Zebrafish embryo and larval care

a detailed examination

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Presentation Overview

1) Embryo collection - Proper rinsing and media

2) The first 24-hrs - handling, sorting and storage

3) 48-hpf to 72-hpf- clean-up and hatching

4) 4-dpf to 5-dpf - Swim-up, transfer to holding cage and first feed

5) First Feeding

Collect in a sieve or strainer, and **rinse thoroughly with embryo medium** to remove feces, scales, and other debris.

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What is Embryo Medium?
(a.k.a egg water, embryo water, E2, E3, etc.)

- It's essential qualities are:
  - Water
    - adequate purity (RO, DI, distilled, filter sterilized, etc)
    - Chemically defined (no chlorine, chloramines, nitrogen, phosphorus, etc.)
    - Biologically inert- not from the fish system!
  - Salts (ionic compound) that impart:
    - Adequate pH (~7.5)
      - Typically from a salt (buffer) such as Soduim Bicarbonate ($NaHCO_3$) a salt composed of sodium ions and bicarbonate ions
    - Adequate conductivity (500-1000uS)
      - Typically from a Sodium Chloride (NaCl)

Step 2:

Sorting the eggs to remove the embryos from the non-fertilized eggs

choose your tool wisely!

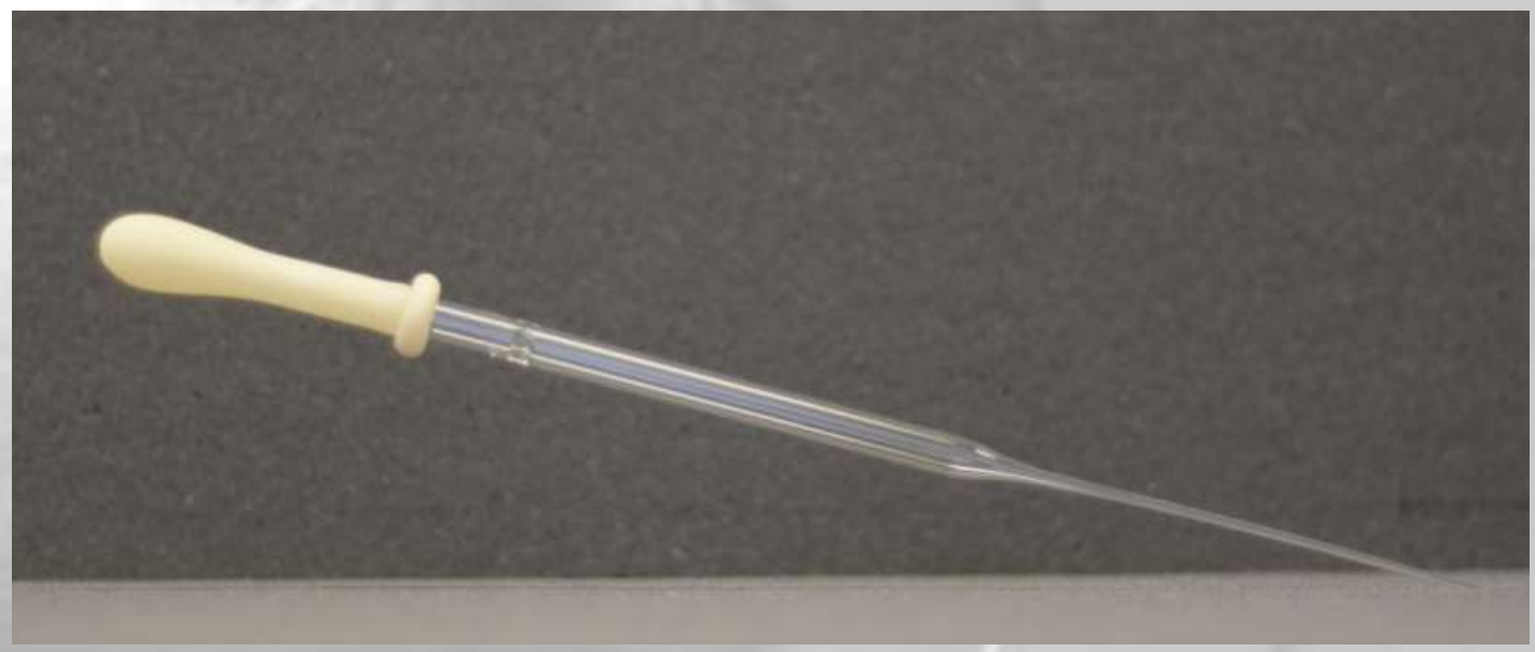
Pipette Pump Bel-Art F37898-0000

Transfer pipette 3mL

The first 24-hrs

choose your tool wisely!

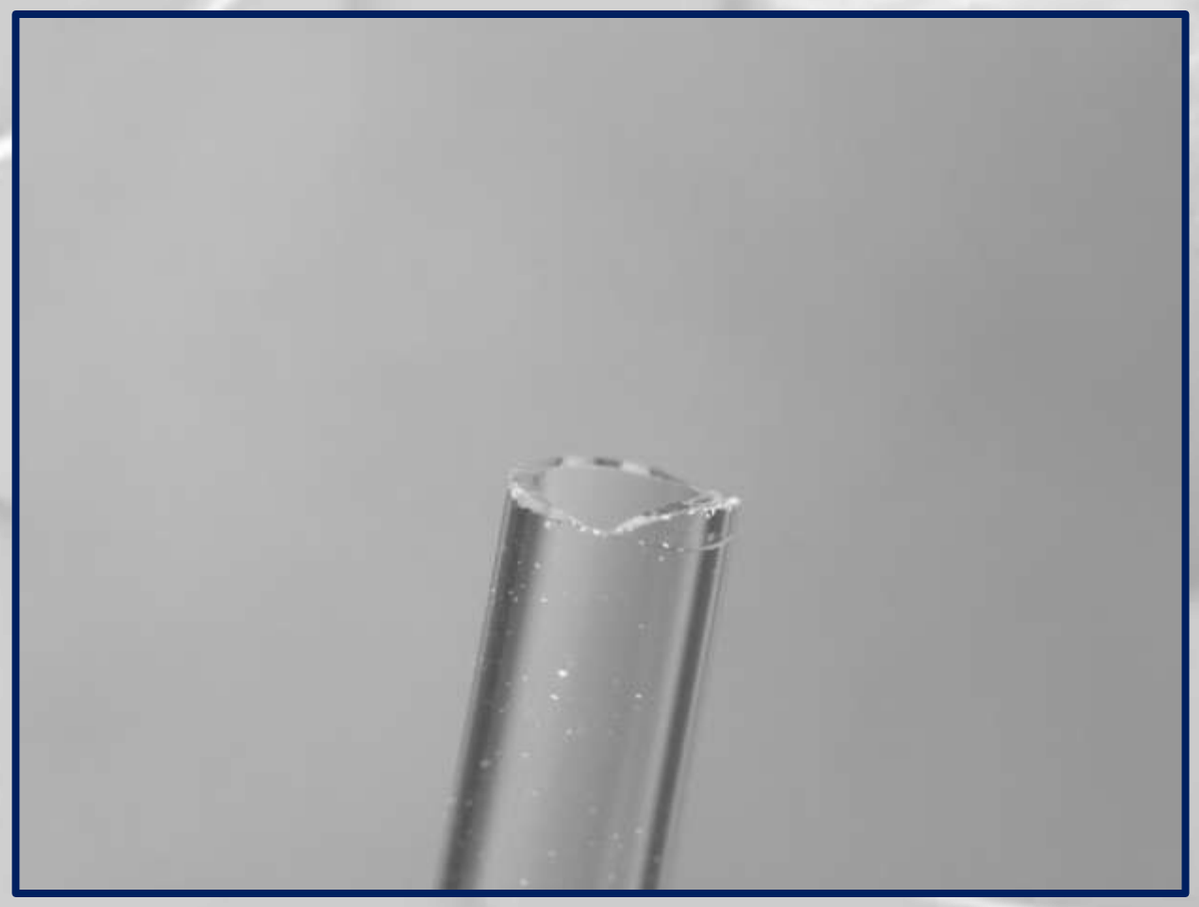
Pipette Pump
Bel-Art F37898-0000

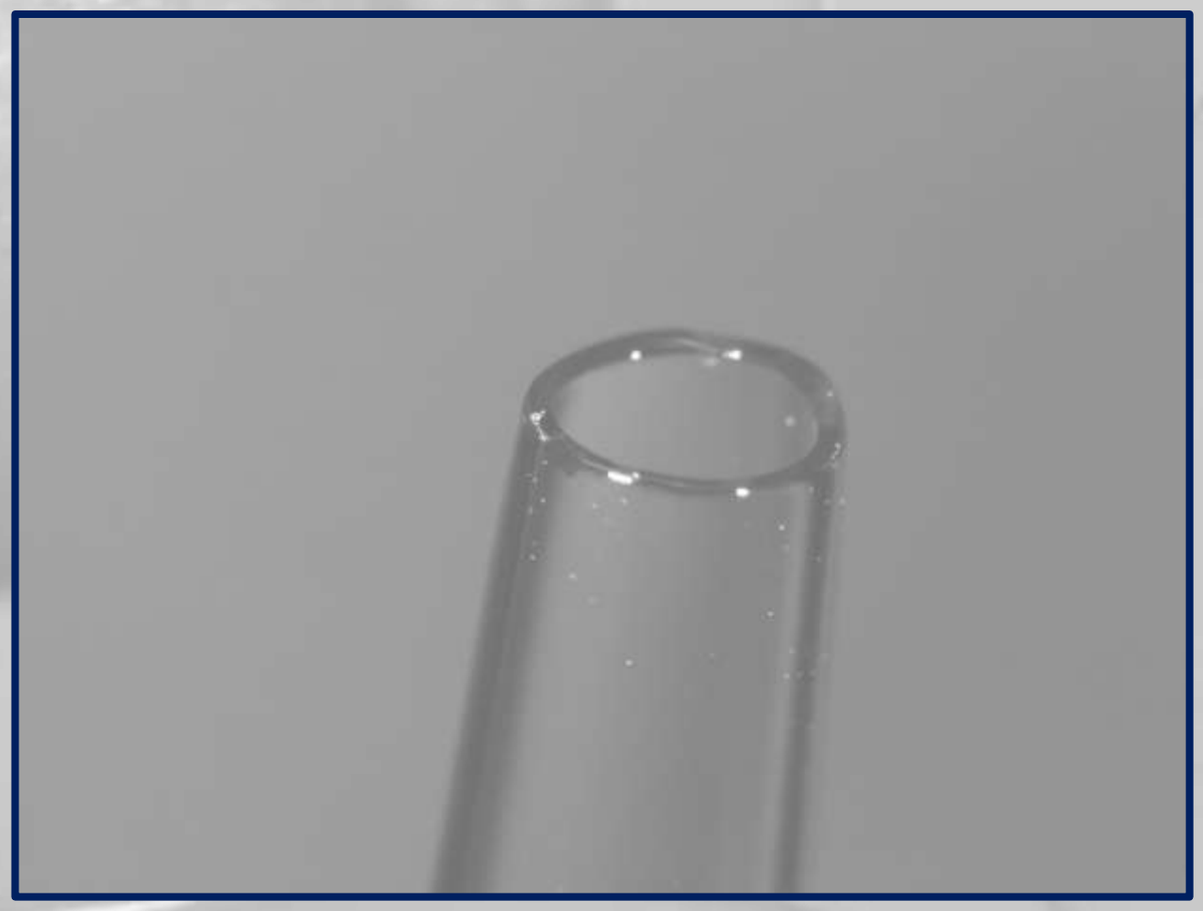


Pasteur transfer pipette
(Fisher Scientific Item #NC9993639)

Pasteur transfer pipette

(Fisher Scientific Item #NC9993639)

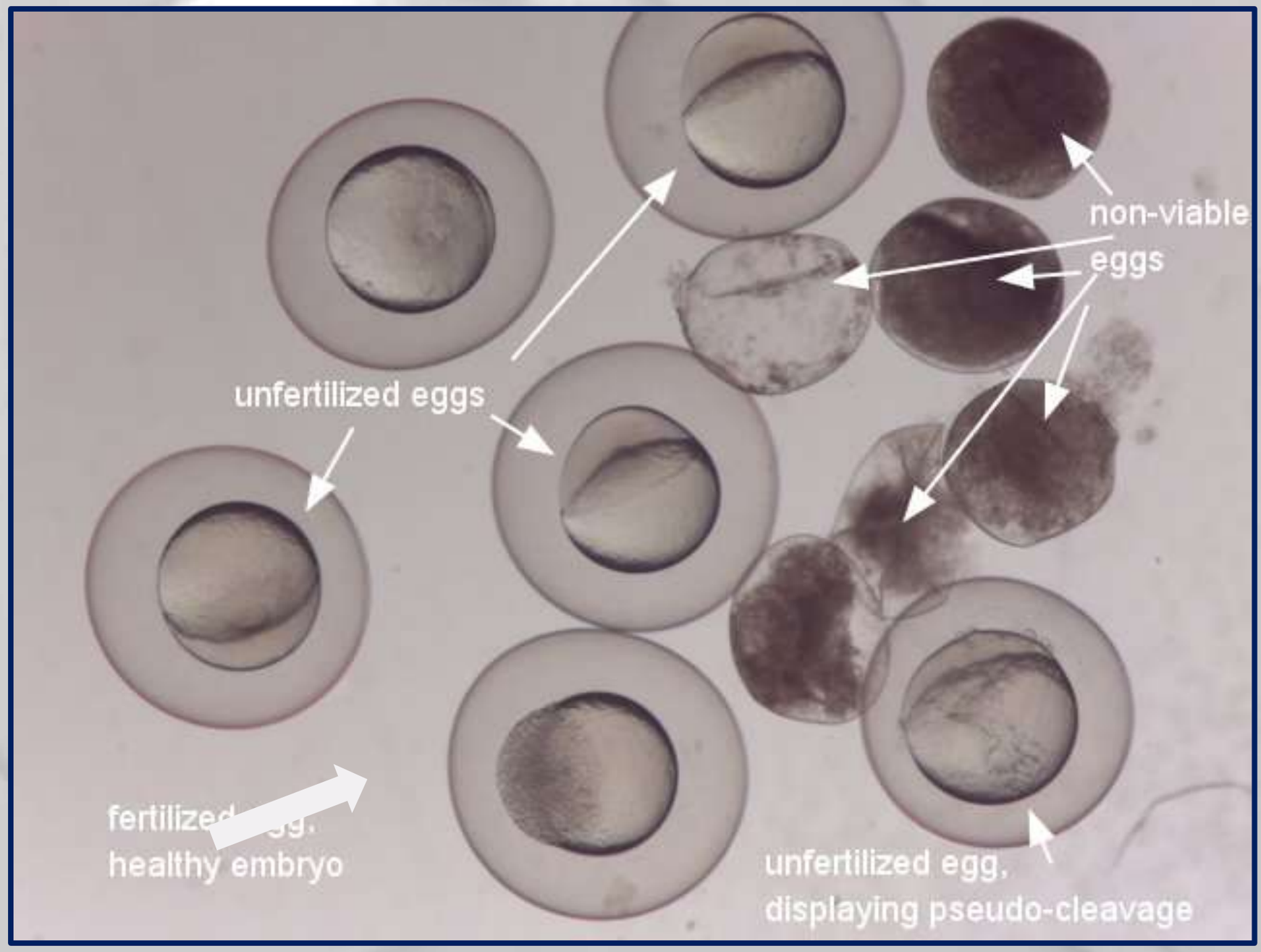




out of the box vs. fire-polished

Clean-up of embryos is critical to success

0-dpf cleanup of embryos



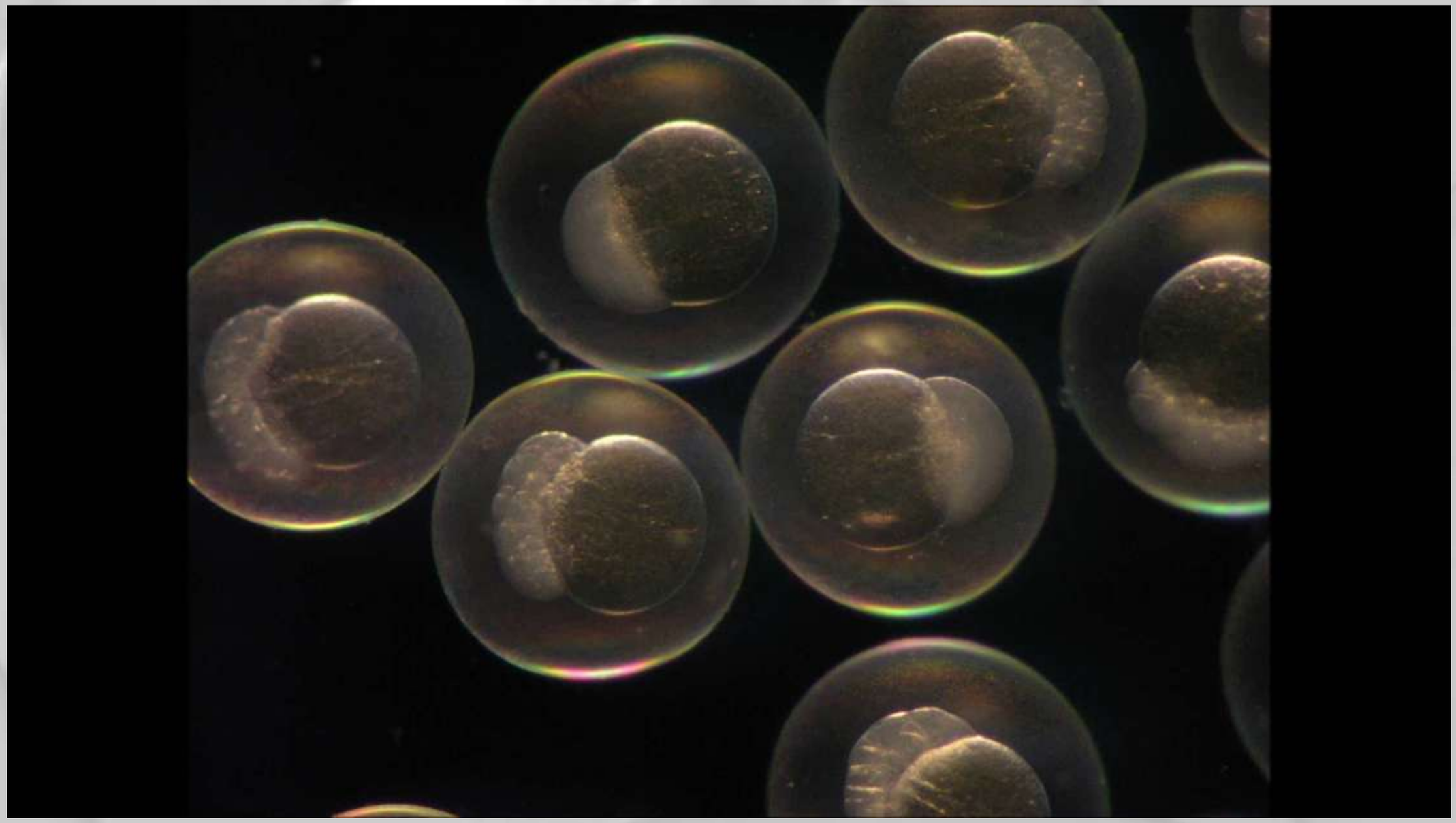
The first 24-hrs

What actually happens to those non-fertilized embryos and those that die of normal attrition?

21-hrs of development in 24-seconds

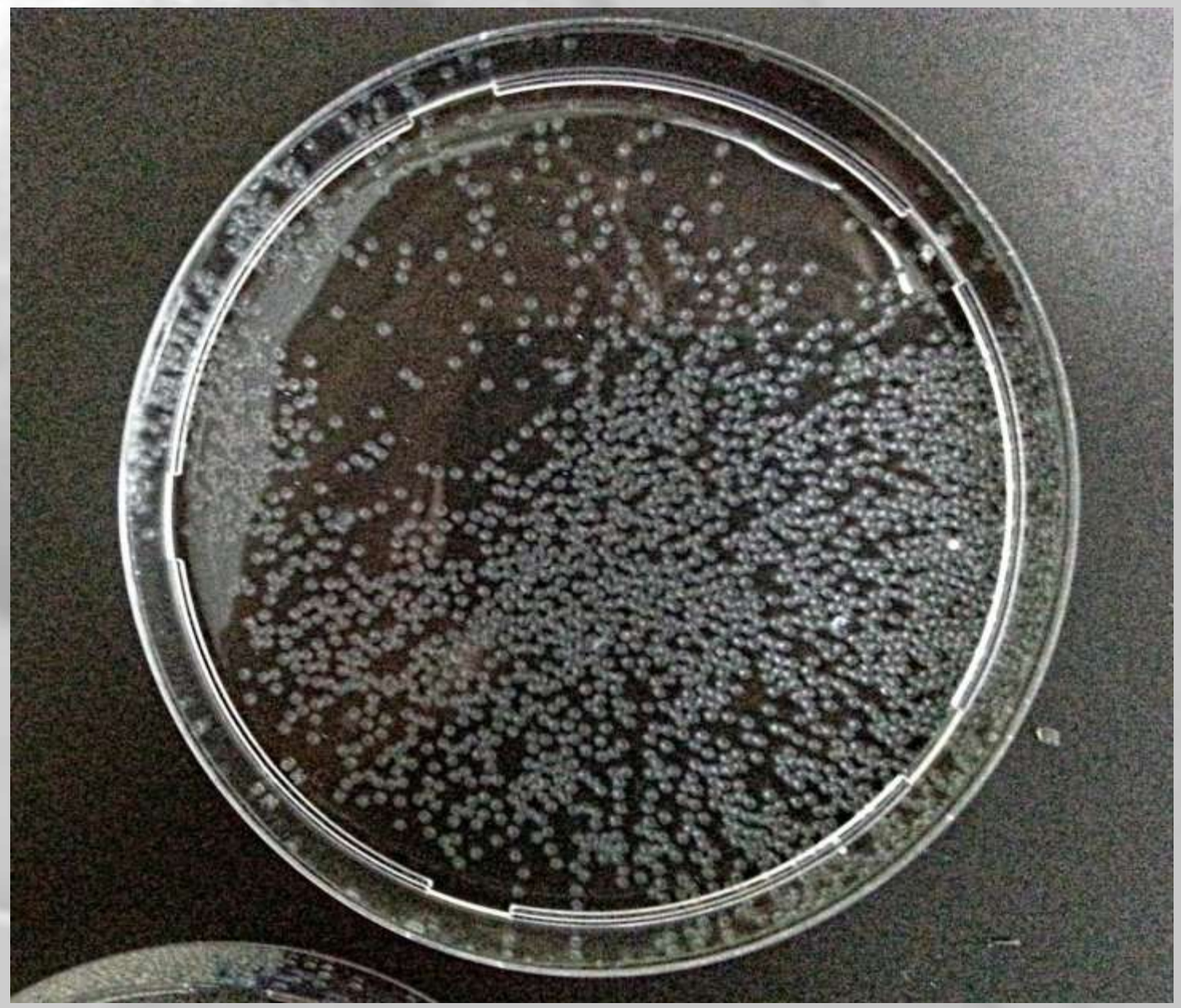
Time-lapse in 5-min intervals.

Start: 11:42AM
End: 08:22AM



Recommended storage density of embryos:

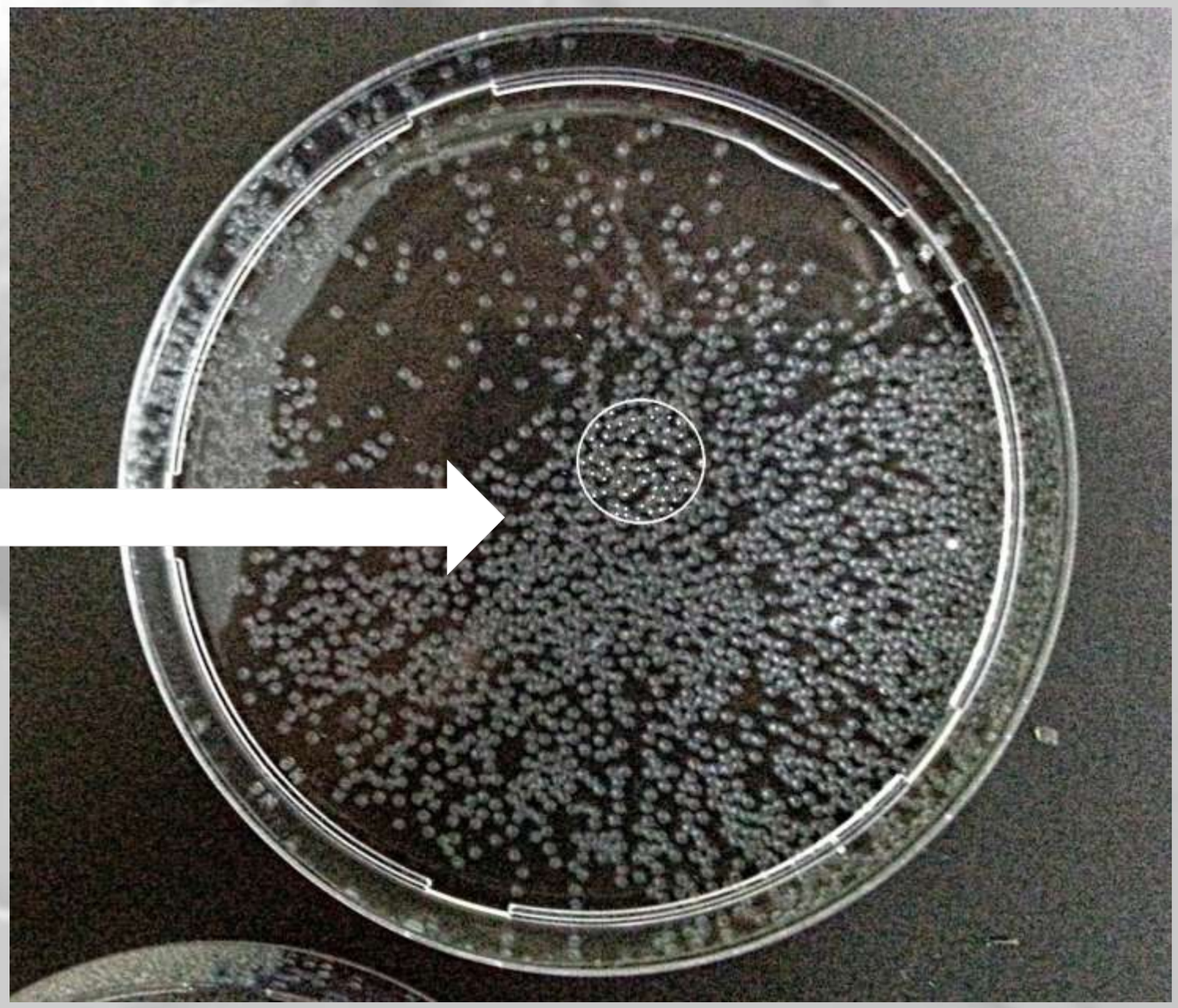
- 50-embryos per Petri dish (~50ml)



Recommended storage density of embryos:

- 50-embryos per Petri dish (~50ml)

THIS is what 50-embryos looks like



good embryo care is critical for success

- Best practice is to move healthy embryos to a new dish with fresh media
- Use of Methylene blue is most common anti-fungal used in zebrafish culture
- Very little is required
- Too much can stain tissues and interfere with imaging and in-situ results
- Not needed after chorion detritus is removed
- Is detrimental to live feeds if transferred into container for first-feeding

The first 24-hrs through day 3

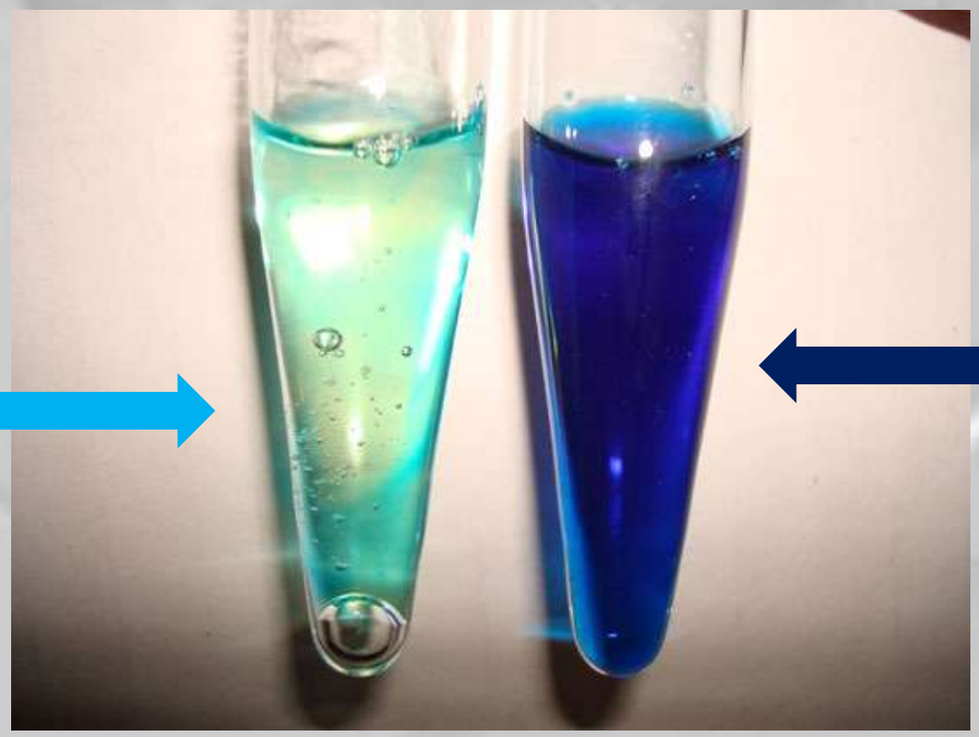
Methylene Blue Stock solution

- 1 g methylene blue powder ***(M9140 SIGMA-ALDRICH)***
- 1 L Reverse Osmosis or suitably pure water

Store at Room temperature

Only 1 to 2 drops of the stock solution is needed for each Petri dish of embryos!

Less is more!



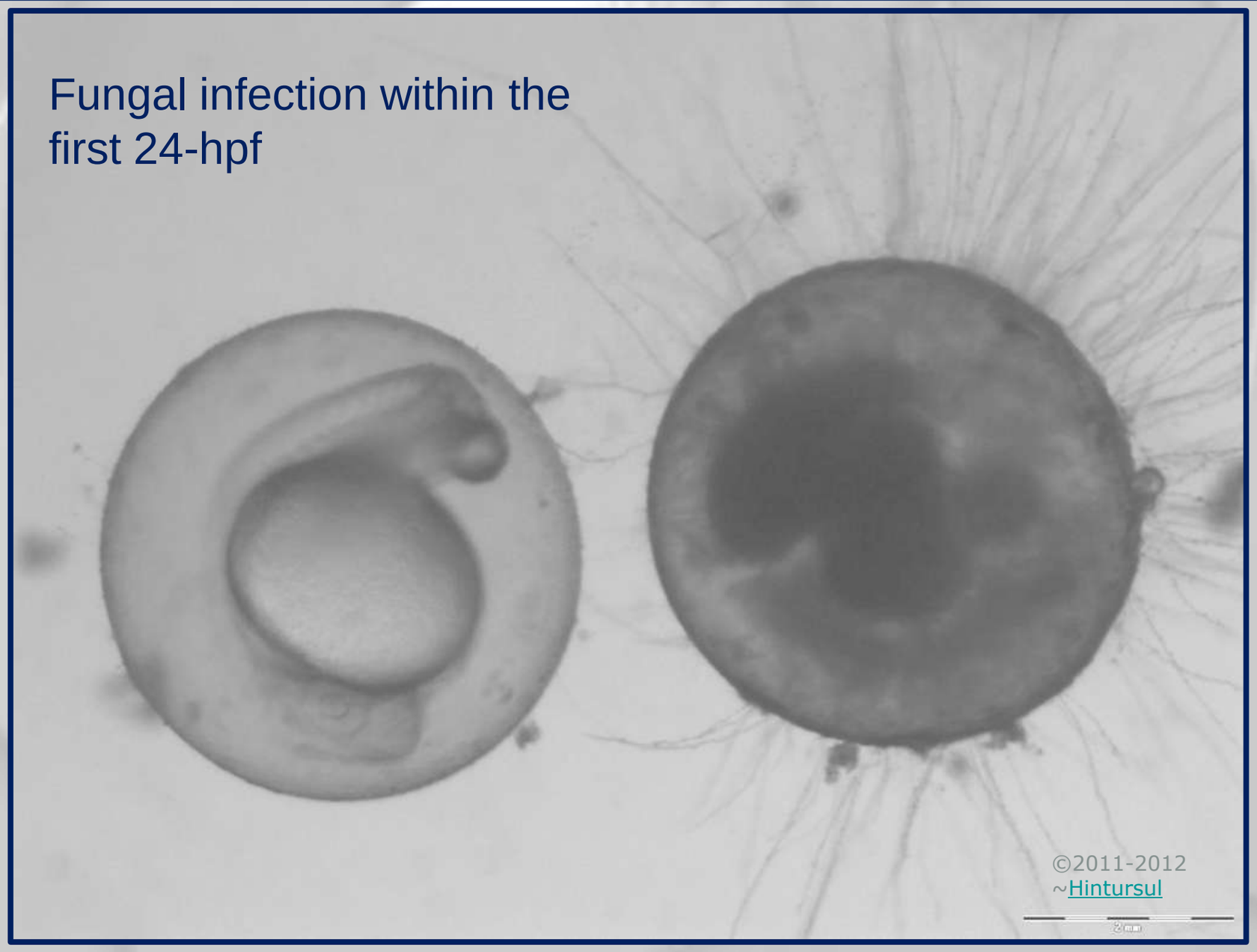
Much too much!

Consequences of failing to do a good job?

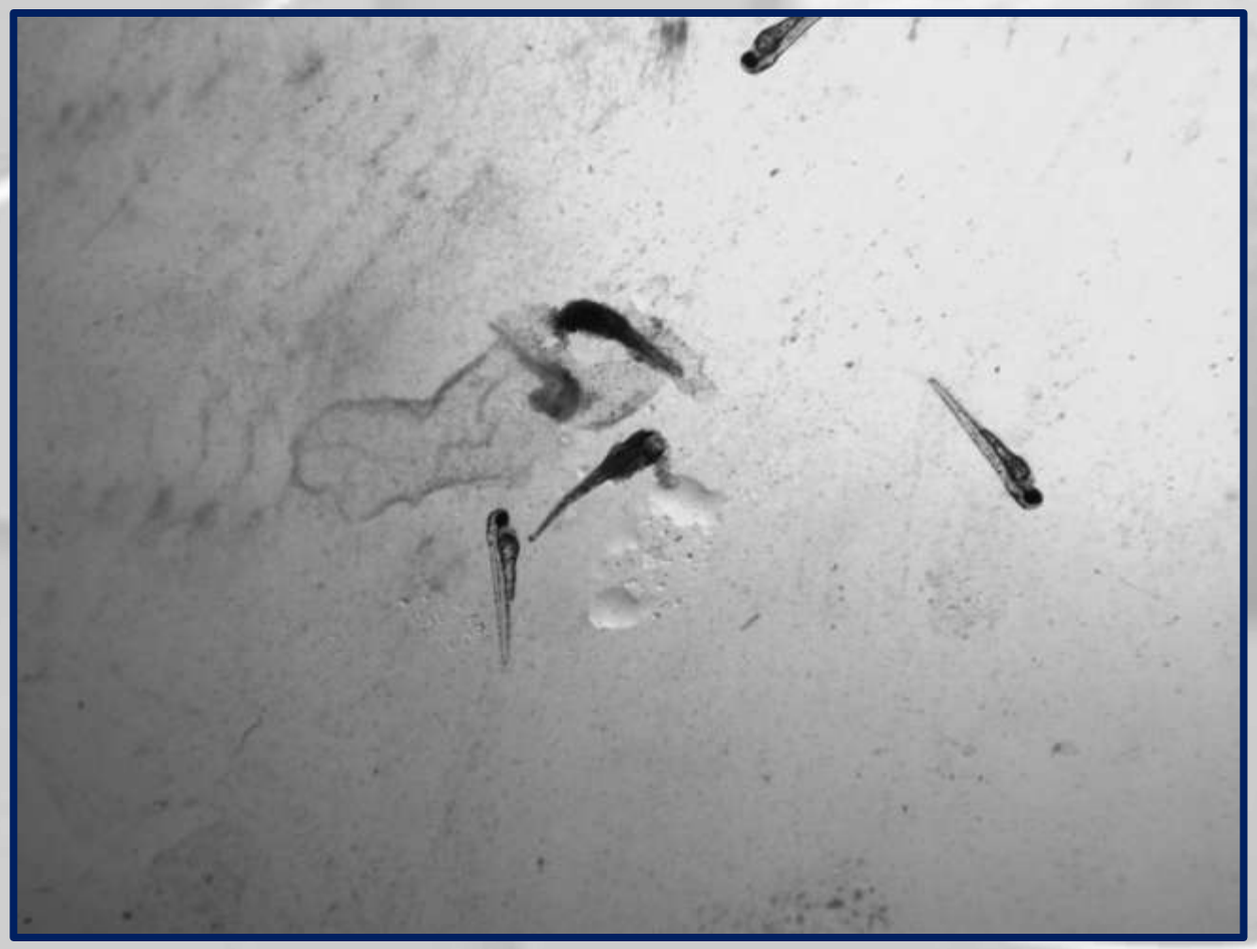
- Fungi – serious losses of embryos
- Fouling of water in dish resulting in serious losses- can extend beyond a single dish!
- Protozoan blooms
- hypoxia

What happens if fungi is not prevented (you don't use methylene blue)?

Fungal infection within the first 24-hpf



Look familiar?



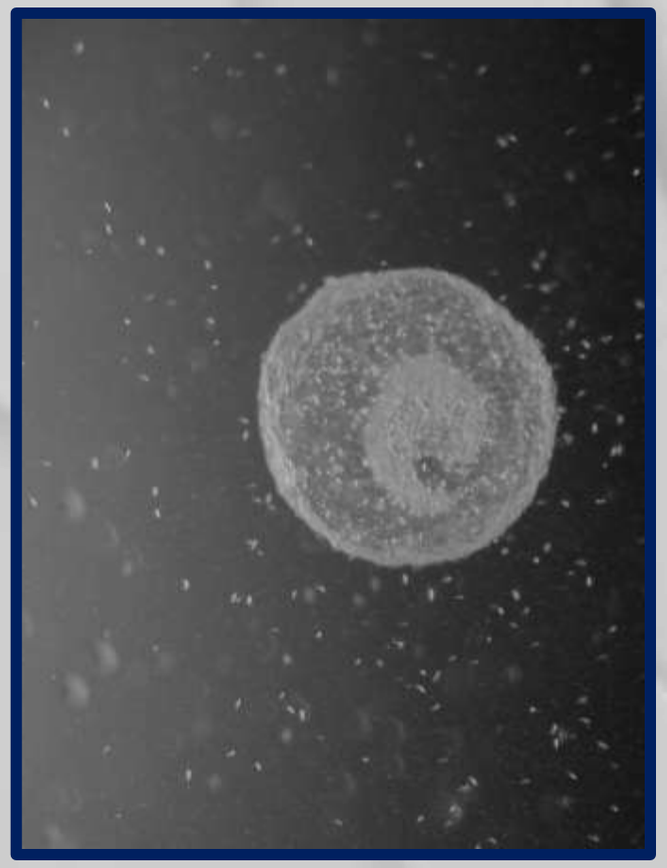
•*Coleps* feeds on bacteria, algae, flagellates, living and dead ciliates, animal and plant tissues.

•*Coleps* uses toxicysts, poison it carries to capture its prey from its oral area.

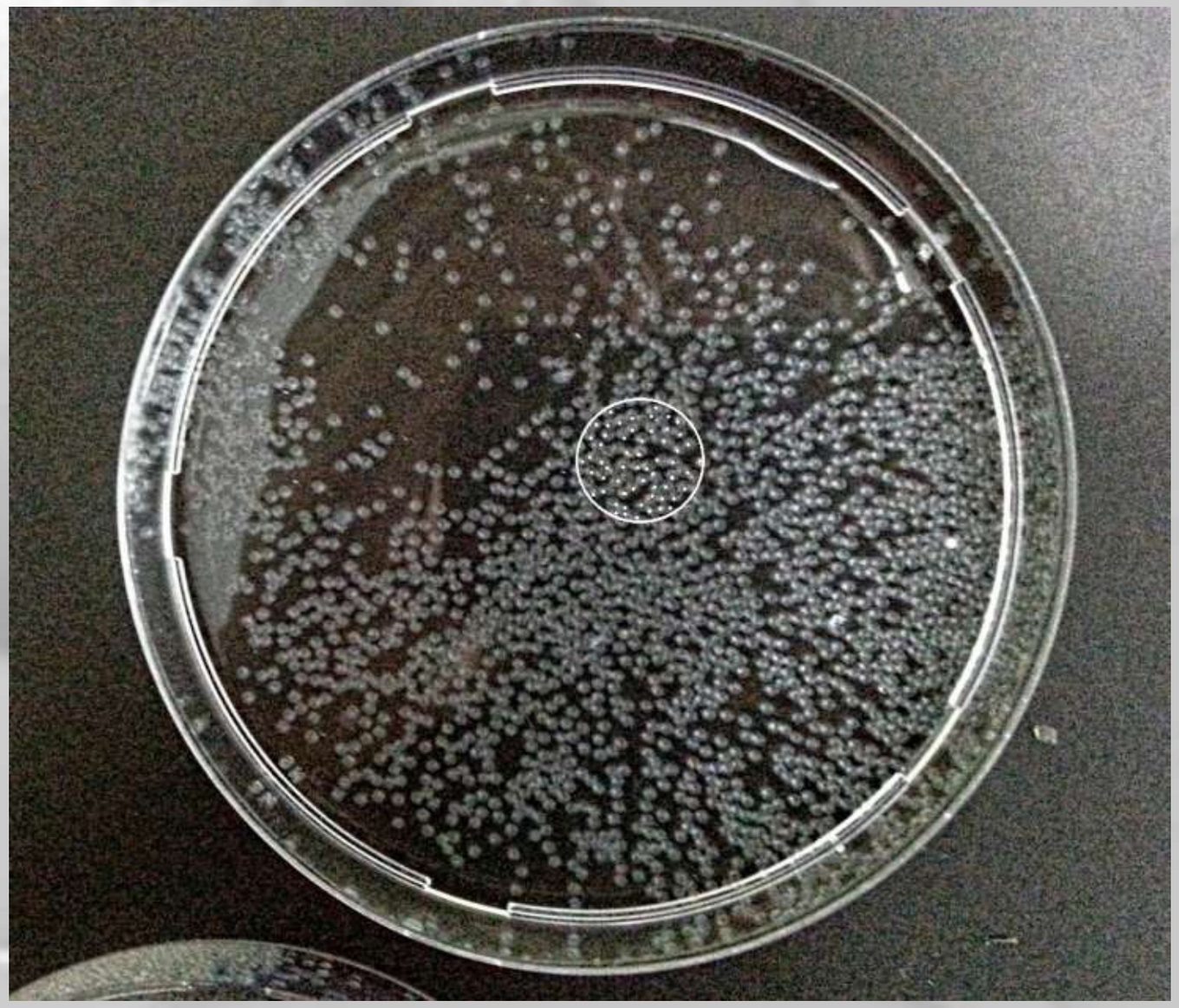
•It extrudes tube-like structures to force toxicysts into its prey and wait until its prey becomes paralyzed.

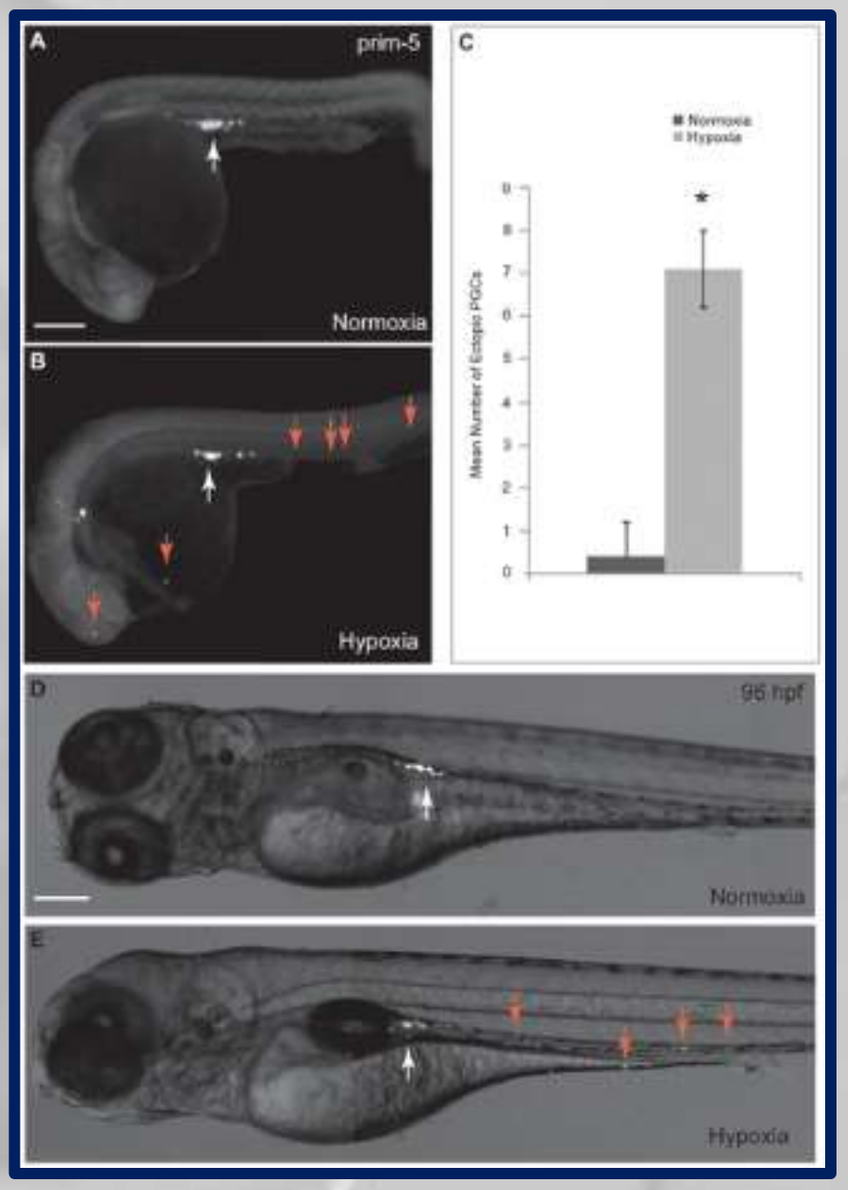
•These toxicysts, however, takes about 5–10 minutes to be effective on the prey of the *Coleps* and it separates itself from the prey during this time.

•If there are numerous *Coleps* hunting for the same prey, some *Coleps* will cling to its prey until the toxicysts become effective and fragment the prey, consuming only few parts



What kinds of things can happen if you crowd the embryos?





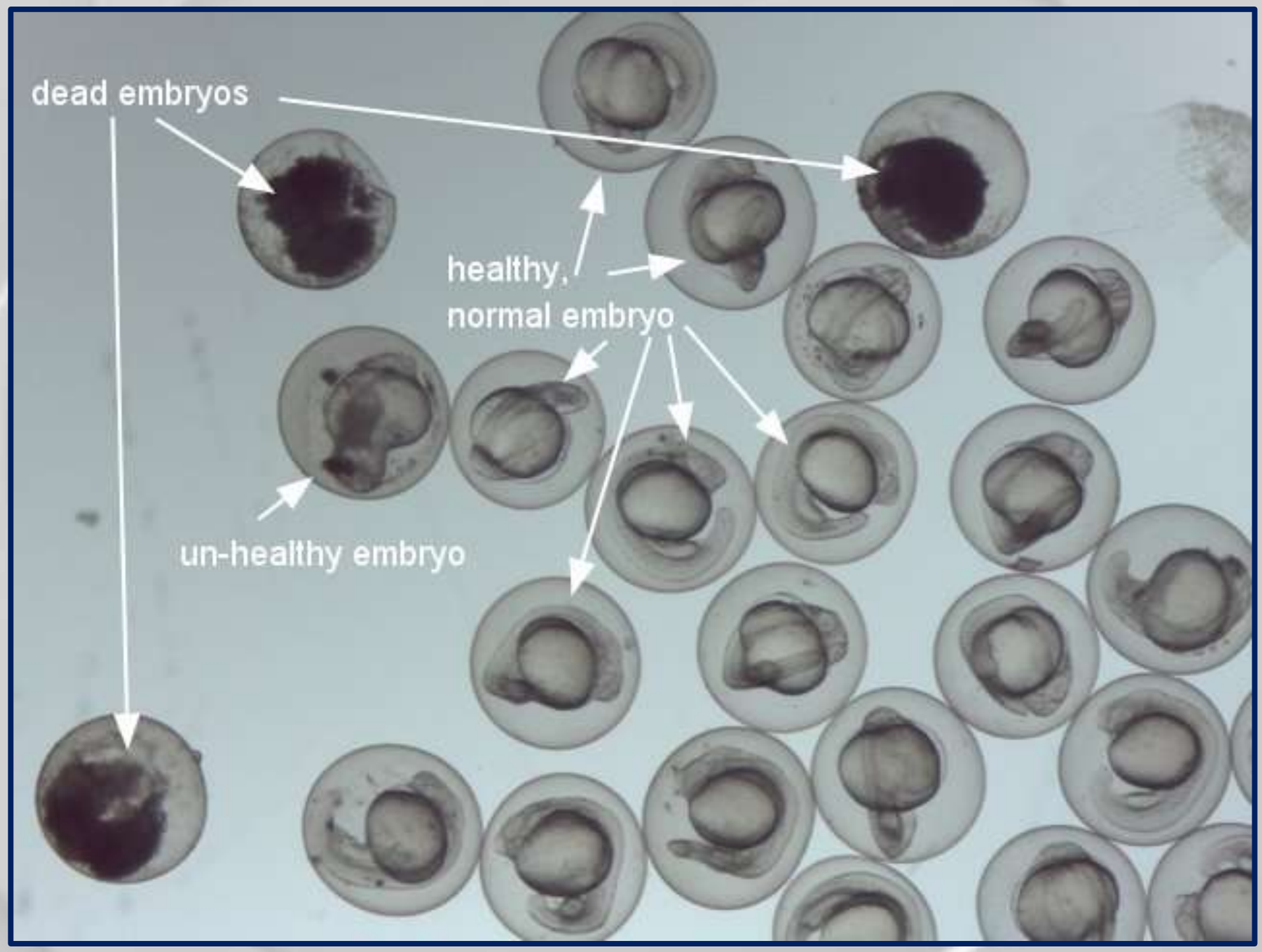
good embryo care is critical

hypoxia

Effects of hypoxia are wide-ranging. From developmental retardation and abnormalities to primordial germ cell migration defects, and disruption of pathfinding of forebrain neurons. more than +25.7K hits on Google scholar “zebrafish hypoxia”

Day 2

Approx.
24-hpf

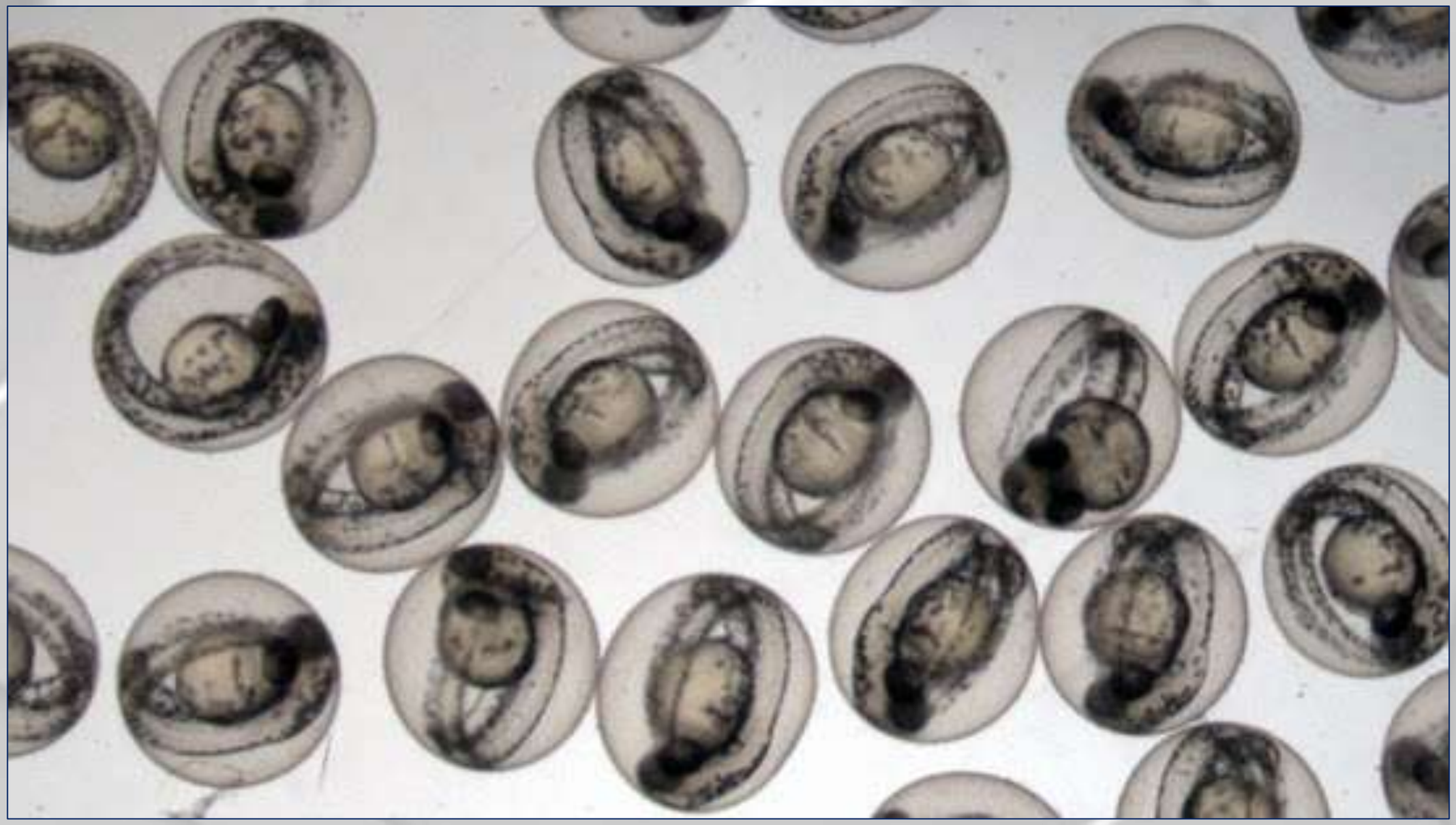


Continued, diligent cleanup
Move embryos to new dishes

48-hpf to 72-hpf

Continued, diligent cleanup of embryos

Approx 48-hpf



Continued, diligent cleanup

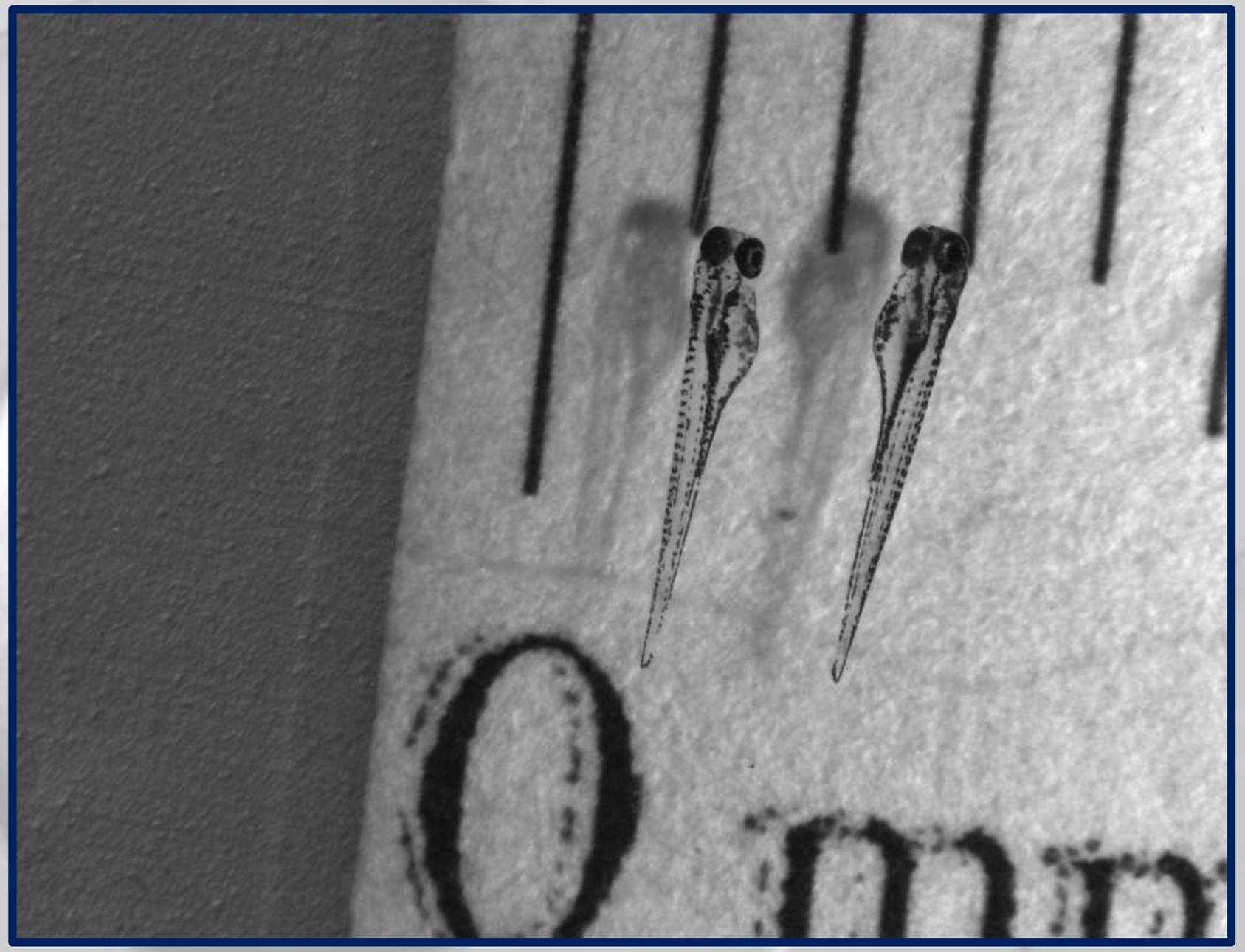
Move embryos to new dishes

Approx. 72-hpf

Continued, diligent cleanup
Move embryos to new dishes

according to Zfin

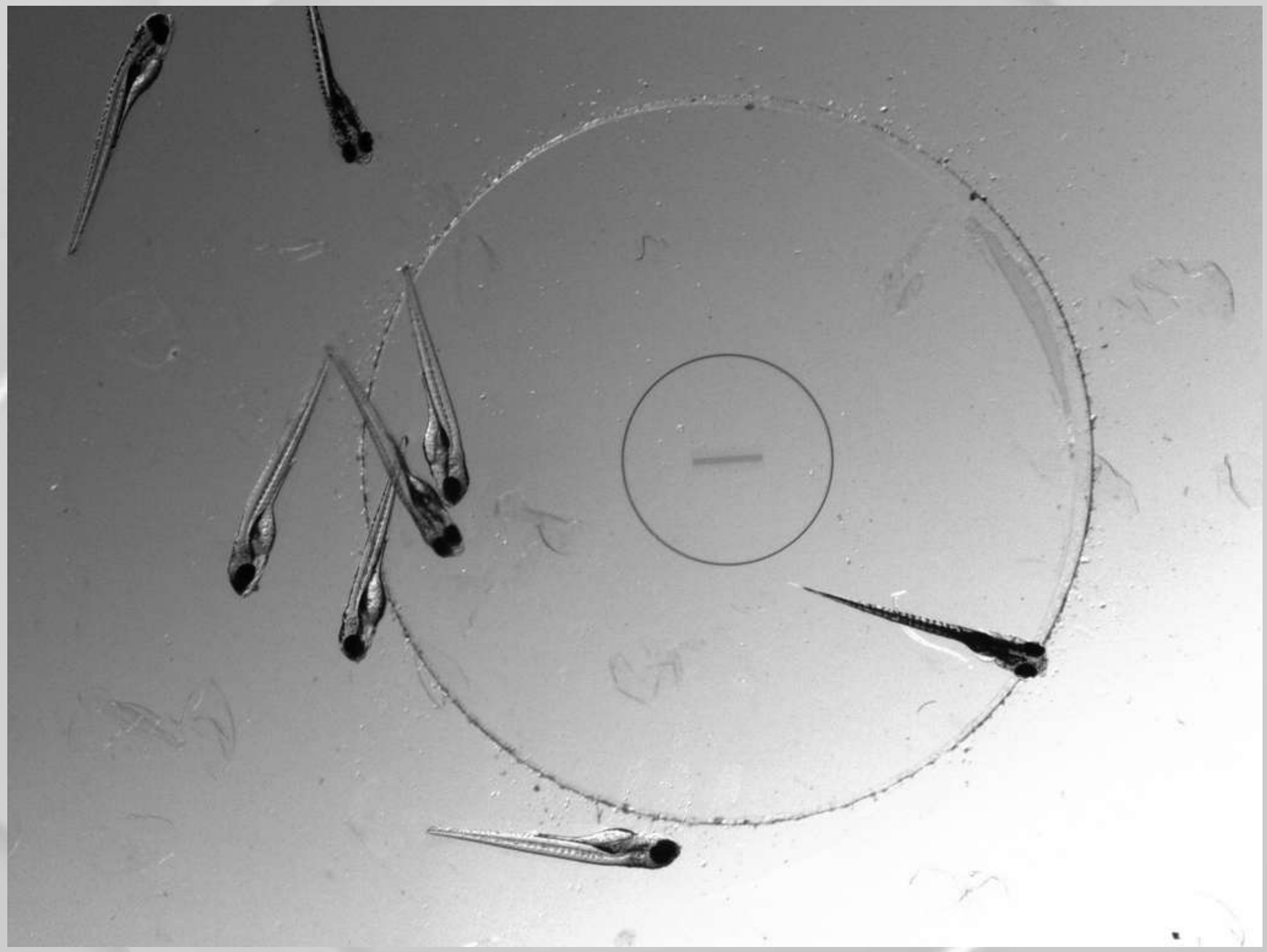
Hatching (48 - 72 h)	Long-pec	48 h	EL = 3.1 mm; elongated pectoral fin buds
	Pec-fin	60 h	EL = 3.3 mm; pectoral fin blades
Larval	Protruding-mouth	72 h	3.5 mm total body length
	Day 4	96 h	3.7 mm total body length
	Day 5	120 h	3.9 mm total body length; 6 teeth
	Day 6	144 h	4.2 mm total body length
	Days 7-13	168 h	4.5 mm total body length; 8 teeth
	Days 14-20	14 d	6.2 mm total body length; 10 teeth
	Days 21-29	21 d	7.8 mm total body length
Juvenile	Days 30-44	30 d	10 mm total body length; adult fins/pigment
	Days 45-89	45 d	14 mm total body length; 12 teeth
Adult (90 d - 2 y)		90 d	Breeding adult



clear definitions

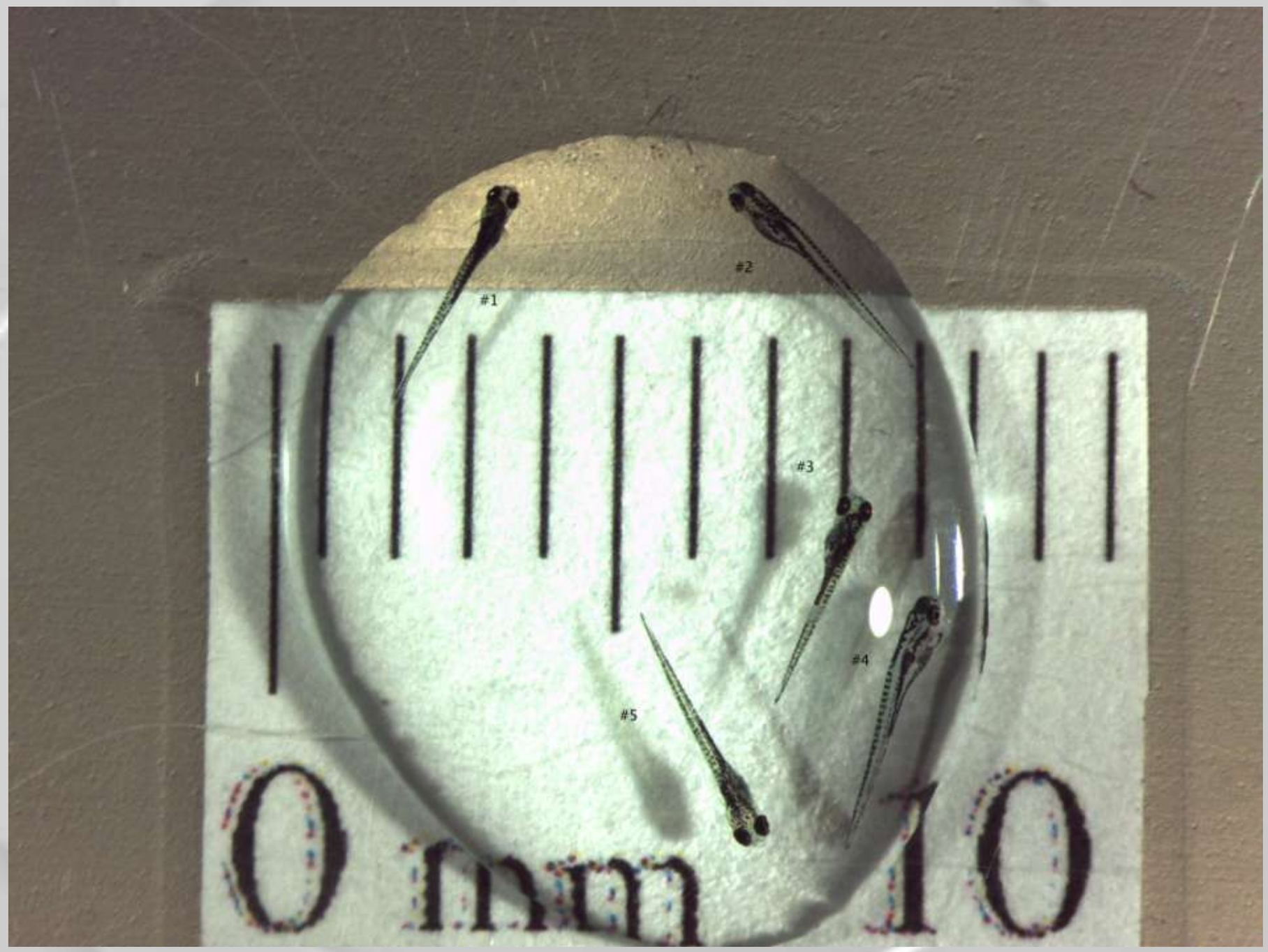
embryo & larvae

Approx. 4-dpf



Continued, diligent cleanup of embryos. Move larvae to new dishes!

Approx. 5-dpf



Continued, diligent cleanup of embryos. Move larvae to new dishes!

Days 4 and 5

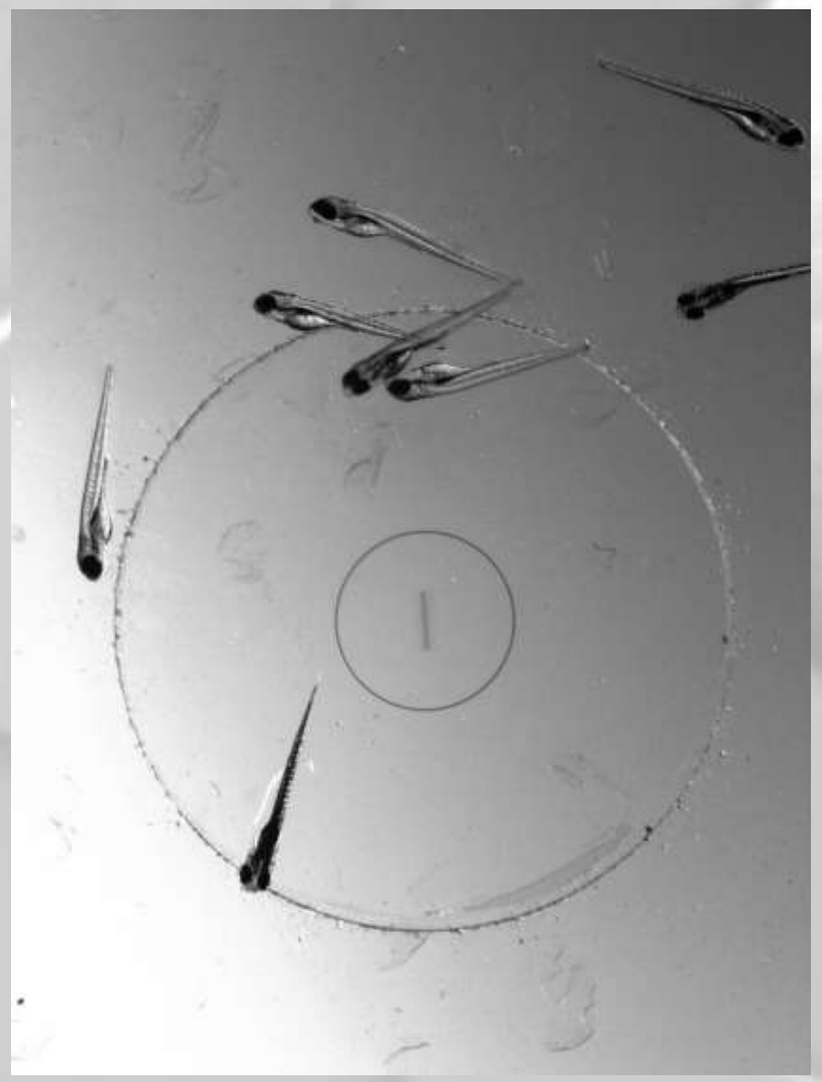
When to start feeding??

time is not the best or absolute answer

- *Some fish lines exhibit delayed gas-bladder inflation (example: casper, albino)*
- *+75% of larvae should be swimming in water column and have inflated gas bladders before offering food*

Days 4 and 5

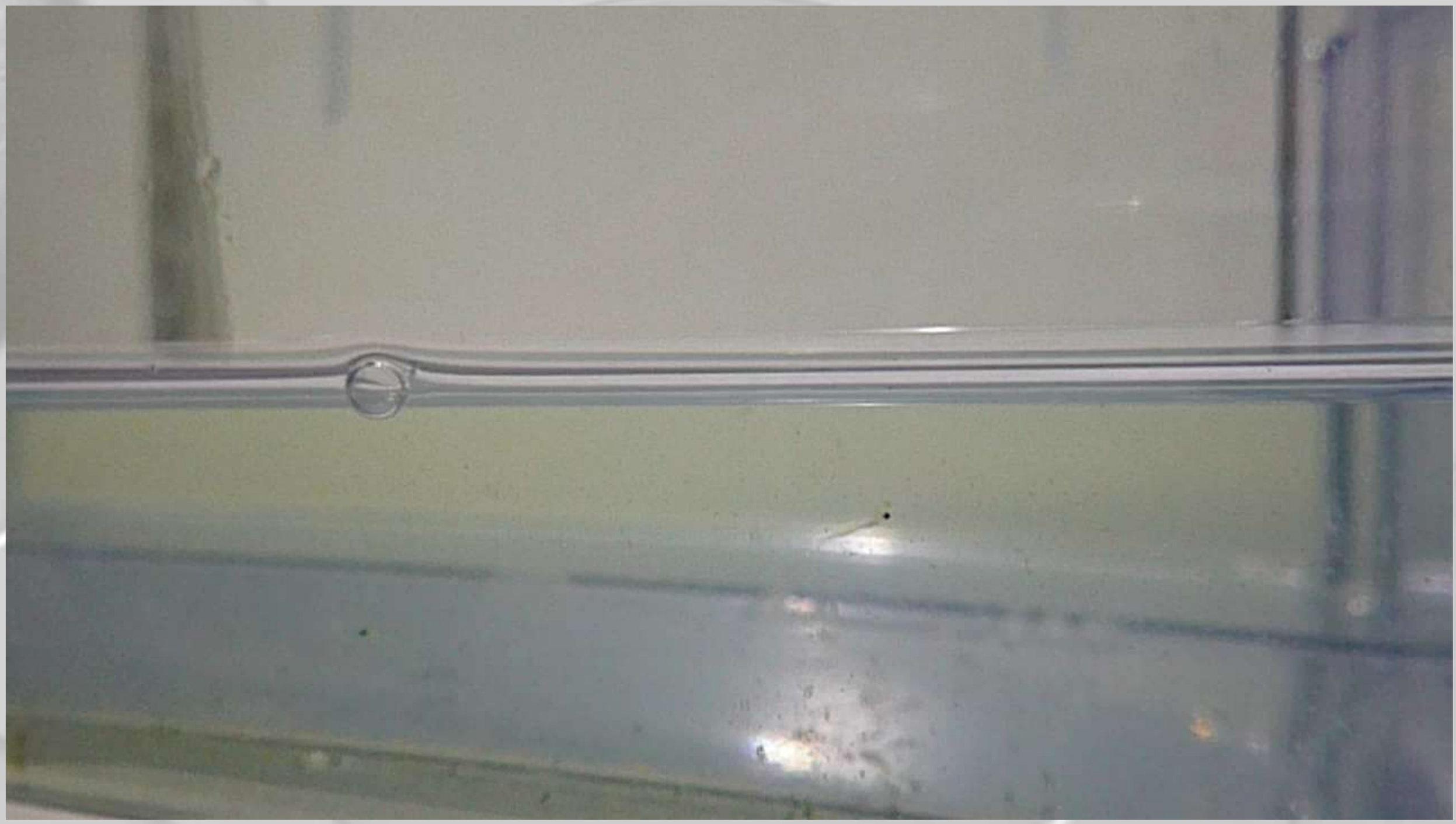
When to start feeding??



Too early

Larvae attempting “swim-up” using cement-like excretion to attach to tank wall

and too much water



Too early

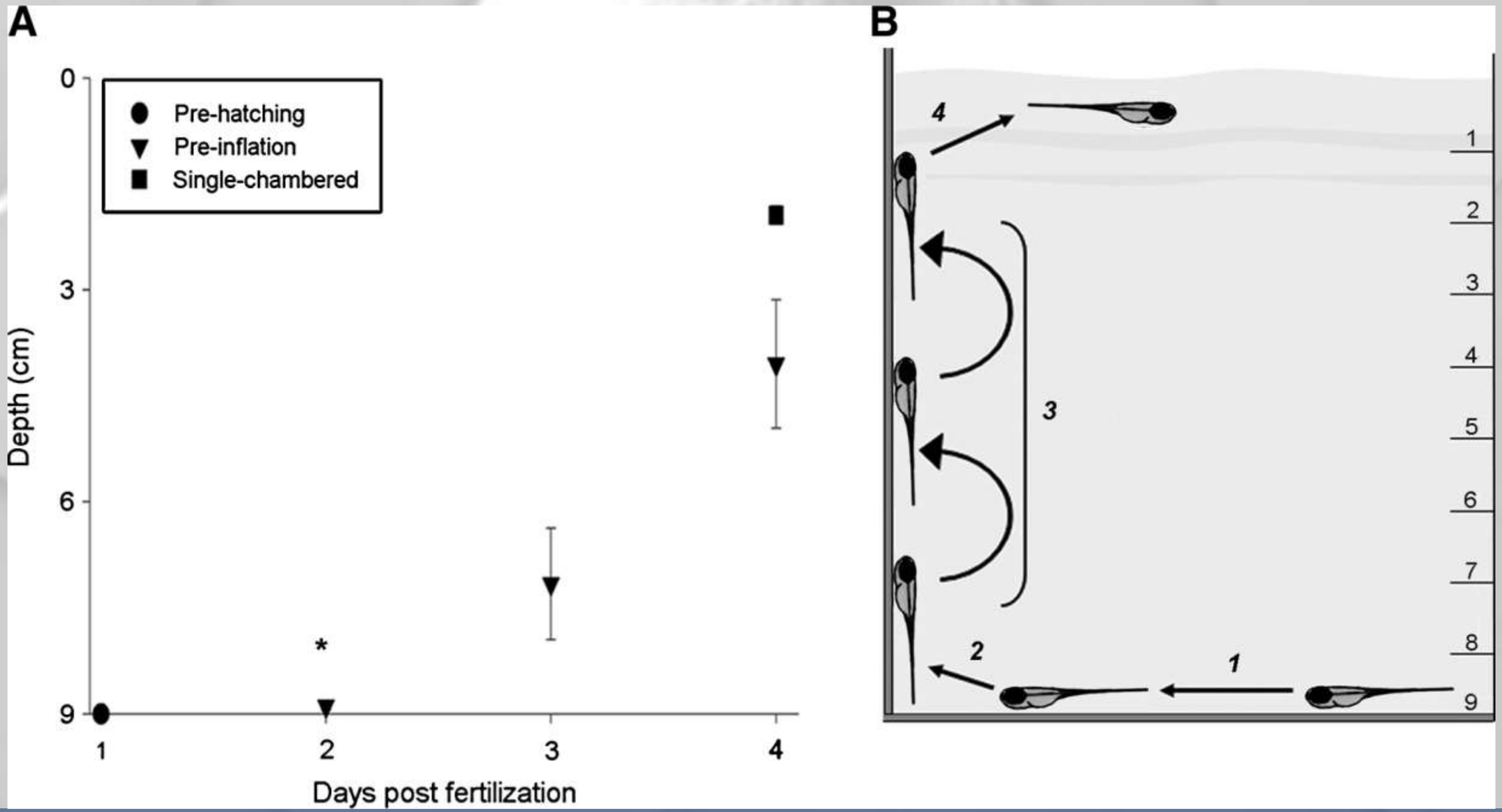
Without inflated gas-bladders, the fish will fail to feed and survive 6-dpf

Just right

these fish have inflated gas-bladders and are capable of swimming in all levels of the water column. 6-dpf

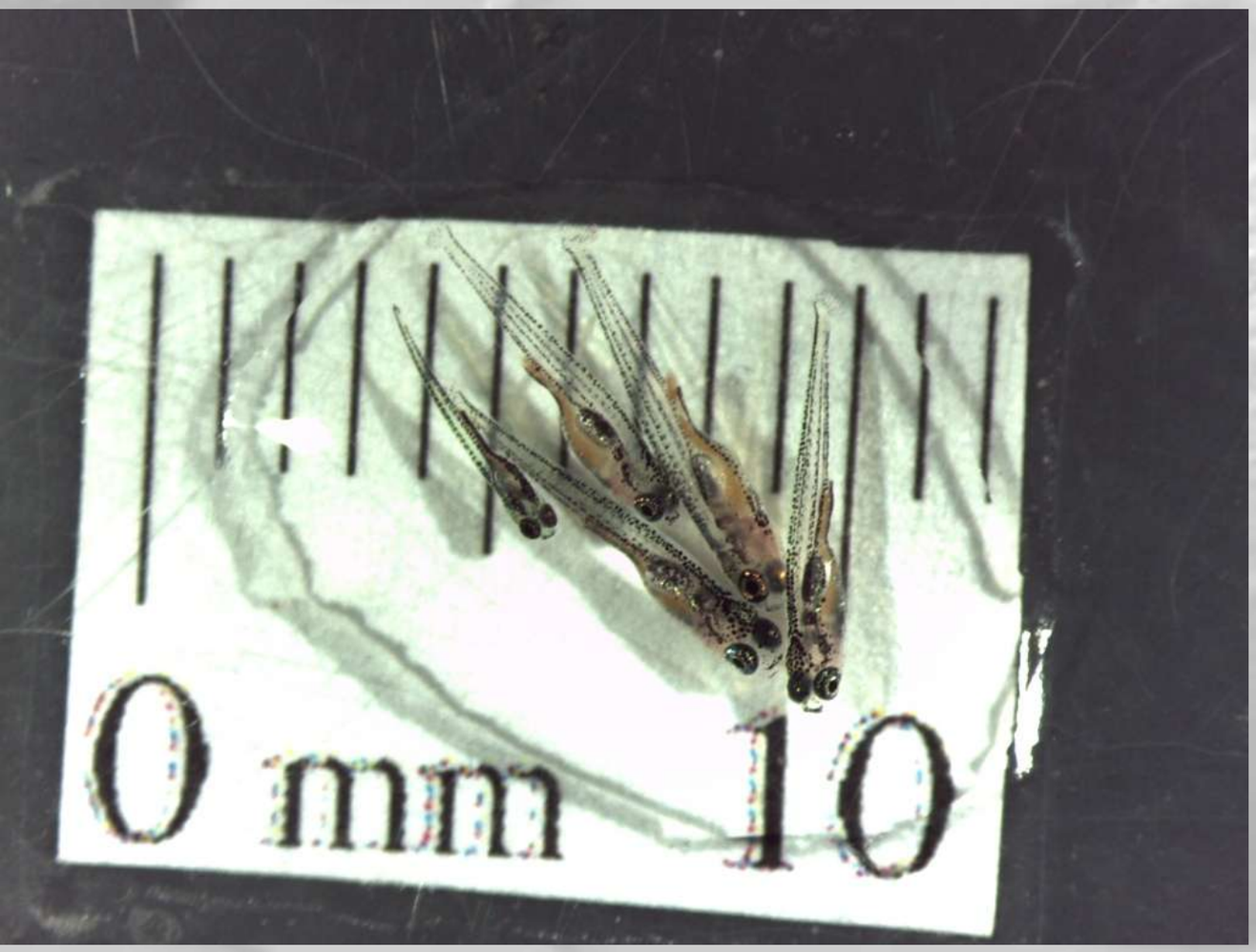
Days 4 and 5

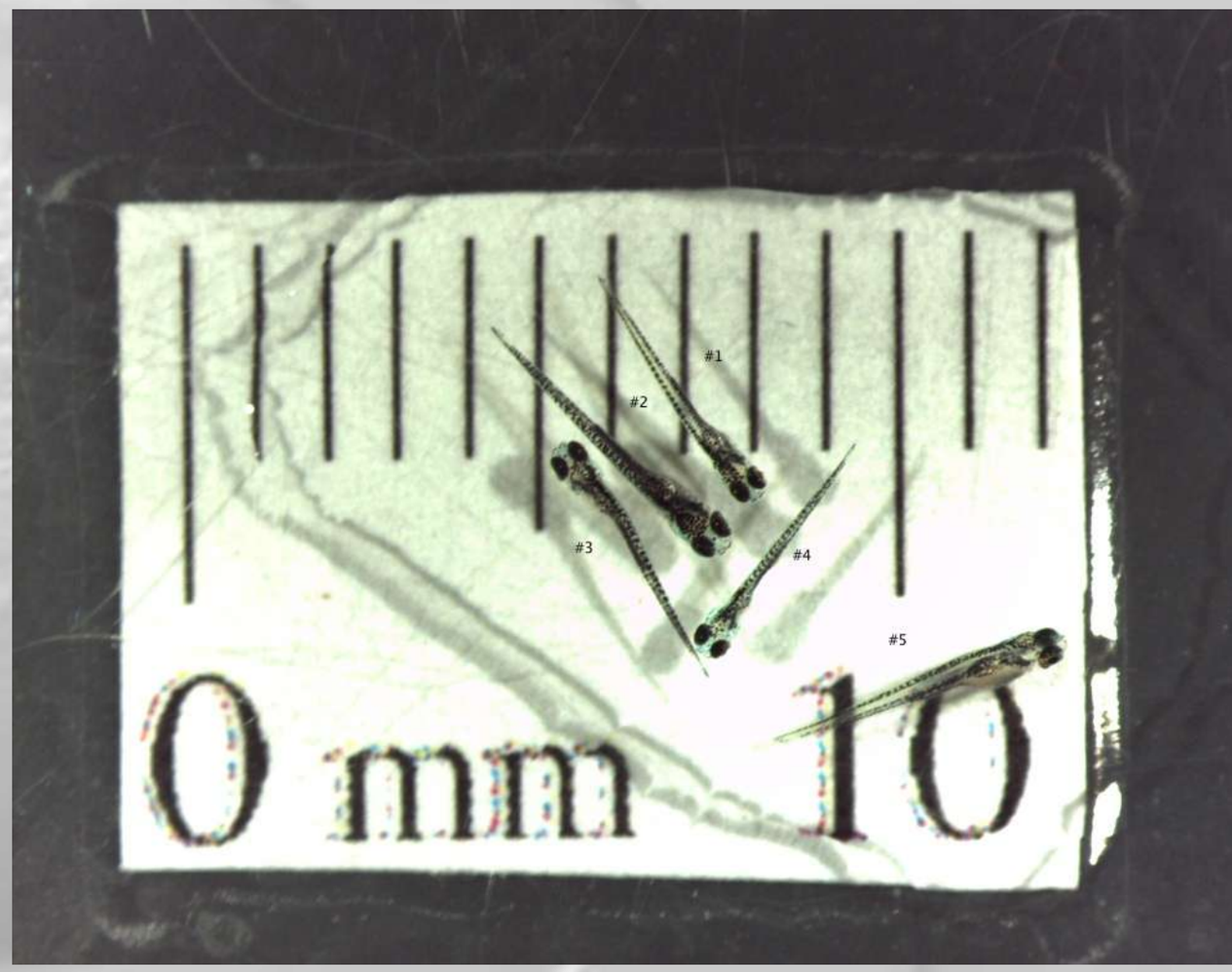
Understanding swim-up



these fish have inflated gas-bladders and are capable of swimming in all levels of the water column.

When to start feeding??

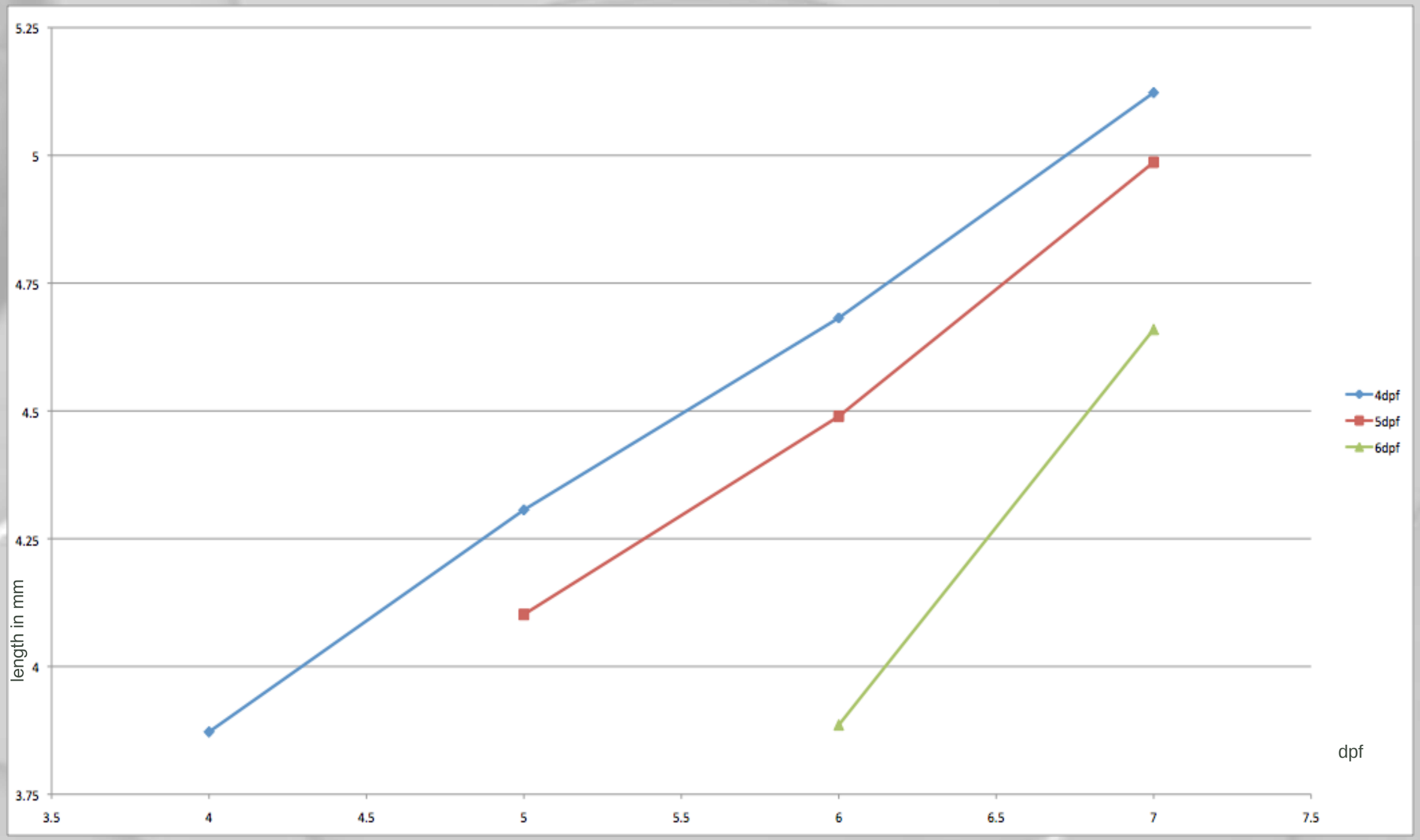




Waiting too long can be disasterous

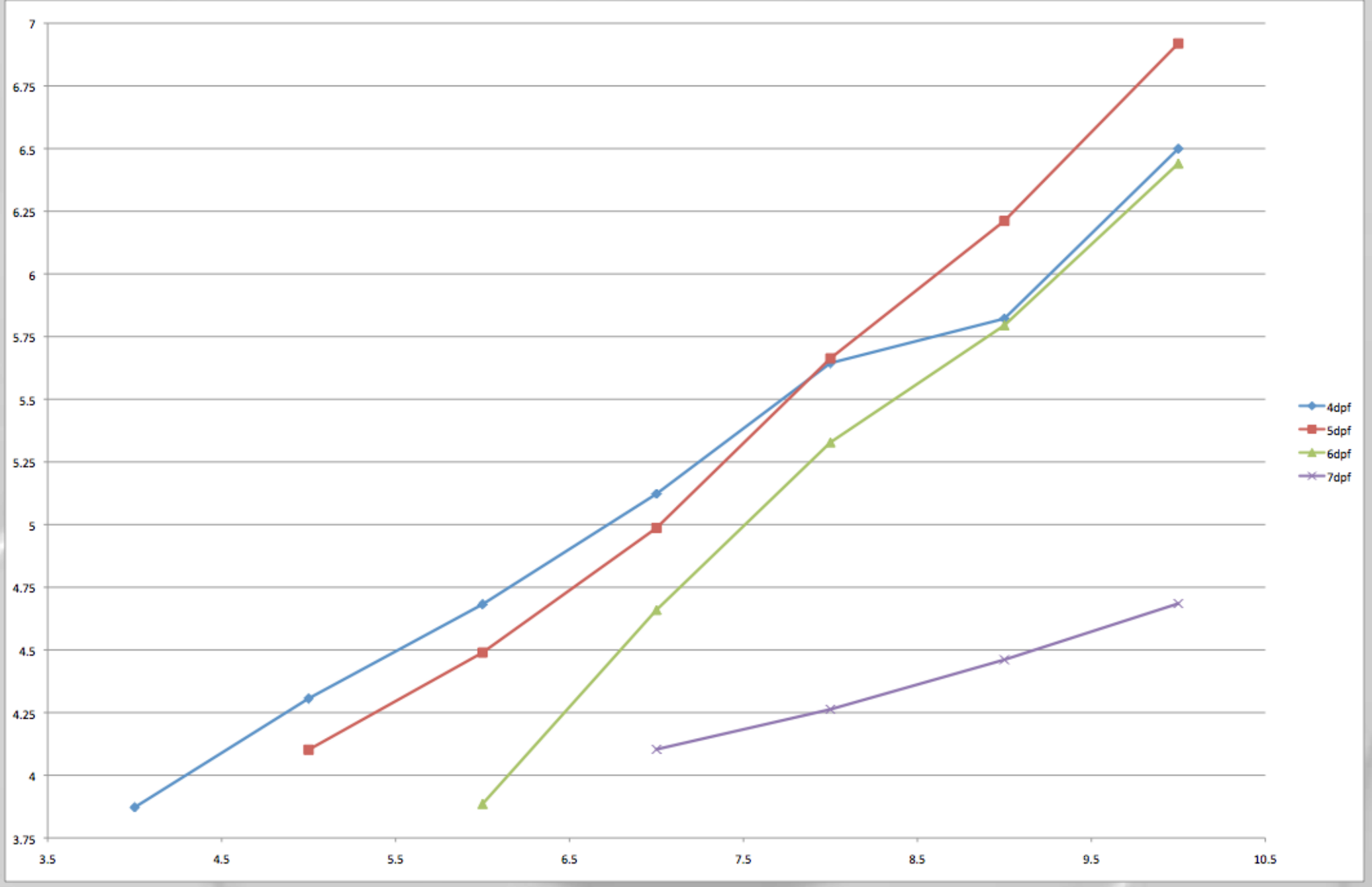
both are: 10dpf , same clutch

the left, offered food (rotifers) at 4pdf; on the right offered food at 7dpf



When to start feeding

time isn't the best or absolute answer



When to start feeding

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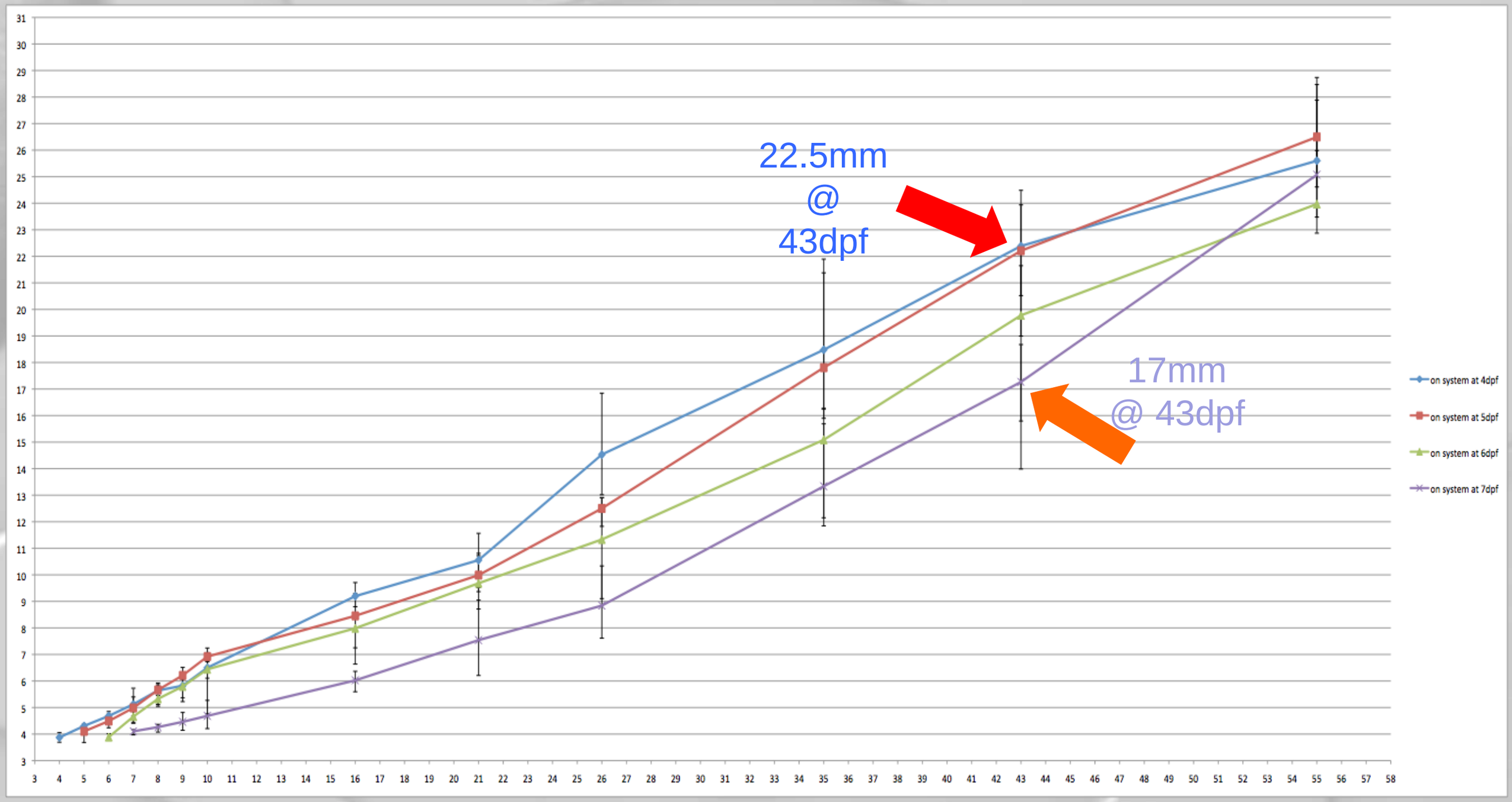


When to start feeding

time isn't the best or absolute answer

feed choices

live, and prepared

live diets

- Artemia – costly, unknown provenance and potential for importing disease, and other untoward effects
- **Rotifers – currently the superior choice**

prepared diets:

what to look for when making a choice

aquaculture feeds vs. hobbyist feeds

- more digestible protein sources and bio-availability
- fatty acid profiles tuned to warm-water vs. cold-water fish
- carotenoids (antioxidants)
- differing buoyancies (dispersal differences) –highly engineered!
- traceability

post-metamorphic feeds

prepared diets

How to apply dry feeds?

<u>top fed (dry)-</u>

- superior
- The way these feeds are designed to be used

<u>Liquified- or mixed into water:</u>

- Poorest choice
- water soluble vitamins are gone before you get it to the fish

Leaching of water soluble vitamins from feeds upon hydration

TABLE 2

Effect of vitamin leaching from experimental diet 2 after a period of 30 s in water (n = 1)

Vitamin	Concentration before contact with water	Percentage vitamin loss in 30 s
	mg · kg dry diet^{-1}	
Pyridoxine	49	6.1
Pteroyl-monoglutamic acid	25	16.0
Choline	3700	27.0
Pantotenic acid	76	47.4
Ascorbic acid	470	66.0
Cyanocobalamin	0.6	90.0

From Pannevis and Earle, *J. Nutrition 1994*

Leaching of water soluble protein from feeds upon hydration

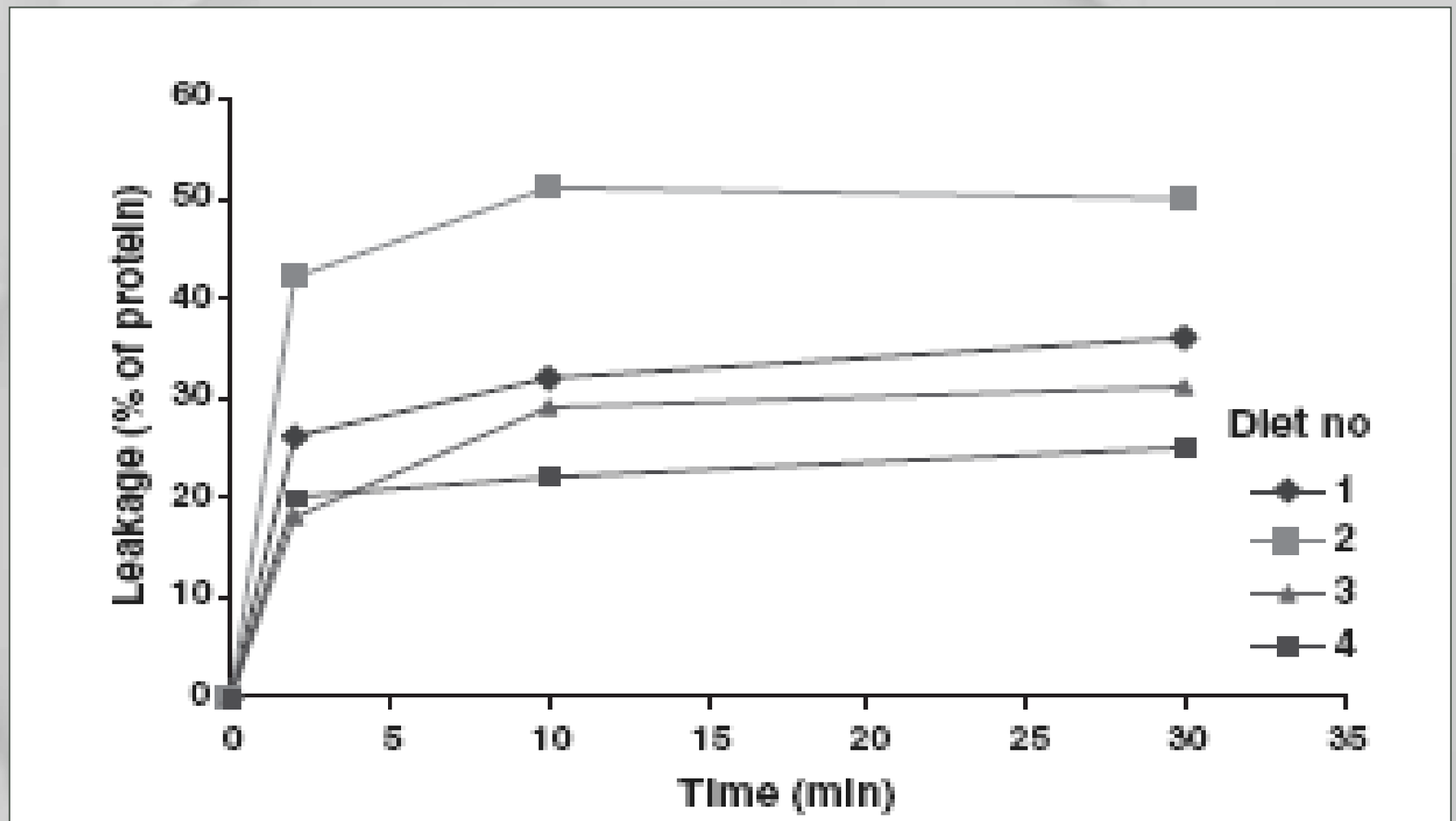


Figure 1 Leaching of crude protein (%) from formulated larval feeds. Diets 1 and 4 are experimental feeds, diet 2 and 3 are commercial feeds. All diets were micro-bound. Leaching was measured by incubating 1 g of diet in 100 mL seawater for variable time intervals. Protein leached to the water was measured as N × 6.25 after filtration and partly evaporation of the water phase (Hamre 2006).

From Kvale et al. , *Aquac. Nutrit.,* 2007

Effect of temperature on lipids in feeds

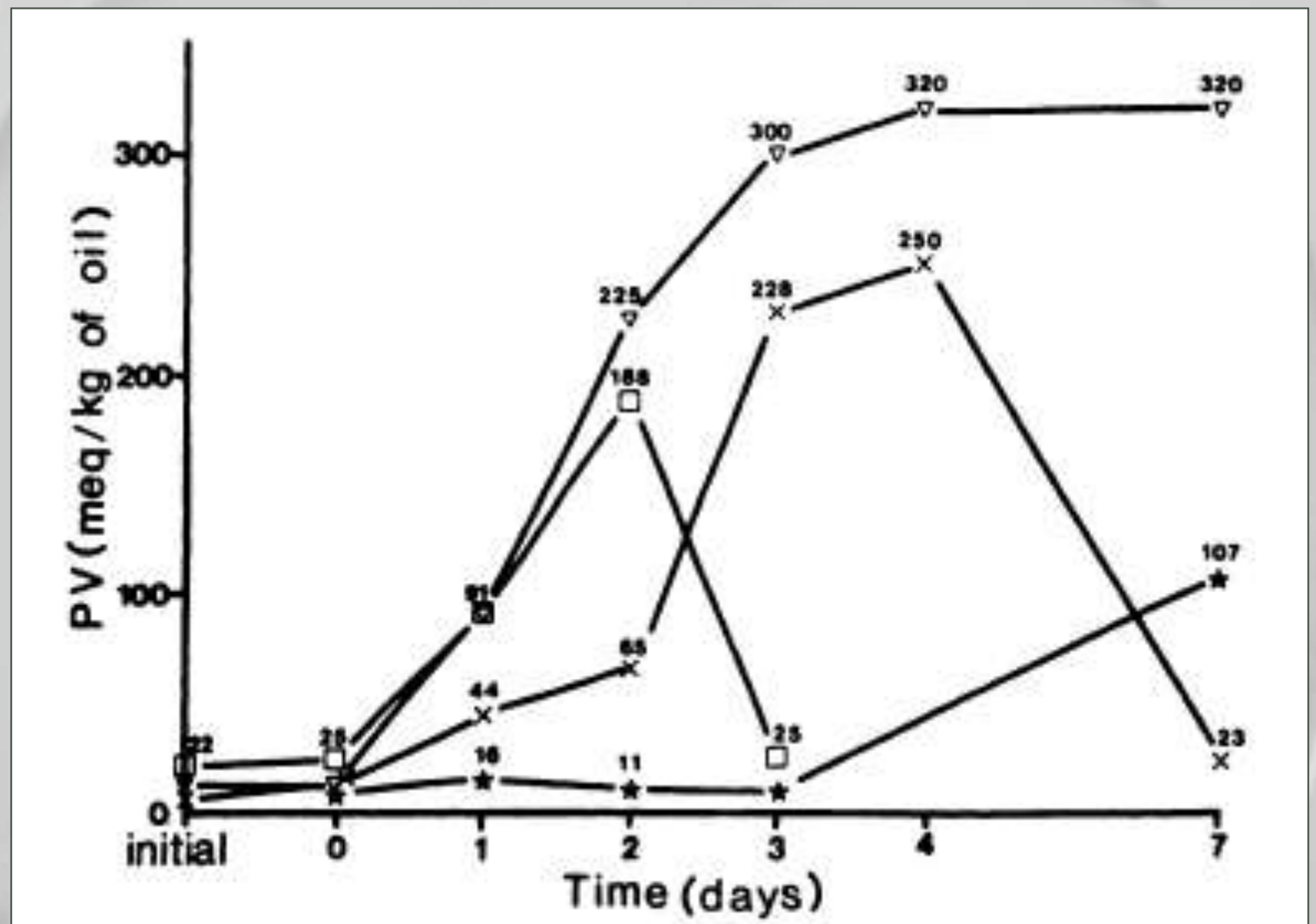
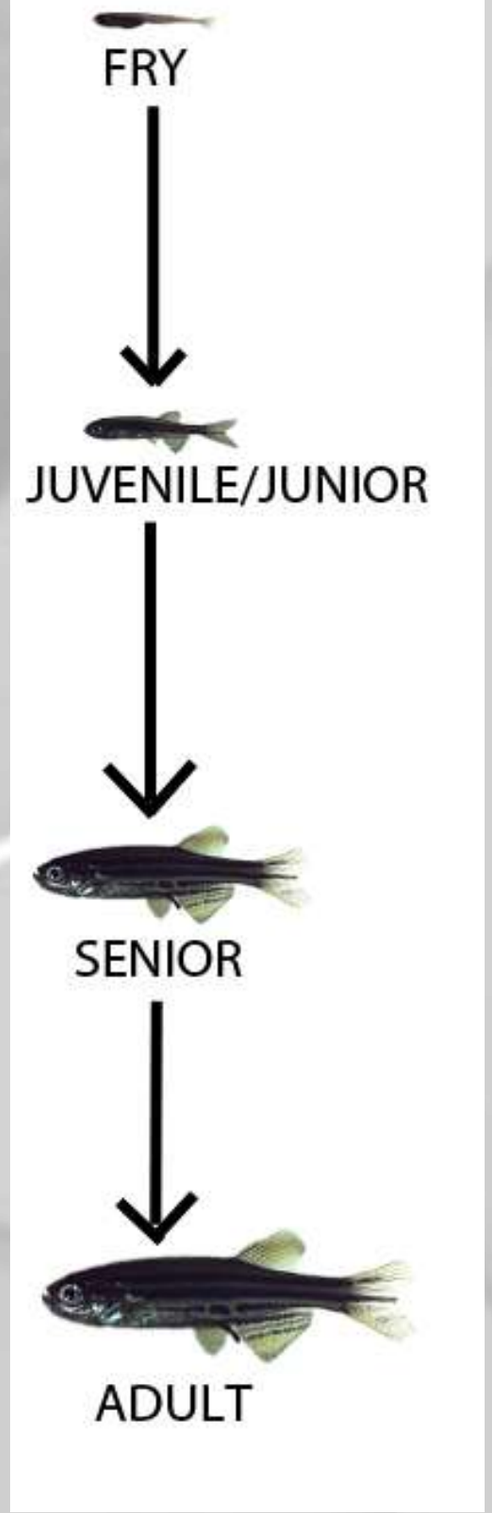


FIGURE 1 Peroxide value of menhaden oil before and after incorporation into purified diets following exposure to air at room temperature for up to 7 d. Menhaden oil with 0.02% TBHQ (★——★) and without added antioxidant, trial 1 (□——□), trial 2 (X——X) and trial 3 (▽——▽).

From Fritsche and Johnston, *J. Nutrit.* 1988

Let them eat…

your observations are the basis for diet changes



graphic tools

empower the staff and researchers to make decisions and avoid making mistakes

Images like these can reflect you feeding practices, and avoid problems with offering the wrong feed type or amount to your fish.

In the past, I have printed these on transparency sheets, and then laminated them for use in the fish room.

Proper Feeding Frequencies to:

- provide adequate nutrition
- exploit the rapid-growth potential of the model

- Larvae - constant
- Juvenile - high frequency
- sub-adult - high frequency

Thanks

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

Kathleen, Lillian and Finley Sanker-Sanders
Christian Lawrence