RESEARCH

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. Hererin, 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 (NDLIl) of females but in NHd and NDLIl 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.

Key Words

- kisspeptin
- social stress
- sexually dimorphic distribution
- yellowtail clownfish
- RNAscope

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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*/*Kiss1*) 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

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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 burtoni*), 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-breeders are in the subordinate position. In the absence of female fish, the subdominant male individual will undergo sexual reversal and become the dominant female, and one 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

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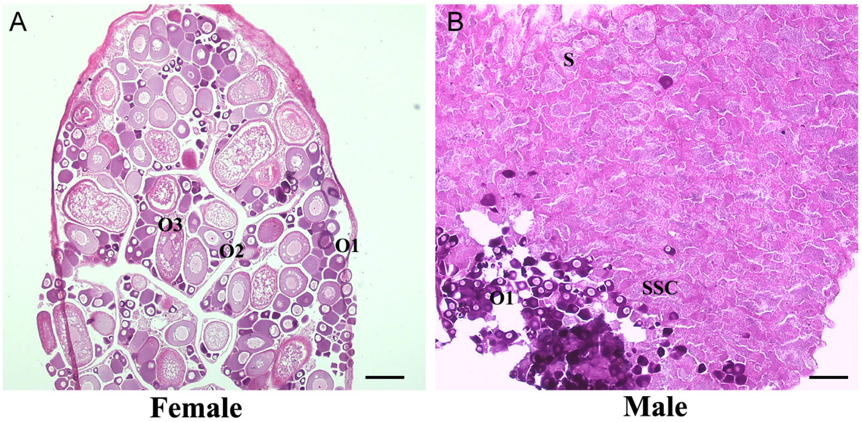

Figure 1
Histological slices were used to distinguish females and males according to the level of gonadal development in yellowtail clownfish. (A) The histological slices of the female gonad; (B) the histological slices of the male gonad. O1, oocytes in primary growth stage; O2, oocytes in cortical vesicle stage; O3, oocytes in vitellogenesis stage; SSC, secondary spermatocytes; S, spermatozoon. Scale = 200 µm.

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

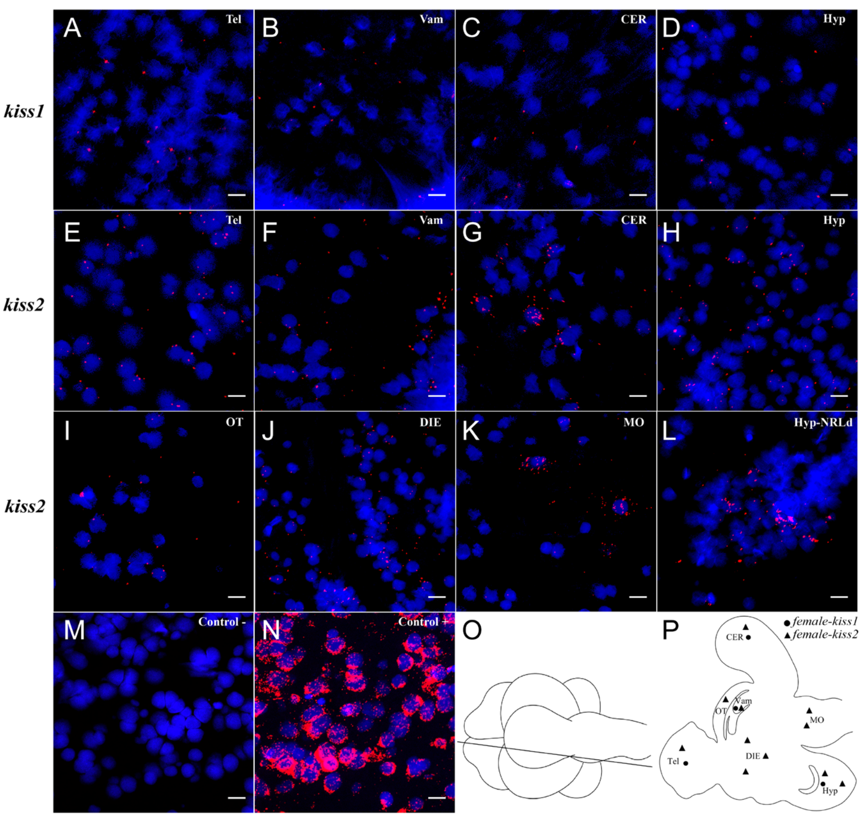

Figure 2
The sagittal distribution of *kiss1* and *kiss2* mRNA in female yellowtail clownfish brain. (A, B, C and D) The *kiss1*-expressed brain regions in sagittal planes of the female brain; (E, F, G, H, I, J, K and L) the *kiss2* expressed brain regions in sagittal planes of the female brain; (M) 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.

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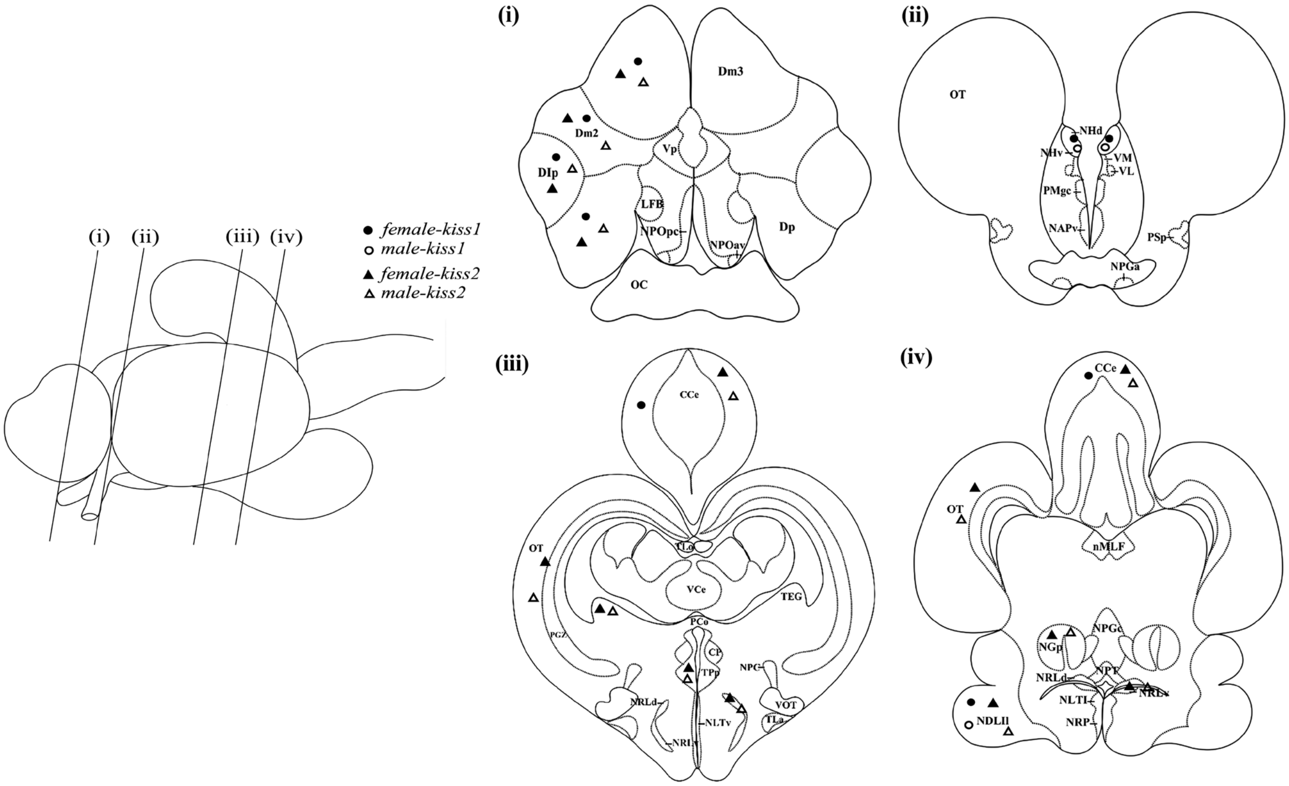

Figure 3

The distribution of *kiss* mRNA in the female and male brains of yellowtail clownfish in coronal planes. Black circles, the distribution of *kiss* mRNA in the female brain; open circles, the distribution of *kiss* mRNA in the male brain; black triangles, the distribution of *kiss* mRNA in the female brain; open triangles,the distribution of *kiss* mRNA in the male brain. CCe, corpus of the cerebellum; Cp, central posterior thalamic nucleus; Dlp, lateral posterior part of the dorsal telencephalic area; Dm2, subdivision 2 of the medial dorsal telencephalic area; Dm3, subdivision 3 of the medial dorsal telencephalic area; Dp, posterior portion of the dorsal telencephalon; NAPv, anterior periventricular nucleus; NDLIl, lateral part of the diffuse nucleus of the inferior lobe; NGp, posterior part of glomerular nucleus; NHd, dorsal habenular nucleus; NHv, ventral habenular nucleus; NLTI, inferior part of the lateral tuberal nucleus; NLTv, ventral part of the lateral tuberal nucleus; nMLF, nucleus of the medial longitudinal fasciculus; NPC, central pretectal nucleus; NPGa, anterior preglomerular nucleus; NPGc, commissural preglomerural nucleus; NPOav, anteroventral part of the parvocellular preoptic nucleus; NPOPc, parvocellular part of the parvocellular preoptic nucleus; NPT, posterior tuberal nucleus; NRLd, dorsal part of the nucleus of the lateral recess; NRLv, ventral part of the nucleus of the lateral recess; NRP, nucleus of the posterior recces; LFB, lateral forebrain bundle; OC, optic chiasm; OT, optic tectum; PCo, posterior commissure; PGZ, periglomerular gray zone; PMgc, gigantocellular part of the magnocellular preoptic nucleus; PSp, parvocellular superficial pretectal nucleus; TEG, tegmentum; TLa, nucleus of the torus lateralis; TLo, torus longitudinalis; TPp, periventricular nucleus of the posterior tuberculum; VCe, valvula of the cerebellum; VL, ventrolateral thalamic nucleus; VM, ventromedial thalamic nucleus; VOT, ventral optic tract; VP, postcommissural part of the ventral telencephalon.

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.

Table 1 Name of targets and catalog number of probes used for present study.

RNAscope-probe	Cat No.
Acl-kiss1-C1	1044931-C1
Acl-kiss2-C1	1044941-C1
Aoc-actb2-C1	1045881-C1
Negative control probe-DapB	310043

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,

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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 (NDLIl) 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 NDLIl 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

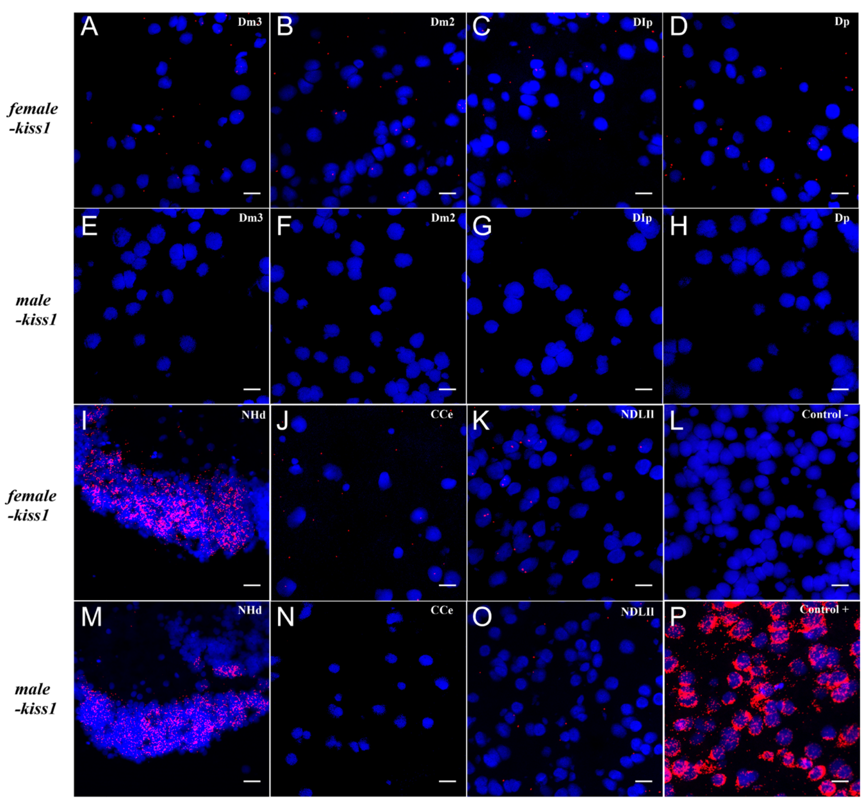

Figure 4
The coronal distribution of *kiss1* mRNA in female and male yellowtail clownfish brain. (A, B, C and D) and (I, J and K) The *kiss1*-expressed brain regions in coronal planes of the female brain; (E, F, G and H) and (M, N and O) the *kiss1*-expressed brain regions in coronal planes of the male brain; (L) negative control; (P) positive control. Dm3, subdivision 3 of the medial dorsal telencephalic area; Dm2, subdivision 2 of the medial dorsal telencephalic area; DIp, lateral posterior part of the dorsal telencephalic area; Dp, posterior portion of the dorsal telencephalon; NHd, dorsal habenular nucleus; CCe, corpus of the cerebellum; NDLII, lateral part of the diffuse nucleus of the inferior lobe. Scale = 20 µm.

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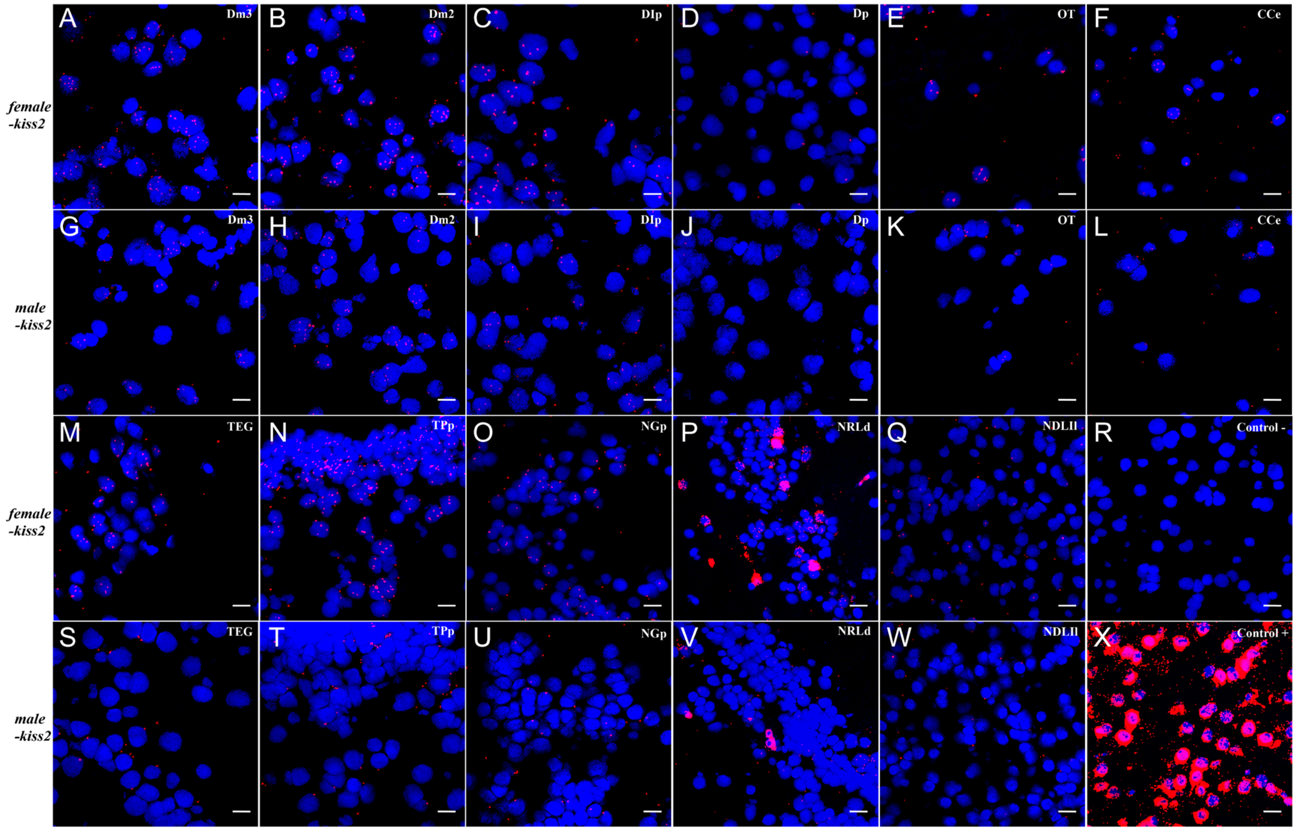

Figure 5
The coronal distribution of *kiss2* mRNA in female and male yellowtail clownfish brain. (A, B, C, D, E and F) and (M, N, O, P and Q) the *kiss2*expressed brain regions in coronal planes of the female brain; (G, H, I, J, K and L) and (S, T, U, V and W) the *kiss2*-expressed brain regions in coronal planes of the male brain; (R) negative control; (X) positive control. Dm3, subdivision 3 of the medial dorsal telencephalic area; Dm2, subdivision 2 of the medial dorsal telencephalic area; Dlp, lateral posterior part of the dorsal telencephalic area; Dp, posterior portion of the dorsal telencephalon; OT, optic tectum; CCe, corpus of the cerebellum; TEG, tegmentum; TPp, periventricular nucleus of the posterior tuberculum; NGp, posterior part of glomerular nucleus; NRLd, dorsal part of the nucleus of the lateral recess; NDLII, lateral part of the diffuse nucleus of the inferior lobe. Scale = 20 µm.

(ESD) (33). 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 hypothalamus (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 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 seabream (*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 *kiss*1 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 NDLIl 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 Trevisan CM, Montagna E, De Oliveira R, Christofolini DM, Barbosa CP, 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 Aerle 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 (*Carassius auratus*). *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 (*Oryzias latipes*). *Endocrinology* 2010 **151** 1751–1759. (https://doi.org/10.1210/en.2009-1174)
8 Selvaraj S, Kitano H, Fujinaga Y, Ohga H, Yoneda M, Yamaguchi A, Shimizu A & Matsuyama 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 (*Dicentrarchus 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 L & 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.2015.10.008)
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 17α-ethinylestradiol in rare minnow *Gobiocypris rarus*. *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 Bohórquez MO, Mechaly AS, Hughes LC, Campanella D, Ortí G, Canosa LF & Somaza GM. Kisspeptin system in pejerrey 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, *Chrysiptera cyanea*, 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, Anthonysamy 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* 2022 **48** 419–436. (https://doi.org/10.1007/s10695-022-01055-7)
17 Wang B, Mechaly 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 & Trudeau 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, Trudeau 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 & Matsuyama 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* 2018 **9** 147. (https://doi.org/10.3389/fendo.2018.00147)
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 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. (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, Matsunaga T, Yamamoto N, Yamada S, Tsukamura H, Maeda KI & Oka Y. Identification of KiSS-1 product kisspeptin and steroid-sensitive sexually dimorphic kisspeptin neurons in medaka (*Oryzias latipes*). *Endocrinology* 2008 **149** 2467–2476. (https://doi.org/10.1210/en.2007-1503)
25 Song Y, Chen J, Tao B, Luo D, Zhu Z & Hu W. Kisspeptin2 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 Whirledge S & Cidlowski JA. A role for glucocorticoids in stress-impaired reproduction: Beyond the hypothalamus and pituitary. *Endocrinology* 2013 **154** 4450–4468. (https://doi.org/10.1210/en.2013-1652)
27 Kinsey-Jones JS, Li XF, 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.1365-2826.2008.01807.x)
28 Grone BP, Maruska KP, Korzan WJ & Fernald RD. Social status regulates kisspeptin receptor mRNA in the brain of *Astatotilapia 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)
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)
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.13911)
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 hermaphroditic yellowtail clownfish, *Amphiprion clarkii*. *Gene* 2020 **745** 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)
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)
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)
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)
38 Kanda S & Oka Y. Evolutionary insights into the steroid sensitive kiss1 and kiss2 neurons in the vertebrate brain. *Frontiers in Endocrinology* 2012 **3** 28. (https://doi.org/10.3389/fendo.2012.00028)
39 Escobar S, Servili A, Espigares F, 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)
40 Ogawa S & Parhar IS. Biological significance of kisspeptin–kiss 1 receptor signaling in the habenula of teleost species. *Frontiers in Endocrinology* 2018 **9** 222. (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)
42 Shimizu Y, Tomikawa J, Hirano K, Nanikawa Y, Akazome Y, Kanda S, Kazeto Y, Okuzawa K, Uenoyama Y, Ohkura S, *et al.* Central distribution of kiss2 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)
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)
44 Zhao L, Bakke 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)
45 Higuchi Y, Soga T & Parhar IS. Social defeat stress decreases mRNA for monoamine oxidase A and increases 5-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)
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)
47 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. *General and Comparative Endocrinology* 2010 **169** 48–57. (https://doi.org/10.1016/j.ygcen.2010.07.008)
48 Ogawa S, Ng KW, Xue X, Ramadasan PN, Sivalingam 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)

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