Analysis of Hematocrit Levels in Walleye *Sander vitreus* from Similar Waterbodies

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Walleye *Sander vitreus* are a popular fish among anglers and hold a prominent place in Minnesotan's identity as their state fish. Because of this, multiple management practices are used to ensure the continued health of their populations within Minnesota waters. In this study, the process of analyzing hematocrit levels was used to determine if the analysis of hematological parameters could be a useful tool in detecting differences in population health. To evaluate this, blood samples were acquired from age-0 and age-1 fish from two similar lakes and run through a hematocrit centrifuge to separate platelets from plasma to read the hematocrit percentage. The results from these samples were analyzed using a two-sample T-test. There was a significant difference between the average hematocrit levels of 42.7% in Lake Bemidji and 36.2% in Lake Plantagenet ($P < 0.01$). The cause of which has not yet been determined and will require additional research.

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Introduction

In Minnesota, Walleye are a species actively managed by Natural Resource Departments due to its assignment as the state fish in 1965 and its high popularity among anglers. Because of its high demand, different management techniques are implemented to maintain the stability and integrity of Walleye populations in Minnesota, including but not limited to supplemental stocking and yearly population assessments via age-0 survival rate estimates and CPUE via gill and trap net surveys (Jennings et al. 2005). However, a possible more in-depth way to measure the health of a population is through the measurement of hematocrit, the packed red blood cell (RBC) volume in a sample of blood. Levels of hematocrit are often connected to environmental and physiological factors such as oxygen availability, food consumption, stress levels, and outside influence from other organisms. Hematocrit levels have been seen to decrease when fish are exposed to prolonged starvation and abnormally high-water temperatures and increase rapidly alongside increasing levels of cortisol, the stress hormone. Therefore, it may be valuable for researchers to have the ability to evaluate hematocrit levels as part of hematological parameters, as it would allow insight into the health and wellbeing of a singular organism or an entire population and help detect changes to their environment around them (Rios et al. 2004, Sheikh and Ahmed 2016, Zeigeweid and Black 2010).

Lake Bemidji and Lake Plantagenet are both lakes greater than 400 hectares within the Mississippi River system in Northern Minnesota, with a Walleye population managed by the MNDNR. Similar in depth, (max depth 23.2 m in Bemidji and 19.8 m in Plantagenet) and water clarity (1.5 and 1 m, respectively), these lakes are on the same Walleye stocking schedule, both stocked with fry every other year. Both lakes also have a Walleye catch rate/gill net well above the ranges found in similar lakes and are popular lakes for anglers, producing higher annual Walleye harvest rates (MNDNR 2021). Though similar in these regards, Lake Bemidji exists within a highly populated urban area with five boat access launch points for anglers, and Lake Plantagenet is within a rural area and is only boat accessible through one access point. The objective of this study is to analyze the hematocrit levels in age-0 and age-1 Walleye sampled from these separate waterbodies to determine if the environments in which they live are different enough to cause observable hematological change.

Methods

Walleye were collected from Lake Bemidji and Lake Plantagenet via boat electrofishing on September 14th and 16th, 2021, and were measured while on the water to determine age class with assistance from MNDNR biologists. Age-0 and age-1 Walleye were then separated into respective containers and transported to the lab. Fish were then moved to prepared tanks with oxygen bubblers and

water movement. Blood samples were collected as soon as possible and within 24 h after arrival at the lab. Total length and wet weight measurements were recorded for each Walleye before blood collection. Fish were placed in a ventral position and a small puncture was made just underneath the V where the gill flaps meet the body. After wiping away the first layer of blood, gentle pressure was applied to aid blood flow. Fish were held horizontally, and an open end of a 75 mm heparin-lined micro-hematocrit tube was gently pushed into the incision site. Blood began to flow into the tube via capillary action. The tube continued to fill until it was at least 3/4ths of the way full. Once full, the tube was immediately sealed with a wax sealing medium.

The filled hematocrit tube, seal pointing outward was placed into a hematocrit centrifuge and balanced with either an empty micro-hematocrit tube or another blood-filled tube on the direct opposite side. The inner cover was locked in place over the tubes by turning the dial so the lines were perpendicular to the inner cover. The outer cover was then closed, ensured by an audible click. The centrifuge was run for five minutes and allowed to fully stop on its own before covers were opened. After centrifuging, the blood samples had separated with red blood cells collected at the bottom of the tube, and plasma at the top.

The hematocrit percentage was read using a hematocrit reader table by lining up the top of the wax seal with the bottom line of the table, and the meniscus of the plasma with the top of the table. Following the line on the table that meets with the RBC meniscus, a hematocrit percentage was provided on the side of the table. All percentages were recorded and run through a two-sample t-test to determine if the difference between samples was statistically significant.

Please note that in this study, the fish samples were returned to the local DNR office for use in their population assessments. All fish sampled were bagged and frozen before being returned for DNR research purposes and no methods of non-lethal blood sampling were explored.

Results

The average hematocrit levels for the two sampled lakes were 42.7% in Lake Bemidji and 36.2% in Lake Plantagenet ($P < 0.01$; Figure 1). This difference in hematocrit levels is not likely related to differences in condition between the two sampled populations (Figure 2).

Discussion

There is no current agreed-upon baseline for what an optimal hematocrit level is within Walleye.

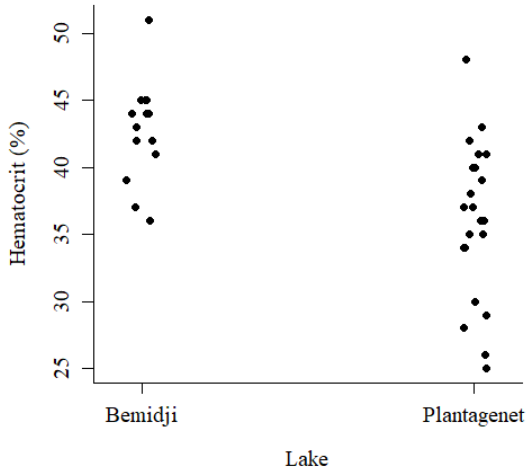

Figure 1. Average hematocrit levels within Bemidji and Plantagenet in age-0 and age-1 Walleye. 42.7%, 36.2% ($P < 0.01$).

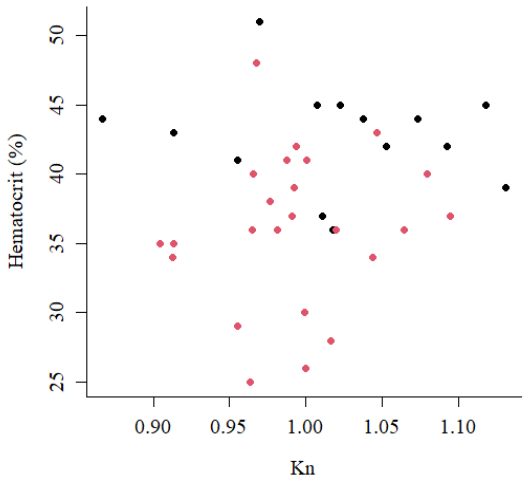

Figure 2. Kn estimates for sampled populations within Lake Bemidji and Plantagenet. Black – Bemidji, Red – Plantagenet.

There are no current studies that imply either of the hematocrit levels within these populations are inherently unhealthy and the average hematocrit levels seen in Lake Bemidji and Lake Plantagenet are similar to other optimum levels observed in other species. Atlantic salmon *Salmo salar* have an observed optimal hematocrit of 44-49%, Trout (*Salvelinus fontinalis, Salmo trutta, Oncorhynchus mykiss*) optimal levels are 39-53% depending on species, and catfish *Clarias sp.* optimal levels range from 30-45% (Sandnes et al. 1988, Sneizko et al. 1960, Yanuhar et al. 2021). Compared to these studies, the average levels of 42.7% and 36.2% fall

well within other observed ranges, however, they still significantly differ from each other.

There is a multitude of factors that may play into why the hematocrit levels between Lake Bemidji and Lake Plantagenet differ from one another. One of the possible factors that may be attributed to this being the aforementioned higher levels of urbanization surrounding Lake Bemidji, and more specifically, the noise pollution that may be created. Because Lake Bemidji has more boat access points, higher population density, and closer proximity to major roads, the threat of noise pollution and therefore chronic noise stress on fish populations within the waterbody is higher than within the waters of Lake Plantagenet. In marine studies on Sea Bass *Dicentrarchus labrax* and Gilthead Sea Bream *Sparus aurata*, the impact of acoustic stimulus and noise pollution on physiological parameters was assessed by Buscaino et al. (2010) and Celi et al. (2015). The results from these studies showed that as fish were exposed to the noise frequency produced by water vessel traffic, levels of both cortisol and hematocrit were largely increased. The production of cortisol is a stress response which in turn increases hematocrit levels. Though the environmental and physiological effects of prolonged vessel noise pollution on underwater life has been explored in marine systems, very little research has been conducted on freshwater populations. Recorded underwater decibel levels from both waterbodies would need to be evaluated to confirm or reject this possibility.

Another stressor that may cause this difference in hematocrit levels is also connected to Lake Bemidji's proximity to urban areas and more specifically, major roadways. Considering the northern location of these lakes, winter months are times of road salting, often using an 8-10% salt sand mix. As the weather warms and snow melts, runoff from imperviable surfaces such as roadways and sidewalks that have accumulated salt concentrations over the winter drain through storm drains or directly off roadways and bridges into the lake system. The higher introduction of salt into Lake Bemidji may influence physiological factors that could alter hematocrit levels. Increases in salt levels can interfere with osmo/ionregulation, metabolism, increase the activity of the antioxidant defense system and cause issues in regulating plasma and blood glucose (Meland et al. 2010, Tollefsen and Teien 2015). This not only would affect the metabolic and antioxidant defense system, causing hematocrit levels to increase to counteract the higher usage of energy and oxygen, but also cause stress, leading to a rise in cortisol levels. Lake Plantagenet has less proximity to major roads and paved impermeable roadways, lessening the amount of salt-containing runoff directly entering the system.

Though the above-mentioned physiological stressors have the possibility to cause the differences in average hematocrit levels within the sampled population from Lake Bemidji and Lake Plantagenet, more research involving aquatic noise pollution, water chemical analysis, and continued hematocrit sampling will be needed to fully determine the cause.

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