



Proceeding Paper

Danio rerio: A Sustainable Model for Monitoring Pollutants in Aquatic Environments [†]

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Abstract: Since the beginning of humanity, many sectors have produced different chemicals. These chemicals are the main causes of environmental pollution and have become a global problem with irreversible effects in terms of health. Because of this, countries have set a target of “a pollution-free planet”. We need to determine target-specific strategies to both eliminate pollution and protect health. To date, traditional methods of monitoring in receiving aquatic environments have been used; however, they do not provide information on toxic levels of pollutants. For this reason, researchers have focused on “bio-indicator” or “bio-monitor” organisms. Since these organisms are in equilibrium with the aquatic environment, they can also be considered an integrated sampling tool and may indicate potential contamination. *Danio rerio* (zebrafish) is considered a promising model organism for single health studies in terms of its biological structure. This review aims to present *Danio rerio*'s characteristics, susceptibility to environmental pollutants, and risks associated with pollutants in the aquatic environment.

Keywords: aquatic environment; bio-indicator; *Danio rerio*; environmental pollutants



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1. Introduction

Environmental change poses a devastating risk to human and environmental health. Environmental pollution is the most difficult challenge for all countries in the world, as it affects all living things and ecosystems under the concept of one health [1–3]. A rapid study of water conditions is necessary for monitoring, assessing, and addressing this global health hazard. Bio-indicators or biological monitors can monitor water quality changes in real-time. Zebrafish (*Danio rerio*) is an ideal bio-indicator for detecting environmental changes due to its biomedical equipment, widespread geographic distribution, and well-characterized specific properties against environmental pollutants [4,5]. *Danio rerio*, which is used to determine the toxicity (teratogenicity, cardiotoxicity, neurotoxicity, hepatotoxicity, and nephrotoxicity, etc.) effect of many chemicals, has also recently been used in the detection and research of diseases [6]. Considering the increasing use of zebrafish as an experimental animal against a wide variety of chemicals and pollutants in toxicity studies, it is also of great importance to improve laboratory conditions in terms of reproductive quality. Despite the zebrafish's numerous advantages, this model organism has several limitations. As a model for monitoring the toxicity of environmental pollutants, zebrafish have some disadvantages, such as low sensitivity and inconvenient statistical experiments [3–6].

In general, a good animal model should have all or most of the following characteristics: (i) structural simplicity that also incorporates the basic cellular processes that more complex organisms have; (ii) easy accessibility for research; (iii) easy and economical to manufacture in the laboratory; (iv) prone to genetic changes; and (v) if possible, have a relatively small and stable genome [7]. The most popular fish species preferred

in experimental processes are listed as *Danio rerio*, *Carassius auratus*, *Oryzias latipes*, *Poecia Reticulata*, *Gasterosteus aculeatus*, *Oncorhynchus mykiss*, *Takifugu rubripes*, and *Xiphophorus hellerii*. Research on zebrafish has become more popular in the last decade. *Danio rerio*'s high fertility rate (ability to fertilize approximately 200–300 eggs every 5–7 days), economic maintenance, and ease of genetic modification make *Danio rerio* an alternative and valuable vertebrate model compared to other species [8]. The use of zebrafish as a model for toxicity studies is carried out according to standards. These standards are the Zebrafish Toxicity Test, listed as British standard BS/EN/ISO 7346-3-1998, German standard DIN/EN/ISO 7346-3-1998, and Chinese standard GB/T13267-91, OECD n 203-236-473-487-489-490 [9,10]. *Danio rerio* (Cyprinidae family: freshwater teleost) is considered a model organism in many research areas, especially in health and pollution detection. In this study, we describe the criteria for the use of this fish species as a model for research in environmental toxicology and argue that *Danio rerio* is an ideal bio-indicator for detecting the toxicity of pollutants.

2. Methodology

A literature search related to zebrafish used as a model in ecotoxicity assessments was conducted in databases such as Web of Science, Science Direct, Scopus, and PubMed. The following keywords were used in the search process: “Zebrafish” and “Ecotoxicity”, “*Danio rerio*” and “Acute toxicity”, “Zebrafish development” and “Fish assay”, and “Zebrafish embryo test” and “Exposure”, etc. A total of 225 articles were reviewed in all databases. Studies that did not comply with the study’s purpose were not included in the evaluation. Of the 225 articles published until January 2023, 92 were thoroughly reviewed. Each article used in the study was compiled according to the following parameters: (i) publication year; (ii) the type of pollutant; (iii) exposure factors; (iv) toxicity; (v) stage of development; (vi) types of tests; and (vii) ecotoxic effects (Figure 1) [1,11,12].

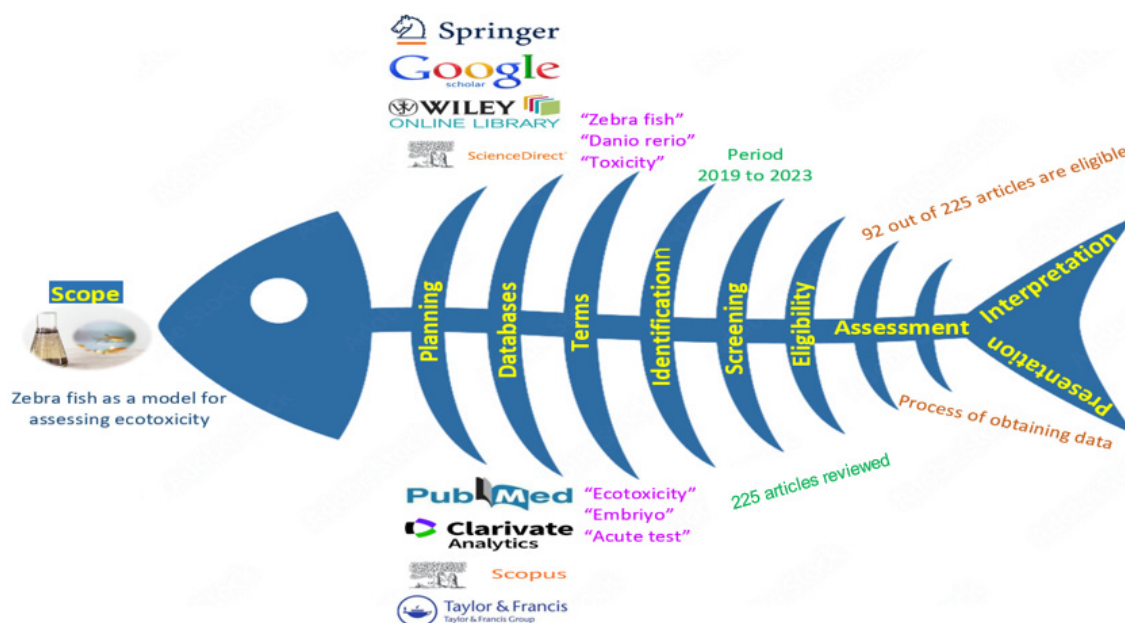


Figure 1. Systematic review methodology.

3. Advantages and Disadvantages of Zebrafish in Ecotoxicity Tests

Zebrafish is a successful monitoring model applied in both medicine (drug production, disease diagnosis, treatment, etc.) and environmental pollution (determination of the effect level of pollutants) [13]. Zebrafish has been widely used in ecotoxicity studies in recent years due to its advantageous features, such as reproduction process and developmental stage [14]. The most important feature that distinguishes zebrafish from other model creatures is that it is used in both sexes, which has brought zebrafish to the forefront in ecotoxicity tests [1]. One of the main advantages of this model is that experimental studies

can be carried out in the embryo process [13]. Additionally, embryos (laying from egg: 48–72 h (hpf); organ formation: 120 hpf; adulthood: 3 months) grow rapidly [15,16]. Rapid development is an excellent feature in toxicity studies with animal models as it allows instant monitoring of toxic exposure [17]. All genetic features of zebrafish are stored in a database (www.zfin.org, accessed on 1 March 2023). In addition, 70% of zebrafish genes are similar to human genes [8]. In recent years, zebrafish has been evaluated as an alternative ecotoxicity model to replace mice and other fish species. It has proven to be a bio-indicator that complies with the 3R (Reduce, Reuse, and Recycle) concept, as tests with zebrafish are economical, provide fast results, and the presence of a large number of embryos reduces the chemical requirements [18]. Among the disadvantages of zebrafish, it can be said that different reactions occur according to the sex type, and the embryo development pools are large.

4. Development and Distribution of Zebrafish

The zebrafish was described in 1882 by Francis Hamilton, who found it near the Ganges River in India [19]. After George Streisinger first used the zebrafish to study vertebrate development in the 1980s, it became one of the most important laboratory animals with unprecedented speed [8]. The zebrafish is native to most of the Indian subcontinent, from Pakistan in the west through India, Nepal, and Bangladesh to Myanmar in the east. Zebrafish can be found in a wide variety of habitats. The adult zebrafish is 2 to 4 cm long, and its body is characterized by zebra-like stripes (Figure 2).



Figure 2. Adult zebrafish illustration.

In toxicological studies, especially in order to determine the toxic levels of environmental pollutants, parameters close to the natural environment of zebrafish should be provided in the laboratory environment. In this context, water quality parameters come to the fore (Table 1) [20–23]. The zebrafish is found in rivers, streams, canals, and rice fields in India, Pakistan, Bangladesh, Nepal, Myanmar, and Bhutan [22]. Wild zebrafish distribution includes Brazil, Colombia, Malaysia, Sri Lanka, Thailand, and the United States [21,23]. Native zebrafish is found in more than 3000 institutions in more than 100 countries and is a popular aquarium fish [22,24]. In research studies with zebrafish in the literature, China (26.7%), Brazil (14.8%), France (12.5%), Germany (8.5%), India (6.3%), and Italy (5.1%) take the lead [1]. The University of Oregon in the United States is home to the Zebrafish International Resource Center (ZIRC), the world's first and largest zebrafish resource center located on the Zebrafish Information Network (ZFIN). According to data from the Federation of European Aquaculture Producers (FEAP), this type of fish is produced at a rate of 1.8% (<https://feap.info/>, accessed on 1 March 2023).

Table 1. Physico-chemical properties of water for lab-raised zebrafish.

| Parameters | Unit | Permissible Limits | Optimum Limits |
|--|------|--------------------|----------------|
| Temperature | °C | 6.5–42 | 26 |
| Hardness | mg/L | 72–198 | 100 |
| pH | - | 5.5–9.0 | 7.7 |
| NaCl | ppt | 0.1–14 | 0.65 |
| Ammonia (NH ₄ ⁺) | mg/L | 0–0.05 | 0.02 |
| Nitrite (NO ₂ ⁻) | mg/L | 0–0.5 | 0.2 |
| Nitrate (NO ₃ ⁻) | mg/L | 15–200 | 50 |
| Phosphate (PO ₄ ³⁻) | mg/L | 150–300 | 250 |
| Dissolved Oxygen | mg/L | 5.5–8.0 | 7.5 |
| Conductivity | µS | 200–4000 | 1250 |

5. Zebrafish-Based Experimental Applications

In addition to fish species, experiment design is an important factor in ecotoxicity studies. The type of exposure (in vivo or in vitro), exposure route, exposure times, and stage of development are considered experimental parameters in data generation [13]. The majority of tests are performed in vivo (90.3%) compared to in vitro tests (9.7%) (Figure 3). Embryos (7.3%) and larvae (32.1%) are used less frequently than adults (55.8%) in ecotoxicity evaluation according to the development process (Figure 3). In terms of exposure route and duration, water (94.9%) and hour (51.1%) are evaluated at the highest rates, respectively (Figure 3). In Table 2, toxicity studies for which zebrafish are the models are given.

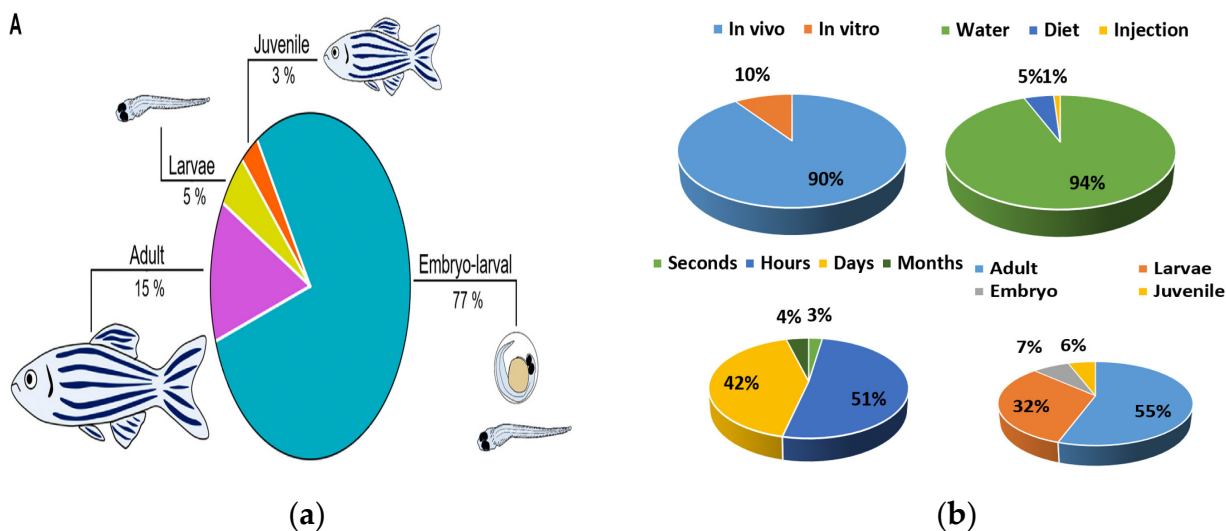


Figure 3. (a) *Danio rerio* development; (b) type of studies in vivo and in vitro, zebrafish exposure route, exposure time, and developmental stages (Adapted from Refs. [1,11]).

Table 2. Different toxicity studies in the literature.

| Test Type | Exposure-Pollutant | References |
|---------------------------------------|---------------------|------------|
| Zebrafish embryo test | Crude oil component | [25] |
| <i>Danio rerio</i> long-term exposure | Metformin | [26] |
| Zebrafish antagonistic test | Deltamethrin | [27] |
| Adult zebrafish behavioral test | Cannabinoids | [28] |
| Early life stage zebrafish assay | Sediment | [29] |
| Acute-Chronic test | Lead | [30] |
| Early life stage zebrafish assay | Sodium Hypochloride | [31] |
| Combined toxicity test | Agrochemicals | [32] |
| Adult zebrafish behavioral test | Triclosan | [33] |

6. Results

The zebrafish's small size, resistance to biotic and abiotic conditions, rapid development process, short reproduction period, compatibility with laboratory conditions, easy supply, economy, high fertility, ability to manipulate embryos, and genetic similarity with humans increase the importance of this species for toxicity studies. Thanks to these advantages, zebrafish can be used instead of other living models in scientific research and can support the 3R rule. As a result of studies researched in international databases, it is thought that different uses of this species will be widespread, and it will be a key model organism in the future.

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