Effects of Environmental Factors on Gametogenesis and Reproductive Endocrine System in the Dojo Loach, *Misgurnus anguillicaudatus*

（ドジョウの配偶子形成と生殖内分泌系に及ぼす環境要因の影響）

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Reproduction is one of life's most important processes that help a species to continue its generation by recruiting a new offspring. Thus, reproductive information are all essential in understanding fish population dynamics in order to manage and control the wild stock and to develop a technique for seed production. Information about reproductive biology is crucial to study effects of environmental problems such as global warming, endocrine disrupting chemicals and habitat distraction, so that the study of reproduction has practical as well as academic importance. However, there are many species, including loaches, for which accurate basic data on their reproductive biology and consequences of environment effects and its mechanisms are not well documented. Therefore, the objectives of the studies were to investigate the annual reproductive cycle, effects of temperature and photoperiod on the gonadal maturation at different season, effect of prolonged exposure to various temperatures and photoperiod on reproductive status of dojo loach and to clarified the endocrine mediation between the environmental factors and brain pituitary gonad axis in dojo loach, *Misgurnus anguillicaudatus*. It will be important to understand the specific role of environmental factors in driving gametogenesis and spawning to address the mismatch that may occur with global climate change.

Reproductive cycle and characteristics of gametogenesis in the dojo loach

Dojo loach reproductive cycle and gametogenesis was characterized based on evidence from gonadal histology and steroid hormones. Both females and males showed characteristics of multiple spawner having extended spawning period from May to August and April to September, respectively, a season with long photoperiod and warm temperature, and accompanied by high gonadosomatic indices (GSI) and elevated levels of plasma steroids testosterone (T), estradiol-17β (E_2) for female, 11-ketotestosterone (11-KT) for male and 17, 20β-dihydroxy-4-pregnen-3-one (DHP). Gametogenesis is controlled by energy accumulation (high HSI), environmental factors, and the endocrine reproductive axis. The changes in plasma levels of sex steroids were correlated with the annual gonadal cycle, and suggesting that these hormones regulate the process of oocyte and testicular development (chapter II).

Environmental regulation of the reproductive success in dojo loach

To investigate the relative importance of photoperiod and temperature on controlling gametogenesis and the magnitude of changes in the gametogenic stages, experiments were set at different season of the year using several photoperiod and temperature. In summer (July to September), fish exposed to a short photoperiod (10L: 14D) at various temperatures (20, 25 and 30ºC) showed low E_2, GSI and intense gonadal regression which leads to the termination of the spawning period. During autumn and early winter groups

from long photoperiod and warm temperature displayed high GSI, T, E_2 and fully enhanced vitellogenesis. In spring (March to May) vitellogenic fish exposed to 15, 25 and 30ºC either to long (14L: 10D) or short photoperiod (10L: 14D), and water temperature is the driver of gonadal final maturation; vitellogenesis is maintained at 15ºC, reached spawning stage at 25ºC, however ovarian regression is observed at high temperature (30ºC) which indicates that the temperature sensitive stage may occur during the transition from late vitellogenic stage to initiation of final maturation. The mechanisms of high water temperature inhibition of gonad maturation appeared to be associated with impairment of plasma steroid secretion and action. Collectively, temperature and photoperiod play proximate cues in the regulation of annual reproduction at different stages in this species (chapter III).

Effects of Long term exposure to photoperiod and water temperature on maturation in the dojo loach

Female fish exposed to the 18L & 25ºC group during the end of spawning season produced the highest reproductive performance and having high GSI, T, E2 and DHP, maintaining their matured ovaries continuously. The 18L & 30ºC groups displayed vitellogenic oocytes but did not reached mature stage and plasma sex steroid are low and erratic. Fish exposed to 35ºC for 40days followed by 25ºC for the remaining 4months showed slow recovery in ovarian recrudescence from the high temperature damage. Thus, high temperature limits the physiological response of gonad to increased photoperiod. Therefore, using photoperiod (18L) and temperature (25ºC) manipulation, it is possible to induce maturation and spawning as required (chapter IV).

Molecular cloning and characterization of gonadotropin subunits in dojo loach

The three cDNAs GPHα, FSHβ and LHβ subunits consisted of 809, 471 and 947 nucleotides encoding peptides of 119, 128 and 141 amino acids, respectively were identified. Like other cyprinids, both the cysteine and N-linked glycosylation sites in the GPHα, FSHβ and LHβ subunits are located at the conserved position. The deduced amino acid sequence of each mature subunit displayed high homology with those of other teleost, especially cypriniformes, implying their close phylogenetic relationship. The gene expression of FSH and LHβ subunits exposed to long photoperiod and warm temperature displayed high expression in similar pattern to the steroid level and GSI than those exposed to the low temperature and short photoperiod. These indicate that environmental effects are mediated through gonadotropin gonad axis. These results can have importance to develop hormone therapies to override the effect of high temperature at pituitary level (chapter V).

General discussion

To conclude, the thesis represents novel research which explores in detail about reproductive cycle, and the photoperiodic and temperature window of opportunities that maintain individuals in reproductive active and provided valuable information into the intrinsic links of brain-pituitary-gonadal and sex steroids. Further more, the thesis discusses and proposes possible options to improve aquaculture by reducing the effect of increasing temperature. Thus, manipulation of photoperiod and temperature offer relatively simple and environmental friend methods for altering reproductive time and hence providing out-of season supplies of egg and fry for commercial culture. This will make a major contribution to development of aquaculture of the species without the necessity of relying upon natural, wild spawning (chapter VI).