

Influence of rapid changes in salinity and temperature on the mobility of the rotifer *Brachionus plicatilis*

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Abstract

The rotifer *Brachionus plicatilis* can grow in a wide range of salinities and temperatures, but rapid shifts in both salinity and temperature may result in immobilized, non-swimming rotifers. The goal of this study was to examine the effect of perturbations in temperature and salinity on the swimming pattern of the rotifer.

Only slight changes in mobility were observed when rotifers were exposed to changes in temperature (from 20 °C to 8–30 °C) and to an increase in salinity (from 20‰ to 30‰). When the salinity was reduced to 15‰ and 5‰, the proportion of mobile rotifers was reduced to 50% and 5%, respectively. The rotifers were throughout more resistant to perturbations in temperature than to those of salinity.

Combined temperature and salinity perturbations compared to perturbations in each factor separately suggested a synergetic effect of temperature and salinity on the rotifers locomotion. Transfer from cultivation conditions to low salinity (5‰) and high temperature (28 °C) resulted in very low percent of mobile rotifers (0–10%). However, if the temperature was reduced to 8 °C concomitant with the changes in salinity, the percent of mobile rotifers was 85%.

Rotifers use a high share of their metabolic energy for locomotion, and it is therefore not surprising that perturbations in salinity and temperature may result in partial or complete immobilization.

Introduction

The rotifer *B. plicatilis* tolerates salinities ranging from 1 to 97‰ (Walker, 1981). The species is an osmoconformer (Epp & Winston, 1977), meaning that a change in ambient salinity results in a corresponding change in the concentration of the body fluids. The rotifer exhibits, however, a slight but consistent trend towards hyperosmolarity at low salinities. Both temperature and salinity will directly influence the reproductive rate of the rotifer, and the response in general depends on strain and environmental conditions (Miracle & Serra, 1989).

It has been observed that *B. plicatilis* may become immobilized upon sudden shifts in salinity or temperature. In order to reduce the perturbations in temperature and salinity when the rotifers are fed to fish larvae or are transferred to other growth conditions (e.g., inoculation), the rotifer has been produced at 20 °C and 20‰ salinity in our laboratory. These cultivation conditions are a compromise between the optimal production conditions and the problems related to immobilization of rotifers.

The aim of this study was to examine the effect of perturbations in temperature and salinity on rotifer locomotion. Both strength and duration

of the immobilization period, which follow perturbations, may be important in larval rearing.

Material and methods

The rotifer *B. plicatilis* (SINTEF-strain, length 250 μm) was cultivated in 250 liters conical vessels at 20‰ seawater salinity and 20 °C. The cultures (200 l) were aerated in order to secure mixing and sufficient oxygen supply, and were fed once each day with 5–30 g bakers yeast (wet weight) and 0.5–3 g capelin oil (10% by weight). After 10 days of cultivation, 10% of the culture volume was each day replaced by 20‰ seawater resulting in a semi-continuous culture. The rotifers were used in experiments when growth balanced dilution (steady state of growth), and the specific growth (μ) rate of the rotifers was calculated from

$$\mu = \ln(N_1/N_0)/t + \ln(D + 1)/t,$$

where N_1 is the rotifer numbers just before dilution (day 1) and N_0 is the rotifer numbers after dilution one day earlier (day 0), t is the time between dilution (here, $t = 1$ day) and D is the dilution rate of the culture defined as

$$D = dV/(V - dV),$$

where dV is the daily replaced volume and V is the culture volume.

The specific growth rate in semi-continuous cultures can be used as a measure of the physiological state of the rotifers, and the method was used in the present experiments in order to maintain constant physiological state of the rotifers. Their average specific growth rate was $0.12 \pm 0.03 \text{ day}^{-1}$, corresponding to 27% of the maximum growth rate.

The effect of four different temperatures (8-, 15-, 20- and 28 °C) and five different salinities (5-, 10-, 15-, 20-, and 30‰) were investigated. The various salinities were prepared by mixing seawater (33‰) with freshwater. The rotifers were collected on a 90 μm nylon filter and immediately transferred to a beaker just added 300 ml water of the appropriate salinity and temperature. Rep-

resentative samples (1 ml, two replicates) for determination of the fraction of mobile (and immobile) rotifers were randomly taken from the beaker with a pipette and transferred to a petridish after 1-, 2-, 4-, 8-, 16-, 32-, 64-, and 124 min. The beaker was mixed just before sampling. The number of mobile and immobile rotifers in the subsamples (1 ml) were counted in microscope immediately after sampling.

We define mobile rotifers as swimming individuals whereas immobilized rotifers are non-swimming individuals and individuals which attached their foot to the bottom of the petridish.

Results

The rotifers became temporarily immobilized when they were exposed to a sudden change in either temperature or salinity. This is given in Fig. 1, which shows that a reduction in salinity from 20 to 5‰, resulted in severe immobilization of rotifers. Most individuals recovered within 30 min, but >20% of the rotifers remained immobilized even after 100 min. The effect of a sudden reduction in temperature from 20 to 8 °C was less pronounced, and most of the rotifers recovered within few minutes.

Results presented in Fig. 1 revealed that the percent of mobile rotifers varied with time after perturbation. We therefore defined observations made after 4 and 60 min as short-term and long-term immobilization, respectively, and these are shown in the following figures. The percent of mobile rotifers dropped slightly after transferring them to 8 °C, but the animals recovered mobility within 4 min. The swimming pattern of the rotifers indicated, however, inhibition of locomotion, and a major fraction of the rotifers resided on, or close to, the bottom of the petridish.

The short-term effect of changing salinity while keeping the temperature constant at 20 °C was more pronounced than for temperature (Fig. 2). Both transfer to higher and lower salinities resulted in a reduced percent of mobile rotifers, but only a small fraction of rotifers became immobilized after transfer to higher salinity. Transfer to

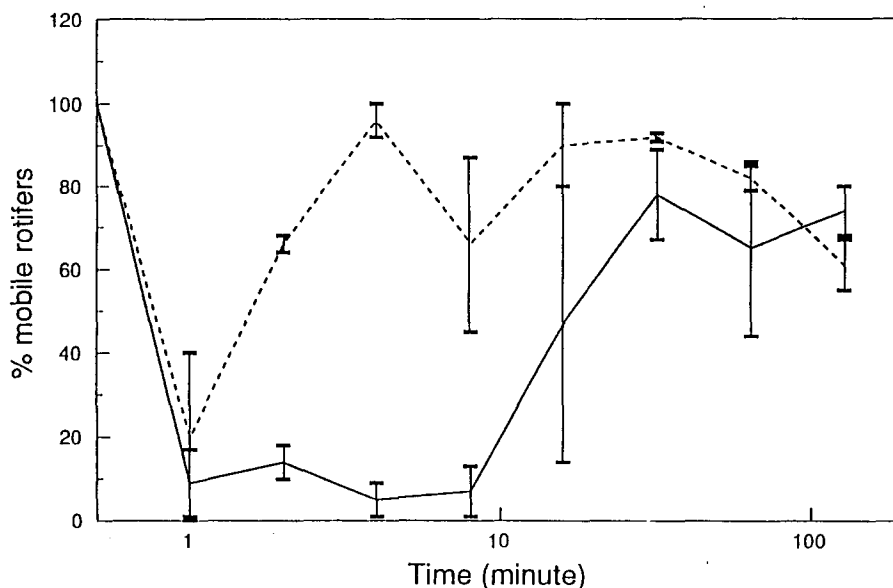


Fig. 1. Fraction of mobile rotifers after transfer from culture conditions (20‰, 20 °C) to low temperature (8 °C, 20‰, dashed line) or low salinity (5‰, 20 °C, solid line).

5 and 10‰ gave pronounced and immediate reductions in mobility, and a high fraction of rotifers remained immobilized even after 60 min (Fig. 2B).

The handling stress during transfer did not influence the mobility of the rotifers. Controls which were taken from and transferred to cultivation conditions (indicated by arrows, Fig. 2) exhibited no reduction in mobility.

The effect of changing temperature and salinity at the same time is also illustrated in Fig. 2. Short-term immobilization (Fig. 2A) was most pronounced when the temperature was increased to 28 °C along with a reduction in salinity, and all the rotifers became immobilized upon transfer to 5 and 10‰ salinity. The rotifers were more resistant to reduced salinities at 8 °C and 15 °C than if the temperature was kept constant or increased. Sudden increases in salinity had only a minor effect on rotifer mobility, and it was independent of the temperature and was stable with time.

The long-term immobilization effect (Fig. 2B) after perturbation in temperature and salinity was far more moderate than the short-term effect. The fraction of mobile rotifers remained low for

> 60 min after transfer to low salinity and high temperature (28 °C). Further observation indicated that this treatment resulted in high mortality.

The swimming pattern of the rotifers was irregular after transfer to low salinity, and the mobile fraction given in Fig. 2 was higher than the fraction of well performing, swimming rotifers. The rotifers frequently attached their foot to the walls of the glass beaker, and performed irregular rotations. Other individuals swam in circles while rotating in an irregular manner.

The results suggested a synergetic effect of temperature- and salinity perturbations on rotifer locomotion. The numbers given in Table 1 expresses the additive synergetic effect 60 min after exposure (SYN) (Miracle & Serra, 1989), and were estimated as

$$\text{SYN} = (I_{s+t} - I_o) - (I_t - I_o) - (I_s - I_o).$$

I_{s+t} is the fraction of immobilized rotifers after sudden perturbation in both temperature (from 20 to t °C) and salinity (from 20 to s ‰), I_o is immobilized fraction in controls, I_s is the immobilized fraction after a change in salinity from 20 to s ‰ while the temperature is kept constant, and

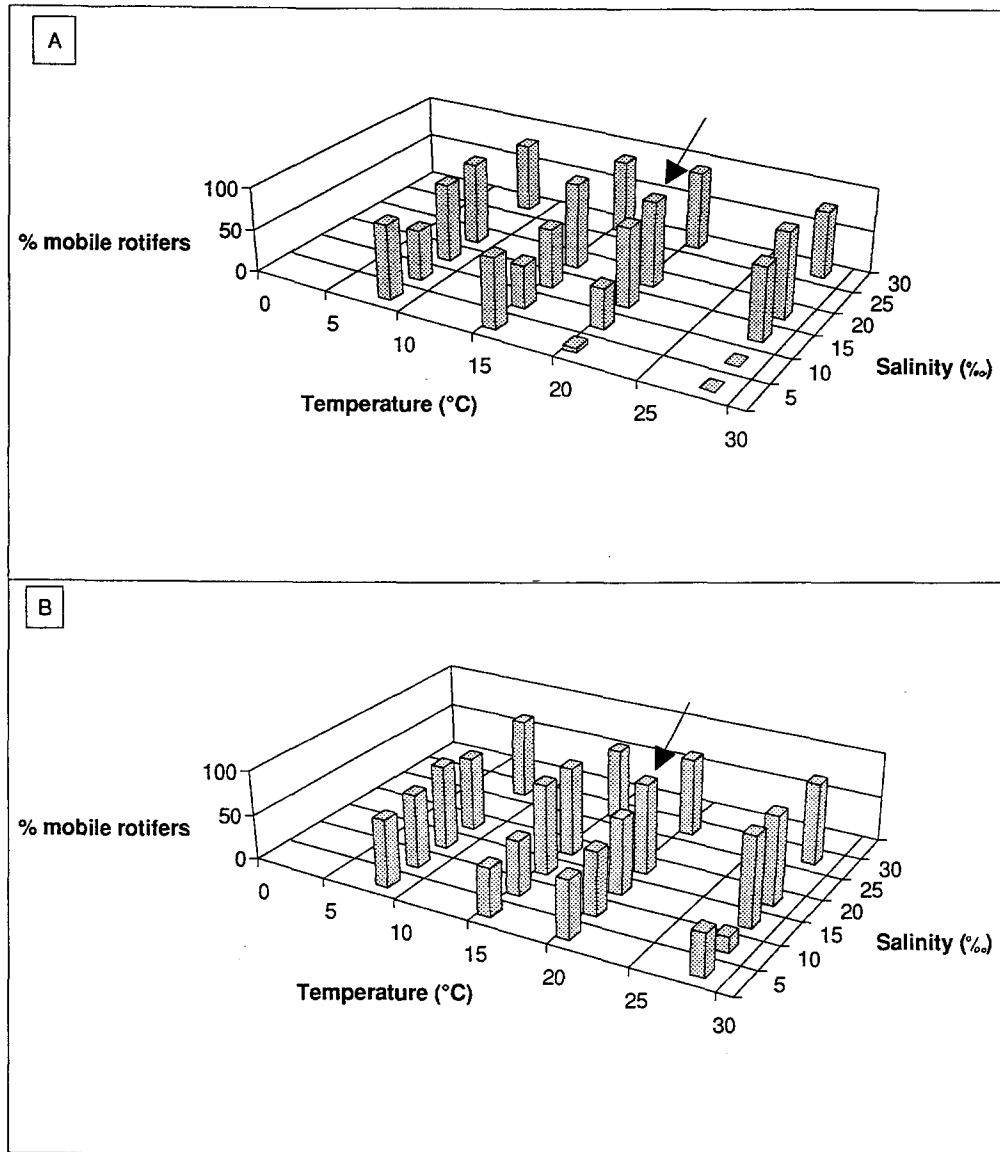


Fig. 2. Fraction of mobile rotifers (%) after rapid transfer to different salinities and temperatures. The arrow indicate cultivation condition. A: short-term effect (4 min) of perturbation and B: long-term effect (60 min).

I_{s+t} is the fraction after a change in temperature from 20 to t °C while the salinity is kept constant. Positive values of SYN indicates increased immobilization for combined changes compared to the additive effect of changing one by one factor. Negative values indicate that the additive effect is more pronounced than combined exposure.

The calculations suggested a positive synergetic effect of temperature and salinity when the salin-

ity was reduced below 15‰ while the temperature was increased to 28 °C. On the other hand, the synergetic effect of temperature and salinity was negative for transfer to both higher and lower salinities when rotifers were transferred to 8 °C. The effects obtained for 15 °C at various salinities were lower than for higher and lower temperatures.

Table 1. Synergetic effect of shifts in salinity and temperature on locomotion of *B. plicatilis* (see text). Rotifers were cultivated at 20 °C and a salinity of 20‰.

Temp. °C	Salinity ‰			
	5	10	15	30
28	+ 17	+ 53	- 17	- 5
15	+ 9	+ 8	- 18	+ 10
8	- 38	- 29	- 29	- 20

Discussion

The transitions in temperature and salinity to which rotifers were exposed to in the present study are within the range that the rotifer (*B. plicatilis*) is able to grow and reproduce. Its ability to reproduce at 5‰ is questionable (Lubzens *et al.*, 1985), but all temperatures are probably adequate (Olsen *et al.*, 1993).

Few reports are available on the effects of fluctuations in temperature and salinity on rotifer locomotion. It has been demonstrated that shifts in temperature affects the swimming speed of rotifers (Snell *et al.*, 1987), and that rapid changes in salinity may result in immobilization although gradually adapted rotifers may tolerate a wide range of salinities (1–97 g l⁻¹; Walker, 1981). It is also known that rotifers uses a high share (> 60%) of their metabolic energy for locomotion (Epp & Levis, 1980). This is probably because ciliate mediated locomotion is inefficient in animals as big as the rotifers.

It is generally known that changes in temperature and salinity will result in increased metabolism in aquatic animals, and protein metabolism plays an important part (Schmidt-Nielsen, 1985). Rapid changes in temperature can also be anticipated to give enhanced metabolism. The metabolism following a pronounced reduction in temperature may be lower than before the shift, but higher than the equilibrium metabolism at the given low temperature. Perturbations in temperature will probably also affect protein metabolism in the rotifers (Chochrane *et al.*, 1991).

These physiological effects of fluctuations in temperature and salinity on metabolism, together

with the fact that rotifers use a high share of their metabolic energy for locomotion, makes it likely that immobilization or other temporary changes in the swimming pattern may be a result of pronounced fluctuations in both temperature and salinity. Our data do not allow further speculations on the physiological mechanism of the synergetic effect.

The results suggested that rapid changes in salinity were more critical for the rotifers than changes in temperature, although these factors cannot be quantitatively compared. However, this finding is not surprising because fluctuations in salinity will have other effects on the rotifers than the metabolic effect discussed above. Pronounced reductions in salinity may result in cell damage and leakage of body fluids, and in turn in enhanced mortality. The ability of *B. plicatilis* to tolerate pronounced changes in salinity is related to its ability to tolerate considerable variation and perturbations in the concentration of its body fluids. This trait is quite common in brackish water animals (Potts & Parry, 1964), but the range of tolerance in *Brachionus plicatilis* is very wide (Epp & Winston, 1977).

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