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Effect of water viscosity on the population growth of the rotifer *Brachionus plicatilis* Müller

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Abstract

We measured the relative viscosity of water taken from a 500 ml rotifer batch culture against natural sea water using a capillary type viscometer (Ostwald type) during 30 days. The relative viscosity ranged from 1.054 to 1.148. We observed a negative correlation between population growth rate and viscosity. We experimentally tested the effect of viscosity on reproduction, swimming activity and ingestion rate of *Brachionus plicatilis*. By dissolving methyl cellulose, the relative viscosity of natural sea water (salinity 22 ppt) was increased from 1.0 (control group) to 1.022, 1.031, 1.078 and 1.169. At higher viscosity, individually cultured rotifers at 25 °C that were fed *Nannochloropsis oculata* showed slower swimming, lower ingestion rate and lower fecundity than control. By increasing the relative viscosity from 1 to 1.169, the swimming activity index decreased from 28.3 to 4.9 (1 mm^2 square grids/30 s), the ingestion rate decreased from 1.13 to 0.34 ($\times 10^3$ cells ind^{-1} h^{-1}), and the number of offspring per amictic female decreased from 20.8 ± 3.0 to 8.1 ± 3.7 (mean $\pm$ SD).

Introduction

Viscous forces predominate over inertial forces for microorganisms with a low Reynolds number. For microorganisms, moving in water is like swimming in a pool of molasses for a human (Mann & Lazier, 1991). Theoretical mechanisms of particle retention in fluid dynamic environments have been useful in clarifying feeding mechanisms of copepods (Koehl & Strickler, 1971) and rotifers (Rubenstein & Koehl, 1977; Starkweather, 1995).

Monogonont rotifers inhabit eutrophic waters where the viscosity of water is greater than that of oligotrophic waters. The euryhaline rotifers, *Brachionus plicatilis* and *B. rotundiformis*, have become popular as models for investigating fundamental principles of aquatic ecology, as live food in aquaculture and as test animals for eco-toxicological research. Population growth of rotifers can decrease due to an increase in free ammonia in the culture (Yu & Hirayama, 1986) or an increase in certain bacteria and protozoa (Maeda & Hino, 1991). It is theoretically predicted that food ingestion in rotifers could be promoted by water turbulence due to a decrease of the boundary layer around the body surface of rotifers. On the other hand, rotifer swimming or food ingestion would be suppressed by an increase in water viscosity. Information is scarce, however, on the effect of such an environment on the physiological state of cultured rotifers. In this study, we examined (1) the relation between growth rate of rotifers in a laboratory culture and viscosity of the culture and (2) the effect of changes in water viscosity on growth, reproduction, feeding and swimming behavior of the rotifer *Brachionus plicatilis*.

Materials and methods

B. plicatilis (Tokyo strain) used in these experiments was cultured under steady-state conditions. *Nannochloropsis oculata* grown in modified Erd–Schreiber medium (Hagiwara et al., 1994) was used as food. Algal density in rotifer cultures was adjusted daily to replenish 7×10^6 cells ml^{-1}. Culture temperature was 25 ± 1 °C, salinity was 22 ppt and photoperiod at 0 L: 24 D. Salinity was adjusted by diluting natural sea water collected at Nomo Fisheries Station, Nagasaki University with distilled water.

The effect of viscosity on rotifer reproduction in a batch culture

B. plicatilis was mass cultured in a 500 ml glass beaker for 29 days. The culture was initiated with 4 rotifers /ml. A 20 ml water sample for viscosity measurement was collected daily, and frozen at −30 °C after GF/C (Whatman) filtration. A 5 ml water sample was also collected to determine rotifer density under a stereomicroscope. Water viscosity was estimated by using a capillary type viscometer (Ostwald type, 0.5 mm tube diameter, Vidrex, Fukuoka, Japan). The viscometer was placed in a water bath (25 ± 1 °C) for viscosity measurement (Yamato Kagaku Co., Tokyo, Japan). A 5 ml GF/C (Whatman) filtered sample was added to the viscometer capillary and kept for 10 min. After the tested sample was acclimated to 25 °C, the water sample was released inside the capillary tube and the time required for the water mass to fall between two menisci (about 3 ml volume) was monitored. This procedure was repeated ten times for each sample. Between measurements of each sample, the viscometer was washed and rinsed with distilled water and 99% ethanol. Falling time (seconds) in the capillary tube was 106.65 ± 0.18 and 112.57 ± 0.33 (mean ± SD) for distilled water and natural sea water (diluted to 22 ppt salinity by distilled water), respectively. The relative viscosity of culture water sample was calculated as a ratio of the falling time of the culture sample against that of 22 ppt sea water.

Regression analysis was conducted to examine the relation between culture water viscosity and daily population growth ratio of rotifers.

Effects of viscosity on rotifer performance

We tested the effect of viscosity changes on the reproduction, swimming speed, ratio of swimming to attached individuals and ingestion rate of *B. plicatilis*.

Viscosity of the experimental sea water was adjusted by the addition of methyl cellulose (13–18 mPa s of a 2% aqueous solution at 20 °C, Wako Chemical Co, Tokyo, Japan) to 0.0125, 0.025, 0.05 and 0.1%. The relative viscosity against 22 ppt diluted sea water was 1 (control, no addition of methyl cellulose), 1.022, 1.031, 1.078 and 1.169. These media were used to determine the effect of sea water viscosity on rotifer fecundity, swimming speed, and ingestion rates. The relative viscosity was measured by a viscometer (see previous section).

Amictic females carrying first laid eggs were prepared following the protocol described by Satuito & Hirayama (1986). Rotifers were individually transferred to 2 ml *N. oculata* suspension medium in multiwell plates (Iwaki 24 well tissue culture plate, Tokyo, Japan). Ten rotifers were tested at each viscosity. Maternal females were transferred to newly prepared *N. oculata* suspension medium once a day. After transfer, neonates were counted and removed. The number of offspring produced during the lifetime as well as an intrinsic rate of natural increase (Birch, 1948) was calculated. The swimming activity at different viscosities was determined in 24- and 96-hour-old rotifers by the method reported by Snell et al. (1987). One minute after the experimental rotifer was transferred to the test medium, the number of 1 mm^2 grids trespassed by a rotifer during a 30 s observation period was monitored and used as an index of rotifer swimming activity.

The ratio of swimming to attached rotifers was determined 24 h after the inoculation of 20 rotifers into 10 ml medium at the varying viscosities. Three replicates for each treatment were prepared with newborn and egg-bearing females.

Ingestion rates (cells $rotifer^{-1}$ h^{-1}) under different viscosity conditions were determined directly on batch cultures. From an exponentially grown 500 ml rotifer culture, 1000 rotifers were pipetted into screw cap glass vials containing 5 ml medium that was adjusted to the tested viscosities. *N. oculata* was added to adjust the cell density to 7×10^6 cells/ml. Three replicates were prepared, as well as controls that contained *N. oculata* alone. Cultures were kept under total darkness. After one hour, 0.5 ml 10% formaldehyde solution was added and the algal cell density was counted in a hemacytometer. The ingestion rate was computed using the following equation (Fuller & Clarke, 1936; Nimura, 1963):

$$G_t = V(C_t - C_0)\frac{\log C_t' - \log C_t}{\log C_t - \log C_0},$$

where, G_t is food uptake per animal during time t, C_0 and C_t are food densities of rotifer cultures at time t and 0, respectively, and C_t' is the food density of control cultures (without rotifers) at time t.

One-way analysis of variance (ANOVA) using Systat 7.0 (SPSS Inc.) was conducted to identify significant effects of culture water viscosity on reproduction, swimming activity and ingestion rate. Multiple comparison was conducted using Tukey HSD to determine which treatments were significantly different.

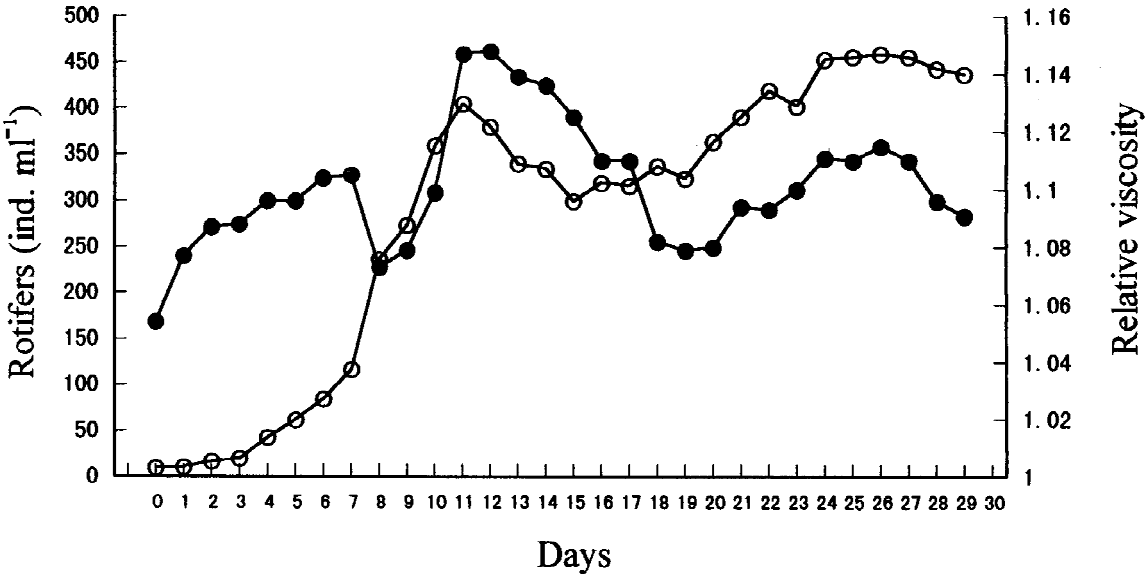

Figure 1. Population growth of *B. plicatilis* (open circles) and relative water viscosity (closed circles) during 29 day culture period.

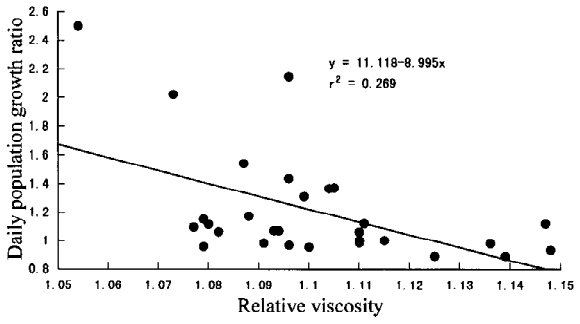

Figure 2. Correlation between viscosity of culture water and daily population growth ratio of *B. plicatilis*.

Table 1. Mean and SD of life span and number of offspring, and intrinsic rate of natural increase (r) of *B. plicatilis* reared in sea water (22 ppt in salinity) with different viscosities by dissolving methyl cellulose. Values that do not share a superscript are significantly different ($p < 0.05$, Tukey HSD test)

Relative vicosity	n	Life span	Number of offspring	r
1	10	8.0±0.0^a	20.8±3.0^a	0.853
1.022	10	7.7±0.5^a	19.4±2.0^a	0.917
1.031	10	7.7±0.5^a	13.1±2.2^b	0.748
1.078	10	8.0±0.0^a	11.8±2.9^b	0.784
1.169	10	6.3±2.0^a	8.1±3.7^c	0.640

Results

Figure 1 presents daily data of rotifer population size and water viscosity in a 500 ml culture. Through the inoculation of rotifers at 10 ind ml^{-1} and feeding of *N. oculata* at 7×10^7 cells ml^{-1} on day 0, culture viscosity increased 1.054 times. The R.V. value increased for the initial 7 days, and it decreased when rotifers grew exponentially on day 8. After day 8, viscosity changes corresponded to the population growth pattern. The highest viscosity was 1.148 on day 13 when rotifer density was 380 ind/ml. Negative correlation was detected between daily population growth rates and R.V. values (Figure 2, $y = 11.118 - 88.995x$, $r^2 = 0.269$, df = 28, $F = 10.25$, $P < 0.01$).

Life table characteristics of amictic females cultured in sea water at different viscosities are presented in Table 1. Viscosity of culture water significantly affected the life span and number of offsprings (ANOVA: life span – $F = 5.62$, df = 4, $P < 0.01$; number of offsprings – $F = 36.03$, df = 4, $P < 0.01$). There were no significant differences in life span of amictic females except when cultured at R.V. = 1.169. Compared to controls, the number of offspring was significantly reduced at viscosities higher than 1.031. The intrinsic rate of natural increase (r) ranged between 0.640 (R.V. = 1.169) and 0.917 (R.V. = 1.022).

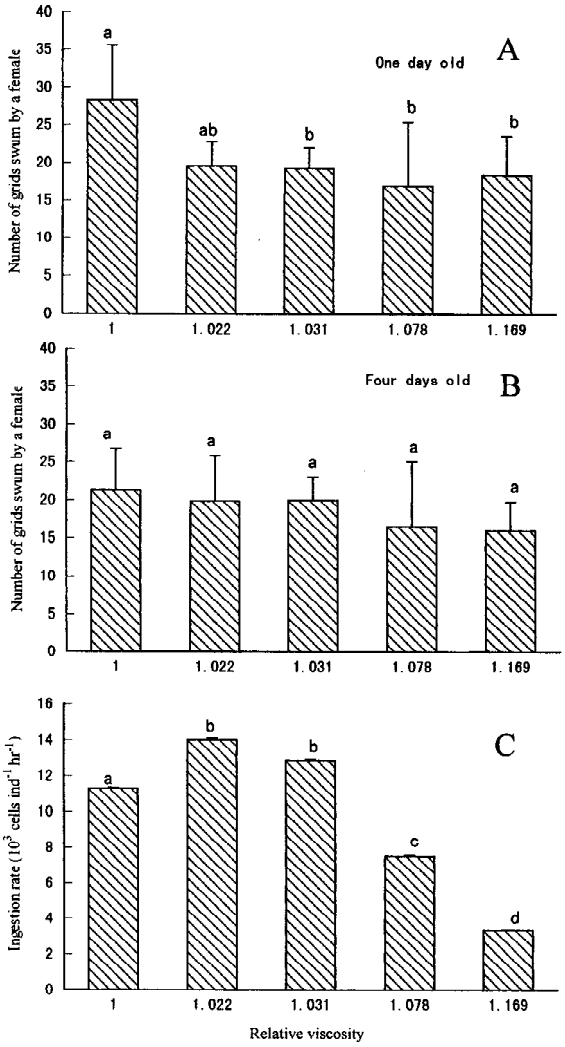

Figure 3. Number of 1 mm^2 grids swum by one day old (A) and four days old (B) *B. plicatilis* females at different viscosities, and ingestion rate (cells ind^{-1} h^{-1}) at different viscosities (C). Vertical bars indicate standard deviation. Values that do not share a superscript are significantly different ($P < 0.05$, Tukey HSD test).

The number of l mm^2 grids swum by a rotifer is shown in Figure 3. Viscosity significantly influenced the swimming speed for 24-hour-old rotifers (ANOVA, $F = 4.21$, df = 4, $P < 0.01$). Such effect was detected only against the control (R.V. = 1). Rotifers swam slower when the relative water viscosity (R.V.) was higher than 1.031. The number of grids swum by a rotifer did not differ when the R.V. was higher than 1.022. For 96-hour-old rotifers it did not differ among treatments. The ratio of swimming to attached individuals was not affected by culture viscosity in either newborn or egg-bearing females.

Ingestion rates were also significantly affected by the change of culture water viscosity (Figure 3; ANOVA, $F = 165.32$, df = 4, $P < 0.01$). Ingestion decreased when rotifers were exposed to media with R.V. at 1.078 and 1.169. It was higher than the control, however, at 1.022 and 1.031.

Discussion

In the batch culture condition where rotifer density changed from 10 to 460 ind/ml, the viscosity of culture water ranged between 1.054 and 1.148. Although we obtained data from one trial only, we could observe the increase of viscosity during the rotifer culture period. Rotifer population growth was negatively correlated with the relative viscosity of culture water. From Figure 2, it appears that lower viscosity (less than 1.11) more strongly affected rotifer reproduction. Only small changes occur with increasing the viscosity from 1.11 to 1.15. Factors other than viscosity, such as free ammonia concentration (Yu & Hirayama, 1986) and bacterial flora (Yu et al., 1990), should be taken into account in explaining the low population growth in the batch culture. On the other hand, predominance of vitamin B_{12} producing bacteria stabilizes rotifer cultures (Yu et al., 1994).

The highest R.V. value 1.169 that we tested in individual culture experiments was equivalent to that observed in the rotifer mass culture (Figure 1). The suppressed rotifer population growth at the higher viscosity was also observed in the individual culture experiment (Table 1). Both lifespan and offspring number of individually cultured amictic females decreased with an increase of viscosity.

In order to clarify the mechanism of the lower rotifer reproduction at the higher viscosity, we examined swimming speed and ingestion rate of rotifers. These are recognized as indicators to assess physiological stress on rotifers from a variety of environmental factors (Snell et al., 1987; Juchelka & Snell, 1994) and as important components in the energy budget of rotifers that determines reproductive potential. A decrease in swimming speed was observed for younger rotifers (Figure 3) having a lower Reynold's number which are more susceptible to the effect of viscosity increase. The ingestion rate decreased markedly when R.V. was more than 1.078 (Figure 3). These data indicate that the increase of culture viscosity functions as sublethal stress for rotifers that decreases their reproductive ability. In our experiment, the ingestion rate

of rotifers was faster than that of the control (R.V. = 1) when R.V. was 1.022 and 1.031. The mechanism of this action is unknown, but similar results were reported by Juchelka & Snell (1994) who measured ingestion rates when rotifers were exposed to several types of toxicants. Former publications (Hirayama & Ogawa, 1972; Hirayama et al., 1973; Starkweather, 1988) also met with the difficulty of directly correlating *B. plicatilis* population growth with its ingestion rate at different food densities.

A problem encountered in this study was that the regulation of water viscosity can be adjusted only by dissolving chemicals which may have a toxic effect on animals. In addition to methyl cellulose, we also employed sodium alginate to regulate water viscosity. However, the addition of sodium alginate resulted in 50–60% mortality of rotifers during the initial three days. With methyl cellulose, there were no significant differences in life span of amictic females except when cultured at R.V. = 1.169. Methyl cellulose is generally a non-toxic compound which is widely used for human food processing and cosmetics. We suspect that dissolved peptides may cause an increase in viscosity in rotifer culture water. We are attempting to clarify the characteristics of these peptides and to reduce their effect on the R.V. of culture water by the addition of various enzymes.

Phytoplankton mucus may kill marine fish by clogging their gills (Matsusato & Kobayashi, 1974). The mucilage produced by diatoms restricted fish trawling (Boalch & Harbour, 1977). Jenkinson (1986) explained these mechanisms by investigating non-newtonian property of laboratory phytoplankton cultures. The relative viscosity of sea water collected from an area where abundant decomposing macroalgae existed was 1.015 (M. Hamada, pers. comm.). In this experiment, the inoculation of rotifers on day 0 at 10 ind ml^{-1} and feeding of *N. oculata* at 7×10^6 cells ml^{-1} immediately caused a 5.4% increase in culture viscosity. The viscosity coefficients of freshwater and seawater (36 ppt S) increase from 0.802 and 0.866 mPa s to 1.794 and 1.890 mPa s, respectively, when the water temperature decreases from 30 to 5 °C (Miyake & Koizumi, 1948; Ohgushi, 1981). We conducted the current experiments at 25 °C, since most of the rotifer mass cultures are maintained around this temperature. In order to understand the rotifer life cycle in nature, however, it is of interest to continue research at lower temperatures where viscous forces predominate. Such a research also provides information for live food culturing conducted at colder regions.

Rotifers are widely used as a live food source for feeding fish larvae. Production of larvae, therefore, depends on the productivity and stability of rotifer cultures. Factors that cause instability of the rotifer mesocosm have been reviewed by several authors (Hirayama, 1987; Maeda & Hino, 1991). Among them, an increase of free ammonia, a change of bacterial flora and contamination by protozoan species are critical. Several methods, such as measurements of egg ratio (eggs per female) and swimming activity, have been established for the rapid assessment of the rotifer mass culture status (Snell et al., 1987). Current study suggests that the measurement of water viscosity can also be a means of detecting stress on rotifers by environmental conditions, where viscous substances are comparatively abundant. Recent developments enable rotifer mass cultures to reach more than 10 000 ind ml^{-1} mainly because of the availability of ample amounts of a preserved phytoplankton diet (Yoshimura et al., 1996, 1998). Increase of viscosity could be more significant in such an extreme environment.

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