

Physiology and endocrinology studies on reproduction of blacktip grouper
(*Epinephelus fasciatus*): role of water temperature and photoperiod
アカハタの繁殖に関する生理・内分泌学的研究: 水温と光周期の役割

長崎大学大学院水産・環境科学総合研究科
Pham Thanh Nhan

Brain-pituitary-gonad (B-P-G) axis has been known that regulate the maturation. Besides, water temperature and photoperiod strongly affect to fish endocrine system, closely related to gonadal development and spawning. Blacktip grouper is considered as target marine species for aquaculture in many Asian countries, particularly in Japan. The purposes of this study were to (1) determine the growth, sexual transition, maturation, and its related physiological and endocrine changes from juvenile to puberty, (2) seasonal changes photothermal condition and its effects to gonadal development and endocrine system, (3) induce maturation by artificial photothermal condition, (4) spawning characteristic and its related to water temperature.

Chapter I: Growth, sexual transition, and maturation of blacktip grouper; its related to physiological and endocrine changes from juvenile to puberty

To obtain the basic information about growth, sexual transition, gonadal development and age of puberty and roles of endocrine system during these progress, juveniles of blacktip grouper were reared during 4-year period from 2017 to 2021 in captive condition under natural water temperature regime. Changes in water temperatures, growth, gonadosomatic (GSI), gonadal development, plasma 17 β -estradiol (E₂), 11-ketotestosterone (11-KT) levels were investigated during the experimental period. Our results showed that maturation and spawning occurred in almost blacktip groupers at 3 years of age, although some females matured at 2 years. Plasma E₂ levels increased rapidly to high levels during puberty at 3 years of age, especially during the late stage of vitellogenesis at the tertiary yolk stage and reduced at the ripe and ovulated stages prior to spawning. The gonadal somatic index also showed an increase consistent with the rise in plasma E₂ levels. Plasma 11-KT levels increased dramatically during sex change and in males. The significant decrease in plasma E₂ levels and remarkable increase in plasma 11-KT were the key factors for the progress of sex change in these fish. Although this species had a protogynous hermaphrodite pattern, when individuals born in the same year were reared together, some individuals were capable of directly becoming males without experiencing a mature female stage

Chapter II: The reproductive cycle of female blacktip grouper in Nagasaki, Japan

Water temperature and photoperiod are known to play very critical roles during the reproductive cycle. The development of gonad and changes in endocrine systems are strongly influenced by seasonal photothermal condition. This study aims to determine the seasonal change in gonadal development and plasma E₂ levels throughout the year, and to understand the relationship between them and water temperature and photoperiod. We conducted the experiments that rearing adult blacktip grouper in net cage under natural photothermal condition during one-year period from July 2018 – July 2019 and collected wild blacktip grouper from May – June 2020 at coastal areas in Nagasaki. The results showed that vitellogenesis started after May water temperature was approximately 20 °C and photoperiod was 14 h/day; maturation and spawning occurred in June –

July when water temperatures were at 24 – 26 °C and photoperiod was at 14 h/day. Plasma E₂ levels. were high at vitellogenesis stage fish at spawning season.

Chapter III: Induce maturation of blacktip grouper by artificial photothermal condition

Blacktip grouper started vitellogenesis and matured in accordance with the increase of photothermal condition from spring and summer. Artificially suitable high temperature and long photoperiod could induce maturation in non-spawning season of many fish species, including grouper. In this study, we carried out three experiments with different manners of artificial photothermal manipulations in non-spawning season (i) December 2018 – May 2019; (ii) February – July 2020; (iii) February – May 2021. Fish matured after two months when water temperature increased artificially at 1 °C/week from 18 °C – 25 °C during February – April and then spawning several times at 25 °C. By contrast, 20 °C and 28 °C was not suitable for maturation.

Chapter IV: Spawning characteristics of blacktip grouper and its related to water temperature

This study was aimed to determine the spawning period, water temperature, spawning characteristics: number of eggs, buoyancy rate, fertilization rate, hatching rate; as well as spawning behavior; the ability to improve buoyancy and fertilization rate in higher depth tank was checked. We conducted three experiments at spawning season under natural water temperature (i) 5 m³ – 1m depth tank, June – October 2018; (ii) 1.5 m³ – 1m depth tank, June – October 2020; (iii) 7 m³ – 2 m depth tank, June – October 2020. Main spawning period were in June and July when water temperatures were 24 – 27 °C, spawning were not observed when water temperatures were at 27 – 29 °C in August. Buoyancy and fertilization rate were higher at 7 m³ – 2 m depth tank.

General discussion and future direction

We obtained information for gonadal development, age of early puberty at 2 years old and puberty at fully mature at almost female at 3 years of age with high plasma E₂ levels at this age. This is very important information in broodstock management in seed production in hatchery. Male that transformed directly from immature female at age of two consisted of developed stage at Sz and milky semen with mature and active sperm with fertilize the ovulated egg at natural spawning with high fertilization rates. This phenomenon is very important and helpful in broodstock management in seed production due to the fact that we could obtain functional male at younger age directly from juvenile while in the wild male appeared from 5 years of age.

Under high artificial photothermal treatment, all fish matured after two months when water temperature increased artificially with low speed and continuously at 0.5 °C/ 3 days (1 °C/ week) from 18 °C – 25 °C within 2 months and then spawning several times when kept at 25 °C.

The future direction will be (i) the measurement the gene expression of reproductive hormones in BGP axis to clarify the changes in endocrine system during maturation and the effects of photothermal condition to BGP axis; (ii) artificial photothermal manipulation on puberty and maturation of blacktip grouper.

With the findings of this PhD study and future plan, we have been trying to obtain and clarify the physiology and endocrinology on reproduction of blacktip grouper and roles of water temperature and photoperiod to maturation and spawning of this species. We hope that we can contribute to the success and development of seed production as well as aquaculture of the blacktip grouper.