Guidelines of CPCSEA for Experimentation on Fishes

Government of India
Ministry of Fisheries, Animal Husbandry and Dairying
Department of Animal Husbandry and Dairying
Committee for the Purpose of Control and Supervision of Experiments on Animals
(CPCSEA)

2021

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CONTENTS

1. INTRODUCTION

Fishes have long been considered useful indicators of environmental and ecological integrity. As a result, they are increasingly being employed as physiological and biomedical models in life science research. Amongst the most aquatic species, Zebrafish are routinely being used as experimental models as environmental indicators and as substitutes for higher sentient laboratory animals for use in pharmacological, toxicological, genetic, and biomedical research and testing. It is known that fish can also perceive stimuli that are damaging or potentially damaging to normal tissue (noxious stimuli) like mechanical pressure, extremes of temperature, and corrosive chemicals. However, their capacity to experience adverse conditions associated with pain as in mammals has been debated. The use of fish as a replacement for mammals as experimental subjects has been enhanced over the years. Different fisheries, institutes and universities use various inland and marine fish, including Cyprinids like the Indian major carps (IMC), Salmonids like Rainbow and Brown trout for both aquaculture and experimental purposes besides Zebrafish.

Humane procedures involving holding, handling, and sampling fish are quite essential to reduce pain or discomfort experienced by experimental subjects. The investigators, animal ethics committees, facility managers, and animal care staff need to be well informed about the care and use of fish for experimental, research, or teaching purposes. These guidelines would enable them to assist in providing proper care and humane use of fish in experimental procedures and to avoid unnecessary infliction of pain or suffering on these creatures.

The use of fish in research, teaching, and testing requires specific ethical considerations that need to be followed. Fish should be treated with respect equal to the other vertebrate species and must be used only if the non-animal models have failed, not available, or not possible. The least likely numbers of fish should be used in such a way that the quality of the experiment is not compromised, whether it is for a laboratory or a field trial. Pilot studies should be preferred for conducting an investigation *in vitro* than *in vivo* if any. Fish should be maintained in an environment that will match to their natural habitat to keep them in optimal health and wellbeing conditions, besides maintaining consistency with the demands of the experimental protocol. Fishes should not be subjected to any kind of noxious stimulus (pain or distress unless required by the experimental procedure). Further following points should be followed while conducting experiments on fishes:

1. Experimental protocols should be framed for the use of fish in research, teaching, or testing, and prior approval of IAEC must be obtained before the commencement of experimentation on fish.

2. Training should be provided to all the personnel (investigators, technical staff, and students) involved in experimentation with fish. The outcome of the training must be evaluated for competence.

3. It is the responsibility of the investigators to comply with occupational health and safety regulations for the protection of personnel from known or suspected physical and biological hazards as the case may be. While conducting experiments involving fish pathogens, biosafety guidelines should also be kept in mind.

4. Due care must be taken while working with zoonotic pathogens from fish.

2. GOAL

The goal of these Guidelines is to promote the humane care and use of fish in research, testing, and training with the primary objective of providing specifications that will enhance quality in the pursuit of the advancement of biological knowledge relevant to humans, animals, and fish. Good Laboratory Practices (GLP) are intended to assure quality maintenance and welfare of fish used in laboratory studies while conducting biomedical research and product testing.

3. FISH PROCUREMENT

Acquisition, exchange, transport of, or research on fish must be carried out strictly as per the guidelines, and the personnel must be familiar with relevant legislation. Compliance with the policies governing the capture of fishes and their transfer from one water body or jurisdiction to another should be adhered to as it may lead to introduction of new pathogens.

Investigators must acquire fish as per the CPCSEA guidelines from wild habitat, commercial hatcheries, breeding farms, establishments registered with CPCSEA. Various species of fish listed in **Annexure 1.**

Conditions for Procurement:

1. The use of any kind of piscicidal compounds should be discouraged for fish acquisition from natural bodies. Instead, anesthetic agents with minimal impact on the environment and non-target species should be considered.

2. Methods like electro fishing and netting should be carried out, ensuring with minimum impact on fish being caught.

3. Minimal morbidity and mortality must be ensured while capturing, transportion, and handling of wild fishes.

4. The risk of escape and the accidental introduction of exotic/infectious diseases and other detrimental outcomes resulting from the introduction of unknown or non-native species must be taken into account.

5. If fish are procured from registered vendors, research organizations, national or international repositories, their health status, and preferably known genetic

history should also be obtained.

6. Consistent husbandry and management practices should be adopted with developed SOPs in the fish facilities like in any other laboratory animal production facilities.

7. Each consignment of fish should be inspected for compliance with procurement specifications, and the fish should be quarantined and stabilized according to procedures appropriate for the species and circumstances.

4. QUARANTINE AND ACCLIMATION

1. Quarantine is the separation of newly received fish from those already in the facility until the health and possibly the microbial status of the newly acquired fish have been determined. Effective quarantine minimizes the chance of introduction of pathogens into the established stock.

2. For maintenance and housing of different fish species, separate rooms and separate attendants are required. Sick fish must be isolated and kept separately, and a separate set of personnel should be identified for taking care of these sick fish, and other workers should be restricted from entering into the facilities unless otherwise required. After handling these fish, they should not be handling any other fish in the facilities.

3. Once the caught or acquired fish reaches its destination lab or field, they must be kept under quarantine for two weeks period and allowed to acclimatize to the new conditions.

4. Under no circumstances fish from two or more different sources should be mixed. All acquired fish should be quarantined separately, and the quarantine areas should be under constant vigilance. All morbidities and mortalities must be recorded and good record-keeping must be practiced to detect and respond to any health problems of fish during quarantine period.

5. The quarantine duration should be appropriate to assure the health of the fishes (usually 14 days). However, the period of quarantine may be extended if fish are suspected of any infection for further tests. On confirmation of any infectious disease, they should not be allowed to continue in the facility either for experiments or for breeding purposes.

5. AQUATIC FACILITIES

a. Facilities

Facilities for aquatic species must be designed in such a way to minimize stress to the fish being reared for experimental or breeding purposes. Quality water appropriate to species (brackish, sea, or freshwater) should be chosen to enable

efficient operation of the facility and ensure a safe working environment for personnel.

b. Water Quality

Water used for fish culture should be clean and available in abundance. It should be regularly tested and adequately treated to remove contaminants and pathogens. Adequate water supply of suitable quality should be provided for the fish at all times.

c. Engineering and design

1. The assistance of the experienced civil engineers and subject experts should be involved in designing and construction of aquatic facilities. Corrosion-free and water-resistant construction materials should be used for the construction of fish housing facilities. The use of toxic materials in aquatic facilities should be strictly avoided. However, if they require to be used as part of experimentation and testing, they should be permitted to use in minimum quantities judiciously with proper justification and permissions and, the list of those toxic material should be readily available to the staff to rule out the intolerable effects.

2. To ensure the adequate ventilation of aquatic areas, air handling systems must be in place with humidity controllers and to guarantee minimum transfer of aerosol between tanks. Vibration and noise producing machinery should preferably be isolated from fish housing areas.

3. Ensure installation of quality electrical systems suitable for operation in moist environments. The electricals must include proper grounding and ground fault interrupters on all circuits. All electrical wires conduits should be fixed safely away from water pipelines, and from water circulation areas. The use of extension cords should strictly be avoided to prevent accidents.

4. Electrical components and equipment should be located outside splash zones and housed in moisture-proof enclosures. Electrical fixtures should be secured well with gaskets to prevent the incursion of water and should be located away from pipe runs. Alternate systems, including pumps, motors and critical aeration programming systems should be duplicated to ensure 100 percent back up and to overcome failures and service interruptions.

5. Light and dark cycles should be maintained within the facility to adjust light wavelengths and intensities appropriate for the species where it is known. Where lighting is needed for people to work in the room, it should be restricted in its dispersion and be placed at a lower level than the tank surface.

6. All aquatic facilities should have an emergency contingency plan for maintaining aerated and filtered water and assuring the continuation of life support in case of any kind of water crisis.

d. Housing

1. Aquatic housing environments should be designed to meet the established physical and behavioural requirements for fish wellbeing like shelter, social grouping, overhead cover, and lighting.

2. The shape, colour, depth, and volume of tanks should be appropriate for the species and life stage being held.

3. The tanks used should have smooth, inert, sealed interior surfaces with a facility of self-cleaning and regular cleaning.

4. To prevent the fish from jumping out of the tanks, the tanks need to be adequately covered with suitable nets.

5. The wet lab should have a constant supply of clean water, adequate light and well-placed electricals for connecting aerators and water heaters.

6. Use of extension boards should be avoided.

7. Controlled feeding should be practiced to prevent wastage of feed that may invite fish diseases. Likewise, re-circulatory systems must meet the basic requirements along with constant power supply to prevent fish loss. Besides, the basic infrastructure and clean water, re-circulatory systems and raceways should have restricted entry.

6. FACILITY MANAGEMENT, OPERATION AND MAINTENANCE

a. Security and Access

Access to fish facilities should be designed to minimize traffic through the area. Access should be restricted to delineate personnel involved in the maintenance of the facility, caring for the fish, and those using the facilities for experiments or teaching.

b. General Maintenance of the Facility

1. Sufficient staff must be available for care and management of fish facility round the year for both routine and emergency needs.

2. All architectural and engineering specifications and drawings of the facility should be available to those in charge of running the facility, as well as all operating manuals of special equipment such as pumps, chillers, and computer control systems.

3. Aquatic facilities must have written maintenance schedules explicitly developed for the facility.

4. Facilities should be kept in a clean and orderly manner. Tanks/tubs/boxes, lids, baffles, water sumps and plumbing should be disinfected with tested disinfectants before and after every experiment, ensure no residual contents of disinfectants after cleaning.

5. The staff responsible for operating an aquatic facility should have the specialized knowledge, experience, and training for proper functional, operational, and maintenance of the water systems.

c. Environmental Monitoring and Control

1. Environmental monitoring system is essential for aquatic facilities and should be designed to suit the water management system.

2. Water quality must be monitored and maintained within acceptable parameters for the species being held (**Annexure 2**).

3. Fishes should not be subjected to rapid changes in temperature, particularly to rapid increases in temperature.

4. Water quality monitoring systems should be able to detect and react to changes in water quality and give alarm before they become life-threatening to fish housed in the system.

5. Water quality parameters should be regularly monitored at an appropriate frequency in the facility to permit predictive rather than reactive management of crises (**Annexure 3**).

6. Good water quality measuring equipment should be available in adequate numbers within the facility and they should be regularly calibrated and well maintained.

7. Records of water quality, testing should be maintained and retrievable for retrospective analysis if any problem is encountered.

8. Fishes should be kept in water with an adequate concentration of oxygen.

9. Aquatic systems are susceptible to acute or chronic super saturation. This may lead to gas super saturation resulting in serious problems. Individuals responsible for operating aquatic systems should be made aware of its consequences to mitigate such issues.

10. Changes in pH may influence other water quality parameters; therefore, pH of the water being used in the facility should be properly maintained all times at a stable and optimal level.

11. Measures should be in place to prevent the accumulation of free Ammonia

and nitrites as they are toxic to fishes.

12. Changes in salinity should be made slowly and with attention to the physical status of the fishes. This procedure is known to be stressful for fishes.

13. If there is a doubt that hazardous materials or infectious agents have accidentally entered the water system, it should be isolated and tested.

14. Chemicals used in the facility should be safely stored away from the aquatic housing area and the water supply.

15. Following are the Physical parameters and normal values for laboratory Zebra fish (Source: Zebra fish in Biomedical Research; Biology, Husbandry, Diseases and Research Applications. American College of Laboratory Animal Medicine series, 2020).

Sr. No.	Parameters	Normal Values
1	Room temperature (^{o}C)	28-29
2	Relative humidity (%)	70
3	Light intensity (Lux)	300
4	Photoperiod (Light : Dark)	14:10
5	Noise/vibration	Free (if possible)

7. HUSBANDRY

a. Density and carrying capacity

Fish species should be housed in clean experimental tanks at an optimum density to ensure their wellbeing while meeting experimental parameters. For the maintenance of a particular species, an ideal environment must be developed using performance-based criteria. Such environmental conditions should be followed for the said fish in the future. Moreover, under no circumstances, established maximum densities should not be exceeded (**Annexure-4**).

b. Record-keeping and Documentation

1. Standard Operating Procedures (SOPs) should be developed for fish procurement, quarantine, breeding, embryo disinfection/bleaching, larviculture, feeding, live feed preparation, water parameter monitoring, sanitation of tanks, rooms and equipment.

2. Records of every cleaning, maintenance and experimental procedure must be

maintained and a checklist should be used for each group of fish.

3. Wellbeing in fishes should be monitored daily and written records should be maintained along with basic physical and behavioral parameters. Any observational changes should be recorded and investigated to identify the cause and for corrections.

c. Food, feeding and nutrition

1. Fish feed should be purchased from reliable sources that manufacture as per the standards formulation. However, if the feed produced in house, it should be in accordance with the published nutrient requirements for the species.

2. Purchased feed must be in sealed bags, that has proper label with date of manufacture, expiry and information of its contents, analysis / guarantee details etc. In case of procured feed, small aliquots should be retained for independent testing if required.

3. Feed must be stored in dedicated storage areas and ensure the storehouse is pest-free and dark. The temperature and humidity of feed storage room must be controlled to ensure the feed's nutritional quality and prevent fungal growth and spoilage. Feed for daily consumption should be kept in sealed-top containers to protect it from humidity and light and frequently replaced with fresh feed from storage.

4. Feed must be provided adequately suitable to the life stage of fish at appropriate time points for proper growth, development, reproduction and good health leaving minimum scope for leftover uneaten feed as that would result in fouling (Annexure 5). However, uneaten feed should be siphoned off to prevent spoilage of water quality. Fish should be closely observed to determine their response to feed and signs of either over or underfeeding must be recorded.

5. Medicated feeds must only be used in exceptional conditions under the supervision of a fisheries/aquaculture expert.

d. Broodstock and Breeding

There should be appropriate holding systems and environmental conditions for the species being maintained. The importance of environmental cues for the maintenance or manipulation of endogenous reproductive rhythms should be in place for effective breeding. If possible, rational genetic management of broodstock should be employed. A strict disease and health control program should be implemented to ensure the production of healthy progeny and prevention of disease transmission through water sources, fish, or eggs.

1. Breeding

Zebrafish are generally kept under laboratory conditions designed to replicate perpetual summer. In wild habitat, depending upon food availability and temperature, they can breed all year round (Spence et al., 2006), with females generally producing eggs once every one to three days. Darkness allows the Zebrafish to rest and the return of light will trigger fish to breed (Vargesson, 2007). A layer of marbles, closely spaced rods, or mesh can be used to cover part or the whole of the bottom of the tank to prevent the fish from eating their eggs once laid (Matthews *et al* 2002). Females consistently spawn more frequently and produce larger clutches of eggs with some males than others. A good clutch consists of between 70 and 300 eggs, of which at least 80% are fertilized (Brand *et al.,* 2002).

A generalized description of the techniques and some of the equipment used in relation to the spawning process can be found in Lawrence (2007).

Typically: A small (typically <1L) plastic mating cage or box with a mesh or grill bottom is placed inside a slightly larger container that is filled with water; breeding pairs or small groups of fish are added to the box in the evening; when the fish spawn (usually the following morning), the fertilized eggs fall through the 'floor' of the inner box (which means the fish are prevented from eating them). It is possible for both males and females to reach sexual maturity within three months of hatching. Although establishments may begin using fish for breeding from this age (Kurtzman, 2010), initial batches of eggs from such young females may not be of optimal quality. The highest number of embryos is reported to be obtained from fish between 6 and 18 months of age (Vargesson, 2007). The mating behaviour of Zebrafish seems to be influenced by the exposure of mating partners to one another during the 24 hours before spawning begins (at sunrise), with males stimulated to perform courtship behaviour by the detection of female gonadal hormones in the water (Delaney *et al.,* 2002). Source: Reed and Jennings, 2010

2. Raising of larvae

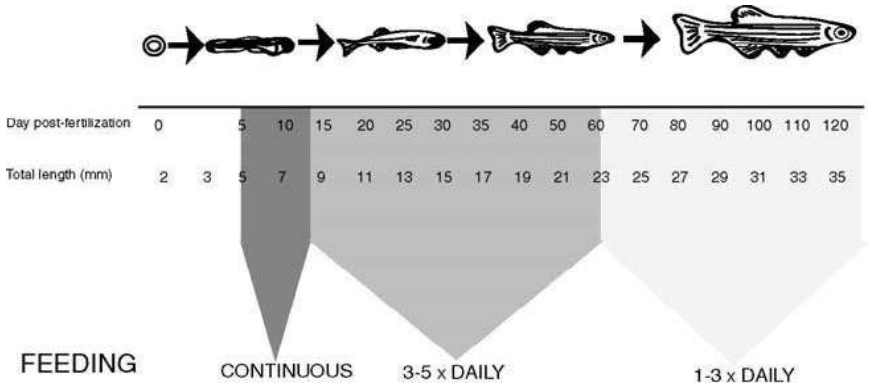
Fertilized eggs are kept in an incubator (~28.5 °C) for 72 hr until the larvae are hatched. The embryos are reared in embryo medium; a.k.a. EM3 (NaCl, 13.7 mM; KCl, 0.54 mM; $MgSO_4$, 1.0 mM; $CaCl_2$, 1.3 mM; Na_2HPO_4, 0.025 mM; KH_2PO_4, 0.044 mM; $NaHCO_3$, 4.2 mM) (Avdesh et al., 2012).

The different stages of the Zebrafish life cycle (Reed and Jennings, 2010) have been broadly established as follows (Fleming, 2007):

i. 0-72 hours post-fertilization - Embryos
ii. 72 hours to 13 days post-fertilization - Early larvae
iii. 14 days to 29 days post-fertilization - Mid larvae
iv. 30 days to 3 or 4 months – Juveniles
v. When sexually mature – Adults

Feeding of larvae should commence from 5 dpf (days post hatching). Young larvae can be fed with dry food of ~100 microns in size (e.g., ZM100) or live food such as

paramecium and rotifers (which stimulates growth). The food size can slowly be increased to 200 microns (e.g. ZM200) or 300/400 microns (e.g. ZM300). A population of adult fish should be around 6-7 fish per liter of water. This practice is recommended to better maintain BOD (Biological Oxygen Demand) to the tanks (Avdesh *et al.,* 2012). The relationship between feeding frequencies and development in Zebrafish is given below **(Source: Lawerence, 2011)**:



3. System Maintenance

Re-circulatory system:

Zebrafish are kept in a re-circulating water system that continuously treat and sterilizes the system water (effluent) before it re-enters into the aquaria to maintain the water quality required for a healthy aquatic environment. The re-circulating system also helps to filter excess food and fish excreta. Different companies provide circulating Zebrafish systems. A set of different kinds of filters are used in the system 120-micron filter pad, 50- micron canister filter, biological filter, activated carbon absorption filter and UV disinfection. The filters need to be changed regularly (Avdesh et al., 2012).

The other important features of Zebrafish rearing system that needs due care for wellbeing of Zebrafish are provided below:

Sl. No.	Features	Details
1	Lighting	A cycle of 14 hours light, and 10 hours dark has been advised and would appear to be common practice (Matthews et al., 2002; Brand et al., 2002). Ideally, wherever artificial lighting is used, a gradual brightening/dimming period of around 20-30 minutes in the morning and evening can be incorporated. Light triggers Zebrafish to breed, so periods of darkness are important for allowing fish to rest (Vargesson, 2007; Brand et al., 2002). Francis (2008) states that, one of the fastest ways to ensure fish prevent laying eggs, is to leave the lights on all the time.

2	Noise and other disturbances	It has also been suggested that spawning in these fish may be affected if it is very noisy or if there is lot of nearby movement or activity (Vargesson, 2007).
3	Water Depth	Zebrafish are often described as surface-living fish, yet field studies show that they occupy the whole of the water column, with no significant difference in their distribution according to depth (Spence et al., 2006). It has been recommended that as long as tanks have a 'relatively large surface area,' water depth does not have to exceed 25cm (Brand et al., 2002). Elsewhere it has been suggested that for spawning, just 10cm water depth in a 50-litre tank should be provided for three adult males and two females (Andrews, 1999).
4	Volume and population density	20 eggs/embryos per 100ml water. 20 young larvae per 400ml up to juvenile stage. Growing juvenile fish and holding adults - 5 fish per litre. For breeding, a pair can be kept overnight in 1.5 litres, or 6 fish in 2 to 3 litres of water (Matthews et al., 2002) 5 fish per litre in systems possessing filters and a biofilter, as long as there is good water exchange, good feeding regime and good water quality. For breeding purposes it is best to have less fish per tank (2-3 fish per litre). In a tank that does not have filters or a biofilter, the maximum number should be 1 or 2 fish per litre (Vargesson, 2007). In large-scale re-circulating systems, families of sibling adult fish are kept in serial tanks at densities of 5 adult fish per litre (60 fish/12 litres) (Brand et al.,2002). 25 fish in 45 litres (~10 gallons) (Westerfield, 2000)
5	Temperature	A widely used standard temperature for developmental studies is 28.5°C (Matthews et al., 2002) An ideal temperature for both breeding and development of the embryos is 28.5°C (Bilotta et al., 1999)
6	Cleaning	Standing water tanks maintained by manual water changes can be equipped with filtration units that will continually remove unwanted material from the water (Matthews et al., 2002). If a third of the water is replaced each day by siphoning up debris from the bottom of the tank, a separate tank filtering system may not be required. If a filter is used, about half the water will need to be changed at least once a week (Westerfield, 2000). Cleaning strategies should be designed to minimize disturbance and distress to the fish. Disinfectants should be used with extreme caution.

7	Tank material	Tanks used to hold Zebrafish are usually made of polycarbonate, high-quality glass or acrylic material (Matthews et al., 2002).
8	Colour and transparency	Glass and other transparent-walled containers have the advantage of allowing easy observation and monitoring of the fish, but a disadvantage in that movements of staff and equipment outside the tank can disturb them. On the other hand, opaque, or very dark colours can lead to hygiene problems since contamination may not be obvious (The Berlin Workshop 1994). A container colouration of medium blue is probably best. Consideration should be given to placing tanks on a dark surface which will prevent light emanating from below, as it is suggested that fish prefer this to light coloured surfaces (Brand et al., 2002).
9	Food type and feeding regime	Zebrafish larvae chase and catch their prey (e.g. Paramecium) in a process that appears to be predominantly visually guided (McElligott & O'Malley, 2005). Dry food alone is not sufficient to keep fish in good breeding conditions. Therefore it is necessary to supplement it with live or frozen food. The most commonly used additional live food is Artemia nauplii, Rotifers, paramecium. Alternatively, or in addition to Artemia, Drosophila larvae or different types of frozen food that are available from aquaculture supply stores can be used. Live or frozen food (e.g. tubifex, Daphnia and Chironomus larvae) that has been harvested from freshwater systems that also harbour fish, should be avoided, as it may be a source of pathogens. On the other hand, salt-water-dwelling articulates are safe (e.g. frozen adult Artemia and krill). A typical feeding regimen is to feed adult fish tanks twice a day (once at weekends). Adult fish that have to be kept for longer periods of time without breeding require very little feeding (e.g. twice a week, preferably with live food). Two weeks of rich feeding will bring them back into breeding condition again (Brand et al., 2002). Newly hatched Zebrafish can eat Paramecium (800µm x 80µm), as well as a variety of prepared foods, infusoria and rotifers (Matthews et al., 2002). Once fish reach one month of age: flake food supplemented with live food such as Artemia. Adult fish being prepared for breeding: live food (Howells and Betts, 2009).

10	Egg harvesting	There are a number of techniques associated with the procurement of eggs. The main ones are: 1. Natural mating 2. Manual expression ('squeezing') of eggs from females for in vitro fertilization. A good clutch consists of between 70 and 300 eggs, of which at least 80% are fertilized (Brand et al., 2002). On the basis of current knowledge, a minimum interval of a week should usually be allowed between episodes of breeding in females.

Source: Reed and Jennings, 2010

To decontaminate the fishnet, spray with 70% ethanol, rinse in water, and let it dry before re-using. It should be noted that UV filter disinfection dose rate is ~110 mJ/cm^2 at the beginning of the lamp life and the dose rate decreases over the course of time, hence it is necessary to replace the globe even when it appears to still be functional (Avdesh et al., 2012)

4. Water Quality Parameters

The optimal water quality parameters for raising Zebrafish are provided below:

Sl no	Parameter	Optimum Range
1	Alkalinity	50-150 mg/L CaCO3
2	pH	6.8-7.5 (6.0-8.5 tolerated)
3	Temperature	26-28.5 °C
4	Hardness	50-100 mg/L CaCO3
5	Unionized ammonia	<0.02 mg/L
6	Nitrate	50 mg/L
7	Nitrite	<0.1 mg/L
8	Dissolved Oxygen	>6.0 mg/L
9	Salinity	0.5-1 g/L
10	Conductivity	300 -1,500 µS

Source: Avdesh et al., 2012

8. VETERINARY CARE

1. Adequate veterinary care must be provided and it is the responsibility of a veterinarian or a fish health care professional, or a person who has adequate training or experience in handling fish/ aquaculture.

2. Regular observation of fish can be accomplished by someone other than a veterinarian or a fish health care professional or a person who has adequate training or experience in handling fish/ aquaculture; however, a mechanism of direct and frequent communication should be adopted so that timely and accurate information on problems in fish health and well being is conveyed to the concerned professional.

3. The veterinarian or a fish health care professional or a person who has adequate training or experience in handling fish/ aquaculture can also help the establishment in designing appropriate policies and procedures for ancillary aspects of health care, such as the use of appropriate methods to prevent and control diseases (e.g. vaccination and other prophylaxis, disease monitoring and surveillance, quarantine and isolation), operative and post-operative care, diagnosis and treatment of diseases as well as injuries. Reviewing protocols and proposals, fisheries and welfare, monitoring occupational health hazards containment and zoonosis control programs; and supervising nutrition and sanitation.

9. MONITORING, DIAGNOSIS AND CONTROL OF DISEASE

1. All fish should be observed for signs of illness, injury, or abnormal behaviour by designated staff. As a rule, this should occur daily, but more-frequent observations might be warranted, during experimentation or when fish are ill or have a physical deficit. It is imperative that, appropriate methods be in place for disease monitoring and diagnosis.

2. Post-mortem examination and signs of illness, distress, or other deviations from normal health conditions in fish should be reported promptly to ensure appropriate and timely delivery of veterinary care. Fish that show signs of a contagious disease should be isolated from healthy fish.

3. The isolation, quarantine and stabilization programs for newly arrived fish are necessary to provide time to assess their health status, allow, them to recover from the stress of shipment and an opportunity to adapt to their new environment. The extent of these programs depends on several factors, including species and source of the fish as well as their intended use.

4. Only fish of defined health status should be used in research and testing unless a specific, naturally occurring or induced disease state is being studied. Systems should be established to protect fish within the institution from

exposure to diseases.

5. Transgenic and mutant fish may be particularly susceptible to diseases and may require special protection to ensure their health. Systems to prevent spread of disease may include facility design features, containment/isolation tanks, and use of standard operating procedures. Training of fish care personnel and research staff is essential to prevent spread of fish diseases.

6. Disease surveillance is a major responsibility of the veterinarian/fish care staff and should include routine monitoring of fish for the presence of parasitic and microbiological agents that may cause overt or inapparent disease. The type and intensity of monitoring necessary will depend upon the professional veterinary judgment and the species, source, use and number of fish housed and used in the facility.

7. Diagnostic laboratory services must be available and used as appropriate. Laboratory services should include necropsy, histopathology, microbiology, hematology (**Annexure 6**), clinical pathology (**Annexure 7**), serology, parasitology, and other routine or specialized laboratory procedures, as needed. It is not necessary that all of these services be available within the institutional facilities. Facilities from other laboratories with appropriate capabilities can be used.

8. Fish showing the signs of infectious/contagious diseases must be isolated from others by placing them in isolation units in separate rooms appropriate for the containment of the agents of concern. In certain circumstances, when an entire group of fish are known or suspected to be exposed or infected, it may be appropriate to keep the group intact during the time necessary for diagnosis and treatment, for taking other control measures, or for completion of a project.

9. The veterinarian must have authority to use appropriate treatment or control measures, including euthanasia following diagnosis of fish disease or injury. If possible, the veterinarian should discuss the situation with the principal investigator to determine a course of action consistent with experimental goals. However, if the principal investigator is not available, or if agreement cannot be reached, the veterinarian must have authority to act to protect the health and wellbeing of the institutional fish stock.

10. It is mandatory that all facilities must have a fish health monitoring program. For disease prevention, strategic measures should include:
    i. a program for the detection and management of disease conditions and monitor water quality problems related to physiological stress,
    ii. a system for regular monitoring and reporting of health assessment,
    iii. Details on the strategic application of disease control measures, like quarantine, immunization, and prophylactic treatments a formal written

delegation of responsibility to be given to the fish health care professional for the management and monitoring of morbidity and mortality in the facility.

iv. In order to have correct control measures, due consideration on early diagnosis and identification of the causal agents besides factors leading to stress must be introduced.

v. As a result of experimental stress, due consideration must be given to identify clinical as well as subclinical pathogens.

vi. After any potentially stressful event, fish must be closely monitored.

vii. Fish should be handled only by competent or trained personnel in order to avoid or minimize potential injury and pain. Morbidity and mortality in the experimental fish as a result of compromise in osmoregulation, systemic acidosis, and opportunistic infections of damaged skin because of handling and traumatic injuries should be reduced to a minimum.

viii. Standard Operating Procedures must be in place for standard treatment methods with inclusion of defined endpoints if fish are adversely affected. Health management procedure must exclude behavioral interactions such as aggression.

10. FISH HEALTH

A good understanding of Zebrafish biology and behaviour, including diseases, clinical signs and treatments, is necessary to minimize suffering or death. Zebrafish should be regularly monitored for signs of ill health. The following are a few of the important resources for information on diseases in Zebrafish (Reed and Jennings, 2010):

1. Zebrafish International Resource Center - Disease Manual http://zebrafish.org/zirc/health/diseaseManual.php
2. Laboratory Animal Medicine (2002) (Second edition) American College of Laboratory Animal Medicine Series
3. The Laboratory Fish (2000) - Gary K. Ostrander (editor) Academic Press, San Diego

The maximal recorded life-span of Zebrafish in the laboratory is 5½ years, though an average of 3½ years has been reported (Gerhard et al., 2002). In laboratories, these animals are routinely kept for 18 months to two years, after which they are considered to be of lower reproductive value and also prone to develop diseases. In the wild, there is little evidence that individuals survive more than a year or two. This may be due to predation or parasites (Spence, 2007).

The following are important resources for those using Zebrafish:

1. Zebrafish Husbandry Association (ZHA) www.zhaonline.org
2. British Association for Zebrafish Husbandry (BAZH) www.bazh.co.uk
3. Zebrafish Information Network - the Zebrafish model organism database (ZFIN) http://zfin.org/zf_info/dbase/db.html
4. Zebrafish International Resource Center (ZIRC) http://zebrafish.org/zirc/home/guide.php

5. European Zebrafish Resource Centre (EZRC) https://www.ezrc.kit.edu/

The following book will also be of interest:

1. The Laboratory Zebrafish (2010) Claudia Harper & Christian Lawrence; CRC Press, Boca Raton, USA.
2. Zebrafish, Practical Approach (2005), Edited by Christiane Nusslein-Volhard and Ralf Dahm,Oxford University Press.
3. Zebrafish in Biomedical Research. Biology, Husbandry, Diseases and Research Applications. Academic press (2020)

11. PERSONNEL AND TRAINING

Fish care programs require technical and husbandry support. Institutions should employ people trained in fishery science or provide both formal and on-the-job training to ensure effective implementation of the programs.

1. The selection of staff particularly working in fish sheds, farm, fish aquarium and those involved in transportation, is a critical component in the management of a fish facility.

2. The staff must be provided with all required protective clothing (gloves, gumboots, other footwear etc.) while working in fish house. Facilities should be provided for change over with lockers, washbasin, toilets and bathrooms to maintain personal hygiene. It is also important to have a regular medical check-up programs arranged for the workers to ensure that they have not picked up any zoonotic infection and also that they are not acting as a source of transmission of infection to the fish.

3. Initial in-house training of staff at all levels is essential. A few weeks must be spent on the training of the newly recruited staff, teaching them the handling techniques, live diet preparation and feeding cleaning of tanks/tubs/boxes/ racks, and importance of hygiene, disinfection and sterilization. They should also be made familiar with the behavioural activities of normal healthy and sick fish so that, they will be able to spot the sick fish during their daily routine check-up of tanks.

12. PERSONAL HYGIENE

1. It is essential that the fish health care staff maintain a high standard of personal cleanliness. Facilities and supplies for meeting this obligation should be provided with appropriate Personnel Protective Equipment (PPE) e.g. showers, change of uniforms, footwear etc.

2. Clothing suitable for use in the fish facility should be supplied and laundered by the institution. A commercial laundering service can be engaged for

services; however, institutional facilities should be used to decontaminate clothing exposed to potentially hazardous microbial agents or toxic substances. It is acceptable to use disposable gloves, masks, head covers, coats, overalls and shoe covers. Personnel should change clothing as often as is necessary to maintain personal hygiene. Outer garments worn in the fish shed should not be worn outside the fish facility.

3. Washing and showering facilities appropriate to the program should be available. Personnel should not be permitted to eat, drink, smoke or apply cosmetics and perfumes in fish rooms. A separate area or room should be made available for these purposes.

13. FISH EXPERIMENTATION INVOLVING HAZARDOUS AGENTS

1. Institutions should have policies governing experimentation with hazardous agents. Ensure Institutional Bio-safety Committee whose members are knowledgeable about hazardous agents for taking care of safety issues.

2. Since the use of fish in such studies requires special considerations, the procedures to be used and the facilities must be reviewed by both the Institutional Bio-safety committee and Institutional Animal Ethics Committee (IAEC). Disposing of tissues and fluids from such used fish must also be appropriately governed as per the laid standard practices in the bio-safety regulations.

14. DURATIONS OF EXPERIMENTS

No fish should be used for experimentation for more than two years unless adequate justification is provided.

15. PHYSICAL RESTRAINT

1. Minimum physical restraint of fish for physical examination, collection of samples, and a variety of other clinical and experimental manipulations can be accomplished manually or with devices of suitable size and design to minimize stress and avoid injury.

2. Prolonged restraint of any fish, should be avoided unless essential to the research objectives.

3. Following points should be considered during handling and restraining fish:

    i. Fish should be handled by competent individuals trained in methods that do not cause distress and injury.
    ii. The use of restraint devices sometimes essential for the welfare of the fish and safety of the handler. Restraint devices should be used to the

minimum extent, for the minimum period required to accomplish the purpose of the experiment and be appropriate for the fish.

4. The following are important guidelines for the use of restraint equipment:

   i. Restraint devices cannot be used simply as a convenience to handle fish. The period of restraint should be minimum to accomplish the research objectives. Staff should be given training to adapt to the equipment prior to initiation of experimentation.

   ii. Provision should be made for observation of fish at appropriate intervals. Veterinary care should be provided if symptoms of stress or illness observed during restraint period. On noticing any illness, or severe behavioural changes fish should be removed temporarily or permanently from restraint related protocols.

16. LOCATION OF FISH FACILITIES TO LABORATORIES

1. Fish facilities should be physically separated from personnel areas such as offices, break room, training and education rooms. This will ensure good fish husbandry practices and ensure human comfort and prevent disease transmission.

2. Fish are very sensitive to their living conditions. It is important that they shall be housed in an isolated building located as far away from human habitation as possible and not exposed to dust, smoke, noise, wild rodents, and insects. The building, aquatic systems, tanks/cages and environment of fish rooms are the major factors, which affect the quality of fish.

3. This separation can be accomplished by having the fish rooms separately. Careful planning should make it possible to place fish rooms adjacent to or near laboratories, but separated from them by barriers such as entry locks, corridors, or floors.

4. While planning experimental fish facility, space should be well divided for different activities. The fish rooms should occupy about 50-60% of the total constructed area and the remaining area should be utilized for services such as stores, washing areas, office and staff, machine rooms, isolation, quarantine and corridors. The environment of fish rooms (Macro- Environment) and fish tank/cage (Microenvironment) are factors on which the production and experimental efficiency of the fish depends. Since fish are very sensitive to environmental changes, sharp, fluctuations in temperature, humidity, light, sound and ventilation should be avoided.

17. FUNCTIONAL AREAS

1. The size and nature of a facility will determine whether areas for separate services and functions are required or not. As a minimum, the exclusive Fish areas require to ensure:

a. Separation of species or isolation of individual projects when necessary;
b. Receipt and quarantine, and isolation of fish; and
c. Separate housing for breeding and experimentation

2. In facilities that are small and intend to maintain very few fish or plans to house fish under specialized conditions (e.g., facilities exclusively used for housing germfree colonies) may not require all the functional areas as listed below. However, a full-fledged independent functional facility with comprehensive multidimentional activities having different species requires them. Professional judgment must be exercised when developing a practically useful system for fish facility based on the need and the intended use.

a. Sufficient rooms for housing different species.

b. Areas or rooms for activities such as surgery, necropsy, preparation of special diets, experimental manipulations, treatment, and sample collection, diagnostic laboratory, small procedures and containment facilities for handling experiments with infectious / pathogenic organisms.

c. Separate rooms and Equipment, for handling of hazardous biological, physical, or chemical agents.

d. Specialized laboratories for handling tissues for histopathology and microbiology, health and genetic monitoring and molecular diagnosis laboratories.

e. Room for formulation and storage of Pharmaceuticals, biologics, and supplies.

f. Space for office administration, staff supervision, and room for maintaining records, control rooms for building management.

g. Change rooms, Shower areas, lockers and toilets for animal care personnel at entry and exit.

h. Area for washing and sterilization of equipment and supplies.

i. Separate room and area for receiving and storage of feed

j. Temporary holding areas for used and cleaned tanks / tubs /boxes and equipment.

k. Area for repairing of utensils and equipment.

l. Area to store wastes and discard material prior to disposal or incineration.

m. There should be adequate and appropriate storage areas for fish tanks, filters, feed, and other accessories.

18. PHYSICAL FACILITIES

The physical condition, size and design of fish facilities depend on the scope of institutional research activities. Fish to be housed physically close to the rest of the institution. A well planned and properly maintained facility is an important element in good fish care and to carryout successful research programs. The following points should be considered for housing facility for Fishes:

a. Housing facility should be compatible with the needs of the species to be housed.
b. Housing Facilities should be designed and operated to facilitate control of environmental factors to exclude vermin and limit contamination associated with the housing of fish, delivery of food, water, and the entry of people and other animals.
c. Housing Facilities should be maintained in good condition. Walls and floors should be constructed with durable materials with surfaces that can withstand to regular cleaning and disinfection.
d. Housing Facilities should be kept clean and tidy and operated to achieve maximum possible hygiene.
e. There should be a pest control programme.
f. Sprays and deodorants that mask the odours should not be used in fish facilities as they may expose fish to volatile compounds which can alter metabolic processes. In addition, deodorants must not be used as a substitute for good cleaning practices and good ventilation.
g. Cleaning practices should be monitored on a regular basis to ensure effective hygiene and sanitation. This may include visual inspection, monitoring water temperatures and microbiological testing of surfaces after cleaning.
h. There should be proper water supply and drainage.
i. There should be adequate contingency plans to cover such emergencies as flooding and fire, or the breakdown of lighting, heating, cooling or ventilation.
j. In the interest of disease prevention and general fish welfare, access to the Housing Facilities by unauthorized persons should be restricted.

1. **Building Materials** should be selected to facilitate efficient and hygienic operation of fish facilities. Durable, moisture-proof, fire-resistant, seamless materials are most desirable for interior surfaces including vermin and pest resistance.

2. **Corridor(s)** should be wide enough to facilitate the movement of personnel as well as equipment and should be kept clean.

3. **Utilities** such as water lines, drain pipes, and electrical connections should preferably be accessible through service panels or shafts in corridors outside the fish sheds.

4. **Fish room Doors, the material used** should not attract rust and should be vermin and dust proof. They should fit properly within their frames. Door closures may also be provided. Rodent barriers can be provided in the doors of the fish facilities/sheds.

5. **Floors** should be monolithic smooth, moisture-proof, nonabsorbent, antiskid, resistant to wear, acid, solvents and should able to with stand to the regular use of detergents and disinfectants. It should be solid with minimum number of joints, strong enough to support water-filled fish tanks / units, equipment, and stored items without becoming gouged, cracked, or pitted.

6. **Drains** are essential in all rooms wherever fish are housed. The rooms can be maintained satisfactorily by wet vacuuming or mopping with appropriate disinfectants or cleaning compounds. Floors should be given sufficient slope towards drain traps and they should be fitted with SS "P" traps with additional mesh to avoid vermin and wild rodent entry.. The slope in the room should allow rapid removal of water and drying of surfaces.

7. **Walls & Ceilings** should be free of cracks, unsealed utility penetrations, or imperfect junctions with doors, ceilings, floors and corners.

8. **Surface materials** should be capable of withstanding scrubbing with detergents, disinfectants and the impact of water under high pressure.

9. **Materials** used for construction of roof should cater needs of local climatic condition to provide comfort to the fish.

10. **Storage Areas:** Separate storage areas should be designed for feed, unclean and cleaned fish tanks, tubs, boxes, rack, utensils and materials that are not in use. Refrigerated storage, separated from other cold storage, is essential for storage of fish carcasses and fish tissue waste prior to disposal.

11. Facilities for Sanitizing Equipment and Supplies: Area for sanitizing tanks,tubs/boxes racks, utensils and ancillary equipment is essential with

adequate water supply.

12. **Experimental Area:** All experimental procedures in fish should be carried out in a separate area away from the place where fish are housed.

19. SANITATION AND CLEANLINESS:

1. Sanitation is an essential activity in a fish house. Fish tanks, corridors, storage spaces, and other areas should be properly cleaned with appropriate detergents and disinfectants as often as necessary to keep them free of dirt, debris, and harmful agents of contamination.

2. Tankstubs/boxes and racks should be sanitized before fish are placed in them. Fish tanks, tubs/boxes, racks, utensils and accessories such as lids, baffles, filters should be washed and sanitized frequently to keep them clean and contamination and odour free.

3. Disinfection can be accomplished with appropriate chemicals that are time tested for toxic products. Tanks, tubs, boxes, racks and accessories should be rinsed with fresh and sterile water for making them free of chemicals prior to use. Periodic microbiologic monitoring is recommended to determine the efficacy of disinfection or sterilization procedures.

4. Some means for sterilizing equipment and supplies, such as an autoclave or gas sterilizer, is essential when experiments are performed with pathogenic organisms. Routine sterilization of tankstubs/boxes, is also essential besides care is taken to use clean materials from reliable sources. Where hazardous biological, chemical, or physical agents are used, a system of regular equipment monitoring is recommended.

20. ASSESSING THE EFFECTIVENESS OF SANITATION

1. Sanitation practices should be monitored appropriately to ensure the effectiveness of the process and materials being used for cleaning; it can include visual inspection of the materials, monitoring of water temperatures, or microbiologic monitoring.
2. A decision to change the frequency of tanktubs / rack washing should be based on factors such as the loss of transparency due to algae growth, formation of rust, biofilm or accumulation of fecal/uneaten feed in the tanks/tubs.

21. WASTE DISPOSAL

1. Wastes should be removed regularly and frequently. All waste should be collected and disposed off in a safe and sanitary manner. Filter pads, bags,

cartridges and charcoal should be treated with a bleach solution before disposal. Fish carcasses, tissue, blood and any infectious materials should be incinerated. Incinerators should be in compliance with all central, state, and local Public Health and Pollution Control Board regulations.

2. Waste containers containing animal tissues, carcasses, and hazardous wastes should be lined with leak-proof, disposable liners. If wastes must be stored before removal, the waste storage areas should be separated from other storage facilities and free of flies, cockroaches, rodents, and other vermin. Cold storage is necessary to prevent the decomposition of biological wastes. Hazardous wastes should be rendered safe by disinfection, decontamination, or other appropriate means before they are disposed off from the fish facility.

22. EXPERIMENTAL PROCEDURES

a. Handling and Restraint

1. Personnel involved in handling fish should undergo extensive training to ensure that, they are well trained to avoid injury and morbidity to fishes during care.
2. Restraint and handling should be carried out in a way to avoid visual stimulation and damage to their mucus-skin barrier.
3. As far as possible, direct light and rapid changes in temperature and lighting should be avoided, and fish should not be kept in the air continuously for more than 30 seconds.
4. Only adequately trained and competent individuals should be permitted to work with dangerous emergency items for which appropriate first aid must be available.

b. Restricted Environments

1. Fish should be held in restricted environments that are non-stressful as far as possible.
2. The environment should comply with the constraints of the experimental design.

c. Surgery

1. Individuals with appropriate training should only be allowed to carry out surgery in fish.
2. In order to achieve the best outcome of surgery, procedure of surgical technique and suture should be practiced on inanimate material or dead specimens until perfection is attained so that there are good chances for the recovery of fish following surgery.
3. Surgical sites should be arranged in a fashion so that tissue damage and contamination of wound areas is minimised.
4. Asepsis and disinfection should be given due importance while performing any kind of surgery. Sterile instruments must be used to avoid contamination of wound while maximizing the healing.

5. Water quality should be maintained at a high level, with minimal bacterial and organic burden during prolonged surgery. In order to minimize shock caused by differences in temperature, pH, and electrolytes water for anesthesia, water should be from the same source as the tank water.
6. Use of anesthetics is recommended for experiments where noxious stimuli are expected and experiments involving extensive handling or to minimize trauma and physiological stress.
7. Documented anesthetics should be used on the basis of their ability to provide predictable results, like immobilization, analgesia, rapid induction and recovery, besides ensuring safety for the fish and the operators (**Annexure 8**).
8. Anesthetics should be first tested on a small sample of fish. The anesthetic capability should be determined in local water conditions, as well as the species, life stage, and size of the fish before the experiment.
9. Personnel working with anesthetic agents in fish must be adequately trained and protected with recommended personal protective equipment (PPE) as per the requirement of the experiment.
10. Incisions on the lateral line should be avoided rather than follow the longitudinal axis of the fish.
11. Non-hygroscopic strong and inert monofilament suture material and atraumatic needles should be used for closing the incisions.
12. In Zebrafish tail fin clipping for genotyping or experimental purposes should be carried out under sedation or light anesthesia.
13. Following surgery, fish must be closely observed and monitored both under laboratory or applicable field situations.
14. Post-surgery, fish should be held in an environment that reduces or eliminates intraspecific interactions in tanks, besides meeting appropriate living conditions.
15. Post-surgery, the use of prophylactic antibiotics should be cautiously evaluated. For maintaining common groups of recovering fish, factors, such as size differences, ability to feed or exclude other fish from feed and, agonistic behavior, should be considered in experimental design.

d. Administration of Compounds and Devices by Various Routes

1. For oral administration of compounds, the dose rate of a compound to be administered should not exceed 1% of the bodyweight (1 mL/100 g).
2. While injecting intramuscular, the needle should be introduced in spaces between the scales. Large dorsal epaxial and abdominal muscles should be preferred avoiding the lateral line and ventral blood vessels.
3. For intraperitoneal (IP) injections care should be taken to avoid penetration in to abdominal viscera as it may be inflammatory and lead to formation of adhesion.
4. Implants should be implanted using sterile techniques, biocompatible, and aseptic.

e. Tagging and Marking

1. Marking methods should preferably be non-invasive and must be minimized.

It must be ensured that the marking should remain visible for the entire duration of the study.

2. Adverse effects of marking and tagging can lead to behavioral changes, changes in physiology or survival of individual experimental fish should be carefully considered. Pilot study should be carried out if effects of markings are not known for a particular species.
3. Marking techniques causing substantial injuries to tissue, like, branding, tattooing or clip- ping important fins should be avoided but under exceptional circumstances, it may be used by an investigator after providing adequate justification to animal ethics committee indicating why alternative methods cannot be used.

f. Collection of Body Fluids

To restrain fish for collection or cannulation purposes, sedation or anesthesia should be used. However, there may be alteration in physiological parameters like serum glucose and several hormone levels.

g. Endpoints and Criteria for Early Euthanasia

1. Possible pain and distress should be eliminated or mitigated by the investigators on their experimental subjects whenever it is achievable employing good scientific practice.
2. For studies involving potential pain or distress, pilot studies should be conducted to identify desired clinical signs that may be used as an endpoint for establishing appropriate monitoring.
3. Parameters must be set to document assessment of health status when experiments with defined early pre-lethal endpoints are to be conducted.
4. Criteria for early euthanasia should be clearly defined for studies where morbidity and mortality are expected.

h. Monitoring

Experimental fish must be monitored daily at least once which may depend on the requirement of study as well as the time of morbidity. Moreover, the frequency of monitoring should be synchronized in such a way that there should be ample time for the removal of the fish before severe morbidity. Experiments with predicted high mortality should be monitored more frequently.

i. Exercise to Exhaustion

Exhaustion studies in fish must strictly comply as per the guidelines. Fish used for experiments involving the forced swimming should be subjected to minimum distress to the point of exhaustion in conjunction with negative reinforcement.

j. Environmental Extremes

Earliest endpoint possible should be used for fish subjected to studies involving exposure to environmental extremes.

k. Genetically Modified Fish

1. As a result of genetic alteration, genetically modified fishes may have changes in physiology and anatomy and hence there is a need to monitor them closely.
2. Unless going through extensive safety assessment and certification for the authorization of sale, genetically modified fishes must not be permitted to enter the food or feed chain. Such fish can only be introduced with the prior permission of the competent authority, or a Committee for the care and use of animals.

23. EUTHANASIA

Euthanasia should be resorted to events where fish are required to be sacrificed to reduce suffering or to limit spread of infections or for termination as part of an experiment or for other ethical reasons. The procedure should be carried out quickly and painlessly in an atmosphere free from fear or anxiety. For accepting the euthanasia method as humane, it should have an initial depressive action on the central nervous system for immediate insensitivity to pain. The choice of a method will depend on the nature of study and species to be killed (Annexure 9). The method should in all cases, meet the following requirements:

a. Death, without causing anxiety, pain or distress with minimum time lag phase.
b. Minimum physiological and psychological disturbances.
c. Compatibility with the purpose of the study and minimum emotional effect on the operator
d. Location should be separate from animal rooms and free from environmental contaminants.
e. Two-step euthanasia should be practiced in fishes. An initial step involving anesthesia enabling to reach the point of loss of equilibrium, followed by a physical or chemical method to cause brain death.
f. Physical technique of euthanasia used for killing fish, should involve physical destruction of brain tissue by pithing or crushing the brain. However this requires the additional permission from IAEC.

24. DISPOSITION OF FISH AFTER STUDY

a. Consumption of Fish

Fishes to be used for food that were subjected to sedation or anesthesia should be held for adequate withdrawal time before being killed and consumed. In such cases the veterinarian should verify that the fish is safe for consumption.

b. Release of Fish to Wild

As a rule of thumb, fish kept in captive environments and used for research must not be released into the wild. Release into the wild can only be permitted as per the National or territorial regulations. Researchers should ensure death of the embryos, larvae or adult fish before disposal. Flushing of embryos and larvae into the sink should be discouraged and disposal is carried out as per state regulations for the biomedical waste. It is also recommended to cover water outlets in sink with wire mesh.

25. PEST CONTROL

Adaptation of Programs designed to prevent, control, or eliminate the presence of or infestations by pests are essential in the fish rooms. Best results can be achieved by giving contracts to people/firm specialized in pest control.

26. EMERGENCY, WEEKEND AND HOLIDAY CARE

There should be an institutional policy for the care of fish by qualified personnel every day, including weekends and holidays, to safeguards their well being including emergency health care. In the event of an emergency, institutional security personnel and fire or police officials should be able to reach responsible persons for the fish facility. That can be enhanced by prominently posting emergency procedures, names, or telephone numbers in fish facilities or by placing them in the security department or near telephone. A disaster plan that takes into account both personnel and fish should be prepared as part of the overall safety plan for the fish facility.

27. RECORD KEEPING

It is essential that fish House should maintain following records:

1. Fish House plans, which includes typical floor plan, all fixtures etc.
2. Fish House staff record - both technical and non - technical
3. Health record of staff and fish
4. All SOPs relevant to experiments, care, breeding and management of fish
5. Breeding, stock, purchase and sales records
6. Minutes of institutional Animals Ethics Committee Meetings
7. Records of experiments conducted with the number of fish used (copy of Form D)
8. Mortality, Postmortem Record, wherever required.
9. Clinical record of sick fish.
10. Training record of staff involved in fish activities
11. Water, feed and bedding materials analysis report
12. Health monitoring Records.

28. STANDARD OPERATING PROCEDURES (SOPs) / GUIDELINES

The Institute should maintain SOPs describing procedures/methods adapted with regard to fish Husbandry, maintenance, breeding, fish facility activities and experimentation.

SOP should contain the following items:

1. Name of the Author
2. Title of the SOP
3. Date of approval

4. Reference of previous SOP on the same subject and date (Issue no and Date)
5. Location and distribution of SOP's with the sign of each recipient
6. Objectives
7. Detailed information of the instruments used in relation with fish with methodology (Model no., Serial no., Date of commissioning, etc)
8. The name of the manufacturer of the reagents and the methodology of the analysis pertaining to fish
9. Normal value of all parameters
10. Hazard identification and risk assessment

29. TRANSPORT OF FISH:

Before fish are transported from one locality to another, they must undergo health assessment. Appropriate regulatory approval and permits must be in place before any transfer.

a. The transport of fish from one place to another is very important and must be undertaken with due care. The main considerations for transport of fish are mode of transport, size and type of containers, density in containers, food and water during transit, protection from transit infections, injuries and stress.

b. The mode of transport of fish depends on the distance, seasonal and climatic conditions and the species of fish. Fish can be transported by road, rail or air taking into consideration of the above factors. In any case the transport stress should be avoided and the containers should be of appropriate size so as to enable the fish to have a comfortable movement and protection from possible injuries. Sometimes injuries can be avoided by reducing space and decreasing the time of transportation. The food and water should be provided in suitable containers or in suitable form to ensure that they get adequate food and more particularly fluid during transit. The transport containers (tanks, tubs, boxes) should be of appropriate size and only a permissible number of fish should be accommodated in each container to avoid overcrowding and infighting (Annexure 10).

30. ANIMAL ETHICS

All scientists working with fish must have a deep ethical consideration for the fish they are dealing with. From the ethical point of view, it is important that such considerations are taken care at the individual level, at institutional level and finally at the national level. Interaction amongst people working in fish house should be organized regularly to discuss ethical issues favoring the wellbeing of fish.

31. GENETICALLY ENGINEERED / MODIFIED TRANSGENIC FISH

Transgenic fish are those fish, into whose germ line the foreign gene(s) have been introduced, whereas knockout fish are those whose specific gene(s) have been disrupted leading to loss of function. These fish can be bred to establish transgenic fish strains. Transgenic fish are used to study the biological functions of specific genes, to develop fish models for diseases of humans or animals to produce therapeutic products, vaccines and for biological screening, etc. They can be either developed in the laboratory or procured for R&D purpose from registered scientific/academic institutions or commercial firms generally from abroad with approval from appropriate authorities.

For initiating a colony, the breeding stock must be procured from established breeders or suppliers ensuring their genetic makeup and health status of fish is known. In case of an inbred fish line, the characters of the line with their gene distribution and the number of inbred generation must be known for further propagation. The health status should indicate their origin, e.g. conventional, specific pathogen free or transgenic, gnotobiotic or knockout stock.

32. MAINTENANCE

Housing, feeding, ventilation, lighting, sanitation and routine management practices for such fish are similar to those for the other fish species as given in guidelines. However, special care must be taken for transgenic/gene knockout fish where the fish can become susceptible to diseases and special conditions of maintenance are required due to their altered metabolic activities. The transgenic and knockout fish carry additional genes or lack few genes compared to the wild population. To avoid the spread of the genes in to wild population, care should be taken to ensure that they are not inadvertently released in the wild to prevent cross-breeding with other fish.

The transgenic and knockout fish should be maintained in clean room environment or in fish isolators.

33. DISPOSAL

The transgenic and knockout fish should be first euthanized and then disposed off as described elsewhere in the guidelines. A record of disposal and the manner of disposal should be kept as a matter of routine.

Annexure 1: Common species of Fish

Sl. No.	Common name	Scientific name	Category	Habitat	IUCN status	Age at Maturity	Size at Maturity	Fecundity	Feeding Habit	Max Total Length @age	Max Body Weight @age
1	Catla	*Catla catla*	Carps	Freshwater	Least Concern (LC)	2 years	2kg (avg)	100000-200000/kg BW	Surface feeder, Omnivorous	180 cm	40 kg
2	Rohu	Labeo rohita	Carps	Freshwater	LC	2 years	Male-46 cm, female-51cm	109000 to 535000 eggs/kg	Column/bottom feeder/ planktonivore	200 cm	45 kg
3	Mrigal	*Cirrhinus mrigala*	Carps	Freshwater	VU	2years	34.9 cm	100000 to 150000 eggs/kg	Illiophagous, (feeding on the bottom on decayed vegetation)/ filter feeder	100 cm	12.7 kg
4	Reba	*Cirrhinus reba*	Carps	Freshwater	LC	1 year	13.5 cm	20722 to 211200 eggs/kg	planktonivore	30cm	500 g in 1 year

5	Bata	*Labeo bata*	Carps	Freshwater	LC	1 year	14.12cm -male	192785 eggs/kg	It is bottom dwellers and takes rotten	61 cm	181.35g (Hossen
							14.60cm -female (400-500 g)		plant, algae and plankton		*et al.*, 2018)
6	Fringe-lipped Carp	*Labeo fimbriatus*	Carps	Freshwater	LC	2 years	33.6-74 cm	64800 to 526000eggs /kg	Herbivorous	91 cm	>450 g 1 year (Mohant a *et al.)*
7	Calbasu	*Labeo calbasu*	Carps	Freshwater	LC	3 years	40cm - male , 45cm - female	8,76,000 eggs/kg	feed on plants, filamentous algae and diatoms	91.2 cm	600 kg-Powai lake

8	Pengba	*Osteobrama belangeri*	Carps	Freshwater	Near threaten ed (NT)	2+ years	0.6-0.8kg male and 0.8-1.0 kg female	65,136 egg/kg	Omnivorous (algae, aquatic plants, zoo plankton, diatoms etc)	31cm	
9	Common Carp	Cyprinus carpio	Carps	Freshwater	Vulnerable (VU)	3-4 years	25 - 36 cm	100,000 and 300,000 eggs per kg	Zooplankton feeder in the juvenile stage and a benthic feeder later on	120cm	40 kg
10	Grass Carp	Ctenopharyngodon idella	Carps	Freshwater	Not evaluate d (NE)	3-8 years	50-86 cm	100000 to 2000000eggs/kg	Herbivorous-aquatic plants and submerged grasses; detritus, insects and other invertebrate.	150 cm	45 kg at 21 years age

11	Silver Carp	Hypophthalmichthys molitrix	Carps	Freshwater	NT	3-4 yeas	3-8 kg	0.8-1.0 lakh eggs/kg	Planktivores, filter feeder	140 cm	50 kg
12	Magur/Walking Catfish	Clarias magur	Catfish	Freshwater	Endangered (EN)	End of 1st year	100-150 g	3600±274.16 to 9539±746.50 eggs	omnivorous, opportunistic feeder, feeding on both living and dead matter	46 cm	250 gm
13	Singhi/ Stinging Catfish	*Heteropneustes fossilis*	Catfish	Freshwater	LC	1 year		1500-2000 eggs/ gm ovary	Omnivorous	31 cm	Grows to 200 g
14	Giant River-Catfish	*Sperata seenghala*	Catfish	Freshwater	LC	2nd year	40-50 cm	1,31,820-4,28,376 eggs	Column feeder /Carnivore	224 cm (Mishra, 1959)	
15	Freshwater Shark	*Wallago attu*	Catfish	Freshwater	NT	2 year	45-49.5 cm	30,000-40,000 eggs/kg.	Juveniles feed mainly on insects; adults feed on smaller fish, crustaceans, and mollusks.	240 cm	45 kg (Talwar and Jhingran, 1991)

16	Pabda Catfish	*Ompok pabda*	Catfish	Freshwater	NT	1 year	12.9 cm-male and 13.9 cm female	2460-5986 (Gupta et al., 2014)	Omnivore	30 cm (Shafi and Quddus, 2001)	30-35g weight at 7-8 months of age
17	Chital	*Chitala chitala*	Featherbacks	Freshwater	Threate ned	2-3 year	75 cm	5286 eggs/kg body weight	An obligate, typically nocturnal, predator feeding	122 cm	2 kg in 243 days (Rahmat ullah *et al.*, 2009)
18	Bronze Featherback	*Notopterus notopterus*	Featherbacks	Freshwater	LC	2nd year	18-35 cm	1,235-1,377 eggs (340-349 mm)	Feed on insects, fish, crustaceans and some young roots of aquatic plants	60 cm	315 g

19	Mola Carplet	*Amblypharyngodon mola*	carps	Freshwater	LC	1.5-2 years	5-7.8 cm	940 to 7210 eggs (Pal & Mahapatra, 2016)	Surface feeder and planktonivore	20 cm	3.64 g-male 6.32 g female (Gupta et al.,2014)
20	Climbing Perch	*Anabas testudineus*	Perciform	Freshwater	Vu	1 year	8-10cm	7,989 to 12,758 eggs (Ziauddin *et al.*,2016)	Omnivorous	25 cm	117.27 g
21	Ticto Barb	*Puntius ticto*	carp	Freshwater	Vu	1st year	4.30 cm	12461 to 15105 eggs (Bithy et al.,2012)	Surface feeder and it feeds on Diatom, Algae, Crustaceans, Rotifer, insects etc	12.5 cm	24 g (Hossain *et al.*, 2012)
22	Silver Hatchet Chela	*Chela cachius*	carp	Freshwater	Not evaluated (NE)	1-2 yr	7-8 cm	4326 to 9017 eggs	Feed on aquatic invertebrates	12 cm (Shafi and Quddus 2001).	3.22 g Yeasmin *et al.*, 2015

23	Striped Murrel / Snakehead	*Channa striata*	Snakeheads	Freshwater	LC	2 years	18 cm	10,000-15,000 eggs/kg	Omnivore- Feed on fish, frogs, snakes, insects, earthworms, tadpoles	100 cm	3 kg
24	Spotted Murrel / Snakehead	*Channa punctata*	Snakeheads	Freshwater	LC	1st year	11-23 cm	2,200 to 33,873 eggs	Carnivore; voracious and predatory to small fish and fries	31 cm (Talwar and Jhingra n 1991)	1.5 kg Archarya and Iftekhar (2000)
25	Flower Murrel / Bullseye Snakehead	Channa marulius	Snakeheads	Freshwater	LC	2-3 year (Kilambi 1986)	11.1-18.1 cm and 14.4-70.5 g	2116-11332 eggs	Piscivorous, carnivorous and cannibalistic	183 cm (Shrestha, T.K., 1990.)	30.0 kg (Talwar and Jhingran 1991)
26	Dwarf Murrel / Snakehead	Channa gachua	Snakeheads	Freshwater	NE		11-15.5cm and 25-53gm	925 to 3450 eggs	Obligate predator feeding mostly on smaller fishes and insects	28.8 cm (sun *et al.*,2016)	201.40 g (sun *et al.*,2016)

27	Nile Tilapia	*Oreochromis niloticus*	Cichlid	Freshwater	LC	5-6 months	20-39 cm	Few hundred to 2000 eggs/ batch	Omnivorous mainly feed on phytoplankton	60 cm	4.3 kg
28	GIFT Strain	*Oreochromis niloticus*	Cichlid	Freshwater	LC	4-5 months	205 g	400-500 eggs/female /batch	Omnivorous mainly feed on phytoplankton		1kg in 10 months Max 5 kg
29	Red Tilapia	Oreochromis niloticus	Cichlid	Freshwater	LC	4-6 months			Omnivorous		264 g in 1 yr (Siddiqu i and Harbi,1 9 95)
30	Mozambique Tilapia	*Oreochromis mozambicus*	Cichlid	Freshwater	NT	2-5 month	15.4 cm	235 to 390 eggs/gm bw	Omnivorous	39.0 cm	1.1 kg
31	Golden Mahseer	*Tor putitora*	Mahseer	Cold water	EN	4+ year	31-58 cm	7076 to 18525 eggs	Omnivorous, feeding on fish, zooplankton, dipteran larvae and plant matter	275 cm	54 kg

32	Tor Mahseer	*Tor tor*	Mahseer	Cold water	Data deficien t (DD)	3rd year	34-38 cm	7000 to 101600 eggs	Omnivorous, feed on algae, water beetles and crustaceans	200 cm	9 kg
33	Deccan Mahseer	*Tor khudree*	Mahseer	Cold water	EN	1-1.5 years	180 mm (320 g)- male 280 mm (740 g)- femal e	7,500-17,500 eggs	Feeds on plants, insects, shrimps and mollusks	54.5 cm	22.5 kg
34	Chocolate Mahseer	*Neolissochilu s hexagonolepi s*	Mahseer	Cold water	Near Threate ned (NT)	3 yrs	22.5 cm	3500	Omnivorous	120 cm TL	11.0 kg
35	Common Snow Trout	*Schizothorax richardsonii*	Trout	Cold water	Vulnera ble (VU)	2 yrs	17.5 cm for male and 250m m in	30000 - 50000	Herbivorous	60.0 cm TL	

							female				
36	Rainbow Trout	*Oncorhynchus mykiss*	Trout	Cold water	Not Evaluated	2-3 yrs	15 cm	200 - 13000	Omnivorous	122 cm TL	25.4 kg
37	Brown Trout	*Salmo trutta*	Trout	Cold water	Least Concern (LC)	1-2 yrs of age for males and 2-3 yrs of age for females	19.3 cm	1 000 -2 000	Carnivorous	140 cm SL	50 kg
38	Brook Trout	*Salvelinus fontinalis*	Trout	Cold water	Not Evaluated	2- 3 yrs	13 cm	Upto 5000	Omnivorous	86.0 cm SL	8.0 kg
39	Mountain Trout	*Barilius vagra*	Trout	Cold water	Least Concern (LC)		6.5 cm	800-3000 (Saxena et al., 2016)	Carnivorous	15.6 cm TL	
40	Gheur	*Barilius bendelisis*	Trout	Cold water	Not evaluated		10.07 cm	320 to 4000 (Saxena et al., 2016)	Planktivorous	22.7 cm TL	

41	Grey Mullet	*Mugil cephalus*	Mullet	Brackish water	Least Concern (LC)	2-4 yrs	35.4 cm	0.4 to 5.2 million	Planktivorous	100.0 cm SL	12 kg
42	Goldspot Mullet	*Mugil parsia*	Mullet	Brackish water	Not Evaluated		9.5 cm	66,000 and 2,06,000	Omnivorous	40 cm TL	
43	Milkfish,	*Chanos chanos*	Chanidae	Brackish water	Least Concern (LC)	3-5 yrs	91.8 cm	3.1-5.7 Million eggs	Herbivorous	180 cm SL male/ 124.0 cm SL	14.0 kg
44	Asian Seabass	*Lates calcarifer*	Bass	Brackish water	Not Evaluated	3–4 yrs	29 - 60 cm	2.1 to 7.1 million eggs	Carnivorous	200 cm TL male	60.0 kg
45	Tiger Bass	*Terapon jarbua*	Bass	Brackish water	Least Concern (LC)	2-3 yrs	13.0 cm,	13,475 to 115,920	Omnivorous	36.0 cm TL	
46	Green Chromide Pearlspot	*Etroplus suratensis*	cichlid	Brackish water	Least Concern (LC)	2 yrs	15.0 cm	1300-6000	Planktivorous	40.0 cm TL	
47	Indian pampano	*Trachinotus mookalee*	Pampano	Marine water	Least Concern (LC)	1 yr	47.6 cm	80,000 to 184,000	Carnivorous	90.0 cm TL	8.1 kg

48	Orange-spotted Grouper	*Epinephelus coioides*	Grouper	Marine water	Least Concern (LC)	2-3 yrs	48.3 cm	43000 - 470000	Carnivorous	120 cm TL	15.0 kg
49	Pink ear emperor	*Lethrinus lentjan*		Marine water	Least Concern (LC)	2 yrs	27.7	80000-85000	Carnivorous	52.0 cm TL	
50	Spotted Scat,	*Scatophagus argus*	Scat	Marine water	Least Concern (LC)	1 yr	17 cm	300000 - 600000	Omnivorous	38.0 cm TL	
51	Long Whiskers Catfish	*Mystus gulio*	Catfish	Marine water	Least Concern (LC)		56 g and 6.5 cm,	19000-24000	Carnivorous	46.0 cm TL	
52	Striped Dwarf Catfish	*Mystus vittatus*	Catfish	Marine water	Least Concern (LC)		9.0 cm,	12,000-14,000	Omnivorous	21 cm TL	
53	Silver Pompano,	*Trachinotus blochii*	Pampano	Marine water	Least Concern (LC)	2-3 yrs	58 cm	133 000 - 800 000	Carnivorous	110 cm TL	3.4 kg
54	Cobia	*Rachycentron canadum*		Marine water	Least Concern (LC)	1-2 yrs	43 cm	1,935,000-5,500,000	Carnivorous	200 cm TL	68 kg
55	Giant Freshwater Prawn,	*Macrobrachium rosenbergii*	Prawns	Freshwater	Least Concern (LC)	4-7 month	Mature at 140 - 150 mm length and 35-	20,000–100,000 eggs	Zooplanktivorous	30 cm	

							40 g weight				
56	Indian River Prawn	*Macrobrachium malcolmsonii*	Prawns	Freshwater	Least Concern (LC)		Maturity at100-190 mm for female And male at 170-205 mm	3,500 to 80,000 no	Omnivorous	23 cm for male and 20 cm for female	
57	Tiger Prawn/ Black Tiger Shrimp	*Penaeus monodon*	Shrimp	Marine	Not Evaluated	10-12 month	Male mature at 37 mm carapace length and 35 g body weight and females mature at 47 mm carapace length	2-10 lakhs	Carnivorous	33.6 cm TL	250.00 g

							70 g of BW				
58	Indian White Prawn/Shrimp	*Penaeus indicus*	Shrimp	Marine	Not Evaluated		12.2 cm	437,000 – 550,000 eggs	Omnivorous	18.4 cm TL for male and 22.7 cm TL for female	35.00 g
59	Pacific White Shrimp	*Litopenaeus vannamei*	Shrimp	Marine	Not Evaluated	Onwards at the age of 6–7 months	Males become mature from 20 g and females from 28 g	100 000–250 000 eggs	Omnivorous	23.0 cm TL	
60	Indian Freshwater Crab	*Barytelphusa cunicularis*	Crabs	Freshwater	Not Evaluated		Male maturity at 35 mm carapace length, while female attains at 38	234 eggs and 987 eggs (RF Pathre, M Patil - World journal of Zoology, 2010)	Omnivorous		

							mm carapace length.				
61		*Maydelliathelphusa lugubris*	Crabs	Freshwater	Least Concern (LC)				omnivores		
62		*Maydelliathelphusa falcidigitis*	Crabs	Freshwater	Data deficient				omnivores		
63	Mud Crab	*Scylla serrata*	Crabs	Marine	Not Evaluated		Maturity at 80 mm carapace width	.5 to 2.5 million eggs	Omnivorous	28.0 cm CW	3.0 kg
64	Three-spot Swimming Crab	*Portunus sanguinolentus*	Crabs	Marine	Not Evaluated		*Maturity at* 8.1 – 9.6 cm CW	961000 - 2250000 eggs	Carnivorous	15–20 cm	
65	Blue Swimming Crab	*Portunus pelagicus*	Crabs	Marine	Not Evaluated		Maturity at 31 to 49 mm carapace length	270,000 to 1,880,000 eggs	Carnivorous	20.0 cm CW	
66	Scalloped Spiny	*Panulirus*	Lobster	Marine	Least Concern		sexual maturity	1,20,544 to 4,49,585 egg	omnivorous	31.0 cm	

	Lobster	*homarus*			(LC)		at 55 mm carapace length	s		TL	
67	Ornate Spiny Lobster	*Panulirus ornatus*	Lobster	Marine	Least Concern (LC)		Sexual maturity at 90 mm CL (250 mm TL)	5,18,181 to 19,79,522 eggs	omnivorous	50.0 cm TL	6.5 kg
67	Ornate Spiny Lobster	*Panulirus ornatus*	Lobster	Marine						500 mm	
68	Slipper Lobster	*Thenus orientalis*	Lobster	Marine	LC						
69	Blood Calm	*Anadara granosa*	Clams	Marine			20-24 mm	518,400 - 2,313,200 eggs	Filter feeder	73.4 mm	
70	Asiatic Hard Clam	*Meretrix meritrix*	Clams	Marine			21-26 mm	318,400 to 3,825,000 eggs/individual	Filter feeder	91 mm	
71	Black Clam	*Villorita*	Clams	Marine	LC		20-		Filter feeder	52 mm	

		cyprinoides					25mm				
72	Short-neck Clam	*Paphia malabarica*	Clams	Marine			11-20mm		Filter feeder	55 mm	
73	Backwater Clam	*Meretrix casta*	Clams	Marine			11-17mm		Filter feeder	55 mm	
74	Green Mussel	*Perna viridis*	Mussels	Marine		2-3 month	15.5-28mm	0.5-10 million	Filter feeder	160 mm	
75	Brown Mussel	*Perna indica*	Mussels	Marine		2-3 month	15- 28 mm	0.5- 10 million	Filter feeder	120 mm	
76	Indian Backwater Oyster	*Crassostrea madrasensis*	Oyster	Marine		3-4 month	12-14 mm (M) 22-26 mm (F)	10-15 million eggs	Filter feeder	128 mm	
77	Rock Oyster	*Saccostrea cucullata*	Oyster	Marine		4-5 month	29 mm male 33 mm female		Filter feeder	20 cm	
78	Pearl	*Pinctata*	Oyster	Marine		Less than	28 mm		Filter feeder	9 cm	70 gm

	Oyster	*fucata*				1 yr	male 38 mm female				
79	Window pane Oyster	*Placuna placuna*	Oyster	Marine		2-3 month	53 mm		Filter feeder	157 mm	
80	Freshwater pearl mussel	*Lamelidens marginalis*	Mussel	Freshwater					Filter feeder	120 mm	
81		*Lamelidens corrianus*	Mussel	Freshwater					Filter feeder		
82		*Parreysia corrugata*	Mussel	Freshwater	LC				Filter feeder	80 mm	
83	Gold fish	*Carrasius auratus*	Ornamental	Freshwater		225-230 days		2000-3000 eggs/ kg	omnivorous	45cm	
84	Guppy	*Poecilia reticulata*	Ornamental	Freshwater		10-20 weeks	15-18 mm	20-100 young ones	omnivore	Male 2.5-3.5 cm Female 5-6 cm	

85	Koi carp		Ornamental	Freshwater	LC	3-4 months		2000-3000 eggs	Omnivore	100 cm	
86	Molly	*Poecilia sphenops*	Ornamental	Freshwater	LC	12-16 weeks	25-40 mm	30-70 young one	omnivore	Male 7-8 cm female 9 cm	
87	Siamese fighter	*Beta splendens*	Ornamental	Freshwater		3 month		200-300 eggs	carnivore	7 cm length	
88	Dwarf Gourami		Ornamental	Freshwater		12-14 week	4-5 cm	400-600 eggs	omnivore	5.5 cm	
89	Swordtail	*Xiphophorus helleri*	Ornamental	Freshwater	LC	6-8 weeks	25-30 mm	20-100 young ones	Omnivore	Male 6-7 cm Female 7-9 cm	
90	Red Line torpedo barb	*Sahyadria denisonii*	Ornamental	Freshwater	EN		85.33±1.52 mm for males and 95.66±1.15 mm	376- 1098 eggs	Omnivores	162 mm male 132 mm female	

							for females				
91	Angel fish	*Pterophylum scalare*	Ornamental	Freshwater		10 month		1000 eggs	Omnivore	15 cm	
92	Parrot fish	*Scarus Psittacus*	Ornamental	Freshwater			10cm male 15 cm female		herbivore	34 cm	904 gm
93	Indian Glassfish		Ornamental	Freshwater	LC		2.58 cm	500-6506 eggs	Carnivore	9 cm	
94	Goby	*Glossogobius giuris*	Ornamental	Freshwater		Less than 1 yr	9.5cm	8050-10700 eggs	Carnivore	50 cm	
95	Loach	*Botia Dario*	Ornamental	Freshwater	LC		7.32 cm (M) 7.89 cm (F)	5,245-53,754 eggs	Omnivore	13.9 cm	32.1 gm
96	Clown fish	*Amphiprion ocellaris*	Ornamental	Marine		18 months	58- 65 mm male 70-80 mm female	300-350 eggs	omnivores	11 cm	

97	Damsel fish (Dascyllus trimaculatus)	*Abudefduf saxatilis*	Ornamental	Marine	LC		31-40 mm female 51-60 mm male	809-9634 ova	omnivore	14 cm	
98	Moorish Idol	*Zanclus cornutus*	Ornamental	Marine	LC				omnivores	22 cm	
99	Rabbit fish	*Siganus cannaliculatus*	Ornamental	Marine	LC	Less than a year	10.6 cm male 11.6 cm female	166000 to 1000000 eggs	Omnivorus		
100	Sea Goldie	*Pseudanthias squamipinnis*	Ornamental	Marine	LC				carnivore	15 cm	

Annexure 2: Water quality parameters, normal values, testing frequency and management for Zebrafish (Source: Zebrafish in Biomedical Research; Biology, Husbandry, Diseases and Research Applications. American College of Laboratory Animal Medicine series, 2020).

Sr. No.	Parameters	Normal Range	Testing Frequency	Management
1	Water temperature (^oC)	26-29 (Optimal 28.5)	Daily	↑- Start water cooling ↓- Start water warming
2	pH	7-8	Daily	↑- Slow addition of HCl ↓- Slow addition of $NaHCO_3$
3	Conductivity (uS)/salinity (ppt)	200-3000/ 0.5-2	Daily	↑- Slow addition of RO/DI ↓- Slow addition of Salts
4	Hardness (ppm)	100-200	Weekly	↑- Slow addition of RO/DI ↓- Slow addition of Salts
6	Alkalinity (ppm)	50-75	Weekly	↑- Slow addition of RO/DI ↓- Slow addition of $NaHCO_3$
7	Dissolved Oxygen (ppm)	6-8 (near saturation)	Weekly	↑- Increase water aeration ↓- Reduce water aeration
8	Chlorine (ppm)	zero	Regular	↑- Vigorous aeration Sodium thiosulphate Chemical filtration

Annexure 3: Nitrogenous compounds/gases, normal values, testing frequency and management (Source: Zebrafish in Biomedical Research; Biology, Husbandry, Diseases and Research Applications. American Collegeof Laboratory Animal Medicine series, 2020)

Sr. No.	Nitrogenous compounds and gases	Normal value	Testing Frequency	Management when crossed acceptable levels
1	Total Ammonia Nitrogen (ppm)	0- <1	Weekly	Remove waste solids, increase water exchange, check pH and alkalinity
2	Nitrite NO_2 (ppm)	0 - <0.5	Weekly	Remove waste solids, increase water exchange, check pH and alkalinity, slow addition of sea salt solution
3	Nitrate, NO_3 (ppm)	<50	Weekly	Increase water exchange
4	Carbon dioxide (ppm)	Close to zero, prompt action at 15 ppm	Weekly	Increase aeration, remove waste solids, increase water exchange, ventilate area

Annexure 4: Stocking density for different stages and in different capacity tanks

Sr. No.	Fish tanks capacity (commercially available)	Recirculation system (5-10 fish/L)	Purpose
1	0.8	1-2 adults (IDs)	Isolation, nursery
2	1.1	1-2 adults (IDs)	Isolation, nursery
3	1.4-1.5	1-2 adults (IDs)	Isolation, nursery
4	1.8	Up to 18	Adult group housing, isolation, nursery
5	2.8-3.0	Up to 28-30	Adult group housing, isolation, nursery
6	3.5	Up to 35	Adult group housing, isolation, nursery
7	6.0	Up to 60	Adult group housing, nursery
8	8.0	Up to 80	Adult group housing
9	9.0	Up to 90	Adult group housing
10	10.0	Up to 100	Adult group housing
11	11.0	Up to 110	Adult group housing

*In static water maximum density should not cross more than 1-2 fish/L water.

Source:

1. Lawrence C and T. Mason (2012) Zebrafish Housing Systems: A Review of Basic Operating Principles and Considerations for Design and Functionality. ILAR, Vol, 53(2),p179-191.

2. Brand M, Volhard CN, Dahm R (2002) Keeping and raising Zebrafish. In: Volhard CN (ed) Zebrafish: A Practical Approach. Oxford University Press, New York, p1-37.

3. Matthews M, Trevarrow B, Matthews J (2002) A virtual tour of the guide for Zebrafish users. Lab Anim 31:34-40.

Annexure-5: Different types of Zebrafish foods, presentations, pellet size and feeding stages.

Sr. No.	Types of feeds	Presentation	Size of pellets or live foods	Culture and harvesting	Life stage of Zebrafish
1	Micro-pellet	Dry powder	50-100 um	No	Early and mid-larvae
2	Micro-pellet	Dry powder	100-200 um	No	Mid-larva, Juvenile
3	Micro-pellet	Dry powder	200-500 um	No	Juvenile, Adults
4	Micro-pellet	Dry powder	400-700 um	No	Adults
4	Brine shrimp Artemia	Dry cysts	400-500 um* (length)	Yes	Larval, juvenile and adults
5	Rotifers	Culture	100-210 um & 130-340 um	Yes	Larval, juvenile and adults
6	Paramecium	Culture	50 x180 um (Width x length)	Yes	Larval, juvenile and adults

Source:

1. Zebrafish in Biomedical Research; Biology, Husbandry, Diseases and Research Applications. American College of Laboratory Animal Medicine series, 2020).

2. Gemma food, www.zebrafish.skrettingusa.com

Annexure-6: Hematological parameters for laboratory Zebrafish:

Sr. No.	Blood cells	Normal range (%)	Mean± SD (%)
1	Lymphocytes	71-92	82.95 ± 5.47
2	Monocytes	5-15	9.68 ± 2.44
3	Neutrophils	2-18	7.10 ± 4.75
4	Eosinophils	0-2	0.15 ± 0.53
5	Basophils	0-2	0.13 ± 0.40
6	RBC (cells/microl)	3.02×10^6	--

Source:

1. Murtha JM, Qi W, Keller ET. Hematologic and serum biochemical values for Zebrafish (Danio rerio). Comp Med. 2003;53 (1):37-41.

2. Zebrafish in Biomedical Research; Biology, Husbandry, Diseases, and Research Applications. American College of Laboratory Animal Medicine series, 2020).

Annexure-7: Serum biochemical parameters for adult Zebrafish.

Sr. No.	Serum biochemicals	Normal range (%)	Mean± SD (%)
1	Albumin (g/dL)	2.7-3.3	3.0 ± 0.2
2	Globulins (g/dL)	1.3-2.8	2.1 ± 0.6
3	Total Proteins (g/dL)	4.4-5.8	5.2 ± 0.5
4	Glucose (g/dL)	62.0-91.0	82.2 ± 12.0
5	ALP (U/L)	0.0-10.0	2.0 ± 4.5
6	ALT (U/L)	343.0-410.0	367.0 ± 25.3
7	Amylase (U/L)	1898.0-3195.0	2331.4 ± 520.6
8	Total bilirubin (mg/dL)	0.2-0.6	0.38 ± 0.1
9	BUN (mg/dL)	3.0-4.0	3.2 ± 0.4
10	Creatinine (mg/dL)	0.5-0.9	0.7 ± 0.2
11	Calcium (mg/dL)	12.3-18.6	14.7 ± 2.3
12	Phosphorus (mg/dL)	20.3-24.3	22.3 ± 1.5
13	Potassium (mEq/L)	5.2-7.7	6.8 ± 1.0

Source:

1. Murtha JM, Qi W, Keller ET. Hematologic and serum biochemical values for Zebrafish (Danio rerio). Comp Med. 2003;53 (1):37-41.

2. Zebrafish in Biomedical Research; Biology, Husbandry, Diseases and Research Applications. American College of Laboratory Animal Medicine series, 2020).

Annexure 8: Anaesthetic agents used for Zebrafish (Source: Zebrafish in Biomedical Research; Biology, Husbandry, Diseases and Research Applications. American College of Laboratory Animal Medicine series, 2020)

Sr. No.	Anesthetic agents (Drugs)	Anesthetic Doses (mg/L)	Stage of Anesthesia
1	Tricaine methasulphonate	50 mg/L 50-100 mg/L 100-200 mg/L	Sedation Light Anesthesia Surgical Anesthesia
2	Benzocaine	25-100 mg/L 35 mg/L	Light Anesthesia Light Anesthesia
3	2-Phenoxyethanol	200-300 ul/L	Light Anesthesia
4	Clove oil (eugenol or isoeugenol)	2-5 mg/L 60-100 mg/L	Sedation Surgical Anesthesia
5	Metomidate hydrochloride	2-4 mg/L 6-10 mg/L	Sedation Light Anesthesia
6	Lidocaine hydrochloride	300 mg/L 325 mg/L	Light Anestheia Surgical Anesthesia
7	Tricaine methasulphonate and Isoflurane	65 ppm + 65 ppm 175 ppm + 175 ppm	Light Anesthesia Deep Anesthesia
8	Hypothermia	12 ^{0}C 10 ^{0}C 0-4 ^{0}C	Sedation Light Anesthesia Anesthesia

Experimental procedures

1. Sedation: ENU mutagenesis imaging
2. Light Anesthesia: Weighing, gill and skin scrap
3. Surgical Anesthesia: Gill, tail fin biopsy, recovery surgery
4. Deep Anesthesia: Nonrecovery surgery

Annexure 9: Euthanasia agents and doses for Zebrafish (Source: Zebrafish in Biomedical Research; Biology, Husbandry, Diseases and Research Applications. American College of Laboratory Animal Medicine series, 2020).

Sr. No.	Euthanasia agents (Drugs)	Euthanasia Doses (mg/L)	Observations and comments
	Adult Zebrafish		
1	Tricaine methasulphonate	>200 mg/L	For 10 min after cessation of opercula movement
2	Clove oil (Eugenol)	>100 mg/L	For 10 min after cessation of opercular movement
3	Lidocaine hydrochloride	>400 mg/L	For 10 min after cessation of opercular movement
4	2-Phenoxyethanol	≥800 mg/L	For 5 min
5	Hypothermia	0-4 ^{0}C	For 5 min
	Larval fish		
1	Tricaine methasulphonate	>1800 mg/L >1800 mg/L	For larvae 3-8 dpf with exposure for over 1 h For larvae 3-8 dpf followed by an adjunctive method of euthanasia
2	Clove oil (Isoeugenol)	>1500 mL/L	For larvae 14 dpf with exposure for at least 20 min after cessation of heartbeat
3	Hypothermia	0-4 ^{0}C	Followed by an adjunctive method (bleach/decapitation/

			maceration) after exposure for at least 20 min after cessation of heart beat for larvae <14 dpf Maintained in cold solution for at least 12h for larvae ≤14 dpf if no secondary adjunctive method used

Annexure-10: Specification for transport of laboratory Zebrafish by road, rail and air (*Source: Zebrafish in Biomedical Research; Biology, Husbandry, Diseases and Research Applications. American College of Laboratory Animal Medicine series, 2020).*

	Adults	**Embryos**	**Cryopreserved sperm**
Stocking density	5 fish per litter	One embryo/1.5 ml	---
Transport Medium	Fresh fish water (oxygenated)	E2 medium	Cryo medium
Exterior packing material	Styrofoam box (wall thickness 1.5-2″) place in cardboard box	Styrofoam box (wall thickness 1.5-2″) place in cardboard box	Plastic jacket or shipping cartoon
Inner container	1.Double polythene bag (3-4 mil) 2.Cubitainer with screw cap (1-20 L capacity)	Tissue culture flasks with a solid sealed screw cap	Dry shippers (vacuum-insulated Dewar) with cryo-vial box.
Absorbent material	Absorbent bench or spill pads	Absorbent bench or spill pads	NA
Filler	Bubble wrap, packing peanuts, air packs, or other water-resistant materials	Bubble wrap, packing peanuts, air packs, or other water-resistant materials	NA
Temperature*	RT	RT	Vapor phase LN_2
Feed withhold time	24 hours prior to shipment	NA	NA

- Temperature: heat packs are advised in exterior packing (not in immediate contact with fish container) for adults and embryos during extreme cold months.

Label on outer box

- Live Fish
- Keep at Room Temperature
- Handle with Care
- Do Not X-ray
- Nontoxic
- Non-hazardous
- No Commercial Value

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