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# INTRASPECIFIC PHEROMONE DISCRIMINATION AND SUBSTRATE MARKING BY ATLANTIC SALMON PARR

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**Abstract**—Attraction and preference behavior patterns shown by Atlantic salmon (*Salmo salar* L.) parr towards intraspecific odors were measured. Salmon parr were attracted to tank water in which relatives of their own strain were held but were also attracted to tank water containing another strain of their species. When given a choice between the two types of tank water, water containing their own strain odor was significantly favored over water containing conspecific but nonfamiliar odor, revealing that pheromones are present at intraspecific levels in salmonid juveniles. Competitive experiments with various extracts suggest that the active compounds are most likely produced in the liver and are voided via the intestinal tract, as determined by presence of attractants in intestinal contents and bile. Intraspecific discrimination was not detected with extracts from either skin surface mucus or blood plasma. Extracts from gravel that had been kept below the fish in their rearing tanks, however, induced a strain-related preference behavior. This suggests an ability for substrate marking by salmonid fishes, presumably mediated by deposition of fecal material. Strain discrimination and substrate marking are discussed in relation to stationary behavior and homing of fishes within discrete populations in natural systems.

**Key Words**—*Salmo salar*, olfaction, pheromones, substrate marking, feces, bile, skin mucus.

## INTRODUCTION

Salmonid fishes living in streams demonstrate a substantial degree of regional stability throughout their life, (Miller, 1954; Saunders and Gee, 1964; Edmundson et al., 1968; Hesthagen, 1978; Cargill, 1980; Harcup et al., 1984).

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When moved from their "home area," most of the fishes will return to that area whether displaced upstream or downstream. Downstream displacement, however, was found by Miller (1954) to result in a more precise homing in cutthroat trout (*Salmo clarki* L.). Accordingly, it was suggested that the sense of smell was the most important factor in homing since fish displaced downstream could smell their home area continuously.

Regional stability and homing after displacement in streams have also been demonstrated in several nonsalmonid species of fishes (Gerking, 1953, 1959; Stott, 1967). In sensory deprivation experiments, using longear sunfish (*Lepomis megalotis megalotis* Raf.), Gunning (1959) demonstrated that fishes with an impaired olfactory sense moved at random in the stream, whereas blinded individuals homed as quickly and accurately as controls. The precision of intact fish homing in the upstream direction was also found to be greater than that of those moving downstream. Homing was therefore concluded to be maintained by olfactory mechanisms.

The above summary indicates that fishes within streams search for their home range through rheotactic behavior mediated by olfaction, and they apparently recognize their "home area" by some characteristic odor. The question then arises: what is the origin of the characteristic odors used by fishes when homing and when maintaining regional stability?

Hasler and Wisby (1951) suggested that because of local differences in soil and vegetation of the drainage basin, each stream has a unique chemical composition. During smolt transformation and seaward migration, the young salmon were presumed to become "imprinted" to the distinctive odor of their home stream (see also: Hasler et al., 1978; Hasler and Scholz, 1983). When returning as mature adult salmon, they would proposedly remember this "imprinted" odor and use it as a cue for homing when migrating through the homestream network. The "imprinting" hypothesis, however, has been closely associated with the process of smolt transformation (Hasler and Scholz, 1983) and does not, therefore, explain the phenomena of regional stability and homing after displacement in juvenile fishes (Stabell, 1984).

The pheromone hypothesis for anadromous salmonids (Nordeng, 1971, 1977) proposes that homing migrants return to their home area by detecting population-specific pheromones secreted by downstream migrating smolt and young relatives resident in the stream. The hypothesis has been supported through behavior studies with mature anadromous char (*Salvelinus alpinus* L.) by Selset and Døving (1980), demonstrating that char have the ability to detect the odor of young relatives and that they prefer the odor of relatives to that of nonrelated conspecifics.

By positive rheotaxis olfactometry, it has recently been demonstrated that Atlantic salmon (*Salmo salar* L.) parr are also able to discriminate between substances secreted by strains of conspecifics (Stabell, 1982). Accordingly, it was suggested that intraspecific odors could be responsible for the regional stability of juveniles in streams and that these odorants could well be identical to those attracting mature migrants to the spawning grounds. Studies of positive rheotactic behavior in parr towards conspecific odors could therefore provide insight into adult homing behavior.

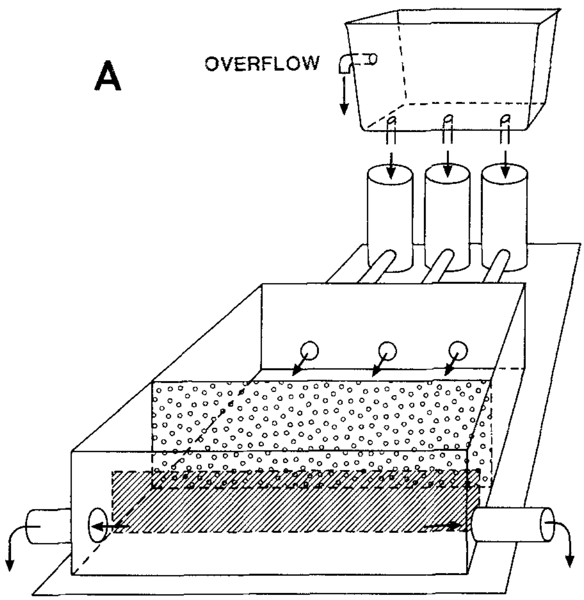
The intention with the present study was to further investigate the preference and attraction phenomena of Atlantic salmon parr towards conspecific odors. In particular, it was of interest to evaluate possible pathways for the secretion of the intraspecific signal substances and to test for substrate marking by fishes related to regional stability and homing.

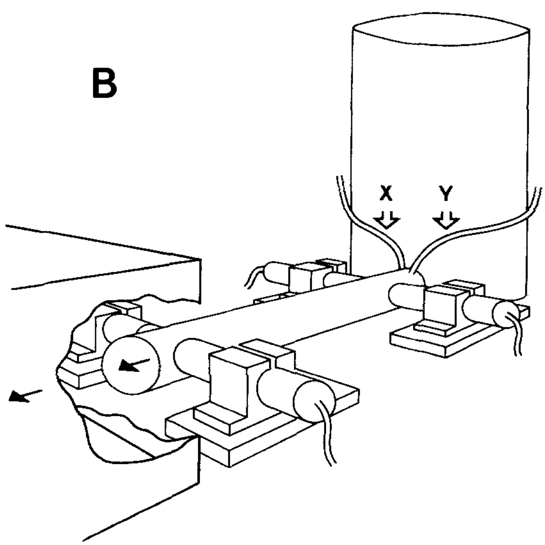
## METHODS AND MATERIALS

*Study Animals.* Atlantic salmon (*Salmo salar* L.) parr were used in the study. The parr originated from the river Sandvikselv, Akershus County, southeastern Norway; the river Imsa, Rogaland County, southwestern Norway; and from the river Altaelv, Finnmark County, northern Norway. After artificial fertilization, the eggs were hatched and the fish raised at the Research Station for Freshwater Fish, belonging to the Directorate for Wildlife and Freshwater Fish, at the Imsa River. The fishes were subsequently transported to aquarium facilities at the University of Oslo, where the experiments were conducted. The Altaelv parr were all siblings of the brood year 1981 and were transported to Oslo in May 1982. The parr from Sandvikselv and from Imsa were of the brood year 1982 and arrived at the University in September 1982. Of these fish, the Imsa parr belonged to one sibling group, while the Sandvikselv parr came from a group where ova and milt from several spawners had been mixed. The three strains of fish were reared in separate tanks supplied with tap water and fed with commercially available dry pellets from automatic feeders.

*Test System.* A fully automated test system was used in the experimental series to measure preference behavior and upstream movement aroused by chemical stimuli. The aquarium part of the system was of the two-choice, parallel-entrance type, as described by Selset and Døving (1980). In the current study the aquarium part was slightly modified with regard to water-flow characteristics and test sample injections, according to the description by Stabell (1982).

The system involves a main chamber with three freshwater inlets, of which the fish were allowed to choose between the two outermost (Figure 1A). The middle inlet was used to ensure good separation of water flow within the main chamber, and fish were prevented from entering that inlet by a plastic net cover. Tap water was introduced from an overflow reservoir into three separate chambers in the upstream end of the system, ensuring equal flow from each of the inlets (3 × 3 liters/min). A water level of 8 cm was used in the system, giving a main chamber volume of 28.3 liters.


A
OVERFLOW


B
X
Y

The test substances were introduced to the system at the upper end of the inlet tubes, as shown in Figure 1B, and added by hydrostatic pressure to the background flow through solenoid valves at an approximate ratio of 1:60 in volume. When a test sample ($X$) was introduced to one of the inlets, a reference sample ($Y$) was introduced to the other and vice versa. Movements of fish into the inlets were registered by photocells (IR light) located at the lower and upper end of each inlet (Figure 1B). Test performances were controlled and monitored by a microcomputer connected to an interface with in–out ports and a printer.

*Odor Sampling.* Fish odors were sampled by siphoning water from fish rearing tanks (volume: 500 liters, flow: 10 liters/min) containing approximately 100 fish that had been undisturbed for more than 12 hr. Skin mucus was sampled from the surfaces of the fish using an aspirator flask, according to the description by Stabell and Selset (1980). Skin mucus, intestinal contents, blood plasma, and bile were all sampled from the same fish. Each type of material was pooled from five fish and subsequently divided for use in two 4-hr test series. Material not used for immediate testing was frozen and stored at −20°C.

Test solutions from skin mucus as well as intestinal contents were prepared by adding 20 ml of tap water to each of the sampled materials. After careful mixing, the solutions were centrifuged and the supernatant pipetted off. Another 20 ml of tap water was added to the residue and the procedure repeated. The supernatants from both water extracts were then pooled for use in the tests. Ethanol extracts from each type of material were prepared in a similar manner to that described for water extracts. Before using in the tests, however, ethanol was removed in a rotary evaporator and the residues redissolved in tap water.

Gravel retained by a 2-cm sieve and washed in water was introduced under the fish in the rearing tanks upon arrival of the 1982 brood years. Approximately 20 liters of gravel were used in each tank. After two months, the gravel was removed from the tanks and washed carefully with running water to remove food residues and feces. It was then spread out on water-absorbing paper on the laboratory bench to dry. After a week, methanol extracts were made from the

FIG. 1. (A) Overview of the aquarium part of the test system; flow directions are shown by solid arrows. City tap water is introduced to the upper overflow tank, ensuring equal flow from each of the three inlets. Entrance by the fish into the middle inlet is prevented by a grid. A perforated wall in the downstream end of the main chamber prevents fish from moving downstream, and an overflow wall (hatched) secures a constant level of water in the system. (B) Close-up of a freshwater inlet showing positions of photocells in the downstream and upstream ends. Positions of tubes for test sample injections, denoted X and Y, are shown by open arrows in the upstream end of the inlet. Test samples are introduced through solenoid valves, and test performance is controlled and monitored by a microcomputer.

material covering the gravel surface. Five liters of methanol was used to wash the gravel from each tank. Methanol extracts were subsequently treated in the same manner as that previously described for the ethanol samples.

*Experimental Procedures.* Test samples were introduced to the inlet tubes for periods of 20 min followed by pauses of 10 min. Every half hour the samples were switched between the inlets in order to balance possible preferences of the fish for a particular side of the aquarium. Because of possible side preferences, two subsequent half-hour test periods were treated pairwise in the evaluation of data.

Four hours of testing, consisting of four pairs of half-hour test periods, were run as a series. This time limitation for each test series was because of the storage capacity of the test-sample reservoirs. Several 4-hr test series were usually necessary to obtain a satisfactory number of choices within each type of test. Ten fish were used simultaneously in the test system, and no fish were ever used in more than two successive 4-hr test series.

*Data Evaluation.* The two photocells used for each inlet allowed two different events to be evaluated: (1) search: only the beam of the downstream photocell is broken, i.e., the fish enters the lowermost part of the inlet and then backs out; (2) ascent: the beam of the downstream photocell is broken followed by that of the upstream cell, i.e., the fish moves all the way up through the inlet tube.

In random choices, the fish will choose equally between the two inlets (Selset and Døving, 1980; Stabell, 1982), and the binomial model can therefore be used for probability calculations (Siegel, 1956). The activity score (AS) obtained for each event (ascents or search) is given by the expression:

$$AS = \frac{N_t - N_r}{N_t + N_r} \times 100$$

where $N_t$ and $N_r$ are the number of choices in favor of the test substance and the reference substance, respectively (Olsén, 1985). The data are presented as column graphs for the two events, each representing the activity score for the total number of trials performed.

Probabilities for random choice are given in the figures. The probabilities are given as the lowest score obtained for each experiment at the 5, 1, or 0.1% levels. All probabilities given are one-tailed. The results were regarded to be within significant levels of attraction or repulsion when the probability of being generated by random choice was found equal to, or less than, 5%.

## RESULTS

*Fish Odors in Tank Waters.* The results obtained from tests with fish odors in tank waters are presented in Figure 2. Fish from the Sandvikselv strain were used as test fish in all three experiments of this type, which took place from October 10 to November 3, 1982. When water from the tank containing fish of the Sandvikselv strain was tested against tap water without any salmon odor, the fish significantly preferred the tank water (Figure 1A). The results refer to both parameters tested. Of 93 ascents performed, 55 were in favor of the tank water, giving an activity score (AS) of +18.3. This result represents a significant level of attraction ($P = 0.0495$). For search behavior, 468 of 820 trials performed were in favor of the tank water containing the related fish, giving an AS of +14.2, which is highly significant ($P < 0.00003$).

The test fish also demonstrated a preference for tank water over tap water when water from a tank containing an unfamiliar strain of salmon parr was used (Figure 1B). Of 122 ascents recorded, 77 were in favor of the tank water, giving an AS of +26.2 ($P = 0.0025$); from a total of 1333 searches, 725 were also in favor of the tank water (AS = +8.8; $P = 0.007$).

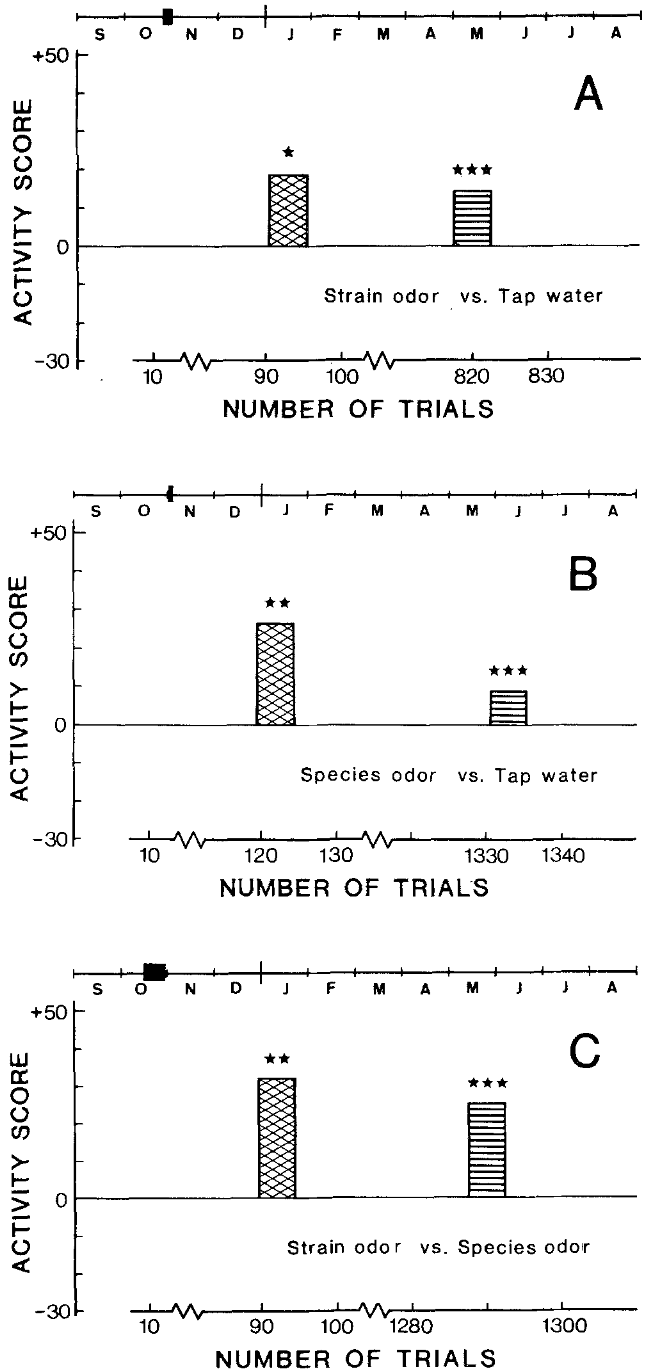
When tank water containing the related Sandvikselv strain of fish was tested against tank water containing the unfamiliar Imsa strain that had previously been found attractive, the Sandvikselv fish highly favored water from the related strain to that from the unfamiliar one (Figure 2C). Of 92 ascents recorded altogether, 61 were in favor of the familiar odor (AS = +32.6; $P = 0.0013$). For search, 806 of 1290 trials were in favor of the familiar odor (AS = +25.0), demonstrating that this result was also at a highly significant level ($P \ll 0.00003$).

*Odorants in Excretion Products from Fish.* Competition experiments with extracts from skin mucus of the Sandvikselv strain versus similar extracts of the Imsa strain are presented in Figure 3A. Fish from the Sandvikselv strain were used as test fish. In the middle of November 1982, fish from the Sandvikselv strain were found to discriminate between water extracts for the search parameter only. Of 70 ascents recorded, only 32 were in favor of the related strain (AS = −8.6; $P = 0.274$), whereas 470 of 880 searches were directed towards the familiar strain (AS = +6.8; $P = 0.023$).

In the middle of June 1983, however, no significant preferences for either strain was found in competition experiments with water extracts from skin mucus (Figure 3A). Fish from the Imsa strain were used as test fish this time. Sixty-one ascents from a total of 128 were in favor of the related strain (AS = +4.7; $P = 0.33$), and 258 of 514 searches were similarly directed (AS = +0.4; $P = 0.484$).

Ethanol extracts from skin mucus of the two strains mentioned above, tested in July 1983 with the Sandvikselv strain as test fish, elicited neither of the preference behaviors (Figure 3A). Only 33 ascents were registered in total, of which 15 were in favor of the material from the related strain (AS = −9.1; $P = 0.36$). Of 199 searches, 96 were in favor of the related strain (AS = −3.5; $P = 0.334$).

Experiments with extracts from intestinal contents are presented in Figure


S O N D J F M A M J J A
+50
0
-30
ACTIVITY SCORE
A
Strain odor vs. Tap water
10 90 100 820 830
NUMBER OF TRIALS
B
Species odor vs. Tap water
10 120 130 1330 1340
C
Strain odor vs. Species odor
10 90 100 1280 1300

3B. When water extracts from intestinal material were run in the middle of November 1982 with fish from the Sandvikselv strain as test fish, no preference behavior was detected (Figure 3B). Seventy-seven ascents were recorded; 35 of them were in favor of the related strain (AS = −9.1; $P$ = 0.248). Of 963 searches, 464 were in favor of the related strain (AS = −3.6; $P$ = 0.138).

When tested in June 1983, however, an ability among the Sandvikselv parr for intraspecific discrimination of water extracts from intestinal material was revealed (Figure 3B). Although a significant level was not found among ascents, 28 of 45 trials were in favor of the familiar odor (AS = +24.4; $P$ = 0.068). For the search parameter, 124 of 205 trials were in favor of the related strain, revealing a highly significant level of preference (AS = +21.0; $P$ = 0.0017).

Ethanol extracts from intestinal contents also elicited preference behavior of the Imsa fish towards material from the familiar strain (Figure 3B). Twenty-eight ascents of 42 were found in favor of the familiar strain, giving a significant level of preference (AS = +33.3; $P$ = 0.022). For the search parameter, however, a repellent effect from the familiar strain was found. Of 1460 trials, only 633 were directed towards the native material, revealing a highly significant repellent effect (AS = −13.3; $P \ll 0.00003$).

*Origin of Odorants.* Results from competition experiments using bile from two genetic strains of parr are presented in Figure 4A. Fish from the Sandvikselv strain were used as test fish. Very few ascents were recorded during the experimental period which took place in June 1983. Only 28 trials were registered in total, and 16 were in favor of the extracted material from the familiar strain (AS = +14.3; $P$ = 0.284). For search, however, 254 trials were recorded, of which 142 were directed towards the familiar material (AS = +11.8; $P$ = 0.034). This result suggests that the strain-specific substances demonstrated in tank water and intestinal contents of fish may originate from the liver.

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FIG. 2. Activity scores for ascents (cross-hatched bars) and search (horizontal lined bars) in test series with water from fish-rearing tanks, presented at the total number of trials recorded for each event. (A) Test with tank water from the Sandvikselv strain against tap water as reference. (B) Test with tank water from the Imsa strain against tap water as reference. (C) Test with tank water from the Sandvikselv strain against tank water from the Imsa strain as reference. Fish from the Sandvikselv strain were used as test fish in all three test series. Ascents are the movements of fish up through the inlet tubes. Search is the entrance into the downstream part of inlets only. Activity scores for each event are given as the difference between trials in favor of the test sample and trials in favor of the reference sample, expressed as percent of the total number of trials. Probabilities for random choice are given in the figures. The lowest probability obtained among the 5, 1, and 0.1% levels are in each case given by one, two, or three asterisks, respectively. Black vertical bars in the upper part of each figure indicate experimental periods in 1982.

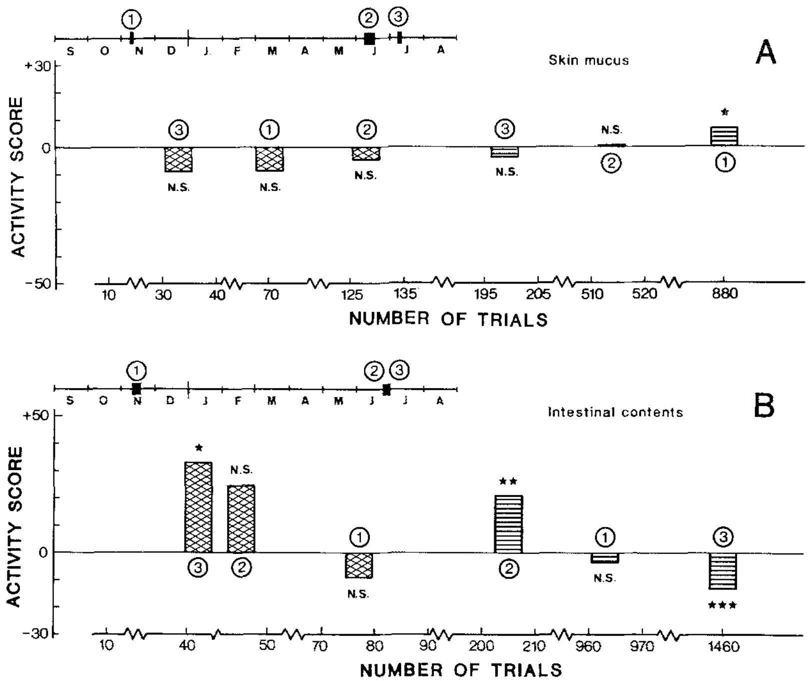


FIG. 3. Activity scores for ascents (cross-hatched bars) and search (horizontal lined bars) in test series with extracts from skin mucus and intestinal contents. The tests were performed with extracts from a familiar versus an unfamiliar strain and are presented at the total number of trials recorded for each event. (A) Tests with extracts from skin mucus. (B) Tests with extracts from intestinal contents. Encircled number one represents experiments performed in 1982 with water extracts from crude material. Encircled number two represents experiments performed in 1983 with water extracts from crude material. Encircled number three represents experiments performed in 1983 with ethanol extracts from crude material. The strain of test fish used in each case and experimental design for each type of test performed are given in Table 1. General figure explanations are given in the legend to Figure 2.

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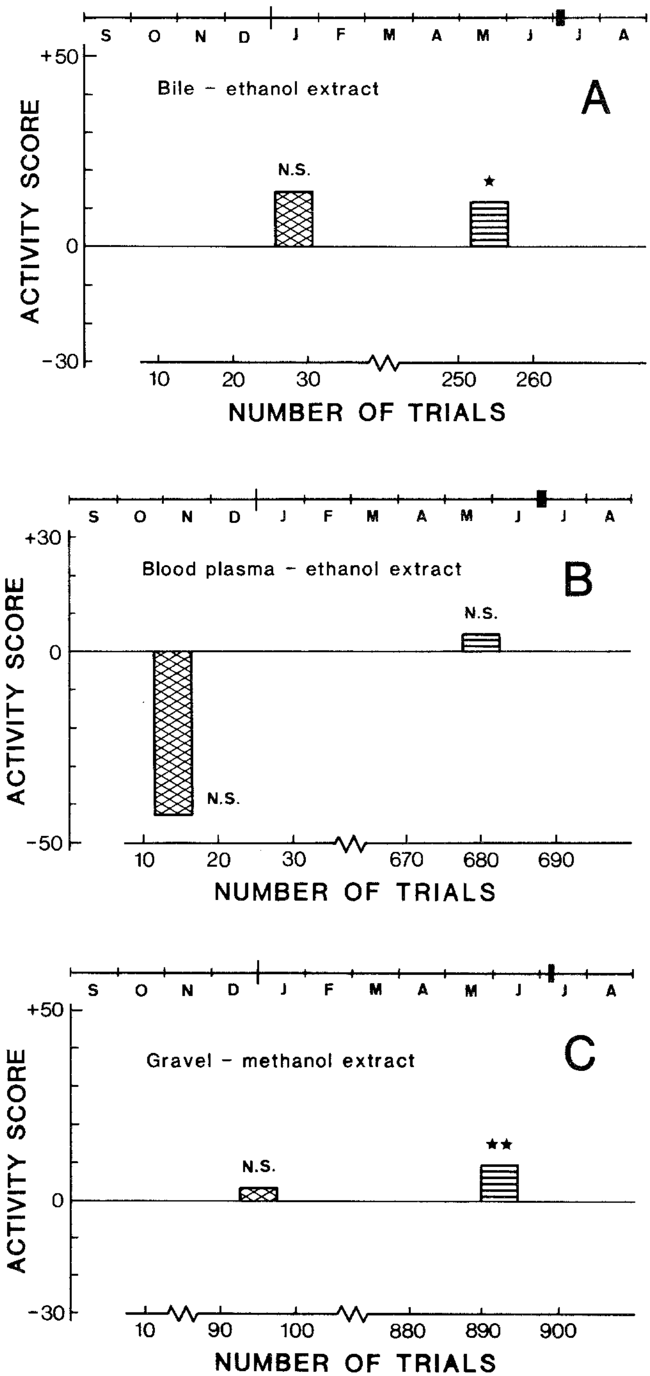
FIG. 4. Activity scores for ascents (cross-hatched bars) and search (horizontal lined bars) in test series with ethanol or methanol extracts from various sources. The tests were performed with extracts from familiar versus an unfamiliar river strain. (A) Competitive test with extracts from bile. (B) Competitive test with extracts from blood plasma. (C) Competitive test with extracts from gravel which had been retained for two months in rearing tanks below fish from two separate river strains. Strains of test fish used in each case and experimental design for each type of test performed are given in Table 1. General figure explanations are given in the legend to Figure 2.


S O N D J F M A M J J A
+50
Bile – ethanol extract
A
N.S.
★
0
-30
ACTIVITY SCORE
10 20 30 250 260
NUMBER OF TRIALS
S O N D J F M A M J J A
+30
Blood plasma – ethanol extract
B
N.S.
0
N.S.
-50
ACTIVITY SCORE
10 20 30 670 680 690
NUMBER OF TRIALS
S O N D J F M A M J J A
+50
Gravel – methanol extract
C
★★
N.S.
0
-30
ACTIVITY SCORE
10 90 100 880 890 900
NUMBER OF TRIALS

Experiments with extracts from blood plasma elicited no preference behavior of the fish from the Imsa strain (Figure 4B). Only 14 ascents were recorded in total in June 1983, with four in favor of the familiar strain (AS = −42.9; $P$ = 0.092); 355 searches of 680 were directed in a similar manner (AS = +4.4; $P$ = 0.134). This suggests that the attractant material found in the bile is secreted through the intestinal tract and not transported in the blood to be secreted elsewhere.

The Imsa parr demonstrated preference behavior when extracts from gravel were tested in June 1983 (Figure 4C). Of 892 searches recorded, 487 were in favor of the extract from gravel kept below their strain (AS = +3.2; $P$ = 0.0034). For ascents, 49 of 95 trials were in favor of the gravel extract from the tank containing familiar fish (AS = +9.2; $P$ = 0.417). Since the gravel used in the tanks originated from the same source, the results suggest that each strain of fish is able to mark the substrate of its living environment.

*Activity of Fish.* All experiments presented in this paper were performed within the relatively narrow temperature range of 7.3–9.3°C (Table 1). Activity of the fish, however, as shown by the data, appears to be independent of temperature. This suggests that variations obtained in activity of the fish must be related to parameters other than temperature.

When tank waters were used, the activity per hour of ascents ranged from 7.7 to 15.3, and for search from 107.5 to 166.6 (Table 1). Activity was found to be at the highest level when water containing an unfamiliar strain of fish was used. When waters from tanks containing fish were introduced to both entrances in competition tests, the activity was found to be at the lowest level. Regardless of the test type performed with tank waters, the ascents-to-search ratio remained relatively stable.

In tests with water extracts from skin mucus and intestinal contents (Table 1) a higher activity in search seems to have been present in November than in June. In general, test samples made from extracts also resulted in a lower activity than tank waters in the tests. For skin mucus, tests with ethanol extracts appear to have reduced the overall activity compared to tests with water extracts, whereas similar extraction from intestinal contents apparently had the opposite effect. Ethanol extracts from bile and blood plasma (Table 1) induced a very low activity in ascents, while search did not deviate apparently from that found for other organic solvent extracts. For gravel extracts, the fish demonstrated a behavior that did not deviate from normal activity.

## DISCUSSION

The data presented in this paper reveal that Atlantic salmon parr demonstrate preference behavior towards, and are attracted to, water conditioned by conspecifics. In competition experiments they favor water conditioned by their

TABLE 1. SUMMARY OF PREFERENCE TESTS PERFORMED AND ACTIVITY RELATIONSHIP FOR BEHAVIORAL PARAMETERS MEASURED[a]

| Figure | Sample origin | Type of test | Test Fish | Experimental Period | Activity per hour | | A/S ratio | T (°C) |
|---|---|---|---|---|---|---|---|---|
| | | | | | Ascents | Search | | |
| 2A | Tank water | S vs. TW | S | Oct. 28–Nov. 11 | 13.3 | 117.1 | 0.11 | 9.0 |
| 2B | Tank water | I vs. TW | S | Nov. 02–Nov. 03 | 15.3 | 166.6 | 0.09 | 8.6 |
| 2C | Tank water | S vs. I | S | Oct. 15–Oct. 27 | 7.7 | 107.5 | 0.07 | 9.3 |
| 3A | Skin mucus | S vs. I | S | Nov. 09–Nov. 11 | 5.4 | 67.7 | 0.08 | 8.1 |
| 3A | Skin mucus | I vs. S | I | June 09–June 16 | 6.4 | 25.7 | 0.25 | 7.3 |
| 3A | Skin mucus, ethanol extract | S vs. I | S | July 09–July 11 | 1.7 | 10.0 | 0.17 | 8.0 |
| 3B | Intestinal content | S vs. I | S | Nov. 11–Nov. 17 | 3.9 | 48.2 | 0.08 | 8.0 |
| 3B | Intestinal content | S vs. I | S | June 25–June 27 | 2.3 | 10.3 | 0.22 | 7.8 |
| 3B | Intestinal content, ethanol extract | I vs. S | I | June 28–June 29 | 2.3 | 81.1 | 0.03 | 7.7 |
| 4A | Bile, ethanol extract | S vs. I | S | July 04–July 07 | 1.4 | 12.7 | 0.11 | 7.9 |
| 4B | Blood plasma, ethanol extract | I vs. S. | I | July 01–July 04 | 0.7 | 34.0 | 0.02 | 7.9 |
| 4C | Gravel, methanol extract | I vs. A | I | July 07–July 09 | 4.8 | 44.6 | 0.11 | 7.9 |

[a] S = Sandvik River strain; I = Imsa River strain; A = Alta River strain; TW = tap water.

related strain to that conditioned by another genetic strain of their species. The findings reveal that salmon parr secrete substances specific for their strain and that they are able to discriminate between these chemical cues.

The secretion of signal substances seems restricted to the gastrointestinal tract and appears to originate from the liver, the largest "chemical factory" in the vertebrate body. Evidence has also been provided for substrate marking by salmonid fishes, presumably mediated by deposition of fecal material. In the following sections, the behavior towards chemical signals will be considered, together with aspects of physiology and ecology related to the ability of strain discrimination and substrate marking in salmonid fishes.

*Determination of Chemically Mediated Rheotaxis.* Fish denied olfaction demonstrate a preference for directing the body towards water currents (Høglund and Åstrand, 1973), i.e., positive rheotaxis is maintained in the absence of olfactory cues. In blind tests related to preference experiments, fish will also move upstream in the absence of any known chemical stimulus (Selset and Døving, 1980; Stabell, 1982). It is therefore difficult to give a direct measure for attractive responses due to chemical stimuli in the determination of fish behavior. The problem has been solved indirectly in behavior experiments by comparing the rate of activity of the fish to stimulants added simultaneously. In the current study, it has been possible to separate the behavior towards chemical stimuli into two events by using a double set of photocells in each tube (Figure 1B).

When held under abnormal and crowded conditions, it has previously been demonstrated that salmon parr secrete substances to which their relatives demonstrate an avoidance response when given an alternative and unscented choice of water (Stabell, 1982). Furthermore, confronted with a choice of odors from two stressed groups of fish, the parr demonstrated a preference for chemical cues secreted by their related strain. Both search and ascents were found within significant statistical levels of preference in the report mentioned, even when "repellent" substances were present in the samples tested. Tests with sample extracts in the current study, however, apparently produced a shift in preference behavior towards the search parameter. When organic solvents were used for sample extractions, ascents were found to work only once as a functional parameter in the tests. The shift in preference behavior was also found to coincide with a general decrease in activity when sample extracts were used, the decrease being greatest after treatment with organic solvents, while the ratio of ascents to search was not found to be systematically affected. A decrease in attractive responses in mature char, probably similar to the one obtained here, was also reported by Selset and Døvig (1980) when test samples had been treated by chemical means. Therefore, the extraction procedure rather than the presence of repellents seems responsible for the decrease in activity and the shift in preference behavior found in this study.

All pretreated samples in the present study were dissolved in small quantities of water before testing. In order to function as substrate markers, as will be discussed in a subsequent section, water solubility of the topical signal substances must be expected to be very low. The small volumes of water used for extraction of the active material may, therefore, have been a determining factor for the observed shift in behavior because of the low concentrations of the applied stimulus. Additional "trapping" of active compounds within precipitated material when organic solvents were used for extraction may possibly explain the special behavioral effects found following such treatment. Accordingly, both ascents and search must be viewed as parameters suitable for detecting preference behavior. While the ascent parameter is probably that which most strongly expresses attractive properties, it is suggested that search may work alone as a functional parameter in determination of chemically mediated rheotaxis when dealing with low stimulus concentrations.

*Production and Secretion of Odorants.* Preference tests with tank waters, each containing a separate genetic group of salmon parr, demonstrate that the fish secrete odorants specific for their strain. The observation that salmonid juveniles secrete intraspecific pheromones supports the results from several earlier reports in the field (Nordeng, 1971, 1977; Selset and Døving, 1980; Quinn and Busack, 1985; Quinn and Tolson, 1986; Olsén, 1985, 1986). Skin mucus was initially suggested to be the source of salmonid pheromones (reviewed by Stabell, 1984), but the origin of the attractive chemical components was questioned by Stabell and Selset (1980) because of a demonstrated contamination by intestinal juices of mucus collected in the customary way. Selset and Døving (1980), who found no behavioral response in mature Arctic char to "pure" skin mucus, also questioned skin mucus as a source for salmonid pheromones.

In the current study, ascents were never found below the 5% probability level in tests with water extracts from skin mucus. As for the search parameter, on one occasion only, in late autumn, an intraspecific preference for skin mucus was obtained. Since intestinal contents gave no behavioral responses at that time of the year, the result is difficult to interpret. Comparative physiologic indicators for presence of attractive substances in vertebrate skin mucus are, however, given in the literature. In female garter snakes (*Thamnophis sirtalis parietalis*), blood plasma has been shown to contain pheromones arousing sexual behavior in males (Garstka and Crews, 1981). As in this study, the pheromones from the mentioned reptiles are produced in the liver but are transported in the blood and secreted by mucus glands present between the surface scales. Extracts from blood plasma in the current study, however, revealed no preference or attractant properties. If mucus cells in salmonid fishes secrete attractant substances, the production must consequently be suggested to take place within the same cells. Possible attractant properties of skin mucus in salmonid fishes obviously await further investigation. Altogether, however, the data reported here appear to support the preliminary conclusion that the topical signal substances do not originate from skin mucus.

Tests with water extracts from intestinal contents of different salmon strains presented here demonstrate that the fish are able to discriminate among various compounds found in the gastrointestinal tract. The observed variation in odorants secreted cannot be attributed to food ingested, since the different strains of fish were fed by food pellets of common origin. Secretion of intraspecific pheromones through the intestinal tract by juvenile Atlantic salmon is in accordance with earlier observations with Arctic char (Selset and Døving, 1980). The experiments performed with ethanol extracts from intestinal contents in the present study were found to result in a significant preference level for ascents, while repellent effects were obtained for the search parameter. This was the only time during the study that contrary results were found for the parameters used, and an interpretation would therefore appear futile. Ethanol extracts from the bile, however, gave significant preference levels in search towards the familiar strain, supporting the conclusion by Selset (1980) that salmonid pheromones may originate from the liver and could be of a steroid nature.

*Substrate Marking and Regional Stability.* Atlantic salmon parr apparently mark their living environment with scents of an intraspecific nature, as demonstrated in this study. The odorants in question are most probably secreted within fecal material and may be deposited passively on the substrate by the bottom-dwelling parr. The chemical compounds representing the specific odors have been demonstrated to withstand dried-up conditions for at least a period of time, but they must also hold properties of low solubility due to the continuous flow of water in natural environments. Since the holding tanks in the current study were continuously exposed to flowing water, and the gravel washed by water before drying, a low solubility of the compounds in question seems to have been confirmed.

It has been suggested by some authors that salmonid fishes may leave residual scent on the spawning site long enough to secure homing by returning migrants (Larkin, 1975; Kristiansen, 1980; Selset, 1980; reviewed by Stabell, 1984). Foster (1985) also demonstrated in tank experiments that lake trout (*Salvelinus namaycush*) would preferently spawn on artificial reefs scented with material taken from eggs and excretion products of young fish. It would seem unlikely, from the point of view of energy costs, that juvenile fish have developed several parallel systems of pheromone production. The above reports, therefore, together with the data presented here, strongly indicate a common chemical basis for regional stability in juveniles and the mechanisms underlying homing of adult migrants to specific spawning grounds.

*Ecological Aspects of Olfactory Recognition.* Intraspecific recognition among juvenile siblings of Atlantic salmon, originating from different river strains, was demonstrated by Stabell (1982). It was suggested that the events observed among the parr were based on the same mechanisms of recognition as the one attracting mature migrants to the spawning grounds. Quinn and Busack (1985) also demonstrated an ability among juvenile coho salmon (*O. kisutch*) to recognize and prefer chemical cues secreted by siblings. They suggested that the most obvious adaptive values of kin recognition were to encourage schooling and thereby improve predator avoidance. Facilitated inbreeding avoidance among adults was also suggested as a possible resulting effect. The conclusions reached by Quinn and Busack (1985), however, appear to contradict the events generally observed in nature, since bottom-dwelling, territorial behavior in juvenile stream-living salmonids, together with specific return to native spawning grounds by adult maturing fish, are well documented phenomena. In the current study, fish other than pure sibling groups were used, indicating that strain recognition may work on additional levels to that of siblings and suggesting that a hierarchic order of chemical recognition may be at work within discrete populations of salmonids.

The fish in this study were able to discriminate between intestinal water extracts in June but not in the middle of November. This finding may result from a late autumn decline of the behavioral response, the olfactory sensitivity, or the secretory activity of the fishes. Since, however, high behavioral activities were still recorded in November, and olfactory sensitivity in electrophysiological recordings have been found present also during the winter (G. Thommesen, Department of General Physiology, University of Oslo, personal communication), it is suggested that the results are due to a decline or absence of pheromone secretion outside the migrating season.

Selset and Døving (1980) reported an ability of mature Arctic char to discriminate between conspecific odorants secreted within the intestinal contents of juvenile fish. The present study reveals that juvenile salmonids also have the ability to discriminate between conspecific odorants of intestinal origin from juveniles. An olfactory-dependent intraspecific recognition in young fish, together with substrate marking in the "home" area by juveniles, thus extends the view of the functional role of pheromones in the ecology of salmonid migration.

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## REFERENCES

CARGILL, A.S. 1980. Lack of rainbow trout movement in a small stream. *Trans. Am. Fish. Soc.* 109:484–490.

EDMUNDSON, E., EVEREST, F.E., and CHAPMAN, D.W. 1968. Permanence of station in juvenile chinook salmon and steelhead trout. *J. Fish. Res. Board Can.* 25:1453–1464.

FOSTER, N.R. 1985. Lake trout reproductive behavior: Influence of chemosensory cues from young-of-the-year by-products. *Trans. Am. Fish. Soc.* 114:794–803.

GARSTKA, W.R., and CREWS, D. 1981. Female sex pheromone in the skin and circulation of a garter snake. *Science* 214:681–683.

GERKING, S. D. 1953. Evidence for the concepts of home range and territory in stream fishes. *Ecology* 34:347–365.

GERKING, S.D. 1959. The restricted movement of fish populations. *Biol. Rev.* 34:221–242.

GUNNING, G.E. 1959. The sensory basis for homing in the longear sunfish, *Lepomis megalotis megalotis* (Raf.). *Invest. Indiana Lakes Streams* 5:103–130.

HARCUP, M.F., WILLIAMS, R., and ELLIS, D.M. 1984. Movements of brown trout, *Salmo trutta* L., in the River Gwyddon, South Wales. *J. Fish Biol.* 24:415–426.

HASLER, A.D., and SCHOLZ, A.T. 1983. Olfactory Imprinting and Homing in Salmon. Investigations into the Mechanism of the Imprinting Process. Springer Verlag, Berlin.

HASLER, A.D., and WISBY, W.J. 1951. Discrimination of stream odours by fishes and relation to parent stream behavior. *Am. Nat.* 85:223–238.

HASLER, A.D., SCHOLZ, A.T., and HORRAL, R.M. 1978. Olfactory imprinting and homing in salmon. *Am. Sci.* 66:347–355.

HESTHAGEN, T. 1978. Stationary behavior of stream-living trout (*Salmo trutta* L.) and young salmon (*Salmo salar* L.) in a stream in north Norway. (in Norwegian). *Cand. real.* thesis. Institute of Biology and Geology, University of Tromsø, Norway, 87 pp.

HØGLUND, L.B., and ÅSTRAND, M. 1973. Preferences among juvenile char (*Salvelinus alpinus* L.) to intraspecific odors and water current studied with the fluviarium technique. *Rep. Inst. Freshwat. Res. Drottningholm* 53:21–30.

KRISTIANSEN, H. 1980. Migration and life history of grayling, *Thymallus thymallus* (L.) in Lake Mjøsa. (in Norwegian) *Cand. real.* thesis. Institute of Zoology, University of Oslo, v + 148 pp.

LARKIN, P.A. 1975. Some major problems for further study on Pacific salmon. *Int. North Pac. Fish. Comm. Bull.* 32:3–9.

MILLER, R.B. 1954. Movements of cutthroat trout after different periods of retention upstream and downstream from their homes. *J. Fish. Res. Board Can.* 11:550–558.

NORDENG, H. 1971. Is the local orientation of anadromous fishes determined by pheromones? *Nature* 233:411–413.

NORDENG, H. 1977. A pheromone hypothesis for homeward migration in anadromous salmonids. *Oikos* 28:155–159.

OLSÉN, K.H. 1985. Chemoattraction between fry of Arctic charr [*Salvelinus alpinus* (L.)] studied in a Y-maze fluviarium. *J. Chem. Ecol.* 11:1009–1017.

OLSÉN, K.H. 1986. Chemoattraction between juveniles of two sympatric stocks of Arctic charr [*Salvelinus alpinus* (L.)] and their gene frequency of serum esterases. *J. Fish Biol.* 28:221–231.

QUINN, T.P., and BUSACK, C.A. 1985. Chemosensory recognition of siblings in juvenile coho salmon (*Oncorhynchus kisutch*). *Anim. Behav.* 33:51–56.

QUINN, T.P., and TOLSON, G.M. 1986. Evidence of chemically mediated population recognition in coho salmon (*Oncorhynchus kisutch*). *Can. J. Zool.* 64:84–87.

SAUNDERS, R.L., and GEE, J.H. 1964. Movements of young Atlantic salmon in a small stream. *J. Fish. Res. Board Can.* 21:27-36.

SELSET, R. 1980. Chemical methods for fractionation of odorants produced by char smolts and tentative suggestions for pheromone origins. *Acta Physiol. Scand.* 108:97–103.

SELSET, R., and DØVING, K.B. 1980. Behavior of mature anadromous char (*Salmo alpinus* L.) towards odorants produced by smolts of their own population. *Acta Physiol. Scand.* 108:113–122.

SIEGEL, S. 1956. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, New York.

STABELL, O.B. 1982. Detection of natural odorants by Atlantic salmon parr using positive rheotaxis olfactometry, pp. 71–78, *in* E.L. Brannon and E.O. Salo (eds.). *Proceedings of the Salmon and Trout Migratory Behavior Symposium, June 1981.* University of Washington, Seattle.

STABELL, O.B. 1984. Homing and olfaction in salmonids: A critical review with special reference to the Atlantic salmon. *Biol. Rev.* 59:333-388.

STABELL, O.B., and SELSET, R. 1980. Comparison of mucus collecting methods in fish olfaction. *Acta Physiol. Scand.* 108:91-96.

STOTT, B. 1967. The movements and population densities of roach (*Rutilus rutilus* L.) and gudgeon (*Gobio gobio* L.) in the river Mole. *J. Anim. Ecol.* 36:407-423.