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Olfactory Discriminating Ability of Lacustrine Sockeye and Masu Salmon in Various Freshwaters

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ABSTRACT—In order to study the olfactory discriminating ability of lacustrine sockeye salmon (*Oncorhynchus nerka*) and masu salmon (*O. masou*), the integrated olfactory nerve response to various freshwaters was recorded by electrophysiological techniques. In both species independent of sex and gonadal maturity, each freshwater caused a different olfactory response. Source and effluent waters of the culture pond at Toya Lake Station (the source and culture pond waters) evoked the minimum and maximum response magnitude, respectively. In cross-adaptation experiments, the culture pond water abolished all secondary responses to other freshwaters, and no freshwater abolished the secondary response to the culture pond water. The concentration response study revealed that the minimum concentration (threshold) to induce response to the culture pond water after adaptation to Lake Toya water was between 0.1 and 1.0%. The present study indicates that the olfactory organ of lacustrine salmonids may discriminate different intensities of various freshwater odors.

INTRODUCTION

It is generally hypothesized that adult salmon primarily use olfactory cues during their homing migration when they migrate from the coastal area to the natal river. Since the olfactory homing hypothesis was introduced by Hasler and Wisby (1951), the importance of olfactory function to the salmonid homing migration was reported by many behavioral studies (Hasler and Scholz, 1983; Stabell, 1992). The electrophysiological studies also reported the relationship between the olfactory bulbar response (EEG) to natal stream water and the homing behavior of salmonids (Hara *et al.*, 1965; Ueda *et al.*, 1967, 1971; Satou, 1974; Kaji *et al.*, 1975; Ueda, 1985). These studies examined the magnitude of EEG or differences of frequency spectrum as the exclusive parameter. Although the maximum magnitude of EEG was used as the index of the response to the odor of natal stream water, these magnitude has been considered to be independent of the recognition of natal stream water (Hara, 1994).

Bodznick (1975, 1978a) recorded the EEG responses and the single olfactory bulb unit response in juvenile sockeye salmon (*Oncorhynchus nerka*) indicating that these responses showed the different properties to natural water and amino

acids. Nevitt *et al.* (1994) recorded the responses to phenyl ethyl alcohol (PEA) and amino acids from olfactory receptor neuron of coho salmon (*O. kisutch*) by means of patch-clamp technique and indicated that the olfactory receptor neurons isolated from PEA-exposed salmon showed higher sensitivity to PEA than non-exposed fish. However, few studies examined the response of primary olfactory neuron activity to various freshwaters.

In the present study, olfactory discriminating ability was examined in two lacustrine salmonids, sockeye salmon (*O. nerka*) and masu salmon (*O. masou*), whose habitat was freshwater throughout their life history. The integrated olfactory nerve response to various freshwaters was recorded as an index to the olfactory discrimination of various freshwater odors. Cross-adaptation experiments to two different kinds of freshwater as well as a concentration response analysis estimating minimum concentration (threshold) value were also carried out.

MATERIALS AND METHODS

Experimental animals

Lacustrine sockeye and masu salmon were used in this study. The sockeye salmon were obtained from two different sources; some fish were reared from eggs at Toya Lake Station for Environmental Biology, and some fish were caught in Lake Shikotsu during their homing migration. The latter fish were transferred to the station and maintained for one week in the outdoor culture pond supplied with a continuous flow of spring water. The gonadal maturity was estimated by gonadosomatic index (GSI: gonad weight/body weight × 100). They

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were divided into three groups; (1) mature fish from Lake Shikotsu (male: fork length (FL) 25.95 ± 2.03 cm, GSI 4.11 ± 1.36 , $n=7$; female: FL 26.17 ± 0.55 cm, GSI 13.32 ± 2.15 , $n=6$), (2) mature female from Toya Lake Station (FL 31.22 ± 4.72 cm, GSI 11.09 ± 4.87 , $n=4$), (3) immature female from Toya Lake Station (FL 25.22 ± 0.56 cm, GSI 0.54 ± 0.23 , $n=5$). Two mature male masu salmon (FL 22.75 ± 0.07 cm, GSI 6.14 ± 1.74) and 6 immature male masu salmon (FL 21.66 ± 1.37 cm, GSI 0.85 ± 0.32) reared at Toya Lake Station were also used. The mature fish were tested from September to November, 1995 and 1996, the immature fish were tested from April to June, 1996.

Olfactory nerve response recording

The integrated olfactory nerve response was recorded using the electrophysiological technique of Sveinsson and Hara (1990). The fish were immobilized with an intramuscular injection of gallamine triethiodide (Sigma, St. Louis, MO, USA; 3 mg/kg body weight) and locally anesthetized with lidocaine at the wound and head fixation points. The gills were perfused through the mouth with aerated solution containing tricaine-methane sulphate (MS 222, Sigma; 70 mg/l) which was never in contact with the olfactory rosette. Exposed parts of the fish were covered with wet tissue and kept moist throughout the experiment. The skin and a part of skull were removed to expose the olfactory bulbs and the proximal part of olfactory nerve. The olfactory nerve responses were recorded from twin tungsten electrodes inserted in the olfactory nerve. A grand electrode filled with 3 M KCl-agar (2%) bridged to an Ag-AgCl electrode was placed lightly on the dorsal skin. Electrodes were not removed throughout the experiments. The signal was amplified by AC preamplifier (MOD. DAM-5A, W-P Instruments, Sarasota, FL, USA) with a bandpass of 300–3 KHz and integrated by the hand-made electric integrator (time constant=0.3 s). Integrated olfactory nerve responses were recorded by a pen recorder.

Olfactory stimulants

The irrigating and stimulating solutions were applied to the olfactory epithelium through a stainless steel tube. Freshwaters used for stimulation were as follows: water from Lake Toya (lake water), source and effluent waters of the culture pond at Toya Lake Station (source and culture pond waters), and waters from River Poromoi and River Sobetsu (Poromoi and Sobetsu waters) that flow into Lake Toya. Effluent water from the Shikotsu Salmon Hatchery (hatchery water), which is the natal river for the sockeye salmon in Lake Shikotsu, was also applied. Each water was drew just before the experiments, the water temperature was left at room temperature, and was applied to the olfactory epithelium at a constant flow rate (11 ml/min). The magnitude of olfactory response was measured from the spontaneous level to the peak, and standardized to the response to 10^{-5} M L-serine dissolved in distilled water. This amino acid was chosen because it consistently induces a large response in fish olfactory organ.

Cross-adaptation experiment

First, the olfactory epithelium was exposed to distilled water (DW) for 3 min, then one freshwater was applied until the response was stabilized, then the other freshwater was applied, and finally DW was applied again.

Statistics

Data were expressed as mean \pm standard deviations or errors. Statistical analyses were calculated by two-way factorial analysis of variance (ANOVA) and Fisher's PLSD test.

RESULTS

Each freshwater produced a different magnitude of electrophysiological response that was recordable from the olfac-

tory nerve in the lacustrine sockeye salmon (Fig. 1A) and masu salmon (Fig. 1B). In sockeye salmon reared at Toya Lake Station, the culture pond water and the hatchery water evoked the maximum response magnitude, and the source water elicited the minimum response magnitude, independent of gonadal maturity. The response to the lake water was lower than that to the Poromoi water. In masu salmon, the culture pond water also evoked the maximum response magnitude, regardless of gonadal maturity. In sockeye salmon caught

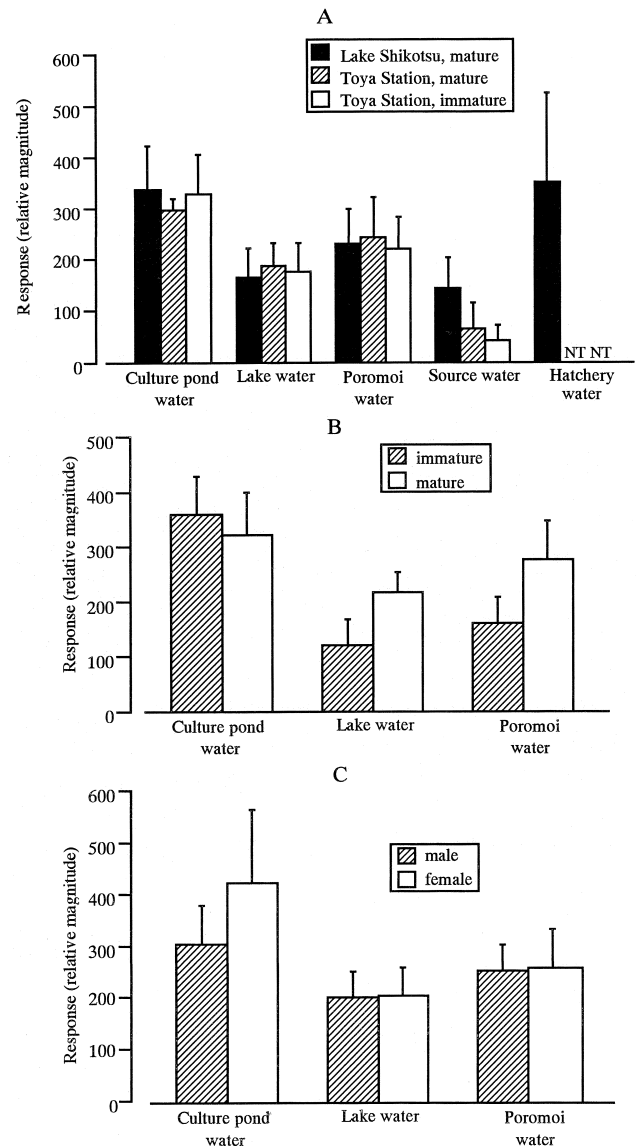


Fig. 1. Relative magnitude of integrated olfactory nerve responses to various freshwaters in sockeye salmon (A) and masu salmon (B). And comparison between male and female sockeye salmon caught in Lake Shikotsu (C). Magnitude of the response is expressed in % of response to 10^{-5} M L-serine dissolved in distilled water. In fish caught in Lake Shikotsu, there are significant differences between the culture pond water and other test waters ($n=13$, ANOVA: $p < 0.0001$; Fisher's PLSD test: $p < 0.05$), and no significant differences between male and female ($p > 0.05$, $n=13$). Vertical bars represent standard deviations. NT; not tested.

in Lake Shikotsu, significant differences were observed between two waters (the culture pond and hatchery) and other test waters ($n=13$, ANOVA: $p<0.0001$; Fisher's PLSD test: $p<0.05$), but no differences were detected between the culture pond water and the hatchery water. The magnitude of response to test waters was compared between male and female of sockeye salmon from Lake Shikotsu, and there were no significant differences among sex ($n=13$, Fisher's PLSD test: $p>0.05$, Fig. 1C).

Figure 2 shows the typical olfactory nerve response of mature female sockeye salmon (A) and mature male masu salmon (B) reared at Toya Lake Station to various freshwaters in the cross-adaptation experiment. No differences in typical olfactory nerve response to freshwater were observed between sockeye and masu salmon. In the first application, responses did not decrease to the spontaneous level within 2 min. The culture pond water abolished all secondary responses to other freshwaters in both sockeye and masu salmon, and no freshwaters abolished the secondary response to the culture pond water. The Poromoi water abolished the secondary

response to the lake and Sobetsu waters.

The magnitude of olfactory responses to the culture pond and Poromoi waters was compared after acclimation to the lake water in sockeye salmon (Fig. 3). The responses to the culture pond water were greater than those to the Poromoi water. Mature and immature fish reared at the station showed a lower response magnitude than mature fish caught in Lake Shikotsu ($n=16$, Two-way ANOVA: $p=0.028, 0.043$).

Concentration response curves for the olfactory nerve responses to the culture pond water diluted with the lake water were examined in three immature sockeye and masu salmon from Toya Lake Station (Fig. 4). First, the olfactory epithelium was exposed to the lake water for 5 min, then a concentration series of the culture pond water diluted with the lake water was applied and the secondary response was recorded. All tested sockeye salmon responded to 1% culture pond water, but in some fish there was no response to 0.1% culture pond water. Masu salmon did not respond to 0.1% culture pond water.

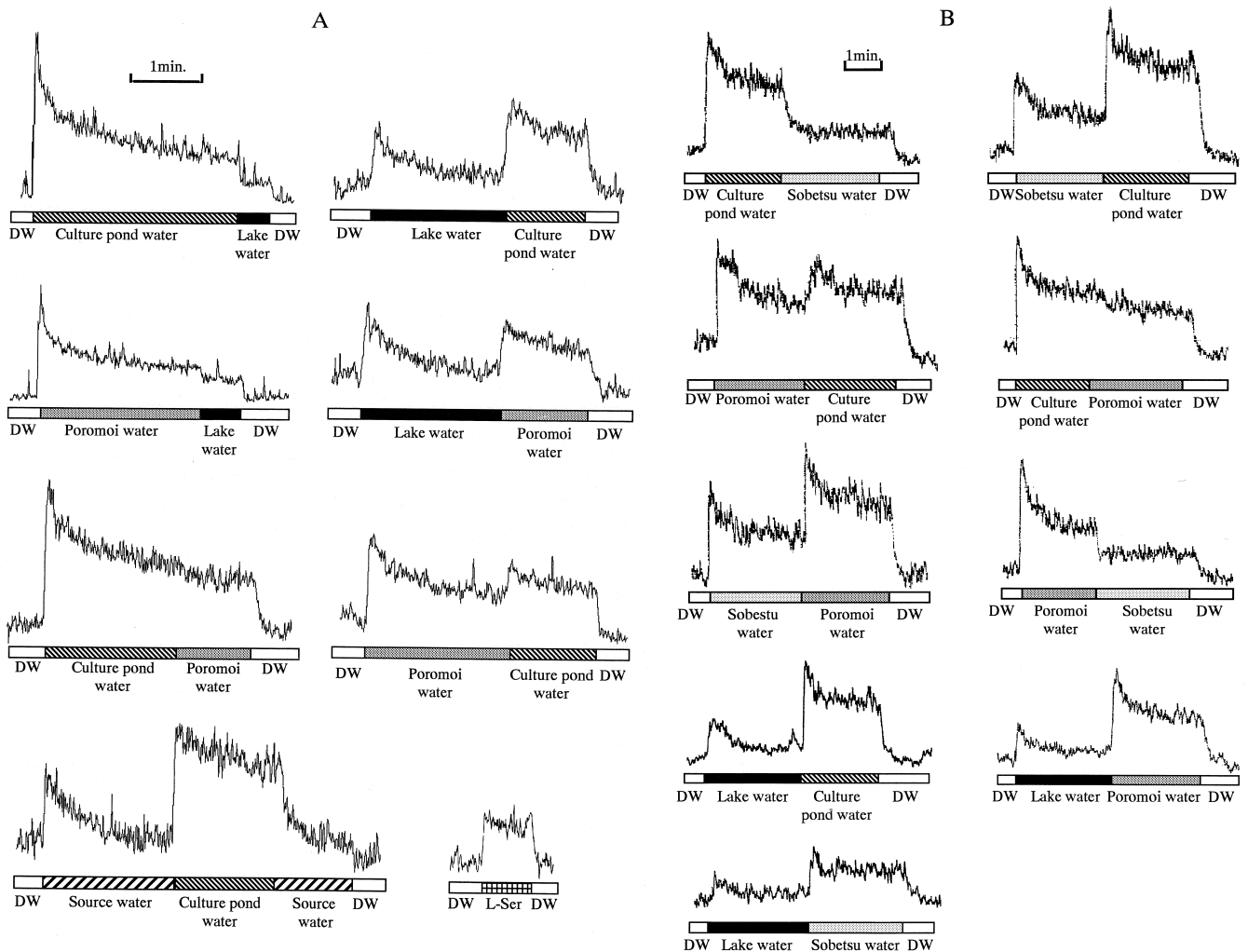


Fig. 2. Typical integrated olfactory nerve response in mature female sockeye salmon (A) and mature male masu salmon (B) reared at Toya Lake Station in the cross-acclimation experiments. The bar at the bottom of each record represents various freshwaters and the duration of stimulation.

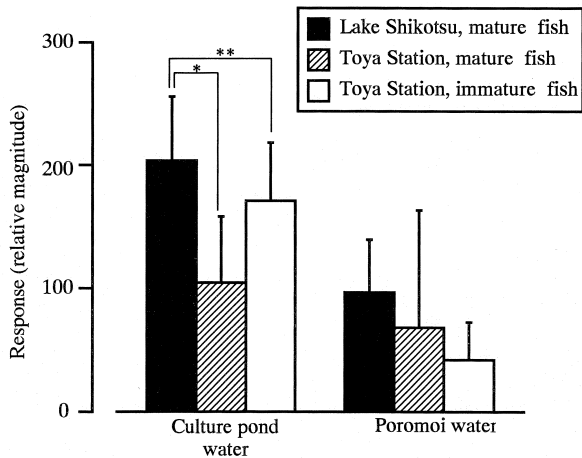


Fig. 3. Relative magnitude of integrated olfactory nerve responses to the culture pond and Poromoi waters after adaptation to the lake water in sockeye salmon. Magnitude of the response is expressed in % of response to 10^{-5} M L-serine dissolved in distilled water. Significant differences ($n=16$, Two-way Factorial ANOVA: $*p=0.028$, $**p=0.043$) are found between fish caught in Lake Shikotsu and fish reared at Toya Lake Station. Vertical bars represent standard deviations.

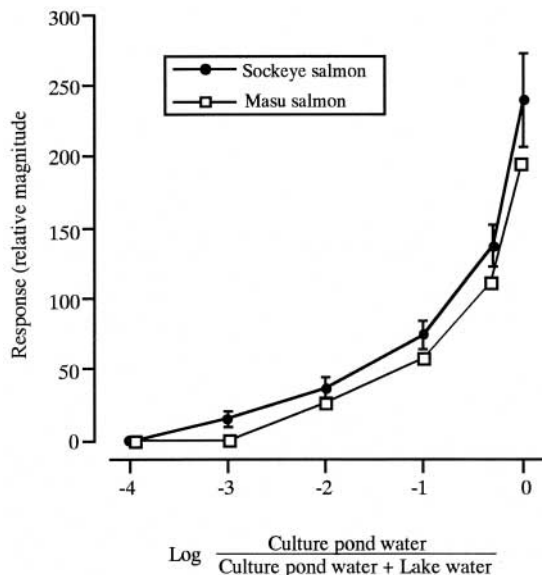


Fig. 4. Concentration response curves for the olfactory nerve responses in three immature female sockeye and immature male masu salmon reared at Toya Lake Station to the culture pond water diluted with the lake water demonstrating mean (symbols) and standard errors (vertical bars). Magnitude of the response is expressed in % of response to 10^{-5} M L-serine dissolved in distilled water.

DISCUSSIONS

The present study shows that the olfactory organs of lacustrine sockeye and masu salmon elicit different response properties to various freshwaters independent of sex and gonadal maturity. The relative magnitude of olfactory nerve responses in lacustrine salmonids suggests that each freshwater has different intensity of odors. Ueda *et al.* (1971) dem-

onstrated that sockeye salmon were able to discriminate odor differences using olfactory bulbar response (EEG) recording methods. It was not clear, however, what kinds of odorants were discriminated by the olfactory system. By behavioral analyses, Idler *et al.* (1961) reported that natal river odors were volatile, heat-labile, neutral and dialyzable, while other studies proposed that the non-volatile fraction was important (Fagerlund *et al.*, 1963; Bodznick, 1978b). Cooper *et al.* (1974) reported that the stimulatory portion of the natal river water was non-volatile by means of the recording EEG activities. Spectral analysis of EEG suggested that the home water stimulant(s) was absorbed on activated carbon and ion-exchange resin, insoluble in petroleum-ether, dialyzable, non-volatile, and heat-stable (Ueda, 1985). Chemicals which elicit the response from the olfactory receptor cells are amino acids, steroids, bile acids, and prostaglandins (Hara, 1994). The chemical composition of freshwater odorants that allow salmon to discriminate the natal river are now investigating in our laboratory, and our recent study suggests that amino acids are one of the important components for the determination of various freshwaters.

In chum salmon, lower-frequency components in EEG activity was important index for the recognition of the stream as their home stream (Ueda, 1985). Nevitt *et al.* (1994) reported that the olfactory receptor cell responses of PEA-exposed salmon were larger than those of non-exposed salmon suggesting that these cells have a high sensitivity to the natal river water odor. If olfactory receptor cells form the memory of the information related to the recognition of the home stream, the information of their natal river water should be reflected in the amplitude, in the frequency spectrum and/or in the patterns of the response to home stream in the olfactory nerve. The response to a great variety of the odorants contained in natural waters may set off the fewer specific odor response in the response magnitude. Further studies are needed to characterize the nervous areas where the memory of natal river water odors is imprinted.

In sockeye salmon, the culture pond water elicited the largest olfactory response among various freshwaters. The fish reared at Toya Lake Station, however, showed less sensitivity to the culture pond and Poromoi waters than the fish caught in Lake Shikotsu when they were acclimated to the lake water. It is possible that some environmental factors and/or genetic strain differences may affect the olfactory sensitivities. The olfactory systems of salmonids reared at Toya Lake Station may be adapted or acclimated to the culture pond water. Various odorants are thought to be released from fish, e.g. amino acids, steroids, and prostaglandins (Hara, 1994). Several studies have suggested that juvenile salmonids produce population-specific odors or pheromones which might be detectable by the adults during homing migration (Nørdeng, 1971, 1977; Groot *et al.*, 1986; Quinn and Tolson, 1986). It has also been demonstrated that sex steroids and prostaglandins have effects on the olfactory epithelium of salmonids by acting as sexual pheromones (Moore and Scott, 1992; Moore and Warning, 1996). These odorants may modify the

source water as to make the culture pond water more detectable to the olfactory system.

In both sockeye and masu salmon, the minimum concentration (threshold) to induce the olfactory nerve response to the culture pond water after adaptation to the lake water was between 0.1 and 1.0%. This threshold level suggests that the olfactory discriminating ability of salmonids during homing migration must have a function within a limiting distance from the natal river. It has been discussed that sensory organs other than the olfactory organ are used by salmonids when they are migrating far from the natal river. Recently, we proposed that visual cues are important for orientation during homing migration in lacustrine sockeye salmon (Ueda *et al.*, 1998).

In summary, the olfactory organs of lacustrine salmonids have an ability to discriminate different intensities of odors in various freshwaters. Of particular interest is the relation between this ability and the olfactory hypothesis on salmonid homing migration. The chemical characters of the natal river odorants which must be imprinted to the olfactory system of juvenile salmonids are presently unknown, and are now under investigation using the cross-adaptation experiments in the lacustrine salmonids.

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