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Review

Rearing Conditions and Automated Feed Distribution Systems for Zebrafish (*Danio rerio*)

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Abstract: Zebrafish (*Danio rerio*) is a well-established animal model, used in a number of research areas. In the last decade, it has also emerged as a tool to evaluate the effects of diets and dietary components and to test novel paradigms in nutrigenomics, nutrigenetics, and nutritional physiology. Despite its worldwide use, the standardization of the zebrafish rearing conditions, including daily nutritional and good feed management practices, is not yet achieved. This is surprising when compared with what is available for other reared animals, such as rodents or other (e.g., commercial) fishes. To date, a major applicative goal in zebrafish nutritional physiology research is to define common, standard, and reproducible protocols of rearing and feeding conditions to generate reliable and comparable results among research laboratories. This review aims to focus on limitations and disadvantages of the current rearing and feeding practices and on some recent technological solutions provided by research groups and/or biotech companies in the field of facility design, with emphasis on automated feeding distribution systems. A general overview of some common schemes of zebrafish husbandry is also given.

Keywords: automated distribution; feeding; rearing systems; zebrafish

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1. Introduction

Zebrafish (*Danio rerio*) is a freshwater teleost (ord. Cypriniformes; fam. Danionidae) that has been used in home aquaria for many years; however, during the last three decades, it has become a key model in a variety of human-biology-related research areas, from biomedicine to toxicology [1,2], from human diseases to therapeutic drugs screening [3,4]. Its use back to fish biology as a tool for complementing research in aquaculture and commercial fish production processes [5,6] has enhanced and further extended its experimental relevance as an animal model.

Zebrafish genome shares a high degree of synteny with both lower and higher vertebrate (from teleost fish to human) genomes [7,8]. Its sequence is fully accessible [9], a condition shared by many other teleost fishes, e.g., Japanese fugu (*Fugu rubripes*), green-spotted pufferfish (*Tetraodon nigroviridis*), medaka (*Oryzias latipes*), or three-spined stickleback (*Gasterosteus aculeatus*). Moreover, various established approaches in genetic manipulation make zebrafish transgenic lines available to date [10,11]. Among fish models, zebrafish is most likely the only one offering a very complete panel of experimental advantages, such as easy rearing and breeding in captivity, including very short generation time (≈3 months), large number of eggs (100–200 eggs/clutch), transparency during egg and larval period, and maturation of organogenesis in the larval stage (i.e., organs and systems are all functional making the larva physiologically comparable to the adult) [12]. The advantages of the experimental model go together with a community of zebrafish researchers spread worldwide and a robust and rather advanced technological support on the zebrafish-rearing aquaria systems.

Recently, zebrafish has started to emerge as a model for evaluating the direct effects of administered dietary components on functional diet–gene interactions and for exploiting novel approaches in nutrigenomics, nutrigenetics, nutritional physiology, and immunity [13]. In fact, depending on their presence, availability, and storage, dietary compounds can temporarily alter gene and protein expression (e.g., acting as co-factors within the relevant metabolic systems). These effects are more relevant in poorly stored nutrients such as water-soluble vitamins. Additionally, they can have longer lasting impacts on gene expression as essentially permanent genome alterations, which can occur, for example, for dietary components altering mutation rates or genome methylation patterns [14]. In addition, in this context, dietary amino acids have been shown to be effective in zebrafish [15,16]. On the other hand, variations in individual genetics can affect nutrient needs and tolerances, requiring individualized diet recommendations [14]. Taken together, the results obtained in zebrafish could be translated to aquaculture nutrition research and to relevant commercial species, such as Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*), among others. However, such translation requires in-depth comparative knowledge on the physiology and the biology of the species in question to assess the compatibility of the translation.

Despite the zebrafish use worldwide in the laboratory, the standardization of its rearing conditions, including daily nutritional requirements and good feed management practices, is still poorly studied [17]. To some extent, this is surprising when compared with what is available for other animal models, including terrestrial vertebrates such as rodents [18], or aquatic species such as tilapia [19], channel catfish (*Ictalurus punctatus*) [20], or common carp [21], among others. The reason for the lack of standardization lies perhaps in the fact that zebrafish is such an easy fish to keep in home aquaria that the optimization of standard conditions has never been evaluated as necessary, although it is obvious that parameters such temperature, feed composition, etc., will affect zebrafish like all other animals, regardless of its robustness.

This review aims to focus on the limitations and disadvantages of the current zebrafish rearing and feeding practices and, in this respect, on (some of) the most recent technological solutions provided by biotech companies and/or research groups in the field of facility design. To better understand critical issues, an overview of the most common schemes of zebrafish husbandry is given.

2. Standard Rearing Conditions/Parameters

Wild zebrafish is a freshwater teleost fish with origins in South Asia, where it lives in a wide variety of natural habitats, including irrigation ditches and rice fields, man-made fish ponds, upper reaches of rivers, and even fast flowing hill streams [22–26]. Mimicking the zebrafish wild environment is a good approach to minimize sub-optimal rearing conditions, which result in increasing energy towards maintaining homeostasis, rather than on growth, gamete production, and immune function, thus leading to a decrease in growth performance, number, quality of offspring, and survival [27].

On the basis of these considerations, in the laboratory, zebrafish is usually maintained in low moving and slightly acidic to slightly alkaline (pH 6.5–8.0) waters (in natural habitats pH can vary from 6 up to 10) [25–27] with relatively high clarity (≈35 cm) [27], under 14:10 h light:dark cycle [27] and a temperature of 28.5 °C [28], which is the almost universally cited temperature for normal development. With respect to adults, zebrafish embryos and larvae are generally reared in a solution (E3 medium (also known as egg water)) containing all key ions (5 mmol/L NaCl, 0.17 mmol/L KCl, and 0.33 mmol/L $MgSO_4$) at low salinity levels and methylene blue (0.5 mg/L) to reduce fungal infections [29]. With the development (and size increase), larval, juvenile, and adult zebrafish are gradually reared in tanks housed in designed culture systems (see Section 3. Designed culture systems) under the standard conditions stated above.

For detailed information about zebrafish rearing procedures, please refer to, among others, the reviews by Lawrence et al. [27,30], Aleström et al. [29], Watts et al. [31], and Lee et al. [32]. The most relevant conditions and parameters reported in the cited reviews for zebrafish rearing are summarized in Table 1.

Table 1. Parameters and recommendations for zebrafish rearing.

Specific Parameter	Recommendations
Temperature	Adult zebrafish exhibit tolerance for a wide range of water temperatures (24.0–29.0 °C). For zebrafish embryos and larvae, the recommended rearing temperature is 28.5 °C. Lower temperatures may slow down the development.
pH	For both larval and adult zebrafish, a pH range from 6.5 to 8.0 is recommended.
Light/dark cycle	Fourteen hours day (light) and 10 h night (dark) is generally recommended for both adult and larval zebrafish. The light/dark cycle does not seem to affect zebrafish embryonic development.
Salinity (total concentration of ions dissolved in water)	A range 0.25–0.75 part per thousand (ppt) is recommended for adult and larval zebrafish.
Conductivity (the quantity of sodium and chloride or calcium and carbonate)	In recirculating water systems, a 150–1700 µS/cm range is recommended.
Oxygen and NH_3	In recirculating water systems, dissolved oxygen levels are kept at approx. 7.8 mg/L at 28.0 °C. Levels of total ammonia, nitrites, and nitrates are generally kept less than 0.1, 0.3, and 25 mg/L, respectively.
Hardness (concentration of divalent ions, such as Ca^{2+} and Mg^{2+}, and carbonate, such as $CaCO_3$ and $MgCO_3$)	A range between 75 and 200 mg/L (generally above 100 mg/L) is generally recommended.
Density	Adult sexually mature zebrafish are recommended to be maintained in a range between 3 and 12 fish/L. Zebrafish embryos are cultured in 9 cm Petri dishes at a stock density of up to 100 embryos/35 mL. Larval zebrafish from 6 to 16 days post-fertilization (dpf) are recommended to be raised in small tanks with no water flow at a density up to 60 larvae/L.

3. Designed Culture Systems

The development of common husbandry schemes for zebrafish has improved together with the use of this fish in laboratories to ensure not only that next fish generations are produced but also that vigor and robustness of the strains are preserved. Yet, basic standard protocols for zebrafish rearing in laboratory conditions are still designed to improve fast growth and high reproduction rates rather than to address adequate nutrient requirements and overall fish health and welfare [14].

To standardize zebrafish rearing procedures, many different culture systems have been developed based on the specific needs of laboratories or institutions. Generally, these systems are referred as (a) flow-through, (b) recirculating, or (c) static renewal [31,33,34].

The flow-through system is the least common design among research laboratories, although it offers several advantages when performing nutritional experiments. The principle involves large volumes of water (filtered or unfiltered) that are quickly pumped in the facility tanks and thereafter returned to the environment, usually after remediation, with no appreciable amounts of waste materials and, whether accumulated, removal does not need great effort. Once established, the system does not require expensive costs to operate. Furthermore, it also represents the best approach to minimize the build-up of nitrogenous wastes in zebrafish tanks. However, the tank water quality depends mostly on the source and, thus, it follows a high probability of including pollutants or toxicological elements, which can negatively affect zebrafish wellness and growth. Flow-through systems have been used for maintaining zebrafish in the laboratory by Hohn and Petrie-Hanson [35] and Streisinger et al. [36], for example.

The basic concept of flow-through system has been used for the development of microfluidic systems for culturing of zebrafish embryos and larvae. These systems are aimed at supporting adequately embryonic and/or larval fish-raising under controlled experimental conditions, while a unique flow-through solution moves to all the raised individuals. Most of the traditional and common raising practices are, in fact, still performed manually, which imply time-consuming operations, human errors, and limited reproducibility [37]. A microfluidic system for embryonic and larval culture, where most tasks are performed automatically without external events that can disturb embryo development, has been developed [37]. Such a device allows one to transport, immobilize for imaging, continuously deliver reagents and drugs while real-time observations are performed, and retrieve zebrafish embryos post-analysis in a more efficient way compared with conventional static cultures [37]. Despite zebrafish larvae showing a more complex shape and active swimming compared with immobilized embryos, which makes the fabrication of suitable systems more difficult, microfluidic devices have also been made for this stage. Examples of microfluidic systems for raising of embryonic and larval zebrafish developed by research groups are given in Table 2. More detailed information of the microfluidic systems can be found in the review of Khalili and Rezai [38]. The next design, called recirculating system, is the most commonly used in laboratories and gives investigators the ability to control water quality and even eliminate undesired factors, such as over-exceeded ions and toxic elements. Nearly all the culture systems commercially available for zebrafish are the recirculating type and most of them have been developed as an open-formula to satisfy specific needs of the laboratories. The general scheme of a recirculating system includes the collection of tap water, previously subject to filtration, in a common filter unit which pumps water into a large reservoir (header tank). From there, the water is distributed from the top by gravity flow into each row and into each single tank where the flow rate can be adjusted according to the size, number, and age of the fish. Then, the water returns to the common filter unit for recirculation. The water source can be treated using a variety of filter systems such as charcoal filters, reverse osmosis, or ion-exchange columns, and specific ions can be added. Therefore, the main advantage of the recirculation systems includes the possibility of providing high-quality water and a high exchange rate in the tanks. Moreover, synthetic sea salt can be dissolved to achieve appropriate conductivity. However, highly purified water requires knowledge of culture engineering technologies, water management, and maintenance costs. The purification of recirculating water can be done by different mechanisms, such as the use of mechanical filters and clarifiers in combination with biological filters, ultraviolet sterilization, protein skimming, and ozonation (or combinations thereof). However, all these systems, particularly the biological filtration, often require an initial adjustment period, whereby appropriate levels of autotrophic nitrifying bacterial populations need to be established. If water parameters, such as alkalinity, hardness, and pH are not monitored and corrected as needed, filter efficiencies will fluctuate over time. Examples of recirculating systems are the open-design solutions proposed by Burg et al. [39], Paige et al. [40], and Nema and Bhargava [41,42].

Table 2. Examples of flow-through-based microfluidic systems for raising of embryonic and larval zebrafish.

Microfluidic System	Description
Wielhouwer et al. [43]	On-chip culturing of more than 100 zebrafish embryos for real-time imaging, thanks to three borosilicate glass layers bonded together and two sets of flow-through systems for the circulation of buffer medium and warm water.
Zhou et al. [44]	A chip with the exact well interspacing of a 96-well plate designed for entrapping, culturing, and treatment of zebrafish embryos. The chip is composed of 12 microscale clusters, an array of 21 embryos traps, inlet and outlet ports, and a suction channel that exerts a force to immobilize embryos.

Table 2. *Cont.*

Microfluidic System	Description
Akanji et al. [37]	A chip for a one-step automatic loading, hydrodynamic positioning, trapping, and long-term immobilization of single embryos.
Bischel et al. [45]	A device with branching channels for manual loading, positioning, and orientation of 3–5 dpf zebrafish larvae, allowing both dorsal and lateral view of the fish.
Lin et al. [46,47]	A device consisting of two side-by-side horizontal channels bridged by a series of short, tapered channels and of a hydrodynamic force continuously applied. This force allows the loading and the immobilization of larvae which, once entrapped, can act as a plug directing the following larvae towards the empty channels in a sequential manner.

In small scale, static renewal systems are inexpensive and effective. In this design, fish are kept in small tanks or raceways, and the water is incrementally replaced at levels ranging from 5 to 50% of volume exchange per day. However, water exchange can have consequences, mainly related to disturbing events to zebrafish in culture. Given the high rates of water exchange, water quality will become inconsistent. Thus, these systems are not recommended for use with large populations of zebrafish. Examples of static renewal systems use are in Choi et al. [48], Maximino et al. [49], and Miller et al. [50].

4. Feeding Requirements

Dietary lipids in fish diets represent the main conventional energy source, especially in carnivorous species, although low efficiency rates and different growth performance, wellness, and body compositions among species are generally found [31]. In addition, fish diets do not require specific dietary carbohydrate levels [51]. Thus, proteins remain the most relevant dietary compounds in formulated diets. Notably, fish require higher levels of dietary proteins compared with terrestrial-farmed vertebrates, though this consideration needs to not be taken as absolute. In fact, fish and terrestrial vertebrates differ only in relative protein concentrations for achieving maximum growth rate, and such difference is explained by a lower basal energy needed for fish [31]. On these premises, fish reared under intensive aquaculture conditions are fed with common feedstuffs balanced to supply all the essential nutrients (protein, lipids and, carbohydrates, as well as minerals and trace elements) vital for growth, reproduction, overall wellness, and health [31].

The increased demand and costs for feedstuffs push the need for alternative protein sources for formulating new functional diets meeting nutritional, economic, and environmental requirements. For example, soybean meal has gradually been implemented as an alternative to common feedstuff such as fish meal and fish oil, but the results are not fully satisfactory. In fact, plant-derived proteins may contain anti-nutritional factors such as protease inhibitors, tannins, and saponins that can negatively affect growth and health performance of both omnivorous [52,53] and carnivorous species [54–56]. In this context, nutritional programming is made to counteract the negative effects of plant proteins (mostly intestinal inflammatory events) and to maintain acceptable growth rates and feed efficiency values at high fishmeal substitution levels [57].

4.1. Formulated Diets

Currently, several different formulated diets are available for zebrafish, including commercial dry feeds and live feed such as *Artemia* nauplii, rotifers (*Brachionus* sp.), *Paramecium caudatum*, and *Tetrahymena*. Among these, dry diets are generally assumed to be nutritionally complete, whereas live feed stimulates the associated predatory (fish–prey capture) behavior [30,58,59].

Zebrafish dry diets can be classified based on ingredient and nutrient composition: while some diets are used for specific nutrient requirements under determined experimental conditions, others have commercial applications and are designed for large-scale production [31].

At the time of the complete development of the gut (at approx. 5 days post-fertilization, dpf) [60,61], *Paremecium*, rotifers, and *Artemia* nauplii are usually administered as first feed because they are useful for increasing survival and early growth, as indicated by various authors [31,62]. Early zebrafish larvae prefer to consume *Paramecium* and rotifers due to their small diameter, but not *Artemia* [29]. In this respect, Best et al. [63] showed the effective use of a static rotifer–larvae "polyculture" methods for feeding 5–9 dpf zebrafish. Naturally, since live food is a common vector of pathogens, care must be taken to minimize their spread.

After a period of early development (which generally spans from a few days to several weeks), artificial feeds are introduced in zebrafish larval diet [31]. The administered commercial feeds can be used in two different ways, which are as supplement to live diets or as the sole food source [27].

Currently, the most standard and widespread protocols for zebrafish nutrition include the administration of live feed combined with processed feed (usually as fish flake) or specific diets containing fish oil and fish meal, which have been shown to be sub-optimal in many ways [64,65]. While live food such as *Artemia* nauplii does not negatively affect fish growth and health (especially non-hatching decapsulated *Artemia* cysts) in juvenile and adult zebrafish [66], the administration of rotifers and *Paramecium* as live food shows large variability in nutritional profiles [67,68], as well as the risk of spreading pathogens [69] and toxic compounds, which may negatively affect fish health status [70]. For these reasons, live food as the sole source of nutrients is not recommended, and a combination with artificial diets containing the remaining nutrients is preferred. As for live food, the use of flake feeds for juvenile and adult zebrafish is problematic as well, since determining the precise ingredients and the nutritional profile is difficult. Likewise, their poor stability in water means soluble nutrients leach into the water rather than being ingested by the fish [71]. Furthermore, formulated diets may contain potentially harmful compounds such as genistein and soy isoflavone, which have been shown to be an estrogen mimic in both fish and mammals [72,73]. Therefore, based on this information, the administration of live food just after opening the intestinal canal and the gradual combination with the dry feeds represents the most recommended option to raise zebrafish.

Despite a larger number of commercial dry feeds for zebrafish been commercialized in the last decades [74,75], the standardization of zebrafish feeding protocols has not yet occurred, and its development represents a great challenge. Compared with rodent diets, open formulations for zebrafish are not available, with the consequences that many nutrients (and antinutrients) are not established, and the fidelity of many scientific experimentations is decreased. Moreover, the presence of many different dry diets commercially available leads to different and confounding results. As an example, Siccardi et al. [76] showed that five different commercially available fish feeds and two laboratory-prepared diets produced differences in fundamental growth responses. Similar results were also reported by Fowler et al. [77]. The availability and use of multiple commercially available diets, each often characteristically used by a particular research laboratory and across different life stages (larvae, juveniles, and adults), underscore the confusion that would inevitably result from the variance of results ascribed in the scientific literature [78].

4.2. Feeding Management

The lack of standardization of zebrafish feeding protocols not only concerns the great consideration for implementing factors affecting the daily nutritional requirements [27,56,79], but also includes feeding management practices, which are equally important and should be designed by taking into consideration the nutrient and physical properties of the diet. Many studies are used to report both feed amount and daily/weekly feeding regime (feed ratio and frequency), but each study follows its own personal scheme, and the direct effects of the various feed management criteria on specific outcomes have seldom been investigated [31]. Feeding ratio is the amount of diet administered per group or per group of individuals, which is usually referred to as grams per individual or percentage of body weight [31]. Feeding frequency, on the other hand, is defined as the number of times feeding is provided (ratio per individual per unit time) [31]. Both practices are often determined by the availability and the economic resources of the operators [31], thus significantly affecting zebrafish nutrition, especially when using formulated diets [65,80,81]. Therefore, it is not surprising to find a wide variety of feeding frequencies, ranging from once per day to several times per day or even during the night. Moreover, the feeding frequency should also be adjusted for different sizes and ages of zebrafish with the aim of reducing the suspension time in the water before ingestion [31]. Furthermore, the common practice in laboratories is to feed zebrafish *ad libitum* (i.e., the animals are offered as much food as they want), which can be followed by leaching from uneaten food and mixing of feeds with fecal material in the bottom of the tanks, thus reducing much of the ability to quantify feed intake—an essential practice in determining daily nutrient requirements—as well as water quality and, thus, fish welfare [31]. In addition to feeding frequency and ratio, feeding time (the time of day or night when the diet is provided) is also greatly affected by the operators [31]. For example, feeds can be administered any time of the day or night, depending on the operators' availability. Since feeding time can affect zebrafish behavior and feed intake [82], specific time(s) of feeding should be standardized and reported. Fish, in fact, do not usually feed continuously in the wild, but consume meals at certain times of the day or night, exhibiting distinct daily feeding rhythms [83], and although no preferential feeding times have been reported, food-anticipatory activities when fed on a fixed schedule have been shown [84]. Moreover, if multiple ratios per day are provided, the amount of each food ratio should be determined in order to optimize feed ingestion at specific times [31], since ingestion of a ratio may vary depending on the time per day (or night).

To fully assess the potentiality of zebrafish as a model and its increasing use in research laboratories, facilities, and biotech companies (which invariably implies highly controlled fish rearing conditions), the need to combine novel standard diets which satisfies all nutrient requirements with a controlled and reproducible administration setup, far and independent from variables introduced by the operators, is increasingly urgent. As a starting point, an initial reference growth curve is already established [17].

5. Automated Feeding Systems

In the last decades, both (from) single research groups and (to) biotech companies have developed automated systems for zebrafish including feeders. This has been accomplished to meet adequate rearing and nutrient conditions, improve zebrafish health and welfare, and implement new tools in the culture facilities to reach standardization and/or removal of human error factors. Specifically, in the field of feeding supply, attention has been given to standardize feeding practice, including feeding ratio, frequency, and time, that matches digestive biology and normal feeding behavior of zebrafish. As mentioned above, feeding and feed management practices have many and critical implications for the success of rearing zebrafish in a facility. Moreover, the simple act of daily feeding zebrafish in a facility with hundreds or even thousands of individual tanks has many important practical implications, from growth and reproductive performance of the fish stocks to the percentage of labor devoted to carrying out such operations [27]. Feeding management also impacts fish welfare and health in captivity as well as reproducibility and statistical reliability of results obtained by different laboratories. Therefore, the development of standardized systems is increasingly needed, and greater attention to improve the knowledge of physiological and behavioral basis and a constant monitoring is required.

The behavior related to feeding management has been shown to be highly affected by the circadian rhythms, which are endogenous and persistent rhythmic activities under constant environmental conditions driving the synchronization of feeding rhythm and locomotor activity to feeding regime. In this context, zebrafish has already been widely used in different studies, including daily rhythms of locomotor activity in adults and larvae [85], synchronization of activity rhythms to light and temperature cycles [86], and daily rhythms of reproduction [83]. Searching for food, for example, usually takes place when the abundance of prey increases and the risk of predation is reduced [87], and, from a physiological point of view, the nutrient search and utilization mostly depend on the digestive and metabolic states of the fish [88]. In this context, swimming and locomotor activities are probably two of the most widely studied behavioral variables [30] which, coupled with the feeding rhythm, are found to be highly affected by light. For this, both variables represent two main behavioral factors determining feeding time in many fish species [83]. Thus, del Pozo et al. [87] developed a new self-feeder system with a food-demand sensor suitable for zebrafish to investigate the daily pattern of feeding and locomotor activities, their endogenous nature under constant conditions, and their synchronization to feeding regime. The observations indicated that zebrafish usually display nocturnal feeding rhythms, whereas the locomotor activity rhythms are located mostly in the diurnal range.

In the context of home aquaria, where the number of individuals and cages to feed in captivity is more reduced, automated and practical equipment allowing for the control the delivery of food amounts, such as rotating barrel fish feeder and peristaltic pump, have been commercialized. However, when such devices are translated from small-scale to large-scale systems, their inefficiency and limitations emerge, which are, among others: (i) one feeder needed for one tank, thus resulting in a difficult application to housing systems composed of hundreds of tanks; (ii) not eliminating the need of cleaning a large number of devices on a daily-to-weekly basis, which may result in a very time-consuming and high-cost labor activity; and (iii) feeder(s) needed to be set to control different granulometry feeds. The automatization of the protocols can improve the standardization of feeding management practices and remove manual feeding, which represents the major parameter affecting zebrafish wellness, growth, and reproduction, with a clear lack of control over the delivered quantities [88]. Different feeding regimens and feeding amounts can, in fact, affect growth and reproductive performance in wild-type zebrafish [47] and, as an example, modulate melanoma tumor onset in a p53/BRAF zebrafish line [89], respectively. Moreover, especially in facilities with many tanks, the lack of automated systems leads to the appearance of musculoskeletal disorders among technicians in charge of feeding the reared zebrafish [90]. In recent years, advanced technologies such as machine vision, acoustic technology, and sensor data fusion have gradually been applied to large-scale and refined aquaculture [91,92]. These technologies might be considered for implementation in the zebrafish rearing and feeding systems.

In this context, at the University of Padova (Padova, Italy), a novel automatic dispenser for fast delivery of zebrafish food has been developed and optimized. The so-called multiple fishtank feeding doser (https://www.knowledge-share.eu/en/patent/multiple-fishtank-feeding-doser/, accessed on 4 May 2016) (Inventors: Francesco Argenton and Luigi Pivotti [93]) (see also [94]) allows for regulation of the amount of food provided according to the fish number, age, size, weight, and experimental setup. In this way, the dispenser avoids the most common problems related to the activity of operators such as overfeeding, which leads to the accumulation of an excess of food at the bottom of the tanks and negatively affects water filters and fish health, or underfeeding, which leads to impaired fish growth and reproductive fitness. The system consists of a small and practice pneumatic device that delivers pulsated amounts of food in one second, thus allowing the administration of a precise amount of food in a precise amount of time to large-scale systems with hundreds of individual tanks. Furthermore, the designed dispenser allows one to save not only food, but also time, as it produces no waste around the feeding hole and, thus, less cleaning is required. This also allows more time available for other husbandry-related tasks, such as cleaning and monitoring.

Another interesting solution is presented by the semi-automatic dispenser for solid and liquid food in aquatic facilities developed by Candelier et al. [90]. The system represents an intermediate solution between manual and fully automated systems, keeping the assets of both approaches while eliminating most of their drawbacks. The semi-automatic dispenser is battery-powered and portable with a low footprint, able to deliver dry solid or liquid food in a modular manner. It is able to displace all the weight of liquid in a self-supporting reservoir on caster wheels and it does not require a specific operator to trigger and to remain in operation. More importantly, the dispenser can deliver either fixed quantities, operate on controlled quantities, or obtain information on the number of individuals in each tank via near-field communication and automatically deliver the exact amount of food intended.

Similar to Candelier et al. [90], Tangara et al. [95] developed two custom-made open-source semi-automatic and low-cost feeding systems for dry and live food to be implemented in zebrafish facilities. For the delivery of *Artemia*, the system is based on an electric pump capable of sucking live *Artemia* and delivering them to the housed tanks. The rate of delivery can be adjusted by regulating the pump's speed. The pump is triggered by a sensitive and light-weight button held by the person in charge of feeding. On the other hand, the delivery of dry feed in the form of granules is based on a system using standard electronic components. Food delivery can be triggered using three different methods to avoid repetitive movements. These are two sensitive buttons close to the natural proximity of the fingers and contact sensors which are activated as soon as the device is in contact with the lid of the housed tank.

Yang et al. [96] have coupled an automatic feeding system to a Noldus EthoVision video-tracking system able to control the production of obese zebrafish by applying a short-term overfeeding period protocol and analyzing the metabolic changes during aging and overfeeding.

Moreover, automated feed distribution allowed Doyle et al. [97] to develop an automated apparatus for rapid zebrafish conditioning paradigms using Arduino microprocessors. This system allows for control of the delivery of auditory or visual stimuli to groups of different aged zebrafish (juveniles or adults) reared in their home tanks in a conventional facility. After the conditioned stimuli, precise amounts of food are administered through an automatic feeder, and the responses are recorded using video cameras and analyzed using the software ImageJ (Wayne, Rasband, National Institutes of Health, Bethesda, MD, USA) or Matlab (The Mathworks Inc., Natick, MA, USA).

Unlike Candelier et al. [90] and Tangara et al. [95], the solution Zebrafish Automatic Feeder (ZAF) developed by Lange et al. [98] represents a fully automated solution for feeding. The basic operating principle of ZAF is very simple: it consists of a servo motor that rotates a food canister to dispense food into a container directly filled with water. The food–water mixture is then distributed to the tanks using pumps and a manifold tubing system. The ZAF system consists of three main modules: (i) electronics, (ii) tubing and pumps, and (iii) food preparation which synergistically allow for a constant amount of food to be delivered in all tanks and to modify the dosage by adjusting the food container opening as well as the degree of servo rotation. The system provides standardized diets to all the housed tanks, is cost-efficient, and easy to build. Furthermore, the advanced version of ZAF, called ZAF^{+}, also allows a precise control of food distribution and a function of fish density per tank.

In this context, a further contribution is given by the automated feeding system called Tritone, designed and produced by Tecniplast S.p.A. (Buguggiate, Varese, Italy) (Inventors Marco Brocca and Giovanni Frangelli) [99]. The Tritone is a robotic system able to deliver multiple dry diets (up to four different diets), different in granulometry and composition, as well as liquid diets (generally containing *Artemia* nauplii). This solution represents a suitable approach for feeding experiments, since the flexibility to test different diets in a standardized manner is allowed, thus avoiding the variability introduced by the operators. The dosing device, in fact, is equipped with an automatic coupling and release means that alternatively retain or release a pin solidly associated with the container bottle. The spout of the dispenser is located close to the feeding hole in order to get a highly efficient food deliver. Different diet granulometries are provided by different diameters of the spout connected to the dispenser, and particle sizes up to 600 µm do not affect feed delivery. The tank-specific automated food administration is allowed by special codes located at the bottom of the tanks where all information is coded. Thus, the operators can determine and schedule specific diets for specific tanks housed in the facility according to their precise requirements. Feeding time(s) and feeding ratio(s) can also be decided by the operators. Moreover, the system is supplied with a video camera housed directly on the head of the dispenser so as to remotely control correct operation and, in particular, correct positioning of the dispensing system. The position of the video camera also allows the monitoring of zebrafish behavior and, thus, the possibility of improving the automated food distribution.

All the automated feeding systems are summarized in Table 3.

Table 3. Summary of automated feeding systems for zebrafish.

Automated Feeding System	Description
del Pozo et al. [81]	A self-feeder system with an infrared photocell acting as a food-demand sensor (high costs).
Argenton and Pivotti [87]	A small and practical pneumatic device delivering food (low costs).
Candelier et al. [84]	A semi-automatic dispenser for solid and liquid food (low costs).
Tangara et al. [89]	An open-source semi-automatic feeding system for dry and live food (low costs).
Yang et al. [90]	An automatic feeding system coupled with an EthoVision video-tracking system (high costs).
Doyle et al. [91]	An automatic feeder of precise amounts of foods (low costs).
Lange et al. [92]	A fully automated solution which provides standardized amounts of diets (high costs).
Brocca and Frangelli [93]	A robot able to deliver multiple dry and liquid diets (high costs).

6. Conclusions and Future Challenges

When analyzing aspects of fish nutrition, such as those related to the effects of diets, nutrients, molecules, etc., it is not easy to generate highly significant datasets; this is often due to the absence of common and standardized rearing and feeding conditions for the raised animals. The intensive and increasing use of zebrafish as a well-established animal model in many different fields of the biological, biomedical, toxicological, and environmental research thus makes necessary the development and implementation of new automated systems that allow one to obtain as highly controlled rearing and feeding conditions as possible, and, in parallel, to reduce human labor and remove human errors.

To date, the standardization of rearing and feeding protocols by adopting semi-automatic, automatic, or even robotic feed distribution solutions is a necessary goal to achieve in zebrafish nutritional research. The improvement of these technologies in conjunction with research laboratories and industry, combined with the thorough comprehension of the regulatory networks supporting the alimentary function(s), on one hand, and to the optimal formulation of experimental and commercial feeds, on the other, will significantly extend the potentialities of the zebrafish as a tool to evaluate the effects of diets, dietary components, ingredients or single nutrient molecules, and to test novel hypotheses in nutrigenomics, nutrigenetics, and nutritional physiology.

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