PRIMARY RESEARCH PAPER

Can unique amino acid profiles guide adult salmon to natal streams? A comparison of streams sampled prior to and after the arrival of adult Pacific salmon

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Abstract Homing is an essential aspect of the evolutionary ecology of salmon, and the final stages are guided by odors learned prior to migration from natal sites. Amino acids (AAs) have been hypothesized to provide olfactory information sufficient for homing. We sampled water from five tributaries to Lake Aleknagik, Alaska prior to and after the arrival of adult sockeye salmon to determine whether the AA composition or concentration differed among streams, or changed after adult salmon entered the streams and began spawning. The dominant AAs were generally similar before and after salmon entered, although some shifts in relative contribution were noted. However, there were dramatic increases in concentration of all AAs after the arrival of salmon. Analysis of AA concentrations and relative proportions indicated that clustering of samples in multivariate trait space depended on timing (pre- or post-arrival of salmon) but not among streams. The shift in AA composition in post-arrival samples was consistent with odors from the salmon (e.g., eggs). Collectively, these data are inconsistent with the idea that AAs alone differ sufficiently among streams throughout the migration period to guide homing, though they may contribute to more complex combinations of odorants.

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Introduction

The great majority of Pacific salmon (genus *Oncorhynchus*), Atlantic salmon, *Salmo salar* (Linnaeus, 1758), and their relatives return to spawn at their natal sites rather than at other sites, and this homing is fundamental to their biology, management and conservation (Moulton, 1939; Thompson, 1959). Homing results in reproductive isolation and reduced gene flow among populations, leading to the evolution of myriad population-specific differences in phenotypic traits (Taylor, 1991; Fraser et al., 2011; Quinn, 2018) and independent population dynamics.

Homing to the natal site or site of previous breeding is known in many fish species including white suckers, *Catostomus commersoni* (Lacepède, 1803) (Werner, 1970; Werner & Lannoo, 1994), snook, *Centropomus undecimalis* (Bloch, 1792) (Adams et al., 2011), northern redbelly dace, *Phoxinus eos* (Cope, 1861) (Massicotte et al., 2008), walleye, *Sander vitreus* (Mitchill, 1818) (Hayden et al., 2018), American shad, *Alosa sapidissima* (Wilson, 1811) (Nichols, 1960; Melvin et al., 1986), and Allis shad, *Alosa alosa* Linnaeus, 1758 (Martin et al., 2015). Salmonids, in particular, are an ideal model system for investigating homing behavior.

The homing of salmonids in the open ocean is poorly understood but has long been recognized as separate from the process of identifying the natal stream (Hasler, 1966), which is guided by olfaction (Hasler & Scholz, 1983). Early experiments indicated that the attractive compounds in the home stream water could be chemically fractionated but little progress was made in determining which compounds were most important (Fagerlund et al., 1963; McBride et al., 1964). Elegant experiments later revealed that exposure of juveniles to novel synthetic chemicals, morpholine and phenethyl alcohol, was sufficient to enable them to locate streams scented with those odors at maturity in coho salmon, *Oncorhynchus kisutch* (Walbaum, 1792) (Scholz et al., 1976), brown trout, *Salmo trutta* Linnaeus, 1758 (Scholz et al., 1978a), and rainbow trout, *O. mykiss* (Walbaum, 1792) (Scholz et al., 1978b). These experiments established the generality of the imprinting phenomenon but did not address the question of what natural odors salmon learn. As an alternative to the hypothesis that odors from biota and geology unique to each stream provided the key odors, Nordeng (1971, 1977) built on the idea that odors of juvenile salmon in streams might guide returning adults (White, 1934), and proposed that adults are guided by population-specific pheromones released from the skin mucus of juveniles. Subsequent experiments indicated that bile acids and amino acids (AAs) were likely involved in distinguishing among groups of conspecifics (Døving et al., 1980; Olsén, 1986), though these odors may not be essential to guide homing salmon (Brannon & Quinn, 1990).

Recent studies have emphasized the potential role of dissolved free AAs in salmon imprinting and homing (Yamamoto et al., 2010, 2013). Such AAs could come from many sources in streams (Thomas, 1997), including biofilm on substrate (Costerton et al., 1994; Ishizawa et al., 2010) and sediment pore water (Thomas & Eaton, 1996). Importantly, the chemical cues that drive salmon homing must be robust to seasonal and annual variation. For example, in many species juvenile salmon imprint in spring and the adults return in fall, after one, two, three or more years at sea, although the seasonal timing of natal stream emigration and homing vary considerably between salmon species and populations (Quinn, 2018). Moreover, some adults ascend streams devoid of adult conspecifics whereas others ascend the same stream a few days, weeks, or months later, when the stream likely differs in many chemical features. Biofilm and AA profiles may be affected by temperature, nutrients, sunlight, and other seasonal features of streams, but the digging of nests in the gravel by salmon themselves also causes dramatic short-term increases in fine sediment transport (Hassan et al., 2008), reductions in benthic invertebrate density (Hildebrand, 1971; Minakawa & Gara, 2003) and biofilm (Minakawa & Gara, 1999; Moore & Schindler, 2008), and alters hyporheic exchange (Buxton et al., 2015). In addition to the possible role of odors from juvenile salmonids in streams and alteration in stream chemistry related to digging by adult salmon, the adult salmon themselves can represent a very large biomass, so AAs and other organic molecules from the mucus on their bodies (Stabell & Selset, 1980), excretion, gametes released into the water column (Dittman & Quinn, 2020), and decomposition of their carcasses (Hood et al., 2007) might also change the chemistry of the water. Thus the first salmon returning to a stream each year may experience water bearing odors that differ from the odors experienced by salmon arriving a few weeks later, after other salmon have arrived, spawned, and disrupted the substrate. Despite such changes, throughout the migration period the salmon must be able to distinguish their natal rivers from others nearby, so some odor constituents and qualities must be consistent.

The purpose of this study was to sample the AAs in five streams that support sockeye salmon [*Oncorhynchus nerka* (Walbaum, 1792)] spawning and flow into a single lake. We hypothesized that the presence of spawning adult salmon would alter the amino acid profiles in these streams relative to pre-salmon profiles. We determined whether (1)

the AA profile differed among the streams (including samples collected before and after the arrival of adult salmon; henceforth, "pre-arrival" and "post-arrival"), (2) the proportions or overall AA concentrations changed post-arrival, (3) the streams were more different from each other pre-arrival than post-arrival, as might be expected if odors from salmon, their gametes, or some feature of their presence were responsible for the shifts in AA composition, and (4) samples collected post-arrival clustered with AA compositions that emanate from ovulatory salmon eggs (Dittman & Quinn, 2020).

Materials and methods

Study site

We sampled five small streams, all tributaries to Lake Aleknagik in the Wood River system of southwestern Alaska (Fig. 1; Table 1). Adult sockeye salmon ascend the Wood River from Bristol Bay in late June and early July, complete final maturation in the lake, and ascend streams to spawn from mid-July to late August (Doctor et al., 2010). In addition to sockeye salmon, the study streams are occupied by smaller resident fishes, including rainbow trout, Dolly Varden, *Salvelinus malma* (Walbaum, 1792), Arctic char *S. alpinus* (Linnaeus, 1758), coho salmon, coastrange sculpin, *Cottus aleuticus* Gilbert, 1896, and
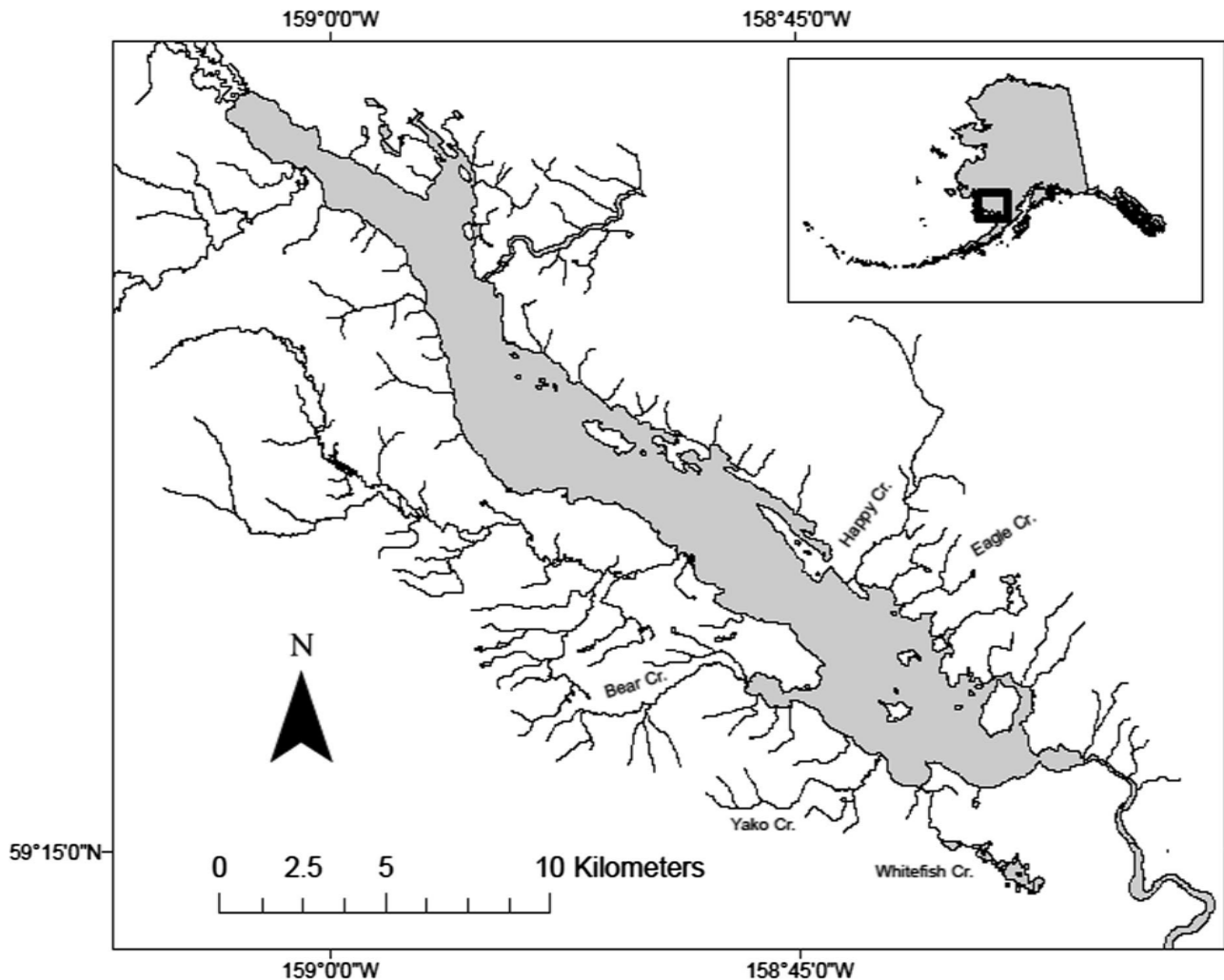

Fig. 1 Map of Lake Aleknagik, Alaska, showing the locations of the five tributaries sampled for AAs. The lake's outlet, the Wood River, is at the lower right (southeast) side, draining into Bristol Bay via the Nushagak River

Table 1 For each of the five study streams, the upper rows indicate the mean wetted width and depth (from Quinn et al., 2001), and discharge (m³/sec) of the five study streams during the summer period of salmon spawning and sampling, measured repeatedly during the summer from 2008 to 2015 (University of Washington, unpublished data).

Sample	Bear	Whitefish	Eagle	Happy	Yako
Mean width (m)	5.1	3.4	2.4	4.7	4.2
Mean depth (cm)	18.8	25.3	21.6	26.2	22.6
Mean discharge (m³/sec)	0.78	0.36	0.10	0.67	0.61
Pre-arrival					
Date	12-Jul	10-Jul	10-Jul	12-Jul	10-Jul
No. adult salmon	0	0	0	5253	0
Discharge	0.14	0.30	0.06	0.40	0.11
Salmon/discharge	0	0	0	13,035	0
Total AA conc. (nM)	66.79	113.82	135.14	237.75	88.2
Post-arrival					
Date	28-Jul	28-Jul	24-Jul	28-Jul	28-Jul
No. adult salmon	3,951	1,402	400	14,172	7703
Discharge	0.12	0.22	0.05	0.15	0.15
Salmon/discharge	32,122	6,491	7,843	95,114	51,013
Total AA conc. (nM)	569.54	410.17	1,745.27	1,806.25	697.53

The lower rows indicate the numbers of adult sockeye salmon counted in each stream on the dates of sampling (pre-arrival and post-arrival), the stream discharge on that date, the number of salmon per $m^3 \ s^{-1}$ (salmon/discharge), and the total concentration of AAs (sum of all 17 individual AAs measured)

slimy sculpin, *C. cognatus* Richardson, 1836; Quinn, unpublished data) but support very low densities of other species of Pacific salmon (Pess et al., 2014). These streams contain high densities of sockeye salmon embryos during most of the year, and spawning adults in mid-summer (Quinn et al., 2001).

Water collection

The pre-arrival water samples were collected on 10 or 12 July, 2014, at end of the period when sockeye salmon fry are emerging from gravel nests and emigrating from their natal streams to Lake Aleknagik (Abrey, 2005), but before returning adults arrive. The post-arrival samples, during the salmon spawning period, were collected on 24 or 28 July, 2014. Some mature sockeye salmon entered Happy Creek earlier than usual in 2014, and 5253 were counted when the pre-arrival samples were collected for this stream on 12 July but far more (14,172) were counted on the post-arrival collection date. These dates were characterized by typical flows at that time of year (Table 1), as there were no conspicuous rain events that would affect discharge. At each stream, on each date, duplicate water samples were collected at the same location just upstream of the mouth in the main channel, prior to any human presence that day. Nitrile gloves were worn during all sample collection to avoid contamination by AAs from human skin. For each replicate sample, water was collected with a sterile 60 ml syringe, rinsed three times with stream water, from approximately 5–10 cm below the surface. Water was then filtered through 0.20 μm Millipore filter into pre-labeled, sterile 50 ml conical tubes, immediately placed on ice and then frozen at − 20 °C within a few hours of collection.

Egg water

The composition of AA odors emanating from salmon eggs was determined using eggs from mature female sockeye salmon collected in the tributaries of Lake Aleknagik (Dittman & Quinn, 2020). Briefly, ovulated eggs were soaked in local well water (4 °C) for 1 h, stored at − 20 °C, and analyzed for AA composition using the same laboratory and techniques used in this study.

Amino acid analysis

Dissolved free AA analysis (L-form) was conducted by the Protein Structure Core Facility at University of Nebraska on a Hitachi L-8800A analyzer. Samples were first reduced in volume using a lyophilizer, then filtered through a 3000 Da MW filter (Pall Inc.) and the flow-through evaporated until dry. Samples were then dissolved in 100 μl of 0.02 N HCl and 50 μl was injected onto the AA Analyzer. Norleucine was used as an internal standard to correct for dilution errors. The lowest detectable concentration of amino acids was approximately 1.5 nM.

Statistical analyses

Multivariate analyses were conducted on both raw AA concentrations and AA proportions (i.e., percent concentration). A permutation-based analysis of variance (PERMANOVA) was used to identify differences among groups within the AA observations, consisting of two replicates from samples collected on each of the 10 sampling events (5 streams, each sampled twice—once pre-arrival and once post-arrival of salmon). PERMANOVA was selected for this purpose because it doesn't require specific assumptions about the distribution of the data and can be applied to any suitable multivariate distance measure. Dissimilarity matrices across the 17 distinct AAs were calculated based on appropriate multivariate distance measures using Bray–Curtis distances for raw amino acid concentrations and Aitchison distances for relative concentrations. Based on the lack of differences between replicates indicated by PERMANOVA (see Results section), we report the average values as concentrations and as percentages (Table 2), and display them in graphs, but the variation between replicates was incorporated in the complete analyses testing for differences among streams and between samples collected pre-arrival and post-arrival of salmon.

Non-metric multi-dimensional scaling (NMDS) was used to visualize the ordination of water samples in multivariate trait space, and to identify: (1) whether samples clustered more closely within streams regardless of the presence of salmon, or whether the presence or absence of salmon was the dominant source of variation, (2) which AAs contributed most to these differences, and (3) whether samples collected post-arrival clustered more closely

Table 2 Average concentration and relative percentage of AAs, averaged among five streams prior to the arrival of sockeye salmon and during the salmon spawning season (pre-arrival and post-arrival, respectively)

Amino acid	Concentration (nM)		Relative percentage	
	Pre-arrival	Post-arrival	Pre-arrival	Post-arrival
Glycine	14.4	178.9	11.2	17.1
Threonine	14.0	99.3	10.9	9.5
Serine	13.5	163.7	10.5	15.6
Glutamine	12.6	74.7	9.8	7.1
Cysteine	11.8	23.8	9.2	2.3
Phenylala-nine	10.0	54.2	7.8	5.2
Alanine	8.5	115.2	6.6	11.0
Isoleucine	7.4	28.1	5.8	2.7
Asparagine	7.3	47.5	5.7	4.5
Valine	5.4	61.5	4.2	5.9
Tyrosine	5.0	24.5	3.9	2.3
Leucine	4.9	38.3	3.8	3.7
Arginine	4.2	16.0	3.3	1.5
Lysine	3.9	61.1	3.0	5.8
Proline	3.3	23.3	2.6	2.2
Histidine	2.3	22.7	1.8	2.2
Methionine	0.0	13.1	0.0	1.3
Sum	128.3	1045.8		

with AA compositions that emanate from ovulatory salmon eggs (Dittman & Quinn, 2020). NMDS iteratively searches for the most parsimonious (i.e., lowest stress) ordination of observations in trait space based upon the distance or dissimilarity among multivariate observations. NMDS was conducted for both absolute and relative AA concentrations, and with 2–8 dimensions. However, because stress was below 11% for 2 dimensions (2.4% for absolute and 7.5% for relative concentrations, and 10.6% for relative concentrations including analyses of egg odor), results from NMDS with two dimensions are described and illustrated below. NMDS for samples including eggs was only conducted on relative, rather than absolute, concentrations because the egg samples were created using an arbitrary water volume and number of eggs in the laboratory and the absolute concentrations likely do not reflect absolute concentrations apparent in natural streams.

Finally, the nature of our sampling (i.e., 2 replicates for each combination of stream and date) did

not allow us to use the above techniques to examine possible differences between streams separately for pre-arrival and post-arrival samples. As an alternative, we calculated Pearson correlation coefficients (CCs) using the percent composition data between all 10 pairs of streams (e.g., Happy vs. Yako, Happy vs. Eagle, Eagle vs. Yako, etc.) using the data collected pre-arrival, and then repeated the procedure using the post-arrival data. We compared the CCs to determine whether, in general, the streams were more similar prior to the arrival of salmon than after salmon had arrived. Similarly, we used CCs to assess whether the proportions of AAs in each stream pre- and post-arrival were similar.

Results

Averaged among the streams, water samples collected before salmon arrived included 16 of the 17 AAs; only methionine was not detected in the chemical analysis (Table 2; Supplemental Table 1). The mean total AA concentrations were much higher post-arrival compared to the pre-arrival samples in the same streams (1045.8 vs. 128.3 nM). The increases in total concentration were evident in all streams (12.9-fold in Eagle, 8.5-fold in Bear, 7.9-fold in Yako,

7.6-fold in Happy, and 3.6-fold in Whitefish (Fig. 2)), and for all AAs, ranging from 15.8-fold increase in lysine to a 2.0-fold increase in cysteine. Methionine was not detected pre-arrival so a fold increase could not be calculated but post-arrival samples averaged 13.1 nM.

Significant differences between the replicates from each of the 10 sampling events were not detected by PERMANOVA on either the absolute concentrations ($Pr (>F)=0.79$) or the relative concentrations ($Pr(>F)=0.34$). In addition, differences among streams were not detected in absolute ($Pr(>F)=0.78$) or relative concentrations ($Pr(>F)=0.75$). PERMANOVA indicated significant differences in both absolute ($Pr(>F)=0.001$) and relative ($Pr(>F)=0.001$) amino acid composition between pre- and post-arrival treatments. When relative amino acid compositions between pre-arrival, post-arrival, and salmon egg treatments were compared with PERMANOVA, a significant difference among treatments was detected ($Pr(>F)=0.001$).

When averaged among the five streams (Table 2; Fig. 2), the dominant AAs pre-arrival were glycine (11.2%), threonine (10.9%), and serine (10.5%); followed by a gradual reduction in contribution down to the lowest detectable AA, histidine (1.8%). Post-arrival of salmon, glycine (17.1%) and serine (15.6%)
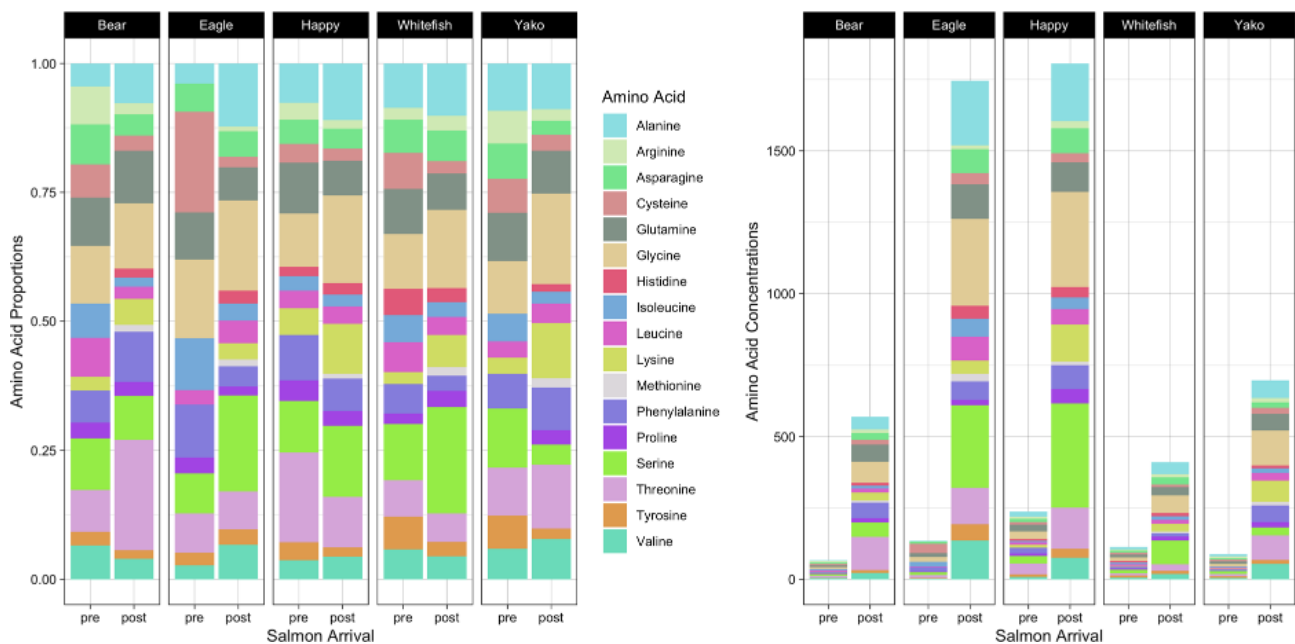

Fig. 2 Relative proportions (left panel) and total concentrations (nM—right panel) of 17 AA, detected in five different streams (Bear, Eagle, Happy, Whitefish, and Yako creeks) before and after arrival of spawning salmon (designated "Pre/Post")

remained prominent, and the pre-arrival composition of all AAs were positively correlated with the post-arrival contributions ($r = 0.731$, $P < 0.001$). However, highly significant differences were detected between pre- and post-arrival samples in absolute and relative concentrations ($Pr(>F) = 0.001$ in both cases; Fig. 2). The differences between pre- and post-arrival samples resulted from combinations of AAs (Table 2), and most prominently the increases in lysine, glycine, serine, and alanine, and the decreases in cysteine, arginine, phenylalanine, and isoleucine (Table 2).

Ordination of samples in multivariate trait space with NMDS revealed that samples clustered more closely based on the presence or absence of salmon across streams (Fig. 3, panels b, d), rather than among streams (pooling samples collected pre- and post-arrival of salmon for each stream: Fig. 3, panels a, c). These results were similar whether absolute or relative AA concentrations were used as the response variable. For absolute AA concentrations,

the first NMDS dimension was most directly associated with the increase in total concentration of all AAs (Fig. 3a), and primarily discriminated between the samples collected pre- and post-arrival. The first NMDS dimension for the relative AA proportions indicated that the pre- and post-arrival samples were best discriminated by increasing proportions of methionine and alanine, and decreasing proportions of cysteine and isoleucine (Fig. 4, panels c, d).

When the relative AA compositions of replicate samples from streams pre- and post-arrival were ordinated using NMDS in conjunction with samples of salmon eggs, samples grouped between pre-salmon, post-salmon, and egg treatments, rather than within river systems (Fig. 4). When viewed in multivariate trait space, the relative amino acid compositions of post-salmon stream water samples were closer to the relative amino acid composition of salmon egg samples. This suggests that the relative amino acid concentration observed across all streams sampled after

Fig. 3 Non-metric multi-dimensional scaling (NMDS) plots showing the ordination of each sample (point) in multivariate (AA) trait space. (1) all samples (pre- and post-salmon) from each stream (Eagle, Yako, Happy, Whitefish, and Bear creeks: panels **a**, **c**; and (2) all samples from all streams pooled into either pre- or post-salmon arrival collections: panels **b**, **d**). For each comparison, panels **a** and **b** display the absolute concentrations, and panels **a** and **d** display the relative concentrations
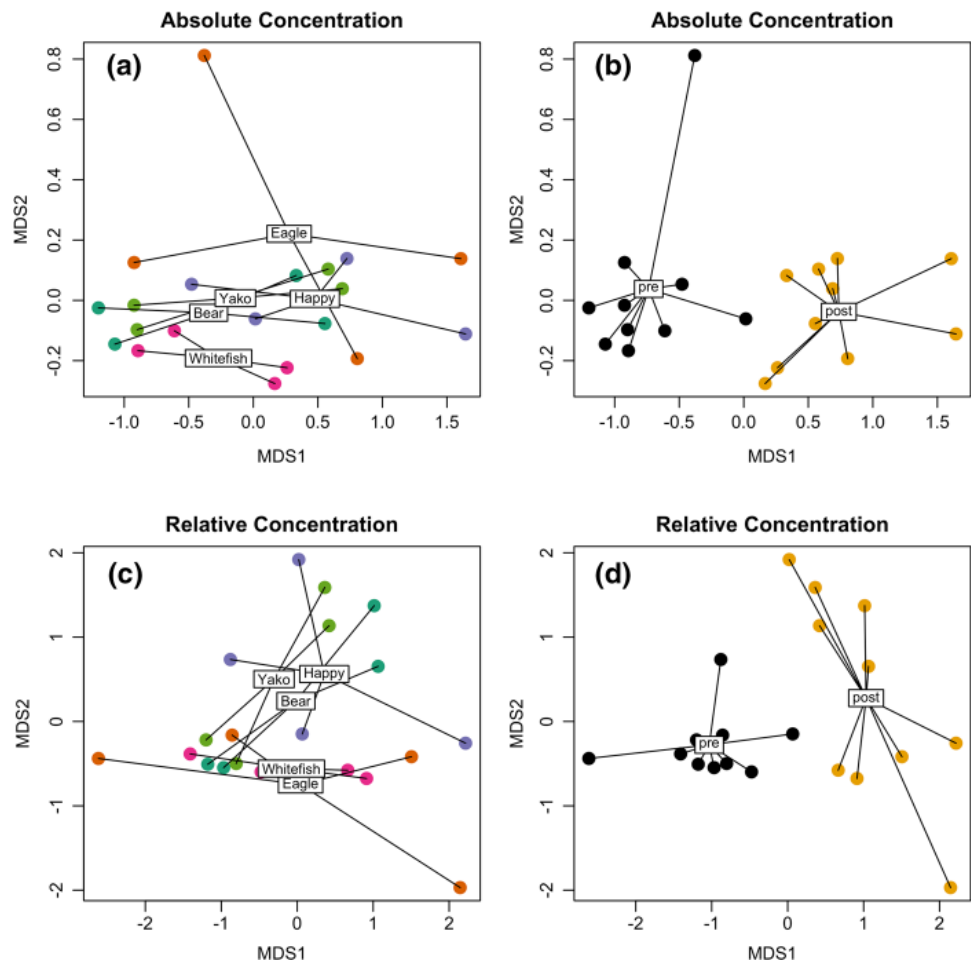
Fig. 4 Comparison of loadings relative concentrations for 17 different AAs (arrows) on the first and second NMDS dimension, relative to the ordination of samples (points) collected from (i) each stream (Eagle, Yako, Happy, Whitefish, and Bear creeks, combining both pre- and post-arrival salmon collections) and (ii) samples from all streams pooled into either pre- or post-salmon arrival groups. Each panel also displays AA composition of water in which sockeye salmon eggs had been soaked ("eggs," data from Dittman and Quinn 2000). Panels **a** and **c** color points for individual samples based on origin (i.e., stream or egg), while panels **b** and **d** color points based on treatment (pre-arrival, post-arrival, egg). NMDS loadings (blue arrows) have been overlaid on panels **c** and **d**
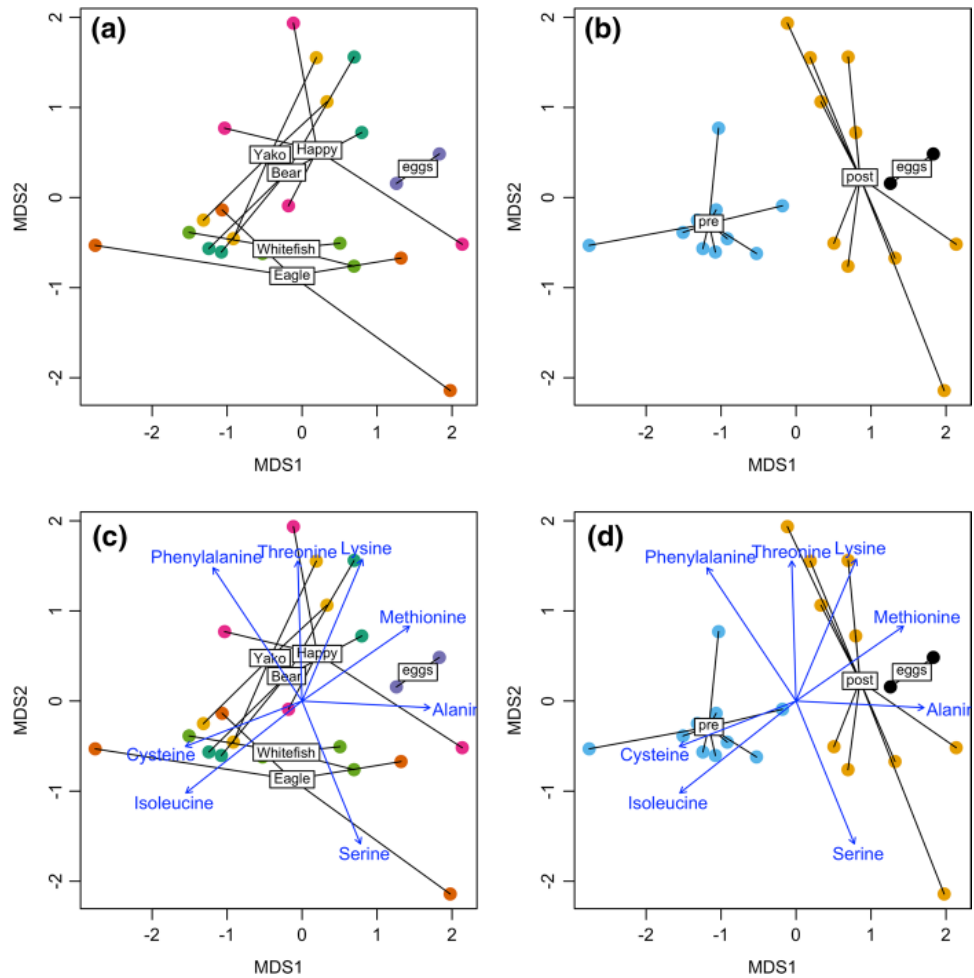
the arrival of salmon more closely reflected the amino acid signature of salmon egg samples.

These results were consistent with the analysis of CCs between pairs of streams. Overall, the mean CC between stream pairs increased post-arrival of salmon (mean $r = 0.70$) relative to pre-arrival CCs (mean $r = 0.63$). However, the patterns of correlation were complex; five pairs of streams had higher CCs and five had lower CCs post-arrival (Fig. 5). CCs varied considerably between stream pairs ranging from a high of 0.85 (Whitefish/Yako) to a low of 0.33 (Happy/Eagle) pre-arrival of salmon and a high of 0.98 (Whitefish/Happy) to a low of 0.47 (Whitefish/Yako) post-arrival (Fig. 5). While there was a tendency for the AA composition in streams on the west side of the lake (Fig. 1) to be more highly correlated with one another pre-arrival of salmon, there were few obvious patterns associated with AA composition and environmental parameters (width, depth, discharge) or salmon density (Table 1).
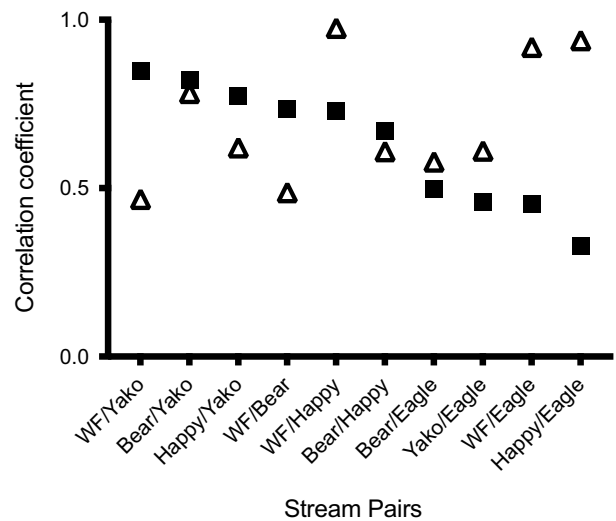

Fig. 5 Pearson correlation coefficients of percent AA composition data between all pairs of streams before (solid squares) and after (open triangles) salmon arrival

Discussion

We quantified AA concentrations and relative proportions in a suite of streams used for spawning by sockeye salmon to compare differences among streams, as might be used by juveniles to learn the unique odors of the stream for later homing as adults. We did not detect significant differences among the streams, but observed dramatic changes in the AA concentration and composition between samples collected prior to and after the arrival of adult salmon. The collections prior to the arrival of salmon occurred when emergent fry were leaving their natal tributaries and presumably imprinting. These results are inconsistent with the hypothesis that AAs can provide sufficiently unique signals to guide homing to natal streams. The operating assumption behind the study is that the great majority of sockeye salmon home to their natal tributary rather than exchange freely among these and other nearby populations. This assumption is supported by a large body of literature on homing in salmon in general (Dittman & Quinn, 1996; Keefer & Caudill, 2014), and by the consistent differences among at least some of these specific populations in age, size at maturity, and morphology (Quinn et al., 2001), timing of spawning (Lin et al., 2008), and gene frequencies (Larson et al., 2014). Indeed, marking studies indicate that sockeye salmon home preferentially to regions within their natal stream where they were incubated and emerged (Quinn et al., 2006) or released as fry (Barnett et al., 2019). Thus, streams must have sufficient differences in chemical composition that embryos or fry, exposed to these chemicals during incubation and when they emerge to migrate to their nursery lake, can successfully identify them several years later when they return as adults.

The absence of significant statistical differences in AA composition among the Lake Aleknagik streams does not necessarily preclude the possibility that salmon can detect and utilize subtle differences in AA compositions for homing. The salmonid olfactory system can certainly detect and discriminate unique AAs at the concentrations we observed (Hara, 1976, 1994). However, the behavioral ability of many fish species including salmon (Yamamoto & Ueda, 2009); brown bullhead, *Ameiurus melas* (Rafinesque, 1820) (Valentincic et al., 2011), and zebrafish, *Danio rerio* (Hamilton, 1822) (Miklavc & Valentincic, 2012) to discriminate closely-related, complex mixtures of

AAs has not been clearly demonstrated. Furthermore, homing salmon must not only be able to distinguish the odor signature of their stream relative to other proximate streams but also must be able to generalize the odorant signature of their natal stream as unique despite seasonal and annual changes in the chemical composition of these signatures (e.g., Yamamoto et al., 2013). Returning adult sockeye salmon are not only able to identify their natal tributaries in Lake Aleknagik but even specific reaches within these tributaries where they emerged as fry years earlier (Quinn et al., 2006). Given the relative similarity between AA composition in the streams we studied and the dramatic, somewhat unpredictable changes in AA composition post-arrival arrival of spawning salmon, it seems unlikely that the composition of AAs alone is sufficient to identify their natal streams consistently over the course of the spawning season.

Several studies have demonstrated that salmon can learn AAs as homing cues (Yamamoto et al., 2010; Havey et al., 2017; Armstrong et al., 2022) and it is likely that AAs contribute in natural systems to the suite of chemicals imprinted by alevins in the gravel or juveniles prior to leaving the streams. A variety of chemical compounds including AAs, bile acids, and minerals in natural river systems have all been hypothesized as potential homing cues (Bett & Hinch, 2015) and may contribute to the olfactory signature that salmon require to distinguish their natal stream. Many aspects of the chemistry of streams are dynamic, differing from spring to fall, as well as from year to year, in addition to the presumably even greater differences among streams. This is especially true for AAs, which may be metabolized quickly in many lotic ecosystems (Thomas, 1997). Given the temporal complexity and substantial noise associated with chemical signals in rivers, the ability of salmon to routinely home to their natal stream is both remarkable and enigmatic. It is likely that a combination of chemicals provides a consistent overall chemical odorscape that may be important, and the most concentrated chemicals may not necessarily be the most important for imprinting and homing. Somehow, the essence of the stream is detected and remembered by juveniles in a combination of peripheral and central processes (Nevitt et al., 1994; Dittman et al., 1997), and some combination of these chemicals help adult salmon later identify the stream years later, despite variation in river flow and chemistry.

We detected a dramatic increase in the overall concentration of AAs after salmon entered their natal streams to spawn. Such an increase might make streams easier to detect by a salmon swimming in the lake but not necessarily make the streams easier to distinguish from one another. Tributary AA composition clustering was most strongly associated with the presence or absence of salmon across streams (Fig. 3) suggesting that the presence of salmon increased the similarity of AA profiles in all streams, perhaps making discrimination of individual streams, based on AA composition, more challenging. However, all AAs (and other chemicals) may not be equally important, alone or in combination, as clues for the salmon, and higher chemical concentrations might not facilitate discrimination. For example, humic substances, released from the stream substrate during salmon migration and spawning, may bind free AAs (Lytle and Perdue 1981), and humic acids can hinder detection of odors by fishes (Hubbard et al., 2002; Mobley et al., 2020), and might, similarly, interfere with detection of imprinted odors. We also detected a change in the relative (proportional) composition of AAs, such that the streams were more similar to each other post-arrival of salmon, though in each stream the proportions of the different AAs were positively correlated between the two time periods. Whether quantified as absolute or relative concentrations, the AA composition of tributary water samples was more similar across streams pre- or post-arrival of salmon, than within streams across the salmon spawning season.

The post-arrival samples were collected before all salmon had arrived that season, so at least some salmon would have to identify their home stream when the AAs were very different from those experienced by conspecifics a few weeks earlier. In addition to the orientation challenge posed by the changes in AA composition and especially concentration toward the end of the season might pose for homing salmon, there is the question of how the presence of adult salmon makes the tributaries more similar. The digging of redds for spawning by females mobilizes fine sediment and silt (Kondolf et al., 1993), and displaced periphyton and benthic invertebrates also drift downstream (Field-Dodgson, 1987; Minakawa & Gara, 1999, 2003; Peterson & Foote, 2000; Moore & Schindler, 2008). The resulting change in stream chemistry after the arrival of salmon might tend to make streams more similar within a region, such as Lake Aleknagik, if there were similar species of insects, periphyton, and decomposed terrestrial vegetation. Indeed, AAs released from biofilms have been proposed as sources of homing cues for salmon in Japanese streams (Ishizawa et al., 2010), and the AAs (serine and glycine) that increased the most in the Lake Aleknagik tributaries after salmon arrived were the most abundant AAs released from biofilms in the Japanese streams. This may help explain the positive correlation we observed between the AA composition identified in the Teshio River, Japan in May and September of two different years and the water in Lake Toya, Japan (Yamamoto et al., 2013) with the averaged proportions of the five Lake Aleknagik streams ($r = 0.72$, $P < 0.01$). Such correlations, from water bodies several thousand km apart, suggest that the AAs may reflect, in large part, the general biota of the streams such as periphyton and detritus (Costerton et al., 1994; Thomas, 1997; Ishizawa et al., 2010).

A second possible source of cues that might make streams more similar after salmon enter is the salmon themselves. AAs might be released from the skin of adult salmon (Stabell and Selset 1980), their gametes during spawning (Dittman & Quinn, 2020), and their decomposing bodies (Hood et al., 2007), all of which might tend to make the streams more similar to each other. We did not obtain samples of AAs from a full suite of sockeye salmon-related sources but the shift in AA composition from pre- to post-arrival was in a direction (within multivariate trait space) toward the samples from sockeye salmon eggs (Fig. 4). Moreover, 3 of 5 AAs (alanine, lysine, serine) with the highest concentrations in water from eggs (Dittman & Quinn, 2020) were partially responsible for the clustering of post-salmon samples (Fig. 4). However, we do not infer that the eggs were the source of the change—only that the change was consistent with a greater representation of AAs that were found in samples of ovulatory salmon eggs. Further samples, from water bathing live and decomposing adult salmon, might help assess the extent to which the salmon contribute to the changes in AA composition. Other salmon species are sufficiently scarce in the Wood River system, relative to sockeye salmon (Pess et al., 2014), that their effect would likely be much smaller in these streams, but multiple salmon species commonly spawn in sympatry, which might further complicate the chemical signals. On the other

hand, salmon can distinguish conspecifics of their own population (Groot et al., 1986; Quinn & Tolson, 1986), so odors from fish that arrive early may help guide those arriving later, but this has yet to be fully demonstrated.

The imprinting and homing ability of salmon is indeed remarkable when considered as a challenge to pattern learning and recognition. As juveniles they learn the chemical signature of a stream in a sufficiently generalized manner that they can distinguish it from others that they detect along the homeward migration years later as adults, despite changes in that signature on multiple temporal scales. Our results indicated that the AA composition of streams is diverse but does not, alone, seem sufficiently and consistently different to guide homing, though it likely plays a part in the process. Further investigations into the generality of these dramatic AA changes in other river systems or in hatcheries as adult salmon return to spawn, and the changes in chemistry of streams related to specific sources (e.g., periphyton, insects, and fishes, including the salmon themselves) will provide further insights into this ability.

Declarations

Conflict of interest The authors have no competing financial or non-financial interests to disclose. The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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