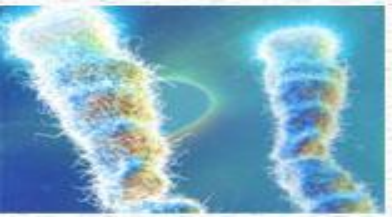
ZEBRAFISH (*DANIO RERIO*)

Fonte: Braunbeck *et al.* (2004)




ZIRC
Zebrafish International Resource Center

Rank	Countries/Territories	Records	No. of institutes doing zebrafish research	Records/Institute	Percent[a]
1	USA	8196	877	9.35	47.625
2	Germany	1871	359	5.21	10.869
3	England	1393	180	7.74	8.094
4	Japan	1284	228	5.63	7.451
5	People's Republic of China	1151	255	4.51	6.703
6	Canada	902	113	7.98	5.243
7	France	877	219	4.00	5.098
8	Netherlands	501	77	6.51	2.914
9	Spain	449	138	3.25	2.601
10	Taiwan	431	84	5.13	2.509
11	Singapore	421	34	12.38	2.445
12	Italy	419	167	2.51	2.433
13	Australia	322	74	4.35	1.866
14	South Korea	275	99	2.78	1.593
15	Switzerland	251	54	4.65	1.454
16	Sweden	238	34	7.00	1.379
17	Belgium	215	47	4.57	1.246
18	Norway	208	43	4.84	1.211
19	Israel	180	30	6.00	1.043
20	Scotland	176	17	10.35	1.020
21	India	170	50	3.40	0.991
22	Brazil	151	51	2.96	0.875
23	Austria	131	27	4.85	0.765
24	Chile	103	16	6.44	0.597
25	Portugal	103	39	2.64	0.597

[a]Total percent is more than 100 because many articles have authors from different countries.

Legislazione italiana

Decreto legislativo 4 marzo 2014, n. 26. Attuazione della direttiva 2010/63/UE sulla protezione degli animali utilizzati a fini scientifici. (14G00036) (GU Serie Generale n.61 del 14-3-2014). note: Entrata in vigore del provvedimento: 29/03/2014.

ALLEGATO *I*

Elenco degli animali di cui all'articolo 10, comma 1

1. Topo *(Mus musculus)*
2. Ratto *(Rattus norvegicus)*
3. Porcellino d'India *(Cavia porcellus)*
4. Criceto siriano (o dorato) *(Mesocricetus auratus)*
5. Criceto cinese *(Cricetulus griseus)*
6. Gerbillo della Mongolia *(Meriones unguiculatus)*
7. Coniglio *(Oryctolagus cuniculus)*
8. Cane *(Canis familiaris)*
9. Gatto *(Felis catus)*
10. Tutte le specie di primati non umani
11. Rana [*Xenopus* (*laevis*, *tropicalis*), Rana (*temporaria*, *pipiens*)]
12. Pesce zebra (*Danio rerio*)

ART. 1 -OGGETTO E AMBITO DI APPLICAZIONE

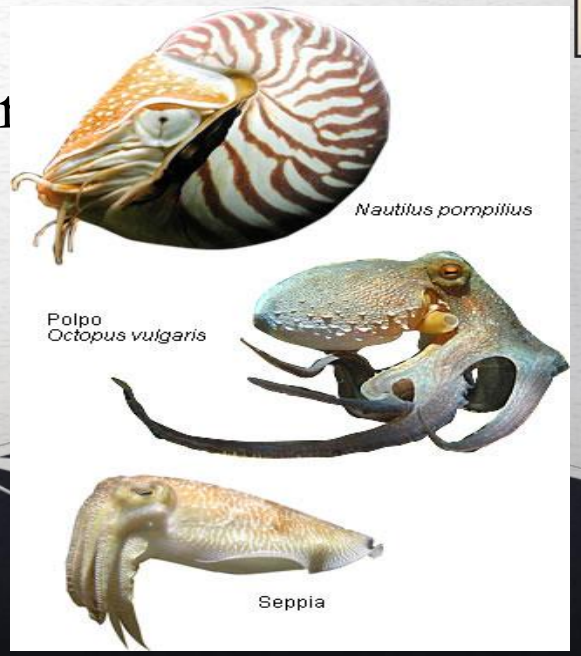
a) animali vertebrati vivi non umani, comprese:

1. forme larvali capaci di alimentarsi autonomamente;

2. forme fetali di mammiferi a partir

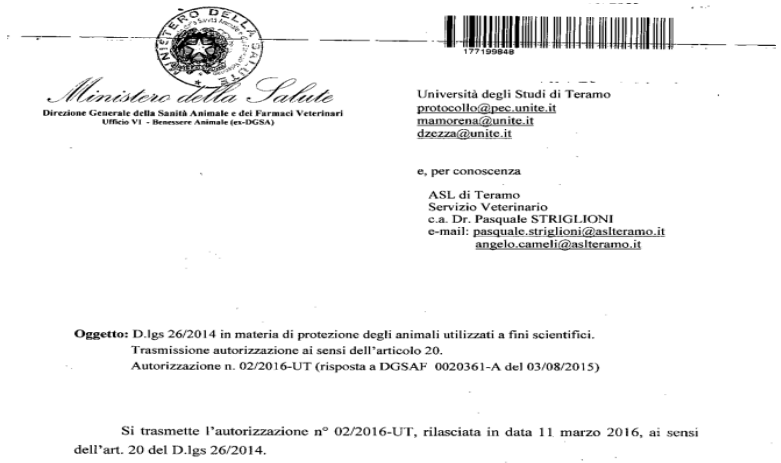
dall'ultimo terzo del loro normale sviluppo;

a) cefalopodi vivi.



UNITE

Stabilimento utilizzatore per la specie zebrafish



Ministero della Salute
Direzione Generale della Sanità Animale e dei Farmaci Veterinari
Ufficio VI - Benessere Animale (ex-DGSA)

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e, per conoscenza

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Oggetto: D.lgs 26/2014 in materia di protezione degli animali utilizzati a fini scientifici.
Trasmissione autorizzazione ai sensi dell'articolo 20.
Autorizzazione n. 02/2016-UT (risposta a DGSAF 0020361-A del 03/08/2015)

Si trasmette l'autorizzazione n° 02/2016-UT, rilasciata in data 11 marzo 2016, ai sensi dell'art. 20 del D.lgs 26/2014.

Università degli Studi di Teramo

Zebrafish Facility

REGISTRO ANIMALI ALLEVATI

Codice identificativo aziendale: 041TE294

Registro n. 1 consegnato il 01/03/2016

Il registro è composto da 50 fogli, a pagine affiancate e numerati progressivamente dal n. 01 al n. 50.
Il registro è a disposizione delle Autorità Sanitarie

Stabilimento allevatore per la specie zebrafish

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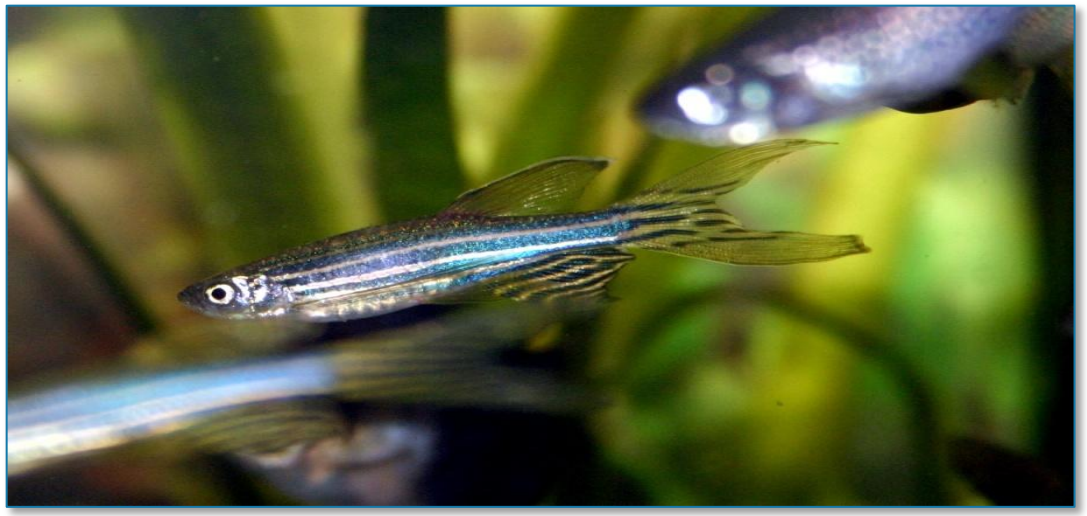
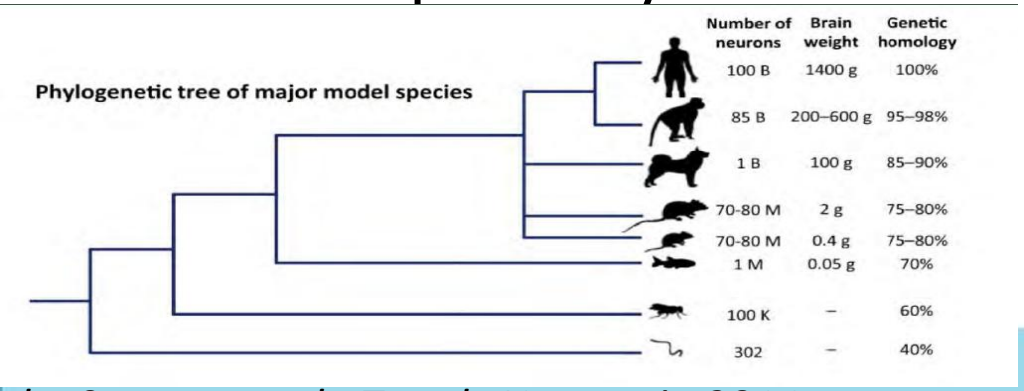
What is Zebrafish ?

- ***Danio rerio***
- tropical fresh water fish
- Cartilaginous fish
- Origin Ganges and Brahmaputra river basin
- Male and female
- Good model of replacement (common molecular pathways with mammals)



Last common ancestor with humans was 445 million years ago: far more remote from humans than other animal models such as rodents

Scientific classification	
Kingdom:	Animalia
Phylum:	Chordata
Class:	Actinopterygii
Order:	Cypriniformes
Family:	Cyprinidae
Genus:	*Danio*
Species:	***D. rerio***



by Stewart et al., Trends Neurosci., 2014

ZFIN
Research | General Information | ZIRC
The Zebrafish Model Organism Database

Genome sequencing

- The **Wellcome Trust Sanger Institute** - zebra genome sequencing project
- **2009**- Institute of Genomics & Integrative Biology, New Delhi
- 17 April 2013- zebrafish reference genome sequence was published

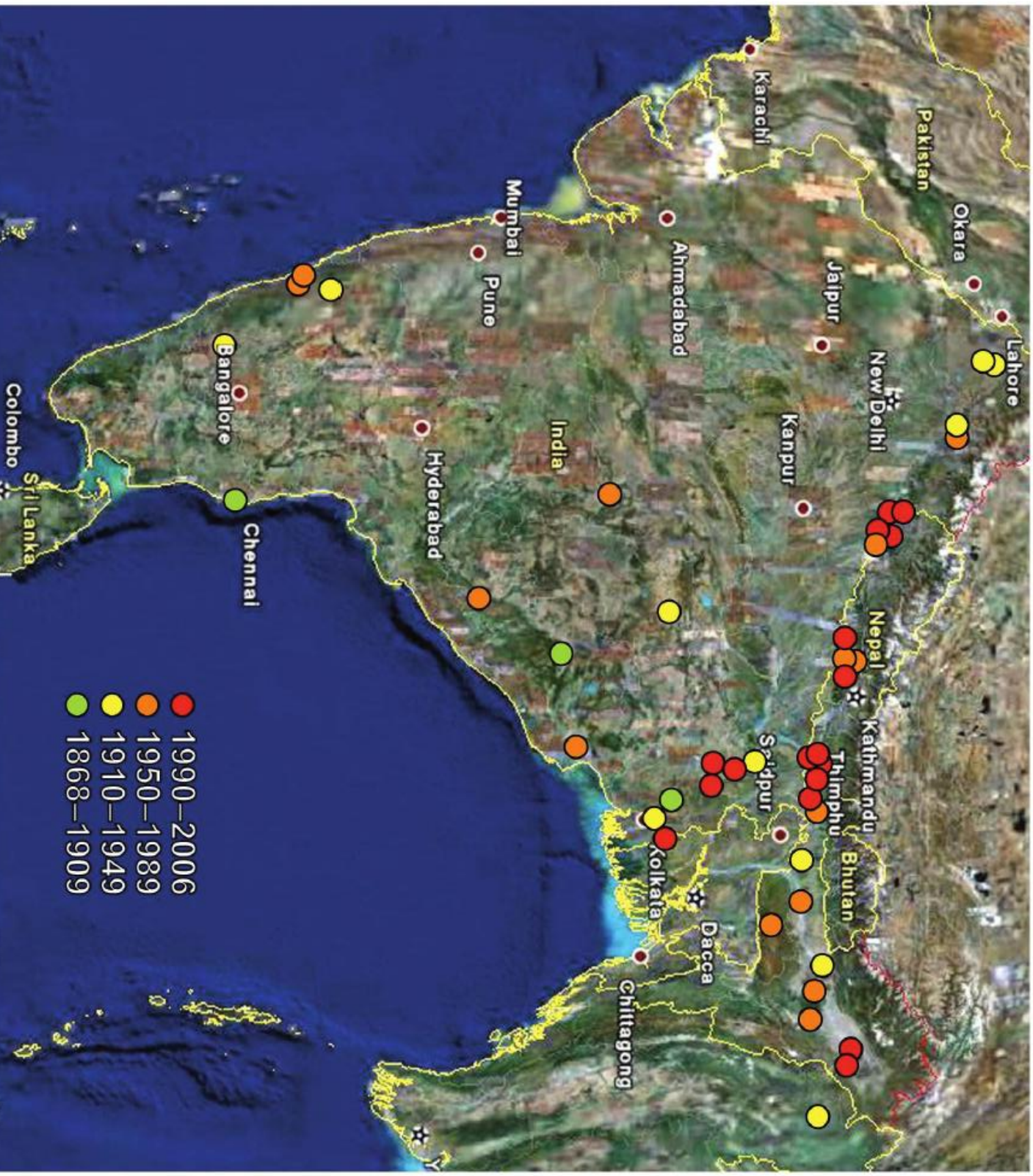
INTRODUCTION

Embryo **Larva** **Adult**

Zebrafish (*Danio rerio*):

- Family *Cyprinidae*
- Freshwater fish (Asia)
- Length 4-5 cm (adults)
- Males and females. Sexual dimorphism in zebrafish is minimal
- Adult develops stripes that run along the length of the body and look blue in colour.

Where is zebrafish in the world



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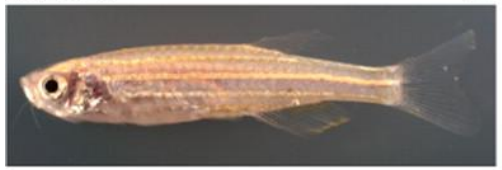
Natural habitats of zebrafish

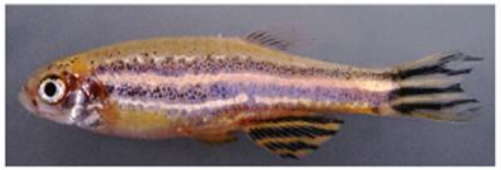
still water (currents, 0 m–sec to 0.1 m–sec) at 27° C to 34° C and pH 7.9–8.2; widths of water bodies ranged from 1 to 12 m, and depths ranged from 16 to 57 cm; water was relatively clear (transparent to 35 cm).5-6 months for sexual maturation.

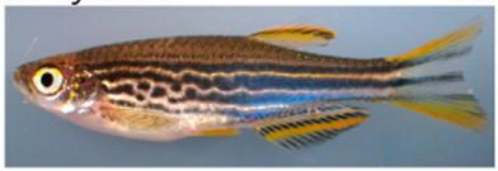
Engeszer RE, et al. Zebrafish, 4:21-40, 2007

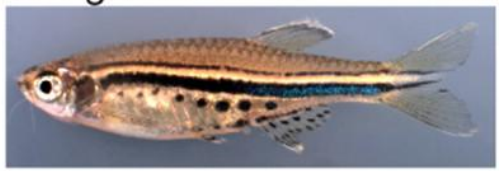
Zebrafish strain

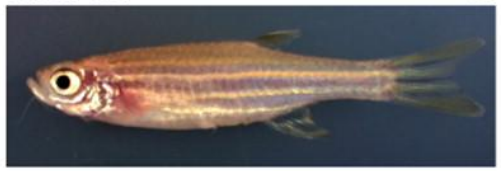
- Wild type
- Mutant
- Transgenic

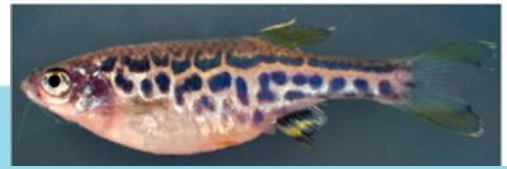


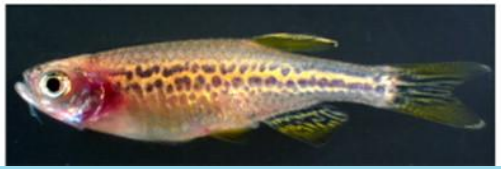




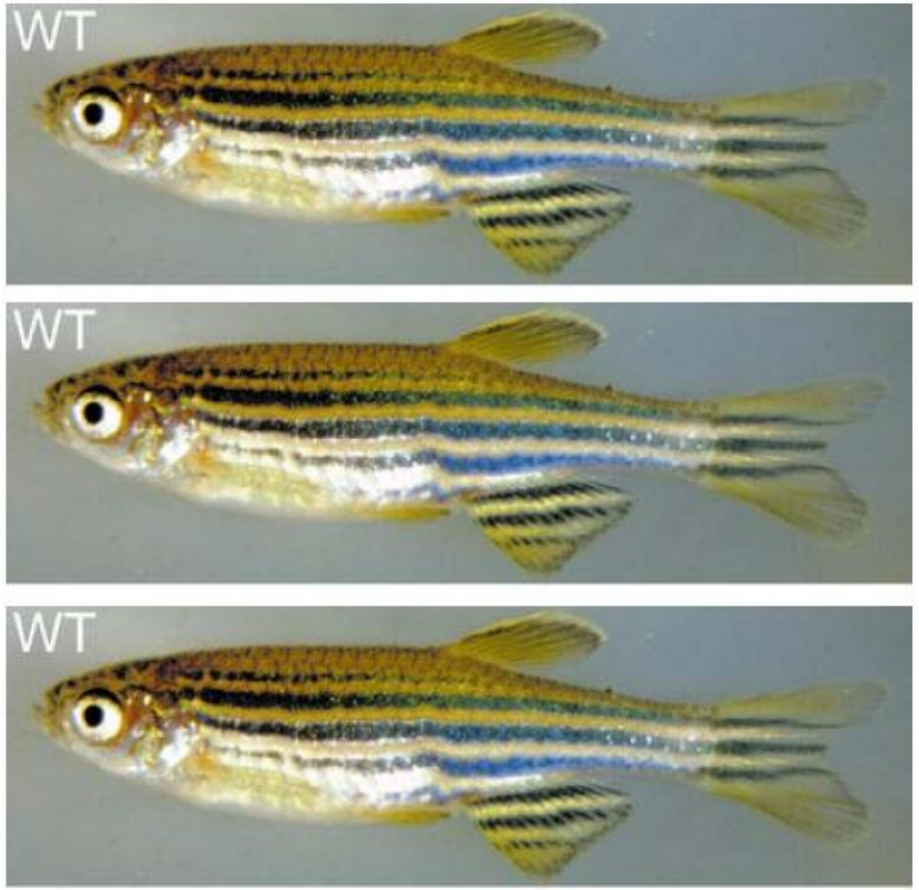








Laboratory strains



AB

(pet shop derived)

TÜ

WIK (Wild type India Kolkata)

They may look the same, but are genetically different!
WIK was used for mapping mutations from the first mutagenesis screens

Zebrafish breeding and reproduction

Fonte: Braunbeck *et al.* (2004)

General Features

General Features	Benefits
Appearance -Dimension ~4 cm -Salient distinguishable features of male and female -Often transparent adult bodies	Large number can be kept easily and cheaply in lab Good model for visualization of cellular activity
Habitat -Fresh water fish - Tropical fish	Universally available
Feeding -Omnivorous	Low cost of maintenance
Reproduction -Female spawns every 2-3 days -Breeds all year round -Several hundreds of eggs produced in single clutch -External fertilization	Large number of offspring- good for batch variation studies Easy availability of eggs

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The habitat in the lab…

recirculating systems

Lab habitat: physico-chemical properties

Water Quality
Temperature
pH
Ammonia (NH3 /NH4)
Nitrite (NO2)
Nitrate (NO3)
Dissolved Oxygen (DO)
Conductivity

Found in two recent studies:

Temperature:	12.3 - 38.6 °C
pH:	5.9 - 9.8
Conductivity:	10 - 271 µS

In the lab:

26-29 °C

6.5-8.0

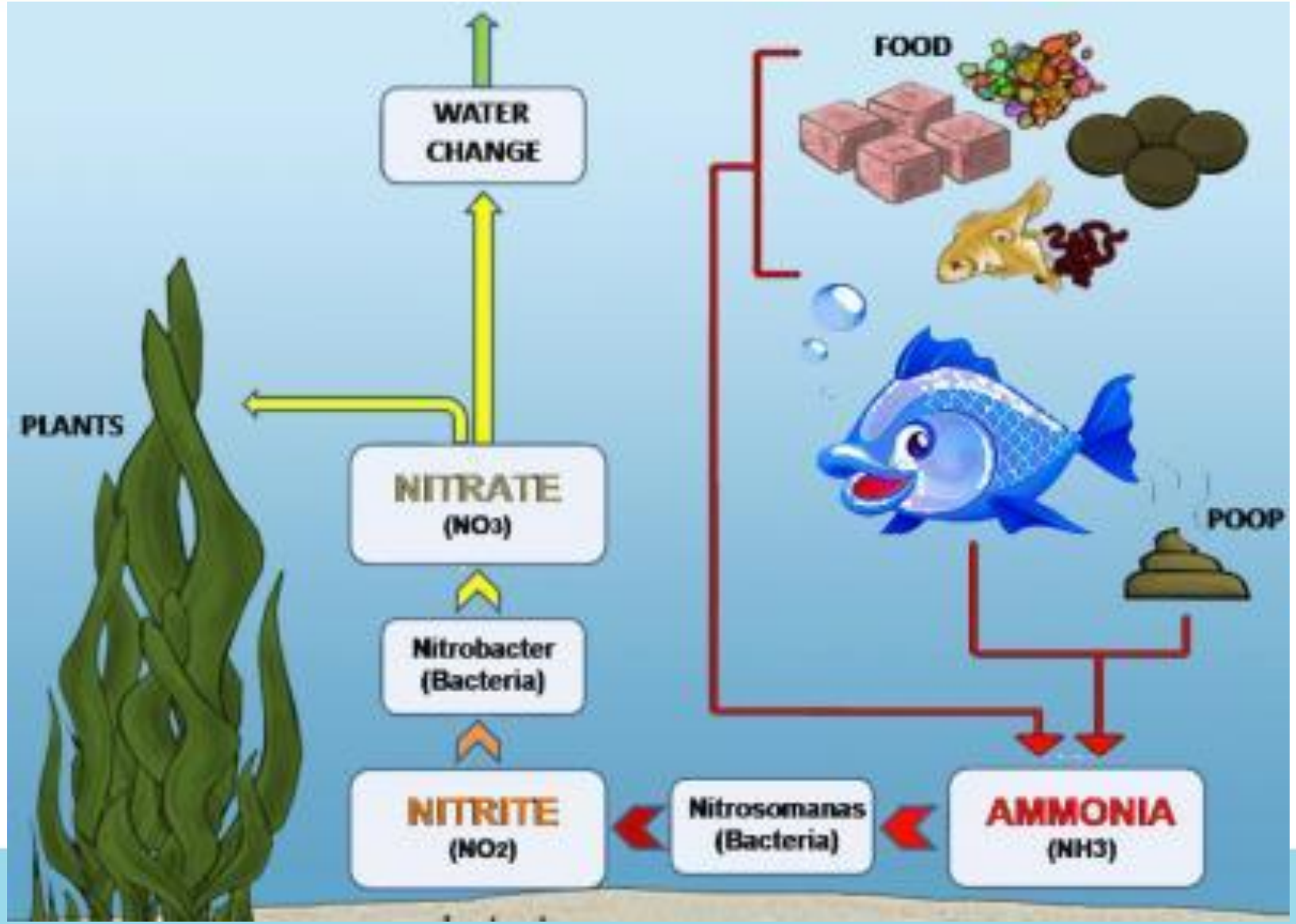
250-600 µS

Light 14hours/day

Quite tolerant – in line to what was observed in the laboratory

Targeted values: NO_3^- <2.5 mg/l, NO_2^- < 0.025 mg/l

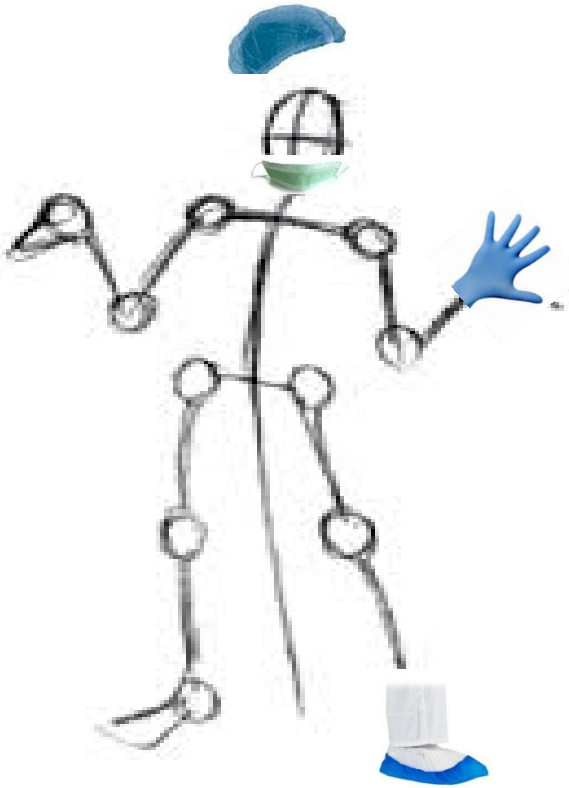
Nitrogen cycle



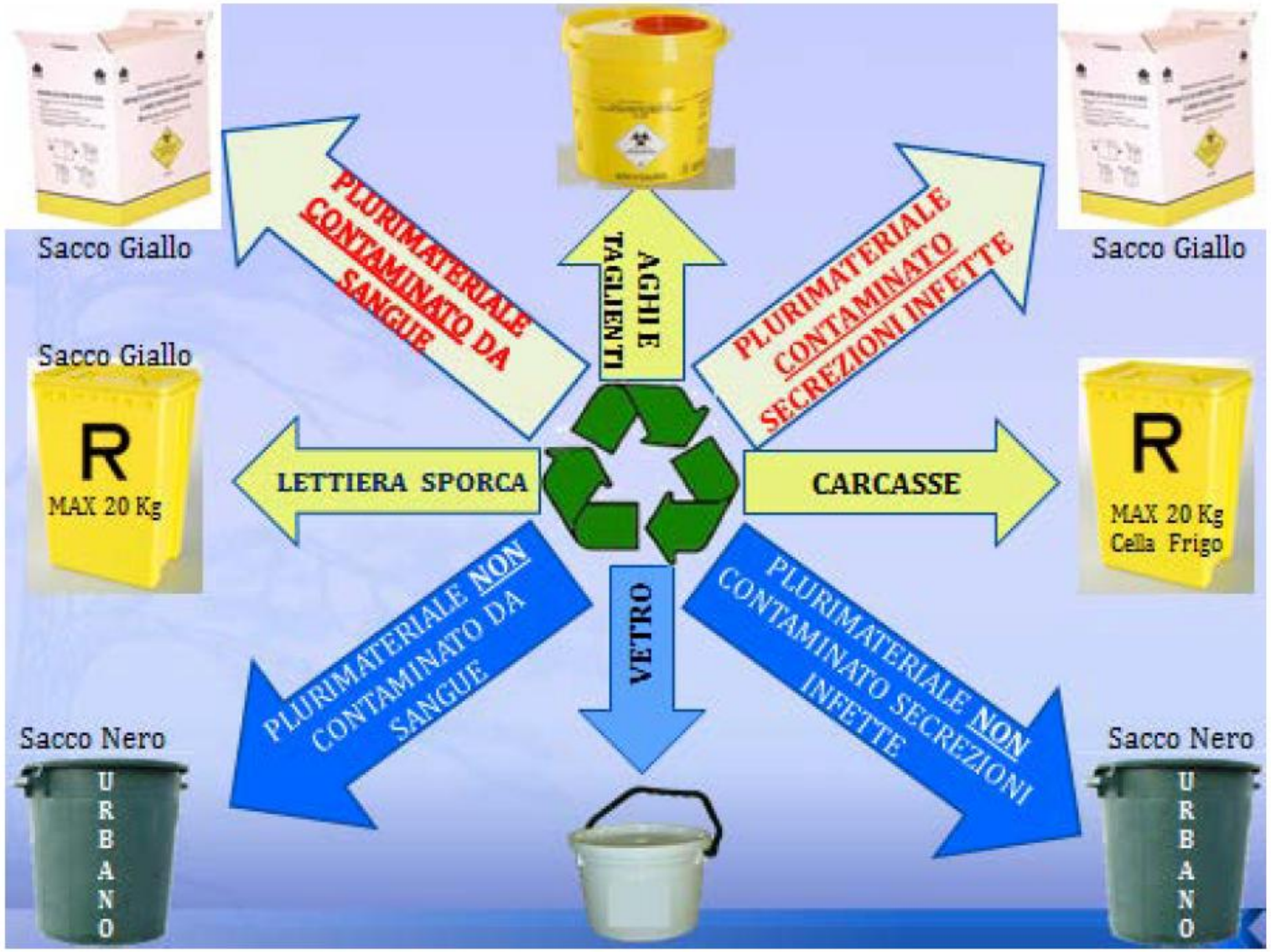
Internal biosecurity

- Use nets in one tank only
- Set good temperature in the room
- Light cycle
- Sanitize equipments between use
- Keep sentinel fish!!!!!

Use personal protective equipment (PPE)



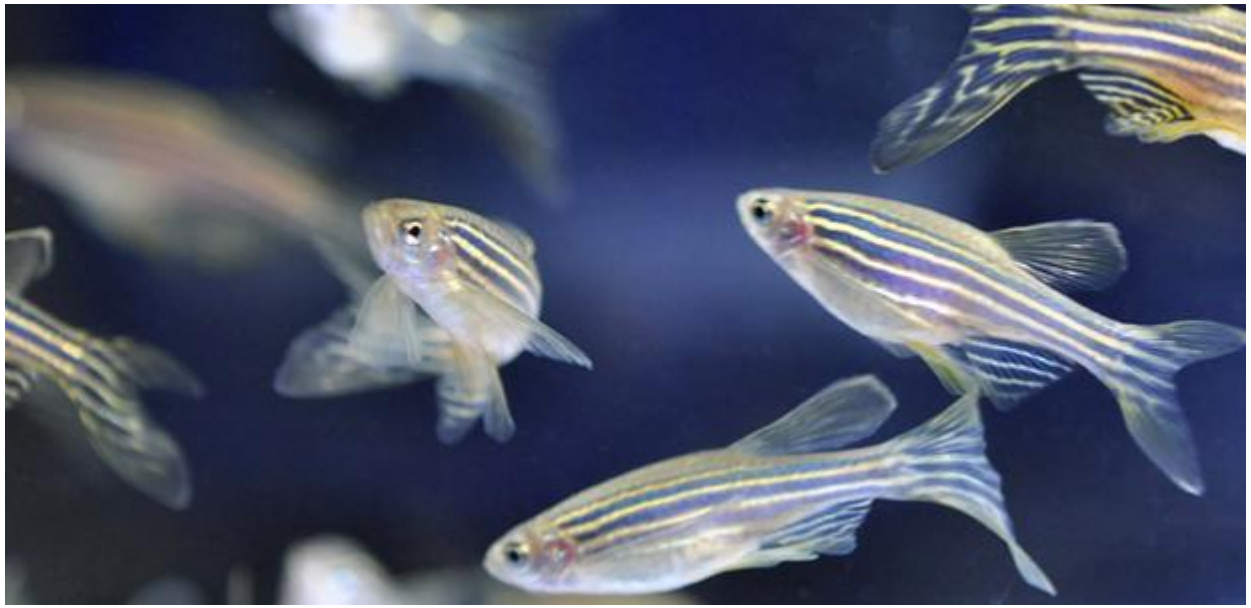
Smaltimento Rifiuti - Procedura



Biosecurity
Quarantine

- **All fish from outside sources**
- **Isolated from central facility**
  - Tanks, racks, water system
  - Equipment, nets, glassware, etc.
- **Observation of adult fish**
  - Observe adults 2-3 weeks prior to breeding
- **Euthanatize or treat sick fish**
- **Surface sanitize embryos**
  - Highly recommended

Behaviour

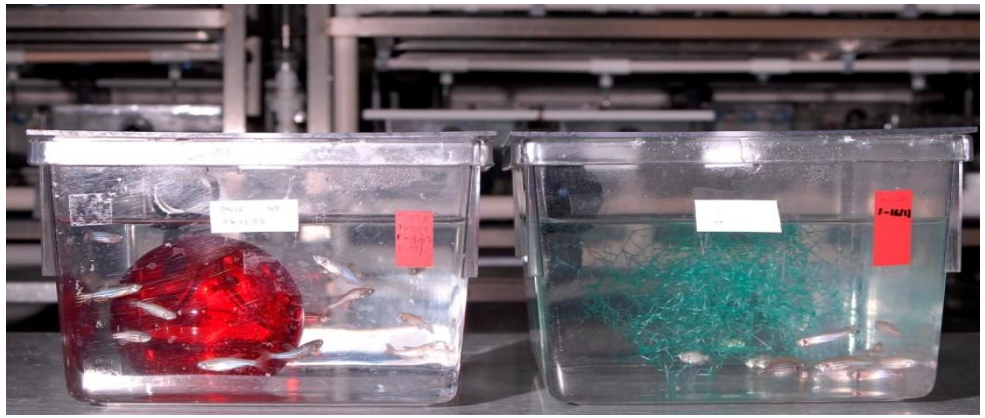


https://www.youtube.com/watch?v=F7JNB2k_S00

- Omnivorous and diurnal fish
- Good swimmer
- Show a territorial behavior and establish dominance
- Breed in large number, important social interaction
- Many behaviors are relatively similar between adult and larval zebrafish models
- Use of video-tracking software

Environmental enrichment

- Improve welfare.
- Promote natural behaviour.
- Reduce the stereotypy.



Common enrichment: plastic grass or plastic leaves or Artemia or marbles

Important : should be no toxic

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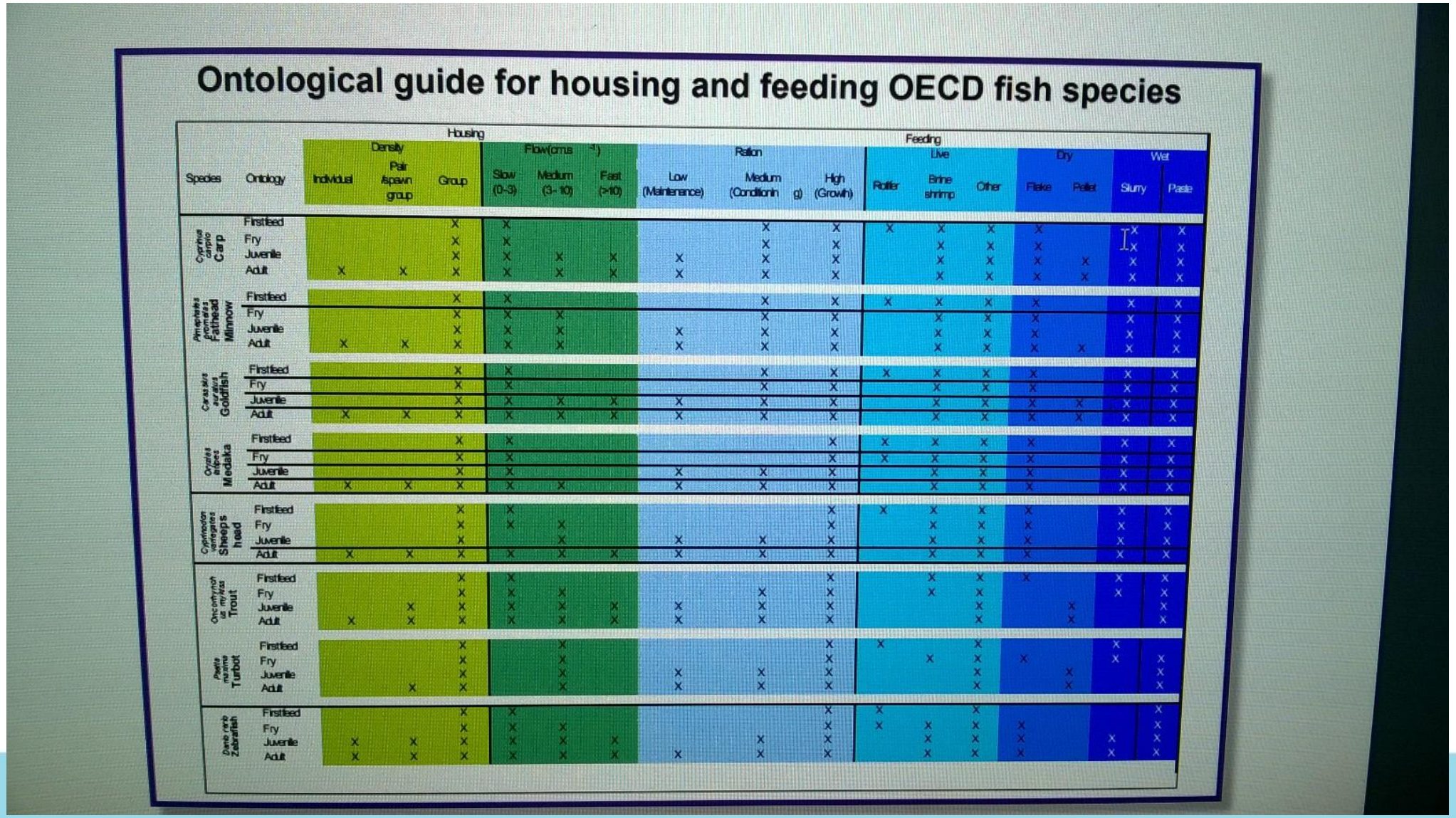
Ontological guide for housing and feeding OECD fish species



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Diet of zebrafish

Food in the nature

Insects (like ants) that have fallen into the water

crustacean larvae and other zooplankton and phytoplankton

prefer to feed at surface – upward gaping mouth

but chase prey also across the water

Artemia larvae as live food = tank enrichment!

Food in the lab

Feeding

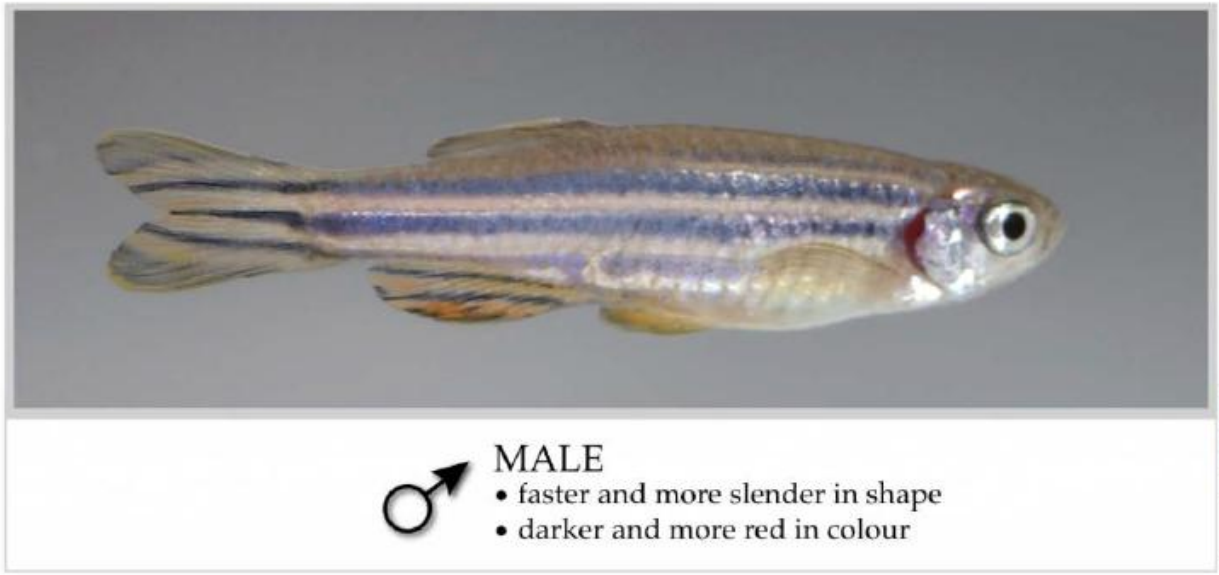
- **Larval Diets**
  - Dry - micro-capsule
  - Live - Paramecia, *Artemia* naupli, rotifers
- **Adult Diets**
  - Dry - Flake, pellet, crumble, freeze dried
  - Live - *Artemia* (brine shrimp)
  - Varied diet recommended
- **2-4 feedings/day**
  - Do Not Overfeed

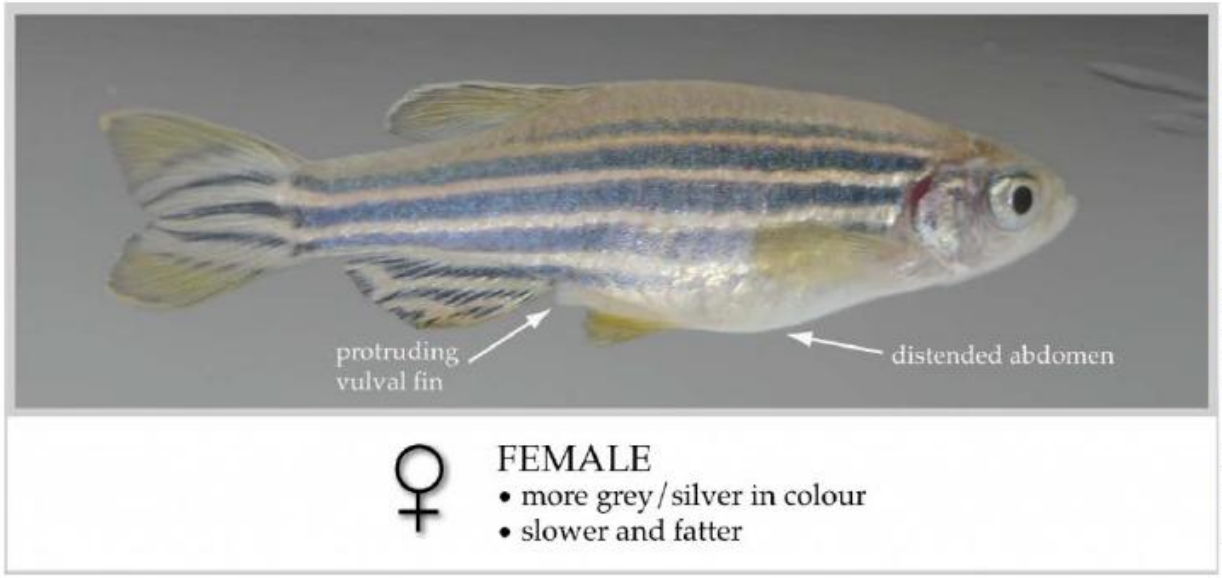
The zebrafish

Danio rerio Hamilton 1822

zebrafish
Zebrabärbling
poisson-zèbre
pez cebra

(in older literature also *Brachydanio rerio*)





http://emblog.embl.de/ells/ish2012/files/2012/06/Zebrafish_female_male-1024x991.png

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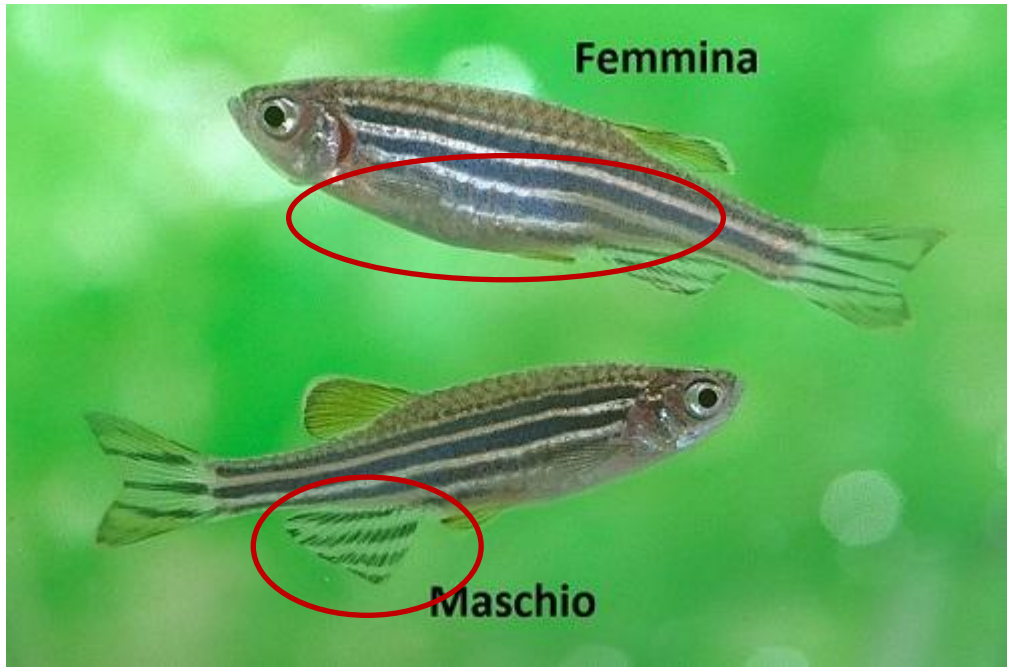
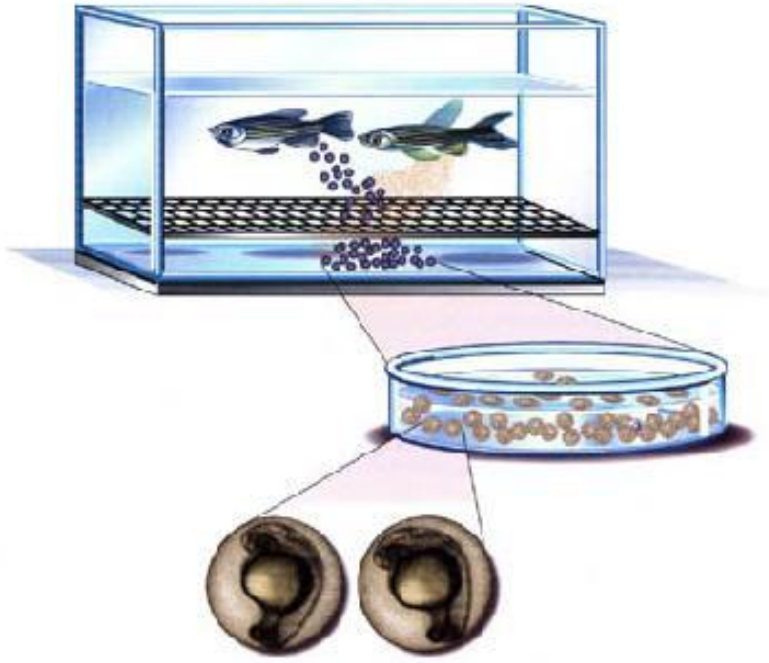
Males and femals

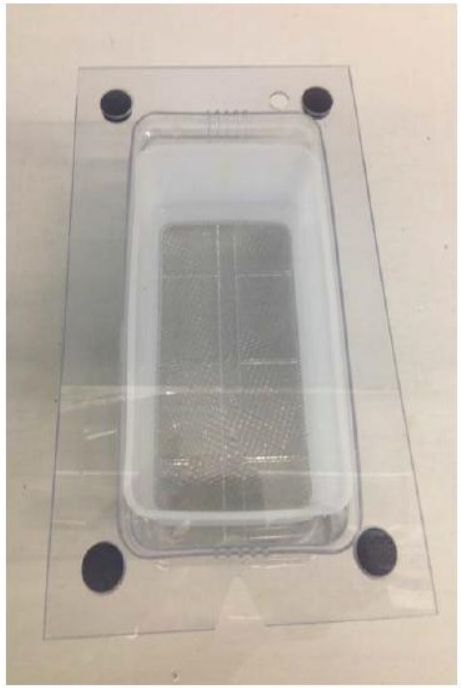


REPRODUCTION

- In the afternoon put females and males together into a mating tank (1:1 o 2:1 or all togheter)
- Leave alone fish, do not disturb
- Remove the partition if present
- Zebrafish typically lay eggs within the first two hours after the lights come on
- Do not over breed and allow 1-2 weeks between breeding events



REPRODUCTION

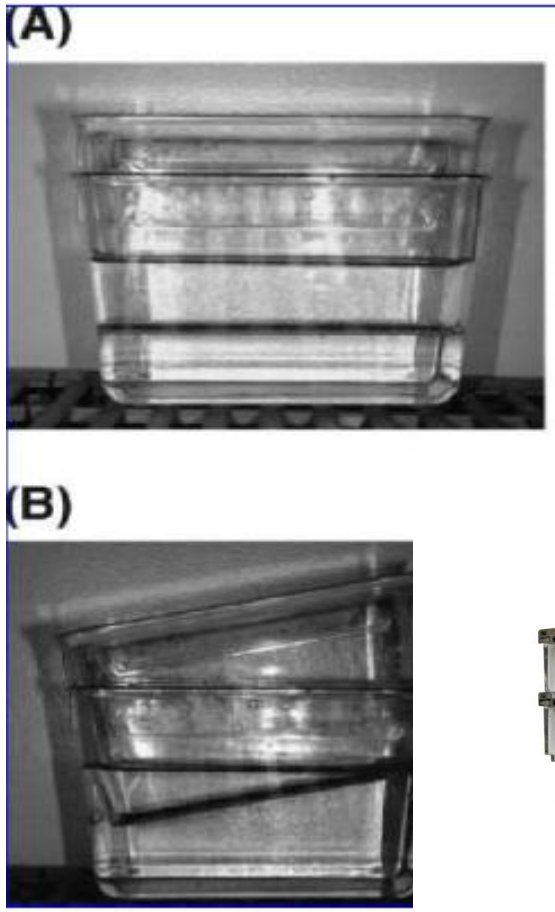


Typical breeding cage for single couple

Set up fish late in the afternoon – they will wait with mating until the next morning

Or

Separate male and female, put them together early next morning – generates precisely dated eggs

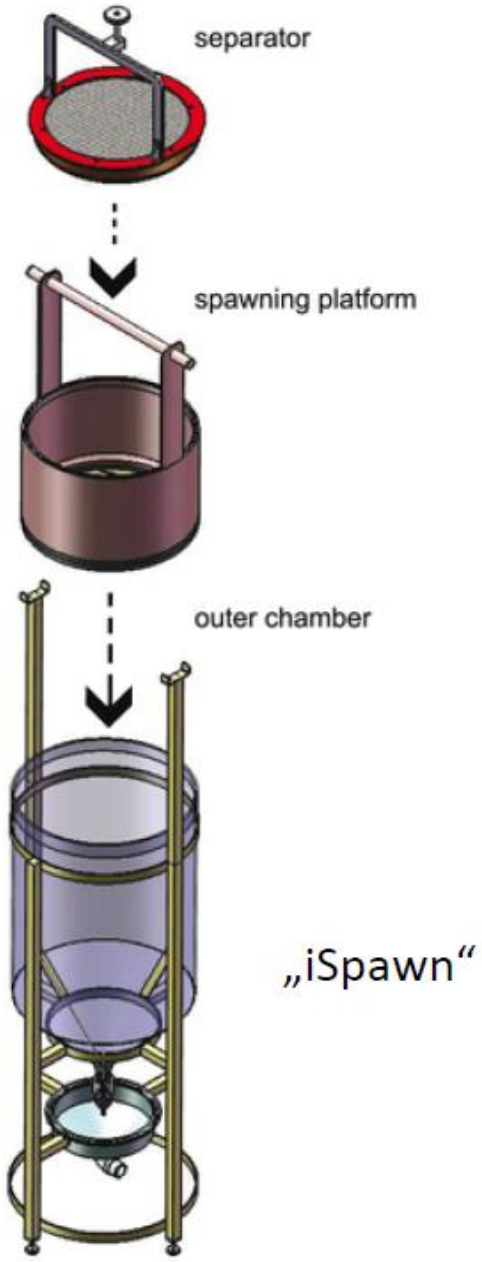


https://www.youtube.com/watch?v=Wb1mEfACpY4

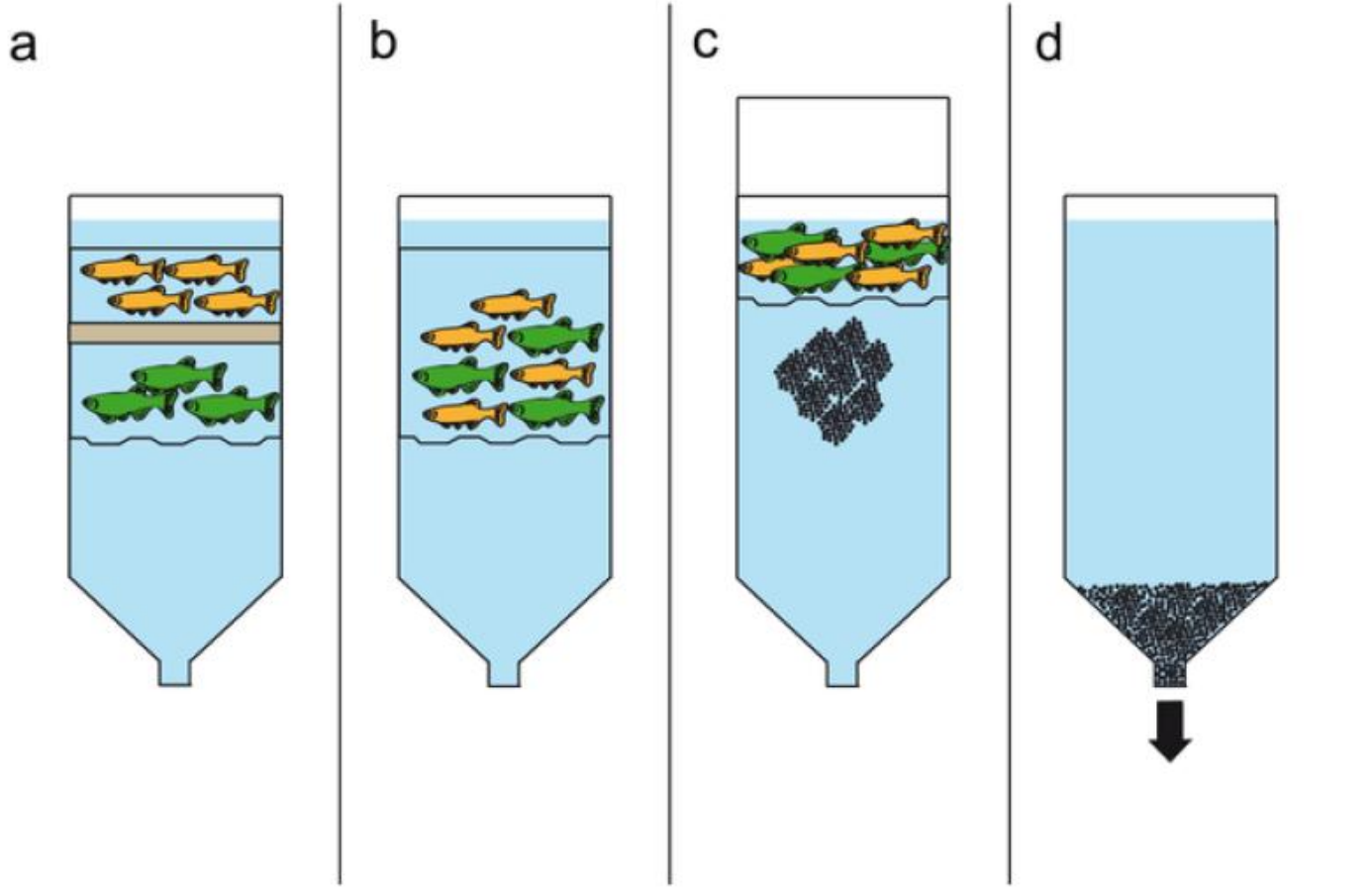
https://www.youtube.com/watch?v=PUNN7YzC4Zo

https://www.youtube.com/watch?v=-aecw9Hx3DU&t=5s

Mating – practical part



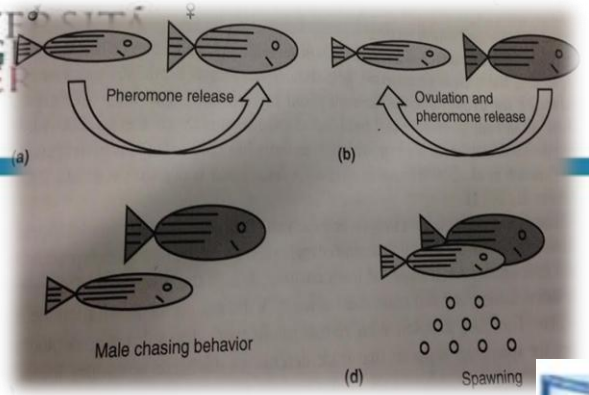
„iSpawn“

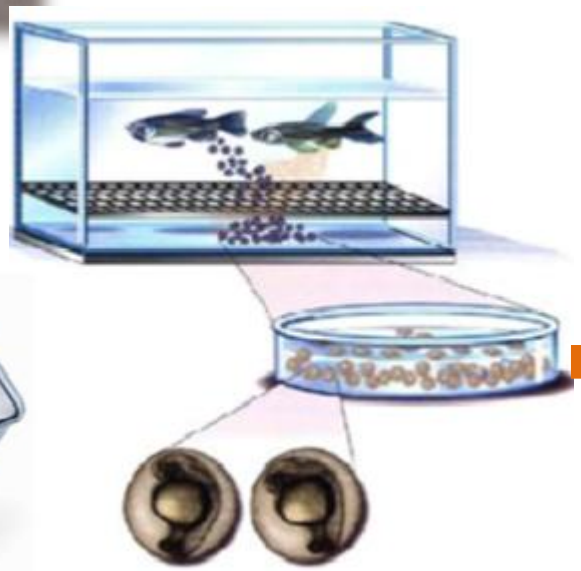


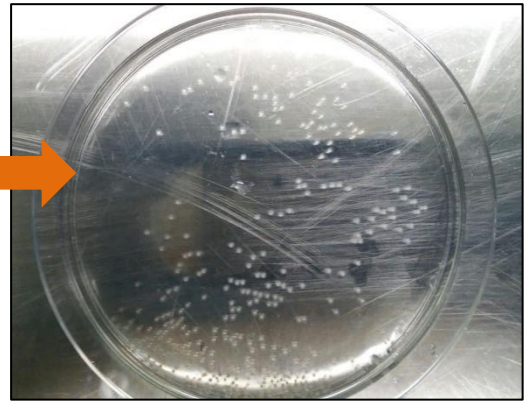
separation „sex on the beach“ egg harvesting

Stress

- As a result of:
  - handling,
  - disturbance,
  - poor water quality,
  - overcrowding,
  - aggression from other fish
  - loud sudden noise







Breeding

Stimoli scatenanti la riproduzione: stimoli olfattivi (Ref: C. Lawrence) e contatto fisico.

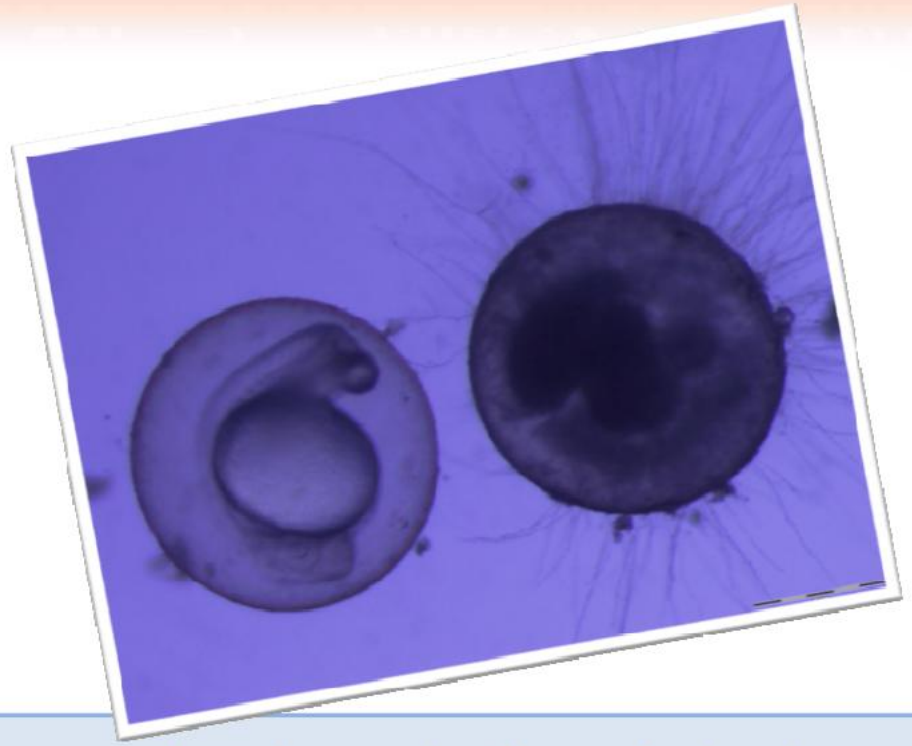
COLLECT THE EGGS AND INCUBATION

Incubazione degli embrioni

Passaggio fondamentale spesso sottovalutato

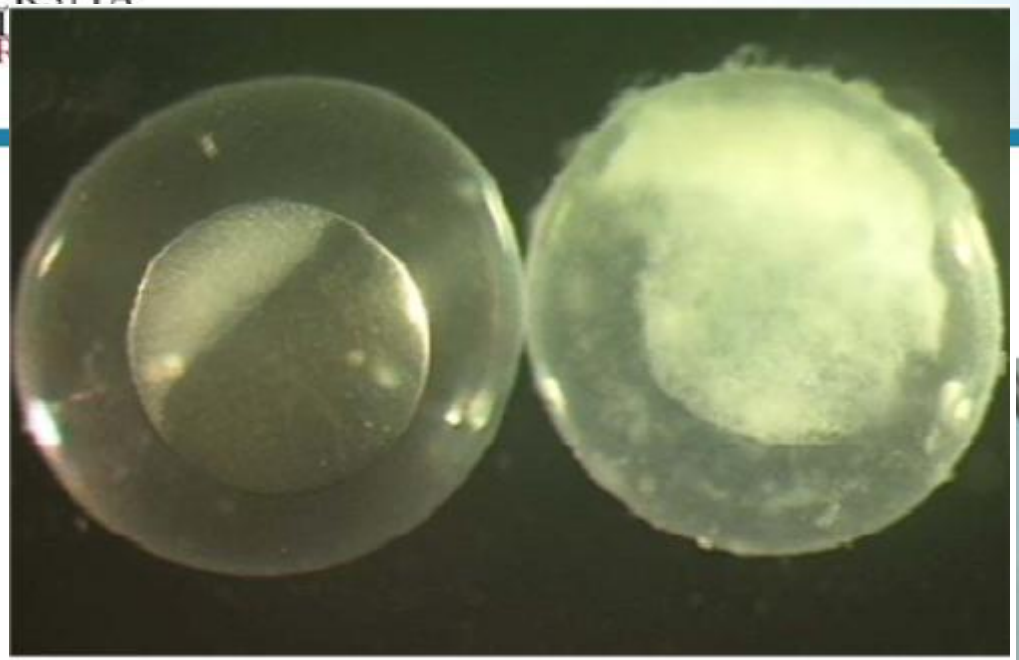
Breeding

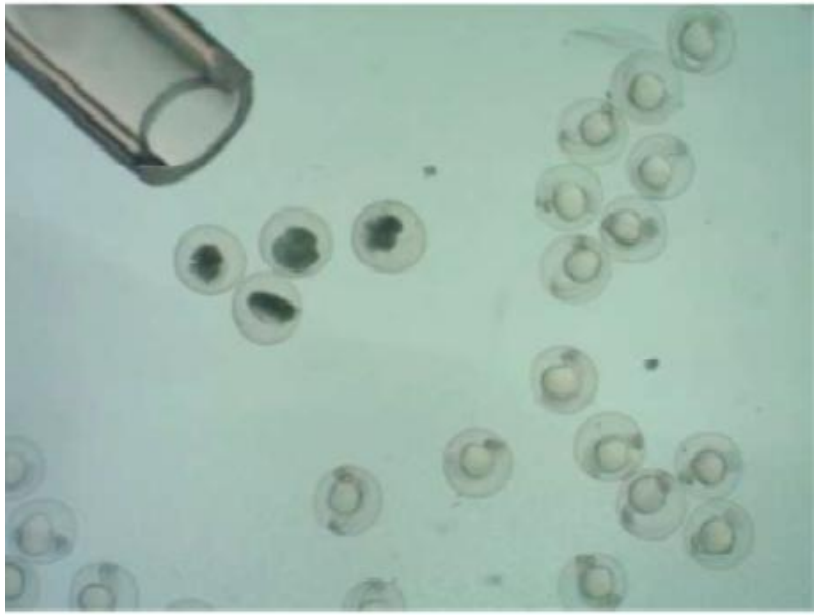
Raccolta degli embrioni



PROBLEMS? FUNGI

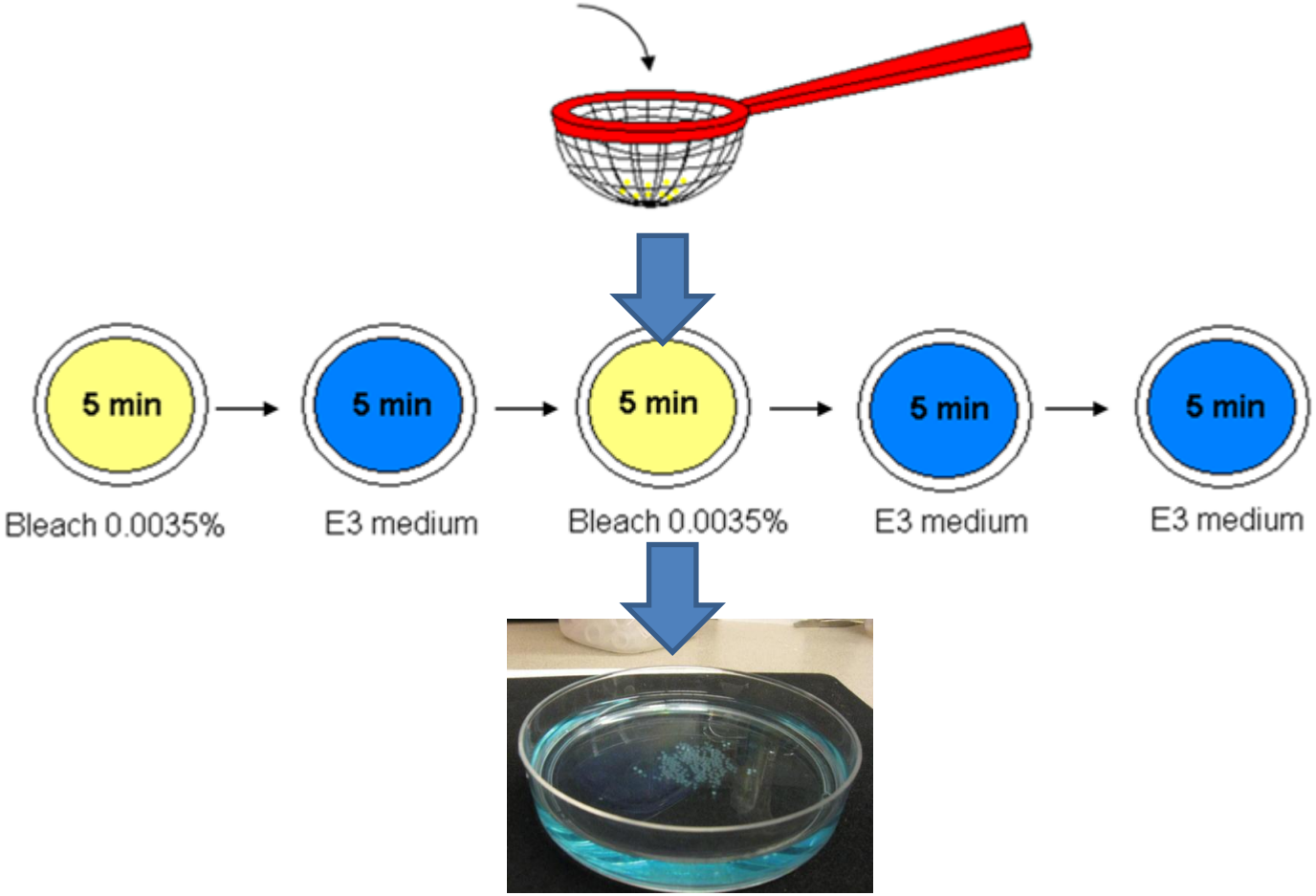
PROTOZOA





HYPOXIA

Collection of eggs



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Different uses of zebrafish eggs