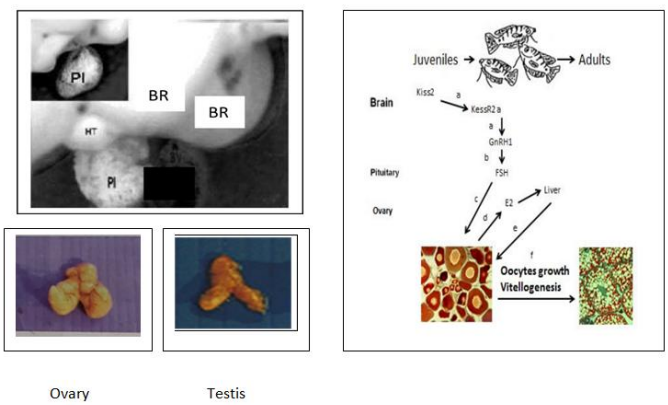
Correlation between Kisspeptin 2 and its Receptors in Blue Gourami Fish (*Trichogaster trichopterus*), and its Role in Reproduction

Gad Degani [a*]

DOI:

ABSTRACT

Expression of kisspeptin 2 (Kiss2) and Kiss receptors (Kiss1r [GPR54] and Kiss2r) was examined in blue gourami fish. The degree of similarity between the cDNA sequences of Kiss2 and Kiss1r in blue gourami compared to various other fish species was low, whereas there was little variation in Kiss2r sequences among the species. To investigate the expression of *Kiss2*, *Kiss1r*, and *Kiss2r* in the female blue gourami brain, quantitative PCR amplification was used to determine relative mRNA levels in juvenile and mature females before vitellogenesis and during vitellogenesis, respectively. Results showed significant differences in their transcription levels, and a model is proposed for regulation of vitellogenesis by Kiss2 in blue gourami.



[a] *School of Science and Technology, Tel-Hai Academic College, Upper Galilee-12210, Israel*.
[b] *MIGAL–Galilee Technology Center, KiryatShmona-11016, Israel.*
**Corresponding author: E-mail: gregoryyd@gmail.com, gad@migal.org.il;*

Graphical Abstract: Gonadotropic brain-pituitary-gonadal (BPG) axis and hypothalamic-pituitary-gonadal (HPG) axis. BR, brain; PI, pituitary gland; HT, hypothalamus; Kiss, kisspeptin; KissR, Kiss receptor; GnRH, gonadotropin-releasing hormone; FSH, follicle-stimulating hormone; E2, estradiol. The HPG axis is suggested to be influenced by the interaction of these hormones.

Keywords: Kisspeptin 2; kisspeptin receptors; blue gourami; vitellogenesis; gonads.

1. INTRODUCTION

Kisspeptin (Kiss), a member of the RFamide peptide family, has gained considerable attention as a potential regulator of reproductive functions in teleosts [1] and other vertebrates. In mammals, the *Kiss1* gene produces various peptides, including Kiss-54, Kiss-14, Kiss-13, and Kiss-10, which are generated by breaking down the Kiss precursor. All of these peptides share a common core sequence—Kiss-10, which allows them to bind to their G-protein coupled receptor GPR54 (also termed Kiss1r) [2,3]. Initially identified as a metastasis suppressor in mammals, Kiss is now recognized as a key player in the neuroendocrine control of reproduction.

Kiss1 is responsible for controlling the hypothalamic-pituitary-gonadal (HPG) axis, which affects gonadotropin-releasing hormone (GnRH) receptors [3,4] in the caudal hypothalamus. It regulates the release of the pituitary gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which further regulate gametogenesis [5] via steroids [6,7]. In teleosts, Kiss involvement in reproduction varies, and further studies are required due to the large systematic class and high variations of hormones involved in reproduction. The potential roles of the kiss/kissr systems in the control of the reproductive axis have also been investigated in nonmammalian vertebrates [8]. The model fish zebrafish has two Kiss genes (*Kiss1* and *Kiss2*) and two Kiss receptors (*Kiss1r* and *Kiss2r*) [9,3], similar to the lamprey (*Petromyzon marinus*) [9], medaka (*Oryzias latipes*) [10], goldfish (*Carassius auratus*) [11] and sea bass (*Morone saxatilis*) [12]. The kiss/kissr systems are presumed to be critical regulators of reproduction in fish and other nonmammalian vertebrates [8].

Blue gourami fish (*Trichogaster trichopterus*) belong to the suborder Labyrinthici, characterized by the presence of an air-filled breathing cavity located above the gills under the operculum, in the family Anabantidae [13,14]. The blue gourami is a useful model for studying the impact of hormonal regulation on reproduction, as it is male-dependent, and has multiple spawnings and asynchronous ovary development [15]. Its hormonal control of reproduction, oogenesis, and spermatogenesis can be influenced by both pheromones and environmental factors [16,17]. Researchers can study and control each stage of its gonadal development separately in a laboratory setting. Studies have been conducted on the blue gourami by Jackson et al. [15,18] and Degani [19,20], among others.

Previous studies have reported the secretion and gene-expression patterns of βFSH, βLH [21], and growth hormone (GH), as well as sex steroid secretion, during gonadal development in male and female blue gourami [16,22,13]. The mRNA levels of GnRH1, GnRH2, and GnRH3 [23] have also been measured during oogenesis in this fish and are associated with FSH and LH transcription. The HPG axis has been suggested to be influenced by these hormones' interactions [24].

The mRNA expression of pituitary adenylate cyclase-activating polypeptide (PACAP) has been measured in blue gourami during different reproductive stages, and its role in regulating the transcription of pituitary hormones was investigated by Levy and Degani [25] and Levy et al. [23]. In that research, the focus was on the brain peptide Kiss2 and two Kiss receptors (Kiss1r and Kiss2r) that are involved in reproduction during vitellogenesis in the blue gourami. Specifically, the study compared the mRNA levels of Kiss2 and the two Kiss receptors in juvenile vs. adult females.

2. MATERIALS AND METHODS

Blue gourami fish were obtained from Ma'abarot fish farm in Kibbutz Ma'abarot, Israel. Average body weight of mature females was 6.64 ± 0.55 g, and of juvenile females, 0.96 ± 0.13 g—significantly different between the two groups. The fish were grown in containers under controlled temperature and light conditions, and were fed a diet supplemented with live food. Female fish at different stages of gonadal development were used for the study. Brain samples were collected from 2-month-old females at different stages of previtellogenesis and 4-month-old females in high vitellogenesis (Figs. 1 and 2). The fish were anesthetized and their fork length and body weight were measured. The brains were removed and frozen for further analysis. The study focused on examining the levels of Kiss1 and 2, and Kiss1r and Kiss2r mRNA in the brain (Fig. 1) [19].

2.1 Histological Analysis

Samples of the gonads were fixed in Bouin's solution and prepared for light microscopy by staining 6-mm thick paraffin sections with hematoxylin and eosin, as previously described [15] (Fig. 2).
Sequence analysis

Total RNA was extracted from whole brains of female fish. The brains were placed in RNAlater at 4oC for 20 h, and diencephalon/midbrain sections were trimmed for analysis of Kiss1, Kiss2, Kiss1r, and Kiss2r. The brains were immediately frozen in liquid nitrogen and stored at -80oC until RNA extraction using Trizol reagent (Invitrogen) and the RNeasy® Mini Kit (Qiagen) according to the manufacturer's protocol. First-strand cDNA synthesis was performed using the QuantiTect Reverse Transcription Kit (Qiagen) on 0.5 to 2 mg of total RNA, following the manufacturer's instructions [23].

Fig.1. Fish were sedated and decapitated to remove their brains. The brains were divided sagittally into two hemispheres and frozen in 1.5-ml tubes containing RNAlater at a temperature of -25°C pending further analysis. (A) Female fish. (B) Anesthetized fish. (C) Fish is dissected to observe the development of its ovaries. (D–G) Upper portion of the brain is removed. (H, I) Brain is placed in a tube containing RNAlater

The cloned fragments were used to create gene-specific primers for 3' and 5' rapid amplification of cDNA ends (RACE) PCR (Table 1). The resulting cDNA sequences were then assembled and analyzed using various software packages. The signal-peptide cleavage site was determined using the SignalP V1.1 program [26], and multiple sequence alignments and phylogenetic cluster analysis were conducted [26] using the ClustalX computer program. To ensure accuracy, at least three independent clones were sequenced for each cDNA sequence.

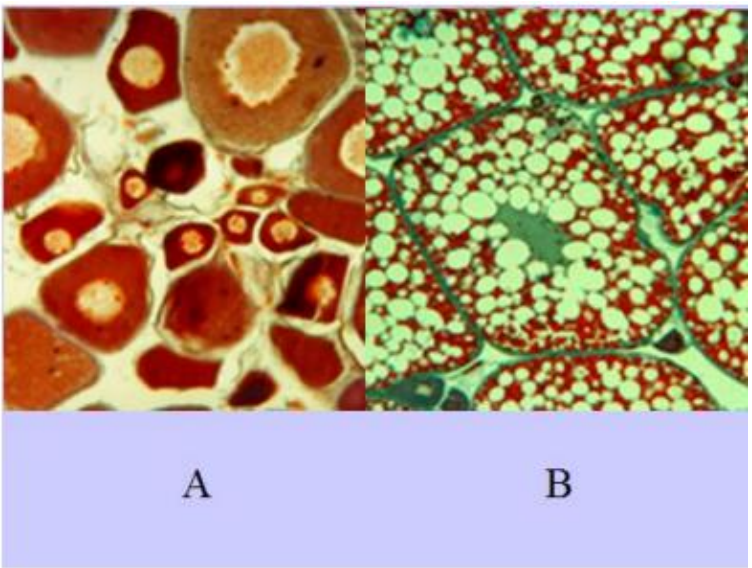


Fig. 2. Females were segregated into two categories based on their stage of gonadal development. (A) Young females who have not yet started producing yolk (previtellogenesis). (B) Females at a later stage of development, with advanced yolk production (high vitellogenesis)

2.2 Real-Time PCR

The amount of *Kiss1*, *Kiss2*, *Kiss1r*, and *Kiss2r* cDNA was measured in the brains of individual fish in a primary culture of dispersed brain cells, using a NanoDrop® ND-1000 instrument (Thermo Scientific). The mRNA levels were compared to that of *β-actin* as the reference gene [27], and the amount of mRNA decay was calculated using methods from Muller et al. [28] and Pfaffl [29].

The 2-ΔCt method was used to determine the relative amount of each gene, where ΔCt is the difference between the cycle thresholds of the target gene and *β-actin*. To confirm the accuracy of this method, brain cDNA samples were diluted and gene-amplification efficiency was compared using Muller et al.'s [28] method by plotting ΔCt versus log template. Different primers were used to amplify *Kiss2*, *Kiss1r*, and *Kiss2r* in various species (blue bass, puffer fish; medaka and zebrafish primers are shown in Table 1). Linear regression was used to analyze the plots, and an R^2 value of 0.99 was considered acceptable for this study.

Table 2 shows the cDNA sequences of *Kiss2*, *Kiss2r*, and *Kiss1r* in blue gourami. Comparisons of these sequences with homologous sequences from other fish species are shown in Figs. 3, 4, and 5.

Table 1. Primers used in this study for *Kiss2*, *Kiss1r*, and *Kiss2r* in blue gourami brain. (But These Are From Other Species?)

Species	Gene	Synthesis direction	5'-3' sequence
Zebrafish	Kiss1Receptor	Forward	TCCGTTCAGAAGCACTGTGG
Zebrafish	Kiss1Receptor	Reverse	TATTTCCACCTTCGGTGCTC
Zebrafish	Kiss2 receptor	Forward	GTCATTAAAAACCAGCAGATGAAGAC
Zebrafish	Kiss2 receptor	Reverse	GTGGTGCACACAGACAGAGCCA
Medaka	Kiss2	Forward	GGTTGTGCTCGTGCTGTGC
Medaka	Kiss2	Reverse	CAGAGTCGTCCTCGCTCCTG

Table 2. cDNA sequences *Kiss2*, *Kiss2r*, and *Kiss1r* in blue gourami

	Trichogaster trichopterus sequences
Kiss2	CTCCGCTGTTGTGTCTCGCKCGGGGAGCGCYGGCCTGTGAATCTYCCCTCCTGGTGGTGG
	ATTCAGCTCTWGCAWTAWCGGGAGGCAGSAACGMCCGAGCTSGGTGCTMCCGTGGGGGGC
	ATTTYCYGTTTGWGGCCCGACTCGAAAGCCCRACCCAGGTAKGYGGACTTGKTGCMCAGC
	TGTGAKGGGCTYTMACWYTAAGMGAAMTGSACACAGGRSGTTRWGTACKGAGAACAYKGA
	CTCATAGARGGWSRTCAAGAACAGKACAATGACGGAGCGAGGACGACTYTGGTGACTTTG
	GCTTCATGTTTGGAGGCAACAGACAGCAACAGGACAGAAACATTCCTAGAGGAAATGACA
	TAGTGCTAGACCTGGAGGTCACCCTAGAAGAGGTGTATTCTGGGAACTTTGTGGAGGTTG
	TACGTAACAAACCCATAGCCAAAGAAGCTCCTGGCAAGAGGAAGTGTAACTGCAGACAGG
	AGCGAGGACGACTCTG
Kiss2r	ATTTATGTCGCCCCCACTTTGCACGCACGCACACACAGARRAATCGYTGTGGCTSTGTST
	GTGTGCACCACAG
Kiss1r	CTWWCRRRCMSSWASCWTGGCYTTMRRGGMCGCMACGCCGAGATMAGTTTGTGGCTGCAGAGGAGCGCAACAACAAT

2.3 Statistical Analysis

Data are expressed as means ± SE. To analyze the differences between experimental groups, a t-test was conducted. In addition, the mRNA levels were compared using one-way ANOVA (F) to identify any significant differences. Statistical significance was set at $p < 0.05$.

3. RESULTS

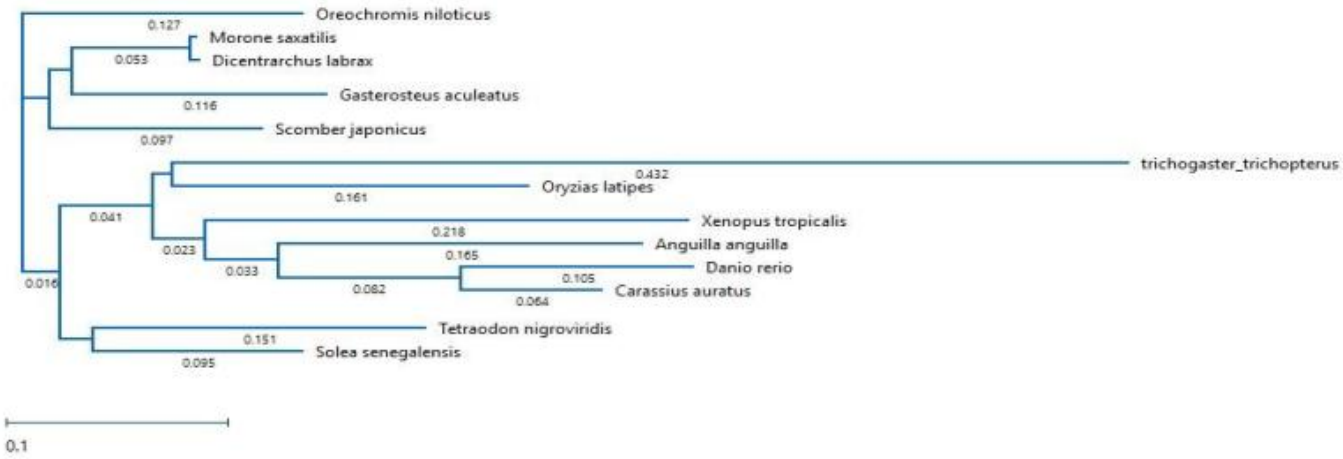
The partial gene sequence of *Kiss2* (Table 2) was compared to *Kiss2* sequences in other fish species (Fig. 3). There was relatively low similarity between the

nucleotide and amino acid sequences of blue gourami *Kiss2* and those of other fish species (Fig. 3). A higher degree of similarity was observed between *Kiss2r* of blue gourami and of other fish species (Fig. 4). Similarly, the nucleotide and amino acid sequences of blue gourami *Kiss1r* showed low similarity to those of other fish species (Fig. 5). To investigate the expression of *Kiss1*, *Kiss2*, *Kiss1r*, and *Kiss2r* in female brains, total RNA was subjected to qRT-PCR and compared to the β-actin gene, which served as an internal control (Fig. 6). However, *Kiss1* was not clearly identified with these methods, and further detailed study is required to determine if it is present and expressed in the brain. Amplification of β-actin in the brain samples showed clear expression (Fig. 6), indicating no RNA degradation of the prepared RNA. The relative mRNA levels of *Kiss2*, *Kiss1r*, and *Kiss2r* were determined in brains of previtellogenic juvenile females and mature females in advanced vitellogenesis (see Fig. 2). Significant differences were observed between *Kiss2* and *Kiss2r* expression levels in the juvenile vs. mature females (Figs. 7 and 8). Mean mRNA levels of *Kiss2* in previtellogenesis were significantly higher than those of oocytes in high vitellogenesis (Fig. 7). The mRNA levels of *Kiss2r* were also higher in juveniles (Fig. 8), in accordance with previous studies. No significant difference was found in *Kiss1r* mRNA levels between mature and juvenile females (Fig. 9). The results supported the hypothesis that *Kiss2* affects *Kiss2r*, which plays a role in controlling reproduction, consistent with previous studies in other fish species. However, the number of Kiss peptides and receptors involved in reproduction varies among different fish species and is not always clear. Other studies have also investigated neurosecretory Kiss hormones in various fish species, such as lamprey, medaka, goldfish, and sea bass (Table 6).

Table 3. *Kiss2* mRNA sequences used for alignment and phylogenetic tree construction

Order	Species	Accession no.
Cypriniformes	*Carassius auratus*	GQ141877.1
Cypriniformes	*Danio rerio*	NM_001142585.1
Beloniformes	*Oryzias latipes*	NM_001160441.1
Perciformes	*Dicentrarchus_labrax*	FJ008915.1
Perciformes	*Oreochromis niloticus*	NM_001279468.1
Perciformes	*Scomber japonicus*	GU731673.1
Perciformes	*Morone saxatilis*	GU351865.1
Perciformes	*Trichogaster trichopterus*	According to this study.
Pleuronectiformes	*Solea senegalensis*	HM116743.1
Gasterosteiformes	*Gasterosteus aculeatus*	KT202354.1
Tetraodontiformes	*Tetraodon nigroviridis*	KT202353.1
Anguilliformes	*Anguilla anguilla*	LT844561.1
Anura	*Xenopus tropicalis*	NM_001162860.1

A


Oreochromis niloticus
0.127
Morone saxatilis
Dicentrarchus labrax
0.053
Gasterosteus aculeatus
0.116
Scomber japonicus
0.097
trichogaster_trichopterus
0.432
Oryzias latipes
0.161
0.041
Xenopus tropicalis
0.218
0.023
Anguilla anguilla
0.165
0.033
Danio rerio
0.105
0.082
Carassius auratus
0.064
0.016
Tetraodon nigroviridis
0.151
Solea senegalensis
0.095
0.1

B

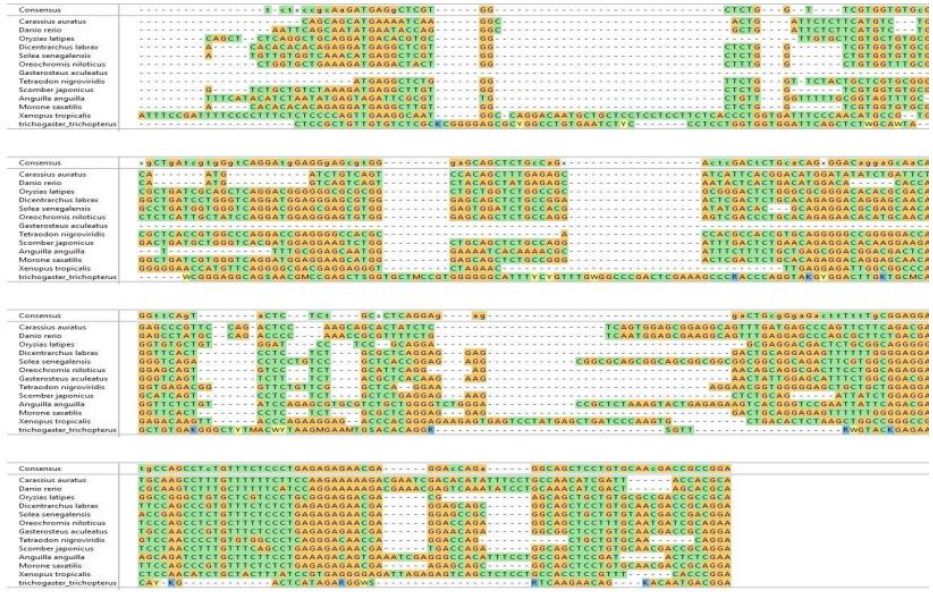


Fig. 3. (A) Phylogenetic tree of fish *Kiss2* cDNA sequences, generated with DNA Star MegAlign PRO MAFFT. *T. trichopterus* sequence is from the current study; all other sequences are from NCBI Genbank, with accession numbers provided in Table 3. (B) Alignment of *Kiss2* sequences from different fish species

Table 4. *Kiss2r* cDNA sequences used for alignment and phylogenetic tree construction

Order	Species	Accession no.
Cypriniformes	*Carassius auratus*	FJ465140.1
Cypriniformes	*Danio rerio*	EU047918.1
Perciformes	*Dicentrarchus_labrax*	JN202446.1
Perciformes	*Scomber japonicus*	JX982323.1
Perciformes	*Trichogaster trichopterus*	According to this study.
Gasterosteiformes	*Gasterosteus aculeatus*	KT261496.1
Tetraodontiformes	*Tetraodon nigroviridis*	KT261495.1
Anura	*Xenopus tropicalis*	NM_001171825.1

A

B

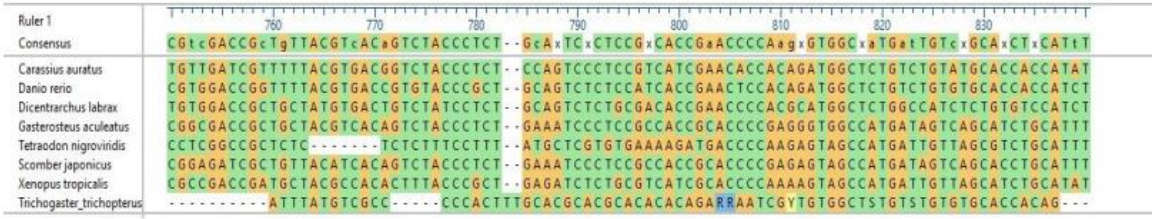


Fig. 4. (A) Phylogenetic tree of *Kiss2r* in vertebrates, generated using DNA Star MegAlign Pro Clustal Omega. *T. trichopterus* sequence is from the current study; all other sequences are from NCBI Genbank, with accession numbers listed in Table 4. (B) Alignment of *Kiss2r* sequences from different fish species

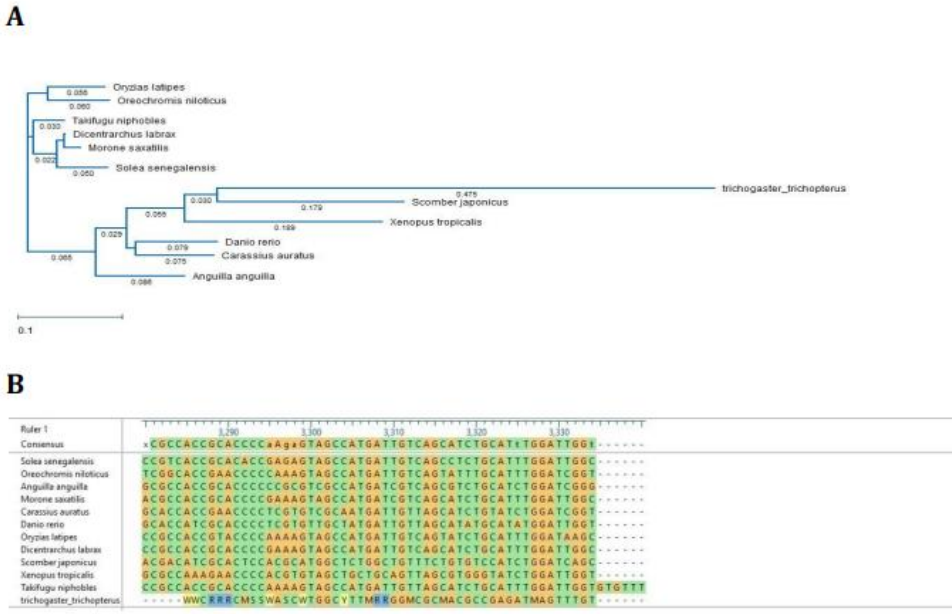


Fig. 5. (A) Phylogenetic tree of vertebrate *Kiss1r* cDNA sequences, generated with DNA Star MegAlign PRO MAFFT. *T. trichopterus* sequence is from the current study; all other sequences are from NCBI Genbank, with accession numbers provided in Table 5. Units of branch length represent nucleotide substitutions per site. (B) Alignment of *Kiss1r* sequences from different fish species

Table 5. *Kiss1r* mRNA sequences used for alignment and phylogenetic tree construction

Order	Species	Accession no.
Cypriniformes	*Carassius auratus*	FJ465139.1
Cypriniformes	*Danio rerio*	NM_001105679.2
Pleuronectiformes	*Solea senegalensis*	EU136710.1
Perciformes	*Oreochromis niloticus*	AB162143.1
Perciformes	*Morone saxatilis*	GU351869.1
Perciformes	*Dicentrarchus_labrax*	JQ839286.1
Perciformes	*Scomber japonicus*	JX982322.1
Perciformes	*Trichogaster trichopterus*	According to this study.
Anguilliformes	*Anguilla anguilla*	FR667382.1
Beloniformes	*Oryzias latipes*	XM_004079431.3
Tetraodontiformes	*Takifugu niphobles*	AB548356.1
Anura	*Xenopus tropicalis*	NM_001170514.1

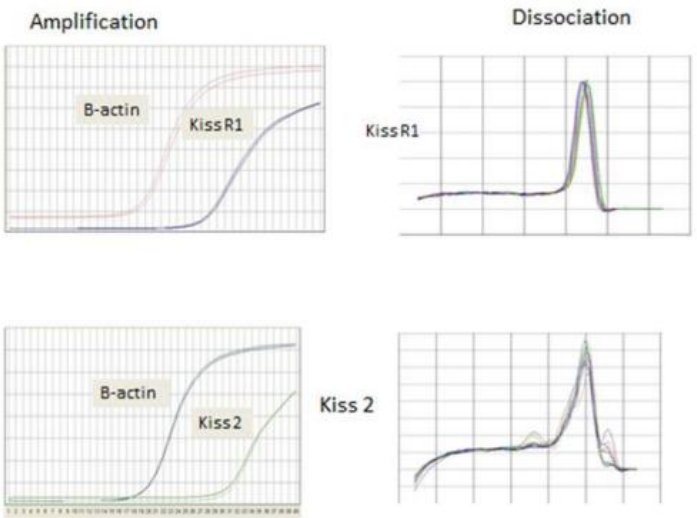


Fig. 6. Results of quantitative PCR for *Kissr1* and *Kiss2* compared to the β-actin gene. Dissociation for *KissR1* occurred at cycle 36, and for *Kiss2* at cycle 25. The primers used for *Kiss1r* were: F1 (TCCGTTCAGAAGCACTGTGG) and R1 (TATTTCCACCTTCGGTGCTC); and for *Kiss2*: F1 (GTCATTAAAAACCAGCAGATGAAGAC) and R1 (GTGGTGCACACAGACAGAGCCA)

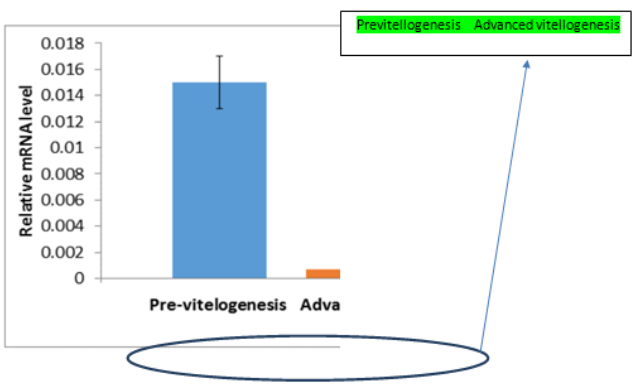


Fig. 7. mRNA levels of *Kiss2* in the brain of female blue gourami measured at different stages of oogenesis: previtellogenesis and advanced vitellogenesis. Each histogram shows the average of independent measurements (mean ± SE). Significant differences in mRNA levels were found between the two stages of oogenesis

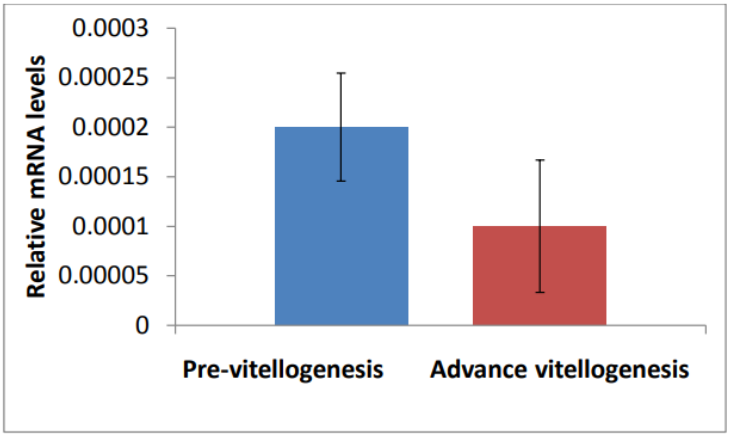


Fig. 8. mRNA levels of *Kiss2r* in the brain of female blue gourami measured at different stages of oogenesis: previtellogenesis and advanced vitellogenesis. Each histogram shows the average of independent measurements (mean ± SE). Significant differences in mRNA levels were found between the two stages

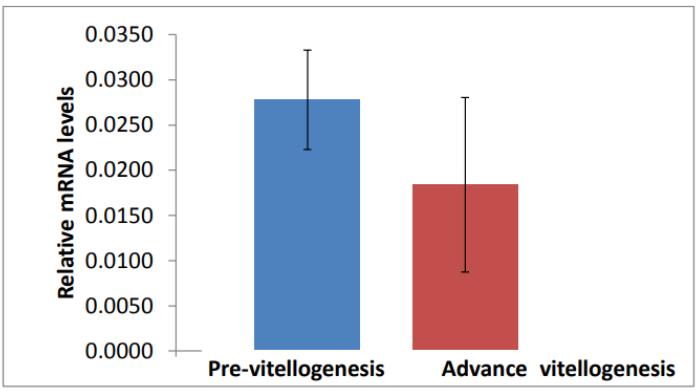


Fig. 9. mRNA levels of *Kiss1r* in the brain of female blue gourami measured at different stages of oogenesis: previtellogenesis and advanced vitellogenesis. Each histogram shows the average of independent measurements (mean ± SE). There was no significant difference in mRNA levels between the two stages of oogenesis

Table 6. Compilation of investigations of the neurosecretory kiss hormones and receptors in different fish species

Species	Kiss1	Kiss2	Kiss1r	Kiss2r	References
Zebra fish (*Danio rerio)*	+	+	+	+	Ogawa et al., [30]
Chub mackerel (Scomber japonicus)	+	+	-	-	Mechaly et al., 2013
Striped bass (Morone saxatilis)	+	+	+	+	Mechaly et al., 2013
Senegalese sole (Solea senegalensis)	-	+	+	+	Mechaly et al., 2009, 2013
Medaka (Oryzias latipes)	+	+	+	+	Kanda, 2012
European Sea Bass (Dicentrarchus labrax)	+	+	+	+	Escobar et al., 2013
Grass puffer (Takifugu niphobles)	-	+	-	+	Tena-Sempere et al., 2012
Three-spined stickleback (Gasterosteus aculeatus)	-	+	+	+	Tena-Sempere et al., 2012
European eel (Anguilla anguilla)	+	+	+	+	Tena Sempere et al., 2012
Tetraodon (Tetraodon nigroviridis)	-	+	-	+	Tena-Sempere et al., 2012
Goldfish (Carassius auratus)	+	+	+	+	Tena-Sempere et al., 2012
Blue gourami (Trichopodus trichopterus)	-	+	+	+	According to this study

4. DISCUSSION

We examined the expression of *Kiss2*, *Kiss1r*, and *Kiss2r* in female blue gourami during vitellogenesis—a crucial stage in fish oogenesis [5]. Our findings lend support to the notion that Kiss2 affects the function of Kiss2r, which plays a role in regulating reproduction. This conclusion is consistent with earlier research conducted on various fish species [1]. Nonetheless, the results from different fish species are not entirely consistent, and the number of Kiss peptides and receptors involved in reproduction in the fish brain is not always well-defined [9, 31]. For instance, in zebrafish, two Kiss genes (*Kiss1* and *Kiss2*) and two kiss receptors (*Kiss1r* and *Kiss2r*) were identified in the brain [9,3]. The Kiss neurosecretory hormones have also been examined in other fish species, such as lamprey (*Petromyzon marinus*) [9], medaka (*Oryzias latipes*) [10], goldfish (*Carassius auratus*) [11], and sea bass (*Dicentrarchus labrax*) [12] (Table 6). In blue gourami, pheromones have a chemical signal effect [16,7] on oogenesis via the HPG axis. Fish pheromones (steroid glucuronides), which are soluble in water, as detected by gas chromatography mass spectrometry, radioimmunoassay and thin-layer chromatography, are found in the gonads and in water in which the fish were maintained [7,30]. The pheromones of blue gourami males affect the female's brain hormones and gonadotropins, which control vitellogenesis and oocyte maturation. Pheromones affect the female's brain-pituitary-gonadal axis and oogenesis [17]. Our hypothesis is that pheromones and environmental factors affect the expression of Kiss2, Kiss1r and Kiss2r, described in the present study, as well as GnRH, which controls gonadotropins (FSH and LH) [23-25]. The hypothesis is supported by the present study, indicating that blue gourami Kiss2 and Kiss2r expression during vitellogenesis and GnRH are involved in the control of oogenesis [25].

According to Jackson et al. [18], specimens classified as being in high vitellogenesis have the highest levels of FSH mRNA. Moreover, the final stage of vitellogenesis may involve GnRH1, FSH, and GH [25]. In grass puffer, sexual maturation is influenced by temperature during the spawning season, as demonstrated by Shahjahan et al. [32] who found that temperature affects the expression of *Kiss1r* and *Kiss2r*, *GnRH*, and gonadotropin subunit genes. In zebrafish, Kiss2-expressing neurons are located in both the dorsal and ventral hypothalamus, whereas Kiss1-expressing cells project solely into the interpeduncular and raphe nuclei and strongly express Kiss1r. The present study revealed differences in the expression of *Kiss1r*, *Kiss2r*, and *Kiss2* during vitellogenesis, but there was no significant difference in *Kiss1r* expression between previtellogenesis and high vitellogenesis in blue gourami. Our interpretation of the results is that just before vitellogenesis begins and is regulated by Kiss2, Kiss2r, GnRH1, FSH, and estradiol (as depicted in Fig. 10), Kiss2 and Kiss2r transcription may synthesize these hormones.

The HPG axis hormones are responsible for regulating vitellogenesis [18,25,17]. Essentially, the blue gourami relies on males for reproduction and has asynchronous ovary development, which leads to lower mRNA levels in high vitellogenesis compared to previtellogenesis [22]. In zebrafish, Kiss2-expressing

cells are primarily found in the dorsal and ventral hypothalamus and extensively project into various regions, such as the sub-pallium, preoptic area, thalamus, ventral and caudal hypothalamus, and mesencephalon. *Kiss2r* mRNA is strongly expressed in all of these areas, and the Kiss2 fibers innervate the ventral forebrain and closely interact with GnRH3 neurons [13]. Estradiol treatment in juvenile fish leads to an increase in Kiss2 and Kiss2r expression, which supports the current study's findings that both Kiss2 and Kiss2r have high expression in juveniles that decreases in mature females toward the end of vitellogenesis, regulated by estrogen [13,14,17].

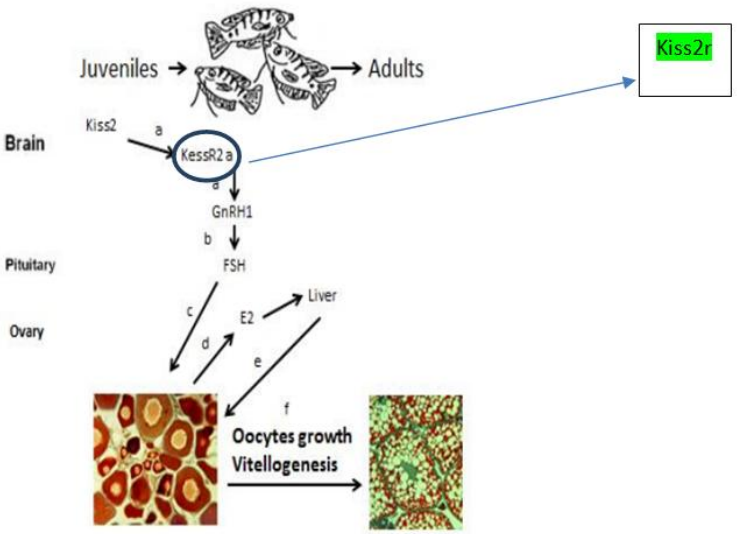


Fig. 10. Proposed qualitative model that illustrates the Kiss2 mechanism responsible for regulating vitellogenesis in blue gourami. The model integrates findings from various studies, including the present one (a), as well as Levy and Degani [24,25] (b), Degani and Boker [20] and Degani [22] (c), Mananos et al. [33] and Degani et al. (1999) (c), Degani [6] and Degani and Boker [20], Degani et al. [13] and Jackson et al. [15] (d), Degani et al. [13] and Jackson et al. [15] (e), and Degani [23] and Jackson et al. [15] (f)

Levy and Degani [12] put forward a hypothesis on the involvement of certain brain hormones—GnRHs (GnRH1, GnRH2, and GnRH3) and PACAP—in the process of oogenesis in blue gourami. They arrived at this hypothesis through studies on hormone control of blue gourami reproduction. The novelty of their findings lies in the suggestion that Kiss2 and its receptors may transmit signals to GnRH1, thereby influencing the release of hormones that play a role in regulating oogenesis. However, further research is needed to establish the validity of this hypothesis. In Fig. 10, we propose a model for controlling vitellogenesis through the brain, which incorporates Kiss2 and its receptors in blue gourami.

5. CONCLUSION

Please insert conclusion section here. Conclusion part is mandatory part of a paper.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Oakley AE, Clifton DK, Steiner RA. Kisspeptin signaling in the brain. Endocr. Rev. 2009;30:713-743.
2. Muir AI, Chamberlain L, Elshourbagy NA, Michalovich D, Moore DJ, Calamari A. AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. J. Biol. Chem. 2001;276:28969-28975.
3. Servili A, Le Page Y, Leprince J, Caraty A, Escobar S, Parhar IS, Seong JY, Vaudry H, Kah O. Organization of two independent kisspeptin systems derived from evolutionary-ancient Kiss genes in the brain of zebrafish. Endocrinology. 2011;152:1527-1540.
4. Shahjahan M, Kitahashi T, Parhar IS. Central pathways integrating metabolism and reproduction in teleosts. Front. Endocrinol. 2014;5:1-17.
5. Yaron Z, Levavi-Sivan B. Hormonal control of reproduction and growth: endocrine regulation of fish reproduction. In: Encyclopedia of Fish Physiology: From Genome to Environment (Editor: A.P. Farrell). Academic Press, San Diego, CA. 2011;1500-1508.
6. Degani G. The effects of human chorionic gonadotropin on steroid changes in Trichogaster trichopterus. Comp. Biochem. Physiol. A. 1990;96:525-528.
7. Degani G. The effect of sexual behavior on oocyte development and steroid changes in Trichogaster trichopterus. Copeia. 1993;4:1091-1096.
8. Beck BH, Fuller SA, Peatman E, McEntire ME, Darwish A, Freeman DW. Chronic exogenous kisspeptin administration accelerates gonadal development in basses of the genus Morone. Comp Biochem Physiol A Mol Integr Physiol. 2012;162:265–273
9. Lee YR, Tsunekawa K, Moon MJ, Um HN, Hwang JI, Osugi T, Otaki N, Sunakawa Y, Kim K, Vaudry H, Kwon HB, Seong JY, Tsutsui K. Molecular evolution of multiple forms of kisspeptins and GPR54 receptors in vertebrates. Endocrinology. 2009;150:2837-2846.
10. Kitahashi T, Ogawa S, Parhar IS. Cloning and expression of kiss2 in the zebrafish and medaka. Endocrinology. 2009;150:821-831.
11. Li S, Zhang Y, Liu Y, Huang X, Huang W, Lu D, Zhu P, Shi Y, Cheng CHK, Liu X, Lin H. Structural and functional multiplicity of the kisspeptin/GPR54 system in goldfish (*Carassius auratus*). J. Endocrinol. 2009;201:407-441.
12. Espigares F, Rocha A, Gómez A, Carillo Zanuy S. Photoperiod modulates the reproductive axis of European sea bass through regulation of kiss1 and gnrh2 neuronal expression. Gen. Comp. Endocrinol. 2017;240:35-45.

13. Degani G. Effect of gonadotropin and steroids on vitellogenesis during ovarian development in Trichogaster trichopterus. J. Aquacult. Trop. 1994;9:15-23.
14. Degani G. Blue Gourami (*Trichogaster trichopterus*) Model for Labyrinth Fish. Laser Pages Publishing, Jerusalem, Israel; 2001.
15. Jackson K, Abraham M, Degani G. Oocyte maturation triggered by the male in a tropical fish Trichogaster trichopterus. J. Morphol. 1994;220:1-9.
16. Becker D, Galili N, Degani G. GCMS-identified steroids and steroid glucoronides in gonads and holding water of Trichogaster tricopterus (Anabantidea, Pallas 1770). Comp. Biochem. Physiol. B. 1992;103:15-19.
17. Degani G. Oogenesis control in multi-spawning blue gourami (*Trichogaster trichopterus*) as a model for the Anabantidae family. Int. J. Sci. Res. 2016;5:179-184.
18. Jackson K. Goldberg G, Ofir R, Abraham M, Degani G. Blue gourami (*Trichogaster trichopterus*) gonadotropic subunits (I & II) cDNA sequences and expression during oogenesis J. Mol. Endocrinol. 1999;23:177-187.
19. Degani G. Interaction of Sexual Behavior and Hormone Gene Expression in the Labyrinthici Fish Blue Gourami (*Trichogaster trichopterus*) during Reproduction. Scientific Research Publishing, Wuhan, China; 2022.
20. Degani G, Boker R. Sensitivity to maturation inducing steroids and gonadotropin in the oocytes of blue gourami Trichogaster trichopterus, (Anabantidae, Pallas, 1770) Gen. Comp. Endinocrinol. 1992;85:430-439.
21. Degani G, Gal E, Vaya J. *In vitro* biosynthesis of steroids in ovary of female *Trichogaster trichopterus* (Pallas, 1770). Comp. Biochem. Physiol. B. 1994;109:715-723.
22. Degani G, Schreibman MP. Pheronome of male blue gourami and its effect on vitellogenesis, steroidogenesis and cells in pituitary of female J. Fish Biol. 1993;43:475-485.
23. Levy G, David D, Degani G. Effect of environmental temperature on growth- and reproduction-related hormone gene expression in the female blue gourami (*Trichogaster trichopterus*). Comp. Biochem. Physiol. A. 2011;160:381-389.
24. Levy G, Degani G. Evidence for a reproduction-related function of pituitary adenylate cyclase-activating polypeptide-related peptide (PRP) in an Anabantidae fish. J. Mol. Endocrinol. 2011;46:101-110.
25. Levy G, Degani G. Involvement of GnRH, PACAP and PRP in the reproduction of blue gourami females (*Trichogaster trichopterus*). J. Mol. Neurosci. 2012;48:9730-9738.
26. Bonfield JK, Smith K, Staden R. A new DNA sequence assembly program. Nucleic Acids Res. 1995;23:4992-4999.
27. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29:9-45.
28. Muller PY, Janovjak H, Miserez AR, Dobbie Z. Processing of gene expression data generated by quantitative real-time RT-PCR. Biotechniques. 2002;32:1372-1379.
29. Pfaffl MW. Quantification strategies in real-time PCR. In: A-Z of Quantitative PCR (Editor: S.A. Bustin). International University Line (IUL) Biotechnology, La Jolla, CA,. 2004;87-112.

30. Ogawa S, Nathan FM, Parha IS. Habenular kisspeptin modulates fear in the zebrafish. Proc. Natl. Acad. Sci. USA. 2014;111:3841-3846.
31. Pasquier J, Kamech N, Lafont AHV, Rousseau K, Dufour S. Molecular evolution of GPCRs: kisspeptin/kisspeptin receptors. J. Mol. Endocrinol. 2014; 52:101-117.
32. Shahjahan M, Motohashi E, Doi H, Ando H. Elevation of Kiss2 and its receptor gene expression in the brain and pituitary of grass puffer during the spawning season. Gen. Comp. Endocrinol. 2010;169:48-57.
33. Mananos E, Zohar Y, Degani G. The relationship between gonadotropin and sexual behavior of male *Trichogaster trichopterus* (Pallas). Indian J. Fisheries. 1997;44:239-246.

Biography of author(s)

Please replace this photo with a clear photo. This photo is not proper

Prof. Gad Degani
School of Science and Technology, Tel-Hai Academic College, Upper Galilee-12210, Israel.
MIGAL–Galilee Technology Center, KiryatShmona-11016, Israel.

He has research and academic experience in gene expression mechanisms in hormonal control of fish growth and reproduction, animals in the ecological system and environmental effects on habitat selection and molecular markers in fish. His area of research includes applied scientific research in the fields of animal biotechnology, and environmental and agriculture sciences. He has published more than 250 articles and 6 books.

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
International Journal of Zoological Investigations, 3(2): 106-122, 2017.

Peer-Review History: During review of this manuscript, double blind peer-review policy has been followed. All manuscripts are thoroughly checked to prevent plagiarism. Minimum two peer-reviewers reviewed each manuscript. After review and revision of the manuscript, the Book Editor approved only quality manuscripts for final publication.