Zebrafish (*Danio rerio*) as model system

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Zebrafish taxonomy

Euteleostomi (bony vertebrate, like human)

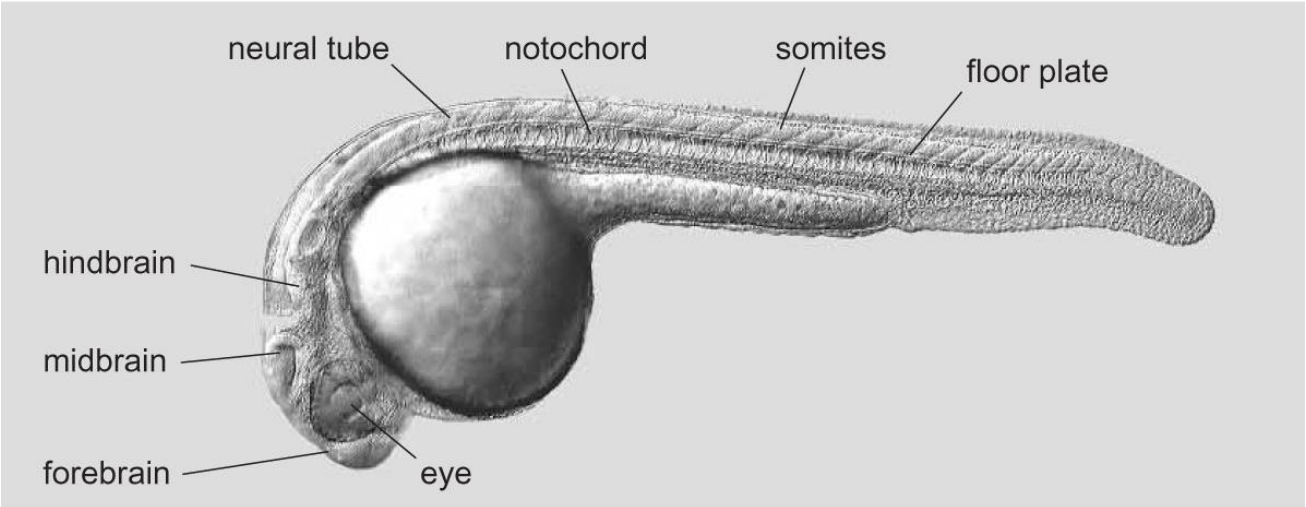
Actinopterygii (ray-finned fish, „Strahlenflosser“, tetrapods belong to the clade of lobe-finned fish „Fleischflosser“)

Teleost („echte Knochenfische“)

Cyprinidae („Karpfenfische“)

Zebrafish as model system

representative of its animal group	good
relevance for humans	good
many progeny	excellent
progeny all year round	excellent
fast generation time	OK
easy to house/culture	good
small (but not too small)	good
cheap	good
fast embryonic development	excellent
external development	excellent
transparency (imaging!)	excellent
accessible for embryological techniques (transplantations)	good
diploid (or haploid): ability to identify mutants	good
small genome	OK
inbred lines	poor
forward genetics	excellent
reverse genetics	excellent
RNAi	?
transgenesis	excellent
pluripotent stem cell culture (ES cells)	NO
knock-ins/outs	in development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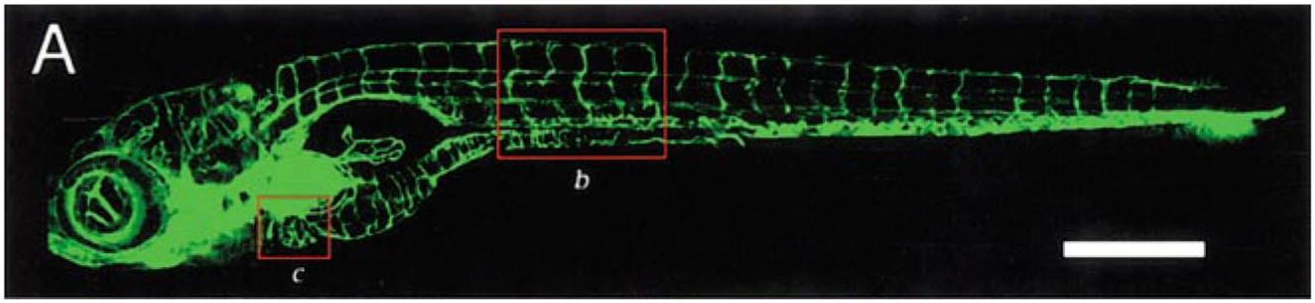
forebrain
eye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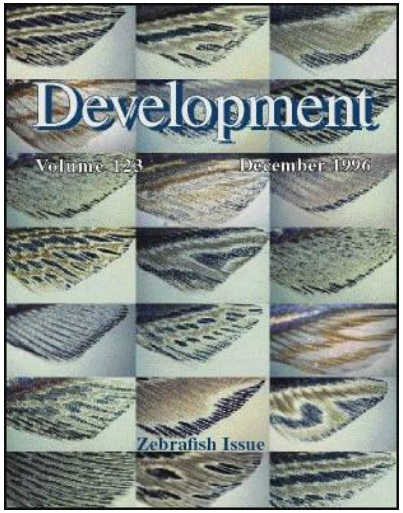
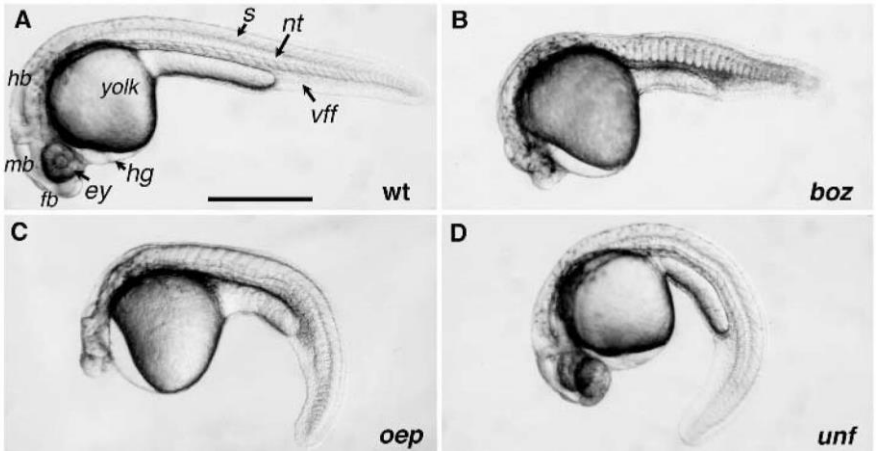

Development
Volume 123
December 1996
Zebrafish Issue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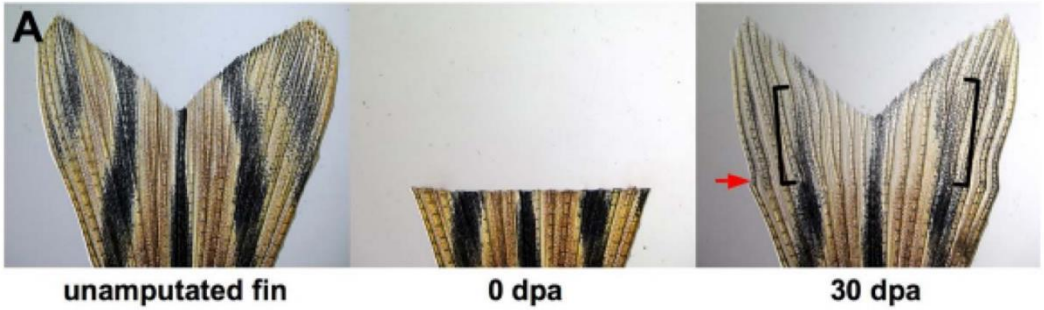
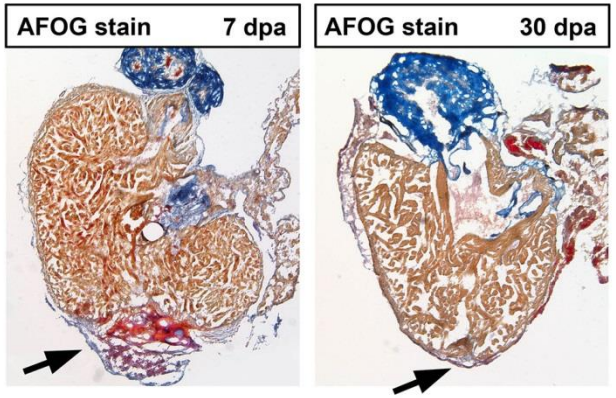
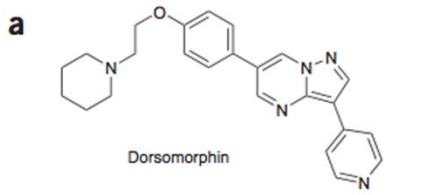
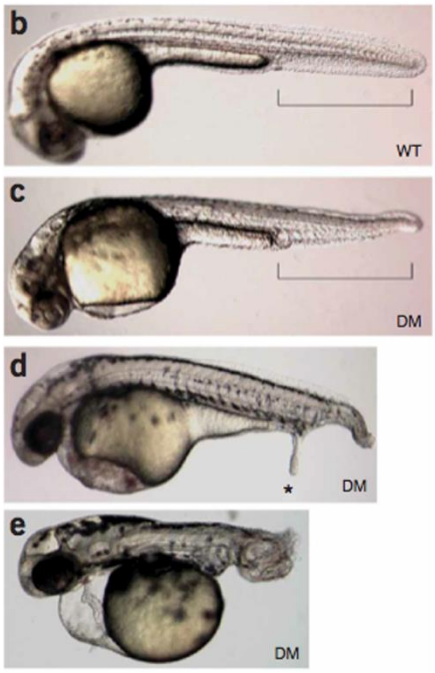
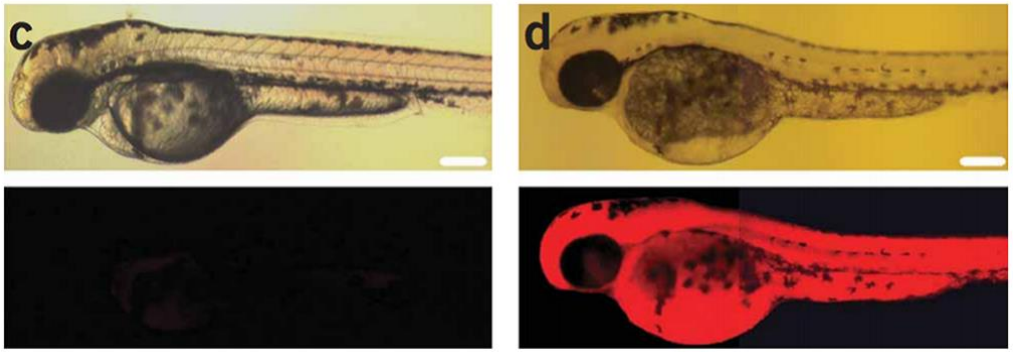










Transgenic mercury probe.

Yang, Y. K.; Ko, S. K.; Shin, I.; Tae, J.Nat. Protoc.2007, 2, 1740

Habitat

- freshwater fish
- indigenous to South Asia (India, Bangladesh, Nepal, Myanmar, Pakistan)
- prefer shallow, slow-flowing or still water (ponds)
- in the wild found at 16.5 to 33 °C, slightly alkaline pH (7.9-8.2)
- form shoals
- life-span in the wild unclear

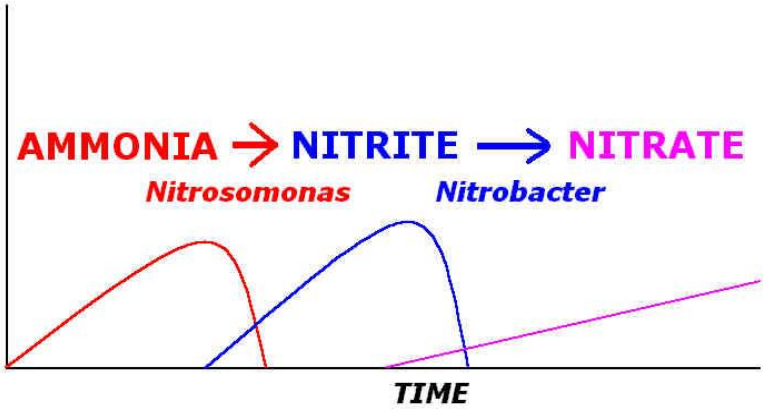
Housing in the lab

- re-circulating freshwater systems (5-10% water exchange per day)
- reverse osmosis water (RO) + defined amount of salts
  OR: mixture of tap water (in Germany!) + RO water + salts

Water parameters

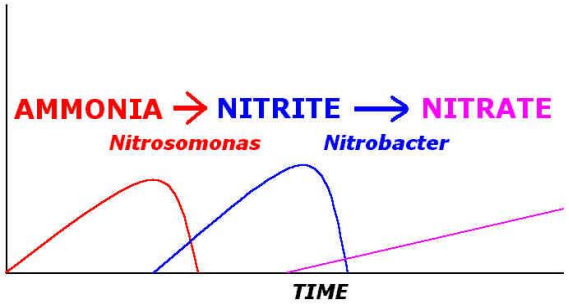
Parameter	Represents	Target	Comments	Controlled by
Conductivity	Total ion concentration	200 – 1000 µS Weidinger: 350µS (Siemens)	Higher might help to reduce energetic cost (osmotic balance); but not good to use salts that contain a lot of NaCl	Addition of $CaCO_3$ & $MgCO_3$ + trace elements And/or „Red Sea Salt“, „Instant Ocean“ or the like
Total hardness (GH)	All multivalent ions, particular Ca^{2+} and Mg^{2+}	5 ° dH	Too little causes bone and other defects	
Carbonate (temporary) hardness (KH)	Bicarbonate (HCO_3^-)	3 ° dH	Too much causes limescale	
Copper		0	Is toxic	No use of copper pipes!
Phosphate (PO4)		< 5 mg/l	Fish don‘t care much, but high concentrations favor algae growth	Amount and type of food.
pH		7 - 8 Weidinger: 7.3		Sodium bicarbonate (usually pH needs to be brought up)
Temperature		24 – 30 °C (28.5°C) Weidinger: 27°C	Compromise between fast growth and bearable climate in room.	Air temperature needs to be similar to minimize evaporation.
Oxygen		At saturation: 7.8 mg/l at 28°C		Water flow, recirculation of water in storage tank

Nitrogen cycle



N excreted by fish (urine, feces) ends up in the water. Needs to be dealt with. Bacteria in the filters metabolize it.

Nitrogen cycle



Formula	English	Deutsch	relevance	Target	Comment
NH_3	Ammonia	Ammoniak	Very toxic	Cannot be measured	Should not accumulate, since it converts to NH_4^+ But: at high pH it might accumulate!
NH_4^+	Ammonium	Ammonium	harmless	< 0.02 mg/l	Harmless, but should not accumulate if bacteria-mediated nitrogen cycle works
NO_2	Nitrite	Nitrit	Toxic	< 10 mg/l	
NO_3	Nitrate	Nitrat	Rel. harmless	< 50 mg/l	Remove by water exchange

Disease

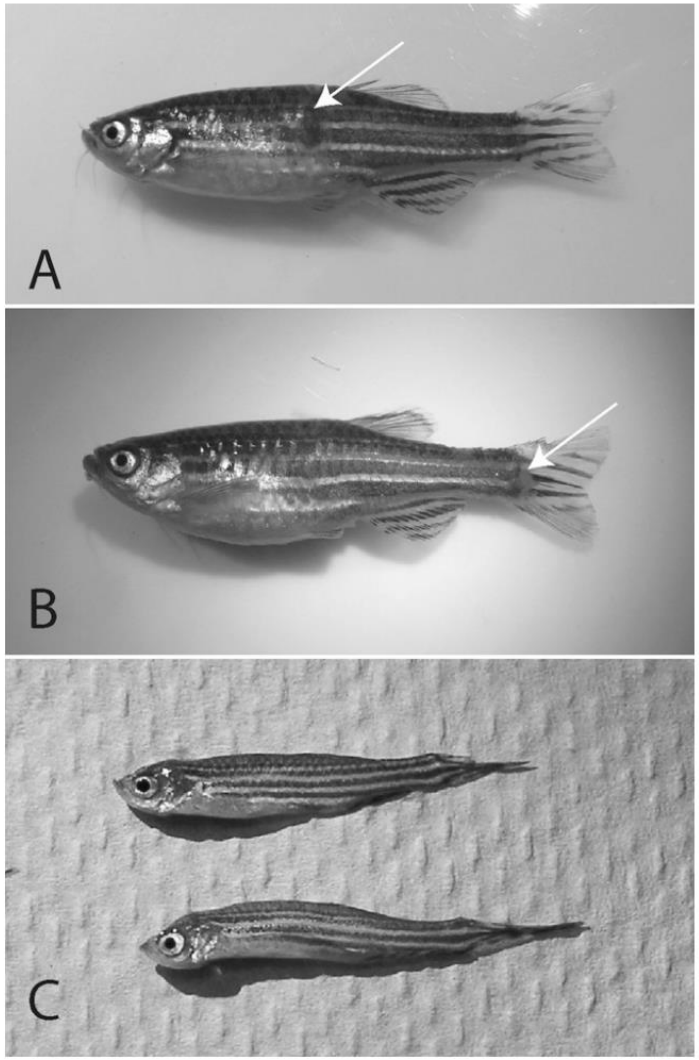


Figure 1 (**A, B**) External lesions (arrows) associated with *Mycobacterium marinum* infection in zebrafish. (**C**) Severe emaciation associated with *Mycobacterium haemophilum* infection.

Table 1 ***Mycobacterium*** **species known to infect zebrafish in research facilities**

Species	Source
Mycobacterium abscessus	Astrofsky et al. (2000); Watral and Kent (2007)
Mycobacterium chelonae	Astrofsky et al. (2000); Kent et al. (2004); Whipps et al. (2008)
Mycobacterium chelonae–like	Kent et al. (2004); Whipps et al. (2007a)
Mycobacterium fortuitum	Astrofsky et al. (2000)
Mycobacterium haemophilum	Whipps et al. (2007b)
Mycobacterium marinum	Watral and Kent (2007)
Mycobacterium peregrinum	Kent et al. (2004)

Whipps et al. (2012), ILAR 53, 85-105.

Hygiene

Recirculating water is **filtered** and **sterilized**

1. debris is allowed to settle in sump
2. water is coarsely filtered through filter mats (which also contain bacteria)
3. water is fine filtered in pressurized filters
4. water is UV sterilized

Hygiene

Cleanliness

1. feces and left-over food is removed from bottom of tanks
2. tank surfaces and lids are kept clean
3. removable tanks are washed (dishwasher) regularily
4. NO plants (real or artificial) are used
5. snails can be used to manage algae

Health management

Monitoring

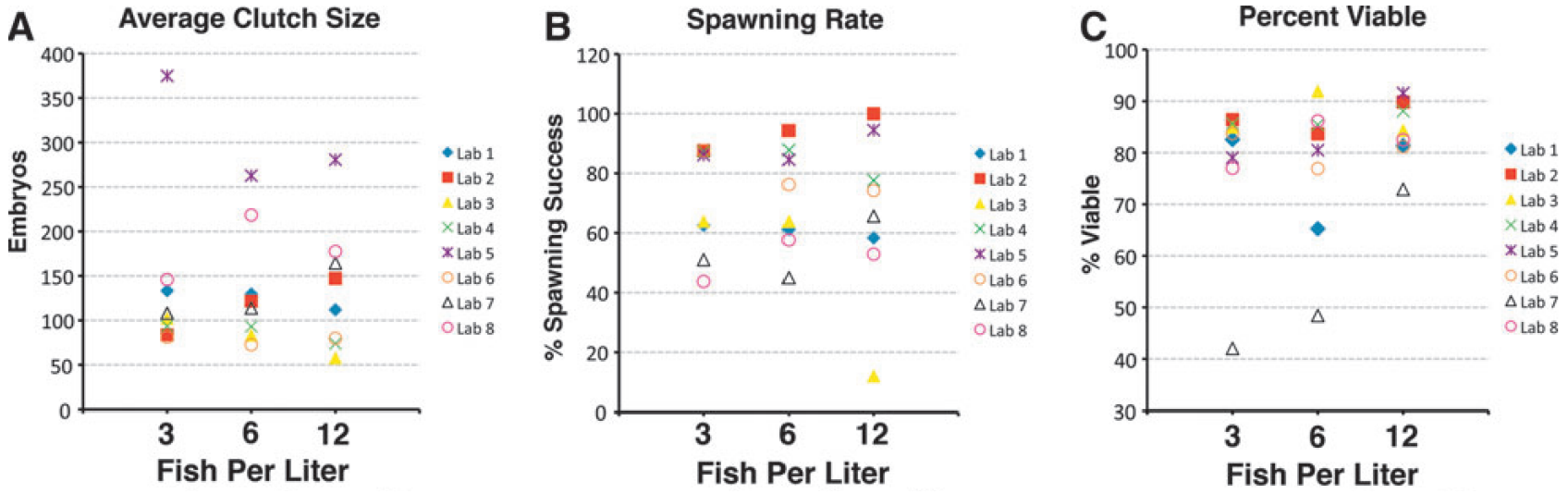
- fish status is monitored 2x daily
- dead or sick fish are removed (euthanised) immediately
- sick fish are submitted to pathology services (ZIRC, Oregon)

Precautions

- **stress is kept at a minimum** (water parameters, nutrition, density)
- fish are never imported into main facility > quarantene > only embryos are transfered
- old fish are euthanized (> 2 years)
- embryos are bleached to remove parasites from chorions
- no street shoes and/or disinfection mats

Stocking density

- Zebrafish are social, form shoals > don‘t like to be kept individually for long periods of time
- Zebrafish diplay social order, in particular males. 2 males kept together will fight > avoid that, rather keep individually or in larger groups.



Castranova et al., Zebrafish 2011.

- Fish well-being as measured by reproductive success is not adversely affected by high stocking density (12 fish per liter).
  Weidinger: Usually 5/liter, permit to use 7/liter.

Food

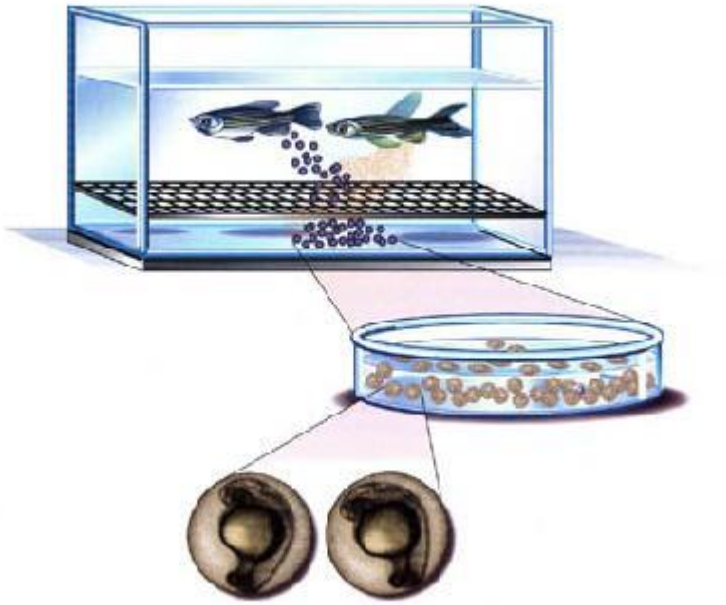
- Fish dry food flakes (eg. Tetramin)
  must be refrigerated and administered dry.
- Live food: artemia brine shrimp.
  purchased as cysts. Hatch within 48h in aerated high salt water.
- Adults: 1-3 times a day. Can easily survive for 7 days without food.

Breeding

- day-night cycle (12-14 h day, 10-12 h night)
- fish spawn in the morning (till noon)
- male + female must spend the night together
- rel. small space (1 l per pair)
- they eat their progeny!

variations:

- timed egg lay via separation of male + female
- mass-spawning: eg. 4 males + 4 females
- in vitro fertilization



Carlos Manuel Díaz

Larval rearing

- Embryos/larvae survive on yolk for 5 days.
- Day 5 – 12: Continuous food supply is best, no or very slow water flow.
- Different foods based on size of larvae:
  paramecia (easily cultured protozoa) OR rotifers (Protostomia)
  dry foods of increasing grain size, as often as possible

Growth rate depends heavily on nutrition and stocking density.
(Weidinger: 10 larvae/liter)

Sexual maturity can be reached within 5 weeks, usually within 2.5 months.

Anesthesia

MS222 (**tricaine** methanesulfonate, Ethyl 3-aminobenzoate)

- inhibits sodium ion channels
- acts systemically in fish
- widely used in aquaculture, large safety margin
  (EC50 up to 50 times higher than dose for anethesia, dpends on species)
- is rapidly taken up via gills
- only approved anethetic in USA and Germany

- use at 0.02%
- in egg or adult fish water (E3 usually for embryos)
- can reduce pH > adjust with NaOH to 7
  (Weidinger: 25x Tricaine stock in 20mM Tris pH 7)

Anesthesia

Induction

- add fish to beaker or petridish containing MS222
- **level 1:** light sedation
  reaction to visual and tactile stimuli reduced
- **level 2:** deep sedation
  *no reaction to visual and tactile stimuli, **reduced** opercular movement*
- **level 3:** partial loss of equilibrium
  *erratic swimming, **increased** opercular movement, still reaction to pressure*
- **level 4:** loss of equilibrium
  *no movement, **reduced** opercular movement, no reflexes*
  stage for surgical interventions
- **level 5:** shallow opercular movement, decreased heart rate
- **level 6:** no opercular movement > will soon lead to death

Matthews, M. & Varga, Z. M. Anesthesia and euthanasia in zebrafish. *ILAR journal / National Research Council, Institute of Laboratory Animal Resources* **53**, 192-204, (2012).

Anesthesia

Maintenance

- it‘s OK if adult fish reach stage 5 for a few minutes > all recover
- for prolonged anethesia: perfusion (water flow through mouth over gills)

- short interventions (< 1min): fish on glass or plastic surface
- longer interventions: put fish on damp sponge

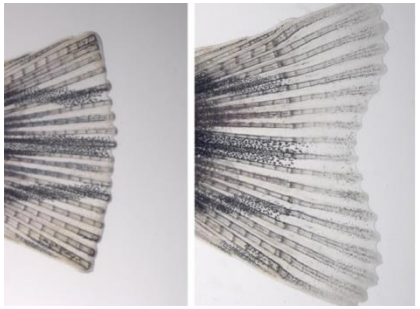
Recovery

- transfer fish to large volume of embryo or fish water
- monitor: if adult fish has not recovered (begun to swim) within 3 minutes > use tranfer pipette to blow water over gills

Common surgical interventions

Partial fin amputation

- caudal fin is amputated at 50% of its length with scalpel
- bleeding stops within seconds
- fish behavior (swimming, feeding, mating) is not impaired
- fin regenerates within 2-3 weeks



Intraperitoneal & retroorbital injection

Heart injury

- ventricular resection
- cryoinjury

Common features

- wounds are small > no wound care necessary
- infections are extremely rare > no sterile environment necessary
- **Precautions:** Isolation, addition of methylene blue (suppresses fungal and bacterial growth), addition of STRESS COAT®, which forms synthetic slime coating

Humane killing

MS222 overdose

- 0.2% in buffered fish water
- dead when operculum movement has stopped for > 5 minutes

Rapid cooling

- icewater (no chunks of ice which could burn skin)
- shown to be faster & less stressful (fewer signs of distress) than MS222
  (J Am Assoc Lab Anim Sci. 2009 Nov;48(6):785-9.)
- illegal in EU

Resources

Zebrafish International Resource Center (ZIRC), University of Oregon

protocols for husbandry, pathology services, source for wild-type and transgenic / mutant fish lines

European Zebrafish Resource Center, Karlsruhe Institute for Technology, ezrc.kit.edu

European repository for fish lines, screening facility

Zebrafish model organism database (ZFIN). zfin.org

Info on fish lines (transgenic, mutant), research reagents (antibodies, morpholinos), genome annotation

Zebrafish husbandry organisation. zhaonline.org

Non-profit, promotes husbandry standards through education & research

European Society for Fish Models in Biology and Medicine (EuFishBioMed)

promotes collaboration and exchange between fish labs