

2023-2025 Sabbatical Research Report

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Sabbatical Proposal Title: **Enhancement of LSSU Education by Strengthening Research Capability with a Zebrafish Model System**

Executive Summary:

Thank you very much for granting me two semesters of sabbatical leave to conduct the proposed research during two separate terms (Fall 2023 and Fall 2024). During this sabbatical period, I have completed several manuscripts—seven of which have been published in peer-reviewed journals, while three others are currently under revision or in press.

In addition, I successfully established a zebrafish culture system at LSSU, which has significantly supported both the Biology and Environmental Chemistry programs. Using this zebrafish model, I trained five LSSU students, who acquired valuable knowledge and skills through their senior research projects. These students also secured LSSU Undergraduate Research Funds and presented their findings at several national and regional scientific conferences.

Moreover, I had the opportunities to visit my collaborators at the University of Maryland at Baltimore, the University of Michigan at Ann Arbor, the Yellow Sea Fisheries Research Institute, and the Institute of Oceanology at the Chinese Academy of Sciences. These visits allowed me to gain hands-on research experience and acquire new technical skills, which will further strengthen future collaborations and support potential research grant applications.

The details of my accomplishments are outlined below:

1. Establishment of Zebrafish Culture System at LSSU:

After being awarded a two-semester sabbatical, I began researching suitable zebrafish culture systems and ultimately placed an order—including the main system and related accessories—through **AQUATIC ENTERPRISES** (www.aquaticenterprises.com). Upon

the system's arrival, we assembled and installed all components, ensuring the system operated smoothly. This process was carried out with the assistance of a biology student, **Andrew Pothoven**. Throughout the setup and testing phases, we gained valuable first-hand experience, developed a deeper understanding of the system, and improved our troubleshooting skills.

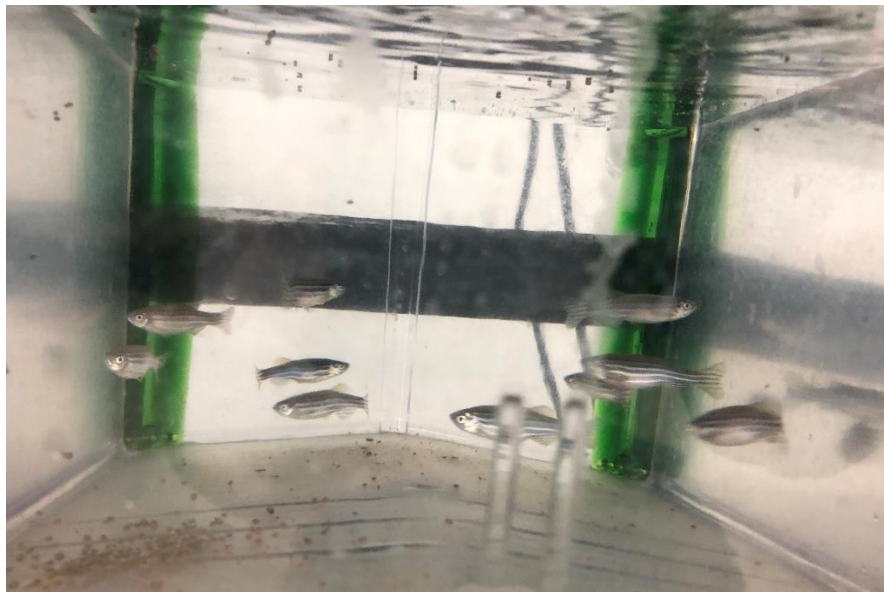


To gain more knowledge about zebrafish culture—including routine system maintenance, nutritional requirements, spawning, and larval husbandry—I began by self-studying

through relevant websites and instructional videos (please see the **Appendices**). I then conducted several on-site visits to zebrafish facilities at institutions such as the **University of Michigan**, the **University of Maryland**, and various research centers in China. These visits provided hands-on experience and a deeper understanding of practical techniques, as well as exposure to real-world zebrafish research activities. More importantly, they allowed me to connect with the zebrafish research community, fostering valuable relationships and enabling me to receive guidance and suggestions from experienced scientists across the U.S. and around the world.

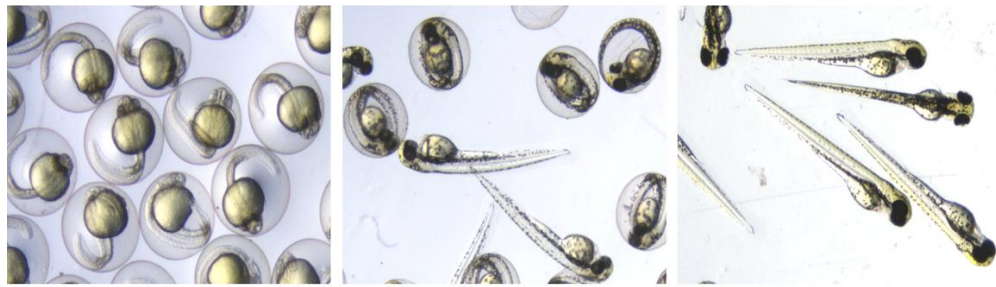
2. Zebrafish culture at LSSU

After gaining sufficient knowledge about zebrafish culture, we introduced two dozen adult zebrafish into our system from the Zebrafish International Resource Center (ZIRC) (www.zebrafish.org) to test its functionality, and the system performed well. We later ordered additional dozens of adult zebrafish with different genetic lineages, all of which have remained healthy and active under our routine daily care (See the image below).

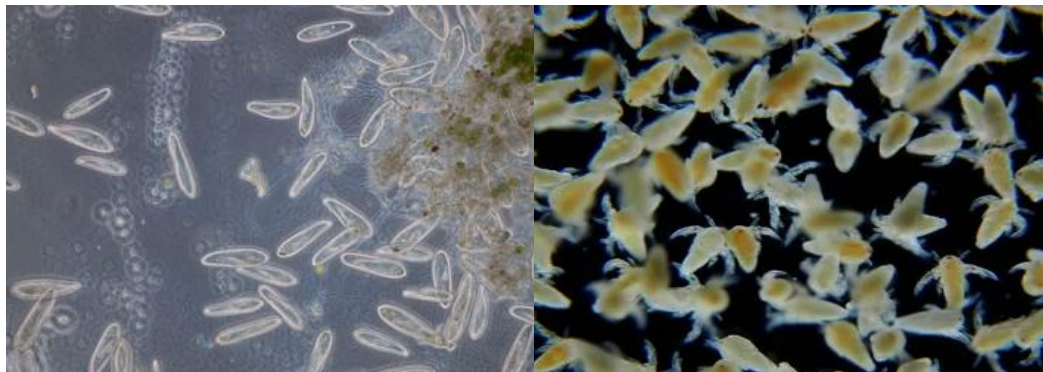


Next, we began culturing and maintaining zebrafish embryos and larvae to gain hands-on experience with delicate nursery care and to closely observe developmental stages. One of the most important practical skills we acquired was culturing live food, which

is essential for larval zebrafish at different developmental stages. This included *Paramecia*—microscopic, single-celled protists—for early-stage larvae (from 4 to 21 days post-hatching), and brine shrimp for older larvae (over 21 days post-hatching). We experimented with various culture conditions to optimize the growth and harvesting of these essential live foods. Through feeding practices, we also gained valuable hands-on experience in larval zebrafish husbandry. Several embryos have successfully developed and matured into adult zebrafish in our lab at Lake Superior State University (LSSU).



Zebrafish embryos at various developmental stages



Paramecia

Brine shrimp

3. Instruction and Research by Using Zebrafish Model

3.1 Involvement of LSSU students

During the past two years, there are 5 Biology students have been involved in the culture and care practice of zebrafish and using them for their senior research projects, three of them have received **LSSU Undergraduate Research Funds**:

– **Andrew Pothoven,**

- Senior, graduated in Spring 2025,
- Majored in Biology and Fish & Wildlife Management
- 2 years research work with zebrafish, by taking Biol. 399, Biol. 499
- Research work: **Determining the Effects of Sediments from Stamp Sands alone Lake Superior on Zebrafish, *Danio rerio***
- **Awarded LSSU Undergraduate Funds (Fall 2023: \$460; and Fall 2024: \$295)**

– **Elaine Book,**

- Senior, graduated in Spring 2025,
- Majored in Biology (Animal Biology Concentration)
- 2 years research by taking Biol. 399, Biol. 499
- Research Work: **Effects of Temperature and PFAS Pollutants on Zebrafish Embryo Development**
- **Awarded LSSU Undergraduate Funds (Spring 2025: \$500)**

– **Sydney Kapushinski**

- Senior, graduated in Spring 2025
- Majored in Biology (Animal Biology Concentration)
- 1 year research experience by taking Biol. 389 internship
- Research work: **Nutrition requirement of Zebrafish, *Danio rerio***

- **Awarded LSSU Undergraduate Funds (Spring 2024: \$485)**
- **Emily Hall**
 - Current Senior
 - Majored in Biology (pre-Vet)
 - 1 year research experience by taking Biol. 399, ongoing for another year to take Biol 499
 - Research Work: **Genetic and Developmental Divergence: A comparative Study of Wild and Lab-Bred Zebrafish Embryos**
- **Alyssa Kozlowski**
 - Current Senior
 - Majored in Biology (pre-Med)
 - 1 year research experience by taking Biol. 399, ongoing for another year to take Biol 499
 - Research work: **Zebrafish as a Model to Study Alzheimer's Disease**
 - Applied LSSU Undergraduate Research Fund, but her proposal was not awarded, will be resubmitted after revision.

3.2 Presentations in National/Regional Conferences/Symposia:

Two senior students, **Andrew Pothoven** and **Elaine Book** presented their research findings in the 9th LSSU Senior Research Symposium held at LSSU in April 25th 2025 (**see Appendices**). Meanwhile, two current senior students Emily and Alyssa also presented their proposed research in their junior presentation classes, and finished writing their research proposals.

In addition, Andrew also presented his findings based on the research with zebrafish at LSSU in the following national and regional conference/meetings:

- 1) Andrew Pothoven, Jun Li, Dereck Wright, 2025. **Determining the Effects of Sediments from Stamp Sands alone Lake Superior on Zebrafish, *Danio rerio*.** Presented in the 2025 World Aquaculture Society (WAS) Aquaculture Conference, held at New Orleans, LA (Oral presentations).
- 2) Andrew Pothoven, Jun Li, 2024. **Determining the Effects of Sediments from Stamp Sands alone Lake Superior on Zebrafish, *Danio rerio*.** Presented in the 2024 America Aquaculture Conference, held at San Antonio, TX.
- 3) Andrew Pothoven, Jun Li, 2024. **Determining the Effects of Sediments from Different Water Bodies in Michigan on Zebrafish, *Danio rerio*.** Presented in the 2024 Wisconsin and Minnesota Aquaculture Conference, held at Red Cliff, WI.

It is worth noting that the research findings presented by LSSU undergraduates at national scientific conferences received highly positive attention from audiences, including aquaculture professionals and zebrafish husbandry experts from across the country. These accomplishments not only highlighted the quality of research conducted by LSSU students and faculty but also brought greater visibility and recognition to the university.

4. Other Scholarly Research and Publications

In addition to establishing a new zebrafish culture system and applying this model organism for research at LSSU, I also continued my collaborative research efforts, particularly focusing on completing ongoing projects and conducting data analysis. These additional efforts have resulted in several peer-reviewed publications, as listed below:

- 1) Li, WX., Wang, XH., Lin, YJ, Zhou YY, Li J, Zhang XY, & Chen XH. 2023. Large yellow croaker (*Larimichthys crocea*) mitofusin 2 inhibits type I IFN responses by degrading MAVS via enhanced K48-linked ubiquitination. *Mar Life Sci Technol.* **5**, 359–372 (2023). <https://doi.org/10.1007/s42995-023-00189-8>
- 2) Li, WX., Wang, XH., Lin, YJ, Zhou YY, Li J, Zhang XY, & Chen XH. **Correction:** Large yellow croaker (*Larimichthys crocea*) mitofusin 2 inhibits type I IFN responses

- by degrading MAVS via enhanced K48-linked ubiquitination. **Mar Life Sci Technol** (2024). <https://doi.org/10.1007/s42995-024-00247-9>
- 3) Liu S, Wang W, Jia T, Xin L, Xu T, Wang C, Xie G, Luo K, **Li J**, Kong J, Zhang Q. 2023. *Vibrio parahaemolyticus* becomes lethal to post-larvae shrimp via acquiring novel virulence factors. **Microbiology Spectrum** 11:e00492-23. <https://doi.org/10.1128/spectrum.00492-23>
 - 4) Han XQ, Cui ZW, Ma ZY, Wang J, Hu YZ, **Li J**, Ye JM, Tafalla C, Zhang YA, Zhang XJ. 2024. Phagocytic Plasma Cells in Teleost Fish Provide Insights into the Origin and Evolution of B Cells in Vertebrates. **J Immunol**. 2024 Sep 1; 213(5):730-742. doi: 10.4049/jimmunol.2400182.
 - 5) Zhou Z, Huang Y, Hao R, Yang C, Abarike ED and Li J (2024) Editorial: Population and action mechanism of immune cells in fish. **Front. Immunol**. 15:1425155. doi: 10.3389/fimmu.2024.1425155
 - 6) Mu LL, Li JD, Lin ZY, Zeng QL, Deng L, Wu SQ, **Li J**, Yin XX, Ye JM. 2025. MBL regulates phagocytosis and bactericidal activity of macrophages by triggering AKT/NF- κ B/Rab5A axis occurred early in vertebrate evolution. **J Immunol**. 2025, vkaf028, <https://doi.org/10.1093/jimmun/vkaf028>.
 - 7) Xu TT, Xia JT, Wang C, Li YX, **Li J**, Zhang QL. 2025. Horizontal and vertical transmission of covert mortality nodavirus in the marine fish, **Aquaculture**, 607 (2025) 742620, <https://doi.org/10.1016/j.aquaculture.2025.742620>.
 - 8) Wang J, Dong WJ, Tian TT, Wu CS, Zhang XY, Hu YZ, Pan YR, Han XQ, **Li J**, Tafalla C, Zhang YA, Zhang XJ. 2025. Cell communication and signaling: Decision on Cold-blood vertebrates evolved regulatory B cells to participate in inflammatory diseases. **Cell Communication and Signaling**. *Under revision*.
 - 9) Wu LT, Ye JM, **Li J**. 2025. The linkage between innate and adaptive immunity: Recent advances in antigen recognition, processing and presentation in fish. **Aquaculture and Fisheries**. *In press*.

- 10) Qiu R, Li J, Jiang CJ, Yu Y, Li DD, Xie XH, Lei Y, Yao LG. 2025. Antibacterial activity of baicalein against *Aeromonas hydrophila*: in vitro and in vivo evaluation. **Frontiers in Microbiology**. *Under Revision*.

The overall scientific collaborations and scholarly activities have not only significantly strengthened the reputation of LSSU faculty as active scientists on a global scale—as evidenced by research publications—but have also enhanced LSSU’s educational capacity by ensuring that faculty continually incorporate current research knowledge and skills into the curriculum. Through these scholarly pursuits, I have remained at the forefront of emerging scientific questions, which has continuously sharpened my critical thinking skills and deepened my understanding of the rapid advancements in biological sciences. These efforts have greatly benefited students by providing them with up-to-date knowledge and laboratory techniques (Instruction), as well as more accurate and informed academic advising to help them select meaningful research projects and plan for graduate school or future career paths (Advising).

As a conclusion, I believe my sabbatical activities successfully achieved the proposed goals:

- 1) To develop a new on-campus zebrafish model system that enhances faculty and student research capabilities;
- 2) To make significant scientific contributions to the field of aquatic animal health and elevate the reputation of LSSU faculty as biologists and educators;
- 3) To integrate current research knowledge and methodologies into the LSSU curriculum.