Dependence between temperature and clearance rate of *Balanion comatum* **Wulff***

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Abstract

The dependence between temperature and clearance rate of the ciliate *Balanion comatum* Wulff 1919 was assessed in the coastal zone of the southern Baltic Sea. Five in situ experiments were carried out with the use of wheat starch as a surrogate of food particles. The clearance rate rose from 1.4 to 7.0 μl cell^{-1} h^{-1} with a temperature rise from 8 to 19°C. *B. comatum* preferred particles of size 1.9–4.4 μm, and the clearance rates calculated for the preferred particles were consistently higher than those measured for the whole range of particles ingested (Wilcoxon's signed rank test, p = 0.04). The exponential dependence between temperature and clearance rates for preferred particles was statistically significant ($R^2 = 0.86$, p = 0.02) and enabled the Q_{10} coefficient to be calculated. This amounted to 2.9 and lay within the range of typical values. The linear dependence (also drawn for preferred particles) demonstrated a higher significance ($R^2 = 0.91$, p = 0.02), indicating the linear dynamics of the process.

1. Introduction

The physiology of all organisms is affected by temperature; this parameter is therefore used as a steering function in ecological models.

The complete text of the paper is available at http://www.iopan.gda.pl/oceanologia/

In the case of ciliates it was demonstrated that temperature accelerates both ciliate growth (Müller & Geller 1993, Montagnes et al. 2003) and feeding rates (Dolan & Coates 1991), modifying energy flow through a ciliate community.

The aim of this study was to assess the dependence between the clearance rate of the common marine ciliate *Balanion comatum* Wulff 1919 and ambient temperature. *B. comatum*, redescribed by Jakobsen & Montagnes (1999), is a cosmopolitan marine ciliate. Common both in coastal and offshore waters, also in the brackish Baltic Sea (Witek 1998, Setälä & Kivi 2003), it grazes on nanoflagellates (Jakobsen & Hansen 1997, Rychert 2008).

Experiments were conducted under natural conditions with wheat starch added to water and used as a surrogate of food particles. Starch particles were observed inside ciliates after staining with Lugol's solution. The volume of water cleared of starch particles (clearance rate, μl cell^{-1} h^{-1}) was plotted against environmental temperature to check the statistical significance of the regression. The Q_{10} coefficient is most convenient for ecological modelling (e.g. Brush et al. 2002); the exponential dependence was therefore determined. However, according to Montagnes et al. (2003), growth rates of protists seem to increase linearly with temperature. Consequently, the author checked whether a linear model would fit the experimental data better.

As demonstrated previously (Rychert 2008), *B. comatum* preferentially ingests particles from 3.1 to 4.4 μm in size. Consequently, the author separately assessed the clearance rates for all particles ingested and for those of the preferred size.

2. Material and methods

Between 2007 and 2009, five in situ experiments were carried out in the coastal zone of the southern Baltic Sea at two stations: one located near the town of Ustka (54°35'N, 16°50'E; 2 experiments) and the other in the small village of Poddąbie (54°38'N, 16°59'E; 3 experiments). The water at both stations was brackish – the salinity ranged from 6.6 to 7.7 per mil. (slightly less than the typical value for the open waters of the southern Baltic: 7.5–8.0 per mil.). Experiments were done at different seasons and ambient temperatures (8–19°C).

Wheat starch was used as food particles, previously applied in such studies by Kivi & Setälä (1995). Its usage is very convenient because Lugol's solution simultaneously fixes ciliates and stains starch particles – they turn dark. The starch suspension was prepared as follows: (i) the starch was soaked overnight, (ii) filtered (10 μm) to exclude larger particles, and (iii) preserved with antibiotics: penicillin G (100 000 i.u. l^{-1}) and streptomycin

(100 mg l^{-1}) (Weisse 1989). The stock suspension was kept in a refrigerator (4°C) and used up within 6 months. Three starch preparations were used. Every time, before use, the suspension was gently stirred with a magnetic stirrer (30 rpm for 1 h). Subsequent analyses proved the efficiency of this method for preventing the particles from forming clumps. Small volumes (a few to twenty microlitres) of the stock suspension were used for preparing the working solutions. After dilution to the working concentration (at least 1000 times), the antibiotics did not influence community metabolism, as demonstrated by five comparisons of oxygen consumption by marine pelagic water with (OC$_1$) and without diluted antibiotics (OC$_2$): (OC$_1$ = 0.98 × OC$_2$, R^2 = 0.98, p = 0.001). Before every experiment, the starch solution was stained with Lugol's solution and analysed under an inverted microscope equipped with a camera and software for image analysis. Every time the abundance of particles and their size distribution (classes: 1.25 μm, 2.50 μm, 3.75 μm. . . 10.00 μm) were analysed (a few thousand particles in 20 fields of view). For the sake of compatibility, particles were categorized into size classes in the same way as in previous studies (Rychert 2008), in which measurements were carried out using a graticule with an elementary scale equal to 1.25 μm.

Clearance rates were measured during incubation with a known concentration of surrogate food particles. For each experiment 5 to 6 dilutions with gradually changing starch concentration, ranging from 10^2 to 10^4 particles ml^{-1} were prepared. Next, one treatment, in which cells of *B. comatum* ingested no more than one particle per cell on average, was chosen for analysis.

All bottles with sea water (200 ml each) were incubated for half an hour on an anchored experimental set-up deployed in the coastal zone of the Baltic Sea. All experiments were carried out between 11:00 and 14:00 hrs (around noon). Samples were taken before and after the incubations and immediately fixed with acid Lugol's solution (a low concentration – 0.5%). Samples were stored in a refrigerator (4°C) and analysed under an inverted microscope (Utermöhl 1958) within one month. All measurements were done manually with the image analysis system. Starch particles inside ciliates were categorized into 8 size classes: 1.25 μm, 2.50 μm, 3.75 μm. . . 10.00 μm (as above). Because some *B. comatum* cells contained dark inclusions prior to incubation (most probably food particles like flagellates), two analyses were performed: before and after incubation, the difference being treated as due to starch particles ingested during the experiment. Typically, 50–70 cells in every sample were analysed (the minimum number of specimens was 23). Additionally, the abundance of natural food – nanoflagellates – was determined in the samples taken

before experimental incubations. This was done under an epifluorescence microscope after staining with primulin (Caron 1983).

3. Results

Balanion comatum ingested particles ranging from 1.25 μm to 6.25 μm, and preferably from two size classes, 2.50 μm and 3.75 μm. Because of the classification into arbitrary size classes, the preferred particle size in practice ranged from 1.9 to 4.4 μm. The clearance rate for the whole range of particles ingested generally rose from 1.4 to 6.4 μl cell^{-1} h^{-1} with a temperature increase from 8 to 19°C (Figure 1); however, the dependence was non-significant (both linear and exponential models).
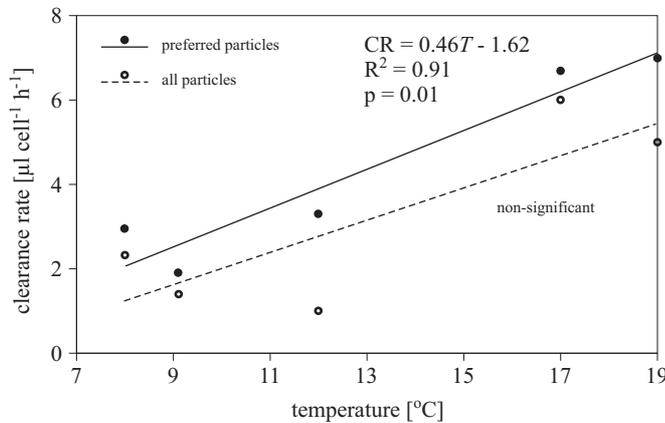
Figure 1. Clearance rate (CR) of *Balanion comatum* Wulff as a function of ambient temperature (T). The relation is statistically significant only for particles of preferred size

Consistently higher estimates (Wilcoxon's signed rank test, p = 0.04) were obtained for particles of preferred size (1.9–7.0 μl cell^{-1} h^{-1}, the same temperature range). This clearance rate (for preferred particles) rose significantly with temperature (Table 1). The linear approximation was

Table 1. Dependence between temperature (T, °C) and clearance rate (CR, μl cell^{-1} h^{-1}) of *Balanion comatum* Wulff determined for starch particles of preferred size (2.50 and 3.75 μm)

Model	Equation	Statistical significance
linear	$CR = 0.46\,T - 1.62$	$R^2 = 0.91$, p = 0.01
exponential	$CR = 0.95\,\exp^{0.11\,T}$	$R^2 = 0.86$, p = 0.02

statistically highly significant ($R^2 = 0.91$, p $= 0.01$), whereas the exponential model yielded a lower significance ($R^2 = 0.86$, p $= 0.02$). Q_{10} calculated with the exponential model amounted to 2.9 and lay within the range of typical values.

4. Discussion

As the studies were carried out under natural conditions (temperature, irradiance, wave motion), the measured clearance rates were most probably very close to the natural ones. Starch particles are typically used as a surrogate food for oligotrichs and choreotrichs (Heinbokel 1978, Kivi & Setälä 1995), that is, filter-feeders that ingest particles rather unselectively. Therefore, the application of starch particles for studying organisms from the genus *Balanion* might be considered risky because of their selective, raptorial mode of feeding (Müller & Schlegel 1999). However, it should be mentioned that starch is not such an unnatural food surrogate as e.g. latex beads. The author's previous studies (Rychert 2008) indicated that *B. comatum* did ingest wheat starch particles. Clearance rates measured in this study were slightly higher than the *B. comatum* clearance rates of up to 2.8 μl cell^{-1} h^{-1} during incubation in 15°C reported by Jakobsen & Hansen (1997).

In this study the preferred particles were from size classes 2.50 μm and 3.75 μm (that is, particles between 1.9 μm and 4.4 μm), which was partly consistent with the previous study (Rychert 2008), indicating that *B. comatum* preferred particles of about 3.75 μm in size (3.1–4.4 μm). In both studies the preferred size of particles was lower than that described by Jakobsen & Hansen (1997), who observed that *B. comatum* ingested flagellates ranging from 4 to 10 μm and preferred flagellates of size about 8 μm. The author cannot give an explanation for this discrepancy.

The main problem that could affect the accurate determination of clearance rates was the concentration of natural food. According to Jakobsen & Hansen (1997) *B. comatum* shows a Holling type II functional response (Holling 1959). However, regardless of the type of functional response, maximal clearance rates, or rather values approaching maximal ones, could be observed at low food concentrations. The experiments involved the addition of starch particles, but no further manipulation was undertaken to change the concentration of natural food. The functional response published by Jakobsen & Hansen (1997) demonstrated that *B. comatum* exhibited saturated feeding for a food concentration equal to about 2000 food particles ml^{-1}. In this study the combined abundance of flagellates and starch particles of preferred size (only the results for preferred particles turned out to be statistically significant) did not exceed 700 ml^{-1}.

Therefore, the concentration of food particles was located over the initial slope of the functional response, which confirms the reliability of the results.

Another possible problem could be the rather long incubation (half an hour), which could theoretically lead to the digestion of some starch particles. A similar species, *B. planctonicum*, digests flagellates within 20–33 minutes (Kenter et al. 1996). However, it could be expected that the digestion of a dense starch particle takes more time than the digestion of a cryptophyte cell. Thus, digestion would lead only to a slight underestimation of the clearance rate, if any.

An increase in clearance rates with temperature was also observed in the case of other ciliates e.g. *Strobilidium spiralis* (Rassoulzadegan 1982). Most probably, the increase in the clearance rate with temperature is due to an acceleration of the swimming speed. Acceleration of swimming speed with temperature was previously demonstrated for ciliates by Jones & Goulder (1973). The value of Q_{10} determined in the present study (2.9) indicates that the swimming speed could increase almost threefold after a temperature rise of $10°C$. The results presented here could also be useful in the construction of mechanistic models of microbial food webs. For example, Q_{10} could specify a possible increase in grazing pressure after the increase in temperature caused by a global warming. However, the linear dependence demonstrated a greater significance than the exponential model. This indicates, like the study by Montagnes et al. (2003), that the dynamics of some ecophysiological processes is linear and that the use of Q_{10} could lead to uncertain estimates.

Acknowledgements

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