Rotifer (*Brachionus rotundiformis*) Culture Technology for Hatchery Production in Singapore

Marine Aquaculture Centre
1. Background

2. Key challenges of rotifer culture in Singapore

3. Analysis & Solutions
   - Unstable rotifer treatment
   - Quality rotifer diet
   - Good management protocol
   - Recirculating Rotifer Culture System
   - A.I. for rotifer counting

4. Summary
Part One

Background

- Rotifer introduction
- Types of rotifer culture in Singapore
Rotifer Introduction

- Rotifers are used extensively in aquaculture
  - Optimal size for fish larvae, shellfish, corals and other filter feeders
  - Good nutritional profile if properly enriched
  - High reproduction rate
  - Easy to culture

![Diagram of Rotifer](image)

Fig. 2.2. Morphology and inner organisation of a Brachionus sp. female (left) and male (right). a, dorsal antenna; b, bladder; bt, buccal tube; c, corona; e, eye; eg, egg; f, foot; fg, foot gland; g, central ganglion; la, lateral antenna; m, mastax; mu, muscle; o, oesophagus; ov, ovary; p, prostate; pe, penis; s, sensory cirri; sg, stomach gland; st, stomach; t, toe; te, testis; tr, trophy; v, vas deferens. (From Koste & Shiel 1987. Reproduced from Invertebrate Taxonomy, Volume 7 with permission of CSIRO Publishing.)
**Rotifer Introduction**

**Commonly Used Species**

**B. plicatilis (L)**
- 171-238µm
- Low-temperatures 20-25°C
- Euryhaline

**B. rotundiformis (S)**
- 121-162µm
- High-temperatures 28-35°C
- Low-salinities
Rotifer Life Cycle

Asexual and Sexual

Nearly all the rotifers seen in nature are females

Males occur only for short periods

Life span of amictic females: 7 -12 days

Number of eggs for amictic females produced per day: ~5 eggs

Schematic explanations of sexual and asexual cycles of reproduction of rotifer Brachionus (Lubzens and Zmora 2003)
Water Quality & Feed

Water Quality

1. Dissolved oxygen → Above 4ppm
2. pH → 7.5 – 8.5
3. Unionized Ammonia → less than 1ppm
4. Salinity → 10-25 ppt
5. Temperature → 28-35°C

Feed

1. Rotifers are filter feeders
2. Prey size spectrum → 1.4 – 21µm in diameter
3. Need to feed every 4 hours
4. Food preferences → microalgae, yeast, bacteria
Types of Rotifer Culture in Singapore: Outdoor Pond

Tank size: 20 m²

Density: 20-50 ind/ml

Productivity: 10 mil rotifer produced per m³ per day

Manpower: 0.39 hr to produce 100 mil rotifer per day

Ponds needed to start production: 3-5

Pond size: 120 m²

Pond size: 1200 m²
Types of Rotifer Culture in Singapore: Indoor Batch Culture

- Tank size: 1 m³
- Tank size: 2.5 m³
- Tank size: 8 m³

Density: 200-500 ind/ml
Productivity: 100mil rotifer produced per m³ per day
Manpower: 0.87 hr to produce 100 mil rotifer per day
Tanks needed: 5-8
Key Challenges of Rotifer Culture in Singapore

- Maintain rotifer culture stability
- Producing rotifers with good nutrition
- Producing hygienic rotifers
Maintain Rotifer Culture Stability

1\textsuperscript{st} rotifer crash in 2017

2\textsuperscript{nd} and 3\textsuperscript{rd} rotifer crash in 2017

4\textsuperscript{th} rotifer crash in 2017

5\textsuperscript{th} rotifer crash
Producing Rotifers with Good Nutrition

1. **Docosahexaenoic Acid (DHA)**
   An essential fatty acid that accumulates in the brain of fish during early development where it increases neural functions.

2. **Eicosapentaenoic Acid (EPA)**
   EPA is the primary mediator of cellular inflammation.

3. **Arachidonic Acid (AA)**
   AA being the preferred substrate and producing eicosanoids of higher biological activity.
Producing Hygienic Rotifers

Bacteria: harmful types cause unexpected mortality to rotifers; detrimental effect on fish larvae.

Vibrio: pathogenic to fish when introduced to fish larvae tanks with cultured rotifers.

Fungal: reduce productivity of rotifer mass cultures and abnormal mortality.

Ciliates: may release growth-inhibiting substance into the water and retard fish growth.
Part Three

Analysis & Solutions

1. Treatment for Rotifers with Unstable Growth
2. Proper Enrichment of Rotifers
3. Good Management Protocol
4. Recirculating Rotifer Culture System
5. A.I. for Rotifer Counting
Treatment for Rotifers with Unstable Growth

**Signs of unstable rotifer culture**

<table>
<thead>
<tr>
<th></th>
<th>Healthy rotifer culture</th>
<th>Unhealthy rotifer culture</th>
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<tbody>
<tr>
<td>Egg carriers</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Movement</td>
<td>Active</td>
<td>Lethargic</td>
</tr>
<tr>
<td>Ciliates</td>
<td>None or few</td>
<td>A lot</td>
</tr>
<tr>
<td>Clumps</td>
<td>No</td>
<td>A large number</td>
</tr>
<tr>
<td>Gut content</td>
<td>Full</td>
<td>Empty</td>
</tr>
</tbody>
</table>

**Avg swimming speed of rotifer**

- **Active rotifers**: 1.3 mm/s, 1.5 mm/s, 0.6 mm/s
- **Slow-moving rotifers**: 0.4 mm/s
1 Treatment for Rotifers with Unstable Growth

Reasons for unstable rotifer:

➢ Poor water quality

➢ Pathogenic bacteria infection

➢ Ciliates and copepod

➢ Over or under-feeding

• Infected rotifers cause a detrimental effect on fish larvae
Unstable rotifer culture treated with live microalgae at MAC

- The unstable rotifer culture recovered and became stable
- Grew from 300mil to 1 billion within 8 days
- However, rotiflers remained unstable in the control tank
- Microalgae has positive effect on the bacterial load in rotifer culture tank

Solution: Live Microalgae Treatment

![Graph showing rotifer number (mil) vs. date with data points for co-feeding with algae and without algae (control).]
Concentrated Microalgae Instead of Live Microalgae

At Mac we concentrate live microalgae for ease of storage, transport and use

Benefits:

- Concentrated microalgae is alive compared to commercially-available freeze-dried microalgae
- Not harmful to fish larvae
- Easy to transport
- Can be stored for up to 6 months or more

Growth chart of resuspended microalgae (mil/ml)
Proper Enrichment of Rotifers

Enrichment includes:
- Short-term enrichment
- Long-term enrichment

Enrichment food:
- Microalgae
- Oil emulsions
- Formulated diets

Commercial enrichment products:
- Fresh Chlorella
- Instant microalgae
- AlgaMac
- Selco® S.parkle
3 Good Management Protocol

➢ Treatment of unstable rotifer culture
  • Immediate adding of live microalgae

➢ Prevention of disease
  • Treat water with filter and UV
  • Personal hygiene
  • Equipment and culture area disinfection

Uronema

Euplotes

~18-28 µm

~80 µm
Recirculating Rotifer Culture System

- 2.5m$^3$ culture tank
- 45µm net
- Protein skimmer
- Bio filter
- Peristaltic pump
Rotifer Net Clogging Issue

Issue: Clogging

- Rotifer net is easily clogged by debris and foam.

Solution: Movable screen

- Strong aeration helped to clean the net, and eliminate the number of rotifers going though the net.

Video of movable screen under water
Recirculating Rotifer Culture System

Advantage:

➢ More hygienic
➢ Higher density
➢ Higher productivity
➢ Less manpower
➢ Stable
## Comparison of Different Culture Systems

<table>
<thead>
<tr>
<th></th>
<th>Outdoor pond culture</th>
<th>Indoor batch culture</th>
<th>RAS continuous culture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume:</strong></td>
<td>1200 m³</td>
<td>2 m³</td>
<td>2.5 m³</td>
</tr>
<tr>
<td><strong>Density:</strong></td>
<td>20-50 ind/ml</td>
<td>200-500 ind/ml</td>
<td>800-1000 ind/ml</td>
</tr>
<tr>
<td><strong>Productivity:</strong></td>
<td>10 mil</td>
<td>100 mil</td>
<td>200 mil</td>
</tr>
<tr>
<td>(rotifer produced per m³ per day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Manpower:</strong></td>
<td>0.39 hr</td>
<td>0.87 hr</td>
<td>0.39 hr</td>
</tr>
<tr>
<td>(produce 100 million rotifer per day)</td>
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High Density Rotifer in Recirculating Rotifer Culture System

- High density: 3000-4000 ind/ml
- Compact
- Stable
- Continuous supply
- High productivity
Improvement in Rotifer Growth Rate

Average daily growth rate

- Mar-18: 10%
- Apr-18: 13%
- May-18: 23%
- Jun-18: 47%
- Jul-18: 38%
- Aug-18: 40%
- Sep-18: 64%
Others: Protocols to Enhance Rotifer Growth Rates (On going)

Preliminary observations:
➢ Rotifer grew 1000 times from 4mil to 4bil within 11 days at 10ppt.
➢ Daily growth rate is 150%, which is higher than 30ppt.

Benefits:
➢ Save manpower
➢ Save cost
➢ Increase productivity
➢ Reduce preparation time

<table>
<thead>
<tr>
<th></th>
<th>10ppt</th>
<th>30ppt</th>
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<tbody>
<tr>
<td>Daily growth rate</td>
<td>150%</td>
<td>95%</td>
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Rotifer growth with different salinity
A.I. for Rotifer Counting

*(Developed in collaboration with GovTech)*

- A mobile app software which is able to assess and analyse rotifer culture health from photos taken of rotifer samples.

**Advantage:**

- Faster counting of rotifers
- Saves time and manpower

*(QR code to software will be updated)*
Summary & Future study

- Summary
- Future Study
Produce rotifers intensively and consistently on a small footprint is important for Singapore hatcheries.

Live microalgae treatment is one of the solutions to treat unstable rotifer culture.

Recirculating Rotifer Culture improves productivity and can support large-scale hatchery production.
Future Study

1. Protocols to enhance rotifer growth rates
2. Transfer of technology to local fish farm
Thank you!