Timing of imprinting to natural and artificial odors by coho salmon (Oncorhynchus kisutch)

Andrew H. Dittman, Thomas P. Quinn, and Gabrielle A. Nevitt

Abstract: Juvenile salmon learn odors associated with their home stream before seaward migration and use these odor memories for homing as adults. Experimental evidence has indicated that this olfactory imprinting occurs during a sensitive period associated with surges in plasma thyroxine levels during Parr–smolt transformation; however, many natural migration patterns clearly indicate that home stream learning must occur before the sensitive period at Parr–smolt transformation. To examine the timing of olfactory imprinting, we exposed hatchery-reared coho salmon (Oncorhynchus kisutch) to either natural odors or an artificial odorant (β-phenylethyl alcohol) at specific developmental stages and tested their adult behavioral responses to these odors. Our results confirmed the importance of the Parr–smolt transformation as a sensitive period for olfactory imprinting: only salmon exposed to β-phenylethyl alcohol at the smolt stage demonstrated an increased attraction to this odorant as adults. We found little evidence that salmon learned homing clues prior to the smolt stage. Results further suggested that migration, and the associated stimuli, may also be important for optimal imprinting. Therefore, the absence of presmolt learning in this study may be due, in part, to the stable environment and restrictions on migration associated with hatchery rearing.

Résumé : Avant leur migration vers la mer, les juvéniles de saumons mémorisent les odeurs associées à leur cours d'eau natal et utilisent ces souvenirs pour leur remontée à l'âge adulte. On a démontré expérimentalement que cette imprégnation olfactive survient au cours d'une période sensible associée à des hausses momentanées des teneurs en thyroxine plasmatique au cours de leur transformation de tacan en smolt. Toutefois, les profils de migration de nombreuses populations de saumons sauvages indiquent clairement que la mémorisation des odeurs du cours d'eau natal doit survenir avant la période de sensibilité de la transformation de tacan en smolt. Afin d'étudier la chronologie de l'imprégnation olfactive, nous avons exposé des saumons cohos (Oncorhynchus kisutch) élevés en pisciculture soit à des odeurs naturelles, soit à un arôme artificiel (alcool β-phénylétanol) lors de stades spécifiques du développement, et nous avons testé les réponses comportementales de poissons adultes à ces odeurs. Nos résultats ont confirmé l'importance de la période de la transformation de tacan en smolt comme période de sensibilisation par imprégnation olfactive : seuls les saumons exposés à l'alcool β-phénylétanol au stade de smolt présentaient à l'âge adulte un attract prononcé pour cet arôme. Très peu de résultats indiquaient que le saumon mémorisait des caractéristiques de son cours d'eau natal avant le stade de smolt. En outre, les résultats suggéraient que la migration et les stimuli associés à celle-ci pourraient être aussi importants que l'imprégnation olfactive. Par conséquent, l'absence de cas de mémorisation précédant le stade de smolt dans cette étude peut s'expliquer en partie par le milieu stable et les entraves à la migration caractérisant l'élevage en pisciculture.

Introduction

Salmonids are well known for their ability to return to their river of origin to spawn. Prior to their seaward migration, juvenile salmon are thought to learn site-specific odors emanating from the vegetation and soil of their natal stream and later use these odor memories for homing (Wisby and Hasler 1954; Hasler and Scholz 1983). This imprinting phenomenon was elegantly demonstrated by Hasler and his colleagues, who showed that juvenile coho salmon (Oncorhynchus kisutch) exposed to synthetic chemicals, such as morpholine or β-phenylethyl alcohol (PEA), could be attracted into an unfamiliar stream scented with one of these chemicals during their spawning migration 1.5 years later (Cooper et al. 1976; Scholz et al. 1976). Subsequent laboratory experiments led Hasler and Scholz (1983) to hypothesize that juvenile salmon imprint on home-stream odors during a single sensitive period of development, the Parr–smolt transformation (PST). This life stage is characterized by physiological, morphological, and behavioral changes that ready salmon for life at sea. Many of these changes are associated with surges in the hormone thyroxine that occur during smolting (Dickhoff et al. 1978; Dickhoff and Sullivan 1987) and these high thyroxine levels

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are thought to be involved in olfactory learning (Hasler and Scholz 1983; Morin et al. 1989a, 1989b).

While experimental evidence with both hatchery- and laboratory-reared fish indicates that the PST is important for imprinting, the complex freshwater migration patterns of wild salmon suggest that olfactory imprinting can also occur prior to smolting. For example, sockeye salmon (Oncorhynchus nerka) fry typically emerge from their natal stream gravel and immediately migrate downstream to a lake where they live for 1 or 2 years before smolting and migrating to sea. During their homing migration, adults migrate past the outlet stream and lake where they smolted and return to their natal stream to spawn (e.g., Varanavskaya et al. 1994). Similarly, coho and chinook salmon (Oncorhynchus tshawytscha) often make extensive migrations in freshwater prior to the PST (e.g., Peterson 1982; Murray and Rosenau 1989; Scrivener et al. 1994) yet still home to their natal sites.

The natural migratory patterns of juvenile salmon in freshwater and subsequent natal stream homing led Harden Jones (1968) and Brannon (1982) to propose that juvenile salmon learn a series of olfactory way points as they migrate through freshwater to the sea and later retrace this odor sequence as adults. Such a scenario might explain the homing patterns observed in salmon released from locations other than their rearing site. Juvenile salmon transported to a different river system or long distances downstream within the same river system tend to return as adults to their release site (e.g., Donaldson and Allen 1957; Jensen and Duncan 1971; Solazzi et al. 1991; reviewed in Lister et al. 1981). On the other hand, the closer the release site is to the rearing site, the higher the percentage of salmon returning to their rearing site as adults (Lister et al. 1981; Johnson et al. 1990). This suggests that salmon may initially return to their site of release, but if they can then detect the odors of their rearing site they will migrate there. This hypothesis is supported by the finding that coho salmon smolts displaced downstream prior to release can learn the odor characteristics of both their rearing site and their downriver release site (Quinn et al. 1989).

Most studies investigating a sensitive period for home-stream imprinting in salmonids have focused on the ability of juveniles to learn site-specific odors either just prior to, or during PST (reviewed in Hasler and Scholz 1983). However, juvenile salmon learn odors during earlier developmental periods. Morphological evidence suggests that the salmonid olfactory system is functional as early as hatching (Brannon 1972; Hara and Zielinski 1989). Soon after emergence, salmon are able to learn odors associated with specific habitats (e.g., lake versus river water; Brannon 1972; Bodzien 1978) and odors from other fishes (e.g., Quinn and Hara 1986; Courtenay 1989). Courtenay and Morin, cited in Courtenay (1989), demonstrated that juvenile coho salmon exposed to morpholine shortly after or even before hatching retained a memory of this odorant over a year later. However, it is not known whether odor memories established in early development are maintained until maturity and used for homing. In the present study, we examined the timing of olfactory imprinting for homing by exposing juvenile coho salmon to natural odors or to an artificial odorant, PEA, as either embryos, parr, or smolts or continuously throughout their lives. We subsequently tested whether these fish retained memories of these odors by examining the behavioral responses of mature and immature fish 17 months later.

Materials and methods

Fish and experimental approach

On November 30 and December 1, 1987, we collected and fertilized 70,000 eggs from adult coho salmon that had returned to the University of Washington hatchery (UWH) (Figs. 1A, 1B). The resulting embryos were subsequently divided into the experimental lots described below. Salmon were reared under an accelerated feeding and temperature regime that allowed them to smolt in the first spring after hatching (Brannon et al. 1982). Stages of development during the PST were determined by changes in morphology and coloration, and the ability of fish to maintain blood plasma sodium levels within 5% during a seawater challenge (Clarke and Blackburn 1977; Sullivan 1986; Dittman et al. 1994). Between May 9 and 20, salmon were dark with distinct parr marks and were unable to maintain plasma sodium levels within 5% (freshwater exposure: 155.9 ± 0.8 mequiv. Na+/L plasma, N = 8; seawater exposure: 179.7 ± 2.7 mequiv. Na+/L plasma, N = 12). Thus, juvenile salmon exposed to odors during this period were parr. Between June 15 and 25, 1988, salmon were elongate and silvery and were able to maintain plasma sodium levels after seawater exposure (freshwater exposure: 152.9 ± 1.2 mequiv. Na+/L plasma, N = 10; seawater exposure: 159.0 ± 1.7 mequiv. Na+/L plasma, N = 8). Thus, juvenile salmon exposed to odors during this period were smolts.

Three sets of experiments were conducted to test the ability of juvenile coho salmon to imprint during distinct developmental stages. First, we assessed the ability of salmon to learn the artificial odorant PEA as a homing clue by exposing fish to PEA at the alevin, parr, or smolt stages and later examining the responses of mature fish to water scented with this odorant. Second, we assessed the ability of juvenile coho salmon to learn natural site-specific odors by manipulating the rearing water that they experienced during the embryo, parr, and smolt stages prior to release and examining their subsequent homing patterns as adults. Third, we compared the learning and homing ability of salmon exposed to a single water source throughout their lives (embryo to adult stages) versus salmon exposed to this water source only through PST.

Exposure to PEA at specific life stages

The ability of coho salmon to learn PEA as a homing clue was assessed by exposing juveniles to PEA at specific developmental stages and later examining the responses of mature fish to this odorant. Twenty thousand fertilized eggs were divided into four lots and reared separately in UWH water until hatching was completed in late January, 1988. The alevin, parr, and smolt exposure groups were continuously exposed to 10£ M PEA (Sigma Chemical Co., St. Louis, Mo.) from January 29 to February 20, from May 9 to 20, and from June 15 to 24, respectively. A fourth group was maintained as a PEA-naive control. After PST, the members of each group were given a distinct fin clip, transferred to the Seward Park hatchery (SPH) (Figs. 1A, 1B), and reared communally until they matured in fall 1989. Mature fish were tested for responsiveness to PEA in two separate controlled field studies described below.

Big Beef Creek experiment

PEA recognition experiments were conducted in a two-choice maze constructed in a controlled-flow spawning channel located at the University of Washington Big Beef Creek Research Station, Kitsap Peninsula, Washington (Fig. 1). Big Beef Creek is outside the Lake Washington watershed and its water was unfamiliar to all experimental fish. The two-choice maze and experimental protocol have been described previously (Dittman et al. 1994). Briefly, the maze was 39 m long with the extreme upstream section divided into two arms by a barrier extending downstream from the channel inlet. Traps in each arm allowed fish to move upstream into either arm but did not allow them to leave.

On November 11, 1989, 25 mature, tagged salmon from each
Fig. 1. (A) The location of the study sites in the Puget Sound region of Washington state, U.S.A. (B) An enlarged view of the Puget Sound region showing the location of study sites at Big Beef Creek, Issaquah Creek, the University of Washington hatchery (UWH), the Seward Park hatchery (SPH), and release locations for displacement experiments. (C) An enlarged view of the Issaquah Creek study site.

PEA exposure group were transported from SPH and released into the maze. Fish were allowed to move freely within the maze until they entered a trap. Each day, trapped individuals were removed and identified and their arm choice was recorded. Twice each week, new fish from each treatment were released into the maze to maintain the original density. We continued this procedure until 110 fish from each treatment had been tested. From November 11 to 14, no PEA was pumped into the maze and fish making choices during this period were designated as PEA-absent controls. Between November 15 and December 20, PEA was continuously pumped into the inlet of arm B of the maze to give a concentration of $10^{-7}$ M PEA. We also tested whether immature fish of the same age and PEA exposure history (i.e., cohorts that did not mature in 1989) would respond differently to PEA than mature fish. For these experiments, 10 immature salmon from each treatment were released into the maze on December 13 and monitored as described above.

The responses of the experimental groups in the presence and absence of PEA were compared using chi-square contingency analysis. We assumed that all fish were acting independently. Fish that did not enter either trap were excluded from the analysis of arm choice but were tallied for comparison between mature and immature fish.

**Issaquah Creek experiment**

We also measured PEA recognition in a natural stream setting, the
confluence of Issaquah Creek and its east fork in Issaquah, Washington (Fig. 1C). Issaquah Creek flows into Lake Sammamish in the Lake Washington watershed 3.4 km below the confluence of the two stream. On the main fork, the Washington State Department of Fisheries operated a weir and salmon hatchery 1.6 km upstream from the confluence with the east fork. During this study, salmon migrating up the main fork could not pass the weir and had to enter the hatchery. No traps or weirs were located on the east fork. The flow in the main fork was approximately 8–10 times greater than in the east fork, although flows fluctuated considerably during this study.

We expected that in the absence of any familiar odors (i.e., PEA), most fish either would migrate up the larger (main) fork or would not migrate upstream at all. On October 10, 1989, 1 week before releasing fish at the study site, we began pumping PEA into the east fork to establish a concentration of $10^6-10^7$ M PEA in this creek. The PEA pump was adjusted daily to maintain this concentration throughout the study. On October 17, 100 mature fish from each of the four exposure groups (PEA-naive and alevin-, parr-, and smolt-exposure) were released into Issaquah Creek at a point 1.2 km below the confluence of the two forks. Fish from each treatment group were tagged with numbered, color-coded disc tags so that treatment group and individual identity could be determined from a distance. Fish migrating up the main fork of Issaquah Creek were monitored by recovering tagged fish in the hatchery return pond and surveying the stream from the confluence to the hatchery ladder. Fish that migrated up the east fork were monitored three times per week by surveys of the stream from the confluence to a point 4.0 km upstream. Treatment group, identification number, and location were recorded for all tagged fish sighted. Both forks were monitored from October 19 until November 12. The proportion of PEA-exposed fish choosing the PEA-scented east fork versus the main fork was compared with the proportion of PEA-naive fish choosing either fork by using Fisher's exact test.

**Exposure to natural odors at specific life stages**

For these experiments, salmon were reared and released at the SPH, upstream from the UWH (Fig. 1B), except for brief exposures to UWH water during specific developmental stages. Upon release as smolts in 1988, these fish migrated to sea and their return patterns were examined in 1989 and 1990. Because returning adult salmon must swim within about 100 m of the UWH to reach the SPH (Fig. 1B), we hypothesized that salmon that had learned the odors of UWH during their brief exposure there would return to UWH.

Fertilized embryos for this experiment were divided into four groups of 10,000 fish and initially reared in dechlorinated Seattle city water at the University of Washington. On January 7, 1988, the parr-, smolt-, and non-UWH exposure control groups were transferred as eyed embryos to the SPH for further incubation. Between January 7 and March 13, 1988, the embryo-exposure group was reared in UWH water (supplemented with water pumped from the hatchery's adult homing pond) and then transferred to the SPH for further rearing until release as smolts. During this exposure period, fish in the embryo-exposure group experienced UWH -- homing pond water for approximately 3 weeks as unhatched embryos, 3–4 weeks as alevins, and 1–2 weeks as free-swimming fry. The parr- and smolt-exposure groups were transferred from SPH and exposed to UWH hatchery--homing pond water from May 9 to 20 (parr), and from June 15 to 25 (smolts), respectively. After exposure to UWH water, fish were transferred back to SPH and reared until release. The final group, non-UWH exposure control, never experienced UWH water prior to release from SPH. Table 1 summarizes the exposure protocol for the different treatment groups. Prior to release, fish that had not smolted (as determined by color and morphology) were culled. The remaining fish in each treatment group (approximately 7000 fish per group; average weight 17.53 g) were given a distinct coded wire tag and released into Lake Washington from the SPH on July 3, 1988.

Although adult University of Washington coho salmon returned primarily in 1989, the 2nd year after their release to the Lake Washington watershed, we monitored both the UWH and SPH for returning coded-wire tagged fish from 1988 through 1990. The number and location of experimental fish captured in both the commercial and sport fisheries was also estimated from the coded wire tag data base (Markay et al. 1990, 1991; Markay and Anderson 1992).

**Odor exposure duration: embryo-to-smolt versus embryo-to-adult exposure**

Typically, salmon only experience the odors of their natal sites prior to or during the PST. However, we tested whether exposure to an odor source beyond the smolt stage, without any seaward migration, would influence the tendency of mature salmon to return to that odor source. To do this, the homing tendency of salmon exposed to an odor source through the PST and released to migrate to sea was compared with that of salmon exposed to an odor source during their entire lives without any seaward migration.

**Embryo-to-adult exposure**

On July 7, 1988, eyed embryos were transferred from dechlorinated Seattle city water to the SPH and reared until maturity in fall 1989. Upon maturation, we tested the homing ability of these fish by displacing them a short distance downstream from SPH (Fig. 1B) and monitoring their tendency to return to the SPH. To determine whether the adult return to the hatchery was guided by olfaction rather than other sensory modalities, we made some of these fish anosmic.

On October 4, 1989, we anesthetized (MS-222; Argent) and tagged 135 mature salmon with numbered tags. Thirty-five fish were made anosmic by injecting softened wax into both nares. The wax rapidly solidified and blocked water access to the olfactory epithelium. To control for handling stress, the remaining 100 fish were treated similarly by injecting warm water into the nares (sham-operated fish). After a 48-h recovery period, the 100 sham-operated fish were transported and released from shore approximately 1.0 km north of the SPH. To ensure that anosmic fish did not simply follow sham-operated fish back to the hatchery, the anosmic fish were held for an additional 24 h before transport and release. The trap at the SPH was monitored daily for returning fish. To determine whether the anosmia operation affected survival, four anosmic and four sham-operated fish were retained at the hatchery for observation.

**Embryo-to-smolt exposure**

For comparison with the salmon reared exclusively in the hatchery,
Fig. 2. Behavioral responses of mature coho salmon to β-phenylethyl alcohol (PEA) in the Big Beef Creek two-choice arena. Salmon were exposed to PEA at the developmental stage indicated or never experienced PEA (control). Open bars show the proportion of salmon choosing arm B in the absence of added PEA. Shaded bars show the proportion of salmon choosing arm B in the presence of PEA metered into arm B. Numbers above bars indicate the total number of fish choosing either arm A or B.

Test this experiment was also conducted using anosmic and sham-operated salmon displaced downstream after completing their natural homing migration. Because the SPH does not maintain a coho salmon run, these experiments were conducted at the UWH with salmon that were released as smolts and had returned to the hatchery after 1.5 years at sea. On October 26, 1988, 20 anosmic and 20 sham-operated tagged salmon were transported approximately 5 km downstream and released (Fig. 1B). The UWH homing pond was surveyed each day thereafter for tagged fish. As above, four anosmic and four sham-operated fish were maintained in the hatchery to assess the impact of the anosmia operation on health and survival.

Results

Exposure to PEA at specific life stages
In the absence of PEA, mature salmon tested in the experimental channel at Big Beef Creek entered arm A of the arena in greater numbers than arm B (p < 0.05 for all groups) and the proportion of fish entering arm A was the same for all PEA-exposure groups (χ^2 = 0.17; p < 0.99; Fig. 2). When we subsequently metered 10^-7 M PEA into the less preferred arm (arm B), the choices of salmon in the PEA-naïve (p < 0.90), alevin-exposure (p < 0.99), and parr-exposure (p < 0.95) groups were indistinguishable from their responses in the absence of PEA (Fig. 2). In contrast, mature salmon that had been exposed to PEA as smolts showed a greater tendency to enter arm B when PEA was present than when it was absent (χ^2 = 3.97; p < 0.05; Fig. 2). Of the 440 mature fish tested, 357 (81.1%) entered one arm or the other during the experiment. None (0.0%) of the 40 immature salmon from the four PEA-exposure groups entered either arm of the arena over the 7 days of testing. They remained primarily in the downstream section of the arena but appeared to be healthy and active.

The responses of mature PEA-exposed fish to PEA in Issaquah Creek were similar to the responses of their cohorts tested at Big Beef Creek. For all treatment groups, the majority of fish that migrated upstream were recovered in the Issaquah Creek hatchery (Table 2). However, the proportion of fish choosing the PEA-scented east fork varied according to their PEA exposure history. The proportion of mature salmon choosing the PEA-scented east fork was the same (1/26; 0.04%) for PEA-naïve fish and fish exposed to PEA as alevins. On the other hand, a larger proportion of the salmon exposed to PEA as smolts migrated up the PEA-scented fork (9/33; 27.3%) compared with controls (p = 0.018). The parr-exposure group showed a slightly greater tendency to ascend the PEA fork (5/29; 20.8%) than controls (p = 0.12). The majority of fish released below the stream confluence were not recovered. Some of these fish probably moved downstream and into Lake Sammamish, where several tagged fish were recovered. All but one fish observed in the east fork was sighted on more than one occasion. Fish originally found in the east fork were never subsequently captured at the hatchery located on the main fork.

Exposure to natural odors at specific life stages
Only salmon that experienced the UWH water as parr and smolts returned to the UWH (Table 3). Fish exposed to UWH water as embryos and control fish that never experienced UWH water returned exclusively to the SPH. Poor return rates, however, precluded statistical analysis. Of the 28 000 fish released as smolts from the SPH, only 35 mature fish returned to the SPH and UWH. The low return rates to the hatcheries were paralleled by poor returns from fishery samples

Table 2. Numbers of adult coho salmon recovered in either the β-phenylethyl alcohol (PEA)-scented east fork or the main fork (control) of Issaquah Creek after release 1.2 km below the confluence of the two streams.

<table>
<thead>
<tr>
<th>Recovery site</th>
<th>Treatment groups</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Main fork</td>
<td></td>
</tr>
<tr>
<td>(control)</td>
<td>25</td>
</tr>
<tr>
<td>East fork</td>
<td></td>
</tr>
<tr>
<td>(PEA-scented)</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: Salmon were exposed to 10^-7 M PEA at three different developmental stages: alevin, parr, and smolts. Control fish never experienced PEA.

Table 3. Patterns of homing displayed by adult coho salmon reared and released at the Seward Park hatchery (SPH) except for brief exposures to University of Washington hatchery (UWH) water at one of three different developmental stages: embryo, parr, and smolt.

<table>
<thead>
<tr>
<th>Recovery site</th>
<th>Treatment</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>SPH</td>
<td>14</td>
</tr>
<tr>
<td>UWH</td>
<td>0</td>
</tr>
<tr>
<td>Commercial and sport fisheries</td>
<td>13</td>
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</tbody>
</table>

Note: Control fish never experienced UWH water.
(Table 3), suggesting low survival rather than impairment of homing.

Odor exposure duration: embryo-to-smolt versus embryo-to-adult exposure
The percentage of mature salmon returning to their rearing site after displacement was lower for fish that had lived there throughout their lives than for fish released as smolts and allowed to migrate naturally. Only 18 (18.0%) of the 100 sham-operated salmon that had spent their entire lives at the SPH returned to that facility after being displaced only 1.0 km away (Fig. 3). The time to return varied between 1 and 60 days. Eighteen days after release, two of these fish were recovered 18 km away in the UWH homing pond. None of the 35 anosmic fish displaced from Seward Park returned to the SPH. No difference in survival was detected between anosmic (21.2 ± 1.5 days, mean longevity ± standard deviation (SD)) and sham-operated (20.0 ± 0.9 days) fish maintained at the hatchery during the course of this study.

In the parallel study of salmon exposed to UWH water as juveniles only, 14 (70%) of the 20 sham-operated salmon released downstream from the UWH returned to that hatchery within 5 days and 18 salmon (90%) returned within 15 days (Fig. 3). The two salmon (10%) that did not return were captured in the commercial gill-net fishery in Lake Union. In contrast, none of the anosmic salmon returned to the UWH pond up to 60 days after release and none were reported captured in the fishery (Fig. 3). One anosmic fish was observed in the outflow of the UWH but did not enter the facility. There was no difference in survival between treatment groups for fish maintained in hatchery raceways (longevities were 14.7 ± 0.9 days for anosmic and 15.7 ± 1.0 days for sham-operated fish). Experimental manipulations were not the cause of mortality in either the lifelong or juvenile exposure experiments. The timing of death in both experiments was consistent with the natural mortality occurring after maturation and spawning.

Discussion
Timing of imprinting
Our PEA and water source exposure experiments indicated that the end of the PST (smolt stage) is a particularly important period for olfactory learning in coho salmon reared in a hatchery. In two separate experiments, mature salmon that were exposed to 10^{-7} M PEA for only 10 days as smolts displayed an increased attraction to PEA-scented water when tested 17 months later. Similarly, the return patterns of salmon exposed to UWH water as smolts, but released from the SPH, suggested that some of these fish had learned and were attracted to the UWH water. Two of the five returning fish that were exposed to UWH water as smolts were recovered at the UWH. In contrast, 100% of the 14 fish in this experiment and all 82 fish in a previous study (Brannon and Quinn 1990) that were reared and released at SPH without any UWH water exposure bypassed the UWH and returned to the SPH. Long-term olfactory learning during the smolt stage was also indicated by biochemical and electrophysiological measurements of increased peripheral olfactory sensitivity to PEA in fish from the group exposed to PEA as smolts (Dittman 1994; Nevitt et al. 1994).

While coho smolts clearly learned olfactory homing clues, there was no evidence for such learning by embryos in these experiments, even though embryos were exposed to both natural water and PEA for 2–8 weeks longer than the smolt-exposure group. Similarly, the evidence for imprinting by parr was weak. One of the seven returning fish that were exposed to UWH water as parr did return to the UWH as an adult. There was also a slightly increased tendency for salmon exposed to PEA as parr to migrate up the PEA-scented fork of Issaquah Creek. On the other hand, there was no indication of parr learning in the Big Beef Creek experiment and in all cases the homing responses of fish exposed to odors as parr were weaker than those of smolt-exposure fish and did not differ significantly from those of controls. These findings agree with previous studies implicating smolting as a critical period for olfactory imprinting (Hasler and Scholz 1983). Scholz (1980) found that coho salmon did not imprint on artificial odorants prior to PST and he proposed that olfactory imprinting in coho salmon is associated with elevated thyroid hormone levels that occur during PST. Similarly, Morin et al. (1989a) found that Atlantic salmon, Salmo salar, had an optimal period for long-term olfactory learning 21–28 days after the onset of PST, coinciding with peak levels of thyroid activity (Morin et al. 1989b).

Our results also suggest that zero-age coho smolts (i.e., 6 months old) provide an appropriate time-saving model system for studying the timing of imprinting. Coho salmon normally experience the PST as 18-month-old fish but under appropriate rearing conditions this process can be accelerated by 12 months (Brannon et al. 1982). The embryonic and parr stages were not affected by the accelerated rearing regime. Changes in morphology, behavior, and seawater adaptability all indicated that zero-age fish successfully smolted and were able to imprint and home accurately (Quinn et al. 1989; Brannon and Quinn 1990; the present study). There are physiological differences between zero-age and yearling smolts (e.g., reduced thyroxine surges in zero-age smolts) but these differences have no
obvious effects on imprinting and homing ability (Dittman et al. 1994).

**Influence of sexual maturity**
The absence of any evidence of presmolt imprinting is interesting because Courtenay (1989) found that coho salmon exposed to the artificial odorant morpholine as prehatch embryos, alevins, or fry responded to this odorant in behavioral tests 2–4 months later. Furthermore, Courtenay’s fish maintained an olfactory memory for morpholine more than 1 year after exposure, as indicated by heart rate reductions in the presence of this odorant (Courtenay and Morin; cited in Courtenay 1989). The differences between our results may reflect the different developmental status of salmon tested for odor recognition (i.e., mature versus immature) because the long-term odor memory demonstrated by Courtenay (1989) may not have been associated with reproductive homing. Presmoltts learn odors associated with specific habitats (e.g., lake versus river water) (Brannon 1972; Bodzick 1978) and odors from other fishes (e.g., Quinn and Hara 1986; Courtenay 1989), but it is not known whether such odor memories are important for homing.

In our Big Beef Creek experiments, immature salmon did not respond to PEA-scented water regardless of their PEA exposure history. Whether this reflects an inability to recognize PEA or a lack of motivation to respond to PEA is not clear but it does indicate that mature and immature salmon respond differently to odors learned as juveniles. Consistent with our results, Hasler and Scholz (1983) reported that only mature coho salmon responded behaviorally to imprinted odorants. Electrophysiology and heart rate conditioning experiments also indicated that olfactory sensitivity to imprinted odorants was greatest during maturation (Cooper and Hasler 1973; Hasler and Scholz 1983). It is important to note, however, that homing and maturation are not inextricably linked. Many populations of salmon migrate back to their river of origin months before maturing (e.g., spring chinook salmon and summer steelhead, *Oncorhynchus mykiss*). In these races, fish may home hundreds of kilometres upriver to the general vicinity of their natal stream, then hold in the main-stem river for several months before resuming their homing migration to their spawning site (e.g., Berman and Quinn 1991).

**Presmolt imprinting**

*Sequential imprinting*
The apparent lack of presmolt imprinting in these experiments may be due, in part, to the methods we and others have used to assess odor recognition and attraction. As part of the sequential imprinting hypothesis, Harden Jones (1968) proposed that during their homing migration, salmon retrace their seaward migration by first seeking the last odor they experienced before going to sea, then the next-to-last odor, and so on, in sequence until they arrive back at the natal site. If this is correct, the salmon in our study may have imprinted prior to smolting but did not respond to these odors because they did not first experience the odors they learned as smolts. This explanation is consistent with our PEA experiments and data from displacement experiments on the Columbia River in which salmon that had been released upriver from their rearing site as smolts migrated past the rearing site as adults and subsequently returned to the release site (Slatick et al. 1988; Pascal et al. 1995). In one Columbia River experiment, however, juvenile coho salmon released upstream of their hatchery returned as adults to their rearing hatchery, not their release site (Johnson et al. 1990). In our study, 3 of the 35 adults returning to Lake Washington after release from SPH as juveniles were recovered at the UWH but it is uncertain whether these fish entered the UWH when they first encountered UWH water, or, rather, first returned to the vicinity of the SPH and then began searching for UWH water.

**Influences of thyroid hormones**
Another factor that may have contributed to the lack of any observed presmolt learning is the different rearing environments experienced by hatchery-reared and wild fish and the resulting differences in thyroid hormone production. Although a direct link between elevated plasma thyroxine levels and imprinting has been questioned (Dittman et al. 1994), thyroid hormone signalling has been repeatedly implicated in olfactory imprinting (e.g., Hasler and Scholz 1983; Morin et al. 1989a, 1989b). Scholz (1980) demonstrated that presmolt salmon were unable to learn artificial odors unless they were injected with thyroid stimulating hormone to raise thyroid hormone levels. Similarly, Morin et al. (1989a) found that Atlantic salmon had an optimal period for long-term olfactory learning 21–28 days after the onset of the PST, coinciding with peak levels of thyroid activity (Morin et al. 1989b).

Nishioka et al. (1985) hypothesized that the stable rearing conditions in most hatcheries (e.g., constant temperature, flow rate, water source) may provide insufficient environmental stimuli for optimal thyroid development. Under such conditions, hatchery fish may experience only an endogenously controlled smolting-related increase in thyroxine levels. In contrast, wild salmon experience various environmental changes that may elicit multiple thyroxine surges throughout the period of freshwater rearing (Nishioka et al. 1985). Exposure to novel water, changing lunar cycles, increased water velocity, and temperature fluctuations have all been linked to increased thyroid activity (Grau et al. 1981; Dickhoff et al. 1982; Iwamoto 1982; Youngson and Simpson 1984; Nishioka et al. 1985). Therefore, if thyroid hormones are involved in imprinting, the different thyroid hormone profiles of hatchery and wild fish might explain the apparent inconsistencies between experimental evidence obtained with hatchery-reared fish indicating that PST is a critical period for imprinting and studies with wild fish that indicate that salmon must also learn at other times.

**Role of migration in imprinting**
While our results indicated that the end of the PST (smolt stage) is a sensitive period for olfactory imprinting, the poor homing performance of salmon exposed throughout their lives to SPH water suggests that exposure to a water source during PST may not, in itself, be sufficient for imprinting. Only 18 of the 100 salmon displaced just 1.0 km downstream were recovered at the SPH, 2 fish were recovered at the UWH (which they had never experienced), and the time to return to the SPH was as high as 60 days. In contrast, 90% of the salmon that were released as smolts, and experienced both seaward and homing migrations, returned to their home hatchery within 15 days after displacement. As expected, none of the anomic fish
returned to their home hatchery, confirming that the olfactory sense is required for salmon homing (Wisby and Hasler 1954). The differences in the return rates of salmon displaced from the SPH and UWH were not likely due to any inherent problems with the SPH as a return site. For example, all of the returning coho salmon reared and released from the SPH as smolts returned to that hatchery at maturity (Brannon and Quinn 1990; the present study).

The most likely factor influencing the return patterns was the different migratory experience of each group as juveniles. We hypothesize that there is a sensitive window (i.e., PST) during which salmon must migrate for optimal imprinting to occur. Thus, salmon held for their entire lives at the SPH and not allowed to migrate during the PST exhibited impaired homing compared with fish released from the UWH as smolts and allowed to migrate naturally. Salmon released from hatcheries as smolts show a greater tendency for homing than those released after the smolt stage (chinook salmon: Unwin and Quinn 1993; Pascual et al. 1995; Atlantic salmon: Hansen and Jonsson 1991). Furthermore, displacement experiments in which smolts are trucked downstream for release have indicated that while some fish return to their natal site, the majority return as adults to the area where they began their downstream migration (Slatick et al. 1988; Quinn et al. 1989).

How might migration be involved in imprinting? During migrations, swimming rates often increase and salmon move into new environments of differing water chemistry, temperatures, and flow rates. All these factors can stimulate thyroxine production (Dickhoff et al. 1982; Nishioka et al. 1985) and migrating salmon may therefore experience elevated thyroxine levels that may facilitate olfactory imprinting (see above). This may also explain why wild salmon apparently learn odors as fry (perhaps when they migrate from their incubation sites) but hatchery-reared salmon show no evidence of such learning.

Concluding remarks
In summary, our results confirm the importance of the parr–smolt transformation as a sensitive period for olfactory imprinting but suggest that exposure to a water source alone is not sufficient for optimal imprinting. There was little evidence for presmolting learning of homing clues by hatchery-reared fish. Because salmon in the wild often learn home site odors prior to smolting, the lack of any observed early learning in our study may be due to the uniformity of hatchery rearing conditions and lack of migration. Finally, our results suggest that while migration may not be absolutely required for olfactory imprinting, the combination of stimuli associated with migration and physiological changes involved in smolting may be important for optimal imprinting and homing.

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