



11th International Course Care and Use of Laboratory Animals: mice, rats and zebrafish

Zebrafish Biology and Husbandry

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Learning outcomes

To get knowledge and skills on the monitoring of zebrafish welfare

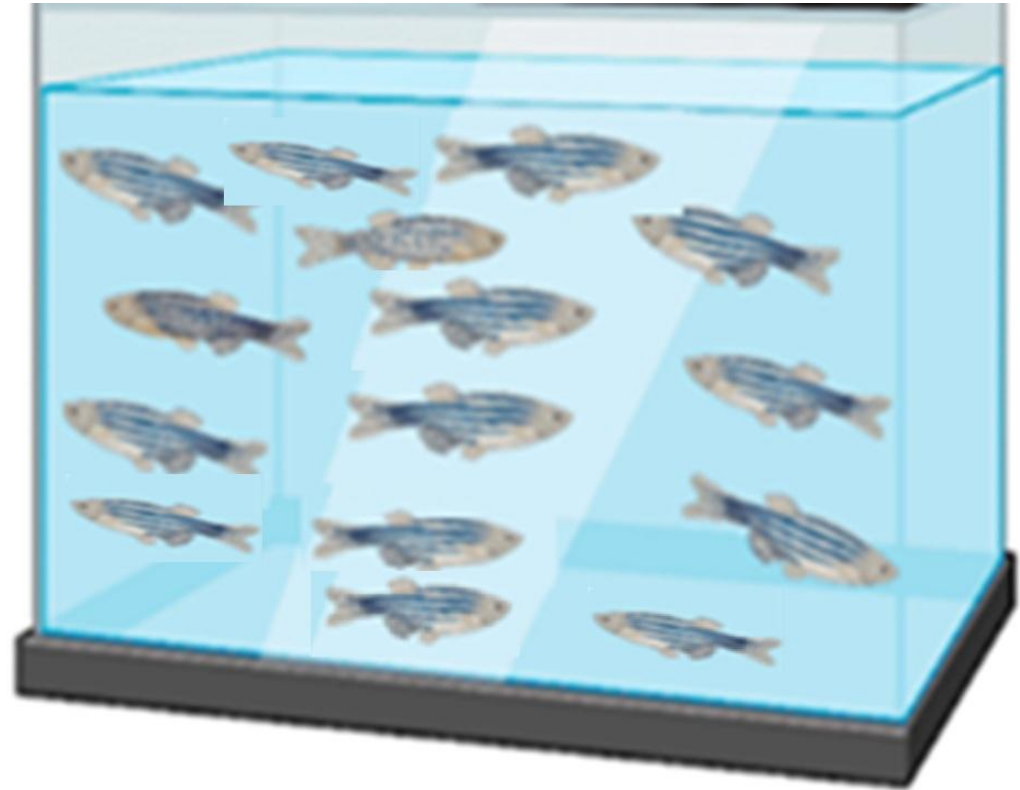
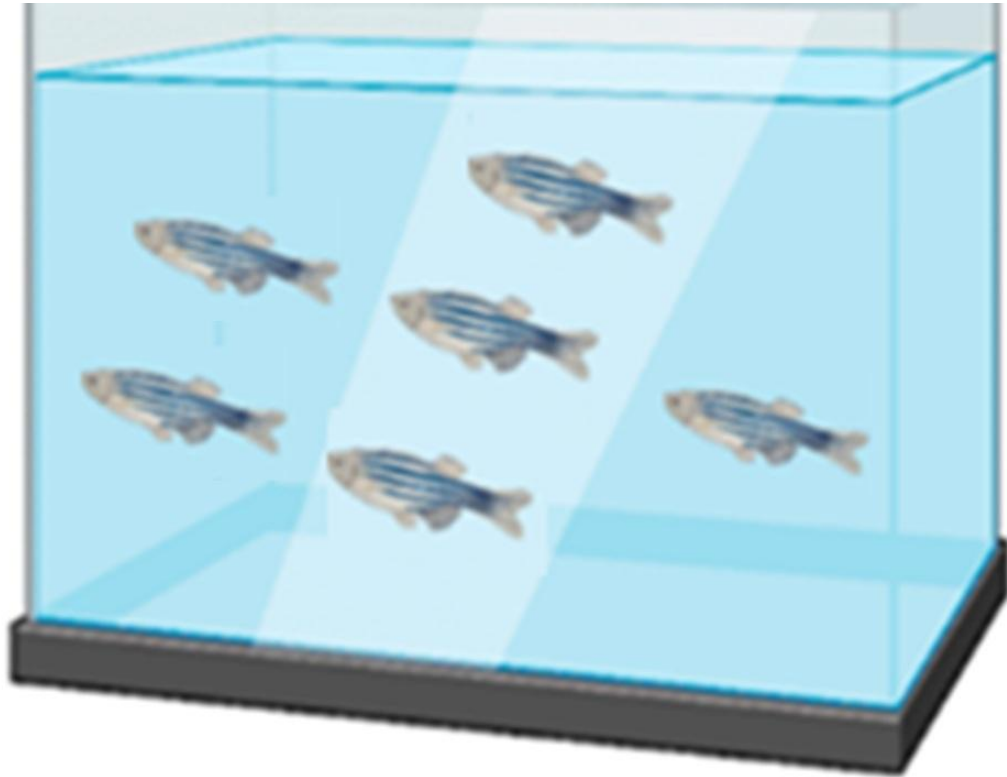
To be competent in the measuring and interpretation of water quality parameters (input welfare indicators)

To be competent in zebrafish handling and sex identification

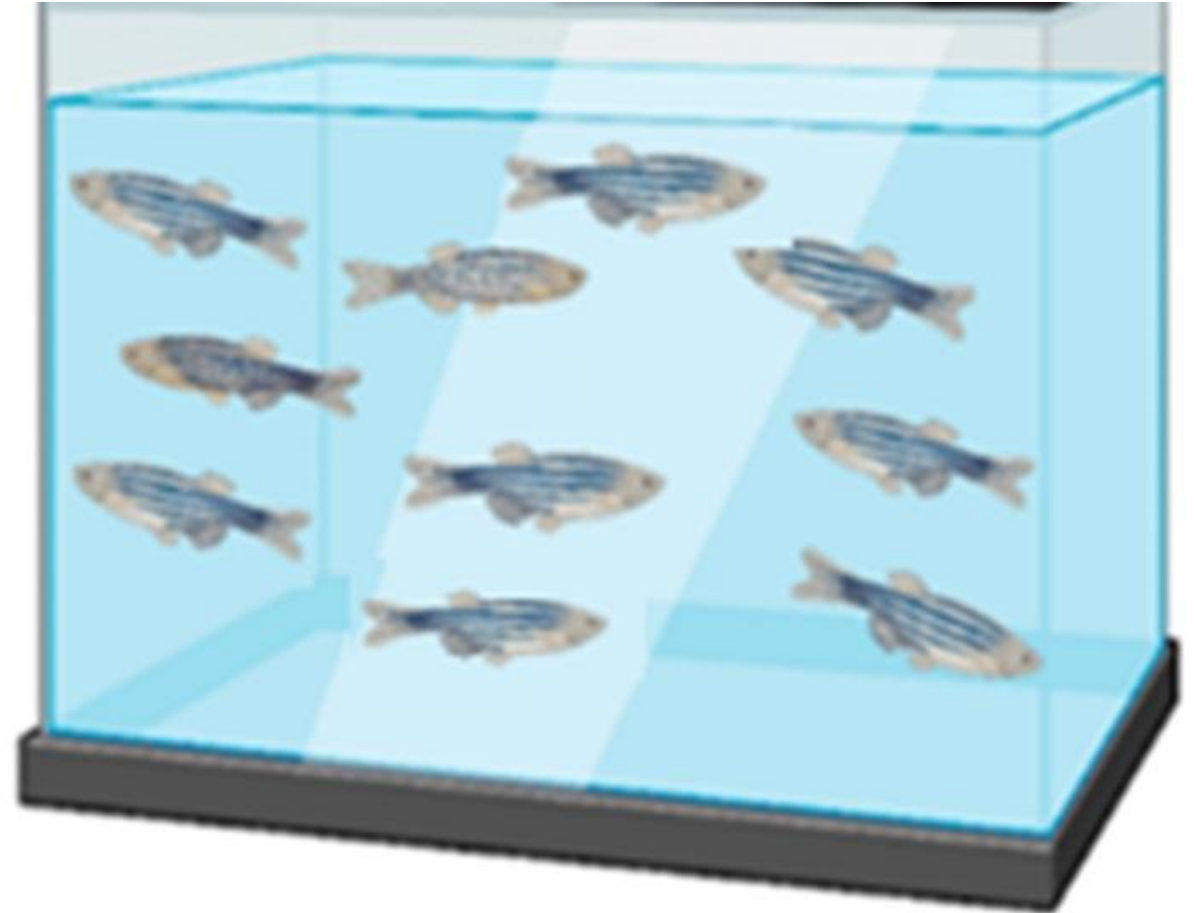
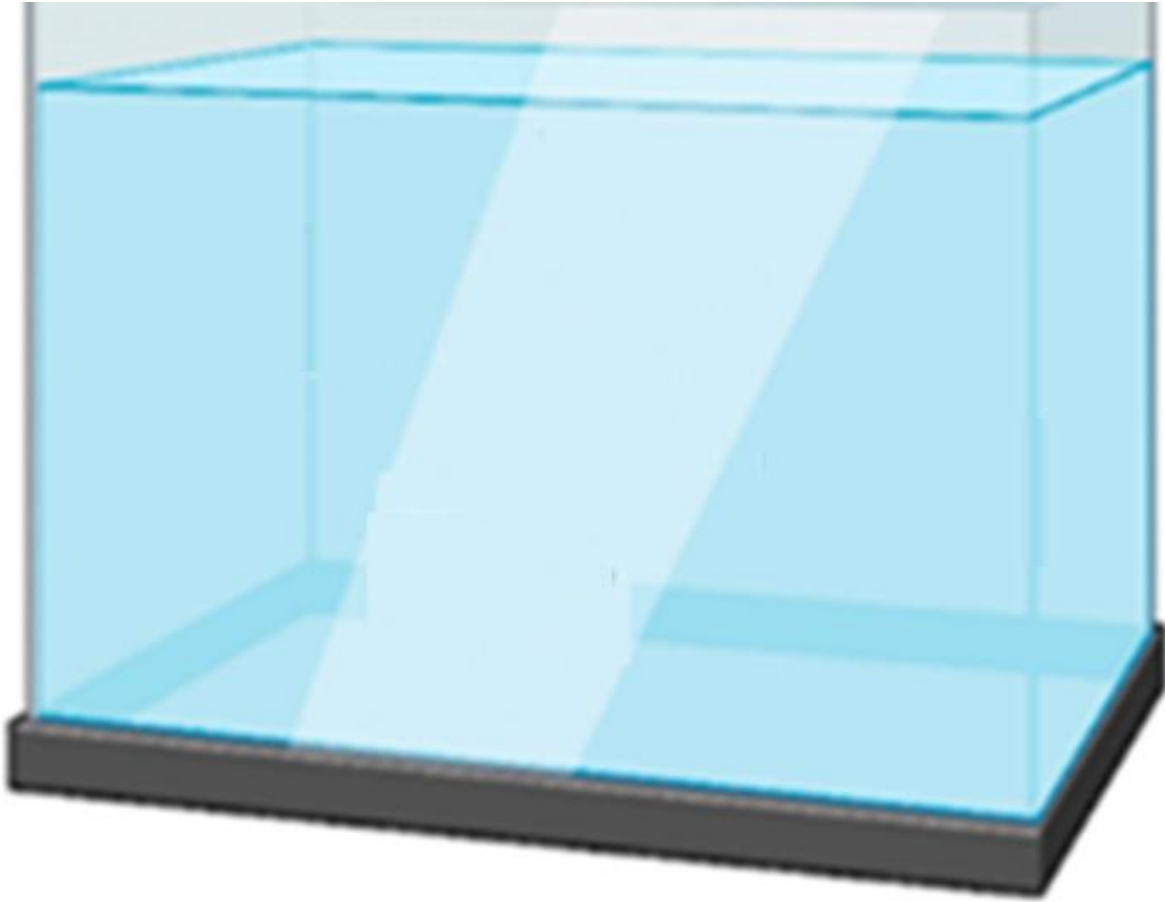
To understand the critical steps of anaesthesia and to get skills in the proper use of anaesthetics

To get training for intraperitoneal injections of drugs

Part 1: 2 tanks (6 fish and 15 fish, respectively)

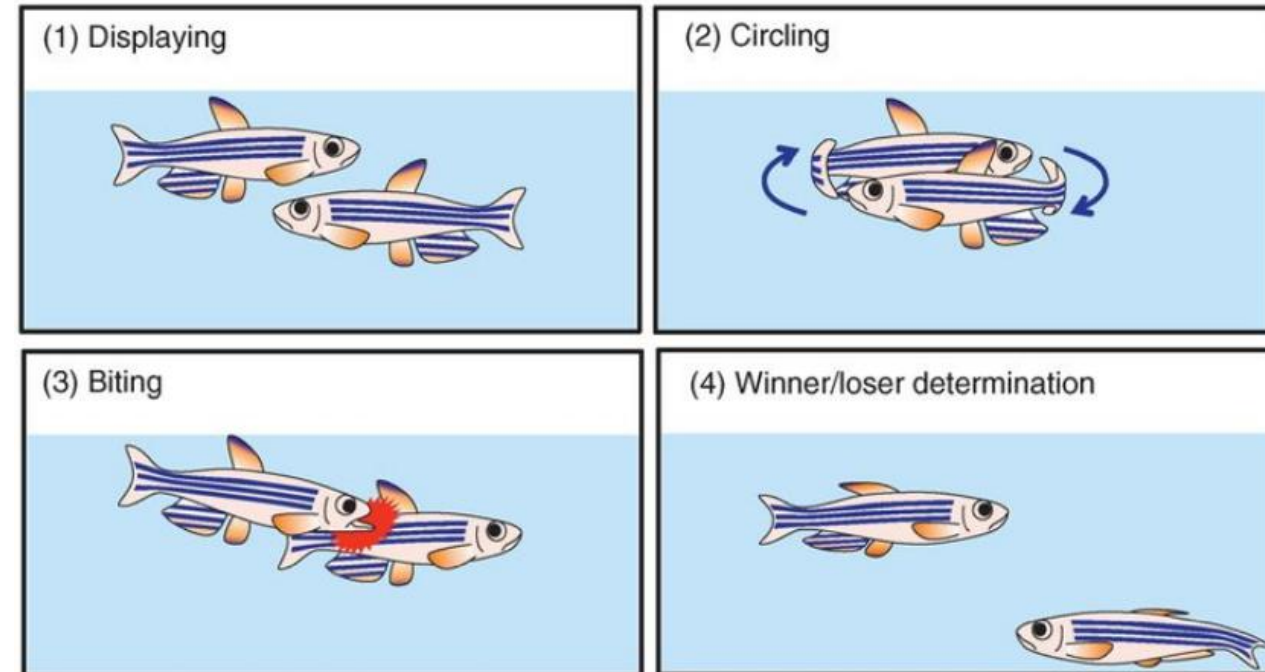
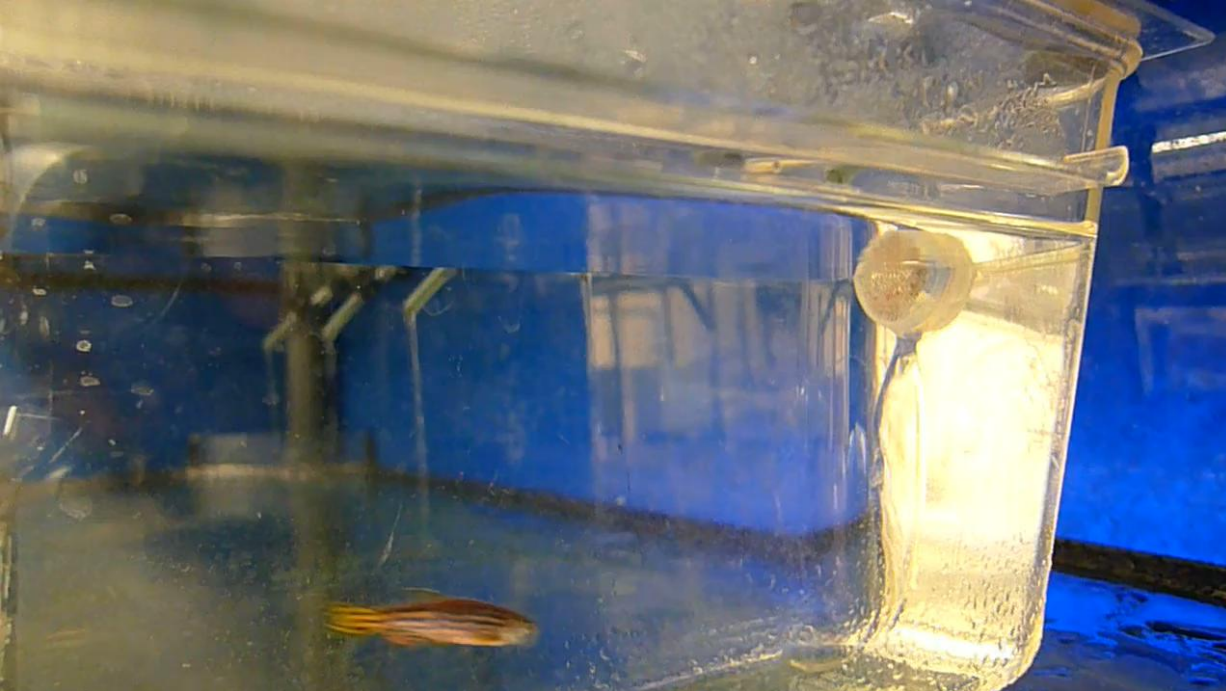


Part 2: 2 tanks (20 fish and 0 fish respectively)



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

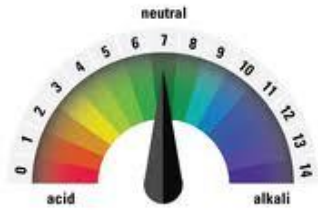
Water parameters

- Temperature
- pH
- Salinity
- Dissolved oxygen
- Nitrogenous wastes



Water quality parameters - Recommendations

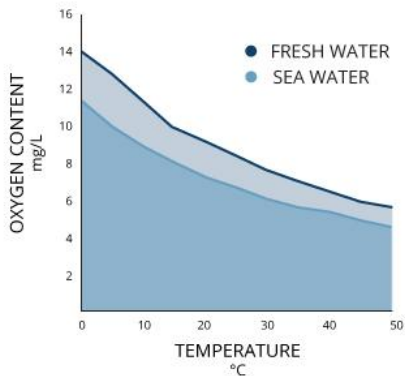
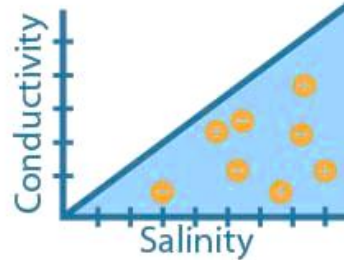
- Optimum temperature 28°C



pH scale

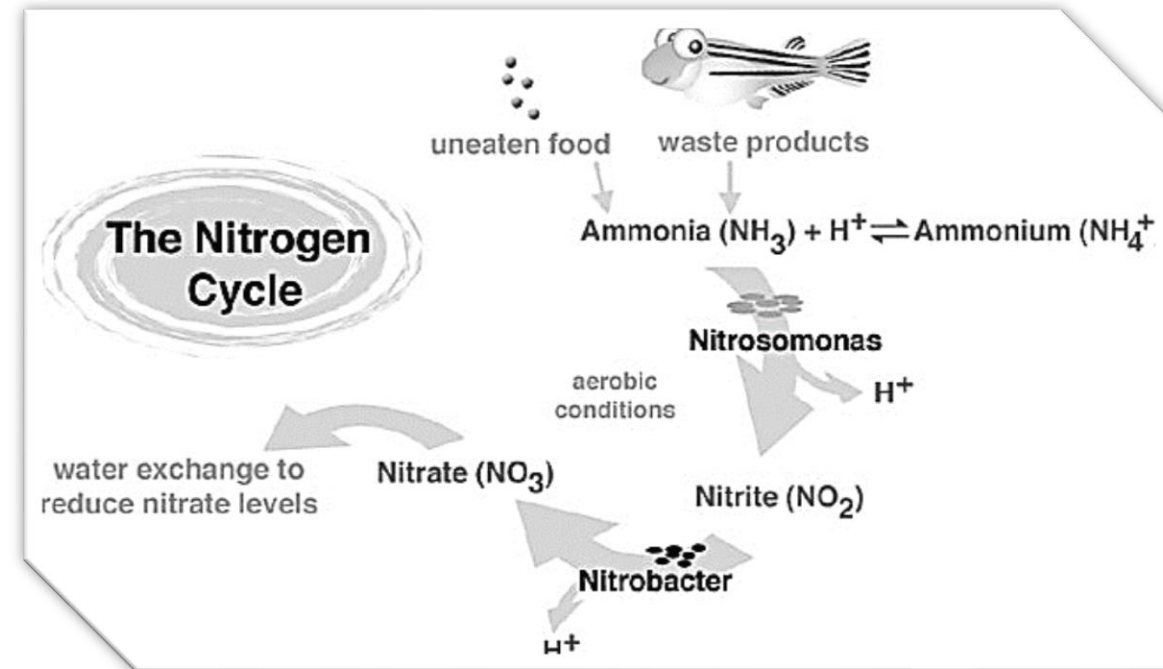
pH: between 6.5 – 8.0

- Conductivity: 150-1700 $\mu\text{S}/\text{cm}^2$
- salinity within 0.25 – 0.75 ppt



>5 mg/L
Optimum ~7.8 mg/L at 28.0 °C

- Ammonia < 0,1 ppm
- Nitrite < 0,3 ppm
- Nitrate < 25 ppm



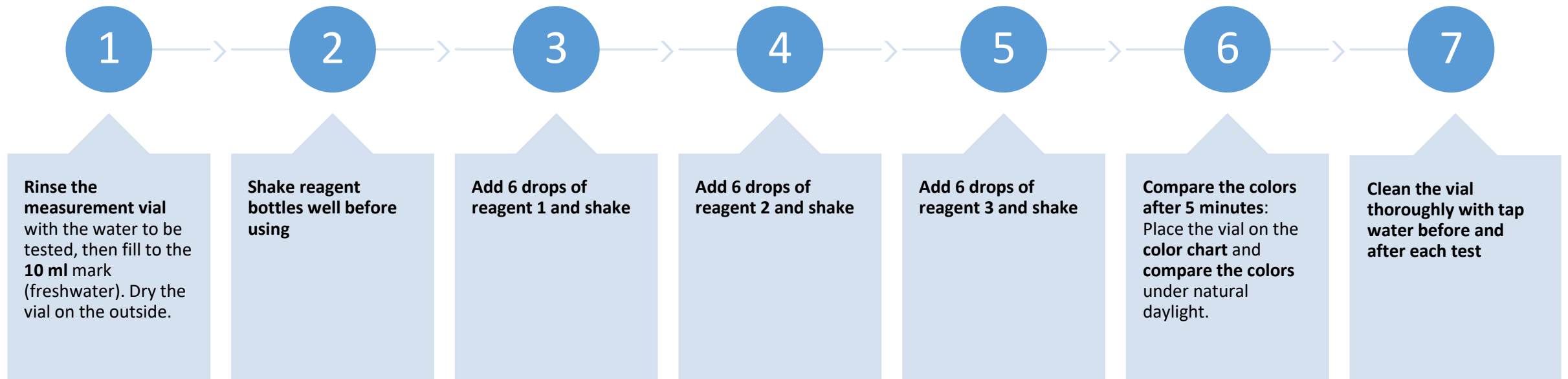
Conversion by nitrifying bacteria

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!





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					actual NH_3 level in mg/l
	7	7.5	8	8.5	9	
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 (NO₂)

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

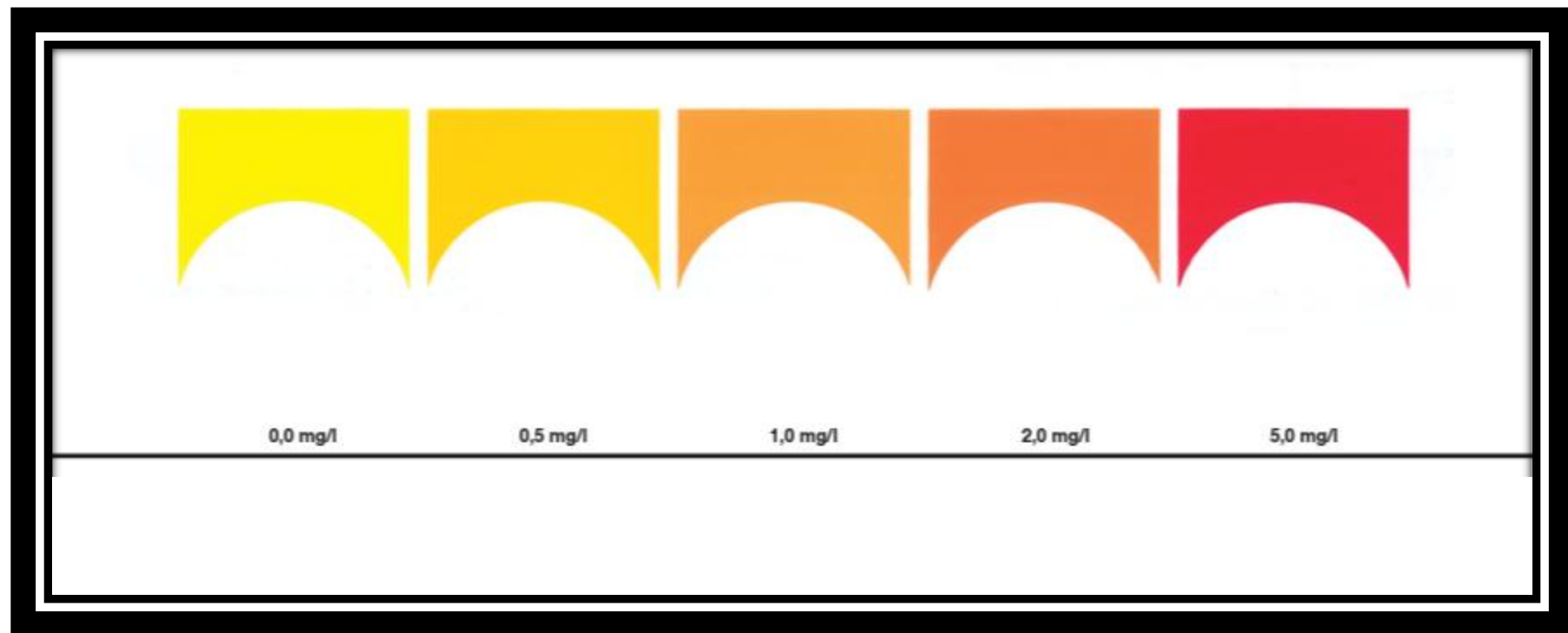
Add 5 drops reagent 1 and shake the vial until the liquid is evenly distributed

3

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

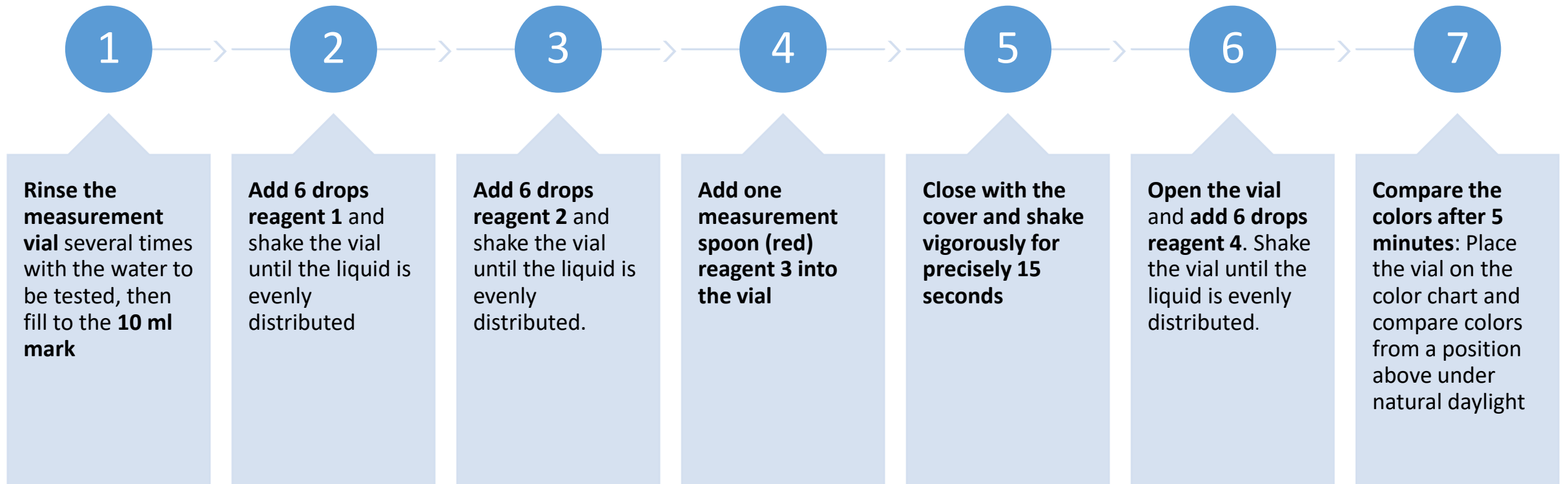
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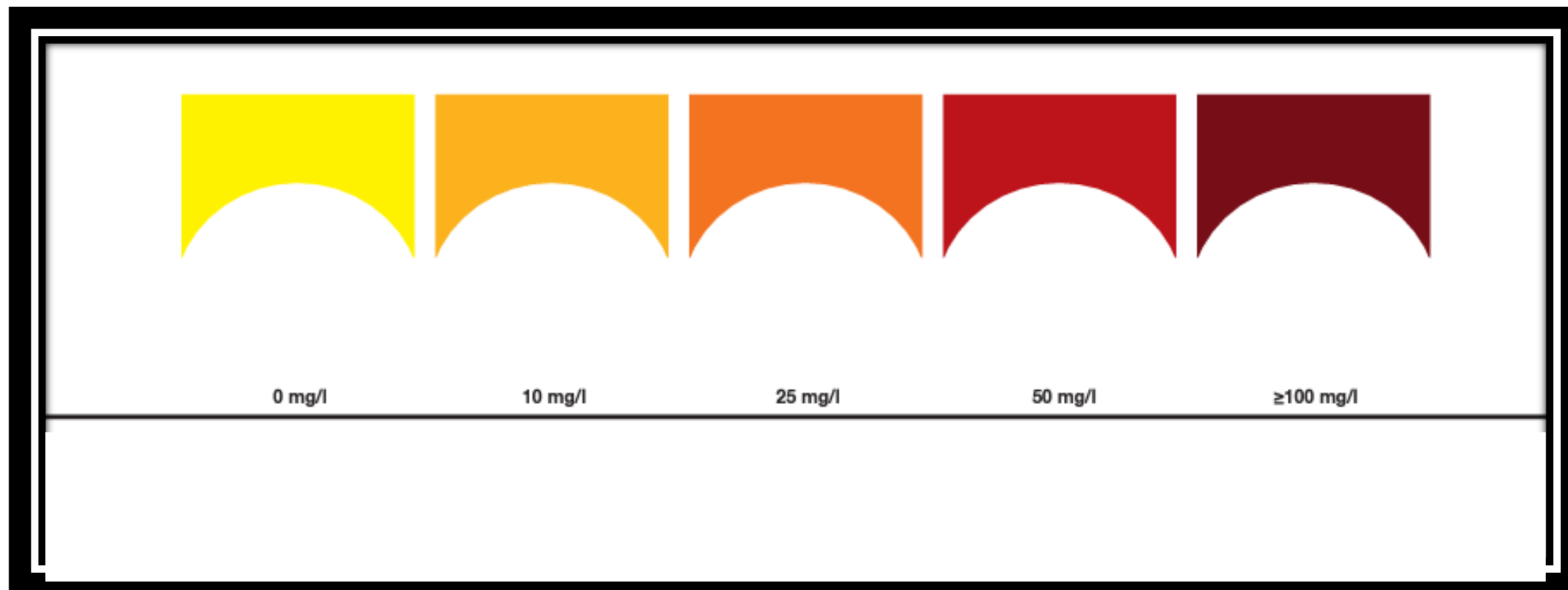
Compare the colors after 5 minutes: Place the vial on the color chart and compare colors from a position above under natural daylight

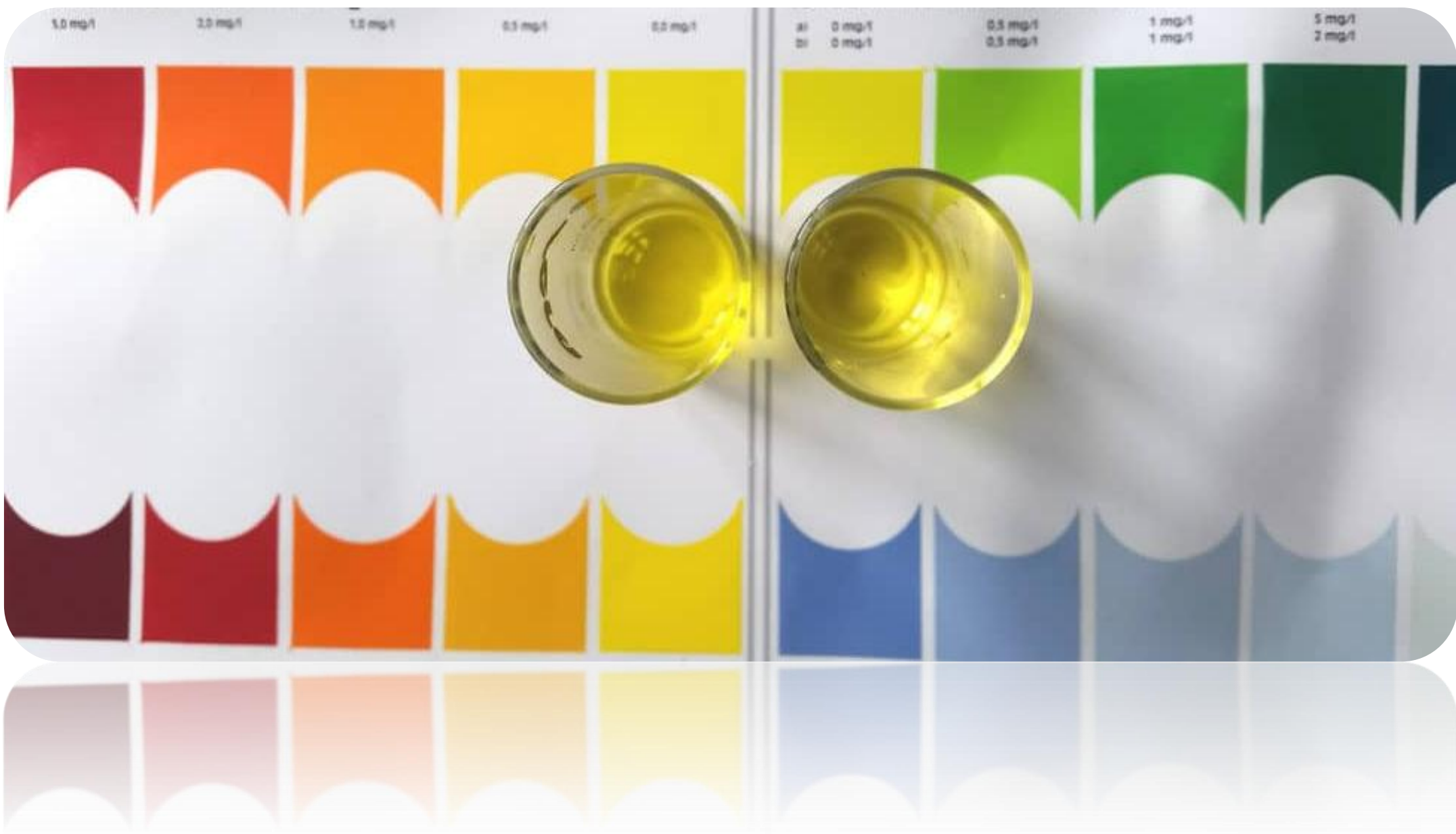


Nitrate test (NO_3)

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





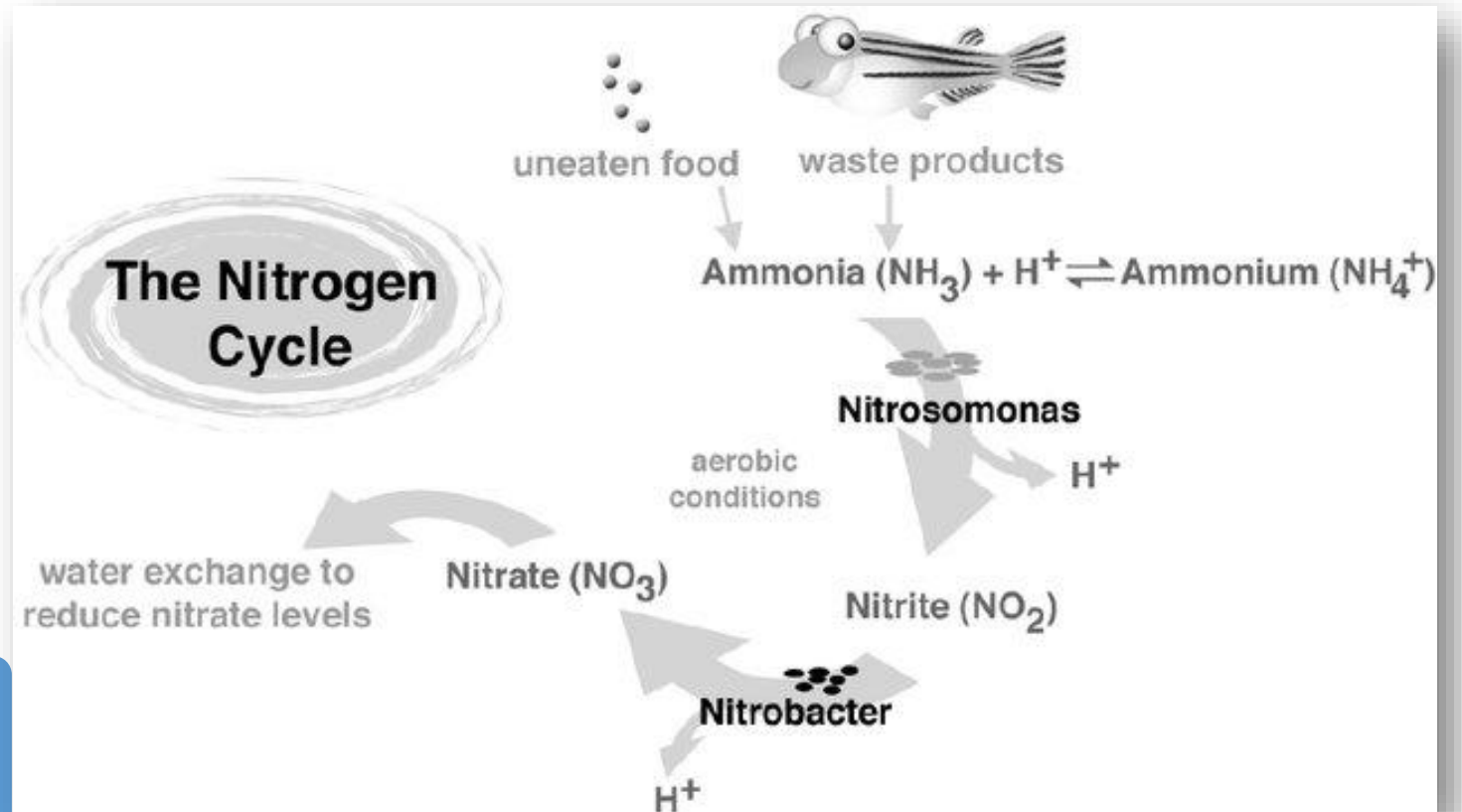


Higher values of
nitrogenous wastes
indicates problems with

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).
4. Add 6 drops of reagent 1 and shake
5. Add 6 drops of reagent 2 and shake
6. Add 6 drops of reagent 3 and shake
7. Compare the colors after 5 minutes
under natural daylight. Avoid direct sunlight.

3. fill to the 5 ml mark.
4. Add 5 drops of reagent 1 and shake
5. Add 5 drops of reagent 2 and shake
6. Compare the colors after 5 minutes under natural daylight. Avoid direct sunlight.

3. fill to the 10 ml mark
4. Add 6 drops reagent 1 and shake
5. Add 6 drops reagent 2 and shake
6. Add one measurement spoon (red) reagent 3
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

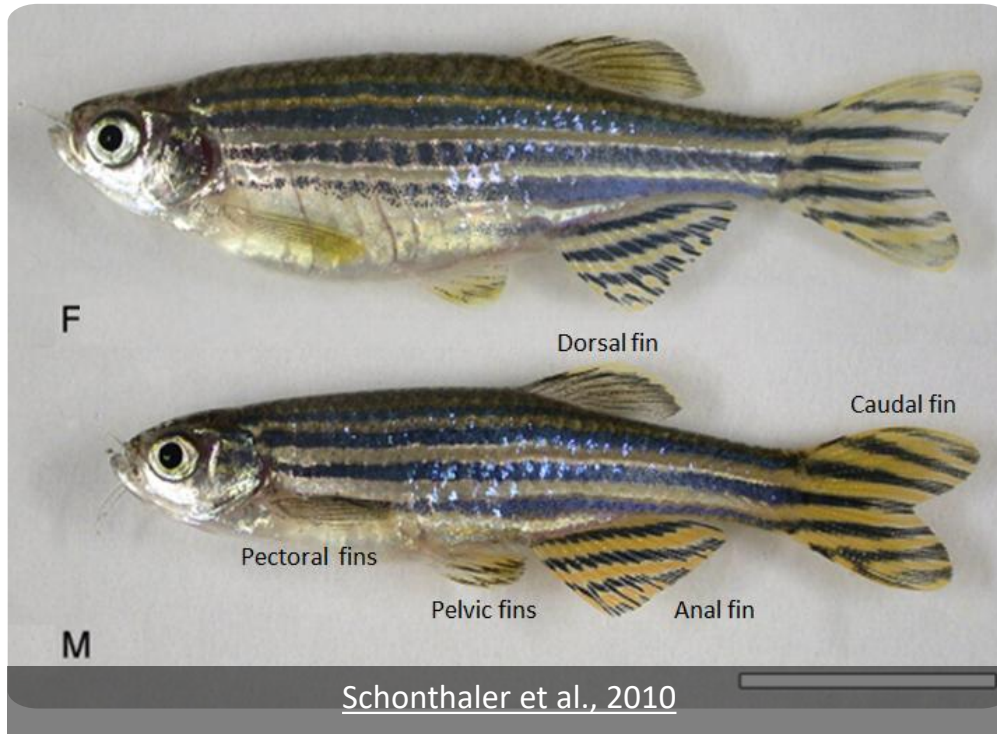
- **Handling,**
- **sex identification,**
- **anaesthesia,**
- **intraperitoneal injection in rubber fish**

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**

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,
- 10 ml pipet,
- anaesthetic solution,
- anaesthetic tank,
- recovery tank,
- sponges,
- Syringes,
- rubber fish

Breeding of Zebrafish

Pairwise breeding:

Transfer **one female** and **one male fish** separated to **opposite sides** of the breeding tank



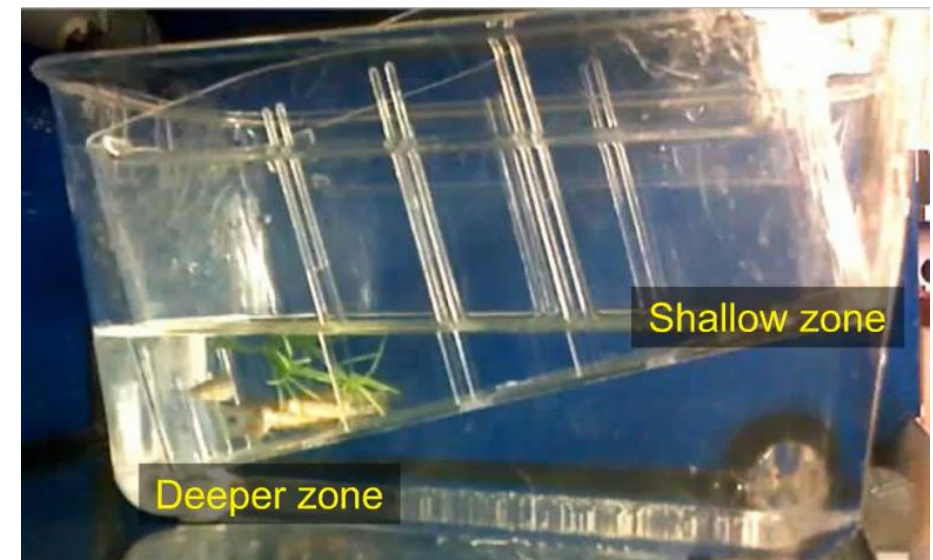
Leave them 2-3 min and then, **remove the plastic divider** and **place it in one side**, between the two parts of the breeding tank, so that you can create a deeper and a shallower zone in the tank



Finally, **the fish** will be used for the **anaesthesia**



<https://youtu.be/wWR2-D3xV64>



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:

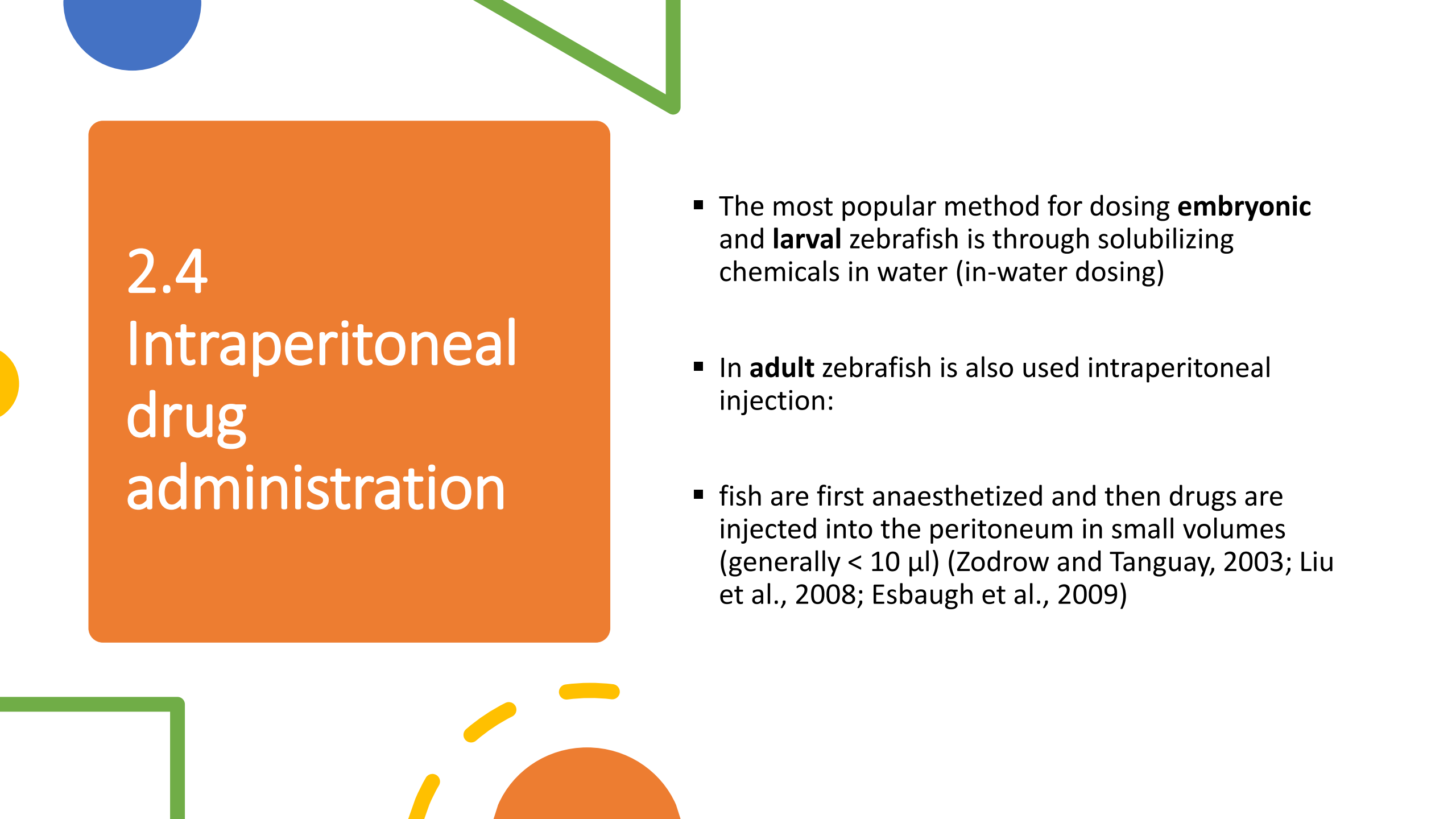
- achieve the required **depth and duration of anaesthesia**
- cause **minimum distress** to the animal
- be **free from undesirable side effects**
- allow a smooth and **uncomplicated recovery**
- cause minimal interference with the purpose of the research procedure

- **MS-222 (Tricaine)**: is the most widely used fish anesthetic and induces a very rapid and deep anesthesia (dosage: 25-100 mg/L)
- **Add 8,5 ml stock MS-222 in 200 ml tank water**

Anesthesia

<https://youtu.be/50qaWPGDmkl>

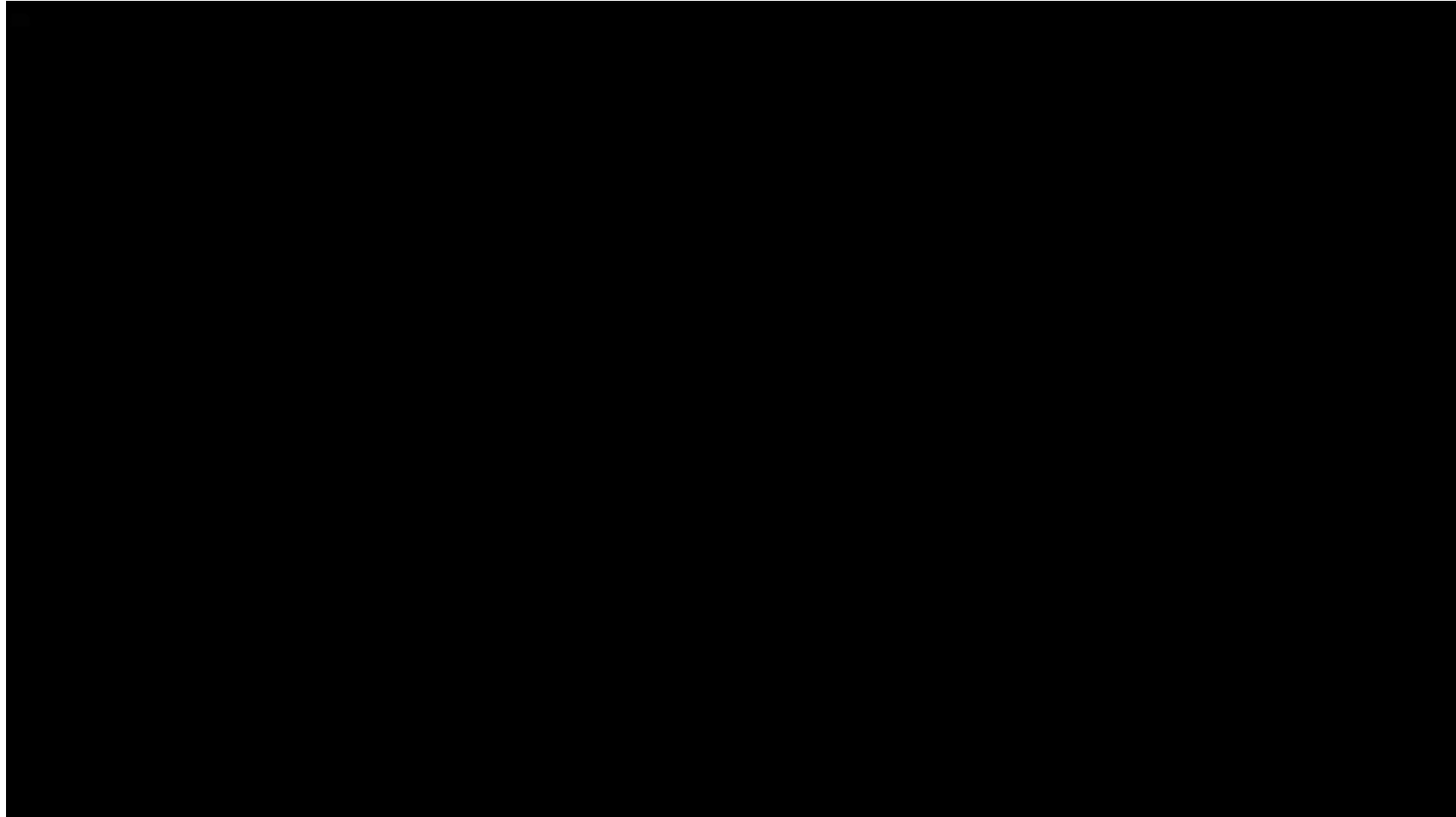
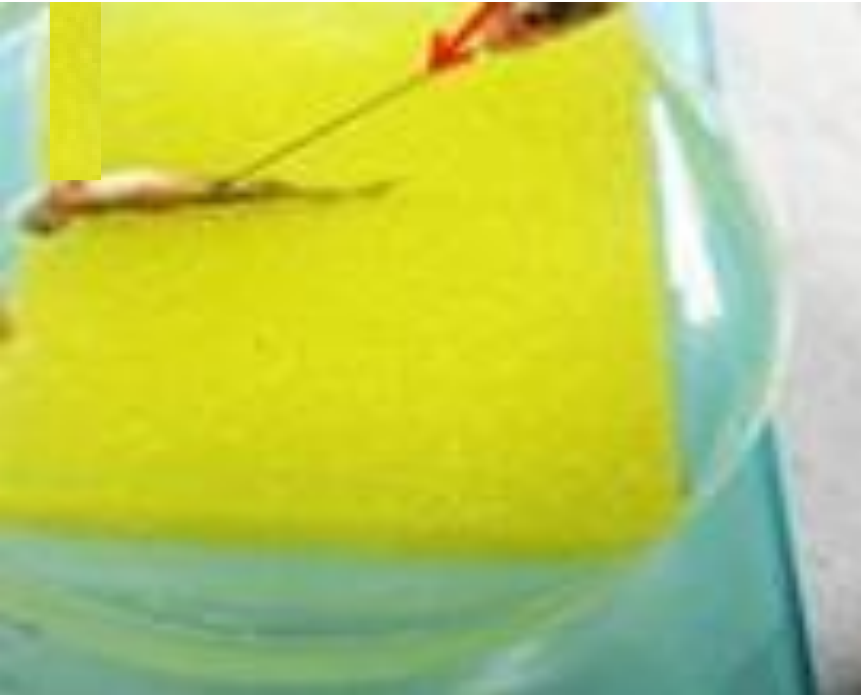


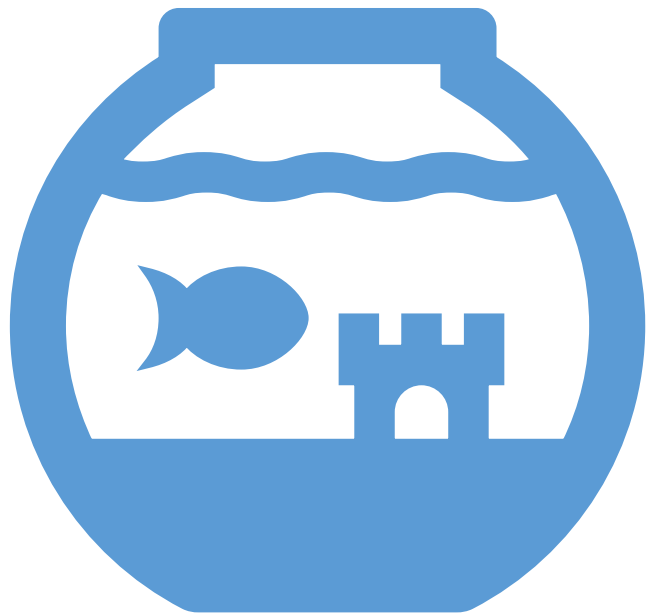


2.4 Intraperitoneal drug administration

- 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

Welfare indicators

<https://www.socrative.com/>

login as Student

Room Name : FANOURAKI

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Let's practice

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