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Multiplicity of gonadotropin-releasing hormone in fish

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Abstract
Gonadotropin-releasing hormone (GnRH) is the major neuroendocrine hormone regulating reproduction in vertebrates, including finfish. In recent years, presence of GnRH and their function in reproduction has been confirmed in shellfish, crustaceans and molluscs. Interestingly, shellfish GnRH do not cluster within finfish GnRH, suggesting diversity in *gnrh* genes and their evolution in animal kingdom. GnRH analogues have been synthesized chemically and successfully used in induced reproduction of finfish. Studies on GnRH potency in induced maturation and spawning of shellfish are limited. The present paper report the recent information of shellfish GnRH cDNAs that have been successfully isolated in number of molluscan species, and would help in the farming of shellfish under controlled conditions. Also, the paper highlights the scintometric mapping of GnRH research in fish, suggesting rapid accumulation of information on fish GnRH and their importance in fish reproductive biology and endocrinology.

Keywords: GnRH, finfish, shellfish, reproduction

Introduction

Gonadotropin-releasing hormone is a neuropeptide hormone produced in the brain and is involved in diverse functions, including reproduction in the vertebrates and invertebrates. Vertebrates possess multiple GnRH forms that are classified as GnRH-I, GnRH-II, and GnRH-III (Okubo *et al*., 2003; Chen and Fernald, 2008; Okubo and Nagahama, 2008; Matsuyama *et al*., 2013; Muñoz-Cueto *et al*., 2020) [59, 11, 61, 43, 48]. At least two GnRH forms are present in the brain of all vertebrates [(commonly cGnRH-II (GnRH-II), plus one of either GnRH-I or GnRH-III)] (Amano *et al*., 1997; Karigo and Oka, 2013) [4, 32]. Teleost fish of the order Perciformes were the first group of vertebrates in which three GnRH forms were found based on immunological and molecular characterization: salmon GnRH (sGnRH; GnRH-III), chicken GnRH-II (cGnRH-II; GnRH-II), and seabream GnRH (sbGnRH; GnRH-I) (Powell *et al*., 1994, 1996; Dubois *et al*., 2002; Lethimonier *et al*., 2004; Kah *et al*., 2007; Okubo and Nagahama, 2008; Selvaraj *et al*., 2013) [67-68, 13, 41, 31, 61, 78]. Particularly, GnRH neurons distributed in the preoptic area (POA) and the hypothalamus (HYP) regions of the brain are shown to be involved in the stimulation of pituitary gonadotrophic hormones (GtHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Yamamoto *et al*., 1998; Schulz *et al*., 2001; Swanson *et al*., 2003; Shahjahan *et al*., 2010) [103, 74, 90, 81]. These pituitary GtHs control the process of gametogenesis by activating the steriodogenic pathways resulting in the production of sex steroid hormones (Senthilkumaran *et al*., 1999; Nagahama and Yamashita, 2008; Rajakumar and Sethilkumaran, 2020) [79, 50, 70]. Unlike mammals, teleost fish lack a functional hypophyseal portal system and neuronal axonal fibers directly innervate anterior pituitary regions, where FSH and LH producing cells are localized. Recent studies indicate three molecular GnRH forms exist in the brain of cartilaginous elasmobranch fish (Powell *et al*., 1986; Wright and Demski, 1991; King *et al*., 1992; Gaillard *et al*., 2018) [69, 101, 36, 18].

Presence of GnRH and GnRH-like peptides has been demonstrated in primitive vertebrates, the lampreys and invertebrates, crustaceans and molluscs (Kavanaugh *et al*., 2008; Sharker *et al*., 2020) [33, 82]. Phylogenetic analysis show that these GnRH forms are falling under separate outgroups, resulting from duplications of an ancestral GnRH gene followed by point mutations, and that some forms have been lost in certain vertebrate lineages (Sherwood *et al*., 1986; Okubho and Nagahama, 2008) [83, 61]. Interestingly, neuroanatomical studies have demonstrated wide distribution of multiple GnRH forms in the brain of vertebrates and invertebrates, suggesting diverse functions of GnRH peptides.

GnRH-I, GnRH-II and GnRH-III forms in Teleost and Cartilaginous Fish

GnRH-I form is present in majority of teleost species (Sherwood *et al*., 1997; Fernald and White, 1999; Okubho and Nagahama, 2008) [84, 15, 61]. Presence of teleost GnRH-I form confirmed in the elasmobranch fish (Gaillard *et al*., 2018) [18]. Teleosts share the mammalian GnRH (mGnRH) form in the GnRH1 group with other vertebrates. However, different variant of GnRH-I form has been reported in medaka, *Oryzias latipes* (Okubo *et al*., 2000) [57]; pejerrey, *Odontesthes bonariensis* (Guilgur *et al*., 2003, 2009; Montaner *et al*., 2001) (pjGnRH or mdGnRH) [25, 27, 47]; herring, *Clupea harengus pallasi* (hrGnRH) (Carolsfeld *et al*., 2000; Sukhan *et al*., 2013) [10, 87] and whitefish, *Coregonus clupeaformis* (wfGnRH) (Vickers *et al*., 2004) [98]. Interestingly, the GnRH-I form is missing in some fish species where the hypophysiotropic function is accomplished by GnRH-III (Kobayashi *et al*., 1994; Kobayashi *et al*., 1997; Okuzawa and Kobayashi, 1999; Volkoff and Peter, 1999) [39, 40, 62, 99].

GnRH-II is represented in all vertebrates examined to date by chicken GnRH-II (cGnRH-II) form. GnRH-II is the midbrain tegmentum (MT) form, with a neuronal population in the MT region, mainly in nuclei of the medial longitudinal fasciculus (nMLF). However, fibers project widely into different brain regions, suggesting a role in neuromodulation (Amano *et al*., 1997; Okuzawa and Kobayashi, 1999; Munoz-Cueto *et al*., 2020) [4, 62, 48]. GnRH-II shows the widest pattern of distribution and found in all major Gnathostome lineages. GnRH-II has also been detected in the pituitary of goldfish, striped bass, zebrafish, African catfish, European eel, and Senegalese sole where direct innervation of the pars distalis occurs as in other teleosts; however, in the goldfish and zebrafish, the origin of the pituitary GnRH-II has been mapped to the mid-brain GnRH2 neurons, suggesting hypophysiotropic function of GnRH-II in these species (Yu *et al*., 1997; Kim *et al*., 1995; Gothilf *et al*., 1996; Kah *et al*., 2007; Servili *et al*., 2010; Goos *et al*., 1995, 1997) [105, 34, 23, 31, 80, 21-22]. GenBank accession nos. of finfish expressing three GnRH forms are shown in Table 1. The list of primers used for isolating GnRH-I variants in teleost fish are presented in Table 2.

GnRH-III is a teleost-specific form, expressed in neuronal populations mainly in the olfactory bulb (OB), terminal nerve ganglion (TNG) region, and POA. GnRH-III is characteristic to teleosts all generating the same fish-specific peptide. Like GnRH-II, GnRH-III axonal fibers project into different brain regions, suggesting a role in neuromodulation. Multiple functions have been suggested for different forms of GnRH. GnRH-III has also been detected in the pituitary of fish expressing two only GnRH-II and GnRH-III forms (King *et al*., 1990; Okubo *et al*., 1999; Suetake *et al*., 2000; Targersen *et al*., 2002; Muñoz-Cueto *et al*., 2020) [36, 58, 86, 93, 48]. Both GnRH-II and GnRH-III neurons also innervate to regions of peripheral and autonomic nervous system, suggesting diverse functions (Yu *et al*., 1997; Chen and Fernald, 2008; Umatani and Oka, 2019) [105, 11, 97]. Primers used for cloning GnRH-II and GnRH-III cDNAs in teleosts are presented in Table 3. The amino acid sequences of functional GnRH decapeptides in teleosts and elasmobranchs are shown in Table 4.

Functions of multiple GnRH forms

The functions of multiple GnRH forms have been revealed through immunocytochemical distribution of GnRH-immunoreactive (-ir) neurons and axonal fibers projecting to different regions of the brain and pituitary gland (Amano *et al*., 1997; Parhar, 1997; Yamamoto *et al*., 1998; Okubho *et al*., 2003; Muñoz-Cueto *et al*., 2020) [4, 65, 102, 59, 48]. Our previous studies in different fish species belonging to order Perciformes confirmed the presence of three GnRH forms using immunological and molecular cDNA cloning method (Selvaraj *et al*., 2009, 2012a,b; Nagase *et al*., 2010; Kitano *et al*., 2012; Sukhan *et al*., 2013; Imanaga *et al*., 2014) [75, 76-77, 51, 38, 87, 29]. Immunological characterization and neuroanatomical distribution of different GnRH forms revealed that GnRH-I-ir neurons are localized in the preoptic area and hypothalamic regions with –ir fibers projecting to the anterior pituitary region, where FSH and LH-ir cells are localized (Selvaraj *et al*., 2009) [75]. Sparse distribution of –ir fibers were detected in different brain regions with prominent immunodetection in the preoptic area and hypothalamus. GnRH-II-ir and GnRH-III-ir neurons were localized in the midbrain tegmentum and olfactory bulb regions, respectively. In contrast to GnRH-I-ir, GnRH-II and GnRH-III-ir fibers were detected in different regions suggesting their function in neuromodulation (Selvaraj *et al*., 2009) [75]. In the chub mackerel (*Scomber japonicus*) and Jack mackerel (*Trachurus japonicus*), seabream GnRH (sbGnRH) was the GnRH1 form (Selvaraj *et al*., 2012; Imanaga *et al*., 2014) [75, 29]. In the bambooleaf wrasse (*Pseudolabrus sieboldi*) and Japanese anchovy (*Engraulis japonicus*), medaka GnRH and herring GnRH were found to be GnRH1 forms (Nagase *et al*., 2010; Sukhan *et al*., 2013) [51, 87].

Expression changes of *gnrh* mRNAs and GnRH peptides during seasonal reproductive and spawning cycles indicated that in the females, brain *gnrh1* mRNA levels were low during immature phase when only perinucleolar oocytes were dominant in the ovary. Higher level of *gnrh1* mRNA was found during early and late vitellogenic phases, when the vitellogenic oocytes with yolk granules and oil droplets were prominent in the ovary. During post-spawning stage, no significant changes in the gnrh1 mRNA level were recorded. During immature, maturing and mature phases, GnRH1 peptide levels did not show any statistically significant differences; however, levels were higher during post-spawning stages. Messenger RNA and peptide levels of GnRH2 and GnRH3 did not show any significant differences between the maturity stages analyzed during the seasonal reproductive cycle. Similar, expression profiles were observed in the males. These results clearly indicated that GnRH1 form is involved in the seasonal reproduction of chub mackerel (Selvaraj *et al*., 2012a) [76].

During spawning cycle, mRNA levels of all three *gnrh* forms fluctuated suggesting involvement of multiple GnRH forms during the final oocyte maturation and spawning stages. Interestingly, levels of *gnrh2* and *gnrh3* mRNAs were significantly higher during germinal vesicle stage suggesting their involvement in prespawning stages of chub mackerel spawning cycle (Selvaraj *et al*., 2012b) [77]. The results are in agreement with immunocytochemical distribution of GnRH2 and GnRH3 immunoreactive fibers to different regions of the brain, indicating their role as neuromodulator (Selvaraj *et al*., 2009) [75]. During post-ovulation stage, only GnRH1 mRNA level was found to be higher suggesting their involvement in the progression of late vitellogenic follicles to final oocyte maturation, likely through stimulation of pituitary gonadotropins. Peptide levels of all three GnRH forms peaked during hydration stage of the spawning cycle, in agreement with the rise of *gnrh* mRNA levels during germinal vesicle stage, suggesting the machinery of transcription and translation active during these phases of final oocyte maturation, when the fish is ready to undergo mating process and ready to release the gametes. After spawning, lower GnRH peptide levels were observed in the brain and pituitary (Selvaraj *et al*., 2012b) [77].

In the Jack mackerel (*Trachurus japonicus*) that exhibit severe reproductive dysfunction in captivity, *gnrh1* mRNA expression was remarkably lower in the captive fish at all reproductive stages than in the wild fish. Levels of *gnrh2* and *gnrh3* mRNAs did not show any significant fluctuation, suggesting the dominant involvement of GnRH1 form in this carangid fish (Imanaga *et al*., 2014) [29]. In the bambooleaf wrasse and Japanese anchovy expressing different GnRH1 forms, *gnrh1* mRNA levels were higher in mature fish, compared to immature stage (Nagase *et al*., 2010; Sukhan *et al*., 2013) [51, 87]. In Indian pearlspot (*Etroplus suratensis*), *gnrh1* mRNA levels did not show any significant differences between reproductive stages analyzed, suggesting possibility of fluctuation of GnRH peptide levels in the brain and pituitary (Ezhilarasi *et al*., 2020, communicated) [14]. Analyses of messenger RNA and peptide levels performed in several teleosts clearly indicate that higher GnRH expression correlate with an increase in pituitary gonadotropins and circulating sex steroids, suggesting the activation of reproductive brain-pituitary-gonad axis (reviewed in Muñoz-Cueto *et al*., 2020) [48].

Research on function of GnRH in Indian fish species has revealed conservation of GnRH function in reproduction (Halder *et al*., 1995; Bhattacharya *et al*., 2002; Swapna *et al*., 2005, 2008; Nandi *et al*., 2007; Rather *et al*., 2015) [28, 6, 92, 53, 71]. Triploid catfish (*Heteropneustes fossilis*) exhibit significant decrease in size and number of GnRH-ir neurons of preoptic area and low immunoreactivity in pituitary in comparison to their diploid full-sibs during the late pre-spawning phase of ovarian cycle, suggesting reduced responsiveness of GnRH neurons to environmental cues required for gonadal development in triploids (Tiwary *et al*., 2002) [94]. In an Indian major carp, mrigal (*Cirrhinus mrigala*), intense GnRH-ir was observed in several olfactory receptor neurons (ORNs) and their axonal fibers as they extend over the olfactory nerve, spread in the periphery of the olfactory bulb (OB), and terminate in the glomerular layer, suggesting the involvement of GnRH in olfaction. GnRH immunoreactivity showed a seasonal pattern with highest in the prespawning phase, with significant reduction in the fiber density in the fish of spawning and the regressive phases (Biju *et al*., 2003) [7]. Further, ontogenic analyses indicated GnRH immunoreactive fibers are distributed in the olfactory nerve layer in the periphery of the bulb and glomeruli-like innervation was clearly established in 5 days old larvae of mrigal. The innervation to the olfactory bulb showed a considerable increase in GnRH immunoreactivity in 9 and 19 days old larvae. However, GnRH immunoreactivity in non-migratory as well as migratory components gradually diminished and disappeared altogether by the age of 68 days, suggesting GnRH functioning as a neurotransmitter (Biju *et al*., 2005) [8]. In the rohu, GnRH pathway genes have been revealed (Sahu *et al*., 2015) [73].

GnRH-IV in Lampreys

In lamprey three members of the GnRH family of neuropeptides have been identified: lamprey GnRH-I (lGnRH-I), lamprey GnRH-II (lGnRH-II), and lamprey GnRH-III (lGnRH-III). Lamprey GnRH forms has been demonstrated in number of species: lGnRH-I *Petromyzon marinus* (Sea lamprey); lGnRH-III *Mordacia mordax* (Australian lamprey); lGnRH-III *Geotria australis* (Pouched lamprey); lGnRH-III *Ichthyomyzon unicuspis* (Silver lamprey); lGnRH-III *Petromyzon marinus* (Sea lamprey); lGnRH-III *Lampetra appendix* (American brook lamprey); lGnRH-III *Lampetra tridentalis* (Pacific lamprey); lGnRH-III *Lampetra richardsoni* (Western brook lamprey) and lGnRH-III *Ichthyomyzon fossor* (Northern brook lamprey) (Kavanaugh *et al*., 2008; Sower and Baron, 2011; Nozaki *et al*., 2000; Tobet *et al*., 1995) [33, 85, 55, 95].

In the lamprey GnRH decapeptides amino acid residues 1, 2, 4, 9, and10 are common with the Gnathostome GnRH. Molecular phylogenetic analysis of the GnRH transcripts lGnRH-I and -III were initially classified as a fourth group of vertebrate GnRH peptides; however, recent approaches based on synteny analysis of GnRH paralogs suggest that lGnRH-I and -III belong likely to the type 3 GnRH group. lGnRH-II shows the highest similarity with Gnathostome sequences and it is suggested to be a direct descendant of an ancient GnRH form reported in vertebrates (Kavanaugh *et al*., 2008; Decatur *et al*., 2013) [33, 12]. Table 5 shows the amino acid sequences of GnRH decapeptides in lampreys.

Using antiserum generated against peptides of lamprey GnRHI-III, GnRH-II immunoreactive nerve fibers were found to originate from cells in the arc-shaped hypothalamic/preoptic areas and end at the neurohypophysis, suggesting similar morphological distribution (preopticohypophysial GnRH tract) as demonstrated in teleosts. The same study found distribution of GnRH cells in the hypothalamus and medulla oblongata regions, using insitu hybridization (Kavanaugh *et al*., 2008) [33]. Interestingly, lamprey GnRH-I and lamprey-III mRNAs are colocalized in the same cells in the preoptic nucleus/hypothalamic regions of adult lampreys as reported for GnRH1 and GnRH2 mRNAs colocalization in the preoptic area and hypothalamus of teleost fish (Root *et al*., 2005; Kavanaugh *et al*., 2008) [72, 33].

GnRH-V in Crustaceans and Molluscs

The existence of GnRH-like immunoreactive materials was demonstrated in shellfish including crustaceans (Guan *et al*., 2014; Suwansa-ard *et al*., 2016) [24, 88], bivalves (Pazos and Mathieu, 1999) [66], gastropods (Young *et al*., 1999; Zhang *et al*., 2000, 2008; Kim *et al*., 2017) [103, 105-106, 35] and cephalopods (Iwakoshi *et al*., 2002) [30]. Molluscan and other protostomian GnRHs have been allocated to the group GnRH V. Immunocytochemical studies have demonstrated GnRH-like peptide in crustaceans, and observations are particularly plentiful in molluscs, including bivalves, gastropods and cephalopods (reviewed in Treen *et al*., 2012; Sharker *et al*., 2020) [96, 82]. Like vertebrate GnRHs, molluscan GnRH has been shown to be involved in reproduction (Osada and Treen, 2013) [64]. Phylogenetic analysis showed that crustacean GnRH falls in a separate group (Suwansa-ard *et al*., 2016) [88]. GenBank accession nos. of shellfish expressing GnRH is shown in Table 6, and the amino acid sequences of functional GnRH peptide regions in different molluscan species are presented in Table 7.

Scintometric Mapping of GnRH Research in Fish

Scintometric mapping of publications on GnRH in fish using Web of Science for last twenty years indicated that highest number of research papers appeared in General and Comparative Endocrinology followed by Endocrinology, Fish Physiology and Biochemistry, Comparative Biochemistry and Physiology Part A Molecular Integrative Physiology and Frontiers in Endocrinology. The details of number of papers, publisher, impact factor and citescore are presented in Table 8. The list of top ten institutions who have published their work on GnRH in fish in different journals and their impact factors and citescore are shown in Table 9. National Institutes of Health, USA, Ministry of Education Culture Sports Science and Technology, Japan, National Natural Science Foundation of China, Japan Society for the Promotion of Science and Natural Sciences and Engineering Research Council of Canada are the leading funding agencies promoting GnRH research in fish (Table 10). The leading authors of GnRH research in fish are Prof. M. Matsuyama of Kyushu University, Japan who retired very recently followed by Prof. Munoz-Cueto Ja of University of Cadiz, Spain; Prof. Ishwar Parhar of Monash University, Malaysia; Prof. Levavi-Sivan of The Hebrew University of Jerusalem, Israel; Prof. Yamaguchi of Kyushu University, Japan (Table 11).

Table 1: GenBank accession nos. of finfish expressing three GnRH forms

Teleost fish	GenBank Accession Nos.			References
	gnrh1	***gnrh2***	***gnrh3***	
African cichlid fish, *Haplochromis burtoni*	AF076963	AF076962	AF076961	White and Fernald, 1998 [100]
Atlantic croaker, *Micropogonias undulatus*	AY324668	AY324669	AY3246670	Mohamed *et al*., 2005 [44]
Barfin flounder, *Verasper moseri*	AB066360	AB066359	AB066358	Amano *et al*., 2002 [5]
Bambooleaf wrasse, *Pseudolabrus sieboldi*	KC896411	KC896412	KC896413	Nagase *et al*., 2010 [51]; Kitano *et al*., 2010 [38]
Black porgy, *Acanthopagrus schlegelii*	EU099997	EU099996	EU117212	An *et al*., 2008 [3]
Chub mackerel, *Scomber japonicus*	HQ108193	HQ108194	HQ108195	Selvaraj *et al*., 2012a [76]
Cobia, *Rachycentron canadum*	AY677175	AY677174	AY677173	Mohamed *et al*., 2007 [45]
European sea bass, *Dicentrarchus labrax*	AF224279	AF224281	AF224280	Gonzalez-Martinez *et al*., 2001 [20]; Zmora *et al*., 2002 [107]
Gilthead seabream, *Sparus aurata*	U30320	U30325	U30311	Powell *et al*., 1994 [67]
Grass puffer, *Takifugu niphobles*	AB531127	AB531128	AB531129	Shahjahan *et al*., 2010 [81]
Grey mullet, *Mugil cephalus*	AY373450	AY373451	AY373449	Nocillado and Elizur, 2008 [54]
Medaka, *Oryzias latipes*	NP_001098169	NC_019863	AB041335	Okubo *et al*., 2000 [57], 2002 [60]
Spotted catshark, *Scyliorhinus canicula*	MH468810	MH468811	MH468812	Gaillard *et al*., 2018 [18]
Sea lamprey, *Petromyzon marinus*	AF14448.1	AF144481, DQ457017	AY052628	Suzuki *et al*., 2000 [89]; Kavanaugh *et al*., 2008 [33]

Table 2: Nucleotide sequence of primers used in *gnrh1* cDNA cloning

GnRH 1 forms	Degenerate Primer Sequence	References
Seabream GnRH	DP Fw 5'CATATGGGGAAG(AG)GGGA(AC)CTGGACG3' DP Rv 5'GGATCCTCATTT(TC)TT(AG)TAXGTTCTG(TG)GTCC 3'	Zmora *et al*., 2002 [107]*
Seabream GnRH	DP1 5' CARCAYTGGTCITAYGGICTIAG 3' DP2 5' TAYGGICTIAGYCCIGGIGG 3'	Amano *et al*., 2002 [5]; Mohamed *et al*., 2005 [44]; Mohamed *et al*., 2007 [45]
Herring GnRH	DP1 Fw 5'CARCAYTGGTCICAYGGNCTNTC3' DP2 Fw 5'CARCAYTGGTCICAYGGNCTNAG3' DP3 Fw 5'TTRAGYCCIGGHGGVAARAG3'	Abraham, 2004 [1]
Whitefish GnRH	DP1 5' CAGCACTGGTCGTATGVATG 3' DP2 5' ATGAAVCCTGGAGGVAAGAGR 3'	Vickers *et al*., 2004 [98]
Pejerrey GnRH	5'-CAGCACTGGTCITWYGGICTG-3' 5'-TGGTCITWYGGICTGAGYCCW GG-3'	Guilgur *et al*., 2007 [27]
Mammalian GnRH	5'-CAGCACTGGTC(CT)TA(CT)GGICT(GC)AG-3' 5'-GGICT(GC)AGICCIGG(GC)GGCAAG-3'	Gharaei *et al*., 2010 [19]

*Primers designed from GnRH associated peptide region
R = A or G; Y = C or T; V = A or C or G; N or I = A or C or G or T; M= A or C; H = T or C or A

Table 3: Nucleotide sequence of primers used in *gnrh2* and *gnrh3* cDNAs cloning

GnRH forms	Degenerate Primer Sequence	References
chicken GnRH	DP Fw CA(AorG)CA(CorT)TGGTCNCA(CorT)GGNTGG DP Rv 5' CA(CorT)TCICCIGC(CorT)TC(AorG)CA 3'	Gothilf *et al*., 1996 [23]
	DP Fw 5'CATATGGGCAAGAGGGAACTGGACTCTTT3' DP Rv 5'GGATCCTCACTTCCTTCTGGAGCT3'	Zmora *et al*., 2002 [107]*
	DP1 5'CARCAYTGGTCICAYGGITGGTA3' DP2 5'CAYGGITGGTAYCCIGGIGG3'	Vickers *et al*., 2004 [98]; Mohamed *et al*., 2005 [44], 2007 [45]
salmon GnRH	DP1 5'CARCAYTGGTCITAYGGITGGYT3' DP2 5'TAYGGITGGYTICCIGGIGG3'	Amano *et al*., 2002 [5]; Mohamed *et al*., 2007 [45]
	DP Fw 5'T (AorT) (AorG)T(AorG)CTGGTG(GorT)TG(GorT)TG 3' DP Rv 5'ATTA(CorT)AT(CorT)(AorG)ATAGGT(CorT)T(AorC)AG3'	Gothilf *et al*., 1996 [23]
	DP1 5'CAGCACTGGTCGTATGGVTGG3' DP2 5'GGCTACCTGGAGGRAAGAGAA3'	Mohamed *et al*., 2005 [44];
	DP Fw 5'CATATGGGGAAGAGAAG(CT)GTGGGAGA3' DP Rv	Zmora *et al*., 2002 [107]*

	5’GGATCCTCA(TA)TT(AG)TT(CA)GGGAACCT(CT)TT(CT)TT(CT)TT3’	

*Primers designed from GnRH associated peptide region
R=A or G; Y=C or T; V = A or C or G; N or I = A or C or G or T

Table 4: Amino acid sequences of GnRH decapeptides in teleosts and elasmobranchs

Position	1	2	3	4	5	6	7	8	9	10
Teleost GnRH I forms										
sbGnRH	pGln	His	Trp	Ser	Tyr	Gly	Leu	Ser	Pro	Gly-NH$_2$
mGnRH	—	—	—	—	—	—	—	Arg	—	—
pjGnRH	—	—	—	—	Phe	—	—	Ser	—	—
cfGnRH	—	—	—	—	His	—	—	Asn	—	—
hrGnRH	—	—	—	—	His	—	—	Ser	—	—
wfGnRH	—	—	—	—	—	—	Met	Asn	—	—
Elasmobranchs										
scGnRH	—	—	—	—	His	—	Trp	Arg	—	—
dfGnRH	—	—	—	—	His	—	—	Leu	—	—
GnRH II forms										
cGnRHII (teleost)	pGln	His	Trp	Ser	His	Gly	Trp	Tyr	Pro	Gly-NH$_2$
cGnRH-II (elasmobranch)	—	—	—	—	Phe	Asp	Tyr	Arg	—	—
GnRH III forms										
sGnRH (teleost)	pGln	His	Trp	Ser	Tyr	Gly	Trp	Leu	Pro	Gly-NH$_2$
sGnRH (elasmobranch)	—	—	—	—	Phe	Asp	—	—	—	—

(sbGnRH, Seabream GnRH; mGnRH, Mammalian GnRH; pjGnRH or mdGnRH, Pejerrey or Medaka GnRH; cfGnRH, catfish GnRH; dfGnRH, dogfish GnRH; hrGnRH, Herring GnRH; wfGnRH, Whitefish GnRH; scGnRH, Spotted catshark)

Table 5: Amino acid sequences of functional GnRH decapeptides in lamprey GnRH forms

Position	1	2	3	4	5	6	7	8	9	10
lGnRH-I	pGln	His	Tyr	Ser	Leu	Glu	Trp	Lys	Pro	Gly-NH$_2$
lGnRH-II	—	—	Trp	—	His	Gly	—	Phe	—	—
lGnRH-III	—	—	Trp	—	His	Asp	—	—	—	—

Table 6: GenBank accession nos. of cDNAs encoding GnRH mRNAs in crustaceans and molluscs

Species	GenBank Accession No.	References
Crustaceans		
Giant freshwater prawn, *Macrobrachium rosenbergii*	KT765022	Suwansa-ard *et al*., 2016 [88]
Gastropods		
Gray garden slug, *Deroceras reticulatum*	KY659277	Ahn *et al*., 2017 [2]
Greenlip abalone, *Haliotis laevigata*	KP719129	Nuurai *et al*., 2016 [56]
Abalone, *Haliotis asinine*	KP719130	Nuurai *et al*., 2016 [56]
California sea hare, *Aplysia californica*	EU204144	Zhang *et al*., 2008 [105]
Giant triton snail, *Charonia tritonis*	KY287981	Bose *et al*., 2017 [9]
Great pond snail, *Lymnaea stagnalis*	MN385595	Fodor *et al*., 2020 [17]
Japanese disc abalone or Pacific abalone, *Haliotis discus Hanoi*	MK089558	Sharker *et al*., 2020 [82]; Funayama *et al*., 2018 [16]
Bivalves		
Pacific oyster, *Crassostrea gigas*	HQ712119	Treen *et al*., 2012 [96]
Japanese scallop, *Mizuhopecten yessoensis*	AB486004	Osada *et al*., 2009*; Nagasawa *et al*., 2015 [52]
Cephalopods		
Japanese spineless cuttlefish *Sepiella japonica*	KP982885	Cao *et al*., 2015*
Kisslip cuttlefish, *Sepia lycidas*	LC550284	Murata *et al*., 2021[49]
Swordtip squid, *Uroteuthis edulis*	AB447557	Onitsuka *et al*., 2009 [63]
Pharaoh Cuttlefish, *Sepia pharaonis*	MT211953	Zhu *et al*., 2020*
Common octopus, *Octopus vulgaris*	AB037165	Iwakoshi *et al*., 2002 [30]

*References obtained from the GenBank database

Table 7: Amino acid sequences of functional GnRH peptide regions in molluscs

Species	1	2	3	4	5	6	7	8	9	10	11	12
Sea hare	pGln	Asn	Tyr	His	Phe	Ser	Asn	Gly	Trp	Tyr	Ala	Gly-NH$_2$
Abalone	—	—	—	—	—	—	—	—	—	His	—	—
Garden slug	—	—	—	—	—	—	—	—	—	—	Pro	—
Triton snail	—	—	—	—	Tyr	—	—	—	—	His	Pro	—
Oyster	—	—	—	—	—	—	—	—	—	—	Pro	—
Scallop	—	—	Phe	—	Tyr	—	—	—	—	Gln	Pro	—
Cuttlefish and Squid	—	—	—	—	—	—	—	—	—	His	Pro	—

Table 8: Top ten journals in which papers specifically to fish gonadotropin-releasing hormones (GnRHs) published between the years 2000 and 2019 and their impact factors and citescore

Journal titles	No. of papers	Publisher	Impact factor	Cite Score
General and Comparative Endocrinology	78	Elsevier	2.445	2.52
Endocrinology*	16	Oxford Academic	3.8	3.62
Fish Physiology and Biochemistry	15	Springer	1.729	1.82
Comparative Biochemistry and Physiology Part A Molecular Integrative Physiology	14	Elsevier	2.142	2.24
Frontiers in Endocrinology	12	Frontiers Media S.A.	3.634	3.51
Aquaculture	11	Elsevier	3.022	3.42
Aquaculture research	10	John Wiley & Sons	1.502	1.70
Plos One	9	Public Library of Science	2.776	3.02
Biology of Reproduction	8	Society for the Study of Reproduction	2.960	2.61
Aquatic Toxicology	7	Elsevier	3.794	4.19

*Merged with the Journal of Molecular and Cellular Endocrinology in November 2016

Table 9: List of top ten institutions who have published their work on GnRH in fish in different journals and their impact factors and citescore

Name of the Institution	No. of papers	Top Journal	Impact factor	Cite Score
Kyushu University	17	General and Comparative Endocrinology	2.445	2.52
Centre National de la Recherche Scientifique CNRS	16	General and Comparative Endocrinology		
Universidad de Cadiz	15	General and Comparative Endocrinology		
University of Tokyo	15	Endocrinology	3.8	3.62
University of Warmia Mazury	15	Animal Reproduction Science	1.817	1.92
		Aquaculture	3.022	3.42
		Aquaculture International	1.455	1.70
Monash University	14	Frontiers in Endocrinology	3.634	3.51
Chinese Academy of Sciences	13	Aquaculture Research	1.502	1.70
Hebrew University of Jerusalem	12	Endocrinology	3.8	3.62
		General and Comparative Endocrinology	2.445	2.52
Polish Academy of Sciences	12	Animal Reproduction Science	1.817	1.92
		Aquaculture	3.022	3.42
		Aquaculture Research	1.502	1.70
University System of Maryland	11	General and Comparative Endocrinology	2.445	2.52

Table 10: Top ten funding agencies who have funded for GnRH research in fish

Name of the agency	No. of papers	Top Journal
National Institutes of Health, USA	51	General and Comparative Endocrinology
Ministry of Education Culture Sports Science and Technology, Japan	47	
National Natural Science Foundation of China	44	General and Comparative Endocrinology and Comparative Biochemistry and Physiology with same number of papers
Japan Society for the Promotion of Science	27	General and Comparative Endocrinology
Natural Sciences and Engineering Research Council of Canada	27	
National Science Foundation	19	
European Union	15	
Consejo Nacional de Investigaciones Cientificas y Tecnicas Conicet	14	
NIH National Institute of Neurological Disorders Stroke	14	Endocrinology
Monash University	11	Frontiers in Endocrinology

Table 11: Leading authors who have authored and coauthored research articles on GnRH in finfish and shellfish

Name of the authors	No. of papers	Top Journals
Matsuyama M	13	General and Comparative Endocrinology, Comparative Biochemistry and Physiology, Zoological Science with equal number of papers
Munoz-Cueto Ja		General and Comparative Endocrinology
Parhar IS		Frontiers in Endocrinology
Levavi-Sivan B	12	Endocrinology and General and Comparative Endocrinology
Yamaguchi A		General and Comparative Endocrinology, Comparative Biochemistry and Physiology, Zoological Science with equal number of papers
Kucharczyk D	11	Animal Reproduction Science and Aquaculture International
Selvaraj S		General and Comparative Endocrinology, Comparative Biochemistry and Physiology, Zoological Science with equal number of papers
Zarski D		Aquaculture and Aquaculture International with equal number of papers
Cejko Bi	10	Animal Reproduction Science, Aquaculture Research, and Journal of Applied Ichthyology with

		equal number of papers
Nyuji M		General and Comparative Endocrinology, Comparative Biochemistry and Physiology, Zoological Science with equal number of papers
Ohga H		
Tsutsui K		General and Comparative Endocrinology
Zohar Y		Biology of Reproduction, Endocrinology and General and Comparative Endocrinology with equal number of papers

Conclusion and Future Perspectives

GnRH is well conserved in finfish and shellfish and shown to be indispensable in animal reproductive. In teleosts, two group of fish can be recognized with GnRH-I form absent in cyprinid and salmonid fish and GnRH-I variant varying in number of perciform, clupeiform, atheriniform fish. Globally, diversification of aquacultured species is preferred for supporting the decline of natural fish population. It is likely that *gnrh* cDNAs will be isolated in number of species in the near future to understand their role in reproductive cycle and to develop GnRH analogues for undertaking breeding programmes in captivity. Amino acid in different positions of functional GnRH decapeptide region may be considered for developing higher activity of GnRH analogues in inducing pituitary gonadotropins. Emerging studies indicate novel recombinant gonadotropin-releasing hormone associated peptide as a spawning inducing agent for fish (Mohammadzadeh *et al*., 2020) [46]. Kisspeptin suggested to be an upstream regulator of GnRH in number of fish species and their functional peptide regions shown to be potent in activating the reproductive axis. It is likely that functional peptides of active forms of kisspeptins and GnRHs for induced breeding in commercial aquaculture will be promoted in the future.

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