Short Paper

Triploid male masu salmon *Oncorhynchus masou* shows normal courtship behaviorS. Kitamura,*¹ H. Ogata,*¹ and H. Onozato*¹

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Triploid female fish have less developed ovaries than diploids, while the testes of triploid males are partially developed, and in some species, they are as large as those of diploids.¹⁻⁷⁾ In masu salmon *Oncorhynchus masou* the average gonadosomatic index of triploid males is significantly lower than that of diploids, and the triploid testes contained no spermatozoa in the breeding season, yet steroid hormone and GTH levels in the triploid males are not significantly different from those in the diploids,^{8,9)} as is the case of rainbow trout *Oncorhynchus mykiss*.⁴⁾ Moreover, secondary sexual characteristics such as changes in the body coloration and shape were observed in triploid male masu salmon. If this is the case, triploid males as well as diploids might show normal sexual behaviors toward females. In this study, we examined whether triploid male masu salmon could display normal courtship behavior ('quivering') toward an ovulating female (diploid) in an aquarium.

The test animals used in this experiment were masu salmon of the Amago strain *Oncorhynchus masou rhodurus*. Triploid males were two years old and diploids were yearling. Fish were raised on a commercial trout diet in indoor glass tanks (30×60×30 cm in size, WT: 13–16°C) and outdoor experimental ponds (1×4×1 m in size, WT: 13–16°C). Triploidy was induced by hydrostatic pressure at 650 atm for a duration of 6 min, 15–30 min after fertilization.⁸⁾ Triploids were identified just before the present study by comparing major axes of red blood cells (RBC) with those of diploid controls, by an image analyzer (Ibas 2000, Zeiss Co, Ltd.) The triploid males used in this study gave mean RBC major axes of 18.5–20.0 µm compared with values of 14.8–15.8 µm for the diploids. Although the triploid males showed external secondary sexual characteristics, no sperm was observed in their testes. By contrast, the diploids used here were spermiated and sperm was easily expressed by slight pressure to the body wall. The body lengths of diploids and triploids ranged from 19.0 to 24.5 cm and from 25.0 to 28.5 cm, respectively.

The experimental aquarium was 45×90×45 cm in size, with a sufficient depth of gravel to allow for nest building. Precooled well water (15°C) was provided at a velocity of about 3 cm/s through the aquarium during experiments. A triploid or diploid male was placed in the aquarium with an ovulating diploid female. The behavior of male fish was recorded using a video camera recorder.

Male masu salmon showed two different patterns of 'quivering'. One was basically the same pattern as has been observed in other salmonids,^{8,10)} that is, male approaches the female from down stream, and vibrating his body on one side of the female. The other pattern, which was observed specifically in masu salmon of the Amago strain and the Yamame strain *Oncorhynchus masou masou*,¹¹⁾ is that male arches and strongly vibrates his body upstream or on one side of the female. The frequencies of both quiverings displayed by male were counted for 1 h from the time the first quivering was displayed. The experiments were

conducted October–November 1989, using 10 and 5 different pairs of diploid male–diploid female pairs and triploid–diploid pairs, respectively.

The triploid and diploid males both began their quiverings 10–30 min after being placed in the aquarium with an ovulating female. The mean total numbers of quiverings in the triploids and diploids were 83.6/h (range: 54–122/h) and 40.7/h (range: 9–79/h), respectively. The number of the former was significantly higher than that of the latter ($P < 0.05$, by Mann-Whitney U-test).

Lincoln and Scott⁴⁾ found that a thin, watery milt containing motile spermatozoa was expressed from triploid males of rainbow trout, however, levels of androgens in the triploid and diploid males were not significantly different. Nakamura *et al.*^{8,9)} also found natural secretions of GTH and steroid hormones in triploid male masu salmon at maturation. Normal quivering behaviors by triploid males observed in this study are probably due to the natural activity of steroid hormones within the fish. The reason for triploid males showing quiverings more than twice that of diploids is unclear at present. Difference in the age between the triploid and diploid males used here may have been one of the reasons.

In this study, nest digging behavior by ovulating females was often observed, but females failed to spawn under the present experimental conditions. Judging from the result obtained here, it is very likely that a female would spawn with triploid male in a natural environment. Development of the fertilized eggs does not proceed beyond hatching, even if the sperm of triploid fish are capable of fertilizing eggs.^{4,7)} Exhibition of reproductive behavior by triploid male would mean infertile mating. Therefore, triploid male fish should not be released from rearing pond into the natural environment.

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*¹ Inland Station, National Research Institute of Aquaculture, Tamaki, Mie 519–04, Japan (北村章二, 尾形博, 小野里 坦: 水産庁養殖研究所).

*² M. Nakamura, F. Tsuchiya, M. Iwabashi, K. Suzuki, and Y. Nagahama: Jpn. Soc. Sci. Fish. Biannual Meeting in Spring 1987 (Abstr. 323, in Japanese).