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Title	Responses of immature male masu salmon parr to the urine of mature males
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Citation	Ichthyological Research, 53(2), 182-184 https://doi.org/10.1007/s10228-005-0323-1
Issue Date	2006-05
Doc URL	https://hdl.handle.net/2115/14418
Rights	The original publication is available at www.springerlink.com
Type	journal article
File Information	IR 04083.yambe.pdf

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Responses of immature male masu salmon parr to the urine of mature males

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Suggesting running head: Immature male masu salmon respond to male urine

Type of paper: Short Report

Paper volume: 8 papers in text, with 1 figure.

Abstract Responses of male masu salmon *Oncorhynchus masou* parr to mature male urine were investigated in a Y-maze trough. Immature masu salmon had fewer entries into the channel conditioned by mature male urine than by control water. This phenomenon may suggest that immature males avoid sexually active males in spawning season.

Key words Behavior· Immature male parr· Mature male urine· Masu salmon· Pheromone

In many fishes, chemical communication using urine play an important role in social interactions, such as recognition of siblings, individuals, and sex (Olsén, 1987; Yamazaki, 1990; Katsel et al., 1992; Moore et al., 1994). In the salmonid fishes, female urine has been remarkably studied over the last decade. The urine of ovulated female masu salmon *Oncorhynchus masou* and female rainbow trout *Oncorhynchus mykiss* have been also reported to be a source of male-attracting pheromones that attract conspecific males (Yambe et al., 1999; Yambe and Yamazaki, 2001). On the other hand, little attention has been given to the effects of mature male urine as chemical signals for the salmonid males. Male urine may also contain something to act toward other individuals. We examined whether mature male urine has signals inducing behavioral reactions in immature and mature male masu salmon.

Materials and Methods

Masu salmon has two types of life history. Most males are a river-resident type called "precocious male or parr," reaching 15 to 25 cm in total length. A portion of males and most females are an anadromous type that grow to 60 cm in total length. The masu salmon were obtained from Hokkaido Salmon Hatchery, Mori, Hokkaido. Their spawning season is from September to October in northern Japan. In all behavioral tests, precocious males (parr) were used as test fish, because they were small in size and easier to handle and maintain than anadromous types. The 0^+ year immature males of masu salmon parr, which later became mature [n = 140, 150-250 mm fork length (FL)], were used as the test fish in May and October 2000. Three 1^+ year anadromous mature males (1-2 days after spermiation, FL ± SD = 365.7 ± 24.8 mm) and three 1^+ year anadromous immature males (361.9 ± 39.6 mm FL) with spermatids and spermatocytes were used as the sources of urine in September 1999 and May 2000, respectively. They were catheterized to collect the urine as described previously (Yambe et al., 1999). Urine solutions were made by diluting 500 µl urine with 300 ml of distilled water (DW). The volume of the urine used in experiments was calculated from the rate of urination

reported by Curtis and Wood (1991). Three hundred ml of well water (WW) was used as control solutions.

One pair of mature or immature male parr was tested to the urine of adult-sized mature or immature males. Full details of behavioral experiments using a Y-maze trough (290 cm distance x 35 cm width, 10 cm in water depth, 50 l/min in flow of each channel; see Yambe et al., 1999: fig.3) with a mirror to observe the downstream section followed Yambe et al. (1999). In short, two test fish were acclimated for 15 min in the downstream section. After the acclimation period, one of three pairs of test solutions was simultaneously introduced from separatory funnels for a 2 min period into each channel. The three pairs of test and control solutions were (1) DW and WW, (2) mature male urine (MMU) and WW, and (3) immature male urine (IMU) and WW. The experiment was started by opening the gate, which was in the middle of the trough, 12 s after starting to introduce the test and control solutions. The total number of entries of the two test fish into each channel, chase or attack, and retreat were recorded for 6 min after opening the gate. The entries that appeared to be caused by agonistic behavior were also included as the frequencies of these agonistic interactions were low and had no statistical effect. After each trial, the side receiving test and control solutions were switched to avoid location bias so that both channels were alternately conditioned by test solutions. The two test fish were changed after a trial. The number of entries of the two test fish into each channel was pooled for the respective trials and compared by a Wilcoxon-signed rank test between the test and the control channels. The number of retreat of the two test fish in the downstream section was pooled for the respective trials and compared by a Mann Whitney *U*-test between immature and mature males exposed to the same sample. The significance level was set at $P = 0.05$. To confirm the gonadal condition in the immature males, six randomly selected immature males were dissected and observed histologically to check the developmental state.

Results

Mature and immature males showed no preference between the two channels conditioned by DW and WW ($P > 0.05$, Fig. 1). They also showed no preference when given a choice between the channels conditioned by IMU and WW ($P > 0.05$, Fig. 1). Contrary to our expectation, the responses of the mature males were not significantly different between the channels conditioned by MMU and WW ($P > 0.05$, Fig. 1a). However, immature male parr had significantly fewer entries into the channel conditioned by MMU than into the channel conditioned by WW ($P = 0.011$, Fig. 1b). They sometimes retreated with the face turned forward and shaking their head in the plume of MMU in the downstream section (Table 1). Frequency of retreat in immature exposed to MMU tended to be more than that of mature males ($P = 0.0512$). Immature male parr had small testes and showed no sign of spermiation (with many spermatocytes and only a few spermatids, mean gonad somatic index ± SD = 0.84 ± 0.54).

Discussion

Immature male preferred the channel conditioned by water to the channel conditioned by MMU. Chemical signals in the urine would be lined up as the most important candidate for the interpretation. Female urine induces positively the endocrine or behavioral responses of male salmon (Vermeirssen et al., 1997; Yambe et al., 1999). However, unexpected results suggesting a negative reaction have also been reported on sex pheromones. For example, precocious male chinook salmon *Oncorhynchus tshawytscha* has been suggested to avoid the water conditioned with 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P) (Dittman and Quinn, 1994). Bjerselius et al. (1995) showed that spermiating male crucian carp *Carassius carassius* avoided 17,20β-P implicating interactions for benefit to spawning males. In land vertebrates, Rasmussen et al. (2002) reported that juvenile males of Asian elephant avoided the odors secreted from adult males. Therefore, there is the possibility that

not only female urine but also male urine induce behavioral responses in male masu salmon.

Sneaking tactics is known to be common phenomenon in salmonid males during spawning (Maekawa and Onozato, 1986; Hutchings and Meyer, 1988). Perhaps mature males release chemical signals inducing such interaction with other individuals in the spawning. Here we propose a pair of hypotheses for the present result although it may be difficult to relate the laboratory results to the natural situation. Immature males exposed to mature male urine may avoid unexpected attacks by sexually active males. On the other hand, mature male urine may estrange cannibal males (egg eaters) from mature males, when viewed from mature males.

This paper may suggest that mature male urine contains alarm pheromones. Further study needs to clear the detail functions and the chemical substance in mature male urine. There may be new contexts of chemical communications in fish.

Acknowledgements We are grateful to A. Goto and K. Arai (Hokkaido University) for their advices, to K. Imada and Y. Sasaki (Hokkaido Fish Hatchery) for providing materials. This study was supported by grants for young scientists from Hokkaido Foundation for the Promotion of Scientific & Industrial Technology (No. 046 to H. Y.), Northern Advancement Center for Science & Technology (No. 057 to H. Y.) and the 21st Century COE program of the University of the Ryukyus.

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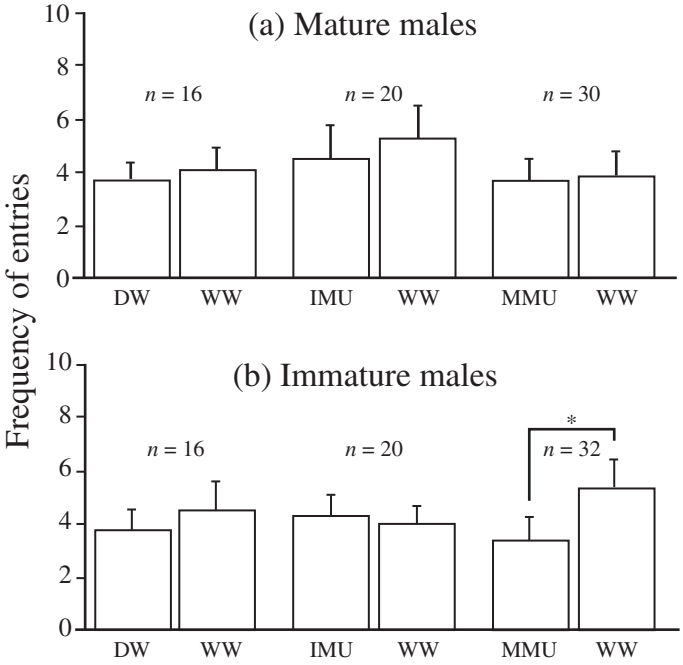
Figure legend

Fig. 1. Frequency of entries of spermiating mature (**a**) and immature (**b**) male masu salmon parr to the water conditioned by distilled water (*DW*), well water (*WW*) and the urine of adult male masu salmon in the Y-maze trough. *Vertical bars* show standard error of mean. *n*, number of each trial. *Asterisk* shows a significant difference ($P < 0.05$). *IMU*, immature male urine; *MMU*, mature male urine

Table 1. Frequency of retreat in test fish in the downstream section when male urine was introduced into the Y-maze trough

Test fish	Sample	Mean	SD	Range
Mature	Water	0.06	0.25	0-1
	IMU	0.10	0.31	0-1
	MMU	0.37	0.72	0-2
Immature	Water	0.00	0.00	0-0
	IMU	0.05	0.22	0-1
	MMU	0.72	0.85	0-3

IMU, immature male urine; MMU, mature male urine


(a) Mature males
(b) Immature males
Frequency of entries
10
8
6
4
2
0
n = 16
n = 20
n = 30
n = 32
*
DW
WW
IMU
WW
MMU
WW