Sexually dimorphic distribution of kiss1 and kiss2 in the brain of yellowtail clownfish, *Amphiprion clarkii*

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**Abstract**

Kisspeptin system was shown to be a key factor in mediating social stress and reproduction. Yellowtail clownfish, *Amphiprion clarkii*, is a hermaphrodite fish, whose sex determination and gonadal development are affected by the social status of individuals. The yellowtail clownfish is a fantastic animal model to explore sex determination, but the social status and precise distribution of kiss mRNAs in the brain of this species are unknown. Herein, a novel in situ hybridization technique, RNAscope, was used to investigate the distribution of kiss1 and kiss2 expressions in the brain of yellowtail clownfish. The coronal planes of brain showed that the kiss1 signal was mainly present in dorsal habenular nucleus (NHd) and kiss2 mRNA was widely expressed in telencephalon, midbrain, and hypothalamus, especially in dorsal part of the nucleus of the lateral recess (NRLd). Additionally, kiss1 and kiss2 signals have sexually dimorphic distribution. The kiss1 mRNA was distributed in NHd, the telencephalon, and lateral part of the diffuse nucleus of the inferior lobe (NDLII) of females but in NHd and NDLII of males. kiss2 signals were stronger in females than that in males. The distribution of kiss1 and kiss2 neurons in NHd of habenula and NRLd of hypothalamus may suggest that kiss genes associate environmental signaling and reproductive function in yellowtail clownfish.

**Introduction**

Kisspeptin is an upstream regulator of the reproductive axis (hypothalamic–pituitary–gonadal, HPG axis) (1). Kisspeptin interacts with its receptor, KissR (G protein-coupled receptor 54, GPR54), resulting in the release of the gonadotropin-releasing hormone (GnRH) and further regulating the gonadotropic hormone (GtHs, including luteinizing hormone and follicle-stimulating hormone) secretion (2). The GtHs act on the gonads and affect sexual differentiation and gonadal development in teleosts (3). Furthermore, kisspeptin system is also involved in modulating certain cancers and vascular dynamics (4).

Kisspeptin is encoded by one gene (*KISS1/KissI*) in mammals, whereas two paralogous genes, kiss1 and kiss2, have been identified in almost all teleosts, including Nile tilapia (*Oreochromis niloticus*), zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), chub mackerel (*Scomber japonicus*), rohu (*Labeo rohita*), Siberian sturgeon (*Acipenser baerii*), sapphire devil (*Chrysiptera cyanea*), rare minnow (*Gobiocypris rarus*), pejerrey (*Odontesthes bonariensis*), sea bass (*Dicentrarchus labrax*), orange-spotted grouper (*Epinephelus coioides*), and goldfish (*Carassius auratus*) (5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16). Additionally, only one kisspeptin-encoding gene...
was identified in several pleuronectiforms (17). Utilizing RNA sequencing and genomics technology, the multiple gene encoding kisspeptin will be identified in more teleosts (18, 19). Different expression patterns of kiss1 and kiss2 indicate the distinct physiological functions they would play. In chub mackerel, kiss1 is mainly expressed in the brain, whereas kiss2 is expressed in the brain, pituitary, and testis (20). In yellowtail clownfish (Amphiprion clarkii), the highest kiss1 expression level is detected in the liver, but kiss2 is mainly expressed in the cerebellum, pituitary, and hypothalamus (21). In rare minnow with estradiol treatment, both kiss1 and kiss2 are increased in the female brain but suppressed in the male brain (11). In zebrafish, kiss1 is mainly expressed in the habenula (vHb) and kiss2 signals are distributed in the dorsal zone (Hd), the posterior tuberal nucleus (nPT), and the ventral (Hv) (22). The brain is regarded as the organ where kisspeptin genes primarily act, and the habenula mainly regulates circadian rhythm and stress response (23). The sexually dimorphic distribution of kiss-positive cells in the brain is reported in medaka and zebrafish (24, 25). Under reproductive conditions, more nucleus ventral tuberis (NVT) KISS-1 neurons are observed in male medaka than females (24). In zebrafish, kiss2-positive cells are identified in the pituitary of females but not males (25).

The sexual reversal of hermaphroditic teleosts is associated with social stress and gonadal development (26). Acute and chronic stress with corticosterone decrease Kiss1 but increase Kiss1r expression in the medial preoptic area (mPOA) and the arcuate nucleus (ARC) of female rats (27). In the African cichlid fish (Astatotilapia burtonii), the male is a subordinate individual in the group, whose reproductive activity is inhibited and the expression of kiss1r is lower throughout brain (28). Recent studies have shown that kiss2 but not kiss1 is involved in the regulation of social stress and the gonad development in yellowtail clownfish (21). Social stress may directly act on the kisspeptin signal system via glucocorticoid and then participate in the regulation of gonadal differentiation and sexual reversal. However, the distribution of two kiss-expressing neurons in the brain of sexual reversal teleosts has been poorly studied.

The yellowtail clownfish is a protandrous hermaphroditic teleost whose sexual development can be regulated by its social status (29). In general, there is only one dominant female individual and one subdominant male with reproductive function in the group, while non-breeder will become the mate of the subdominant male individual (30). Social sex determination of yellowtail clownfish is a specific reproductive phenomenon regulated by the social stress and HPG axis (31). The yellowtail clownfish is regarded as a suitable model to study the mechanism of social sex determination (32).

In the present study, we would explore the sexually dimorphic distribution of kiss1 and kiss2 mRNA in the brain of yellowtail clownfish using RNAscope in situ hybridization. The research on the distribution of kiss-expressing brain regions is the basis for elucidating the association between environmental cues and reproductive function.

Materials and methods

Animals

Sexually mature yellowtail clownfish were purchased from a local aquarium (Haikou city, Hainan, China). Fish were fed with commercial feeds twice a day (08:30 and 17:30 h) in culture system with circulating seawater for acclimatization. Water temperature was maintained at ranges from 26°C to 28°C and the photoperiod was a 12 h light:12 h darkness cycle. After acclimatization for a week, fish were anaesthetized with 0.05% MS222 (Sigma). The gonad and brain of each individual were fixed in Bouin’s solution (Sigma) and 4% paraformaldehyde fix solution (Sigma), respectively.

This study protocol was reviewed and approved by Hainan University Institutional Animal Use and Care Committee, approval number HNUAUCC-2021-00014.

Histological sex identification and tissue preparation

The fixed gonad of each yellowtail clownfish was embedded in paraffin after ethanol dehydration and xylene transparency. The gonadal tissues were cut into 5 μm paraffin slices and stained with hematoxylin and eosin and then observed by microscope to determine sex of each individual (Fig. 1).

All fixed brains were dehydrated through diethyl pyrocarbonate (DEPC)-treated PBS with 30% sucrose gradients and embedded in Tissue-Tek OCT (Sakura Finetechnical, Tokyo, Japan). The brain tissues of female (n = 3) were cut into 10 μm sagittal slices on Superfrost® Plus Microscope Slides (Fisher Scientific). Depending on the distribution of kiss1 and kiss2 signals in the female...
The brain of yellowtail clownfish in sagittal planes (Fig. 2O), the brain tissues of female and male (n = 3) were cut into 10 μm coronal slices (Fig. 3), respectively. The level of slices in the sagittal and coronal drawing view of yellowtail clownfish brain was separately shown in Figs 2P and 3. All manipulations were RNase-free.

**Fluorescent in situ hybridization**

RNAscope probes were designed with reference to the kiss1 (GenBank No.: MK368701) and kiss2 (GenBank No.: MK368702) genes of yellowtail clownfish and listed in Table 1. Fluorescent in situ hybridization (FISH) was provided by RNAscope® Multiplex Fluorescent Reagent Kit (Advanced Cell Diagnostics, Hayward, USA). Briefly, the cleared slices were incubated with hydrogen peroxide for 10 min at room temperature and then treated in boiling 1× RNAscope® Target Retrieval Reagents after washing with RNase-free water. The slices were washed with RNase-free water and ethanol to ensure complete drying at room temperature. RNAscope® Protease Reagents were dropped onto the slices and treated at 40°C for 30 min. After washing, the brain tissue slices of yellowtail clownfish were incubated with the probe solution in ACD HybEZ™ II

**Figure 1**

Histological slices were used to distinguish females and males according to the level of gonadal development in yellowtail clownfish. (A) The histological slices of the female gonad; (B) the histological slices of the male gonad. O1, oocytes in primary growth stage; O2, oocytes in cortical vesicle stage; O3, oocytes in vitellogenesis stage; SSC, secondary spermatocytes; S, spermatozoon. Scale = 200 μm.

**Figure 2**

The sagittal distribution of kiss1 and kiss2 mRNA in female yellowtail clownfish brain. (A, B, C and D) The kiss1-expressed brain regions in sagittal planes of the female brain; (E, F, G, H, I, J, K and L) the kiss2 expressed brain regions in sagittal planes of the female brain; (M) negative control; (N) positive control; (O) the level of the slices in the sagittal drawing view of female yellowtail clownfish brain; (P) the distribution of kiss1 and kiss2 in the female brain of yellowtail clownfish in sagittal planes. Tel, telencephalon; Vam, medial division of valvula cerebelli; CER, cerebellum; Hyp, hypothalamus; OT, optic tectum; DIE, diencephalon; MO, medulla oblongata; Hyp-NRLd, dorsal part of the nucleus of the lateral recess. Scale = 20 μm.
Hybridization System (ACD Bio-Techne, USA) at 40°C for 2 h and washed twice in 1× RNAscope® wash buffer. Slices were sequentially immersed in AMP-1 and AMP-2 reagent at 40°C twice for 30 min each and finally immersed in AMP-3 reagent at 40°C twice for 15 min each. RNAscope® HRP-C1 signal was developed and employed TSA® Plus Cy3 (Perkin Elmer) to mark probe. All treated slices were incubated with DAPI for 30 sbefore being washed and cover coverslips with Prolong Gold Antifade (Thermo Fisher Scientific) mounting medium. Images were captured by fluorescence confocal microscopy (Nikon ECLIPSE Ti2) and analyzed on selected regions by NIS-Elements AR 5.30.02.

### Results

**Distribution of kiss1 and kiss2 mRNA in the brain of yellowtail clownfish**

kiss1 and kiss2 signals were detected in the sagittal planes of yellowtail clownfish brain (Fig. 2). In the female brain,
the *kiss1*-positive signal was observed in the telencephalon (Tel), medial division of valvula cerebelli (Vam), cerebellum (CER), and hypothalamus (Hyp) (Fig. 2A, B, C and D). The *kiss2* mRNA was the whole brain distributed and mainly expressed in Tel, optic tectum (OT), Vam, CER, diencephalon (DIE), medulla oblongata (MO), and Hyp (Fig. 2E, F, G, H, I, J, K and L). Based on the distribution of *kiss1* and *kiss2* signals in the female brain of yellowtail clownfish in sagittal planes (Fig. 2P), four coronal slices were selected for the next studies (Fig. 3).

**Sexually dimorphic distribution of *kiss1* mRNA in the brain of yellowtail clownfish**

The distribution of *kiss1* positive signals was marked in the coronal drawing view of yellowtail clownfish brain (Fig. 3). In females, the *kiss1* signal was highly expressed at the dorsal habenular nucleus (NHd) of the habenula and lateral part of the diffuse nucleus of the inferior lobe (NDLII) of hypothalamus, as well as minimally distributed in subdivision 3 of the medial dorsal telencephalic area (Dm3), subdivision 2 of the medial dorsal telencephalic area (Dm2), lateral posterior part of the dorsal telencephalic area (Dip), and posterior portion of the dorsal telencephalon (Dp) regions of the telencephalon (Fig. 4A, B, C, D, I, J and K). In males, the *kiss1* mRNA was abundantly distributed at NHd in the habenula and low expressed at NDLII of the hypothalamus. Compared with females, *kiss1* was not detected in other brain regions of males (Fig. 4E, F, G, H, M, N and O).

**Sexually dimorphic distribution of *kiss2* mRNA in the brain of yellowtail clownfish**

The distribution of the *kiss2*-positive signals was marked in the coronal drawing view of yellowtail clownfish brain (Fig. 3). In females, *kiss2* transcripts were widely distributed in the telencephalon, midbrain, and hypothalamus, especially in the dorsal part of the nucleus of the lateral recess (NRLd) (Fig. 5A, B, C, D, E, F, M, N, O, P and Q). In males, *kiss2*-signaling molecules were found in the telencephalon, midbrain, and abundantly distributed at NRLd of the hypothalamus (Fig. 4G, H, I, J, K, L, S, T, U, V and W). The similar distribution of *kiss2* mRNA was observed between males and females, whereas the stronger signal intensity of *kiss2* was found in females than in males.

**Discussion**

Sex determination of teleosts includes genotypic sex determination and environmental sex determination...
ESD has been deeply studied, but regulatory mechanisms in fish with the more complex social sex determination are still poorly understood (34, 35). Kisspeptin/GPR-54 signaling system is speculated as the key integrator between environmental cues and reproduction (2). In the present study, we investigated the distribution of kiss1 and kiss2 genes in the brain of both female and male yellowtail clownfish by RNAscope.

In mammals, kisspeptin neurons are mainly localized in the anteroventral periventricular (AVPV), the periventricular nucleus (PeN), and the arcuate (ARC) hypothalamic nucleus (1). Kisspeptin neurons show the wide distribution in brain of teleosts. In the zebrafish brain, kiss1 neurons are located in the ventromedial habenula and periventricular hypothalamic nucleus; the kiss2 neurons are distributed in the preoptic area (POA), midbasal hypothalamus, posterior tuberous nucleus, and periventricular hypothalamic nucleus (22, 36). In medaka, cells expressing kiss1 mRNA are mainly found in the habenula, hypothalamus, NVT, and nucleus posterioris periventricularis (NPPv). The distribution of medaka kiss2 neurons is similar to that in zebrafish (36). The distribution of kiss1 neurons in goldfish resembles that in zebrafish, and kiss2 mRNA is mainly expressed in the POA, nucleus lateralis tuberis (NLT), and nucleus recessus lateralis (NRL) (37, 38). In the present study, both kiss1- and kiss2-expressing cells were mainly distributed in the Tel, Vam, CER, and Hyp regions, while kiss2 signals were detected in the OT, DIE, and MO regions compared with kiss1. The kiss2 mRNA is more widely distributed in the brain of yellowtail clownfish than kiss1 mRNA. Moreover, kiss1 showed a high-intensity signal in NHd of the habenula and kiss2 was highly expressed at the NRLd of the hypothalamus. In African clawed frog (Xenopus Laevis), kiss gene signals are found in the ventral hypothalamic (VH), but kiss2 has more excess expression in the POA than kiss1 (36). The hypothalamus is considered a region of upstream regulation of the reproductive axis. The habenula, involved

![Image of a diagram with coronal distributions of kiss2 mRNA in the brain of yellowtail clownfish](https://doi.org/10.1530/EC-22-0136)
in behavioral responses related to pain, stress, anxiety and sleep, has the most conserved structure in the brain of vertebrates and is the main region of kiss1 distribution in teleosts such as zebrafish, medaka, goldfish, and European seabass (Dicentrarchus labrax) (22, 24, 38, 39, 40). Thus, yellowtail clownfish habenular kiss1 may be related to environmental and metabolic signals. In addition, kiss1r is detected in GnRH neurons of tilapia, suggesting that Kiss1 has a potential role in regulating reproduction (41).

The distribution of kiss mRNA is sexually dimorphic. In the NVT of medaka, males have a greater number of kiss1 neurons than females (24). Yellowtail clownfish kiss2 exhibited stronger signals in NRLd of the hypothalamus of the female than the male. Furthermore, our results showed that the kiss1 mRNA has a broader distribution pattern in females than in males. In red seabeam (Pagrus major), kiss2 mRNA is mainly found in the NRLd and NRLv parts of hypothalamic nucleus recessi lateralis, and it has high expression in mature males compared with the male after spawning (42). In European seabass, kiss2r mRNA is detected in GnRH neurons (22). Moreover, kisspeptin-2 is more effective in regulating gonadotropin synthesis compared to kisspeptin-1 in zebrafish and medaka (36). Therefore, kiss2 might be associated with reproductive function.

Briefly, kiss1 is more widely distributed in females, and kiss2 is less abundant in males than females, implying that kiss1 and kiss2 might have different functions between sexuality and social status, and the lack of kiss2 mRNA leads to the delay of gonadal development. The previous studies showed that kiss1 and kiss2 show different expression patterns in yellowtail clownfish individuals under different social statuses, and kiss2 is considered to be the key regulatory gene in reproductive function (21). In goldfish, the GRE domain is found in the promoter region of kiss gene, suggesting that kisspeptin may be regulated by glucocorticoid receptor (GR) (43). It is reported that the ventromedial hypothalamic nucleus (VMH) has steroidogenic factor 1 (SF1; also known as Nr5a1) neurons, suggesting that glucocorticoids are associated with kisspeptin neurons in the hypothalamic region, especially kiss2 neurons (44). Moreover, different social status individuals with divergent cortisol levels are observed in Nile tilapia (45). Our previous study showed that GR2 is more sensitive to cortisol than GR1 in yellowtail clownfish (32). GR genes also show the sexually dimorphic expression in the brain of medaka, in which GR has high expression in several preoptic and thalamic nuclei of females (46).

In the present study, kiss2 had higher positive signals in primary brain nuclei, which may suggest that kiss genes have disparate functions among different brain regions. Furthermore, only one kiss gene (kiss2) is reported in Nile tilapia and puffer fish (Takifugu niphobles) (47, 48). Therefore, kiss2 might have an important role in reproductive function, whereas kiss1 may be involved in sensing environmental signals and metabolism (22). The results of the present research further support this hypothesis.

**Conclusion**

Sexually dimorphic distribution of kiss genes in the brain of yellowtail clownfish is studied. The kiss1 mRNA had wider and stronger signal intensity in female individuals than in males. The distribution of the kiss2-positive brain region was similar in both females and males, but the signal intensity was stronger in females. In our results, kiss1/kiss2 signals were detected in ND1ll and NRLd of hypothalamus implicating the possible involvement of kiss genes in reproductive regulation. Moreover, the kiss1 signals detected in habenula suggest that kiss1 may be associated with environmental and metabolic signals, such as social stress, pain, and anxiety. At last, kiss genes involved in environmental cues and reproductive function may be key regulators of sex reversed fish with ESD.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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**Data availability statement**

The data sets that were analyzed during the current study are available from the corresponding author on reasonable request.

**Author contribution statement**

Zhang Yan-yu, Zhang Xian, and Wang Qian contributed to the study design. Zhang Xian, Bu Shao-yang, Zhang Wei-wei, Li Tian-xiu, Zheng De-cai, and Huang Ze-xiang contributed to the acquisition of data. Zhang Yan-yu, Zhang Xian, and Bu Shao-yang performed statistical analyses. Zhang Yan-yu and Wang Qian drafted the manuscript. Zhang Yan-yu, Zhang Xian, Bu Shao-yang, Zhang Wei-wei, Li Tian-xiu, Zheng De-cai, Huang Ze-xiang, and Wang Qian contributed to data interpretation, provided critical revisions, and approved the final version of the manuscript.

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References

1 Ogawa S & Parhar IS. Anatomy of the kisspeptin systems in teleosts. General and Comparative Endocrinology 2013 181 169–174. (https://doi.org/10.1016/j.ygcen.2012.08.023)

2 Treviran CM, Montagana E, De Oliveira R, Christofolini DM, Barbosa CR, Crandall KA & Bianco B. Kisspeptin/GPR54 system: what do we know about its role in human reproduction? Cellular Physiology and Biochemistry 2018 49 1259–1276. (https://doi.org/10.1159/000493406)

3 Wu F, Zhang X, Zhang W, Huang B, Liu Z, Hu C & Wang D. Expression of three gonadotropin subunits in Southern catfish gonad and their possible roles during early gonadal development. Comparative Biochemistry and Physiology: Part A, Molecular and Integrative Physiology 2009 153 44–48. (https://doi.org/10.1016/j.cbpa.2008.12.013)

4 Oakley AE, Clifton DK & Steiner RA. Kisspeptin signaling in the brain. Endocrine Reviews 2009 30 713–743. (https://doi.org/10.1210/er.2009-0005)

5 Van Aere R, Kille P, Lange A & Tyler CR. Evidence for the existence of a functional Kiss1/Kiss1 receptor pathway in fish. Peptides 2008 29 57–64. (https://doi.org/10.1016/j.peptides.2007.10.018)

6 Li S, Zhang Y, Liu Y, Huang X, Huang W, Lu D, Zhu P, Shi Y, Cheng CH, Liu X, et al. Structural and functional multiplicity of the kisspeptin/GPR54 system in goldfish (Cassiope aurata). Journal of Endocrinology 2009 201 407–418. (https://doi.org/10.1677/JOE-09-0016)

7 Mitani Y, Kanda S, Akazome Y, Zempo B & Oka Y. Hypothalamic Kiss1 but not Kiss2 neurons are involved in estrogen feedback in medaka (Orzysias latipes). Endocrinology 2010 151 1751–1759. (https://doi.org/10.1210/en.2010-09117)

8 Selvaraj S, Kishan H, Fujinaga Y, Oneda M, Yamaguchi A, Shimizu A & Matsuysau M. Molecular characterization, tissue distribution, and mRNA expression profiles of two Kiss genes in the adult male and female chub mackerel (Scomber japonicus) during different gonadal stages. General and Comparative Endocrinology 2010 169 28–38. (https://doi.org/10.1016/j.ygcen.2010.07.011)

9 Escobar S, Felip A, Zanuy S & Carrillo M. Is the kisspeptin system involved in responses to food restriction in order to preserve reproduction in pubertal male sea bass (Dicentarchus labrax)? Comparative Biochemistry and Physiology: Part A, Molecular and Integrative Physiology 2016 199 38–46. (https://doi.org/10.1016/j.cbpa.2016.05.005)

10 Saha A, Pradhan A, Sengupta S, Nayak M, Samanta M, Sahoo I & Giri SS. Molecular characterization of two kiss genes and their expression in rohu (Labeo rohita) during annual reproductive cycle. Comparative Biochemistry and Physiology: Part B, Biochemistry and Molecular Biology 2016 191 135–145. (https://doi.org/10.1016/j.cbpb.2015.09.008)

11 Yang Y, Gao J, Yuan C, Zhang Y, Guan Y & Wang Z. Molecular identification of Kiss/GPR54 and function analysis with mRNA expression profiles exposure to 17a-ethinylestradiol in rare minnow Gobioicypris sarus. Molecular Biology Reports 2016 43 737–749. (https://doi.org/10.1007/s11033-016-4014-y)

12 Guo Y, Wang Q, Li G, He M, Tang H, Zhang H, Yang X, Liu X & Lin H. Molecular mechanism of feedback regulation of 17β-estradiol on two kiss genes in the protogynous orange-spotted grouper (Epinephelus coioides). Molecular Reproduction and Development 2017 84 495–507. (https://doi.org/10.1002/mrd.22800)

13 Tovar Bohorquez MO, Mechaly AS, Hughes LC, Campanella D, Ortí G, Canosa LF & Somaza GM. Kisspeptin system in pejenever fish (Odontesthes bonariensis) characterization and gene expression pattern during early developmental stages. Comparative Biochemistry and Physiology: Part A, Molecular and Integrative Physiology 2017 204 146–156. (https://doi.org/10.1016/j.cbpa.2016.11.014)

14 Imamura S, Hur SP, Takeuchi Y, Badruzzaman M, Mahardini A, Rizky D & Takemura A. The mRNA expression patterns of kisspeptins, GnRHS, and gonadotropins in the brain and pituitary gland of a tropical damselfish, Chrysipetra cyanus, during the reproductive cycle. Fish Physiology and Biochemistry 2020 46 277–291. (https://doi.org/10.1007/s10695-019-00715-5)

15 Ogawa S, Sivalingam M, Anthonlysamy R & Parhar IS. Distribution of Kiss2 receptor in the brain and its localization in neuroendocrine cells in the zebrafish. Cell and Tissue Research 2020 379 349–372. (https://doi.org/10.1007/s00441-019-03089-5)

16 Xu S, Wang M, Li Y, Tang N, Zhang X, Chen H, Zhang S, Liu Y, Wang J, Chen D, et al. Cloning and expression of kiss genes and regulation of feeding in Siberian sturgeon (Acipenser baerii). Fish Physiology and Biochemistry 2020 48 419–436. (https://doi.org/10.1007/s10695-022-01055-7)

17 Wang B, Mecahly AS & Somoza GM. Overview and new insights into the diversity, evolution, role, and regulation of kisspeptins and their receptors in teleost fish. Frontiers in Endocrinology 2022 13 862614. (https://doi.org/10.3389/fendo.2022.862614)

18 Somoza GM, Mechaly AS & Truevel VL. Kisspeptin and GnRH interactions in the reproductive brain of teleosts. General and Comparative Endocrinology 2020 298 113568. (https://doi.org/10.1016/j.ygcen.2020.113568)

19 Sivalingam M, Ogawa S, Truevel VL & Parhar IS. Conserved functions of hypothalamic kisspeptin in vertebrates. General and Comparative Endocrinology 2022 317 113973. (https://doi.org/10.1016/j.ygcen.2021.113973)

20 Ohga H, Selvaraj S & Matsuysau M. The roles of kisspeptin system in the reproductive physiology of fish with special reference to chub mackerel studies as main axis. Frontiers in Endocrinology 2019 9 147. (https://doi.org/10.3389/fendo.2019.00047)

21 Zhang H, Zhang Y, Guo Y, Zhang X, Wang Q, Liu X & Lin H. Kiss2 but not kiss1 is involved in the regulation of social stress on the gonad development in yellowtail clownfish, Amphiprion clarkii. General and Comparative Endocrinology 2020 298 113551. (https://doi.org/10.1016/j.ygcen.2020.113551)

22 Servilli A, Le Page Y, Leprince J, Caraty A, Escobar S, Parhar IS, Seong YM, Vaudry H & Koh O. Organization of two independent kisspeptin systems derived from evolutionary-ancient kiss genes in the brain of zebrafish. Endocrinology 2011 152 1527–1540. (https://doi.org/10.1210/en.2010-0948)

23 Namboodiri VMK, Rodriguez-Romaguera J & Stuber GD. The habenula. Current Biology 2016 26 R873–R877. (https://doi.org/10.1016/j.cub.2016.08.051)

24 Kanda S, Akazome Y, Matsuynaga T, Yamamoto N, Yamada S, Tsukamura H, Maeda KI & Oka Y. Identification of KiSS-1 product KiSS-1/1 product in goldfish (Oryzias latipes). Endocrinology 2006 149 2467–2476. (https://doi.org/10.1210/en.2006-1503)

25 Song Y, Chen J, Tao B, Luo D, Zhu Z & Hu W. Kiss2 regulates hormone expression in female zebrafish (Danio rerio) pituitary. Molecular and Cellular Endocrinology 2020 513 110858. (https://doi.org/10.1016/j.mce.2020.110858)

26 Whirlhege S & Cidkowski JA. A role for glucocorticoids in stress-impaired reproduction: Beyond the hypothalamus and pituitary. Endocrinology 2013 154 4450–4466. (https://doi.org/10.1210/en.2013-1632)

27 Kinsey-Jones JS, Li XE, Knox AMI, Wilkinson ES, Zhu XL, Chaudhary AA, Milligan SR, Lightman SL & O’Byrne KT. Down-regulation of hypothalamic kisspeptin and its receptor, Kiss1r, mRNA expression is associated with stress-induced suppression of luteinising hormone secretion in the female rat. Journal of Neuroendocrinology 2009 21 20–29. (https://doi.org/10.1111/j.1365-2632.2008.01807.x)

28 Grone BP, Maruska KP, Korzan WJ & Fernald RD. Social status regulates kisspeptin receptor mRNA in the brain of Astotolatilapia burtoni. General and Comparative Endocrinology 2010 169 98–107. (https://doi.org/10.1016/j.ygcen.2010.07.018)

29 Miura S, Komatsu T, Higa M, Bhandari RK, Nakamura S & Nakamura M. Gonadal sex differentiation in protandrous anemone
fish, Amphiprion clarkii. *Fish Physiology and Biochemistry* 2003 28 165–166. ([https://doi.org/10.1023/B:FISH.0000030513.05061.88](https://doi.org/10.1023/B:FISH.0000030513.05061.88))

30 Hattori A & Yanagisawa Y. Life-history pathways in relation to gonadal sex differentiation in the anemonefish, *Amphiprion clarkii*, in temperate waters of Japan. *Environmental Biology of Fishes* 1991 31 139–155. ([https://doi.org/10.1007/BF00001015](https://doi.org/10.1007/BF00001015))

31 Chen J, Xiao L, Peng C, Ye Z, Wang D, Yang Y, Zhang H, Zhao M, Li S, Lin H, et al. Socially controlled male-to-female sex reversal in the protogynous orange-spotted grouper, *Epinephelus coioides*. *Journal of Fish Biology* 2019 94 414–421. ([https://doi.org/10.1111/jfb.13991](https://doi.org/10.1111/jfb.13991))

32 Zhang Y, Zhang H, Wang J, Zhang X, Bu S, Liu X, Wang Q & Lin H. Molecular characterization and expression patterns of glucocorticoid receptor (GR) genes in protandrous hermaphrodite yellowtail clownfish, *Amphiprion clarkii*. *Gene* 2020 745 144651. ([https://doi.org/10.1016/j.gene.2020.144651](https://doi.org/10.1016/j.gene.2020.144651))

33 Capel B. Vertebrate sex determination: evolutionary plasticity of a fundamental switch. *Nature Reviews. Genetics* 2017 18 675–689. ([https://doi.org/10.1038/nrg.2017.60](https://doi.org/10.1038/nrg.2017.60))

34 Conover DO. Temperature-dependent sex determination in fishes. *Temperature-Dependent Sex Determination in Vertebrates* 2004 11 20.

35 Santidrián TP & Spotila JR. Temperature-dependent sex determination in sea turtles in the context of climate change: uncovering the adaptive significance. *BioEssays* 2020 42 2000146. ([https://doi.org/10.1002/bies.202000146](https://doi.org/10.1002/bies.202000146))

36 Kitahashi T, Ogawa S & Parhar IS. Cloning and expression of kiss2 in the zebrafish and medaka. *Endocrinology* 2009 150 821–831. ([https://doi.org/10.1210/en.2008-0940](https://doi.org/10.1210/en.2008-0940))

37 Kanda S, Karigo T & Oka Y. Steroid sensitive kiss2 neurones in the goldfish: evolutionary insights into the duplicate kisspeptin gene-expressing neurones. *Journal of Neuroendocrinology* 2012 24 897–906. ([https://doi.org/10.1111/j.1365-2826.2012.02296.x](https://doi.org/10.1111/j.1365-2826.2012.02296.x))

38 Kanda S & Oka Y. Evolutionary insights into the steroid sensitive kiss1 and kiss2 neurones in the vertebrate brain. *Frontiers in Endocrinology* 2012 3 28. ([https://doi.org/10.3389/fendo.2012.00028](https://doi.org/10.3389/fendo.2012.00028))

39 Escobar S, Serrulli A, Espigares E, Gueguen MM, Brocal I, Felip A, Gómez A, Carrillo M, Zanuy S & Kah O. Expression of kisspeptins and kiss receptors suggests a large range of functions for kisspeptin systems in the brain of the European sea bass. *PLoS ONE* 2013 8 e70177. ([https://doi.org/10.1371/journal.pone.0070177](https://doi.org/10.1371/journal.pone.0070177))

40 Ogawa S & Parhar IS. Biological significance of kisspeptin–kiss 1 receptor signaling in the haremula of telesost species. *Frontiers in Endocrinology* 2018 9 222. ([https://doi.org/10.3389/fendo.2018.00222](https://doi.org/10.3389/fendo.2018.00222))

41 Parhar IS, Ogawa S & Sakuma Y. Laser-captured single digoxigenin-labeled neurons of gonadotropin-releasing hormone types reveal a novel G protein-coupled receptor (GpR54) during maturation in cichlid fish. *Endocrinology* 2004 145 3613–3618. ([https://doi.org/10.1210/en.2004-0395](https://doi.org/10.1210/en.2004-0395))

42 Shimizu Y, Tomikawa J, Hirano K, Nanikawa Y, Akazome Y, Kanda S, Kazeto Y, Okuzawa K, Uenoymaya Y, Ohkura S, et al. Central distribution of kiss 2 neurons and peri-pubertal changes in their expression in the brain of male and female red seabream Pagrus major. *General and Comparative Endocrinology* 2012 175 432–442. ([https://doi.org/10.1016/j.ygcen.2011.11.038](https://doi.org/10.1016/j.ygcen.2011.11.038))

43 Wang Q, Sham KWy, Ogawa S, Li S, Parhar IS, Cheng CHK, Liu X & Lin H. Regulation of the two kiss promoters in goldfish (Carassius auratus) by estrogen via different Erα pathways. *Molecular and Cellular Endocrinology* 2013 375 130–139. ([https://doi.org/10.1016/j.mce.2013.04.023](https://doi.org/10.1016/j.mce.2013.04.023))

44 Zhao L, Rakke M, Krimkevich Y, Cushman LJ, Parlow AF, Camper SA & Parker KL. Steroidogenic factor 1 (SF1) is essential for pituitary gonadotrope function. *Development* 2001 128 147–154. ([https://doi.org/10.1242/dev.128.2.147](https://doi.org/10.1242/dev.128.2.147))

45 Higuchi Y, Soga T & Parhar IS. Social defeat stress decreases mRNA for monoamine oxidase A and increases S-HT turnover in the brain of male Nile tilapia (Oreochromis niloticus). *Frontiers in Pharmacology* 2018 9 1549. ([https://doi.org/10.3389/fphar.2018.01549](https://doi.org/10.3389/fphar.2018.01549))

46 Kikuchi Y, Hosono K, Yamashita J, Kawabata Y & Okubo K. Glucocorticoid receptor exhibits sexually dimorphic expression in the medaka brain. *General and Comparative Endocrinology* 2015 223 47–53. ([https://doi.org/10.1016/j.ygcen.2015.09.031](https://doi.org/10.1016/j.ygcen.2015.09.031))

47 Shahbajian M, Motohashi I, Doi H & Ando H. Elevation of Kiss 2 and its receptor gene expression in the brain and pituitary of grass puffer during the spawning season. *General and Comparative Endocrinology* 2010 169 48–57. ([https://doi.org/10.1016/j.ygcen.2010.07.008](https://doi.org/10.1016/j.ygcen.2010.07.008))

48 Ogawa S, Ng KW, Xue X, Ramadasan PN, Sivalingham M, Li S, Levavi-Sivan B, Lin H, Liu X & Parhar IS. Thyroid hormone upregulates hypothalamic kiss2 gene in the male Nile tilapia, Oreochromis niloticus. *Frontiers in Endocrinology* 2013 4 184. ([https://doi.org/10.3389/fendo.2013.00184](https://doi.org/10.3389/fendo.2013.00184))

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