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The behavioural homing response of adult chum salmon *Oncorhynchus keta* to amino-acid profiles

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Adult chum salmon *Oncorhynchus keta* homing behaviour in a two-choice test tank (Y-maze) was monitored using a passive integrated transponder (PIT)-tag system in response to river-specific dissolved free amino-acid (DFAA) profiles and revealed that the majority of *O. keta* showed a preference for artificial natal-stream water and tended to stay in this maze arm for a longer period; natal-stream water was chosen over a nearby tributary's water, but not when the *O. keta* were presented with a non-tributary water. The results demonstrate the ability of *O. keta* to discriminate artificial stream waters containing natural levels of DFAA.

Key words: artificial stream waters; dissolved free amino acids; homing behaviour; PIT tag; Y-maze.

INTRODUCTION

The ability of Pacific salmonids to successfully return to their natal stream following outmigration to and rearing in the ocean is a critical component of the success of the group (Groot & Margolis, 1991; Dittman *et al.*, 1996). Homing rates for adult salmonids to their natal streams may be as high as 90% (Quinn, 1997). While homing at sea may require geomagnetic navigation and possibly the use of lunar cues (Hasegawa, 2012; Putman *et al.*, 2013), homing within catchments is attributed to olfactory mechanisms that depend on juvenile imprinting to natal stream-specific odours (Hasler & Scholz, 1983; Nevitt *et al.*, 1994; Dittman & Quinn, 1996; Ueda, 2011). As returning adults, salmonids are thought to recall an olfactory memory and discriminate differences in stream odours to locate their respective natal streams (Cooper *et al.*, 1976; Hasler & Scholz, 1983; Dittman & Quinn, 1996).

Natal stream odours in the form of dissolved free amino acids (DFAA) may be a key distinguishing factor in natal-stream recognition (Shoji *et al.*, 2000, 2003; Yamamoto *et al.*, 2010). Yamamoto *et al.* (2010) successfully imprinted juvenile lacustrine sockeye salmon *Oncorhynchus nerka* (Walbaum 1792) from Lake Toya in Hokkaido,

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Japan, with a 14 day exposure to the amino acid L-proline. Two years after the imprinting process, the matured *O. nerka* were given a behavioural choice between proline water and natural lake water and a significant majority of the *O. nerka* chose the proline water, suggesting that the *O. nerka* were able to set and later retrieve and use olfactory memory of the amino acid as an olfactory guidance cue. It is unlikely, however, that a single amino acid would provide sufficient resolution for homing and it is likely that a more comprehensive DFAA profile, comprising multiple amino acids, is used. For DFAA imprinting and recall to be effective as a guidance mechanism, natal-stream DFAA must exhibit seasonal and annual stability or predictability since salmonids may take up to 6 years to return to their natal stream for spawning (Hamner *et al.*, 2004). In this study, DFAA from the Chitose River (natal stream) and Toyohira River (off-path tributary stream, non-natal stream) were characterized and the concentrations used to make artificial stream waters (ASW) used in a two-choice test tank (Y-maze; Scholz *et al.*, 1976) to test the behavioural homing responses of adult male chum salmon *Oncorhynchus keta* (Walbaum 1792) from the Chitose River to DFAA profiles using a passive integrated transponder (PIT)-tag system to monitor their selective movement accurately and on a fine scale.

MATERIALS AND METHODS

DFAA COMPOSITION ANALYSES

To determine the DFAA content of water to be used in the choice experiments, water samples were collected from the second bridge of Chitose River, the Nango Dori Bridge of Toyohira River and spring water of the Sapporo Salmon Museum (www.sapporo-park.or.jp; Fig. 1). All water samples were collected from the middle of each bridge on the main channel to ensure well mixed samples from September to November 2014 (Hemond & Fechner-Levy, 2000). DFAA composition was determined using an ultra-high performance liquid chromatography (UHPLC) system (LaChrom Elite, Hitachi High-Tech Corporation; www.hitachi-hightech.com/global) capable of fluorescently detecting 19 amino acids (alanine: Ala; arginine: Arg; asparagine: Asn; aspartic acid: Asp; cysteine: Cys2; glutamine: Gln; glutamic acid: Glu; glycine: Gly; histidine: His; isoleucine: Ile; leucine: Leu; lysine: Lys; methionine: Met; phenylalanine: Phe; proline: Pro; serine: Ser; threonine: Thr; tyrosine; Tyr; valine: Val). The amino-acid peaks were separated by a Zorbax column (Agilent; www.chem-agilent.com) at 37° C in a column oven (Elite LaChrom L-2300; Hitachi High-Tech Corporation). The resulting chromatogram was analysed with a compatible software system (EZChrom Elite 3·1.8 dJ; www.gls.co.jp/glsoft/vs_elite). DFAA total quantities were obtained for each sample from the UHPLC analysis and molar percentages were calculated. There were some fluctuations of Chitose River DFAA profiles between September to October and November. Similar fluctuations were also observed in the Teshio River (Yamamoto *et al.*, 2013).

Y-MAZE BEHAVIOUR EXPERIMENTS

A fibreglass Y-maze test tank (CRP Hokkaido; www.mapion.co.jp) was constructed at the Sapporo Salmon Museum in Sapporo, Japan. The tank consisted of a holding arena (3·0 m × 0·6 m × 3·0 m) connected to the base of two parallel arms (each 9·0 m × 0·6 m × 0·6 m) elevated at a 15° angle to simulate downstream water flow into the holding arena. Each arm was fed with 20 l min^{-1} filtered well water that contained insignificant amounts of DFAA according to UHPLC analysis. A 20 l polypropylene bottle was used to drip reconstituted DFAA water into the head end of each arm as appropriate to the test conditions. The holding arena and arms were covered with opaque fibreglass sheets to limit external visual interference. To detect *O. nerka* entry frequencies and timings, each arm was fitted with PIT tag radio frequency

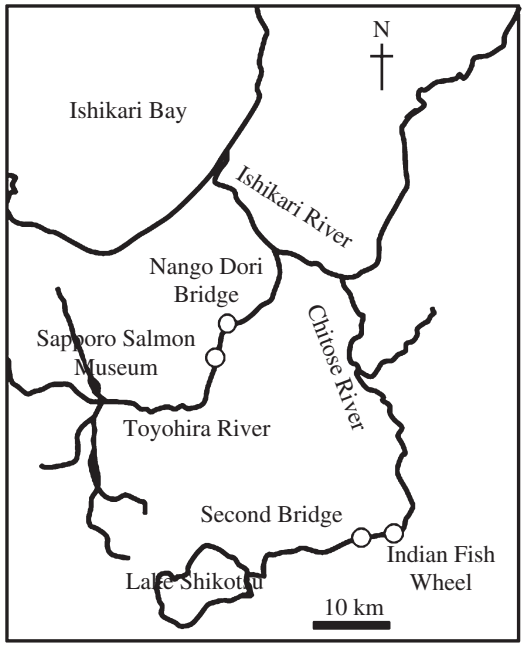

FIG. 1. Map showing the water sampling sites (Nango Dori and Second bridges, Sapporo Salmon Museum) and the site of *Oncorhynchus keta* collection (Indian Fish Wheel) in Ishikari River catchment, Hokkaido, Japan.

identification (RFID) antennas (Biomark; www.biomark.com) at 2 and 4 m from the holding arena. Antennas were connected to a PIT-tag reader (RM-310, Biomark), which was configured to continuously detect tags. Two hours prior to experimental initiation, highly concentrated DFAA were dripped from the 20 l bottles down each Y-maze arm (left and right) and water flow was initiated to produce ASW with molar percentages and total concentrations identical to those of the Toyohira and Chitose Rivers from September to November 2014, which is the time period when the *O. keta* were collected.

EXPERIMENTAL ANIMALS

Adult, matured (spermiated) male *O. keta* were collected from the Indian Fish Wheel at the Chitose Salmon Museum and transferred to the Sapporo Salmon Museum (Fig. 1). They were anaesthetized for 2 min with 0·05% clove oil (FA 100, Tamura Medicine Inc.; www.animal.ds-pharma.co.jp), measured for fork length (L_F) and then laterally tagged in the pelvic region with a 12-mm PIT tag using a rapid implant gun (MK-25, Biomark) before being placed in the Y-maze holding area for a 1-h acclimation period. Removable screens were placed at the base of each arm adjacent to the holding arena to prevent *O. keta* entry during the acclimation period. After the 1-h acclimation period, the screens were gently removed to allow *O. keta* movement into the arms. Each experiment was run during the evening for 3 h (starting at 1800 or 2200 hours) during the period of most activity in wild *O. keta* (Daum & Osborne, 1998; Ishida *et al.*, 2001). Experiments were run with one *O. keta* per trial with the following water options presented: Chitose ASW *v*. control water (32 fish) with ASW based on September to October DFAA concentration and Chitose *v*. Toyohira ASW (20 fish) with ASW based on November DFAA concentrations (Fig. 2). Each fish was tested twice by switching the experimental water between the arms to account for any biases between the arms yielding a total of 44 trials where *O. keta* entered the choice arms for the Chitose ASW *v.* control water and 25 trials where *O. keta* entered a choice arm for the Chitose *v.* Toyohira experiments. Only trials where an *O. keta* entered at least one of the choice arms were included in the analyses.

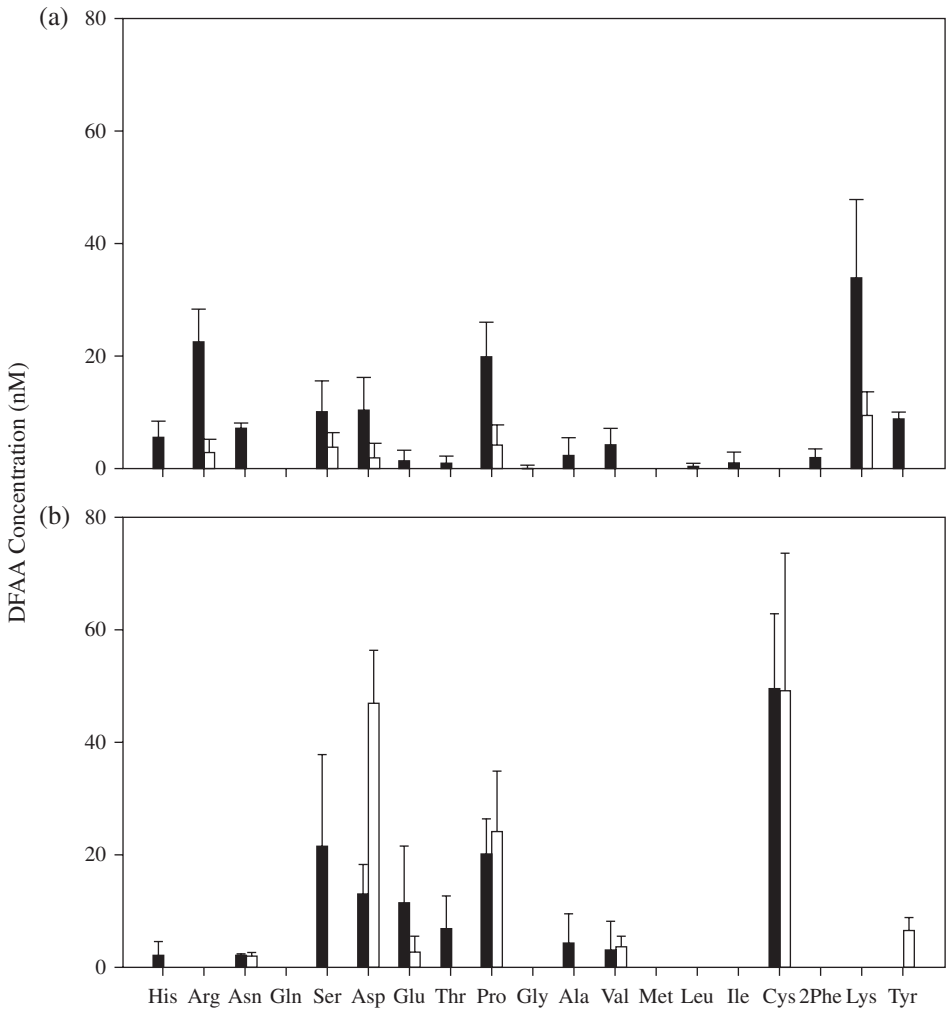

FIG. 2. Comparison of the mean + S.D. dissolved free amino-acid (DFAA) concentrations from (a) September and October for Chitose River (■) and Sapporo Salmon Museum (□) and (b) November 2014 for Chitose River (■) and Toyohira River (□). These profiles from the actual sites were used to constitute artificial sea water.

The total number of entries, duration of each entry and total duration spent in each arm were compared with Wilcoxon analysis to accommodate non-normally distributed paired data sets. Differences were considered significant at P-values $< 0{\cdot}05$.

ETHICAL NOTE

This study (16-8) was approved to carry out in accordance with the Guide for Care and Use of Laboratory Animals in Field Science Center for Northern Biosphere, Hokkaido University and Japanese Governmental Law (No. 105) and Notification (No. 6). To avoid stress during experiment, *O. keta* were anaesthetized for 2 min with 0·05% clove oil, measured for L_F and then laterally tagged in the pelvic region with a 12 mm PIT tag using a rapid implant gun before being placed into the Y-maze holding area for a 1-h acclimation period. All *O. keta* were decapitated immediately after the experiment.

RESULTS

The choice between Chitose ASW and control water yielded no significant differences in the total number of entries detected (Wilcoxon $Z = 10{\cdot}263$, $P > 0{\cdot}05$), the total time duration spent in each arm ($Z = -0{\cdot}667$, $P > 0{\cdot}5$), or the mean time per entry ($Z = -1{\cdot}642$, $P > 0{\cdot}05$). For the experiments offering the choice between Chitose ASW (the home stream analogue) and Toyohira ASW (the off-path tributary analogue) (Fig. 3), the median number of entries into Chitose ASW (45 entries) did not differ from Toyohira ASW (four entries) (Wilcoxon $Z = -1{\cdot}835$, $P > 0{\cdot}05$), although there was a clear trend toward a difference. Total duration of time spent in the Chitose ASW water (median 11·0 min) was 198% greater (Wilcoxon $Z = -2{\cdot}435$, $P < 0{\cdot}05$) than time spent in Toyohira ASW (5·6 min). The mean duration of each entry into the Chitose ASW maze arm (median 1·7 min) was 132% greater (average; $Z = -2{\cdot}215$, $P < 0{\cdot}05$) than Toyohira ASW (median 1·28 min).

DISCUSSION

When comparing the two Y-maze treatment groups, *O. keta* showed a stronger inclination toward Chitose ASW (home stream) compared with Toyohira ASW, but with no clear preference for Chitose ASW over control water. This somewhat contradictory result could have been a result of *O. keta* exploratory behaviour towards an unfamiliar water source (the control). As Chitose *O. keta* need to pass the confluence of the Toyohira River before reaching their natal river, it is plausible that the tested *O. keta* recognized the Toyohira ASW as a known incorrect natal waterway and thus showed a preference for the home stream profile from the Chitose River. Since the control water is spring water from the Sapporo Salmon Museum and the compositions of DFAA are simpler than Chitose ASW, *O. keta* might have been unable to discriminate this simple control composition of DFAA from the home stream ASW provided. These results suggest that the *O. keta* are capable of detecting the DFAA profiles, but that responses may be complicated and influenced by a comparator function.

Despite the differences found among treatment groups, the level of selection indicators (*e.g.* duration of stay) was relatively modest and many *O. keta* showed substantial movement within the behavioural choice maze. The data are similar to those seen in other studies (Yamamoto & Ueda, 2009; Yamamoto *et al.*, 2010, 2013) and may reflect the need for *O. keta* to frequently compare waters to be able to discriminate among them. Additionally, the time of day of the experiments, when wild *O. keta* are most active, may have caused *O. keta* to be more likely to continue to move among the choices rather than make a selection and remain in place. Some *O. keta* also did not choose to move into choice arms, which may reflect their level of reproductive maturity; spermiated *O. keta* represent those *O. keta* at the end of the migration and they may have become less motivated to express upstream migration.

Previous studies exploring the electro-olfactogram (EOG) responses for salmonids have shown distinct responses to reconstituted lake or river ASW which contained DFAA quantities similar to the Ishikari River catchment (Shoji *et al.*, 2000; Yamamoto & Ueda, 2009; Yamamoto *et al.*, 2013) and in this study it was possible to document a finer scale understanding of the movements and entries into a similar behavioural choice situation. As the EOG response thresholds were high in previous studies, it

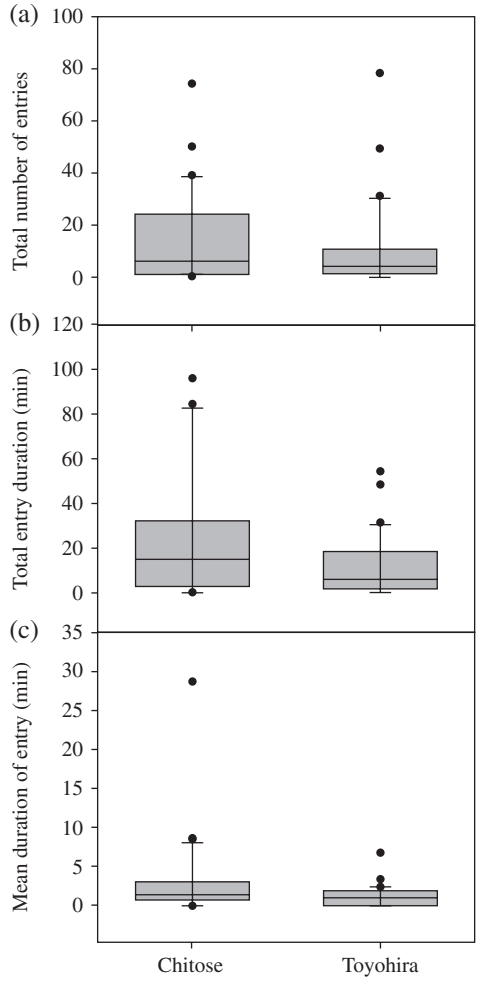

FIG. 3. Y-maze test tank results for the Chitose artificial stream water (ASW) *v*. Toyohira ASW treatment groups. (a) The number of entries made per trial for those trials where at least one maze arm was entered, (b) the total duration of time spent in each of the maze arms if an *O. keta* entered the arm and (c) the duration per attempt in each arm. Boxes indicate the distribution of the data such that the lower boundary of the box indicates the 25th percentile, the line within the box marks the median, and the upper boundary of the box indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles; ●, outliers.

seemed conceivable that salmonids are capable of detecting lower DFAA concentrations that would support the use of less abundant DFAA for salmonid natal-stream recognition. In the present study, DFAA levels were used appropriate to wild conditions and showed detectability sufficient to elicit behavioural responses.

DFAA probably play vital roles in salmonid homing and imprinting. Even with the vast research devoted to salmonid migrations (Dittman & Quinn, 1996; Shoji *et al.*, 2003; Hasegawa, 2012; Putman *et al.*, 2013), the precise mechanisms and behavioural attributes guiding salmonid navigation continue to puzzle physiologists and ethologists

alike, but progress is being made towards understanding the relationship between importance of olfactory cues and how this relates to migratory behaviour. In this study, the short-term DFAA profiles of a Hokkaido salmonid river were characterized and this information used to describe behavioural responses in *O. keta*. It was also demonstrated that artificial maze systems can be useful in further exploring olfactory guidance and behaviour in adult salmonids.

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