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Zebrafish as an Emerging Model System in the Global South: Two Decades of Research in Brazil

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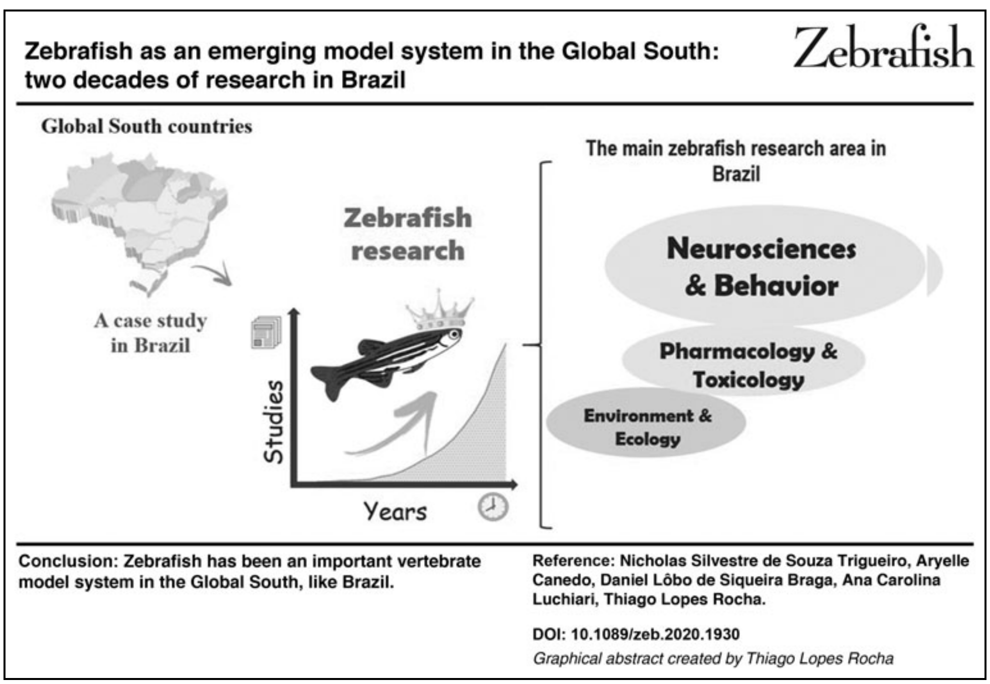
Abstract

The zebrafish (*Danio rerio*) is an emerging model system in several research areas worldwide, especially in the Global South. In this context, the present study revised the historical use and trends of zebrafish as experimental models in Brazil. The data concerning the bibliometric parameters, research areas, geographic distribution, experimental design, zebrafish strain, and reporter lines, as well as recent advances were revised. In addition, the comparative trends of Brazilian and global research were discussed. Revised data showed the rapid growth of Brazilian scientific production using zebrafish as a model, especially in three main research areas (Neuroscience &and Behavior, Pharmacology and Toxicology, and Environment/Ecology). Studies were conducted in 19 Brazilian states (70.37%), confirming the wide geographic distribution and importance of zebrafish research. Results indicated that research related to toxicological approaches are widespread in Global South countries such as Brazil. Studies were performed mainly using *in vivo* tests (89.58%) with adult fish (59.75%) and embryos (30.67%). Moreover, significant research gaps and recommendations for future research are presented. The present study shows that the zebrafish is a suitable vertebrate model system in the Global South.



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Keywords: *Danio rerio*, animal model, research networks, bibliometric analysis

Introduction

The zebrafish, *Danio rerio* (Hamilton 1822), a small tropical freshwater fish, has been an established model in scientific research for more than 100 years.[1] In the early 1980s, the first zebrafish mutant was produced by George Streisinger at the University of Oregon.[2] In recent decades, zebrafish emerged as a suitable model system in many research areas, such as developmental and reproductive toxicity,[3] genetics, toxicology, and stress,[4,5] cancer and other human diseases,[6,7] the discovery of new biotechnology products and environmental applications,[8–10] innate immune response and antiviral research,[11] as well as nanomedicine[12] and nanotoxicology.[13]

D. rerio is an attractive model system due to its low-cost maintenance, external fertilization, large number of offspring, transparent embryos, short life cycle, rapid development, primary organs developed by 5 days postfertilization and numerous homologies between fish and humans (Fig. 1).[14–17] These characteristics make zebrafish embryos a viable animal model, considering the 3R (Replacement, Reduction, and Refinement) criteria. The embryos can replace the use of adult animals in regulatory testing, and reduce the number of animals for *in vitro* and *in vivo* tests due to their high sensitivity. Furthermore, tests with early developmental stages involve less-invasive procedures (less stress for the animal) due to the transparency of the embryos, as well as exposure through the water.[10,18,19] Additionally, there are databases where genetic information, such as genetic markers, different lines, and the zebrafish genome, are available online (i.e., Ensembl and Zfin).[20,21]

Despite the emerging literature on zebrafish as a global model system in recent years, reviews on their use have focused on the advantages of the model system or global trends.[13,22] On the other hand, there is a lack of critical analysis of the current research trends on the use of zebrafish in the Global South. Although Global South countries are considered to have poor infrastructure and weak economy, some have a strong science and technology capacity, such as Brazil, China, and India.[23–25] Thus, a scientometric analysis concerning zebrafish in the Global South contributes to describing the historical use of zebrafish and recent research trends. As reported by Gheno *et al.*,[26] the trend of Brazilian scientific research with zebrafish is different from that of the global scientific community. However, knowledge concerning zebrafish research in recent years in the Global South remains limited.

In this context, the present study aimed to evaluate the use of zebrafish (*D. rerio*) as a model system for research in the Global South, with Brazil as a case study. The study summarized the data available in the scientific literature concerning scientometric patterns, such as year of publication, journals, keywords, authorships, country interactions, the field of science, and study distribution throughout the country. In addition, the methodological aspects and major scientific areas (Neurosciences and Behavior, Pharmacology and Toxicology, and Environment/Ecology) of these studies are discussed, and the comparative trends of Brazilian and global research are presented.

Methodological Approach

A literature search was performed on the ISI Web of Science website using the following keywords: ‘‘zebrafish’’ OR ‘‘*Danio rerio*’’ AND ‘‘Brazil’’ (Fig. 2). The survey was conducted on February 27, 2020, and a total of 788 articles

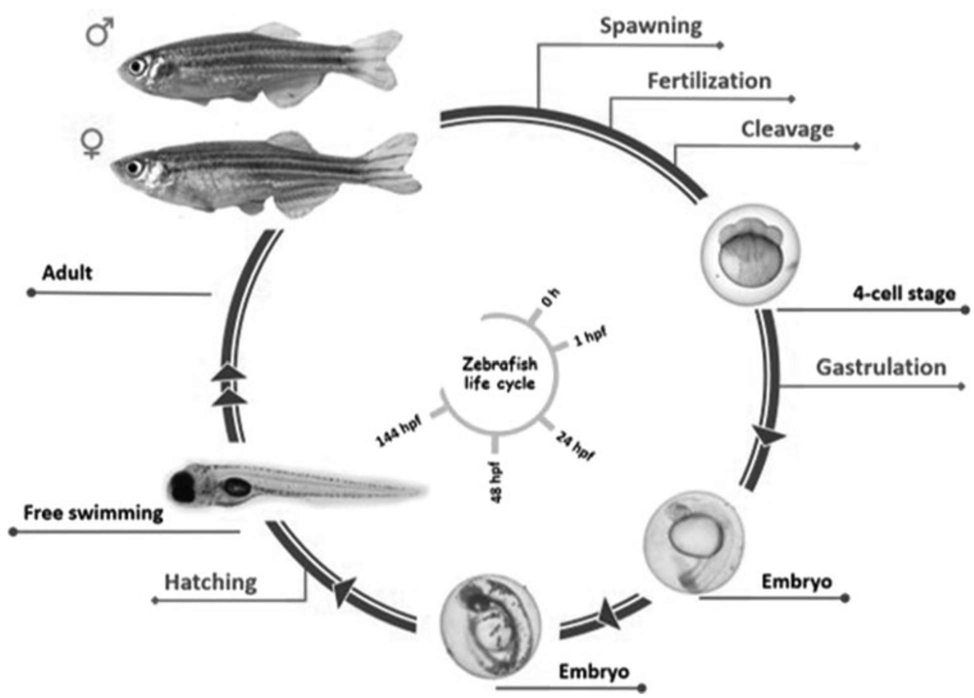

FIG. 1. The zebrafish (*Danio rerio*) life cycle.

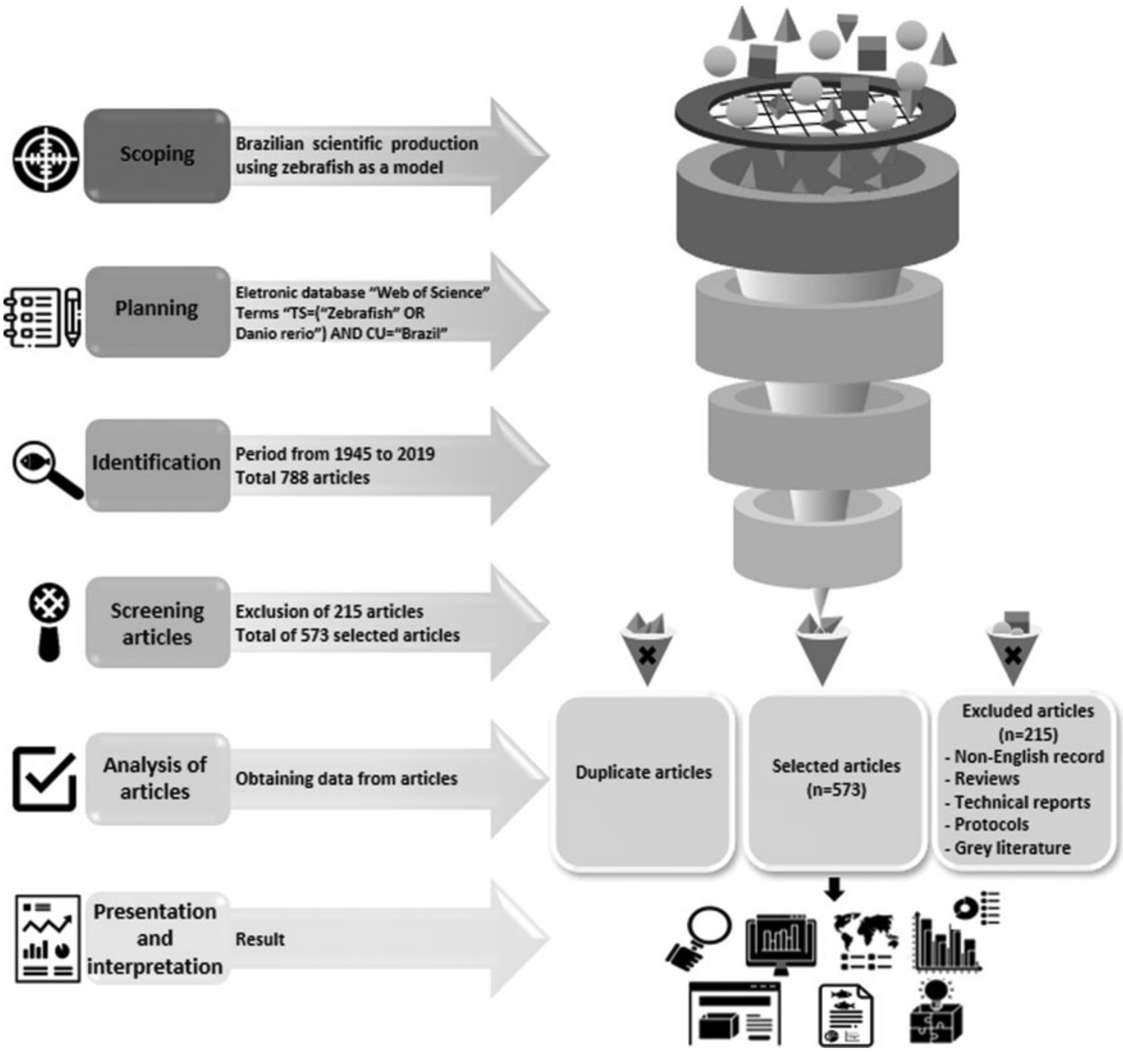

FIG. 2. Flowchart for performing a systematic review of zebrafish in Brazil.

were found. Non-English records, reviews, technical reports, protocols, and gray literature (abstracts and academic theses) were excluded. After article selection according to the exclusion criteria, a total of 573 articles were selected and summarized according to the following parameters: (1) year of publication; (2) geographical distribution (corresponding author's location); (3) keywords; (4) journal; (5) field of science and technological classification (based on the data available on Master Journal list subcategories; https://mjl.clarivate.com); (6) experimental condition (*in vitro*, *in vivo*, and *ex vivo* tests)[27]; (7) developmental stage (embryo, larvae, juvenile, and adult) based on which developmental stage was used at the beginning of the experiment[16,28] (Fig. 1); and (8) zebrafish strain and reporter lines, and cell lines.

The geographical distribution (Brazilian state) of studies was plotted using QGis 3.12 software. The author's keywords network, authors collaboration network, and country collaboration network were obtained from the Bibliometrics shiny interface (Biblioshiny).[29] To better understand the increase in article production over the years, the absolute and cumulative number of articles were log-transformed, and linear regression analyses conducted. Regressions between the normalized number of articles and year of publication were performed in GraphPad Prism 8. The data were organized in Excel 2013 (Microsoft®) and the graphs created in GraphPad Prism 8.

Results and Discussion

Historical analysis

The first article using zebrafish as an animal model in Brazil, entitled "Morphometric Study of the Regeneration of Individual Rays in Teleost Tail Fins,"[30] was published in the Journal of Anatomy in 1999. Although Gheno *et al.*[26] proposed the first study with zebrafish in Brazil was carried out in 1996,[31] it described the muscle development of the tambaqui, an Amazonian fish species (*Colossoma macropomum*). Kinth *et al.*[22] showed an increase in zebrafish global research since 1996.

By December 2019, the number of articles using *D. rerio* as an animal model in Brazil was 573 (Fig. 3A). An increasing number of articles occurred after 2013, the same year the zebrafish genome was sequenced, and the Fish Embryo Toxicity test was established by the OECD.[17,32] In 2014, the National Council for the Control of Animal Experimentation (CONCEA), an institution affiliated with the Ministry of Science, Technology, and Innovation in Brazil, established

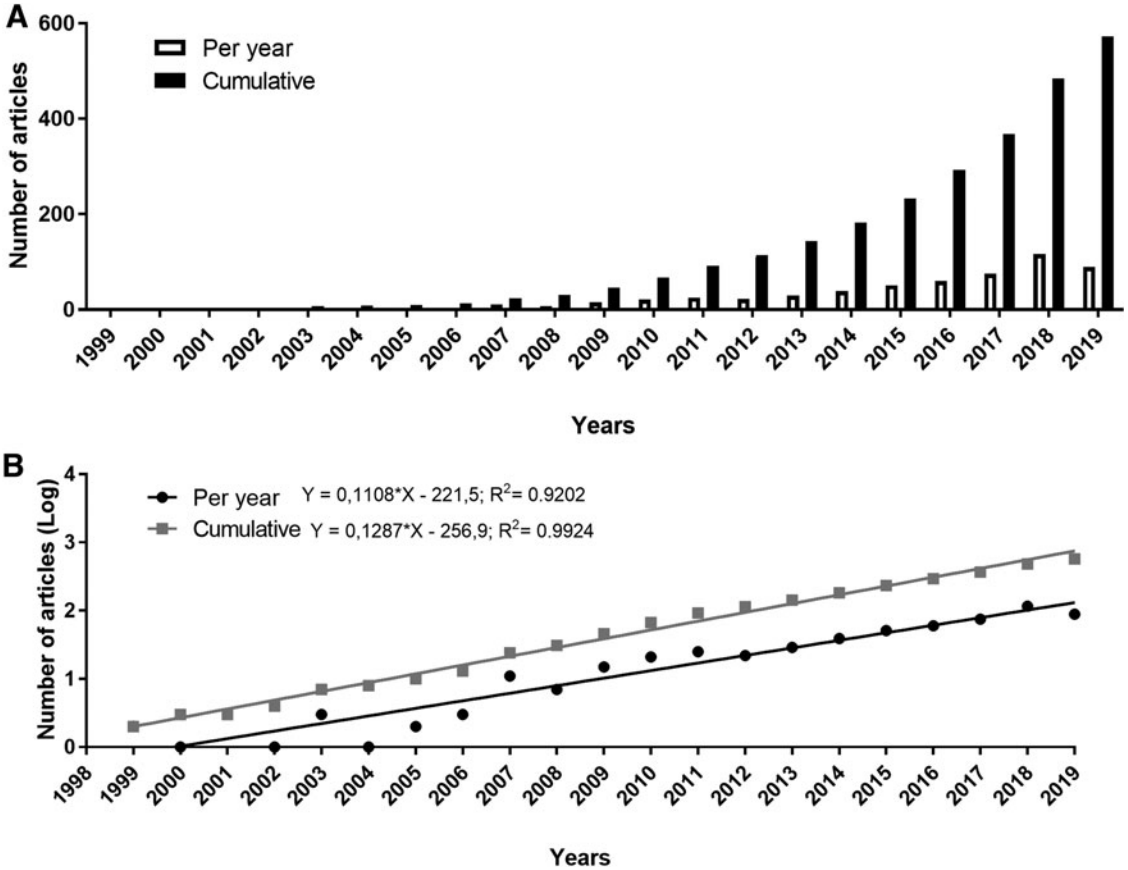

FIG. 3. Absolute and cumulative number of articles per year (**A**). Linear regression with normalized absolute and cumulative numbers (Log) of articles per year (**B**).

Normative Resolution No. 7/2014, which regulates the use of alternative animal research models based on the 3R concept (Gheno *et al.*[26]). Moreover, in 2015, the Instituto Butantan created a zebrafish platform to organize the zebrafish research data in Brazil (www.cetics.com.br/plataformas/zebrafish). The absolute ($\text{Log}Y = 0.1108 \times X - 221.5$; $r^2 = 0.9202$; $p < 0.0001$) and cumulative ($\text{Log}Y = 0.1287 \times X - 256.9$; $r^2 = 0.9924$; $p < 0.0001$) number of articles increased over time (Fig. 3B), confirming the rapid growth of Brazilian scientific production using zebrafish as a model system.

Brazilian studies with zebrafish have been published in a wide range of journals ($n = 203$) with an average impact factor of 2.98 ± 1.11 (0.15–25.45). Studies have been published mainly in the following journals: Comparative Biochemistry and Physiology C—Toxicology & Pharmacology (4.36%); Zebrafish (4.36%); Pharmacology, Biochemistry, and Behavior (3.32%); Behavioral Brain Research (3.14%); Chemosphere (2.62%); American Journal of Human Genetics (2.44%); Scientific Reports (2.44%); Aquatic Toxicology (2.27%); Environmental Science and Pollution Research (2.27%); and PLoS One (2.27%) (Supplementary Table S1). This diversity of journal confirms that zebrafish can be used in different areas of Brazilian scientific research.

Geographic distribution

The spatial distribution of studies using zebrafish as a model in Brazil is presented in Figure 4A. Nineteen Brazilian states (70.37%) conducted studies with zebrafish, mainly Rio Grande do Sul (RS; $n = 253$; 54.41%), São Paulo (SP; $n = 63$; 13.55%), Pará (PA; $n = 23$; 4.95%), Rio de Janeiro (RJ; $n = 22$; 4.73%), Santa Catarina (SC; $n = 21$; 4.52%), Rio Grande do Norte (RN; $n = 16$; 3.44%), Distrito Federal (DF; $n = 12$; 2.58), Goiás (GO; $n = 11$; 2.37%), and Ceará (CE; $n = 10$; 2.15%) (Fig. 4A). According to Cross *et al.*,[33] in a report from Clarivate Analytics to *Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior* (CAPES), 7 of the top 10 states with studies on zebrafish were among the 10 Brazilian states with the highest number of published articles in all areas.

In recent years, there has been an increasing number of studies in Midwest, North, and Northeastern Brazil. For example, since 2013, 10 states have started publishing, all from these 3 regions (Alagoas—2019, Amazonas—2013, Amapá—2017, Ceará—2015, Goiás—2016, Maranhão—2017, Mato Grosso do Sul—2016, Piauí—2019, Rio Grande do Norte—2013, and Sergipe—2018). Moreover, some states began publishing again from that date; for example, Santa Catarina published for the first time in 2005, and only again 9 years later (2014). Similarly, the Federal District and Minas Gerais took 7 years to publish articles on zebrafish (the former first published in 2009 and once again in 2016, and the latter in 2010 and 2017). This demonstrates the importance of investigating the zebrafish genome, particularly OECD test guideline 236, which concerns using fish embryos for toxicity tests. In addition, it indicates that Midwest, North, and

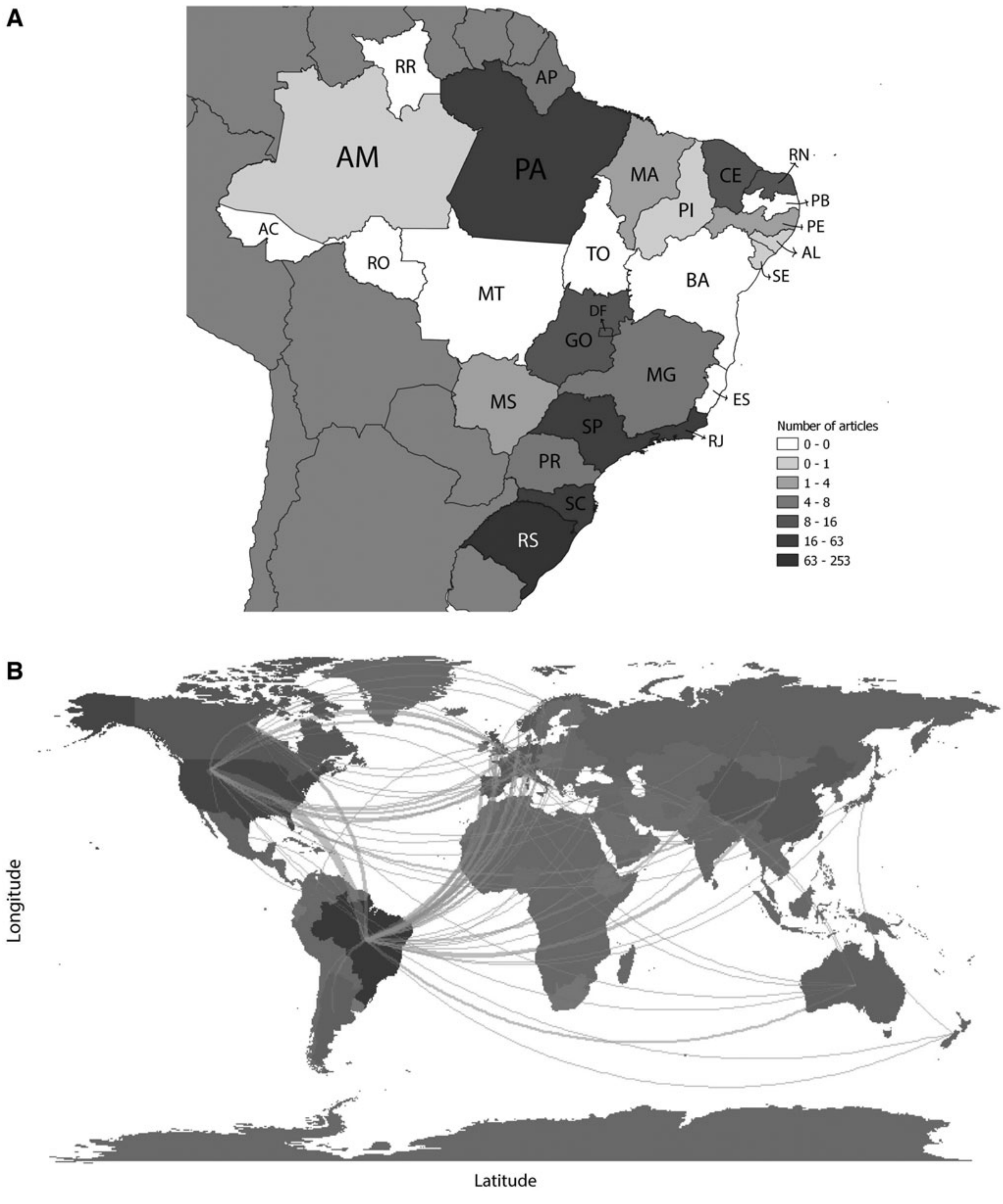

FIG. 4. Geographic distribution of studies concerning the use of zebrafish in Brazil **(A)**. The map shows the number of articles per Brazilian state; AC, Acre; AL, Alagoas; AM, Amazonas; AP, Amapá; BA, Bahia; CE, Ceará; DF, Federal District; ES, Espirito Santo; GO, Goiás; MA, Maranhão; MG, Minas Gerais; MS, Mato Grosso do Sul; MT, Mato Grosso; PA, Pará; PB, Paraíba; PE, Pernambuco; PI, Piauí; PR, Paraná; RJ, Rio de Janeiro; RN, Rio Grande do Norte; RO, Rondônia; RR, Roraima; RS, Rio Grande do Sul; SC, Santa Catarina; SE, Sergipe; SP, São Paulo; and TO, Tocantins. Graduated world map indicating collaborations (*Color* and borders) **(B)**.

Northeastern Brazil are increasingly publishing articles, given that five of the top 10 publishing states are from these regions. These data also reinforce the idea of spatial heterogeneity in scientific production, since the South and Southeastern regions have a higher concentration.[34] However, the increase in published articles in the Northeastern, North, and Midwest regions shows a decentralization of scientific publications. The same decentralization in scientific production was observed in several other countries, such as China, France, Mexico, Spain, the United Kingdom, and Russia.[34–37]

The Brazilian scientific production using zebrafish was carried out in collaboration mainly with Brazilian (64.05%) than foreign researchers (35.95%). The countries that most collaborated with Brazil are Global North countries, especially the United States ($n = 115$; 27%), United Kingdom ($n = 32$; 7.51%), and Portugal ($n = 25$; 5.87%) (Supplementary Table S2 and Fig. 4B). The Global South countries that most collaborated with Brazil were China ($n = 17$; 3.99%), Argentina ($n = 8$; 1.88%), and Russia ($n = 7$; 1.64%) (Supplementary Table S2 and Fig. 4B). It is important to highlight that China and Russia are part of BRICS, a group of developing countries, including Brazil. Argentina borders Brazil, and is part of Mercosul. The United States is also an important trading partner of Brazil, with significant scientific relations. Luukkonen *et al.*[38] reported that historical, social, geopolitical, and economic factors are important in country-to-country collaborations. The official language of Portugal and Brazil is Portuguese, which facilitates collaboration between these two countries. Luukkonen *et al.*[38] also underscore that countries with less scientific infrastructure may be intellectually dependent on developed countries. This is confirmed by the fact that only 1 of the top 10 collaborating countries is a developing nation (China) (Supplementary Table S2).

Network analysis

The coauthorship network was analyzed using 50 nodes and the Bibliometrix R Package. Results indicate nine main F5 c research groups (Fig. 5A). The first group has published several articles on Neurosciences and Behavior, mainly using zebrafish as an animal model to test psychoactive compounds. Carla Denise Bonan, Mauricio Reis Bogo, and Denis Broock Rosemberg from Pontifícia Universidade Católica do Rio Grande do Sul and Universidade Federal de Santa Maria have made significant pioneering contributions and collaborations in this research area. The second largest group also published many articles in Neurosciences and Behavior. Three researchers belonged to the highest producing coauthorship network: Angelo Luis Piato, Gessi Koaski, and Leonardo José Gil Barcellos from Universidade Federal do Rio Grande do Sul and Universidade de Passo Fundo. The last author appeared twice in the analysis due to the different ways his name was presented in the articles (Gil Barcellos, LJ and Barcellos, LJG) (Fig. 5A). The third and fourth groups are composed of four and five authors, respectively, and collaborated considerably with the Bonan's and Barcellos' groups. Furthermore, five small independent groups were observed (Fig. 5A), which develop different research in human genetic studies, neurophysiology, and ecotoxicology. It should be noted that emerging research groups were also observed in different Brazilian states (Fig. 5A).

The keyword analysis was performed with 30 nodes (Fig. 5B). The co-occurrence network with the author's keywords demonstrated that ''zebrafish'' and ''*Danio rerio*'' are standard terms used in articles, and show the focus of research and methodologies in these articles. Most keywords are related to toxicology, revealing a trend in Brazilian research with zebrafish. The largest group of words contain not only the term ''zebrafish,'' but also words related to xenobiotic agents (copper and ethanol) and their effects on behavioral tasks and physiological analysis (oxidative stress, serotonin, inflammation, and acetylcholinesterase) (Fig. 5B). The upper group is directly connected to the Brown group, with words related to toxicity research in general and other possible outcomes of toxicity tests, such as genotoxicity, embryotoxicity, neurotoxicity, and nanotoxicity (Fig. 5B). Another large group of words is the bottom group, used mainly in research that specifically focuses on neurobiology and the effects of psychiatric drugs and other chemicals on the brain (adenosine deaminase, NTPdase, ectonucleotidase, brain); these keywords are connected to Bonan's group, as shown in Figure 5A. Other group of words consists of only two keywords: cortisol and stress (Fig. 5B), indicating research on stress mediated by cortisol. The keywords, growth hormone and gene expression, make up another small group; these keywords concern the Marins LF's research group, which studies GH transgenic zebrafish (Fig. 5A). The last group presented in network analysis is the animal model group, which uses other animal models (mouse and chicken); this group specifically concerns developmental biology studies that compare other vertebrate models.

Research areas

The three most popular scientific areas using the zebrafish as a model in Brazil are Neurosciences and Behavior, Pharmacology and Toxicology, and Environment/Ecology (Supplementary Table S3). Although these three subcategories are very different from one another, they have common toxicological aspects. Thus, in the Global South, research on toxicological approaches are common. This becomes more evident when analyzing the journals with the highest number of published articles from Brazil, where the top five journals publish toxicology studies (Supplementary Table S1). In the future, studies that use zebrafish as an animal model may diversify, due to the decline in the hegemony of consolidated scientific centers and growth of Global South countries, which have a different research focus regarding *D. rerio*.[35,39] For example, in China, the zebrafish is used mainly in research on Biochemistry and Molecular Biology, Toxicology, and Environmental Sciences & Ecology.[39]

Zebrafish as a model in neurosciences and behavior. Neuroscience and behavior are the largest zebrafish research area in Brazil, with 120 articles published by 2019, corresponding to 20.9% of total Brazilian production. In a similar evaluation, neuroscience was the second largest area in Brazil in 2016.[26] In a study on the top subjects of zebrafish research worldwide published 7 years ago, neuroscience ranked 4th.[22] The first article in the neuroscience field was published in 2006, increasing to 31 studies in 2013, and quadrupling in 2019. Only 30% of all neuroscience and behavioral studies involved collaboration with foreign scientists, 67.5% of

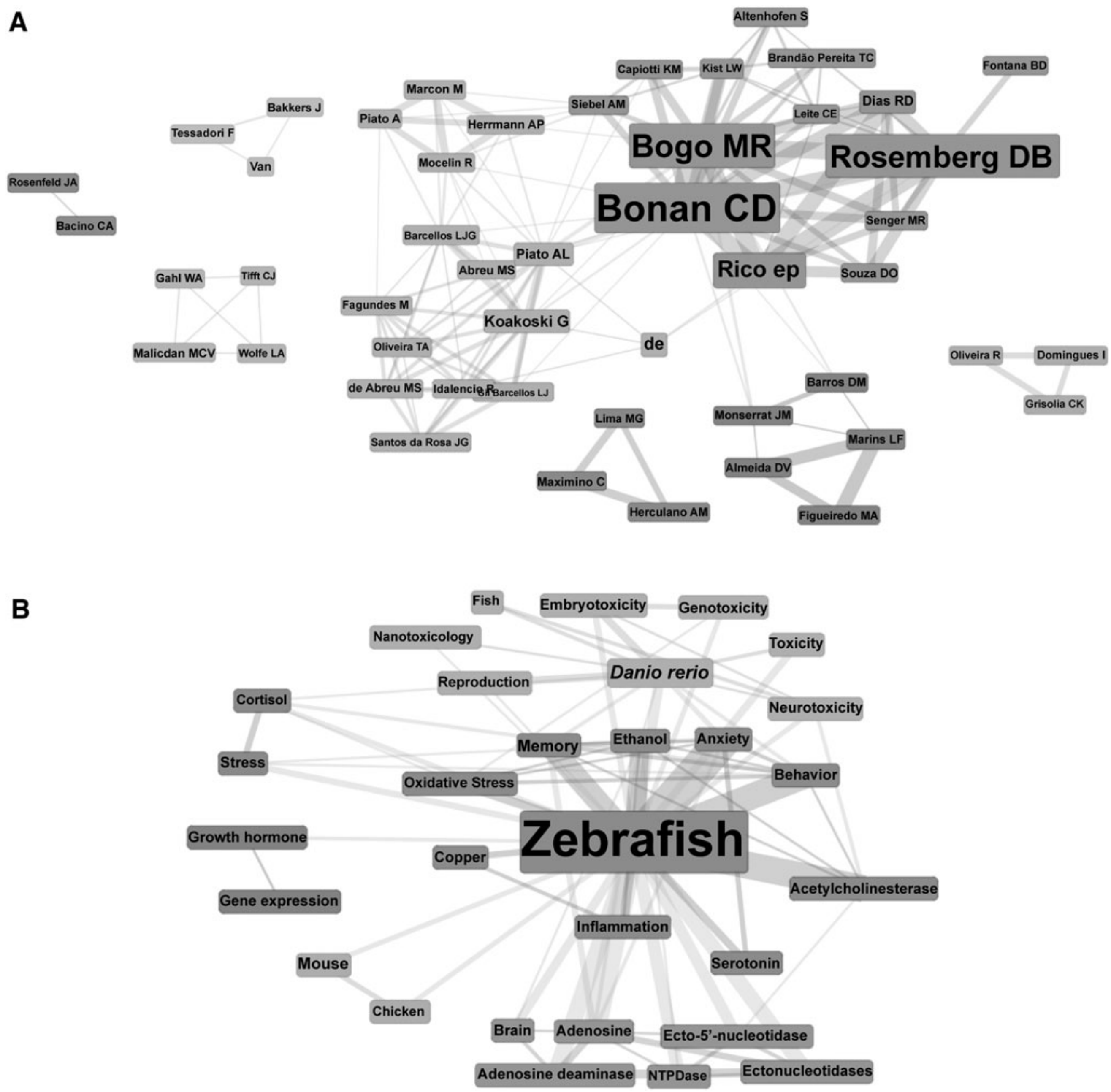

FIG. 5. Network analysis with 50 nodes of author co-occurrence **(A)** and 30 nodes of author's keyword co-occurrence **(B)**.

whom were from the United States. These data suggest Brazilian autonomy in neuroscience and behavioral research, which may soon increase further due to the emergence of new research groups in the last few years, distributed in 10 Brazilian states in 2016[26] and only 3 years later, in 18 states across the country (Fig. 4A).

Zebrafish and the human brain exhibit high molecular and structural homology.[40] This feature attracts the attention of researchers for the zebrafish as a model in neuroscience and behavioral studies. Conserved vertebrate genetics provide major brain regions with similar neurochemistry and connectivity, and several studies have shown the nervous system structure and function homology between zebrafish and humans, such as basal ganglia-,[41,42] cortex,[43] amygdala,[44] and hippocampus-like circuits.[43–45] The brain and its neural circuits modulate behavior, which is studied as the ultimate manifestation of brain functioning. In this regard, establishing the functional homology between human and zebrafish behavior allows us to use the fish as a model for understanding several human mental disorders.

In neuroscience and behavior, most studies address the neuropharmacology of the zebrafish, focusing on the basic biology of human neurological conditions. The zebrafish is an excellent model for studying the function of different neurotransmission systems, and has also been used as a neuroscience model in studies on neurodegenerative diseases and testing of drug candidates.[46–48] Neurological and neuropsychiatric disorders are highly prevalent in the population, compromising quality of life and increasing public health spending. The drugs available for the treatment of these disorders, although useful, have numerous side effects, and a considerable portion of the population does not respond to these treatments. As a result, the zebrafish has become an essential tool in investigating the etiology of these diseases and in the search for new pharmacological targets.[49]

Numerous studies in Brazil still evaluate more basic behavioral responses and approaches to social behavior, fear, learning, memory, stress, and sleep, among others.[50–53] Although not directly related to the neuroscience translational perspective, these studies create an important baseline for the use of zebrafish as a model in several research areas, thereby contributing to the improvement of the neuroscience approach. A model usually represents a complex problem in a simple and uncomplicated manner while maintaining its reliability. In the case of the biological model, validity is the most important issue.[54] In this sense, face validity refers to the similarity between the animal model and natural human responses. For instance, studies investigating the zebrafish circadian rhythm have shown its diurnal activity and nocturnal resting period,[55] comparable to other diurnal beings, such as humans. The model must undergo construct validity assessment to determine how it mimics the condition being tested.

For example, in anxiety tests, not only does the behavior shown by the model need to match the human response, but the physiological manifestation of the anxiety response should also be comparable. In this case, anxiety changes the same neurotransmitter and hormone levels in zebrafish and humans, making it a suitable animal model. Finally, predictive validity shows how animal performance predicts the condition being tested. In Brazil, numerous neuroscience and behavioral studies have used the zebrafish as a model to understand the effects of coffee,[56,57] ayahuasca,[58,59] and alcohol.[60–63] This field is gaining importance worldwide, and new research partnerships are being established based on the know-how gained by Brazilian researchers. Neuroscience and behavioral studies in Brazil have contributed to the understanding of neurodevelopmental disorders and neurodegenerative diseases. They have been used as references for other studies, thereby contributing to scientific progress.

Zebrafish as a model in pharmacology and toxicology. Zebrafish possesses several characteristics that encourage its use as a model in pharmacology and toxicology. These include high synteny with the human genome, optical clarity of embryos, highly developed immune system, rapid life cycle, and ease of genetic modification, among others.[64] In Brazil, the zebrafish has been used to characterize the mechanism of action and toxicity of several chemicals, such as metals,[65–67] pesticides,[68–70] pharmaceutical compounds,[71–73] and nanomaterial.[74–76]

Pharmacology & Toxicology is the second largest area researched in Brazil, and accounts for 16.9% of the articles published up to 2019, contrary to what is observed worldwide, where more developmental biology, cellular biology, molecular biology, biochemistry, and genetics studies have been published.[22,26] Nevertheless, the fact that Brazil does not follow international publication trends makes it a reference in Pharmacology and Toxicology since scientific production is increasing and new research groups emerging.[26]

In pharmacology, zebrafish has been used as an alternative model for screening drugs effective in the treatment of diseases, such as cancer,[77] genetic kidney diseases,[78] epilepsy,[79] Parkinson's disease, and other movement disorders.[80] Moreover, in the last few years, many transgenic, knockdown and mutant lineages have been created for pharmacological studies.[77,80] In Brazil, the zebrafish has been used for preclinical analysis of cancer drugs,[81–84] toxicological analysis of medicinal plant extracts,[85–87] and mainly in studies on several antipsychotic drugs.[88–90] A number of studies in this area focus on the toxicological effects of natural toxins, such as cyanotoxins[91,92] and cnidarian toxins[93,94]; the latter and snake venom toxins may be useful for new drug development.[95,96] The growing research in Brazil is important because it helps develop new treatments for diseases.

Nanomaterials are used in medicine,[97] environmental applications,[98] cosmetics,[99] biotechnology,[100] engineering,[101] and electronics,[100] among others. Rising nanomaterial production has been accompanied by their increasing release into the environment, categorizing nanomaterials as emerging pollutants.[102,103] In this context, *D. rerio* is a suitable model for nanotoxicity.[13,104] Half of the studies using nanomaterials in Brazil investigated the toxicity of carbon nanotubes with different functionalizations[75,105–109] and the toxicity of TiO_2 nanoparticles and multiwalled carbon nanotube hybrids.[110] Others studied the toxicity of silver nanoparticles in zebrafish and their bacterial communities,[111] iron oxide nanoparticles,[74,76] graphene oxide associated with organic matter[112] and the interaction of fullerene and arsenium in zebrafish liver cells.[113]

In the last few decades, nanomedicine has become an essential field in developing treatments for diseases such as cancer.[114] Zebrafish exhibits many advantages that make it an efficient model in research fields such as nanomedicine. These include the possibility of obtaining optical images, the large number of individuals for sampling, analysis of specific organs allowing the study of drug distribution through the body, in addition to the 70% homologous genes between zebrafish and humans.[12,115] In Brazil, Singulani *et al.*[116] reported the potential of a nanostructured lipid system in association with dodecyl gallate for treating paracoccidioidomycosis.

Zebrafish as a model in environment and ecology. In Brazil, Environmental/Ecological research using zebrafish as a model represents 10.3% of scientific production, and is the third largest research field. Brazil is considered a reference for other countries in this area since Environment/Ecology is less studied worldwide.[26] The first study on Environment/Ecology was conducted in 2000,[117] using zebrafish as a biomonitor to evaluate landfill leachate toxicity, with a significant increase in the number of studies after 2016. Nevertheless, growing concern about the environmental impacts caused by urbanization, industrialization, agriculture, and pollutants released into the aquatic environment, air, and soil require regular analysis of the impact of human activities on the environment.

Zebrafish has been used as a model system to assess the ecotoxicological impact of several chemicals in laboratory and field samples. Multiple biomarker responses in zebrafish have been used to assess the toxicity and quality of environmental samples. Several morphological, biochemical, genetic, molecular, cytological and tissue-level biomarkers have been used to determine the ecotoxicity of environmental samples in embryos[10,118] and adult zebrafish.[119] Zebrafish embryos have been widely used in ecotoxicological research, mainly due to test guideline no. 236, recommended by the OECD.[32] Although this guideline recommends 96 h of exposure, the zebrafish embryotoxicity test (ZET) could be prolonged to 120 and 144 h of exposure. New parameters to evaluate sublethal effects have been incorporated into the ZET protocol, such as spontaneous contraction frequency (SCF), heartbeat, hatching rate, and morphometric analysis.[10,13,120]

Molecular, biochemical, and behavioral biomarkers of adult zebrafish and zebrafish hepatocytes (ZFL) were used to assess the water quality of the Mirim-Patos lagoon, Rio Grande do Sul (RS) state, Southern Brazil.[121] Gene expressions (*cyp1a*, *hsp70*, *cat*, *sod1*, *tsh*, *cyp19a1a*, *cyp19a1b*, *cyp26b1*, *casp8*, *sox2*, *cyb561d2*, and *thrb*) of zebrafish embryos were used to evaluate the toxicity of the water samples from two rivers (Dourados and Brilhante) in the Upper Paraná River basin, southern Mato Grasso do Sul state.[118] Furthermore, biochemical biomarkers (i.e., antioxidant enzymes and AChE activity), behavioral tests (novel tank test and light/dark test), and the micronucleus test of adult zebrafish were used to assess the toxicity of heavy metals commonly found in the groundwater of the Serra Geral Aquifer System, Santa Catarina state.[119] Multiple biomarker responses in zebrafish embryos and larvae (mortality rate, hatching rate, SCF, heart rate, frequency of morphological changes, and morphometric parameters) were recently applied to assess the ecotoxic effects of effluents from Brazilian wastewater treatment plants in Goiânia, Midwest Brazil.[10] The revised data showed that zebrafish is a suitable model in ecotoxicological studies in Brazil. On the other hand, further studies are needed to characterize the differential response and sensibility of zebrafish and native fish.

Methodological aspects

Zebrafish studies have been conducted mainly using *in vivo* tests ($n=533$; 89.58%) in comparison with *in vitro* tests ($n=54$; 9.08%) or *ex vivo* tests ($n=8$; 1.34%) (Fig. 6A). Studies using *ex vivo* analysis were performed with a testis, focusing on the effects of hormones on spermatogenesis.[122–124] *In vivo* tests used mainly adult animals ($n=337$; 59.75%), followed by embryos ($n=173$; 30.67%), larvae ($n=36$; 6.38%), and juveniles ($n=18$; 3.19%) (Fig. 6B). The high number of embryos used in experiments is mainly due to OECD guideline 236,[32] which regulates the use of fish embryos for toxicity tests.

Research with zebrafish in Brazil was conducted with 76 different strains or reporter lines (Supplementary Table S4). The most widely used strain was the wild type (short fin; $n=239$; 38.49%), followed by AB strain ($n=31$; 4.99%), F0104—a GH transgenic ($n=20$; 3.22%), Longfin ($n=15$; 2.41%), and Tübingen ($n=13$; 2.09%). On the other hand, many articles did not record which strain was used (212 articles; 34.14%), which is problematic because different strains may respond differently to the research stimulus and cause bias.[125–127] Furthermore, 30 zebrafish lines contained green fluorescent proteins (GFP), which were employed to facilitate the observation of malformations/teratogenicity caused by chemicals and track development features in zebrafish.[128]

The most widely used tissues for *in vitro* analysis were brain ($n=27$; 48.21%) and liver ($n=19$; 33.93%), followed by embryonic ($n=4$; 7.14%) and ovarian ($n=3$ articles; 5.36%) (Fig. 6C). However, most of the articles using zebrafish cells for *in vitro* tests did not record the cell line ($n=20$; 30.19%) (Fig. 6D). ZF-L cells were employed in 16 papers and ZEM-2S in 4 (30.19% and 24.53%, respectively); these are zebrafish liver and embryonic tissue cell lines (Fig. 6D). Finally, 13 of these articles used cells obtained directly from wild animals (Fig. 6D).

Conclusion and Perspectives

This study presents the current scenario of Brazilian scientific research using zebrafish. It describes the most important research groups, collaborations, scientific areas of

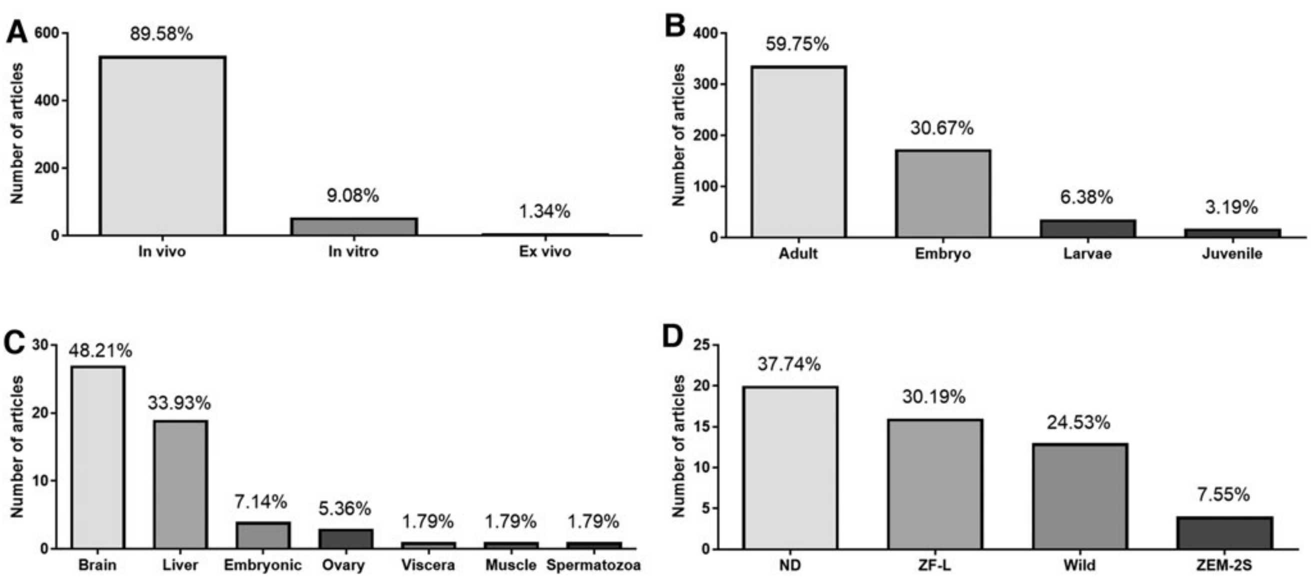

FIG. 6. Methodological aspects of studies concerning the use of zebrafish in Brazil. The number of articles according to *in vivo*, *in vitro*, or *ex vivo* tests **(A)**, developmental stages **(B)**, tissues analyzed by *in vitro* tests **(C)**, and cell lines used by *in vitro* tests **(D)**.

significant interest, and the trends and impacts of national production. In this study, we analyze the evolution of zebrafish research in Brazil and compare it to worldwide scientific production. We highlight the recent increase in the number of new research groups established in the different Brazilian states. The main research interest is separated into three areas (Neurosciences and Behavior, Pharmacology and Toxicology, and Environment/Ecology), and the involvement of toxicological aspects is described.

The largest zebrafish research area in Brazil is neuroscience and behavior, which has contributed to understanding neurological diseases, and the brain circuitry and behavioral manifestations of several conditions. Together with the second largest area of Brazilian zebrafish research (pharmacology and toxicology), Brazilian scientific production has contributed to the development of novel treatments and several drug screenings before pharmacological validation that have proved to be of significant global importance.

The major international collaborations of Brazilian researchers are with North America and Europe, where scientific production has been published in high-impact journals and received considerable attention. Collaboration is an essential aspect of Brazilian production and positively affects the quality of the study, both in terms of intellectual contribution and the possibility of publishing in higher impact journals. With the increase in the spatial distribution of zebrafish research groups in Brazil, mainly in the Northeastern, North, and Midwest regions, scientific collaborations gained status. Moreover, the creation of the national zebrafish platform and the national zebrafish symposium are substantially contributing to the exchange of knowledge and improved research quality in the country, which will undoubtedly resonate in zebrafish science worldwide. Based on the revised data, the following issues need to be considered to promote the use of zebrafish as a model system in Brazil:

(1) development of a cost-effective, easy-to-build, and easy-to-maintain zebrafish housing system;
(2) more (eco)toxicological studies with zebrafish under environmentally relevant conditions;
(3) development of software for behavior analysis and other biomarkers;
(4) development of protocols, including the fluorescent staining of specific cellular uptake and trafficking mechanisms;
(5) establishment of OMIC technologies and molecular markers for the analysis of zebrafish embryos and larvae;
(6) development of patient-specific zebrafish disease models;
(7) encouragement of public policies for the import and export of different zebrafish strains or reporter lines (i.e., transgenic and gene-edited animals);
(8) improvement of the integrative Brazilian zebrafish database;
(9) encouragement of national and international collaboration.

Disclosure Statement

The authors reported no potential conflicts of interest.

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Supplementary Material

Supplementary Table S1
Supplementary Table S2
Supplementary Table S3
Supplementary Table S4

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