PIL ABK Fish bolt-on

Paul Schroeder BSc (Biol.), MSc (Fish Sc.), DVM, CertVEPH, PhD, MIFM, MRCVS

01/04/25

AGENDA

Bolt-on Fish Course
Programme

09.00 Welcome & introductions
09.15 Intro to fish biology (all species)

10.25 Comfort break

10.40 Husbandry, biosecurity & disease prevention

11.50 Comfort break

12.05 Anaesthesia & Schedule 1
12.35 Minor procedures & severity assessment
12.55 Intro to fish welfare & refinement

13.30 Lunch break

Assessments
Marine fish assessment (20 mins)
Freshwater fish assessment (20 mins)
Zebrafish assessment (20 mins)

PIL bolt-on Fish

Introduction to fish biology and the most popular research species

Fishes

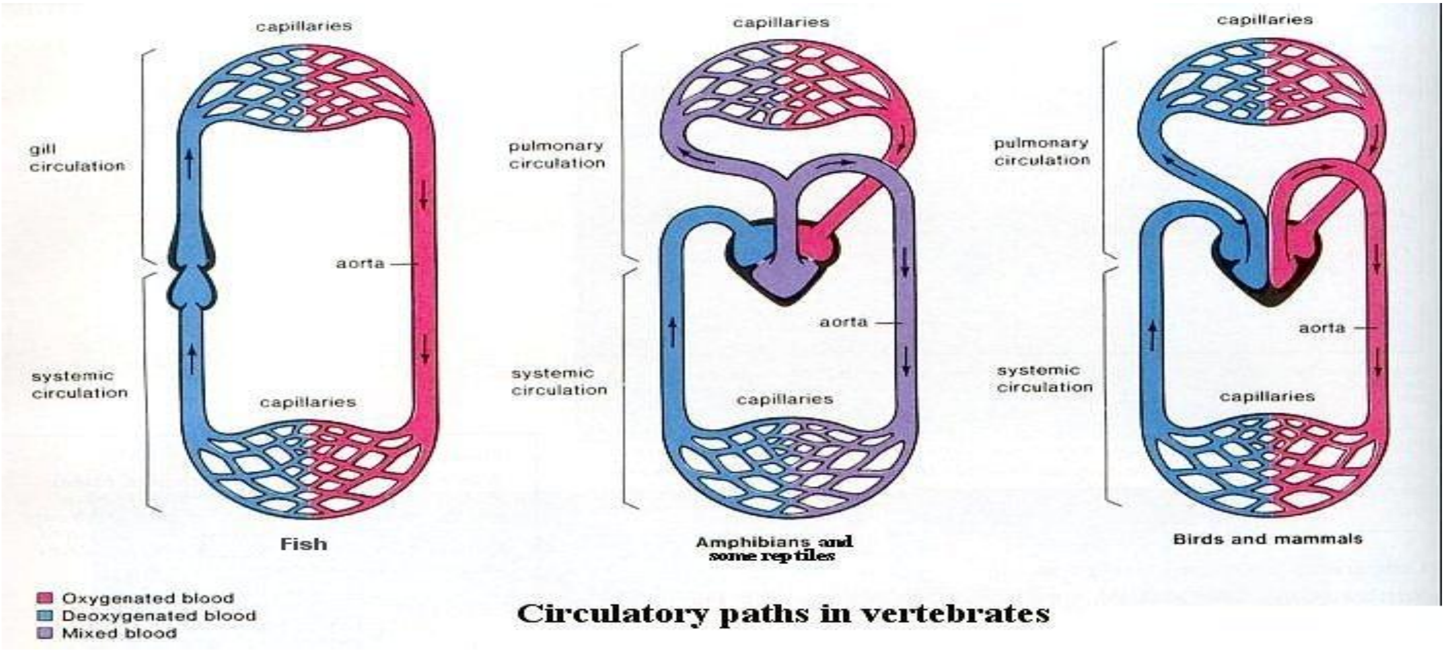
Suitable definition: “Craniate, gilled ectothermic aquatic animals with limbs in the shape of fins and lacking digits. These morphological traits can have evolved homologously or analogously.”

Edward Branson, Fish Welfare (2008)

Not a systematic term!

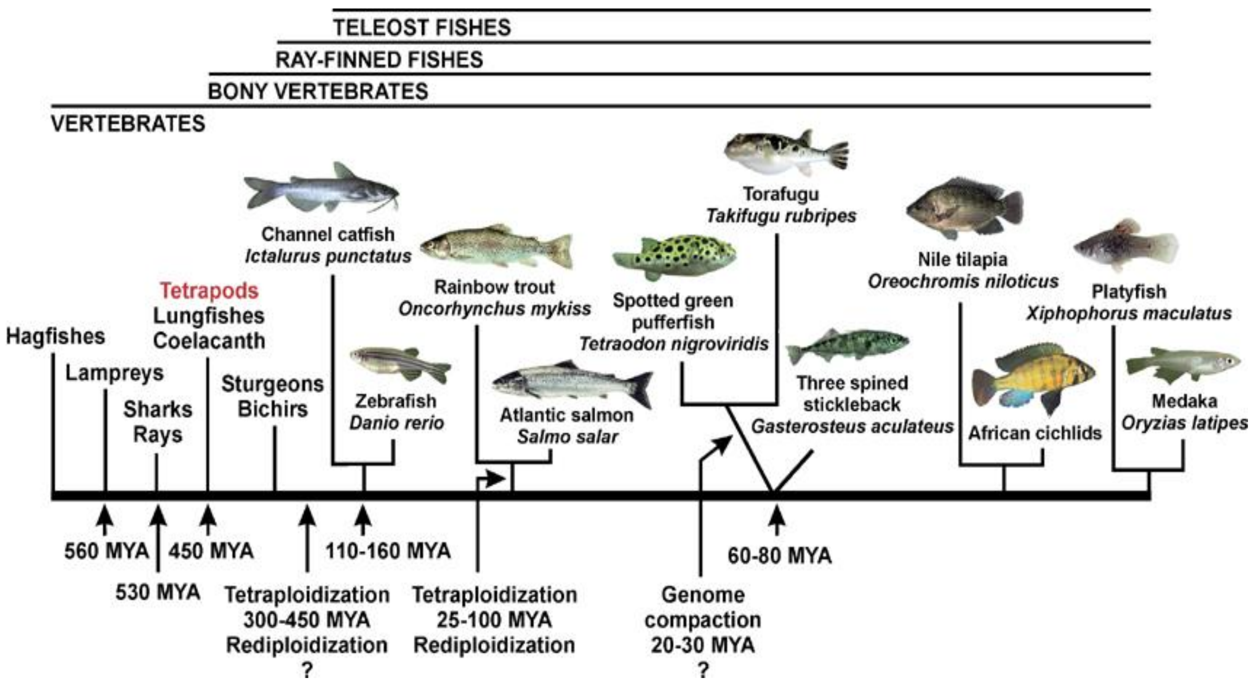
UNIVERSITY OF LIVERPOOL

Overview of fish biology



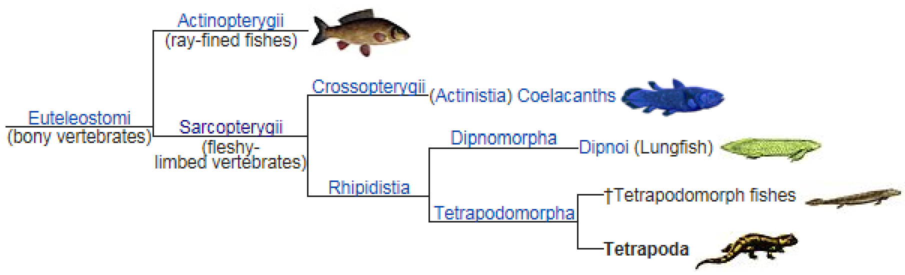
www.Nature.com

Overview of fish biology



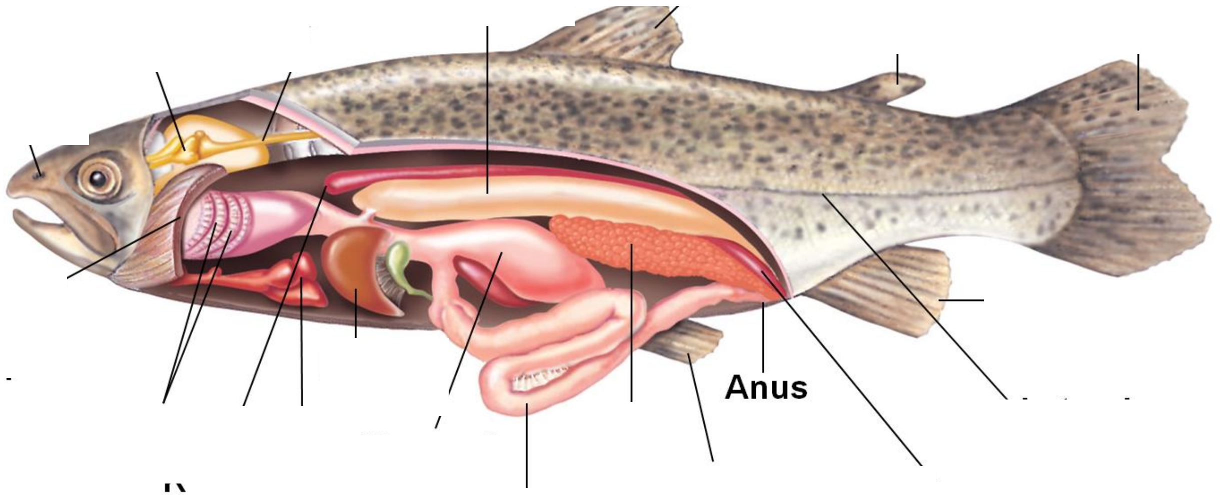
www.Nature.com

Humans are fish…

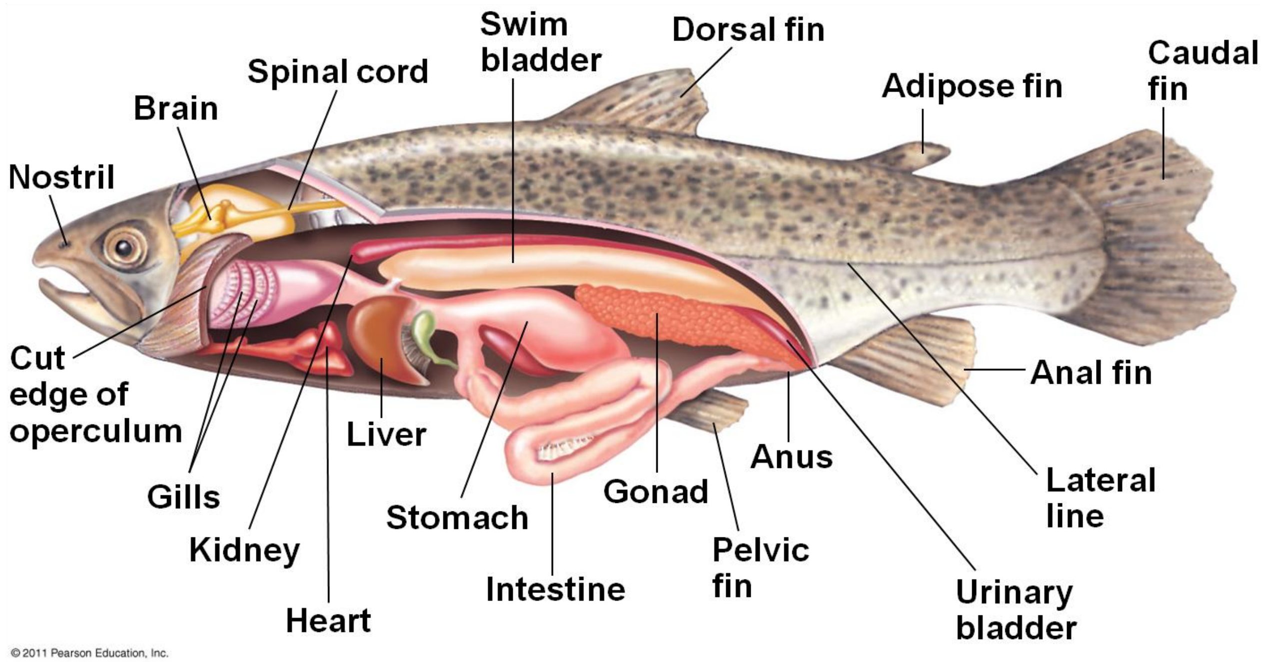


Cladistically all tetrapods (including mammals) are fish as they stem from lobe finned fishes emerging during the Devonian period

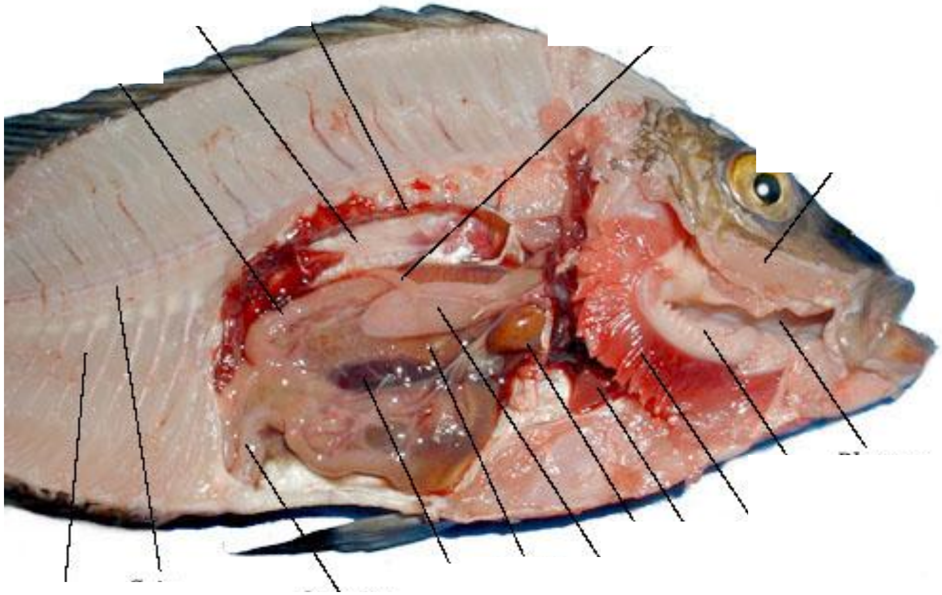
Fish Anatomy



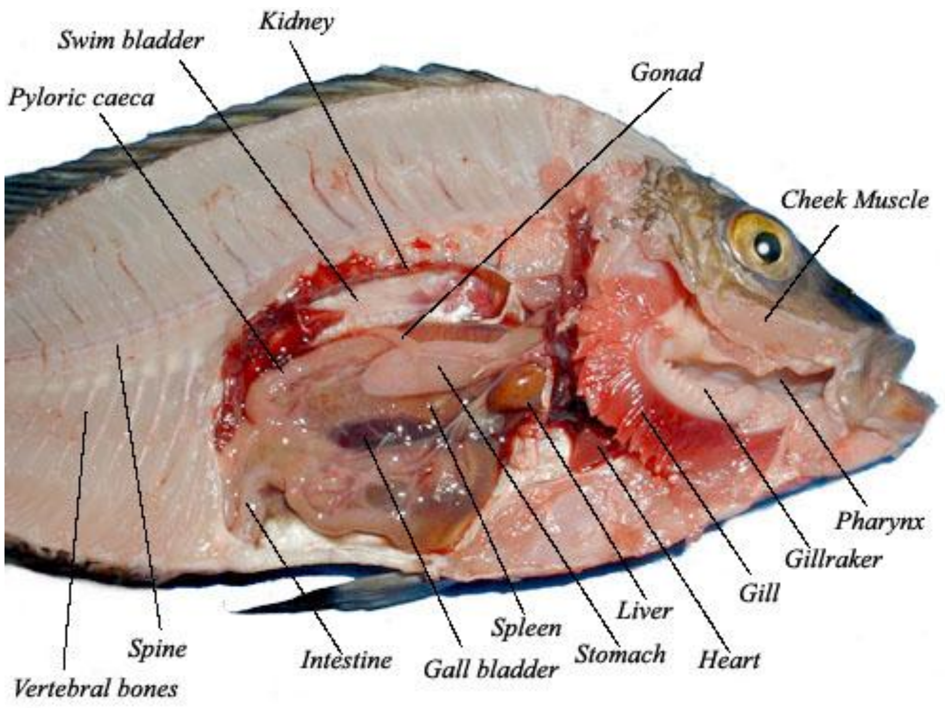
Fish Anatomy



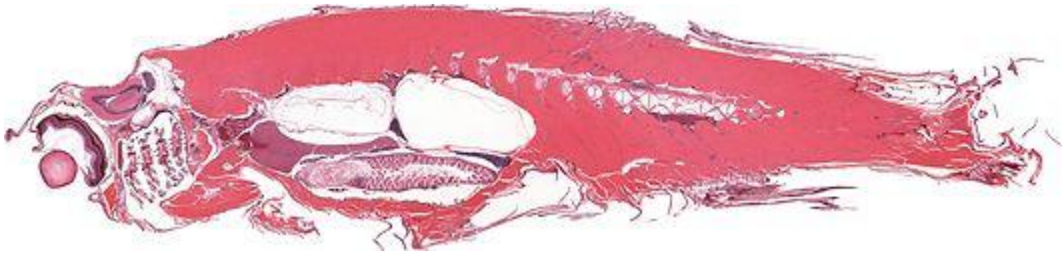
Fish Anatomy



Fish Anatomy



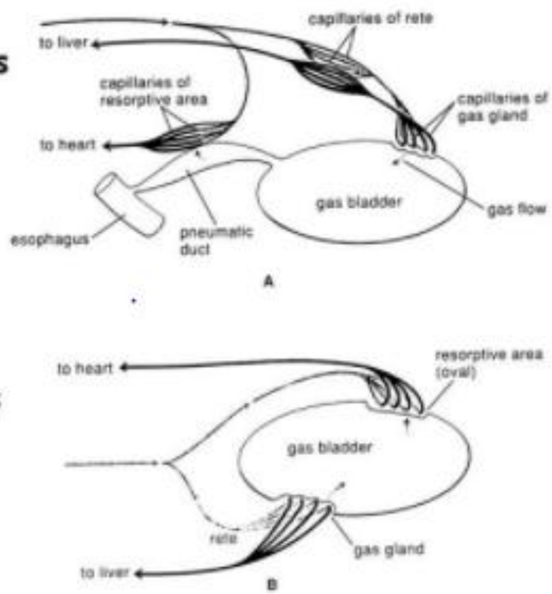
Fish Anatomy

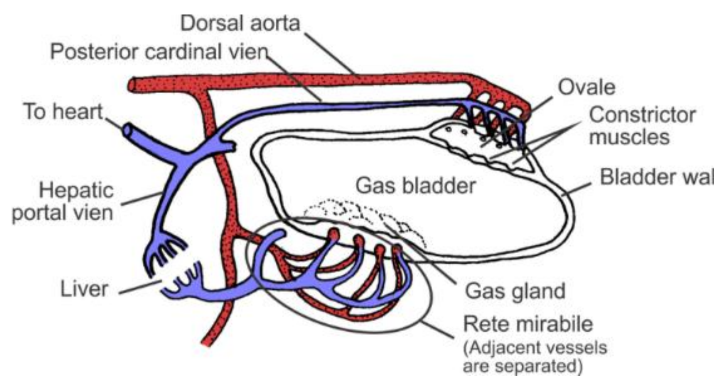


Some (more or less) unique anatomical features

physostomous

physoclistous



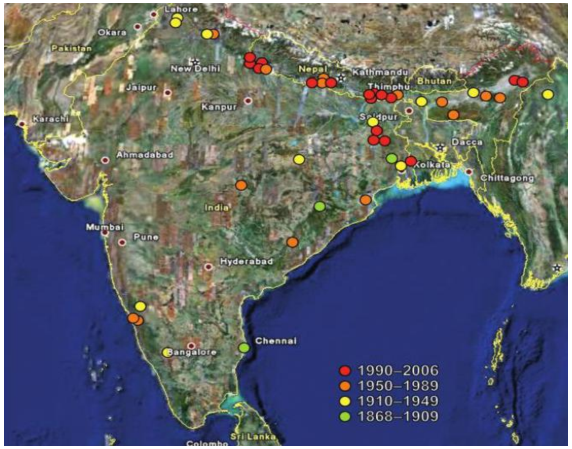


Some (more or less) unique morphological and physiological features

- No thoracic diaphragm
- No middle ear
- No limbs or limb girdles
- Swim bladder (most teleosts)
- Bony fin rays without skeletal support
- Otoliths
- Heart consists of single chamber
- 95% of gas exchange through gills, 5% through skin

Zebrafish

- Zebrafish (*Danio rerio*) – small **shoaling** members of Cyprinidae family (carps & minnows)
- Max total length 50mm, weight up to 1.8g
- **Sexually mature 12 weeks after hatching**
- **Photoperiodic breeders – right after sunrise**
- **Females spawn every 2-3d, producing clutches of 100-200 eggs**
- **Larvae hatch 2-3d pf at 28.5°C**
- Live up to 5 years
- Natural geographic range: South and South East Asia (Pakistan to Myanmar)
- Habitat: well oxygenated (>5mg dissolved O_2 /l) freshwater - slow flowing streams, flooded plains, rice paddies; water temperature range 14-34°C (in captivity - ideally 25-29 °C)
- Diet: copepods & other small crustaceans, insects & insect larvae, zooplankton…



Zebrafish use

- Zebrafish popular aquarium species before development of ZF research model
- Fast development (motile transparent larvae 48h post fertilization), rapid wound regeneration, cheap husbandry → ideal for toxicology, surgical models and genetic research
- 2nd most common (appr. 15% of total) animal used in scientific procedures
- As vertebrates, zebrafish are protected under ASPA from the time they can feed independently (day 5 p.f)
- some husbandry recommendations in Home Office CoP (max 5 fish/litre, unspecified structural enrichment)

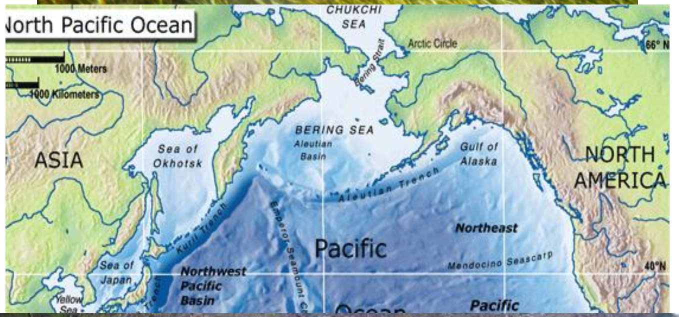
Zebrafish


UNDERSTANDING ANIMAL RESEARCH
ZEBRAFISH IN THE WILD
VS ZEBRAFISH IN THE LAB

Freshwater fishes

Rainbow Trout

- *Onchorhynchus mykiss* (formerly *S gairdneri*)
- Originally N Pacific Ocean and N American & N Asian tributaries only
- Freshwater but optionally anadromous (steelhead)
- Considerable size range depending on region & migratory habit – 35-100cm; 0.4-9kg,
- Omnivorous, very adaptable diet (mostly invertebrates)
- **Maturity at 2-3 years;** spawning all year (AC) or **once/year (wild)**
- Usually live for 5-6 (max. 11) years
- Temp. range 4-20°C, in captivity 10-15°C
- Popular and resilient aquaculture species



Trout in research facilities

- Ecotoxicology

(Usually juvenile trout)

- Diet studies
- Behavioural work
- Vaccine research (model species for all salmonids)
- "Swim up" 2 weeks post-hatching

Carp

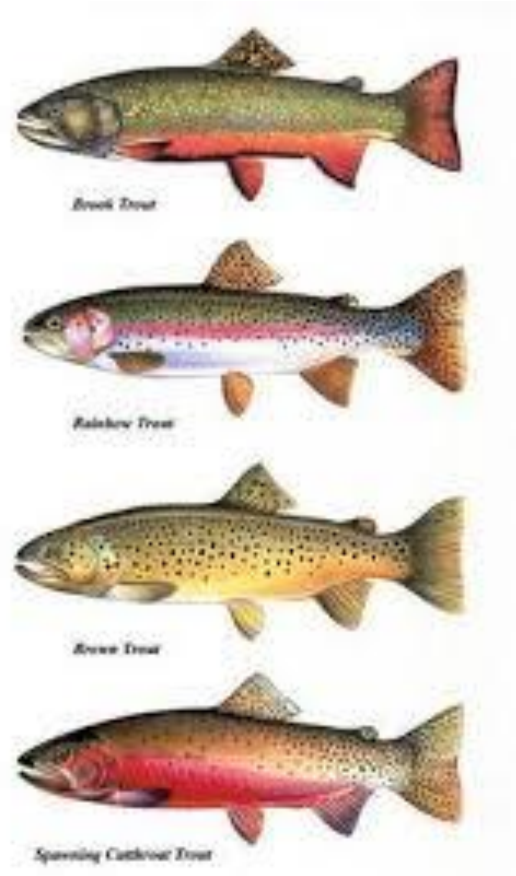
- *Cyprinus carpio*
- Introduced to Europe from Central Asia by Romans; spread to N Europe in early Middle Ages
- First kept in ponds around monasteries as alternative to meat during fasting periods
- All wild carp in Europe are feral form of cultured carp
- Live in freshwater; lakes, ponds and rivers, slightly brackish (max salinity 0.5%) rivers
- Life span up to 50 years; up to 120 cm (50kg)
- Shoaling
- **Omnivorous: aquatic and terrestrial invertebrates e.g. insects and insect larvae, earthworms and crustaceans, water plants**
- Popular AC species in SE Asia, due to fast growth rate (2-4% BW/day
- Females reach sexual maturity at 20,000 degree days (600d), spring spawners, clutches with up to 300,000 eggs
- Tolerant of ↓O_2
- Temp. range 4-30°C, in captivity 14-23°C (aquaculture 20-28°C); indoor systems require cooling

Carp in research facilities

- Usually kept small
- Ecotoxicology (usually juvenile/small fish)
- Physiological studies (1980s)
- Diet studies
- Genetic research
- Hatching 3dpf; free swimming 6dpf

Trout & Carp

- Both can tolerate range of salinity levels
- Not Schedule 2
- Sourcing should be done from fish farms with strict biosecurity
- Strict hygiene & health screening essential, parasites & viral infections particularly problematic



Marine species

European seabass

- Protected under ASPA from 6dph (7-10 days pf)
- **Temperature** 15-25ºC
- **Diet:** rotifera, artemia & nauplii from d6ph;
- D40-50ph: transfer to nurseries & transition from live prey to granulates
- Commercial fish meal & fish oil pellets
- In the wild: slow growing species that takes several years to **reach full adulthood (4-7y)**
- Wild seabass can grow to **1m/12kg (females); 7kg (males)**
- AQ: 1.5-2 years to slaughter (350-500g)
- Seasonal migration from **summer coastal feeding to winter offshore spawning grounds**

European seabass

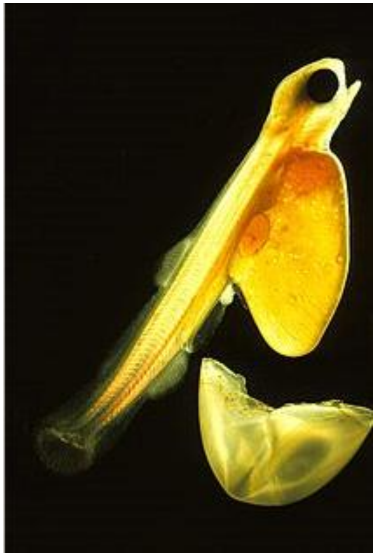
- *Dicentrarchus ibrax*
- Salt- and brackish coastal waters; NW Atlantic incl. Mediterranean
- “Pioneer” species for marine aquaculture (sustainable seafood, aquaponics)

Regulated procedures UK:

- Nutritional research
- Physiological studies
- Marine ecotoxicology

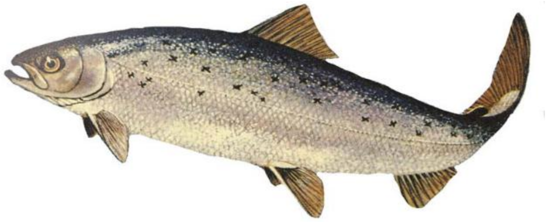
Atlantic Salmon

- **Atlantic Salmon** (*Salmo salar*)



- N Atlantic and tributaries;
- Anadromous (landlocked forms exist)
- "Classic" commercial aquaculture species (since early 1980s)
- Hatcheries & nurseries (FW)
- Grow out/fattening (SW) in sea pens/sea cages;
- Or combined egg to table aquaculture facilities

- Slaughter weight 3kg after 1.5-2 (organic: 3) years
- Perennial disease issues: sealice, ISA etc.



Wild caught species

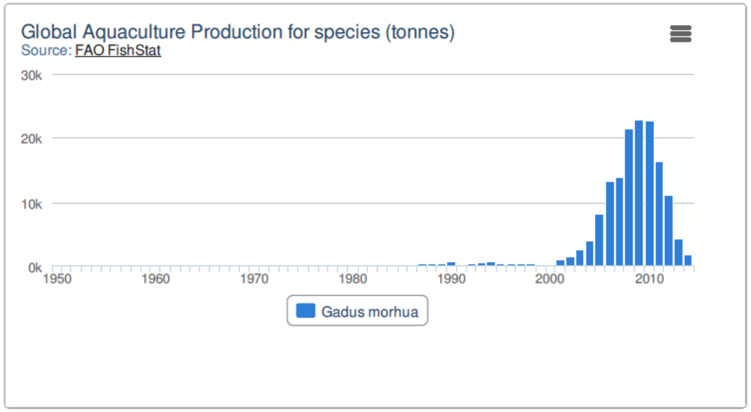
- e.g. **spiny dogfish** (Squalus acanthias)

- Temperate coastal waters S & N hemisphere
- Sexual maturity >20yo
- Ovoviviparous
- Caught by anglers & trawl bycatch
- Optimal temperature 7-14ºC
- Poor survival in conventional recirc systems

- e.g. **Atlantic cod** (Gadus morua)

- Continental shelf Atlantic N of 40` latitude
- Mature at 3-5yo
- Usually young fish (<1.5yo) caught by coastal angling but heavy transfer losses
- Adults survive in conventional recirc systems (11-15ºC)
- Can be prone to barotrauma

POLE needs to be specified in PPL application!



Marine fish

- Can usually tolerate wide range of salinities as they are excellent osmoregulators
- In saltwater ammonia and nitrite has less toxicity relative to FW but still needs to be monitored; high stocking densities can produce extreme levels!!
- Sourcing?
  Not Sch2 - e.g. commercial AQ
  Wild caught – POLE
- Health issues – hygiene, C&D, screening to limit parasites & viral diseases
- Grading - Separating by size cohorts to minimize aggression and cannibalism
- Feeding - Give sufficient quantities to avert aggression and competition stress
- Wild caught fish – risk of barotrauma

Husbandry:

housing, breeding & production, nutrition, transport, GMOs, biosecurity, disease prevention

General - Water quality

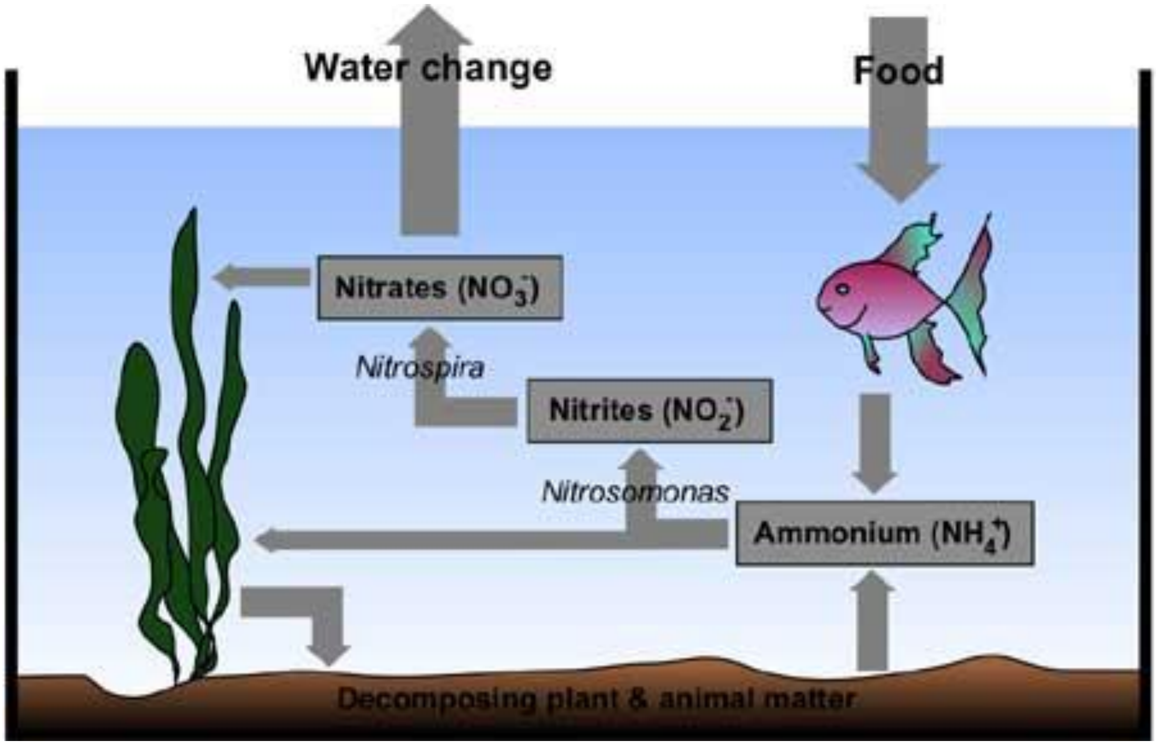
- Fish ectothermic, water passing **by** and passing **through**
- There is nowhere to hide if anything is wrong with tank water

Water quality

- PH 6.8-7.6 (ZF); 6.8-8.0 (trout) optimal;
- Temperature 26-29° (ZF); 22-24° (seabass); 8-15° (salmon)
- Oxygen above 5-6mg/l
- Conductivity 3-500 ms
- Salinity 1-3 ppt

Buffering to resist changes in pH; 50-150mg/L $CaCO_3$

Water quality

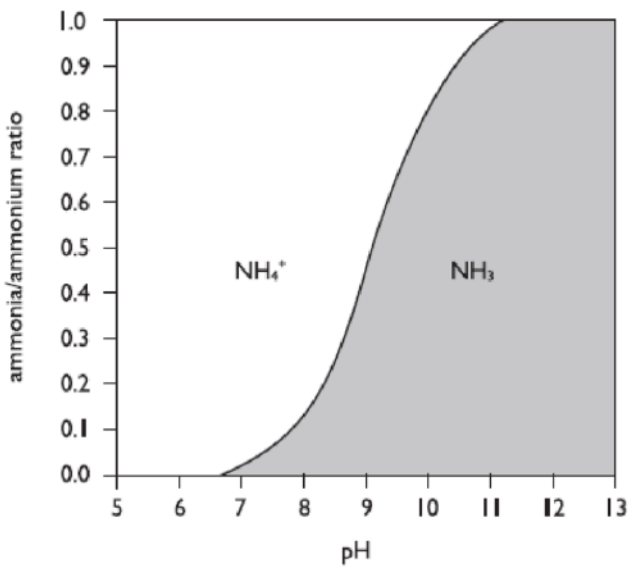


Ammonia, nitrite and nitrate

- Either dissolved as ammonium ions (NH_4^+) or more dangerously as free ammonia (NH_3).
- Levels of free ammonia increase with increased pH or temperature.
- Ideally<0.06ppm, toxic>0.3ppm.
- Nitrite less toxic than ammonia.
- Toxic from 0.5 mg/l; death at levels>10mg/l
- Nitrate depends on bacterial nitrification (final product in the nitrogen cycle); toxic from 100mg/l
- Recommended maximum 50mg/l

pH & denitrification monitoring

- $NH3/NH4^+$
- $NO2^-$
- $NO3^-$



(Brennan 2011)

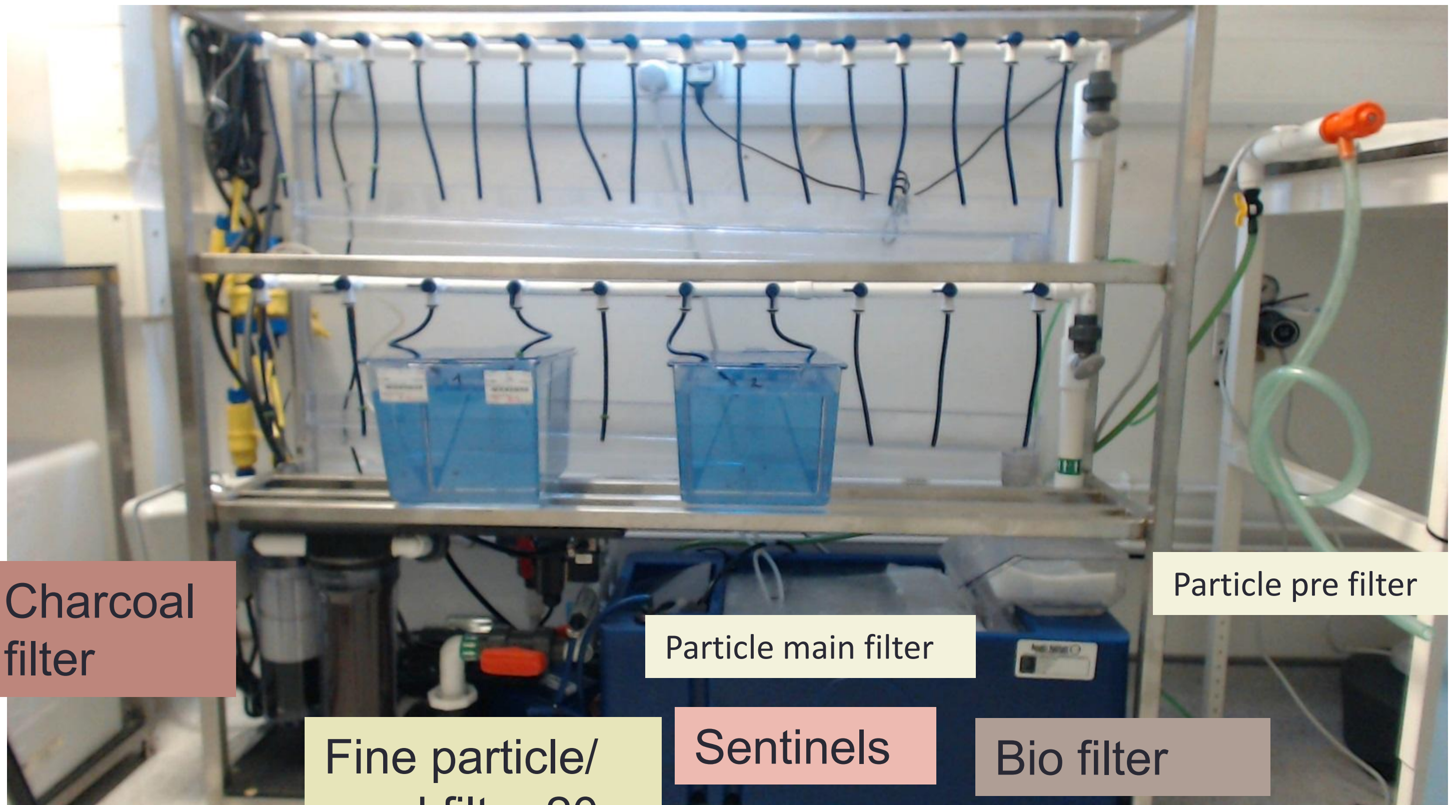
Temperature

- Fish should be kept “as close to natural environmental temperature” (CoP) yet this is 14-34°C *(Zebrafish in the wild; Engeszer et al. 2004)*
- **Zebrafish**
  - Optimum temperature of 26-29°C
  - Above 31C and below 25C → decreased breeding performance.
  - Room temperature at 25°C as temperature buffer.
- **Rainbow trout**
  - 12-20°C
  - Room temperature 14°C
- **Common carp**
  - 14-20°C
  - Room temperature 14-17°C

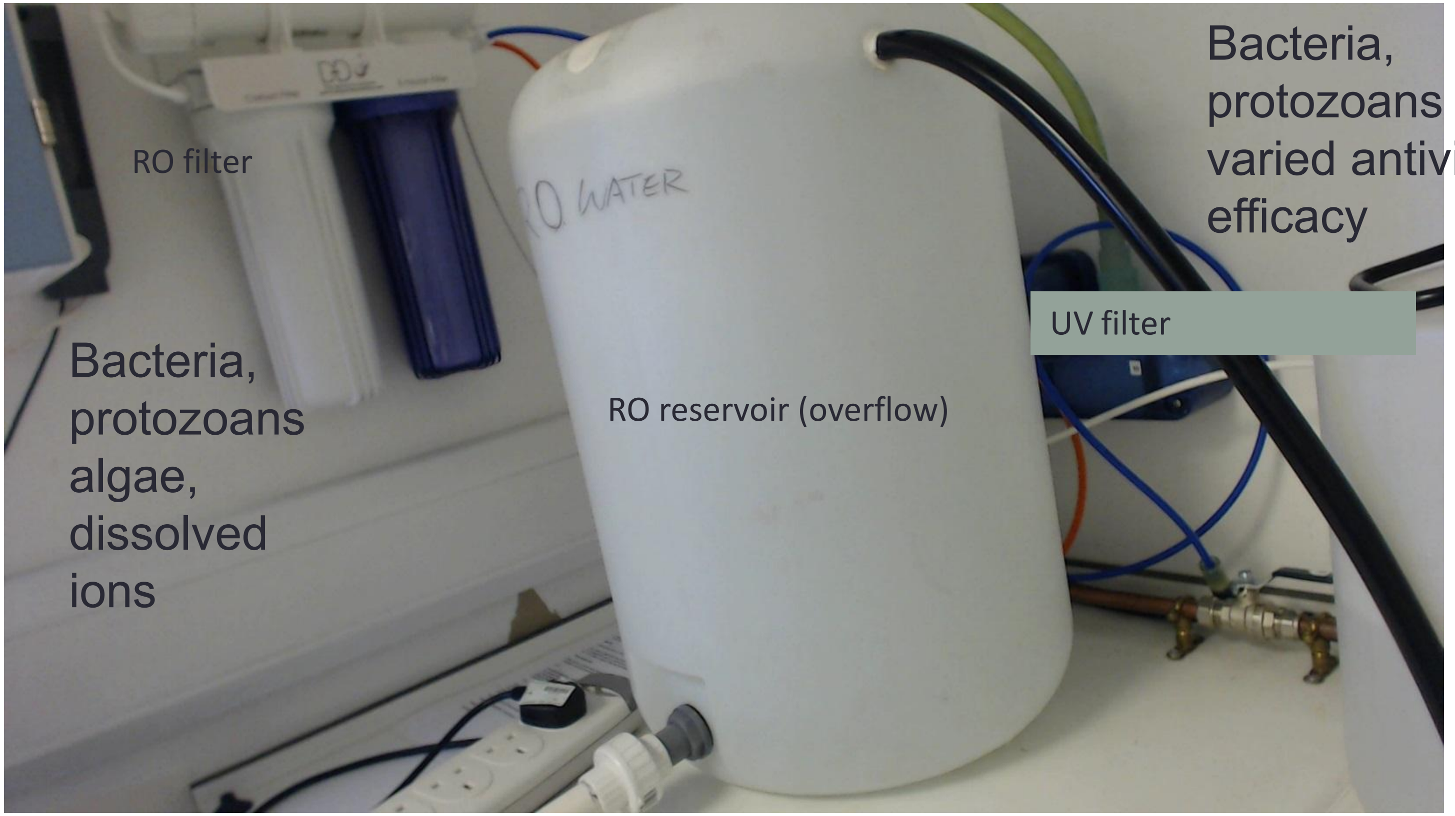
Temperature change

- Osmo-regulatory dysfunction.
- Suppression of immune system.
- Loss of equilibrium.
- High temp. - Increased activity.
- Low temp – Ataxia and coma.

Filtration


Charcoal filter
Particle pre filter
Particle main filter
Fine particle/ sand filter 20-50µm
Sentinels
Bio filter

Filtration


RO filter
Bacteria, protozoans algae, dissolved ions
RO reservoir (overflow)
Bacteria, protozoans algae, varied antiviral efficacy
UV filter

Housing - zebrafish

- For ZF - facility sizes vary from one tabletop rack to those with tens of thousands of tanks (ZF Resource Center Karlsruhe, Sanger Institute).

Housing - zebrafish

Housing – carp and rainbow trout

- For trout - facility setup depends on age and size of fish.
- Require water flow and often active cooling
- Usually separated into age cohorts
- Modified aquaculture systems or bespoke recirc tank assemby
- Filter systems similar to ones used for ZF but often lacking UV filtration (especially in cold water systems <14°)
- Avoid solitary housing

Housing – marine aquaculture spp.

- As for most obligatorily marine species – salinity 20-30%.
- May require water flow and **always** active cooling esp. for young stock (6-8°C, N Atlantic seabass 10-12°C)
- Juveniles & adults 10-12 (15)°C
- Usually, modified aquaculture systems with circular tanks
- High-capacity filter systems usually lacking UV filtration

Nutrition

Feeding – presentation, frequency, hazards

- Controlled ration vs ad libitum
- Timed vs continuous
- Frequent (several times/day) vs infrequent (once/day or less)
- Pellet/commercial vs live/natural feeds
- Depends on species requirements: energy expenditure (breeding, activity levels & metabolic state as well as digestive morphology)
  - Example: ZF – controlled ration, timed, frequent, commercial & live
  - Example: Juvenile carp (cool water system) infrequent, commercial, timed ad libitum
  - Example: Juvenile carp (warm water system) frequent, natural & commercial, timed, controlled ration

Fish pellet forms

Commercial formula for adult ZF and medaka – “slow sinking” <1mm micropellets

Aquaculture pellets – almost 100% fish meal & fish oil

Koi pellets – mix of fish meal, fish oil, poultry byproduct, wheat, soy

Feed related hazards

- Overfeed
  - Obesity
  - Accumulation & degradation in tank edges, overflows
  - Overload of physical & biological filtration process; displacement & overgrowth of denitrifiers by decomposition bacteria
  - Increased resistance for effluent leg of recirc system, slowing down water replacement
  - Can lead to increased circulating microorganisms/pathogens – as fine waste food particles & pigments may pass through physical & biofilters, reducing efficacy of UV filtration through shading

Feed related hazards

- Live feed as vector for parasites

Paramecium, Rotifers & Artemia

  - cultured in freshwater, veritable "bacterial soups," shown to support diverse microbial communities, including *Vibrio*.
  - Commonly utilized zooplankton species indiscriminately feed on bacteria, it is entirely possible that they would then have infective potential if they ingested pathogens and then in turn were themselves ingested by fish.
  - Documented cases not limited to *Vibrio*: *"Paramecium caudatum*, a commonly utilized prey item for first feeding zebrafish, experimentally enhanced transmission of both *Mycobacterium chelonae* and *M. marinum* to zebrafish larvae"

Watts et al. (2016)

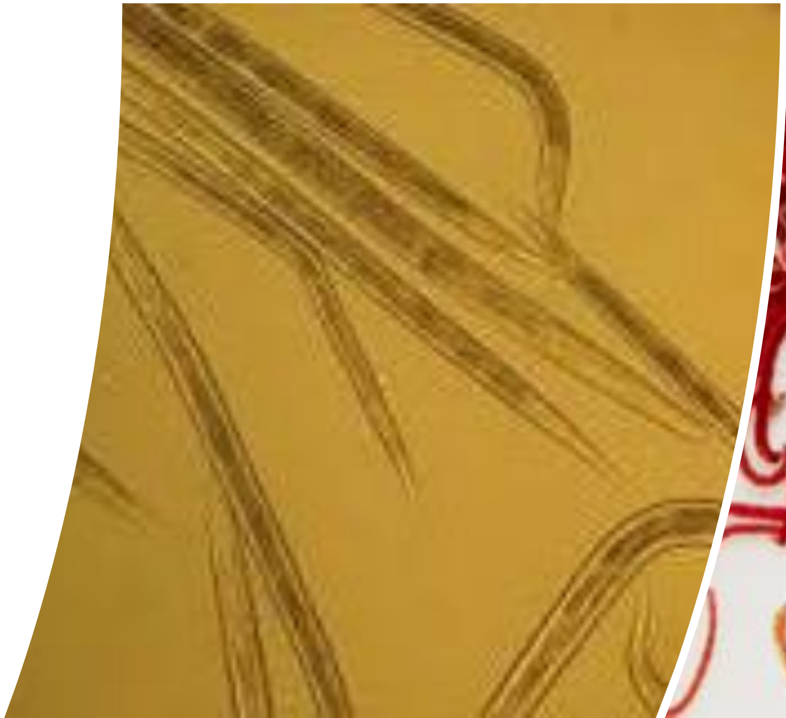
Zebrafish/medaka/guppy nutrition

- Minimum requirement:
- ZF & medaka < 10-12 days typically fed Paramecium or rotifers
- ZF & medaka > 10-12 days dried flakes 000 & rotifers with feed particle size increased progressively from then & transitioning to Artemia live feed by 21dpf
- Guppies: fry formula or paramecium <3weeks, flakes for older fish
- Live feeds with artemia at least 2-3 times per week (ideally daily)

AVOID non standard live feeds!

AVOID OVERFEEDING!!

Non-standard live feeds



- Panagrellus
- Bloodworm
- Tubifex
- Daphnia
- …

Trout/salmon/seabass nutrition

- Adult salmonids and bass feed on variety of invertebrates e.g., freshwater shrimp, aquatic and terrestrial insects, and small fishes.

- Fry can be weaned onto an artificial diet.
  - Starter feeds, starting from when approximately 50% have reached the 'swim-up' stage.
  - When most fish actively feeding, give 10% of their weight daily for 2–3weeks.
  - When fish reach 15–25 mm long feeding based on temperature and fish size.
  - Overfeeding must be avoided.
  - Fish moved to larger tanks to reduce density as they grow.

- Adults fed pelleted diet as a % of body weight (typically 1-2%) depending on how much growth you want!

Fish handling, capture & restraint

- Fish are invariably stressed through all forms of handling
- Struggle during capture in wild and captive environment
- Significant damage to eyes, gills, epidermis & scales can result from rough handling, followed by osmoregulatory & neurological pathology
- For larger ornamental or AC species, sedation may be necessary prior to netting

Capture & netting

- Some management activities e.g. cleaning of the tank, moving fish to another tank, require netting.
- Suitable non-abrasive/knotless net
- Attempt to reduce chasing with a net during capture.
- Ensure that fish are out of water for the shortest possible time.
- Well-established routines should be applied to the care to both minimize the frequency of cleaning and the moving of fish.
- Zebrafish, medaka, guppies and other small ornamental breeds are caught and transferred from tank to tank by the use of soft non-abrasive nets to minimise any stress and damage to scales and skin.
- Any skin damage should be avoided as this may lead to infection.

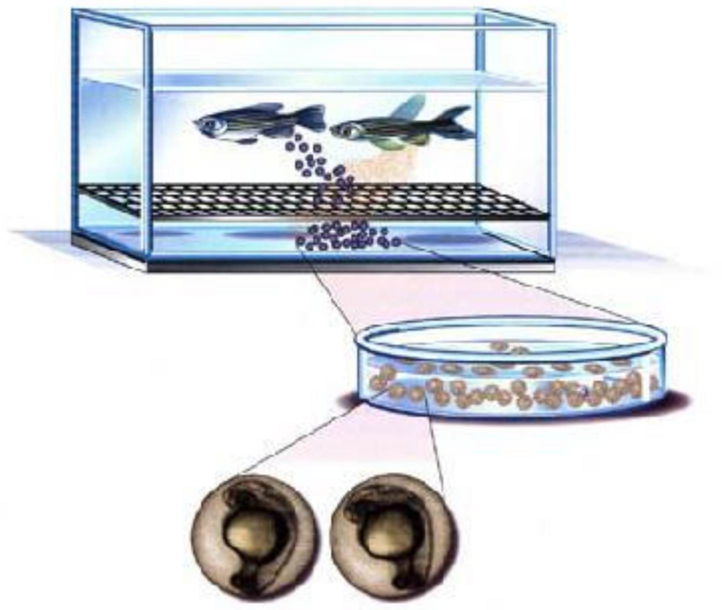
Handling & restraint

- Handle with clean, soap free, moist hands or gloves.
- Place animals onto plastic covered foam or soft moist cloth to avoid skin abrasions.
- Larger fish may require restraining device for quick out-of-water procedures

Breeding/production systems

Breeding systems - ZF

- "Low tech" breeding systems or fully automated nurseries
- Install screens to stop egg predation
- Install enrichment cues to encourage spawning
- Select and monitor brood stock
- Natural or in vitro fertilization



Breeding systems - ZF

- Select fertilized eggs
- Keep in fry tanks or deep petri dishes
- Frequent water change & removal of dead eggs/larvae
- Feed Paramecium from day 5 pf

Fish Transport

- ZF, medaka, sticklebacks, juvenile carp - adults or juveniles in bags (system water) in insulated boxes
- For medaka & ZF>3 months no more than 5 fish/l
- Air pocket must be locked into bag!
- Post transport quarantine
- Careful of any **netting damage- skin surface delicate!**

<u>Large fish</u>

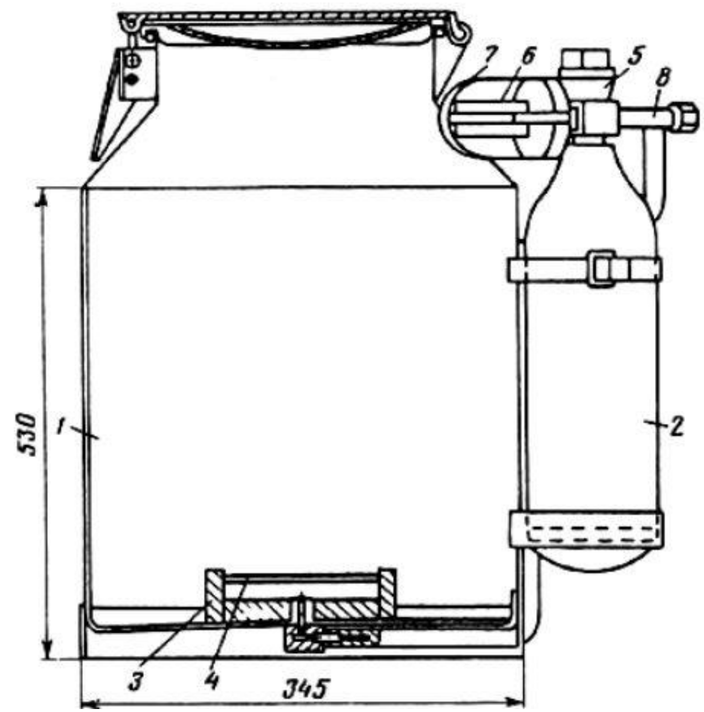
- Barrelled in groups or bagged individually as above - lower temperature (within species appropriate range) for longer transport to reduce oxygen demand. Ideally no longer than 2h
- Consider alternative methods for short distances – pipes, chutes

Transport

- Transport stressful to all fish species, resulting immunosuppression and ↓ oxygen can lead to high transport mortality
- Higher oxygen demand with higher temperatures
- Published loading rates in need of revision
- Allow for temperature acclimation pre and post transport

Weight (in kg) of channel catfish that can be transported per litre of 18°C water (Piper *et al.*, 1982)

Number of fish (per kg)	Transit period (in h)		
	8	12	16
2	0.75	0.66	0.57
4	0.71	0.57	0.41
9	0.60	0.49	0.35
110	0.41	0.30	0.24
276	0.35	0.26	0.21
552	0.26	0.21	0.18
1 100	0.21	0.20	0.15
2 200	0.15	0.12	0.08
22 000	0.02	0.02	0.02



Piper et al. (1982)

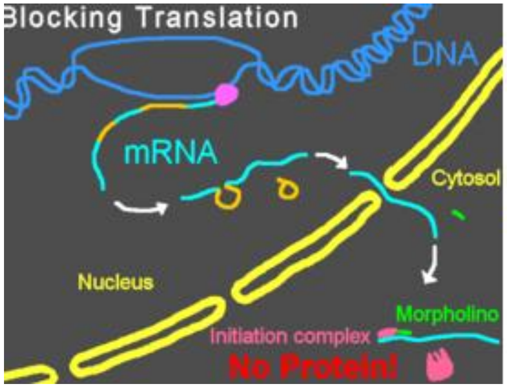
Genetic modifications

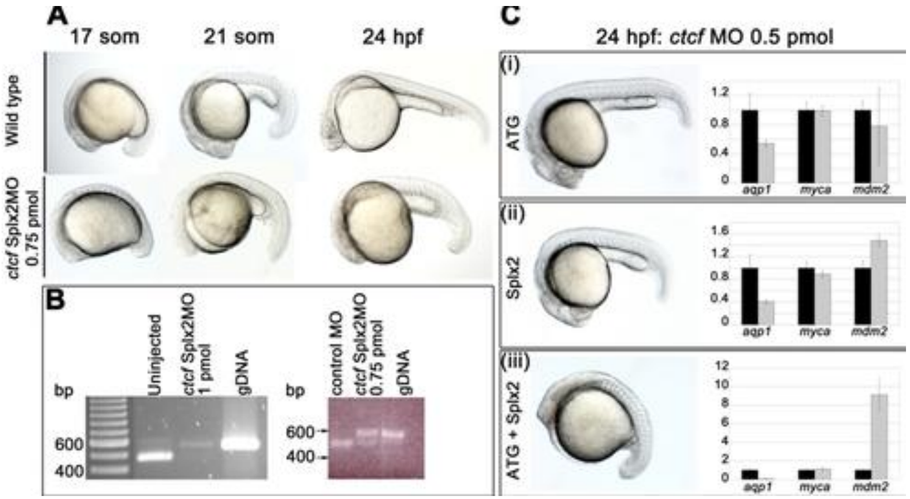
- Morpholinos (knockdowns)
- Transgenics
- Knockouts

Genetic modifications

- Morpholinos (knockdowns)

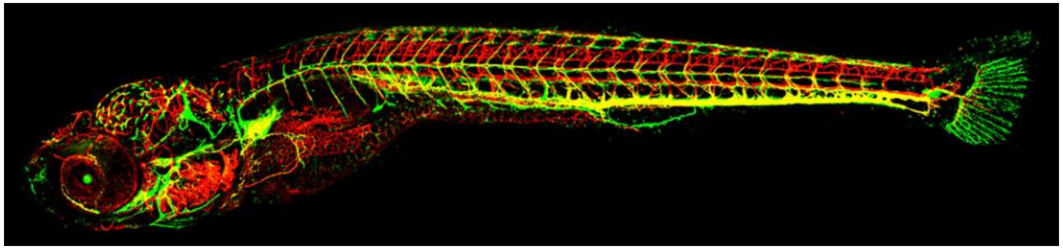
used in molecular biology to modify gene expression. DNA bases attached to a backbone of methylenemorpholine rings. Morpholinos block access of small (~25 base) specific sequences of the base-pairing surfaces of ribonucleic acid (mRNA). Morpholinos are used as research tools for reverse genetics by knocking down gene function.

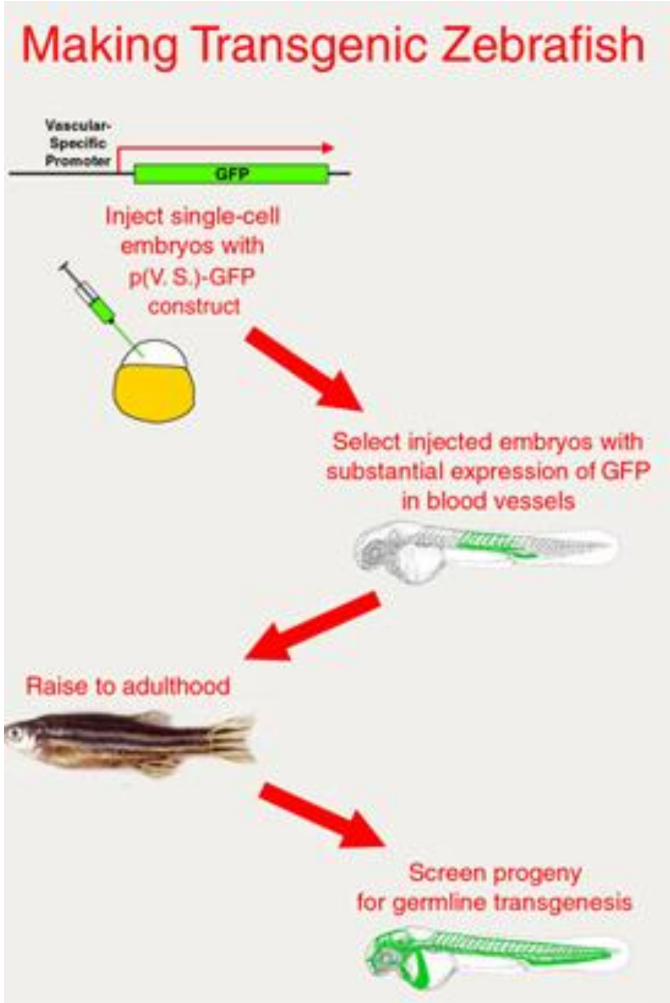




Genetic modifications

Transgenics

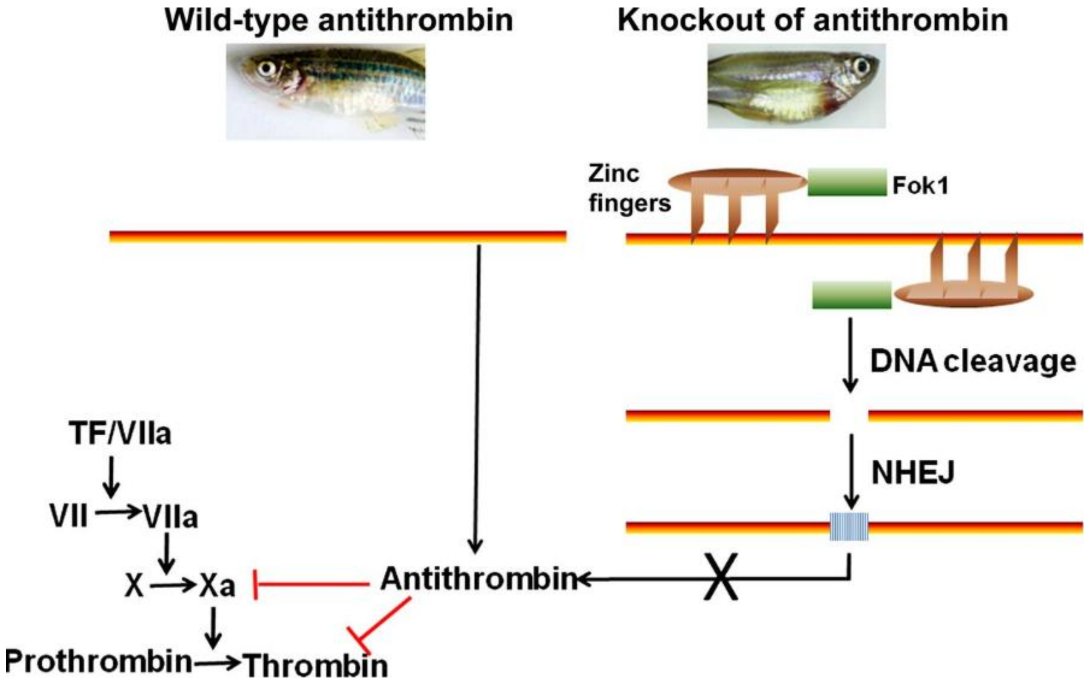




Genetic modifications

Knockouts

- ↑membrane permeability with electroporation before chemically induced DNA cleavage



Husbandry – biosecurity & disease prevention

Diseases

General

- Relatively little knowledge on ZF diseases
- No “known viral pathogens” in research facilities (but some suspicious cases)
- Several persistent infections with high morbidity but low mortality, affecting many facilities.
- As with other laboratory animals used in research, imperative to conduct studies with disease-free fish (Kent 2009).
- Unlike rodent models, where there are many certified specific pathogen free (SPF) strains, it is early days still for SPF stocks of zebrafish

Examination

- External - **Fish behavior**

- Flashing (rubbing on the sides of tanks), may indicate external parasites.

- Cessation of feeding, lethargy, loss of equilibrium, abnormal position in the aquarium (e.g., at surface or bottom).

- Abnormal respiratory pattern (gill damage, other stress).

- whirling or spiralling swimming often indicates neurological damage.

Further examination

- **Necropsy** conducted on diseased fish that are collected while still alive
- Several affected fish should be examined whenever possible
- Include apparently normal, asymptomatic fish to detect early pathological changes
- Dead fish – CAVEAT post-mortem autolysis.

Further examination

Further examination

- **Close up examination** with stereo dissecting microscope.
- Surface abnormalities (e.g., frayed fins, cloudy eyes, ulcers, skin discolorations, parasites, and tumors).
- Prepare wet mounts of skin mucus/scales by scraping surface of fish with glass cover → put on slide; reduce light and lower condenser for higher contrast

Preservation for diagnosis (zebrafish.org)

Preservation methods of fish tissues and their uses in fish disease diagnostic examinations. +++ = optimal; ++ = satisfactory in most cases; + = suboptimal, can be used if no other tissue available; 0 = useless.

	Live	Iced	Frozen	Preserved *
Parasitology	+++	++	+	+
Bacteriology	+++	+	+	0
Virology	+++	++	+	0
Toxicology	+++	++	+++	0 to +++
Histology	+++	+	+	+++
Electron Microscopy	+++	+	0	+++
PCR	+++	++	+++	+++

* Preserved in formalin-based fixative (e.g., Dietrich's Fixative) for histology, glutaraldehyde-based fixative for electron microscopy, 95% ethanol for PCR testing.

Disease

- Infectious/parasitic – protozoan, fungal, viral, algal bacterial, myxozoan, crustacean
- Factorial e.g. red mark syndrome
- Captivity-linked e.g. triggerfish aphagia

Protozoan/fungal infections

- **1. Microsporidiosis**

- *Pseudoloma neurophilia* (Matthews et al. 2001)

- "Skinny disease"
- Scoliosis


A
B


G

Protozoan/fungal infections

- Most “skinny” fish and up to 1/3rd of healthy fish infected → infection routinely in normal, healthy appearing fish
- stress causes increased severity of the infection
- **Transmission:** obligate intracellular fungus-like parasites which produces infectious and resistant spore

Protozoan/fungal infections

- Transmission via the ingestion of infective spore stage;
- Fry fish are very susceptible, and may show more acute form
- Transmitted by feeding on infected carcases, but also via water in recirc system!

Protozoan/fungal infections

- **Diagnosis –** histology; spores ovoid to pyriform, with a prominent posterior vacuole, and average 5.4 x 2.7 um.
- Xenomas within the spinal cord and hindbrain.
- PCR tests (non-invasive test based on PCR of water and eggs in development).
- Pre-screening all brood fish by PCR → Pseudoloma free stock.

Protozoan/fungal infections

- **Control and Treatment –** *UV* at 30-50,000 µWsec/cm^2 kills the parasite and thus prevents it transmission in a recirculating system.
- Fumagillin as oral treatment for ornamental fish and aquaculture species (eel, salmon; originally developed for honeybee microsporidiosis).
- Microsporidian spores resistant to disinfectants!
- chlorine levels routinely used by zebrafish facilities (i.e., 25 or 50 ppm for 10 min) not effective for killing spores, especially those persisting in eggs.

Protozoan/fungal infections

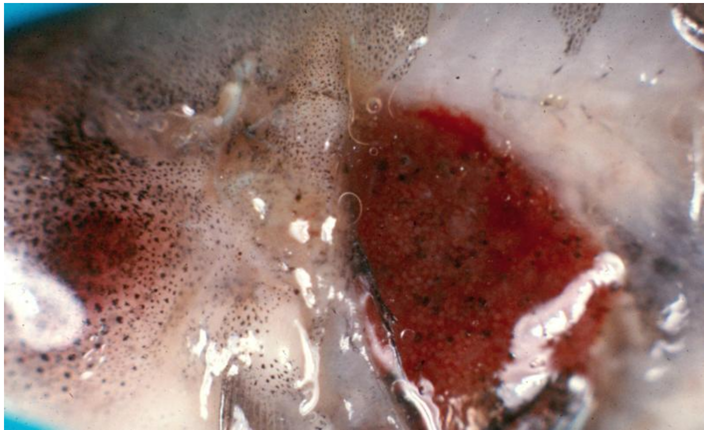
- **Ichthyophthirius (white spot/Ich)**
  - Most common disease in FW ornamentals
  - Excessive mucus production, labored breathing, and lethargy
  - White, raised nodules on the skin ("white spot“)

Protozoan/fungal infections

- **Treatment/control** - Ich penetrates the epithelium hence difficult to eradicate with external baths or dips.
- Formaldehyde 1:5000; Methylene blue
- Multiple treatments!
- UV filters upstream & downstream, especially in recirc systems
- Quarantine racks with separate recirc/flow through system

Bacterial infections

- **Mycobacteriosis (Fish TB)**
- Chronic, systemic bacterial infections by various *Mycobacterium* species
- *M. marinum*, *M. abscessus*, *M. chelonae*, *M. fortuitum*, *M. peregrinum* and *M. haemophilum* associated with mycobacteriosis in zebrafish



Bacterial infections

- usually chronic with low level mortality
- rarely acute or peracute with severe losses in ZF colonies.
- Factors responsible for the differences yet to be determined (strain, species, water quality, stress etc.).

Bacterial infections

- **Zoonosis**! "Fish handlers disease"(usually extremities) which needs aggressive antibiotic treatments, can even be lethal to immune compromised individuals
- **Wear gloves!** Wash hands after coming in contact with water containing fish, and avoid exposing open lesions to aquarium water and fishes.
- Most cases of human mycobacteriosis associated with fishes caused by *M. marinum*.

→ check that *M. marinum* is covered by AFB scan (if applicable)

Bacterial infections

Aquatic health screening – rationale & epidemiology

- What do we screen

- (Epidemiological) Population, a group of conspecific individuals sharing a defined space or medium, so that they have an approximately equal chance of contact with a hypothetical pathogen

Aquatic health screening – rationale & epidemiology

Zebrafish Real-Time PCR Panels

AS - Aseptic swab or aseptic stab | E - Environmental sample (biofilm, sediment, detritus) | F - Feces | SS - Skin swab | T - Tissue (kidney, liver, spleen) | WF - Whole fish

	Preferred Sample Types	Mycobacterium	Essential	Comprehensive	FELASA / AALAS Quarterly	FELASA / AALAS Annual
Mycobacterium abscessus	WF, E	●	●	●	●	●
Mycobacterium chelonae	WF, E	●	●	●	●	●
Mycobacterium fortuitum	WF, E	●	●	●	●	●
Mycobacterium haemophilum	WF, E	●	●	●	●	●
Mycobacterium marinum	WF, E	●	●	●	●	●
Mycobacterium peregrinum	WF, E	●	●	●	●	●
Mycobacterium saopaulense	WF, E	●	●	●	●	●
Mycobacterium gordonae	WF, E	●	●	●	●	●
Edwardsiella ictaluri	WF, E		●	●		●
Pseudocapillaria tomentosa	WF, E		●	●	●	●
Pseudoloma neurophilia	WF		●	●	●	●
Flavobacterium columnare	WF, E			●		●
Ichthyophthirius multifiliis	WF			●		●
Infectious spleen and kidney necrosis virus (ISKNV)	WF			●		●
Piscinoodinium pillulare	WF			●		●
Pleistophora hyphessobryconis	WF			●		●
Myxidium streisingeri	WF, E		●	●		●
Zebrafish picornavirus (ZfPV1)	WF, F		●	●		●
Redspotted grouper nervous necrosis virus (RGNNV)	WF			●		●
Saprolegnia brachydanis	WF			●		
Aeromonas hydrophilia	WF, E			●		

What do we screen

Aquatic health screening – rationale & epidemiology

- What do we screen

Zebrafish Microbiology Panel

AS - Aseptic swab or aseptic stab | E - Environmental sample (biofilm, sediment, detritus) | F - Feces | SS - Skin swab | T - Tissue (kidney, liver, spleen) | WF - Whole fish

Pathogen	Preferred Sample Type(S)
Aeromonas dhakensis	AS, E, T, WF
Aeromonas hydrophilia	AS, E, T, WF
Edwardsiella ictaluri	AS, T, WF
Flavobacterium columnare	SS, T, WF
Plesiomonas shigelloides	AS, E, T, WF
Pseudomonas aeruginosa	AS, E, T, WF
Pseudomonas fluorescens	AS, E, T, WF
Saprolegnia spp.	SS, E, T, WF

Aquatic health screening – rationale & epidemiology

- Why do we screen?

Reactive

- Understanding cause of acute mortality event
- Morbidity
- Failure to thrive
- Known biosecurity breach

Aquatic health screening – rationale & epidemiology

- Why do we screen?

Preventive

- ID pathogens before clinical manifestation
- ID pathogens because clinical manifestations other than death are unlikely to be spotted by anyone
- ↓ Statistical noise (screening for infections which result in confounding subclinical conditions)
- Protect human health (screening for zoonotic pathogens such as *Mycobacterium* spp., *Anisakis* etc.)
- Quality assurance of genetic integrity, especially inbred strains bred and maintained in facility
- Environmental factors (quality of feed, water, and bedding; lighting; noise; etc) that can affect colony health.

Aquatic health screening – rationale & epidemiology

- How do we screen?

Indirect (e.g., tank water)

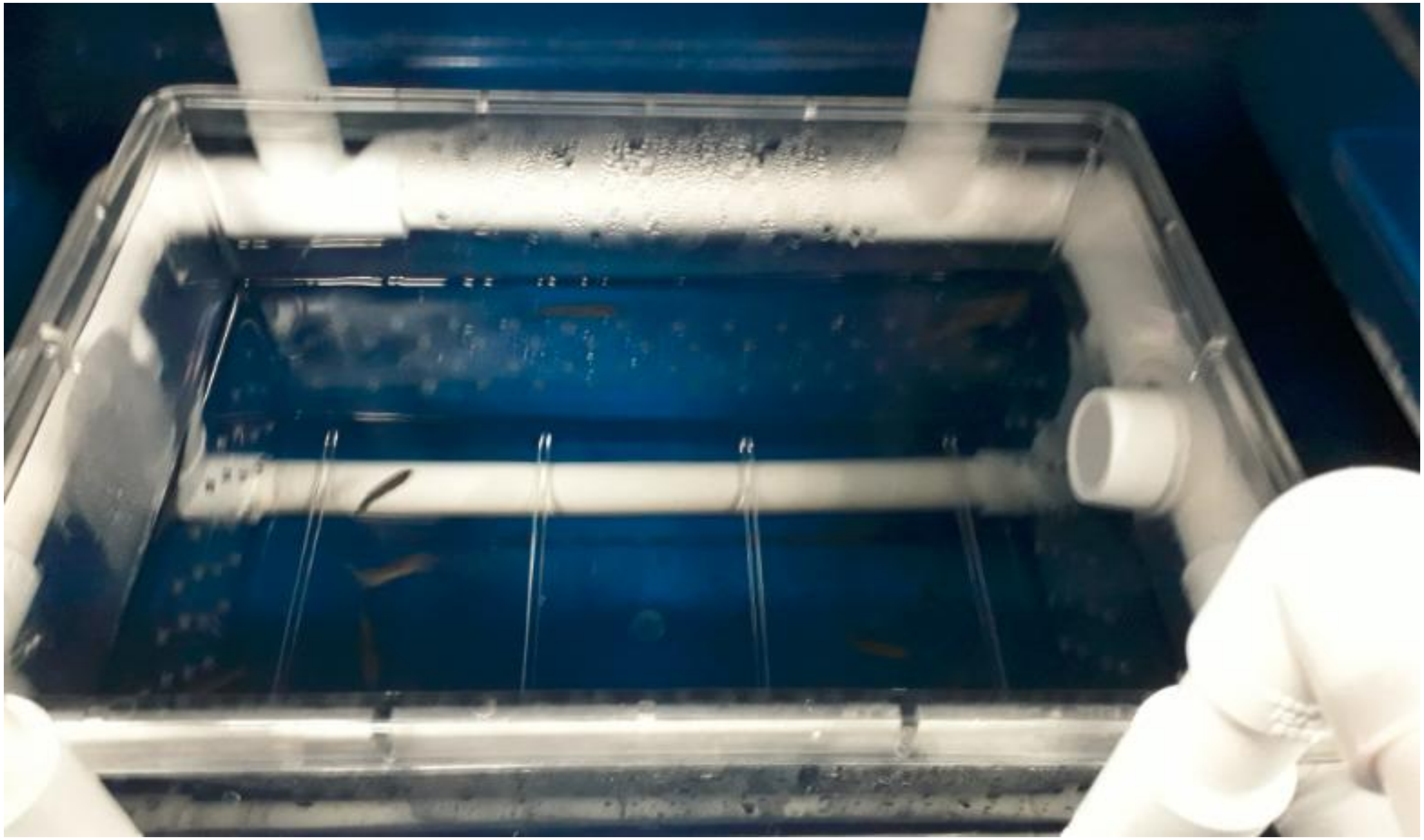
Direct:

- non lethal, non invasive (e.g. faeces)
- Non lethal, minimally invasive (e.g. mucus swab from gills or skin)
- Non lethal, moderately invasive (e.g. scale extraction, peripheral blood sample, gill biopsy, fin clip, stripping)
- Non lethal, highly invasive (e.g. liver biopsy, surgical egg removal)
- Lethal (whole fish or tissue samples)

Aquatic health screening – rationale & epidemiology

If samples are to be sent away, live fish usually preferred e.g. CR Health Surveillance 360 Diagnostics™

Testing Type	Required Sample Type(s)
Signature Health Inspection	65 live fish selected randomly from the population
Diagnostic Investigation	10 live fish: ideally some, or all, of the fish submitted will be moribund, or displaying signs of disease. Please also health history of the animal/colony, being as detailed as possible.
Histopathology	Live fish or fish fixed in 10% neutral buffered formalin (euthanized immediately before fixing, with belly *carefully* slit to allow optimal fixation)
Aerobic Culture	Live fish
Molecular Testing	Live fish, frozen fish, or fish preserved in RNAlater or ethanol (euthanized immediately before preservation, with belly *carefully* slit to allow optimal preservation)
Virus Isolation	Live fish

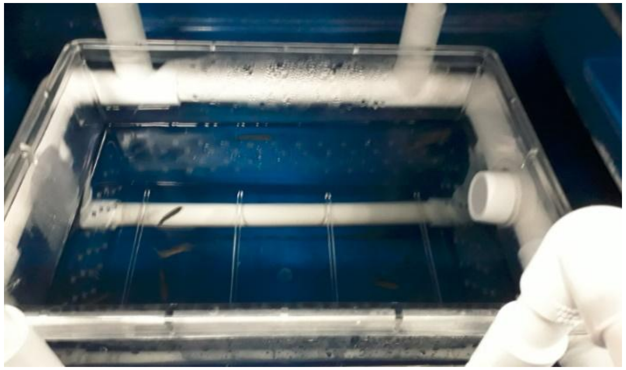


Aquatic health screening – rationale & epidemiology

- Preventive screening

Sentinel or randomly chosen tank?

- Depends on facility design
- How strongly are we convinced that our filtration system and gradual water replacement significantly reduces circulating pathogens?

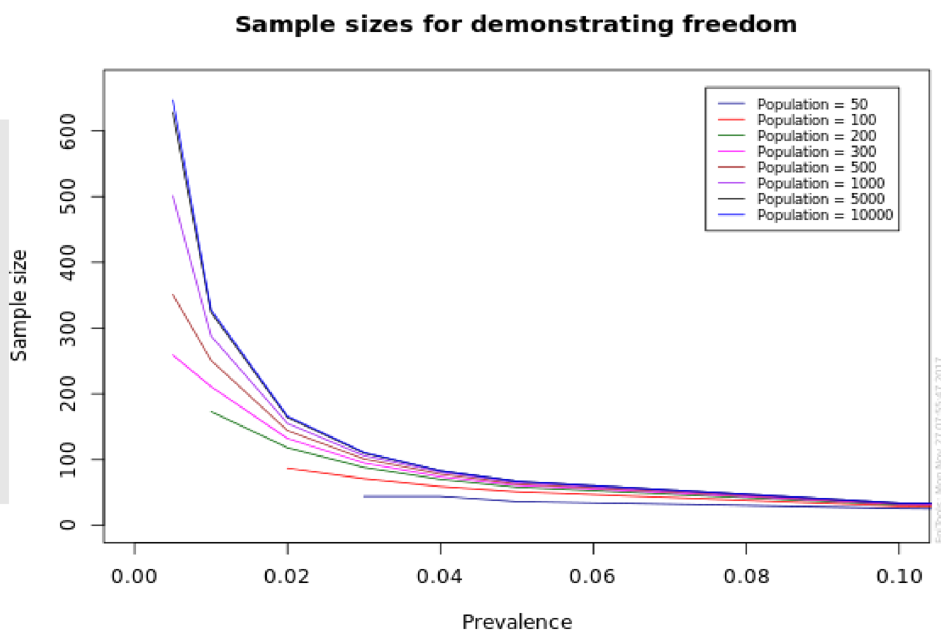


Required sample sizes

It is a numbers game and depends on:

- Prevalence
- Test sensitivity
- Desired confidence interval

Signature Health Inspection	The Signature Health Inspection provides a 95% confidence level in detection, assuming pathogen prevalence is at least 5%. This approach to health monitoring in aquatic systems is based on the recommendations of the Office International Des Epizooties (OIE) and the American Fisheries Society-Fish Health Section.



Required sample sizes for demonstrating disease freedom

e.g. Pseudoloma PCR assay, 99.4% specificity, 85% sensitivity, CI95%

	Prevalence = 0.005	Prevalence = 0.01	Prevalence = 0.02	Prevalence = 0.03	Prevalence = 0.04	Prevalence = 0.05	Prevalence = 0.1	Prevalence = 0.2
Population = 50				46	46	38	27	16
Population = 100			92	75	63	54	31	17
Population = 200		183	125	93	74	61	33	17
Population = 300	275	223	139	100	78	64	34	18
Population = 500	372	266	153	107	82	67	35	18
Population = 1000	531	305	164	112	85	69	35	18
Population = 5000	665	343	174	117	88	71	36	18
Population = 10000	685	348	175	117	88	71	36	18
Population = 1e+05	703	352	177	118	89	71	36	18
Population = 1e+06	705	353	177	118	89	71	36	18

Required sample sizes for demonstrating disease freedom

- The lower the prevalence, the higher the required sample size for any given test sensitivity
- The lower the test sensitivity, the higher the required sample size for any given prevalence
- The size of population under surveillance only affects sample sizes up to n=10,000

Anaesthesia & Sch1

Definitions

- 96% of fish species gilled teleosts
- UK 2019 annual returns - 80% of regulated fish procedures on ZF; rest variety of spp. incl. trout, stickleback, cave fish



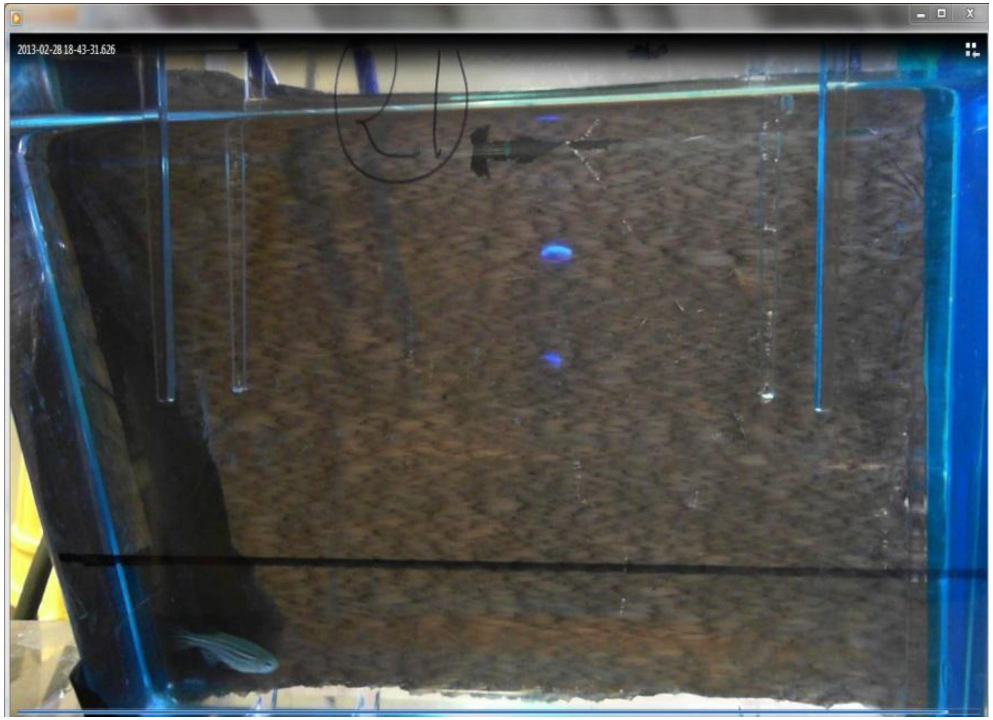
Definitions

Non-immersion fish anaesthesia:

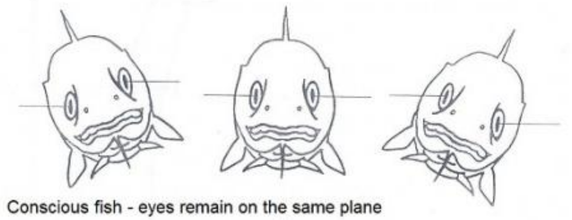
- i.v. - restraint issues
- i.c. – visceral damage
- i.m. – (dorsal musculature) – leakage
- oral – precise dosing difficult, absorption?

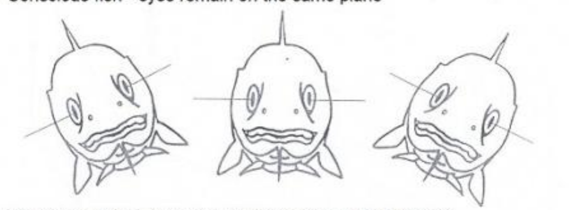
Main method of choice for fish

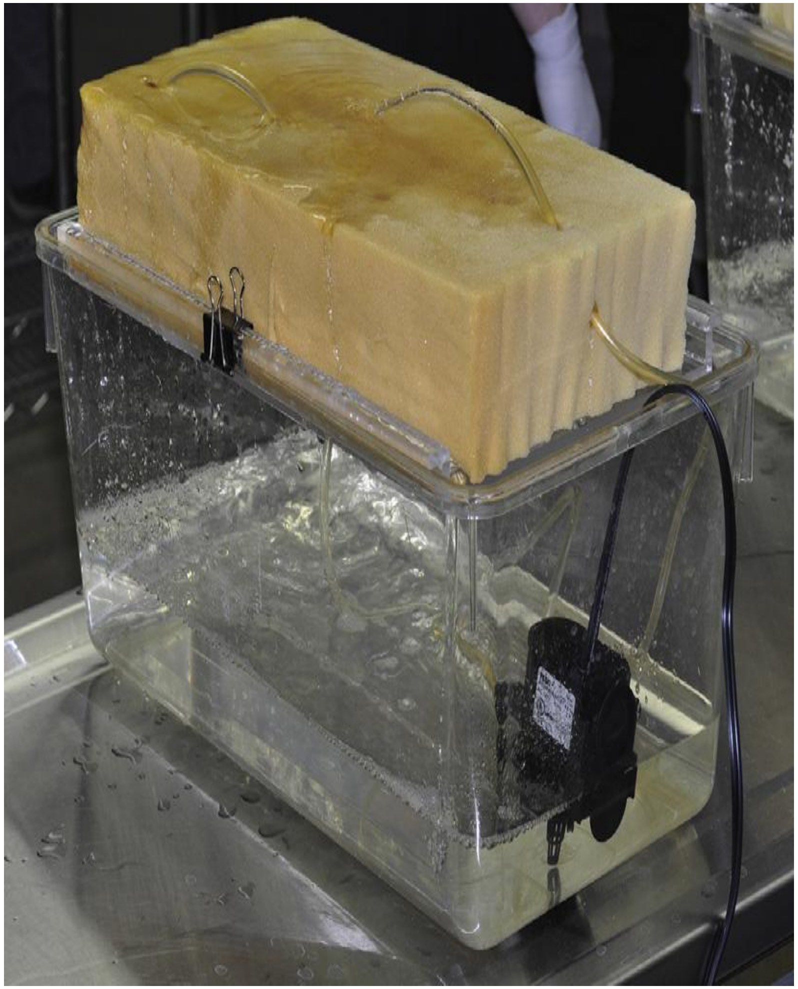
- anaesthesia: immersion (water medication)

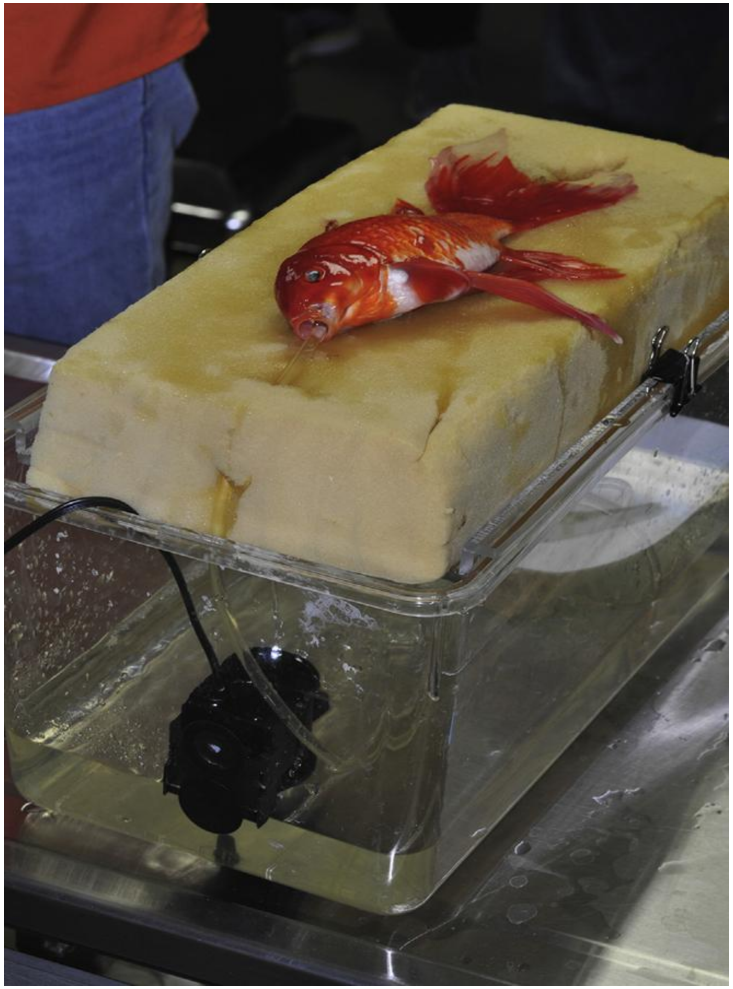


	Mammal	Fish (immersion route)
Premed	Anti-emetic Anticholinergic Sedatives Anaesthetic potentiators Analgesics	?
Short acting anaesthetic	Inhalant anaesthetic Propofol	MS222, benzocaine, 2POH, isoeugenol
Longer acting anaesthetic	α2, diss. agents, opioids etc..	
Adjusting	↓↑Inhalant conc. ↑Top-up with injectant	↑Top-up immersion bath with anaesthetic stock solution; ↓bags of anaesthetic-free water if using reservoir
Monitoring	Righting reflex Spinal reflexes Ocular reflexes Respiration Temperature Pulse O_2; CO_2	Righting reflex Spinal reflexes Ocular reflexes Respiration
Reversal	Opioids, α2	n/a; "gill flushing"


Conscious fish - eyes remain on the same plane


Unconscious fish - eyes remain fixed when fish is rotated





Pre-anaesthetic considerations

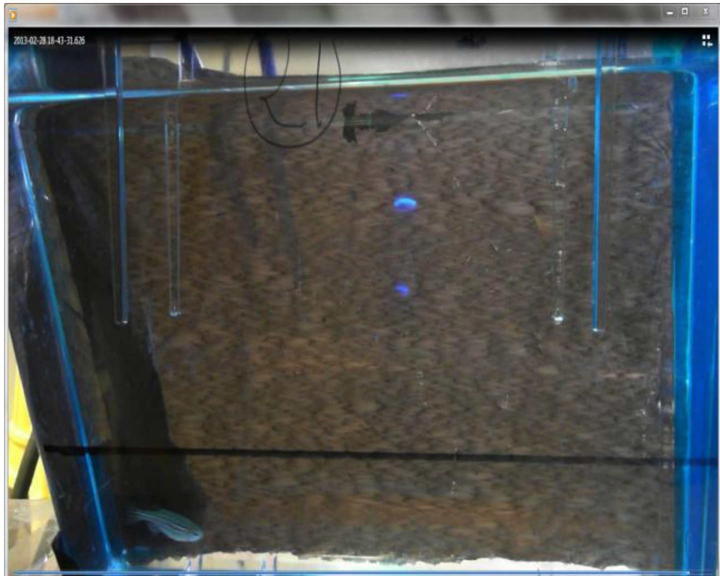
Routes of administration and pharmacokinetic considerations:

- Immersion drug uptake mostly gills,
- Generally very little through olfactory tract and skin
- But species dependent with less scaled fish absorbing higher proportion through skin
- E.g. for electric eels more quinaldine may be absorbed through skin than gills (Neiffer & Stamper 2007)
- Permeability of skin to immersion drugs varies between animals and with age

Pre-anaesthetic considerations

Routes of administration and pharmacokinetic considerations:

- Skin only permeable to water and small, nonpolar molecules limited by mucus and scales
- Gills with varying absorption - lipophilic compounds will diffuse across gills but not ions with mol weight > 100;
- even with ideal absorption 95% of immersion anaesthetic wasted!



Pre anaesthetic considerations

- Fasting -12/24/48h (WF cost?)
- Health?
- Preparation of transport, anaesthetic and recovery tanks
- Oxygenation of water
- Out of water procedure?
- poikilo/endothermy? Could temperature become an issue?

Pre anaesthetic considerations

Peri-procedural parameters to be monitored:

- Opercular beat rate
- Swimming behaviour: coordination, activity level
- Righting reflex (maintenance of equilibrium?)
- Responsiveness to tactile stimuli (blunt forceps)
- Eye roll
- Return-to-feeding time (post recovery)

Criteria for suitability of anaesthetic (Brown, 1994/2010):

Induce stage III anaesthesia within 3 mins

- Offer a 90s window for out-of-water procedure
- be safe to fish when used for 30 mins
- allow full recovery within 5 mins
- Rapid metabolisation or secretion
- Zero or short withdrawal period*

*degree-days, calculated by multiplying mean daily water temperature by total number of days on which temperature was measured; for any off-label use 500 d/d is minimum withdrawal period established by European Economic Commission Directive No. 82/2001

Anaesthesia in fish – anaesthetic agents

Agent	Dose to induce surgical anaesthesia	Properties
Benzocaine	25-200mg/l	+ Rapid uptake - Hypoxia Efficacy affected by water temperature --> poor safety margins for warm water species; Poor safety margin for salmonids
Buffered tricain (MS 222)	100-300mg/l	+ Rapid uptake through gills due to high lipid solubility, inexpensive - Induction of hypoxia; Effects on CV system / vasoconstriction make long term anaesthesia hard to manage, Sensitive to light
Clove oil + polysorbate 80 (Aqui-S®)	15-25mg/l	+ No withdrawal period, very fast recovery for lower to medium doses - Small safety margin when using higher concentrations, interactions with local anaesthetics
2-Phenoxyethanol	200-800mg/l	+ Inexpensive, easy to dose, Fast onset - Questionable analgesic properties, oily consistency requires mixing with water
Buffered quinaldine sulphate	25-40mg/l	+ Very fast induction and recovery - Expensive, poor safety margin in some species

Anaesthesia in Fish - stages

Stage	Plane	Description	Signs	Dose required (MS 222, mg/l, 3-5 mins)
I	1	Light sedation	Reduced motion, responsive to stimuli, ↓ventilation possible	20-30
	2	Deep sedation	Same as 1 but less responsive to stimuli, some analgesia	50-80
II	1	Light anaesthesia	Loss of equilibrium, still some response to tactile stimuli, good analgesia, ventilation↓↓	100 (Leopard) 125 (AB)
	2	Deeper anaesthesia		125-150 (?)
III	n/a	Surgical anaesthesia (formerly "stage 4")	As in II but total loss of responsiveness to stimuli, ventilation ↓↓↓ and very shallow	125-200
IV	1	Anaesthetic overdose	Gasping, no other signs of ventilation	250-300
	2	Medullary collapse	Total absence of opercular movements, death (after 15-30mins)	>400

adapted for zebrafish from *own data, 2011-14*, *Treves-Brown (2000) Applied Fish Pharmacology* and *Roberts (2010) Fundamentals of Ornamental Fish Health*

Anaesthesia in fish – recommended species' doses

- MS 222 (buffered tricain)
  - ZF: 100-200mg/l
  - Trout: 80-180mg/l
  - Carp: 30-200mg/l
  - Medaka: 200-300mg/l

- Benzocaine
  - ZF: 50-100mg/l
  - Trout: 25-35mg/l
  - Carp: 50mg/l

Why such differences?

- Mucus layer (gill, tegument) and scale cover vary from fish to fish

- MS 222 is fat soluble

Getting the basics right?

-Oxygenation
-Buffering
-Temperature ($\Delta T<1^{o}/h$) – thermal buffer?
-Water volume – min of 1l

European ZF procedure survey 2016 (n=98; Lidster et al. 2017)

- 93% of respondents buffer to neutral pH
- The depth of anaesthesia most commonly monitored by response to stimuli, visual assessment, ventilation rate and posture.
- >50% of respondents report adverse effects during MS222 anaesthesia incl. rapid swimming, escape responses, gasping and avoidance
- Terminating laboratory fish is necessarily a two step process but in 17% of cases death was not confirmed.
- 48% did not use any methods of refinement

ZF, guppies & medaka - use cameras!

Humane killing & Sch1 – legal context

Schedule 1:

- List of methods for humanely killing (euthanizing) animals, approved by the Home Office
- Under section 21 of the Animals (Scientific Procedures) Act 1986 (2012) Amendment Regulation
- There are two sections:

(i) Table A for all animals other than foetal, larval and embryonic forms

(ii) Table B for foetal, larval and embryonic forms

Euthanasia / Sch 1

Which of these methods is consistent with ASPA 1986 (2012 Amendment Regulation) Schedule: 1, listing humane killing methods for protected species?

5.2kg salmon: concussion with heavy metal rod then destruction of the brain

Yes – ALTHOUGH, BEFORE 2018, ANIMAL WOULD HAVE EXCEEDED WEIGHT LIMIT FOR THIS METHOD

Euthanasia / Sch 1

Which of these methods is consistent with ASPA 1986 (2012 Amendment Regulation) Schedule 1, listing humane killing methods for protected species?

1 yo Zebrafish: netting, concussion by "snipping" index finger onto cranium, followed by checking reflexes for vital signs

NO – CHECKING REFLEXES NOT AN ACCEPTABLE METHOD FOR CONFIRMING DEATH

Euthanasia / Sch 1

Which of these methods is consistent with ASPA 1986 (2012 Amendment Regulation) Schedule 1, listing humane killing methods for protected species?

4 wo Zebrafish: netting, injection of 2ml/kg pentobarbital with 35 gauge needle, followed by destruction of the brain

YES – CONSISTENT WITH SCH1

Euthanasia / Sch 1

Which of these methods is consistent with ASPA 1986 (2012 Amendment Regulation) Schedule 1, listing humane killing methods for protected species?

3mo rainbow trout: electrocution with 24V/14A, followed by destruction of the brain

NO – ELECTROCUTION NOT IN SCH1

Euthanasia / Sch 1

- Physical methods – concussion, then destruction of the brain
- Non physical methods – Overdose of anaesthetic
- Injection of pentobarbital - 0.06mg/g body weight
- Immersion bath using range of anaesthetics:

Euthanasia / Sch 1

- MS 222 (>500mg/l, 15-45 min)
- 2POH (>1500mg/l, 15-30 min)
- Benzocaine (>250mg/l, 30-45 min)

Euthanise individually!

Euthanasia / Sch 1

When destruction of the brain is not an option

- Permanent cessation of circulation (only ascertainable when heart is exposed e.g. during cryoinjury procedures)
- 30min overdose followed by rapid freezing (needs PPL authority)
- 5min overdose followed by perfusion (needs PPL authority)
- If none of these are practicable, contact ASRU

Severity considerations

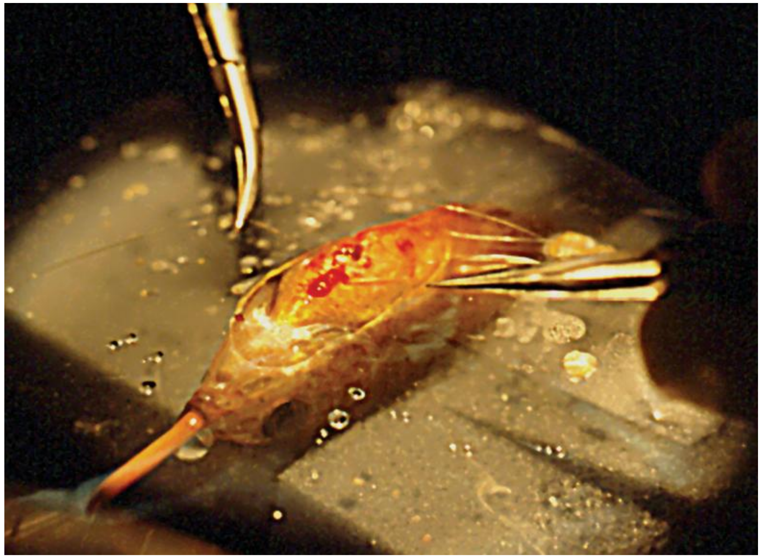
- (Sub – threshold)
- Non-recovery
- Mild
- Moderate
- Severe

Severity considerations – examples

1. Control animals in genetic study → Sch1?
2. ZF: 16 hours food retention effecting slight behavioural change?
3. ZF: Cardiac excision procedure with 10-15% mortality?
4. Carp: Fin clip under anaesthesia?
5. ZF: VIE implant under anaesthesia?
6. Medaka: superficial mucus swab without anaesthesia?
7. Rainbow trout: blood sample for health screening ?
8. Fish anaesthetised for surgical implantation of PIT tag in coelom

Minor procedures and aseptic technique

- Injection sites
- Fin clipping & other ID systems
- Aseptic technique
- Post op care

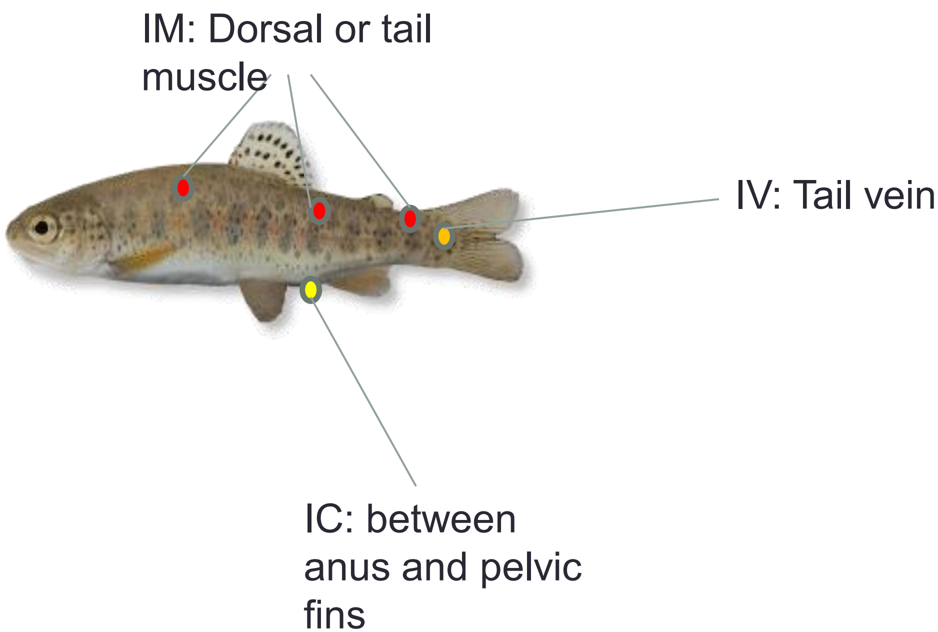


Harms (2005)

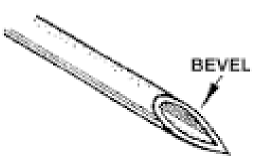
Injection sites

- IM – 85% of fish may be muscle so easy enough to find! E.g. dorsal musculature,
- IC – along midline between pectoral fin and anus, fish in dorsal recumbency
- IV – lateral caudal peduncle, tricky in smaller fish (blind technique)

Injection techniques



BEVEL ALWAYS POINTS UPWARDS!!!

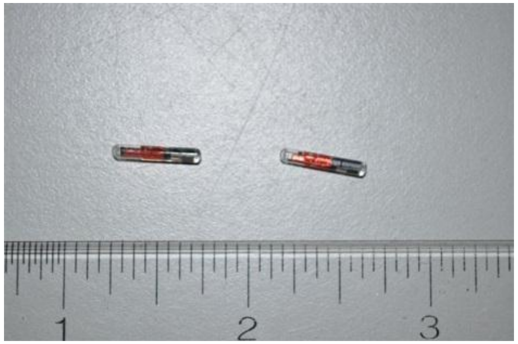


Fin clip and other minor procedures (e.g., scale removal)

- For batch ID or for genetic material
- Size 10/15 curved scalpel – remove max 50% of tailfin distal of forking
- 2-6 weeks to full regeneration (ZF)
- Anaesthetize to allow at least 60s out-of-water procedure
-
- Aseptic technique similar to that in other species: sterile nets; sterile scalpel/surgical scissors; sterile drape or fine kitchen towel; sterile system water for squirting/drenching BUT DON'T DISINFECT SKIN; JUST SWAB EXCESS MUCUS
- Batch surgery – use bead sterilizer or autoclave
  - in between (or have several sets of sterile
  - instruments ready

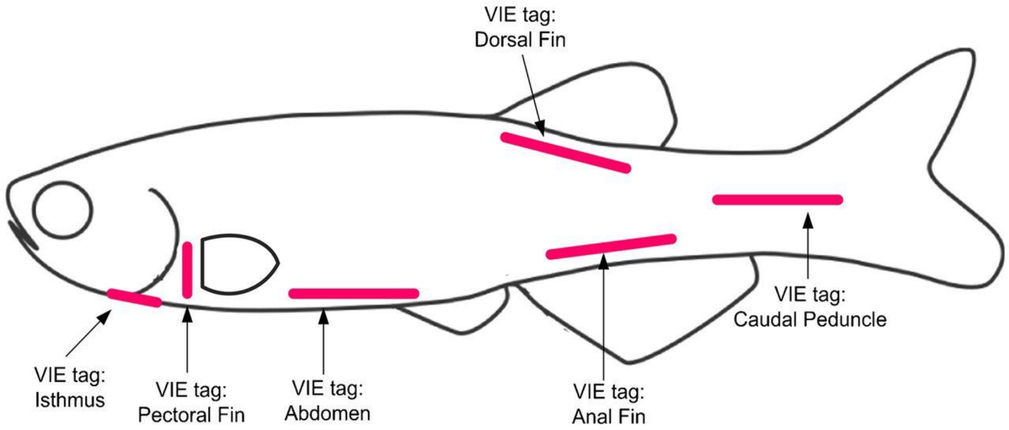
Tagging – Passive integrated transponder (PIT)

- First developed to monitor salmon movements in the 1980s
- 8-12mm long, not suitable for fish < 15cm TL
- Less invasive alternative preferable (VIE, even fin clip
  - for smaller fish)
- Can exceed limits of “minor procedure” e.g. surgical implantation of PIT tag in coelom



Tagging – Visible implant elastomer (VIE)

- Implant applied subcutaneously in several sites
- Batch tagging, no additional data stored electronically (unlike PIT tag)
- Smaller and thinner than PIT tag



Post surgery

- Check on full recovery/feeding
- Analgesia: lidocaine 2-5mg/l

Some final legal considerations

- Protection from free swimming stage – use minimum; guppies protected from day one!
- Prospective **&** reported severity – surgical procedures often “severe”
- Refinement obligation
- Sch1 with completion!!
- Sch2!!!

Between evidence base & speciesism – paradigms and debates on fish welfare

Fishes as subject of animal welfare science

11,000,000 – 80,000,000 fish/year

10,000,000-20,000,000 fish per/year

4,000,000,000,000 fish per year

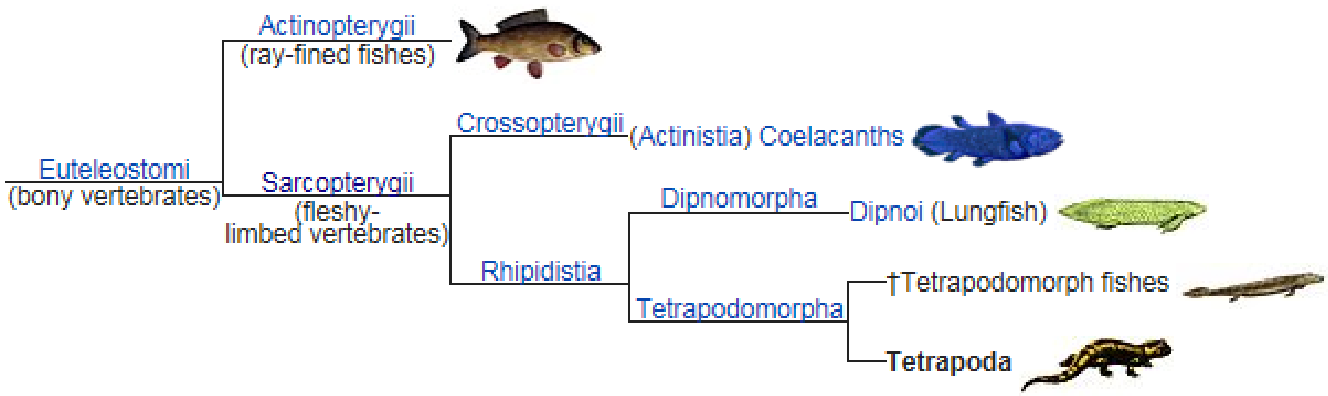
Fish in laboratories

- 480 000 fish procedures in UK 2019, 82% of which ZF (HO returns, 2020)
- Worldwide estimate: >3,250 institutes in >100 countries work with zebrafish and >5 million are used per year

Lidster K, Readman GD, Prescott MJ, Owen SF (2017) International survey on the use and welfare of zebrafish in research. Journal of Fish Biology doi:10.1111/jfb.13278

Humans are fish…

- (cladistically) as all tetrapods, including mammals, stem from lobe finned fishes emerging during the Devonian period



First steps in recognising pain sensitivity in fish & the perennial debate on sentience

General principles in fish welfare – pain sensitivity?

Sentience - "the capacity to feel, perceive, or experience subjectively"

Prof.dr. F.J. Verheijen (1920 -)

Gewoon hoogleraar De vergelijkende fysiologie vanaf 1 augustus 1975

General principles in fish welfare –pain sensitivity?

Verheijen (1983):

- Hooked and released 30 juvenile carp:
- Reactions to hook – spitting, headshaking (even with slack line) – "pain" ?
- Buoyancy changes due to expulsion of spitgas when line was pulled taught

General principles in fish welfare – pain sensitivity?

Verheijen (1983):

- Different behavioural pattern when line was kept slack
- ↑ time to feeding, erratic behaviours – fear, anxiety (?)

General principles in fish welfare – pain sensitivity?

Verheijen (1983):

- Work was partially funded by the Dutch Angling Society who subsequently refused permission for international publication of results;
- First mentioned in New Scientist feature in 1987; now considered seminal work

General principles in fish welfare – pain sensitivity?

Sneddon (2003):

- 3 nociceptor types on head of rainbow trout, A-δ and C fibres in trigeminal ganglion
- nociceptive events could change the motivational state of trout & post nociceptive behaviours after injection of acetic acid and bee venom:
- Lip rubbing, ↓↑OBR, ↓activity

“Why fish do not feel pain”

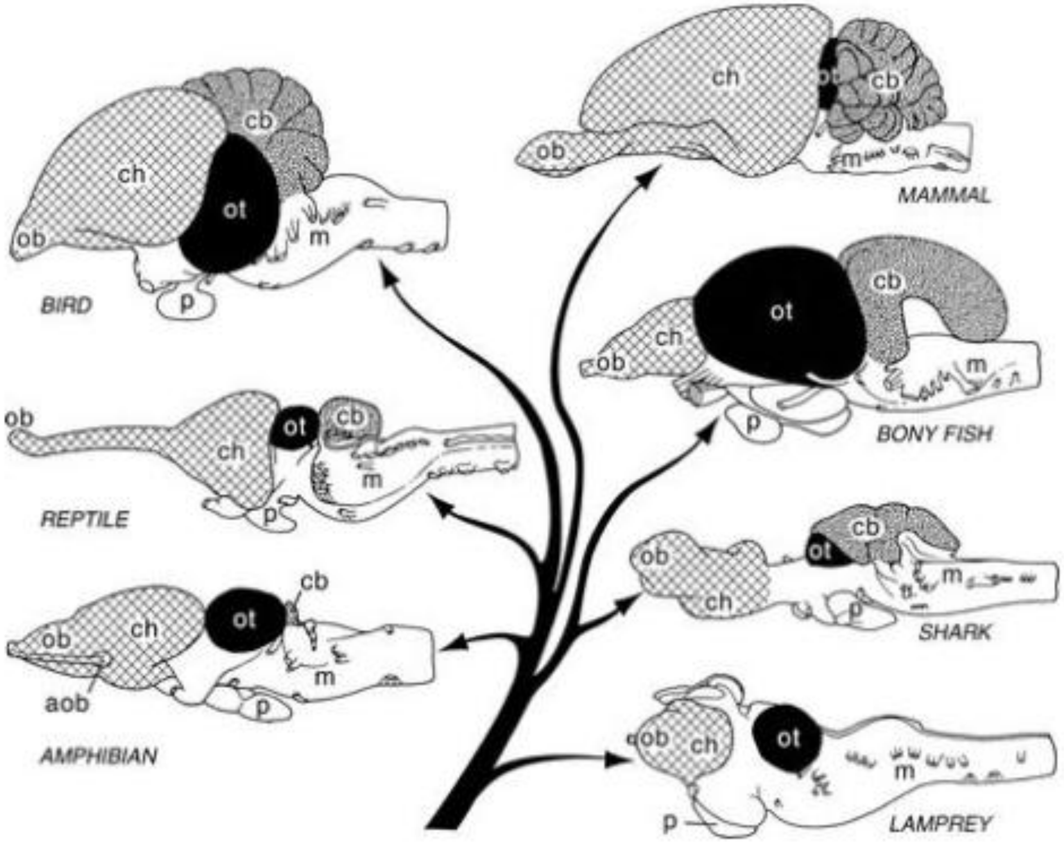
Brian Key (2016)

“Accepting at face that fish may feel pain (…) comparable to believing causal association between MMR vaccination and the development of autism in children”

“Why fish do not feel pain”

Brian Key (2016)

- Devastating consequences through legislative restrictions on fish-related activities with potentially serious negative implications for
- native subsistence fishing,
- human food supply
- economic development
- “Benefit of the doubt” argument can quickly lead to unsupported anthropomorphic conclusions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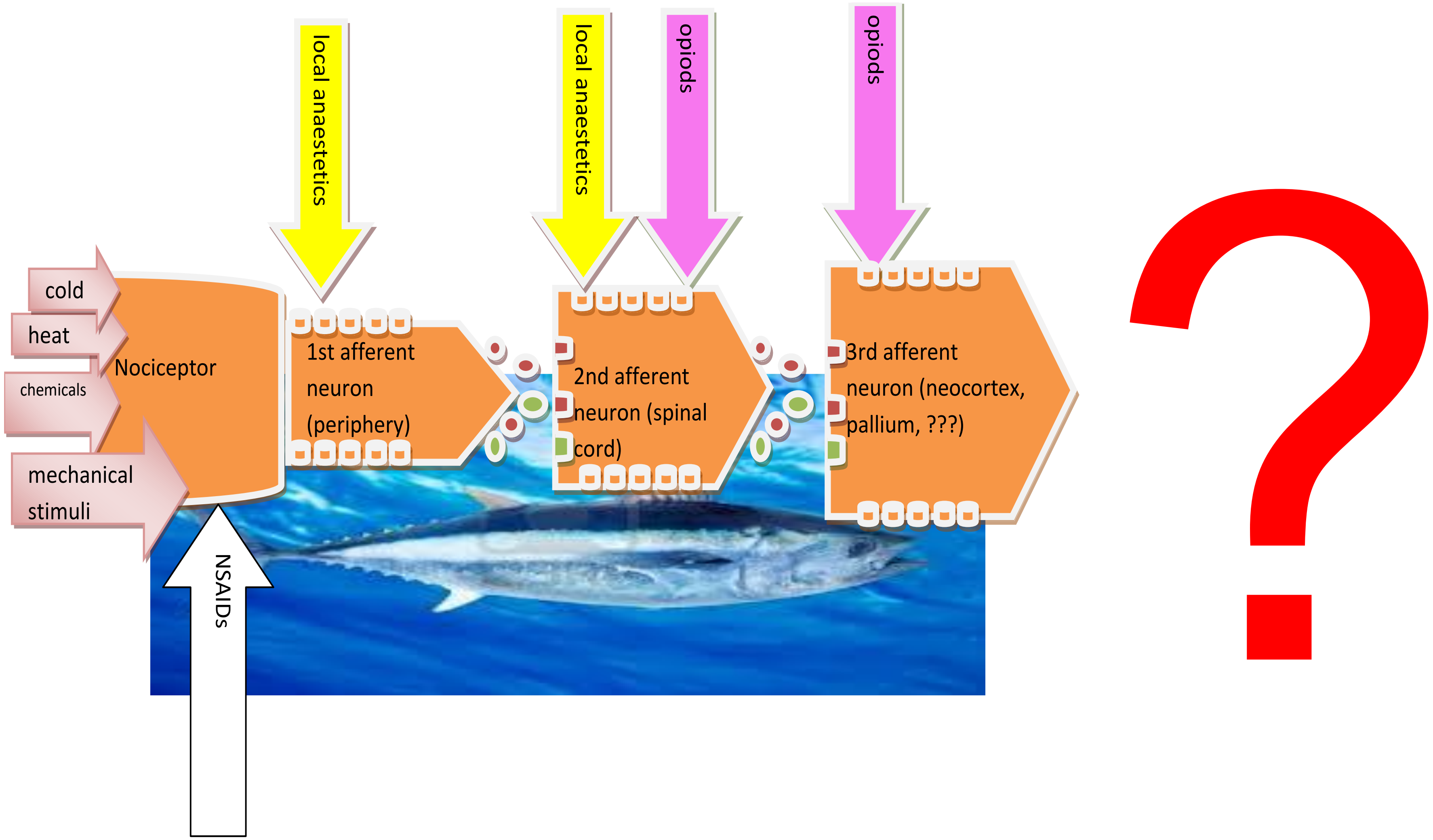
local anaestetics
local anaestetics
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Nociceptor
1st afferent neuron (periphery)
2nd afferent neuron (spinal cord)
3rd afferent neuron (neocortex, pallium, ???)
NSAIDs
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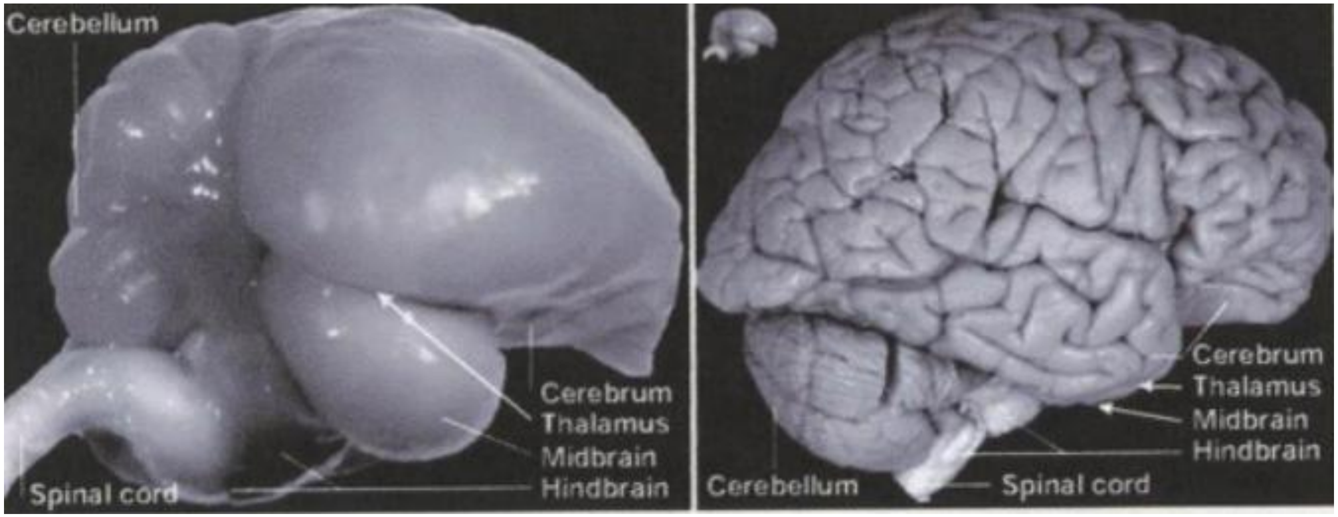
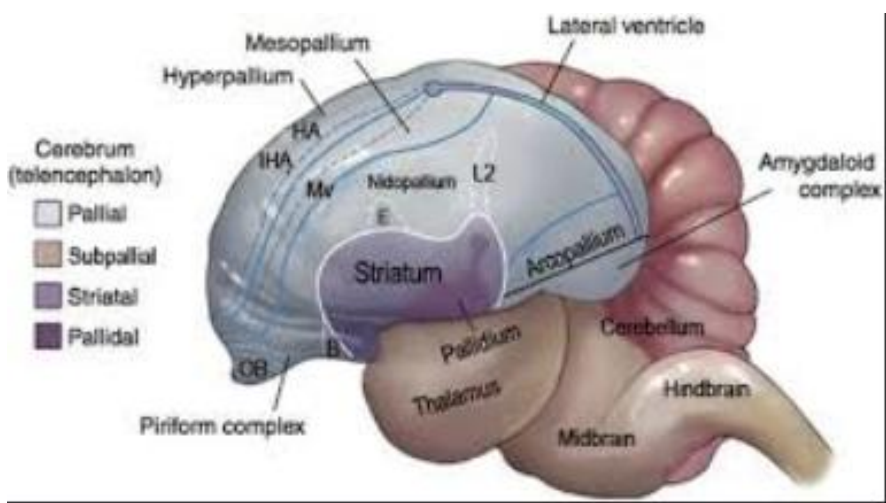
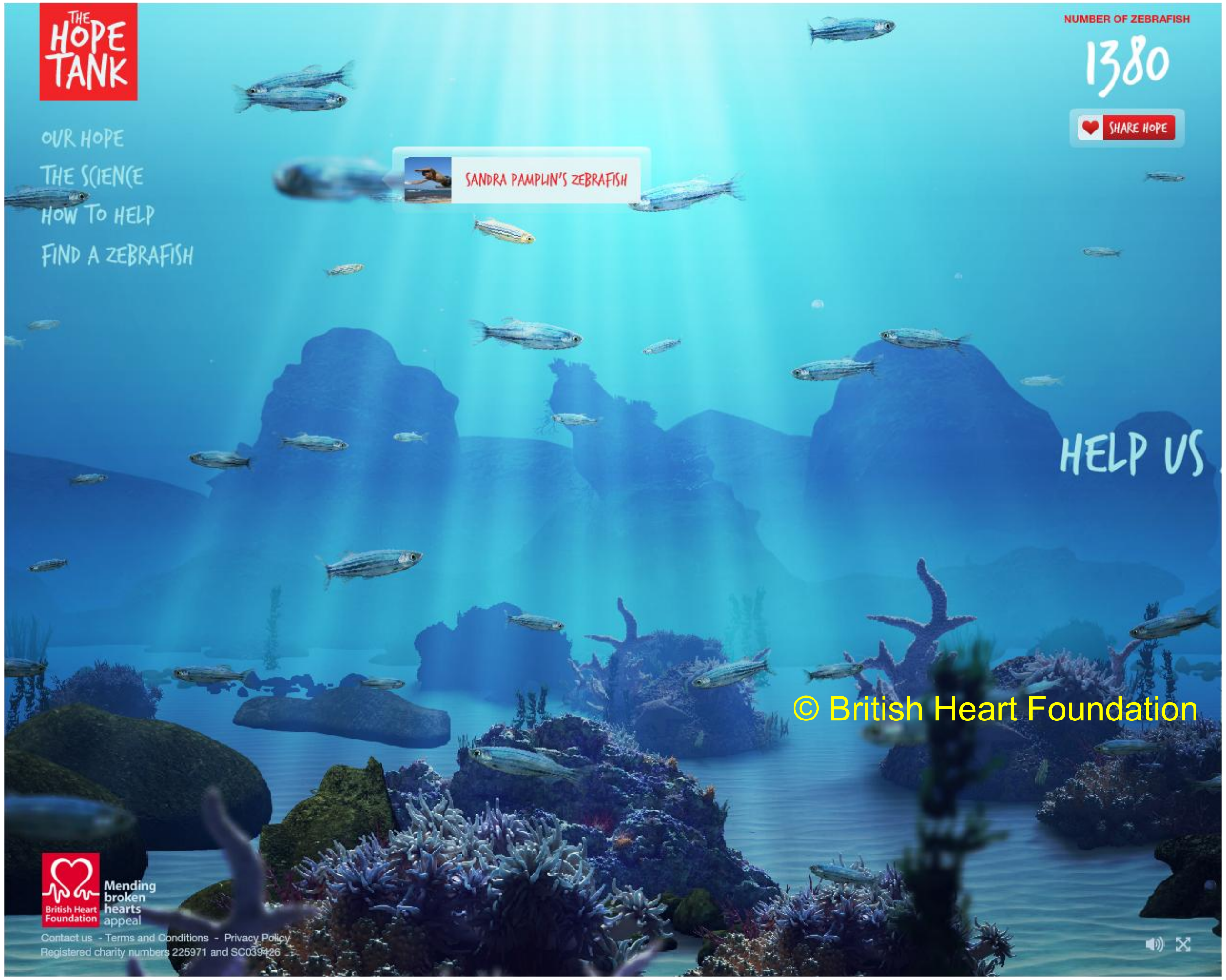


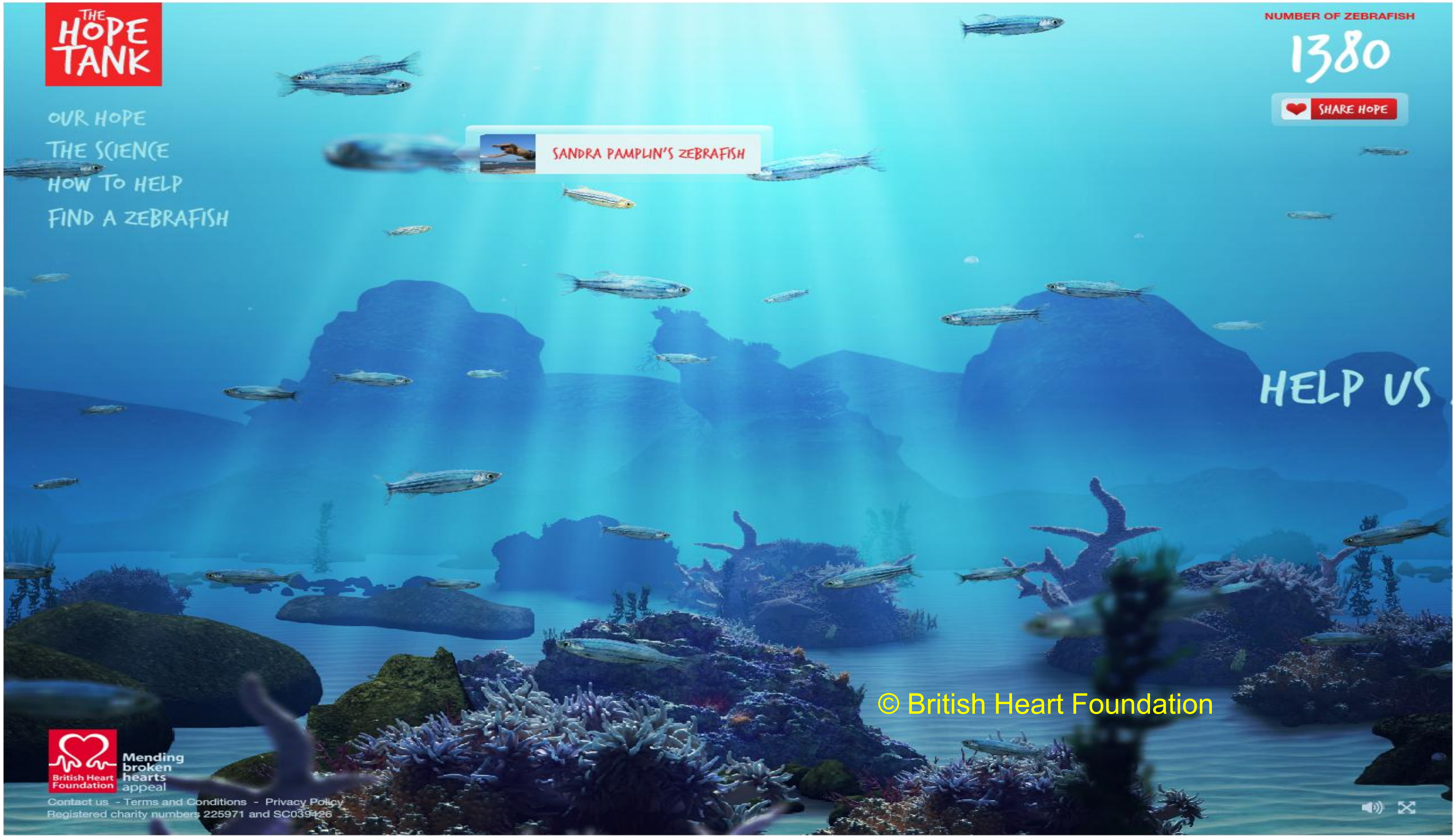
Jarvis et al. (2005)



Speciesism


THE HOPE TANK
OUR HOPE
THE SCIENCE
HOW TO HELP
FIND A ZEBRAFISH
SANDRA PAMPLIN'S ZEBRAFISH
NUMBER OF ZEBRAFISH
1380
SHARE HOPE
HELP US
© British Heart Foundation
British Heart Foundation
Mending broken hearts appeal
Contact us - Terms and Conditions - Privacy Policy
Registered charity numbers 225971 and SC039426


NUMBER OF FERRETS
34
SHARE THE GIFT OF HEARING
Lend me an ear appeal
National Deaf Children's Society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British Heart Foundation
Mending broken hearts appeal
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Registered charity numbers 225971 and SC039426
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Conclusion

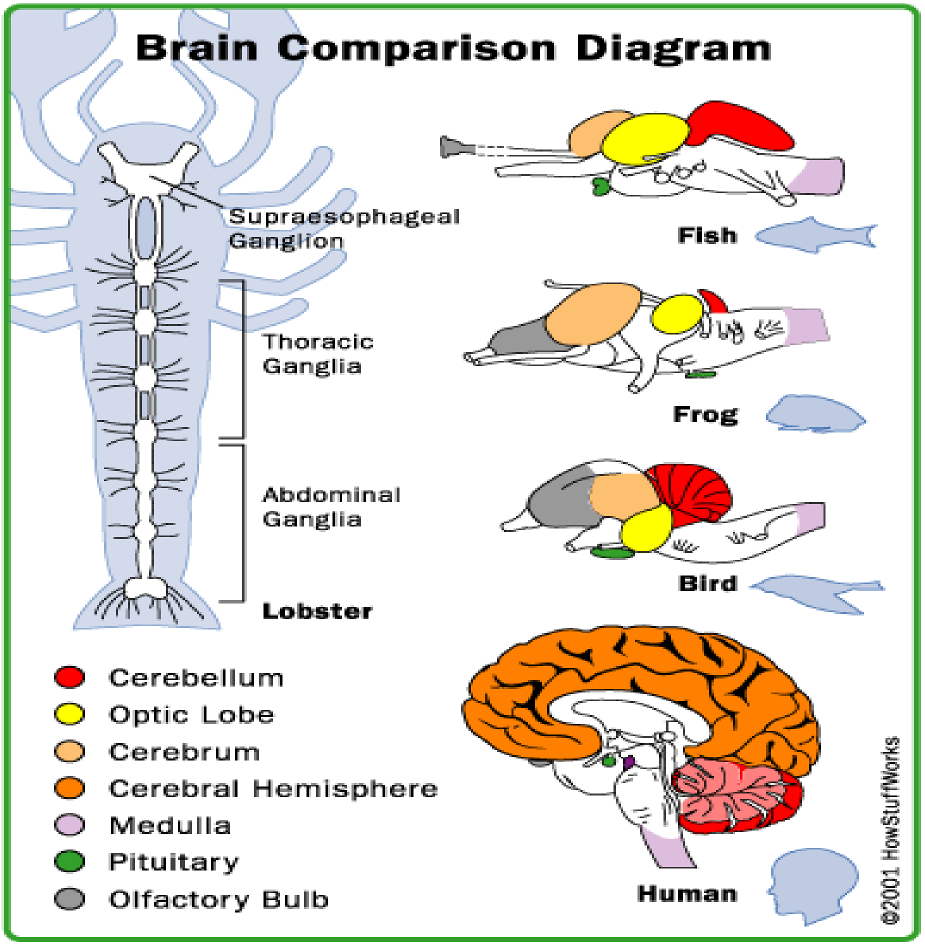
The Göttingen Minipig is increasingly being selected for all aspects of pharmaceutical research

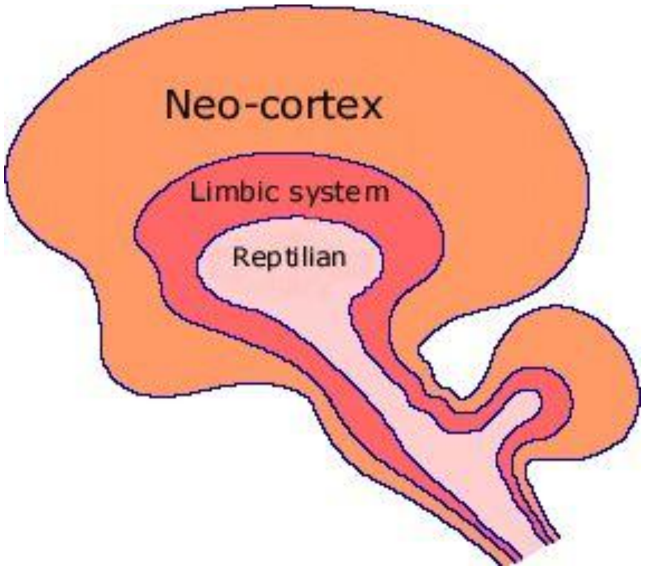
- The smallest of all commercially available mini- and micropigs
- Available in large uniform groups
- Well-defined and managed genetics
- Well-defined health status
- More and more available background data
- Easy to fulfil natural needs, socialize and train for scientific procedures
- More accepted by the general public

Speciesism

- Assigning certain value on membership of a specific genetic clade/species/taxon
- For example – special protection for dogs, cats & horses under European laboratory animal legislation (but not pigs, psittacines..)

Fish – sentient species?





brainevolution.html

Fish – sentient species?

- Birds and reptiles would be equally incapable of any “higher” response as they do not possess a neocortex
- Different species can use different neurological structures and systems to handle the same functions
- E.g. avian pallium => functional rather than structural homologies between avian and mammalian brains

Jarvis, Güntürkün et al. 2005

The perennial debate…

Key, Brian (2016) [Why fish do not feel pain](#)
Animal Sentience 2016.003

Author Website
http://www.uq.edu.au/sbms/staff/brian-key

Abstract

Abstract: Only humans can report feeling pain. In contrast, pain in animals is typically inferred on the basis of nonverbal behaviour. Unfortunately, these behavioural data can be problematic when the reliability and validity of the behavioural tests are questionable. The thesis proposed here is based on the bioengineering principle that structure determines function. Basic functional homologies can be mapped to structural homologies across a broad spectrum of vertebrate species. For example, olfaction depends on olfactory glomeruli in the olfactory bulbs of the forebrain, visual orientation responses depend on the laminated optic tectum in the midbrain, and locomotion depends on pattern generators in the spinal cord throughout vertebrate phylogeny, from fish to humans. Here I delineate the region of the human brain that is directly responsible for feeling painful stimuli. The principal structural features of this region are identified and then used as biomarkers to infer whether fish are, at least, anatomically capable of feeling pain. Using this strategy, I conclude that fish lack the necessary neurocytoarchitecture, microcircuitry, and structural connectivity for the neural processing required for feeling pain.

Fish do not feel pain and its implications for understanding phenomenal consciousness

Brian Key

Received: 14 April 2014 / Accepted: 6 December 2014 / Published online: 16 December 2014

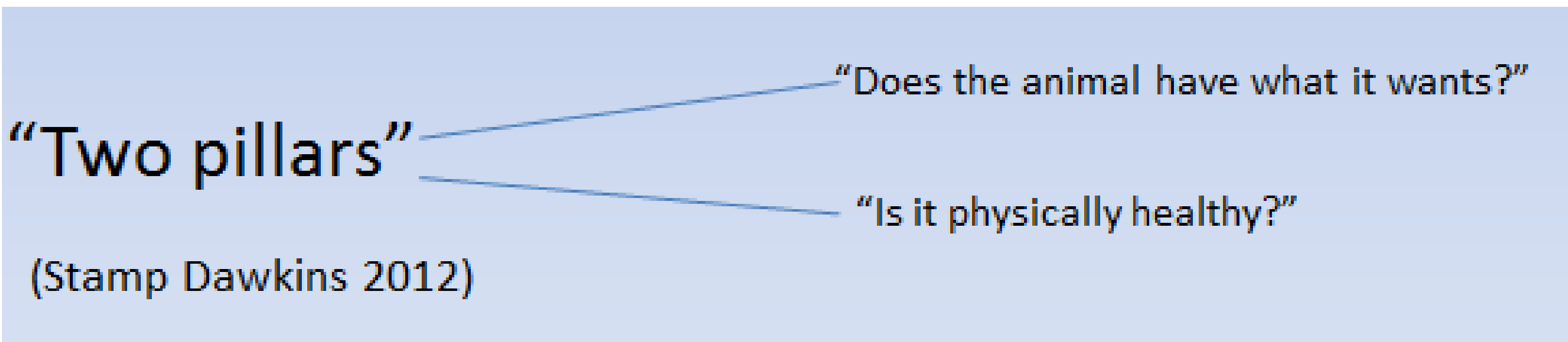
Abstract Phenomenal consciousness or the subjective experience of feeling sensory stimuli is fundamental to human existence. Because of the ubiquity of their subjective experiences, humans seem to readily accept the anthropomorphic extension of these mental states to other animals. Humans will typically extrapolate feelings of pain to animals if they respond physiologically and behaviourally to noxious stimuli. The alternative view that fish instead respond to noxious stimuli reflexly and with a limited behavioural repertoire is defended within the context of our current understanding of the neuroanatomy and neurophysiology of mental states. Consequently, a set of fundamental properties of neural tissue necessary for feeling pain or experiencing affective states in vertebrates is proposed. While mammals and birds possess the prerequisite neural architecture for phenomenal consciousness, it is concluded that fish lack these essential characteristics and hence do not feel pain.

e human brain that is directly responsible

Speciesism

- Assigning certain value on membership of a specific genetic clade/species/taxon

State of (zebra)fish welfare today

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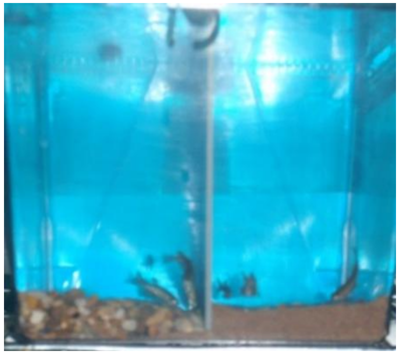
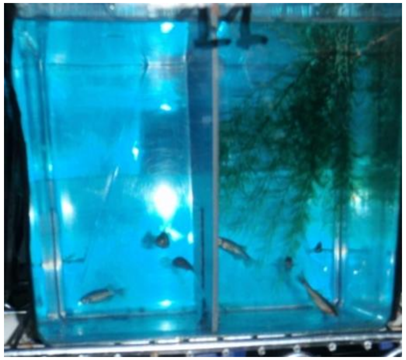
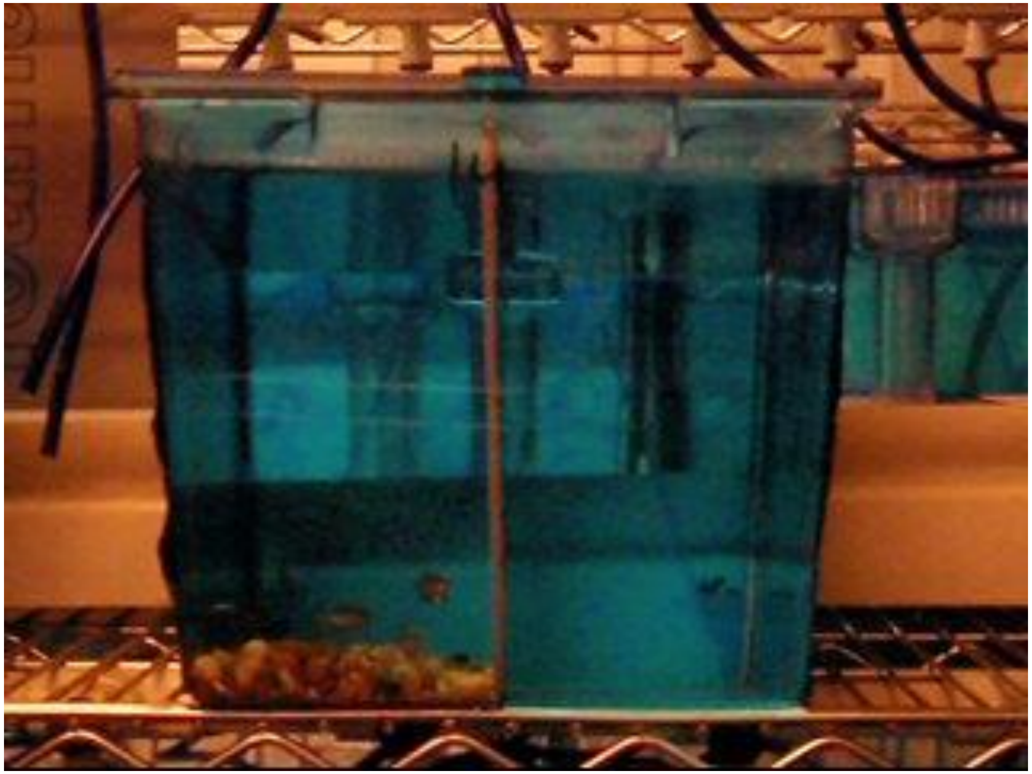
Does the animal have what it wants?

Paul Schroeder, Soffia Jones, Iain S Young and Lynne U Sneddon (2014): What do zebrafish want? Impact of social grouping, dominance and gender on preference for enrichment. Laboratory Animals ***48 (4)*** *328-327*





- Most enrichment cues significantly preferred over barren environment except airstones (significantly avoided
- Images of (gravel) substrate alone sufficient to elicit preference behaviour, attracting occupancy rates almost as high as the actual substrate



Is the animal healthy?

- Microsporidiosis
- *Pseudoloma neurophilia.* “skinny fish disease” or scoliosis
- 90-95% prevalence in European ZF facilities (estimate, QM Diagnostics, 2015) with 10-30% of healthy fish and 90% of emaciated fish infected (Matthews et al. 2001)
- **Diagnosis –** histology; PCR (water, eggs)
- **Control** - Pre-screening all brood fish by PCR → Pseudoloma free stock.

State of (zebra)fish welfare today

"Two pillars"
- "Does the animal have what it wants?"
- "Is it physically healthy?"

(Stamp Dawkins 2012)

"The only animal species which we can justifiably use for invasive surgical research procedures is the dog"

Prof Eddie Clutton (2013)

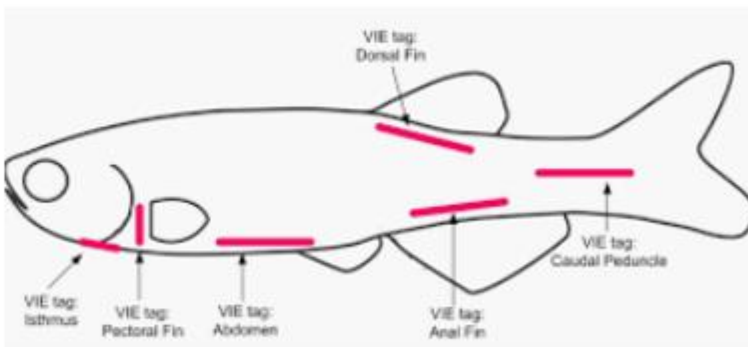
Questions

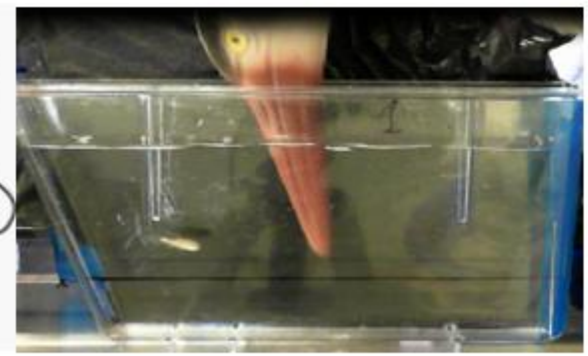
Break out session 2

- **PIL AB Live Webinar - Break out session**

PIL AB Live Webinar - Break out session

For a series of elastomer implants (3mm x 0.2mm, left picture) for tagging 200 zebrafish (AB wild type, all female, 6mo, avg. 2.8cm and 0.95g) in an experimental setup exploring visual memory and acquired avoidance learning, it has been decided to sedate the fish with MS 222 at 80mg/l, with subcutaneous injection of two implants achieved through a trochary device, one into the dorsal aspect, 3mm ventral to the spine and another diagonally, from a point just distal of the anal opening towards the caudal peduncle.





The tagging protocol constitutes a new addition to this study which commenced 3 months ago and so far has not required a Project License. The principal investigator does not expect any change to this status quo.

Over the next 15min, please consider each of the questions below. Each participant will only be asked to present their deliberations for one question but please go through the other tasks so that you can contribute to the ensuing discussion.

Tasks

- Does the tagging process necessitate any change in the licensing requirements? Explain your reasoning.
- Comment on the utility of the anaesthetic/sedative protocol. Where would you set the threshold between sedation and anaesthesia?
- So far, the attempted trigger for avoidance learning was bright halogen lights emitted at high frequency. This has not yet met any of the expectations; therefore it has been decided to inject alarm pheromone and a visual stimulus (mock predator, right picture) into the tank water instead. Regardless of the issues raised in tasks (1) and (2), does this affect licensing requirements?