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Evaluating Behavioral Responses of Spawning-phase Walleyes to Odors of Rivers and Other Walleyes

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Abstract

Many fish species show a remarkable ability to home to streams for spawning and fishery managers have applied that knowledge to restore or rehabilitate populations of some species. Walleye *Sander vitreus* show strong homing tendencies and the ability to select their natal river from other streams. The role of olfactory cues has been hypothesized but never tested in Walleye and many aspects of Walleye migratory behavior associated with spawning are poorly understood. The objectives of our study were: 1) to determine if spawning-phase Walleye from the Ford River in Michigan favored the odor of water from their home stream over that of a nearby river; and 2) to examine effects of male and female Walleye (conspecific) odors on water choice preferences of spawning-phase male Walleyes to learn whether such odors may attract adult Walleyes into rivers. Flow-through flume assays with two side-by-side channels were used to evaluate behavioral preferences of spawning-phase Walleye to river and conspecific odors. Odors of Ford River in 2021 trials and conspecific males in 2019 trials (when analyzed by individuals but not groups) influenced the behavior of male Walleye. However, follow up work during 2022 did not detect any responses to Ford River water or male-conditioned water. Additional work is needed to determine the possible role of olfactory cues in the homing behavior of spawning-phase Walleye.

INTRODUCTION

Many fish species show a remarkable ability to home to natal streams for spawning and fishery managers have applied that knowledge to restore or rehabilitate populations of some species. The concept of rearing fish using methods that allow olfactory (odor) imprinting and encourage fish to return to their natal stream is largely based on research on Pacific salmon *Oncorhynchus* spp., which are well known for their ability to home using odors learned as juveniles. Pacific salmon imprint on the unique odor of their stream during critical early-life stages (e.g., hatch, alevin, and emergence) and later during smoltification, and then use these odors to migrate back to their home stream as spawning adults (Dittman et al. 2015). Interestingly, Pacific salmon may even follow odors back to the vicinity of the specific redd where they hatched (Dittman and Quinn 1996; Dittman et al. 2015). Imprinting young salmon on water from streams they want the fish to return to as adults is being employed by fishery managers as part of a Kokanee Salmon *Oncorhynchus nerka* recovery program in Lake Sammamish Washington (Lake Sammamish Kokanee Work Group 2013) and could be appropriate for other conservation and supplementation hatchery programs. Imprinted stream odors and other olfactory cues are thought to guide spawning migrations in various fishes, but direct evidence remains limited to Pacific salmon and a few other species (Bett and Hinch 2016; Cathcart 2021).

Pacific salmon are not the only fishes that show strong homing tendencies and distinct ability to select their natal river from other streams. Walleye is a native top-level predator and rehabilitation of Walleye populations has been a priority of fisheries managers in the upper Great Lakes (Fielder and Baker 2004; Zorn and Kramer 2012). Tag-based studies of Lake Michigan Walleyes showed strong homing to spawning habitats, with over 95% of tagged Walleyes in northern Green Bay returning to the same spawning areas where originally tagged (Zorn and Schneeberger 2011) and 83% fidelity for Walleyes spawning in southern Green Bay tributaries (Dembkowski et al. 2018). Strong fidelity of Walleyes to spawning sites has also been shown in acoustic telemetry studies in Lakes Huron, Erie, and Ontario (Hayden et al. 2018; Elliott et al. 2023) and Lake Michigan (Izzo et al. 2023), and via otolith microchemistry work in Lake Erie (Chen et al. 2020). Early-life imprinting likely occurs for Walleyes because eggs and larvae typically reside in spawning streams for 1-2 months before out-migrating (Horrall 1981; Todd 1990; Stepien et al. 2015). Alternately, identification of spawning waters may occur at the adult stage with adult fish being drawn to the odors of other adult spawning-phase Walleyes (MacLean and Evans 1981; Stepien et al. 2015). Recent lab-based studies have uncovered mechanisms by which spawning-phase Lake Trout *Salvelinus namaycush* and Sea Lamprey *Petromyzon marinus* use olfaction to locate spawning areas (Buchinger et al. 2015a; Buchinger et al. 2015b). Using the approach and methods of Buchinger et al. (2015a; 2015b) with spawning-phase Walleye could provide an efficient means to better understand use of olfaction by adult Walleyes and contribute to Walleye rehabilitation efforts.

General ideas for how fish navigate to spawning grounds using smell often focus on either the basic smell of the river (imprinting) or scents (e.g., pheromones) of conspecifics (i.e., adult males or females of their species) already in the river. Testing the ability of spawning-phase Walleyes to discriminate between odors of potential spawning waters and their responses to odors of conspecifics formed the basis of this research. Specifically, constituent interest in stocking spring fingerling Walleyes to enhance the Walleye spawning run in the Ford River, a tributary to northern Green Bay in Lake Michigan, raised the question of whether Walleyes stocked in the Ford River could, as spawning adults, discriminate between odors of the Ford River and nearby Escanaba River. Our study objectives were to determine if spawning-phase Ford River Walleyes: 1) favored the odor of Ford River water (their home stream) over Escanaba River water, or Lake Huron water used in the lab; and 2) were attracted to odors of conspecifics.

METHODS

Study Locations and Walleye Population

We evaluated preference of Ford River Walleyes for Ford River water over water from the Escanaba River, whose outlet is about 16 km away. Both rivers drain into northern Green Bay, Lake Michigan; the Ford River directly into northern Green Bay proper (45.67686, -87.14280) and the Escanaba River into the southern end of Little Bay de Noc (45.77812, -87.06169) (Figure 1). Both rivers support substantial adfluvial populations of Walleyes, with the Ford River spawning run estimated at 16,748 Walleyes in 2011 and the Escanaba River run at 10,791 fish in 2008 (Zorn 2021). Additionally, these rivers were chosen due to constituent interest in using stocking to enhance the Walleye population in the Ford River. The Ford River was stocked, usually biennially, with fingerlings from 1988 to 2004 and fry in 2014 to 2017 (Michigan Department of Natural Resources [MDNR] Fisheries Division, unpublished data). We expect that most Walleyes used in this study were adults resulting from natural reproduction and had the opportunity to imprint on the Ford River given the lack of recent Walleye stocking in the Ford River, the abundance of the river's spawning run in 2011 (Zorn 2021), and evidence of annual natural reproduction of Walleyes in the 2011 Ford River spawning population estimate (Zorn 2021; MDNR unpublished data). Water choice trials were run at the United States Geological Survey's Hammond Bay Biological Station (HBBS) near Hammond Bay, Michigan along the shoreline of Lake Huron. HBBS is supplied with water from Lake Huron.

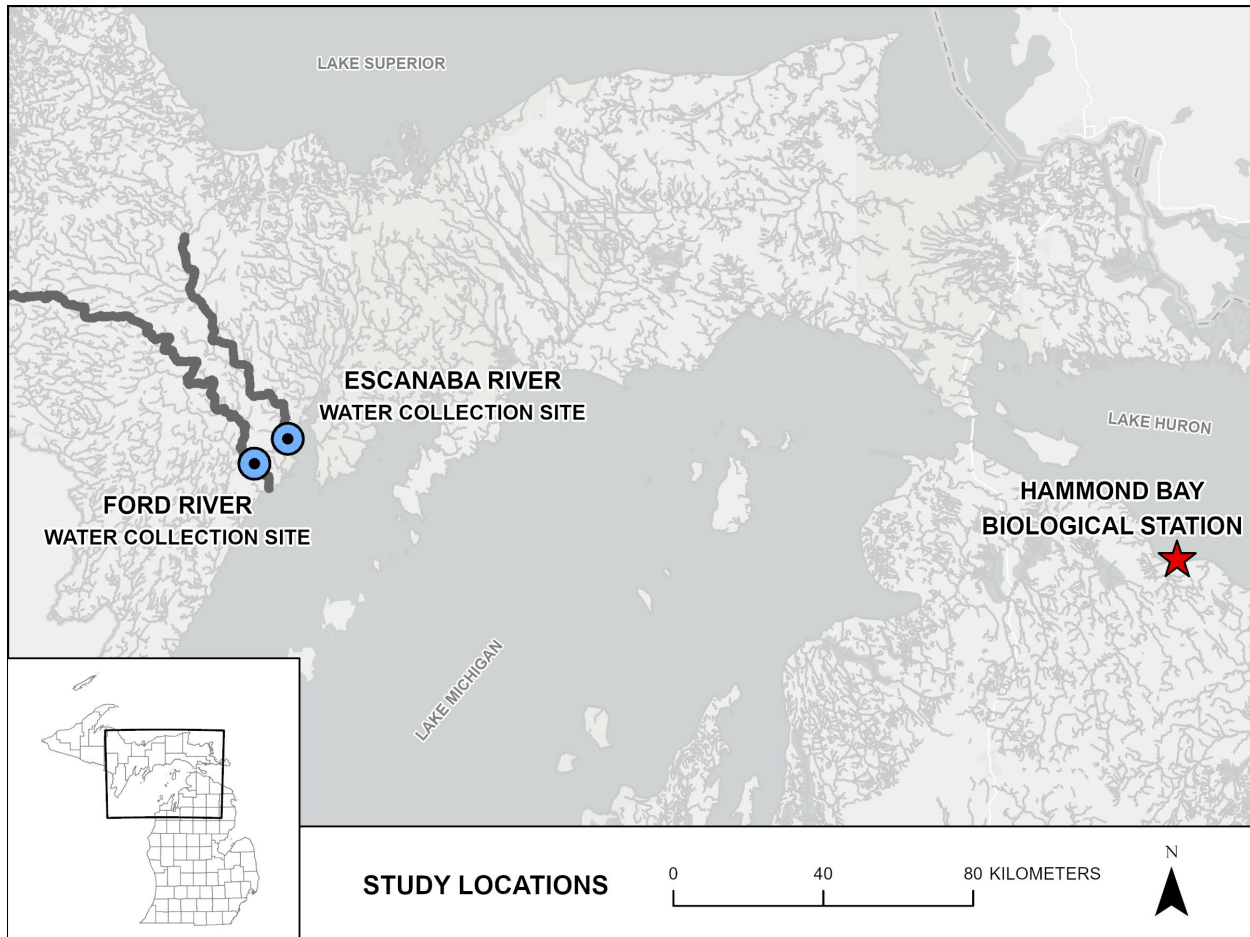


FIGURE 1. Locations of Ford and Escanaba rivers in northern Green Bay and the Hammond Bay Biological Station. Adfluvial Walleyes used in the study were collected from the Ford River. Water for water choice trials came from upstream locations in these rivers (see text for details) and Lake Huron, which supplies the Hammond Bay Biological Station laboratory where water choice trials were conducted.

Walleye Used In Experiments

Spawning-phase Walleyes were obtained from the Ford River on 17 April 2019, 5 April 2021, and 14 and 20 April 2022 via daytime boat electrofishing. Upon arrival to HBBS, fish used in 2019 experiments were briefly immobilized using 0.08% (by volume) clove oil, measured, and implanted with a 23 mm passive integrated transponder tag (PIT; Oregon RFID, Portland, Oregon) via a small incision in the abdomen. 31 males (48.8 ± 16.1 cm, 1.59 ± 0.63 kg, mean \pm SD) were used in behavioral experiments. Four males and four females (63.63 ± 5.45 cm, 3.00 ± 1.02 kg, mean \pm SD) were used to provide odor. To reduce handling and stress we did not PIT tag fish in 2021 and 2022. Rather, we tagged fish with external streamer tags after first use in the experiment. We used 22 males in behavioral experiments in 2021 and did not measure them to avoid handling-related stress. In 2022 we used 20 males (52.43 ± 9.60 cm, 1.36 ± 0.66 kg, mean \pm SD) in behavioral experiments, with four males and four females (61.16 ± 7.16 cm, 2.43 ± 0.77 kg, mean \pm SD) providing odor. Each year, fish were separated by sex and held in 600 L flow-through tanks supplied with aerated Lake Huron water at ambient temperature. Walleyes used for this project were returned to the Ford River each spring after experiments ended. All experimental animals were used with approval from the Michigan State University Animal Use and Care Committee (PROTO201800064 and PROTO202100198).

Experimental Procedures

Water choice trials evaluated responses of spawning phase Ford River Walleyes to odors from different water sources and effluent of tanks holding conspecifics. River water used for water choice trials was obtained from the Ford River at the US-2 bridge (45.735095, -87.192578) and the Escanaba River just upstream of the most downstream dam on the river (45.795756, -87.080269), and was delivered to HBBS and held in river-specific tanks for use in experiments (Figure 2). Both locations were upstream of where Walleyes congregate for spawning, making these water sources unlikely to possess conspecific odors. River water was held in tanks and applied to the channel during water choice trials at 0.5 L min^{-1} using dosing pumps. The four Walleyes serving as conspecific odor donors were held in approximately 600 L of water (four males in one tank, four females in another), with an inflow rate of 1 L min^{-1} , and the odor was similarly applied to the channel during trials at 0.5 L min^{-1} . The HBBS was supplied with Lake Huron water that originated from a deep-water intake and provided an additional water source to evaluate in water choice trials. Experimental procedures, comparisons, and analyses changed iteratively between years due to changes in the experimental facility and unanticipated findings in 2019 and in response to the previous year's results. These changes are outlined by year in the following paragraphs.

2019 Experiments

Seven sets of binary water choice comparisons were made in 2019: 1) Lake Huron water versus Lake Huron water (to test for a left vs. right channel preference); 2) Escanaba River water versus Lake Huron water (to test for preference of Lake Huron water “spiked” or “conditioned” with river odor to Lake Huron water used in the HBBS lab); 3) Ford River water versus Lake Huron water; 4) Ford River water versus Escanaba River water; 5) Male conditioned (i.e., water from the tank holding four odor-providing males described above) Lake Huron water versus Lake Huron water; 6) Female-conditioned Lake Huron water versus Lake Huron water; and 7) Male-conditioned Lake Huron water versus female-conditioned Lake Huron water. River water experiments occurred first each night, with conspecific odor experiments occurring last. Description of experimental set-up, procedures and data analysis used in 2019 follow.

Experiments during 2019 occurred in the original HBBS wet laboratory using the experimental set-up and methods successfully used to study responses of Lake Trout to conspecific odors (Buchinger et al., 2015a; 2017). Two duplicate (but entirely separate) flumes were used to evaluate behavioral preferences of spawning phase Walleye for one of two odors presented during a trial. Each flume had two side-by-side channels at their upstream ends (Figure 2), from which source water from Lake Huron and odorants (e.g., Ford River, Escanaba River, male or female conspecific) entered. A layer of 10–20 cm diameter rock substrate (“reef”) overlaid with horizontal PIT antennae occurred in the upstream end of each side-by-side replicate channel (Figure 2). The rocks held the PIT antenna in place and simulated spawning substrate. Each channel was supplied with odors via a dosing pump (Vivosum 3000 L per h submersible pump). Each flume was $9.1 \text{ m} \times 1.85 \text{ m} \times 0.6 \text{ m}$ and reefs were $1.5 \text{ m} \times 0.85 \text{ m} \times 0.13 \text{ m}$. Water depth, velocity, and flow were 0.42 m, 0.02 m s^{-1} , and 932 L min^{-1} . Dye tests confirmed that there was little mixing across the channel divider and that odors partially dispersed throughout the flume downstream of the channel divider (the choice point). Water flowed through the system and there was no recirculation.



FIGURE 2. One of two replicate flumes used for water choice experiments in 2019. Top image (looking upstream) shows areas where odors were applied, and bottom image looks downstream. Small boxes at upstream end are dosing pumps for applying odors and round tanks on left held Walleyes. All trials occurred at night with all lights off.

Water choice experiments were conducted from 18 April 2019 to 3 May 2019 (water temperatures ranged from 3.0 to 5.7 °C), with each day's experiments proceeding as described below. Groups of Walleye (three to four individuals per group) were held in an acclimation area downstream of the reefs for at least 1 h prior to an experiment. Two experiments were conducted each night. Experiments occurred at night because Walleyes commonly spawn in darkness (Becker 1983) and to avoid influences daytime conditions and activities in the lab environment might have on Walleye behavior. Odor application for the first experiment began 30 min after sunset. Fish were released by removing a gate between the acclimation and the experimental areas 1 h after sunset and allowed to swim freely throughout the entire experimental setup. The PIT antennae over each reef recorded when fish were near each odor treatment and was used to calculate the duration of time each fish spent in the channel's odor. After 3 h, the first experiment ended. The fish were gently directed back to the acclimation area and odor tubes flushed with Lake Huron water. After 30 min, odor application for the second experiment began, and after another 30 min, the fish were released from the acclimation area and data collected as in the first experiment. Only the first 3 h of the data from each second experiment were analyzed, though fish from the second experiment remained in the flume until the next day. The same group of Walleyes was repeatedly used in subsequent nights to evaluate responses to different treatments.

Data were evaluated using paired t-tests of the seconds spent in each of the side-by-side channels. One analysis treated individuals as the unit of replication and, due to the possibility that individuals within a group did not move independently, a second analysis treated a group as the unit of replication. For example, one trial during which four fish were observed was analyzed as $n = 4$ for the individual analysis (with the data being the time in seconds that an individual spent in a channel) or alternatively as $n = 1$ for the group analysis (with the data being the summed time all four fish spent in a channel). Using these approaches to the analysis, the total statistical replicates were $n = 20-24$ for each set of individual analyses and 6-7 for each set of group analyses. Previous similar studies on Lake Trout found consistent results using analysis by group and individual (Buchinger et al., 2015a; 2017).

2021 and 2022 Experiments

Experiments during 2021 and 2022 followed a similar method with some modification. All portions of the study occurred in the newly constructed HBBS wet laboratory after the original laboratory was demolished in 2019. In the new facility, a single raceway (3 m wide) was divided into two duplicate flumes (1.5 m x 10 m), each separate from the other (Figure 3). The upstream end of each flume was divided into two channels for fish to choose between (0.75 m wide x 1.5 m long). To increase the concentration of odors that Walleyes were exposed to during trials, water depth, velocity, and flow were reduced from 2019 values to 0.3 m, 0.01 m s⁻¹, and 270 L min⁻¹ and the rate of odor application was increased from the 2019 rate (0.5 L min⁻¹) to 2 L min⁻¹. Dye tests confirmed that there was little mixing across the channel divider and that odors partially dispersed throughout the flume downstream of the choice point. Water flowed through the system and there was no recirculation. Fish were not PIT tagged during 2021 or 2022, all observations were made using night vision cameras, and only one fish was tested per trial. Two sets of experiments were conducted each night.

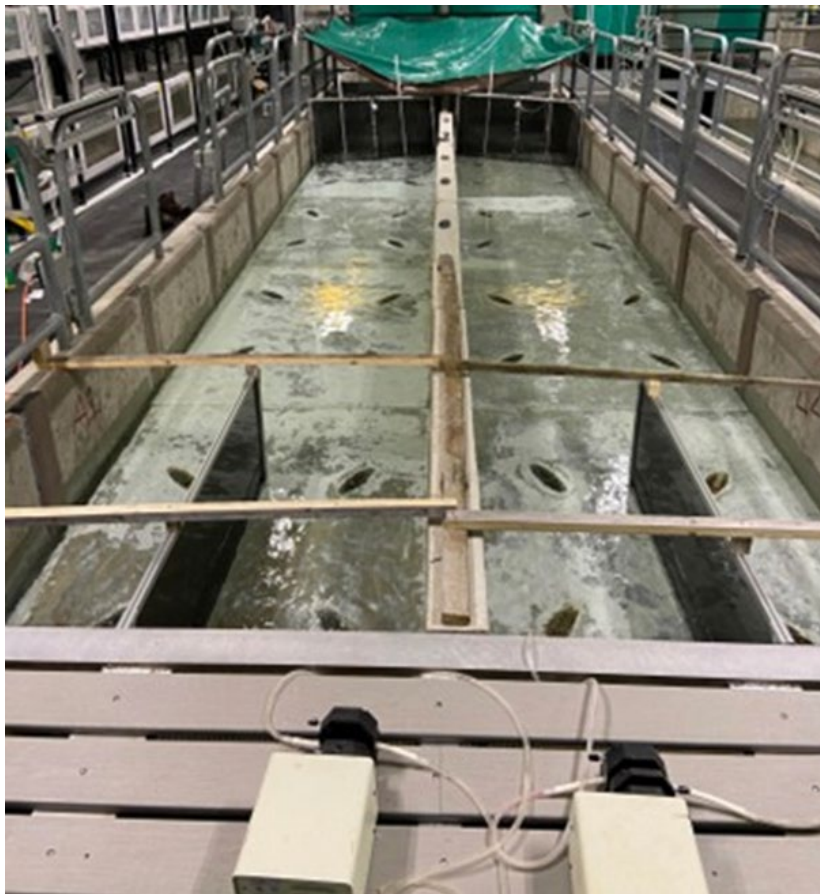


FIGURE 3. Replicate flumes used for water choice experiments in 2021 and 2022. Top image looks upstream to where odors were applied and shows pool that was blocked off midway through 2021 trials, while bottom image looks downstream. Small boxes at upstream end of channel are dosing pumps. All trials occurred at night with all lights off.

Two series of experiments were conducted during 2021, building on the 2019 channel bias and river odor preference findings. However, experimental procedures changed due to a channel bias preference that had not been observed in previous work using the 2019 experimental setup (Buchinger et al. 2015a). The first series occurred from 9 April 2021 to 20 April 2021. Trials began with the lights in the flume room being turned off at sunset, and 30 min later one fish was placed in each flume to acclimate. After a 30-minute acclimation, the amount of time a fish was visually observed in the left and right channels was recorded while no odors were being applied. Observation ended after 30 min and odor application began. The odors were allowed to disperse through the flume for 15 min with no observation and then fish were observed for 30 min while odors were applied. Finally, fish were removed, and the flume flushed with Lake Huron water for 15 min before starting the second set of trials. During the first series of experiments, flumes included a small pool at the downstream end, which allowed the fish to remain in the flume during the day to limit handling and reduce stress. However, many fish failed to exit the pool area during experiments which resulted in their exclusion from the analysis. To prevent this concern, access to the pool was eliminated for the second series of experiments. The second series was conducted from 21 April 2021 to 2 May 2021, during which water temperatures ranged from 4.5 to 5.4 °C. Given the lack of significant preference of Walleyes for Ford River water (their home tributary) over Lake Huron water in 2019, we decided to more rigorously test their water choice preferences to ensure any observed preference could not be attributed to the experimental setting. Therefore, the only treatments tested in 2021 were Ford River water versus Lake Huron water and strictly Lake Huron water in both channels of the flume to provide a negative control. The side of and order of treatments were generally alternated, though some treatments were applied more often on one side versus the other.

Experiments during 2022 followed the same methods as the second series of experiments from 2021, and were conducted from 19 April to 8 May, during which water temperatures ranged from 1.2 to 5.0 °C. During 2022, treatments were: 1) Ford River water versus Lake Huron water; 2) Male-conditioned Lake Huron water versus Lake Huron water; and 3) Female-conditioned Lake Huron water versus Lake Huron water.

Water choice preferences for 2021 and 2022 experiments were statistically analyzed by comparing the total time an individual spent in each channel before odor application (control period) and during odor application (treatment period). Times in the control channel before odor application, the experimental channel before odor application, the control channel during odor application, and the experimental channel during odor application were used to calculate indices of preference based on previous research on olfactory cues in Sea Lamprey (Li et al. 2002) and Lake Trout (Buchinger et al. 2020). Trials were excluded if the fish did not enter both channels during the pre-treatment control period and if they did not enter either channel during the treatment period. Indices are calculated as the time spent in a channel during the treatment period divided by the total time spent in that channel during the control and treatment period combined. Analyzing the data using indices of preference accounts for any bias fish showed for one channel irrespective of odor treatment. The indices of preference were then compared using a Wilcoxon signed-rank test.

RESULTS

Results are reported by year due to annual changes in the experimental facility, procedures, and trials conducted.

2019

Analysis of data on Walleye response to odors produced conflicting results when individuals versus groups were used as units of replication. Neither conspecific nor river odors influenced male behavior when data were analyzed for groups of Walleyes (paired t-tests; $df = 6-7$; $P > 0.05$). However, when the

2019 data were analyzed by individuals, males spent less time in the channel conditioned with male odor but showed no consistent responses to river waters (Figure 4). Walleye spent more time in the channel treated with Escanaba River water than the channel treated with Ford River water ($t = -2.62$; $df = 23$; $P = 0.01$). There was no consistent preference between Escanaba River water and Lake Huron water, Ford River water and Lake Huron water, or the left and right channels when no odors were applied (paired t-tests; $df = 21-24$; $P > 0.05$; Figure 4).

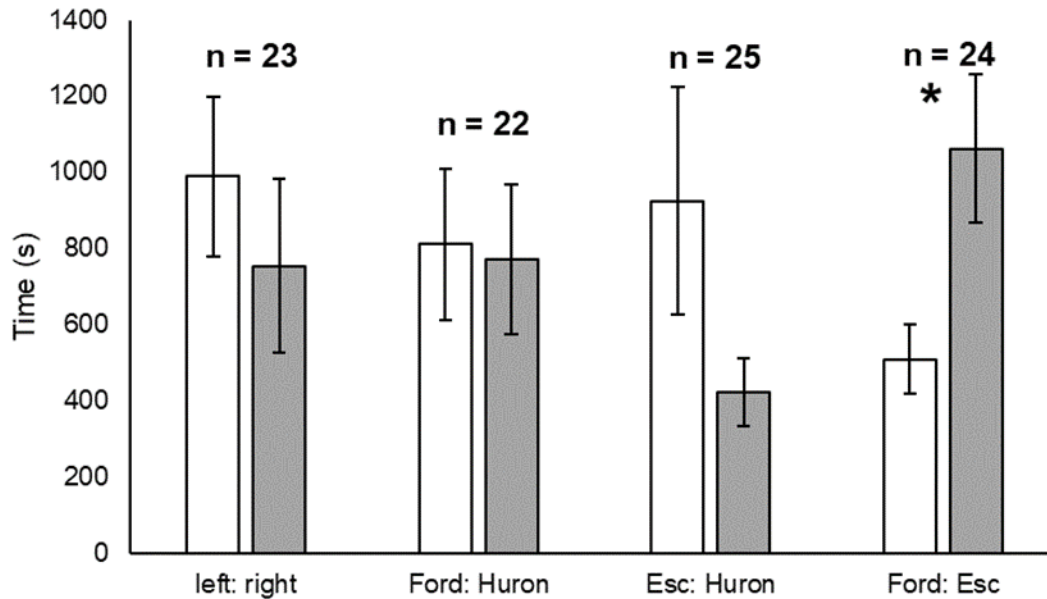
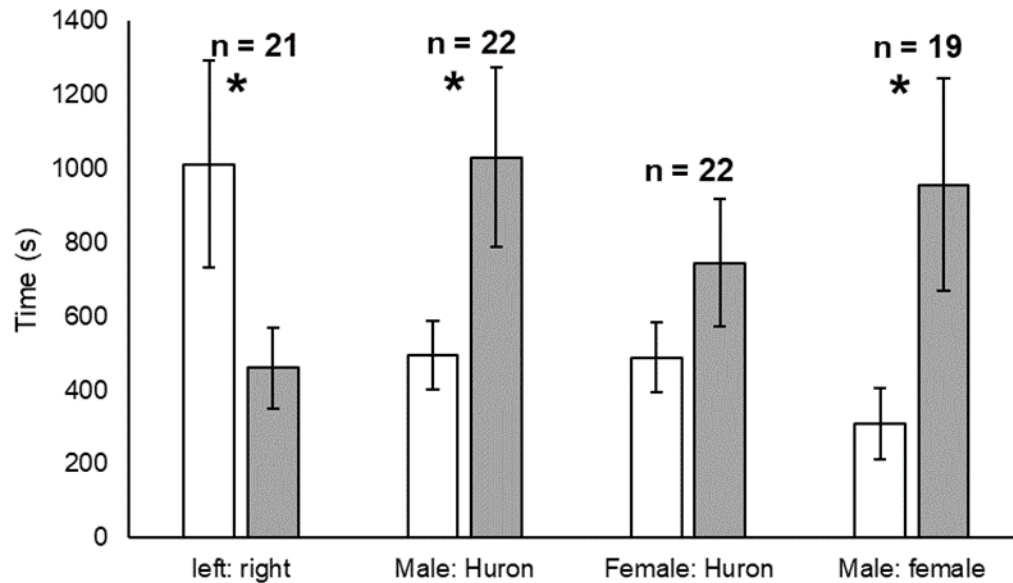


FIGURE 4. Mean number of seconds (\pm one standard error) male Ford River Walleyes spent over reefs in channels where different river odors were applied during 2019. Results illustrated include analyses of individuals used as replicates with n representing the number of individuals analyzed. Asterisks indicate significant differences at $P < 0.05$ for pairwise t-test comparisons between channel (left or right) when no odors were applied and among water sources (Ford, Escanaba, or Lake Huron). No comparisons were significant when groups were used as replicates.

In the conspecific odor tests, males spent less time in the channel treated with male odor than channels treated with Lake Huron water ($t = -2.55$; $df = 21$; $P = 0.02$) or female odor ($t = -2.87$; $df = 18$; $P = 0.01$; Figure 5A). Males also spent more time in the left channel when no odors were applied ($t = 2.38$; $df = 20$; $P = 0.03$), with the bias particularly evident in the left experimental flume, hereafter flume A (Figure 5A). Because the potentially confounding effect of the left-channel bias, the statistical analysis for conspecific odors was repeated using data only from trials conducted in the right experimental flume, hereafter flume B. In flume B (Figure 5B), males spent equal time in the left and right channels ($t = -1.07$; $df = 10$; $P = 0.31$) and female and Lake Huron channels ($t = 1.01$; $df = 9$; $P = 0.34$). Males spent less time in the male channel compared to the Lake Huron channel ($t = -3.08$; $df = 11$; $P = 0.001$) and tended to spend less time in the male channel compared to the female channel ($t = -1.77$; $df = 11$; $P = 0.11$) (Figure 5B). In total, 30 of 124 observations of individuals for river waters tests and 33 of 117 observations of individuals in conspecific odor tests were excluded because fish did not enter either channel.

A



B

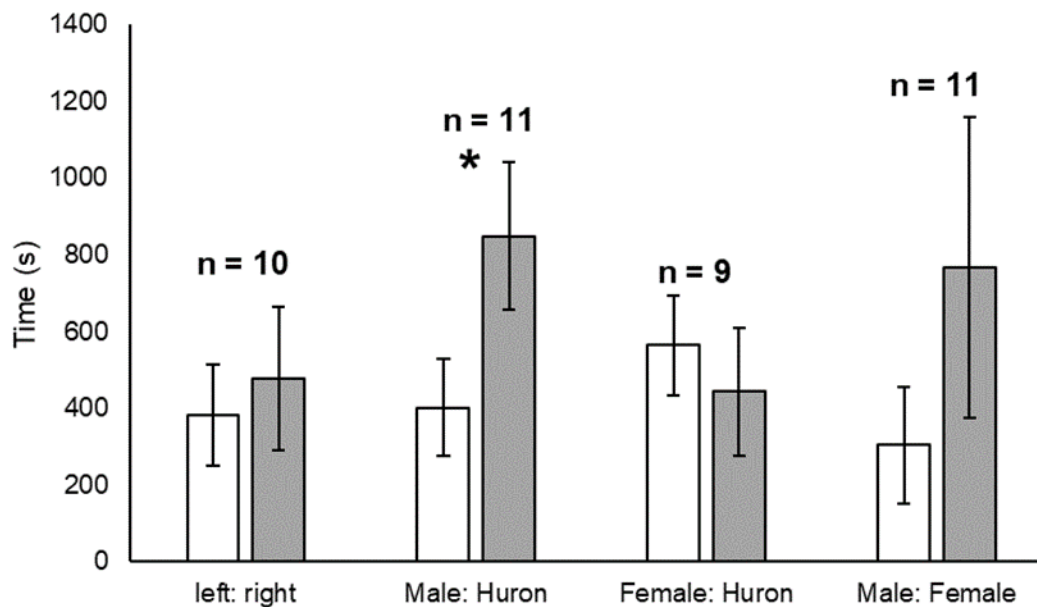


FIGURE 5. Mean (\pm one standard error) number of seconds male Ford River Walleyes spent over reefs (in channels) where different conspecific odors were applied during 2019. Results shown for A) both flumes and B) flume B only. Asterisks indicate significant differences at $P < 0.05$ for pairwise comparisons. Comparisons occurred between channel (left or right) when no odors were applied, and Lake Huron lab water with no odors added (Huron) and water conditioned with conspecific (male or female) odors. Data were analyzed using individuals as replicates with n indicating the number of individuals analyzed. No comparisons were significant when groups were used as replicates.

2021

Nineteen male Walleyes were used to test for a left vs right channel bias with both channels receiving Lake Huron water. Nine individuals explored the assay sufficiently for inclusion in the analysis. Four of nine Walleyes spent more time in the left channel (Wilcoxon signed-rank test; $P = 0.57$). Trial results from 2021 indicated no channel bias in either flume, so results from both flumes were included in analyses.

The same nineteen males were tested individually in 2021 to evaluate preferences for Ford River water versus Lake Huron water, following up on 2019 results showing a lack of preference for Ford River water over Lake Huron water used in the lab. Only seven males explored the assay (i.e., both channels in the flume) sufficiently to be included in the analysis, and six of those seven males spent more time in Ford River water versus water from Lake Huron (Wilcoxon signed-rank test; $P = 0.047$) (Figure 6).

Notably, most successful trials (i.e., suitable for analysis) were from 21-April through 25 April, with fish becoming largely inactive after this period. Eight additional trials were conducted using conspecific odors on 1 May and 2 May, but only one fish was active.

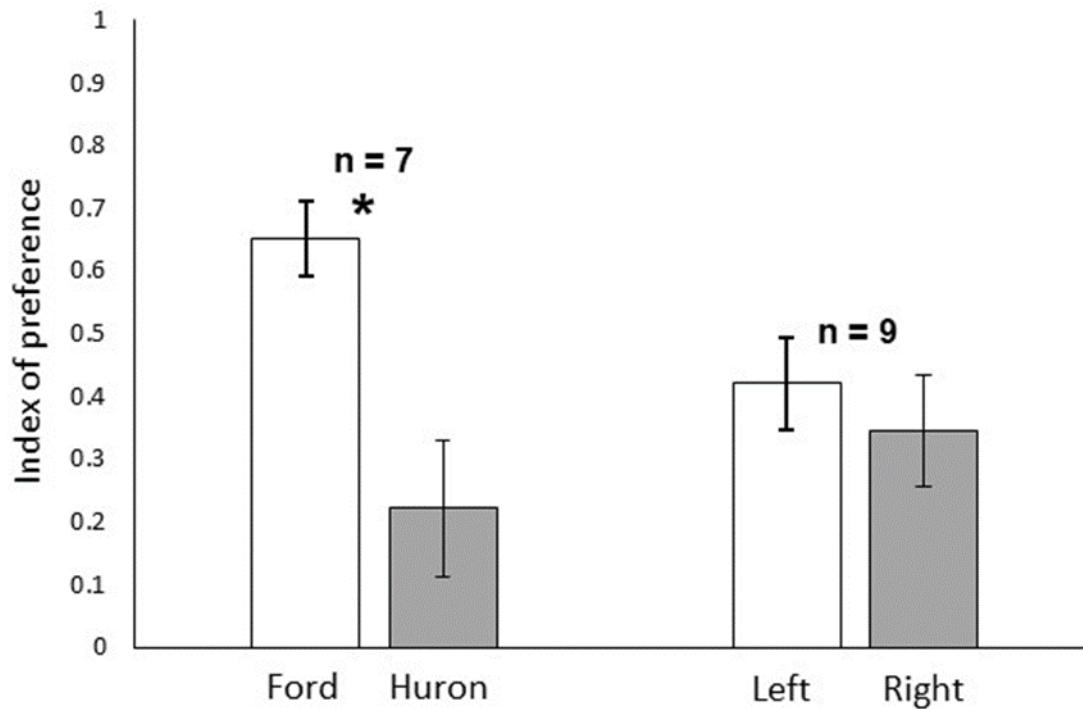


FIGURE 6. Preferences of male Ford River Walleyes for Ford River water versus Lake Huron water and assessment of left vs. right channel bias during 2021 trials. Bars show the mean index of preference for each channel and error bars represent \pm one standard error of the mean. Asterisk indicates $P < 0.05$ as determined using Wilcoxon signed-rank tests. The number of individuals analyzed is indicated by n.

2022

Experiments during 2022 focused on confirming the Ford River water versus Lake Huron water choice results from 2021 trials and testing for responses of male Walleyes to male and female conspecific odors. Eighteen male Walleyes were tested individually for responses to Ford River water versus Lake Huron water, with all but two exploring the assay sufficiently to be included in subsequent analysis. Only five of the sixteen males spent more time in the channel activated with Ford River water versus Lake Huron water (Wilcoxon signed-rank test; $P = 0.14$) (Figure 7).

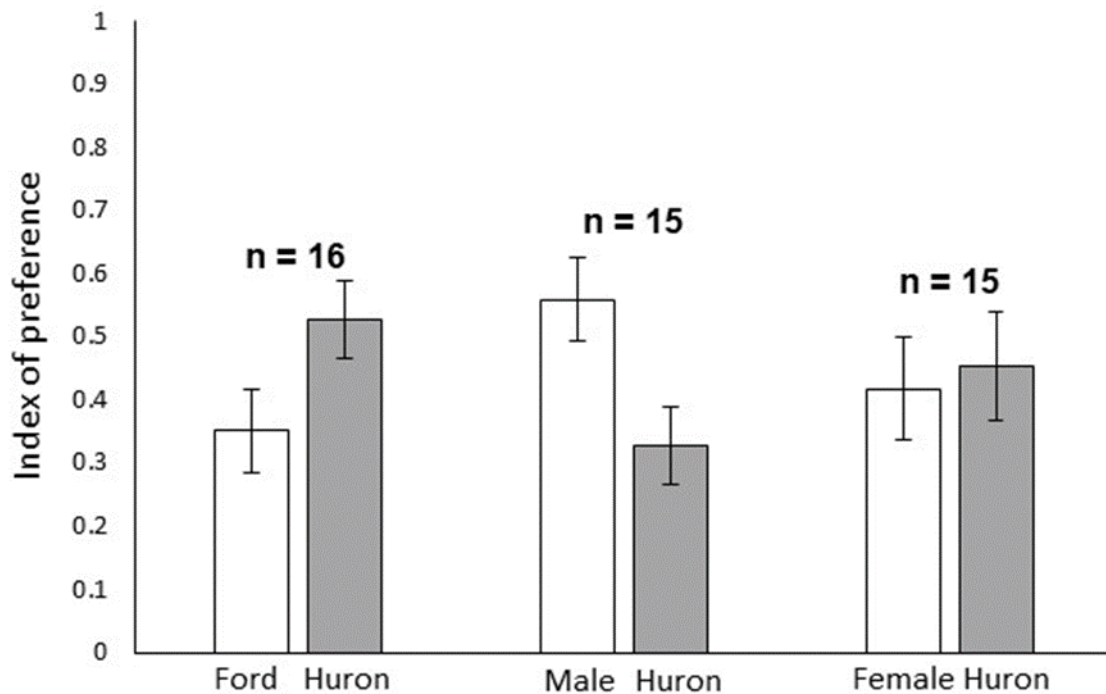


FIGURE 7. Ford River male Walleye preferences for water conditioned with odors from the Ford River, male Walleyes or female Walleyes versus Lake Huron water during 2022 experiments. Bars show the mean index of preference for each channel and error bars represent \pm one standard error of the mean. Wilcoxon signed-rank tests indicated no comparisons were significantly different ($\alpha = 0.05$). The number of individuals analyzed is indicated by n.

Twenty males were tested for responses to male-conditioned water versus Lake Huron water, with fifteen exploring the assay sufficiently to be included in subsequent analysis. Ten of the males spent more time in the channel activated with male-conditioned water versus Lake Huron water (Wilcoxon signed-rank test; $P = 0.08$) (Figure 7). Twenty males were tested for responses to female-conditioned water versus Lake Huron water, with fifteen exploring the assay sufficiently to be included in subsequent analysis. Nine of the 15 males spent more time in the channel activated with female-conditioned water versus Lake Huron water (Wilcoxon signed-rank test; $P = 0.76$) (Figure 7).

DISCUSSION

Our investigation indicated spawning-phase male Walleyes may respond to olfactory cues from their spawning river and conspecifics. Odors of Ford River in 2021 trials and conspecific males in 2019 trials (when analyzed by individuals) influenced the behavior of male Walleyes. However, follow up work during 2022 did not detect any responses to Ford River water or male-conditioned water. Additional work is needed to determine the possible role of olfactory cues in the spawning behavior of Walleyes.

Our results from three years of research were largely inconclusive and did not provide support for the hypothesis that adult Walleyes use olfaction to distinguish between their natal stream and other waters, including nearby potentially suitable spawning rivers. We hypothesized that Walleye might imprint on the odor of natal rivers during the first months of life, using olfaction to return to them as adults for spawning. However, we observed no significant preference by Ford River Walleyes for Ford River water in our 2019 trials, instead observing some preference for water from the Escanaba River (Figure 4). With previous research suggesting over 80% of Green Bay Walleyes return to the river where they were originally tagged (Zorn and Schneeberger 2011; Dembkowski et al. 2018; Izzo et al. 2023), it seems unlikely the Ford River Walleyes used in 2019 experiments might have preferred Escanaba River because they were strays from the Escanaba River.

Attraction to Escanaba River water and neutral response to Ford River water may be spurious results produced by issues with the experimental setting and procedures (discussed below). Male Walleyes in the 2021 experiments preferred Ford River water over Lake Huron water (Figure 6), providing some support that early life imprinting guides Walleyes to their natal streams for spawning. However, follow up experiments during 2022 using the same approach used during 2021 did not indicate any attraction to Ford River water (Figure 7). In summary, our results cannot confirm or reject the hypothesis that Walleyes home to spawning rivers solely using river odors. Note, our river odor tests were largely comparing effects of river odors themselves because river water was sourced from upstream areas receiving relatively little use (in the case of the Ford River) or no use (in the case of the Escanaba River) by spawning adfluvial Walleyes (Figure 1).

Results of the 2019 conspecific experiments did not confirm or reject the hypothesis that spawning-phase Walleyes use the odor of other spawning adults to locate spawning streams. Walleye spawning run surveys in northern Green Bay rivers show males typically spend much of the spawning period in spawning rivers while females enter rivers just prior to spawning and depart shortly after spawning (Zorn 2021). Therefore, we reasoned males may follow the odors of other males into spawning rivers. Indeed, evidence that spawning male Lake Trout are attracted to the odor of other males indicates male odors may guide spawning aggregations (Buchinger et al. 2015a). Instead, we found that male Walleye avoided the scent of other males, whether presented adjacent to Lake Huron water or female odor (Figure 5). Note that we observed response to male odors when data were analyzed using individual males as the unit of replication, but not when groups of males were used as the unit of replication. Analyzing the data with individuals as replicates provides a more powerful statistical method but assumes individuals do not influence each other during the experimental trial, which may not have been the case. Follow up experiments during 2022 using an individual-based assay did not show a significant response to male odor but hinted towards a preference for male odor (Figure 7). Though male Walleye may use chemical signals to mediate aggressive interactions during competition for mates (da Silva et al., 2021), our results provide only weak, preliminary support for a role of male odors and should be considered bearing in mind the conflicting results between analysis methods and years, and issues with the experimental setting and procedures (discussed below).

Potential Issues With Experiments

Several issues may have confounded the 2019 experiments. First, we observed a significant preference for the left channel during conspecific odor trials, despite our use of methods and a facility where successful conspecific odor studies had occurred previously (Buchinger et al. 2015a). Avoidance of male odor was still significant when data from flume A was excluded from analyses (Figure 5B). Second, the concentration of river water may have been below the detection threshold of Walleye. River water was pumped at a rate that yielded a concentration of approximately 0.1 % river water, assuming the applied river water mixed completely in the channel being treated with the odor. As mixing was likely not complete, concentrations higher or lower than 0.1 % likely occurred in different portions of the channel. Regardless, the concentration was much lower than that used in most studies on imprinting, which often test responses to natal water that is undiluted by imprinting fish to artificial odorants easily recreated at high concentrations (Bett and Hinch 2016). Testing with undiluted natal water was not logistically possible for our study because the distance between study rivers and the laboratory was over 320 km (Figure 1) and frequency of trips required for shuttling water from study rivers for undiluted comparisons would have been well beyond our limited budget for the study. Importantly, responses to natal water diluted to 0.1 % have previously been reported (Sutterlin and Gray 1973). Third, experiments during 2019 may have been confounded by social interactions among Walleyes. We based our methods off a similar study on Lake Trout olfactory cues which observed positive responses to conspecific cues by groups of Lake Trout (Buchinger et al., 2015a; 2017). However, it is possible that social interactions among fish may override or reshape the influence of odors on behavioral movements of individual spawning-phase Walleyes.

We adjusted our experimental design in 2021 to address the potential issues that arose during 2019 but encountered new challenges. First, we evaluated responses to odors using a method that directly accounts for any possible side bias by comparing time spent in a channel before and during odor application. Second, we increased the concentration of odor applied by lowering the water level and velocity in the flume (lower total volume in which odors were being diluted) and applying river water at a higher rate; the estimated concentration increased approximately 13 times, from 0.1 % river water used during 2019 to nearly 1.5 % river water. Third, we tested Walleye individually to prevent any social interactions among Walleye that might influence responses to odors. However, we experienced a major challenge with fish being inactive during many of our trials. To reduce stress, fish were left in the raceway while trials were not being conducted and this required a small area with greater depth at the end of the raceway. Unfortunately, many fish in the first several days of trials failed to leave this pool. We then adjusted the method to restrict fish's access to the pool and then observed responses to Ford River water. However, fish activity began to decline again before other treatments could be tested. This inactivity may have been due to the spawning period of Ford River Walleyes having concluded. For example, spawning run estimate data for adfluvial Walleye in the Ford River and other Green Bay tributaries suggest spawning runs in the area often occur in early to mid-April and may largely conclude by May (Zorn 2021). Even though water temperatures in the HBBS lab remained suitably cold (e.g., < 6°C) for the duration of experiments due to the lab's Lake Huron water supply, the time period when olfactory cues potentially guide spawning-related movements might have passed during later portions of some trial periods.

We conducted water choice trials at water temperatures that previous studies in the region suggest are conducive to Walleye migration to spawning locations or active spawning (Zorn and Schneeberger 2011; Zorn 2021). Nevertheless, wild Ford River Walleyes being held in HBBS lab may have become increasingly stressed over time (showing altered behavior) due to handling or being contained in a laboratory environment for an extended period. In addition to somewhat different temperatures than the

river, the lab environment was entirely different and foreign in numerous respects (e.g., lighting, sound, currents, physical structure of environment, etc.), so multiple factors could have affected behavior of Walleyes we studied.

Trials during 2022 again indicated contradictory results, with no responses observed to either Ford River water or conspecific-conditioned water, suggesting further refinement of our approach is needed for future work. In light of these findings, questions of whether Walleye fingerlings stocked in the Ford River imprint on its odor and will predictably return there after maturing still remain unresolved.

SUMMARY

We designed and tested several experimental configurations for assessing adult Walleye responses to odors and tested responses of spawning males to odors from conspecifics and their home river. Experiments from 2019 provided conflicting results that should be considered carefully given the potential issues discussed above. Experiments during 2019 informed our approach for 2021 (and beyond). The 2021 design indicated a response to river water after incorporating possible side biases, testing males individually, and using a higher river odor concentration (approximately 1.5 %); however, trials during 2022 using the same approach did not detect significant responses to any odors tested. Importantly, our experiments do not provide strong evidence for or against a potential role of imprinting to water or conspecific odors by Walleyes as we did not have a positive control to confirm our tests could reliably detect a response to odors. Walleye may rely more on other cues (e.g., vision) or may not show natural behavior patterns in the laboratory environment, especially immediately after being collected from the wild during the spawning period. Future studies on olfactory cues in Walleye may benefit from using hatchery-raised individuals which may be more acclimated to the laboratory environment. Alternately, field studies to observe behaviors in a more natural environment (e.g., telemetry) could potentially be designed to elucidate imprinting and water choice preferences.

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REFERENCES

- Becker, G.C. 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison.
- Bett, Nolan N., and S. G. Hinch. 2016. Olfactory navigation during spawning migrations: a review and introduction of the Hierarchical Navigation Hypothesis. *Biological Reviews* 91:728-759.
- Buchinger, T. J., W. Li, and N. S. Johnson. 2015a. Behavioral evidence for a role of chemoreception during reproduction in lake trout. *Canadian Journal of Fisheries and Aquatic Sciences* 72:1847-1852.
- Buchinger, T.J., M. J. Siefkes, B. S. Zielinski, C. O. Brant, and W. Li. 2015b. Chemical cues and pheromones in the sea lamprey (*Petromyzon marinus*). *Frontiers in Zoology* 12, 32.
<https://doi.org/10.1186/s12983-015-0126-9>
- Buchinger, T.J., J.E. Marsden, T.R. Binder, M. Huertas, U. Bussy, K. Li, J.E. Hanson, C.C. Krueger, W. Li, and N.S. Johnson. 2017. Temporal constraints on the potential role of fry odors as cues of past reproductive success for spawning lake trout. *Ecology and Evolution* 7:10196–10206.
- Buchinger, T.J., W. Li, and N.S. Johnson. 2020. Behavioural responses of female lake trout *Salvelinus namaycush* to male chemical stimuli and prostaglandin F₂ α . *Journal of Fish Biology* 97:1224–1227.
- Cathcart, C. 2021. Olfactory activation: imprinting as an emerging frontier in the conservation of non-salmonid migratory fishes. *Fisheries* 46: 513–518.
- Chen, K.Y., S.A. Ludsin, B.J. Marcek, J.W. Olesik, and E.A. Marschall. 2020. Otolith microchemistry shows natal philopatry of walleye in western Lake Erie. *Journal of Great Lakes Research* 46:1349–1357.
- da Silva, M. C., A. V. M. Canário, P. C. Hubbard, and D. M. F. Gonçalves. 2021. Physiology, endocrinology, and chemical communication in aggressive behaviour of fishes. *Journal of Fish Biology* 98:1217–1233.
- Dembkowski, D. J., D. A. Isermann, S. R. Hogler, W. A. Larson, and K. N. Turnquist. 2018. Stock structure, dynamics, demographics, and movements of walleyes spawning in four tributaries to Green Bay. *Journal of Great Lakes Research* 44:970–978.
- Dittman, A. H., and T. P. Quinn. 1996. Homing in Pacific salmon: mechanisms and ecological basis. *Journal of Experimental Biology* 199:83–91.
- Dittman, A. H., T. N. Pearsons, D. May, R. B. Couture and D. L. G. Noakes. 2015. Imprinting of hatchery-reared salmon to targeted spawning locations: a new embryonic imprinting paradigm for hatchery programs. *Fisheries* 40:114–123.
- Elliott, C.W., M.S. Ridgway, E. Brown, and B.L. Tufts. 2023. High degree of individual repeatability found in the annual migrations of walleye (*Sander vitreus*) in eastern Lake Ontario. *Journal of Great Lakes Research* 49:725–736.
- Fielder, D. G., and J. P. Baker. 2004. Strategy and options for completing the recovery of walleye in Saginaw Bay, Lake Huron. Michigan Department of Natural Resources, Fisheries Division, Special Report 29. Ann Arbor.

- Hayden, T.A., T.R. Binder, C.M. Holbrook, C.S. Vandergoot, D.G. Fielder, S.J. Cooke, J.M. Dettmers, and C.C. Krueger. 2018. Spawning site fidelity and apparent annual survival of walleye (*Sander vitreus*) differ between a Lake Huron and Lake Erie tributary. *Ecology of Freshwater Fish* 27:339–349.
- Horrall, R. M. 1981. Behavioral stock-isolating mechanisms in Great Lakes fishes with special reference to homing and site imprinting. *Canadian Journal of Fisheries and Aquatic Sciences* 38: 1481–1496.
- Izzo, L. K., D. Dembkowski, T. Hayden, T. Binder, C. Vandergoot, S. Hogler, M. Donofrio, T. Zorn, C. Krueger, and D. Isermann. 2023. Spawning locations, movements, and potential for stock mixing of Walleye in Green Bay, Lake Michigan. *North American Journal of Fisheries Management* 43:695–714.
- Lake Sammamish Kokanee Work Group. 2013. Conservation supplementation plan for Lake Sammamish late-run (winter-run) Kokanee, King County, Seattle, Washington. <https://your.kingcounty.gov/dnrp/library/water-and-land/salmon/kokanee/late-run-supplementation-plan-2013.pdf>. (June 2024).
- Li, W., A.P. Scott, M.J. Siefkes, H. Yan, Q. Liu, S.S. Yun, and D.A. Gage. 2002. Bile acid secreted by male sea lamprey that acts as a sex pheromone. *Science* 296(5565):138–141.
- MacLean, J. A., and D. O. Evans. 1981. The stock concept, discreteness of fish stocks, and fisheries management. *Canadian Journal of Fisheries and Aquatic Sciences* 38:1889–1898.
- Sutterlin, A. M., and R. Gray. 1973. Chemical basis for homing of Atlantic salmon (*Salmo salar*) to a hatchery. *Journal of the Fisheries Research Board of Canada* 30:985–989.
- Stepien, C.A., Sepulveda-Villet, O.J., and Haponski, A.E. 2015. Comparative genetic diversity, population structure, and adaptations of walleye and yellow perch across North America. In *Biology and culture of percid fishes*. Springer. pp. 643–689.
- Todd, T. N. 1990. Genetic differentiation of walleye stocks in Lake St. Clair and western Lake Erie. U. S. Fish and Wildlife Service, Fisheries and Wildlife Technical Report 28.
- Zorn, T. G. 2021. Assessment of walleye spawning runs in tributaries to the upper Great Lakes and factors potentially influencing the magnitude of spawning runs. Michigan Department of Natural Resources, Fisheries Report 33, Lansing.
- Zorn, T. G., and NLMMU (D. Kramer). 2012. Walleye management strategy for Little Bay de Noc, Lake Michigan. Michigan DNR Fisheries Division.
- Zorn, T.G., and P.J. Schneeberger. 2011. Habitat and fish community changes in the Michigan waters of Green Bay 1988-2005. Michigan Department of Natural Resources, Fisheries Research Report 2096, Ann Arbor.

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