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学位論文内容の要旨

Dissertation Abstract

博士の専攻分野の名称：博士（水産科学） 氏名：(Mutea Fridah Gacheri)

学位論文題目
Title of dissertation

**Studies on the production of sex-reversed genetic females (pseudomales)
and all-female monosex populations in black rockfish (*Sebastes schlegelii*)**

(クロソイ (*Sebastes schlegelii*) における性転換遺伝雌（偽雄）
及び全雌単一性集団の作出に関する研究）

Sex-control technologies in aquaculture have become an important tool for enhancing production efficiency. Black rockfish (*Sebastes schlegelii*), an economically important species, displays sexual dimorphism, with growth-related dimorphism in particular presenting significant challenges for sustainable aquaculture. Specifically, females grow faster than males. The establishment of all-female monosex populations (AFMSP) represents a significant advancement in black rockfish aquaculture, as it promotes faster growth and more uniform body size, thereby enhancing production efficiency and overall profitability.

The ultimate goal of this study is to establish fundamental sex-control technologies that will contribute to the advancement of efficient and sustainable aquaculture of black rockfish. To achieve this goal, the present study aimed to assess the feasibility of indirect feminization in this species by crossing pseudomales (i.e., sex-reversed genetic females) with normal females and the sex of the progeny confirmed through PCR-based sex genotyping and histological analyses. This study addresses: (Chapter 2) production of pseudomales in black rockfish through oral administration of 17 α -methyltestosterone (MT) and letrozole (LTZ); (Chapter 3) gonadal mRNA expression of P450 aromatase (*cyp19a1a*) and anti-Müllerian hormones (*amha/amhy*) through the pseudomale production, and (Chapter 4) establishment of an AFMSP through an indirect feminization method using the pseudomales. Chapter 2:

Two sex-reversal trials in genetic females were sequentially conducted through an oral administration of 10 mg MT/kg diet (1st pseudomale production), and either 10 mg MT/kg diet or 20 mg LTZ/kg diet (2nd pseudomale production), for 50 days starting from 15 days post parturition (dpp). Genetic sex was determined by PCR-based sex genotyping, amplifying the duplicated anti-Müllerian hormone (*amha* and *amhy*) genes. Genetic females (XX) displayed a single ~400 bp *amha* band in agarose electrophoresis, whereas genetic males (XY) displayed two bands (~400 and ~600 bp; *amha* and *amhy*, respectively). The effect of MT and LTZ were assessed through histological analyses, as well as the PCR-based sex genotyping. Results of two trials revealed that all individuals (100%) of the treatment groups were phenotypic males, while control individuals (no treatment) exhibited nearly 1:1

sex ratio with complete concordance between phenotypic sex and genetic sex, indicating successful sex reversal from genetic females to phenotypic males in both treatment groups. No significant differences in body size and testicular stages were observed between the genetic males and females within each of the MT- or LTZ-treated group. This contrasts with the notable differences in body size found in the control males and females at 745 dpp, possibly due to early maturation in males. In contrast, late maturation in females is likely to encourage faster growth, enhancing breeding profitability in black rockfish aquaculture.

Chapter 3:

Quantitative real-time reverse transcription PCR (qPCR) were developed for measuring *amh* (*amha/amhy*) and *cyp19a1a* expression in the gonads of genetic females and males, as well as of pseudomales, during different stages of gonadal development following the time when their gonads have initiated morphological differentiation. The *amha/amhy* expression in testicular tissue of the phenotypic males in the control group, as well as *amha* transcripts in the MT and LTZ groups (pseudomales), increased as testicular growth and spermatogenesis progressed. The control genetic females exhibited inferior *amha* expression. The *cyp19a1a* expression increased as ovarian growth progressed from the chromatin-nucleolus stage at 251 dpp to the late peri-nucleolus stage at 748 dpp in the control genetic females, while were kept at low levels in pseudomales, as well as in genetic males of control and MT/LTZ groups, thus exhibiting the typical molecular sex dimorphism in black rockfish. These patterns of *amh* and *cyp19a1a* provided a basis to understand the molecular mechanisms underlying sexual differentiation, as well as gonadal development and maturation, in control and sex-reversed groups of black rockfish.

Chapter 4:

Two trials on the production of AFMSP were conducted using sperm obtained from MT- (1st trial) or LTZ- (2nd trial) pseudomales. During the 1st trial, A female broodstock inseminated with sperm from the MT pseudomales produced larvae (monosex group), likewise a female inseminated with control genetic males (control group). In both groups, there were no significant differences observed in the growth indices. The larvae of the monosex group collected at parturition in the 1st trial were subjected to PCR-based genetic sexing analyses; this confirmed the production of all genetic female progeny by amplifying a single band (~400 bp). The control group larvae produced either single (approximately 400 bp) or double (approximately 400 and 600 bp) bands. In the 1st trial, all individuals from the all-female progeny group exhibited 100% normal ovarian differentiation, resembling the genetic females in the control group, indicating successful AFMSP production. Males in the control group exhibited typical testicular structures. During the 2nd trial, females inseminated with sperm from the LTZ pseudomales produced larvae. All the larvae from the females appeared to be all genetic female progeny after amplifying a single ~400bp band in the PCR-based genetic sexing, indicating successful production of AFMSP.

The establishment of AFMSP in black rockfish was achieved for the first time through indirect feminization methods using two lines of pseudomales produced by either MT- or LTZ-administration. In addition to preventing unwanted production of slow-growing males, the remarkably faster growth in females can lead to higher market value and profound economic growth. This study marks a crucial step towards a large-scale all-female culture of this species, ultimately contributing to sustainable and efficient aquaculture practices.