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Biology of selected Clarias catfish species used in aquaculture

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Abstract

Catfish are important organisms used extensively in aquaculture. For this review, we are focusing on specific species of catfish in the genus *Clarias*, primarily *Clarias batrachus*, *C. gariepinus*, and *C. macrocephalus*, because of their widespread use in aquaculture and the considerable amount of information that has been amassed on different aspects of their biology. This review summarizes information available on topics ranging from the taxonomy and geographic distribution of these catfish to work done on them at the genetic, molecular biological and genomic levels. This information may be useful in future efforts to grow and expand their use in aquaculture.

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Introduction

The species known collectively as catfish represent a diverse group of organisms. These organisms are broadly classified as fish mainly because of their anatomical features and tendency to live primarily in aquatic environments. However, catfish are also well known to have a specialized organ to breathe atmospheric oxygen (Agnese and Teugels, 2005) and the ability to use their pectoral fins to “walk,” in some cases over extensive distances, in terrestrial environments (Li et al., 2018a). Some catfish species have also exhibited positive and negative chemotaxis when specific stimuli are present in the terrestrial environment (Bressman et al., 2020).

Catfish are very important in aquaculture (Dunham and Elawad, 2018). Around the world, in recent years, these fish have been among the top contributors to global aquacultural production (Adeleke et al., 2021; Naylor et al., 2021). In Asia, the annual production of these fish exceeds 200 million tons (Nguyen 2021a and references therein). In the US, catfish have a long history of commercial importance (Dunham and Elawad 2018), and they currently constitute the most significant portion of US domestic aquaculture production (Hedge et al., 2021).

For a general review of the biology of all species described as catfish, readers are referred to the comprehensive work of Dunham and Elawad (2018). In this review, we focus primarily on the biology of a few specific catfish species in the genus *Clarias*, partly because of their widespread use and importance in aquaculture. These species represent a vital economic resource for aquaculture farms that range from small, individual operations to massive, state-sponsored enterprises (Khedkar et al., 2016). Some of these same catfish species have also been shown to have great potential to enhance the use of aquaponics in some areas of the world (Knaus et al., 2020).

Primarily for three species in this genus, *C. batrachus*, *C. gariepinus*, and *C. macrocephalus*, a considerable range of information on these species' biology and aquacultural value is already known. Here we will first review information about taxonomic identification and the systematic relationships of *Clarias* species, including their origin and current global distribution. For reviews and discussion of higher-level taxonomic and systematic relationships of significant groups of teleost fishes that include the catfish, readers are referred to the work of Kappas et al. (2016). Next, we will review knowledge available about the genetics and molecular biology of *Clarias* populations and species, along with genome-level information where it is available. Also, as a topic related to the genetics of these species, we will review work done in studies dealing with the formation and use of hybrid and polyploid catfish for aquaculture. Finally, we will also look at studies emphasizing different aspects of growth, nutrition, and environmental tolerance in these species in aquaculture applications.

Taxonomy, origins, and geographic distribution of *Clarias* species

From a taxonomic perspective, the genus *Clarias* is one of approximately 16 genera in the family Clariidae (Teleostei, Siluriformes) (Baisvar et al., 2021). The Clariidae family is considered to be Asian in origin, but species in the genus *Clarias* are known to occur on at least three continents (Agnese and Teugels 2005). The earliest descriptions of individual species of *Clarias* can be found going back to the works of Linnaeus in 1758 (see Ng and Kottelat 2008 for a review of early reports). Over the years, several revisions of the systematic relationships of the species found in this genus have been carried out, but currently, at least 61 known species are known (Maneechot et al., 2016).

In terms of natural habitats, *Clarias* species are typically found inhabiting inland water bodies within broad geographic areas of Africa and Asia. However, the ability to make clear and unambiguous identification of species found in different parts of the world has been particularly challenging for several reasons (Teugels 1984, Ozouf-Costaz et al., 1990). African forms show extensive variation in external morphology and osteology (Agnese and Teugels 2005). Within Asian forms, studies have also identified considerable diversity, to the point

where it has been suggested that the Asian species should be assigned to a separate genus (Ng and Hadiaty 2011). It is also worth noting that the dynamics of *Clarias* species composition in certain geographic localities can change rapidly. For example, Ng et al. (2014) reported that in Singapore, *C. batrachus*, which was recently the most common catfish found in this region, has now largely been displaced by *C. gariepinus* after it was introduced sometime in the mid-1990s.

Many controversies regarding identification appear to stem from heavy reliance on morphological traits for descriptions and classifications of species in this genus. A wide range of different morphological characters have been used in catfish identification (see Agnese et al., 1997; Khedkar et al., 2014a; Ng and Hadiaty, 2011; Ng and Kottelat, 2008 for characters used). These characters can be challenging to use because they are all derived from adult-stage specimens and require precise biometric measurements of specific characters that can be compared to holotype specimens (Ng and Hadiaty 2011). In addition to the well-known fact that there is always some variation in the morphological characters used, it is often unclear how this variation should be incorporated into the identification process. Differences of opinion on classification led, for example, Ng and Kottelat (2008) to argue that the variation seen in some of these characters indicated that more than one species had been confused under the name *C. batrachus*. They suggested that Catfish from Java should retain the neotype designation of *C. batrachus* based on a combination of morphological character measurements; they proposed that the non-Javanese catfish should be designated as four different species. More recently, Ng and Hadiaty (2011) also stated that based again on the use of a combination of morphological characters, the designation of a new species of walking catfish in Malaysia, named *C. microspilus*, was warranted. Overall, as stated by Khedkar et al. (2014a), it can be concluded that many of these morphological characters can vary over such a wide range that their usefulness in distinguishing one species from another is diminished.

Finally, in any discussion of using morphological characters to identify *Clarias* species, researchers must also keep in mind that several of these species are known to naturally be capable of forming hybrids, at least some of which show intermediate morphologies (Hashimoto et al., 2016). And while these hybrids may have useful applications in aquaculture practices (reviewed in a later section), it is clear that this may also contribute to difficulties in relying on morphological characters for identification purposes.

Molecular markers for species identification and analysis of systematic relationships

To further develop work on species identification and clarification of evolutionary relationships amongst these fishes, the use of alternative characters, in particular those based on molecular markers, should be of great value. As with many other species, some early work in this area has been done with allozyme markers (discussed below). Still, more contemporary molecular markers have been used for some *Clarias* species, as described here. The DNA-based markers used for identification purposes have mainly been derived from mitochondrial DNA CO1 sequences in a DNA barcoding approach. Still, other molecular markers have been used for species identification and clarification of systematic relationships between species and populations.

Allozyme markers for population and species relationships. Studies of variation in allozyme or isozyme markers in *Clarias* species, primarily for analyzing population relationships, have been done independently or in conjunction with other features, such as morphological characters. For example, Rognon et al. (1998) looked at morphological and allozyme variation in African populations of *C. gariepinus* and *C. anguillaris*. This study found that while both marker types could distinguish some population groups based on geographic differences, the allozyme data also showed complex patterns of genetic variation within the *C. gariepinus* samples. Agnese et al. (1997) looked at four different types of markers (morphometry, allozymes, microsatellites, and mitochondrial RFLPs) in the same two species from Senegal. They found that a combined approach using three assays could reliably distinguish these two

species in this case. Na-Nakorn et al. (2002) found that isozyme markers could be used to make clear distinctions among four *Clarias* species found in Thailand.

Molecular markers for DNA barcoding. For identification purposes, focusing on Indian clariids, Devassy et al. (2016) used a DNA barcoding approach (Hebert et al., 2003) based on COI sequences to look at genetic variation within and between populations of *C. magur*, *C. dussumieri*, and *C. gariepinus*. This analysis showed that despite the morphological similarities between *C. dussumieri* and *C. gariepinus*, apparent differences between these two species could be resolved using this molecular approach. Their results were also used to reconstruct phylogenetic relationships of these *Clarias* species relative to others available from GenBank. The potential value of using DNA barcoding to resolve confusion when relying on morphological identification can also be seen in a study of the marketing of aquaculture products in India. *C. batrachus* is a highly preferred fish in India because of its taste and reported medicinal properties. However, this species is often limited availability, and it has been suggested that many traders were routinely substituting products from *C. gariepinus* because of morphological similarities. In a survey of market samples sold as *C. batrachus* in India, Khedhar et al. (2014a) used DNA barcoding for species identification. They showed that up to 99% of the market samples sold as *C. batrachus* were, in fact products from *C. gariepinus*, although this catfish is supposedly banned as an exotic species in India.

Other mitochondrial markers in species and populations. At the population level, using DNA sequences from the mitochondrial D-loop region, Barasa et al. (2016) looked at the genetic diversity and structure of populations of *C. gariepinus* from two lakes in Kenya. A similar approach was used to examine D-loop variation in an expanded set of African lake populations (Alal et al., 2021). This study found relatively little gene flow and sharing of mitochondrial haplotypes between these populations, resulting in genetically distinct populations in these habitats.

In studies of wild populations of *C. batrachus* in India, Khedkar et al. (2016) also looked at genetic variation using mitochondrial cytochrome B and D-loop regions. This study found low levels of genetic variation among eight populations from both northern and southern areas and minimal evidence for any genetic structure in the populations. This study also confirmed previous results showing a lack of extensive genetic diversity among Indian populations of *C. batrachus* using RAPD genetic markers (Khedkar et al., 2010).

Microsatellite and SSR markers. Several studies employing these markers have mainly focused on genetic variation within two *Clarias* species, *C. batrachus* and *C. macrocephalus*. In *C. batrachus*, Srivastava et al. (2016, 2017) looked at SSR markers in multiple populations within India, and they found high levels of heterozygosity and significant variation among the sampled populations. Nazia and Azizah (2014a) identified several microsatellite markers from *C. macrocephalus*. They showed that these markers could characterize levels of genetic variation in populations of this species from They also found that some of the microsatellite markers from *C. macrocephalus* could cross amplify in at least two other *Clarias* species, namely *C. batrachus* and *C. meladerma*, to potential aid in the genetic characterization of these species as well (Nazia and Azizah 2014b). More recently, Wachirachaikarn and Na-Nakorn (2019) used microsatellites to examine the genetic diversity of *C. gariepinus* hatchery stock populations in Thailand. They found evidence for significant genetic differentiation among the sampled populations.

Genome level characterizations

Most of the molecular work described previously relied on the analysis of individual DNA markers. Their markers were chosen, for the most part, on the availability of appropriate primers and amplification parameters for successful PCR rather than their relationship to relevant biological parameters such as fecundity, fertility, longevity, environmental tolerances, etc. There can be little doubt that a better understanding of the genetic basis of these more complex aspects of the phenotype, especially those relevant to aquacultural practices, may

be better achieved through the simultaneous analysis of multiple genes, or better yet, through the analysis of complete mitochondrial or nuclear genomes. The following sections will review work already done in this area.

Mitochondrial genome sequencing. Studies of the “mitogenome,” including the complete characterization of mitochondrial DNA and genes, have been carried out in *C. batrachus* (Kushwaha et al., 2015) in *C. gariepinus* by Han et al. (2015) and in *C. fuscus* by Zhou et al. (2015) and Yang et al. (2016). These studies reported basic features, including the overall mitogenome size, ranging from 16,508 to 16,525 base pairs (depending on the species), A+T content, and the identification of coding, tRNA, and rRNA genes in the D-loop or control region for each species. Chand et al. (2021) directly compared the mitogenomes of *C. batrachus* and *C. gariepinus*. They described variation in 12 of 13 proteins encoded by these genomes that might lead to functional and stability differences.

Studies by Han et al. (2015) and Kushwaha et al. (2015) also compared the mitogenomes of other catfish species using DNA sequences available in public databases. In these comparative studies, Han et al. (2015) found that the mitogenome of *C. gariepinus* was very similar overall to that of the mitogenomes of four other *Clarias* species. Kushwaha et al. (2015) found a few differences in the *C. batrachus* mitogenome compared to a broader range of data from 24 catfish species.

Nuclear genome sequences. To date, complete genome sequence studies have been carried out for *C. batrachus* (Li et al., 2018), *C. magur* (Kushwaha et al., 2021), and *C. macrocephalus* (Duong et al., 2020; Ma et al., 2021)). The work on *C. batrachus* from Li et al. (2018) provided an estimate of the total genome size (~821 Mb), the estimated number of genes (22,914), and the percent of repetitive DNA sequences found in this species (30.3%). The study of *C. magur* from India by Kushwaha (2021) reported annotation of 19,279 genes and a higher percentage of repetitive DNA (43.72%) than that of *C. batrachus*. However, as described previously, the extent to which these species’ designations correctly reflect distinct taxonomic differences is still controversial (Ng and Kottelat, 2008; Khedkar et al., 2014b). These studies used slightly different methodologies for generating the basic datasets, and each used specimens of various origins (Florida vs. India, respectively). For *C. macrocephalus*, the work of Duong et al. (2020) generated a dataset indicating a larger total genome size (883 Mb) and an intermediate number of annotated genes (21,124). The work of Ma et al. (2021) on this same species will be discussed in more detail below.

These studies also aimed to understand better the genes and proteins involved in the evolution of air-breathing and other terrestrial adaptations seen in *C. batrachus* and other catfish. For this purpose, the researchers used methods to directly compare the genome datasets of *Clarias* species to similar datasets derived from non-air-breathing fish species. This was accomplished using comparative genome studies to identify genes that are either unique to one species or another or find common genes that differ at least in expression levels.

Using this approach, Li et al. (2018a) found that *C. batrachus* had 15 copies of the myoglobin (*mb*) gene compared to only 1 or 2 copies of this gene found in other fish genomes. This research group also looked at how genes involved in related processes such as elastic fiber formation, oxygen binding, transport, angiogenesis, ion homeostasis, and acid-base balance differed from non-air-breathing fish. The study of the *C. magur* genome by Kushwaha et al. (2021) identified genes in several categories relevant to environmental and terrestrial adaptation, such as vision, locomotion, olfactory and vomeronasal receptors, thermoregulation, osmoregulation, air-breathing ability, and detoxification. Several of these genes were declared to be either unique or duplicated in the genome of *C. magur* compared to other teleostean species. In work on *C. macrocephalus*, Duong et al. (2020) described two genes, *mb* (as described above) and an olfactory receptor gene (*ora1*), as being associated with adaptations to air-breathing ability and a semi-terrestrial lifestyle.

Ma et al. (2021) reported on a study of both the *C. macrocephalus* genome and transcriptome to identify a gene in this air-breathing catfish that were either not present or

differentially expressed in the genome of the channel catfish, *Ictalurus punctatus*, a species that does not possess a similar air-breathing organ. Their analysis found more than 1,000 genes in the *C. macrocephalus* genome absent in *I. punctatus*. This collection identified three top candidate genes specific for air-breathing adaptation. These included the same myoglobin gene (*mb*) described above, a neuroglobin gene *ngb*, and an alpha hemoglobin gene *hbae*. These genes are all associated with oxygen-carrying, oxygen-binding, and heme-binding type functions.

Transcriptome studies. Another approach to analyses done at the genome level involves focusing on the transcriptome. The transcriptome is derived from RNA transcripts of expressed genes only. One advantage of this approach is that it yields a much smaller dataset than the complete genome. The disadvantage of this approach, however, is that the set of RNA transcripts produced is constantly changing, necessitating the analysis of multiple transcriptomes. However, this can also be turned into an advantage by allowing researchers to focus on even more refined subsets of genes that are expressed in different ways, such as on a sex-specific basis, during specific stages of the life cycle, and/or under specific environmental conditions (among many others).

Several transcriptome studies focusing on genes expressed in specific tissues and times of development have been carried out using material from different *Clarias* species. Most of these have focused on patterns of gene expression in particular tissues. For example, one of the earliest studies of this type was reported using material derived from the spleen of *C. batrachus* by Singh et al. (2013). This material described the identification of several genes involved in immune system functions. Gene expression in the spleen was also considered using material from *C. gariepinus* by Li et al. (2019). This study also described several genes involved in immune system function, especially after challenging fish (by inoculation) with exposure to *Aeromonas veronii*, a pathogen known to cause severe problems in aquaculture environments.

Studies of the transcriptome derived from liver material have been described in *C. macrocephalus* by Chatchaiphan et al. (2017). Chatchaiphan et al. (2017) looked at gene expression profiles in the livers of diploid and triploid catfish. This study found that the vast majority of the genes (> 90%) they considered were expressed at similar levels, despite the ploidy differences. Arinez et al. (2020) looked at liver transcriptome profiles of two isolates of this species from the Philippines to identify possible candidates for reintroduction or the establishment of new aquaculture populations. They found that fish from one of the populations (southern Philippines) exhibited up-regulation of several genes related to growth and potential resistance to environmental stressors, suggesting that these fish would be a better candidate for any reintroduction attempts.

Transcriptome studies have also been done using brain and gonadal tissue from *C. magur* (Agarwal et al., 2020) and gonadal tissue only from *C. fuscus* by Lin et al. (2021). The analysis of the material from *C. magur* (Agarwal et al., 2020) were done to potentially identify unique and differentially expressed genes related to reproductive activity in catfish. They described in detail some genes that were upregulated in expression in testes of males and the role these genes might play in regulating milt release in captive males. In *C. fuscus*, the analysis of the gonadal transcriptome by Lin et al. (2021) revealed several genes involved in the reproductive process, which might also provide further insights into mechanisms of sex determination in these fish.

Characterizations of individual genes

A few individual genes have been cloned and further characterized in the *Clarias* species described here. In *C. gariepinus*, for example, Kanjanaworakul et al. (2016) reported on the cloning and characterization of the myostatin gene, a regulatory gene known to be involved in growth inhibition. Using a partial cDNA sequence derived from this gene, they were able to monitor the level of expression of the myostatin gene at different stages of development in

these fish, with the highest level being detected in larvae at one-month post-hatch. They were also able to show that the DNA sequence of this gene from *C. gariepinus* was largely conserved in other *Clarias* species. This is relevant because, in a non-Clarid catfish, the CRISPR gene editing method has been used to modify the myostatin gene expression (Khalil et al., 2017) to promote increased muscle cell development and overall body weight. Because of the conservation of this gene in *Clarias* species (Kanjaworakul et al., 2016), similar efforts should be feasible here as well. Other genes involved in growth control have also been identified in channel catfish using a genome-wide scanning method, as reported by Li et al. (2018b). This may help identify similar genes in *Clarias* species in future work.

Yadav et al. (2017) published a study on the expression of the RTF2h gene under oxidative stress in *C. magur*. This gene enables DNA repair mechanisms to work during DNA replication in other organisms. These authors wanted to examine possible changes in the expression of this gene in catfish subjected to hypoxia, a condition known to induce DNA damage. They cloned this gene in *C. magur*, and using RT-PCR analysis, they showed elevated expression in the spleen after short-term exposure to hypoxic conditions. After long-term exposure, they found high levels of expression in the spleen, liver, and head kidney but down-regulation of expression in the brain.

Using the technique of identifying expressed sequence tags (ESTs), Singh et al. (2012) created a cDNA library of genes in spleen material from *C. batrachus*. Their goal was to look for immune-related genes that were highly expressed genes in this organ. A total of 65 genes associated with immune functions were identified, most of which were related to stress or chemical responses. A partial cDNA putatively corresponding to a *thrombomodulin* gene, known to be associated with the immune response, was identified for the first time in any teleost fish.

Finally, studies have also looked at other genes in growth and reproduction in *C. gariepinus*. Imron et al. (2020) conducted a selection experiment on growth control in this species. After three generations of selection, they reported seeing increased growth and improvement in several aquaculture-related parameters in farmed *C. gariepinus* in Indonesia. Their selected strain showed a 10-40% improvement in growth compared to existing local strains. This strain also showed correlated improvement for other parameters, including productivity, shorter growing period, and higher survival rates when challenged by *Aeromonas hydrophyla*, a bacterial pathogen known to cause disease problems at the juvenile stage of development. An analysis of the heritability for reproductive traits such as relative fecundity, average egg weight, sperm concentration, and percent live sperm was described by Srimai et al. (2019) for *C. gariepinus*. They found relatively low values for heritability for several traits, suggesting that genetic selection for improving these traits was not likely to be effective.

Chromosomes and karyotypes

Chromosome analysis represents another level of genomic representation, and several studies have reported on the chromosome karyotypes of *Clarias* species. The diploid numbers of chromosomes found in these species range from 50 to 56, and the karyotypes consist mainly of metacentric and submetacentric chromosomes (Baisvar et al., 2021; Maneechot et al., 2016; Nagpure et al., 2000). The nucleolus organizing region (NOR) has been identified within the karyotype on specific chromosomes in different species (Baisvar et al., 2021). This same study also reported the location for the 18s rDNA gene in *C. magur* (also known as *C. batrachus*). A study by Pandey and Lakra (1997) also provided evidence for B or *supernumerary* chromosomes in this species. For *C. macrocephalus*, a preliminary genetic linkage map showing the location of 134 AFLP markers, distributed among 31 linkage groups, was published by Poompuang and Na-Nakorn (2004).

Regarding sex chromosomes and sex determination mechanisms, multiple systems may be found within *Clarias* species. Two studies of *C. batrachus* (Ozouf-Costa et al., 1990; Pandey and Lakra, 1997) have reported that females are the heterogametic sex with a ZZ-ZW type

system of sex determination. In *C. macrocephalus*, however, the possibility of males being the heterogametic sex was described in a study by Nguyen et al. (2021a). Here, evidence was provided for the presence of multiple male-linked loci identified through a genome-wide SNP analysis in this species. Finally, in *C. gariepinus*, SNP markers showing evidence for linkage to both male and female sex phenotypes were found, suggesting that either a ZZ/ZW or an XX/XY system of sex determination may exist in this species (Nguyen et al., 2021b).

Hybrids

The ability to form hybrids between different species within the same genus is generally somewhat unusual in evolution. However, in aquaculture, hybrids are routinely generated and employed, especially with catfish. For example, 70% of all catfish grown for food production are hybrids (Dunham and Elasad 2018). Within the genus *Clarias*, a considerable amount of work has been done evaluating the use of hybrids in aquaculture applications. For example, hybrids between *C. macrocephalus* and *C. gariepinus* have been routinely produced to improve broodstock for aquaculture. In Thailand, these fish now account for over 90% of the annual aquaculture production (Na-Nakorn et al., 2004). Some of these hybrids may have escaped into and colonized natural river systems (Na-Nakorn et al., 2002), or they may have intentionally been transplanted from one region to another as part of aquacultural development (Khedkar et al., 2014b). Hybrids have also been formed using one species from the genus *Clarias* being crossed with species from another genus, such as the genus *Heterobranchus*.

Interspecies hybrids. Interspecific hybrids between *C. macrocephalus* and *C. gariepinus* have been cultured in Thailand for more than 20 years (Na-Nakorn et al., 2004, Koolboon et al., 2014). While these crosses have not always consistently shown the type of positive effects of heterosis seen in some other cases of hybrid formation, the hybrid fish produced do appear to combine the meat quality of *C. macrocephalus* with the high growth rate and environmental tolerances that *C. gariepinus* is known for (Koolboon et al., 2014). Some improvement in growth-related parameters of hybrids was also seen in a study of reciprocal crosses between *C. gariepinus* (an exotic species) and *C. jaensis* (a native species) in Cameroon (Tiogue et al., 2020), and similar results were seen in hybrids between *C. gariepinus* and *C. anuillaris* in Nigeria (Akinwande et al., 2012). Finally, Na-nakorn et al. (2004) also showed that hybrids could be used to induce the formation of triploid fish. These fish are generally sterile, and if the hybrid fish escape from captivity, this could help avoid the possibility of introgression of non-native genes from introduced species into natural populations.

Hybrids between species of different genera. In a few cases, mainly for their potential in aquaculture applications, hybrid catfish have also been produced between species of other genera. For example, crosses have been carried out between *C. gariepinus* and the species *H. bidorsalis* (Olanyi et al., 2017). Reciprocal crosses have also produced viable intergeneric hybrids between *C. gariepinus* and *H. longifili* (Legendre et al., 1992). In these cases, the morphology of the hybrids was intermediate between the two parental strains. However, the hybrid fish also showed some developmental problems, such as the delayed acquisition of sexual maturity and abnormalities in gonadal development. A study of hybrids between the same two species by Olufeagba et al. (2016) reported significantly higher survival of the hybrids compared to either of the pure strains. Still, for parameters related to growth, fish from the parental strains were rated better than the hybrids. Finally, Olanyi et al. (2017) developed and evaluated hybrids between *C. gariepinus* and another *Heterobranchus* species (*H. bidorsalis*). Still, they also found no consistent improvement in the quality of the hybrid fish compared to fish from the parental strains.

Polyploids

Reports of polyploid forms of different organisms, defined as those retaining sets of chromosomes beyond the usual diploid number (triploids, tetraploids, etc.), can be found in

the literature. Polyploids are somewhat well known amongst several lower teleosts, a group that includes the Siluform catfish (Leggatt and Iwama, 2003). And in addition to that, polyploidy is known to occur naturally at relatively rare frequencies in various organisms; for aquaculture applications, methods have been developed to artificially induce changes in ploidy in several fish species (Piferrer et al., 2009).

One reason for the interest in using polyploid fish in aquaculture is that as fish become sexually mature, they usually decrease their body growth rates as they devote energy towards gonadal development. Polyploid fish, however, may avoid these limitations because they are generally sterile. As a result, these animals may show generally increased growth rates and improved organoleptic qualities important to consumers (Chatchaiphan et al., 2016). Sterile animals also potentially provide greater ease of containment and avoidance of potential problems associated with genetic impacts of escaped farm fish (Piferrer et al., 2009).

Polyploids have been produced in a few *Clarias* species, primarily using *C. gariepinus*. Specifically, in *Clarias* species, most of these studies involve using methods to induce triploid forms to study the effect this might have on the growth rate, nutritional enhancement, or tolerance to environmental pollutants (Karami et al., 2016, 2018). In these experiments, polyploids were usually generated events using a cold shock treatment at 4-5°C for 15-40 minutes immediately (within 2 or 3 minutes) after fertilization to disrupt normal chromosome segregation (Na-Nakorn et al., 2004; Karami et al., 2010; Chatchaiphan et al., 2016; Normala et al., 2021). Although 70 to 90% of the hatching embryos treated this way are triploid, cold shock treatments typically reduce the overall hatch rates of these embryos compared to controls (Na-Nakorn et al., 2004; Chatchaiphan et al., 2016). Okomoda et al., (2020) also described electric shock as an alternative method to generate polyploidy in *C. gariepinus*. They reported similar success rates for triploid induction, ranging from a low of 10% to a high of 85%. The higher frequency success rates were achieved using increased electric voltages and longer exposures, but these results were also negatively correlated with hatchability.

Overall, the triploid catfish produced in these experiments have not generally shown meaningful improvements in growth or life cycle parameters compared to diploid fish (Karami et al., 2010; Hassan et al., 2018). However, Chatchaiphan et al. (2016) did show with *C. macrocephalus* showed some differences in triploid performance depending on the strain of diploid parents.

Environmental tolerances

A better understanding of environmental tolerances in both natural and artificial situations may also be critical for properly managing *Clarias* catfish in aquaculture. For aquaculture applications, the effects of stocking density on various aspects of the health and welfare of juvenile specimens of *C. gariepinus* were described by van de Nieuwegiessen et al. (2008 and references therein). In general, they found that stocking densities between 1125 and 2375 fish/m³ were optimal for balancing productivity with the desire to avoid chronic stress on the fish. A more recent study by Wenzel et al. (2021) assessed the impact on growth parameters of juvenile *C. gariepinus* under four different levels of potassium (K) concentrations that may be used to adjust the water pH in recirculating aquaculture systems. They found that the juvenile fish did best under K concentrations ranging between 200 and 400 mg per liter.

In terms of other types of chemical tolerance, *Clarias* catfish have been used to test the effects of exposure to several pollutants known to be found in natural river systems. Using *C. gariepinus*, for example, Ogbeide et al. (2019) found significant impacts on gill morphology and liver damage in collections of these catfish from a pesticide-impacted river. Other studies have used hybrid and polyploid *Clarias* catfish as test subjects in laboratory experiments, such as using hybrids from crosses of *C. gariepinus* to *C. macrocephalus* to test the possible effects of exposure to chemicals such as chlorpyrifos and carbaryl (Somnuek et al., 2009). These researchers found that exposure to chlorpyrifos produced more than a 12-fold increase in the expression of acetylcholinesterase, an enzyme involved in nerve cell transmission, in these

fish. However, carbaryl exposure did not significantly impact the expression of this same gene in the catfish. Karami et al. (2016) compared the effects of phenanthrene exposure to juvenile diploid and triploid *C. gariepinus* catfish. They found that after a relatively short exposure (96h) to this compound, significant increases in the expression level of several genes were noted. These included *ftz-f1*, a gene known to play a critical role in early development, along with the expression of the gene encoding the enzyme tryptophan hydroxylase. Elevated expression of two other genes, alkaline phosphatase and lactate dehydrogenase, was also noted.

Conclusions

The rich resource that catfish species in the genus *Clarias* provide for aquaculture in several countries worldwide has led to a sizable accumulation of information about the biology of these species. Many applications in aquaculture have also taken advantage of some less conventional aspects of the biology of these catfish, such as the apparent ease of forming hybrids between different species and the ability to generate polyploid forms containing multiple sets of chromosomes. Genome-level studies have also made significant progress in identifying essential genes underlying other novel aspects of catfish biology, including the ability to move and breathe air in terrestrial environments. There can, however, be little doubt that there is still much more to be learned about these fascinating species and that their contributions as valuable resources in aquaculture will continue to grow.

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