1 ABCD of Zebrafish Culture

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Abstract

Zebrafish is not a new name as model organism for biomedical researchers around the world. Though institutional zebrafish culture facilities usually cater to the need of researchers for the zebrafish supply and maintenance, such facilities are very limited globally. Therefore, researchers are compelled to culture zebrafish in their lab. The literature and science behind zebrafish culture and care are scattered and limited which makes zebrafish culture a daunting task and might have discouraged new researchers from embracing zebrafish as an animal model. Therefore, this chapter has presented an overview of information on the know-how and science behind zebrafish culture. First, a note on static, flow-through and recirculating systems is described as these are used to maintain zebrafish in lab conditions. Each system has a different utility in rearing zebrafish. Apart from commercial systems, cost-effective, open-design options are also discussed to suit custom requirements of the researchers. Next, a discussion on the factors affecting the health of zebrafish and their optimum range required for the well-being of zebrafish is presented. Finally, the role and types of zebrafish diets are described. Overall, this chapter provides the readers a comprehensive guide on the maintenance of zebrafish for research purposes with ease.

Keywords

Zebrafish culture · Diet · Open-design · Recirculating · Vertebrate model

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1.1 Zebrafish: A Brief Background

During the early nineteenth century, Dr. Francis Buchanan (later known as Francis Hamilton), a Scottish surgeon, first described zebrafish in his book when he was stationed in West Bengal during his visit to India with East India Company. He presented an account of ten *Danio* species he sampled from the paddy fields in and around the West Bengal area (Hamilton 1822). However, zebrafish as a vertebrate-animal model was popularized by Dr. George Streisinger from the University of Oregon during the 1970s (Streisinger et al. 1981).

Zebrafish belongs to phylum Chordata, family Cyprinidae and genus *Danio*. Its scientific name is *Danio rerio*. Zebrafish is a freshwater fish with natural habitat along the regions of river Ganges and Brahmaputra especially in India, Nepal, Bangladesh and Myanmar (Spence and Smith 2006). Zebrafish has been reported predominantly in paddy fields, shallow ponds or river drainages with slow moving water at various sites in northern Indian states present along the bank of Ganges (McClure et al. 2006). The overall size of the adult zebrafish is so small that it can fit in a 1.5 mL Eppendorf tube. It has got its name "zebrafish" due to the presence of black and white stripes on the lateral side of its body. Zebrafish shows sexual dimorphism (having morphologically distinct male and female) which makes it easy for researchers to visually inspect and select them for mating purpose.

1.2 Zebrafish: A Vertebrate-Animal Model

To understand various life processes including any deviation from the normal course leading to the onset and progression of the disease, a model organism is required. Animal models are also required to test the therapeutics' effectiveness before it can be used for human purposes.

Now zebrafish is not a new name in the field of biomedical research. Since the availability of zebrafish transgenic and tools to study them, several labs have started to embrace this model over other mammalian models which is quite evident from the ever-increasing number of publications that employ zebrafish in their research. Zebrafish has served the need for a suitable animal model ranging from cell-based studies to the whole organism behavioural studies (Sarasamma et al. 2017). The genome of zebrafish is also fully sequenced. Its sequence revealed that the zebrafish and humans had a common ancestor nearly 450 million years ago (Woods et al. 2000). Though zebrafish is a model organism of choice, it has a few limitations. The following are the pros and cons of using zebrafish (Lawrence 2007; Spitsbergen and Kent 2003).

1.2.1 Pros

- The maintenance of a large number of zebrafish in small area is possible with little effort and cost.

- Zebrafish shows sexual dimorphism, meaning the male and females have morphologically distinct features and can be identified with a visual inspection.
- Zebrafish show high fecundity which means they can produce a large number of eggs during each mating.
- The zebrafish being a vertebrate animal shows fast and ex vivo development in which growth of the embryo occurs outside the body of mother but inside a protective layer called the chorion. This is a useful characteristic that makes developmental stages amenable for modifications by external agents like toxins, chemicals, etc. by either microinjection or via bath incubation.
- The size of various developmental stages is small, in which embryo and larval stages are optically transparent. This property is useful for optical imaging experiments.
- The genome of zebrafish shows significant sequence homology with humans especially with the disease-associated genes.
- Tools of genetic engineering are available to generate transgenic zebrafish.
- The zebrafish being small in size is compatible with the existing high-throughput screening platforms.
- Zebrafish have a well-developed sensory system. They are diurnal animals similar to humans having more cones in their eyes (unlike rodents that are nocturnal and have more rods in their eyes). Zebrafish also shows a large repertoire of behaviour similar to humans which makes them suitable for neurobehavioural studies.

1.2.2 Cons

- Our understanding of zebrafish immune system is still limited compared to other vertebrate animals like rats/mice.
- The microbiological profile of zebrafish is also limited. Moreover, the zebrafish grown in culture facilities are susceptible to microbial pathogens like mycobacterium, microsporidia, etc. The microsporidia infection is challenging to clean from recirculating systems.
- The breeding of zebrafish is highly dependent on external factors like water quality, light, etc. which are sometimes difficult to control and may affect spawning.
- We have a limited understanding of the zebrafish diet. Though several commercial pellet-based diets are available, they are far from being ideal.
- Zebrafish embryos are not differentiated into distinct male and female sexes at the time of birth; therefore, gender-based studies at embryonic stages are not possible.
- Many of the organs/structures like the breast, hippocampus, amygdale, etc. are structurally not distinct in the zebrafish and its brain. Thus limiting their use in investigations where questions related to these structures are investigated.

1.3 Steps in Zebrafish Culture

1.3.1 Acquisition and Maintenance of Zebrafish

Interestingly, zebrafish is becoming one of the popular aquarium species among pet enthusiasts due to its robust nature and the availability of various fluorescent variants called GloFish™ which express green, yellow and red fluorescent proteins in their muscles (Linney and Udvadia 2004; Shafizadeh et al. 2002). These transgenic fishes glow in the dark when irradiated with UV light. These and other wild-caught strains are frequently available in local pet shops in various countries. However, other specific strains of zebrafish are available with and can be procured from "The Zebrafish Information Network (ZFIN)" at the University of Oregon, Eugene, USA. Common strains which are popular among zebrafish researchers are AB strain, AB/Tuebingen (AB/TU) strain, Tübingen strain (TU, short fin), Tüpfel long fin (TL), etc. For zebrafish genome sequencing TU strain was used. Currently, zebrafish are cultured and maintained using the following types of systems. The applicability of these systems depends on the experimental need. The distinction between these systems mainly lies in the mechanism of water exchange (Bhargava 2018).

1.3.1.1 Static System

Figure 1.1a shows the overview of the static system. It is a tank filled with water having large number of adult zebrafish (for better reproductive health of zebrafish higher stocking density i.e. upto 19 fishes/L, should be avoided). The size of the tank may vary. Water exchange in this housing system is a single pass and discontinuous, which means that water is added once with no water exchange over a defined period (generally 1–3 days). After the intended period, the complete water is replaced (often manually) by freshwater and this cycle continues. The water quality depends on the number of zebrafish housed per litre of water. Water quality also deteriorates over time; therefore, it requires constant monitoring. Though this system is very cost-effective to set up, cleaning is time- and labour-intensive. This system can be used to maintain a small population of adult zebrafish only for a limited time due to quick change in water parameters (pH change and nitrogen content build-up). Sometimes, to enrich the tank environment, one can use artificial plants and clay items in the tank. For quarantine purposes, this system is very convenient. The static system is also best suited to rear zebrafish larvae. It is because the static system design ensures that the food is available for maximal duration in the tank for growing larvae to feed upon. In the past, several studies have used this type of system for zebrafish maintenance in the lab (Miller et al. 2013).

1.3.1.2 Flow-Through System

Figure 1.1b shows the overview of the flow-through system. In this system, water exchange is continuous with a single pass. It means that the clean water, which is stored in an overhead tank, is allowed to pass through the zebrafish tanks via an inlet port continuously. The outlet port discards the overflow water from the zebrafish tank, thus making this system continuous in which water enters and leaves

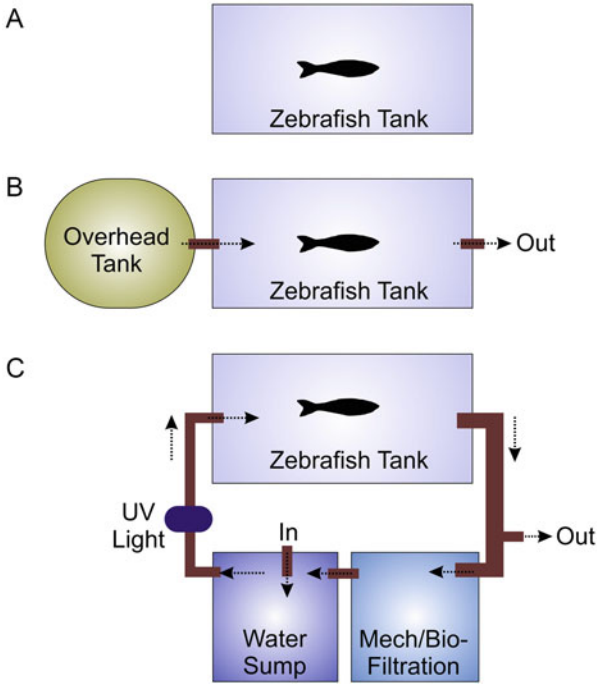

Fig. 1.1 Schematics of zebrafish maintenance systems. (**a**) Static system is a tank-based system in which water exchange is discontinuous and single pass. (**b**) Flow-through system offers continuous water exchange in which water passes zebrafish tank singly. Continuous water supply is maintained using an overhead tank which hold filtered water. Water is discarded after it leaves (out) zebrafish tank. (**c**) Recirculating system is a continuous system in which water exchange occurs multiple times. It requires daily exchange of ~10% water in the water sump. It also requires installation of mechanical and biological (mech/bio) filtration unit to filter out particulate matter and to lower nitrogen content in the recirculating water

the zebrafish tanks singly. As the water passage occurs singly, this system is devoid of any biological filter to check the nitrogen build-up. It is easy to maintain water quality in the zebrafish tank as freshwater is continuously being fed into the system; however, water wastage is relatively high in such system as the water is not being recycled after it leaves the zebrafish tank. This system is useful to maintain a pathogen-free zebrafish colony. Few studies have used this system in their lab for research (Hohn and Petrie-Hanson 2007).

1.3.1.3 Recirculating System

Recirculating system is an ideal system to maintain adult zebrafish in lab conditions because water exchange is continuous and multipass (Fig. 1.1c). In this system, water is not discarded but fed back in the system multiple times after passing through mechanical and biological filters and UV treatment chamber. Thus, the recirculating system reduces water wastage significantly as compared to the flow-through system.

To save space, the system can be made in the form of an upright rack with multiple shelves to hold several zebrafish tanks (of appropriate size) in a compact space. Zebrafish larvae can also be reared in the recirculating system with few modifications, e.g. slow water exchange and fine-mesh baffle installation in the zebrafish tanks. Due to several advantages, nearly all the commercially available zebrafish maintenance systems are recirculating systems with modular rack assembly. Table 1.1 shows comparative components of recirculating systems offered by major commercial suppliers. The only limitation of commercially available recirculating systems is their significantly high cost, which may prohibit many start-up labs or young researchers with limited funding to establish zebrafish lab with such systems. Therefore, to ease these and other limitations, "open-design" options were also built and explored in past. Under this, progress was made to replicate the function of a commercial recirculating system using custom-designs/parts. The main purpose of open-design efforts was to reduce the system cost and to equip the specific lab in custom designing the setup for their own requirement. Recirculating systems offer regulating different parameters like space requirement, water quality, salinity, sediment removal, minimizing water wastage, scale-up of zebrafish holding capacity, etc. with ease. Therefore, many researchers have already employed such systems (commercial or open-design) in their lab for research (Castranova et al. 2011; Nema and Bhargava 2017).

Major Components of the Recirculating System

Due to several advantages, nearly all the zebrafish research facilities worldwide rely on the use of rack-based recirculating system to maintain zebrafish. If cost is the prohibiting factor, one can even construct their rack-based recirculating systems to suit their custom requirement in a cost-effective manner using open-design options. A recirculating system has several components (Bhargava 2018) whose functions are listed below:

1. **Rack**: Rack is often made with metal-angle parts that provide a space and strength to hold various components.
2. **Pipes and connectors**: For water recirculation in the system, PVC pipes of schedule 40 or 80 are used. There are two types of pipes used in the recirculating systems. One is ascending pipe (1/2 in. diameter) which circulates water from the water sump to the zebrafish tanks. The other is descending (collection) pipe (1 or 2 in. diameter) which collects water from the zebrafish tanks and transports it to the water sump via filtration module. To feed the water from ascending pipe to each zebrafish tank, connectors/valves with flexible tubing may be used (narrow tubing with push-fit connectors).
3. **Filtration units**: The water collected from zebrafish tanks has both particulate and dissolved matter in it. Therefore, it requires appropriate filtration before the water can be fed back into the system sump. The particulate matter is filtered using mechanical filtration. These are sieve-based filters often made with polyesters/sponge. Activated charcoal in a nylon bag is placed in the water sump to remove odour and dissolved traces of chlorine gas in water. Biological

Table 1.1 Popular commercial suppliers of zebrafish culture system

Type of units	Properties	Popular commercial setup			
		Tecniplast aquatic solutions	Yakos65	Aquaneering Inc	Thoren aquatic systems Inc.
Zebrafish holding tanks	Capacity (in litres)	1.0, 3.5, 8.0	1.5, 3.0, 10.0	0.8, 1.4, 1.8, 2.8, 6.0, 9.5	1.0, 3.0
	Self-cleaning	Yes	Yes	Yes	Yes
	Autoclave compatible	Yes	Yes	Yes	Yes
	Materials	Polysulfone, polycarbonate	Polycarbonate	Polycarbonate	Aquatic compatible plastic
	Tank colour	Blue	Blue	Green	Transparent or light grey
Water sump/ reservoir	Capacity (in litres)	Variable	Variable	Variable	Variable
	Materials	NL[a]	NL[a]	NL[a]	NL[a]
Structural components	Rack material	Plastic coated, movable shelves, adjustable structure, rubber feet and identification marks	PVC coated, corrosion resistant	Stainless steel	Steel
	Pipes (inlet, outlet and connectors)	Grey PVC pipe, flow controllers	White PVC pipe, flow control nozzles	PVC pipe, flow regulators	Grey PVC plastic
	Lights and auto-timer	Lights (with automation)	Lights (with automation)	Lights (with automation)	NL[a]
	Pump	Variable frequency, submerged, an additional backup pump	External	External	External
	Heaters	Heating coils	Auto controlled heater	Digital control heater	Automated heaters
	Aeration	Air diffusers	Air diffusers	Air diffuser	Air diffuser in linear position

(continued)

Table 1.1 (continued)

Type of units	Properties	Popular commercial setup			
		Tecniplast aquatic solutions	Yakos65	Aquaneering Inc	Thoren aquatic systems Inc.
Filtration and disinfection	Mechanical	Fine mechanical filtration	Yes	Yes	Yes
	Chemical (activated charcoal)	Yes (pre-fill compact)	Yes	Yes (in line fitting)	Yes
	Biological (Bioballs)	Yes	Yes	Yes	Yes
	UV (disinfection)	Yes	Yes	Yes	Yes
	Other	Drum filter optional	Gravitational filtration system and pre-filter washable pads	Fluidized bed filtration, pad filters	Bag filters
Accessories	Accessory parts	Breeding and auto feeding systems, biochemical probes, cooling unit etc.	Stand-alone racks in various size, French-SMOFY technology for noise reduction, reduced electricity consumption	Chillers, dosing systems, dissolved oxygen probe, brine shrimp hatchers, breeders, etc.	Low water sensor, block filter indicator and auto fill valve, different modular rack designs
Contact info	Web links	http://www.aquaticsolutions.it/	https://www.yakos65.com/	http://www.aquaneering.com/	http://www.thoren.com/

[a]*NL* not listed

filtration is used to remove ammonia and lower the nitrate/nitrite (nitrogen content) in the water. It is done using biomedia/bio-balls or gravels (wet/dry or wet/wet method). This media will allow growth of the bacteria, e.g. *Nitrosomonas* sp., *Nitrobacter* sp., *Pseudomonas* sp., *Alcaligenes* sp., etc., which use dissolved nitrogen contents and reduce its amount in the system water. UV assembly will further reduce the number of actively growing microbial species. UV assembly is installed in the ascending pipe so that water entering the zebrafish tank is first sterilized in the UV light. The zap-dose/kill-dose depends on the wattage of the UV lamp and duration of the UV light exposure.

4. **Chambers/tanks**: A water reservoir chamber (water sump) and tanks (to hold zebrafish) are used in the recirculating system. Commercial zebrafish tanks are self-cleaning tanks that are made of polycarbonate material. The water sump is also made of polycarbonate material. It houses a pump, carbon filter, heater and/or chillers, aerators, pH sensor, etc. Tanks made with food-grade plastic are commonly used in the open-design zebrafish tanks. Size of the zebrafish tanks may vary based on the need of the individual lab (ranging from 2 to 8 L). The utility of self-cleaning feature in commercial zebrafish tanks is enormous as it significantly reduces build-up of particulate matter at the bottom of zebrafish tanks. For the first time, our work has extended the auto-cleaning feature to custom-made zebrafish tanks and found that the installation of "self-cleaning unit" in zebrafish tanks results in efficient removal of sediments from the bottom of zebrafish tanks. The design of our self-cleaning unit was also found to be independent of tank geometry (Nema and Bhargava 2016).
5. **Accessories**: It includes components like light, cooling, feeding, temperature sensing, etc. Though these components are "essential", they are not required to be an integrated part of zebrafish recirculating rack. Bright lights can be provided using standard ceiling lights in a room, e.g. LED lights, CFL lights, etc. To maintain ~29 °C during summers, cooling is required. Cooling can be provided by installing the zebrafish recirculating rack in a temperature-controlled room or a room with installed AC/water cooler. During winters, heating of the system water is required to maintain the appropriate temperature. This can be done by installing an underwater heater in the water sump. Automatic feeding machines can dispense a pre-set amount of pellet-based food and thus reduce manual labour. However, it will add to the extra cost of zebrafish maintenance.

1.3.2 Breeding of Zebrafish in Lab Conditions

Zebrafish are dimorphic animals (male and female sexes are present in different bodies) with external fertilization. For mating in the lab conditions, male and female adult zebrafish in 2:1 ratio are placed in the dark for overnight in a mating chamber. The dark phase is essential as it is required to enhance melatonin production in zebrafish which is shown to regulate sexual behaviour in zebrafish (Lima-Cabello et al. 2014). The mating chamber is a tank with a barrier (barrier can be a base with glass balls/marvel or a sieve/net) to prevent the access of adult zebrafish to the fertilized eggs as they tend to feed on them. The next day, spawning (a process of egg laying) is stimulated by light and fertilized eggs are collected at the bottom of the tank. Sometimes, a physical partition can also be used to separate males and females in the mating chamber for overnight to stimulate the mating process and increase the chances of spawning. In our lab, we have observed a good spawning rate even without physically separating males and females during overnight period.

It is visually easy to distinguish fertilized eggs from unfertilized ones. Fertilized eggs have a clear chorion (the protective layer around a central mass of cells), whereas unfertilized eggs are greyish and uniform (in contrast, dead eggs are white and cloudy). Zebrafish produce several eggs during each mating process; however, the clutch size (number of eggs per spawning) may vary between batches, strains and environmental conditions. A detailed comparative account on the variety of breeding and husbandry methods is discussed by others elsewhere (Tsang et al. 2017).

1.3.3 Care of Zebrafish

For the well-being of zebrafish, maintenance of various physical parameters are required. The water conductivity (300–1500 μS), pH (6.6–8.2, with ideal pH ~7.5), temperature (24–35 °C with ideal temperature 28 ± 2 °C), oxygen concentration (4–6 mg/L), photoperiod (14 h light, 10 h dark), nitrogen load (ammonia, 0 mg/L; nitrite, <10 mg/L; nitrate, <75 mg/L), UV-C light zap dose (20,000 $\mu W/cm^2$ to kill common bacteria and viruses) and ~10% water exchange daily are the optimum requirements which can also be adjusted by the individual labs to suit their requirements (Bhargava 2018; Avdesh et al. 2012; Kwong et al. 2014; Lawrence and Mason 2012). In recirculating systems, a small portion of water needs to be exchanged daily in the sump to reduce the nitrogen load and build-up of chemicals leaching out from the plastic components, e.g. bisphenol A or bisphenol S. Over time, zebrafish tanks may show the growth of algae and biofilms on its wall. The growth of algae can be retarded using blue/green shades of zebrafish tanks, but the removal of biofilms is only possible through mechanical scrubbing (Bhargava 2018).

Crowding of developing zebrafish (higher stocking density of zebrafish beyond 19 fishes/L water) has been linked to stress-induced perturbation of sex differentiation (Ribas et al. 2017). Others have found that stocking density of adult zebrafish at 5 fishes/L is optimal to express their normal behaviour without the influence of cortisol (Pavlidis et al. 2013).

Zebrafish is also susceptible to microbial infections in which mycobacteriosis and microsporidiosis are the most common infections caused by genus *Mycobacterium* and obligate parasite *Pseudoloma neurophilia*, respectively (Sanders et al. 2012; Whipps et al. 2012). The biofilm is shown to increase the chances of mycobacteriosis. If a zebrafish is infected, the infected fish should be immediately removed and discarded followed by complete cleaning of the system to prevent microbial transmission to other healthy fishes.

Importantly, whenever a fresh stock of zebrafish is procured from pet shops or transferred between labs, it is essential to first quarantine them in a separate tank(s) for at least 2–4 weeks before transferring them to the main housing system. Steps in quarantine are explained in more detail by others elsewhere (Borges et al. 2016).

1.3.4 Diet for Zebrafish Developmental Stages

Even though diet plays a vital role in the health and well-being of zebrafish of all ages, the actual nutritional requirement for them remains elusive. The zebrafish can feed on anything, but in larval stages, opening and strength of their mouth limits the size of food particles they can ingest. To this end, there are both live, natural and artificial/synthesized particulate size-based diets that are available to rear larval stages.

1.3.4.1 Live, Natural Lab-Grown Diet

Most labs grow infusoria culture to feed first feeding stage (FFS) larvae (Lawrence 2011). It is a broth culture with collection of actively growing unicellular organisms, e.g. algae, amoeba, ciliates, etc. As the maintenance of pathogen-free infusoria is a difficult task, few labs use freshwater-based defined rotifer culture/polyculture or polyculture with type L saltwater rotifers to feed FFS larvae (Aoyama et al. 2015; Best et al. 2010; Lawrence et al. 2016). The artemia culture can provide a live feed for the advanced stage zebrafish larvae (Gonzales Jr. 2012).

1.3.4.2 Commercially Available Formulated Diet

As a solution to the difficult infusoria diet, few companies are offering ready-to-use, defined size-based particulate formulated diet mainly for the FFS and advanced stage zebrafish larvae. These are compared in more detail in Table 1.2. Commercially available diets too have few limitations like different strains of zebrafish may have a varied nutritional requirement which may not be possible to supplement with a single commercial diet. Also, companies may change the product composition without notice, and the overall growth and survival may be affected if given only formulated diets (Goolish et al. 1999; Watts et al. 2012). Therefore, an option for both commercial and live feed should be used.

Feeding Regime for Zebrafish

1. From 0 to 5 dpf stage: No external feed is required. The developing embryo gets its supply from the yolk located in the yolk sac present on the ventral side of the body.
2. 6–8 dpf stage (FFS larvae): In this stage, yolk content is depleted significantly, and larvae require exogenous feeding for their survival which primarily consists of paramecium, rotifers or artemia. Some users have also tested the utility of artificial diet and found that portion of live feed can be replaced by continuous application of artificial diet without compromising the overall growth and survival of zebrafish larvae (Carvalho et al. 2006). Of note, a study found no adverse

Table 1.2 Nutritional profile of commercial feed suitable for First Feeding stage (FFS) larvae and other stages

S. No.	Companies	Product name	Lowest particle size (μm)	Nutritional analysis (%)					Remarks
				Protein	Lipid	Fibre	Ash	Moisture	
1	Sparos	Zebrafeed	<100	60	14	<1	9	8	Four types of diets are available: Zebrafeed (<100 μm); Zebrafeed (100–200 μm); Zebrafeed (200–400 μm); Zebrafeed (400–600 μm).
									https://www.sparos.pt/
2	Salt Creek Inc.	Fish Starter Diets	<200	57	14	5	11	7	Microfeast Feed at Bartlesville, OK, is acquired by Salt Creek Inc. Microfeast L-10 diet is now branded as Progression 1 (<200 μm). Higher particle sizes are also available: Progression 2 (200–300 μm); Progression 3 (300–500 μm); Progression 4 (500–800 μm).
									http://www.saltcreekinc.com/products/progfish.htm
3	Skretting	Gemma Micro	50–100	59	14	<1	14	–	Four types of diets are available: Gemma Micro 75: 50–100 μm; Gemma Micro 150: 100–200 μm; Gemma Micro 300: 200–500 μm; Gemma Micro 500: 400–700 μm.
									http://zebrafish.skrettingusa.com/collections/all
4	Zeigler Bros. Inc.	Larva Z Plus	<50	50	15	2	8	12	Five types of diets are available: 1 (<50 μm) Zoea 1 to Zoea 3; 2 (<100 μm) Zoea 3 to Mysis 3; 3 (100–150 μm) Mysis 3 to PL 3; 4 (150–250 μm) PL 3 to PL 6; 5 (250–450 μm) PL6 to PL 12[a].

									https://www.zeiglerfeed.com/shrimp/hatchery/larva-z-plus/
5	INVE Aquaculture	FRiPPAK FRESH micro encapsulated feed	5–30	52	15	3	–	10	Diet comes in three formats: #1 CAR (5–30 μm); #2 CD (30–90 μm); #3 CD (90–150 μm). This product can be procured from primo aquaculture.
									https://www.primo.net.au/shop/Prawn-Hatchery-Feeds/frippak-fresh
6	Tetra	Tetramin Tropical Flakes	Variable	47	10	3	–	6	Flakes can be converted into powder and filtered using sieve of desired size before use.
									http://www.tetra-fish.com/
7	Ocean Star International (OSI)	BSF (Brine Shrimp Flakes)	Variable	53	9	2	11	9	Flakes can be converted into powder and filtered using sieve of desired size before use.
									http://www.oceanstarinternational.com/BSFRedJungle454g.htm
8	Ocean Nutrition	Brine Shrimp Plus Flakes	Variable	55	15	<1	4	8	Flakes can be converted into powder and filtered using sieve of desired size before use.
									http://www.oceannutrition.com/dry-foods-detail/brine-shrimpplus-flakes.html

[a]Feed particles are constituted by crustacean larvae stages, e.g. Zoea (Z), Mysis (M) and Phyllosoma Larva or Post larva (PL)

effect on the growth and survival of zebrafish larvae even when there was a delay in initial feeding of exogenous diet to larvae until 8 dpf (Hernandez et al. 2018).

3. 9–11 dpf stage: The larvae can be fed twice per day with an artemia-based live feed (Farias and Certal 2016).
4. 12–30 dpf stage: The diet at this stage entirely consists of artemia fed three to four times per day (Farias and Certal 2016).
5. 31–60 dpf stage: At this stage, the diet consists of a combination of artemia and flakes/crushed pellets. The frequency can be two to four times per day (Wilson 2012).
6. Beyond 61 dpf stage to adult stage (~3 months old and beyond): At this stage, they can be fed with a normal adult diet made of commercial pellets.

1.4 Conclusion

The maintenance of zebrafish in a lab conditions is a daunting task as it requires proper care and attention. To understand the ABCD of zebrafish culture, a brief account on various steps right from zebrafish acquisition from the pet/commercial supplier to the options available to maintain them in lab conditions is presented in this chapter. For maintenance, several commercial to open-design systems are discussed. Health of the zebrafish can be influenced by water, dissolved oxygen, nitrogen content, toxins, housing density and pathogenic microbes. These environmental factors are also briefly discussed in the section related to the care of zebrafish health. Finally, the diet of zebrafish is discussed from the perspective of its developmental stage. In this section, the options of undefined infusoria-based diet to defined commercial pellet-based diet are presented and compared. Both types of diets (live and artificial) have their unique advantages and limitations due to the varied nutritional requirements of zebrafish at various developmental stages. It has also been demonstrated that the diet has a direct role in regulating the survival and reproductive health of the zebrafish which is a critical step in zebrafish rearing in lab conditions.

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