

VHSV Research Products Influencing Management and Regulatory Decisions



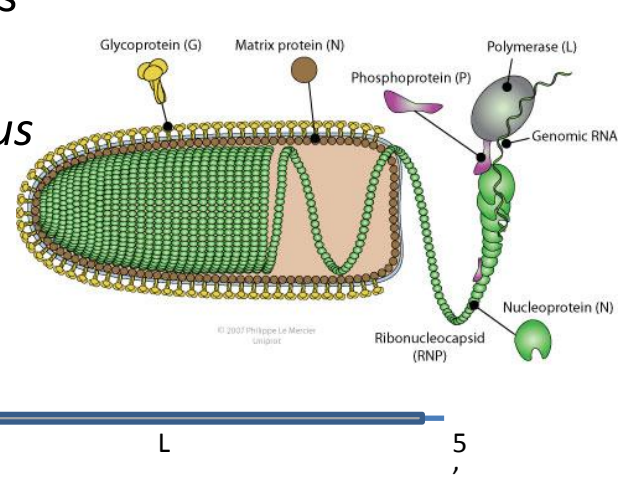
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MP45B-14

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VHSV background

- Viral Hemorrhagic Septicemia virus (VHSV)
- Rhabdovirus genus *Novirhabdovirus*
- Bullet shaped viral particles
- Negative sense ssRNA
- 11,000 nucleotides



Viral hemorrhagic septicemia virus (VHSV) is a rhabdovirus endemic in fish from oceans of the northern hemisphere and freshwaters of Europe. There are four genotypes of VHSV. Types I, II, and III occur in Europe. Types IVa and IVb occur in North America.



- VHSV has caused extensive losses of cultured and wild fish and has most recently become established in the Great Lakes. Fish kills caused by VHSV have alarmed the public and provoked government attention on pathogen introductions into US waters.
- Our NY Sea Grant research project was one of the first extramurally funded efforts to study the emergence of VHSV type IVb. The research has had a number of significant impacts.

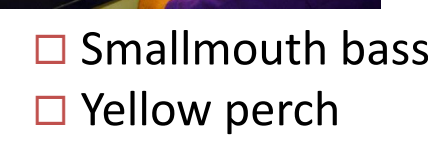


Outbreak investigations

- In 2006 and 2007 significant VHS mortalities among muskellunge, round gobies, freshwater drum, smallmouth bass, gizzard shad, and yellow perch occurred.
- Healthy fish survey was conducted in St. Lawrence River by SUNY-ESF during late May to early June 2006.
- Samples frozen and transported to Cornell for processing.
- Approximately 300 fish from 18 locations along the St. Lawrence River near the Thousand Islands Biological Station.



- VHSV confirmed in these healthy fish:
 - Black crappie
 - Bluegill
 - Brown bullhead
 - Channel catfish



- Smallmouth bass
- Yellow perch

- A pilot survey of New York State baitfish conducted in 2006 detected high copy numbers of VHSV.
 - Healthy baitfish minnows collected.
 - Four pooled samples from three sites for VHSV testing (5 fish each)
 - Confirmed VHSV:
 - Bluntnose minnows: St. Lawrence River
 - Emerald shiners: Niagara River
 - Emerald shiners: Lake Erie



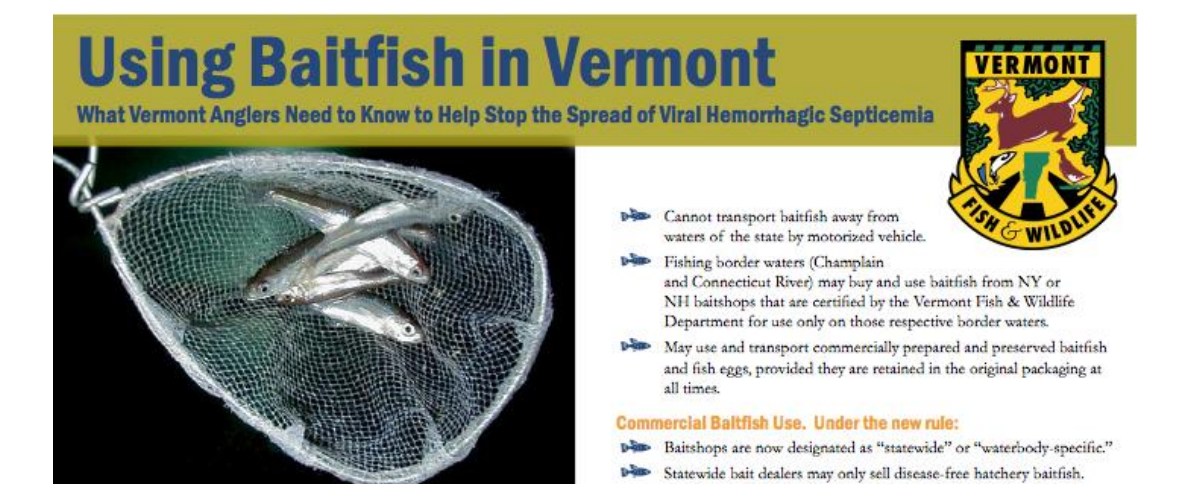
Fish health regulations in response to VHS



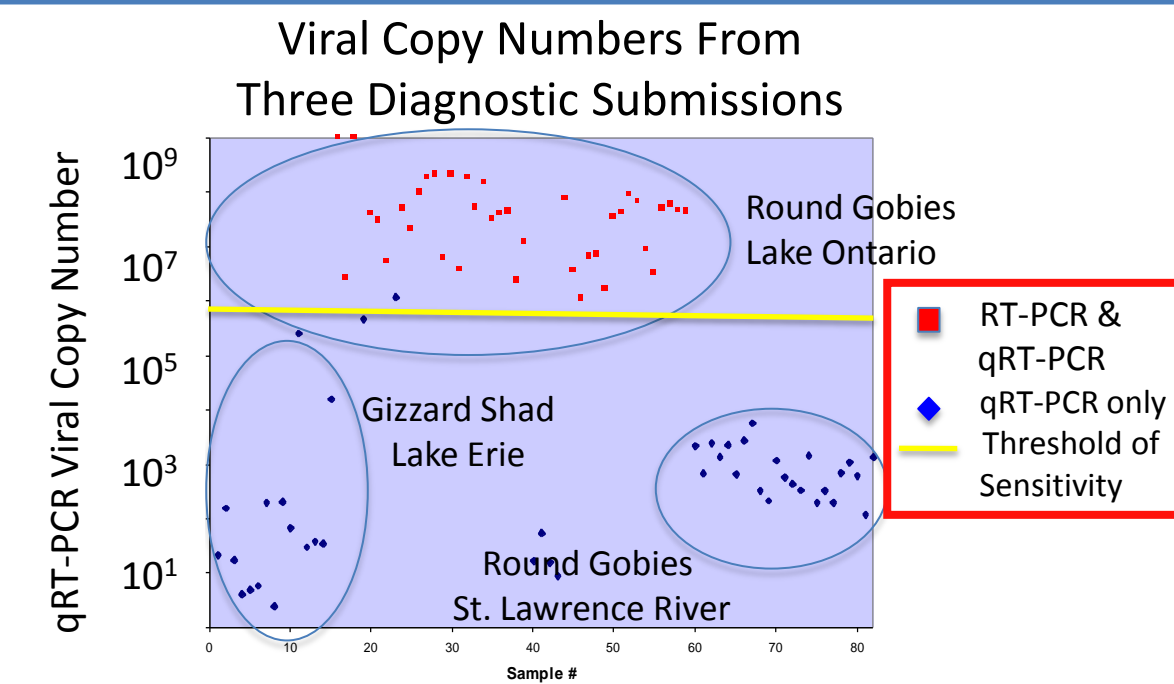
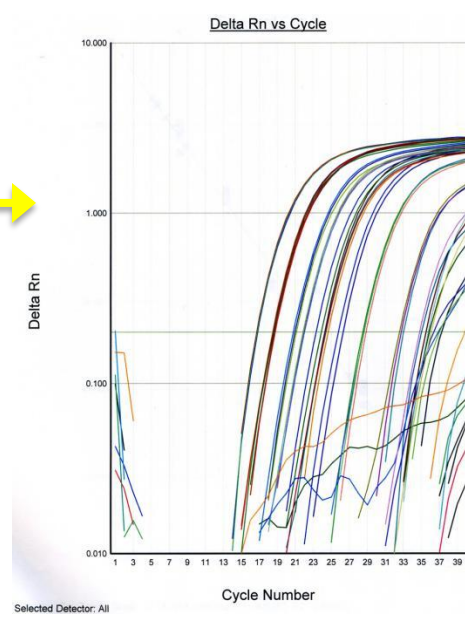
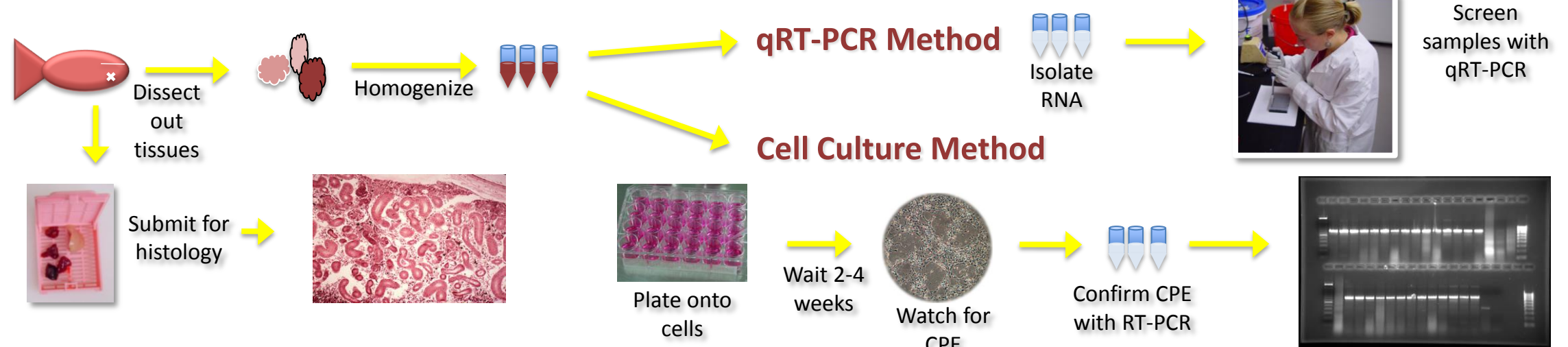
Viral Hemorrhagic Septicemia in the Great Lakes July 2006 Emerging Disease Notice



Questions and Answers About Viral Hemorrhagic Septicemia (VHS) Federal Order November 2006



Rapid, sensitive diagnostic qRT-PCR developed

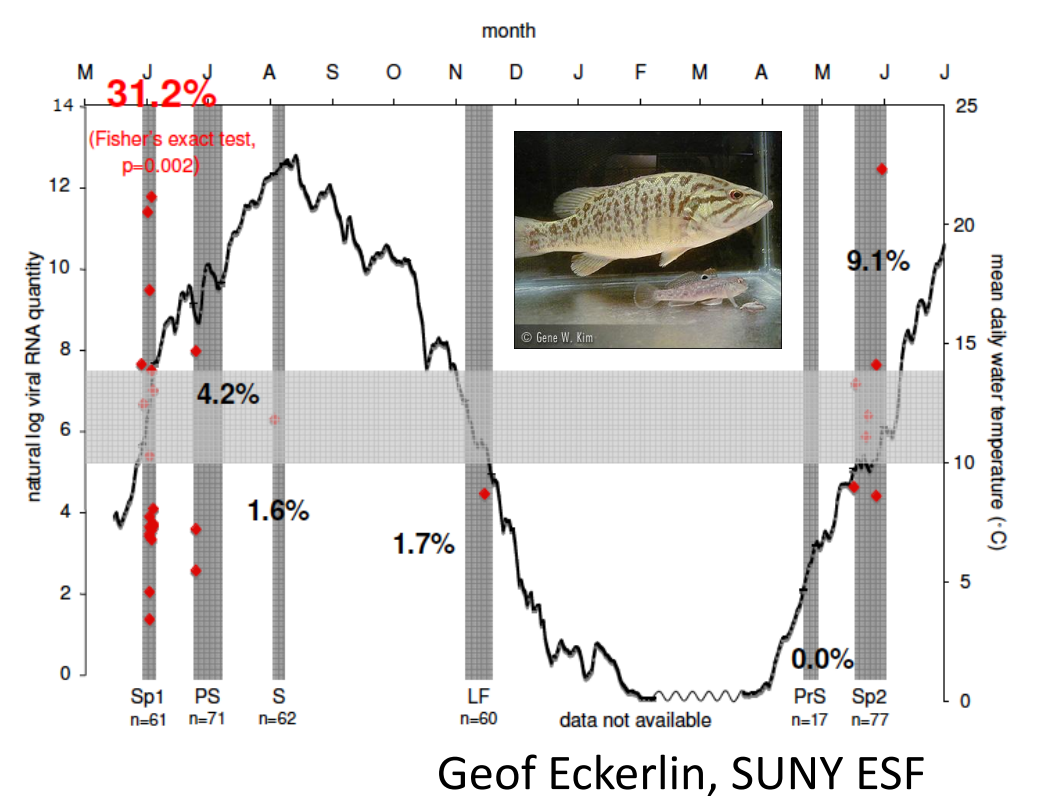


qRT-PCR assay evaluated for regulatory use

- 2008 RESOLUTION: The United States Animal Health Association requests that the USDA APHIS, Veterinary Services evaluate and validate the Canadian (all strains) and/or Cornell (strain IVb) polymerase chain reaction (qRT-PCR) assay for the detection of VHSV.
- The test will be used to monitor the spread of VHSV in wild fish and to satisfy VHSV interstate movement requirements for regulated species of fish as determined by USDA APHIS.
- Published Cornell method in Hope et al. 2010 titled: Comparison of quantitative RT-PCR with cell culture to detect viral hemorrhagic septicemia virus IVb (VHSV IVb) infections in the Great Lakes. *Journal of Aquatic Animal Health* 22(1): 50-61.

Seasonal studies

- Smallmouth bass (SMB) and round gobies (RG) surveyed for 15 months
- Strong yearly VHSV outbreak trend observed
- Results can be used to focus monitoring effort
- VHSV affected juvenile SMB more than adults
- Smallmouth may be selecting RG as prey



Round goby may be a reservoir host

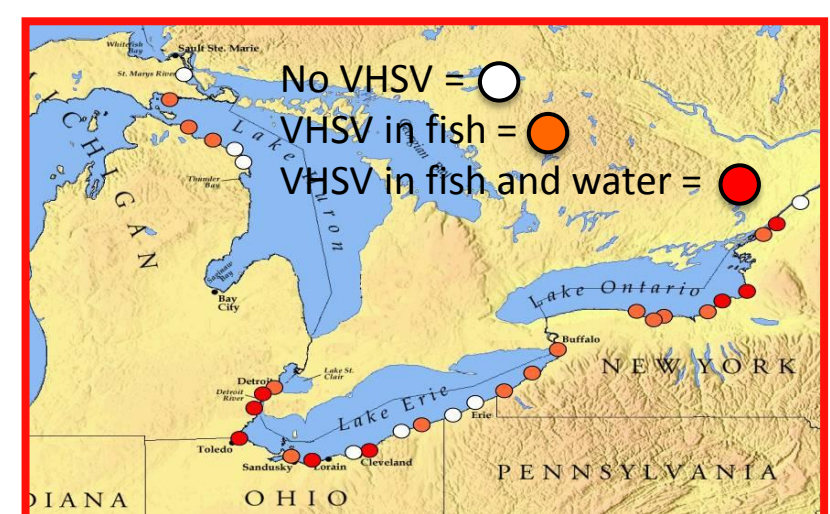
- Round goby densities follow same general spatial pattern as VHSV prevalence
- High densities, serially stressed
- Highly affected by VHSV, though still hyper-abundant
- Aggressive, social, and highly integrated into food web
- Virus present among RG during pre-spawn when not found among SMB
- Extremely high viral levels (1.4 X 10⁷)



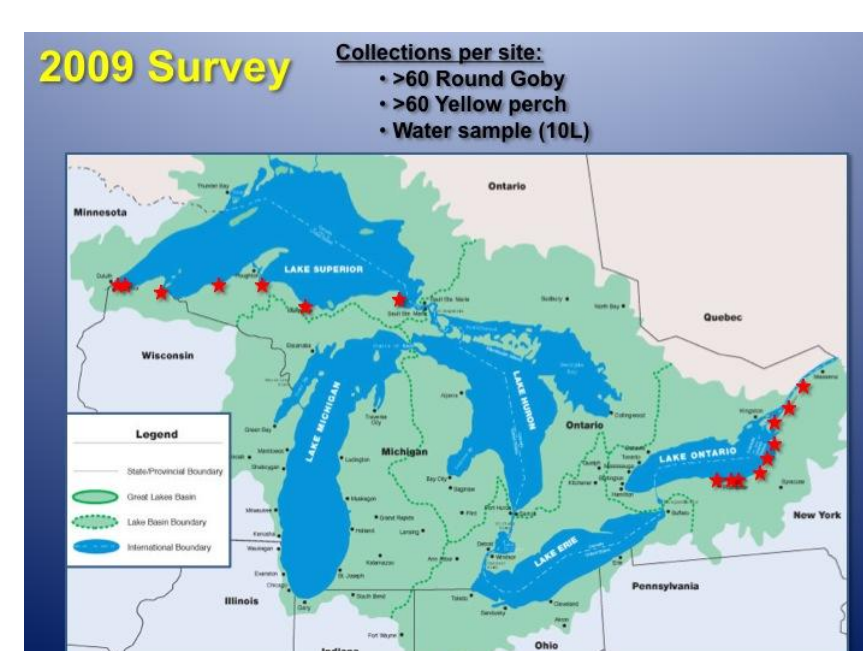
Optimal testing window established

- Bilateral VHSV Surveillance Working Group made up of the Canadian Food Inspection Agency, Aquatic Animal Health Division; Great Lakes Fish Health Committee; USDA APHIS; and USFWS has proposed VHSV sampling be conducted in the spring when water temperatures are rising, or in the fall when water temperatures are dropping.
- Ideally, aiming for water temperatures above 2 and below 20°C.
- Targeting surveillance to population segments with higher expected prevalence or risk of infection can reduce surveillance costs.
- Our studies of the effects of environmental variables like temperature and seasonality has helped management agencies to understand the epidemiology of VHS.

Great Lakes surveillance



- 2008 Survey Results
 - VHSV is widespread in fish and water samples in the lower Great Lakes region even in the absence of disease.
 - VHSV can be detected at sub-clinical levels by qRT-PCR and cell culture.



- 2009 Survey Results
 - The results from 874 fish sampled from 7 sites in Lake Superior showed low level infections from 4 of the tested sites.
 - These are the first VHSV positive samples from Lake Superior.
 - Carrier-level infections continued to be found in Lake Ontario (9/9 sites).



VHSV detected in water and healthy carriers

- Surveillance has identified susceptible species that might serve as reservoirs or carriers. These endemic reservoirs among free-ranging fish pose a long-term threat to Great Lakes fisheries.
- The results will further our understanding of how the virus is maintained and transmitted in natural systems and can be used to predict future effects upon wild species.
- The current distribution of infected carriers are risks for aquaculture managers to consider in their decision making.
- We demonstrated that pathogen distribution information could be developed quickly and is clearly needed for aquatic ecosystem conservation, management of affected populations, and informed regulation of the worldwide trade of aquatic organisms.

Infection trials

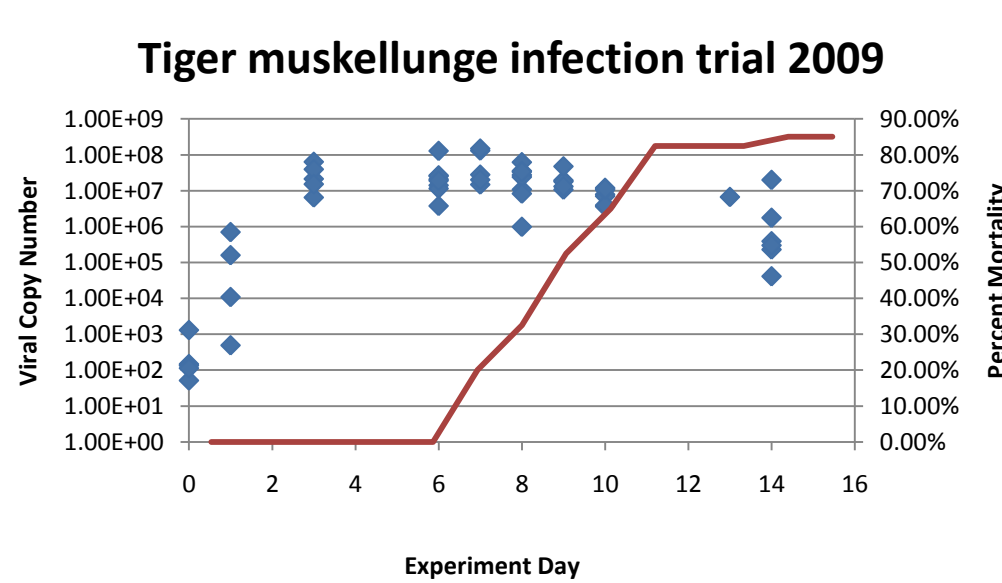
2009 Tiger Muskellunge trials

- Injected with 10⁶ pfu/fish IP
- Temperature 10 °C ± 0.5
- Thermal shock: 15 °C to 10 °C
- Immunosuppression of fish
- Ideal temperature for viral replication



- Multiple multifocal hemorrhages noted externally on body surfaces and gills, with occasional intraocular hemorrhages.
- Internal hemorrhages seen on swim bladder and peritoneum as well as on omental fat or GI tract.
- Kidneys swollen and friable.
- Intramuscular hemorrhages seen.
- All samples were positive by cell culture and qRT-PCR.

Tiger Muskellunge Mortality Curve



Aquacultured species susceptibility varied

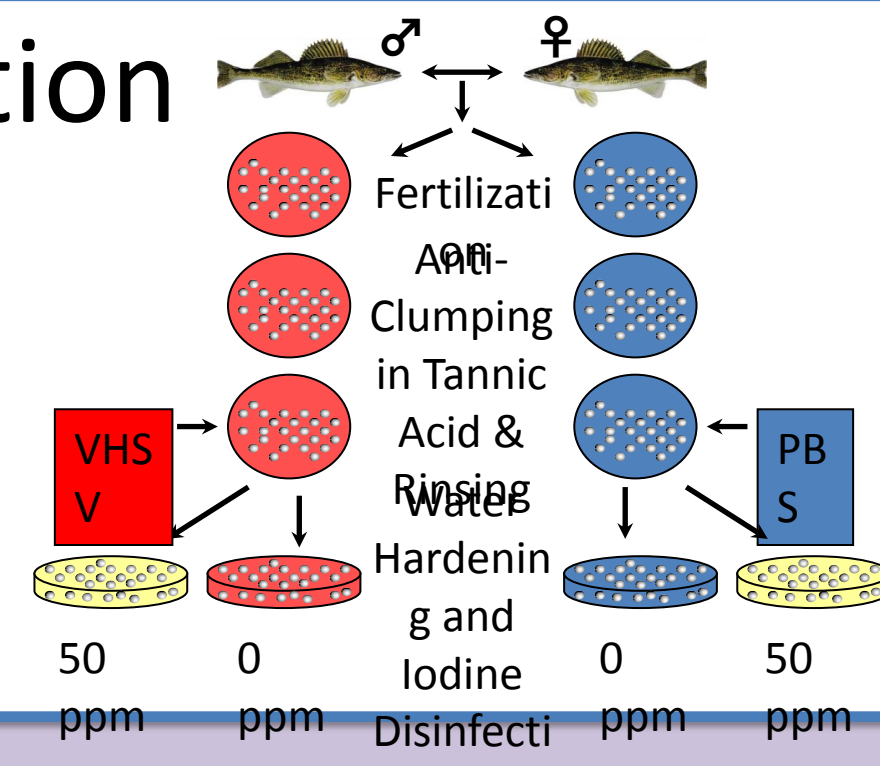
- Species susceptibility is of great importance to aquaculture and natural resources regulatory agencies.
- Comparative susceptibility of tiger muskellunge, fathead minnows, emerald shiners, channel catfish, rainbow trout, Atlantic salmon, walleye, and hybrid striped bass to VHSV type IVb is being provided to the Extension/Outreach team members for inclusion in VHSV workshops and in web-based materials such as The VHS "EDUCATION ALLIANCE" (www.aphis.usda.gov/animal_health/animal_dis_spec/aquaculture/downloads/vhs_education_alliance.pdf).



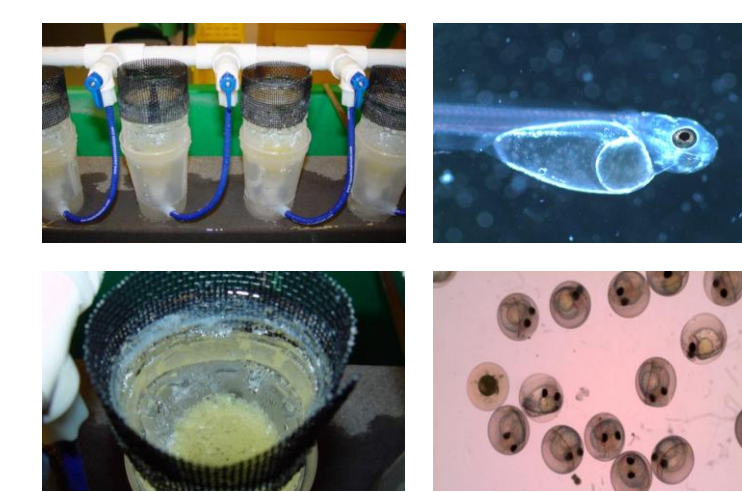
Walleye egg disinfection



- Our latest NY Sea Grant funded effort will establish an effective egg disinfection procedure for cool water fish species.



Mini hatching jars for walleye eggs



VHSV detected in iodophore treated and untreated groups

Day	Cell Culture				QRT-PCR			
	0 ppm	50 ppm	0 ppm	50 ppm	0 ppm	50 ppm	0 ppm	50 ppm
0	+	+	+	+	+	+	+	+
1	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+
End	+	+	+	+	+	+	+	+

Effective iodophore dose tested

- Previously, effectiveness of the iodophore disinfection technique was uncertain for the eggs from cool water fish species like walleye.
- Clearly, 50 ppm iodophore egg treatment for 30 minutes may not be adequate.
- The results from Year 1 of this project have been described to scientists and policy makers from NYSDEC, USDA APHIS, USGS, USFWS, and numerous universities this year.
- We have also worked closely with our NY Sea Grant fisheries specialist, Dave Mac Neill on a variety of efforts involving VHSV outreach, including our egg disinfection trials.