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Author(s)	NAGATA, Warren D.
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Long-Term Acclimation of a Parthenogenetic Strain of *Brachionus plicatilis* MÜLLER to Subnormal Temperatures II. Effect on Clearance and Ingestion Rates

Warren D. NAGATA*

Abstract

Clearance and ingestion rates of the rotifer *Brachionus plicatilis* feeding on *Chlorella saccharophila* were determined after one year of acclimation to 10 and 20°C, using both standard and time series experimental designs. Ingestion rates were found to increase linearly with higher algal densities, while clearance rates were found to be independent of algal concentration, within the tested range of 1.1 to 20.2×10^6 cells ml^{-1}. No critical concentration or plateau of ingestion was noted in this study, indicating superfluous feeding at high algal concentrations. Q_{10} values of clearance and ingestion rates were 1.11 and 1.75 for the standard trials, and 2.18 and 1.79 for the time series trials; thus indicating either a very moderate influence of temperature on clearance and ingestion rates between 10 and 20° C, or some degree of adaptation of these processes to the lower acclimation temperature.

Introduction

The rotifer *Brachionus plicatilis* has risen to primary importance as a live food organism in the larval culture of marine fish and crustacean species (Hirata, 1979; Fujita, 1983). This species has been described by Walker (1981) as a nonselective, polyphagous member of the filter feeding planktonic rotifers, whose diet may include various types of algae as well as bacteria and yeasts. In Japan this rotifer is usually mass-cultured fed on a marine species of *Chlorella*, with either baker's or marine yeast as a latter supplement (Hirata, 1979; Imada, 1983).

This paper is the second in a series examining the effects of long-term acclimation of a parthenogenetic strain of this species to a temperature below its normal environmental range. In this study the effects of low temperature acclimation on the clearance or filtration rates and the ingestion rates of *B. plicatilis* feeding on *Chlorella saccharophila* were observed.

Materials and Methods

The monogonont rotifer, *Brachionus plicatilis* MÜLLER and the Chlorophycean, *Chlorella saccharophila* var. *saccharophila* KRUGER, were obtained from the same source and cultured under similar conditions as previously described (Nagata, submitted for publication). Following one year of acclimation to 10 or 20°C, clearance and ingestion rate experiments were carried out under two experimental designs.

* Laboratory of Planktology, Faculty of Fisheries, Hokkaido University
(北海道大学水産学部浮游生物学講座)

In the first series rotifers were taken from either 500 ml or 1 l stock cultures, and resuspended into various concentrations of *C. saccharophila*. Several hours prior to the introduction of the rotifers, log phase algae were centrifuged from their medium and resuspended in modified ASP medium (Hirata and Nagata, 1982). The rotifer densities in the combined culture were adjusted to the same as those of the stock cultures (ca. 100 ind ml^{-1}), thus avoiding artificial concentration of the animals. Following a two hour acclimation period to a temperature of either 10 or 20 ± 0.2°C, salinity of 20 ppt, and light intensity of 1 klux, the culture was slowly pipetted into a 100 ml flask, then tightly stoppered. A control flask containing the same algal culture after removal of the rotifers with a 40 μm plankton filter was also prepared. The flasks were then placed in an incubator and rotated over their longitudinal axes through a radius of 10 cm at 5 rpm for an experimental period of 3 h. Dissolved oxygen, measured with a Y.S.I. 58 D.O. meter, remained above 4.00 mg l^{-1} at the end of each trial; thus oxygen was never limiting to the rotifers. Prior to and immediately after both the acclimation and experimental periods at least eight replicate counts of algal density were made with a hemocytometer under a light microscope at 400×, equipped with Nomarski interference contrast optics. Despite the tediousness of this method, it allowed easy discrimination of cell fragments and partially digested cells from healthy uningested cells; thus the former were not included in enumeration. Whole partially digested cells lacked the characteristic fluorescence of growing ones.

A time series design was incorporated in the second set of experiments. In this series the methodology was similar to the previous one, however the glass stopper of the experimental flask was replaced with a silicon stopper fitted with a sampling port, which allowed the periodic sampling of the algal culture devoid of rotifers. The acclimation period was reduced to one hour, and the culture was sampled at 30 min intervals throughout a 4 h experimental period.

Clearance rate is defined as an estimate of the volume of water processed by a rotifer per unit time during which it is actively feeding; while ingestion rate will denote the number of cells consumed by a rotifer per unit time (Starkweather, 1980). These rates were calculated by the equations of Gauld (1951) and Hargis (1977) as presented in Schlosser and Anger (1982). Corrections for changes in algal concentration of the control flasks during the incubation period were made in the calculation of clearance rates, while the geometric means of algal concentration were used in the calculation of ingestion rates.

Results

The results of the first series of experiments are presented in Table 1. Clearance rates ranged from 0.90 to 2.44 μl ind^{-1} h^{-1}, while ingestion rates ranged from 35 to 345 cells ind^{-1} min^{-1}. The values for the 2 h acclimation period showed much variability, however no distinct pattern in relation to those obtained for the experimental period were observed. The acclimation data were therefore excluded from subsequent calculations. Mean values of clearance and filtration rates at high and low algal concentrations at 20°C, and at 10°C were then calculated. As can be seen in Table 1 the mean clearance rates did not appreciably differ from the pooled value of 1.63 ± 0.47 μl ind^{-1} h^{-1}, however ingestion rates vary considerably between

Table 1. Clearance and ingestion rates of *B. plicatilis* at 20 and 10°C, and various algal densities. Values for acclimation period given in brackets.

Trial	T°C	Rotifer density (ind ml^{-1})	Algal density (10^4 cells ind^{-1})	Clearance rate (μl ind^{-1} h^{-1})	Ingestion rate (cells ind^{-1} min^{-1})
1	20	70	19.50 (22.70)	1.83 (1.07)	345 (264)
2	20	71	18.90 (21.71)	1.81 (0.88)	334 (210)
3	20	85	17.10 (23.80)	1.44 (1.94)	290 (554)
4	20	89	17.80 (20.00)	1.06 (0.66)	243 (185)
5	20	149	8.80 (12.40)	1.39 (1.16)	223 (185)
6	20	96	18.20 (20.30)	1.24 (0.56)	302 (174)
7	20	81	4.99 (6.86)	1.55 (1.97)	87 (156)
8	20	103	3.32 (4.58)	0.90 (1.60)	45 (107)
9	20	84	4.79 (5.49)	2.23 (0.82)	113 (59)
10	20	91	3.85 (4.73)	1.87 (1.13)	85 (73)
11	20	106	3.88 (5.67)	2.28 (1.96)	112 (162)
12	20	96	3.06 (3.88)	1.27 (1.11)	52 (69)
13	20	89	4.57 (8.38)	2.44 (3.40)	120 (313)
14	20	98	5.21 (9.37)	1.73 (3.10)	116 (354)
15	10	115	1.31 (2.39)	1.90 (2.63)	35 (89)
16	10	99	2.69 (3.96)	1.53 (1.96)	54 (105)
17	10	119	1.69 (2.74)	1.80 (2.08)	44 (89)
18	10	102	3.23 (4.58)	0.99 (1.82)	48 (119)
19	10	92	3.58 (4.62)	1.79 (1.41)	77 (88)
$\bar{X}$(20°C 1- 6)		93	16.72±3.98	1.46±0.31	290± 49
$\bar{X}$(20°C 7-14)		94	4.21±0.80	1.78±0.53	91± 29
$\bar{X}$(10°C)		105	2.50±0.98	1.60±0.37	54± 16
$\bar{X}$(total)				1.63±0.47	143±108

Table 2. Time series determinations of clearance (μl ind^{-1} h^{-1}) and ingestion rates (cells ind^{-1} min^{-1}) in *B. plicatilis*. Ingestion data within brackets.

Trial	T°C	Initial algal density*	Acclimation period (min)		Experimental period (min)								
			30	60	15	30	60	90	120	150	180	210	240
A I.	20	7.55	6.39 (661)	4.88 (403)	4.70 (337)	7.05 (449)	3.38 (188)	3.87 (187)	2.16 (93)	5.43 (201)	12.28 (328)	4.09 (76)	— —
II.	20	5.53	2.69 (204)	5.94 (382)	6.09 (328)	3.20 (157)	4.09 (180)	17.23 (493)	2.78 (53)	4.09 (69)	1.86 (28)	12.53 (146)	4.49 (38)
III.	20	4.35	— —	3.42 (241)	2.63 (153)	5.49 (289)	4.69 (207)	1.40 (53)	5.19 (169)	4.42 (114)	10.62 (190)	3.84 (48)	1.59 (18)
IV.	20	5.82	—	3.53 (311)	— —	0.97 (74)	4.10 (279)	1.35 (82)	3.11 (169)	1.43 (70)	3.15 (140)	1.29 (52)	0.49 (19)
B I.	10	2.41	4.56 (181)	2.96 (97)	— —	2.19 (64)	3.44 (97)	2.13 (47)	3.11 (61)	0.76 (14)	3.84 (61)	2.23 (30)	0.80 (10)
II.	10	5.67	5.73 (557)	2.58 (203)	0.47 (34)	1.85 (132)	1.83 (122)	0.60 (37)	0.94 (57)	0.53 (31)	0.49 (28)	3.65 (186)	2.64 (144)
III.	10	4.50	6.57 (443)	0.32 (19)	0.26 (15)	2.96 (163)	1.22 (63)	0.52 (26)	0.54 (26)	3.14 (141)	2.43 (96)	0.81 (30)	5.56 (177)
IV.	10	3.92	4.19 (305)	0.18 (12)	1.22 (77)	7.07 (398)	0.96 (48)	0.37 (18)	1.10 (51)	4.55 (178)	2.10 (68)	3.41 (95)	3.07 (71)

*Algal densities expressed as cells ind$^{-1} \times 10^4$.

groups. Thus clearance rates appear to be little affected by algal concentration and temperature within the tested ranges, while ingestion rates seem to be greatly affected by these parameters. Q_{10} values determined for the low algal 20°C and 10° C data were 1.11 and 1.75 for clearance and ingestion rates, respectively.

The results of the time series trials are seen in Table 2, and Fig. 1 and 2. The most obvious feature is seen in the great variability in clearance and ingestion rates over the time course within a trial. Clearance rates ranged from 0.49 to as high as 17.23 μl ind^{-1} h^{-1} at 20°C, and from 0.18 to 7.07 μl ind^{-1} h^{-1} at 10°C; while ingestion rates ranged from 18 to 661 cells ind^{-1} min^{-1} at 20°C, and 10 to 557 cells ind^{-1} min^{-1} at 10°C. Again data from the acclimation periods were quite variable, however a general trend of rapid decline in ingestion rates, and to a lesser extent in

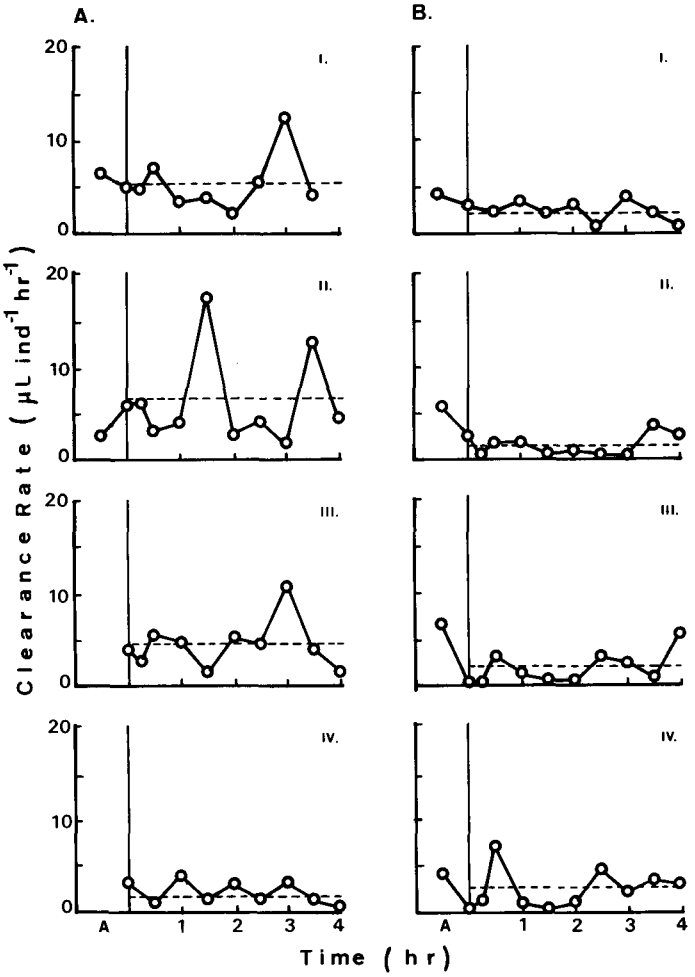

Figure 1. Time series profiles of clearance rates of *B. plicatilis* at 20°C (A) and 10°C (B). Vertical lines mark end of acclimation and start of experimental period. Broken lines mark mean clearance rates.

clearance rates, was seen over the 1 h duration in all but one of the trials. At 20°C large peaks in clearance rate over 10 μl ind^{-1} h^{-1} were present in all except the last trial. The 10°C trials also showed large periodic peaks in clearance rate, but to a much lesser magnitude. Where two peaks occurred in either the clearance or ingestion rate profiles within a trial, they were usually separated in time by 2.0 to 2.5 h. This may indicate some form of periodicity in feeding activity of this rotifer. A general trend of decrease in ingestion rates over the duration of the experimental period was also seen in the 20°C trials, however was much less evident at 10°C.

Mean values of clearance and ingestion rate determined over the experimental period were: 4.71 ± 2.01 μl ind^{-1} h^{-1} and 152 ± 45 cells ind^{-1} min^{-1} at 20°C, and 2.21 ± 0.52 μl ind^{-1} h^{-1} and 85 ± 29 cells ind^{-1} min^{-1} at 10°C. Much of the variation exhibited by the 20°C clearance rate mean can be attributed to the last trial. Here

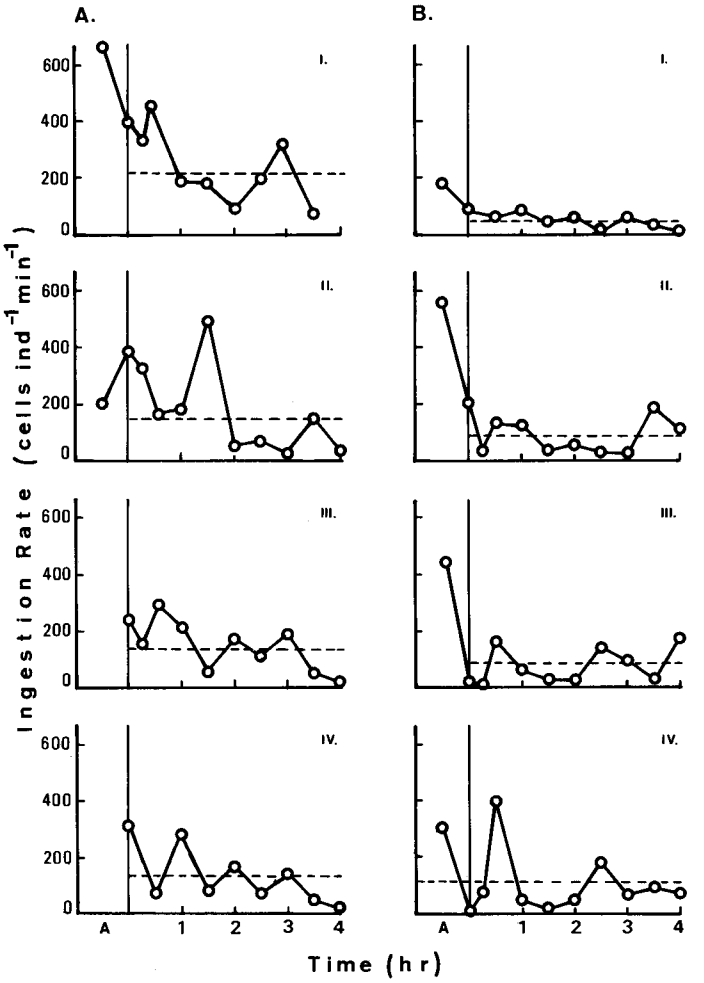

Figure 2. Time series profiles of ingestion rates of *B. plicatilis* at 20°C (A) and 10°C (B). Vertical lines mark end of acclimation and start of experimental period. Broken lines mark mean ingestion rates.

a low mean clearance rate (1.99 ± 1.28 μl ind^{-1} h^{-1}), comparable to those at 10°C, was accompanied by a high ingestion rate (111 ± 83 cells ind^{-1} min^{-1}). Q_{10} values of 2.18 and 1.79 were obtained for clearance and ingestion rates, respectively. Thus a more evident temperature effect on clearance rates, which was masked in the previous series, was seen in this one; while the effect on ingestion rates remained fairly constant.

For a more detailed analysis of the effect of algal concentration on clearance and ingestion rates, those data from both series were regressed against rotifer specific algal densities (i.e. the number of algal cells available to each rotifer at a given time) in various combinations. No significant regressions were obtained for clearance rate vs. algal density, however four very significant regressions were obtained for ingestion rate vs. algal density. These are listed below.

Series 1, 10 and 20°C data: $r^2=0.94$ $Y=15.69X+22.43$ $P<0.05$ $n=19$
Series 2, 10 and 20°C data: $r^2=0.64$ $Y=26.85X-4.97$ $P<0.05$ $n=8$
Series 1 and 2, 20°C data: $r^2=0.91$ $Y=14.50X+43.22$ $P<0.05$ $n=18$
Series 1 and 2, 10°C data: $r^2=0.75$ $Y=17.08X+12.36$ $P<0.05$ $n=9$

These were further examined by analysis of covariance to test the degree of similarity between them. The initial test for homogeneity of variances, Bartlett's test (Zar, 1974), yielded heterogeneous variances at $P<0.05$, but homogeneity at $0.005<P<0.01$; thus the significance tests were done using the lower probability level (Underwood, 1981). In the first significance test the slopes of the above regressions were found to be equal ($F=3.12$, $0.025<P<0.05$). The elevations were also found to be the same ($F=0.0009$, $P>0.25$). Finally, the regressions were confirmed to be coincident by an overall test ($F=1.55$, $0.10<P<0.25$). Thus criteria for pooling the data from the above regressions was arrived at, and the subsequent relation ($Y=15.40X+29.44$, $r^2=0.92$, $P<0.05$, $n=27$) obtained. The plot of this regression is shown in Fig. 3. Therefore it can be seen that ingestion rates of *B. plicatilis* were highly dependent on algal concentration; increasing in a

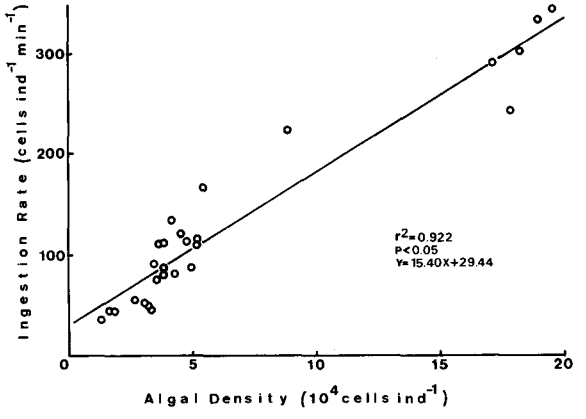

Figure 3. Dependence of ingestion rates on rotifer specific algal densities in *B. plicatilis*, for pooled data from 10 and 20°C.

linear fashion in response to algal density throughout the tested range.

Discussion

Clearance and ingestion rates have probably been the most widely studied physiological parameters of the planktonic Rotifera. Reviews of these two parameters from both field and laboratory rotifer populations feeding on a wide array of food organisms can be found in Pourriot (1977), Starkweather (1980), Walker (1981), Schlosser and Anger (1982), and Hirayama (1983). The latter two reviews concentrated on studies with *B. plicatilis*; and that of Schlosser and Anger further examined the effects of variations in the methodology used in studying these parameters.

Mean clearance rates obtained in this study were within the range of 1 to 10 μl ind^{-1} h^{-1} for planktonic rotifers, in general, at 20-25°C (Starkweather, 1980); and 0.38 to 10.00 μl ind^{-1} h^{-1} for *B. plicatilis* at 20-28°C (Hirayama, 1983). The peak clearance rates from the time series trials exceeded these ranges (i.e. 17.23 μl ind^{-1} h^{-1}), but were still well below the rate of 50 μl ind^{-1} h^{-1} obtained for *Brachionus calyciflorus* (Starkweather and Gilbert, 1977).

In addition, these clearance and ingestion rates are comparable to those of Hirayama and Ogawa (1972) for *B. plicatilis* also feeding on a marine form of *Chlorella* sp. They observed clearance rates of 0.12 to 6.0 μl ind^{-1} h^{-1} and ingestion rates from 30 to 250 cells ind^{-1} min^{-1} at algal densities of 1.0 to 24.0×10^6 cells ml^{-1} and a temperature of 25°C. However, a number of methodological effects can be found in their experimental design, which have been discussed in Schlosser and Anger (1982).

Hirayama and Ogawa (1972) found clearance rates to decrease with increasing algal densities, while ingestion rates remained constant at algal densities over 2.1×10^6 cells ml^{-1}. Contrary to these results, in this study clearance rates were determined to be independent of algal density, as no significant inverse relations were obtained between these two parameters. However, ingestion rates increased proportionally with higher algal densities, within the tested range (1.08 to 20.21×10^6 cells ml^{-1}). Schlosser and Anger (1982) also observed independence of clearance rates, and a linear dependence of ingestion rates on algal densities. The majority of the tested algal concentrations in this study were well over the critical concentration (2.2×10^6 cells ml^{-1}) from the study of Hirayama and Ogawa, but no critical concentration or plateau of ingestion were evident, indicating the continuous ingestion of food in proportion to its availability (Starkweather, 1980). Similar results were obtained by Dewey (1976; from Starkweather and Gilbert, 1977) for *B. plicatilis* feeding on *Isochrysis galbana*; and for *B. calyciflorus* feeding on the yeast species, *Rhodotorula glutinis* (Starkweather and Gilbert, 1977). The latter authors hypothesized that this may have been an effect of cell size, since very small cells were used in both studies. The cell size of *C. saccharophila* (cell diameter of 3.5 μm) lies between those of the above two food types. Thus the critical food concentration for such small cells may occur at much higher levels than the maximum tested in these studies, about 117 μg dry wt ml^{-1} in this study and 100 μg dry wt ml^{-1} in the study of Starkweather and Gilbert (1977).

The effect of temperature on clearance and ingestion rates in *B. plicatilis* was

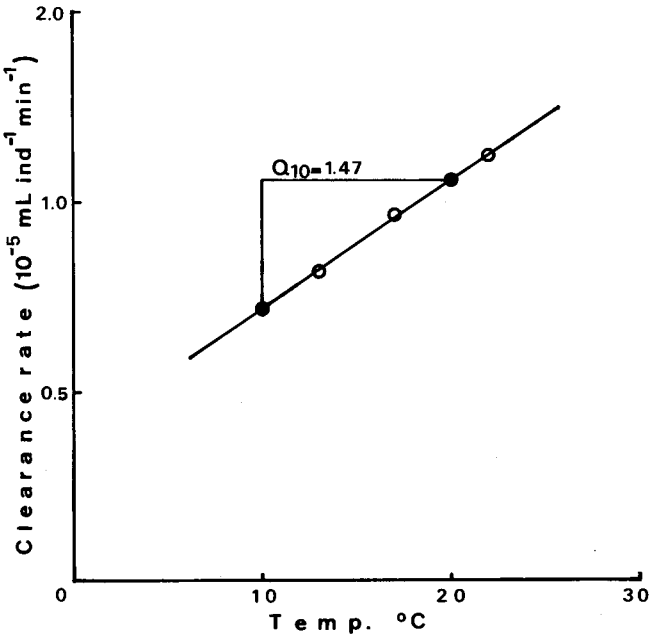

Figure 4. The dependence of clearance and ingestion rates on temperature. Modified from Hirayama and Ogawa (1972). Open circles -original data ; closed circles -derived data.

also examined by Hirayama and Ogawa (1972). From their Fig. 6 (shown in Fig. 4), mean clearance rates were calculated for the pooled data for 13 through 22° C, a regression of the means plotted, and values for 10 and 20°C estimated. The derived Q_{10} for clearance rates within this temperature range was 1.47, a value between those of the present study. However the relations between temperature, and clearance and ingestion rates may not be linear, but curvilinear functions ; such as the exponential relation described for pooled cladoceran ingestion rates by Peters and Downing (1984). From their relation a Q_{10} value of 1.86 was derived, which fell short of Prosser's theoretical value of 2 to 3, based on individual species responses (Prosser, 1973). Only the clearance rate Q_{10} from the time series trials in this study fell within this range, while the other Q_{10} values were short of it. This may indicate some degree of adaptation to the lower temperature. Schlosser and Anger (1982) observed an increase in clearance rates of 42% with a 5.5°C rise in temperature from 18°C ; but no apparent temperature effect on clearance and ingestion rates was seen over a similar temperature range in the study of Starkweather and Gilbert (1977).

The time series trials in this study have shown that clearance and ingestion rates of *B. plicatilis* can vary greatly over the duration of an experiment, with constant rates being the exception rather than the mean. Thus the regulation of these rates over short time spans has been demonstrated ; whose underlying mechanisms probably involve changes in the frequency of beating of the coronal cilia, in addition to the three mechanisms of fine control described by Gilbert and Starkweather (1977).

Short-term experiments of 1 h or less would probably only measure one peak of an activity spectrum, leading to both over and underestimations of these rates.

Schlosser and Anger (1982) found mean clearance and ingestion rates to decline in a curvilinear fashion with longer experimental periods, which they attributed to a saturation effect. However, the initial high rates which they measured in short term trials probably resulted from the effects of previous starvation, since food was not provided during the 1 h acclimation period. The very high ingestion rates seen during the acclimation period in this study (Fig. 2) most likely illustrate a similar response. Furthermore, the slope of their curve may have been exaggerated due to the enumeration of fecal materials and cell fragments by their Coulter Counter method (Peters and Downing, 1984). Schlosser and Anger did in fact acknowledge a counting error at the lower ends of their particle spectra.

In conculsion it appears that clearance and ingestion rates of *B. plicatilis* are still influenced by a temperature effect, even after long-term acclimation to a subnormal temperature. However the intensity of this effect is variable, and may indicate a small degree of adaptation to the lower temperature. This effect on ingestion rates in particular may also be superseded by other factors such as that of algal density. Nevertheless, the effect was at most moderate in comparison to the effect of low temperature on growth and reproduction, reported previously (Nagata, submitted for publication).

The implications of these findings for the mass culture of this species are obvious, with it requiring less food at the lower temperature. As a conservative estimate of daily consumption, an adult amictic female *B. plicatilis* weighing 0.42 μg dry weight (Hirata and Nagata, 1982) could ingest 174, 960 cells or 1.01 μg dry weight of *C. saccharophila* per day at 20°C ; and 98,640 cells or 0.57 μg dry weight at 10°C. This ration being equivalent to 2.6$\times$ and 1.4$\times$ dry body weight at the two temperatures, respectively. A thorough analysis of feeding energetics of *B. plicatilis* at these acclimation temperatures will, nevertheless, await further study.

Acknowledgements.

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