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NOTES ON PLATYMONAS IMPELLUCIDA (McLachlan&Parke) SPECIES IN MONO-ALGAL CULTURES

Pia Elena Mihnea

Romanian Institut for Marine Researches Constanţa/Romania

A b s t r a c t

A new species found in the Black Sea, Platymonas impellucida McLachlan & Parke, is described. Were analysed the rithms of its growth in culture conditions. Nine variants of the same culture medium were used; the environmental conditions were maintained constant and identical for each alternative. It was attempted a comparison of natural populations with the experimental ones, and the data regarding differences found were discussed.

The dinamics of the Chlorophyta group did the subject of some previous observations (MIHNEA, 1969). Indeed, in the abundent phytoplanktonic material, sampled during the 1968 Spring we found a lot of species belong to this group. Some of these species were isolated in mono-algal cultures.

Our observations on these cultures are liable to get usable to explain the path-ways of their evolution in marine phytoplankton.

The growing rithms of the monocultures were compared with quantitativ data of the natural populations.

Platymonas impellucida is a new species for the Black Sea phytoplankton. It was described also as a new species for world algological flora by McLACHLAN & PARKE in 1967.

Material and methods

This species was isolated from a sample collected on June 6th, 1968, in front of the Agigea Laboratory, at 5m depth, and it was cultivated in ErdSchreiber medium.

The culture media used were nine alternatives of the PM type ; these differed one from other as to their component levels (MIHNEA and LAURENZI, in press).

We considered necessary to maintain the temperature close to that of the marine environment at the time of sampling (that was 21°to 22°C), a constant 0_2 level (achieved through an uninterrupted air bubbling), as well as light conditions of 2,500 lx. The cultures were done in air - tight vessels, thus preventing evaporation, hence an increase in salinity level.

The morphology of Platymonas impellucida have been investigated under direct light and phase contrast.

Results and discussions

The Platymonas genus belongs to the Chlorophyta Phylum the Prasinophyceae Class, Pyramimonadales Order.

With regrad to morphological features of this species that were described by J. McLACHLAN and M. PARKE, we found them wholly, at the species sampled from the Black Sea; namely the eliptical form, ovoid, convex-concave with visible constriction in the median area, wich can be observed when the cell is looked on the narrow face (Plate I, fig. 3). The same, a starch shell is not developed around the pyrenoid.

McLACHLAN and PARKE showed the variable size of the cells,

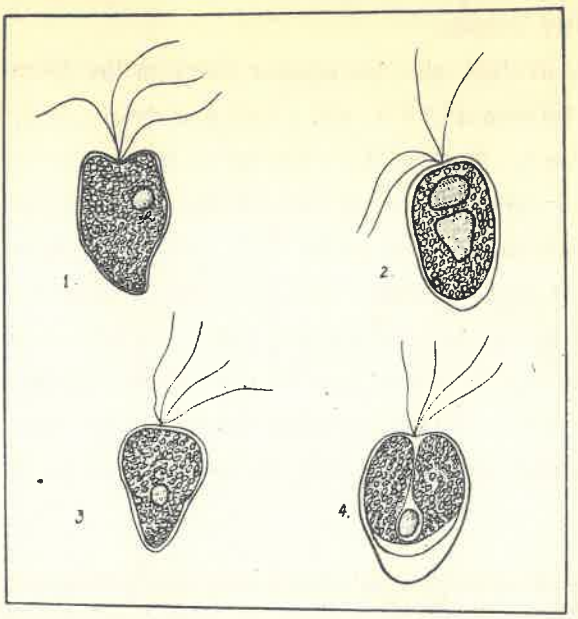


Plate I - 1-2 : The free cell stage of the Platymonas impellucida species; 3 : lateral view of a cell (to note the median construction); 4 : cell prior to divisionary stage

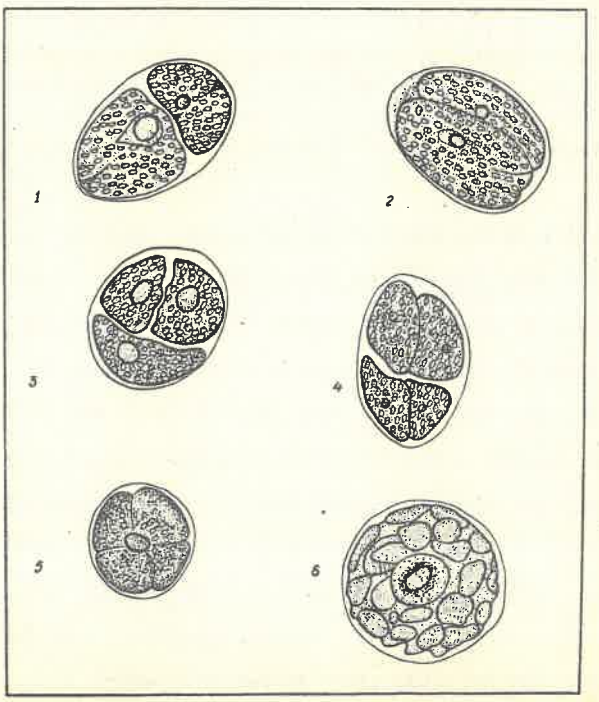


Plate II - 1-5 : Division stages with 2-to 4 cells in the theca ; cyst

comprised between 14 μ and 23 μ in lenght, and 8 μ - 17 μ in width for the Puerto Rico waters.

The average size for species living in the Black Sea is comprised between between 15, 68 μ and 17, 92 μ in length, and 11, 02 μ to 13.53 μ in width (Table I). Studying the evolution of these cultures, we have noted that their size varied depending upon culture age; this reflected the various stages of accumulation thus, in the first week, the size varied between 14.70 μ and 16.70 μ in length, and 5.14 μ to 13.96 μ in width, absolute values. Starting with the 30th culture day, cells size werw visibly bigger: 18.37 μ to 30.00 μ in length, and 16.17 μ to 29.00 μ in width, absolute values, too. It results that the absolute values for the cells size from the Black Sea species were higher than the values cited by the above authors (Table I).

Table I

Average value of cells size in Platymonas impellucida cultivated on the nine culture media used (average obtained on 80 individuals)

The cell mean lenght /width in microns	Culture medium								
	Pm^{A}	Pm^{B}	Pm^{C}	Pm^{2}	Pm^{3}	Pm^{4}	Pm^{5}	Pm^{6}	Pm^{7}
	15, 68	17, 009	17, 92	16, 38	16,003	16,01	16,03	15,97	16, 49
	11, 35	13, 53	12,66	13,39	11, 33	11,02	11,46	12,45	12, 02

Just like in the other Prasinophyceae, motility is ensured by a flagellar system. The Platymonas impellucida has four flagellae of which size were two-thirds of the algal length. The cells assumed giratory motion, moving fowards with their flagellae; they have also the possibility to move backwards too, but much slowly (McLACHLAN and PARKE, 1967).

From ourself observations on samples proceed from the Black Sea, results that the motion depends upon the development degree, just like for cell size. A young culture moves very quickly; starting with the 28th culture day, that is when the size was larger, the cells were moving much slower. Moreover, when the culture regressed, the flagellar system also disappeared and the cells enter in the rest stage.

Direct division was the only multiplication system described by

the authors of the species (McLACHLAN and PARKE, 1967), and found by us, too.

The presence of "division" forms with theca and free forms was observed all over the growth period. The number of "division" forms were small in lag. phase and increased during the exponential phase of the development curve. The lower number of the stages with theca was recorded within the stationary phase. Two to four cells were usualy obtained, by the division process; the cells had the same size. We observed, also, cases when the division gave cells of unequal size. We couldn't detect the resons inducing the appearance ot two to four cells after the mythotic division, nor the factors determining the division time, because the phenomenon occured in cells of widely varying size.

The cysts which are the resistant forms, appeared in the stationary, phase, being very numerous in the descending stage of the population. In fresh medium, the cysts germinated, resulting in two flagel-cells. The size of the resting forms was comprised between 28 μ and 38 μ

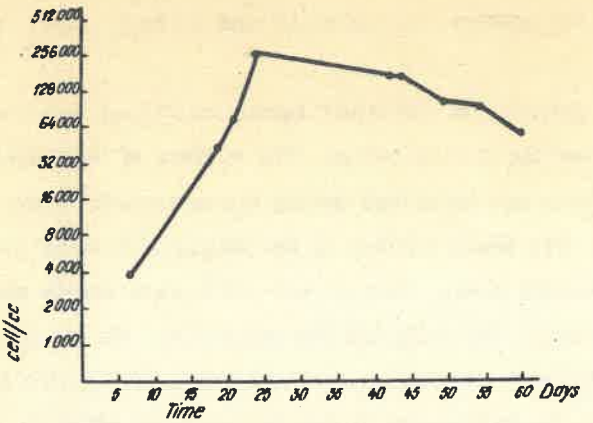
I shall not insist on the inner morphology, as this was described by the above authors.

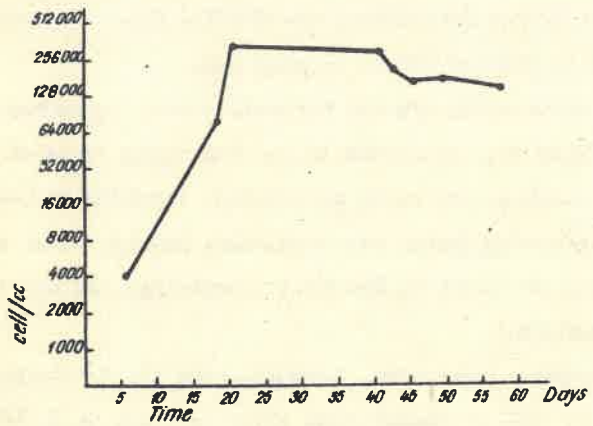
A typical marine form, mentioned for the La Parruera waters, Puerto Rico, for Gulf of Naples (data given by Prof. A. A. Allem) in the Black Sea waters it can be quoted - for the moment - at Romanian shores, namely in front of the Agigea Station, near the Mamaia coast, and in Midia-Cape waters.

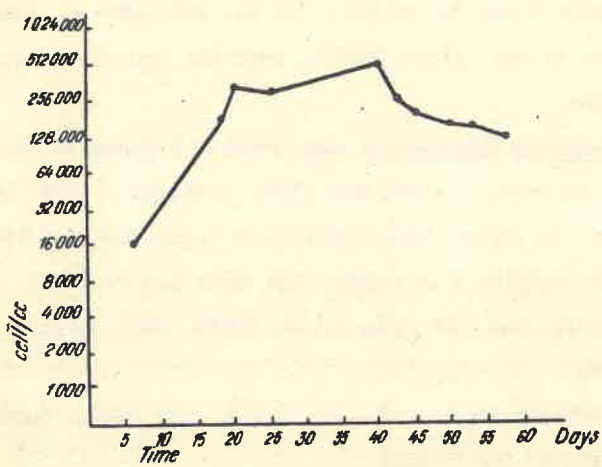
Platymonas impellucida was found in samples collected late in Spring and in Summer, in 1968 and 1969, at depths of 5m, 10m, and 20m, only quite near the shore. Quantitatively it varied between 148 cells per cc (1968 Summer, Agigea, 5 m depth), 230 cells per cc (1968, Autumn, Mamaia, 10m depth), and 480 cells per cc (1969, July, Agigea, 5 m, 10m, and 20m depth).

As already mentioned, nine media were used, varying as to the amount of additional substances.

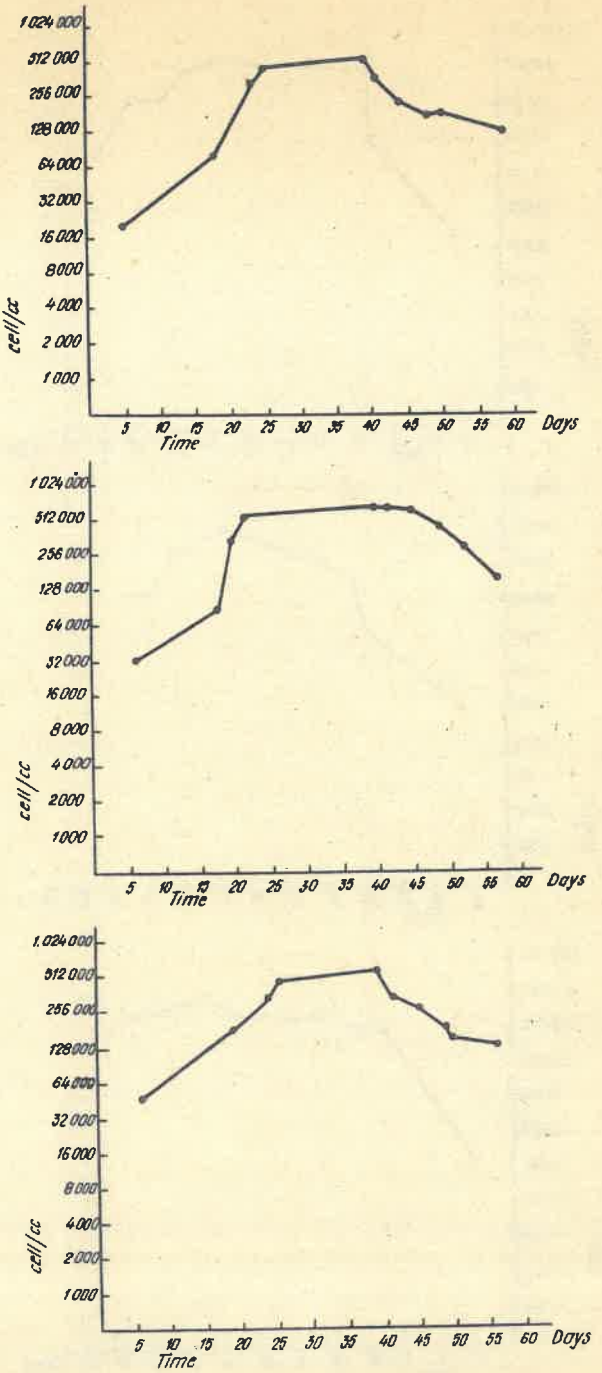
From the analysis of the growth curves of the cultures investigated (Diagram 1-9), it resulted that the best suited culture media were



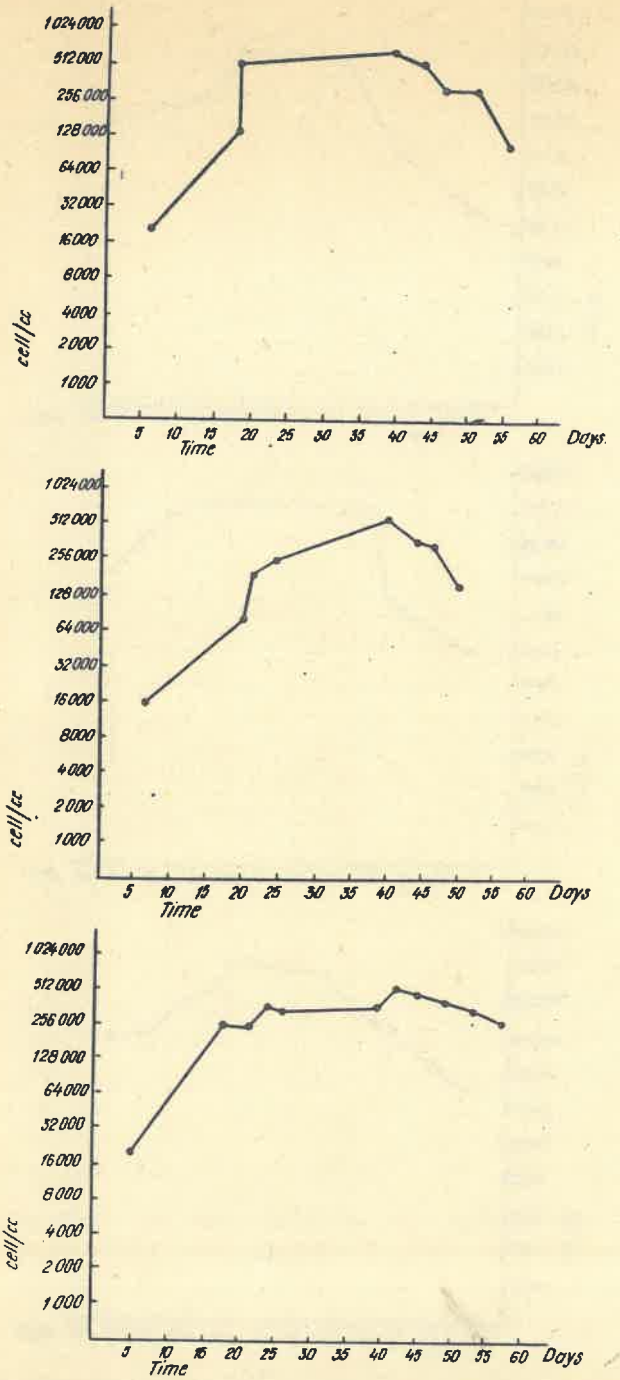




Diagrams 1-3 - Development of the Platymonas culture in various media of the PM type : 1:PM^{a}; 2 : PM^{b}; 3 : PM^{c}

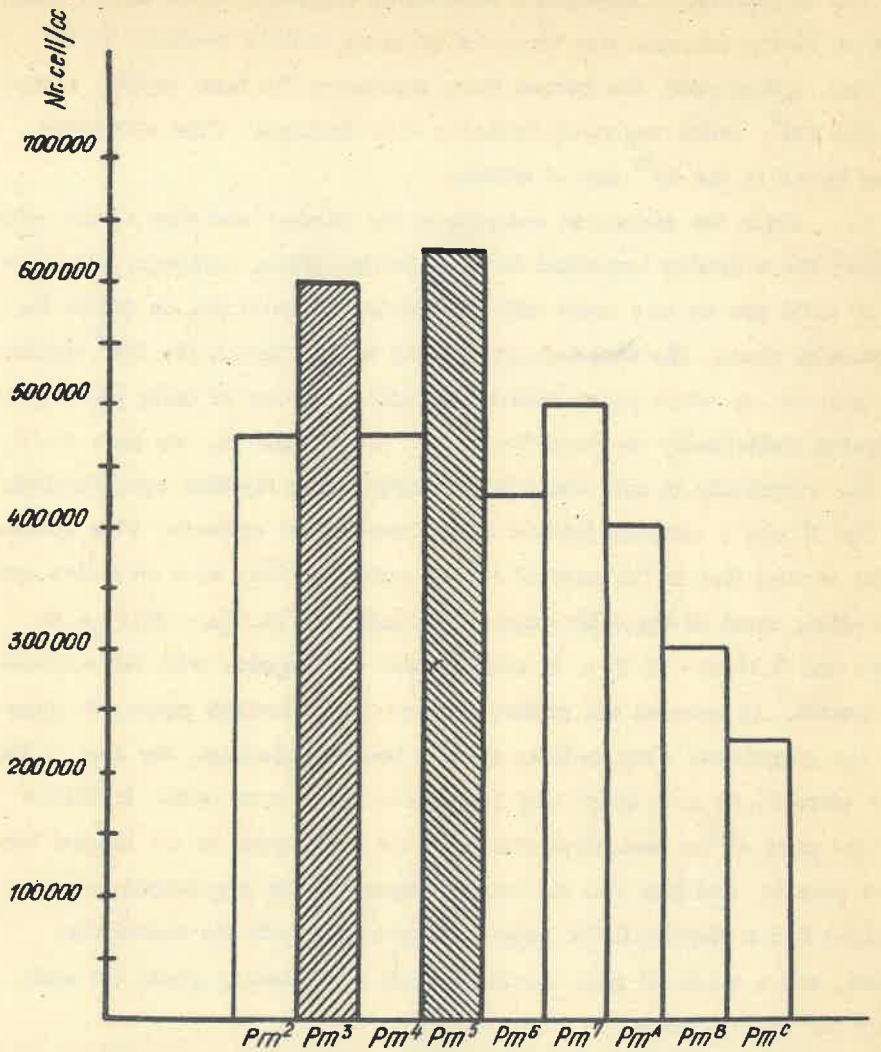


Diagrams 4–6 – Development of the Platymonas culture in various media of the PM type: 4 ; PM_1^2: 5 : PM^3; 6 : PM^4



Diagrams 7-9 - Development of the Platymonas culture in various media of the PM type: 7 : PM^5; 8 : PM^6; 9 : PM^7

the PM^3 and PM^5 as their concentration level allowed a satisfactory growth during the exponential phase, reaching also the highest density on cc. The average number of cells per cc for the peach development period



Graph Nr. 10 The growth medium

Diagram 10 - Graphical representation of the average values of number of the cell/cc in peak growth period for the nine culture media used

was 600, 373 cells per cc for the PM^3 medium, and of 650, 253 cells per cc for the PM^5 culture medium (Diagram 10).

Mention should be made of the fact that each of the experimental media were inoculated originally with 200 cells per cc.

It also observed that the development curves had generally a short lag phase, although culture media inoculation was performed with an old culture. The exponential phase was comprised between the 5th and the 25th day of culture. It assumed a progresive character up to the 18th day, when an abrupt increase was recorded at every culture medium, to the 25th day. Afterwards, the curves were stationary for most media, excepting the PM^{6}, which registred further a slow increase. This stationary phase lasted to the 40th day of culture.

From the statistical analysis of the number and size of the cells resulted the following important fact: in decline phase, although, the number of cells per cc was equal with the number of cells per cc within the exponential phase, the biomass gave higher value. Taking the PM^{5} medium like witness, in which we recorded the highest number of cells per cc, and analysing statistically the variation in cell size (Table II), we have noted that the variability in cell size did not depend upon medium concentration, but that it was a common feature of all investigated cultures. This observation showed that in the case of a very young culture, with an active multiplication, most of the cells were of the order of 14.70μ - 16,70μ in length and 5.14μ - 13.96μ in width (which corresponds with the exponential phase). As soon as the culture entered the stationary phase, it appeared age phenomena : the cellular content became abundant, the size of the cells were 18.00μ in length and 14.00μ to 18.37μ in width. It follows that the peak of the numerical value did not correspond to the biggest biomass amount, and just like the natural phytoplankton populations, these cultures had a photosynthetic peak (corresponding with the exponential phase), and a ponderal peak (corresponding to stationary phase but espe. cially the decline phase).

The natural populations studied in June, July, and August were quantitatively lower than the other phytoplanktonic organisms ; the highest number recorded was of 480 cells per cc (July, at 10 m depth), as against 3,200 cells per cc recorded for the Exuviaella cordata, 2,500 cells per cc for Cyclotella caspia and 800 cells per cc for Chlamydomonas intermedia. The population was maintained at a density of 230 cells per cc, with small shifts all over the entire investigated period.

Table II

Statistical analysis of the cell size variation (in microns), for the Platymonas cultures, compared with the PM^5 media taken as witness; n: number of cells measured; X : aberage; E S: standard error; p: probability

(p) / F (n°)		Culture medium								
Length / width		Pm^A	Pm^B	Pm^C	Pm^2	Pm^3	Pm^4	Pm^5	Pm^6	Pm^7
18th day of culture	n	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00
	x	13, 44	13, 37	14, 33	13, 59	14, 22	14, 99	13, 74	13, 04	13, 00
	E S	0, 80	0, 70	0, 02	0, 30	0, 90	0, 80	0, 80	1, 80	0, 70
	p	0, 26	0, 35	0, 70	0, 11	0, 24	1, 10		0, 35	0, 70
	n	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00
	x	11, 17	8, 96	9, 99	10, 36	11, 17	9, 55	10, 65	10, 16	9, 40
	E S	0, 80	0, 80	0, 40	0, 20	0, 70	0, 70	0, 80	1, 20	0, 80
	p	0, 44	1, 47	0, 77	0, 28	0, 48	0, 88		0, 33	1, 10
24th day of culture	n	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00
	x	14, 25	14, 77	14, 47	14, 40	13, 08	14, 55	12, 93	12, 86	13, 96
	E S	0, 02	0, 02	0, 30	0, 01	0, 50	0, 40	0, 90	0, 80	0, 30
	p	1, 34	1, 60	1, 54	1, 38	0, 13	1, 54		0, 66	0, 77
	n	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00
	x	9, 70	11, 16	9, 69	9, 99	8, 30	10, 28	9, 62	9, 40	10, 28
	E S	0, 50	0, 60	0, 40	0, 60	0, 50	0, 80	0, 80	0, 50	0, 60
	p	0, 83	1, 55	0, 66	0, 35	1, 32	0, 57		0, 22	0, 66
40th day of culture	n	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00
	x	21, 46	23, 68	22, 63	23, 15	21, 82	19, 99	22, 85	22, 34	22, 26
	E S	1, 70	2, 60	0, 70	1, 30	0, 70	3, 09	1, 70	0, 90	1, 50
	p	0, 48	0, 26	0, 11	0, 13	0, 55	0, 79		0, 26	0, 22
	n	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00	10, 00
	x	13, 96	17, 47	16, 90	15, 62	13, 66	14, 69	15, 14	18, 29	15, 94
	E S	0, 02	2, 90	0, 80	0, 90	0, 20	1, 70	0, 50	2, 70	0, 80
	p	2, 20	0, 72	0, 82	0, 44	1, 76	0, 24		0, 11	0, 66

The "in situ" development, as well as the evolution of the algae in culture media, raised the following questions: which of the causes inducing in other species a low number of individuals under natural conditions are valid also for Platymonas ?

From data recorded so far by other authors (FOGG, 1966; RICE, 1954 ; ROUND, 1966), as well as from our own observations

(MIHNEA, 1969), it results that the above phenomenon was due do the following causes :

- being a newly established species in the Black Sea it had not sufficient time to adapt itself completely to the conditions existing there ;

- the competition between various vegetal organisms gets possible the maintenance of one of them at a quantitatively low level ;

- active consumption of the unicellular algae by organisms belonging to the second foodchain level conceals the phenomenon of intense multiplication by the former.

In order to determine the real causes of the low quantitative situation of the investigated species, in situ, sugestions are made to continue the ecological studies. On the other hand, the high division rates point the possbility to use Platymonas i. in mass-culture.

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