Studies on environmental factors associated with stress response and modulation of sex determination in pejerrey Odontesthes bonariensis

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

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論文題目 Title	Studies on environmental factors associated with stress response and modulation of sex determination in pejerrey *Odontesthes bonariensis* ペヘレイのストレス応答および性決定に及ぼす環境要因の影響に関する研究		

The pejerrey *Odontesthes bonariensis* is a brackish water fish native from South America that is economically important in its native distribution range and at the same time a promising candidate for aquaculture in several countries where it has been introduced, including in Japan. The pejerrey presents a combination of genotypic sex determination (GSD) and environmental sex determination (ESD) in the form of strong temperature-dependent sex determination (TSD). Temperatures of 17 and 29 °C during the first 5 weeks after hatching (wah) lead to feminization and masculinization of XY and XX individuals, respectively, whereas at intermediate temperatures (24-25 °C) there is a high concordance between genotypic and phenotypic sex. Masculinization by high temperatures in pejerrey is associated with thermal stress as indicated by increased cortisol titers, and cortisol buildup indirectly leads to the synthesis of 11-Ketotestosterone (11-KT), a potent androgen, because cortisol inactivation and 11-KT synthesis are mediated by the same enzyme, HSD11B2 (11β-hydroxysteroid dehydrogenase type 2). Stress, cortisol, and androgens have been implicated in the ESD of other fish species in response to salinity, background color, or the social environment, so it is plausible that pejerrey may show also other forms of ESD in addition to TSD. If this is the case, knowledge on other forms of ESD and their interactions would be critical to predict the sex ratios of natural pejerrey populations and to control the sex in farmed stocks. However, little is known on alternative sex-determining environmental factors in pejerrey. In this context, I designed a series of experiments to evaluate the occurrence of other forms of ESD and their relationship with stress responses in this species. The tested factors were background color, rearing density/space availability, and water salinity.

The first chapter examined the effect of background color and included rearing fish throughout the critical period of sex determination in tanks with black, gray, dark blue, light blue, green, red and white walls. Two trials were conducted, each with the progeny from a single cross between a male and female of known genotype (XX-XY). Fish were reared at an intermediate temperature (24 ± 0.5 °C) and low density (10 and 15 larvae/L in the first and second trials, respectively). The sex reversal rate of each group was inferred from the results of histological analysis of the gonads and detection of *amhy* gene as the genotypic sex marker at the end of 14 weeks. In addition, whole-body cortisol and 11-KT titers were analyzed by ELISA. The results showed no statistical differences among the sex ratios of fish reared in different background colors. On the other hand, groups of fish reared in tanks with different colors had

clearly different body coloration and some groups also presented statistically different cortisol and 11-KT titers. However, none of these could be clearly correlated with the sex ratios. Based on these observations, it is suggested that background color can potentially affect the physiology of pejerrey young including processes related to skin color adaptation and probably also the stress axis, but the stimulus was not strong enough as to lead to sex reversal.

The second chapter examined the effects of rearing density/space availability. This study used two different pejerrey progenies, each one from a different XX-XY pair of parents. The larvae were stocked immediately after hatching in meshed containers of three different volumes (6.4, 1.6 and 0.4 L) at three different densities (15, 62 and 250 larvae/L). All containers shared the same rearing water within a large water bath kept at 24 ± 0.5 °C. In addition, to evaluate if the fish could perceive the presence of conspecifics and the rearing density through visual information, each trial included also two containers (1.6 and 6.4 L) with mirror-finish, reflecting walls in order to visually simulate a high rearing density. Fish were sampled individually as they attained a minimum standard length of 32 mm and analyzed as described above for the background color experiment. The Akaike Information Criterion was used to select among models that took into consideration rearing density and volume (available space) separately or in combination. The results indicated an interaction of available space and rearing density with a higher frequency of masculinization at the smallest volumes and/or highest densities, but the contribution of rearing density was by far more important than that of available space. Whole-body cortisol and 11-KT titers generally increased with rearing density and/or a reduction in rearing volume, particularly in XY fish, although not all paired comparisons were statistically significant. The mirror wall containers produced higher rates of masculinized individuals (sex-reversed XX males) than containers with non-reflecting walls. Overall, these observations suggest that the fish visually perceived and may have felt stressed by the excessive proximity of conspecifics or with limitation of space and that high rearing density is a clear stressor and a form of ESD for pejerrey.

The third chapter examined the effects of water salinity. Fish were reared from hatching until complete sex determination and gonadal differentiation under six salinity concentrations (0, 0.05, 0.1, 0.3, 1 and 3 %). All concentrations were prepared with artificial sea water salts and dechlorinated tap water. These concentrations cover the range of salinities that pejerrey can experience in their natural habitat and include the levels considered as most suitable for survival of this species (0.1-0.3 %). Larvae were reared for 10 weeks at the experimental salinities and then transferred to 0.3 % for further rearing until sampling. Sex ratios were determined at the end of the experiment as in previous chapters. Whole-body hormone (cortisol and 11KT) and gene expression (*hsd11b2*) analyses were conducted at 2, 4, and 2, 4, 6, 8 wah, respectively. The results indicated that the percentage of males among XX fish increased stepwise between 0.3 and 1% salinity whereas in XY larvae the percentage of males increased gradually between 0 and 1-3% salinity. The cortisol levels at 2 wah were lowest at 1 and 3% for both genotypes whereas at 4 wah there was no relation to water salinity. In case of 11-KT, the levels at 2 wah were higher at 0% for XX but the same trend was not evident in XY genotypes; at 4 wah the levels were

variable and unrelated to salinity concentration. Transcription of *hsd11b2* increased transiently between 2 and 8 wah in some XX fish at 0, 1 and 3 % salinity and in XY fish at 1 and 3 %. Thus, although there was no clear association between cortisol, 11KT titers, and sex ratios, masculinization was clearly promoted at the highest water salinities. The fact that a stress response could not be demonstrated in the male-biased groups may be related to the rapid hormone clearance by HSD11B2 action and/or a role of cortisol in osmoregulation. This finding emphasizes the need to explore alternative molecular pathways such as those involved in osmoregulation and thyroid axis that could potentially interact with the sex determination mechanism in pejerrey.

In summary, the results of the above experiments show that other environmental variables besides temperature can also act as stressors for young pejerrey and affect its gonadal sex differentiation. For some factors, the magnitude of the stress they induce when operating singly may not be enough to cause sex reversal. Thus, this study also emphasizes the possibility of interactions (either synergistic or antagonistic) among environmental factors that might affect sex determination in pejerrey. This, in turn, points to the need to examine also other variables (e.g. photoperiod, pH, dissolved oxygen) that may be equally conducive to stress and hence may affect sex determination in pejerrey in order to accurately predict and manage the sex ratios of wild and farmed populations.