Chem. Senses 25: 533–540, 2000

Amino Acids Dissolved in Stream Water as Possible Home Stream Odorants for Masu Salmon

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Abstract

It is well established that salmon return to their home stream by sensing the odors of the stream water. In this study we have attempted to identify the home stream odorants used by masu salmon in Lake Toya. The salmon in Lake Toya return to the home stream which flows into the lake after lake life for 2–3 years. Besides water from the home stream, waters from two other streams which flow into Lake Toya were also used in the experiments. We analyzed the compositions of amino acids, inorganic cations and bile acids in waters from the three streams. Application of mixtures of inorganic cations or bile acids, reconstituted based on the compositions of the stream waters, to the olfactory epithelium induced only very small responses. On the other hand, application of mixtures of amino acids induced large responses. The response to artificial stream water reconstituted based on the compositions of amino acids and salts closely resembles that to the corresponding stream water. Cross-adaptation experiments with three combinations of the mixtures were carried out. The response pattern for each combination closely resembled that to the corresponding combination of stream waters. Based on the results obtained, we concluded that amino acids dissolved in the home stream water are possible home stream odorants.

Introduction

Salmon have an amazing ability to migrate thousands of kilometers from the ocean to the home stream to spawn after ocean life for a number of years. In a previous paper, we showed that salmon use visual cues for long-range navigation (Ueda *et al.*, 1988). For navigation in local waters, Hasler and Wisby proposed the olfactory hypothesis (Hasler and Wisby, 1951; Wisby and Hasler, 1954), i.e. salmon memorize the odors of water of the home stream where they spent their river life and return to the home stream by sensing the odors. Many field studies carried out under various conditions have supported the olfactory hypothesis (Cooper and Hirsch, 1982; Hasler and Scholz, 1983; Stabell, 1984, 1992). Behavioral and electrophysiological studies using coho salmon showed that imprinting with a synthetic odorant such as phenyl ethyl alcohol could also be done (Nevitt *et al.*, 1994; Dittman *et al.*, 1996).

It was shown that application of home stream water to the olfactory epithelium of homing salmon induced a large olfactory bulbar response (Hara *et al.*, 1965; Ueda *et al.*, 1967; Hara, 1970). Later, it was shown that not only the home stream water, but also waters from other streams induced olfactory bulbar responses in salmon (Ohshima *et al.*, 1969; Ueda *et al.*, 1971; Dizon *et al.*, 1973; Bodznick, 1975). Ueda *et al.* (Ueda *et al.*, 1971) reported that temporal changes in each component spectral pattern within the olfactory bulbar response of himé salmon were characteristic of odors of different stream waters and the salmon could discriminate odors of different streams by the spectral pattern. Attempts to identify the home stream odorants were made based on the spectral pattern of the olfactory bulbar response. The odorants were insoluble in petroleum ether, dialyzable, non-volatile and heat stable (Ueda, 1985). Cooper *et al.* (Cooper *et al.*, 1974) also reported that the home stream odorants were non-volatile.

Unlike olfactory organs of terrestrial animals, fish olfactory organs respond only to limited species of chemicals. In general, amino acids are potent odorants for fish. The salmon olfactory organ responds sensitively to various species of amino acids. For example, rainbow trout respond to 10^{-8} M L-serine and 10^{-7} M L-alanine (Caprio, 1982, 1988;

Hara, 1982, 1992). Since the chemical properties of amino acids fit those of the home steam odorants described above, there is the possibility that the odorants are amino acids. In the present study, we attempted to test the possibility that the home stream odorants are amino acids.

Usually masu salmon stay in their home river for 1 year and a few months after hatching and then go down to the sea. After living in the sea for 1 year, the fish return to their natal river, where they stay for a few months before finally reach the spawning area. Masu salmon in Lake Toya offer a good system for studying homing salmon, i.e. the salmon return to the home stream which flows into the lake after lake life for 2–3 years.

In the present study, we analyzed the compositions of amino acids, inorganic cations and bile acids in waters from three streams which flow into Lake Toya. Application of mixtures of inorganic cations or of bile acids to the salmon olfactory epitehlium induced very small responses. We compared the olfactory responses to mixtures of amino acids reconstituted based on the amino acid compositions in stream waters with those to stream waters. The results showed that the olfactory response to the reconstituted water closely resembled the response to the corresponding stream water, indicating that amino acids are possible home stream odorants. It seems that salmon return to the home stream by sensing differences in compositions of amino acids dissolved in stream waters.

Materials and methods

Fish

Electrophysiological experiments were carried out using 2- or 3-year-old masu salmon (*Oncorhynchus masou*, 18–25 cm) reared in the culture pond of the Toya Lake Station for Environmental Biology. The culture pond is supplied with well water and the water flowing out from this pond goes to Lake Toya though a small stream. Hence, this stream is the home stream for the masu salmon.

Collection of stream waters and lake water

Stream waters were collected from the Toya Lake Station stream (Station), the Poromoi stream (Poromoi) and the Soubetsu stream (Soubetsu) at the beginning of October. The sites for collection were the center of the flow near the stream mouth. The lake water was collected at a site 100 m offshore from the Toya Lake Station. The waters collected were rapidly filtered with a membrane filter (pore size 0.45 µm) and used for electrophysiological experiments and chemical analysis. Waters stored in a deep freeze (–80°C) were also used for the experiments.

Chemical analysis

Water samples concentrated by rotary evaporation were used for analysis of amino acids and inorganic cations. For analysis of bile acids, the waters were subjected to extraction with solid phase extraction cartridges (Waters Sep-Pak tC_{18}; Environmental Cartridges). Analyses of amino acids, inorganic cations and bile acids were carried out with an amino acid analyzer (Hitachi L-8500), an ICP emission spectral analyzer (Hitachi P-5200) and a bile acid analysis system (JASCO LCSS-905), respectively. Bilepak-II and Enzymepak-HSD (Jasco) were used for separation and detection of bile acids, respectively.

Electrophysiological recordings

Olfactory responses were recorded from the olfactory nerves according to Sveinsson and Hara (Sveinsson and Hara, 1990). Fish were used without distinguishing female and male. In brief, fish were immobilized with galamine triethiodide (3 mg/kg body wt) and locally anesthetized with lidocaine at the wound and head fixation points. The gills were perfused with aerated cold water containing MS-222 (70 mg/l) during the experiment. Artificial freshwater (AFW) was constantly flowed over the olfactory epithelium during the period of stimulation. Olfactory nerve responses were recorded using twin tungsten electrodes inserted in the olfactory nerve and were integrated by an electrical integrator (time constant 0.3 s).

Chemicals and solutions

AFW to perfuse the olfactory epithelium contained 0.5 mM NaCl, 0.05 mM KCl, 0.4 mM $CaCl_2$ and 0.2 mM $NaHCO_3$ (pH 6.9). The temperature of all stimulating and adapting solutions was kept at 10–13°C. The amino acids, amino acid-related substances, bile acids and inorganic salts used to prepare the artificial stream waters were purchased from Wako Pure Chemical Industries (Tokyo), Sigma Chemical Co. (St Louis) and Nacalai Tesque (Kyoto).

Results

In the experiments, 2- or 3-year-old masu 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. Besides the home stream water, waters from the Poromoi stream and the Soubetsu stream, which flow into Lake Toya, were used for the experiments. In the Poromoi and Soubetsu streams masu salmon breed naturally. In the middle of September to early October, mature masu salmon return to these streams from the lake.

Figure 1 shows olfactory nerve responses to waters from the three streams. The magnitudes of the responses to waters from these streams were comparable to that to 0.1 mM L-serine used as a control. Under natural conditions the olfactory epithelium of the fish is adapted to the water of Lake Toya and fish recognize water from the stream in the vicinity of the mouth of the stream. Hence, in the experiments for Figure 2, the epithelium was adapted to water from Lake Toya before application of waters from the

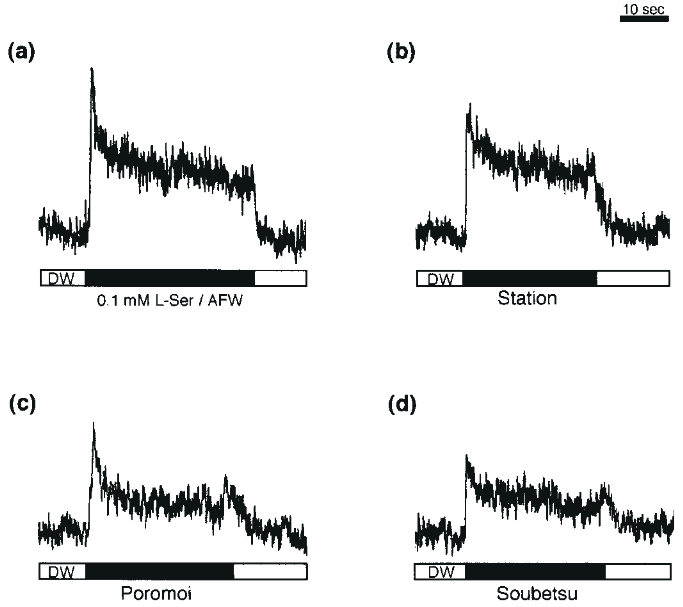

Figure 1 Typical integrated olfactory nerve responses of masu salmon to 0.1 mM L-serine (L-Ser) dissolved in AFW **(a)**, water flowing from the culture pond at the Toya Lake Station (Station) **(b)**, the Poromoi stream water (Poromoi) **(c)** and the Soubetsu stream water (Soubetsu) **(d)**. These responses were obtained from the same fish. The bar at the bottom of each record represents the duration of stimulation with the solution indicated. The stimulating solutions were applied after the responses to the adapting solution (deionized water, DW) reached a spontaneous level.

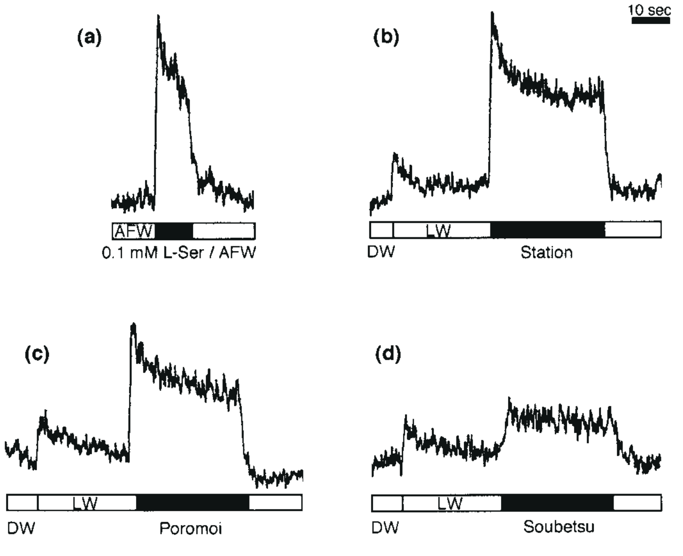

Figure 2 Typical integrated olfactory nerve responses to stream waters after the olfactory epithelium was adapted to the lake water (b–d). **(a)** The response to 0.1 mM L-serine dissolved in AFW was recorded as a control response. The Toya Lake Station stream water (Station) **(b)**, the Poromoi stream water **(c)** and the Soubetsu stream water **(d)** were applied after the response to the lake water was adapted to a spontaneous level. All the responses were obtained from the same fish.

streams. As seen from the figure, waters from the streams induced large responses under these condition.

To test the possibility that the home stream odorants are amino acids, we analyzed the amino acid compositions of waters from the streams. The compositions of amino acids varied greatly among the streams (Figure 3).

We also analyzed the compositions of inorganic cations in waters from the streams (Table 1). The order of magnitude of cation contents was Ca > Na > Mg > K for each stream. Figure 4 shows the olfactory responses to mixtures of salts reconstituted based on the salt compositions of the streams. The magnitudes of the responses to the salt solutions were much less than that to 0.1 mM L-serine.

Figure 5 shows the olfactory responses to artificial stream waters reconstituted based on the compositions of amino acids and salts shown in Figure 3 and Table 1. Each response closely resembles the response to the corresponding stream water shown in Figure 2.

We also analyzed the compositions of bile acids, which are considered to be pheromones for certain salmon, in waters from the streams (Table 2). Water from the Poromoi stream contains various species of bile acids. On the other hand, waters from the Soubetsu stream and the Toya Lake Station stream contain only one and two bile acids, respectively. Application of mixtures of bile acids and salts reconstituted based on the data shown in Tables 1 and 2 induced only small olfactory responses (Figure 6). The experiments for Figures 4–6 were repeated with at least four fish. Figure 7 shows the average values of relative magnitudes of responses to reconstituted mixtures of salts, amino acids + salts and bile acids + salts based on the compositions in the three stream waters. Here the magnitude of the response to each natural stream water is taken as unity. The magnitude of the response to artificial stream water containing amino acids and salts is close to that to the corresponding natural stream water. Salts themselves contribute only partly to the response. Comparison of the response to salts with the response to salts + bile acids indicates that bile acids essentially do not contribute to the response.

We carried out cross-adaptation experiments among olfactory responses to waters from different streams. Figure 8 shows the results of the experiments with three combinations of the streams. On left of the figure, the order of application of the stream water was reversed. Each combination gave a characteristic response pattern. Cross-adaptation experiments were also carried out with artificial stream waters, which were prepared based on the compositions of amino acids and salts. As shown in Figure 9, the response patterns to the artificial stream waters closely resembled those to the corresponding stream waters shown in Figure 8.

Discussion

Application of waters from three streams to the olfactory epithelium of masu salmon in Lake Toya induced large olfactory responses. Similar olfactory responses were obtained by application of stream waters reconstituted

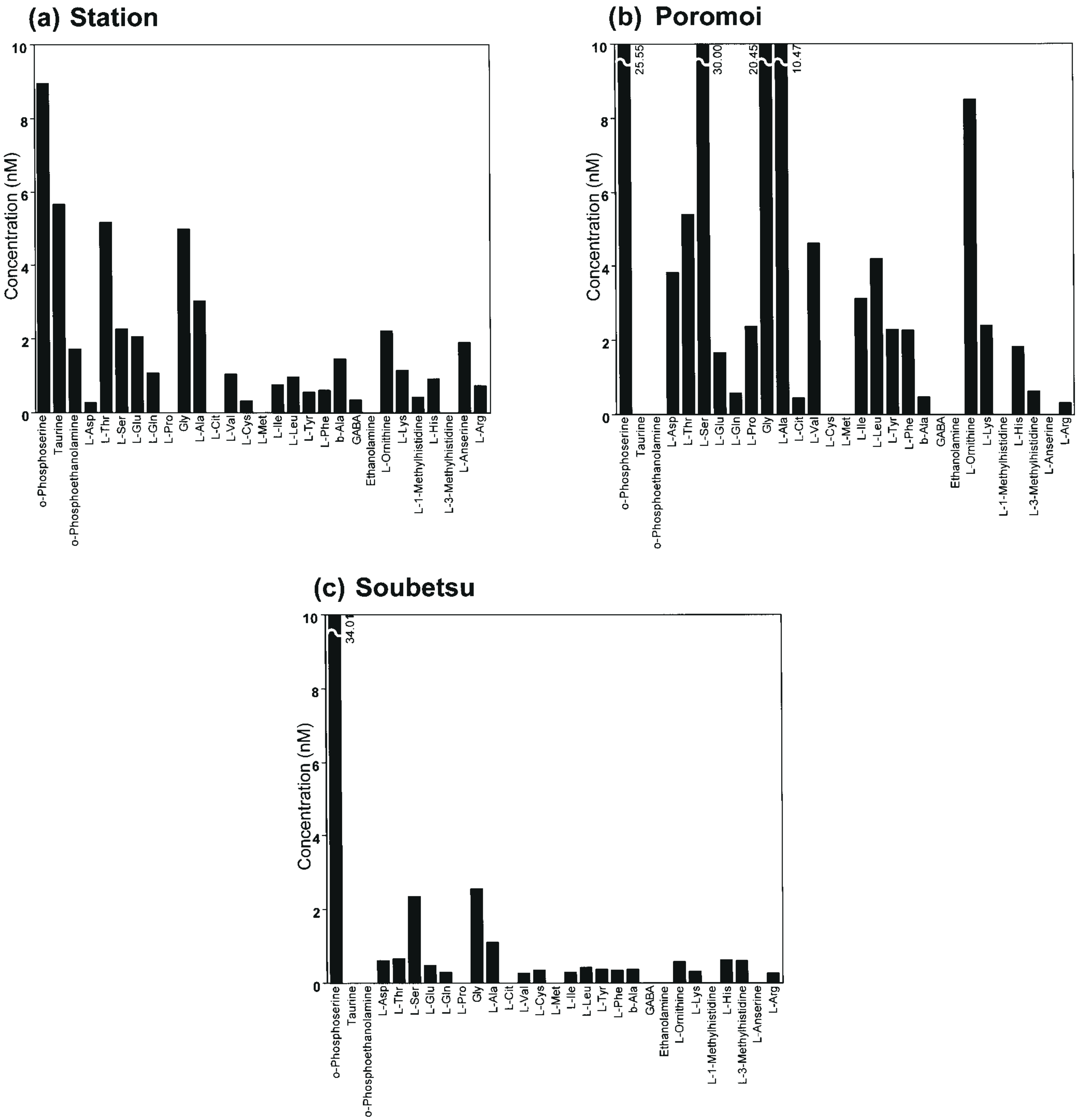

Figure 3 The concentrations of amino acids and related substances in stream waters. The vertical line of the graph shows the concentration (nM) of each amino acid.

based on the compositions of amino acids determined. The cross-adaptation experiments with three combinations of the stream waters were carried out and it was found that each combination gave a characteristic response profile. Cross-adaptation experiments were also carried out with reconstituted stream waters, which were prepared based on the compositions of amino acids and salts. The response

Table 1 Concentrations of main inorganic cations (mg/l)

	Na	K	Ca	Mg
Station	8.31	0.41	17.37	5.19
Poromoi	8.22	0.87	15.36	4.09
Soubetsu	6.63	0.34	8.86	2.26

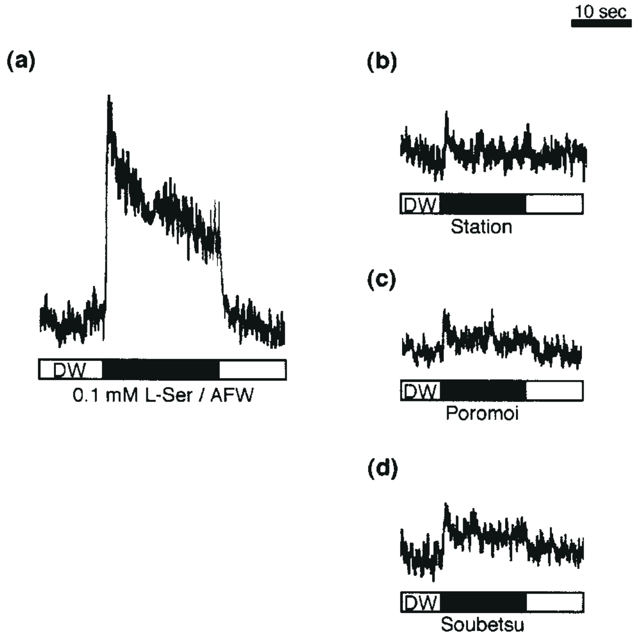

Figure 4 Typical integrated olfactory nerve responses to mixtures of inorganic salts reconstituted based on the compositions shown in Table 1 **(b–d)**. The chlorides were used as inorganic salts. **(a)**. The response to 0.1 mM L-serine dissolved in AFW was recorded as a control response. All the responses were obtained from the same fish.

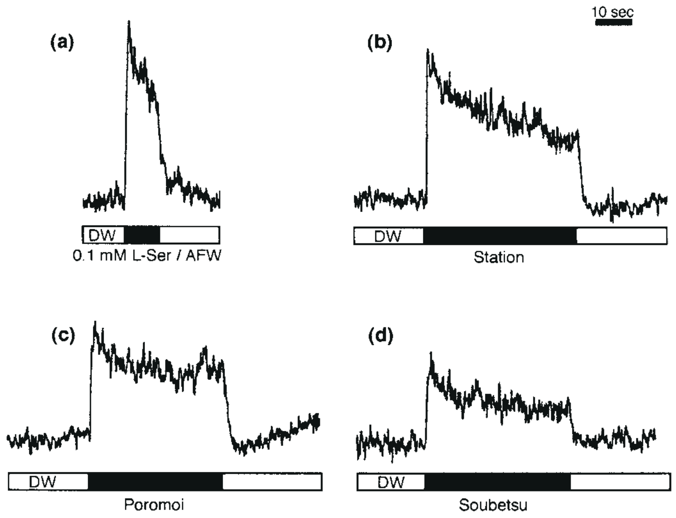

Figure 5 Typical integrated olfactory nerve responses to artificial stream waters reconstituted with amino acids and salts based on the compositions shown in Figure 3 and Table 1 **(b–d)**. The pH values of the artificial stream waters were adjusted to 7.0. It was confirmed that the pH values of the natural stream waters tested were near neutrality. **(a)** The response to 0.1 mM L-serine dissolved in AFW was recorded as a control response. All the responses were obtained from the same fish.

Table 2 Concentrations of the bile acids (pM)

	Station	Poromoi	Soubetsu
Glycoursodeoxycholic acid	–	240	–
Tauroursodeoxycholic acid	–	3570	–
Ursodeoxycholic acid	–	–	–
Glycocholic acid	–	184	–
Taurocholic acid	3580	429	296
Cholic acid	–	–	–
Glycochenodeoxycholic acid	–	257	–
Taurochenodeoxycholic acid	958	247	–
Glycodeoxycholic acid	–	178	–
Taurodeoxycholic acid	–	–	–
Chenodeoxycholic acid	–	–	–
Deoxycholic acid	–	–	–
Glycolithocholic acid	–	–	–
Taurolithocholic acid	–	–	–
Lithochenocholic acid	–	–	–

The concentrations of 15 bile acids, the main bile acids secreted by organisms into stream waters. – indicates that the concentration is below the detection limit.

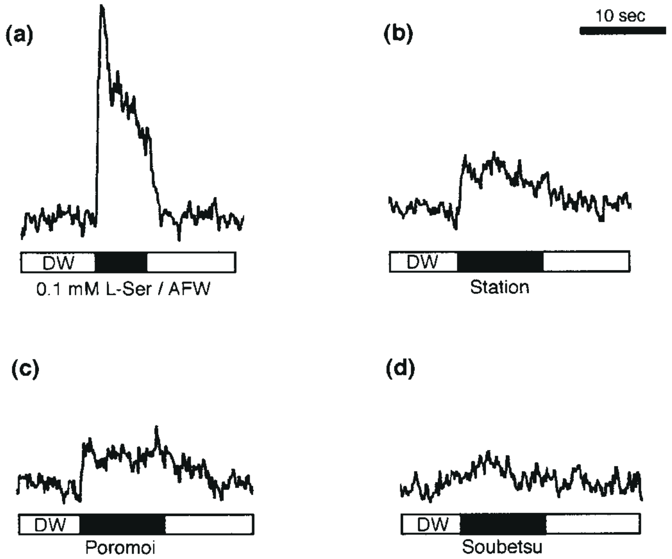

Figure 6 Typical integrated olfactory nerve responses to mixtures of bile acids and inorganic salts based on the compositions shown in Figure 3 and Table 1 **(b–d)**. The pH values of the mixtures were adjusted to 7.0. **(a)** The response to 0.1 mM L-serine dissolved in AFW was recorded as a control response. All the responses were obtained from the same fish.

profiles for the reconstituted stream waters closely resembled those of the corresponding stream waters. Salts themselves contribute only partly to the responses to waters and, hence, amino acids mainly contribute to the olfactory responses to stream waters. The chemical properties of amino acids fit those of the home stream odorants suggested by previous electrophysiological studies (Ueda *et al.*, 1971; Kaji *et al.*, 1975; Ueda, 1985).

Not only water from the home stream but also waters from other streams induced large olfactory responses in masu salmon. Figures 1 and 2 seem to indicate that the magnitude of the olfactory response to water from the home

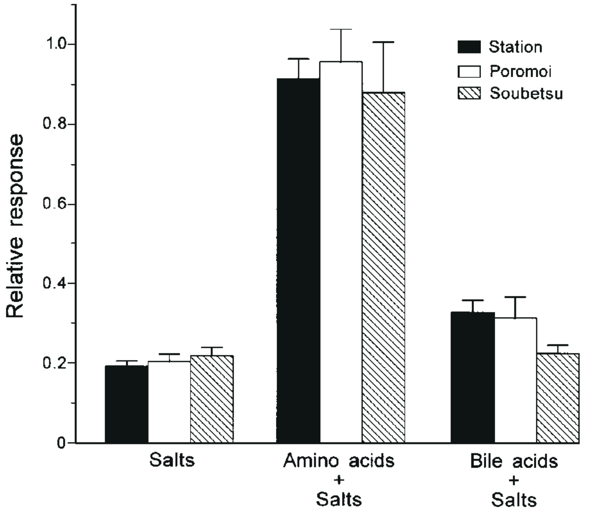

Figure 7 Average values of relative magnitudes of responses to reconstituted mixtures of salts, amino acids + salts and bile acids + salts based on compositions in the three stream waters. The values are means ± SE of data obtained from at least four fish. In the figure, the magnitude of the response to each natural stream water is taken as unity. The peak height of the response is taken as magnitude of the response.

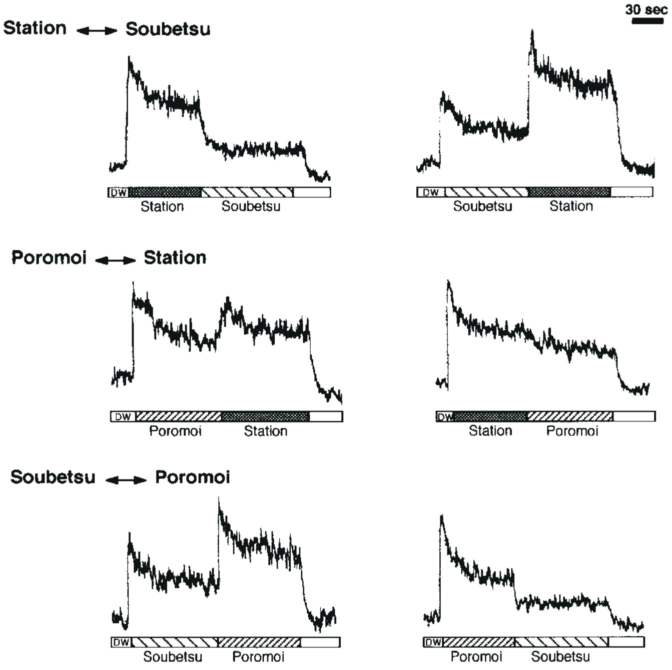

Figure 8 Typical integrated olfactory responses to natural stream waters in cross-adaptation experiments between the Toya Lake Station stream, Poromoi stream and Soubetsu stream. The records shown on the right are those obtained when the order of application of waters was reversed. DW was used as the adapting solution.

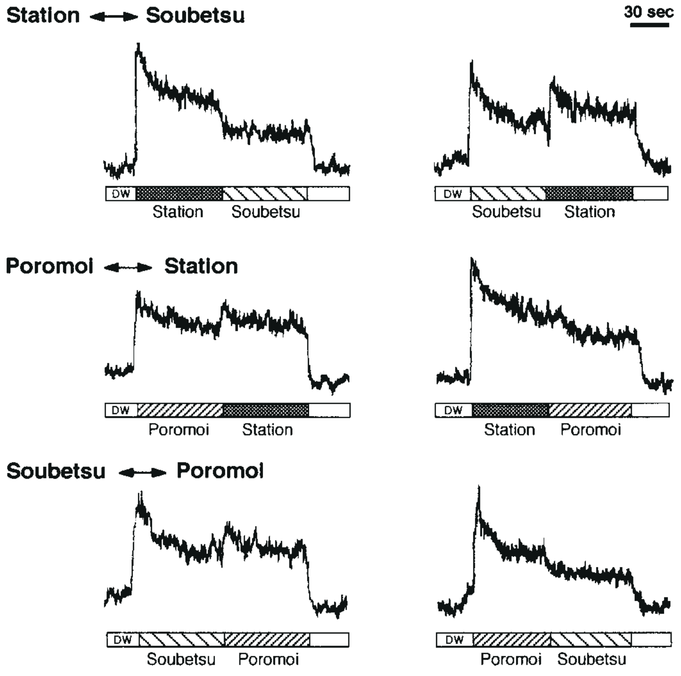

Figure 9 Typical integrated olfactory responses to artificial stream waters in cross-adaptation experiments. Each artificial stream water contains amino acids and salts at the concentrations shown in Figure 3 and Table 1. The records shown on the right are those obtained when the order of application of waters was reversed. DW was used as the adapting solution.

stream (the Toya Lake Station stream) is higher than that to other stream waters. However, this seems to be an artifact. This notion is supported by the following results: olfactory responses of lacustrine sockeye salmon captured in Lake Shikotsu to waters from three streams were quite similar to those of the salmon captured in Lake Toya (Sato *et al.*, 2000).

Hence, masu salmon do not specifically sense a strong odor from the home stream water. It seems that the olfactory system of masu salmon discriminates the quality of odors of various stream waters. Ueda *et al.* reported that temporal changes in the frequency components within the olfactory bulbar response were characteristic of the stimuli with different stream waters (Ueda *et al.*, 1971; Ueda, 1985).

The present results show that the amino acid compositions vary greatly among different stream waters. Since the olfactory responses of masu salmon to stream waters are induced mainly by amino acids dissolved in the water, salmon discriminate stream waters by sensing differences in amino acid compositions.

It seems that amino acids in stream waters derive from living organisms, including plants. Probably the species of organisms in and near the streams vary among streams and hence the amino acids compositions vary greatly among streams. Amino acids in the mucus on the fish body surface may partly contribute to the formation of the home stream odors (Hara *et al.*, 1984), but large variations in the amino acid compositions among streams cannot be explained only in terms of the mucus amino acids. It seems that most of the amino acids in stream waters come from other organisms.

It is known that the characteristic tastes of foods are determined by the compositions of amino acids contained in the foods; the characteristic tastes of many foods, such as fish, crab, scallop and sea urchin, are due to different combinations of amino acids (Fuke and Konosu, 1991). We can easily discriminate differences in their tastes. Hence, it is likely that salmon discriminate differences in amino acid compositions.

Bodznick (Bodznick, 1978) reported that calcium ions in the home stream water play an important role in olfactory discrimination by sockeye salmon. The present results, however, show that inorganic cations, including calcium ions, contribute only partly to the salmon olfactory response and are not a major component of the home stream odorants.

It was proposed that salmon such as Arctic char and Atlantic salmon return to the stream by sensing pheromones released from young salmon living in the stream. Since the olfactory organ of salmon responds sensitively to bile acids, bile acids may play a role as pheromones in homing migration (Nordeng, 1971; Døving *et al.*, 1974, 1980). When masu salmon return to the home stream, both young and resident form masu salmon are usually living in the stream. Hence, we analyzed bile acids in the streams which flow into Lake Toya. Application of mixtures of bile acids, reconstituted based on their compositions in the streams, to the salmon olfactory epithelium elicited a small response. The following result also does not support the pheromone hypothesis: waters from the Chitose river where abundant chum salmon (*Oncorhynchus keta*) run did not contain detectable bile acids (T. Shoji *et al.*, unpublished data). Hence, in masu salmon and chum salmon, bile acids do not contribute to the olfactory response to stream waters. Li *et al.* (Li *et al.*, 1995) reported that the adult sea lamprey olfactory organ could discriminate between unique bile acids released by conspecific larvae. It may be likely that bile acids play a role in recognizing fish of the same strain and formation of a group.

Acknowledgements

We thank Japan Spectroscopic Co. Ltd and the Center for Instrumental Analysis, Hokkaido University for their technical assistance. This work was supported by a grant from The Japan Science Society and Grants-in-aid for Science Research on Priority Areas (A) from the Ministry of Education, Sciences, Sports and Culture, Japan.

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Accepted March 10, 2000