Physiological Study on Imprinting and Homing Related Olfactory Functions in Salmon

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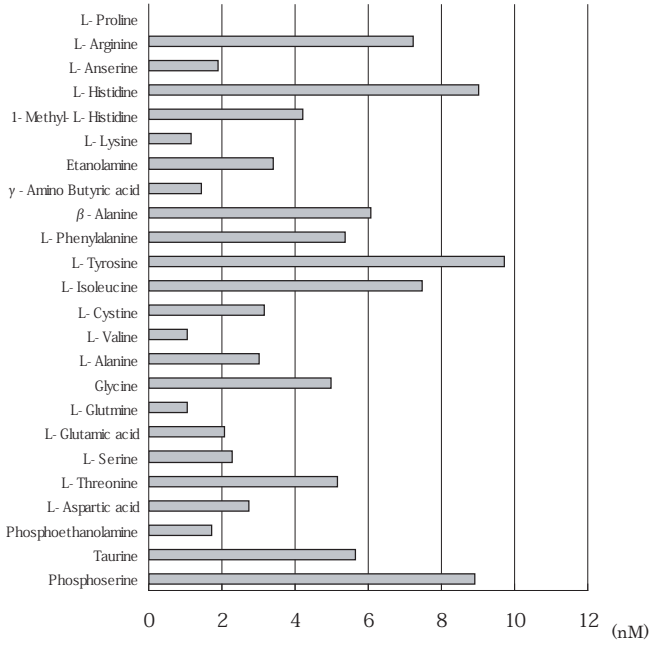
Keywords: Amino acids, imprinting, odorants, home stream, homing migration, salmon

As for the upstream homing migration of salmon from the coastal sea to the home stream, the olfactory hypothesis proposed by Hasler and Wisby (1951) has been discussed with many behavioral and electrophysiological studies (Hasler and Scholz 1983; Ueda 1985; Stabell 1992; Dittman et al. 1996; Ueda and Shoji 2002). However, these odor substances of home stream are still unknown. From our recent electrophysiological experiments, we proposed that amino acids dissolved in the home stream water were possible home stream odor substances for salmon (Shoji et al. 2000). Additionally, we carried out behavior experiments to test whether amino acid mixtures have attractive effects on male chum salmon (*Oncorhynchus keta*) and lacustrine sockeye salmon (*O. nerka*) of both sexes upstream selective movement or not by means of the two-choice test tank. Of 44 male chum salmon tested, 28 fish were found in one of the choice arms and 24 (86%) of these fish were in the arm running their artificial home stream water containing the amino acid mixture of their home stream (Shoji et al. 2003). In sockeye salmon, of 151 fish tested, 61 fish showed upstream movement to one of the choice arms and 47 (76%) of these fish were found in the arm running the artificial home stream water containing amino acid mixture of their home stream. From these results of electrophysiological experiments and behavior experiments, we proposed that amino acids dissolved in the home stream water were possible home river substances for salmon.

Juvenile salmon are considered to be imprinted by site-specific odors associated with their home stream and adult salmon utilize these "odor" memories for homing. This imprinting phenomenon was demonstrated on juvenile coho salmon (*O. kisutch*) by imprinting them with the artificial odorants β-phenylethyl alcohol (PEA) or morpholine (Cooper et al. 1976; Scholz et al. 1976; Dittman et al. 1996). Additionally, Nevitt et al. (1994) showed that olfactory receptor cells of coho salmon that had been imprinted with PEA have a higher sensitivity to PEA than that of non-imprinted fish. We have a test of whether lacustrine sockeye salmon can be imprinted by one amino acid that is absent in natural home stream water.

Fig.1. The concentration of amino acids and related substances in the Toya Lake Station river water. The line of the graph shows the concentration (nM) of each amino acid.



In the experiment, one-year-old lacustrine sockeye salmon cultured in the pond of the Toya Lake Station were used. The pond water flows into the lake through a small stream. Hence, this stream is the home stream for the salmon. We analyzed the compositions of amino acids in water from Toya Lake Station stream water (Shoji et al. 2000). Water from Toya Lake Station stream contains various species of amino acids, but does not contain L-Proline (Pro) (Fig. 1), thus we tested whether lacustrine sockeye salmon might be able to be imprinted by Pro.

Imprinting procedure was carried out according to Nevitt et al. (Nevitt et al. 1994). One-year-old sockeye salmon hatched and reared at the Toya Lake Station were used in the experiments from March to July. Fish were divided into experimental and control groups. Fish in the experimental group was imprinted for two weeks by adding Pro to the water intake at

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concentrations of 10^{-6} nM from March to July. Control fish were never exposed to Pro. Experimental (Pro-exposed) and control groups (Pro-naïve) were marked and transferred to a common outdoor fresh water rearing Toya Lake Station. To test whether salmon can be imprinted by Pro, electro-olfactogram (EOG) response to the experimental water was measured. We compared sensitivity to Pro of imprinted fish with non-imprinted control fish. EOG were obtained by using an experimental technique according to Evans and Hara (1985). Odor-evoked EOGs were recorded by using a pair of glass microelectrodes filled with 2% agar-saline and bridged to Ag-AgCl electrodes by 3-M KCl. The recording electrode was placed along the midline of the rosette at the base of the large, posteriormost lamella with the aid of a stereomicroscope mounted on a boom stand. A reference electrode was placed in the skin above the rosette. A separate ground was placed in the muscle near the tail. The differential signal was amplified (500×) and filtered (100-Hz low-pass) with an amplifier (MOD. 3000, A-M Systems, Inc, Carlsberg, WA, USA). The signal was then integrated by an electrical integrator.

From March to June, the responses of Pro imprinted fish to the experimental water were greater than that of non-imprinted fish. In July, however, the responses to experimental water is smaller than other months. There is no significant difference in the average values of relative magnitudes of responses to experimental water in the Pro imprinted groups. There is no significant difference in the average values of relative magnitudes of responses to experimental water in the Pro imprinted groups. These results suggest that sockeye salmon may be imprinted by one amino acid (Pro), and may be imprinted not only during parr-smolt transformation but also either before or after parr-smolt transformation.

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