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The 100 Most Cited Articles in Zebrafish: a Bibliometric Perspective

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ABSTRACT

The 100 most cited articles in zebrafish were published between 1990 and 2015, with 70% published after 2000. The most cited article received 7379 citations, while the least received only 405. The 100 most cited articles were published in 39 different journals with *Development* having the highest citations (n = 11,966) as well as the highest total publications (n=21). Eighteen authors listed four or more articles, while the USA was found to be the most prolific country, producing 81 articles, and having the most affiliated institutions and the top research funders. The most frequent keywords were "zebrafish" followed by "*Danio rerio*", "blastula", "cleavage", "embryogenesis", "mesoderm", "notochord", and "somitogenesis". This bibliometric data render a historical perspective on the advancement of zebrafish research, and facilitate mapping potential authors, institutions and countries for future collaborations as well as important key topics for future research.

INTRODUCTION

Zebrafish (*Danio rerio*) is a teleost fish in the Cyprinidae family under Actinopterygii (ray-finned fishes) class. It is a small tropical freshwater fish that originates in Northern India's Ganges River and its tributaries **(Tavares *et al.*, 2013)**. The zebrafish is one of the most popular models in various research fields such as developmental biology, drug discovery, cancer, molecular genetics, toxicology, human and animal health research, as well as aquaculture research **(Hason & Bartůněk, 2019; Khan & Alhewairini, 2019; Teame *et al.*, 2019; Hayati *et al.*, 2020; Jørgensen, 2020)**. The main advantages of using zebrafish are summarized as follow: early-life optical transparency, a short generation period, continuous and numerous egg production, rapid

development, a wide spectrum of mutant/transgenic lines, the ease with which genetic manipulations can be conducted and comparatively low cost of facilities **(Jørgensen, 2020)**.

Human and zebrafish have many physiological and genetic similarities, including brain, muscles, gastrointestinal tract, vessels, and innate immune system **(Lieschke *et al.*, 2001; Weinstein, 2002; Guyon *et al.*, 2007; Gore *et al.*, 2012; Kalueff *et al.*, 2014; Kanungo *et al.*, 2014; Zhao *et al.*, 2016)**. The majority of tissues and organs in both humans and zebrafish are identical except for the lungs, prostate and breast **(Khan & Alhewairini, 2019)**. The first human illness detected using zebrafish was a low haemoglobin production resulting from a mutation in the gene coding for ALAS2 **(Chitramuthu, 2013)**. Numerous other mutations in haematological disorders **(Brownlie *et al.*, 1998; Berman *et al.*, 2012)**, cardiovascular diseases **(Sehnert *et al.*, 2002)**, cancers (**Patton *et al.*, 2005; Liu and Leach, 2011**), muscle disease **(Lin, 2012)**, neurological disorders **(Gama Sosa *et al.*, 2012)** including Parkinson's disease **(Sarath *et al.*, 2016)**, anxiety, and posttraumatic stress disorder **(Chakravarty *et al.*, 2013)** have been found to display similar phenotype to human disease. In terms of aquaculture research, various infections including: tuberculosis, streptococcal and salmonella are studied using zebrafish disease models **(Van der Sar *et al.*, 2004)**.

The zebrafish is emerging as an increasingly successful model for various translational researches, and the number of worldwide publications is rapidly growing over time. The bibliometric analysis enables the researcher to study the growth and trend of a particular research field and helps in identifying prominent authors, institutes, countries etc. Up till now, only two bibliometric studies are detected on zebrafish research output. The first study, "Mapping of zebrafish research: a global outlook", was published in 2013 by **Kinth *et al.* (2013)**. The second recently published article which was entitled "Zebrafish as an emerging model system in the Global South: two decades of research in Brazil" by **Triguero *et al.* (2020)**. The first article used the Web of Science database for the period from 1945 to December 2012 to map significant publication patterns and development in zebrafish research, and to evaluate the research performance from multiple perspectives. The second article also used the Web of Science database to evaluate the use of zebra fish as a model system for research in the Global South, with Brazil as a case study. They reported rapid growth of Brazilian scientific production using the zebrafish model, especially in three main areas such as neuroscience and behavior, pharmacology and toxicology, and environment/ecology research. The article also showed that research related to toxicological approaches are widespread in Global South countries such as Brazil. The present study, however, used the Scopus database to identify the 100 most cited articles in worldwide research related to zebrafish using a bibliometric approach.

MATERIAL AND METHODS

In the present study, a literature search was done in the Scopus database as of November 2020. The terms ''zebrafish'' OR ''zebra fish'' OR ''*Danio rerio*'' in the article title were used to search relevant articles related to zebrafish research. The retrieved documents were structured in decreasing order of the number of citation counts

and the 100 most cited articles were recorded. There were no restrictions in terms of document type, source type, language and publication date or status.

Harzing Publish or Perish (www.harzing.com) was used to calculate the citation metric. Moreover, Microsoft Excel 2016 was to evaluate characteristics of citation (authors, title, year of publication, journal, and citations); and VOSviewer software version 1.6.15 (Center for Science and Technology Studies of Leiden University, Netherlands) was utilized to create and visualize the bibliometric network.

RESULTS

Type of document, number of citations and citation density

Of the 100 most cited articles, 89 were original articles, 9 were review papers, and 2 were letters. All the articles were written in English. The citation metric of the 100 most cited articles is shown in Table (1). The articles were published between 1990 and 2015, with 70% published after 1999 (Figure 1). The articles received 72,777 total citations, with h-index and g-index of 100 each. The years 2005 and 2008 recorded the highest number of publications out of the 100 most cited articles (n = 9 and 10, respectively).

Table 1: Citation Metric.

Publication years	1990-2015
Citations	72777
Cites/year	2347.65
Cites/paper	727.77
Authors/paper	5.83
h-index	100
g-index	100

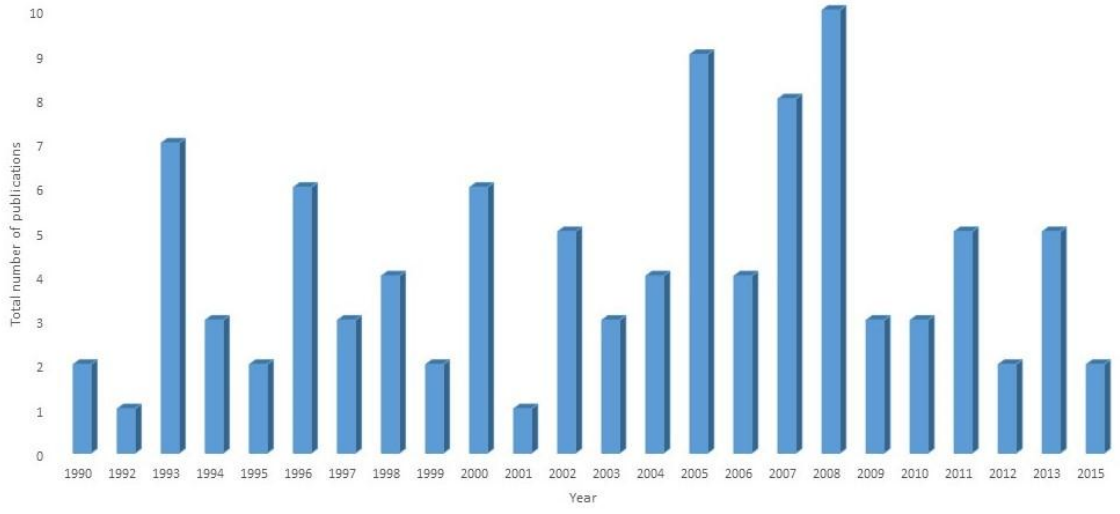

Figure 1: Distribution of 100 most cited articles.

Each of the top 15 articles received over 1,000 citations. The article of **Kimmel *et al*. (1995)** entitled: "Stages of embryonic development of the zebrafish" in the *Developmental Dynamics* journal received a total of 7,379 citations which was ranked first. **Nasevicius and Ekker (2000)** article, "Effective targeted gene knockdown in zebrafish", in the *Nature Genetics* journal was ranked second and received 1,985 citations. Articles by **Howe *et al.* (2013)** and **Hwang *et al*. (2013)** were cited 1711 and 1688 times, respectively. The articles were then ranked in order of their citation density (the total number of citation divided by the number of years in which the article was published). The article with the highest citation density (295.16) was of **Kimmel *et al.* (1995)**, whilst the lowest citation (14.23) was of **Trevarrow *et al.* (1990)**.

Journals characteristics

A total of 23936 documents on zebrafish research appeared in 160 journals. Journals of the 100 most cited articles are listed in Table (2) in a descending order based on the total number of publications. The articles appeared in 39 separate journals. *Development* published the largest number of articles (n = 21), followed by *Science* (n = 10), *Nature* (n = 8), and *Cell* (n = 6). Those four journals were responsible for 45% of the most cited articles in zebrafish research. Twenty three out of 39 journals (58.8%) published only one article each in the 100 most cited article list. SCImago Journal Rank (SJR) in Scopus provides an option to the impact factor (IF) or average citations per document over two years, abbreviated as "Cites per Doc. (2y)". The SJR of the journals ranged between 0.769 and 24.698 (mean of 7.018). The journal with the highest SJR (28.619) was *Nature Reviews Genetics*, which contributed two articles.

Table 2: The journals in which the 100 most cited articles were published

Journal Title	TP	TC	Publisher	Cite Score	SJR 2019	SNIP 2019
Development	21	11966	The Company of Biologists Ltd.	8.8	3.658	1.430
Science	10	9043	American Association for the Advancement of Science	45.3	13.110	7.521
Nature	8	7016	Springer nature	51.00	14.047	8.820
Cell	6	3667	Elsevier	58.7	24.698	7.114
Nature Biotechnology	5	3870	Springer nature	31.5	12.565	5.715
Proceedings of The National Academy of Sciences of The United States of America	5	2611	National Academy of Sciences	15.7	5.165	2.689
Current Biology	3	1761	Elsevier	13.8	3.958	2.318
Nature Genetics	3	3064	Springer Nature	45.2	19.795	6.001
Blood	2	987	American Society of Hematology	18.6	5.416	3.215
Developmental Biology	2	1895	Elsevier	5.9	1.813	0.920
Developmental Cell	2	1003	Elsevier	15.1	5.220	2.193
Genome Research	5	992	Cold Spring Harbor	16.8	9.245	2.534

			Laboratory Press			
Journal of Neuroscience	2	938	Society for Neuroscience	10.5	3.701	1.528
Nature Reviews Drug Discovery	2	1369	Springer Nature	35.5	7.018	9.489
Nature Reviews Genetics	2	1801	Springer Nature	73.5	28.619	9.126
Neuron	2	878	Elsevier	24.9	9.527	3.351
ACS Nano	1	608	American Chemical Society	23.5	6.131	2.522
Acta Biochimica et Biophysica Sinica	1	415	Oxford University Press	3.2	0.672	0.500
ALTEX : Alternativen zu Tierexperimenten	1	425	Springer Nature	8.4	1.270	1.529
Behavioral Brain Research	1	661	Elsevier	5.3	1.179	0.901
Biological Reviews	1	553	Wiley-Blackwell	19.3	4.974	3.939
Cell Host and Microbe	1	421	Elsevier	27.6	7.168	3.022
Cell Research	1	497	Springer Nature	19.8	6.141	2.276
Cell Stem Cell	1	672	Elsevier	35.7	9.340	3.906
Current Opinion in Genetics and Development	1	440	Elsevier	10.0	3.809	1.244
Developmental Dynamics	1	7379	Wiley-Blackwell	5.0	1.559	0.924
Environmental Science and Technology	1	438	American Chemical Society	12.6	2.704	2.060
Immunity	1	408	Elsevier	37.4	11.977	4.273
ISME Journal	1	490	Springer Nature	17.3	5.073	2.384
Journal of Morphology	1	448	Wiley-Blackwell	2.7	0.681	0.800
Nanotechnology	1	669	Institute of Physics Publishing	6.1	1.026	0.868
Nature Cell Biology	1	505	Springer Nature	25.3	9.916	3.005
Nature Immunology	1	498	Springer Nature	29.0	9.283	3.703
Nature Protocols	1	1438	Springer Nature	20.4	7.649	3.026
Nucleic Acids Research	1	624	Oxford University Press	21.1	8.907	3.545
PLoS ONE	1	608	Public Library of Science	5.2	1.023	1.205
Small	1	440	Wiley-Blackwell	15.7	3.717	1.695
Toxicological Sciences	1	864	Oxford University Press	6.2	1.175	1.037
Zebrafish	1	415	Mary Ann Liebert	3.5	0.769	0.591

Note: TP=total number of publications; TC=total citations

Authors

A total of 160 researchers contributed to the 100 most cited articles. Zon, L. I. authored nine articles followed by Schier A.F. (8 articles), Kimmel C.B., Nüsslein-Volhard C and Stainier DYR (6 articles each). Fifty-four articles had 1-5 authors in collaboration, while thirty articles had 6 to 12 authors in collaboration and the remaining sixteen articles had more than 12 authors in collaboration. Figure (2) shows a collaboration network that was created for the authors who contributed at least 4 or more articles of the 100 most cited articles. In the collaborative network, only 17 out of 160 most successful authors were linked together. The number of articles published by each

author is represented by the node size, while the number of publications of two researchers has co-authored, and is represented by the joining lines.

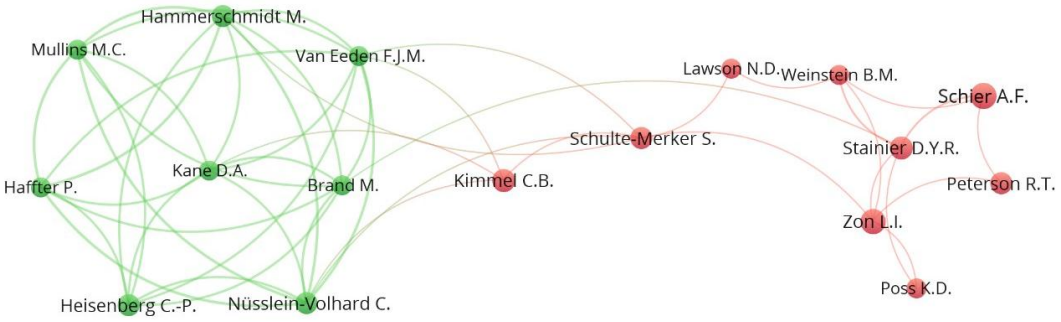

Figure 2: Co-author contribution with four or more articles with their network in the top 100 most cited articles. Seventeen authors were connected while one author was not connected to each other

Countries of origin, institutions and funding bodies

The 100 most cited articles on zebrafish are originated from 23 different countries. The most prolific country was the United States with 81 articles, followed by Germany (n = 20), the United Kingdom (n = 13), the Netherlands (n = 8), France (n =5), Japan (n = 5), Canada (n =3), China (n =3), Denmark (n =3), Spain (n =3), Australia (n = 2), South Korea (n = 2), and the rest of the countries**.** Figure (3) shows a collaboration network of the countries that was created with a threshold of 2 or more collaborations. The number of international collaboration was the largest in both the United States and Germany.

The institution with the highest number of articles was led by the University of Oregon in the USA (n = 17). This was followed by the Howard Hughes Medical Institute in the USA (n = 13), and ten articles each from Harvard Medical School in the USA, Max Planck Institute for Developmental Biology in Germany, Massachusetts General Hospital in the USA, and Children's Hospital, Boston in the USA.

The top organizations in the USA that funded zebrafish research were the National Institutes of Health (n = 21), Eunice Kennedy Shriver National Institute of Child Health and Human Development (n = 12), National Institute of General Medical Sciences (n = 8), National Heart, Lung, and Blood Institute (n = 6), March of Dimes Foundation and National Center for Research Resources (n = 5 each).

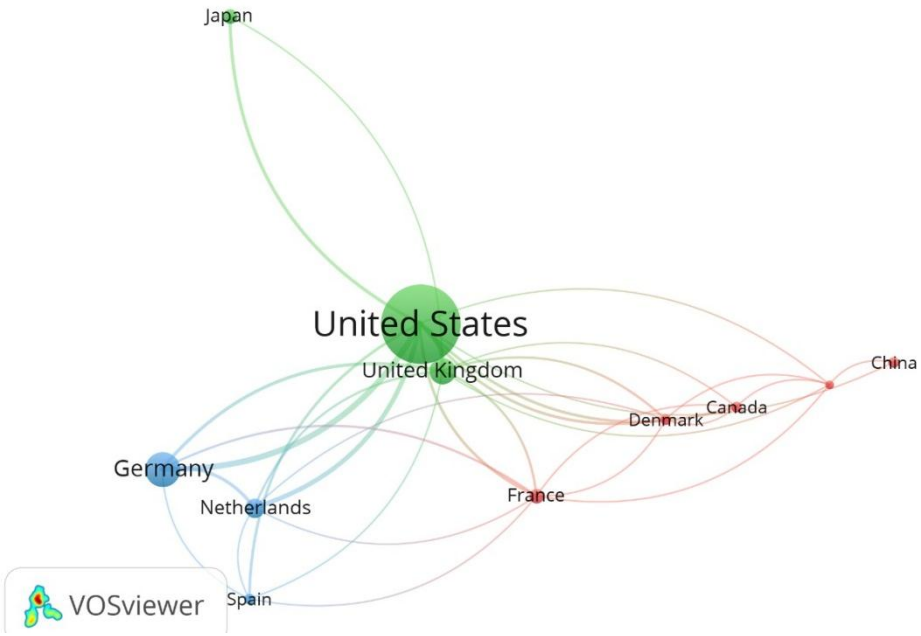

Figure 3: Visualization map of country co-authorship (international collaboration) for publications related to zebrafish

Keywords of the top-cited studies

A total of 188 author keywords were identified, with 165 of them appearing only once. Figure (4) shows the network analysis of keyword co-occurrence. "Zebrafish" was the largest node, with 23 occurrences, followed by "*Danio rerio*" (5 occurrences), "blastula", "cleavage", "embryogenesis", "mesoderm", "notochord", and "somitogenesis" (each with 3 occurrences).

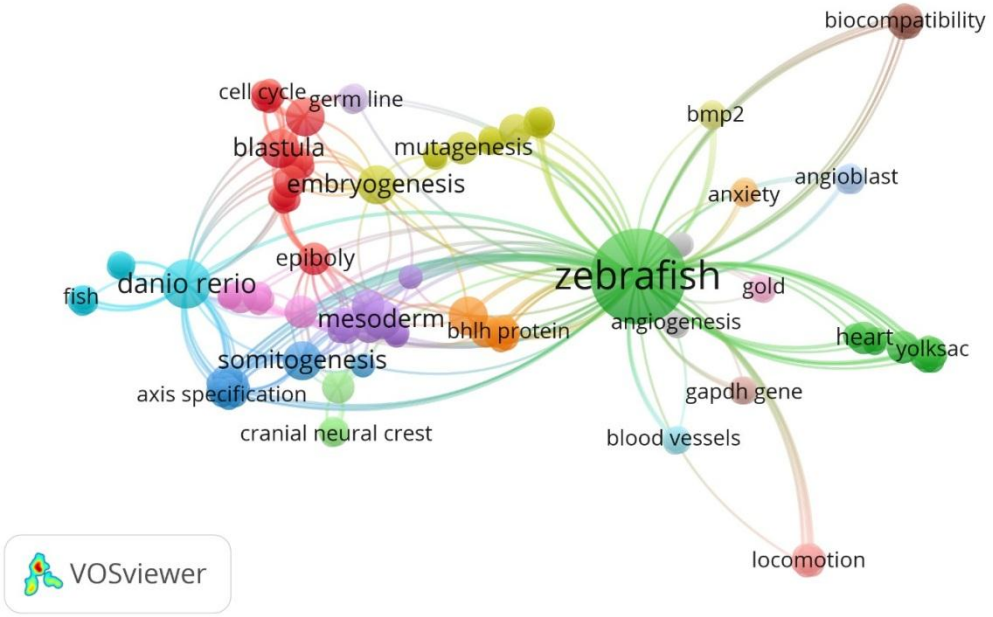

Figure 4: Keyword co-occurrence network of the 100 top-cited articles

DISCUSSION

One of the techniques for determining the most influential articles in a specific field is to use citation analysis. As a mark of distinction for any article, the total citation is acknowledged. An article that has earned 100 or more citations is categorized as a classic paper **(Garfield, 1996)**. In this study, a total of 1626 articles were cited more than 100 times, while 15 articles were cited more than 1000 times. All the 100 most cited articles on zebrafish had more than 400 citations.

As **Kinth *et al.* (2013)** previously stated there has been a growing pattern of publication since the 1990s. The oldest and latest publication was respectively dated from 1990 and 2015. Articles of the last five years were included in the present study, and this could be due to several reasons. Older articles typically had plenty of time to reach a global audience and were therefore more likely to be cited than those that were recently published, irrespective of their scientific merit. Consequently, although highly significant, the recent publications would not be reflected in citation analysis. Moreover, landmark trials received less citation because their findings were widely acknowledged that their sources or contributors were frequently forgotten over time. This is referred to as the effect of 'obliteration by incorporation' **(Gupta *et al*., 2020)**.

To correct the time bias, citation density was calculated, which is the total number of citations divided by the number of years after the publication of each article. The article of **Kimmel *et al.* (1995)** entitled ''Stages of embryonic development of the zebrafish'' published in *Developmental Dynamics*, received the largest number of citations (7379) as well as the highest citation density (295.16). The second top-cited article, "Effective targeted gene 'knockdown' in zebrafish" **(Nasevicius & Ekker, 2000)**, moved down to the sixth rank based on citation density, while "The zebrafish reference genome sequence and its relationship to the human genome" **(Howe *et al.*, 2013)** and "Efficient genome editing in zebrafish using a CRISPR-Cas system**" (Hwang et al., 2013)** were ranked third and fourth based on total citation, but they were ranked second and third based on citation density.

The 100 most cited articles were published in 39 journals, and *Development* contributed to the highest number of articles (n = 21), followed by *Science* (n = 10), *Nature* (n = 8), and *Cell* (n = 6). Those four journals are responsible for 45% of the most cited articles in zebrafish research. *Development* that focused on developmental aspects was also among the top 3 journals reported by **Kinth *et al.* (2013**) in their bibliometric analysis published in 2013. Other journals such as *Science* and *Nature* are multidisciplinary journal, and *Cell* is a journal which covers subjects like biochemistry, genetics and molecular biology. These may suggest the shift of focus of subject areas in research related to zebrafish.

Regarding publications by specific authors, Zon LI from the Harvard Medical School, Boston, United States ranked first with nine articles in the list as the first author. This was followed by Schier AF from Harvard University, Cambridge, United States (8 articles), Kimmel CB Form Max Planck Institute for Developmental Biology, USA, and Nüsslein-Volhard C and Stainier DYR, both from Max Planck Institute, Germany (6 articles each). Those authors (except for Nüsslein-Volhard C) were earlier reported to be the 25 most prominent authors by **Kinth *et al.* (2013)**. More than half of the most cited articles had 1-5 contributing authors, while thirty articles had 6 to 12 contributing authors and the

remaining sixteen articles had more than 12 contributing authors. Other authors noted a similar trend in their bibliometric analyses **(Gondivkar *et al.*, 2018; Ahmi *et al.*, 2020; Patil *et al*., 2020)**.

In this study, academic institutions in the United States contributed more than 80% of the articles. This is in agreement with bibliometric studies in other disciplines **(Patil *et al*., 2020)**. The current analysis of the co-authorship network showed that most authors who collaborated with authors affiliated either to the same organization or country. Significant collaboration amongst researchers was from the USA, Germany, UK and Netherlands. The top affiliated institutions and the top research funders were mostly from the USA.

The analysis of keywords was conducted to acquire information on patterns of zebrafish research. About 23 out of 188 keywords were identified to appear more than once, and the most frequent was "zebrafish". This was followed by "*Danio rerio*", "blastula", "cleavage", "embryogenesis", "mesoderm", "notochord", and "somitogenesis". Both "zebrafish" and "*Danio rerio*" were earlier listed as the most frequent keywords **(Kinth *et al.*, 2013; Trigueiro *et al.*, 2020)**. Other top keywords were related to the development, and it is expected that the most frequent keywords will evolve with time as already observed in the top journals.

CONCLUSIONS

This is the first article to report the 100 most cited articles in zebrafish research using a bibliometric approach. The list of journals in which the 100 most cited articles were published will guide and benefit future academic pursuits in zebrafish research. This study also maps potential authors, institutions and countries for future collaborations as well as important key topics for future research.

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