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EMBRYONIC TOXICITY: SYSTEMATIC REVIEW OF SHORT-TERM EXPOSURE EFFECTS OF RARE EARTH ELEMENTS IN *Danio rerio* (ZEBRAFISH)

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Abstract

Rare earth elements (REEs) are widely used in industries due to their unique chemical and physical properties, but are not found in concentrated or pure forms in nature. Their extensive use, long and large-scale mining and poor e-waste management have led to increasing environmental contamination, particularly in aquatic ecosystems. REEs are considered emerging pollutants, and their exact ecosystem impacts are inconclusive. Hence, a systematic review was carried out to comprehend the biological effects of REEs using the early stage of the model organism, zebrafish (*Danio rerio)*. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) strategy was used to conduct a comprehensive literature review. Databases including ResearchGate, PubMed, Google Scholar, and Science Direct were searched for peer-reviewed research articles between 2000 and 2025. The published articles were collected, screened, and reviewed on the effects of REEs in the embryonic stages of zebrafish. Only nine articles became eligible and were analysed for effects, including hatching rate, mortality, malformations, body length, swimming speed, and coagulation on zebrafish embryos exposed to different concentrations of REEs. One study showed significant effects on heart rate, while significant effects of malformations have been investigated by four studies upon exposure to REEs. Significant effects on swimming speed were observed in one study and five articles reported significant effects on hatching rate and time. Three studies investigated the survival and mortality of zebrafish embryos, but only one article showed a significant effect. Three studies investigated the significant effect on body length of zebrafish embryos, and one study reported on the significant effect on coagulation. Based on the systematic review, it can be concluded that acute exposure to REEs in zebrafish embryos caused a significant decrease in heart rate, body length, swimming speed, hatching rate, survival and a significant increment of malformations, hatching time, mortality, and coagulated embryos. Therefore, based on the review, it can be concluded that acute exposure to REEs caused significant toxic impacts on early life stages of embryos. However, a significant knowledge gap exists, as only selected growth effects have been studied in 14 selected REEs using the zebrafish embryonic model.

Keywords: PRISMA Analysis, Rees Short-Term Exposure, Zebrafish Embryos

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Introduction

Rare earth elements (REEs) belong to the f-block of the periodic table and include a total of seventeen elements: the fifteen lanthanides along with yttrium and scandium, as defined by the International Union for Pure and Applied Chemistry (Balaram, 2019; Rim, 2016). These elements, which are considered a subgroup of transition metals, share similar physical and chemical characteristics (Lora-Benítez et al., 2024). Based on their ionic radii and physicochemical properties, REEs are divided into two main categories: light rare earth elements (LREEs) and heavy rare earth elements (HREEs) (Wang et al., 2024; Brouziotis et al., 2022). Although REEs are relatively abundant in the Earth's crust, they are not found in concentrated deposits or in their pure metallic forms (Zepf, 2013; Balaram, 2019). Instead, they occur naturally, combined with the complex in various mineral forms, including oxides, phosphates, silicates, halides, and carbonates (Zepf, 2013; Balaram, 2019), making them challenging to obtain, which contributes to their classification as "rare earth materials" (Dinh et al., 2022; Lora-Benítez et al., 2024)

In recent years, REEs have gained significant attention as a valuable material resource (Wang et al., 2024). Because of their crucial role in the development of conventional industries and technological breakthroughs, REEs are frequently described to as "industrial vitamins" and a "treasury" of novel materials (Wang et al., 2024; Rim, 2016). The applications of REEs depend on their unique chemical and physical characteristics (Lazaris et al., 2023). Since REEs improve energy efficiency, durability, miniaturisation, speed, and thermal stability, they have become essential in a variety of fields, including medical, petrochemical engineering, defence, agriculture, and environmental protection (Feng et al., 2023). REEs are commonly used in luminescent materials, biomedical imaging contrast agents, automotive catalytic converters, anti-cancer medicines, high-performance permanent magnets, nuclear radiation detectors, and even in fertilisers (Cui et al., 2012). Beyond these advanced uses, REEs are also found in everyday products such as metal alloys, ceramics, electronic devices, dyes and high-tech devices like smartphones, laptops, TVs, and portable media players (Lazaris et al., 2023). The glass industry is currently the largest consumer of REE raw materials. Furthermore, REE compounds are also crucial in developing LED lighting, rechargeable batteries for electric and hybrid vehicles, renewable energy technologies such as wind turbines, as well as lasers (Balaram, 2016, 2019; Rim, 2016; Feng et al., 2023). Their increasing industrial popularity is mainly due to catalytic, chemical, electrical, metallurgical, nuclear, magnetic, luminescent, and optical properties (Cui et al.,2012).

Global demand for these elements has steadily increased, indicating an annual growth rate of about 5% by 2020 (Jowitt et al., 2018). Despite their growing demand, less than 1% of REEs are recovered through recycling, with most ending up as waste and leaving the material cycle. This increased usage has raised concerns about their release into the environment and the potential risks to human and animal health (Feng et al., 2023). REEs can enter the body through inhalation, ingestion, or dermal contact (Brouziotis et al., 2022; Wang et al., 2024). Activities such as long-term and large-scale mining, industrial and medical applications, and improper e-waste disposal have significantly contributed to environmental contamination and global health risks (Wang et al., 2024; Lora-Benítez et al., 2024). As a result, REEs have recently been identified as "emerging pollutants" (Lora-Benítez et al., 2024; Feng et al., 2023).

As their industrial demand and mineral extraction continue to grow, REEs are increasingly being released into the environment, particularly into aquatic systems (Cui et al., 2012). In general, the concentration of REEs has been detected in measurable quantities in surface water, pore water, sediments, and ultimately in aquatic vegetation, such as macrophytes (Lora-Benítez et al., 2024). Freshwater systems near mining and industrial zones are especially susceptible to contamination.

Recent studies have investigated how REEs are absorbed and bioaccumulated in tissues, including gills, liver, kidneys, and muscle, in aquatic organisms primarily through water, sediment contact, and trophic transfer via the food web. The bottom-dwelling and benthivorous fish like cyprinids, as well as shellfish and molluscs, are especially vulnerable to REE exposure (Lora-Benítez et al., 2024).

Some research has shown that REEs are cytotoxic and genotoxic (Chen et al., 2020). Fish embryos and larvae are generally the most vulnerable stages to pollutants, but the effects of REEs on developing embryos have not been well studied (Cui et al., 2012). Research has shown that REEs can cause liver damage in common carp (*Cyprinus carpio*), and studies on biodistribution reveal that REEs tend to accumulate in the fish's internal organs, bones, and muscles (Cui et al., 2012).

REEs, including lanthanides, can interfere with important developmental processes during the embryonic stage (Lazaris et al., 2023). The eggshell does not provide complete protection against metal penetration. The effects vary depending on metal concentration and exposure period (Lazaris et al., 2023).

Zebrafish (*Danio rerio*), a member of the Cyprinidae family, are widely used as a model species to assess the risks of potentially toxic substances in the aquatic ecosystem (Chen et al., 2020; Lora-Benítez et al., 2024). Over the past two decades, zebrafish have gained popularity as a high-throughput model for studying acute toxicity, assessing the ecological risks of pollutants, evaluating chemical toxicity and the effects of nanomaterials (Chen et al., 2020; Karthiga et al., 2018; Lora-Benítez et al., 2024). Zebrafish embryos or larvae (Chen et al., 2020) are particularly sensitive to low-level environmental pollutants (Cui et al., 2012). Zebrafish present multiple experimental advantages, including their small size, low maintenance cost, high fecundity, transparent embryos, rapid development, high reproducibility, and a fully sequenced genome with approximately 70% similarity to the human genome (Khan et al., 2024; Yang et al., 2009; Karthiga et al., 2018; Lazaris et al., 2012). Other than that, their developmental stages are well-characterised, which allows for the clear identification of any abnormalities resulting from exposure to toxic substances (Lora-Benítez et al., 2024). These combined traits have established zebrafish as a highly effective and widely used model organism for tracking behaviour and physiological changes in toxicological and biomedical research (Yang et al., 2009; Karthiga et al., 2018; Rosa et al., 2022). Several studies have investigated the impact of REEs on this model, reporting effects on growth and locomotor behaviour (Lora-Benítez et al., 2024).

However, research specifically evaluating the toxicity of REEs in zebrafish embryos remains limited (Lora-Benítez et al., 2024). Although documented health effects in aquatic species include impaired growth, altered behaviour, oxidative stress, reproductive toxicity, and histopathological damage, current knowledge remains fragmented. Furthermore, most studies have only investigated a few individual REEs, leaving significant gaps in understanding the full spectrum of their ecological and toxicological impacts. This highlights the need for more comprehensive assessments to evaluate the risks of REE exposure in freshwater ecosystems.

This review aims to systematically evaluate the biological effects of short-term exposure to REEs on zebrafish embryos by analysing published literature. A systematic review involves selecting existing studies based on clearly defined criteria and rigorously examining their content (Li et al., 2018; Ribeiro et al., 2019; Wassenaar et al., 2017). Systematic reviews in the fields of physiology and medicine commonly adhere to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009). This structure comprises four primary stages: identification, screening, assessment of eligibility, and final inclusion of relevant literature. Studies that fulfil the predefined qualitative criteria are selected for in-depth analysis, and the most suitable among them may also be used for quantitative evaluation (Li et al., 2018; Ribeiro et al., 2019).

Since early-life exposures are critical in an organism's survival and growth, literature on early-life exposure effects on survival, hatching, locomotion and malformations was systematically reviewed using the PRISMA approach to scrutinise the effects of REEs in the popular toxicology animal model of zebrafish (*Danio rerio).*

Methodology

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) approach was used to conduct the systematic review of the published literature (Trifu et al., 2022). Research Gate, PubMed, Google Scholar, and Science Direct were used to obtain peer-reviewed research articles from 2000 to 2025. Published articles were collected, screened, and reviewed following the four sequential steps of PRISMA analysis, viz identification, screening, eligibility, and inclusion.

In the "identification" step, literature was searched in each database by entering keywords such as rare earth elements (REEs), toxicity, zebrafish, hatching time, malformation, growth, and locomotion separately (Trifu et al., 2022). During the "screening", duplicate articles were removed, and titles and abstracts were carefully reviewed for relevance to the research question. Suitable articles were chosen by reading abstracts. Initially selected articles were further studied by reading the complete text, and articles meeting the exclusion criteria were removed. Most qualitatively suitable and eligible articles were included for analysis (Trifu et al., 2022).

Inclusion and exclusion criteria were used in recruiting articles for the review. Inclusion criteria: research articles that report on (1) early life stages, especially embryos of wild-type zebrafish (*Danio rerio*), (2) short-term exposures, (3) growth-related outcomes such as body weight, body length, malformation and locomotion, (4) only external effects were observed.
Exclusion criteria: articles were excluded if (1) they were duplicated, (2) elements tested other than REEs, (3) they used other model organisms except zebrafish (*Danio rerio*), or (4) internal and cellular effects were excluded.

In the eligibility step, articles deemed irrelevant were excluded after reviewing the full text. In addition, the most suitable articles were included in the final qualitative analysis.

Results

Research articles were searched using individual keywords such as REEs, toxicity, zebrafish, hatching time, malformation, growth, and locomotion. Research articles were collected from Science Direct (n =104), Research Gate (n =1297), Google Scholar (n =15400) and PubMed (n=753). After screening, only two articles were recruited from Science Direct, which focus only on the embryonic stage of zebrafish. From Research Gate, only two papers were included by focusing on the external effects of zebrafish embryos. Of the two papers selected from ResearchGate, one article was removed due to duplication in ScienceDirect. A total of 318 articles were included from Google Scholar, focusing on external effects and short-term exposures. Among them, only three research articles were recruited for review based on the inclusion criteria. Forty-three articles on zebrafish were selected from PubMed after excluding articles on other model organisms. Of them, 21 articles were selected based on the embryonic stage of zebrafish. From the 21st, two articles were excluded due to a lack of toxicity data. Remaining 19 articles were screened by reading the full text. At the eligibility step, 11 articles were selected, and eight articles were removed due to the internal effects of zebrafish embryos. The remaining

11 articles were selected based on the embryonic stage of zebrafish and focused solely on the effects of rare earth elements on zebrafish embryos. Accordingly, three articles met the eligibility criteria for the systematic review on external effects of embryonic stage of zebrafish exposure to REEs. Finally, nine articles were selected for qualitative analysis, specifically two articles from ScienceDirect, one article from ResearchGate, three articles from Google Scholar, and three articles from PubMed.

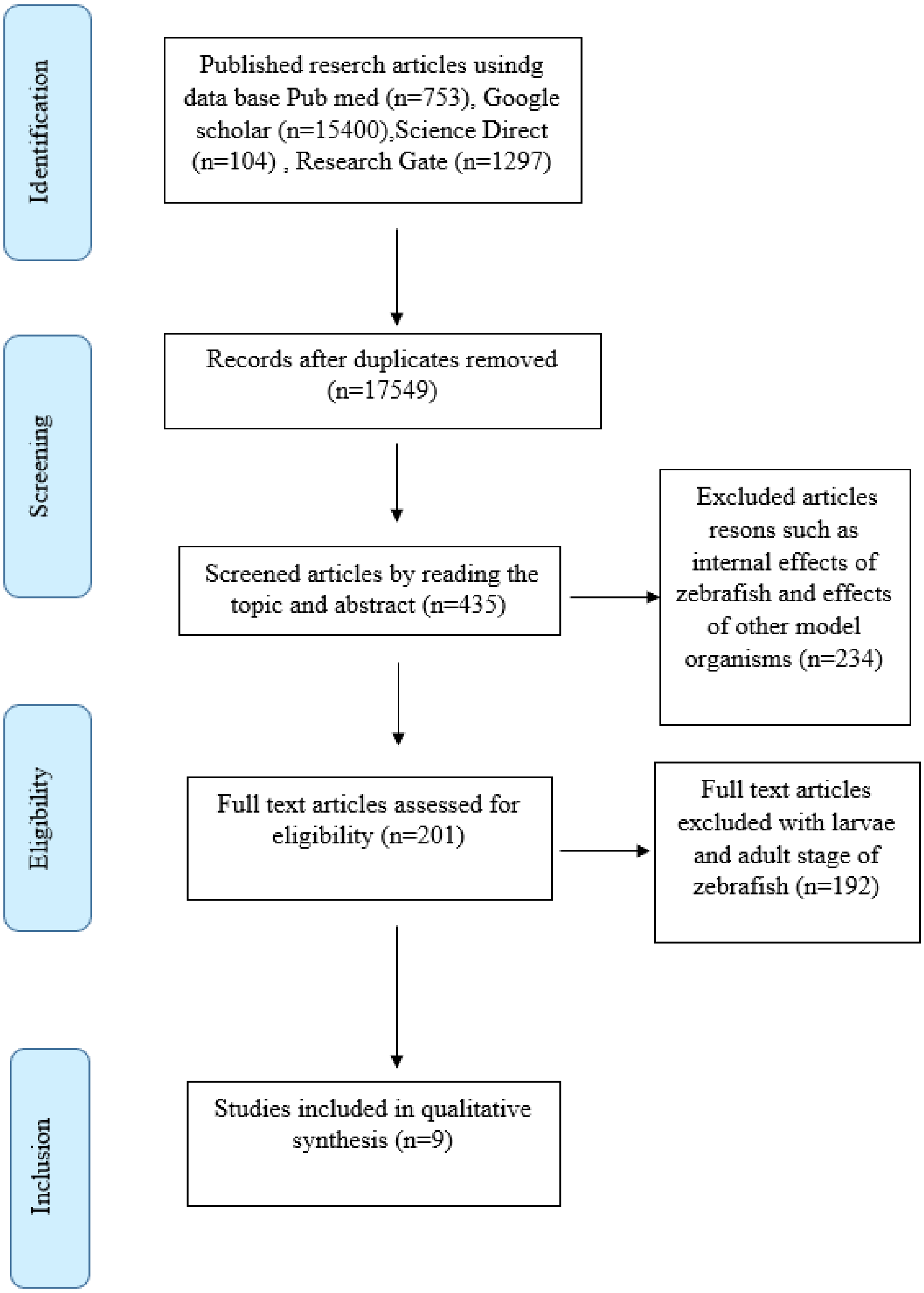

Figure 01: PRISMA flow diagram indicating the search strategy, inclusion and exclusion criteria of scientific literature for systematic review and meta-analysis (Adopted from Moher et al, 2009).

These research articles investigated the external effects, including hatching rate, mortality, malformations, body length, swimming speed, and coagulation of zebrafish embryos exposed to different concentrations of REEs. One study investigated heart rate (Chen et al., 2020), while four studies have examined malformations in zebrafish embryos following exposure to REEs (Cui et al., 2012; Chen et al., 2020; Karthiga et al., 2018; Khan et al., 2019). One study investigated the changes in swimming speed when exposed to light rare earth elements (LREEs) and heavy rare earth elements (HREEs) in zebrafish embryos (Lin et al., 2022). Five studies were on the hatching rate and time of zebrafish embryos upon exposure to REEs (Cui et al., 2012; Karthiga et al., 2018; Lazaris et al., 2012; Lazaris et al., 2023; Khan et al., 2024). Three studies investigated the survival and mortality of zebrafish embryos (Cui et al., 2012; Karthiga et al., 2018; Khan et al., 2024). Three studies investigated the effect of REEs on body length (Cui et al., 2012; Lazaris et al., 2012; Khan et al., 2024), while one study examined the coagulation of embryos upon exposure to REEs (Lora-Benítez et al., 2024).

Significant effects on heart rate (Chen et al., 2020) were reported under REEs exposure, while significant effects of malformation were reported in four studies (Cui et al., 2012; Karthiga et al., 2018; Khan, L. U. et al., 2019; Chen et al., 2020). Another study observed significant effects on swimming speed (Lin et al., 2022), while four other studies examined the effects on hatching rate and time (Karthiga et al., 2018; Khan et al., 2024; Lazaris et al., 2012; Lazaris et al., 2023). Three studies investigated the survival and mortality of zebrafish embryos, but only one article reported a significant effect (Karthiga et al., 2018). Three studies have reported on a significant effect on body length of zebrafish embryos (Cui et al., 2012; Lazaris et al., 2012; Khan et al., 2024). One study investigated the significant effect on coagulation of zebrafish embryos (Lora-Benítez et al., 2024).

Table 01: Summary of Studies on Effects of Rare Earth Element Exposure in Early Life of Zebrafish *(Danio rerio*).

Author	**Life cycle stage**	**Rare earth elements**	**Exposure period**	**Exposure concentration**	**Effects/ observations.**
(Cui et al., 2012)	Embryos	La^{3+} and Yb^{3+}	8, 24, 32, 48-60, 72 h.	(0, 0.01, 0.1, 0.3, 0.5, 1.0) mg/L	Hatching rate decreased. Mortality increased. Caused malformations (body oedema, bent tail, and yolk arc oedema). Larval body length decreased.
(Lazaris et al., 2012)	Embryos	$EuCl_3 \cdot 6H_2O$	72 hpf	(0, 0.05, 0.5, 5, 50, 100, 200, 320, 400, 500) mg/L	Increased median hatching time and overall hatching duration. Decreased mean standard length.
(Karthiga et al., 2018)	Embryos	Zirconium oxide nanoparticles (ZrO_2.NPs)	24 – 96 hpf	(0, 500, 1000, 2000, 3000, 4000, or 5000) mg/L	Increased mortality. Retardation of hatching. Caused malformations

					(yolk-sac and pericardial oedema, tail bent, axis bent, and spinal curvature).
(Khan et al., 2019)	Embryos	Fe_3O_4@ SiO_2/GdOF:xCe^{3+},y Tb^{3+} (x = 5; y = 10 mol %) nanoparticles	96 h	(1, 10, and 100) mg/L	Caused malformations (spinal curvature).
(Chen et al., 2020).	Embryos	Neodymium oxide (Nd_2O_3)	120 h	(0, 50, 100, 200, 400 and 800) mg/L	Gradually increased malformation rate. Reduced heart rate.
(Lin et al., 2022)	Embryos	Lanthanum(La), Cerium (Ce), Praseodymium(Pr), Neodymium(Nd), Samarium (Sm), Europium (Eu), Gadolinium(Gd), Terbium(Tb), Dysprosium (Dy), Holmium (Ho), Erbium (Er), Thulium(Tm), Ytterbium (Yb), Lutetium (Lu)	24 h and 96 h	(0 1) mg/L	Eu: increased the swimming speed. Yb and Lu: reduced the swimming speed. Ce and Pr: disrupted the PMR activity by increasing the movement in light conditions. HREE elements (Tb, Dy, Er, Tm, Yb, and Lu): Altered the PMR.
(Lazaris et al., 2023)	Embryos	Europium (III) chloride hexahydrate ($EuCl_3 \cdot 6H_2O$)	10,72 hpf	(0, 0.05, 0.5, 5, 50, 100, 200, 320, 400 and 500) mg/L	Increased mean-hatching time.
(Khan et al., 2024)	Embryos	Ln-UCNP solution (NaYF4:Yb, Er)	120 hpf	(0, 0.1, 1, 10, and 100) mg/L	Reduced embryo survival. Reduced body length.
(Lora-Benítez et al., 2024)	Embryos	Nd (neodymium chloride (III) hexahydrate ($NdCl_3 \cdot 6H_2O$) and Y (yttrium chloride (III) hexahydrate ($YCl_3 \cdot 6H_2O$)	24, 48, 72, and 96 h.	(6.4, 16, 40, 100, 250) mg/L	Increased embryo coagulation.

Table 02: Summary of Studies that Reported on Statistically Significant Effects on Zebrafish *(Danio rerio)* Upon Exposure to Rare Earth Elements.

Articles	The concentration that caused a significant effect	Significant outcomes
(Cui et al., 2012)	1.7304 mg/L Yb^{3+} ≥17.304 mg/L Yb^{3+} 13.89 mg/L La^{3+} ≥ 13.89 mg/L La^{3+}	Increased larval mortality. Decreased body length of larvae. The toxicity of Yb^{3+} to the hatching embryos was dose-dependent. Increased larval mortality. Decreased the body length of larvae. The toxicity of La^{3+} to the hatching embryos was dose-dependent.
(Lazaris et al., 2012)	≤ 100 mg/L $EuCl_3 \cdot 6H_2O$ ≥ 100 mg/L $EuCl_3 \cdot 6H_2O$ 500 mg/L $EuCl_3 \cdot 6H_2O$	Increased median hatching time. Increased mortality. Decreased standard body length. 100% mortality of embryos.
(Karthiga et al., 2018)	0.5–5 mg/L ZrO_2NPs 0.5–1 mg/L ZrO_2NPs ≥1 mg/L ZrO_2NPs 1 and 2 mg/L of ZrO_2NPs 3, 4, or 5 mg/L ZrO_2NPs	Higher malformation rates. Increased mortality and hatching delay. Unhatched and dead embryos. Increased mortality in embryos. 100% mortality.
(Khan et al., 2019)	100 mg/L Gd, Ce, Tb intact nanoparticles 50 and 100 mg/L Gd, Ce, Tb intact nanoparticles	Caused malformation and oedema. Hatching rate was varied.
(Chen et al., 2020).	Nd_2O_3 ≥ 200 mg/L	Dose-dependent increase in mortality and malformation rate. Decreased heart rate.

(Lin et al., 2022)	1 mg/L of LREEs and HREEs.	The significant difference for LC_{50} between LREEs and HREEs acclimate that most of the tested LREEs (La to Gd) are more toxic than HREEs (Tb to Lu).
(Lazaris et al., 2023)	0.05 mg/L of $EuCl_3 \cdot 6H_2O$	Dose-dependent increase in median-hatching time and total duration of hatching. Continuous exposure at 72 hpf resulted in a longer hatching delay compared to exposure at 10 hours post-fertilisation (hpf).
(Khan et al., 2024)	1 - 10 mg/L (NaYF4:Yb, Er) UCNPs 1, 10, and 100 mg/L (NaYF4:Yb, Er) UCNPs	A significant difference in hatching rate. Reduced body length.
(Lora-Benítez et al., 2024)	250 mg/L concentration of Nd (neodymium chloride (III) hexahydrate ($NdCl_3 \cdot 6H_2O$) 250 mg/L concentration of Y (yttrium chloride (III) hexahydrate ($YCl_3 \cdot 6H_2O$)	Induced the coagulation of embryos at 24h. Induced the coagulation of all the embryos at 24 h.

Malformations: There were four studies on REEs-induced malformations, such as body length, bent tail, yolk arc oedema, yolk-sac and pericardial oedema, tail bent, axis bent, and spinal curvature in zebrafish embryos.(Cui et al., 2012; Karthiga, et al., 2018; Khan, et al., 2019; Chen et al., 2020). Chen et al. (2020 reported on different levels of malformations induced by different concentrations of Nd_2O_3 exposure. Central malformations were decreased, a bent tail was present, and cardiac oedema was observed. Nd_2O_3 nanoparticles-induced malformations are dose-dependent in concentrations greater than 200 mg/L (Chen et al., 2020).

Another study using Gd, Ce, and Tb at 100 mg L^{-1} in a nanoplatform has reported REE-induced, yet statistically insignificant, levels of malformations, such as spinal curvature (5.53%) and oedema (19.44%), in zebrafish larvae. Additionally, no significant results were observed for mortality, total body length, yolk sac size, and oedema in these studies (Khan et al., 2019).

According to Karthiga. et al. (2018) found that exposure to ZrO2 nanoparticles in the concentration range of 0.5–5 mg/L significantly increased malformations of the yolk sac and pericardial oedema, as well as tail bending, axis bending, and spinal curvature, compared to the control group. However, higher concentrations, such as 1000 mg/L of ZrO_2 NPs, have been tested and found to cause acute malformations in treated embryos, including yolk-sac and pericardial oedema, tail bent, axis bent, spinal curvature, and coagulation of unhatched embryos. Cui et al. (2012 showed that La at the concentration ≥ 13.89 mg/L La^{3+} and Yb at the concentration ≥ 17.304 mg/L Yb^{3+} induced embryonic malformations such as oedema, bent tail, and yolk arc oedema. However, abnormalities such as tail detachment, eye development, somite formation, heartbeat, circulatory system, pigmentation, and optic capsule were not observed in treated embryos.

Heart rate: One study investigated the effects of 200 mg/L Nd_2O_3 on the heart rate of zebrafish embryos. With 200 mg/L of Nd_2O_3, the heart rate was reduced by 10–30% to the control. The mean heart rate of control embryos at 120 hpf was 96 beats per minute (bpm), while the mean heart rate of Nd_2O_3-exposed embryos was 72 bpm (Chen et al., 2020).

Swimming speed: One study investigated the swimming speed of zebrafish embryos when exposed to 1 mg/L lanthanides for 24 h. LREE and HREE lanthanides could induce alteration in one or more zebrafish locomotion endpoints, such as distance, burst, and rotation. In the LREE group, La exposure increased the average burst count, and Eu increased the activity in all behaviour endpoints (distance, burst, and rotation) (Lin et al., 2022). However, Yb and Lu (HREE group) constantly reduced all three endpoints tested. Moreover, HREE such as Ho and Er increased the total distance activity but reduced the burst count. LREEs, such as Nd and Sm, and HREEs, such as Er, resulted in higher rotation activity compared to the control. Several Lanthanides significantly affected the PMR (photomotor response) of zebrafish, as indicated by the travel distance between dark and light conditions. From the LREE group, two lanthanides, such as Ce and Pr, disrupted the PMR activity by significantly increasing the movement in light conditions. However, HREEs such as Tb, Dy, Er, Tm, Yb, and Lu significantly altered the PMR, which decreases in darkness and increases in light (Lin et al., 2022).

Hatching rate and time: Five studies investigated the effects on hatching rate and time of zebrafish embryos when exposed to REEs (Cui et al., 2012; Lazaris et al., 2012; Karthiga et al., 2018; Lazaris et al., 2023; Khan et al., 2024). Lazaris et al. (2023) analysed the hatching rate using only live and successfully hatched embryos. Continuous exposure to high concentrations, such as 50, 100, 200, and 320 mg/L of Eu at 72 hours post-fertilisation (hpf), resulted in an extended hatching time compared to the control group. Exposure to much higher Eu concentrations, such as 200, 320, 400, and 500 mg/L at 10 hpf, resulted in further extension of hatching time (Lazaris et al., 2023). The second study showed that embryos exposed to 1000 mg/L ZrO_2NPs showed a significant hatching delay compared to the control. However, 1000 mg/L of ZrO_2NPs showed a lack of embryonic hatchability, caused developmental toxicity, and severe hatching retardation effects on embryonic zebrafish (Karthiga et al., 2018). As per the third study, exposure to Eu significantly increased the median hatching time and overall duration of hatching (Lazaris et al., 2012). The fourth study investigated the effects on hatching rates by a solution containing ≤ 13.89 mg/L La^{3+} or ≤ 17.304 mg/L Yb^{3+} and did not report on any significant effects. However, the toxicities of La^{3+} and Yb^{3+} on the hatching of zebrafish embryos were dose-dependent (Cui et al., 2012). In the fifth study, a significant difference in hatching rate was observed between the 1 and 10 mg/L NaYF4:Yb, Er UC-NPs exposure groups and the control group (Khan et al., 2024).

Survival or mortality: Three studies investigated the survival of zebrafish embryos (Cui et al., 2012; Karthiga et al., 2018; Khan et al., 2024). One study investigated zebrafish embryos at 48-hpf and 120-hpf following exposure to NaYF4: Yb, Er, UC-NPs. Although survival was reduced at a concentration of 100 mg/L compared to the control group, the difference was not statistically significant (Khan et al., 2024). According to Karthiga et al. (2018), the concentration of 1000 and 2000 mg/L ZrO_2 NPs in the treated groups showed significant effects on mortality in zebrafish embryos. At 96 hpf, 1000 and 2000 mg/L of ZrO_2 NPs demonstrated mortality of 55% and 80%, respectively. However, 3000, 4000, and

5000 mg/L ZrO_2-NPs treated groups showed 100% mortality at 72 hbf. The third study reported on a dose-dependent mortality of larvae with La and Yb exposures (Cui et al., 2012).

Body length: Three studies investigated the effects of REEs on the body length of zebrafish embryos (Cui et al., 2012; Lazaris et al., 2012; Khan et al., 2024). One study showed that the body length of zebrafish was significantly reduced with exposure to NaYF4:Yb, Er, UC-NPs at concentrations of 1, 10, and 100 mg/L, respectively, compared to the control group (Khan et al., 2024). The second study on Eu has reported on significantly reduced length of newly hatched larvae under high concentrations. Continuous exposure to 100mg/L Eu resulted in a significant decrease in the standard length of newly hatched larvae in a dose-dependent manner (Lazaris et al., 2012). In the third study, solutions containing 13.89 mg/L - La or 17.304 mg/L - Yb significantly decreased the body length of larvae compared to the control group. There was no significant difference in the body length between La and Yb treatment groups (Cui et al., 2012).

Embryo coagulation: The zebrafish embryos were exposed to different Nd concentrations between 24 and 96 hours. All the embryos coagulated 24 hours following the exposure to 250 mg/L of Nd, resulting in death. Mortality rates for lower doses of 16 mg/L, 40 mg/L, and 100 mg/L, at 96 hours were 30%, 45%, and 75% respectively. The 6.4 mg/L of Nd exposure showed a viability of above 90% which is the same as the controls. Thus, the embryos were unaffected by Nd lower than 6.4 mg/L (Lora-Benítez et al., 2024). A study with 250 mg/L of Yttrium exposure for 24 hours has shown similar effects as Nd (Lora-Benítez et al., 2024).

Discussion

REEs are often considered "emerging contaminants" due to their increasing presence in the environment, resulting from the rise in mining and their use in modern technologies. Their environmental release has been acclaimed to impose substantial ecological and health risks to all biota. Recent studies have focused attention on the developmental toxicity of REEs, particularly in aquatic model organisms like zebrafish *(Danio rerio)* embryos. Short-term REEs exposure in zebrafish embryos has been linked to a range of external abnormalities. However, the existing literature on the developmental toxicity of REEs in zebrafish embryos is inconclusive. To date, comprehensive studies evaluating the effects of all REEs on zebrafish embryos have not been conducted.

Here, a systematic review was carried out using existing literature on the effects of short-term exposure to REEs during the embryonic stages of zebrafish, identifying, evaluating, and synthesising relevant studies to provide a comprehensive and unbiased summary. Available research has primarily focused on a subset of elements, including La, Yb, Eu, Zr, Gd, Ce, Tb, Nd, Pr, Sm, Dy, Ho, Er, Tm, and Lu.

Among these elements, Nd, La, Yb, and Zr exposure has been shown to increase mortality rate in zebrafish embryos in a dose-dependent manner (Chen et al., 2020; Cui et al., 2012; Karthiga et al., 2018). According to Lazaris et al. (2012), early exposure to Eu has caused relatively low embryonic mortality, while the second day post-fertilisation observed a substantial increase in mortality. This mortality is linked to impaired cardiac function, which results in reduced metabolic activity rate and depletion of energy resources (Lazaris et al., 2012).

Embryos exposed to Nd have shown to induce disruptions in normal embryonic development and dose-dependent malformations (Chen et al., 2020; Karthiga et al., 2018). Findings from the early 2000s also support these observations, where Gd exposure inhibited normal embryonic and skeletal development in marine species. Accordingly, these findings conclude that the exposure to Nd causes teratogenic effects in zebrafish (Chen et al., 2020). Recently, Khan et al. (2024) observed that oral exposure to multifunctional REE nanoparticles, containing Gd, Ce, and Tb, specifically led to a marked increase in nanoparticle accumulation in the gastrointestinal tract of zebrafish embryos (Khan et al., 2019). Supporting these findings, uncoated Yb and Er containing upconversion nanoparticles and zirconia nanoparticles significantly affect the growth and development of zebrafish embryos (Khan et al., 2019; Karthiga et al., 2018).

Exposure to Nd and Eu in zebrafish embryos resulted in cardiac arrhythmias and a significant delay in heart development (Chen et al., 2020; Lazaris et al., 2012). The cardiotoxicity of REES is potentially associated with activation of apoptotic pathways. Additionally, Gd^{3+} has altered the contractility and electrophysiological properties of cardiomyocytes in rat heart models, supporting the notion that similar toxic effects are observed in zebrafish (Chen et al., 2020).

Zebrafish typically hatch between 48 and 72 hpf, and are considered as a critical stage for embryonic development. According to the embryo toxicity test conducted by Cui et al. (2012), exposure to La and Yb delayed zebrafish embryo hatching. Similarly, even the lowest concentration of Eu caused significant changes in hatching time and hatching success (Lazaris et al., 2012, 2023). The delayed hatching could be explained by impaired cardiac function, resulting from reduced metabolic activity and depletion of energy resources (Lazaris et al., 2012).

REEs in 3^+ state/ions have shown acute toxicity to zebrafish embryos, and their developmental toxicity is attributed to disruption of calcium homeostasis. Ca is vital for cellular processes in zebrafish, and due to the similar ionic radius of REE^{3+} (9.6–11.5 nm) to Ca^{2+} (9.9 nm), but with a higher charge, REE^{3+} can competitively replace Ca. This substitution may interfere with Ca^{2+}- dependent physiological processes in developing zebrafish embryos, contributing to impaired growth and developmental abnormalities. In addition to calcium interference, other possible mechanisms may contribute to REE^{3+} toxicity. In general, pre-hatching embryos develop hatching glands located on their head, which secrete chorionase, a protein enzyme essential for breaking down the eggshell and facilitating the hatching process (Lazaris et al., 2023). These enzymes are transient and sensitive to environmental factors (Cui et al., 2012). Therefore, exposure to certain metals can impair the development and function of these glands, potentially disrupting transcription and translation processes, leading to decreased synthesis of critical proteins such as chorionase (Lazaris et al., 2023). However, during the initial embryonic developmental phase immediately after fertilisation, swelling of the egg is observed due to the absorption of water. If REEs are present in the surrounding medium, they may penetrate the embryo, altering the permeability and structural integrity of the chorion (Lazaris et al., 2023).

For example, in 2012, Cui et al. found that exposure to La^{3+} and Yb^{3+} could lead to calcium deficiency, impairments in intracellular free calcium levels, deficits in spatial memory, and a reduction in the enzymatic activity of protease (Cui et al., 2012). Moreover, under neutral pH, La^{3+} and Yb^{3+} can undergo hydrolysis, forming hydroxides that may block chorion pore canals, limiting oxygen supply to developing embryo (Cui et al., 2012). Also, Eu^{3+} ions may competitively bind to embryonic tissues, replacing Ca^{2+} and Mg^{2+} ions at specific molecular binding sites. Although Eu^{3+} shows less competitive affinity for Mg^{2+} than Ca^{2+}, its similar ionic radius allows interference with Mg^{2}-dependent metabolic pathways during embryonic differentiation, inhibiting growth and developmental processes (Lazaris et al., 2023)

Based on Lin et al. (2022), analysis of locomotion and PMR data revealed that HREEs generally induced greater disruption in zebrafish movement compared to LREEs. Prior findings also indicated that certain HREEs, including Lu, Eu, Tb, Ho, and Tm, have higher ecological toxicity that accounts for both elemental abundance and potential to release (Lin et al., 2022). Notably, the developmental toxicity of the HREE - Yb^{3+} on zebrafish embryos or larvae was significantly greater than that of the LREE - La^{3+} (Cui et al., 2012). This increased toxicity may be attributed to the smaller ionic radius of Yb^{3+} compared to La^{3+}, despite having the same charge, which allows Yb^{3+} to form more stable complexes with biological molecules (Evans, 1990), thereby enhancing its toxic effects. Although La and Yb caused a lesser acute toxicity to zebrafish embryos than common heavy metals like Cu and Cd, heavy REEs such as Yb were found to be more toxic than light REEs such as La in zebrafish embryo models (Cui et al., 2012).

In contrast, toxicity trends in *Hyalella azteca* showed a decrease from La to Er, but this pattern reversed with the three heaviest REEs (Tm, Yb, Lu). Additionally, LREE such as Gd exhibited comparatively lower toxicity than some HREEs, suggesting variability among REEs and the need for further study. Differences in toxicity rankings across studies highlight that observed effects depend heavily on both the test organism and the specific toxicological endpoint, leaving the broader question of whether HREEs are inherently more toxic than LREEs unresolved (Lin et al., 2022).

Conclusion and recommendations

This review concluded that even short-term exposure to REEs caused several external effects, including malformations, abnormalities in heart rate, mortality, reduced body length, and delayed hatching in zebrafish embryos. However, the studies conducted so far have only focused on 15 out of the 17 REEs. Among these, Changes in swimming speed were evaluated for 14 REEs. Embryonic malformations and changes in hatching rates in zebrafish embryos have been investigated for the majority of REEs, followed by an examination of their effects on mortality. Abnormalities in heart rate, body length and embryo coagulation have been tested for a limited number of REEs. Overall, these findings emphasise the limited and selective nature of existing research, highlighting the need for broader toxicological evaluations across all REEs on Zebrafish embryos.

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