp.

SMITH, F.E. 1954. Quantitative aspects of population growth. *In* E.J.Boell (ed.): Dynamics of Growth Processes, pp: 277-294. Princeton University Press, Princeton.

STIVEN, A.E. 1962. The effect of temperature and feeding on the intrinsic rate of increase of three species of *Hydra*. Ecology, **43**: 325-328.

THEISEN, B.F. 1966. The life history of seven species of ostracodes from a Danish brackish-water locality. Meddr. Danm. Fisk.- og Havunders., N.S., **4**: 215-270.

THUN, WOLF VON. 1966. Eine Methode zur Kultivierung der Mikrofauna. Veröff. Inst. Meeresforsch. Bremerh., Sonderband II: 277-280.