Practical Rotifer Culture for Zebrafish Facilities

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Advantages of rotifers as first feed

- Appropriate size for first-feeding zebrafish (120 – 350 μm at maturity, depending on strain)
- Swimming activity stimulates larval feeding
- Rotifer gut enzymes and bacterial flora enhance immature larval digestive functions
- Good nutrition — easy to enrich specific components, e.g. HUFAs, sterols, vitamins
- “Polyculture” technique retains rotifer nutritional value in larval tank, saves labor, doesn’t foul tank
- Easy and economical to reliably produce very large quantities, continuously, at high densities
- Rotifers and feeds commercially available
Brachionus spp. = “L” or “S” type rotifers

- “Saltwater” rotifers, native to estuaries and inland saline environments
- Can thrive in a wide range of salinities
- Can breed in salinities as low as 3 ppt
- Harvest rates of up to 50% per day
- Less likely to harbor freshwater pathogens
- Proven effective for freshwater fish
  - Laboratory Zebrafish
  - Spotfin Shiners
  - Yellow Perch
**Brachionus** spp. = “L” or “S” type rotifers

Rotifers swim and feed by the beating of cilia that are grouped into the **corona**, surrounding the mouth.

The “**foot**” has adhesive glands, for attachment.

The single **red eye** is visible in the right-hand specimen and in one developing embryo.

**Eggs** are carried until they hatch, but eggs are physiologically independent.
Rotifers are efficient feeders

- Rotifers are equipped with a “mastax” chewing mechanism that can break cell walls of algae.
- They can digest such foods much more efficiently than other small aquatic animals and protozoa.
Brachionus “birds & bees”

- A vigorous culture is ALL females
- Eggs are produced by parthenogenesis (no males needed) so the offspring are genetically identical
- Only when a culture is severely stressed (temperature, salinity, pH, low oxygen, high ammonia), are males and sexual females produced
  - Males remain small, lack a digestive system
  - They mate with sexual females, which produce special eggs, “resting cysts,” that can survive for several years
  - Sexual reproduction is extremely rare in well-managed cultures — you should never see it
B. plicatilis production dynamics

- From egg to producing eggs in ~18 hours
- Lifespan 6-14 days (depends on temperature)
- Eggs produced only for the first ~5 days, most vigorous egg production first 2 – 3 days
- Filter feeders, feed mostly on microalgae
- Food passage time through gut ~45 minutes
- Can consume 10% of their dry weight per hour
- Can convert feed biomass to rotifer biomass at an efficiency exceeding 30%
Harvest Rate and Productivity

A high daily harvest is very beneficial

- Only young rotifers produce many eggs
- Accumulation of older rotifers steadily reduces average reproductive rate
- Senescent rotifers are less nutritious
- 25 – 50% daily harvest removes old rotifers, decreases average age, and thus maintains maximum reproduction and vigor
- Vigorous cultures can be “ramped up” much more quickly when more rotifers are needed
Optimal culture conditions

- **Temperature**: L-type 26 – 27°C (tolerate 10 – 30°); S-type 32 – 35°C (tolerate 20 – 40°)
- **Salinity**: ∼15 g/L (seawater is ∼35 g/L)
  - Can tolerate 3 – 40 g/L
- **Oxygen**: ∼8 mg/L (atmospheric saturation)
  - Can tolerate ∼1 mg/L
- **pH**: 7.0 – 8.5
  - Can tolerate 6.5 – 9.0
- **Ammonia (NH₃)**: <1 mg/L
  - Easier to achieve at lower pH
The rotifer culture system

1. Culture vessel – cylindrical or conical
   - Typically 15 – 150 L (1000+ L in aquaculture)

2. Aeration
   - Ample, no chemical/microbial contamination

3. Feed supply
   - Refrigerated feed reservoir

4. Feed delivery and dosing control
   - By hand, or peristaltic pump controlled by timer

5. Waste removal
   - “Floss” to trap solid waste
   - Ammonia neutralizer
Rotifer culture vessels

- 5-Gallon Bucket
- Inverted 20-Liter Carboy
- 200-liter tank

Photo: E. Sanders
Feed supply & dosing control

*Feed dosed by hand*
- Minimum 2x/day
- Limits culture to low density

*Automated feeding*
- Reduces labor input
- Easier to manage staff days off
- More reliable feeding
- More consistent feed delivery
- Easily varied to meet variable need
- Higher feeding rates possible
  - Does not overload system
  - Supports higher production

Photo: E. Sanders
Waste removal

Rotifer Floss ~ 1 cm thick

Sodium hydroxy-methanesulfonate ammonia neutralizer

Aerator "chimney"
Dense cultures require dense feeds

- High-density rotifer cultures (1 – 5 million/L) supply ample larval feed from a small footprint
- High-density rotifer cultures require high-density, high-quality feeds
- Liquid algae (natural rotifer food) concentrates
  - The right particle sizes for rotifers
  - Much “cleaner” cultures than emulsions or dry feeds
  - Incorporation of optimal algae strains provides highest nutritional value (HUFAs, sterols, vitamins) for larvae
  - Dependable & economical food source
  - Suitable for automated feeding
Feeds for Zebrafish labs

RotiGrow Plus
Multi-algae feed optimizes nutritional value of rotifers for larval fish

RGComplete
Same multi-algae formula with ammonia neutralizer and pH buffer – ideal for small-scale (e.g. bucket) culture
Compact Culture System

Floss carrier
Aerator
Mounting clips

Simple construction for easy assembly

Daily maintenance:
1. Remove “T”
2. Flush retained waste from floss
3. Brush inside surfaces of bucket
4. Replace “T”
Harvesting rotifers

Collect rotifers on 40 μm screen

Flush rotifers from screen

Dilute rotifers into convenient volume of water at larval tank salinity
Daily routine

- **HARVEST! Or at least 25% water change**
- **Flush solid waste from floss**
- **Brush inside of tank**
- **Check feed delivery system**
- **Record system parameters**
  - Temperature
  - pH
  - NH₃
  - Count rotifers and eggs
    - Egg ratio usually should be >20%
Weekends and Holidays

- **Feed every day**
- **Harvest may be skipped**
  - If resulting density will not be too high
    - Reduce density at start of break
    - Reduce feed rate during break
    - Reduce temperature of culture
- **But — Every day confirm that system is operating normally**
  - Aeration, temperature, feed delivery
Oops! — But I thought ............

- **Backup supply**
  - Concentrated rotifer suspension may be kept refrigerated for 1 week or more
  - Use for feeding or to restart culture

- **More rotifers (up to hundreds of millions) can be obtained overnight from Reed Mariculture**
Best, J., Adatto, I., Cockington, J., James, A., Lawrence, C.
A novel method for rearing first-feeding larval zebrafish: polyculture with Type L saltwater rotifers (*Brachionus plicatilis*).

Lawrence, C., Sanders, E., Henry, E.
Methods for culturing saltwater rotifers (*Brachionus plicatilis*) for rearing larval zebrafish.
*Zebrafish*. 9, 140-146 (2012).

The Complete and Updated "Rotifer Polyculture Method" for Rearing First Feeding Zebrafish.

The list of culture materials presented here can be downloaded at:
http://www.jove.com/pdf-materials/53629