NEONICOTINOID BIOACCUMULATION IN BLUEGILL AND BULLHEAD WITHIN LAKE JULIA AND LAKE ZUMWALDE

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***Abstract*—Brown Bullhead *Ameiurus nebulosus*, Yellow Bullhead *Ameiurus natalis,* and Bluegill *Lepomis macrochirus* are unique in their stark differences. Bullhead are scaleless bottom feeders that can tolerate high pollution and low O_2 conditions. In contrast, Bluegill are scaled, aggressive nesters and are less tolerant of high pollutants or low-oxygen conditions. Among these pollutants include a class of chemicals called neonicotinoids (hereafter referred to as neonics). Neonics are a class of insecticides primarily used within agriculture and pest management. Due to the effectiveness, application has escalated on a global scale. They have been well documented by many toxicology assessments. Although these compounds are known endocrine disruptors in fish, little research has examined them in Minnesota lakes. The objective of this study was to determine neonic concentration in Bullhead and Bluegill livers. A secondary objective of this study was to test whether pollution levels influence neonic concentration in Bullhead and Bluegill livers. An ELIZA kit was used on fish livers to evaluate whether pollution levels predict neonic concentration. Neonics were detectable in Yellow & Brown Bullhead from both study areas ($P < 0.22$). Bullhead imidacloprid concentration was 2.03 ± 0.90 ng/g (mean ± SD). Neonics were also detectable in Bluegill from both study areas ($P < 0.017$). Bluegill neonic concentration was 3.05 ± 0.53 ng/g (mean ± SD). Laboratory analyses have demonstrated that neonics can be detected in freshwater ecosystems located in Minnesota. The data found in this study suggests that pollution levels play an important role in neonic bioaccumulation.**

INTRODUCTION

Neonics are a class of chemicals used as insecticides. These chemicals were first developed in the 1980s, and their use became more prevalent in the 1990s. As the name suggests, neonics are related to the chemical nicotine with some minor differences. Neonics are nicotinic acetylcholine receptor agonists (nAChRs), and they bind robustly to nicotinic acetylcholine receptors in the nervous system of insects. This causes nervous system stimulation at low concentrations. When exposed to higher concentrations, neonics can cause insects to go into paralysis, which ultimately results in death (Goulson 2013).

Neonics bind more strongly to insect nicotinic acetylcholine receptors (nAChRs) when compared to vertebrate receptors. This means that neonics are overall more toxic to invertebrates (Goulson 2013). As a result of these factors, neonic use has become prevalent in pest management, agriculture, horticulture, forestry, aquaculture, and urban household pest control products. In the United States, 79–100% of all corn acres and 34–44% of soybean acres are also planted using neonic coated seeds. Despite its extensive application in agriculture, the use of neonics in the United States is strongly upheld in the biotic environment (Douglas and Tooker 2015).

Freshwater invertebrates are generally more sensitive to pesticide exposure than terrestrial invertebrate counterparts (Krupke and Tooker 2020). A shocking example in recent history can showcase the destructive nature of neonics. From 1993 to 2019, neonic application and biotic effects have been documented in Lake Shinji, Japan. Analysis from Yamamuro et al. (2019) revealed an 83% decrease in zooplankton biomass. Severe reduction of zooplankton has resulted in a complete population crash of the Japanese Smelt *Hypomesus nipponensis*. Before 1993, the annual smelt harvest reached about 240 tons. By the late 2000s, this figure had fallen to just 22 tons in a single year. (Yamamuro et al. 2019).

Many lakes in Minnesota share similar agricultural watershed characteristics seen in Lake Shinji, Japan. It is possible that pesticides may be affecting fish-based index of biological integrity (FIBI) scores through direct and indirect influence. Indirect influence tends to be a more common trend with pesticide application. Reduction of freshwater invertebrate prey for insectivorous fish can lead to reduced fish abundance. Knowing these factors are vital when revolving around aquatic ecosystem considerations. Having healthy and balanced native fish populations are of cultural, economic, and social importance to the constituents of Minnesota (MDA 2019).

The effects of neonics continue to become less of a mystery. In 2013, a joint toxicity assessment of acetamiprid and thiamethoxam, two neonics, was conducted for the species Asian Carp *Catla catla*. In this assessment, one group of fish was exposed to neonic concentrations in water, and the other group was not. After 96 hours, blood and gill tissue samples from both groups were collected and analyzed. The results showed that fish exposed to neonics will have significant drops in their plasma protein levels, plasma electrolyte levels, and a decrease in gill ATPase activity. A decrease in ATPase activity within the gills leads to oxidative stress. The reduction of plasma proteins and electrolytes increases physiological stress in fish. As a result of neonic exposure, osmoregulation in *Catla catla* becomes significantly more difficult (Veedu et al. 2022).

Neonics have been proven to persist in the environment in many forms. They have been found in soil, dust, wetlands, groundwater, nontargeted plants, vertebrate prey, foods common to the American diet, which includes wild and aquacultured marine species (Cimino et al. 2016). Humans are now at the front lines when it comes to neonic exposure. A study conducted by (Zhang and Lu 2022) has quantified pesticide concentration in human urine. Concentrations ranging between 1.1 to 5.6 ng/mL have been found in various countries and age groups across the globe. These findings have led many experts to question the integrity of pesticide application on the landscape. Present case studies have correlated health risks with neonic exposure. Some of these health risks include the following: Disruption of insulin and glucose homeostasis in adults non-diabetic, decreased sperm motility among healthy men, increased serum lipid molecules, and urinary oxidative stress. These established factors have sparked widespread controversy and debate amongst the public. Neonicotinoids are an issue that is increasingly gaining global attention (Zhang and Lu 2022).

The primary objective of this study is to determine if neonicotinoid (imidacloprid) concentrations exist within Brown Bullhead *Ameiurus nebulosus*, Yellow Bullhead *Ameiurus natalis,* and Bluegill *Lepomis macrochirus* livers. A secondary objective of this study was to test whether pollution levels influence neonic concentration in Bullhead and Bluegill livers.

METHODS

Lake selection. — Two Minnesota lakes have been selected for this study that have large variations in characteristics such as trophic status and contributing watershed land use and size. Lake Julia (04016600; MNDNR 2025; Map 1) was selected for having a less productive trophic status and its relatively small contributing watershed with minimal land use identified as cultivated agriculture. Alternatively, Lake Zumwalde (73008900; MNDNR 2025; Map 2) was selected for having a much more productive trophic status and its relatively large contributing watershed with a relatively high percentage of land use identified as cultivated agriculture.

Lake Julia is a 206.9-hectare lake with 3.9% of the surrounding land cover being influenced by agricultural cultivation (Homer et al.). This lake serves as a control variable, representing a minimally disturbed aquatic system. Lake Zumwalde is a 51.84-hectare lake that is heavily influenced by agricultural disturbance. Approximately 68.4% of the lakes surrounding land cover is influenced by cultivation (Homer et al. 2012). This lake lies on the Sauk River Chain of Lakes and is hyper eutrophic. This lake serves as the dependent variable, representing a disturbed aquatic system.

Sample harvesting. — Finding the concentration of neonics required the harvesting of Brown Bullhead, Yellow Bullhead, and Bluegill. This was done through strategic angling on both lakes. A Hummingbird Helix 5 was employed to detect the presence of Bullhead and Bluegill. When angling, handmade glow in the dark jigs were utilized on both lakes. These jigs were strategically selected to help aid with the catch and harvest of Bullhead. Bullhead are bottom feeders and are known to have obsolete retinas. Using glow in the dark lures helped enhance whatever visual ability the Bullhead possessed. Bullhead are distinguishable and known for their gifted barbels. Barbels can track various vibrations and scents within the benthic zone. Based on these considerations, garlic-scented night crawlers were affixed to the hook. When angling, an ultralight rod was used with a Shimano Sienna 2500 reel. 3.629 kg test Berkley Trilene line was used to withstand the strength of Bullheads. Both Bullhead and Bluegill were captured between depths of 2.13m and 4.57m.

Brown Bullhead from Lake Julia and Lake Zumwalde were primarily captured from the hours of 2000-0200. Bluegill were caught from the hours of 1600-2000 on both lakes. Eight Brown Bullhead and eight Bluegill were angled from Lake Julia (47°40.532'N, 94°53.581'). Eight Yellow Bullhead, and eight Bluegill were angled from Lake Zumwalde (45°26.212'N 94°29.528'W). Once the fish were caught, total lengths were measured for all twenty-four fish. The Bullhead and Bluegill were then neutralized humanely by a swift blow to the cranium severing the caudal vein. After mortality was achieved, all fish were frozen immediately for preservation purposes. It is important to note that all sampling and collection were carried out in accordance with all 2026 Minnesota fishing regulations.

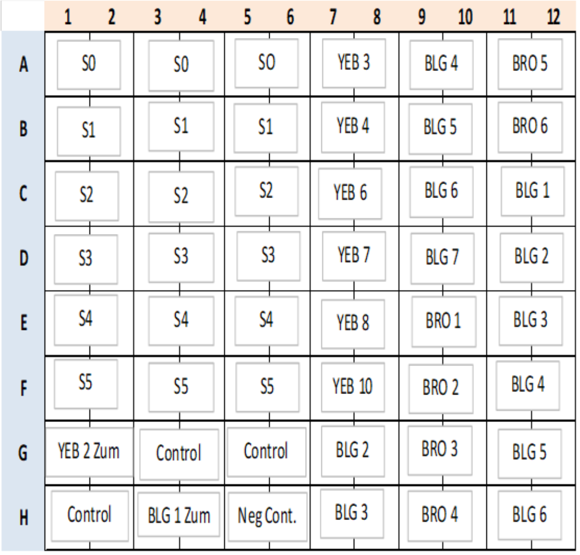
Map 1. Aerial view of where sampling occurred on Lake Julia. Brown Bullhead and Bluegill samples were obtained from this area. Eutrophication is not present from agricultural practices (04016600 MNDNR 2025).

Map 2. Aerial view of where sampling occurred on Lake Zumwalde. Yellow Bullhead and Bluegill samples were obtained from this area. Eutrophication and disturbances from agricultural and practices are noticeable (73008900 MNDNR 2025).

Solid Phase Extraction (SPE). — The next step involved thawing the fish from both lakes. Once thawed, all fish livers were removed using a scalpel. The masses of the livers were taken immediately after extraction. Livers were then grouped to their respective species and lakes before being refrozen. Before testing for pesticides, Solid Phase Extraction (SPE) was a requirement for sample preparation. To accomplish this, an SPE and ELIZA detection procedure needed to be modeled from existing literature. A study conducted by (Frew and Grue 2012) provided a foundational method on how to extract neonics in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Creating procedures for neonic testing needed to be thorough and experimental in order to be successful. Above all, sanitary conditions needed to be present at all times. Maintaining this rigorous integrity prevented cross contamination and matrix effects.

A mortar and pestle were used to crush the individual livers. Once a liquid was obtained, 0.100 g of liver sample was measured and added to its perspective beaker. Phospholipids and proteins need to be removed to prevent matrix effects. To do this, 0.100 g of liver sample was measured from each fish and then homogenized in a detergent called Triton X-100. Before adding Triton X-100, a blend of (4.0 mL of 20.0 mM Triton X-100) needed to be created. A ratio of 0.100 g liver tissue to 5 mL Triton X-100 was used for homogenization. Once the homogenate was mixed, an incubation period of 6 hours at 50–55 °C was required. After incubation, all samples required general centrifuging. Homogenate supernatant (2 mL) was then added to individual C18 micro spin columns for each sample. All the supernatant liquid would be discarded leaving the C18 spin columns empty. To finish (SPE), 2 mL aliquots of 70% ethanol were centrifuged through each individual spin column. The ethanol was then collected and stored into its proper sample vial. Once centrifugation was complete, all vials evaporated in a fumigation hood for 48 hours. Once the vials were completely dry, all samples were refrigerated for preservation. (Frew and Grue 2012).

ELISA kit testing. — Imidacloprid can be detected using an ELISA kit. The kit provides instructions on how to prepare diluted samples (GSD 2024). The centrifuged liver samples differed from the sample protocol in ELISA kit and required reconstitution. Reconstitution of the samples involved adding 220 µL of sample diluent to evaporated vials. A total of 32 out of 96 wells were used to determine this sample dilution protocol. In addition, 64 wells would be used for the remainder of the test. All standards, controls, negative controls, and fish samples would be tested in duplicates to ensure precision (Figure 1).



	1 2	3 4	5 6	7 8	9 10	11 12
A	S0	S0	S0	YEB 3	BLG 4	BRO 5
B	S1	S1	S1	YEB 4	BLG 5	BRO 6
C	S2	S2	S2	YEB 6	BLG 6	BLG 1
D	S3	S3	S3	YEB 7	BLG 7	BLG 2
E	S4	S4	S4	YEB 8	BRO 1	BLG 3
F	S5	S5	S5	YEB 10	BRO 2	BLG 4
G	YEB 2 Zum	Control	Control	BLG 2	BRO 3	BLG 5
H	Control	BLG 1 Zum	Neg Cont.	BLG 3	BRO 4	BLG 6

Figure 1. Testing protocol schematic used for ELIZA kit testing.

The first step of running the test involves adding the standards, control solutions, and negative controls to the wells. Next, the unknown liver samples were added with a known imidacloprid concentrate. Once the samples were placed in the well, the known imidacloprid concentrate, and the unknown fish liver concentrate competitively inhibited. The color intensity of each well corresponded to the concentration present. A yellow coloration indicated low absorbance in the fish livers. If a clear color was seen, an indication of high imidacloprid absorbance was present in the livers. Once the test was complete, a plate reader was used to determine imidacloprid absorbance. From here, an imidacloprid calibration curve was constructed in Excel (Figure 2).

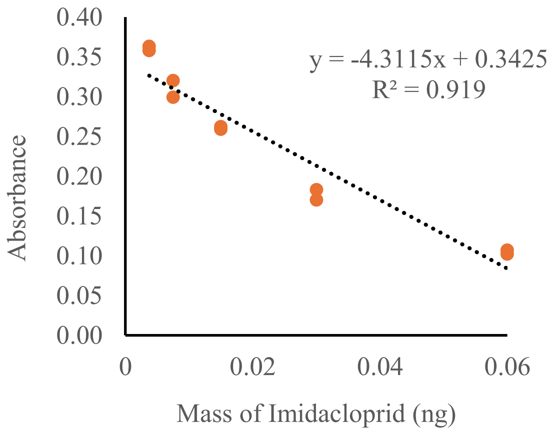


Figure 2. Standard calibration curve constructed for stoichiometric calculations.

RESULTS

Bullhead results. — The results from Brown Bullhead in Lake Julia and Yellow Bullhead in Lake Zumwalde demonstrated a small contrast. Bullhead from Lake Julia had lower imidacloprid concentration. On the other hand, Bullhead from Lake Zumwalde had slightly higher imidacloprid concentration ($P < 0.22$; Figure 3). Bullhead imidacloprid concentration was 2.03 ± 0.90 ng/g (mean ± SD; Figure 4).

Bluegill results. — Bluegill from Lake Julia and Lake Zumwalde demonstrated slight variation. Bluegill from Lake Julia had a low neonic concentration. Bluegill from Lake Zumwalde had a slightly higher neonic concentration ($P < 0.017$; Figure 3). Bluegill neonic concentration was 3.05 ± 0.53 ng/g (mean ± SD; Figure 4).

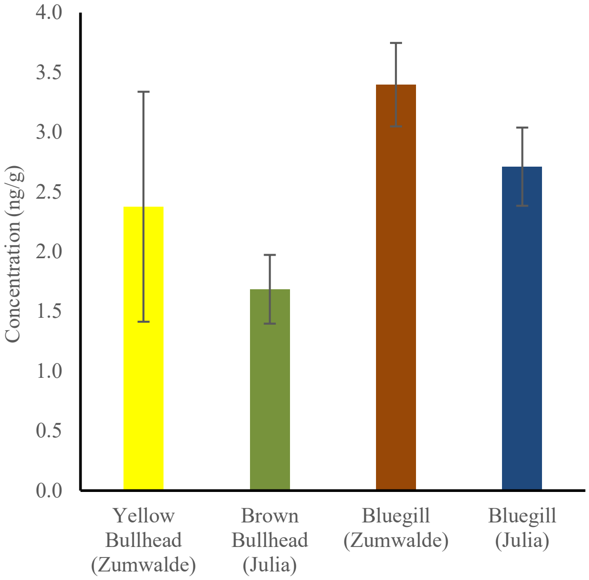


Figure 3. Average liver concentrations of imidacloprid found in each fish species (ng/g).

DISCUSSION

This study found evidence of neonic bioaccumulation in fish from Lake Zumwalde and Lake Julia. This suggests that neonics persist under a range of environmental conditions. The presence of neonics in both disturbed and undisturbed lakes from agriculture suggests that contamination may be more widespread than previously hypothesized. Notably, concentrations detected in Lake Zumwalde and Lake Julia were lower than the numbers reported from the Owena River Basin in Nigeria (Adegun et al. 2020). In that study, the authors documented measurable neonic levels across six fish species, including the African sharptooth catfish (*Clarias gariepinus*). Overall, neonic concentrations in their samples ranged from 0.09 to 0.63 nmol/g (≈10–160 ng/g) across all species examined. In this study, Bluegill from Lake Zumwalde exhibited higher concentrations than Bluegill from Lake Julia. Yellow Bullhead from Lake Zumwalde exhibited higher concentrations than Brown Bullhead from Lake Julia. This pattern alone suggests pollution levels do influence the bioaccumulation of neonics within fish.

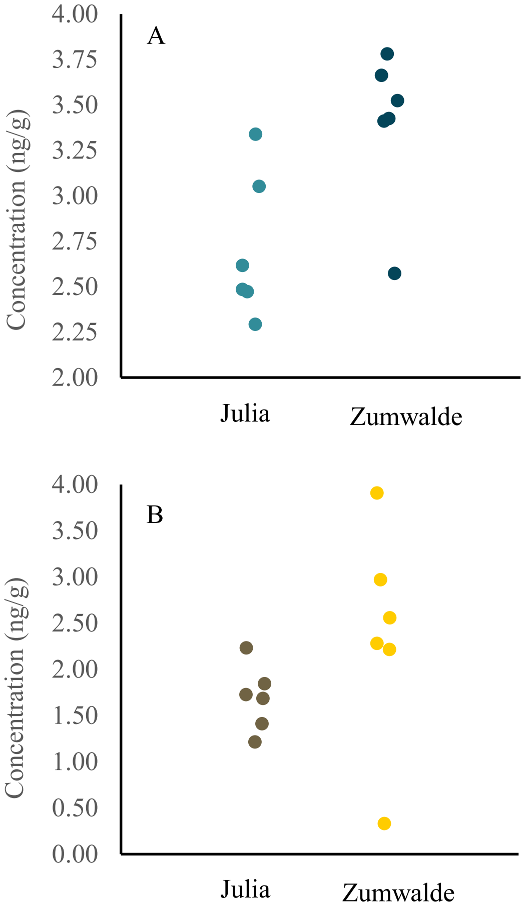


Figure 4. Measurements of imidacloprid concentration (ng/g) in the livers of (A) 12 Bluegill and (B) 12 Bullhead captured from Lake Julia and Lake Zumwalde in Fall of 2025.

Another important factor to consider is behavioral characteristics. Neonics are primarily absorbed through the gills, making habitat use and activity patterns key determinants of exposure (Xu et al. 2023). Gill absorption may vary depending on environmental conditions and species behavior. Bullhead typically inhabit murky and sandy substrates within warm water. As benthic bottom feeders, they congregate in areas with low water movement. Bullhead have the ability to tolerate polluted and low-oxygen environments. Similarly, Bluegill favor shallow, vegetated areas and weedy bays of smaller lakes. As nesters, they tend to favor warm water with mild turbidity. Bluegill will inhabit murky and sandy substrates located within lakes and rivers. Bluegill are less tolerant of polluted and low-oxygen environments (Becker 1983). Collectively, differences in morphology, behavior, and habitat use can influence the degree of neonic bioaccumulation among observed species.

Age differences may also contribute to bioaccumulation. In Minnesota, Bluegill can live up to fourteen years (Tomcko and Pierce 2001). Brown Bullhead can live up to nine years (Rubec and Quadri 1982), and Yellow Bullhead can live up to seven years (Becker 1983). Age differences between the three species could affect overall neonic bioaccumulation. Future studies should include aging both species to further examine this relationship.

Brown Bullhead and Bluegill from Lake Julia had a low concentration of neonics. Remarkably, neonics still find their way into undisturbed systems. While the levels are not high, this could become a public health concern. Seeing these results questions the integrity of other lakes in Minnesota. When comparing Lake Julia to Lake Zumwalde, the differences in concentration levels were not as sharp as predicted. Agricultural runoff is common in Lake Zumwalde but largely absent in Lake Julia. The small contrast between these two systems highlights a potential pattern of pollution influence. If this trend holds true, northern Minnesota lakes may be less susceptible to neonic contamination than those in the southern region. This study also indicates that agricultural pollution may not be the sole factor influencing neonic concentrations.

Increasing sample sizes and incorporating a broader range of species in future studies could enhance the robustness of findings. Expanding the number of lakes examined would further strengthen spatial comparisons. Additionally, analyzing other tissues such as scales, whole body homogenates, gills, blood, and plasma alongside the liver could provide deeper insight. This broader tissue approach may reveal organ-specific differences that are overlooked when solely focusing on liver analysis. Tag and recapture methods, combined with hormonal monitoring, could yield important information about sublethal and physiological responses to exposure. Overall, this project has established a strong foundation for understanding neonics in Minnesota lakes. Continued research is essential to uncover the far-reaching impacts of neonicotinoid contamination on fish, human health, and ecosystems worldwide.

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