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Bull Trout (Salvelinus confluentus) can detect conspecific pheromones in a two choice Y-maze

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Introduction

Bull Trout (Salvelinus confluentus, Figure 1) were tested in a two choice Ymaze to determine if they could be attracted to population specific pheromones (PSPs produced by other members of the same Bull Trout population) or conspecific pheromones (CSPs produced by a different Bull Trout population). This experiment was conducted to determine the potential for using Bull Trout pheromones to attract other Bull Trout into a fish trap below Albeni Falls Dam.

Albeni Falls Dam, located on the Pend Oreille River downstream of Lake Pend Oreille, Idaho does not have a fish ladder, so if Bull Trout that spawn in tributaries of the lake entrain over the dam, they are unable to return to their natal (home) stream. The U.S. Army Corps of Engineers recently funded a trap-andhaul facility to collect Bull Trout below the dam and transport them above it. The objective was to support homing of Bull Trout to their natal tributaries and improve population demographics in each of them.

We conducted radio-tracking studies of 12 Bull Trout implanted with temperature/pressure sensitive transmitters and released below Albeni Falls Dam in 2015 to observe their interactions with the trap. Eleven of them passed within a few meters of the trap entrance but none of them entered it. Hence, we conducted the present study to determine the efficacy for using Bull Trout pheromones to attract free swimming Bull Trout below Albeni Falls Dam into the trap.

Methods

- Two stocks of Bull Trout, one from the Metolius River (MR), OR and a second from the Pack River (PR), ID were tested to determine if they could detect pheromones from the same natal population (population specific pheromones PSP) or from a different (conspecific) population of Bull Trout (CSP). [Each fish was implanted with a PIT tag].
- Prior to testing, each stock was held in a separate raceway at the Kalispel Tribal fish hatchery in 9°C well water.
- Fish were put into the containment compartment of the Y-maze and accessed either arm through a plywood weir (See Figure 2). Well water at 9 °C was supplied to both arms of the Y-maze, and one arm was randomly selected (via coin toss) to be supplied with pheromones from one of the stock tanks. Temperature and discharge of each arm were matched to within 0.1°C and 0.01 l/s (checked at least 3X daily).
- A dye test was used to determine how mixed the odors were and the amount of time to clear odor out of each arm (See Figure 3).
- Four types of tests were conducted with each stock:
 - 1. No treatment supplied to either arm (Control Trials)
 - 2. PSPs added to well water in one arm and blank in the other arm (PSP Trials)
 - 3. CSPs added to well water in one arm and blank in the other arm (CSP Trials)
 - 4. PSPs added to well water in one arm and CSPs added to well water in the other arm (PSP-v-CSP Trials)
- Fish were tested until they remained continuously in one arm for 30 minutes or for 1 hour if they moved back and forth into both arms. Two stop watches were used to keep track of the amount of time spent in each arm. [Note: This was backed up using a PIT tag array comprised of two antennas (at downstream and upstream ends) of each arm].
- Time spent in each arm was recorded on an Excel spreadsheet. Arm preference was calculated by dividing the amount of time spent in each arm by the total time in both arms of the maze. Preferences of 55/45, 60/40, 70/30, 80/20, and 90/10 percent were tabulated, and the data for the 70/30 group was tested statistically.
- Categorical data about the preference for a particular arm was tested using a Chi Square (χ^2) Goodness of Fit Test. This test compared the frequency at which fish entered each arm to a theoretical distribution that assumed they had randomly selected an arm. The calculated χ^2 value for each test was compared to the critical value for $\alpha = 0.05$ (5.991). If calculated $\alpha < 5.991$, we accepted the null hypothesis (that the observed and theoretical distributions were uniform). If calculated $\alpha \ge 5.991$, we rejected the null hypothesis and accepted one of two alternatives: 1) fish preferred an arm scented with a particular PSP or CSP pheromone if the fish selected that arm more frequently than the control arm, or 2) fish preferred a control arm to one scented with a particular PSP or CSP if the fish selected the control arm more frequently than it selected the pheromone arm.
- The amount of time that fish spent in each arm during a particular test was compared using a non-parametric Kruskal-Wallis test which determined if the mean time spent in each arm was uniform or different. The H-statistic was calculated by assigning a rank to each individual time spent in each arm of the maze for a particular experiment. The ranked values from one arm were compared to the ranked values for the other arm to determine if the means were uniform. The value calculated for the H-statistic was compared to the critical value of $\alpha = 0.05$ at df (n₁ - 1, n₂ - 1). We accepted the null hypothesis (that there was no difference between the means) if the calculated H-statistic was less than the critical value. We rejected the null hypothesis if the calculated H-statistic was greater than the critical value.







Bull Trout (Salvelinus confluentus) can detect conspecific pheromones in a two choice Y-maze Hannah M. Coles¹, Allan T. Scholz¹, Raymond Ostlie², Paul Spruell¹, Mark Paluch¹, and Jason Connor² ¹Eastern Washington University Fisheries Research Center and ²Kalispel Tribe of Indians Department of Natural Resources

Figure 1. Bull Trout (*Salvelinus Confluentus*). Illustration by Joseph Tomerelli.

Figure 2. Schematic diagram of Y-maze, with two 602 liter stock tanks (housed fish producing MR and PR pheromones). Flexible hoses on each stock tank could be adjusted to supply pheromones to either arm of the maze.

Figure 3. Dye test in Y-maze: Rhodamine-B (red) and florescent yellow/green Bright Dye were introduced into left and right simultaneously: Indicates discharge in both arms was well matched.

Chi Square Goodness of Fit Test (See Table 1)

- p > 0.05 (= 0.091), n = 28.
- p > 0.05 (= 0.097), n = 27.
- +PSP arm].
- +CSP arm]
- +CSP arm]

- because $\chi^2 = 4.90 \ (< 5.991) \ (a) \ 2 \ df. \ p > 0.05 \ (= 0.086), \ n = 31.$

the $\alpha = 0.05$ level.

	Chi Squar	e (χ ²) Good	ness	of F	it Te	st		
Test Type	Fish Stock	Arm Pref.	Obs.	Exp.	Ν	df	χ^2	p
С	PR	1	13	9.33	28	2	4.79	0.09
		2	11	9.33				
		No Pref.	4	9.33				
	MR	1	8	9.00	27	2	4.67	0.09
		2	5	9.00				
		No Pref.	14	9.00				
	PR	+PSP	18	9.00	27	2	14.00	0.00
		-PSP	3	9.00				
PSP		No Pref.	6	9.00				
	MR	+PSP	21	9.33	28	2	21.94	
		-PSP	3	9.33				*
		No Pref.	4	9.33				
	PR	+CSP	18	8.67	26	2	15.07	0.00
		-CSP	4	8.67				
CSP		No Pref.	4	8.67				
	MR	+CSP	21	10.33	31	2	16.72	<00
		-CSP	4	10.33				*
		No Pref.	6	10.33				
PSP-v-CSP	PR	+PSP	8	9.67	29	2	2.96	0.22
		+CSP	14	9.67				
		No Pref.	7	9.67				
	MR	+PSP	15	10.33	31	2	4.90	0.08
		+CSP	5	10.33				
		No Pref.	11	10.33				

Kruskal-Wallis (H-Statistic) Test (See Table 2)

- 0.05. PR fish spent 2.8 x more time in arm with PR PSPs than arm without PR PSPs.
- spent 2.7 x more time in arm with CSPs than arm without CSPs.

- fish spent 1.5 x more time in arm with MR PSPs than arm with CSPs.

Results

• PR (control trials) had no preference for either arm because $\chi^2 = 4.79$ (< 5.991) (a) 2 df.

MR (control trials) had no preference for either arm because $\chi^2 = 4.67$ (< 5.991) (a) 2 df.

• PR (PSP trials) preferred the arm with PR PSPs over the arm without PR PSPs because $\chi^2 = 14.00 \ (> 5.991) \ (a) \ 2 \ df. \ p < 0.05 \ (= 0.001), n = 27 \ [18 \ of the \ 27 \ (66.6\%) \ fish \ selected \ the$

• MR (PSP trials) preferred the arm with MR PSPs over the arm without MR PSPs because $\chi^2 = 21.94 (> 5.991)$ @ 2 df. p < 0.05 (< 0.001), n = 28 [21 of the 28 (75%) selected the +PSP

• PR (CSP trials) preferred the arm with CSPs (MR PSPs) over the arm without CSPs because $\chi^2 = 15.07 \ (> 5.991) \ @ 2 df. \ p < 0.05 \ (= 0.001), n = 26 \ [18 of the 26 \ (69.2\%) fish selected the$

• MR (CSP trials) preferred the arm with CSPs (PR PSPs) over the arm without CSPs because $\chi^2 = 16.72 \ (> 5.991) \ @ 2 df. p < 0.05 \ (< 0.001), n = 31 \ [21 of the 31 \ (67.7\%) fish selected the$

• PR (PSP-v-CSP trials) had no preference for either arm with PR PSPs or CSPs (MR PSPs) because $\chi^2 = 2.96 (< 5.991)$ @ 2 df. p > 0.05 (= 0.227), n = 29.

• MR (PSP-v-CSP trials) had no preference for either arm with MR PSPs or CSPs (PR PSPs)

Table 1. Chi Square (χ^2) Goodness of Fit Test calculated values for all four tests conducted in the Y-maze with PR and MR stocks. P-values marked with an asterisk (*) are significant at

• PR (control trials) mean time spent in Arm 1 = mean time spent in Arm 2. H statistic = 0.250, n = 56, df ($n_1 = 27$, $n_2 = 27$), p = 0.617. Result not significant at $\alpha 0.05$.

• MR (control trials) mean time spent in Arm 1 = mean time spent in Arm 2. H statistic = 0.326, n = 54, df ($n_1 = 26$, $n_2 = 26$), p = 0.566. Result not significant at $\alpha 0.05$.

• PR (PSP trials) mean time spent in arm with PR PSPs \neq mean time spent in arm without PR PSPs. H statistic = 18.781, n = 54, df ($n_1 = 26$, $n_2 = 26$), p < 0.001. Result significant at α

• MR (PSP trials) mean time spent in arm with MR PSPs \neq mean time spent in arm without MR PSPs. H statistic = 24.168, n = 56, df ($n_1 = 27$, $n_2 = 27$), p < 0.001. Result significant at α 0.05. MR fish spent 2.9 x more time in arm with MR PSPs than arm without MR PSPs. • PR mean time spent in arm with CSPs (MR PSPs) \neq mean time spent in arm without CSPs. H statistic = 12.671, N = 52, df (n_1 = 25, n_2 = 25), p < 0.001. Result significant at α 0.05. PR

• MR mean time spent in arm with CSPs (PR PSPs) \neq mean time spent in arm without CSPs. H statistic = 22.577, N = 62, df (n_1 = 30, n_2 = 30), p < 0.001. Result significant at α 0.05. MR spent 2.6 x more time in arm with CSPs than arm without CSPs.

• PR mean time spent in arm with PR PSPs = mean time spent in arm with CSPs (MR PSPs). H statistic = 1.873, n = 58, df ($n_1 = 28$, $n_2 = 28$), p = 0.171. Result not significant at α 0.05. • MR mean time spent in arm with MR PSPs \neq mean time spent in arm with CSPs (PR PSPs). H statistic = 6.315, n = 62, df (n_1 = 30, n_2 = 30), p = 0.012. Result significant at α 0.05. MR

Table 2. Kruskal-Wallis H Test calculated values for all four tests conducted in the Y-maze for each PR and MR stocks. P-values marked with an asterisk (*) are significant at the $\alpha = 0.05$ level.



Bull Trout detected pheromones produced by other Bull Trout because when no Bull Trout pheromones were added to either arm in control tests, neither MR nor PR Bull Trout had a preference for either arm. However, if either CSPs or PSPs were added to one of the arms in experimental tests, both MR and PR Bull Trout had a statistically significant preference for the arm with pheromones added. Results of experimental tests with CSPs added to one arm and PSPs added to the other were somewhat ambiguous. χ^2 Goodness of Fit tests indicated that neither MR nor PR Bull Trout had a preference for either arm. Kruskal-Wallis tests indicated that PR Bull Trout spent equal time in both CSP and PSP arms, whereas MR Bull trout spent statistically more time in the PSP arm (contained MR pheromones) than the CSP arm (contained PR pheromones). However, both groups of fish spent more time in the arm scented with MR pheromones than PR pheromones.

MR B

Thus, we conclude that Bull Trout can detect CSPs, but it is unclear if they can differentiate between CSPs and PSPs. (The MR Bull Trout were ~ 4X the size of the PR Bull Trout, and 5 fish from each group were supplied to the stock tanks that were the source of pheromones, so perhaps both stocks spent more time in the MR arm than the PR arm because it contained a greater concentration of pheromones).

Stabell (1987) found that Atlantic Salmon (Salmo salar) in a Y-maze were attracted to water that contained their own relatives (PSPs) to blank water, but were also attracted to water that contained a different population of Atlantic Salmon (CSPs) to blank water. When given a choice between an arm that contained PSPs and one that contained CSPs, they choose the arm that contained PSPs a significantly greater percentage of the time. This study indicated that Atlantic Salmon could discriminate between PSPs and CSPs, and that when given a choice between them they preferred the arm scented with PSPs.

Quinn and Tolson (1986) tested Coho Salmon (Oncorhynchus kisutch) from two different natal populations in British Columbia in a Y-maze. Results showed that fish preferred an arm that contained water scented with fish from their own river (PSPs) to an arm scented with fish from the other river (CPSs).

Dams without fish ladders, e.g., Albeni Falls Dam on the Pend Oreille River, fragment Bull Trout spawning populations by blocking the migration of individuals that entrain below the dam back to natal tributaries above the dam. Small spawning population sizes can cause a loss in genetic variation when Bull Trout are unable to return to their natal spawning tributaries. Redd counts in natal tributaries surrounding Lake Pend Oreille, Idaho have declined in recent years, so restoring connectivity will likely improve population demographics in each of them (Bouwens and Jakubowski 2016).

Bull Trout also prefer water $\leq 16^{\circ}$ C and do not survive well at temperatures $\geq 18^{\circ}$ C. Water temperature of the Pend Oreille River below Albeni Falls Dam reaches $\geq 23 - 24$ °C by August (lethal for Bull Trout), so restoring connectivity at Albeni Falls Dam will also likely increase survival of Bull Trout by providing them with a cold water thermal refuge $\leq 12^{\circ}$ C in the thermocline or hypolimnion of Lake Pend Oreille.

A trap-and-haul facility was recently installed below Albeni Falls Dam to capture Bull Trout and transport them above the dam but to date has been ineffective in attraction of Bull Trout through the entrance. Our results indicate that it may be possible to improve the attraction of Bull Trout into fish trap entrances by adding pheromones. Moreover, our results indicated that Bull Trout from any natal population are attracted to CSPs produced by a different natal population. Beginning in 2019, we plan to pump water laden with Bull Trout pheromones through the entrance of the trap to determine if it improves the efficacy of Bull Trout attraction into it. We intend to compare the number of Bull Trout entering the trap on randomly selected control days (when Bull Trout pheromones are absent in the trap) and experimental days (when Bull Trout pheromones are present in the trap).

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Kruskal-Wallis H Test						
Fish Stock	Arm	Time (±SD) (min)	# Pref.	Ν	Η	р
PR	1	17.2 ± 13.5	15	56	0.250	0.617
	2	15.2 ± 13.2	13	50		
MR	1	19.2 ± 10.3	14	54	0.326	0.566
	2	17.6 ± 10.5	13	54	0.520	0.500
PR	+PSP	25.7 ± 9.5	21	51	18.781	<0.001 *
	-PSP	9.1 ± 11.9	6	34		
MR	+PSP	27.0 ± 7.9	25	56	24.168	<0.001
	-PSPS	9.3 ± 9.9	3	50		*
PR	+CSP	24.3 ± 10.8	20	50	12.671	<0.001 *
	-CSP	9.0 ± 12.1	6	54		
MR	+CSP	26.3 ± 8.7	27	67	22 577 <0.	<0.001
	-CSP	10.2 ± 10.7	4	02	22.577	*
PR	+PSP	14.4 ± 13.3	11	50	1 972	0 171
	+CSP	19.8 ± 12.4	18	20	1.0/3	0.1/1
MR	+PSP	23.0 ± 9.0	19	62	6.315	0.012*
	+CSP	15.7 ± 11.7	12			

Discussion

it pheromones than i it pheromones.					
Group	MR arm (min.)	PR arm (min.)			
Ill Trout	19.8 ± 12.4	14.4 ± 13.3			
ull Trout	23.0 ± 9.0	15.7 ± 11.7			

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Literature Cited