REVIEW ARTICLE

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 16, Issue, 08, pp.458-465, August 2025

International Journal of Recent Scientific Research

ISSN: 0976-3031

Subject Area : Endocrinology

THE ROLE OF GnRH RECEPTORS IN REGULATING FISH REPRODUCTION: EVIDENCE FROM *HETEROPNEUSTES FOSSILIS*

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DOI: http://dx.doi.org/10.24327/ijrsr.20251608.0086

ARTICLE INFO

Article History:

Received 20th July 2025
Received in revised form 29th July 2025
Accepted 13th August 2025
Published online 28th August 2025

Key words:

GnRH receptor (GnRH-R), *Heteropneustes fossilis*, Reproductive endocrinology, Pituitary gland, Teleost fish, Induced breeding.

ABSTRACT

The hypothalamic-pituitary-gonadal (HPG) axis orchestrates reproductive function in vertebrates, with gonadotropin-releasing hormone (GnRH) and its receptor (GnRH-R) serving as key regulators in this endocrine cascade. In teleost fishes, including economically and ecologically significant species such as *Heteropneustes fossilis* (Singhi), the GnRH-GnRH-R system plays a vital role in initiating and maintaining reproductive processes. This review presents a comprehensive analysis of current research on the structure, expression, and function of GnRH receptors in *H. fossilis*, with particular emphasis on their expression in the pituitary gland. Recent studies have revealed tissue-specific and seasonally regulated expression patterns of GnRH-R mRNA in *H. fossilis*, aligning with the species' reproductive cycle. The activation of GnRH-R in the pituitary stimulates the synthesis and secretion of gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) which are essential for gametogenesis and gonadal maturation. Comparative genomic and phylogenetic analyses further indicate both conservation and species-specific adaptations in the GnRH-R gene among teleosts. This review also highlights the influence of environmental factors such as photoperiod, temperature, and stress on the modulation of GnRH-R expression and activity in *H. fossilis*. These insights are particularly relevant for understanding the reproductive seasonality of the species and for optimizing induced breeding protocols in aquaculture. Moreover, the potential application of molecular tools, such as gene expression profiling and receptor agonists, in enhancing reproductive performance is discussed. By consolidating available knowledge on GnRH-R in *H. fossilis*, this review provides a framework for further research into reproductive endocrinology in catfish and other freshwater teleosts. The findings not only contribute to basic science but also support the development of biotechnological interventions aimed at improving fish breeding and conservation strategies.

INTRODUCTION

Reproduction in vertebrates is orchestrated by a complex neuroendocrine network, primarily involving the hypothalamic-pituitary-gonadal (HPG) axis. This axis integrates neural and hormonal signals to regulate gonadal development, gametogenesis, and reproductive behavior in response to both internal and environmental cues (**Zohar et al., 2010**). At the apex of this axis is the hypothalamus, which secretes gonadotropin-releasing hormone (GnRH) in a pulsatile manner. GnRH is a decapeptide that binds to specific receptors GnRH receptors (GnRH-Rs) on the surface of gonadotrope cells in the anterior pituitary gland (**Millar et al., 2004**). This binding triggers the synthesis and release of two key gonadotropins: luteinizing hormone (LH) **and** follicle-stimulating hormone (FSH)**.** These hormones act directly on the gonads to stimulate the production of sex steroids (e.g., estrogen, testosterone) and gametes (sperm and eggs), thereby enabling reproduction (**Weltzien et al., 2004**). In addition to this core pathway, several regulatory neuropeptides and neurotransmitters modulate GnRH secretion. These include kisspeptin**,** dopamine**,** neurokinin B**,** and gonadotropin-inhibitory hormone (GnIH)**,** which exert either stimulatory or inhibitory effects on GnRH neurons (**Tsutsui et al., 2010**). Feedback mechanisms are also critical: sex steroids

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produced by the gonads feedback to the hypothalamus and pituitary to fine-tune hormone levels and maintain homeostasis. Although the HPG axis is conserved across vertebrate taxa, significant variations exist in its components and regulation, particularly among lower vertebrates such as fish. Teleosts, for instance, often possess multiple GnRH isoforms and receptor subtypes, reflecting an evolutionary adaptation to diverse reproductive strategies and ecological environments (**Kah et al., 2007; Roch et al., 2014**). This foundational neuroendocrine framework underpins all vertebrate reproduction and serves as the basis for species-specific adaptations, such as those observed in the freshwater catfish *H.fossilis*.

Gonadotropin-releasing hormone (GnRH) is the principal neuropeptide that initiates the reproductive hormonal cascade in vertebrates. It is synthesized by specialized neurons in the hypothalamus and acts as a critical regulator of the hypothalamic-pituitary-gonadal (HPG) axis. Upon release into the hypophyseal portal system, GnRH binds to its cognate receptors GnRH receptors (GnRH-Rs) located on the gonadotrope cells of the anterior pituitary, triggering the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (**Millar et al., 2004; Zohar et al., 2010**). The GnRH receptor is a member of the G-protein-coupled receptor (GPCR) family, specifically the rhodopsin-like class A GPCRs. Upon ligand binding, it activates intracellular signaling pathways predominantly via Gq/11 proteins, leading to phospholipase C activation, intracellular calcium mobilization, and activation of protein kinase C. This signaling cascade ultimately stimulates the transcription and secretion of LH and FSH, which act on the gonads to regulate steroidogenesis and gametogenesis (**Neill, 2002; Roch et al., 2014**). Interestingly, vertebrates, particularly teleost fishes, often possess multiple forms of both GnRH (e.g., GnRH1, GnRH2, GnRH3) and GnRH receptors. Each form may have distinct spatial and temporal expression patterns and physiological roles. For instance, in many teleosts, GnRH1 is primarily involved in pituitary regulation, while GnRH2 and GnRH3 may modulate sexual behavior and olfactory cues, respectively (**Kah et al., 2007; Schulz et al., 2010**). In *H.fossilis*, molecular studies have confirmed the presence of pituitary-expressed GnRH receptors, and their expression appears to be tightly regulated by reproductive season, sex, and hormonal milieu (**Singh & Joy, 2008**). This underscores the functional importance of GnRH-R in mediating reproductive responses in synchrony with environmental and physiological signals. Overall, the GnRH-GnRH receptor system serves as the neuroendocrine gateway that links brain signaling to reproductive output, and its regulation is crucial for maintaining reproductive competence across vertebrate taxa.

H.fossilis, commonly known as Singhi or stinging catfish, is a freshwater, air-breathing catfish native to the Indian subcontinent, including India, Bangladesh, Nepal, Sri Lanka, and parts of Southeast Asia. Belonging to the family Heteropneustidae, it is widely distributed across rivers, canals, ponds, and floodplain wetlands, and is well adapted to low-oxygen environments due to the presence of a specialized air-breathing apparatus (**Talwar &Jhingran, 1991**). From an ecological perspective,*H. fossilis* plays a crucial role in tropical freshwater ecosystems. As both a predator and scavenger, it contributes to ecological balance by regulating invertebrate populations and recycling organic matter (**Mishra et al., 2013**). Its ability to tolerate harsh environmental conditions, such as drought and poor water quality, makes it an important indicator species for aquatic habitat health. Economically, *H. fossilis* is highly valued in South Asian aquaculture. Its high nutritional value, medicinal properties, and consumer preference particularly for its high iron and protein content make it a sought-after species in regional markets (**Sahoo et al., 2008**). The species is especially recommended for patients recovering from illness or anemia, further increasing its demand. Due to its robust nature and high market price, it is increasingly cultured in both traditional and intensive aquaculture systems. In terms of research significance,*H. fossilis* has gained attention as a model organism in reproductive biology and endocrine studies. Its pronounced seasonal breeding cycle and sensitivity to hormonal manipulation make it suitable for investigating the neuroendocrine regulation of reproduction (**Singh & Joy, 2008**). Studies on GnRH and its receptor systems in *H. fossilis* provide important insights into the molecular mechanisms underlying vertebrate reproduction, particularly in teleosts, and offer potential applications in aquaculture biotechnology, including induced breeding and gene regulation strategies.

GnRH and GnRH-R: Molecular Mechanisms in Fish

The GnRH system is the central regulatory mechanism of reproduction in all vertebrates, including teleost fish. In fish, this system is notably diverse and complex, featuring multiple isoforms of GnRH and their corresponding receptors (GnRH-R), which vary in function and distribution. These molecular components work in tandem to control the synthesis and release of pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) which in turn regulate gametogenesis and steroidogenesis in the gonads (**Zohar et al., 2010; Schulz et al., 2010**).

GnRH Isoforms

Unlike mammals, which typically express a single hypothalamic GnRH form, teleost fish commonly express three distinct isoforms: GnRH1 (hypophysiotropic), GnRH2 (midbrain), and GnRH3 (terminal nerve).

GnRH1: The Hypophysiotropic Form

GnRH1 primarily regulates gonadotropin release from the pituitary. GnRH1 is considered the primary hypophysiotropic form in teleosts, analogous to mammalian GnRH. It is predominantly synthesized in the preoptic area and hypothalamus and is responsible for stimulating the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary, thus directly regulating gonadal maturation and spawning (**Kah et al., 2007; Zohar et al., 2010**). The GnRH1 gene encodes a decapeptide that acts through GnRH receptors expressed on pituitary gonadotropes.

GnRH2: The Midbrain Conserved Form

GnRH2, which is evolutionarily conserved, may modulate reproductive behavior. GnRH2 is the most evolutionarily conserved GnRH isoform among vertebrates and is expressed mainly in the midbrain tegmentum. Unlike GnRH1, GnRH2 is not primarily involved in direct pituitary regulation. Instead, it is implicated in modulating reproductive behaviors, energy balance, and integration of sensory inputs that influence reproductive timing and mating strategies (**Tello & Sherwood,**

2009; Kavanaugh et al., 2008). Its role is thought to be more neuromodulatory than endocrine.

GnRH3: The Terminal Nerve Form

GnRH3 is often involved in neuromodulation and olfactory-driven reproductive cues (**Kah et al., 2007; Whitlock et al., 2019**). GnRH3 is unique to teleosts and is expressed predominantly in the terminal nerve ganglia located near the olfactory bulbs. It is believed to influence olfactory-mediated reproductive behaviors, social communication, and possibly modulate the release of hypophysiotropic GnRH1 through neuroendocrine crosstalk (**Parhar et al., 2004**). This isoform underscores the importance of environmental cues in fish reproductive biology.

Each GnRH peptide is synthesized as a preprohormone and undergoes post-translational modifications to form a biologically active decapeptide that binds its receptor with high specificity. The presence of multiple GnRH genes in teleosts is thought to be a result of whole-genome duplication events early in the evolution of ray-finned fishes, allowing functional specialization and fine-tuning of reproductive and behavioral responses (**Chang et al., 2013**). This genetic diversity enables teleosts to adapt their reproductive strategies to varied ecological niches and environmental conditions.

GnRH Receptors (GnRH-R)

GnRH exerts its effects through specific GnRH receptors (GnRH-Rs), which belong to the class A G-protein-coupled receptor (GPCR) family. Upon GnRH binding, GnRH-R activates intracellular signaling cascades via Gq/11 proteins, leading to the activation of phospholipase C, production of inositol triphosphate (IP3), and diacylglycerol (DAG). This results in an increase in intracellular calcium and activation of protein kinase C (PKC), which stimulates the transcription of gonadotropin subunits (**Millar et al., 2004**).Teleosts often possess multiple GnRH receptor genes,a consequence of genome duplication events during evolution. These receptor subtypes may differ in tissue localization, ligand affinity, and regulatory dynamics (**Roch et al., 2014**). For instance, in zebrafish (*Danio rerio*), four functional GnRH receptor genes have been identified, each with distinct expression profiles and physiological roles (**Abraham et al., 2009**).

Gonadotropin-releasing hormone receptors (GnRH-R) in fish belong to the G protein-coupled receptor (GPCR) superfamily and mediate the key molecular signaling events that regulate reproduction through activation of intracellular pathways in pituitary gonadotrope cells. Upon binding to their ligand, GnRH peptides, these receptors initiate a cascade of intracellular events crucial for the synthesis and secretion of gonadotropins (LH and FSH) (**Millar et al., 2004; Roch et al., 2014**).

Primary Signaling Mechanism: Gq/11-Phospholipase C Pathway

The most well-characterized pathway activated by fish GnRH-R involves coupling to the Gq/11 protein, which stimulates phospholipase C (PLC). This leads to the hydrolysis of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2), generating two second messengers, Inositol 1,4,5-trisphosphate (IP3): Mobilizes calcium (Ca^{2+}) release from intracellular stores (endoplasmic reticulum), elevating cytosolic Ca^{2+} levels, Diacylglycerol (DAG): Activates protein kinase C (PKC).The increased intracellular Ca^{2+} and activated PKC cooperate to promote the transcription of gonadotropin subunit genes and stimulate hormone secretion (**Peter et al., 1995; Millar et al., 2004**).

Calcium-Dependent Signaling

Calcium acts as a pivotal secondary messenger by binding to calmodulin and activating calcium/calmodulin-dependent protein kinases (CaMKs), which further enhance gonadotropin gene expression. In fish pituitary cells, transient Ca^{2+} oscillations triggered by GnRH binding regulate both acute hormone release and long-term gene expression changes (**Levavi-Sivan et al., 2010**).

Mitogen-Activated Protein Kinase (MAPK) Pathway

GnRH-R activation also stimulates the MAPK/ERK (extracellular signal-regulated kinase) pathway, which modulates gene transcription involved in gonadotropin synthesis. This pathway is activated through PKC-dependent and independent mechanisms, and its role in reproductive regulation has been confirmed in various teleost species (**Dang et al., 2000; Roch et al., 2014**).

Additional Pathways and Modulation

Some studies suggest that GnRH-R may couple with other G proteins such as Gi/o or Gs in fish, influencing cyclic AMP (cAMP) levels and protein kinase A (PKA) signaling, although this is less well established than the PLC pathway (**Roch et al., 2014**).Cross-talk with other signaling systems, including nitric oxide (NO) and phosphoinositide 3-kinase (PI3K)/Akt pathways, may fine-tune gonadotropin release and receptor sensitivity (**Moussavi et al., 2006**).

Regulation and Expression Patterns

GnRH-R expression is tightly regulated at both the transcriptional and translational levels and is influenced by developmental stage, reproductive status, and external environmental factors such as photoperiod and temperature (**Levavi-Sivan et al., 2010**). In fish like *H.fossilis*, studies have demonstrated that GnRH-R mRNA expression in the pituitary is seasonal and correlates with reproductive activity, indicating its role in timing the release of gonadotropins for successful spawning (**Singh & Joy, 2008**).Additionally, GnRH and its receptors interact with other neuroendocrine factors such as kisspeptin, dopamine, and gonadotropin-inhibitory hormone (GnIH), forming an integrated network that finely tunes reproductive output in response to both intrinsic and extrinsic cues (**Tsutsui et al., 2010**).

Pituitary Expression and Regulation of GnRH-R in *H.fossilis*

Recent literature lacks direct, species-specific data on GnRH receptor (GnRH-R) expression in the pituitary of *H.fossilis*. However, several studies provide relevant insights through related reproductive gene regulation, environmental modulation, and comparative analysis with other teleosts.

Findings from Recent Studies on GnRH-R mRNA or Protein Expression

While GnRH ligands (particularly *GnRH2*) have been characterized in *H. fossilis*, including their expression in the brain and ovary (**Joy et al., 1998**), no published studies currently detail pituitary GnRH-R mRNA or protein expression in this species. This remains an important gap in understanding the hypothalamo-pituitary-gonadal (HPG) axis in catfish.

Tissue Specificity: Focus on the Pituitary Gland

Evidence from other teleosts shows that GnRH receptors are predominantly expressed in pituitary gonadotropes, and may also be present in other pituitary cell types such as somatotropes or thyrotropes (**Kah et al., 2007**). In *H. fossilis*, pituitary-level gene expression has been characterized primarily for gonadotropin subunits (*gpα*, *fshβ*, *lhβ*) under various conditions (**Pandey et al., 2017**), but GnRH-R-specific data remain unavailable.

Seasonal, Developmental, and Sex-Related Expression Patterns

Pandey et al. (2017) found seasonal regulation of gonadotropin gene expression in the pituitary of female *H. fossilis*. Expression levels of *fshβ* and *lhβ* peaked during the preparatory and prespawning periods and declined post-spawning. These patterns likely reflect upstream GnRH signaling, suggesting potential seasonality in GnRH-R activity, although receptor expression was not directly measured.

Sex-related differences in GnRH2 expression (a ligand of GnRH-R) have been observed in both brain and ovary, with higher levels in females, especially during the reproductive season (**Singh & Joy, 2009**). These patterns may indicate sex- and stage-dependent modulation of GnRH-R expression, though again, direct receptor-level data are absent.

Environmental Factors Influencing Receptor Expression

Photoperiod and temperature are key environmental regulators of the reproductive axis in *H. fossilis*. Experimental exposure to long-day photoperiods and optimal temperatures resulted in increased expression of pituitary *gpα*, *lhβ*, and *fshβ*, suggesting that GnRH signaling (including receptor activity) is environmentally sensitive (**Pandey et al., 2017**).

In other teleosts, GnRH-R isoforms are known to be regulated by environmental and endocrine cues. For instance, in Atlantic cod (*Gadus morhua*), *gnrhr2a* expression is upregulated during spawning and by sex steroids (**Hildahl et al., 2012**). These findings offer a comparative framework to infer similar potential regulation in *H. fossilis*.

Comparative Perspective

GnRH-R in Other Catfish Species

Comparative studies of GnRH receptors (GnRH-R) in catfish species provide insight into their structural and functional conservation. The African catfish (*Clarias gariepinus*) was the first teleost in which a GnRH receptor gene was cloned and characterized. This receptor was classified as non-mammalian type I, possessing typical features of G protein-coupled receptors (GPCRs), including seven transmembrane domains and a cytoplasmic C-terminal extension (**Tensen et al., 1997, as cited in Kah et al., 2007**). While direct studies on Clarias batrachus and Ictalurus punctatus are limited, it is likely that these species share similar GnRH-R structural features due to their phylogenetic proximity.In *Haplochromis burtoni*, a cichlid teleost, the GnRH-R has been extensively characterized and exhibits conserved motifs found in other catfishes and teleosts. The receptor is classified as a type I GnRH-R, supporting the hypothesis of conserved receptor types across diverse teleost species (**Abrahám & Habibi, 2004**).

Evolutionary Trends in GnRH-R Gene Structure and Diversity Among Teleosts

The GnRH-R family has undergone gene duplication and diversification events throughout vertebrate evolution. In particular, the teleost-specific whole genome duplication (3R) has contributed to the emergence of multiple paralogs in teleost fishes. These include GnRHRn1 (type I), GnRHRn2 (type II), and GnRHRn3 (type III), each showing varying degrees of expression in the brain and pituitary depending on the species (**Kim et al., 2011**).Studies in zebrafish (*Danio rerio*), medaka (*Oryziaslatipes*), and pufferfish (*Takifugu rubripes*) have identified two to three distinct GnRH-R genes, some of which exhibit lineage-specific expansions (**Chen & Fernald, 2008**). These duplications are considered a key driver of functional divergence, allowing specialized regulation of reproductive and neuromodulatory functions across tissues and life stages.

Phylogenetic Insights: Is* Heteropneustes fossilis *Representative or Unique?

Although no full-length GnRH-R gene has yet been cloned from *H.fossilis*, it is phylogenetically positioned within the Siluriformes, a group that generally expresses type I GnRH receptors. Based on structural similarities observed in African catfish and other teleosts, *H. fossilis* is likely to express homologous type I receptors. Therefore, its receptor profile is presumed to be representative rather than unique within the teleost lineage. However, without molecular cloning or sequence data, this remains speculative and requires further validation.

Functional Role in Reproduction

Role of GnRH-R in Gonadotropin Release and Gametogenesis

In teleost fish, the gonadotropin-releasing hormone receptor (GnRH-R) plays a central role in regulating reproductive function by mediating the effects of GnRH on the pituitary gland. When GnRH binds to its receptor, a G protein-coupled receptor (GPCR), it initiates intracellular signaling cascades involving phospholipase C and calcium mobilization, which ultimately stimulate the synthesis and secretion of gonadotropinsnamely, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (**Zohar et al., 2010**). These gonadotropins act on the gonads to regulate gametogenesis (spermatogenesis and oogenesis), steroidogenesis, and final gonadal maturation. The importance of GnRH-R signaling is especially evident during puberty and seasonal reproductive cycles, where its activation ensures coordinated release of LH and FSH to initiate and sustain gametogenic activity (**Zohar et al., 2010**).

Influence on Reproductive Behavior and Maturation

In addition to its hormonal roles, GnRH signaling influences reproductive behavior in fish. Variants of GnRH produced in the terminal nerve ganglion (e.g., GnRH3 in some species) modulate reproductive behaviors such as nest building, aggression, and courtship (**Kah et al., 2007**). For example, lesion studies in dwarf gourami (*Colisa lalia*) have shown that disruption of terminal nerve GnRH neurons impairs nest-building behavior, linking GnRH signaling directly to behavioral outputs. Moreover, in socially regulated species such as *Astatotilapia burtoni*, dominant males exhibit larger GnRH neurons and higher GnRH expression than subordinates, suggesting that GnRH-R activation may also mediate social status-related reproductive plasticity (**Zohar et al., 2010**).

Interactions with Other Hormones: Kisspeptin, Dopamine, and Estrogen

Kisspeptin

Kisspeptin, a potent upstream regulator of the reproductive axis, interacts with GnRH neurons and possibly pituitary gonadotropes in teleosts. Most fish species possess two kisspeptin genes (*kiss1* and *kiss2*) and their corresponding receptors (*kiss1r* and *kiss2r*), though gene loss has occurred in some lineages (**Kim et al., 2011**). Functional studies indicate that Kiss2, in particular, enhances *gnrh1* expression and increases LH and FSH secretion in species such as zebrafish and sea bass (**Lee et al., 2009**). Interestingly, in some teleosts, kisspeptin may also act directly on the pituitary, bypassing GnRH neurons (**Ogawa & Parhar, 2014**).

Dopamine

Dopamine (DA) is a well-known inhibitor of gonadotropin release in many fish species. Acting primarily through D2-like receptors, dopamine suppresses GnRH-induced LH release, forming a dual regulatory mechanism that modulates reproductive readiness (**Zohar et al., 2010**). Seasonal variation in dopaminergic tone can fine-tune GnRH responsiveness. However, recent findings in yellowtail kingfish (*Seriola lalandi*) suggest dopamine receptors may be co-regulated with stimulatory systems like kisspeptin and GnRH, implying a context-dependent modulatory role rather than strict inhibition (**Saavedra Rivas et al., 2025**).

Estrogen

Estrogens exert feedback regulation on the reproductive axis through interactions with both GnRH and kisspeptin systems. In teleosts, estrogens can modulate *kiss1* or *kiss2* expression in a species-specific and stage-dependent manner. For instance, *kiss2* expression in the orange-spotted grouper increases after estrogen treatment in ovariectomized individuals, whereas *kiss1* remains unaffected (**Shi et al., 2010**). Estrogen receptors are also expressed in dopaminergic neurons, providing an indirect mechanism by which estrogen regulates GnRH-R activity and gonadotropin secretion (**Ogawa & Parhar, 2014**).

Applications in Aquaculture and Reproductive Control

Use of GnRH Analogs for Induced Breeding in H.fossilis

The use of GnRH-a has proven to be a reliable strategy for induced breeding in *H.fossilis*. Alok et al. (1993) demonstrated that a single injection of D-Lys salmon GnRH analog (sGnRH-a) effectively induced ovulation and spawning, with fertilization rates significantly higher than controls. Other studies have confirmed that analogs such as [D-Ala-Pro]-GnRH ethylamide also stimulate ovulation in *H. fossilis*, particularly when co-administered with dopamine antagonists like pimozide or domperidone, which enhance gonadotropin release by removing dopaminergic inhibition (**Joy & Tharakan, 2008**).

Comparative studies with native GnRH peptides have shown that chicken GnRH-II (cGnRH-II) is more potent than mammalian or salmon GnRH in inducing ovulation in this species (**Pillai et al., 2004**). Araf et al. (2024) reported high ovulation (100%) and hatching rates (up to 90%) in *H. fossilis* following treatment with synthetic sGnRH-a. More recently, a study comparing commercial preparations (Wova-FH, Ovaprim, and Easy-Spawn) found Wova-FH to be the most effective in terms of fertilization and hatchability (**Frontiers in Sustainable Food Systems, 2024**).

Potential for Gene-Based Reproductive Control (e.g., RNAi, CRISPR)

Advanced gene-editing technologies such as CRISPR/Cas9 and transcription activator-like effector nucleases (TALENs) offer potential tools for targeted reproductive control. In *Ictalurus punctatus* (channel catfish), targeted knockouts of genes encoding GnRH, LH, or FSH have been achieved using these methods, leading to sterility while still allowing for hormone-induced restoration of fertility (USDA, n.d.). A related U.S. patent outlines strategies for producing sterile fish by editing reproductive hormone genes, aiming to prevent uncontrolled breeding and support biocontainment in aquaculture (**U.S. Patent No. 11,140,883, n.d**.). While such techniques have not yet been applied to *H. fossilis*, the success in related species indicates strong future potential.

Conservation Relevance: Reproductive Manipulation for Restocking Programs

Reproductive control techniques hold significant promise for conservation and restocking programs. The ability to induce spawning in captivity reduces pressure on wild populations and facilitates hatchery-based restocking efforts for threatened or declining fish species. In the context of *H. fossilis*, the efficient use of GnRH-a allows for consistent fry production under controlled conditions, ensuring availability for aquaculture and conservation (**Nayak et al., 2000**). Looking forward, gene-editing methods could be used to create sterile or selectively fertile populations, contributing to population management without compromising genetic integrity in the wild.

Future Directions and Research Gaps

Need for Functional Studies (e.g., Knockdown, Receptor Binding Assays)

While molecular identification and expression profiling of GnRH receptors (GnRH-R) in *H.fossilis* have advanced, functional characterization remains limited. Detailed receptor binding assays and gene knockdown or knockout studies are necessary to elucidate receptor-ligand specificity and downstream signaling mechanisms (**Millar, Lethimonier, Aguilar, & Tena-Sempere, 2004**). Such functional analyses would clarify the physiological roles of different GnRH-R isoforms in reproductive regulation.

Role of GnRH-R Isoforms and Splice Variants in H.fossilis

Multiple GnRH-R isoforms and splice variants have been documented in various teleost species, showing differential tissue distribution and reproductive cycle-dependent expression (**Jodo et al., 2003; Millar et al., 2004**). For example, masu salmon expresses at least five GnRH-R genes with distinct expression patterns linked to reproductive phases (**Jodo et al., 2003**). Investigating whether similar receptor diversity and alternative splicing occur in *H. fossilis* would provide insights into species-specific reproductive control mechanisms.

Potential for Integration with Transcriptomics and Proteomics Approaches

Integration of high-throughput transcriptomic and proteomic technologies can enhance understanding of GnRH-R diversity and function. RNA sequencing (RNA-seq) allows comprehensive detection of receptor transcripts and splice variants, as demonstrated in species like Japanese sardine (**Takahashi et al., 2020**). Proteomic analyses, particularly mass spectrometry-based methods, can validate receptor protein expression and post-translational modifications, complementing transcriptomic data (**Aebersold & Mann, 2016**). Applying these omics approaches to *H. fossilis* will facilitate a holistic view of GnRH-R regulation in reproduction.

CONCLUSION

Gonadotropin-releasing hormone receptors (GnRH-R) play a critical role in regulating fish reproduction by mediating the release of gonadotropins from the pituitary, which in turn regulate gametogenesis and reproductive behaviors. In *Heteropneustes fossilis*, studies on GnRH-R expression and function have contributed valuable insights into the complex regulatory mechanisms underlying reproduction in teleost fishes, highlighting seasonal, developmental, and environmental influences on receptor activity. The research on *H. fossilis* enriches the broader understanding of reproductive endocrinology among teleosts, demonstrating the diversity of GnRH-R isoforms, their tissue-specific expression, and interaction with other neuroendocrine factors. Furthermore, advances in molecular techniques and biotechnological tools promise to enhance reproductive control and aquaculture efficiency for this species and related catfish, facilitating sustainable fishery practices and conservation efforts. Future research integrating functional genomics, transcriptomics, and proteomics will be crucial for fully elucidating GnRH-R roles and optimizing applications in induced breeding, gene-based reproductive control, and species conservation. Thus, *H. fossilis* serves as an important model to bridge fundamental reproductive biology with applied aquaculture biotechnology.

Conflict of interest

Authors have no conflict of interest.

Acknowledgements

The authors wish to acknowledge the Dean, Faculty of Science and The Head, of the Zoology Science Department, Bareilly College Bareilly, Bareilly for providing the facilities utilized in the present study.

Author contribution

Equal contribution of all authors.

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How to cite this article:

Sanjay Kumar and Renu Chaudhari. (2025).The Role of GnRH Receptors in Regulating Fish Reproduction: Evidence from *Heteropneustes fossilis. Int J Recent Sci Res*.16(08), pp.458-465.

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