

# Data on the knowledge of *Peridinium palatinum* Lauterborn (Dinophyta) in Körös area (SE, Hungary)

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## *Abstract*

*Peridinium palatinum* belongs to the group of algal species of which we have only a few taxonomical and ecological data, although in some running and standing waters of Hungary it can be found in large numbers.

The aim of our study, accomplished from 1990 to 1996, was revealing the occurrence of this species. Our results suggest that the occurrence of *P. palatinum* has been influenced by two factors: water temperature and the organic matter concentration of water. Comparing eleven types of waters in which it either appeared or not, it could be established that *P. palatinum* occurs in waters that exceed a relatively high level of organic matter concentration. Concerning water temperature *P. palatinum* is a typically winter early spring organism, because the temperature are important in the excystment and the developing of vegetative cells. Consequently, its occurrence can well indicate relatively high concentrations of organic nutrients during the winter season in Hungarian freshwaters.

**Keywords:** *Peridinium palatinum*, oxbow, excystment.

## *Introduction*

Seasonal phytoplankton succession has been the focus of thousands freshwater and marine studies, but few studies have examined the background planktonic algal appearance in winter season (Hobbie 1973; Klaff et al. 1975; Lewis 1974, Maeda & Ichimura 1973, Reynolds et al. 1983, Saija & Sakamoto 1964, Trimbee & Harris 1984, Wetzel 1966, Willén 1961).

Although cells of *P. palatinum* are relative conspicuous and readily recognisable among the dinoflagellate, the biology of *P. palatinum* has not been intensively studied to date. Mainly the investigations concentrate on the morphological features and tried to find it in different waters without to focus on the environmental and biological background of

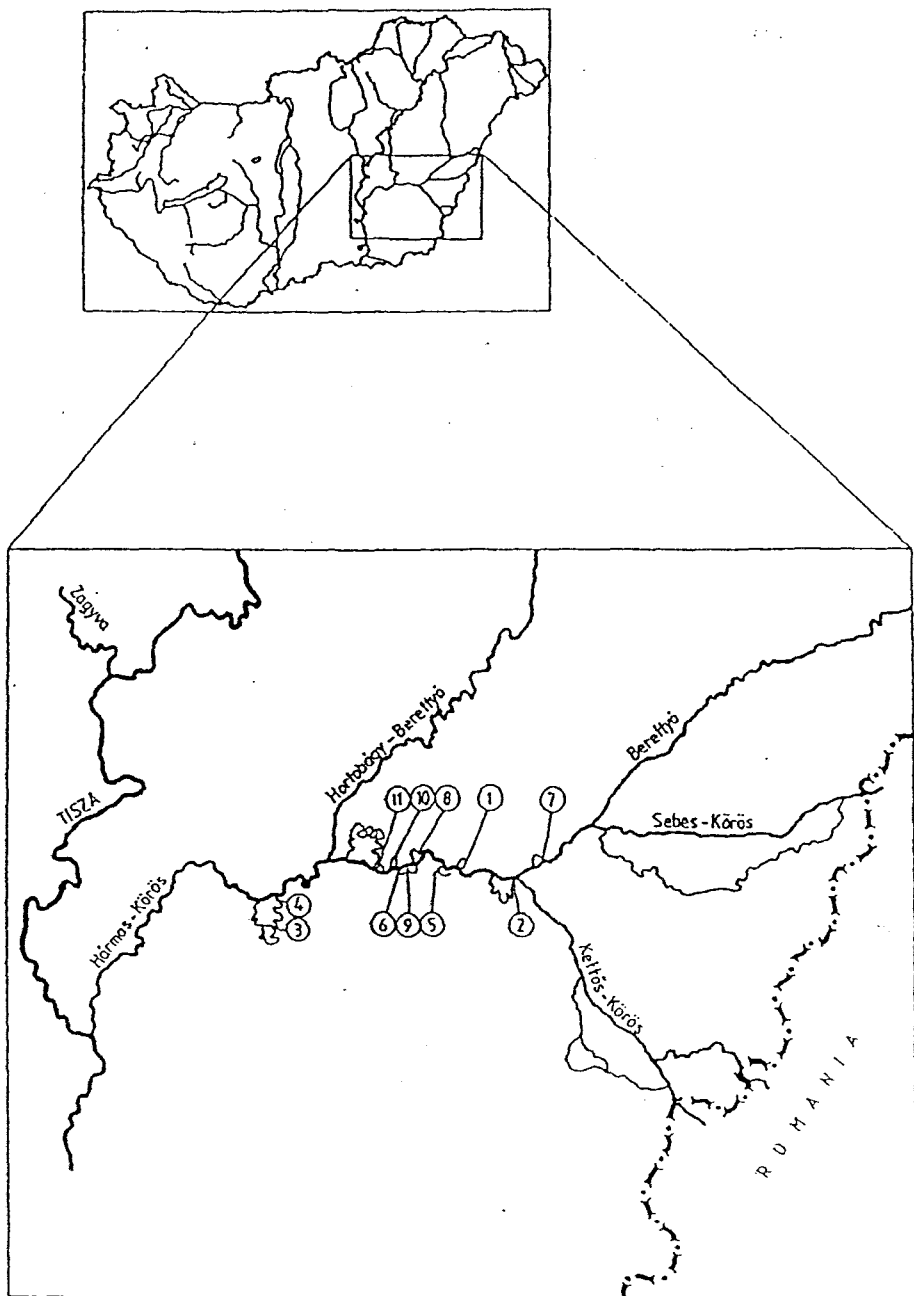


Fig. 1. Sketch map of sampling sites. 1. Siratói-Holt-Körös, 2. Félhalmi-Holt-Körös, 3. Szarvasi-Holt-Körös, 4. Szarvasi-Holt-Körös, 5. Torzsási-Holt-Körös, 6. Endröd-Középső-Holt-Körös, 7. Folyáséri-Holt-Körös, 8. Németszugi-Holt-Körös, 9. Fűzfászugi-Holt-Körös, 10. Templomzugi-Holt-Körös, 11. Kecskészugi-Holt-Körös

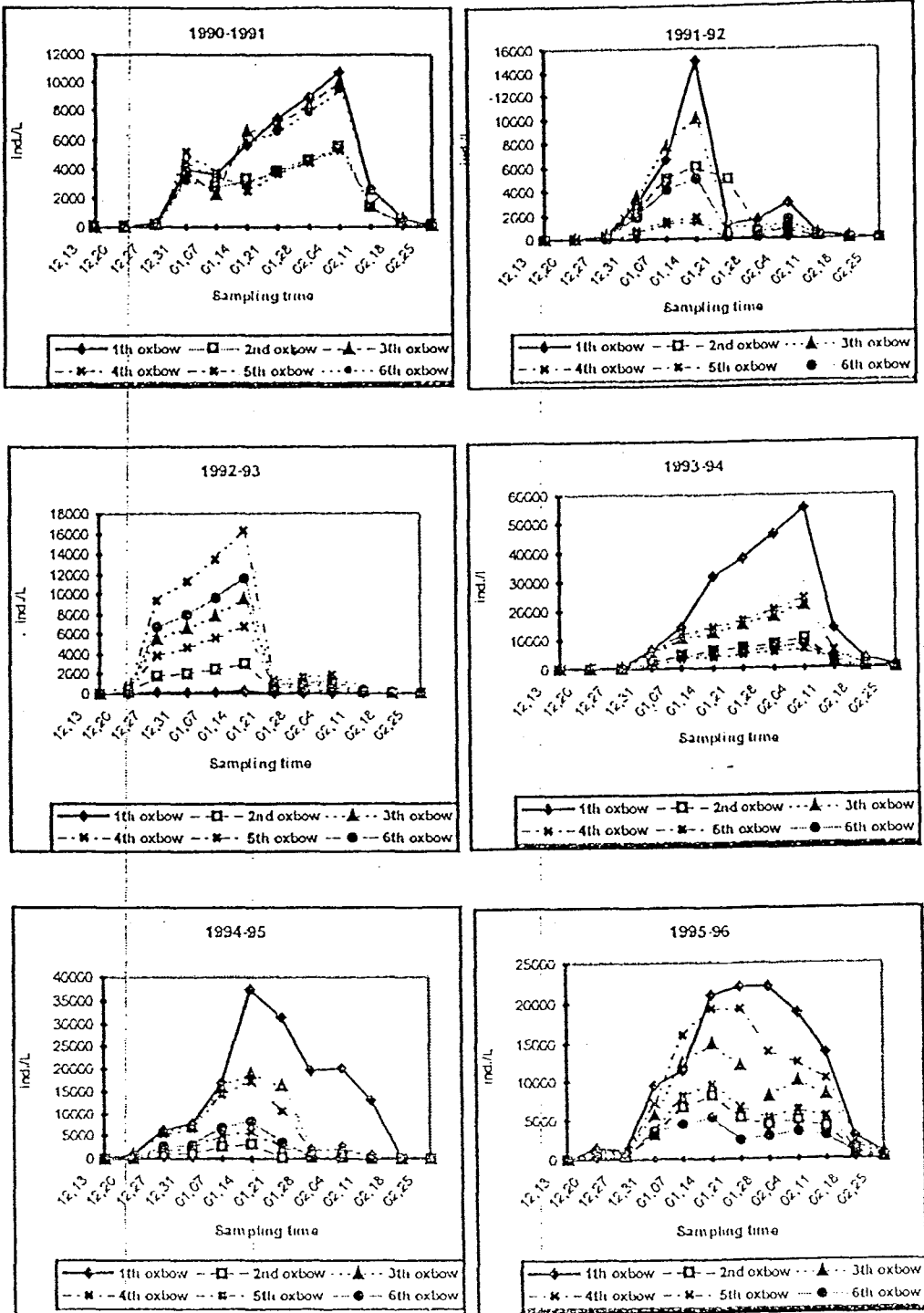


Fig. 2. The individuum number of *P. palatinum* in 1<sup>st</sup>-6<sup>th</sup> studied oxbows

its appearance (Huitfeld-Kaas 1900, Lemmermann 1900, West 1909, Lindemann 1919, 1928, Lefevre 1932, Popovsky & Pfiester 1990). These books and papers mentioned that the *P. palatinum* can be sampled in the cooler region in Europe and in cooler season in other countries. There is no publication on the regularities of appearance of *P. palatinum*, although it can be found regularly in very large numbers in some running and standing waters of Hungary and in some Central-European countries (Austria, Slovakia, Rumania and).

Experimental studies using laboratory cultures have attempted to model the indication and the importance of different dinoflagellate species (Eppley et al. 1968, Stoch 1973, Pfiester 1975, Kamykowski & Zentara, 1977; Heaney & Furnass 1980, Sako et al. 1985.). We used the culture to determine the importance of temperature of excystment with reference to the appearance in the observed oxbows. Activation of cyst by cold treatment is known in *Peridinium cinctum*, *Peridiniopsis cunningtonii* and *Woloszynskia pseudopalustre* and *Woloszynskia apiculata* culture. In the present study, we used this dark-cold method to observe the *P. palatinum* cysts and determine the optimum conditions for excystment.

The present paper describes some experiments with cultures, and field samples of the *P. palatinum* to describe the main factors which can influence its occurrence.

### *Study sites, material and method*

Changes in the annual population densities of the *P. palatinum* in eleven adjacent Hungarian oxbows are summarised over the 6 year period of 1990-1996 (Fig. 2). The samples were taken twice per month at same days between December and February 1990-1996 where the appearance of *P. palatinum* was to be expected. The studied oxbows are situated in of the Körös area near the Hármas-Körös river and Kettős-Körös river (SE, Hungary) (Fig.1). The sampling was done in near the deepest part in the oxbows. Water samples used for chemical analyses and algal counts were collected using a weighted plastic tube from the 0-5 m layer. A sub-sample was immediately fixed in the field with Lugol's iodine for subsequent algal counts by the Uterhmöhl inverted microscope technique. The microscopical investigation was performed with a Jenamed-2 microscope and an Axiovert-100 inverted microscope by using phase-contrast and Nomarski-contrast technology. The oxbows vary in size from 4 to 199 ha, in average depth from 1.5 to 2.5 m and in volume from 75.000 to 4,300.000 m<sup>3</sup> (Table 1.)

Excystment: After the dark-cold treated cyst were washed with distilled water, each of the fifty cysts were inoculated into a hole of microtest plate which contained enriched Carefoot's medium (Carefoot 1968). The plates were incubated under various conditions of temperature, from 4°C to 18°C for 10 days (Fig.3.). The percentage of excystment was

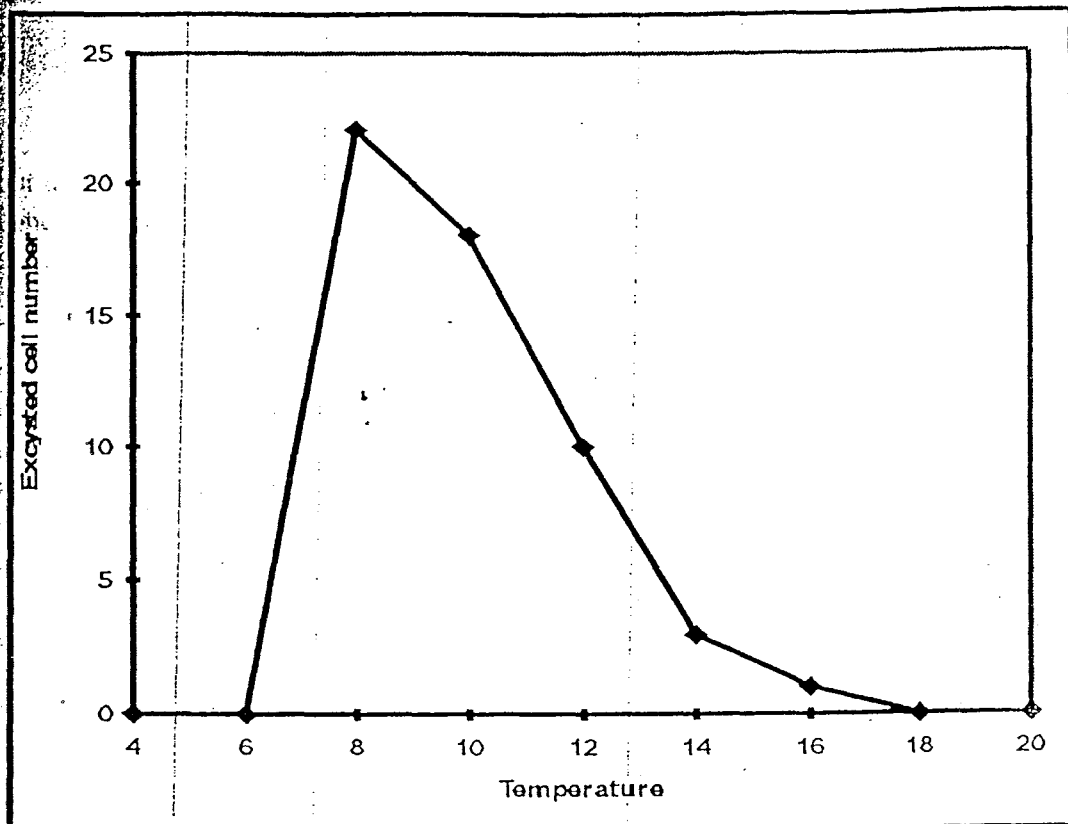


Fig. 3. The results of excystment investigation

determined by counting the number of cells excysted from each holes under the above mentioned inverted microscope. After their excystment they placed on different temperature, from 4°C to 18°C and the vegetative cell were counted after 7 days to determine, which could be the optimum temperature for the vegetative cells. The standard condition for the culture was 10,000 lx and a 14:10 h light-dark cycle in an enriched Carefoot's medium.

Different chemical components were measured in the waters (Table 3a., b.). The Table 3a., b. show the average values of chemical components and their standard deviations. Six of the sampling places (1<sup>st</sup>-6<sup>th</sup> oxbows) received domestic waste from villages situated nearby them so that they were highly meso/eutrophic. Five other sampling places (7<sup>th</sup>-11<sup>th</sup> oxbows) did not get any mineral and organic nutrients by inflow.

|                                 | 1 <sup>st</sup> Siraói-Holt-Körös | 2 <sup>nd</sup> Félhalmi-Holt-Körös | 3 <sup>rd</sup> Szarvasi-Holt-Körös I. | 4 <sup>th</sup> Szarvasi-Holt-Körös II. | 5 <sup>th</sup> Torzási-Holt-Körös | 6 <sup>th</sup> Endrőd-Közpső-Holt-Körös |
|---------------------------------|-----------------------------------|-------------------------------------|--|---|------------------------------------|--|
| Length (km)                     | 4.9                               | 9                                   | 39.2                                   | 29.2                                    | 29.2                               | 1.2                                      |
| Average width (m <sup>2</sup> ) | 57                                | 44                                  | 68                                     | 68                                      | 0.668                              | 46                                       |
| Area (ha)                       | 28                                | 39                                  | 199                                    | 199                                     | 199                                | 6  |
| Volume (m <sup>3</sup> )        | 280000                            | 970000                              | 4300000                                | 4300000                                 | 180000                             | 120000                                   |
| Average depth(m)                | 1.8                               | 2.5                                 | 2.2                                    | 2.2                                     | 2.2                                | 2  |

|                          | 7 <sup>th</sup> Folyáséri-Holt-Körös | 8 <sup>th</sup> Németszugi-Holt-Körös | 9 <sup>th</sup> Füzfászugi-Holt-Körös | 10 <sup>th</sup> Templomzugi-Holt-Körös | 11 <sup>th</sup> Kocsészugi-Holt-Körös |
|--------------------------|--------------------------------------|---------------------------------------|---------------------------------------|---|--|
| Length (km)              | 1.7                                  | 2                                     | 2.3                                   | 2                                       | 1.1                                    |
| Average width (m)        | 30                                   | 60                                    | 442                                   | 45                                      | 325                                    |
| Area (ha)                | 5                                    | 12                                    | 10                                    | 10                                      | 4                                      |
| Volume (m <sup>3</sup> ) | 75000                                | 264000                                | 200000                                | 200000                                  | 110000                                 |
| Average depth(m)         | 1.5                                  | 2.2                                   | 2                                     | 2.1                                     | 1.7                                    |

Table 1. The parameters of studied oxbows

| Temperature of living cell culture (°C) |   |   |      |      |      |      |     |    |    |
|---|---|---|------|------|------|------|-----|----|----|
| Excystment temperature(°C)              | 4 | 6 | 8    | 10   | 12   | 14   | 16  | 18 | 20 |
| 4                                       | 0 | 0 | 0    | 0    | 0    | 0    | 0   | 0  | 0  |
| 6                                       | 0 | 0 | 0    | 0    | 0    | 0    | 0   | 0  | 0  |
| 8                                       | 0 | 0 | 22±3 | 19±3 | 30±3 | 12±2 | 3±0 | 0  | 0  |
| 10                                      | 0 | 0 | 12±1 | 18±2 | 13±1 | 3±0  | 3±0 | 0  | 0  |
| 12                                      | 0 | 0 | 4±1  | 5±1  | 10±1 | 3±0  | 4±1 | 0  | 0  |
| 14                                      | 0 | 0 | 4±0  | 5±0  | 10±1 | 3±1  | 4±1 | 0  | 0  |
| 16                                      | 0 | 0 | 2±0  | 5±0  | 5±1  | 5±1  | 1±0 | 0  | 0  |
| 18                                      | 0 | 0 | 0    | 0    | 0    | 0    | 0   | 0  | 0  |
| 20                                      | 0 | 0 | 0    | 0    | 0    | 0    | 0   | 0  | 0  |

Table 2. Excysted cell were incubated under various conditions of temperature, from 4°C to 20°C.

At six sample places (1<sup>st</sup>-6<sup>th</sup> oxbows) out of 11 the *P. palatinum* occurred each year but in 5 places (7<sup>th</sup>-11<sup>th</sup> oxbows) it could not be found. The Table 3a., b. show the average concentration of chemical components. Based on water chemistry the 11 oxbows can be separated into 2 distinct groups (Table 3a., b.). 1<sup>st</sup>-6<sup>th</sup> sampling places were meso/eutrophic characterized by little higher TP, ion level and organic matter concentration (Table 3a., b.) 7<sup>th</sup>-11<sup>th</sup> sampling places exhibited similarly lower levels of TP and organic matter concentration were therefore comparably oligo/mesotrophic (Table 3a., b.).

In 1<sup>st</sup>-6<sup>th</sup> sample places: The individual number of *P. palatinum* ranged from 0 to 55200 ind./ml (Fig. 2.). The pattern of change in densities of *P. palatinum* varied widely among the 5 years. The growth curves of this species varied considerably among the 1<sup>st</sup>-6<sup>th</sup> oxbows (Fig. 2.). The patterns of individual numbers can be divided into two types. On the one hand the first type (i) consisting of an increasing part, a first bloom, a minimum, a second bloom, and a general decrease (1990-91, 1991-1992, 1992-93,) of growth curve. On the other hand, the second type (ii) showed a slowly increasing part, a bloom and general decreasing part in 1993-94, 1994-95, 1995-1996 (Fig 2.). In 1990-1991 season the first bloom was smaller (5.700 ind./L.) than the second one (12.000 ind./L.), contrary in 1991-1992 and 1992-1993 season the first bloom were higher (15.000 ind./L., 18.500 ind./L.) than the second (3.100 ind./L, 2.200 ind./L.)

The maximum individual number (55.000 ind./L.) was found in 04. 02. 1993. The longest appearance, during eleven weeks was in 1995-1996., In the other years the *P. palatinum* can be found in the observed waters during 7 or 8 weeks.

Excystment process: The cyst starts to grow when the temperature was minimum 8°C (Fig. 3.) in the culture. Under this temperature the excystment was not observed (Fig. 3.). The maximum excystment was at 8 °C (32 cells: from 50) (Fig. 3.). The excysted cell number decreased by the increasing of the temperature (Fig. 3.). The maximum temperature that makes the excystment possible is at 16 °C - 3 cells excysted from fifty -, show by the culturing results (Fig. 3.).

Vegetative cells in culture: The microtest plates were incubated under various conditions of temperature, from 4°C to 20°C for 10 days. After their excystment the excysted cells were put under different temperature, from 4 °C to 20 °C and the vegetative cell were counted after 7 days. Fewer than 8°C and above 16 °C living of the vegetative cells was not observed in the culture. (Table 2.). Each temperature the vegetative cell number was lower then the excysted cell number at the given temperature. (Fig. 3. and Table. 2.). For example the excysted cell number was 32 at 8°C and the vegetative cell number was 22 at this temperature. The maximum cell number (30 cells) found in case of then the cyst were excysted at 8 °C and they put at 12 °C. At 14°C and 16 °C the vegetative cell number were extremely low (they varied from 1 to 5), except when the cells excysted at 8 °C (12 cells).

| Variable                      | Units               | 1 <sup>st</sup> oxbow |        | 2 <sup>nd</sup> oxbow |        | 3 <sup>rd</sup> oxbow |        | 4 <sup>th</sup> oxbow |        | 5 <sup>th</sup> oxbow |        | 6 <sup>th</sup> oxbow |        |
|-------------------------------|---------------------|-----------------------|--------|-----------------------|--------|-----------------------|--------|-----------------------|--------|-----------------------|--------|-----------------------|--------|
|                               |                     | Mean                  | Std.D. | Mean                  | Std.D. | Mean                  | Std.D. | Mean                  | Std.D. | Mean                  | Std.D. | Mean                  | Std.D. |
| H <sup>+</sup>                | pH                  | 7.2                   | 0.2    | 7.5                   | 0.2    | 7.5                   | 0.2    | 7.4                   | 0.2    | 7.5                   | 0.2    | 7.5                   | 0.1    |
| ALK                           | µeq l <sup>-1</sup> | 153.9                 | 9.7    | 290.6                 | 10.0   | 147.3                 | 13.4   | 171.4                 | 11.4   | 188.1                 | 10.2   | 174.2                 | 10.1   |
| Ca <sup>2+</sup>              | mg l <sup>-1</sup>  | 2.1                   | 0.2    | 3.7                   | 0.4    | 2.1                   | 0.4    | 2.9                   | 0.2    | 3.1                   | 0.2    | 3.1                   | 0.2    |
| Mg <sup>2+</sup>              | mg l <sup>-1</sup>  | 0.36                  | 0.03   | 0.44                  | 0.04   | 0.61                  | 0.03   | 0.65                  | 0.02   | 0.42                  | 0.02   | 0.40                  | 0.03   |
| Na <sup>+</sup>               | mg l <sup>-1</sup>  | 0.61                  | 0.05   | 0.82                  | 0.04   | 0.60                  | 0.08   | 0.62                  | 0.05   | 0.92                  | 0.07   | 0.56                  | 0.03   |
| K <sup>+</sup>                | mg l <sup>-1</sup>  | 0.2                   | 0.02   | 0.27                  | 0.02   | 0.23                  | 0.02   | 0.25                  | 0.02   | 0.18                  | 0.02   | 0.15                  | 0.02   |
| NH <sub>4</sub> <sup>+</sup>  | mg l <sup>-1</sup>  | 0.01                  | 0.002  | 0.03                  | 0.002  | 0.03                  | 0.001  | 0.02                  | 0.001  | 0.02                  | 0.002  | 0.02                  | 0.002  |
| SO <sub>4</sub> <sup>2-</sup> | mg l <sup>-1</sup>  | 2.18                  | 0.3    | 1.82                  | 0.2    | 1.96                  | 0.1    | 2.12                  | 0.3    | 2.08                  | 0.2    | 2.01                  | 0.3    |
| NO <sub>3</sub> <sup>-</sup>  | mg l <sup>-1</sup>  | 0.69                  | 0.1    | 0.52                  | 0.2    | 0.55                  | 0.1    | 0.63                  | 0.1    | 0.52                  | 0.09   | 0.52                  | 0.11   |
| TP                            | mg l <sup>-1</sup>  | 0.91                  | 0.07   | 1.65                  | 0.09   | 1.73                  | 0.04   | 1.62                  | 0.11   | 1.01                  | 0.07   | 1.63                  | 0.08   |
| COD <sub>Cr</sub>             | mg l <sup>-1</sup>  | 26.2                  | 2.7    | 27.9                  | 3.0    | 28.7                  | 3.1    | 27.4                  | 3.2    | 28.6                  | 3.3    | 26.4                  | 3.0    |
| COD <sub>Mn</sub>             | mg l <sup>-1</sup>  | 15.2                  | 3.7    | 16.1                  | 3.2    | 16.4                  | 2.8    | 14.6                  | 2.1    | 13.9                  | 2.4    | 13.9                  | 2.1    |

| Variable                      | Units               | 7 <sup>th</sup> oxbow |        | 8 <sup>th</sup> oxbow |        | 9 <sup>th</sup> oxbow |        | 10 <sup>th</sup> oxbow |        | 11 <sup>th</sup> oxbow |        |
|-------------------------------|---------------------|-----------------------|--------|-----------------------|--------|-----------------------|--------|------------------------|--------|------------------------|--------|
|                               |                     | Mean                  | Std.D. | Mean                  | Std.D. | Mean                  | Std.D. | Mean                   | Std.D. | Mean                   | Std.D. |
| H <sup>+</sup>                | pH                  | 7.4                   | 0.1    | 7.2                   | 0.1    | 7.3                   | 0.1    | 7.5                    | 0.1    | 7.2                    | 0.1    |
| ALK                           | µeq l <sup>-1</sup> | 185.1                 | 9.9    | 207.6                 | 12.4   | 198.4                 | 11.1   | 204.6                  | 12.6   | 174.5                  | 10.4   |
| Ca <sup>2+</sup>              | mg l <sup>-1</sup>  | 3.0                   | 0.3    | 2.4                   | 0.2    | 3.2                   | 0.3    | 3.0                    | 0.3    | 3.7                    | 0.4    |
| Mg <sup>2+</sup>              | mg l <sup>-1</sup>  | 0.49                  | 0.02   | 0.51                  | 0.02   | 0.46                  | 0.02   | 0.71                   | 0.03   | 0.40                   | 0.03   |
| Na <sup>+</sup>               | mg l <sup>-1</sup>  | 0.52                  | 0.04   | 0.64                  | 0.04   | 0.53                  | 0.03   | 0.72                   | 0.06   | 0.54                   | 0.05   |
| K <sup>+</sup>                | mg l <sup>-1</sup>  | 0.12                  | 0.01   | 0.14                  | 0.01   | 0.22                  | 0.02   | 0.37                   | 0.02   | 0.29                   | 0.03   |
| NH <sub>4</sub> <sup>+</sup>  | mg l <sup>-1</sup>  | 0.01                  | 0.002  | 0.02                  | 0.002  | 0.04                  | 0.002  | 0.03                   | 0.002  | 0.03                   | 0.002  |
| SO <sub>4</sub> <sup>2-</sup> | mg l <sup>-1</sup>  | 2.11                  | 0.2    | 2.35                  | 0.3    | 2.49                  | 0.3    | 2.38                   | 0.3    | 2.51                   | 0.3    |
| NO <sub>3</sub> <sup>-</sup>  | mg l <sup>-1</sup>  | 0.69                  | 0.2    | 0.68                  | 0.2    | 0.62                  | 0.2    | 0.76                   | 0.2    | 0.47                   | 0.1    |
| TP                            | mg l <sup>-1</sup>  | 0.62                  | 0.02   | 0.64                  | 0.02   | 1.21                  | 0.04   | 0.33                   | 0.01   | 0.46                   | 0.02   |
| COD <sub>Cr</sub>             | mg l <sup>-1</sup>  | 14.1                  | 2.4    | 14.2                  | 2.3    | 14.6                  | 1.9    | 13.9                   | 1.2    | 12.9                   | 1.0    |
| COD <sub>Mn</sub>             | mg l <sup>-1</sup>  | 5.3                   | 1.0    | 4.1                   | 0.9    | 4.2                   | 1.0    | 3.9                    | 0.6    | 3.2                    | 0.6    |

Table 3.a., b. Chemical parameters of studied oxbows

Chemical components: 1<sup>st</sup>-6<sup>th</sup> sampled oxbows differed from 7<sup>th</sup>-11<sup>th</sup> sampled oxbows mainly in dissolved COD<sub>Mn</sub> and COD<sub>Cr</sub>. In all the sample places of 1<sup>st</sup>-6<sup>th</sup> where *P. palatinum* could be found the dissolved COD<sub>Mn</sub>, was above 8.7 mg/L the lowest mean value was 13.9 mg/L, and dissolved COD<sub>Cr</sub> was above 16.2 mg/L and the lowest mean value was 26.2 (Tables 3a.). In waters where *P. palatinum* did not occur even the highest values of dissolved COD<sub>Mn</sub> hardly reached 6.3 mg/L., the highest mean value was 5.3 and the dissolved COD<sub>Mn</sub> did not exceed the minimum values of waters where *P. palatinum* could be found (Table 3a., b.).



According to the literature, (Huitfeld-Kaas 1900, Lemmermann 1900, West 1909, Lindemann 1919, 1928, Lefevre 1932, Popovsky & Pfiester 1990,) the only thing known about this species that it can be found in cool water and this species is cosmopolitan in the cooler region in Europe. Exactly the background and limits of *P. palatinum* occurrence are not known.

The result of this culturing and field study suggests that decrease of water temperature is sufficient for *P. palatinum* to occur. It can also be stated that, during the six year study period, in the water bodies the species occurred we could find it year by year. This phenomenon enables us to suppose that the occasional occurrence is not typical of this species since the absence of *P. palatinum* in some waters could not result from any mistakes in sampling.

Two thermal optima may exist for *P. palatinum*, one is 8 °C for excystment and second is 12 °C for the vegetative cells. By the culturing investigations it seems to be that the *P. palatinum* can grow under a relatively wide range of temperatures from 8°C to 16 °C, but the cells are absolutely sensitive for the low temperature, under 8 °C and sensitive for the increasing of the temperature. At the "higher" temperature (14 °C) the vegetative cell number are extremely low.

We can state that the occurrence of this species is influenced by the water temperature but by some other factor as well which make their occurrence possible. In the course of our study we compared the water bodies where *P. palatinum* occurred to those where it was absent. There were two types of oxbows: in the first type *P. palatinum* can be found (1<sup>st</sup>-6<sup>th</sup> oxbows) and in the second it does not occur (7<sup>th</sup>-11<sup>th</sup>).

One of the most difficult aspects of interpreting population development in the studied oxbows, is that chemical constituents of the water were generally similar the each other in the given year. The only exception was the organic material concentration measured by dissolved COD<sub>Mn</sub> and COD<sub>Cr</sub>. Although the organic matter availability is usually not considered a limitation to dinoflagellate growth, but all modes of nutrition have been documented in dinoflagellates: autotrophy, heterotrophy, saprotrophy, predation and parasitism (Elbrachter 1991, Schnepf & Elbrachter 1992). The field study investigations have been suggested that the organic matter requirement for normal growth may be important and essential for *P. palatinum*. The presence or absence of large *P. palatinum* populations can result in marked differences in dissolved COD concentration. Comparing the two types of waters in which it appeared or not it could be established that *P. palatinum* occurred in those where the organic matter concentration exceeded a relatively high level. *P. palatinum* consumes an organic material using it for assimilation and/or energy generating processes.

Our results suggest that the occurrence of *P. palatinum* has been influenced by two very important factors: water temperature and organic matter concentration of water.

Consequently its occurrence has been bound to waters relatively rich in organic nutrients and can indicate well relatively high concentrations of organic matter in waters during the winter season. Our opinion, fundamental to an understanding of the ecology of dinoflagellates during such events is a consideration of the excystment process, developing vegetative cells and connect it with field investigations to understand which can be a background of their appearance. In this study we tried to apply the and use to find some environmental factors which can be help to understand the appearance of a hardly known but common freshwater dinoflagellate, *Peridinium palatinum*.

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