LHRHa-INDUCED SPAWNING OF THE EASTERN LITTLE TUNA *EUTHYNNUS AFFINIS* IN A 70-M^3 LAND-BASED TANK

Takeuchi Y.*, Sato K.º, Yazawa R.º, Yoshikawa H.*, Iwata G.º, Kabeya Nº., Shimizu S. º and Yoshizaki G.º

*Research Center for Advanced Science and Technology, Tokyo University of Marine Science and Technology, 670 Banda, Tateyama, Chiba 294-0308, Japan.
FAX: +81 470 20 9021 e-mail: yutakat@kaiyodai.ac.jp
ºDepartment of Marine Biosciences,3 Tokyo University of Marine Science and Technology, Tokyo, Japan.

Introduction:

If small surrogates could be used to produce gametes of Pacific bluefin tuna *Thunnus orientalis* (PBT), then large quantities of PBT seeds could be produced in small facilities over a short period of time. With its small-body size, short generation time, and physiological similarities to PBT, the eastern little tuna (ELT) is considered to be well suited as a candidate for transplanting donor PBT germ cells. However, the technology for inducing spawning in ELT maintained in captivity is not yet well developed. We therefore attempted to control the reproduction of ELT in a 70-m^3 land-based tank (7 m in diameter, 1.8 m deep) by hormonal administration over an extended period.

Methods:

To investigate gonadal development in 1- to 2-year old ELT, the gonads of fish that died during the rearing period were processed for histological analysis. In addition, in the summer of 2010, cholesterol pellets containing LHRHa (L4513, Sigma, St. Louis) at a dose of 100 µg/kg (body weight, BW) were implanted into the dorsal muscles of 2-year old ELT. A non-invasive sex steroid assay using clipped fins was used to determine the sex of the broodstock and whether LHRHa administration affected the amount of 11-keto testosterone (11-KT) and 17β-estradiol (E2). For each spawning event, the time of spawning, the number of eggs collected, and the fertilization and hatching rates were determined. To identify the number of broodstock that participated in spawning events and the spawning frequency of each individual, DNA extracted from the clipped fin samples of the broodstock and hatched larvae were analyzed using three microsatellite loci.

Results:

ELT juveniles (BW: approx. 300 g; total length (TL) 20 cm) caught in the wild on August 2008 at Kushimoto in Wakayama Prefecture, Japan, were reared in a pen until June 2009. Thirty-two 1-year-old ELT were then transferred to a 70-m^3 land-based tank at Tateyama Station in Chiba Prefecture and reared for 15 months until September 2010 when there were 14 fish (BW: approx. 2 kg; TL: 50 cm). In August 2010, ovaries and testes obtained from dead fish contained vitellogenic oocytes with a diameter of 400 µm and sperm, respectively. No spawning behavior was observed in the ELT reared in the tank without the hormonal treatment. In September 2010, LHRHa-pellets were implanted into nine fish (five females and four males) on the same day. Three days post-implantation, fertilized eggs were collected by an egg collector. Spawning events occurred between 15:30 and 19:30 and continued for 9 days and the number of eggs collected in a day ranged from 50,000 to 170,000. The water temperature during this period ranged from 23.7 to 26.6˚C. The mean fertilization and hatching rates were 86.8% and 34.4%, respectively. The larvae started to feed on rotifers at 3 days post-fertilization and grew up normally. At least 30 larvae were analyzed by microsatellite DNA markers at each spawning event and the parents of 20.2% (76 out of 376) of the larvae were identified. Parentage analysis revealed that three of the five implanted females produced viable offspring, and one of these females participated in up to nine consecutive spawning events. The sires consisted of two implanted males and one non-implanted male, suggesting that the LHRHa administration was not always necessary for inducing spawning behavior in ELT males. In most cases, the offspring of one female were sired by two males. Video recording of spawning events showed the horizontal spawning dash of a female accompanied by one or two males followed by the release of gametes. Using an enzyme-linked immunosorbent assay, LHRHa treatment was shown to elevate 11-KT and E2 levels in the parental broodstock for up to two weeks after implantation.

Conclusion:

This study showed that LHRHa administration was effective for inducing spawning in ELT maintained in captivity and for producing high quality eggs. Multiple spawning events by both males and females were observed. We therefore propose that ELT may be well suited for use surrogate broodstock for the production BLT seeds via xenogenic germ cell transplantation. We are currently focusing on rearing xenotransplanted ELT juveniles harboring donor-derived BTL germ cells in their gonads.