

Research Final Report Guidelines

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Final reporting requirements consist of:

1. A project abstract to help the reader quickly ascertain the project’s purpose, including the main results and conclusions, for posting to the Great Lakes Fishery Trust’s (GLFT) public website
2. A narrative response to the GLFT final report questions
3. A financial report accompanied by financial documentation verifying expenditures (form and instructions attached)
4. Attachments to include (a) copies of manuscripts accepted or submitted for publication (if applicable) and (b) any other technical reports developed as a result of the grant.
5. A brief project summary in 75 words or less

Project ABSTRACT

**Title:** Identity and Function of Lake Trout Pheromones

**Abstract Body:**

The role of mating pheromones in lake trout *Salvelinus namaycush* remains poorly understood due to a lack of information on their source and identity. We investigated the chemical identities of male and female pheromones that may guide reproduction in lake trout. We first reviewed the literature to identify and compare the approaches taken to identify pheromones in fish. Second, we integrated these approaches to identify several candidate pheromones in lake trout. Chemical analyses of one component indicated it is released at high rates by males but not females. Behavioral assays indicated two compounds may elicit responses in spawning lake trout, but additional research is needed given low activity of fish in our experiments. Continued research on the role of pheromones and other mating cues will inform restoration of self-sustaining and genetically diverse populations of lake trout.

Final Narrative Report Guidelines

* **Project Title:** Identity and Function of Lake Trout Pheromones
* **Grantee Organization:** Michigan State University
* **Project Team:** Tyler J. Buchinger, Ugo Busy, Ke Li, Belinda Huerta, Sonam Tamrakar, Weiming Li- Michigan State University. Nicholas S. Johnson- U.S. Geological Survey
* **Contact Person**: Weiming Li, Michigan State University, liweim@msu.edu
* **Grant Amount**: $237,773.00
* **Start and End Dates:** 01/01/2017- 12/31/2020
* **Key Search Words**: Lake trout

Background/Overview

1. *Briefly summarize the project description as outlined in the original proposal.*

The major goal of restoring self-sustaining and genetically diverse populations of lake trout is hindered, in part, by a poor understanding of cues involved in reproduction (Zimmerman and Krueger 2009). Previous research indicated pheromones released by males and females are among the cues that facilitate reproduction (Buchinger et al., 2015). The goal of this project was to characterize the identity and function of pheromones used by lake trout during reproduction. Our objectives were to determine the structure of 1) male lake trout pheromones and their function in guiding aggregation and spawning and 2) female lake trout pheromones and their function in synchronizing spawning. To characterize pheromones in lake trout, we employed an approach integrated with various chemical, physiological, and behavioral methods.

1. *Briefly summarize any significant changes to the work performed in comparison to the originally proposed and funded plan of work. If changes were made, describe how they affected your ability to achieve the intended outcomes for the work.*

We made no significant changes to our plan of work.

Outcomes

*Please characterize key outcomes of the project related to knowledge, training, relationships, and practice. Not all projects will have outcomes of all types.*

1. *To what extent and how (if at all) did this research project advance scientific knowledge of the issue?*

This research project advanced knowledge of pheromones in lake trout and more broadly. First, we defined the framework of approaches used to characterize pheromones in fish, which includes targeted screening, metabolomics, and fractionation (Li et al., 2018; attached). We then applied facets of this framework to characterize pheromones in lake trout. Starting with the targeted screening approach, we investigated prostaglandin F2α (PGF2α) as a sex pheromone in lake trout because extensive evidence indicates it acts as sex pheromone in numerous species of fish. Our results indicate PGF2α has little behavioral effect on females (Buchinger et al., 2020; attached). We continued to investigate PGF2α as a pheromone that acts on males, but the chemical analyses of our final year have been delayed due to the COVID-19 pandemic. Next, we used the metabolomics approach to identify potential compounds of interest in water collected from males. Our previous experiments indicated spawning male and juvenile lake trout release a compound that attracts males and females (Buchinger et al., 2015). Using high-resolution mass spectrometry (HRMS), we found the same molecule was the most abundant peak in male odor and the third most abundant peak in juvenile odor but essentially absent from female odor. Based upon the mass to charge ratio, we predicted the peak corresponded to the bile acid taurocholic acid (TCA). We then collected large quantities of male urine, which contains attractive compounds (Buchinger et al., 2020), and sought to isolate and identify the major components. Doing this, we identified TCA as one of the most abundant components of the male urinary excrets. Lastly, quantification of TCA in tissues, bodily fluids, and conditioned water unveiled that only males have high concentrations of TCA in their urine and conditioned water. Taken together, our results indicate release of TCA is sexually dimorphic and specialized, and implicate it as a putative male pheromone in lake trout. As TCA is not specific to lake trout, our discovery inspired us to challenge the generalization that pheromones are species specific and review the reasons to expect animals to use pheromones that are not species specific (Buchinger and Li, 2020).

1. *To what extent and how (if at all) did this project contribute to the education and advancement of graduate or undergraduate students focused on Great Lakes fishery issues?*

This project supported the training of a post-doctoral researcher but did not directly contribute the any graduate or undergraduate education.

1. *To what extent and how (if at all) did this work help you or others on your team build new relationships with others in the research or management communities?*

This project strengthened existing relationships between Michigan State University, the U.S. Geological Survey’s Hammond Bay Biological Station (HBBS), and various state and federal fish hatcheries, but did not directly involve any new relationships.

1. *To what extent and how (if at all) do the findings have action implications for fishery managers? If the research has direct management implications, do you have any knowledge of use of the findings by managers? If the research does not have direct management implications at this stage, to what extent did the research advance the process of identifying management responses to critical issues?*

Our results help address gaps in the understanding of lake trout reproduction and inform possible future management actions that involve lake trout pheromones, but do not have immediate action implications for fishery managers.

1. *Considering the above or other factors not listed, what do you consider to be the most important benefits or outcomes of the project?*

We consider the most important outcomes of the project to be 1) identification of a possible male pheromone in lake trout, 2) two publications that advance the broader understanding of pheromone identification and function, and 3) project leadership training for a post-doctoral researcher.

Related Efforts

1. *Was this project a standalone effort, or was there a broader effort beyond the part funded by the GLFT? Have other funders been involved, either during the time of your GLFT grant or subsequently?*

This project was part of our ongoing research on olfaction in lake trout. This work began with funding from the Great Lakes Fishery Commission. During this project, the Great Lakes Fishery Commission continued to provide significant support, especially for the chemical analyses.

1. *Has there been any spinoff work or follow-up work related to this project? Did this work inspire subsequent, related research involving you or others?*

The results of this project continue to inspire follow-up research. In late 2020, we conducted an experiment to determine the effect of sea lamprey parasitism on pheromone release by male lake trout. Likewise, we plan to continue investigating lake trout pheromones in autumn 2021.

Communication/Publication of Findings

1. *List publications, presentations, websites, and other forms of formal dissemination of the project deliverables, tools, or results, including those that are planned or in process.*

**Planned publications**

Buchinger TJ, U Bussy, K Li, B Huerta, S Tamrakar, NS Johnson, W Li. Evidence that male lake trout release taurocholic acid as an aggregation pheromone.

Buchinger TJ, S Tamrakar, NS Johnson, W Li. Possible priming effects of prostaglandin F2α on male lake trout.

**Publications**

Buchinger TJ, W Li, NS Johnson. 2020. Behavioural responses of female lake trout to male chemical stimuli and prostaglandin F2α. *Journal of Fish Biology*. 97: 1224-1227.

Buchinger TJ, W Li. The evolution of (non)species-specific pheromones. 2020. *Evolutionary Ecology.* 34: 455-468.

Li K, TJ Buchinger, W Li. 2018. Discovery and characterization of natural products that act as pheromones in fish. *Natural Product Reports*. 35:501-513.

**Conference Presentation**

Buchinger TJ, W Li, NS Johnson. Conspecific olfactory used by lake trout during reproduction. *Oral presentation*. Symposium: Sensing the Environment: Molecules to Populations, International Congress on the Biology of Fishes. July 2018. Calgary, Canada.

**Press coverage**

Interview on: *Insights Into Northeast Michigan*, WBKB 11. Fall 2019. <http://www.wbkb11.com/local-programming/insights-into-northeast-michigan>

1. *Please characterize your efforts to share the findings of this research with state, federal, Tribal, and interjurisdictional (e.g., Great Lakes Fishery Commission) agencies charged with management responsibilities for the Great Lakes fishery. If other audiences were priority for this research, please characterize your outreach efforts to those audiences as well. (Please note: You may wish to consult midterm reports in which specific audiences for the findings, and means of outreach to these audiences, were identified.)*

To date, our efforts to communicate our findings primarily rely on scientific publications and presentations. As we finalize our ongoing chemical analyses (delayed by COVID-19) and continue this line of research, we will continue to disseminate our findings through scientific publications and presentations, and communicate directly with lake trout managers through lake committee technical meetings when warranted.

1. *Please identify technical reports and materials attached to this report by name and indicate for each whether you are requesting that GLFT restrict access to the materials while you seek publication. (Please note that the maximum amount of time during which GLFT will restrict access to the results of funded research is 18 months, unless notified that more time is needed.)*

Attachments include:

* + - 1. Buchinger TJ, W Li, NS Johnson. 2020. Behavioural responses of female lake trout to male chemical stimuli and prostaglandin F2α. *Journal of Fish Biology*. 97: 1224-1227.
			2. Buchinger TJ, W Li. The evolution of (non)species-specific pheromones. 2020. *Evolutionary Ecology.* 34: 455-468.
			3. Li K, TJ Buchinger, W Li. 2018. Discovery and characterization of natural products that act as pheromones in fish. *Natural Product Reports*. 35:501-513.
			4. The financial report.
			5. A brief summary of the project.
1. *Manuscripts. Grantees submitting one or more publications or pending publications in lieu of a standalone technical report must submit a cover memo that confirms that all aspects of the funded research are incorporated in the published work, and in cases of multiple publications, identifies or crosswalks the grant-funded objectives to the published article containing results.*

 The objectives of our funded project were to characterize putative male and female pheromones. Results from our work towards characterizing putative female pheromones are reported in Buchinger et al., 2020 and in the planned publication “Possible priming effects of prostaglandin F2α on male lake trout.” Results from our work towards characterizing putative male pheromones are reported in the planned publication “Evidence that male lake trout release taurocholic acid as an aggregation pheromone.”

1. *Compilation reports. Grantees working on several related subprojects under a single grant may submit a series of subproject reports rather than a single, integrated report. However, grantees must submit a cover sheet or introduction that outlines and crosswalks grant objectives with the location of the results in the compilation document.*

Not applicable.

Discussion

In this project, we 1) defined the integrated framework of approaches used to identify pheromones in fish, 2) identified TCA as a putative male pheromone in lake trout, 3) challenged the generalization that most pheromones are species specific, and investigated the role of PGF2α as a sex pheromone in lake trout. Our work advances the fundamental understanding of pheromones across animals and provides specific knowledge of pheromones in lake trout.

Our review of the literature on fish pheromones revealed three general approaches to identifying the molecules that act as pheromones (Li et al., 2018; attached). Most efforts to identify pheromones in fish use targeted screening of known compounds predicted to act as pheromones based upon their physiological function (*e.g.* hormonal pheromones; Stacey 2015). In contrast, most of the extensive studies on insect pheromones rely on bioassay-guided fractionation, which focuses on chemical characterization of compounds found to elicit responses in behavioral or physiological assays. Recently, researchers seeking to identify pheromones in fish have also employed bioassay-guided fractionation and, in doing so, expanded the chemical templates of pheromone molecules used by fish (Li et al., 2018). Lastly, the full quantitative comparison of samples known to contain pheromone compounds to a negative control (*i.e.* metabolomics) offers a third approach which, to our knowledge, has not yet been leveraged in efforts to identify pheromones in fish. Here, we integrated each of these approaches in our quest to identify pheromone in lake trout.

The results from our integrative approach implicate TCA as a putative male pheromone in lake trout (Buchinger et al., in prep). Consistent with behavioral evidence for an attractive compound released by males and juveniles but not females (Buchinger et al., 2015), metabolomics indicated TCA is a major component of the chemical profile of males and juveniles. In parallel, chemical isolation molecules in male urine unveiled TCA as one of the major components. Lastly, quantification of TCA confirmed it was released by males via their urine, with an estimated release rate of approximately 400 µg/h. Importantly, additional behavioral tests are needed to demonstrate a behavioral function of TCA as an aggregation pheromone. Nevertheless, that TCA might act as a component of the male pheromone fits our working model of lake trout pheromone communication. Closely related Arctic char (*S. alpinus*) appear to use the bile acids, including TCA, released by stream-resident juveniles to locate spawning streams (Nordeng, 1971; Selset and Doving, 1980). Similarly, spawning lake trout are attracted to juvenile odor (Buchinger et al., 2015; 2017) and prefer spawning reefs baited with fry feces (Foster, 1985), which consist in part of TCA (Zhang et al., 2001). However, fry feces are unlikely useful as an odor cue for lake trout searching for spawning sites because juveniles in most existing populations emigrate from spawning reefs long before adults return (Buchinger et al., 2017). Previous observations that male and female lake trout are attracted to the odor of males and juveniles but males do not discriminate between the male and juvenile odor prompted the hypothesis males release some of the same molecules as juveniles to effectively take over the role in guiding spawning site selection (Buchinger et al., 2015). Our discoveries that males, but not females, release TCA at high rates via a specialized route (*i.e.* urine) and females are attracted to male bile (Buchinger et al., 2020) lend further support to our overall hypothesis.

The possibility TCA acts as an aggregation pheromone in lake trout led us to question the assumption that most pheromones are species specific (Buchinger and Li 2020). Across taxa, pheromones are incredibly diverse and can be highly species specific (Symonds and Elgar 2008). In fact, the remarkable diversity of pheromones leads to expectations that pheromones are generally or even fundamentally species specific (*e.g.* Brennan and Zufall, 2006; Bussell and Vosshal, 2012). However, pheromones need not be species specific by definition and many are shared among species (Wyatt 2014; Buchinger and Li, 2020). Animals can use convergent or homologous pheromones due to shared aspects of their evolutionary history, physiology, or ecology. Likewise, species isolation via other chemical or multi-modal cues, and temporal or spatial barriers can alleviate selection for pheromone divergence (Buchinger and Li, 2020). In the case of lake trout, we predict that, if indeed TCA acts as a pheromone, species specificity is achieved via other chemical or multimodal cues (*i.e.* acoustic signals; Johnson et al., 2018) or simply differences in the magnitude of the TCA signal produced by an aggregation of male lake trout versus that released via feces of other fishes.

Whether lake trout use PGF2α as a sex pheromone remains unclear. Although closely related Arctic char appear to use PGF2α as a male sex pheromone (Sveinsson and Hara 1995) and lake trout detect PGF2α as a potent odorant (Hara and Zhang, 1998), we observed no preference of females for 1x10-10 or -11 M PGF2α (Buchinger et al., 2020). We postulate this difference may be due to the reproductive behavior of lake trout, in which, unlike Arctic char, males follow females immediately prior to the act of spawning (Muir et al., 2012). Accordingly, we hypothesize PGF2α may act as female sex pheromone in lake trout, as is the case in goldfish and many other species (Stacey 2015). Indeed, we observed slight (nonsignificant) preferences of male lake trout for PGF2α; however, fish in these experiments exhibited poor responses to positive controls (see below), rendering the experiment inconclusive. Notably, PGF2α may also act as a priming pheromone that stimulates the endocrine system of males (Stacey 2015). We are currently analyzing samples from an experiment testing this hypothesis after experiencing delays related to the COVID-19 pandemic.

Our project faced the significant challenge of poor behavioral responses to positive controls. Studies on fish pheromones often struggle with variation in behavioral responses (Johnson and Li, 2010). Early in our research on lake trout pheromones, we repeatedly observed consistent and robust behavioral responses to water conditioned with males or juveniles (Buchinger et al., 2015; 2017; 2020). These experiments relied on Seneca strain fish provided immediately prior to spawning by the Sullivan Creek National Fish Hatchery. Unfortunately, these fish were not available immediately before spawning for much of our project. Our attempts to hold these fish at Hammond Bay Biological Station or to use other fish (*e.g.* wild fish) resulted in poor responses to positive controls. Likewise, we observed only weak responses to putative pheromone components TCA and PGF2α. An additional confounding variable in our experiments was the transition from the HBBS laboratory in which we had previously had success to a newly constructed laboratory. These challenges reiterated the benefits of integrative research, as our chemical and physiological experiments made significant advances in our understanding of lake trout pheromones.

Much remains unknown about the role of olfactory or other cues that facilitate reproduction in lake trout. Our results add to mounting evidence that olfactory cues from conspecifics play an important role. Future research should leverage the knowledge produced in this project to further describe pheromone communication in lake trout, but also consider imprinted odors from kin (Gerlach et al., 2008) or natal sites (Dittman et al., 2015) as well as auditory (Johnson et al., 2018), visual (Muir et al., 2012) or other cues. Understanding the role of these cues will inform restoration of self-sustaining and genetically diverse populations of lake trout (Muir et al., 2013).