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COLLEGE OF ARTS AND SCIENCES

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March 19, 2009

Edwin J. Hammett, Executive Director
Ohio Lake Erie Commission
One Maritime Plaza, 4th Floor
Toledo, OH 43604

Dear Ed,

I am enclosing a final report for the Lake Erie Protection Fund grant (project SG-3307). Included with the report is the final accounting, abstract, report, and copy of a manuscript under review in the *Journal of Great Lakes Research*. The small grant from the Lake Erie Protection Fund was essential to rapid response to the detection of VHSV in Lake Erie fish populations in 2006. From our sampling in 2007 and 2008, we have determined that infection is wide-spread in yellow perch near the outlet of the Chagrin River during the early Spring, but we did not document the level of mortality expected of these high levels of infection.

Sincerely,

A handwritten signature in cursive script that reads "Joseph F. Koonce".

Joseph F. Koonce
Professor and Chair

LAKE ERIE PROTECTION FUND

SMALL GRANT - FINAL ACCOUNTING

Grant Number: SG 330-07

v2008

Budget Categories	Original Budget	Funds Spent	Current Balance	Matching Funds
A. Salaries & Wages				
	4000.00	4000.00	0.00	7339.12
B. Fringe Benefits				
				2129.34
C. Total Salaries & Benefits (A+B)				
	\$4,000.00	\$4,000.00	\$0.00	\$9,468.46
D. Non-expendable Equipment				
E. Expendable Materials & Supplies				
supplies	490.00	1772.15	-1282.15	76.01
shipping	100.00	507.85	-407.85	
F. Travel				
G. Services or Consultants				
Lab Testing fees	4500.00	2810.00	1690.00	
H. Computer Costs				
I. Publications/Presentations				
J. All other direct costs				
K. Total Direct Costs (C thru J)				
	\$9,090.00	\$9,090.00	\$0.00	\$9,544.47
L. Indirect Costs				
10% TDC	909.00	909.00	0.00	954.45
Total Costs (K + L)				
	\$9,999.00	\$9,999.00	\$0.00	\$10,498.92

Ohio Lake Erie Commission
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I certify that the grant expenditures listed and descriptions of the charges are true and accurate to the best of my knowledge. These expenditures represent approved grant costs that have been previously paid for and for which complete documentation is on file.

Project Director Authorizing Agent Fiscal Agent	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 80%; text-align: center;"><i>Joseph D. Kooney</i></td> <td style="width: 20%; text-align: center;">Date</td> </tr> <tr> <td style="text-align: center;"><i>[Signature]</i></td> <td style="text-align: center;">3/4/2009</td> </tr> <tr> <td style="text-align: center;"><i>[Signature]</i></td> <td style="text-align: center;">03-12-2009</td> </tr> <tr> <td style="text-align: center;"><i>[Signature]</i></td> <td style="text-align: center;">3/6/09</td> </tr> </table>	<i>Joseph D. Kooney</i>	Date	<i>[Signature]</i>	3/4/2009	<i>[Signature]</i>	03-12-2009	<i>[Signature]</i>	3/6/09
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<i>[Signature]</i>	03-12-2009								
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The Prevalence of VHSV in Yellow Perch

A Technical Report to the Ohio Lake Erie Commission, Ohio Lake Erie Protection Fund

LEPF Project: SG-3307

Principal Investigator: Dr. Joseph Koonce
(joseph.koonce@case.edu)
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2080 Adelbert Rd
Cleveland, OH 44106-7080

Date:

Abstract

Viral Hemorrhagic Septicemia Virus (VHSV) infects wild and hatchery fish in Europe, Japan, and the Great Lakes and Pacific regions of North America. The virus was first detected in Lake Erie in 2006, when a spring fish kill was attributed to VHSV infection. To determine the infection pattern of VHSV, we sampled yellow perch, *Perca flavescens*, during the Spring, Summer, and Fall in 2007 and 2008 in the Central Basin of Lake Erie using bottom trawls in nearshore, mid-depth, and offshore locations near the Chagrin River. The Ohio Department of Agriculture's Diagnostic Laboratories and the U.S. Fish and Wildlife Service's La Crosse Fish Health Center tested for VHSV from homogenized samples obtained from yellow perch kidney, spleen, and brain. At each lake sample location, we also measured temperature, dissolved oxygen, and conductivity. In both years, the infection period occurred during a three-week period starting in the last week of spawning to early June. A high proportion of adult male and female yellow perch tested positive for VHSV during the infection period in our sample population. Infection appeared to be associated with temperatures between 12-18°C and with significantly higher yellow perch densities during spawning. These results suggest that yellow perch are susceptible for VHSV infection starting at the end of spawning and continuing for

three weeks while temperatures are below 20°C. No large mortalities of yellow perch occurred during the VHSV infection period in 2007 and 2008.

Activities and Timeline

Viral hemorrhagic septicemia virus was first detected in Lake Erie in 2006, when a spring fish kill was attributed to VHSV infection. The virus has high mortalities associated with infection (Meyers and Winton 1995) and has become great concern for the Great Lakes since it infects commercial and recreational fish. This project aimed to provide a descriptive analysis of VHSV infection in yellow perch, *Perca flavescens*, in Lake Erie in 2007 and 2008. Specifically by investigating 1) biological parameters of the fish such as age, sex, length, and weight and 2) collecting abiotic environmental parameters such as pH, conductivity, percent dissolved oxygen saturation, spatial distribution of infection, and fish density at each sampling site.

In coordination with a current Ohio Department of Natural Resources (ODNR) yellow perch survey we sub-sampled yellow perch from the ODNR's sampled population in the vicinity of the Chagrin watershed, approximately 625 km², in the central basin. We chose this sample site because of the large number of yellow perch found dead during the VHSV infection of 2006. For each fish, we measured total length to the nearest mm and weight to the nearest 0.1g. We recorded physical condition, sex, and determined age from otoliths. Fish ranging between 110 and 130 mm were considered yearlings. For VHSV infection we extracted kidney and spleen in 2007, 14 May to 29 October, and kidney, spleen, and brain in 2008, 21 April to 29 July, at nearshore, 19 ≤ 35 ft; mid depth, 36 ≤ 49 ft; and offshore, 50 ≤ 60 ft; locations. Pooled samples of 5 containing individuals in 2007 and 2 – 5 individuals in 2008 (2 individuals 14 May to 2 June and 5 individuals from 21 April to 5 May, 24 June, and 28 July) of the same sex and similar length were shipped overnight on ice to La Crosse Fish Health Center and Ohio Department of Agriculture Diagnostic Laboratory to test for VHSV.

In 2007, we estimated the probability of individual infection (p) as binomial function of the number of fish per pool (eqn 1).

$$p = 1 - (1 - P)^{1/v} \quad (1)$$

where P is the probability of a positive pools and v is the number of individuals per pool. The lower bound for a 95% confidence interval on the probability that all pools are positive (L_1) is determined by the standard confidence interval for a binomial population parameter: (eqn 2).

$$L_1 = \frac{\varepsilon}{\varepsilon + F_{0.025, 2, 2\varepsilon}} \quad (2)$$

where ε is the number of replicate pools.

At all trawling locations, we collected temperature, percent dissolved oxygen saturation, pH in 2007, and conductivity profiles in the water column with a YSI sonde (model 6620 V2). We considered surface values to be averages of measurements between 0.1 m and 0.8 m, while bottom values are the average of the last three values 0.5 m from the bottom. Latitude and longitude were also recorded at the beginning of each trawl snode. From trawling data, we calculated the fish density as catch per hectare (CPHT), which is the ratio the number of fish caught at the sample site to the product of width, duration, and velocity of the trawl. Trawl durations ranged from 4 to 10 minutes. The spawning season was considered to be 7 May to 29 May in 2007 and in 2008 1 May to 21 May (Knight, ODNR, unpublished data, pers.comm). We classified all trawl surveys to be pre-spawn before these dates and post-spawn after these corresponding dates in 2007 and 2008. We used a one-way ANOVA for unequal variance to determine the statistical significance of patterns of variability in densities during pre-spawn, spawn, and post-spawn by location using CPHT.

In 2007, we collected a total of 80 pools of which 48 pools were adults, 16 pools were yearlings, and 16 were young-of-the-year (YOY) and in 2008, we collected a total of 36 pools of

adult fish age-2 and older. For a 3 week period, 22 May to 6 June 2007 and 20 May to 2 June 2008, all adult pools tested positive for VHSV. Before and after this time period, all adult pooled samples tested negative. Yearlings and YOYs all tested negative for VHSV. In 2007, for twelve replicate pools of five fish, the lower bound on a 95% confidence interval for the all positive pools (Eq. 2) was 0.735, which corresponds to a 95% confidence interval of 0.23 to 1.00 probability of infection. A very small percentage of sampled fish exhibited typical clinical signs of infection, ie. external hemorrhaging, but the vast majority of fish appeared to be healthy. Infection with VHSV did not appear to be associated with the sex of the fish. On the two days that we detected the virus in fish, all adult males and females tested positive for the virus, and later summer sample dates had similar male and female percentages as dates with positive fish.

Only temperature and dissolved oxygen showed trends during the observation periods. The difference between surface and bottom temperatures increased steadily from early spring to the end of the summer. Dissolved oxygen saturation was relatively stable throughout the sample period at the surface, but began to decrease from the summer to fall. Temperatures ranged between 12-18°C during the 3 week infection period. Both in 2007 and 2008 ANOVA results suggested significant differences in densities between locations, nearshore, mid depth, and offshore ($P < 0.001$, $P < 0.001$) and by trawling date ($P = 0.001$, $P < 0.001$).

The project design included water analysis to detect VHSV; however, we were unable to run water samples since we did not have the facility and laboratory equipment or find a laboratory willing to run these samples. The main focus of the project aimed to determine the prevalence of VHSV in the yellow perch population at our sample site. A high proportion of fish tested positive for VHSV. In 2007 all pools during the infection period were sent only to La Crosse and tested positive and in 2008 both testing labs received tissue samples. All pools

except two sent to ODA tested positive and all pools sent to La Crosse tested negative. We estimated prevalence of infection for VHSV in 2007; however, with the contradictory results from the two labs in 2008 we were unable to calculate a reliable estimate of prevalence.

Work Products

This project employed one master student, Michelle Kane, and provided two undergraduates, Bryan Kinter and Greg Bergquist, independent research projects necessary for degree completion. The Cleveland Metroparks Zoo provided volunteers and research supplies. We also worked closely with the ODNR's Fairport Fisheries Research Station and provided ODNR with VHSV results and updates. We presented two posters at the Ohio Fish and Wildlife Conference in 2008 and one power point presentation at the Fish Health Committee Meeting in 2009. A paper has been composed and submitted for publication in the Journal of Great Lake's Research which is appended to this technical report.

References

Meyers, T. R. and J. R. Winton. 1995. Viral Hemorrhagic Septicemia Virus in North America. *Ann. Rev. Fish Diseases* 5:3-24.

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Viral Hemorrhagic Septicemia Virus Infection in Yellow Perch, *Perca flavescens*, in Lake Erie.

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All correspondences are to be directed to Michelle Kane

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Abstract

Viral Hemorrhagic Septicemia Virus (VHSV) infects wild and hatchery fish in Europe, Japan, and the Great Lakes and Pacific regions of North America. The virus was first detected in Lake Erie in 2006, when a spring fish kill was attributed to VHSV infection. To determine the infection pattern of VHSV, we sampled yellow perch, *Perca flavescens*, during the spring, summer, and fall in 2007 and 2008 in the central basin of Lake Erie using bottom trawls in nearshore, mid-depth, and offshore locations near the Chagrin River. The Ohio Department of Agriculture's Diagnostic Laboratories and the U.S. Fish and Wildlife Service's La Crosse Fish Health Center tested for VHSV from homogenized samples obtained from yellow perch kidney, spleen, and brain. At each lake sample location, we also measured temperature, dissolved oxygen, and conductivity. In both years, the infection period occurred during a three-week period starting in the last week of spawning to early June. A high proportion of adult male and female yellow perch tested positive for VHSV during the infection period. In our sample population, infection appeared to be associated with temperatures between 12-18°C and with significantly higher yellow perch densities during spawning. These results suggest that yellow perch are susceptible for VHSV infection starting at the end of spawning and continuing for three weeks while temperatures are below 20°C. No large mortalities of yellow perch occurred during the VHSV infection period in 2007 and 2008.

Keywords: VHSV; infection period; spawning; yellow perch

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3 Introduction
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6 Viral hemorrhagic septicemia virus (VHSV) is a fish pathogen that was first detected in
7 fish populations of Lake Erie in 2006 (Phillips, 2007). VHSV is an enveloped negative-strand
8 RNA virus belonging to the *Novirhabdovirus* genus of the *Rhabdoviridae* family (Einer-Jensen *et*
9 *al.*, 2004). The virus is spread by urine and female sex products. Gills are thought to be the
10 main portal of infection to the fish (Meyers *et al.* 1995, Lorenzen *et al.* 2000). Because the virus
11 is associated with an adult fish death rate of 25-75% and up to a 100% juvenile fish death rate,
12 there has been substantial concern about the effect of the virus on the health of fish populations
13 in the lake (Meyers *et al.* 1995).
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25 VHSV was first identified in 1962 Denmark in cultured rainbow trout fish farms and was
26 linked to a severe outbreak (Einer-Jensen *et al.*, 2004). Since its first detection in Denmark,
27 researchers sequenced and mapped VHSV into four genotypes (Snow *et al.*, 1999). To this date,
28 all VHSV detected in the Great Lakes region have been identified as belonging to IVb
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Department of Natural Resources (ODNR) issued a news release announcing the detection of VHSV in muskellunge gametes in Clear Fork Reservoir, which located in Richland and Morrow counties (ODNR, pers. comm.). These were the first VHSV positive samples found extending outside the Great Lakes watershed.

Because the 2006 Great Lakes survey found high levels of infection and mortality in Ohio's Lake Erie watershed, our studies focused on the prevalence of VHSV infection in the Lake Erie Central Basin yellow perch, *Perca flavescens*. To determine the distribution and timing of infection, we sampled yellow perch in the vicinity of the Chagrin River and investigated the associations between infection and fish age, sex, water quality variables. Our preliminary results indicated that there is a small window of infection starting the last week of spawning to early June when temperatures are between 12°C and 19°C. During this time period adult male and female fish were infected with the virus at all depth stratum of sampling locations. Temperature during the infection period appeared to be most associated with infection.

Methods

The Chagrin site is approximately 625 km² and is located in Ohio waters of the Central Basin (Fig. 1) of Lake Erie. We sampled yellow perch with a Yankee two-seam bottom trawl from 14 May to 29 October in 2007 and from 21 April to 29 July in 2008. In both years, we sub-sampled fish for detection of VHSV in tissues. For each fish, we measured total length to the nearest mm and weight to the nearest 0.1g. We recorded physical condition and sex and determined age from otoliths. Fish ranging between 110 and 130 mm were considered yearlings. In 2007, we pooled groups of five individuals with two to thirteen replicates per trawl. All pools

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consisted of fish of the same sex and similar length. Extracted tissues in 2007 included only kidney and spleen and in 2008, kidney, spleen, and brain. All tissue samples were extracted and homogenized in Hank's buffered salt solution and submitted to the U.S. Fish and Wildlife Service's La Crosse Fish Health Center (La Crosse) or the Ohio Department of Agriculture's Diagnostic Laboratory (ODA). Between each sample pool, we cleaned dissecting instruments and trays with 70% isopropanol or ethanol.

We estimated the probability of individual infection (p) as binomial function of the number of fish per pool (eqn 1).

$$p = 1 - (1 - P)^{1/v} \quad (1)$$

where P is the probability of a positive pools and v is the number of individuals per pool. The lower bound for a 95% confidence interval on the probability that all pools are positive (L_1) is determined by the standard confidence interval for a binomial population parameter: (eqn 2).

$$L_1 = \frac{\varepsilon}{\varepsilon + F_{0.025, 2, 2\varepsilon}} \quad (2)$$

where ε is the number of replicate pools.

At all trawling locations, we collected temperature, percent dissolved oxygen saturation, and conductivity profiles in the water column with a YSI sonde (model 6620 V2). We considered surface values to be averages of measurements between 0.1 m and 0.8 m, while bottom values are the average of the last three values 0.5 m from the bottom. Latitude and longitude were also recorded at the beginning of each trawl.snode From trawling data, we calculated the fish density as catch per hectare (CPHT), which is the ratio the number of fish caught at the sample site to the product of width, duration, and velocity of the trawl. Trawl durations ranged from 4 to 10 minutes. We demarcated three zones for the trawl samples: nearshore, from $19 \leq 35$ ft; mid depth, $36 \leq 49$ ft; and offshore from $50 \leq 60$ ft. The spawning season was considered to be 7

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3 May to 29 May and in 2008 1 May to 21 May (Knight, ODNR, unpublished data, pers.comm).

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5 We classified all trawl surveys to be pre-spawn before these dates and post-spawn after these
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7 corresponding dates in 2007 and 2008. We used a one-way ANOVA for unequal variance to
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9 determine the statistical significance of patterns of variability in densities during pre-spawn,
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11 spawn, and post-spawn by location using CPHT.
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14 Results

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17 In 2007, we collected a total of 80 pools from 15 May to 29 October of which 48 pools
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19 were adults, 16 pools were yearlings, and 16 were young-of-the-year (YOY). On 22 May and 6
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21 June, all adult pools tested positive for VHSV (Table 1 and Fig. 2). Yearlings and YOYs all
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23 tested negative for VHSV. For twelve replicate pools of five fish, the lower bound on a 95%
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25 confidence interval for the all positive pools (Eq. 2) was 0.735, which corresponds to a 95%
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29 confidence interval of 0.23 to 1.00 probability of infection. Before and after this time period, all
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31 adult pooled samples tested negative. A very small percentage of sampled fish exhibited typical
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33 clinical signs of infection, ie. external hemorrhaging, but the vast majority of fish appeared to be
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35 healthy. Only temperature and dissolved oxygen showed trends during the observation period.
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37 The difference between surface and bottom temperatures increased steadily from early spring to
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39 the end of the summer (Table 2). Dissolved oxygen saturation was relatively stable throughout
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41 the sample period at the surface (Table 2). ANOVA results suggested significant differences in
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43 densities between locations, nearshore, mid depth, and offshore ($P < 0.001$) and by trawling date
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45 ($P = 0.001$). The highest density of fish for mid depth and offshore occurred during the
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47 spawning and the highest density for nearshore occurred the week before spawn (Fig. 3A).
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52 Because VHSV can be detected in ovarian fluids (Meyers *et al.*, 1995), we calculated the
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54 percentage of males and females on sampling dates. Infection with VHSV did not appear to be
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associated with the sex of the fish. On the two days that we detected the virus in fish, all adult males and females tested positive for the virus, and later summer sample dates had similar male and female percentages as dates with positive fish (data not shown).

In 2008, we collected a total of 36 pools of adult fish age-2 and older during expected pre-infection and 24 post-infection periods, and 82 pools during expected infection period. La Crosse received samples during the expected pre-infection period, ODA and La Crosse received samples during the expected infection period, and ODA received samples during the expected post-infection period (Table 1). During the expected pre- and post-infection periods, all pools tested negative for infection. During the expected infection period, results varied. All pools on 14 May sent to La Crosse and ODA tested negative. On 20 May 10 out of 11 pools sent to the ODA tested positive, but 12 pools sent to La Crosse tested negative. On 28 May 10 out of 11 pools sent to ODA tested positive and 12 pools sent to La Crosse tested negative. While on 2 June all 12 pools sent to ODA tested positive and all 12 pools sent to La Crosse tested negative (Table 1). A very small percentage of sampled fish exhibited clinical signs of infection, but the vast majority of fish appeared to be healthy.

In 2008, the difference between surface and bottom temperatures increased with depth strata and season (Table 2). Both surface and bottom dissolved oxygen saturation decreased as temperature increased and generally did not show variation among locations except on the water surface recordings on 25 June (Table 2). ANOVA results showed significant difference in density by location ($P < 0.001$) and trawling date ($P < 0.001$). The highest density for mid shore locations occurred during the first two weeks of spawning while for offshore locations, highest catches occurred the week before spawn. Nearshore density remained relatively the same throughout the whole sampling period (Fig. 3B).

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Discussion

Results from 2007 and 2008 VHSV sampling surveys determined the time period of VHSV infection in yellow perch and factors associated with infection. Detection of infection started post-spawn and lasted for three weeks. Factors associated with infection include temperature and density. Cases of infection occurred at all sampling locations, suggesting distribution of fish did not play a role in the infection pattern.

Prior to infection, yellow perch were consistently exposed to higher densities in all depth locations, especially right before spawning and mid-way through spawning (approximately 3.5 weeks). We did not detect infection until the last week of spawning when densities were lower than previous weeks. Because VHSV is not detectable until 4 to 6 days after exposure at medium doses of virus, $10^{3.5}$ PFU/mL, (Kocan *et al.* 1997), it is reasonable to assume that spawning aggregations contributed to the epizootic. Hershberger *et al.* (1999), for example, proposed that prolonged crowding of pacific herring increases the probability of VHSV exposure in water. Also, previous research on infectious hematopoietic necrosis, another fish rhabdovirus, suggested that fish density had a direct relationship with prevalence after the exposure of an infected individual in rainbow trout (Ogut *et al.*, 2004). Webemeyer (1970) demonstrated that elevated stress is associated with infection in fish; therefore, the aggregate effect of increased fish densities and physiological stress that occur during spawning may contribute to a greater susceptibility to infection.

Water temperature during post spawn also appeared to be associated with VHSV infection. In the post-spawning interval, the temperatures are within the optimal range of viral multiplication. Both surface and bottom temperatures recorded below 10°C during spawning

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while post spawn temperatures recorded between 12-18°C. VHSV transmission occurs in water temperatures between 1-12°C with optimum growth at 15°C. The IVb strain is best isolated between 15-18°C, with growth efficiency decreasing when temperatures are above 20°C (Meyers *et al.* 1995, USGS, 2007). Other environmental parameters did not appear to be associated with infection, but dissolved oxygen saturation is lower during the infection period and in the summer compared to pre-spawn and spawning.

With the La Crosse 2007 results, we expected all or at least most pools from 2008 to be positive for VHSV during the three-week period between the end of May and early June; therefore, we decreased our sample size from five individuals per pool to two to better estimate the proportion of VHSV infected individuals. Because we randomly selected samples to go to either lab, it is unlikely that all the positive fish sampled on the same date and from the same trawl were sent to ODA. Between years, sampling differed only in pool size (N=5 in 2007, N=2 in 2008). ODA follows The World Organization of Animal Health (OIE) protocol and used the RTG-2 cell line, while La Crosse follows the American Fisheries Society (AFS) protocol and used the EPC cell line. Both protocols recommend both of these cell lines for VHSV testing. For the European VHSV viral strains, RTG-2 and BF-2 proved to be more sensitive for VHSV growth in cell culture instead of EPC cell lines (Lorenzen *et al.* 1999). However, it is recommended that the EPC cell line be used to culture the North American IV and IVb strains, because it has a higher sensitivity compared to other cell lines used for testing (USGS, 2007). Adding further to the complexity of the 2008 results are the ODNR results for freshwater drum (*Aplodinotus grunniens*). ODNR sent freshwater drum and emerald shiners on 20 May for VHSV testing to La Crosse. Their pools consisted of the standard five fish and came from the

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same location as our yellow perch samples (Insley, ODNR, pers. comm.). Freshwater drum tested positive for VHSV on 20 May and 4 June while emerald shiners tested negative.

It is not known why this inconsistency in the results occurred, and the discrepancy cannot be answered within the context of this research, but this difference brings up serious concerns and questions about the exact methods used to detect the virus within different Great Lakes species. It is not clear whether the sensitivity and specificity of VHSV testing change with each species or if the virus sequence variability among species is sufficient to cause problems with cell line sensitivity. Generating more viral sequences in different fish species may aid in determining if the virus sequence is changing enough between species to cause such a discrepancy.

Although we detected very high levels of infection in yellow perch, we did not observe the high mortality rates seen in laboratory experiments with other fish species (Isshiki *et al.* 2001), nor did we observe a repeat of the mortality pattern observed in 2006. The VHSV infection we observed in yellow perch after the 2007 and 2008 spawning seasons thus seems to affect only morbidity. Bioenergetics analysis of yellow perch demonstrated that maximum growth occurs at about 18°C at the end of May (Kitchell and Stewart, 1977), which is during the infection period. Assuming that fish will eat less while infected implies that the growth rate of yellow perch infected with VHSV could be reduced. Follow up studies of the bioenergetics of VHSV infected yellow perch would thus seem to be a high priority.

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Figure legends

Fig. 1 Chagrin Study Site.

Fig. 2 Fish Density and location of positive and negative VHSV fish during 2007 and 2008 infection period. Negative results are black dots and positive are white crosses respectively. Crossed hatch areas represent multiple pools from the same location. 29 May density map includes 28 May and 2 June VHSV sampling dates. Contours represent 5 m depth strata.

Fig. 3 Average density of yellow perch per week. The average density, in catch per hectare trawled (CPHT), for 2007 (A) and 2008 (B) for nearshore (Near), midshore (Mid), and offshore (Off) locations on each trawling date from pre to post spawn are graphically presented in this figure. *Sp* indicates spawning and *Inf* indicates infection period. The 1 June, 2008 infection date is not included since it was a VHSV sampling date, thus no density data was collected.

Table 1 VHSV results for 2007 and 2008. For 2007 and 2008 kidney and spleen (KS) and brain (B) pooled samples are listed and separated by laboratory, La Crosse Fish Health Center (La Crosse) and the Ohio Department of Agriculture (ODA), and by positive or negative results on each date of collection. A number listed under positive represents the number of pools found positive for VHSV and likewise for negative results. Dashes indicate no pools tested positive or negative on sampling date. 2007 results include adults (age 2+), yearlings, and young of the year yellow perch while 2008 results include only adult yellow perch.

Table 2 2007 and 2008 surface and bottom temperature and % DO at each depth strata at sampling locations on trawling dates. Dates with missing values indicate insufficient data for analysis or a VHSV sampling date.

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Figure 1
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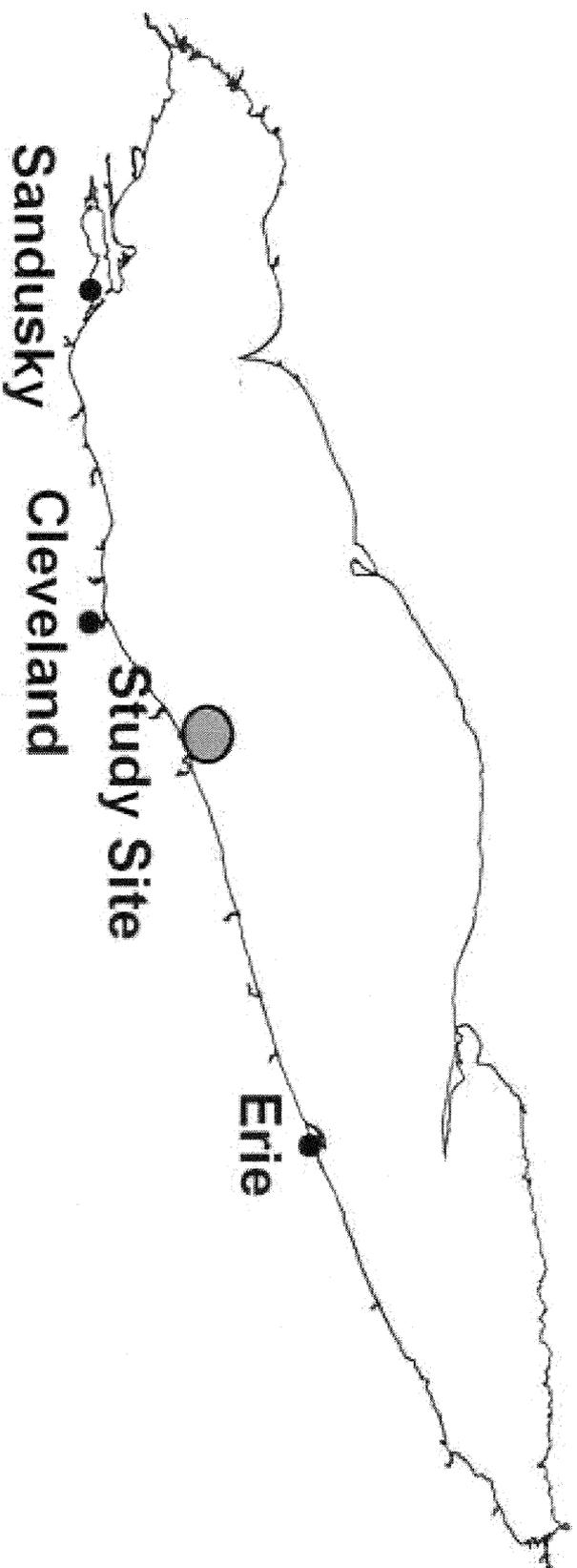


Figure 2
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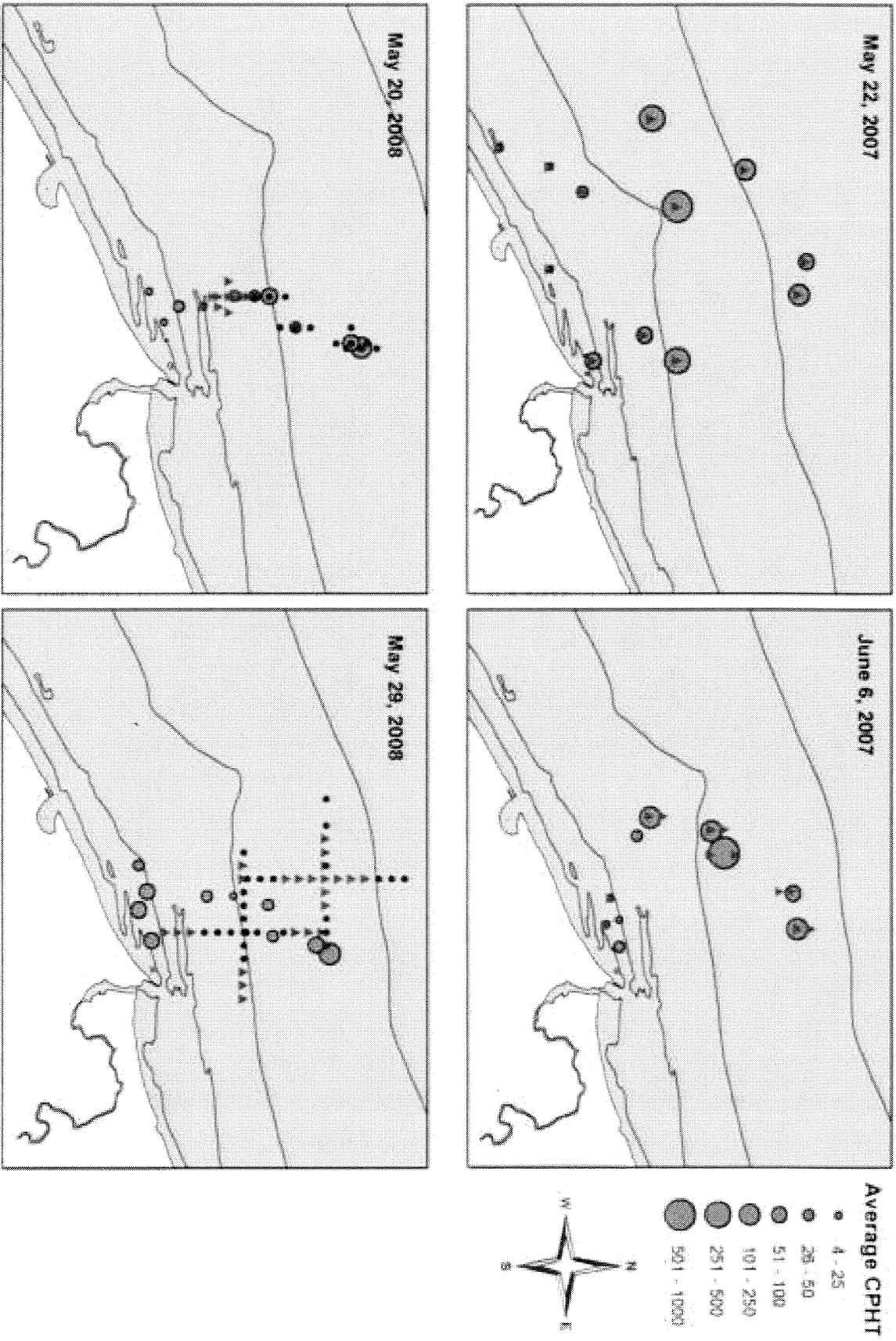


Figure 3

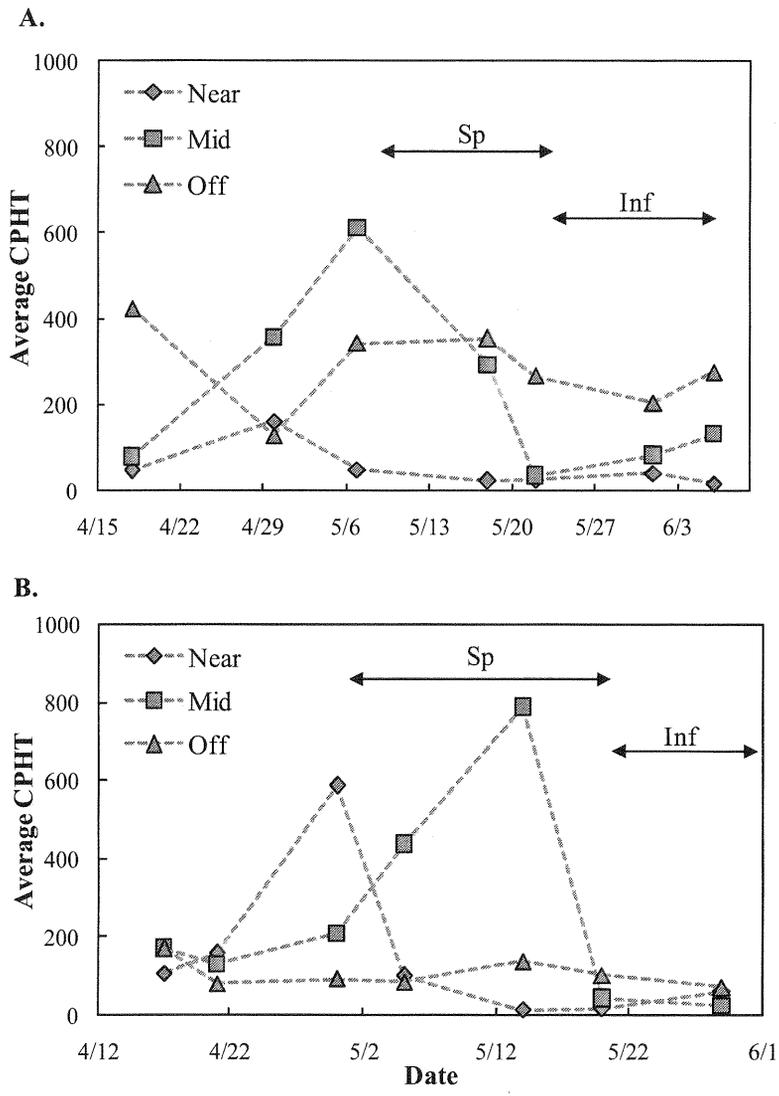


Table 1

Date	Lab	Tissue	Number fish/pool	Total Number fish	Number Pools	Positive	Negative
2007							
May 15	La Crosse	KS	5	30	6	-	6
May 22	La Crosse	KS	5	25	5	5	-
June 6	La Crosse	KS	5	60	12	12	-
June 18	La Crosse	KS	5	60	12	-	12
July 5	La Crosse	KS	5	10	2	-	2
Jul 23	La Crosse	KS	5	60	12	-	12
Sept 5	ODA	KS	5	65	13	-	13
Oct 29	ODA	KS	5	65	13	-	13
2008							
April 21	La Crosse	KS & B	5	30	12	-	12
April 30	La Crosse	KS & B	5	30	12	-	12
May 5	La Crosse	KS & B	5	30	12	-	12
May 14	La Crosse	KS	2	24	12	-	12
	ODA	KS	2	22	11	-	11
May 20	La Crosse	KS	2	24	12	-	12
	ODA	KS	2	22	11	10	1
May 28	La Crosse	KS	2	24	12	-	12
	ODA	KS	2	24	12	10	1
June 2	La Crosse	KS	2	24	12	-	12
	ODA	KS	2	24	12	12	-
June 24	ODA	KS & B	5	30	12	-	12
July 28	ODA	KS & B	5	30	12	-	12

Table 2

		Surface				Bottom				
		Depth Strata (m)				Depth Strata (m)				
Date		5	10	15	20	Date	5	10	15	20
2007										
Temp °C	April 18	5	4.3	4.1		April 18	5	4.3	4	
	April 30	9.7	8.75	8		April 30	8.8	7.8	6.65	
	May 7	9.8	9.8	9.8		May 7	9	8.1	7.8	
	May 18	11.4	10.2	9.6		May 18	10.1	9.5	9.1	
	May 22	12.7	12.3	11.8	11	May 22	12.4	11.3	9.5	6.6
	June 6	19.25	17.25	17.1		June 6	19.3	17.0	16.2	
	June 18	20.65	19.65	20.71	20.86	June 18	14.9	9.3	7.7	12.6
	July 18	23.95	24	23.33	22.63	July 18	22.9	22.8	19.4	11.1
	July 23	22.74	22.57		22.09	July 23	22.3	21.5	19.4	10.9
	Spetember 5			23.7		Spetember 5			20.64	
					October 29		14.4			
% DO Saturation	April 18					April 18				
	April 30					April 30				
	May 7					May 7				
	May 18	95	96	98		May 18	90	92	92	
	May 22	99	101	102		May 22	99	90	92	
	June 6	100.367	100.5	101.975		June 6	101.3	97.7	91.4	
	June 18	90.82	107.3	100	103.385	June 18	57.9	83.1	79.3	88.4
	July 18	98.04	98.9565	102.369	103.385	July 18	87.3	88.4	73.9	64.4
	July 23	98.6	98.5		104.1	July 23	88.5	92.8		68.2
	Spetember 5			100		Spetember 5			90	
					October 29					
2008										
Temp °C	April 21	9.35	9.59	7.66		April 21	6.82	5.44	5.67	
	April 30	8.95	8.77	6.91		April 30	8.02	7.72	6.57	
	May 5	11.08	9.75	9.66		May 5	10.95	9.47	8.91	
	May 14	11.57	12.04	10.14		May 14	10.13	9.59	8.63	
	May 20		12.73	11.55		May 20		12.59	11.55	
	May 29	14.16	13.67	14.54		May 29	13.37	12.56	11.89	
	June 2			14.53		June 2			13.57	
	June 25	21.84	21.32	20.49		June 25	11.014	16.034	13.09	
	July 23				23.84	July 23				12.69
	% DO Saturation	April 21	109.842	113.03	115.50		April 21	107.42	110.10	106.70
April 30		108.40	105.03	107.89		April 30	110.33	113.50	122.50	
May 5		105.70	97.19	103.30		May 5	105.70	101.70	105.77	
May 14		100.94	99.70	99.46		May 14	99.57	100.83	101.93	
May 20			91.90	93.50		May 20		93.100	100.63	
May 29		92.85	94.58	96.11		May 29	95.200	92.267	87.033	
June 2				57.22		June 2			99.133	
June 25		96.01	45.46	81.00		June 25	77.600	58.67	66.800	
July 23					100.73	July 23				30.67