University of Crete
Dept. of Biology

10th International Course Care and Use of Laboratory Animals: mice, rats and zebrafish

Zebrafish Biology and Husbandry

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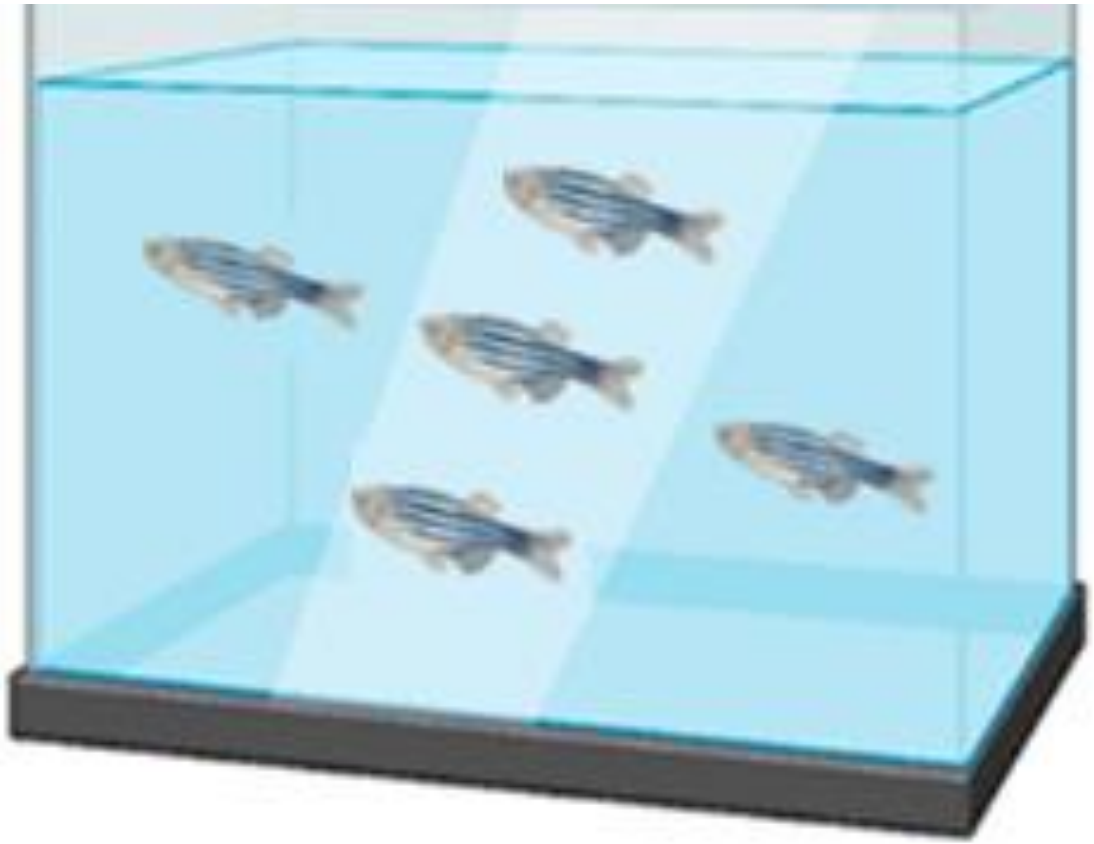
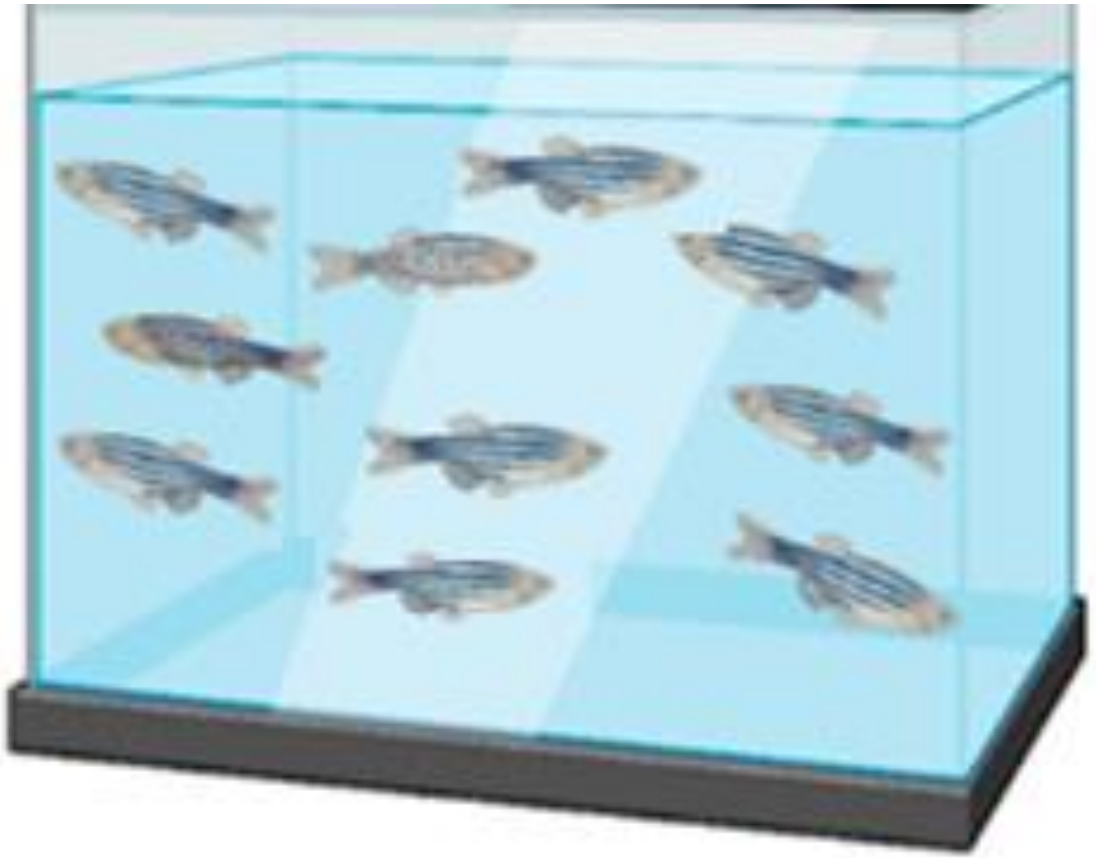
Heraklion, 3-4th June 2024

Learning outcomes

- To get knowledge and skills on the monitoring of zebrafish welfare
- To be competent in the measuring and interpretation of water quality parameters (input welfare indicators)
- To be competent in zebrafish handling and sex identification
- To understand the critical steps of anaesthesia and to get skills in the proper use of anaesthetics
- To get training for intraperitoneal injections of drugs

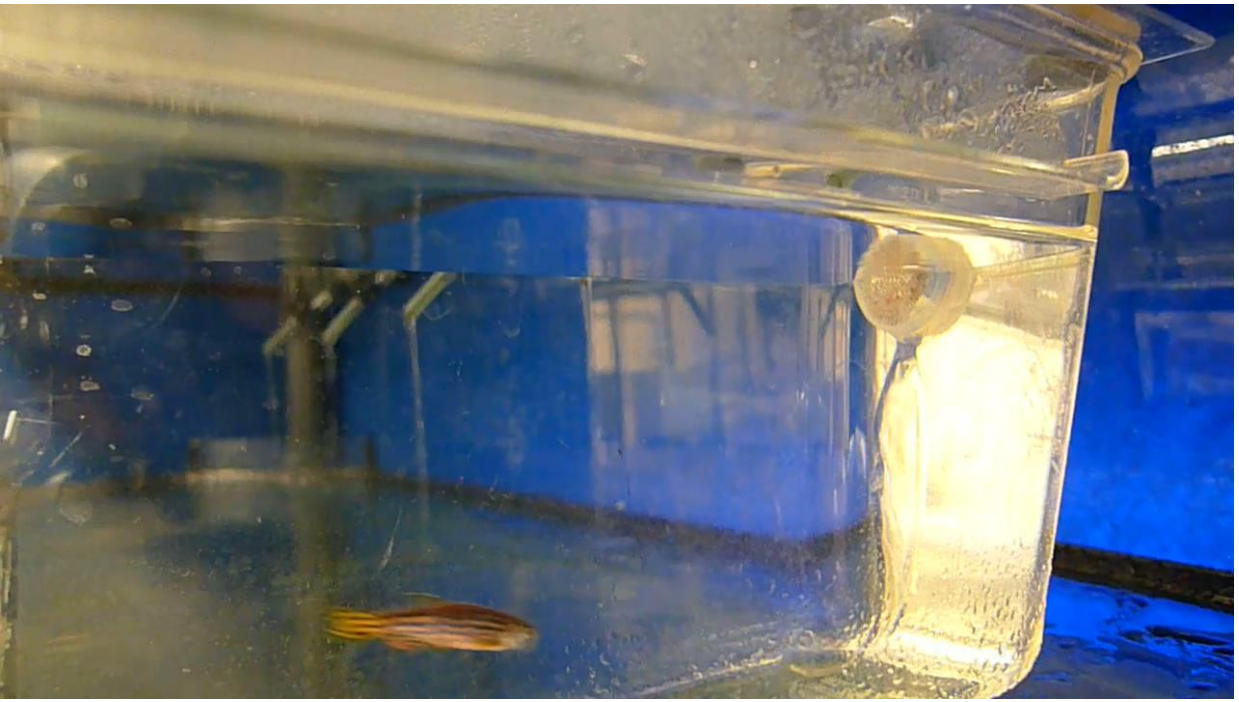
In the bench – 2 tanks/group

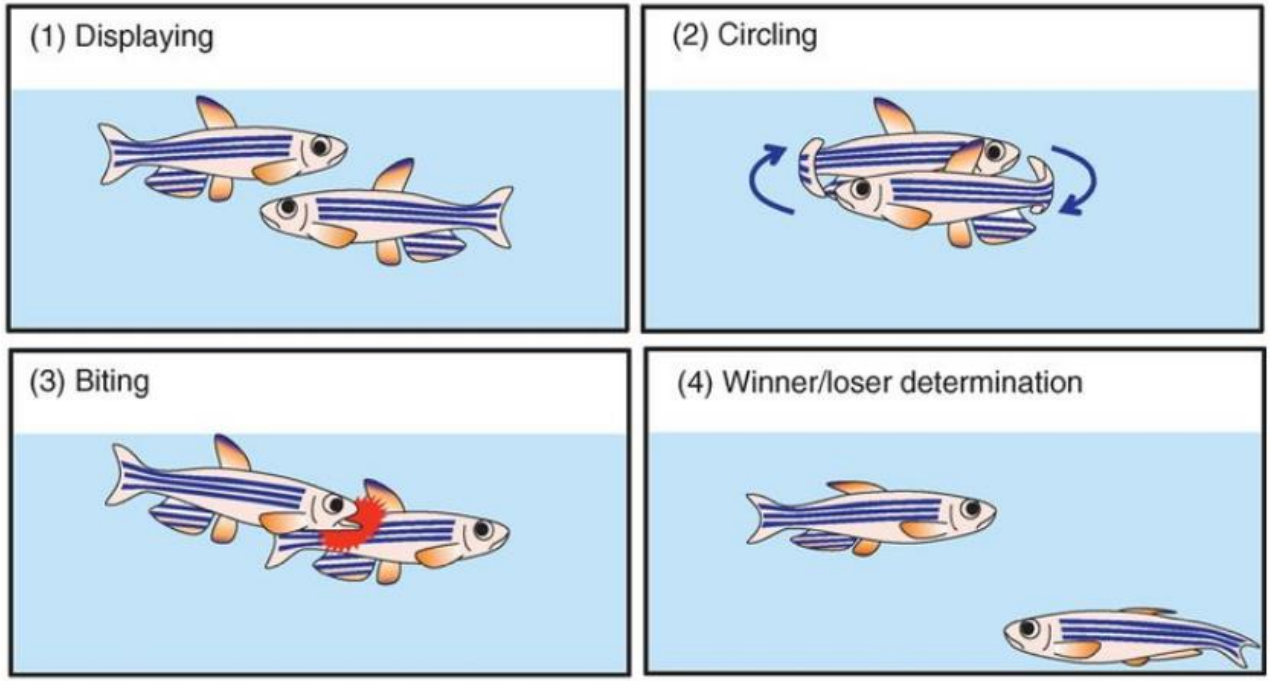




Stocking density

- In modern fish facilities, equipped with efficient and standardized water quality measures, fish are often maintained at densities of **4–10 adult fish/L**
- **Higher stocking densities** have been associated with **crowding stress and poor water quality**
- **Lower stocking densities** are associated with the emergence of **dominant and submissive behavior** in zebrafish, which leads to **aggressive behavior** and **elevated stress** and consequently reduced welfare





Water quality parameters

1st part of the practical session

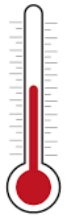
Water parameters

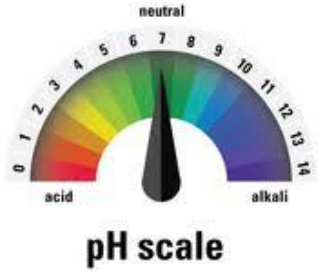
- Temperature
- pH
- Salinity
- Dissolved oxygen

- Nitrogenous wastes

Water quality parameters - Recommendations

- **Optimum temperature 28°C**

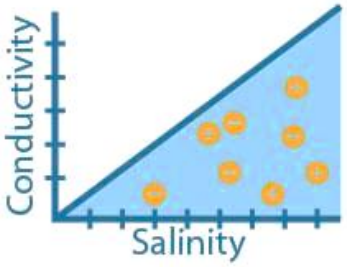


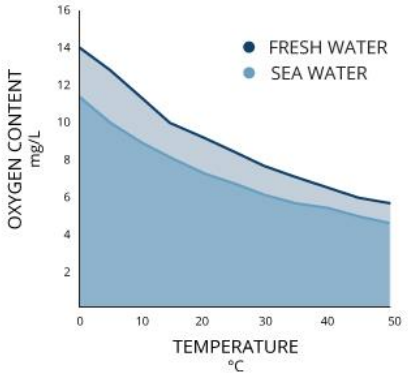


pH scale

- **pH: between 7.0 – 8.0**

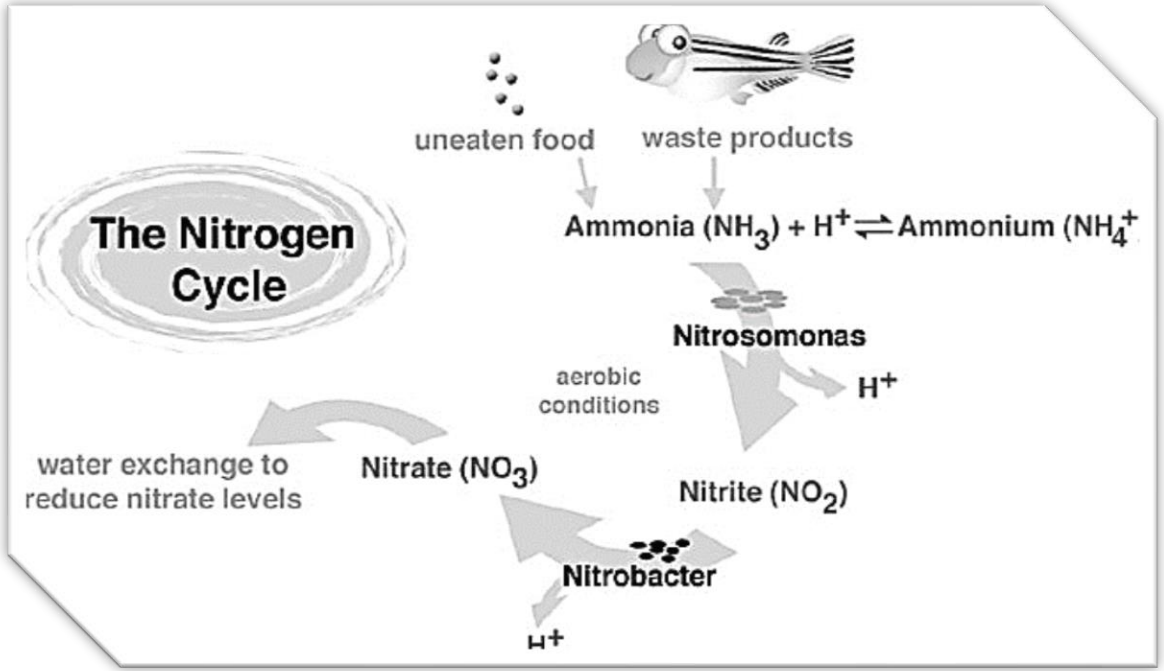
- **salinity** within **0.25 – 0.75 ppt**





~7.8 mg/L at 28.0 °C

- **Ammonia 0 ppm**
- **Nitrite 0 ppm**
- **Nitrate < 25 ppm**



Conversion by nitrifying bacteria

Procedure

- Demonstration of how to use the probes to measure water parameters and

- Demonstration of how to use the kits to measure nitrogenous wastes

Ammonium test (NH_4/NH_3) – Your task

Instructions for use: Shake reagent bottles well before using!
Do not allow tested water to contact tank water!

1. **Rinse the measurement vial** with the water to be tested, then fill to the **10 ml** mark (freshwater). Dry the vial on the outside.
2. **Shake reagent bottles well before using**
3. **Add 6 drops of reagent 1 and shake**
4. **Add 6 drops of reagent 2 and shake**
5. **Add 6 drops of reagent 3 and shake**
6. **Compare the colors after 5 minutes**: Place the vial on the **color chart** and **compare the colors** under natural daylight.
7. **Clean the vial thoroughly with tap water before and after each test**

a)	0 mg/l	0,5 mg/l	1 mg/l	5 mg/l	10 mg/l
b)	0 mg/l	0,5 mg/l	1 mg/l	2 mg/l	5 mg/l

Ammonium/Ammoniak-Test (NH_4/NH_3)

NH_4	pH value					
	7	7.5	8	8.5	9	actual NH_3 level in mg/l
0.5 mg/l	0.003	0.009	0.03	0.08	0.18	
1 mg/l	0.006	0.02	0.05	0.15	0.36	
2 mg/l	0.01	0.03	0.11	0.30	0.72	
5 mg/l	0.03	0.09	0.27	0.75	1.80	
10 mg/l	0.06	0.17	0.53	1.51	3.60	

= harmless

= harmful with long-term exposure

= acutely toxic

color chart:
a) freshwater
b) marine water

Nitrite test (NO_2)

Instructions for use: Shake reagent bottles well before using!
Do not allow tested water to contact tank water!

1

Rinse the measurement vial several times with the water to be tested, then fill to the **5 ml mark**

2

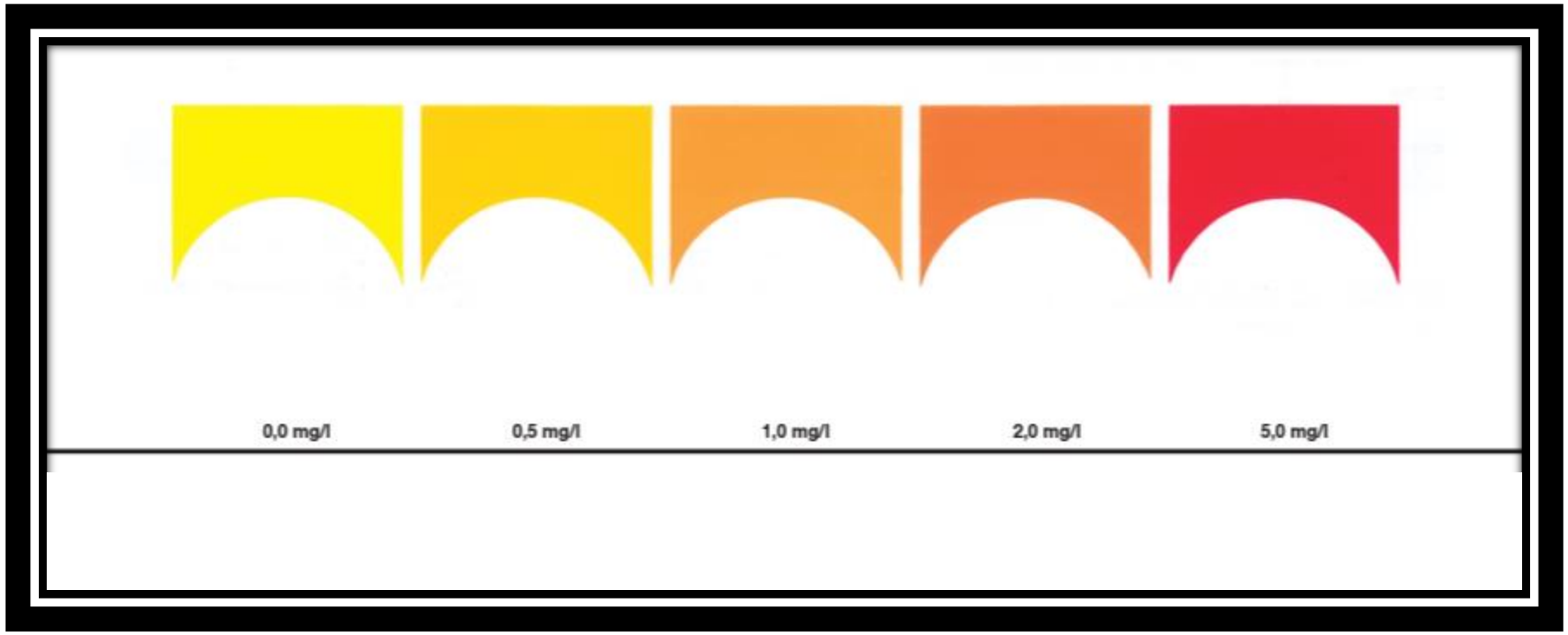
Add 5 drops reagent 1 and shake the vial until the liquid is evenly distributed

3

Add 5 drops reagent 2 and shake the vial until the liquid is evenly distributed.

4

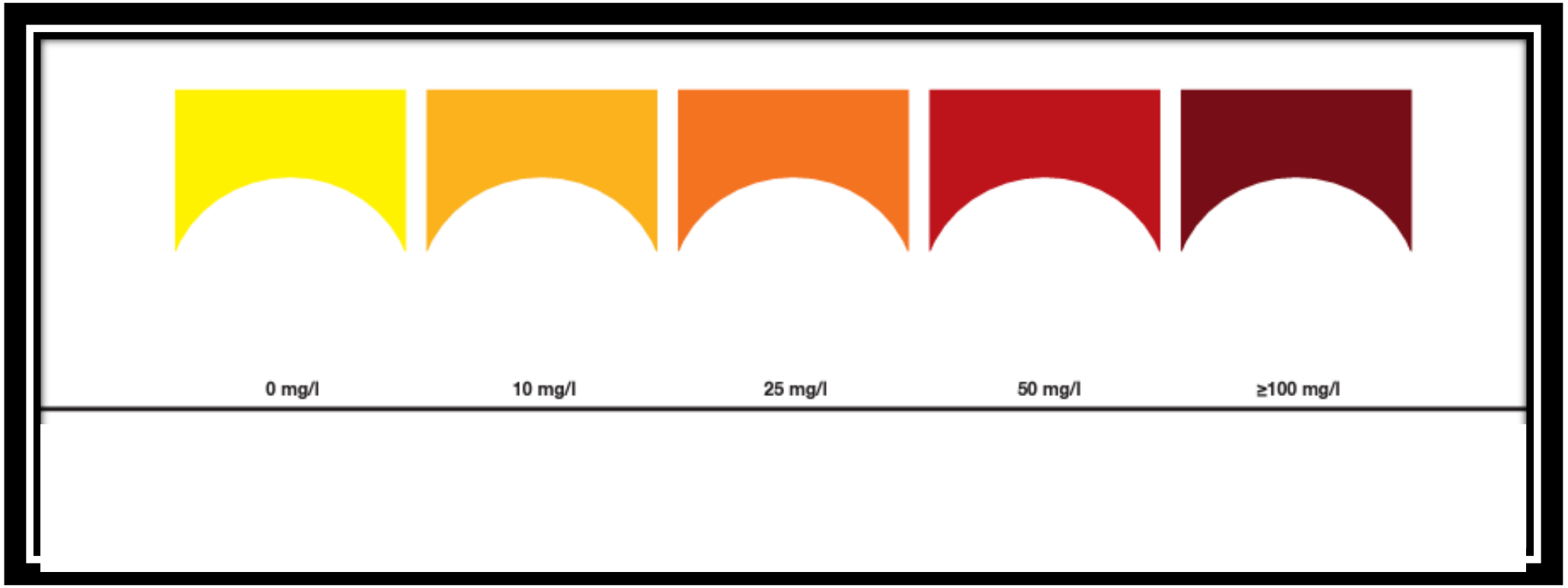
Compare the colors after 5 minutes: Place the vial on the color chart and compare colors from a position above under natural daylight

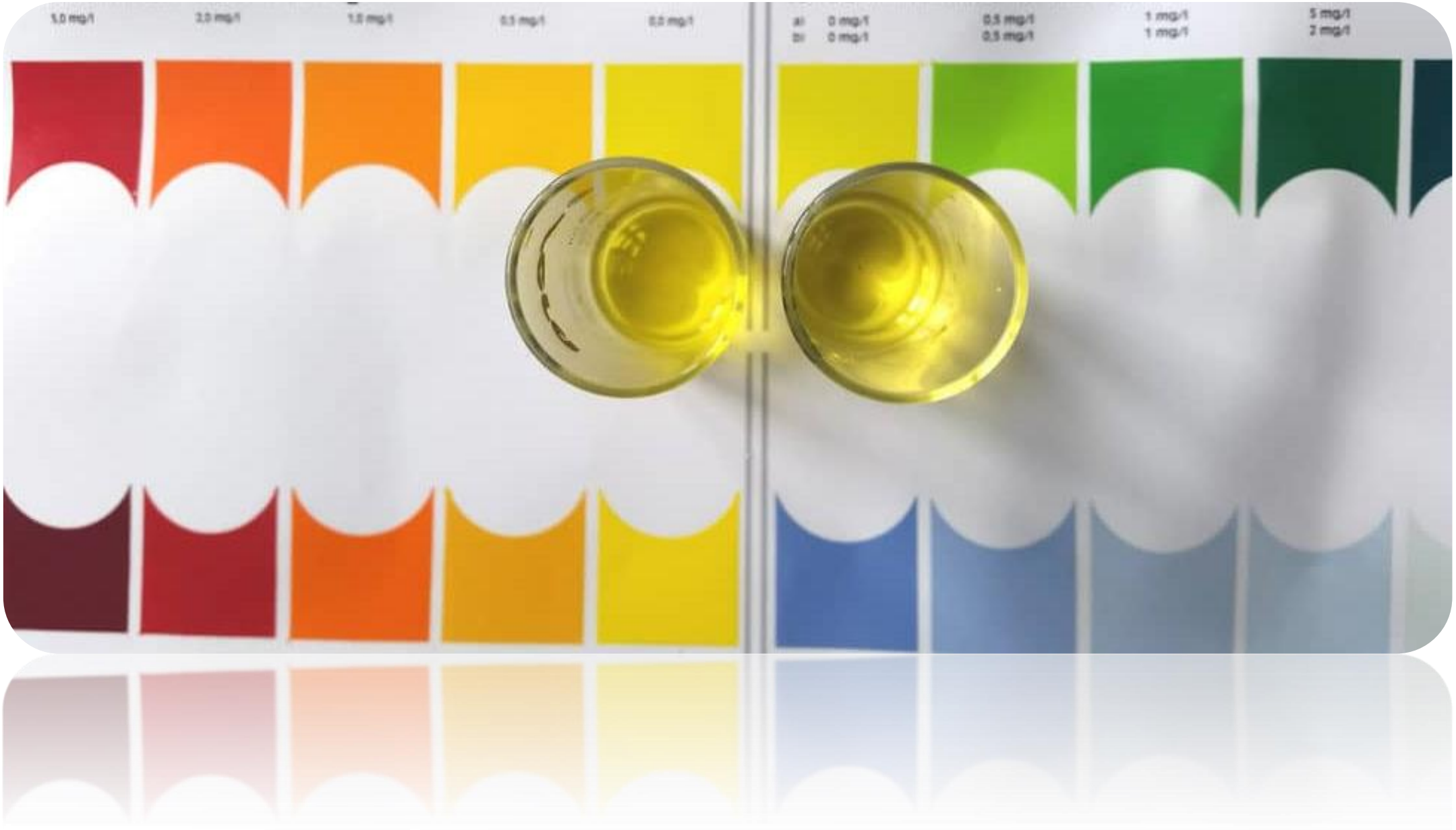

0,0 mg/l
0,5 mg/l
1,0 mg/l
2,0 mg/l
5,0 mg/l

Nitrate test (NO_3)

Instructions for use: Shake reagent bottles well before using!
Do not allow tested water to contact tank water!

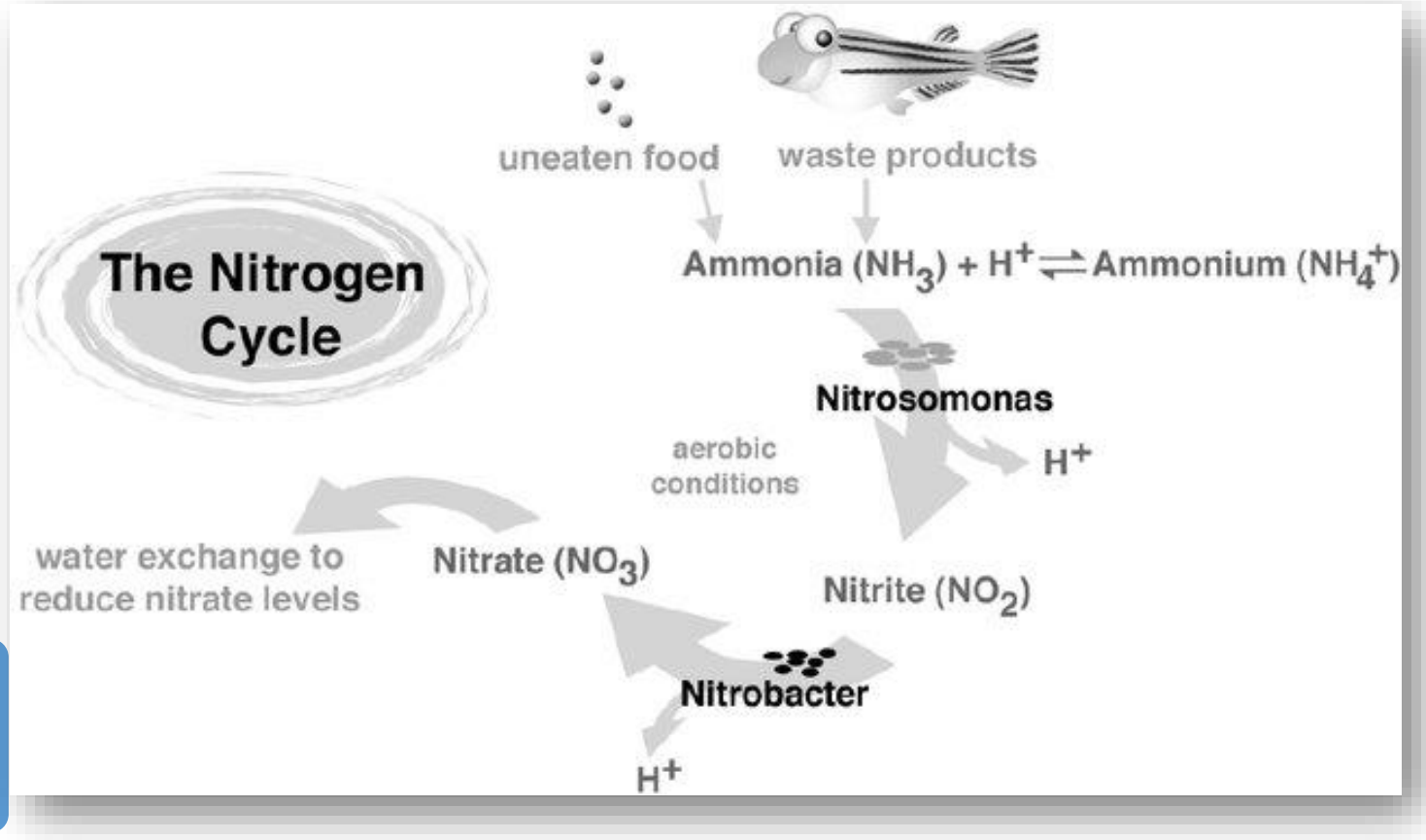
1. **Rinse the measurement vial** several times with the water to be tested, then fill to the **10 ml mark**
2. **Add 6 drops reagent 1** and shake the vial until the liquid is evenly distributed
3. **Add 6 drops reagent 2** and shake the vial until the liquid is evenly distributed.
4. **Add one measurement spoon (red) reagent 3 into the vial**
5. **Close with the cover and shake vigorously for precisely 15 seconds**
6. **Open the vial** and **add 6 drops reagent 4**. Shake the vial until the liquid is evenly distributed.
7. **Compare the colors after 5 minutes**: Place the vial on the color chart and compare colors from a position above under natural daylight


0 mg/l
10 mg/l
25 mg/l
50 mg/l
≥100 mg/l


1,0 mg/l
2,0 mg/l
1,0 mg/l
0,5 mg/l
0,0 mg/l
a)
b)
0 mg/l
0 mg/l
0,5 mg/l
0,5 mg/l
1 mg/l
1 mg/l
5 mg/l
2 mg/l

Higher values of nitrogenous wastes indicates problems with

- the quantity of food
- the biological filter (nitrifying bacteria)
- The renewal water rate



Ammonium test (NH_4/NH_3)	Nitrite test (NO_2)	Nitrate test (NO_3)

1. Shake reagent bottles well before using!

2. Rinse the measurement vial several times with the water to be tested

3. fill to the 10 ml mark (freshwater).	**3. fill to the 5 ml mark**.	**3. fill to the 10 ml mark**
4. Add 6 drops of reagent 1 and shake	**4. Add 5 drops of reagent 1** and shake	**4. Add 6 drops reagent 1 and shake**
5. Add 6 drops of reagent 2 and shake	**5. Add 5 drops of reagent 2** and shake	**5. Add 6 drops reagent 2 and shake**
6. Add 6 drops of reagent 3 and shake	**6. Compare the colors after 5 minutes** under natural daylight. Avoid direct sunlight.	**6. Add one measurement spoon (red) reagent 3**
7. Compare the colors after 5 minutes under natural daylight. Avoid direct sunlight.		**7. Close with the cover and shake vigorously for precisely 15 seconds**
		8. Add 6 drops reagent 4 and shake
		9. Compare the colors after 5 minutes under natural daylight. Avoid direct sunlight.

Clean the vial thoroughly with tap water before and after each test

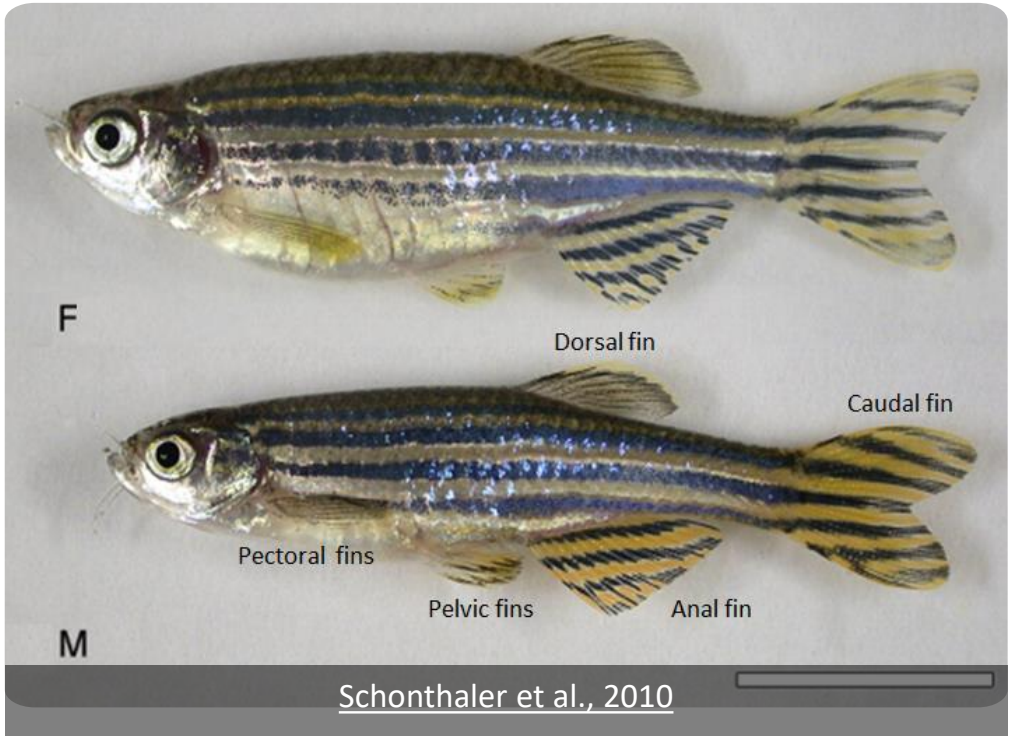
2nd part of the practical session

- Handling,
- sex identification,
- anaesthesia,
- intraperitoneal injection in rubber fish

2.1 Fish handling

Fish used in research, must be treated with the respect accorded to other vertebrate species.

- use of **anaesthesia** to minimize stress and pain
- **minimize handling time** of the fish
- Always **use a net** to catch the fish
- **Keep fish wet** while handling them. This prevents damage to the fish's protective **mucous** surface
- All animals should be approached in a **calm, quiet and confident manner**.
- Limit the time of **air exposure (max 30 seconds)**
- Wear **gloves**



Schonthaler et al., 2010

Breeding of Zebrafish

- **Females** can be distinguished from males because of their **bigger underbelly**.
- **Males** can also be distinguished from females because they are **more slender and darker in colour**.
- Moreover, males have **more yellow coloration in the anal fin** compared to females

Preparation of work space

- Apply filter paper on the bench
- Make sure that you have all the equipment that you will need on your bench

Equipment/group

- Nets (2 sizes),
- 1 breeding tank,
- 1000 µl pipet,
- anaesthetic solution,
- anaesthetic tank,
- recovery tank,
- sponges,
- Syringes,
- rubber fish

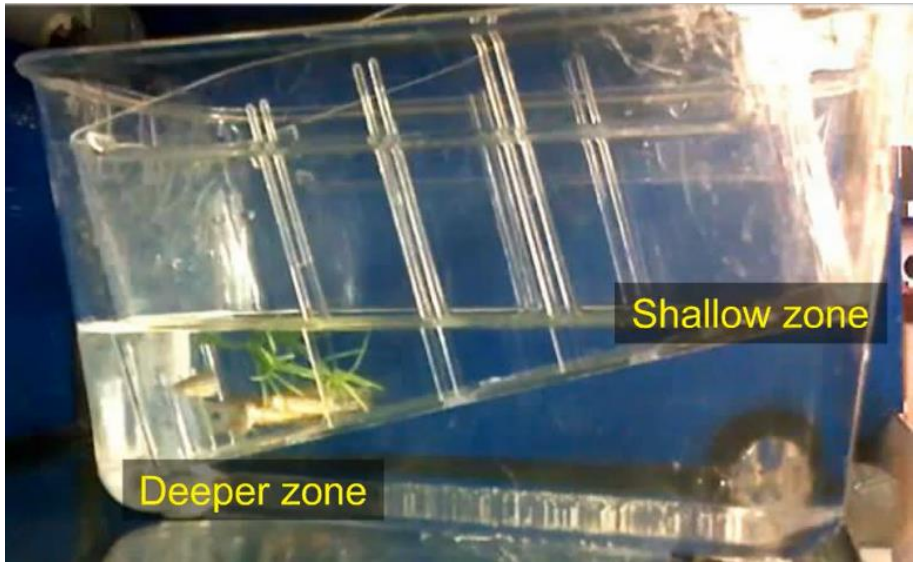
Breeding of Zebrafish

Pairwise breeding: Transfer **one female** and one or **two male fish separated to opposite sides** of the breeding tank

Leave them 2-3 min and then, **remove the plastic divider** and **place it in one side**, between the two parts of the breeding tank, so that you can create a deeper and a shallower zone in the tank

Finally, **return 2 of the fish** back to the recovery tank. The **3rd fish** will be used for the **anaesthesia**

https://youtu.be/wWR2-D3xV64



Christian Lawrence et al., 2012

2.3 Anaesthesia

Anaesthesia is generally defined as a **state caused by an applied external agent resulting in a loss of sensation** through depression of the nervous system

The efficacy of most anesthetics is affected by

- Species
- age
- body size
- the density of fish in the bath
- water quality

it is imperative that **preliminary tests** be performed with small numbers of fish to determine the **most appropriate** anaesthetic, the optimal **dosage** and **exposure time**.

Anaesthesia

Animals are anaesthetized to **provide analgesia and lack of awareness** so that painful or stressful procedures can be undertaken humanely (e.g., blood sampling, injection, surgery, manipulation).

Anaesthesia can also provide a **means of restraining an animal** so that it is not distressed by prolonged immobilization.

In general, an **anaesthetic agent should**:

- achieve the required **depth and duration of anaesthesia**
- cause **minimum distress** to the animal
- be **free from undesirable side effects**
- allow a smooth and **uncomplicated recovery**
- cause minimal interference with the purpose of the research procedure

- **MS-222 (Tricaine):** is the most widely used fish anesthetic and induces a very rapid and deep anesthesia (dosage: 25-100 mg/L)

- **Add 4,2 ml stock MS-222 in 100 ml tank water**

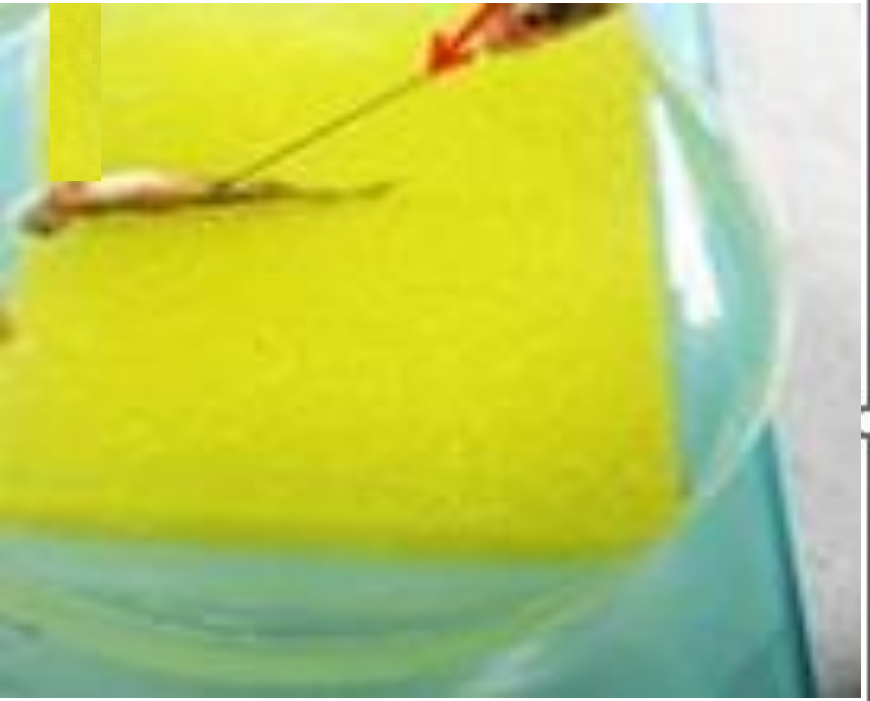
Anesthesia

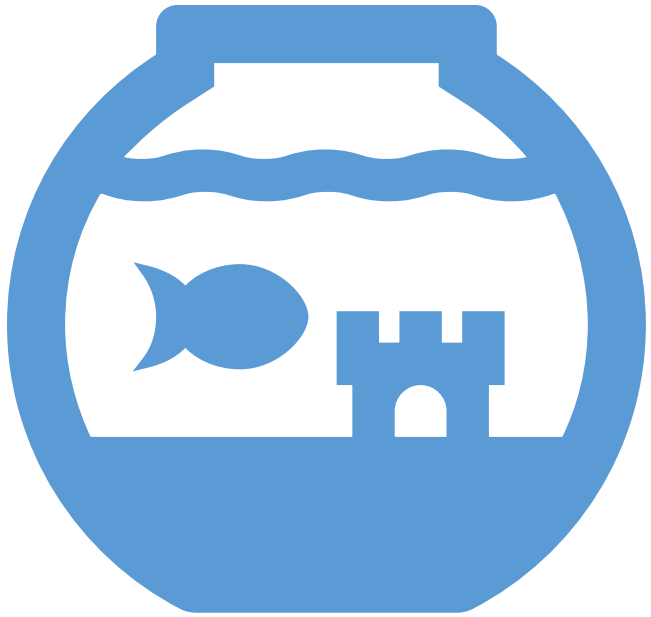
https://youtu.be/50qaWPGDmkI

2.4 Intraperitoneal drug administration

- The most popular method for dosing embryonic and larval zebrafish is through solubilizing chemicals in water (in-water dosing)
- In adult zebrafish is also used intraperitoneal injection:
- fish are first anaesthetized and then drugs are injected into the peritoneum in small volumes (generally < 10 µl) (Zodrow and Tanguay, 2003; Liu et al., 2008; Esbaugh et al., 2009)

Intra-peritoneal injection





Your task / simulation

- Fill the small shallow tank with water
- Get the sponge fully wet by squishing it into the water
- Place the rubber fish in the slit correctly, with the belly up and the gills kept wet
- Use the syringe to intraperitoneal inject a small volume of water

Let's practice

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