Review

Advances in Reproductive Endocrinology and Neuroendocrine Research Using Catfish Models

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Abstract: Catfishes, belonging to the order siluriformes, represent one of the largest groups of freshwater fishes with more than 4000 species and almost 12% teleostean population. Due to their worldwide distribution and diversity, catfishes are interesting models for ecologists and evolutionary biologists. Incidentally, catfish emerged as an excellent animal model for aquaculture research because of economic importance, availability, disease resistance, adaptability to artificial spawning, handling, culture, high fecundity, hatchability, hypoxia tolerance and their ability to acclimate to laboratory conditions. Reproductive system in catfish is orchestrated by complex network of nervous, endocrine system and environmental factors during gonadal growth as well as recrudescence. Lot of new information on the molecular mechanism of gonadal development have been obtained over several decades which are evident from significant number of scientific publications pertaining to reproductive biology and neuroendocrine research in catfish. This review aims to synthesize key findings and compile highly relevant aspects on how catfish can offer insight into fundamental mechanisms of all the areas of reproduction and its neuroendocrine regulation, from gametogenesis to spawning including seasonal reproductive cycle. In addition, the state-of-knowledge surrounding gonadal development and neuroendocrine control of gonadal sex differentiation in catfish are comprehensively summarized in comparison with other fish models.

Keywords: catfish; sex differentiation; gonadal development; gametogenesis; neuroendocrine regulation

1. Introduction

Catfish (order Siluriformes) are diverse groups of ray-finned fish that are mostly benthic or bottom dwellers [1] and are named so for their prominent barbells that resembles a cat’s whiskers. They represent one of the largest groups of freshwater fishes. They are scaleless and are defined by features of the skull, spine in front of their fins and swim bladder. Catfish have widely been caught and farmed for food, due to high protein content, for hundreds of years across many continents. In addition, some species are also reared as ornamental fish or research animals due to more adaptability for artificial spawning and culture. Several air breathing catfish (family- Claridae) consisting of about 48 species [2] together with Heteropneustidae and shark catfish (Pangasiidae) species are widely cultured in the Asia and the Africa due to relatively higher fecundity, high tolerance to hypoxia, etc. Some of the other widely cultured species includes channel catfish, Ictalurus punctatus and blue catfish, I. furcatus. Additionally, genus Cryptopterus contains various small and transparent catfishes described as glass catfish [3].

Catfishes also undergo a seasonal reproductive cycle characterized by distinct stages [preparatory, pre-spawning, spawning, post-spawning and resting] in subtropical countries including India controlled by a hormone regulatory pathway primarily involving gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicular stimu-
lating hormone (FSH), growth hormone, melatonin, and sex steroid hormones [4,5]. Thereby, a gonadotropin (GTH) surge usually facilitates spontaneous oocytes maturation, ovulation or spermiation in nature. However, catfish usually do not spawn or spermiates in laboratory or culture conditions [6–8]. Apart from these, neuroendocrine factors such as, neurotransmitters and neuropeptides, also play a crucial role in neuroendocrine control of gonadal development and maturation [9]. Testosterone (T) and 17β-estradiol (E2) exert a primary role in gonadal development locally, by several positive and negative feedback actions at the levels of brain and pituitary across endocrine axis [10]. Evidently, spawning strategies for catfish can be divided into two main categories: natural and artificial spawning wherein artificial spawning is performed by inducing females to ovulate with hormones, followed by which eggs are hand-stripped and fertilized in vitro.

As endocrine system regulates gonadal development, growth, and reproduction, hence, fish endocrinology has been the focus of various studies for basic understanding of these physiological events and for advances in aquaculture. Over the decades, any fish species have been used to study various aspects of endocrinology in vivo. Several genome editing and transgenesis studies have also been done to understand the complexity of endocrine functions and regulation in fish. This review summarizes the present knowledge and key evidence on catfish being used as research models for studying fish endocrinology. To begin with, key evidence of neuroendocrine control of gonadal development and sex determination/differentiation are discussed followed by understanding of steroidogenic regulation in catfish. Key findings on how catfish models have been used to understand gene regulation and function using gene knock out/transient gene knock down through short interfering RNA (siRNA) are listed. Furthermore, wherever necessary the research findings from catfish models were compared with other teleostean counterparts for comprehensive review of literature.

2. Neuroendocrine Regulation-GnRHs

Teleost fish are excellent models to study neuroendocrine control of reproduction. Fibres synthesize LH and FSH from anterior pituitary under the control of hypothalamus GnRHs to regulate early gametogenesis, steroidogenesis and ovulation/spermiation. Hence, puberty is governed by GnRH and certain gonadal steroids. GnRH release is controlled by several neurotransmitters and neuropeptides. Multiple forms of GnRH have been identified and localized in brains of most of the non-mammalian vertebrates, including, fish [11–13]. In the African catfish, C. gariepinus, two genomic isoforms of GnRH have been characterized till date [11] along with two forms of GnRH receptors with varied tissue distribution but no differences in ligand selectivity [14]. The first teleostean GnRH receptor was isolated from the African catfish [15]. Since the discovery of GnRH in vertebrates over three decades, considerable progress has been made towards understanding of the neuroendocrine control of gonadal development and reproduction in mammals and fish which has been reviewed extensively by Zohar et al. [16]. Molecular cloning/characterization of GnRH2 precursor cDNA and its regulation by ovarian steroids were demonstrated in the stinging catfish, Heteropneustes fossilis [17]. Furthermore, the stimulatory and inhibitory interactions between GnRH- neuropeptides, including neuropeptide Y (NPY) and GnRH- neurotransmitters, including DA and γ-aminobutyric acid (GABA) reviewed and demonstrated well by Trudeau [10] using goldfish model. The effects of 5-hydroxytryptamine (5-HT), GABA and NPY on in vitro release of GnRH have been well demonstrated in a perciform fish [18]. In addition to this, the functional significance of GnRH–kisspeptin (a neuropeptide encoded by the kiss gene, the “gatekeeper” of puberty) in teleostean reproduction and their associated receptors have been reviewed by Gopurappilly et al. [19] including various catfish models. After identification of kiss2 and GnRH2 in the stinging catfish, H. fossilis, [17,20], a recent study demonstrated that kiss2-GnRH2 signaling is involved in photo-thermal-mediated mechanisms controlling reproduction in the catfish [21]. Evolution of kiss functions in teleost along
with the common regulatory mechanism of hypothalamo-hypophyseal gonadal ((HHG) axis has been also reviewed by Kanda [22]. Taken together, these complex systems stimulate gametogenesis and sexual behaviors through the activation of HHG axis in teleosts including catfish.

In addition to HHG axis, endocrine feedback system at the thyroid axis also contributes to homeostasis maintenance, growth, differentiation, and reproduction in teleost including catfish [23,24]. Hence, thyroid hormone (TH) also plays a critical role in brain development/function. THs are also known to modulate reproductive system during different developmental stages in fish [25] and several catfish models have been extensively used over the decades to decode the underlying mechanisms of endocrine control of reproduction and to identify various markers associated functionally across the endocrine axes.

2.1. GTH Duality

GTH, a glycoprotein hormone, stimulates gonadal maturation and development in most of the vertebrates. In many teleosts, including salmonids and rainbow trout, two types of GTHs, GTH-I (FSH-like) and GTH-II (LH-like) have been characterized [26–30] which are equipotent in stimulating E₂ production hence, stimulating steroid synthesis although localized in separate cells. However, primitive teleost such as eel [31,32] and catfish [33–35], only a single GTH (GTH-II) has been characterized which is known to regulate the entire process of gonadal development. The possibilities implicating about the absence of FSH-like GTH-I in catfish has been attributed by Joy [36]. The African catfish FSH-R responded clearly to the highly purified African catfish LH when expressed in a mammalian cell line [37] and the channel catfish FSH-R responded to human chorionic gonadotropin (hCG) although the response was weaker than when challenged with human FSH [38,39].

Furthermore, GnRH’s role in the stimulation of LH synthesis in catfish has been reviewed by Schulz et al. [40]. In line with this, it has been reported that the pituitary gonadotrophs are known to be activated strongly during initiation of spermatogenesis in the African catfish, Clarias gariepinus [41].

In addition, seasonal cyclicity of GTH-II has been demonstrated in various catfish species with standardized protocols as well as comparison with nuclear E₂ receptor binding [42,43]. However, since there is no distinction of GTH-I and GTH-II, it is referred as GTH-II or LH in these catfish species.

2.2. Neurotransmitters, Neuropeptides and GnRH–GTH Axis

Neurotransmitters such as, catecholamines (CA)-dopamine (DA), norepinephrine (NE), adrenaline (A) and serotonin are low molecular weight organic nitrogen compounds. In terms of synthesis, packaging, release, and degradation, the amine neurotransmitters fall somewhere between the properties of other small-molecule neurotransmitters and those of the neuropeptides. Neurotransmitters such as monoamines, amino acids and peptides are known to involve in the neuroendocrine control of reproduction.

2.2.1. Serotonin

Serotonergic system plays a critical role in orchestrating HHG axis to promote gonadal growth in vertebrates including fish. Enzyme, tryptophan hydroxylase (tph), is a crucial rate-limiting enzyme for serotonin synthesis. Selective up regulation of tph expression and serotonin levels in brain has been shown in XY male tilapia and abolition of such a phenomenon leads to complete sex reversal during early development [44] which was evident by para-chlorophenylalanine (pCPA) (a tph blocker) treatment [45,46]. Such a phenomenon was also well demonstrated in catfish with gender differences where in pCPA skewed the population towards females by initiating ovarian differentiation [47].
A single injection of pCPA decreased the content and activity of serotonin in *Channa punctatus* [48]. Similarly, pCPA reduced hypothalamic serotonin level and impaired GnRH and LH secretion in the Atlantic croaker [49]. Furthermore, in fishes, serotonergic system can be modulated by a variety of chemical substances and environmental factors. For example, diurnal variations in the serotonin content and turnover in response to melatonin have been demonstrated in *C. punctatus* [50] and in *H. fossilis* [51,52]. In teleost, serotonin receptors have been identified and characterized in several species in peripheral as well as gonadal tissues, as reviewed by Prasad et al.[53]. Furthermore, high hypothalamic monoamine oxidase (MAO) activity with a relatively high turnover of serotonin has been observed during recrudescence in catfish, relating to high temperature and breeding activity [54,55]. In addition, the involvement of serotonin and MAO has been well demonstrated in feedback regulation of E$_2$ in catfish [52,56–58]. The half-life analysis and turnover of MAO (using pargyline) were conducted to reveal its involvement in E$_2$-modulated feedback regulation of GnRH-GTH axis [58]. Ovariectomy-induced changes in plasma levels of GTH partly mediated by MAO activity and E$_2$ feedback action on serotonin metabolism were also observed in a seasonal-dependent manner [56–58]. The role of serotonin in fish reproduction including studies in catfish except a few recent reports [59,60] has been extensively reviewed by Prasad et al. [53].

2.2.2. CAs

CA, an important component of monoaminergic system in the hypothalamus, modulates the levels of GnRH with subsequent release GTHs in teleosts including catfish [57,61,62]. The CAs include L-DOPA, DA and NA, all of which plays decisive roles in various physiological processes to control reproduction. In the African catfish, dopamine acts as an endogenous inhibitor of GnRH-stimulated GTH release during spermatogenesis and vitellogenesis [63,64].

Among the CAs, DA exerts an inhibitory control on GTH while NA stimulates GTH by regulating GnRH synthesis in teleost [65,66]. Additionally, negative feedback by sex steroids also involves in activation of inhibitory DA system [10]. In the Indian stinging catfish, high temperature decreases DA activity and increases NA activity, which is a stimulatory signal for GTH-II [57]. Mamba and Senthilkumaran [67] demonstrated gfra-1 plausibly entrains GnRH-GTH either directly or indirectly, by partially targeting CA-ergic activity. In addition, another study in catfish demonstrated catecholamines (CE) related enzymatic changes in during GnRH analogue-induced ovulation and suggesting E$_2$ modulation of catechol-O-methyltransferase (COMT) activity [68]. Ovariectomy and/or E$_2$ replacement also modulated hypothalamic COMT activity in catfish. In addition, season-specific changes in hypothalamic COMT demonstrated indicating its involvement in CA/CE mediated control of GTH [69]. Enzyme tyrosine hydroxylase (th) regulates the levels of GnRH in brain and GTHs in the pituitary. In *H. fossilis* brain, th activity and its correlation with the annual reproductive cycle [70] is well demonstrated and is known to be modulated by cyclic AMP-protein kinase A and protein kinase C [71]. Furthermore, sex-specific differential expression of th was observed in early developmental stages in male and female catfish brain that correlating with CAs [62]. Furthermore, a study in the Indian catfish demonstrated sexual dimorphism in the th-positive neurons in the preoptic area of the brain [72]. In some air breathing catfish species, coexisting in sub-tropical waters, there is seasonality in the dominance of the CA during the reproductive cycle wherein DA content and turnover were found to be high during the resting phase and decreased as breeding season progressed with a concomitant increase in NE turnover [57] unlike goldfish wherein the DA inhibitory tone is high. The turnover studies were explicitly performed using α-MPT to depict content and turnover of CA in catfish. Furthermore, NE was high in pre-spawning phase and A was high in spawning phase but not in resting phase. In line with this, administration of a single high dose of GnRH analogue facilitated induced spawning and the periovulatory changes of monoaminergic system has been well demonstrated for the first time in catfish. Furthermore,
precise action of CA on GTH- release has been well studied using specific blockers/precursors in ovariectomized catfish [57,66,69]. Overall, photoperiod, temperature, and E2-negative feedback act on CA to regulate GTH secretion.

2.2.3. GABA

GABA is an important amino acid neurotransmitter. Studies in teleost, including goldfish, rainbow trout and catfish, had confirmed the presence of the metabolic enzymes of GABA in fish brain [73–76]. A pioneering investigation partially characterized the GABA receptor [77] followed by the demonstration of an uptake system in the brain of channel catfish [78]. In teleosts, including the Indian catfish, GABA is known to stimulate GTH-II release during puberty (independent of the DA system) and its distribution in catfish forebrain showed seasonal variation which could be altered negatively upon ovariectomy and restored upon E2 replacement [65,79]. A recent study in catfish demonstrated the role of laser puncture exposure on gonad maturation by examining GABA release in the brain [80].

2.2.4. Neuropeptide Y

NPY, a 36 amino-acid neuropeptide, is involved in various physiological and homeostatic processes including stimulation of appetite. NPY has been identified and demonstrated in several fish species including the I. punctatus, C. batrachus and C. gariepinus [81–85]. Increase in NPY during fasting is consistent with results in mammals [86] and fish models, including channel catfish [87]. Significance of NPY in the regulation of GnRH–LH axis was demonstrated by Subhedar et al. [88] using C. batrachus, also known as C. magur. Involvement of NPY and NPYY1 receptors was evident in regulation of GnRH–LH complex and GH cells in catfish pituitary [82,83]. However, all these studies showed localization pattern of NPY using heterologous antisera. It is important to use homologous system to delineate the localization pattern precisely. In line with this, Sudhakumari et al. [85] precisely localized NPY transcript and protein in the preoptic area of the brain in C. gariepinus using homologous system. In addition, the authors demonstrated higher expression of NPY in the brain during pre-spawning phase as compared to other reproductive phases. Transient silencing of NPY-esiRNA (directly into the brain) decreased the expression of tph2, cfGnRH, th, hsd3b in brain and LH-b/GTH-II in pituitary in addition to several ovary-related transcripts indicating NPY’s role in ovarian development through GnRH-GTH axis. Thus, the authors established possible interaction of NPY with GnRH-GTH axis.

2.3. Brain Sex Differentiation/Dimorphism

Studies on pubertal development have been conducted in various fish species including catfish [35,89,90] suggesting that sex steroids regulate the development of the HHG axis in teleost. Furthermore, its correlation with testicular function has been reviewed by Blázquez and Trudeau [91]. Gonadectomy during later stages of gonadal recrudescence increases LH secretion in several teleost including the African catfish and the Indian catfish which can be restored by treatment with testosterone/E2 [66,92–95]. Ovarian aromatase, cyp19a1a, is known to be involved in conversion of androgens to estrogens and is also known for its role in sex reversal [96]. However, teleost also produce brain aromatase, encoded by the cyp19a1lb and synthesize high amounts of neuroestrogens [97] plausibly along with the action of its related transcription factors such as ftzf1 and foxl2 [98] as seen in catfish, leading to “Brain sex differentiation”. In teleost, most of the earlier reports tend to suggest that gonadal sex differentiation drives brain sex differentiation which has been reviewed extensively by Senthilkumaran et al. [99]. Nevertheless, the influence of brain serotonergic system on gonadal sex development in catfish is well demonstrated indicating the existence of “Brain sex differentiation” in teleosts in-
cluding catfish. However, yet the brain sex changes are questioned as a “consequence” or “cause” to gonadal sex determination/differentiation [44,47,89].

Additionally, teleost models including catfish have been used extensively to study neurotoxicity [100] and neuroendocrine disruption [101]. Neurotoxicity studies are important to identify promising neuroprotective agents for example, ascorbic acid for Al-induced neurotoxicity which was demonstrated using C. gariepinus [102]. In line with this, Mamta and Senthilkumaran [67] used 1-methyl-1,2,3,6-tetrahydropyridine (MPTP), to demonstrate the interaction of GDNF and DA-ergic system in catfish brain. In addition, controlled release of sex steroids using osmotic pump altered brain GnRH1 and CA-ergic system dimorphically in the African catfish providing insights into the reproductive toxicity of sex steroid analogues during gonadal recrudescence [103]. The schematic representation on neuroendocrine control of reproduction in catfish has been depicted in the Figure 1.

Figure 1. Schematic representation of neuroendocrine control of reproduction in catfish.
3. Gonadogenesis

Gonad, in most fish species including catfishes, has bipotential fates to form ovary or testis depending upon a sex determination/differentiation cue [96,104,105] various factors after which gonadal differentiation and further development of gonad takes place. Some hermaphrodite fishes can change their sex uni-directionally or bi-directionally during their life cycle however, catfishes show gonochoristic pattern. Sex differentiation in fish is characterized by differential expression of related genes [106–109]. However, environmental cues, such as, temperature also plays a crucial role in sex differentiation in a few fish species including C. gariepinus [110] and I. punctatus [111]. Environmental sex determination in fish has been reviewed by Baroller et al. [112].

3.1. Sex Determination/Differentiation, Gonadal Development and Growth

In mammals, the discovery of sex determining region Y, SRY gene, demonstrated its crucial role in testicular development [113,114]. However, the same has not been identified in fish except for a study involving identification of Y-chromosome specific molecular markers in a cyprinid fish using sry-specific PCR primers [115]. In fish, dmy or dmrt1b (duplicate copy of dmrt1) was found to be master sex determination gene, which was identified in the Japanese medaka, Oryzias latipes [116,117] as well as in O. curvinotus [118]. Following which, dmrt1 have also been identified as testis-related gene in Cynoglossus semilaevis [119] and with multiple forms in catfish [120]. Thereafter, several studies were performed in various fish species including catfish to indentify crucial sex determination/differentiation genes [121] wherein several candidate genes for sex determination/differentiation were elucidated, for example, amhy in the Nile tilapia, Oreochromis niloticus [122], the Patagonian pejerrey, Odontesthes hatcheri [123] and O. bonariensis [124]; amhr2 in Takifugu rubripes [125]; sdY in the rainbow trout, Oncorhynchus mykiss [126]; gsdf and sox3 in O. luzonensis and O. dancaen [127,128]. The cellular, molecular and physiological aspects of sex determination/differentiation in teleost have been reviewed by Sandra and Norma [129]. Furthermore, epigenetic characterization of sex chromosomes were examined in two species of bullhead catfish (Amblycipitidae), Liobagrus marginatus and L. styani [130]. The genetic and epigenetic processes involved in regulation of sex-change in fish have been well reviewed by Ortega-Recalde et al. [131]. Additionally, sex determination/differentiation and feminization in the Southern catfish, S. meridionalis [132] and the channel catfish, I. punctatus [133] has been reviewed wherein gsdf and cxcl12 plausibly initiated testicular differentiation as demonstrated in channel catfish. The genetic basis of sex determination/differentiation in fishes has been reviewed by Nagahama [134]. Several genes involved in sex determination/differentiation, such as dmrt1, sox9, foxl2, bcar1 [135], and cyp19 in catfish have been also identified. In addition to this, another study catfish revealed the role of ckit in germ cell proliferation, development, and maturation during gonadal recrudescence [136]. Dimorphic expression of various transcription factors and steroidogenic enzyme genes has been demonstrated by Raghuveer et al. [47] during critical period of gonadal differentiation in catfish. Detailed analysis of various genes involved in sex differentiation in catfish has been reviewed [137].

3.2. Gonadal Recrudescence and Sex Reversal

Most of the fishes exhibit seasonal cycle in reproduction in the subtropical and tropical countries. The release of gametes from the body into the surrounding water is called spawning in fishes. Some fishes are daily breeders (such as zebrafish) and some spawn during a specific season/period (seasonal/annual breeders like catfish) due to several environmental cues. During the breeding season of the species, the gonads attain full maturity followed by spawning takes place. Gonadal recrudescence occurs after spawning subsequently to entrain seasonal/reproductive cycle. The breeding season and hence the spawning period is extremely variable among the bony fishes. Some seasonal breeders spawn only once (catfishes), others twice (common carp), while still others may
spawn several times during a year. Catfishes, generally, spawn upon annually during monsoon in the subtropical countries. Additionally, bony fishes can reverse their sex according to various environmental/social cues during their lifetime [96,138,139], however, it varies from species to species. Concepts and mechanisms involving sexual plasticity and gametogenesis in fishes including catfish has been covered extensively in “Sexual Plasticity and Gametogenesis in Fishes” by Senthilkumaran [140] and co-authors. Despite these, clear information about gonadal differentiation in sex-changing fishes remains limited. In catfish, female-to-male sex reversal has been achieved by fadrozole (aromatase inhibitor) and tamoxifen (estrogen receptor antagonist) treatment [141–143] as well as with pulsatile treatment of methyltestosterone (MT) and ethynyl estradiol (EE_2) was demonstrated by Raghuveer and Senthilkumaran [120]. Furthermore, functional feminization of the channel catfish, _I. punctatus_, was demonstrated through the treatment of estrogen diet [142,143]. Hence, estrogens, in teleost, are responsible for ovarian differentiation and feminization although the detailed mechanism involved remains elusive. However, potential androgens like 11-ketotestosterone (11-KT), MT and even non-aromatizable androgen [144] also produced female dominant populations in blue catfish and channel catfishes suggesting that no hormonal treatment could direct masculine sex determination [143,145]. Incidentally, treatment of MT occasionally resulted in intersex in catfish [120]. Hormonal induction of sex-reversal in fish including catfish has been extensively reviewed by Pandian and Kirankumar [146].

4. Gamete Maturation

Gonadal maturation is a critical event wherein gonads undergo cyclic morphological and physiological changes to produce functional gametes during the spawning phase with the help of several gene/factors and hormones. Artificial induction is used to advance the maturation of gonad in seasonal breeders (like catfish and eel) during the off-breeding season. This was first time demonstrated by Miura et al. [147] using the Japanese eel wherein, hCG injection could induce spermatogenesis. As hCG shares the same receptor as the LH, studies were carried out to use hCG or ovaprim to advance gonadal development/maturation in teleost instead of GnRH analogues [148–150]. All these techniques have been adopted from the first discovery of ‘LinPe’ technique for induced breeding in fishes. This has been well established in several catfish [62,151]. In fact, controlled release of hCG via osmotic pump resulted in off-season breeding in catfish [150].

4.1. Final Oocyte Maturation

Final oocyte maturation (FOM), in fishes, is promoted by the maturation inducing steroid, 17α,20β-dihydroxy-4-pregn-3-one (17α,20β-DP) which is produced in ovarian granulosa cells by _hsb20b_, a key enzyme that initiates maturational events [152]. Furthermore, in teleost, shift in steroidogenesis from E_2 to 17α, 20β-DP seems to be a crucial step during oocyte maturation [153,154]. Eventually, promoter motif analysis of _hsb20b_ in catfish and rainbow trout demonstrated that _hsb20b_ type B of rainbow trout had no promoter activity while _hsb20b_ type A of rainbow trout and catfish _hsb20b_ promoters showed basal promoter activity, wherein, cAMP responsive elements were the key regulators along with _crebs_ [155] which was also indentified in the promoter motif of _cyp19a1a_ [156,157]. Additionally, _cyp19a1a_ expression is also crucial to understand the molecular mechanisms that precede ovarian differentiation/development. In vertebrates including teleost, _foxl2_ is one of the earliest markers of ovarian differentiation. Furthermore, it was evident in teleost including catfish that, _add4bpf/sf-1, foxl2_ and _ptz-f1_ regulated _cyp19a1a1b_ expression directly or indirectly in various fish species including catfish [97,156,158–163]. Furthermore, CAMP regulated _hsb20b_ up-regulation in catfish [155]. A single form of _creb_ was identified and characterized in _C. gariepinus_ during FOM unlike multiple forms in the Nile tilapia, _O. niloticus_ [164]. In this line, studies in common carp, suggested plausible roles for _ptx_ and _thoc3_ in ovarian growth, maturation/recurdence
upon functional analysis [165,166]. However, such an observation is yet to be investigated in any catfish species. Transcriptional interaction of Pax2 on wnt5 also attributed to ovarian development in catfish explicitly [167] indicating multiple regulatory factors involved in gonadal function. Another report compared oocyte maturation of teleost with mammals to explicitly describe the phenomenon [168]. In fact, several of these studies in catfish were well complemented with enzyme activity assays to substantiate gene expression analysis authenticating downstream action [169].

Variety of hormones/metabolites/neurotransmitters showed oocytes maturation effects in addition to maturation-inducing hormone (MIH) in catfish species. This included cortisol, vasotocin (VT), CEs and CAs [61,170–178]. Both GTH and ovarian steroids modulate VT levels in catfish to influence follicular growth, ovulation, and spawning [174,175,179]. Incidentally, serotonin also induces oocyte maturation in fish and mollusks [180–185] which is yet to be explored in any catfish species. Despite these findings, MIH remains to be 17α,20β-DP in catfish too like some teleosts [176]. Catfish do not spawn in captivity without induction that may perhaps explain presence of various oocyte maturation inducing agents in vivo.

4.2. Sperm Maturation

In the African catfish, testicular development includes four stages that are distinguished by the presence of spermatogonia alone; spermatogonia and spermatocytes; spermatogonia, spermatocytes and spermatids; and finally, all germ cell stages, including spermatozoa [35,89]. In fish, GTHs show prominent steroidogenic potency at the onset of spermatogenesis and during rapid testicular growth and its receptor has been localized in testicular tissue and in also in the milt of channel catfish, or in the seminal vesicles of the African catfish [37–39]. Maturation-inducing steroids such as 17α,20β-DP have been implicated in sperm maturation of teleosts to some extent including catfish [140,186]. Moreover, steroids T and 11-KT (a potent androgen in fishes) are responsible for sperm maturation and testicular development [186]. As described in the previous section, dmrt1 along with other factors are known to be the molecular players in testicular differentiation and gamete maturation. In addition, several findings suggested that w1l, add1bp/sf-1, nr2c1, gata4, sox3, sox9, sycp3 and pfpdz1 have a potential role in the testicular development, maintenance, and recrudescence in catfish by favoring spermatogenesis [187–191]. However, studies on transcriptional networks between nr2c1 and other factors are necessary to demonstrate their interaction during testicular development and spermatogenesis.

5. Steroidogenic Enzyme Gene Regulation, Transcription Factors and Co-Modulators

Several genes/factors have been identified in teleost implicating their crucial roles in gametogenesis and gonadal steroidogenesis and most of which are regulated directly/indirectly by pituitary GTHs [137]. Steroidogenesis starts with rate-limiting transport of cholesterol into mitochondria [192] mediated by steroidogenic acute regulatory protein (StAR). StAR gene has been identified and characterized in teleosts, including rainbow trout, the African catfish and medaka [193–195]. Enzyme, cyp11a1, is involved in the conversion of cholesterol to pregnenolone, which thereby initiating the whole process of steroidogenesis including production of active steroids like 17α,20β-DP, T, 11-KT and E2[168,196] via action of several steroidogenic enzymes genes which has been well identified and characterized in many teleosts including catfish and their associated transcription factors as evident from promoter motif analysis of the steroidogenic enzymes which has been reviewed in detail by Rajakumar and Senthilkumaran [169].

In addition to these, over a decade, next generation sequencing (NGS) techniques has been widely utilized for the identification of sex-related candidate genes and genetic markers using catfish models including red tail catfish [197]; the Hong Kong catfish [198]; amur catfish [199]; channel catfish [200,201]; yellow catfish [202–205] and the Indian and the African catfish [unpublished data] by investigating gonadal transcriptome. These
studies have provided a valuable genomic resource for further investigating the genetic basis of sex determination/differentiation and would aid in understanding more about sex-controlled breeding in catfish with a scope to extend this information to other teleost species.

6. Gene Knockout/Knockdown/siRNA Based Transient Gene Silencing

In the last few decades, there have been major advances in the field of gene/protein expression analysis to delineate their function in the organism. Many of the expression analysis techniques have been standardized in teleost including quantitative PCR, western blot, northern blotting, reporter assays, and high-throughput techniques like RNAseq and microarrays together with localization techniques such as in situ hybridization for mRNA and, immunohistochemistry/cytochemistry and immunofluorescence for protein.

However, in recent years, the field of reverse genetics has been evolving widely with the development of novel genome editing technologies, such as RNA interference (RNAi), zinc finger nucleases (ZFN) and plasmids, morpholinos, TALEN and CRISPR/Cas9 for functional analysis including targeted gene knockdown and knockout in various species including zebrafish, tilapia, and catfish [206–218]. Morpholinos, on the other hand, provide better specificity than RNAi (siRNA/shRNA/esiRNA) by decreasing the possibility of catastrophic off-target antisense effects [219], and has been widely used for studies in zebrafish and goldfish [217,218]. However, use of these technologies in catfish model has not been explored due to year long duration for development to maturation. Nevertheless, future studies need to be performed on this line to obtain novel information. In many animal models including catfish, RNA knockdown can be achieved more feasibly using siRNA, shRNA or esiRNA. In catfish, in vivo and in vitro transient gene silencing using PEI mediated siRNA/shRNA/esiRNA has been standardized and well established at tissue and cellular levels in gonads and brain as well as at animal level in our laboratory using various fish models including catfish [67,85,136,165,188,220,221] to functionally characterize many important factors related to teleostean reproduction. In addition, Senthilkumaran [168] compared mammalian and piscine oocyte maturation with a note on sperm maturation citing the involvement of hsd20b vis-à-vis 17α,20β-DP in addition to T and 11-KT [140,222]. In line with these, more detailed knock-down analysis can be performed. Orchestration of various genes during different stages of gametogenesis/gonadogenesis of catfish has been schematically represented in the Figure 2.
7. Future Perspectives

Sex determining genes are the master switches controlling the sex determination/differentiation in vertebrates including fishes. Catfishes have been used for decades now, to identify and characterize crucial genes and factors in reproduction and neuro-endocrine control of reproduction. Important findings from such studies have been summarized in the Table 1.

Table 1. Studies in catfish species: Identification of crucial genes/factors in reproduction and its neuroendocrine regulation.

| Catfish species | Nature of study | Markers (genes/factors/hormones) studied | Highlights | References |
|-----------------|-----------------|------------------------------------------|------------|-----------|
| C. magur (C. batrachus) | Neuroendocrine regulation | th | Female specific high expression of th in brain during early development. | [62] |
| | | th | Sexual dimorphism in the hypophysiotropic th-positive neurons in the preoptic area associated with LH cells. | [72] |
| | Neuroendocrine-reproductive axis | GTH-II | Development of a heterologous radioimmunoassay for GTH-II and indication of a dynamic positive/negative feedback relationship between gonadal steroids and GTH-II. | [42] |
| | | MAO | Estimation of MAO activity in gonads during different reproductive phases with a sudden decline after spawning. | [54] |
| | | COMT | Changes in ovarian OE, OE-2-H and COMT depicts stimulation of CE synthesis and degradation during GnRH-induced ovulation. | [68] |
| | | NPY | NPY receptors are involved in the secretagogue effects of NPY on LH and GH cells in the pituitary similar to mammalian Y1 receptors. | [82] |
| | Promoter motif | sox3, hsd11b | Sox3 binds to hsd11b promoter and transactivates to | [191] |
| Analysis                                                                 | Gene(s)          | Description                                                                                                                                                                                                 | Reference(s) |
|------------------------------------------------------------------------|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| Reproductive endocrinology                                             | cyp11a1          | Exposure of MT and EE during testicular development showed lower cyp11a1 levels in the testis and brain indicating a certain feedback intervention.                                                        | [196]        |
|                                                                         | nr2c1            | Expression during pre-spawning phase and localization of nr2c1 transcripts in sperm/spermatids.                                                                                                | [187]        |
| Transient gene silencing                                              | wt1, ad4bp/sf-1, gata4 | Transient silencing of wt1-esiRNA downregulated ad4bp/sf-1 and gata4 expression, along with steroidogenic enzyme genes related to androgen production.                                                       | [188]        |
| Transient gene silencing, promoter motif analysis                      | pax2, wnt4, wnt5 | Synchronous expression of pax2 and wnt5 during the ovarian development and recrudescence. pax2 siRNA treatment reduced the expression of ovarian development like signaling molecules– wnt4/5. Transcriptional interaction of Pax2 on wnt5. | [167, 220]   |
| Neuroendocrine regulation                                             | cGnRH-II, cfGnRH | cGnRH-II is the more potent GTH-II secretagogue than cfGnRH.                                                                                                                                              | [11]         |
|                                                                         | cfGnRH-R1, cfGnRH-R2 | cfGnRH-R1 showed higher affinity than cfGnRH-R2 for cGnRH-II, cfGnRH.                                                                                                                                | [14]         |
|                                                                         | DA, GnRH, GTH, LH-RHa | DA inhibited GnRH- induced GTH release.                                                                                                                                                                | [64, 92]     |
|                                                                         | tph, 5-HT        | Male specific expression of tph in preoptic area of hypothalamus during early development.                                                                                                               | [47]         |
|                                                                         | gfra-1           | Transient silencing of gfra-1-siRNA downregulated brain specific genes and MPTP exposure indicated an interaction between GFRα-1 and DA-ergic system.                                              | [67]         |
| Promoter motif analysis                                                | cyp19a1b, ftz-f1, foxl2 | Synchronous expression of cyp19a1b, ftz-f1 and foxl2 in the brain with high ftz-f1 and foxl2 expression in the female brain.                                                                                  | [98]         |
|                                                                         | CRE, cAMP, hsd20b | Identification of CRE in hsd20b promoter and its modulation by cAMP implicating its role in FOM.                                                                                                         | [155]        |
| Reproductive endocrinology                                             | dmr1a, dmr1b, dmr1c, MT | Identification of multiple dmr1s as testis-specific markers upon MT treatment.                                                                                                                              | [120]        |
|                                                                         | StAR             | Elevation of StAR during hCG-induced oocyte maturation, in vitro and in vivo.                                                                                                                              | [194]        |
|                                                                         | cGnRH-II, GTH-II, cfGnRH | Increase in 11-KT after cGnRH-II and cfGnRH treatment in 24 and 39 week-old fish respectively.                                                                                                          | [90]         |
|                                                                         | GTH              | Castration resulted in increased plasma GTH levels, decreased GTH content in pituitary, T and androstenedione (aromatizable androgens) could abolish the castration-induced increase in plasma GTH and restored pituitary GTH content however, non-aromatizable androgens could not. | [93]         |
|                                                                         | CAs, GnRH-I, E, MT, 11-KT | Controlled release of sex steroids modulates GnRH and CAs activity dimorphically. Brain-related transcripts were elevated after estrogenization as compared to androgenization. | [103]        |
|                                                                         | cyp19a1a, cyp19a1b | cyp19a1a plays critical role during ovarian differentiation and demonstration of female specific expression of brain cyp19a1b during ontogeny.                                                       | [158]        |
| Transient gene silencing                                              | NPY              | Significant decrease in expression of ovary-related transcripts after NPY-esiRNA transient gene silencing indicating a role of NPY in ovary through cfGnRH-GTH axis.                                                | [85]         |
|                                                                         | c-kit, 11-KT, T  | Decrease in 11-KT and T levels c-kit esiRNA silencing.                                                                                                                                                       | [136]        |
|                                                                         | sycp3            | sycp3-esiRNA transient gene silencing affected the                                                                                                                                                    | [189]        |
expression level of various testis-related genes.

| Neuroendocrine regulation | GTH, DA, 5-HT, NE, CE, COMT | Preovulatory decrease in DA content with rise in 5-HT and NE levels. | [8] |
|----------------------------|-------------------------------|---------------------------------------------------------------|-----|
| hFGnRH2, kiss2            | Characterization of brain kiss2 and hFGnRH2. Kiss2-GnRH2 signaling is involved in photo-thermal-mediated mechanisms controlling reproduction. | [17, 20, 21] |
| GTH, DA, NE, A            | 5-HT, NE and A are stimulatory to GTH secretion. Hypothalamic 5-HT content and turnover were inhibited after pCPA and melatonin treatment but the content and turnover of CAs were not. However, α-MPT treatment decreased the content and turnover of DA, NA, and A. | [52] |
| E2, GTH, MAO              | Half-life analysis and turnover study of hypothalamic MAO. E2 exerts feedback regulation of GTH. | [58] |
| DA, NE, A, VT             | Physiological changes in VT are differentially regulated by CAs wherein DA inhibits and NE/A stimulates vasotocin (VT). | [61] |
| GTH-II                    | Ovariectomy-induced rise in GTH-II was regulated by activation of hypothalamic serotonergic and suppression of dopaminergic mechanisms. | [66] |
| th, E2, pKA, pKC, cAMP    | E2 modulated the short-term activation of brain th activity differentially and th activity could be positively correlated with the annual reproductive cycle. | [70, 71] |
| GABA, GTH-II, E2          | GABA regulates GTH-II secretion even when dopamine receptor function is inhibited. | [79] |
| GTH, E2, NE(5R)           | High NE(5R) levels in pituitary, followed by hypothalamus and telencephalon in all the seasons. Ovariectomy exerted a strong negative feedback on GTH secretion in the prespawning phase. | [43] |
| 5-HT, MAO                 | Day-night variations of 5-HT and MAO are photoperiod-dependent and are controlled during the gonadal preparatory phase of the annual reproductive cycle. | [51] |
| 5-HT, MAO                 | High hypothalamic activities of 5-HT and MAO during recrudescence and day-night variations during the early and mid-preparatory phase. | [55] |
| E2, 5-HT, MAO             | E2 modulates MAO activity and alters hypothalamic 5-HT in seasonally dependent manner. | [56] |
| DA, NE, A, E2             | E2-negative feedback act on the CA to modulate GTH secretion. | [57] |
| COMT, E2                  | COMT content increased with progress of ovarian recrudescence in all the brain regions and declined after spawning. Mammalian GnRH analogue injection increased ovarian OE-2-H at 8h and restored to control level after egg-stripping at 16h whereas ovarian OE and COMT activity was significantly decreased at 8h. | [68, 69] |
| VT, isotoxin, E2, T, progesterone, hCG, PGF2α, PGE2 | Immunocytochemical distribution of VT. Steroid hormones and hCG modulated brain and ovarian VT dynamics. Like hCG, VT had differential effects on ovarian steroidogenesis. VT induced FOM/ovulation through the VT receptors and activation of VT secretion and ovarian recrudescence by long photoperiod and high temperature. | [172–177] |
| DA, NA, A, propranolol    | NA modulated FOM through β-adrenergic mechanism, implicating a neural control of oocyte maturation/ovulation | [178] |
| Reproductive endocrinology | E2, T, cortisol | E2 acted as a precursor for estrogen synthesis and cortisol enhanced estrogen-induced vitellogenin synthesis. | [171] |
| Species       | Gene-editing | Regulation | Observation                                                                                     | Reference |
|--------------|--------------|------------|-------------------------------------------------------------------------------------------------|-----------|
| *I. punctatus* | Neuroendocrine-reproductive axis | ccLHR, ccFSHR | Characterization of ccLHR and ccFSHR. LH, a key regulator of periovulatory maturational events, and seasonal changes in ovarian expression of the ccFSHR. (peaked at the onset of ovarian recrudescence and decreased prior to spawning). | [38,39] |
| *C. punctatus* | NGS          | amh, dmyr1, dmyrta2, dmyrta3a, among others | Identification of male-biased genes.                                                              | [200]     |
| *P. fulvidraco* | NGS          | hsd20b, sox9a, spago, fgfbp2, dmr11, cyp17a1, igfbpi2, among others | Identification of sex-related genes.                                                             | [204]     |
| *A. seemanni* | Neuroendocrine regulation | 5-HT, th | Localization of 5-HT positive neurons in the pineal stalk.                                       | [59]      |
| *S. nigrovinctris* | Neuroendocrine regulation | 5-HT, th | th1-expressing dopamine cells (unlike th2-expressing ones) do not co-localize with 5-HT.          | [59]      |
| *M. cavasius* | Neuroendocrine regulation | 5-HT | Melatonin inhibited reproductive activity through modulation of serotonergic activity.           | [60]      |
| *M. wyckiioides* | NGS          | amhr2, gnrh, gnrhr, cyp19a, igf1, igf2, taur, pcdh16, gent3, among others | Identification of 19 differentially expressed genes in the pituitary, annotated to 32 signaling pathways related to gonad development. | [197]     |
| *C. fuscus* | NGS          | cyp17a1, cyp11c1, hsd3b1, hsd17b1, hsd17b2, igfii2, tgfii3, among others | Identification of sex-related genes.                                                             | [198]     |
| *S. asotus* | NGS          | amh, dmyr1, fgfr1a, wt15a, tab3, lnnl3, among others | Identification and sex-specific expression of candidate genes.                                   | [199]     |

However, up to now, Sry and dmy have been the only sex-determining genes isolated in mammals and medaka [114,116], but neither the Sry nor dmy homolog, other than dmyr1 as testis-specific gene in autosomes, has ever been isolated in any other fish species, including in catfish. However, Y-chromosome specific molecular markers have been identified using sry-specific PCR primers in cyprinid fish, *Puntius conchonius* [115]. Experimental evidence demonstrating clarifying the amh function and other candidate genes in sex determination isless explored in catfish. Additionally, in the studies involving identification and characterization of steroidogenic enzyme genes using fish models, most of the time data stops at gene expression analysis through quantitative PCR. However, studies, from our laboratory, on localization, enzymatic assays and protein quantification indicated a robust way of analyzing the enzyme genes not only to distinguish tissue level activities but also seasonally [169]. As most of the catfish species do not
spawn naturally under laboratory conditions, studies comparing GTH induced models together with the use of advanced NGS techniques might lead to discovery/identification of crucial players in spawning and might provide new insights to understand its molecular mechanisms. This makes the use of seasonally breeding catfish unique and advantageous for such studies. Moreover, identification and characterization of novel sex determination related genes which are crucial to understand the masculinization/feminization mechanisms will help and promote aquaculture immensely across teleost including catfish.

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**Abbreviations:**

MPTP \( \quad \) 1-methyl-1,2,3,6-tetrahydropyridine  
11-KT \( \quad \) 11-ketotestosterone  
17α,20β-DP \( \quad \) 17α,20β-dihydroxy-4-pregnen-3-one  
E\(_2\) \( \quad \) 17β-estradiol  
5-HT \( \quad \) 5-hydroxytryptamine  
A \( \quad \) adrenaline  
CA \( \quad \) catecholamines  
COMT \( \quad \) catechol-O-methyltransferase  
CE \( \quad \) catecholestrogens  
DA \( \quad \) dopamine  
EE\(_2\) \( \quad \) ethynyl estradiol  
FOM \( \quad \) final oocyte maturation  
FSH \( \quad \) follicular stimulating hormone  
GABA \( \quad \) γ-aminobutyric acid  
GTH \( \quad \) gonadotropin  
GnRH \( \quad \) gonadotropin-releasing hormone  
hCG \( \quad \) human chorionic gonadotropin  
HHG \( \quad \) hypothalamo-hypophyseal-gonadal  
LH \( \quad \) luteinizing hormone  
MT \( \quad \) methyltestosterone  
MIH \( \quad \) maturation-inducing hormone  
MAO \( \quad \) monoamine oxidase  
NPY \( \quad \) neuropeptide Y  
NGS \( \quad \) next generation sequencing  
NE \( \quad \) norepinephrine  
pCPA \( \quad \) para-chlorophenylalanine  
RNAi \( \quad \) RNA interference  
StAR \( \quad \) steroidogenic acute regulatory protein  
siRNA \( \quad \) short interfering RNA
T  
testosterone
TH  
thyroid hormone
tph  
tryptophan hydroxylase
th  
tyrosine hydroxylase
VT  
vasotocin
ZFN  
zinc finger nucleases

References
1. Bruton, M.N. Alternative life-history strategies of catfishes. *Aquat. Living Resour.* **1996**, *9*, 35–41. https://doi.org/10.1051/alr:1996040.
2. Ng, H.H.; Kottelat, M. The identity of Clariasbatrachus (Linnaeus, 1758), with the designation of a neotype (Teleostei: Claridae). *Zool. J. Linn. Soc.* **2008**, *153*, 725–732.
3. Ng, H.H.; Kottelat, M. After eighty years of misidentification, a name for the glass catfish (Teleostei: Siluridae). *Zootaxa* **2013**, *3630*, 308–316.
4. Goos, H.; Senthilkumar, B.; Joy, K.P. Neuroendocrine integrative mechanisms in the control of gonadotropin secretion in teleosts. In *Comparative endocrinology and reproduction*, Joy, K.P.; Krishna, A.; HaldarC. (Eds.), Narosa/Springer Verlag, New Delhi/Berlin. **1999**, pp. 114–137.
5. Trudeau, V.L.; Spanswick, D.; Fraser, E.J.; Lariviere, K.; Crump, D.; Chiu, S.; MacMillan, M.; Schulz, R.W. The role of amino acid neurotransmitters in the regulation of pituitary gonadotropin release in fish. *Biochem. Cell Biol.* **2000**, *78*, 241–259.
6. Goos, H.; De Leeuw, R.; Burzawa-Gerard, E.; Terlou, M.; Richter, C. Purification of gonadotropic hormone from the pituitary of the African catfish, Clariasgariepinus (Burchell), and the development of a homologous radioimmunoassay. *Gen. Comp. Endocrinol.* **1986**, *63*, 162–170. https://doi.org/10.1016/0016-6480(86)90152-8.
7. Senthilkumar, B. Neuroendocrine Regulation of Gonadotropin Secretion in the Female Catfish, Heteropeustes Fossils (Blocb): Environmental and Hormonal Influences on Hypothalamic Monoaminergic Activity. Ph.D. Thesis, Banaras Hindu Univ-variety, Varanasi, India, 1995.
8. Joy, K.P.; Senthilkumar, B.; Sudhakumari, C.C. Periovulatory changes in hypothalamic and pituitary monoamines following GnRH analogue treatment in the catfish Heteropeustesfossils: A study correlating changes in plasma hormone pro-files. *J. Endocrinol.* **1998**, *156*, 365–372.
9. McGuire, N.L.; Bentley, G.E. Neuropeptides in the gonads: From evolution to pharmacology. *Front. Pharmacol.* **2010**, *1*, 114.
10. Trudeau, V.L. Neuroendocrine regulation of gonadotrophin II release and gonadal growth in the goldfish, Carassius auratus. *Reprod. Reprod.* **1997**, *2*, 55–68.
11. Schulz, R.W.; Bosma, P.T.; Zandbergen, M.A.; Van Der Sanden, M.C.; Van Dijk, W.; Peute, J.; Bogerd, J.; Goos, H.J. Two gonadotropin-releasing hormones in the African catfish, Clariasgariepinus: Localization, pituitary receptor binding, and gonadotropin release activity. *Endocrinology* **1993**, *133*, 1569–1577. https://doi.org/10.1210/endo.133.4.8404596.
12. Sherwood, N.M.; Lovejoy, D.A.; Coe, I.R. Origin of mammalian gonadotropin-releasing hormones. *Endocr. Rev.* **1993**, *14*, 241–254.
13. Sherwood, N.M.; Parker, D.B.; McRory, J.E.; Lescheid, D.W. Molecular evolution of growth hormone-releasing and gonadotropin-releasing hormone. *Fish Physiol.* **1994**, *13*, 3–66.
14. Bogerd, J.; Diepenbroek, W.B.; Hund, E.; van Oosterhout, F.; Teves, A.C.; Leurs, R.; Blomenrohr, M. Two gonadotropin-releasing hormone receptors in the African catfish: No differences in ligand selectivity, but differences in tissue distribution. *Endocrinology* **2002**, *143*, 4673–4682.
15. Tensen, C.; Okuzawa, K.; Blomenrohr, M.; Rebers, F.; Leurs, R.; Bogerd, J.; Schulz, R.; Goos, H. Distinct efficacies for two endogenous ligands on a single cognate gonadoliberin receptor. *Eur. J. Biochem.* **1997**, *243*, 134–140.
16. Zohar, Y.; Muñoz-Cueto, J.A.; Elizur, A.; Kah, O. Neuroendocrinology of reproduction in teleost fish. *Gen. Comp. Endocrinol.* **2010**, *165*, 438–455.
17. Chaube, R.; Rawat, A.; Sharma, S.; Senthilkumar, B.; Bhat, S.; Joy, K. Molecular cloning and characterization of a gonadotropin-releasing hormone 2 precursor cDNA in the catfish Heteropeustesfossils: Expression profile and regulation by ovarian steroids. *Gen. Comp. Endocrinol.* **2019**, *280*, 134–146. https://doi.org/10.1016/j.ygcen.2019.04.021.
18. Senthilkumar, B.; Okuzawa, K.; Gen, K.; Kagawa, H. Effects of serotonin, GABA and neuropeptide Y on seabream gonadotropin releasing hormone release in vitro from preoptic-anterior hypothalamus and pituitary of red seabream, Pagrus major. *J. Neuroendocrinol.* **2001**, *13*, 395–400.
19. Gopurappilly, R.; Ogawa, S.; Parhar, I.S. Functional Significance of GnRH and Kisspeptin, and Their Cognate Receptors in Teleost Reproduction. *Front. Endocrinol.* **2013**, *4*, 24. https://doi.org/10.3389/fendo.2013.00024.
20. Chaube, R.; Sharma, S.; Senthilkumar, B.; Bhat, S.; Joy, K. Identification of kisspeptin2 cDNA in the catfish Heteropeustesfossils: Expression profile, in situ localization and steroid modulation. *Gen. Comp. Endocrinol.* **2020**, *294*, 113472. https://doi.org/10.1016/j.ygcen.2020.113472.
21. Chaube, R.; Sharma, S.; Senthilkumar, B.; Bhat, S.G.; Joy, K.P. Expression profile of kisspeptin2 and gonadotro-bin-releasing hormone2 mRNA during photo-thermal and melatonin treatments in the female air-breathing catfish Hetero-peustesfossils. *Fish Physiol. Biochem.* **2020**, *46*, 2403–2419.
22. Kanda, S. Evolution of the regulatory mechanisms for the hypothalamic-pituitary-gonadal axis in vertebrates—hypothesis from a comparative view. Gen. Comp. Endocrinol. 1999, 117, 120-128. https://doi.org/10.1016/s0016-6480(98)00638-2.

23. Swapna, I.; Rajasekhar, M.; Supriya, A.; Raghuvetter, K.; Sreenivasulu, G.; Rasheeda, M.; Majumdar, K.; Kagawa, H.; Tanaka, H.; Dutta-Gupta, A.; et al. Thiourea-induced thyroid hormone depletion impairs testicular recrudescence in the air-breathing catfish, Clariasgariepinus. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 2006, 144, 1-10. https://doi.org/10.1016/j.cbpa.2006.01.017.

24. Swapna, I.; Senthilkumar, B. Thyroid hormones modulate the hypothalamo–hypophyseal–gonadal axis in teleosts: Molecular insights. Fish Physiol. Biochem. 2007, 33, 335-345. https://doi.org/10.1007/s10695-007-9165-2.

25. Neto, A.T.; Rodrigues, M.D.S.; Habibi, H.R.; Nóbrega, R.H. Thyroid hormone actions on male reproductive system of teleost fish. Gen. Comp. Endocrinol. 2008, 163, 230-236. https://doi.org/10.1016/j.ygcen.2008.04.023.

26. Suzuki, K.; Kawauchi, H.; Nagahama, Y. Isolation and characterization of two distinct gonadotropins from chum salmon pituitary glands. Gen. Comp. Endocrinol. 1988, 71, 292-301. https://doi.org/10.1016/0016-6480(88)90257-2.

27. Suzuki, K.; Kawauchi, H.; Nagahama, Y. Isolation and characterization of subunits from two distinct salmon gonadotropins. Gen. Comp. Endocrinol. 1988, 71, 302-306.

28. Kawauchi, H.; Suzuki, K.; Itoh, H.; Swanson, P.; Naito, N.; Nagahama, Y.; Nozaki, M.; Nakai, Y.; Itoh, S. The duality of teleost gonadotropins. Fish Physiol. Biochem. 1989, 5, 29-38.

29. Swanson, P. Salmon gonadotropins: reconciling old and new ideas. In: Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish, Scott A.P.; Sumpter J.P.; Kime D.E.; Rolfe M.S., (Eds.) 1991, Sheffield, UK: FishSymp; 91; 2-7.

30. Swanson, P. (1997). Pituitary gonadotropins and their receptors in fish. In Proceedings of XIII International Congress of Comparative Endocrinology, Yokohama, Japan, 1997pp. 841-846.

31. Quéréat, B.; Jutisz, M.; Fontaine, Y.A.; Cournis, R. Cloning and sequence analysis of the cDNA for the pituitary glycoprotein hormone α-subunit of the Japanese eel, Anguilla japonica, and increase in their mRNAs during ovarian development induced by injection of chum salmon pituitary homogenate. J. Mol. Endocrinol. 1996, 16, 171–181.

32. Rebers, F.E.M.; Tensen, C.P.; Schulz, R.W.; Goos, H.T.; Bogerd, J. Modulation of glycoprotein hormone α- and gonadotropin βII-subunit mRNA levels in the pituitary gland of mature male African catfish, Clariasgariepinus. Fish Physiol. Biochem. 1997, 17, 99-108.

33. Koide, Y.; Noso, T.; Schouten, G.; Peute, J.; Zandbergen, M.; Bogerd, J.; Schulz, R.; Kawauchi, H.; Goos, H. Maturational gonadotropins. Mol. Cell. Endocrinol. 1990, 71, 253–259.

34. Nagae, M.; Todo, T.; Gen, K.; Kato, Y.; Young, G.; Adachi, S.; Yamauchi, K. Molecular cloning of the cDNAs encoding pituitary glycoprotein hormone α- and β-subunits of the Japanese eel, Anguilla japonica, and increase in their mRNAs during ovarian development induced by injection of chum salmon pituitary homogenate. J. Mol. Endocrinol. 1996, 16, 171–181.

35. Rebers, F.E.M.; Tensen, C.P.; Schulz, R.W.; Goos, H.T.; Bogerd, J. Modulation of glycoprotein hormone α- and gonadotropin βII-subunit mRNA levels in the pituitary gland of mature male African catfish, Clariasgariepinus. Fish Physiol. Biochem. 1997, 17, 99–108.

36. Schütz, R.; Renes, I.B.; Zandbergen, M.A.; Van Dijk, W.; Peute, J.; Goos, H.H.T. Pulbetal Development of Male African Catfish (Clariasgariepinus). Pituitary Ultrastructure and Responsiveness to Gonadotropin-Releasing Hormone. Biol. Reprod. 1995, 53, 940–950. https://doi.org/10.1095/biolreprod53.4.940.

37. Joy, K.P. Role of central monoamines in regulation of gonadotropin-II secretion. In Neural Regulation in the Vertebrate Endocrine System; Springer: Boston, MA, USA, 1999; pp. 111–126. https://doi.org/10.1007/978-1-4615-4805-8_8.

38. Bogerd, J.; Blomenrhr, M.; Andersson, E.; Van Der Putten, H.; Tensen, C.; Vischer, H.; Granneman, J.; Janssen-Dommerholt, C.; Goos, H.; Schulz, R. Discrepancy Between Molecular Structure and Ligand Selectivity of a Testicular Follicle-Stimulating Hormone Receptor of the African Catfish (Clariasgariepinus). J. Biol. Reprod. 2001, 64, 1633–1643. https://doi.org/10.1095/biolreprod64.6.1633.

39. Kumar, R.S.; Ijiri, S.; Trant, J.M. Molecular biology of channel catfish gonadotropin receptors: 1. Cloning of a functional luteinizing hormone receptor and preovulatory induction of gene expression. Biol. Reprod. 2001, 64, 1010–1018.

40. Kumar, R.S.; Ijiri, S.; Trant, J.M. Molecular biology of the channel catfish gonadotropin receptors: 2. Complementary DNA cloning, functional expression, and seasonal gene expression of the follicle-stimulating hormone receptor. Biol. Reprod. 2001, 65, 710–717.

41. Schulz, R.W.; Vischer, H.F.; Cavaco, J.E.B.; Santos, E.M.; Tyler, C.R.; Goos, H.T.; Bogerd, J. Gonadotropins, their receptors, and the regulation of testicular functions in fish. Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 2001, 129, 407–417.

42. Schulz, R.W.; Zandbergen, M.A.; Peute, J.; Bogerd, J.; Van Dijk, W.; Goos, H.J. Pituitary Gonadotrophs are Strongly Activated at the Beginning of Spermatogenesis in African Catfish, Clariasgariepinus. Biol. Reprod. 1997, 57, 139–147. https://doi.org/10.1095/biolreprod57.1.139.

43. Joy, K.P.; Singh, M.S.; Senthilkumar, B.; Goos, H.T. Pituitary-gonadal relationship in the catfish Clariasbatrachus (L): A study correlating gonadotrophin-II and sex steroid dynamics. Zool. Sci. 2000, 17, 395–404.

44. Kirubagaran, R.; Senthilkumar, B.; Sudhakumari, C.C.; Joy, K.P. Seasonal dynamics in gonadotropin secretion and E2-binding in the catfish Heteropneustesfossilis. Fish Physiol. Biochem. 2005, 31, 183–188. https://doi.org/10.1007/s10695-006-0022-5.

45. Sudhakumari, C.; Senthilkumar, B.; Raghuvetter, K.; Wang, D.; Kobayashi, T.; Kagawa, H.; Krishnaiha, C.; Dutta-Gupta, A.; Nagahama, Y. Dimorphic expression of tryptophan hydroxylase in the brain of XX and XY Nile tilapia during early development. Gen. Comp. Endocrinol. 2010, 166, 320–329. https://doi.org/10.1016/j.ygcen.2009.11.009.

46. Tsai, C.-L.; Wang, L.-H. Effects of gonadal steroids on the serotonin synthesis and metabolism in the early developing tilapia brain. Neurosci. Lett. 1999, 264, 45–48. https://doi.org/10.1016/s0304-3940(99)00160-3.
46. Tsai, C.-L.; Wang, L.-H.; Chang, C.-F.; Kao, C.-C. Effects of Gonadal Steroids on Brain Serotonergic and Aromatase Activity During the Critical Period of Sexual Differentiation in Tilapia, Oreochromis mossambicus. J. Neuroendocrinol. 2001, 12, 894–898. https://doi.org/10.1046/j.1365-2826.2000.00536.x.

47. Raghuvееrer, K.; Sudhakumaran, C.; Senthilkumararan, B.; Kaga, H.; Dutta-Gupta, A.; Nagahama, Y. Gender differences in tryptophan hydroxylase-2 mRNA, serotonin, and 5-hydroxytryptophan levels in the brain of catfish, Clariasgariepinus, during sex differentiation. Gen. Comp. Endocrinol. 2011, 171, 94–104. https://doi.org/10.1016/j.ygenen.2010.12.003.

48. Joy, K.P.; Khan, I.A. Pineal-gonadal relationship in the teleost Channa punctatus (Bloch): Evidence for possible involvement of hypothalamic serotonergic system. J. Pineal Res. 1991, 11, 12–22.

49. Khan, I.A.; Thomas, P. Disruption of Neuroendocrine Control of Luteinizing Hormone Secretion by Aroclor 1254 Involves Inhibition of Hypothalamic Tryptophan Hydroxylase Activity. Biol. Reprod. 2001, 64, 955–964. https://doi.org/10.1095/biolreprod64.3.955.

50. Khan, I.A.; Joy, K.P. Diurnal variations in hypothalamic monoamine levels in the teleost Channa punctatus (bloch) in response to melatonin under two photothermal conditions. Fish Physiol. Biochem. 1988, 5, 187–190. https://doi.org/10.1007/bf01874795.

51. Senthilkumararan, B.; Joy, K.P. Effects of photoperiod alterations on day-night variations in hypothalamic serotonin content and turnover, and monoamine oxidase activity in the female catfish, Heteropneustesfossilis (Bloch). Fish Physiol. Biochem. 1994, 13, 301–307. https://doi.org/10.1007/bf00003434.

52. Senthilkumararan, B.; Joy, K.P. Effects of melatonin, p-chlorophenylalanine, and α-methylparatyrosine on plasma gonadotropin level and ovarian activity in the catfish, Heteropneustesfossilis: A study correlating changes in hypothalamic monoamines. Fish Physiol. Biochem. 1995, 14, 471–291.

53. Prasad, P.; Ogawa, S.; Parhar, I.S. Role of serotonin in fish reproduction. Front. Neurosci. 2015, 9, 195.

54. Katti, S.R.; Sathyanesan, A.G. Monoamine oxidase activity in the gonads of Clariasbatrachus (L) in relation to reproductive cycle. Biol. Rhythm. Res. 1986, 17, 207–211.

55. Senthilkumararan, B.; Joy, K. Annual Variations in Hypothalamic Serotonin and Monoamine Oxidase in the Catfish Heteropneustesfossilis with a Note on Brain Regional Differences of Day-Night Variations in Gonadal Preparatory Phase. Gen. Comp. Endocrinol. 1993, 90, 372–382. https://doi.org/10.1006/gcen.1993.1093.

56. Senthilkumararan, B.; Joy, K.P. Effects of ovariectomy and oestradiol replacement on hypothalamic serotonergic and monoamine oxidase activity in the catfish, Heteropneustesfossilis: A study correlating plasma oestradiol and gonadotrophin levels. J. Endocrinol. 1994, 142, 193–203. https://doi.org/10.1677/joe.0.1420193.

57. Senthilkumararan, B.; Joy, K.P. Changes in hypothalamic catecholamines, dopamine-β-hydroxylase, and phenylethanolamine-N-methyltransferase in the catfish Heteropneustesfossilis in relation to season, raised photoperiod and temperature, ovariectomy, and estradiol-17β replacement. Gen. Comp. Endocrinol. 1995, 97, 121–134.

58. Senthilkumararan, B.; Joy, K. A Turnover Study of Hypothalamic Monoamine Oxidase (MAO) and Effects of MAO Inhibition on Gonadotropin Secretion in the Female Catfish, Heteropneustesfossilis. Gen. Comp. Endocrinol. 1995, 97, 1–12. https://doi.org/10.1006/gcen.1995.1001.

59. Rosner, E.; Chagnaud, B.P.; Willlimann, M.F. Serotonin systems in three socially communicating teleost species, the grunting toadfish (Allenbatrachusgrunniens), a South American marine catfish (Ariopisseemanni), and the upside-down catfish (Synodontisgrivertinis). J. Chem. Neuroanat. 2020, 104, 101708.

60. Badruzaman, M.; Ikegami, T.; Amin, A.R. Shahjahan Melatonin inhibits reproductive activity through changes of serotonergic activity in the brain of freshwater catfish (Mystuscasavarius). Aquaculture 2020, 526, 735378. https://doi.org/10.1016/j.aquaculture.2020.735378.

61. Singh, R.K.; Chaube, R.; Joy, K.P. Differential and reproductive stage-dependent regulation of vasotocin secretion by catecholamines in the catfish Heteropneustesfossilis. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 2013, 166, 619–626. https://doi.org/10.1016/j.cbpa.2013.09.005.

62. Mamta, S.K.; Raghuvееrer, K.; Sudhakumaran, C.-C.; Rajakumar, A.; Basavaraju, Y.; Senthilkumararan, B. Cloning and expression analysis of tyrosine hydroxylase and changes in catecholamine levels in brain during ontogeny and after sex steroid analogues exposure in the catfish, Clariasbatrachus. Gen. Comp. Endocrinol. 2014, 197, 18–25. https://doi.org/10.1016/j.ygenen.2013.11.022.

63. Peter, R.E.; Trudeau, V.L.; Sloley, B.D. Brain regulation of reproduction in teleosts. Bull. Inst. Zool. Acad. Sin. Monograph 1991, 16, 89–118.

64. De Leeuw, R.; Van’t Veer, C.; Goos, H.T.; Van Oordt, P.G.W.J. The dopaminergic regulation of gonadotropin-releasing hormone receptor binding in the pituitary of the African catfish, Clariasgariepinus. Gen. Comp. Endocrinol. 1988, 72, 408–415.

65. Popesku, J.T.; Martyniuk, C.J.; Menningen, J.; Xiong, H.; Zhang, D.; Xia, X.; Cossins, A.R.; Trudeau, V.L. The goldfish (Carassiusauratus) as a model for neuroendocrine signaling. Mol. Cell. Endocrinol. 2008, 293, 43–56. https://doi.org/10.1016/j.mce.2008.06.017.

66. Senthilkumararan, B.; Joy, K. Effects of Administration of Some Monoamine-Synthesis Blockers and Precursors on Ovariectomy-Induced Rise in Plasma Gonadotropin II in the Catfish Heteropneustesfossilis. Gen. Comp. Endocrinol. 1996, 101, 220–226. https://doi.org/10.1006/gcen.1996.0024.

67. Mamta, S.K.; Senthilkumararan, B. GDNF family receptor α-1 in catfish: Possible implication to brain dopaminergic activity. Brain Res. Bull. 2018, 140, 270–280.
68. Senthilkumaran, B.; Joy, K.P. Periovulatory changes in catfish ovarian oestradiol-17β, oestrogen-2-hydroxylase and catechol-O-methyltransferase during GnRH analogue-induced ovulation and in vitro induction of oocyte maturation by catecholestrogens. J. Endocrinol. 2001, 168, 239–247. https://doi.org/10.1677/joe.0.1680239.

69. Joy, K.P.; Senthilkumaran, B. Annual and diurnal variations in, and effects of altered photoperiod and temperature, ovariectomy, and estradiol-17β replacement on catechol-O-methyltransferase level in brain regions of the catfish, Heteropneustes fossilis. Comp. Biochem. Physiol. Part C Pharmacol. Toxicol. Endocrinol. 1998, 119, 37–44.

70. Chaube, R.; Joy, K. Brain tyrosine hydroxylase in the catfish Heteropneustes fossilis: Annual and circadian variations, and sex and regional differences in enzyme activity and some kinetic properties. Gen. Comp. Endocrinol. 2003, 130, 29–40. https://doi.org/10.1016/s0016-6480(02)00529-4.

71. Chaube, R.; Joy, K.P. Estrogen regulation of in vitro brain tyrosine hydroxylase activity in the catfish Heteropneustes fossilis: Interactions with cAMP-protein kinase A and protein kinase C systems in enzyme activation. Gen. Comp. Endocrinol. 2005, 141, 115–125.

72. Saha, S.; Patil, S.; Singh, U.; Singh, O.; Singru, P.S. Sexual dimorphism in the hypophysiotropic tyrosine hydroxylase-positive neurons in the preoptic area of the teleost, Clarias batrachus. Biol. Sex Differ. 2015, 6, 23.

73. DeRopp, R.S.; Kastl, L.H.; Forst, A. Comparative effects of temperature on brain enzymes in goldfish (Carassius auratus) and mouse (Mus musculus). Comp. Biochem. Physiol. 1970, 37, 123–125.

74. Sugden, P.H.; Newsholme, E.A. Activities of choline acetyltransferase, acetylcholinesterase, glutamate decarboxylase, 4-aminobutyrate aminotransferase and carmine acetyltransferase in nervous tissue from some vertebrates and invertebrates. Comp. Biochem. Physiol. 1977, 66C, 89–94.

75. Martinoli, M.-G.; Dubourg, P.; Geffard, M.; Kah, O. Distribution of GABA-immunoreactive neurons in the forebrain of the goldfish, Carassius auratus. Cell Tissue Res. 1990, 260, 77–84. https://doi.org/10.1007/bf00297492.

76. Su, Y.Y.T.; Wu, J.-Y.; Lam, D.M.K. Purification of l-glutamic acid decarboxylase from catfish brain. J. Neurochem. 1979, 33, 169–179. https://doi.org/10.1111/j.1471-4159.1979.tb11719.x.

77. Mathis, C.A.; Tunnicliff, G. The \( \gamma \)-aminobutyric acid receptor in catfish brain. Comp. Biochem. Physiol. Part C Pharmacol. 1984, 78, 479–481.

78. Malizia, L.A.; Tunnicliff, G. Uptake of \( \gamma \)-aminobutyric acid by catfish brain. Comp. Biochem. Physiol. Part C Pharmacol. 1987, 87, 37–40.

79. Joy, K.P.; Tharakan, B.; Goos, H.T. Distribution of \( \gamma \)-aminobutyric acid in catfish (Heteropneustes fossilis) forebrain in relation to season, ovariectomy and E2 replacement, and effects of GABA administration on plasma Gonadotropin-II level. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 1999, 123, 369–376.

80. Kusuma, P.S.W.; Hariani, D. The role of laser puncture exposure on gonad maturation mechanism of catfish (Clarias sp.) through \( \text{Ca}^{2+} \), PKC and GABA neurotransmitter. Egypt. J. Aquat. Res. 2017, 43, 303–305.

81. Leonard, J.B.; Waldbieser, G.C.; Silverstein, J.T. Neuropeptide Y Sequence and Messenger RNA Distribution in Channel Catfish (Ictalurus punctatus). Mar. Biotechnol. 2001, 3, 111–118. https://doi.org/10.s10260000050.

82. Mazumdar, M.; Lal, B.; Sakharkar, A.J.; Deshmukh, M.; Singru, P.S.; Subbedar, N. Involvement of neuropeptide Y Y1 receptors in the regulation of LH and GH cells in the pituitary of the catfish, Clarias batrachus: An immunocytochemical study. Gen. Comp. Endocrinol. 2006, 149, 190–196.

83. Mazumdar, M.; Sakharkar, A.J.; Singru, P.S.; Subbedar, N. Reproduction phase-related variations in neuropeptide Y immunoreactivity in the olfactory system, forebrain, and pituitary of the female catfish, Clarias batrachus (Linn.). J. Comp. Neurol. 2007, 504, 450–469.

84. Carpio, Y.; León, K.; Acosta, J.; Morales, R.; Estrada, M.P. Recombinant tilapia Neuropeptide Y promotes growth and antioxidan defenses in African catfish (Clarias gariepinus) fry. Aquaculture 2007, 272, 649–655. https://doi.org/10.1016/j.aquaculture.2007.08.024.

85. Sudhakumari, C.C.; Anitha, A.; Murugananthkumar, R.; Tiwari, D.K.; Bhasker, D.; Senthilkumaran, B.; Dutta-Gupta, A. Cloning, localization and differential expression of Neuropeptide Y during early brain development and gonadal recrudescence in the catfish, Clarias gariepinus. Comp. Biochem. Endocrinol. 2017, 251, 54–65.

86. Marks, J.L.; Li, M.; Schwartz, M.; Porto Jr, D.; Baskin, D.G. Effect of fasting on regional levels of neuropeptide Y mRNA and insulin receptors in the rat hypothalamus: An autoradiographic study. Mol. Cell. Neurosci. 1992, 3, 199–205.

87. Peterson, B.C.; Waldbieser, G.C.; Riley Jr, L.G.; Upton, K.R.; Kobayashi, Y.; Small, B.C. Pre-and postprandial changes in orexigenic and anorexigenic factors in channel catfish (Ictalurus punctatus). Gen. Comp. Endocrinol. 2012, 176, 231–239.

88. Subbedar, N.; Gaikwad, A.; Biju, K.C.; Saha, S. Role of neuropeptide Y (NPY) in the regulation of reproduction: Study based on catfish model. Fish Physiol. Biochem. 2005, 31, 167–172. https://doi.org/10.1007/s10695-006-0020-7.

89. Schulz, R.; Van Der Corput, L.; Janssen-Dommerholt, J.; Goos, H.J.T. Sexual steroids during puberty in male African catfish (Clarias gariepinus): Serum levels and gonadotropin-stimulated testicular secretion in vitro. J. Comp. Physiol. 1994, 164, 195–205. https://doi.org/10.1007/bf00354080.

90. Schulz, R.W.; Van Der Sanden, M.C.A.; Bosma, P.T.; Goos, H.J.T. Effects of gonadotrophin-releasing hormone during the pubertal development of the male African catfish (Clarias gariepinus): Gonadotrophin and androgen levels in plasma. J. Endocrinol. 1994, 140, 265–273. https://doi.org/10.1677/joe.0.1400265.
91. Blazquez, M.; Bosma, P.; Fraser, E.; Van Look, K.; Trudeau, V. Fish as models for the neuroendocrine regulation of reproduction and growth. Comp. Biochem. Physiol. Part C: Pharmacol. Toxicol. Endocrinol.1998, 119, 345–364. https://doi.org/10.1016/s0742-8413(98)00023-1.

92. De Leeuw, R.; Goos, H.T.; Van Oordt, P.G.W.J. The dopaminergic inhibition of the gonadotropin-releasing hormone-induced gonadotropin release: An in vitro study with fragments and cell suspensions from pituitaries of the African catfish, Clariasgariepinus (Burchell). Gen. Comp. Endocrinol.1986, 63, 171–177.

93. De Leeuw, R.; Wurth, Y.A.; Zandbergen, M.A.; Peute, J.; Goos, H.J.T. The effects of aromatizable androgens, non-aromatizable androgens, and estrogens on gonadotropin release in castrated African catfish, Clariasgariepinus (Burchell). Cell Tissue Res.1986, 243, 587–594. https://doi.org/10.1007/bf00218066.

94. Klenke, U.; Zohar, Y. Gonadal regulation of gonadotropin subunit expression and pituitary Lh protein content in female hybrid striped bass. Fish Physiol. Biochem.2003, 28, 25–27. https://doi.org/10.1023/b:fish.000030465.56876.97.

95. Kobayashi, M.; Stacey, N.E. Effect of ovariectomy and steroid hormone implantation on serum gonadotropin levels in female goldfish. Zool. Sci.1990, 7, 715–721.

96. Devlin, R.H.; Nagahama, Y. Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. Aquaculture2002, 208, 191–364. https://doi.org/10.1016/s0044-8486(02)00057-1.

97. Blazquez, M.; Somoza, G.M. Fish with thermolabile sex determination (TSD) as models to study brain sex differentiation. Gen. Comp. Endocrinol.2010, 166, 470–477.

98. Sridi, P.; Chaitanya, R.K.; Dutta-Gupta, A.; Senthilkumar, B. Ftz-f1 and foxl2 up-regulate catfish brain aromatase gene transcription by specific binding to the promoter motifs. Biochem. Biophys. Acta BBA Gene Regul. Mech.2012, 1819, 57–66.

99. Senthilkumar, B.; Sudhakumari, C.C.; Mamta, S.K.; Raghuvase, K.; Swapna, I.; Murugananthkumar, R. Brain sex differentiation in teleosts: Emerging concepts with potential biomarkers. Gen. Comp. Endocrinol.2015, 220, 33–40.

100. Kim, J.-H.; Yu, Y.-B.; Choi, J.-I. Toxic effects on bioaccumulation, hematological parameters, oxidative stress, immune responses and neurotoxicity in fish exposed to microplastics: A review. J. Hazard. Mater.2021, 413, 125423. https://doi.org/10.1016/j.jhazmat.2021.125423.

101. Kar, S.; Sangem, P.; Anusha, N.; Senthilkumar, B. Endocrine disruptors in teleosts: Evaluating environmental risks and biomarkers. Aquac. Fish.2021, 6, 1–26. https://doi.org/10.1016/j.aaf.2020.07.013.

102. Khalil, S.R.; Hussein, M.M. Neurotransmitters and neuronal apoptotic cell death of chronically aluminum intoxicated Nile catfish (Clariasgariepinus) in response to ascorbic acid supplementation. Neurotoxicology2015, 51, 184–191.

103. Mamta, S.-K.; Sudhakumari, C.; Kagawa, H.; Dutta-Gupta, A.; Senthilkumar, B. Controlled release of sex steroids through osmotic pump alters brain GnRH1 and catecholaminergic system dimorphically in the catfish, Clariasgariepinus. Brain Res. Bull.2020, 164, 325–333. https://doi.org/10.1016/j.brainresbull.2020.08.022.

104. Volt, J.-N.; Nanda, L.; Schmid, M.; Sehartl, M. Governing Sex Determination in Fish: Regulatory Putsches and Ephemerall Dictators. Sex. Dev.2007, 1, 85–99. https://doi.org/10.1159/000100030.

105. Pennan, D.J.; Piferrer, F. Fish gonadogenesis. Part I: Genetic and environmental mechanisms of sex determination. Rev. Fish. Sci.2008, 16, 16–34.

106. Yamamoto, T.O. Sex differentiation. In Fish Physiology, Hoar, W.S.; Randall, D.J. (Eds.), Academic Press, 1969, New York, Vol. III, pp. 117–175.

107. Nakamura, M.; Kobayashi, T.; Chang, X.T.; Nagahama, Y. Gonadal sex differentiation in teleost fish. J. Exp. Zool.1998, 281, 362–377.

108. Baroiller, J.-F.; Guiguen, Y.; Fostier, A. Endocrine and environmental aspects of sex differentiation in fish. Cell. Mol. Life Sci.1999, 55, 910–931. https://doi.org/10.1007/s000180050344.

109. Baroiller, J.F.; Guiguen, Y. Environmental and environmental aspects of sex differentiation in gonochoristic fish. Genes Mech. Vertebr. Sex Determ.2001, 91, 177–201.

110. Santi, S.; Rougeot, C.; Toguyen, A.; Gennotte, V.; Keibe, I.; Melard, C. Temperature preference and sex differentiation in African catfish, Clariasgariepinus. J. Exp. Zool. Part A Ecol. Integr. Physiol.2017, 327, 28–37.

111. Patiño, R.; Davis, K.B.; Schoore, J.E.; Uguz, C.; Strüssmann, C.A.; Parker, N.C.; Simco, B.A.; Goudie, C.A. Sex differentiation of channel catfish gonads: Normal developmental and effects of temperature. J. Exp. Zool.1996, 276, 209–218. https://doi.org/10.1002/(sici)1097-010x(19961015)276:3<209::aid-jez5>3.0.co2-r.

112. Baroiller, J.; D’Cotta, H.; Saillant, E. Environmental Effects on Fish Sex Determination and Differentiation. Sex. Dev.2009, 3, 118–135. https://doi.org/10.1159/00023077.

113. Koopman, P.; Munsterberg, A.; Capel, B.; Vivian, N.; Lovell-Badge, R. Expression of a candidate sex-determining gene during mouse testis differentiation. Nature1990, 348, 450–452. https://doi.org/10.1038/348454a0.

114. Sinclair, A.; Berta, P.; Palmer, M.S.; Hawkins, J.R.; Griffiths, B.L.; Smith, M.J.; Foster, J.W.; Frischaufl, A.-M.; Lovell-Badge, R.; Goodfellow, P.N. A gene from the human sex-determining region Y encodes a protein with homology to a conserved DNA-binding domain. Nature1990, 346, 240–244. https://doi.org/10.1038/346240a0.

115. Kirankumar, S.; Anathy, V.; Pandian, T. Hormonal induction of supernmale golden rosy barb and isolation of Y-chromosome specific markers. Gen. Comp. Endocrinol.2003, 134, 62–71. https://doi.org/10.1016/s0016-6480(03)00218-1.

116. Matsuda, M.; Nagahama, Y.; Shinomiya, A.; Sato, T.; Matsuoka, C.; Kobayashi, T.; Morrey, C.E.; Shibata, N.; Asakawa, S.; Shimizu, N.; et al. DMY is a Y-specific DM-domain gene required for male development in the medaka fish. Nature2002, 417, 559–563. https://doi.org/10.1038/nature751.
117. Nanda, I.; Kondo, M.; Hornung, U.; Asakawa, S.; Winkler, C.; Shimizu, A.; Shan, Z.; Haaf, T.; Shimizu, N.; Shima, A.; et al. A duplicated copy of DMRT1 in the sex-determining region of the Y chromosome of the medaka, Oryziaslatipes. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11778–11783. https://doi.org/10.1073/pnas.182314699.

118. Matsuda, M.; Sato, T.; Toyazaki, Y.; Nagahama, Y.; Hamaguchi, S.; Sakaizumi, M. Oryziascurvifinis has DMY, a gene that is required for male development in the medaka, O. latipes. *Zool. Sci.* **2003**, *20*, 159–161.

119. Cui, Z.; Liu, Y.; Wang, W.; Wang, Q.; Zhang, N.; Lin, F.; Wang, N.; Shao, C.; Dong, Z.; Li, Y. Genome editing reveals dmrt1 as an essential male sex-determining gene in Chinese tongue sole (*Cynoglossussemilaevis*). *Sci. Rep.* **2017**, *7*, 42213.

120. Raghuvare, K.; Senthilkumar, B. Identification of multiple dmrt1s in catfish: Localization, dimorphic expression pattern, changes during testicular cycle and after methyltestosterone treatment. *J. Mol. Endocrinol.* **2009**, *42*, 437–448. https://doi.org/10.1677/jme-09-0011.

121. Kobayashi, Y.; Nagahama, Y.; Nakamura, M. Diversity and Plasticity of Sex Determination and Differentiation in Fishes. *Sex. Dev.* **2013**, *7*, 115–125. https://doi.org/10.1159/000342009.

122. Shirak, A.; Seroussi, E.; Cnaani, A.; Howe, A.E.; Domokhovsky, R.; Zilberman, N.; Kocher, T.; Hulata, G.; Ron, M. Amh and Dmrt2 Genes Map to Tilapia (*Oreochromismpp*.) Linkage Group 23 Within Quantitative Trait Locus Regions for Sex Determination. *Genetics* **2006**, *174*, 1573–1581. https://doi.org/10.1534/genetics.106.059030.

123. Hattori, R.S.; Murai, Y.; Oura, M.; Masuda, S.; Majhi, S.K.; Sakamoto, T.; Fernandino, J.I.; Somoza, G.M.; Yokota, M.; Strüssmann, C.A. A Y-linked anti-Müllerian hormone duplication takes over a critical role in sex determination. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 2955–2959.

124. Yamamoto, Y.; Zhang, Y.; Sarida, M.; Hattori, R.S.; Strüssmann, C.A. Coexistence of genotypic and temperature-dependent sex determination in pejerrey (*Odontesthesamia*). *PLoS ONE* **2014**, *9*, e102574.

125. Kamiya, T.; Kai, W.; Tasumi, S.; Oka, A.; Matsunaga, T.; Mizuno, N.; Fujita, M.; Suetake, H.; Suzuki, S.; Hosoya, S.; et al. A Trans-Species Missense SNP in Amhr2 Is Associated with Sex Determination in the Tiger Pufferfish, Takifugurubripes (Fugu). *PLoS Genet.* **2012**, *8*, e1002798. https://doi.org/10.1371/journal.pgen.1002798.

126. Yano, A.; Guyomard, R.; Nicol, B.; Jouanno, E.; Quillet, E.; Kloppe, C.; Cabau, C.; Bouchue, O.; Fostier, A.; Guiguen, Y. An Immune-Related Gene Evolved into the Master Sex-Determining Gene in Rainbow Trout, *Oncorhynchusmykiss*. *Curr. Biol.* **2012**, *22*, 1423–1428. https://doi.org/10.1016/j.cub.2012.05.045.

127. Myoshio, T.; Otake, H.; Masuyama, H.; Matsuda, M.; Kuroki, Y.; Fujiyama, A.; Naruse, K.; Hamaguchi, S.; Sakaizumi, M. Tracing the emergence of a novel sex-determining gene in medaka, *Oryziaslzonculus*. *Genetics* **2012**, *191*, 163–170.

128. Takehana, Y.; Matsuda, M.; Myoshio, T.; Suster, M.L.; Kawakami, K.; Shin-I, T.; Kohara, Y.; Kuroki, Y.; Toyoda, A.; Fujiyama, A.; et al. Co-option of Sox3 as the male-determining factor on the Y chromosome in the fish *Oryziaslincens*. *Nat. Commun.* **2014**, *5*, 4157. https://doi.org/10.1038/ncomms5157.

129. Sandra, G.-E.; Norma, M.-M. Sexual determination and differentiation in teleost fish. *Rev. Fish Biol. Fish.* **2010**, *20*, 101–121. https://doi.org/10.1007/s11160-009-9123-4.

130. Chen, J.; Fu, Y.; Xiang, D.; Zhao, G.; Long, H.; Liu, J.; Yu, Q. XX/XY heteromorphic sex chromosome systems in two bullhead catfish species, *Liobagrusmarginatus* and *L. styani* (*Ambliglyptidae*, Siluriformes). *Cytogenet. Genome Res.* **2008**, *122*, 169–174. https://doi.org/10.1159/000163099.

131. Ortega-Recalde, O.; Goikoetxea, A.; Hore, T.A.; Todd, E.V.; Gemmell, N.J. The Genetics and Epigenetics of Sex Change in Fish. *Annu. Rev. Anim. Biol.* **2020**, *8*, 47–69. https://doi.org/10.1146/annurev-animal-021419-083634.

132. Liu, Z.H.; Zhang, Y.G.; Wang, D.S. Studies on feminization, sex determination, and differentiation of the Southern catfish, *Silurusmeridionalis*—A review. *Fish Physiol. Biochem.* **2010**, *36*, 223–235. https://doi.org/10.1007/s10695-008-9281-7.

133. Patino, R. Sex Differentiation, Gonadal Sex Differentiation, and Sex Control in Channel Catfish. *Sex. Dev.* **2018**, *12*, 477–494. https://doi.org/10.1002/sexdev.21229.

134. Nagahama, Y. Molecular mechanisms of sex determination and gonadal sex differentiation in fish. *Fish Physiol. Biochem.* **2005**, *31*, 105–109. https://doi.org/10.1007/s10166-005-7590-2.

135. Bao, L.; Tian, C.; Liu, S.; Zhang, Y.; Elaswad, A.; Yuan, Z.; Khalil, K.; Sun, F.; Yang, Y.; Zhou, T.; et al. The Y chromosome sequence of the channel catfish suggests novel sex determination mechanisms in teleost fish. *BMC Biol.* **2019**, *17*, 1–16. https://doi.org/10.1186/s12915-019-0627-7.

136. Laldinsangi, C.; Senthilkumar, B. Expression profiling of c-kit and its impact after esiRNA silencing during gonadal development in catfish. *Gen. Comp. Endocrinol.* **2018**, *266*, 38–51. https://doi.org/10.1016/j.ygcen.2018.04.004.

137. Sudhakumari, C.C.; Senthilkumar, B. Expression profiling of various marker genes involved in gonadal differentiation of teleosts: Molecular understanding of sexual plasticity. In: *Sexual Plasticity and Gametogenesis in Fishes*; Senthilkumar, B., (Ed.), Biomedical Inc: New York, NY, USA, 2013; Chapter XXIV, pp. 401–422.

138. Avise, J.; Mank, J. Evolutionary Perspectives on Hermaphroditism in Fishes. *Sex. Dev.* **2009**, *3*, 152–163. https://doi.org/10.1159/000223079.

139. Mank, J.; Avise, J. Evolutionary Diversity and Turn-Over of Sex Determination in Teleost Fishes. *Sex. Dev.* **2009**, *3*, 60–67. https://doi.org/10.1159/000223071.

140. Senthilkumar, B. *Sexual Plasticity and Gametogenesis in Fishes*; Senthilkumar, B., Ed.; Biomedical Inc: New York, NY, USA, 2013.

141. Chan, S.; Yeung, W. 4 Sex Control and Sex Reversal in Fish Under Natural Conditions.*Fish Physiol.* **1983**, *171*, 222–222. https://doi.org/10.1016/s1546-5098(08)60304-0.
165. Gupta, Y.R.; Senthilkumaran, B. Identification, expression profiling and localization of thoc in common carp ovary: Influence of thoc3-siRNA transient silencing. Gene2020, 732, 144350. https://doi.org/10.1016/j.gene.2020.144350.

166. Gupta, Y.R.; Senthilkumaran, B. Common carp pentraxin gene: Evidence for its role in ovarian differentiation and growth. Gen. Comp. Endocrinol. 2020, 290, 113398. https://doi.org/10.1016/j.ygcen.2020.113398.

167. Prathibba, Y.; Senthilkumaran, B. Expression of wnt4/5 during reproductive cycle of catfish and wnt5 promoter analysis. J. Endocrinol. 2017, 232, 1–13.

168. Senthilkumaran, B. Recent advances in meiotic maturation and ovulation: Comparing mammals and pisces. Front. Biosci. 2011, 16, 1898–1914. https://doi.org/10.2741/3829.

169. Rajakumar, A.; Senthilkumaran, B. Steroidogenesis and its regulation in teleost-a review. Fish Physiol. Biochem. 2020, 46, 803–818. https://doi.org/10.1007/s10695-019-00752-0.

170. Sundararaj, B.I.; Goswami, S.V.; Lamba, V. Some aspects of oocyte maturation in catfish. J. Steroid Biochem. 1979, 11, 701–707. https://doi.org/10.1016/0022-4731(79)90003-7.

171. Sundararaj, B.I.; Goswami, S.V.; Lamba, V.J. Role of testosterone, estradiol-17β, and cortisol during vitellogenin synthesis in the catfish, Heteropneustesfossilis. Gen. Comp. Endocrinol. 2008, 159, 214–225. https://doi.org/10.1016/j.ygcen.2008.09.003.

172. Joy, K.P.; Singh, V. Functional interactions between vasotocin and prostaglandins during final oocyte maturation and ovulation in the catfish Heteropneustesfossilis. Gen. Comp. Endocrinol. 2013, 186, 126–135. https://doi.org/10.1016/j.ygcen.2013.02.043.

173. Singh, V.; Joy, K.P. Immunocytochemical localization, HPLC characterization, and seasonal dynamics of vasotocin in the brain, blood plasma and gonads of the catfish Heteropneustesfossilis. Gen. Comp. Endocrinol. 2008, 159, 390–397. https://doi.org/10.1016/j.ygcen.2006.01-0078-4.

174. Chaube, R.; Singh, V.; Joy, K.P. Changes in vasotocin levels in relation to ovarian development in the catfish Heteropneustesfossilis. Gen. Comp. Endocrinol. 2011, 174, 15–21.

175. Chaube, R.; Singh, R.K.; Joy, K.P. Changes in vasotocin levels in relation to ovarian development in the catfish Heteropneustesfossilis exposed to altered photoperiod and temperature. Fish Physiol. Biochem. 2015, 41, 1173–1186. https://doi.org/10.1007/s10695-015-9781-4.

176. Mishra, A.; Chaube, R.; Joy, K.P. An in vitro study on noradrenergic modulation of final oocyte maturation in the catfish Heteropneustesfossilis. Theriogenology 2018, 114, 1–6. https://doi.org/10.1016/j.theriogenology.2018.03.009.

177. Mishra, A.; Joy, K.P. Effects of gonadotrophin in vivo and 2-hydroxyoestradiol-17β in vitro on follicular steroid hormone profile associated with oocyte maturation in the catfish Heteropneustesfossilis. J. Endocrinol. 2006, 189, 341–353. https://doi.org/10.1677/joe.1.06686.

178. Kadam, A.L.; Koide, S.S. Inhibition of serotonin-induced oocyte maturation by aSpisula factor. J. Exp. Zool. 1990, 255, 229–243. https://doi.org/10.1002/jex.1402550212.

179. Iwamatsu, T.; Taya, Y.; Sakai, N.; Terada, Y.; Nagata, R.; Nagahama, Y. Effect of 5-hydroxytryptamine on steroidogenesis and oocyte maturation in pre-ovulatory follicles of the medaka Orzyiaslatipes: (medaka-steroidogenesis/5-hydroxytryptamine/foillice/oocyte maturation). Dev. Growth Differ. 1993, 35, 625–630.

180. Cerda, J.; Petrino, T.R.; Lin, Y.-W.P.; Wallace, R.A. Inhibition of Fundulusheteroclitus oocyte maturation in vitro by serotonin (5-hydroxytryptamine). J. Exp. Zool. 1995, 273, 224–233. https://doi.org/10.1002/jex.1402730307.

181. Tanabe, T.; Osada, M.; Kyozuka, K.; Inaba, K.; Kijima, A. A novel oocyte maturation arresting factor in the central nervous system of scallops inhibits serotonin-induced oocyte maturation and spawning of bivalve mollusks. Gen. Comp. Endocrinol. 2006, 147, 352–361. https://doi.org/10.1016/j.ygcend.2006.02.004.

182. Tomy, S.; Saikrithi, P.; James, N.; Balasubramanian, C.; Panigrahi, A.; Otta, S.K.; Subramoniam, T.; Ponniah, A. Serotonin induced changes in the expression of ovarian gene network in the Indian white shrimp, Penaeus indicus. Aquaculture 2016, 452, 239–246. https://doi.org/10.1016/j.aquaculture.2015.11.003.

183. Trant, J.M.; Thomas, P. Isolation of a novel maturation-inducing steroid produced in vitro by ovaries of Atlantic croaker. Gen. Comp. Endocrinol. 1989, 75, 397–404. https://doi.org/10.1016/0016-6480(89)90174-3.

184. Schulz, R.W.; de Franca, L.R.; Lareyre, J.J.; LeGac, F.; Chiarini-Garcia, H.; Nobrega, R.H.; Miura, T. Spermatogenesis in fish. Gen. Comp. Endocrinol. 2010, 165, 390–411.

185. Murugananthkumar, R.; Akhila, M.; Rajakumar, A.; Mamta, S.; Sudhakumari, C.; Senthilkumaran, B. Molecular cloning, expression analysis and transcript localization of testicular orphan nuclear receptor 2 in the male catfish, Clariasbatrachus. Gen. Comp. Endocrinol. 2016, 239, 71–79. https://doi.org/10.1016/j.ygcen.2015.10.009.

186. Murugananthkumar, R.; Senthilkumaran, B. Expression analysis and localization of wt1, ad4bp/sf-1 and gata4 in the testis of catfish, Clariasbatrachus: Impact of wt1-siRNA silencing. Mol. Cell. Endocrinol. 2016, 431, 164–176. https://doi.org/10.1016/j.j.mce.2016.05.006.

187. Laldinsangi, C.; Senthilkumaran, B. Identification, cloning and expression profile of sycp3 during gonadal cycle and after siRNA silencing in catfish. Gene Rep. 2018, 10, 54–65. https://doi.org/10.1016/j.genrep.2017.10.009.
190. Dan, C.; Lin, Q.; Gong, G.; Yang, T.; Xiong, S.; Xiong, Y.; Huang, P.; Gui, J.-F.; Mei, J. A novel PDZ domain-containing gene is essential for male sex differentiation and maintenance in yellow catfish (Peleobagrus fulvidraco). *Sci. Bull.* 2018, 63, 1420–1430. https://doi.org/10.1016/j.scib.2018.08.012.

191. Rajakumar, A.; Senthilkumaran, B. Sox3 binds to 11β-hydroxysteroid dehydrogenase gene promoter suggesting transcriptional interaction in catfish. *J. Steroid Biochem. Mol. Biol.* 2016, 158, 90–103. https://doi.org/10.1016/j.jsbmb.2016.01.003.

192. Stocco, D.M. The role of the STAP protein in steroidogenesis: Challenges for the future. *J. Endocrinol.* 2000, 164, 247–253. https://doi.org/10.1677/joe.0.1640247.

193. Geslin, M.; Auperin, B. Relationship between changes in mRNAs of the genes encoding steroidogenic acute regulatory protein and P450 cholesterol side chain cleavage in head kidney and plasma levels of cortisol in response to different kinds of acute stress in the rainbow trout (*Oncorhynthus mykiss*). *Gen. Comp. Endocrinol.* 2004, 135, 70–80. https://doi.org/10.1016/s0016-6480(03)00283-1.

194. Sreenivasulu, G.; Sridevi, P.; Sahoo, P.K.; Swapna, I.; Ge, W.; Kirubagarasan, R.; Dutta-Gupta, A.; Senthilkumaran, B. Cloning and expression of STAP during gonadal cycle and hCG-induced oocyte maturation of air-breathing catfish, Clariasgariepi-nisi. *Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol.* 2009, 154, 6–11.

195. Nakamoto, M.; Fukasawa, M.; Tanaka, S.; Shimamori, K.; Suzuki, A.; Matsuda, M.; Kobayashi, T.; Nagahama, Y.; Shibata, N. Expression of 3β-hydroxysteroid dehydrogenase (hsd3b), star and ad4bp/sf-1 during gonadal development in medaka (*Oryziaslatipes*). *Gen. Comp. Endocrinol.* 2012, 176, 222–230.

196. Rajakumar, A.; Senthilkumaran, B. Expression analysis of cyp11a1 during gonadal development, recrudescence and after hCG induction and sex steroid analog in the catfish, Clariasbracthus. *Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol.* 2014, 176, 46–47. https://doi.org/10.1016/j.cbpb.2014.07.007.

197. Wu, J.J.; Zhou, Y.L.; Wang, Z.W.; Li, G.H.; Jin, F.P.; Cui, L.L.; Gao, H.T.; Li, X.P.; Zhou, L.; Gui, J.F. Comparative transcriptome analysis reveals differentially expressed genes and signaling pathways between male and female red-tail catfish (*Mystus-wycki-oides*). *Mar. Biotechnol.* 2019, 21, 463–474.

198. Lin, X.; Zhou, D.; Zhang, X.; Li, G.; Zhang, Y.; Huang, C.; Zhang, Z.; Tian, C. A First Insight into the Gonad Transcriptome of Hong Kong Catfish (*Clariasfuscus*). *Animals* 2021, 11, 1131. https://doi.org/10.3390/ani11041131.

199. Shen, F.; Long, Y.; Li, F.; Ge, G.; Song, G.; Li, Q.; Qiao, Z.; Cui, Z. De novo transcriptome assembly and sex-biased gene expression in the gonads of Amur catfish (*Silurusasotus*). *Genomics* 2020, 112, 2603–2614. https://doi.org/10.1016/j.jgeno.2020.01.026.

200. Sun, F.; Liu, S.; Gao, X.; Jiang, Y.; Perera, D.; Wang, X.; Li, C.; Sun, L.; Zhang, J.; Kaltenboeck, L.; et al. Male-Biased Genes in Catfish as Revealed by RNA-Seq Analysis of the Testis Transcriptome. *PLoS ONE* 2013, 8, e68452. https://doi.org/10.1371/journal.pone.0068452.

201. Zeng, Q.; Liu, S.; Yao, J.; Zhang, Y.; Yuan, Z.; Jiang, C.; Chen, A.; Fu, Q.; Su, B.; Dunham, R.; et al. Transcriptome display during testicular differentiation of channel catfish (*Ictalurus punctatus*) as revealed by RNA-Seq analysis. *Biol. Reprod.* 2016, 95, 19.

202. Zhang, J.; Ma, W.; Song, X.; Lin, Q.; Gui, J.-F.; Mei, J. Characterization and Development of EST-SSR Markers Derived from Transcriptome of Yellow Catfish. *Molecules* 2019, 14, 16402–16415. https://doi.org/10.3390/molecules191016402.

203. Jing, J.; Wu, J.; Liu, W.; Xiong, S.; Ma, W.; Zhang, J.; Wang, W.; Gui, J.-F.; Mei, J. Sex-Biased miRNAs in Gonad and Their Potential Roles for Testis Development in Yellow Catfish. *PLoS ONE* 2014, 9, e107946. https://doi.org/10.1371/journal.pone.0107946.

204. Lu, J.; Luan, P.; Zhang, X.; Xue, S.; Peng, L.; Mahbooband, S.; Sun, X. Gonadal transcriptomic analysis of yellow catfish (*Peleobagrusfulvidraco*): Identification of sex-related genes and genetic markers. *Physiol. Genom.* 2014, 46, 798–807. https://doi.org/10.1152/physiolgenomics.00088.2014.

205. Chen, X.; Mei, J.; Wu, J.; Jing, J.; Ma, W.; Zhang, J.; Dan, C.; Wang, W.; Gui, J.-F. A Comprehensive Transcriptome Provides Candidate Genes for Sex Determination/Differentiation and SSR/SNP Markers in Yellow Catfish. *Mar. Biotechnol.* 2015, 17, 190–198. https://doi.org/10.1007/s10126-014-0960-7.

206. Doyon, Y.; McCammon, J.M.; Miller, J.C.; Faraji, F.; Ngo, C.; Katibah, G.E.; Amora, R.; Hocking, T.D.; Zhang, L.; Rebar, E.J.; et al. Heritable targeted gene disruption in zebrafish using designed zinc-finger nucleases. *Nat. Biotechnol.* 2008, 26, 702–708. https://doi.org/10.1038/nbt1409.

207. Sander, J.D.; Cade, L.; Khayter, C.; Reyon, D.; Peterson, R.T.; Joung, J.K.; Yeh, J.-R.J. Targeted gene disruption in somatic zebrafish cells using engineered TALENs. *Nat. Biotechnol.* 2011, 29, 697–698. https://doi.org/10.1038/nbt.1934.

208. Hruscha, A.; Krawitz, P.; Rechenberg, A.; Heinrich, V.; Hecht, J.; Haass, C.; Schmid, B. Efficient CRISPR/Cas9 genome editing with low off-target effects in development. *Development* 2013, 140, 4982–4987.

209. Dong, Z.; Ge, J.; Xu, Z.; Dong, X.; Cao, S.; Pan, J.; Zhao, Q. Generation of myostatin B knockout yellow catfish (*Ta-chysurusfulvidraco*) using transcription activator-like effector nucleases. *Zebrafish* 2014, 11, 265–274.

210. Li, M.; Wang, D. Gene editing nuclease and its application in tilapia. *Sci. Bull.* 2017, 62, 165–173. https://doi.org/10.1016/j.scib.2017.01.003.

211. Khalil, K.; Elayat, M.; Khalila, E.; Daghsh, S.; Elaswad, A.; Miller, M.; Abdelrahman, H.; Elsayed, K.; Odin, R.; Drescher, D.; et al. Generation of Myostatin Gene-Edited Channel Catfish (*Ictalurus punctatus*) via Zygote Injection of CRISPR/Cas9 System. *Sci. Rep.* 2017, 7, 7301. https://doi.org/10.1038/s41598-017-07223-7.

212. Zhu, B.; Pardeshi, L.; Chen, Y.; Ge, W. Transcriptomic Analysis for Differentially Expressed Genes in Ovarian Follicle Activation in the Zebrafish. *Front. Endocrinol.* 2018, 9, 593. https://doi.org/10.3389/fendo.2018.00593.
213. Li, J.; Ge, W. Zebrafish as a model for studying ovarian development: Recent advances from targeted gene knockout studies. *Mol. Cell. Endocrinol.* 2020, 507, 110778. https://doi.org/10.1016/j.mce.2020.110778.

214. Simora, R.M.C.; Xing, D.; Bangs, M.R.; Wang, W.; Ma, X.; Su, B.; Khan, M.G.Q.; Qin, Z.; Lu, C.; Alston, V.; et al. CRISPR/Cas9-mediated knock-in of alligator cathelicidin gene in a non-coding region of channel catfish genome. *Sci. Rep.* 2020, 10, 22271. https://doi.org/10.1038/s41598-020-79409-5.

215. Fricke, T.; Smalakyte, D.; Lapinski, M.; Pateria, A.; Weige, C.; Pastor, M.; Kolano, A.; Winata, C.; Siksnys, V.; Tamulaitis, G.; et al. Targeted RNA Knockdown by a Type III CRISPR-Cas Complex in Zebrafish. *CRISPR J.* 2020, 3, 299–313. https://doi.org/10.1089/crispr.2020.0032.

216. Qin, Z.; Li, Y.; Su, B.; Cheng, Q.; Ye, Z.; Perera, D.A.; Fobes, M.; Shang, M.; Dunham, R.A. Editing of the Luteinizing Hormone Gene to Sterilize Channel Catfish, Ictalurus punctatus, Using a Modified Zinc Finger Nuclease Technology with Electroporation. *Mar. Biotechnol.* 2016, 18, 255–263. https://doi.org/10.1007/s10126-016-9687-7.

217. Trede, N.S.; Langenau, D.M.; Traver, D.; Look, A.T.; Zon, L.I. The use of zebrafish to understand immunity. *Immunity* 2004, 20, 367–379.

218. Tokumoto, T. Identification of membrane progestin receptors (mPR) in goldfish oocytes as a key mediator of steroid non-genomic action. *Steroids* 2012, 77, 1013–1016. https://doi.org/10.1016/j.steros.2012.04.006.

219. Summerton, J.E. Morpholino, siRNA, and S-DNA Compared: Impact of Structure and Mechanism of Action on Off-Target Effects and Sequence Specificity. *Curr. Top. Med. Chem.* 2007, 7, 651–660. https://doi.org/10.2174/156802607780487740.

220. Prathibha, Y.; Senthilkumaran, B. Involvement of pax2 in ovarian development and recrudescence of catfish: A role in steroidogenesis. *J. Endocrinol.* 2016, 231, 181–195. https://doi.org/10.1530/joe-16-0103.

221. Anitha, A.; Senthilkumaran, B. Role of sox30 in regulating testicular steroidogenesis of common carp. *J. Steroid Biochem. Mol. Biol.* 2020, 204, 105769. https://doi.org/10.1016/j.jsbmb.2020.105769.

222. Sreenivasulu, G., Senthilkumaran, B., Sridevi, P., Rajakumar, A., Rasheeda, M. K. Expression and immunolocalization of 20β-hydroxysteroid dehydrogenase during testicular cycle and after hCG induction, in vivo in the catfish, Clarias gariepinus. *General and comparative endocrinology* 2012, 175(1), 48–54.