Live Feeds for Zebrafish

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Presentation Summary

- *Live feed Advantages/disadvantages*
- *Most common live feed choices*
  - *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*
- *Frozen/Freeze Dried*

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

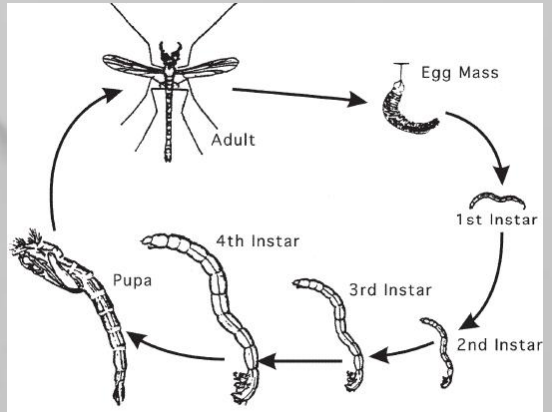
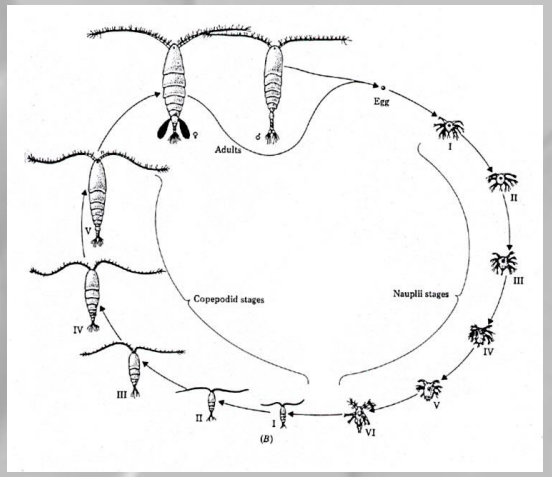
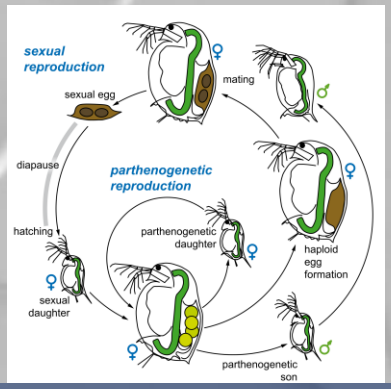


Figure 1. Chironomid life cycle (adapted from Walker 1987).





Live Diets

Advantages

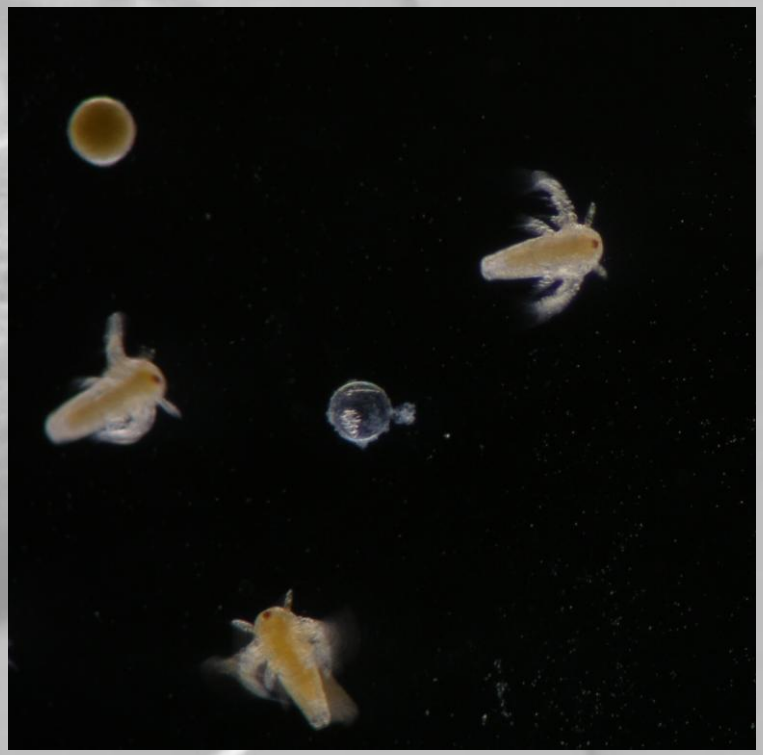
- Amenability to mass culture
- Good nutritional profiles (particularly when enriched)
- Digestible
- Attractive (motility, smell, color, shape)
- Zebrafish are adapted to feed on it, and have co-evolved with it

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: *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 and Cons

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

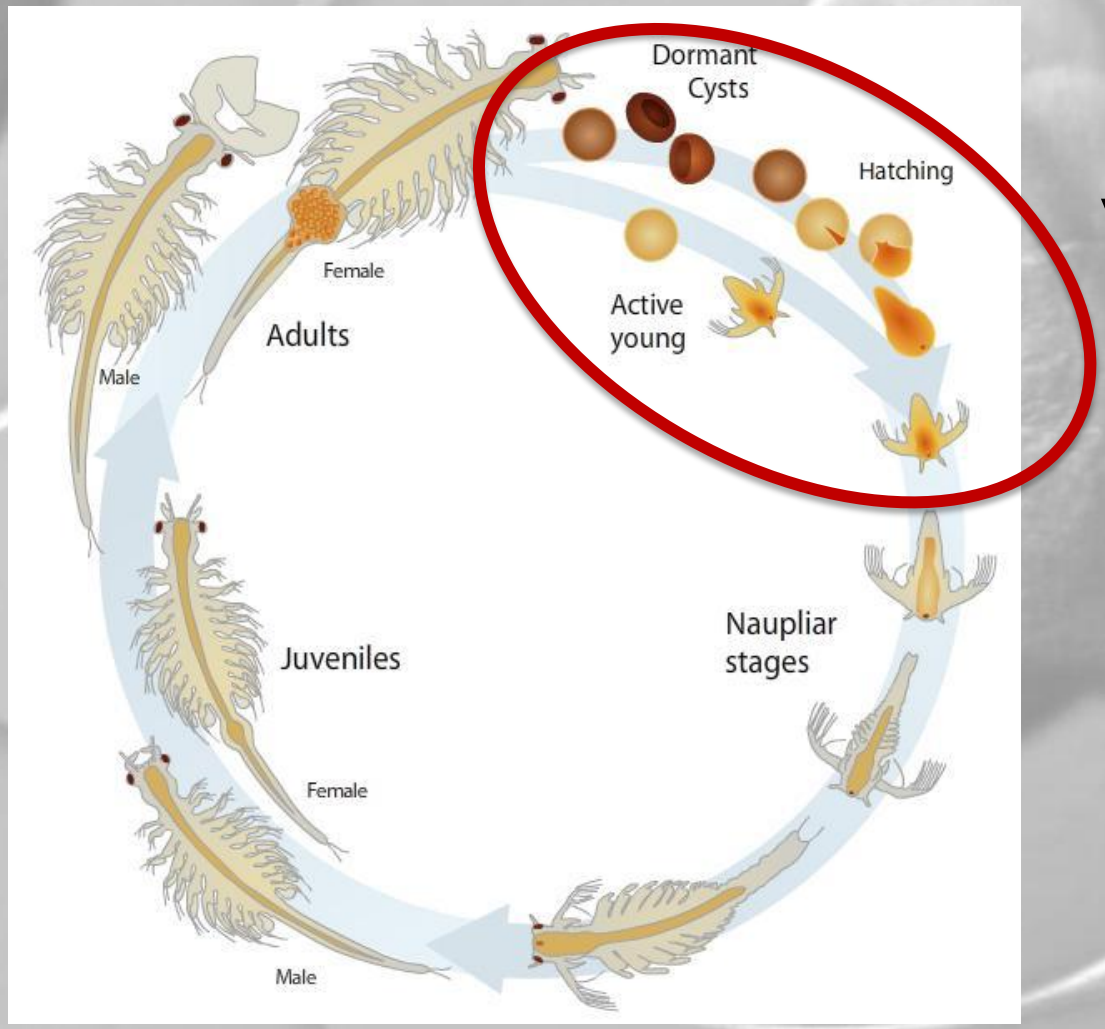
- Decapsulation necessary to sanitize and increase digestibility of un-hatched cysts
- Can be very labor intensive
- Protracted holding time required for enrichment
- Ever increasing cost
- Most are too large for 5-dpf zebrafish
- Can be source of fish pathogens and other biological contaminants

Live feed types: *Artemia*

Cons

Can be source of fish pathogens and other biological contaminants

Live feed types: *Artemia*



Life Stages

Very limited use in zebrafish labs

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

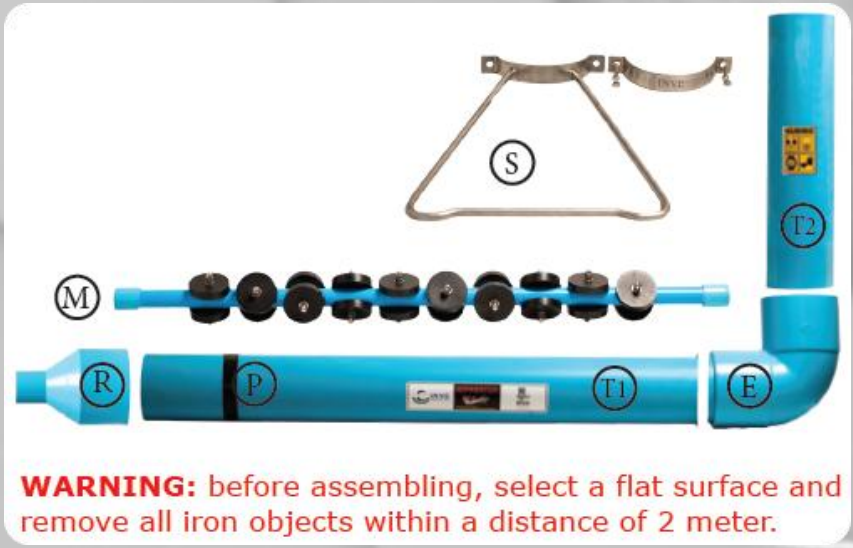
Live feed types: *Artemia*

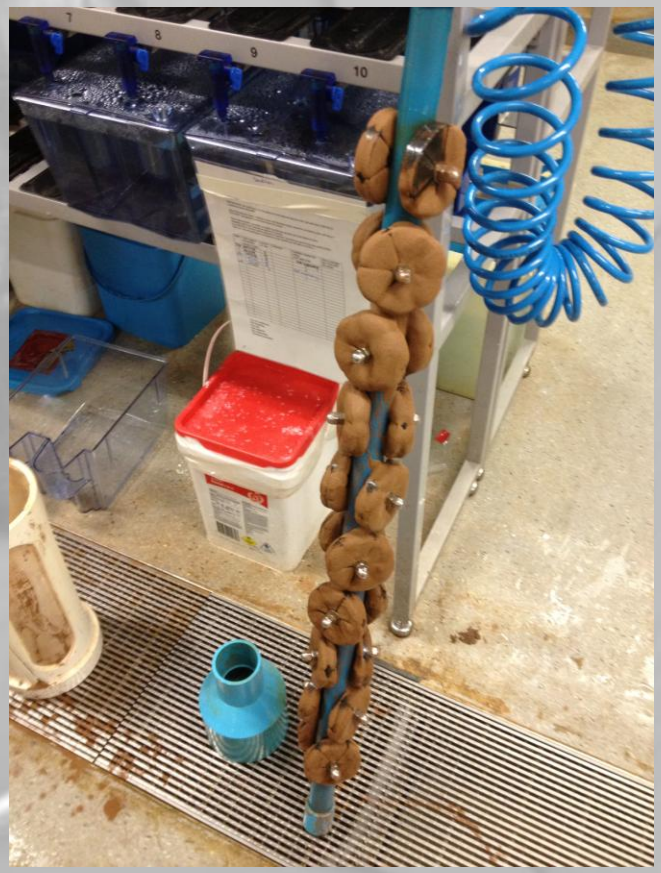
Alternatives to the Traditional Artemia Paradigm

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

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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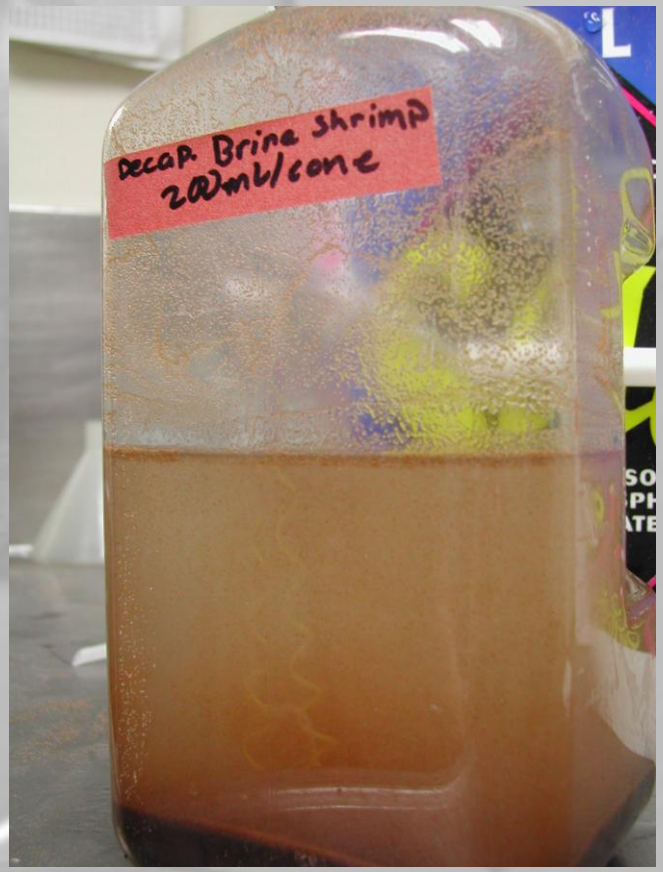
Live feed types: *Artemia*

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

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

Live feed types: *Artemia*

Typical Process in Zebrafish Labs: Decapsulation

Live feed types: *Artemia*

If you use *artemia* be aware of:

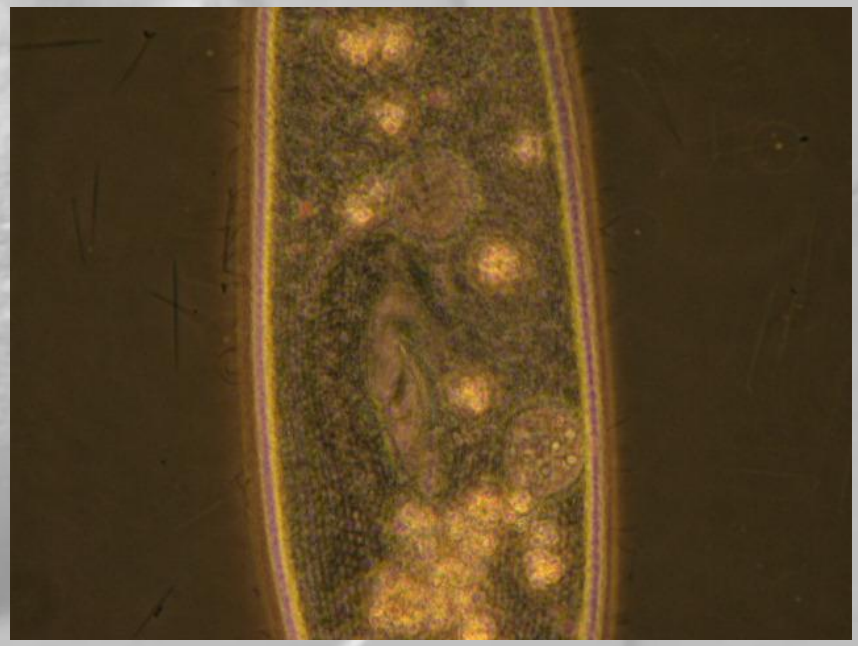
- Discrepancies and variation in harvest/concentration
- Storage Methods are Critical
  - Fragile and easily suffocated
  - Will continue to grow and molt
- Wild Variation in feed-out amounts between staff
- It is a wild captured organism, and is prone to be contaminated with organic materials and pathogenic 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

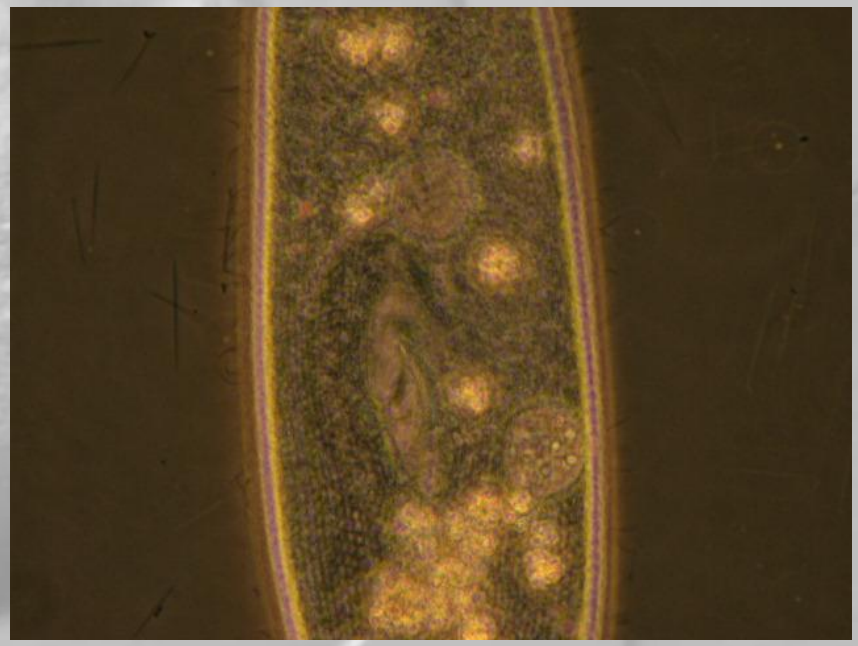


Live feed types: *Paramecium*

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

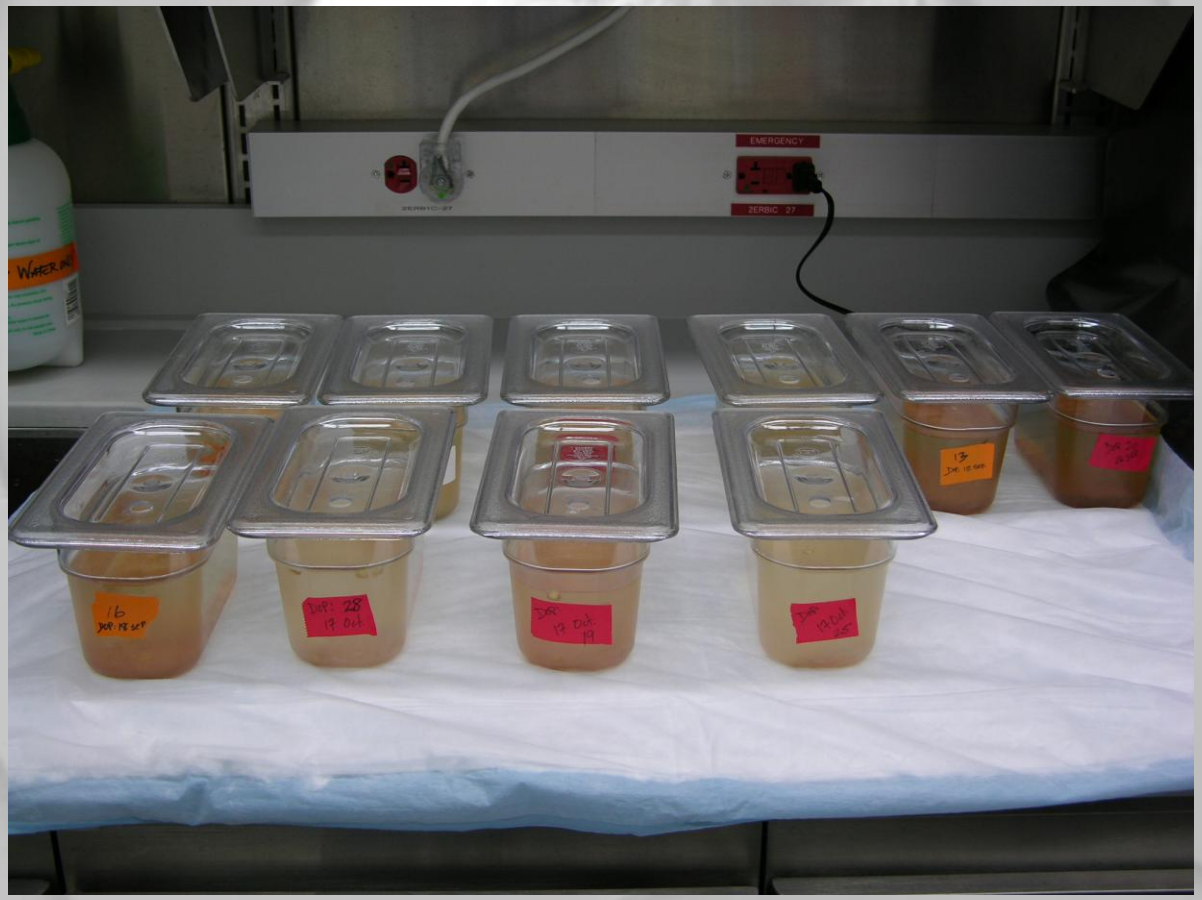
Live feed types: *Paramecium*

- 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:

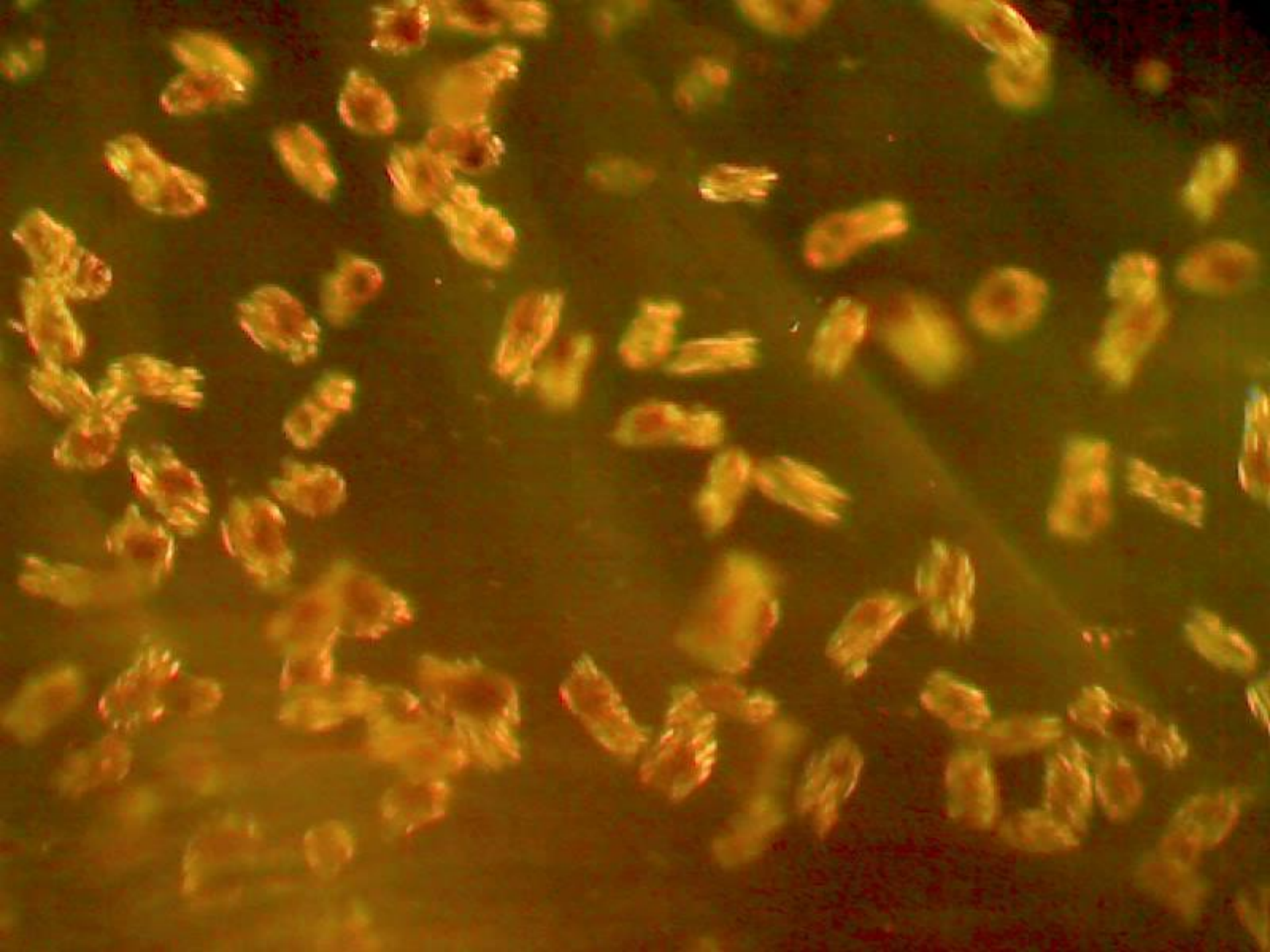
Rotifers (*Brachionus* sp.)

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



TYPICAL ROTIFER CULTURE SYSTEMS IN ZEBRAFISH LABS

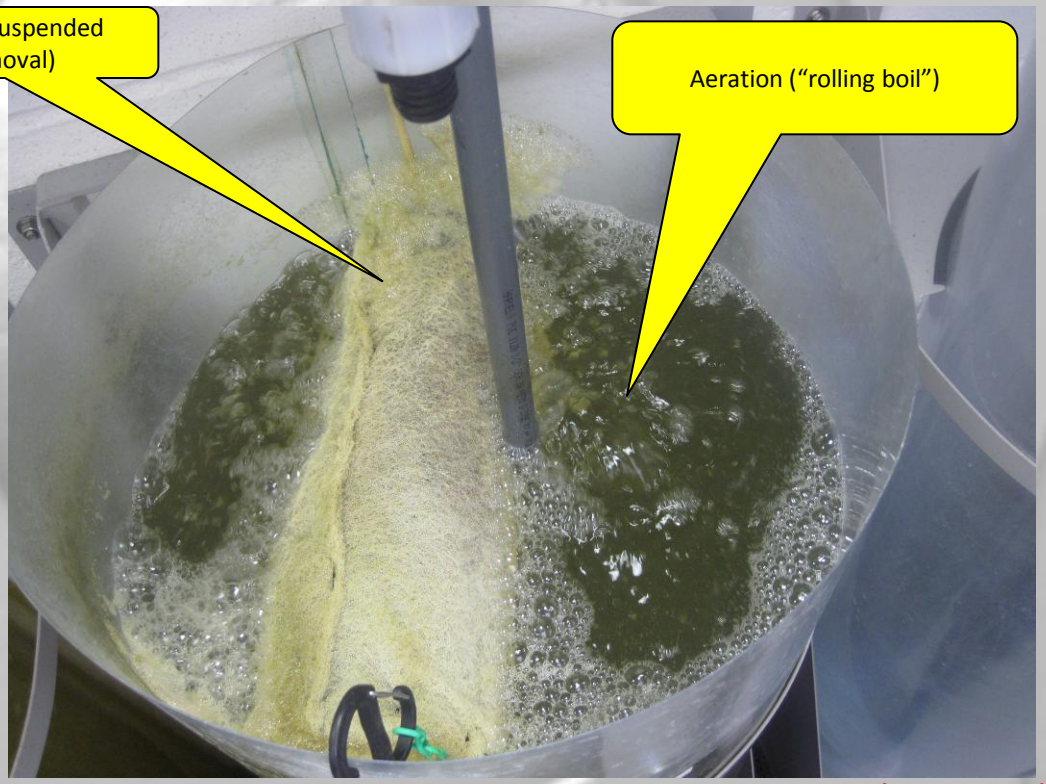
TYPICAL ROTIFER CULTURE SYSTEMS IN ZEBRAFISH LABS

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
- 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)



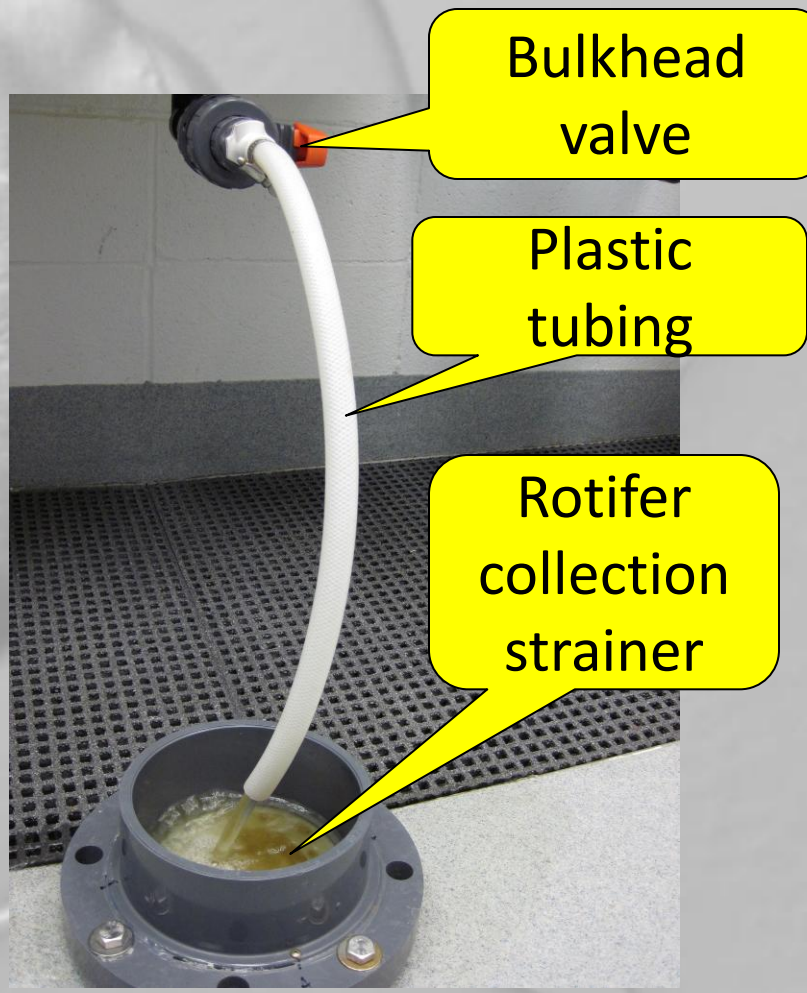
CULTURE (FROM ABOVE)

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.

Simplification of culture process (early stages) using rotifers

- Egg Collection, thorough rinse with egg-water>
- cleanup+methyl blue>
- 28.5C>
- 24hpf cleanup>
- 48hpf ceanup>
- 3dpf cleanup, no more methyl blue>
- 4dpf check H2O quality>
- 5dpf, check swimming/gas-bladders are +75%, place in ~1" of H2O and feed>
- 6dpf - 14dpf add rotifers if needed, add H2O, etc.

How much (many) rotifers?

1) In aquaculture there is one unit of measure that is consistently used to communicate how much live feed is offered to (larval) fish- that being **prey density** (# of prey items/ml is very common when talking about microscopic organisms).

2) While related in some ways, the question I most often get from zebrafish labs is "how many rotifers should I feed my baby fish?"

How much (many) rotifers?

According to Nutrient Requirements of Fish and Shrimp, published by the National research Council of the National Academies (2011)

"Certain prey densities seem to be effective across a number of larval fish species (e.g. 10-20-rotifers per mL); however, optimum prey density may vary with the species. Ontogeny, size of prey, and culture system (Lee and Ostrowski, 2001)

How much (many) rotifers?

According to Nutrient Requirements of Fish and Shrimp, published by the National research Council of the National Academies (2011)

"The best way to determine optimal densities is to monitor both number of larval prey at intake and density of prey in the culture system to avoid under and over feeding (Palmer et al,. 2007). Underfeeding retards larval growth and development, whereas, overfeeding can result in reduced capture success and can also lower water quality…(Lee and Ostrowski, 2001)."

How much (many) rotifers?

# of tanks (3.5L) to feed rotifers to	# of L needed for feedout (30mL/tank)	# rotifers (M) needed for feedout
300	9	31.5
275	8.25	28.875
250	7.5	26.25
225	6.75	23.625
200	6	21
190	5.7	19.95
180	5.4	18.9
170	5.1	17.85
160	4.8	16.8
150	4.5	15.75
140	4.2	14.7
130	3.9	13.65
120	3.6	12.6
110	3.3	11.55
100	3	10.5
90	2.7	9.45
80	2.4	8.4
70	2.1	7.35
60	1.8	6.3
50	1.5	5.25
40	1.2	4.2
30	0.9	3.15
20	0.6	2.1
10	0.3	1.05

How to determine your feed (rotifers, artemia, paramecia, prepared diets) dosages?

After you have a method that is working for you, *then* analyze what you have been doing and develop tables, charts, etc.

- ~1000-rotifers (L-type) per fish, ~30000/tank/day

Results:

- This is my “ideal” situation. Less will work fine.
- Rotifers may not need to be added to the tanks every day- sometimes the rotifers bloom in the tank, eliminating the need to add more.

Typical Zebrafish Facilities use 2 different, but similar, methods of employing rotifers as a first-food item for larval fish.

- Polyculture method
  - We will briefly review
  - At least 3-papers published describe methodology and anticipated results
    - Lawrence et al 2015, Best et al 2010, Lawrence et al 2016,
- Incremental Feeding
  - We will explore in detail here
  - Widely used across all aquaculture disciplines

Polyculture method

- Overview
  - Combining into one tank:
    - rotifers
    - algae (rotifer food)
    - Larvae
  - Total water level changes very little over time

Polyculture method

- Pros
  - Low level of labor and involvement- perceived
  - Good survival expected
  - Shorter duration than incremental
- Cons
  - Still requires daily addition of rotifers
  - Not possible to see fish until end of polyculture phase due to algae
  - Reported methods use *more* rotifers than incremental feeding
  - Fish are smaller at end of phase than incremental method
  - HUGE differences between labs in terms of methods

Polyculture method

- HUGE differences between labs in terms of methods

publication info			Rotifer Information		Statistics	
			rotifers/fish/day	Prey Density (rotifers/ml in larval rearing		
paper ref.	year published	journal	typical max	typical max	reported survival %	sample size (n=)
Lee Ostrowski,	2001	Aquaculture	1000	20	N/A	N/A
Markovich Brown	2005	WAS Abstract	3000	unknown	42	4
allen wallace sisson	2016	Zebrafish	321	6	82	2
best et al	2010	Zebrafish	2664	333	94	5
martins et al	2016	Zebrafish	2571	180	N/A	N/A
lawrence et al	2015	Zebrafish	10667	800	98	6
lawrence et al	2016	JOVE	18675	747	95	1
Hedge et al	2015	WAS Poster	2500	200	N/A	N/A
big lab	current	N/A	1000	unknown	95	N/A
Aoyama	2015	Zebrafish	153	22	91.1	9

Incremental method

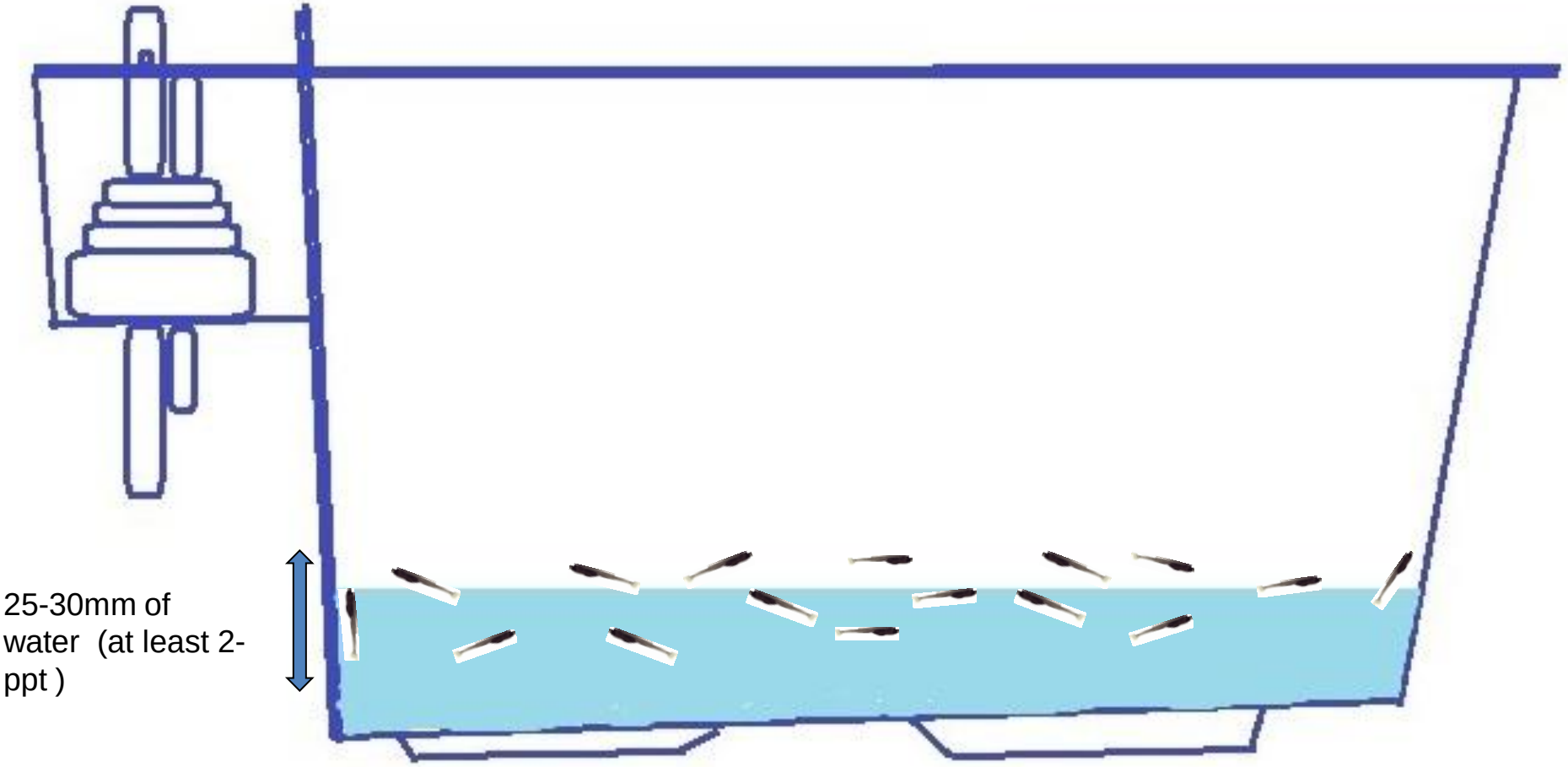
- Overview
  - Combining into one tank:
    - Rotifers (gut-loaded with algae)
    - Larval fish

Incremental method

- Pros
  - Low level of labor and involvement
  - Good survival expected
  - More constant prey density
  - Few rotifers needed
  - Larval fish are visible at all times
- Cons
  - requires daily addition of rotifers
  - Requires regular addition of water to culture

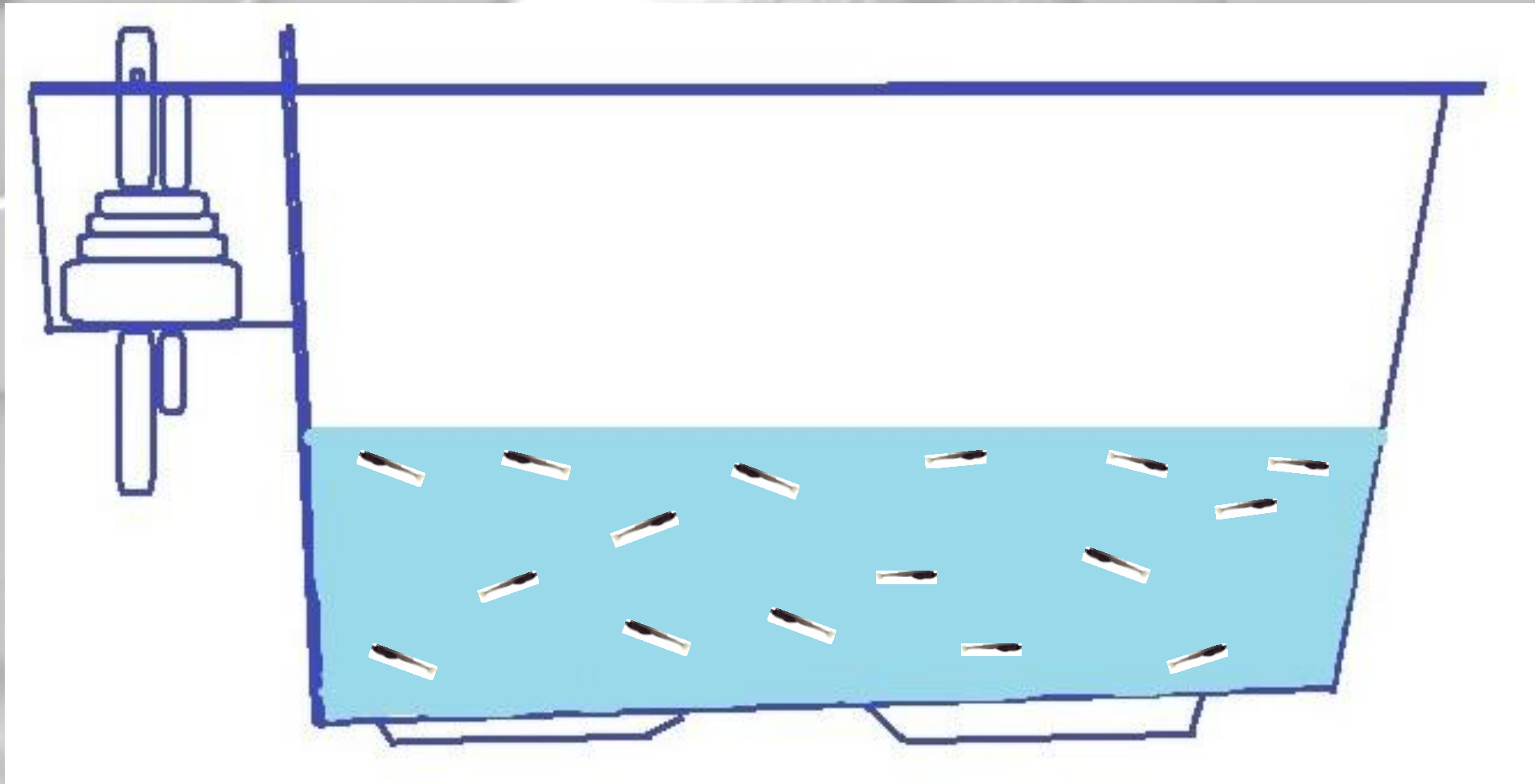
Incremental method

- Larval fish 5-6dpf
  - Rotifers (1000 per larval fish/day)



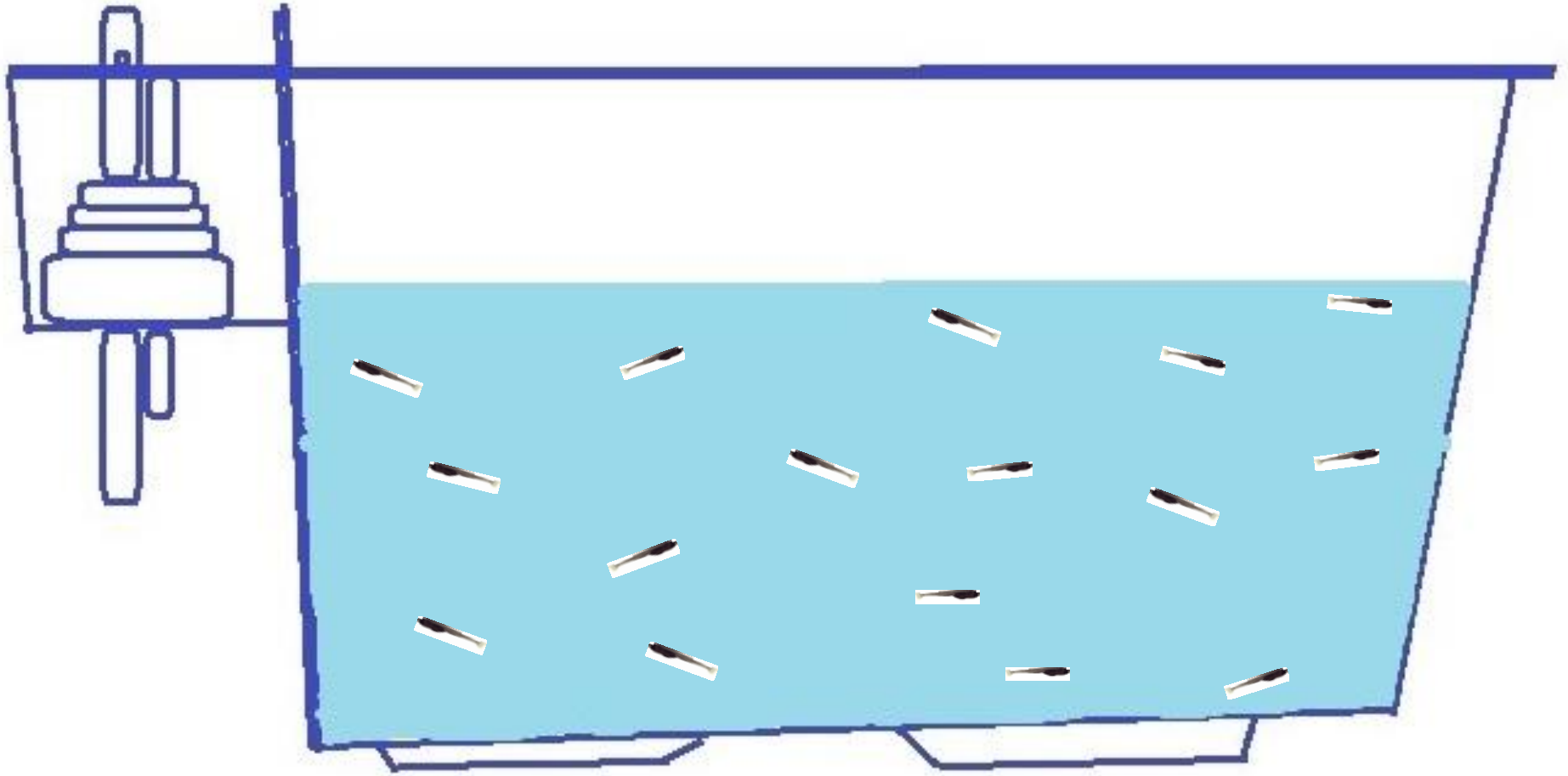
Incremental method

- Larval fish 7-8dpf
  - Rotifers (1000 per larval fish/day)
  - Add 25-30mm water (at least 2ppt)



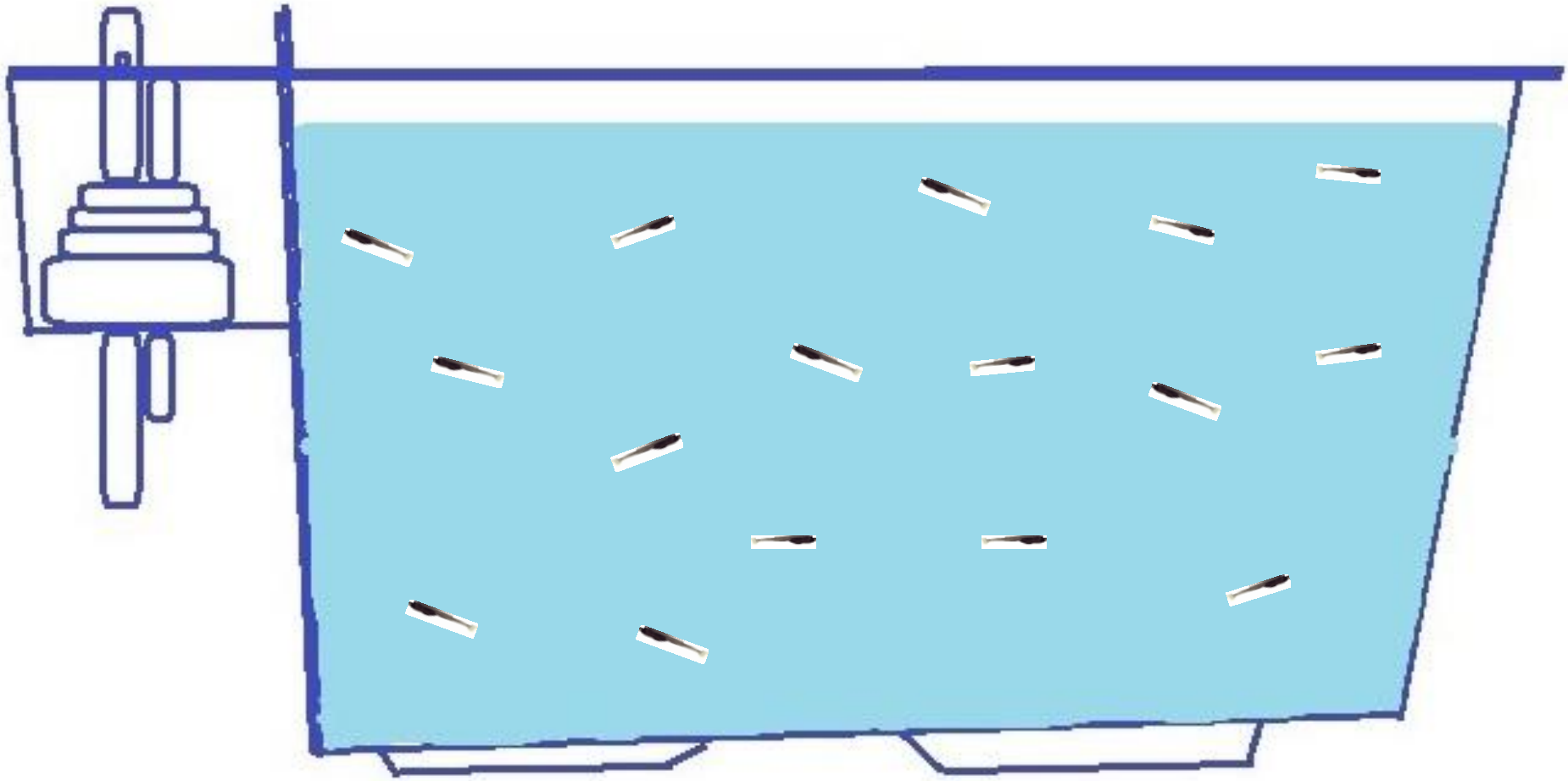
Incremental method

- Larval fish 9-10dpf
  - Rotifers (1000 per larval fish/day)
  - Add 25-30mm water (at least 2ppt)



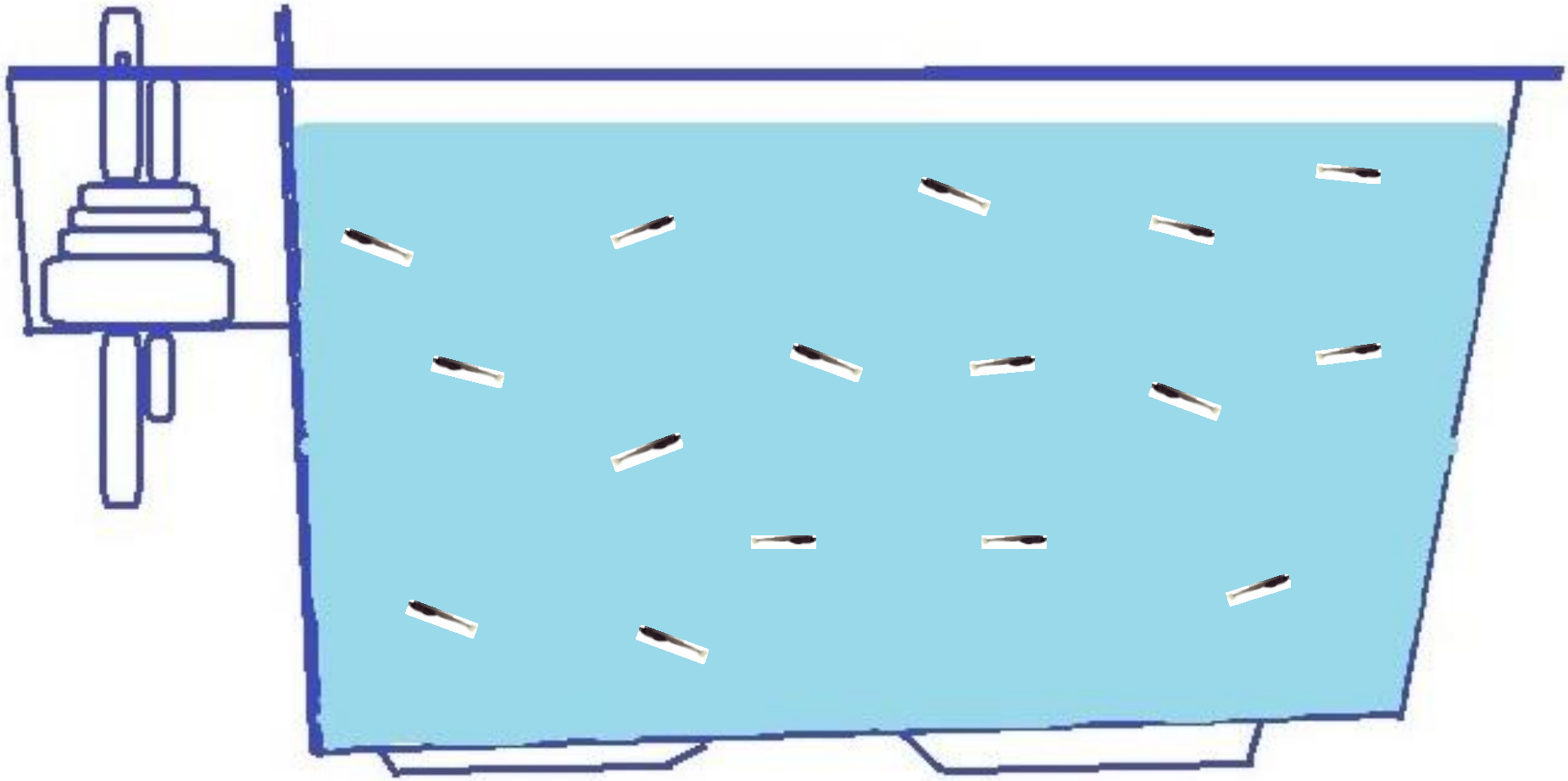
Incremental method

- Larval fish 9-10dpf
  - Rotifers (1000 per larval fish/day)
  - Add 25-30mm water (at least 2ppt)



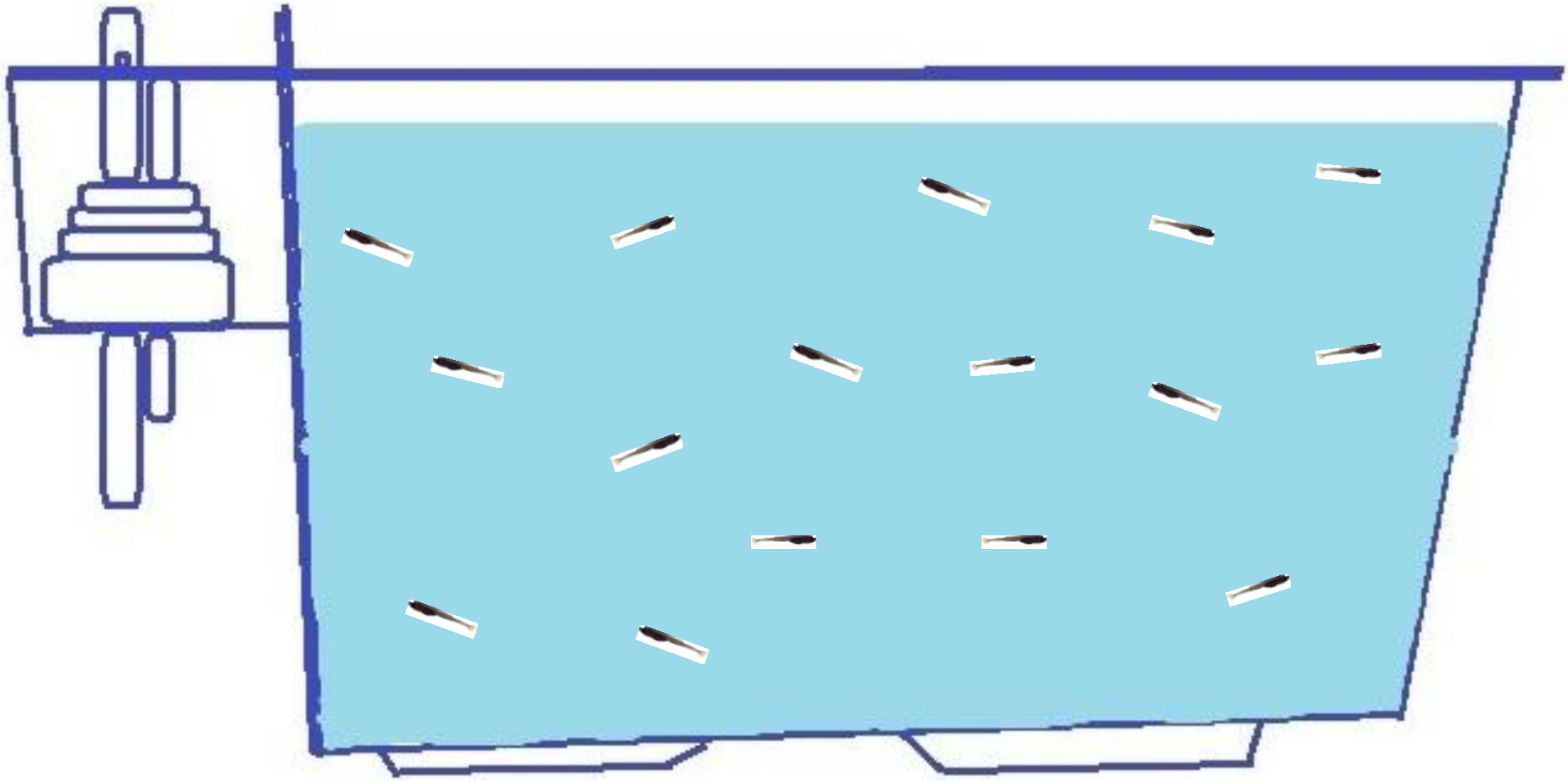
Incremental method

- Larval fish 11-12dpf
  - Rotifers (1000 per larval fish/day)
  - Add 25-30mm water (at least 2ppt) to flush waste



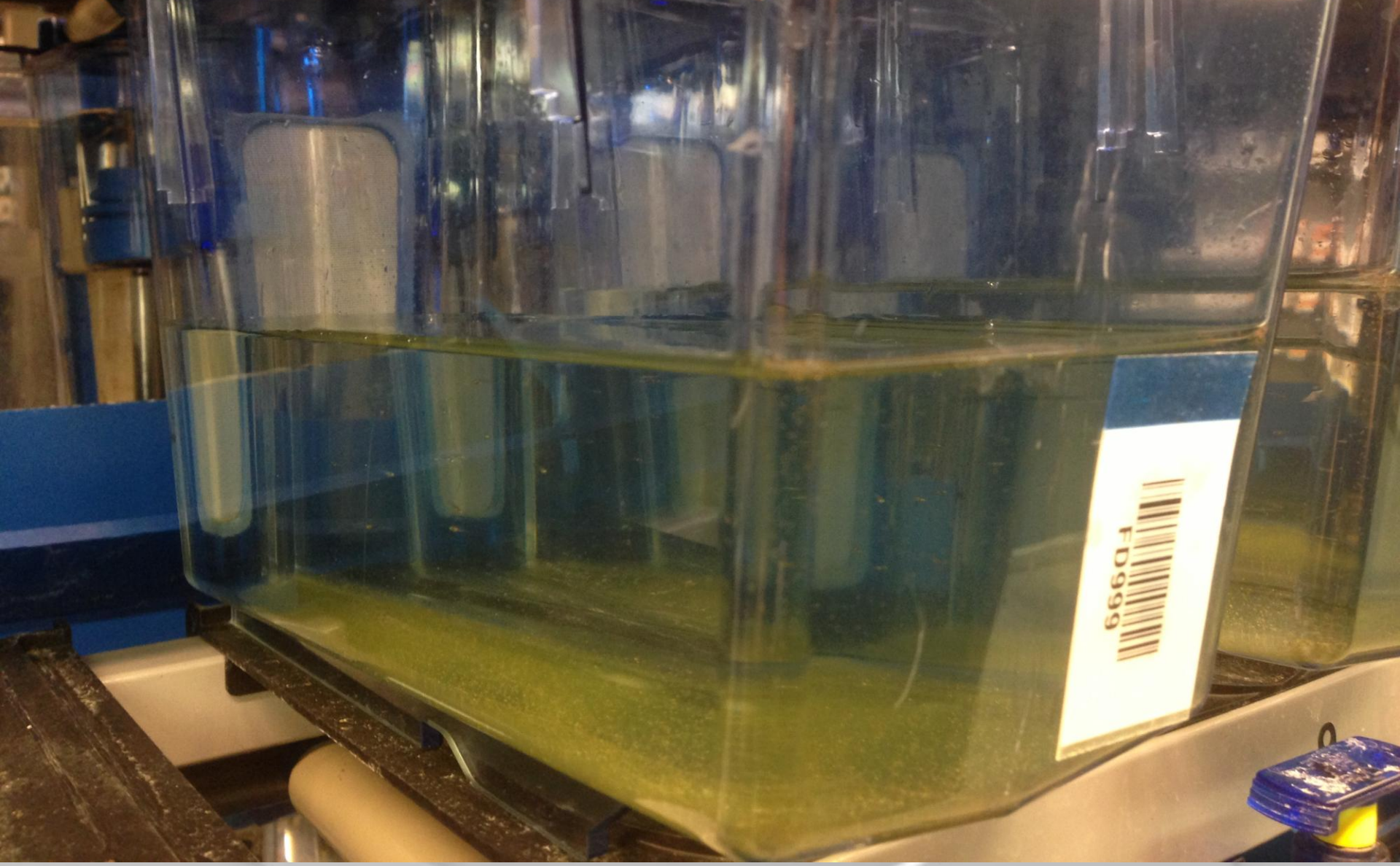
Incremental method

- Larval fish 13-14dpf
  - Rotifers (1000 per larval fish/day)
  - If fish readily eat prepared food, start low-flow and feed both dry-food and rotifers



Incremental method

- >14-dpf
  - Metamorphosis has occurred
  - Fish are no longer larval
  - Initiate gentle flow
  - Feed with prepared food
  - Supplement with rotifers



larval fish tanks with rotifers

larval fish tanks with rotifers

larval fish tanks with rotifers

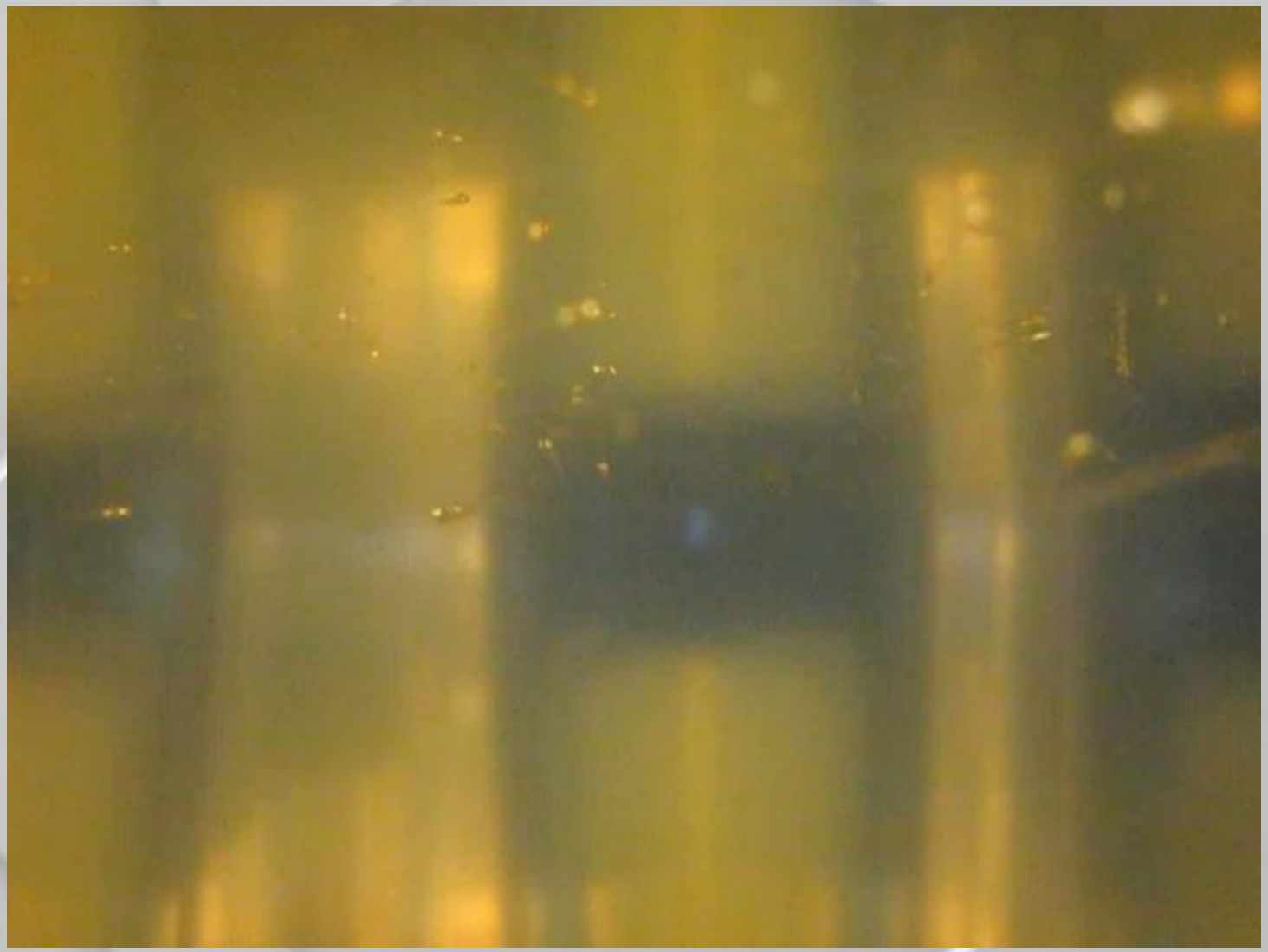
Common Problems in the early stages of raising zebrafish

Trust your senses and your intuition

- use your nose and your eyes!

If it smells bad.....
If it looks milky/hazy/cloudy...
If there is a scum or a slick.....

These conditions require an **ACTION** from you
What actions do you take?



rotifer fed larvae stressed due to scum on water

What Can be Done?

To break up scum, flush out fouled water, and add oxygen you must initiate water exchange.

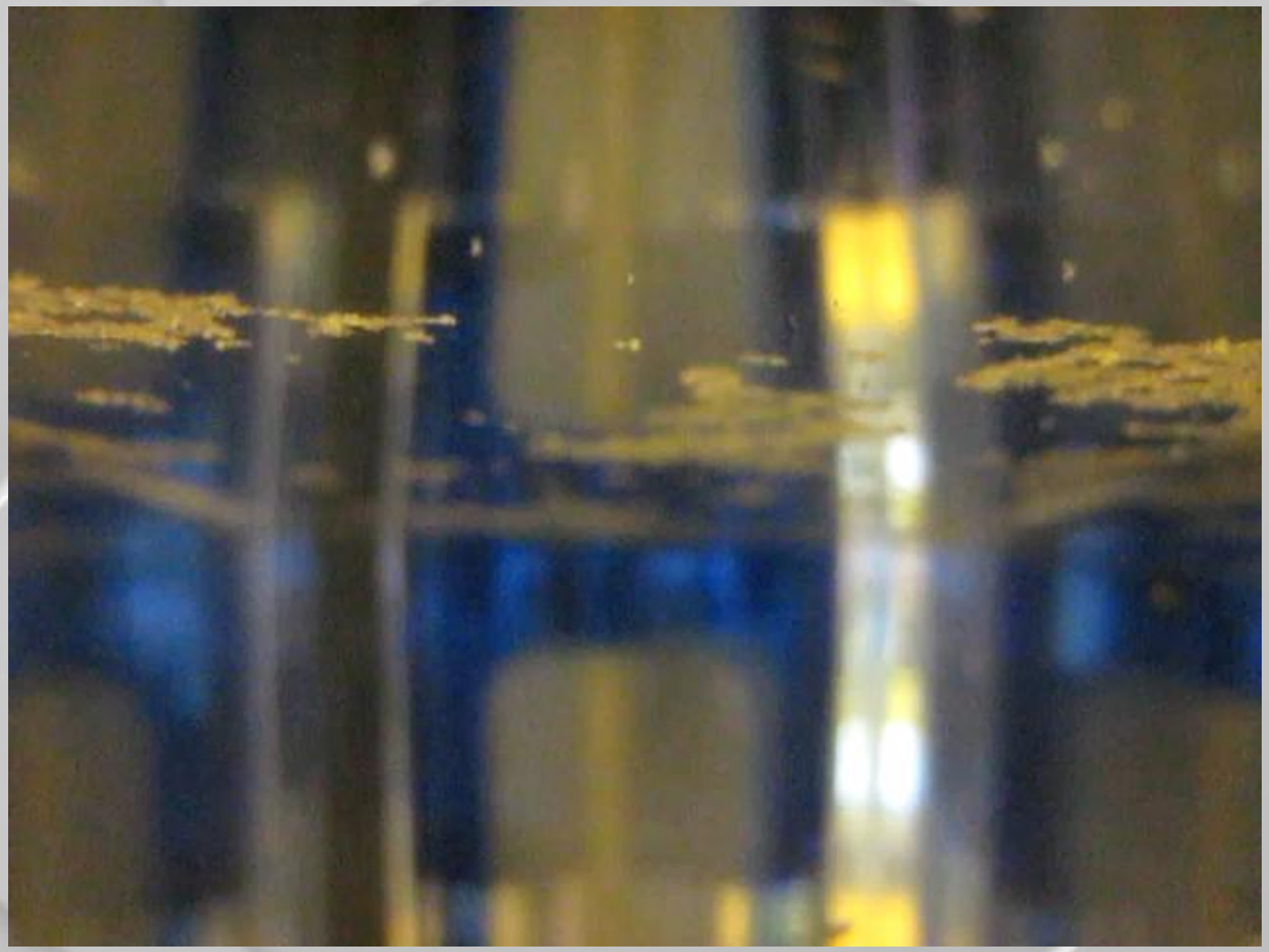
First, best option:

- Initiate water flow
  - Slow and easy is preferable, but not always possible
  - Finish by slowly pouring out water to return the tank to the original fill level

What Can be Done?

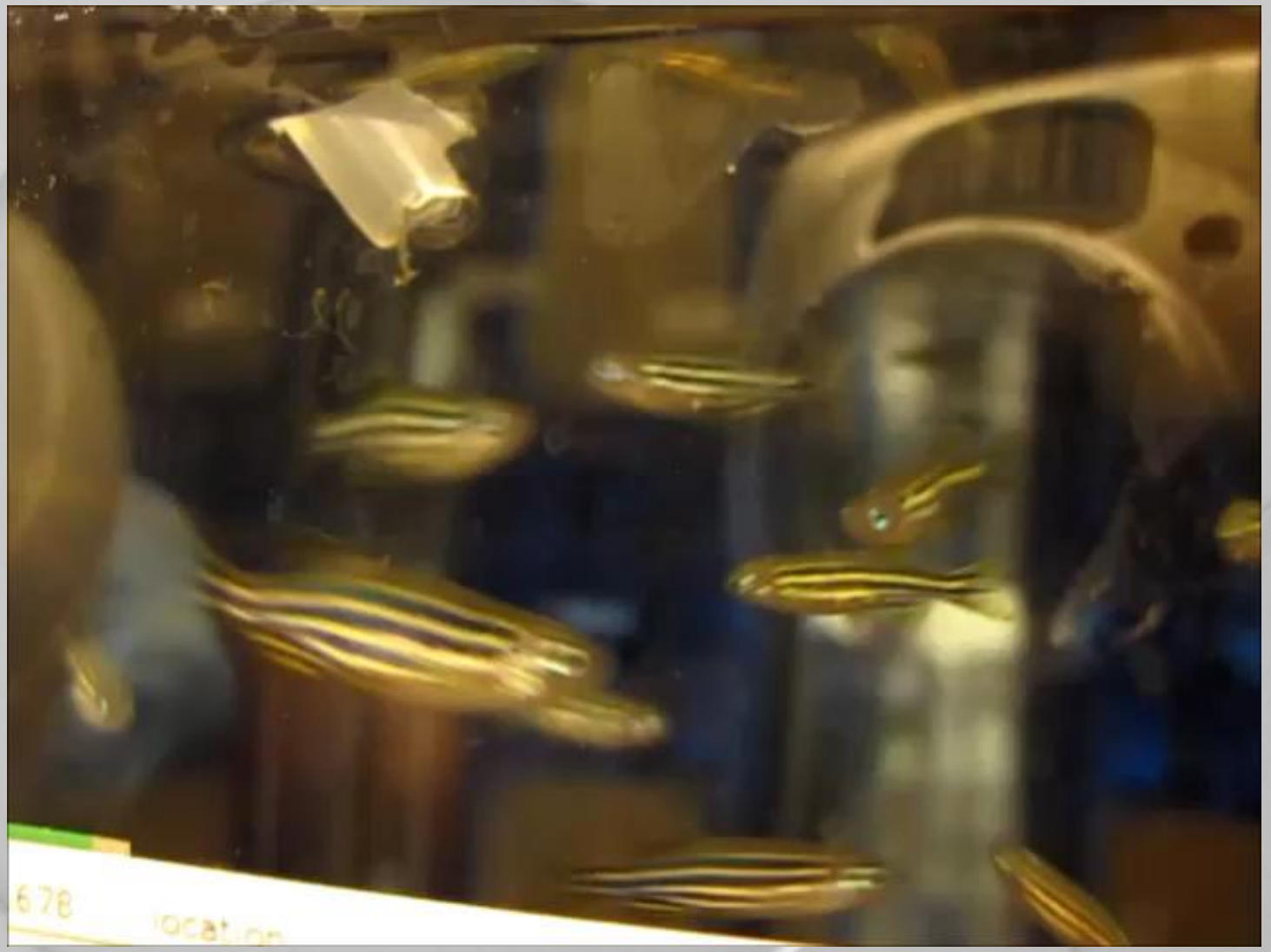
Alternatives to flushing with water:

- To remove scum only: use bristles of a paint brush to skim the offending material from the surface
  - A stack of folded paper towels can work as well
- Light aeration without air-stone can break up scum also



Please note difference in water surface between slow-stream to rapid drip

Can larger fish actually see and eat rotifers?



- Young adults frenzying on rotifers

Live Feed Types: 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

Thanks

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

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