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THE NUTRITION RATES OF PERITRICHOUS CILIATES (Ciliophora, Peritrichia) IN CONDITIONS OF THE TREATMENT FACILITIES OF ZHYTOMIR (UKRAINE)

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SYNOPSIS

The article deals with testing of the method of peritrichous ciliates nutrition rate evaluation using the square number of food vacuoles (SNFV) index. The indexes were judged for most common in treatment facilities for peritrichous species: *Vorticella striata* f. *octava, Epistylis plicatilis, E. bimarginata, Opercularia phryganeae* and *V. convallaria*. The dependence of the nutrition rates of peritrichous ciliates on the temperature, operating conditions of treatment facilities and season were estimated. The method is recommended for control of sewage disposal plants operating efficiency as well as in bioassay.

INTRODUCTION

The peritrichous ciliates are commonly known as indicators of quality of sewage treatment in aerotanks (B a n i n a , 1983). It is believed that the work of treatment facilities is unsatisfactory if peristomes of peritrichs are closed or even half-closed.

However, according to our observations, the above mentioned conditions of peritrich peristomes, wherein the ciliates practically terminated to feed, took place only under the emergency conditions in the aerotanks. Therefore the approach prevents to appreciate the quality of treatment facilities work in the ordinary nonemergency situations.

Thus for the task of such solution the closer method of peritrich feeding rate estimation is necessary.

There are a number of laboratory methods for quantifying of grazing rates of protists (Sher & Sher, 1992). The decreasing of specific prey abundance or increasing of contrasting particles concentration in the food vacuoles in a defined time unit are in common usage for such objectives (Railkin, 1981, 1982; Burkovsky, 1984; Sher & Sher, 1992). However, for the ecological investigations it is more likely to use the methods for estimation of ciliates nutrition rates *in vivo* than *in vitro*.

The different phagocytal indexes based on the ciliate cell volume estimation were developed for that (Railkin, 1982; Burkovsky, 1984). However, this approach was founded on the extrapolation of the findings of laboratory investigation to the natural matters, and its results are sufficiently approximate.

Alternatively, the nutrition rate in ciliates was expressed as an average number of food vacuoles production by cell in a time unit (Hoffman et al., 1974). Finally Railkin (1982) has shown that the closest quantitative characteristic of ciliate nutrition rate is square number of food vacuoles (SNFV) rather than their average number.

Based on the Railkin's approach we have developed the specific method for estimating of peritrich ciliates nutrition rate in conditions of the treatment facilities (Konstantynenko, 2007; Konstantynenko and Dovgal, 2007). In this paper we present the results of usage of the method in conditions of Zhytomir (Ukraine) treatment facilities.

MATERIAL AND METHODS

The samples of activated sludge were collected in treatment facilities of Zhytomir (Ukraine) during 2004-2007. For the determination of the SNFV the activated sludge with ciliates was conditioned for 2 hours in the glass-wares. It was observed (Konstantynenko and Dovgal, 2007) that during 2 hours peritrichs conserve the nutrition rate identical to that for conditions in locality from which they were collected - 2.5 ml of sludge was placed into Petri dishes with diameter 3.5 cm. Than in 0.01 ml of black Indian ink was added to each sample and after ten-minute exposition the dishes were placed under microscope and numbers of food vacuoles which were developed by peritrichs were estimated.

The five most common in aerotanks of Zhytomir peritrich species were used as models: *Vorticella striata* f. *octava* Dujardin, 1841; *Epistylis plicatilis* Ehrenberg, 1831; *E. bimarginata* Nenninger, 1948; *Opercularia phryganeae* Kahl, 1935. *V. convallaria* (Linnaeus, 1758). The several zooids from one colony were included into sample in colonial species.

The ciliate cell size measurements were carried out under magnifications ×150 and ×600.

The Bryantseva's and Kurilov's (2003) algorithms were in use for the estimations of ciliate cells volumes and mass.

The individual cell masses were estimated using the equation:

$W = V \cdot \rho$,

where "W" is the mass of one individual in mg, "V" – cell volume in μm^3 (estimated with different formulas depending on the shape of the cell) and "ρ" is the cell specific gravity (in $mg \cdot \mu m^{-3}$) which in case of ciliates may equate to 1 (Bryantseva and Kurilov, 2003).

The daily rations of peritrichs were calculated according to the following equation:

$I = 0{,}648 \cdot W^{0.8}$,

where "I" is the maximal daily ration of one individual in mg (Burkovsky, 1984).

RESULTS AND DISCUSSION

1. The dependence of the nutrition rates of peritrichous ciliates on the temperature

In our experiments the one-way ANOVA test does not display the sufficient relation between temperature and peritrichous cell volumes as well daily rations while the increasing of the square numbers of food vacuoles was tested to all investigated species. At that the nutrition rate shows an increase up to 30^0 C, and it follows that the value of SNFV somewhat decreases (Fig. 1). This suggests an assumption that temperature range from 20 to 25^0C is optimal for the most common peritrichs inhabiting the aerotanks.

As for species differences in the estimated index (Table 1) in the *V. striata* f. *octava* which have smallest cell mass ($0.1 \cdot 10^{-4}$ mg) the value of SNFV was minimal whereas in *O. phryganea* with the greatest cell mass ($1.29 \cdot 10^{-4}$ mg) the value of SNFV was not maximal. In contrast the greatest nutrition rate was characteristic for *E. plicatilis.* However, the maximal value of daily ration ($0.79 \cdot 10^{-4}$ mg) has been observed exactly in *O. phryganea* but these results from "I" which is a function of cell mass "W".

Based on the SNFV estimation it has also been found that in colonial species (*E. plicatilis, E. bimarginata* and *O. phryganeae*) the nutrition rate per separate zooid has been superior to solitary species (*V. striata* f. *octava* and *V. convallaria*) under the same temperature (Table 1). It seems likely that team-work of peristomes of all zooids of the colony is favourable to increasing the effectiveness of food particles sedimentation.

Table 1. The individual cell masses and characteristics of the grazing activity in peritrichous ciliates from sewage disposal plants.

Species	Sample size (N)	Mass of ciliate cell (in mg·10^{-4})	SNFV	Maximal daily ration (in mg·10^{-4})
Epistylis plicatilis	41	0.61±0.08	134.05±21.40	0.42±0.04
E. bimarginatus	63	0.44±0.02	51.52±8.15	0.33±0.01
Opercularia phryganea	78	1.29±0.04	98.30±11.58	0.79±0.02
Vorticella convallaria	12	0.20±0.03	36.08±8.74	0.18±0.02
V. striata f. *octava*	42	0.10±0.01	3.62±0.37	0.10±0.01

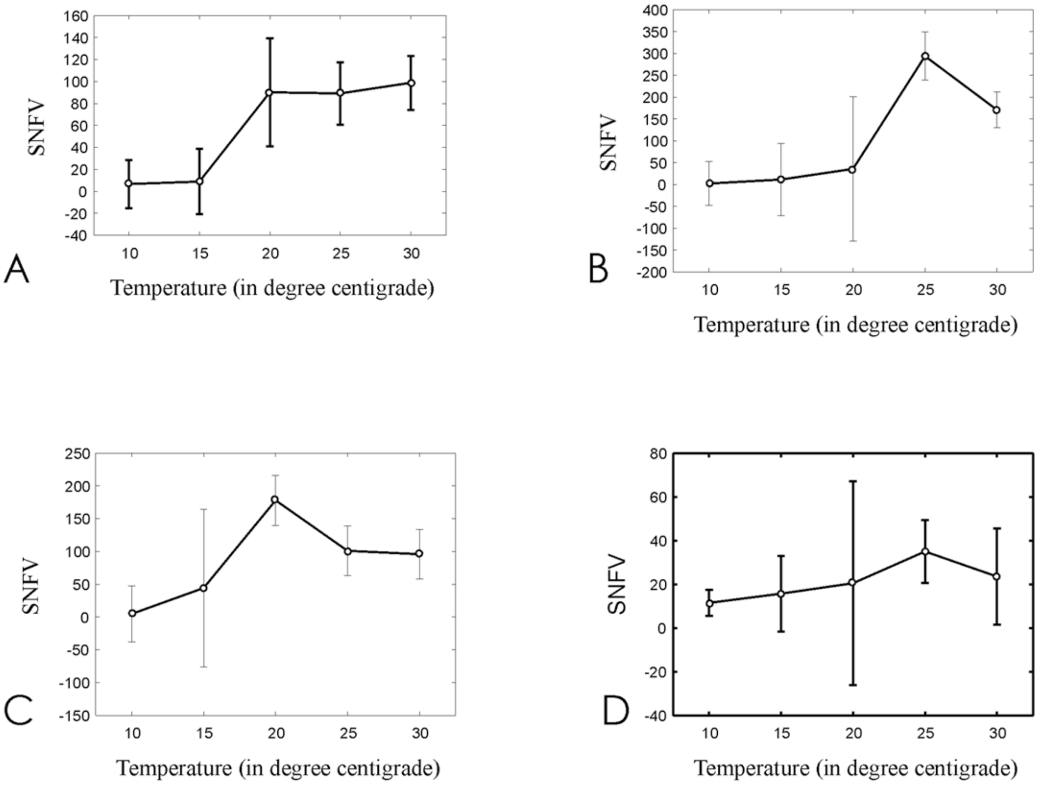

Fig. 1. The square number of food vacuoles dependence on temperature in *Epistylis bimarginatus* (A), *E. plicatilis* (B), *Opercularia phryganea* (C) and *Vorticella striata* f. *octava* (D); one-way ANOVA results.

2. The dependence of the nutrition rates of peritrichous ciliates on the operating conditions of treatment facilities

Except at physiological state of the organisms living in activated sludge the treatment facilities performances activated sludge indexes (the ratio of activated sludge volume (in ml) desilted during 30 min to 1 g of sludge dry substance), dissolved oxygen, dissolved organic matter concentrations and pH.

It has been found for all species that there is a positive correlation between SNFV and dissolved oxygen (Fig. 2) whereas the correlation coefficients between SNFV and sludge index (Fig. 3) and between SNFV and pH (Fig. 4) were negative.

However different peritrichous species variously reacted to the mentioned factors. Thus in *V. microstoma, O. phryganeae, V. striata* f. *octava* and *V. convallaria* the correlations between nutrition rate and sludge index were low and uncertain (Fig. 3, B-E). At the same time *E. plicatilis* was found as good indicator to the changes of this parameter (Fig. 3, A).

In 2004. the breakdowns in operating practices were reported in treatment facilities of Zhytomir what become apparent in sufficient increasing the concentrations of ammonium ions (from 1.88-2.08 to 11.00 mg per litre) and suspension particles (from 11.6-17.5 to 40.7 mg per litre).

In the above mentioned conditions the nutrition rates of peritrich ciliates were more than once as compared with normal operating practices. Thus in *V. striata* f. *octava* value of SNFV was 38.47 under normal conditions and 7.00 under troubles (Fig. 5, A). In *V. microstoma* these indices were 27.65 and 4.94 (Fig. 5, B), in *V. convallaria* 20.30 and 5.00 (Fig. 5, C), in *O. phryganeae* 115.05 and 6.06 (Fig. 5, D) whereas in *E. plicatilis* 213.71 and 21.89 respectively (Fig. 5, E).

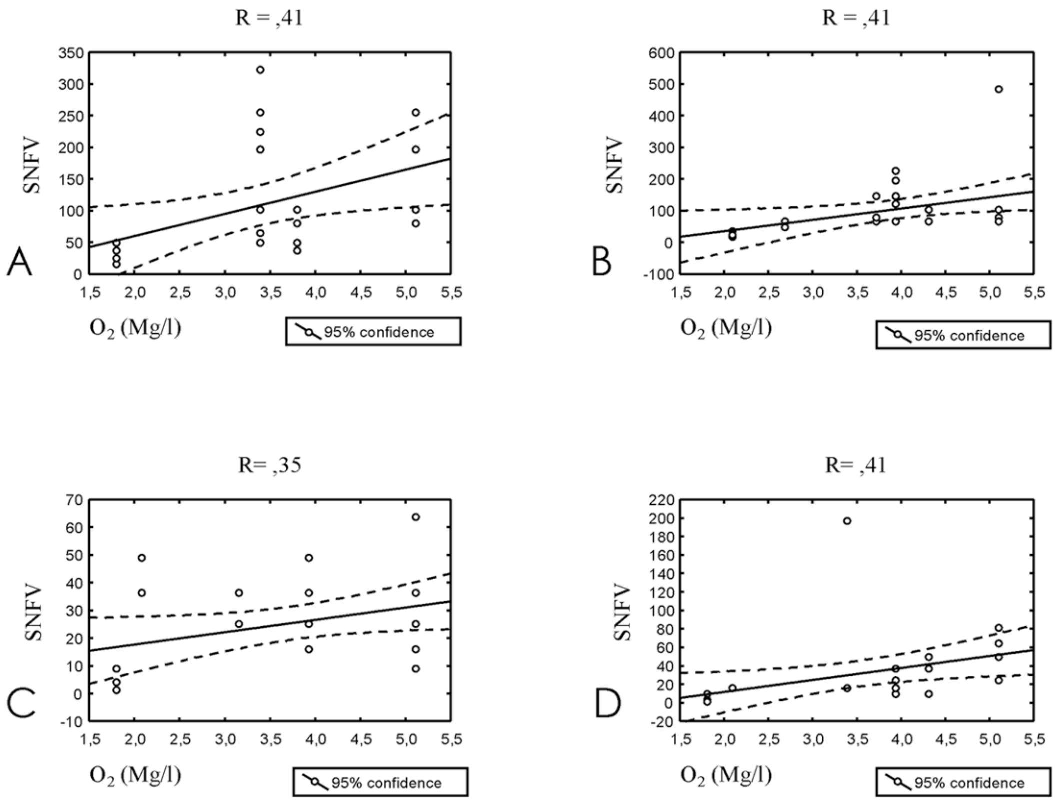

Fig. 2. The dependence between dissolved oxygen concentration in activated sludge and square number of food vacuoles in *Epistylis plicatilis* (A), *Opercularia phryganea* (B), *Vorticella convallaria* (C) and *V. microstoma* (D); correlation analysis results.

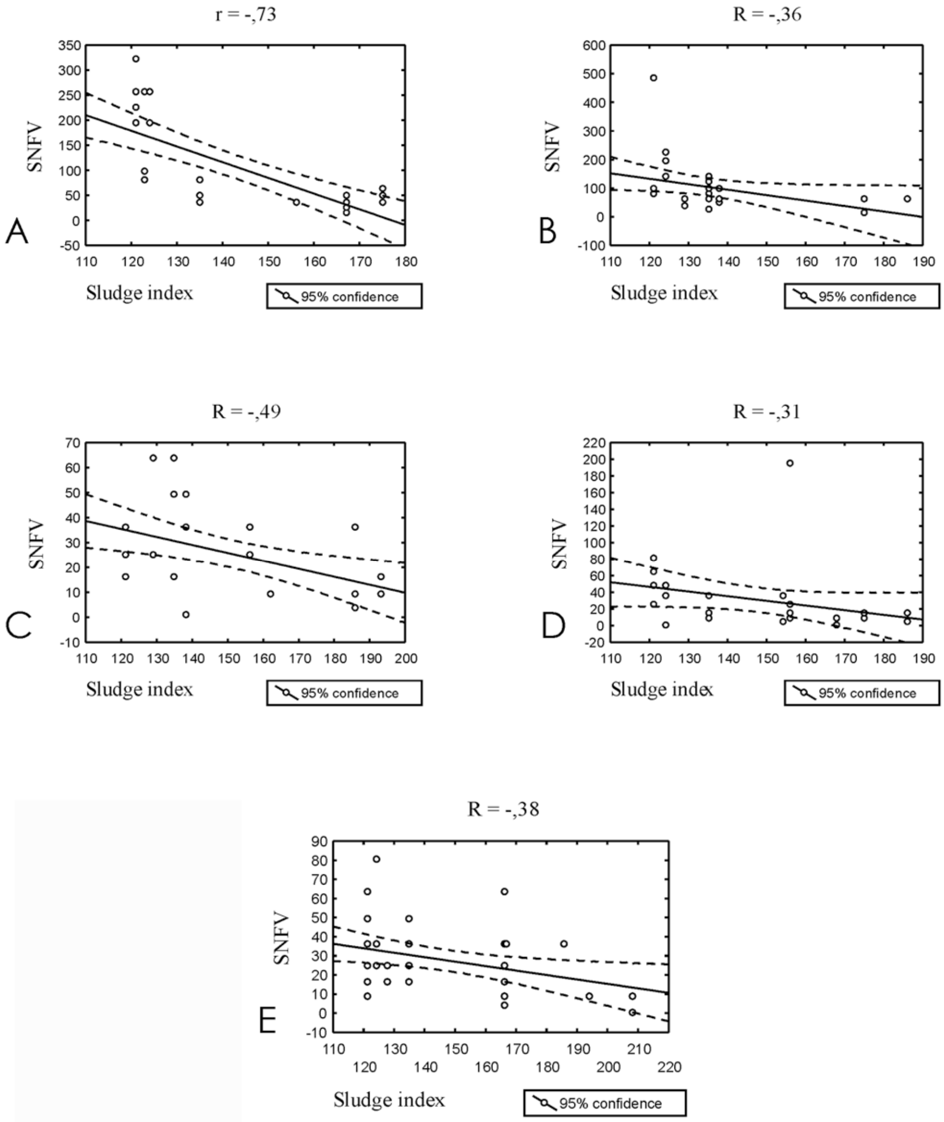

Fig. 3. The dependence between activated sludge index and square number of food vacuoles in *Epistylis plicatilis* (A), *Opercularia phryganea* (B), *Vorticella convallaria* (C), *V. microstoma* (D) and *V. striata* f. *octava* (E); correlation analysis results.

3. The dependence of the nutrition rates of peritrichous ciliates on the season

The less sizable changes of conditions, for example temperature are characteristic for the treatment facilities than for the natural water bodies. Thus in the investigated aerotanks the temperature range in different seasons was from +7.5 to +23.5 degrees Centigrade. Nevertheless, some upward trends in nutrition rates were observed in several peritrich species. Thus in *V. striata* f. *octava* (Fig. 6, E) and *V. microstoma* (Fig. 6, D) the values of SNFV are increasing in summer and autumn whereas in *E. plicatilis* the positive trend was reported from spring to autumn (Fig. 6, A).

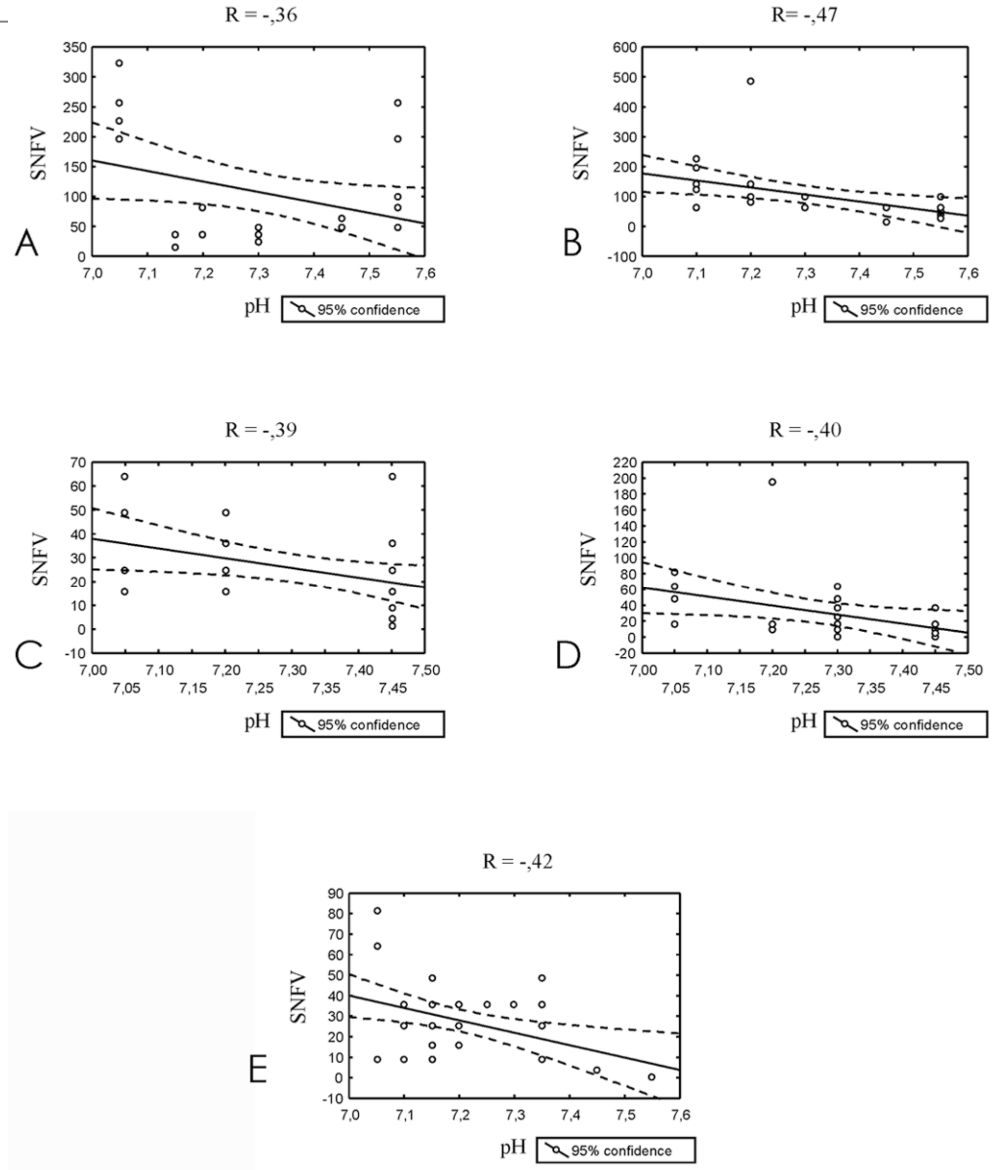

Fig. 4. The dependence between pH and square number of food vacuoles in *Epistylis plicatilis* (A), *Opercularia phryganea* (B), *Vorticella convallaria* (C), *V. microstoma* (D) and *V. striata* f. *octava* (E); correlation analysis results.

The observed dependences were insignificant and they lack statistical support. At the same time in *V. convallaria* (Fig. 6, C) and *O. phryganeae* (Fig. 6, B) the dependence between nutrition rates and season was not reported.

The observed seasonal changes in sizes of zooids in investigated species were quite significant (Fig. 7).

It is our opinion that seasonal changes in aerotanks are long-term whereas peritrich ciliates efficiently react by their nutrition rates on the more rapid changes of the environment factors. In such a situation the changes of the ciliate cell sizes, mass or daily rations may turn out more indicative.

As for the square number of food vacuoles this index can advise for performance control of the treatment facilities, optimization of the operating conditions and in bioassay.

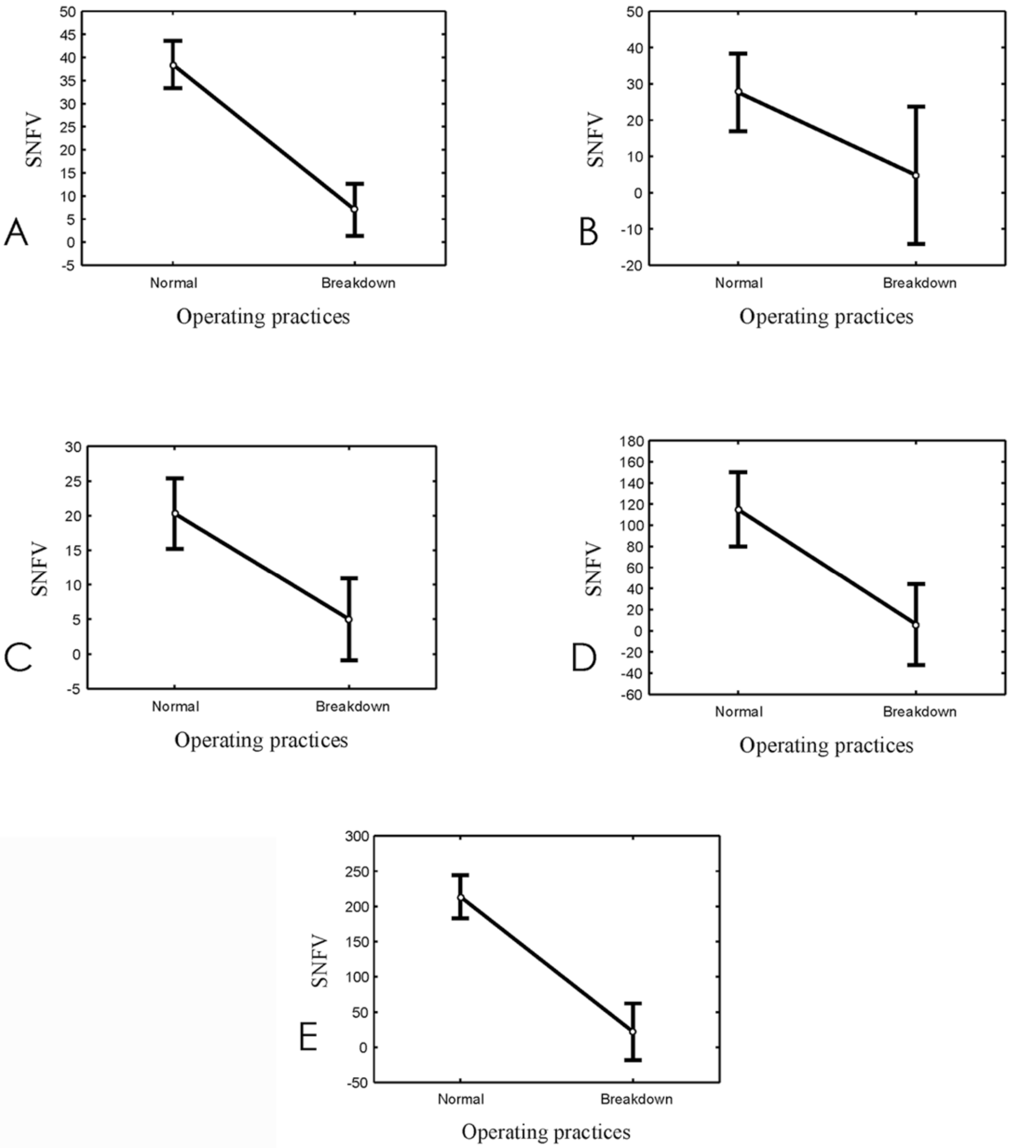

Fig. 5. The square number of food vacuoles dependence on operating practice conditions in *Vorticella striata* f. *octava* (A), *V. microstoma* (B), *Vorticella convallaria* (C), *Opercularia phryganea* (D) and *Epistylis plicatilis* (E); one-way ANOVA results.

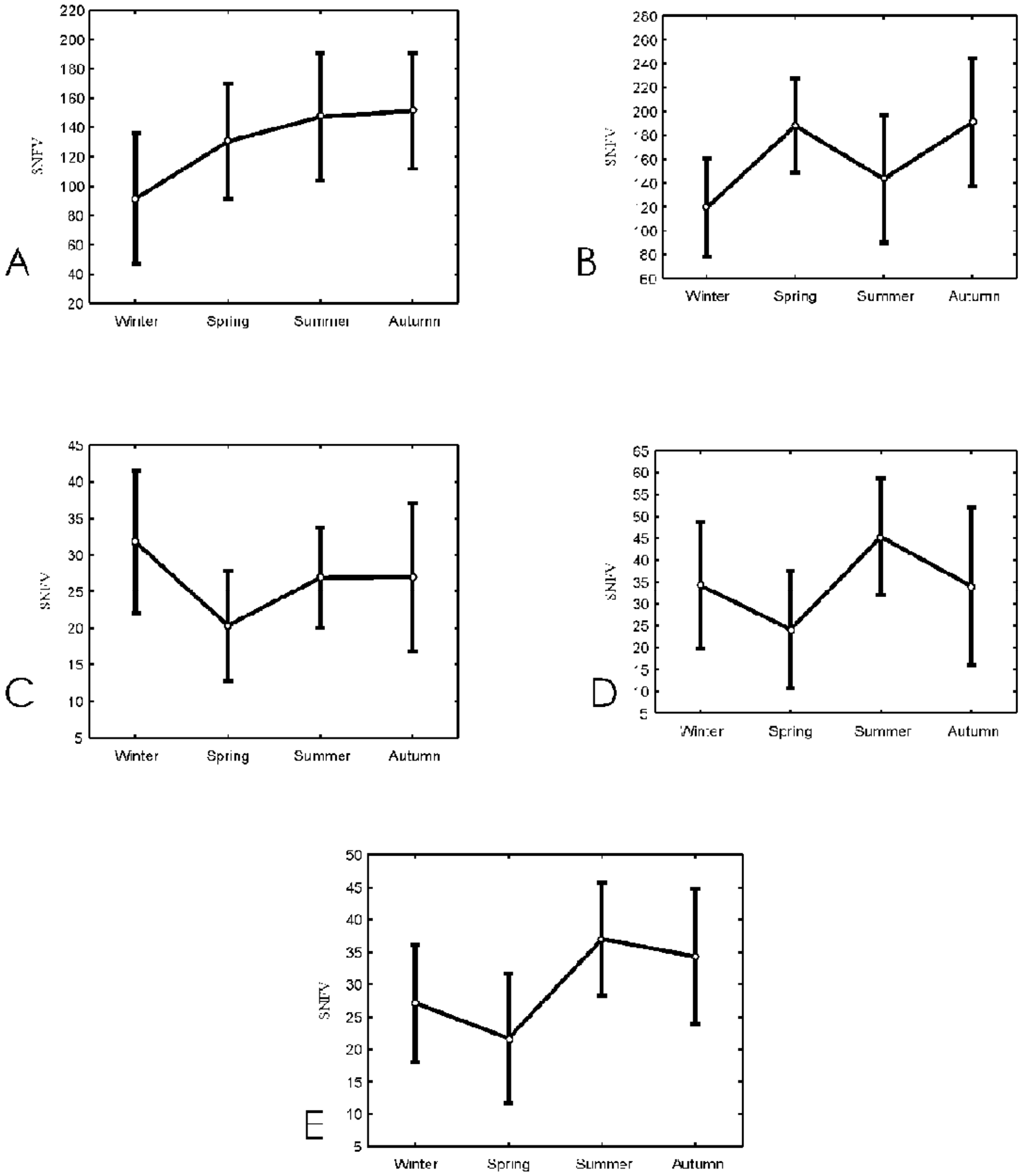

Fig. 6. The square number of food vacuoles dependence on the season in *Epistylis plicatilis* (A), *Opercularia phryganea* (B), *Vorticella convallaria* (C), *V. microstoma* (D) and *V. striata* f. *octava* (E); one-way ANOVA results.

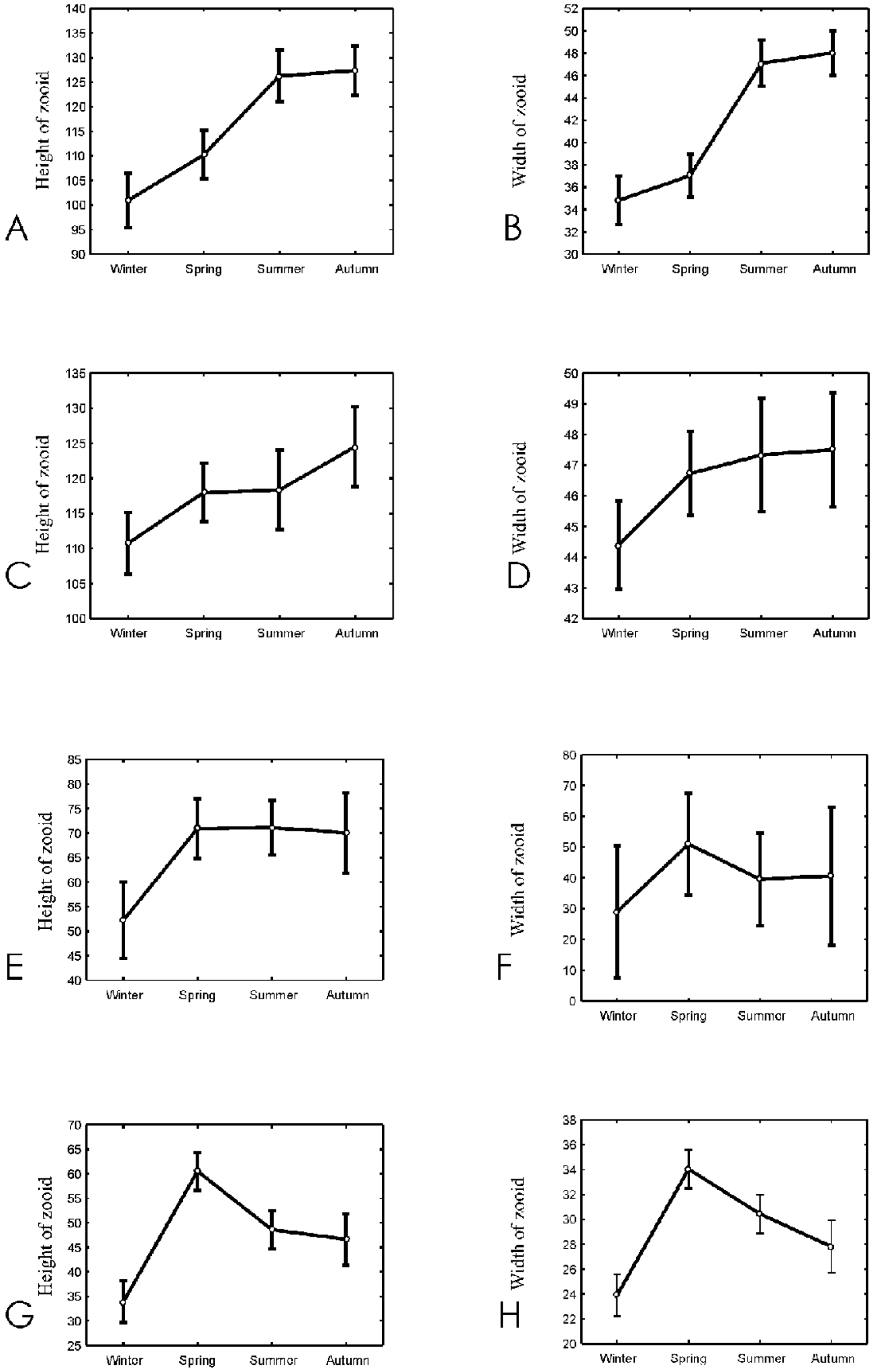

Fig. 7. The seasonal changes of the sizes of zooids on the season in *Epistylis plicatilis* (A, B), *Opercularia phryganea* (C, D), *Vorticella convallaria* (E, F) and *V. microstoma* (G, H); one-way ANOVA results.

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