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Experimental Evidence for Olfactory Imprinting by Sockeye Salmon at Embryonic and Smolt Stages

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Abstract
Anadromous salmonids have an extraordinary ability to migrate back to their natal streams to spawn as adults, but the mechanisms underlying this ability are not completely known. Many experiments indicate that salmon imprint on natal odors at the smolt stage prior to seaward migration, but the life history and population genetics of some species, notably Sockeye Salmon Oncorhynchus nerka, suggest that imprinting also occurs during the period between hatching and emergence from the gravel as fry. To test the hypothesis that Sockeye Salmon imprint during this period, we exposed juveniles to a mixture of odorants during either the alevin or smolt stage. The smolt exposure group was further divided into different exposure durations (6 weeks, 1 week, and 1 d) to evaluate the duration of odor exposure needed for imprinting during that stage. Imprinting was assessed by testing fish as mature adults in two-choice mazes containing unfamiliar water with and without the mixture of odorants. Fish exposed either as alevins or for 6 weeks as smolts both spent significantly more time in the odor-scented arm than control fish (unexposed to the odors as juveniles). Fish exposed to odors for 1 week or 1 d as smolts showed similar but weaker responses. Concurrent measures of gill Na⁺/K⁺-ATPase activity and plasma thyroxine confirmed that the fish exposed as smolts were undergoing parr–smolt transformation during exposure. We conclude that Sockeye Salmon imprinted as both alevins and smolts and that longer periods of odor exposure yielded greater behavioral responses to odors as adults, though specific times within the parr–smolt transformation period may be more sensitive to imprinting than others.

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Pacific salmon (genus: *Oncorhynchus*), Atlantic Salmon *Salmo salar*, and their relatives include many anadromous species that spawn in freshwater, rear in lakes and streams as juveniles, migrate to sea, and then return (home) to their natal sites to spawn (Quinn 2005; Jonsson and Jonsson 2011). Prior to leaving freshwater, juvenile salmon imprint on certain chemical cues in the water that will direct their migration back to their spawning grounds as adults (Hasler and Scholz 1983). This process has been a model for similar olfactory imprinting in other fishes, including White Suckers *Catostomus commersonii* (Werner and Lannoo 1994), anemonefish (Dixson et al. 2014), and other vertebrates such as sea turtles (Grassman et al. 1984). There is, therefore, a broad need to understand how the imprinting process operates and, in particular, whether salmon imprint at a single or multiple times prior to entry into the ocean.

A large body of work on Coho Salmon *O. kisutch* and Atlantic Salmon has revealed that the parr–smolt transformation (PST) is a critical period for imprinting to natal stream water (Hasler and Scholz 1983; Morin et al. 1989a; 1989b; Morin and Døving 1992; Dittman et al. 1996). However, the life histories of Coho and Atlantic Salmon differ from those of some other salmonid species, rearing for one or more years in the natal stream system before starting the process of PST (McCormick et al. 1998; Quinn 2005; Jonsson and Jonsson 2011). These species often migrate short distances downstream and use off-channel habitats, typically in the fall (e.g., Coho Salmon: Peterson 1982; Atlantic Salmon: McCormick et al. 1998), but the smolts generally migrate to sea from the same river where they were spawned. In contrast, Sockeye Salmon *O. nerka* fry emerge from tributary and outlet streams or emerge from lake beaches in late spring and typically migrate directly to a nursery lake to rear for 1 or 2 years before migrating to sea as smolts (Burgner 1991; Quinn 2005). This migration from the natal site at an early life stage implies that imprinting to natal water occurs during or before emergence to ensure homing to the stream of origin. Evidence from field experiments indicates that Sockeye Salmon can home at exceptionally fine spatial scales to the site where they incubated and emerged as fry (Quinn et al. 2006). Studies of the genetic population structure of Sockeye Salmon also provide evidence for strong natal stream fidelity as different spawning tributaries typically represent discrete breeding units. (Beacham et al. 2006; Habicht et al. 2007; Gomez-Uchida et al. 2011). Thus, the pattern of imprinting in Sockeye Salmon may involve both the sites of incubation and emergence, as well as the site of PST and seaward migration. Such migration from natal sites is not limited to Sockeye Salmon, however, because some nonanadromous trout also migrate from natal streams to lakes as fry, returning to the streams to breed years later (e.g., Rainbow Trout *O. mykiss*: [Northcote 1962]; Cutthroat Trout *O. clarkii* [Raleigh and Chapman 1971]).

We examined the timing and duration of olfactory imprinting in juvenile Sockeye Salmon, testing the hypothesis that imprinting takes place at the incubation—emergence and smolt stages. Juveniles were exposed to a mixture of amino acids and phenyl-ethyl alcohol (PEA) as alevins or as smolts and for varying lengths of time during the PST, and they were subsequently assessed at maturity using behavioral assays to determine the life stage and duration of exposure needed to elicit olfactory recognition in adults. We also quantified the PST process in these fish by measuring plasma thyroxine (T4) and gill Na+/K+-ATPase (NKA) activity to place the imprinting results in the context of sensitive physiological windows for imprinting during smolting.

**METHODS**

**Study population and experimental design.**—Okanogan River Sockeye Salmon primarily spawn in October in the upper Okanogan River, a tributary to Osoyoos Lake spanning the border of Washington, USA, and British Columbia, Canada, (Gustafson et al. 1997). Fry emerge in March or April, migrate to the lake and rear there, typically for 1 year (Kendall et al. 2010), before their seaward migration in mid-to-late May (Gustafson et al. 1997). Most fish return to spawn after 2 years at sea, although some return after only a single year (Kendall et al. 2010). The fish used in these experiments were the offspring of adults collected at Wells Dam on the Columbia River during their homing migration. On October 30, 2000, adults were spawned at the Cassimer Bar Hatchery and 4,000 eyed embryos (representing four crosses of one female × two males each) were transferred to the Northwest Fisheries Science Center hatchery in Seattle, Washington on November 30, 2000, to initiate the study. The population was divided into three experimental groups based on when the salmon were exposed to odors: alevin–emergent fry, smolt, and control (never exposed to the test odors). The smolt group was further divided into groups exposed to odors for 1 d, 1 week, or 6 weeks to assess the importance of exposure duration for successful imprinting. The imprinting odor for these experiments was a mixture containing L-glutamate, L-arginine, and L-threonine, and phenyl-ethyl alcohol (PEA; all from Sigma-Aldrich, St Louis, Missouri) prepared in Milli-Q purified water. These amino acids and PEA were chosen because they have all been identified as potent odors for fish or have been used for previous imprinting studies (Hasler and Scholz 1983; Morin et al. 1989a, 1989b; Dittman et al. 1996; Shoji et al. 2000). The three amino acids used in this study represent odors that activate distinct receptor types in the olfactory epithelium (Hara 1992). Each group was exposed to the odorant mixture by metering a stock solution into the rearing water using a peristaltic pump to generate a final continuous concentration of 100 nM of each odorant.

Experimental fish were reared initially at the Northwest Fisheries Science Center in dechlorinated Seattle city water with temperatures ranging between 8°C and 10°C. Fish hatched in January 2001 and were ponded in early March.
The alevin–emergent fry group was continuously exposed to the odorant mixture from February 1 to March 5, 2001, a period spanning yolk utilization, absorption, and emergence. Fish were transferred to the University’s Big Beef Creek (BBC) hatchery, near Seabeck, Washington in August 2001 and reared in well water (constant 10°C) for the remainder of the experiment. Subsets of the smolt exposure group were exposed to the odorant mixture for 6 weeks (April 15 to May 24, 2002), 1 week (May 17–24, 2002), or 1 d (May 24, 2002). All treatment groups were maintained separately until after the PST (May 31, 2002) and then marked by treatment using both passive integrative transponder (PIT) tags and fin clips and reared communally in 4.5-m-diameter tanks with no further exposure to the imprinting odors. The fish initiated sexual maturation in 2003, as evidenced from changes in color and morphology and confirmed by postmortem examination of gonad development.

Smolt physiology.—Beginning in February 2002, 24 fish (4–5 fish/treatment) were sampled every 3 weeks to assess the timing of the PST. Each sampling date, fish were euthanized in a buffered solution of tricaine methanesulfonate (250 mg/L; Argent Chemical Laboratories, Redmond, Washington), measured for fork length (mm) and weight (0.1 g). Blood was immediately collected from the caudal vein using heparinized Natelson tubes (Fisher Scientific Inc., Pittsburgh, Pennsylvania), centrifuged (15 min at 10,000 × gravity) to isolate plasma, and frozen on dry ice for later analysis of thyroxine (T4) concentration. A small portion of gill tissue was collected, placed in 0.5 mL of ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH = 7.3), and snap frozen on dry ice for later assessment of gill Na+/K+-ATPase activity (NKA; assayed using the method of McCormick 1993) as an indicator of smolting. All samples were subsequently stored at −80°C until further analysis. Plasma T4 levels were measured by radioimmunoassay using the method of Dickhoff et al. (1982). Anti-L-T4 antibody was from Accurate Chemical and Scientific Corporation (Westbury, New York) and 125I-labeled T4 was from Perkin–Elmer (Waltham, Massachusetts).

Behavioral testing.—The behavioral responses of mature adult Sockeye Salmon (115 males, 119 females) to the imprinting odor mixture were tested in three two-choice mazes at the BBC facility between November 18 and December 9, 2003. Each maze consisted of a fiberglass raceway (3.05 × 1.22 × 1.22 m) with a divider separating the upstream 0.76 m of each raceway into two separate arms (Figure 1). Each arm was supplied by a head tank (0.91 × 0.61 × 0.91 m) feeding into the side. Previous studies have demonstrated that the expression of natal water choice is stronger when imprinted odors are presented to the fish in unfamiliar background water rather than the water in which they had been reared (Dittman et al. 1996). To provide unfamiliar background water for testing (rather than the well water in which they had been reared), ambient stream water was pumped from a side channel of Big Beef Creek (hereafter, BBC water) into each head tank, and the flow was maintained at approximately 150 L/min in each arm. Prior to testing, fish were acclimated for 1–2 d in holding tanks supplied with BBC water to allow them to adjust to the different temperatures between the rearing well water (10°C) and testing BBC water (5–8°C).

To start a trial, a mature fish (37–47 cm FL) was moved from the holding tank into the downstream section of the maze and allowed to acclimate for 30 min. The experimental group to which the fish belonged was unknown to the observer during the trial. During the acclimation period, a screen placed at the downstream end of the divider prevented fish from entering either arm. During the last 5 min of the acclimation period and for the remainder of the trial, the mixture of test odors (PEA, l-arginine, l-glutamate, and l-threonine) was pumped into one arm of the raceway to generate a final concentration of 100 nM of each odorant. After the acclimation period, the screen was lifted and the fish was allowed to swim freely for 40 min. Fish were observed from behind a blind at the upstream end of the maze, which prevented any

FIGURE 1. Schematic drawing of the two-choice maze for testing adult salmon. Arrows indicate direction of water flow. Water flowed into each upper rectangular tank, then into the large rectangular raceway. A divider separated the upstream 0.76 m to create two “choice” areas. The acclimation screen separated the downstream acclimation area from the choice areas to prevent fish from accessing those areas prior to the start of a trial.
disturbance during the trials. Four metrics of choice were recorded: first and last arms entered, number of entries into each arm, and time spent in each arm. To determine if behavioral responses changed over time, the data from the first 10 min, middle 20 min, and last 10 min were examined separately. The periods for analysis were established a priori before any trials began. At the end of each trial, the peristaltic pump for the odor mixture was turned off and the fish was removed, scanned for a PIT tag and fin clip to determine its experimental group, and measured for fork length. Reproductive status (spermiating, ovulating) was also determined at this time. To remove residual odors, each maze was flushed with BBC water for 5–10 min between trials. At the end of each day, the mazes were drained and scrubbed and the odor input tube was switched to the opposite arm for the next day’s trials to eliminate any bias associated with inherent arm preferences. Fish that did not enter either arm (31 total) during the 40 min trial were excluded from all analyses.

Data analysis.—Data for the first and final arms entered during each period (0–10 min, 10–30 min, 30–40 min) were analyzed using two-tailed Fisher exact tests comparing the experimental group to the responses of the control fish. The proportion of time spent in the odor arm versus the arm with only background water were compared between experimental groups and the control group using a two-tailed t-test after the proportions were normalized using an arcsine-square-root transformation (Zar 1984). The number of entries into the odor arm versus background water arm for each fish was compared using a paired t-test.

To assess whether the behavioral responses of fish changed across the three observation periods, we first compared the proportion of time a fish spent in the odor arm between the three periods using two-way ANOVA with odor exposure history and time period the effects modeled. We also compared the total number of arm entries per minute by two-way repeated measures ANOVA with odor exposure history and period as main effects, and then conducted Tukey post hoc analyses for pairwise comparisons among all treatment groups and times.

The trials of fish exposed to the experimental odors at the smolt stage were, in a sense, also a positive control because these fish should be undergoing the PST, which is the developmental period widely recognized as the time of olfactory imprinting. It was therefore important to determine whether the fish were in fact undergoing the PST when they were exposed to the odors. To do so, seasonal changes in plasma T4 and NKA during the PST were analyzed using ANOVA and followed by Tukey post hoc analyses with sample date the main factor. These physiological measures are standard, well-established assays for evaluating the readiness of salmon to transition to seawater and are linked to other aspects of the PST developmental process (Zaugg and McLain 1972; Hoar 1976; Beckman et al. 2000). Samples were taken from late February through early June, bracketing the period of odorant exposure for all groups. In all cases, significance was set at α = 0.05. All analyses were performed using Prism (Graphpad, La Jolla, California).

RESULTS

To examine the timing of the PST and its relationship to the periods of odor exposures and imprinting, we examined the physiological processes prior to, during, and after the exposure periods. Plasma T4 levels began to rise in late March to early April and peaked in mid-May, near the time of the 1-week and 1-d odor exposures (Figure 2). Changes in NKA lagged slightly behind changes in plasma T4, rising in April and peaking in mid-May. The 6-week odor exposure corresponded with the PST, while the 1-week and 1-d exposures occurred near the end of the PST period (Figure 2).

Adults from the alevin and 6-week smolt exposure groups spent a significantly greater percentage of their time in the odor arm (alevin: 60.1% ± 5.2 [mean ± SE], 6-week smolt: 58.5% ± 4.9) than did control fish (46.4% ± 4.89) during the first 10 min of the trials (Figure 3A). Fish exposed to the odors for 1 week or 1 d during smolt ing spent 52.0 ± 6.2% and 50.7 ± 7.2%, respectively, of their time in the odor arm, but their responses did not differ from those of controls. Similarly, fish exposed to the imprinting odors as alevins or for 6 weeks during smolt ing made more entries into the odor-scented arm than the background arm (Figure 3B). Fish exposed to the

![Figure 2](image-url)
odors for shorter periods during smolting and control fish did not enter the odor-scented arm more frequently than the background arm.

Other measures of odor attraction also suggested that previous exposure to imprinting odors influenced the behavioral responses of mature adults. Fish exposed to odors for 6 weeks and 1 week during the PST (but not alevin-exposed or 1-d PST fish) were more likely to be located in the odor arm at the end of the first 10-min period than odor-naive control fish (Table 1). None of the odor exposure groups demonstrated a greater tendency than control fish to enter the odor-scented arm first ($P \geq 0.318$). We observed no differences between male and female Sockeye Salmon for any odor preference measurement.

We observed that the responses of fish to imprinting odors tended to change over the course of the behavioral trial. For all four experimental odor exposure groups, the percentage of time spent in the odor arm declined across the three periods ($F_{2, 543} = 3.67$, $P = 0.026$) but remained approximately the same over time for the control group, suggesting an extinction of positive responses to odors over time (Figure 4A). In addition, overall activity of the experimental fish, measured by the number of entries into each arm of the maze, increased as the trial progressed for all treatments (ANOVA: $F_{2, 300} = 30.5$, $P < 0.0001$; Figure 4B) from an overall average $\pm$ SE of $0.73 \pm 0.07$ entries/min in the first 10 min to $1.042 \pm 0.05$ in the middle period and $1.16 \pm 0.04$ in the final 10 min, indicating increased exploratory behavior over time. There was no effect of odor exposure on the changes in entry frequency ($F_{4, 150} = 1.075$, $P = 0.371$). The combination of these results indicated a more directed response in the first 10-min period, and greater, but less directed activity during the remainder of the trial.

**DISCUSSION**

**Timing of Exposure Stage/Life History**

Sockeye Salmon exposed to a cocktail of odors containing PEA and amino acids during either the alevin or smolt...
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FIGURE 4. (a) Mean time spent in the arm scented with imprinting odorants relative to the total time spent in both arms (%) and (b) mean number of entries into both arms of the two-choice maze during each time period for the five experimental odor-exposure groups. The error bars represent standard errors of the mean. For each treatment, differences between time periods were assessed by two-way ANOVA followed by post hoc Tukey multiple-comparison tests. Asterisks indicate significant differences \( P \leq 0.05 \) in time spent or the number of entries relative to those for the initial time period. Fish that entered neither arm during the trial were excluded from the analysis.

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stages learned and remembered these odorants, as indicated by their responses as maturing adults up to 2.5 years after exposure. These results suggest that both the alevin and smolt stages are especially important periods for successful olfactory imprinting in this species. Specifically, the timing of imprinting demonstrated in this study is consistent with the life history patterns and migratory timing of the Okanogan River Sockeye Salmon used in this experiment. In the wild, natal site imprinting in tributaries must occur prior to fry emergence and downstream migration to Lake Osoyoos which typically begins in early March (Gustafson et al. 1997), and this behavior is consistent with the timing of alevin imprinting (February 1 to March 5) demonstrated in this study. Similarly, smolt out-migration from Lake Osoyoos occurs in mid-to-late May (Peven 1987), consistent with the PST physiology and smolt imprinting period we observed. We specifically targeted these periods for study because they are typical of the life history of Sockeye Salmon (Quinn 2005), although it is also possible that other developmental stages that we did not examine may also be sensitive windows for imprinting.

Previous laboratory studies with other salmon species (e.g., Coho Salmon and Atlantic Salmon) had indicated that the PST, when salmon initiate their seaward migration, is the primary developmental period in which successful olfactory imprinting occurs (Hasler and Scholz 1983; Dittman et al. 1996; Morin et al. 1989a; 1989b). The differences in temporal sensitivity to imprinting observed here and in earlier studies may reflect differences in the life history patterns of the salmon species studied. Many of the earlier studies used Coho and Atlantic Salmon, which have relatively simple freshwater life history patterns typically rearing in the natal stream before starting the process of PST and downstream migration (McCormick et al. 1998; Quinn 2005; Jonsson and Jonsson 2011). In contrast, species such as Pink Salmon \( O. gorbuscha \) and Chum Salmon \( O. keta \) migrate to the ocean shortly after emergence from their incubation site, suggesting imprinting at an early juvenile stage. Similarly, Sockeye Salmon emerge from their natal gravel in tributary and outlet streams or emerge from incubation sites on lake beaches in late spring and migrate directly to their nursery lake to rear for 1 or 2 years before migrating to sea as smolts (Burgner 1991; Quinn 2005). Upon return, the adults home to their natal streams and beaches with very high fidelity, often at fine spatial scales relative to their incubation site, as evidenced by population genetic analysis (Habicht et al. 2007; Gomez-Uchida et al. 2011) and field experiments with marked fish (e.g., Quinn et al. 2006). This early migration from the natal site suggests that imprinting must occur during or before emergence for Sockeye Salmon to ensure homing to the stream or beach of origin.

While the life history of Sockeye Salmon differs from that of the archetypal life history of Coho and Atlantic Salmon, the freshwater migratory patterns of other salmonid species that have extended freshwater rearing can also involve early migrations from the natal site, suggesting that imprinting must occur early in the life history of other Pacific salmonids as well, including Rainbow Trout (Northcote 1962), Cutthroat Trout (Rayleigh and Chapman 1971), and Chinook Salmon \( O. tshawytscha \) (Hamann and Kennedy 2012). Moreover, Coho
Salmon often migrate downstream to non-natal tributaries and off-channel habitats prior to smolting (Peterson 1982; Anderson et al. 2013; Shrimpton et al. 2014) or enter estuarine waters in the fall, yet home as adults to their natal sites (Bennett et al. 2015). One possible explanation for the discrepancy between the timing of imprinting sensitivity determined in laboratory studies and the imprint timing inferred from the life history of these animals may be the use of hatchery-reared salmon. Hatcheries provide an unnaturally stable rearing environment (e.g., constant temperature, flow rate, water source) and no opportunity for movement, and therefore the normal processes associated with imprinting may not be expressed (Dittman and Quinn 1996; Nevitt and Dittman 1998). We have previously hypothesized that the timing of imprinting sensitivity is a function of both innate, developmentally regulated periods for olfactory imprinting and a response to novel environmental stimuli typically associated with migration (Dittman and Quinn 1996). Therefore, our results may indicate that for Sockeye Salmon, with a genetically fixed life history pattern, the need for early imprinting is essential and is expressed even when fish are reared in the hatchery. Species with more plastic life histories, on the other hand, may only demonstrate early imprinting in response to novel stimuli experienced in the wild and not under the stable conditions in most hatcheries. This is consistent with our findings here and an earlier report that nonanadromous Sockeye Salmon imprinted at the alevin stage (Tilson et al. 1994).

Mechanistically, imprinting is believed to be associated with increases in thyroid hormone levels that occur during distinct developmental periods, and for wild fish, during periods of movement and novel environmental stimuli (Dittman and Quinn 1996; Dittman et al. 2015). For example, there is a distinct developmental surge in plasma T4 levels during PST (Dickhoff et al. 1978) that has been linked to successful olfactory imprinting (Hasler and Scholz 1983; Morin et al. 1989a, 1989b; Tilson et al. 1994). The increases in plasma T4 levels and successful imprinting we observed during PST are consistent with these earlier studies (Figure 2). However, these results contrast with another study of imprinting in nonanadromous Sockeye Salmon (kokanee) demonstrating a sensitive windows for imprinting that did not coincide with increases in plasma T4 levels during PST (Yamamoto et al. 2010). We were unable to measure T4 levels at the alevin stage in this study, however, Tilson et al. (1994) demonstrated that sensitive windows for imprinting in kokanee coincided with elevated levels of thyroid hormones that occurred during hatching and emergence.

**Duration of Exposure**

The duration that fish are exposed to odors may be important for successful imprinting. The experimental fish we exposed to imprinting odors for 1 week or 6 weeks as smolts successfully imprinted to these odors, but imprinting could not be demonstrated in smolts exposed to odors for only 1 d. Previous, laboratory assessments of the time required for Sockeye and Coho Salmon to imprint indicated that exposure periods ranging from 10 d to 6 weeks as smolts were sufficient for successful imprinting (Hasler and Scholz 1983; Dittman et al. 1996; Yamamoto et al. 2010), but exposures less than 14 d did not elicit electrophysiological responses consistent with imprinting (Yamamoto et al. 2010). Most Sockeye Salmon rear in a nursery lake for 1 or 2 years prior to the PST and seaward migration (Quinn 2005), so a long period of imprinting during PST is not inconsistent with their life history.

It is also important to acknowledge that our results are consistent with the hypothesis that the duration of exposure may be as important as the timing of exposure. Alevins exposed to imprinting odors for 32 d and smolts exposed for 39 d (6-week group) demonstrated the strongest behavioral attraction to exposure odors (Figure 3). One-week and 1-d smolt exposures elicited less robust behavioral attraction in adults. It is possible that longer exposures at any juvenile developmental stage might have generated successful imprinting. However, extensive life history and experimental data from previous studies (Hasler and Scholz 1983; Quinn 2005) suggest that there are specific sensitive developmental windows for imprinting linked to increased thyroid hormone status (Hasler and Scholz 1983; Morin et al. 1989b; Dittman et al. 2015).

We are reluctant to definitively conclude that Sockeye Salmon are incapable of imprinting to odors during brief exposure periods because controlled laboratory experiments such as this may not fully capture the complex physiological processes associated with natural migrations. The two-choice maze affords the ability to determine if odorants have been learned and elicit a behavioral response, but it is difficult to make inferences from the absence of responses and to understand the ecological relevance of responses observed in an artificial maze. Indeed, many studies of salmon released into natural river systems suggest that relatively short exposure time is sufficient for fish to learn site-specific olfactory cues that they can utilize for successful homing. For example, Coho Salmon held as smolts for 36–48 h in spring water prior to release homed accurately to this water source as adults (Jensen and Duncan 1971), and Snake River Sockeye Salmon and steelhead smolts released directly into tributaries migrated quickly downstream but most successfully imprint and homed to their release site (Hebdon et al. 2004; Clarke et al. 2010). Together, these results suggest that longer odor exposure periods may facilitate successful imprinting and that acclimation for a period of time prior to release may improve homing fidelity. However, previous studies on the importance of acclimation timing for successful imprinting in steelhead and Chinook Salmon have produced mixed results, suggesting that homing site fidelity may improve with acclimation (e.g., Clarke et al. 2010) or have no effect (e.g., Kenaston et al. 2001; Clarke et al. 2016).
Behavioral Responses to Odorants

Johnsen and Hasler (1980) hypothesized that salmon return to their natal river by demonstrating positive rheotaxis in the presence of imprinted cues but negative rheotaxis in the absence of these cues. Results from our behavioral trials are partially consistent with this idea as salmon that had previously been exposed to imprinting odors initially moved upstream into the odor-scented arms while fish that never experienced the imprinting odors showed no differential rheotactic responses. However, the attractive responses of odor-exposed fish declined during the course of the behavioral trials, suggesting that salmon may experience physiological adaptation to continuous olfactory stimuli or behavioral habituation in the absence of any positive feedback (progress toward natal river, spawning habitat, mates) from their movement. Similar results have been observed in other species wherein continuous exposure to attractive pheromones resulted in diminished neuronal responses and behavioral attraction (Rumbo and Vickers 1997; Judd et al. 2005) and extinction of responses to stimuli if rewards were removed (e.g., Kimber et al. 2014).

Implications for Artificial Propagation

In addition to furthering our understanding of the imprinting process in species such as Sockeye Salmon with complex life histories, there are also important implications of this work for the use of artificial propagation in salmon recovery and conservation. Appropriate reintroduction timing and duration may be an important factor in the success of conservation hatcheries and captive broodstock programs that seek to reintroduce salmon to their native environment. Hatchery-reared salmon that are released at inappropriate life stages or after insufficient periods of exposure to release site water tend to stray at higher rates (e.g., Quinn 1993), thus jeopardizing the recovery efforts of these programs.

One well-known example of the use of artificial propagation to increase a declining wild population is the Snake River Sockeye Salmon captive broodstock program initiated in 1991 as part of efforts to recover the endangered population Sockeye Salmon of Redfish Lake in the Stanley basin, Idaho (Kline and Flagg 2014). Under this program, progeny from the captive broodstock hatcheries have been reintroduced into Redfish Lake at many different life stages, including planting eyed embryos, net pen and direct in-lake releases of presmolts, smolt releases into the lake outlet and nearby streams, and releases of captively reared adults to spawn naturally (Kline and Flagg 2014). This spread-the-risk strategy for reintroduction of salmon into the wild was developed, in part, out of concern that Sockeye Salmon released as smolts at the lake outlet might not have the same opportunity to imprint as fish produced from more natural strategies (e.g., eyed egg plants and prespawn adult releases) or released in lakes as presmolts (Berejikian et al. 2004). However, the successful return of adults that were initially reared at a distant downstream hatchery and then released as smolts from the lake outlet suggests that exposure during the PST was at least sufficient for successful homing to Redfish Lake (Kline and Flagg 2014). Interestingly, these results also suggest that salmon will bypass a distant site they experienced at early life stages to return to their release site.

Using data from PIT-tagged fish, which allowed for automated detection at dams on their migratory route (Prentice et al. 1990), Peterson et al. (2014) reported that 30–70% of the adult Redfish Lake Sockeye Salmon that passed Lower Granite Dam, the last PIT tag monitoring site before Redfish Lake, did not return to the Stanley basin. While it is likely that the majority of this loss is attributable to mortalities associated with temperature and migratory delays (Keefer et al. 2008; Crozier et al. 2014), straying resulting from incomplete imprinting may also contribute to this loss.

While managers of captive rearing programs must balance trade-offs between postrelease survival and appropriate periods of imprinting acclimation, our results suggest that earlier releases and longer acclimation may be beneficial for successful imprinting and homing. Our results also confirmed the potential importance of imprinting Sockeye Salmon at an early life stage to increase recognition of natal-site cues as mature adults and provide support for management strategies that seek to exploit imprinting during embryonic stages, thereby facilitating improved homing of hatchery-reared salmon (Dittman et al. 2015). It is also important to recognize that there is considerable diversity in spawning habitats, juvenile ecology, and freshwater migration patterns among Sockeye Salmon populations and additional studies of imprinting in other populations are warranted.

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