

Effect of dimethyl sulfoxide (DMSO), sodium hydroxide (NaOH), acetone, and ethanol on the population growth, mictic female production, and body size of the rotifer *Brachionus plicatilis* Muller

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Dimethyl sulfoxide (DMSO), sodium hydroxide (NaOH), acetone, and ethanol which are commonly used as solvents for steroid and thyroid hormones were tested for their effect on rotifer population growth, mictic female production, and body size. Each chemical was tested at 0.2%, 0.4%, 0.6%, 0.8% and 1% of the 5-ml *Nannochloropsis oculata* suspension (7×10^6 cells/ml). Initially, 5 rotifers each carrying one amictic egg were exposed in these concentrations at 25°C in dark condition for 48 hours. Thereafter, rotifers were counted and transferred to a new culture medium without the chemical on day 2, 4, 6, and 8. Body size was measured on day 8.

DMSO at 0.2% and 0.4% significantly increased rotifer population growth while 0.4, 0.6, 0.8, and 1% caused an increase in mictic female production. NaOH at 0.4% and 0.8% significantly increased population growth while 0.8% caused an increase in mictic female production. Acetone at 0.8% and 1% caused a decrease in population growth but it had no effect on mictic female production. Ethanol caused a decrease in population growth in all tested concentrations but it did not affect mictic female production. Body size of DMSO-, NaOH-, acetone-, or ethanol-treated rotifers was not significantly different from that of the control. Acetone may be used as a solvent at concentrations lower than 0.8%, whereas DMSO and NaOH may be used as high as 1% without adverse effects. Ethanol caused adverse effects as low as 0.2% and is therefore not suitable as solvent in experiments with rotifer populations.

Keywords: Rotifera, *Brachionus plicatilis*, dimethyl sulfoxide, sodium hydroxide, acetone, ethanol, hormone solvents, population growth, mixis, body size

Introduction

We were interested in testing the effect of peptide, steroid and thyroid hormones on the population growth, mictic female production, and body size of the rotifer *Brachionus plicatilis*. Prior to conducting such experiments, it was necessary to determine the toxicity of hormone solvents on rotifers. Steroid hormones are not water soluble, thus solvents must be used. Dimethyl sulfoxide (DMSO) is a solvent which is often used for drugs in animal studies.^{1,2)} For rotifer research using prostaglandins DMSO was used as solvent.³⁾ Sodium hydroxide (NaOH) is used as solvent for triiodothyronine.⁴⁾ Generally, ethanol is for used for many hormones such as ecdysone and estradiol. However, Hagiwara et al.⁵⁾ have found that ethanol strongly inhibits sexual reproduction of the rotifer *Brachionus plicatilis*, thus, acetone was tested as a possible alternative.

In toxicity studies, the LC₅₀ is commonly used as a test criterion, but in our experiments population growth and mictic female production was monitored every 2 days for 8 days.

Results of this experiment are useful not just for hormone experiments, but for other related experiments with rotifers such as in ecotoxicological research where enzyme substrates need to be dissolved by dimethyl sulfoxide (DMSO) for example.

Materials and Methods

DMSO, NaOH, acetone, or ethanol was added to a 5-ml *Nannochloropsis oculata* suspension (7×10^6 cells/ml) in screw-capped bottles at 0.2, 0.4, 0.6, 0.8, and 1%. Five amictic females of *B. plicatilis* (NH3L strain) bearing one egg each were placed in 5 ml *N. oculata* suspension in 22 ppt

filtered and autoclaved seawater with the chemical at the designated concentration. Three replicates were prepared for each concentration and a control lacking the chemical. In the NaOH treatments, pH was measured immediately after the addition of NaOH and after 48 hours.

After 48 hours at 25°C in darkness, rotifers were transferred to a fresh culture medium lacking solvent with an *N. oculata* suspension of 7×10^6 cells/ml. During transfer, rotifers were counted and classified as: 1) non-ovigerous females, 2) ovigerous amictic females, and 3) ovigerous mictic females. This procedure was carried out every 2 days until day 8.

Upon termination of the experiment on day 8, lorica length and width of 10 egg-bearing females from each replicate ($n=30$ for each treatment) were measured using a compound microscope at $100\times$ magnification with ocular micrometer. For easy measurement, rotifers were fixed with 6.5% HCl which had no effect on body size.⁶⁾

Data on population density was log transformed and the mictic level (%) square root transformed to perform an analysis of variance (ANOVA). A one-way ANOVA using Statview 4.1 was calculated to identify significant differences among treatment means on each day. A repeated-measures ANOVA was done to determine if the average effect of each treatment was stable over 8 days. Fisher's protected least significant difference (PLSD) was calculated to determine which treatment means were significantly different.

Results

Population growth was significantly higher in 0.2% and 0.4% DMSO treatments and 0.4% and 0.8% NaOH, but significantly lower than the control in 0.8% and 1% acetone. All ethanol concentrations significantly reduced rotifer population growth (Fig. 1). Comparing day 8 density of treated rotifers with that of the control (Fig. 2) showed that the population density in 0.2% and 0.4% DMSO is 1.9 and 1.6 times the control, respectively, whereas in 0.4% and 0.8% NaOH, population density was 3.5 and 3.2 times the control, respectively. In contrast, population density of rotifers in 0.8% and 1% acetone and in all tested concentrations of ethanol was less than half of the control.

Addition of NaOH to make solutions of 0.2, 0.4, 0.6, 0.8, and 1% yielded pH of 9.93, 10.29, 10.36, 10.42, 10.44, respectively, which were higher than control pH (7.72). After 48 hours, the pH in the 0.2–1% treatments fell to 8.93–10.14.

Figure 3 shows the mictic level (based on the area of mictic females over the total ovigerous females) of rotifers in each chemical treatment. Mictic female production was significantly higher in 0.4, 0.6, 0.8 and 1% DMSO, but significantly lower in 0.6% ethanol. A one-way ANOVA on ethanol showed that at day 6, mictic female production was significantly lower ($P < 0.01$) at 0.4, 0.6, 0.8 and 1% ethanol, but a repeated measures ANOVA showed no significant difference. This means that the average effect of ethanol on mictic female production was not consistent over 8 days. The concentrations of acetone tested did not affect mictic female production. DMSO, NaOH, acetone, and ethanol may have caused small decreases in body size, but the difference was not statistically significant (Fig. 4).

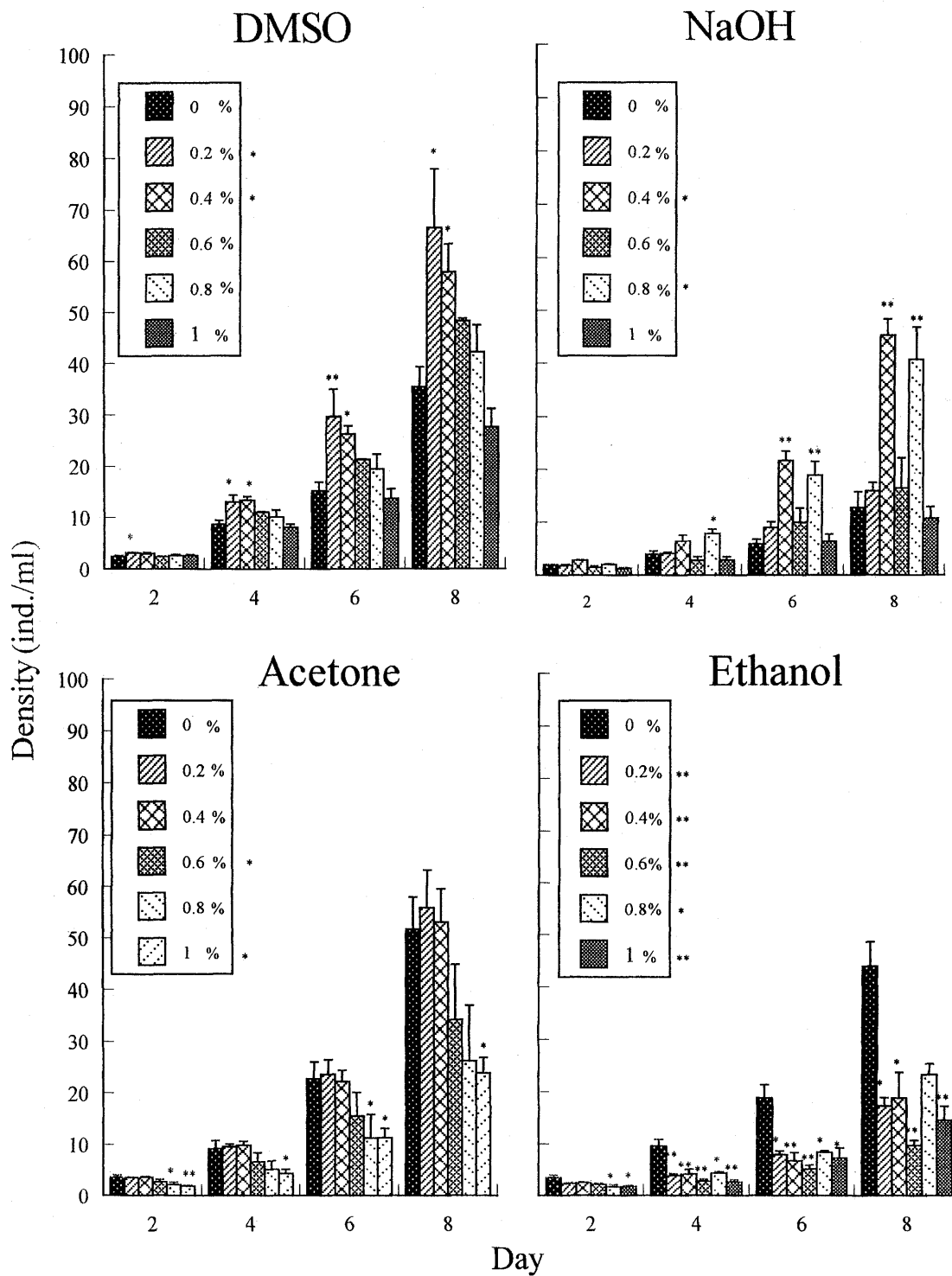


Fig. 1. Population growth of *B. plicatilis* treated with dimethyl sulfoxide (DMSO), sodium hydroxide (NaOH), acetone, and ethanol. Significant level: * P < 0.05, ** P < 0.01.

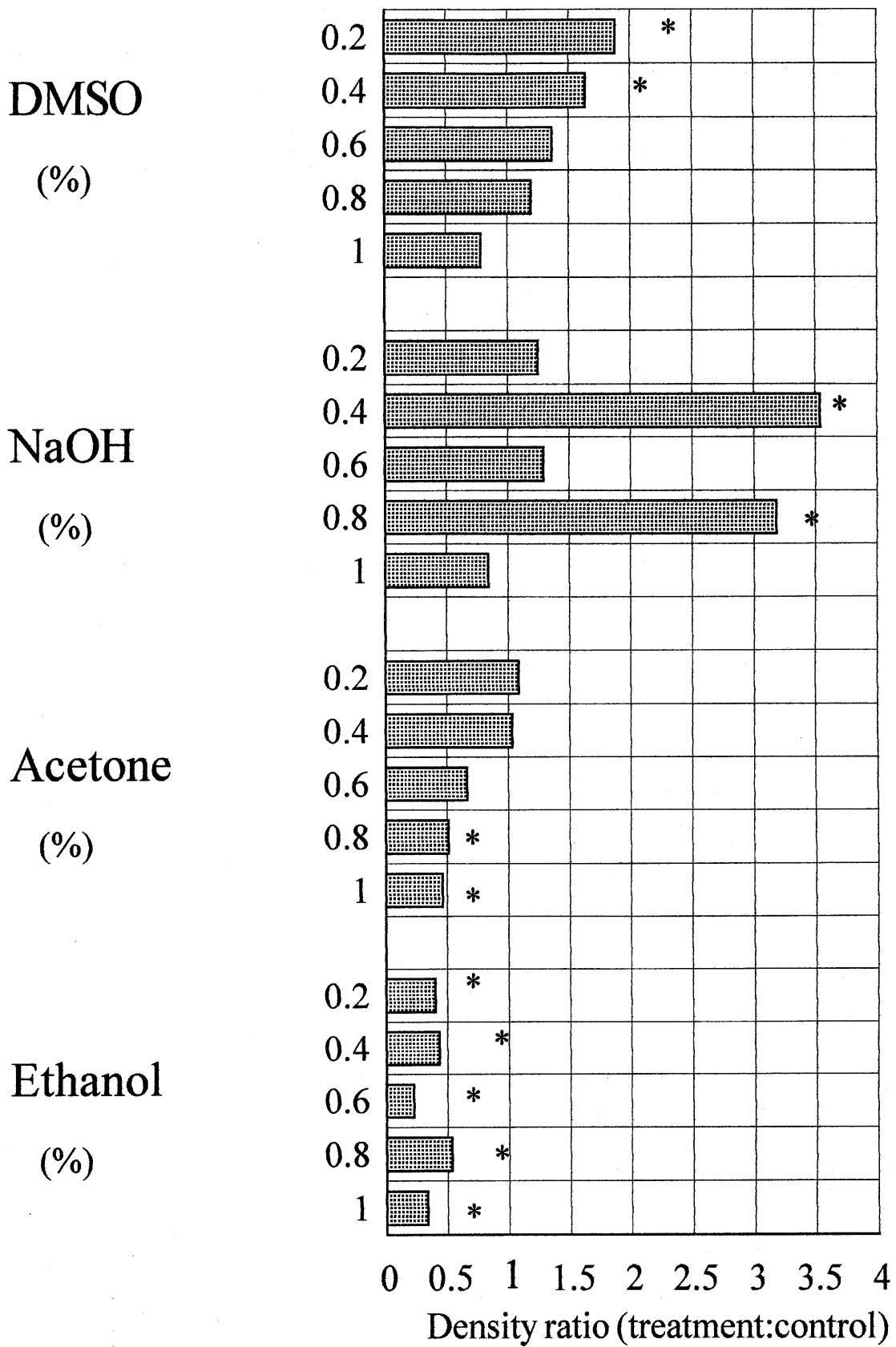


Fig. 2. Comparison of the final (day 8) density of rotifers in each concentration of chemical and the control (value 1). Significant level: * P<0.05.

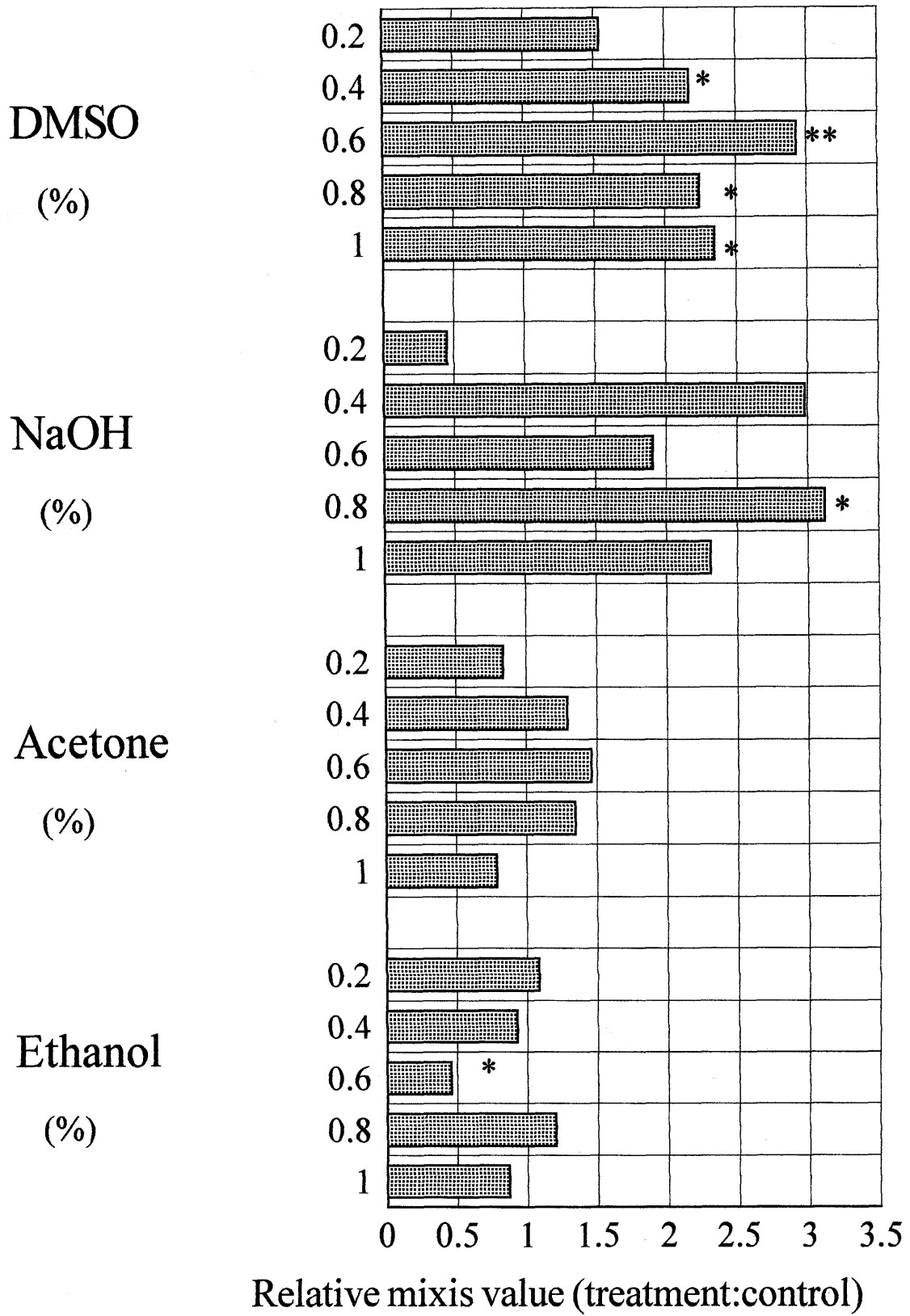


Fig. 3. Comparison of the mictic level in each concentration of chemical and the control (value: 1).
 Significant level: * P < 0.05, ** P < 0.01.

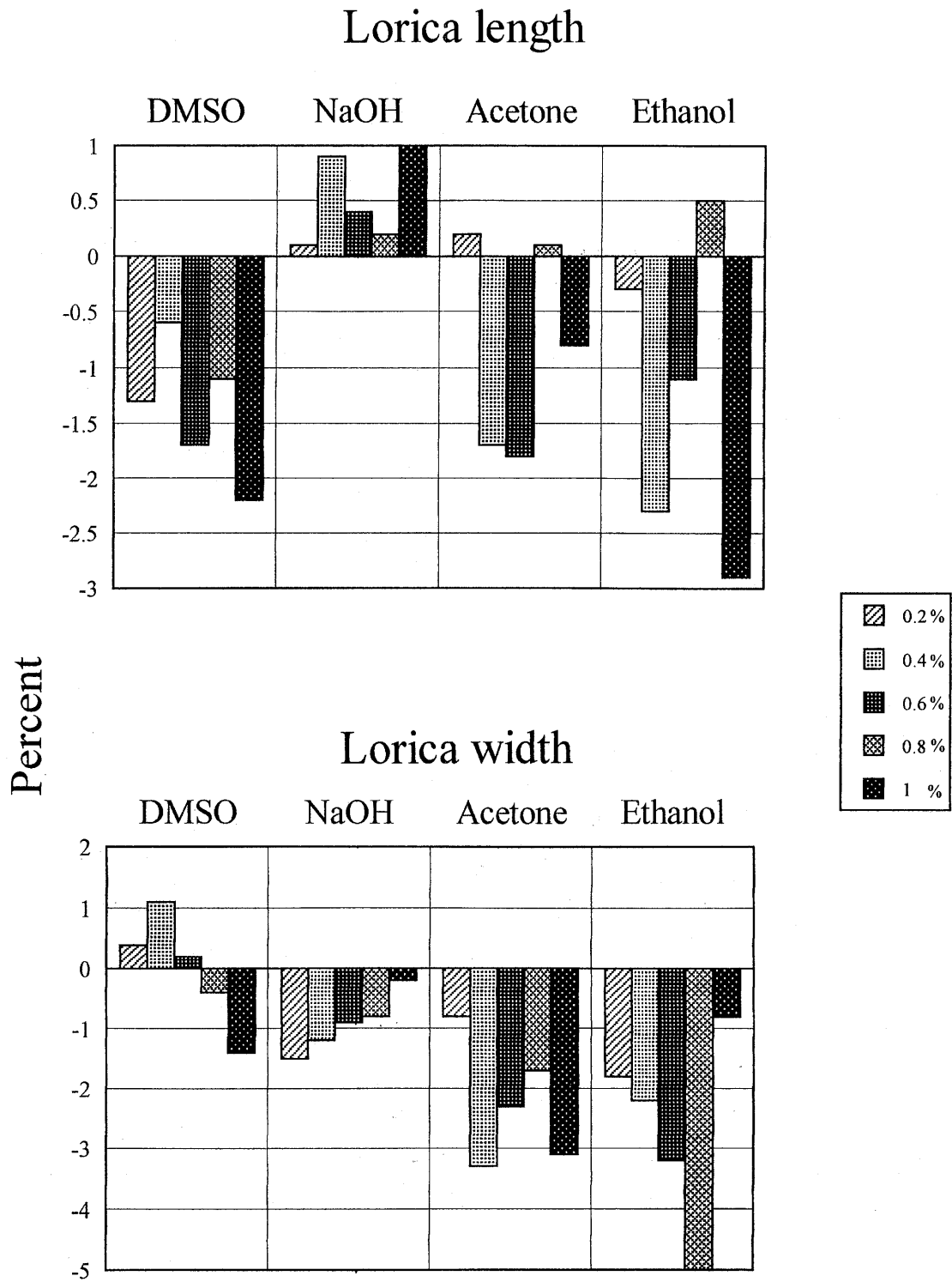


Fig. 4. Percent increase or decrease in body size of *B. plicatilis* treated with dimethyl sulfoxide (DMSO), sodium hydroxide (NaOH), acetone, and ethanol.

Discussion

DMSO

The results indicate that DMSO caused the increase in rotifer population growth and mictic female production. In general, mictic female production is active during the exponential growth phase.⁷⁻⁹⁾ But higher mictic female production suppresses rotifer population growth rate because of the less number of amictic females, thus at 0.6-1% DMSO the population density was low although mictic female production was high.

DMSO is a cryoprotective agent for organisms during freezing;¹⁰⁾ particularly it has been used as a cryoprotectant for freezing rotifer embryos.^{11,12)} Beddig et al.¹¹⁾ also tested the tolerance of amictic females to DMSO at 0.84, 1.27, 1.69, and 2.53 M. At 1.27 M and above, the adults were dead after 24 h. These concentrations are much higher than the concentrations we tested in our experiments (0.002-0.01 M).

DMSO is also used as a solvent for the enzyme substrates in ecotoxicological studies using the freshwater rotifer *Brachionus calyciflorus*.¹³⁾ At final concentrations ranging from 2.5 (M to 17.2 (M, there was an adverse effect on the survival of *B. calyciflorus*. In our experiments with the marine rotifer *B. plicatilis*, DMSO even at 1% (0.01 M) had no significant effect on population growth. This also implies that *B. plicatilis* may be used as a test animal in ecotoxicological studies of marine environments.

NaOH

NaOH has been used as a solvent for triiodothyronine in fish experiments⁴⁾ without positive or negative effect, but in our experiments, 0.4% and 0.8% enhanced rotifer population growth. Since population growth was not affected even when pH was as high as 10.44 at 1% NaOH, this implies that *B. plicatilis* is tolerant of high pH.

Ethanol and Acetone

Ethanol is also a commonly used solvent in toxicity testing. Ethanol toxicity has been demonstrated by Takahashi et al.¹⁴⁾ to the zooplankters *Daphnia magna* and *Ceriodaphnia dubia*. *C. dubia* is 1.9 times more sensitive to ethanol than *D. magna* at 20°C and 2.4 times more sensitive to ethanol than *D. magna* at 24°C. It was therefore recommended that <0.5 mL/L ethanol be used for *D. magna* static acute test, but ethanol is not suitable for *C. dubia*. The effects of ethanol on mating adhesion in *Chlamydomonas* have been investigated by Forest.¹⁵⁾ Low concentrations (from 0.095 to 0.95%) caused an increase in adhesion after 10 minutes, with adhesion peaking at 0.58% ethanol. Above these concentrations, inhibition of adhesion was observed, with greater inhibition caused by either increasing the ethanol concentration or the

length of time gametes were incubated in ethanol. In our experiments, we found that even at 0.2% ethanol, rotifer population growth and mictic female production were adversely affected.

The difference in toxicity of ethanol and acetone to rotifer may be attributed to the difference in the conditions in which they are produced by microbial metabolism. Acetone is produced under aerobic conditions while ethanol is produced under anaerobic conditions.¹⁶⁾ Since rotifers live and are cultured under aerobic conditions, the presence of acetone in their culture medium is natural for rotifers, but ethanol is not. In polluted waters where conditions are often anaerobic, ethanol production could be high enough to cause toxicity. Recently, it has been found that acetone is produced by a marine *Vibrio* species.¹⁷⁾

In conclusion, acetone is more suitable as a hormone solvent than ethanol at concentrations lower than 0.8% whereas DMSO and NaOH are useful as high as 1% without adverse effects on *B. plicatilis* in seawater.

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シオミズツボウムシの増殖, 両性生殖誘導及びサイズに与える DMSO, NaOH, アセトン, エタノール添加の影響

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不溶性のステロイドホルモンや重金属化合物等の溶剤として用いられる, ジメチルスルホキシド, 水酸化ナトリウム, アセトン, エチルアルコール (ワムシ培養への添加濃度は各々0.2, 0.6, 0.8, 1.0%) が, ワムシの生殖特性とサイズにどのような影響を与えるか検討した。0.2%および0.4% DMSO の添加によってワムシの増殖は促進され, 一方0.4%以上の濃度では両性生殖誘導率が高くなった。NaOH を0.4, 0.8%添加するとワムシ増殖率は高くなった。エタノールの添加はいずれの濃度でもワムシの増殖を阻害したが, 0.6%以下のアセトン添加ではワムシの生殖特性に対する影響はみられなかった。いずれの溶剤もワムシのサイズに影響を与えることはなかった。以上の結果はワムシのホルモン作用や毒性評価試験の生物材料としてワムシを使用する場合の基礎知見となるものである。