



Review article



DNA methylation in fish of the cichlid family: A systematic review

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ABSTRACT

Cichlidae is considered one of the most diverse families of freshwater fish in the world. The representatives of this group are known for their ecological and behavioral diversity. DNA methylation is an epigenetic process that involves the addition of methyl groups to DNA, affecting gene expression without altering the DNA sequence. This process has been widely studied in various organisms. The occurrence of abnormal DNA methylation events in Cichlidae may play an important role in these fish's adaptation to different environments. This article presents a review of DNA methylation in fish of this family. For this purpose, a systematic approach was used to search for relevant research in three academic databases: ScienceDirect, Scopus, and Web of Science. Initially, 50 articles were selected based on inclusion criteria, of which 22 were included in this review. This process allowed the identification of the most relevant and high-quality studies on the topic. From the 22 selected articles, a keyword co-occurrence visualization map was created, making it possible to investigate the current state of knowledge about DNA methylation in Cichlidae. The review highlights that DNA methylation is crucial for these fish's adaptation to different environments, influencing gene expression and behavior. Environmental factors, such as temperature, pH, and oxygen, affect this epigenetic modification. Furthermore, DNA methylation can also be impacted by exposure to toxic substances, which may compromise the health and survival of Cichlidae. This review allows understanding the implications of these studies for the conservation and management of these species, as it provides insights into how fish adapt to environmental changes and the risks associated with pollution, as well as providing information for future research.

1. Introduction

Epigenetics is a rising field of study that deals with heritable changes in gene expression that do not involve alterations in DNA sequence, and the mechanisms that shape such changes are often sensitive to environmental factors [1]. Among the known epigenetic mechanisms are DNA methylation, histone modification, and regulation by non-coding RNAs (ncRNAs) such as microRNA (miRNA),

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interfering RNA (siRNA), small nucleolar RNAs (snRNA), X-inactivation RNA (XiRNA), and long non-coding RNA (lncRNA) [2–5]. Epigenetic studies related to ecology and evolution are particularly focused on DNA methylation, as it is known to have greater stability across generations [6]. Beyond gene expression regulation, DNA methylation also acts in other biological processes, such as genome and chromosomal structure stabilization [7], X chromosome inactivation [8], genomic imprinting [9], and gene silencing [10].

DNA methylation, which involves the formation of 5-methylcytosine (5 mC), is considered the dominant methylation pattern in eukaryotes [6] and occurs in the presence of enzymes called DNA methyltransferases (Dnmt1, Dnmt3a, and Dnmt3b) [11]. The enzymes Dnmt3a and Dnmt3b are responsible for de novo methylation, while Dnmt1 maintains existing methylation, commonly being called the maintenance enzyme [12]. Although DNA methylation is well understood, active and passive demethylation, which removes 5 mC, is less explored [13]. Active demethylation is conducted by translocation enzymes called Ten-eleven translocation (Tet1, Tet2, Tet3) and Thymine DNA Glycosylase (TDG), which act by catalyzing the oxidation of 5 mC creating intermediates such as 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) until cytosine returns to its unmethylated state, or by removing methylated bases, acting together with DNA repair mechanisms. Passive demethylation occurs during DNA replication, in the absence of Dnmts activity [13]. In fish, DNA methylation usually occurs in CpG islands and is more frequent than in mammals and birds, highlighting the relevance of these mechanisms in fish genomes [14,15].

Hu and Barrett (2017) [16] report that DNA methylation is an epigenetic mechanism that affects gene expression and phenotypic plasticity in complex ways in plants and animals through environmental factors, such as the proportion of males and females in a given population [17]. This complex phenotypic plasticity is observed differently in plants and animals. For example, DNA methylation targeting in vertebrates typically occurs in gene bodies, while CpG islands in promoter regions are generally unmethylated, and in plants, it occurs mainly in repetitive DNA elements and transposons (TEs) [18]. Generally, environmental factors associated with methylation result from anthropogenic activity and global climate changes [19]. The environmental variables that can cause epigenetic alterations most frequently studied are pH [20], oxygen and temperature [21,22], food or nutrients [23], and heavy metals [24], the latter through pollution or contamination by agro-industrial, domestic, and mining waste.

Fish of the Cichlidae family are considered important models for genetic and evolutionary studies due to their high ecological and behavioral diversity [25,26], adaptive radiation, and phenotypic plasticity [27]. Studies on Cichlidae play a crucial role in understanding biodiversity, aquatic ecology, aquaculture, animal behavior, evolutionary genetics, and biological conservation [28,29]. Additionally, a significant number of these fish have commercial interest, being used for food and ornamental purposes through fishing and captive breeding [30,31]. Understanding DNA methylation in cichlid fish can provide important insights into the biology of these animals.

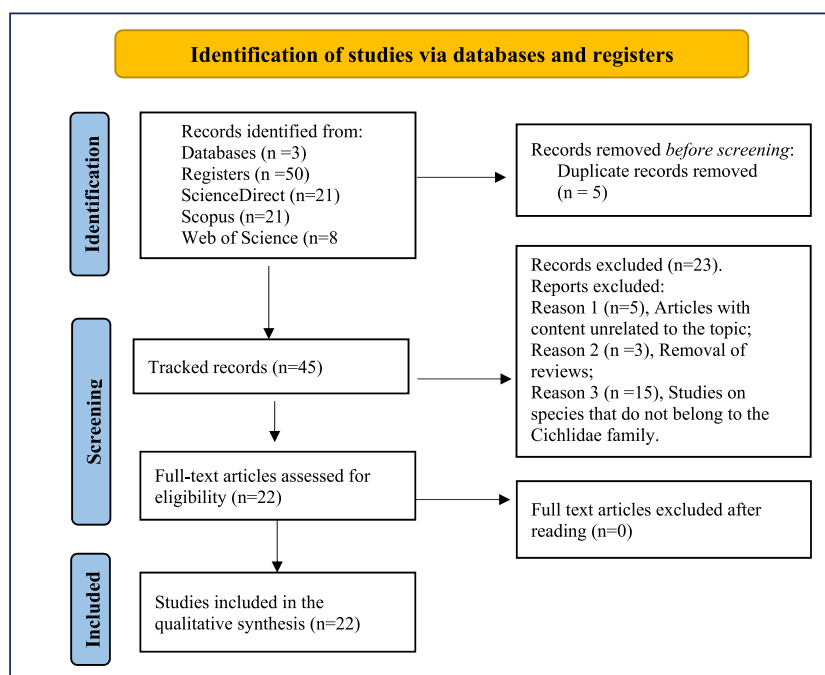


Fig. 1. Flow diagram of the literature screening process.

This review aims to synthesize current knowledge about DNA methylation in fish of the Cichlidae family. We will address how environmental factors can influence DNA methylation in these animals and how this can affect their gene expression and behavior. We will also discuss the implications of these studies for the conservation and management of these species. Through the analysis of relevant literature, we hope to provide a comprehensive overview of this research field, identifying gaps that deserve future investigation.

2. Methods

Three independent reviewers conducted the study selection, observing the risk of bias to ensure validity and overcome potential distortions that may occur during selection, according to the Cochrane Handbook for Systematic Reviews of Interventions [32,33]. Initially, titles, abstracts, and keywords were analyzed to identify potentially relevant studies. Subsequently, the selected studies were evaluated in full to determine their final inclusion in the review process. Conflicts were discussed, and a fourth author made the final decision.

2.1. Search strategy

This systematic review was conducted according to PRISMA guidelines [34]. The literature was retrieved from Scopus, Science Direct, and Web of Science platforms. The terms and Boolean operators used for the search were: “Cichlid” and “DNA methylation”.

2.2. Selection criteria

Articles were first selected based on titles, abstracts, and keywords. Then, refinement tools were used: (1) limit to articles; (2) removal of duplicates; (3) removal of reviews; (4) removal of studies on species not belonging to Cichlidae. Subsequently, literature data were extracted according to selection criteria and organized and stored in libraries on the Rayyan platform [35], where selection criteria were applied. Studies conducted in any country or geographical region and all articles published and available online until December 2023 were considered for analysis. Our systematic searches were based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) literature selection process (Fig. 1).

2.3. Data analysis

In this research, when synthesizing the selected journals, crucial information was considered, such as authors, publication year, source data, keywords, and the type of methodology used. The Rayyan platform was employed for qualitative analysis, while VOSviewer was used for quantitative evaluation. After selecting articles on the Rayyan platform, a table was created containing information about authors, publication year, theme, and findings, with the aim of exploring relevant content about DNA methylation in Cichlidae. This enabled the formation of topic sets for our discussion.

Bibliometric analysis was performed, a quantitative method that employs mathematical and statistical techniques to evaluate the interrelationships and impacts of publications in a specific research area, providing a comprehensive view of a vast set of academic works [36]. After selecting articles in Rayyan, VOSviewer software was used for processing bibliometric data. Distance-based maps of keywords were generated, where a shorter distance indicates a stronger relationship between terms. The lines drawn between items indicate these relationships, and items with greater prominence in the graph are considered of greater importance within the investigated context. A minimum requirement of two occurrences per keyword per article was considered, which resulted in a total of 384 keywords, of which 95 met the necessary threshold for forming 4 sets of relationships.

3. Results & discussion

A total of 50 articles were identified through the search strategy, of which 5 were duplicates, 23 were excluded during our screening because they were not related to DNA methylation studies in Cichlidae or were review articles. Finally, 22 studies were included and compared as they addressed themes related to the effect of epigenetics in Cichlidae aquaculture, influence of pH and temperature on DNA methylation for sex determination and differentiation in Cichlidae, effect of hypoxia on DNA methylation in Cichlidae, and exposure to xenobiotic pollutants on DNA methylation in Cichlidae (Table 1).

3.1. Bibliometric analysis

3.1.1. Keyword co-occurrence

The analysis of keyword co-occurrence assumes that words that typically appear together have a thematic relationship. VOSviewer works with these aggregated relationships and highlights the strong connection between items [36]. Four sets of keywords related to the same content were observed, with a progressive increase in research between 2016 and 2020 (Fig. 2). From 2016 to 2017, the first set of keywords (blue color) stands out, addressing the influence of environmental impacts on DNA methylation and oxidative stress in Cichlidae. The main keywords in this set include: “water pollutants”, “oxidative stress”, “animal cell”, “5-methylcytosine”, “environmental exposure”, and “malondialdehyde”. Between 2017 and 2018, the second set of keywords (pink color) points to research focused on DNA methylation and its relationship with sexual differentiation. The main keywords in this period are: “gene expression”,

Table 1

Systematization of 22 articles on DNA methylation in cichlid families resulting from research in Scopus, Science Direct and Web of Science.

Authors	Titles	Finding
Fuzinatto et al. (2015) [37]	Oxidative stress and hypermethylation induced by exposure of <i>Oreochromis niloticus</i> to complex environmental mixtures of river water from Cubatão do Sul, Brazil	There was a significant increase in lipid peroxidation and DNA methylation in most samples, indicating that the river water induces oxidative damage and hypermethylation in <i>Oreochromis niloticus</i> .
Hu et al. (2021) [38]	Effects of long-term cadmium exposure on growth, antioxidant defense and DNA methylation in juvenile Nile tilapia (<i>Oreochromis niloticus</i>)	Prolonged exposure to cadmium in juvenile Nile tilapia reduced growth, caused oxidative damage, and decreased global DNA methylation.
Flohr et al. (2012) [39]	Effects of exposure to soluble fraction of industrial solid waste on lipid peroxidation and DNA methylation in erythrocytes of <i>Oreochromis Niloticus</i> , as assessed by	Textile residues caused significant increases in lipid peroxidation and DNA methylation levels, with variations between 300 % and 700 % compared to the control. Metal-mechanical and cellulose residues also led to elevations in both biomarkers, suggesting that these soluble fractions can cause oxidative damage and alter DNA methylation, serving as potential indicators of toxicity.
Sun et al. (2016) [17]	Global DNA Methylation Changes in Nile Tilapia Gonads during High Temperature-Induced Masculinization	The masculinization induced by high temperatures in Nile tilapia significantly increases DNA methylation in the gonads, affecting critical genes for sexual differentiation, such as nr0b1a and hsd17b8.
Driscoll et al. (2020) [40]	Epigenetic regulation of gonadal and brain aromatase expression in a cichlid fish with environmental sex determination	In <i>Pelvicachromis pulcher</i> , early exposure to pH influences sex and alternative male forms. The cyp19a1 gene, which is crucial for sex determination, exhibits elevated expression of the A copy in the ovaries and is subject to strong epigenetic regulation in the gonads.
Crispo et al. (2020) [41]	Quantifying genome-wide cytosine methylation in response to hypoxia in the gills, muscle, and brain of an African cichlid fish	Cytosine methylation (5 mC) in <i>Pseudocrenilabrus multicolor</i> varies across tissues and is influenced by hypoxia, with higher levels of 5 mC observed in the gills of fish reared under low-oxygen conditions, suggesting a role for methylation in phenotypic plasticity in response to environmental changes.
Vernaz et al. (2021) [42]	Mapping epigenetic divergence in the massive radiation of Lake Malawi cichlid fishes	The divergence of the methylome among cichlid species from Lake Malawi is associated with changes in the transcription of ecologically relevant genes, suggesting a significant epigenetic contribution to adaptive diversification.
Konstantinidis et al. (2021) [43]	Epigenetic mapping of the somatotrophic axis in Nile tilapia reveals differential DNA hydroxymethylation marks associated with growth	Tissue-specific hydroxy methylation in the somatotrophic pathway may regulate growth-related genes, contributing to phenotypic plasticity through epigenetic regulation.
Yao et al. (2022) [44]	Integrated analysis of DNA methylome and RNA transcriptome during high-temperature-induced masculinization in sex-undifferentiated Nile tilapia gonad	Sex determination mechanisms in fish, such as in Nile tilapia (GSD + TE), can be influenced by high temperatures during thermosensitive periods, resulting in sex reversal. Differentially methylated regions were identified in the genome of gonads from tilapia exposed to high temperatures, suggesting that DNA methylation plays a crucial role in temperature-induced masculinization.
Podgorniak et al. (2019) [45]	Differences in the fast muscle methylome provide insight into sex-specific epigenetic regulation of growth in Nile tilapia during early stages of domestication	Growth in Nile tilapia is influenced by epigenetic regulation, with DNA methylation patterns varying between males and females of different sizes. Growth-related genes, such as map3k5 and akt3 in females, and gadd45g and ppargc1a in males, showed specific methylation patterns, suggesting that epigenetic regulation of growth involves distinct gene networks between the sexes.
Hu et al. (2020) [46]	Genome-Wide DNA Methylation and RNA Analysis Reveal Potential Mechanism of Resistance to <i>Streptococcus agalactiae</i> in GIFT Strain of Nile Tilapia (<i>Oreochromis niloticus</i>)	<i>Streptococcus agalactiae</i> causes economic losses in tilapia farming, and differences in resistance to this bacterium have been observed in genetically improved tilapia. Analysis revealed that resistant fish had increased DNA methylation in the spleen, and four immune-related genes were identified as key factors in the epigenetic mechanisms of resistance.
Lenkov et al. (2015) [47]	Epigenetic DNA Methylation Linked to Social Dominance	In the cichlid <i>Astatotilapia burtoni</i> , manipulation of DNA methylation showed that high levels of methylation increase the likelihood of social ascension, suggesting an important role for methylation in regulating social behavior.
Hilliard et al. (2019) [48]	Genome-wide effects of social status on DNA methylation in the brain of a cichlid fish, <i>Astatotilapia burtoni</i> .	The social status of <i>Astatotilapia burtoni</i> affects DNA methylation in the brain, indicating its role in modulating biological plasticity. These results open new hypotheses for future studies and help to understand the connections between behavioral, neural and transcriptional plasticity.
Li et al. (2014) [49]	Differential expression analysis of genes involved in high-temperature induced sex differentiation in Nile tilapia	High temperatures negatively affect the expression of genes related to sexual differentiation, such as CYP19A1A and FOXL2, in Nile tilapia, and alter the expression of DNA methylation genes and heat shock proteins.
Cardoso et al., 2019) [50]	Epigenetic DNA Modifications Are Correlated With B Chromosomes and Sex in the Cichlid <i>Astatotilapia latifasciata</i>	The B chromosomes in <i>Astatotilapia latifasciata</i> have distinct epigenetic patterns, influencing the loss of 5-methylcytosine (5 mC) in female gonads and 5-hydroxymethylcytosine (5hmC) in male muscle. These chromosomes affect RNA transcription and may explain sex-related variations in cellular physiology.

(continued on next page)

Table 1 (continued)

Authors	Titles	Finding
Antunes et al. (2021) [51]	Early social deprivation shapes neuronal programming of the social decision-making network in a cooperatively breeding fish	Early social experiences altered the neurogenomic profile in multiple nodes of the SDMN network, showing that the early life social environment has lasting effects on neurophysiological development.
Nedoluzhko et al. (2021) [52]	The first mitochondrial 5-methylcytosine map in a non-model teleost (<i>Oreochromis niloticus</i>) reveals extensive strand-specific and non-CpG methylation	Mitochondrial DNA methylation was found in Nile tilapia, mainly in non-CpG contexts, with higher methylation in the negative strand and in the D-loop region, suggesting an epigenetic role in the regulation of mitochondrial metabolic flexibility in fish.
Zhong et al. (2014)	DNA methylation of pituitary growth hormone is involved in male growth superiority of Nile tilapia (<i>Oreochromis niloticus</i>)	Methylation of the growth hormone (GH) gene promoter in the pituitary regulates GH expression and influences growth rate in Nile tilapia, with higher methylation observed in females, resulting in lower growth compared to males.
Shen et al. (2021) [53]	Promoter methylation and expression of the rassf4 gene in Nile tilapia (<i>Oreochromis niloticus</i>)	The rassf4 gene from <i>Oreochromis niloticus</i> was specifically expressed in the ovaries, with the protein localized to follicular cells. The low methylation of the promoter region in the ovaries (13.28 %) and the high methylation in the testes (92.85 %) indicate that this region is crucial for rassf4 transcription, establishing a basis for future studies on its function.
Alvarado et al. (2015) [28]	Social Crowding during Development Causes Changes in GnRH1 DNA Methylation	In the cichlid <i>Astatotilapia burtoni</i> , we found that methylation of the GnRH1 gene, crucial for reproduction, varies during development and after social stress, suggesting social control of development through DNA methylation.
Wang et al. (2021) [11]	Dnmt3aa but Not Dnmt3ab Is Required for Maintenance of Gametogenesis in Nile Tilapia (<i>Oreochromis niloticus</i>)	Mutation of dnmt3aa in tilapia resulted in lower sperm quality, increased apoptosis, and reduced 5-methylcytosine in the gonads, while dnmt3ab did not affect gonadal development, suggesting that dnmt3aa is essential for gametogenesis.
Wang et al. (2017) [10]	Epigenetic control of cyp19a1a expression is critical for high temperature induced Nile tilapia masculinization	Cyp19a1a DNA methylation is lower in women and increases significantly with exposure to high temperatures, which may promote masculinization.

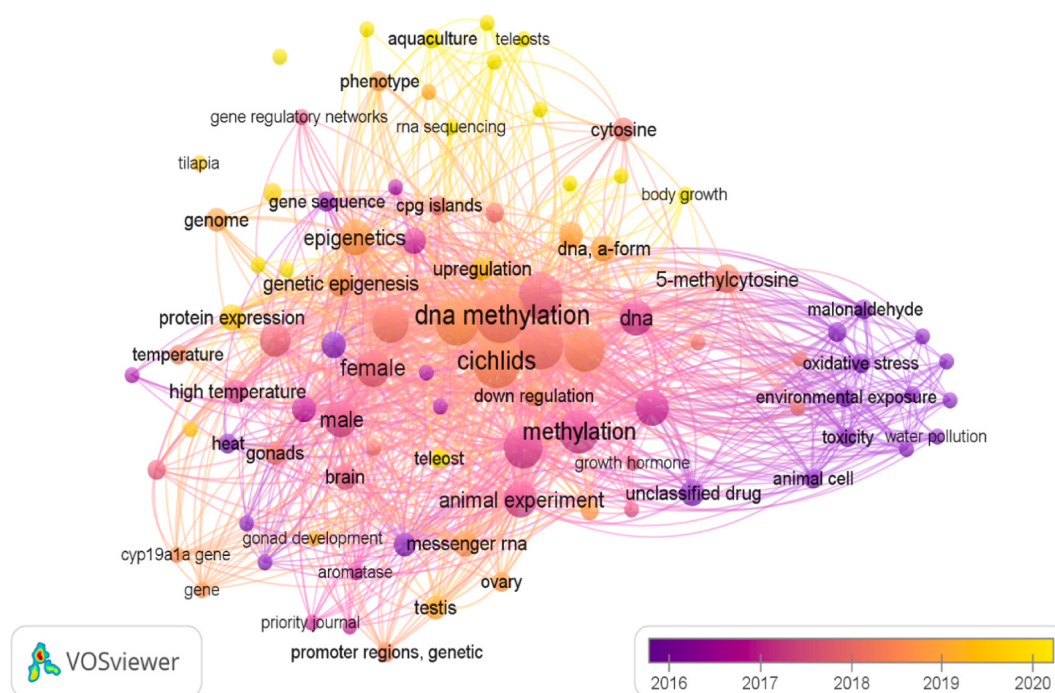


Fig. 2. Network visualization map of keyword co-occurrence based on the number of articles selected, considering the period from 2016 to 2020. The distance between terms shows the strength of the relationship (link strength), and the colors indicate a set of keywords with a high degree of simultaneous occurrence (cluster). The most prominent items are more frequent in database searches.

“protein expression”, “high temperature”, “cyp19a1a gene”, and “regions promoter”.

During the period from 2018 to 2019, the third set of keywords (red color) indicates the most frequent item in database research and greater focus on studies about epigenetic mechanisms and gene regulation in cichlids, emphasizing DNA methylation and its

biological implications. The main keywords include: “DNA methylation”, “epigenesis”, “epigenetic”, “cichlids”, “down regulation”, “gene regulation”, and “CpG Islands”. Finally, from 2019 to 2020, the fourth set of keywords (yellow color) was observed, representing topics currently being studied. These terms include “DNA modifications”, “Aquaculture”, “Molecular Evolution”, “Epigenetics”, “Genetics”, and “Bioinformatics”. This set reflects a concentration of research conducted on the application of epigenetic and genetic knowledge, both in the study of molecular evolution and in the development of sustainable aquaculture practices. Additionally, the increasing use of bioinformatics for analyzing and interpreting large volumes of genomic data highlights the importance of computational tools in understanding these modifications and biological processes.

3.1.2. Co-occurrences of most cited authors

According to the graph (Fig. 3), the most cited authors are: Wang et al. (2017) [10], Li et al. (2014) [49], and Sun et al. (2016) [17], whose research addressed the influence of temperature on DNA methylation regarding gene expression alteration in Nile tilapia masculinization. Although Li et al. (2014) [49] was the third most cited author, considering the publication year in relation to the other authors mentioned above, they were the first to discover that high temperatures induce sexual differentiation in Nile tilapia, affecting gene expression in the gonad and brain. High temperature negatively regulated genes such as CYP19a1a (aromatase enzyme), FOXL2 (essential for ovarian formation and maintenance) and others, but increased the expression of CYP11b2 gene in the ovary (involved in sterility and fertility regulation) and DNMT1 (DNA Methyltransferase 1) in the testis. Although the results are interesting and useful for understanding sexual differentiation in Nile tilapia and sustainable and efficient aquaculture management, additional studies are needed to validate and expand the findings, such as increasing sample numbers to ensure result robustness and including tilapia from different geographical origins to verify if there are variations in response to high temperature.

In this context, Wang et al. (2017) [10] was the second author who made notable contributions in their research, discovering that epigenetic control of cyp19a1a expression is essential for high temperature-induced masculinization of Nile tilapia. Specifically, they found that DNA methylation levels in the cyp19a1a promoter were almost double in males compared to females at 8 months of age. They also discovered that exposure to high temperatures significantly increased DNA methylation levels in the cyp19a1a promoter of Nile tilapia, which can override original genetic influences and cause masculinization. However, it is necessary to consider other factors, for example, expanding the analysis to include other genes related to sexual development and thermal stress response.

Finally, Sun et al. (2016) [17] is the most cited author, indicating possible leadership in terms of significant contributions to the field of DNA methylation in cichlids, as their research provided a comprehensive analysis of global genome methylation patterns in XX and XY gonads after high temperature-induced masculinization. This pioneering work provided a more complete view of epigenetic alterations during sexual differentiation, establishing foundations for subsequent studies.

3.2. Bibliographic analysis

3.2.1. Effects of epigenetics in aquaculture

The application of epigenetic studies in aquaculture (Fig. 2) can lead to more sustainable and efficient practices; for an excellent

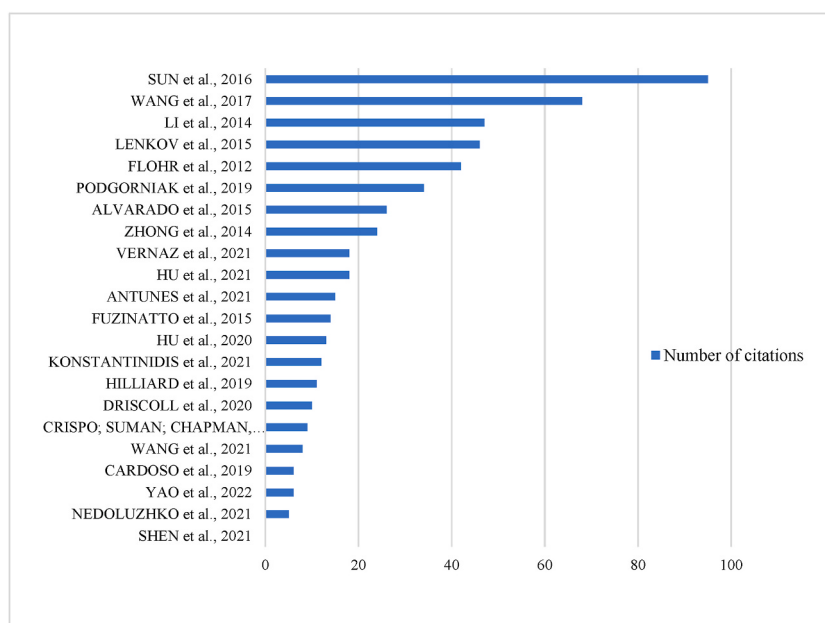


Fig. 3. Graph representing the most cited authors of the 22 articles included according to the percentage value of strength in the period from 2014 to 2022.

review, see Gavary et al. 2017 [54]. Epigenetic manipulations, such as modification of DNA methylation patterns, can be used to improve desirable characteristics in cultivated species, such as disease resistance, high reproductive capacity, stable XX-XY sex determination system, and rapid growth [55,56]. In this review, it was found that Nile tilapia (*Oreochromis niloticus*) is an example of a Cichlidae family species that can benefit from these approaches, given its importance in global aquaculture [30].

Regarding disease resistance, Laith et al. (2017) [57] revealed in their studies that Tilapia is very sensitive to *Streptococcus agalactiae* bacteria, with infections at various ages resulting in rapid dissemination and high mortality, especially at temperatures above 30 °C. The observed symptoms were: water floating, loss of balance, erratic movements, abdominal bleeding, and tail ulceration. *S. agalactiae* is a pathogenic Gram-positive bacterium found in humans, cattle, and fish [58].

Genome-wide DNA methylation studies and RNA analysis showed a methylation mechanism that generates potential resistance to *S. agalactiae* contamination in Nile Tilapia species in controlled environments [46]. In the study, researchers analyzed genome-wide methylations, comparing methylation levels in gene bodies (the part of the gene that is transcribed into RNA) and promoter regions (which control gene activation). They found that cell gene bodies have higher CpG methylation levels (a type of DNA sequence) than promoter and downstream regions (which are after the gene). The result shows a similar methylation pattern that occurs in various species such as humans [59] and goats [60]. Additionally, they also noticed that in other fish species [61], methylation in the promoter region was higher than in the gene body, which also corroborates what was found in the study, thus opening paths for more investigations into how these mechanisms work in different contexts and species.

Growth is another crucial characteristic in aquaculture, but genetic research addressing QTL (quantitative trait loci) related to growth has been limited, and genomic work has enabled discoveries of new genes that regulate growth. Studies related to the identification of new genes affecting growth were recorded in catfish [62], and in Cichlidae, it was discovered in Nile tilapia [49]. Studies on epigenetic regulation in fish and crustaceans showed that DNA methylation differences influence growth characteristics. For example, in Nile tilapia fish, increased methylation of the growth hormone gene promoter was found in females, suggesting epigenetic regulation of growth between sexes [2,43,45].

Although epigenetic research offers promising opportunities to enhance aquaculture, it's important to recognize and understand the associated negative effects. For example, the complexity of epigenetic mechanisms can lead to unpredictable results, affecting not only target genes but also other essential biological processes in animals [54,63]. This reinforces the idea that DNA methylation is an important factor in growth regulation in various aquatic species.

3.2.2. DNA methylation and its effects on behavior (social status) and phenotypic plasticity in the Cichlidae family

Social status hierarchies are common among vertebrates and significantly impact quality of life, with high-ranking individuals having greater access to resources and reproductive advantages. Conversely, individuals of lower status face greater stress, reproductive limitations, and disease vulnerability [47]. Social position and behavior are influenced by neuroendocrine processes and gene expression, shaped by the social environment. Epigenetic mechanisms, such as DNA methylation, promote behavioral and physiological adaptations, allowing adjustments to social dynamics and possible modification of hierarchies [47].

Hilliard et al. (2019) [48] reinforce this idea by showing that social stress caused by overcrowding during development alters DNA methylation in the GnRH1 gene, crucial for reproductive axis regulation, affecting gene expression and influencing reproductive processes in *A. burtoni* males. Alvarado et al. (2015) [28] corroborate this idea, showing that social stress can also modify the epigenetic profile of *A. burtoni*. The authors demonstrate that social interactions affect DNA methylation in the GnRH1 gene during development, which can directly impact fish reproductive behavior and physiological responses. This study highlights that the social environment, especially stress generated by resource competition, is an important factor in epigenetic modulation of genes involved in reproduction.

Lenkov et al. (2015) [47] further advance this discussion, demonstrating that these epigenetic changes in the GnRH1 gene occur not only during development but are also rapidly modulated by social interactions in adults. GnRH1 methylation in *A. burtoni* males is a dynamic process that adjusts according to individuals' social status, regulating rapid transitions between dominant (D) and non-dominant (ND) males. This study highlights epigenetic plasticity as a crucial mechanism for social adaptation, allowing these fish to adjust their behavior and physiology in response to changes in the social environment.

3.2.3. Influence of pH and temperature on DNA methylation for sex determination and differentiation in Cichlidae

The aquatic environment is highly dynamic, and parameters such as temperature and pH can directly influence fundamental biological processes, including epigenetic regulation [40]. In the context of DNA methylation, these environmental factors modulate the expression of genes associated with sexual differentiation and development [11].

In Cichlidae species, DNA methylation is a key mechanism for regulating phenotypic plasticity, particularly in sex determination, where small changes in environmental conditions can influence the path of sexual development [10,17]. Research has shown that pH alterations can affect the ionization of nucleophilic residues participating in cytosine methylation, while temperature can directly alter the activity of methyltransferase enzymes, such as DNMTs, promoting or inhibiting methylation of specific DNA regions [11]. These authors further explain that extreme temperatures can dysregulate these enzymes' function, causing hypomethylation or hypermethylation in promoter regions of genes critical for sexual differentiation, such as the *cyp19a1a* gene, which regulates estrogen production. In extreme heat scenarios, for example, hypomethylation can inhibit the expression of important genes, altering sexual development in temperature-sensitive fish. This can have a direct impact on population dynamics, especially in species with temperature-dependent sex determination (TSD) or combined with genetic factors (GSD + TE) [10].

Yao et al. (2022) [44] provide a complementary view by researching the epigenetic and transcriptomic effects of elevated temperature on Nile tilapia (*Oreochromis niloticus*) masculinization. The integrated analysis of methylome and transcriptome revealed that

temperature increase induces significant changes in DNA methylation in genes related to gonadal development and sexual differentiation, favoring masculinization in sexually undifferentiated fish.

3.2.4. Effect of hypoxia on DNA methylation in Cichlidae

Aquatic hypoxia is intensifying more rapidly than aquatic organisms can adapt, primarily due to increased water temperature caused by climate change. Hypoxia impairs aquatic animal growth, as low oxygen deficiency (OD) concentration causes stress, reduces appetite, and increases vulnerability to females [64]. In fish, the effects of hypoxic stress caused significant alterations in blood parameters, as well as in the functioning of the liver, kidneys, and other organs [65]. This results in increased fish mortality and causes changes in the food chain, compromising ecosystem balance. Generally, the hypoxia-inducible factor (HIF) is essential for fish response to hypoxia, regulating genes related to homeostasis, glucose metabolism, angiogenesis, and cell proliferation. Meanwhile, nuclear factor κ B (NF- κ B) controls genes involved in apoptosis, inflammation, and other stress responses, being activated by stimuli such as cytokines and growth factors. Both play central roles in adaptation to environmental stress [65].

However, hypoxia has been showing effects on DNA methylation in fish and different perspectives on how this environmental stress affects gene regulation [41]. In an earlier study, Crispo and Chapman (2011) [66] investigated how oxygen levels affect morphological and physiological plasticity in cichlids of the species *Pseudocrenilabrus multicolor* in different environments. They observed that fish raised under low oxygen conditions had deeper heads and shorter bodies. This morphological variation was attributed to both direct effects of oxygen availability and indirect effects resulting from increased gill size.

In a complementary study, Crispo et al. (2020) [41] Fig. 4 deepened this investigation with the same species, quantifying cytosine methylation (5 mC) across the genome in response to hypoxia in the gills, muscles, and brain of these cichlids. The results revealed that the amount of 5 mC varied significantly among tissues, especially in the gills, suggesting that epigenetics may play a crucial role in adaptation to extreme environmental conditions, such as low oxygen availability.

3.2.5. Exposure to xenobiotic pollutants in DNA methylation

Given that industrial effluents and solid waste contain complex compositions, such as heavy metals, trace elements, dioxins, furans, phenols, chlorides, and cyanides [67], water pollution research can be conducted using fish. This is because these organisms can accumulate such pollutants both through direct contact with chemical substances present in water and through ingestion of other organisms that also accumulate them. In this context, DNA methylation emerges as a useful marker to measure pollution effects on fish [24,68]. Some research has shown that the availability of xenobiotic pollutants influences DNA methylation in Cichlidae family fish. Flohr et al. (2012) analyzed the effects of exposure to the soluble fraction of industrial solid waste on lipid peroxidation and DNA methylation in Nile Tilapia (*O. niloticus*) erythrocytes, evaluating malondialdehyde (MDA) quantification and 5-methyldeoxycytosine (5mdC) rates, and the results indicated that soluble fractions of these industrial wastes can cause oxidative damage and modify DNA methylation in *O. niloticus*. Thus, MDA and 5mdC rates proved to be effective exposure biomarkers that can be used to evaluate the toxicity of industrial solid waste soluble fractions. Hu et al. (2021) [38] also analyzed effects of prolonged cadmium exposure on growth, antioxidant defense, and DNA methylation (negative regulatory expression DNMT3a and DNMT3b and positive expression TET1 and TET2) in *O. niloticus* juveniles. Their results demonstrate that prolonged Cd (cadmium) exposure significantly reduced growth and antioxidant capacity of this species, causing oxidative damage and decreasing global DNA methylation.

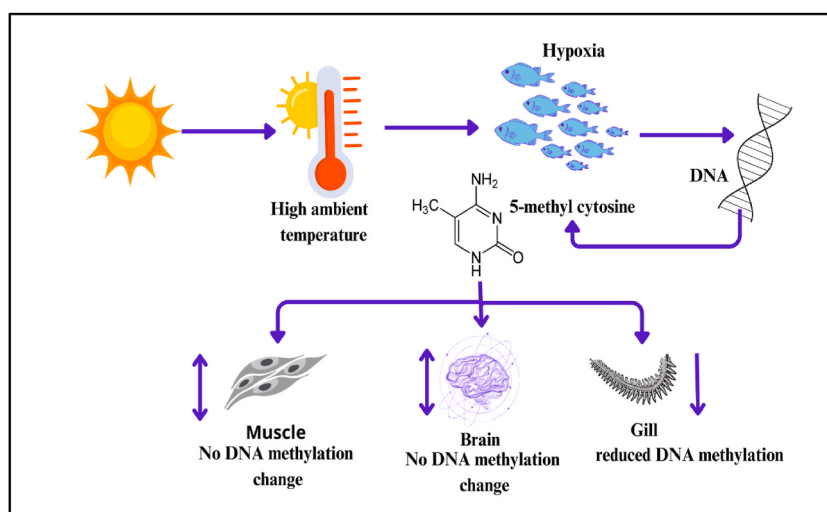


Fig. 4. Effect of hypoxia on epigenetic modification in several organs of the species *Pseudocrenilabrus multicolor*. High temperatures can increase aquatic hypoxia affecting epigenetic modifications in fish. Hypoxia induced a significant decrease in DNA methylation in gill tissue, while in muscle and brain tissues, there were no significant differences.

4. Conclusion

Based on studies of DNA methylation in Cichlidae family fish, the main conclusions are: DNA methylation plays an important role in adapting cichlid fish to different environments. DNA methylation can affect gene expression and behavior of cichlid fish. Environmental factors, such as temperature, pH, oxygen, and nutrients can influence DNA methylation in Cichlidae family fish. DNA methylation can be affected by exposure to toxic substances, which may have implications for the health and survival of Cichlidae family fish.

5. Future perspectives of DNA methylation in Cichlidae for conservation research

DNA methylation, one of the most investigated epigenetic mechanisms, is fundamental in regulating gene expression and adaptation to environmental influences [69]. Recent studies on DNA methylation in Cichlidae species have provided important discoveries for the biology and conservation of these fish.

5.1. Expansion to field studies

Although much of the DNA methylation research in Cichlidae has been conducted with African species under controlled conditions, particularly with Nile tilapia [53,70–72], which may not accurately reflect epigenetic processes occurring in natural environments, there is an urgent need to expand these studies to include other Cichlidae species in their natural habitats, such as neotropical species. Extending these analyses to different species in various environments can reveal habitat-specific methylation patterns and offer a more holistic understanding of epigenetic response to environmental changes. This is particularly relevant for threatened or declining species or those that haven't been studied, where conservation interventions may be more critical.

5.2. Applications in conservation

DNA methylation can be a powerful tool for monitoring the health and adaptability of Cichlidae populations to environmental changes, such as pollution and climate change [38,73]. Future research can focus on identifying epigenetic changes linked to resistance to environmental stressors. This can provide important information for management programs and conservation strategies. Additionally, DNA methylation can help detect the presence of specific pollutants in the aquatic environment and assess their biological impacts on different Cichlidae species.

5.3. Genetic and epigenetic conservation

Traditionally, genetic conservation has focused on maintaining genetic diversity [74–76], but including epigenetic data can provide a more complete view of populations' adaptive capacity. DNA methylation influences the expression of genes linked to adaptive characteristics essential for survival in fragmented or degraded habitats [5,16]. Thus, conservation programs that integrate epigenetic and genetic data can be more applied and effective in preserving biodiversity and resilience of Cichlidae populations.

CRediT authorship contribution statement

Elias Welengane: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Luan Felipe da Silva Frade:** Investigation, Formal analysis, Conceptualization. **Luana Beatriz Sales Pinon:** Formal analysis. **Carlos Eduardo Vasconcelos dos Santos:** Formal analysis, Conceptualization. **Adauto Lima Cardoso:** Supervision, Funding acquisition, Formal analysis, Data curation. **Luís Adriano Santos do Nascimento:** Resources, Funding acquisition, Cesar Martins, Investigation, Funding acquisition. **Renata Coelho Rodrigues Noronha:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Availability of data and materials

Data will be made available on request. For requesting data, please write to the corresponding author.

Ethics approval and consent to participate

Ethical approval and consent to participate are not applicable to this study.

Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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