Effects of 2,4-D application on early life stages of fish

Background

The aquatic herbicide 2,4-D is frequently used to control Eurasian watermilfoil and other invasive plant species in Wisconsin. Although 2,4-D is typically applied in the spring and early summer, which is when many native fish species spawn, there has been little research on whether the herbicide affects early life stages of Wisconsin fish. Over several research studies, this project evaluated the effects of exposure to ecologically relevant 2,4-D concentrations on the early life stages of several freshwater fish species.

Fathead Minnow Reproduction and Development

The goal of these two research studies was to assess if exposure to 2,4-D affects fathead minnow reproductive biology and early development. Fathead minnows are native to Wisconsin and are an ecologically important species. The researchers studied the effects of 2,4-D on fathead minnow reproduction, development, and survival at multiple stages of their early life cycle in the first study.

After findings indicated larvae were sensitive to 2,4-D exposure, researchers conducted a second experiment on fathead minnow larvae to narrow down the critical exposure window (i.e., the point in the early life cycle that 2,4-D exposure has the greatest effect) and explore if the negative effects seen in the first experiment were due to inert ingredients in the commercial herbicide products used or 2,4-D itself.

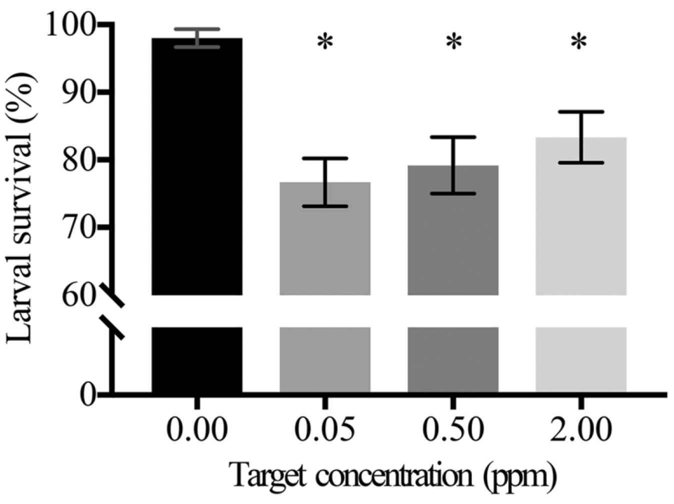

Figure 1. Fathead minnow larval survival when exposed to different concentrations of 2,4-D (Dehnert et al. 2018).

Research Methods

- Fathead minnow (*Pimephales promelas*) eggs, larvae and fertile adults were exposed to different concentrations of 2,4-D in both studies (0, 0.05, 0.5, and 2 ppm).
- The herbicide concentrations used in the experiments were below the maximum labeled application rates and consistent with observed concentrations of 2,4-D in Wisconsin lakes after herbicide treatments.
- Survival was monitored weekly in both studies. Surviving larval fish were counted, weighed, and measured after 30 days.
- The first experiment used two commercially available 2,4-D herbicide products, and the second experiment used the two commercial 2,4-D products and pure 2,4 -D.

Summary of Results

- Exposing larvae to both pure 2,4-D and the two commercial formulations resulted in significantly lower larval survival rates in both studies at concentrations as low as 0.05 ppm (Fig. 1).
- Reproductive abnormalities were also seen in adults exposed to the commercial formulations in the first study.
- The researchers identified a potential critical window of exposure to 2,4-D from fertilization to 14 days after hatching in the second study.
- There was no significant difference in survival after exposure to pure 2,4-D versus the two commercial formulations in the second study. This suggests that observed effects were due to exposure to 2,4-D itself.

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Northern Wisconsin Field Study

After the previous laboratory studies reported 2,4-D exposure can negatively affect larval fish survival, researchers wanted to determine if the effects of 2,4-D could also be seen on early life stages of fish and zooplankton exposed to a 2,4-D commercial formulation in a natural lake environment.

Photo credit: Bob Korth

Research Methods

- Larval fish and zooplankton communities were sampled on six Wisconsin lakes from 2015 – 2017, three of which received a whole-lake 2,4-D treatment in 2016.
- Zooplankton size, density and diversity, larval fish abundance, diversity, feeding, growth and size structure, and juvenile fish abundance and mortality were compared between treated and untreated lakes.

Summary of Results

- No significant responses that could be linked directly to 2,4-D exposure were observed in larval fish, juvenile fish or zooplankton populations.
- These results do not indicate that 2,4-D exposure has no effects on fish and zooplankton, only that there were no statistically significant effects detected in the metrics used by the researchers. Potential effects could have been masked by inherent natural variation in fish and zooplankton populations and the small number of lakes used in the study.

Multiple Freshwater Fish Species

The purpose of this laboratory study was to determine if larvae of other native fish species are also sensitive to 2,4-D exposure and, if so, whether there was a link between closely related fish species and their sensitivity to 2,4-D exposure.

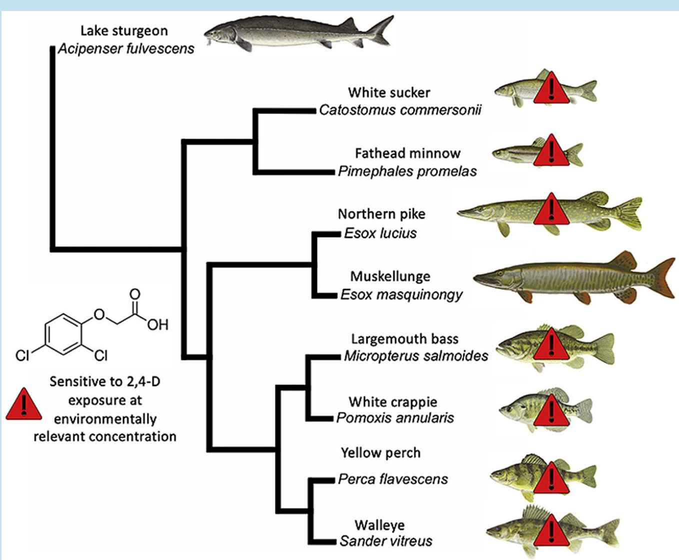

Figure 2. Phylogenetic tree showing the evolutionary relationships among 8 fish species tested in the study (Dehnert et al. 2021).

Research Methods

- Eight freshwater fish species were exposed to a commercially available 2,4-D formulation as embryos, larvae and juveniles.
- Fish were exposed to either 0, 0.05, 0.50, or 2 ppm 2,4 -D, which is below the maximum application rate allowed in Wisconsin lakes.
- Survival was monitored weekly. Surviving embryos, larvae and juveniles were assessed for deformities and growth and compared to fish from untreated control tanks.

Summary of Results

- Six out of the eight species showed a decrease in embryo or larval survival after 2,4-D exposure. Combined with the previous research on fathead minnow larvae, at least seven native Wisconsin fish species were observed to be sensitive to 2,4-D exposure during early life stages.
- Affected species weren't always closely related to other affected species (Fig. 2), meaning that 2,4-D can impact a wide variety of fish communities.

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Zebrafish and Yellow Perch Larval Survival and Behavior

After the previous laboratory studies showed impacts from 2,4-D exposure on larval survival for multiple native Wisconsin fish species, researchers wanted to know if behaviors essential to larval survival would also be affected by 2,4-D exposure.

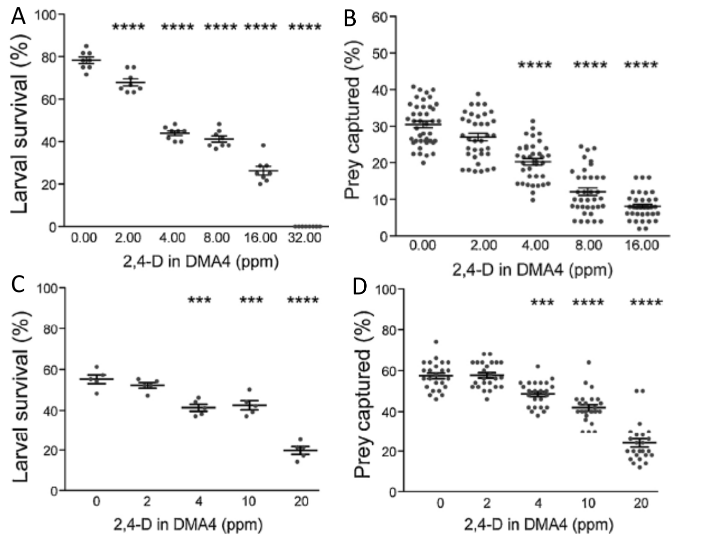

Figure 3. Larval survival and prey capture in zebrafish (A & B) and yellow perch (C & D); (Dehnert et al. 2019).

Research Methods

- In laboratory tanks, zebrafish (*Danio rerio*) and yellow perch (*Perca flavescens*) embryos and larvae were exposed to a range of ecologically relevant concentrations of either pure 2,4-D or a commercially available 2,4-D formulation. Survival and deformities were measured daily.
- The larvae were then evaluated on their prey capture success rate. Nervous system function and other visual– and motor-based skills essential to larval survival were also measured in zebrafish larvae.

Summary of Results

- Exposure to both pure 2,4-D and a 2,4-D commercial formulation impaired prey capture and reduced survival in both zebrafish and yellow perch larvae (Fig. 3).
- Larval survival followed a dose response curve pattern, meaning higher concentrations of 2,4-D resulted in decreased larval survival.
- Zebrafish larvae exposed to 2,4-D during visual system development had normal motor skills but impaired visual skills, suggesting that 2,4-D may affect visual system development in larvae.

Yellow Perch Associative Learning

Building on previous research, which showed feeding behavior impairments in zebrafish and yellow perch larvae, this study sought to home in on the mechanism of impairment in yellow perch and see if the same results would be found in the next early life stage (i.e., juveniles).

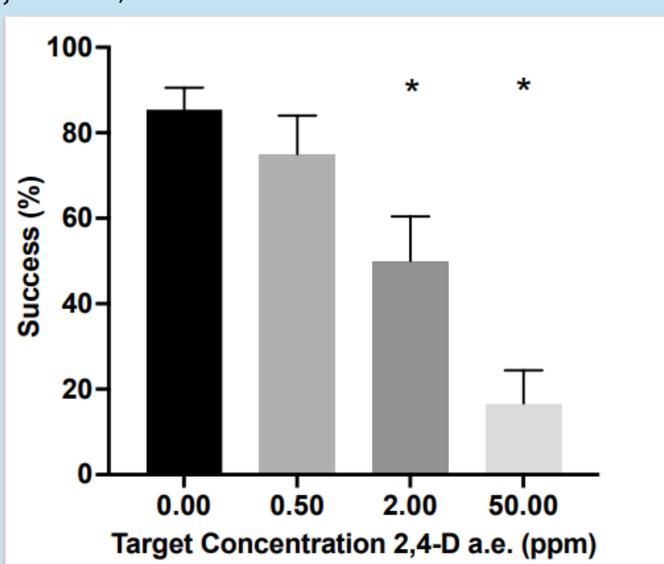

Figure 4. Feed associated learning behavior success rate of juvenile yellow perch exposed to different concentrations of 2,4-D. (Anton et al. 2021).

Research Methods

- Juvenile yellow perch (*Perca flavescens*) were exposed to a 2,4-D commercial formulation at 0, 0.5, 2 and 50 ppm 2,4-D.
- The fish were then tested on their ability to perform a food-based visual learned behavior, which is a proxy for how well the fish can hunt for prey.
- Food consumption, startle response and locomotion were also measured to see if any of those mechanisms could explain the results of the learned behavior test.

Summary of Results

- Juvenile yellow perch were significantly worse at completing the learned behavior when exposed to 2 and 50 ppm 2,4-D (Fig. 4).
- There was no difference in food consumption, startle response, or locomotion between exposed and unexposed fish.
- These results suggest that it could be more difficult for juveniles exposed to these concentrations of 2,4-D to hunt for prey in the wild, although the mechanism causing the impairment is still unknown.

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Fish and Zooplankton Community Response

In 2012, DNR fisheries biologists and scientists noticed failures in fish recruitment (the process of young fish transitioning to older life stages) on Lake Ellwood, WI. The lake had an extensive history of 2,4-D treatments, which previous laboratory studies showed can negatively influence early life stages of fish. Researchers monitored the fish and zooplankton communities in Lake Ellwood after herbicide treatments ceased in 2013 to test whether the communities would rebound and to potentially identify causes of the recruitment failures.

Photo credit: Bob Korth

Research Methods

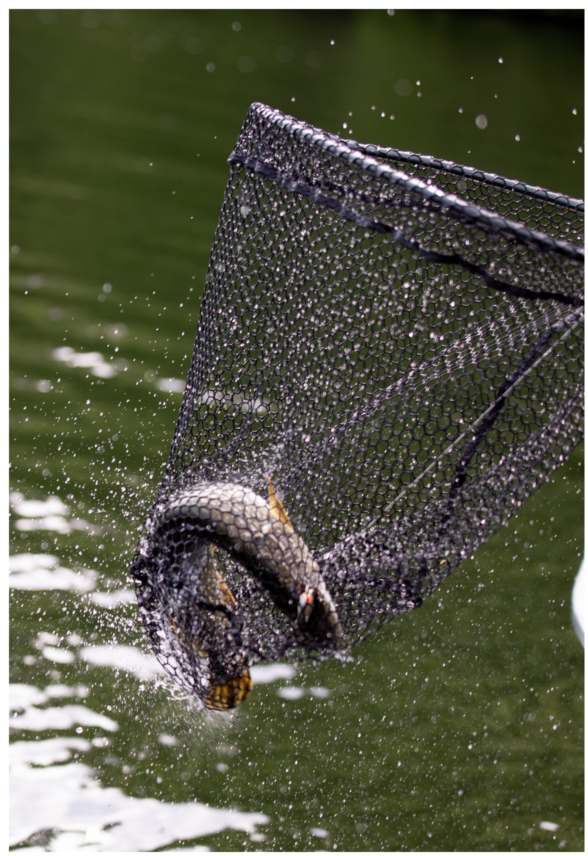
- Adult and juvenile fish were sampled in Lake Ellwood during 2013—2019. The data collected were used to measure natural recruitment and adult/juvenile relative abundance. Zooplankton density was also measured.
- Fish and zooplankton community compositions were then compared between Lake Ellwood and two reference lakes with no prior herbicide treatments.

Summary of Results

- Relative abundances of several fish species at early life stages rapidly rebounded after treatments ceased, suggesting that years of continuous 2,4-D treatments may have been hampering fish recruitment in Lake Ellwood. Zooplankton density also rapidly increased then stabilized to reference levels.
- Aquatic vegetation density and water quality parameters did not change in Lake Ellwood post-treatment. Declines in the fish and zooplankton communities pre-2013 were likely not due to habitat loss from the chemical treatments.

Potential Applications

Altogether, the results of these studies suggest that exposure to 2,4-D, even at concentrations legally permitted by the Environmental Protection Agency for use in Wisconsin lakes, can have a negative effect on the survival, behavior, and cognition of early life stages of fish. Not only can exposure to 2,4-D directly affect survival of fish larvae themselves, but it can also negatively influence skills that are vital for the survival and wellbeing of early life stages of fish, such as hunting for prey and predator avoidance. Researchers also found that 2,4-D can negatively influence a wide variety of fish species. Scheduling the timing of 2,4-D treatments after fish have completed spawning and larval growth stage development may be beneficial in minimizing non-target influences on these organisms. In addition, using an integrated pest management (IPM) approach, which considers the use of all available management options including non-chemical management strategies, can also help guide future aquatic plant management activities in a way that minimizes harm to aquatic ecosystems and ensures long-term protection of Wisconsin's natural resources.



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Related Publications and Resources

Anton, B.J. et al. 2021. Subchronic impacts of 2,4-D herbicide Weedestroy®AM40 on associative learning in juvenile yellow perch (*Perca flavescens*). Aquatic Toxicology 237:105909.

DeQuattro, Z.A. and Karasov, W.H. 2016. Impacts of 2,4-Dichlorophenoxyacetic acid aquatic herbicide formulations on reproduction and development of the fathead minnow (*Pimephales promelas*). Environmental Toxicology and Chemistry 35(6):1478-1488.

Dehnert, G.K. et al. 2018. Effects of low, subchronic exposure of 2,4-Dichlorophenoxyacetic acid (2,4-D) and commercial 2,4-D formulations on early life stages of fathead minnows (*Pimephales promelas*). Environmental Toxicology and Chemistry 37(10):2550-2559.

Dehnert, G.K. et al. 2019. 2,4-Dichlorophenoxyacetic acid containing herbicide impairs essential visually guided behaviors of larval fish. Aquatic Toxicology 209:1-12.

Dehnert, G.K. et al. 2021. Impacts of subchronic exposure to a commercial 2,4-D herbicide on developmental stages of multiple freshwater fish species. Chemosphere 263:127638.

Rydell, N.J. 2018. Effects of 2,4-D herbicide treatments used to control Eurasian watermilfoil on fish and zooplankton in northern Wisconsin lakes. Master's Thesis. University of Wisconsin, Stevens Point, Wisconsin.

Rydell, N.J. et al. 2019. Effects of 2,4-D herbicide treatments used to control Eurasian watermilfoil on fish and zooplankton in northern WI lakes. Presentation at Wisconsin Lakes and Rivers Convention.

Schleppenbach, B.T. et al. 2022. Fish and zooplankton community responses to the cessation of long-term invasive Eurasian watermilfoil (Myriophyllum spicatum) chemical treatments in a north-temperate, USA Lake. Fishes 2022, 7(4):165.

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