Ovarian Fluid Pheromone Testing

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Acknowledgments

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Introduction and background

Chemoreception has been known widespread in the animal kingdom. The insects represent the most extensively studied examples of chemoreception in the animal kingdom (Carde, 1990; Chapman, 2000), and scientific research on the theme of chemical communication mediating various behaviors has led to the development of economically and ecologically important tools for the management of insect species of interest. In fish, pheromone-mediated behaviors, reproductive behaviors in particular, have been well documented (Liley and Stacey, 1983) but little information on the chemical identity of pheromones involved is available, except for a few studies where the chemical identity of the chemosensory cues was elucidated in goldfish (Sorensen and Stacey, 1999), catfish (Resink et al., 1989), and sea lamprey (Li et al., 1995; Li et al., 2002; Sorensen et al., 2005).

Chemical communication systems mediated by pheromones in the various animal groups have been offering useful biological tools in manipulating populations of interest (Carde, 1990; Li et al., 2003). The basic characteristics of pheromones, species-specificity and low threshold for responses, make them very useful in applying to fisheries management, once they are identified. In particular, better understanding of pheromone identities involved in critical life history stages could offer a possible target for population manipulation. In fisheries management pheromones could elicit significant physiological and behavioral responses in fish.

Ample evidence exists to support the notion that sturgeon rely heavily on their chemosensory system for feeding behaviors, homing, and male-female interactions (Bruch and Binkowski, 2002; Dadswell, 1979; Kasumyan, 1993, 1999; 2002; Kynard and Horgan, 2002). Chemical communication in sturgeon has been described in European species and some Atlantic coast species. In the earlier works by Kasumyan (1993, 1999), it was demonstrated that males are attracted by female postovulatory pheromones. In Russian sturgeon *A. gueldenstaedti* and starred sturgeon, *A. stellatus*, the odor of ovarian fluid from ovulatory females was found to be a highly effective stimulant for male sturgeon, demonstrating presence of chemical communication mechanisms mediated by sex pheromones (Kasumyan, 1993, 1999). It was also demonstrated in the shortnose sturgeon, *A. brevirostrum* that males can discover mature females by the odor (Kynard and Horgan,

2002). Field observations on white sturgeon in the spawning grounds showed that spawning activity begins after females move on to the site and begin ovulating, which indicates initiation of male spawning behaviors triggered by unknown chemical cues from ovulatory females (Bruch and Binkowski, 2002).

Stocking, habitat protection, and habitat restoration are the focus of recovery efforts in the Nechako River. In particular, the Nechako White Sturgeon Recovery Initiative has been working to create suitable gravel substrate for spawning. However, there is a potential need to draw spawning adults to the spawning ground to make such habitat restoration efforts complete. Based on biological information from other sturgeon species, it can be speculated that white sturgeon also use chemical cues for their behavioral interactions during migration and reproduction. A better understanding of chemosensory mechanisms mediated by chemical cues could offer managers a scientific background for the formulation of the most efficient white sturgeon restoration strategies in the Nechako River. In this project, we examined whether sturgeon were attracted to female ovarian fluid in the natural environment.

Objective

To test the hypothesis that ovarian fluid can act as an attractant to mature adult white sturgeon in a river environment.

Methods

1. Ovarian fluid collection

Ovarian fluid was collected from Nechako White Sturgeon Conservation Hatchery wild brood stock. Ovulation was induced by double injection of GnRH on May 25 – 26 and on May 31 – June 1, 2015, which is typical timing of wild spawning activity in Nechako River. Ovarian fluid was stored for less than 24 hrs in -20 °C.

2. Application of ovarian fluid and tracking of white sturgeon

Ovarian fluid was dosed into Nechako River at strategic locations, during a time when white sturgeon (*Acipenser transmontanus*) spawning activity is typically observed. Prior to dosing the river, the technical working group searched the Nechako River spawning reach for radio-tagged white sturgeon using Lotek SRX800 mobile receiver. Time, location, radio frequency, maximum signal strength, and radio code were recorded for each unique fish detected in the area. If a radio code was detected multiple times, but within 30 seconds of a previous detection it was only recorded once and a running tally of minutes detected was recorded. If a radio code was detected once, and then the same code was detected more than 30 seconds after the previous detection it was recorded as a new detection.

Ovarian fluid dosing occurred in areas that were approximately 200 m upstream of radio tagged fish detections. The fluid was pumped into the river using a Masterflex peristaltic pump (Cole-Parmer, Vernon Hills, IL, USA) calibrated for ovarian fluid in the laboratory, using approximately 15 m of tubing, and a benthic mooring. Tubing of approximately 4 mm I.D. was used to ensure that some eggs present in ovarian fluid did not block the flow of ovarian fluid. The delivery/nozzle tubing was attached to the mooring approximately 1 m above the foot of the mooring using a combination of metal hose clamps and duct tape. Video recordings were collected using Shark Marine underwater camera, and recorded to MiniHD tapes using Canon Vixia HV30 (Canon, Japan). Tape footage was subsequently digitized.

Experiment 1

Releases during experiment 1 occurred ~6.4 downstream of the Burrard Avenue Bridge, based on telemetry based locations of fish. During the first trial of experiment 1 ovarian fluid flow rate was held constant at 20 ml/min. During the second trial of experiment 1 the

ovarian fluid flow rate was modified to 100 ml/min for the first three minutes, and then down-regulated to 20 ml/min for the remainder of the fluid was released. Trial 1 ovarian fluid dosing commenced at 13:09:05 on May 27th 2015, and ended at 13:39:17, for a total duration of 30 minutes and 12 seconds, and total ovarian fluid dose of 603 ml. Trial 2 (Table 2) of experiment 1 started at 13:48:25 in the same location as trial 1 and lasted for a total duration of 16 minutes and 37 seconds, and a total ovarian fluid volume of 572.4 ml.

Table 1. Summary of doses used the present pheromone study

Experiment	**Doses and application duration**	**Locations and conditions**
Exp 1 Trial 1	20 ml/min	0436351 easting, 5986881 northing, zone 10 river width = 143m, depth = 3.8m
Exp 1 Trial 2	100 ml/min for 3 mins, 20 ml/min for the rest of experiment	
Exp 2 Trial 1	100 ml/min for 5 min, 25 ml/min for 40 min	0433657 easting, 5986944northing, zone 10, River width = 145m, depth = 3.1m
Exp 2 Trial 2	200 ml/min for 5 min, no further release	

Experiment 2

A second experiment was carried out on June 2, 2015. It replicated the first experiment except the dosing rate was increased to test a hypothesis that greater concentration would result in increased white sturgeon attractiveness. Experiment 2 also consisted of two trials meant to retest the objective hypothesis using faster ovarian fluid flow rates, thus slightly more concentrated dosing. Experiment 2 occurred at a predetermined location just upstream of an 18-receiver VPS array used for precisely locating acoustically tagged fish (see Beardsall and McAdam 2016). The dosing location was chosen for two reasons; 1) because it was within a known spawning location for white sturgeon, and 2) because of the possibility of attracting an acoustically tagged white sturgeon, in which case the VPS would collect detailed movement data of the attraction process. During the first trial of experiment 2 ovarian fluid flow rate started at 100 ml/min for the first 5 minutes, and then reduced to 25 ml/min for 40 minutes. During trial 2 ovarian fluid flow rate started at 200 ml/min for 5 minutes, and then no more ovarian fluid was pumped for the remaining 25 minutes of the trial. Experiment 2, trial 1 started on June 2nd, 2015 at 17:22:19. Trial 2 began at 18:04:36.

Biological data and spawning likelihood are provided for radio tagged fish detected during the experiment. Fish were considered spawners if they had been tagged in 2015 and their gonads were mature, or if they had been captured and tagged in 2014 and their gonads were near maturity. Fish were considered possible spawners if they had been captured and tagged previous to 2014, and they were large enough to be considered mature adults. Fish were considered unlikely spawners if they had been captured and tagged in 2015 and their gonads were not mature, or if they had been captured and tagged in 2014 and their gonads were mature and ready to spawn in 2014.

3. Data analysis

River discharge data for the days of experiments were obtained from Canada water office (https://wateroffice.ec.gc.ca/report/report_e.html?mode=Graph&type=realTime&stn=08JC001&dataType=Real-Time&startDate=2015-06-01&endDate=2015-06-06&prm1=47&y1Max=&y1Min=&prm2=5&y2Max=&y2Min=).

Results

River Flow Conditions and Dilution Factor

During the experiments, water discharge at the experimental sites was approximately 675 m^3/s. Assuming ovarian fluid applied to the river become totally mixed with river water, dilution factors for each dose can be estimated. At 20 ml/min, 100 ml/min, and 200 ml/min doses, it was estimated that dilution factors were 2.25 x 109, 400x106, and 200x106, respectively.

Experiment 1

Experiment 1 was conducted on May 27th, 2015, and consisted of two experimental trials. Five radio tagged white sturgeon were detected between river kilometer (rkm) 130 and 135 (Table 1), and two of those fish were located relatively close together (~200 m separation). Due to the presence of two fish relatively close together, the technical working group decided to move the experiment 1 dosing location upstream, just out of detection range of those two fish (codes 42 and 38).

Three unique radio codes were detected between 13:16:00 and 13:18:00, however these radio code detections were low signal strength. Code 64 was detected for less than a minute, code 15 was detected for one minute, and code 18 was detected for just over two minutes. Code 13 was detected pre-dosing, and remained within the detection range for nearly the entire dosing period of trial 1. Code 13 was typically low signal strength thus relatively far from the dosing location, however that fish appeared to move close to the dosing location at 13:28:08 (92 signal strength) and at 13:35:26 (131 signal strength).

Before reporting underwater video results, it should be noted that water clarity was extremely low making it difficult to identify fish more than an estimated 0.5 m away from the lens. In addition it was also difficult to extract snapshots of clearly identifiable fish, but when watching the video in motion there are certainly instances of fish moving within frame. Underwater video footage revealed a small, unidentifiable fish present in frame at 13:25:25. At 13:25:54 the snout of a large fish (suspected white sturgeon) enters the top left frame of the video, but water clarity prevents a 100% certain identification. At 13:26:25 another white sturgeon-shaped fish moves from left to right across the video frame, and again from right to left across the frame at 13:26:42. A northern pikeminnow (*Ptychocheilus oregonensis*) enters the frame at 13:29:12 (Figure 1), and repeatedly enters the video frame inspecting the pheromone nozzle until 13:29:34.

Figure 1. Snapshot of northern pikeminnow (*Ptychocheilus oregonensis)* captured approximately 20 minutes after the start of experiment 1, trial 1.

A northern pikeminnow is identified within frame again at 13:30:09, and continues to come in and out of frame until 13:33:25. At 13:34:00 a relatively large fish with a white outline along the ventral edge of the body moves from the top of the frame towards the bottom and slightly towards the right edge of the frame. A scute row is not clearly visible, but this is another suspected white sturgeon captured on frame. A similar shape appears on frame at 13:34:36 (Figure 2), but the fish is too far away to clearly identify.

Figure 2. Snapshot of a suspected white sturgeon in center of frame; unfortunately water clarity makes it difficult to identify the fish at distance.

Northern pikeminnow continue to enter and exit the video frame for the remainder of the experiment. At 13:35:25 two pikeminnow are seen within frame (Figure 3).

Figure 3. Snapshot captured at the end of Experiment 1 Trial 1 containing two northern pikeminnow.

Table 2. Summary of white sturgeon (WSG) radio detections and underwater video footage identifications during experiment 1 trial 1

Experiment 1 - Radio and Video Tracking Summary					
Time (24hr)	**Radio Freq.**	**Radio Code**	**Power**	**Duration (min)**	**Comments**
			Predose		
11:51:00	149.52	62	50	NA	Male, unlikely spawner, 206.2 cm TL, 2015-04 release
11:54:00	149.52	65	97	NA	Male, possible spawner, 174.4 cm TL, 2012-10 release
11:57:00	148.6	32	94	NA	Female, spawner, 272.5 cm TL, 2015-05 release
12:05:00	149.52	42	109	NA	Male, possible spawner, 248.4 cm TL, 2014-05 release
12:06:00	149.52	38	104	NA	Female, unlikely spawner, 236 cm TL, 2015-05 release
13:05:00	149.52	13	<50	35	Male, possible spawner, 202.4 cm TL, 2011-05 release
			Trial 1 - Start at 13:09:05		
13:16:15	149.52	64	<50	<1	Female, possible spawner, 213.8 cm TL, 2012-10 release
13:16:15	149.52	15	<50	1	Male, possible spawner 218.1 cm TL, 2011-04 release
13:17:50	149.52	18	<50	2	Male, possible spawner, 275 cm TL, 2011-04 release
13:25:25	NA	NA	NA	<1	Small unidentifiable fish in video frame for a couple seconds
13:25:54	NA	NA	NA	<1	Snout of large fish (suspected WSG) briefly in frame
13:26:25	NA	NA	NA	<1	Suspected WSG
13:26:42	NA	NA	NA	<1	Suspected WSG
13:28:08	149.52	13	92	35	Male, possible spawner, 202.4 cm TL, 2011-05 release
13:29:12	NA	NA	NA	<1	NPM inspects/feeds at pheromone release nozzle
13:30:09	NA	NA	NA	3	NPM intermittantly inspects/feeds at pheromone release nozzle
13:34:00	NA	NA	NA	1	Suspected WSG
13:35:25	NA	NA	NA	<1	Two NPM in frame
13:35:26	149.52	13	131	35	Male, possible spawner, 202.4 cm TL, 2011-05 release

NPM = Northern pikeminnow ; WSG = white sturgeon

Code 18 was detected at 13:53:02, for just over three minutes and peak signal strength of 64, then two unique fish (codes 44 and 16) were detected at 13:54:50 and 13:55:20 respectively, for less than one minute. Code 15 was detected at 14:03:25, just prior to the end of trial 2 at 14:05:02.

Table 3. Summary of white sturgeon (WSG) radio detections and underwater video footage identifications during experiment 1 trial 2.

Experiment 1 - Radio and Video Tracking Summary					
Time (24hr)	**Radio Freq**	**Radio Code**	**Power**	**Duration (min)**	**Comments**
Trial 2 - Start at 13:48:25					
13:50:12	NA	NA	NA	<1	NPM intermittantly inspects/feeds at pheromone release nozzle
13:50:45	NA	NA	NA	<1	Suspected WSG
13:50:50	NA	NA	NA	<1	WSG clearly identified; only in frame for a second
13:51:04	NA	NA	NA	<1	NPM briefly enters frame
13:53:02	149.52	18	64	3	Male, possible spawner, 275 cm TL, 2011-04 release
13:53:52	NA	NA	NA	<1	WSG briefly crosses upper right corner of video frame
13:54:50	149.52	44	<50	<1	Male, unlikely spawner, 243.8 cm TL, 2014-05 release
13:55:20	149.52	16	<50	<1	Female, possible spawner, 221.1 cm TL, 2011-05 release
13:55:53	NA	NA	NA	<1	NPM briefly enters frame
13:58:38	NA	NA	NA	<1	WSG briefly holds position downstream of nozzle
14:01:30	NA	NA	NA	<1	NPM aggressively feeds from nozzle
14:03:25	149.52	15	<50	1	Male, possible spawner, 218.1 cm TL, 2011-04 release

NPM = Northern pikeminnow ; WSG = white sturgeon

During the first three minutes of trial two's video footage the ovarian fluid was relatively obvious when discharging from the nozzle, as one might expect at a flow rate 5x higher than trial 1. One northern pikeminnow entered the video frame at 13:50:12 and continued to enter and exit the frame for 10 seconds. At 13:50:45 a large fish (suspected

white sturgeon) briefly crosses the top right corner of the video frame, and then at 13:50:50 a white sturgeon can be clearly identified in the top right corner of the video frame (Figure 4).

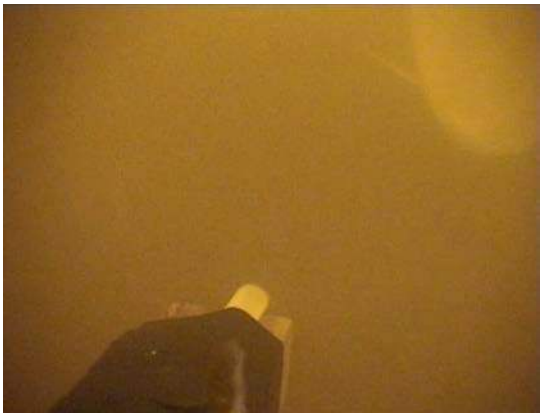

Figure 4. Snout and barbell of a white sturgeon are clearly identified in the top right corner of the video frame 2 minutes and 25 seconds after trial 2 initiated.

A pikeminnow enters the video frame and inspects the nozzle at 13:51:04 for several seconds. At 13:53:52 a white sturgeon briefly crosses the upper left corner of the video frame (Figure 5).

Figure 5. A white sturgeon briefly crosses the upper left corner of the video frame at 13:53:52, or 5 minutes and 27 seconds after the start of trial 2.

A pikeminnow inspects the nozzle at 13:55:53. A white sturgeon once again enters the video frame at 13:58:38, and holds its position very briefly in the center of the video frame just downstream of the nozzle (Figure 6).

Figure 6. A white sturgeon briefly holds its position downstream of the pheromone nozzle at 13:58:38, or 10 minutes and 13 seconds after the start of trial 2.

At 14:01:30 a pikeminnow aggressively feeds from the nozzle, making direct contact with its mouth (Figure 7).

Figure 7. Northern pikeminnow aggressively feeding from the pheromone nozzle during experiment 1 trial 2.

Experiment 2

The pump was inactive between 17:27:22 and 17:28:02 due to switching the flow rate on the pump control interface. Seven unique white sturgeon were detected between 17:29:12 and 17:45:10, but all for short durations and very low power (less than a minute; see table 3).

Trial 2 consisted of one large dose of ovarian fluid, and then passive telemetry to monitor subsequent activity. No radio tagged fish were detected after the single large dose of ovarian fluid. No fish were captured on underwater video during experiment 2

Table 4. Summary of white sturgeon (WSG) radio detections and underwater video footage identifications during experiment 2 trial 1.

Experiment 2 - Radio and Video Tracking Summary					
Time (24hr)	**Radio Freq**	**Radio Code**	**Power**	**Duration (min)**	**Comments**
Trial 1 - Start at 17:22:19					
17:29:13	148.6	15	<50	<1	Female, unlikely spawner, 250 cm TL, 2014-05 release
17:29:13	148.6	44	<50	<1	No database record
17:29:13	148.6	30	54	<1	Female, spawner, 238 cm TL, 2015-05 release
17:29:14	148.6	24	<50	<1	Male, unlikely spawner, 227.4 cm TL, 2014-05 release
17:29:14	148.6	34	<50	<1	Female, spawner, 252.5 cm TL, 2015-05 release
17:30:06	148.6	18	<50	<1	Male, unlikely spawner, 245 cm TL, 2014-05 release
17:32:06	148.6	23	<50	<1	Male, spawner, 241.2 cm TL, 2014-05 release
17:45:10	148.6	58	<50	<1	No database record

NPM = Northern pikeminnow ; WSG = white sturgeon

Discussion

During Experiment 1 trial 1 a total of 4 unique radio tags were detected and 4 events of suspected white sturgeon came within video frame. Only one radio tag detection reached a signal strength above 100 indicating only that radio tagged fish (code 13) swam relatively close to the pheromone release location; the other radio tags were not homing into the exact pheromone release location. Despite low power signals on most of the radio detections, the fact that new tags entered the detection zone as short as 7 min and 10 sec after releasing ovarian fluid suggests radio tagged sturgeon were attracted towards the release location. Perhaps the concentration or flow-rate of the ovarian fluid (20ml/min during trial 1) release was too small to allow sturgeon to pinpoint the release location. Most radio tag detections were also very brief (typically less than one minute) suggesting the tagged fish quickly became disinterested in locating the source of ovarian fluid.

During experiment 1 trial 2 there were a total of 4 unique radio tags detected. The first clear identification of a white sturgeon on video occurs 2 minutes and 20 seconds after the start of the trial, while the ovarian fluid flow rate was at 100ml/min. One radio tag (code 18) was detected for 3 consecutive minutes indicating this fish was interested in locating the ovarian fluid source, although radio signal power remained low (maximum 64) perhaps indicating the fish did not accurately pinpoint the release location. A white sturgeon was identified in the video footage 50 seconds after code 18 was first detected. There is not enough information to identify the fish in the video footage as the fish tagged with radio code 18. However, the timing of code 18 detections and the positive identification of a white sturgeon on video suggest white sturgeon code 18 was able to pinpoint the release location despite the low power signal.

All of the white sturgeon identified on camera spent a very brief amount of time within frame. This may suggest the fish became disinterested with the ovarian fluid alone. Perhaps there are multiple cues that are necessary to elicit a spawning behavior pattern in white sturgeon. White sturgeon activity continues throughout the entire duration of trial 2. One sturgeon was clearly identified on camera approximately 10 minutes after trial 2 initiated and a radio tagged fish was detected for one minute approximately 15 minutes after trial 2 initiated.

Video footage clearly shows Northern pikeminnow are attracted to the odour source and displaying feeding behavior towards the ovarian fluid source. It is plausible to speculate that Northern pikeminnow could have evolved to recognize chemical cues from

ovarian fluid as prey cues that they can use to track down the region where white sturgeon eggs are laid. Other possibility could be that Northern pikeminnow are attracted to amino acids or protein components that are common in ovarian fluid (Rime et al., 2004). When combined with high density of Northern pikeminnow in the study area, evidenced by frequent appearance in the video, it warrants further investigations on the potential roles of Northern pikeminnow in predation on eggs and larval sturgeon, given that Nechako River white sturgeon population is currently experiencing recruitment failure, but that the specific contributing factors are still unclear.

From annual egg collection projects it is clear white sturgeon are engaging in the act of spawning, however those fertilized eggs do not appear to survive to juvenile ages. One possible explanation is a high predation rate from other species, such as Northern pikeminnow or bull trout. Video results from this project suggest Northern pikeminnow are especially adept at locating white sturgeon ovarian fluid release locations, and actively feed on it. It would be reasonable to assume Northern pikeminnow would also feed on fertilized and unfertilized eggs, as well as white sturgeon larvae.

No appearance of white sturgeon in video footage during our Experiment 2 was surprising because Experiment 2's release location was in an area where white sturgeon are typically detected via radio tracking, and a known spawning location. Therefore, radio detections during this experiment should be interpreted cautiously. Five radio detections occurred nearly within the same second, and one of those radio codes appears to be erroneous because there is no database record for that radio tag. Although the detection pattern appears odd, the other 4 tags detected at 17:29:13/14 are plausible detection codes. The burst of radio detections in Experiment 2 suggests a large initial dose (e.g. 100 ml/min for 5 min) attracted a group of white sturgeon near the release location, but only briefly. Interestingly, 3 of the 6 fish detected in Experiment 2 trial 1 were mature fish ready to spawn in 2015 (confirmed by visually inspecting gonad development during tagging events in 2015). Those radio-detected fish also had low power signals, which suggests the tagged fish did not swim close enough to be captured on video.

The total lack of sturgeon detections (both video and radio) during Experiment 2 trial 2 was an unexpected result. While the lack of video detections may not be surprising, the lack of radio detections after a large initial dose suggests any sturgeon that did sense the ovarian fluid was not able to track it back to the release location, perhaps due to an absent "scent trail". Another possible explanation for the lack of on-camera identifications is the video

camera may not have been pointing downstream thus making it nearly impossible to capture approaching fish.

The doses of potential chemical cues used in the present study were not unusual given the dilution factors of 2.25x 10, 400x 10, and 200x10. If ovarian fluid contains any potential cues at a concentration of 10^{-3}M, the final pheromone concentrations downstream could range between 10^{-11} M and 10^{-13} M, which is not unusual given that fish are known to have highly sensitive olfaction: for example, sea lamprey can smell sex pheromone as low as 10^{-13} M (Johnson et al., 2009). However, there are two other factors to consider: 1) we do not know actual concentrations of potential pheromones in ovarian fluid; 2) White sturgeon's olfactory sensitivities are largely unknown. Therefore, it is premature the doses used were within the white sturgeon's olfactory capacity. Since it is well known that olfactory sensitivities are species specific, more conservative approaches may need to be considered in the future experiment.

Although the present study may suggest a possibility that ovarian fluid contain chemical stimuli, attracting mature males to the odour source, further studies are required to confirm the presence of attractive behavior mediated by the pheromonal compounds originating from ovarian fluid. First, not knowing the potency of the potential pheromonal compounds in ovarian fluid, it was virtually impossible to develop an appropriate dosing protocol for the experiment. Apparently, the doses used in the present study were not optimal in keeping male sturgeon occupied during the experiment. By considering the dilution factor in the river, the final concentrations of the potential pheromones needs to be adjusted and white sturgeon's responses to the varying concentrations can be examined. In addition, it will be advisable to devise a mode of application in order to sustain pheromone concentrations in the river and create concentration gradient that white sturgeon can use to zoom in to the odour source.

One limitation in the present study was that white sturgeon's movements were not traceable with a high resolution, making it difficult to determine whether fish were truly attracted to the odour source or not. By setting up radio receivers near the relase site, the behavioral resolution can be improved.

In conclusion, this pilot study provided positive results for ovarian fluid acting as an attractant to adult sturgeon in a river environment. Experiment 1 resulted in a greater number of white sturgeon identifications on camera, perhaps because the release location was chosen based on confirmed presence of multiple white sturgeon downstream. In

addition the camera was clearly pointed downstream in Experiment 1. The high density of Northern pikeminnow at the ovarian fluid release location should be investigated further. Future experiments should test whether Northern pikeminnow are actively locating the release location of ovarian fluid, if they're simply at such a high density that they are captured on video by chance. Releasing a control fluid (river water) in addition to ovarian fluid and comparing the number of Northern pikeminnow captured on video should be a sufficient test. Experiment 2 also demonstrated adult white sturgeon near the area ovarian fluid was released, however no fish were captured on camera suggesting the fish were not interested, or unable to locate the ovarian fluid release location. The lack of video identifications may simply reflect a poorly set-up camera, thus future experiments need to ensure the mooring is deployed in such a way that ovarian fluid release nozzle and video camera are facing downstream. Future experiments should also manipulate the flow rates of ovarian fluid. The complete lack of detections in experiment 2 trial 2 suggest white sturgeon require a minimal "scent trail" to actually locate an area where ovarian fluid has been released. In addition to flow rate manipulations it may be interesting to provide additional spawning cues in an attempt to keep white sturgeon in the release location after they have found it.

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