NATURA MONTENEGRINA, Podgorica, 2012, 11(3): 555-561

THE ESTIMATION OF THE SESSILE MICROORGANISM NUMBERS BY MEANS OF EVALUATION OF THE RELATIVE ABUNDANCES OF THEIR MIGRATORY STAGES

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Key words:
sessile microorganisms, abundance, flowing water, migratory stages.

SYNOPSIS

The data on the density of sedentary microorganisms which obtained resulting of exposition of the experimental substrates often do not reflect the actual abundance of these organisms due to the effects of current water. The laboratory test which based on a comparison of slopes of linear regressions has been proposed for obtaining of comparable results on relative abundance for such organisms under different velocity of flowing water.

INTRODUCTION

Factors as water flow can significantly distort the data on abundance at artificial substrates (such as the glass slides) the sessile microorganisms like protozoans (Holm, 1921, 1925; Rieder, 1936). These circumstances cause sufficient difficulties of the objective assessment of the numbers of these organisms in conditions of presence or absence of current water (Dovgal, 1990).

This is especially significant in process of the assessing of water quality with use the saprobic indices when estimating of relative abundances of indicator species including sessile protists (Sladeĉkova & Sladeĉek, 1966) are in use (Foissner, 1992). This influence of water flow is also necessary to take into consideration when estimating the role of motile stages of sessile microorganisms (swarmers or buds) in the plankton.

However it is often extremely difficult to estimate the abundance of motile stages when they are scarce or exist in a free state for a short time. In addition the motile stages are unknown in many species of sessile organisms. This is especially

true in cases of estimation of mobile stages (swarmers and telotrochs) in sessile ciliates such as suctorians (class Suctorea) or peritrichs (subclass Peritrichia).

Under such investigations the methods of indirect estimations were used. Thus Holm (1925) has collected the equal in volume (500 cm^3) samples of water and placed them in identical vessels and covered of bottoms and walls of the vessels by cover glasses (microglasses). For completeness of ciliate number estimating Holm also covered by cover glasses the surface film of water. After exposure in term that necessary for suctorian metamorphosis Holm has estimated the number of adults (trophonts) of suctorians. However this method was failed to comparative estimating of abundance the suctorian ciliates in the conditions of different velocity of current water.

The similar to Holm's method was used of Braiko and Dalekaya (1984). The numbers of ciliate swarmers at the glass slides in theirs experiments were estimated every 6, 24 and 48 hours respectively. The volumes of water samples in the experiments were 8 liters. However, unlike to Holm these authors do not use the cover glasses placed on the surface film so the accuracy of their results was probably lower than in Holm's work.

In contrast to the above-mentioned authors Dovgal (1990) believed that rate of colonization of substrate by swarmers of sessile ciliates (suctorians) is proportional to their concentration in the plankton. Therefore Dovgal used the Holm's method but with some differences. Similar to Holm, Dovgal collected the equal in volume samples of water from sites with the presence and absence of water flow and placed them in identical vessels. The latter author compared the values of the angular coefficients of linear regressions obtained from data of the colonization experiment. However the method of comparison of the coefficients of linear regressions was not mentioned in the Dovgal's work.

METHODS

Process of substrate colonization by sessile ciliates (Suctorea) was investigated in the valley of Desna river near Oster (Chernigov region, Ukraine) from September 20 to 27 of 1986 (station I) and near village Pogreby in the vicinity of Kiev (Ukraine) from September 3 to 8 of 1987 (station II) (Dovgal, 1990). For the estimation the influence of water flow rates on the abundance of ciliates the experimental substrates were exposed in river as well as in adjacent former river-bed. The water temperature at the station I was +14° C, depth 0.5 m, flow velocity 0.2 ms^{-1}. The water temperature at station II was +16° C, depth 0.3 m, flow velocity 0.3 ms^{-1}. 160 of 18X18 mm cover glasses or 48 of 76X26 mm glass slides were placed in waterbodies vertically and (in cases of lotic conditions) parallel to the flow and exposed at each site during 6 days. 26 of microglasses (18x18 mm) and 8 of

glass slides (76x26 mm) were examined every day and the relative abundances of ciliates were calculated and expressed in individual cells per m^2. The obtained data were approximated by the logistic equation.

For comparative estimates of the rates of colonization in different conditions a linear data transformations into their natural logarithms with following linear approximations were used.

The laboratory experiments for estimations of the relative abundance of ciliate swarmers in conditions of different water flow have been pursued.

The 10 liters of water at a time were taken from Desna River and from its former river-bed (station II) and placed in identical vessels for this purpose. In the either of the two vessel were placed the same number (60) of identically (vertical) oriented glass slides (76x26 mm). The 5 glass slides were examined every four hours and numbers of ciliate trophonts were calculated and expressed in individuals per m^2. The duration of the experiment was 48 hours (Dovgal, 1990). Than the data were lineary approximated but transformations into logarithms were not performed in this case.

For the comparison of the angular coefficients of linear regressions (slopes) the analysis of covariance (ANCOVA) (Sokal & Rohlf, 1995) has been used.

The statistical data were processed using the software package PAST 2.13 (Hammer et al., 2001).

RESULTS AND DISCUSSION

Our data provide support for Holm's (1921, 1925) observation that the density of ciliates on the substrate is appreciably dependent on the flow velocity at the same time of exposure (Fig. 1). In addition the water flow velocity significantly affects the rates of colonization of the substrates (Fig. 2). The following equations were obtained by means of the linear approximations of the colonization data: $Y = 3.732 - 0.1734X$ under absence of water flow; $Y = 5.1147 + 0.1249X$ under flow velocity 0.2 ms^{-1} and $Y = 5.2127 + 0.8383X$ under flow velocity 0.3 ms^{-1}, where ***Y*** is the density of ciliates in specimens per m^2 and ***X*** is duration of exposition in days.

As evident from these equations the greatest differences were found in the rates of substrate colonizations. The angular coefficients of the regression lines were -0.1734, 0.1249, and 0.8383, respectively. The comparison of the slopes by means of analysis of covariance (ANCOVA) confirmed these differences with a high degree of confidence (the Fisher's criterion (F) was 36.39 under p=0.00001).

Considering that in natural conditions the complex of factors influencing the density of sessile microorganisms in lotic waterbodies (Dovgal & Kochin, 1997; Dovgal, 1998, 2008; Dovgal & Pesic, 2012) and some of these factors remains a

mystery the relation between flow velocity and the rate of colonization of the substrate also remains unknown as a rule.

For estimating of likely influence of water course on ciliate density the laboratory experiment was conducted with samples from station II as mentioned above (Fig. 3). The following equations were obtained by means of the linear approximations: $Y = -1.6364 + 2.7545X$ under the absence of flow and $Y = -34.8364 + 2.2212X$ under a flow velocity 0.3 ms^{-1}, where ***Y*** is the density of ciliates in specimens per m^2 and ***X*** is duration of exposition in hours.

As can be seen from these equations the angular coefficients were 2.7545 and 2.2212 in the absence and presence of flow respectively and the differences between the rates of colonization are essentially absent in case when the influence of the factor of water flow was eliminated. The ANCOVA test also confirmed the absence of differences in the rates of colonization (F=0.3793 under p=0.6997).

However the essential difference between the intertcept coefficients of these equations which were -1.6364 under the absence of flow and 34.8364 under the presence of flow came to our attention. This difference is very well supported statistically (F=92.86 under p=0.00001). This in particular indicates that the ciliate swarmers from the sample with absence of flowing water begin to colonize the substrates about 15.7 hours early than in the sample from the river.

In our opinion there are several possible explanations for this. For example this difference could be due to different spatial distribution of ciliate swarmers in samples but small volumes of experimental vessels almost exclude this possibility.

It seems more likely that the numbers of swarmers of different ciliate species are randomly distributed in the samples. In case if in the any sample the migratory stages of species which characterized by a shorter period of location in the plankton are dominated the process of colonization of the substrates can begin somewhat earlier here than in the sample with long term existing buds.

It is highly plausible that the situation when occurs the combined effect of several unverifiable factors influencing on the intertcept coefficients. As a result the usage of intertcept coefficients until now is unusable for comparison of the relative abundance of ciliate swarmers.

The slopes of the regression lines in turn adequately reflect the relative concentrations of sessile microorganism migratory stages in the plankton. Therefore in the subject under discussion the relative abundances of ciliate swarmers in sites without flow and with water flow velocity of 0.3 ms^{-1} were about the same. In our opinion this indicates the approximately equal abundance of ciliate trophic stages too. This allows recommending the comparison of the angular coefficients obtained in laboratory by method which being discussed for estimating of relative abundances of sessile microorganisms under different conditions in lotic waterbodies.

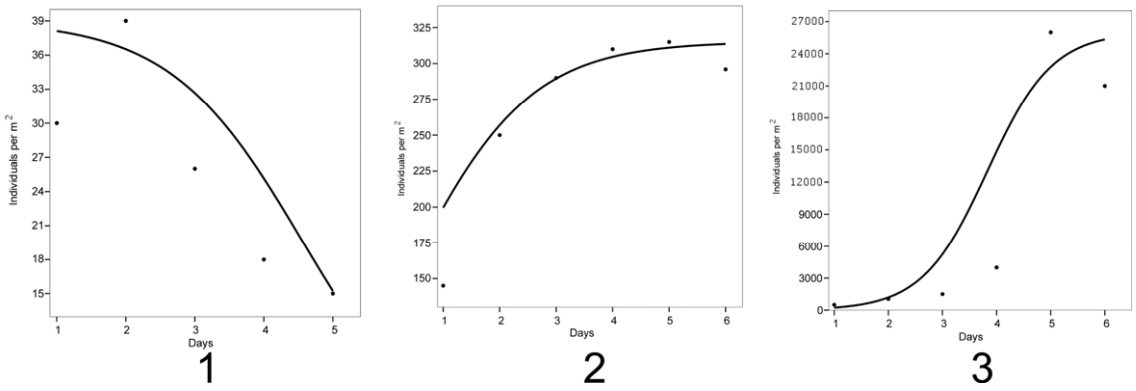

Figure 1: The dynamics of substrate colonization by suctorian ciliates under different water flow velocities (1 – stagnant water; 2 - water velocity is 0.2 ms^{-1}; 2 – water velocity is 0.3 ms^{-1}).

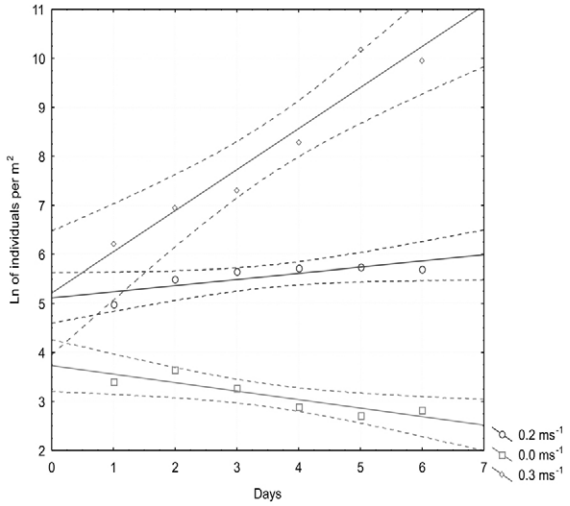

Figure 2: The linear approximations of the dynamics of substrate colonization by suctorian ciliates under different water flow velocities (the confidence belts indicated under p=0.95).

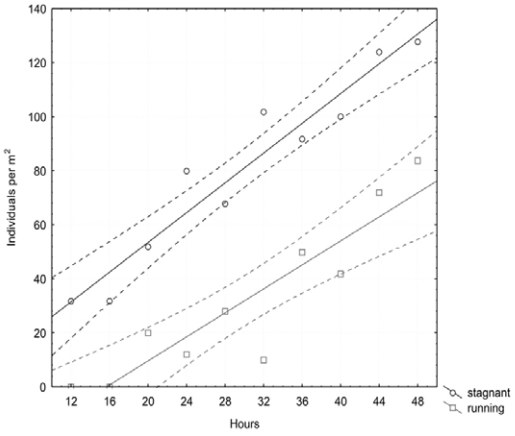

Figure 3: The linear approximations of the dynamics of substrate colonization by suctorian ciliates in samples of water from waterbodies with absence and presence of current (results of laboratory experiments; the confidence belts indicated under p=0.95).

CONCLUSIONS

By this means the following laboratory test is recommended:

1. Collect from the sites which in comparison the standard volumes of water (10 liters is recommended) without filtration or any other concentration.

2. Place in identical vessels with samples of water an equal numbers of vertical oriented glass slides.

3. Take out at regular intervals of time the same number of glass slides for following estimating of the density of settled adult stages of sessile microorganisms. It is recommended to remove the fouling organisms at the one side of the glass slide.

4. The obtained data must be approximate by equation of linear regression and the angular coefficients must be compared using ANCOVA test.

5. Use the angular coefficients obtained as an index of relative abundance of sessile microorganisms.

The recommended duration of the experiment is from 5 to 7 days depending on the duration of metamorphosis of studied organisms. It should be better to use the sampling experiment to determine the necessary general number of glass slides; number of glass slides which necessary for every examination and the duration of the interval between examinations.

The approach allows introducing a correction for the effect of water flow on the data about abundance the sessile microorganisms at artificial substrates. It is better to use as a control the samples of water from stagnant areas of rivers or from floodplains which connected to the river. In the absence of differences in the rates of colonization in the compared samples it is better to calculate the density of sessile organisms based on data obtained in the stagnant waterbody.

In the case when absent the possibility of the performance of the above mentioned test (especially under assessment of water quality in flowing waters with the saprobity indexes using sessile microorganisms as indicators) it is recommended to use a separate score scales of relative abundance of indicator species for each flow velocity.

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Received: 19 June 2012.