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DENSITY AND SIZE OF MEDIUM IN POPULATIONS OF *PARAMECIUM CAUDATUM**

Paramecium cultured in media of different sizes attains maximum density in the smallest media. This phenomenon is far less distinct if the media of different sizes possess a constantly maintained ratio of volume/surface.

For a large number of years data have been given in protozoology which provide grounds for assuming that both excessively large relative volume of the culture medium (considerable thinning of population) and small relative volume (considerable density of the population) are unfavourable to the development of its numbers. This rule is clearly evident in the six phases of population growth, beginning with inoculation of the medium by a small number of individuals: "(1) a stationary period, (2) a lag period of increasing rate of growth, (3) a logarithmic period of constant relative rate of growth, (4) a period of declining rate of growth, (5) a period of equilibrium of numbers and finally, (6) a period of declining numbers" (Richards 1941). It can easily be seen that at first the reduction in the reserve of space in the developing culture exerts a favourable effect on its development, since it passes from the stationary phase (1) to phase of intensive growth (2) and (3); then a further decrease in the relative volume of the medium begins to exert an unfavourable effect and the culture passes from the phase of intensive growth (3) to periods of declining (4) – (6).

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Research limited to the beginning of the history of *Protozoa* populations led to the formation of the autocatalysis and allelocatalysis concept (Robertson 1927), and investigations concentrated exclusively on periods of declining to the theory of the aging of the cultures (Maupas 1886 and 1888); theories were therefore formed explaining the growth of population in *Protozoa* by endogenic factors. Dimitrowa (1932) drew attention to the role of the culture medium in general, and other authors emphasised the role of definite variations in the medium: of food requirements (Chejfec 1929), of pH (Darby 1930) and of redox potential (Jahn 1934). Complete confirmation is given in the papers by Grębecki and Kuźnicki (1956a and b) of the correctness of all the interpretations explaining the phases of development of *Protozoa* populations by the influence of exogenic factors, and later (Grębecki 1961a and b) it was found that the same conditioning of the medium by the developing population causes both the initial stationary phase and the final declining phases. This is in agreement with the views put forward by Allee (1934) that both the under-density of a population and its overdensity, are unfavourable. It was therefore shown that there is an optimum relative volume of the culture, that is, an optimum ratio of number of individuals to the volume of the medium.

The effect of the relative volume of the medium (density of the culture) on different manifestations of life, both individual and population, is a well-known general ecological rule, usually termed Allee's rule.

Data forming evidence that the productivity of the population depends on the medium size have recently been accumulated in the Institute of Ecology of the Polish Academy of Sciences. Using mice and *Tribolium* as examples, it was found that the productivity – understood as the number of individuals per unit of medium (volume or surface) – is greater in smaller total medium sizes (Petrusewicz and Trojan 1963, Petrusewicz, Prus and Rudzka 1963).

As far as the authors are aware, the influence of the total medium size on the density of population has never been investigated in the case of cultures of *Protozoa*. A series of experiments was therefore undertaken, intended to show that differences exist in the density of populations of *Protozoa* depending on total medium volume, if other living conditions and also initial density are the same.

1. METHODS

All the experiments were carried out on cultures of *Paramecium caudatum* originating from the clonal line maintained in the Department of General Biology of the M. Nencki Institute of Experimental Biology in Warsaw.

The initial cultures were fed on milk. The *Paramecia* were washed with tap water (pH 7.1) before the experiment and then diluted to the required density.

The experimental cultures were kept in a room. Temperature during the experiment was from 20°–22°C. The light conditions of all the simultaneously maintained cultures were uniform. The experimental cultures were fed daily with milk, always maintaining a slightly opaque medium. A slight excess of food was therefore constantly maintained, which ensured uniformity of food requirements independently of the existing density of the culture. The initial density of all the cultures (independently of their total size) was 50 individuals per 1 ml.

Despite the fact that every attempt was made to achieve uniformity of culture conditions, the values of absolute figures obtained in different experiments (i.e. at different times) cannot be compared. The numerical results, however, are completely comparable for the replications (repeats) carried out at the same time, i.e. within the scope of each definite experiment. This is proved by the values of the variances ($CV\%$), since although the repeats were not numerous ($n=10$) the values of the variances are not great; they vary within limits of 4% – 19%.

The following information was obtained: 3 samples, each containing 0.1 ml of the liquid, were taken from each culture, and all the individuals counted in each sample, and the mean value for the given population calculated. The mean figure from all the replications was next calculated. Ten cultures of each type were always set up. In the majority of the experiments measurements were made every 3 days, while the cultures were maintained for 33–42 days. The populations under observation reached equilibrium level during the period from the 9 - 27th day. Observations were therefore never interrupted before peak numbers had been attained. One experiment only (experiment 1b) was maintained for 140 days, while samples were taken every 10 days.

2. ANALYSIS OF MATERIAL

2.1. Populations in a medium of uniform shape (experiments 1a and 1b)

In the first series of experiments (exp. 1a) 10 cultures of *P. caudatum* were set up, of the following volumes: 50 ml, 100 ml, 200 ml, 400 ml, 800 ml and 1600 ml (a total of 60 cultures). The culture vessels for all the six volumes were so chosen as to maintain a similar ratio between the base of the vessel and the height of the column of liquid (const. $\phi : V$).

The results of experiment 1a show that:

1) greater total numbers are attained in larger media (Tab. I). The increase in total abundance is not, however, as rapid as the increase in the medium, and as a result;

2) density (measured by the number of individuals per 1 ml) is greater in smaller media (Fig. 1, Tab. I). Average density is to a certain extent a measure

Numbers (*N*), density (*D* – number of individuals per 1 ml), *CV*%, and relative density (*RD*% – percent of the density at 100 ml) in respect to the different size of medium. Average for 18–33rd days of observation, non-buffered medium (experiment 1a).

Tab. I

Size of medium (ml)	*N*	*D*	*CV*%	*RD*%
50	59 550	1 191	10.6	105
100	113 500	1 135	15.0	100
200	214 000	1 070	4.7	83
400	297 200	743	6.9	65
800	513 600	642	3.8	56
1600	608 000	380	19.7	33

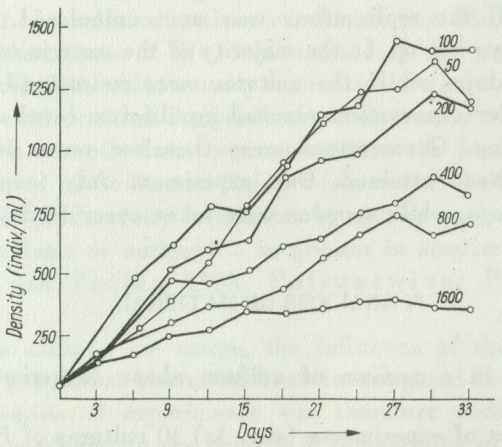

Fig. 1. Density changing with different medium sizes (experiment 1a – non buffered medium)

of productivity. The largest media have therefore the lowest productivity. In the 1600 ml medium it is scarcely 20% of the productivity of the 100 ml medium.

The smallest of the media examined – 50 ml, is the exception to this rule, as far as the regularity of the curve is concerned (Fig. 1). The average density, however, is slightly greater than in the 100 ml medium (Tab. I).

Lower productivity of the population in greater volumes of medium may take place in two different ways: either populations in a large medium attain the maximum of their development at a lower density than in a small medium, or

the rate of development of a large population is slowed down and the same maximum level of density should be attained but far later. The previous experiments, lasting only 33 days, did not exclude either of these two possibilities. The experiments were therefore repeated, extending the observation period to 140 days (experiment 1b), but were limited to 3 volumes of cultures (100 ml, 400 ml and 1600 ml), while density was measured only once every 10 days.

The results (Fig. 2) show that the lower productivity of a population cultured in a larger volume is caused by the maximum of density occurring at

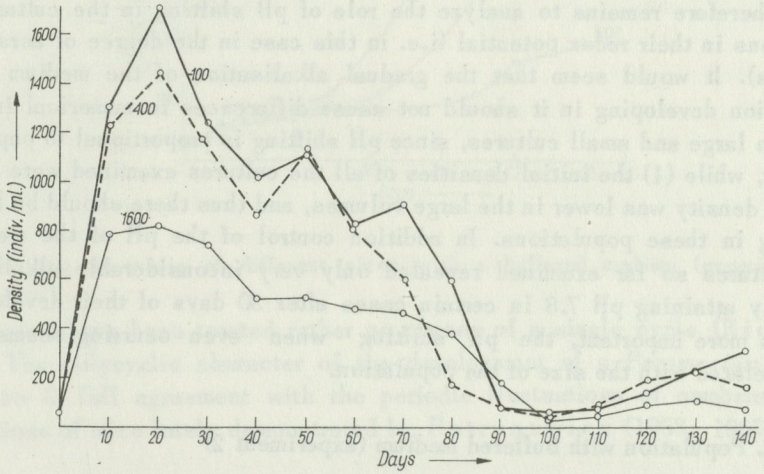

Fig. 2. Dynamics of density in a non-buffered medium in long-lasting cultures (experiment 1b)

a far lower level, while no extension of the duration of each phase of growth is found. On the contrary, the almost ideal synchronisation of variations in the numbers of all the populations examined is a striking phenomenon. Independently of culture size, and therefore of its numbers, the maximum of growth occurred in this experiment on the 20th day, and the minimum of numbers – on the 100th day; the decrease in numbers between the 20th and 100th day was temporarily arrested in almost all the cultures about the 50th and about the 70th day of the life of the population.

The question arises as to what is the cause of the phenomenon revealed, that is, the lasser productivity of the *Paramecium* populations developing in larger media. To generalise it may be imagined that the cause here may be either (1) changing living conditions (here deteriorating) with the increase in the medium, or (2) changing population organisation with the increase in the absolute numbers of the population.

Research undertaken so far on the factors limiting the free development of populations of *Protozoa* (Chejfec 1929, Darby 1930, Jahn 1934, Grębecki and Kuźnicki 1956a and b) demonstrated the role of food requirements, of pH, of redox potential and of osmotic pressure in the culture medium.

In the present study the food supply was uniform in all the cultures examined, since a slight excess of food was constantly maintained.

As stated previously (Grębecki and Kuźnicki 1956a and b) the osmotic pressure in the culture medium rises very slowly and cannot be the cause of the differences in population growth evident as early as during the first few days of their development.

It therefore remains to analyze the role of pH shifting in the cultures and variations in their redox potential (i.e. in this case in the degree of aeration of cultures). It would seem that the gradual alkalisation of the medium by the population developing in it should not cause differences in numerical increase between large and small cultures, since pH shifting is proportional to population density, while (1) the initial densities of all the cultures examined were uniform and (2) density was lower in the large volumes, and thus there should be less pH shifting in these populations. In addition control of the pH of the media of the cultures so far examined revealed only very inconsiderable alkalisation, scareely attaining pH 7.8 in certain cases after 30 days of their development. What is more important, the pH shifting when even ocurring seems not to be correlated with the size of the population.

2.2. Population with buffered medium (experiment 2)

Beside the considerations given above, in order to check whether variations in pH are not responsible for different population densities, the experiment described above was repeated, buffering the culture medium (in medium volumes of 100 ml, 400 ml, 1600 ml) with the neutral phosphate buffer after Dryl (1961).

The results of this experiment (Fig. 3) show that despite the stabilisation of the pH of the medium, larger populations attain a lower density than small ones. The only difference between the course of the growth curves of populations remaining in a non-buffered medium (Fig. 1 and 2) and those developing in a buffered medium (Fig. 3) consists in the fact that in the second case the growth curves are far more regular approaching to the theoretical curves of an exponential growth, which would seem quite natural and obvious, since pH and ion relations in the culture liquid have been stabilised.

Finally, this experiment on the one hand completely confirmed the previous result, and on the other showed that the difference sought for between populations small and great in the total number of *Paramecia* does not depend on pH shifting.

The course of curves of density obtained from the longlasting experiments,

that is experiment 1b and experiment 2, which lasted both 140 days (cultures with buffered medium) gives also evidence that probably the history of a *Paramecium* culture is polycyclic; up to now the periods of growth of a population

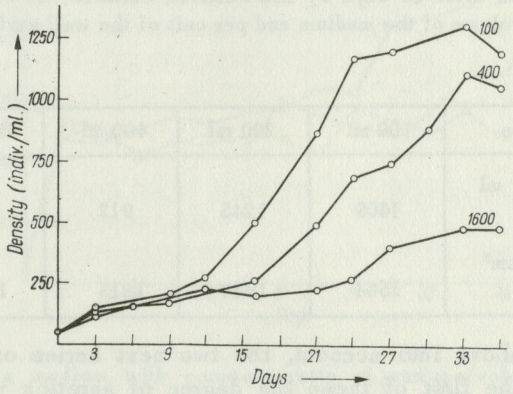

Fig. 3. Density in media of different sizes with a buffered medium (experiment 2)

of *Protozoa* have been treated rather as phases of a single cycle (Richards 1941). The polycyclic character of the development of a *Paramecium* culture would be in full agreement with the periodic fluctuations of numbers in the populations of mice lately demonstrated by Petrusewicz (1958, 1960, 1962).

2.3. Populations in a medium with a constant ratio of volume to surface

As has already been emphasised, all the experiments so far made were conducted in vessels of uniform shape, i.e. each culture was of cylindrical shape with a uniform ratio of basis to altitude. In these circumstances the increase in surface does not keep up with the increase in volume. Two kind of surface would seem to be particularly important to the growth of a *Paramecium* population: 1) the free air/water interface, and 2) the surface of the walls of the vessel.

The free upper surface determines the degree of aeration of the culture medium. It can therefore be calculated that cultures 400 ml in volume, and even more so 1600 ml in volume, were far more weakly aerated than those 100 ml in volume.

The size of the area of the walls of the vessel is important on account of the fact that in a normally developing culture, a relatively small quantity of *Paramecia* swims freely, the greater part forming dense aggregations attached to the walls. As a result, in a larger culture, even if there are fewer individuals

per unit of volume of the medium, the number of individuals calculated per unit of the wall surface may be even greater. This regularity is shown in Table II.

The density reached after 30 days by non-buffered cultures, as calculated per unit of volume of the medium and per unit of the wall surface

Tab. II

Size of culture	100 ml	200 ml	400 ml	800 ml	1600 ml
Individuals per ml of medium	1409	1245	912	687	381
Individuals per cm^2 of wall	1564	1943	1815	1669	1079

Taking the above into account, the two next series of experiments were carried out. In the first of these the degree of aeration was made equal by means of placing the larger cultures in flatter vessels, so that regardless of the volume, the column of liquid was always 6 cm. In the second series the surface of the walls also was rendered uniform by placing the appropriate number of test tubes into larger vessels, so that the ratio of the surface of the walls to the volume of the medium was always the same as that in the vessels containing cultures 100 ml in volume.

Density (*D*) and relative density in percent (*RD%*) in populations with constant aeration (experiment 3) and with constant aeration and constant ratio of wall surface/volume of medium (experiment 4). Average for 3 - 42nd days (Fig. 4)

Tab. III

Size of medium (ml)	Experiment 3		Experiment 4	
	D	*RD%*	*D*	*RD%*
100	918	100	857	100
400	895	97	684	80
1600	833	91	606	71

Comparison of data (Tab. III, Fig. 4) shows that the regularity found in the previous experiments (smaller productivity of larger media) is confirmed despite the fact that the aeration and ratio of wall surface to volume have been rendered uniform. The decrease in density is, however, far smaller. In the previous experiments the average density in the 1600 ml medium was 35–54% of the density in the 100 ml whereas with a constant degree of aeration and constant

ratio of wall surface to volume the corresponding values are 71–91%. The difference in average density with 100 ml and 1600 ml in the 4th experiment

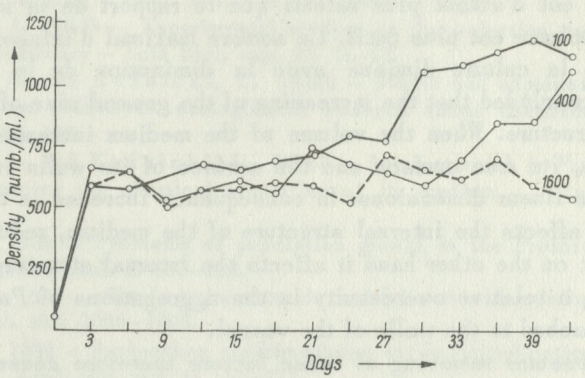

Fig. 4. Density in a medium with constant ratio of surface/volume (experiment 4)

is statistically significant at the level of .001 (checked by the Student test), while in the 3rd experiment it proved to be non-significant.

3. DISCUSSION OF RESULTS

It was found in all the experiments that with the other factors uniform, density is regularly less in greater volumes. The density attained determines productivity to a certain degree it may therefore be stated that the productivity of larger media is smaller. These results fully agree with the results of experiments carried out on white laboratory mice (Petrusewicz and Trojan 1963) and on *Tribolium castaneum* and *T. confusum* (Petrusewicz, Prus, Rudzka 1963).

Discussion was made of the environment factors which might be responsible for this and pH and food were excluded. The experiments made with media of different size but with a constant ratio of surface to volume show that (1) aeration and wall surface affected the results of experiments made in vessels of similar shape, but (2) did not completely discarded, but only considerably weakened. the decrease in density with increase in the size of the medium.

The influence of the ratio surface/volume and aeration is clearly demonstrated by the fact a far smaller decrease in density was found with increasing size of the medium, when a constant surface/volume ratio was maintained. It may therefore be stated that the failure of increase in surface to keep up with an increase in volume did in fact prove to be a factor suppressing the growth of populations which are greater in their absolute size.

This conclusion which fully agrees with the views of Jahn (1934) is also confirmed in certain observations made by Mędrkiewiczówna (1921) in relation to *Colpidium colpoda*. This authoress writes: "..... le développement des infusoires est d'autant plus ralenti que le rapport de la surface libre du liquide à son volume est plus petit. Le nombre maximal d'infusoires dans 1 cm^3 du liquide de la culture diminue avec la diminution de la surface libre".

It may be concluded that the increasing of the general size of medium affects its internal structure. When the volume of the medium increases as a cube of its linear size, its free surface and the surface of its walls increase only as a square of the linear dimensions. In consequence increase in the general size of the culture affects the internal structure of the medium, reducing the degree of its aeration; on the other hand it affects the internal structure of the population, involving a relative overdensity in the aggregations of *Paramecium* which are usually attached to the walls of the vessel.

The experimental removing of these factors therefore causes considerable levelling out of differences in the density of large and small populations, but does not completely remove them. Even in media with uniform ratio of surface/volume density exhibited some tendencies to decrease with an increase in volume.

Not being able to find a complete explanation of the phenomena described in this study in medium conditions only, it may be assumed that the ecological structure of the population, which may be different with different absolute numbers, is also responsible for them. It may also be imagined, for instance, that not only its density, but also its dynamic density (i.e. frequency of the individuals to meet) affects the growth of population, and the latter certainly increases with the absolute number of individuals (i.e. together with the size of the culture).

It is possible that the results obtained may be of practical significance for culturing *Paramecium* in laboratory conditions by demonstrating that it is better to maintain a great number of small cultures than a few cultures great in size, in order to obtain abundant experimental material.

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ZAGĘSZCZENIE A WIELKOŚĆ ŚRODOWISKA W POPULACJACH *PARAMECIUM CAUDATUM*

Streszczenie

Wykonano szereg doświadczeń mających na celu wykazać, czy wzrost liczebny populacji *Paramecium caudatum* i odpowiadająca mu produktywność hodowli zależy od bezwzględnej wielkości kultury. Celem drugiej części pracy była próba identyfikacji czynników odpowiedzialnych za powstające różnice produktywności małych i dużych hodowli.

Informacje o wzroście liczebnym populacji uzyskiwano drogą regularnych pomiarów liczby osobników przypadającej na 1 ml środowiska. Gęstość wyjściowa wszystkich hodowli wynosiła zawsze 50 osobników na 1 ml, zmienne zaś były ich objętości oraz — w konsekwencji — ogólne, bezwzględne liczby osobników. Wyrównano warunki pokarmowe, cieplne i oświetlenie dla wszystkich hodowli.

Pierwsza seria doświadczeń obejmowała 6 wielkości kultur od 50 ml do 1600 ml, zakładanych w środowisku wody wodociągowej i utrzymywanych w naczyniach o jednakowym kształcie, przy stałym stosunku wysokości słupa cieczy do wolnej powierzchni. Wyniki przedstawione w Tab. I i na Fig. 1 wskazują, że aczkolwiek ogólna liczba osobników w hodowlach większych jest wyższa, to jednak gęstość jest w nich regularnie niższa. Największą produktywnością cechują się więc hodowle o najmniejszej objętości ogólnej. Doświadczenie to prowadzono przez 33 dni i zakończono je tuż po osiągnięciu przez hodowlę maksimum wzrostu. Drugi eksperyment, trwający 140 dni i obejmujący (podobnie jak wszystkie następne doświadczenia) hodowle o objętości 100 ml, 400 ml i 1600 ml, potwierdził poprzedni rezultat oraz dowiódł, że niższa wydajność populacji hodowanej w większej objętości środowiska wyraża się obniżeniem poziomu maksimum wzrostu, a nie jego opóźnieniem (Fig. 2).

W poszukiwaniu czynników wywołujących zaobserwowane różnice wydajności hodowli sprawdzono rolę zmian pH środowiska powodowanych przez rozwijające się pierwotniaki. W tym celu powtórzono eksperymenty w środowisku buforowym o pH 7.0. Jedynym rezultatem zbuforowania środowiska jest osiągnięcie większej regularności krzywych wzrostu, charakter różnic w ich przebiegu pozostaje jednak nie zmieniony (Fig. 3).

Wyliczenie wykazuje, że różnice wydajności małych i dużych hodowli stają się niezbyt istotne, jeżeli gęstość podać w przeliczeniu na 1 cm^2 powierzchni kultury, zamiast przeliczać ją na 1 ml jej objętości (Tab. II). Powierzchnia ścianek bocznych jest istotna z tego względu, że na nich tworzą się tigmotaktyczne skupienia osiadających pierwotniaków; niemniej ważna jest swobodna powierzchnia górna, bo decyduje ona o natlenieniu środowiska. Biorąc pod uwagę, że przy zachowaniu niezmiennego kształtu naczynia wzrost powierzchni nie nadąża za wzrostem objętości, hodowle duże mogą być: 1) niedotlenione, 2) przegęszczone na ściankach mimo niższej liczby osobników na 1 ml środowiska.

Powtórzono wobec tego eksperymenty w kulturach o tak dobranym kształcie, aby mimo różnic objętości zachować stałą wysokość słupa cieczy (tzn. aby wyrównać natlenienie). W następnej serii doświadczeń wyrównano ponadto także powierzchnię ścianek bocznych zanurzając w większych naczyniach odpowiednią liczbę probówek. W wyniku tych zabiegów uzyskano bardzo znaczne zniwelowanie różnic w produktywności dużych i małych kultur (Tab. III i Fig. 4), co wskazuje, że istotnie wynikały one w wielkiej mierze ze zróżnicowania struktury środowisk.

Nie udało się jednak uzyskać zupełnego uniezależnienia produktywności hodowli od jej ogólnej objętości. Utrzymująca się różnica może więc mieć związek ze strukturą samej populacji, np. z tym, że dynamiczna gęstość populacji prawdopodobnie zależy od jej ogólnej bezwzględnej liczebności.

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