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SPATIAL AND TEMPORAL VARIABILITY IN POST-LARVAL YELLOW PERCH DENSITY

by

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Steve Hauschildt

Yellow Perch *Perca flavescens* are important as forage for other species and for sport fishing in many northern lakes. However, estimating post-larval Yellow Perch populations can be difficult because of many environmental factors that cause unexplained variation. The objective of this study was to help reduce unexplained variation by determining a post-larval Yellow Perch population density estimate on Blackduck Lake, MN, provide guidance to help determine the number of trawls required for trawling-based recruitment indices to achieve varying levels of precision, and test for the effects of wind speed and direction on post-larval Yellow Perch spatial variability. This study estimated a density of 0.45 fish/m^3 (0.58 SD) during the sampling period (26 Jun – 07 Jul 2017). It was determined that between 10-15 trawls produced a precise density estimate; however, trawls should be taken over multiple days in varying wind speeds to avoid over/under estimation. Trawling should also be performed in-line with wind direction to ensure non-bias estimates are calculated from both upwind and downwind sectors. This study determined wind speed and direction had a significant influence on the distribution of post-larval Yellow Perch, as more fish were caught in the downwind sector until winds reached 15 kmph. At 15 kmph, fish densities were equal in the upwind and downwind sectors of the lake. Wind did not have a significant influence though on how post-larval Yellow Perch were distributed by total length. During high wind events, more fish were found in the upwind sector of the lake, suggesting that post-larval fish are being moved laterally out of non-towable areas. From the results of this study it appears Yellow Perch must continually relocate back into shallow areas after each high wind event moves them out of the non-trawlable areas, until they are strong enough to resist the wind.

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INTRODUCTION

In many northern lake ecosystems Yellow Perch *Perca flavescens* are an important species for both forage and sport fishing. As a result, many aquatic studies require precise and accurate estimates of post-larval Yellow Perch densities. However, these estimate are difficult to obtain because environmental factors can cause spatial and temporal variation in the fish distribution within a lake (Sogard 1997). Fluctuating environmental conditions during this critical period also ensures that an individual fish has a low chance for survival into adulthood (Sogard 1992; Sogard 1997; Fitzgerald et al. 2004; Forney 2011). Most researchers agree year-class strength is established during the first year of a cohort and that many environmental factors likely affect year class strength (Urho 1996). These environmental factors can cause unexplained variation in estimates of fish density (Hansen et al. 2007; Quist et al. 2009; Brown et al. 2012).

Further complicating matters, is the fact that post-larval Yellow Perch have both pelagic and littoral phases as they develop. Yellow Perch spawn when water temperatures reach 7-13 $^{\circ}$C, and hatch usually occurs approximately 27 days after spawn (Mansueti 1964; Harmon 2011). Newly hatched Yellow Perch are approximately 6 mm in total length (Mansueti 1964). After hatching, larval Yellow Perch will stay in the littoral zone for 1-2 weeks until the yolk sac has been absorbed (Harmon 2011). As larval Yellow Perch continue to develop they move from littoral to pelagic areas to avoid littoral predators and to access pelagic zooplankton (Nordqvist 1914; Noble 1968; Houde 1969; Coles 1981; Dettmers et al. 2005). They typically stay in the pelagic area for 4-8 weeks. This pelagic phase is the optimal time to use larval nets, such as neuston trawls, for sampling in lake ecosystems (Whiteside et al. 1985; Harmon 2011). Post-larval Yellow Perch around 40 mm in length migrate back to the littoral zone in search of larger food (Sissenwine 1984).

During the post-larval pelagic phase, wind speed and direction likely influence spatial variation in larval fish densities. Houde (1969) reported Yellow Perch under 9.5 mm can only maintain position when currents are less than 3.0 cm/sec. A study by Brodnik et al. (2016) further reported that Yellow Perch use the wind as a passive transport to the pelagic area of a lake to feed on zooplankton. As Yellow Perch feed on zooplankton, the wind can create an accumulation of phytoplankton and zooplankton on the downwind side of a lake (Thackeray et al. 2004; Blukacz et al. 2009; Cyr 2017). Since fish are more susceptible to transport by wind during the post-larval phase, this study attempted to determine how wind effects the special distribution and neuston net trawling of post-larval Yellow Perch. Therefore, this study focused on accomplishing three objectives: (1) determine a post-larval Yellow Perch population density estimate on Blackduck Lake, MN, (2) provide guidance to help determine the number of trawls required for trawling-based recruitment indices to achieve varying levels of precision, and (3) test for the effects of wind speed and direction on post-larval Yellow Perch spatial variability.

METHODS

To accomplish these three objectives, a nearby lake was selected that had sufficient numbers of post-larval Yellow Perch available for sampling. Blackduck Lake in Beltrami County located in north-central Minnesota, is 10.87 km^2, contains 5.56 km^2 of littoral area, 19.89 km of shoreline, and has a maximum depth of 8.53 m. An island located in the center of the lake provides some sheltering from the effects of the wind. The Minnesota Department of Natural Resources conducted trap and gill net surveys on Blackduck Lake every 3-6 years from 1983–2012 and found more Yellow Perch than any other species in every survey during this time-period. Additionally, neuston trawls in prior years on Blackduck Lake consistently returned strong numbers of post-larval Yellow Perch for sampling.

Procedures for neuston net operations followed the methods of Brown and Cheng (1981) but were adapted for a modified neuston net measuring 1 x 2 x 4.9 m, constructed with 500 *u*m mesh. Damage to post-larval fish was reduced by controlling the length of tow duration (1 min), speed (4 kmph), and mesh size (Nichols and Thompson 1991; Hopcroft et al. 2005). The boat was maneuvered in a zig-zag pattern to keep prop wash away from the net (Brown and Cheng 1981).

Neuston net sampling took place every other day over a two-week period, from 26 Jun - 07 Jul 17, for six days of total sampling (Figure 1). Each day 56-60 trawls were conducted. All trawls were conducted in a random pattern across Blackduck Lake where water depth was greater than 3 m. Upon completion of the trawl, the net was quickly retrieved to avoid loss of post-larval fish from inside the net. As the neuston net was pulled into the boat, the mesh of the net was dipped in and out of the water to rinse any Yellow Perch into the collection cup. Contents of the collection cup were strained through hand-held nets to remove excess water and placed into 500 mL bottles with 95% ethanol alcohol for later analysis. Fish were counted and up to 30 fish had total length measured from each tow. Latitude and longitude were recorded at the start of each trawl and used with GIS mapping software.

DATA ANALYSIS

To accomplish the first objective of this study, a density estimate was constructed to determine how many fish per m^3 were in Blackduck Lake. To establish a density estimate for Blackduck Lake, the following formulas were used:

$$\text{net area} = 1 \times 2 \text{ m}$$

$$\text{tow speed} = \frac{4000}{60} \text{ m/min}$$

$$\text{tow time} = 1 \text{ min}$$

$$\text{volume (m}^3) = 2 \text{ m}^2 \text{ (net area)} \times 66.67 \text{ m/min (tow speed)} \times 1 \text{ min (tow time)}$$

$$\text{fish density } \left(\frac{\text{fish}}{\text{m}^3}\right) = \frac{\text{fish/trawl}}{\text{net area} \times \text{tow speed} \times \text{tow time}}$$

Average fish density was then calculated for each day of trawling, as well as a total average density estimate that included all trawls from the six days of sampling.

To accomplish the second objective a mean fish density and 95% confidence interval was calculated varying the number of trawls (sample size) from 3 – 120, with the sample size corresponding to the number of trawls randomly selected from the pool of all 354 trawls done in this study. This process was replicated 1,000 times for each sample size. Finally, a mean fish density, mean upper confidence limit (UCL), and mean lower confidence limit (LCL) were calculated from these 1,000 replicates for each sample size. To further clarify the process, the sample size for 3 is below.

$$\text{total trawls} = 354 \text{ neuston net trawls}$$

$$\text{subsample of } 3 = \frac{\text{3 ransom neuston net trawls}}{3} \; and \; 95\% \text{ confidence interval}$$

$$\text{mean fish density of } 3 = \frac{\text{1,000 subsample of 3}}{1{,}000} \; and \; 95\% \text{ confidence interval}$$

process repeated for each sample size from 3 -120

This provided a figure demonstrating the expected level of precision over the range of sample sizes typically used in fish research or management.

To accomplish the third objective, geographic information system (GIS) mapping software was used to show how wind affected post-larval Yellow Perch on Blackduck Lake for visual representation only, not for statistical purposes. A Kriging method took known data from each trawl point and created the best prediction of values in between those known points; this creates a smooth pattern in the GIS map. Kriging was used to create interpolated map layers that visually showed the spatial and temporal variation in fish densities (Figure 2) or mean fish total length within Blackduck Lake (Figure 3). To test for the effects of wind on the daily spatial distribution of post-larval Yellow Perch, five daily time-periods of different lengths were used to calculate mean wind speed and direction. The closest weather instrumentation to Blackduck Lake was located at the Bemidji Regional Airport. Wind speed and direction recorded there were downloaded from the National Oceanic and Atmospheric Administration (NWS 2018). For the first time-period, mean wind speed and direction were calculated from the start of the first trawl to the end of the last trawl, for each individual day. For the three-hour time-period wind speed and direction were calculated from three hours prior to the first trawl, to the end of the last trawl. Mean wind speed and direction were calculated for the 6, 12, and 24-hour time-periods in the same manner, with the hours prior to the first trawl corresponding to the different time-periods. This data was then used to create upwind and downwind sections for Blackduck Lake.

Upwind and downwind sectors of the lake were created to determine if the wind moved more post-larval Yellow Perch into the downwind half of Blackduck Lake. A mean wind direction was created for each time-period, for each day of trawling, as described previously. GIS maps were used to display the mean wind directions, and a center point of the lake was selected (Figure 4). A line for each mean wind direction was then placed on the map, dissecting through this center point. Another line, perpendicular to the wind direction was then added through the center point to create two halves, or upwind and downwind sectors of the lake. As the wind direction changed, the perpendicular line changed with it. Even though each individual trawl point does not change location, the sector it was in could change depending on which half it was located in.

Negative binomial models were then used to determine if wind had a significant effect on the number of post-larval Yellow Perch in each trawl. Six candidate models, one for each of the time periods described previously and an intercept only model, were developed. In all models, other than the intercept only model, fish numbers were a function of wind speed interacting with wind direction. Whether the trawl was in upwind or downwind sector represented wind direction in the model. Akaike's information criterion (AIC) was used to determine the best supported model (Akaike 1973). An AIC score two units lower than other scores was chosen as the best supported model (Richards et al. 2011).

Linear regression was used to determine if wind arranged post-larval Yellow Perch in the upwind and downwind sectors of Blackduck Lake based on fish total length. Six candidate models, including an intercept model, were again developed where mean fish total length at trawl location was a function of wind speed interacting with wind direction which was represented by whether the trawl was done in the upwind or downwind sector. The AIC score that was two units lower than other scores was again the best supported model selected (Richards et al. 2011).

RESULTS

A total of 21,409 post-larval Yellow Perch were sampled from 354 modified neuston net trawls (Table 1). Using all 354 trawls produced a post-larval Yellow Perch density estimate of 0.45 fish/m^3 (0.58 SD). Daily density estimates for 26 Jun – 07 Jul ranged from 0.19 fish/m^3 (0.24 SD) to 0.71 fish/m^3 (0.57 SD) on 03 Jul and 26 Jun, respectively (Figure 5). In addition to determining a density estimate for Blackduck Lake, all 354 neuston net trawls were used to demonstrate the expected level of precision over the range of sample sizes using 95% confidence limits. The 95% confidence interval (CI) declined sharply as the number of trawls increased from three (95% CI = -0.62-1.54) to nine (95% CI = 0.07-0.83). Between 10 (95% CI = 0.09-0.82) and 15 (95% CI = 0.16-0.75) trawls the confidence interval began to stabilize and after 20 (95% CI = 0.20-0.71) trawls there was little change in the confidence interval width (Figure 6).

The distribution of post-larval Yellow Perch in Blackduck Lake was significantly influenced by wind speed and direction. The 24-hour model was best supported (Table 2) and indicated that as wind speeds increased, fish count also increased in both sectors of the lake. However, there were consistently more post-larval Yellow Perch located in the downwind sector of the lake when wind speeds were below 15 kmph. When wind speeds reached 15 kmph the post-larval Yellow Perch in both the upwind and downwind sectors had equal densities. When wind speeds exceeded 15 kmph, the predicted mean fish count in the downwind half of the lake rose marginally from 66.45 to 78.84. After 15 kmph there was a cross-over point where fish count become higher in the upwind sector instead of the downwind sector. As wind speed increased from approximately 15 to 17 kmph predicted mean fish count increased from 54.49 to 106.09, respectively, in the upwind sector of the lake (Figure 7).

Although wind speed and direction had a significant influence on post-larval Yellow Perch distribution, wind speed and direction did not have a significant influence on distributing fish within Blackduck Lake by size. The TL (mm) ~ Intercept model had stronger support than all other wind-based models (Table 3). To better understand why wind did not affect size, the 24-hour wind-period model was again used to demonstrate how wind speed affected average total length (Figure 8). The upwind half of the lake consistently had larger post-larval Yellow Perch when wind speeds were above 10 kmph; however, the greatest difference in predicted fish total length between the upwind and downwind sectors of the lake was only 0.065 mm at 17 kmph (Figure 8). Most of the Yellow Perch captured as part of this study had lengths ranging from 21 – 28 mm (25.5 mean; 1.17 SD; Figure 9). Daily median total lengths can be seen in Figure 10.

DISCUSSION

In this study, daily density estimates of post-larval Yellow Perch ranged from 0.19-0.71 fish/m^3. Since variability can be affected by many factors, consideration must be given to spatial and temporal factors affecting densities (Fulford et al. 2006). There are many spatial and temporal factors that affect Blackduck Lake, but this study found wind had a statistically significant effect on the variability of daily density estimates. Hettler et al. (1997) also studied variability in daily abundance of larval fishes and determined that wind often resulted in density estimates that were double or triple the estimate of the day prior. Like this study, Blukacz et al. (2009) determined days with higher winds produced higher daily plankton estimates with positively buoyant phytoplankton accumulating on the downwind side of the lake.

Larval trawling in varying wind speeds and directions should be incorporated into sampling procedures to ensure that over/under estimation of post-larval Yellow Perch populations does not occur. Another option may be to standardize annual sampling dates around the same wind speeds in addition to sampling on the same date each year. The survival of these post-larval Yellow Perch can often affect the variability of annual recruitment, so it is important to get an accurate density estimate in this larval phase (Cyr 1992). In this study, changes in wind speed and direction likely caused daily density estimates to change, the higher the wind speed, the higher the number of fish sampled. The accuracy of density estimates should be improved by sampling in multiple wind speeds especially on days with high wind speeds, and by sampling equally in the upwind and downwind sectors of the lake.

Wind direction and speed had a significant effect on the spatial distribution of post-larval Yellow Perch in Blackduck Lake. Herb et al. (2016) quantified how wind-sheltering from land and trees affected wind-wave energy on Minnesota lakes and found that typically 24 hours of wind works best for time scale wave models. All wind model time-periods used in this study had relatively the same wind direction, except the wind direction for the 24-hour wind-period (Table 4). The wind speed from this 24-hour wind-period affected spatial distribution more than all other time-periods in this study. Weber et al. (2011) determined that most freshwater systems are too small to have strong consistent currents. Therefore, it is possible that post-larval Yellow Perch required longer than 12 hours to redistribute themselves on Blackduck Lake. Calmer winds at night would allow larval Yellow Perch time to relocate back to pelagic waters, possibly for predator avoidance or optimal energy intake.

In this study when wind speeds increased the density estimates of post-larval Yellow Perch also increased in both sectors of the lake. It is possible that on days with higher wind speeds, fish were moved laterally from nearshore habitats back into the pelagic area of the lake increasing fish density in those areas. This suggests that high wind events may cause post-larval Yellow Perch to migrate into the non-trawlable area multiple times. Eventually, post-larval Yellow Perch will reach a size where they can maintain their position, instead of just passively drifting (Beletsky et al. 2007). But because of wind speeds and the direction, larval Yellow Perch may not be able to reach the desired location until they become larger, especially in years with frequent wind events. If it was safe to sample with neuston nets in winds above 17 kmph, the densities of both sectors of the lake would likely even out.

Moyle and Cech (2004) determined Yellow Perch use behavioral thermoregulation to move to warmer temperatures to maximize growth, and several studies have suggested Yellow Perch select warmer waters in the epilimnion layer of a lake (Dettmers et al. 2005; Isermann and Willis 2008; Martin et al. 2011). Martin et al. (2011) found the highest pelagic densities of post-larval Yellow Perch were in the top 1 m of water. Larval perch were found in depths down to 11 m in Lake Michigan, but densities sharply reduced after 1 m. When post-larval Yellow Perch in the top 1 m of water become demersal, they swim either into shallower areas or deeper water as their diet shifts to benthic invertebrates (Wahl et al. 1993; Dettmers et al. 2005; Beletsky et al. 2007; Isermann and Willis 2008). So even though this suggests that more post-larval Yellow Perch are located within the top 1 m of water, this alone does not explain how wind causes an increase in post-larval Yellow Perch density.

Langmuir circulation was also researched to determine if water movement could have caused fish densities to rise during high winds. Patterns of alternating helical currents can form in surface waters when wind speeds reach between 7.2-10.8 kmph, mixing the top layer of water and interfering with larval fish maintaining position (Wetzel 2001). The higher the wind speed the higher the wave height, and the larger Stokes drift that is created. Stokes drift will force objects with heavier density down into the water column, but buoyant objects like post-larval Yellow Perch with expanded swim bladders remain in the convergence point between helical currents (Mansueti 1964; Wetzel 2001). Waves on Blackduck Lake were never more than 0.14 m while towing, possibly because of a sheltering effect. Using Drews (2013) wave height conversion, the highest winds in this study were 17 kmph (4.7 m/s) and able to produce a 0.3 m wave. Bascom (1964) determined there is a 1:7 ratio for wave peak height to wavelength, and waves can affect movement in water at a depth of half the wavelength. So, the 0.3 m wave could create a 2.1 m wavelength and move fish at a depth of 1.05 m. Winds were not strong enough to create Langmuir circulation during this study. This suggests the increase in post-larval Yellow Perch densities during high winds did not come from below the 1 m depth, but laterally from non-trawlable areas. Since Langmuir circulation did not happen during this study and almost half of Blackduck Lake is littoral area, this strengthens the theory of post-larval Yellow Perch being moved laterally out of non-trawlable areas as the wind increases in speeds.

Although wind affected the spatial distribution of post-larval Yellow Perch, there was not a significant influence on how post-larval Yellow Perch of various sizes were distributed in Blackduck Lake. The post-larval Yellow Perch sampled on Blackduck Lake were mostly between the 20 – 28 mm and had a unimodal distribution, suggesting the Yellow Perch hatched in a short time period. This short hatch period is supported by Isermann and Willis (2008) who determined Yellow Perch populations in many lakes have brief spawning and hatch periods, resulting in a single annual cohort with very similar lengths. In addition to a brief hatch period, post-larval Yellow Perch also shift to demersal prey around 24-31 mm (Wahl et al. 1993; Dettmers et al. 2005). If sampling had continued past 7 Jul 17 more fish above 28 mm may have been sampled.

Results from this study provide evidence to suggest sampling with a neuston net in a lake the size of Blackduck Lake is a proficient method of obtaining an early, accurate density estimate. Larval abundance estimates can give management an early understanding of population sizes since there is a significant relationship between post-larval and juvenile abundance (Anderson et al. 1998). Neuston nets can be a proficient gear to provide these early abundance estimates of post-larval Yellow Perch. However, when estimating abundance estimates care must be taken in selecting an appropriate sample size, low sample sizes affect accuracy, and high sample sizes waste time and resources. This study produced a precise estimate with only 10 - 15 trawls and there was not a significant increase in precision beyond 15 trawls.

In conclusion, a density estimate of 0.45 fish/m^3 (0.58 SD) was calculated during the sampling period (26 Jun – 07 Jul 2017) on Blackduck Lake, MN. The daily density estimate was higher on days with higher winds, and between 10-15 modified neuston trawls produced a precise density estimate. This study determined that wind influenced the distribution of post-larval Yellow Perch, but it did not significantly distribute fish by total length. Shortly after hatching, post-larval Yellow Perch moved into the pelagic area of Blackduck Lake, when larval Yellow Perch became larger, they moved back into littoral areas. But instead of larval Yellow Perch moving into non-trawlable areas and staying there, this study suggests that larval Yellow Perch are continually moved back into the pelagic area of the lake during high wind events, mixing with fish in the pelagic area. Then when wind speeds decreased, post-larval Yellow Perch had an opportunity to either swim back into shallow areas or lower in the water column. Eventually post-larval Yellow Perch should reach a size where they could swim against currents from higher winds. Wind speed and direction should be used to determine upwind and downwind sectors of the lake. Then an equal number of larval trawls should be performed in both sectors of the lake. The results of this study determined that a combination of wind speeds and directions moved post-larval Yellow Perch in Blackduck Lake, and it appears that spatial and temporal variation is more important to the recruitment of post-larval Yellow Perch than previously thought.

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Table 1. Measurements taken from six days of post-larval Yellow Perch trawling on Blackduck Lake. Numbers within parenthesis represent standard deviation.

Trawl Day	Fish Sampled	Fish/m^3	Mean Total Length (mm)
26 Jun 17	5654	0.71 (0.57)	23.27 (1.98)
29 Jun 17	3320	0.44 (0.42)	23.44 (2.13)
01 Jul 17	2504	0.32 (0.48)	23.75 (2.29)
03 Jul 17	1541	0.19 (0.24)	24.15 (2.35)
05 Jul 17	5075	0.63 (0.72)	24.14 (2.57)
07 Jul 17	3315	0.42 (0.58)	24.28 (2.77)

Table 2. Models and AIC scores used to test for the effect of wind on the distribution of post-larval Yellow Perch in Blackduck Lake.

Model	AIC	Δ
Fish (#) ~ Up/Downwind Half 24hr × Wind (kmph) 24hr	3551.94	0
Fish (#) ~ Up/Downwind Half 12hr × Wind (kmph) 12hr	3590.39	38.45
Fish (#) ~ Up/Downwind Half 6hr × Wind (kmph) 6hr	3605.58	53.65
Fish (#) ~ Up/Downwind Half 3hr × Wind (kmph) 3hr	3610.22	58.29
Fish (#) ~ Up/Downwind Half × Wind Speed (kmph)	3614.42	62.48
Fish (#) ~ Intercept	3615.34	63.41

Table 3. Models and AIC scores used test for the effect of wind on how post-larval Yellow Perch of various sizes were distributed in Blackduck Lake.

Model	AIC	Δ
TL (mm) ~ Intercept	1115.98	0
TL (mm) ~ Up/Downwind Half 3hr × Wind (kmph) 3hr	1119.06	3.08
TL (mm) ~ Up/Downwind Half × Wind (kmph)	1119.09	3.11
TL(mm) ~ Up/Downwind Half 6hr × Wind (kmph) 6hr	1120.53	4.56
TL (mm) ~ Up/Downwind Half 12hr × Wind (kmph)12hr	1120.92	4.94
TL (mm) ~ Up/Downwind Half 24hr × Wind (kmph) 24hr	1121.84	5.86

Table 4. Mean wind speeds and directions for the 24-hour time-period during trawling (26 Jun 17 – 07 Jul 17) on Blackduck Lake. Numbers within parenthesis represent standard deviation.

Date	Mean 24 hr Wind Speed (kmph)	Mean 24 hr Wind Direction (Azimuth Degrees)
26 June 2017	16.93 (7.3)	305^o (18.27)
29 June 2017	14.17 (3.8)	12^o (116.35)
01 July 2017	10.92 (6.81)	266^o (35.59)
03 July 2017	8.21 (5.75)	206^o (117.44)
05 July 2017	13.98 (7.49)	207^o (50.22)
07 July 2017	16.24 (7.77)	306^o (28.25)

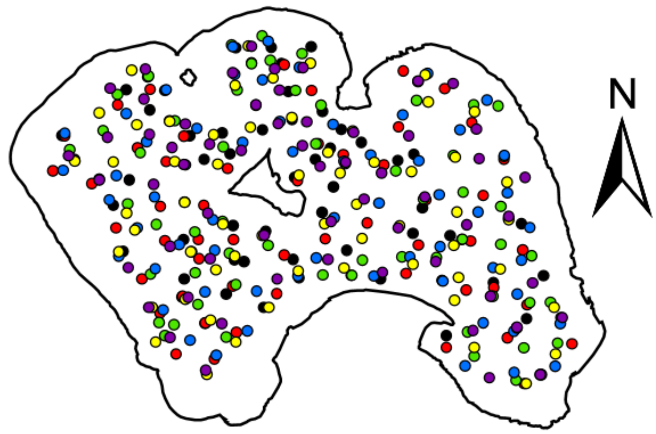

Figure 1. Post-larval trawl point locations on Blackduck Lake, MN. Each day of trawls are represented by the following colors: 26 Jun – black, 29 Jun – red, 01 Jul – green, 03 Jul – blue, 05 Jul – yellow, and 07 Jul – purple.

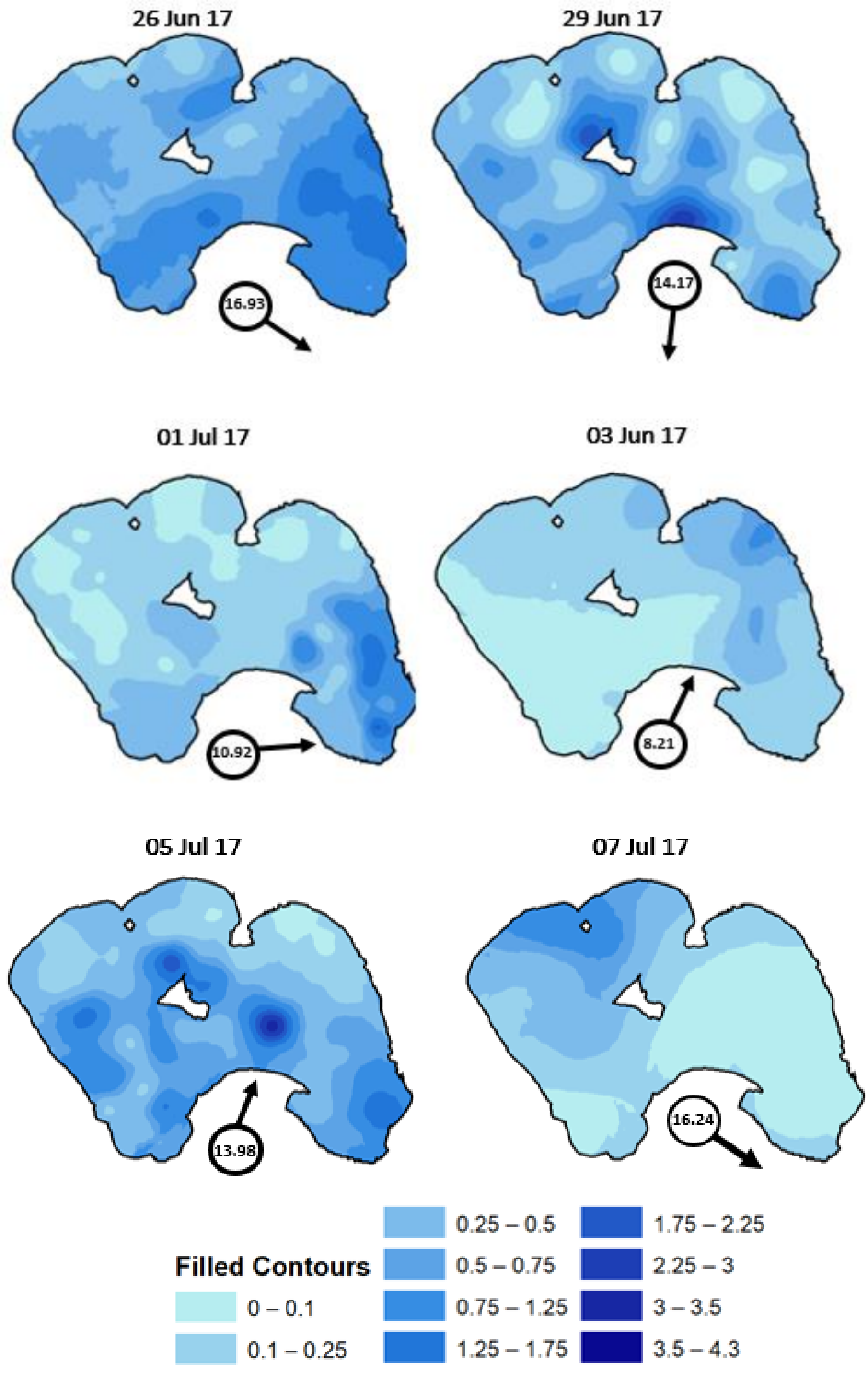

Figure 2. Daily post-larval Yellow Perch densities from Blackduck Lake between 26 Jun – 07 Jul 17, shown with wind speed (kmph) and direction arrow. GIS Maps were created with a Kriging method, using the number of fish/m^3 from each trawl on that specific day to create the contours of the map.

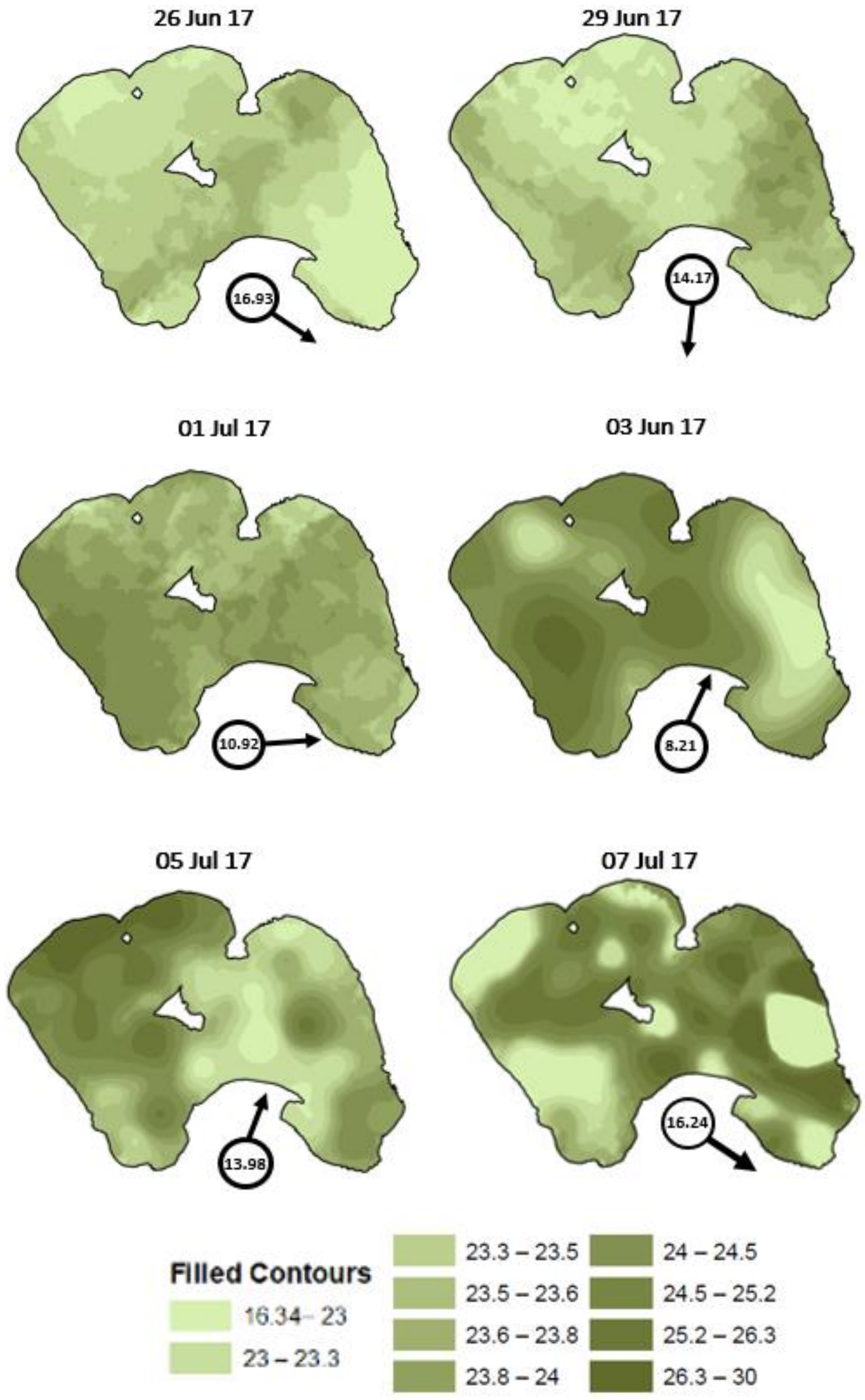

Figure 3. Daily mean total length (mm) of post-larval Yellow Perch from Blackduck Lake between 26 Jun – 07 Jul 17, shown with wind speed (kmph) and direction arrow. GIS Maps were created with a Kriging method, using the mean total length from each trawl on that specific day to create the contours of the map.

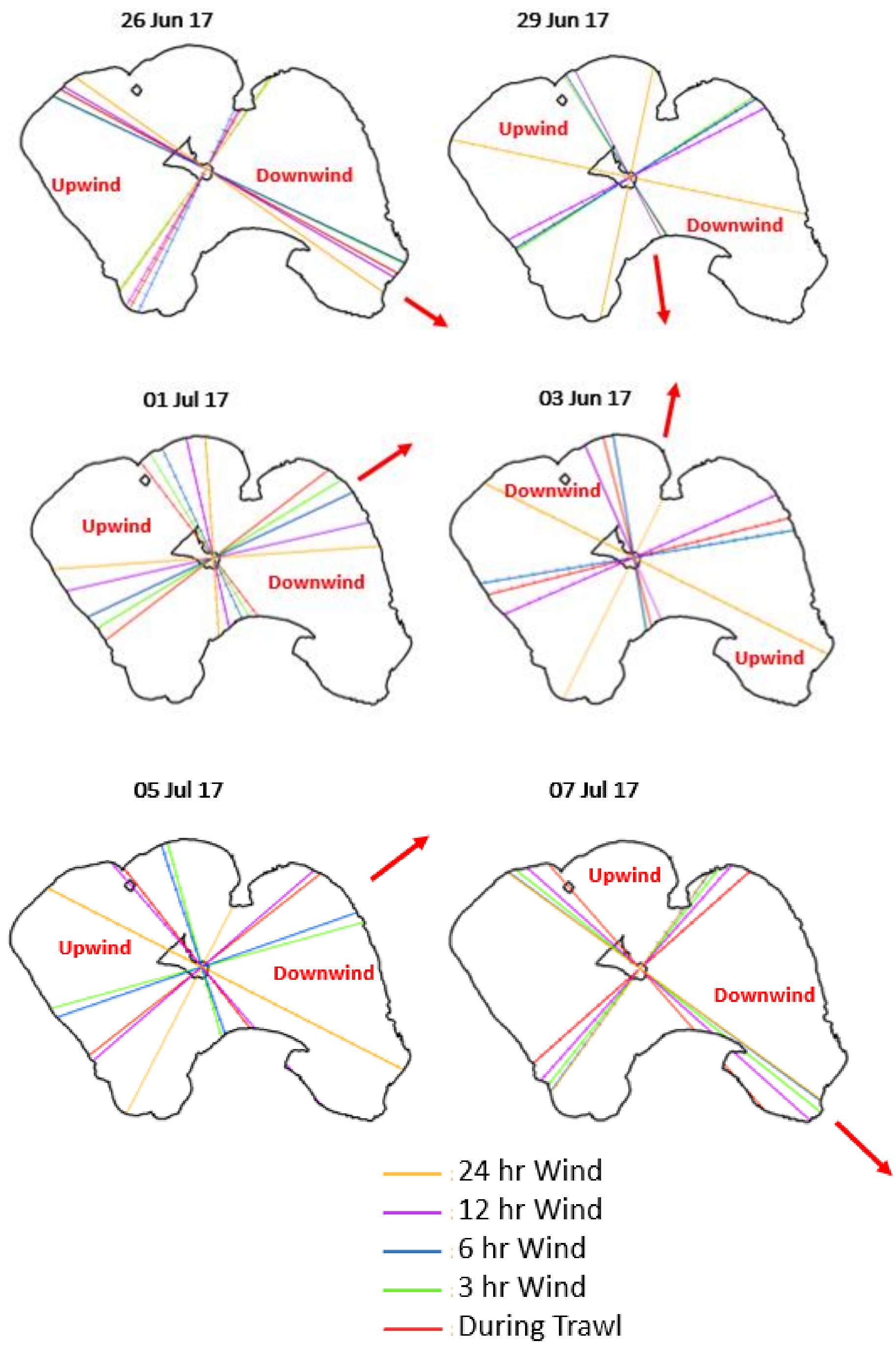

Figure 4. Changes in mean wind direction during the six sample periods on Blackduck Lake. Also included are the perpendicular lines (dashed) that were used to separate the lake into a downwind and upwind half for each of the respective time periods. Time-periods were created by adding the amount of the time-period to before the start of the first trawl, until the end of the last trawl on that specific day.

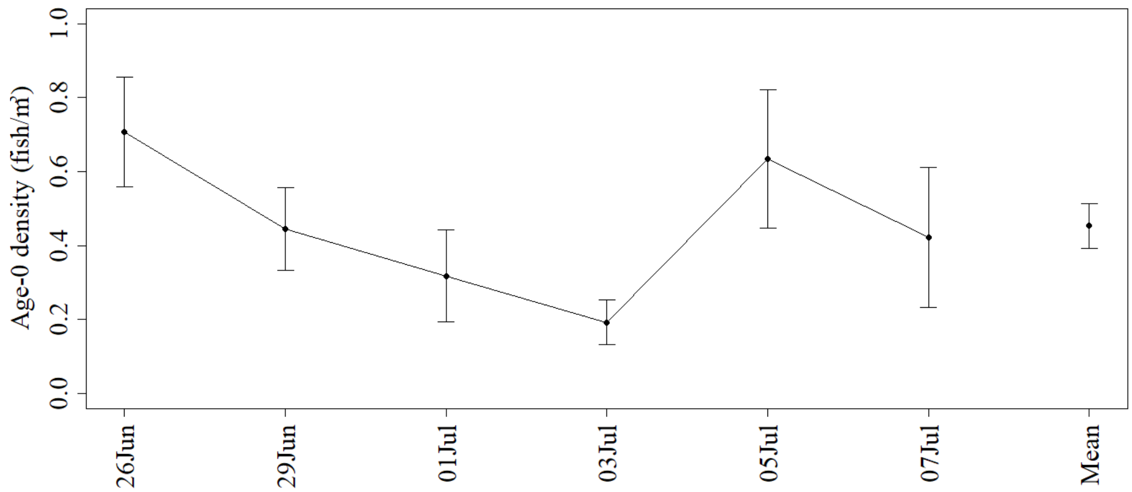

Figure 5. Daily density (fish/m^3) estimates demonstrating the inaccuracies that can develop when using a single day of trawling. Trawls from 26 Jun – 07 Jul 17 were included in the mean density estimate for Blackduck Lake. Black bars represent 95% CI.

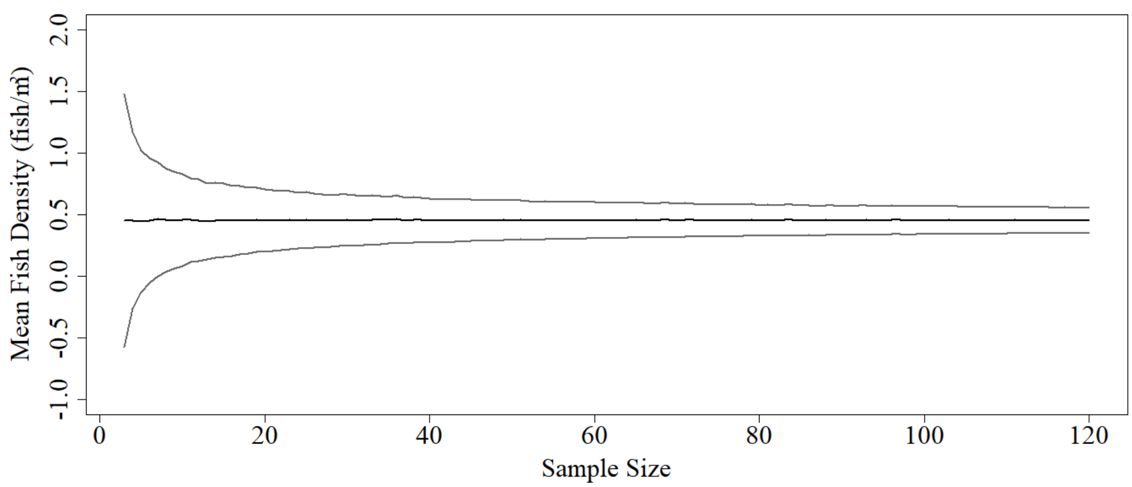

Figure 6. Expected level of precision for mean post-larval Yellow Perch density across sample sizes (number of trawls) ranging 3 to 120. The black line represents the mean fish density and the grey lines represent the confidence interval width. Trawl data was collected from Blackduck Lake, MN between 26 Jun – 07 Jul 17.

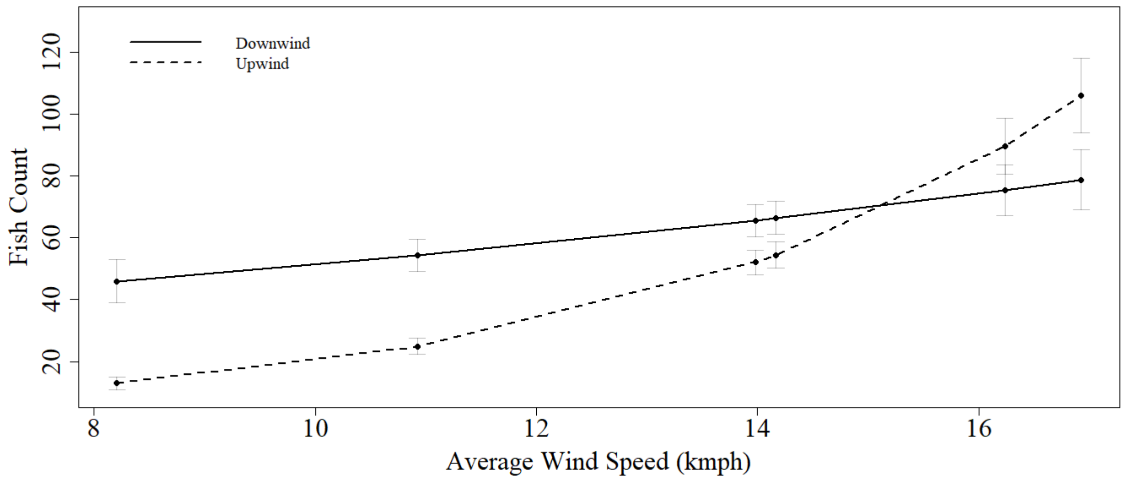

Figure 7. Change in predicted mean fish count in the upwind and downwind sectors of Blackduck Lake, as affected by varying wind speeds (kmph) during the 26 Jun – 07 Jul 17 sampling period. The 24-hr wind-period model determined that wind speed and direction had a significant influence on the distribution of post-larval Yellow Perch, error bars represent standard error.

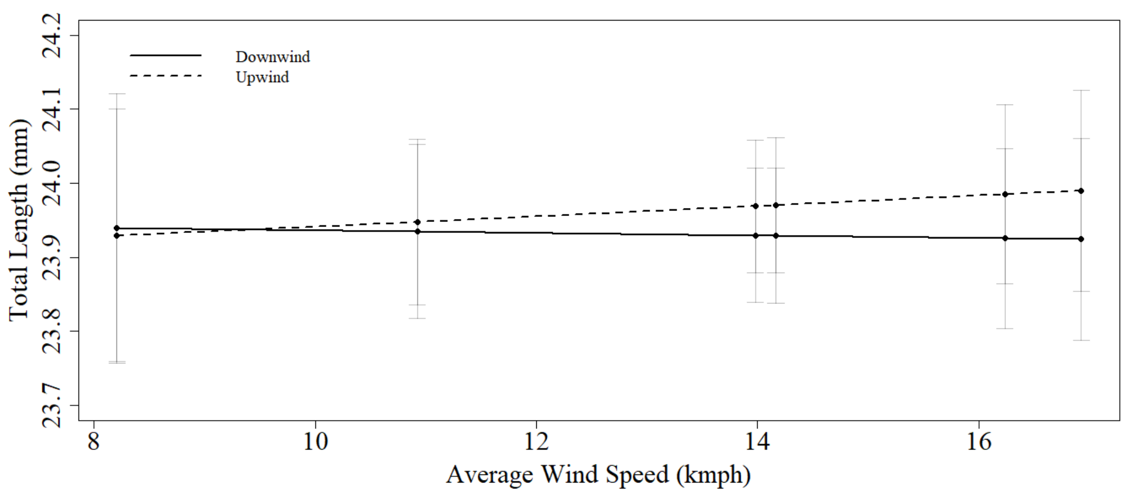

Figure 8. Relationship between predicted post-larval Yellow Perch mean total length (mm) and wind speed (kmph) in the upwind and downwind sectors in Blackduck Lake, error bars represent standard error.

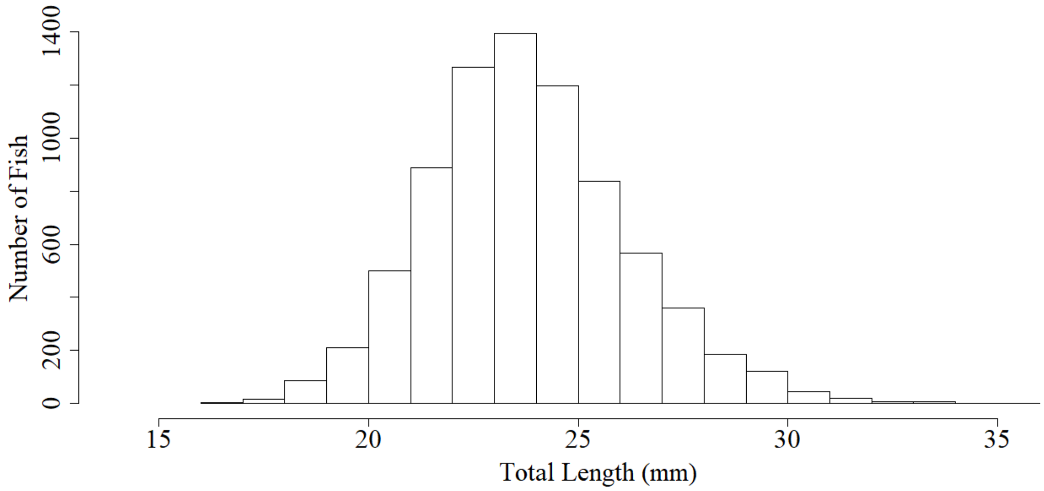

Figure 9. Distribution of post-larval Yellow Perch total lengths (mm) for fish captured via neuston trawl on Blackduck Lake from 26 Jun – 07 Jul 17.

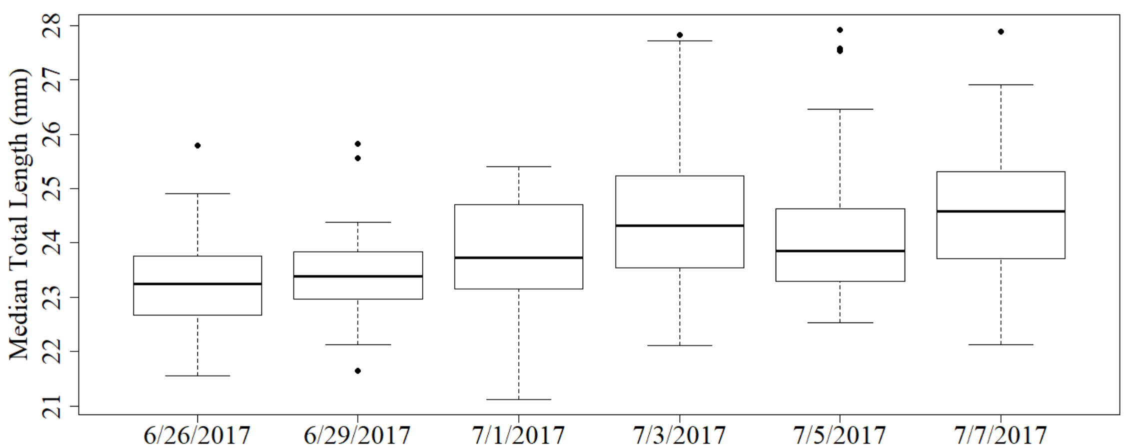

Figure 10. Daily median and interquartile ranges of post-larval Yellow Perch total lengths (mm) for fish captured via neuston trawl in Blackduck Lake from 26 Jun – 07 Jul 17.

APPENDIX A: Daily Photographic Documentation of Larval Yellow Perch

In-depth knowledge related to early developmental history of fish is required to develop stocking assessments and management plans for a species of fish (Victor and Brothers 1981). Accurate age readings are necessary to form growth and mortality rates and have been used in many types of analysis requiring age or elapsed time (Campana 2001). The key to identifying larval fishes is to accurately identify specific features and understand how larval fishes develop. Auer's (1982) work on identifying larval fishes in the Great Lakes Basin was of interest to this study. With the use of illustrations, Auer (1982) documented what larval fish should look like as they grow, by identifying distinguishing physical features, pigmentation spots, measuring external body parts and how many myomeres are present at each growth stage (Auer 1982). This study's objective focused on documenting the initial growth stages of larval Yellow Perch with digital photographs to improve Auer's (1982) prior illustrations.

To photograph larval fish, Yellow Perch eggs were collected from Lake Julia, MN and raised in stock tanks near the shore of Lake Bemidji, MN. Upon hatching, fish were removed and separated into different chambers by the date of their hatch. Each day fish were removed from the hatchery system and placed in an iodine solution for daily photographs. All photographs and measurements were taken with a Unitron microscope (Z850, Unitron, Commack, NY), using an ocular micrometer, and Micrometrics SE Premium laboratory microscope camera (318CU 3.2 MP, Micrometrics, NYC, NY) with imaging software. Both ocular micrometer and the operating system were calibrated before photographs were taken. It is assumed that the feeding of larval Yellow Perch was unsuccessful, resulting in only 13 days of photographs. Digital photographs can be seen below.

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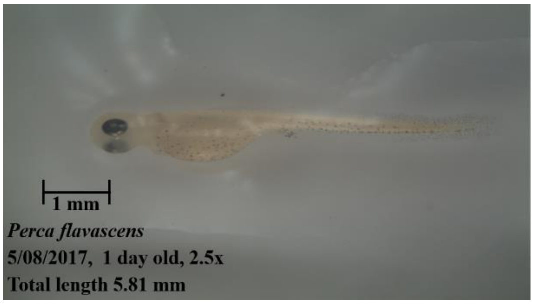
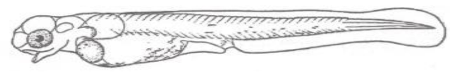
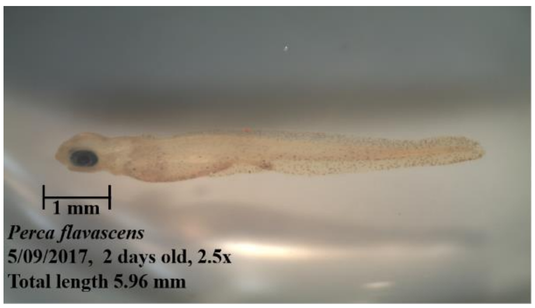
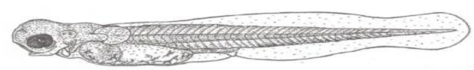
PHOTOS. Daily growth photos.



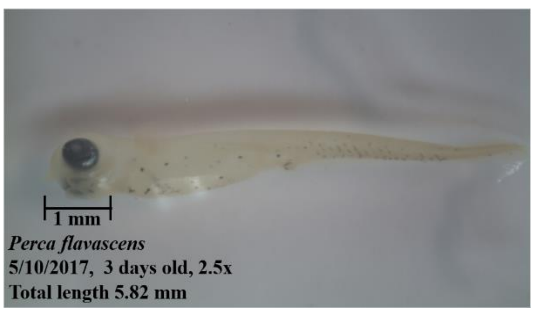


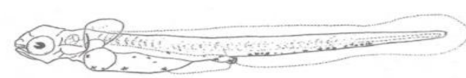
Day 1 Yellow Perch, and 5.6 mm illustration (Auer 1982).



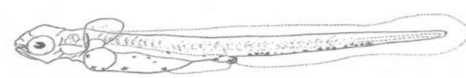


Day 2 Yellow Perch, and 5.8 mm illustration (Auer 1982).

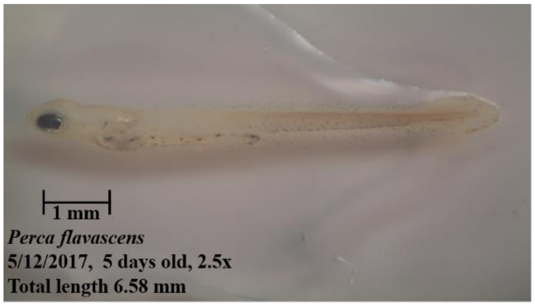


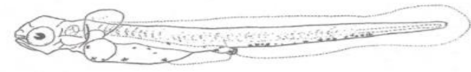


Day 3 Yellow Perch, and 5.8 mm illustration (Auer 1982).

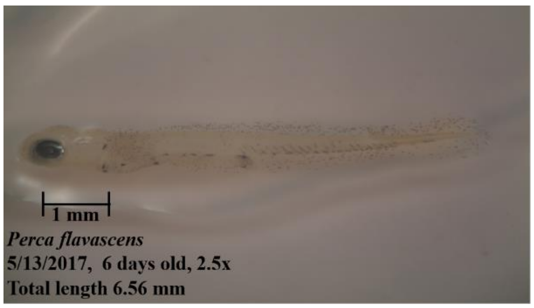


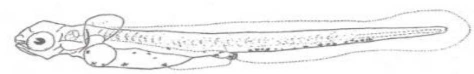
Day 4 Yellow Perch, and 5.8 mm illustration (Auer 1982).



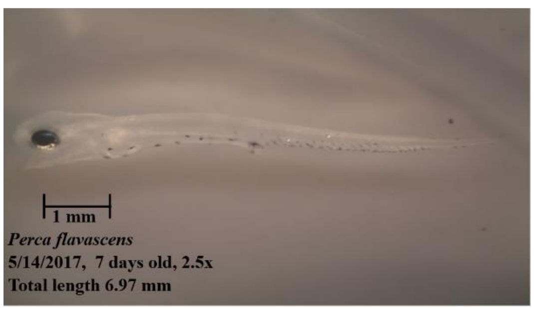


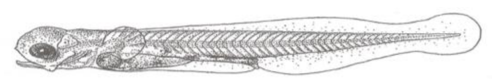
Day 5 Yellow Perch, and 5.8 mm illustration (Auer 1982).



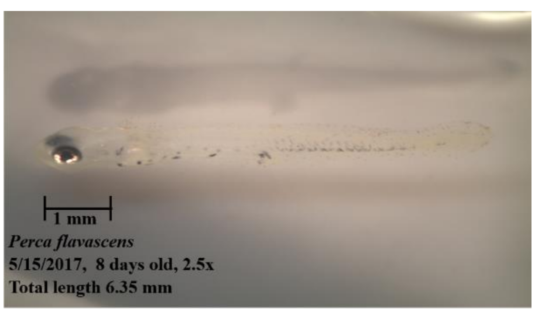


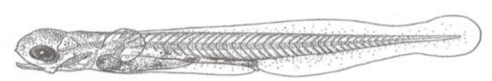
Day 6 Yellow Perch, and 5.8 mm illustration (Auer 1982).



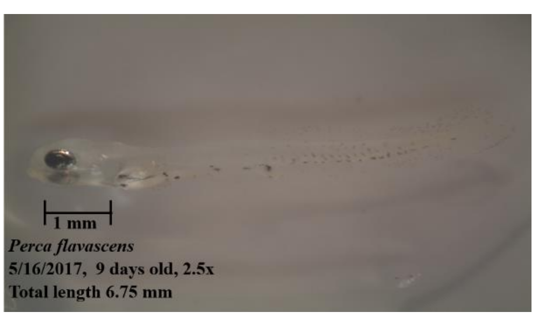


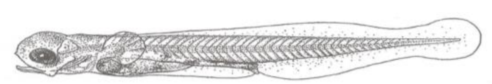
Day 7 Yellow Perch, and 6.8 mm illustration (Auer 1982).



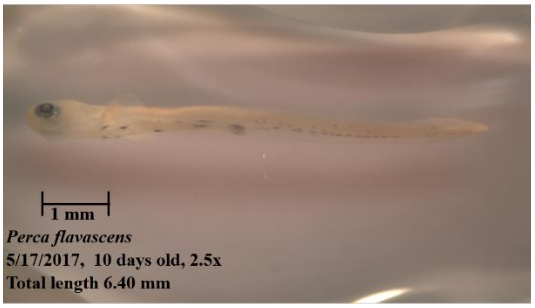


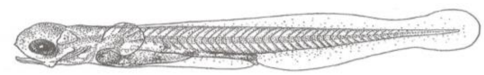
Day 8 Yellow Perch, and 6.8 mm illustration (Auer 1982).



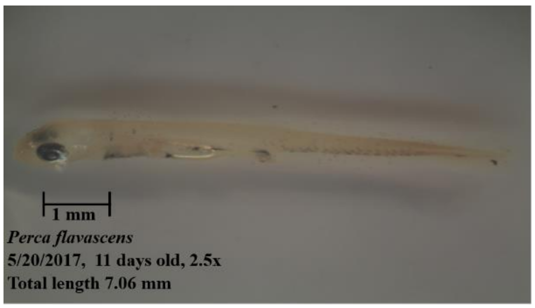


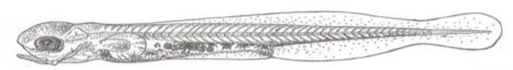
Day 9 Yellow Perch, and 6.8 mm illustration (Auer 1982).



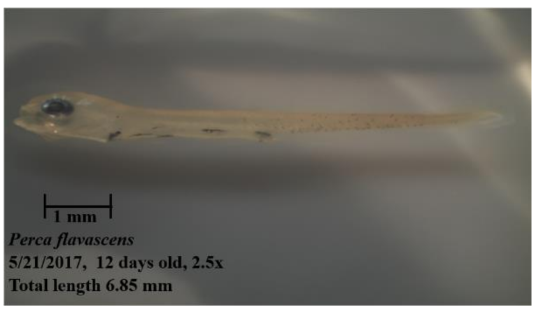


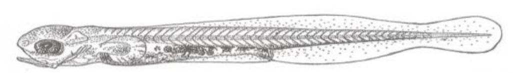
Day 10 Yellow Perch, and 6.8 mm illustration (Auer 1982).



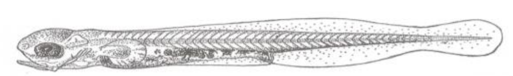


Day 11 Yellow Perch, and 7.0 mm illustration (Auer 1982).





Day 12 Yellow Perch, and 7.0 mm illustration (Auer 1982).



Day 13 Yellow Perch, and 7.0 mm illustration (Auer 1982).

APPENDIX B: Documenting the Effects of Synthetic Compounds in Surface Water on the Hatch and Growth Rates of Larval Yellow Perch

A wide variety of synthetic chemicals are used in modern life and these chemicals enter the aquatic environment, affecting organisms that live there. Synthetic chemicals are manmade chemicals that pollute the environment, and at least one synthetic chemical has already been found in 47 out of 50 tested lakes throughout Minnesota (Ferrey 2013). Synthetic chemicals from fertilizers, insecticides, domestic uses, and industrial uses can mimic the effects of hormones in fish, affect fish behavior, and reproduction due to chemical exposure (Barber et al. 2007; Schoenfuss et al. 2008; Painter et al. 2009; Ferrey 2011). Synthetic chemicals can affect the behavioral and physiological aspects of fishes in lower concentrations and can collapse a fish population (Kidd et al. 2007). The purpose of this study was to test surface water where different cocktails of synthetic chemicals may occur. This study tested hatch and growth rates of Yellow Perch cultured in: waters in forested areas (Forest), waters impacted by agricultural inputs (Agricultural), wastewater treatment plant (WWTP) inputs, and waters impacted by privately owned septic systems (Septic). All sites chosen for this study were from a latitude of N 47° (Table B1).

Yellow Perch hatch rate, growth rate, and spinal curvature measurements were tested to determine if they differed significantly among the previously described test groups. The major assumption here is the amounts of synthetic chemicals differs among treatment groups and that is what would cause any change in response variables. To accomplish this objective, fertilized Yellow Perch eggs were collected from Lake Bemidji shortly after spawn. Each of the twelve aquariums contained 11 L of water sampled from one of 12 locations. Each of the four treatment areas: forested, agricultural, WWTP, and septic treatment areas each had water sampled from three locations. Aquariums were placed in a climate-controlled room where water was gradually warmed from 7.9 to 15.6°C over 10 days to simulate natural Yellow Perch hatch temperatures. Skeins of 100 eggs were placed into each aquarium. Aquarium setup followed Schwartz et al. (2010) method with aeration in each tank. A YSI professional plus recorded water temperature in °C, the pH of the water, and dissolved oxygen in mg/L inside all aquariums. To simulate natural conditions outside, the controlled climate room was given light for 13 hours and darkness for 11 hours. After ten days, all hatched fish were counted, measured for total length, and spinal curvature was measured (mm) as a surrogate of spinal deformity. Fish were observed using a Unitron microscope (Z850, Unitron, Commack, NY) and photographed with a Micrometrics camera.

There was not a significant difference in hatch rates (Kruskal-Wallis; χ^2 = 1.61, df = 3, P = 0.66) or spinal curvature measurements (Kruskal-Wallis; χ^2 = 2.21, df = 3, P = 0.53) among the four tested types of surface water areas (Figure B1). This does not necessarily mean the water is free of synthetic chemicals. Normally, water near WWTP's are a significant source of synthetic chemicals, since multiple household chemicals can accumulate in treatment plants. What does stand out in this study is the WWTP hatch rate is larger than any other tested areas. Although water was not tested for synthetic chemicals, it is possible that synthetic chemicals like hormones may be causing the larger hatch rate in the WWTP water (Ferrey 2013). Only two fish were found with spinal deformities (Figure B2), one fish was found in the privately owned septic system treatment group (0.28 mm of curvature), and one fish was found in the WWTP treatment group (0.30 mm of curvature).

There was a significant difference in growth rates (TL) (Kruskal-Wallis; χ^2 = 9.81, df = 3, P = 0.02) and a multiple comparison test determined the difference was between septic and WWTP groups (Figure B3). Ferrey (2013) tested 50 random lakes in Minnesota and determined that lakes influenced by either septic systems or a WWTP had the highest number of synthetic chemicals present. In this study, the septic treatment group had the highest median TL and the largest quartile range of any tested area (6.03 mm). It is unknown what caused the higher growth rates in the septic treatment group, but synthetic chemicals are a possibility. It is also unclear what caused the WWTP group to have a larger quartile range and low growth rate, but synthetic chemicals are again the probable cause. Synthetic chemicals serve a purpose, but when these chemicals are moved out of the area they were intended for, they cause a multitude of issues. This is possibly what happened in this study, synthetic chemicals of some type are interfering in the normal hatch and growth of larval Yellow Perch. Future studies should continue to test the effects of synthetic chemicals on hatch rates, growth rates, and spinal curvature measurements of fishes in northern lake ecosystems and determine if there is a larger issue in northern lake ecosystems.

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Table B1. Lake Identification and acreage from four different types of sites, waters in forested areas (Forest), waters impacted by agricultural inputs (Agricultural), wastewater treatment plant (WWTP) inputs, and waters impacted by privately owned septic systems (Septic Systems).

Type	Lake	Pop.	km^2	Latitude	Longitude
Forrest	Lindgren Lake	N/A	0.28	N 47.674559°	W 94.817512°
Forrest	Drury Lake	N/A	0.13	N 47.608410°	W 94.595653°
Forrest	Nelson Lake	N/A	0.13	N 47.651917°	W 94.619722°
Agricultural	Nebish Lake	N/A	0.29	N 47.772216°	W 95.868157°
Agricultural	Silver Creek	N/A	N/A	N 47.718593°	W 95.472727°
Agricultural	Ruffy Brook	N/A	N/A	N 47.746201°	W 95.413220°
WWTP	Lake Bemidji	14,594	26.69	N 47.467799°	W 94.877260°
WWTP	Clearwater River (Bagley)	1,400	N/A	N 47.526383°	W 95.382229°
WWTP	Bagley Drainage	1,400	N/A	N 47.519717°	W 95.378083°
Septic Systems	Grace Lake	N/A	3.48	N 47.410784°	W 94.760030°
Septic Systems	Turtle River Lake	N/A	7.04	N 47.589421°	W 94.749947°
Septic Systems	Lake Irvine	N/A	2.48	N 47.454051°	W 94.887966°

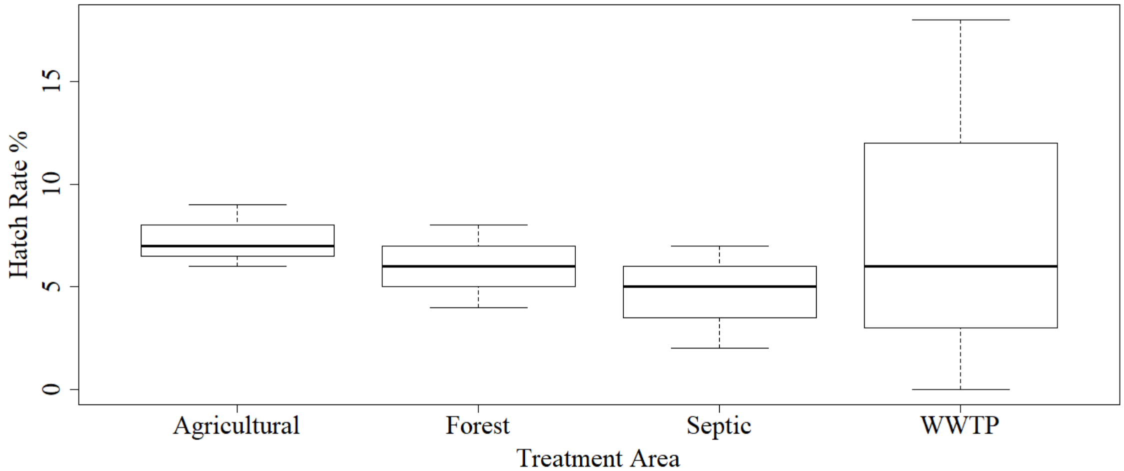

Figure B1. Yellow Perch hatch rates from four different test groups: waters impacted by agricultural inputs (Agricultural), water in forested areas (control), waters impacted by privately owned septic systems (Septic), and wastewater treatment plant (WWTP) inputs. Each tank started with 100 Yellow Perch eggs, Kruskal-Wallis test determined no significant difference was found between test groups ($\chi^2 = 1.61$, df = 3, $P = 0.66$).

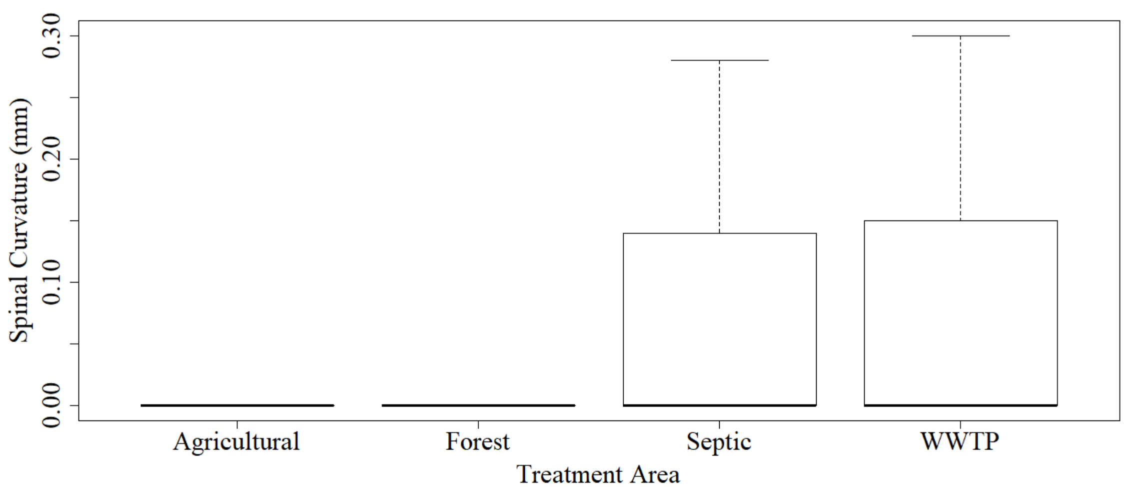

Figure B2. Yellow Perch spinal curvature measurements from four different test groups: waters impacted by agricultural inputs (Agricultural), water in forested areas (control), waters impacted by privately owned septic systems (Septic), and wastewater treatment plant (WWTP) inputs. Each tank started with 100 Yellow Perch eggs, Kruskal-Wallis test determined no significant difference was found between test groups ($\chi^2 = 2.21$, df = 3, $P = 0.53$).

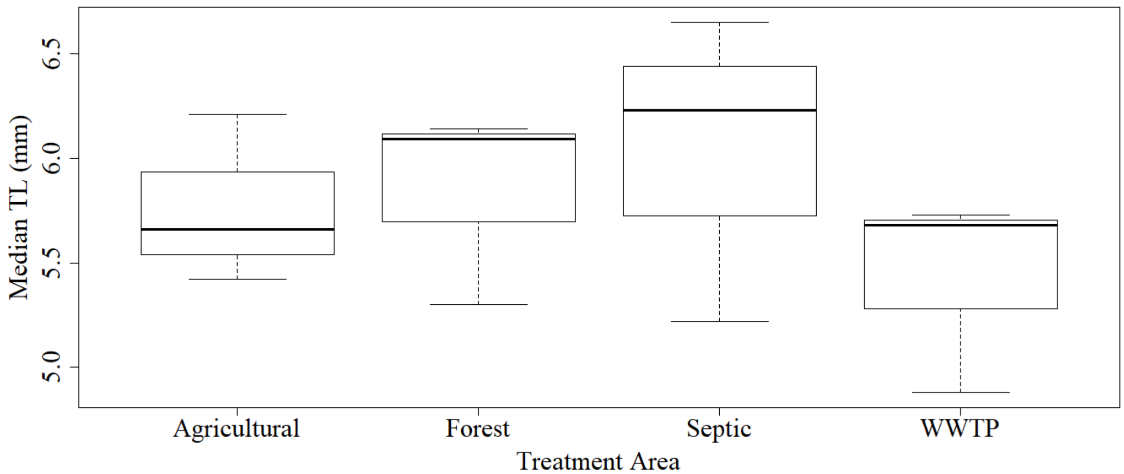

Figure B3. Yellow Perch growth rates measured from all fish that hatched after ten days, from four test groups: waters impacted by agricultural inputs (Agricultural), water in forested areas (control), waters impacted by privately owned septic systems (Septic), and wastewater treatment plant (WWTP) inputs. Each tank started with 100 Yellow Perch eggs, a significant difference was found between the septic and WWTP groups (χ^2 = 9.81, df = 3, P = 0.02).

APPENDIX C: Detecting Estrogen Mediated Endocrine Disruption in Yellow Perch using a Perciform Vitellogenin ELISA Kit

Compounds with estrogenic properties are found in pesticides, plastics, birth control, and a variety of other products that can disrupt the reproductive health of many fish species including the Yellow Perch *Perca flavescens* (Kime 1998; Tyler et al. 1998; Van Der Kraak 1998; Guillette and Gunderson 2001; Nash et al. 2004). Male fishes normally produce androgens (testosterone and 11-ketotestosterone) which stimulates the production of sperm in the testes (Moyle and Cech 2004). Female fishes produce estrogen (17β-estradiol; E_2) which simulates vitellogenin (phosphoglycolipopeptide yolk precursor) in the ovary which leads to the production of eggs (Moyle and Cech 2004). Estrogenic compounds can interfere with the functioning of cell signaling pathways during development and reproduction, causing feminization of male fishes (Colborn et al. 1993; Moyle and Cech 2004; Kidd et al. 2007). After these fishes are exposed to estrogenic compounds, they can begin to have abnormal behavior, eggs in their gonads, and can cause sterility of male fishes further complicating reproduction. Biomarker (vitellogenin) concentrations can be measured by testing homogenized fish livers where vitellogenin is synthesized and can then be detected with assay kits created specifically for different species.

An enzyme-linked immunosorbent assay (ELSIA) was used to determine how source water affected the vitellogenin (VTG) production in Yellow Perch. Adult Yellow Perch (51) were collected by hook and line from six lakes in northern Minnesota: Bemidji, Pike Bay, Benjamin, Little Turtle, Julia, and Big Turtle. Fish were weighted, lengths recorded, livers extracted and weighted, separated by sex and lake, and then processed with ELSIA. Assay procedures for this study followed the exact instructions as set by TECOmedical Group Perch (Perciformes) Vitellogenin ELISA-kit TE-1035 (2016). Chemical reagents and standards were first mixed and placed in vials to prepare for the creation of a standard curve to judge VTG results from this test. A calibration curve was created from known VTG concentrations in fish livers. Matrix solution, prepared standards, prepared controls, and pre-diluted samples were placed into ELISA-kit wells. Samples were incubated on a shaker, aspirated with diluted wash buffer, new reagents added, and then this procedure was repeated for varying lengths of time. Finally, color reaction was measured with a plate reader within 10 minutes of adding the stop solution. Using the calibration curve as a guide, the vitellogenin concentrations from the fish livers were then able to be determined.

The VTG levels of homogenized livers from male Yellow Perch ranged from 13.75 to 2,030.14 ng of VTG and were high across all lakes sampled (Figure C1). A Kruskal-Wallis test was used to determine there was no significant difference in VTC levels in male ($\chi^2 = 0.38$, df = 3, $P = 0.95$) or female ($\chi^2 = 5.97$, df = 5, $P = 0.31$) fish from each lake (Figure C2, Figure C3). In lakes Bemidji, Benjamin, and Julia the Yellow Perch VTG levels were higher in males than in females (Figure C1.). Nash et al. (2004) determined that 2-10 ng/L of the pharmaceutical ethynylestradiol affected fecundity and concentrations of ethynylestradiol above 10 ng/L affected reproductive behavior. So according to the concentrations in the study of Nash et al. (2004), the concentrations in this study are enough to cause behavioral issues in Yellow Perch. Male and juvenile fish produce very little VTG, so the levels found in this study suggest estrogenic compounds are found in lakes Bemidji, Benjamin, and Julia.

To understand how VTG changed with Yellow Perch mass, the weight of fifty-one male and female Yellow Perch (g) were compared to the VTG levels in fish livers (ng) using linear regression. There was not a significant difference in the VTG levels in sampled male Yellow Perch ($F = 3.3$, $P = 0.08$), but VTG levels appeared to increase as the mass of the fish increased (Figure C4). There was also not a significant difference in the VTG levels in female Yellow Perch ($F = 1.17$, $P = 0.29$), but VTG levels appeared to increase as the mass of the fish increased (Figure C5). Since these fish were sampled in the pre-spawn timeframe of April, female fish would be producing eggs and therefore have higher VTC levels. Although VTG levels increased as both male and female Yellow Perch increased in size, only female Yellow Perch should have increased VTG levels since the number of eggs produced normally increases with size.

The homogenized liver samples from Yellow Perch in this study were compared to homogenized liver samples from other fishes to understand how post-larval Yellow Perch in northern Minnesota may be affected by estrogenic compounds. First results from this study were compared to homogenized livers from male Zebrafish *Danio rerio* (Zhang et al. 2016). Zebrafish were exposed to three different exposure rates (0.01, 0.1, 1.0 mg/L) of a synthetic compound called perfluoroalkyl acid (PFAA). Non-exposed male fish had a rate of 753.45 ng/g of VTG in their livers. When exposed to the dose rates of PFAA, the VTG in male Zebrafish livers rose to 857.19 ng/g (0.01 mg/L dose rate), 868.11 ng/g (0.1 mg/L dose rate), and 862.65 ng/g (1.0 mg/L dose rate) of VTG (Zhang et al. 2016). The results from this study were also compared to homogenized liver samples from Japanese Medaka exposed to 30 ng and 200 ng of 17β-estradiol (Nilsen et al. 2003). Control female fish had 1,215.10 ng/ml of VTG in fish liver, and un-exposed male fish had 110.23 ng/ml of VTG in fish liver (Nilsen et al. 2003). When Nilsen et al. (2003) exposed male Japanese Medaka fish to the two exposure rates of 17β-estradiol, their livers had 164.45 ng/ml of VTG in fish liver (30 ng), and 814.51 ng/ml of VTG in fish liver (200 ng). All male Yellow Perch in this study had a VTG rate higher than 900 ng/g in their liver, indicating that all male Yellow Perch in this study were exposed to a larger dose of estrogenic compound than the highest dose rates by Zhang et al. (2016) and Nilsen et al. (2003). Where types of fish and chemicals used may be different in Nilsen et al. (2003) and Zhang et al. (2016), the male fish in all studies had high VTG levels.

Types of estrogenic compounds in this study were not determined; however, sources of estrogenic compounds in this study may be explained based on location. High VTG levels in male Yellow Perch may be explained by the Bemidji WWTP discharging directly into the Mississippi River, then flowing 240 m into Bemidji Lake. Septic system drainage fields on Lake Julia, Big Turtle, Benjamin, and Bemidji may be seeping down into the lakes from the surrounding high banks. Finally, Lake Benjamin is not connected to any river and has few private residents, so its high level of VTG is interesting since it seems to be separated away from possible pollution sources.

It is suggested that fish in this study have been exposed to some type of unknown estrogenic compound. More study is needed on these lakes to determine where the estrogenic compounds are coming from and the identity of the estrogenic compound(s). More ELISA testing should take place first before expensive chemical testing is done. Chemicals need to be identified so that their source can be located, and solutions found. Recommendations would also suggest the local area be educated on estrogenic compounds and their effects on fishes, wildlife, and humans.

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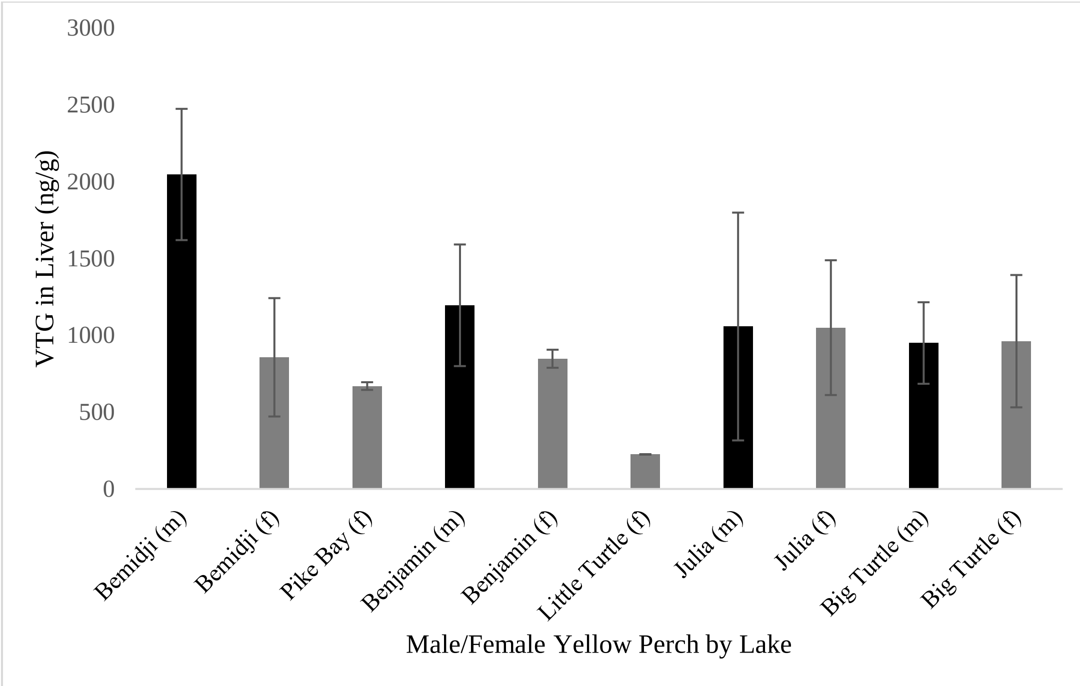

Figure C1. Vitellogenin levels from adult male and female Yellow Perch livers (ng) from six northern Minnesota lakes.

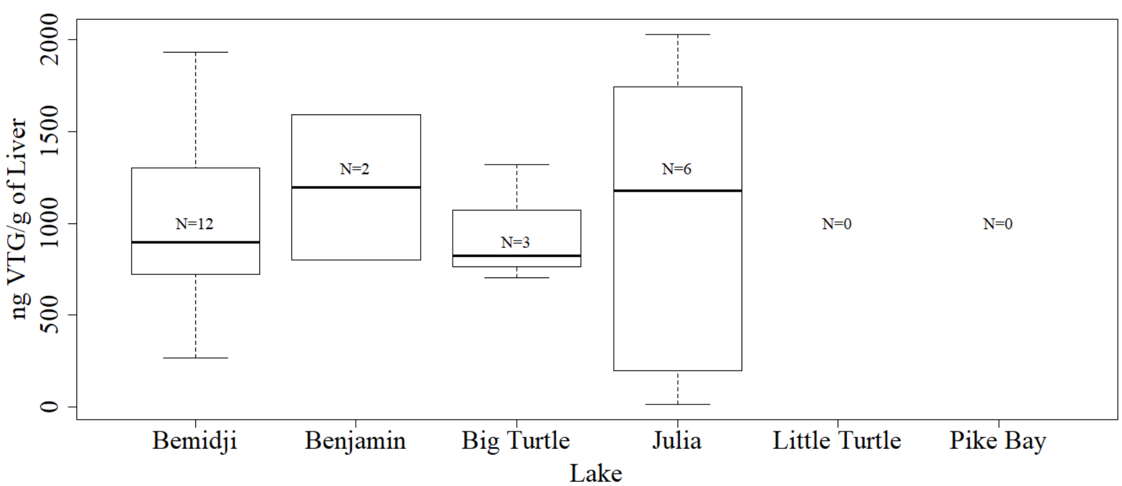

Figure C2. Vitellogenin levels from adult male Yellow Perch livers (ng) from six northern Minnesota lakes. No significant difference was found among male fish in lakes (Kruskal-Wallis; $\chi^2 = 0.38$, df = 3, $P = 0.95$).

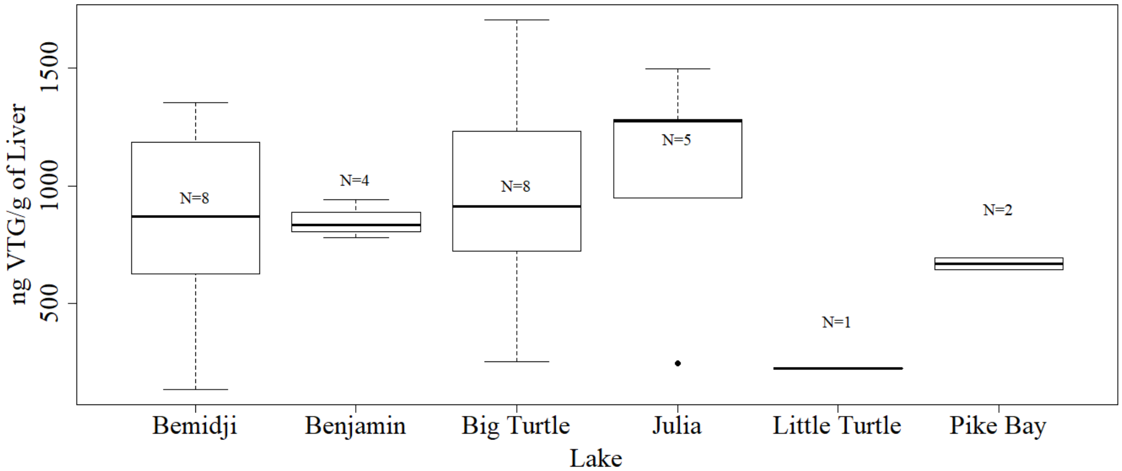

Figure C3. Vitellogenin levels from adult female Yellow Perch livers (ng) from six northern Minnesota lakes. No significant difference was found among female fish in lakes (Kruskal-Wallis; $\chi^2 = 5.97$, df = 5, $P = 0.31$).

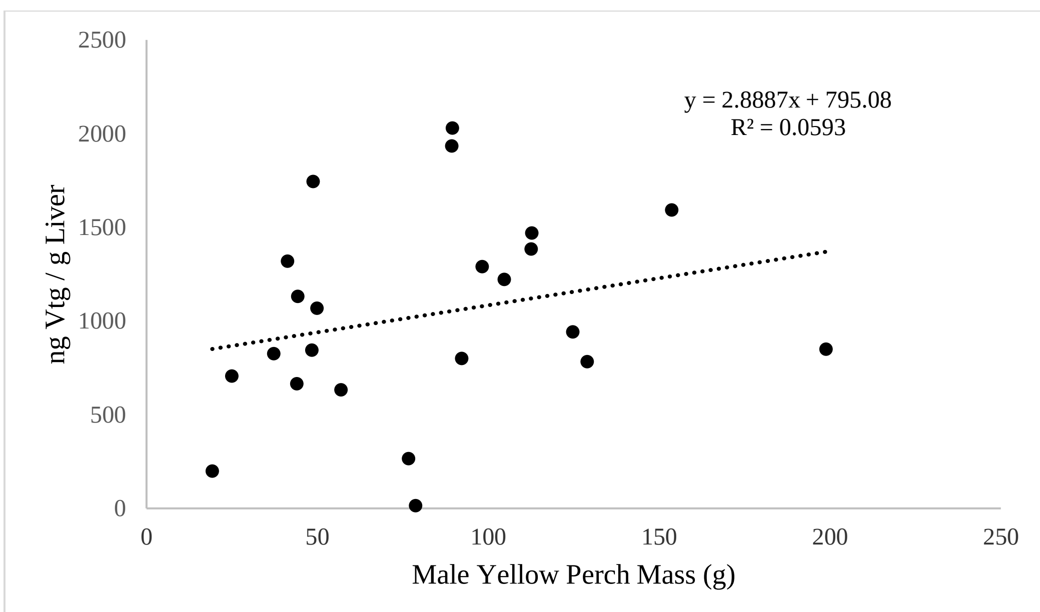

Figure C4. Vitellogenin levels from fish liver (ng) compared to the mass of male Yellow Perch, suggesting there is no significant realtionship between vitellogenin levels and size ($P = 0.08$).

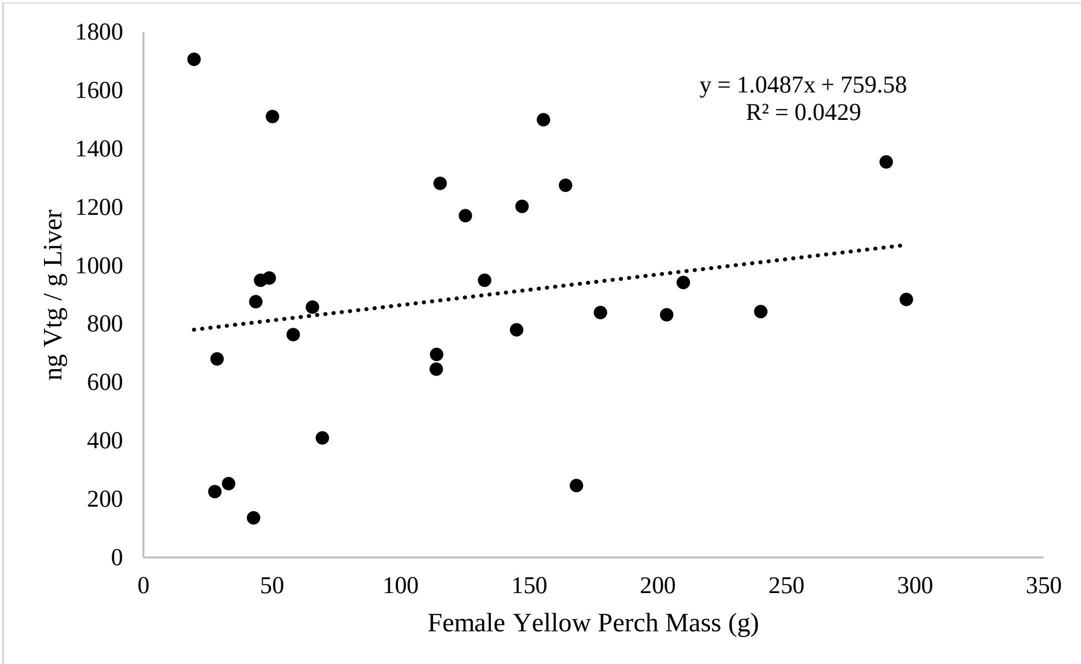

Figure C5. Vitellogenin levels in fish liver (ng) compared to the mass of female Yellow Perch, suggesting there is no significant realtionship between vitellogenin levels and size (P = 0.29).