Possible influence of social stress on sex differentiation in Japanese eel

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INTRODUCTION

The mechanisms of sex determination of many teleost species have been under investigation. In many cases, it has been shown that environmental factors are involved in sex determination, modulating the expression of the genetic component. During early development, eels undergo distinct external morphological and ecological changes. Sex differentiation occurs during late juvenile development i.e. after pigmentation or during upstream migration. Under culture conditions, Japanese eel, Anguilla japonica, differentiate mostly into males, while sex ratio is impartial in the natural river. The environmental factors influencing on gonadal sex differentiation of eel remains to be determined.

In the present study, we observed behavioural and physiological characteristics of the eel during gonadal sex differentiation in particular environmental and social stress in cultured conditions that might affect on sex differentiation.

MATERIALS AND METHODS

Experimental fish
Japanese glass eels were obtained from the coastal waters of Japan and reared in indoor tank with recirculated water, 28 ± 1 °C controlled thermostatically until use. Underyearing eels were used for each experiment according to their growth 6-20 cm body length (BL).

Histology
The gonads were fixed in Bouin’s fluid solution and embedded in paraffin. They were cut into 5 μm sections and stained with hematoxilin-eosin solution for light microscope observation.

Behavioral observations
Swimming activity was analyzed by counts of number of swimming eels in upper and middle layers of a tank in day (8:00, 12:00, 16:00) and night time (20:00, 0:00, 4:00). Rheotaxis was measured up- or down-stream movement in test flow by an infra-red ray monitoring system for 24 h. Preference of aggregate or solitary life was determined by the counts of number of eels in a burrow (20 eels for 20 burrows in a test tank) 5 times in a day. The frequency of nipping behavior was recorded for 10 minutes at intervals of 2 h during the night time (18:00-4:00) as an index of aggressiveness.

The effect of exogenous estrogen
In order to determine susceptible period of feminization of the gonad by exogenous estrogen, effects of dietary estradiol-17β at a dose of 20 mg/kg diet on the feminization of the gonads in cultivated eels were investigated at various stages of growth (7-19 cm BL).

The effect of temperature and pH
The fish were exposed to varying constant temperatures (15, 21, 27, 32 and 36°C) and pH (4.8, 7.0 and 8.5) during the period of sex differentiation. They were reared until presumed sex determined stage (20-23 cm BL), followed by collecting the gonads for subsequent microscopic identification of sex.

The effect of stocking density
The fish (20 cm BL) were assigned to each glass water tank (60 × 30 × 30 cm) at stocking density of 1, 2, 4 and 8 fish, respectively. They were acclimated to those conditions for 2 weeks. At the end of the experiment, fish were sacrificed by electroshock for blood samples. The concentration of serum cortisol was determined by time-resolved fluoroimmunoassay (Yamada et al. unpublished).

RESULTS

Sex differentiation of Gonads
Morphological sex differentiation of the gonads was observed at 15-23 cm body length (BL). A few females appeared, but more than 80% of 287 yellow eels differentiated to male and 6-21% of the eel showed hermaphroditic gonad under aquarium condition.

Hormone sensitive period for feminization
Dietary estradiol-17β treatment induced feminization
when it was given before and after the stage of morphological sex differentiation of gonad in 11 cm to 20 cm BL eels, thus the susceptible period of feminization by estrogen was determined.

The effect of temperature and pH on sex differentiation
Various levels of temperature and pH did not affect the sex ratios, resulting the high proportions of males (75-90%). Temperature and pH are not likely to be one of the environmental factor influencing the sex differentiation in eel.

Changes in upstream migratory behavior
The swimming activity of glass eel and yellow eel increased with growth up to 12 cm in BL in an experimental tank, then it reached to the minimum at 16 cm BL stage. Upstream movement in a water-flow test tank was maximum in 12 cm BL yellow eel, however, the upstream swimming was minimized in 16 cm and 20 cm yellow eels. Aggregation in a burrow disappeared after 9 cm in BL and the eels sifted to solitary life (Fig. 1). Frequency of aggressive behavior sharply increased with growth up to 16 cm yellow eel.

CONCLUSIONS
Susceptible period to estrogen for feminization was between 11 and 20 cm BL. This hormone sensitive period is earlier than the morphological sex differentiation, indicating the initial stage of testicular differentiation. In several species displaying environmental sex determination, the timing and duration of hormone sensitive period is similar to the temperature sensitive period. Therefore, elucidation of this critical period will be important to analyze the effect of certain environmental factor on sex differentiation in eel.

Changes in upstream migratory behavior indicate that gonadal sex differentiation of eel occurs in the period of ecological transition from upstream migratory phase to settlement phase. This result suggest that the importance to examine the ecological influence to the cultured eels that differentiate mostly into male.

Higher level of plasma cortisol was observed in aggregate eels in a tank, suggesting that high density induces a chronic stress. In high-density culture of eels, agonistic interactions have been implicated in causing social stress, leading stunting, mortality and cannibalism. Eel is naturally a solitary and territorial species so stress by social interactions in culture condition may affect on sex differentiation.

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REFERENCES