GREAT LAKES FISH HEALTH COMMITTEE

2008 Annual Meeting
Windsor, Ontario
January 29-31, 2008

Minutes
(with attachments)

Submitted By:
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GREAT LAKES FISHERY COMMISSION
2100 Commonwealth Blvd
Ann Arbor, Michigan 48105-1563

Great Lakes Fish Health Committee
2008 Annual Meeting

Windsor, Ontario
January 29-31, 2008

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List of Attendees

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Final Draft Agenda - Great Lakes Fish Health Committee Meeting

Windsor Hilton, Windsor, Ontario
January 29-31, 2008

January 29, 2008 Agenda – Start Time 10 AM

Opening remarks and introductions

1. Decision Item – 2008 Meeting Minute Approval – Andy Noyes, NY DEC 1/29 10:00 AM – 15 minutes

Minutes are near completion. Related documents and files will be included and sent to John Dettmers for web posting. The committee agreed that we have no need to produce paper copies since minutes are available on the web. Perhaps decision items should be formatted for easy access. The committee discussed what format should be used for drafting the minutes

Action Item: Minutes will get posted to the web very much like technical committee minutes. Lake Superior committee minutes are a suitable model to follow; the table of contents are more elaborate than we've done and key decision points will be included. The committee agreed to transition to a new format (see Lake Superior minutes for example).

2007 minutes are approved.

2. Decision Item – Secretary Election – Gary Whelan, MI DNR 1/29 10:15 AM – 15 minutes

Greg Wright suggested that we reorganize our current committee structure and create an "assistant chair" to replace the "secretary" position. The host agency will provide staff to record meeting minutes and the assistant chair will help the chair in normal committee business. John Dettmers suggested that the commission might provide a secretary to record minutes. The Terms of Reference would have to be changed and the proposed change would have to be sent to the CLC and approved. Minutes would be sent to the assistant chair for committee distribution.

Action Item: Abolish the existing GLFHC Secretary position and replace with an assistant chair. The assistant chair would serve for a two year term

which would alternate with the term of the chair. The agency hosting the meeting will provide staff to record the meeting minutes.

All Agreed.

The committee then moved to elect an assistant chair. Beth Wright was nominated approved by the committee. The motion is effective as soon as the CLC approves the recommendation.

3. Decision Item – Basin Wide Coolwater Egg Disinfection Protocols – Gary Whelan, MI DNR 1/29 10:30 AM – 30 minutes

Packets provided on page 3.

Decision Item 1: Does the committee support the idea of this basin wide protocol to be adopted. Ken Stark noted that PA may choose not do so because of walleye concerns.

All other agencies approved.

Decision Item 2-1: The committee agreed to adopt a protocol where egg disinfection was to be performed during water hardening when possible and after water hardening otherwise.

All Agreed.

Decision Item 2-2: The committee was given several disinfection scenarios (depending on fish species). Greg Wright suggested that we use a "minimum set of recommendations" which gives each agency some latitude to do more. Each item was discussed and a number of agencies thought these recommendations need to be modified and we need efficacy data to support the need for performing egg disinfection. The starting point should be simple and include language stating that these guidelines currently are not well supported with data.

Committee agreed to the following:

2a. Iodophor treatment for 30 minutes at 50 ppm during water hardening when possible. Must use fish-free water for egg transfer.

2b. If water-hardening disinfection is not possible or fish free water is not used, 100 ppm iodophor treatment for 10-15 minutes after water hardening should be performed.

2c. For all inter-hatchery egg transfers, use 2b

All agreed.

3. GLFHC recommends that pH should be buffered to ensure that pH does not vary more than 0.3.

All Agreed. All member agencies agree with some changes.

4. Discussion Item – Development of a Broodstock Transfer Protocol – Dave Meuninck, MI DNR 1/29 11:00 AM – 30 minutes

Background: Skamania steelhead swim to through the Berrien Springs fish ladder (MI) on their way to Indiana to spawn and 700 fish are used for Indiana egg collections. About half of the steelhead that pass through Berrien Springs will not continue their way to the South Bend trap (IN) before brood harvest operations end. Genetic diversity of this summer-run strain needs to be maintained, so a large number of brood collected in Barrien Springs is important. The new APHIS regulation prohibits the transfer of interstate fish yet these fish would eventually swim to Indiana on their own anyway, so can Indian be granted a waiver? Discussion followed relating to the nuances of the APHIS rule. A map of the region was shown and the river section in question spanning Indiana and Michigan was highlighted.

Dave will request waiver for APHIS rule and wanted to get committee support. The committee supported request. A response from the GLFHC will then be sent to APHIS.

5. Discussion Item – Development of a Fry Sampling Protocol – Gary Whelan, MI DNR 1/29 11:30 AM – 30 minutes

Gary Whelan addressed whether our committee should develop a protocol for fry and egg sampling? Discussion followed relating to the current bluebook method and logistics of working with small fish. The importance of early stage detection is important for fish, such as walleye, where fry stocking is vital. For viral assays, maintaining minimum dilution of the

sample is critical, so the size of the fish is important. No additional technical information is needed here since BB and OIE protocols already cover this situation. At some point we need to consider the differences between BB and OIE, as they relate to the Model Program.

6. Information Item - IAGLR Fish Disease Workshop – Stephen Riley, USGS – Ann Arbor 1/29 2 PM – 15 minutes

Stephen Riley briefly discussed the IAGLR workshop and he distributed information.

7. Information Item/Presentation – "Fish disease ecology in the Great Lakes: overview of GLFC research theme" – Stephen Riley, USGS-Ann Arbor 1/29 2:15 PM – 30 minutes

(powerpoint)

Stephen Riley presented information relating to fish population impact due to specific fish diseases. He addressed different types of disease sources and related them to different types of changes to the ecosystem. Many assumptions were listed relating to preexisting effect of disease and his list of diseases of recent concern included VHS, BKD, EMS, and Botulism. To determine if disease incidence is increasing, we must have better baseline disease data. Anthropogenic changes (invasive species introduction, human introductions, etc.) are important when studying this issue. He is developing a model to characterize disease impacts of certain diseases and used botulism as an example. He then discussed how VHS infection rates may be effected by physical criteria, such as temperature and biological criteria, such as fish age. Research pre-proposals were already submitted to the GLFC.

8. Decision Item – Research Priorities – Gary Whelan, MI DNR 1/29 3:00 PM – 30 minutes

Gary asked the committee whether our research priorities need to be revised. Discussion followed as to what role this document has. The GLFT uses our list to evaluate proposals and establishing a top-3 priority list is important. But the list hasn't been changed for several years and may need to be updated. We may assign to sub-committee between now and summer. Today we can simply come up with top 3-4 items, then hand the remainder off to a subcommittee. The current order is as follows with new ranking

#1 (nonlethal testing):
-Move to #3

#2 (EMS): This item has already been heavily studied and should drop down to the end.
-Move to #6.

#3 (VHS IVb): This is the most important issue we have
-Move to #1

#4 (new pathogens):
-Move to #4.

#5 (What events occur to make a pathogen harmful)
-Move to #5

New item: Develop assessment for pathogen surveillance
- Move to #2.

Note that the commission won't fund monitoring programs, so that should be a consideration when generating a list of research priorities. Targeted epidemiological surveillance proposals must still be based on a hypothesis driven design.

Action Item: Research Priority list has been changed. A subcommittee was assembled to take existing research priorities and develop new list. Subcommittee includes Greg Wright, Ken Phillips, Dave Meuninck. Date to have draft is May '08.

9. Discussion Item – Fishery Research Board Proposals – John Dettmers, GLFC 1/29 3:30 PM – 30 minutes

John summoned input from the committee on a research proposal by Marcogliese. The hypothesis suggests that baitfish will be driving force for pathogen introduction into naïve fish populations. Members support this approach because this type of study addresses "early warning" disease research.

Committee endorses for full proposal.

10. Information Item/Assistance Request – Research Project – Sue Marcquenski, WI DNR 1/29 4:00 PM – 15 minutes

Sue outlined her desire to develop nonlethal technique for EED testing. She contacted Ron Hedrick (U.C.-Davis) to develop a PCR method for EED detection. She now needs archived lake trout tissues to send to Hedrick. The skin seems to be the prime target tissue, although ovarian fluid pellets would be suitable. Sue will compile a list of information to be sent to Hedrick.

11. Information Item – IJC Fish Disease Workshop – John Dettmers, GLFC 1/29 4:15 PM – 15 minutes

John Dettmers announced that an I.J.C. sponsored workshop on fish diseases will be held in Toronto on March 12-13, 2008. The purpose of the meeting is to discuss the current state of VHS and what to expect in the future. Discussion items may include locating research funding and identifying researchers to accomplish specific tasks. Probable attendees may include researchers having interest in future funding.

12. Information Item – AFS VHSv Workshop – Beth Wright, OMNR 1/29 4:30 PM – 15 minutes

In the upcoming AFS meeting in Ottawa, a VHS symposium is being organized by the OMNR and 16 presentations have already been accepted. Beth Wright asked for a member to speak on behalf of the GLFHC to discuss our approach and accomplishments. Issues may include what we have done regarding disinfection, management strategies, public awareness, or other topics. Gary Whelan has already submitted a proposal for the committee and will make a presentation. The committee members need to provide him with information to discuss.

13. Information Items – Cysts and Vesicles in Great Lakes Fish – Sue Marcquenski WI DNR 1/29 4:45 PM – 15 minutes

Sue discussed a few disease investigations that she has seen recently. She first showed pictures of emerald shiners with skin hemorrhages. Cysts in viscera contain large numbers of bipolar spores similar to *M. cerebralis*. She explained that this seems to be a new finding. Next she explained that increased *I. multifiliis* infections in coho salmon lead to increased furunculosis and BKD infections this year. She also observed Chinook

salmon with fluid filled cysts in the G.I. tract. Other committee members suggested that this may be the result of a *Carnobacterium* sp. infection, also seen in bloater chubs. *Carnobacterium* causes nephrocalcinosis and pseudokidney disease.

Wednesday 1/30 Starting at 8:15 AM – VHSv Session

14. Information Item/Presentation - "VHSV research and surveillance in Michigan: Lessons learned." – Dr. Mohamed Faisal, MSU 1/30 8:15 AM – 30 minutes

Dr. Faisal summarized the decline of many animal species around the world and suggested that the decline of many animal populations is related to disease. Examples were given for lobsters, crab, shrimp, oysters, clams, menhaden, sea turtles, and sea mammals. In the Great Lakes, BKD, EED, WD, and furunculosis have had prominent impacts on fish populations. Since 2001, many new diseases have been identified in the Great Lakes. In flavobacteria family, at least 67 different species have been identified. Other new diseases include streptococcus species in sturgeon, "bearded" muskies, koi herpesvirus in carp, *Phoma herarum* in Chinook salmon, Heterosporis in many species, *Triaenophurus* cestodes in yellow perch, Piscirickettsia in several fish species, a microsporidiean parasite in mottled sculpin, and pseudokidney disease. We had previously seen various retroviruses and herpesviruses in walleye that cause tumors. The newest bacterial disease to the region is *Pantoea agglomerans*, a plant pathogen which has been found in diseased brown trout.

VHS was first identified in 2003 in Lake St. Claire and has been isolated in other Great Lakes since. Taxonomic studies characterizing the G (glycoprotein) and N genes show that the Great Lakes strain is a new lineage and is distinct from other known strains. Michigan isolates have recently been sequenced and some heterogeneity exists, but all isolates are related. Since then, VHS has spread inland in New York, Wisconsin and Michigan, but distribution is spotty and spread is slow. So far, 25 fish species have been infected. In lab experiments, Koch's postulates were fulfilled in muskies, where clinical signs were seen experimentally and virus was isolated. VHS targets the endothelial lining of blood vessels. The LD_{50} data for muskellunge was 2.21 PFU/mL and the time to death was 3 to 5 days. Experimentally infected largemouth bass showed classic clinical disease, but were less susceptible than muskellunge, 3.4 PFU/mL and mortality occurred in 8-14 days. Lake trout were less susceptible than both

muskellunge and bass and clinical signs included hemorrhaged muscle. An order of species susceptibility was proposed of those species tested.

(Most to least susceptible)
1. Muskellunge
2. Largemouth Bass
3. Brook Trout
4. Brown Trout
5. Rainbow Trout
6. Lake Trout
7. Coho Salmon
8. Chinook Salmon

VHS disease can be peracute with rapid mortality and no clinical signs. There seems to be a similar pattern of observable disease in various species; epidermal petecchia, hemorrhaged fins, pale gills, enlarged liver, muscle hemorrhage, and swim bladder hemorrhage. Ascites was only found in largemouth bass and oral hemorrhage in muskellunge and largemouth bass. Future surveillance should include all age groups to address age related susceptibility.

The distribution of VHS in the Great Lakes is interesting, being found throughout Lake Huron, yet only the northwest corner of Lake Michigan. And VHS distribution appears in distinct pockets, rather than a homogeneous distribution, probably related to where fish assemblages are found. In cell culture, two negative passages were not always enough, a third passage was sometimes required. In all, we lack qualified aquatic animal health labs in the field and greater funding for this research is needed. We also need to make better use of VHS publicity and develop a master plan for disease control strategies.

15. Agency VHSv Updates – Each Agency 1/30 8:45 AM – 30 minutes
- **a. 2007 VHSv Updates**
- **b. Spring Surveillance Plan**
- **c. Coolwater Production Updates**
- **d. Spring 2008 Plans**

New York: The New York State DEC imposed regulations in November, 2006 which require testing of eight fish pathogens. In 2007, state hatchery inspections were conducted of all fish lots and only *A. salmonicida* was

found in Chinook salmon at the Salmon River hatchery. The DEC also conducted 130 inspections (~13,000 fish) of licensed private bass and trout hatcheries in New York. One private hatchery tested positive for IPN and all others were negative. All commercial bait operators required testing by private inspection laboratories. The state tested 55 lakes or rivers for the same eight pathogens, collecting 30 predator and 30 prey species from each site. To date, VHS has been found at six different locations, all on Great Lakes waters. Cornell has conducted 30 wild fish disease investigations in 2007 and those results were discussed. Two VHS-related research projects were initiated in 2007. The first addressed walleye and muskellunge egg survival in iodophor during water-hardening and the results indicated that survival of both species was excellent at the suggested concentration of 50 ppm iodophor. The second study looked at iodophor efficacy in destroying VHS. To date, no conclusions can be made and further study will be needed. In 2008, two separate surveillance projects will be conducted. For the APHIS program, 30 sites within the Upper Hudson River basin will tested for VHS only. The other project will be a continuance of the statewide program that was begun in 2007, where an additional 30 statewide sites will be tested for 8 pathogens. DEC funded inspections of private hatcheries will also be suspended in 2008.

Pennsylvania: In 2006, the use of Lake Erie esocids was discontinued. Lake Erie steelhead did have IPN (3% prevalence), but no VHS. All non-salmonid production lots were inspected and no VHS found. Non-salmonid brood lots were scheduled to be inspected, but could not due to various reasons. Surveillance will be the same in 2008. Plans for conducting an APHIS surveillance study are underway. No other changes in the agency's cool water program are expected. A quarantine order is in place for the Lake Erie drainage and an education program is being developed to educate bait dealers.

Ohio: VHS related fish mortalities were less in 2007 than 2006. Significant surveillance of inland waters found no VHS in 2007, although Lake Erie surveillance revealed LMBV in bass and VHS in yellow perch. No pathogens were detected from fish from the Ohio River or other brood sources. All walleye are collected from inland water sources and the state plans to continue the moratorium on Great Lake collections. Iodophor disinfection experiments are planned with muskies and hybrid stripers for 2008. All domestic brood stock were tested for VHS and that will continue. OHDAG has made plans for using APHIS money to do statewide VHS

surveillance in 22 locations (170 fish/site). Location selection was based on by boating traffic and proximity to hatcheries.

Ontario: The ministry placed a restriction on bait fish harvest in January, 2007. This was revised in Apr 2007 to use a VHS Management Zone approach. A VHS management zone was defined for drainage waters to the first impassable barrier to fish (this does not include fishways). Commercial harvest of baitfish is permitted but transport and sale has restrictions. Aquaculturists are urged to use caution with respect to broodstock selection. Disinfected eggs may be moved outside the VHS Management Zone only if disinfected during water hardening following OMNR BMP. Ovidine is not currently a registered drug in Canada, so the private sector must comply with the appropriate regulatory issues. The ministry suspended walleye egg collections from the Bay of Quinte when VHS first appeared. Public outreach includes a VHS website and many fact sheets. There is a plan to move fish diseases into invasive species program in order to get better funding. In 2007, there was just one mortality event associated with VHS which occurred in Hamilton Harbor and included two VHS-positive freshwater drum. Fish collections for two OMNR surveillance projects are being coordinated and testing will take place at the DFO labs (CFIA-led project) and Univ of Guelph (Roz Stephenson) (OMNR-led project). In the Thames River, 1 pool of 5 largemouth bass tested positive for VHS. The fish were collected live and showed no signs of disease. The ministry is currently addressing funding options for future surveillance.

Michigan: In 2007, 163 lots were inspected (7300 fish) and VHS was only found in one new location, Budd Lake. Swann Lake was the only location where VHS was found in follow-up surveillance from previously positive locations. VHS sampling data for the region will be included in the GIS project. Of the 25 fish species reported positive for VHS, 17 were reported in Michigan. Their management philosophy for VHS is to "contain and control" and improved outreach is important. Eggs from wild, cool water species were disinfected with iodophor and VHS was not detected. The agency has secured $134,000 APHIS money to sample 113 different locations. Changes in biosecurity include the construction of truck disinfection stations near hatchery grounds, ballast water restrictions, and large vessels are not permitted to move between major management areas. The regulation package was approved in June. They chose not to close wild harvest, but rather create regulations to manage them. Fish from VHS-positive waters can only go into VHS-positive waters and fish from pathogen free waters can be used anywhere. There was great demand for

certified minnows in 2007 and little demand for uncertified bait. Three geographic regions have been defined in the state; VHS positive, VHS surveillance, and VHS free. There is great need for improved public information regarding VHS. In cool water egg collections, the agency still collects adult fish from the Great Lakes. Disease testing plans include sampling adults before and during spawning, and fry before stocking. Walleye will come from Bay de Noc and Muskegon River and muskellunge from inland sources. Eggs will be incubated at the Thompson hatchery and fish will be reared in non-drainable ponds. Muskellunge will be reared at the Wolf Lake hatchery and cultured in an isolation building.

Indiana: Dylan Sickles is no longer hatchery supervisor. In 2007, wild brood sources for walleye, muskellunge and steelhead were tested and no VHS was detected. Disease investigations included one LMBV fish kill, and another fish kill in Wabash where shovelnose sturgeon epizootic persisted for 30 days. In the Barbee chain of lakes, a small muskellunge mortality occurred, but VHS was not detected. In all, 430 fish were inspected from wild collections. In hatchery inspections, 2250 fish from 22 species were tested and no Model Program pathogens were detected. APHIS funded work was discussed. For 2008, testing of Lake Michigan tributaries will occur. Cool water propagation will continue as before and hatchery biosecurity will be continually refined. Public outreach and staff education programs are underway.

Wisconsin:

The committee was shown a public affairs message from the Wisconsin DNR website with “Dr. Drum and Mr Fin”. VHS work in Wisconsin was summarized in a power point presentation. They conducted a study to address VHS survival in frozen tissue and virus was still viable after 7 months at -20 C. Lesions from wild, VHS-infected brown trout included enlarged spleen, eye hemorrhage, swollen kidney, and petechiae in visceral fat and swim bladder. Surveillance plans were discussed where a surveillance map was shown to the committee and ‘high risk’ waters to be sampled were highlighted. Emergency regulations were discussed which include draining water all boats and prohibit the transport of live fish from any water. Regulations for bait use were discussed including methods to

preserve bait. Department of Agriculture inspection permits are valid for 30 days and are renewable. These also include crayfish and turtles.

Great lakes brood stock will not be used due to VHS concerns and the sturgeon program will be conducted at 3 other hatcheries. Sturgeon ovarian fluid samples will be used for testing since adults can not be destroyed. Fry stocking will not occur this year.

Minnesota:

BKD testing was the biggest challenge in 2007. Ovarian fluid from brown trout and rainbow trout of Lanesboro tested positive for BKD using ELISA and were confirmed by PCR at the Minnesota DNR Pathology Laboratory. To confirm the results, identical trout samples were sent to the USFWS fish health lab in LaCrosse. Results from the two labs did not agree. The standard culture method was then used and all results were negative. There is uncertainty regarding the validity of these tests. Aeromonas salmonicida was detected in the Crystal Spring hatchery in 2007. APHIS surveillance funds will be used to sample 90 sites in Minnesota, and so far 41 have been completed. Sucker eggs are incubated at the French River hatchery and eggs are collected from St. Louis River adults. Inspections have been done and fish are pathogen free. There are plans to move this program to an inland hatchery and establish a three-year disease history. The St Louis River is no longer the source for walleye; instead the agency is contracting with private operations. For walleye egg disinfection, iodophor was not used during water hardening because of possible toxicity to eggs, so eggs are simply moved to fish-free water after fertilization. If VHS is found in Minnesota, regulations to restrict fish movement will follow. Some laws already exist to prevent importation of bait. A VHS informational pamphlet is being developed for the public.

DFO:

Deferred to the written agency report. The DFO is dealing with a reduction in manpower and are not sure what long term implications will be.

USGS-LaCrosse:

Rick Nelson retired. Over 7000 fish were tested in 200 cases which is twice the effort from the previous year. Some short term staff were added to assist with lab work. SVCV was isolated in carp from pool 8 and reported to

APHIS. In 2008, they plan to continue to assist partner agencies with surveillance efforts.

CORA:

Conducted disease surveillance on three lakes and all were negative. Nets and other equipment are disinfected before being moved to other sites and walleye eggs will be disinfected. No regulations have been imposed to date and disease surveillance will continue in 2008.

16. VHSv Photo Webpage – Sue Marcquenski, WI DNR 1/30 4:30 PM – 15 minutes

Sue Marcquenski discussed an idea for the committee to create an atlas of VHS pictures and post them on the GLFHC web site. We can start with a manageable number of pictures then expand as more pictures become available. All pictures would be captioned with related case information. Technical web details needed to develop a program like this need to be further researched. The committee asked who would edit the pictures before posting and Sue volunteered. The committee needs to compile pictures with related case information and histopathology photos and send to Sue.

Action: Send gross photographs with related case information to Sue by 2/15/08. Sue will compile this and send to the commission by 3/10/08.

Thursday 1/31 Starting at 8:15 AM

17. Information Item – MN Catfish Kill Case - Ling Shen, MN DNR 1/31 8:15 AM - 15 minutes

A Catfish kill (1600 fish) observed along a 9-mile stretch on Red River. A range of skin and visceral lesions were visible, although these were not consistent between fish. A wide assortment of bacteria were isolated but there was no consistency between fish. Etiology is still unknown.

18. Information Item – Validation of Non-Culture Methods for BKD – Diane Elliott, USGS 1/31 8:30 AM - 30 minutes

Diane Elliott compared culture vs. non-culture methods for the detection of *R. salmoninarum*. Specifically, she addressed FAT, ELISA, nPCR, qPCR, and MF-FAT from ovarian fluid vs. enhanced culture methods.
For specificity testing, she used 17 similar organisms (purified) to test against each method. Nonspecific, cross reaction only occurred in ELISA. However, all methods detected positives successfully. In sensitivity trials, culture methods were most sensitive and Elisa was the least. She determined that the order of sensitivity is as follows (most to least sensitive):

Culture>mf-fat>npcr>qpcr>elisa

When sampling directly from kidney tissue, culture methods were much less effective, especially when the tissue is contaminated/infected with other bacteria. But kidney pasteurization greatly improves sensitivity.

In vivo testing, kidney samples were combined from infected fish with uninfected fish. With ELISA, a number of threshold patterns were described where certain highly sensitive strategies will find positives, but result in some false positive, and visa versa. True prevalence is best found with ELISA, but a higher number of false-positives will result from ELISA compared to other methods.

19. Information Item – Non-lethal Methods for the Detection of BKD – Diane Elliott, USGS 1/31 9:00 AM – 30 minutes

A non-lethal test method would enable performance and survival monitoring of infected fish populations. The criteria for testing were discussed. Non-lethal tissues included blood, gills, mucous, fin clip, and kidney biopsy and were analyzed by several methods in three different size groups of Chinook salmon. One concern with this approach was how lethal these non-lethal techniques were. She found that gill and mucous collections were non-lethal, fin and blood collections were more lethal, and kidney biopsies were the most lethal. This study is still underway.

20. Discussion Item – New Pathogen Monitoring Protocol – Sue Marcquenski, WI DNR 1/31 9:30 AM – 30 minutes

Sue Marcquenski proposed that a new pathogen monitoring program should be initiated. Since our regional fisheries are continually inundated with new, invasive species of aquatic organisms, including fish pathogens, we may want to find support to fund a monitoring project. Since there are

certain locations within the Great lakes basin where invasive species seem to arise first, we may want to focus our efforts there. These include Hamilton Harbor in Lake Ontario, Lake St. Claire, and Bay of Quinte in Lake Ontario. APHIS may be the appropriate funding source.

Action: The chair suggested that we discuss this at the summer meeting and include this in a research plan. A subcommittee was selected to develop goals and objectives. The subcommittee includes Sue Marcquenski, Beth Wright, and Greg Wright. The white paper should be drafted by May and ready for the fall CLC meeting.

21. Information Item – Model Program Status – Gary Whelan, MI DNR 1/31 10:15 AM - 15 minutes

The Model Program is near completion. The chair will send the completed draft to Beth by 2/15/08 and then to the committee by 3/7/08. This should be finalized by the summer meeting.

22. Information Item – Coho salmon BKD Diagnostic Work (ELISA vs. culture results) – Sue Marcquenski, WI DNR 1/31 10:30 AM - 30 minutes

Sue summarized some BKD case data that she has compiled over the last few years. In 2004, 36 fish were tested for BKD, 30 were positive in plate culture (SKDM-2 medium) and none were FAT positive. In 2005, 8/60 were positive in culture and 4 positive with ELISA. In 2007, many fish were positive with ELISA, but not culture, which negated the previous trend where culture detected more positives than ELISA. Sue emphasized her lack of confidence in the standard BKD detection procedure. And PCR testing from cultured isolates would be invalid since BKD DNA is found in spent medium, a component of the standard culture method.

23. Information Item – Wild Fish Parasite Database Project – Gary Whelan, MI DNR 1/31 11:00 AM - 15 minutes

This project was funded for two years and currently has several components. The database is still in development and will be web-based. Anyone can enter or look at data. Data allows input on individual fish basis, lot based, or summarized data. Any type of pathogen data can be entered. Data entry forms were shown and explained. Menus are 'pull down' for easy use. The literature search for web design is complete, a prototype will be ready by

March 2008, data entry by summer 2008, and GIS and query engine by fall 2008. Also, a background synonym table will be used to capture older literature.

24. Information Item – American Eel and Atlantic Salmon Importation Update – Beth Wright, OMNR 1/31 11:15 AM – 15 minutes

American eels were stocked last year. Atlantic salmon from 60 pairs of adults were passed through quarantine and a small number were stocked in 2007. Conditions for import permits and disease testing for Sebago strains were met. At Normandale, Lac St. Jean strain fish are also in 'import status' and in quarantine. Disease testing is satisfied for these fish and stress testing is still being considered.

25. Information Item – Other Key Agency Updates – Gary Whelan, MI DNR 1/31 11:30 AM - 15 minutes

Michigan: - Steelhead are now being cultured at the Platte River hatchery. The fish were infected with a myxosporidean parasite similar to Whirling Disease, but were negative with PCR. Those fish were destroyed and all of the fish cultured at Platte River have since tested negative. In 2008, wild fish in the waters above the hatchery will be tested for WD.

Indiana: Inquired about lot determination within a hatchery having multiple water sources.

Ontario: In hatcheries, a newly described diseased called "no mucous skin disease" has been found in walleye. More than 4000 fish were lost and secondary fungal disease was common. Also, a Chlamydia-like organism was found in lake trout and Oxytetracycline therapy was only marginally successful. Sue discussed that a similar situation occurred with the discovery of EED, because they also, had a CLO that did not respond to OTC.

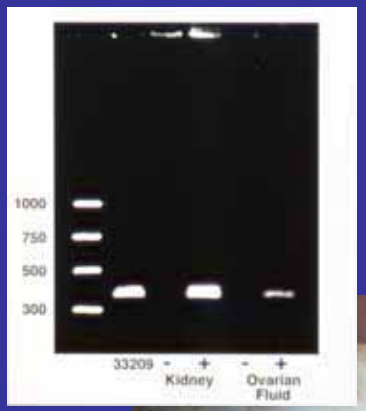
CORA: Alewife population is in decline in the region. Productivity in both Lakes Michigan and Huron are becoming more like Lake Superior.

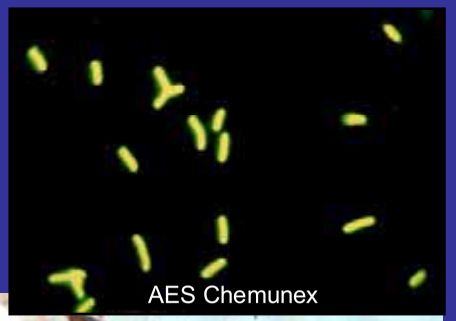
26. Information Item – Summer Meeting and Meeting Task List – Gary Whelan, MI DNR 1/31 11:45 AM - 15 minutes

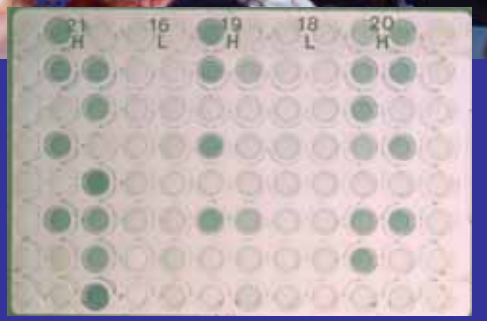
The plan is to have our summer meeting on the Thursday and Friday immediately after the AFS meeting (8/21-22) in Ottawa. A task list will be sent as soon as possible from this meeting. The next winter meeting will be held in NY, preferably during the last week of January, 2009.

USGS
science for a changing world

Validation of Non-culture Methods for *Renibacterium salmoninarum* Detection

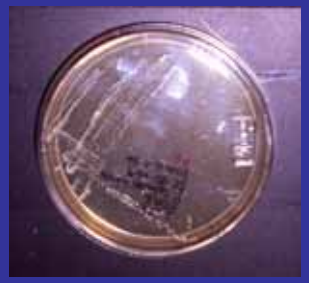






Diane Elliott, Anthony Murray, Connie McKibben, and LynnMarie Applegate

Western Fisheries Research Center, USGS, Seattle



Detection of *Renibacterium salmoninarum* (Rs)

- Because of the extremely slow growth and fastidious nature of Rs, bacteriological culture is unsuitable for rapid detection and quantification of the bacterium from field samples.
- Non-culture methods for Rs detection may aid fishery managers in developing more effective measures for monitoring and controlling bacterial kidney disease (BKD).
- However, questions remain regarding the accuracy of Rs diagnoses based on non-culture methods.

Validation of Non-culture Methods for Rs Detection

- Although comparisons of sensitivity among several non-culture methods for Rs detection have been made, none have been sufficiently extensive or rigorous to validate a particular method.
- To validate a diagnostic method, it must undergo thorough laboratory and field testing of specificity, sensitivity, and repeatability according to standardized published procedures.

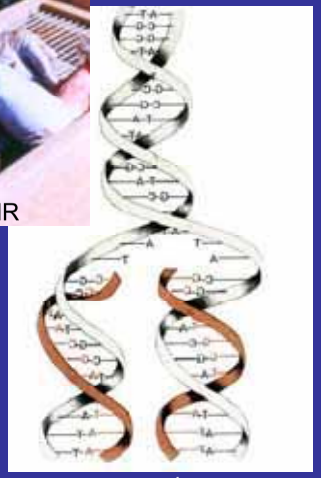
Project Design

- Comparison of existing non-culture Rs diagnostic methods with culture (benchmark standard) for detection of Rs in kidney and ovarian fluid.
- Selection of non-culture methods:
  - Method described in peer-reviewed publication.
  - Standardized protocol has been developed.
  - Appropriate reagents are commercially available.
  - Method is currently in use.

Non-Culture Methods for Detecting Rs in Kidney Tissue

humanvaccine.duke.edu

Photo: Wisconsin DNR



www.genelex.com

- Immunological methods
  - Fluorescent antibody test (FAT)
  - Enzyme-linked immunosorbent assay (ELISA)
- Molecular methods
  - Nested polymerase chain reaction (nPCR)
  - Real-time quantitative PCR (qPCR)

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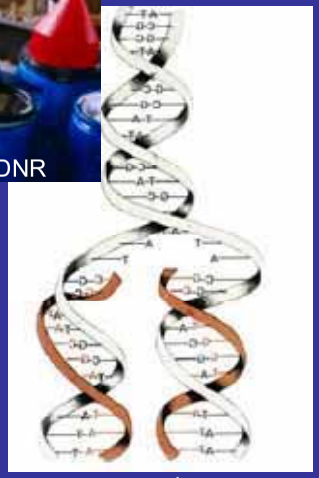
Non-Culture Methods for Detecting Rs in Ovarian Fluid

humanvaccine.duke.edu

Photo: Wisconsin DNR



www.genelex.com

- Immunological methods
  - Membrane filtration-fluorescent antibody test (MF-FAT)
  - Enzyme-linked immunosorbent assay (ELISA)
- Molecular methods
  - Nested polymerase chain reaction (nPCR)
  - Real-time quantitative PCR (qPCR)

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Culture of Rs

- Culture method used was the procedure of Jansson et al. (1996; Dis. Aquat. Org. 27:197-206).
  - Kidney tissue diluted 1:10 in peptone-saline, homogenized, then centrifuged at 2,500 x *g* for 20 min at 4ºC.
  - Supernatant discarded and pellet resuspended 1:2 (w:v) in peptone-saline.
  - 10 µL volumes of resuspended tissue were inoculated in triplicate onto SKDM plates (10-fold serial dilutions made and cultured as necessary).
  - Ovarian fluid also inoculated in triplicate (100 µL volumes of undiluted sample or 10-fold dilutions).

Objectives

www.niehs.nih.gov

- *In vitro* analyses: Compare the relative specificities, sensitivities, and intra- and inter-assay variation of the selected Rs detection methods.
- *In vivo* analyses: Compare the relative abilities of the various detection methods to describe the progress of an Rs infection.
- On the basis of results of *in vitro* and *in vivo* analyses, select non-culture Rs detection methods for future validation studies with field samples.

Specificity Testing

www.bookworld.com

www.cat.cc.md.us

- Selection of non-target organisms:
  - Organisms phenotypically or phylogenetically similar to Rs
  - Other fish pathogenic bacteria
  - Some of bacteria selected have been reported to cross-react in immunological tests for Rs diagnosis

Non-Target Bacteria: Related to Rs

Species	ATCC No.
Arthrobacter globiformis	8010
Arthrobacter protophormiae	19271
Kokuria (Micrococcus) varians	15306
Leifsonia aquatica (Corynebacterium aquaticum)	14665
Micrococcus luteus	4698
Mycobacterium marinum	927
Nocardia asteroides	19247

Non-Target Bacteria: Other Fish Pathogens

Species	ATCC No.
Aeromonas hydrophila	7966
Aeromonas salmonicida	33658
Carnobacterium maltoromaticum (piscicola)	35586
Edwardsiella tarda	15947
Flavobacterium johnsoniae (columnarae)	43622

Non-Target Bacteria: Other Fish Pathogens (continued)

Species	ATCC No.
Flavobacterium psychrophilum	49418
Listonella (Vibrio) anguillarum	68554
Pseudomonas fluorescens	13525
Vibrio ordalii	33509
Yersinia ruckeri	29473

Target Isolates (Rs Strains)

www.noaa.gov

www.nrcs.usda.gov

www.gov.mb.ca

- Strategy: to test Rs isolates from a variety of fish species and geographic locations, with several Great Lakes isolates.
- What do we lack? Isolates from native Great Lakes fish species!

www.zoology.ubc.ca

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Target Isolates: Rs strains

Isolate No.	Fish Species	Geographic Origin
CHLM 91-02b	Chinook	MI
GL-64	Chinook	ON
M05-33K	Steelhead	WI
M04-K35	Coho	WI
M05-BNT	Brown trout	WI

Target Isolates: Rs strains (continued)

Isolate No.	Fish Species	Geogr. Origin
ATCC 33209	Chinook	OR
#684	Brown trout	Norway
GR5	Arctic grayling	MT
MT 239	Atlantic salmon	Scotland
BPA-6031	Sockeye	WA
Willamette	Spr. Chinook	OR

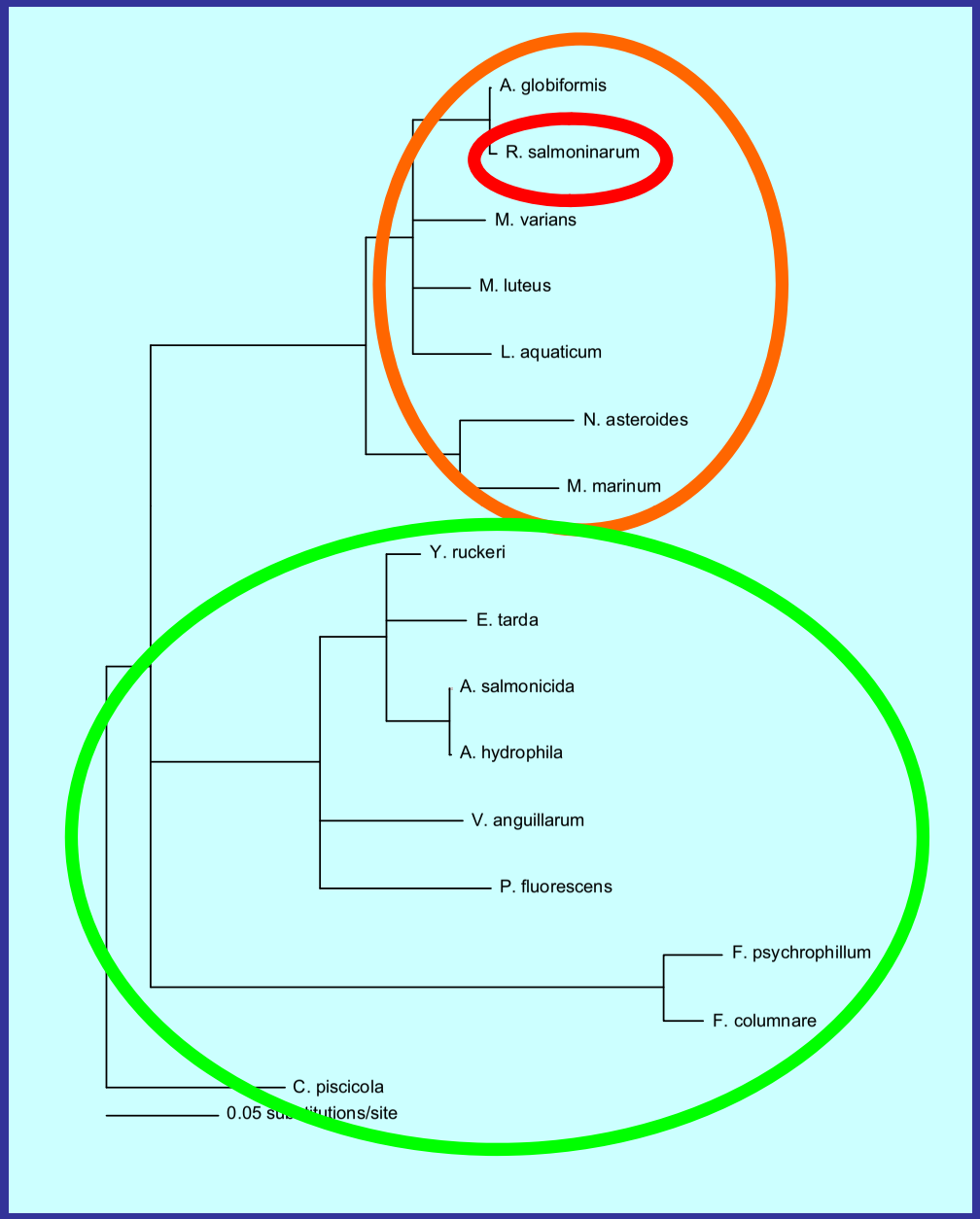
www.cvgs.k12.va.us

Preparation of Stock Bacterial Suspensions

- Stock broth cultures of each isolate were diluted in PBS (saline) to make final concentrations of $1x10^8$, $1x10^7$, and $1x10^6$ bacteria/mL.
- Strongly auto-aggregating species (*Mycobacterium marinum*, *Nocardia asteroides*, and *Vibrio ordalii*) were diluted by weight to make final concentrations of 5 mg/mL, 2 mg/mL, and 1 mg/mL wet weight.
- Wet mounts and Gram stains were made to confirm purity of cultures.

Confirmation of Identity of Bacterial Isolates

- A portion of the 16S ribosomal RNA (16S rRNA) gene of each isolate was sequenced and compared with known sequence.

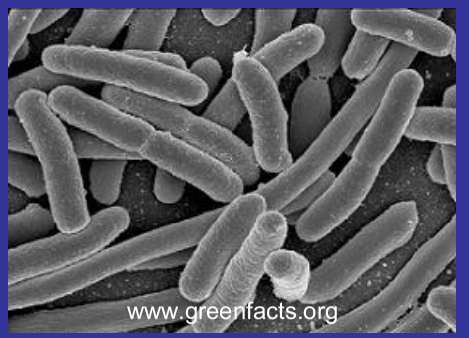


Results of Specificity Testing: Non-Target Species and Rs

Bacteria	No. Isolates Detected/No. Tested			
	nPCR	qPCR	ELISA	MF-FAT
Non-Target	0/17	0/17	0-2/17	0/17
Rs isolates	6/6	6/6	6/6	6/6

Summary: Specificity Testing

- The nPCR, qPCR #1, and MF-FAT did not produce any positive results with any of the non-target bacteria at any concentration tested.
- All of the assays showed positive results with 6 Rs isolates tested thus far.



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Summary: Specificity Testing

- The ELISA showed positive reactions with *Vibrio ordalii* and *Pseudomonas fluorescens* at the highest concentration only (1 x 10^8 bacteria per mL).
- ELISA OD values for both bacterial species were near the positive-negative cutoff.
- Re-testing of *P. fluorescens* at 1 x 10^8 bacteria per mL produced negative ELISA OD values.
- Re-testing of *V. ordalii* at 1 x 10^8 bacteria per mL again produced a positive ELISA OD value near the positive-negative cutoff.

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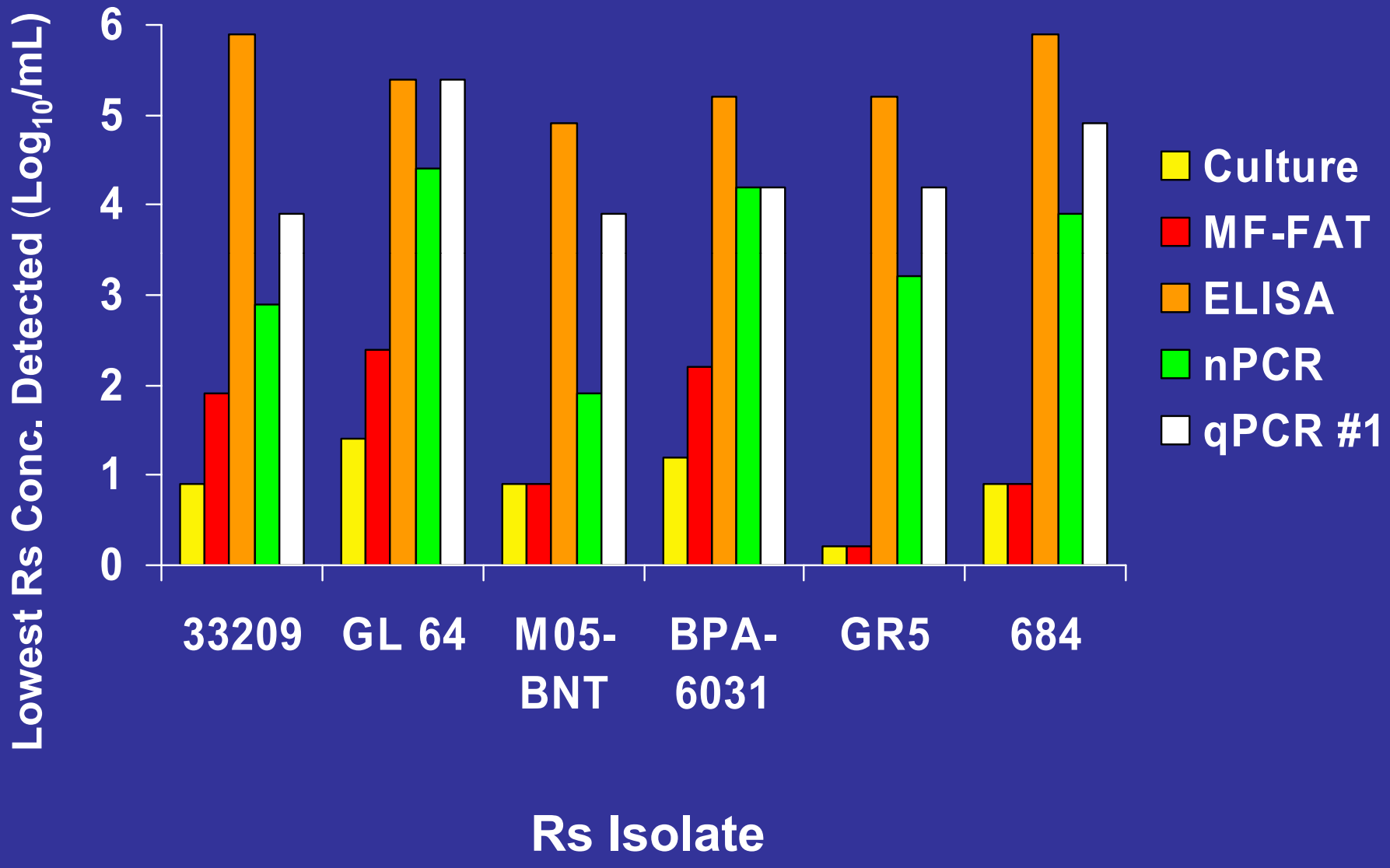
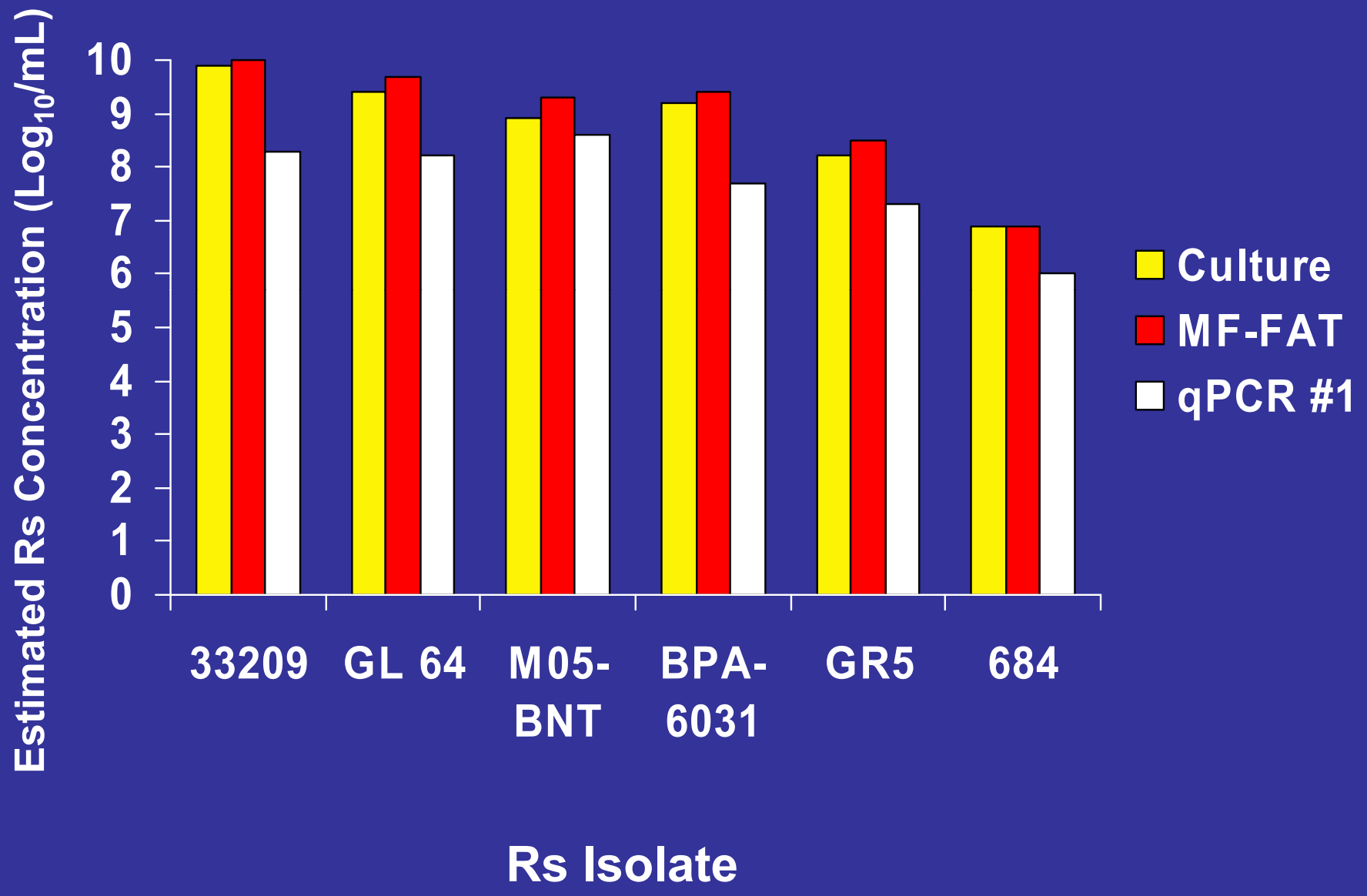
Rs Detection Sensitivity

- Three matrices were used for testing sensitivity of the various assays for Rs detection: PBS, pooled kidney tissue, and pooled ovarian fluid.
  - Kidney tissue and ovarian fluid from individual adult Chinook salmon were tested for Rs by culture, FAT, ELISA and PCR.
  - Samples testing negative by all assays were pooled for seeding with bacteria.
- Target Rs concentrations for seeded samples were between 10^1 and 10^8 bacteria per mL (fluid samples) or per gram (kidney tissue).

Sensitivity Testing: PBS Matrix



Sensitivity Testing: PBS Matrix



Summary: Sensitivity Testing in PBS

- For detection of Rs in a PBS matrix, culture was the most sensitive, followed by MF-FAT, nPCR, qPCR #1, and ELISA.
  - ELISA sensitivity for detection of Rs in fish tissues is likely increased by production of large amounts of soluble proteins by the bacterium over time.
- Starting Rs concentration was slightly over-estimated by MF-FAT and was under-estimated by qPCR.
  - MF-FAT counts both live and dead bacteria.
  - Sample volume tested by qPCR much smaller than that tested in culture.

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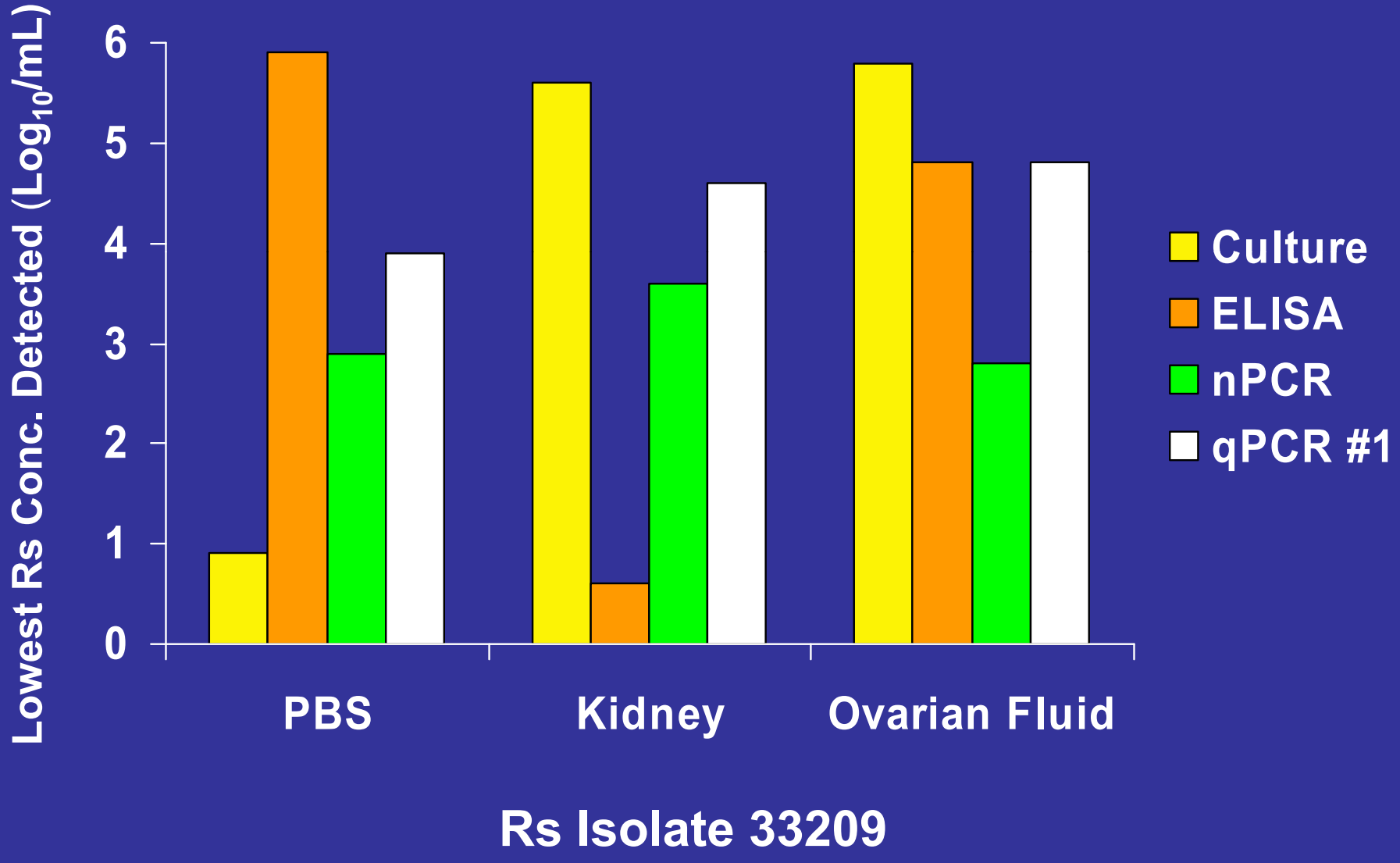
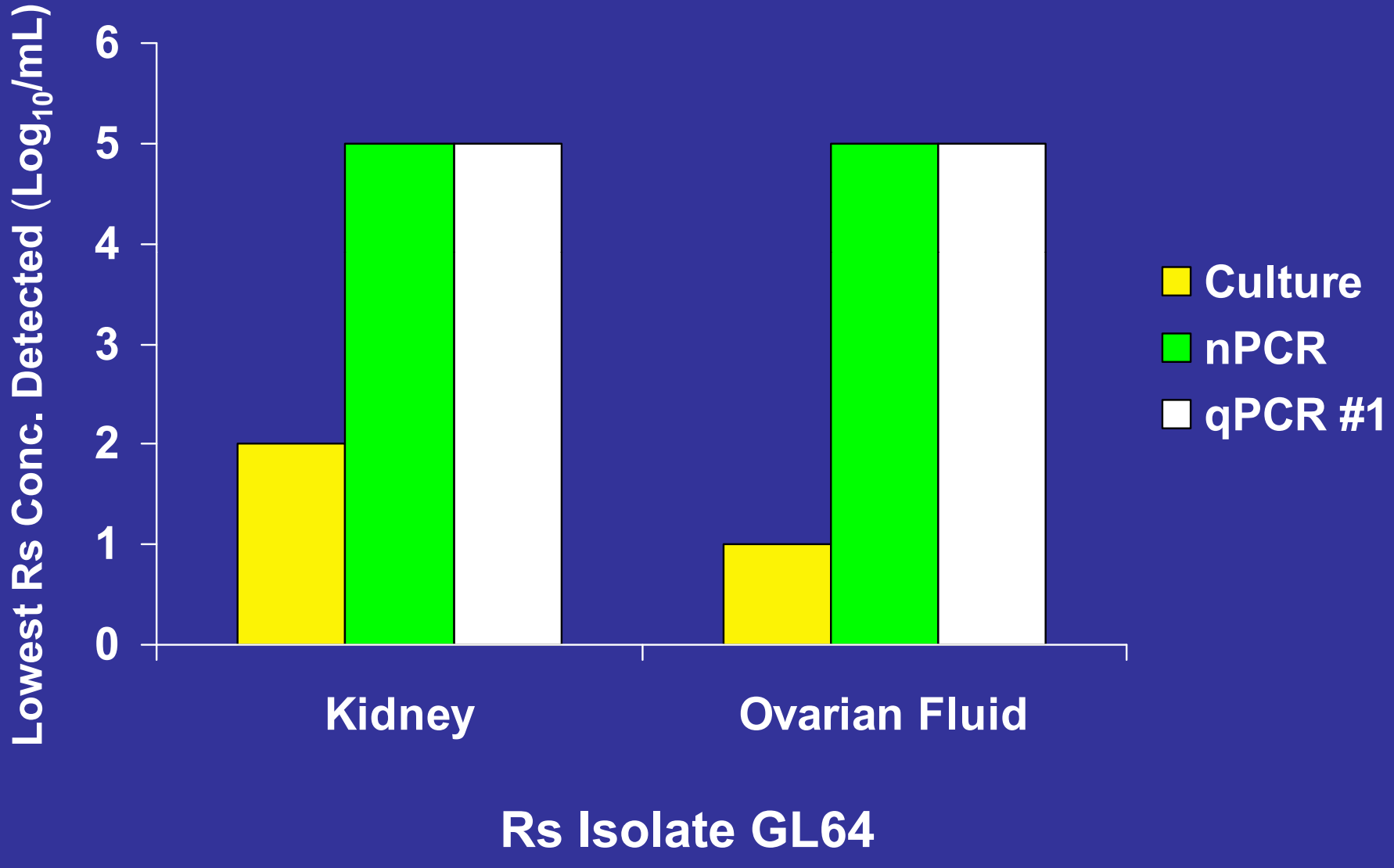
Sensitivity Testing: All Matrices Unpasteurized Kidney and Ovarian Fluid



Sensitivity Testing
Pasteurized Kidney and Ovarian Fluid



Summary: Sensitivity

- Sensitivity of culture for Rs detection was affected by matrix type.
  - Culture was the most sensitive for detection of Rs in PBS (followed by MF-FAT), and the least sensitive for detection of Rs 33209 in unpasteurized kidney and ovarian fluid.
  - Culture sensitivity was affected by contaminating organisms in kidney and ovarian fluid that grew on the plates despite the use of selective medium.
  - Pasteurization of kidney and ovarian fluid greatly reduced contaminating organisms and increased sensitivity of culture.

Summary: Sensitivity

- Sensitivity of ELISA for Rs detection was also affected by matrix type.
  - ELISA was the least sensitive assay for detection of Rs in PBS, and the most sensitive for detection of Rs 33209 in unpasteurized kidney tissue.
  - Testing has thus far corroborated past analyses which have indicated higher sensitivity of ELISA for Rs detection in kidney in comparison to ovarian fluid.

Summary: Sensitivity

- PCR assays appeared less affected by matrix type than did culture and ELISA.
  - Small sample weight/volume used for PCR probably decreased detection sensitivity compared with other assays.
  - At seeding concentrations where Rs was detected by both culture and qPCR #1 in unpasteurized and pasteurized kidney tissue and ovarian fluid, Rs concentrations estimated by qPCR #1 were close (within 1 $\log_{10}$) to those estimated by culture.

In Vivo Analyses: Comparison of Relative Abilities of Assays to Describe Progress of an Rs Infection

- Task 1: Measure the effect of sample size on the prevalence of infection among groups of fish with different Rs prevalences
- Task 2: Monitor the progression of Rs infections

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Task 1: Methods

- Subgroups of Chinook salmon created by randomly combining kidney tissue samples from healthy (uninjected) fish with those previously injected with Rs.
  - Infected fish injected with 1 x 10^6 Rs/fish 15 days prior to sampling.
  - Tissues sampled and randomly assigned to subgroups representing Rs prevalences of 20%, 40%, 60%, 80% and 100%.

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Task 1: Rs Prevalence Groups

Final Prevalence (%)	Number of Fish	
	Infected	Healthy
20	10	40
40	20	30
60	30	20
80	40	10
100	50	0

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ELISA Antigen Levels

Expected Prevalence	N	ELISA Antigen Category (%)			
		Neg	Low	Med	High
20%	50	46%	50%	4%	0
40%	50	38%	48%	12%	2%
60%	50	24%	52%	20%	4%
80%	50	14%	46%	40%	0
100%	49	0	49%	51%	0

Rs Prevalence at Different ELISA Pos/Neg Cutoff OD Values

Expected Prevalence	Prevalence at Pos/Neg Cutoff OD				
	0.064 2SD Pascho	0.068 4SD	0.070 5SD	0.095 Meyers 1993	0.100 Munson 2005
20%	54%	24%	20%	12%	12%
40%	62%	44%	38%	28%	26%
60%	76%	56%	54%	36%	34%
80%	86%	82%	78%	66%	62%
100%	100%	100%	96%	86%	80%

False/True Positives Detected by ELISA

Lowest positive ELISA OD	% True Positives	% False Positives
0.062 (1 SD)	100	79.0
0.064 (2 SD) (Pascho)	99.3	40.0
0.066 (3 SD)	98.7	15.0
0.068 (4 SD)	97.3	7.0
0.070 (5 SD)	94.6	3.0
0.095 (Meyers)	75.8	0
0.101 (Munson)	70.5	0

Rs Prevalence by Other Assays

Expected Prevalence	Observed Prevalence		
	Culture	nPCR	qPCR #1
20%	8%	16%	4%
40%	16%	22%	8%
60%	20%	20%	18%
80%	32%	18%	18%
100%	35%	6%	27%

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False/True Positives Detected by All Assays

Test	% True Positives	% False Positives
ELISA	70.5-100%	0-79%
Culture	37%	0%
nPCR	21%	10%
qPCR	25%	0%

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Summary: *In Vivo* Prevalence Test

- Rs prevalence detected by ELISA was generally higher than prevalences detected by culture, n PCR and qPCR #1 for all prevalence groups.
  - The majority of ELISA-positive fish had low to medium Rs antigen levels.
  - Because Rs produces copious amounts of soluble antigen, the ELISA may have detected antigen produced by small numbers of bacteria.
  - The ELISA can also detect Rs antigen in the absence of an active infection; live bacteria may have been absent from some fish.

Summary

www.ambioscience.com

- Specificity testing has shown little or no cross-reactivity of non-target bacterial species in testing by FAT, nPCR, qPCR #1 and ELISA.
  - Minimal cross-reactivity by ELISA testing with *Pseudomonas fluorescens* and *Vibrio ordalii* at highest concentration (1 x 10^8 bacteria/mL) only.

www-micro.msb.le.ac.uk

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Summary

- Sensitivity testing has given variable results, depending on the matrix used.
  - Culture and ELISA results were most affected by the matrix characteristics.
    - Culture sensitivity was adversely affected by contaminating organisms in unpasteurized kidney tissue and ovarian fluid.
    - ELISA sensitivity was high in unpasteurized kidney tissue, but relatively low in PBS or ovarian fluid.
    - PCR sensitivity was unaffected by matrix type.

Summary

- Sensitivity of assays also has been affected by the weight or volume of sample used for testing.
  - Small sample weight/volume used for PCR likely decreased detection sensitivity compared with other assays.
  - At seeding concentrations where Rs was detected by both culture and qPCR #1 in kidney tissue and ovarian fluid, however, Rs concentrations estimated by qPCR #1 were close (within 1 $\log_{10}$) to those estimated by culture.

Summary

- In an *in vivo* experiment using Rs-injected fish and uninjected fish, the Rs prevalence detected by ELISA was generally higher than prevalences detected by culture, n PCR and qPCR #1 for all prevalence groups.
  - The ELISA may have detected soluble antigen produced by small numbers of bacteria.
  - In some fish, the ELISA may have detected soluble antigen in the absence of live bacteria.
  - Rs detection in fish by ELISA and other assays is being further evaluated in an experiment monitoring the progression of Rs infection following an immersion challenge.

Ongoing Research

- Work is continuing on sensitivity of assays for detection of diverse Rs isolates.
- Other work is testing repeatability of Rs detection by the various assays.
- Research is underway to test the ability of the assays to monitor progression of Rs infections in fish.


Photo: Leslie Dorn

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Viral Hemorrhagic Septicemia Virus: Status in the Great Lakes and Summary of State and Federal Actions

Susan Marcquenski WI DNR

In this talk:

- The virus, its transmission, signs of disease
- Known distribution in the eastern U.S.
- Steps taken to control the spread of the virus:
  - New laws
  - New policies

Many thanks to members of the Great Lakes Fish Health Committee for providing the current laws and policies for their agencies;

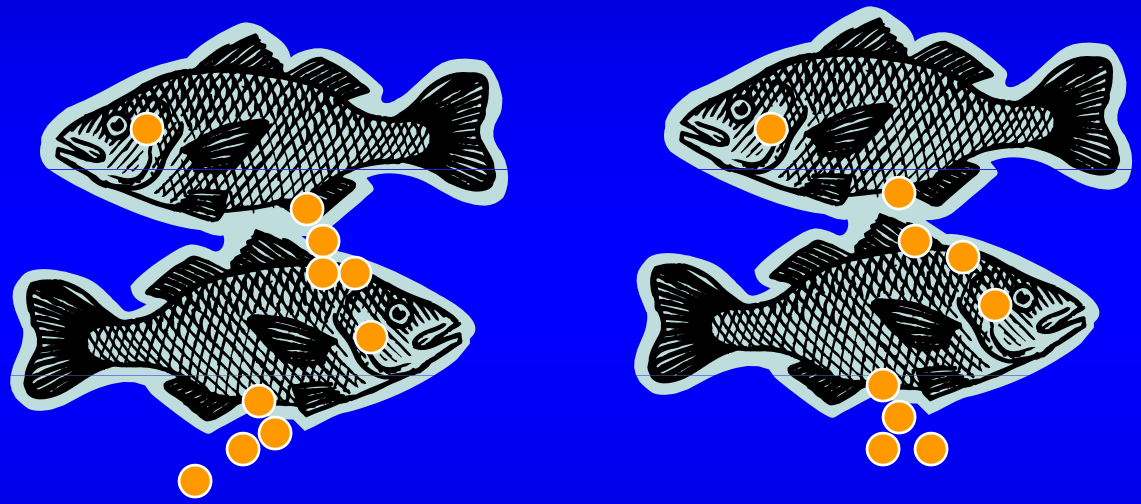
And thanks to Karl Scheidegger for creating the map of the Great Lakes basin.

The Virus

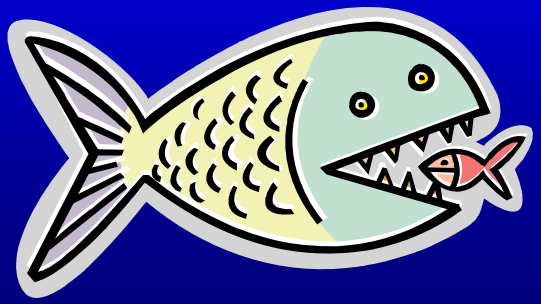
- Was first isolated in 1963, but the disease was known as early as 1938 in farmed rainbow trout in Denmark
- Is present in freshwater and marine species in Europe, West Coast U.S., Japan, East Coast U.S.
- Is not a human health concern
- Can only reproduce if it is inside a cell.

Transmission

- Virus is shed in urine, ovarian fluid and milt
- Virus particles infect the gills and then move to internal organs. Virus survives at least 14 days in water.



- Transmission also occurs when a fish eats an infected fish



The Disease

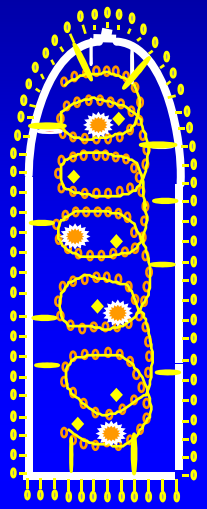
The virus infects internal organs and the cells that line blood vessels, causing severe hemorrhage

The Disease

- Clinical signs include pop-eye, anemia, swollen internal organs

- In WI, VHS detected at water temps 45 to 69 °F
- Fish can produce antibodies against the virus
- Stress is important – spawning stress and others
- VHS must be confirmed by laboratory tests

VHS virus particle



L Polymerase

G Glycoprotein

N Nucleoprotein

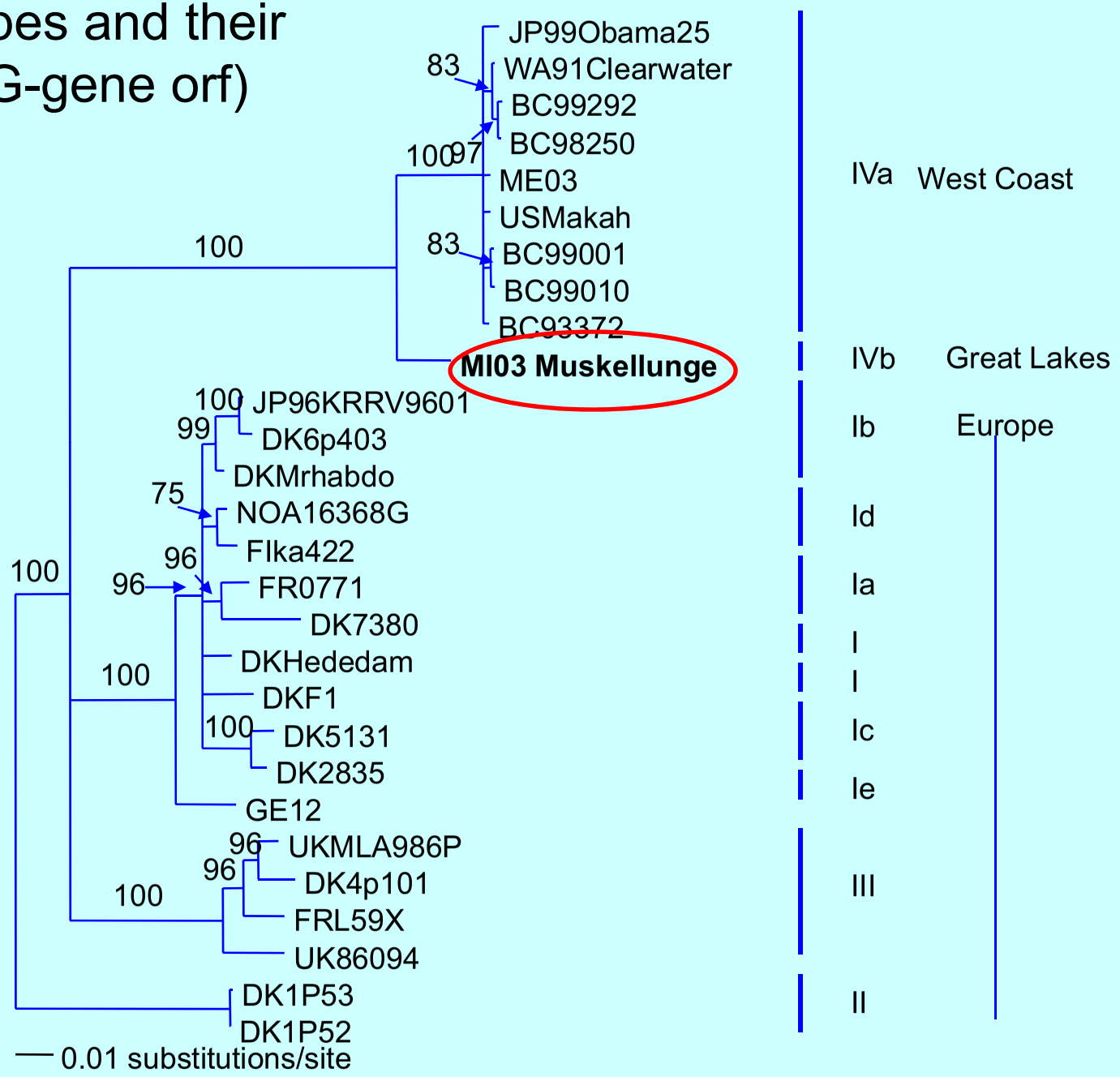
P Phosphoprotein

M Matrix protein

Envelope

VHSV Genome RNA (11 Kbp)

VHSV genotypes and their sublineages (G-gene orf)



Distribution and susceptible hosts of VHS

- 1938-1988: rainbow trout in European fish farms; a few marine species found to be susceptible (natural infections)
- 1988: Spawning Chinook and Coho in Washington
  - Genetically different from the European strain
- 1989- wild Pacific cod and herring

Distribution and susceptible hosts of VHS

- 1989-present Additional viral isolates obtained from fish along entire West Coast and Japan
- Sea-run brown trout, 3 spine stickleback, mummichog from Western Atlantic Ocean, East Coast of Canada and off the coast of Maine


VHSv in the Great Lakes
U.S. Army Corps of Engineers, Detroit District

VHSv confirmed in the Great Lakes

Spring 2005

- Lake Ontario: freshwater drum
- St Lawrence River: musky
- Lake St Clair: musky (sampled in Spring 2003)

VHSv confirmed in the Great Lakes

Spring 2006

Lake Erie: freshwater drum, yellow perch

- also caused disease in many other species

Lake St Clair: spotted musky

- also caused disease in many other species

St Lawrence R.: musky and round goby

VHSv confirmed in the Great Lakes

Fall 2006

Lake Huron

- Swan River: Chinook salmon (broodfish)
- Thunder Bay: Walleye
- Thunder Bay: Whitefish (sampled in 2005)

Where did VHSv come from?

Ballast, movements of wild fish, use of Pacific herring as cut bait, movement of boats are possible sources

VHS in Wisconsin

- Drum, LLBDM May 1, 2007
- Drum, Lake Winnebago May 8 &10
  - Popeye with hemorrhage
  - Slight skin hemorrhages

VHS in Wisconsin

- Drum, LLBDM May 1, 2007
- Drum, Lake Winnebago May 8 &10
  - Liver hemorrhages
  - Enlarged spleen

VHS in Wisconsin

- Smallmouth bass- Sturgeon Bay
  - May 8 2007
  - Health check
  - Popeye with hemorrhage

VHS in Wisconsin

Smallmouth bass- Sturgeon Bay

- Swollen kidney
- Enlarged spleen
- Skin hemorrhages

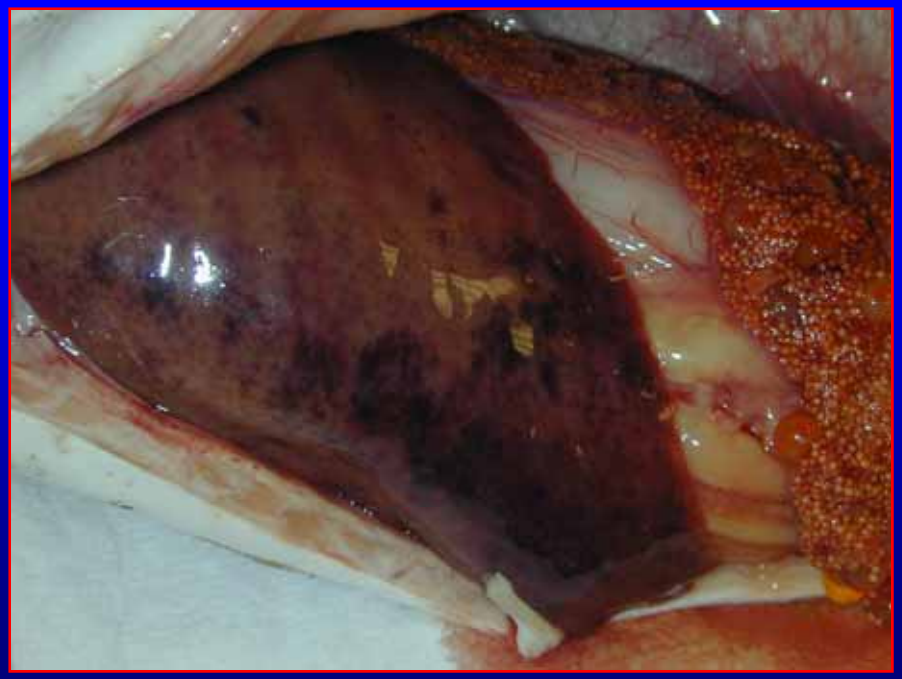
VHS in Wisconsin

- Brown Trout- Algoma/Kewaunee
- May 15 2007
- Dead on the beach (2 fish)
- No external signs

VHS in Wisconsin

Brown Trout- Algoma/Kewaunee

- Liver hemorrhages
- Enlarged spleen
- No muscle hemorrhages



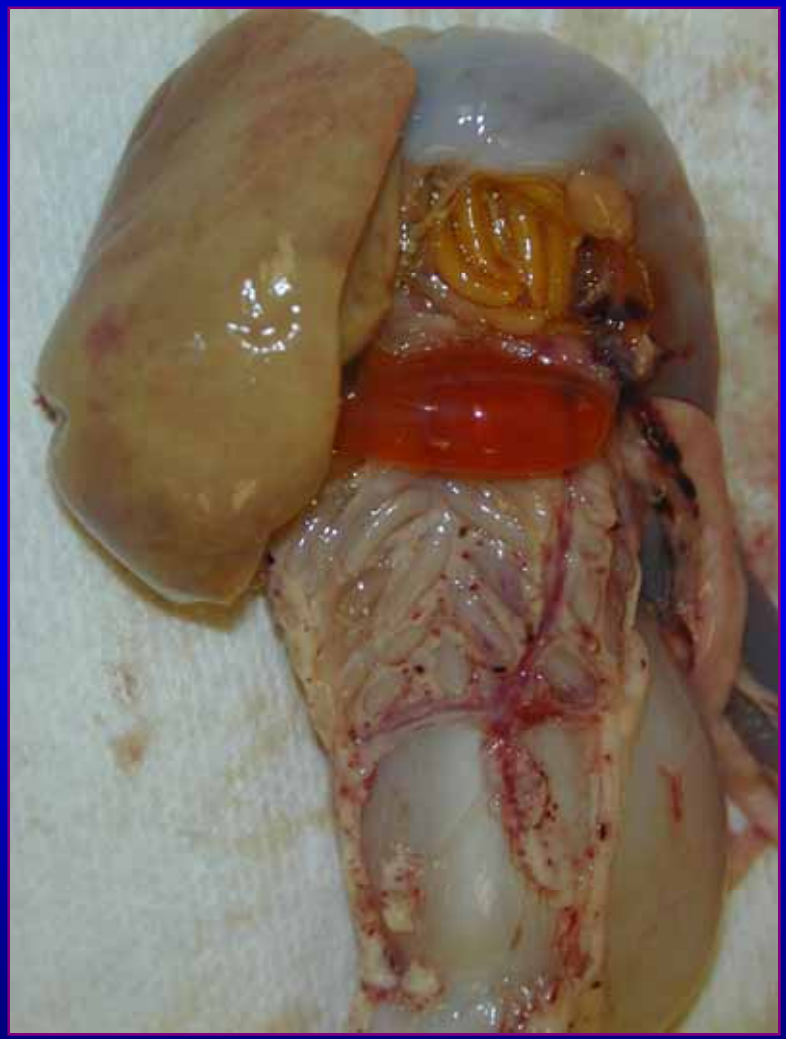
VHS in Wisconsin

- Lake whitefish- Northern Green Bay
- May 22, 2007
- Commercial fishermen observations
- Popeye and skin hemorrhages

VHS in Wisconsin

Lake whitefish- Northern Green Bay

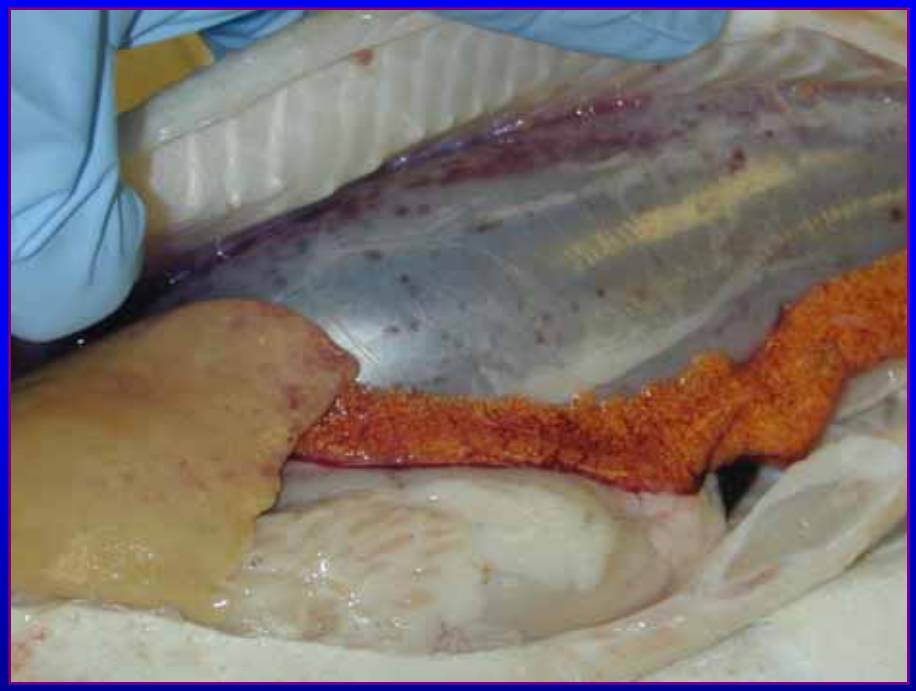
- Pale liver
- Body fat hemorrhages
- Enlarged spleen



VHS in Wisconsin

Lake whitefish- Northern Green Bay

- Swim bladder, liver and kidney hemorrhages



Fish we tested, but no VHS

- Walleye- Wolf R and Lake Winnebago

Fish we tested, but no VHS

Walleye- Wolf R and Lake Winnebago

No internal lesions

Fish we tested, but no VHS

Walleye- Wolf R and Lake Winnebago

Fish we tested, but no VHS

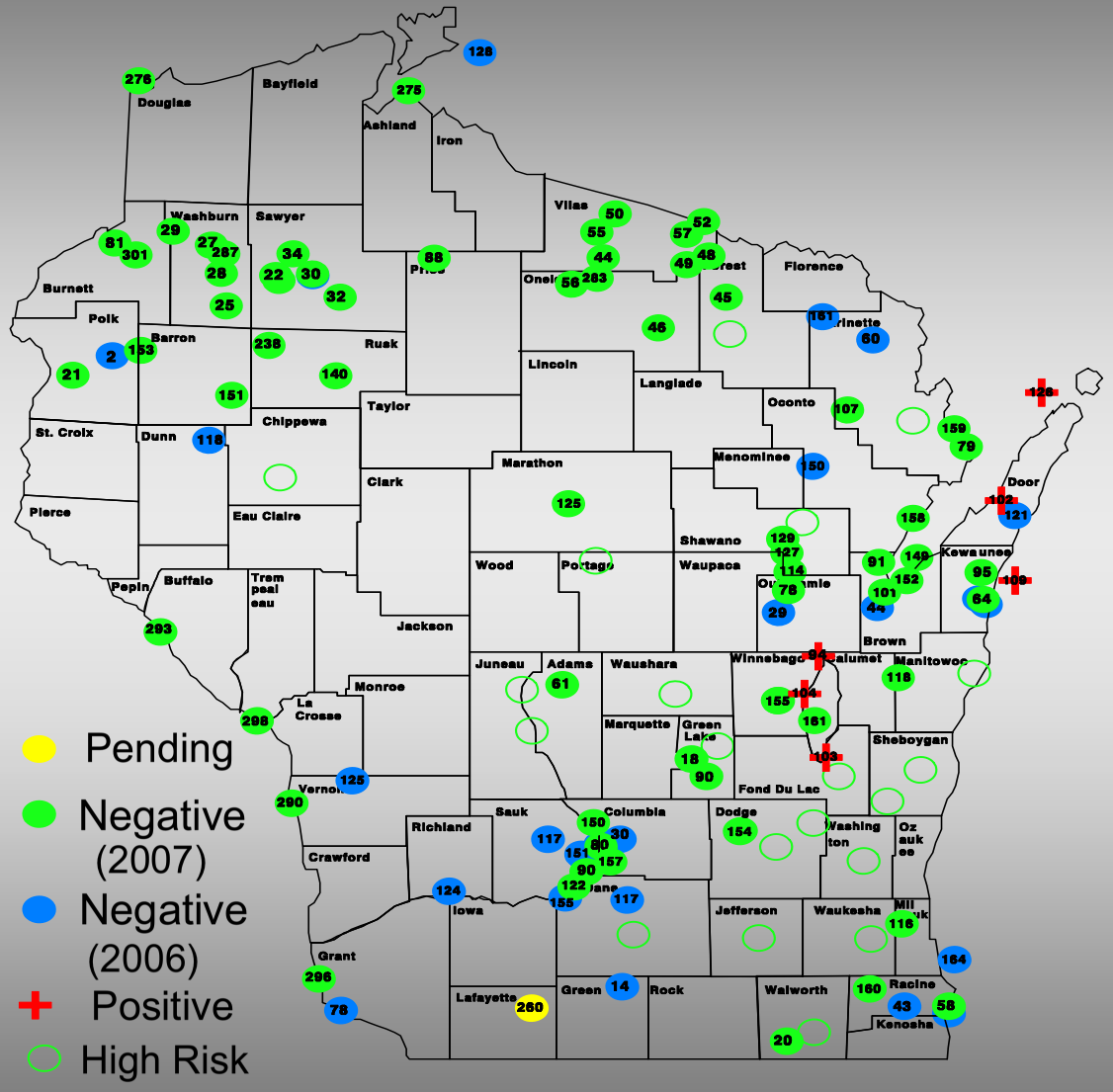
- Channel catfish Lake Winneconne

Fish we tested, but no VHS

Surveillance (60 wild fish per species per location)

- White suckers- Kewaunee R. and Chequamegon Bay
- Round goby- Green Bay and St Louis River
- Western banded killifish- Menomonie R.
- Yellow perch L Michigan Milw., St. Louis R., Chequamegon Bay
- Emerald shiner- Sugar R. Green Bay, Chequamegon Bay and St Louis R.
- Golden shiner- St Louis R.
- Bluegill, drum, LMB- Miss.R.
- Lake whitefish Green Bay, L. Superior
- Bloater chubs- L Michigan Milw. and Sheboygan

VHS Surveillance 2006-08



as of 10/16/2007

VHSv hosts in the Great Lakes (so far)

freshwater drum	lake whitefish	bluntnose minnow
round goby	walleye	Chinook lake trout
gizzard shad	emerald shiner	brown trout steelhead
muskellunge	spottail shiner	burbot white bass
smallmouth bass	black crappie bluegill	northern pike
yellow perch	redhorse	

VHSv distribution in the eastern U.S.

Risks to Fisheries

Restoration of native species

Unpredictable, devastating loss of fish

Wild fish egg collections for hatcheries

Forage collections for hatcheries

Wild fish transfers

Ecosystem effects- energy flow, food web, recruitment of new year classes of fish

What can citizens do?

- Comply with new VHS emergency rules:
  - Do not empty bait buckets in lakes or rivers
  - Do not transfer live fish from one location to another
  - Dewater live wells, bilges, buckets, etc. before leaving boat launches in Wisconsin and before entering Wisconsin if you fish elsewhere
  - Do not bring bait obtained in other states into Wisconsin
- Report fish kills, especially when fish have external hemorrhages

What have state and Federal agencies done?
Official disclaimer

Please contact the fisheries or agriculture agencies in the Great Lakes basin for complete knowledge and interpretation of VHS laws and statutes.

New Federal Laws

USDA APHIS- Fall 2006

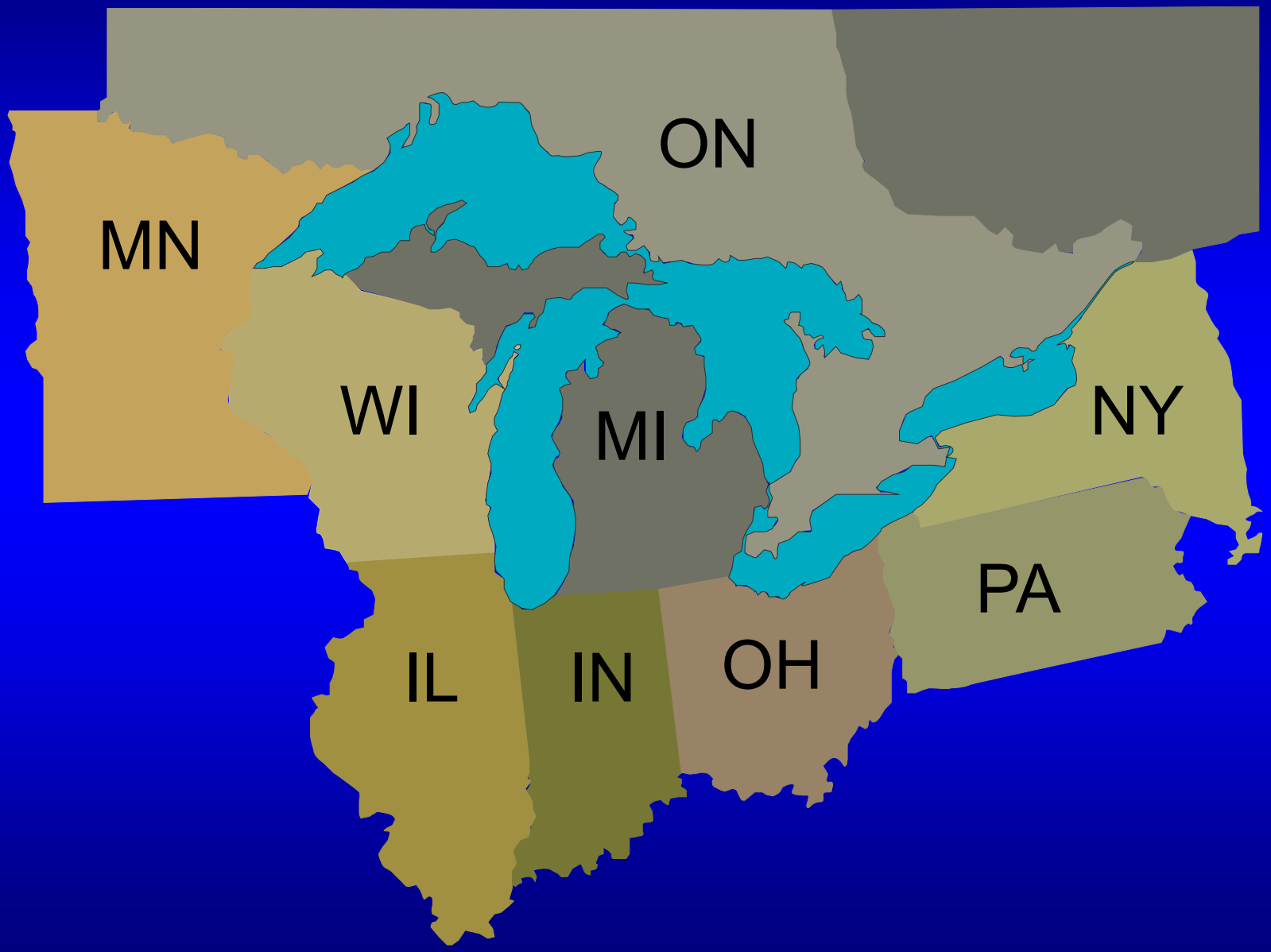
Issued a Federal Order prohibiting the movement of VHS susceptible fish out of the Great Lakes states, with certain exceptions.

The order also prohibited the import of susceptible species from Canada into the U.S.

APHIS is developing interim rules for VHS

DFO/CFIA are developing regulations for VHS in Canada

Geography refresher



New State/Provincial Laws - Authorities

Conservation agency

Dept of Agriculture

New State/Provincial Laws promulgated (so far)

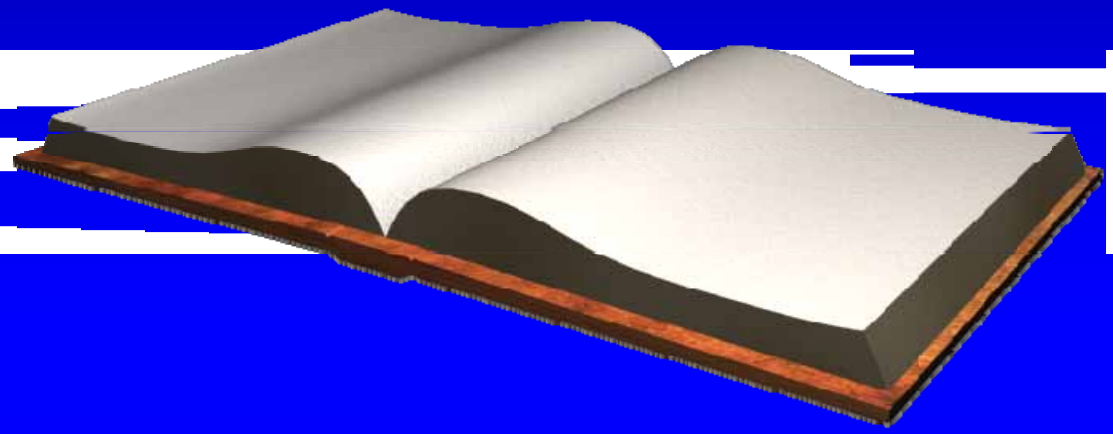
New York

[redacted]

Ohio

Pennsylvania

Michigan

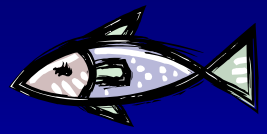


New State/Provincial Laws pertain to:

- VHS +/- zones
- Bait fish
- Game fish
- Water
- Fish farms
- Testing for pathogens

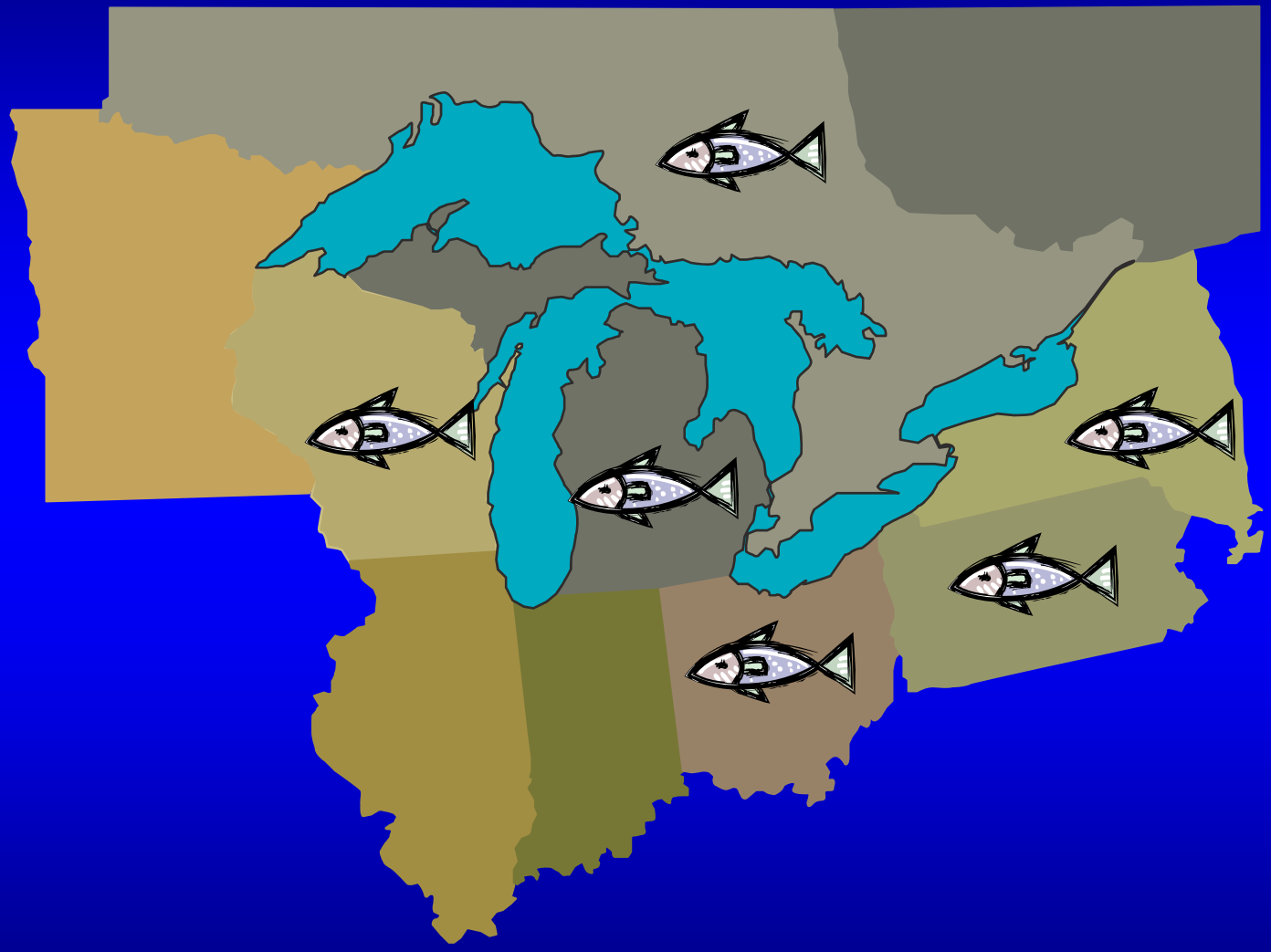
Existing laws also apply to regulating VHS in these categories

VHS + or +/free zones established by law

Bait fish 

- Permits/conditions for harvest, gear
- Conditions for intrastate movement
  - Facility certification (does not apply to fish farms)
- Conditions for use
  - Personal
  - Commercial
- Conditions for sale
  - Within, between zones
  - Health certification, live fish, eggs, dead fish
  - Retail and non-retail sales receipts contain health status information

Bait fish

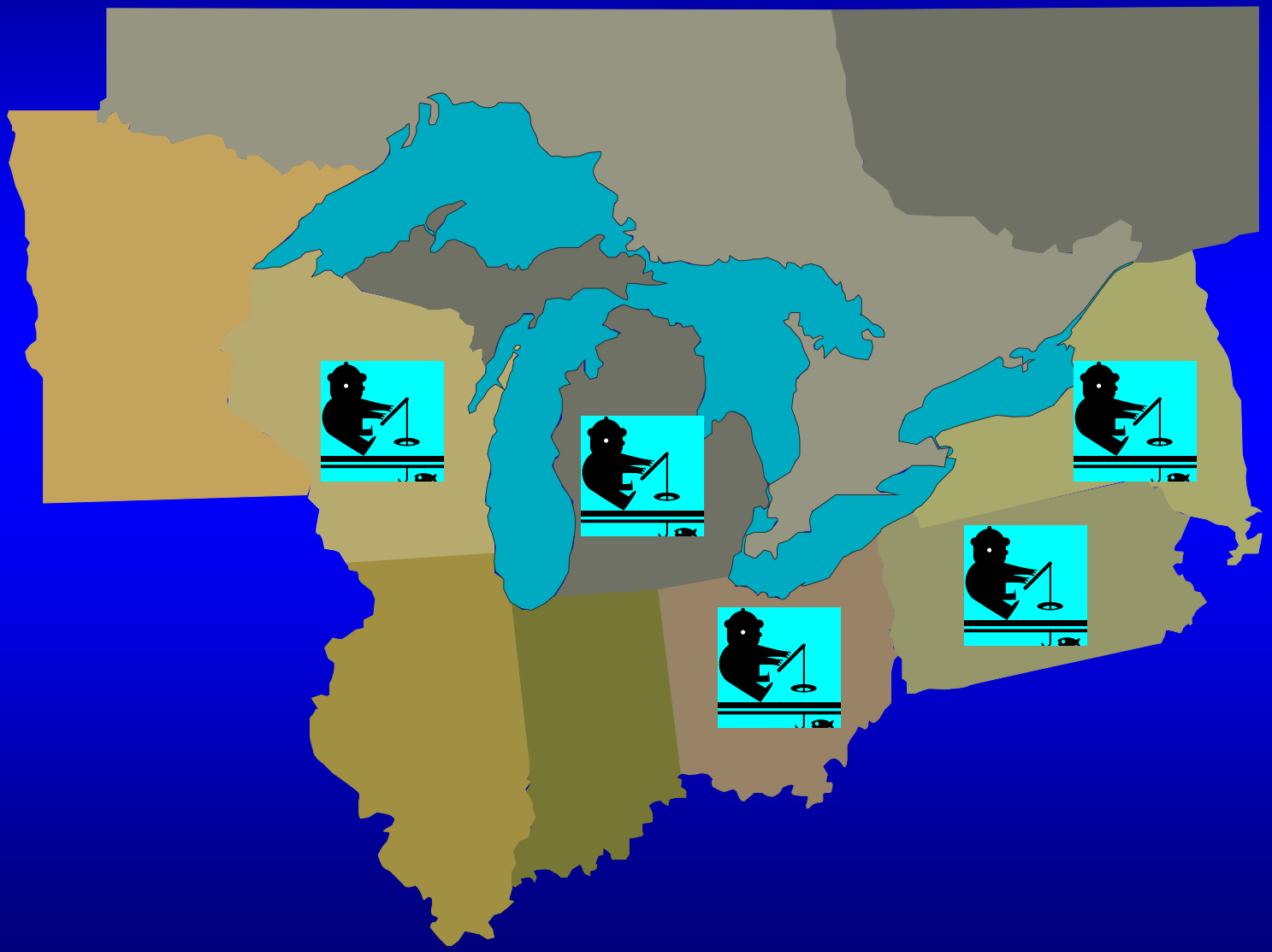


Game fish

Conditions for moving wild game fish

- All species
- VHS susceptible species

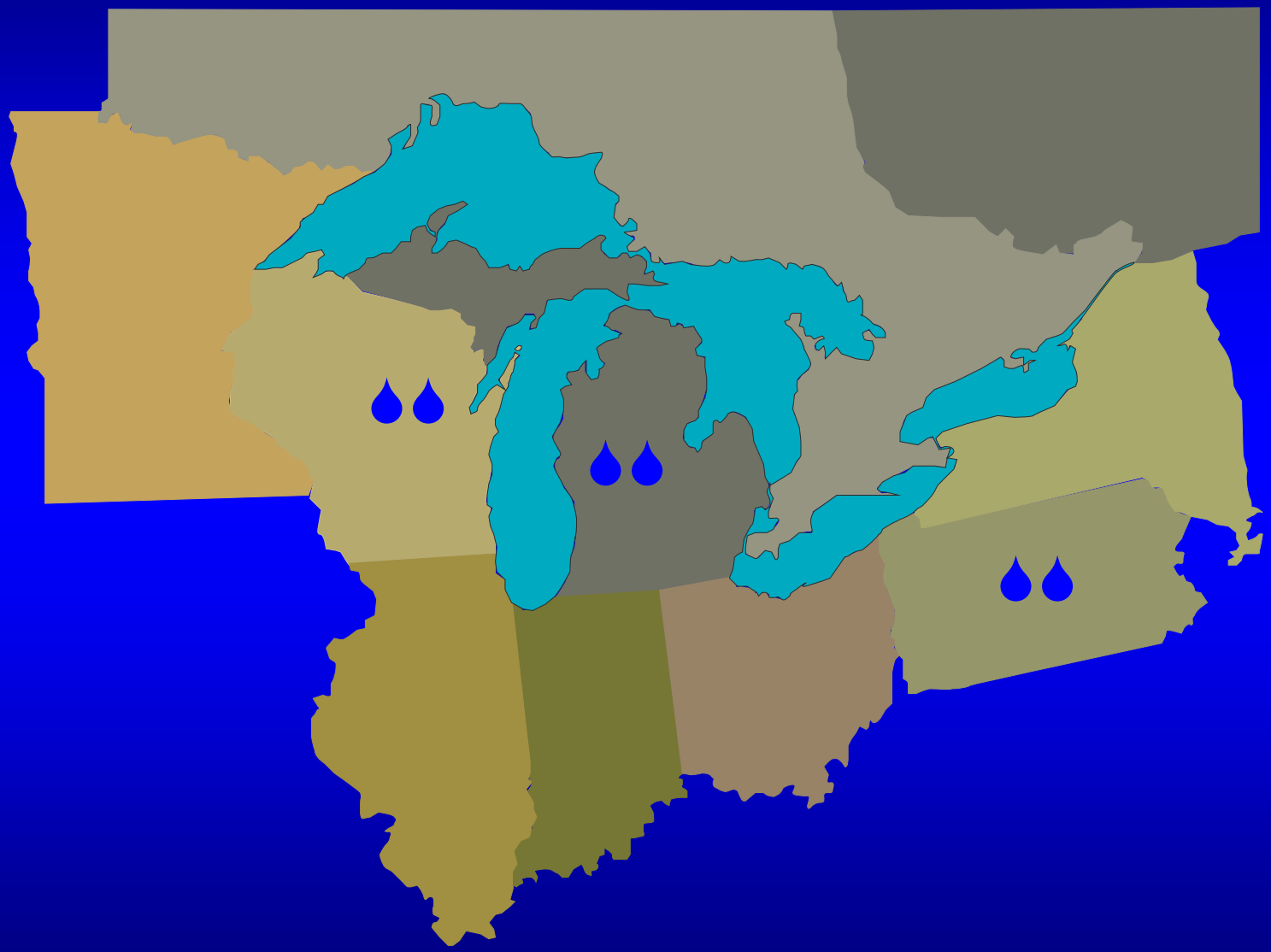
Game Fish



Water

- Drain live wells, bilges, etc. before leaving boat ramps
- Discharge water that contained VHS susceptible fish away from public waters that are outside of VHS + zones

Water

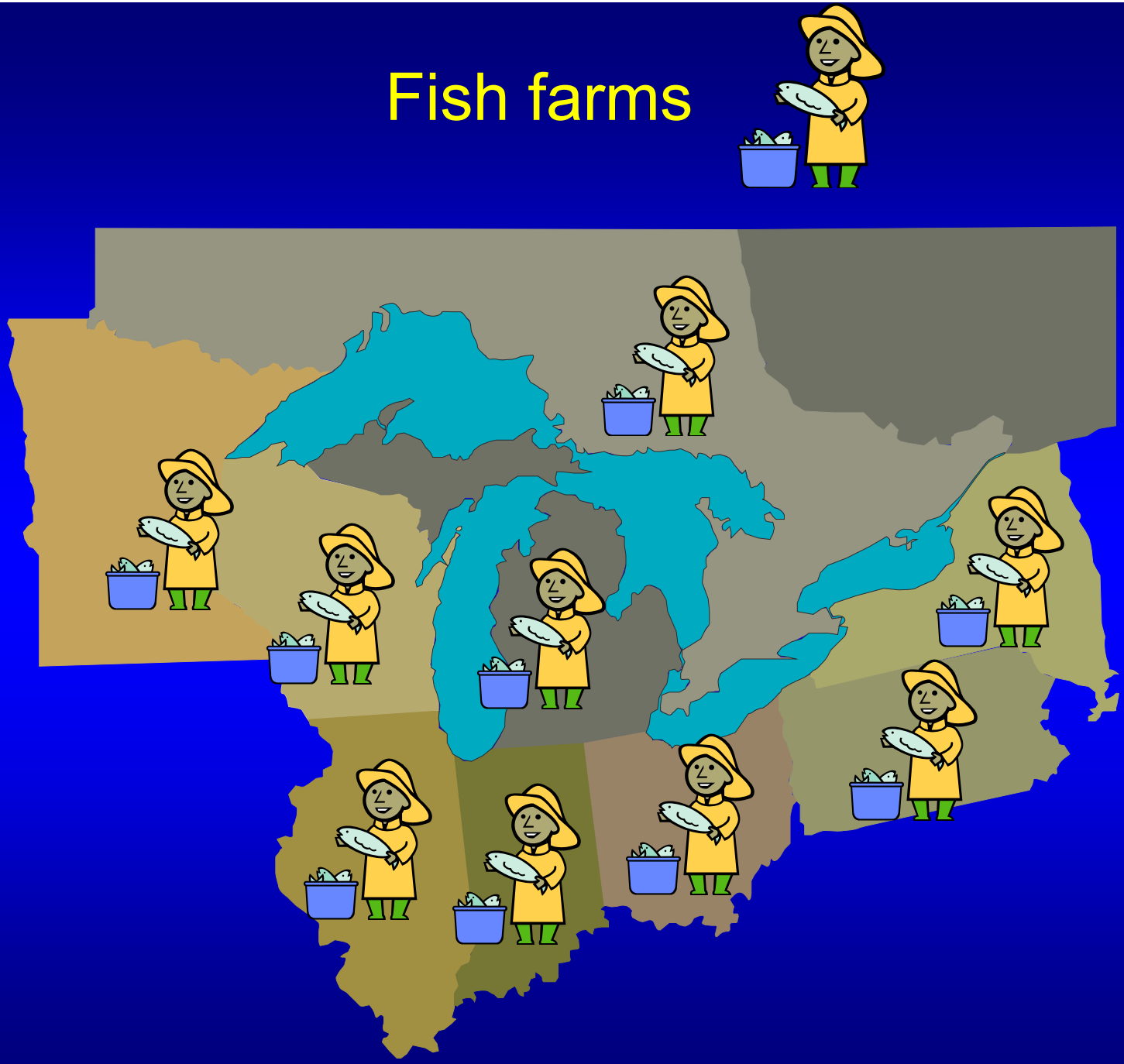


Fish Farms

Conditions for movement

- Farm to farm
- Farm to stocking public waters
- Importation
- All species
- VHS susceptible species
- Selected species

Fish farms

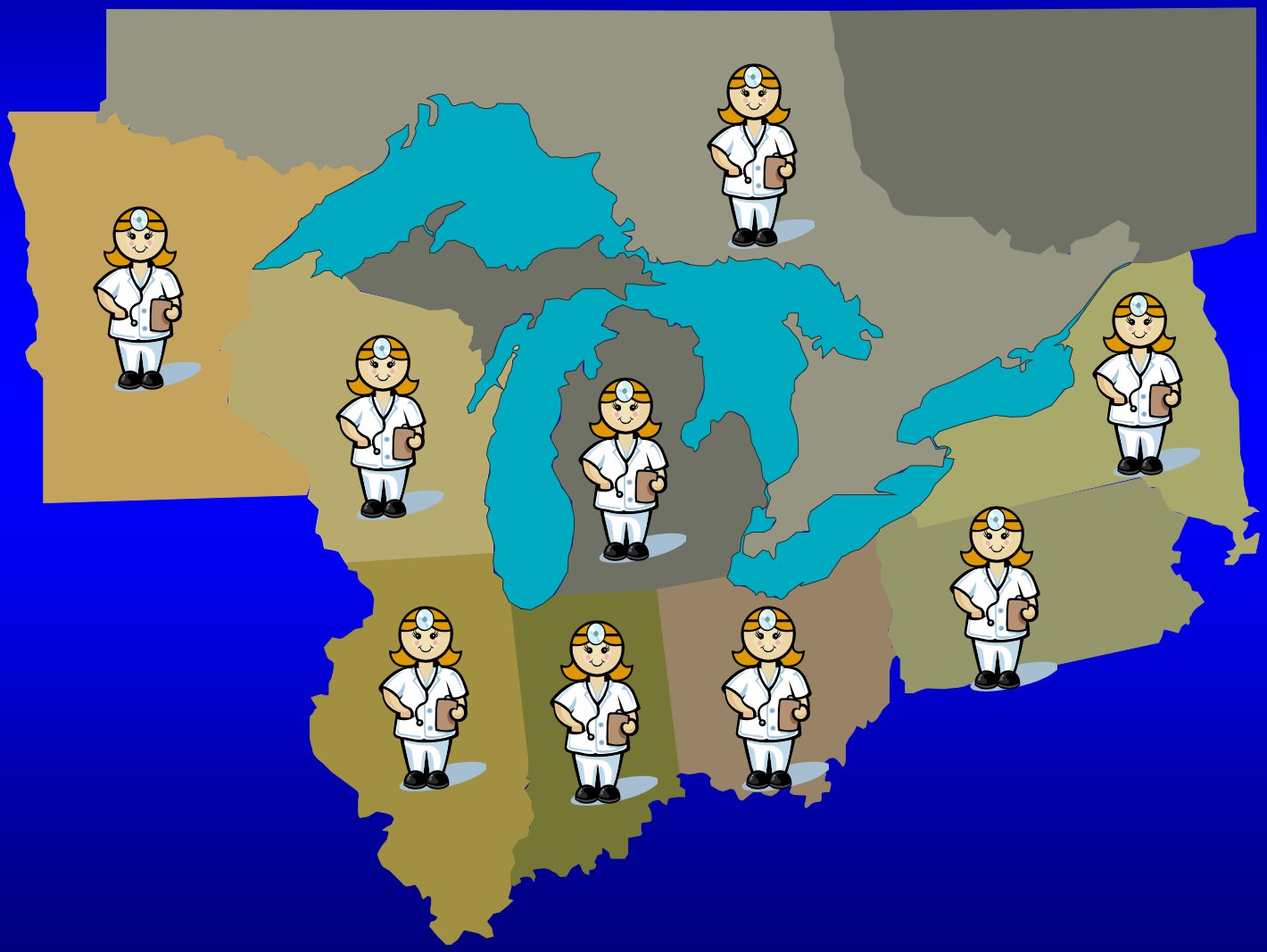


Testing for VHSv and other pathogens

- Conditions of importation
- For intrastate/province movement of fish
- Condition of stocking into public waters
- Condition of sale

May apply to all species, selected species, VHS susceptible species

Testing for pathogens



Wisconsin emergency VHS rules summary (effective 11/3/2007, some exceptions)

- A person may not leave the water with live fish

- All water must be drained from boats and equipment at the boat ramp or before entering the state

Emergency VHS rules summary

Bait

Baitfish and eggs may only be used if:

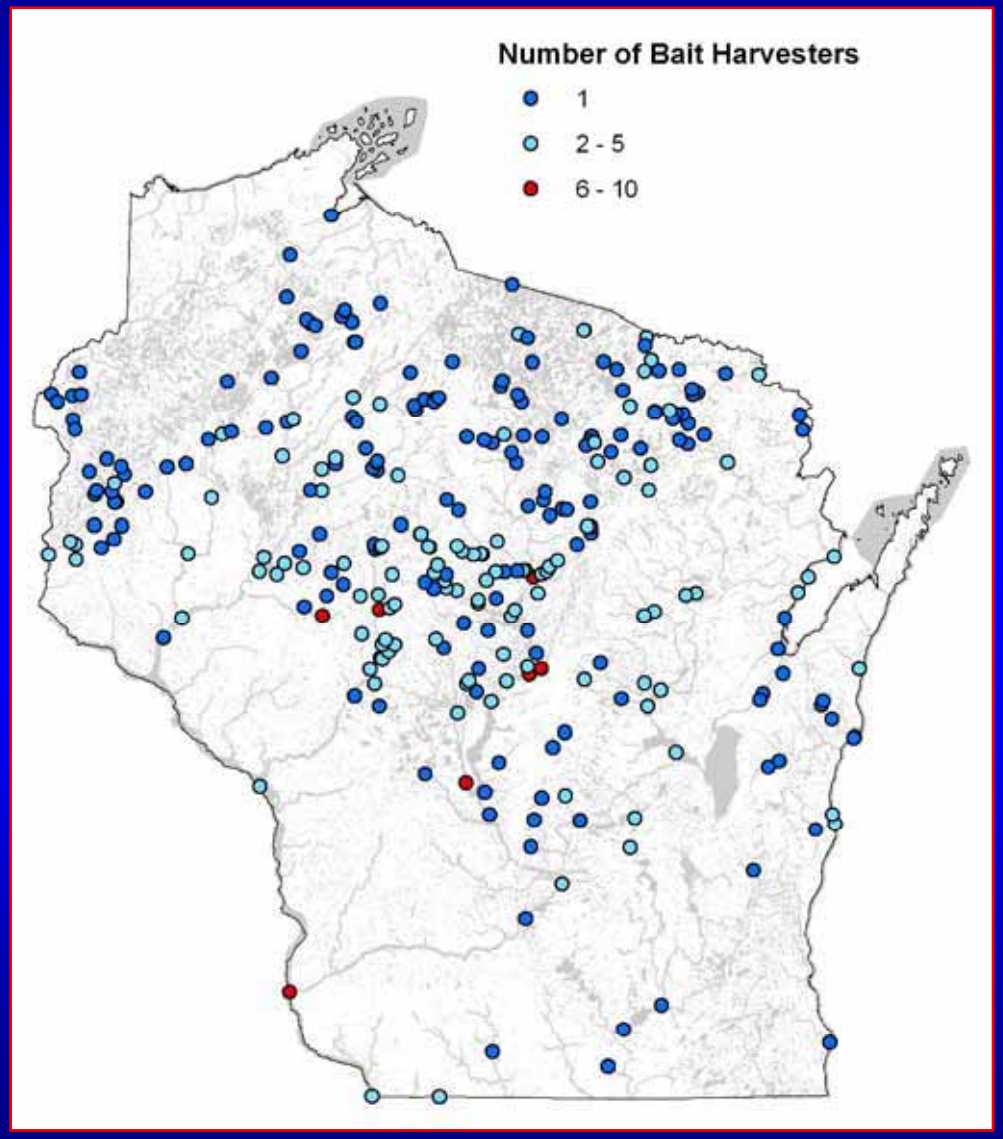
- Purchased from a Wisconsin baitshop (cannot purchase bait in another state and bring it into WI, except on the Miss. R.)
- Caught and used on the same water (fresh or frozen)
- Baitfish and eggs caught in one water for use on another water must be dead and preserved (not just refrigerated or frozen)

Emergency VHS rules summary
Wild bait harvest

Commercial wild bait harvest is by permit only

- permits will not be issued for waters where VHS is present,
- DATCP fish health certificate required to distribute baitfish (emerald & spottail shiners, bluntnose minnow)
- A gear disinfection requirement is in the permit
- Permits are valid for 30 days and are renewable

Locations of Wild Bait Harvest, 2007
~90 individual permitted harvesters



Stream locations are shown at the mouth, harvest may have occurred upstream

Emergency VHS rules summary
Crayfish and turtle harvest

- A fishing or small game license is needed to harvest crayfish and turtles (existing law)
- If dead fish are used as bait:
  - they must come from the same water where the traps are set, or
  - they must be minnows from a Wisconsin bait dealer
  - Or there must be written approval from DNR

New VHS policies for WI DNR

- Disinfection
  - Boats, gear
  - Fish eggs for hatcheries
- Conditions for the use of wild broodfish for hatchery programs
- Biosecurity plans developed for hatcheries
- Isolation facilities for hatcheries
- Surveillance for VHSv
- Wild fish transfers
- Operation of fishways/fish passage
- Increased public education and outreach
- Fishing tournaments
- Conditions for Scientific Collector's permits

Disinfection - 3 C's:

Chemical
Concentration
Contact time

Chlorine 200 ppm for 5 minutes
(⅓ cup bleach in 5 gallons of water)
Iodine 100 ppm for 10-15 minutes
Virkon 1:1000 for 15 minutes
Quaternary ammonium products
as per label
UV light 1-3 X 10^3µW s /cm^2
Heat: 113 °F for 60 minutes or
140 °F for 15 minutes
Steam cleaning
(212 °F for seconds)

DNR VHS website

http://dnr.wi.gov/fish/vhs/

Great Lakes Fish Parasite Database and Atlas Project Update

Dr. Patrick Muzzall,
Michigan State University
and
Gary Whelan
MI DNR Fisheries Division
January 2008

Project Overview and Status

- Literature search for all available information on Great Lakes parasite data
- Relational database to handle all types of fish pathogen data – web based database
  - Individual fish
  - Lots of fish
  - Summarized data from literature or reports
- Linked to GIS with appropriate query engine

Database Structure

Wild Aquatic Organism Pathogen Database Project
SQL/Server Database: DNRS0210-FISH
Last Updated: June 28, 2007 by Chris Larson

FHI_Facility_Collection_Point

	Column Name	Condensed Type	Nullable
🔑	Facility_Name	nchar(50)	NOT NULL
	Facility_Type	nchar(30)	NULL
	Facility_Address1	nchar(30)	NULL
	Facility_Address2	nchar(30)	NULL
	Facility_City	nchar(30)	NULL
	Facility_State	char(3)	NULL
	Facility_Zip	char(7)	NULL
	Facility_Zip4	char(4)	NULL
	Facility_Phone	nchar(10)	NULL
	Facility_Fax	nchar(10)	NULL
	Owner_Manager_Name	nchar(50)	NULL
	Owner_Manager_Title	nchar(30)	NULL
	Facility_County_Name	nchar(15)	NULL
	Facility_Township	nchar(10)	NULL
	Facility_TownRange	nchar(10)	NULL
	Facility_Section	tinyint	NULL
	Facility_TRS	nchar(10)	NULL
	Water_Supply	nchar(35)	NULL
	Last_Update_Entity	varchar(30)	NULL
	Last_Update_DateTime	smalldatetime	NULL

FHI_Water_Supply (FIS)

	Column Name	Condensed Type	Nullable
🔑	Water_Supply	nchar(35)	NOT NULL
	Description	varchar(255)	NULL
	Last_Update_Entity	varchar(30)	NULL
	Last_Update_DateTime	smalldatetime	NULL

FHI_Water_Collection_Point

	Column Name	Condensed Type	Nullable
🔑	Collection_Point_Name	nchar(50)	NOT NULL
	Collection_Waterbody	nchar(50)	NULL
	Collection_Latitude	decimal(9, 7)	NULL
	Collection_Longitude	decimal(9, 7)	NULL
	Collection_County_Name	nchar(15)	NULL
	Collection_Township	nchar(10)	NULL
	Collection_TownRange	char(8)	NULL
	Collection_Section	tinyint	NULL
	Collection_TRS	nchar(10)	NULL
	MI_Georef_X	float(53)	NULL
	MI_Georef_Y	float(53)	NULL
	Location_Narrative	nchar(200)	NULL
	Water_Supply	nchar(35)	NULL
	WaterBody_Key	char(10)	NULL
	Last_Update_Entity	varchar(30)	NULL
	Last_Update_DateTime	smalldatetime	NULL

FHI_Fish_Type (FIS)

	Column Name	Condensed Type	Nullable
🔑	Fish_Type	char(35)	NOT NULL
	Description	text	NULL
	Last_Update_Entity	varchar(30)	NULL
	Last_Update_DateTime	smalldatetime	NULL

FHI_Age (FIS)

	Column Name	Condensed Type	Nullable
🔑	Age_Code	char(10)	NOT NULL
	Description	varchar(255)	NULL
	Last_Update_Entity	varchar(30)	NULL
	Last_Update_DateTime	smalldatetime	NULL

FHI_Fish_Sample

	Column Name	Condensed Type	Nullable
🔑	Inspection_Number	int	NOT NULL
🔑	Sample_Number	int	NOT NULL
	Species_Code	char(6)	NULL
	Fish_Designation	nchar(50)	NULL
	Age_Code	char(10)	NULL
	Fish_Type	char(35)	NULL
	Number_of_Fish_Collected	smallint	NULL
	Number_of_Fish_To_WLHatchery	smallint	NULL
	Number_of_Fish_To_Lab	smallint	NULL
	Number_of_Fish_Tested	smallint	NULL
	Obtained_As	nchar(10)	NULL
	Lab_Sample_Number	nchar(50)	NULL
	Sample_Comments	text	NULL
	Last_Update_Entity	varchar(30)	NULL
	Last_Update_DateTime	smalldatetime	NULL

Domain table for SpecStr_Code is view FIS.SpecStr.

Need to allow for other fish health labs

Project Overview and Status

FHI_Collector (FIS)

	Column Name	Condensed Type	Nullable
🔑	Collector_Name	nchar(50)	NOT NULL
	Collector_Organization	nchar(50)	NULL
	Collector_Address1	nchar(40)	NULL
	Collector_Address2	nchar(40)	NULL
	Collector_City	nchar(30)	NULL
	Collector_State	char(3)	NULL
	Collector_Zip	nchar(12)	NULL
	Collector_Country	char(3)	NULL
	Collector_Telephone	nchar(10)	NULL
	Collector_Email	nchar(50)	NULL
	Last_Update_Entity	varchar(30)	NULL
	Last_Update_DateTime	smalldatetime	NULL

FHI_Fish_Health_Official (FIS)

	Column Name	Condensed Type	Nullable
🔑	Fish_Health_Official_Name	varchar(50)	NOT NULL
	Title	varchar(40)	NULL
	Address1	varchar(40)	NULL
	Address2	varchar(40)	NULL
	Address3	varchar(40)	NULL
	City	varchar(30)	NULL
	State	char(3)	NULL
	Zip5	varchar(7)	NULL
	Zip4	varchar(4)	NULL
	Phone	varchar(10)	NULL
	Fax	varchar(10)	NULL
	Last_Update_Entity	varchar(30)	NULL
	Last_Update_DateTime	smalldatetime	NULL

FHI_Inspection_Purpose

	Column Name	Condensed Type	Nullable
🔑	Inspection_Purpose	nchar(10)	NOT NULL
	Last_Update_Entity	varchar(30)	NULL
	Last_Update_DateTime	smalldatetime	NULL

FHI_Inspection (FIS)

	Column Name	Condensed Type	Nullable
🔑	Inspection_Number	int	NOT NUL
	Collector_Name	nchar(50)	NULL
	Fish_Source	char(10)	NULL
	Fish_Source_Name	nchar(50)	NULL
	Classification	nchar(50)	NULL
	Inspection_Purpose	nchar(10)	NULL
	Date_Collected	smalldatetime	NULL
	Date_To_WLHatchery	smalldatetime	NULL
	Date_At_WLHatchery	smalldatetime	NULL
	Date_To_Health_Lab	smalldatetime	NULL
	Date_At_Health_Lab	smalldatetime	NULL
	Date_Of_Lab_Examination	smalldatetime	NULL
	Fish_Health_Official_Name	varchar(50)	NULL
	MDNR_Fish_Kill_Report_Number	nchar(10)	NULL
	Inspection_Comments	text	NULL
	Last_Update_Entity	varchar(30)	NULL
	Last_Update_DateTime	smalldatetime	NULL

FHI_Fish_Sample_Supplemental

	Column Name	Condensed Type	Nullable
🔑	Inspection_Number	int	NOT NULL
🔑	Sample_Number	int	NOT NULL
🔑	DateTime	datetime	NOT NULL
	Findings	varchar(255)	NULL
	Last_Update_Entity	varchar(30)	NULL
	Last_Update_DateTime	smalldatetime	NULL

FHI_Fish_Subsample

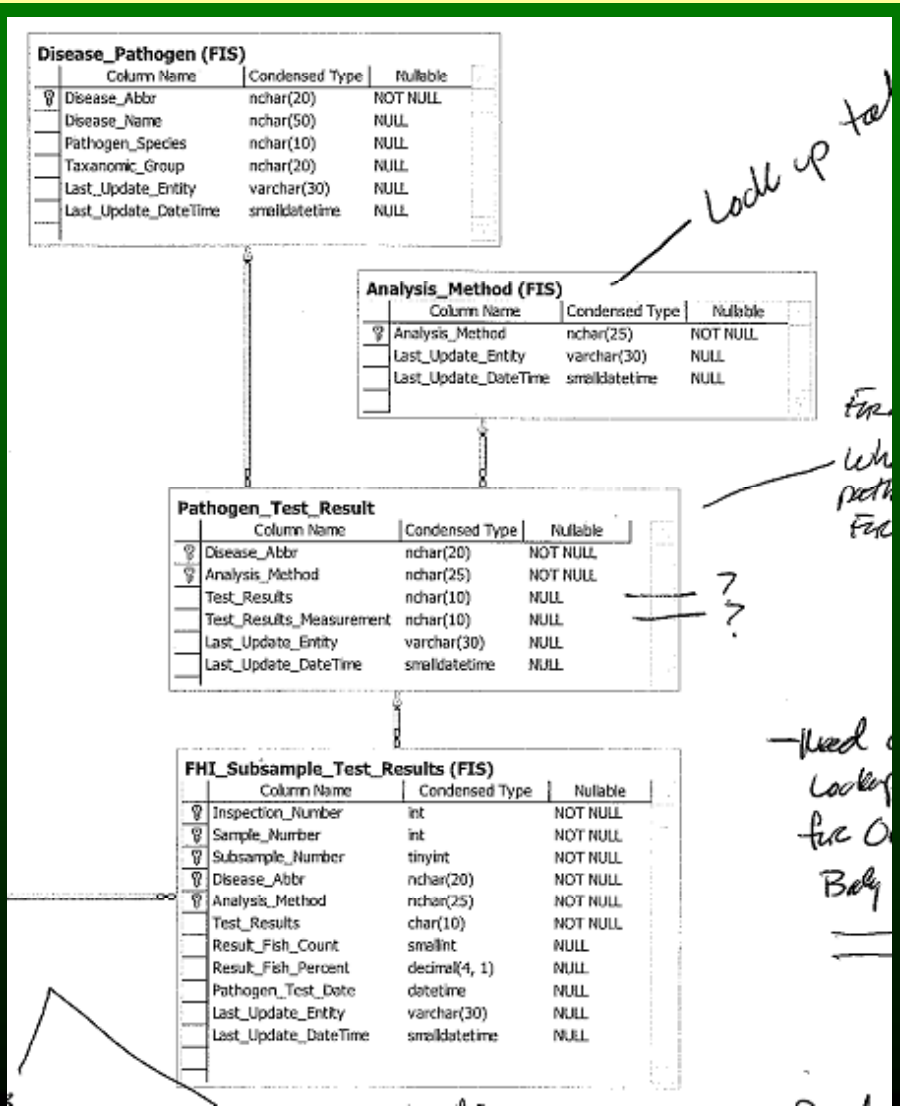
	Column Name	Condensed Type	Nullable
🔑	Inspection_Number	int	NOT NULL
🔑	Sample_Number	int	NOT NULL
🔑	Subsample_Number	tinyint	NOT NULL
	Number_of_Fish	smallint	NULL
	Percentage_of_Sample	decimal(4, 1)	NULL
	Minimum_Length_in_mm	float(53)	NULL
	Maximum_Length_in_mm	float(53)	NULL
	Median_Length_in_mm	float(53)	NULL
	Mean_Length_in_mm	float(53)	NULL
	Minimum_Weight_in_SE	float(53)	NULL
	Maximum_Weight_in_SE	float(53)	NULL
	Median_Weight_in_SE	float(53)	NULL
	Mean_Weight_in_SE	float(53)	NULL
	Lab_Sample_Number	nvarchar(50)	NULL
	Subsample_Comments	char(10)	NULL
	Last_Update_Entity	varchar(30)	NULL
	Last_Update_DateTime	smalldatetime	NULL

These are the fish samples as they were divided down into subgroups for testing at the lab. One individual fish can be in many subsamples.

When"Number of fish" = 1,a single fish was exampled.
When "Number of fish" > 1 and < # of fish in sampe, fish were pooled together for testing.

OK, we will need to

Project Overview and Status



Disease_Pathogen (FIS)

Column Name	Condensed Type	Nullable
Disease_Abbr	nchar(20)	NOT NULL
Disease_Name	nchar(50)	NULL
Pathogen_Species	nchar(10)	NULL
Taxanomic_Group	nchar(20)	NULL
Last_Update_Entity	varchar(30)	NULL
Last_Update_DateTime	smalldatetime	NULL

Analysis_Method (FIS)

Column Name	Condensed Type	Nullable
Analysis_Method	nchar(25)	NOT NULL
Last_Update_Entity	varchar(30)	NULL
Last_Update_DateTime	smalldatetime	NULL

Pathogen_Test_Result

Column Name	Condensed Type	Nullable
Disease_Abbr	nchar(20)	NOT NULL
Analysis_Method	nchar(25)	NOT NULL
Test_Results	nchar(10)	NULL
Test_Results_Measurement	nchar(10)	NULL
Last_Update_Entity	varchar(30)	NULL
Last_Update_DateTime	smalldatetime	NULL

FHI_Subsample_Test_Results (FIS)

Column Name	Condensed Type	Nullable
Inspection_Number	int	NOT NULL
Sample_Number	int	NOT NULL
Subsample_Number	tinyint	NOT NULL
Disease_Abbr	nchar(20)	NOT NULL
Analysis_Method	nchar(25)	NOT NULL
Test_Results	char(10)	NOT NULL
Result_Fish_Count	smallint	NULL
Result_Fish_Percent	decimal(4, 1)	NULL
Pathogen_Test_Date	datetime	NULL
Last_Update_Entity	varchar(30)	NULL
Last_Update_DateTime	smalldatetime	NULL

Project Schedule

- Literature search – complete in Spring 2008
- Database development – prototype in late March 2008
- Data entry – Summer 2008
- GIS and query engine – Fall 2008

Questions? Comments?

Great Lakes, Great Times, Great Outdoors

www.michigan.gov/dnr

Channel Catfish Die Off in Red River in Minnesota

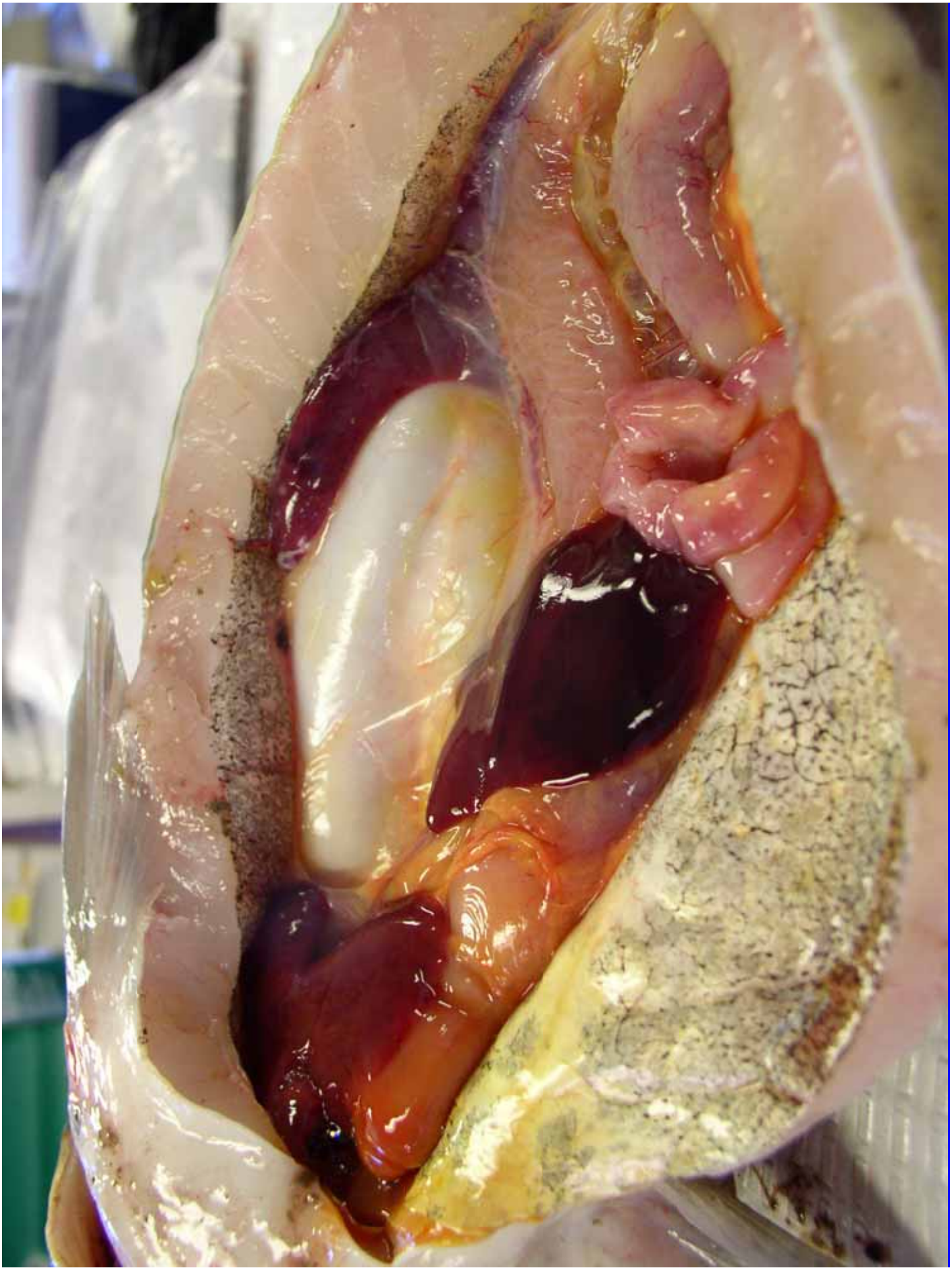
September 2007

Channel Catfish Die Off in Red River in Minnesota

- 1600-1700 dead channel catfish observed along approximately nine-mile stretch of the river
- Ranged in size from about 5 inches to 30 inches
- Reported dead fish by fishermen the last few weeks

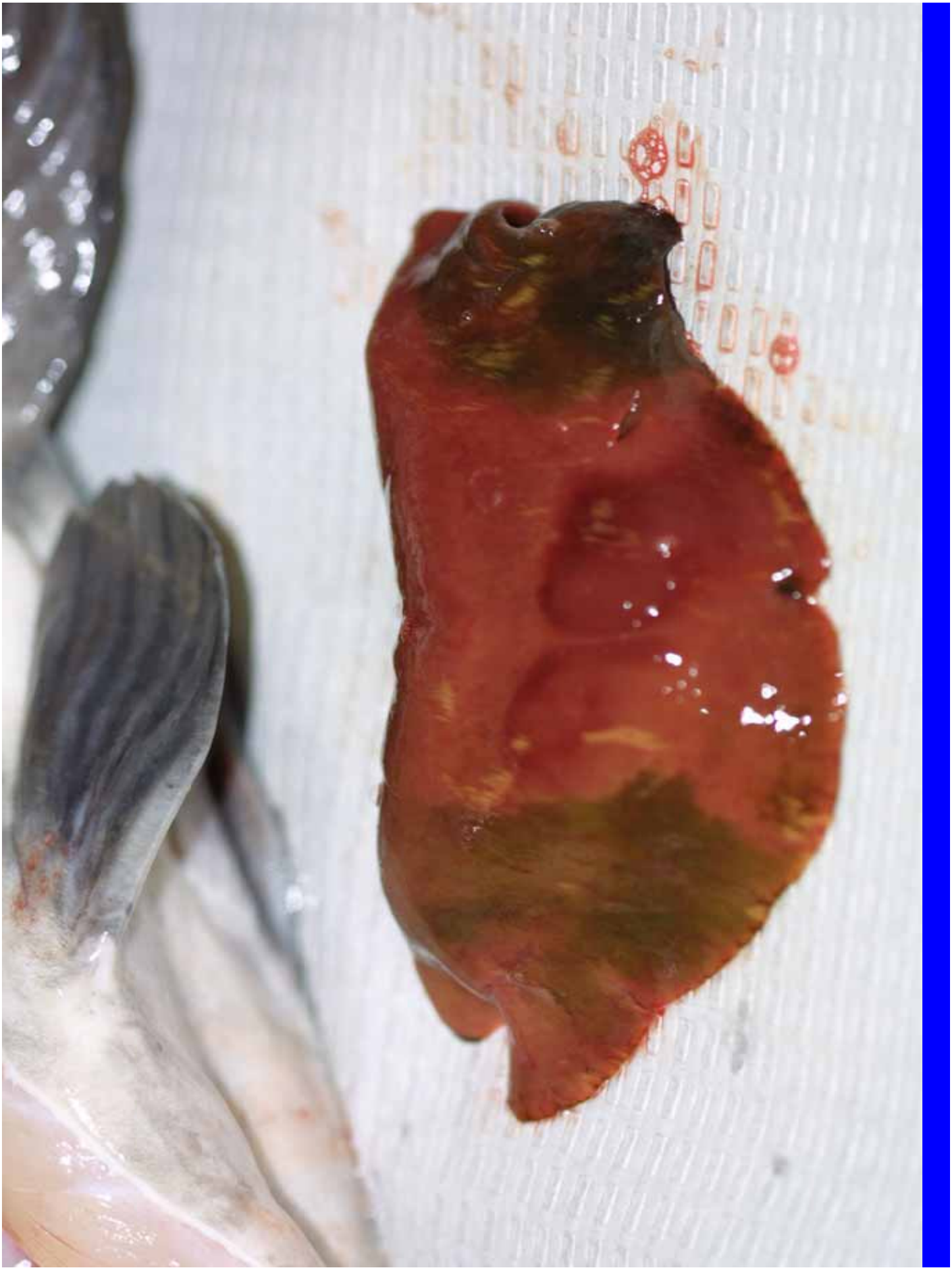
Channel Catfish Die Off in Red River in Minnesota

- Most dead fish were decomposed or highly dehydrated
- One fish were freshly dead and sent to our pathology lab



Channel Catfish Die Off in Red River in Minnesota

- Flavobacterium like Bacteria found on gill smear
- Aeromonas spp.(A. veronii & Aeromonas media like DNA group 5A) isolated from the skin lesions, kidney and intestine
- A. hydrophia from kidney and spleen
- VHSv and CCV negative



Channel Catfish Die Off in Red River in Minnesota

- No Flavobacterium like bacteria on gill smear
- Parasites were found
  - Trichodia
  - Cleidodiscus

Channel Catfish Die Off in Red River in Minnesota

Bacterial Isolation

Skin Lesion

- Pseudomonas Fluorescens
- Pseudomonas viridiflava
- Vibrio metschnikovii

Channel Catfish Die Off in Red River in Minnesota

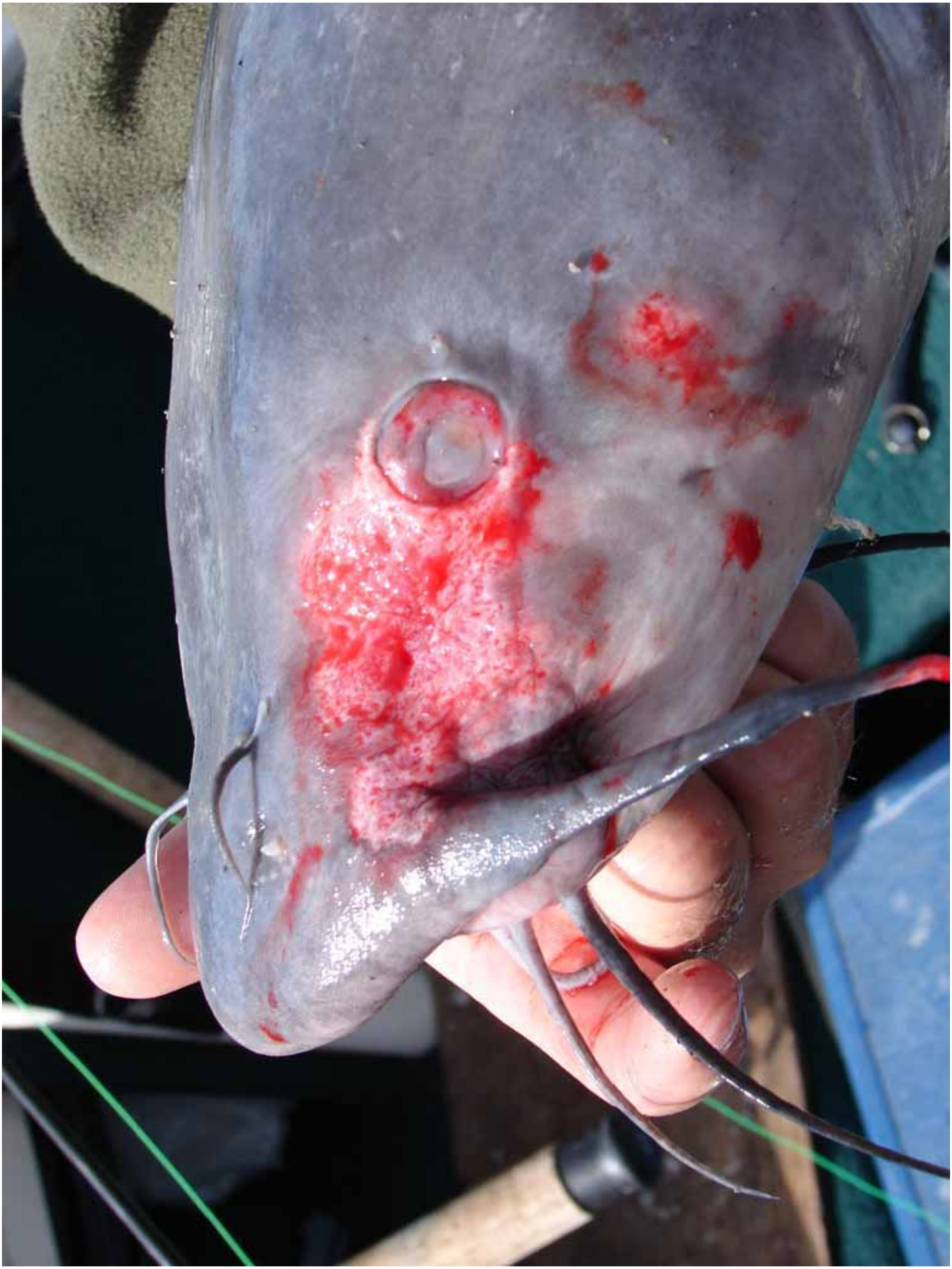
Bacterial isolation

Kidney

- Vibrio metschnikovii

Liver

- Aeromonas schubertii




VHS Sampling
Because we just can't get enough of VHS

But First, A Refresher Course
Fish Health 101

A Guide to the Who, What, Where, When, and Why's of Fish Health

Fish Health Program Priorities

Disease testing required as member of Great Lakes Fish Health Committee

- Annual Inspections of production fish and broodstocks
- Diagnostics
  - Production fish
  - Wild Fish

Pathogens of Concern

- BKD-Bacterial Kidney Disease
- WD-Whirling Disease
- IPN-Infectious Pancreatic Necrosis Virus
- IHN-Infectious Hematopoietic Necrosis Virus
- VHS-Viral Hemorrhagic Septicemia
- ERM-Enteric Redmouth
- Furunculosis

- LMBV-Largemouth Bass Virus
- Heterosporis
- Spring Viremia of Carp
- White Sturgeon Herpesvirus and Iridovirus
- Piscirickettsia

Who does what?

Aquatic Animal Health Lab at Michigan State University

- Annual contract with DNR for fish health services $157,000
  - 200 hours of consultation
  - 250 cases for pathogen analysis
    - Case is a group of fish submitted for testing
    - Inspections are 60 fish
    - Diagnostics are 20 fish, but could be 1-60 fish

Who does what?

- Aquatic Animal Health Lab at Michigan State University
  - Services provided include:
    - Annual inspections of production and broodstock lots
    - Diagnostics
      - Hatchery stocks
      - Feral stocks
    - Treatment Recommendations
    - Reports
    - Consultations
    - INAD Coordination
    - Quarantine Facilities

Who does what?

- DNR-Fisheries
  - Fish Health Program Manager
    - Liaison with MSU to coordinate fish health work
      - Scheduling annual inspections
      - Process requests and schedule diagnostics
      - Process requests for consultations
      - Distribution of recommendation and reports
      - Maintain fish health data base
    - Oversight of fish health budget

Why do we have this new system?

- In the first few years of the contract, MSU encouraged DNR to submit all fish health concerns
  - Provided diagnostic services we hadn't had available in the past
  - Provided a framework for including fish health issues in our work- hatchery and field
- The need to manage the fish health work load became evident early in 2006
  - Lab overwhelmed with case work
  - Requests for work, questions concerning fish health coming from all directions
  - Results of testing not reported timely
  - Required reports not being generated

What does that mean for me?

- DNR-MSU Liaisons work together to coordinate fish health work
  - All fish health requests (diagnostics, fish kills, disease questions, etc) are routed through the DNR liason-Martha to MSU liason- Michelle Gunn
  - All fish health requests are routed through Martha
  - All fish health requests are routed through Martha
  - All fish health requests are routed through Martha

Will everything I request get done?

- Probably, but maybe no
  - Dependent on:
    - Pathogen suspected
    - Affected population (a few fish, one species or many fish and many species)
    - Scope of pathogen (one location or widespread)
- Need to prioritize fish health work given limited resources
  - Production fish and broodstock are priority (GLFHC)
  - Emergency diseases (i.e. VHS) and diseases of concern in Feral stocks are next priority
  - All other requests are evaluated on a case by case basis

This is all good to know, but all I really want to know is what do I need to do to get fish health work done?

You only need to remember a few key things:

- Plan ahead—10 weeks from receipt of samples to report for pathogen lab work
  - Emergencies (fish kills or disease outbreaks) will be handled through the same process but given high priority
  - Fish transfers (water body to water body) require:
    - Approved prescription
    - Sample collection and lab work done as close to the planned transfer as possible

A few key things continued:

- Work through your unit Manager
  - Requests to Martha should come from unit Managers only
  - Requests supported by unit or basin team will receive higher priority
- Submit requests for work on appropriate form electronically to Martha.

A few key things continued:

- Request updates on results of testing to Martha, if past due
- If Martha is not available, contact Jan or Ed—we work as a team. They are copied on all fish health correspondence so can fill in when needed.

In the works:

- Currently working to get this process outlined in a policy/procedure so is readily accessible to all the division
- Forms needed to request fish health work are on the Intranet, in the Fish Health folder

Miscellaneous items:

- Shipping labels that bill directly to fish health budget are available.
- Shipping containers can be purchased if needed, get approval and coding numbers from Martha.
- Questions, comments, suggestions, problems……who else, Martha.

To summarize:

- Plan Ahead
- Work with your unit manager and/or basin team
- Everything goes through Martha
- Patience:
  - This is a new process for everyone
  - Fish Health is not Martha's only responsibility, so patience and understanding are appreciated.
  - Suggestions and comments to improve the process are always welcome.


Now…VHS

Traditional methods of sample collection and processing

- Live fish collected and shipped or delivered to MSU AAHL
- MSU lab staff performs exam and collects tissue samples
- MSU runs test and issues report with results

Changes to this process for VHS

- VHS can be detected in samples that have been frozen
- VHS testing only requires spleen and kidney
- Freezing allows us to:
  - Collect fish over a longer period of time
  - Ship samples in one group
  - Collect tissue instead of whole fish

Why are we modifying the process?

- VHS sampling statewide
  - Surveillance
  - Monitoring
  - Mortality events
- Not all samples can shipped
  - Large fish
- Tissue samplin costs
  - Reduced shipp
  - Reduced trans processing tim

Whole Fish vs. Tissue Sample

- Collect and freeze whole fish
  - The fish being sampled are small— < 12 inches
    - Storage and shipping
  - Situation/location
  - Proximity to MSU

Whole Fish vs. Tissue Sample

- Collect and freeze kidney and spleen tissue
  - Fish being sampled are large— >12 inches
  - Collection and storage of whole fish is not possible

Who will be doing this?

What will you need to collect tissue?

- Training
  - DVD
  - Written procedure
  - Other resources
- Equipment
  - Container with sample collection tools, alcohol, disinfectant, gloves
  - Coolers for shipping
  - Autoclave

Adapting for any location

- Simple
- Minimal equipment
- Easy to use, no matter where it's done

I’ve got the fish, now what?

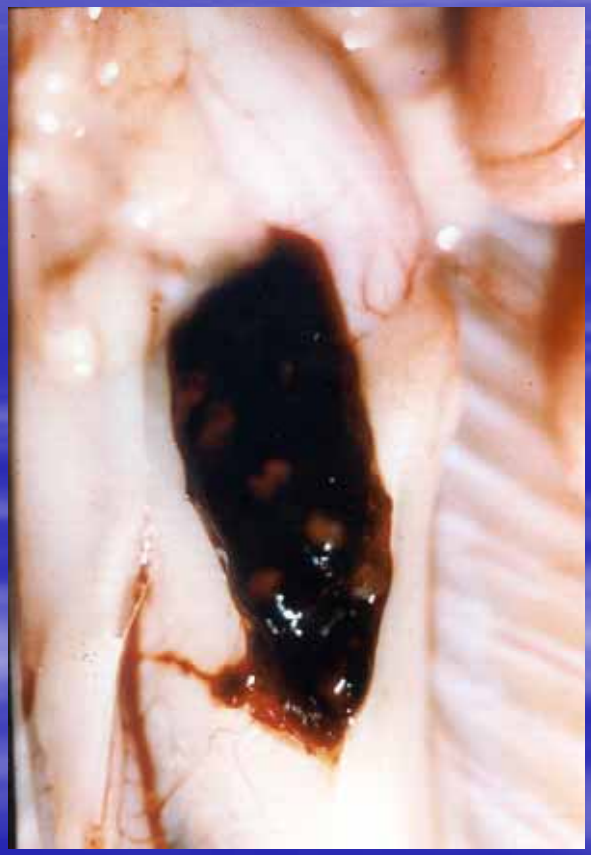
- Fish can be collected and iced to remove samples next day- not preferred
- Do a quick external exam
  - Look for lesions or other abnormalities
  - If you think they are unusual for the species or time of year
    - Take photos or samples
    - Record observations
- Spray fish with alcohol and wipe down

Opening up the fish

- Blunt point to cut fish open
- Make additional cut to create flap
- Reducing contamination:
  - Use scissors or opposite end of forceps to move around organs
  - Only use forceps tips to touch spleen and kidney

Spleen Tissue

- Spleen usually dark red to black
- Can be found:
  - Buried in fat
  - Near junction of stomach and intestine
- Collect whole spleen if small or raisin-sized or pea-sized piece if large—more is not better



Kidney Tissue

- How it looks depends on species
- Air bladder
- Small fish-take whole kidney
- Large fish
  - sample anterior, mid, and posterior sections
  - anterior kidney is most important

Packaging Samples

- Whole fish
  - Small fish—5 per bag
  - Large fish—individual bags

Packaging Samples

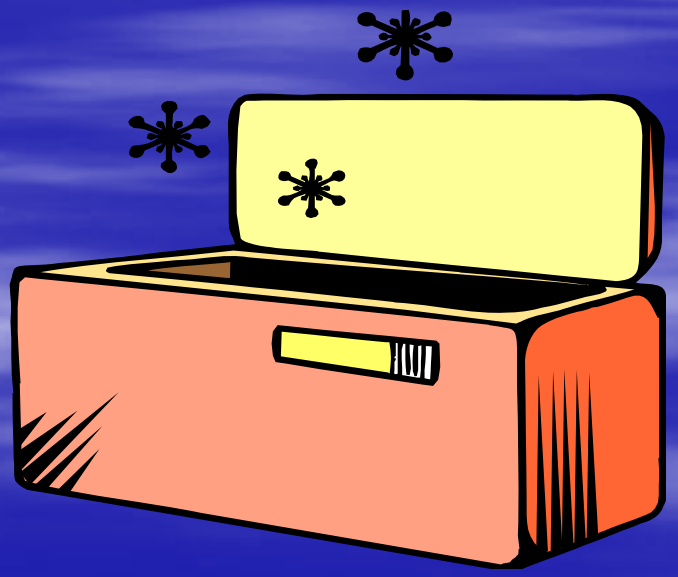
- Tissue samples
  - Only touch inside of whirl-pak with tissue and forceps
  - Put kidney and spleen from 5 fish per whirl-pak
  - Remove excess air from bags before sealing

Cleaning and Disinfecting Tools

- Cleaning and Disinfecting
  - Scrub scissors and scalpels with 1% solution Liquinox
  - Can be soaked up to 1 hr to loosen dried on tissue
  - Rinse and lay out to air dry
- Sterilization
  - Autoclave
    - Marquette, Charlevoix, Alpena, Lake St. Clair, and Wolf Lake

Storing Samples

- Freeze samples immediately
- Store in regular freezer for no longer than 7 days
  - If collecting samples from a group for more than a week, ship samples collected once a week
- Ship to Wolf Lake (unless told to ship to MSU)
- Samples can be stored longer in ultra cold freezer (-80°C)

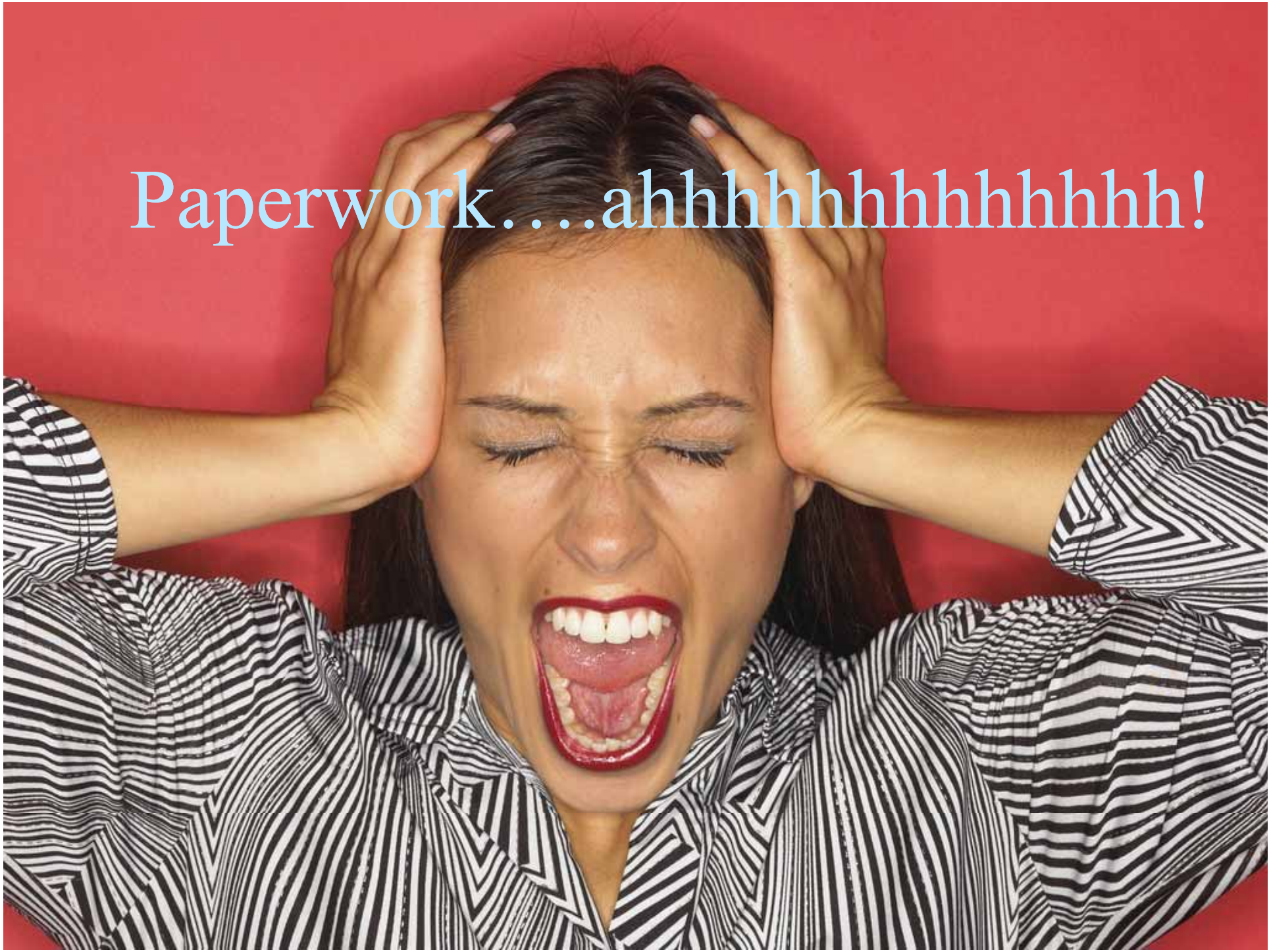


Shipping Samples

- Arrange shipment with Martha before sending
  - Shipping labels that bill directly to fish health
- Samples MUST stay frozen
- Only use styrofoam coolers with 1.5 in walls
- Ship all samples with dry ice
  - Min 3 lbs for whole fish
  - Min 4 lbs for tissue

Shipping Samples

- Put dry ice in paper bag
- Samples in bottom of cooler and dry ice directly on top of them
- Tissue samples should always be packed directly below dry ice
- Fill empty space above dry ice with crumpled newspaper
- Disease Form
  - Email copy of disease form to Martha
  - Include copy with shipment


Paperwork….ahhhhhhhhhhhhhh!

Aquatic Animal Health Laboratory (AAHL)
Director - Mohamed Faisal, DVM, PhD
Room S-112 Plant Biology Building
Michigan State University, East Lansing, MI, 48824

Disease Investigation Form – Wild Fish

Date Collected:		Date Submitted:	
Submitted by:		Phone:	( ) - -
Lake/Stream:		DNR Suffix	

Latitude/Longitude of Collection Site(s) (decimal degrees):

How often are fish collected?

Previous/current stocking?

History of disease and/or treatment?

Anything else?

Location description:

Method of collection:

Scrapings, tagging, etc.?

Species and numbers of fish submitted:

Previous/current stocking?·· _____
History of disease and/or treatment?·· _____
Anything else?·· _____
Location description:·· _____
Method of collection:·· _____
Scrapings, tagging, etc.?· _____
Species and numbers of fish submitted:·· _____
Reason for submitting fish:·· fish kill·· ☐ ····· pre-transfer·· ☐ ····· surveillance·· ☐ ·····¶ → → ··future broodstock·· ☐ ····· clinical·· ☐ ····· VHS·· ☐ ····· other·· _____
Any abnormal behavior:·· _____
If fish kill, how long were they dead and any suspected causes? Additionally, fish kill form should be completed and submitted:·· _____¶
Saving this document for submission:¶ Disease Investigation Form – Location ··three letter code for species collected ··date collected¶ Example:·· Disease Investigation Form – Lake Michigan Grand Haven YEP EMS RGB 5-15-07.doc

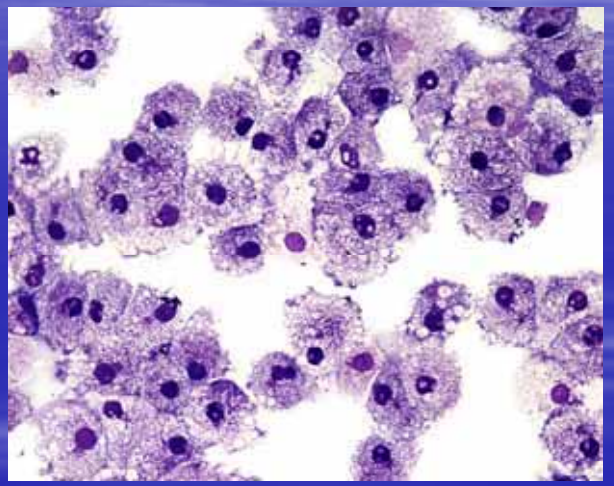
Section Break (Next Page)

- One form for each unique location
  - List all species collected
  - If collected from several spots, list all locations and the corresponding coordinates

VHS Testing 101

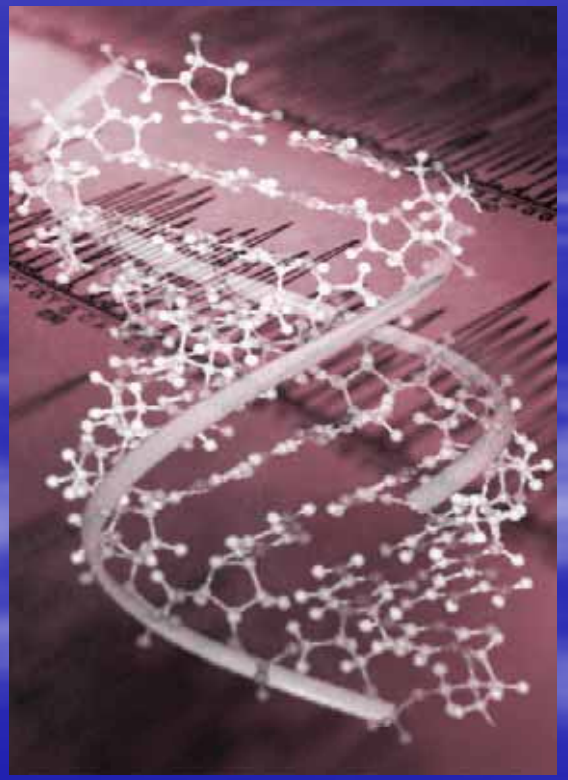
Cell Culture

- **This is still the standard**
  - If VHS negative
    - Results in 4 weeks
  - If there is activity in the culture, several test runs (passes) needed to positively ID the virus present
  - Contamination of samples lengthens time to final results
    - Results could take up to 10 weeks



PCR

- Confirmatory test only
- Not a reliable diagnostic test to date



VHS Results

What do they tell us?

Pathogen Detection Probability for Given Sample Sizes at Different Pathogen Prevalence Levels

	Number of fish in the sample	30	60	200	300	500	1000
Pathogen prevalence	0.001	0.03	0.06	0.18	0.26	0.39	0.63
	0.01	0.26	0.45	0.86	0.95	0.99	1.00
	0.05	0.78	0.95	1.00	1.00	1.00	1.00
	0.1	0.95	1.00	1.00	1.00	1.00	1.00

Pathogen Detection Probability for Given Sample Sizes at Different Pathogen Prevalence Levels

Number of fish in the sample		30	60	200	300	500	1000
Pathogen prevalence	0.001	0.03	0.06	0.18	0.26	0.39	0.63
	0.01	0.26	0.45	0.86	0.95	0.99	1.00
	0.05	0.78	0.95	1.00	1.00	1.00	1.00
	0.1	0.95	1.00	1.00	1.00	1.00	1.00

Pathogen Detection Probability for Given Sample Sizes at Different Pathogen Prevalence Levels

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Pathogen prevalence	0.001	0.03	0.06	0.18	0.26	0.39	0.63
	0.01	0.26	0.45	0.86	0.95	0.99	1.00
	0.05	0.78	0.95	1.00	1.00	1.00	1.00
	0.1	0.95	1.00	1.00	1.00	1.00	1.00

What does a negative VHS result mean for a sample of 60 fish?

- Good confidence with high pathogen prevalence
  - If the true prevalence is > 5%, there is high confidence (95%) in detecting the pathogen
- Less confidence with low pathogen prevalence
  - If the true prevalence is < 1%, there is low confidence (45%) in detecting the pathogen

So why aren't we collecting more than 60 fish?

- VHS prevalence in our populations is not known, but suspect it is currently low (<1%) in many
- Hard to collect 60 fish in some populations, times of year

So why aren't we collecting more than 60 fish?

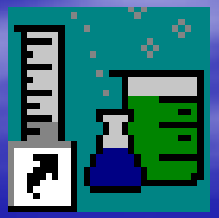
- Sacrifice of 60 individuals may not be desired
  - Low numbers
  - Highly valuable
- May be requesting larger numbers of fish collected to increase our chances of detecting VHS

2007 Summary

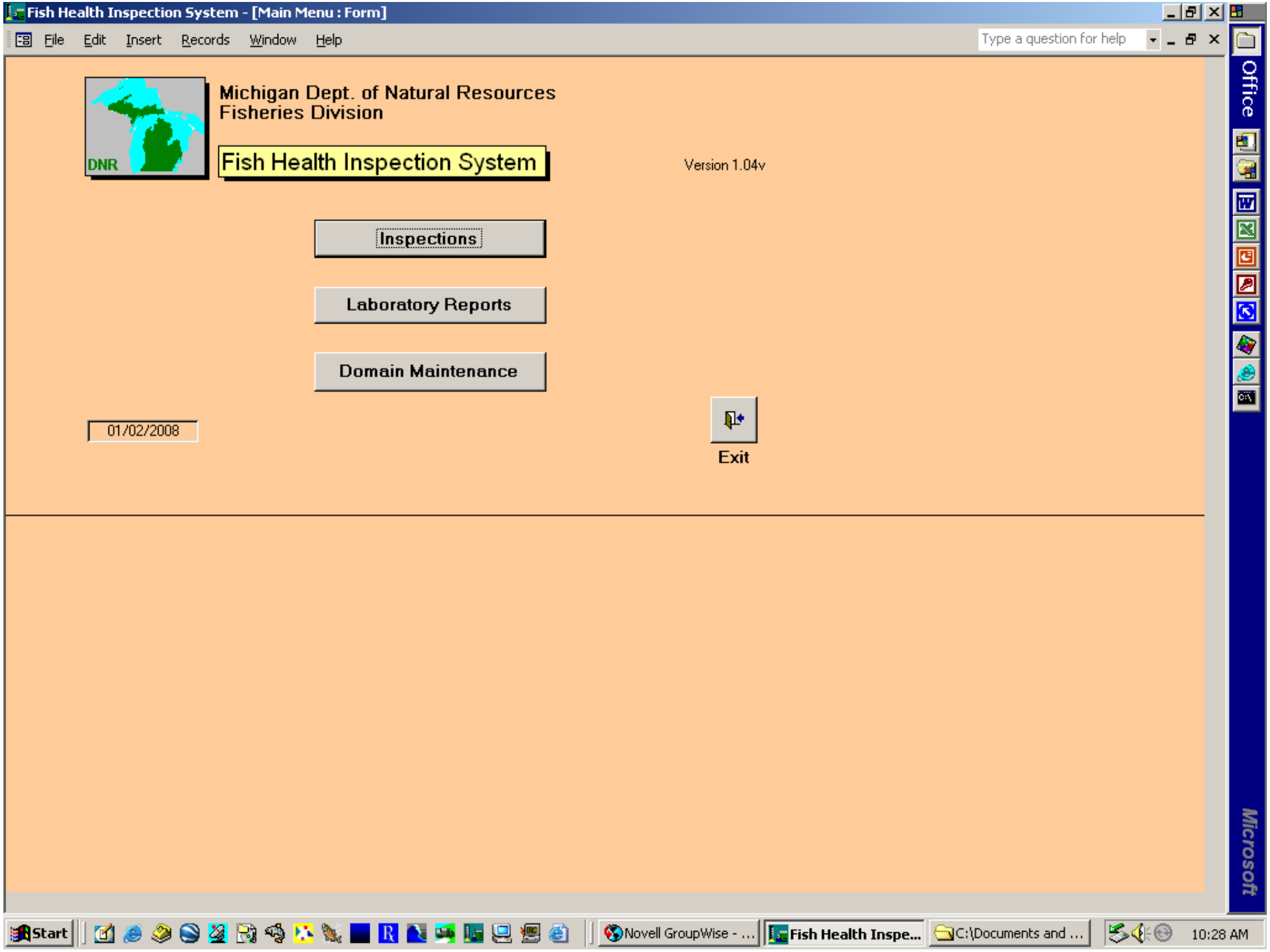
- VHS Cases for 2006-2007: 178 Total
  - Surveillance/Monitoring: 166
  - Fish Kills/Symptomatic Fish: 12
- Sites Sampled: 62
  - Great Lakes sites: 33
  - Inland sites: 29
- Fish Sampled
  - Fish collected: 8,933
  - Species sampled: 36

2007 Summary

- All reports for 2007 VHS sampling have been received
- Reports sent to unit manager that submitted samples and basin coordinator
- Results for all fish health cases entered into Fish Health database



Fish Health Inspections.lnk


Fish Health Inspection System - [Main Menu : Form]
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Type a question for help
DNR
Michigan Dept. of Natural Resources
Fisheries Division
Fish Health Inspection System
Version 1.04v
Inspections
Laboratory Reports
Domain Maintenance
01/02/2008
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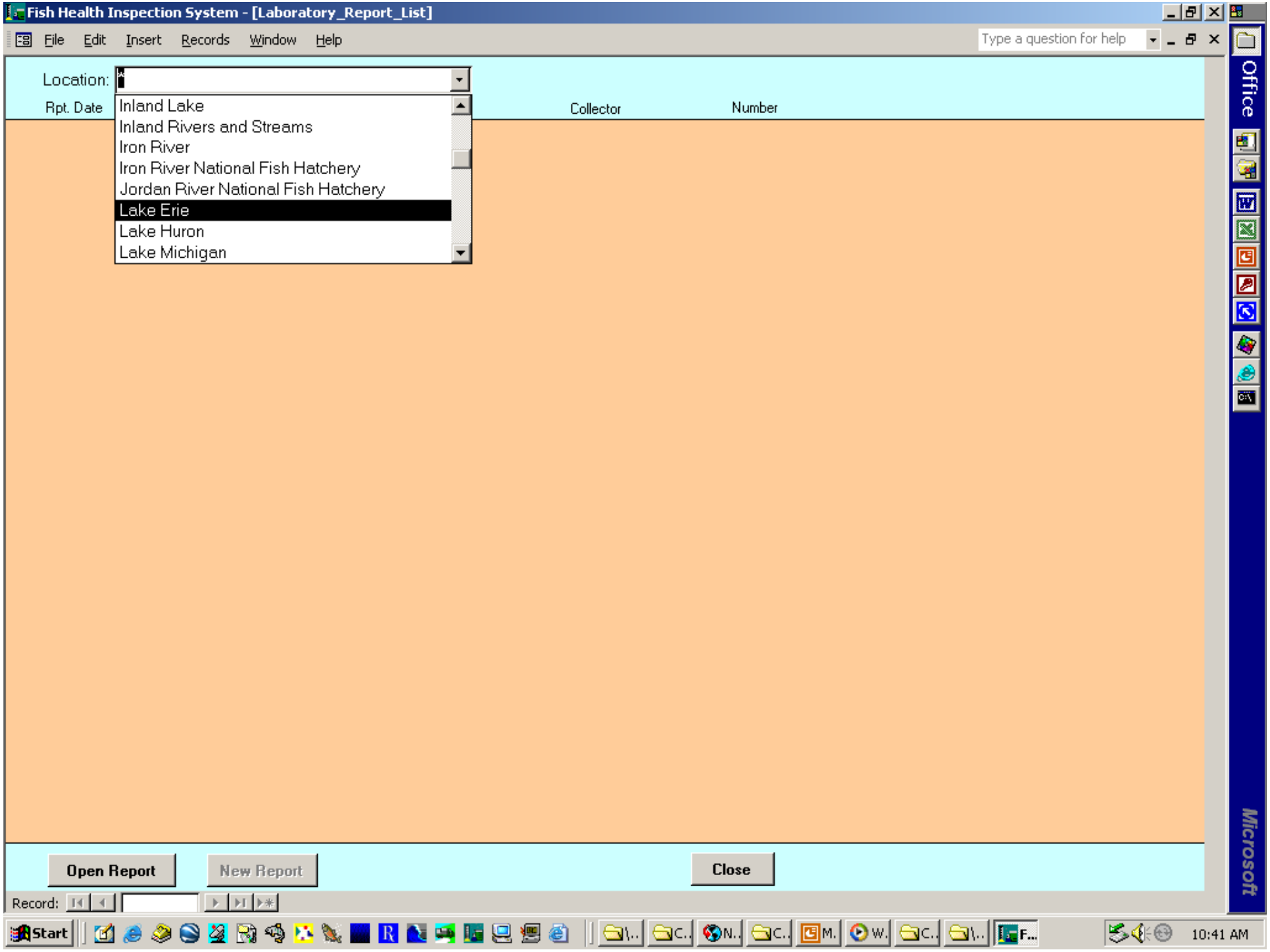
Microsoft
Start
Novell GroupWise - ...
Fish Health Inspe...
C:\Documents and ...
10:28 AM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File Edit Insert Records Window Help
Type a question for help
Location:
Rpt. Date
Collector
Number
Inland Lake
Inland Rivers and Streams
Iron River
Iron River National Fish Hatchery
Jordan River National Fish Hatchery
Lake Erie
Lake Huron
Lake Michigan
Open Report
New Report
Close
Record:
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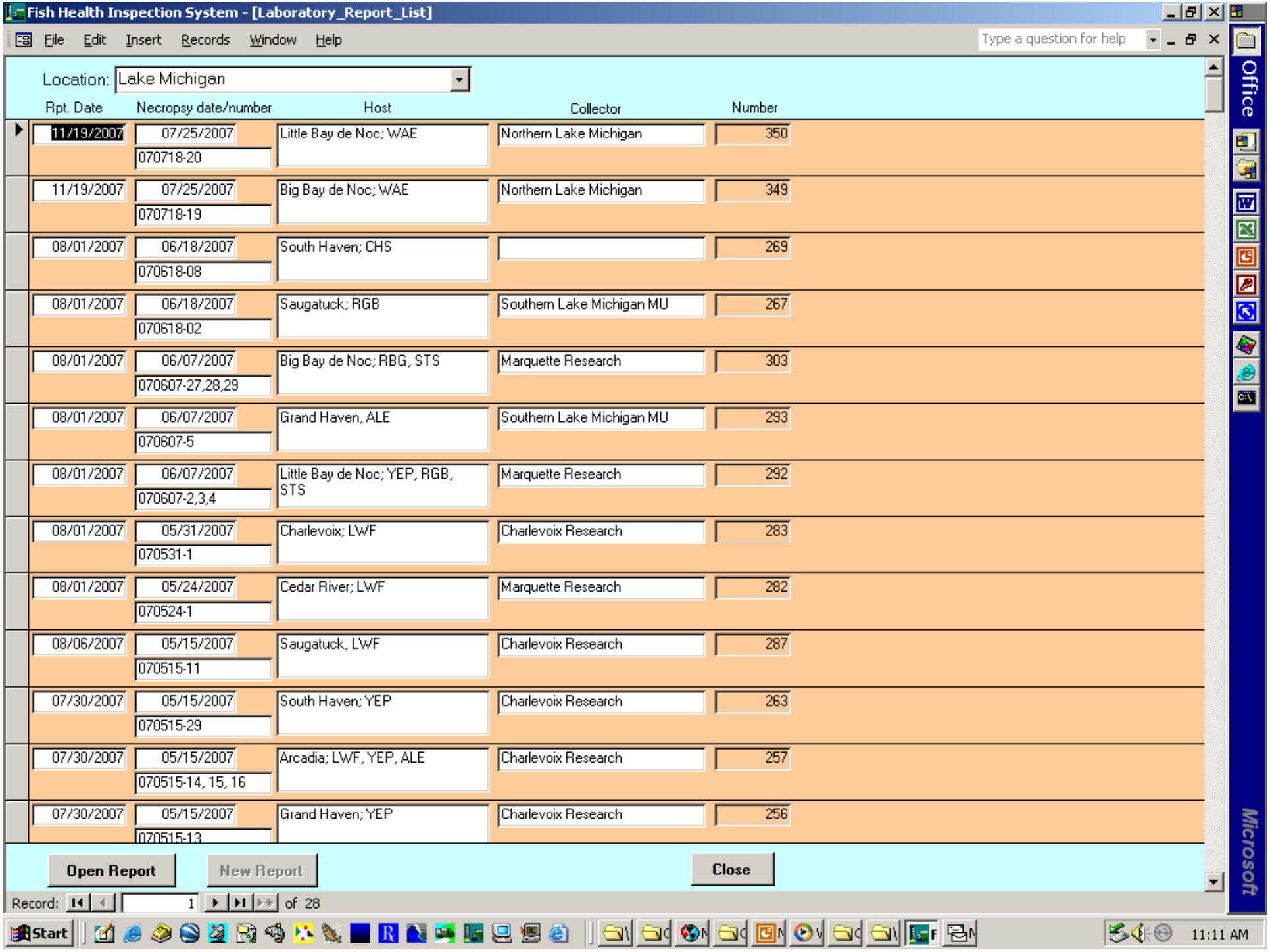
Microsoft
Start
10:41 AM


Fish Health Inspection System - [Laboratory_Report_List]
File Edit Insert Records Window Help
Type a question for help
Location: Lake Michigan
Rpt. Date | Necropsy date/number | Host | Collector | Number
11/19/2007 | 07/25/2007 | 070718-20 | Little Bay de Noc; WAE | Northern Lake Michigan | 350
11/19/2007 | 07/25/2007 | 070718-19 | Big Bay de Noc; WAE | Northern Lake Michigan | 349
08/01/2007 | 06/18/2007 | 070618-08 | South Haven; CHS | | 269
08/01/2007 | 06/18/2007 | 070618-02 | Saugatuck; RGB | Southern Lake Michigan MU | 267
08/01/2007 | 06/07/2007 | 070607-27,28,29 | Big Bay de Noc; RBG, STS | Marquette Research | 303
08/01/2007 | 06/07/2007 | 070607-5 | Grand Haven, ALE | Southern Lake Michigan MU | 293
08/01/2007 | 06/07/2007 | 070607-2,3,4 | Little Bay de Noc; YEP, RGB, STS | Marquette Research | 292
08/01/2007 | 05/31/2007 | 070531-1 | Charlevoix; LWF | Charlevoix Research | 283
08/01/2007 | 05/24/2007 | 070524-1 | Cedar River; LWF | Marquette Research | 282
08/06/2007 | 05/15/2007 | 070515-11 | Saugatuck, LWF | Charlevoix Research | 287
07/30/2007 | 05/15/2007 | 070515-29 | South Haven; YEP | Charlevoix Research | 263
07/30/2007 | 05/15/2007 | 070515-14, 15, 16 | Arcadia; LWF, YEP, ALE | Charlevoix Research | 257
07/30/2007 | 05/15/2007 | 070515-13 | Grand Haven; YEP | Charlevoix Research | 256
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New Report
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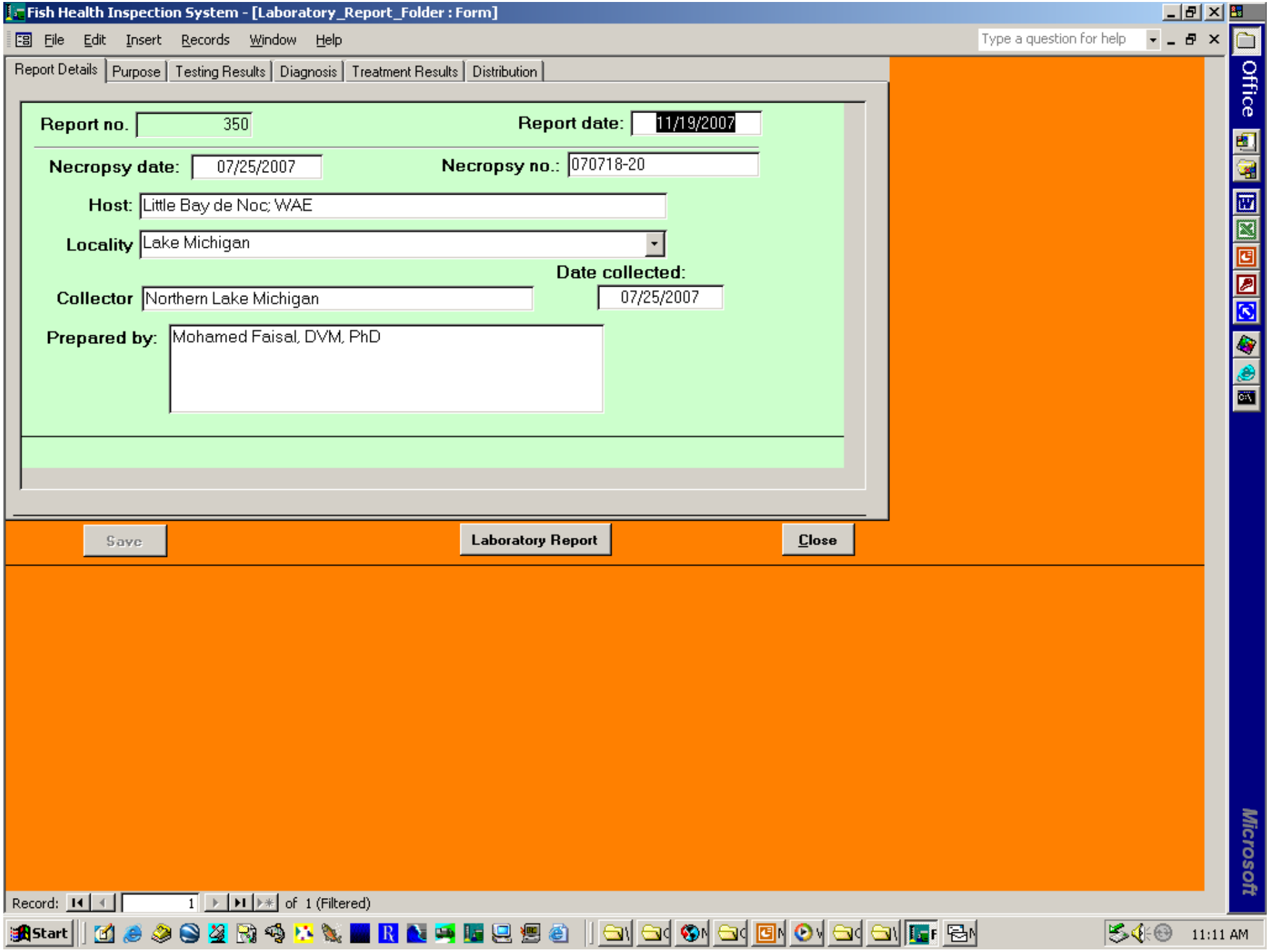
Fish Health Inspection System - [Laboratory_Report_Folder : Form]
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Report Details
Purpose
Testing Results
Diagnosis
Treatment Results
Distribution
Report no.
350
Report date:
11/19/2007
Necropsy date:
07/25/2007
Necropsy no.:
070718-20
Host:
Little Bay de Noc; WAE
Locality
Lake Michigan
Date collected:
Collector
Northern Lake Michigan
07/25/2007
Prepared by:
Mohamed Faisal, DVM, PhD
Save
Laboratory Report
Close
Record:
1
of 1 (Filtered)
Start
11:11 AM
Office
Microsoft

Michigan Dept. of Natural Resources
Fisheries Division

Fish Health Laboratory
Laboratory Report

Wolf Lake State Fish Hatchery
3270 C.R. 652
Mattawan, MI 49071

To:

Report date: 11/19/2007

Necropsy date: 07/25/2007 Necropsy no.: 070718-20

Host: Little Bay de Noc; WAF

Locality: Lake Michigan

Collector: Northern Lake Michigan Date collected: 07/25/2007

Purpose of examination:

2 fins on WAF submitted for VHS surveillance.

Testing results:

Negative for VHS.

Diagnosis:

Treatment results:

Prepared by: Mohamed Faisal, DVM, PhD

cc:

Getting Ready for 2008

- Review Site List
  - Look at sites, month to be sampled, and species
    - Refer to Prohibited Species list and Tier Species list for substitutes
  - If changes need to be made, unit manager should provide those to Martha
- Inventory Supplies
  - Make sure all tools are disinfected and autoclaved
  - Let Martha know if you need any items

Getting Ready for 2008

- Review Sampling and Shipping Procedures
  - DVD
  - Written Instructions
  - This presentation is available on the Intranet
- Shipping Labels

Getting Ready for 2008-Coding

- APHIS Samples
  - Eligible costs—Time, travel, sample handling and data processing, payment for commercial fish, and shipping costs
  - Coding—your own index, 76845 235022 00

- Non-APHIS Samples
  - Coding—your own index, 76841 235021 00

Getting Ready for 2008-Coding

- Coding for other costs—i.e. coolers, dry ice, etc
  - Charge to Fish Health budget
  - 83880 76871 235021 00
  - Send email to Martha, copy to Cheryl Lake that includes item purchased, where it was purchased, date, and amount

Feedback from 2007—How can we make 2008 better ?
(besides not having to do any more VHS sampling)

USGS
science for a changing world

Non-lethal Testing of Juvenile Salmonids for *Renibacterium salmoninarum*

Diane Elliott, Connie McKibben, LynnMarie Applegate, and Sacha Mosterd

Western Fisheries Research Center, USGS, Seattle

Bacterial Kidney Disease Caused by *Renibacterium salmoninarum* (Rs)

- Chronic infection that does not always progress to death.

- Because of the slow course of the disease, monitoring the entire infection cycle can be difficult.

USGS science for a changing world

Why Non-lethal Sampling?

- Non-lethal methods for detection and quantification of Rs in juvenile fish would enable monitoring of their performance and survival after testing.
- Effective non-lethal sampling methods for Rs detection could reduce the need to sacrifice large numbers of individuals in threatened and endangered salmonid populations for pathogen surveys.

Evaluation Criteria for Potential Non-Lethal Methods

- Sampling method is indeed non-lethal
  - Diagnostic test is effective with small tissue samples
- Diagnostic test is specific for pathogen
- Diagnostic test is sensitive for pathogen detection (detects sub-clinical infections)
  - Ability to quantify pathogen load is desirable

Candidate Non-Lethal Samples

- Blood (standard non-lethal sample)
- Gill filaments
  - Procedure developed for non-lethal gill ATPase microassay (Schrock et al. 1994, Trans. Am. Fish Soc. 123:223-229)
- Mucus
  - Procedure developed for non-lethal *Aeromonas salmonicida* detection (Cipriano et al. 1994, Biomed. Lett. 49:229-233)

Candidate Non-Lethal Samples

- Fin clip (standard non-lethal mark)
- Kidney biopsy
  - Procedure developed for non-lethal *Yersinia ruckeri* detection (Noga et al. 1988, Am. J. Vet. Res. 49:363-365

Candidate Testing Methods: PCR Assays

- PCR can use very small tissue samples (~10 mg) in comparison to ELISA, which requires a sample of about 100 mg.
- Published reports have documented specificity of Rs PCR assays.
- Sensitivity of Rs PCR (nested and real-time) reported to be equal to or greater than ELISA.
- Real-time PCR can quantify pathogen loads.

Presentation Outline

- Preliminary studies
  - Survival of fish following non-lethal sampling
  - Comparisons of Rs detection rates in lethal samples (kidney) with non-lethal samples in experimentally infected fish
- Future studies

www.fs.fed.us

Survival Experiments

- Candidate non-lethal samples tested:
  - Blood (caudal vessel, 20-50 µL sample)
  - Gill filaments (2 mm x 3mm sample, 5-10 mg sample)
  - Mucus scraping (dorsal to lateral line, 5-10 mg sample)
  - Fin clip (anal fin, 2 mm x 3mm, 5-10 mg sample)
  - Kidney biopsy (needle biopsy, anterior kidney, 20-50 µL sample)

Survival Experiments

- Non-lethal samples taken from juvenile Chinook salmon at three sizes.
- Fish held for 30 days after sampling.
- Control groups: Anesthetization only group; unhandled group.

USGS science for a changing world

Survival Study Results

	No. Dead/Total (%)		
Treatment	2.8 g fish	8.2 g fish	15.2 g fish
Gill	0/60	5/60 (8)	0/60
Mucus	0/60	6/60 (10)	0/60
Fin	1/60 (2)	6/60 (10)	1/60 (2)
Blood	5/60 (8)	17/60 (28)	3/59 (5)
Kidney	18/60 (30)	32/60 (53)	3/55 (5)
MS-222 only	0/60	1/60 (2)	0/60
Unhandled	0/60	4/60 (7)	0/57

Summary: Survival Experiments

- Gill, mucus and fin samples resulted in the lowest mortality.
- Kidney biopsy and blood samples were less suitable for small fish, especially fish ≤ 8 g.
- Coldwater disease likely contributed to mortality in one experiment (8.2 g average wt. fish).

USGS science for a changing world

Comparison of Non-Lethal and Lethal Methods to Monitor Rs Progression in Fish

- A preliminary experiment is underway to determine optimum sampling times and methodology.
- Chinook salmon (50 g average weight) were challenged by a 24-h immersion in 2 x 10^7 Rs cells/mL.
- Sampling is occurring at 3-week intervals.

USGS science for a changing world

Comparison of Non-Lethal and Lethal Methods to Monitor Rs Progression in Fish

- Sampling scheme:
  - Fish health and condition profile: length, weight, Goede Health Index, hematological tests and histopathology.
  - Evaluation of Rs in gill, mucus, blood and fin samples by nPCR and qPCR (non-lethal methods).
  - Evaluation of Rs in kidney tissue by culture, ELISA, FAT, nPCR and qPCR (lethal sample).

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Future Research

- Comparison of non-lethal and lethal sampling methods for monitoring Rs infections in Chinook salmon populations exhibiting low or high BKD mortality following challenge with Rs.
- Comparison of solid-phase laser scanning cytometry (SPC) with existing methods for detection of Rs in water and ovarian fluid samples.

USGS science for a changing world

Acknowledgements

Funding:

Wisconsin DNR
- Susan Marcquenski

Soos Creek Fish Hatchery (WDFW)

Carson Fish Hatchery (USFWS)

WFRC
- Maureen Purcell
- Dorothy Chase

Viral hemorrhagic septicemia virus in the Great Lakes

James Winton

USGS Western Fisheries Research Center
6505 NE 65th Street, Seattle, WA 98115

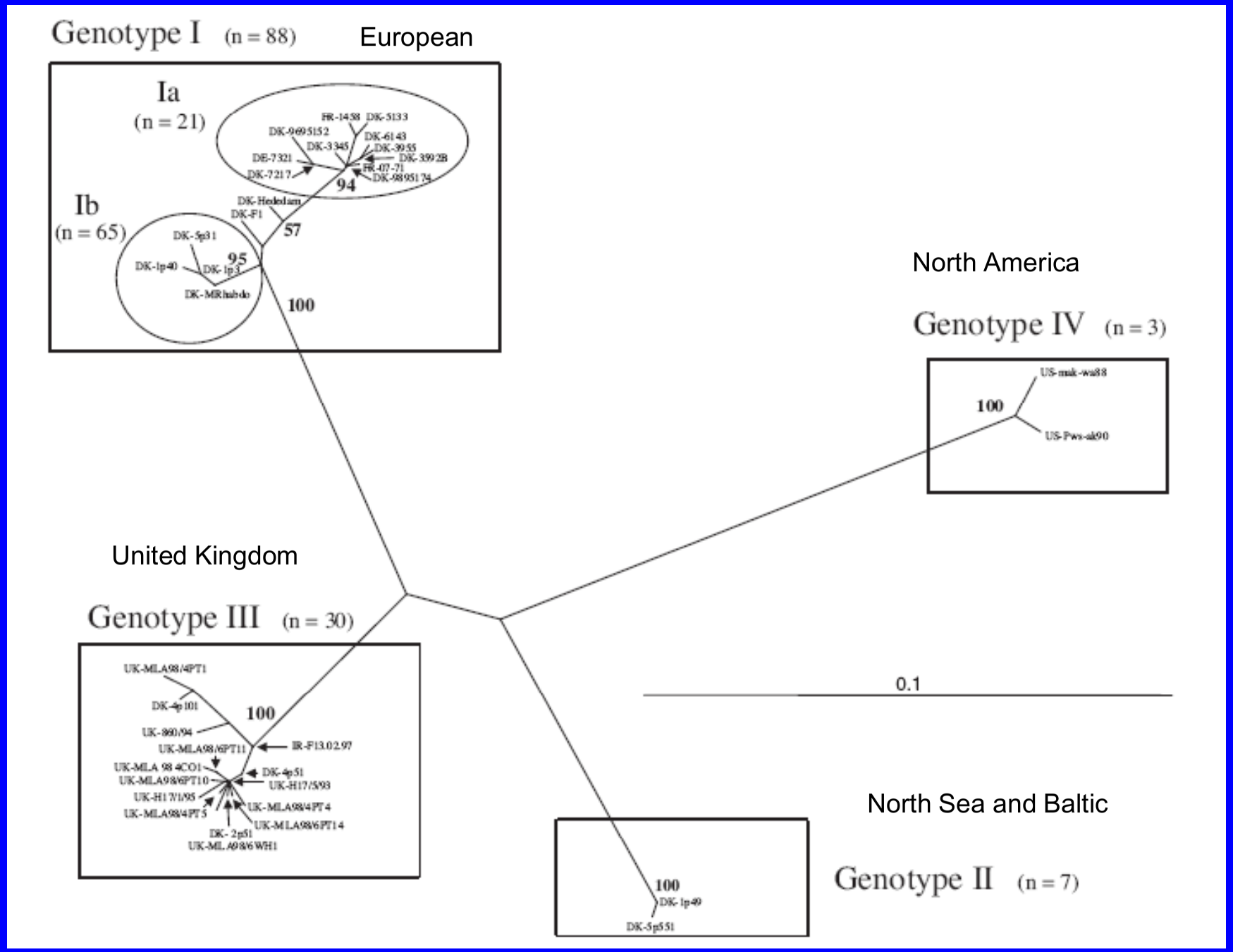
USGS
science for a changing world

Viral hemorrhagic septicemia - 1

- Disease in rainbow trout described by Schaperclaus (1938)
- Evidence for filterable agent (virus) in 1950s
- Virus first isolated in Denmark by Jensen (1963)
- Established cell lines and diagnostic antisera available
- Virus found in increasing number of freshwater species
- Experimental testing of host range
- Curiously, a few isolations occurred from marine fish
- Virus known as an endemic pathogen of freshwater fish mostly affecting (the introduced) rainbow trout in western Europe

Viral hemorrhagic septicemia - 2

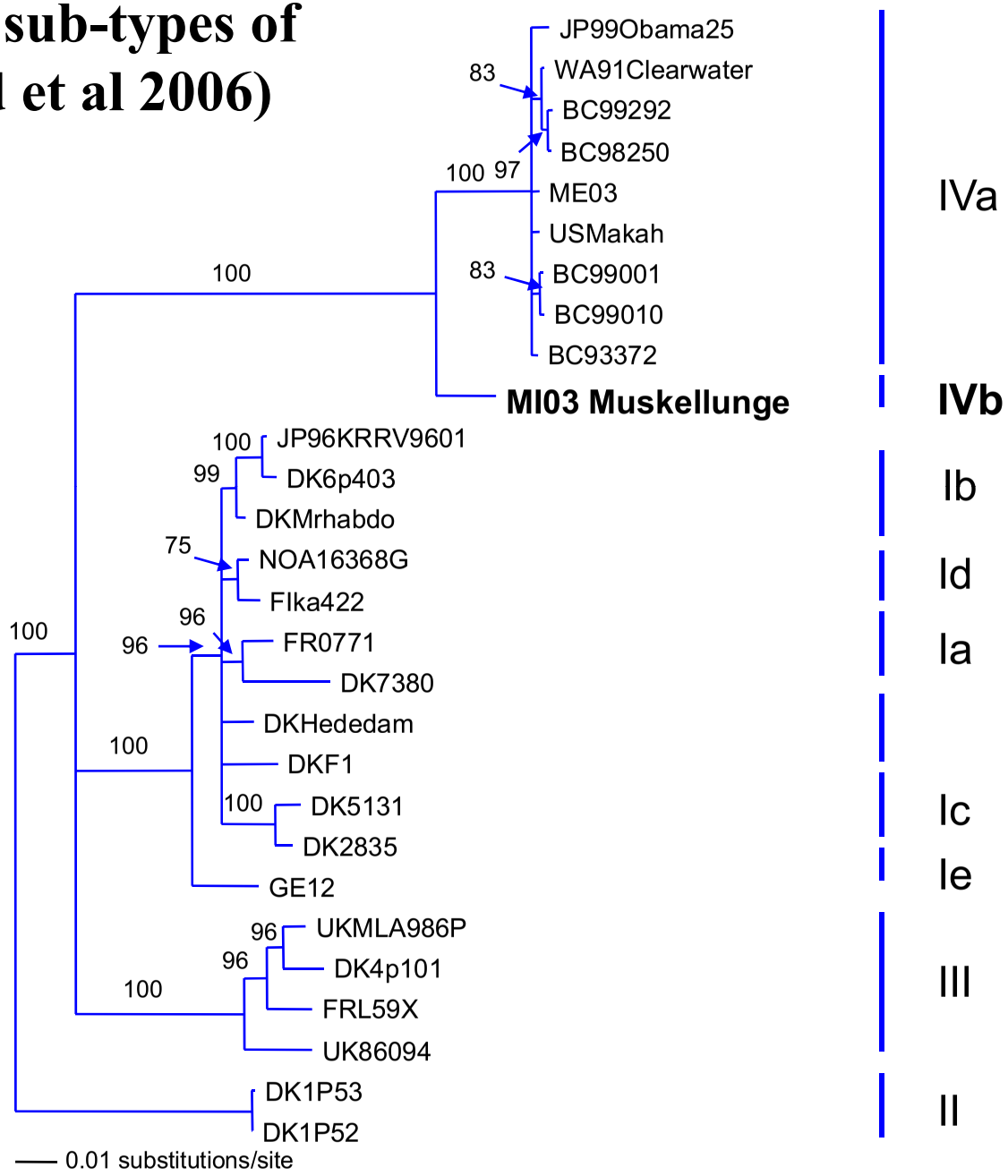
- VHSV isolated in 1988 from normal adult salmonids in WA returning from Pacific Ocean – first report from North America
- Diseased marine fish (cod) in AK and Baltic produced VHSV
- Marine isolates were less pathogenic for salmonids than historic isolates from trout, but highly pathogenic for marine species
- Increased surveillance demonstrates the host and geographic range includes many marine fish species from the west coast of North America, North Sea, Baltic Sea, North Atlantic and Japan
- Sequence analysis reveals four genotypes, largely associated with geographic location
- Until 2005, VHSV thought to be mainly a pathogen of marine fish in North Atlantic and North Pacific Oceans that was introduced to trout farms in Europe via feeding of raw marine fish (e.g. herring) where it adapted to become more virulent for trout


Genotype I (n = 88)
European
Ia
(n = 21)
Ib
(n = 65)
FR-1458
DK-5133
DK-9695152
DK-6143
DK-3345
DK-3955
DE-7321
DK-3992B
FR-07-71
DK-7217
DK-9895174
94
DK-Heidelam
DK-F1
57
DK-5p31
95
DK-1p40
DK-1p3
DK-MRhabdo
100
North America
Genotype IV (n = 3)
US-mak-wa88
100
US-Pws-ak90
United Kingdom
Genotype III (n = 30)
UK-MLA98/4PT1
DK-4p101
100
UK-860/94
UK-MLA98/6PT11
IR-F13.02.97
UK-MLA 98 4CO1
DK-4p51
UK-MLA98/6PT10
UK-H17/5/93
UK-H17/1/95
UK-MLA98/4PT4
UK-MLA98/4PT5
UK-MLA98/6PT14
DK-2p51
UK-MLA98/6WH1
0.1
North Sea and Baltic
100
DK-1p49
DK-5p551
Genotype II (n = 7)

Viral hemorrhagic septicemia - 3

- May 2000 - VHSV isolated from mummichog and 3-spined stickleback from an outbreak on the coast of New Brunswick, Canada – reported as similar to North American genotype IV
- 2002-2004 - similar isolates of VHSV from striped bass and sea-run brown trout in the same region of new Brunswick – Gagne et al. (in press)
- 2005 - VHSV isolated from large outbreak among freshwater drum and other species in Lake Ontario, Canada
- 2005 - virus isolate from diseased muskellunge in Lake St Clair obtained in 2003 shown to be identical to isolate from drum in Lake Ontario and representative of a novel sub-lineage of VHSV (IVb) – Elsayed et al. (2006)

Genotypes and sub-types of VHSV (Elsayed et al 2006)



Viral hemorrhagic septicemia - 4

- 2006 - VHSV type IVb is isolated from multiple species in Lake St. Clair, Lake Erie, Lake Ontario and the St. Lawrence River. Some isolates obtained from large mortalities
- Great Lakes VHSV isolates examined to date (N=18) are within 1 nt of each other (669 nt compared). This low genetic diversity suggests a recent introduction (ballast water or migratory fish)
- The Great Lakes isolates are most like isolates from the Atlantic coast of Canada suggesting a marine origin for the virus
- The 2006 VHSV outbreak in the Great Lakes can be considered as one large epizootic involving many species of fish. Such outbreaks are not atypical among naïve populations following initial exposure to an introduced pathogen
- January 2007 - VHSV reported to have been isolated from Chinook, walleye and whitefish in Lake Huron

Genetic differences among VHSV genotypes

	Percent nt diversity
Within IVa (North Pacific)	0.2 - 0.6
Within IVb (Great Lakes)	0.0 - 0.15
Between IVa and IVb	3.6 - 3.7
IVa vs European strains	13.3 - 15.0
IVb vs European strains	12.6 - 14.2

Questions from Managers

1) Likely effects of the disease on fish populations
2) Likely time the disease can live outside a fish
3) Any thoughts (okay guesses) on which species maybe more sensitive and why
4) Best methods to disinfect fisheries gear, raceways, earthen ponds, and transportation trucks
5) What should we do if a hatchery is found to have an infected lot
6) How are west coast agencies doing surveillance for this virus

Effects of the disease on fish populations

- Virus will become widely established in the GL Basin
- Additional host species will become involved
- Virus will persist in low-level carriers much of the year
- Stressors will exacerbate latent infections and disease
- Disease will occur on an episodic basis – esp. spring
- Recovered fish will become immune leading to disease occurring primarily in younger age classes
- Disease will have population-level effects in some species
- Disease will become less explosive in most years

Likely time the disease can live outside a fish

- Virus is obligate pathogen - must have a host to replicate.
- VHSV is an enveloped RNA virus - not very stable in water or without tissue or organic matter to protect it.
- Research with European strains of VHSV showed highly variable survival in water (up to a few months) depending on water quality and temperature. Needs to be checked for Great Lakes isolates
- Virus bound to organic matter, in sediment or in dead fish will persist much longer.
- Possible role of invertebrate carriers or vectors for some fish rhabdoviruses has been postulated.

Any thoughts (okay guesses) on which species maybe more sensitive and why

- VHSV has a wide host range and should be able to infect a large number of Great Lakes species
- The most affected species should be those that receive a high virus dose while concomitantly stressed or immunosuppressed by non-optimal temperatures, contaminants, or high cortisol levels that are typical at spawning in some species
- Aggregate spawners and species with schooling fry or juveniles will be at greater risk from enhanced transmission and higher infectious dose - especially if simultaneously stressed

Best methods to disinfect fisheries gear, raceways, earthen ponds, and transportation trucks

- The OIE and USFWS have published methods for disinfecting facilities and equipment. These have been used successfully for VHSV
- Chlorine for hard surfaces (tanks, walls, floors)
- Iodine for soft items that will later contact fish (nets, gloves, raingear)
- Lime for earthen ponds
- Burn wooden items

What should we do if a hatchery is found to have an infected lot

- Eggs reared at hatcheries in endemic area - Re-disinfect eggs and rear on virus-free water. Check fry at swim-up.
- Eggs already moved to non-endemic area - Destroy eggs and disinfect facility.
- Fry or fingerlings - Destroy fish and disinfect facility.
- Adults within endemic area - Spawn fish and disinfect eggs. Rear on virus-free water. Check fry at swim-up.
- Until natural host range and species susceptibility of the existing strain is known, make efforts not to promote viral adaptation to new species as happened in Europe.

How are west coast agencies doing surveillance for this virus

- Virological assay of tissues and ovarian fluids from statistically-based sample (or entire) adult salmonid broodstock collected at spawning in most west coast salmon hatcheries.
- Virological assay of tissues from statistically-based sample of juvenile salmonids collected before release from many west coast salmon hatcheries.
- Virological assay of tissues of freshwater and marine fish species collected from any unexplained mortality occurring in the wild or at public or private hatcheries.
- Virological assay of tissues from both freshwater and marine fish species collected during research or other opportunities (e.g. USFWS Wild Fish Health Survey).

Research needs - 1

Epidemiological information

- Understand the current host and geographic range of VHSV in the Great Lakes region
- Determine the potential host range using standard laboratory challenges of important species
- Provide reference laboratory services for fish health laboratories in the region
- Develop a common database for shared epidemiological information

Diagnostic methods

- Improved diagnostic methods for tissues and water samples
- Develop serological or other assays for previous exposure
- Standardize and validate newer diagnostic methods including non-lethal assays

Virus studies

- Determine effects of environmental factors on virus stability and survival
- Investigate the molecular basis of virulence and host range
- Investigate the drivers of virus evolution and host adaptation
- Develop a searchable database of VHSV sequence information

Research needs - 2

Host studies

- Develop a standard research fish model for laboratory experiments
- Determine the effect of size at exposure on mortality and immunity
- Determine the nature and duration of the immune response
- Understand the nature of the carrier state
- Investigate level and duration of virus shedding during outbreaks and by carriers
- Determine effects of anthropogenic and environmental stressors on disease

Tools for managers

- Assess potential effects of management practices on virus spread
- Assess potential effects of commercial and recreational activity on virus spread
- Develop a risk model for managers to identify major pathways of spread

Control methods

- Develop measures to restrict spread into new areas and species
- Develop control measures for use by aquaculture
- Test candidate vaccines for immunization of propagated species

Ontario
VHS update

January 30, 2008
GLFHC Annual Meeting

Elizabeth Wright
Ontario Ministry of Natural Resources

January 2007 - old

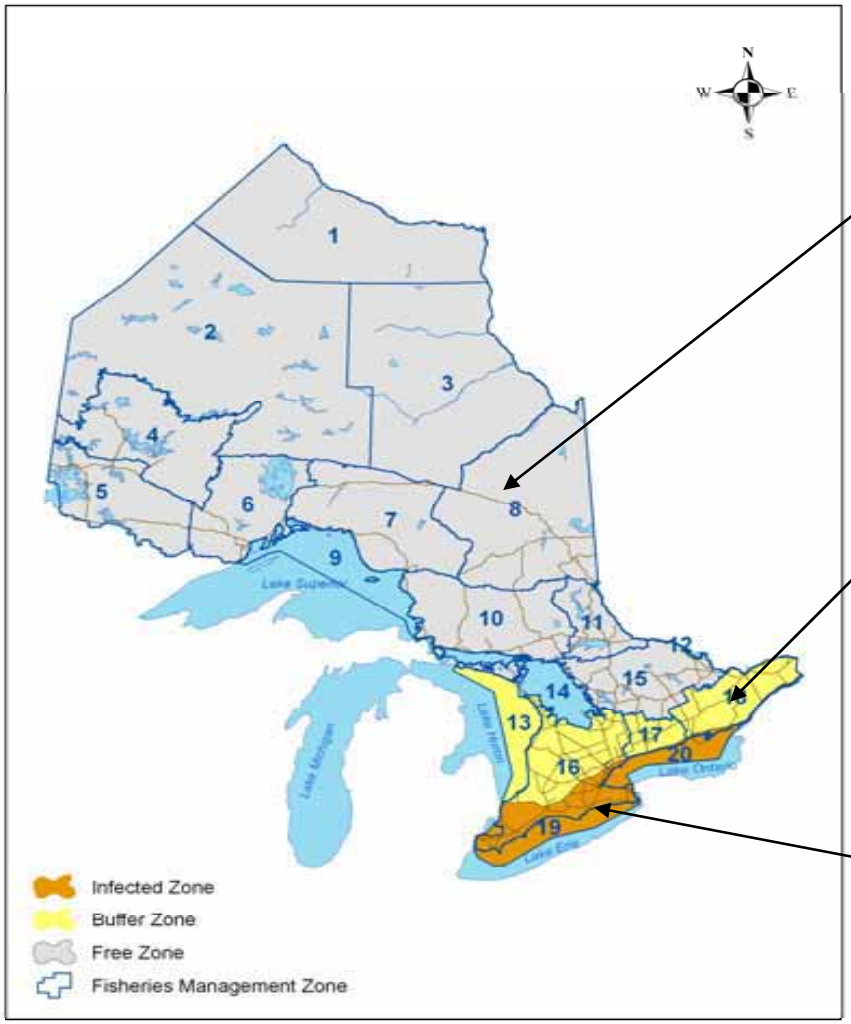
- put bait fish restrictions in place Jan. 8/07

  - large volumes of bait fish harvested in lower Great Lakes (particularly emerald shiners from Lake Erie) transported to holding ponds in southern Ontario and then sold throughout province

  - there was no consultation with stakeholders due to risk of fish being moved quickly

  - 1400 dealers/harvesters selling >3 million dozen baitfish
    - retail value $20M - $37M

Interim VHS Control Zones



<u>Virus-free</u> = 1 – 11, 12 north of 18, and 14,15

<u>Buffer</u> = 13 and north of Hwy 401/402 in 16, 17, 18, portion of 12 contiguous with 18

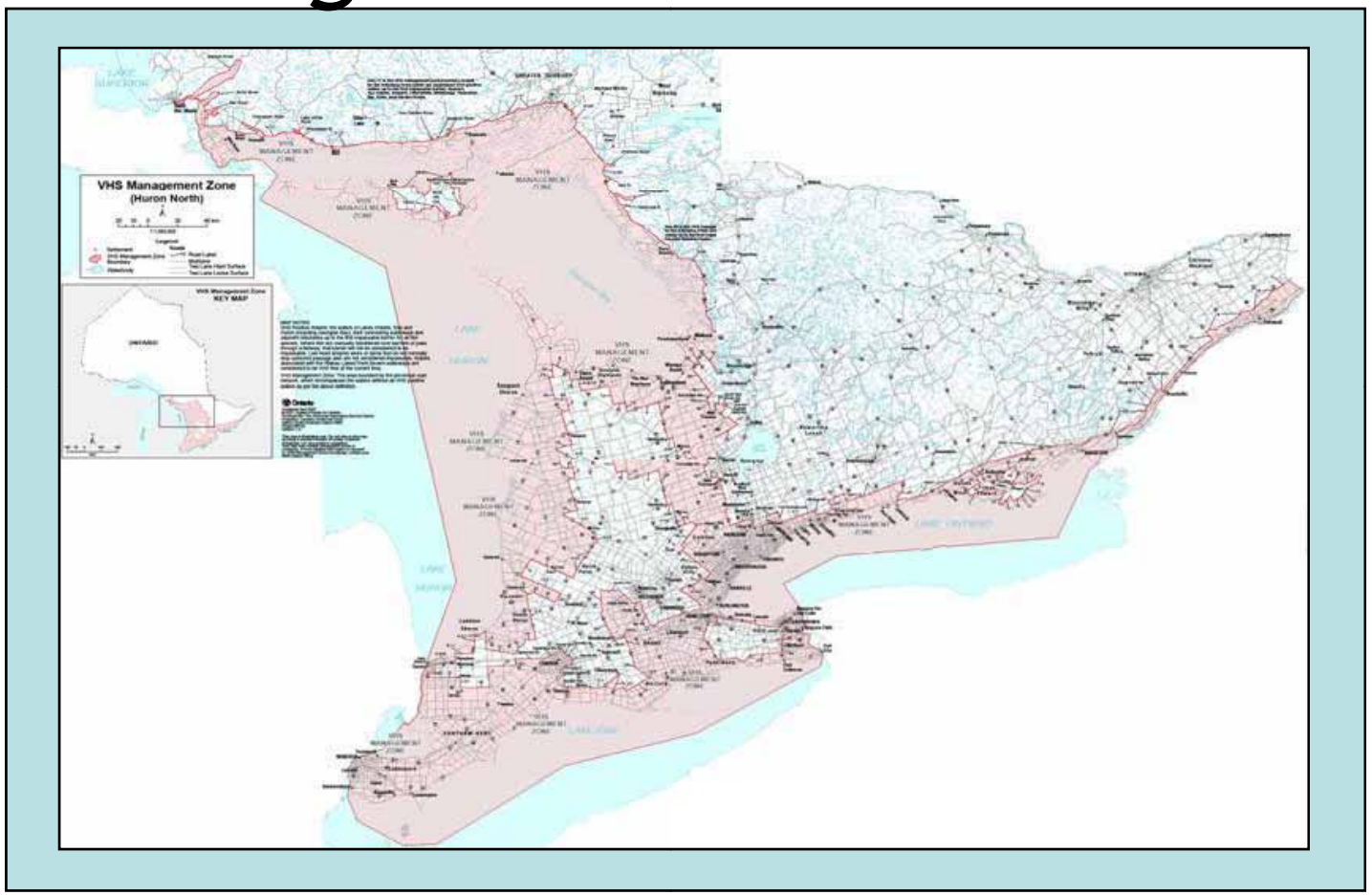
<u>Infected</u> = FMZ 19, 20 and south of Hwy 401/402 in 16,17,18

250

January 2007 - old

- Challenges
    - bait harvesters fished on one side of highway and had ponds on the other
    - no appetite from stakeholders – if fish could naturally reach beyond the highway, why can't fish be trucked?
    - little scientific support for highway boundary
    - no way restrict angler movement of fish
    - enforcement support constrained by established program

VHS Management Zone - Mar 29/08



Contains VHS positive waters in an area bounded by the provincial road network as shown on maps

Regulations

- Live bait fish harvested inside VHS Management Zone is not permitted to be transported out
  - Condition of licence on all bait harvest licences
  - Where risk of VHS spread is low, a risk assessment may enable some transport of bait (through zone)
  - We know bait still moves – enforcement is investigating, close to laying charges
  - No stockpiling of bait in fall 2007 so shortage now

Already have laws prohibiting

- anglers from dumping bait buckets

- import of live bait into Ontario

Restrictions

- New scientific collection permits must address disposal of fish and water if collected inside Zone
- Existing fishways (18-20) are permitted to operate, new activities subject to risk assessment
- Possible future conditions on aquaculture licences (~250 licenced facilities) to address fish origin/live feed
    - Ongoing discussions with legal services
- Possible future restriction on angler harvest/use of bait
    - New ON fishing regulations

VHS Changes in Stocking

Eggs from VHS-positive waters

- 10 MNR hatcheries

- > 50 partner-operated hatcheries and
    - 15-20 collection permits annually for gametes
    - Unknown number of MNR supervised collections

VHS Changes in Stocking

Eggs from VHS-positive waters

Wild Spawn Collection - Salmon and Trout Species

- Eggs disinfected and reared outside Zone can be stocked anywhere
  - Need a vet/Health Canada to sign an Emergency Drug Release to get Ovadine (iodophor)
- All other collections must be reared inside Zone and stocked back into VHS-positive waters
- Condition on collection permits (gametes) and on stocking licences

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VHS Changes in Stocking

Eggs from VHS-positive waters

Wild Spawn Collection – Non-salmonids

- Eggs must be reared inside Zone and stocked back into VHS-positive waters
- Risk assessment/fish health testing on adults and progeny may enable stocking outside Zone
- OMNR supplied walleye eggs collected outside Zone to some partner hatcheries inside Zone in 2007
  - Expecting to do same in 2008

Public Information/Outreach

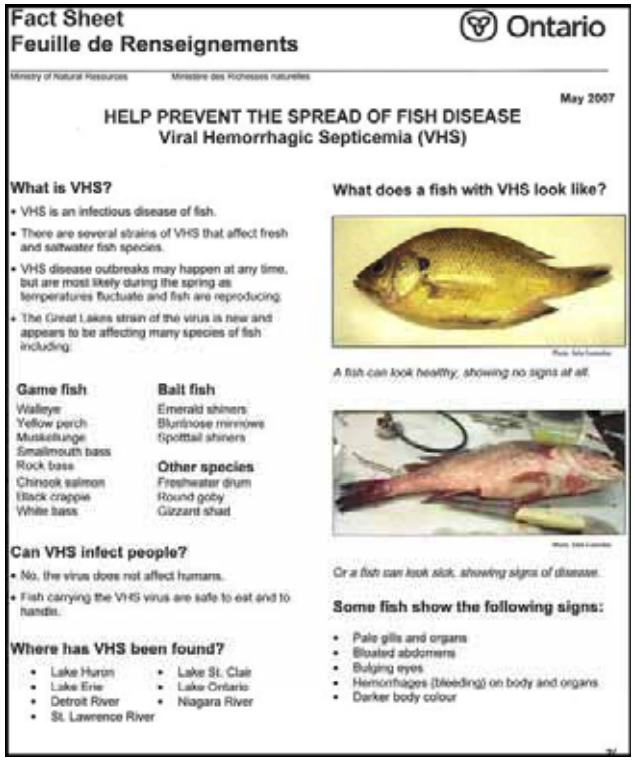
- VHS website
- 5 VHS Fact Sheets

  anglers, bait harvesters, fish farmers,

  aquarists, property owners

- 6th Fact Sheet being translated now

  management actions



Fact Sheet
Feuille de Renseignements

Ontario

Ministry of Natural Resources — Ministère des Richesses naturelles

May 2007

HELP PREVENT THE SPREAD OF FISH DISEASE
Viral Hemorrhagic Septicemia (VHS)

What is VHS?

- VHS is an infectious disease of fish.
- There are several strains of VHS that affect fresh and saltwater fish species.
- VHS disease outbreaks may happen at any time, but are most likely during the spring as temperatures fluctuate and fish are reproducing.
- The Great Lakes strain of the virus is new and appears to be affecting many species of fish including:

Game fish
Walleye
Yellow perch
Muskellunge
Smallmouth bass
Rock bass
Chinook salmon
Black crappie
White bass

Bait fish
Emerald shiners
Bluntnose minnows
Spottail shiners

Other species
Freshwater drum
Round goby
Gizzard shad

Can VHS infect people?

- No, the virus does not affect humans.
- Fish carrying the VHS virus are safe to eat and to handle.

Where has VHS been found?

- Lake Huron
- Lake Erie
- Detroit River
- St. Lawrence River
- Lake St. Clair
- Lake Ontario
- Niagara River

What does a fish with VHS look like?

A fish can look healthy, showing no signs at all.

Or a fish can look sick, showing signs of disease.

Some fish show the following signs:

- Pale gills and organs
- Bloated abdomens
- Bulging eyes
- Hemorrhages (bleeding) on body and organs
- Darker body colour

- Suggestions to slow the spread
- Phone number to report die-offs – 2007 and 2008?

Help stop the spread of invading species

What you should know about VHS

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LAWS TO HELP STOP THE SPREAD OF INVADING SPECIES

Harmful introduced species are often spread unknowingly. As an angler or boater, you should always take precautions to help stop the spread of invading species. The following laws are in place to prevent unauthorized introductions.

POSSESSING LIVE FISH

It is illegal to possess live invasive fish including: round goby, tubenose goby, grass carp, bighead carp, black carp, silver carp and any species of snakehead. If any of these species are caught they should be destroyed and not released back into any waters.

MOVING LIVE FISH

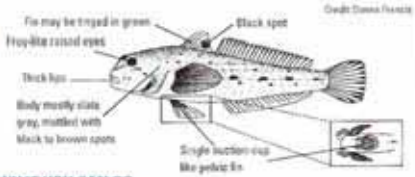
Many new populations of fish have been established through unauthorized stocking. This practice is illegal and can cause great harm to existing fisheries and aquatic ecosystems. **A licence is required for all fish** (including live spawn) **transfers and stocking into Ontario waters, and a licence is required to ship or transport live fish, other than baitfish, taken from Ontario waters.** Also, take care when cleaning smelt. Do not rinse equipment or dump entrails into a lake or river. Fertilized smelt eggs can easily invade new waters.

AQUARIUM FISH

Never release or flush pets, plants or water from aquaria, backyard ponds or water gardens. It is illegal and can harm the environment. If you have an unwanted aquarium pet, you can return it to a local pet store, donate it to a school or contact the Fish Rescue Program at **1-800-563-7711**.

ROUND GOBY

The round goby is frequently caught by anglers. It is just one of the many serious threats to North American waters. Since its discovery in the St. Clair River in 1990, this bottom-dwelling fish has rapidly spread to many areas of the Great Lakes and inland waters. The round goby can displace native fish from optimal habitat, eat their eggs and young and spawn multiple times a season. Anglers should know how to identify the round goby - these aggressive fish are easily caught by hook and line.



WHAT YOU CAN DO...

- Report new sightings. If you catch a round goby do not throw it back alive! Always dispose of your unwanted bait and the contents of your bait bucket or bait bucket water on land or in the trash – never dump your bait bucket into a waterbody.
- Never use gobies as bait – remember it is against the law to use them as bait or have live gobies in your possession.

For more information or to report a sighting, call the **Invading Species Hotline 1-800-563-7711** or visit www.invadingspecies.com.

BY SPREADING THE WORD AND TAKING ACTION AGAINST INVADING SPECIES YOU CAN HELP CONSERVE ONTARIO'S HEALTHY FISHERIES!

WHAT YOU SHOULD KNOW ABOUT VHS

Help Slow the Spread of VHS

Viral Hemorrhagic Septicemia (VHS) is an infectious disease of fish. VHS is not a threat to human health. Fish carrying the VHS virus are safe to eat and handle.

You can help slow the spread of this virus and other invasive species by following the laws outlined above (Laws to Help Stop the Spread of Invasive Species) and the guidelines on page 57 (Keep All Our Lakes Clear).

More information is available at: http://www.mnr.gov.on.ca/MNR/fishing/VHS.html or call:
Natural Resources Information Centre 1-800-667-1940.

2005 Survey of Recreational Fishing in Ontario – Results

Every 5 years, the Ontario Ministry of Natural Resources, in cooperation with Canada's Department of Fisheries and Oceans, conducts a survey of recreational fishing in Ontario. The results of the survey may be viewed online at: http://www.dfo-mpo.gc.ca/communic/statistics/recreational/canada/index_e.htm

Public Information/Outreach

- Bait bucket sticker developed with

  Ontario Federation of Anglers and Hunters

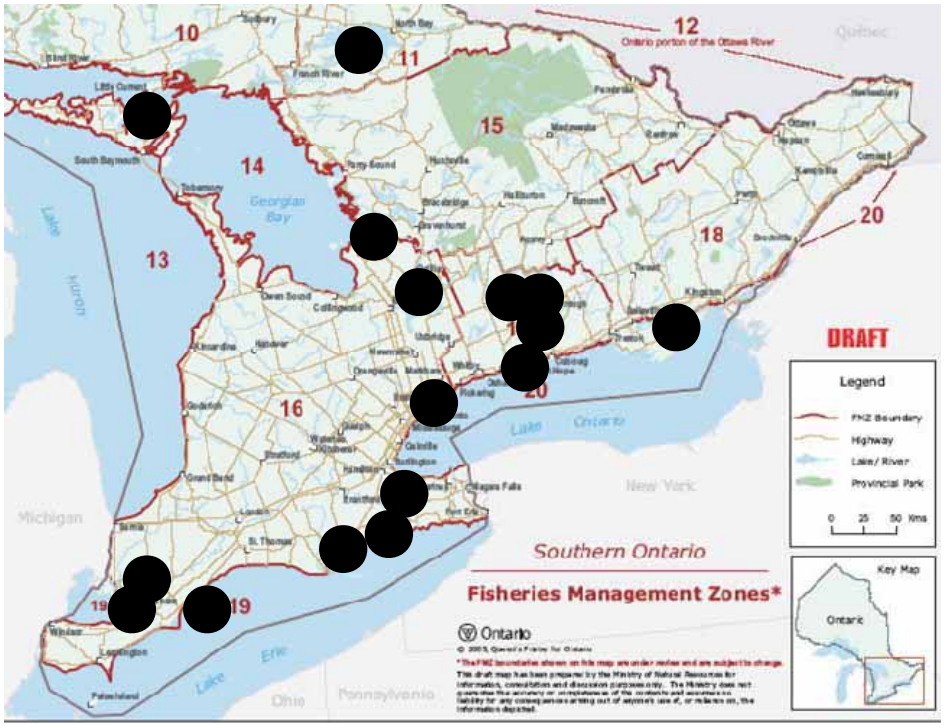
  >300,000 distributed to anglers

Mortalities

May 2007

- Die-off in Hamilton Harbour, Lake Ontario
- Investigated by Fish Pathology Lab, Univ. of Guelph
- VHS found in 2 freshwater drum, confirmed by DFO
- No VHS in 7 other fish
  - channel catfish, brown bullhead, common carp, white sucker, gizzard shad

VHS Surveillance



- OMNR contributed to 2 programs in 2007
- Fish collected mainly in southern Ontario

VHS Surveillance - Federal

- Federal Program led by Canadian Food Inspection Agency (CFIA)
- In partnership with Fisheries and Oceans Canada (DFO), Provincial Ministries in Quebec and Ontario
- 3 spring sites and 7 fall sites sampled in Ontario
    - 1700 fish tested

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VHS Surveillance - Provincial

- OMNR Program funded by Canada-Ontario Agreement Respecting the Great Lakes Basin Ecosystem

- In partnership with Fish Health Lab, University of Guelph

- Collected 989 fish
  6 spring sites and 6 fall sites

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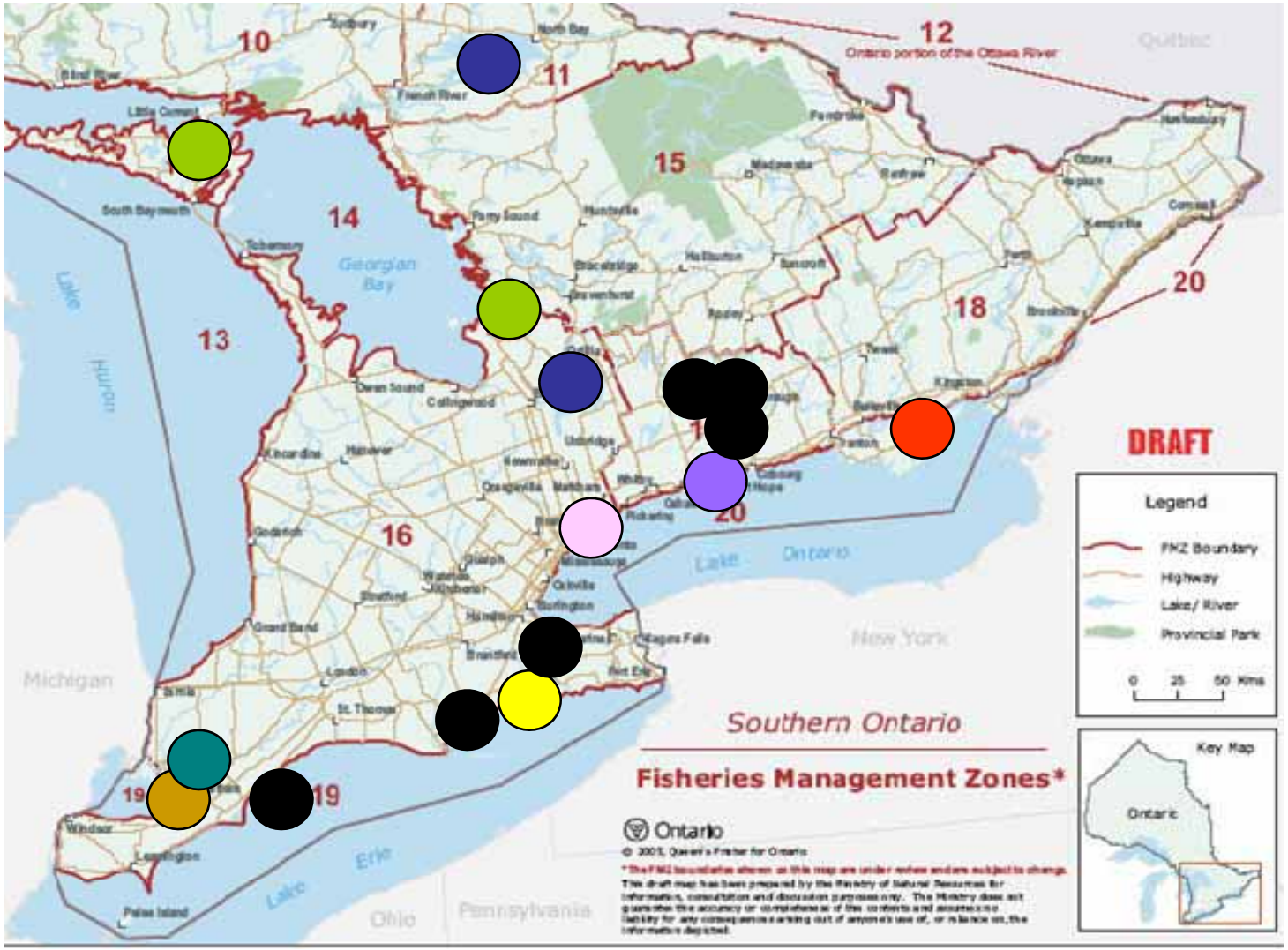
VHS Surveillance – Provincial Results

- VHS was found in Thames River in spring
  - Detected in 1 pool of 5 largemouth bass
  - No signs of disease

- Fall test results were expected in late December

How sites were selected



VHS Surveillance - 2008

- CFIA/DFO – looking at funding and delivery options
  - will do some surveillance in 2008
  - expect approx. same as 2007

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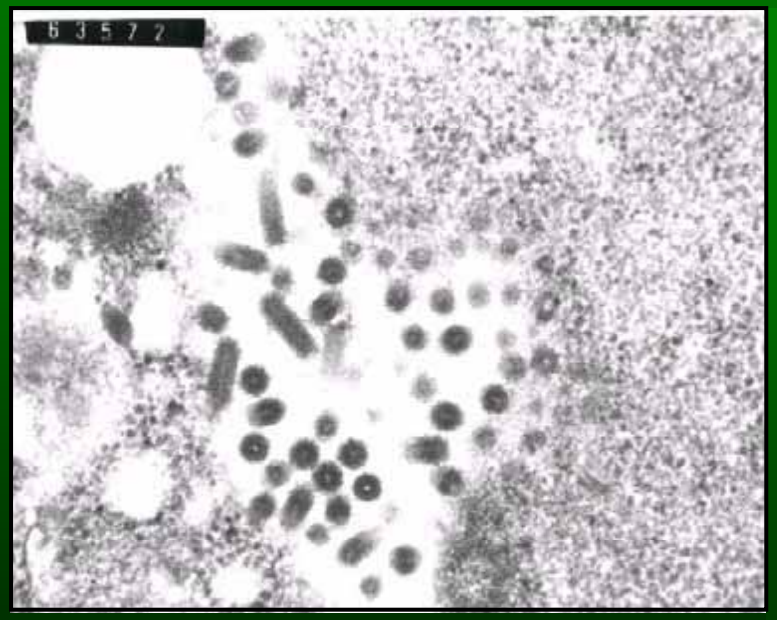
- OMNR Program – no COA funding
  - will do some surveillance
  - How????
  - target sites where bait is harvested/used; possibly lakes with inland commercial fisheries

VHS Surveillance - Challenges

- Coordination
  - 3 prov agencies, 2 federal agencies, 10 MNR offices
- Collection
  - use ongoing programming (location/timing/species)
  - set nets Monday, lift Tues-Weds but must ship Weds
- Sampling happened at lab
  - means increased costs/mass of shipping
  - time consuming/ capacity (1 site per week per lab)
- Couriers
  - delays, different time zones

The Gift That Keeps on Giving: The Continuing Saga of VHS in Michigan



Gary Whelan
MI DNR Fisheries Division
January 2008

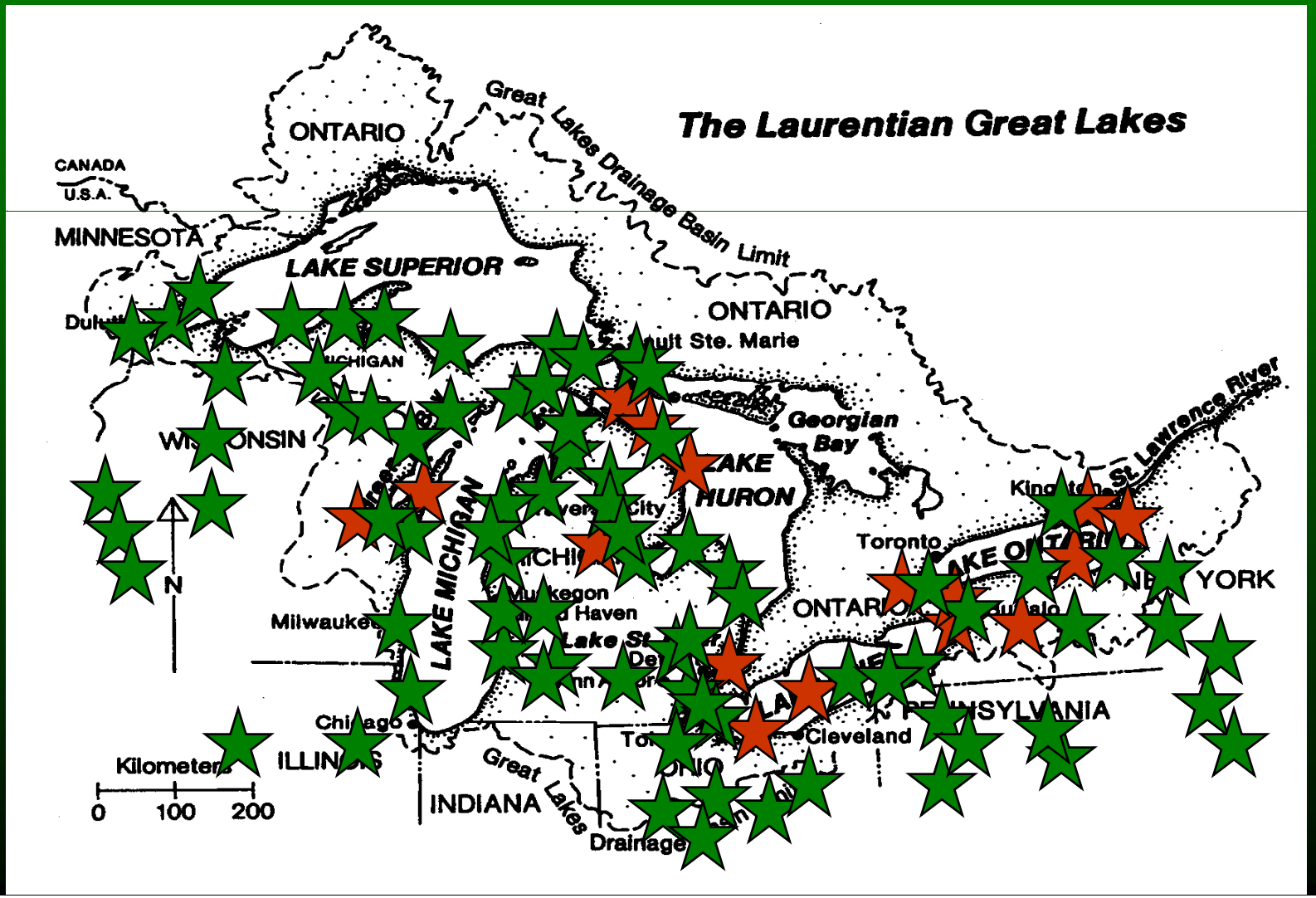
MI DNR VHSv Management Summary for 2007 and 2008

- 2007 VHSv Management Update
  - Surveillance
  - Management Actions
- 2008 Planned VHSv Surveillance
- Spring 2008 Coolwater Production Plans

2007 VHSv Surveillance Summary

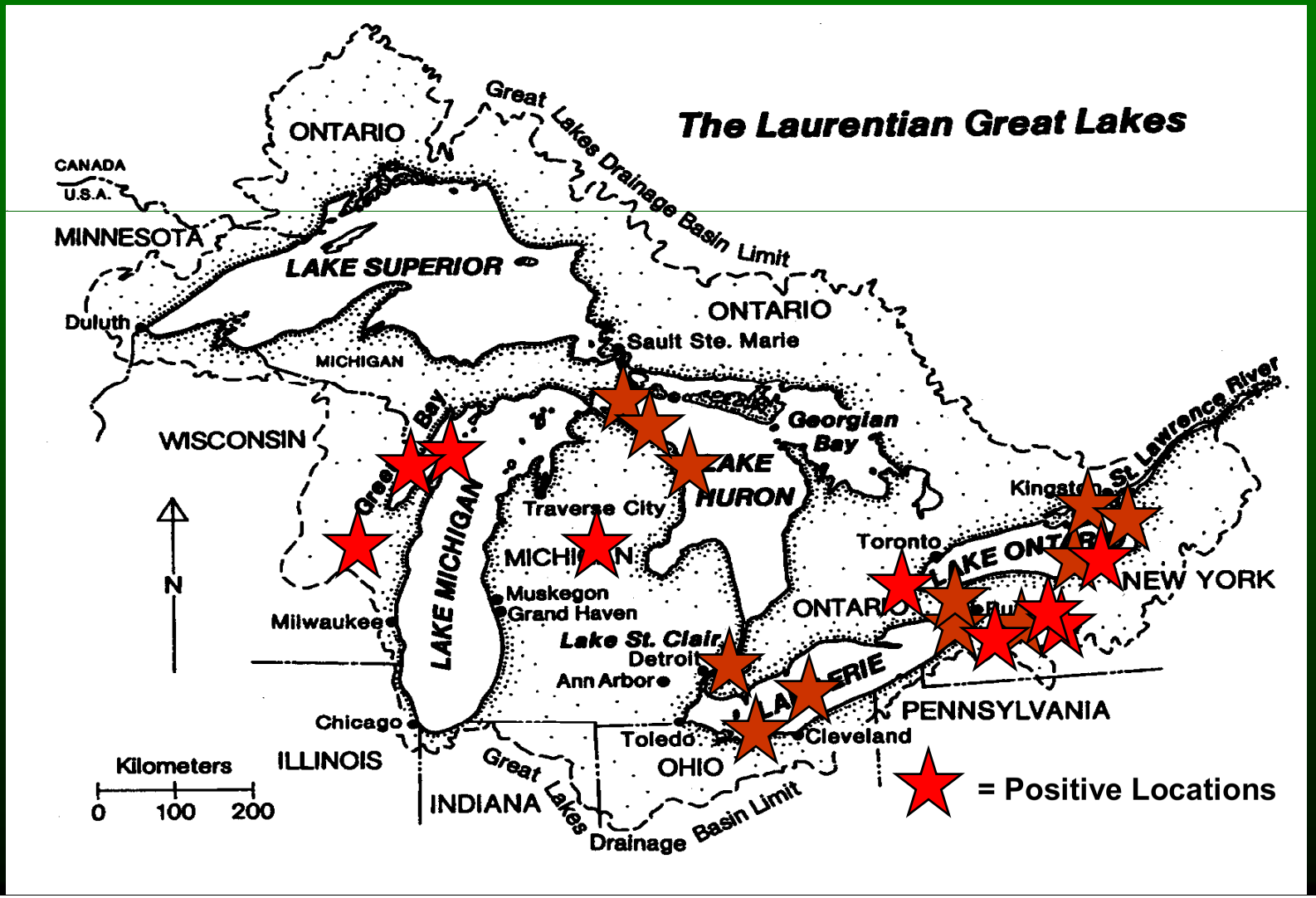
- Collected 163 lots of fish for VHSv surveillance
  - 7355 fish examined from 38 species
  - Only positive fish were found during an epizootic in Budd Lake
- Minnow industry has collected ten additional Great Lakes samples as part of their certification
  - All negative
- No repeat detections or mortalities

Great Lakes Region Sampling for VHS



Great Lakes Distribution of VHS

Early Stages of Invasion



MI VHS Affected Species – 17 of 25

- Large mortalities (6)
  - Gizzard Shad
  - Black crappie, bluegills, yellow perch
  - Freshwater drum
  - Muskies
- Small mortalities (5)
  - Pumpkinseed sunfish, rock bass
  - Smallmouth bass, lake whitefish, walleye
- Detected (6)
  - Smallmouth bass
  - Emerald and spottail shiners
  - Silver and shorthead redhorse
  - Chinook salmon

MI DNR Control Strategy

- Strategy is to contain and slow the spread of most pathogens such as VHS
  - Management actions to slow movement of VHSv
  - Provide time to protect key broodstocks and inland waters and to prepare for pathogen's arrival
    - Need to watch affects in inland waters
  - Allow additional research to be done on susceptibility and transmission
    - Transmission and susceptibility studies are being funded
    - Biosecurity tools in development
      - Egg disinfection methods
      - Facility biosecurity measures

GLFHC Agency VHS Actions – MI DNR Update

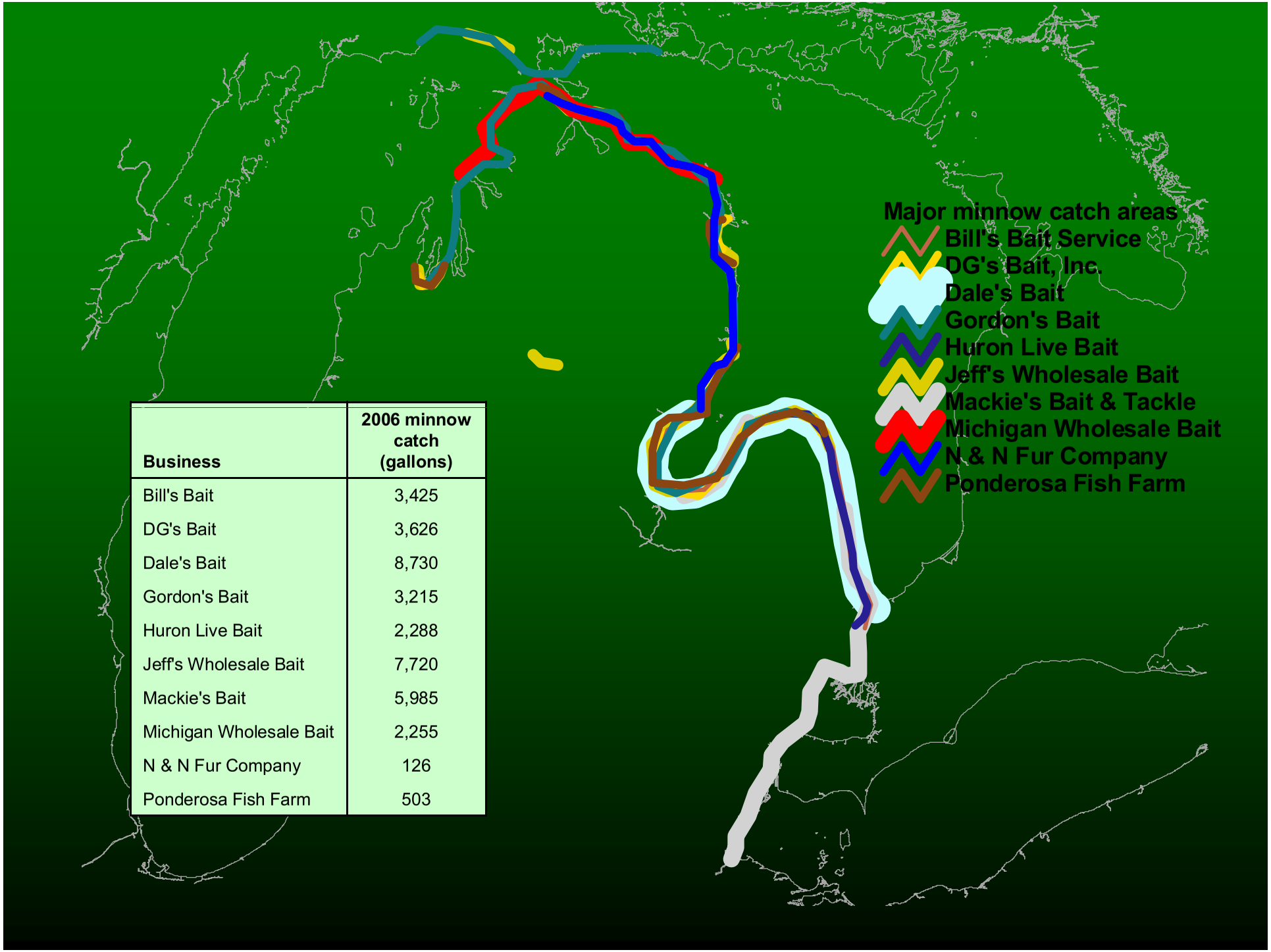
- Pathogen and Disinfection Testing
- Surveillance
- Fish Culture
- Fish Transfers
- Other Management Actions
- Commercial Fishing
- Bait Industry
- Public Information
- Other Measures

GLFHC Agency VHS Actions – MI DNR Update

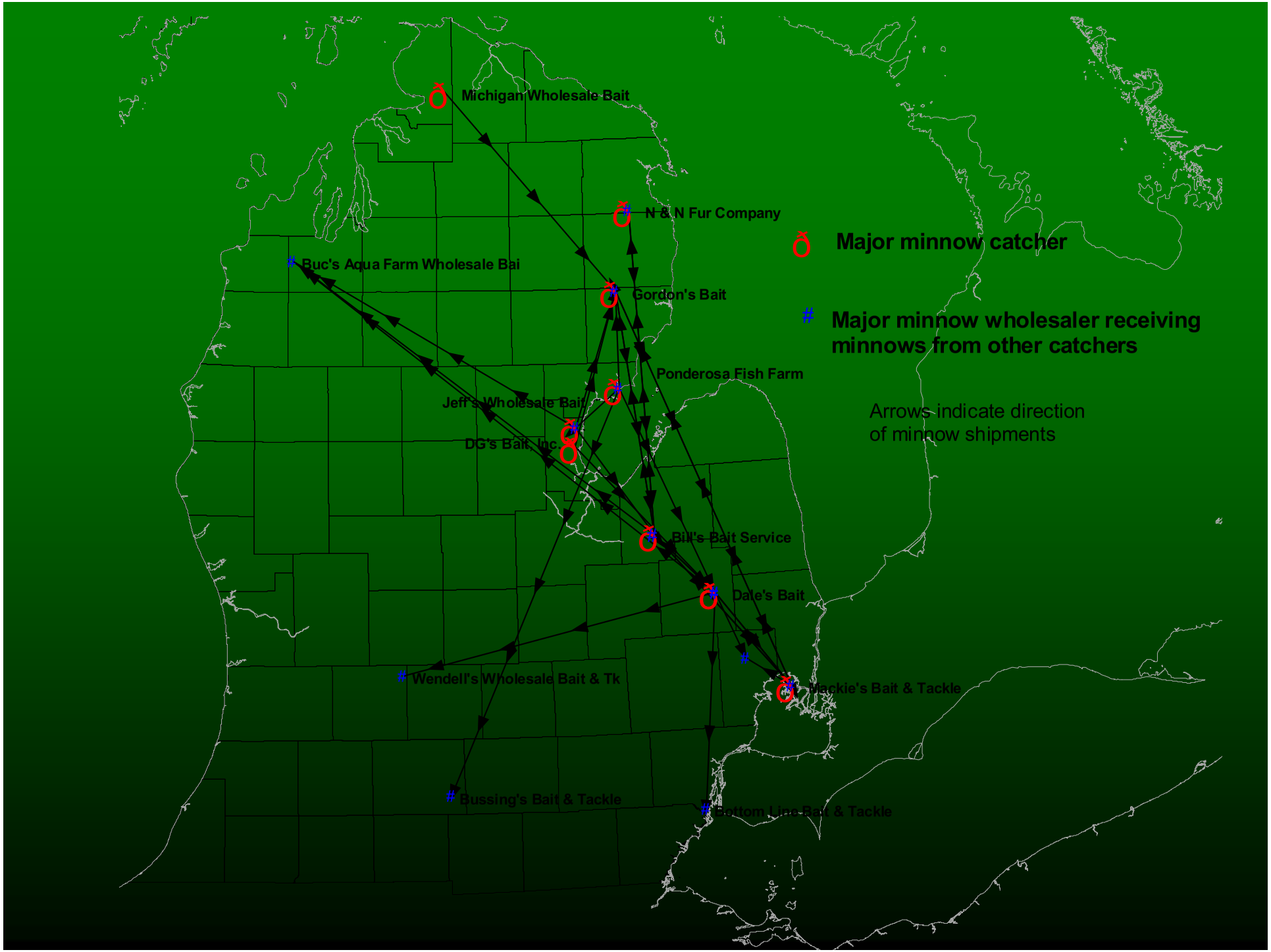
- Pathogen and Disinfection Testing
  - Coolwater fish egg disinfection
    - Disinfection with wild fish
  - Increase lab capacity - MSU
- Surveillance
  - USDA-APHIS Grant
- Fish Culture
  - Biosecurity
  - Increased broodstock testing

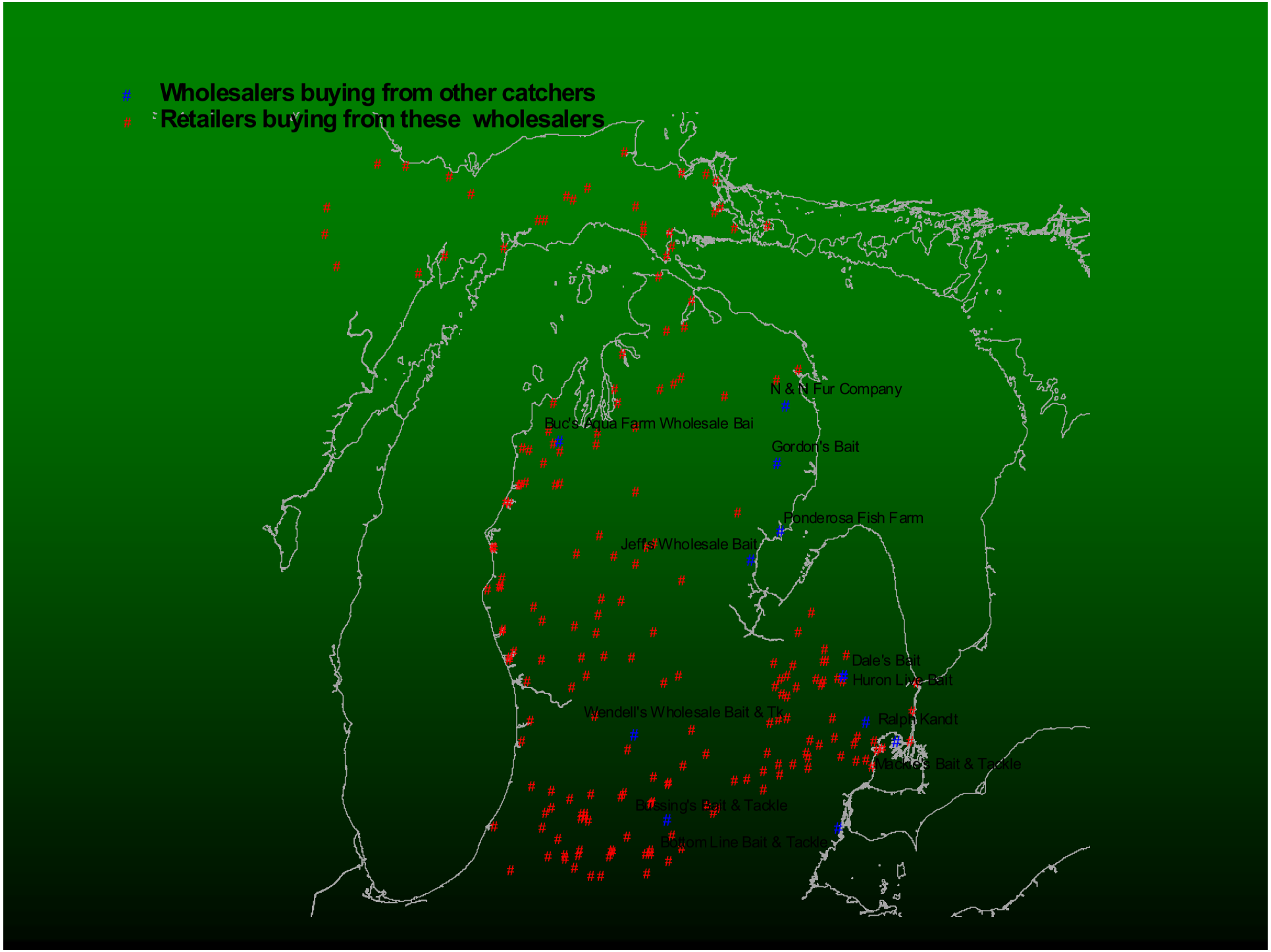
GLFHC Agency VHS Actions – MI DNR Update

- Fish Transfers
  - Prohibition without testing
  - Test timing close to when fish will be moved
- Other Management Actions
  - Large vessels
    - Not between management areas
    - Dry large gear and ballast tank disinfection
  - Disinfection of all gear and boats
    - Contact – 1 cup bleach/ 10 gallons
    - Immersion
      - 20 ppm chlorine – 30 minutes
      - 250 ppm Virkon



Business	2006 minnow catch (gallons)
Bill's Bait	3,425
DG's Bait	3,626
Dale's Bait	8,730
Gordon's Bait	3,215
Huron Live Bait	2,288
Jeff's Wholesale Bait	7,720
Mackie's Bait	5,985
Michigan Wholesale Bait	2,255
N & N Fur Company	126
Ponderosa Fish Farm	503


Michigan Wholesale Bait
N & N Fur Company
Buc's Aqua Farm Wholesale Bai
Gordon's Bait
Ponderosa Fish Farm
Jeff's Wholesale Bait
DG's Bait, Inc.
Bill's Bait Service
Dale's Bait
Wendell's Wholesale Bait & Tk
Buckie's Bait & Tackle
Bussing's Bait & Tackle
Bottom Line Bait & Tackle
Major minnow catcher
Major minnow wholesaler receiving minnows from other catchers
Arrows indicate direction of minnow shipments


Wholesalers buying from other catchers
Retailers buying from these wholesalers
N & N Fur Company
Buc's Aqua Farm Wholesale Bai
Gordon's Bait
Ponderosa Fish Farm
Jeffs' Wholesale Bait
Dale's Bait
Huron Live Bait
Wendell's Wholesale Bait & Tk
Ralph Kandt
Mackie's Bait & Tackle
Bottom Line Bait & Tackle

GLFHC Agency VHS Actions – MI DNR Update

- Other Management Actions
  - Regulations – FO245 Example
    - Prohibited Species
    - Certification and Tracking Process
    - Catch and Release
    - Baitfish and Roe only on hook
    - Drain all live wells and bilges
    - Uncertified bait
      - Positive - Positive
      - Surveillance - Positive or Surveillance
      - Pathogen Free – All waters

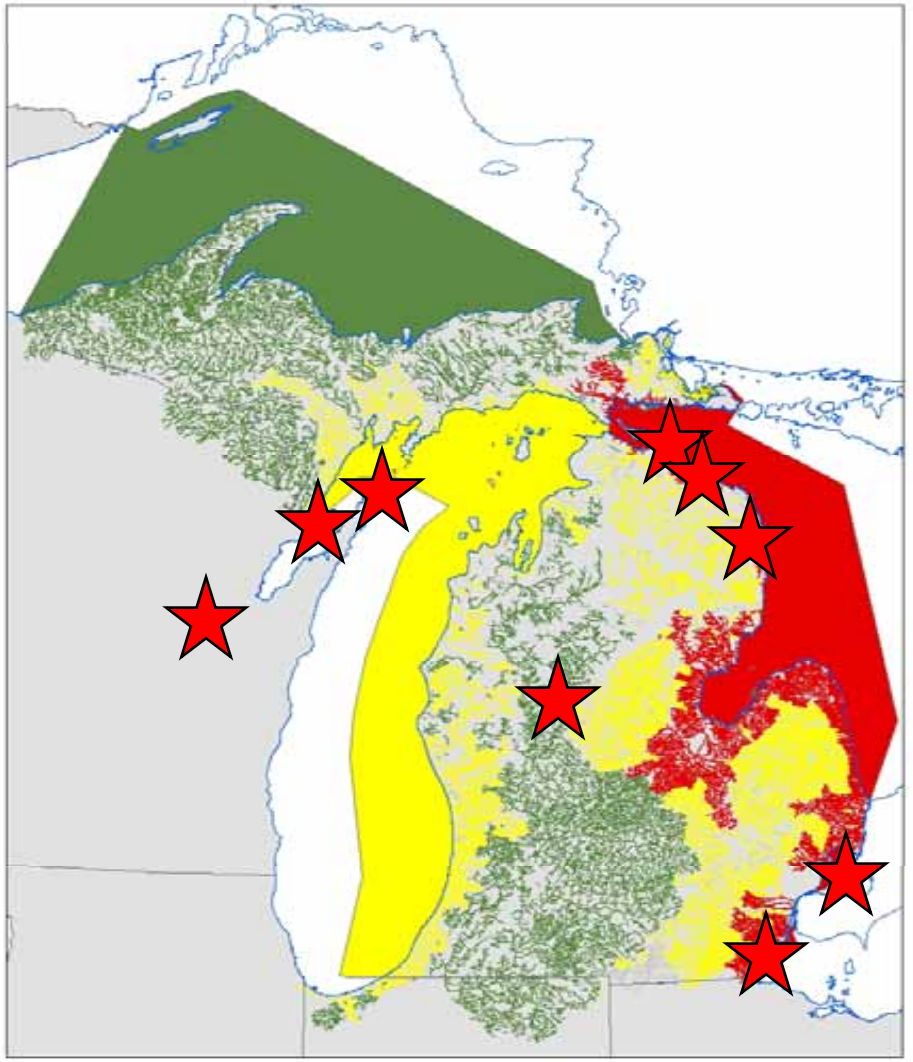
Fish Disease Control Orders
MI DNR VHS Management Areas

- VHS Management Areas

- VHS Positive Area

- VHS Surveillance Area

- VHS Free Area



GLFHC Agency VHS Actions

- Commercial Fishing
  - Live fish movement regulation
- Public Information
  - USDA-APHIS Funds
  - Websites
  - Public Service Announcements
- Other Measures
  - Ballast water
  - Research facilitation

VHS Research Initiatives

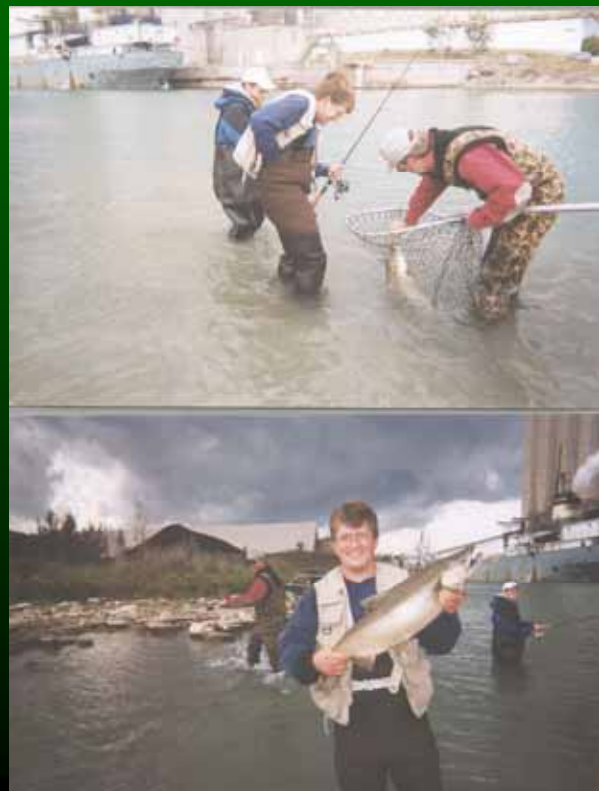
- NCRAC - $157K
  - Iodine Disinfection Effectiveness – LMB and YEP
  - VHS Education – Biosecurity Workshops and HAACP Plans
- CRSEES - $203K
  - MSU – Faisal - $50K – Enhanced Diagnostic Reagents
  - Cornell – Bowser - $153K – PCR-R, CCF and LMB
    - Reference Services
- USDA-APHIS - $1.5 Million
- GLFT - $500-800K
  - Species susceptibility and strain typing

Public Actions to Help Control VHS

- Public Information Focus
  - Do not move live fish from water to water
  - Empty and disinfect live wells and bilge water
  - Clean and disinfect boats and gear when moving between waters
    - Bleach solution (1 cup to 10 gallons water)
    - Drying 4-6 hours in the sun
  - Eggs and Frozen Baits
    - Salt and Borax
      - Short Term – 20 minutes – No effect with up to 100% solution
      - Long Term – 11 days – Significant reduction to below detection
  - Report unusual fish kills to DNR Offices

2008 MI DNR Surveillance Overview

- Sample 113 waters
  - 335 lots of 60 fish (61% USDA-APHIS)
  - 19 Great Lakes lots
  - 8 Great Lakes lots
- All wild broodstock lots
  - Chinook salmon
  - Walleye
  - Muskellunge
  - Coho salmon
  - Steelhead



2008 MI DNR Coolwater Production

- Reduced Program for walleye
- Rear walleye and muskellunge
  - Testing
    - Adults - Pre-spawning and Egg Take
    - Eggs, Fry and Fingerlings
  - Bay de Noc and Muskegon River walleye broodstocks
  - Inland lake muskie broodstocks
- Incubate walleye at Thompson State Fish Hatchery
  - Pseudo-isolation area
  - Only use non-drainable ponds or those with direct drainage to Great Lakes

2008 MI DNR Coolwater Production

- Only rear muskies at Wolf Lake State Fish Hatchery
  - Pseudo-isolation in old hatchery building
- Stocking
  - Back into same Great Lakes waters or connected waters
  - Lake Michigan or Huron drainage lakes without outlets
- Managed risk program

Questions? Comments?

Great Lakes, Great Times, Great Outdoors

www.michigan.gov/dnr

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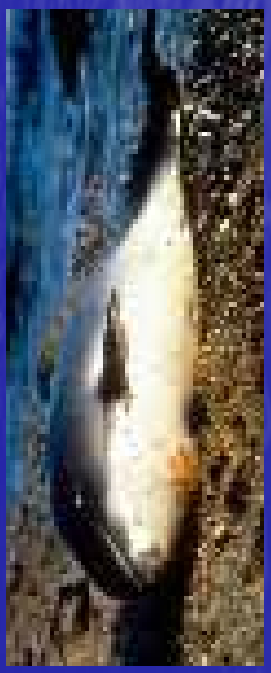
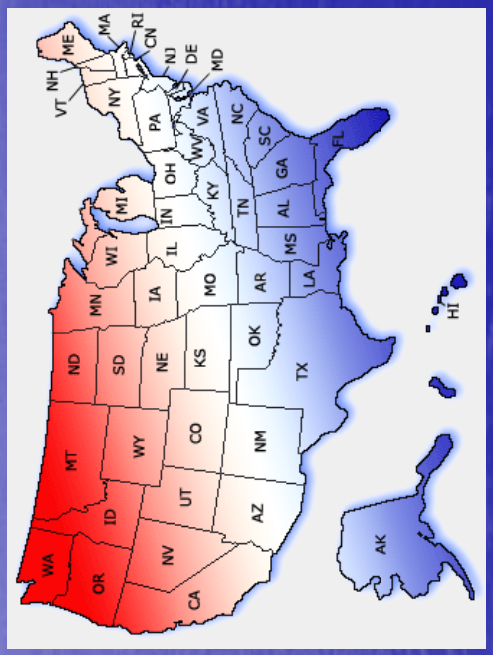
College of
VETERINARY MEDICINE
Michigan State University

Department of Fisheries and Wildlife
Michigan State University

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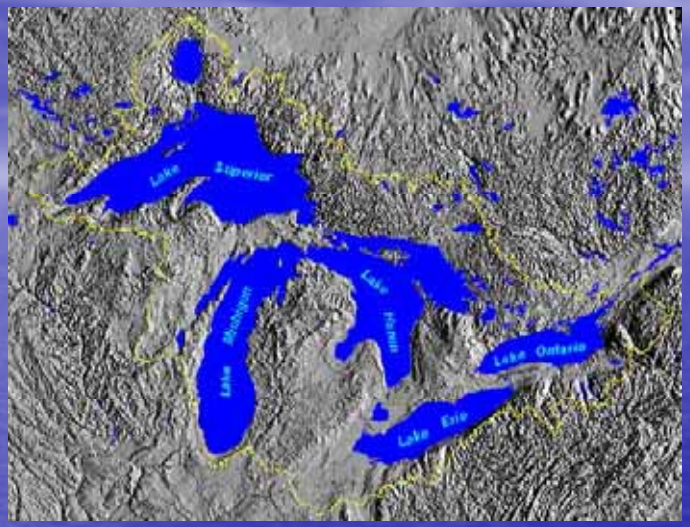
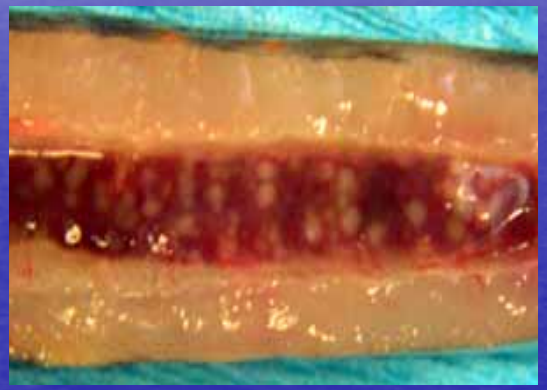
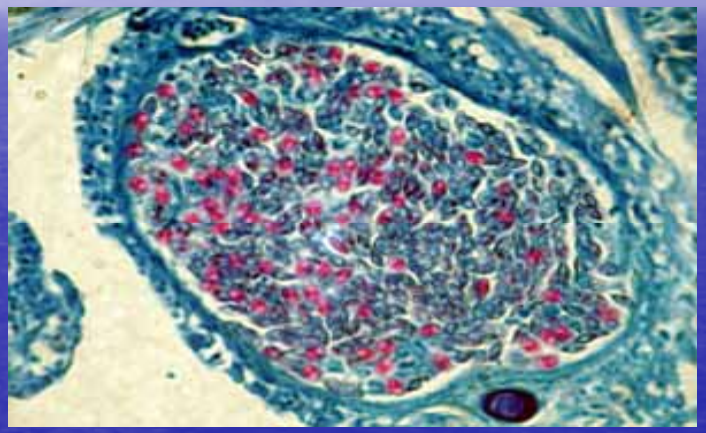


1952-Present: BKD
1970s: *Glugea hertwigi*
1988 BKD Kills in Lake Michigan
1989 EED
1980s-Pre Whirling Disease
1988-Pre Furunculosis
2001-Pre Emerging/resurging/non-reported before

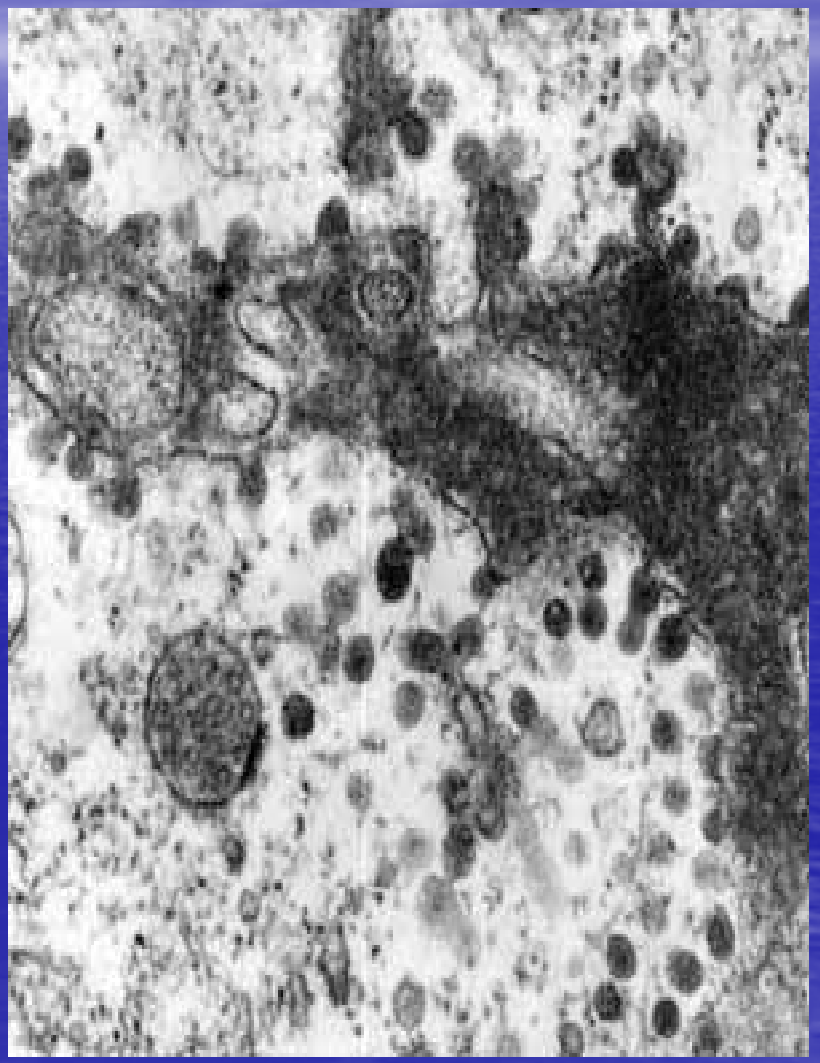
Since 2001



Emerging/Resurging Disease

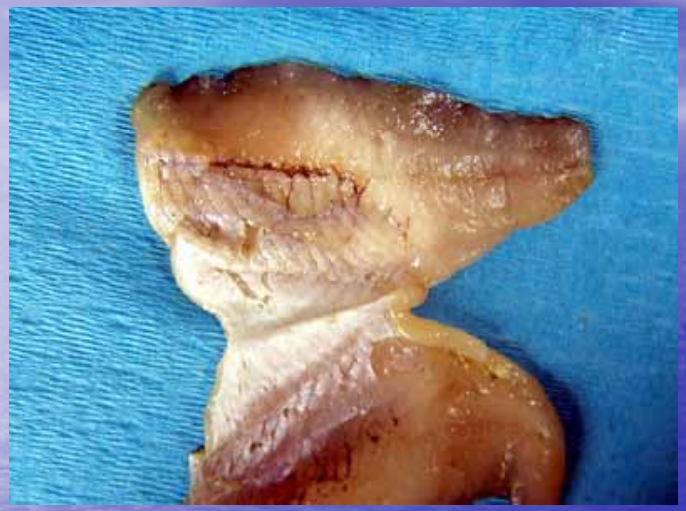


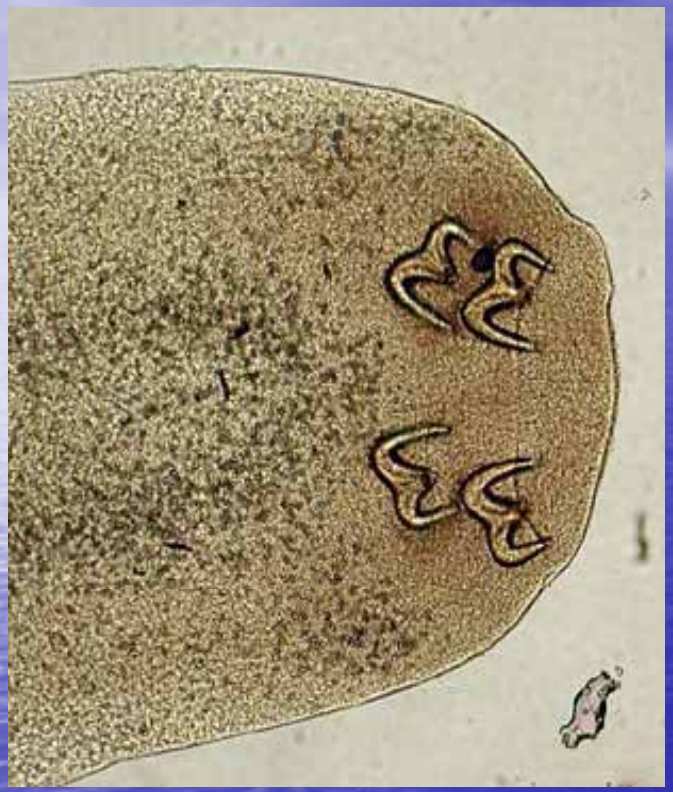
Koi Herpes Virus

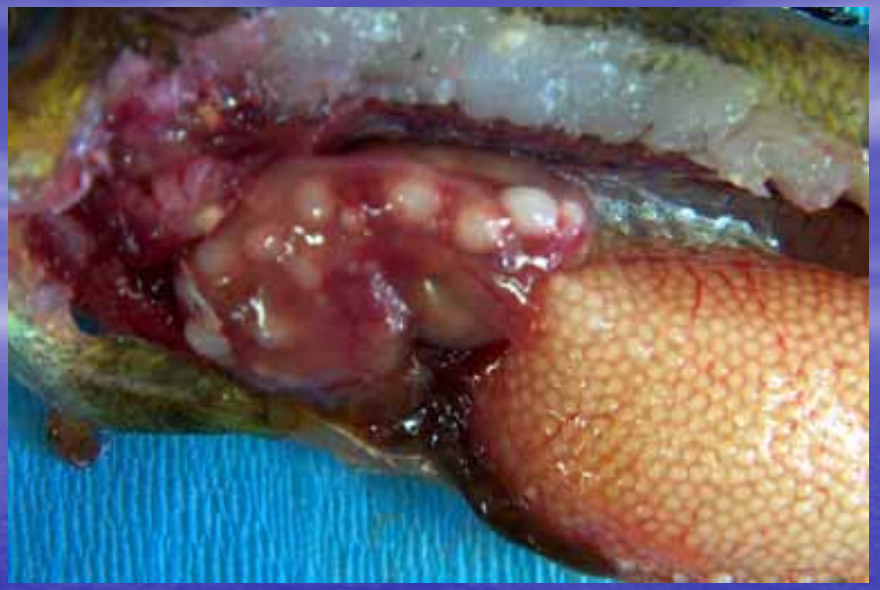


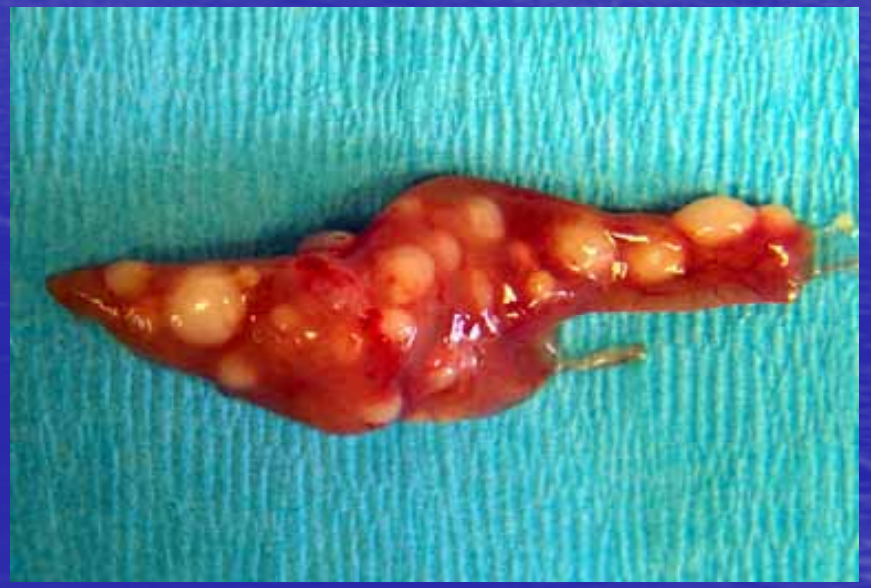
Phoma herbarum in Chinook salmon

Heterosporis

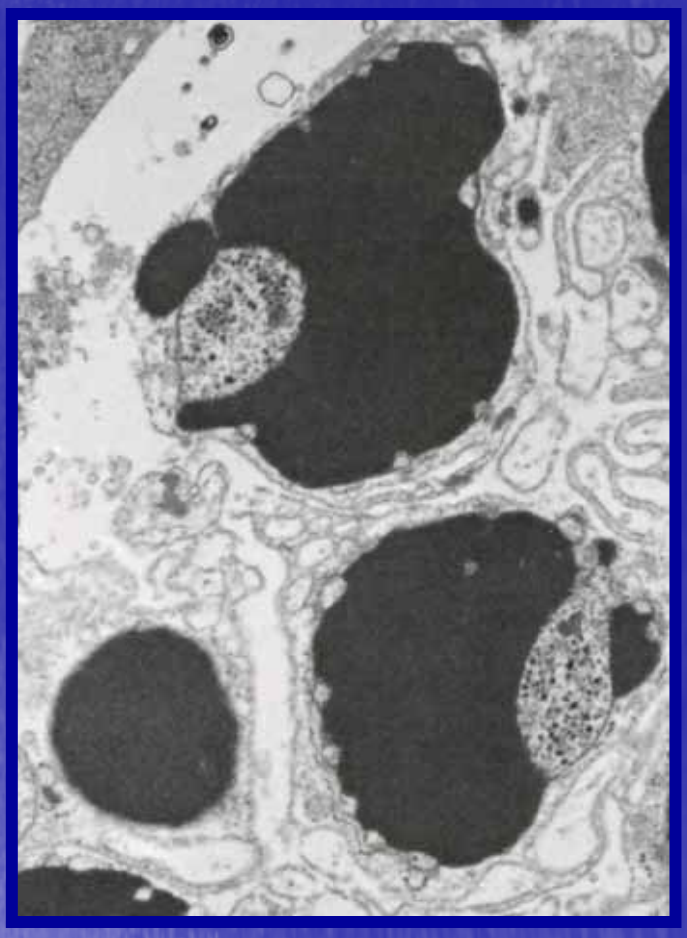




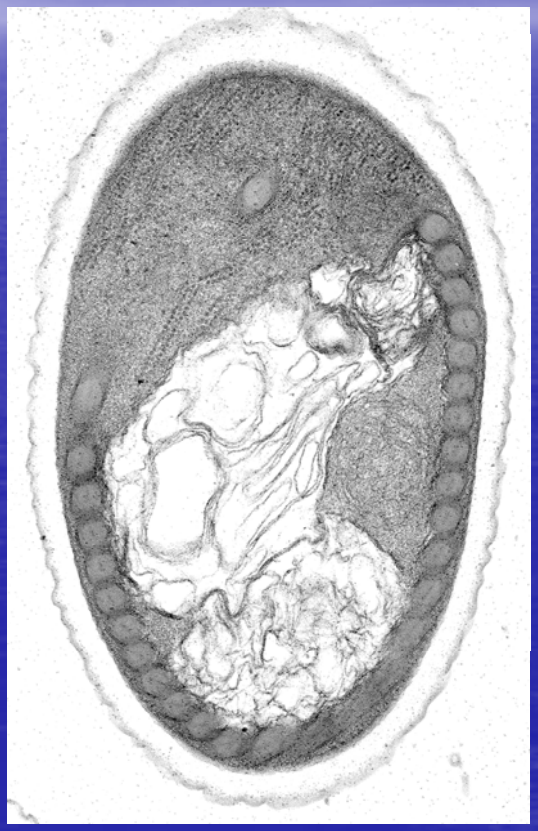


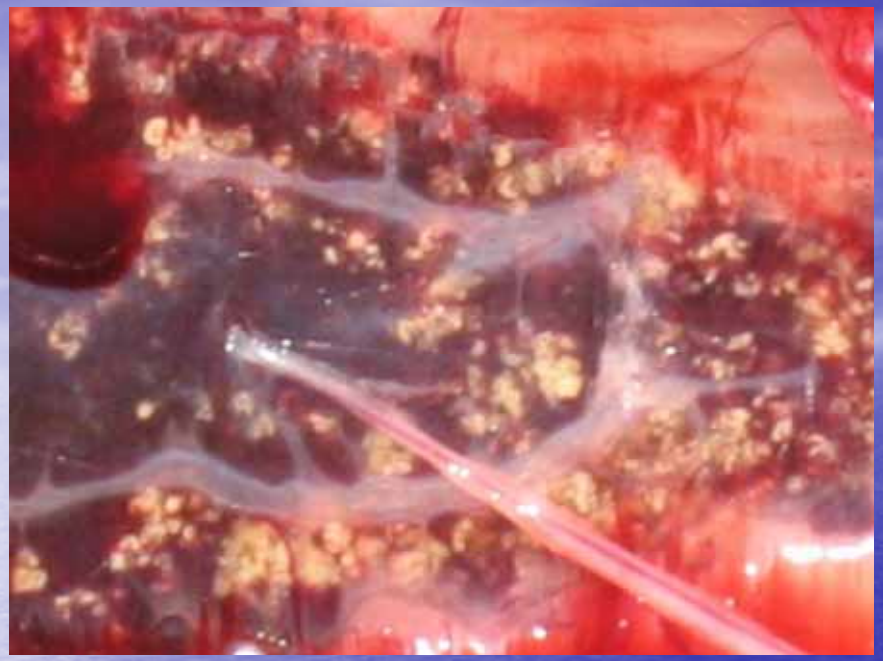


Triaenophora nodulosis

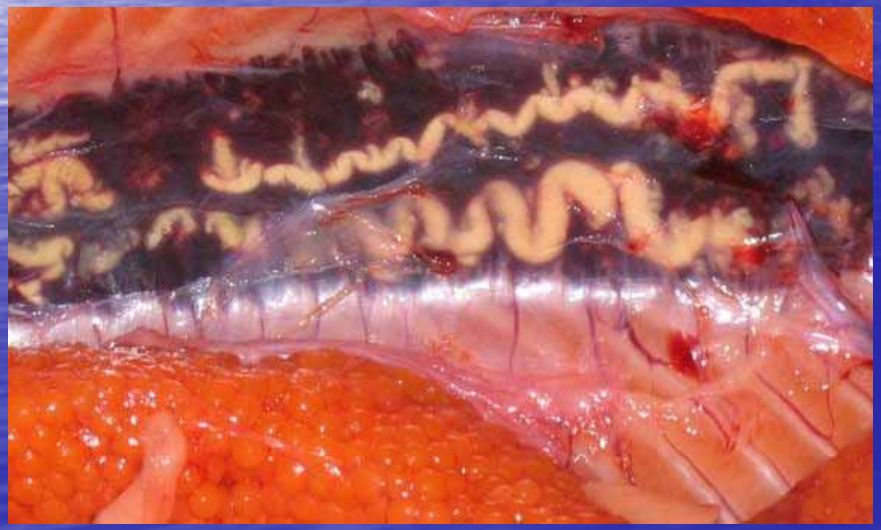


Mottled Sculpin (*Cottus bairdi*)

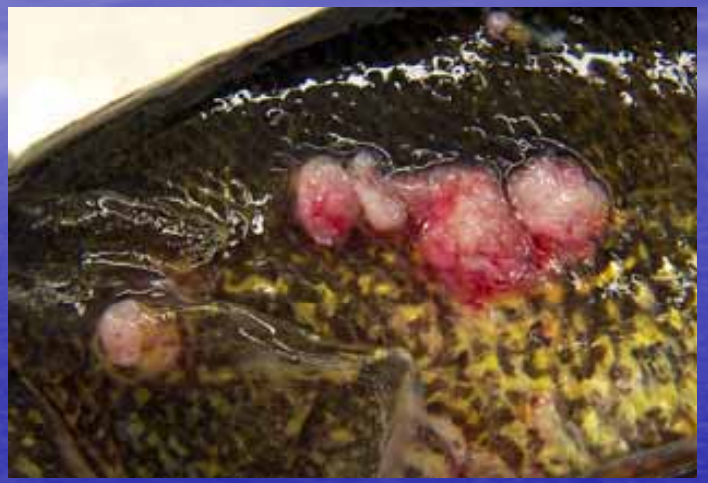


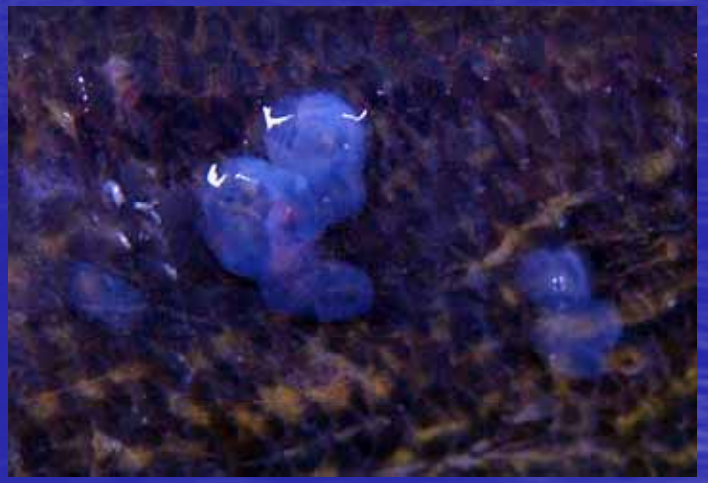


Carnobacterium maltaromaticum

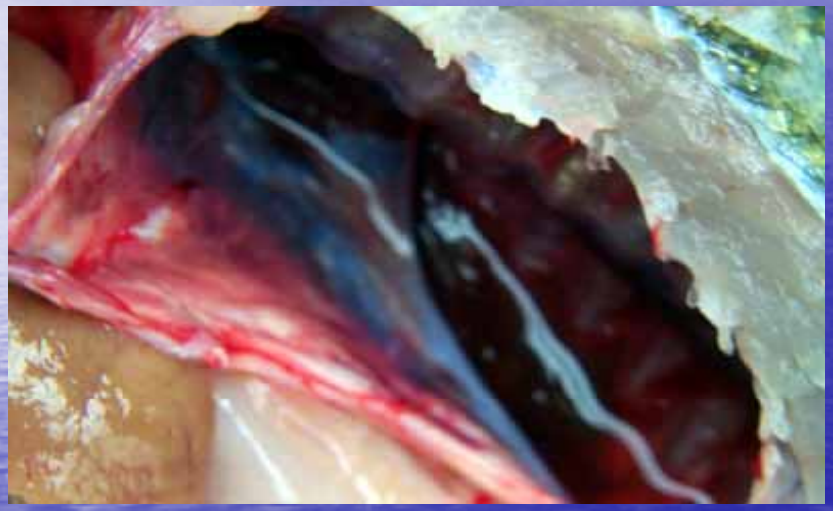


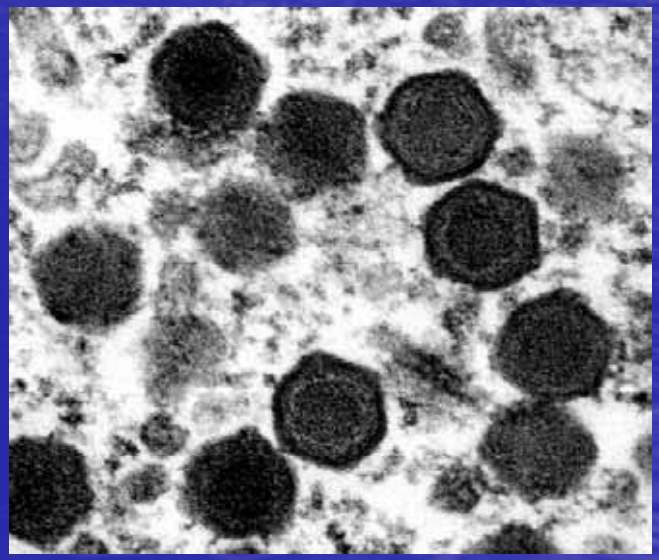
Walleye and skin tumors



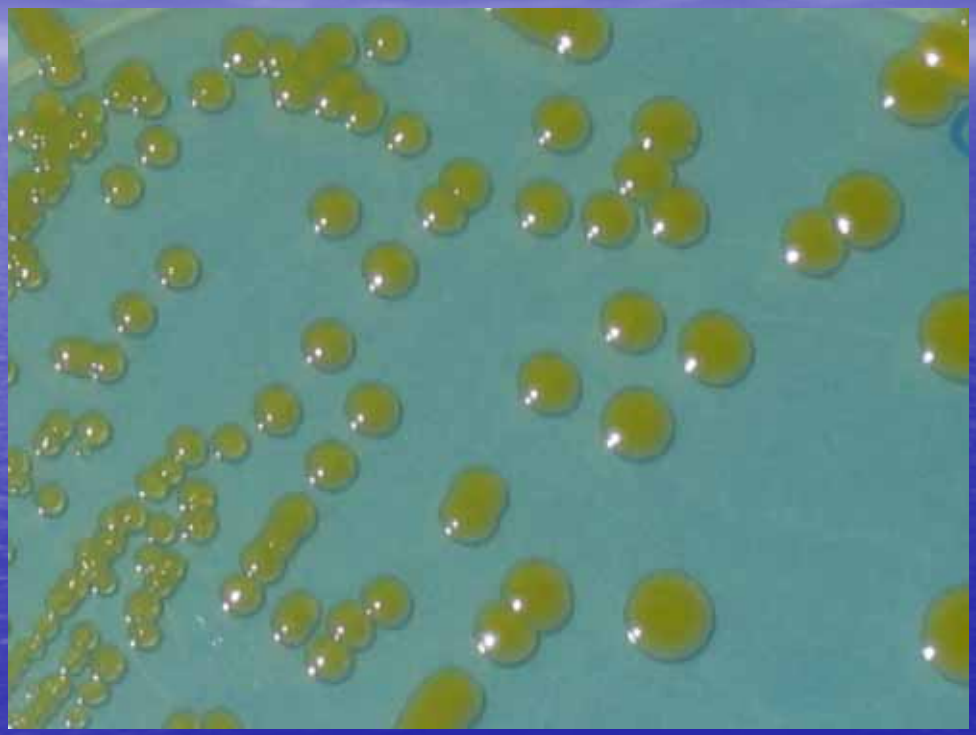


Largemouth Bass Virus





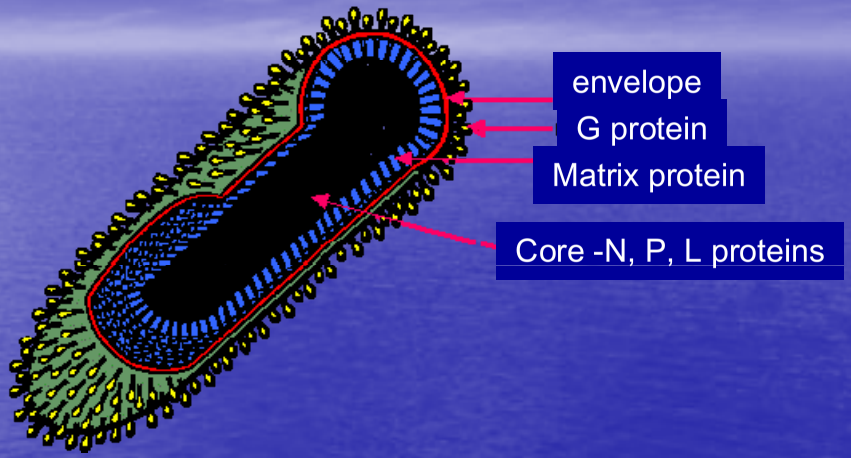
Pantoea Agglomerans



2003: Lake St. Clair Muskies and a Rhabdovirus

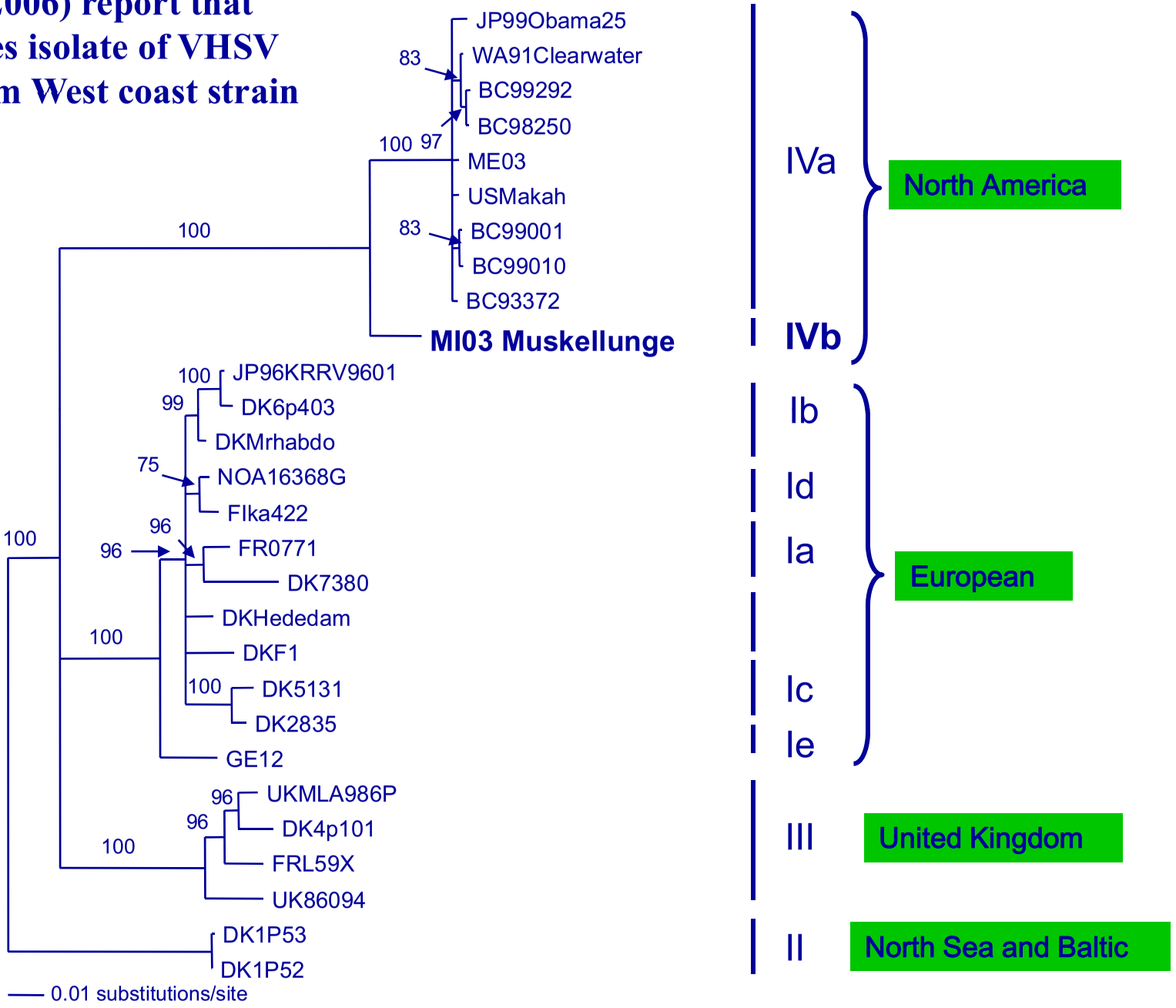
March 14, 2006: Gizzard Shad Mortalities

VHSV Particle and Genes



Negative-sense single-stranded RNA, (11,158 nt total, 6 genes)

MSU-AAHL (2006) report that the Great Lakes isolate of VHSV is different from West coast strain

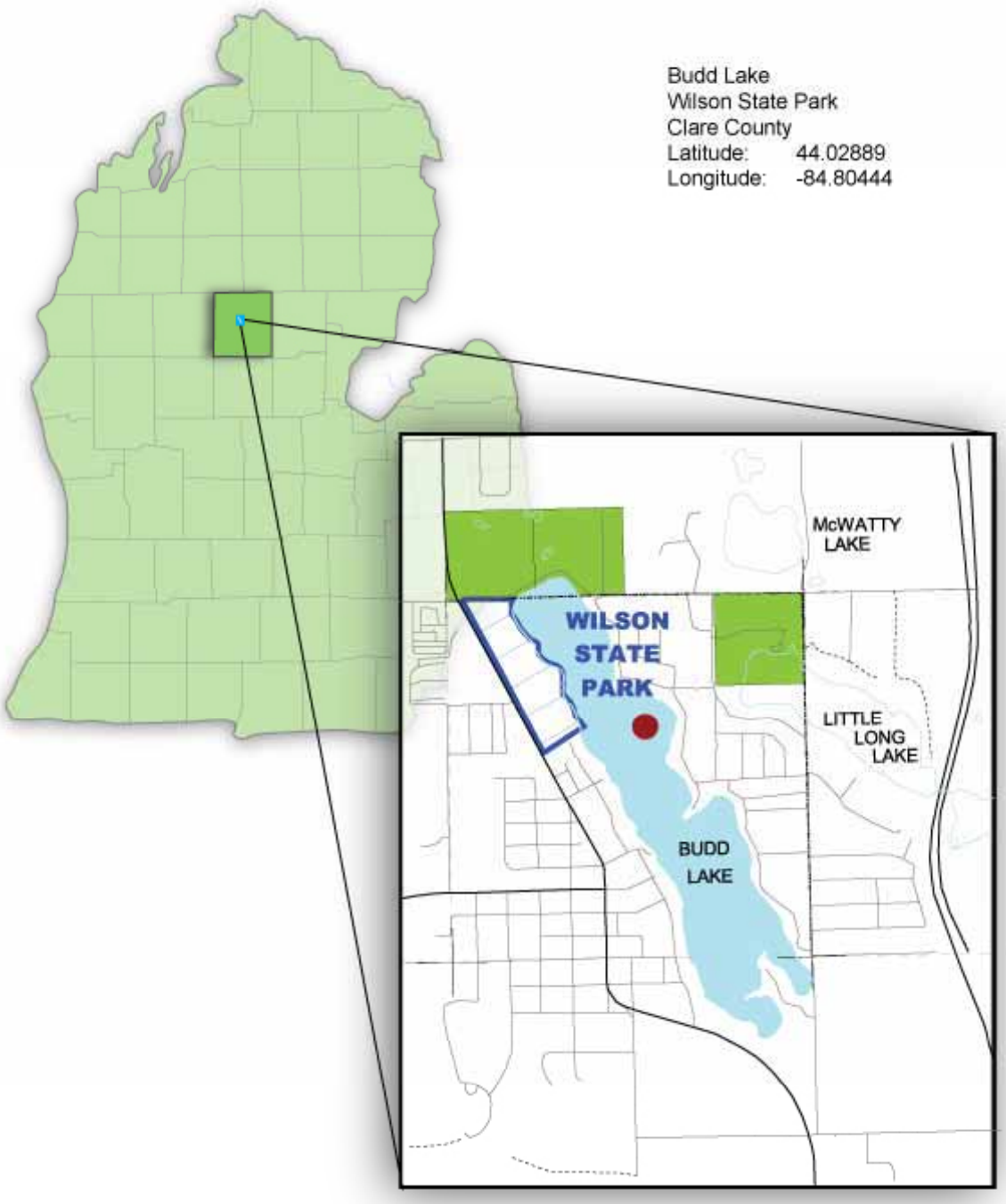


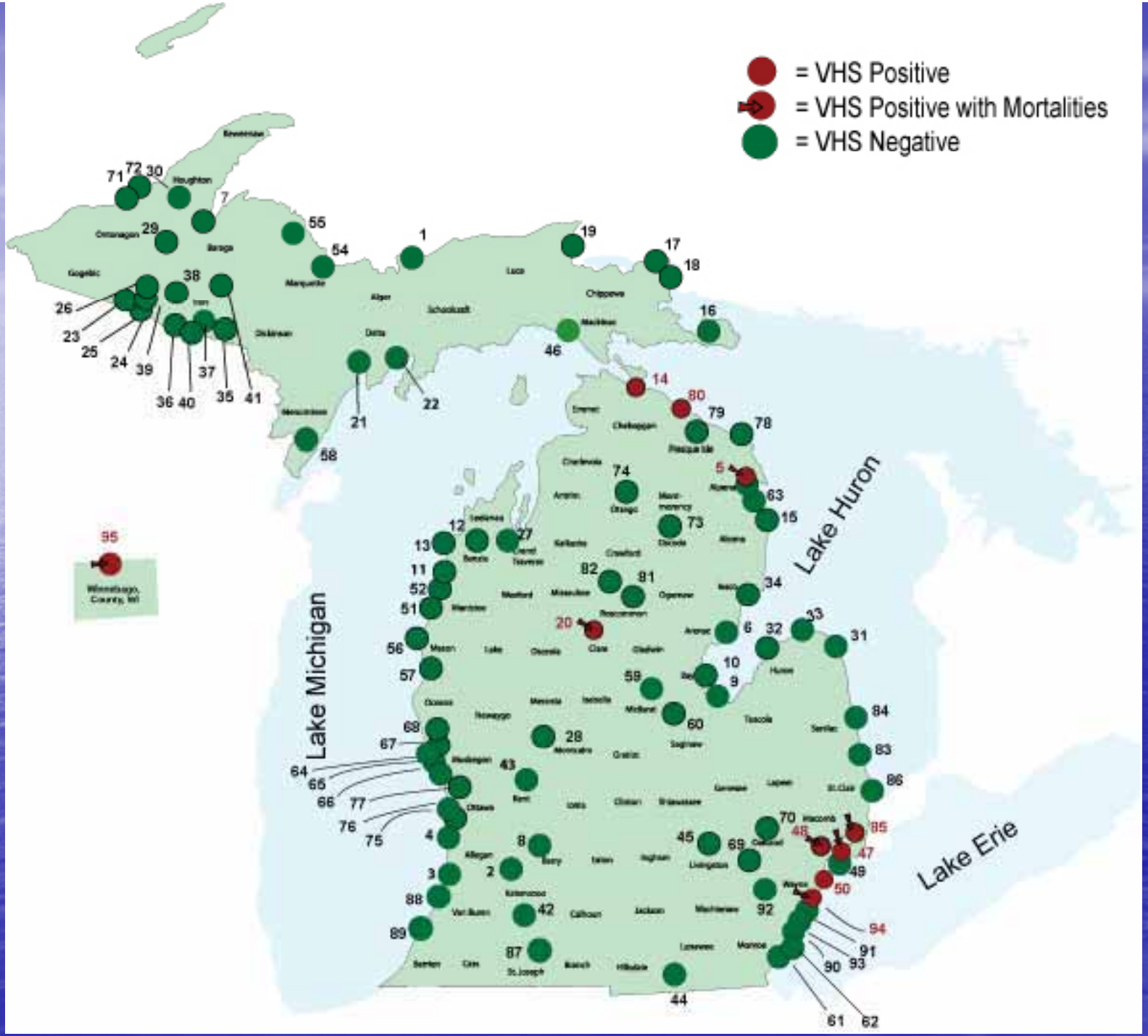
1. All Great Lakes VHSV isolates (2003-2006) are within 3 nt of each other (669 nt G/N), and 67 nt (whole genome).

2. The very low diversity among the isolates suggests a recent introduction.

3. All GL VHSV outbreaks in 2005/06 are one large epizootic involving many species of fish in several lakes.

4. GL fish populations are naïve to this emerging pathogen.


Budd Lake
Wilson State Park
Clare County
Latitude: 44.02889
Longitude: -84.80444
McWATTY LAKE
WILSON STATE PARK
LITTLE LONG LAKE
BUDD LAKE


= VHS Positive
= VHS Positive with Mortalities
= VHS Negative
Lake Michigan
Lake Huron
Lake Erie
Houghton
Ontonagon
Baraga
Marquette
Alger
Schoolcraft
Luce
Chippewa
Mackinac
Delta
Menominee
Gogebic
Iron
Dickinson
Winnebago County, WI

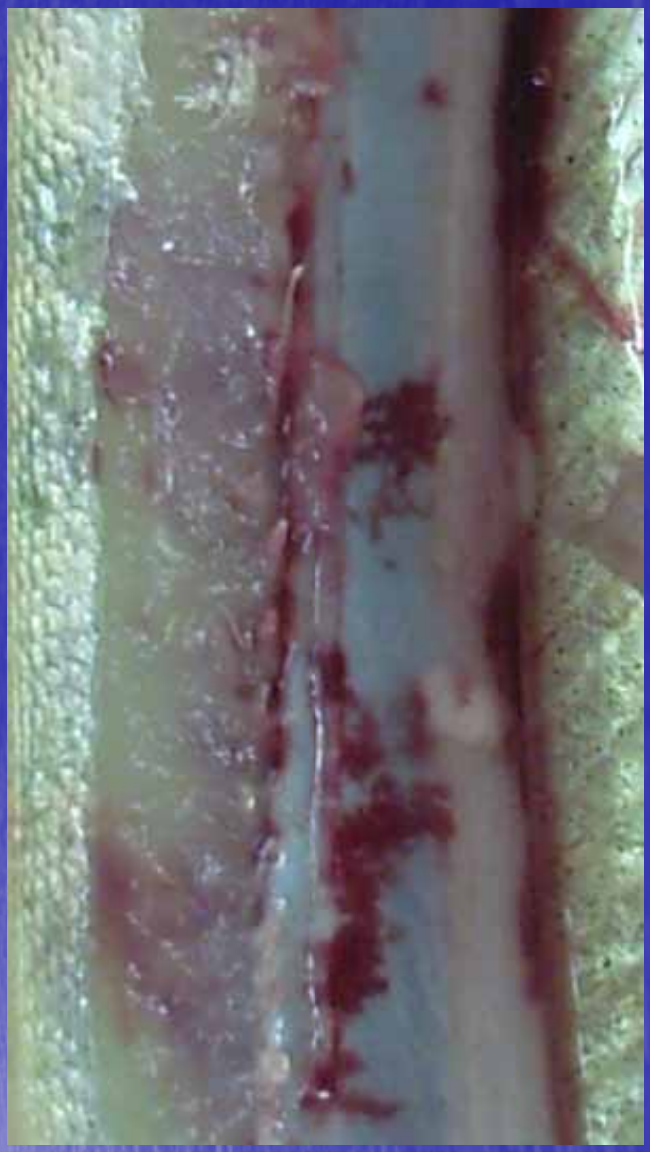
5. VHSV IVb seems to spotty in distribution and its spread westwards is relatively slow.

6. Mortalities were, in most cases, the first signal for the virus presence

6. VHS Ivb Morts

- Large (7)
  - Gizzard Shad,
  - Round gobies
  - Black crappie
  - Bluegills
  - Yellow perch
  - Freshwater drum
  - Muskies
- Small (8)
  - Carp
  - Pumpkinseed sunfish
  - Rock bass
  - Largemouth bass
  - Smallmouth bass
  - Lake whitefish
  - Brown trout
  - Rainbow trout (steelhead)
- Detected (10)
  - Emerald shiner
  - Spottail shiner
  - White suckers
  - Redhorse sp. (2)
  - Walleye
  - Lake trout
  - Burbot
  - Chinook salmon
  - Channel catfish

- 7. Koch's Postulates are fulfilled under standardized experimental conditions



8. Histopathology and electron microscopy demonstrated that the major lesion caused by VHSV IVb is in the endothelial lining of blood vessels.

9. Great Lakes fish species differ in their susceptibility to VHSV IVb

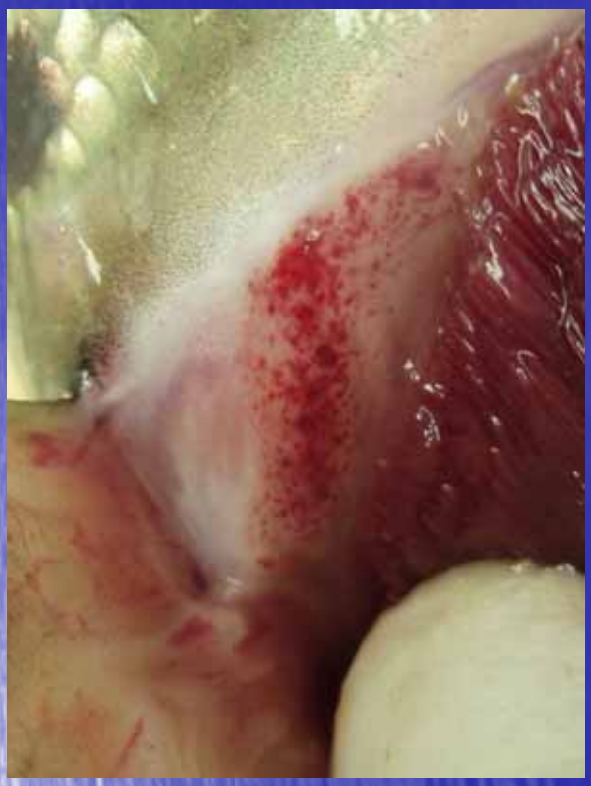
LD_{50} Data – juvenile Muskellunge

$LD_{50} = 10^{-8.5}$ $TCID_{50}$
= 2.21 plaque forming units

Time to death = 5-8 days IP
= 3 days by water challenge

6. VHS IVb Morts

- Large (7)
  - Gizzard Shad,
  - Round gobies
  - Black crappie
  - Bluegills
  - Yellow perch
  - Freshwater drum
  - **Muskies**
- Small (8)
  - Carp
  - Pumpkinseed sunfish
  - Rock bass
  - **Largemouth bass**
  - Smallmouth bass
  - Lake whitefish
  - Brown trout
  - Rainbow trout (steelhead)
- Detected (10)
  - Emerald shiner
  - Spottail shiner
  - White suckers
  - Redhorse sp. (2)
  - Walleye
  - **Lake trout**
  - Burbot
  - Chinook salmon
  - Channel catfish



Largemouth Bass

LD_{50} =1.5 x $10^{-5.5}$ TCID50 = 3.4 x 10^{3} plaque forming units

Time to death = 8-14 days IP

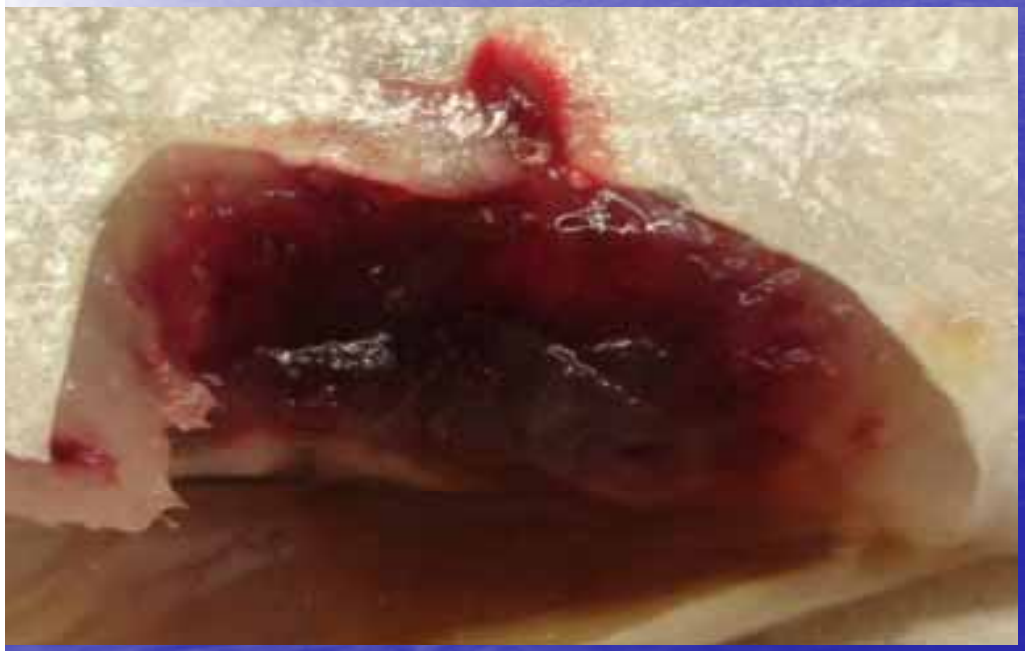
Muskellunge

LD_{50} = $10^{-8.5}$ $TCID_{50}$ = 2.21 plaque forming units

Time to death = 5-8 days IP, 3 days by water challenge

Lake trout

- Two Mortalities



Replication and Morts (all experimental)

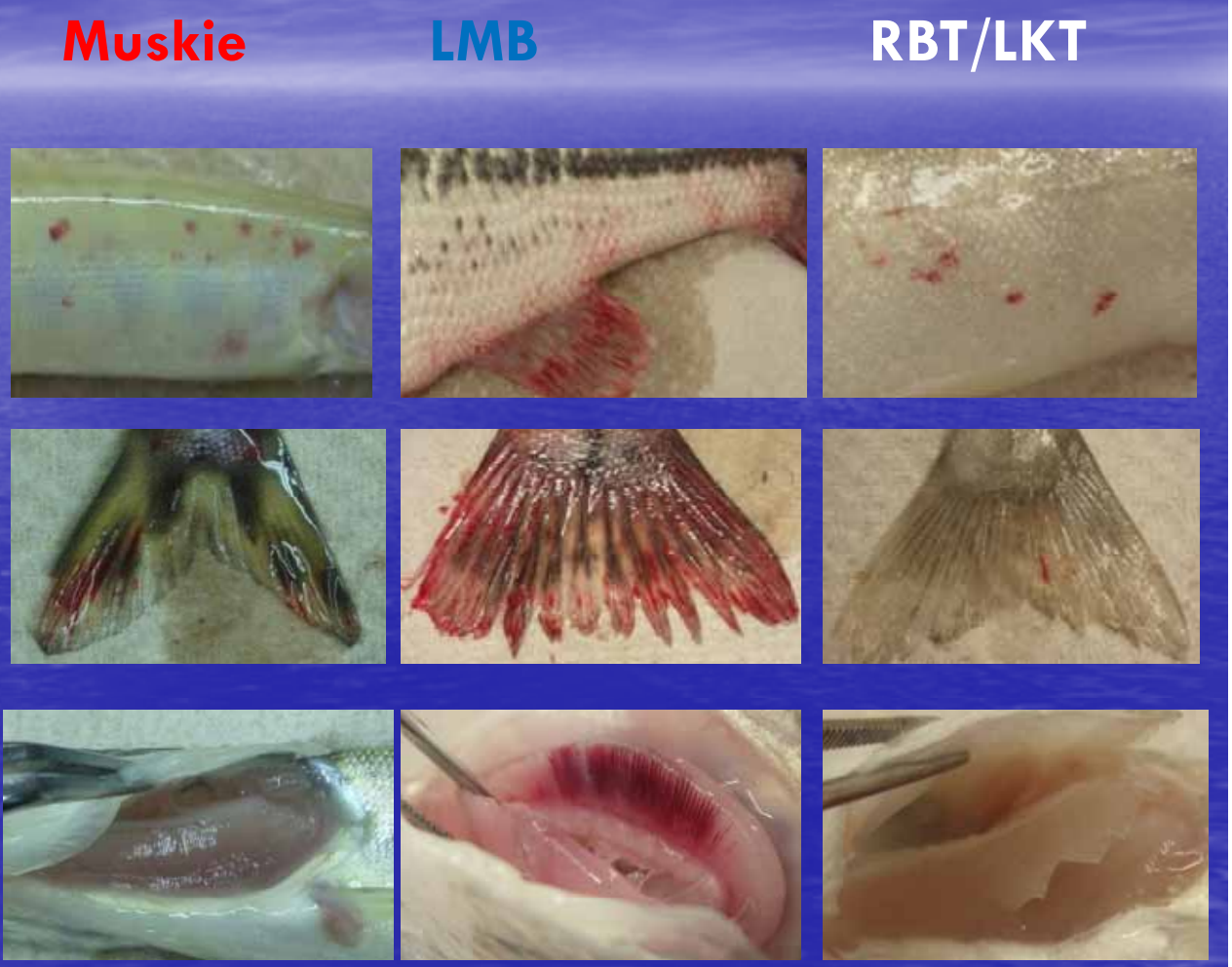
- Muskellunge
- Largemouth bass
- Brook trout
- Brown trout
- Steelheads
- Lake trout
- Coho salmon
- Chinook salmon

10. VHSV Ivb-disease course can run a peracute (mortalities, no signs) and acute form

11. There is virus replication with no disease signs in some species (carrier?)

Similarities in Clinical Signs

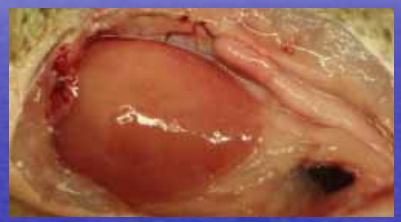
- Similarities
  - Epidermal petechia
  - Fin
  - hemorrhage
  - Pale gills



Similarities in Clinical Signs

- Similarities
  - Enlarged, pale, friable liver

Muskie LMB RBT/LKT


  - Intramuscular hemorrhage

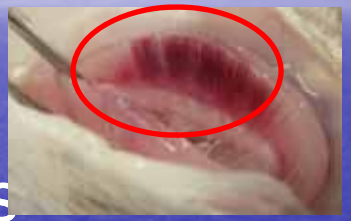


  - Swim bladder serosa hemorrhage



Differences in Clinical Signs

- Differences
  - Congestion and hemorrhage on gills (LMB)
  - Maxillary and mandibular hemorrhage (Muskellunge and LMB)
  - Ascites (LMB)



12. VHSV IVb-disease signs can differ from one species to the other.

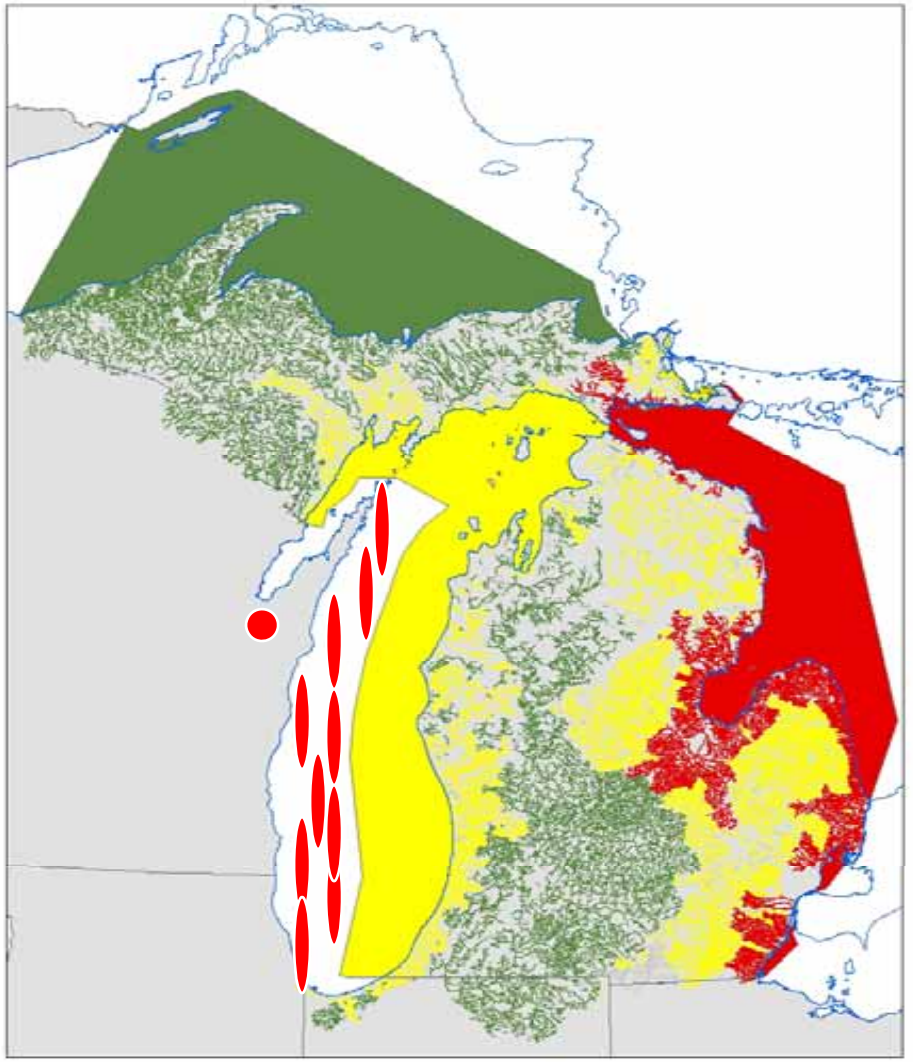
13. VHSV IVb is pathogenic to juvenile fish, yet no massive kills observed

14. Different age groups should be represented in surveillance and monitoring (impact on recruitment unknown)

15. Pathogen trafficking between E&W Lake Michigan is puzzling!!!!

- **VHS Management Areas**

- VHS Positive Area
- VHS Surveillance Area
- VHS Free Area

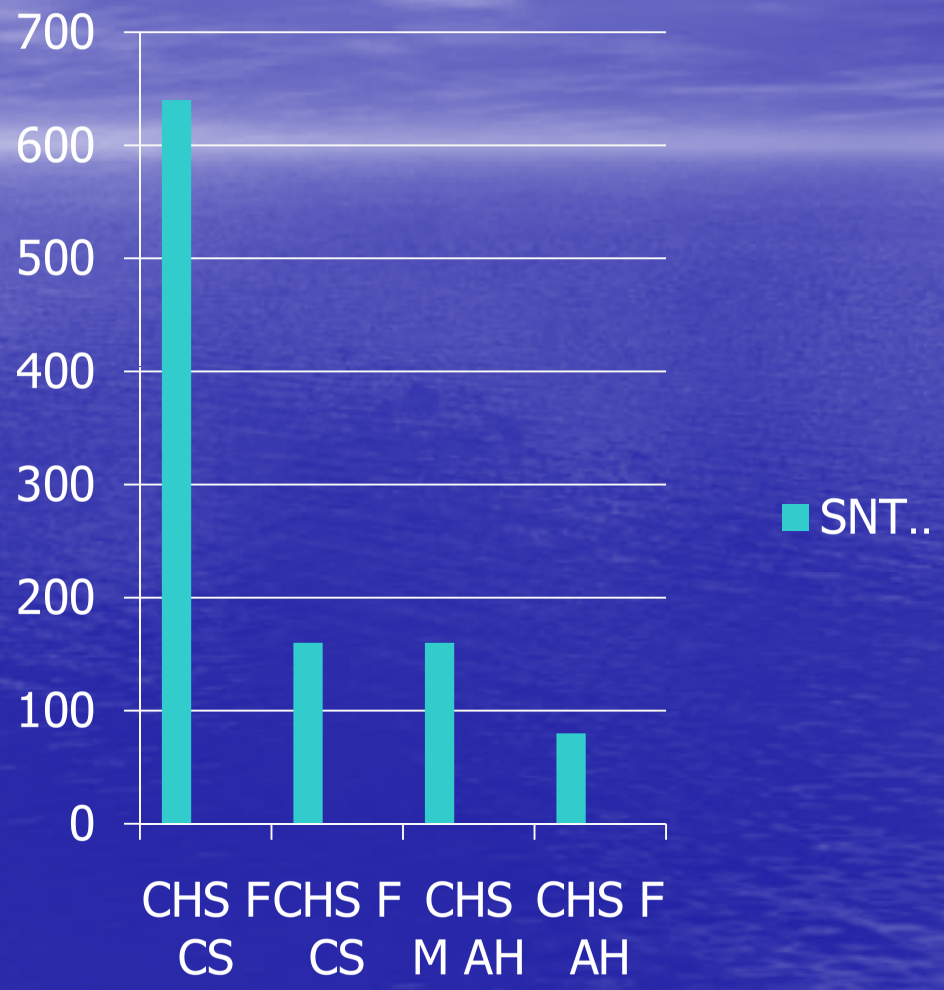


How accurate is our diagnostics

- 16. Two negative passages are not enough

SRW: 1st neg
2nd neg
3rd pos

- 17. Serology testing is needed to determine post-exposure status.



18. Lack of qualified Aquatic Animal Health Professionals and diagnostic laboratories (Pseudo-specialists)

19. Lack of funding for research

20. VHSV IVb is just one of many emerging diseases in the GLB, let's use it's publicity in developing a master plan for disease control strategies

Fish disease ecology in the Great Lakes: overview of GLFC research theme

Stephen C. Riley[1]
Kelly R. Munkittrick[2]
Allison N. Evans[3]
Charles C. Krueger[3]
John M. Dettmers[3]

[1] USGS Great Lakes Science Center, Ann Arbor, MI
[2] University of New Brunswick, St. John, NB
[3] Great Lakes Fishery Commission, Ann Arbor, MI

Research Theme

- GLFC Fishery Research Program
- "Ecosystem Dysfunction and Fish Health"
- 2007-2012
- Theme paper on GLFC website

Riley, S.C., K.R. Munkittrick, A.N. Evans, and C. C. Krueger. In press. Understanding the ecology of disease in Great Lakes fish populations. Aquatic Ecosystem Health and Management.

Goals

- Promote population- and community-level research on disease in Great Lakes fish populations
- Promote investigation of ecological factors associated with fish disease
- Propose conceptual framework

DISEASE

- Threats to fish health which may include communicable diseases, parasites, nutrient deficiencies, and trophically accumulated toxins, but not contaminants.

Central premises

- Health and disease in wild fish populations can be better understood if evaluated in an ecosystem framework
- Functioning healthy ecosystems are more likely to be resilient to disease events than dysfunctional ecosystems

Assumptions

- The biosphere is always changing; these changes will continue to affect Great Lakes aquatic ecosystems.
- Pathogens are natural components of ecosystems and may occur in the absence of disease.
- The prevalence and severity of disease vary temporally and spatially, within which normative conditions can be defined, and are influenced by interactions among hosts, pathogens, and the environment.
- Although diseases may have population-level impacts under some conditions, many pathogens do not exert long-term population-level effects.

Population-level effects of disease

Clearly significant in some cases:

Western gorilla

American Chestnut

Carolina parakeet?

Amphibian declines

Fish diseases of recent concern in the Great Lakes

- Viral Haemorrhagic Septicemia (VHS)
- Botulism
- Thiamine Deficiency Complex (TDC)
- Bacterial Kidney Disease (BKD)

Incidence of disease increasing?

- Some evidence from marine systems
- Botulism, TDC?
- No baseline data in Great Lakes

Anthropogenic impacts?

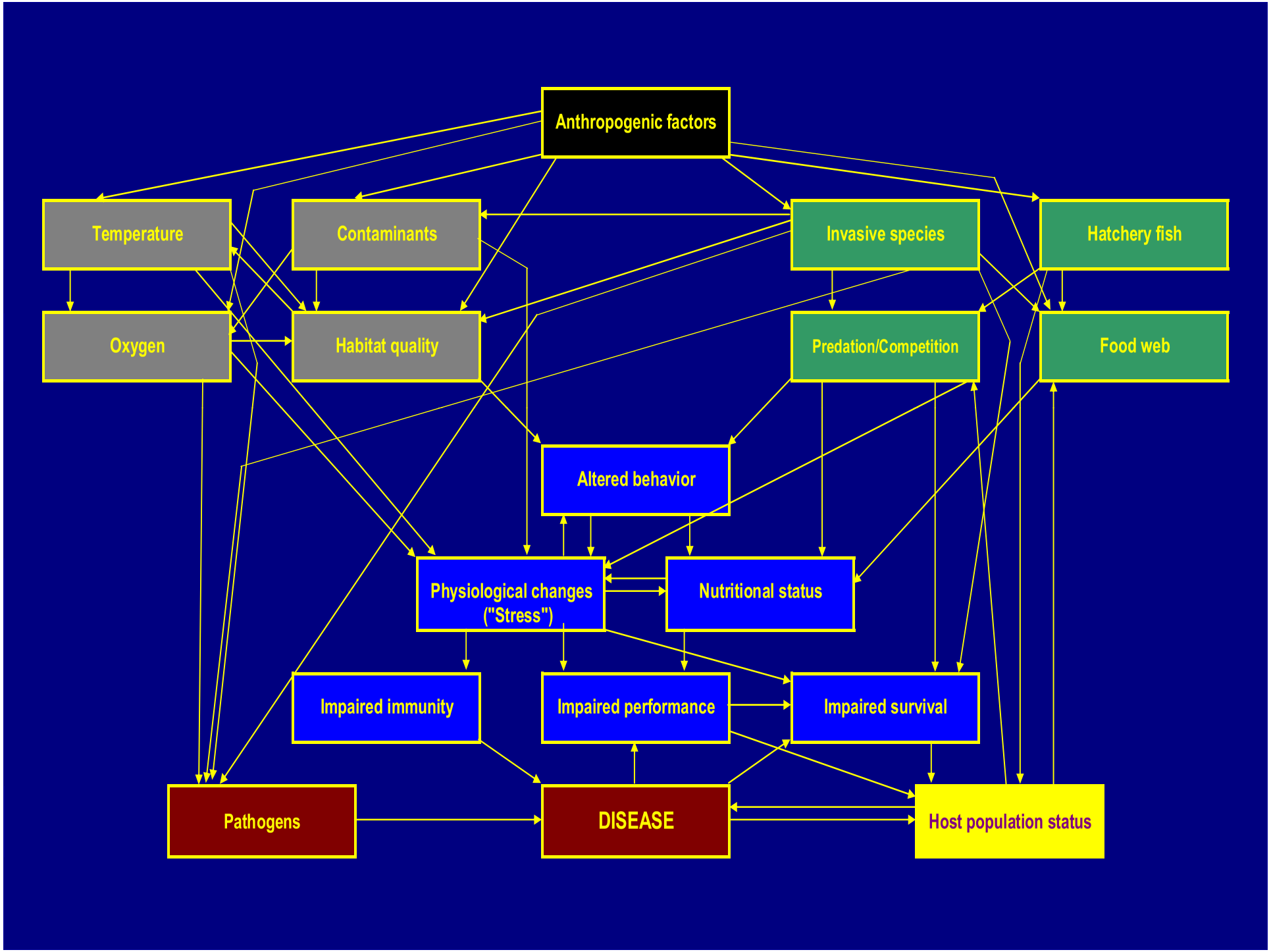
- Climate change
- Invasive species
- Introduced pathogens
- Fishery management

Effects of disease

- Very little known about the population-level effects of disease for fish
- What are risks to wild fish and bird populations?

Methods

- Population or community-level variables?
- Epidemiology and disease dynamics poorly studied in fish populations
- Models may be a good place to start


Anthropogenic factors
Temperature
Contaminants
Invasive species
Hatchery fish
Oxygen
Habitat quality
Predation/Competition
Food web
Altered behavior
Physiological changes ("Stress")
Nutritional status
Impaired immunity
Impaired performance
Impaired survival
Pathogens
DISEASE
Host population status

Type-E Botulism

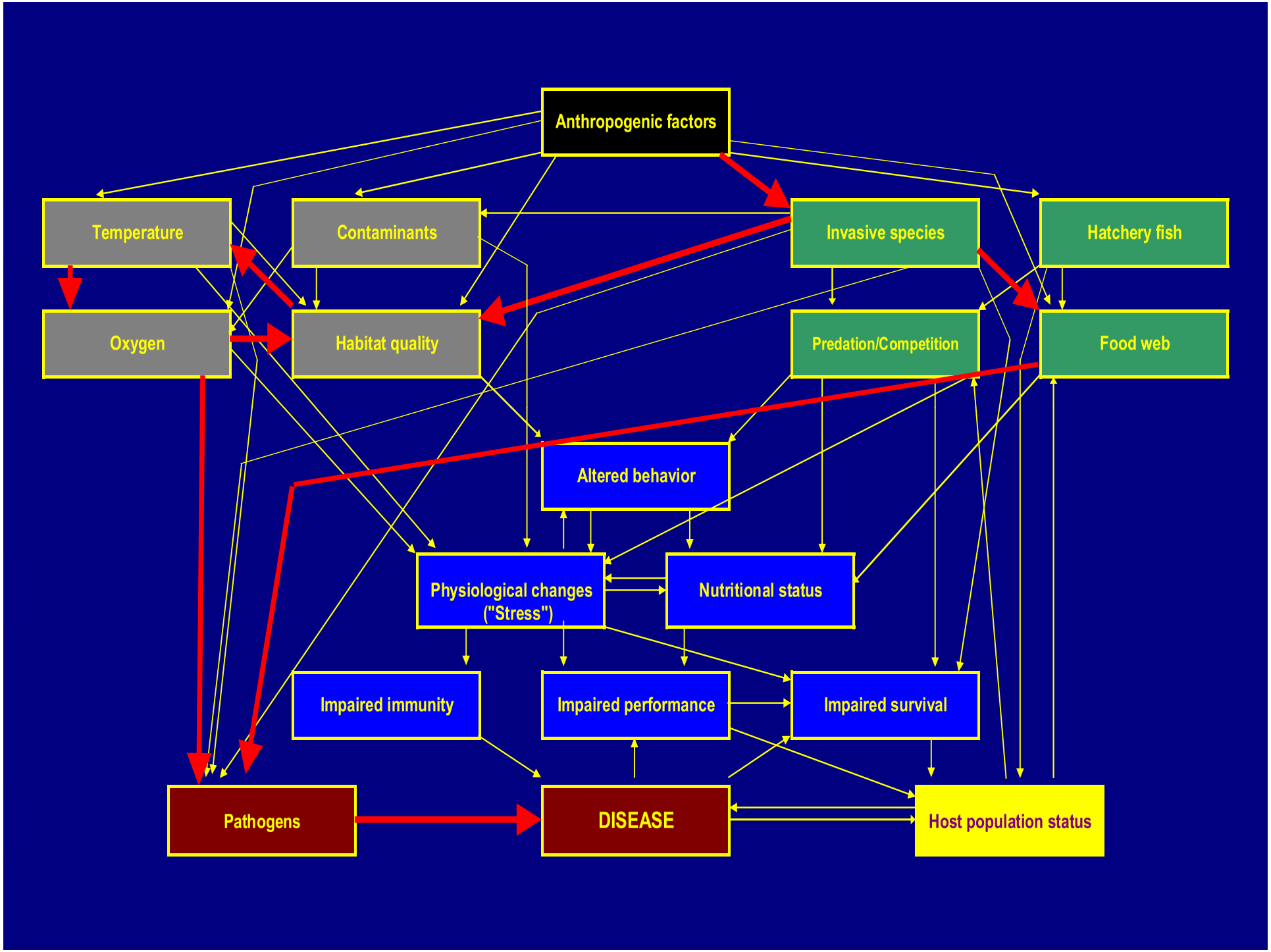
- 50,000+ birds killed in lakes Erie and Ontario alone since 2000 – loons, mergansers, cormorants, grebes, gulls
- Thousands of dead fish, including sturgeon, drum, bass, gobies
- Why has mortality from botulism increased?

chicagotribune.com
Chicago Tribune Web Edition

January 15, 2008

Botulism takes fatal toll on thousands of Great Lakes birds

Botulism and the infamous zebra mussel are blamed for killing birds - from gulls to loons - by the thousands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DISEASE
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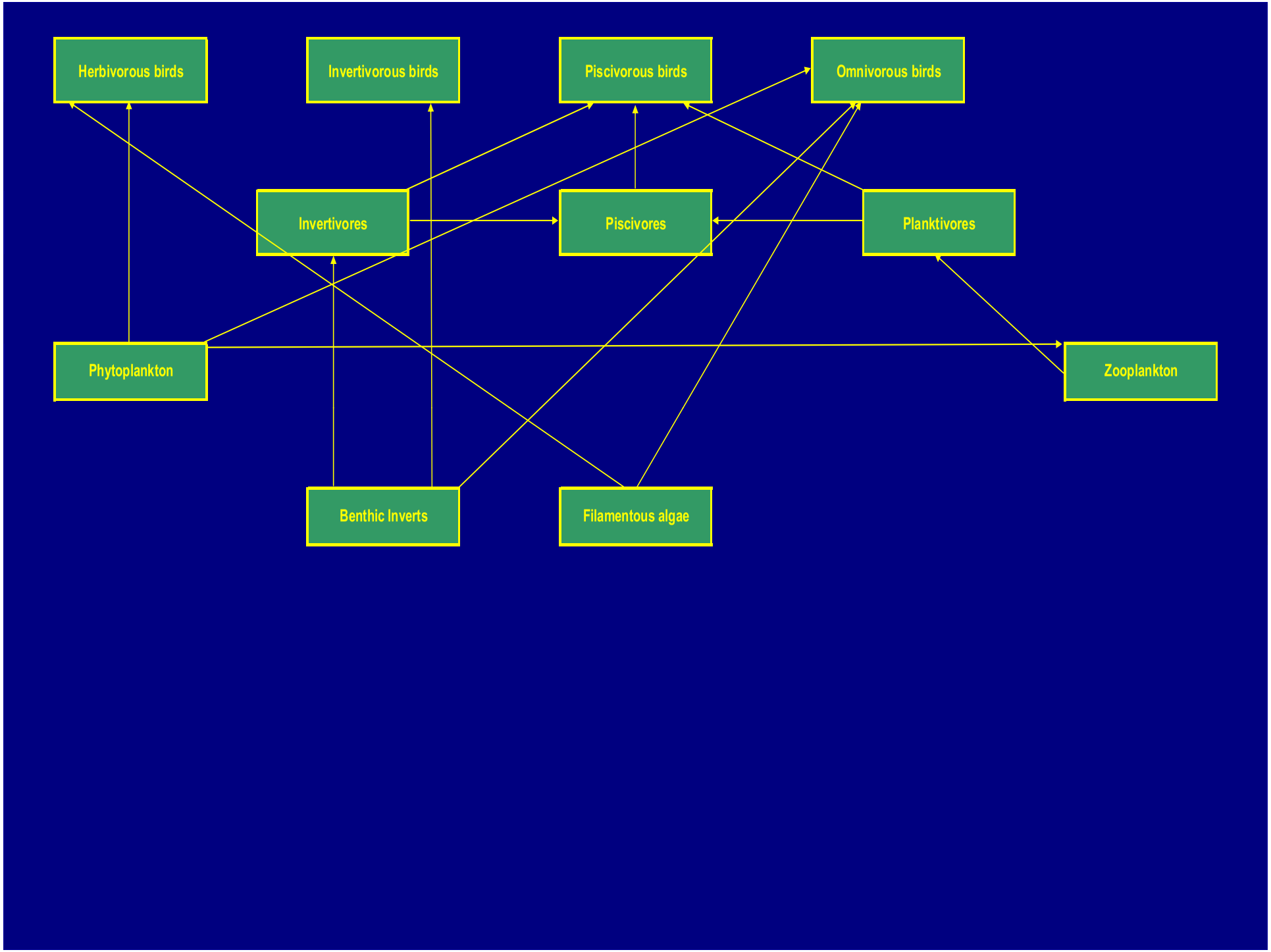
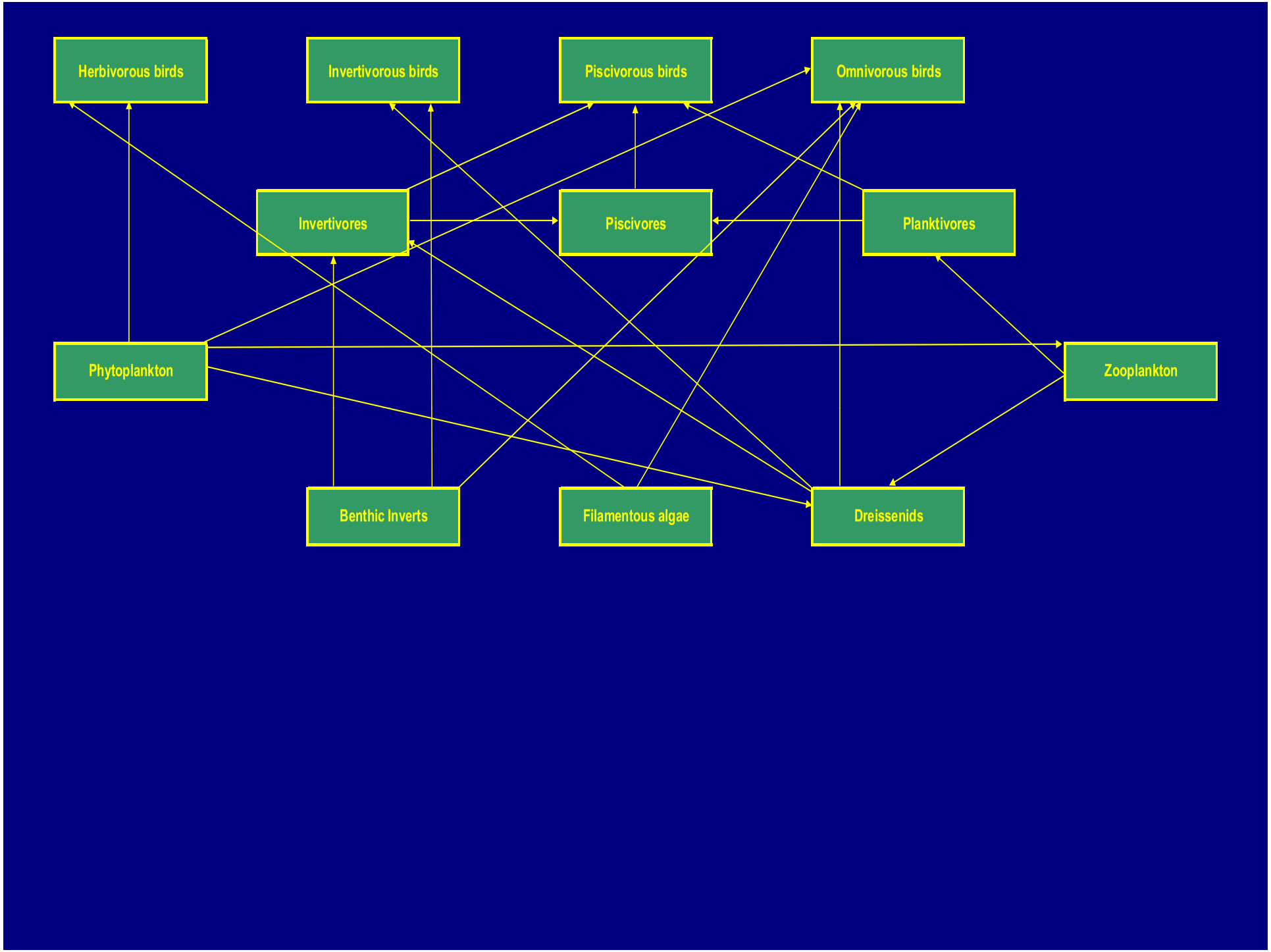

Herbivorous birds
Invertivorous birds
Piscivorous birds
Omnivorous birds
Invertivores
Piscivores
Planktivores
Phytoplankton
Zooplankton
Benthic Inverts
Filamentous algae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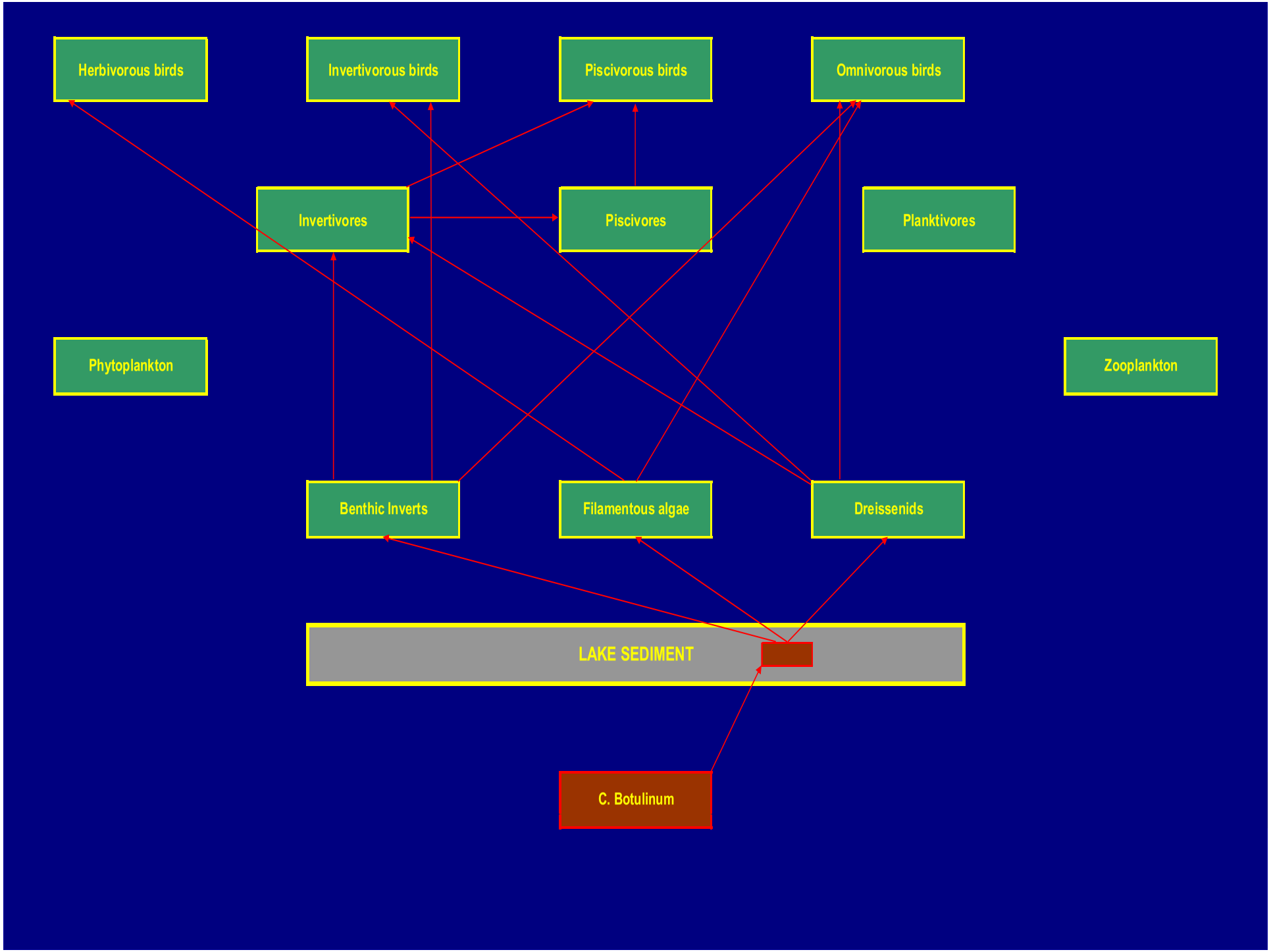
Zooplankton
Benthic Inverts
Filamentous algae
Dreissenids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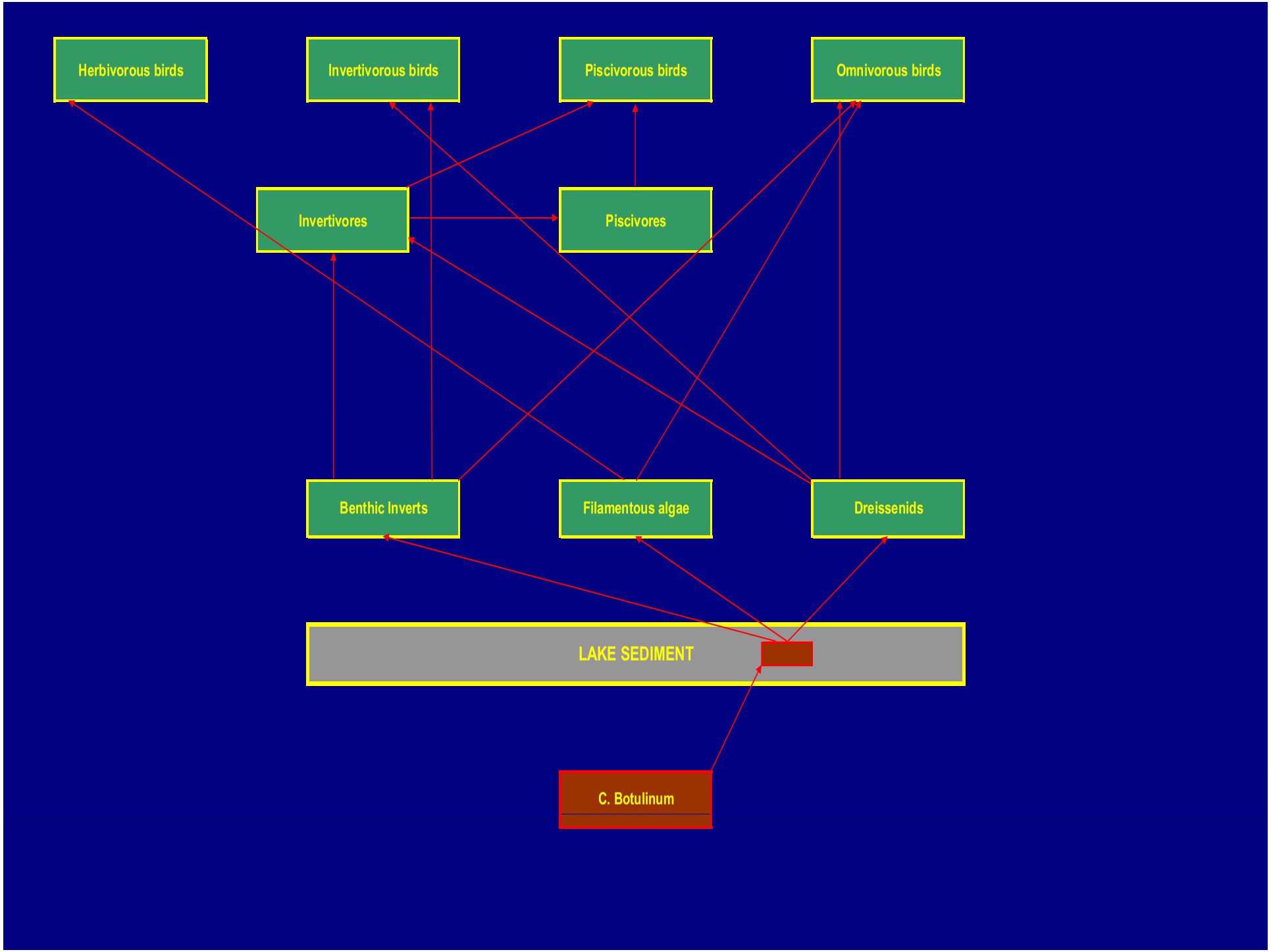
Filamentous algae
Dreissenids
LAKE SEDIMENT
C. Botulinum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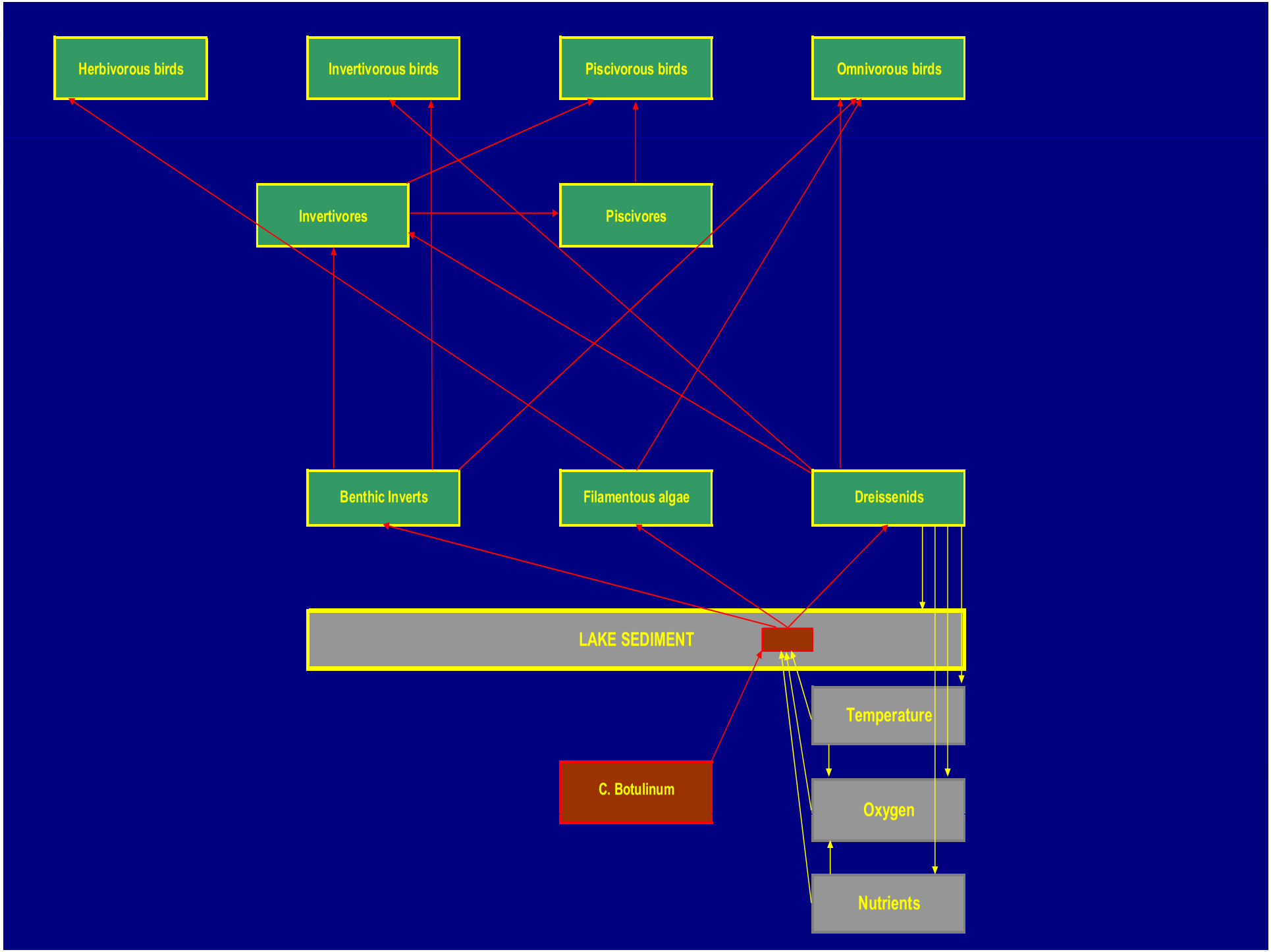
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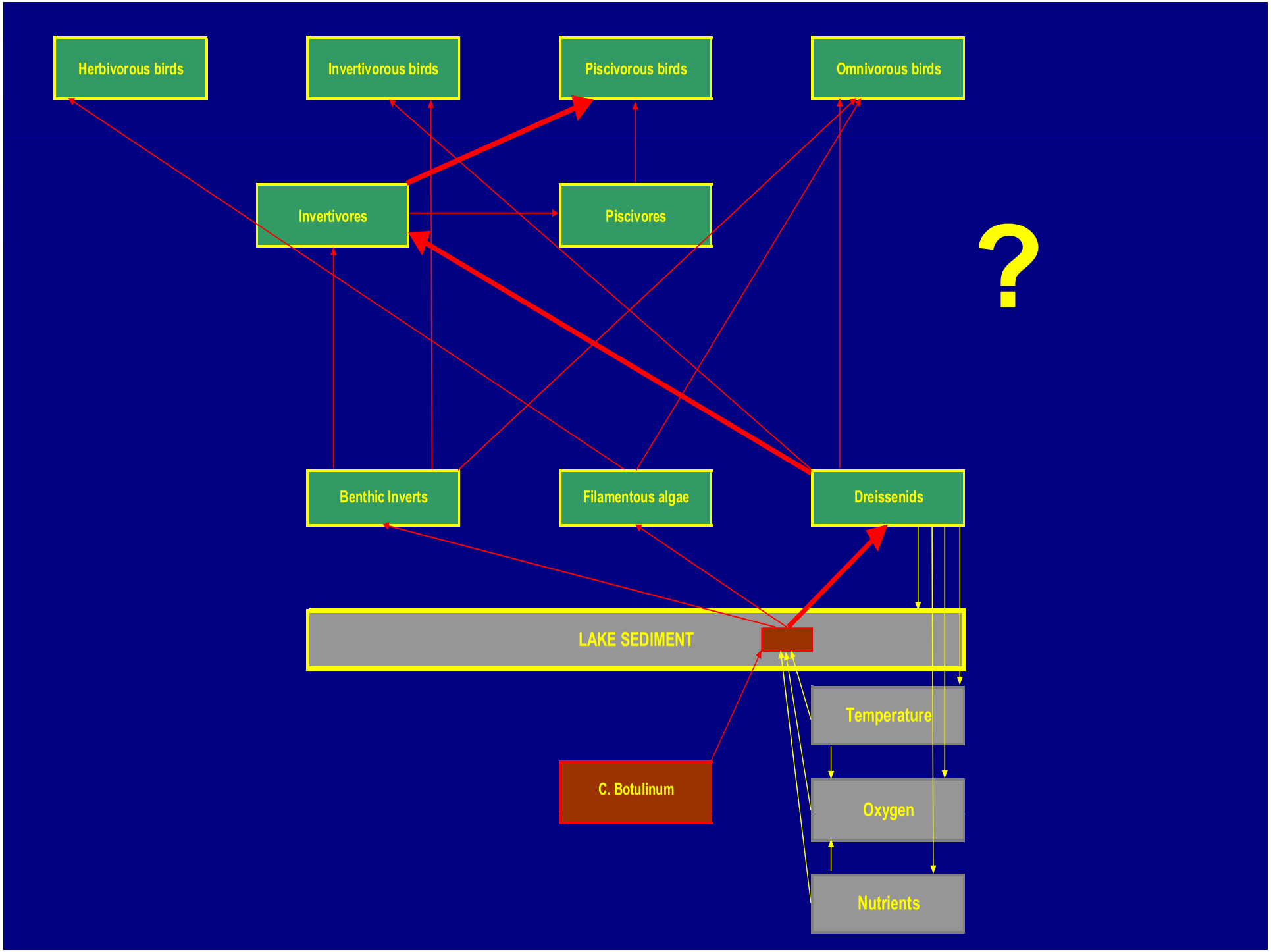
C. Botulinum
Temperature
Oxygen
Nutrients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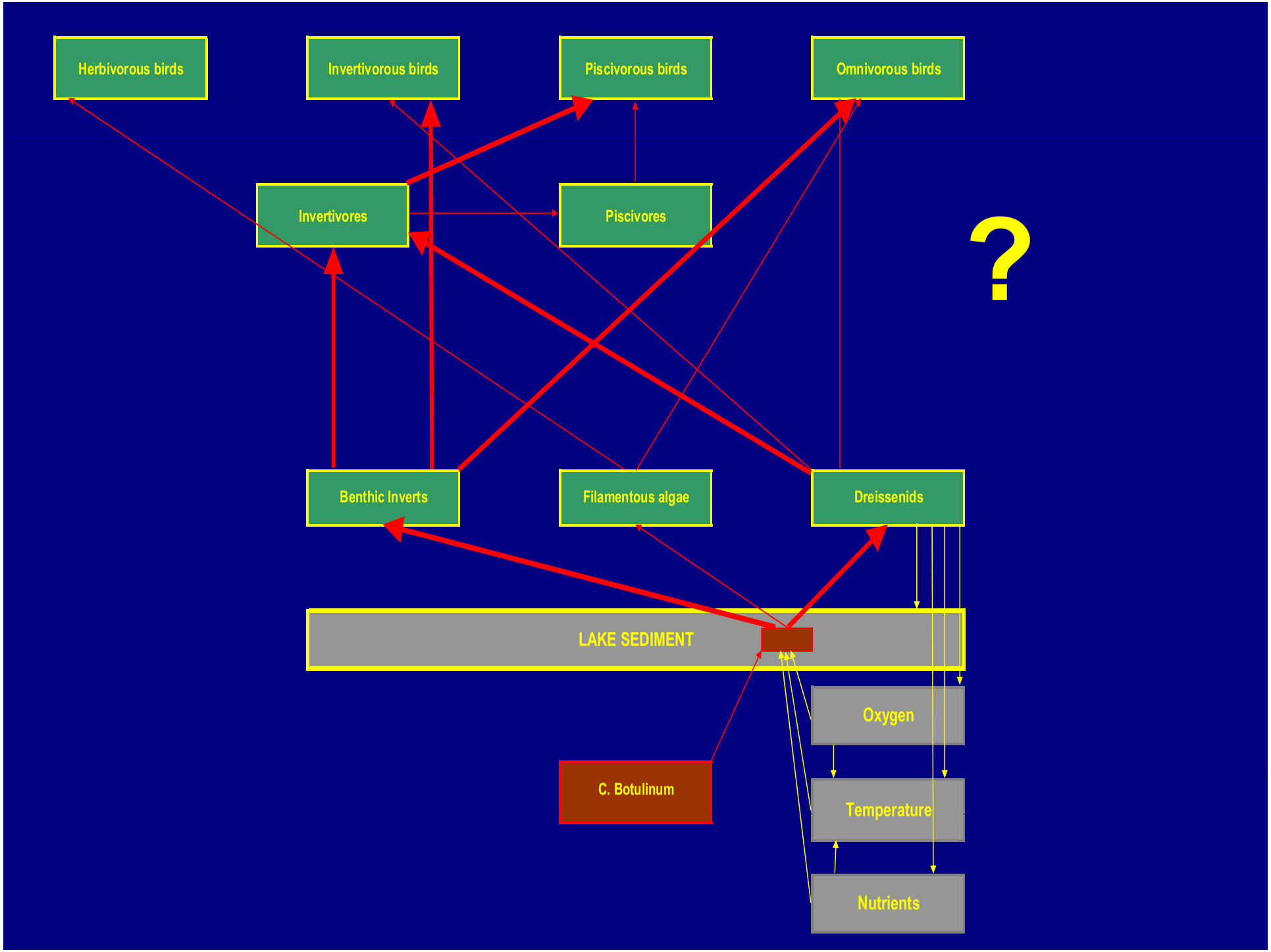
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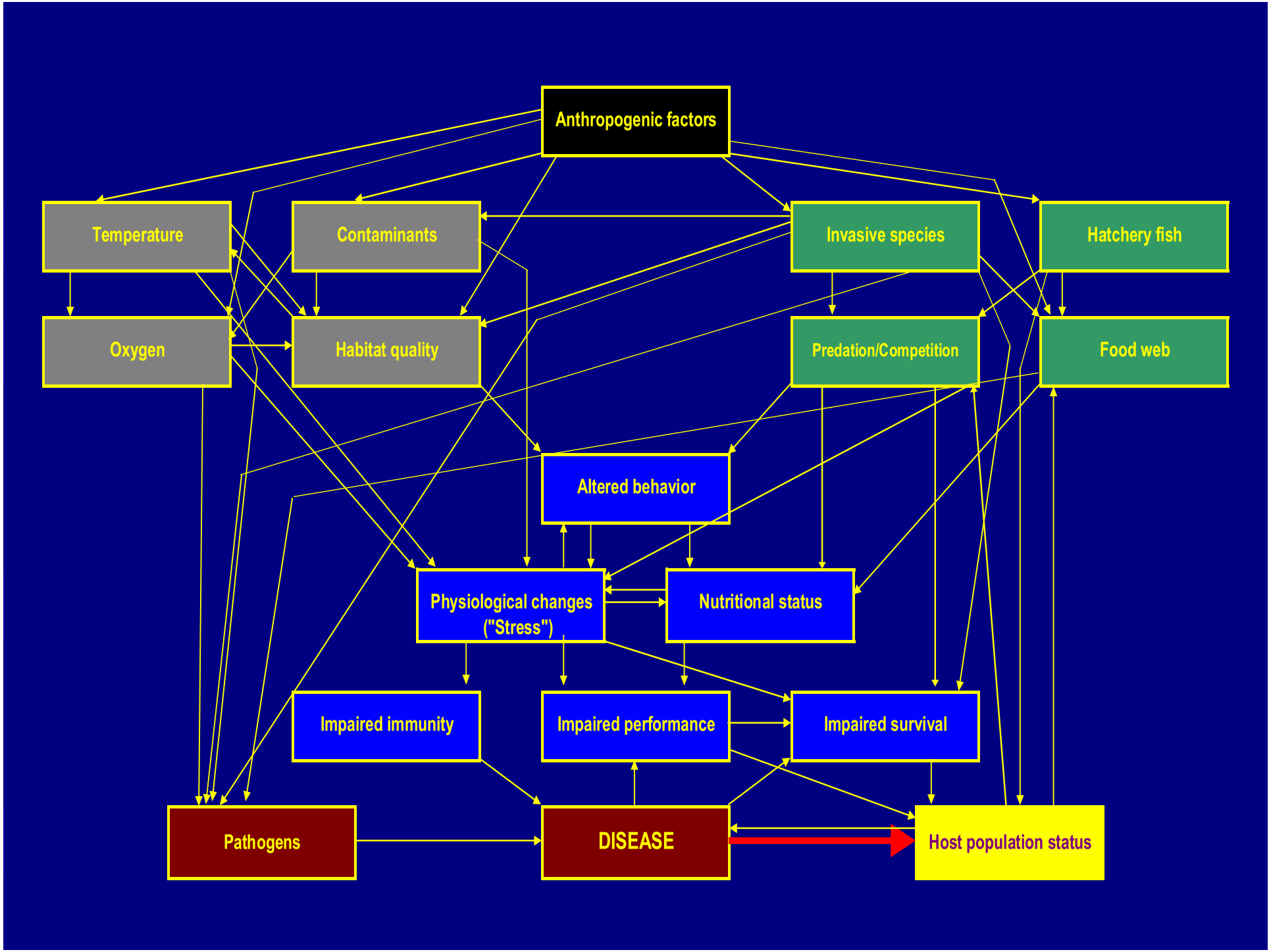
VHS

- May cause mortality in freshwater drum, muskellunge, round gobies, gizzard shad, black crappie, bluegill, white bass, common carp, yellow perch, lake whitefish, walleye, smallmouth bass, rock bass, burbot.
- Mortality greater at lower water temperature.
- Survivors may become carriers.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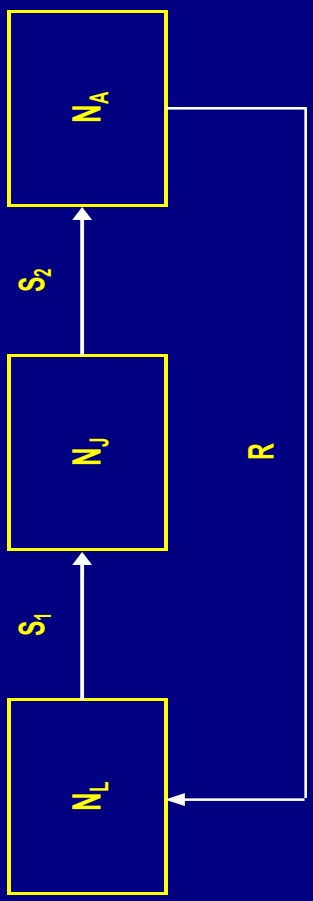
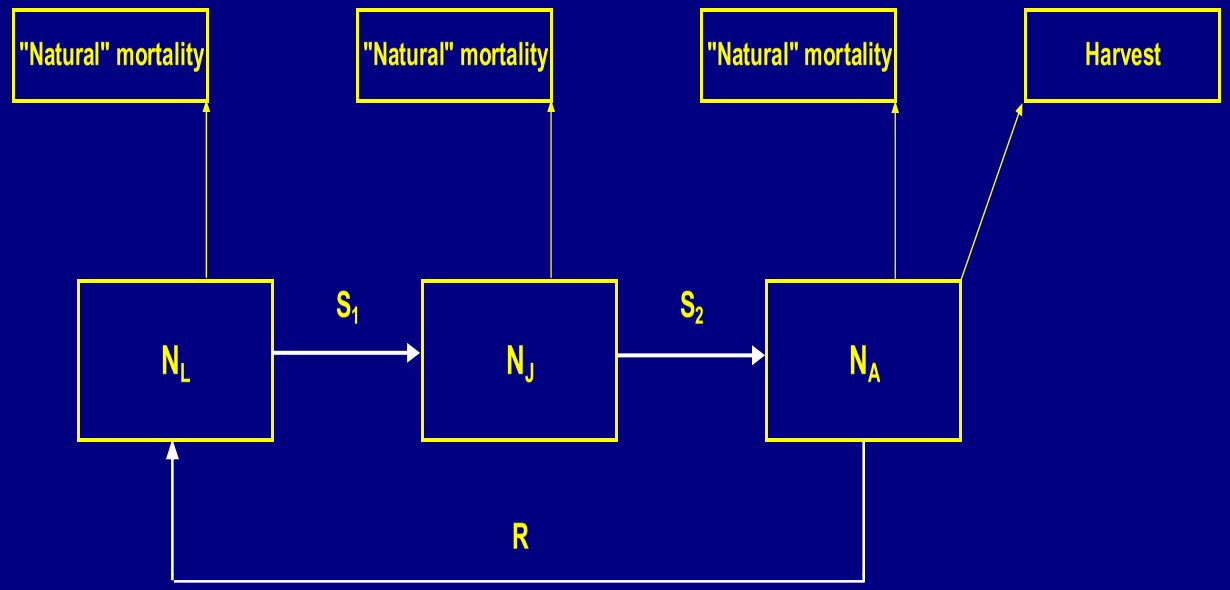
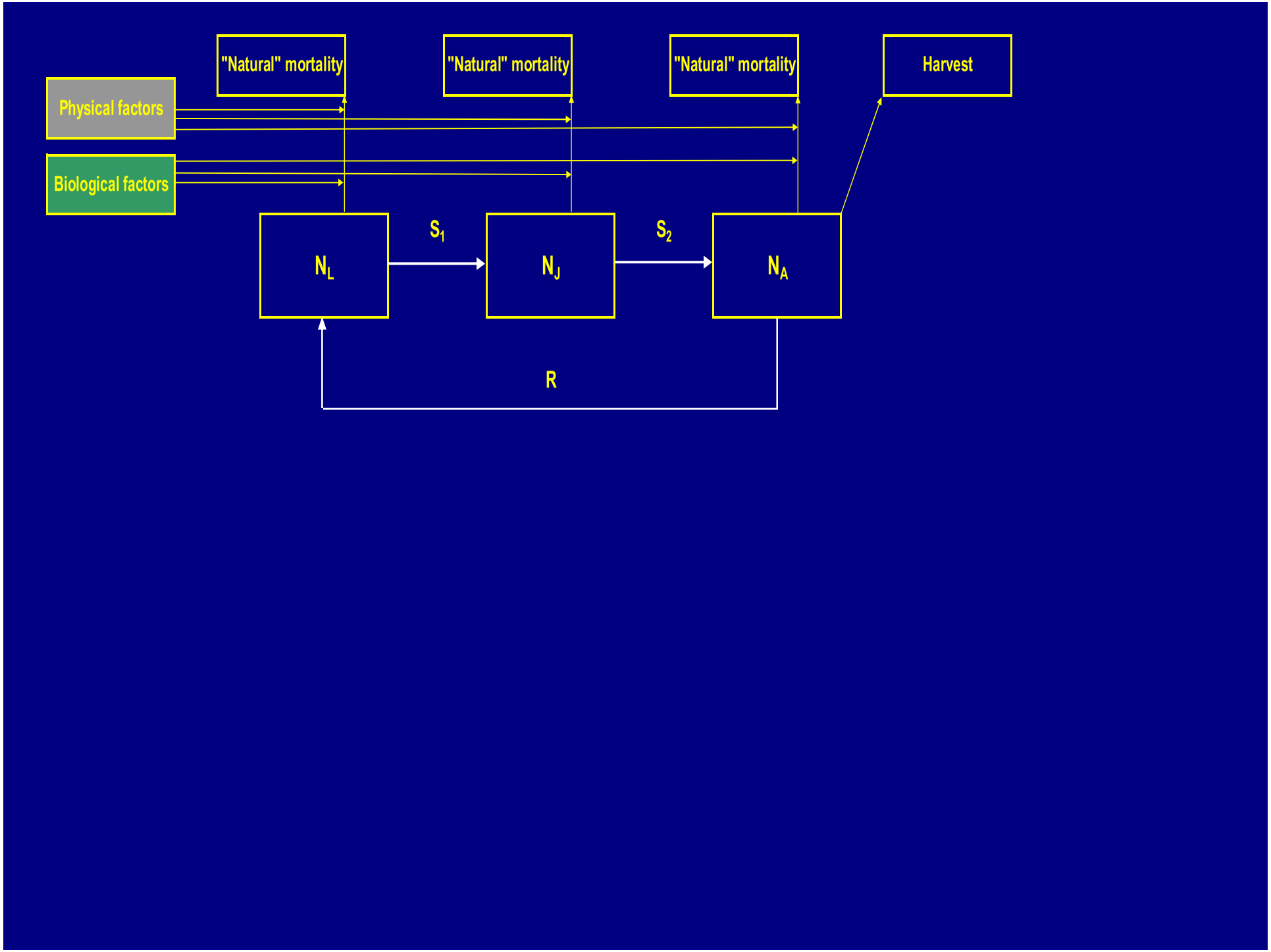
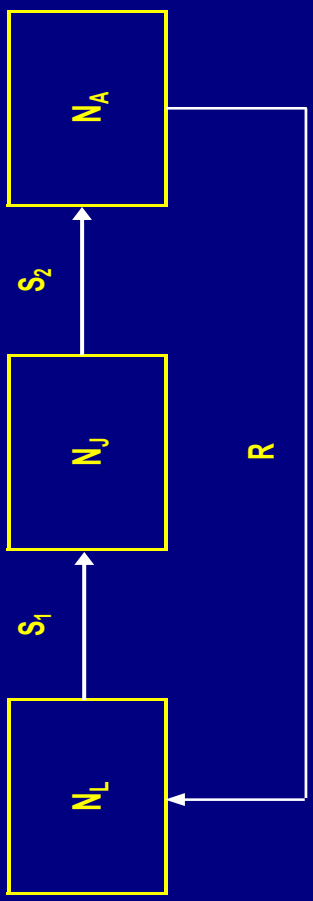
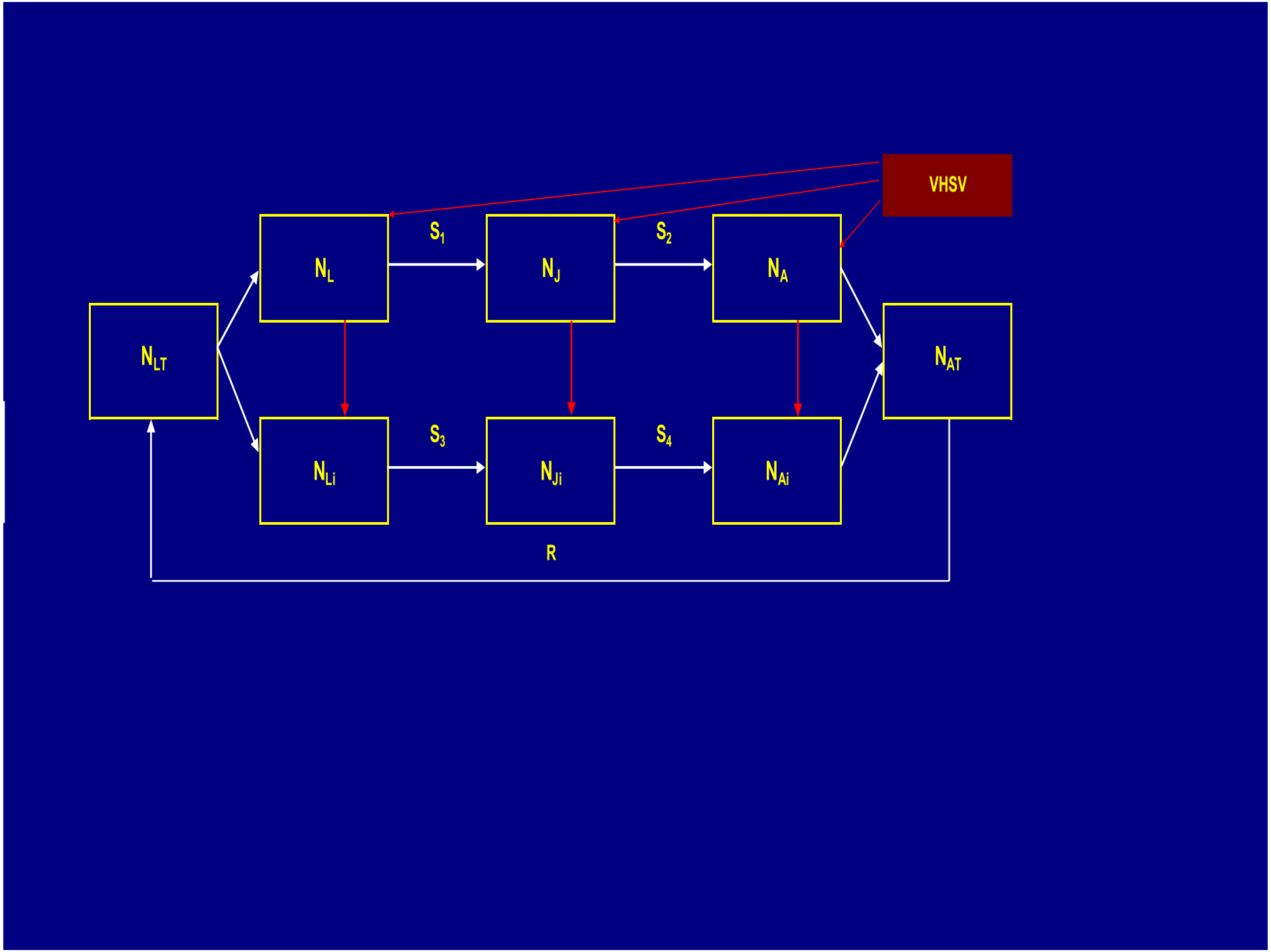
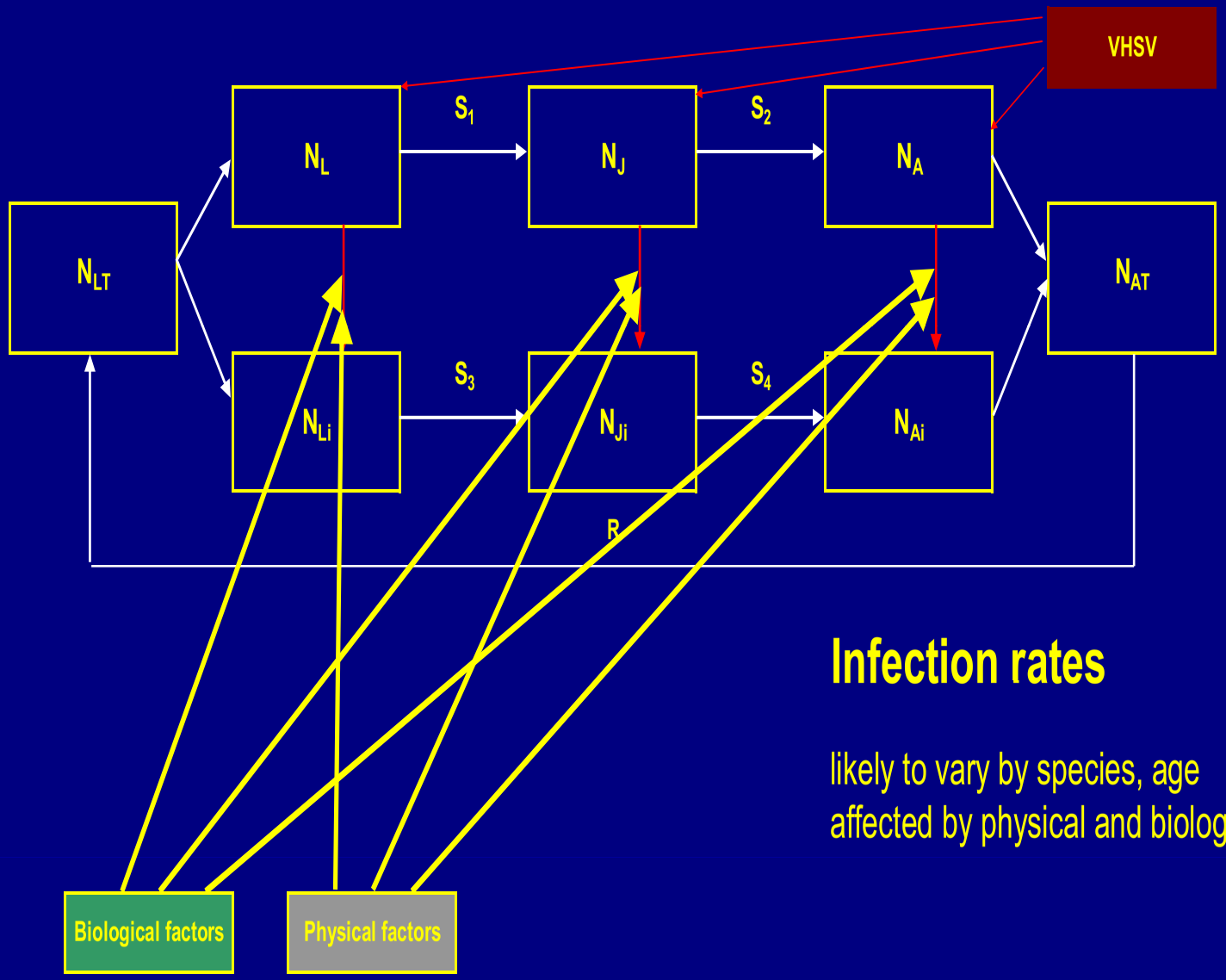

N_A
S_2
N_J
R
S_1
N_L


"Natural" mortality
"Natural" mortality
"Natural" mortality
Harvest
N_L
S_1
N_J
S_2
N_A
R


"Natural" mortality
"Natural" mortality
"Natural" mortality
Harvest
Physical factors
Biological factors
N_L
S_1
N_J
S_2
N_A
R

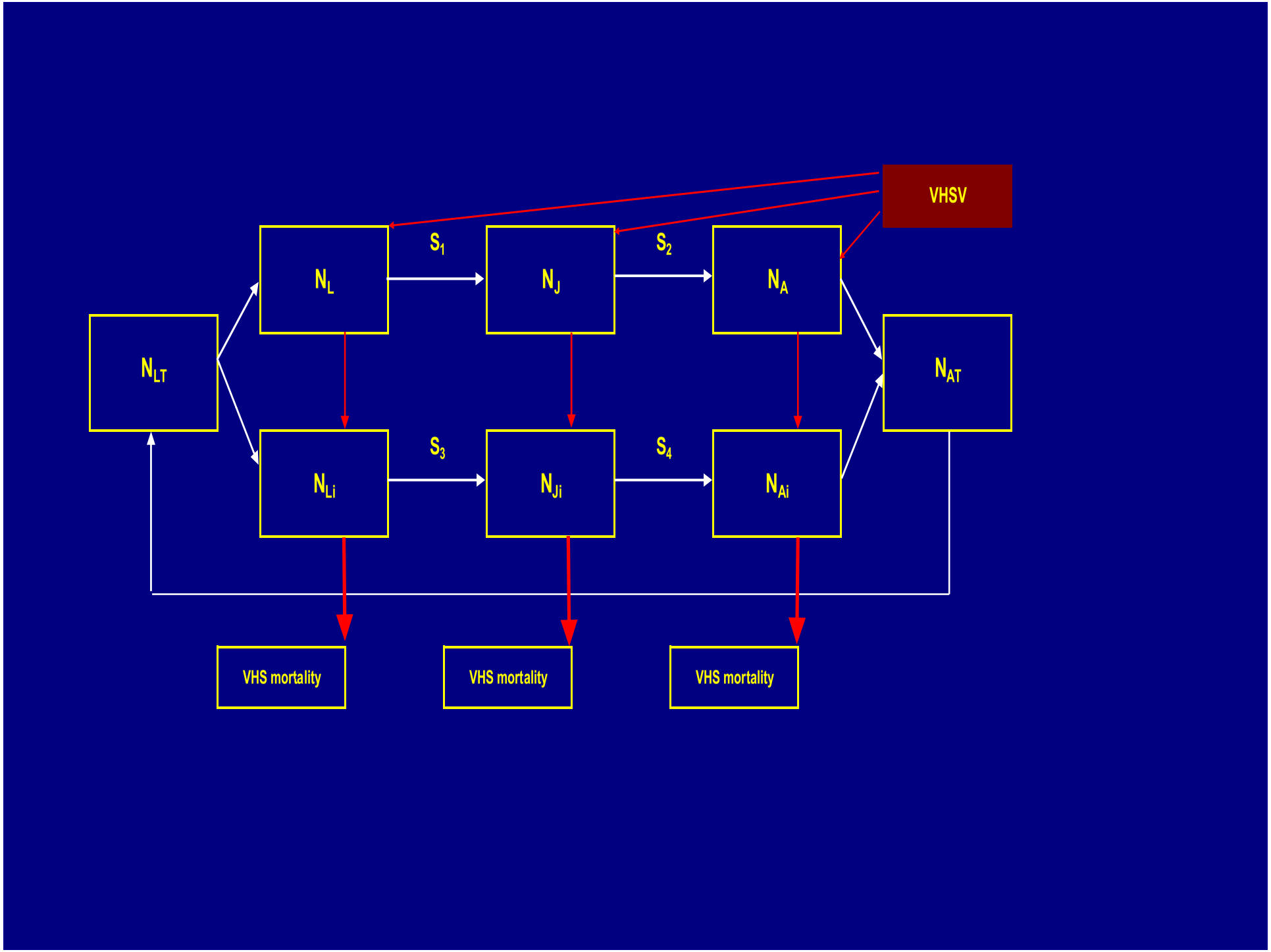

N_A
S_2
N_J
R
S_1
N_L


VHSV
N_L
S_1
N_J
S_2
N_A
N_{LT}
N_{AT}
N_{Li}
S_3
N_{Ji}
S_4
N_{Ai}
R



Infection rates

likely to vary by species, age
affected by physical and biological variables


VHSV
N_L
S_1
N_J
S_2
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N_{AT}
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S_4
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VHS mortality
VHS mortality
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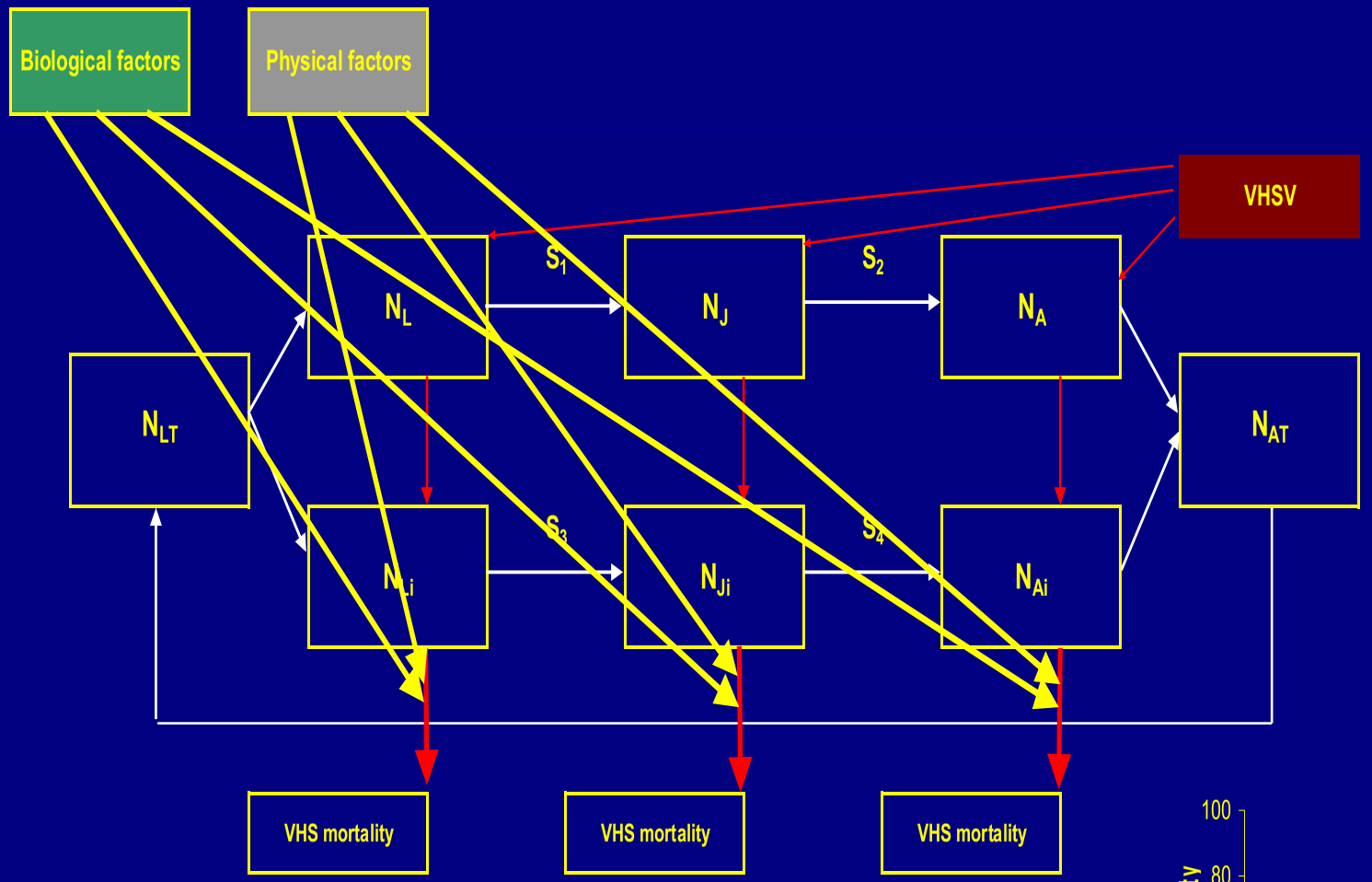
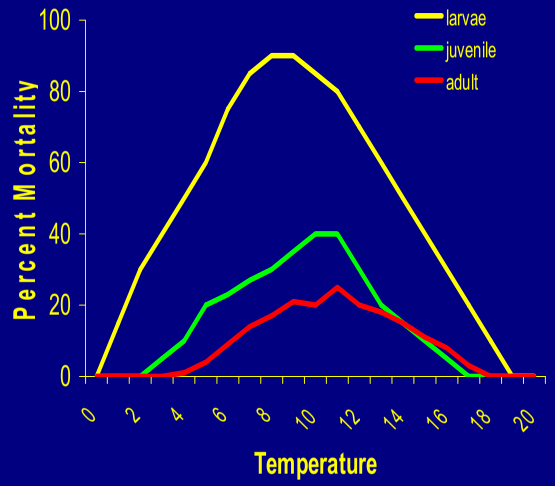


?

Mortality rates

also likely to vary by species, age
affected by physical variables (e.g., temperature)
affected by biological variables (population density)
what proportion of natural mortality? Compensatory?



Summary

- Complex ecological mechanisms may govern the effects of disease on wild fish populations
- GLFC research theme promotes research to address these questions
- Conceptual models may help focus research

Research Proposals

- Preproposals due in January of each year
- www.glfc.org

Workshop

- 5 – 7 November 2008, Ann Arbor
- Focus on building conceptual models
- TDC research summaries