Short-Term Immune Response of Largemouth Bass (*Micropterus salmoides*) to Crude Oil Exposure.

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Introduction and Purpose

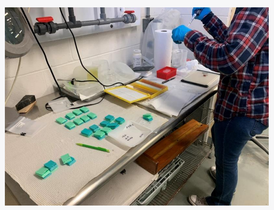
- In North America, crude oil is transported through pipelines and by rail in ever increasing amounts
- In 2019, approximately 1,000 tones of oil were spiled into water systems across the world, the largest occurring in North America
- But the impacts of oil spills in freshwater systems, particularly sub-lethal effects on fish and other aquatic organisms, are significantly understudied
- to fundamentally improve our understanding of how oil spills affect freshwater fish, we designed and conducted an experiment to develop an immune response timeline for largemouth bass exposed to crude oil

Objectives

- Evaluate the effects of oil exposure on the immune response of largemouth bass at: 2, 7, and 14 days post-exposure
- Define the timeline of response to oil exposure for physiological measures of immune function

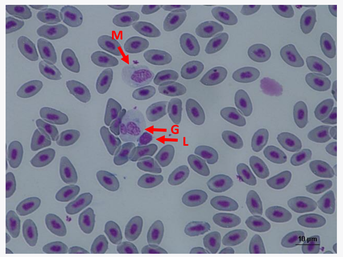
Methods

- Largemouth bass were exposed through intraperitoneal injections to 10% crude oil solution in dimethyl sulfoxide (DMSO) or DMSO alone as a negative control
- Each fish was anesthetized, weighed, then injected (1.0 µL/g body weight) with either DMSO or 10% oil in DMSO. Fish were injected with the same amount of solution to their body weight.
- Fish were held in flow-through tanks (25°C), monitored daily, and euthanized at sampling.
- At necropsy, peripheral blood was taken from the posterior caudal artery and vein with a heparinized needle; triplicate blood smears were made from whole blood; spleens were removed for histological analysis of macrophage aggregates; livers were removed for future EROD analysis; and gills were removed for histological enumeration of chloride cells.
- Blood smears were stained using the Wright Geimsa stain and analyzed using the Nikon 90i and NIS Elements software
- Spleen samples were processed (Leica tp1020) and embedded in wax, sectioned at 4 micrometers, and stained using the Iron, Gomori Prussian Blue Stain. Slides were analyzed using the Nikon 90i and NIS elements software. Aggregates were only counted if there were >5 macrophages present.
- Data was collected on the area of each aggregate in a specific total area of the spleen.
- Total area was collected, and the average macrophage percent was calculated (Macrophage Area/Total Area*100).

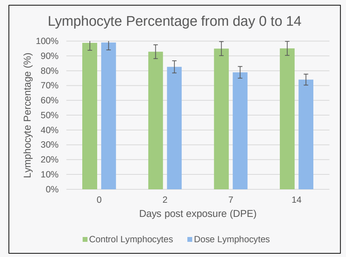


Results and Discussion

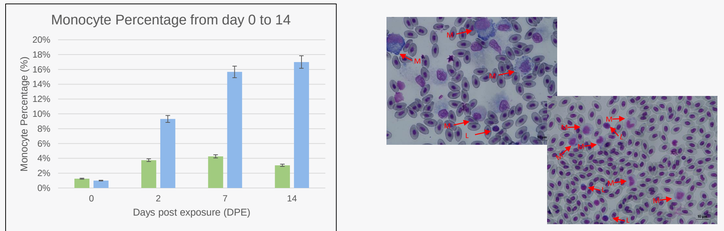
Blood Smear Data



- Monocyte (M) distinguished by the single lobed nuclei surrounded by large granular cytoplasm
- Granulocyte (G) distinguished by a multilobed nucleus surrounded by large granular cytoplasm
- Lymphocyte (L) distinguished by its large dark blue nucleus and small ring of light blue cytoplasm

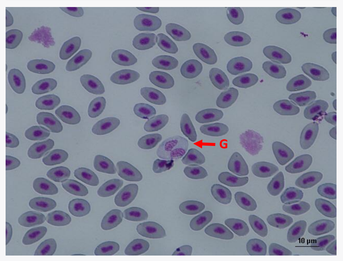


- Percent lymphocytes decreased in exposed bass over 14 DPE.
- Lymphocyte numbers were highest at time zero.
- Throughout the study lymphocyte percentages in the control treatment group remained constant (between 93-95%).
- Lymphocyte percentages decreased in the dosed group from 99% at 0 DPE to 74% by the end of the study at 14 DPE.

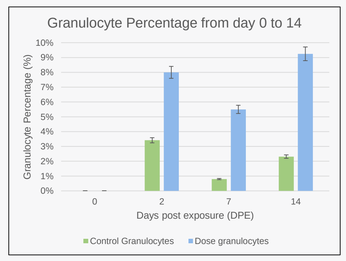


- Monocytes (M) are distinguished by their single lobed nuclei surrounded by large granular cytoplasm.

- Monocyte percentages were lowest at time zero at 1%
- Throughout the study the percentage of monocytes in the control group remained relatively constant at 3-4%
- Monocyte percentages increased in the oil treatment from 1% (0 DPE), 9% (2 DPE), 16% (7 DPE), and 17% (14 DPE)

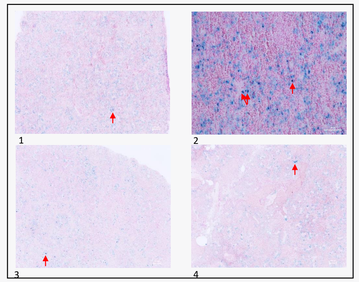


- A singular granulocyte (G) distinguished by a multi lobed nucleus surrounded by large granular cytoplasm

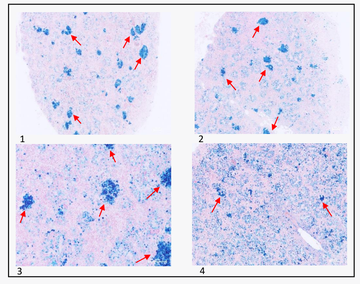


- Granulocyte percentages were lowest at time zero at 0%
- The throughout the study the percentage of monocytes in the control group remained relatively constant (between 1-3%).
- Monocyte percentages increased in the dosed treatment group, starting at 0% at 0 DPE and ending at 9% on 14 DPE.

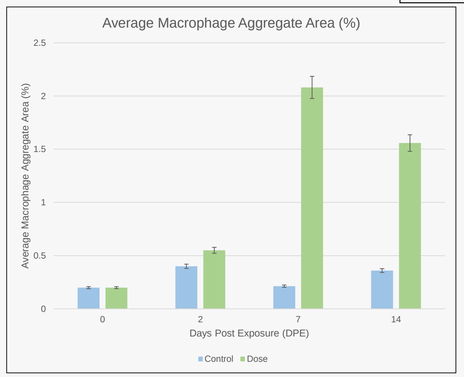
Macrophage Aggregate Data



- All micrographs depict slides of control fish spleens
- Pictures 1 and 3 were taken on the Nikon 90i at 4x resolution
- Pictures 2 and 4 were taken on the Nikon 90i at 10x resolution
- Dark blue stained dots represent macrophage aggregates (not all aggregates are represented by arrows)



- All pictures depict slides of exposed fish spleens
- Pictures 1, 2, and 4 were taken on the Nikon 90i at 4x resolution
- Pictures 3 was taken on the Nikon 90i at 10x resolution
- Dark blue stained dots represent macrophage aggregates (not all aggregates are represented by arrows)



- The average area of macrophage aggregate area in the spleens of oil-exposed fish increased over the course of the study.
- Macrophage aggregates increased in area of the spleen over the course of the study in the oil-exposed fish.
- Macrophage aggregate area stayed relatively constant in the unexposed control fish (0.21- 0.39%) over the course of the study.
- Macrophage aggregate area in oil-exposed fish was lowest at time zero at .12% and still relatively low at 2 DPE (0.55%)
- Spleens of oil-exposed fish contained macrophage aggregate areas which peaked at 7 DPE (2.08%), then decreased slightly to 1.56% at 14 DPE.

Conclusion

These initial changes in peripheral blood cell composition and macrophage aggregations indicate a change in immune status in response to crude oil exposure. Moreover, these results clarify the temporal aspects of immune system responses that occur in largemouth bass after an oil exposure. This information will help determine appropriate timing of fish health assessments after oil spill events.

Future Studies:
Evaluation of chloride cell numbers in gills and ethoxyresorufin-O-deethylase (EROD) analysis are planned to complete this study.

Acknowledgements

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