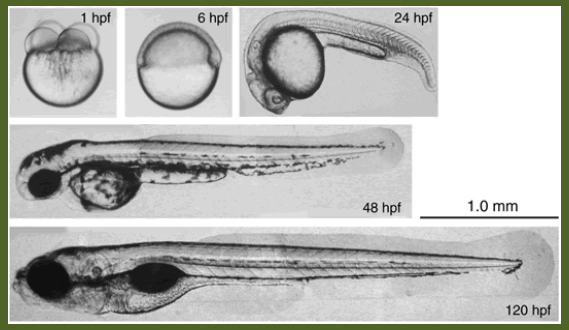
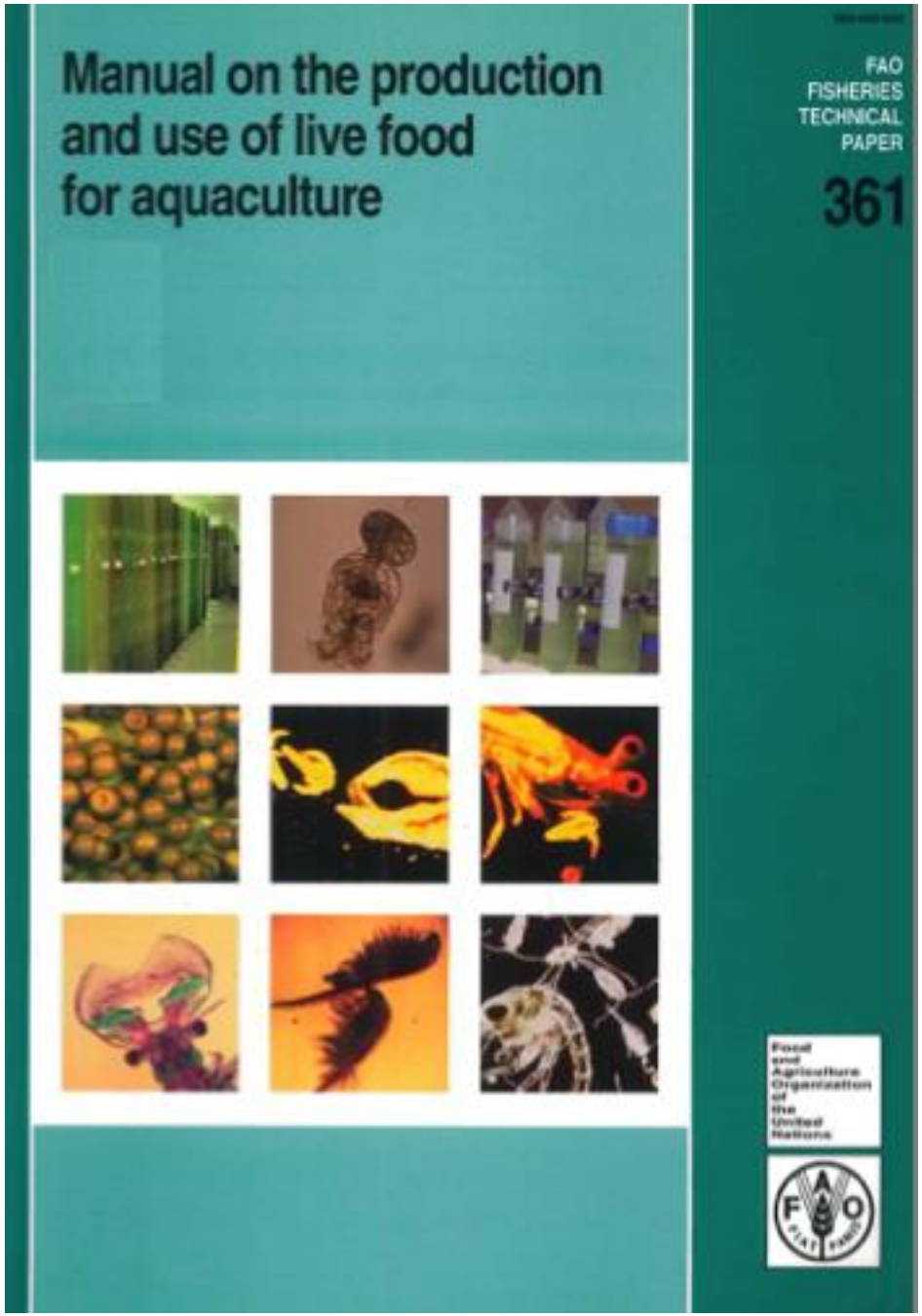
ROTIFERS for ZEBRAFISH:



Primary Literature Cited:

Manual on the production and use of live food for aquaculture
FAO Fisheries Technical Paper. No. 361



Morphology

- Rotatoria (=Rotifera) belong to the smallest metazoa of which over 1000 species have been described

- 90 % of which inhabit freshwater habitats.

- seldom reach 2 mm in body length.

- Males have reduced sizes and are less developed than females

- The body of all species consists of a constant number of cells, the different
    - *Brachionus* species containing approximately 1000 cells ,which should not be considered as single identities but as a plasma area.

- The growth of the animal is assured by plasma increase and not by cell division.

Rotifer Anatomy

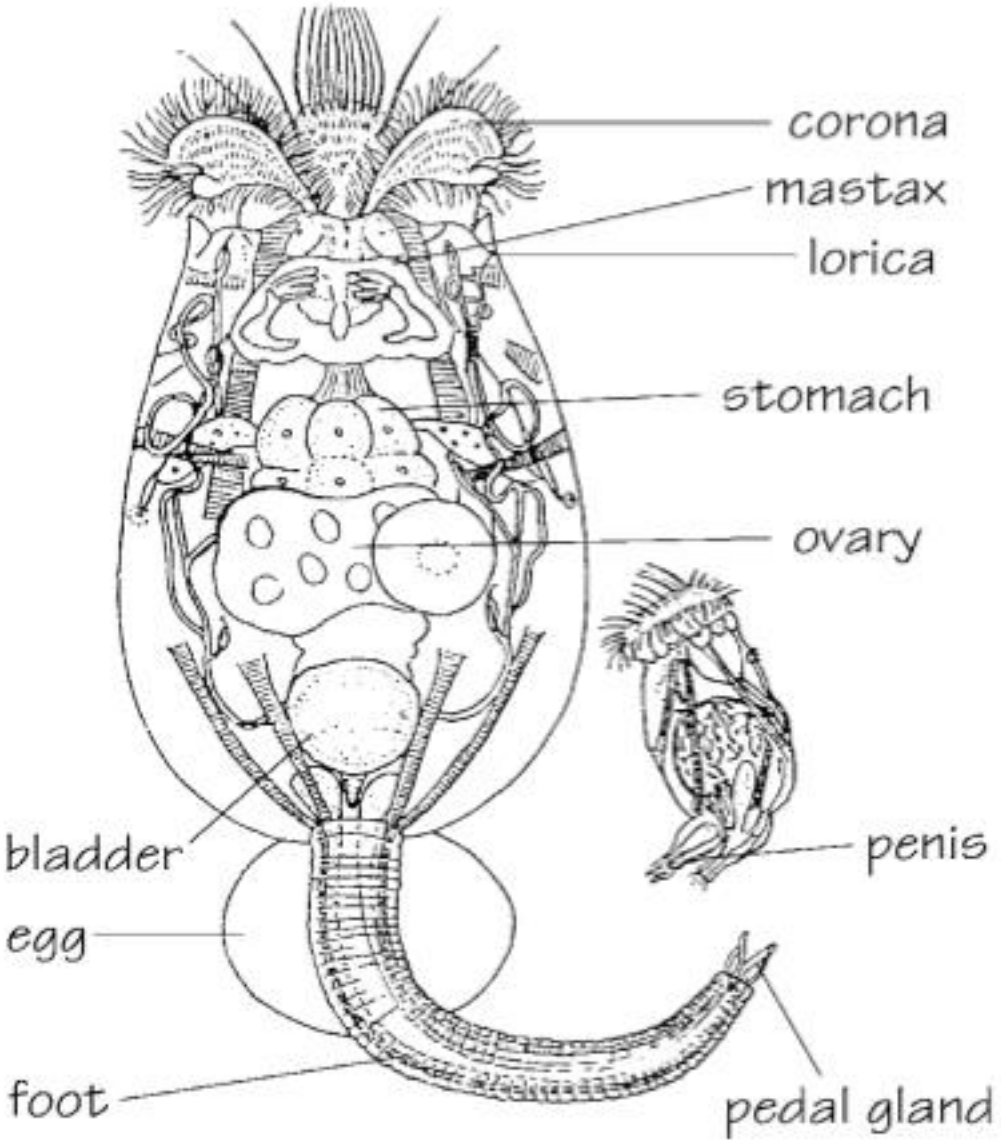


Figure 3.1. ***Brachionus plicatilis, female and male (modified from Koste, 1980).***

Biology and life history

- The life span of rotifers has been estimated to be between 3.4 to 4.4 days at 25 C.
  - Generally, the larvae become adult after 0.5 to 1.5 days and females thereafter start to lay eggs approximately every four hours.

- It is believed that females can produce ten generations of offspring before they eventually die.

- The reproduction activity of *Brachionus depends on the temperature of the environment.*

- *The life cycle of Brachionus plicatilis can be closed by two modes of reproduction*
  - *During female parthenogenesis the amictic females produce amictic (diploid, 2n chromosomes) eggs which develop and hatch into amictic females.*

  - *Under specific environmental conditions the females switch to a more complicated sexual reproduction resulting in mictic and amictic females.*

    - *Although both are not distinguishable morphologically, the mictic females produce haploid (n chromosomes) eggs. Larvae hatching out of these unfertilized mictic eggs develop into haploid males.*

Biology and life history

- *These males* are about one quarter of the size of the female; they have no digestive tract and no bladder but have an over-proportionated single testis which is filled with sperm.

- Mictic eggs which will hatch into males are significantly smaller in size, while the mictic fertilized eggs are larger and have a thick, faintly granulated outer layer.

- These are the resting eggs that will only develop and hatch into amictic females after exposure to specific environmental conditions.
    - These can be the result of changes in environmental conditions eventually creating alternations in temperature or salinity or changing food conditions.

- It should be emphasized that the rotifer density of the population also plays an important role in the determination of the mode of reproduction.

- Although the mechanism is not completely understood, it is generally believed that the production of resting eggs is a survival strategy of the population through

unfavourable environmental conditions such as drought or cold

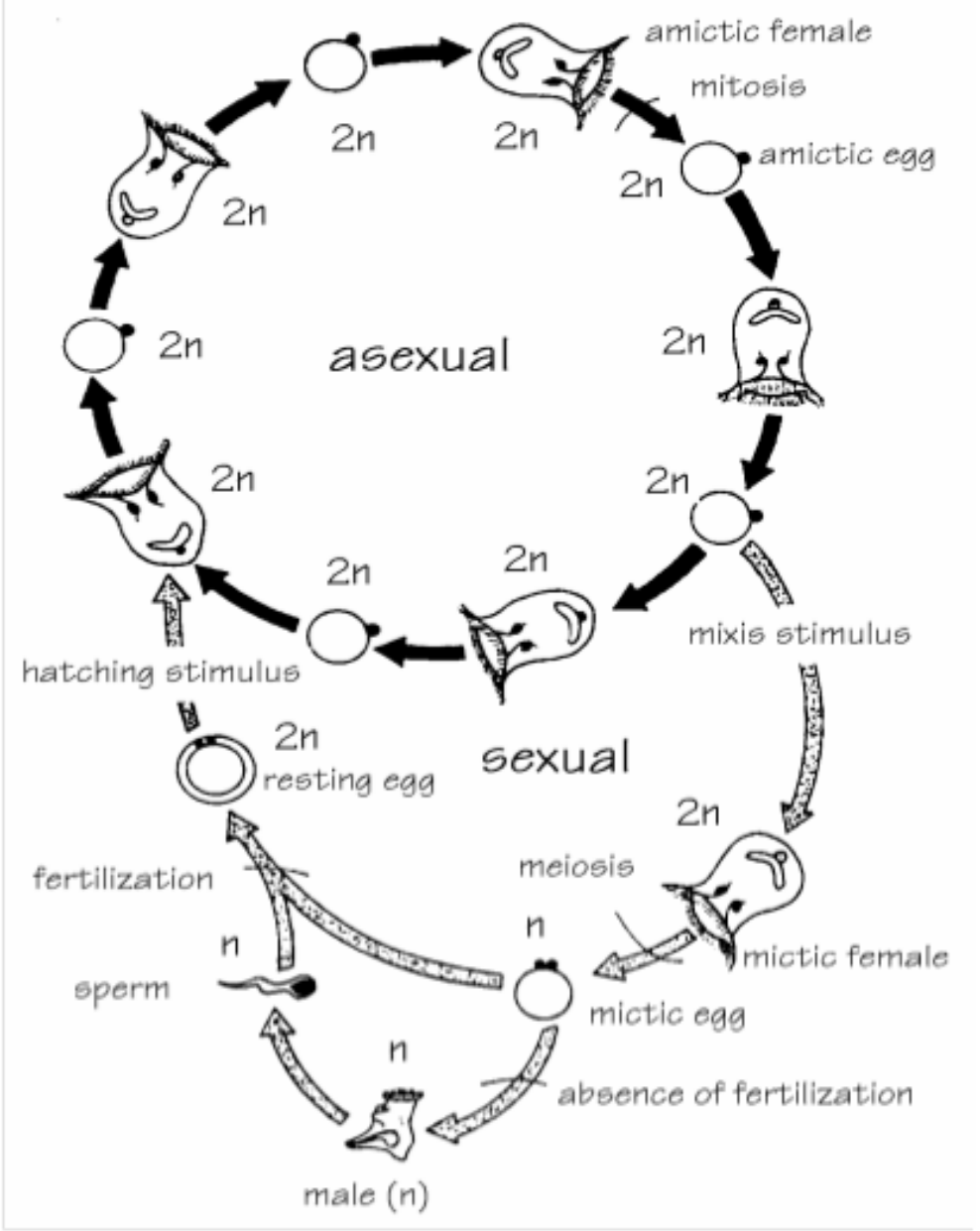
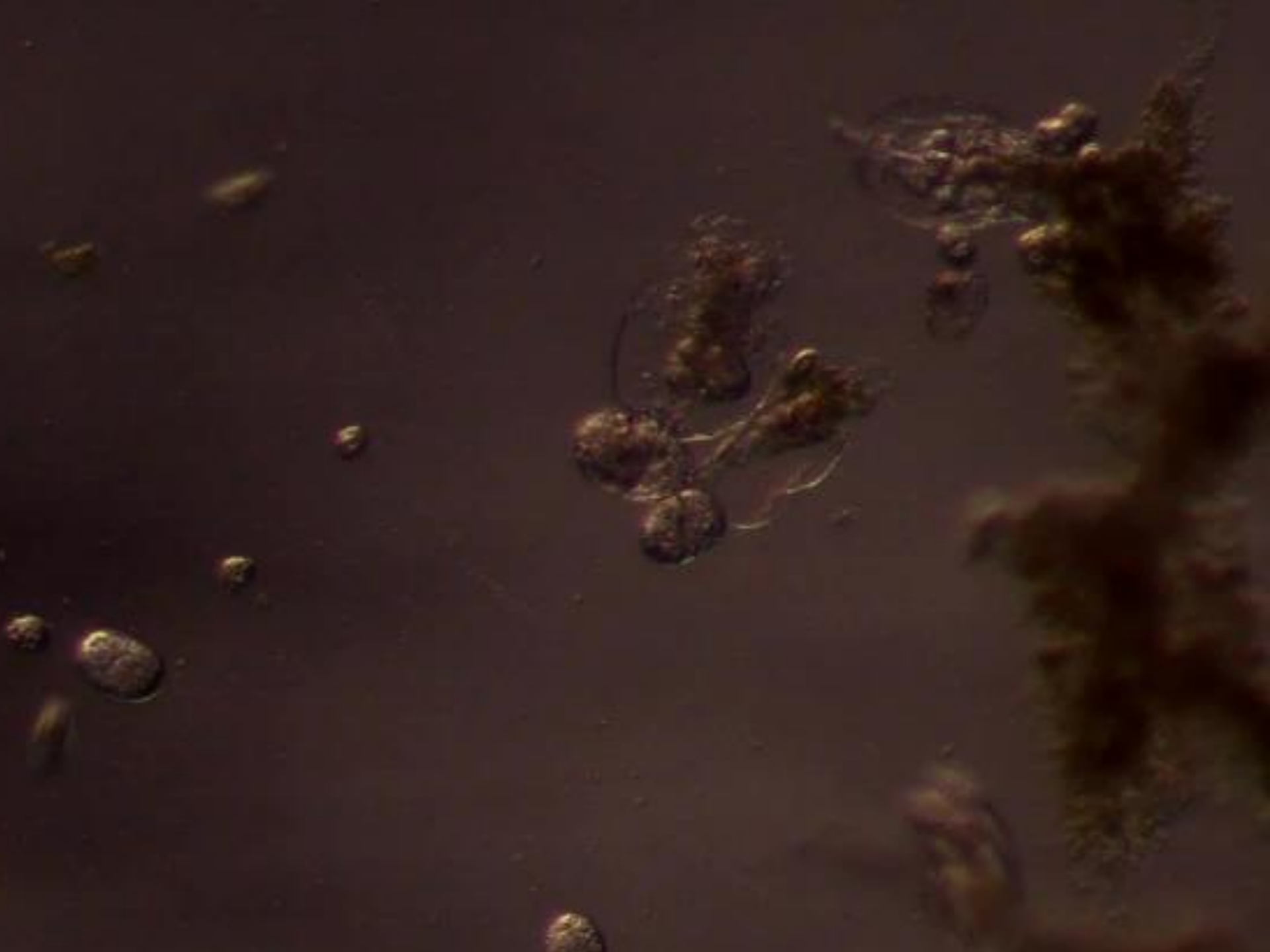


Figure 3.2. Parthenogenetical and sexual reproduction in *Brachionus plicatilis* (modified from Hoff and Snell, 1987).



Strain differences

for use in aquaculture, a simple classification is used which is based on two different morphotypes,

- *Brachionus rotundiformis* or small (S-type) rotifers
- *Brachionus plicatilis* or large (L-type) rotifers.

The S- and L- morphotypes also differ in their optimal growth temperature. The S-type has an optimal growth at 28-35˚ C, while the L-type reaches its optimal growth at 18-25˚ C.

Since contamination with both types of rotifers occurs frequently, lowering or increasing culture temperatures can be used to obtain pure cultures: rotifers at their upper or lower tolerance limit do not multiply as fast and can in this way be out-competed in favour of the desired morphotype.

Culture Methods

Batch vs. Continuous

Batch: Defined Start and End Date

example:

Cone	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	100/ml	200/ml	400/ml	800/ml			
2		100/ml	200/ml	400/ml	800/ml		
3			100/ml	200/ml	400/ml	800/ml	
4				100/ml	200/ml	400/ml	800/ml

advantages: predictable, consistent, quick

disadvantages: crashes leave you with nothing to feed out

Culture Methods

Batch vs. Continuous

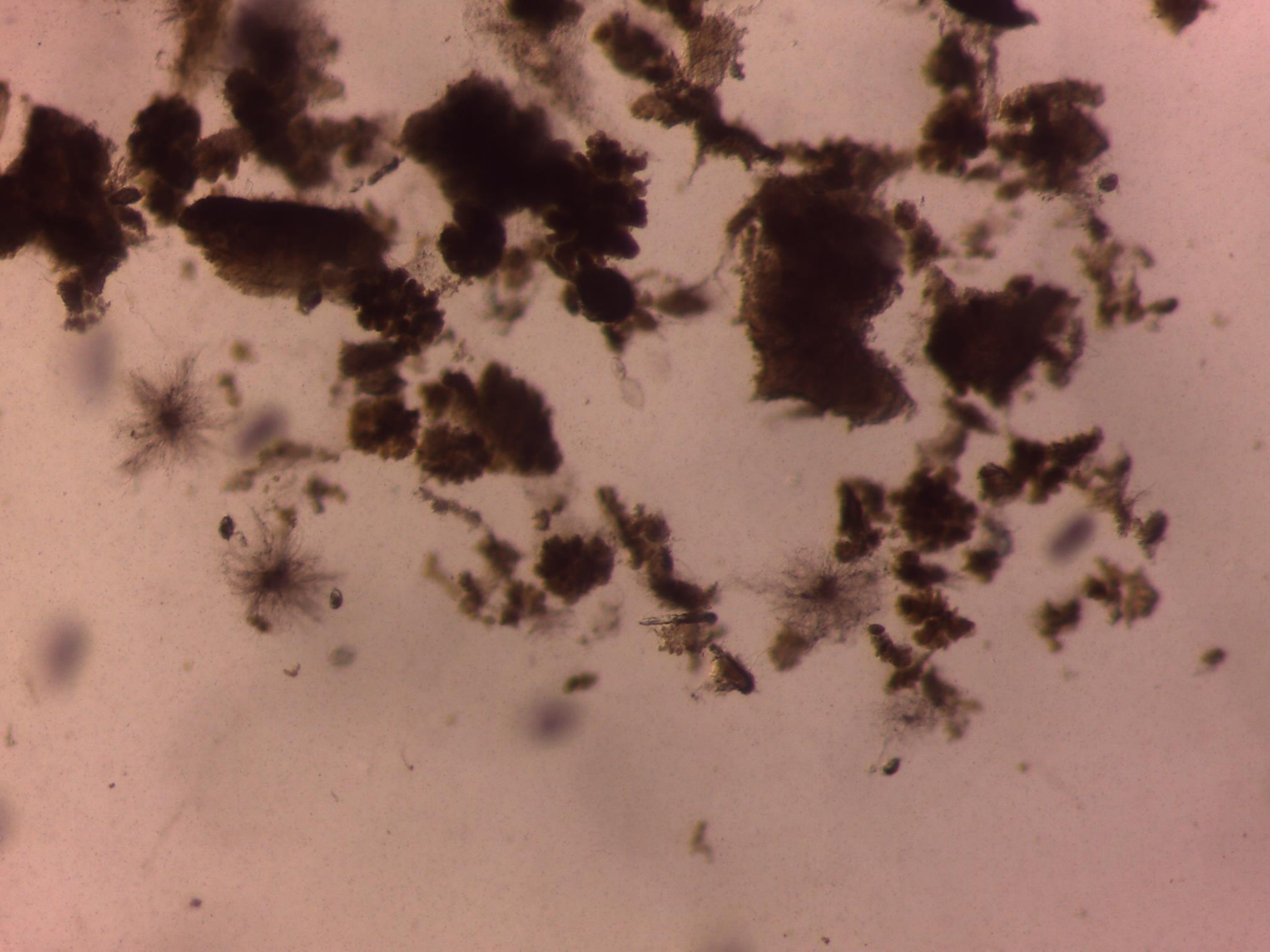
Continuous: near chemostat (perpetual)
- % is removed or water exchanged ~daily
- can skew size profile

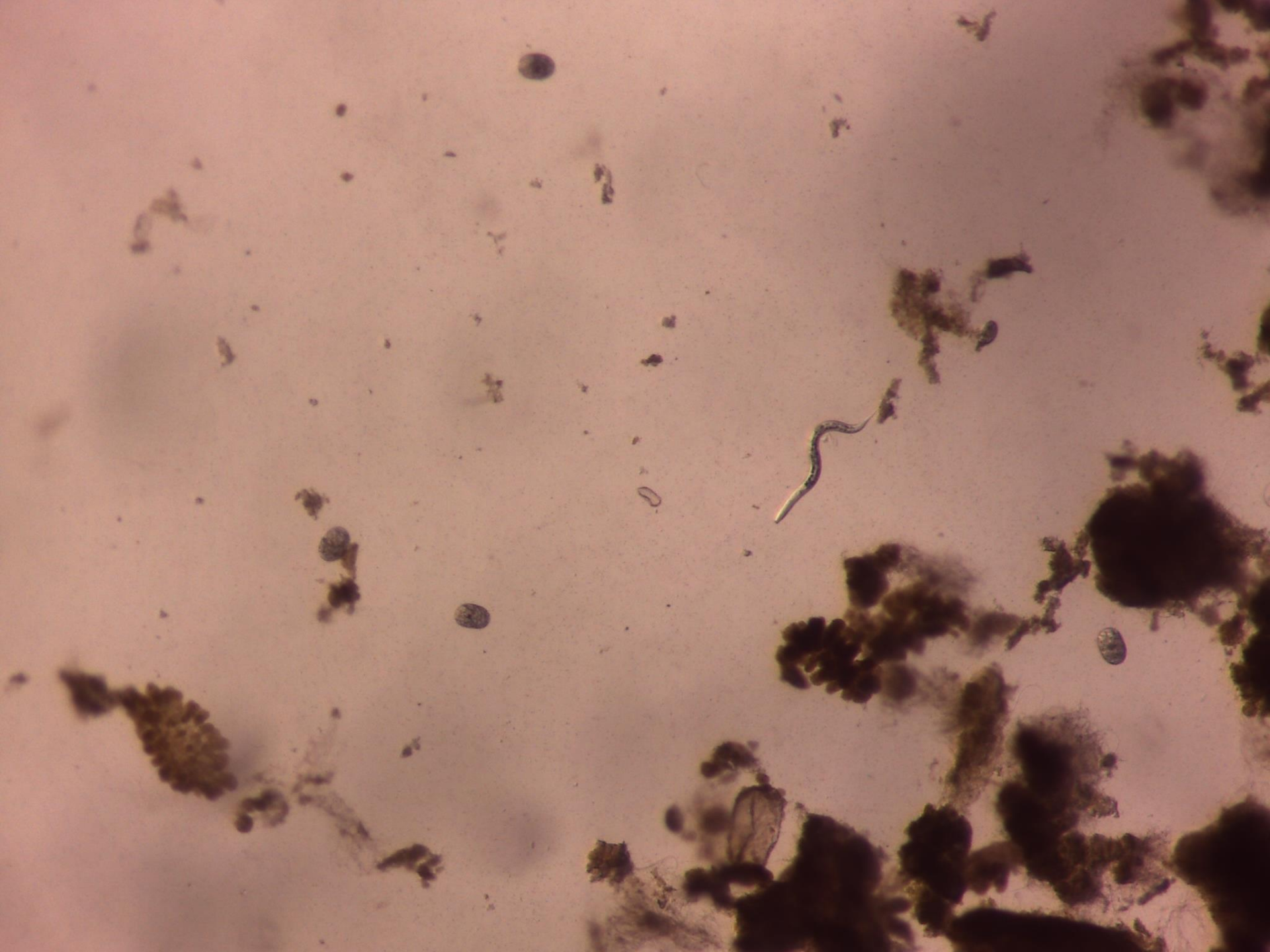
example:

25-50% harvest every day
replace with rotifer-ready water

advantages: crashes do not leave you with nothing to feed out, usually some rotifers left

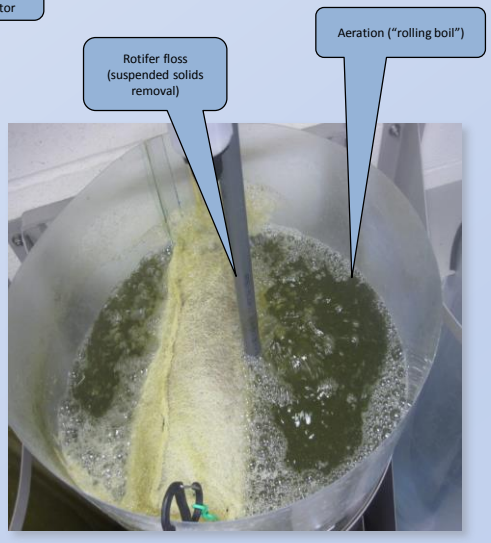
disadvantages:
more labor intensive than batch,
requires more cleaning of cones, floc traps
requires rotifer-ready water to be on-hand or mixed as needed
less predictable,
co-cultres/contaminants are not ever completely removed





1 ROTIFER CULTURE OVERVIEW

CULTURE (FAR VIEW)



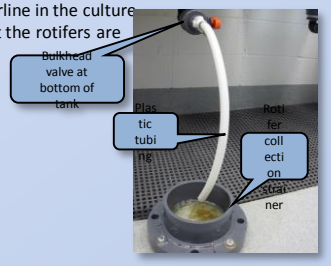
CULTURE (see from above)

2 STARTUP of 200L CULTURE

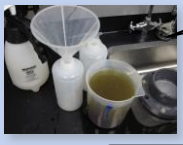
1. Order 5 million rotifers (*Brachionus plicatilis*) from Reed Mariculture (Item# RSK-5M). This will suffice to start a 200L culture
2. Make up 50L of receiving water (18 ppt salinity) in culture tank
3. Upon receipt, float bags in culture tank for 20-30 min to gradually equilibrate temperature
4. Pour contents of bags into tank. Start moderate aeration. Leave undisturbed for an hour
5. Begin feeding, e.g. 1-2 ml of Reed Mariculture RotiGrow per 2-3 hours
6. At 24 hours, increase volume to 100L, continue feeding at same rate
7. At 48 hours increase to 150L, feed 2-4ml of Algae/Rotifer Diet per 2-3 hours
8. At 72 hours increase to 200L and begin daily maintenance routine

3 DAILY ROUTINE

1. Harvest 30% of the culture: Place plastic tubing on end of the culture bulkhead and the other into a 40-55uM mesh strainer. Open up the valve completely. Keep the airline in the culture tank so that the rotifers are uniformly distributed in the water column.



2. Rinse the collected rotifers into 1-4L of pre-mixed 3ppt salinity water.



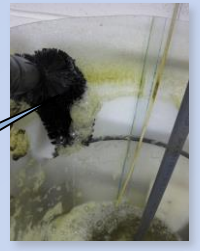
3. Raise dirty floss above tank and allow free water to drain away (~30 sec.)

Clean the floss using a high pressure jet of tap water

When effluent water runs clear, return the floss to the culture tank

4. Scrub entire inside surface of tank with designated brush

This will re-suspend all detritus/solid wastes and send them into circulation so they can be t... by the floss



4 FEEDING THE CULTURE

- The culture is fed automatically using a peristaltic pump dosing from a container of refrigerated algae concentrate
- The pump is controlled by a timer to dose at frequent intervals (preferably at least hourly)
- The feeding setup should be checked daily to ensure that the system is working properly, in particular that the supply of algae is not exhausted and that the supply lines are not clogged

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

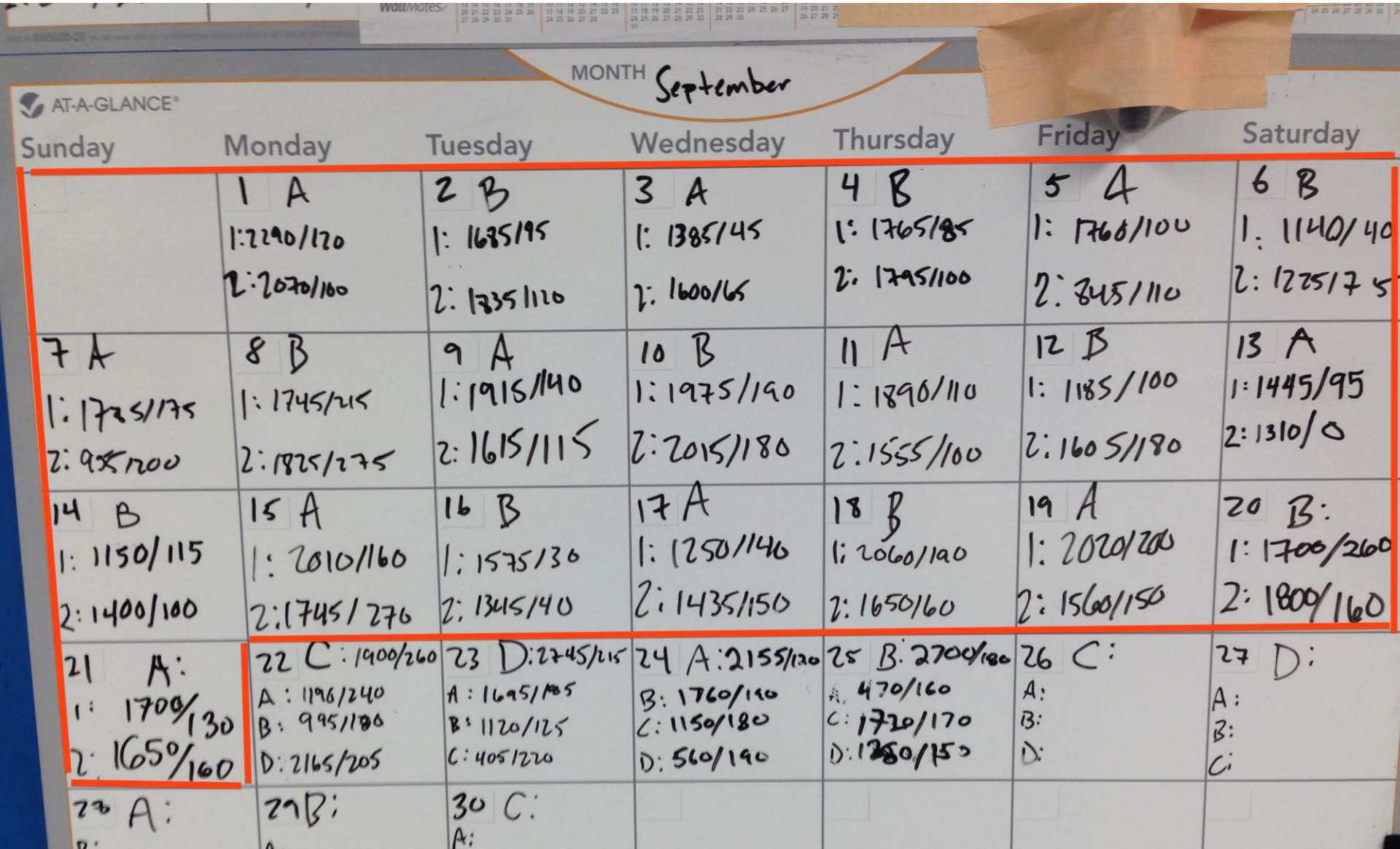
Problem: Dirty culture (very dark, "cloudy", lots of suspended solids)

Solution: Either overfeeding is occurring or many rotifers have died. Check temperature, salinity, pH, ammonia. Ensure that algae supply setting is correct. Floss material also may be need to be washed multiple times per day until excess solids are removed. Algae feeding may also have to be decreased

Problem: Clear culture – little algae in water – rotifers starving

Solution: Ensure that feed line is not clogged and that pump and timer (if used) are working properly. If these are OK, then rotifer density may be high enough to warrant an increase in feeding

Continuous Culture at WASHU




A2
B1
B2

Batch Culture at WASHU

•90% is harvested/concentrated for feed-out

•10% is collected and rinsed, then used to inoculate the cleaned cone.

Day 1	Day 2	Day 3	Day 4
C: 1900/260	D: 2745/215	A: 2155/120	B: 2700/180
A: 1190/240	A: 1695/185	B: 1760/190	A: 470/160
B: 995/170	B: 1120/125	C: 1150/180	C: 1730/170
D: 2165/205	C: 405/220	D: 560/190	D: 1250/150

Feeding of your rotifers

Lots of options:
yeast, rice bran, oil emulsions, live/preserved algae (+10 spp), prepared diets specifically for rotifers

Matter of production needs vs. convenience vs. cost

Delivery Methods:

Manual dosing: scheduled- introduces big variability, needs to be a reflection of rotifers desired, and culture method

Automated: small cost investment, (timer, tubing, pump, food container and mixer), can be geared to reflect desired rotifer production*, or* feeding the *actual* # of rotifers

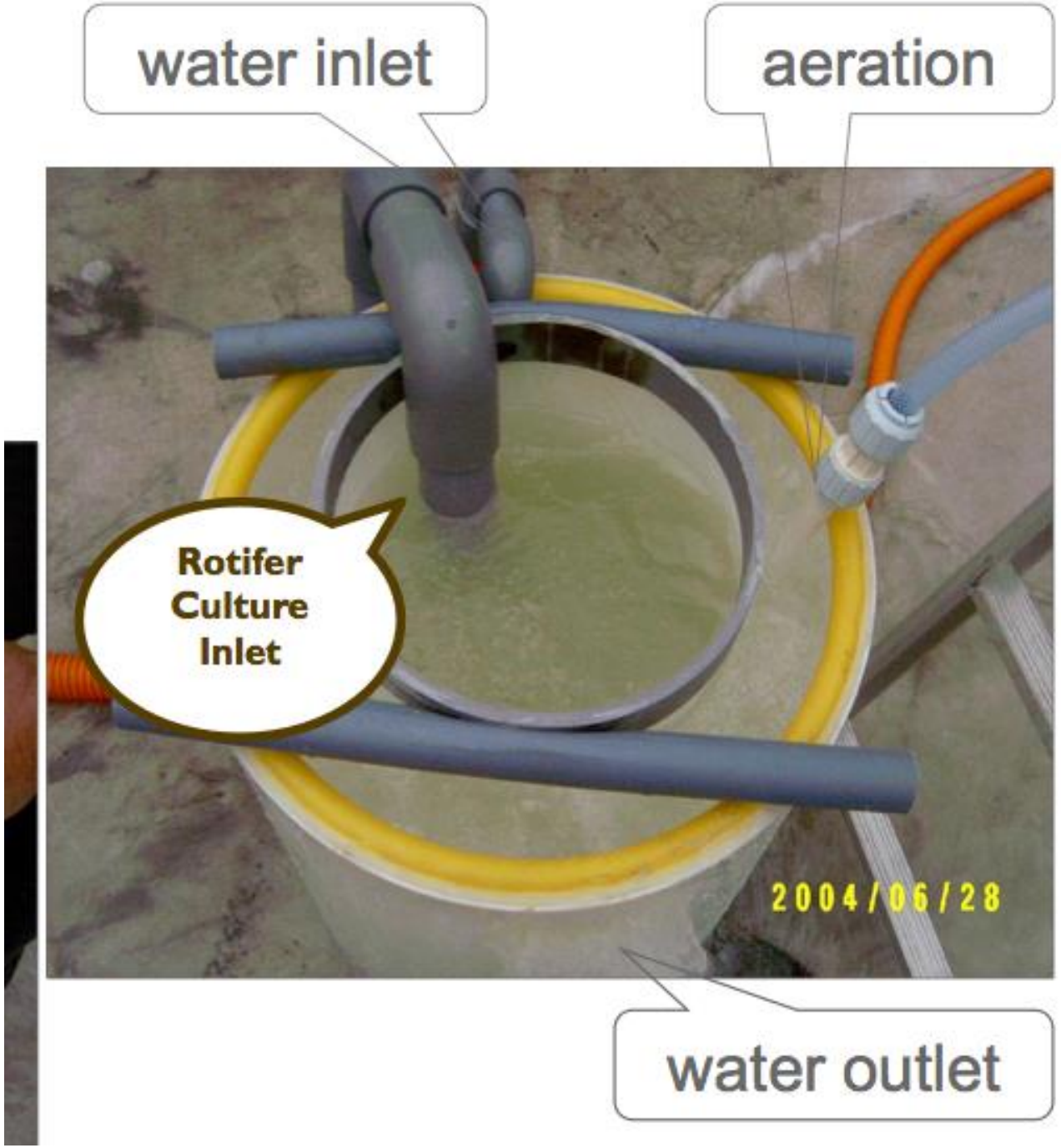
Pre-feedout Enrichment

Follow the recommendations of the product you intend to use

may require 1-4hrs of exposure

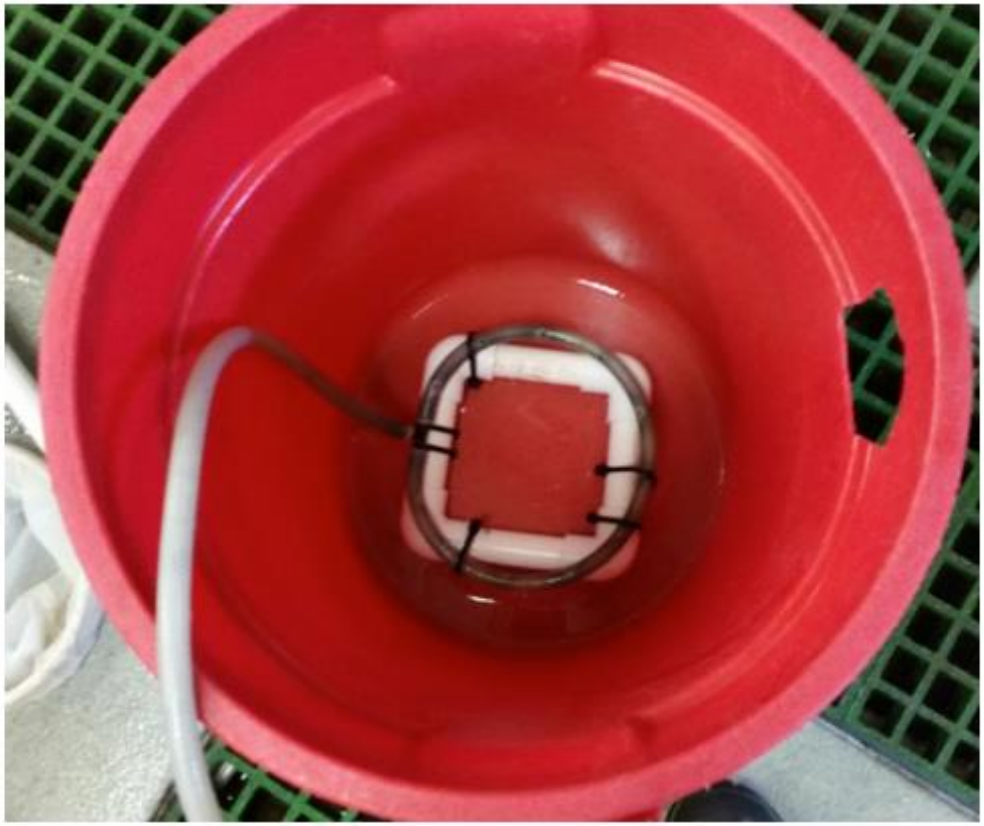
serves to not only enrich the gut of the rotifer, but to provide food for rotifer after being delivered to the tanks

Harvest


water inlet
aeration
Rotifer
Culture
Inlet
2004/06/28
water outlet

Harvest

BUCKET



AIR RING
(drilled holes)

Harvest