Evidence of chemically mediated population recognition in coho salmon (Oncorhynchus kisutch)

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To test the hypothesis that population-specific pheromones guide adult salmonids to their natal streams, juvenile and adult coho salmon (Oncorhynchus kisutch) were tested for chemosensory responses in two-choice tanks. Coho salmon from Quinsam and Big Qualicum rivers, British Columbia, Canada, distinguished their own population from the other. Tagging evidence indicates that straying between these two rivers and a third, geographically intermediate river seldom occurs. Thus, population-specific chemicals constitute a potential source of information for homing coho salmon, though their role vis-à-vis imprinted odors from other sources could not be evaluated.


La sensibilité chimique de saumons argentés (Oncorhynchus kisutch) juvéniles et adultes a été mise à l’épreuve par des tests dans des aquariums présentant un choix de conditions, afin de vérifier l’hypothèse selon laquelle les phénomènes spécifiques à la population guident les salmonsidés adultes vers leur natal natal. Les saumons de la rivière Quinsam et ceux de la rivière Big Qualicum en Colombie-Britannique, Canada, ont été capables de distinguer leur propre population de l’autre. Des expériences de marquage ont permis de démontrer que les échanges sont rares entre ces deux rivières et une troisième rivière intermédiaire entre les deux autres par sa position géographique. Les substances chimiques propres à une population constituent donc une source potentielle d’information lors du homing des saumons argentés, mais leur rôle par rapport aux odeurs imprégnées provenant d’autres sources n’a pu être déterminé.

[Traduit par le journal]

Materials and methods

Introduction

The propensity of Pacific salmon to home to their natal stream for spawning was demonstrated by marking studies (e.g., Poerst 1936). The olfactory occlusion experiment conducted by Wisby and Hasles (1954) indicated that odors guide salmon to their home stream. The reliance on imprinted odors was demonstrated by a series of subsequent experiments (reviewed by Hasler and Scholz 1983). However, Nordeng (1971) revived and elaborated upon White’s (1934) hypothesis that the odors of juvenile conspecifics influence spawning stream selection. Nordeng (1977) proposed that all salmonids are innately attracted to pheromones from their own population and that pheromones from juveniles residing in freshwater or migrating to sea guide homing adults. Electrophysiological (Davies et al. 1974) and behavioral (Selset and Daving 1980) studies with adult Arctic char (Salvelinus alpinus) supported the hypothesis. Stabell (1982) reported that juvenile Atlantic salmon (Salmo salar) could distinguish their own population from another, but the results are open to the alternative explanation of sibling recognition (Quinn and Busack 1985). While the only experimental tests of the pheromone hypothesis with Pacific salmon yielded negative results (Quinn et al. 1983; Brann et al. 1984), Stabell (1984) criticized the small sample size in the experiment of Quinn et al. (1983). However, recent work with coho salmon (Oncorhynchus kisutch) fry has revealed the capacity to distinguish siblings from nonsiblings (Quinn and Busack 1985). Encouraged by this evidence of acute sensitivity to intraspecific differences in chemical traces, we initiated experiments to test the chemosensory responses of adult and juvenile coho salmon to population-specific chemicals.

Experimental subjects

Underyearling coho salmon were taken from hatcheries on Quinsam and Big Qualicum rivers, 80 km apart on the east coast of Vancouver Island, British Columbia, Canada. Both hatcheries enhance populations that existed in those systems prior to hatchery construction. At present, wild spawning and hatchery production exists in both rivers, but separate wild and hatchery lines are not maintained. Two groups of juvenile salmon were collected from each population. Each group contained fish of mixed parentage, but the two groups from each river had been reared apart and no parents were common to both groups. The fish were transported to an experimental hatchery on Rosewall Creek on 16–17 July 1984. Additional juvenile coho salmon used as sources of chemical traces for adult experiments were transported from both hatcheries on 29 September 1984. All fish were held in well water at 8°C and fed a standard salmon diet (Oregon Moist Pellet). Mortalities were rare (~1%) and all fish remained in excellent condition.

Juvenile experiments

The two groups of juvenile coho salmon from the two rivers were held in circular fiber-glass tanks on separated water supplies. They were tested in two 90 cm long, plywood, two-choice tanks filled to a depth of 6 cm (Fig. 1A). The tanks received flows of 1.75 L/min from each of two identical 37-L pastic head tanks. Depending upon the test, the head tanks contained only well water or well water and 300 g juvenile coho salmon. Salmon, tested individually, were placed in the lower end of the tank and their movements were observed by mirror. Each test fish was placed in the two-choice tank and allowed 1 min for acclimation. The first movement into or from a choice arm after the acclimation period initiated a 10-min test period. During the test period, entrances into the right and left arms of the tanks were tallied and the total time spent in each arm was also compiled. After each test, the fish were removed, the water supplies to the arms were switched, and the tanks were drained and refilled for another pair of tests. For statistical analysis, the numbers of entrances into the two arms made by the fish and the amounts of time spent there were compared using paired t-tests. In addition, the data were analyzed nonparametrically by counting the fish that made the majority of their entrances or spent the

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Fig. 1. Scale drawings of the tanks used to test the behavioral responses of (A) juvenile and (B) adult coho salmon to the chemical traces of juveniles of their own and another population.

majority of their time in one arm or the other and comparing these ratios to 1:1 using the χ² test.

Fifteen fish from each river were tested with well water in both head tanks to test for biases in the equipment. Fifty fish from each population were tested with well water versus well water with coho salmon from their own population. Fifty other fish from each population were tested with well water with their own population versus well water with the other population. All tests were conducted between 18 July and 12 August; the fish averaged 2.0 g (Quinsam) and 3.3 g (Big Qualicum).

Adult (jack) experiments

For the experiments with adult salmon, 2-year-old male salmon from the two hatcheries were used. These males, known as jacks, mature 1 year earlier than most males and the females. Jacks were used because their small size makes them easier to transport and maintain than older fish. Jacks that returned to Quinsam and Big Qualicum rivers in fall 1984 were transported to Rosewall Creek for tests. The Quinsam River jacks averaged 32.3 cm (fork length, N = 100) and the Big Qualicum River jacks averaged 32.5 cm (N = 57). They were held in 183-cm circular tanks supplied with well water. They were tested individually outdoors in two 488 × 122 × 122 cm plywood tanks (Fig. 1B). The tanks were filled to 30 cm with well water draining two 244-cm circular tanks containing 8 kg of juvenile coho salmon from the two populations (1102 Quinsam fish, 949 Big Qualicum fish). The head tanks each contributed 10 L/min into the testing tank and an additional 60 L/min well water was introduced into each side of the tanks for a total flow of 140 L/min into each testing tank.

For testing, a fish was placed at the lower end of the tank and observed for 30 min. Entrances into the two sides and time spent there were recorded. As most fish either entered one arm and never left or made several entrances before staying in one arm for the remainder of the test, the last arm entered was used to indicate choice. However, the first choices were also analysed as an additional indicator of response. The numbers of fish choosing water draining the two head tanks were compared with a 1:1 ratio using the χ² test. Because the fish were undergoing sexual maturation, we anticipated that the responses might change over the course of the tests (Hasler and Scholz 1983). Therefore, the data were compiled and analysed daily.

Homing and straying

To interpret the experimental results, we needed to know the extent to which the coho salmon populations are distinct from one another. Large straying rates would make it unlikely that the two populations would have evolved differences that could be detected by chemosensory systems. Moreover, if jacks stray substantially more often than adult coho salmon, the results of behavioral tests on jacks may not accurately represent the total population.

Detection of straying is facilitated by the marking of salmon at these hatcheries and many others in Canada and the United States. Salmon receive an internal coded wire tag (CWT) in the cranium and the adipose fin is excised to signal the tag’s presence. Records of coho salmon returns to the two hatcheries over the past years were examined for evidence of straying. Based on subsampling procedures, the total numbers of strays were estimated (see Results for details). In addition, records from a third hatchery, located on Puntledge River between Quinsam and Big Qualicum rivers, were checked as an additional source of information on straying rates.

Results

Juvenile experiments

The 30 juvenile salmon tested in the control (well water vs. well water) displayed no preference for the left or right arms of the tanks (mean times [minutes:seconds]: left, 2:46, right, 2:40; mean entrances: left, 7.2; right, 6.7). In tests with the chemical traces of their population paired against well water, both Quinsam and Big Qualicum fish were attracted to coho-conditioned water (Table 1). However, the Quinsam River fish responses were somewhat stronger than those of the Big Qualicum River fish.

When the chemical traces of the two populations were paired, the Quinsam River coho salmon were attracted to their own population (Table 1). Big Qualicum River fish were weakly (not significantly) attracted to their own population.

Adult experiments

One hundred Quinsam River coho jacks were tested between 15 October and 9 November. Fish tested early were silver colored and were not ripe. Almost all coho tested in November were ripe, as shown by free-flowing milt and their red gray color. The coho tested early (15 October through 25 October)

<table>
<thead>
<tr>
<th>Test fish</th>
<th>Water</th>
<th>Average no. of entrances</th>
<th>Average time spent (s)</th>
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</thead>
<tbody>
<tr>
<td>BQ</td>
<td>BQ</td>
<td>10.6***(34.5)**</td>
<td>190(32)*</td>
</tr>
<tr>
<td></td>
<td>Well</td>
<td>7.8(15.5)</td>
<td>157(18)</td>
</tr>
<tr>
<td>BQ</td>
<td>BQ</td>
<td>7.6(28.5)</td>
<td>151(27.5)</td>
</tr>
<tr>
<td></td>
<td>Quin</td>
<td>7.2(21.5)</td>
<td>131(22.5)</td>
</tr>
<tr>
<td>Quin</td>
<td>Quin</td>
<td>9.7***(36)**</td>
<td>191**(34)**</td>
</tr>
<tr>
<td></td>
<td>Well</td>
<td>6.7(14)</td>
<td>129(16)</td>
</tr>
<tr>
<td>Quin</td>
<td>Quin</td>
<td>10.0**(37)**</td>
<td>185**(36.5)**</td>
</tr>
<tr>
<td></td>
<td>BQ</td>
<td>7.8(13)</td>
<td>128(13.5)</td>
</tr>
</tbody>
</table>

Note: Average numbers of entrances into channels and time spent were analysed with paired t-tests. Values in parentheses are the numbers of fish that made the majority of their entrances or spent most of their time in one channel or the other; these values were analysed with χ² tests. **, P < 0.001; *, P < 0.05; ***, P < 0.01.
displayed a strong preference for water conditioned by juvenile Quinsam River coho, based on the last channel arm that they entered. Of 43 salmon tested, 31 chose Quinsam conditioned water ($\chi^2 = 8.40, P < 0.01$; Fig. 2). Thereafter, the choices were random (27 Quinsam choices vs. 30 for Big Qualicum conditioned water) and the final, cumulative ratio of 58:42 did not differ ($P > 0.05$) from random. Analysis of first entrances gave a similar result (57:43).

Fifty-seven Big Qualicum jacks were tested from 17 October through 6 November. They initially displayed a weak preference for Quinsam conditioned water (Fig. 3). As the season progressed, the tendency strengthened and the cumulative total indicated attraction to juvenile Quinsam coho (37:20; $\chi^2 = 5.07, P < 0.05$). Analysis of the first entrances yielded the same result (37:20).

In most experimental studies of the sensory bases of homing, salmon that return to a given river are assumed to have left that river years before, but it is usually not possible to exclude the possibility that some are strays. This was the case for the Big Qualicum River fish, but 18 of the fish taken from Quinsam River contained CWTs indicating their origin. All 18 had been released from Quinsam River and the ratio of Quinsam:Big Qualicum choices made by these fish was 10:8, essentially the same ratio of choices displayed by untagged Quinsam River fish.

**Homing and straying**

In 1980, only one marked stray from Big Qualicum River was recovered in Quinsam River. Since only 60% of the coho salmon with adipose fins removed (presumably containing CWTs) were examined for tags, this fish was estimated to represent two strays among the marked fish. A total of 45,328 (6.0%) of the 750,195 fish from that group were tagged. Thus, if the untagged fish strayed as often as the tagged fish, 33 coho from that Big Qualicum River group might have strayed to Quinsam River that year. Using this expansion procedure to correct for subsampling, the 9 strays recovered in Quinsam River Hatchery in 1980 represented 85 total strays. Eleven (13%) of the estimated strays were jacks, representing 0.08% of the year’s total of 13,271 jacks. The 74 estimated adult strays represented 0.33% of the year’s total of 22,100 adult coho salmon. No strays were recorded in Quinsam River in 1981, 1982, or 1983, in spite of large numbers of salmon examined (Table 2).

Between 1983 and 1978, 10 stray coho salmon with CWTs were recovered at Big Qualicum River hatchery. Corrected for subsampling, those 10 fish were estimated to represent 232 strays out of the 386,595 total coho salmon returning in those 6 years. None of the identified strays originated in Quinsam River hatchery. The estimated numbers of jacks and adults that strayed were approximately equal, relative to the total numbers of jacks and adults that returned to the hatchery (jacks, 0.07%; adults, 0.06%). Puntledge River hatchery, located between

**Fig. 2.** Numbers of adult male (jack) coho salmon from Quinsam River ascending the arms of a two-choice apparatus containing the chemical traces of juveniles from Quinsam and Big Qualicum rivers (running total, tallied daily).

**Fig. 3.** Numbers of adult male (jack) coho salmon from Big Qualicum River ascending the arms of a two-choice apparatus containing the chemical traces of juveniles from Quinsam and Big Qualicum rivers (running total, tallied daily).

**Table 2.** Straying of adult (3-year-old) and jack (2-year-old male) coho salmon into Big Qualicum, Puntledge, and Quinsam hatcheries, as indicated by recoveries of coded wire tags. See text for details of the process of estimating total strays from numbers observed.

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<tbody>
<tr>
<td>Adults examined</td>
<td>274,745</td>
<td>53,215*</td>
<td>72,515</td>
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<tr>
<td>Marked adults</td>
<td>28,252</td>
<td>4,196</td>
<td>10,042</td>
</tr>
<tr>
<td>Strays observed</td>
<td>7</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Strays estimated</td>
<td>158</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td>Jacks examined</td>
<td>111,850</td>
<td>26,833*</td>
<td>37,193</td>
</tr>
<tr>
<td>Marked jacks</td>
<td>4,597</td>
<td>4,599</td>
<td>6,257</td>
</tr>
<tr>
<td>Strays observed</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Strays estimated</td>
<td>74</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

*In 1979, the total number of salmon examined at Puntledge hatchery was not separated into jacks and adults; hence, these totals are approximate.
Big Qualicum and Quinsam rivers, received no coho salmon identified as strays from 1978 to 1983 (Table 2).

Discussion

The tests with juvenile coho salmon indicated that members of both populations were attracted to the chemical traces of their own population over water devoid of coho traces. The Quinsam River fish were also attracted to the traces of their population over those of Big Qualicum River fish. The test fish were unrelated (different parents) to the fish conditioning the water and had also been reared apart from them. Thus, the attraction was presumably based on some generalized interpopulation difference. The test fish may have learned the characteristics of their population from their tank mates and generalized this to the members of their population that they had not encountered. This would be analogous to the "phenotype matching" system of sibling recognition (Holmes and Sherman 1982) that seems to control sibling recognition in coho salmon (Quinn and Busack 1985; Quinn and Hara 1986). On the other hand, these results do not exclude the possibility that the fish have the innate ability to distinguish their own population from another, as suggested by Nordeng (1977) and Stabell (1984). The Big Qualicum River coho salmon displayed a weak (not significant) attraction to their population over the Quinsam River fish. The weaker response of this population to the traces of their population is not readily explainable, but a similar pattern of interpopulation differences in chemosensory responses has been found with adult sockeye salmon, Oncorhynchus nerka (Groot et al. 1986).

In the experiments with mature (jack) coho salmon, the Quinsam River fish were attracted to juveniles of their population early in the fall, but responded randomly in the later tests (Fig. 2). Hasler and Scholz (1983) reported that coho salmon responses to home water declined near spawning as reproductive hormone levels changed. These findings emphasize the need to evaluate physiological state and motivation when studying salmon olfactory responses owing to the rapid change from migratory to reproductive behavior. The attraction of Big Qualicum River fish to the chemical traces of Quinsam River juveniles was unexpected. It was not an artifact of the holding tanks, as these were switched during the tests. Diet for the juveniles was standardized and fish from both populations were healthy during the testing period. Since few Big Qualicum River jacks stray into Quinsam River, it clearly is not representative of their homing behavior under natural conditions.

Examination of the CWT records for evidence of straying demonstrated several important points. First, straying rates by jacks and adult coho salmon are not substantially different. This is consistent with the report by Quinn and Fresh (1984) indicating accurate homing by chinook salmon jacks. Thus, it is reasonable to test the homing responses of jacks and obtain results that can be applied to older salmon as well. Second, the overall homing to these three hatcheries was very precise. While there are several sources of potential error in the straying estimates, the fact that jack and adult estimates to all three hatcheries were far below 1% indicates that probably very little genetic exchange occurs among the hatcheries. The lack of genetic exchange could have provided the opportunity for biochemical differences to evolve. If these differences are detectable by salmon and are stable over generations, they could constitute a source of information for homing. Based on the results of the present experimental studies, it is tentatively concluded that coho salmon can distinguish between populations using chemosensory information. However, the results of transplants and experiments in which salmon were imprinted on artificial chemicals (reviewed by Hasler and Scholz 1983) indicate that pheromones are not a prerequisite for homing and may not even be of primary importance in many situations.

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