

Study on the Epigenetic Modulation and Phenotypic Plasticity of Reproduction-Related Genes in Chub Mackerel (*Scomber japonicus*)

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Title : Study on the Epigenetic Modulation and Phenotypic Plasticity of
Reproduction-Related Genes in Chub Mackerel (*Scomber japonicus*)
(マサバ (*Scomber japonicus*) における生殖関連遺伝子のエピジェネテ
ック制御機構に関する研究)

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Thesis Summary

Finfish farming is the primary contributor to global aquaculture production. Among scombrids, chub mackerel (*Scomber japonicus*) is widely consumed. Aquaculture primarily operates in natural environments where external factors significantly influence fish breeding and survival, affecting overall production. Understanding these mechanisms is essential for industry sustainability, especially in the context of climate change. Epigenetic modifications, particularly DNA methylation, play a crucial role in acclimatization and adaptation across generations. DNA methylation fine-tunes gene expression during reproductive maturation by regulating the hypothalamic-pituitary-gonadal axis and sex steroid production, involving proteins like the gonadotropins Fsh, Lh, and aromatases Cyp19a and Cyp19b.

Our study investigates gene expression modulation through DNA methylation and identifies epigenetically influenced regulatory elements in reproduction-related genes. We sampled fish at various maturation stages and collected tissues from the gonad, liver, hypothalamus, pituitary, and brain for DNA and RNA isolation. After bisulfite (BS) conversion, the BS-DNA was used for several epigenetic studies. We optimized a cost-effective protocol for Targeted BS-sequencing and analyzed DNA methylation patterns of the *fshb* and *lhb* promoters in different tissues, identifying differentially methylated CpG sites in the pituitary of mature fish.

We also examined the effects of maturation and sex on DNA methylation and expression of gonad and brain aromatases (*cyp19a* and *cyp19b*, respectively). Methylation was observed in both promoters: *cyp19b* was hypermethylated in the brain regardless of sex and maturation, whereas *cyp19a* promoter methylation in the gonad was influenced by both sex and maturation. In vitro assays confirmed the repressive effect of methylation on *cyp19a* promoter activity, underscoring its regulatory role. We also identified an SF-1 binding site crucial for *cyp19a* promoter activation in chub mackerel. Furthermore, we compared methylation patterns of the *cyp19a* promoter in other fish species with different sexual systems, observing increased methylation levels in the testes' aromatase promoter across species and identifying sexually and species-variable DNA methylation patterns. Our inter-species analysis suggests that, while the global increase in DNA methylation is a commonality, the nuanced patterns are intricately tailored to the specific genetic makeup of each species, contributing to the complexity of the observed phenomenon.

Our findings provide a deeper understanding of the epigenetic regulation involved in fish reproduction. The identified regulatory regions, modulated by DNA methylation, can serve as a baseline for future studies. This foundation can be used to explore how various environmental factors, such as increased temperature or changes in food availability, might influence gene expression, phenotypic traits, and overall fish adaptability.