**Fish in the laboratory**

**Introduction**

Aquatic species are increasingly used in scientific procedures, for fundamental studies such as developmental biology and applied research. Fish represent the second largest group of animals used in research, being second only to mice. Over 14% of the animals used in 2014 were fish, the majority in fundamental studies, but significant numbers in applied studies such as ecotoxicology.

Fish are an adaptable and diverse group, naturally inhabiting a vast range of habitats. Some fish can adapt to be able to live in either salt water or fresh water, and others can change sex depending on the conditions. Fish account for almost half of known vertebrates, with about 28 000 species. Fish can be divided into bony fish (Osteichthyes or teleost fish, 96%), cartilaginous fish such as sharks and rays (Chondrichthyes, < 4%) and jawless fish such as lampreys (Agnatha or cyclostomes, < 1%).

There is a lack of published research into the husbandry needs of many species of fish however satisfactory husbandry practices have been developed through experience. Fish welfare science is an expanding field, and researchers are advised to consult the latest research for up-to-date information on these species. A brief introduction is provided here for three commonly used species, zebrafish (Danio rerio), rainbow trout (Onchorhynchus mykiss) and common carp (Cyprinus carpio).

**Sources of fish**

Commonly used fish species are generally obtained from commercial stocks. High health sources are available for some species (e.g. Zebrafish), but others are only available from commercial fish farms with variable disease status. There is likely to be considerable variation within and between stocks, even in a genetically homogenous population, due to the plasticity seen in fish. Where possible, fish should be sourced from a reputable supplier with a high health status and known genetic makeup.

**General biology**

Fish are ectothermic, and biological processes are temperature-dependent. Ectotherms do not have to maintain body temperature and therefore can survive on much less food than homeotherms.

Reproduction varies with the species, some fish producing large quantities of eggs and providing no protection for the young, while others give birth to live young.

Fish exhibit a wide range of complex behaviour patterns and have different environmental requirements which vary with species. Some species have to be housed individually, whereas others are best kept in groups. An appropriate stocking density is important, but stocking density is not the only factor influencing welfare. Other factors are involved, including water quality and resource availability. Some fish are territorial, and may show aggression if competing for resources. This can be prevented by avoiding defensible resources and visual cues, which can be used to define territories. Covered areas may reduce predator-avoidance behaviour. Fish behaviour is affected by husbandry procedures (e.g. feeding, cleaning), so normal behaviour will not be seen at these times.

**Biological data**

There is significant variation within and between species of fish. Normal values for haematology and biochemistry are generally not useful. Growth depends on numerous factors. Therefore records of biological parameters for the fish in the particular system being used should be kept, to enable meaningful conclusions to be drawn from analysis of any subsequent data.

**Husbandry**

The most important factor in the health of fish is water quality. Water must be clean and aerated. Either fish may be kept in static water systems at low stocking density and the water changed regularly, or kept in circulating systems with biological filters to remove toxins. Tanks can be glass or plastic. Tanks should be kept on a dark surface away from light, and tubing carrying fresh and waste water should be non-transparent, to minimise algal growth.

For all fish some treatment of the water is required. As a minimum, it should be filtered through charcoal to remove organic compounds and chlorine. Salts may need to be added to produce an appropriate pH and conductivity. Aeration is usually required.

Non-filtering systems need considerable space due to the low stocking density and high maintenance requirements. Changing a proportion of the water daily is needed. In recirculating systems water is filtered to remove debris, passed through charcoal and biological filters to remove toxins, then aerated before recirculating. Ultraviolet sterilisation can also be used to reduce microbial growth. If the filters are not working properly, denitrifying bacteria will be inhibited and toxic material may build up.

Biological filters usually consist of boxes of material with a large surface area. These act both as mechanical filters, and provide a large surface area for colonisation with denitrifying bacteria, which degrade toxic ammonium compounds to nitrites, then to nitrates, which is less toxic. Disruption of the bacterial filter can result in a rapid deterioration in water quality. Biological filters can take several weeks to become established.

Regular cleaning of tanks is required in all systems. Tanks can be autoclaved or treated with disinfectant, for example hydrogen peroxide.

**Table 1 Water-quality requirements for fish species**

**Parameter Zebrafish Rainbow trout Common carp**

Temperature (◦C) 18–28 (25–28 optimal) 12–21 14-20

Dissolved oxygen (mg/l) >6 >5 6-8 (can survive

>0.3-0.5)

Carbon dioxide (mg/l) <5 <2 5-8

Ammonia (mg/l) <0.02 <0.0125 <0.05

Nitrite\* (mg/l) <0.5 <0.3 <0.5

Nitrate (mg/l) <50 <20 <80

pH 6–8 6.5–8.5 6.5-9

Conductivity (uS) 300–1500 400–1200 >500

Salinity (g/l) 0.5–1 0.6 <5

\*toxic level of nitrite is dependent also on concentration of other ions especially chloride.

**Water quality**

Water quality affects the health of fish. Effects on behaviour or other indicators of welfare are largely unknown. Table 1 lists acceptable water-quality standards for fish.

As a minimum, in flow-through systems, temperature and dissolved oxygen should be measured at least once daily, with pH and ammonia checked at least weekly. In static systems temperature and dissolved oxygen should be measured daily, and other values depending on the frequency and amount of water exchange.

**Transport**

Small groups of fish can be placed in sealed plastic bags or boxes containing one-third tank water and filled with oxygen. Temperature should be maintained as far as possible. On arrival fish should be acclimatised to the new environment: the water conditions should be as similar as possible, and the temperature in the transport water and receiving tank allowed to equilibrate before the fish are released into the new container. Fish should be closely monitored after transport for signs of ill health.

**Identification**

For some fish it may be possible to identify them from markings. Anaesthesia or sedation may be required for tagging or marking. No single tag or marking method is suitable for all situations. Options include:

* Various types of internal and external tag, including small tags and passive integrated transponder (PIT) tags for subcutaneous and abdominal implantation, are available.
* Subcutaneous injection of small quantities of coloured compounds.
* Freeze branding, tattooing or clipping important fins should only be used if alternative methods are not suitable as these are harmful.

**Feeding**

Fish need relatively little food compared with mammals. Short periods of starvation appear not to be detrimental whereas overfeeding has many adverse effects. Different species have different requirements, and specialised texts should be consulted for detailed information. In general, first feeding, when the recently hatched fish are starting to feed independently, can be a critical period, and particular care must be taken at this time.

**Handling and Techniques**

Handling is stressful for fishes, and routine handling procedures may cause injury or death. Handle very gently, and only when necessary, with appropriate equipment, and keep them moist at all times.

Fish defecate and vomit in response to stress to conserve energy. Fishes should therefore be fasted prior to any procedure, including handling or transport. For small fish (<400 g), 24 h of fasting is sufficient, with 48 h for larger fish. High-quality water for procedures and recovery should be provided so that any gut emptying that occurs does not cause welfare problems.

Skin damage can lead to significant morbidity. In some species the skin is delicate and easily damaged. Handling can also cause myopathy. Anaesthesia or sedation is recommended to prevent damage during procedures. Procedures should be conducted as quickly as possible. Recovery from procedures may take several days, so a suitable period of recovery is required between procedures. Some water additives are available to help maintain the integrity of the skin and aid recovery after procedures.

Fishes should not normally be kept in air continuously for more than 30 s. Gills need to be kept moist.

**Anaesthesia**

Prior to anaesthesia, fish should always be checked to make sure they are healthy. Fish that are not healthy (normal movement and respiratory rate, smooth and unbroken skin, no damage or haemorrhages, scales flat against the body, gills a ‘salmon pink’ colour) should not be anaesthetised. The response of an animal to anaesthesia will depend on several factors, including temperature, species, sex, weight, maturity and fat content.

Fish should be starved for 12–24 h before anaesthesia to prevent regurgitation. Anaesthesia can be achieved by immersion of the animal in a solution of tricaine methane sulphonate (MS222), benzocaine or phenoxyethanol.

**MS222** produces dose-dependent sedation and anaesthesia, depending on the species. The level of anaesthesia depends on water temperature, water hardness, salinity, oxygen concentration and duration of immersion. Smaller fish tend to be more sensitive. Therefore a low concentration should be chosen initially. Water for anaesthesia should be taken from the holding tank. MS222 should be weighed and dissolved in a small quantity of water, which is added to the anaesthetic bath, into which the fish are then placed. The water should be aerated. MS222 can be acidic, and **buffering is essential** to raise the pH to 7–7.5.

Initially there will be increased opercular movement, followed by a reduction in opercular movement, increasing ataxia and loss of balance, then immobility. It may take 10 min for anaesthesia to be induced. Depth of anaesthesia can be judged by respiratory rate and (lack of) response to pinching the tail base. Anaesthesia can be maintained by trickling a lower concentration of anaesthetic over the gills (50–100 mg/l).

Following the procedure the fish should be placed in clean water and will return to consciousness within 1–30 min.

Table 2 gives suggested induction doses for MS222 in different species.

**Table 2 Induction of anaesthesia with MS222**

Species Sedation (mg/l) Light anaesthesia (mg/l) Deep anaesthesia (mg/l)

Zebrafish 30–50 50–100 100–200

Rainbow trout 10–30 30–80 80–180

**2-Phenoxyethanol (2-POH)** is marketed as Aqua-Sed. Dosing is easy as it comes with a measuring pump. One pump per litre of water gives a concentration of 500-700ppm. Fish should be left in the solution only until appropriately anaesthetised, then removed.

**Benzocaine** comes in two forms, a crystalline salt which can be dissolved in water, and a form which must be dissolved in ethanol first. The powder is a respiratory irritant and care should be taken. The efficacy of benzocaine is affected by the size of the fish - smaller fish require lower doses. It is also affected by water temperature. Doses range from 25 - 100 mg/L, with doses for salmonids between 25 - 45 mg/L. Induction time is generally in less than 4 minutes and when fish are placed in clean water, recovery is usually within 10 minutes. The safety margin is widest in cooler temperatures. Fish may retain some locomotor functions throughout all stages of anaesthesia, making this an unsuitable anaesthetic for use in procedures involving surgery.

**Administration of compounds**

***Immersion***

The most common route for administration is via the gills. Compounds are dissolved in a quantity of tank water, usually in a separate smaller vessel. Fish must be observed for signs of ill health during the period of exposure and the water aerated or changed to maintain water quality.

***Oral***

Fishes may be force-fed liquids and semi-solid solutions using rubber tubing and a syringe. Anaesthesia may be necessary to prevent struggling and vomiting. Regurgitation may occur, so fish should be carefully observed following dosing. No more than 1% body weight should be administered orally in a single dose.

***Injection***

Common routes for injection in fish are intravascular, intraperitoneal and intramuscular. Needles should be inserted **between** the scales. Intramuscular injections may be made into the large dorsal epaxial and abdominal muscles, taking care to avoid the lateral line and ventral blood vessels.

Intraperitoneal injections should avoid penetrating abdominal viscera.

Implants and slow release devices can be surgically implanted in the peritoneal cavity or implanted with a trocar into muscle.

**Collection of samples**

Samples may be taken from anaesthetised fish, or collected post-mortem. This must be done very quickly as fish carcases deteriorate very rapidly after death.

***Blood***

Blood sample volumes up to 1 ml/kg of body weight may be collected. The fish must recover their haematocrit prior to subsequent blood collection. Haematocrit recovery times are temperature-dependent and highly variable.

* Tail ablation after euthanasia. The caudal peduncle is severed with a scalpel and blood collected with a capillary tube.
* Caudal venous puncture. The fish is placed on its side and the needle is inserted either in the ventral midline just behind the anal fin and angled slightly forwards, or between the scales just below the lateral line in the middle portion of the tail base. The needle is inserted slowly upwards towards the vertebral column until the bone is reached, then the needle withdrawn slightly and the sample taken.
* Cardiac puncture: this is a difficult technique. The needle is placed perpendicular to the skin and inserted slightly below the gill cover.

**Health and disease**

Fish used for research should be free of diseases. Disease-control measures, such as quarantine, immunisation and prophylactic treatments, and a system of regular health monitoring should be in place. Many diseases in fish can be prevented through good husbandry and biosecurity measures. New stock should be sourced from colonies free from infectious diseases, and quarantined for a suitable period (3 weeks or more) on arrival. Sick fish should be removed from the colony immediately. Avoid mixing fish from different groups. Ideally separate tanks should receive separate supplies of water: systems which circulate water between different tanks facilitate the spread of disease. Shared equipment such as nets should be properly disinfected between tanks.

**Recognition of pain and distress**

Recent studies have identified nociceptors in fish, and fishes respond to noxious stimuli with altered behavioural, physiological and hormonal parameters. The responses seen are similar to those seen in more highly evolved vertebrates. There is increasing evidence that fish have the capacity for long-term suffering, and stress responses can be detrimental. Therefore it is essential to minimise stress when managing fish in the laboratory.

The recognition and evaluation of pain and distress in fishes is not easy. Many fish species are prey animals and do not exhibit overt signs of injury or pain. Animals should be observed closely so as to determine the normal behaviour and appearance of the fish in the particular setting, to facilitate identification of any abnormalities. Reluctance to eat, unusual behaviour, discoloration of the integument and lesions, reddening around the mouth and vent, swimming close to the surface or erratically, inability to maintain posture, swollen abdomen, lethargy, darkening, blue-grey slime or white patches on body, loss of condition, or a cloudy lens in the eye are signs of possible problems.

**Euthanasia**

Schedule 1 permits euthanasia of fish by overdose of anaesthetic, concussion. Overdose of anaesthetic is often achieved by use of a buffered solution of MS222 (tricaine methane sulphonate) at a concentration of at least 250 mg/l. Death usually occurs within 5 minutes, but animals should be kept in the solution for at least 10 min after opercular movements cease. 2-Phenoxyethanol is also suitable for euthanasia at 4x the normal dose, 2000-2800ppm. Fish should be left in the solution for at least an hour.

Fish may also be given an overdose of pentobarbitone (60–100 mg/kg) by intravascular or intraperitoneal injection. Death must be confirmed following an overdose.

For concussion, a blow is administered to the head, which should be followed by pithing or cervical dislocation.

**Zebrafish**

Zebrafish, Danio rerio, are Cyprinids which are widely used research. Many wild-type and genetically modified lines are available (see http://zfin.org for details). They are found naturally in slow-flowing shallow turbid pools in south and south-east Asia, often inhabiting floodplains rather than rivers.

Zebrafish are small (up to 40 mm long) and robust, and large numbers can be kept in the laboratory. Females tend to be larger than males.

Husbandry protocols are not well developed and vary between laboratories. Zebrafish tolerate a range of conditions, but production will be optimized within a set of parameters. Recommended parameters are based on successful practices.

**Behaviour**

Zebrafish are a shoaling species, and establish dominance hierarchies that do not seem to be related to sex or size. Zebrafish can be aggressive at low densities. They are best kept at intermediate densities to avoid stress from overcrowding. Aggression can be reduced by adding complexity to the environment and cover. When mating, male zebrafish may be territorial and pursue females. They release a pheromone when there is damage to the epidermis, which causes alarm in other fish. This is manifest as an increase in shoaling behaviour, and either agitated swimming or freezing on the substratum, reduced appetite and increased aggression. There are behavioural differences between strains.

**Husbandry**

Zebrafish are commonly kept in recirculating systems where several tanks are held on a rack and supplied by a single filtration column. The stocking density depends on the efficacy of filters, the age of the fish and the amount of feeding. In recirculating systems adult fish can be kept at five adult fish per litre.

In non-filtering systems fish must be held at a low density. For periods longer than 10 days, regular water exchange and feeding is needed.

Juvenile fish can be held in static tanks at 60 per 2.5 litres until they are large enough not to pass through filters (2–3 months).

Zebrafish are surface-living, so tank height of 25 cm is sufficient provided there is adequate surface area. A well-fitting clear plastic lid is needed with a small feeding hole.

Zebrafish need temperatures between 25 and 28◦C. The room temperature can be set higher to prevent condensation and growth of mould on tanks. Higher temperatures reduce oxygen levels in water. If temperature falls outside 25–31◦C the fish may not breed or may show abnormal development.

Zebrafish usually have 14 h light/10 h dark cycle. Fish do well in dim light, and bright light encourages algal growth. Red light is often used.

**Feeding**

Zebrafish are omnivorous, eating zooplankton and insects. Adult fish not being bred can be fed twice weekly. For breeding, fish require rich feeding, typically twice daily with dry food, and supplementation with live food twice weekly or more. Avoid overfeeding.

**Breeding**

In the laboratory zebrafish can breed all year round. Zebrafish mate and spawn at dawn. Pairs of fish can be set up in mating tanks in the afternoon or evening, and most fish will then spawn the next morning. Lids are essential as zebrafish can jump. Females can spawn every 2–3 days, and a single clutch typically contains 100–200 eggs, but can contain as many as 700 eggs. There is no parental protection, and parents may eat their eggs.

Hatching occurs between 48 and 72 h post-fertilization at 28.5◦C. Development is very rapid. Larvae initially attach to hard surfaces, attaching to progressively higher levels. Once they reach the surface (2–3 days after hatching) they inflate their swim bladders and begin free feeding. Larvae are kept in petri dishes up to day 5, and are then transferred to a tank, and fed within 48 h. From days 6–15 they can be fed twice daily with a pinch of appropriate food. Overfeeding must be avoided. After 2 weeks the fish can be kept in continuous-flow water systems at a low flow rate so they are not flushed out and fed three times daily with live food. At about 5 weeks dry food is given as well. They grow quickly up to 3 months, when they are transferred into larger tanks.

They reach sexual maturity at about 4 months and will then start breeding. Fish continue to grow until about 18 months.

Initially all gonads develop as ovaries, then in males these begin to develop into testes after 5–7 weeks. Sexual maturity is reached when approximately 23 mm long (about 75 days) but is related to size, rather than age. The mean life span for zebrafish is 42 months.

**Health and disease**

Zebrafish can be affected by a number of diseases, for example velvet disease, fish tuberculosis and nematodes. Fish TB is zoonotic.

*Pseudoloma neurophilia* is a common fungal infection in laboratory zebrafish colonies. Appropriate biosecurity measures, husbandry and breeding strategies, and health-screening protocols should be implemented to minimise the effects of these diseases. Only a small minority of ZF facilities in the UK carry out health screening; for these colonies, prevalence of *Pseudoloma* infection is over 90%.

**Rainbow Trout**

Rainbow trout are salmonids native to North America, but have been introduced widely around the world. They are commonly used in ecotoxicology studies. Rainbow trout are very hardy, tolerating a wide range of environments. Some strains are anadromous (live in the ocean returning to spawn in fresh water), whereas others live permanently in freshwater lakes. They grow rapidly, with freshwater strains reaching 4.5 kg and anadromous strains reaching 7.5–10 kg within 3 years. Rainbow trout live for 5–6 years and can reach 10 kg in weight.

In the laboratory trout may be held in glass or plastic tanks, in circulating water. Circular tanks help maintain a regular current. Tanks are usually 50–60 cm deep.

**Feeding**

In the wild, adult trout feed on a variety of invertebrates and smaller fish. Fry are fed commercial starter feeds, from the age where 50% have reached the ‘swim-up’ stage and are seeking food (2-4 weeks post hatching). They are fed 10% of their weight daily for 2–3 weeks, then when 15–25 mm long feeding is related to temperature and fish size. Fish of 25mm length held at 14oC would typically be fed 2-2.5% of their biomass daily. Again, overfeeding must be avoided.

**Breeding**

Spawning times vary between strains. Wild rainbow trout usually spawn once yearly, in spring, but some cultured strains can spawn all year round. Eggs are laid on gravel nests, covered with gravel and left to mature. Trout rarely spawn naturally outside their native range, and juveniles are usually obtained from hatcheries. After hatching, growth is rapid, with males reaching maturity at approximately 2 years and females 3 years.

**Health and disease**

Rainbow trout may develop a number of infections in the laboratory, including mycobacteria and white spot (see above), sleeping disease, furunculosis and rainbow trout fry syndrome. The presence of disease may only be identified through an increase in mortality. Fry often carry these diseases subclinically, and outbreaks of disease may not be seen until the animals are stressed by procedures.

**Common carp**

The common carp, *Cyprinus carpio* has been farmed since 300BC. It was introduced to Europe from Central Asia by the Romans, and spread to N Europe in the early Middle Ages. They were first kept in ponds around monasteries as an alternative to meat during fasting periods. All wild carp in Europe are feral form of cultured carp. Carp are used as food in many areas, but are regarded as a pest in some regions due to their ability to out-compete native fish stocks. They are notorious for altering their environments, causing serious damage to native duck and fish populations.

Carp are freshwater fish, living in lakes, ponds and rivers, usually with moderately flowing or standing water, but can also inhabit brackish-water estuaries, backwaters, and bays. As schooling fish, they prefer to be in groups of five or more. Common carp have been observed to leap out of the water when threatened.

Carp can live up to 50 years, and can grow up to 120 cm in length and nearly 50kg in weight, although the average size of a common carp is around 40–80 cm and 2–14 kg.

Common carp prefer large bodies of slow or standing water and soft, vegetative sediments. They naturally live in temperate climates in fresh or slightly brackish water with a pH of 6.5–9.0 and salinity up to about 0.5%. They are tolerant of most conditions, including low oxygen levels, which they survive by gulping at the surface.

They can tolerate a wide temperature range, between 4-30°C, and are usually kept between 16-22°C in captivity. They can survive winter in a frozen-over pond, as long as some free water remains below the ice.

**Feeding**

Carp are omnivorous. Adult carp feed on aquatic and terrestrial invertebrates e.g. insects and insect larvae, earthworms and crustaceans. They can eat a herbivorous diet of water plants, but prefer to scavenge the bottom for insects, crustaceans and worms.

Young carp can be reared on pastes or koi starter feeds, combined with live feeds (Daphnia etc.) Larger specimens can be fed koi or carp pellets, but not ad libitum. They can be fed every day or every other day. They can be given live feeds, which may provide an opportunity for natural behaviour, although such supplements may be of questionable nutritive value and risks introducing protozoan or metazoan parasites.

**Breeding**

In temperate zones, female carp need about 11 000 to 12 000 degree-days to reach maturity (e.g. 600 days at 20oC), males mature in a period that is 25-35 percent shorter. European carp spawn when the water temperature reaches 17-18 °C. An adult domestic female can then lay 300,000 eggs in a single spawn. Carp typically spawn in the spring, but can spawn multiple times in a season. Submerged aquatic plants are used as substrata for egg laying.

Embryonic development takes about 3 days at 20-23 °C (60-70 degree-days). Under natural conditions, hatched fry stick to the substrata. About three days after hatching, the swim bladder begins to develop, the larvae swim horizontally, and start to consume tiny particles of food (diameter 150-180 µm, mainly rotifers). The daily growth of young carp can be 2 to 4 percent of body weight in tropical zones. Carps can reach 0.6 to 1.0 kg body weight within one season

**Health and disease**

Carp can be infected with numerous viral, bacterial and parasitic diseases including mycobacteria and white spot (see above), spring viraemia of carp, and koi herpes virus.

Spring viraemia of carp is caused by *Rhabdovirus carpio* . Outbreaks can be seen above 12 ºC; affected animals show erratic swimming, progressing to lethargy, enteritis, oedema, exophthalmia, pale gills, and haemorrhages in skin. This is controlled by elimination of vectors, such as blood sucking parasites, and culling of infected fish

Koi herpes virus causes apathy & separation from the shoal, excessive mucus production, gill discolouration & haemorrhaging, and sunken eyes. This is often subclinical, and may render fish sufficiently immunocompromised to allow secondary (bacterial) infection. Carried by a multitude of vectors, koi herpes is not infectious <4°C and >30°C.

**Marine species**

**Preface**

Marine fish are used in a wide range of regulated procedures, including marine ecotoxicology, nutritional trials and parasitology as well as repeat blood sampling. To adapt to a life in a high salinity environment marine fish species have developed an increased capacity for osmoregulation which in turn makes them interesting subjects for physiological studies. Please note that both salmon and sturgeon are included here as marine; they are both popular research species and despite being migratory between rivers and the sea, they spend most of their lives in marine habitats. Although some marine species may be less susceptible to changes to water quality brought about by a disturbance of the nitrogen cycle, water quality parameters need to be as diligently monitored as with any freshwater species.

**European seabass** *(Dicentrarchus ibrax)*

Biology and use

In the wild, European seabass inhabit Salt- and brackish coastal waters in the NW Atlantic incl. Mediterranean. They have been a popular food species since the antiquity and because of their high value have become “pioneer” species for marine aquaculture. Intensive commercial facilities are prevalent across Eastern (Baltic States) and Southern Europe (Greece) as well as Turkey.

Aquaculture advances and the drive to develop sustainable seafood also feature as main research objectives for this species with focus on nutrition and aquaponics (combination of aquaculture and agriculture). Seabass are an indicator species for functioning coastal habitats and are therefore also used as model species for marine ecotoxicology.

Breeding and husbandry

Because of their aquaculture background European sea bass are usually facility bred. Although higher temperatures (25-26ºC) are favoured in commercial fattening units, optimal conditions in flow-through or recirculation systems may be considerably cooler. Seabass are protected under ASPA from 6dph (7-10 days pf). Their diet should begin with rotifera, artemia & nauplii from d6ph; after d40-50ph transfer to nurseries & transition from live prey to granulates (commercial fish meal & fish oil pellets) can be undertaken. Commercial seabass are fattened for 1.5-2 years to slaughter (350-500g).

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

Biology and use

Atlantic salmon are the most popular aquaculture species in W Europe, S and N America. Their natural habitat is the North Atlantic and tributaries; they are usually anadromous in that they spend most of their adult lives in the sea and only migrate into rivers to spawn. Several landlocked forms exist, most of them in Arctic lakes in N Siberia.

Salmon are the “classic” aquaculture, although this was only scaled up to any commercial level in the 1980s) and this commercial context still dominates much of their use as research species, with a large contingent in procedures targeting the development of vaccines and antiparasitic drugs.

Breeding, husbandry and diseases

Commercial salmon aquaculture has devolved into specialized life stage-specific accommodation with resulting site transfer journeys a genuine welfare concern. “Roe to table” one-site aquaculture facilities also exist but generally hatcheries and grow out pens are in separate locations. Grow out/fattening is usually undertaken in sea pens/sea cages; with a slaughter weight of 3kg reached after 1.5-2 (organic: 3) years. Diet is uncomplicated as it usually relies on 100% commercial formula.

Salmon facilities are fraught with perennial disease issues: pancreatic disease, infectious salmon anaemia and sea lice (the most economically devastating) amongst others. By and large, these issues occur during the “sea” stage, with a more or less open system, high stocking densities (up to 100, 000 fish per sea pen) and resulting issues with biosecurity and biocontainment.

**Sturgeons**

There is a range of relevant species but most popular are Atlantic sturgeon (*Acipenser sturio*) and various hybrids such as Bester (*Acipenser ruthenus*/*Huso huso*). These are not teleosts (bony fish) like salmon, seabass or zebrafish but representatives of a much older taxon with a cartilaginous skeleton, not unlike sharks and rays.

Like salmon, sturgeon migrate between the sea and rivers, spending most of their adult lives in seawater and only migrating up rivers to spawn (anadromous migration pattern). Freshwater forms exist (in Canada and Russia). Sturgeons were a “novel” aquaculture species in the movement towards “sturgeon friendly caviar” since the mid-90s and much of early sturgeon research was dedicated to aquaculture feasibility studies.

Very few single purpose “cultured caviar” production facilities remain today as start-up cost and a variety of disease issues have bankrupted most operators. Remaining sturgeon breeding units usually also cater for a growing ornamentals trade. There are a variety of bespoke breeding systems across Europe (France, Germany, UK) and North America; regulated use today is mainly in research on physiology and evolutionary biology.

**Wild caught species**

Over 30 wild-caught marine fish and cephalopod species are currently used in UK research facilities. The following section can therefore only offer a small glimpse into a diverse and interesting branch of laboratory animal science. There are a variety of reasons why facilities would rely on captured rather than purpose bred research animals and the scope and complexity of the legal connotations are already sufficiently explained in the public domain\*. Many fishes such as sharks are difficult or very expensive to breed yet their use as in-vivo model may be indispensable for physiological or behavioural research. In such cases trapping and transferring animals to research facilities may a feasible option. Apart from the ethical problems relating to the lack of husbandry experience for many wild caught species, conservation issues may also have to be addressed and both of the following species are extremely vulnerable to overfishing.

Spiny dogfish (*Squalus acanthias*)

Spiny dogfish inhabit temperate coastal waters in the S & N hemisphere. These small (80-100cm max) sharks are ovoviviparous (i.e. their eggs are hatched within the body) and they reach sexual maturity only at >20yo which explains why self-sustained breeding systems currently only exist in a handful of public aquaria. They are usually caught by anglers & as trawl bycatch (if they survive long enough). Optimal temperature is 7-14ºC but generally they have shown very poor survival in conventional recirculation systems.

Atlantic Cod (*Gadus morhua*)

Despite a dramatic population collapse in the 1990s (and a resulting three-fold increase in market price), Atlantic cod remains one of the most popular food fish in Western Europe. It geographic range stretches across the Atlantic continental shelf, N of 40` latitude. Sexual maturity is usually reached at 3-5yo. Cod have also been grown in aquaculture facilities although commercially this has never caught on. Usually young fish (<1.5yo) are caught by coastal angling and heavy transfer losses are the norm. Those fish surviving the transfer usually cope well in conventional recirculation (but cooled to 11-15ºC) or flow through systems.

\*https://www.gov.uk/government/uploads/system/uploads/attachment\_data/file/535574/working-with-wild-animals-160706.pdf