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# Effect of rapid changes in temperature and salinity on availability of the rotifers *Brachionus rotundiformis* and *Brachionus plicatilis*

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**Abstract**

Euryhaline rotifers are an important food for rearing marine fish larvae. Their availability to fish larvae in the water column may be reduced if they are transferred to fish larval rearing tanks with different temperatures and salinities. The rotifers, *Brachionus rotundiformis* (formerly called S-type) and *Brachionus plicatilis* (formerly called L-type), were mass-cultured at 23°C and 35‰ salinity, and then abruptly transfered into tanks at different temperatures (18°C, 23°C, 28°C) and salinities (10‰, 15‰, 20‰, 25‰, 30‰, 35‰). Their availability in the water column was then measured in non-turbulent beakers over time. Both species showed initial transfer shock. The number of rotifers 1 h after stocking was approximately 50% of that potentially available, but increased to approximately 75% after 6 h. Availability was reduced when rotifers were transferred from 23°C to 18°C, but was not affected by transfer from 23°C to 28°C and remained relatively constant over time. *B. rotundiformis* was slightly more tolerant at higher temperatures than *B. plicatilis*. Salinity had a greater effect on availability than temperature. Availability of rotifers decreased as salinity was reduced. The availability of rotifers increased over time indicating that some rotifers had acclimated to the transfer conditions. *B. plicatilis* was slightly more tolerant at lower salinity than *B. rotundiformis*. Rotifers should be cultured at lower temperatures and similar salinities to the fish larval rearing tanks or acclimated for at least 6 h to larval rearing conditions before transfer. 

*Keywords:* Rotifers; Shock; Availability; Salinity; Temperature

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## 1. Introduction

Euryhaline rotifers, *Brachionus* spp., are currently essential for intensive culture of marine larval finfish in many hatcheries throughout the world (Kafuku and Ikenoue, 1983; Lubzens, 1987). Rotifers are ideal as a first exogenous food source due to their small size, slow swimming speed and ability to stay suspended in the water column. They are also relatively easy to culture at high densities and can be enriched with fatty acids and antibiotics (Lubzens et al., 1989).

Two different species of rotifer, the small *B. rotundiformis* (formerly called S-type; Segers, 1995) and large *B. plicatilis* (formerly called L-type), are commonly cultured. This allows an optimum size of prey to be offered for selection by different larval stages (Fukusho, 1983; Korunuma and Fukusho, 1987) and thus improve hatchery production (Snell and Carillo, 1984).

A large volume of research has been conducted on the use of rotifers in aquaculture (for reviews see Lubzens, 1987; Lubzens et al., 1989). It is known that temperature and salinity have variable effects on productivity of different strains (Miracle and Serra, 1989); thus, Japanese *B. rotundiformis* are most productive at high temperatures ($>30$°C) while *B. plicatilis* are most productive at lower temperatures ($<25$°C) (Fukusho, 1983; Fukusho and Iwamoto, 1980). Different optima have also been reported for strains originating outside of Japan (Lubzens et al., 1989).

Optimal reproduction at the usual culture temperature of 25°C occurs at any salinity within the range 4–35‰ (Komis, 1992); however, rotifers are generally cultured at salinities between 10‰ and 20‰ (Fulks and Main, 1991). Rotifers are often fed to larval fish cultivated at temperatures and salinities different from the rotifer cultures (Blaxter, 1988; Lubzens et al., 1989). This change in conditions can affect mobility and availability of rotifers (Gatesoupe and Luquet, 1981; Lubzens, 1987; Øie and Olsen, 1993) although no information is available to compare differences between *B. rotundiformis* and *B. plicatilis*. Øie and Olsen (1993) recommend that practical culture conditions for rotifers should be a compromise between their optimal production conditions and the degree of immobilisation that they experience when transferred to larval fish tanks. The effects of transfer shock and sudden changes in temperature on the ability of rotifers to swim in the horizontal plane were assessed by Øie and Olsen (1993). However, quantification of rotifer availability in the water column after they have been harvested from cultures and supplied to larval fish rearing tanks has rarely been assessed.

The density of rotifers in the water column has a significant effect on the feeding success of marine fish larvae by influencing the probability of encounter (Hunter, 1980). It is therefore essential to quantify the number of rotifers effectively available in the water column of the larval-rearing tanks. Insufficient rotifer density decreases larval survival and growth because the energetic requirements of the larvae are not satisfied (Dowd and Houde, 1980; Tandler and Sherman, 1981). An excessive rotifer density can also decrease larval survival and growth by promoting excessive ingestion of rotifers, hence decreased gut retention time and a subsequent reduction in assimilation efficiency (Boehlert and Yoklavich, 1984; Tandler and Mason, 1984). Overfeeding can also lead to

accumulation of nutritionally inadequate rotifers, and can cause decreased survival and growth of fish larvae (Lubzens et al., 1989).

The effect of initial transfer shock on rotifer availability has major implications for feeding larval fish, in particular first feeding stages, which are usually reared with minimal water turbulence to improve prey capture and increase swim bladder inflation (Battaglene, 1995). Rotifers that have been starved for 6 h at high temperatures ($> 20$°C) have lost most of their nutritional value (Hoff and Snell, 1989; Olsen et al., 1989); therefore, rotifers that recover from the transfer and become available to the fish larvae in clearwater culture conditions may be nutritionally deficient.

The aims of our study were: (1) to determine whether rotifer availability changes with temperature and/or salinity following harvest shock and stocking, (2) to determine whether availability of rotifers is constant over time after stocking, and (3) to assess whether availability of *B. rotundiformis* and *B. plicatilis* is similar. The temperatures and salinities chosen for the study are those commonly experienced by rotifers in their transfer from culture tanks to larval marine fish rearing tanks in Australia (Battaglene, 1995).

## 2. Materials and methods

### 2.1. General

Two species of rotifer were used in this study: a strain of *B. plicatilis* (mean length 260 μm; $n = 100$) originating in Hawaii. This strain has been cultured at the Port Stephens Research Centre since 1979 (Battaglene and Talbot, 1989). The other was a strain of *B. rotundiformis* (mean length 135 μm; $n = 100$) originating from Thailand and obtained from Prof. A. Hagiwara, Nagasaki University in 1994.

Both species of rotifer were mass-cultured in flat-bottomed, polyethylene tanks of 1100-l capacity. Aerated seawater of $35 \pm 1$‰, $23 \pm 1$°C and pH range 7.4 to 8.2 was used. Rotifers were cultured using the method of Battaglene and Talbot (1989). Rotifers were fed by draining 20% of the tank volume each day through a 53 μm sieve and then refilling the tank with a log phase microalga culture of either Tahitian *Isochrysis* aff. *galbana* (range $2.0–2.9 \times 10^6$ cells $ml^{-1}$), *Pavlova lutheri* (range $1.8–2.5 \times 10^6$ cells $ml^{-1}$) or *Tetraselmis chuii* (range $0.7–1.1 \times 10^6$ cells $ml^{-1}$). Rotifers removed during sieving were returned to the tank. Compressed baker's yeast, *Saccharomyces cerevisiae*, was fed once per day at 0.75 g $10^{-6}$ rotifers.

### 2.2. Stocking and sampling

The rotifers used for all experiments were harvested from the mass cultures and concentrated through a 53 μm sieve, rinsed with seawater of similar salinity and temperature to prevent transfer shock, and held in a lightly aerated 50-ml measuring

cylinder. The rotifers were always immersed in seawater during sieving. Clear plastic 150-ml beakers were used for all experiments. The volume of concentrate required to provide 25 rotifers $ml^{-1}$ in experimental beakers was determined by a preliminary trial. For this, a 1-ml aliquot of concentrate was autopipetted into each of three beakers filled with 150 ml of seawater, which were then aerated to ensure the rotifers were well mixed. Three 1-ml samples were then taken from each beaker and the rotifers counted under a dissecting microscope. The average number was calculated and the volume of concentrate adjusted to supply approximately 25 rotifers $ml^{-1}$. This volume ranged from 0.43 ml for Experiment 4 to 1.5 ml for Experiment 1.

Rotifer concentrates were then added to each experimental beaker with an autopipette. It took approximately 5 min to stock all of the beakers. Microalgae and aeration were not provided to the beakers in order to maintain rotifer concentration and to emulate conditions of low turbulence commonly experienced in larval fish tanks. Fluorescent light was provided at 400 lx.

After 1 h, three replicate 1 ml samples were taken from each beaker in order of stocking, using 1-ml glass pipettes. The pipette was positioned approximately 10 mm below the water surface near the centre of the beaker and the sample withdrawn. The pipette was then sealed with a plug of plasticine. The sampling was completed in 10 min. The number of live rotifers in each 1-ml pipette was then counted and availability was defined as the number of rotifers in each milliliter. Samples were not returned to the beakers. Further samples were taken at 2, 3, 4, 5 and 6 h after stocking using fresh pipettes. Because the contents of the experimental beakers were not mixed during the experiments, an estimate of the initial stocking density of rotifers was made after the final 6-h sampling. Each beaker was lightly aerated and three 1-ml samples were taken. No mortality of rotifers was observed in any of the experimental treatments.

### 2.3. Effect of rapid temperature change on availability

Experiments determined the effect on their availability over a 6 h period of rapidly transferring *B. rotundiformis* (Experiment 1) and *B. plicatilis* (Experiment 2) from

Table 1
Mean number of available rotifers, for control treatments (23°C, 35‰) in experiments 1–4 giving the number potentially available and those sampled 1 and 6 h after stocking into non-turbulent beakers

| Experiment number | Rotifer (*Brachionus* species) | Potentially available number of rotifers $ml^{-1}$ [a] | Mean rotifers $ml^{-1}$ after 6 h (%)[b] | Mean rotifers $ml^{-1}$ after 6 h (%)[b] |
|---|---|---|---|---|
| 1 | *B. rotundiformis* | $25.1 \pm 0.6$ | $61.7 \pm 4.9$ | $89.5 \pm 9.6$ |
| 2 | *B. plicatilis* | $26.2 \pm 0.6$ | $57.0 \pm 2.3$ | $63.9 \pm 3.5$ |
| 3 | *B. rotundiformis* | $27.6 \pm 0.5$ | $36.4 \pm 4.9$ | $61.7 \pm 9.5$ |
| 4 | *B. plicatilis* | $26.1 \pm 0.6$ | $45.3 \pm 1.9$ | $87.3 \pm 11.1$ |

[a] Values are means ± standard error of three replicate beakers.
[b] Values are percent availability of potentially available number. Data for Experiments 1 and 2 are means ± se of eight replicate beakers. Data for Experiments 3 and 4 are means ± se of four replicate beakers.

ambient culture conditions (35‰; 23°C) to 18°C, 23°C (control) or 28°C (and 35‰). Twenty-four beakers were filled with 150 ml of seawater (35‰). Four beakers were placed in each of six freshwater baths maintained at 18 ± 0.5°C, 23 ± 0.5°C or 28 ± 0.5°C (two baths at each temperature). The initial stocking density of rotifers did not differ significantly between treatments for both Experiment 1 (ANOVA, $F = 0.39$, 2,48 *df*, $P > 0.1$) or Experiment 2 (ANOVA, $F = 0.40$, 2,48 *df*, $P > 0.1$) (Table1).

### 2.4. Effect of rapid salinity change on availability

These experiments determined the effect on their availability over a 6-h period of rapidly transferring *B. rotundiformis* (Experiment 3) and *B. plicatilis* (Experiment 4) from ambient culture conditions to one of six different salinities, 10‰, 15‰, 20‰, 25‰, 30‰ or 35‰ (control). Four replicate experimental beakers with 150 ml of solution were used for each salinity for each experiment. Solutions were made by diluting seawater with rainwater and were measured to the nearest 0.1‰ (Horiba model U-10, Horiba, Kyoto, Japan). The pH of each solution for Experiment 3 was 7.40, 7.46, 7.50, 7.60, 7.85 and 8.02 for 10‰, 15‰, 20‰, 25‰, 30‰ and 35‰ treatments, respectively. For Experiment 4, the pH of each solution was 7.67, 7.69, 7.76, 7.89, 7.90 and 8.12 for 10‰, 15‰, 20‰, 25‰, 30‰ and 35‰ treatments, respectively. The

Table 2
ANOVA of number of available *Brachionus rotundiformis* in the water column 1 to 6 h after rapid transfer from 23°C to 18°C, 23°C and 28°C ($n = 8$ beakers per treatment)

| Source of variation | *d.f* | MS | *F* ratio | *P* |
|---|---|---|---|---|
| (a) ANOVA[1] | | | | |
| Time (T) | 5 | 318.5 | 7.19 | < 0.001 |
| Temperature (TT) | 2 | 2719.9 | 61.4 | < 0.001 |
| Beakers (T x TT) | 126 | 44.3 | 2.05 | < 0.001 |
| T x TT | 10 | 30.6 | 0.69 | NS |
| Residual | 288 | 21.6 | | |
| Total | 431 | | | |

(b) SNK for time[2]

Time (h)

1 2 5 4 3 6

(c) SNK for temperature treatments[2]

Temperature °C

18 28 23

[1]Time and temperature treatments were fixed, beakers random and nested in time and temperature treatment; $n = 3$ replicates; variances were homogeneous (Cochran's test was not significant, $C = 0.04$).

[2]Overall SNK tests for time and temperature treatments. Means ranked from smallest to largest with those underlined not significantly different at $P > 0.05$.

beakers were suspended in a water bath at 23°C. The initial stocking density of rotifers did not differ significantly between treatments for Experiment 3 (ANOVA, $F = 0.70$, 5,48 *df*, $P > 0.1$) or Experiment 4 (ANOVA, $F = 1.14$, 5,48 *df*, $P > 0.1$) (Table 1).

### 2.5. Statistical analyses

Data in Experiments 1 and 2 were analysed using a four-factor, mixed model ANOVA, with time, temperature and block treatments being orthogonal and fixed factors. The fourth factor, beaker, was used to determine the level of variation between beakers within each treatment and was random and nested within time, temperature and block treatments. There was no significant effect of blocks in Experiment 1 ($F = 2.82$, 1,288 *df*, $P > 0.05$) and Experiment 2 ($F = 0.05$, 1,288 *df*, $P > 0.1$) and data were pooled ($n = 8$). Data were then analysed using a three-factor, mixed model ANOVA, with time and temperature treatments (Experiments 1 and 2) or time and salinity

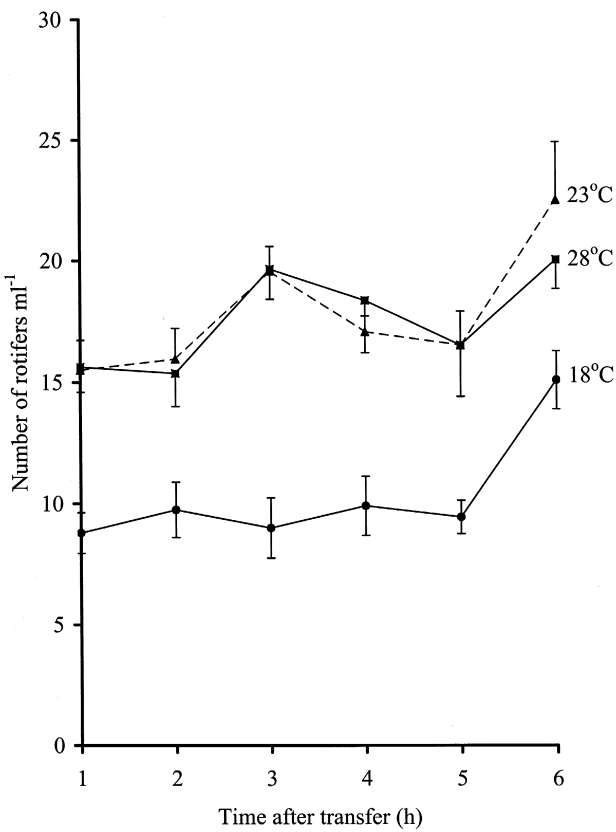


Fig. 1. Mean number of available *Brachionus rotundiformis* in the water column 1 to 6 h after rapid transfer from 23°C to 18°C, 23°C and 28°C. Data are means ± se ($n = 8$). Experiment 1.

treatments (Experiments 3 and 4) being orthogonal and fixed, and beakers were random and nested within treatments. Prior to analysis data in Experiments 3 and 4 were transformed by $(x+1)^{0.5}$ to satisfy the assumption of homogeneity of variance (Cochran's tests $[C]$ after transformation were not significant, $P > 0.05$). Where significant main effects or interaction terms occurred, means were compared by the Student–Newman–Keuls test (SNK).

Table 3
ANOVA of number of available *Brachionus plicatilis* in the water column 1 to 6 h after rapid transfer from 23°C to 18°C, 23°C and 28°C ($n = 8$ beakers per treatment)

| Source of variation | *d.f* | MS | *F* ratio | *P* |
|---|---|---|---|---|
| (a) ANOVA[1] | | | | |
| Time (T) | 5 | 121.4 | 3.6 | < 0.01 |
| Temperature (TT) | 2 | 1129.0 | 33.2 | < 0.001 |
| Beakers (T x TT) | 126 | 34.1 | 1.3 | < 0.05 |
| T x TT | 10 | 155.6 | 4.6 | < 0.001 |
| Residual | 288 | 25.6 | | |
| Total | 431 | | | |

(b) SNK test for time[2]

| Time (h) | Temperature °C | | |
|---|---|---|---|
| 1[a] | 28 | 18 | 23 |
| 2[b] | 18 | 28 | 23 |
| 3[b] | 18 | 23 | 28 |
| 4[a] | 18 | 28 | 23 |
| 5[a] | 28 | 18 | 23 |
| 6[c] | 18 | 28 | 23 |

(c) SNK test for temperature treatments[2]

| Temperature °C | Time (h) | | | | | |
|---|---|---|---|---|---|---|
| 18[a] | 3 | 4 | 2 | 1 | 6 | 5 |
| 23[a] | 1 | 2 | 3 | 6 | 5 | 4 |
| 28[b] | 1 | 2 | 5 | 6 | 4 | 3 |

[1] Time and temperature treatments were fixed, beakers random and nested in time and temperature treatment; $n = 3$ replicates; variances were homogeneous (Cochran's test was not significant, $C = 0.04$).

[2] Separate SNK tests for time and temperature treatments due to the significant interaction terms in the ANOVA. Means ranked from smallest to largest with those connected by a solid line not significantly different at $P > 0.05$.

[a] Data not transformed.

[b] Data transformed by $(x+1)^{0.5}$.

[c] Data transformed by $x^{0.5}$.

## 3. Results

For all experiments, the maximum number of available rotifers in the undisturbed suspensions was always less than the final mixed suspensions (Table 1) indicating that some rotifers experienced transfer shock and fell out of the water column when they were stocked.

### *3.1. Experiment 1: Effect of rapid temperature change on availability of B. rotundiformis*

There was a significant difference in mean number of available rotifers in the water column with time ($P < 0.001$, Table 2). Mean numbers of available rotifers in all treatments remained relatively constant and were not significantly different for the first 5

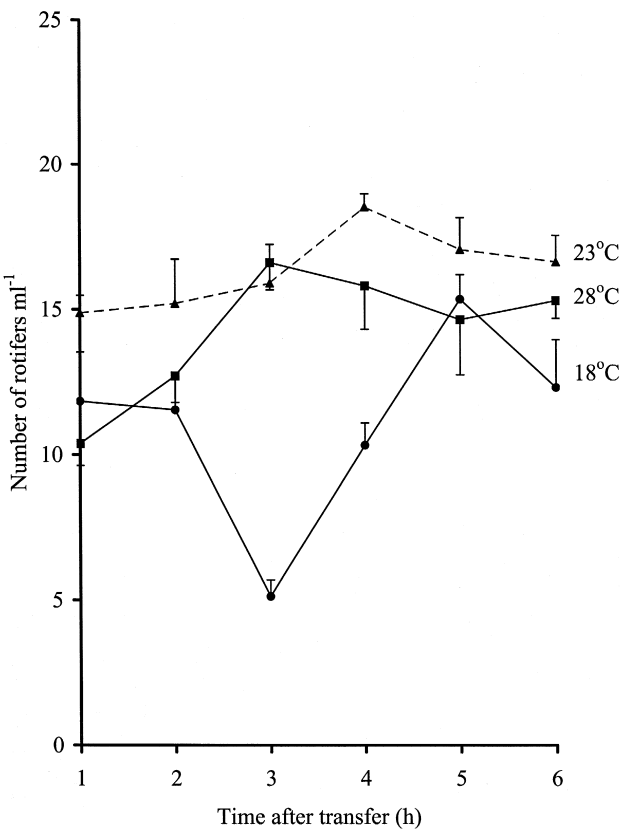


Fig. 2. Mean number of available *Brachionus plicatilis* in the water column 1 to 6 h after rapid transfer from 23°C to 18°C, 23°C and 28°C. Data are means ± se ($n = 8$). Experiment 2.

h after stocking ($P > 0.05$, Table 2, Fig. 1). However, there was a significant increase in the number of available rotifers after 6 h ($P < 0.01$, Table 2, Fig. 1).

The mean available rotifers differed significantly ($P < 0.001$, Table 2) as a result of temperature treatments. Rotifers transferred from 23°C to 18°C had significantly lower mean available numbers ($P < 0.01$, Table 2, Fig. 1) than those transferred to 23°C and 28°C, which did not differ.

### 3.2. Experiment 2: Effect of rapid temperature change on availability of B. plicatilis

There was a significant interaction between time and temperature treatment (identified as T × TT in Table 3), due to the variable and declining number of rotifers in the 18°C treatment. The available number of rotifers was generally greatest at 23°C and showed no significant increase over time ($P > 0.05$, Table 3, Fig. 2). The number of available rotifers in the 28°C treatment was initially lower than that at 23°C, but increased rapidly and after 6 h, the mean available rotifers in the 28°C treatment was not significantly different from the 23°C treatment ($P > 0.05$). The number of available rotifers in the 18°C treatment showed more variability with time and a significant

Table 4
ANOVA of number of available *Brachionus rotundiformis* in the water column 1 to 6 h after rapid transfer from 35‰ to 10‰, 15‰, 20‰, 25‰, 30‰ and 35‰ ($n = 4$ beakers per treatment)

| Source of variation | *d.f* | MS | *F* ratio | *P* |
|---|---|---|---|---|
| (a) ANOVA[1] | | | | |
| Time (T) | 5 | 17.5 | 18.6 | < 0.001 |
| Salinity (S) | 5 | 41.1 | 43.5 | < 0.001 |
| Beakers (T x S) | 108 | 0.9 | 2.2 | < 0.001 |
| T x S | 25 | 0.6 | 0.7 | NS |
| Residual | 288 | 0.4 | | |
| Total | 431 | | | |

(b) SNK for time[2]

| Time (h) | | | | | |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |

(c) SNK for salinity treatments[2]

| Salinity ‰ | | | | | |
|---|---|---|---|---|---|
| 10 | 15 | 20 | 25 | 35 | 30 |

[1]Time and salinity treatments were fixed, beakers random and nested in time and salinity treatment; $n = 3$ replicates; data were transformed to $(x + 1)^{0.5}$ to stabilise variances (Cochran's test after transformation were not significant, $C = 0.06$).

[2]Overall SNK tests for time and salinity treatments. Means ranked from smallest to largest with those underlined not significantly different at $P > 0.05$.

decline ($P < 0.05$, Table 2, Fig. 2) after 3 h from stocking. Generally, the number of available rotifers at 18°C was significantly lower ($P < 0.05$) than both 23°C and 28°C.

### 3.3. Experiment 3: Effect of rapid salinity change on availability of B. rotundiformis

There were significant differences in mean available rotifers in the water column with time ($P < 0.001$, Table 4). In general, availability increased with time at all salinities. The mean available rotifers 1 h after stocking was significantly lower ($P < 0.05$, Table 4, Fig. 3) than all other times. Salinity significantly affected the mean available rotifers ($P < 0.001$, Table 4). In general, availability of rotifers decreased as salinity was reduced. At 1 h after stocking the mean available rotifers increased as salinity was increased (Fig. 3). There was a similar tendency after 6 h; however, rotifer availability had increased in all salinities.

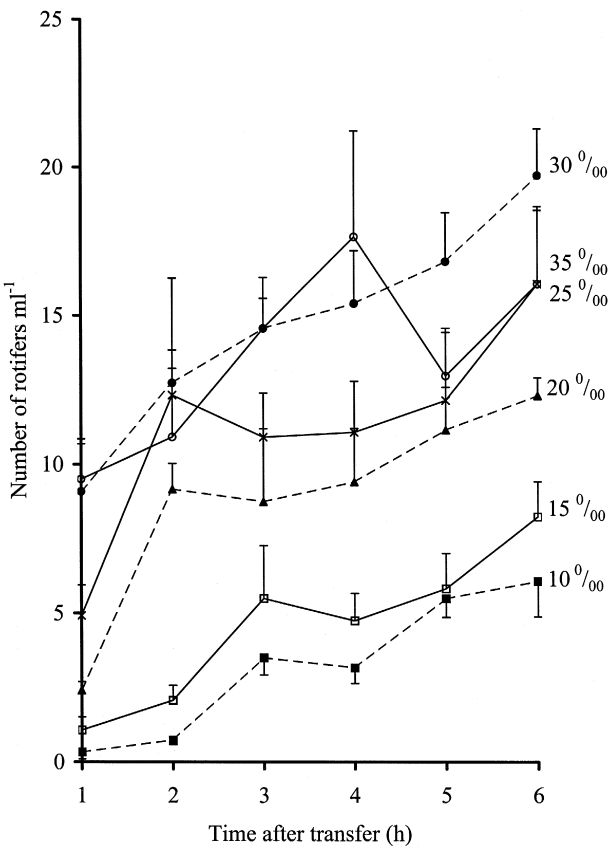


Fig. 3. Mean number of available *Brachionus rotundiformis* in the water column 1 to 6 h after rapid transfer from 35‰ to 10‰, 15‰, 20‰, 25‰, 30‰ and 35‰. Data are means ± se ($n = 4$). Experiment 3.

Table 5
ANOVA of number of available *Brachionus plicatilis* in the water column 1 to 6 h after rapid transfer from 35‰ to 10‰, 15‰, 20‰, 25‰, 30‰ and 35‰ ($n = 4$ beakers per treatment)

| Source of variation | *d.f* | MS | *F* ratio | *P* |
|---|---|---|---|---|
| (a) ANOVA[1] | | | | |
| Time (T) | 5 | 13.0 | 34.6 | < 0.001 |
| Salinity (S) | 5 | 81.1 | 215.4 | < 0.001 |
| Beakers (T x S) | 108 | 0.4 | 1.0 | NS |
| T x S | 25 | 0.6 | 1.6 | NS |
| Residual | 288 | 0.4 | | |
| Total | 431 | | | |

(b) SNK for time[2]

| Time (h) | | | | | |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 6 | 5 |

(c) SNK for salinity treatments[2]

| Salinity ‰ | | | | | |
|---|---|---|---|---|---|
| 10 | 15 | 20 | 25 | 30 | 35 |

[1]Time and salinity treatments were fixed, beakers random and nested in time and salinity treatment; $n = 3$ replicates; data were transformed to $(x + 1)^{0.5}$ to stabilise variances (Cochran's test after transformation were not significant, $C = 0.04$).

[2]Overall SNK tests for time and salinity treatments. Means ranked from smallest to largest with those underlined not significantly different at $P > 0.05$.

### *3.4. Experiment 4: Effect of rapid salinity change on availability of B. plicatilis*

Rotifer availability increased with time ($P < 0.001$, Table 5). The mean number of available rotifers at 1 h after stocking was significantly lower than all other times ($P < 0.001$, Table 5, Fig. 4).

Salinity significantly affected the mean available rotifers ($P < 0.001$, Table 5). Availability of rotifers decreased as salinity was reduced (Table 5, Fig. 4). After 1 h, the mean number of available rotifers was greater as salinity increased to 25‰ with no rotifers being sampled in the 10‰ treatment. The mean available rotifers, in all salinity treatments, increased in number at 6 h and followed a similar trend as in the first hour (Fig. 4).

## 4. Discussion

Rotifers are osmo-conformers and can tolerate salinities ranging from 1‰ to 97‰ (Epp and Winston, 1977; Walker, 1981), but rapid changes in salinity cause shock. Rotifers that have experienced shock sink to the bottom and/or adhere to the sides of

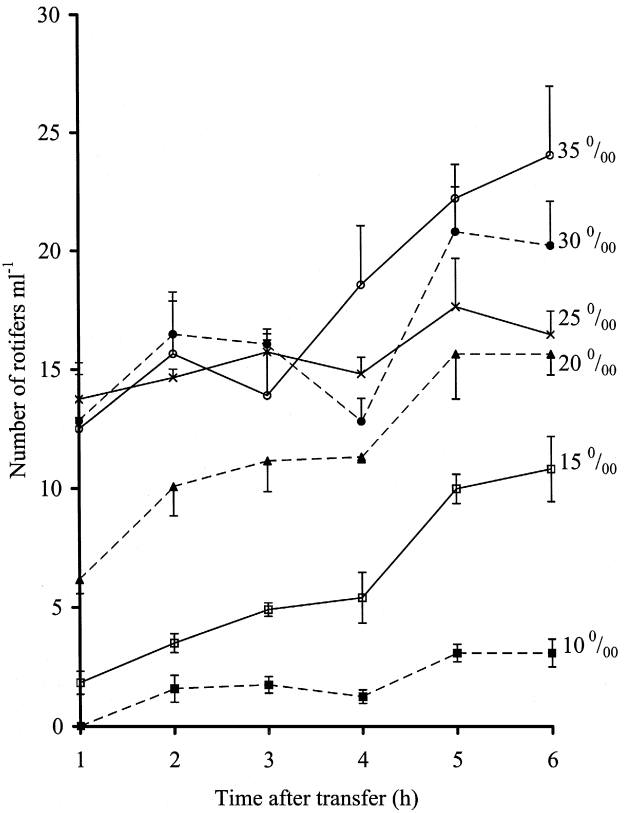


Fig. 4. Mean number of available *Brachionus plicatilis* in the water column 1 to 6 h after rapid transfer from 35‰ to 10‰, 15‰, 20‰, 25‰, 30‰ and 35‰. Data are means ± se ($n = 4$). Experiment 4.

the vessels with their sticky feet (Lubzens et al., 1989). Rapid changes in temperature and salinity also affect swimming activity and oxygen consumption of the rotifer, *B. plicatilis* (Epp and Winston, 1978; Snell et al., 1987; Øie and Olsen, 1993).

Generally, our study showed that swimming activity (= availability) of both species of rotifer increased with time regardless of temperature or salinity treatment, indicating that some rotifers experienced an initial transfer shock. The maximum number of rotifers initially available in the control beakers of each experiment ranged from 36.4% to 61.7% of the homogeneous density (Table 1). We assessed the availability or density of rotifers in the vertical plane of the water column and all sampled rotifers were swimming when counted. In contrast to our results, Øie and Olsen (1993) did not report any effect of transfer shock on mobility of rotifers; but, in their study, mobile or swimming rotifers were assessed in the horizontal plane in a 1-ml droplet on a Petri dish.

Our results suggest that approximately twice as many rotifers may have to be added to compensate for their absence in the water column due to transfer shock, regardless of

temperature and salinity differences. For example, after 6 h the maximum number of available rotifers had increased but did not attain that of the final homogeneous density and ranged from 61.7% to 89.5%.

Rapid changes in temperature affected the availability of rotifers in the water column and both species recovered slowly over time. The *B. rotundiformis* were more tolerant of transfer to increased temperature which follows their preference for higher production temperatures (Komis, 1992). Our results indicate that to maximise rotifer availability, both strains should be cultured at temperatures lower than the recipient larval fish tanks or acclimated for at least 6 h to larval rearing conditions before transfer.

Øie and Olsen (1993) found that a sudden reduction in temperature from 20°C to 8°C caused initial immobilisation of rotifers (SINTEF-strain, length 250 μm), but most recovered within 4 min. Snell et al. (1987) found that maximum swimming activity of the rotifer (Russian strain, see Snell and Carillo, 1984) was recorded at 25–30°C and declined with temperature decrease. Epp and Lewis (1984) showed that absolute swimming speed of six different *B. plicatilis* clones was greatest between 20°C and 32°C and declined at temperatures outside this range.

The effect of salinity on the availability of rotifers in the water column was greater than the effect of temperature. Generally, availability of rotifers decreased as salinity was reduced. The *B. plicatilis* were slightly more tolerant at lower salinity than the *B. rotundiformis* and availability was not affected by rapid transfer to salinities that were 10‰ lower than rotifer cultures. Availability of *B. rotundiformis*, however, decreased when they were rapidly transferred to salinities 5‰ lower than the rotifer culture salinity. The availability of rotifers increased over time indicating that the rotifers had begun to acclimate to the conditions.

Øie and Olsen (1993) found that sudden transfer of rotifers from ambient salinity of 20‰ to 5‰ resulted in their immediate immobilisation. The effect of salinity was also found to be more pronounced than temperature. Recovery from immobilisation was slower, but about 80% of the rotifers had regained mobility after 30 min. The effect of immobilisation was even more pronounced when the temperature was increased to 28°C in conjunction with a reduction in salinity. Transfer to low salinity caused irregular swimming patterns and rotifers often attached their feet to the walls of the beaker. In addition, Epp and Winston (1978) found that rotifer activity and oxygen consumption declined after sudden exposure to low salinity but increased after a period of acclimation.

Water quality parameters such as pH may have influenced availability of rotifers; however, it is most likely that the changes in availability were caused by the different salinities. Epp and Winston (1978) found that changes in pH from 6.5 to 8.5 had no effect on rotifer activity or metabolism. The pH of all solutions in our experiments was within this range.

Our study highlights some difficulties in working with rotifers in small beakers and in particular, separating treatment effects due to the high variability between replicate beakers from the same treatment. We were able to identify variation among beakers by using a nested design. However, it would have been better to reduce the variability by using more replicates for each interval. The limitation was the difficulty in counting the required number of rotifers within the time available before the next sampling. Simi-

larly, it would have been statistically more appropriate to use individual beakers for each estimate of rotifer availability (Underwood, 1981). We have made the assumption that samples taken from the same beaker over time are independent and although we appear to have detected a real response pattern, care should be exercised in interpreting the results.

Care should also be taken before extrapolating our results to the availability of rotifers in rearing tanks for fish larvae. Our experiments were conducted in small, non-turbulent beakers, and mobility and availability of rotifers may be different in larger tanks. The initial transfer shock may also be exacerbated in smaller beakers due to increased edge effects. A greater surface area to volume ratio may have encouraged a higher proportion of the rotifers to attach themselves to the beaker walls. Larval rearing tanks are generally aerated, albeit at low levels for early developmental stages, which may also alter rotifer availability.

Our results indicate that rotifer availability was influenced by, in order of importance, transfer shock, salinity and temperature. There were also differences between availability of *B. rotundiformis* and *B. plicatilis*. Clearly, the results suggest that it is important to monitor rotifer density in fish larvae tanks, particularly because the number of available rotifers in the water column increases after initial transfer. Rapid changes in salinity had a more pronounced effect on availability of rotifers than temperature. Differences in salinity between rotifer culture tanks and larval fish tanks should therefore be avoided or, if they do exist, the rotifers should be acclimated for 6 h at temperature and salinity similar to the larval fish tank. Food must be provided to the rotifers during the acclimation period.

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## References

Battaglene, S.C., 1995. Induced ovulation and larval rearing of four species of Australian marine fish. PhD thesis, University of Tasmania, Australia, 215 pp.

Battaglene, S., Talbot, B., 1989. Mass production of rotifers. Austasia Aquaculture Magazine 3 (11), 6–7.

Blaxter, J.H.S., 1988. Pattern and variety in development. In: Hoar, W.S., Randall, D.J., Donaldson, E.M. (Eds.), Fish Physiology: Part A vol. XI Academic Press, New York, NY, pp. 1–48.

Boehlert, G.W., Yoklavich, M.M., 1984. Carbon assimilation as a function of ingestion rate in larval Pacific herring, *Clupea harengus pallasi* Valenciennes. J. Exp. Mar. Biol. Ecol. 79, 251–262.

Dowd, C.E., Houde, E.D., 1980. Combined effects of prey concentration and photoperiod on survival and growth of larval sea bream, *Archosargus rhomboidalis* (Sparidae). Mar. Ecol. Prog. Ser. 3, 154–181.

Epp, R.W., Lewis, W.M., 1984. Cost and speed of locomotion for rotifers. Oecologia (Berlin) 61, 289–292.
Epp, R.W., Winston, P.W., 1977. Osmotic regulation in the brackish-water rotifer *Brachionus plicatilis* (Müller). J. Exp. Biol. 68, 151–156.
Epp, R.W., Winston, P.W., 1978. The effects of salinity and pH on the activity and oxygen consumption of *Brachionus plicatilis* (Rotatoria). Comp. Biochem. Physiol. 59A, 9–12.
Fukusho, K., 1983. Present status and problems in culture of the rotifer *Brachionus plicatilis* for fry production of marine fishes in Japan. In: Fuentes, H.R., Castillo, J.G., Disalvo, L.H. (Eds.), Advances and Perspectives in Aquaculture. Proceedings of a Symposium, Universidad del Norte, Coquimbo, Chile, September. pp. 361–374.
Fukusho, K., Iwamoto, H., 1980. Cyclomorphosis in size of the cultured rotifer, *Brachionus plicatilis*. Bull. Natl. Res. Inst. Aquacult. 1, 29–37.
Fulks, W., Main, K.L., 1991. Rotifer (*Brachionus plicatilis*) production systems. In: Fulks, W., Main, K.L. (Eds.), Rotifer and Microalgae Culture Systems. Proceedings of a US–Asia Workshop, Honolulu, HA, January 28–31. pp. 3–52.
Gatesoupe, F.J., Luquet, P., 1981. Practical diet for mass culture of the rotifer *Brachionus plicatilis*: application to larval rearing of sea bass *Dicentrarchus labrax*. Aquaculture 27, 149–163.
Hoff, F.H., Snell, T.W., 1989. Plankton Culture Manual. Florida Aqua Farms, Dade City, FL, USA, 126 pp.
Hunter, J.R., 1980. The feeding behaviour and ecology of marine fish larvae. In: Bardach, J.E., Magnuson, J.J., May, R.C., Reinhart, J.M. (Eds.), Fish Behaviour and Its Use in the Capture and Culture of Fishes. ICLARM Conf. Proc. 5, Manila, Philippines. pp. 287–330.
Kafuku, T., Ikenoue, H., 1983. Modern methods of aquaculture in Japan, Developments of Aquaculture and Fisheries Science, 11,Kodansha Tokyo, Elsevier Amsterdam, 216 pp.
Komis, A., 1992. Improved production and utilization of the rotifer *Brachionus plicatilis* Müller, in European sea bream (*Sparus aurata* Linnaeus) and sea bass (*Dicentrarchus labrax* Linnaeus) larviculture. Doctor in Agricultural Sciences Thesis, University of Gent, 277 pp.
Korunuma, K., Fukusho, K., 1987. Rearing of Marine Fish Larvae in Japan. IDRC, Ottawa, 109 pp.
Lubzens, E., 1987. Raising rotifers for use in aquaculture. Hydrobiologia 147, 245–255.
Lubzens, E., Tandler, A., Minkoff, G., 1989. Rotifers as food in aquaculture. Hydrobiologia 186/187, 387–400.
Miracle, M.R., Serra, M., 1989. Salinity and temperature influence in rotifer life history characteristics. Hydrobiologia 52, 81–102.
Øie, G., Olsen, Y., 1993. Influence of rapid changes in salinity and temperature on the mobility of the rotifer *Brachionus plicatilis*. Hydrobiologia 255/256, 81–86.
Olsen, Y., Rodriguez, R.J., Vadstein, O., Jensen, A., 1989. The effect of short term starvation upon the fatty acids in enriched *Brachionus plicatilis*. Hydrobiologia 186/187, 409–413.
Segers, H., 1995. Nomenclatural consequences of some recent studies on *Brachionus plicatilis* (Rotifera, Brachionidae). Hydrobiologia 313, 121–122.
Snell, T.W., Carillo, K., 1984. Body size variation among strains of the rotifer *Brachionus plicatilis*. Aquaculture 37, 359–367.
Snell, T.W., Childress, M., Boyer, E.M., Hoff, F.H., 1987. Assessing the status of rotifer mass cultures. J. World Aquacult. Soc. 18, 270–277.
Tandler, A., Mason, C., 1984. The use of $^{14}C$ labelled rotifers (*Brachionus plicatilis*) in the larvae of gilthead seabream (*Sparus aurata*): measurements of the effect of rotifer concentration, the lighting regime and seabream larval age on their rate of rotifer ingestion. In: Research on Aquaculture. Proceedings of the Second Seminar of the German–Israeli Cooperation in Aquaculture Research, 5–6 March 1984, Hamburg, Germany. Rosenthal, H., Sarig, S. (Eds.), Spec. Publ. Eur. Maricult. Soc. 8 pp. 241–259.
Tandler, A., Sherman, R., 1981. Food organism concentration, environmental temperature and survival of the gilthead bream (*Sparus aurata*) larvae. In: Rosenthal, H., Oren, O.H. (Eds.), Research on Intensive Aquaculture. Proceedings of a German–Israeli Seminar on Aquaculture, 10–11 March 1980, Shefayim, Israel. Spec. Publ. Eur. Maricult. Soc. 6 pp. 237–248.
Underwood, A.J., 1981. Techniques of analysis of variance in experimental marine biology and ecology. Oceanogr. Mar. Biol. 19, 513–605.
Walker, K.F., 1981. A synopsis of ecological information on the saline lake rotifer *Brachionus plicatilis* Müller 1786. Hydrobiologia 81, 159–167.