

PUBERTY IN TELEOST: BIOTECHNOLOGICAL APPLICATIONS

Abstract

Freshwater fish make up a significant portion of the aquaculture business and are crucial to the economics of many nations. But premature or delayed puberty is one of the reasons the aquaculture industry is facing problems because of its negative consequences on growth performance, sexual behavior, and health. Different external (exogenous) and internal (endogenous) regulating mechanisms influence puberty. But the precise mechanism is still unknown. To name a few, internal factors that contribute to the onset of puberty include peptides like Neurokinin B and Kisspeptin, Leptin, miRNAs, the photoperiod hormone Melatonin, and neuroendocrine transmitters like Serotonin, Dopamine, and Gamma-aminobutyric acid and other hormones of the central reproductive axis. Understanding the mechanism would help to control the pubertal onset in fish, ultimately promoting the expansion of the aquaculture sector. Our knowledge of the underlying mechanisms is being greatly advanced by the biotechnological developments in investigating fish puberty. The biotechnological approaches like transcriptomics, genomics, CRISPR/Cas9, etc. have played a significant role in providing the information that we know today. The advancements made till now have been discussed below.

Authors

Nehareeka Dan

Division of Biomedical and Life Sciences
School of Science
Navrachana University
Gujarat, India

Harsh Shah

Division of Biomedical and Life Sciences
School of Science
Navrachana University
Gujarat, India

Parth Pandya

Division of Biomedical and Life Sciences
School of Science
Navrachana University
Gujarat, India
pkpandya.13@gmail.com

I. INTRODUCTION TO PUBERTY IN FISHES

Freshwater fish, such as Tilapia, Rohu, Catla, Carp, Mrigal, and others, play a vital role in the economies of many countries, serving as a staple food for a significant portion of the population. Due to their importance, the aquaculture sector has been rapidly expanding as a sustainable solution to address global food security concerns, particularly in animal food production. In fact, the freshwater aquaculture industry now contributes to over 95% of the overall aquaculture production [Jayasankar, 2018].

The growth of the aquaculture sector relies heavily on the proper breeding and reproduction of fish. In India, fish breeding periods vary, and reproduction occurs periodically, governed by a combination of external environmental factors and internal regulatory mechanisms.

The crucial internal regulatory factor governing reproduction is the molecular mechanism known as the central reproductive axis or the Hypothalamic-Pituitary-Gonadal (HPG) axis. Within this axis, the release of Gonadotropin-releasing hormone (GnRH) from the hypothalamus triggers the production and release of pituitary glycoprotein hormones called gonadotropins, namely luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These gonadotropins then act on the gonads, promoting gametogenesis and steroidogenesis by stimulating the secretion of sex steroids, such as androgens and estrogens. Positioned at the pinnacle of this axis, GnRH neurons regulate reproduction by integrating information from social and environmental cues in conjunction with the hormonal status [Spaziani et al., 2021].

Puberty represents a biological transition that occurs in vertebrates, marking certain physiological changes in their bodies. During this period, individuals exhibit the first signs of sexual maturity and become capable of reproduction. The onset of puberty in vertebrates, including fishes, coincides with the activation of the HPG axis. In teleosts, puberty typically begins after gonadal sex differentiation [Strussmann and Nakamura, 2002], characterized by the commencement of spermatogenesis in males [Schulz et al., 2002] and vitellogenic ovarian development in females [Patino and Sullivan, 2002].

In the context of aquaculture, comprehending the mechanism responsible for initiating puberty and the diverse factors influencing this process is crucial for effectively managing the onset of puberty in fish farming. The biotechnological advancements in studying the puberty in fish are playing a significant role in advancing our understanding of the underlying mechanisms. Some of the key biotechnological approaches that are being utilized are discussed below.

1. Transcriptomics, Genomics and Proteomics: High-throughput sequencing technologies like RNA sequencing (RNA-Seq) and whole-genome sequencing have enabled researchers to study the gene expression patterns and genomic changes associated with puberty in fish. By comparing the transcriptomes and genomes of fish at different developmental stages, scientists can identify genes and pathways involved in the process.

With the help of transcriptomics and genomics, internal determinants have been identified in teleosts during the pubertal onset time. Kisspeptin, encoded by *kiss1*

gene, has been identified as the gatekeeper of the HPG axis by breakthrough researches conducted in 2003. It was noticed that a mutation in the *kiss1r* gene (receptor of kisspeptin) led to delayed puberty and a condition called hypogonadotropic hypogonadism in humans [de Roux et al., 2003; Seminara et al., 2003]. Later on, the gene was then identified in different fish species like Rohu (*Labeo rohita*) [Saha et al., 2016], Zebrafish (*Danio rerio*) [van Aerle et al., 2008], European Sea Bass (*Dicentrarchus labrax*) [Felip et al., 2009], Goldfish (*Carassius auratus*) [Li et al., 2009], Chub Mackerel (*Scomber japonicas*) [Selveraj et al., 2010], Catla (*Catla catla*) [Rather et al., 2016], Medaka (*Oryzias latipes*) [Kanda et al., 2008] and Senegalese Sole (*Solea senegalensis*) [Mechaly et al., 2009], where the levels of *kiss1* were found to be increasing sharply during the pubertal onset time. Growth factor like IGF-1 (insulin-like growth factor-1) plays a role in progression of puberty in teleosts as it was found to directly stimulate the synthesis and release of the growth hormones [Luckenbach et al., 2010; Furukuma et al., 2008]. Leptin, a hormone produced by adipocytes, serves as a signal that communicates the accumulation of lipid stores and the initiation of puberty [Friedman and Halaas, 1998]. Its role in controlling the pubertal onset has been identified in several fishes like Chub Mackerel [Ohga et al., 2017; Ohga et al., 2021; Ohga et al., 2020; Ohga et al., 2021; Ohga et al., 2015], Blunt Snout Beam (*Megalobrama amblycephala*) [Zhao et al., 2015], European Sea bass [Peyon et al., 2001] and Rainbow Trout (*Onchorynchus mykiss*) [Weil et al., 2003] where the transcriptomics data showed that it regulates the gonadotropin secretion and gonadal maturation. Neuropeptide Y (NPY) and Neurokinin B (NKB) have also been identified to stimulate the gonadotropin and growth hormone release in Tilapia [Gur et al., 2002] and Zebrafish [Biran et al., 2012].

In order to investigate the miRNAs that control mRNA expression during the onset of puberty in Atlantic Salmon, an integrated transcriptome analysis of salmon testis was conducted at three different developmental stages: immature stage, pre-pubertal stage and pubertal stage. During the immature stage, the analysis unveiled miRNAs that were already known to be regulated in immature vertebrate testis, such as miR-101, miR-137, miR-92b, miR-18a, and miR-20a. Additionally, miRNAs reported for the first time to be regulated in the testis were also identified, including miR-new289, miR-30c, miR-724, miR-26b, miR-new271, miR-217, miR-216a, miR-135a, miR-new194, and the novel predicted n268. During the transition into puberty stage, distinct expression patterns of miRNAs were observed, some of which were already linked to this process (let-7a/b/c), and others were newly associated with it (miR-15c, miR-2184, miR-145, and the novel predicted n7a and n7b). During the pubertal stage along with the previously discovered miRNAs (miR-20a, miR-25, miR-181a, miR-202, let-7c/d/a, miR-125b, miR-222a/b, miR-190a), new connections of miRNAs (miR-2188, miR-144, miR-731, miR-8157, and the novel n2) were found with the initiation of puberty [Skaftnesmo et al., 2017].

Proteomics involves the study of the entire complement of proteins expressed by an organism. It allows researchers to analyze changes in protein expression and post-translational modifications during puberty in fish. This information complements genomics data and provides insights into the functional aspects of the genes involved.

Western blot, Immunohistochemistry and Immunocytochemistry are a few techniques performed for identification and localization of the protein of interest. An increased expression of kisspeptin in brain and pituitary during pubertal onset time has

been confirmed in the European Sea Bass with the help of immunohistochemical detection [Escobar et al., 2013]. In a study done in Chinese Sucker (*Myxocyprinus asiaticus*), the kisspeptin-gonadotropin releasing hormone system was highly expressed at the onset of puberty, indicating it to be an important signalling pathway for triggering the HPG axis during the pubertal onset time [Su et al., 2020].

- 2. Endocrine Manipulations:** Biotechnological approaches, such as hormone treatment and gene knockdown techniques, have been used to manipulate the endocrine system of fish. By altering hormone levels or disrupting specific genes involved in puberty regulation, the effects on the timing and progression of puberty can be observed.

In Catla fish, administering nano-encapsulated kisspeptin through injections resulted in an upregulation of reproductive genes (GnRH, LH, and FSH) expression, ultimately triggering the initiation of puberty [Rather et al., 2016]. In Nile Tilapia, the expression of *kiss2* was notably higher during the immature stage compared to the mature stage, indicating its potential role in gonadal development. Additionally, external administration of kisspeptin led to increased levels of estradiol and testosterone in the blood plasma, along with elevated expression of GnRH and gonadotropins in the brain [Park et al., 2016]. In 2020, a study involving Senegalese sole utilized an intramuscular injection of kisspeptin, which led to elevated synthesis and secretion of gonadotropins, along with increased testosterone levels in the plasma [Oliveira et al., 2020]. In Chub Mackerel, after giving the treatment of leptin to pituitary cells under *in-vitro* conditions, it influenced the release of gonadotropin, which might be a key signal for onset of puberty [Ohga et al., 2020; Ohga et al., 2021]. The administration of NKB has a notable impact on zebrafish [Biran et al., 2012], goldfish [Qi et al., 2015], and tilapia [Biran et al., 2014], effectively triggering the secretion and production of luteinizing hormone (LH). This indicates that NKB signalling plays a crucial role in regulating the reproductive axis.

- 3. Molecular Markers:** Biotechnology has enabled the discovery and development of molecular markers associated with puberty in fish. These markers can be used for early detection of puberty onset and to study the genetic basis of variations in pubertal timing among different fish populations.

Kisspeptin, Gonadotropin releasing hormone, Insulin like growth factor-1, Leptin, NeuropeptideY, NeurokininB and miRNAs, these are few molecular markers identified during pubertal onset in fishes with the help of the biotechnological advancements. Apart from these there are other markers like Makorin ring finger protein 3 (MKRN3), which was the first gene that was identified to have inhibitory effect on GnRH. The decrease in hypothalamic *mkrn3* expression observed in mice during prepubertal development, as well as the loss of MKRN3 function in patients with precocious puberty, provides compelling evidence supporting the role of MKRN3 in repressing the onset of puberty [Abreu et al., 2013]. Next is Delta-like non-canonical notch ligand 1 (DLK1) whose would result in an increase in the formation of kisspeptin neurons. A study shows that female mice deficient in DLK1 experience pubertal onset [Macedo et al., 2021]. However, the regulation of these two molecules (MKRN3 and DLK1) have yet not been studied in a fish model. This arouses an interest to further investigate the effects of them in a fish model. Then hormonal regulators like Melatonin

and Serotonin have also been observed to have an indirect regulation on the pubertal onset. The results from the Zebrafish study indicate that melatonin, through receptor-mediated action at the brain level, could potentially stimulate the release of hypothalamic GnRH. This mechanism likely involves the *kiss1r* system in the GnRH neurons [Carnevali et al., 2011]. In Orange-spotted grouper (*Epinephelus coioides*), the melatonin receptor, which shows higher expression at night and lower expression during the day, was down-regulated. This down-regulation led to the up-regulation of *kiss2* and GnRH, which exhibit higher expression during the day and lower expression at night [Chai et al., 2013]. On the other hand, studies done on serotonin suggest that its receptors co-expresses with the GnRH neurons and promotes gonadotropin secretion [Norton et al., 2008; Schneider et al., 2012; Khan et al., 1993; Bhattarai et al., 2014; Somoza and Peter, 1991; Khan and Thomas, 1994; Wong et al., 1998]. Further investigation is required to properly understand the mechanism behind the pubertal onset and the interaction between all these molecular markers into it.

- 4. CRISPR/Cas9:** The advent of genome editing, such as this revolutionary clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein (Cas9) technology, presents exciting opportunities to expedite sustainable genetic advancements in fisheries and aquaculture. This technology facilitates the investigation of specific genes involved in puberty and helps researchers understand their roles. Its applications benefit from certain attributes of aquaculture species, such as high fecundity, external fertilization, well-established breeding methods, and efficient larval rearing techniques [Okoli et al., 2022].

The use of CRISPR/Cas9 genome editing enables precise, targeted modifications to the genome of the target species, often involving minor changes. Importantly, this technology does not introduce any foreign DNA, addressing significant public concerns about the safety of genetically modified organisms (GMOs) [Okoli et al., 2022]. In recent times, researchers have extensively investigated the CRISPR/Cas9 system to study gene functions and breeding *in vivo* and/or in cell lines of numerous prominent aquaculture species, encompassing over 20 different species, namely Nile Tilapia, Rainbow Trout, Atlantic Salmon, Common Carp, Rohu, and other species [Gratacap et al., 2019; Blix et al., 2021]. In genome-editing experiments conducted on cultured fishes, the primary focus has been on targeting key genes such as myostatin (*mstn*), which plays a critical role in regulating skeletal muscle growth and fertility, along with sex-related genes like dead end (*dnd*) and doublesex- and mab-3-related transcription factor (*dmrt*) [Hallerman, 2021].

By targeting and disabling the *mstn* gene in various fishes like Common Carp [Zhong et al., 2016], Red Sea Bream [Kishimoto et al., 2018], Channel Catfish [Khalil et al., 2017], the outcome has been notable, leading to a significant increase in skeletal muscle mass (up to 16%), shortened body length, improved feed efficiency, and ultimately promoting better overall growth in the fish. In another study done in the Tiger Puffer (*Takifugu rubripe*), CRISPR is utilized to modify growth-associated traits by disrupting the leptin receptor gene, which regulates appetite. This genetic alteration causes the fish to exhibit increased food consumption [Shinbun, 2021]. The Atlantic salmon, upon the knockout of the germ cell-specific candidate, dead end (*dnd*) gene, exhibited a notable outcome: they remained in an immature state and did not undergo

puberty [Kleppe et al., 2017]. In a recent development, CRISPR/Cas9 technology was used to generate an all-female population of Common Carp by disrupting the Cytochrome P450 17A1 (*cyp17a1*) gene [Zhai et al., 2022].

Studies with respect to puberty with the help of CRISPR/Cas9 techniques in fishes are scarce. Compared to other genome editing technologies, CRISPR/Cas is more cost-effective, simpler to use, and offers higher precision. As a result, it can be harnessed as an innovative breeding technology in fisheries and aquaculture to address wide-ranging challenges effectively.

In conclusion, by combining these biotechnological advancements with traditional physiological and behavioral studies, researchers are making significant strides in unraveling the complexities of puberty in fish. These insights have implications not only for fish biology and aquaculture but also for our understanding of reproductive processes in other vertebrates, including humans.

REFERENCES

- [1] Jayasankar, P. (2018). Present status of freshwater aquaculture in India-A review. *Indian Journal of Fisheries*, 65(4), 157-165. <https://doi.org/10.21077/ijf.2018.65.4.81300-20>
- [2] Spaziani, M., Tarantino, C., Tahani, N., Gianfrilli, D., Sbardella, E., Lenzi, A., & Radicioni, A. F. (2021). Hypothalamo-Pituitary axis and puberty. *Molecular and cellular endocrinology*, 520, 111094. <https://doi.org/10.1016/j.mce.2020.111094>
- [3] Strüssmann, C. A., & Nakamura, M. (2002). Morphology, endocrinology, and environmental modulation of gonadal sex differentiation in teleost fishes. *Fish physiology and biochemistry*, 26(1), 13-29. <https://doi.org/10.1023/A:1023343023556>
- [4] Schulz, R. W., & Miura, T. (2002). Spermatogenesis and its endocrine regulation. *Fish physiology and biochemistry*, 26(1), 43-56. <https://doi.org/10.1023/A:1023303427191>
- [5] Patiño, R., & Sullivan, C. V. (2002). Ovarian follicle growth, maturation, and ovulation in teleost fish. *Fish Physiology and Biochemistry*, 26(1), 57-70. <https://doi.org/10.1023/A:1023311613987>
- [6] de Roux, N., Genin, E., Carel, J. C., Matsuda, F., Chaussain, J. L., & Milgrom, E. (2003). Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proceedings of the National Academy of Sciences of the United States of America*, 100(19), 10972–10976. <https://doi.org/10.1073/pnas.1834399100>
- [7] Seminara, S. B., Messenger, S., Chatzidaki, E. E., Thresher, R. R., Acierno, J. S., Jr, Shagoury, J. K., Bo-Abbas, Y., Kuohung, W., Schwino, K. M., Hendrick, A. G., Zahn, D., Dixon, J., Kaiser, U. B., Slaugenhaupt, S. A., Gusella, J. F., O'Rahilly, S., Carlton, M. B., Crowley, W. F., Jr, Aparicio, S. A., & Colledge, W. H. (2003). The GPR54 gene as a regulator of puberty. *The New England journal of medicine*, 349(17), 1614–1627. <https://doi.org/10.1056/NEJMoa035322>
- [8] Saha, A., Pradhan, A., Sengupta, S., Nayak, M., Samanta, M., Sahoo, L., & Giri, S. S. (2016). Molecular characterization of two kiss genes and their expression in rohu (*Labeo rohita*) during annual reproductive cycle. *Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology*, 191, 135–145. <https://doi.org/10.1016/j.cbpb.2015.10.008>
- [9] van Aerle, R., Kille, P., Lange, A., & Tyler, C. R. (2008). Evidence for the existence of a functional Kiss1/Kiss1 receptor pathway in fish. *Peptides*, 29(1), 57–64. <https://doi.org/10.1016/j.peptides.2007.10.018>
- [10] Felip, A., Zanuy, S., Pineda, R., Pinilla, L., Carrillo, M., Tena-Sempere, M., & Gómez, A. (2009). Evidence for two distinct KiSS genes in non-placental vertebrates that encode kisspeptins with different gonadotropin-releasing activities in fish and mammals. *Molecular and cellular endocrinology*, 312(1-2), 61–71. <https://doi.org/10.1016/j.mce.2008.11.017>
- [11] Li, S., Zhang, Y., Liu, Y., Huang, X., Huang, W., Lu, D., Zhu, P., Shi, Y., Cheng, C. H., Liu, X., & Lin, H. (2009). Structural and functional multiplicity of the kisspeptin/GPR54 system in goldfish (*Carassius auratus*). *The Journal of endocrinology*, 201(3), 407–418. <https://doi.org/10.1677/JOE-09-0016>
- [12] Selvaraj, S., Kitano, H., Fujinaga, Y., Ohga, H., Yoneda, M., Yamaguchi, A., Shimizu, A., & Matsuyama, M. (2010). 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*, 169(1), 28–38. <https://doi.org/10.1016/j.ygcen.2010.07.011>
- [13] Rather, M. A., Bhat, I. A., Gireesh-Babu, P., Chaudhari, A., Sundaray, J. K., & Sharma, R. (2016). Molecular characterization of kisspeptin gene and effect of nano-encapsulated kisspeptin-10 on reproductive maturation in *Catla catla*. *Domestic animal endocrinology*, 56, 36–47. <https://doi.org/10.1016/j.domaniend.2016.01.005>
- [14] Kanda, S., Akazome, Y., Matsunaga, T., Yamamoto, N., Yamada, S., Tsukamura, H., Maeda, K., & Oka, Y. (2008). Identification of KiSS-1 product kisspeptin and steroid-sensitive sexually dimorphic kisspeptin neurons in medaka (*oryzias latipes*). *Endocrinology*, 149(5), 2467–2476. <https://doi.org/10.1210/en.2007-1503>
- [15] Mechaly, A. S., Viñas, J., & Piferrer, F. (2009). Identification of two isoforms of the Kisspeptin-1 receptor (*kiss1r*) generated by alternative splicing in a modern teleost, the Senegalese sole (*Solea senegalensis*). *Biology of reproduction*, 80(1), 60–69. <https://doi.org/10.1095/biolreprod.108.072173>
- [16] Luckenbach, J. A., Dickey, J. T., & Swanson, P. (2010). Regulation of pituitary GnRH receptor and gonadotropin subunits by IGF1 and GnRH in prepubertal male coho salmon. *General and comparative endocrinology*, 167(3), 387–396. <https://doi.org/10.1016/j.ygcen.2009.09.010>
- [17] Furukuma, S., Onuma, T., Swanson, P., Luo, Q., Koide, N., Okada, H., Urano, A., & Ando, H. (2008). Stimulatory effects of insulin-like growth factor 1 on expression of gonadotropin subunit genes and release of follicle-stimulating hormone and luteinizing hormone in masu salmon pituitary cells early in gametogenesis. *Zoological science*, 25(1), 88–98. <https://doi.org/10.2108/zsj.25.88>
- [18] Friedman, J. M., & Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. *Nature*, 395(6704), 763–770. <https://doi.org/10.1038/27376>
- [19] Ohga, H., Hirata, D., Matsumori, K., Kitano, H., Nagano, N., Yamaguchi, A., & Matsuyama, M. (2017). Possible role of the leptin system in controlling puberty in the male chub mackerel, *Scomber japonicus*. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*, 203, 159–166. <https://doi.org/10.1016/j.cbpa.2016.09.009>
- [20] Ohga, H., Ito, K., Kakino, K., Mon, H., Kusakabe, T., Lee, J. M., & Matsuyama, M. (2021). Leptin Is an Important Endocrine Player That Directly Activates Gonadotropic Cells in Teleost Fish, Chub Mackerel. *Cells*, 10(12), 3505. <https://doi.org/10.3390/cells10123505>
- [21] Ohga, H., Ito, K., Matsumori, K., Kimura, R., Ohta, K., & Matsuyama, M. (2020). Leptin stimulates gonadotropin release and ovarian development in marine teleost chub mackerel. *General and comparative endocrinology*, 292, 113442. <https://doi.org/10.1016/j.ygcen.2020.113442>
- [22] Ohga, H., & Matsuyama, M. (2021). In vitro action of leptin on gonadotropin secretion in pre-pubertal male chub mackerel. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*, 253, 110856. <https://doi.org/10.1016/j.cbpa.2020.110856>
- [23] Ohga, H., Matsumori, K., Kodama, R., Kitano, H., Nagano, N., Yamaguchi, A., & Matsuyama, M. (2015). Two leptin genes and a leptin receptor gene of female chub mackerel (*Scomber japonicus*): Molecular cloning, tissue distribution and expression in different obesity indices and pubertal stages. *General and comparative endocrinology*, 222, 88–98. <https://doi.org/10.1016/j.ygcen.2015.06.002>
- [24] Zhao, H., Zeng, C., Yi, S., Wan, S., Chen, B., & Gao, Z. (2015). Leptin Genes in Blunt Snout Bream: Cloning, Phylogeny and Expression Correlated to Gonads Development. *International journal of molecular sciences*, 16(11), 27609–27624. <https://doi.org/10.3390/ijms161126044>
- [25] Peyon, P., Zanuy, S., & Carrillo, M. (2001). Action of leptin on in vitro luteinizing hormone release in the European sea bass (*Dicentrarchus labrax*). *Biology of reproduction*, 65(5), 1573–1578. <https://doi.org/10.1095/biolreprod65.5.1573>
- [26] Weil, C., Le Bail, P. Y., Sabin, N., & Le Gac, F. (2003). In vitro action of leptin on FSH and LH production in rainbow trout (*Onchorynchus mykiss*) at different stages of the sexual cycle. *General and comparative endocrinology*, 130(1), 2–12. [https://doi.org/10.1016/s0016-6480\(02\)00504-x](https://doi.org/10.1016/s0016-6480(02)00504-x)
- [27] Gur, G., Bonfil, D., Safarian, H., Naor, Z., & Yaron, Z. (2002). Pituitary adenylate cyclase activating polypeptide and neuropeptide Y regulation of gonadotropin subunit gene expression in tilapia: role of PKC, PKA and ERK. *Neuroendocrinology*, 75(3), 164–174. <https://doi.org/10.1159/000048234>
- [28] Biran, J., Palevitch, O., Ben-Dor, S., & Levavi-Sivan, B. (2012). Neurokinin Bs and neurokinin B receptors in zebrafish-potential role in controlling fish reproduction. *Proceedings of the National Academy of Sciences of the United States of America*, 109(26), 10269–10274. <https://doi.org/10.1073/pnas.1119165109>
- [29] Skaftnesmo, K. O., Edvardsen, R. B., Furmanek, T., Crespo, D., Andersson, E., Kleppe, L., Taranger, G. L., Bogerd, J., Schulz, R. W., & Wargelius, A. (2017). Integrative testis transcriptome analysis reveals

- differentially expressed miRNAs and their mRNA targets during early puberty in Atlantic salmon. *BMC genomics*, 18(1), 801. <https://doi.org/10.1186/s12864-017-4205-5>
- [30] Escobar, S., Felip, A., Gueguen, M. M., Zanuy, S., Carrillo, M., Kah, O., & Servili, A. (2013). Expression of kisspeptins in the brain and pituitary of the European sea bass (*Dicentrarchus labrax*). *The Journal of comparative neurology*, 521(4), 933–948. <https://doi.org/10.1002/cne.23211>
- [31] Su, S., Li, Q., Li, X., Rong, C., & Xie, Q. (2020). Expression of the kisspeptin/gonadotropin-releasing hormone (GnRH) system in the brain of female Chinese sucker (*Myxocyprinus asiaticus*) at the onset of puberty. *Fish physiology and biochemistry*, 46(1), 293–303. <https://doi.org/10.1007/s10695-019-00717-3>
- [32] Park, J. W., Jin, Y. H., Oh, S. Y., & Kwon, J. Y. (2016). Kisspeptin2 stimulates the HPG axis in immature Nile tilapia (*Oreochromis niloticus*). *Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology*, 202, 31–38. <https://doi.org/10.1016/j.cbpb.2016.07.009>
- [33] Oliveira, C., Fatsini, E., Fernández, I., Anjos, C., Chauvigné, F., Cerdà, J., Mjelle, R., Fernandes, J., & Cabrita, E. (2020). Kisspeptin Influences the Reproductive Axis and Circulating Levels of microRNAs in Senegalese Sole. *International journal of molecular sciences*, 21(23), 9051. <https://doi.org/10.3390/ijms21239051>
- [34] Qi, X., Zhou, W., Li, S., Liu, Y., Ye, G., Liu, X., Peng, C., Zhang, Y., & Lin, H. (2015). Goldfish neurokinin B: Cloning, tissue distribution, and potential role in regulating reproduction. *General and comparative endocrinology*, 221, 267–277. <https://doi.org/10.1016/j.ygcen.2014.10.017>
- [35] Biran, J., Golan, M., Mizrahi, N., Ogawa, S., Parhar, I. S., & Levavi-Sivan, B. (2014). Direct regulation of gonadotropin release by neurokinin B in tilapia (*Oreochromis niloticus*). *Endocrinology*, 155(12), 4831–4842. <https://doi.org/10.1210/en.2013-2114>
- [36] Abreu, A. P., Dauber, A., Macedo, D. B., Noel, S. D., Brito, V. N., Gill, J. C., Cukier, P., Thompson, I. R., Navarro, V. M., Gagliardi, P. C., Rodrigues, T., Kochi, C., Longui, C. A., Beckers, D., de Zegher, F., Montenegro, L. R., Mendonca, B. B., Carroll, R. S., Hirschhorn, J. N., Latronico, A. C., ... Kaiser, U. B. (2013). Central precocious puberty caused by mutations in the imprinted gene MKRN3. *The New England journal of medicine*, 368(26), 2467–2475. <https://doi.org/10.1056/NEJMoa1302160>
- [37] Macedo, D. B., Abreu, A. P., Magnuson, M., Kim, H. K., Mancini, A., Latronico, A. C., ... & Kaiser, U. B. (2021). Pubertal Onset Occurs in Female Mice Lacking Paternally Expressed Dlk1 Despite Lower Leptin and Kisspeptin Levels. *Journal of the Endocrine Society*, 5(Supplement_1), A688-A688.
- [38] Carnevali, O., Gioacchini, G., Maradonna, F., Olivotto, I., & Migliarini, B. (2011). Melatonin induces follicle maturation in *Danio rerio*. *PLoS one*, 6(5), e19978. <https://doi.org/10.1371/journal.pone.0019978>
- [39] Chai, K., Liu, X., Zhang, Y., & Lin, H. (2013). Day-night and reproductive cycle profiles of melatonin receptor, kiss, and gnRH expression in orange-spotted grouper (*Epinephelus coioides*). *Molecular reproduction and development*, 80(7), 535–548. <https://doi.org/10.1002/mrd.22191>
- [40] Norton, W. H., Folchert, A., & Bally-Cuif, L. (2008). Comparative analysis of serotonin receptor (HTR1A/HTR1B families) and transporter (slc6a4a/b) gene expression in the zebrafish brain. *The Journal of comparative neurology*, 511(4), 521–542. <https://doi.org/10.1002/cne.21831>
- [41] Schneider, H., Fritzy, L., Williams, J., Heumann, C., Yochum, M., Pattar, K., Noppert, G., Mock, V., & Hawley, E. (2012). Cloning and expression of a zebrafish 5-HT(2C) receptor gene. *Gene*, 502(2), 108–117. <https://doi.org/10.1016/j.gene.2012.03.070>
- [42] Khan, I. A., & Thomas, P. (1993). Immunocytochemical localization of serotonin and gonadotropin-releasing hormone in the brain and pituitary gland of the Atlantic croaker *Micropogonias undulatus*. *General and comparative endocrinology*, 91(2), 167–180. <https://doi.org/10.1006/gcen.1993.1116>
- [43] Bhattarai, J. P., Roa, J., Herbison, A. E., & Han, S. K. (2014). Serotonin acts through 5-HT1 and 5-HT2 receptors to exert biphasic actions on GnRH neuron excitability in the mouse. *Endocrinology*, 155(2), 513–524. <https://doi.org/10.1210/en.2013-1692>
- [44] Somoza, G. M., & Peter, R. E. (1991). Effects of serotonin on gonadotropin and growth hormone release from in vitro perfused goldfish pituitary fragments. *General and comparative endocrinology*, 82(1), 103–110. [https://doi.org/10.1016/0016-6480\(91\)90301-1](https://doi.org/10.1016/0016-6480(91)90301-1)
- [45] Khan, I. A., & Thomas, P. (1994). Seasonal and daily variations in the plasma gonadotropin II response to a LHRH analog and serotonin in Atlantic croaker (*Micropogonias undulatus*): evidence for mediation by 5-HT2 receptors. *The Journal of experimental zoology*, 269(6), 531–537. <https://doi.org/10.1002/jez.1402690606>
- [46] Wong, A. O. L., Murphy, C. K., Chang, J. P., Neumann, C. M., Lo, A., & Peter, R. E. (1998). Direct actions of serotonin on gonadotropin-II and growth hormone release from goldfish pituitary cells: interactions with gonadotropin-releasing hormone and dopamine and further evaluation of serotonin

- receptor specificity. *Fish Physiology and Biochemistry*, 19(1), 23-34. <https://doi.org/10.1023/A:1007713622603>
- [47] Okoli, A. S., Blix, T., Myhr, A. I., Xu, W., & Xu, X. (2022). Sustainable use of CRISPR/Cas in fish aquaculture: the biosafety perspective. *Transgenic research*, 31(1), 1–21. <https://doi.org/10.1007/s11248-021-00274-7>
- [48] Gratacap, R. L., Wargelius, A., Edvardsen, R. B., & Houston, R. D. (2019). Potential of Genome Editing to Improve Aquaculture Breeding and Production. *Trends in genetics : TIG*, 35(9), 672–684. <https://doi.org/10.1016/j.tig.2019.06.006>
- [49] Blix, T. B., Dalmo, R. A., Wargelius, A., & Myhr, A. I. (2021). Genome editing on finfish: Current status and implications for sustainability. *Reviews in Aquaculture*, 13(4), 2344-2363.
- [50] Hallerman, E. (2021). Genome editing in cultured fishes. *CABI Agriculture and Bioscience*, 2(1), 1-19.
- [51] Zhong, Z., Niu, P., Wang, M., Huang, G., Xu, S., Sun, Y., Xu, X., Hou, Y., Sun, X., Yan, Y., & Wang, H. (2016). Targeted disruption of sp7 and myostatin with CRISPR-Cas9 results in severe bone defects and more muscular cells in common carp. *Scientific reports*, 6, 22953. <https://doi.org/10.1038/srep22953>
- [52] Kishimoto, K., Washio, Y., Yoshiura, Y., Toyoda, A., Ueno, T., Fukuyama, H., ... & Kinoshita, M. (2018). Production of a breed of red sea bream *Pagrus major* with an increase of skeletal muscle mass and reduced body length by genome editing with CRISPR/Cas9. *Aquaculture*, 495, 415-427.
- [53] Khalil, K., Elayat, M., Khalifa, E., Daghash, S., Elaswad, A., Miller, M., Abdelrahman, H., Ye, Z., Odin, R., Drescher, D., Vo, K., Gosh, K., Bugg, W., Robinson, D., & Dunham, R. (2017). Generation of Myostatin Gene-Edited Channel Catfish (*Ictalurus punctatus*) via Zygote Injection of CRISPR/Cas9 System. *Scientific reports*, 7(1), 7301. <https://doi.org/10.1038/s41598-017-07223-7>
- [54] Shimbun Y. (2021). Kyoto Frm Puts Genome-Edited Tiger Pufer on the Table (Japan: Japan News). Available at: <https://the-japan-news.com/news/article/000793605>
- [55] Kleppe, L., Andersson, E., Skaftnesmo, K. O., Edvardsen, R. B., Fjelldal, P. G., Norberg, B., Bogerd, J., Schulz, R. W., & Wargelius, A. (2017). Sex steroid production associated with puberty is absent in germ cell-free salmon. *Scientific reports*, 7(1), 12584. <https://doi.org/10.1038/s41598-017-12936-w>
- [56] Zhai, G., Shu, T., Chen, K., Lou, Q., Jia, J., Huang, J., ... & Yin, Z. (2022). Successful production of an all-female common carp (*Cyprinus carpio* L.) population using *cyp17a1*-deficient neomale carp. *Engineering*, 8, 181-189.