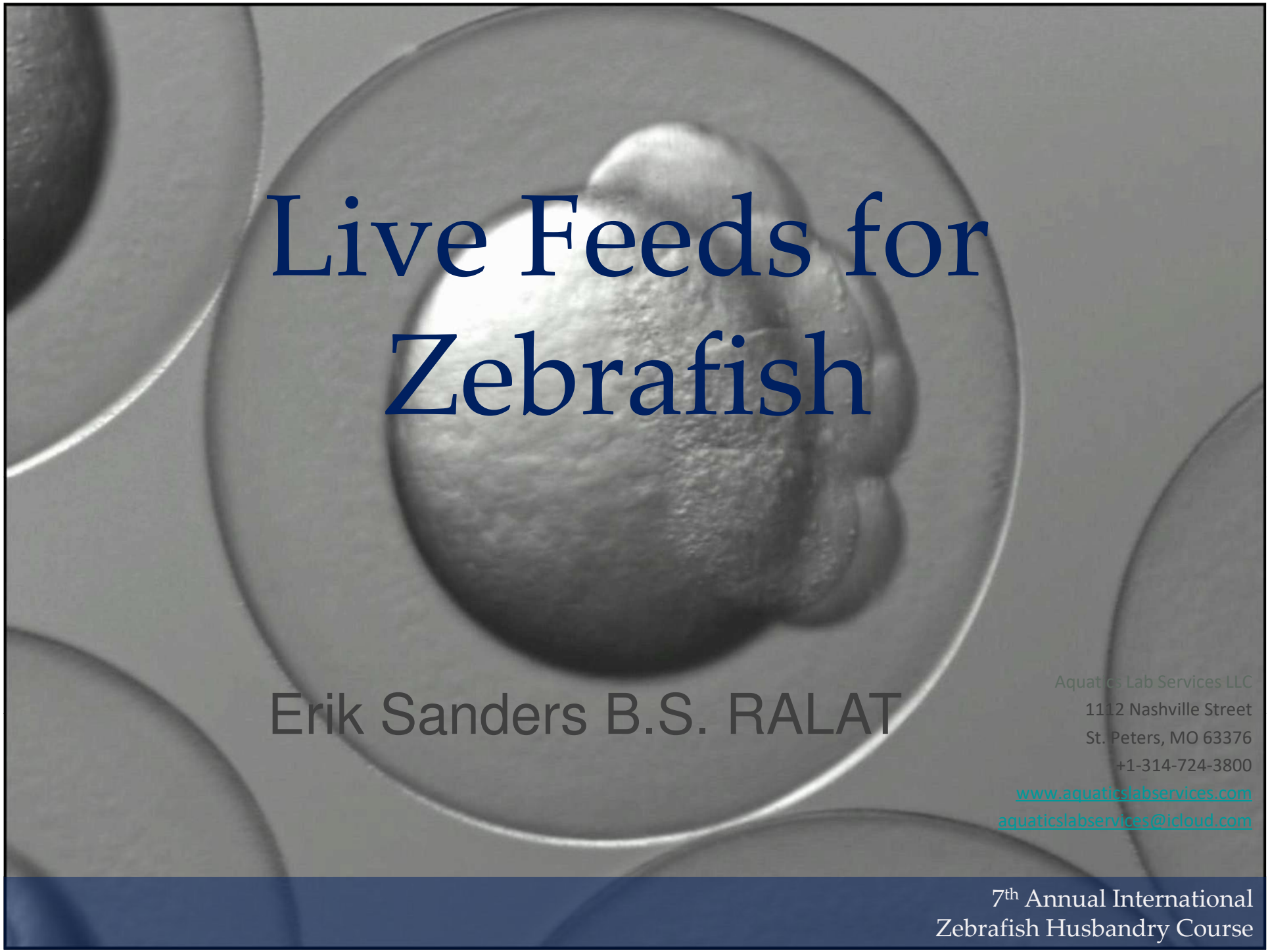


Live Feeds for Zebrafish

Erik Sanders B.S. RALAT

Aquatics Lab Services LLC
1112 Nashville Street
St. Peters, MO 63376
+1-314-724-3800
www.aquaticslabservices.com
aquaticslabservices@icloud.com

7th Annual International
Zebrafish Husbandry Course

Presentation Summary

- *Live feed Advantages/disadvantages*
- *Most common live feed choices*
  - *Frozen/Freeze Dried*
  - *Artemia*
    - *Pros and Cons*
    - *Hatching and feed-out procedure*
  - *Paramecia*
    - *Pros and Cons*
    - *Culture and Feed-out Procedure*
  - *Rotifers*
    - *Pros and Cons*
    - *Culture and Feed-out Procedure*

Live Diets – WHY??

- The main identifiable components of the diet were zooplankton and insects…
- Insects that could be identified to order were primarily dipterans…
- The majority of insects were aquatic species, or aquatic larval forms of terrestrial
- species, with dipteran larvae being particularly common during the monsoon months (June to August).
- …the zebrafish appears to feed chiefly on zooplankton in the water column..

From: Diet, growth and recruitment of wild zebrafish in Bangladesh; Spence et al, Journal of Fish Biology (2007) 71, 304–309 doi:10.1111/j.1095-8649.2007.01492.x

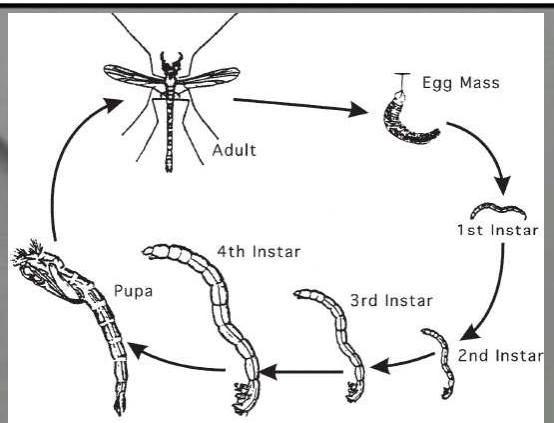
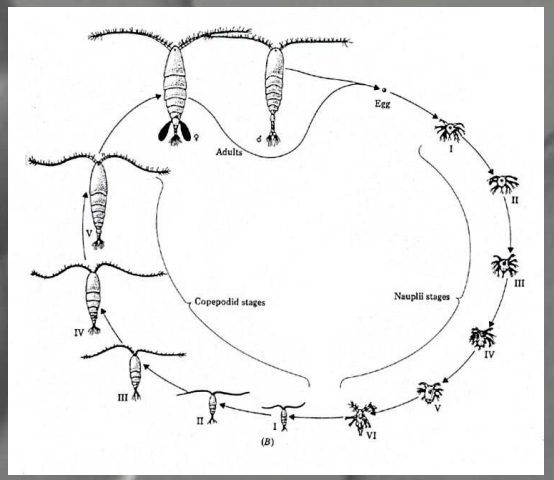
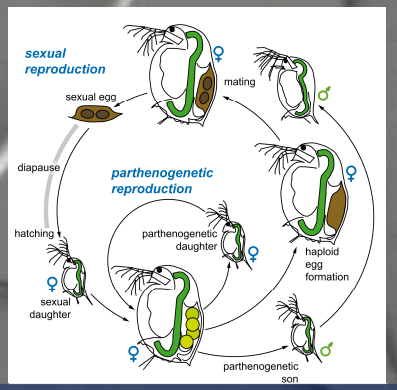


Figure 1. Chironomid life cycle (adapted from Walker 1987).





Live Diets

Advantages

- Amenability to mass production/culture
- Good nutritional profiles (particularly when enriched)
- Digestible
- Attractive (motility, smell, color, shape)
- Zebrafish are adapted to feed on it, and have co-evolved with it

Disadvantages

- Can be variable in nutritional profile
- Can be labor intensive
- Can be a source of pathogens
- Not efficient always as size of fish scales up

Live Feed Types: Frozen or Freeze Dried

- Number of different species available in freeze-dried form.
  - Krill (*Euphasia* sp.), Bloodworms (chironomid larvae), *Tubifex* worms, *Artemia, Daphnia*
- Useful for supplementing diet = deliver nutrients lacking in primary dietary items
- Should be sterilized/irradiated
  - *Tubifex* worms should be avoided; host for parasites

Credit: Argent Chemical Labs

Live feed types: Artemia

- Aquatic crustacean
- Most commonly used live feed for zebrafish of all life stages?
  - Many in the field (mistakenly) believe that the fish "require" it.
- Zebrafish would NEVER encounter artemia in the wild- salinity tolerance does not overlap
- 1.65 Ug dry wt. 1st Instar Nauplii

Live feed types: Artemia

Pros

- Easy to hatch
- Can be enriched
- Readily Available (?)
- Good nutritional profile (some variation within strains)
- Suitable size-distribution for most life-stages of zebrafish

Cons

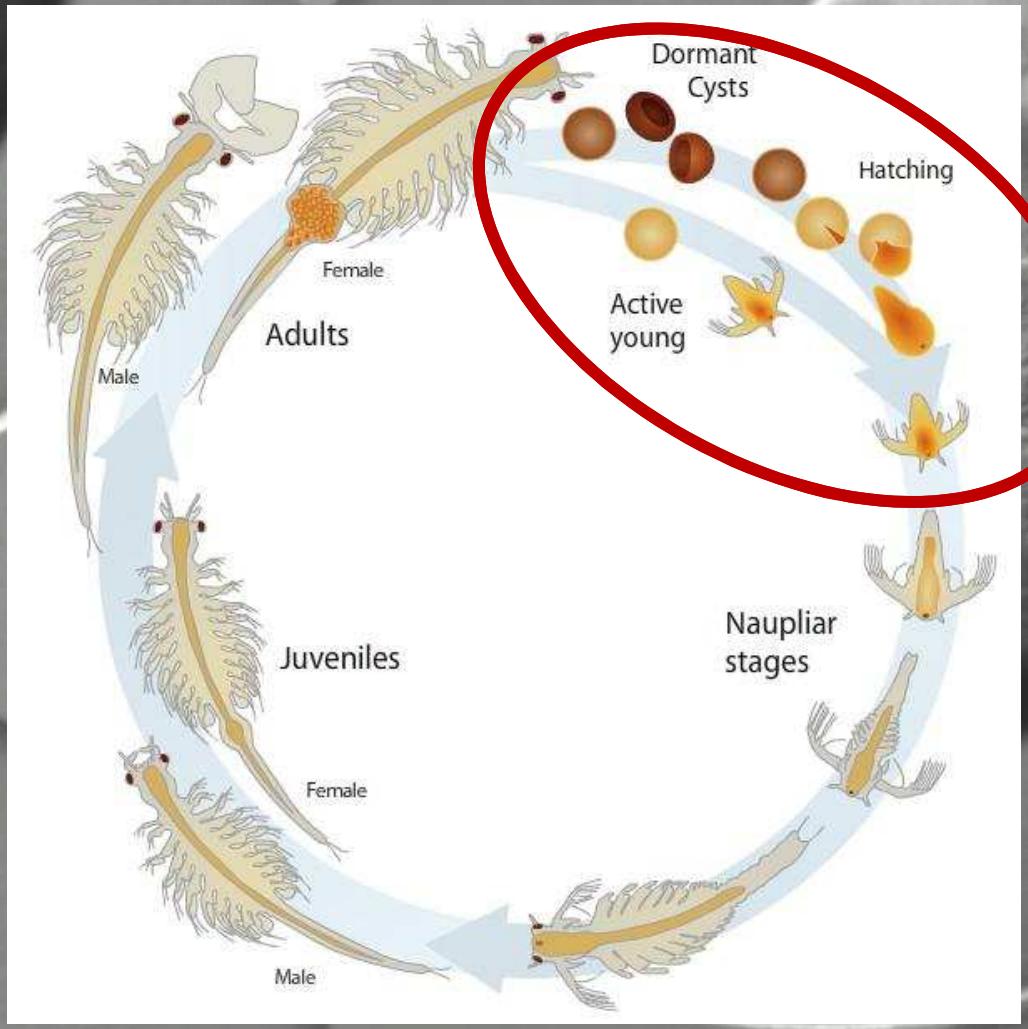
- Decapsulation necessary to sanitize and increase digestibility of un-hatched cysts
- Can be very labor intensive
- Protracted holding time required for enrichment
- Ever increasing cost
- Most are too large for 5-dpf zebrafish
- Can be source of fish pathogens and other biological contaminants

Live feed types: *Artemia*

Cons

Can be source of fish pathogens and other biological contaminants

Live feed types: Artemia



Life Stages (cysts thru 1st instar)

Very limited use in zebrafish labs without additional input into culture operation

Live feed types: Artemia

The physiology and mechanics of hatching

…the metabolism in *Artemia cysts prior to the breaking is a trehalose-glycerol* hyperosmotic regulatory system.
This means that as salinity levels in the incubation medium increase, higher concentrations of glycerol need to be built up in order to reach the critical difference in osmotic pressure which will result in the shell bursting, and less energy reserves will thus be left in the nauplius.

FAO Fisheries Technical Paper 361
Manual on the Production and Use of Live Foods for Aquaculture

Live feed types: Artemia

The physiology and mechanics of hatching

…For reasons of practical convenience natural seawater is mostly used to hatch cysts. However, at 5 g/l salinity, the nauplii hatch faster, as less glycerol has to be built up… and the nauplii have a higher energy content

FAO Fisheries Technical Paper 361
Manual on the Production and Use of Live Foods for Aquaculture

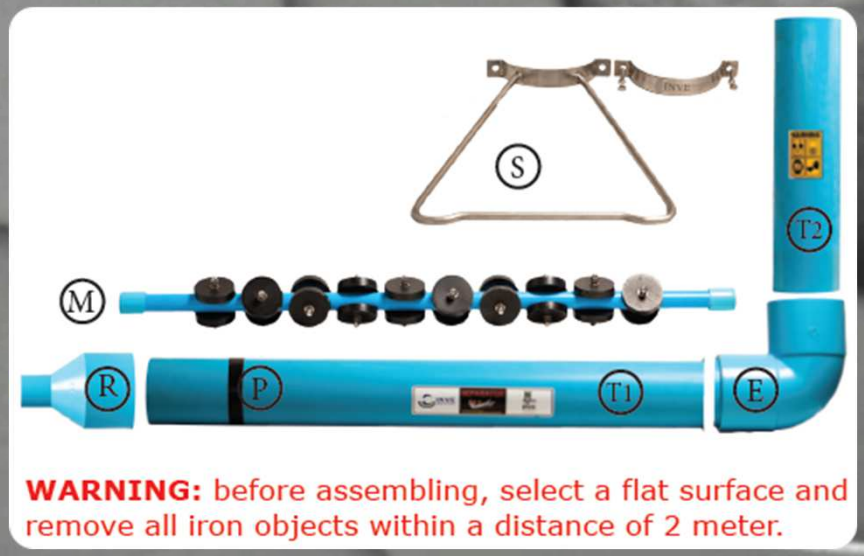
Live feed types: Artemia

Alternatives to the Traditional Artemia Paradigm

Sep-Art

- Polarized cysts stick to magnet
- More equipment costs

Approx. $1030USD/case vs. $588USD Grade A



Live feed types: Artemia

Alternatives to the Traditional Artemia Paradigm

Un-hatchable Cysts

- Pre-decapsulated
- Proven performance with zebrafish (Tye et al 2015)
- No live-prey benefits
- Approx. $212USD/case vs. $588USD Grade A
- Recent experiences indicate presence of toxic residues (arsenic, chromium)- proceed with caution!

Live feed types: Artemia

Typical Process in Zebrafish Labs: Decapsulation

The hard shell that encysts the dormant *Artemia embryo can be completely removed by* short-term exposure to a hypochlorite solution. Decapsulated cysts offer a number of advantages compared to the non-decapsulated ones:

- Cyst shells are not introduced into the culture tanks.
- Nauplii that are hatched out of decapsulated cysts have a higher energy content and
- individual weight (30-55 % depending on strain)
- hatchability might be improved
- Decapsulation results in a disinfection of the cyst material
- Decapsulated cysts can be used as a direct energy-rich food source for fish and shrimp
- For decapsulated cysts, illumination requirements for hatching would be lower

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Live feed types: Artemia

Typical Process in Zebrafish Labs: Storage

The hard shell that encysts the dormant *Artemia embryo can be completely removed by* short-term exposure to a hypochlorite solution.
Decapsulated cysts offer a number of advantages compared to the non-decapsulated ones:

•Cysts can be stored for a few days in the refrigerator at 0-4C without a decrease in hatching

•If storage for prolonged periods is needed (weeks or few months), the decapsulated cysts can be transferred into a saturated brine solution (+350g/l)

•During overnight dehydration in hyper-saline solution (with aeration/mixing to maintain a homogeneous suspension) cysts usually release over 80 % of their cellular water

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Live feed types: Artemia

Typical Process in Zebrafish Labs: Decapsulation

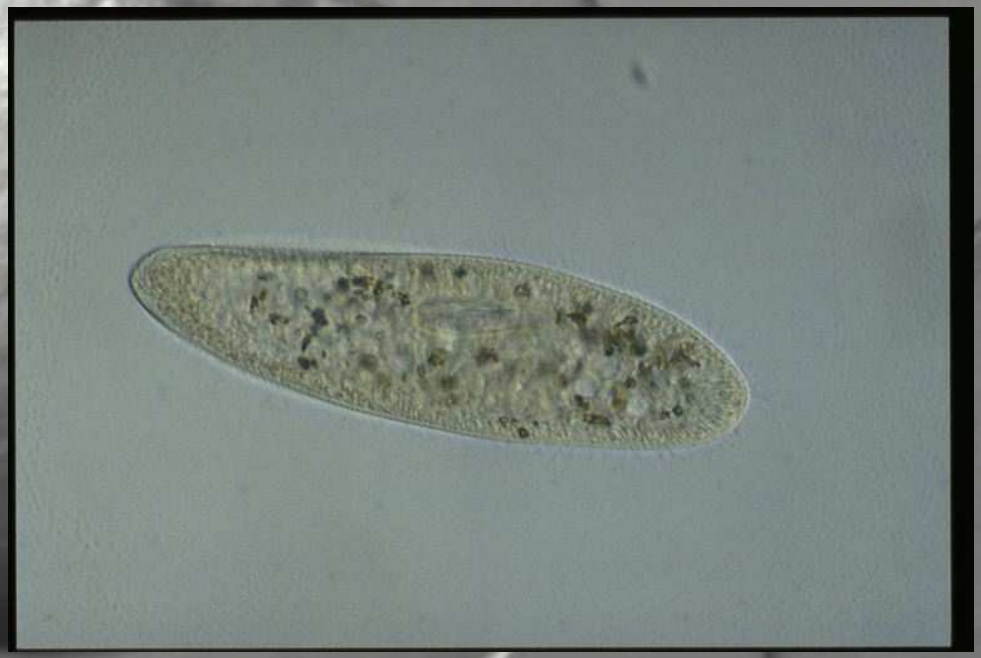
Live feed types: Artemia

If you use *artemia* be aware of:

- Discrepancies and variation in harvest/concentration
- Storage Methods are Critical
  - Nauplii are fragile and easily suffocated- must be aerated
  - Will continue to grow and molt- requiring feed and water exchange
- Variation in feed-out amounts between staff
- It is a wild captured organism, and is prone to be contaminated with organic materials and pathogenic micro-organisms such as *vibrio spp* and *mycobacterium spp.*

Live feed types: Paramecium

- Freshwater ciliate
- Historically used for first feeding zebrafish (wean onto *Artemia*)



Live feed types: Paramecium

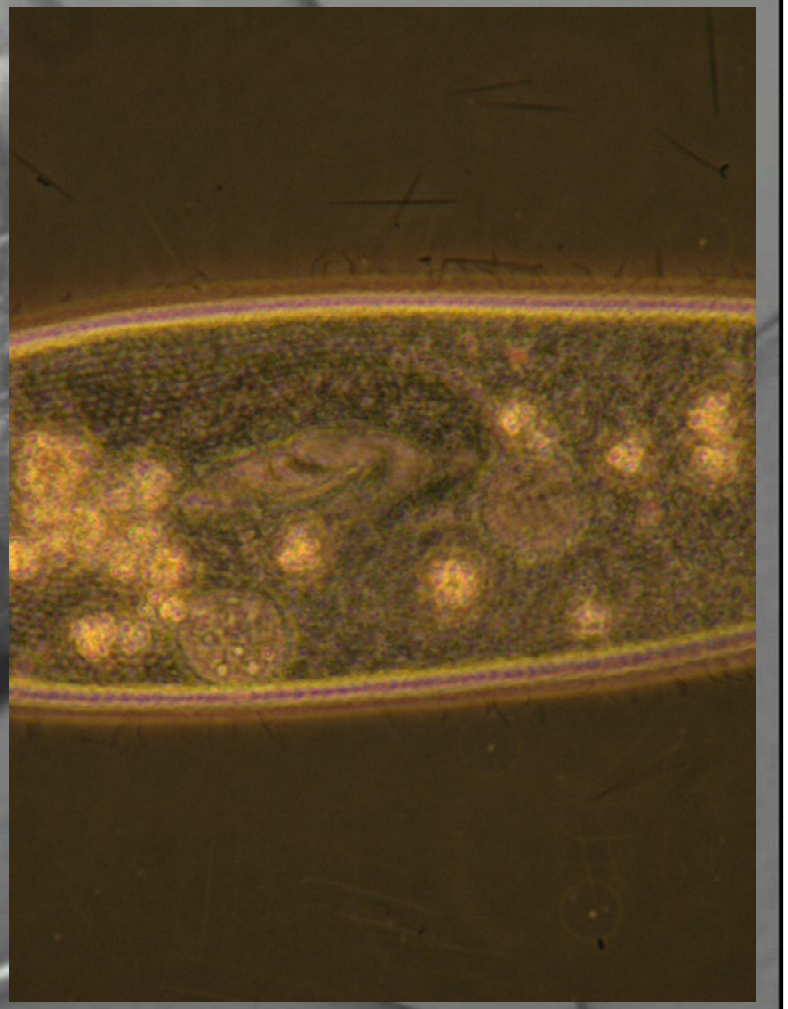
- Known to enhance the transmission of mycobacterium! (Peterson et al 2012)
- Limited nutritional value
- Relatively easy to culture
- Not shown to be effective at bioencapsulation

Live feed types: Paramecium

- Less than ideal swimming behavior
- Commonly contaminated with undesirable organisms

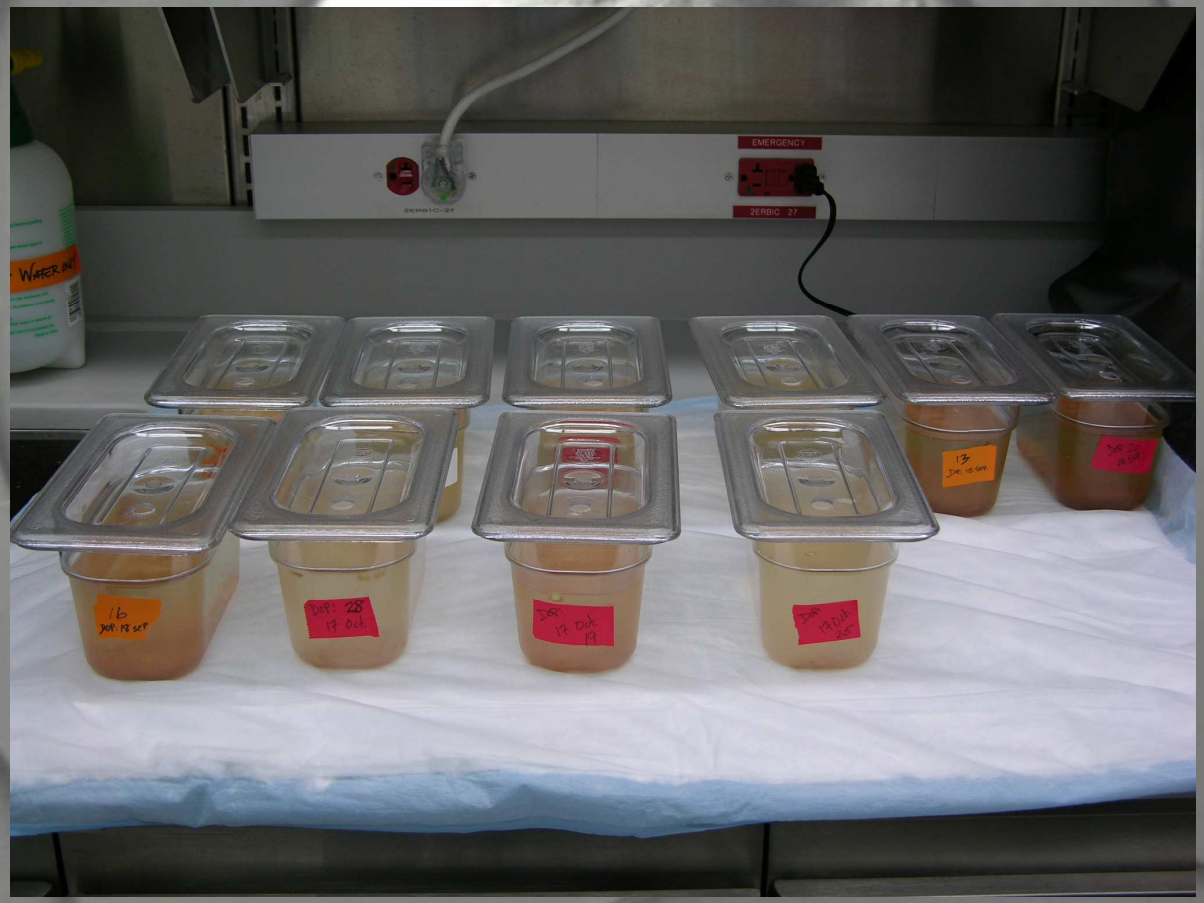
Live feed types: Paramecium

- Lots of planning and anticipation needed to supply large nursery operations
- Comparatively large footprint for small production
- Cultures are often malodorous
- Many steps in preparing culture supplies



Live feed types: Paramecium

- Comparatively large footprint for small production



Live feed types:
Rotifers (Brachionus sp.)

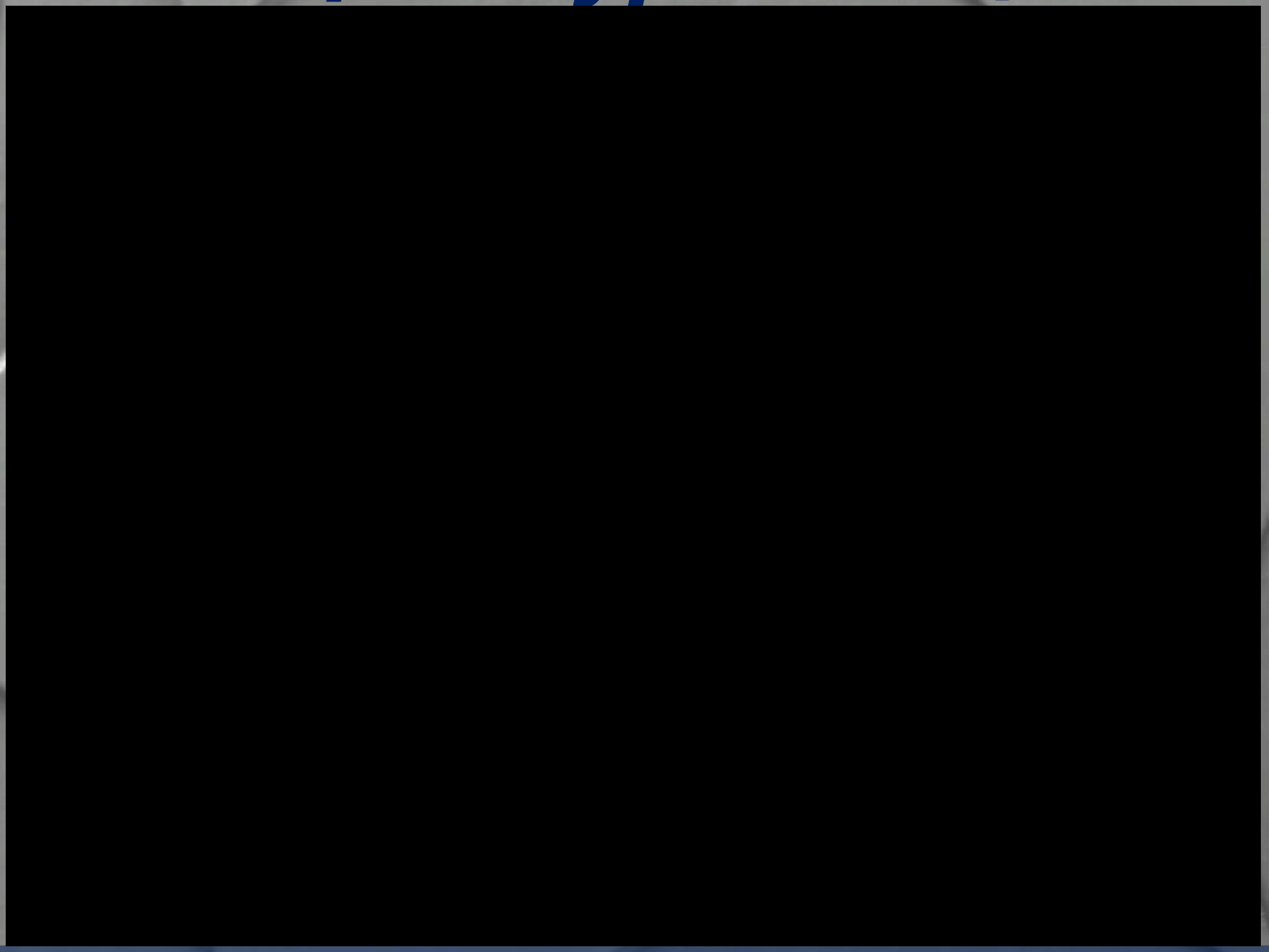
- Aquatic invertebrate (salt & freshwater)
- Approx. 2200 described species
- Extensive history as first-feed in aquaculture
- 0.185 Ug dry wt. (L-type B. plicatilis)
  (~1/9th of an artemia nauplii)

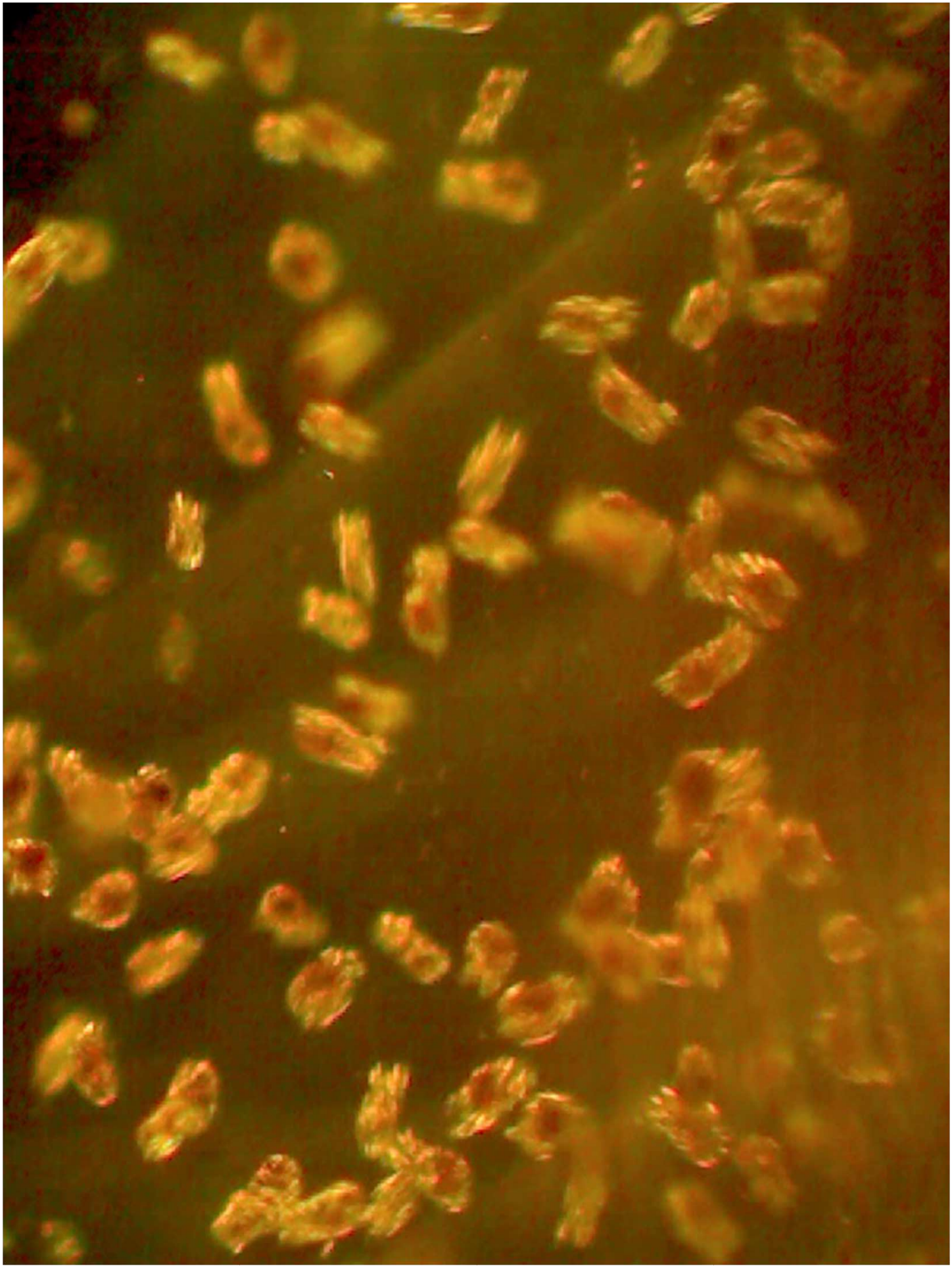
Live feed types:

Rotifers (Brachionus sp.)

- Indiscriminant filter-feeder (will eat anything, including pathogenic organisms, that it can ingest)
- Easy to culture in mass quantities (greater than 1000/ml)
- Very efficient bioencapsulation
- Excellent for first feeding zebrafish
- Slow, lumbering swimmers, perfectly suited for larval zebrafish

Live feed types: Rotifers





Typical Rotifer Culture Systems In Zebrafish Labs

Typical Rotifer Culture Systems In Zebrafish Labs

Rotifer Use Options in Zebrafish Labs

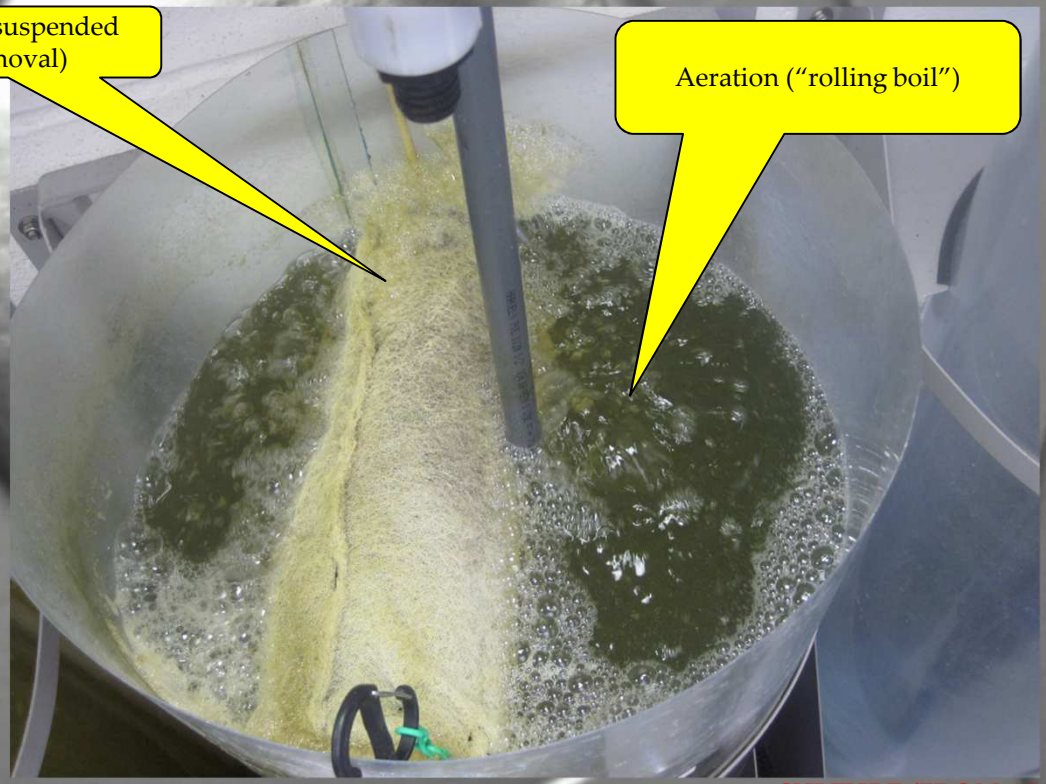
- *Regular Deliveries and Direct Use*
  - Shipped by supplier e.g. Planktovie (FR), Reed Mariculture (USA)
  - Portioned, diluted, and stored in cold (4C) for use each day
  - Appropriate for small operations, and those with very limited lab resources
  - Perhaps single best way to get started evaluating the performance of rotifers as a first feed item for larval zebrafish in your lab

Rotifer Use Options in Zebrafish Labs

- In-House Culture
  - Often the inevitable consequence of Direct Use option given enough time
  - Better control of availability and quality
  - Never stuck at Customs Office

In-house Culture -- Overview--

CULTURE (FAR VIEW)



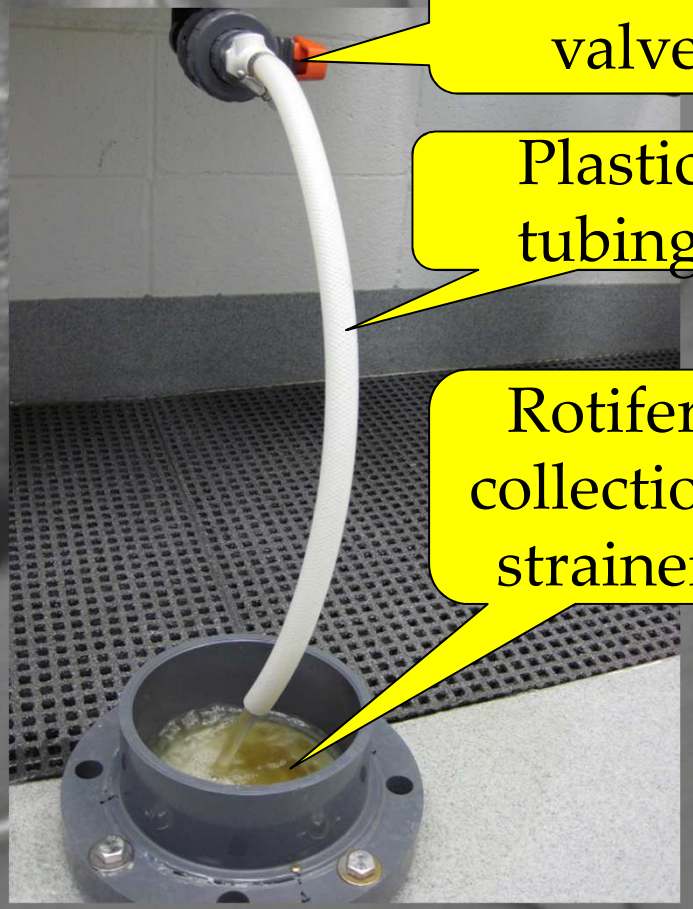
CULTURE (FROM ABOVE)

STARTUP of a Culture

1. Obtain rotifers (*Brachionus spp.*) from reputable source (e.g. Planktovie (FR), Reed Mariculture (USA))
2. Make up 25% of total culture volume receiving water (match salinity of incoming seed culture)
3. Upon receipt, float bag or bags in receiving water for 5-10minutes to equilibrate temperature.
4. Dump contents of bags in receiving water. Start moderate aeration. Leave undisturbed for an hour.
5. Begin feeding, 1-2ml of Algae Concentrate (e.g. RG Complete) per 2-3 hours.
6. At 24 hours, increase volume to 50% of total culture volume , continue feeding at above rate.
7. At 48 hours increase to volume to 75% of total culture volume , feed 2-4ml of Algae Concentrate (e.g. RG Complete) per 2-3 hours.
8. At 72 hours increase to volume to 100% of total culture volume and begin daily maintenance routine.

Daily Routine
-Continuous Culture Method-

1. Harvest approx 30% of the culture.



Daily Routine

1. Rinse the collected rotifers into a beaker
2. Dilute with adequate volume of feed-out ppt water, and distribute per needs
3. Aerate for at least 20-min to allow rotifers to acclimate

Daily Routine

4. Remove dirty floss from tank and flush using high pressure water.
When water runs mostly clear, replace the floss into the culture tank.

Note the green/brown color of dirty floss; saturated with suspended solids

Daily Routine

5. Scrub inside surfaces of tank (entire) with designated brush. This will liberate all detritus/solid wastes and send them into circulation so they can be trapped by the newly flushed floss.

Feeding The Culture

The culture is best fed automatically with a peristaltic pump, which doses from a container of algae/rotifer diet kept at 4C, automatically up to 24x per day

-The feeding setup should be checked daily to ensure that the supply of algae/rotifer diet is not exhausted AND that the supply lines are not clogged.

Reference Information

Rotifer density ratings (per ml):
Very high: >500/ml
High: 200-500/ml
Moderate: 100-200/ml
Low: 25-100/ml
Problematically low: <25/ml

Rotifers require a minimum of:
• a 30% water change per day
• one rotifer floss cleaning (twice is best).

Rotifer culture water consists of:
•10ppt saltwater
•The salinity is best achieved using suitably pure NaCl or a sea salt pre-mix such as Coralife or Instant Ocean

Backup supplies of rotifers should be kept on hand in case of emergencies.
Once every week, collect directly from the culture and place in two clean 1L flasks containing 10 ppt water. Place this at 4C. The rotifers will slow down their metabolism, and can be used as a backup to restart or bolster the culture should there be any problems.

Troubleshooting

Problem: *Low rotifer densities*

Solution: *If possible, do not harvest until densities increase- instead collect rotifers as normal, but return them to the culture tank, effectively giving them a water exchange of 30%. Also clean sides of tank and clean and replace floss.*

Problem: *Dirty culture (very dark, "cloudy", lots of suspended solids)*

Solution: *Ensure algae drip setting is correct. Floss material also may be need to be washed replaced multiple times per day until solids are removed. Algae feeding may also have to be decreased.*

Problem: Clear culture – rotifers starving.

Solution: Ensure that algae drip is not clogged. If there is no problem with drip, densities may be high enough to warrant an increase in feeding.

Thank You

Tecniplast and IWT, the entire Bernardini, Brocca, Frangelli, Nisi, and Sala families.

Kathleen, Robin, Lillian and Finley Sanker-Sanders

Aquatics Lab Services LLC
1112 Nashville Street
St. Peters, MO 63376
+1-314-724-3800
www.aquaticslabservices.com
aquaticslabservices@icloud.com