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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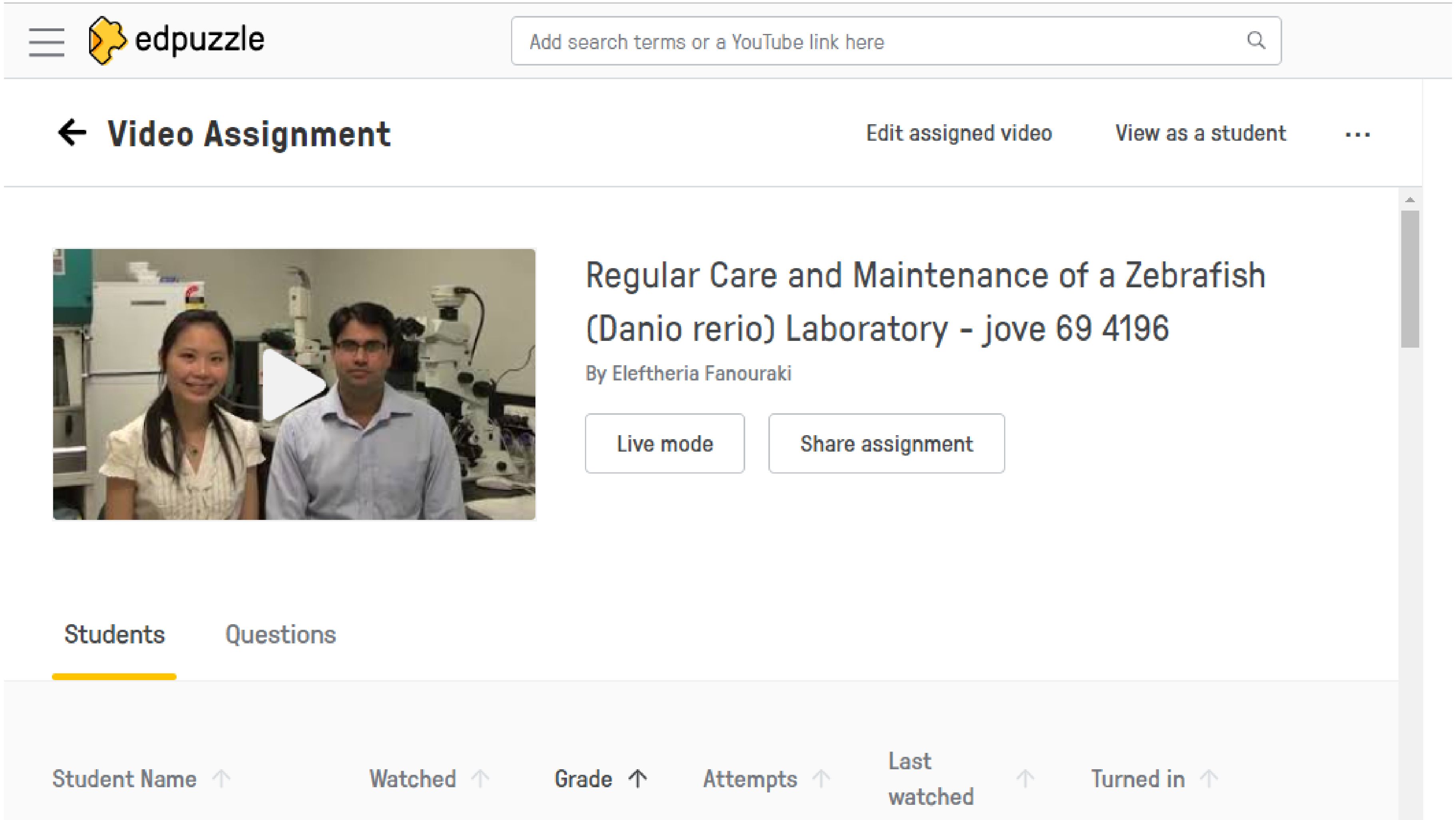
Heraklion, 22th of May, 2024

11 min video with 5 multiple choice questions

https://edpuzzle.com/assignments/644a4669800ebb4340bed334/watch



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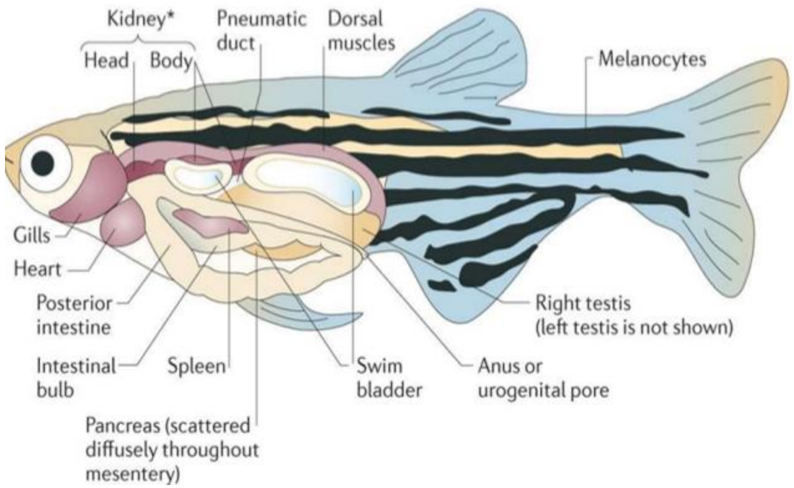
The Zebrafish



- Zebrafish (*Danio rerio*) is a tropical fresh water fish

- Zebrafish is named for the five uniform, horizontal blue stripes which extend to the end of the caudal fin

- It is laterally compressed with its mouth directed upwards

- Common aquaria species

fluorescent fish

The taxonomy of Zebrafish

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

The Zebrafish

Appearance

- Size ~4 cm

- distinguishable features between males and females

Life span

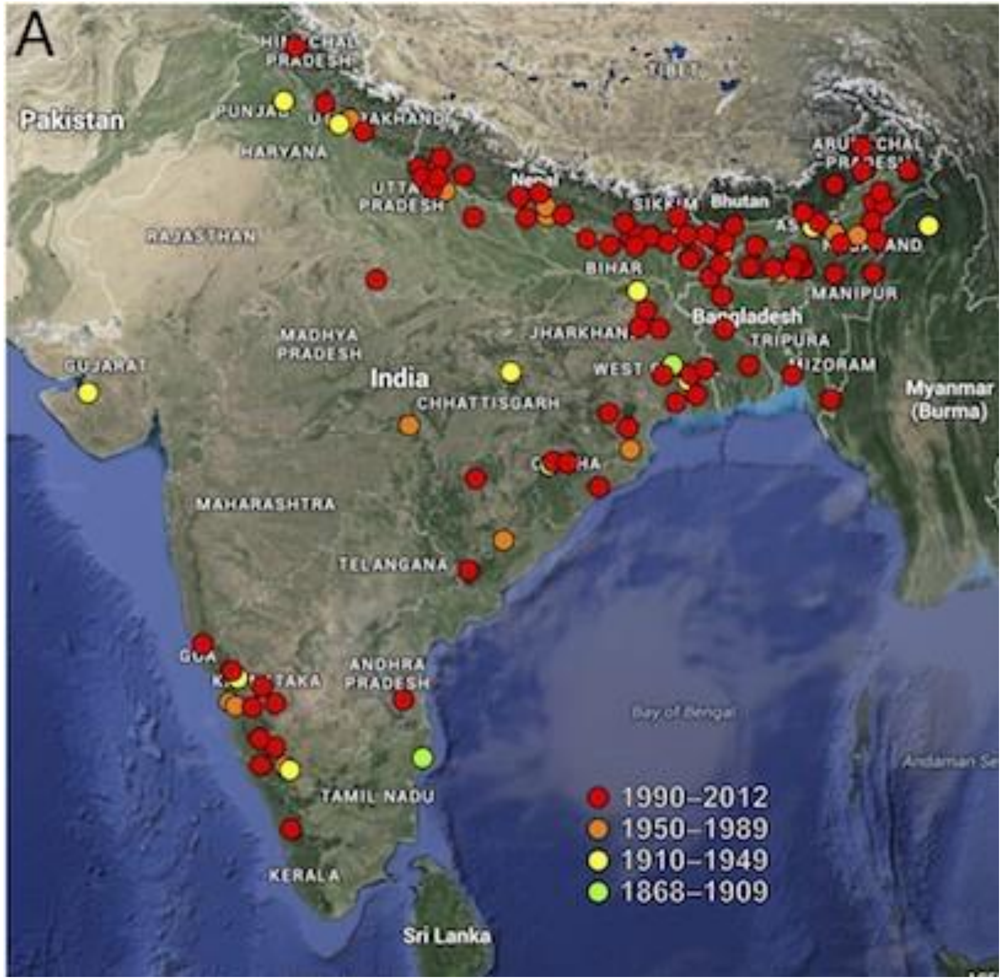
42-66 months (3-5 years)

Habitat

- Fresh water fish

- Tropical environment (22-30^{o}C)

- Native to the streams of the South Asia (India, Bangladesh, Nepal, Myanmar, and Pakistan)



https://elifesciences.org/articles/05635

The Zebrafish

Habitat

- Inhabits streams, ponds and slow moving or still water regions, including rice fields
- Predation pressure has led to the development of shoaling behavior, believed to reduce stress and aggression among fish held in small groups

Feeding

Zebrafish are omnivores, consuming larval and adult insects, small crustaceans and other zooplankton,

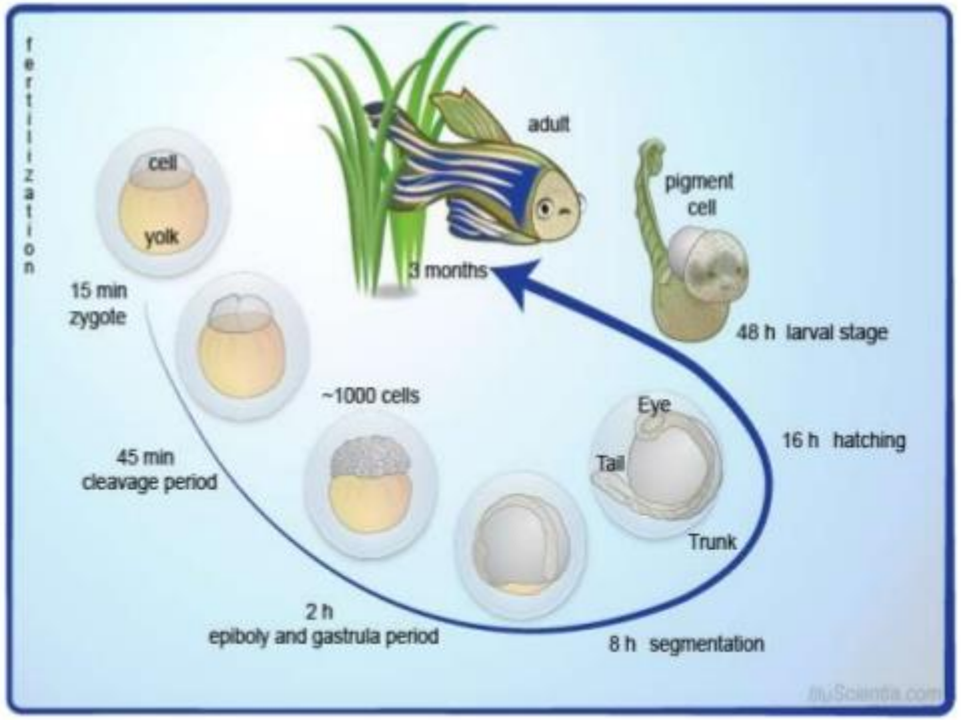
but also algae, plant material and assorted detritus (dead organic material)

https://elifesciences.org/articles/05635

Life cycle of Zebrafish

Zebrafish **breed in small groups**, with females scattering clutches of eggs with no parental care

- **External fertilization**
- Female spawns **every 2-3 days**
- Several **hundreds of eggs** produced every time
- Breeds **all year round**
- **Transparent eggs & embryos**



Zygote
↓
Cleavage
↓
Blastula
↓
Gastrula
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Segmentation
↓
Hatching
↓
Larva
↓
Juvenile
↓
Adult

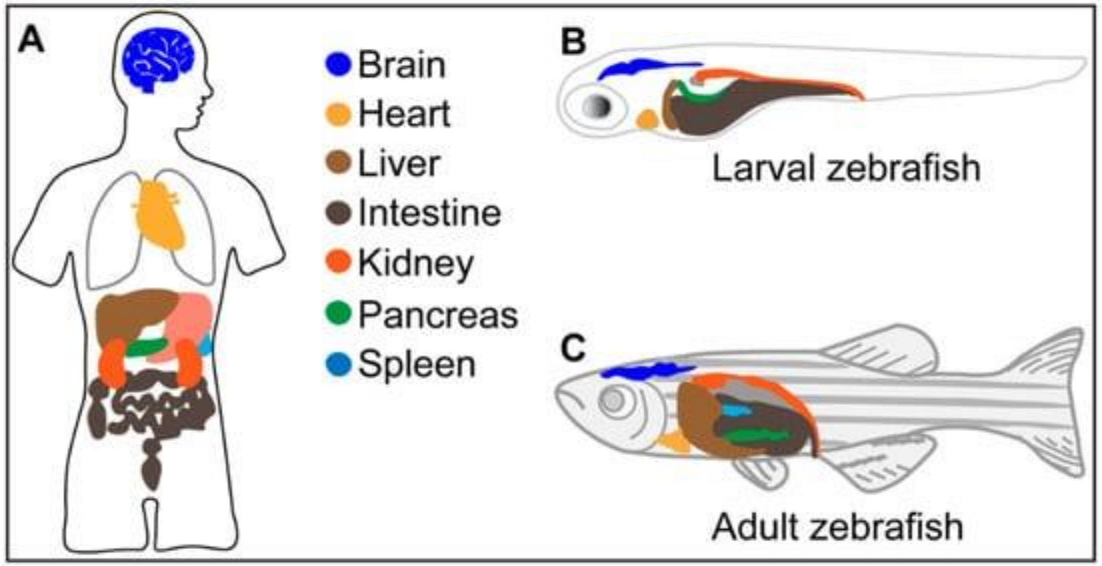
Reproduction is primarily affected by
- **food availability**
- **photoperiod**

A Zebrafish Embryo Develops GIF | Gfycat

Zebrafish as an animal model

Zebrafish is being used widely as an animal model to study fundamental biological questions

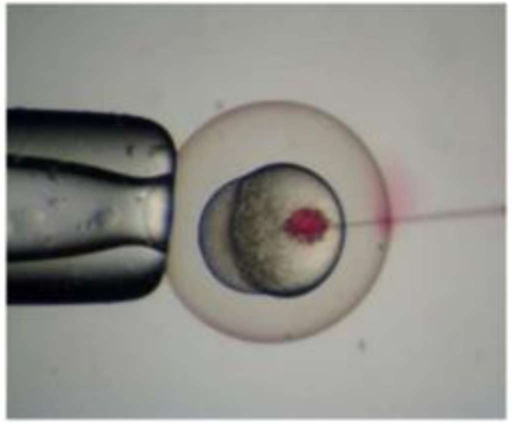
- molecular biology
- developmental biology
- Neurobiology
- Genetics
- Cancer research
- Drug discovery
- Endocrine research
- Stress physiology
- Social interactions
- Biotechnology
- Aquaculture



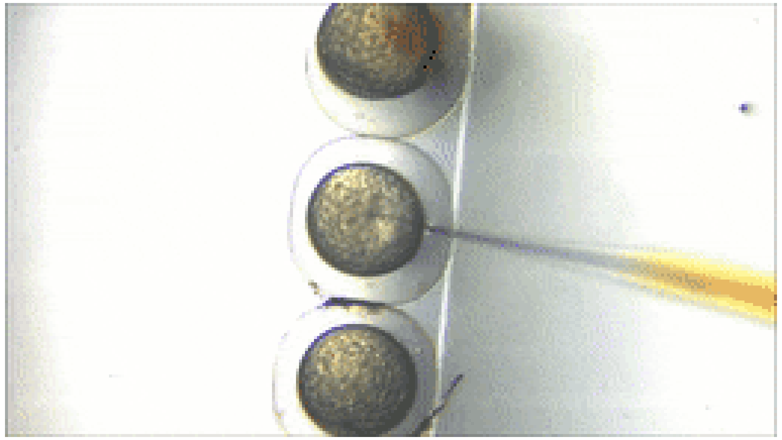
Zebrafish as an animal model

- Universally available
- Small size
- Large number of fish can be kept easily in the lab
- Low cost of maintenance
- Rapid development
- Rapid generation time
- Large number of offspring

Zebrafish as an animal model



- Eggs easy to manipulate (0.7mm)
- Good model for developmental studies (early life stages) due to transparency of the egg
- Extensively Sequenced



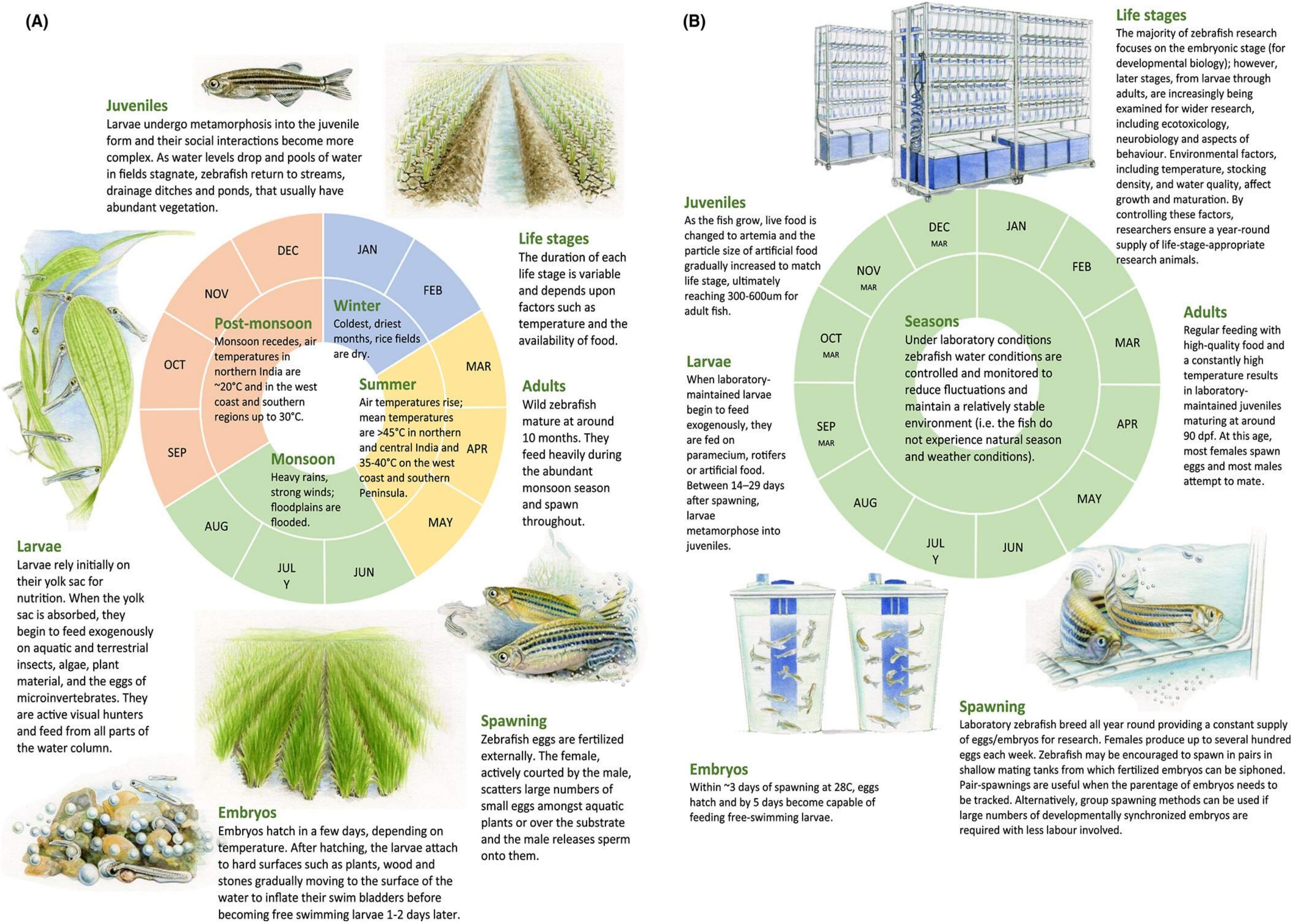
- Although humans may appear to be extremely different than zebrafish,
- 70% of human genes are found in zebrafish and
- 84% of genes known to be associated with human disease have a zebrafish counterpart.

Husbandry of Zebrafish

While conditions in the wild can vary considerably, it is essential to provide stable husbandry conditions in the laboratory,

- Temperature
- Photoperiod
- Water quality parameters
- Stocking density
- Feeding schedules

Improving zebrafish laboratory welfare and scientific research through understanding their natural history



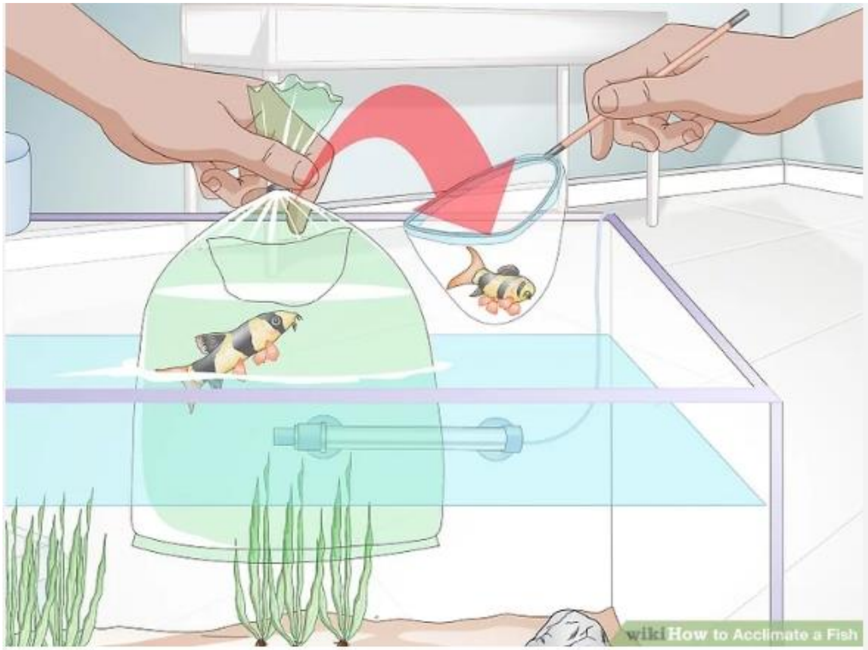
Biological Reviews, Volume: 97, Issue: 3, Pages: 1038-1056, First published: 04 January 2022, DOI: (10.1111/brv.12831)

Husbandry of Zebrafish

Transportation

- Relevant documentation from the exporting facility should be sent prior to the shipment.
- The shortest possible shipment time has to be planned.
- Only bleached embryos and/or healthy juveniles/adult fish can be shipped.

- Upon arrival, animals should be visually checked, equilibrate water temperature and transfer the fish into the reception tank without transferring the water from the transport container.
- In order to avoid ammonia poisoning, it is vital that adult fish are removed with a net from the transport container immediately after temperature adaptation.
- Use a **quarantine** system for raising and monitoring newly imported embryos and adult fish, and ask for health certificate from the sending facility.



Husbandry of Zebrafish

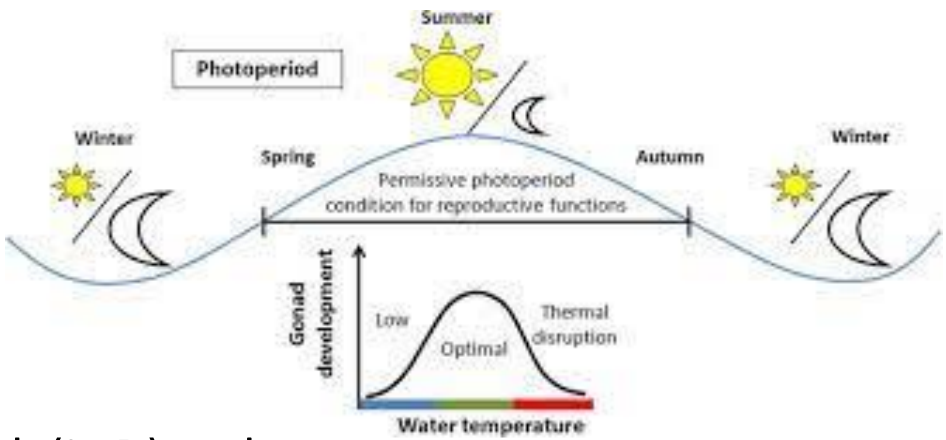
Housing systems

Recirculating water systems for zebrafish should include

- filter systems,
- water chemistry monitoring or regulation capabilities,
- germicidal UV irradiation,
- light and
- temperature control units.

https://journals.sagepub.com/doi/10.1177/0023677219869037

Photoperiod



- In nature, light conditions vary with seasons and weather

- Modern laboratory facilities keep fish exposed to a stable light-dark (L–D) cycle (commonly **14 hours Light:10 hours Dark** or 12 hours Light:12 hours Dark)

- Optionally the exchange between light and dark comes with a **gradual increase and decrease** in light intensity mimicking dusk and sunrise

- The **intensity of light should be as uniform** as possible across tanks and intensities should be adjusted to **between 54 and 334 lux** at the front of the tank

- Using other settings will not affect animal welfare as such, but may influence physiological processes, for example **spawning frequency** (and hence breeding success)

Water quality parameters

- Depending on the local water supply used for recirculating water systems, some laboratories can use **tap water** without major amendments. **Chlorine** still needs to be removed, as levels safe for humans (0.1 mg/L) are **toxic** for fish
- Many facilities only use conditioned **deionised water**.

  Reverse osmosis (RO) is commonly used for deionisation and **sea salt, calcium chloride and sodium bicarbonate** are then added to achieve the desired **salinity, hardness and pH**.
- **Water exchange rate 10% daily**

Water parameters

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

Christian Lawrence, 2007

Water quality parameters: Temperature

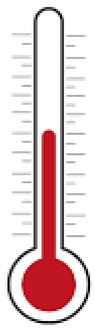
Temperature is one of the most important physical parameters to consider in fish culture operations because of the profound effects it exerts on biological and chemical processes

Fish are **ectothermic animals** and display varying degrees of tolerance to changes in temperature, as well as a more narrow optimum range in which they perform best.

Zebrafish can be classified as **eurythermal**, as they exhibit a tolerance for wide temperature ranges **(12-39ºC)**.

Zebrafish

- **Optimum temperature 28ºC**
- Temperature influences water chemistry and animal physiology and larval development



For standard growth curves, embryos should be kept at a temperature of 28.5 ± 0.5°C to 120 hpf.

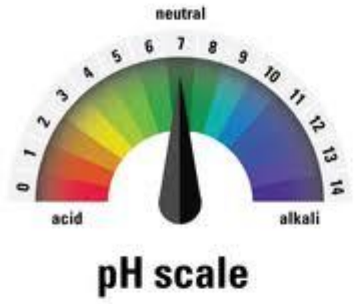
Adjusting the rearing and maintenance temperatures for larvae and adults within a 24–29°C range is recommended.

For **time-limited experiments** a temperature range from **15 to 39°C** is acceptable.

Water quality parameters: pH

Like temperature, the pH of the water in aquatic systems also exerts profound effects on

- biological processes in fish
- the function of the microbial community that supports them



Zebrafish

The maintenance pH that most zebrafish facilities strive for is **between 7.0–8.0**, which is within the general range recommended for freshwater fish

Lee et al., 2022

Laboratory conditions for adult zebrafish					
Parameter	**Wild habitats**	**Tolerance limits**	**Optimal range**	**Recommendations**	**Implications for welfare**
pH	Varies from 5.9 (Engeszer *et al.*, 2007) to 9.8 (Arunachalam *et al.*, 2013)	Lower and upper lethal limits: 3.0 and 12.0 respectively (Zahangir *et al.*, 2015)	7.4–7.5 for reproduction (Alestrom *et al.*, 2019)	7–8 (Hammer, 2020); 6.5–8 (Alestrom *et al.*, 2019)	Exposure to pH near the lower or upper limits damages skin and gills, leads to loss of balance, convulsions and death (Zahangir *et al.*, 2015).

Water quality parameters: hardness

- Water hardness is a measure of the **quantity of divalent ions**, primarily calcium and magnesium in water

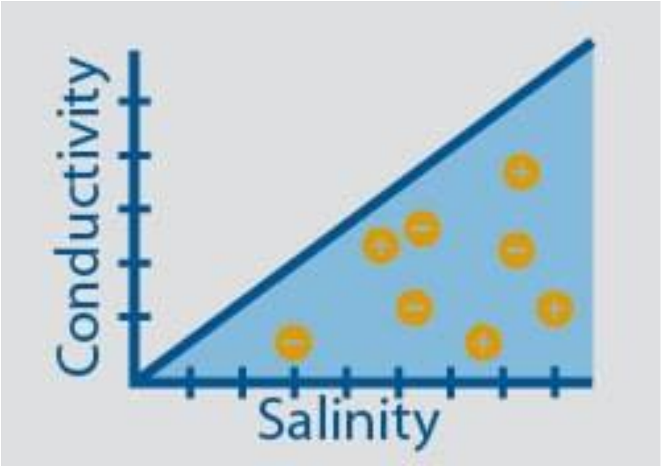
- These ions must be provided to fish in captivity in their water

- These ions are **required by fish for egg, bone and tissue development, osmoregulation and blood clotting**

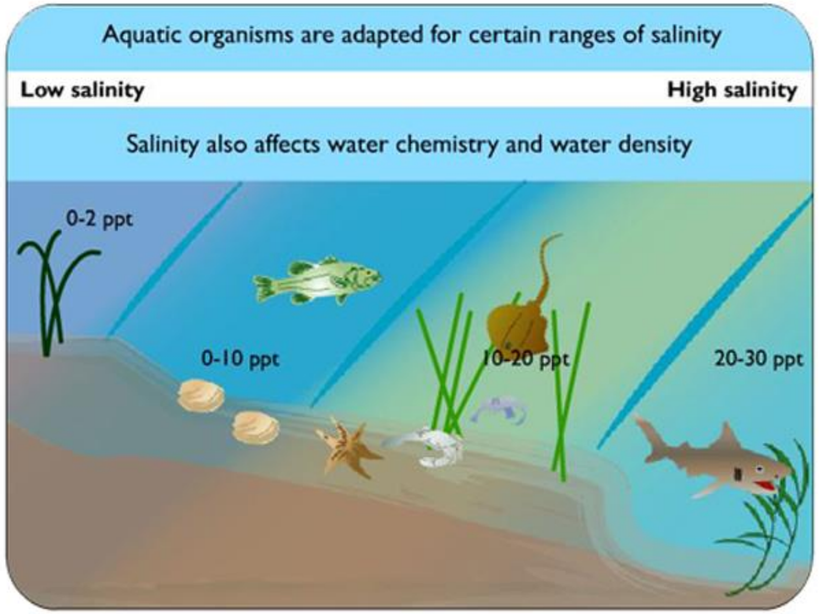
Zebrafish is classified as a **“hard water” species**, preferring hardness values in excess of 100 mg/L $CaCO_3$, so If reverse osmosis water is utilized, then addition of calcium and magnesium salts **is required to bring hardness values within 75–200 mg/L**, the generally recommended range for freshwater aquatic animals

Water quality parameters: salinity

- Salinity is a measure of the mass of **dissolved salts in a given mass of water** and usually expressed as parts per thousand (**ppt**). Salinity is measured using Conductivity (uS/cm) measurements
- Maintaining fish **above or below** their salinity optimum is possible, but because **fish must spend more energy** in doing so, it can compromise growth, survival and reproduction.



- Zebrafish are freshwater fish, but are tolerant of a wide range of salinities
- However, it is better to maintain zebrafish stocks at stable salinities within the **general range of 0.25–0.75 ppt**.



Water quality parameters: dissolved oxygen

- Dissolved oxygen is a highly important parameter in fish cultivation
- Fish require oxygen for **respiration**, and **demand depends** upon a number of factors, including **body size, feeding rate, activity levels, and temperature**
- In general, **small-bodied, tropical fish** such as zebrafish typically have **high metabolic rates** and, therefore, **consume more oxygen per unit weight than larger fish**
- This fact, coupled with their relatively high maintenance temperatures, stocking density, and levels of feed input that are typical of **intensive zebrafish facilities** necessitate that dissolved oxygen levels be maintained **at or just under saturation (~7.8 mg/L at 28.0°C)** to ensure health of the fish

Laboratory conditions for adult zebrafish					
Parameter	Wild habitats	Tolerance limits	Optimal range	Recommendations	Implications for welfare
Dissolved oxygen	Unknown	Levels of 0.8 mg l^{-1} are lethal within 2 days and levels of 0.4 mg l^{-1} are lethal within 12 h (Rees, Sudradjat & Love, 2001)	Unknown	Range from 6 mg l^{-1} (Matthews, Trevarrow & Matthews, 2002) to 7.8 mg l^{-1} (Harper & Lawrence, 2012); with a suggested minimum of 4 mg l^{-1} (Lawrence & Mason, 2012)	Uneaten food, decaying solids and high fish densities can reduce levels (Hammer, 2020). Warning signs include hyperventilation, surface swimming and gulping air (Kramer, 1987); extreme depletion can damage gills, impair growth, cause immunosuppression, and lead to death.

Water quality parameters: nitrogenous wastes

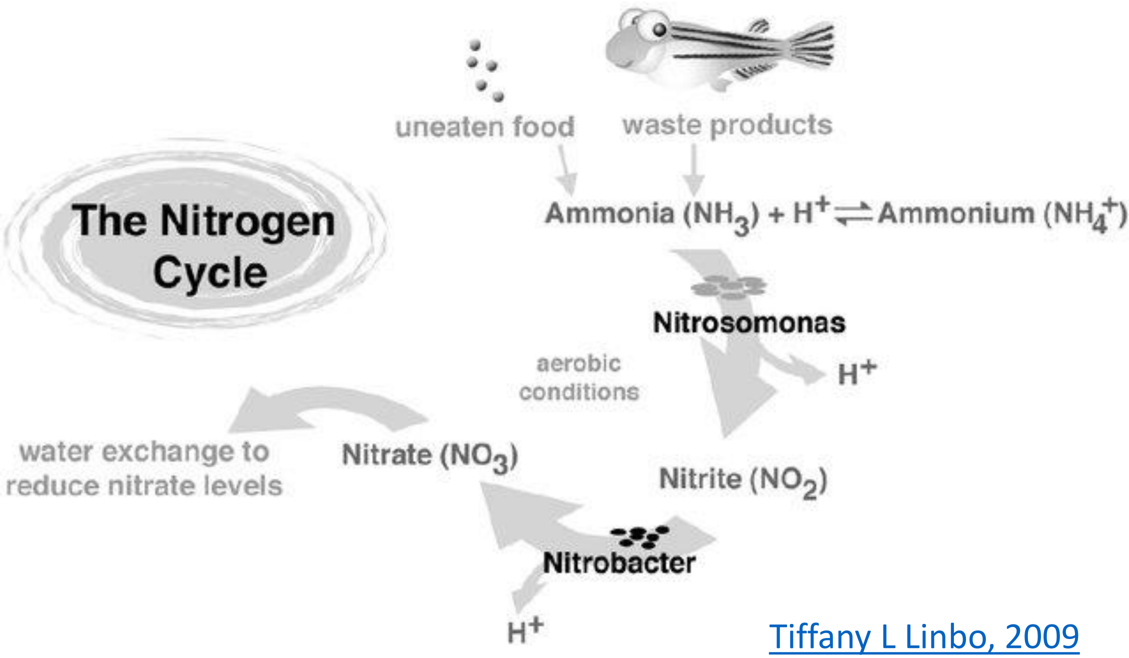
- In freshwater fish, **ammonia is excreted across the branchial epithelium via passive diffusion**, and to a lesser extent, in **feces**
- It is also produced during the **decomposition of decaying organic matter** (i.e. dead fish, uneaten food)

- **Ammonia is highly toxic** and must be eliminated
- this is accomplished by **nitrifying bacteria that oxidize ammonia into nitrate**. The intermediate product of this conversion, nitrite is also toxic to fish
- **10% daily water renewal** prevents accumulation of Nitrate

Zebrafish tolerance to nitrogenous wastes

- Ammonia 0 ppm
- Nitrite 0 ppm
- Nitrate < 10 ppm (ideal for breeding fish)

A well-dimensioned biofilter keeps levels of total ammonia < 0.1 mg/l, nitrite < 0.3 mg/l and nitrate < 25 mg/l



Tiffany L Linbo, 2009

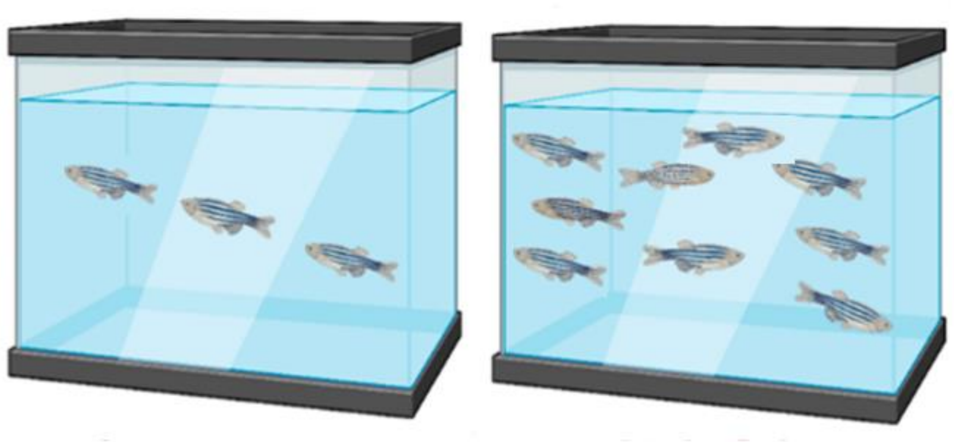
Laboratory conditions for adult zebrafish					
Parameter	**Wild habitats**	**Tolerance limits**	**Optimal range**	**Recommendations**	**Implications for welfare**
Ammonia	Unknown	Levels >1.0 mg l^{-1} are lethal to many fish (Murray, Lains & Spagnoli, 2020) but the specific tolerance limits of zebrafish are unknown	Unknown	As close to 0 mg l^{-1} as possible (Hammer, 2020; Murray *et al.*, 2020)	Highly toxic. Chronic exposure to non-lethal levels can result in immunosuppression and reduced growth (Murray *et al.*, 2020). Acute exposure can cause hyperexcitability, anorexia, and death (Murray *et al.*, 2020).
Nitrite	Unknown	Levels of 386 mg l^{-1} are lethal within 4 days (Voslářová *et al.*, 2008)	Unknown	As close to 0 mg l^{-1} as possible: <0.5 mg l^{-1} (Hammer, 2020); <1.0 mg l^{-1} (Murray *et al.*, 2020)	Warning signs in fish include lethargy, remaining near water inlet, hyperventilating; chronic exposure impairs growth (Murray *et al.*, 2020).
Nitrate	Unknown	Unknown	Unknown	<100 mg l^{-1} (Pereira *et al.*, 2017); <50 mg l^{-1} (Hammer, 2020); <25 mg l^{-1} (Alestrom *et al.*, 2019)	Less toxic than nitrite but may accumulate over time in recirculating systems with high fish densities (Learmonth & Carvalho, 2015). Chronic exposure can damage gills, skin, kidneys, liver and intestines (Pereira *et al.*, 2017).

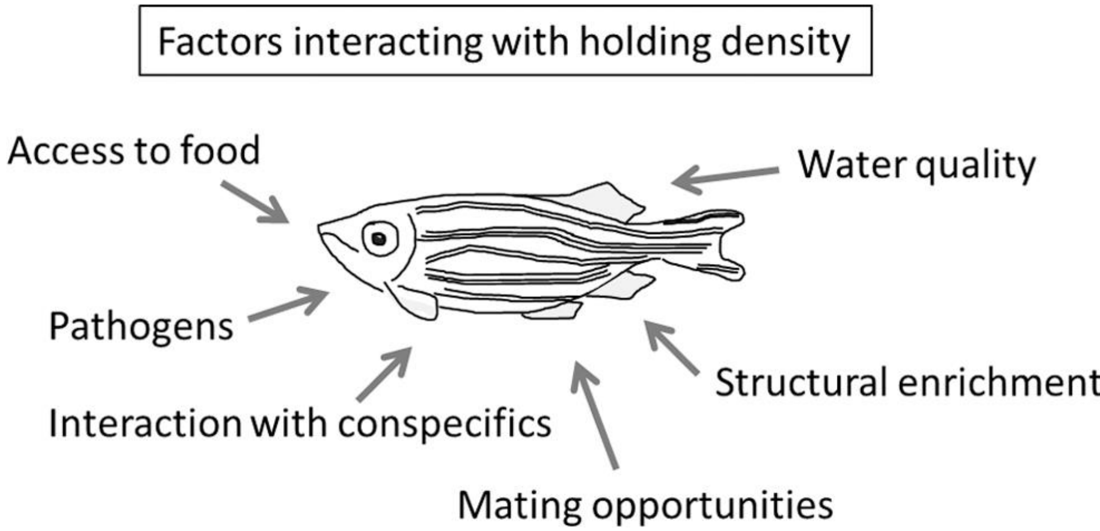
Stocking density

- For long-term housing, a standard of **4–10 adult fish/l** will help to maintain low stress levels and good water quality.

- For **embryos**, a recommended upper limit is **100/35 ml in a 9 cm Petri dish**, and

- for **5–10 dpf larvae**, up to **250/l**. Higher and lower densities, including keeping single fish, can be tolerated for limited periods.



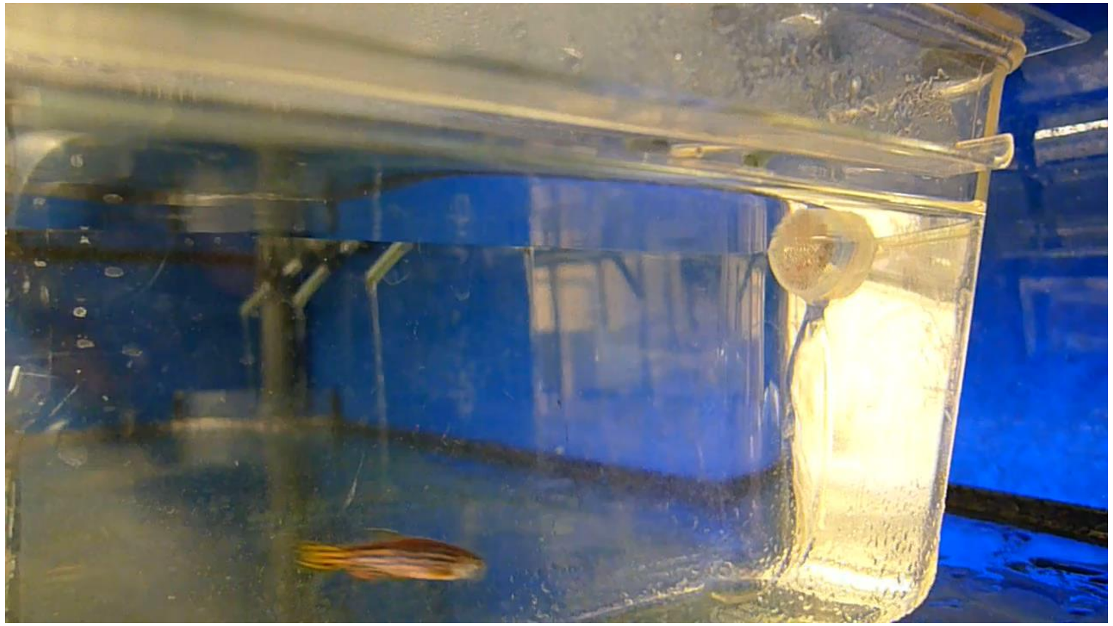


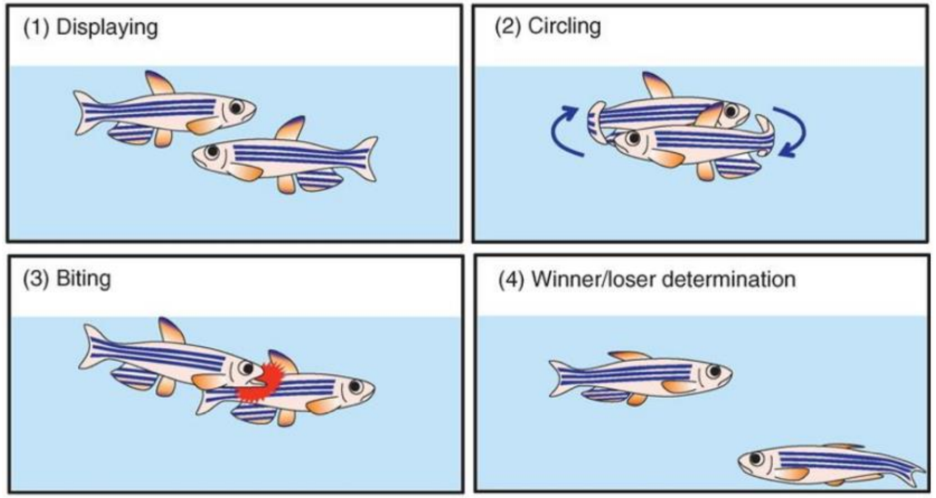
- The holding density does not only correspond to **available space per fish** but will also affect other factors relevant for fish welfare, such as the **access to food** and the resulting **water quality**, including **oxygen levels** and **waste products**.

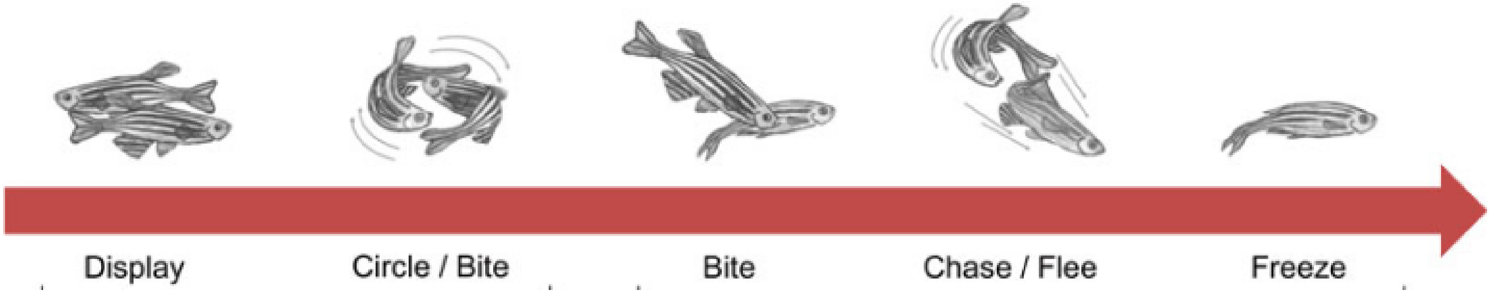
Stocking density

- **Higher stocking densities** have been associated with **crowding stress**

- **Lower stocking densities** have been associated with the emergence of **dominant and submissive behavior** in zebrafish, which leads to **aggressive behavior** and **elevated stress** and consequently reduced welfare







Feeding

- It is generally accepted that a **combination of live feeds and processed dry feeds improves growth**, **sexual maturation and reproductive performance**, all positive indications of well-being
- **Dry feed diets** are generally assumed to be **nutritionally complete**,

  whereas **live feed** and the associated fish **prey-capture behavior has an enrichment effect**

Feedings

2-3 times per day (Depending on the developmental stage)

Larval Diets

- Dry micro-pellets (size: 100-200 microns)
- Live Artemia (*Artemia nauplii*)

Artemia nauplii

Adult Diets

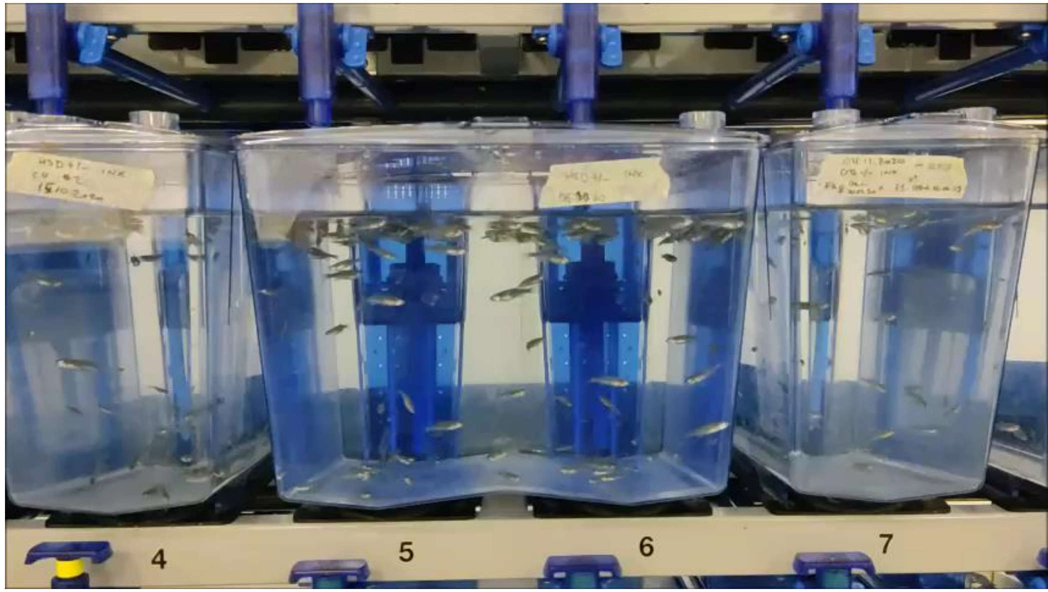
3-5 % of body weight per day

- Dry flakes
- Dry pellets (size: 400-600 microns)
- Live Artemia (*Artemia nauplii*)

Husbandry of Zebrafish

- Decades of experience have proven that maintaining zebrafish is a relatively straightforward task

- In most cases, commercially available **tank systems** are used, which come **fully integrated with**
  filter systems,
  Ultraviolet radiation for water disinfection (UVC) and
  temperature control units



- These **systems contain installed tanks** (glass or polycarbonate), typically between **1 L and 10 L**
  which can be **removed** from the main water supply and
  reconnected again depending on the specific needs

- Most systems rely on a **recirculating water system** in which pumps feed water into the tanks and,
  through overflow system, remove an equal amount of water

8 L tank

3.6 L tank

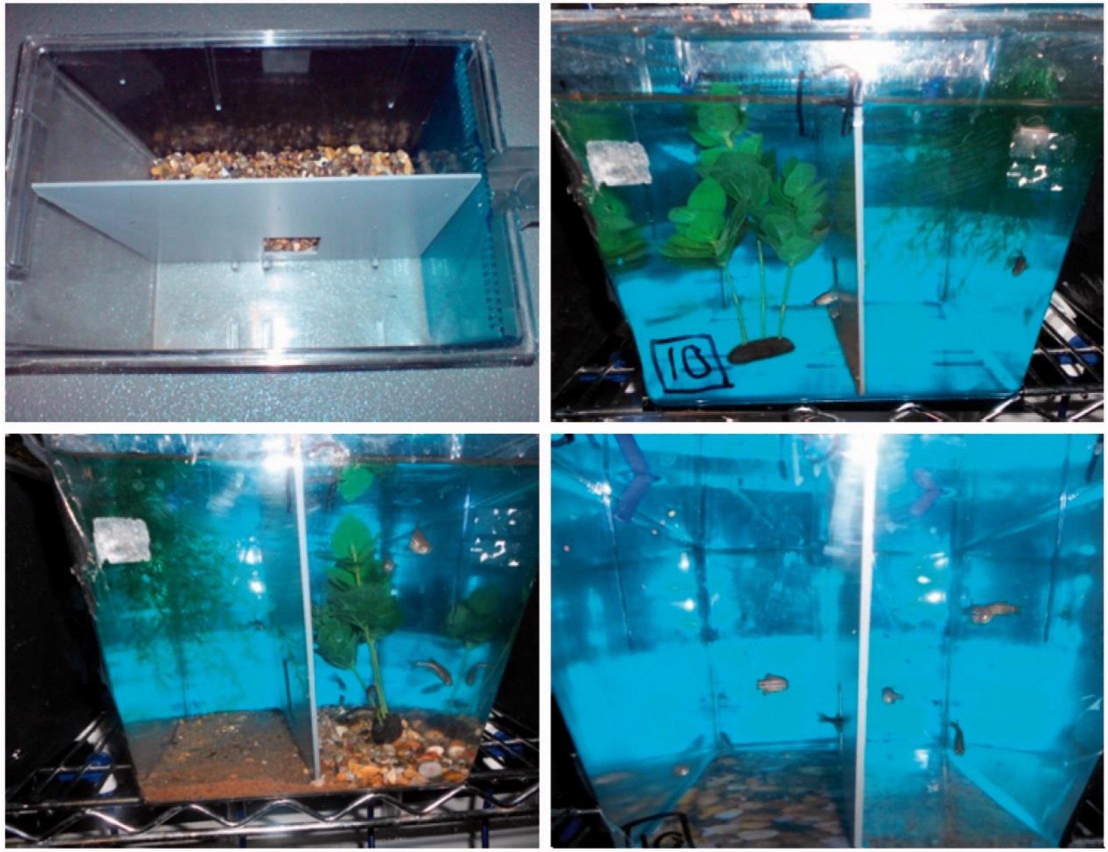
Environmental Enrichment

- Gravel, sand,
- image of gravel or sand,
- artificial plants,

➢ Pairs preferred substrate over barren tanks.

➢ Groups preferred substrates and plants over barren areas;

➢ strong preference for gravel and images of gravel (Schroeder et al.,2014)



Environmental Enrichment

Environmental Enrichment

- flowing water,
- plastic plants used in Wild-caught fish

- ➢ resulted in less cohesive,
- ➢ more aggressive,
- ➢ more active groups (Suriyampola et al., 2017).

- Auditory enrichment (classical music)

- ➢ Led to less anxious,
- ➢ less active
- ➢ no effect on whole-body cortisol (Barcellos et al., 2018)

Environmental Enrichment

- Plastic plants,
- marbles,
- mesh strips,
- PVC pipe,
- various images,
- mirrored paper,
- sight of conspecifics

- Fish preferred mirrored paper and
- sight of conspecifics (Krueger et al., 2020)

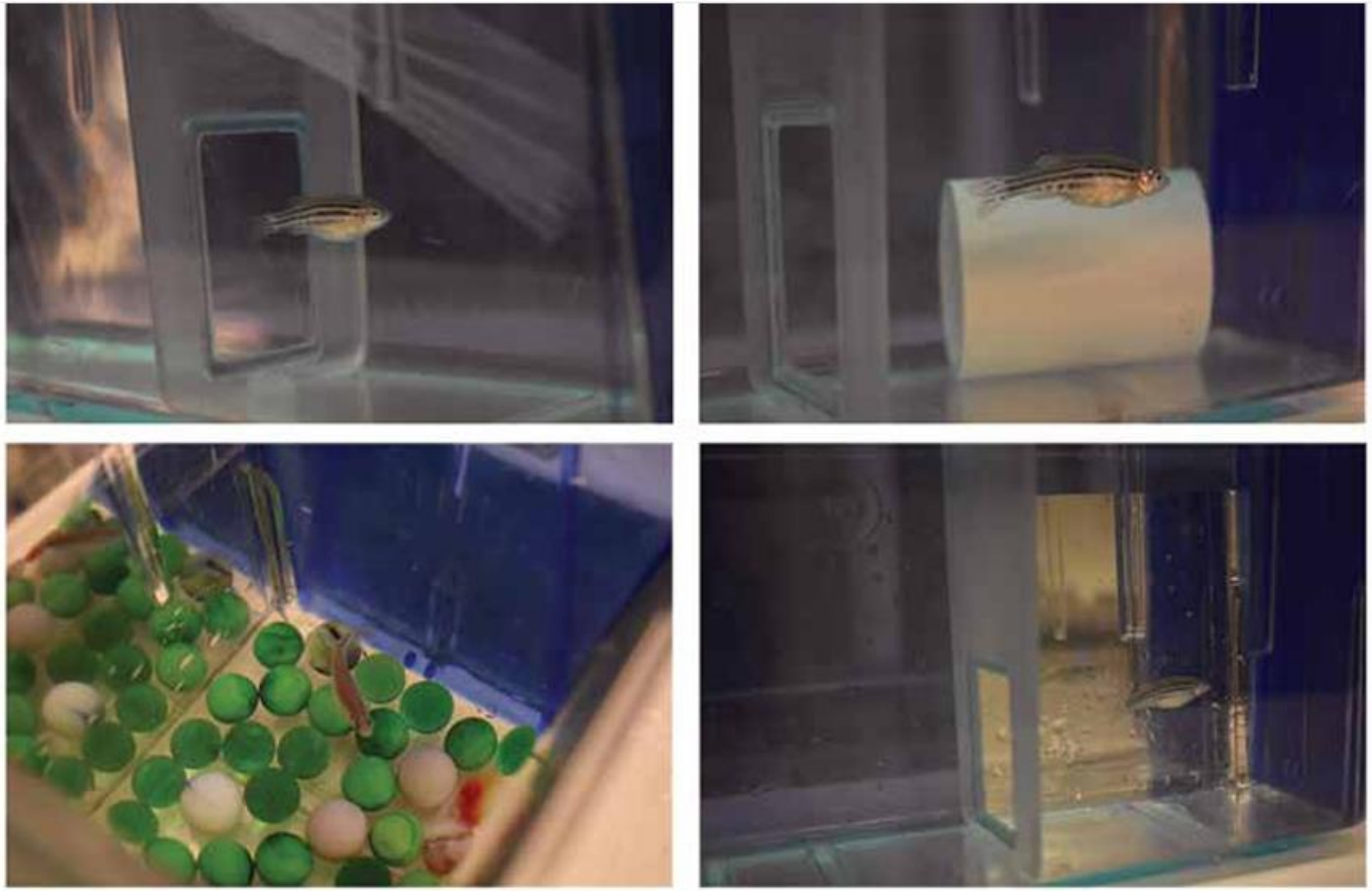
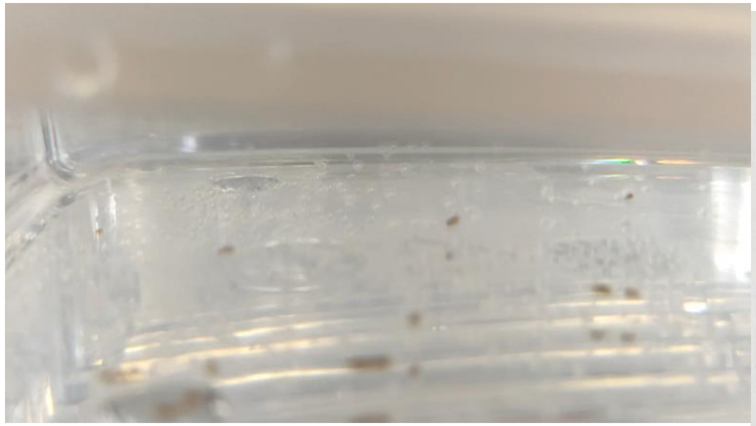


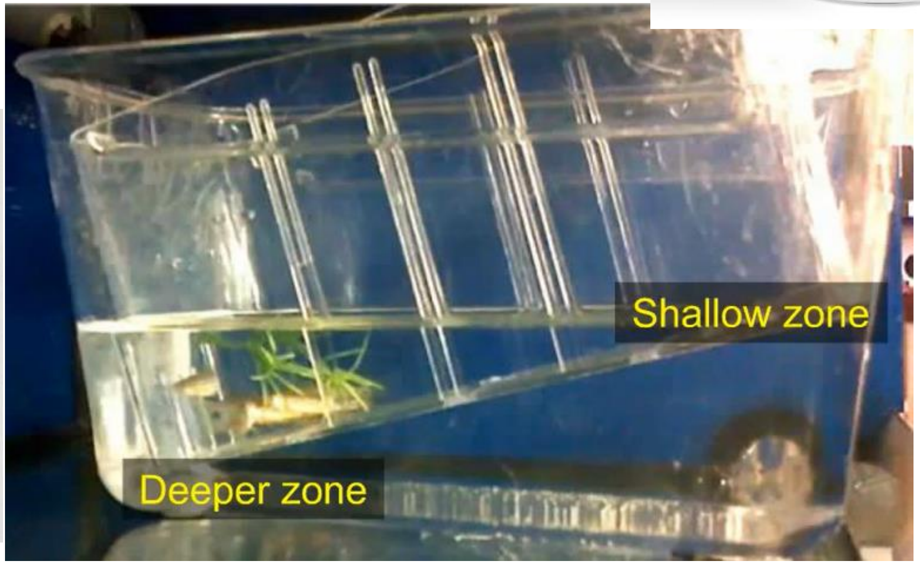
Figure 2. Examples of the inanimate enrichment items evaluated. Clockwise from top left: tulle, PVC pipe, mirrored paper, and marbles.

Breeding of Zebrafish

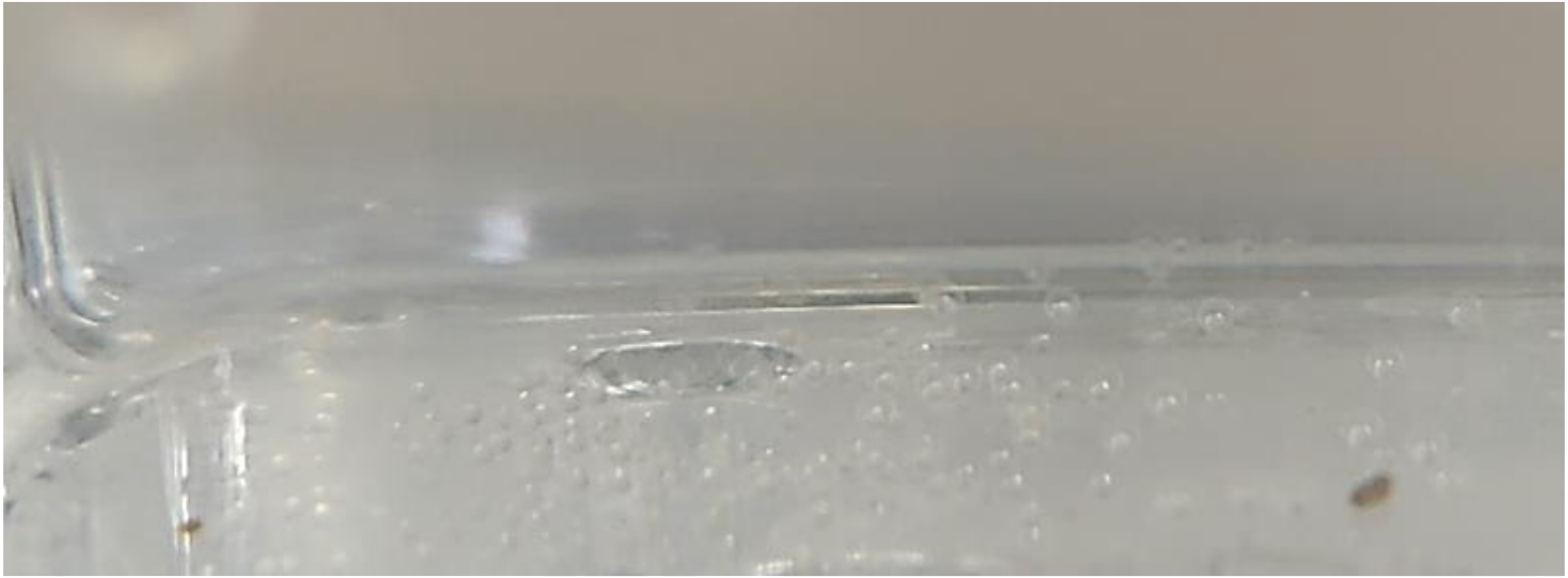
mass spawning device

- **Pairwise breeding**: Transfer **one female and one male** (or other sex proportions) **separated to opposite sides** of the breeding tank, **from the evening before**
- Zebrafish **initiate breeding at dawn**, so the **divider is removed the next morning** shortly after the onset of light.
- **Allow mating for 20 min** so that fertilization can occur and **sufficient number of embryos are laid at the bottom** of the tank





Christian Lawrence et al., 2012



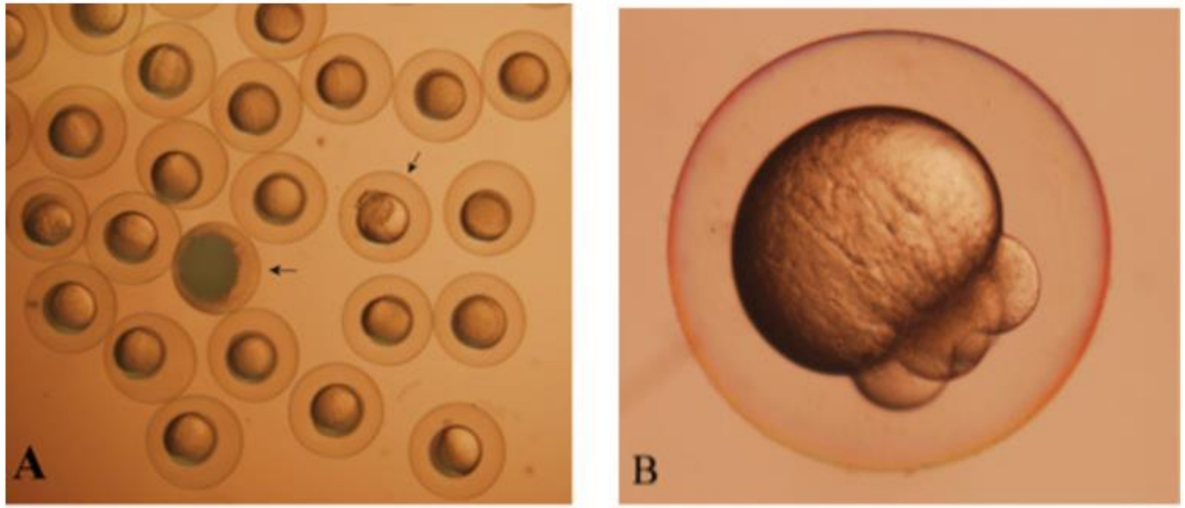
Avoid repeated inbreeding from sibling matings.
Keep records of the dates of fertilisation, the number of fish and key genetic information (transgenesis, mutation).
Follow recommendations by the zebrafish nomenclature committee (see Zebrafish Information Network (ZFIN).

Breeding of Zebrafish

- After 20 min, return the fish to their tanks. **Collect the eggs using a strainer**
- Wash the embryos thoroughly with system water.

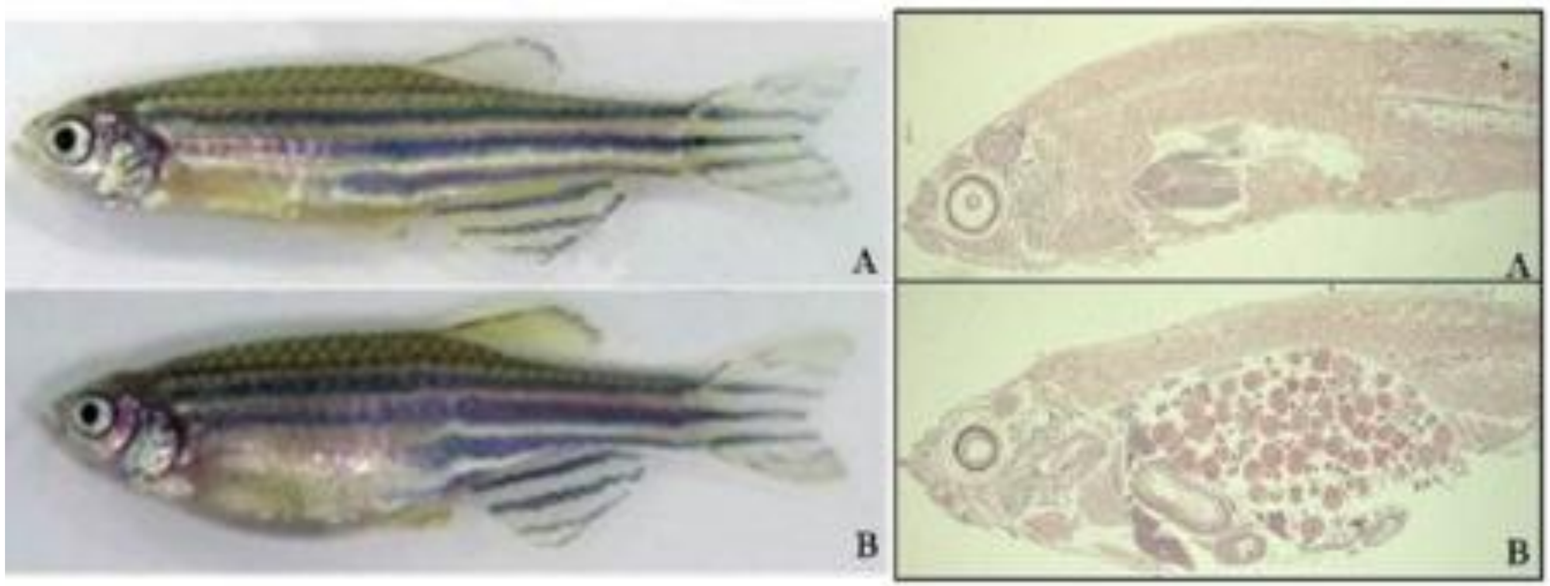
- **Transfer the embryos to a Petri dish or small tank by rinsing the strainer with embryo medium**; a.k.a. EM3 (NaCl, 13.7 mM; KCl, 0.54 mM; $MgSO_4$, 1.0 mM; $CaCl_2$, 1.3 mM; Na_2HPO_4, 0.025 mM; KH_2PO_4, 0.044 mM; $NaHCO_3$, 4.2 mM).

- Embryos can be observed under a **stereoscope**. **Fertilized eggs are then separated** from the unfertilized eggs using a needle and a pipette



Breeding of Zebrafish

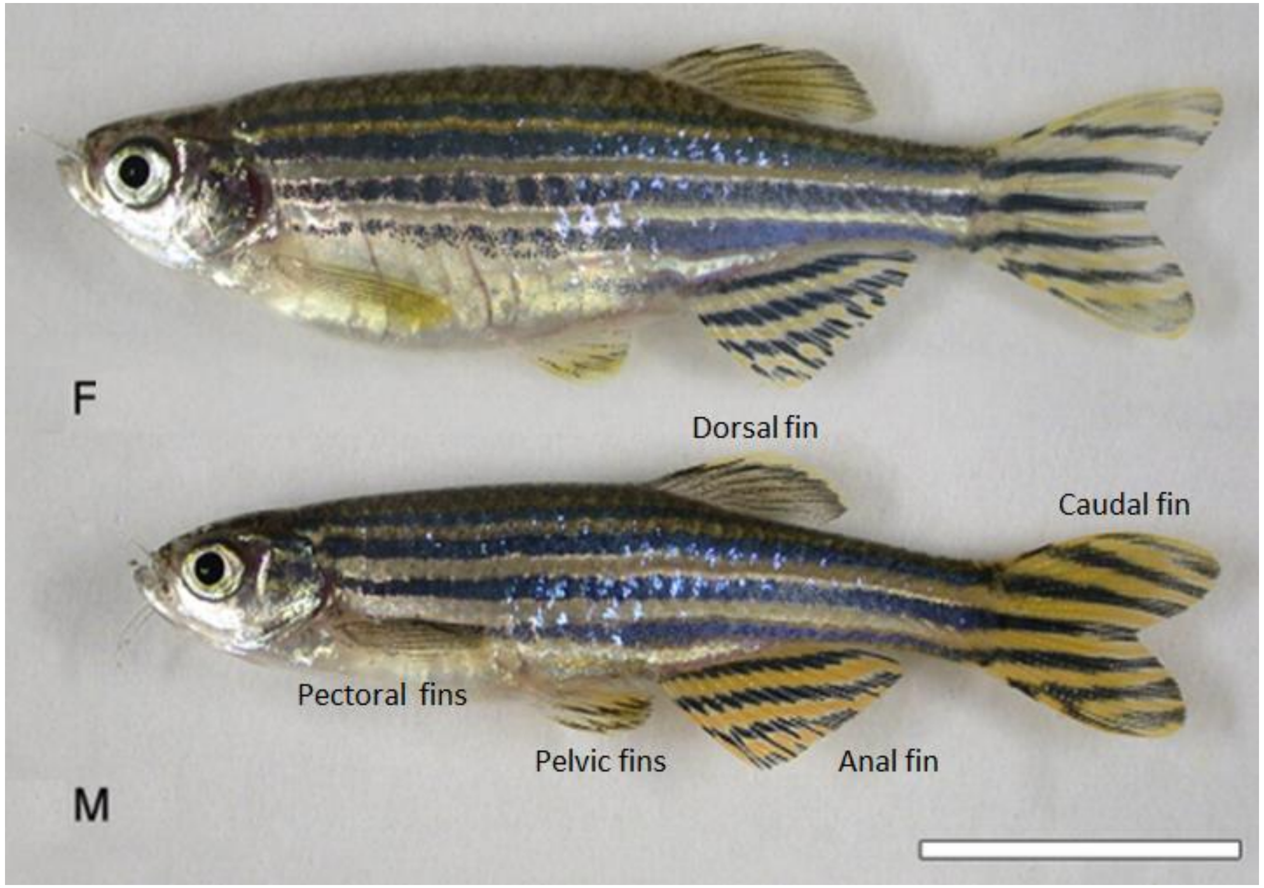
- **Females** can be distinguished from males because of their **bigger underbelly.**



Ricardo Lacava Bailone, et all, 2020

Breeding of Zebrafish

- **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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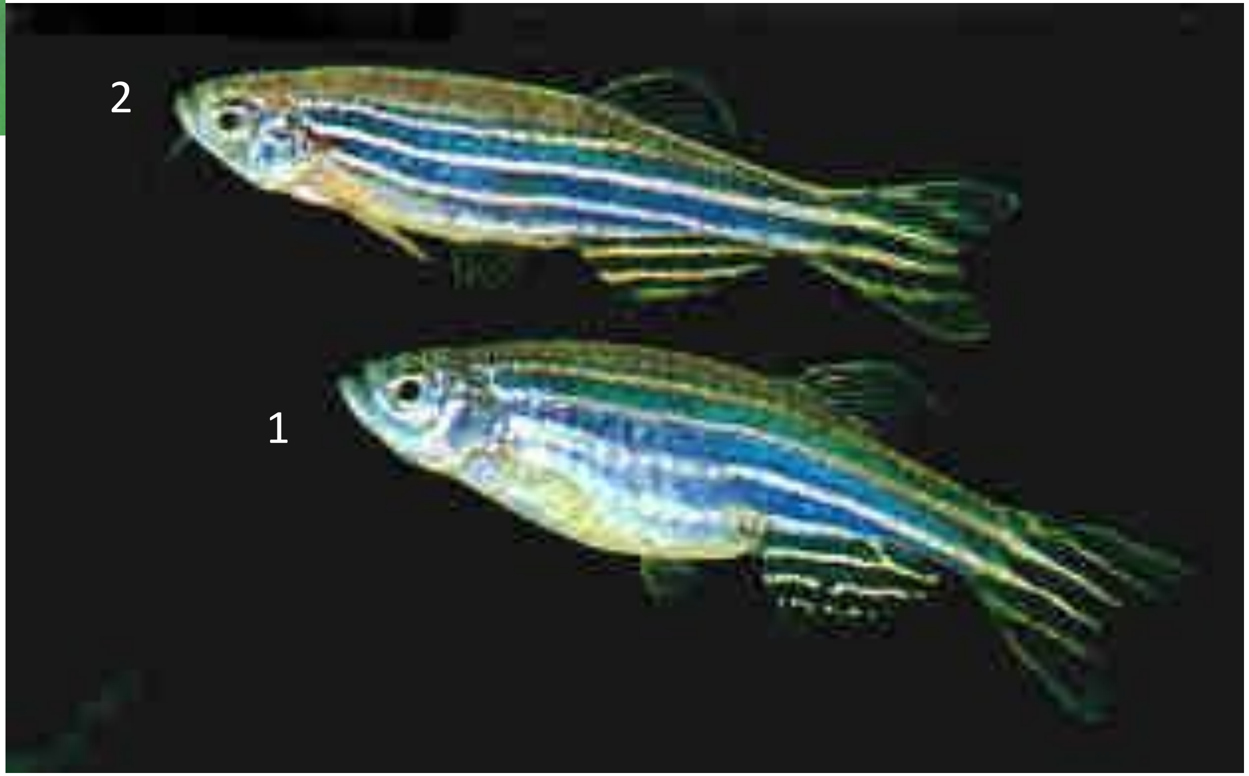
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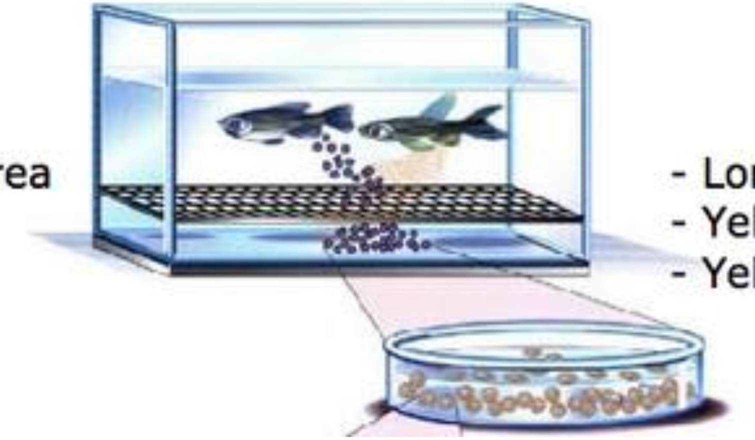
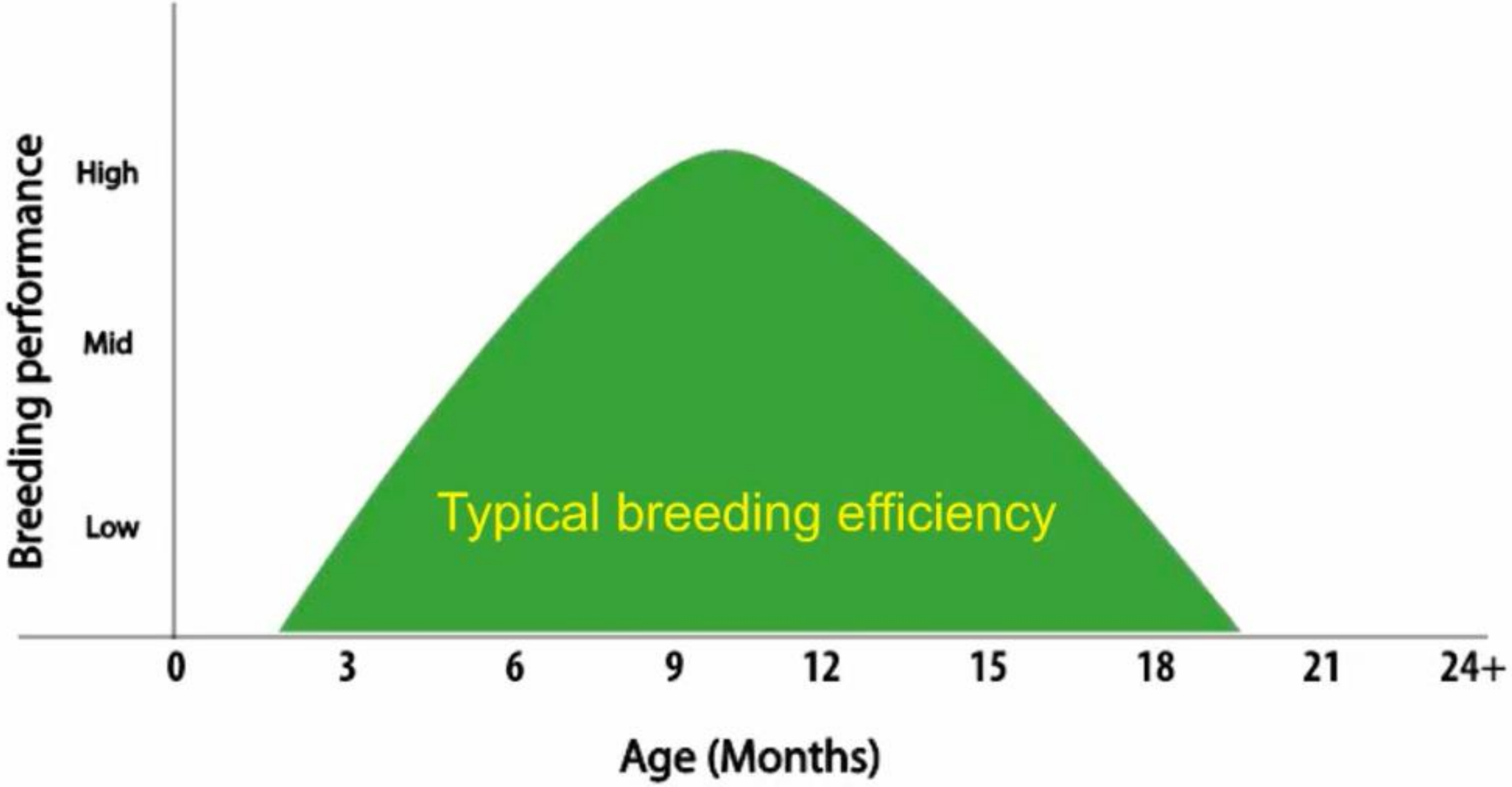
Female

- Wider body with big gut area
- Silver/pink tint
- Silver anal fin



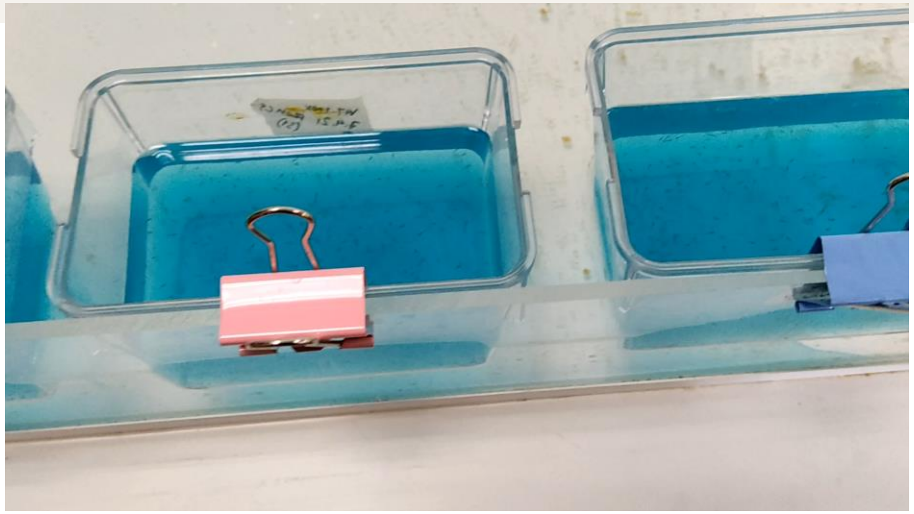
Male

- Long, narrow body
- Yellow/tan tint
- Yellow anal fin



Zebrafish developmental stages

- Embryos settle over the bottom of the Petri dish/tank
- **Fertilized eggs are kept in an incubator (~28.5±0.5°C) for 72 hrs until the larvae are hatched**.
- After hatching, larvae **attach either at the bottom of the tank or at the walls** (feeding from the **yolk salk**)
- At roughly **5 days post fertilisation (dpf)** the **swim bladder** has developed and allows the larvae to **swim freely** in the tank (external feeding)



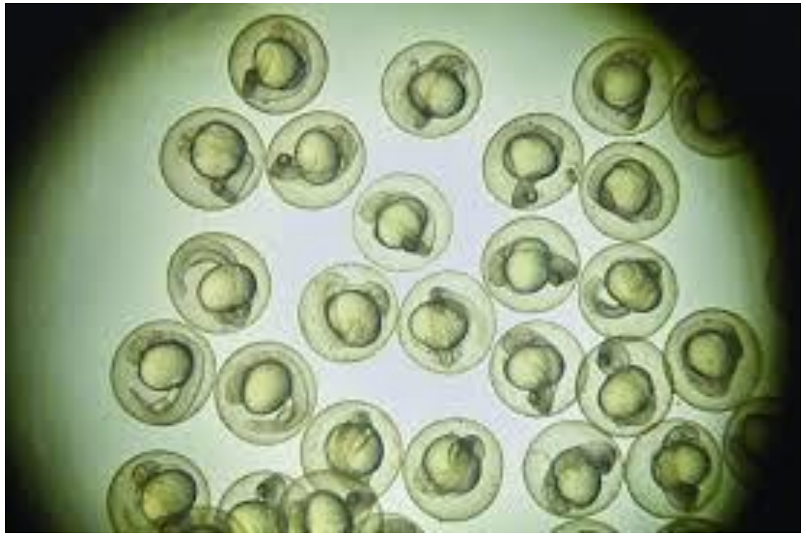
- **Water is changed on a daily basis**. The water change should include **removing dead or diseased larvae** and any other **debris** with a pipette, with **minimum disturbance**

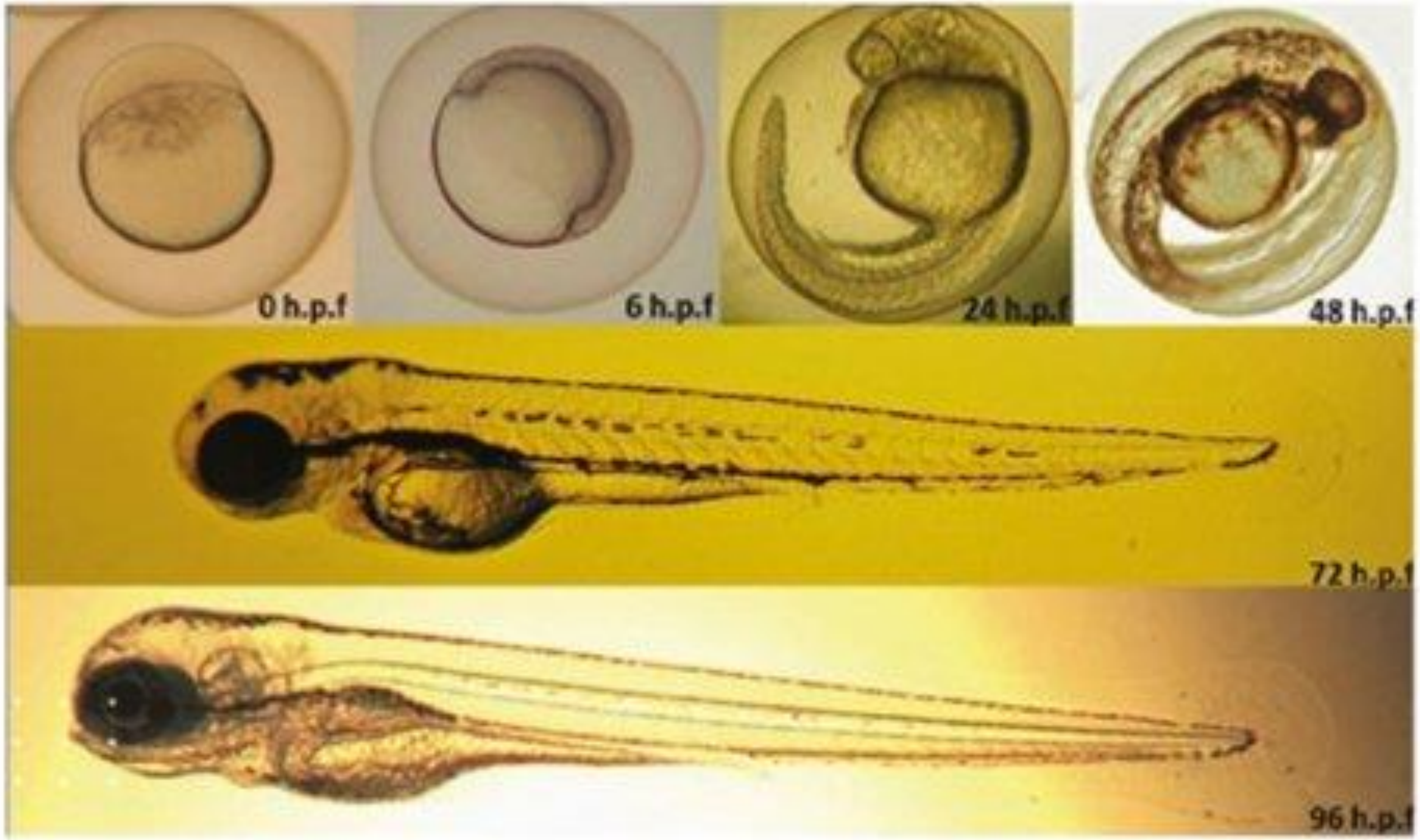
Incubator


TEMP
28.0°C
Set 28.0°C
TIMER
--h: --m
End --.--. --:--
MENU
LIGHT
12 %
15.04.2024 13:02
Final_2-12
Ramp 01
8:27:36
ALARM
min
-10.0°C
max
80.0°C
auto
±5.0 K
GRAPH
eco
PELTIER TECHNOLOGY
ON
OFF

Larval culture

- **After 14 days**, larvae can be shelved into the system, and supplied with a small stream of cycling water (1-2 drops per second). As the larvae grow, water flow can be increased
- It **usually takes 3 months** for the embryos to develop into **sexually mature adults**

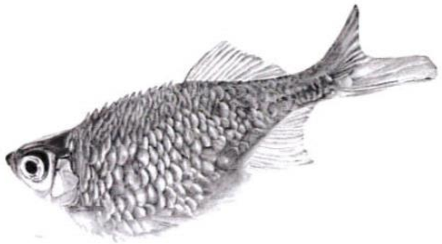




Ricardo Lacava Bailone, et all, 2020

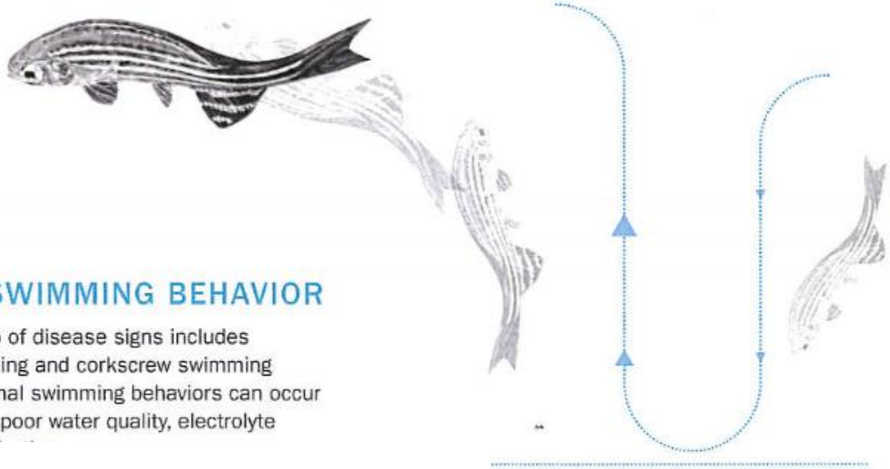
Health and welfare monitoring

Stress and welfare indicators



DROPSY

Fluid build-up in the skin causes the scales to stand upright. Along with fluid accumulation in the coelomic cavity (ascites), this can result from infection or other causes of osmotic imbalance.



ERRATIC SWIMMING BEHAVIOR

This broad group of disease signs includes shimmying, flashing and corkscrew swimming patterns. Abnormal swimming behaviors can occur due to irritation, poor water quality, electrolyte

SKIN DISCOLORATION

Hemorrhage, ulceration, white or dark spots, and darkening or lightening of the skin are common signs of infection in fish.

FRAYED FINS

Trauma, infection and poor water quality contribute to frayed and eroded fins.

EMACIATION

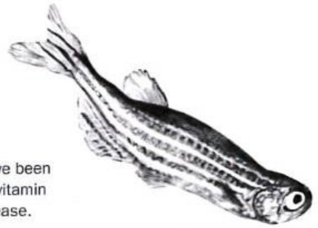
Chronically sick fish often exhibit wasting of muscle and fat reserves due to inappetence.

LETHARGY

Sick fish often express a low level of activity; in particular, a reluctance to feed or reduced avoidance behavior. The fish might also lie on the bottom of the tank.

EXOPHTHALMIA

Also known as "Pop Eye", this arises from systemic inflammation or a space occupying mass behind one or both eyes resulting in protrusion.



CURVATURE OF THE BACK

In fish, lordosis, kyphosis and scoliosis have been associated with developmental disorders, vitamin and mineral imbalance, and infectious disease.

Sanitation of equipment and hygiene

- A **clean environment is essential** for maintaining a high standard of animal health and welfare

- **Avoidance of cross contamination during routine husbandry procedures**, since many diseases can be spread through physical contact between individual fish, tanks and water systems.

- Any piece of **equipment** in physical contact with fish (nets, mating boxes, etc.) should be **dedicated to one specific system and sanitized periodically**

- **During chemical sanitization** (e.g. chlorine, ethanol) care needs to be taken (e.g. **sufficient rinsing with water**) to **avoid contaminating** the water with chemicals

Sanitation of equipment and hygiene

- **Equipment used for quarantine units needs to be isolated** from equipment used at the main facility to reduce cross contamination. As a general rule, staff, material and work movement should be carefully considered so as to reduce contamination.

- **Growth of biofilm on the water surface or algae needs to be monitored** and if any to be removed as it is a source of pathogens

- **Use of gloves and/or appropriate hand disinfection routines are important** in order to avoid cross contamination between fish populations and exposure of facility staff and researchers to **zoonotic** infections.

Fish handling

Safe fish handling is important from an animal **welfare** perspective but also from a **scientific standpoint**, as **fear and stress** responses can result in physiological changes that may :

- **contribute to data variability** and
- **can affect the number of animals needed to achieve statistically significant results**

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

- use of **anaesthesia** to minimize stress and pain
- minimize the **time** that you handle the fish
- Always use an appropriate **net** to catch the fish (size and mesh)

- Limit the time of **air exposure (max 30 seconds)**
- **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**.
- Wear **gloves**

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
- 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 optimal **dosage** and **exposure time**.

Anaesthetics

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

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, 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

Stages of anaesthesia and recovery

Before anaesthesia

3-5 min after addition of MS-222 into the water

After 1min in clean water

Stages of Anesthesia	**Description**
I	Loss of equilibrium
II	Loss of gross body movements but with continued opercular movements
III	As in Stage II with cessation of opercular movements
Stages of Recovery	
I	Body immobilized but opercular movements just starting
II	Regular opercular movements and gross body movements beginning
III	Equilibrium regained and preanesthetic appearance

From Iwama *et al.*, 1989

Euthanasia

The **principles** of euthanasia:

- **Whenever an animal's life is to be taken it should be treated with the highest respect**
- **Humane killing by anaesthetic overdose** can cause immediate unconsciousness and subsequent death (no pain or distress)
- **Require minimum restraint of the animal**
- **Be appropriate for the species, age and health of the animal**
- **Personnel should be trained and competent**

Directive 2010/63/EU

Animals-remarks/methods	Fish	Amphibians	Reptiles	Birds	Rodents	Rabbits	Dogs, cats, ferrets and foxes	Large mammals	Non-human primates
Anaesthetic overdose	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
Captive bolt	X	X	(2)	X	X		X		X
Carbon dioxide	X	X	X		(3)	X	X	X	X
Cervical dislocation	X	X	X	(4)	(5)	(6)	X	X	X
Concussion/percussive blow to the head				(7)	(8)	(9)	(10)	X	X
Decapitation	X	X	X	(11)	(12)	X	X	X	X
Electrical stunning	(13)	(13)	X	(13)	X	(13)	(13)	(13)	X
Inert gases (Ar, N_2)	X	X	X			X	X	(14)	X
Shooting with a free bullet with appropriate rifles, guns and ammunition	X	X	(15)	X	X	X	(16)	(15)	X

Requirements

1. Shall, where appropriate, be used with prior sedation.
13. Specialised equipment required.

Mocho, J.-P.; von Krogh, K.A FELASA Working Group Survey on Fish Species Used for Research, Methods of Euthanasia, Health Monitoring, and Biosecurity in Europe, North America, and Oceania. Biology 2022, 11, 1259. https://doi.org/10.3390/biology11091259

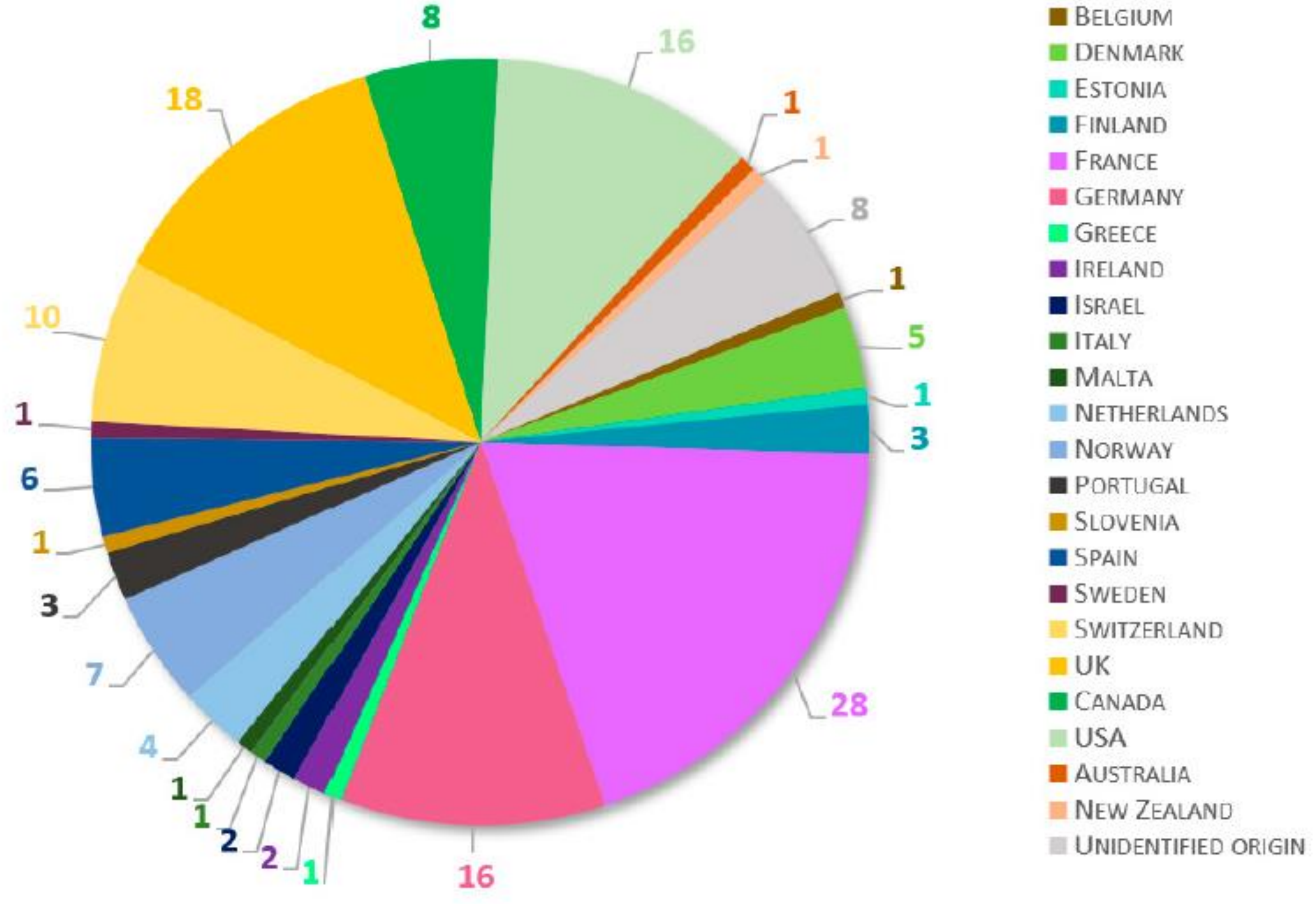
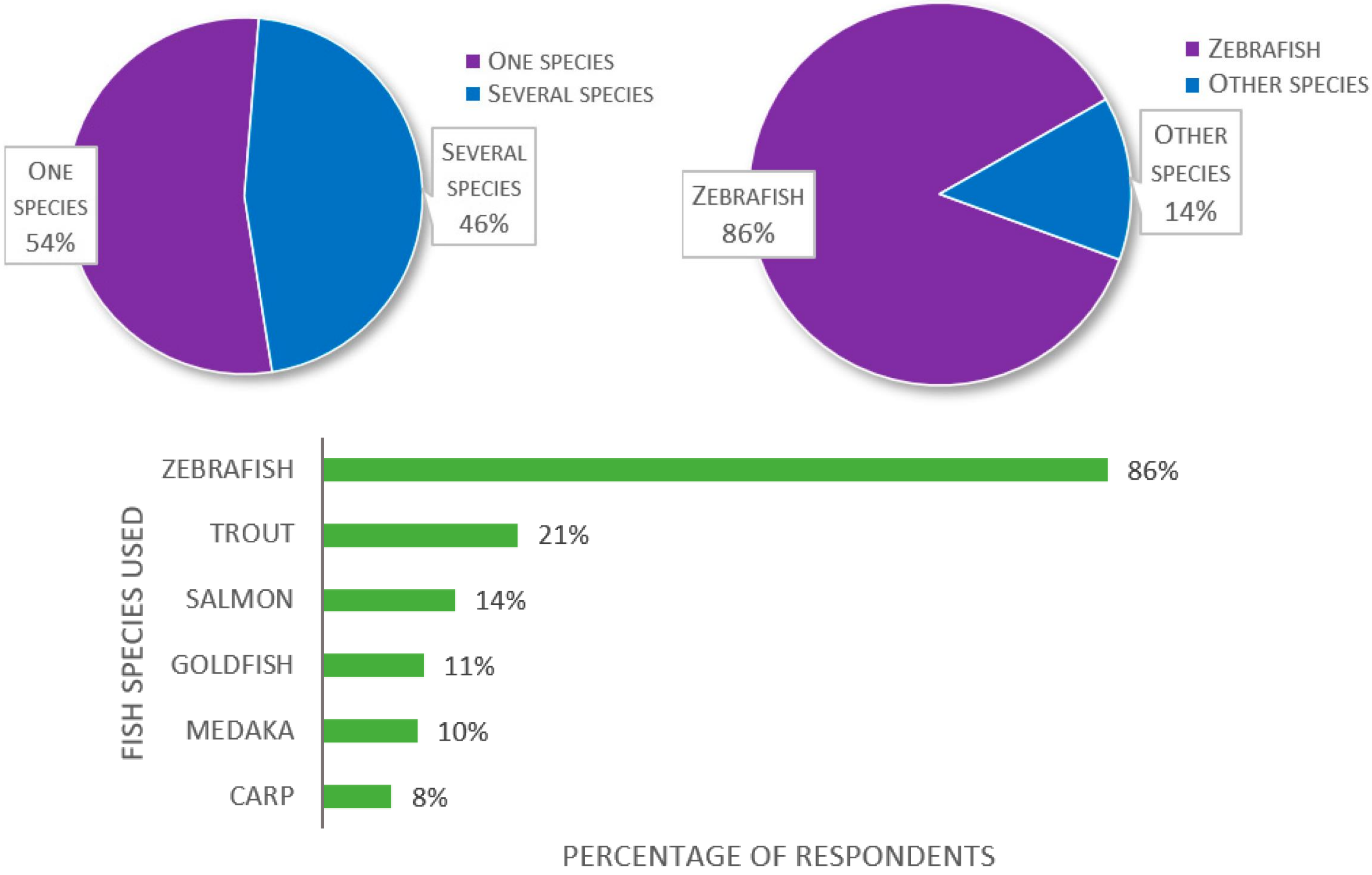
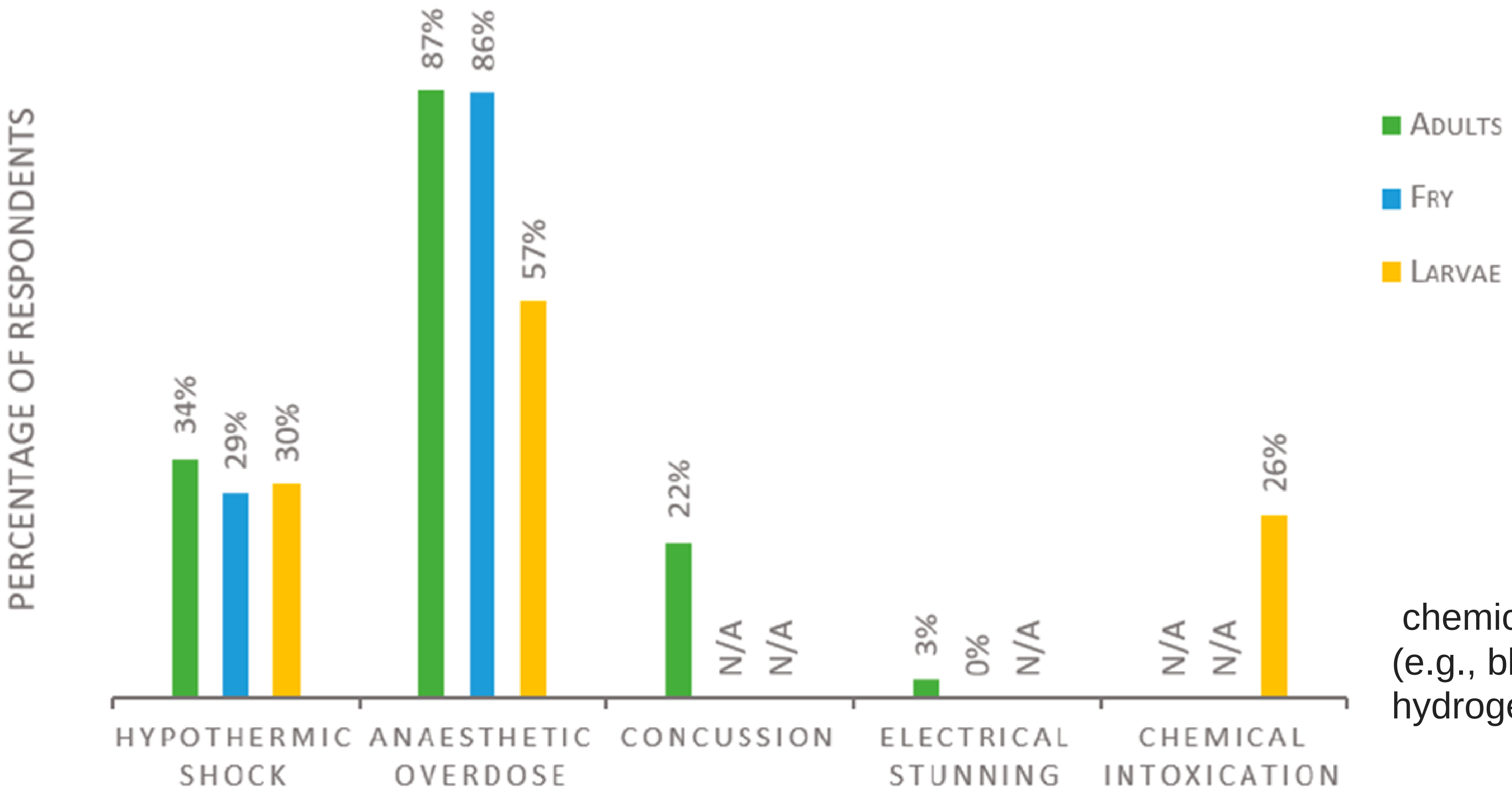


Figure 1. Geographical distribution of laboratories participating in the survey. Total number of contributing laboratories was 145. Eight participants did not declare the location of their facility ("Unidentified origin").



One species
Several species
One species 54%
Several species 46%
Zebrafish
Other species
Zebrafish 86%
Other species 14%
Zebrafish 86%
Trout 21%
Salmon 14%
Goldfish 11%
Medaka 10%
Carp 8%
Fish species used
Percentage of respondents



chemical intoxication (e.g., bleach, ethanol, hydrogen peroxide).

Figure 3. Methods for killing/euthanasia used in fish laboratories according to the developmental stage of the fish, presented as percentage of respondents. Number of respondents was 143, 140, and 138 for adults, fry, and larvae, respectively. Each respondent was allowed to enter multiple answers. N/A; this alternative was not asked for in the survey.

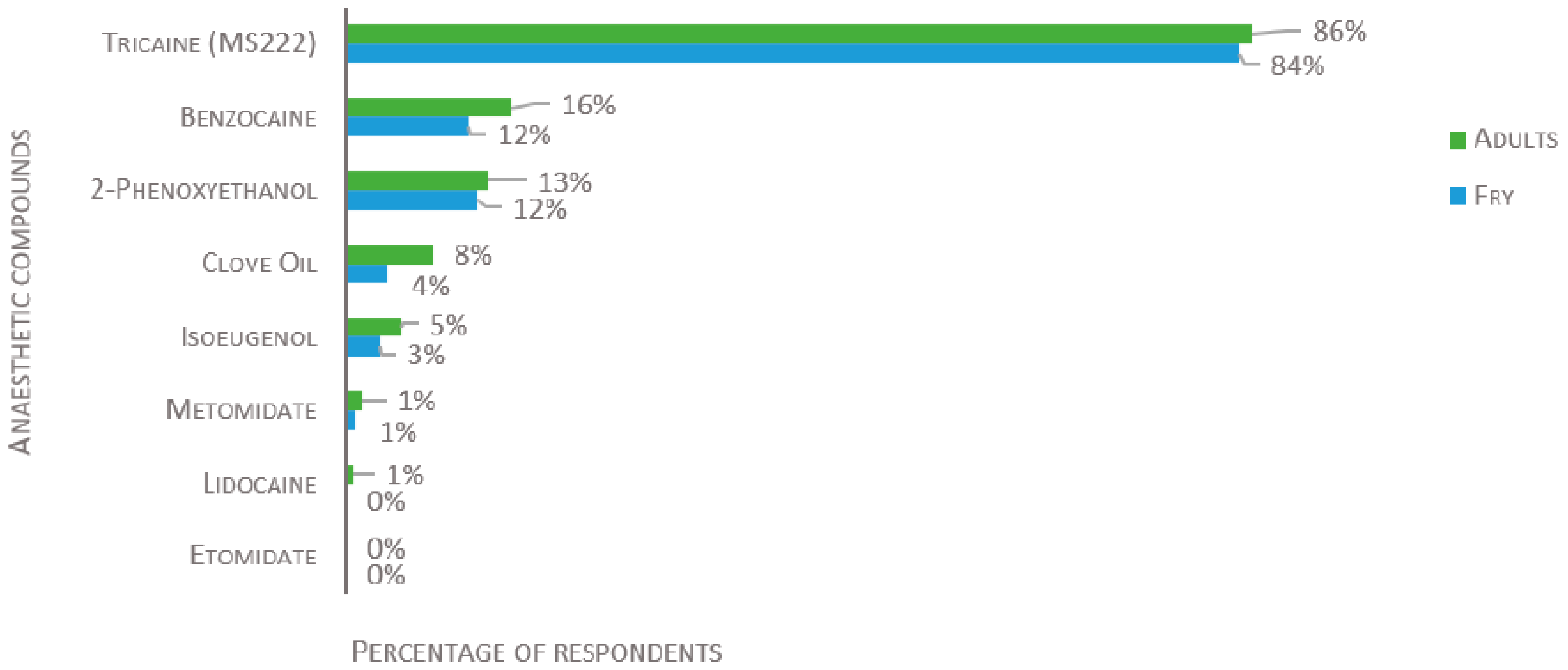


Figure 4. Compounds used to induce an overdose of anesthesia in adults and fry, presented as percentage of respondents (n = 134 for adults and 129 for fry). Each respondent was allowed to select multiple answers.

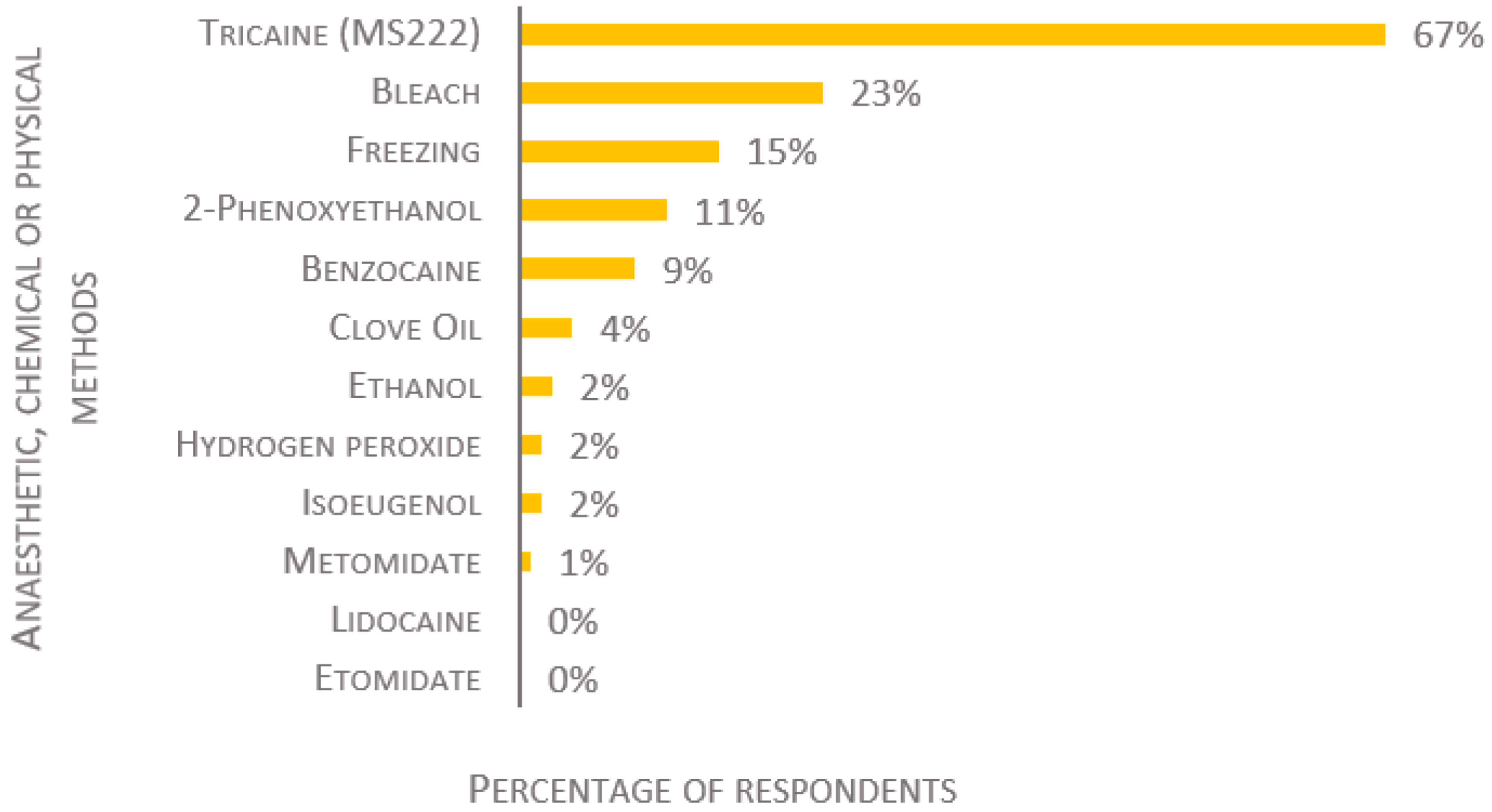


Figure 5. Anesthetic, chemical, and physical methods used to kill larvae, presented as percentage of respondents (n = 124). Each respondent was allowed to select multiple answers.

Conclusions

- **Given the growing importance of the zebrafish as a research model system, it is imperative that standards for its husbandry be developed and applied.**

- **In a laboratory setting the aim is to maintain zebrafish in a 24/7 controlled environment mimicking natural conditions.**

- **Parameters for well-being are reproductive success, growth and the absence of signs of illness or excessive stress.**

University of Crete
Dept. of Biology

Thank you !!!