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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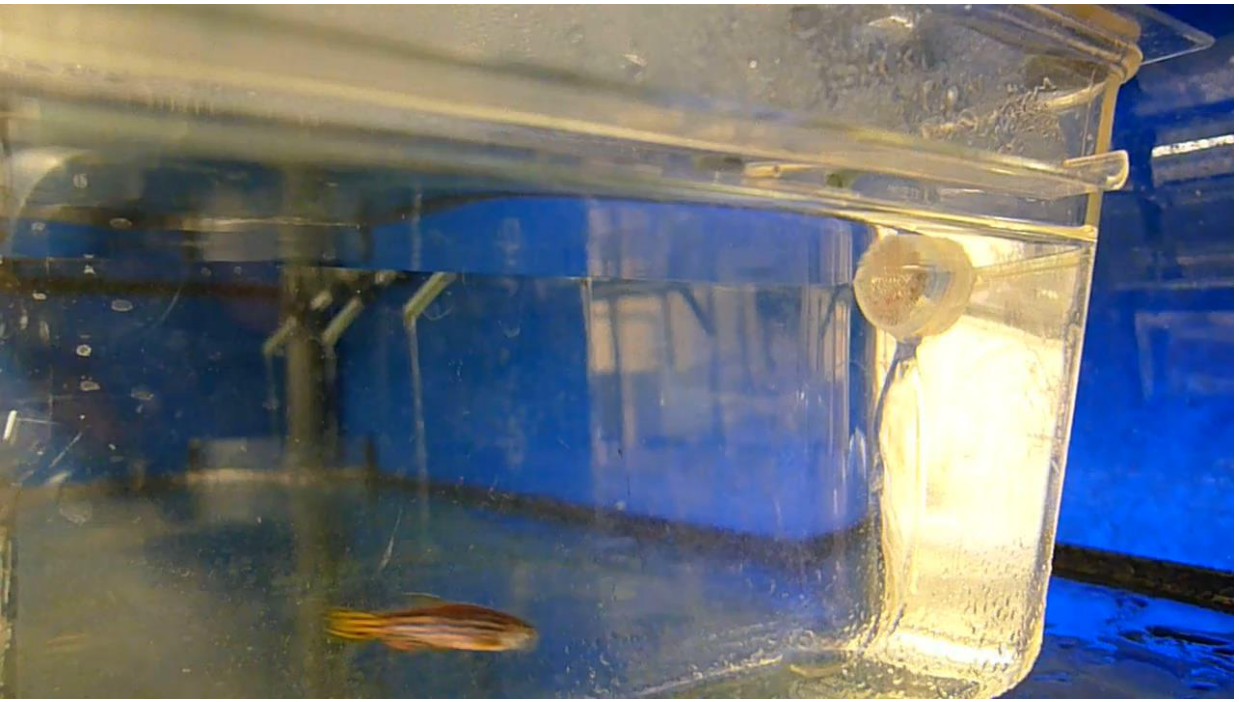
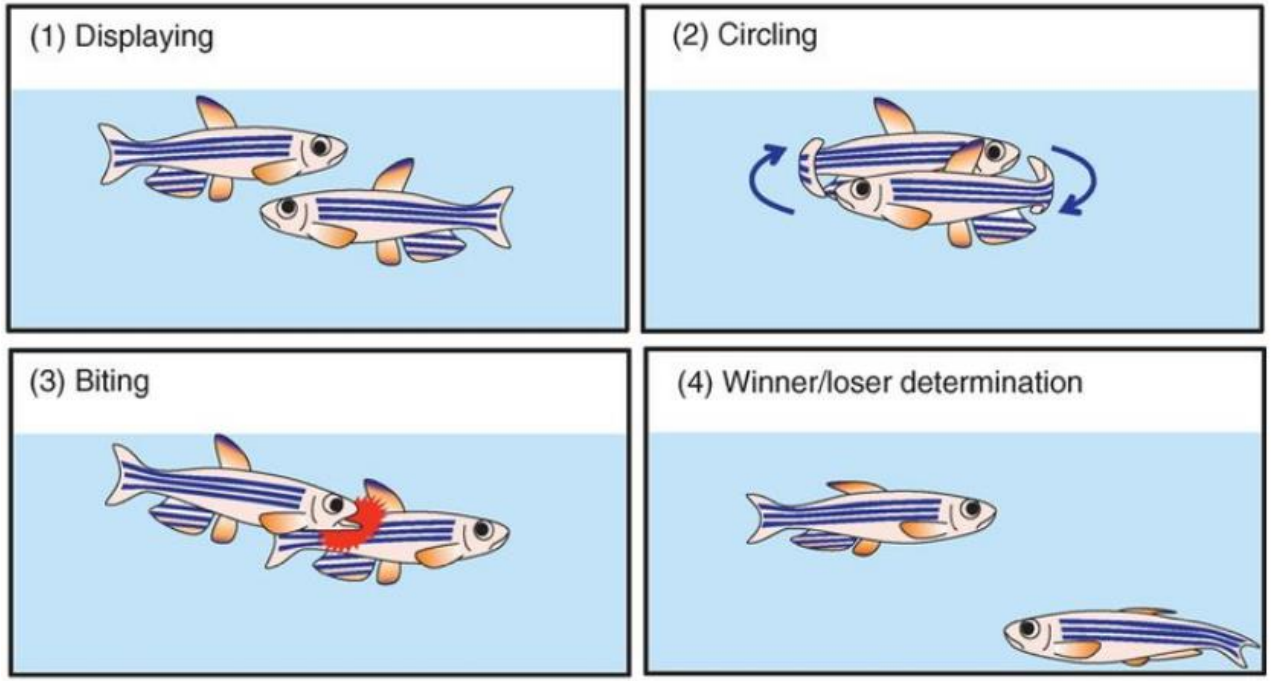
Stocking density

- 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

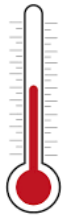
1st part of the practical session

Water parameters

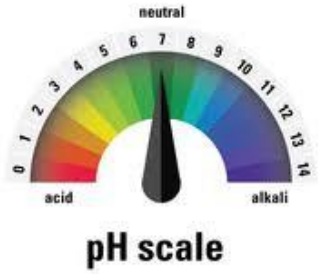
- Temperature
- pH
- Salinity
- Dissolved oxygen
- Nitrogenous wastes

Water quality parameters - Recommendations

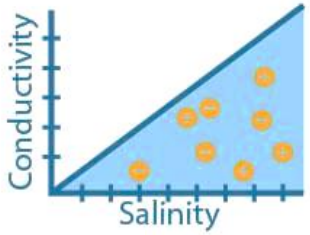
- **Optimum temperature 28°C**



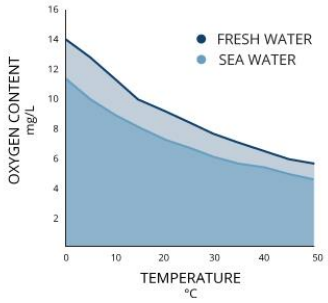
- **pH: between 7.0 – 8.0**



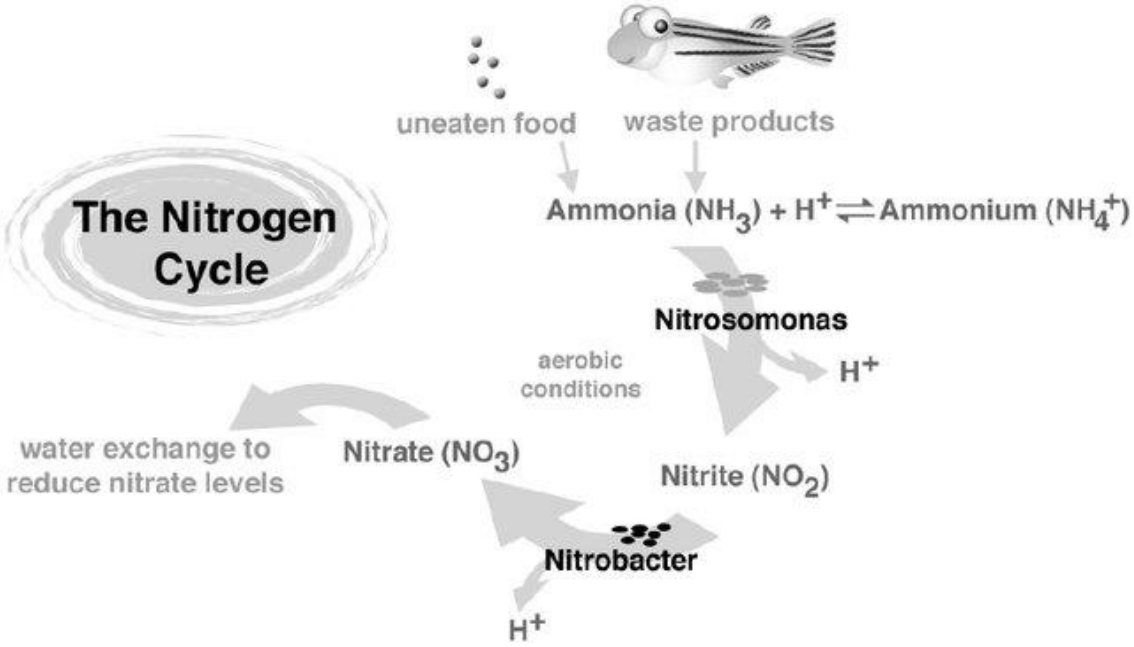
- **salinity** within **0.25 – 0.75 ppt**



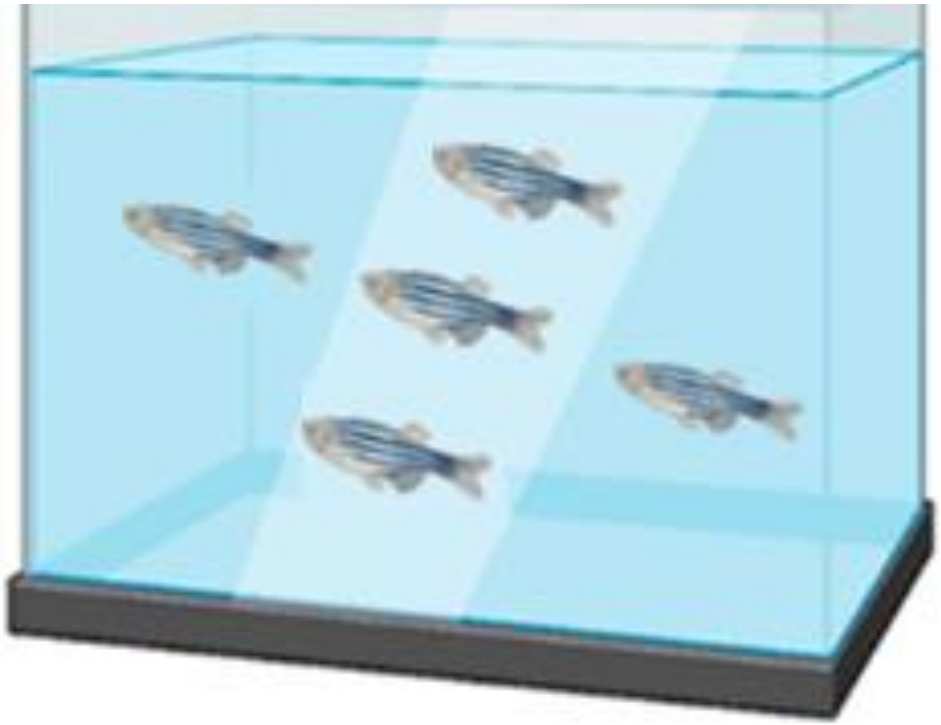
- **~7.8 mg/L at 28.0 °C**

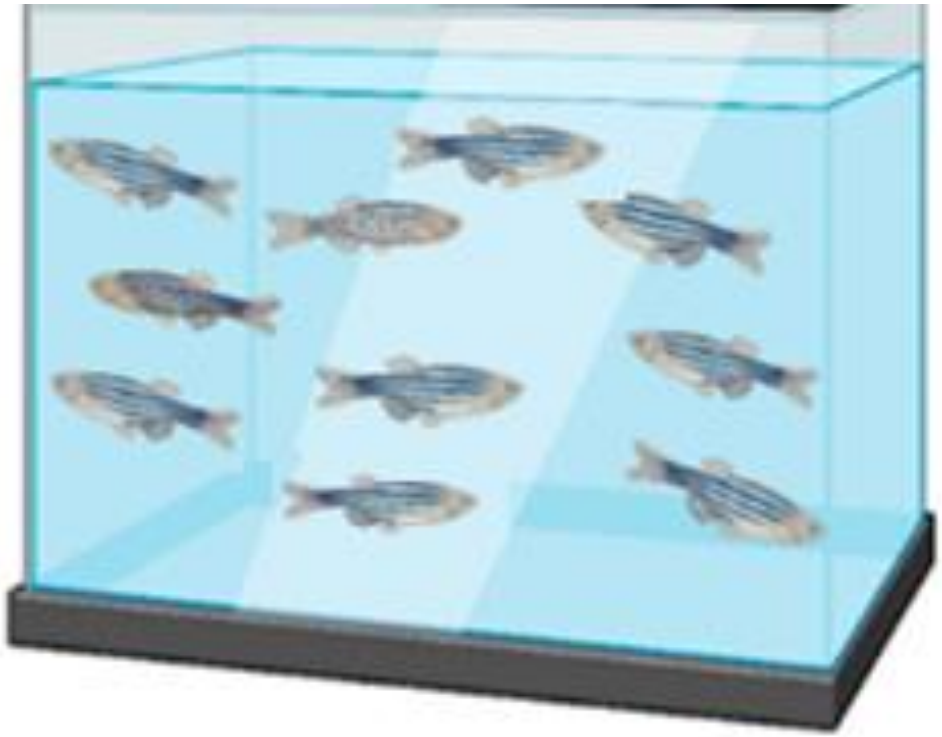


- **Ammonia 0 ppm**
- **Nitrite 0 ppm**
- **Nitrate < 25 ppm**



In the bench – 2 tanks/group





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)

Directions for use: Shake reagent bottles well before using!

Do not allow tested water to contact aquarium or pond 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. Avoid direct sunlight.
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

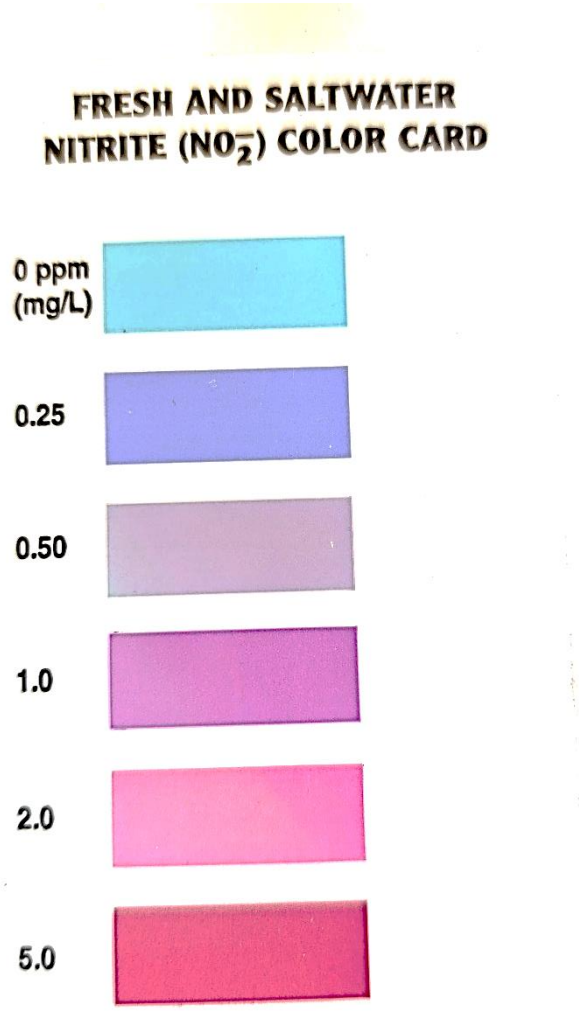
= harmful with long-term exposure

= acutely toxic

color chart:
a) freshwater
b) marine water

Nitrite test (NO_2)

1. **Rinse the measurement vial** with the water to be tested, then fill to the **5 ml** mark.
2. **Add 5 drops of the reagent** into the vial
3. **Cap the vial and shake it for 5 sec** until the liquid is evenly distributed.
4. **Compare the colors after 5 minutes:** Place the vial on the color chart and compare the colors from a position above under natural daylight. Avoid direct sunlight.
5. **Cleaning**: Clean the vial thoroughly with tap water before and after each test.



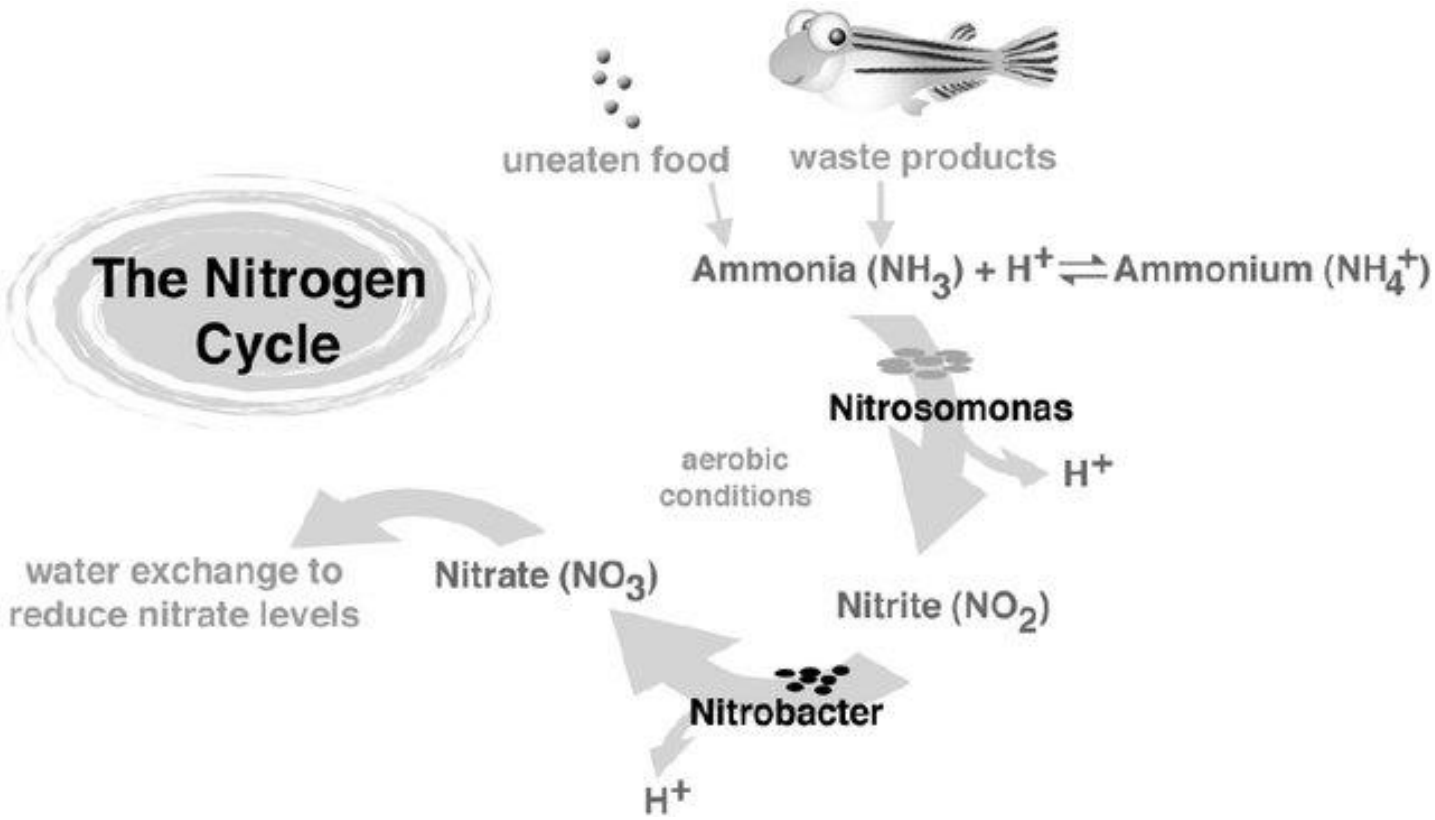
Nitrate test (NO_3)

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
0.2 mg/l
al 0 mg/l
bl 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 biological filter (nitrifying bacteria)
- The renewal of water



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 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 min under natural daylight. Avoid direct sunlight.

3. fill to the 5 ml mark.

4. Add 5 drops of reagent and shake

5. Compare the colors after 5 min under natural daylight.

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 min under natural daylight. Avoid direct sunlight.

Clean the vial thoroughly with tap water after each test

2nd part of the practical session

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

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

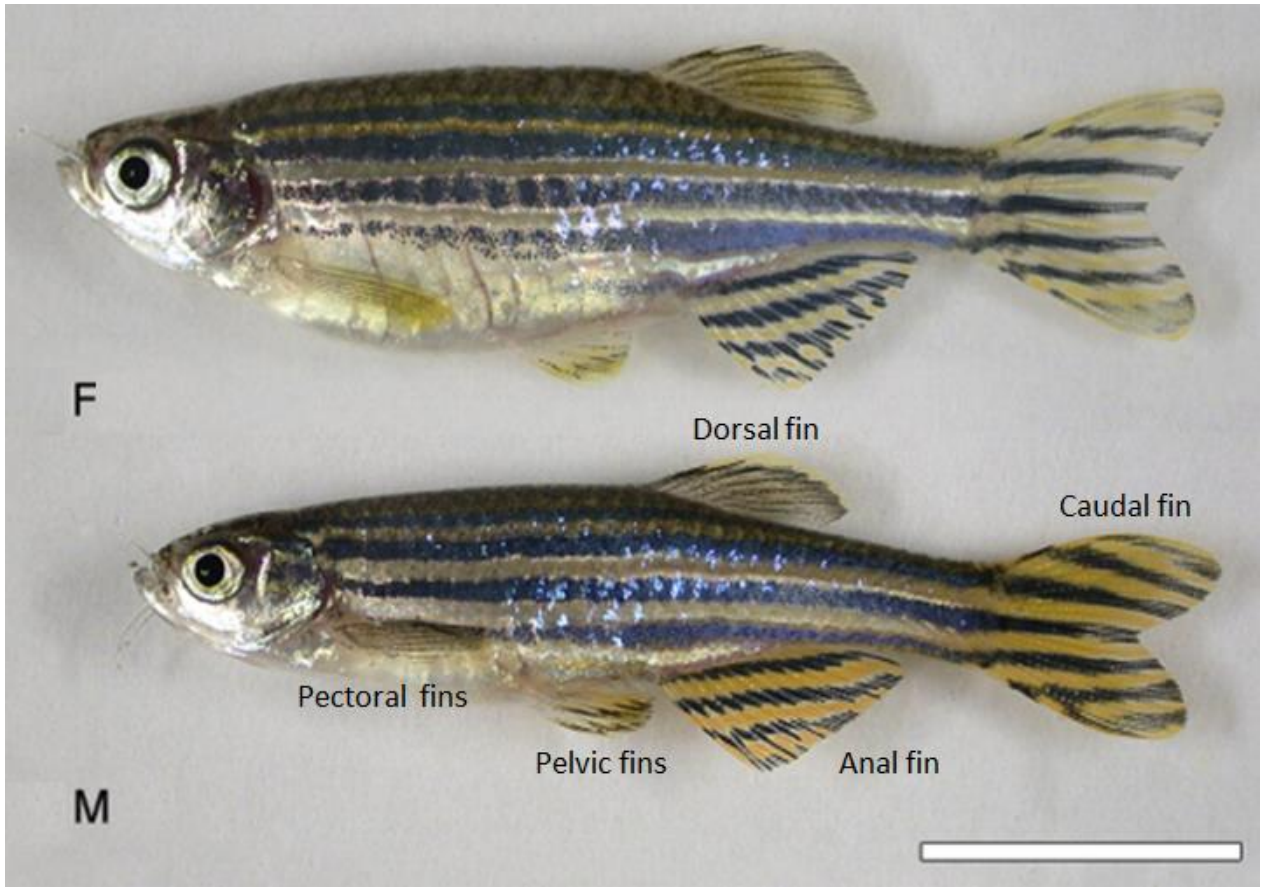
Fish handling

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

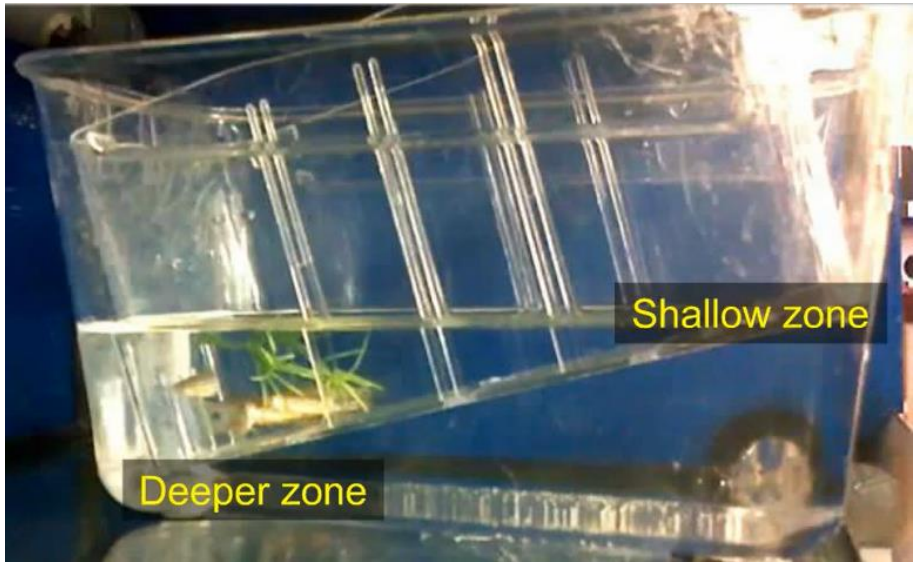


Schonthaler et al., 2010

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

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 are affected by

- species
- 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**.

Anaesthetics

- **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**

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

Anesthesia

https://youtu.be/50qaWPGDmkI

Drug administration - intraperitoneal injection

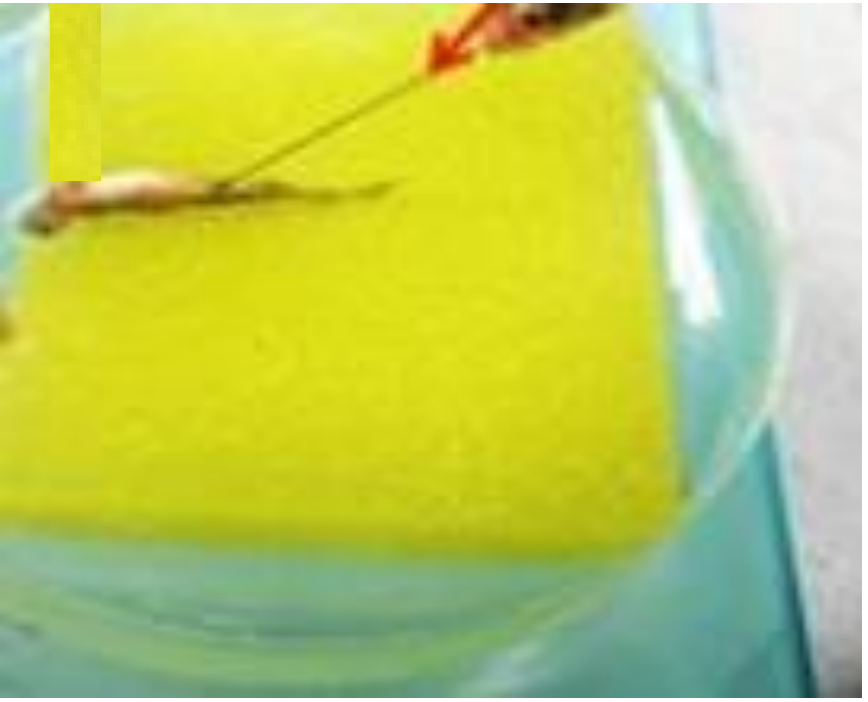
- The most popular method for dosing embryonic and larval zebrafish is through solubilizing chemicals in water (in-water dosing)

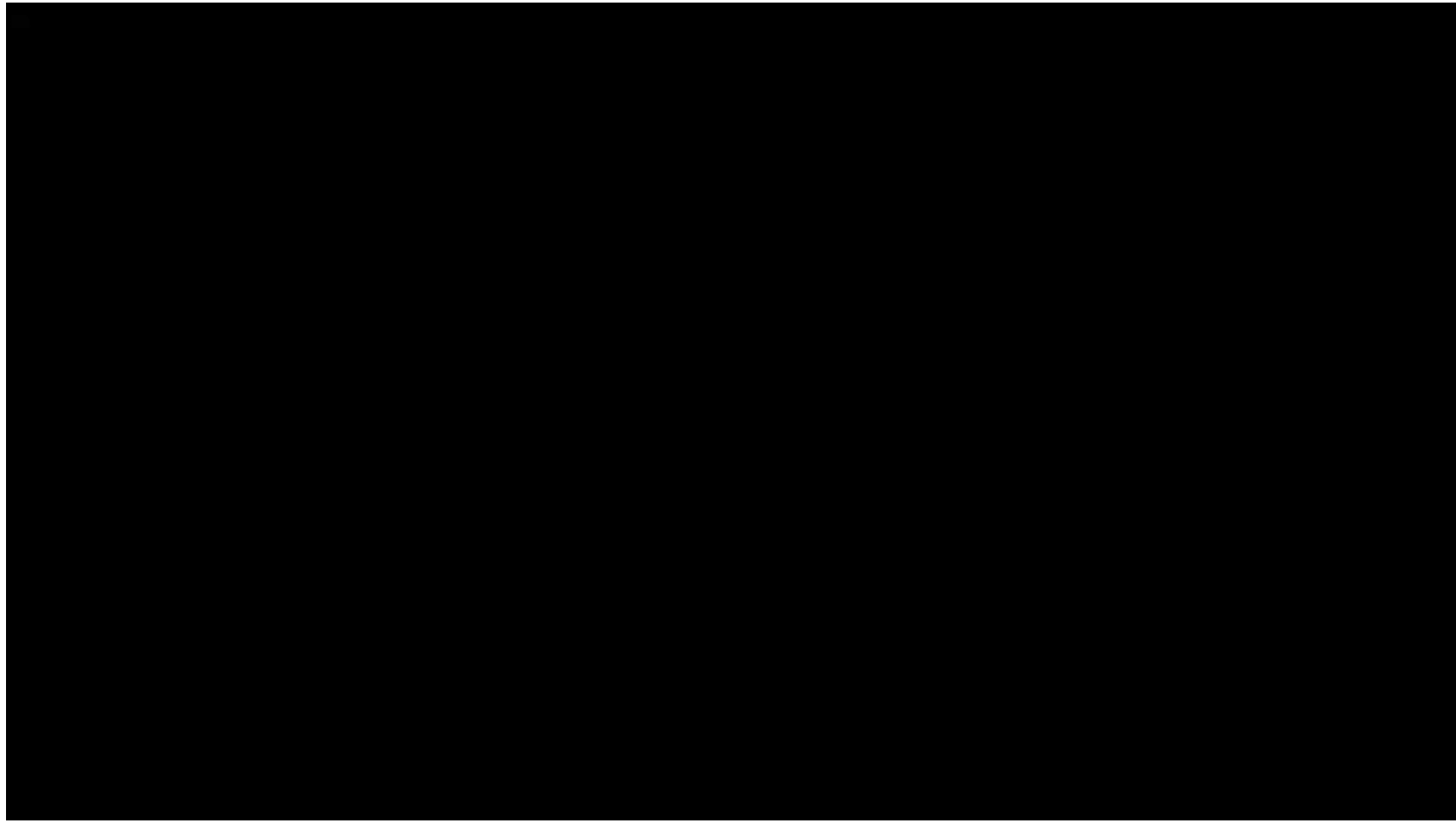
- In **adult zebrafish** is via intraperitoneal injection:

- 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).

- Oral administration for fish have also been reported (DeKoven et al., 1992; Kulkarni et al., 2014, Dayal et al, 2016).

Drug administration - intraperitoneal injection





intraperitoneal injection in rubber fish

- 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

Lets practice