SS-strain Rotifer Culture for Finfish Larvae with Small Mouth Gape

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Introduction

In the aquaculture of temperate marine finfish, the rotifer *Brachionus plicatilis* (L-strain) has been the primary live food for early larval stages. As aquaculture has diversified to include tropical marine species the small strain rotifer, *B. rotundiformis,* is now routinely cultured. Many marine finfish have larvae with a small mouth gape that has required the selection of a super small (SS) strain of *B. rotundiformis.* However, even this SS-strain of rotifer is not an optimal size and a smaller size would be ideal for first-feeding finfish larvae.

The objective of this work was to develop culture techniques that would select for smaller sized rotifers and reduce the average size of rotifers in a population. A typical population of rotifers contains reproductive females of varying sizes with the distribution skewed toward the smaller animals. The aim was to apply selection pressures that would exaggerate this skewed distribution and result in a higher proportion of smaller rotifers.

Methods

Small rotifer body size, as a heritable trait related to the size of amictic and resting eggs, was determined by: measuring amictic eggs attached to rotifers; measuring harvested eggs (amictic and resting); isolating individual eggs to form clonal colonies; and measuring adult rotifers of resulting populations. Initial rotifer populations and clonal lines were maintained at 28°C, 30 ppt salinity and fed *Nannochloropsis oculata* at 3×10^6 cell/ml.

Effects of the environmental factors salinity and diet on rotifer body-size were determined. To examine the effect of salinity on the development rate and final size of rotifers, 30 eggs from a clonal culture were placed in each well of a 24-well plate at three salinities (5, 20 and 30 ppt). Over a 24-hour period following hatching, rotifers were removed hourly from a well (n = 30), measured and the time of appearance of reproductive females was noted.

The effect of diet on rotifer body size was determined by feeding rotifers an equal ash-free, dry-weight ration (equivalent to 3×10^6 cells/ml of *N. oculata*) of algae of different cell mass. Algal mass ranged from 1 pg/cell for *Stichococcus* to 10 pg/cell for *N. oculata,* 170 pg/cell for *Tetraselmis* and 572 pg/cell for *Heterocapsa niei*.

In the first experiment, first laid eggs from a clonal culture were distributed among wells of 24-well plates containing each of the algal diets. Three-day old, F1 rotifers were collected from the resulting populations and measured. In the second experiment, 16 × 1 L rotifer cultures (20 rotifers/ml) were fed with four different algae (4 replicates/algal diet).

Replicates were fed daily and the population adjusted to 20 rotifers/ml. A sample (~40) of harvested, egg-bearing rotifers was measured every second day. After 14 days, the size distribution of the replicate populations was compared to one fed the control species, *N. oculata*.

Advances in Grouper Acquaculture
Edited by M.A. Rimmer, S. McBride and K.C. Williams
ACIAR Monograph 110 (printed version published in 2004)

Results and Discussion

The SS-strain rotifer was isolated from Centenary Lakes, Cairns. At the start of the program, reproductive females had an average lorica length of 151 ± 15 μm and width of 111 ± 10 μm. The distribution was skewed toward smaller sizes with the length of the smallest reproductive female measured being 96 μm (Fig. 1). A poor relationship was found between the length of the parent and the length of its egg ($r^2 = 0.09$); and between the area of the parent rotifer and the area of its egg ($r^2 = 0.24$). The maximum width of amictic eggs averaged 96 ± 11 μm of which 14% were smaller than 85 μm (average –1 SD). Offspring hatched from this sub-group of eggs had an average size of 147 μm and a distribution similar to the initial population. The optimal salinity for hatching and resting eggs was 5 ppt seawater. Resting eggs, collected and sorted into small resting eggs (77 ± 6 μm), hatched to produce females with an average length of 135 ± 9 μm at commencement of egg production. However, selection and culture of the two smallest females (100–120 μm) produced populations with an average body length of 140 and 148 μm (Fig. 2).

Salinity affected the rate of rotifer development. Development was fastest at the lowest tested salinity of 5 ppt and slowed as salinity rose to 20 ppt and 30 ppt (Fig. 3). Rotifers also became reproductive earlier at lower salinity and egg-bearing rotifers appeared before maximal size was attained.

Diet also affected the development of rotifers. After feeding on four equal ration diets of varying particle size (1 pg/cell to 572 pg/cell) for 14 days, the average length and width of the rotifer populations was not significantly different (Fig. 4). However, the distribution of sizes within the populations was different. Rotifers raised on the control diet of *N. oculata* had an average body dimension of 179 μm in length and 140 μm in width. Fifty-six per cent of the population had a body length less than the average and 46% had a body width greater than the average. Rotifers fed *Stichococcus*-like algae, (the smallest diet at 1 pg/cell) had 72% of the population with a body length less than the average length of the control rotifers fed *N. oculata*. Rotifers fed *Tetraselmis* had a larger proportion of wide rotifers with 64% being larger than the average width of those fed the

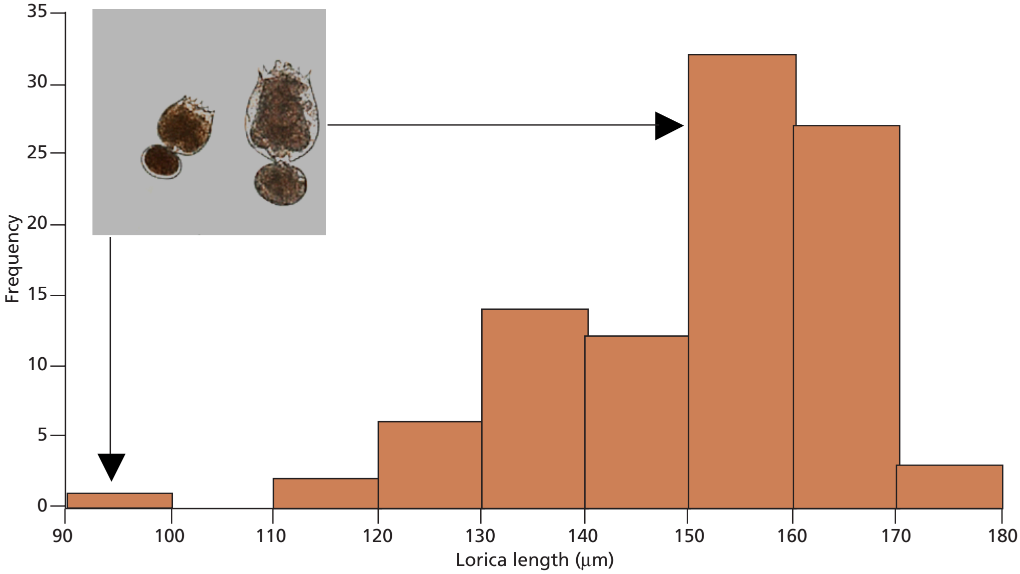

Figure 1. Size distribution of the Cairns isolate of the SS-strain rotifer. Average lorica length = 151 ± 15 μm.

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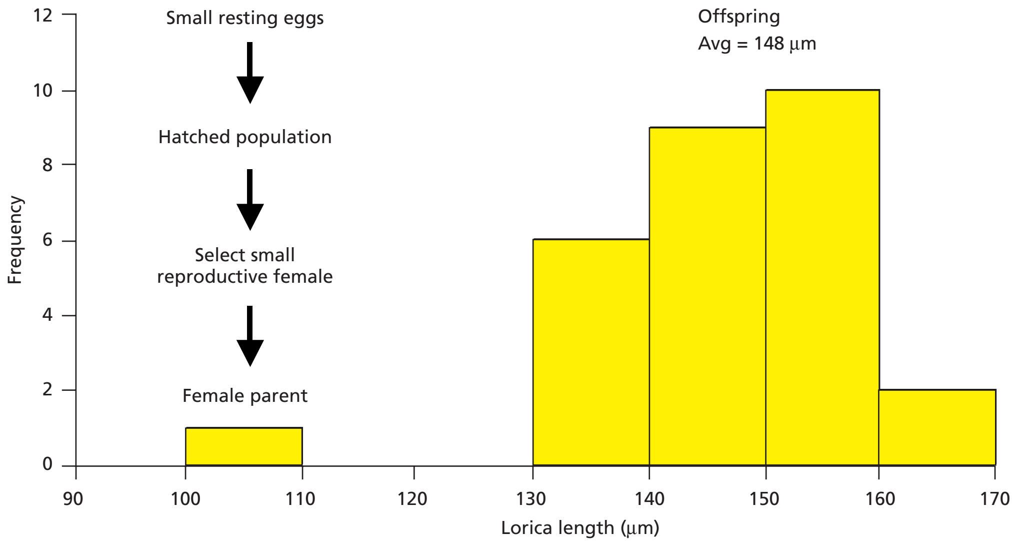

Figure 2. Example of mean size (lorica length) of resulting population of rotifers bred from a single, small parent isolated from a population hatched from smallest resting eggs.

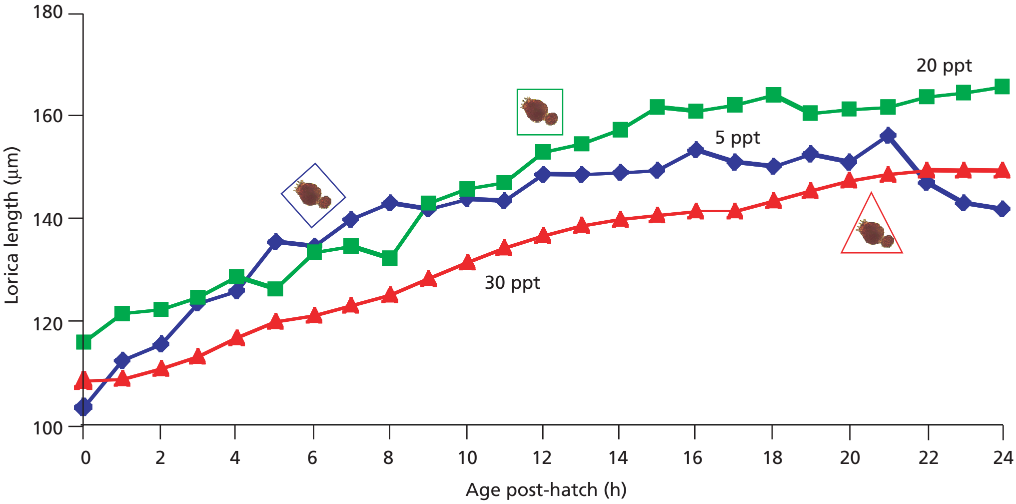

Figure 3. Effect of salinity on the rate of rotifer development (increase in lorica length). Rotifer symbols indicate the time of the first appearance of egg production. All sample points n = 30 rotifers.

control diet *N. oculata*. Rotifers fed the largest size alga, *H. niei,* were similar to those fed the control. This indicates that this alga could be too large for rotifers to ingest so they are feeding on algal cell debris and bacteria.

The results confirm the plasticity of the rotifer lorica and the polymorphism that occurs in populations. Rotifer size may vary by more than 100% between habitats (Ruttner-Kolisko 1977). Increase in rotifer lorica size when a fed diet of large-celled algae (*Tetraselmis*) has been reported (Rumengan et al. 1998). However, Reitan et al. (1997) found differences in lorica length due to different diets that were not large enough to affect their availability to fish larvae. We found diet had no significant effect on the

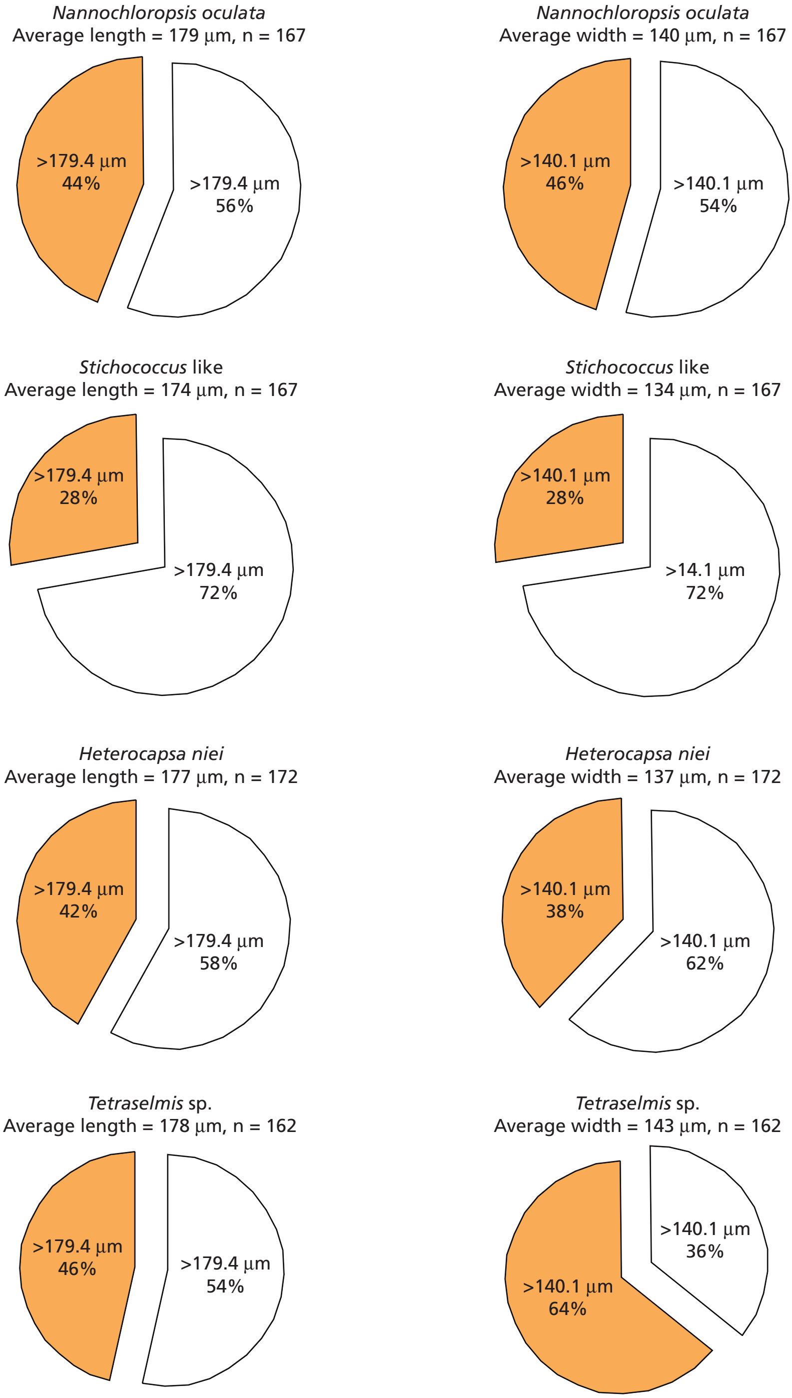

Figure 4. The average size (left: body length; right: body width) and size distribution of populations of rotifers raised for 14 days on equal ration diets of four microalgae of varying particle size (cell mass). Algal cell mass (ash-free, dry-weight/cell) for the control diet *N. oculata* = 10 pg/cell. For the test diets, *Stichococcus*-like = 1 pg/cell, *Tetraselmis* sp. 170 pg/cell and *H. niei* = 572 pg/cell. Size distributions are in relation to the average size (length 179.4 µm and width 140.1 µm) of the control rotifers fed *N. oculata*.

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average size of rotifers but feeding with very small algae did increase the percentage of smaller rotifers within a population. This is beneficial when rotifers are used to feed fish larvae with a small mouth gape.

Conclusions

- Higher percentages of smaller rotifers suitable for first feeding larvae are obtained when rotifers are raised on ultra-small algae such as *Stichococcus*.
- Selection of small resting eggs is more successful than amictic eggs in producing a rotifer population of reduced size.
- Much of the skewness toward smaller sized reproductive rotifers in a population is a result of rotifers reproducing before achieving maximal size. This reduces the chance of successfully reducing the size of rotifers by selecting for small reproductive females since most of these rotifers will continue to increase in size as they mature. Synchronous rotifer cultures are required for this purpose, in which all rotifers have reached maximal size, before the smallest individuals are selected.

References

Rumengan, I.F.M., Warouw, V. and Hagiwara, A. 1998. Morphometry and resting egg production potential of the tropical ultra-minute rotifer *Brachionus rotundiformis* (Manado strain) fed different algae. Bulletin of the Faculty of Fisheries, Nagasaki University, 79, 31–36.

Reitan, K.I., Rainuzzo, J.R., Øie, G. and Olsen, Y. 1997. A review of the nutritional effects of algae in marine fish larvae. Aquaculture 155, 207–221.

Ruttner-Kolisko, A. 1977. Suggestions for biomass calculation of planktonic rotifers. Archiv für Hydrobiologie Beihefte Ergebnisse der Limnologie 8, 71–76.

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