

Resistance of *Paramecium* Species (Ciliophora, Peniculia) to Salinity of Environment

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Summary

The salinity resistance and tolerance of 10 species from the genus *Paramecium* were investigated. The species investigated could be divided into groups in according with their resistance. These groups partly corresponded to the classification of the genus proposed earlier on the basis of morphological features. The ciliates from the "woodruffi" group were more resistant than paramecia from the "aurelia" group. *P. bursaria* and *P. putrinum* (the former "putrinum" group) have lower salinity resistance and tolerance than the species from two other groups. *P. polycaryum* has a salinity resistance and tolerance which corresponded to its "intermediate" position between the "aurelia" and the "woodruffi" groups.

Key words: *Paramecium*, resistance, salinity, taxonomy, tolerance.

Introduction

All species of genus *Paramecium* are able to exist in fresh water biotops. However, in marine biotops paramecia have been found as well (Wichterman, 1986). These ciliates repeatedly were found in oligohaline parts of estuaries, but some of them are able to exist both in polyhaline and in fresh waters (Fokin and Chivilev, 1999).

Among numerous species of *Paramecium* only four, namely *P. woodruffi*, *P. calkinsi*, *P. nephridiatum* and *P. duboscqui* can be considered euryhaline. These species frequently were found on coasts of northern seas (Fokin and Sabaneeva, 1990; Fokin et al., 1995; Fokin et al., 1999a, 1999b), though in the literature they are regarded as uncommon (Wichterman, 1986).

At the beginning of the century the distribution of *Paramecium* in fresh and marine water was investigated by several authors (Chatton and Tellier, 1927; Bullington, 1930; Finley, 1930; Frisch, 1939). They found *Paramecium* only in fresh water or salt water with low concentration, usually less than 1% (Frisch, 1939). Only *P. ambiguum*, *P. calkinsi* and *P. woodruffi* have been found in salt water. The first species is regarded now as nonvalid (Wichterman, 1986).

A number of investigators have tested the ability of *Paramecium* to adapt itself to marine water or to variations of the salt concentration. According to their results *P. caudatum* can survive, when the salt concentration of the culture medium is gradually increased to approximately 1%. The maximum salt concentration to which *P. caudatum* and *P. aurelia* can be transferred directly varies from 0.3 to 0.75 % (Chatton and Tellier, 1927; Finley, 1930). Bullington (1930) investigated the resistance of *P. calkinsi* and *P. woodruffi*. He showed that these ciliates can be transferred directly or in two steps from fresh water to 100% marine water. Thus the existence of at the least two groups of *Paramecium* species which differ in relation to the salinity factor have been shown.

The taxonomy of *Paramecium* species has a long history. Generally, different authors distinguish two species groups within the genus: "aurelia" and "bursaria" (Woodruff, 1921; Wichterman, 1986). Jankowski (1969) recognised three such groups - "putrinum", "woodruffi" and "aurelia". They were regarded by this author as taxonomic subgenera (*Helianter*, *Cypreostoma* and *Paramecium*, respectively) which differ in many morphological characteristics (Jankowski, 1969). All mentioned species of *Paramecium*, which are able to exist in brackish water belong to the "woodruffi" group. *P. polycaryum*,

according to Jankowski, belongs to this group as well. However, according to the results of morphometry and several biological features (Fokin and Chivilev, 1999) this species should be placed somewhere between the “woodruffi” and the “aurelia” groups. The taxonomic relationship of *P. putrinum* and *P. bursaria* still unclear (Fokin and Chivilev, unpublished).

At present, any data on salinity resistance for a number of paramecia are absent. The aim of this study was an analysis of resistance and tolerance range of several species of euryhaline *Paramecium*, as well as the fresh water ones, which are representatives of the different species groups inside the genus.

Material and Methods

The resistance and viability of ciliates acclimated to media of various salinity created with marine salt were investigated. The following species were used in the experiments: *P. caudatum*, *P. jenningsi*, *P. biaurelia*, *P. multimicronucleatum* belonging to the “aurelia” group; *P. calkinsi*, *P. duboscqui*, *P. nephridiatum* and *P. woodruffi* belonging to the “woodruffi” group and three species which have no clear relationship with the other - *P. polycaryum*, *P. bursaria* and *P. putrinum* (Table 1).

Cells were cultivated on lettuce medium inoculated with *Enterobacter aerogenes* (Sonneborn, 1970). The necessary salinity was obtained using artificial marine water (Chubravyi, 1983), diluted in lettuce medium. Before use in the experiment, the ciliates were maintained in fresh or marine lettuce medium not less than one month. At all times the ciliate cultures were fed twice a week.

During the resistance experiments cells were transferred from the medium in which they were kept into a medium of artificial marine water prepared by the above-mentioned recipe with the addition of bidistilled water. Thus a scale of salinity in a range of 5-15 ‰ was obtained for the testing. If the cells of any stock survived in this

salinity, the experiment was repeated in a number of higher salinity ranges from 15-60 ‰.

The experimental cells were contained in depression slides with a volume of 2 ml. The quantity of dead cells was determined under binocular microscope MBS -9 (x24) after 5, 15, 30 etc. minutes. The experiments were made in one repetition for each value of salinity. The volume of material for each experiment was 25-70 cells, usually, 30-40. As a control 25-30 cells were used.

The logits of mortality data obtained on fixed time intervals were calculated. These logits were used to calculate the coefficients of a linear regression equation. These equations were the basis for the calculation of the time of 10 % (LT10), 50 % (LT50) and 90 % (LT90) cell mortality in test salinity.

Except the standard estimation of variability (LT50), received by logit-analysis (Reference book, 1986), the comparative reaction of *P. caudatum* and *P. jenningsi* acclimated to the fresh lettuce medium and salt lettuce medium on the lethal value of salinity was observed. According to these results the amongdecil range (Zhivotovsky, 1991) was calculated. Amongdecil range was obtained as the difference between the values of 90% (LT90) and 10% (LT10) cell mortality. This parameter has allowed to estimate the variability of main part of distribution.

The upper limit of salinity tolerance was defined as the maximal salinity value where the cells acclimated to fresh or salt medium survived not less than 2 day, from the start of the experiment.

Results

The acclimation of the ciliates increased both the time to the beginning of destruction and the time of the life duration (Table 2). The life duration in the upper part of the tested salinity scale (which is estimated by parameter LT90), and the time to cells destruction to the certain value of test salinity were a little different. Apparently, in the

Table 1. *Paramecium* used in experiments

| Species | Stock | Origin | Date |
|-------------------------------|--------|---|------|
| <i>P. caudatum</i> | PK-100 | St. Petersburg district, Russia | 1995 |
| <i>P. jenningsi</i> | IA | Bangalor, India | 1958 |
| <i>P. biaurelia</i> | T10-5 | St. Petersburg district, Russia | 1990 |
| <i>P. multimicronucleatum</i> | GL-5 | Tbilisi, Georgia | 1989 |
| <i>P. calkinsi</i> | OCE4-1 | White Sea, Karelia, Russia | 1994 |
| <i>P. duboscqui</i> | BB2-3 | Baltic Sea, St. Petersburg district, Russia | 1993 |
| <i>P. polycaryum</i> | Pp5-8 | Toljatty, Russia | 1988 |
| <i>P. bursaria</i> | PK-60 | St. Petersburg district, Russia | 1994 |
| <i>P. putrinum</i> | PG-5 | St. Petersburg district, Russia | 1995 |

Table 2. Decil and amongdecil range of destruction time of *P. caudatum* (stock PK-100) and *P. jenningsi* (stock IA)

| Salinity of acclimation | Fresh lettuce medium | | | Marine lettuce medium | | |
|-------------------------|-----------------------------|--------|-----------|-----------------------|--------|-----------|
| Test salinity | LT10 | LT90 | LT90-LT10 | LT10 | LT90 | LT90-LT10 |
| | <i>Paramecium caudatum</i> | | | | | |
| 7 | 95.19 | 142.45 | 47.24 | - | - | - |
| 8 | 81.57 | 105.99 | 24.42 | 192.85 | 392.61 | 199.76 |
| 9 | 81.73 | 105.36 | 23.63 | 138.64 | 243.26 | 104.62 |
| 10 | 51.16 | 85.55 | 31.39 | 113.13 | 177.75 | 64.62 |
| 11 | 33.05 | 67.22 | 34.17 | 81.91 | 136.84 | 54.93 |
| 12 | 26.29 | 56.49 | 30.20 | 92.47 | 148.81 | 56.34 |
| 13 | 39.97 | 60.89 | 20.92 | - | - | - |
| 14 | - | - | - | 58.03 | 120.82 | 62.79 |
| | <i>Paramecium jenningsi</i> | | | | | |
| 7 | 80.29 | 136.63 | 56.34 | - | - | - |
| 8 | 46.09 | 108.00 | 61.91 | - | - | - |
| 9 | 14.97 | 90.02 | 80.35 | 115.71 | 291.49 | 175.78 |
| 10 | 7.74 | 39.12 | 31.38 | 79.68 | 161.06 | 81.38 |
| 12 | 5.98 | 20.62 | 14.64 | 46.67 | 111.37 | 64.70 |
| 13 | - | - | - | 26.66 | 82.40 | 56.34 |
| 15 | - | - | - | 10.97 | 62.64 | 51.67 |

Table 3. Tolerant limits of some *Paramecium* species

| Species | Salinity of acclimation, ‰ | Tolerant limit, ‰ |
|-------------------------------|----------------------------|-------------------|
| <i>P. caudatum</i> | fresh | 3,5 |
| <i>P. caudatum</i> | 5‰ | 6 |
| <i>P. jenningsi</i> | fresh | 3,7 |
| <i>P. jenningsi</i> | 6‰ | 7,5 |
| <i>P. biaurelia</i> | fresh | 4,8 |
| <i>P. multimicronucleatum</i> | fresh | 4,5 |
| <i>P. bursaria</i> | fresh | 4,4 |
| <i>P. putrinum</i> | fresh | 2,1 |
| <i>P. calkinsi</i> | fresh | 15,3 |
| <i>P. nephridiatum</i> | fresh | 18,2 |
| <i>P. polycaryum</i> | fresh | 6,8 |
| <i>P. woodruffi</i> | fresh | 18,7 |

field of large level of the salinity it acts as the unspecific agent quickly resulting in the destruction of cells of stocks. The right part of the diagram corresponds to this area (Fig. 1, 2).

The beginning of destruction shifted and the life duration increased when test salinity reached a certain value (Table 2). The increasing of life duration and the time of destruction did not occur synchronously. The duration of life rose faster than the time of destruction shifted.

The value of amongdecil range was directly connected with the time elapsed from the moment of destruction of the first cell to the moment of destruction of the last one (Table 2). Its values were very similar in the upper part of the tested salinity scale. This parameter increased simultaneously with the increasing of the destruction beginning time (LT10).

The hyperbolic dependence, which was used for the analysis of experimental material has conclusive theoretical and practical advantages. The diagram of hyperbola is limited by asymptotic lines. In the diagram of experimental dependence time of 50 % mortality - test salinity, the vertical asymptotic line should correspond to the salinity at which no cells die. This value is the limit of the tolerant salinity range. Hence processing of experimental data on model of a hyperbolic regression gives certain advantages. It is necessary to note, that the use other regression models, for example, logistic regression, theoretically does not permit the estimation of the salinity at which with cell do not die. In table 3 the values of asymptotic lines are given which in our opinion coincide with upper tolerant limits. The obtained data are very similar to the appropriate values received by other methods (unpublished). The values of the vertical asymptotic line for *P. calkinsi* were 10.9; 15.3; 15.51; 15.7; 15.3 for LT40, LT50, LT60, LT70 and LT80, respectively. For calculation of tolerant limit values it is probably best to use parameters of 50-100 % mortality. Asymptotic line is stabilised as beginning from LT50.

The lines of 50% mortality of the “woodruffi” group (Fig. 5, 7, 8) in scale of growing salinity were very similar to the lines for the “aurelia” group (Fig. 1, 2, 3).

The resistance of *P. multimicronucleatum* and *P. biaurelia* to salinity impact was very similar of *P. jenningsi* and *P. caudatum* resistance (Fig. 2). Despite the fact that the species of the “aurelia” group were very similar with respect to mortal salinity impact (limit 5-6 ‰ during two days are common for them), their tolerance was different.

The maximum upper tolerant salinity limit for *P. caudatum*, *P. jenningsi* and *P. multimicronucleatum* changed insignificantly. The value 8 - 9 ‰ for these ciliates remained insuperable. Another result was obtained for *P. biaurelia*. The maximum upper tolerant limit for this species was 17 ‰. Similar results were received for other

species of the “aurelia” complex - *P. sexaurelia* and *P. primaurelia* (unpublished).

Completely different results were obtained for ciliates, belonging to the “woodruffi” group. *P. duboscqui*, *P. woodruffi*, *P. nephridiatum* and *P. calkinsi* acclimated to fresh lettuce medium had an upper tolerant limit above 8 ‰ (Fig. 4,5,7,8). *P. polycaryum* which had been acclimated for long time in fresh lettuce medium, could not overcome this barrier in the first attempt and, as well as the “aurelia” group species had 100%’s viability only at 6-7 ‰ (Fig. 9). It was possible to acclimate it for 20 ‰ by shifting it first from fresh lettuce medium into 7 ‰ salinity and after one month into a medium with 18 ‰ salinity (Fig. 4).

As a result of consequent acclimation the tolerance of these species considerably rose in both cases (high and low salinity). It is of special interest that *P. calkinsi* and *P. duboscqui* which have been acclimated to 30-35 ‰ not only survive in a medium with 1 ‰ salinity but divided there after two days. Thus, it is possible to stress the existence of considerably high (in comparison with other fresh water paramecia) euryhalinity of the ciliates in the “woodruffi” group.

The lines of the upper limit of tolerance of *P. duboscqui*, *P. calkinsi*, *P. polycaryum* were linear and almost parallel to each other (Fig. 4), that strongly reflects basic similarity of processes of the adaptation to salinity. *P. duboscqui* had the greatest potential tolerance, *P. polycaryum* – the least. The lines of the upper tolerance limits of *P. caudatum* are not parallel to the lines of the “woodruffi” group species which, obviously, indicates differences between these species.

A unique appreciable difference was the expansion of salinity interval of incomplete destruction for the species of the “woodruffi” group. This interval is defined as the difference between the maximal salinity in which survive 100 % of cells survive and the minimal salinity in which all cells are die. So, for *P. calkinsi* in salinity 15-17 ‰ some of cells die, but others have survived for 2 days and reproduced. For the species from other groups of *Paramecium* this interval was significantly less. A change of salinity of 1 ‰ resulted in 100 % destruction of the cells, whereas all cells participated in the experiment earlier survived.

The least resistance to salinity impact was demonstrated by *P. putrinum* and *P. bursaria*. The area of unspecific salinity action for *P. putrinum* and *P. bursaria* moved on the diagrams to the left in comparison with the diagrams for the “aurelia” group (Fig. 6). The time of survival in a medium with 5 - 7 ‰ salinity also was appreciably less. The upper tolerance limit for both species, which were acclimated to the fresh lettuce medium, was located near 3 ‰. This value was the smallest among all investigated species.

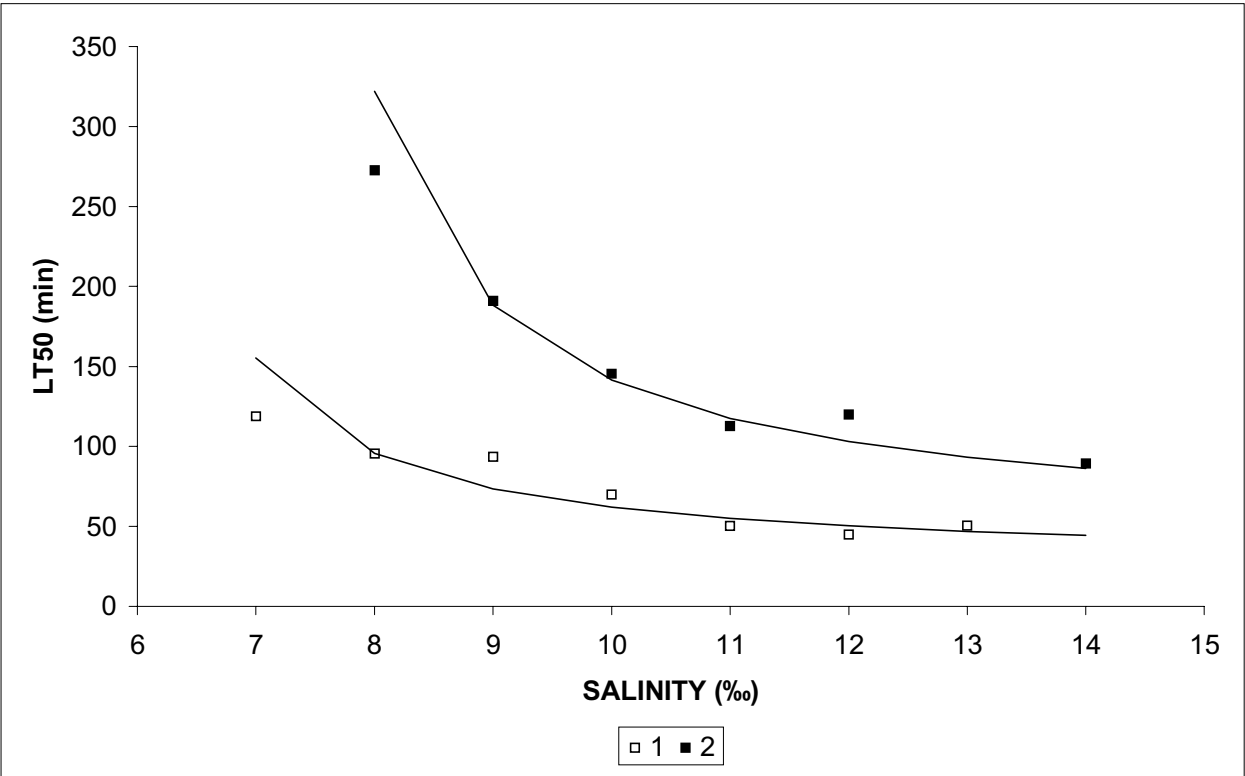


Fig 1. Resistance of *P. caudatum* to salinity impact. Cells acclimated to fresh water lettuce medium (1); cells acclimated to marine lettuce medium (2).

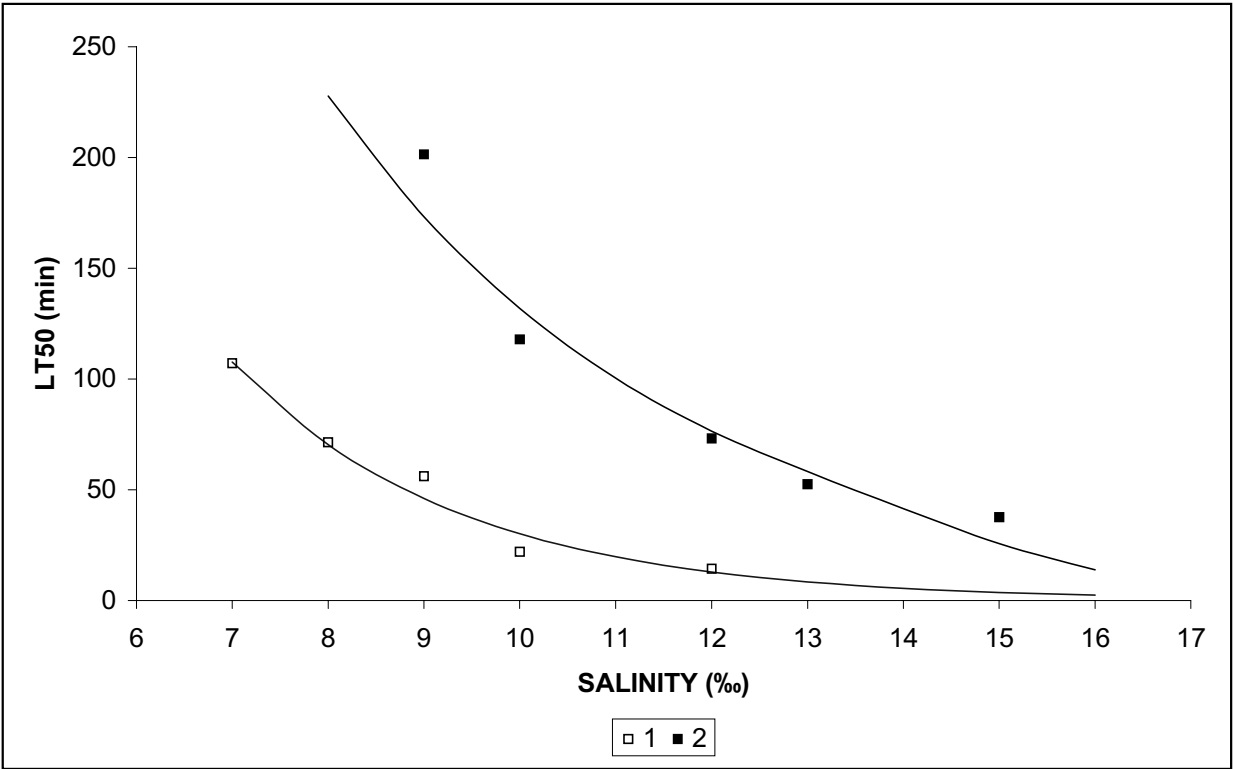


Fig 2. Resistance of *P. jenningsi* to salinity impact. Cells acclimated to fresh water lettuce medium (1); cells acclimated to marine lettuce medium (2).

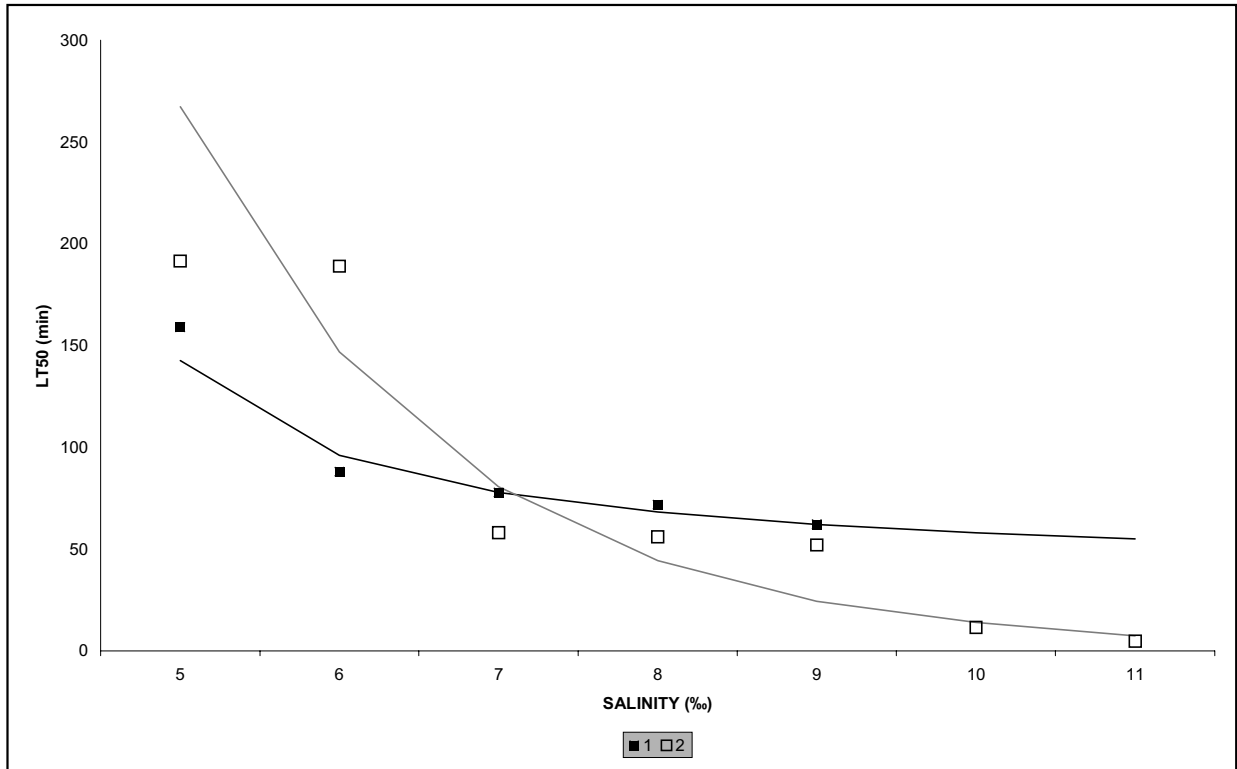


Fig 3. Resistance of *P. multimicronucleatum* (1) and *P. biaurelia* (2) to salinity impact.

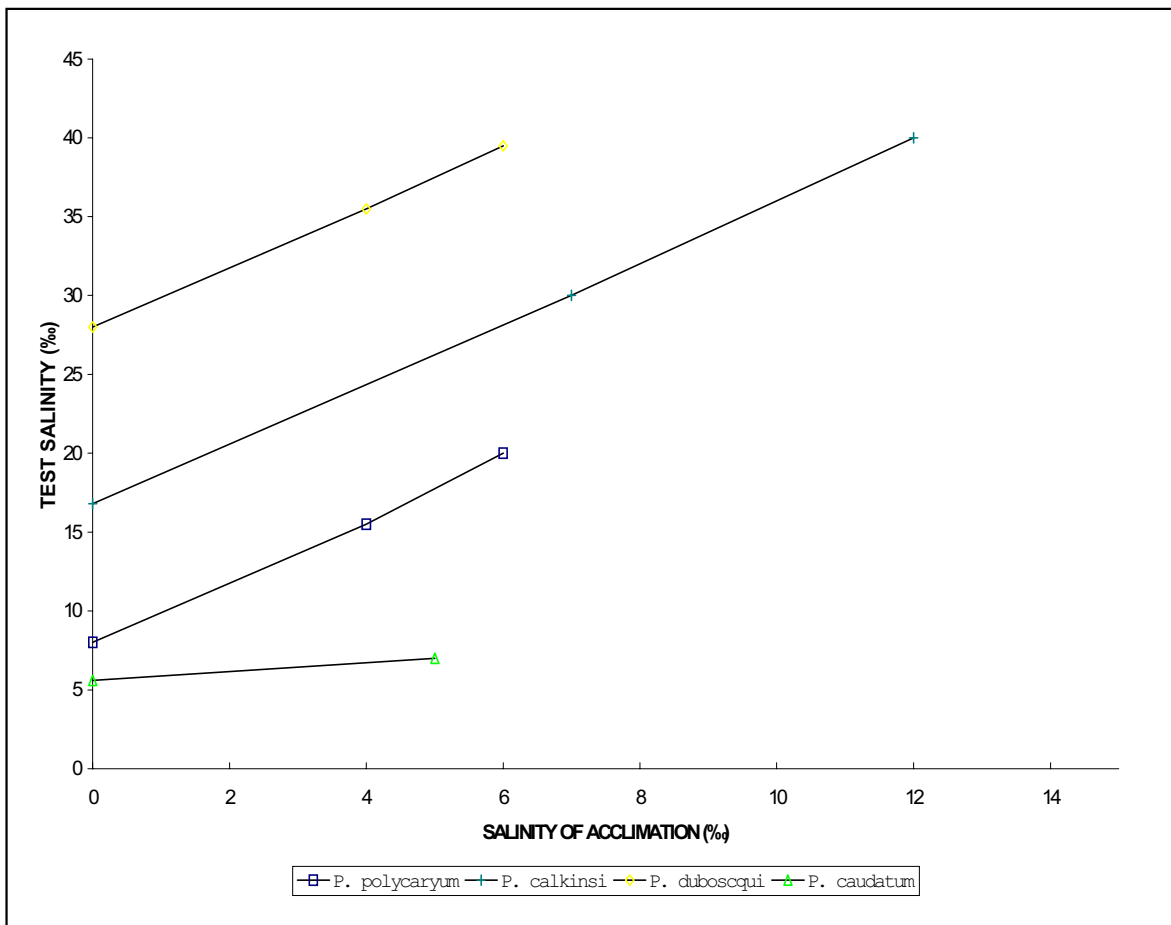


Fig 4. Upper potential tolerant limit for some of *Parametium* species.

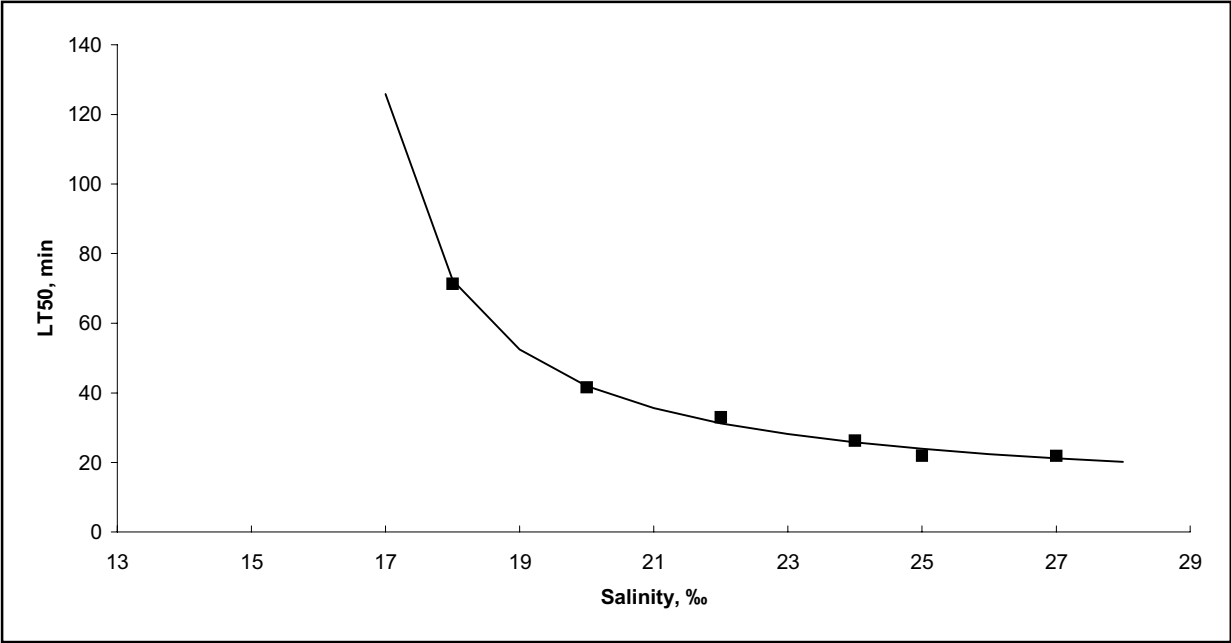


Fig 5. Resistance of *P. calkinsi* to salinity impact.

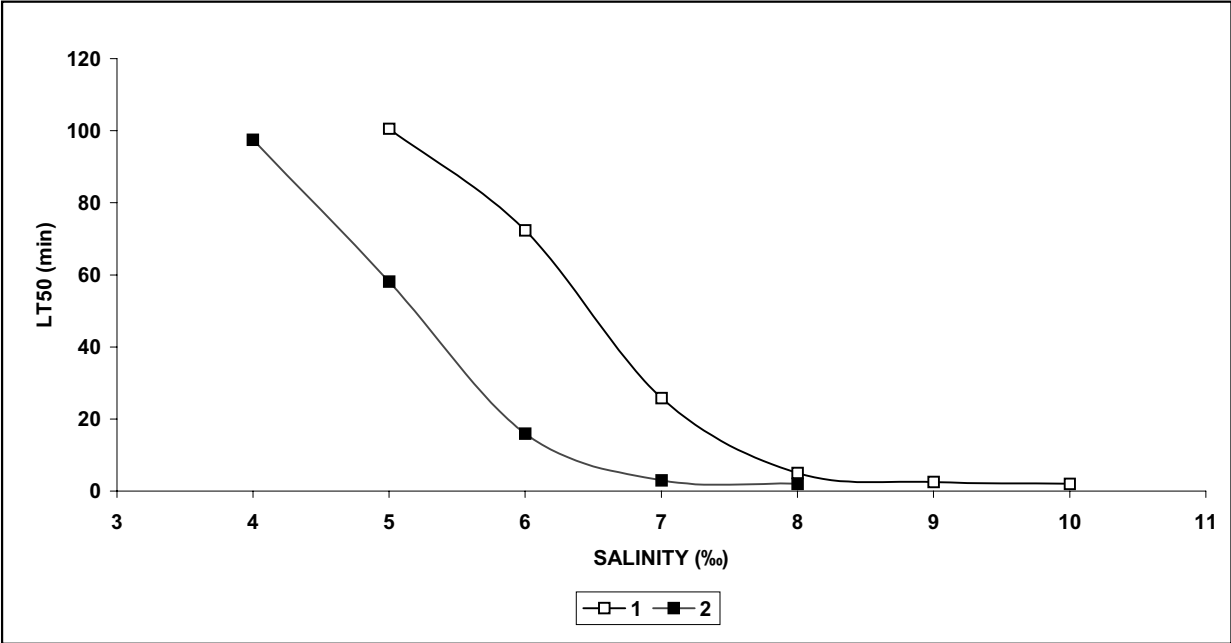


Fig 6. Resistance of *P. bursaria* (1) and *P. putrinum* (2) to salinity impact.

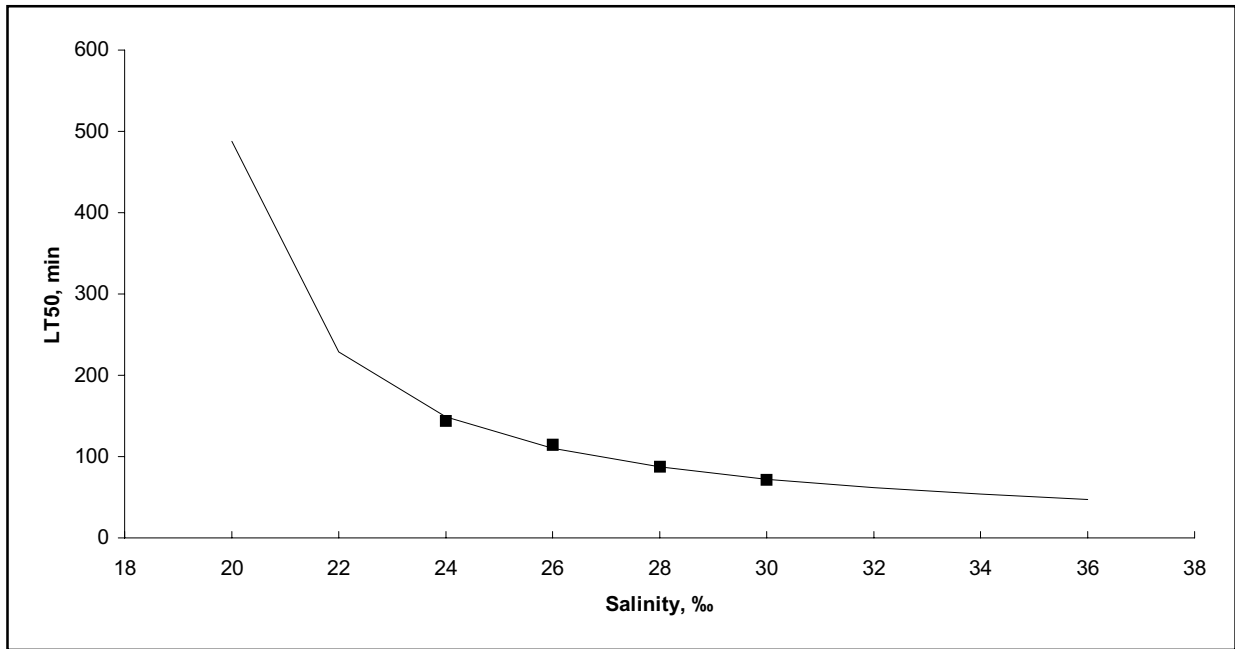


Fig 7. Resistance of *P. nephridiatum* to salinity impact.

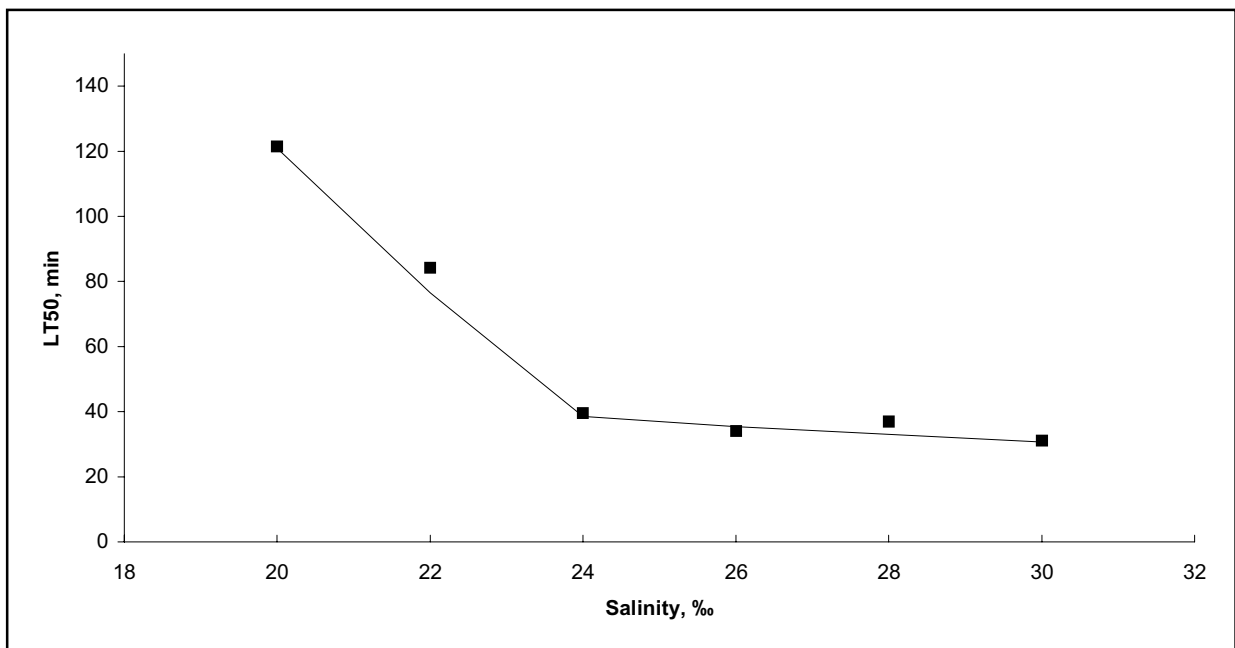


Fig 8. Resistance of *P. woodruffi* to salinity impact.

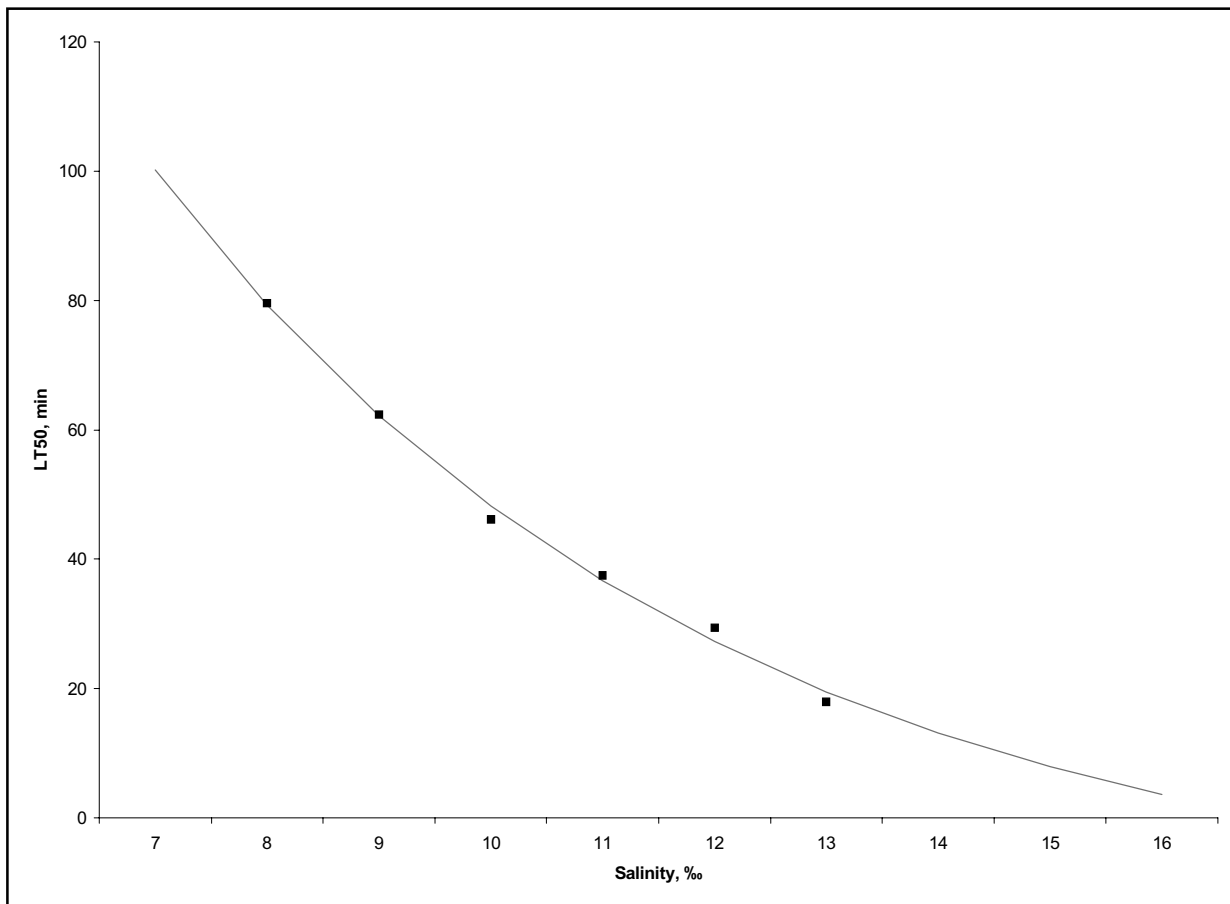


Fig 9. Resistance of *P. polycaryum* to salinity impact.

Discussion

From the data mentioned above the distinctions in salinity reactions between species of different groups of *Paramecium* as well as the similarity in reactions of various species inside of each group are evident.

P. bursaria and *P. putrinum* have the least resistance to salinity impact. They quickly perish upon an insignificant increase in salinity. A similar result was obtained by Gause (1984) regarding *P. bursaria* which, in his experiments, perished already at salinity only a little exceeding 2 ‰. The author supposed that the low salinity resistance of *P. bursaria* is caused by the historical past of this species. Its natural habitat, in Gause's opinion, is slightly mineralised peatbogs. This opinion is not supported in the literature (Wichterman, 1986).

Species of the "aurelia" group tolerate short-term salinity fluctuations much easier. They are able to survive for two days at 5-6 ‰ concentration without previous acclimation.

The species belonging to the "woodruffi" group could easily overcome the barrier 5-8 ‰, which distinguishes marine water and fresh water fauna and was called by Khlebovich (1974) as the critical salinity. These species

can occupy estuaries and some marine biotops. *P. duboscqui*, *P. nephridiatum*, *P. woodruffi* and *P. calkinsi* can without preliminary acclimation penetrate into brackish water bodies.

Salinity reactions of *P. biaurelia* and *P. polycaryum* are very similar. Acclimation for these species seems to be necessary. The critical salinity barrier these paramecia can overcome after long acclimation is only 3 ‰ (unpublished). The upper salinity tolerance limit for these species after acclimation to fresh water lettuce medium is 15-18 ‰. The maximal salinity value, where the cells can divide is 15 ‰. Moreover, after acclimation for 30 days to 15 ‰ in laboratory conditions, a significant part of *P. polycaryum* cells had a cortical breach.

Thus, the data on salinity reactions of the investigated species of the genus *Paramecium* fit well with their grouping, which was made mainly on morphological features (Fokin and Chivilev, 1999). The obtained results provide an opportunity to use ecology-physiological criteria in taxonomy which was seldom possible up to now. According to the data *P. bursaria* and *P. putrinum* probably should be placed more close to each other than it was found by morphometric analysis (Fokin and Chivilev, unpublished).

A lot of studies showed that the cytoplasm of ciliates is hyperosmotic in relation to the external environment. Fresh water metazoans hyperosmolarity is confirmed by many researchers and it was reflected in Bidl's rule (Khlebovich, 1974). Fresh water ciliates, as well as some of metazoan species, are capable of regulations internal ionic composition and, probably, in the same limits - up to 5 ‰. The marine species of ciliates, obviously, are hyperosmotics in wider limits. Tolerance limit of 5 ‰ for fresh water ciliate species and the range of possible osmotic regulation are obviously connected. At the salinity higher than 5 ‰, fresh water species of ciliates after acclimation become poikilosmotic or obtain the ability of osmoregulation in narrow limits. The critical salinity for them is insuperable. The criterion for differentiation between fresh water and marine forms for the majority of ciliate species can be the ability to survive in a medium with salinity 9-10 ‰ after preliminary acclimation to fresh lettuce medium for 3-5 weeks (brackish water samples). In cases where the cells of a species were sampled from an obviously fresh water body, the direct experiments were performed because the species had already acclimated to a fresh medium.

An important feature of euryhaline ciliates is the ability to expand the tolerance range in the process of salinity increase from the fresh environment to the brackish one and, probably, marine. This ability allows the cells which have been acclimated to a greater salinity to be adapted very easy for places with a rapidly varied salinity regime. It is possible to assume that such biotops are occupied mainly by marine populations of euryhaline ciliate species.

All species of the "woodruffi" group were found on coast of the White Sea (Fokin and Sabaneeva, 1990; Fokin et al., 1995). Natural research of the White Sea populations of these species (coasts of Keretsky archipelago, 1983-1997) have permitted the ascertainment of the stability of their existence during a number of years under possible salinity oscillations from 4 up to 45 ‰. These *Paramecium* species are dominating in the majority of investigated biotops together with ciliates of genera *Frontonia* and *Prorodon* within the limits of the specified salinity gradient with variations ciliate species composition of other systematic groups.

The experiments, have been done by us and the field observations are shown, that these species are able to expand their tolerance range with an increase of acclimation salinity. Therefore, the study of salinity adaptations of paramecia can help in our understanding of the biological processes that occur in places of mixed of fresh and marine waters and in biotops with a quickly changing salinity regime.

Stock cultures of euryhaline ciliates are easy picked out from natural populations. The technique of their laboratory maintenance is simple, and basic biological features

are well investigated (Wichterman, 1986; Fokin and Chivilev, 1999, Fokin et al., 1999a, 1999b). At the present time we have created a collection of *Paramecium* stocks isolated from both fresh and brackish water bodies (coasts of Baltic, Northern, White, Barents, Mediterranean seas, Pacific and Atlantic Oceans). All the listed data allow to offer these ciliates together with *Fabrea salina* and possibly some species of *Euplotes* as model objects for the researches of cells' adaptations to salinity impact.

Use of a model of hyperbolic dependence for data processing in salinity resistance experiments is of large interest. The technique described by us can be used for the express tests of salinity estimation of tolerant limits. The importance of this technique probably falls outside the limits of the study only of salinity adaptations, and can be used by aquatic toxicologists.

Acknowledgements

The authors are grateful to Drs. V.V.Khlebovich who made more active the present research and I.S.Plotnikov who has made original program for calculation of hyperbolic regression.

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