Gonadotropin-releasing hormone (GnRH) genes in fish

Thanathip Lamkom

ABSTRACT

The decapeptide gonadotropin-releasing hormone (GnRH) is a neuropeptide which is synthesized in the brain. The GnRH stimulates the synthesis and secretion of gonadotropic hormone (GtH) which triggers steroidogenesis of gonads. Among vertebrates, the highest number of GnRH type (8 types) were identified in fish. *GnRH1*, cfGnRH, hgGnRH, mGnRH, mdGnRH, sbGnRH and wfGnRH were found in the preoptic area of a fish brain, while *GnRH2* (cGnRH-II) and *GnRH3* (sGnRH) were found in the midbrain and telencephalon. Generally two to three types of GnRH were detected in a fish species. A function of *GnRH1* involves regulation of the reproductive system, while *GnRH2* acts as a neurotransmitter. In a species bearing two types of GnRH, *GnRH3* also plays a role in controlling the reproductive system. The bioactive and processing sites of GnRH mRNA showed the highly conserved region. The expression of the GnRH gene was site- and time-specific. It can be concluded that GnRH is involved in the regulation of the hypothalamic-pituitary-gonadal axis of fish. The knowledge on various types and expressions of GnRH are essential for the development of a more efficient GnRHa-induced breeding.

INTRODUCTION

As a result of population growth, global food demand for agriculture and aquaculture products has increased rapidly. Among animals produced, fish is a vital source of high-quality protein providing about 16% of the animal protein to address the demand of the world's population (Tidwell and Allan, 2001). Fish also contains significant amounts of the essential fatty acids, particularly highly unsaturated fatty acid (HUFA) such as eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), which can control triglyceride and decrease blood pressure in humans (Steffens, 1997). Therefore, fish production has risen from 13.96 million tonnes in 1987 to 65.19 million in 2007 (FAO, 2007).

In 2007, total aquaculture production in Thailand reached 519,947 t for coastal aquaculture and 507,284 t for freshwater aquaculture. As aquaculture expands, quantity and availability of fry are required. In the past, traditional aquaculture was based on wild seed collected directly from natural waters, e.g. snakehead (*Channa striatus,* Bloch) and brown spotted grouper (*Epinephelus*

Department of Fisheries, Faculty of Agriculture, Ubon Ratchathani University Warinchamrab, Ubon Ratchathani 34190

malabaricus). For seven striped carp (*Probarbus jullieni*), giant carp (*Catlocarpio siamensis*) and soldier river barb (*Cyclocheilichthys enoplos*), broodstock were collected directly from spawning grounds and used for artificial fertilization. In the early 1970s, hormone-induced breeding techniques were developed to facilitate synchronization of final oocyte maturation (FOM), ovulation, and spermiation in broodstock for stable supply and quality of seed stock. Many kinds of hormone were widely used, such as fish pituitary extract (Na-Nakorn, 1985; Udomkarn and Singsee, 2004), human chorionic gonadotropin (Sukumasavin, 1996), luteinizing hormone-releasing hormone analogue (LHRHa) (Pongchawee and Phasookdee, 2000; Ruensirikul *et al*., 2006), and gonadotropin-releasing hormone analogue (GnRHa) (Prarom *et al*., 2004, Chankaew, 2007). Among the above, GnRH receives extensive attention because it triggers gonad development by targeting the pituitary gland which, as a result, responds by producing gonadotropins. With this action, no feedback mechanism is expected.

Roles of Gonadotropin-releasing hormone (GnRH) in a reproductive system of fish

The reproduction cycle of fish is regulated by environmental factors such as water temperature [e.g. highest ovulation rate is triggered at 24°C in grass carp (Glasser *et al*., 2004); 28°C in channel catfish (*Ictalurus punctatus*) (Phelps *et al*., 2007)], photoperiod [e.g. in Atlantic salmon (*Salmo solar*), high ovulation and spermiation is in the short day period (8 hours) (Björnsson *et al*., 1994), while low sexual maturation occurs in continuous light (Berg *et al*., 1996)] and affected by light intensity [e.g. melatonin, internal factor of reproductive cycle, is sensitive at 1.92×10^{13} photons s^{-1} cm^{-2} in sea bass (*Dicentrarchus labrax*) (Bayarri *et al*., 2002); 3.2×10^{12} photons s^{-1} cm^{-2} in Atlantic cod (*Gadus morhua*) (Vera *et al*., 2010)], and oxygen level [e.g. hypoxia can inhibit final oocyte maturation in common carp (*Cyprinus carpio*) (Wang *et al*., 2008) and Atlantic croaker (*Micropogonias undulates*) (Thomas and Rahman, 2009)]. The appropriate environment stimulus is detected by sensory receptors in the fish brain. Then the hypothalamus is stimulated to release GnRH which subsequently triggers the pituitary gland to produce and release gonadotropin hormones (GtH). GtH plays a role in controlling many gonadal mechanisms such as gametogenesis, vitellogenesis, FOM, ovulation, and spermiation (Figure 1) (Yaron and Sivan, 2006). As such, it can be concluded that GnRH acts as a key neurohormone in the reproductive system of fish by combining the brain, pituitary gland, and gonads together (Brain-pituitary gland-gonad axis).

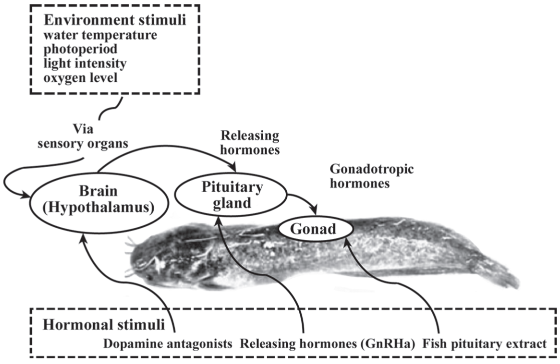

Figure 1. The relationship of environmental and hormonal stimuli with the reproductive system of fish

Source: modified from De Silva *et al*. (2008)

Although a natural form of GnRH could be extracted but it showed low efficiency when applied *in vivo* (Ngamvongchon *et al*., 1992a). Therefore, GnRH analogue (GnRHa) is synthesized and used to stimulate gonadal maturation of broodstock. Empirical data showed the superiority of GnRHa over several other kinds of hormones such as human chorionic gonadotropin (hCG), salmon GnRH (sGnRH), mammalian GnRH (mGnRH), and sGnRH with domperidone. For example, Levavi-Sivan *et al*. (2004) showed that only sGnRH induced the highest FOM and ovulation in silver perch (*Bidyanus bidyanus*) females and increased milt volume in greenback flounder (*Rhombosolea tapirina*) male (Lim *et al*., 2004). Szabó *et al*. (2002) induced ovulation in nase (*Chondrostoma nasus*) using pituitary injection at a dose of 3 mg/kg body weight (BW) and 20 µg GnRHa/ 1 kg female combined with domperidone (DOM) and found that the ovulation index and fertilization rate in GnRHa with DOM (85.5 and 83.5 %) were higher than those of the group receiving pituitary injection (55.9 and 69. %), whereas no female ovulated in the group receiving GnRHa injection alone and the control group. Additionally, GnRH acts as a neurotransmitter involving sexual behavior of vertebrates (Volkoff and Peter, 1999; Temple *et al*., 2003; Gopinath *et al*., 2004) and also stimulates prolactin hormone (Weber *et al*., 1997) and somatolactin hormone secretion (Parhar, 1997).

Types of GnRH and genes

Among vertebrates, fish represents the greatest variation of GnRH (8 types), e.g. mGnRH, chicken type II GnRH (cGnRH-II), sGnRH, catfish GnRH (cfGnRH), sea bream GnRH (sbGnRH), herring GnRH (hgGnRH), medaka GnRH (mdGnRH or pjGnRH) and whitefish GnRH (wfGnRH) (Table 1). The mGnRH was the first form found in fish (Matsuo *et al*., 1971). The sGnRH and cGnRH-II forms were found in salmon (Sherwood *et al*., 1983) and goldfish (Yu *et al*., 1988), respectively. The remaining five GnRH were also found, such as cfGnRH (Bogerd *et al*., 1992; Ngamvongchon *et al*., 1992b), sbGnRH (Powell *et al*., 1994), hgGnRH (Carolsfeld *et al*., 2000), mdGnRH (Okubo *et al*., 2000a), and wfGnRH (Adams *et al*., 2002). The differences of amino acid sequences among GnRH forms and GnRHa are shown in Table 1.

The eight GnRH types are grouped into three major categories, namely *GnRH1, GnRH2 and GnRH3. GnRH1* consists of cfGnRH, hgGnRH, mGnRH, mdGnRH, sbGnRH and wfGnRH types of which neurons were found in the anteroventral preoptic area (POA) (Dubois *et al*., 2002). *GnRH1* releases peptides into the hypothalamo-hypophysial portal vasculature, from which it ultimately reaches the pituitary gonadotrophs (Hoffmann, 2006). *GnRH2* comprises of only chicken type II GnRH of which neurons are localized only in the midbrain (Dellovade *et al*., 1993; Vickers *et al*., 2004), while neurons of *GnRH3* which comprises of only salmon GnRH, were found in the telencephalon (ventral forebrain). In zebrafish (*Danio rerio*), sGnRH and cGnRH-II were found in the telencephalon and midbrain, respectively (Gopinath *et al*., 2004). In barfin flounder and pejerrey fish, sbGnRH, cGnRH-II and sGnRH were detected in the POA, midbrain and telencephalon, respectively (Amano *et al*., 2002a; 2002b; Miranda *et al*., 2003). In some fish species with two GnRH types,

Table 1. The decapeptide amino acids of GnRH in teleost, other vertebrates, and analogue forms (bold = different amino acid)

Group	*Amino acid*										*Reference*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	
Teleost											
Mammalian GnRH	*pGlu*	*His*	*Trp*	*Ser*	*Tyr*	*Gly*	*Leu*	*Arg*	*Pro*	*Gly-NH2*	*Matsuo et al. (1971)*
Salmon GnRH	*pGlu*	*His*	*Trp*	*Ser*	*Tyr*	*Gly*	*Trp*	*Leu*	*Pro*	*Gly-NH2*	*Sherwood et al. (1983)*
Chicken GnRH-II	*pGlu*	*His*	*Trp*	*Ser*	*His*	*Gly*	*Trp*	*Tyr*	*Pro*	*Gly-NH2*	*Yu et al. (1988)*
Catfish GnRH	*pGlu*	*His*	*Trp*	*Ser*	*His*	*Gly*	*Leu*	*Asn*	*Pro*	*Gly-NH2*	*Bogerd et al. (1992)*
Seabream GnRH	*pGlu*	*His*	*Trp*	*Ser*	*Tyr*	*Gly*	*Leu*	*Ser*	*Pro*	*Gly-NH2*	*Powell et al. (1994)*
Herring GnRH	*pGlu*	*His*	*Trp*	*Ser*	*His*	*Gly*	*Leu*	*Ser*	*Pro*	*Gly-NH2*	*Carolfeld et al. (2000)*
Medaka GnRH	*pGlu*	*His*	*Trp*	*Ser*	*Phe*	*Gly*	*Leu*	*Ser*	*Pro*	*Gly-NH2*	*Montaner et al. (2001)*
Whitefish GnRH	*pGlu*	*His*	*Trp*	*Ser*	*Tyr*	*Gly*	*Met*	*Asn*	*Pro*	*Gly-NH2*	*Adams et al. (2002)*
Other vertebrates											
Chicken GnRH-I	*pGlu*	*His*	*Trp*	*Ser*	*Tyr*	*Gly*	*Leu*	*Gln*	*Pro*	*Gly-NH2*	*Miyamoto et al. (1982)*
Mammalian GnRH	*pGlu*	*His*	*Trp*	*Ser*	*Tyr*	*Gly*	*Leu*	*Arg*	*Pro*	*Gly-NH2*	*King et al. (1990)*
Dogfish GnRH	*pGlu*	*His*	*Trp*	*Ser*	*His*	*Gly*	*Trp*	*Leu*	*Pro*	*Gly-NH2*	*Lovejoy et al. (1992)*
Lamprey GnRH-I	*pGlu*	*His*	*Tyr*	*Ser*	*Leu*	*Glu*	*Trp*	*Lys*	*Pro*	*Gly-NH2*	*Sower et al. (1993)*
Lamprey GnRH-III	*pGlu*	*His*	*Trp*	*Ser*	*His*	*Asp*	*Trp*	*Lys*	*Pro*	*Gly-NH2*	*Sower et al. (1993)*
Guinea pig GnRH	*pGlu*	*Tyr*	*Trp*	*Ser*	*Tyr*	*Gly*	*Val*	*Arg*	*Pro*	*Gly-NH2*	*Jimenez-Linan et al. (1997)*
Frog GnRH	*pGlu*	*His*	*Trp*	*Ser*	*Tyr*	*Gly*	*Leu*	*Trp*	*Pro*	*Gly-NH2*	*Yoo et al. (2000)*
GnRHa											
mGnRHa	*pGlu*	*His*	*Trp*	*Ser*	*Tyr*	*Dala*	*Leu*	*Arg*	*Pro*	*NEt*	*Mylonas and Zohar (2001)*
sGnRHa	*pGlu*	*His*	*Trp*	*Ser*	*Tyr*	*DArg*	*Trp*	*Leu*	*Pro*	*NEt*	*Mylonas and Zohar (2001)*

(Ala = alanine, Arg = arginine, Asn = asparagine, Asp = aspartate,
Gln = glutamine, Glu = glutamic acid, Gly = glycine, His = histidine,
Leu = leucine, Lys = lysine, Met = methionine, Pro = proline,
Ser = serine, Trp = tryptophan, Tyr = tyrosine, Val = valine)

both *GnRH1* or *GnRH3* were found in both POA and telencephalon, for example, cfGnRH in African catfish (Bogerd *et al.*, 1994), mGnRH in eel (King *et al.*, 1990), and sGnRH in goldfish (Lin *et al.*, 1997) (Table 2 and Figure 2).

Table 2. The distribution of each GnRH type in preoptic area (P), midbrain (M) and telencephalon (T)

Species	Brain			Reference
	P	M	T	
African catfish (*Clarias gariepinus*)	cfGnRH	cGnRH-II	cfGnRH	Bogerd *et al*. (1994)
Eel (*Anguilla anguilla*)	mGnRH	cGnRH-II	mGnRH	King *et al*. (1990)
Goldfish (*Carassius auratus*)	sGnRH	cGnRH-II	sGnRH	Lin *et al*. (1997)
Herring (*Clupea harengus*)	hgGnRH	cGnRH-II	sGnRH	Carolsfeld *et al*. (2000)
Medaka (*Oryzias latipes*)	mdGnRH	cGnRH-II	sGnRH	Okubo *et al*. (2000a)
Seabream (*Sparus aurata*)	sbGnRH	cGnRH-II	sGnRH	Gothilf *et al*. (1996)
Whitefish (*Coregonus clupeaformis*)	wfGnRH	cGnRH-II	sGnRH	Adams *et al*. (2002)

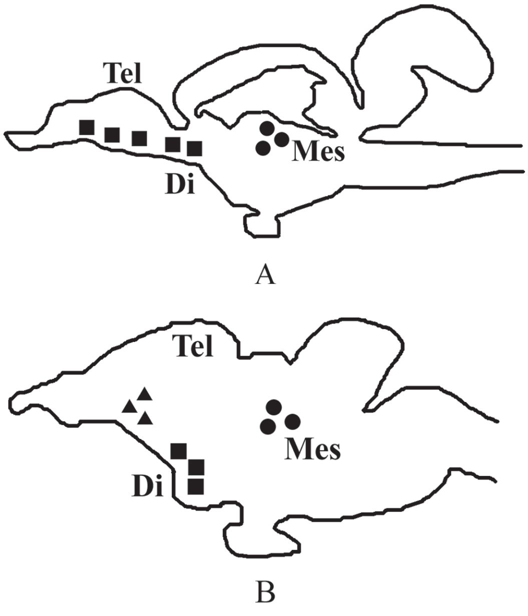

Figure 2. A sagittal section of a fish brain showing localization of each GnRH form; A) sockeye salmon (*O. nerka*) composed of sGnRH (square) and cGnRH-II (circle); and, B) cichlid (*Haplochromis burtoni)* composed of sbGnRH (square), cGnRH-II (circle), and sGnRH (triangle). (Di = Diencephalon, Mes = mesencephalon, Tel = telencephalon)

Source: Dubois *et al*. (2002)

At least two forms of GnRH exist in fish with a maximum of three forms, among which the conserved cGnRH-II is found in every fish species. For example, goldfish (Klausen *et al*., 2001), zebrafish (Powell *et al*., 1996) and common carp (Li *et al*., 2004a, b) have two GnRH forms, *GnRH2* (cGnRH-II) and *GnRH3* (sGnRH), while eel (King *et al*., 1990) and African catfish (Bogerd *et al*., 1992) have *GnRH1* (mGnRH and cfGnRH) and *GnRH2* (cGnRH-II). The brain of tilapia (Parhar *et al*., 1996), sockeye (Andersson *et al*., 2001), barfin flounder (Amano *et al*., 2002a), Pacific herring (Carolsfeld *et al*., 2000), and whitefish (Adams *et al*., 2002) have three types of GnRH (Table 3).

The first study showed that a GnRH-like substance, a hypothalamic factor can stimulate the secretion of pituitary gonadotropic hormone in carp (Breton *et al*., 1971). The characterization of GnRH peptides, salmon and mammalian GnRH were performed in salmon brain by gel filtration and high-performance liquid chromatography (Sherwood *et al*., 1983), while the first

Table 3. Types and number of GnRH peptide in teleost (cII = cGnRH-II, s = sGnRH, m = mGnRH, cf = cfGnRH, hg = hgGnRH, md = mdGnRH, sb = sbGnRH and wf = wfGnRH)

Species	**GnRHM**								**Reference**
	cII	**s**	**m**	**cf**	**hg**	**md**	**sb**	**wf**	
Siruliformes									
African catfish (*Clarias gariepinus*)	✓			✓					Zandbergen *et al*. (1995)
Cypriniformes									
Goldfish (*Carassius auratus*)	✓	✓							Yu *et al*. (1988)
Zebrafish (*Danio rerio*)	✓	✓							Powell *et al*. (1996)
Common carp (*Cyprinus carpio*)	✓	✓							Li *et al*. (2004a)
Anguilliformes									
Eel (*Anguilla anguilla*)	✓		✓						Montero *et al*. (1994)
Salmoniformes									
Rainbow trout (*Oncorhynchus mykiss*)	✓	✓							Okuzawa *et al*. (1990)
Masu salmon (*Oncorhynchus masou*)	✓	✓							Amano *et al*. (1991)
Whitefish (*Coregenus clupeaformis*)	✓	✓						✓	Adams *et al*. (2002)
Clupeiformes									
Herring (*Clupea harengus*)	✓	✓			✓				Carolsfeld *et al*. (2000)
Beloniformes									
Medaka (*Oryzias latipes*)	✓	✓				✓			Okubo *et al*. (2000a, b)
Perciformes									
Seabream (*Sparus aurata*)	✓	✓					✓		Gothilf *et al*. (1996)
Tilapia (*Oreochromis mossambicus*)	✓	✓					✓		Senthikumaran *et al*. (1999)
Pleuronectiformes									
Barfin flounder (*Verasper moseri*)	✓	✓					✓		Amano *et al*. (2002a)

cDNA encoding the prepro GnRH in fish was isolated and characterized from African cichlid (*Haplochromis burtoni*) (Bond *et al*., 1991). Among vertebrates, the full-length GnRH cDNAs have a similar structural organization. The common structure of GnRH cDNA encoding a precursor dacapeptide composes of 3 introns and 4 exons (Suzuki *et al*., 2000). Each exon of the cDNA consists of 1) 5´ untranslated region (5´UTR) and regions encoding following amino acids; 2) 21-23 amino acids (aa) of signal peptide at the N-terminal, 10 aa of bioactive peptide, 3 aa cleavage and processing site (Gly-Lys-Arg) and some parts of GnRH-associated peptide (GAP) (11 aa); 3) 12-43 aa of GAP at the

C-terminal; and, 4) 3´ untranslated region (3´UTR) (King and Millar, 1992; Lethimonier *et al*., 2004; Zmora *et al*., 2002; Clarke and Pompolo, 2005) (Figure 3).

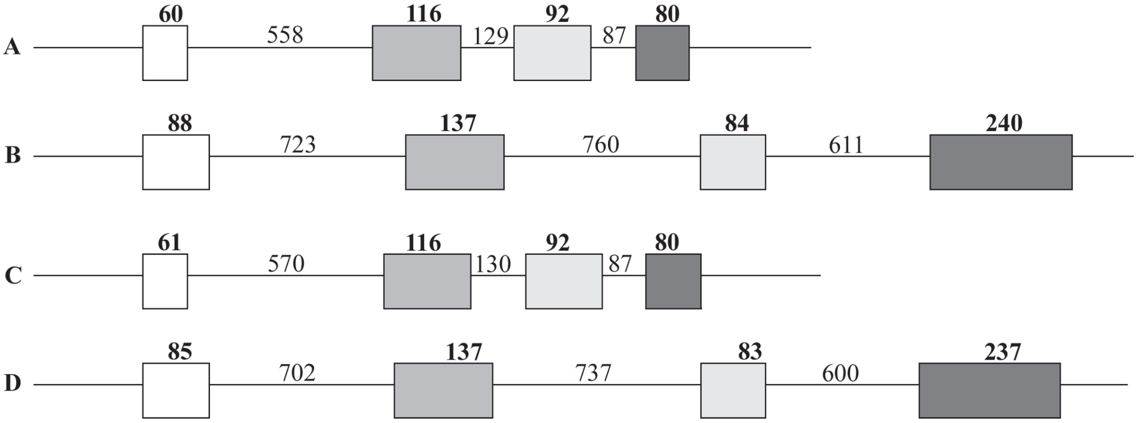

Figure 3. A structure of genomic GnRH gene of channel catfish (*I. punctatus*) (A = catfish type and B = chicken type II), and blue catfish (*I. furcatus*) (C = catfish type and D = chicken type II) (box = exon, line = intron, number = number of nucleotide)
Source: Lamkom (2008)

The GnRH cDNA can be translated into a common prepro-GnRH peptide. The peptide structure deduced from the GnRH cDNA is specific to types of GnRH. The GnRH precursors share amino acid similarity in the bioactive peptide and the cleavage site, while the remaining regions, GAP and 3´UTR are highly diverse (Gopinath *et al*., 2004). The deduced amino acid was aligned using ClustalW multiple sequence alignment algorithm in the software Bioedit and represented that the pro-GnRH cDNAs differ at three positions (Figure 4).

GnRH expression in fish

The spatial expression of GnRH gene is site-specific. The brain is a major organ of expression where GnRH DNA encoding sbGnRH, cGnRH-II, and sGnRH are transcribed into mRNA (Amano *et al*., 2002b). The level of sbGnRH peptide in a pituitary gland of barfin flounder was higher than both sGnRH and cGnRH-II during the testicular maturation (Amano *et al*., 2004). In a spawning season, the sbGnRH peptide in gilthead seabream (*Sparus aurata*) and turbot (*Scophthalmus maximus*) differed significantly from sGnRH about 100-600 and 500 fold, respectively (Powell *et al*., 1994; Andersson *et al*., 2001). It is possible that *GnRH1*, sbGnRH of fish which has three GnRH types, is involved in the regulation of reproduction. The sGnRH of masu salmon and European sea bass (both of which have two GnRH types) is expressed in the olfactory bulb and links with POA and pituitary gland for stimulating GtH secretion. The second type, cGnRH-II in these two species is expressed in the midbrain (Amano *et al*., 2003) (Figure 5).

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channelcfGnRH     -MGIKRALWWMVVC-----VVVLQVSSQHWSHGLNPGGKR--AALQE-TVEE-----MPR 46
bluecfGnRH        -MGIKRALWWMVVC-----VVVLQVSSQHWSHGLNPGGKR--AALQE-TVEE-----MQR 46
catfishcfGnRH     -MGIKRALWWMVVC-----VVVLQVSAQHWSHGLNPGGKR--AVMQE-SAEE-----IPR 46
seabasssbGnRH     -MAAQTFALRLLLVGTLLGTLLGQGCCQHWSYGLSPGGKRELDGLSE-TLGNQIVGSFPH 58
seabreamsbGnRH    -MAPQTSNLWILLLLVVV-MMMSQGCCQHWSYGLSPGGKRDLDSLSD-TLGN-IIERFPH 56
medakamdGnRH      -MVVKTWMPWLLVS-----SVLSQGCCQHWSFGLSPGGKRELKYFPN-TLEN--QIRLLN 51
catfishcGnRH-II   MVHICRLFVVMGMLMF---LSVQFASSQHWSHGWYPGGKREIDVYDPSEVSEE----IKL 53
goldfishcGnRH-II  MVHICRLFVVMGMLLC---LSAQFASSQHWSHGWYPGGKREIDVYDSSEVSGE----IKL 53
channelcGnRH-II   MVSVCRLLLVAALLLC---LQAQLSVSQHWSHGWYPGGKREIDSYSSPEISGE----IKL 53
bluecGnRH-II      MVSVCRLLLVAALLLC---LQAQLSVSQHWSHGWYPGGKREIDSYSSPEISGE----IKL 53
medakacGnRH-II    ---MSRLVLLLGVLLY---VGAQLSQAQHWSHGWYPGGKRELDSF---EVSEE----MKL 47
seabreamcGnRH-II  -MCVSRLVLLLGLLLC---VGAQLSNGQHWSHGWYPGGKRELDSFGTSEISEE----IKL 52
seabasssGnRH      -MEANSRVMVRVLLLA---LVVQVTLSQHWSYGWLPGGKRSVG-----ELEAT----IRM 47
seabreamsGnRH     -MEASSRVTVQVLLLA---LVVQVTLSQHWSYGWLPGGKRSVG-----ELEAT----IRM 47
medakasGnRH       -MDVSSKVVVQVLLLA---LVVQVTLCQHWSYGWLPGGKRSVG-----ELEAT----IRM 47
salmonsGnRH       -MDLSSKTVVQVVMLA---LIAQVTFSQHWSYGWLPGGKRSVG-----ELEAT----IRM 47
goldfishsGnRH     -MEWNGRLLVQLLMLV---CVLEVSLCQHWSYGWLPGGKRSVG-----EVEAT----FKM 47
                              :              ****.*  *****                 :


channelcfGnRH       TSG--YVCDYVDVSPRNKLYRLKDLLTS-----VAEREIGQ------- 80
bluecfGnRH          TSG--YVCDYVDVSPRNKLYRLKDLLTS-----VAEREIGQ------- 80
catfishcfGnRH       SSG--YLCDYVAVSPGNKPFRLKDLLTP-----VAGREIEE------- 80
seabasssbGnRH       VATPCRVLGCAEESPFPKIYRMKGFLDA-----VTDRENGNRTYKK-- 99
seabreamsbGnRH      VDSPCSVLGCVEEPHVPRMYRMKGFIG-------SERDIGHRMYKK-- 95
medakamdGnRH        SNTPCSDLSHLEESSLAKIYRIKGLLGS-----VTEAKNGYRTYK--- 91
catfishcGnRH-II     CNAGKCSFLIPQGRNILKTILLDALTRD------FQKRK--------- 86
goldfishcGnRH-II    CEAGKCSYLRPQGRNILKTILLDAIIRD------SQKRK--------- 86
channelcGnRH-II     CEAGECSYLRPLRTNVLKSILLDALVKE------FQKKK--------- 86
bluecGnRH-II        CEAGECSYLRPLRTNVLKSILLDALARE------FQKRK--------- 86
medakacGnRH-II      CETGECSYMRPQRRSFLRNIVLDALARE------LQKRK--------- 80
seabreamcGnRH-II    CEAGECSYLTPQRRSVLRNILLDALARE------LQKRK--------- 85
seabasssGnRH        MGTG-EVVSLPEEASAQTQERLRPYNVINDDSSHFDRKKRSPNK---- 90
seabreamsGnRH       MGTG-GVVSLPEEASAQTQERLRPYNVIKDDSSPFDRKKRFPNK---- 90
medakasGnRH         MGTG-RVVSLPEDASAQTQERLRQYNLINDGSTYFDRKKRFMSQ---- 90
salmonsGnRH         MDTG-GVMALPEETDAHIPERLRPYDVN---GCHINKEL--------- 82
goldfishsGnRH       MDAGDAVLSIPADSPMEQLLPIHIVNEVDADGLPL-KEQRFPKRRGRV 94
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Figure 4. The alignment of amino acid sequence which is a product of GnRH gene using Clustal W. Sequence access numbers: African catfish: cfGnRH (CAA54970), cGnRH-II (AAB86989); goldfish: sGnRH (BAB18904), cGnRH-II (AAC59858); medaka: sGnRH (BAC06418), cGnRH-II (BAC06417), mdGnRH (NP001098169); seabass: sbGnRH (AAF62898), sGnRH (AAF62899); seabream: sbGnRH (AAA75469), sGnRH (AAA98845), cGnRH-II (AAA75447) from http://www.ncbi.nlm.nih.gov and channel: cfGnRH and cGnRH-II; blue catfish: cfGnRH and cGnRH-II from Lamkom (2008) (bold = bioactive site, italic = processing site)

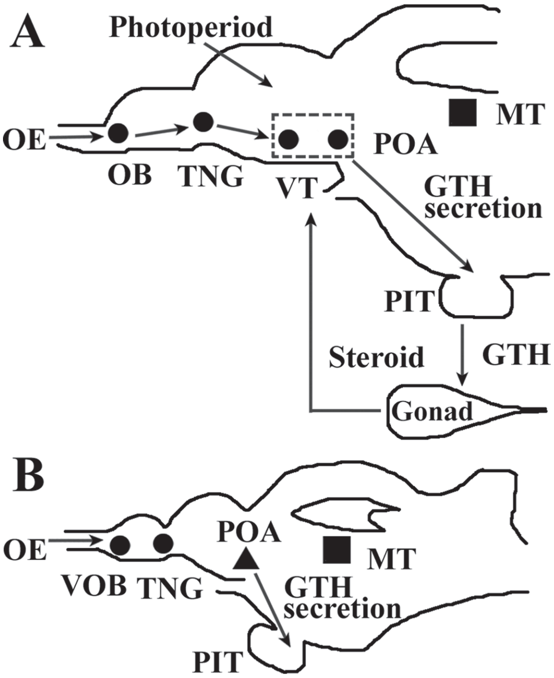

Figure 5. GnRH expression in brain and GtH secretion A) masu salmon (circle = sGnRH and square = cGnRH-II); B) barfin flounder (circle = sGnRH, square = cGnRH-II, and triangle = sbGnRH)(OB = olfactory bulb, OE = olfactory epithelium, MT = midbrain tegmentum, PIT = pituitary gland, TNG = terminal nerve ganglion, VT = ventral telencephalon)
Source: Amano *et al*. (2003)

The function of sGnRH in a reproductive system was explained by Hu *et al*. (2007) who used the antisense sGnRH to bind with sGnRH mRNA for inhibiting the GnRH expression in transgenic common carp (*Cyprinus carpio*). They observed the reduction of GtH resulting in sterilization of fish. Therefore, the sGnRHof fish with two GnRH types may be responsible for gonadal development and maturation. The GnRH mRNA in brain and types of GnRH peptide in the pituitary gland and gonads in other fish is shown in Table 4.

The temporal expression of GnRH gene depends on age of fish and the season. Although in situ hybridization technique shows that the expression of GnRH is observed at two hours post fertilization (Wu *et al*., 2006), the GnRH expression of fry was only found in the brain tissue. However, mature fish show gene expression in all reproductive organs – brain, pituitary gland and gonads (Hu *et al*., 2007). In a spawning season, a level of sbGnRH which is involved in the control of the reproductive system was highly significant compared with both cGnRH-II and sGnRH of red seabream (Okuzawa *et al*., 1997) and European sea bass (Rodriguez *et al*., 2000). The level of sbGnRH in mature gilthead seabream can reach 3-17 folds of cGnRH-II (Holland *et al*., 1998).

Application of GnRH and expression of GnRH mRNA in fish breeding

The function of GnRH on fish reproduction varies remarkably. During ovulation, the levels of sbGnRH and sGnRH reached the highest in both red seabream (*Pagrus major*) and rainbow trout, while the decrease of sbGnRH and increase of sGnRH mRNA levels were observed in red seabream and rainbow trout immature pre-vitellogenic fish (Uzbekova *et al*., 2001;Okuzawa *et al*., 2003). Contrarily, during the period of spermatogenesis in rainbow trout, sGnRH mRNA expression reduced during spermatogonial proliferation and elevated during meiosis due to the effect of anti-proliferative and meiosis-stimulating effect

Table 4. The spatial expression of GnRH mRNA and types of GnRH peptide in various fish species

Species	**Tissue**			**Reference**
	Brain	**Pituitary gland**	**Gonad**	
Zebrafish	sGnRH	-	-	Gopinath *et al*. (2004)
	cGnRH-II	-	-	
Red seabream	sGnRH	-	-	Okuzawa *et al*. (2003)
	cGnRH-II	-	-	
	sbGnRH	sbGnRH	-	
Rainbow trout	sGnRH-1	sGnRH-2	sGnRH-1	Uzbekova *et al*. (2001)
	sGnRH-2	-	sGnRH-2	
Goldfish	sGnRH	cGnRH-II	-	Klausen *et al*. (2001)
Turbot	sGnRH	sGnRH	-	Andersson *et al*. (2001)
	cGnRH-II	sbGnRH	-	
Gilthead seabream	sGnRH	cGnRH-II	-	Holland *et al*. (1998)
	cGnRH-II	sbGnRH	-	
	sbGnRH	-	-	

(Uzbekova *et al*., 2001). Additionally, the rearing condition of broodstock may deplete the levels of GnRH precursor expression, for example, the GnRH1 mRNA levels of wild Senegalese sole (*Solea senegalensis*) females were 2-fold higher than those of the cultured broodstock (Guzmán *et al*., 2009); cultured striped bass failed to reach maturation after vitellogenesis (Mylonas and Zohar, 2001).

Environmental factors, i.e. photoperiod and water temperature, can affect GnRH mRNA levels, for e.g. the levels of sbGnRH mRNA of red seabream elevated with the increase of day length in April (Okuzawa *et al*., 2003), whereas that of European sea bass, turbot and barfin flounder elevated in November, May-July and January-March, respectively (Rodriguez *et al*., 2000; Andersson *et al*., 2001; Amano *et al*., 2004). Other examples include the following: testicular maturation accelerated by a short photoperiod (8L-16D) in masu salmon (Amano *et al*., 1995); the levels of GtH mRNA and steroid hormone of pejerrey (*Odontesthes bonariensis*) increased with a long-day (16 hours) photoperiod (Miranda *et al*., 2008); GnRH mRNA expression increased rapidly at 24°C in red seabream (Okuzawa *et al*., 2003).

The GnRH mRNA levels can also fluctuate depending on the level of steroid hormone. Gonadal steroids such as 17β-estradiol, testosterone and 11-ketotestosterone regulate the reproductive cycle in two directions, either to stimulate or inhibit GnRH mRNA expression, i.e. through positive or negative feedback (Ando *et al*., 2003, 2004; Clarke and Pompolo, 2005). The implantation of the steroid hormones, estradiol and testosterone, into female sea bass during the sexual resting period showed that testosterone had a stronger stimulating effect on the pituitary content of LHβ (Beta-luteinizing hormone). This indicated the positive feedback of the steroid on GnRH and LH synthesis. On the contrary, estradiol reduced mRNA levels of FSHβ (Beta-follicle stimulating hormone) subunit, thus indicating a negative feedback on gonadotropic mRNA levels (Mateos *et al*., 2002). Additionally, the contents of GnRH mRNA depend on the rate of transcription of DNA, translation of mRNA and stability of mRNA in fish brain (Gore and Roberts, 1997). The knockdown of *GnRH2* and *GnRH3* translation after 24 hours post fertilization in zebrafish showed GnRH loss-of-function which eventually affected brain and eye formation during development of fish larvae (Wu *et al*., 2006).

In addition, the efficiency of hormonal -induced breeding depends largely on doses of hormones of which an optimal level varies among species. For example, rose snapper required 75 μg mGnRHa/1 kg female to yield the highest percent of hatching (87.7 %) (Ibarra-Castro and Duncan, 2007), while the 30 μg mGnRHa/1 kg female injected to silver perch stimulated the highest sexual maturity and spawning (Levavi-Sivan *et al*., 2004). The forms of GnRHa may influence gonadal maturation, e.g. the sGnRHa induced the volume of milt and total sperm production in rainbow trout. There was a tendency that a combination of GnRH analogues may increase efficiency of the induction. For example, sGnRHa increased the volume of milt and total sperm production, and mGnRHa accelerated sperm motility in European smelt (Kŕol *et al*., 2009). Additionally, a successful rate of GnRHa-induced ovulation in fish depends on environment factors especially temperature, e.g. Atlantic salmon (*Salmo solar*) injected with 50 μg GnRHa /1 kg female) showed significantly higher ovulation rate at 10.6±1.0°C (ovulation rate = 90 %) than at 6.9±1.0°C (75 %) and 14.3±0.5°C (30 %) (Vikingstad *et al*., 2008).

Not every type of GnRH is involved in fish reproduction. For fish with three GnRH types, hgGnRH, mdGnRH, sbGnRH and wfGnRH play roles in reproduction while cfGnRH, mGnRH and sGnRH function in the reproductive system of fish species with two GnRH types. The other type of GnRH, i.e. cGnRH-II, acts as a neurotransmitter. The expression of each GnRH type depends on species and stage of sexual maturation. Therefore, only GnRH with amino acid sequence homologue to the GnRH type controlling a reproductive system should be used for development of GnRHa for fish breeding purposes. Furthermore, environment stimuli are still important factors for the function of GnRHa. Therefore, knowledge on variations of types and expressions of GnRH should be taken into consideration in order to develop a more efficient GnRHa-induced breeding.

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