GREAT LAKES FISH HEALTH COMMITTEE

2006 Annual Meeting
Madison, Wisconsin
February 21-23, 2006

Minutes
(with attachments)

Submitted By:
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New York State Department of Environmental Conservation

GREAT LAKES FISHERY COMMISSION
2100 Commonwealth Blvd
Ann Arbor, Michigan 48105-1563
Great Lakes Fish Health Committee

2006 Annual Meeting

Madison, WI
February 21-23, 2006

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List of Attendees

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Great Lakes Fish Health Committee
Annual Meeting Agenda
Madison, WI
February 21-23, 2006

Tuesday, February 21, 2006

I. Introduction and meeting information

9:00 AM A. Welcome – *Whelan*

9:15 AM B. Meeting Logistics – *Marcquenski*

9:30 AM C. Attendee Introductions – *Whelan*

9:45 AM D. Committee Business Practices – *Whelan*

II. Research planning

10:00 AM A. Research Objective Development – *Dettmers* (Info.)

10:15 BREAK

10:45 AM B. Revision of GLFHC Research Priorities – *Whelan* (Action)

11:15 AM C. Fish Health Research Pre-proposal Ranking – *Whelan* (Action)

11:45 AM LUNCH

1:00 PM III. Model Program – *Marcquenski* (Action)

2:00 PM IV. Model Program: Coolwater Plan Implementation – *Marcino* (Action/Discuss)

3:00 PM BREAK

3:30 PM V. Fish Hatchery Classification – *Miller-Dodd* (Action/Discuss)

VI. Research Updates

4:00 PM A. BKD Research Update – *Elliott* (Info.)

5:00 PM ADJOURN

Wednesday, February 22, 2006

VII. Research Updates

8:00 AM B. Thiamine Deficiency Research Update – *Honeyfield* (Info.)

8:30 AM C. IPNV Research Update – *McAllister* (Info.)

9:00 AM D. Heterosporis Research Update – *Sutherland* (Info.)

9:30 AM E. Coldwater Disease Bacteria Agar – *Starliper* (Info.)

10:00 AM BREAK

VIII. VHS Updates

10:30 AM A. Lake Ontario – *Miller-Dodd* (Info.)

10:45 AM B. Lake St. Clair –*Whelan/Faisal* (Info.)

IX. Information Items Continued

11:00 AM Lakes Huron and Michigan Food Web Changes – John Dettmer (info)

11:30 AM LUNCH

X. National Planning Updates

1:00 PM F. National Aquatic Animal Health Plan Update – *Amos* (info.)

2:00 PM G. Canadian National Aquatic Animal Health Plan – *Penney* (info.)

3:00 PM ADJOURN

Thursday, February 23, 2006

8:00 AM Non-lethal sampling workshop

Minutes From The Great Lakes Fish Health Committee Meeting
February 21-23, 2006
Madison, Wisconsin

Introduction and meeting information

Welcome (Whelan): Opening remarks highlighted the need for the committee to improve and become more effective.

Meeting Logistics (Marcquenski)

Attendee Introductions (Whelan)

Committee Business Practices (Whelan): Committee chair discussed the lack of interaction and cohesiveness between the GLFHC and other technical committees. Discussion followed and many members described that two meetings would not be possible for a variety of reasons. Much discussion centered on the fact that agency health specialists and administrators deal with agency issues in and out of basin and lack the time, whereas lakes committee biologists deal mostly with issues in the basin. Whelan suggested two face-to-face meetings a year plus conference calls in May and October. From now on, all agenda items should have an issue statement submitted in advance. A single position (opinion) per agency must be taken for GLFHC issues even though agencies may have multiple members and will stick to our time line. Members suggested that travel funding was limited and the lack of cohesiveness with commission is not the result of limited meetings, but time and effort members can afford to dedicate to committee affairs. Chairman ended discussion with statement that we should try to find a compromise.

Research Planning

Research Objective Development (Dettmers): An overview of commission research priorities and technical committee priorities was given. Four principle research theme areas are supported by the commission. In 2005, 86 preproposals were submitted and 6 were funded. A question was asked whether the commission specifies research priorities in advance of proposal submission. Dettmers explained that the commission does not specify research priorities. The lake committees, lake technical committees, and the fish health committee specify research priorities that are used to help evaluate proposals. Dettmers also explained the process that a proposal goes through from submission to eventual funding. Once pre-proposals are submitted, they are evaluated by the Board of Technical Experts (board) to determine which topics are invited to submit full proposals. Once written, full proposals are evaluated by independent peer reviewers. These comments are then summarized and the board recommends a subset of proposals for funding. Finally, the commission makes final funding decisions based on board recommendations and other strategic factors, including the amount of funds available. Proposals must have (1) conceptual frameworks can be tested to explain observed patterns, (2) properly constructed objectives and (3) a strong case for the research must be made.

Research Priorities (Whelan): Whelan suggested that the current research priority list may need to be re-evaluated or reorganized. Discussion followed and VHS-related research needs to be made a high priority. Further discussion centered around the lack of GLFC funding for newly-emergent disease research, like VHS, since monitoring is not likely to be funded by the commission in the absence of testable, process-driven objectives. The approach of long-term budget planning for monitoring programs was suggested because this approach was once a priority and making these programs hypothesis oriented would be more appealing to the commission. Whelan surveyed the committee as to whether the list needs to be (1) left as is, (2) changed, or (3) tabled for later discussion. Result was consensus for tabling for later discussion.

Task: A subcommittee was formed to keep a list of priorities and develop means to revise if needed. Committee includes Sue Marcquenski, Rick Nelson, Dave Meuninck and Greg Wright.

Current Pre-proposal Ranking: Ranking of proposals (top 3) can be found on page 20 of the briefing packet. All members agreed to submit the top three proposals and the order was agreed upon. Ranking was as follows: (1) Densmore and McAllister, (2) Lumsden and Stevenson, (3) McAllister, Densmore and Schill.

Model Program (Marcquenski, Appendix 1): Some cool water sections were merged into the current Model Program as discussed in last year's meeting. The new document will be designated "2006-1". Table of Contents will be changed to accommodate changes made in text. Various changes to the text were discussed. Hatchery classification is now reported annually instead of semi-annually. VHS will remain an emergency pathogen until more is learned about it. Annex II includes the cell culture matrix from the cool water program. Draft in Appendix 1 has all changes made during the meeting.

Task: Sue stated that sections needed to be written for additional pathogens not currently listed in the Model Program. Assignments were as follows: LMBV section is to be done by Plumb, KHV by Noyes, and Piscirickettsia by Faisal.

Research Updates

Bacterial Kidney Disease Update (Elliott, Appendix 2): Diane Elliott gave a summary of two studies she conducted. Study 1 characterized the immune response of three strains of Chinook salmon to two strains of *R. salmoninarum* using ELISA, membrane filter FAT and nested PCR. She found that the Lake Michigan Chinook strain (LM) had higher survival than west coast strains (PWC). A microsatellite parsimony tree was showed that LM salmon originated from Columbia River stock. Cytokine gene expression was higher at first in LM strains then dropped off. Several humoral studies are still ongoing. Results to date indicate that LM salmon are more resistant and clear bacteria faster than PWC fish. Study 2 addresses the validation of non-culture methods to detect BKD. A number of immuno and PCR-based methods were compared. These methods were specific and did not cross-react to detect other pathogens.

Thiamine Deficiency (Honeyfield, Appendix 3) Dale Honeyfield gave a summary of his work. Lake trout fed thiamine deficient diet had depressed erythrocyte, leukocyte and hematocrit

values indicating that thiamine deplete fish may be more susceptible to disease. However, in disease challenge studies, thiamine deplete fish to had lower viral titers. He also noted that wild returning fish had higher thiamine in 2005 than in previous years.

EEDv study (McAllister)–Phil McAllister explained that this study has been terminated because archived EEDv samples were not recoverable.

IPN study (McAllister)–Phil McAllister gave a summary of his IPN study. The first objective was to determine the virulence of various Great Lakes IPN isolates and the second objective was to determine the potential for infection and disease by chronic, low level virus exposure. All work in objective 1 is complete; the genetic tree developed from 40 isolates and the genome was sequenced for isolate comparison. Studies are underway to address objective 2.

Heterosporis Study (Marcquenski, Appendix 4)–Dan Sutherland could not attend but his report was given by Sue Marcquenski. Parasite life cycle, case history and distribution in region were explained. Heterosporis is not passed through digestive tract of cormorants. Other studies indicate that fish are infected from eating infected tissue and may be infected from water bound spores.

Bacterial Coldwater Disease Update (Starliper, Appendix 5 and 6) Cliff Starliper updated committee on GLFC funded BCWD research. He has developed culture medium optimized to culture *F. psychrophilum* and minimally grew contaminating bacteria. The #2 medium was compared to two other media and performed best overall with rapid growth. Fetal bovine serum greatly improves growth and the use of 'metabolite' did not benefit growth. The next step is to refine the medium to select against other yellow pigmented bacteria.

Viral Hemorrhagic Septicemia Updates

Bay of Quinte: Lisa Miller-Dodd explained the freshwater drum kill in the Bay of Quinte. Steven Lord (U. of Guelph) inoculated tissue homogenates from moribund fish onto CHSE-214 and RTG-2 cell cultures and found nothing. The samples were then sent to John Lumsden (U. of Guelph) and inoculated onto other cell lines (FHM and EPC). An infectious agent was observed, isolated, sequenced and found to be similar to the North American, Eastern strain of VHS. Samples were collected from drum at a later date and all were negative. Other fish species (Chinook and others) were then tested collected from same vicinity and all were negative for virus. A news release was not drafted initially, but recommendations were disseminated in the field. Management implications are that Bay of Quinte walleye will not be used for culture use and the OMNR have encouraged minimal use of Bay of Quinte fish for private aquaculturists.

Lake St. Clair (Faisal, Appendix 7). Mohamed Faisal described his investigations of VHS in Lake St. Clair. Trophy-sized muskies had reddened lesions and a Piscirickettsia-like organism was isolated. White vesicles were also found in swim bladder. Samples were inoculated onto 6 cell lines and many developed cytopathic effect. This virus was later identified as a rhabdovirus and confirmed as VHS. Sequence data were compared to other strains and found more closely related to Lumsden's type IV isolate than the western type IV. Gary Whelan then posted these findings on an internet site and submitted a report to the OIE.

Food web shifts in Lake Michigan and Lake Huron (Dettmers, Appendix 8). John Dettmers highlighted major foodweb changes from late 1980's to present. Impacts from gobies were detailed; good prey species, rich in thiamine, but voracious predators on fish egg nests. Indirect impact from zebra and quagga mussels has reduced total zooplankton. Diporeia has declined leading to a reduction in alewife energy density.

National Planning Updates

National Aquatic Animal Health Plan Update (Amos, Appendix 9). Kevin Amos reviewed the National Aquatic Animal Health Plan to the committee. The mission is to develop a plan that is guided by a collaboration among governing agencies. Initially, the plan was developed and specific details are currently under review by working groups.

Canadian National Aquatic Animal Health Plan (Penney, Appendix 10). Rod Penney explained the Canadian Aquatic Animal Health Plan to the committee. The NAAHP is under the authority of the Canadian Food Inspection Agency and was developed to maintain export programs. The plan has a 5-year, 59 million dollar budget. The division of labor between the CFIA and DFO was explained.

Additional Item
Coldwater Model Program: Sue Marcquenski will submit plan with recent revisions, then Gary will pass it on to subcommittee. Internal review will be done by May 1st and sent to the commission by late June.

Coolwater Model Program update (Marcino): Joe Marcino summarized changes to be made in the cool water model program. Joe and John Plumb will be co-authors on the document. Once complete, the document may be posted on the committee web site.

Committee Secretary Term: The term is up for the current secretary. Andy Noyes was nominated and re-elected for another term.

Next Meeting: Ken Stark agreed to host the next annual meeting in Pennsylvania, preferably in late January, 2007

GREAT LAKES FISH DISEASE
CONTROL POLICY AND MODEL PROGRAM

PROTOCOL TO MINIMIZE THE RISK OF INTRODUCING EMERGENCY PATHOGENS WITH IMPORTATION OF SALMONID FISHES FROM ENZOOTIC AREAS

SPECIAL PUBLICATION 93-1

Comment [SM1]: Need a new pub number?

insert current Inside Front Cover text

GREAT LAKES FISH DISEASE CONTROL POLICY AND MODEL PROGRAM

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Citation: ~~Hnath, J. G. [ED.]. 1993~~Marcquenski, S.V., editor. 2006. Great Lakes fish disease control policy and model program (supersedes ~~September~~ the 1993~~1985~~ edition). Great Lakes Fish. Comm. Spec. Pub. 93-1: 1-38.

Comment [SM2]: Do we need to indicate Jim and John are retired? Shodl their agency be identified?

Comment [SM3]: Allison, do youthink we need a new publication number or just put (revised 2006) after the 93-1?

PROTOCOL TO MINIMIZE THE RISK OF INTRODUCING EMERGENCY PATHOGENS WITH IMPORTATION OF SALMONID FISHES FROM ENZOOTIC AREAS

edited by

Rodney W. Horner (retired)

Randy L. Eshenroder (retired)

Citation: Horner, R. W., and R. L. Eshenroder [EDS.]. 1993. Protocol to minimize the risk of introducing emergency pathogens with importation of salmonid fishes from enzootic areas. Great Lakes Fish. Comm. Spec. Pub. 93-1: 39-54.

SPECIAL PUBLICATION 93-1

Great Lakes Fishery Commission
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Comment [SM4]: Need new number

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GREAT LAKES FISH DISEASE CONTROL POLICY AND MODEL PROGRAM

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ABSTRACT. Recognizing the risk of transferring pathogens between cultured and wild fish, including the potential losses of fish products and fishing opportunities to the public and diminished economic returns to Great Lakes communities, the Great Lakes Fishery Commission (GLFC) established a policy that (1) encourages member agencies to work toward controlling the transfer of fish pathogens in the Great Lakes basin, and (2) coordinates the fish-disease control programs of the fish management agencies. To implement this policy, the GLFC's Great Lakes Fish Health Committee (GLFHC) developed a Model Program for achieving fish-disease control objectives in the Great Lakes. The Model Program calls on member agencies to classify salmonid fish hatcheries based upon pathogen history of all lots of fish on each station. Categories of pathogens are established based on their presence (restricted) or absence (emergency) in the Great Lakes basin. Agencies are counseled to undertake all available measures to prevent the introduction of pathogens not yet established in the Great Lakes basin. Specific measures to minimize the spread of or prevent the introduction of pathogens are also recommended. These measures include procedures for detection, treatment, and/or appropriate disposition of affected fish.

INTRODUCTION

Fish-disease control in the Great Lakes basin is the responsibility of those agencies that manage the fisheries resources, while also recognizing the concerns of other stakeholders in this geographic area. The Great Lakes Fish Disease Control Committee (now Great Lakes Fish Health Committee, GLFHC) of the Great Lakes Fishery Commission (GLFC) developed a Control Policy and Model Program to unify and coordinate the fish-disease control efforts of the member agencies. The Control Policy was revised and re-adopted by the GLFC in 1985 and again in 2006. The Model Program sets forth the essential requirements for ~~the prevention~~preventing the introduction and controlling the spread of serious pathogens and includes a system for inspecting and classifying fish hatcheries as well as the technical procedures to be used during these evaluations.

The GLFHC does not seek authority to control fish-diseases ~~control authority~~. The recommendations advanced here are provided as an aid to the member agencies in the development of legislation and regulations. ~~However, d~~Decisions to introduce fish stocks known to be, or suspected of being infected with pathogens of concern (see Annex II), ultimately reside with the respective agency. The GLFHC seeks the advice and counsel of these agencies in the continuing development of fish-disease control programs to assure that such programs are in the best interests of the fishery resources of the Great Lakes.

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CONTROL POLICY

Disease outbreaks may severely affect the efficient propagation of fish and have caused serious losses in fish hatcheries. A potential exists for post-stocking losses and transfer of pathogens to feral and wild Great Lakes fish populations. Disease problems have resulted in reduced survival of stocked fish, cost of production ~~cost~~ increases in excess of 20% ~~or more~~, significant decreases in public fishing opportunity~~y~~ies, and diminished economic returns to Great Lakes communities ~~Disease problems have resulted in reduced survival of stocked fish, production cost increases of 20% or more, significant decreases in public fishing opportunity, and diminished economic returns to Great Lakes communities~~.

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The policy of the GLFC encourages each agency to work toward the control of fish diseases in the Great Lakes basin by:

- developing legislative authority and regulations to allow control of fish diseases and possible eradication of fish pathogens,

- preventing the rearing and release of clinically diseased fish and minimizing the rearing and release of infected fish.

- preventing the importation into the Great Lakes basin of fish infected with emergency pathogens, and

- preventing the transfer within the Great Lakes basin of fish infected with restricted pathogens

The GLFC will strive to coordinate the fish-disease control program of the agencies. To this end, the GLFC endorses and supports the Great Lakes Fish Disease Control Policy and Model Program as a guide for agency program development.

[1] Adopted by the Great Lakes Fishery Commission, 9 May 1985.

MODEL PROGRAM

Section 1. Definitions

Definitions for the purpose of this program are:

"**Clinical sign**" means a visible indication of disease that is readily apparent, overt, or obvious by gross inspection.

"**Closed water supply**" means a water supply that is free of fish and fish pathogens. Generally, enclosed springs and wells are considered closed water supplies.

"**Emergency fish pathogens**" as listed in Annex II means certain pathogens of fish that are transmissible directly or indirectly, from one fish to another, and are not known to exist in the Great Lakes basin.

"**Fish**" means live fish as listed in Annex I, their viable eggs, sperm, or products used for fish foods that have not been so processed as to render them incapable of transmitting either emergency or restricted fish pathogens.

"**Fish-Disease Inspection Report**" means a document (see Annex III) giving evidence of inspections and diagnostic work performed as described in Section 4.

"**Fish hatchery**" means any facility that holds, rears, or releases fish of the species listed in Annex I in the waters of the Great Lakes basin or whose effluent waters drain into the Great Lakes basin.

"**Fish Health Official**" means a fish health specialist who meets the requirements set forth in Section 6.

"**Great Lakes basin**" means the geographical area encompassing Lake Ontario (including the St. Lawrence River from Lake Ontario to the 45th parallel of latitude), Lake Erie, Lake Huron (including Lake St. Clair), Lake Michigan, Lake Superior, their interconnecting waters, and all tributaries to those lakes and waters.

"**Lot**" means a group of fish that originate from the same broodstock during the same year and are reared on the same water source.

"**Member agency**" means each federal, provincial, tribal and state government fishery management or conservation agency that is signatory to the Strategic Great Lakes Fisheries Management Plan (SGLFMP).

"**Open water supply**" means a water supply that may contain fish or fish pathogens. Surface waters (streams, lakes, etc.) and unenclosed springs are generally considered open water supplies.

"**Rearing unit**" means a raceway, pond, tank or other holding container that is used to propagate fish.

"**Restricted fish pathogens**" as listed in Annex II means any of a group of certain pathogens of fish that are transmissible, directly or indirectly, from one fish to another, and are currently known to exist within the Great Lakes basin, but whose geographic range is not widespread.

"**Source**" means any point or place of origin of fish, fertilized eggs or gametes including a fish hatchery or free-ranging spawning population.

"**Transfer**" for purposes of hatchery classification, means a movement of fish or gametes from one location to another within the Great Lakes basin.

Section 2. Basic Obligation

The member agencies shall, where necessary, take all appropriate measures, including the development of legislative authority and regulations, to prevent the introduction of emergency and restricted fish pathogens, to contain them within their known geographic ranges, and to strive for their elimination in accordance with the provisions of this program.

Section 3. Application

The provisions of this Model Program apply to:

a) species of fish identified in Annex I,

b) emergency and restricted fish pathogens as listed in Annex II, and

c) research involving fish infected with or exposed to emergency and restricted fish pathogens.

The provisions of this Model Program shall **not** apply to:

a) fish in transit through the Great Lakes basin that are not released from their original shipping containers, and

b) specimens of fish imported or exported for purposes of diagnostic or inspection services and related laboratory tests provided that all necessary biological containment measures are taken to avoid any dissemination of fish pathogens.

Nothing in this Model Program shall derogate from the right of the member agencies to apply additional measures of inspection, quarantine, depopulation and pathogen eradication for

the control of fish diseases.

Section 4. Fish Disease Inspection Report

A Fish Disease Inspection Report listing the emergency and restricted fish pathogens detected shall include the information prescribed in Annex III. Such reports may be issued only by a Fish Health Official (see Section 6). Each fish hatchery shall be classified on the basis of an annual fish health inspection and other disease or diagnostic work performed in accordance with the plan described in Annex IV.

Section 5. Importation

Fish imported from outside the jurisdiction of a member agency must be accompanied by a Fish Disease Inspection Report or other documents that give equivalent assurance of the state of health of the fish. Such reports or documents must also be prepared and signed by a Fish Health Official in accordance with Sections 4 and 6. Importations of salmonid fish from areas enzootic for emergency pathogens should conform to the Protocol to Minimize the Risk of Introducing Emergency Pathogens with Importation of Salmonid Fishes from Enzootic Areas (pp. XX, this publication) ~~developed by the GLFHC~~.

The goal of the GLFHC shall be that no importation of fish with a record of emergency pathogens will be permitted into the Great Lakes basin. Importations of fish from facilities with restricted disease classifications may be permitted, provided such importations do not result in downgrading of the receiving facility's classification and meet with the requirements stated in Annex IV.

Section 6. Fish Health Officials

Each member agency shall identify, by name, to the chair of the GLFHC, those individuals whom the agency recognizes to be responsible for conducting fish hatchery inspections and the issuance of inspection reports in accordance with this policy. This recognition should also include private fish health inspectors recognized by the state or province in which they perform on-site inspections.

Competence of Fish Health Officials (including members of the GLFHC and others) shall be based on standards set forth by the Canadian Department of Fisheries and Oceans' "Fish Health Protection Regulations Manual of Compliance" (Miscellaneous Special Publication 31, Revised), that requires adequate laboratory facilities and qualified personnel to assure the prompt and accurate conduct of inspection and diagnoses under the procedures set forth in Annex V, or the standards set forth by the Fish Health Section of the American Fisheries Society (AFS).

Fish Health Officials who are not members of the GLFHC ~~shall~~ are encouraged to submit

Comment [SM5]: I meant this to clarify the current policy- not change it. Is this wording acceptable?

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copies of all Fish Disease Inspection Reports to the member agency under whose jurisdiction the inspected hatchery or feral stocks lies. The chair of the GLFHC shall be responsible for the compilation and distribution of current lists of Fish Health Officials. These lists should be updated annually.

Section 7. Other Reports by Member Agencies

1) General Report

At each meeting of the GLFHC member agencies shall present a report describing the status of fish pathogens at their respective facilities (hatcheries, spawning weirs, etc.) as well as wild or feral stocks of fish, the measures adopted for pathogen control, the activities and problems encountered by their Fish Health Officials, and such other information as may be requested to enhance the effectiveness of the Model Program.

2) Hatchery and Feral Broodstock Classification Report

a) SemAiannually (on 30 June and 31 December), each member agency shall provide an updated classification covering all of its hatcheries and feral broodstocks spawned in the wild or held until spawning to the chair secretary of the GLFHC for compilation and distribution to all GLFHC members.

b) Changes in hatchery classifications concerning emergency pathogens and the detection of restricted pathogens as listed in Annex II from new geographic locations shall be immediately submitted to the chair of the GLFHC for compilation and distribution as described above. A copy of the Hatchery Classification Report is shown in Annex VI.

3) Salmonid Importation Report

Annually, (on 31 December) eEach member agency shall provide semiannually (on 30 June and 31 December) an updated list of proposed and known importations of fish, including fertilized eggs and gametes, from outside the Great Lakes basin and a list of proposed and known transfers of fish, including fertilized eggs and gametes within the Great Lakes basin to the chair secretary of the GLFHC for compilation and distribution as above. A copy of the Salmonid Importation Report is shown in Annex VII.

4) Record Maintenance

The chair of the GLFHC, or designee, shall maintain records of the reports submitted. Most records will reside electronically in a GLFC database or on the GLFHC webpages.

Section 8. Amendments to the Model Program

Amendments to this Model Program may be proposed by any GLFHC member agency or by the GLFHC. Any such proposal made by a member agency shall be submitted to the GLFHC

for comments and recommendations. The proposed amendment, together with the comments and recommendations of the GLFHC, shall be communicated to the GLFC for consideration and adoption. Decisions regarding revisions will be made by the GLFC who may seek additional guidance from the Council of Lakes Committee (CLC).

Section 9. Transfer and Release of Fish Within the Great Lakes Basin

The following restrictions apply to transfer and release of fish within the Great Lakes basin:

a) No fish exhibiting serious clinical signs of disease will be released. If daily mortality due to an infectious disease exceeds 0.05% for the week prior to the intended stocking date, fish from that rearing unit will not be stocked until survival improves. Final disposition of these fish will be accomplished in accordance with Annex II.

b) No fish infected with emergency pathogens will be released or transferred.

c) A hatchery with a record of an emergency pathogen must be depopulated and disinfected before new lots of fish are brought in for rearing.

d) ~~For~~ Annex II provides guidelines on the transfer and release of fish from hatcheries or surface waters within the Great Lakes basin ~~with~~ having a record of specific restricted diseases~~, refer to Annex II~~.

ANNEX I
FISH SPECIES COVERED BY THE MODEL PROGRAM

All species and hybrids of the families, Petromyzontidae, Acipenseridae, Anguillidae, Salmonidae, Coregonidae, Escocidae, Cyprinidae, Ictaluridae, Centrarchidae, Percidae, ~~and non-salmonids specifically identified in Annex XX~~ are subject to provisions of the Great Lakes Fish Disease Control Policy and Model Program.

Other species may be added as deemed appropriate by the GLFHC and as consistent with Section -8, Amendments to the Model Program.

ANNEX II

Guidelines for the Control and Management of Pathogens Covered by the Model Program

This document is to be considered as a guide only, and not a regulatory tool that is required to be fully implemented by each agency represented by the GLFHC. The pathogens described in this guide may present a different level of risk for each jurisdiction represented. Full implementation of the recommendations contained in this guide may not be possible or practical at this time. The guide is to be used as a reference with regard to the current state of knowledge on coldwater, warm water and cool water pathogens and the diseases they cause.

Individual agencies may not be able to start testing for each of the emergency and restricted pathogens listed in this guide, due to limited staff and financial resources. However, as members of the GLFHC, each agency must show due diligence with regard to disease prevention and management, and take necessary steps to implement the recommendations to the best of their ability. Thus, it is recommended that each agency initiate an analysis, as resources and time allow, of the risk presented to that agency by each of the pathogens in this guide, starting with emergency pathogens. Factors to consider in these reviews would include quarantine capacity, known range of the pathogen, known range of the susceptible host, socio-economic, ecological and financial impacts associated with diagnostic testing, disease outbreaks and pathogen control, diagnostic method development and capabilities needed for detection and control of the pathogen. Agencies should use the Risk Analysis Tool developed by the GLFHC.

Agencies will also need to consider how implementation of testing for the listed pathogens will coincide with existing legislation and regulation pertaining to disease prevention and management. For example, response plans will be needed for each pathogen being tested, and the necessary legislative authority for possible depopulation and disinfection. In the event of a disease outbreak, response plans must be initiated quickly and efficiently, and will require ~~Response plans will be critical for the timely, controlled and efficient management of pathogen detection and/or disease outbreak, and will allow for~~ a coordinated response between state, provincial and federal governments, universities and private industry.

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As detailed in Section 7, member agencies will annually report the detection ~~occurrence~~ of any of the pathogens listed ~~below~~in this Annex within the Great Lakes basin ~~within the Great Lakes basin~~. Further, every effort should be made by member agencies to ~~encourage~~ require fish health inspectors, diagnosticians, and academic laboratories conducting fish-disease diagnostic work, ~~and private fish health inspectors~~ to report to a member agency the occurrence (within the Great Lakes basin) of any of the pathogens listed ~~below~~ in this Annex~~to a member agency~~.

This Annex provides lists of Emergency and Restricted Pathogens. ~~to a member agency~~. ~~Those pathogens designated as~~ "Emergency Fish Pathogens" are pathogens that have not been detected

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Comment [SM6]: resources?

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within waters of the Great Lakes basin. ~~Those~~ Pathogens~~Those pathogens designated as "Emergency Fish Pathogens" have not been detected within waters of the Great Lakes basin~~. ~~Those pathogens~~ designated as "Restricted Fish Pathogens" are currently enzootic within the Great Lakes basin, but their geographic range may be limited. Changes in hatchery classifications concerning emergency pathogens and the detection of restricted pathogens from new geographic locations shall be immediately submitted to the chair of the GLFHC.

When the Great Lakes Fish Disease Control Policy and Model Program fails to give clear guidance to a member agency concerning pathogens covered by the Model Program ~~to a member agency~~, the member agency should immediately contact the chair of the Great Lakes Fish Health Committee. The chair or a designate will, at his or her discretion, schedule discussions of the problem through the most expedient means for the purpose of providing a consensus decision and appropriate recommendations. These recommendations shall be presented to the concerned member agency. In the interim, the affected fish shall not be released or transferred and every effort should be made to contain the pathogen.

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Emergency Fish Pathogens

The following list gives the name of the emergency disease, the pathogen, the disease acronym, and the two-letter pathogen acronym used in hatchery classification. Specific procedures regarding the importation of fish into the Great Lakes basin are described below for each emergency pathogen. When an emergency disease agent is confirmed in any fish stock under propagation, immediate steps shall be initiated to eradicate the agent from the facility and adjacent waters as authorized by the member agency within jurisdiction. Refer to Chapter 14 in the Great Lakes Fishery Commission Special Publication 83-2 for procedures.

Disease	Pathogen	Disease Acronym	Pathogen Acronym
viral hemorrhagic septicemia	Rhabdovirus	VHS	VE
infectious hematopoietic necrosis	Rhabdovirus	IHN	VH
ceratomyxosis	*Ceratomyxa shasta*	CS	SC*
proliferative kidney disease	*Tetracapsuloides bryosalmonae*	PKD	SP*
infectious salmon anemia	Orthomyxovirus	ISA	VS
spring viremia of carp	Rhabdovirus	SVCV	VC
white sturgeon herpesvirus	Herpesvirus	WSHV	VW
white sturgeon iridovirus	Iridovirus	WSIV	VI
Asian tapeworm	*Bothriocephalus achielognathi*	Asian Tapeworm	SA

Comment [SM7]: I made this up- logic- virus (V) carp (C) ??

Comment [SM8]: Made this up too

Comment [SM9]: Made this up too

Comment [SM10]: Made this up too

* Inspections within the Great Lakes basin do not need to include these pathogens unless the facility being inspected harbors any fish imported from areas where these pathogens are enzootic.

Comment: Given the recent detection of N. A. VHSv in freshwater drum in Lake Ontario, do we want to retain VHSv as an Emergency Fish Pathogen? Or, do we now wish to specify VHSv (Genotype I) as the strain of VHSv to remain on the Emergency Fish Pathogen list, and list VHSv [(N. American) (Genotype IV)] as a Restricted Pathogen

Viral Hemorrhagic Septicemia Virus, Rhabdovirus, VHS, VE

No fish, fertilized eggs, or gametes from any source, unless the source has been found to be free from viral hemorrhagic septicemia virus for three consecutive, annual inspections, should be imported into the Great Lakes basin. If fish are to be imported from a viral hemorrhagic septicemia virus enzootic area, then the "Protocol to Minimize the Risk of Introducing Emergency Disease Agents With Importation of Salmonid Fishes From Enzootic Areas" (pp.) shall apply.

Infectious Hematopoietic Necrosis Virus, Rhabdovirus, IHN, VH

No fish, fertilized eggs, or gametes from any source, unless the source has been found to be free from infectious hematopoietic necrosis virus in three consecutive, annual inspections, should be imported into the Great Lakes basin. If fish, fertilized eggs, or gametes are to be imported from an area where infectious hematopoietic necrosis virus is enzootic, then the "Protocol to Minimize the Risk of Introducing Emergency Disease Agents With Importation of Salmonid Fishes From Enzootic Areas" (pp.) shall apply.

Ceratomyxa shasta, Myxozoan, CS, SC*

No fish, fertilized eggs, or gametes from any source, unless the source has been found to be free from *Ceratomyxa shasta* for three consecutive, annual inspections, should be imported into the Great Lakes basin. If fish, fertilized eggs, or gametes are to be imported from an area where *Ceratomyxa shasta* is enzootic, then the "Protocol to Minimize the Risk of Introducing Emergency Disease Agents With Importation of Salmonid Fishes From Enzootic Areas" (pp??) shall apply. An exception may be made for importation of disinfected eggs because the parasite is not known to be transmitted via eggs.

Tetracapsuloides bryosalmonae, Myxozoan, PKD, SP

No fish, fertilized eggs, or gametes from any source should be imported into the Great Lakes basin, unless the source has been found to be free from *Tetracapsuloides bryosalmonae* for three consecutive, annual inspections., ~~should be imported into the Great Lakes basin.~~ If fish, fertilized eggs, or gametes are to be imported from a location where *Tetracapsuloides*

bryosalmonae is enzootic, the "Protocol to Minimize the Risk of Introducing Emergency Disease Agents With Importation of Salmonid Fishes From Enzootic Areas" (pp.) shall apply. An exception may be made for importation of disinfected eggs because the parasite is not known to be transmitted via eggs.

Infectious Salmon Anemia, Orthomyxovirus, ISA, VS*

Only surface disinfected fertilized eggs may be imported into the Great Lakes basin from areas enzootic for infectious salmon anemia. Sources of eggs within areas enzootic for infectious salmon anemia must be found to be free from the virus for three consecutive annual inspections. In addition to the absence of clinical signs, a combination of two of the following methods (RT-PCR, IFAT, tissue culture using the salmon head kidney cell line, or histopathology) must be used for virus detection. The "Protocol to Minimize the Risk of Introducing Emergency Disease Agents with Importation of Salmonid Fishes from Enzootic Areas" (pp?) shall apply to all importations of surface disinfected eggs from areas enzootic for infectious salmon anemia virus.

NOTE: RT-PCR = reverse transcriptase- polymerase chain reaction
IFAT = indirect fluorescent antibody technique

~~No fish from any source, unless the source has been found to be free form infectious salmon anemia virus for three consecutive, annual inspections, should be imported into the Great Lakes basin. If fish are to be imported from an infectious salmon anemia enzootic area, then the "Protocol to Minimize the Risk of Introducing Emergency Disease Agents With the Importation of Salmonid Fishes From Enzootic Areas" (pp. 39-54) shall apply. An exception may be made in the case of egg importations as the disease agent is not known to be transmitted via eggs.~~

Spring viremia of carp virus, *Rhabdovirus carpio*, SVCV, VC

Spring viremia of carp virus (***Rhabdovirus carpio***) is known to infect several species of carp in Europe, the Middle East, Russia, and the United States. SVC is considered a serious disease in cultured common carp and has caused epizootics in wild carp populations as well. Because pike fry rhabdovirus is closely related to SVCV, and may even be identical, no common carp, northern pike or their eggs, from any European source should be imported into the Great Lakes basin unless the source has been found to be free from spring viremia of carp virus (Rhabdovirus carpio) for three consecutive, annual inspections. Common carp are seldom cultured in North America, but SVCV could adversely affect northern pike populations. Detection of SVCV is by isolation in cell culture with confirmation by serum neutralization or RT-PCR (See OIE 2005).

Comment [SM11]: This is Brian Souter's text from the 2005 meeting.

Comment [SM12]: At the 2005 mtg we needed to check with the AFS FHS Blue book to see if 2 confirming methods are needed. I checked, and IFAT **OR** RT-PCR are accepted as confirming tests (if CPE is present on cell culture), not initial tests. If the Canadian guidance requires 2 tests, could we require tissue culture, and then the 2 confirming tests would be either IFAT, RT-PCR or histopathology?

NO MENTION IN EITHER STURGEON SECTION OR IN ASIAN TAPEWORM SECTION ABOUT INSPECTION AND IMPORTATION CONDITIONS.

White Sturgeon Herpesvirus, Herpesvirus, WSHV, VW

Two strains of white sturgeon herpesvirus, WSHV-1 and WSHV-2, occur in juvenile and adult cultured and wild white sturgeon in California and other areas in the Pacific northwest. Both viruses cause moderate to high mortality in cultured fish. Other species of sturgeon are also susceptible to WSHV. Detection of WSHV is by isolation in cell culture.

White Sturgeon Iridovirus, Iridovirus, WSIV, VI

White sturgeon iridovirus is known only in the Pacific Northwest United States where it is pathogenic to cultured and wild white sturgeon. This virus is also at least mildly pathogenic to lake sturgeon and could affect fishery resources in the Great Lakes basin. Inspection procedures for WSIV involve isolation in cell culture.

***Bothriocephalus achielognathi*, Cestode, Asian Tapeworm, SA**

The Asian tapeworm, *Bothriocephalus achielognathi*, is found throughout the world and there has been one report of it in New York. This parasite primarily affects very young cyprinids which may suffer high mortality but causes little problem in older fish. The effect of this parasite on the fishery resources of the Great Lakes basin should not be great. Detection is by tapeworm identification in wet mounts of material from the anterior intestine.

SHOULD INCLUDE COLDWATER SPECIES EMERGENCY PATHOGENS TO MAKE TABLE COMPLETE. TABLE SHOULD BE REFERENCED IN SECTIONS ABOVE

The following table summarizes the warm and cool water fish species that should be screened for emergency pathogens

Pathogen	Susceptible Species
Spring Viremia of carp virus	Common carp, cyprinids, guppies, northern pike, grass carp, silver carp, bighead carp, crucian carp,
White Sturgeon Herpesvirus	Sturgeon family
White Sturgeon Iridovirus	Sturgeon family

Bothriocephalus	Family cyprinidae. Goby, grass carp, common carp, sleeper, killifish, mosquito fish, humpback chub, roundtail chub, green sunfish, golden shiner, emerald shiner, red shiner, spotfin shiner, fathead minnow, guppy, pike minnow.

* Inspections within the Great Lakes basin do not need to include these pathogens unless importations of fish from enzootic areas are known to have been made or the pathogen was detected within the jurisdiction of a member agency

Restricted Fish Pathogens

Restricted pathogens are enzootic, although possibly geographically limited, within the Great Lakes basin. Every appropriate action should be taken to further reduce the range of restricted pathogens and their prevalence in the basin. The following list gives the name of the disease, the type of pathogen, the disease acronym, and the two-letter pathogen acronym used in hatchery classification. Specific procedures regarding the importation into, and stocking of fish within, the Great Lakes basin are described below for each restricted pathogen. If a restricted pathogen is detected in a new geographic location, the member agency must notify the chair of the GLFHC immediately, who will then inform the GLFHC and the GLFC. If a restricted pathogen is diagnosed as the cause of daily mortality exceeding 0.05% within a week of the desired stocking date, the fish should not be stocked until they have been treated and have recovered from the disease outbreak. Fish infected with restricted bacterial pathogens that are resistant to multiple antibiotics should not be stocked in waters of the Great Lakes basin.

Disease	**Pathogen**	**Disease Acronym**	**Pathogen Acronym**
whirling disease	*Myxobolus cerebralis*	WD	SW
infectious pancreatic necrosis	Birnavirus	IPN	VP
bacterial kidney disease	*Renibacterium salmoninarum*	BKD	BK
furunculosis	*Aeromonas salmonicida*	BF	BF
enteric redmouth	*Yersinia ruckeri*	ERM	BR
epizootic epitheliotropic disease	Herpesvirus	EED	VL**
largemouth bass virus disease	Iridovirus	LMBV	VB
streptococcosis	Streptococcus iniae	STP	BI
Heterosporis	Microsporidan	HM	SH
channel catfish virus	Herpesvirus	CCV	VD
enteric septicemia of catfish	*Edwardsiella ictaluri*	ESC	BE
proliferative gill disease	*Aurantiactinomyxon ictaluri*	PGD	SP

Comment [SM13]: All of the following acronyms are made up- suggest a new one if you like.

** Field diagnostic test not available.

Whirling Disease, *Myxobolus cerebralis,* Myxozoan, WD, SW

No fish from any source (with the exception of disinfected eggs and spore-free i.e. actinospore-free and/or myxospore-free, transport water of well origin), shall be imported into the Great Lakes basin unless the source has been found to be free from detection of the myxozoan parasite *Myxobolus cerebralis* (M.c.), and clinical signs of whirling disease for three consecutive, annual inspections during the preceding two years. If the parasite is confirmed in any hatchery of a member agency, no M.c. positive fish may be stocked into the waters of the Great Lakes basin. All M.c. positive lots of fish must be removed from the infected hatchery. Remaining individual lots on the hatchery may not be stocked until results from three consecutive samplings at the 2% level, conducted at equal intervals during the remaining rearing cycle are known. The interval may not be shorter than 30 days. If the results are negative, the fish may be stocked into waters of the Great Lakes basin. Such an M.c. positive hatchery will carry the (SW) classification until three consecutive negative annual inspections over a two year period at the 5% level of detection for each lot present, have been completed.

Infectious Pancreatic Necrosis Virus, Birnavirus, IPN, VP

No fish, fertilized eggs, or gametes from any source, unless the source has been found to be free from infectious pancreatic necrosis virus for three consecutive annual inspections, shall be imported into the Great Lakes basin. In the event infectious pancreatic necrosis virus is confirmed in any stock under propagation by a member agency, every effort should be made not to release these fish into waters of the Great Lakes basin. However, it is recognized that:

- the virulence of IPNV can vary with virus isolate and host species of fish, and virulent IPNV cannot be distinguished from avirulent IPNV using current diagnostic technology.

- asymptomatic carriers of IPNV can occur and can represent a point source of infection for fish not infected with IPNV.

- release of asymptomatic carriers of IPNV into some of the Great Lakes or tributaries of the Great Lakes may pose a health risk for those salmonids currently not considered to be infected with IPNV.

Therefore, the GLFHC recommends that member agencies and non-member agencies needing to make a decision to stock IPNV-infected fish that do not exhibit clinical signs of the disease, mortality or morbidity associated with IPNV, should conduct a risk assessment (Annex XX) to determine the level of risk associated with a potential stocking action.

Bacterial Kidney Disease, *Renibacterium salmoninarum*, Bacterium, BKD, BK

Since ***Renibacterium salmoninarum*** is enzootic within the Great Lakes basin, harsh restrictions on importation are unrealistic at this time. However, , as part of the GLFHC commitment to reducing the prevalence and intensity of R. salmoninarum in the Great Lakes basin, every effort shall be made not to stock fish that have a high R. salmoninarum prevalence or have clinical signs of the disease.
~~every effort should be made not to import or stock fish with clinical signs of the disease. The GLFHC is committed to reducing the prevalence and intensity of *R. salmoninarum* in the Great Lake basin.~~

Furunculosis, *Aeromonas salmonicida*, Bacterium, BF, BF

No salmonid fishes from facilities where *Aeromonas salmonicida* has been detected shall be transferred to facilities where the bacterium has not been detected in the preceding three consecutive, annual inspections. Disinfected eggs and pathogen free transport water may be transferred to facilities without altering the disease classification of the receiving station. However, every effort should be made not to import or stock fish with clinical signs of the disease.

Enteric Redmouth, *Yersinia ruckeri,* Bacterium, ERM, BR

No fish from facilities or feral populations where *Yersinia ruckeri* has been detected shall be transferred to facilities where the bacterium has not been detected in the preceding three consecutive, annual inspections, Disinfected eggs and pathogen free transport water may be transferred to facilities without altering the disease classification of the receiving station. Every effort should be made not to import or stock fish with clinical signs of the disease.

Epizootic Epitheliotropic Disease Virus, Herpesvirus, EED, VL**

When Epizootic Epitheliotropic Disease virus (EEDV) is detected and confirmed by electron microscopy at a member agency's facility, all fish and eggs at the facility will be destroyed and the facility completely disinfected. Year classes of fish produced during the two-year period immediately following the complete disinfection may only be stocked into Lake Superior, Lake Michigan and Lake Huron. Year classes of fish produced after this two-year period of freedom from detection of EEDV may be transferred to, or stocked in other locations. Eggs collected from wild lake trout in Lake Superior, Lake Michigan or Lake Huron or from a source with a previous history of EEDV must be surface disinfected and held in quarantine or isolation for a minimum of 18 months and stress tested at 8 and 16 months of age to determine if EEDV is present. If the lot is considered EEDV negative, the fish may be stocked or transferred to other hatcheries.

Largemouth bass virus disease, Iridovirus, LMBV, VB

Largemouth bass virus has been reported in wild largemouth bass in many reservoirs and several hatcheries in southern United States and lakes and hatcheries within the Great Lakes basin. While its pathogenicity is not clear, LMBV has been implicated in several epizootics, and therefore, it is prudent to reduce the possibility of further dissemination of the virus. Cell culture and molecular techniques are available for detection of LMBV.

Koi Herpesvirus, Herpesvirus, KHV, VK

Comment [SM14]: Andy, will you write the text?

Channel Catfish Virus Disease, *Herpesvirus ictaluri*, CCV, VD

Channel catfish virus infects young of the year channel catfish during warm summer months. The possibility of this disease causing adverse effects in the Great Lakes basin is minimal because of environmental requirements. However, if very young CCV infected channel catfish were stocked into a recirculating system with water temperatures above 25°C, a high mortality would likely occur. Detection of carrier fish by virus isolation is not dependable, therefore, detection of potential carrier fish is by PCR.

Lymphosarcoma, Virus?, VL?

Lymphosarcoma is a malignancy of esocids in North America, the UK and Europe. Tumors may be present in the muscle and internal organs including the gonads. The disease is thought to be caused by a retrovirus, however this has not been proven. It may be spread by fish to fish contact during spawning, and no evidence yet suggests that it is vertically transmitted. Diagnosis is based on histopathology indicating the presence of neoplastic lymphocytes. It may take up to a year for subclinically infected fish to show external signs. Therefore, northern pike and muskellunge should not be transferred or stocked from locations where lymphosarcoma is known to be present.

Streptococcosis, *Streptococcus iniae*, Bacterium, STP, BI

Streptococcus iniae has become a significant pathogen in intensive tilapia culture, especially recirculating systems, where artificially heated water is used to maintain elevated temperatures. Under adverse conditions the disease can be devastating to tilapia and this

pathogen is also capable of infecting other fish species as well as humans. Limiting its spread is important; however, isolation of the bacterium, which is less than 100% sensitive is the only means currently available for detecting carrier fish.

Enteric Septicemia of Catfish, *Edwardsiella ictaluri*, ESC, BE

Enteric septicemia of catfish is almost exclusively a disease of cultured channel catfish occurring in pond, cage, and recirculating culture systems. Epizootic survivors carry *Edwardsiella ictaluri* which is detectable by isolation. Due to its extensive presence in the catfish industry fish without some exposure to *E. ictaluri* are difficult to find.

Piscirickettsia-like Organisms, Rickettsia, PLO, RP?

Comment [SM15]: Mohamed, will you write the text?

***Heterosporis* sp., Microsporidan, *Heterosporis*, SH**

Heterosporis sp. infects the muscle of fish and is known to occur in a limited number of Wisconsin and Minnesota lakes and eastern Canadian waters of Lake Ontario. Susceptible species include yellow perch, walleye, pumpkinseed, sculpin, trout-perch, rock bass, burbot, northern pike. Under laboratory conditions, rainbow trout, Coho salmon, brook trout, brown trout, lake trout, white suckers, mosquito fish, channel catfish, fathead minnow and largemouth bass can be infected. Although this parasite apparently does not kill fish it does make them unacceptable to the public. Detection of the parasite is made by examining fresh muscle material for evidence of a "freezer burn" or area of white, opaque muscle and by histopathology. Confirmation is by PCR. Susceptible fish species should be examined for the presence of Heterosporis before they are imported or transferred within the Great Lakes basin. Fish from waters where Heterosporis is enzootic should not be transferred to other locations.

Comment [SM16]: This is the advice we gave the CLC a few years ago- is it OK to include here?

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Proliferative Gill Disease, *Aurantiactinomyxon ictaluri*, Haplosporidian, PGD, SP

Proliferative gill disease affects the gills of cultured catfish throughout southern United States. The parasite has a complex life cycle involving bottom dwelling oligochaetes but the possibility of this parasite occurring and/or becoming established in the Great Lakes basin is minimal. Detection is by histological examination of gill tissue.

Comment: If the possibility of this pathogen occurring and/or becoming established in the GLB is minimal then why is it listed as a restricted pathogen?

INCLUDE REFERENCE TO TABLE IN SECTIONS ABOVE

INCLUDE COLDWATER SPECIES RESTRICTED PATHOGENS TO MAKE TABLE COMPLETE.

The following table summarizes the warm and cool water fish species that should be screened for the restricted pathogens.

Pathogen	Susceptible Species
Channel catfish virus	Channel catfish, blue catfish, hybrid Channel catfish
Largemouth Bass virus	Largemouth bass, smallmouth bass, bluegill, spotted bass, suwanee bass, redbreast sunfish, white crappie, black crappie, rock bass.
Koi Herpesvirus	Koi, common carp
Edwardsiella ictaluri	Channel catfish, white catfish, blue catfish, Chinook salmon, rainbow trout
Streptococcus iniae	Tilapia, striped bass, striped bass X white bass, some salmonids
Proliferative Gill Disease	Channel catfish, blue catfish
Piscirickettsial like organism	Muskellunge, coho, Chinook, sakura salmon, rainbow trout, pink salmon and atlantic salmon.
Heterosporis	Yellow perch, northern pike, walleye, trout-perch, burbot, pumpkinseed, sculpin, rock bass, rainbow trout, channel catfish, fathead minnow, largemouth bass.

_____

** Diagnostic test not available. EEDV is presumptively diagnosed by electron microscopy following isopyknic density gradient ultracentrifugation .

ANNEX III
FISH-DISEASE INSPECTION REPORT

To facilitate data retrieval, Each member agency shall use the Fish-Disease Inspection Report (page ?) for submitting inspection data to the GLFHC and GLFC. Contact the GLFC to receive a supply of this report.

~~Each member agency should use the Fish-Disease Inspection Report (page ?) to facilitate data retrieval. Contact the GLFC to receive a supply of this report.~~

Comment [SM17]: An electronic version of this report does not exist. Most agencies have modified the form and have created electronic versions. Since we do not submit the forms to the GLFHC (just the hatchery classification summary), do we need this Annex? We do exchange forms among agencies when fish/eggs are imported.

ANNEX IV
HATCHERY CLASSIFICATION

As resources permit, all ~~salmonid fish hatcheries of~~ member agencies are expected to annually inspect and classify their respective salmonid hatcheries and wild salmonid spawning population used for propagation ~~will be annually inspected and classified~~ for the emergency and restricted fish pathogens in Annex II. ~~Results from the~~ Inspection results will be reported ~~on~~ using the Fish Disease Inspection Report. It is understood that this report indicates the fish health status of a specific facility at the time of inspection. To obtain the most current ~~information about the~~ fish health status of a specific facility, contact the appropriate fish health official. All salmonid fish hatcheries of member agencies and wild salmonid spawning populations used for propagation will be annually inspected and classified for the emergency and restricted salmonid pathogens in Annex II.

Comment [SM18]: Since we are expanding the species included in the MP, shall we just refer tospeacies in Annex1 that are reared in hatcheries and in wild populations used in propagation?

Class A-1

The A-1 classification applies to fish hatcheries only. The following criteria must be met to receive an A-1 classification.

1) All fish rearing water must be obtained from a fish-free water supply such as an enclosed spring or well.

~~2)~~All lots of fish reared at the hatchery must be inspected as described in Annex V for the pathogens listed in Annex II. Three successive negative inspections conducted over a continuous two-year period are required. The two-year period begins with the first negative inspection. ~~Two additional negative inspections performed annually are required to complete the classification process.~~

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~~3)~~2)

~~4)~~3) To maintain an A-1 classification hatcheries must:

- undergo annual inspections.
- remain negative for pathogens listed in Annex II.
- ensure that all fish, fertilized eggs, and gametes (see Section 1) are obtained from sources classified as A-1 or A-2.

Comment [SM19]: Mohamed points out that to remain A-1, fish or gametes should only come from an A-1 facility (not A-2 also)- OK to delete A-2?

NOTE: A new hatchery or a hatchery without a classification record will be classified C, A-1 (see Class C below) for the duration of the two-year inspection process unless pathogens listed in Annex II are detected. When the two-year inspection process is successfully completed without detecting pathogens from Annex II, the hatchery will be classified A-1.

Class A-2

The A-2 classification applies to hatcheries with a surface water supply (lake or stream containing resident fish) and to feral/wild fish populations. The fish in the hatchery or the feral/wild fish populations must be inspected according to guidance in Annex V and must be found free of pathogens listed in Annex II to receive the A-2 classification. The requirements

described in (3) above must be met to maintain an A-2 classification.

NOTE: A hatchery or wild spawning population will be classified C, A-2 (see Class C below) for the duration of the two-year inspection process unless pathogens listed in Annex II are detected. When the two year inspection process is successfully completed without detecting pathogens from Annex II, the hatchery or wild spawning population will be classified as A-2.

Class B

To receive a B classification, hatcheries and wild spawning populations must be inspected for all pathogens listed in Annex II. The B classification is received when one or more of the pathogens listed in Annex II are detected during an inspection or from diagnostic work conducted during the ~~six month~~ inspection reporting period. The pathogen acronym becomes part of the classification designation and the date of the initial isolation of the pathogen follows the acronym. For example, an A-1 hatchery where IPNV was isolated during a health inspection conducted on 6-20-2001 and *Aeromonas salmonicida* was isolated from a diagnostic case on 7-17-2001 would be classified B-VP(6/2001), BF(7/2001) [note the order of pathogens: virus, bacteria, parasite].

In some cases, a classification will be downgraded when fish are transferred from one hatchery to another. For example, a hatchery classified as B-BF (2/2002) will be downgraded to B-BF (2/2~~02~~02),BK-T if fish from a hatchery with a B-BK classification are transferred. Attaching the letter "T" to the classification indicates the downgrading resulted from a transfer, ie. the pathogen was not detected. ~~For example: B-BF (2/2002),BK-T.~~

The following examples illustrate how a hatchery having a B classification can be upgraded.

1) When three consecutive annual inspections fail to detect the specific pathogen, the pathogen acronym is removed from the classification. If no other pathogens have been detected during this period, the hatchery resumes its A-1 or A-2 classification.

2)) When a hatchery is depopulated and disinfected, the classification is changed to A-1 or A-2, follwed by the pathogen acronym(s) and disinfection date in parentheses When three consecutive annual inspections over a continuous two-year period fail to detect the pathogen(s), the pathogen acronym(s) is (are) removed from the classification. If no other pathogens have been detected during this period, the hatchery resumes its A-1 or A-2 classification.. ~~depopulation and disinfection followed by three consecutive, negative, complete, inspections over a continuous two-year period.~~ Using the example above, the hatchery classified B-VP (6/2001), BF (7/2001) would be classified A-1 – (VP, BF) (8/2001) after an ~~April~~ August 2001 disinfection and would remain as such during the two- year inspection period. If, after the two-year inspection period, neither IPNV nor *A. salmonicida*, or any other pathogen listed in Annex II is detected, the classification would revert back to A-1.

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3) In the above example, if the hatchery did undergo depopulation and disinfection and IPNV was still present after the two-year inspection period, the hatchery would be classified B-VP

(date of the most recent detection). This classification would remain until there was a change in the pathogens detected at the hatchery.

NOTE: While a hatchery cannot have a higher classification than the source of its stock, a hatchery with the B classification receiving fish or disinfected eggs from an A-1 or A-2 hatchery would retain its B classification. A hatchery classification will not be altered if surface disinfected eggs and/or pathogen-free transport water are received from sources where horizontally transmitted pathogens (i.e *A. salmonicida*, *Yersinia ruckeri* and *Myxobolus cerebralis*) are known or suspected of being present.

Class C

1) A hatchery without a two year history of annual health inspections will receive the C classification. In addition, a suffix will follow the C, indicating the current fish health status as determined by annual inspections over a two-year period as described above. At the end of the two-year inspection period, the "C" will be dropped and any change to the classification will follow the guidelines above.

2) Hatcheries and wild spawning populations will be assigned a C classification when:
- the disease history is unknown,
- any pathogen listed in Annex II has not been tested for during an inspection,
-the required number of inspections (three annual inspections within a two year period) have not been completed.

Restrictions

Shipments of fish (including fertilized eggs and gametes--see Section 1) between hatcheries will be governed by the classification status of the hatcheries involved. No shipments of fish will be made without prior approval of the receiving authorities whenever such shipment will knowingly downgrade the classification of the receiving hatchery.

At least one inspection for each pathogen listed in Annex II (List of Pathogens Covered by the Model Program), except as noted for *Ceratomyxa shasta*, *Tetracapsuloides bryosalmonae*, and epizootic epitheliotropic disease virus, will be conducted on all lots of salmonid fishes, regardless of age, prior to the transfer or stocking of fish.
Comment: Are there any agency facilities in the U. S. raising susceptible warm and cool-water species along side salmonid species? If there are, will these facilities be required to test susceptible salmonids for pathogens such as *Edwardsiella ictaluri*, *Strep iniae*, the piscirickettsia-like organism and heterosporosis as part of their annual hatchery inspection program?

Comment [SM20]: This does not seem to make sense with the requirement for 3 annual inspections above in order to classify a hatchery. Does anyone remember why this statement is in here?

Also, if it is retained, do we need to add the cool/warmwater pathogens for inspection?

ANNEX V
INSPECTION PROCEDURES AND METHODS OF DIAGNOSIS

Inspection Procedures

Data obtained from inspections are an essential part of this program to control and improve the quality of fish produced at fish hatcheries. Therefore, all hatchery inspections should be conducted in accordance with the current Canadian Manual of Compliance; American Fisheries Society Fish Health Section Blue Book and Inspection Manual; or the Office International des Epizooties (OIE) Diagnostic Manual for Aquatic Animal Diseases.

Comment [SM21]: Brian is this the correct title?

Sample Population

Comment [SM22]: The rest of this section would be deleted.

The following definitions apply to the designation of populations for sampling purposes.

1) The sample population for all fish except those being inspected for whirling disease is determined on the basis of lot and production environment. Lot is defined as those fish that originated from the same brood stock during the same year and that are being raised on the same water source. Example: Two egg shipments of fall-spawning rainbow trout *(Oncorhynchus mykiss*) received in September and December from the same hatchery are considered one lot. Similarly, all spring-spawning rainbow trout from the same source are another lot. However, when one part of the lot is held in an open water supply and the other in a closed water supply, each will be sampled as a separate population. All lots of brood stock of a single species held in the same water supply may be considered one population regardless of the age of the fish.

2) When inspecting for whirling disease, the sample population is defined as all fish in the hatchery held in the same water supply. Samples should be weighted towards the most susceptible species and ages of fish available. Whirling disease spores are difficult to detect in lake trout *(Salvelinus namaycush)* and coho salmon *(O. kisutch)* and in fish larger than 30 cm (12 in.) in length and younger than 160 days.

3) Wild brood stocks must be inspected at least once during the time that eggs are obtained for shipment to a hatchery in the Great Lakes basin. All brood stocks present at the time of inspection will constitute the sample population. The sample size should be large enough to detect diseases at an assumed incidence of infection of 2%. Where it is not feasible to sample wild brood stocks at the 2% level, a smaller sample may be taken at the discretion of the inspecting pathologist after all risks are considered.

Sample Size

For viral, bacterial, and parasitic pathogens, the number of samples to be collected from a given lot is based upon stratified random sampling that provides 95% confidence of detecting a pathogen with an assumed minimum incidence of detectable infection, depending upon conditions, of 2%-5%.

Minimum sample sizes for populations varying from 50 to infinity are:

Population or Lot Size	Sample Size Assumed Incidence	
	2%	5%
50	50	30
100	75	45
250	110	50
500	130	55
1,000	140	55
1,500	140	55
2,000	145	60
4,000	145	60
10,000	145	60
100,000 or greater	150	60

Sample sizes above are minimum. When a pathogen is suspected, larger samples may be necessary and should be taken at the discretion of the inspector.

Sample Collection

Moribund fish and those with clinical signs of disease should be sampled during all inspections. The method of collecting subsamples from rearing units to obtain a representative sample is left to the discretion of the inspector.

For bacterial diseases, sampling of brood-stock populations and production fish should be done on a continual basis throughout the year using moribund and dying fish whenever possible. Hatchery managers can send samples (fixed material) for the detection of Gram-positive *Renibacterium salmoninarum* to agency laboratories on a periodic basis. Training should be provided to hatchery managers in preparing cultured material for diagnosis of Gram-negative bacterial pathogens. Cultures can also be sent to agency laboratories for confirmation of the diagnosis. The annual case history of each designated lot should be compiled by the inspector using accumulated sampling data. The minimum number of samples is left to the discretion of the inspector.

Methods of Diagnosis

The most recent editions of "Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens," developed by the Fish Health Section (FHS) of the AFS, or the "Fish Health Protection Regulations Manual of Compliance" (Miscellaneous Special Publication 31, Revised) of the Department of Fisheries and Oceans, Canada, provide the basis for fish-hatchery inspections and certifications. More-sensitive or more-definitive procedures may be used, but any departures from the basic procedures set forth by the these manuals must be noted on Fish-Disease Inspection Reports. The GLFHC, in an effort to encourage the use of the best possible methods, should be notified of technical advances enhancing the implementation of the Model Program. Procedural changes issued by the FHS or by the Canadian National Registry of Fish Diseases will be incorporated into the program by the GLFHC as appropriate.

ANNEX VI
HATCHERY CLASSIFICATION REPORT

All salmonid fish hatcheries and wild spawning populations of salmonid fishes used for propagation will be annually inspected and classified. . (??? What about the cool and warm water facilities since the tables above refer to such species and facilities???? Information should be recorded using the Hatchery Classification Report (page ?). Contact the GLFC for a copy of the most recent summary report. The Hatchery Classification Report is forwarded by member agencies to the GLFHC secretary by 31 December each year. The information will be amended to the Hatchery Classification database which can be accessed on the GLFC website.

ANNEX VII
~~SALMONID~~ IMPORTATION REPORT

Each member shall provide an updated list of proposed and known importations of fish (including fertilized eggs and gametes--see Section 1). Information should be recorded using the ~~Salmonid~~ Importation Report (page ?). Contact the GLFC to receive a copy of the most recent report. The Importation Report will be forwarded by member agencies to the GLFHC secretary by 31 December each year. The information will be amended to the Importation Report database which can be accessed from the GLFC website.

ACKNOWLEDGMENTS

The Great Lakes Fish Disease Control Policy and Model Program is the product of past and present members of the GLFHC and the editorial staff of the GLFC.

PROTOCOL TO MINIMIZE THE RISK OF INTRODUCING EMERGENCY PATHOGENS WITH IMPORTATION OF SALMONID FISHES FROM ENZOOTIC AREAS

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ABSTRACT. The Great Lakes Fish Disease Control Committee (now Great Lakes Fish Health Committee, GLFHC) was established by the Great Lakes Fishery Commission (GLFC) in 1973 to recommend measures to protect the health of fish in the Great Lakes basin. The GLFHC has representatives from private and governmental sectors concerned with aquaculture and/or fisheries in the basin. Because introductions of pathogens with importation of salmonid fishes from emergency disease enzootic areas are an increasing concern, the GLFHC established a Protocol to reduce risks associated with importation. This Protocol provides guidelines to be followed by federal, provincial, and state-agency members of the GLFHC. Private-sector operators are also encouraged to use the guidelines. The guidelines consist of an outline for a justification and proposal, inspection requirements for developing a history of pathogens in the donor stock and associated fishes, and procedures for quarantine. Conditions for importation, including the need for quarantine, are established. The GLFHC recommends that the GLFC conduct a formal review, assessing risks and benefits, of any proposal by a governmental-member agency for importation of salmonid fishes from emergency disease enzootic areas. The Protocol is published to encourage wide use and acceptance.

INTRODUCTION

The Great Lakes Fish Disease Control Committee (now Great Lakes Fish Health Committee, GLFHC) was established by the Great Lakes Fishery Commission (GLFC) in 1973 to recommend measures to protect the health of cultured- and wild-fish populations in the Great Lakes basin. The GLFHC is comprised of representatives from state, provincial, and federal agencies involved with Great Lakes fishes and from private aquacultural interests. Decisions are made by a consensus of the membership. In 1985, the GLFHC ~~Committee~~ developed a Model Program for controlling fish diseases in the basin. This Model Program was subsequently adopted as a policy of the GLFC, and was updated and republished as a companion to this

document (Hnath 1993).

Increasing national and international interest in importation of salmonid fishes for fisheries management and aquacultural purposes, and the damage caused by disease introductions (Rohovec et al. 1988) indicated that expanded guidelines were needed to protect the health of salmonid fish stocks within the Great Lakes basin. Consequently, the GLFHC recommended at first a ban on importations of salmonid fishes from regions where emergency diseases (see page 11 for a list of emergency fish diseases) were enzootic. For example, importations were banned from the U.S. and Canada west of the Continental Divide, areas where infectious hematopoietic necrosis virus (IHNV) and the parasite *Ceratomyxa shasta*, neither of which are known to occur in the Great Lakes basin, are found.

Viewed from a trade perspective, however, bans may be seen as short-term measures until adequate safeguards can be undertaken. Hence, the GLFHC later developed this Protocol to provide for importation of salmonid fishes from areas where emergency diseases are enzootic. This Protocol applies to federal, provincial, and state-agency members of the GLFHC who propose releases of salmonid fishes from emergency disease enzootic areas into waters under their jurisdiction. Private-sector members of the GLFHC are also encouraged to use it. This Protocol provides guidelines under which importations may be undertaken and establishes procedures for minimizing the associated risks. It is published here to encourage wide acceptance and use. Nothing in the Protocol is intended to supercede or change the intent of the Model Program (Hnath 1993).

JUSTIFICATION AND PROPOSAL

Importations are restricted to fish eggs only. A full written justification should be made available to the GLFC and its cooperators at least six months prior to any proposed importation of salmonid fish eggs from an emergency disease enzootic area outside the Great Lakes states or the Province of Ontario. This stipulation also applies to live salmonid fish eggs imported for research. This notification is consistent with the intent of A Joint Strategic Plan for Management of Great Lakes Fisheries (GLFC 1980), that states:

> Each fishery agency should submit all substantive changes from existing practice to the appropriate lake committee before implementation. . . . Any agency proposal for change which other agencies believe will influence their interests may become the subject of negotiations within lake committees until consensus of affected agencies is achieved.

The proposal and justification for importation should include at least the following items:

[2] Federal, provincial, and state-agency members of the Committee are: Canadian Department of Fisheries and Oceans, U.S. Fish and Wildlife Service, Ontario Ministry of Natural Resources, Illinois Department of Conservation, Indiana Department of Natural Resources, Michigan Department of Natural Resources, Minnesota Department of Natural Resources, New York Department of Environmental Conservation, Ohio Department of Natural Resources, Pennsylvania Fish and Boat Commission, and Wisconsin Department of Natural Resources.

1) species to be introduced,

2) strain,

3) number,

4) location of the donor brood stock,

5) proposed location of introduction,

6) a rationale for the introduction that outlines why the objective cannot be met through utilization of salmonid fish stocks present in the Great Lakes states or the Province of Ontario,

7) information on the strain's preferred habitat,

8) potential for infection from parasites and pathogens, for competition with other fish species in the Great Lakes basin, and for genetic impacts on resident salmonid fishes,

9) reference to previous importation and associated impacts, and

10) a follow-up plan to determine success in relation to objectives and to identify what parasites and pathogens are harbored by the imported fish or their progeny.

Following distribution of a full justification and proposal, the GLFHC recommends that the GLFC conduct a formal review to define the risks of the introduction in relation to the expected benefits. The GLFHC should be consulted as part of the review process. GLFHC members may seek information on the proposed importation in addition to the above items. A formal review is consistent with the Strategic Plan and will help ensure that the interests of GLFC's cooperators are protected. In developing a proposal, importers are advised that the policy of the GLFC under the Great Lakes Fish Disease Control Policy and Model Program (see page 2) is to encourage each cooperating agency to work toward the control of fish diseases in the Great Lakes basin by:

- developing legislative authority and regulations to allow control and possible eradication of fish diseases,

- preventing the release of seriously infected fish,

- discouraging the rearing of diseased fish,

- preventing the importation into the Great Lakes basin of fish infected with emergency pathogens,

- preventing the transfer of fish within the Great Lakes basin of fish infected with restricted pathogens, and

- eradicating fish pathogens, where practicable.

Every effort should be made to avoid importation by using resident salmonid fish stocks from the Great Lakes states or the Province of Ontario. If importation is deemed necessary, every effort should be made to import from areas where annual inspections (see Annex V of the Great Lakes Fish Disease Control Policy and Model Program for inspection procedures) of donor brood stocks and associated salmonid fish stocks in the donor holding facility (captive donors) or in the watershed (wild donors) have been negative for viral hemorrhagic septicemia virus (VHSV) and infectious hemorrhagic necrosis virus (IHNV) for at least five years. The following procedures should be followed for all importations. However, the requirement for quarantine is waived if the five-year stipulation is met. Also, international importations require compliance with the Title 50 Code of Federal Regulations, Part 16, in the U.S. and with Canadian Fish Health Protection Regulations in Canada.

HISTORY OF PATHOGENS IN THE DONOR BROOD STOCK, SOURCE WATERSHED, AND HOLDING FACILITIES

Cultured Salmonid Fishes

Pathogens in Holding Facilities

All salmonid fish stocks in the donor's holding facility must have had at least three inspections during the past two years for all emergency and restricted pathogens (see Annex II of the Great Lakes Fish Disease Control Policy and Model Program for a list of emergency and restricted pathogens) by a recognized Fish Health Official. Inspections should conform to Annex V (Inspection Procedures and Methods of Diagnosis) of the Great Lakes Fish Disease Control Policy and Model Program. The history of inspection must demonstrate the absence of emergency pathogens. Information on the detection of any restricted or other pathogens must also be documented.

Pathogens in Parents

1) At the time of spawning, all parents must be sampled and inspected in conformance with Annex V (Inspection Procedures and Methods of Diagnosis) of the Great Lakes Fish Disease Control Policy and Model Program. No eggs will be accepted for importation if emergency pathogens are detected.

2) If restricted pathogens are detected in the parents, acceptability of eggs will be based on Annex II (Guidelines for the Control and Management of Pathogens) Covered by the Model Program).

3) Following water hardening in suitable concentrations of an organic-iodine disinfectant and a second disinfection at the spawning site, imported eggs must be shipped directly to a quarantine facility for holding prior to release.

Wild Salmonid Fishes

Pathogens in the Source Watershed

1) All salmonid fish species in the source watershed, including the donor stock, must have had at least two consecutive, annual inspections at the time of spawning for all emergency and restricted pathogens by a recognized Fish Health Official using procedures outlined in Annex V (Inspection Procedures and Methods of Diagnosis) of the Great Lakes Fish Disease Control Policy and Model Program. The history of health inspection must demonstrate the absence of VHSV and IHNV. Information on the detection of any restricted or emergency pathogens must be documented.

2) All salmonid fish-culture facilities in the watershed must be inspected as defined above for cultured salmonid fishes.

Pathogens in Parents

1) All parent fish must be killed and sampled at the time of spawning. Inspection for emergency and restricted pathogens should conform to Annex V (Inspection Procedures and Methods of Diagnosis) of the Great Lakes Fish Disease Control Policy and Model Program.

2) No eggs will be accepted for importation if VHSVor IHNV is detected in parent fish or in other salmonid fish inhabiting the donor's watershed.

3) If other emergency or restricted pathogens are detected, acceptability of eggs will be based on Annex II (Guidelines for the Control and Management of Pathogens Covered by the Model Progam) of the Great Lakes Fish Disease Control Policy and Model Program.

4) Following water hardening in suitable concentrations of an organic-iodine disinfectant and a second disinfection at the spawning site, imported eggs must be shipped directly to a quarantine facility for holding prior to release.

QUARANTINE

Facility Design

1) An approved quarantine facility is a physically separated, enclosed culture system that permits the isolation and maintenance of fish while preventing their introduction into the environment. The incoming water source should be from a groundwater supply. If a groundwater supply cannot be found, a closed surface-water supply is acceptable if it is free of fish and treated to be free of all fish pathogens associated with emergency and restricted diseases. All facility effluent must also be treated to prevent the transmission of fish pathogens. The quarantine facility must be physically separated and isolated from all other fish stocks. This separation includes personnel, equipment, and fish feed. Importers are encouraged to submit plans for quarantine facilities to the GLFHC for review.

2) Each quarantine facility should have an egg-receiving area that is isolated from rearing units. Rearing units should also be physically separated from each other. The receiving area may be installed as an isolated part of each rearing unit within the quarantine facility or it may be entirely separate from rearing units. Access should be designed to preclude contamination of rearing units when eggs are delivered. Imported eggs should be brought into the receiving area, surface disinfected, and transferred into a rearing unit where they will remain until quarantine is complete. Contact between personnel in the egg-receiving area and the remainder of the quarantine facility should be avoided. Anyone who is disinfecting eggs would not make transfers directly to anyone who is inside a rearing unit. Transfers would take place through a third person not in contact with the incoming eggs before disinfection. Disinfectant handwashes, footbaths, and appropriate clothing would be utilized by all staff inside each rearing unit and egg-receiving area. All packing materials and water shipped with eggs must be immediately incinerated or chlorine sterilized within the egg-receiving area.

3) Appropriate environmental agencies should be consulted regarding methodologies and procedures available to achieve a rearing-unit effluent free of fish pathogens. Each rearing unit should also have a backup system available to treat effluents in case the primary system fails. It should also have an alarm system to signal a failure of the primary system.

Facility Disinfection

1) Disinfection of the egg-receiving area and rearing unit is required preparatory to each delivery of eggs. These disinfections should proceed according to accepted protocols (Meyer et al. 1983).

2) If emergency or restricted pathogens are detected, procedures for disposal of fish and disinfection of the rearing unit are required as in Annex II (Guidelines for the Control and Management of Pathogens Covered by the Model Program) of the Great Lakes Fish Disease Control Policy and Model Program. If an emergency pathogen is detected, sentinel fish will be used to verify the effectiveness of required disinfections. At least 150 fish of a species and size susceptible to the pathogen(s) detected in the rearing unit will be used as sentinels. These fish will be held for at least 120 days following disinfection. All mortalities of sentinel fish must be monitored. If possible, surviving sentinel fish will be subjected to a heat stress test. All sentinel fish must be disposed of in the manner described in Chapter 14 of Meyer et al. (1983).

Operation and Maintenance

1) **Personnel.** Access to a quarantine facility should be limited to designated personnel. These personnel should be properly trained in operational procedures.

2) **Records.** A fully completed Salmonid Quarantine Report (see Appendix) must accompany each lot of fish held at a quarantine facility. A copy will be submitted to the GLFHC with the semiannual Hatchery Classification Report.

3) **Disinfection Stations.** Each rearing unit must have a disinfection station. This station must include the following: handwashes, footbaths (sunken preferred), and a change of outer clothing (laboratory coats and boots).

4) **Equipment.** Each egg-receiving area and rearing unit will be independent with respect to all equipment and supplies.

5) **Disposal of Daily Mortalities.** Guidelines for inspecting daily mortalities of fish in quarantine are provided in the next section. Daily mortalities not required for inspection must remain in the rearing unit and be placed in an appropriate disinfectant or fixative. These fish must then be bagged for removal from the quarantine facility and disposed of as described in Meyer et al. (1983).

6) **Disinfection Procedures.** Disinfecting solutions will be monitored daily to maintain an effective dose. Outer clothing should be cleaned after each use, and the entire quarantine facility should be routinely cleaned with disinfectants.

Duration of Quarantine

All fish should be quarantined for a minimum period of six months beginning after their first feeding.

INSPECTION AND MONITORING OF FISH

During Quarantine

Fish in quarantine should be monitored and inspected monthly for emergency and restricted pathogens. Daily mortalities need not be assayed unless they are unusual in number or exhibit clinical signs of disease.

In addition to monthly inspections, a heat stress test is required two months prior to the expected release date from quarantine. A minimum of 150 fish should be held for 14 to 21 days at an elevated temperature. All fish must be injected or fed with an immunosuppressant at the beginning of the test. The numbers of fish inspected for emergency and restricted pathogens (normally 150) must be adequate to demonstrate a 2% level of disease prevalence at a 95% confidence level.

Following Quarantine

1) All imported fish should receive a tag or unique mark before planting.

2) Tagging or marking of fish will occur only after all inspection results are known. Tagging and marking will not occur in a quarantine facility.

3) The results of all inspections, vaccinations, tagging, and final destination of fish will be described in the Salmonid Quarantine Report (see Appendix).

ASSESSMENT FOR PATHOGENS IN IMPORTED FISH AFTER RELEASE

Monitoring Plan

An importer must prepare a plan for monitoring introduced fish for emergency pathogens after their release from quarantine. This plan should include isolating at least 300 of the imported fish in captivity to the end of the first generation, or to a maximum of three years for fish with longer life cycles. Annual inspection of captive fish for emergency and restricted pathogens is required and must be based on a statistically valid sample. An inspection at spawning is also desirable.

Response to Emergency Pathogens

If emergency pathogens are detected in captive fish during a monitoring period, all fish in the associated rearing unit must be destroyed and that unit must be disinfected. Any released fish that are recaptured should also be destroyed.

STATUS OF FACILITIES RECEIVING FISH AFTER RELEASE FROM QUARANTINE

The disease classification of a facility receiving fish released from quarantine will be unchanged except where restricted or emergency pathogens are confirmed in released or captive fish. If these agents are not compatible with the disease classification of the receiving facility, that facility's classification will be revised in accordance with Annex IV (Hatchery Classification) of the Great Lakes Fish Disease Control Policy and Model Program.

STATUS OF QUARANTINE FACILITIES FOLLOWING THE QUARANTINE PERIOD AND DISINFECTION

The disease classification of a rearing unit should be consistent with Annex IV (Hatchery Classification) of the Great Lakes Fish Disease Control Policy and Model Program. Disinfection should follow procedures identified in Meyer et al. (1983). In addition, heat stress testing after disinfection is required if emergency pathogens are detected in a quarantine facility or in fish released from quarantine.

ACKNOWLEDGMENTS

This Protocol was developed over a period of several years by the members of the GLFHC, and their foresight, initiative, and perseverance are gratefully acknowledged. One GLFHC member, John Hnath, is singled out for his extensive efforts in coordinating the development of this Protocol. Also noteworthy was Great Lakes Fishery Commission staff support from Margaret Dochoda.

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APPENDIX
SALMONID QUARANTINE REPORT

Copies of the Salmonid Quarantine Report (shown on the next page) are available from the Great Lakes Fishery Commission.

Factors Controlling the Susceptibility of Chinook Salmon to Bacterial Kidney Disease


Leslie Dorn photo

Anthony Murray, Maureen Purcell, Diane Elliott, Stewart Alcorn, Dorothy Chase, and Ronald Pascho

WFRC-Seattle

Introduction

- *Renibacterium salmoninarum* (Rs) is the causitive agent of bacterial kidney disease (BKD) in salmonids.
- Transmission of Rs:
  - Horizontally, fish-to-fish
  - Vertically, from female parent to egg

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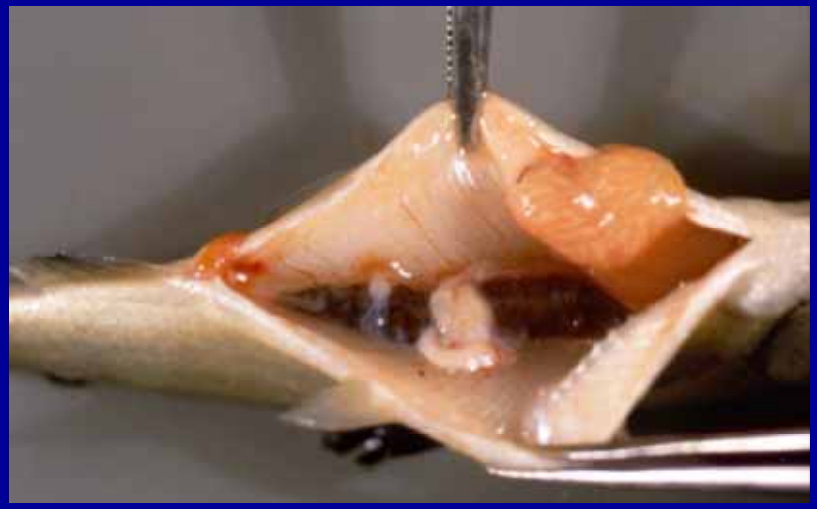
Introduction



- Control of BKD is difficult:
  - Antibiotic chemotherapy is only partially effective.
  - No effective vaccines have been developed.

Introduction

- Development of effective methods for BKD control has been hindered by a lack of understanding of the mechanisms controlling the susceptibility of salmonids to the disease.

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Lake Michigan

Susan Marcquenski photo

- Chinook salmon introduction
  - Initial stocking in 1967
  - Green River (Washington State) putative source

Lake Michigan

- During the late 1980s:
  - Increased Chinook salmon population density
  - Reduction of forage fish population (alewives)
  - Heavy parasite infestations
  - BKD outbreaks in Chinook salmon
  - Collapse of the salmon fishery

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Study Rationale

- Great Lakes Fishery Trust study: characterization of immune response of Chinook salmon to Rs
- Low mortality observed in cohabitation challenges involving Lake Michigan Chinook salmon stocks
- Increased resistance to BKD in Lake Michigan Chinook salmon?

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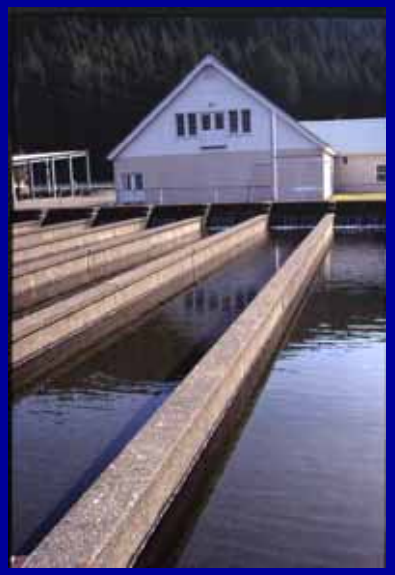
Outline of Initial Studies



- Chinook salmon used:
  - Abernathy fall Chinook (Pacific Coast)
  - Carson spring Chinook (Pacific Coast)
  - Root River fall Chinook (Lake Michigan)

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Outline of Initial Studies

- Rs isolates used:
  - ATCC 33209
    - Pacific Coast – Oregon
  - GL-64
    - Lake Michigan

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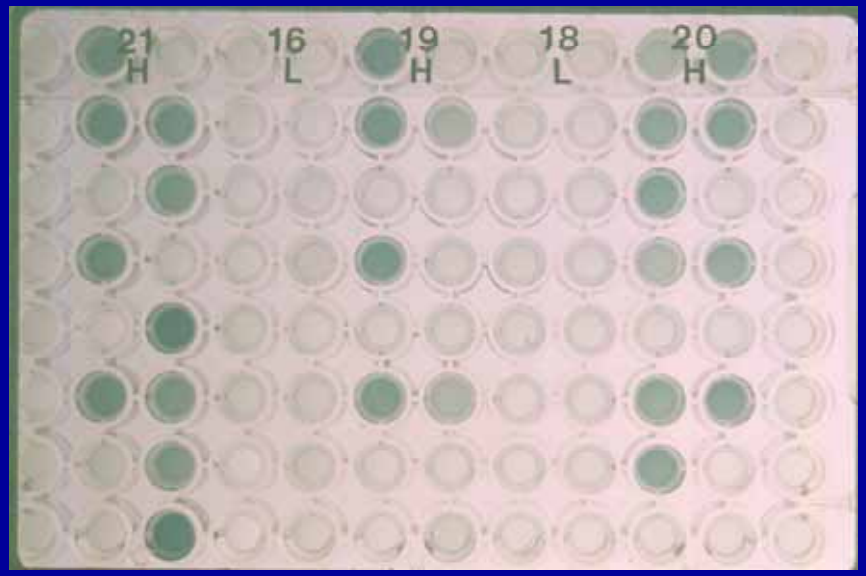
Broodstock Selection



- **ELISA**
  - Detects soluble antigen in kidney and ovarian fluid (OF)
  - Standard method for population screening

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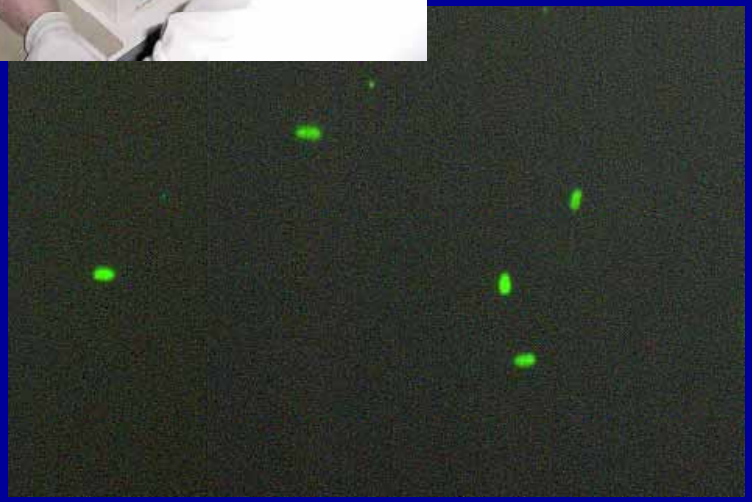
Broodstock Selection



- **Membrane filtration-fluorescent antibody test (MF-FAT)**
  - Direct detection of whole bacteria
  - Increased sensitivity in ovarian fluid

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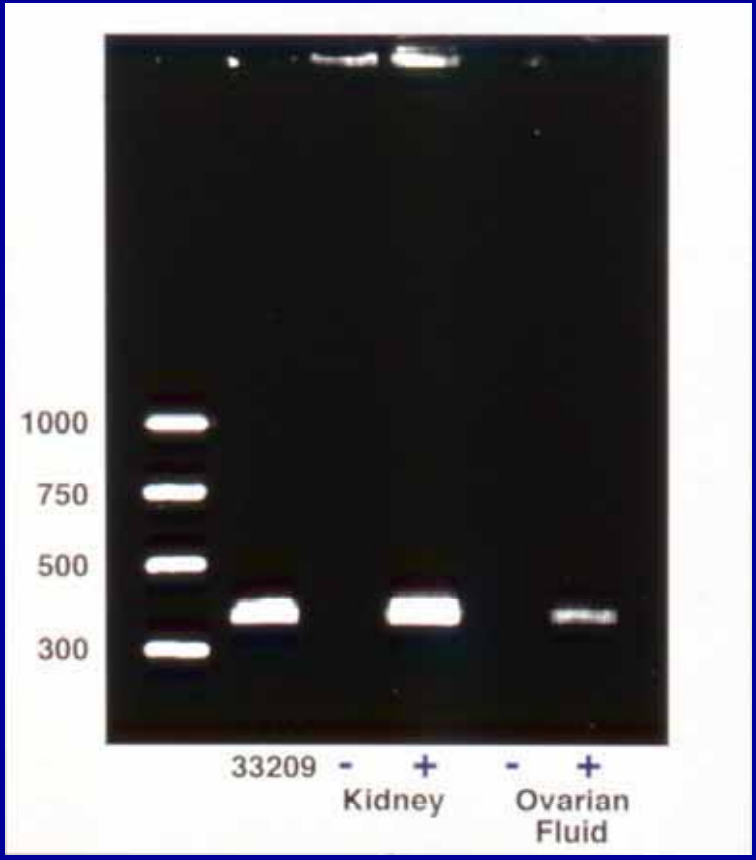
Broodstock Selection



- **Nested PCR**
  - Detects *R. salmoninarum* DNA in kidney and OF

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Broodstock Selection

Susan Marcquenski photo

- Selection criteria
  - ELISA
    - Negative kidney and ovarian fluid
  - MF-FAT
    - Negative ovarian fluid
  - Nested PCR
    - Negative kidney and ovarian fluid

All fish used were considered free of *R. salmoninarum*

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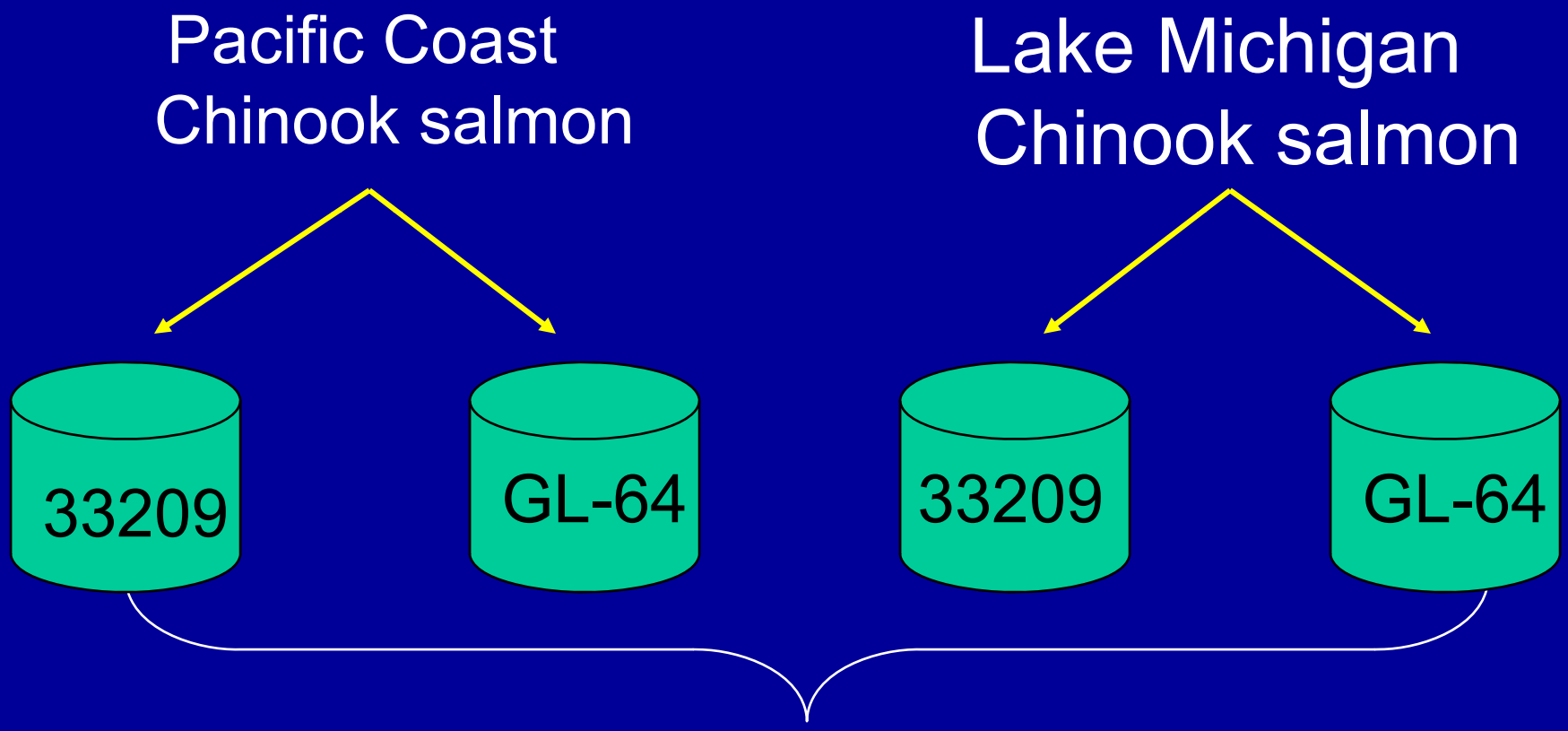
Methods



All fish were injected IP with 1.0 X 10^6 bacteria/fish

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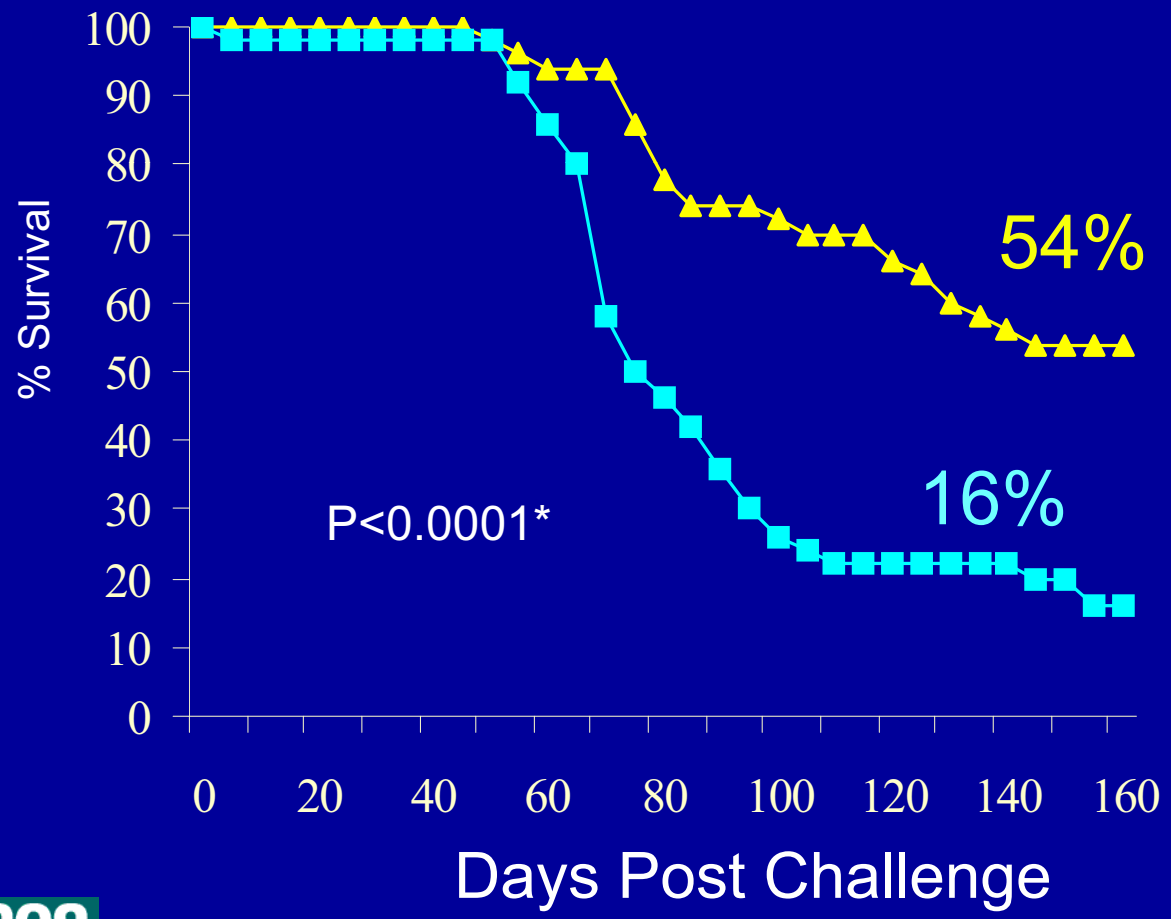
BY 1999 Fall Chinook Salmon Stocks Challenged with 33209



*Log-rank comparison of Kaplan-Meier Survival Curves

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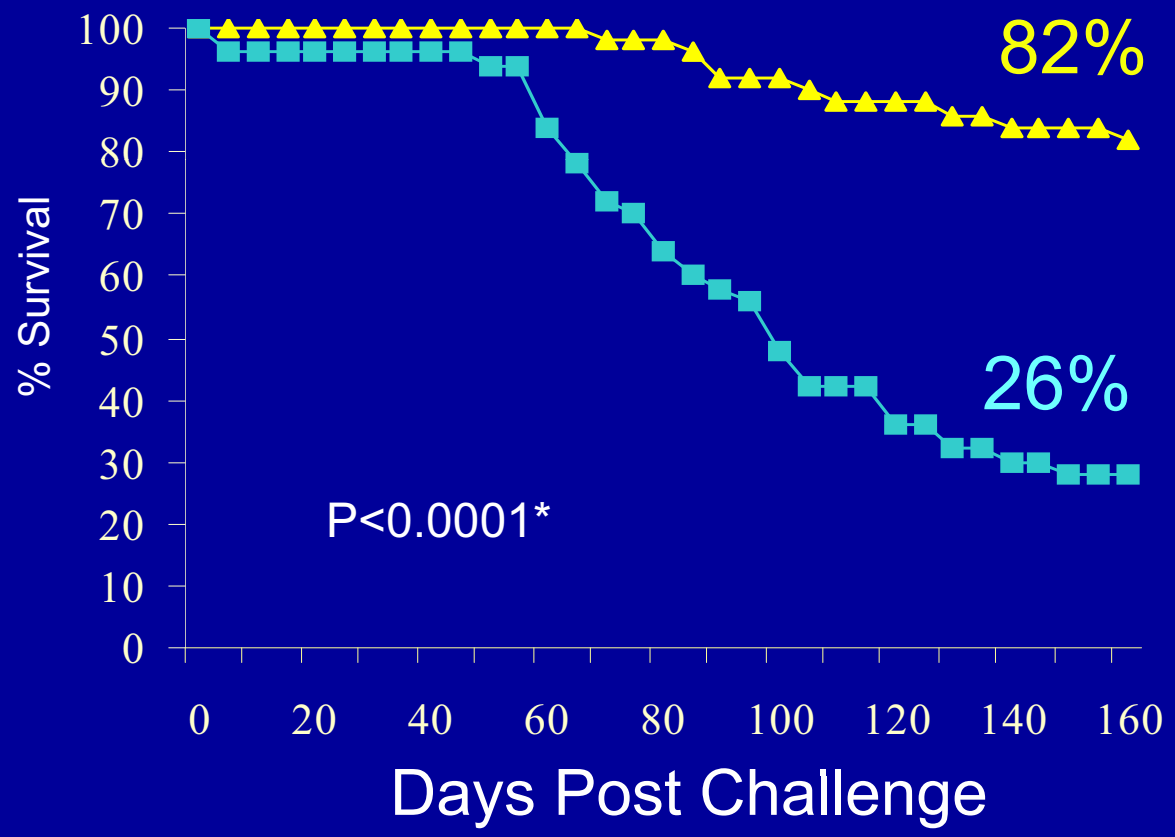
BY 1999
Fall Chinook Salmon Stocks
Challenged with GL-64



*Log-rank comparison of Kaplan-Meier Survival Curves

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Summary of Initial Challenges

- Survival higher for Lake Michigan Chinook salmon than for Pacific Coast Chinook salmon
- Challenge repeated using brood year 2001 and similar results were obtained
  - Pacific Northwest stock used in 2001 was a spring Chinook salmon stock

Summary of Initial Challenges

- Run type (spring vs. fall Chinook) did not affect pattern of disease resistance
- Disease resistance pattern similar for Lake Michigan and Pacific Coast Rs isolates

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Brood Year 2003
Comparison of BKD Disease Resistance

Goals:

- Compare the *R. salmoninarum* resistance of the Lake Michigan Stock (WI) to the putative progenitor stock
- Assess features of disease pathogenesis and immune response in the two stocks

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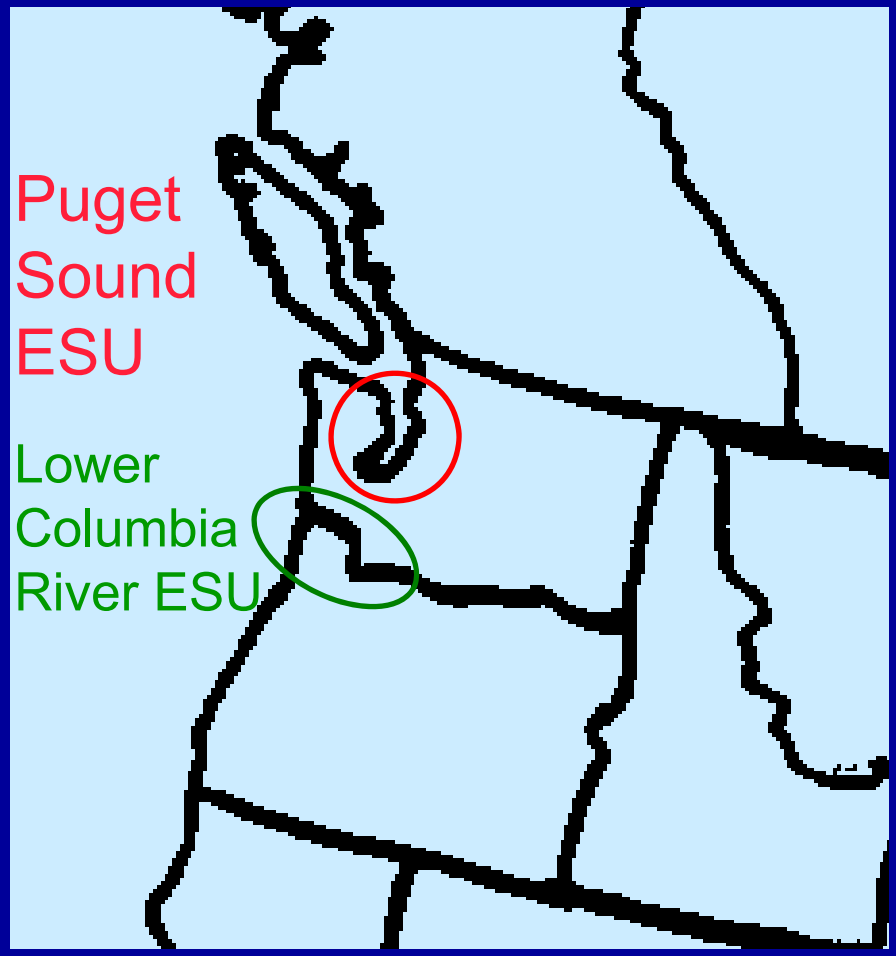
Origin of Contemporary Lake Michigan Chinook Salmon Stock?

- Putative source Green River Washington
  - Puget Sound evolutionary significant unit (ESU)
- Eggs from additional sources may have been used (?)
  - Cowlitz Hatchery Lower Columbia River ESU?



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Origin of Contemporary Lake Michigan Chinook Salmon Stock?

- Lake Michigan stocks genetically very similar to the Green River, WA stock
  - Weeder, Marshall and Epifanio (2005) North. Amer. J. Fish. Manage. 25:861-875
  - 18 allozyme markers examined for 7 different Michigan watersheds

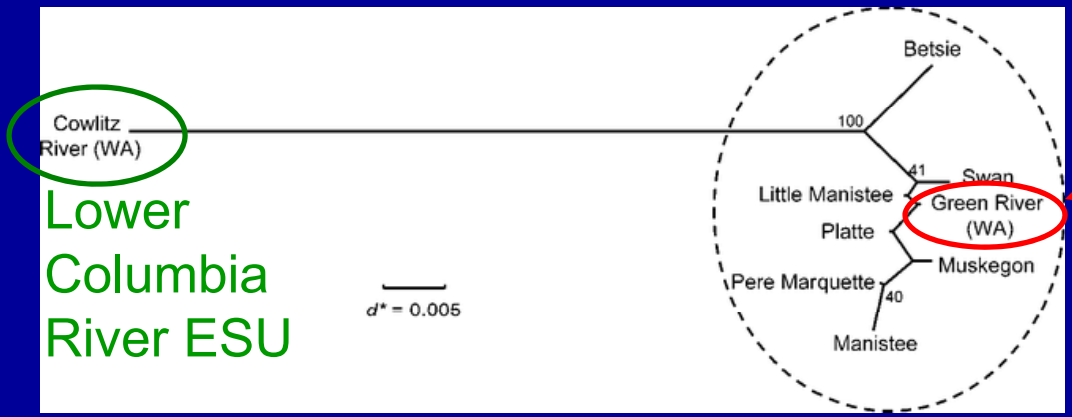


Figure 2 from Weeder et al. (2005)

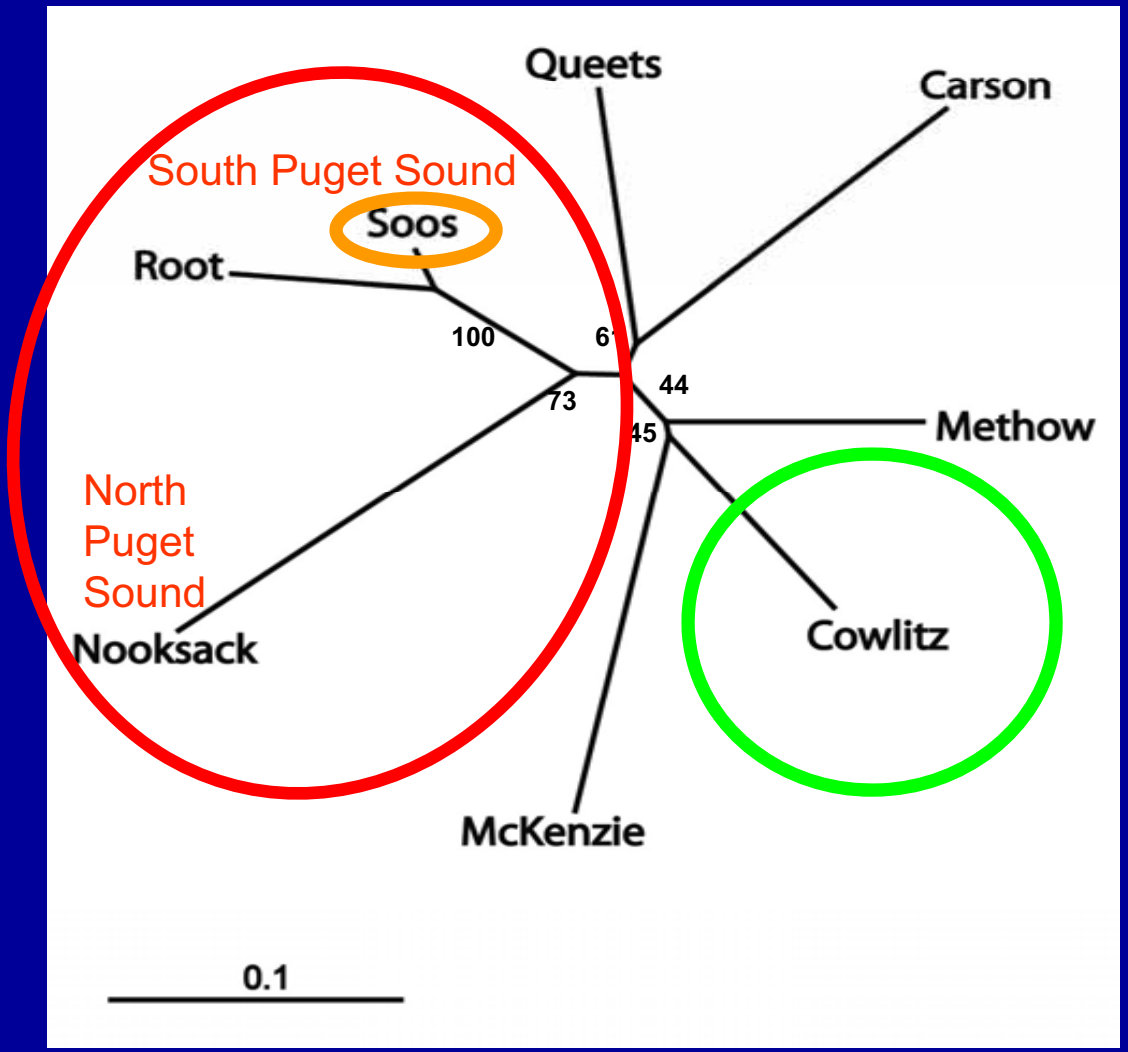
Origin of Contemporary Lake Michigan Chinook Salmon Stock?

- Wisconsin Chinook salmon stocks were not examined by Weeder et al.
- The Root River WI stock should be similar to MI stocks
- To test this assumption:
  - Thirteen microsatellite loci analyzed for the 2003 Root River brood year
  - Compared the Root River WI stock to selected populations in the Coastwide Chinook Salmon Population Genetic Database
    - Coastwide database developed through a collaboration of many different fishery agencies
    - Genetic analysis of Root River stock conducted by Anna Elz and Linda Park (Northwest Fisheries Science Center; NOAA)

Consensus Neighbor Joining Tree using Nei's D_a Genetic Distance using Microsatellite Markers

- Root River WI stock clusters within the Puget Sound ESU

- Most similar to the Soos Creek (Green River) Hatchery Stock



- WI Stock distinct from other ESUs including Lower Columbia River

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Conclusions
Population Genetic Analyses

- Analyses are still ongoing
  - Slight divergence observed between Soos Creek (Green River) and WI stocks
- Additional year classes will be analyzed
- Based on genetic similarity:
  - The Soos Creek hatchery stock (Green River, WA) appears to be appropriate for phenotypic comparisons to Lake Michigan Chinook salmon

Stock Comparison Brood Year 2003

- Lake Michigan fall Chinook (Root River, WI)
- Soos Creek fall Chinook (Green River, WA)
- Carson spring Chinook (Wind River, WA)
  - Used as a positive control for mortality study

Susan Marcquenski photo

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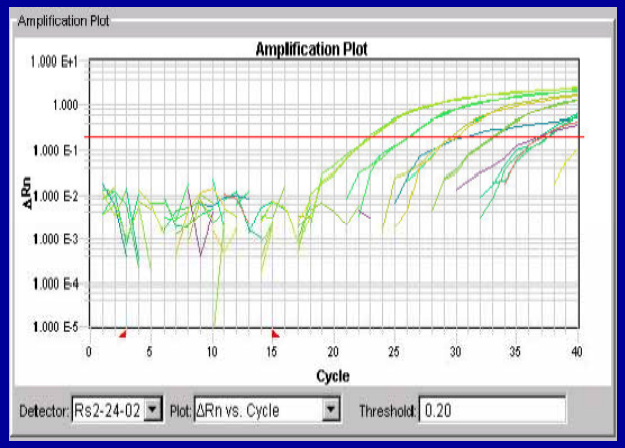
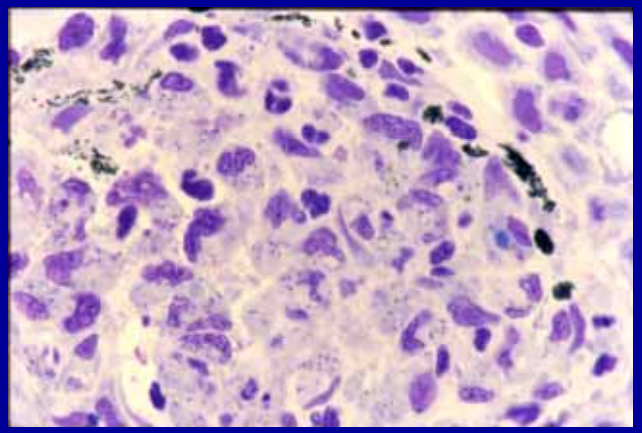
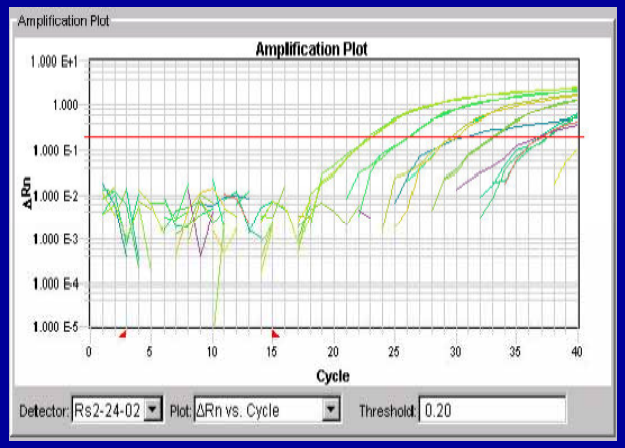
Stock Comparison Brood Year 2003

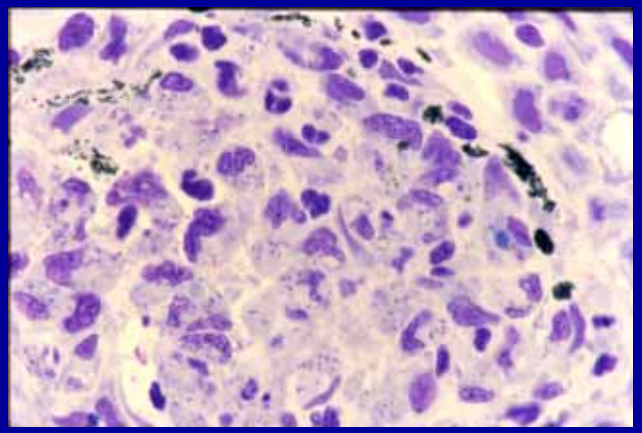




- Disease Resistance Phenotype
  - Measure survival after injection challenge
- Mechanisms of Resistance
  - Immersion challenge
    - *R. salmoninarum* progression/clearance
      - Antigen ELISA, quantitative PCR
  - Host Gene Expression
  - Humoral immune response
    - Antibody ELISA
  - Histopathology

Stock Comparison Brood Year 2003





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    - Antibody ELISA
  - Histopathology

Challenge Procedures

- Bacterial strain: ATCC 33209
- Injection challenge for mortality
  - Fish injected IP with $1x10^6$ bacteria/fish
  - Fish monitored for 160 days and mortality recorded

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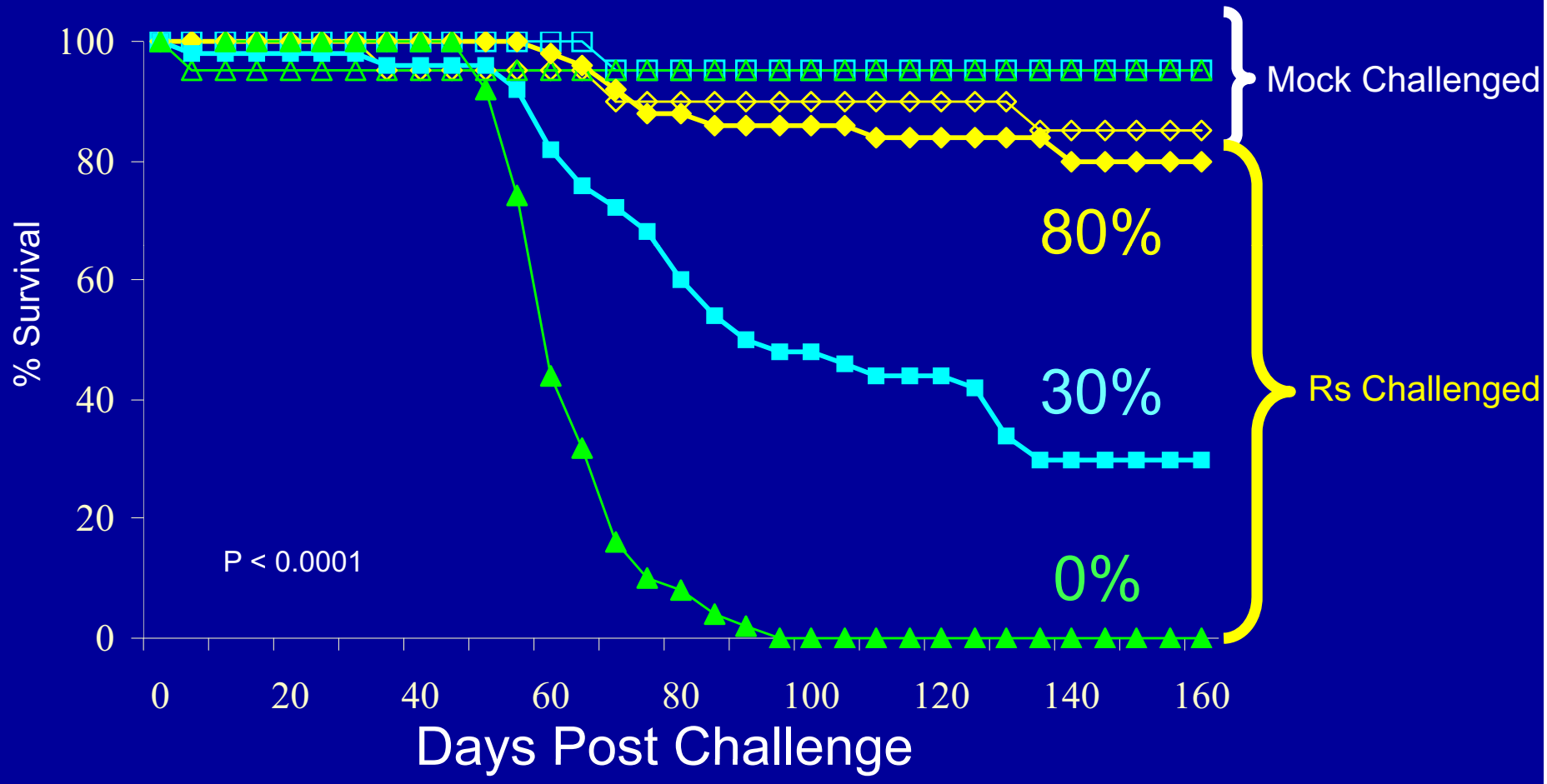
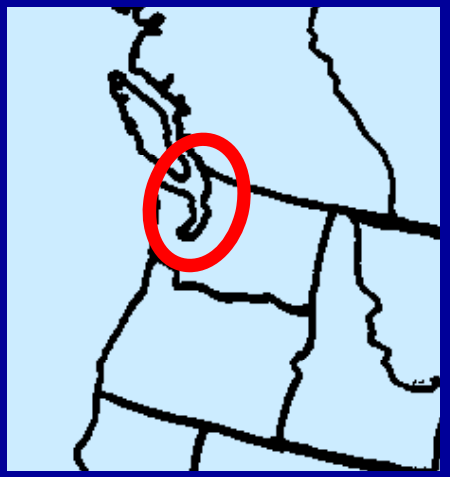
Brood Year 2003
Injection Challenge Results

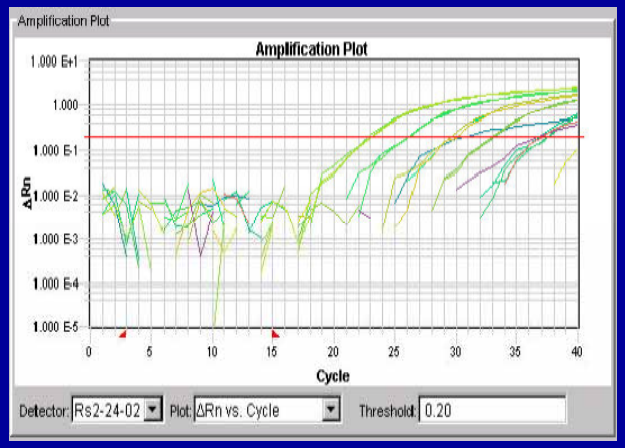


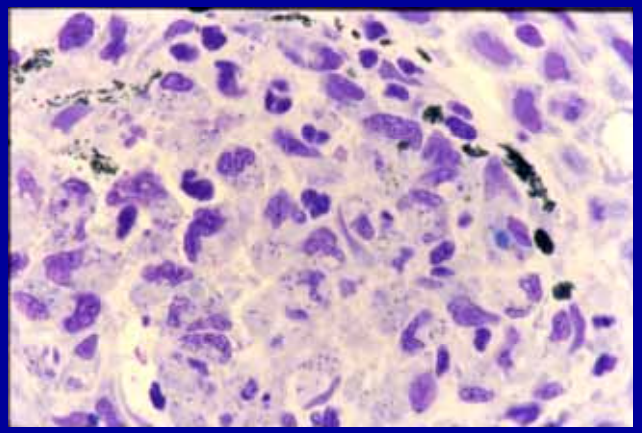
Stock Comparison
Brood Year 2003

- Summary of injection challenge
  - Phenotypic difference in BKD disease resistance between Lake Michigan stock and genetically similar Green River stock



Stock Comparison Brood Year 2003





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  - Host Gene Expression
  - Humoral immune response
    - Antibody ELISA
  - Histopathology

Challenge Procedures
Brood Year 2003

- Bacterial strain: ATCC 33209
- Immersion challenge
  - Fish challenged by 1-hour immersion in $4x10^6$ bacteria/mL
  - Fish monitored for 84 days with periodic sampling

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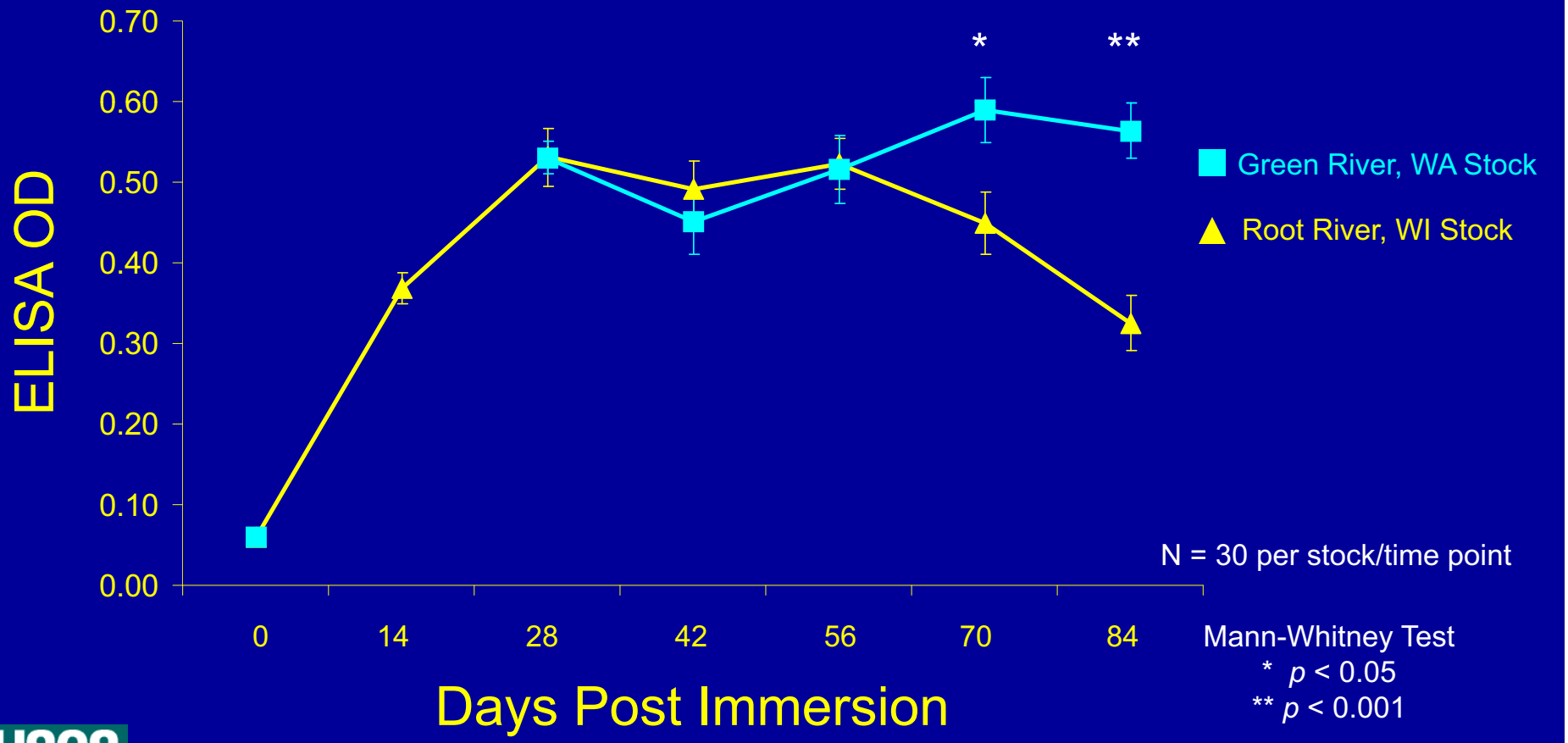
Stock Comparison
Brood Year 2003

Rs Antigen Levels in Kidney after Challenge



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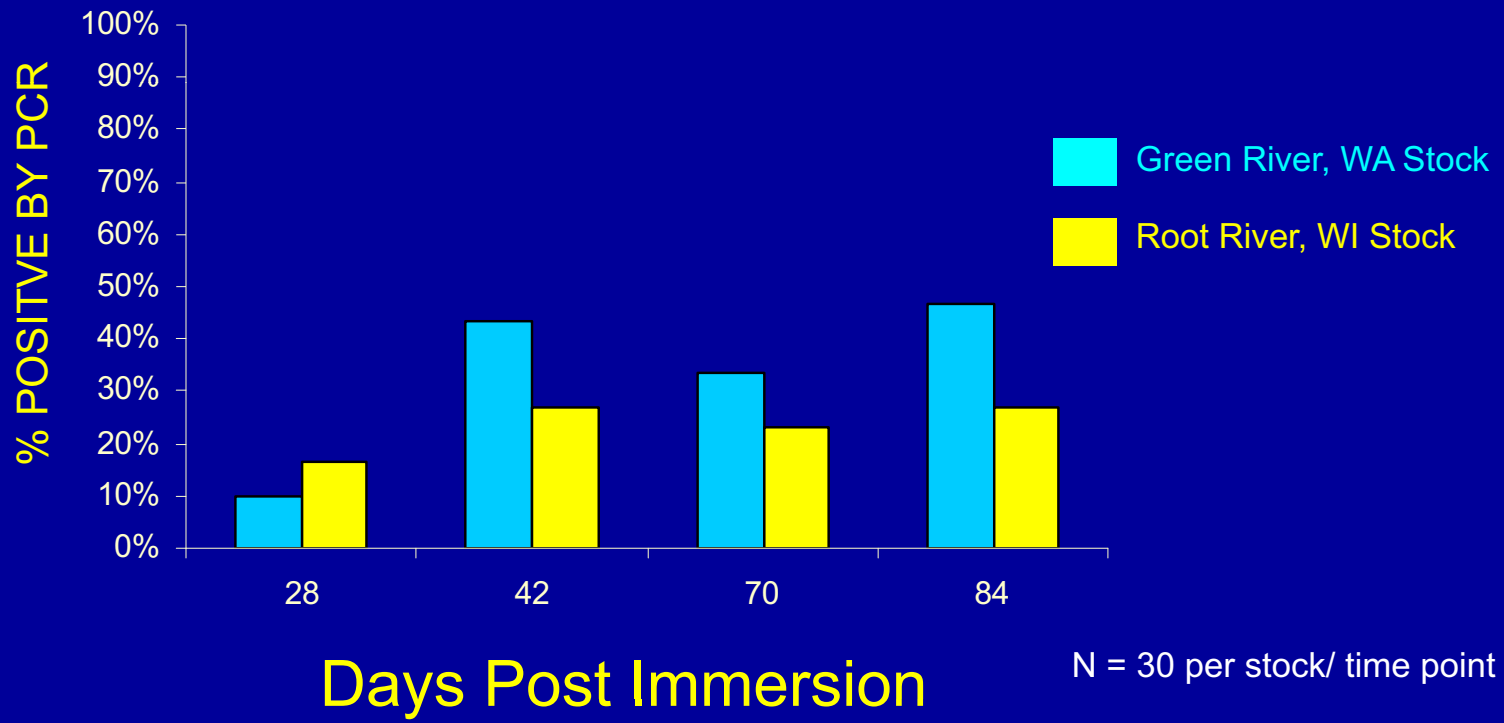
Stock Comparison
Brood Year 2003

% PCR Positive Individuals after Challenge



N = 30 per stock/ time point

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Stock Comparison
Brood Year 2003

- Summary
  - At later time points (≥ 70 days p.i.), the Lake Michigan Chinook salmon group:
    - Exhibited a significant reduction in Rs antigen levels relative to the Green River stock
    - Exhibited fewer PCR positive individuals relative to the Green River stock
      - Quantitative PCR analyses of Rs levels are still ongoing

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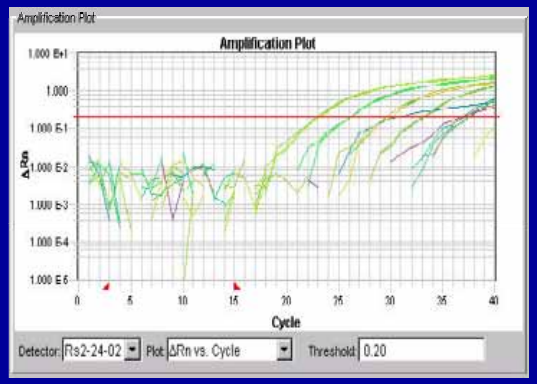
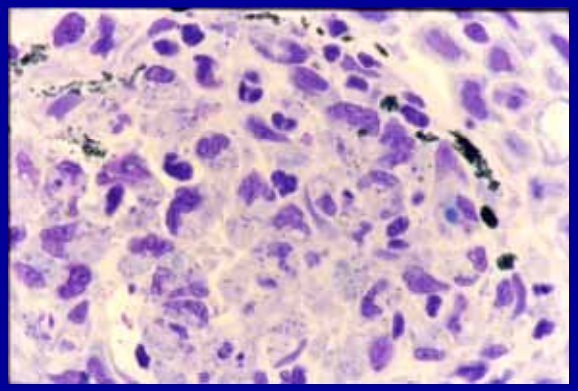
Stock Comparison
Brood Year 2003





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  - Humoral immune response
    - Antibody ELISA
  - Histopathology

Studies still ongoing
Preliminary Host Gene Expression Data

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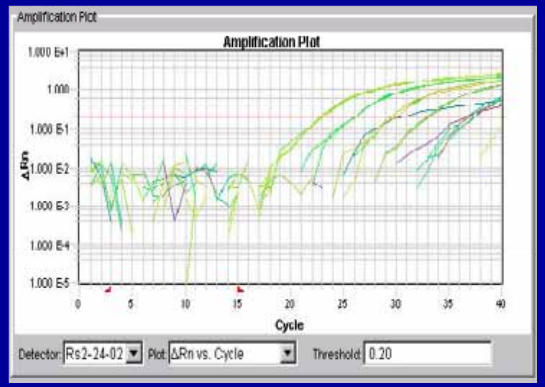
Stock Comparison
Brood Year 2003

- Host Gene Expression Preliminary Data
  - Examined expression of:
    - Pro-inflammatory cytokine (Interleukin 1 beta)
    - Phagocyte function (NADPH cytochrome oxidase phox p40 subunit)



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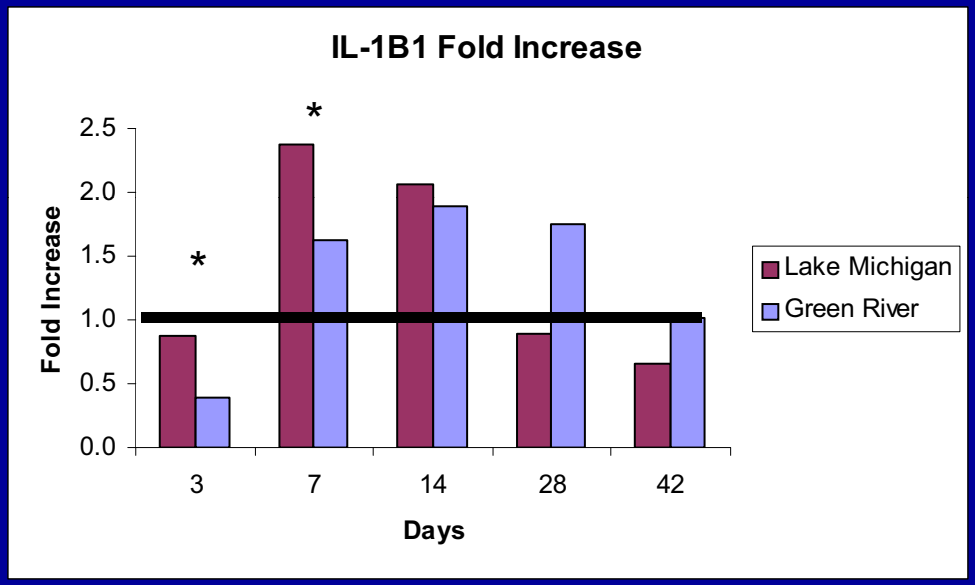
Stock Comparison Brood Year 2003

Expression of Pro-inflammatory Cytokine Gene

- Expression in units of fold change relative to mock group
  - Rs challenged/ Mock challenged
- Significant differences in expression on days 3 and 7



N =10 per group per time point
Groups compared by ANOVA using a Tukey's post-hoc test
*Significance $p < 0.05$

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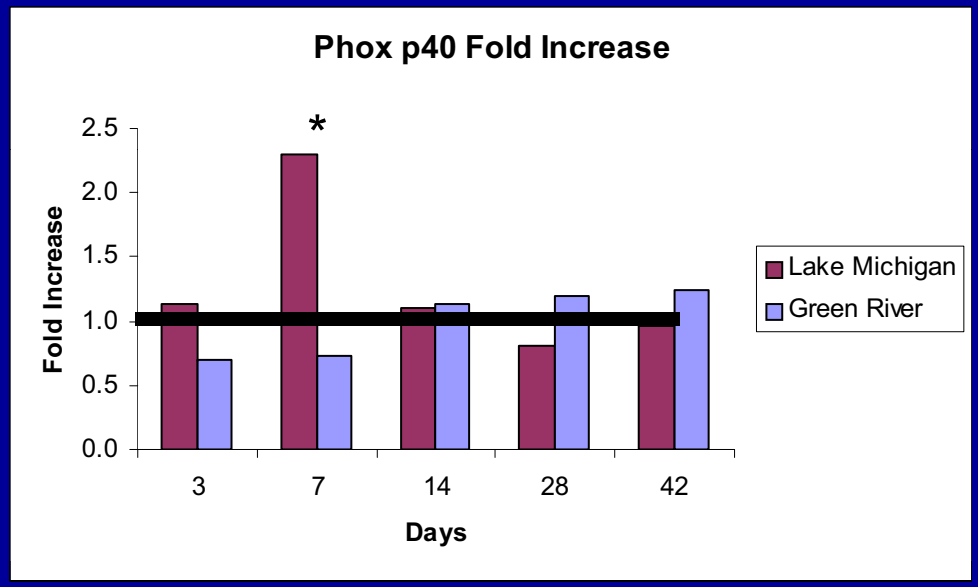
Stock Comparison Brood Year 2003

Expression of Phagocyte Function Gene

- Expression in units of fold change relative to mock group
  - Rs challenged/ Mock challenged
- Significant up-regulation of Phox p40 expresion in Lake Michigan stock on day 7



N =10 per group per time point
Groups compared by ANOVA using a Tukey's post-hoc test
*Significance $p < 0.05$

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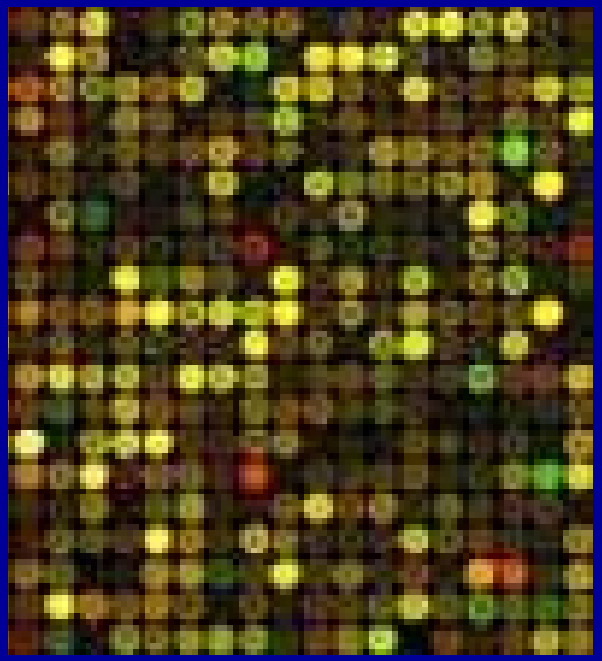
Stock Comparison Brood Year 2003

Host Gene Expression—Ongoing Studies

- Goal of the initial gene expression characterization is to determine a time point for microarray analysis
- Global gene expression changes will be assessed using the 16,000 feature Atlantic Salmon Microarray (GRASP V2)



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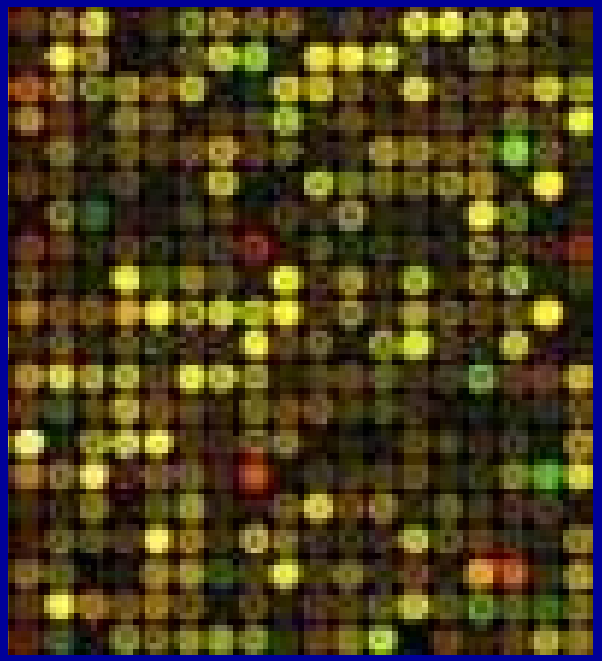
Stock Comparison Brood Year 2003

Host Gene Expression—Ongoing Studies

- Gene expression analyses will provide insight into the host immunological responses to Rs
  - they will not identify the genes ‘controlling’ disease resistance
  - Identifying causal genes requires genetic mapping strategies



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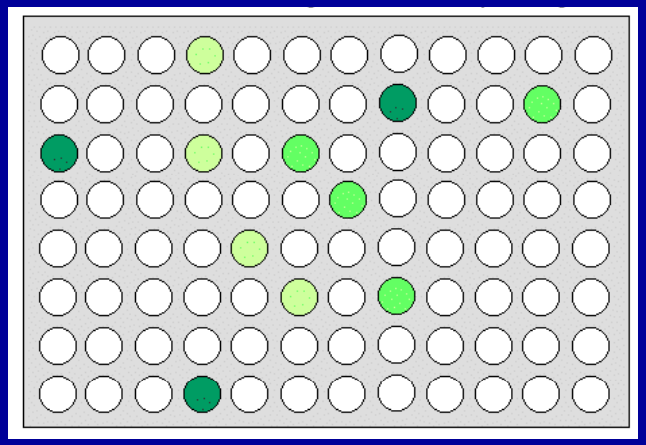
Stock Comparison Brood Year 2003

Other Ongoing Studies



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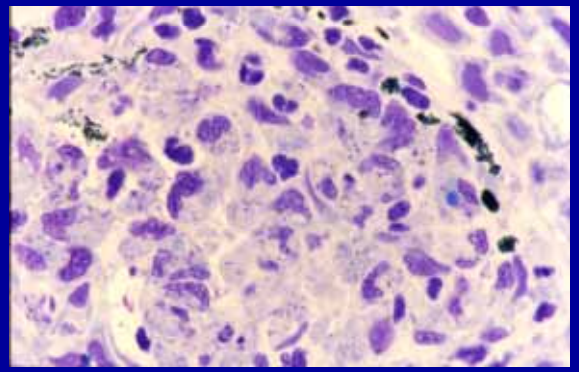

- Humoral immune response
  - Serum ELISA for anti-p57 antibodies
- Histopathology

Conclusions

- Lake Michigan Chinook salmon from WI are more resistant to BKD relative to the genetically similar Green River stock
- The resistant fish appear to have enhanced ability to clear the bacterium
- Ongoing studies seek to understand the host mechanisms of this resistance

Conclusions

- Lake Michigan Chinook salmon from WI are a useful model for studying mechanisms of BKD pathogenesis and host immunity
- In the future, crosses between the Wisconsin and Green River stocks may be useful for mapping and identifying the genes underlying BKD disease resistance


Susan Marcquenski photo

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Additional Areas of Research

- Can broodstock selection for resistance to BKD affect resistance to other diseases?
  - Results of earlier study suggested that broodstock selection based on levels of Rs could lead to increased susceptibilities to both BKD and to another bacterial disease, vibriosis
  - We are examining resistance to vibriosis in Lake Michigan Chinook salmon using 2005 BY fish

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Our Ultimate Goal

- Increase understanding of the mechanisms involved in *R. salmoninarum* resistance for improvement of BKD control measures.

Acknowledgements

WFRC
Lynn Applegate
Carla Conway
Connie McKibben

Soos Creek Fish Hatchery (WDFW)

Carson Fish Hatchery (USFWS)

Wisconsin DNR
Susan Marcquenski
Northeast Region Hatchery and Operations Crew
Kettle Moraine Springs Hatchery
Wild Rose Hatchery
Central Office Fish Health Expeditionary Forces

Funding: Great Lakes Fishery Trust, NOAA, USGS

Microsatellite Analysis of Wisconsin Chinook Salmon Stock

- All loci conformed to Hardy-Weinberg expectations
- Similar levels of heterozygosity

Population	N	Ho	He
Root River	48	0.81	0.79
Soos Creek	160	0.82	0.81

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Evaluation of Immune Function in Thiamine Deficient Lake Trout.

Dale C. Honeyfield, Chris Ottinger, Christine Densmore, and Phil McAllister,
USGS, Leetown Science Center.

USGS
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Support provided by

Great Lakes Fishery Commission

III. GLFC Fish Health Committee Research Priorities

Top research priorities

How severely does Thiamine Deficiency Complex impact important Great Lakes fish stocks and what can be done to minimize the effect?

Numerous Contemporary Fish Diseases of Interest

- BKD
- IPNV
- EEDV
- Furunculosis and others

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What affects immune function?

Evolution

Stress - Cortsol

Nutrition

Lipids	-Eicosanoids
Amino acids	- GSH
Minerals	- Zn, Se
Vitamins	- E, C, beta-carotene

Immune function
is an unexplored area of thiamine deficiency.

Thiamine

Co-factor in metabolic energy pathways
Krebs cycle

Production of ribose for DNA and RNA.
Transketolase in Pentose-phosphate shunt

Lake Trout Rearing

Lake trout (200-250 g) reared on diets that limited body stores of thiamine

but were adequate for growth and survival.

Lake Trout Rearing

2 groups: Adequate and Marginal thiamine.

Marginal group were fed a thiamine deficient diet (CBT) until signs of deficiency were observed.

Tissue sampling phase, fish fed

Adequate thiamine (2 mg/kg feed)

Marginal thiamine (0.4 mg/kg feed).

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	Thiamine	
	Replete	**Depleted**
*Total Erythrocytes (10^6 ul^{-1})	0.93	1.03
*Plasma Protein (g dl^{-1})	7.07	7.88
*Total Leukocytes (10^6 ml^{-1})	29.05	10.90
*Small Lymphocytes (10^6 ml^{-1})	25.70	6.65
*Monocytes (10^6 ml^{-1})	0.14	0.25

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Non-Significant	Thiamine	
	Replete	**Depleted**
Hematocrit (%)	**37.9**	**39.6**
Large Lymphocytes (10^6 ml^{-1})	**0.80**	**0.51**
Polymorphonuclear Cells	**2.30**	**3.59**

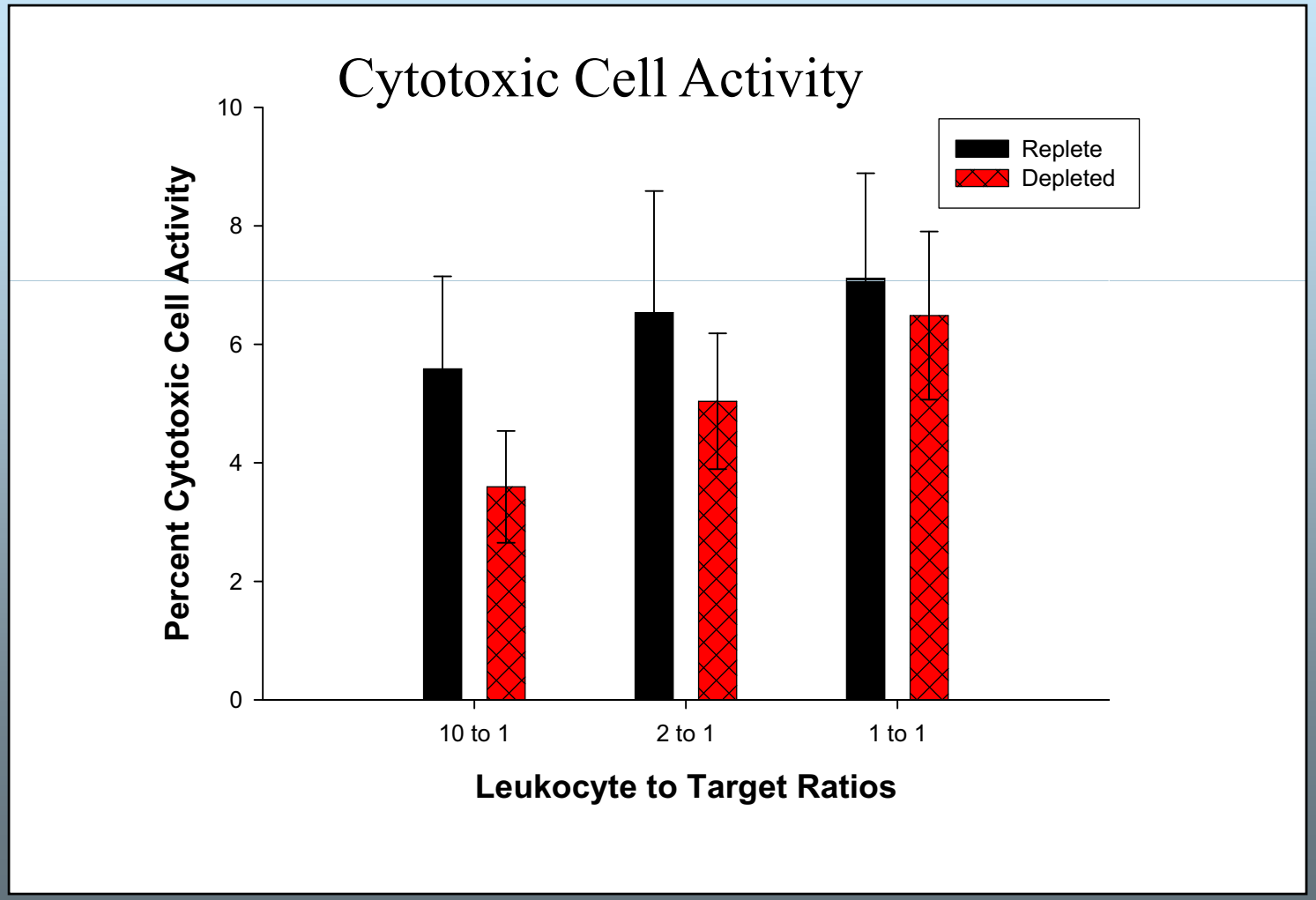
In vitro Immunoassay Methods

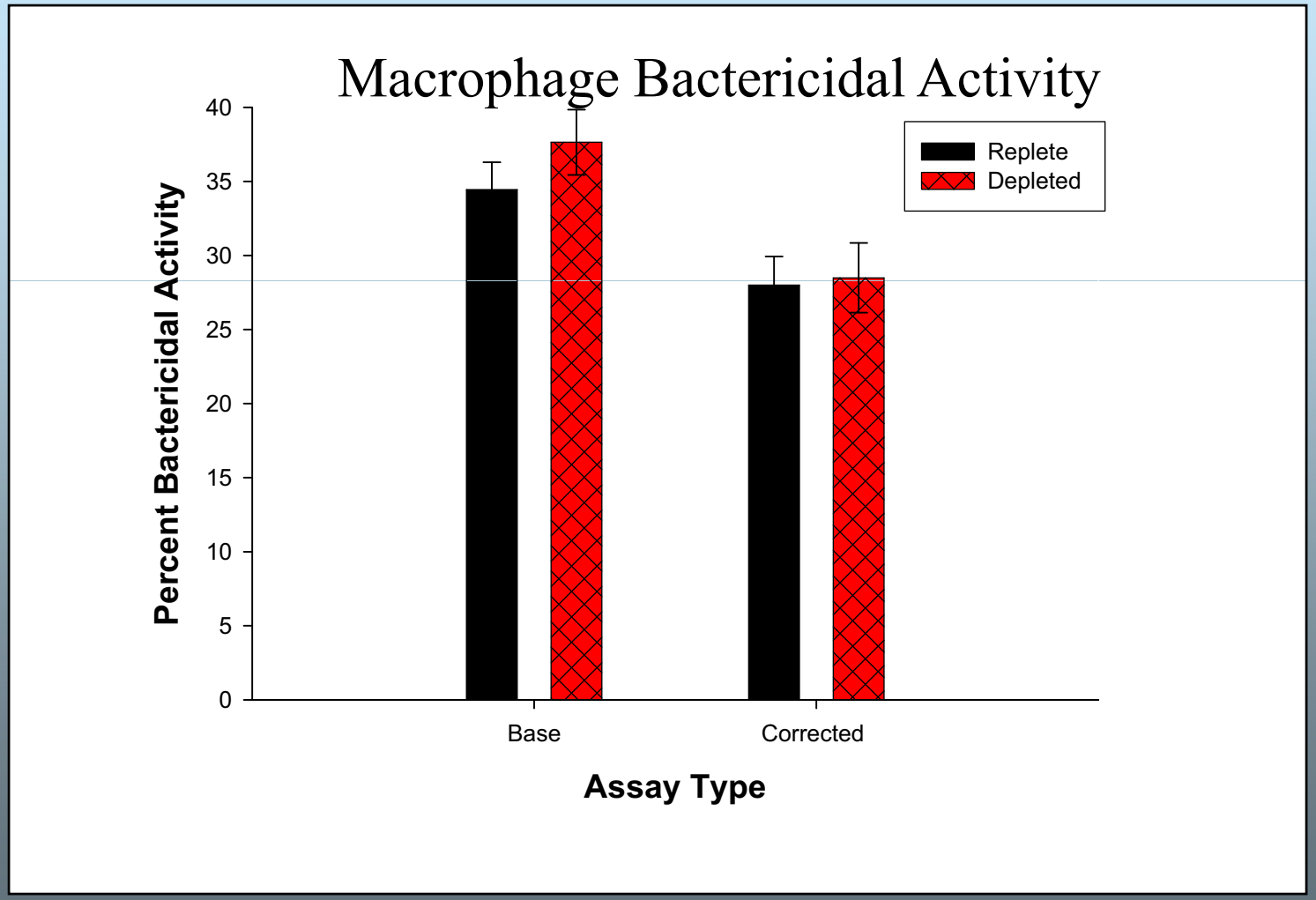
In vitro microplate assays:

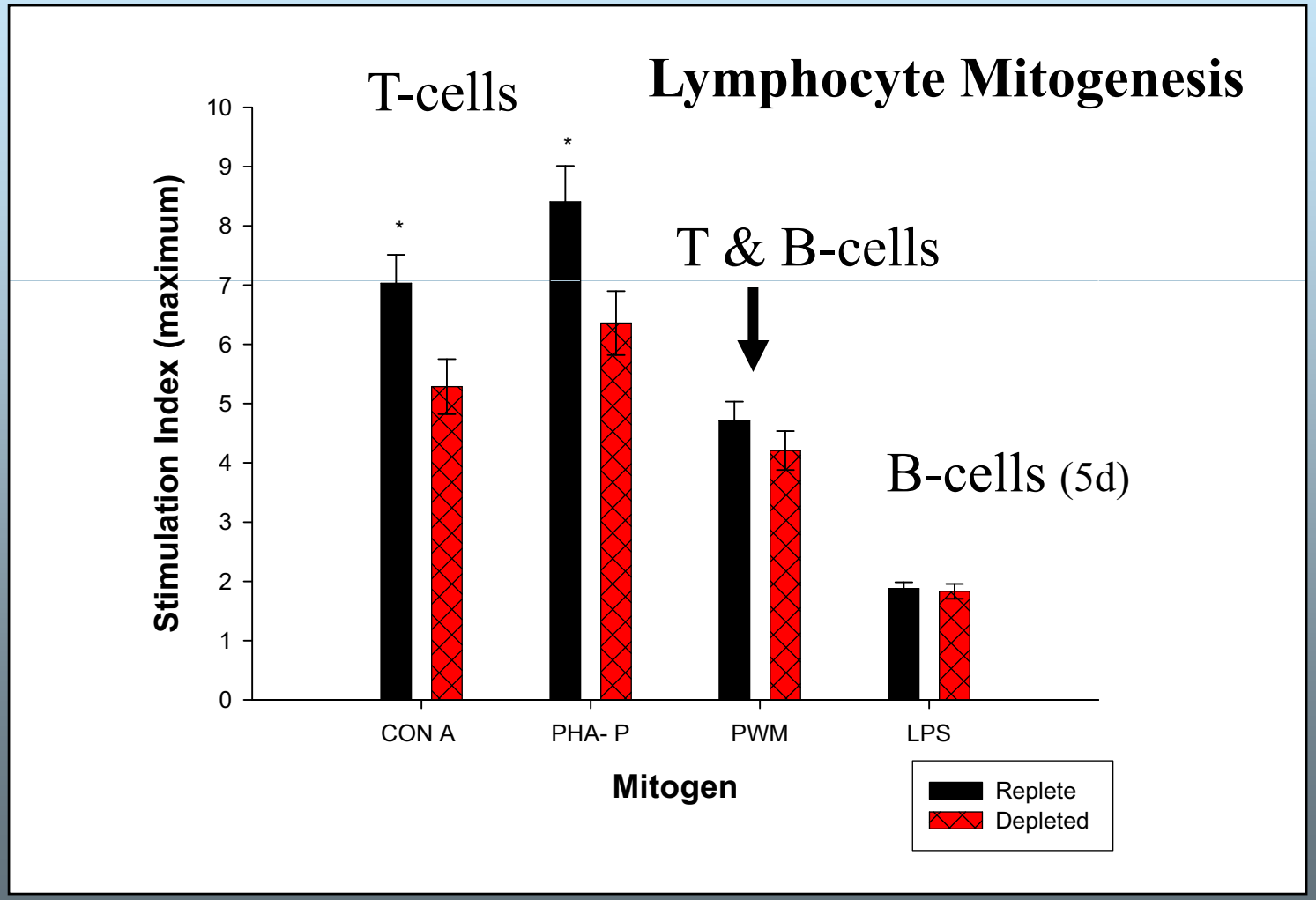
- **Cytotoxic cell activity**
- **Macrophage bactericidal activity**
- **Lymphocyte mitogenesis**

Anterior kidney leukocytes:

- **Purified on Percoll density gradients**


Cytotoxic Cell Activity
Replete
Depleted
Percent Cytotoxic Cell Activity
0
2
4
6
8
10
10 to 1
2 to 1
1 to 1
Leukocyte to Target Ratios


Macrophage Bactericidal Activity
Percent Bactericidal Activity
40
35
30
25
20
15
10
5
0
Replete
Depleted
Base
Corrected
Assay Type


Lymphocyte Mitogenesis
T-cells
T & B-cells
B-cells (5d)
Stimulation Index (maximum)
0
1
2
3
4
5
6
7
8
9
10
*
*
CON A
PHA- P
PWM
LPS
Mitogen
Replete
Depleted

Immune Function Summary

- **Macrophage bactericidal activity and cytotoxic cell activity does not appear to be impacted by the level of thiamine deficiency in this study**

Lymphocyte activity is differentially impacted with T-cell populations exhibiting reduced proliferation following mitogen stimulation.

B-cells do not appear to be impacted.

Implications

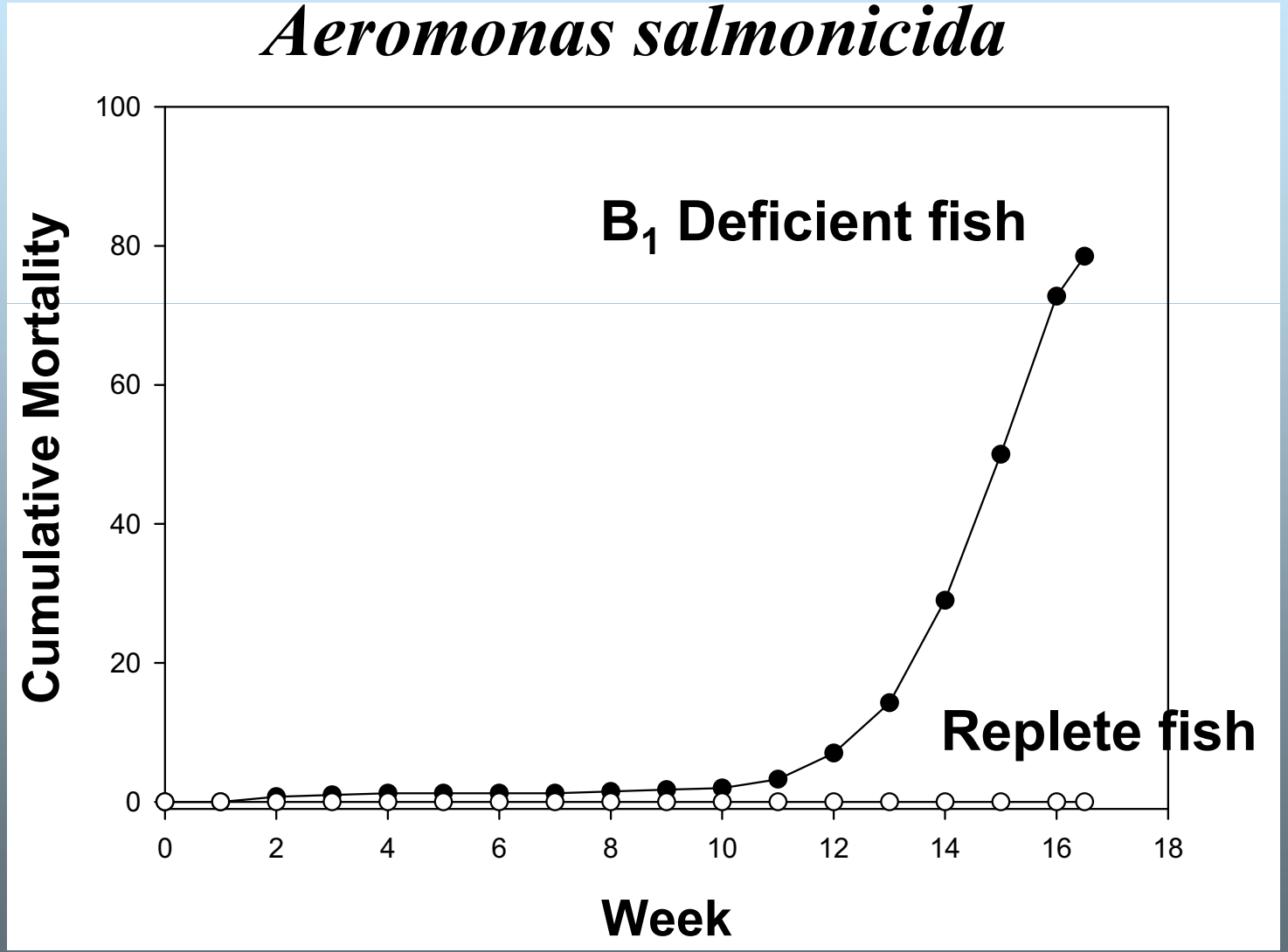
- **T-cells play a critical role in immunity to intracellular pathogens such as viruses.**
- **Thiamine depleted lake trout may be more susceptible to diseases caused by intracellular pathogens (IPNV, BKD).**

Disease Resistance in Thiamine deficiency

Thiamine Deficient and Replete lake trout fry

Aeromonas salmonicida

Viral (IPNV)


Aeromonas salmonicida
Cumulative Mortality
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
Week
B1 Deficient fish
Replete fish

Lake trout Fry Viral Challenge

Challenged with virulent and avirulent IPNV 43-47 days post hatch.

1-21 d post challenge a tendency for deficient fish to have lower median virus titer 100- to 1000-fold lower median virus burden

Lake trout Fry Viral Challenge

22-109 d post primary challenge

Virus-associated mortality was greater than 1-21 d for both groups in both avirulent and virulent virus exposures

In replete cohorts titer decreased ~ 10-fold
In deficient cohorts titer remained consistent.

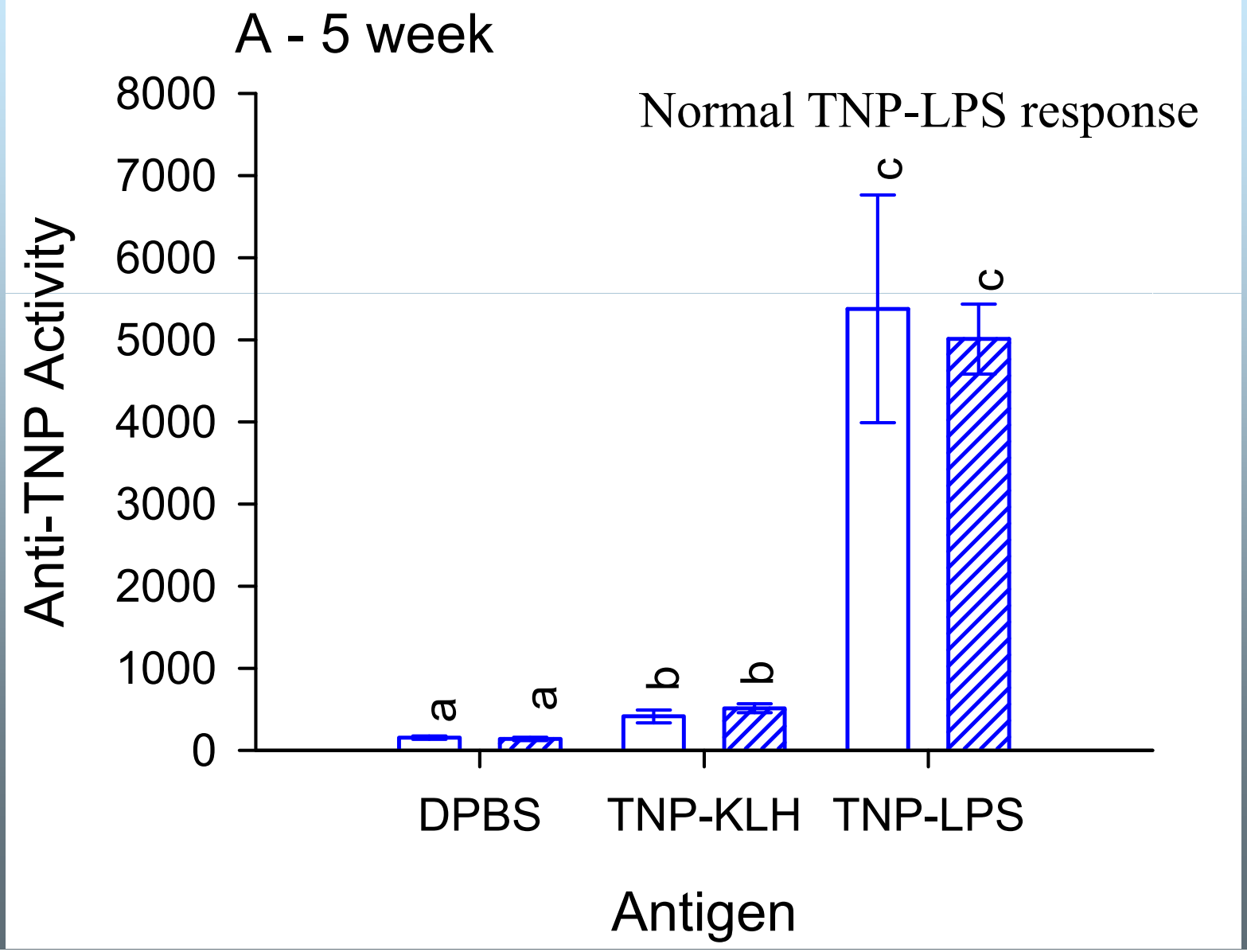
Summary of Viral Challenge

Although trends were evident the results do not strongly support IPNV infection being modulated by thiamine status

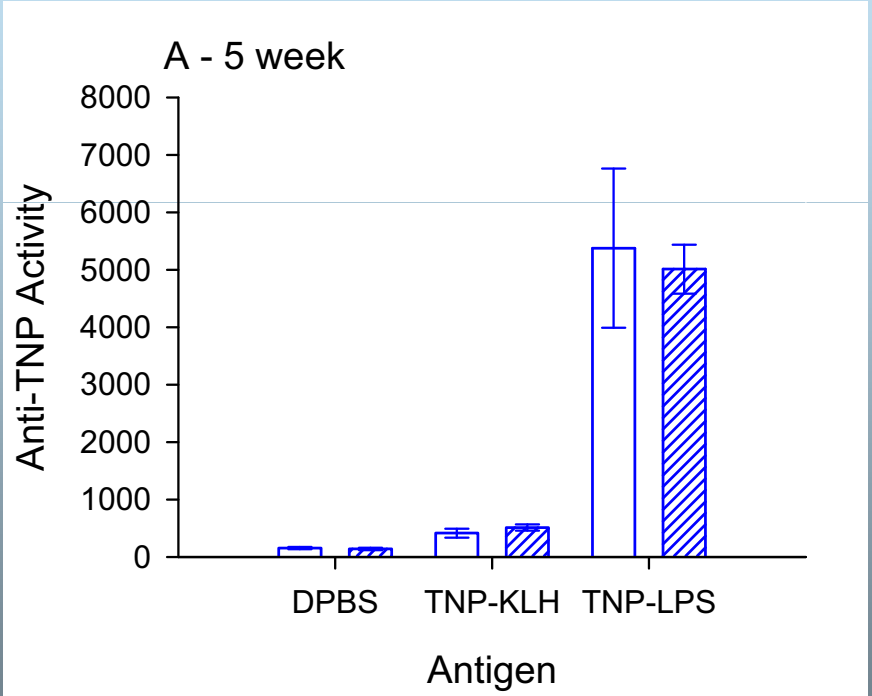
Antibody Production

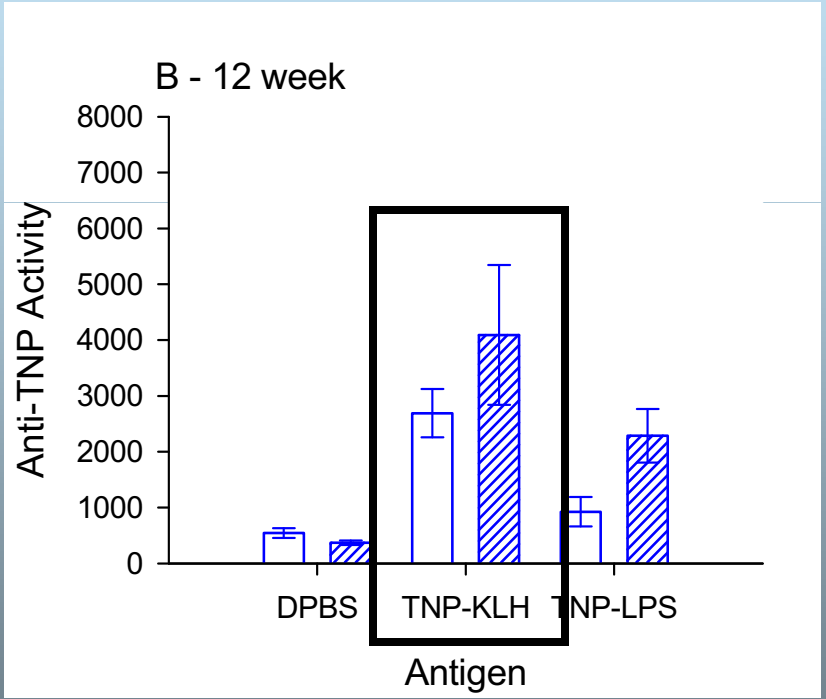
- Thiamine replete and depleted lake trout

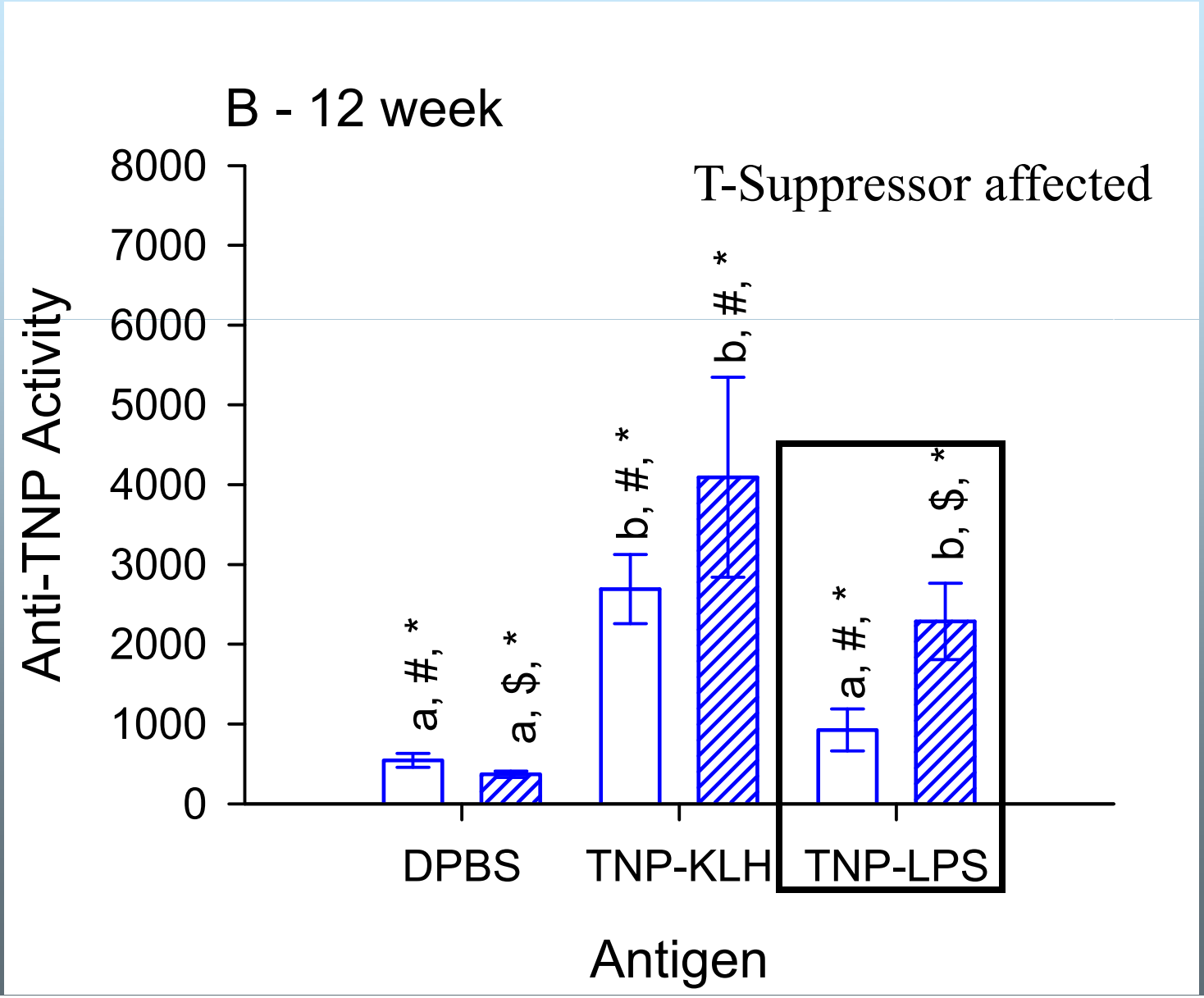
- Fish injected with
- T-cell dependent antigen
- T-cell independent antigen


A - 5 week
Normal TNP-LPS response
Anti-TNP Activity
8000
7000
6000
5000
4000
3000
2000
1000
0
a
a
b
b
c
c
DPBS
TNP-KLH
TNP-LPS
Antigen

T-Helper normal

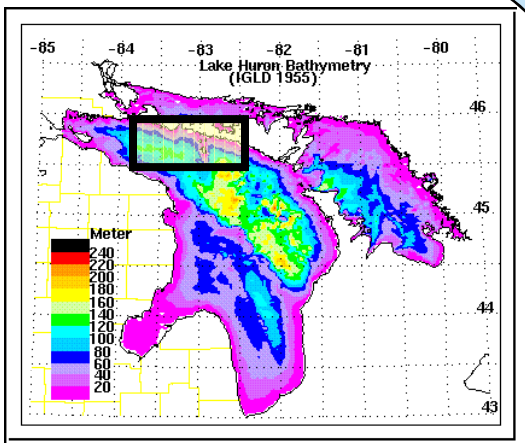





B - 12 week
T-Suppressor affected
Anti-TNP Activity
8000
7000
6000
5000
4000
3000
2000
1000
0
a, #, *
a, $, *
b, #, *
b, #, *
a, #, *
b, $, *
DPBS
TNP-KLH
TNP-LPS
Antigen


2005
Iroquois Bay
Drummond Is.
Frazer Bay
Parry Sound
Hammond Bay
South Bay
Thunder Bay
Six Fathom Bank
Ausauble Pt.
Yankee Reef
Owen Sound
Historic Spawning Grounds

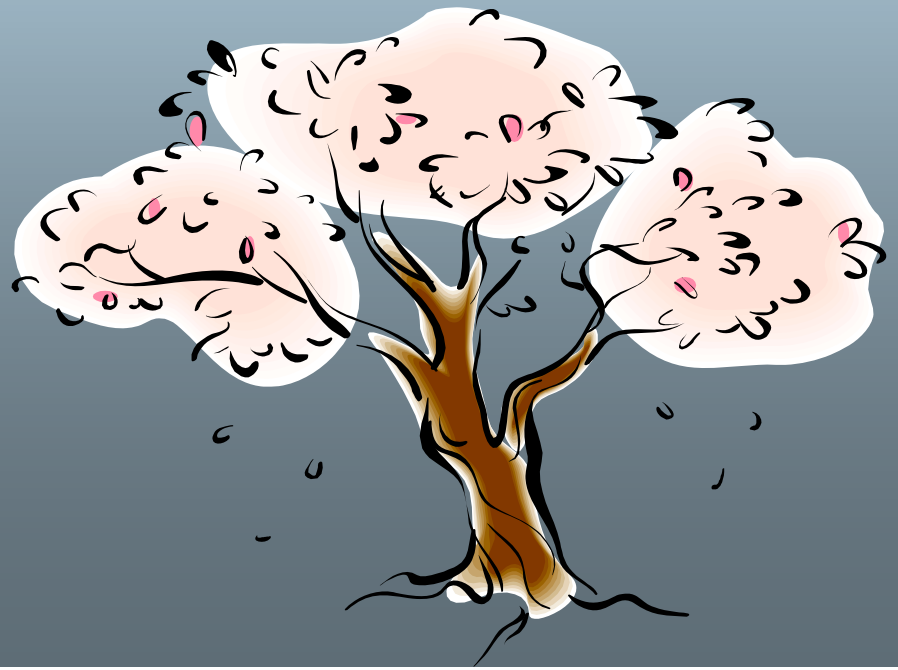
Drummond Island



	'01-'04	'05
n	45	70
Mean	3.86	7.05
nmol/g		
<1.5	17%	0%
<4.0	71%	21%

USGS
science for a changing world

Questions?



1. Cell proliferation is a critical component in immune responses involving lymphocytes. Mitogens are used to test lymphocyte ability to proliferate. Mitogens may be selective or semiselective in terms of the lymphocyte subpopulations they stimulate. Most of what is known about the selectivity is based on mammalian lymphocyte responses but fish responses are thought to follow the same patterns. The selectivity is as follows: Con A – T-cell; PHA-P – T-cell; PWM – T&B-cells; LPS – B-cells.

2. Lymphcyte mitogenesis was measure using ELISA based detection of bromodeoxyuridine incorporated into DNA during cell replication. The first set of assays were performed 2 days post mitogen stimulation based primarly on respnse profile about in previous studies. This time frame work well for CON A, PHA-P, and PWM but not for LPS. In the second set of assay we added an additional time point (Day 5 post stimulation). We were able to pick up the expected LPS response at this time) The data includes Day 2 (CON A, PHA-P & PWM) and Day 5 (LPS) responses. Data presented as mean ± SE (n = CON A replete – 34; CON A depleted – 35; PHA-P replete – 33; PHA-P depleted – 34; PWM replete – 33; PWM depleted – 31; LPS Day 5 replete – 23; LPS Day 5 depleted – 22) Asterisk indicates significant differences.

3. Data suggests that the thiamine depleted fish exhibit a very specific type of dysfunction. T-cell populations seem to be impacted by the deficiency.

USGS
science for a changing world

Sue M comments

Dale, just a quick read, but this is very interesting - especially when we look back on the Chinook die-offs in L. MI in the late 1980's- we have focused on the role of lipids in this die-off, but you and Phil show some info that suggests B1 may have played a role too.

Everyone calls this the "BKD" outbreak, but some fish that died did not have clinical BKD, but they had other bacteria such as Pseudomonas or A. hydrophila, and a lot of hemorrhaging in the intestine from acathocephalus (E. salmonis) infections. We see comparable numbers of E. salmonis now, but the degree of hemorrhage is not as great.

I remember Rod Horner measuring hematocrits of the dying fish and most were less than 10. I don't remember if he measured leucocrits, but I can check back in my records if you want.

Thanks for sharing this! I am not an immunologist, but can at least see the implications...
Sue

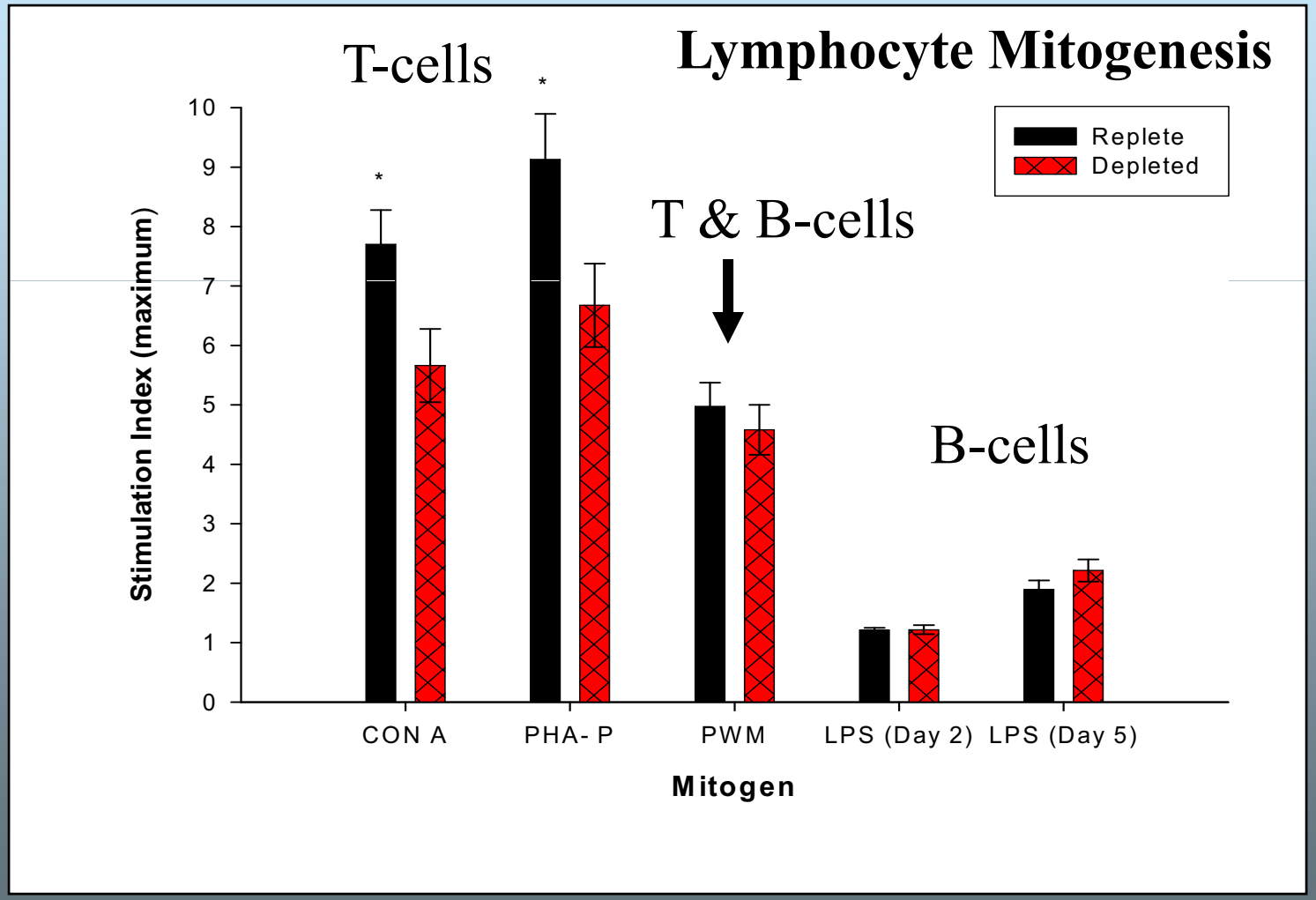
	Thiamine Replete	Thiamine Depleted
* Total Erythrocytes (10^6 ul^{-1})	0.93 ± 0.02	1.03 ± 0.10
Hematocrit (%)	37.9 ± 0.82	39.6 ± 0.99
* Plasma Protein (g dl^{-1})	7.07 ± 0.14	7.88 ± 0.27
* Total Leukocytes (10^6 ml^{-1})	29.05 ± 1.39	10.90 ± 1.83
* Small Lymphocytes (10^6 ml^{-1})	25.70 ± 1.28	6.65 ± 1.88
Large Lymphocytes (10^6 ml^{-1})	0.80 ± 0.15	0.51 ± 0.07
Polymorphonuclear Cells (10^6 ml^{-1})	2.30 ± 0.23	3.59 ± 0.76
* Monocytes (10^6 ml^{-1})	0.14 ± 0.03	0.25 ± 0.04

Summary of Disease Resistance in Thiamine deficiency

Thiamine Deficient and Replete lake trout fry

Deficient – Mortality observed
Replete – No mortality!

Challenged with IPNV
Deficient fry died faster than replete fry


Lymphocyte Mitogenesis
T-cells
T & B-cells
B-cells
Replete
Depleted
Stimulation Index (maximum)
0
1
2
3
4
5
6
7
8
9
10
*
*
CON A
PHA- P
PWM
LPS (Day 2)
LPS (Day 5)
Mitogen

Heterosporis sp. (Microspora: Pleistophoridae): A Parasite from *Perca flavescens*, *Stizostedion vitreum* and *Esox lucius* in Minnesota, Wisconsin and Lake Ontario

Daniel Sutherland, Susan Marcquenski,
Joseph Marcino, Peggy Stelzig, Jiri Lom, Iva Dykova,
Frank Nilsen, Scott Cooper, Hui-Min Hsu,
Wesley Jahns, James Hoyle and Rod Penny

UW-L
University of Wisconsin-La Crosse

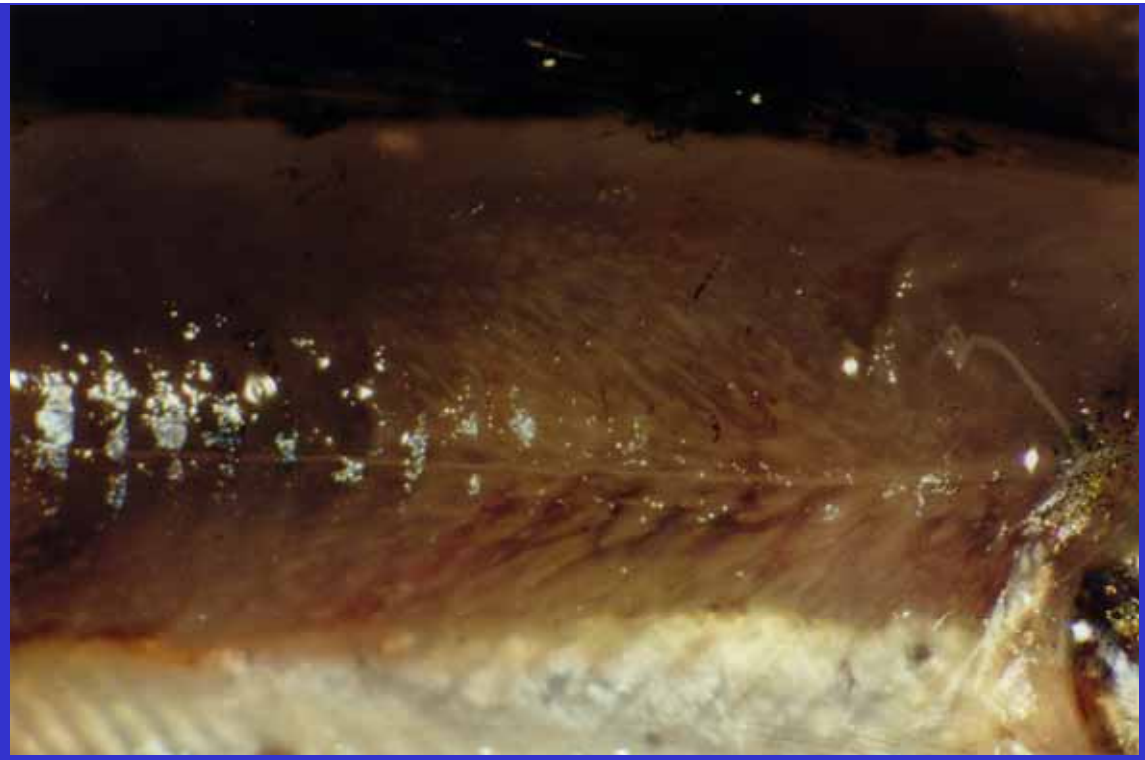
Catfish Lake,
Vilas County, Wisconsin

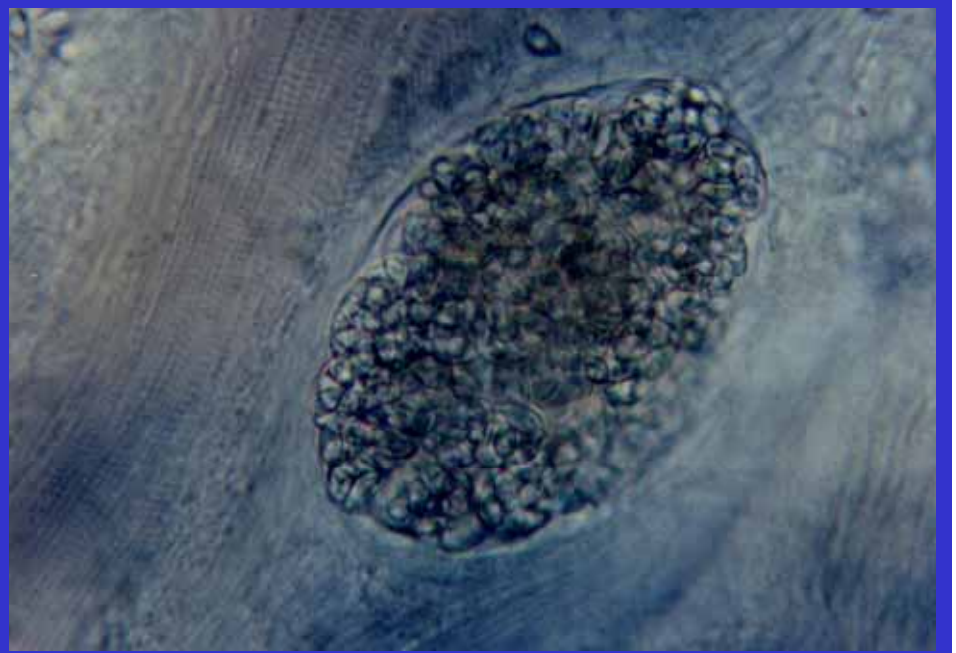
Initial Reports
January, 2000

Yellow Perch

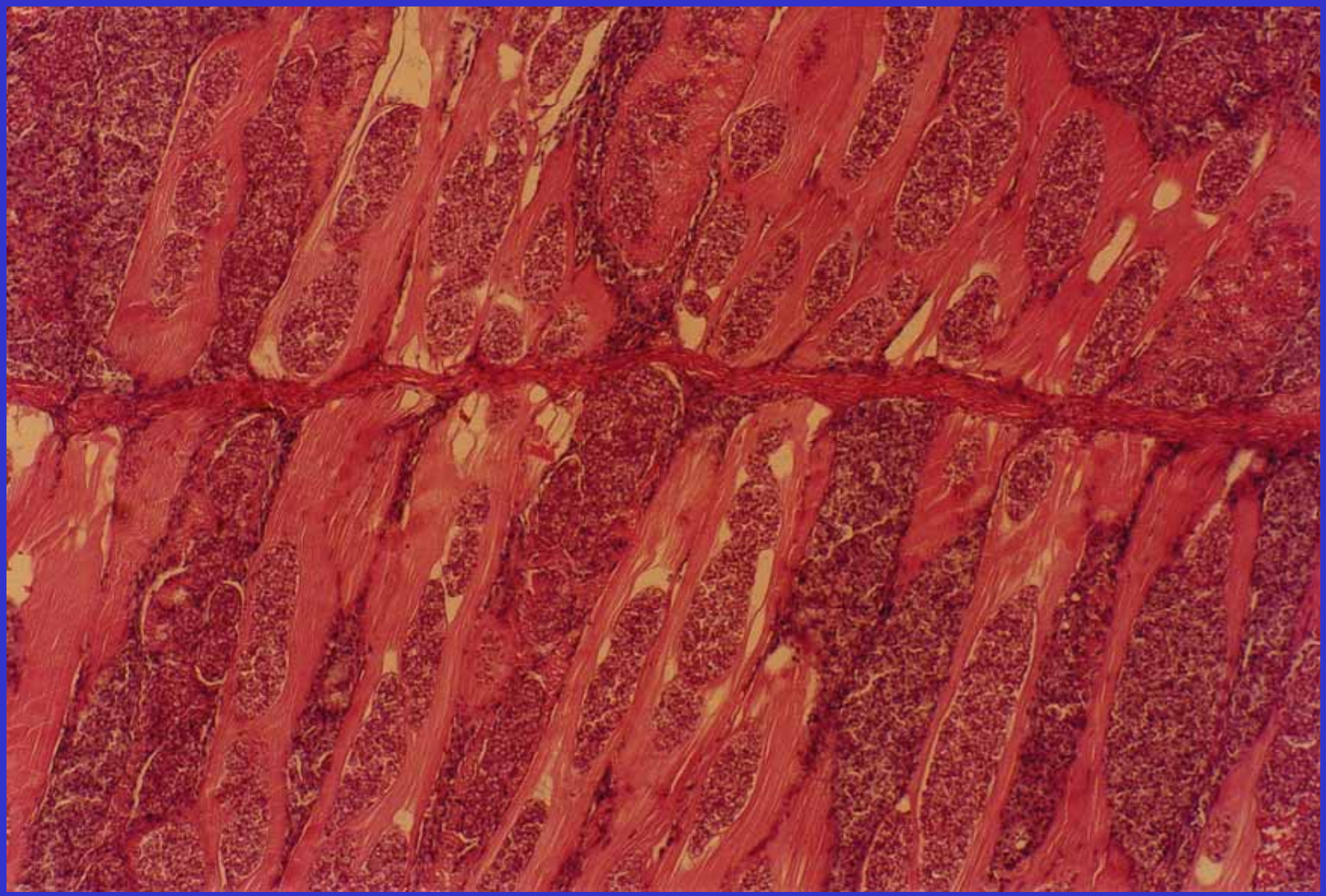
Perca flavescens


INCHES
Hamilton Bell Co., Inc.
MONTVALE, N.J. 07645



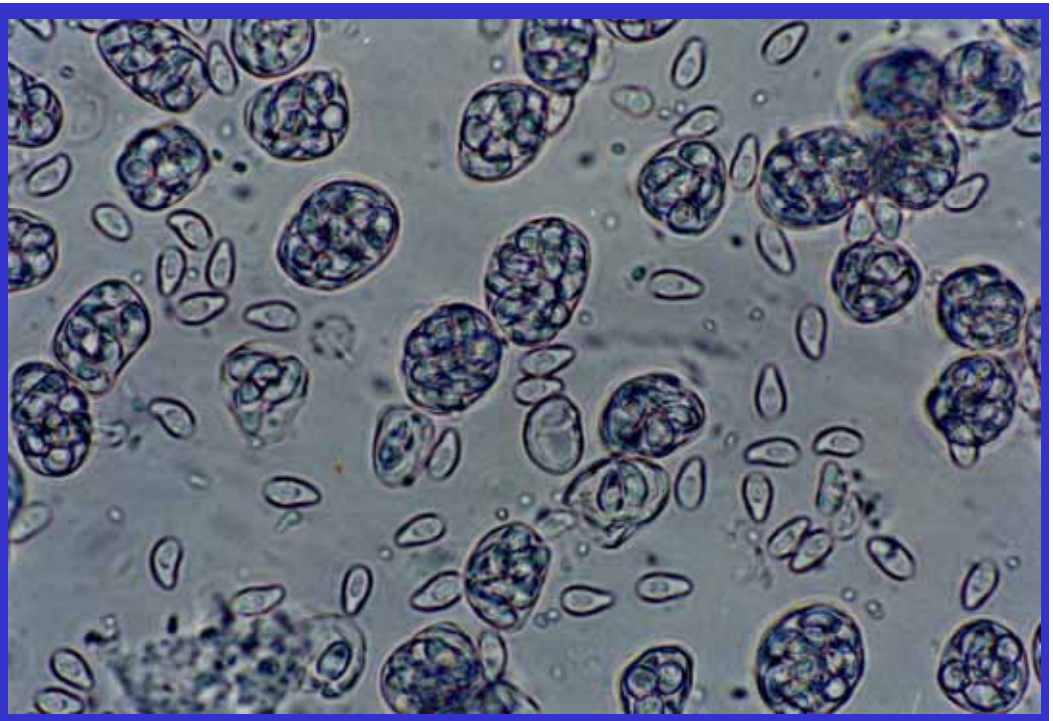


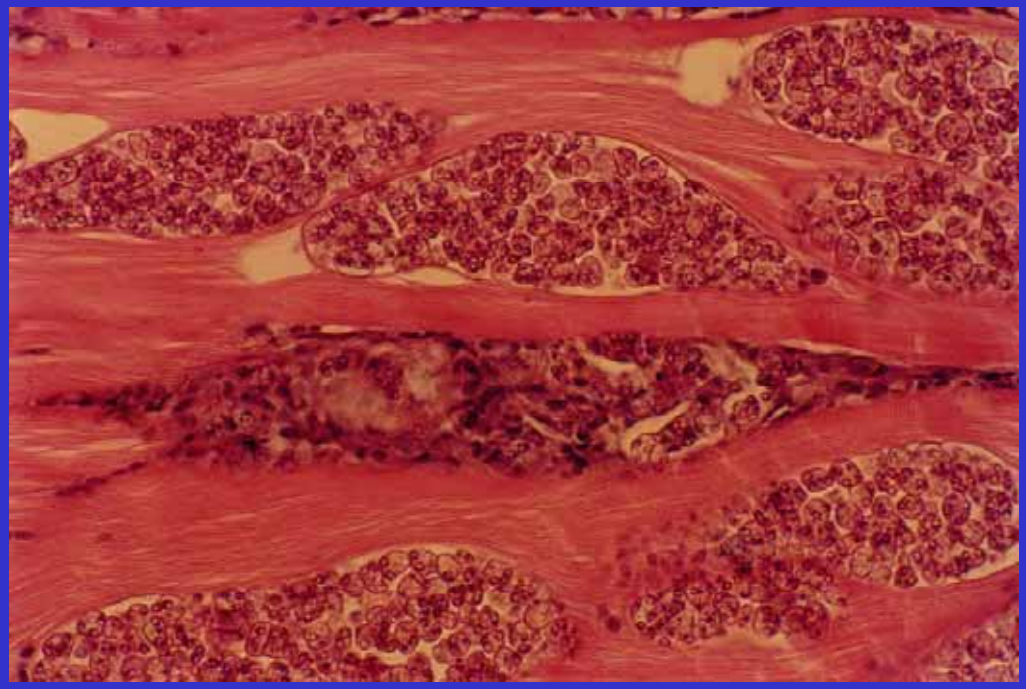
Heavily infected fillets may be mostly parasite

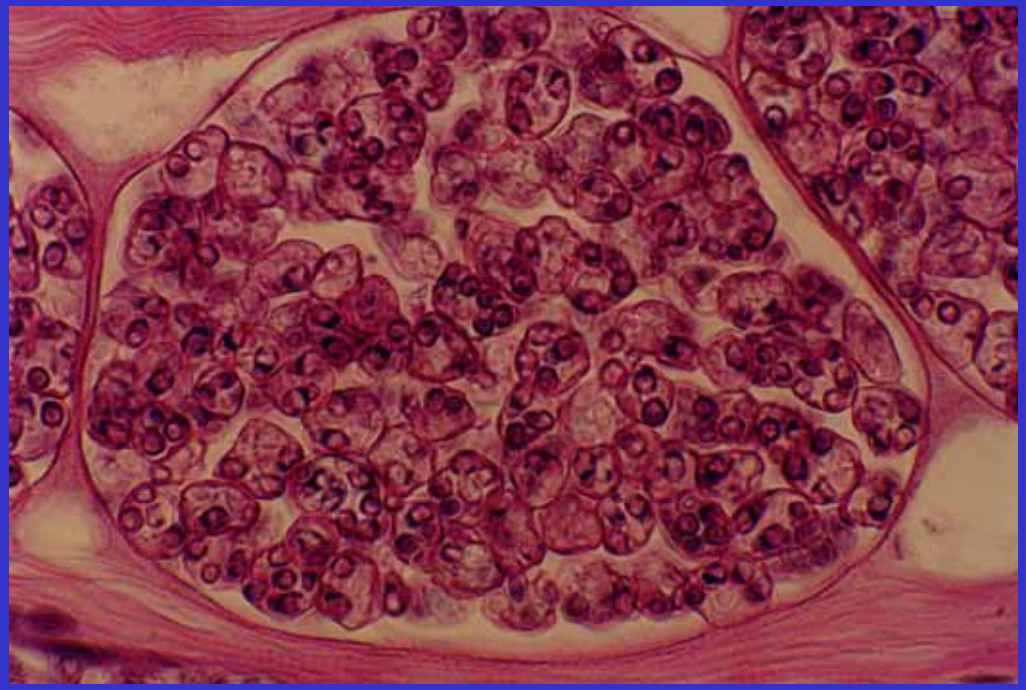


Wet mounts

1. SPV's contain 8 or 16 spores
2. SPV wall fairly resistant to rupturing





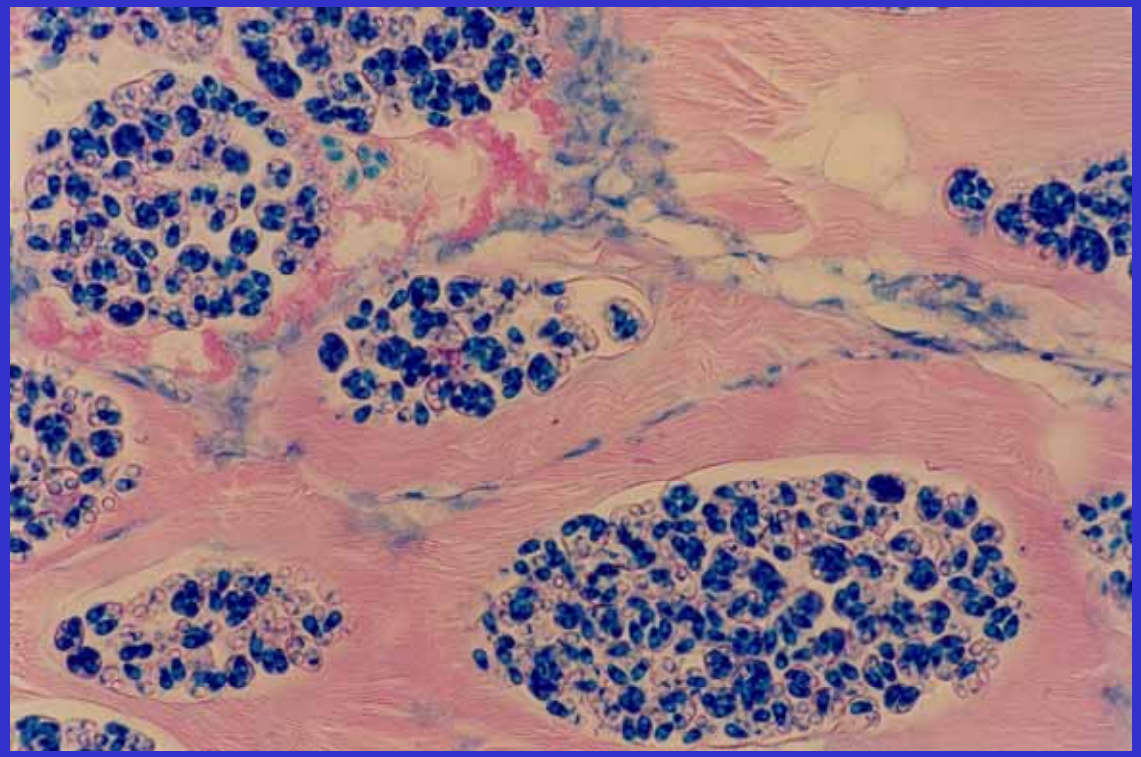


Sporophorocyst

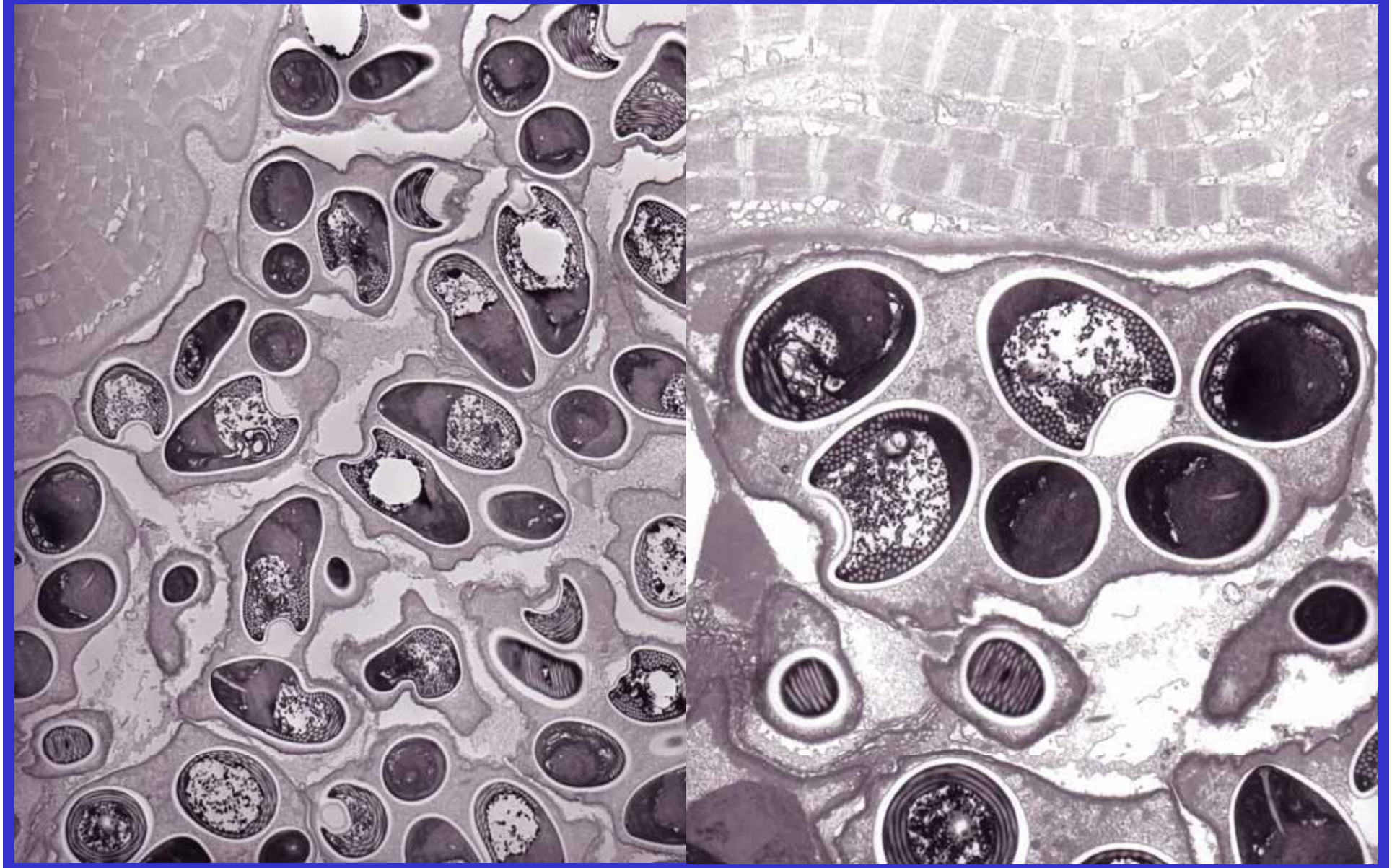
A distinct wall of parasite origin which continues to grow as parasite develops

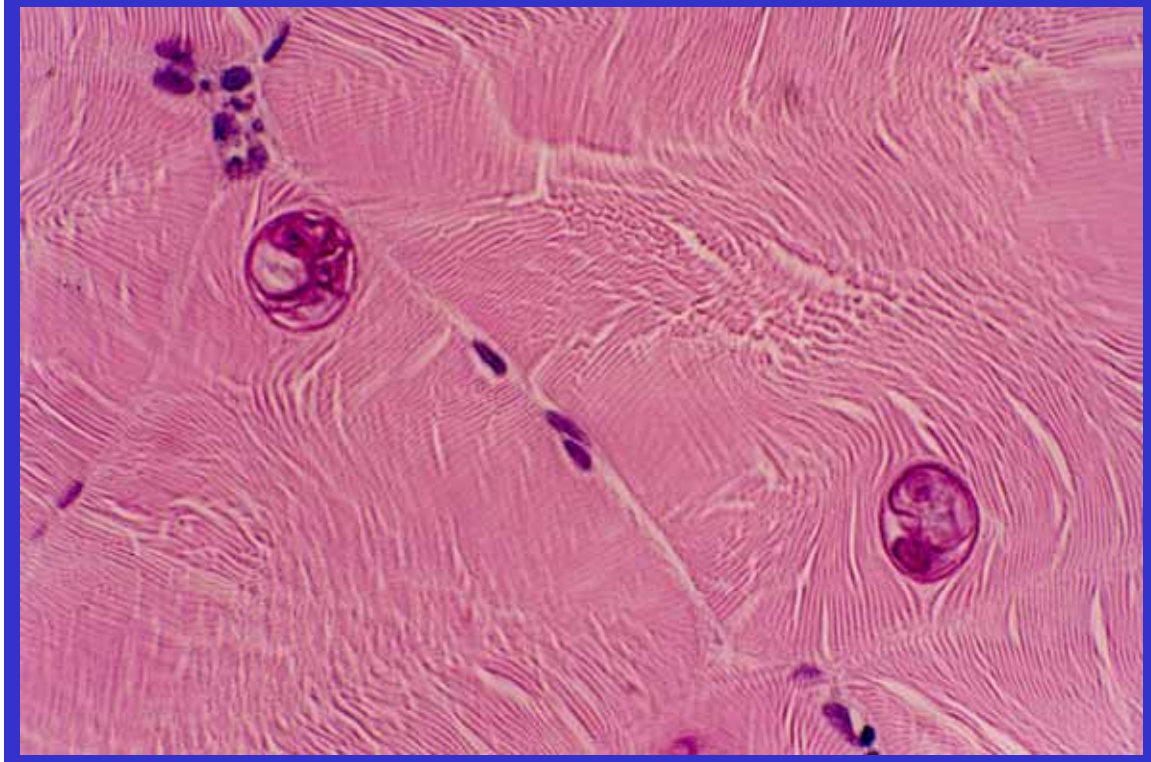
Heterosporis (Microsporida: Pleistophoridae)

Giemsa highlights spores

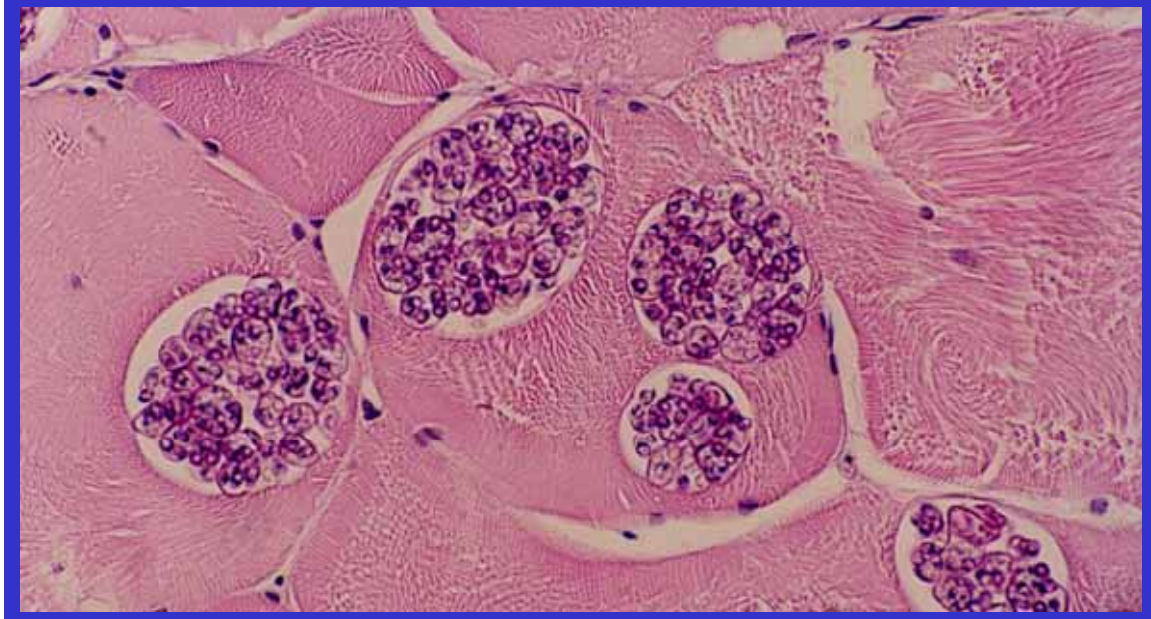


Intact sarcoplasm usually surrounds sporophorocysts

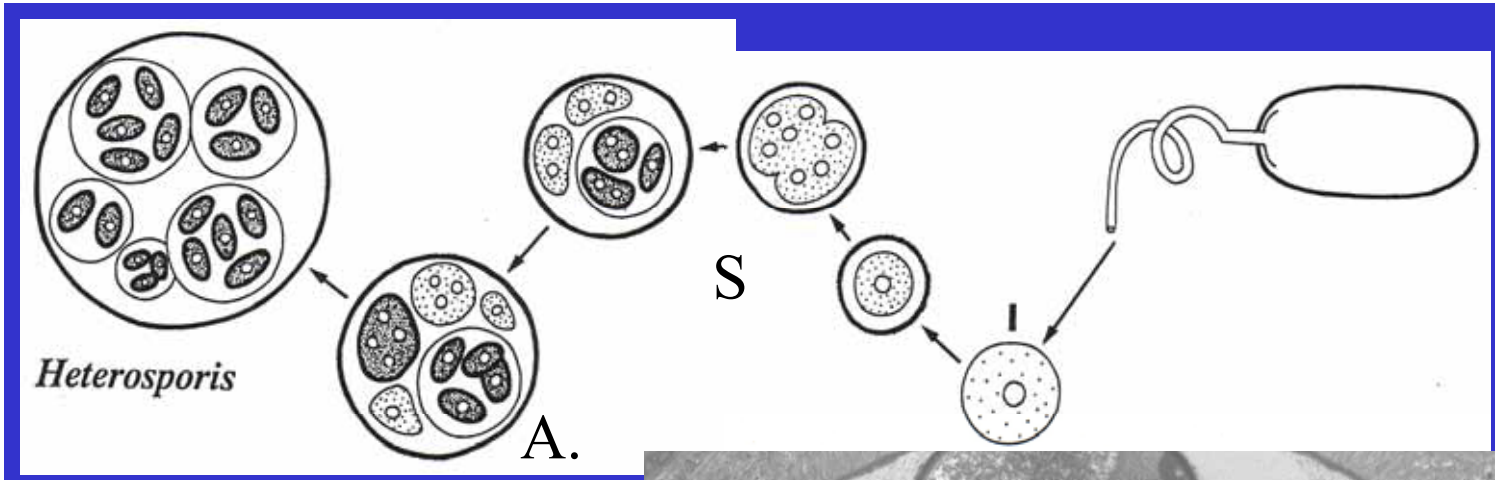




Early developmental stages with both merogonic and sporogonic stages



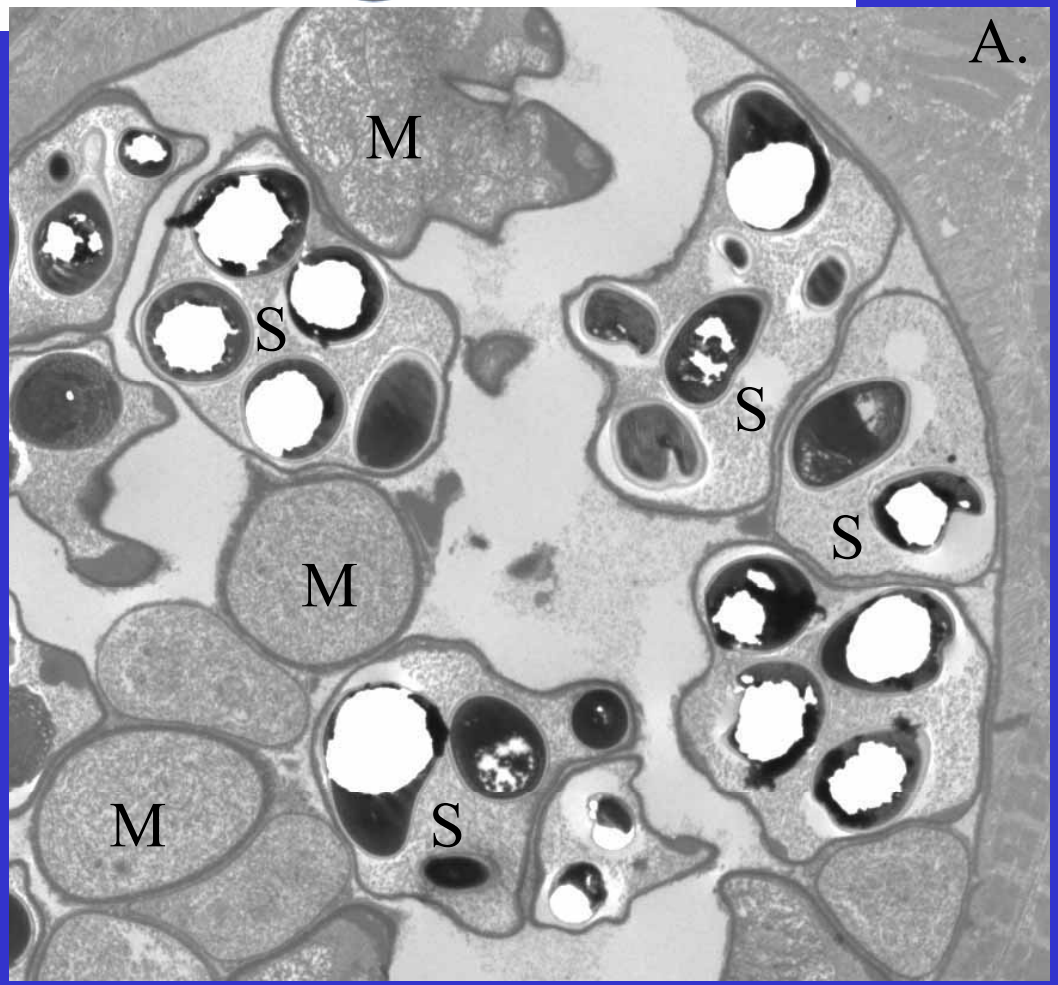
Multiply infected cells

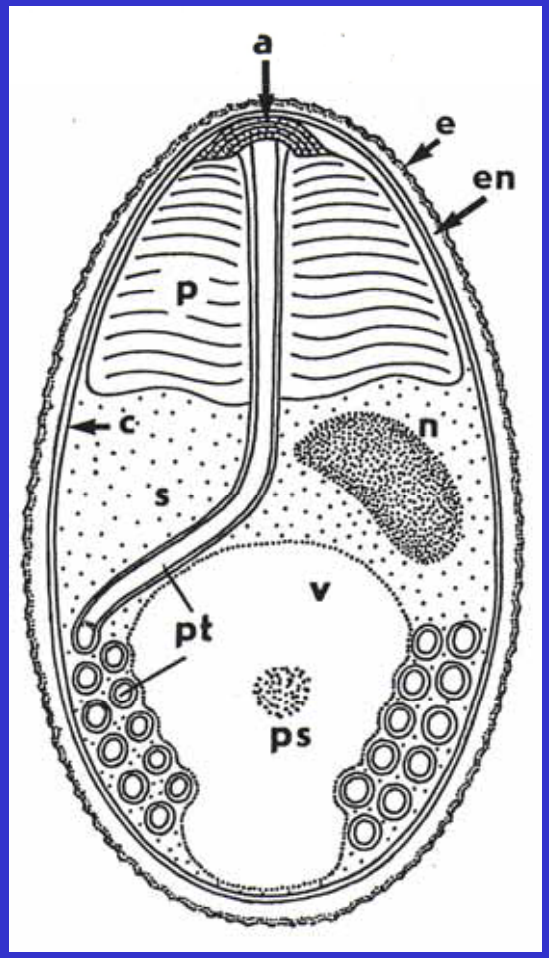


S = Sporogony

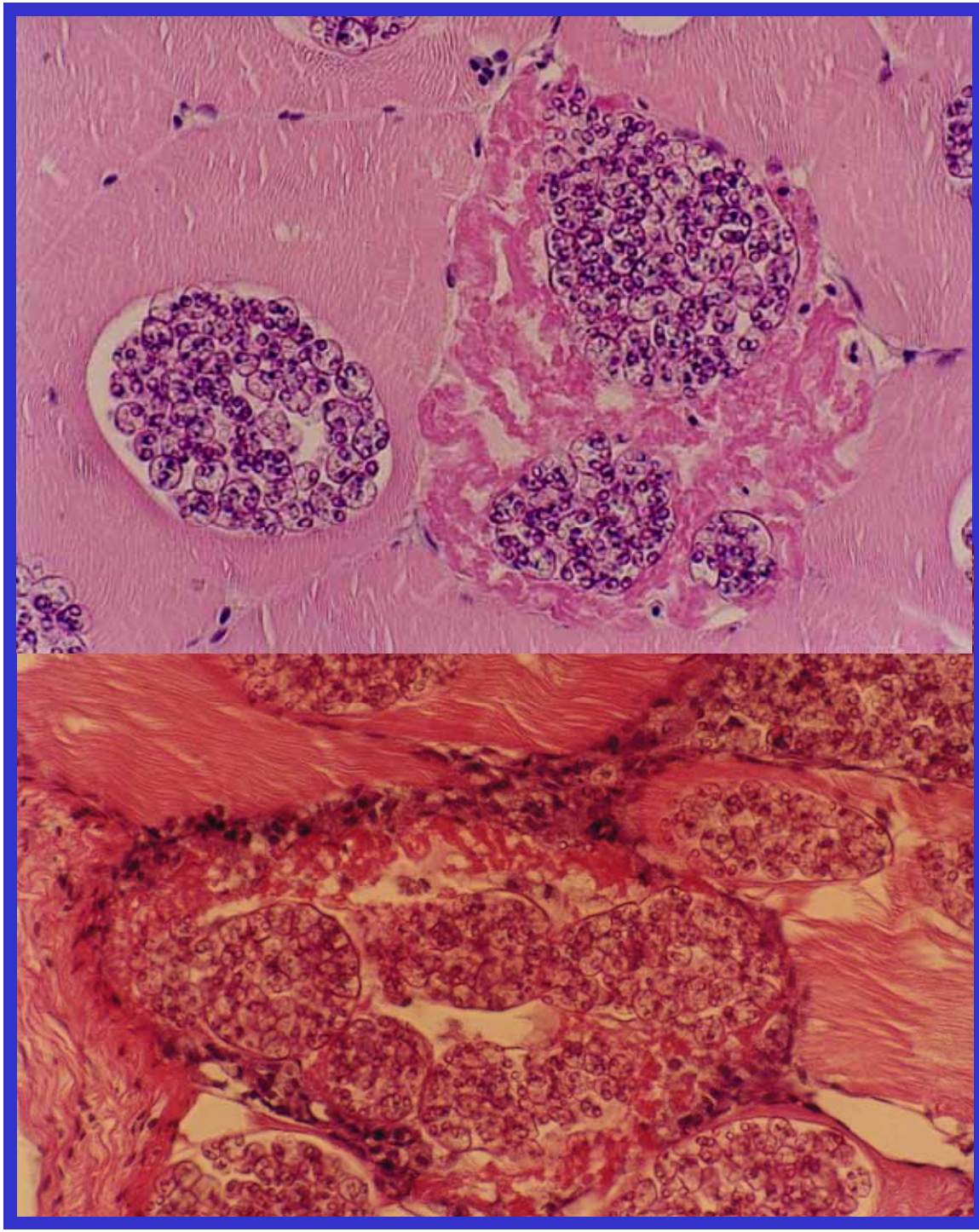
M= Merogony

A hallmark of genus *Heterosporis* is simultaneous merogony and sporogony within same sporophorocyst





e: exospore, en: endospore, c: cell membrane, n: nucleus, v: posterior vacuole, a: anchoring disc, p: polaroplast, ps: posterosome, pt: polar tube

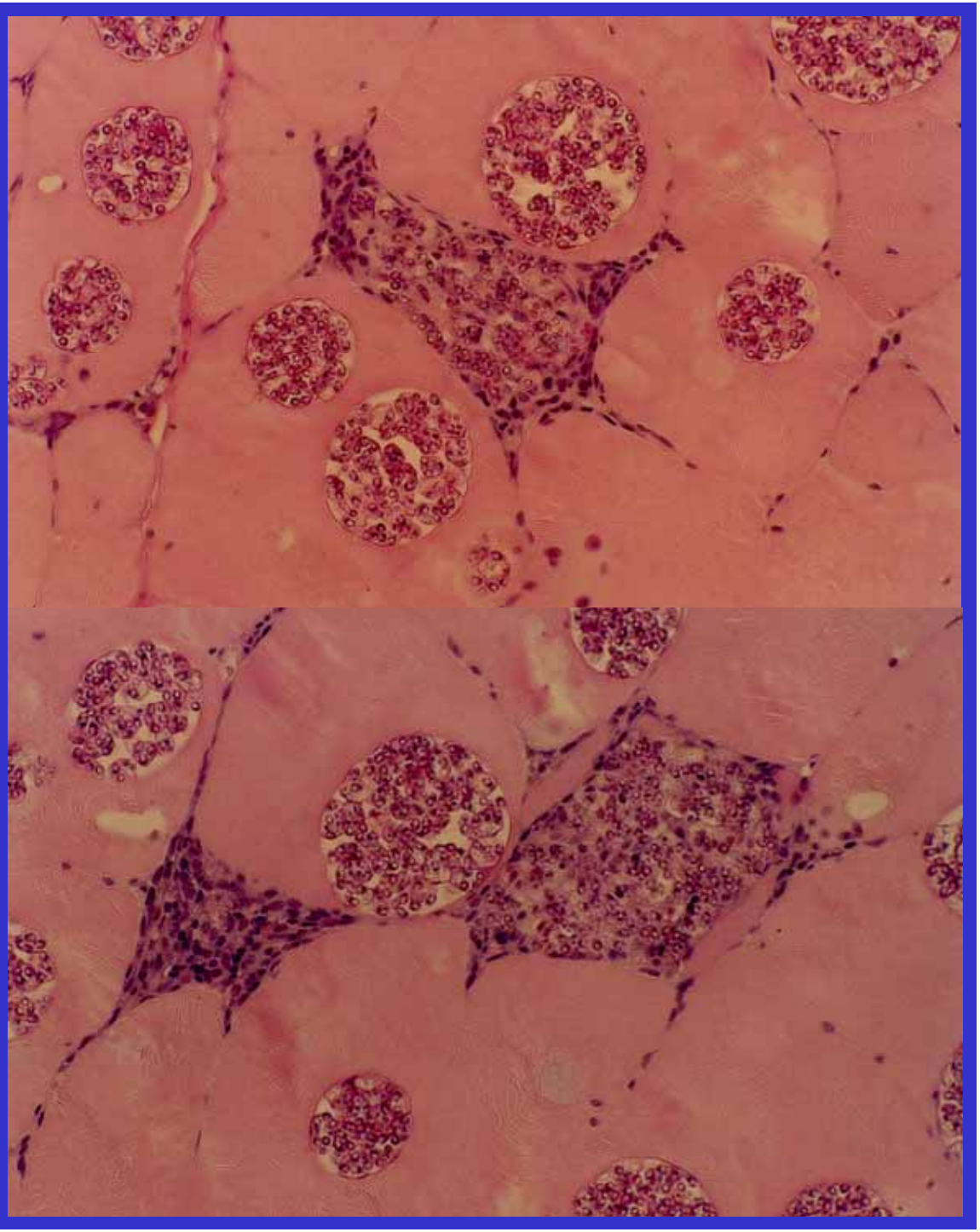


Early skeletal cell necrosis

Necrosis of sarcoplasm, dissolution of sporophorocyst membrane, leukocyte recruitment

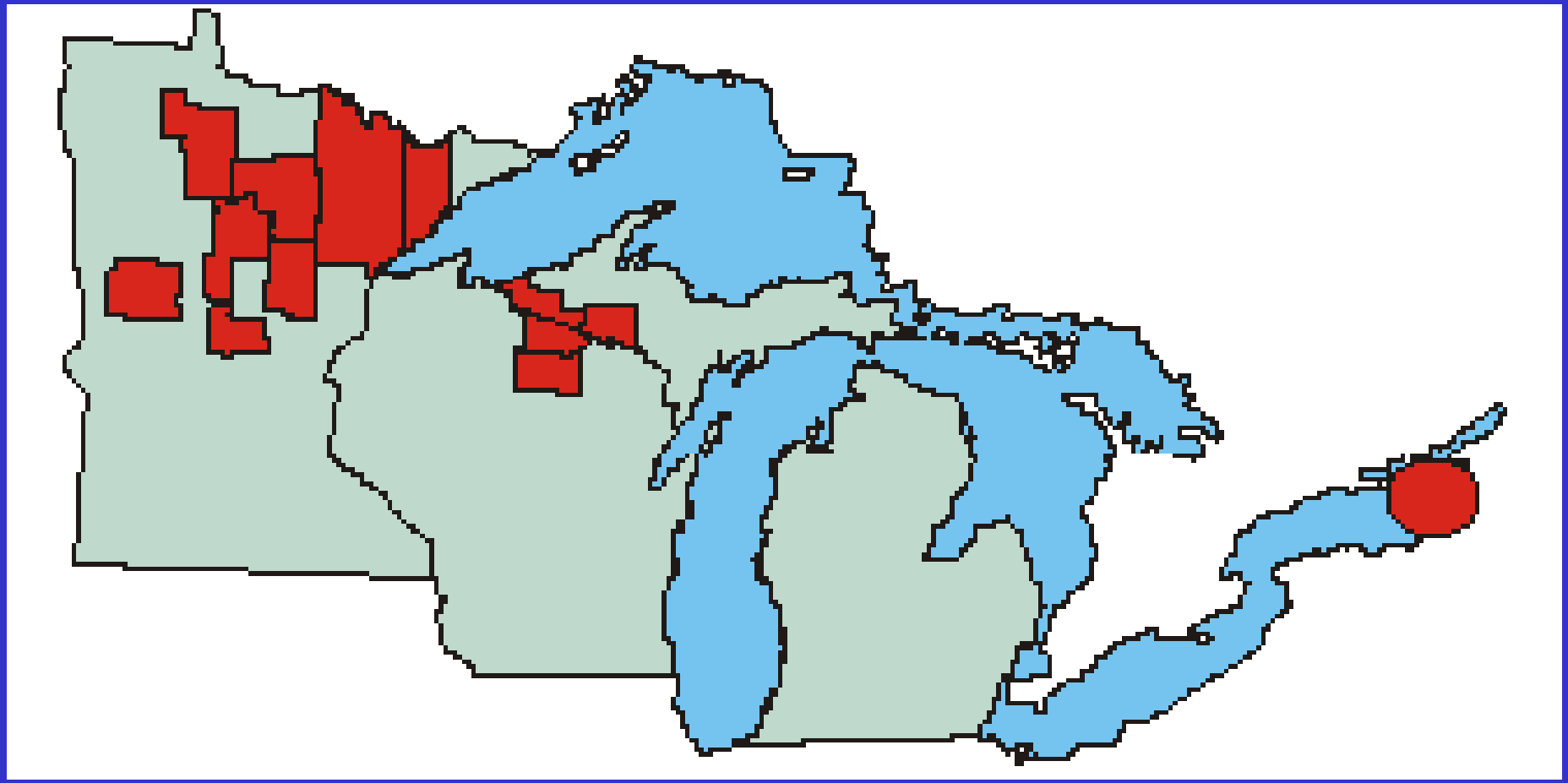
Late skeletal cell necrosis

Sporophorocyst and SPV degeneration liberating individual spores



Three discrete foci of *Heterosporis* infection in North America

Minnesota, NE Wisconsin, NE Lake Ontario



Heterosporis in North America

Yellow perch (*Perca flavescens*)
- Chain of Lakes, Vilas Co., WI
- Robinson Lake, Forest Co., WI
- Leech Lake, Cass Co., MN
- Mille Lacs, Isanti Co., MN
- Lake Vermillion, Isanti Co., MN
- Lake Winnibigosh, Isanti Co., MN
- Bear Lake, Itasca Co., MN
- Moose Lake, Itasca Co., MN
- Northern Lake Ontario, Ontario
- Bay of Quinte, Ontario

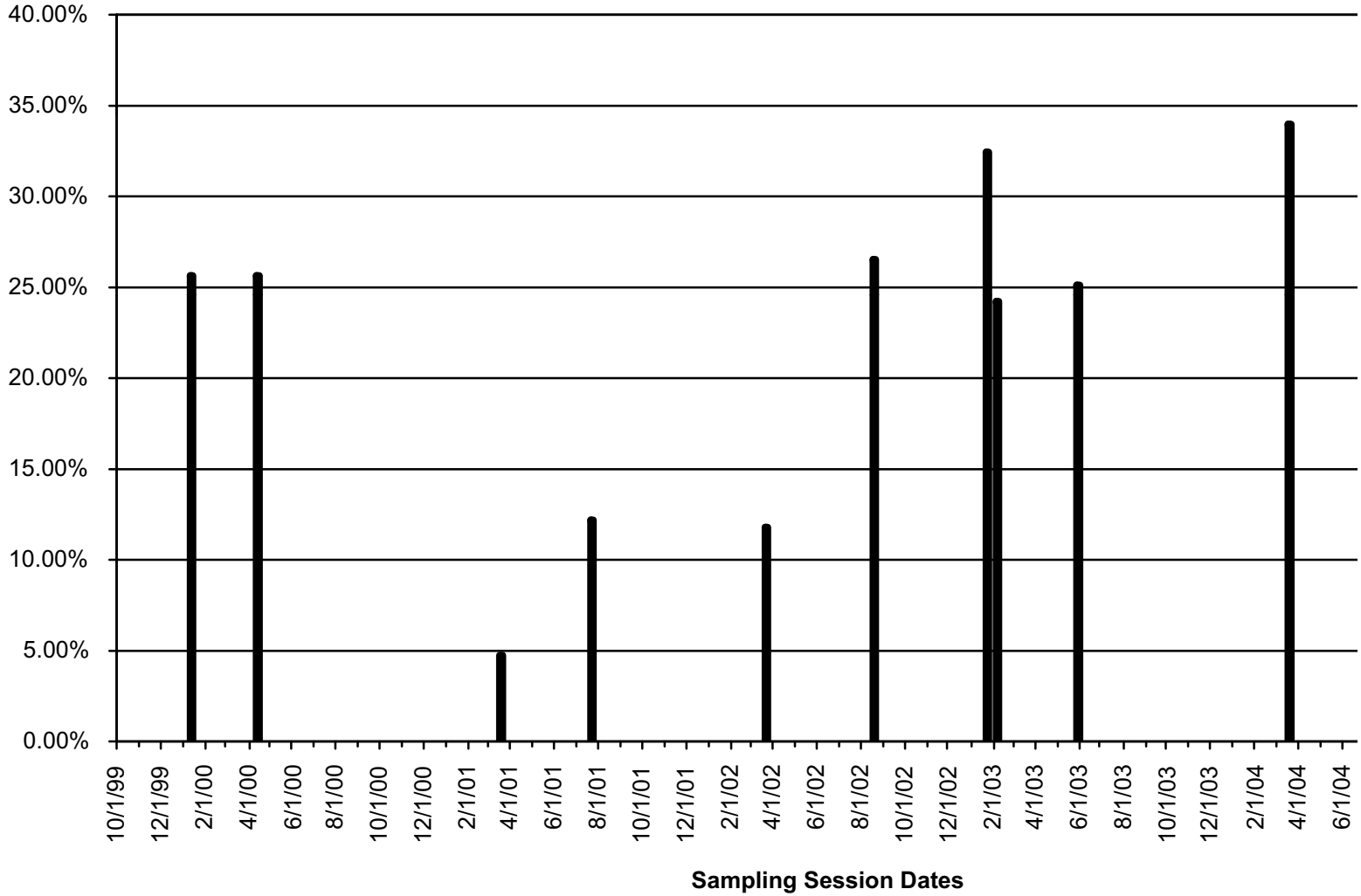
Walleye (*Stizostedion vitreum*)
- Big Sand Lake, Isanti Co., MN
- Chain of Lakes, Vilas Co., WI

Northern Pike (*Esox lucius*)
- Clitheral Lake, Ottertail Co., MN

Catfish Lake, Vilas Co., WI

Mottled sculpin (*Cottus bairdi*)
Burbot (*Lota lota*)
Pumpkinseed (*Lepomis gibbosus*)
Rock Bass (*Ambloplites rupestris*)
Trout Perch (*Percopsis omiscomaycus*)
Pumpkinseed (*Lepomis gibbosus*)

Figure 8. Prevalence of Infected Yellow Perch (Perca flavescens) in Catfish Lake, Wisconsin over time.



Steamboat Lake, MN

Yellow perch (20/212)
Walleye (51)
Northern pike (55)
Bluegill (101)
Pumpkinseed (87)
Rock bass (12)
Largemouth bass (4)
Brown bullhead (27)
Black bullhead (8)
Yellow bullhead (6)
White sucker (53)
Bowfin (4)
Burbot (1)
Lake whitefish (1)

Scattering Rice Lake, WI

Yellow perch (2/71)
Walleye (1)
Northern pike (3)
Bluegill (13)
Pumpkinseed (9)
Rock bass (1/53)
Black crappie (43)
Yellow bullhead (13)
Black bullhead (6)
White sucker (5)
Shorthead redhorse (7)
Bluntnose minnow (7)
Golden shiner (26)
Common shiner (1)
Johnny darter (2)

Catfish Lake, WI

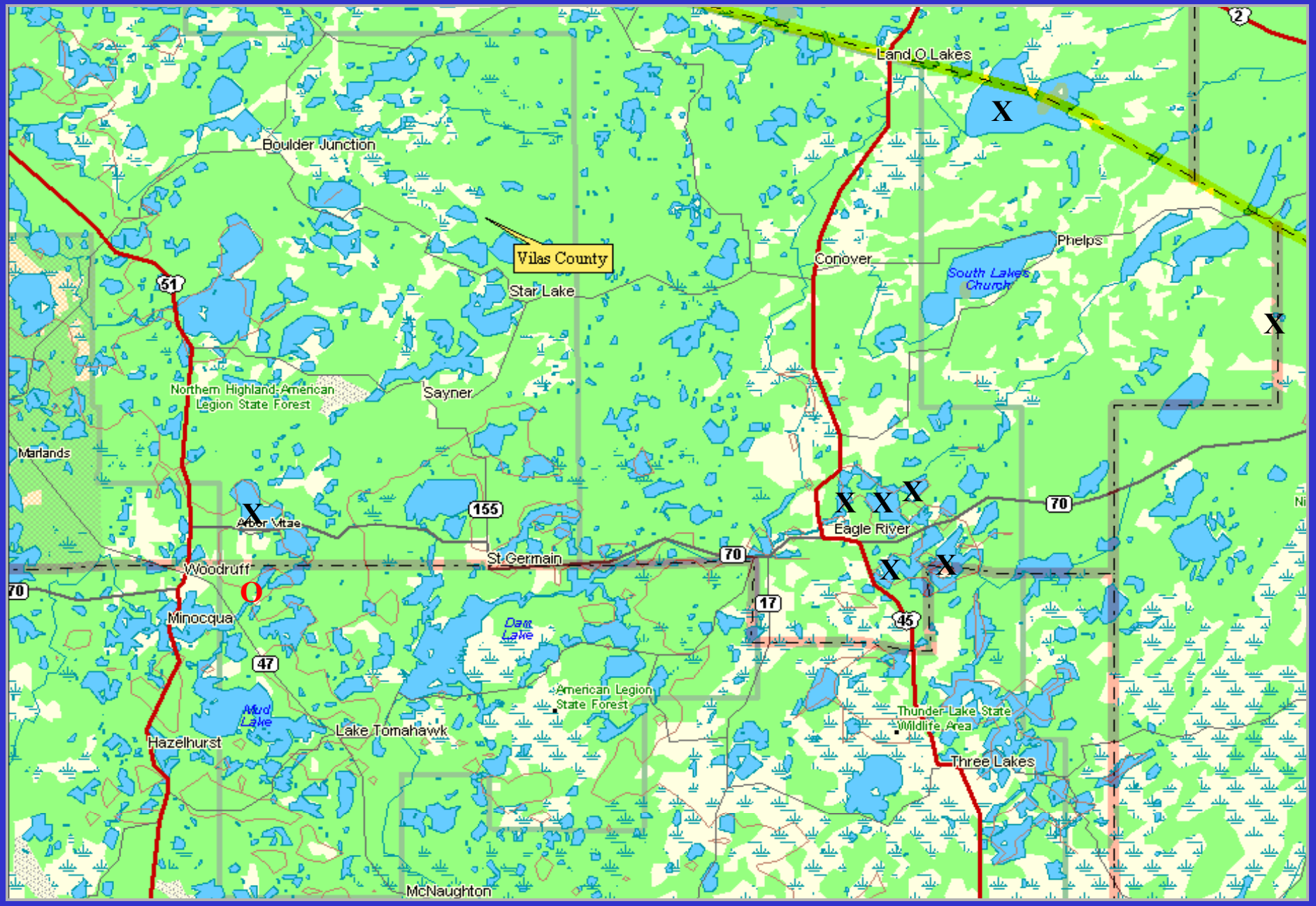
Yellow perch (29/107)
Walleye (1/1)
Northern pike (1)
Bluegill (51)
Pumpkinseed (6/87)
Rock bass (9/112)
Black crappie (37)
White sucker (3)
Golden shiner (8)
Common shiner (3)
Mimic shiner (1)

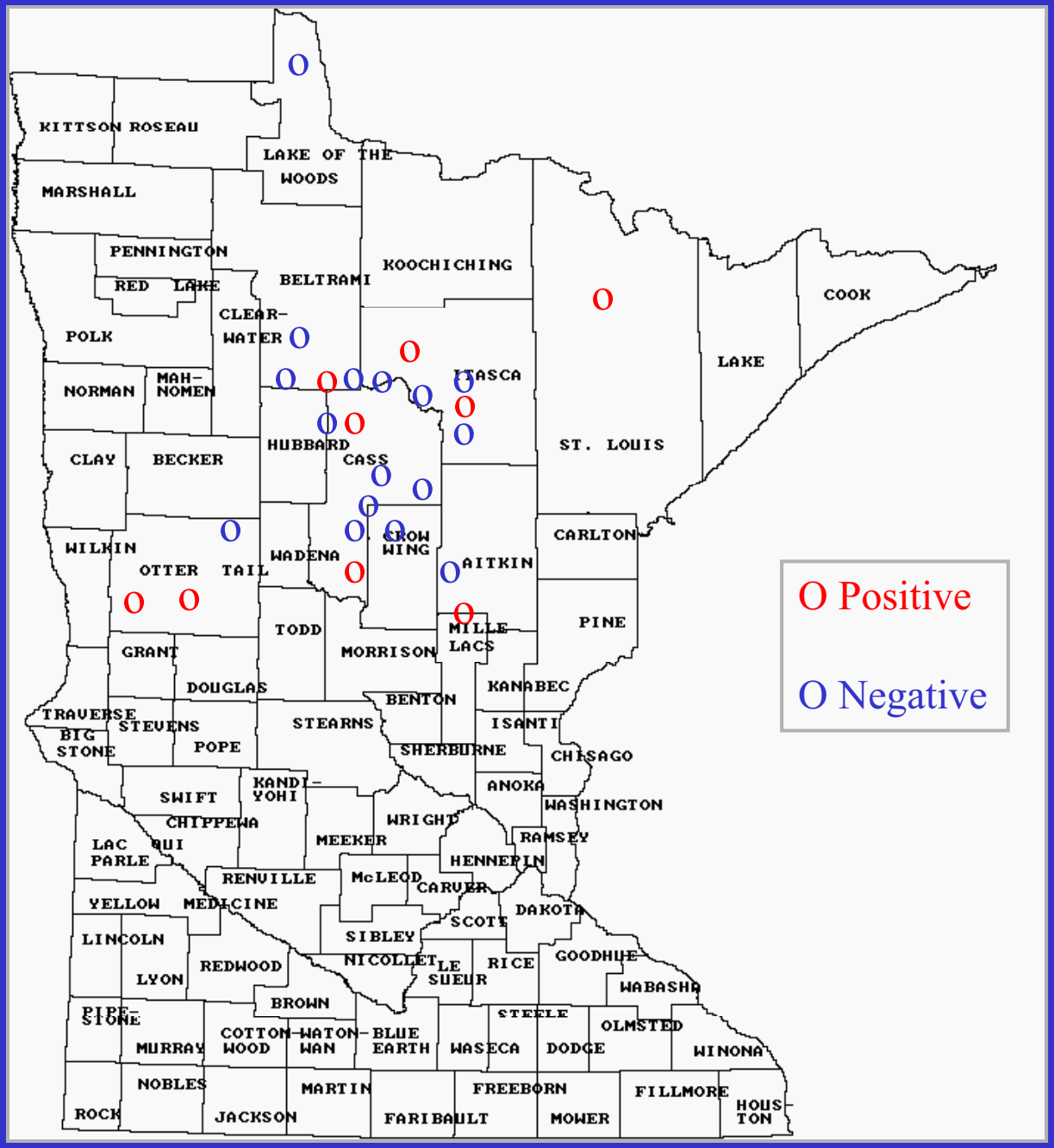
Heterosporis-infected burbot (*Lota lota*) from Catfish Lake, Wisconsin


Bay of Quinte
X
Green Bay

Lake Ontario *Heterosporis* Infections (2004)

Yellow perch	7.32%	(147/2009)
Rockbass	0.98%	(3/305)
Pumpkinseed	1.37%	(2/145)


Land O Lakes
Boulder Junction
Vilas County
Star Lake
Phelps
Conover
South Lakes Church
Northern Highland-American Legion State Forest
Sayner
Marlands
Arbor Vitae
Woodruff
St Germain
Eagle River
Minocqua
Dam Lake
American Legion State Forest
Thunder Lake State Wildlife Area
Three Lakes
Mud Lake
Lake Tomahawk
Hazelhurst
McNaughton
51
155
70
17
45
47
2



Minnesota

Heterosporis-positive locations in 2002

Other *Heterosporis*

Species	Host	Locality
H. anguillarum	*Anguilla japonica*	Japan
H. schuberti	*Pseudocrenilabrus multicolor* (Cichlidae)	Germany
	Ancistrus cirrhosus (Loricariidae)	
H. finki	*Pterophyllum scalare* (Cichlidae)	France
Heterosporis sp	*Betta splendens* (Anabantidae)	Thailand

Experimental Exposures with North American *Heterosporis* (Lom and Dykova)

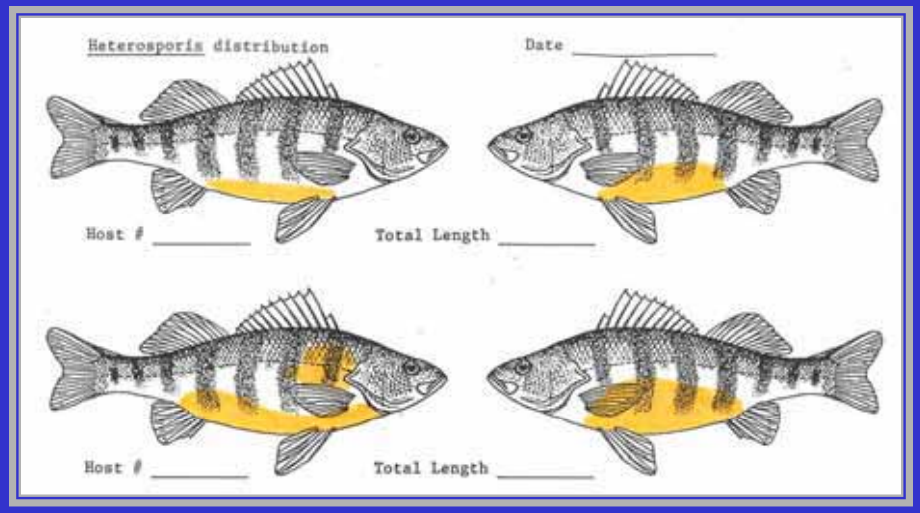
- *Perca fluviatilus*
  - Peroral infection (15/16)
  - I.M. injection (3/5)
  - I.P. injection (0/8?? DPI 14)
- *Carassius auratus*
  - I.M. injection (4/8)
  - I.P. injection (0/8)
- *Cyprinus carpio*
  - I.M. injection (8/8)
- *Oreochromis niloticus*
  - I.P. injection (0/12?? DPI 23)

Laboratory Infections

- Rainbow trout
- Lake trout
- Brown trout
- Channel catfish
- Fathead minnow
- Yellow perch
- Walleye

- Golden shiners
- Northern pike
- Muskellunge*
- Smallmouth bass*

- Brook trout
- Largemouth bass
- Coho salmon
- White sucker
- Bluegill

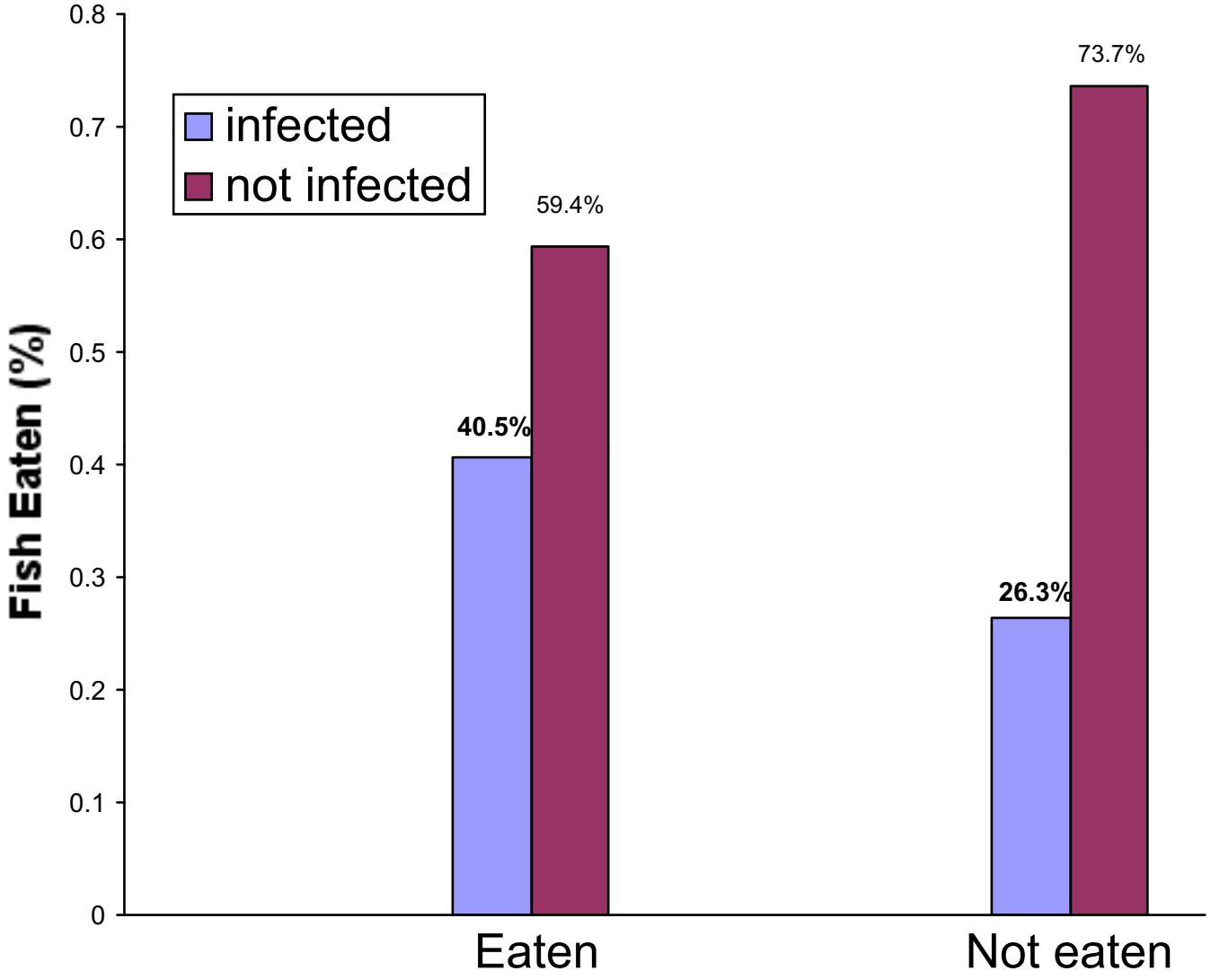


Successful Control Measures

Complete dessication for 24 hours

Freezing at -20 ºC for 24 hours

Immersion in a 2200 ppm bleach solution for 5 minutes (3 cups 6% bleach in 5 gallons water)


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Fish Eaten (%)
infected
not infected
59.4%
40.5%
73.7%
26.3%
Eaten
Not eaten

$p = 0.0085$

Recirculating flume which contains a three foot long clear Plexiglass tube into which fathead minnows were placed to determine swimming stamina of *Heterosporis* infected versus uninfected fathead minnows

Common Loon (*Gavia immer*)

Double-crested Cormorant
(*Phalacrocorax auritus*)

New Yellow Perch Parasite

Heterosporis *sp.* is a newly identified microsporidian parasite of yellow perch. So far, infected perch have been found in the Eagle River Chain of Lakes in Wisconsin and Leech Lake in Minnesota. It has not yet been identified in other fish species in North America. The parasite infects muscle cells which causes the flesh to have a "cooked" or "freezer burn" appearance (white area in the photo above). The parasite infection is **not** visible from the outside of the fish. *Heterosporis* **does not infect people.** However, infected muscle cells reduce the quality and change the texture of the fillet, and therefore people may choose to discard infected fish or at least affected portions of fillets.

Anglers and boaters on the Eagle River Chain of Lakes are encouraged to **take the following actions to help prevent the spread of this parasite:**

1. Infected fish should not be thrown back into the lake; they should be placed in the garbage and taken to a landfill, burned or buried.
2. Thoroughly dry all equipment and boat exteriors before using in other water bodies because the parasite spores may be present in the lake water.
3. Drain all live wells and bilges. Because it is impossible to thoroughly dry these areas, disinfect with one cup bleach in five gallons of water before moving to other water bodies.

The parasite's life cycle is only partially known. As infected fish die and decompose, spores are released into the water and are swallowed by other fish. Muscles just behind the head seem to be infected first. The infection then spreads until the entire fillet is affected. Spores are very hardy and can remain infective for at least a year in water, but they cannot survive under dry conditions.

This information is also provided by the Bureau of Fisheries Management and Habitat Protection at www.dnr.state.wi.us/org/water/fhp/fish/health/disease.htm.

Wisconsin Department of Natural Resources
P.O.Box 7921
Madison, WI 53707-7921

PUB-FH-726-6/00

- Infected fish should not be returned to lake (rather landfill, buried, burned)
- Dry all equipment and boat exteriors
- Drain live wells and bilges
- Disinfect with bleach (one cup /5 gal)

Contacts

- Dan Sutherland (sutherla.dani@uwlax.edu)
- Sue Marcquenski (susan.marcquenski@dnr.state.wi.us)
- Joe Marcino (joe.marcino@dnr.state.mn.us)
- Jim Hoyle (jim.hoyle@mnr.gov.on.ca)

UW-L
University of Wisconsin-La Crosse

Selective and/or Differential Media for Fish Bacterial Pathogens

Clifford Starliper

National Fish Health Research Lab

Leetown, WV

Non-harmful – mucus, blood, feces, sex products; health/pathogen surveys, preventative; selective and/or differential aids extremely beneficial

Diseased – moribund and/or mortality; “lethal” is ok; diagnostics of systemic pathogens; selective and/or differential useful, but not always essential

General Bacteriological growth medium = recipe provides basic nutrients necessary for cultivation

Differential media = general plus a substrate or substrates that certain bacteria either attack or do not. Essentially qualitative; pos/neg responses. Hastens characterization. Requires an indicator system to visualize. Presumptive Aid/Tool.

Selective media = general plus ingredient(s) that inhibit growth of non-desirables. Resistant-sensitive criteria must be met. Indicator system not relevant.

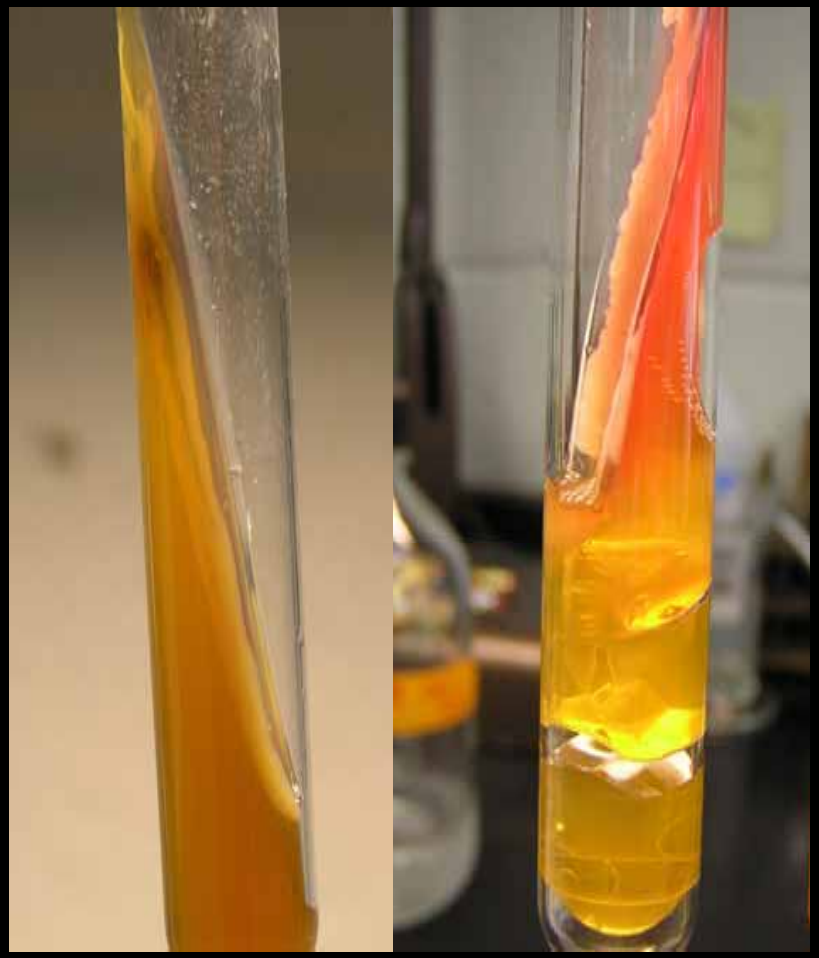
Differential and Selective

Differential

- Sugars – pH
- Proteins/substrates – clear zone
- Protein stain – colored colony

Selection

- Temperature
- Increased salt %
- Nutrient richness of medium
- Bile salts, azide, crystal violet, phenylethanol
- Selenite, tellurite
- Antimicrobials



pH indicator system

H_2S indicator system

Triple Sugar Iron

Beef extract, yeast extract, digest of casein, peptones, sodium chloride

0.1 % glucose

1 % lactose

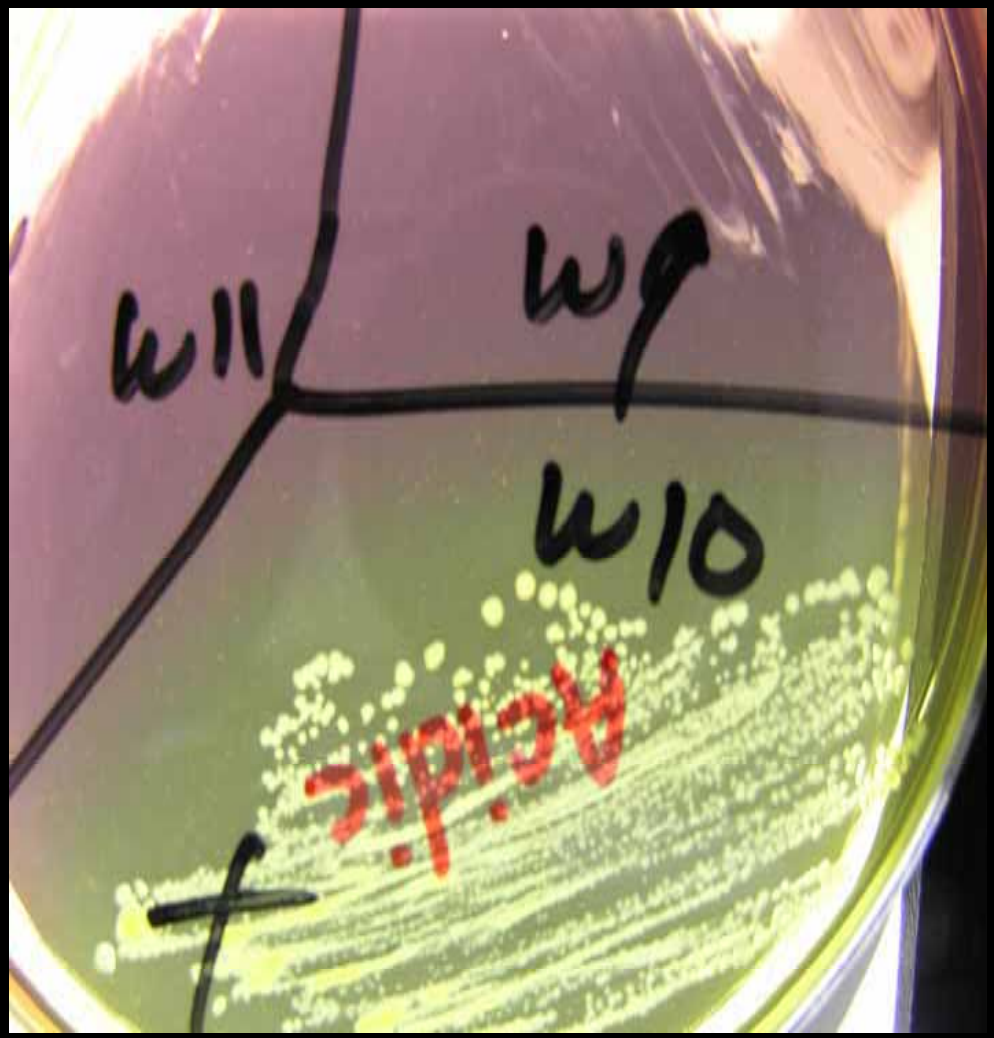
1 % sucrose

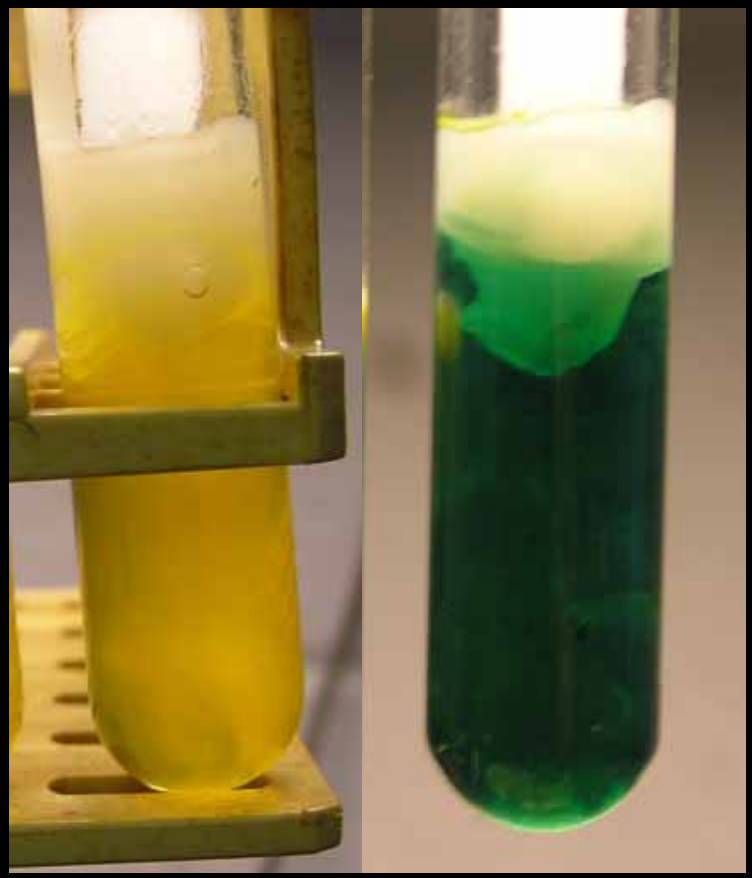
0.0024 % phenol red

Sodium thiosulfate

Ferrous sulfate

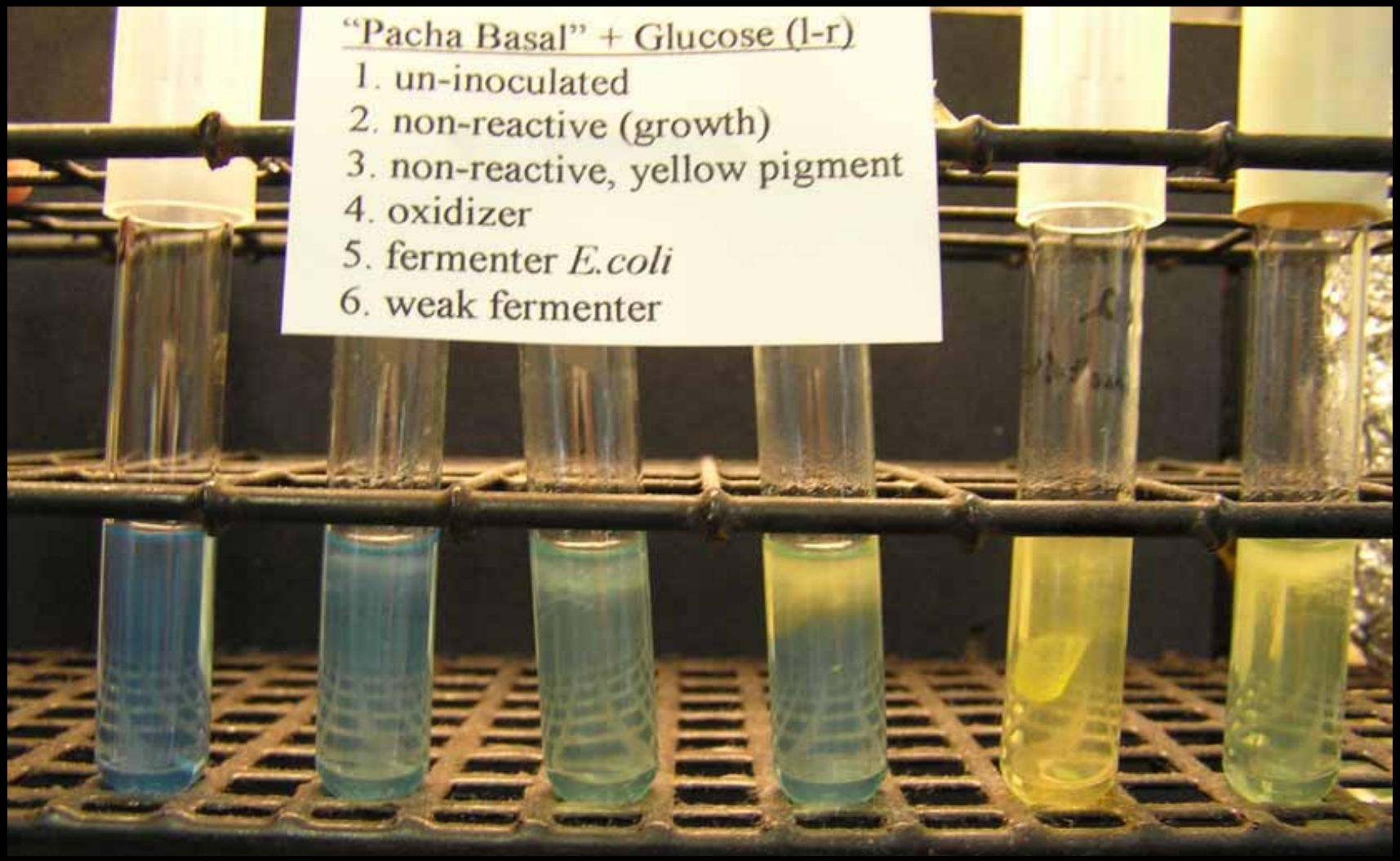
pH Indicators





Modified “Pacha Basal” + Glu Reactions

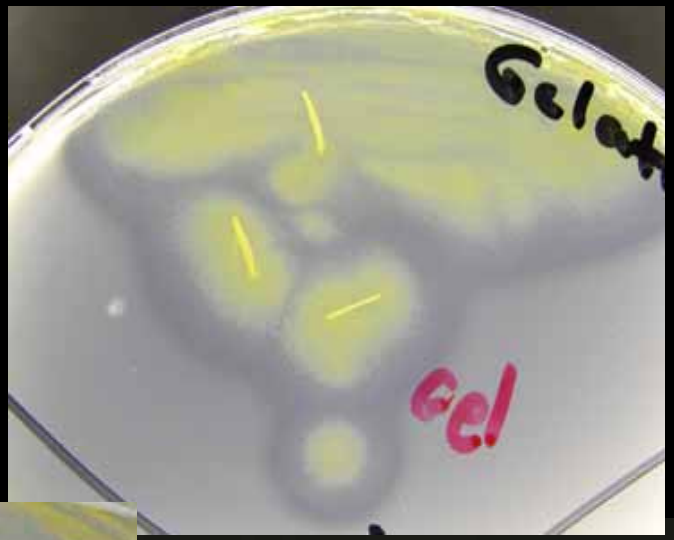
Ingredients fine tuned for sensitiivty

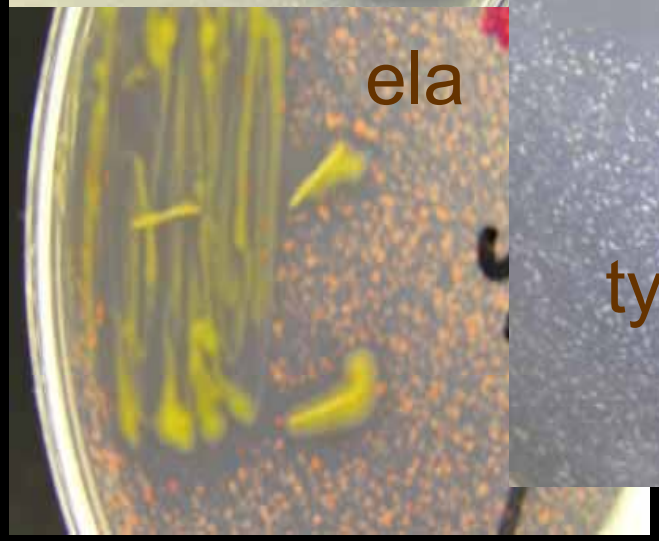


Reagents Necessary. Chemistry for development of a colored product.

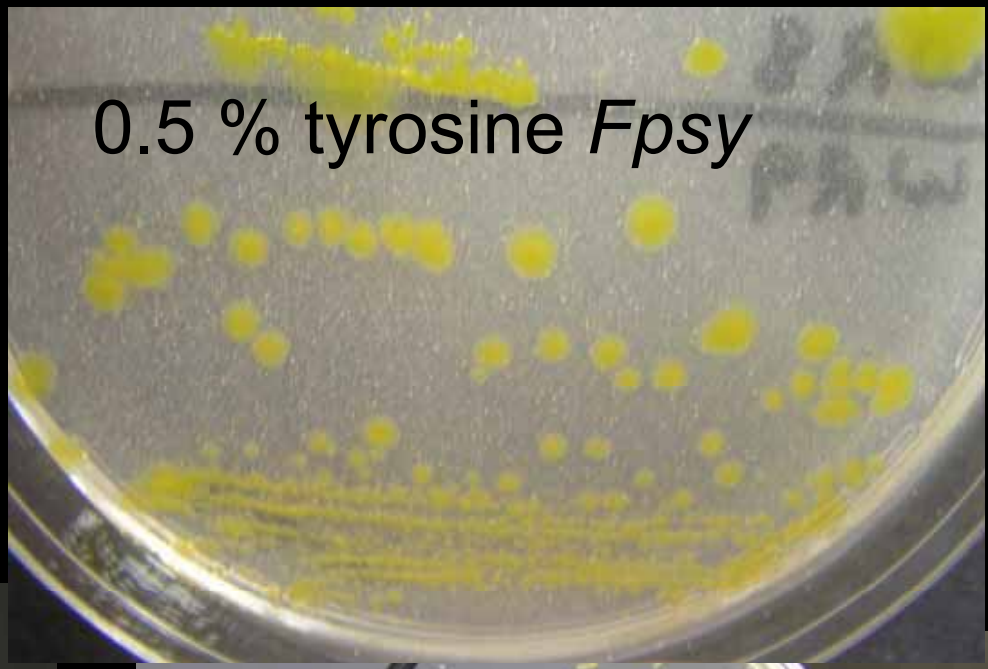
Indole
Methyl Red
Voges-Proskauer
Nitrate Reduction

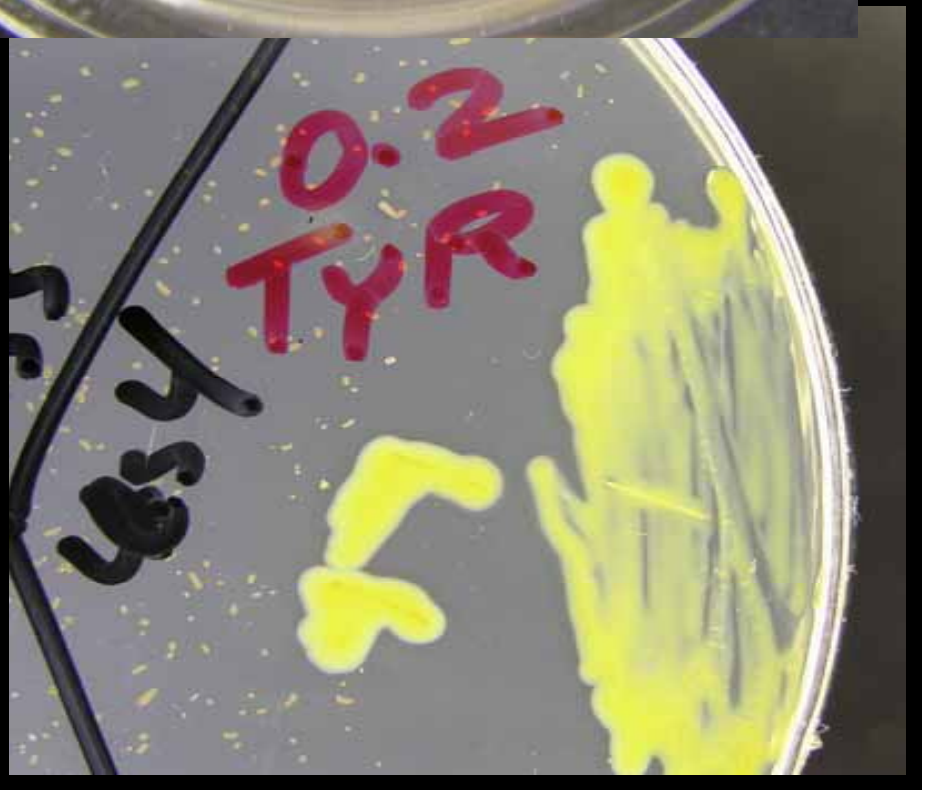
Contrasting Zone of action. Degradation.





Concentration Affects Sensitivity; and therefore Results Reporting





Renibacterium salmoninarum

Tryptone	1 % (peptone)
Yeast extract	0.05
l-cysteine	0.1
serum	10 (charcoal)

Selective agents

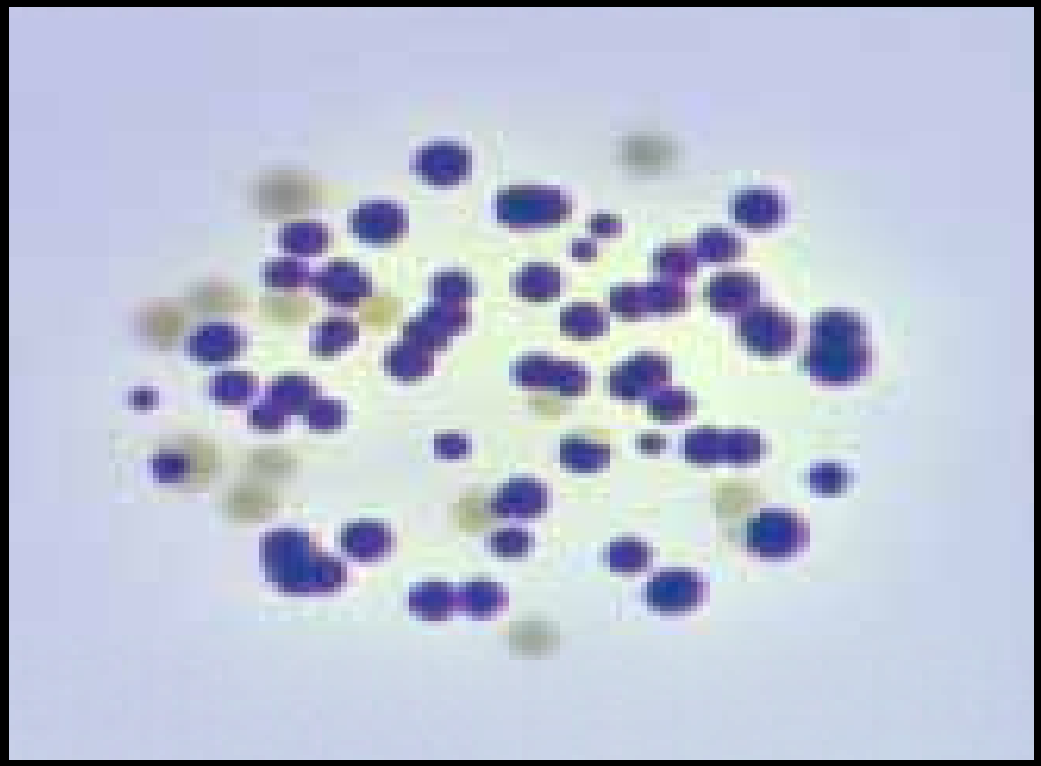
Cycloserine	0.00125
Polymyxin B	0.0025
Oxolinic acid	0.00025
Cyclohexamide	0.005

1.5 % agar; pH 6.8 (6.5)

Austin et al 1983; Daly & Stevenson1985

Aeromonas salmonicida CBB, Congo Red Agar

TSA + Coomassie brilliant blue 0.01 %



Udey 1982; Cipriano and Bertolini 1988

Michel & Faivre 1991: chloramphenicol & methicillin used in an in vivo *A. sal* competition study

Yersinia ruckeri – SW, ROD, XLD, et al.

Ribose – Ornithine – Desoxycholate

Rodgers 1992

	Yeast extract	0.3 %
	Sodium chloride	0.5
selective	Sodium desox.	0.1
selective	SDS	1
non-black	Sodium thiosulfate	0.68
non-black	Ferric Am. Citrate	0.08
yellow colony vs. red medium	Ornithine	0.5
yellow colony vs. red medium	Ribose, Maltose	0.375, 0.75
yellow colony vs. red medium	Phenol Red 7.4	0.008

"SW" Waltman & Shotts, 1984

	Tryptone	0.2
	Yeast extract	0.2
	Sodium chloride	0.5
oleic acid hydrolysis ppt zone	Calcium chloride	0.01
oleic acid hydrolysis ppt zone	Tween 80	1 v/v (pH to color)
green colony on green	Sucrose	0.5
green colony on green	BTB 7.4	0.0003 (0.003)

T-80 in family of fatty acid esters, fatty acid is oleate

Flavobacterium columnare

Shieh medium + tobramycin (Decostere et al 1997)

SCA Hawke & Thune 1992	
Tryptone	0.05 %
Yeast extract	0.05
Beef extract	0.02
Sod. acetate	0.02
Neomycin	5 µg/mL
Polymyxin B	200 IU/mL

GN or Hsu – Shotts Bullock et al 1986	
Tryptone	0.2 %
Yeast extract	0.05
Gelatin	0.3
Neomycin	4 µg/mL

Flavobacterium psychrophilum

Stay Tuned. #2 + antimicrobics

Tryptone	0.5 %
Yeast extract	0.05
Beef extract	0.05
Sodium Acetate	0.02
Mag. Sulfate	0.05
Calcium chloride	0.02
FBS	5

Edwardsiella tarda

Enrich in 2X SS; plate on SS

Salmonella-Shigella Medium

	Peptone & Beef Extract	0.5 % ea
	Sodium Citrate	0.85
Inhibit G+ & many G–	Bile Salts	0.85
	Brilliant Green	0.033
Black centers	Sodium Thiosulfate	0.85
	Ferric Citrate	0.1
Green colonies on red-orange	Lactose	1
	Neutral Red	0.0025

Edwardsiella ictaluri (green)

"SIM" Shotts & Waltman 1990

	Tryptone	1 %
	Yeast extract	1
	Sodium chloride	5
Phenylpyruvic acid and ferric ions = brownish-green	Phenyalanine	0.125
	Ferric amon. citrate	0.12
Non-yellow zone on green	BTB	0.003
	Mannitol	0.35
Selective	Colistin	10µg/mL
	Bile salts	0.1

Motile *Aeromonas* sp.: R-S and SGAP-10C

A. hydrophila: yellow on R-S; maltose pos, H2S, lysine and ornithine neg

minimal	Yeast extract, sodium chloride
AA basic	L-lysine HCl, L-ornithine HCl
sugar acidic	Maltose, BTB, pH 7.0
H_2S	Sodium thioS, L-cysteine, Ferric AC
selection	Sodium deoxycholate, novobiocin

SGAP: starch, glutamate, ampicillin, penicillin

Contrast of pH Indicator

Colony Contrast

Streptococcus iniae. ‘TAOAB’ thalliuim acetate-oxolinic acid-blood. Todd-Hewitt broth spiked with thallium acetate and abx (Nguyen et al 2002)

Pseudomonads. PIA pseudomonas isolation agar

Vibrio spp. TCBS thiosulfate-citrate-bile-sucrose

Vibrio anguillarm. ‘VAM’ salts, bile salts, sorbitol as C-source, ampicillin, etc. (Alsina et al 1994)

Vibrio vulnificus. ‘VVM’ cellobiose as C-source, salts, etc. (Cerdà-Cuéllar et al 2000)

Mycobacteria. Ex. Lowenstein series (e.g. Gruft has abx)

Streptococcus spp. and *Staphylococcus* spp. Azide Blood Agar

Lactobacillus spp. and *Carnobacterium maltaromaticum (piscicola)*: Rogosa SL rich, storebought

Development of an improved medium for primary isolation of *Flavobacterium psychrophilum*

Clifford Starliper, USGS WV

Sue Marcquenski, WI DNR

Andrew Noyes, NY SDEC

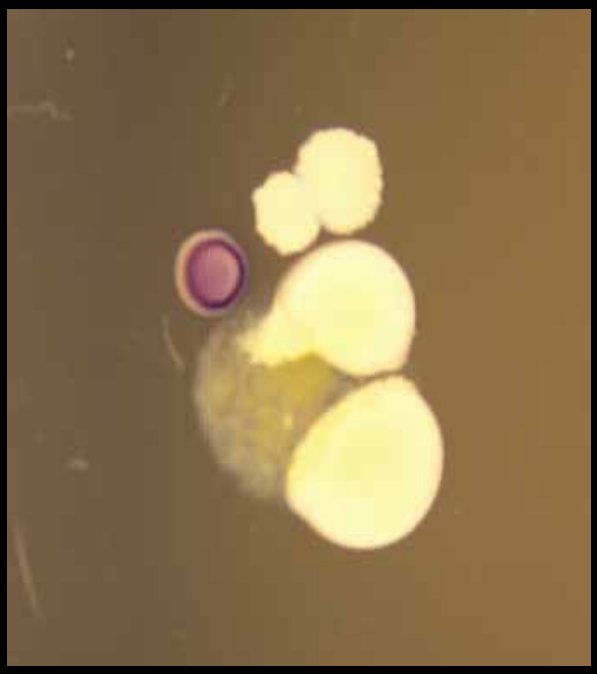
Rod Penney, MNR, ON

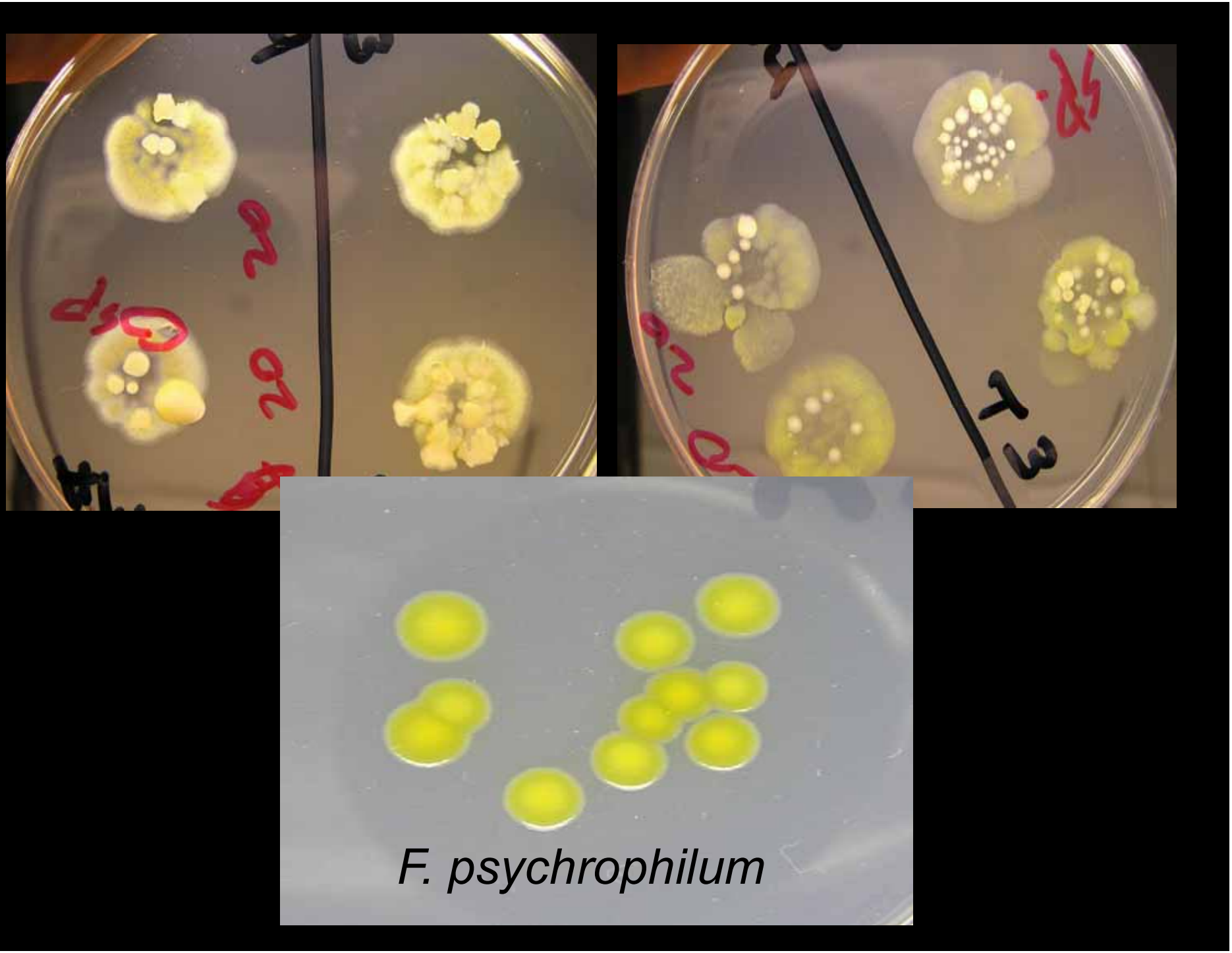
Pamela Whittington, USGS WV

Erin Edge & Kristin Sayler, SU WV

GLFC

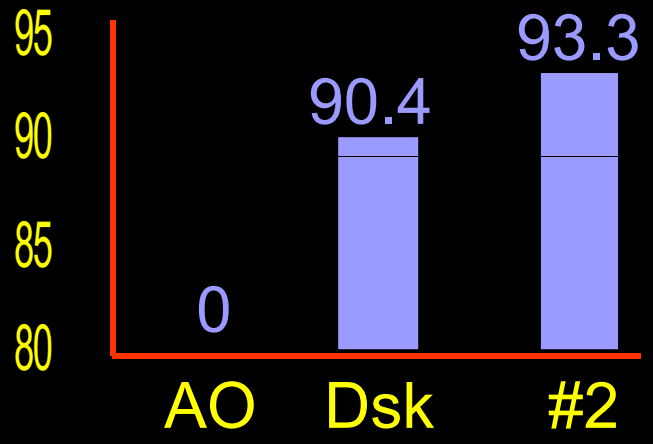
USGS science for a changing world




F. psychrophilum

%'s	AO	Dsk	#2
Tryp	.05	.05	.5
YE	.05	.05	.05
SodA	.02	.02	.02
BE	.02	.02	.05
$CaCl_2$			.02
$MgSO_4$			.05
Serum			5fbs
Galac		.05	
Gluc		.05	
Rham		.05	
S.Milk		.05	

% Recovery of 130+ *F. psychrophilum*, -70°C



Improved Medium

1- Enhance the growth of *F. psychrophilum* (desired) on primary isolation plates

   * Increase colony size (i.e. 'luxurious)
   * Reduce time until recognized colonies

2- Inhibit the growth of contaminants (undesired)

   * Antimicrobial(s) supplement

3- Ease of preparation

4- Inexpensive

Uses

Confirmed etiology

Cultures for sensitivity; minimize Abc resistance development

Diagnostic tool

Preventative tool; screening and follow morts; quicker intervention while fish continue to feed

Inexpensive

% positive	WI n=10	PNW n=20	East n=25
CO, cat, shift	100	100	100
grow ↑ media	Vneg	0	V
gel, cas, lysis	100	100	100
tyrosine	70	55	80
starch, xan, $ChSO_4$, indole	0	0	0
No_3 reduction	80	5	53
elastin-ase	10	0	64
18 sugars	70 (1-9)	5 (7 hit)	4 (3 hit)
6,16,24,30°C	+++V	++VV	++VV

Anacker and Ordal 1959. J Bact 78:25 “Cytophaga”

Tryptone: 0.4 %, 0.5 %

Bernardet & Kerouault 1989. AEM 55:1796; Lorenzen 1993. BEAFP 13:64; Brown et al. 1997. DAO 29:213; Rangdale et al. 1997. Aqua 158:193

Fetal Calf Serum (10%) European

Obach & Baudin-Laurencin 1991. DAO 12:13

Horse Serum (5%) European

Michel et al. 1999. ResM 150:351

Newborn Calf Serum (source?)

Lorenzen 1993 BEAFP 13:64 (5%); Brown et al. 1997 DAO 29:213 (0.5%)

Sugars/Carbohydrates/Protein

Daskalov et al. 1999. LAM 28:297

Calcium/Magnesium

Holt 1987 PhD. and other authors

Trace Elements Solution

Lewin & Lounsbery. 1969. JGM 58:145

“weak” Agar Concentrations

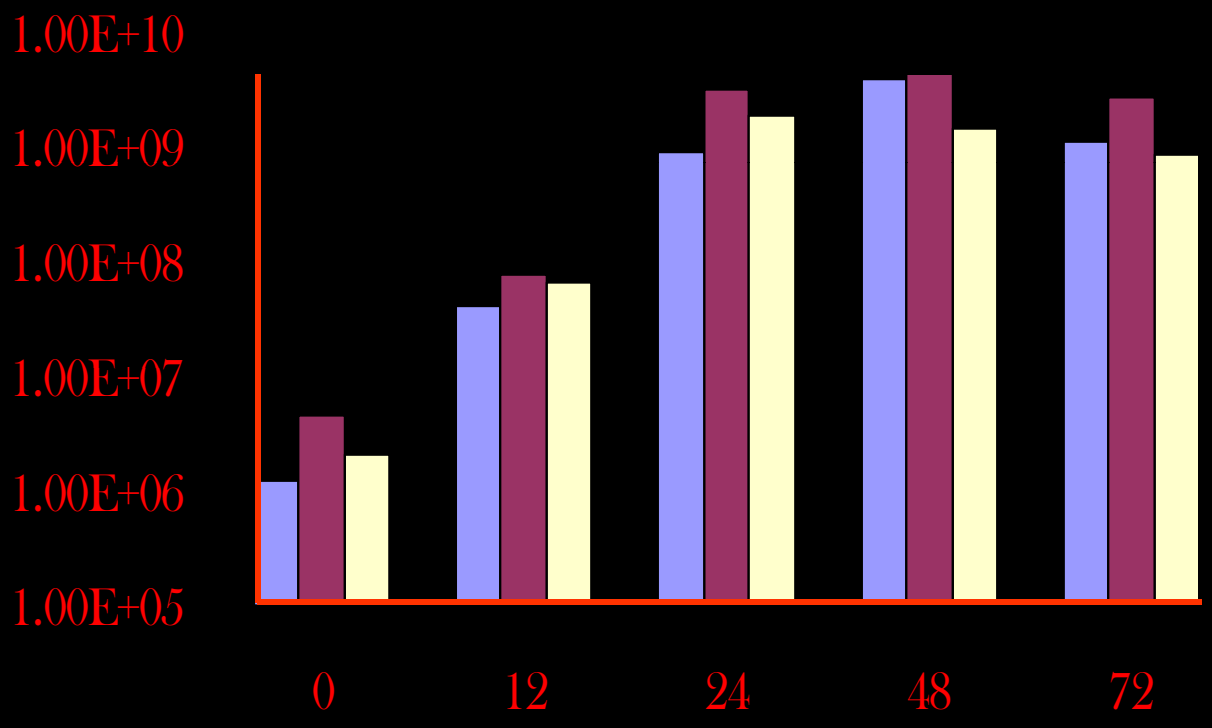
%'s	AO	Rgd	Brn	Lrz	Mch	Dsk	#2
Tryp	.05	.5	.4	.5	.5	.05	.5
YE	.05	.05	.04	.05	.05	.05	.05
SodA	.02	.02		.02	.02	.02	.02
BE	.02	.05		.02	.02	.02	.05
$CaCl_2$			.02				.02
$MgSO_4$			.05				.05
Serum			.5nbc	5nbc	5h(fc)		5fbs
Galac						.05	
Gluc						.05	
Rham						.05	
S.Milk						.05	
TrcEle					L&L		

Three media for Evaluation. Broth and Agar. Always made in 1 L batches. %'s given.

pH's=7-7.2	AO Anacker & Ordal 1959	EAO+FBS Michel et al. 1999*	#2
Tryptone	0.05	0.5	0.5
Yeast Extract	0.05	0.05	0.05
Beef Extract	0.02	0.02	0.05
Sod. Acetate	0.02	0.02	0.02
Calcium Cl			0.02
Magnes. SO_4			0.05
Serum		5 FBS (H)	5 FBS

*trace element pack deleted.

Initial Trials of *F. psychrophilum*** grown in experimental medium (#2), diluted in tryp-ye, plated on #2. Excellent growth 24-48 h. Mean cfu/mL



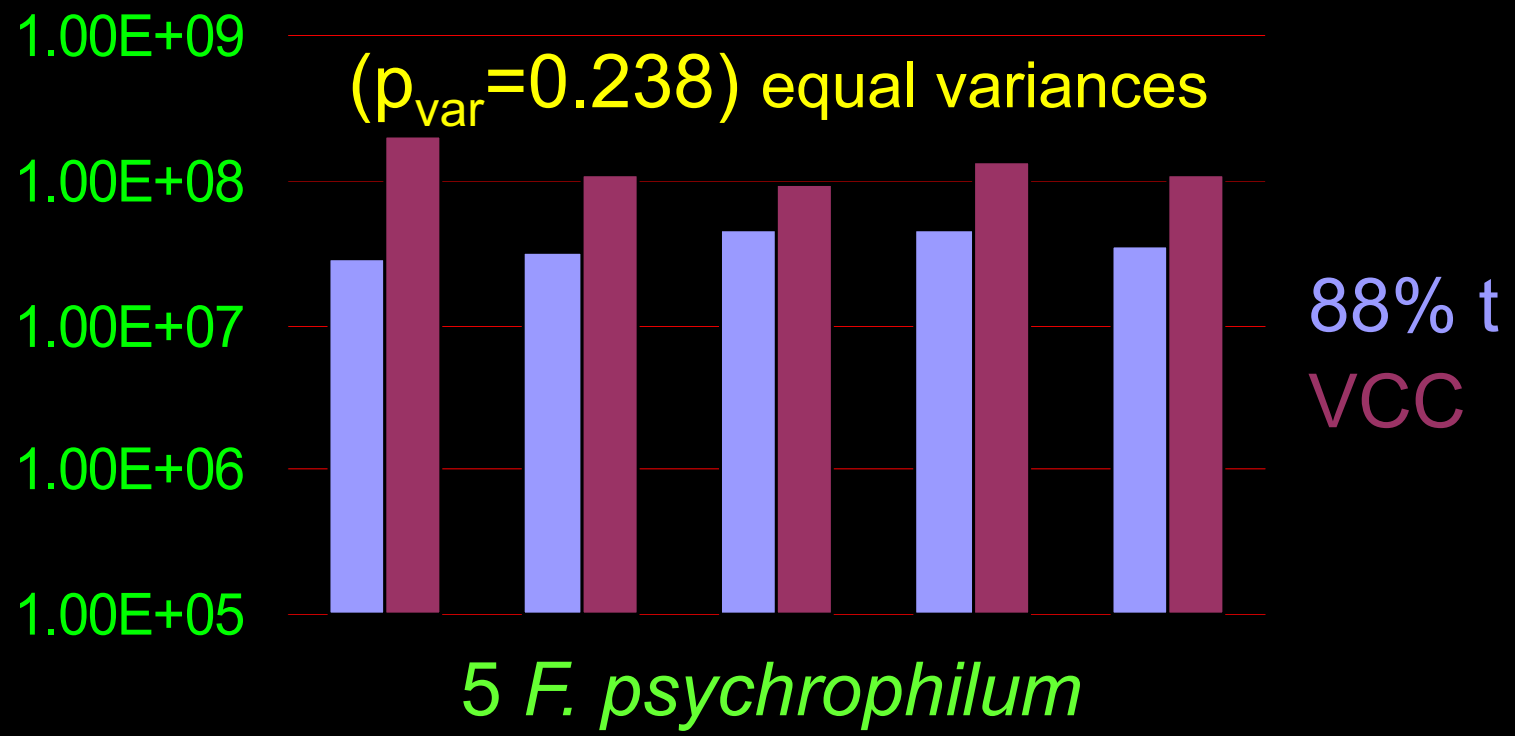
**Three geog locales. Wash n=7; Wisc n=4; Eastern n=8

1mL (-70°C) + 5mL #2 broth, 48 h, 16°C

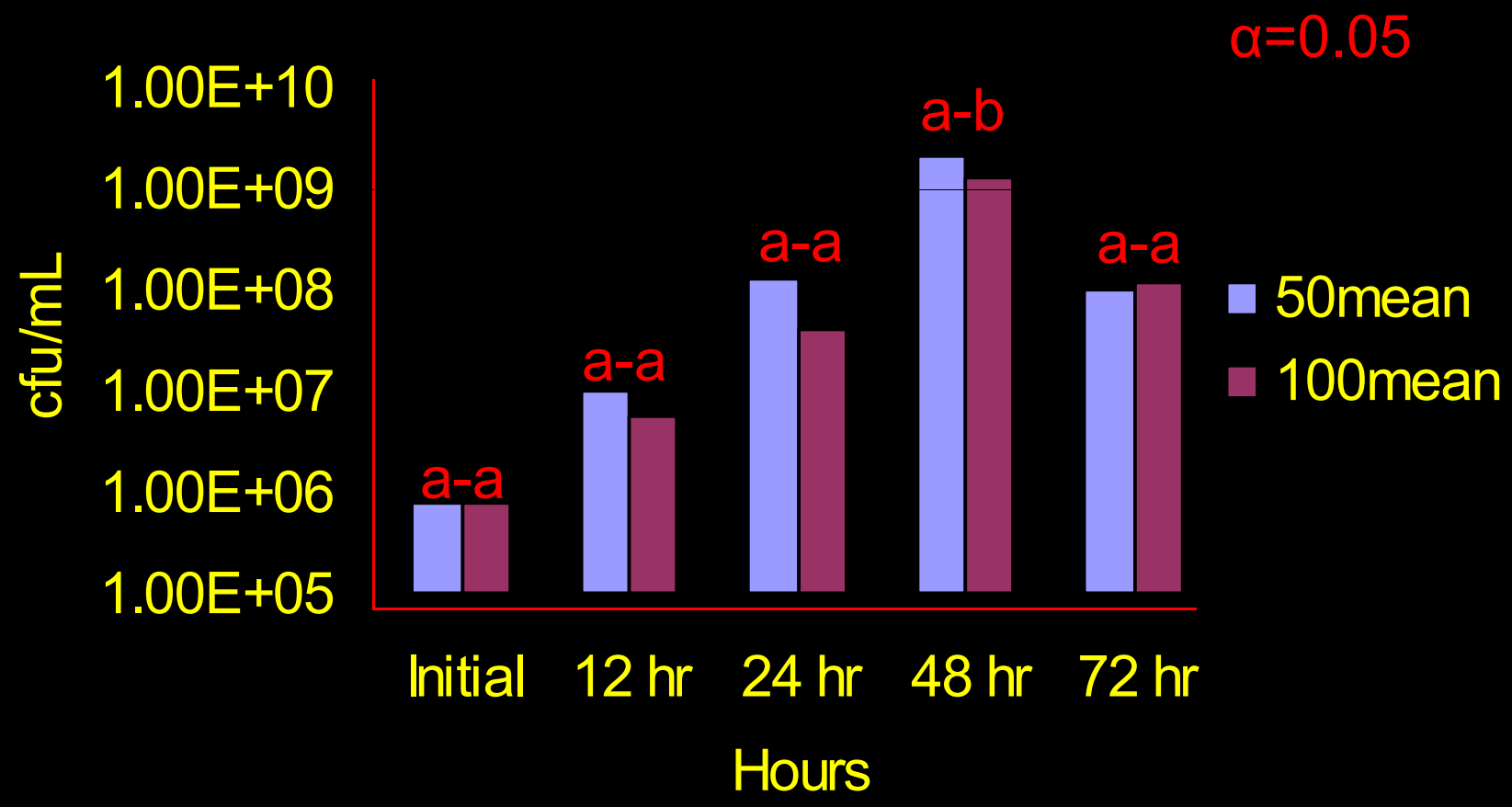
0.5 mL into 5 mL #2 broth, 48 h, 16°C

88%t @ 525λ
VCC diluted in tryp-ye

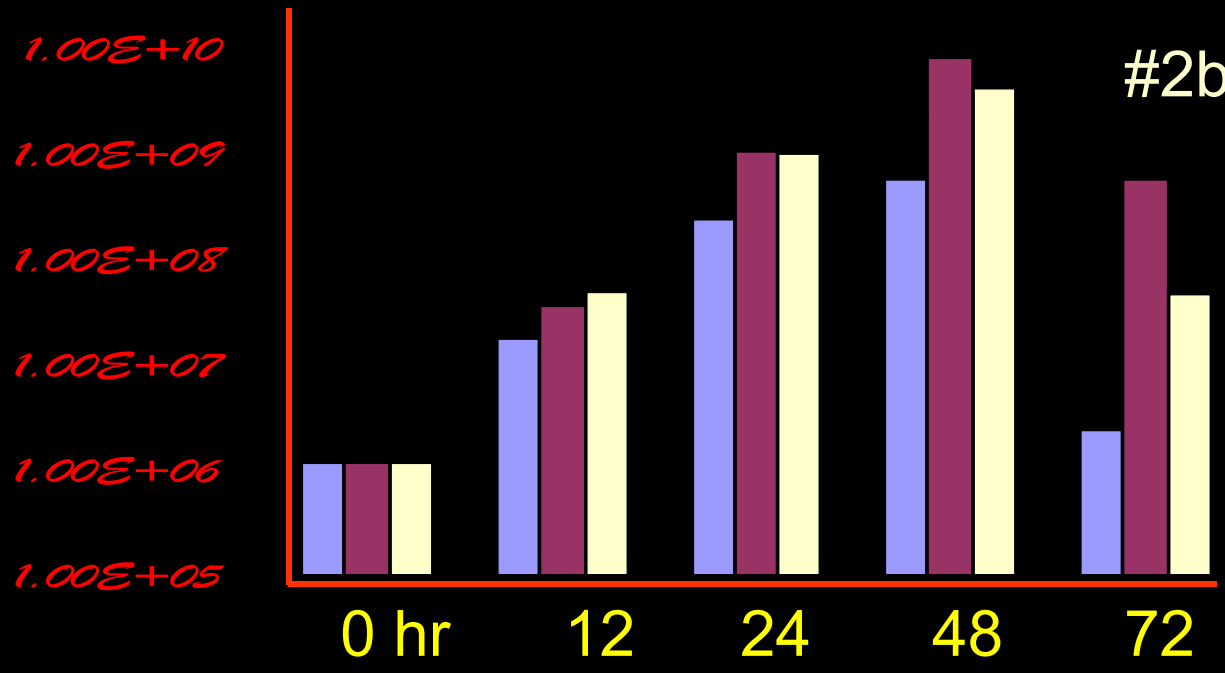
VCC diluted in tryp-ye
(1% to flasks)



F. psychrophilum: 50 vs. 100 mL in a 250 mL Flask @ 16C, 120 rpm n=3 isolates



Effect of cell diluent on cell viability. “Isotonic” vs. #2 basal vs. tryp-ye (0.1-0.05%). Three *F. psychrophilum* in #2 on #2.



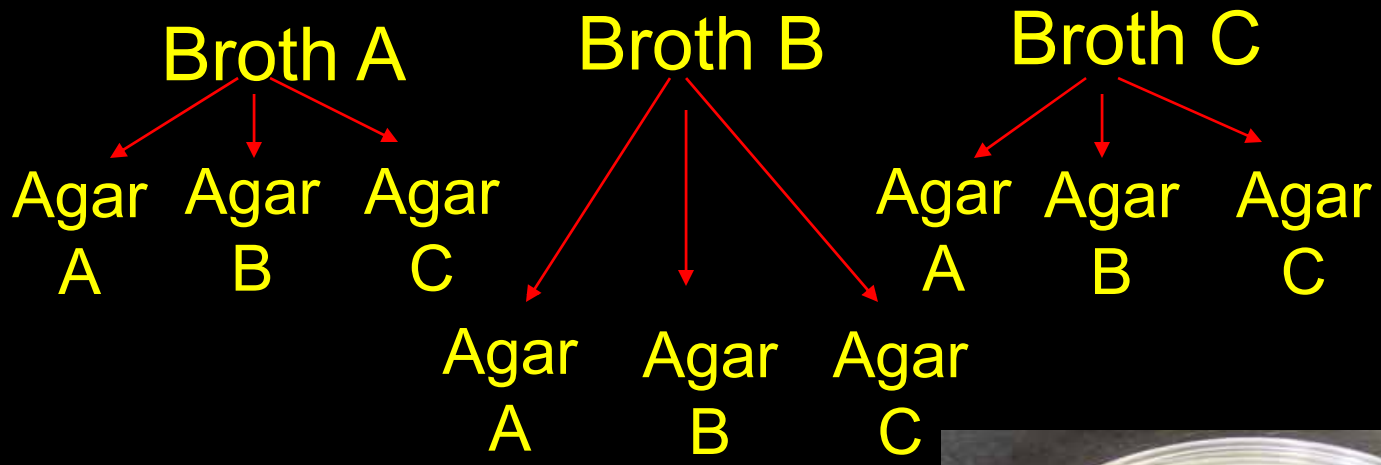
48h p=0.028*

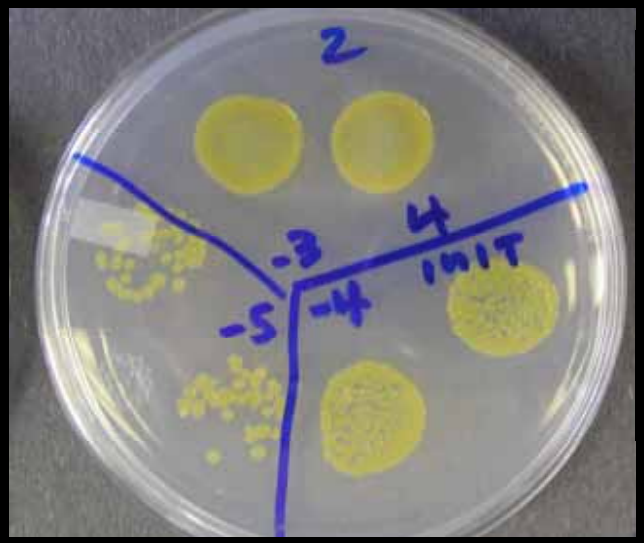
PBS	a
tryp-ye	b
#2bsl	ab

* Only other…In Cyto & on Cyto system at 72 h: p=0.0241
tryp-ye a…#2bsl b…PBS b

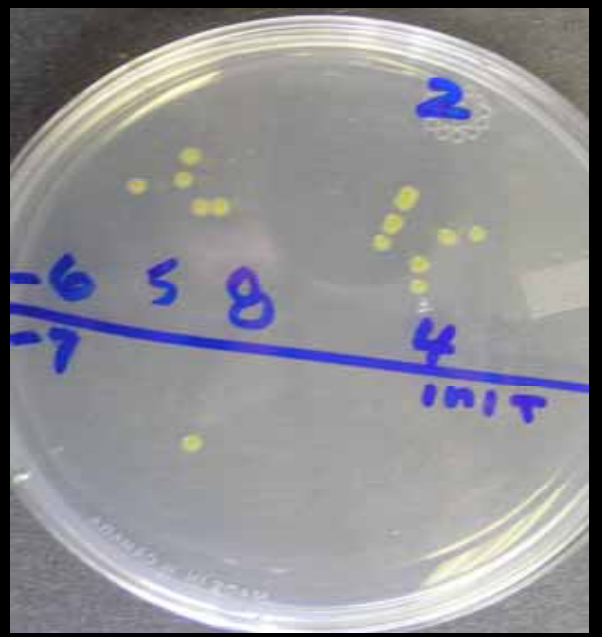
Ex: Experimental Design--

F. psychrophilum isolate 1% 50mL (std inoculum)

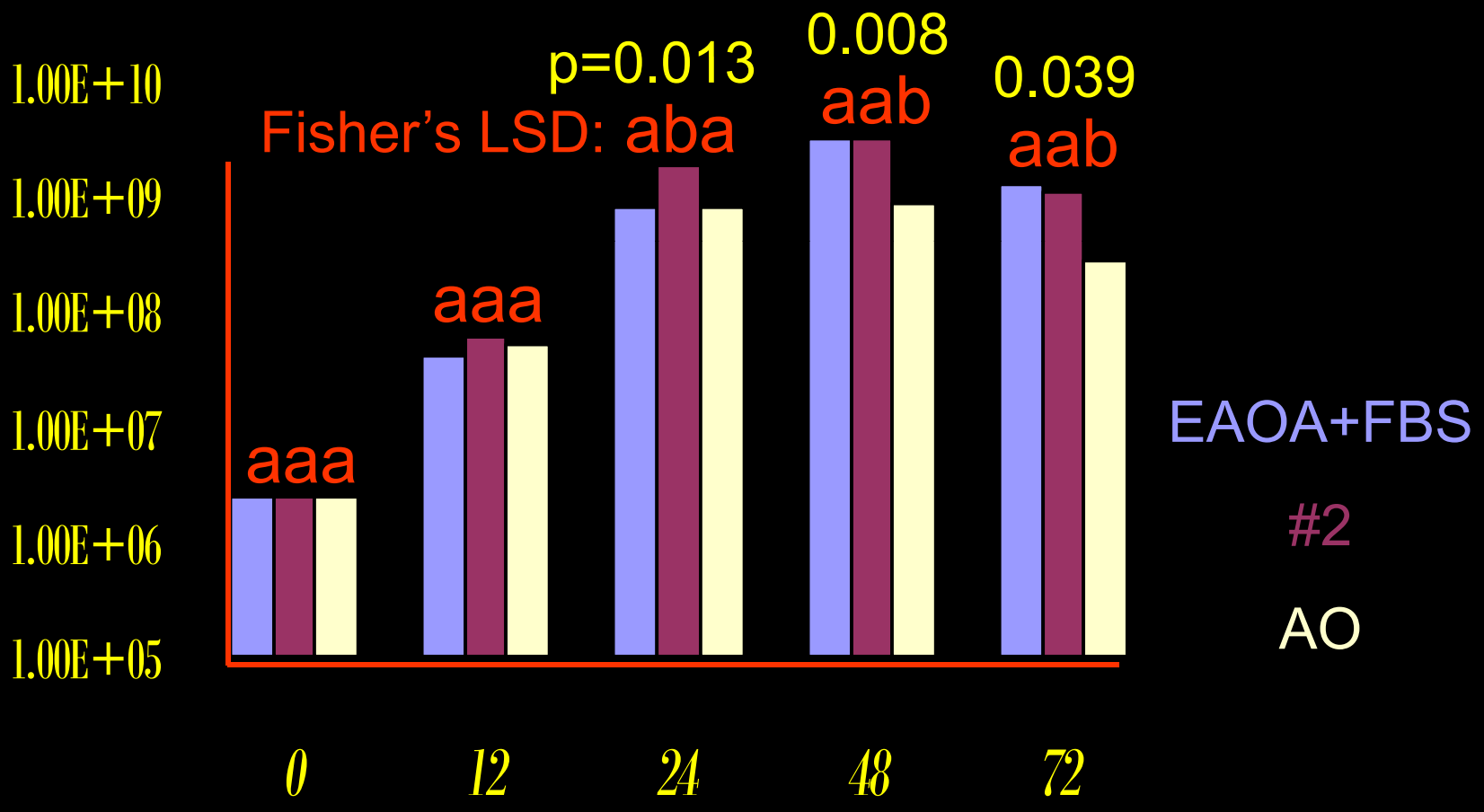




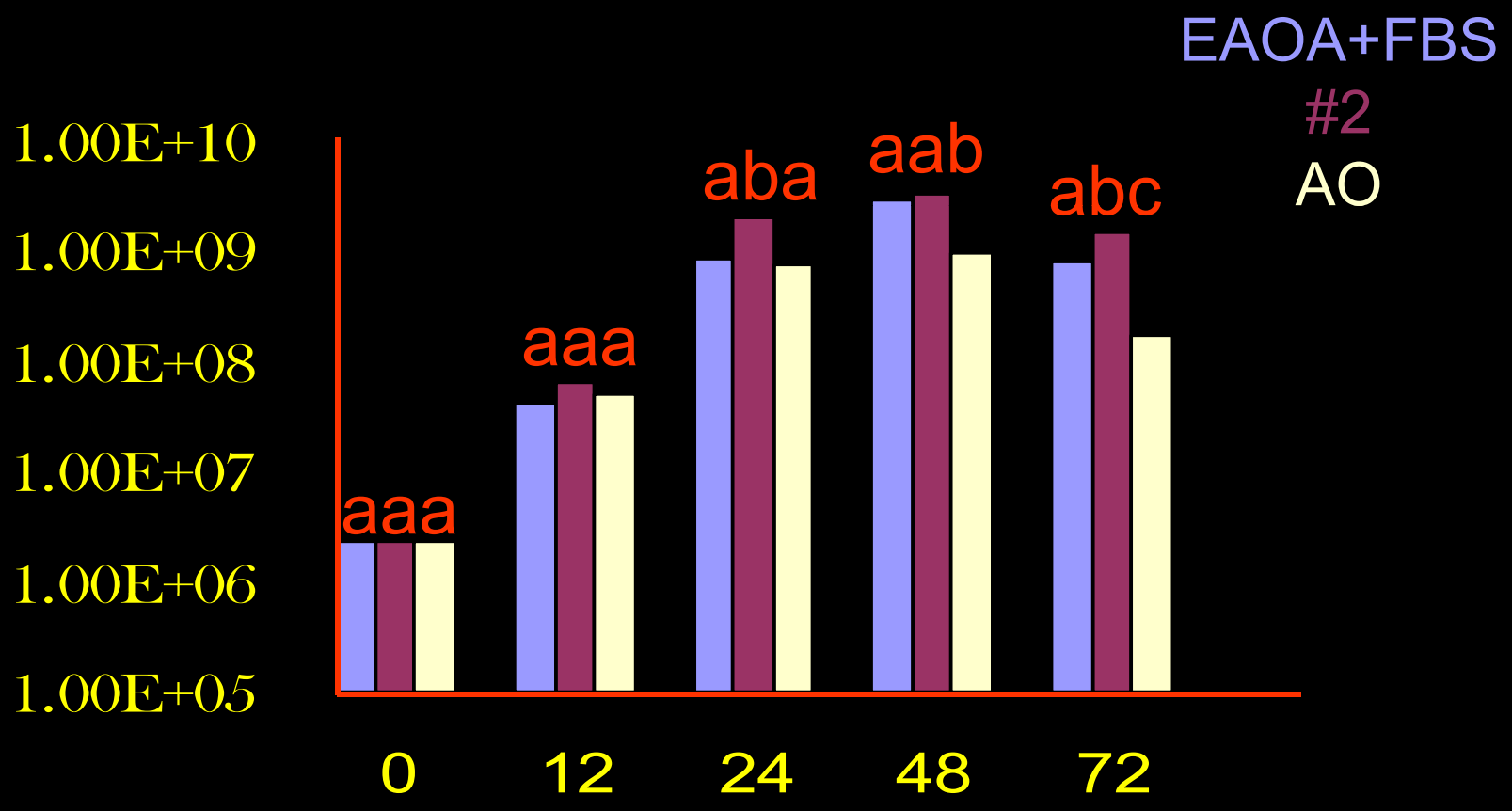
VCC
12 h
24 h
48 h
72 h



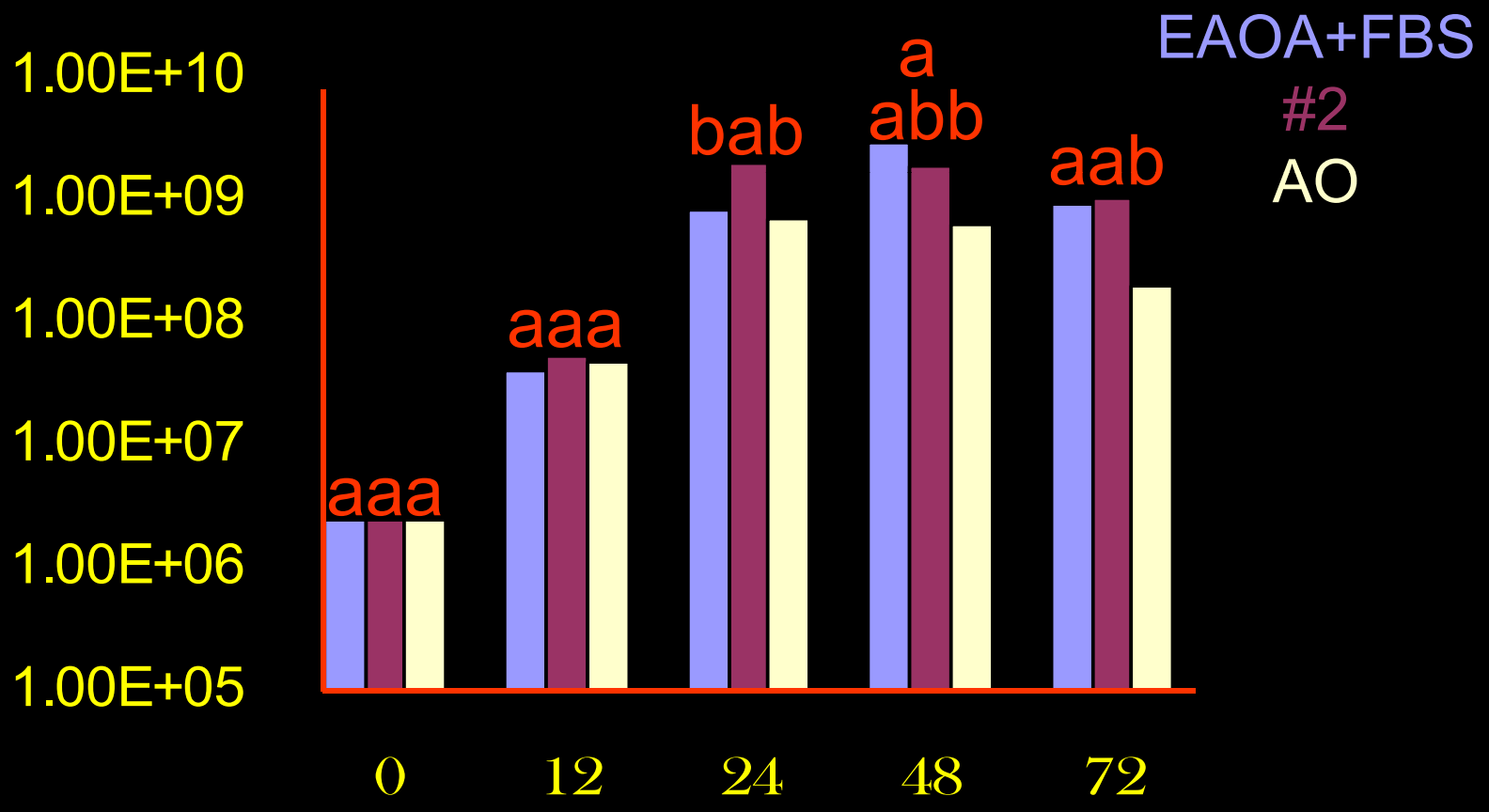
Best performing broth medium. Means for n=18 *F. psychrophilum*. All plated on #2 agar.



Best performing broth medium. Means for n=18 *F. psychrophilum*. All plated on EAOA+FBS.



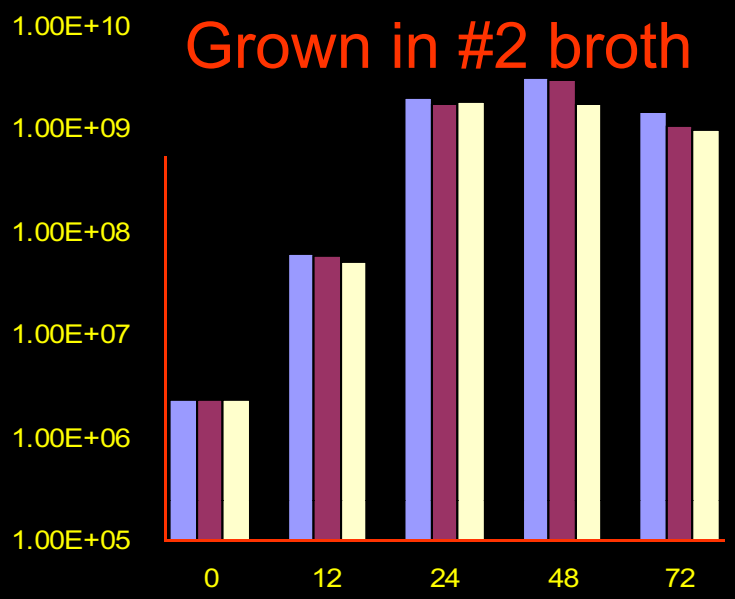
Best performing broth medium. Means for n=18 *F. psychrophilum*. All plated on AO Cytophaga.

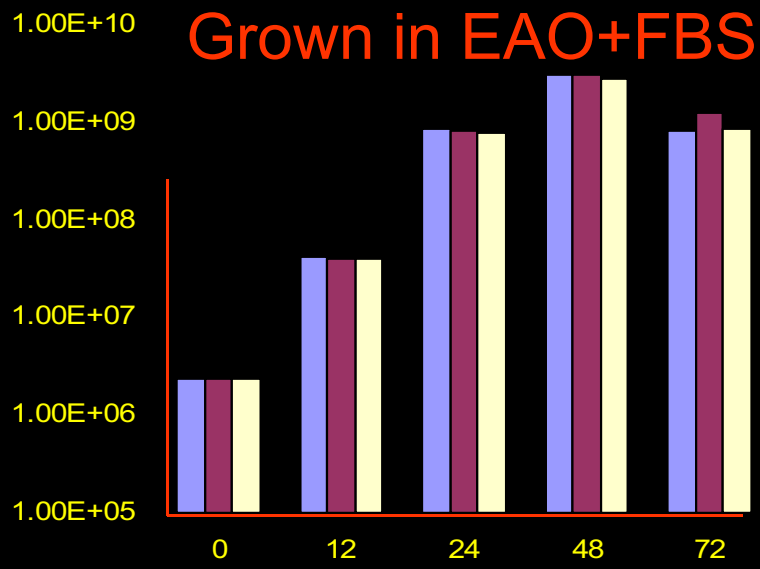


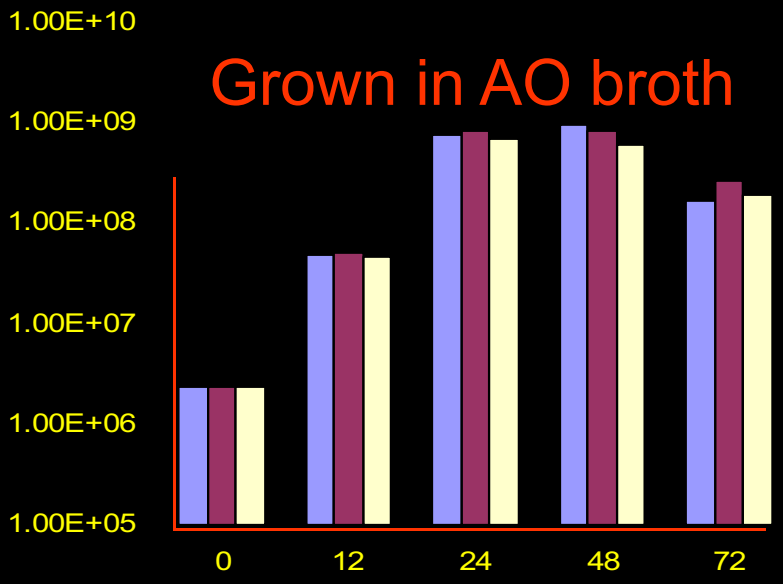
Best performing medium in agar format? n=18 *F.psy.*

*No quantitative Significant Difference**. ANOVA 0.05*

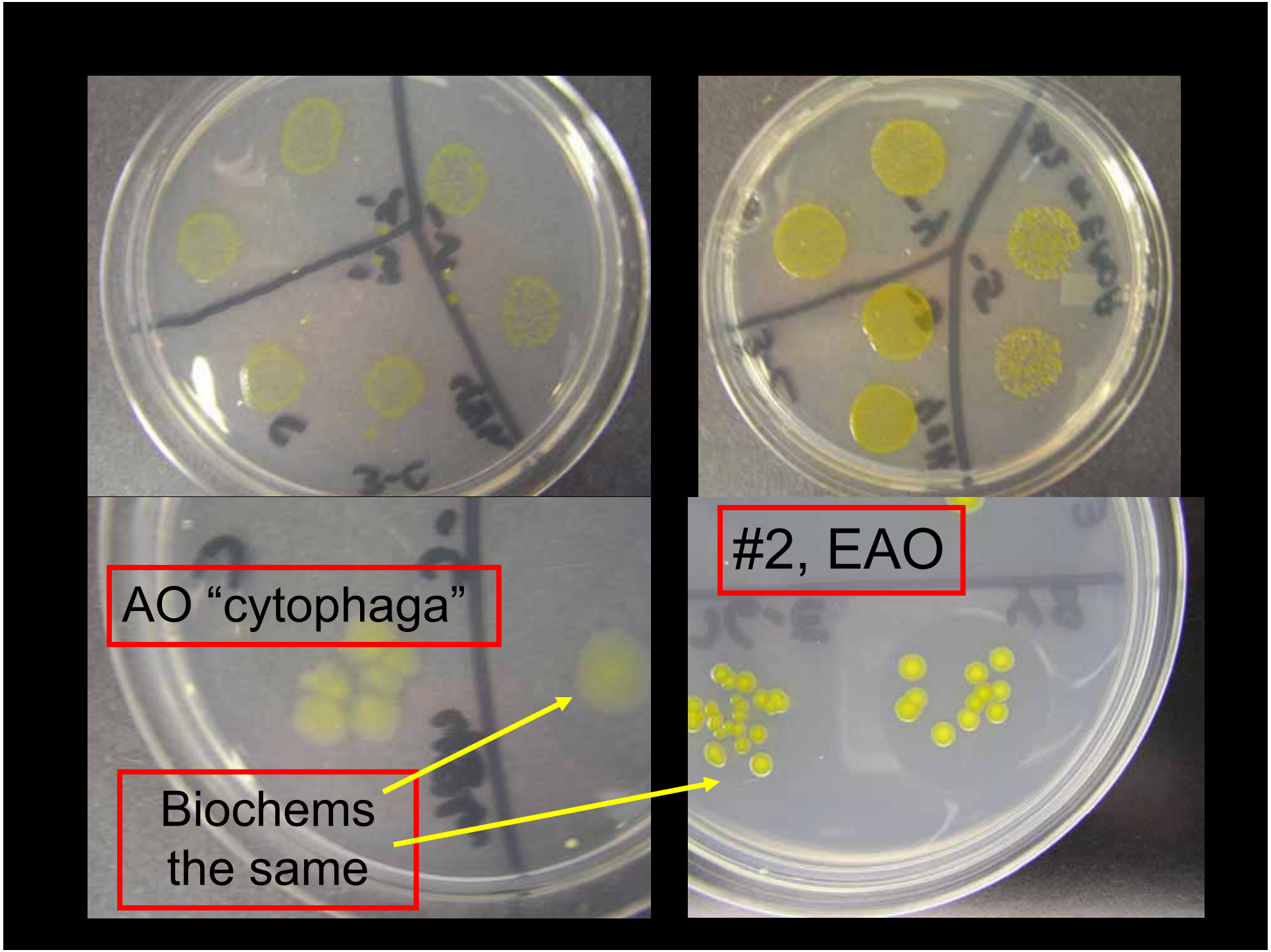
EAO+FB, #2, AO





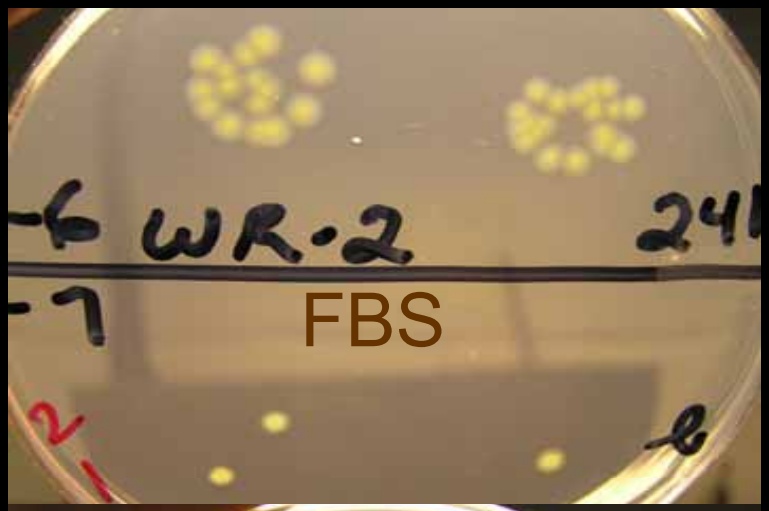


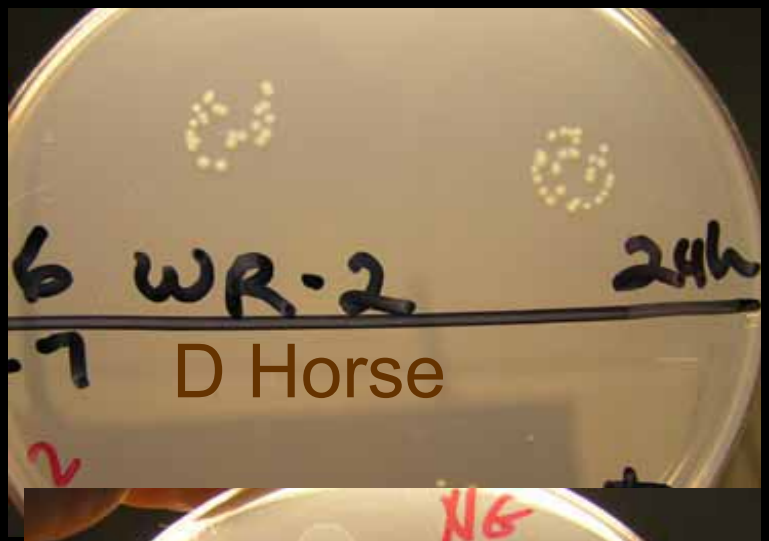
**NG's on EAO+FBS

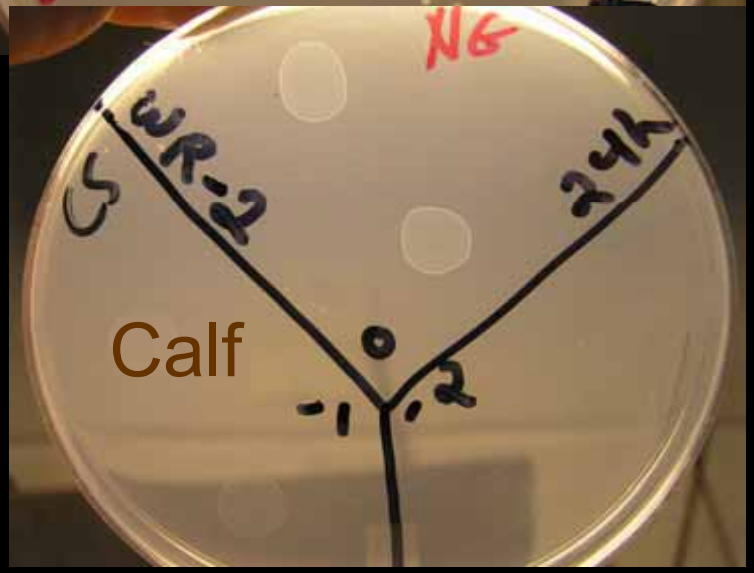

AO “cytophaga”
Biochems
the same
#2, EAO

Best is #2. Varying %'s had no effect: sodium acetate, magnesium, calcium (tryptone, YE, BE)

Cambrex Bio Science & Atlanta Biologicals







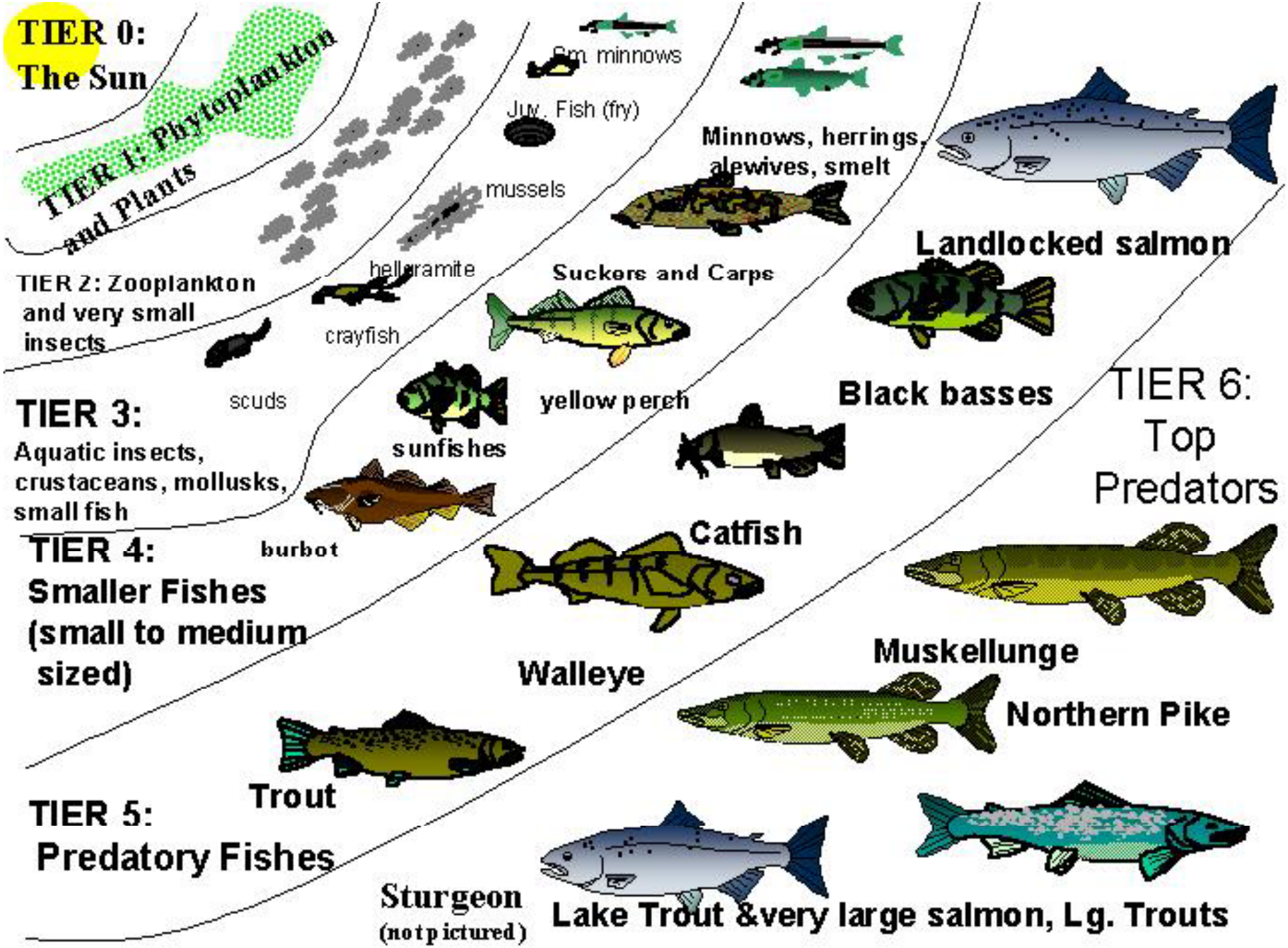
* With many isolates-reps, colonies counted on #2 a full day sooner than on AO
* Changed *F. psychrophilum* colony morphology on #2 is dependant on solid medium, not broth
* Serum reduces surface tension vs. AO
* Broth pH's were 7.6 – 8.0 after 72 h (should be, i.e. indicator of good culture growth)
* Did not demonstrate a benefit of metabolite
* Screen for selective agents. Test these in appropriate concentrations as additive(s) to #2 to inhibit/retard growth of non-desirables

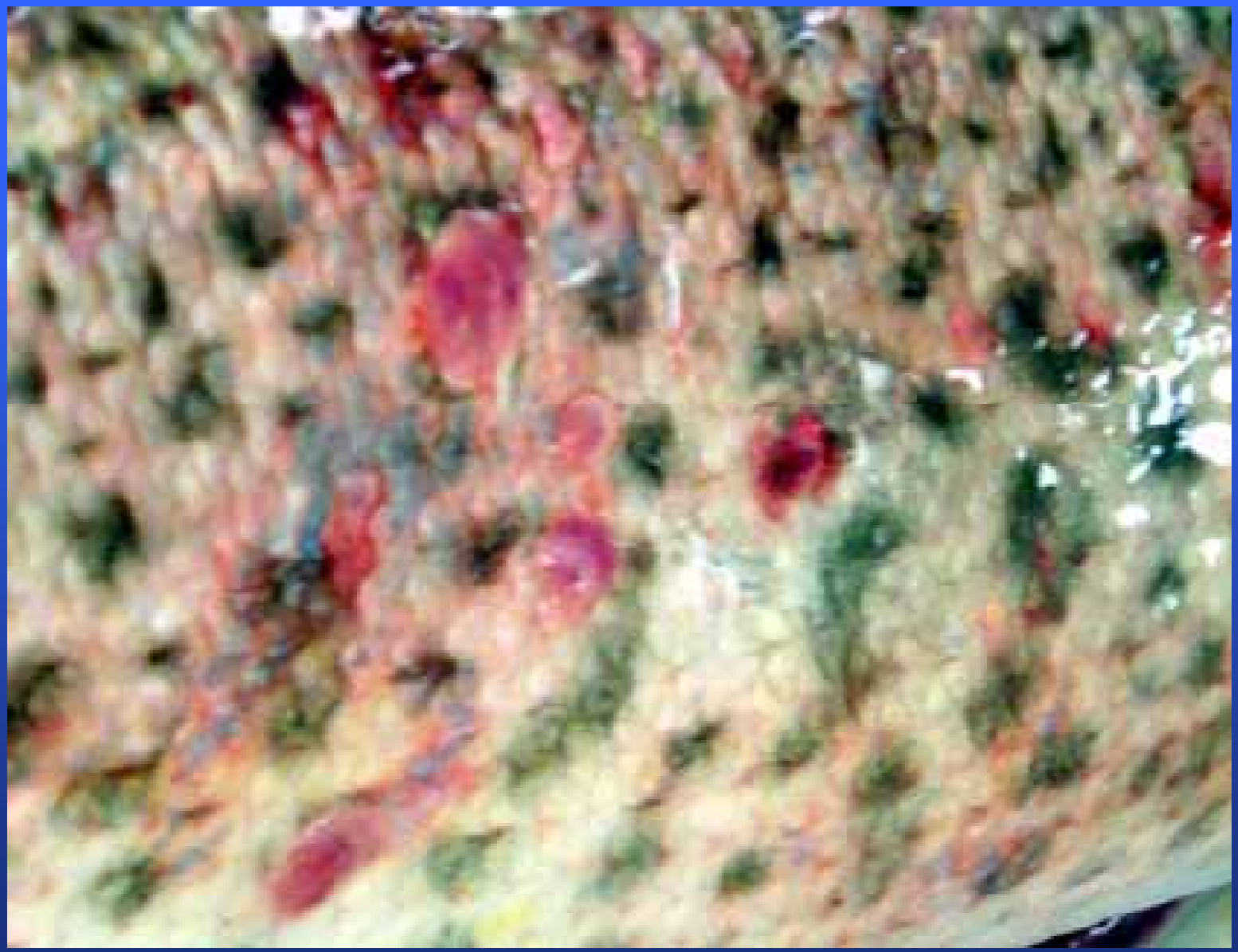
Mohamed Faisal, Gary Whelan, Michael Thomas, Ehab Elsayed, Kathryn Ambrose, Thomas Loch

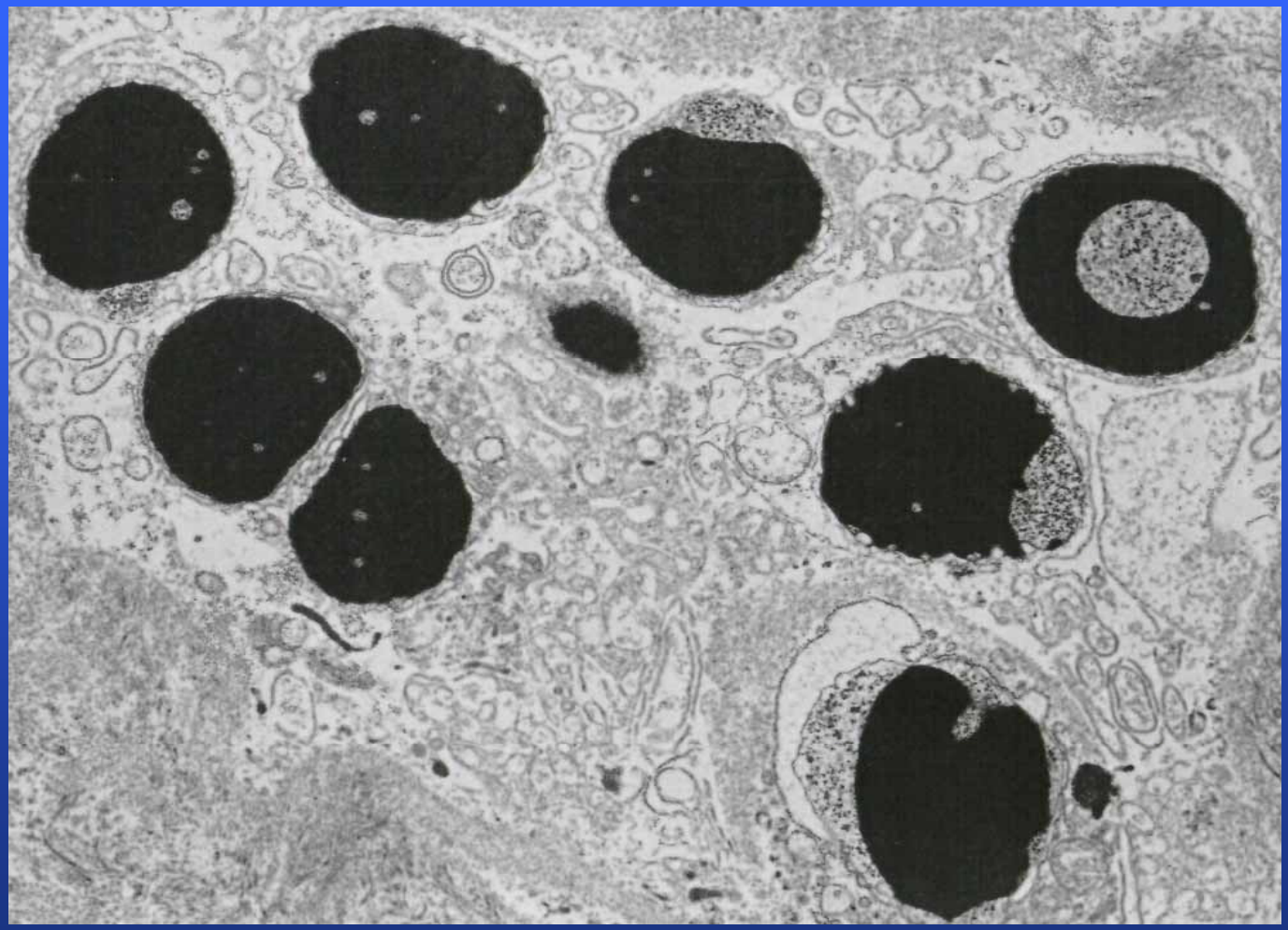
College of VETERINARY MEDICINE
Michigan State University

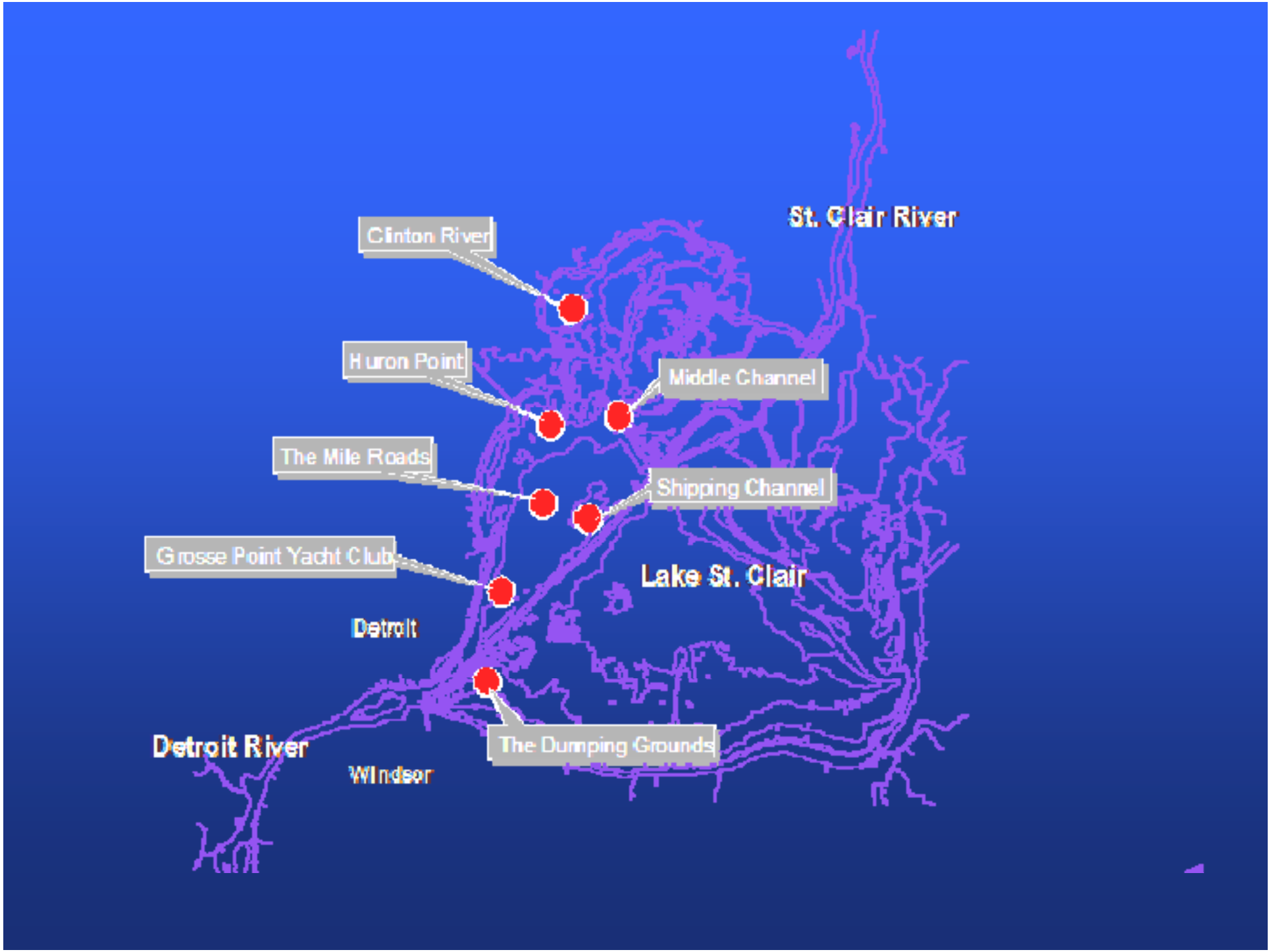
Department of Fisheries and Wildlife
Michigan State University

Department of Natural Resources

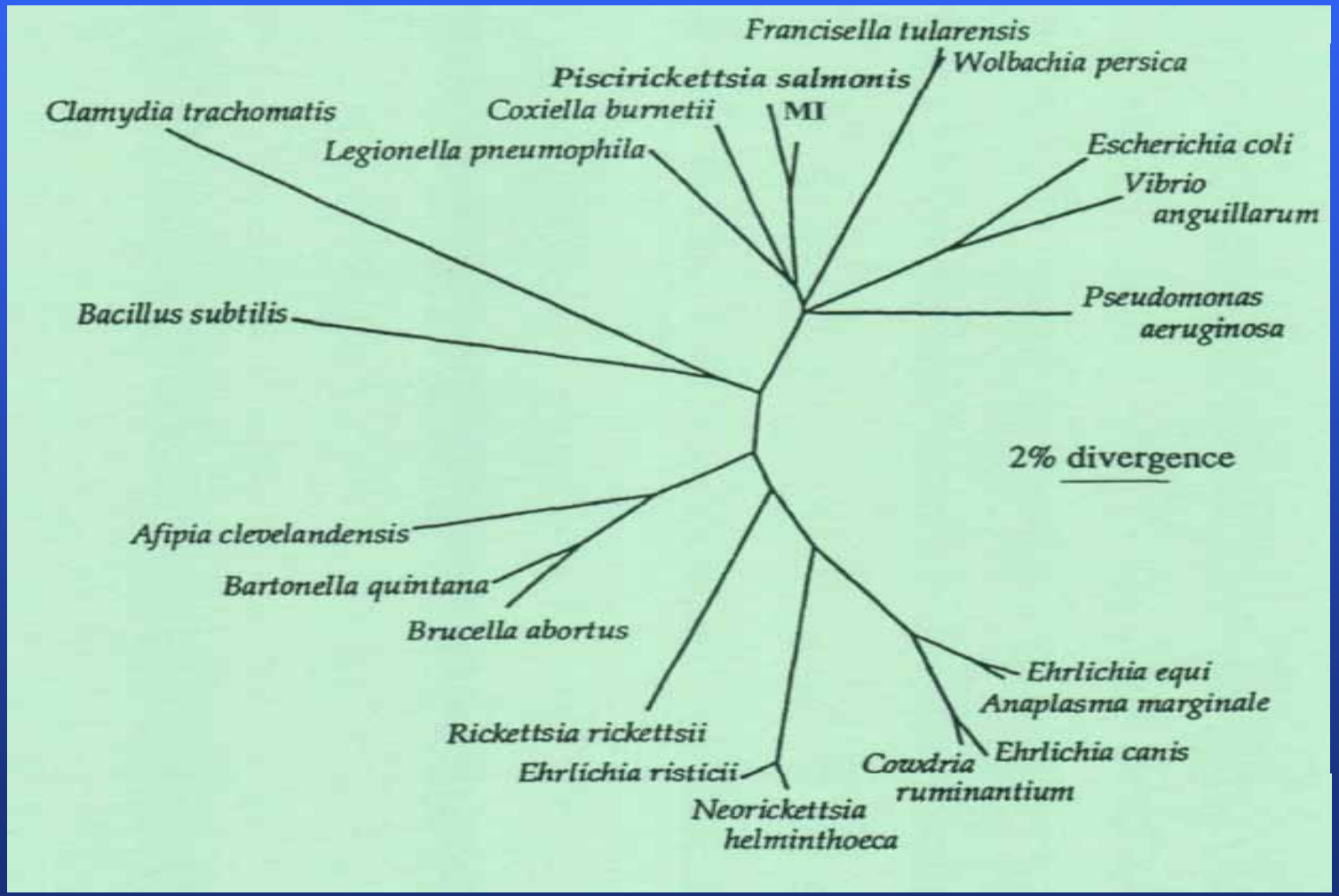

TIER 0:
The Sun
TIER 1: Phytoplankton and Plants
TIER 2: Zooplankton and very small insects
TIER 3:
Aquatic insects, crustaceans, mollusks, small fish
TIER 4:
Smaller Fishes (small to medium sized)
TIER 5:
Predatory Fishes
TIER 6:
Top Predators
Sm. minnows
Juv. Fish (fry)
mussels
hellgramite
crayfish
scuds
sunfishes
burbot
Trout
Sturgeon
(not pictured)
Minnows, herrings, alewives, smelt
Landlocked salmon
Suckers and Carps
yellow perch
Black basses
Catfish
Walleye
Muskellunge
Northern Pike
Lake Trout &very large salmon, Lg. Trouts

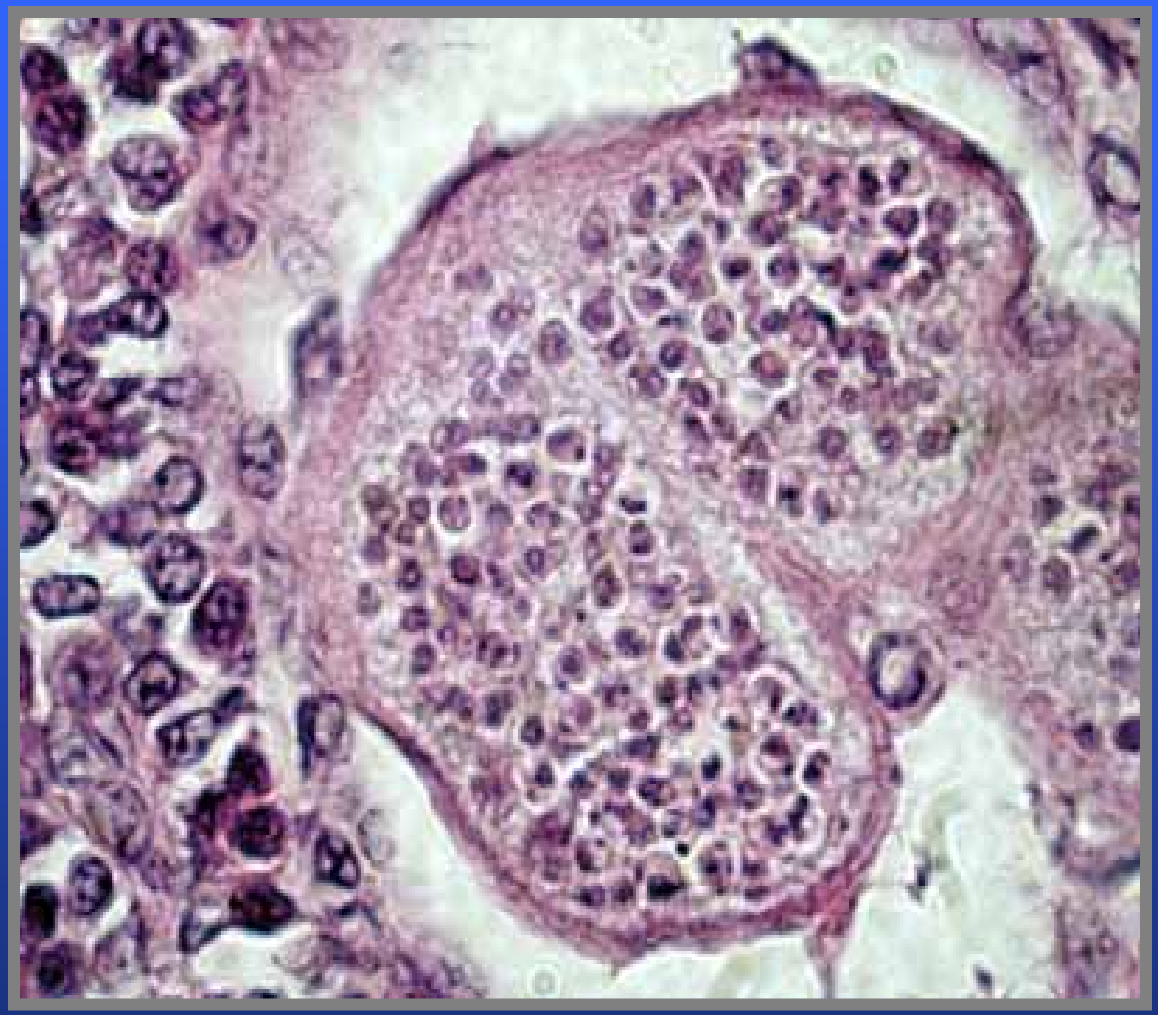




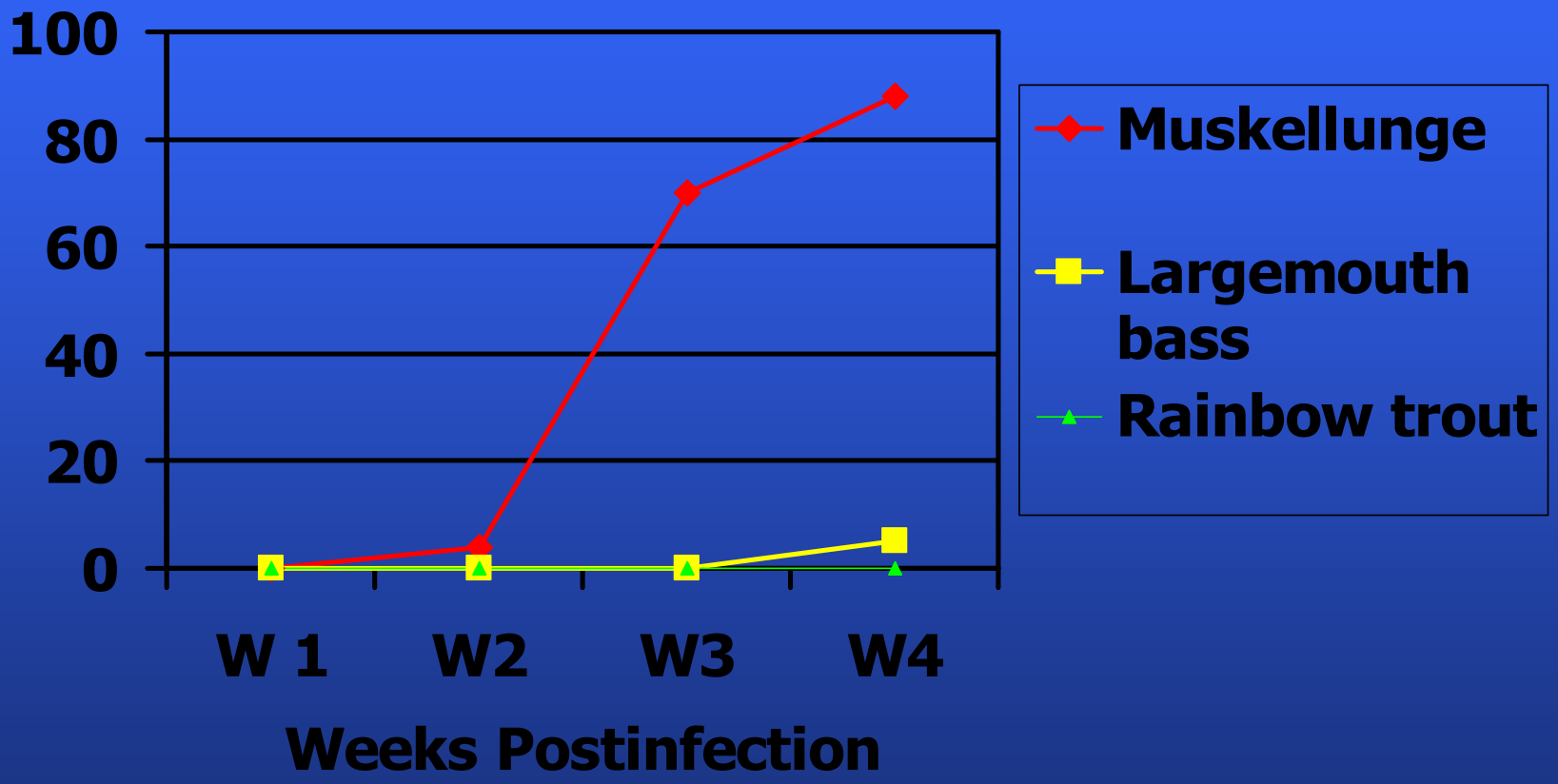

St. Clair River
Clinton River
Huron Point
Middle Channel
The Mile Roads
Shipping Channel
Grosse Point Yacht Club
Lake St. Clair
Detroit
The Dumping Grounds
Detroit River
Windsor

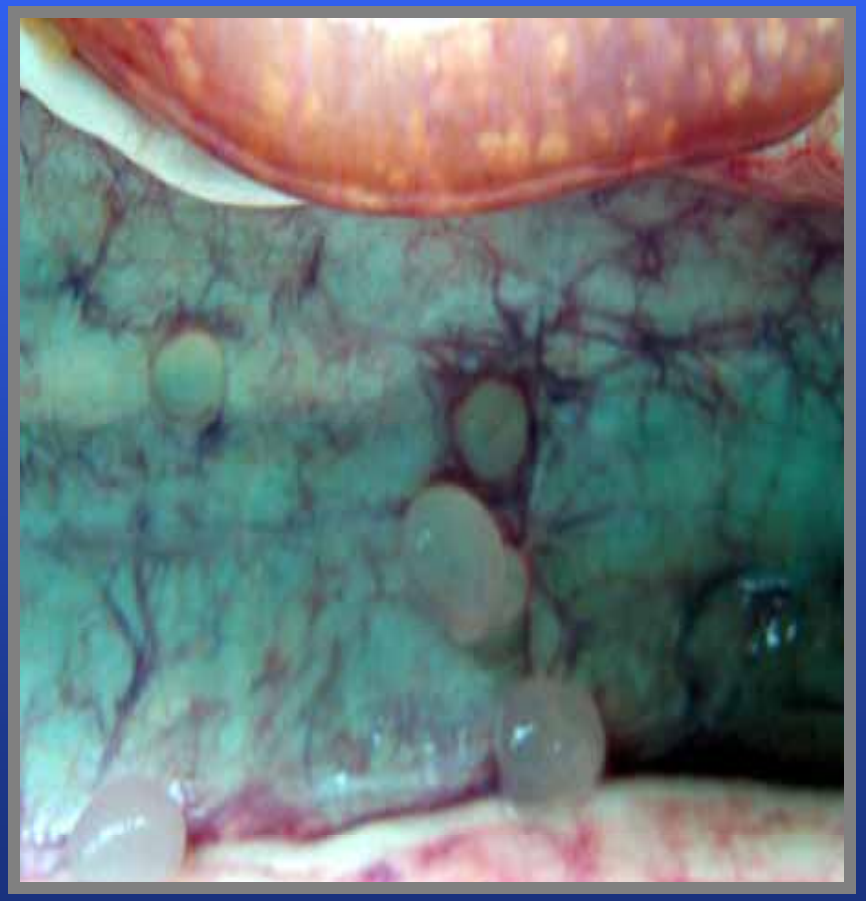
Phylogenetic Analysis Based upon 16S, ITS, and 23S rDNA Sequencing

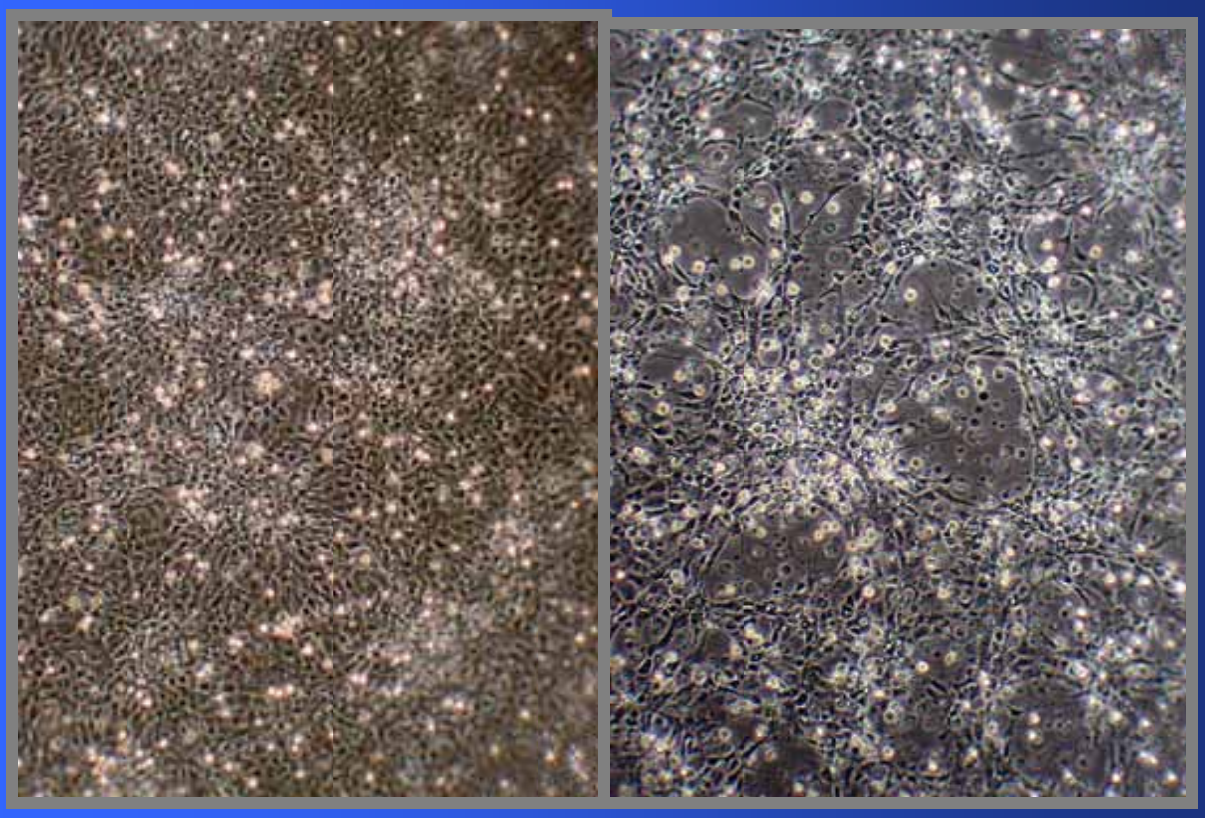


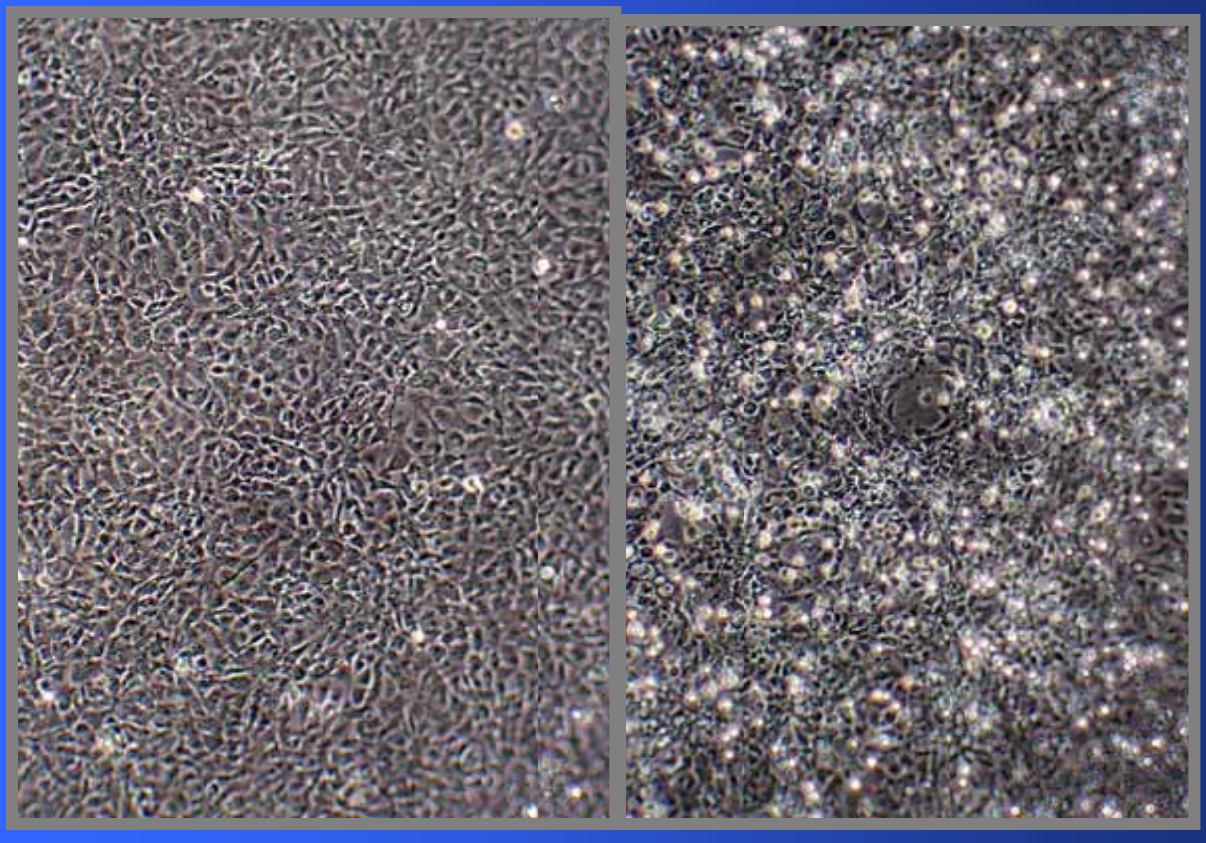


Cumulative Mortalities

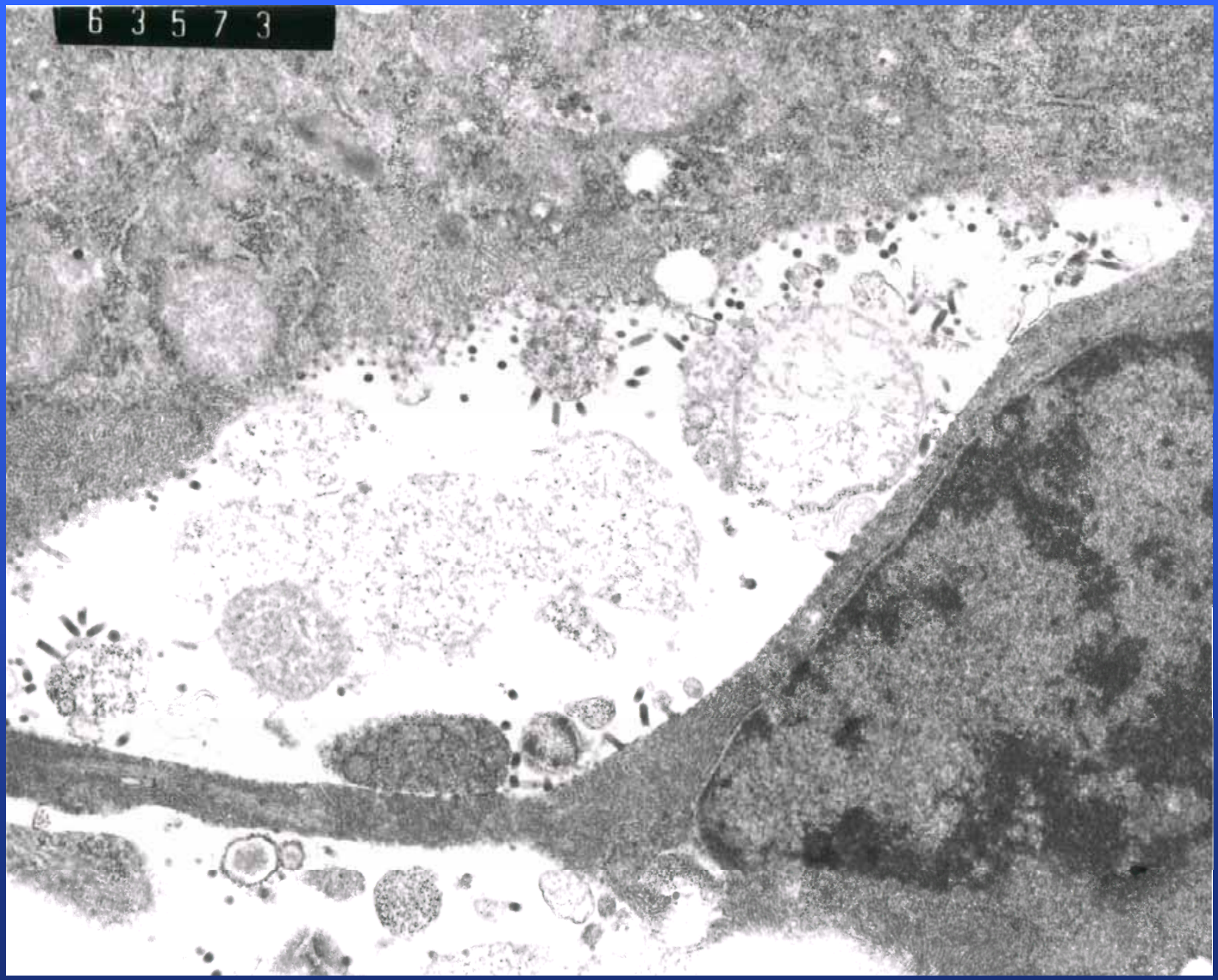


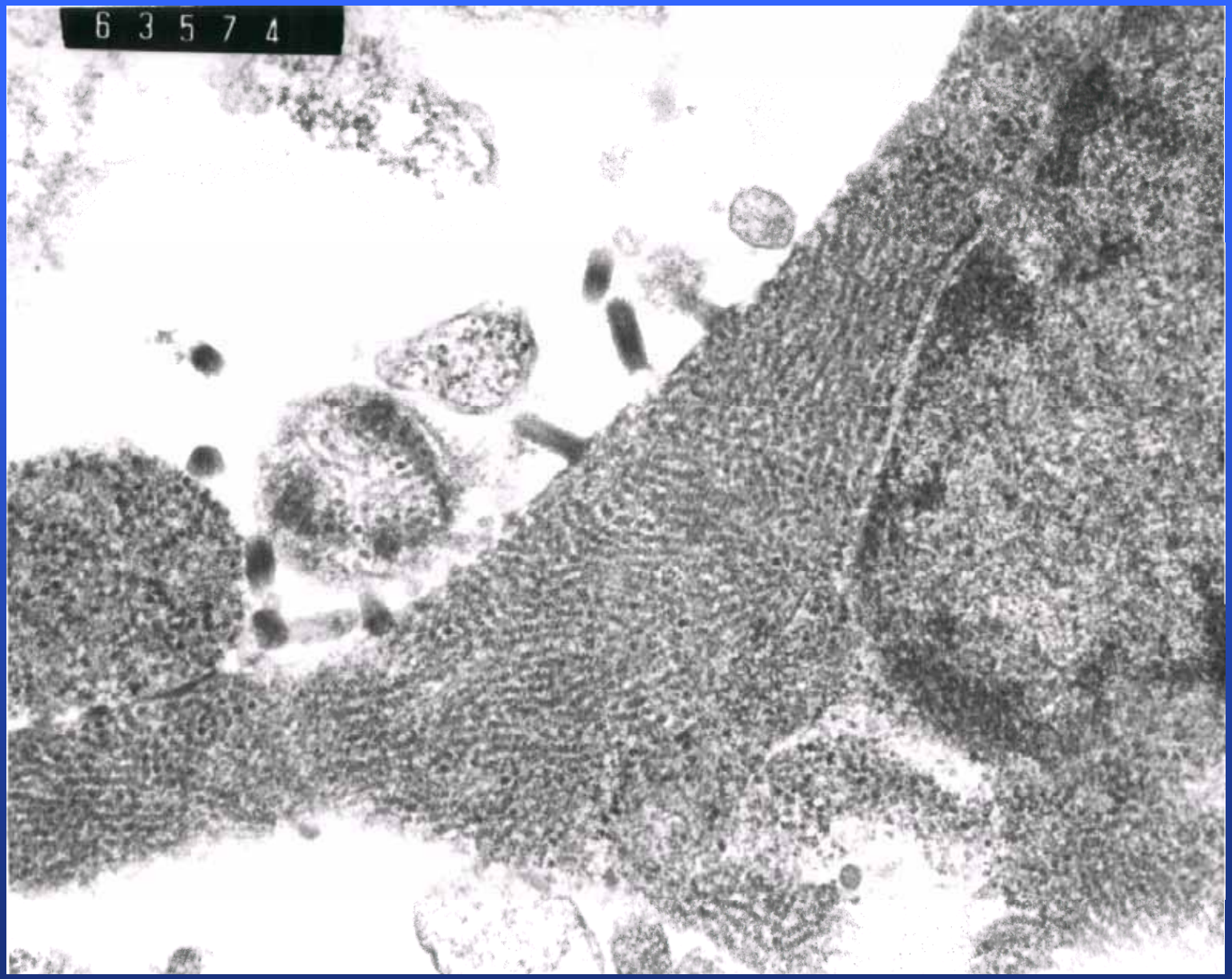


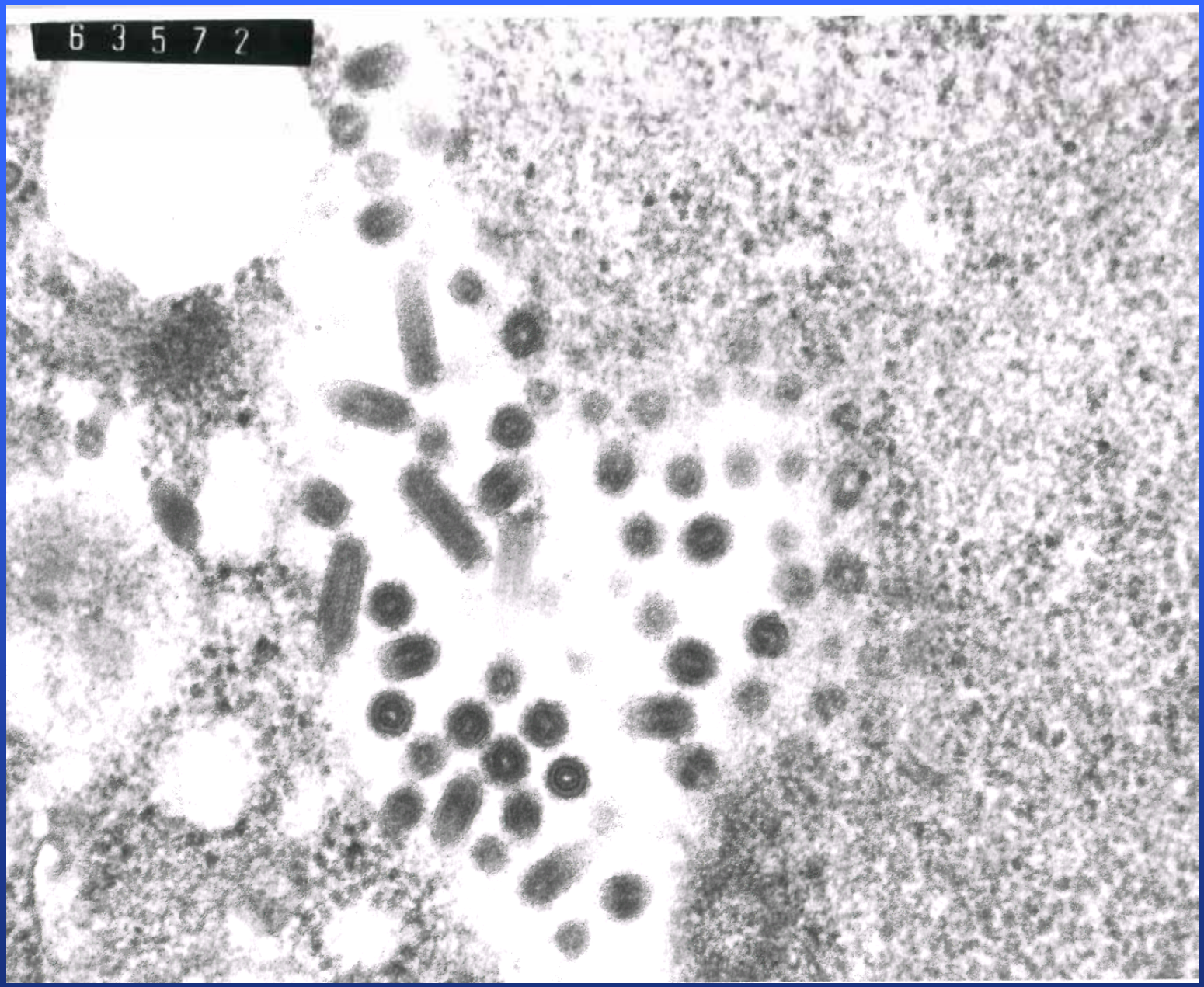




6 3 5 6 2


6 3 5 7 3


6 3 5 7 4


6 3 5 7 2

USGS Western Fisheries Research Center

Dr. James Winton

Dr. William Batts

Muskellunge Isolate

- **RT-PCR:**

  Central region of the glycoprotein (G) gene

- **Viral Hemorrhagic Septicemia Virus:**

  Family: *Rhabdoviridae*

  Genus: *Novirhabdovirus*

VHSV in the marine environment:

- **North America:**
  - **Meyers et al., 1992**
  - **Meyers & Winton 1995**
  - **Hedrick et al., 2003**
- **Europe**
  - **Dixon et al 1997**
  - **Mortensen et al., 1999**
  - **King et al., 2001:**

Japan

- **Takano et al., 2001**

- **Europe: Genotypes I (a-e), II and III**
- **North America:**

  Genotype IV (low genetic diversity, Hedrick et al 2003).

- **Muskellunge VHSV Sequenced:**
  - **G gene (1609 nt)**
  - **N gene (1386 nt)**

Muskellunge VHSV

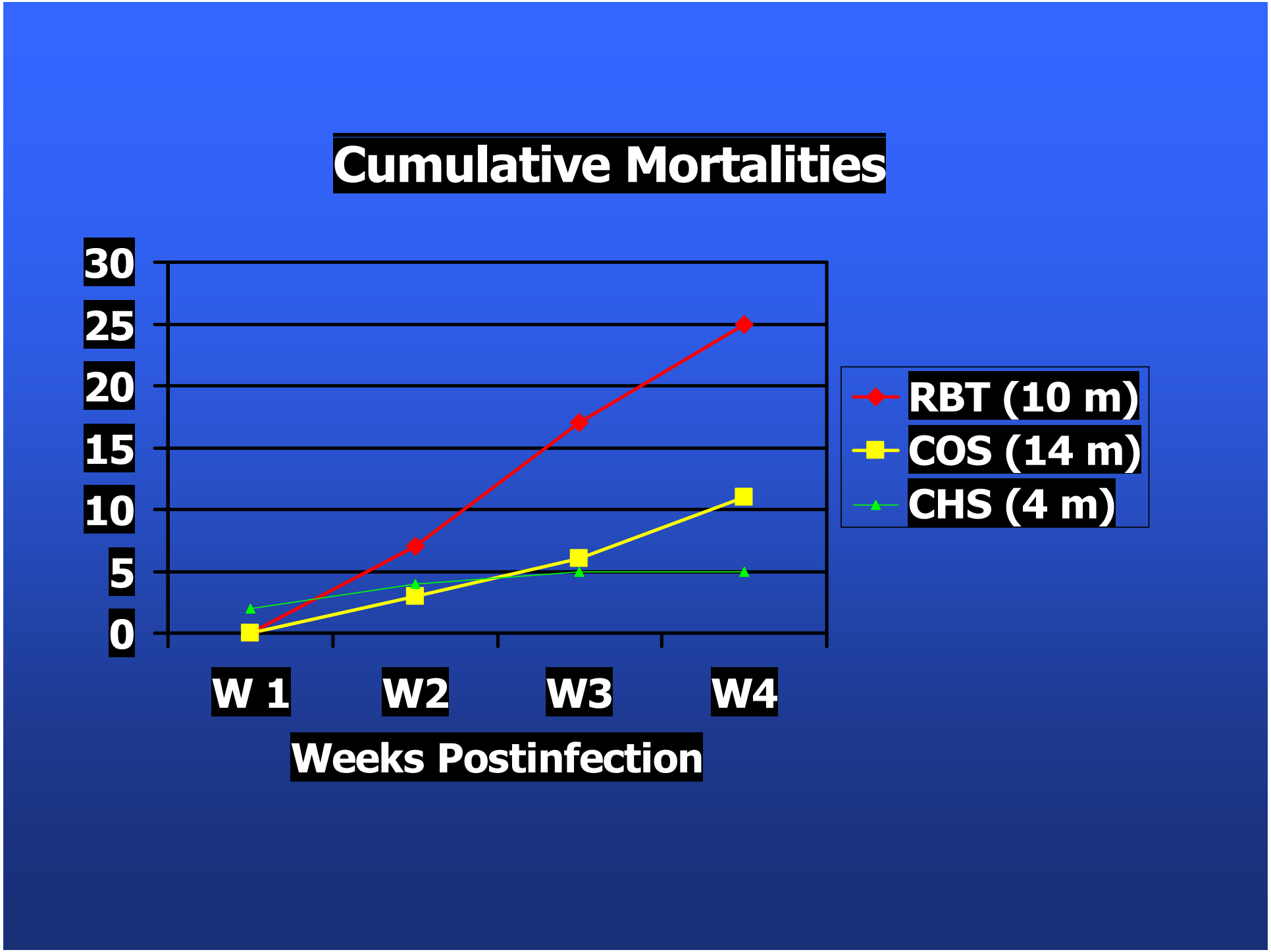
- Clearly distinct from European I-III genotypes
- There are sequence differences between Muskellunge VHSV and Pacific NA and Japan.

Canadian East Coast Isolates:

Dopazo et al (2002): Greenland halibut

Olivier (2002): Mummichog in New Brunswick

Genotype		Fish species
I		RBT
I	a	RBT
I	b	Japanese flounder, Atlantic cod, Atlantic herring
I	c	RBT
I	d	RBT
I	e	RBT
II		Sprat, Atlantic herring
III		Whiting, eel, turbot, Norway pout
IV	a	COS. ATS, Japanese flounder, Pacific sardine, Pacific herring
IV	b	Muskellunge


Cumulative Mortalities
30
25
20
15
10
5
0
W 1
W2
W3
W4
Weeks Postinfection
RBT (10 m)
COS (14 m)
CHS (4 m)

DEPARTMENT OF NATURAL RESOURCES
DNR
MICHIGAN
Great Lakes
Fishery Trust
UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY
NOAA
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
U.S. DEPARTMENT OF COMMERCE

Recent Food-web Shifts in Lakes Michigan and Huron: Implications for Fish Health?

Outline

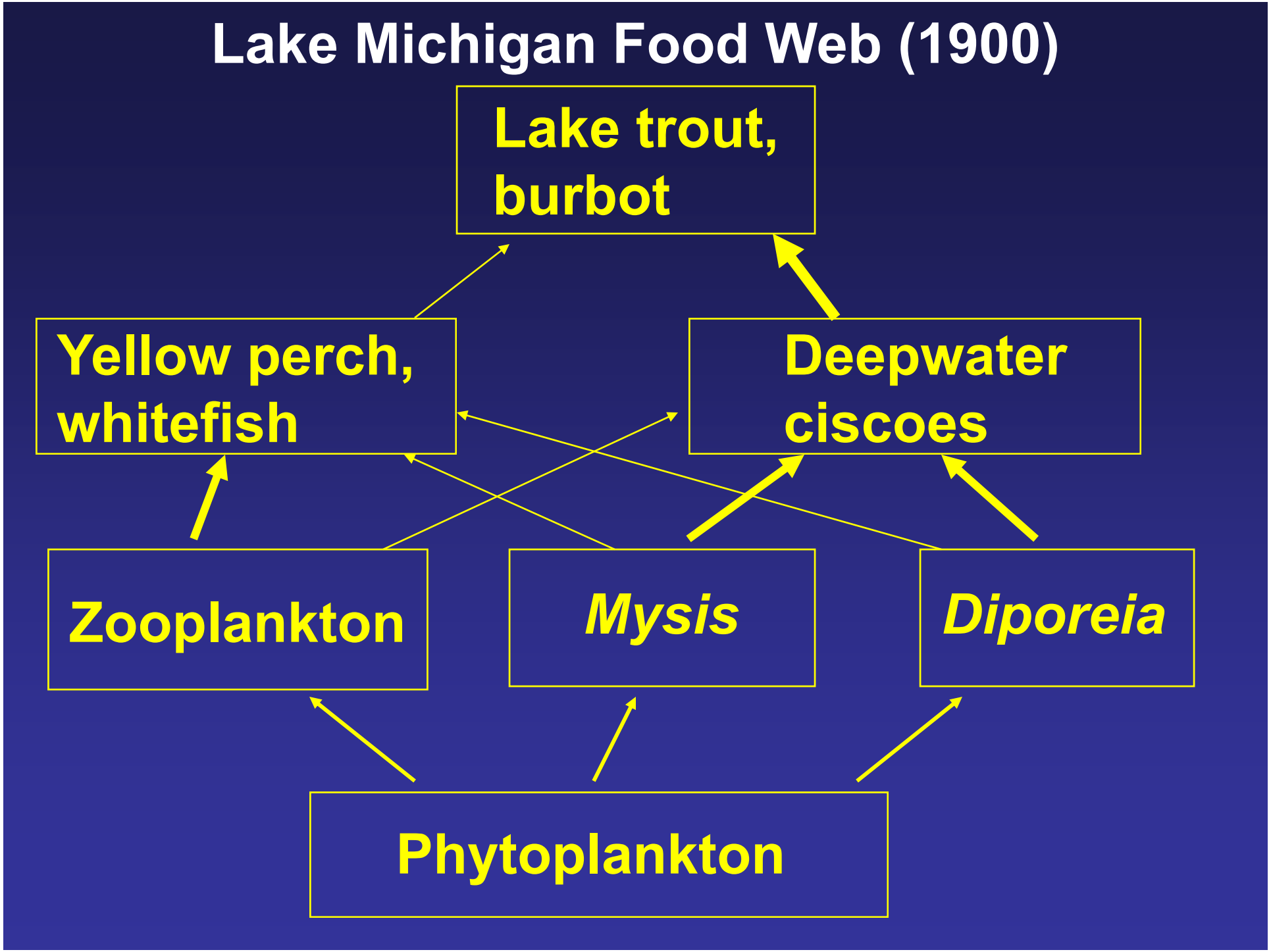
I. Food-web changes, past 20 yr

II. Direct impacts

III. Indirect impacts

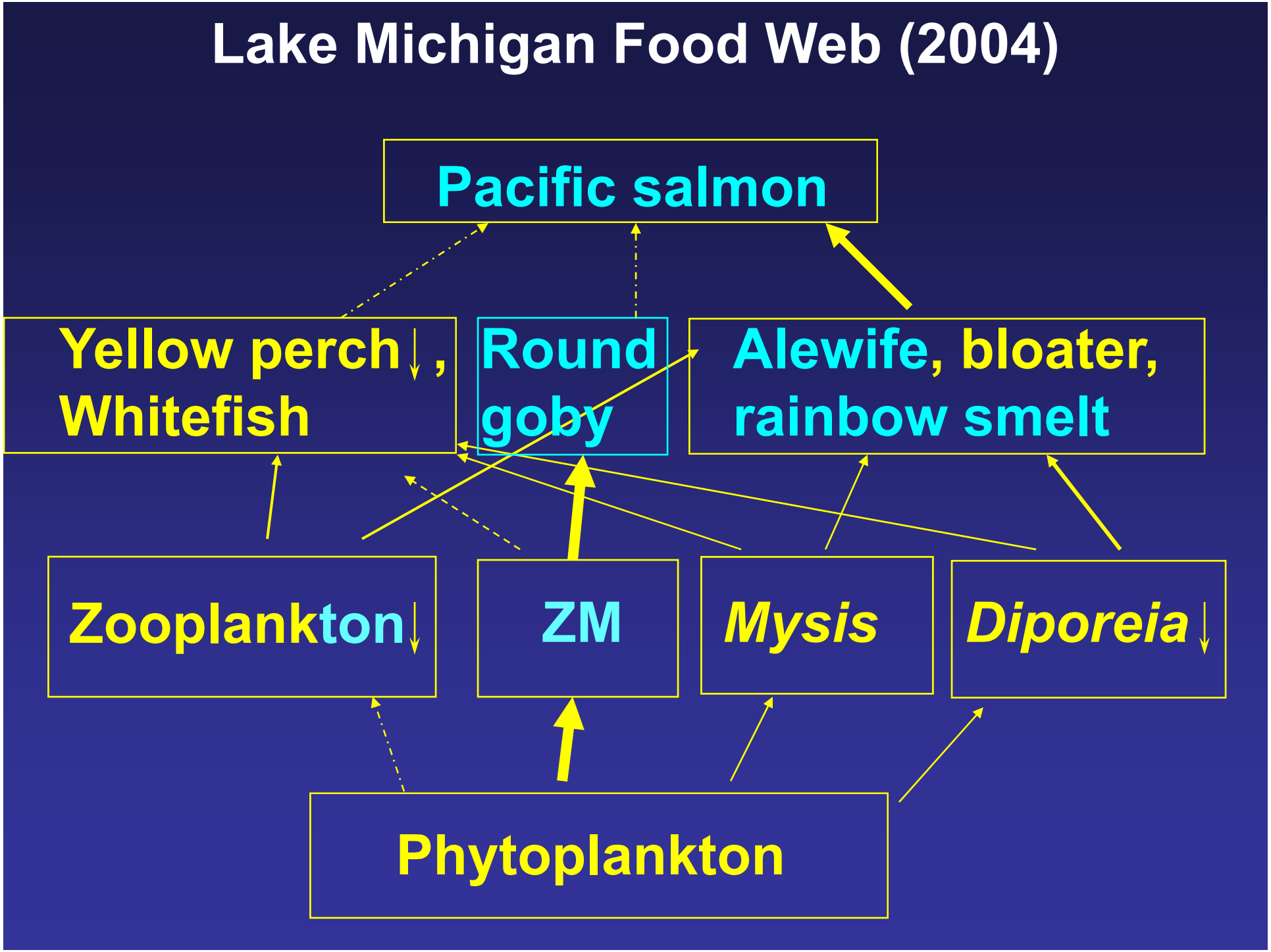
IV. Fish health implications

Lake Michigan Food Web (1900)

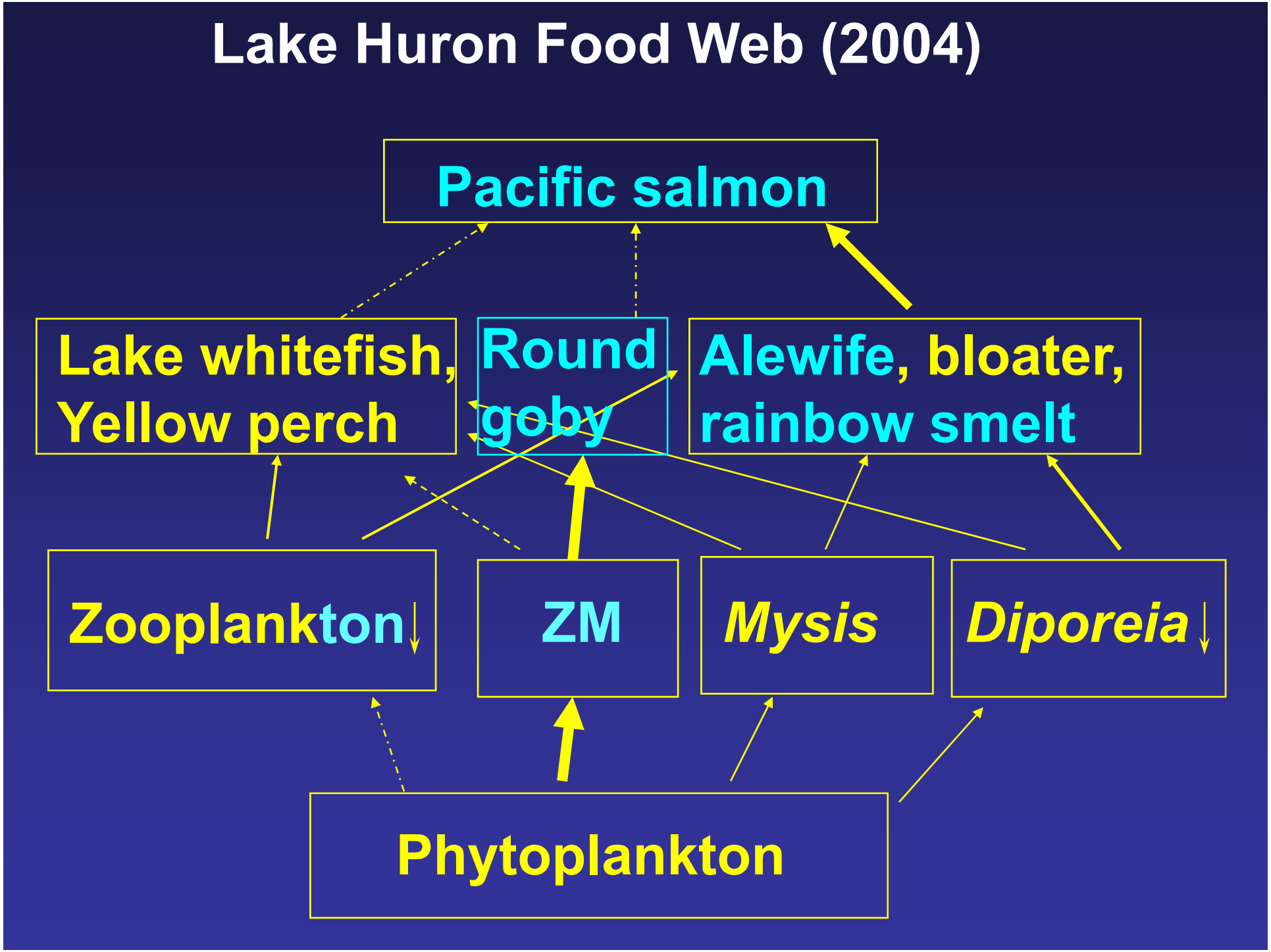


Invasive Species Timeline

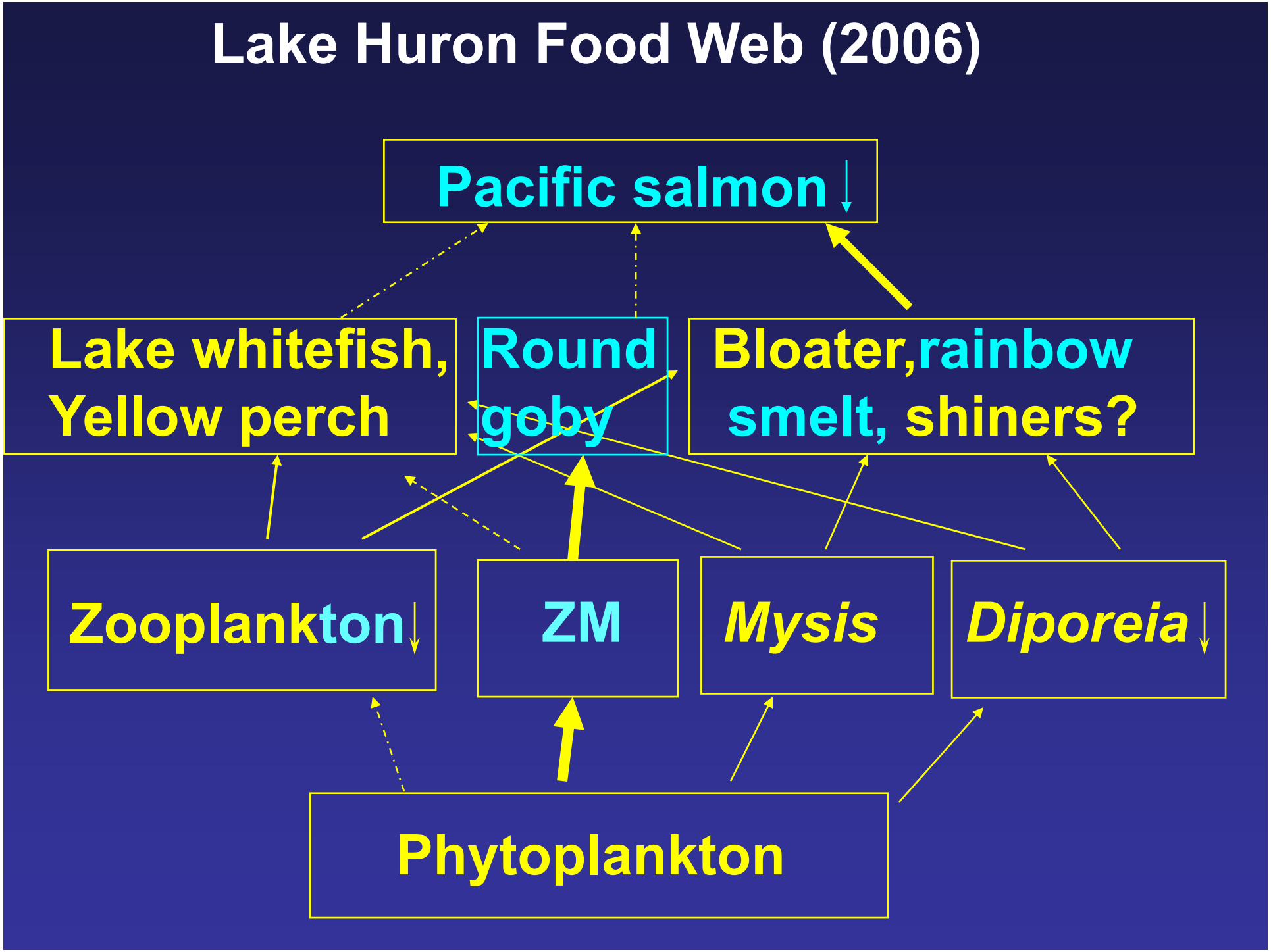
1985	1986	1989	1992	1999	2000
Rainbow smelt, Alewife, Sea lamprey, Pacific salmon	Spiny water flea	Zebra mussel	Round goby	Fishhook flea	


Lake Michigan Food Web (2004)
Pacific salmon
Yellow perch↓, Whitefish
Round goby
Alewife, bloater, rainbow smelt
Zooplankton↓
ZM
Mysis
Diporeia↓
Phytoplankton

Lake Huron Food Web (2004)



Lake Huron Food Web (2006)



Outline

I. Food-web changes, past 20 yr

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IV. Fish health implications

Direct Impacts: Round goby

- Serves as an important prey species
  - For yellow perch, smallmouth bass
  - Lake trout, coho salmon
- Rich in thiamine compared to alewife
  - May reduce EMS symptoms
- Bioaccumulation of contaminants
- Strong negative interactions with natives
  - Predation on lake trout eggs, fry
  - Eliminates some nearshore fishes

Outline

I. Food-web changes, past 20 yr

II. Direct impacts

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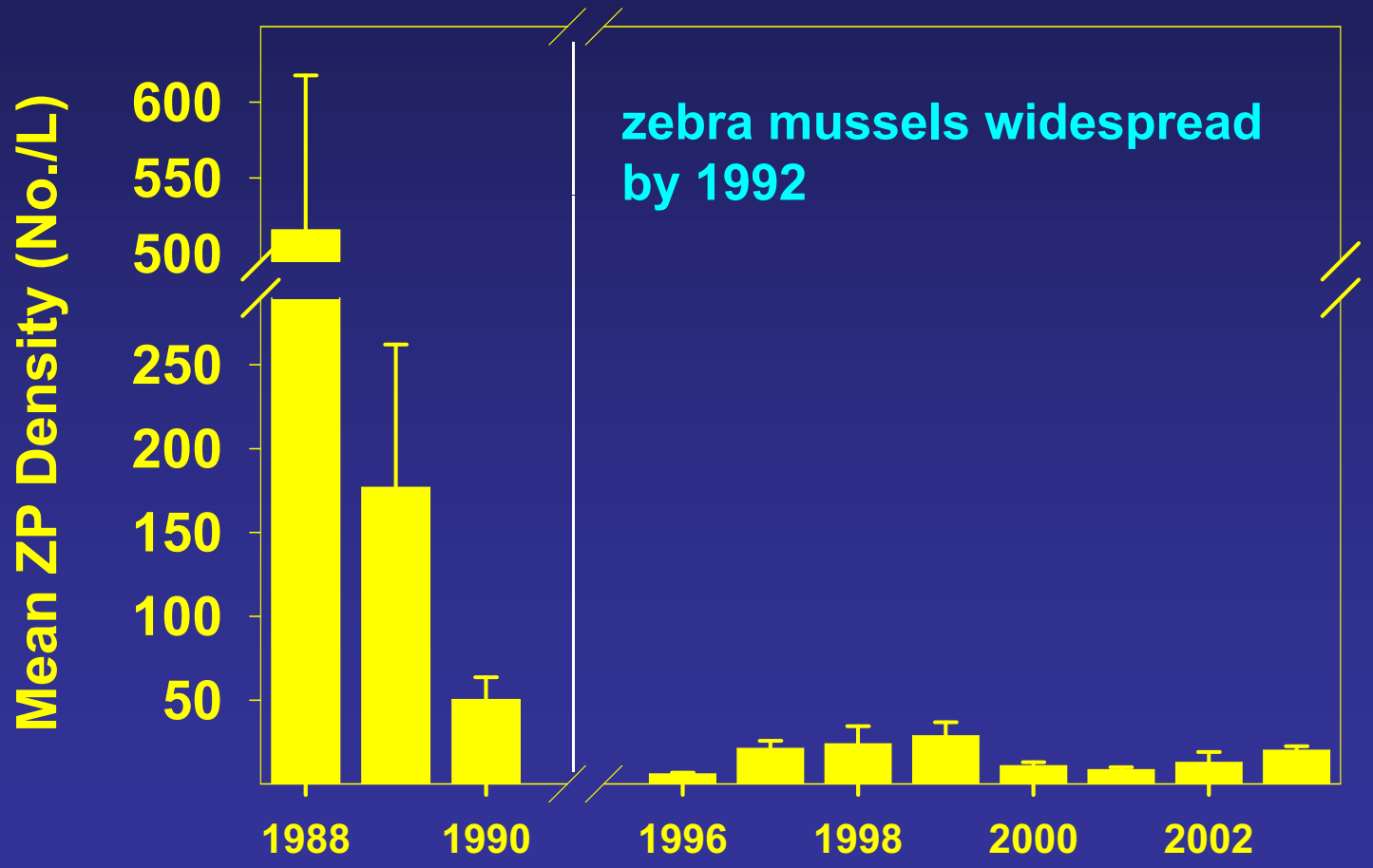
Food Web Shifts

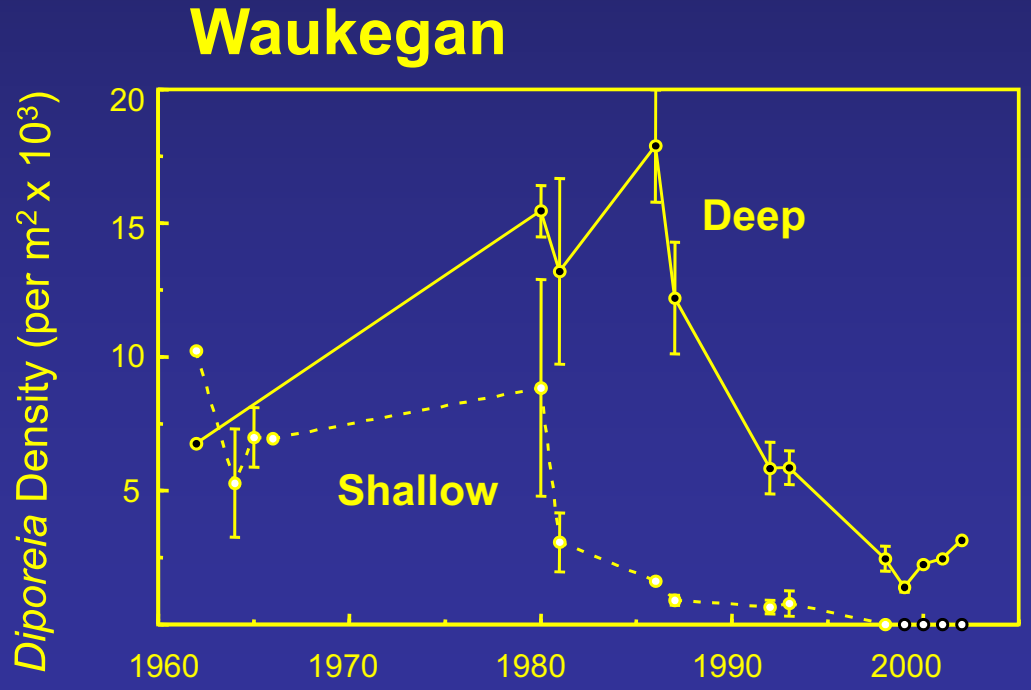
- **Reduced zooplankton**

Illinois waters
Dettmers et al. 2003 and unpublished data

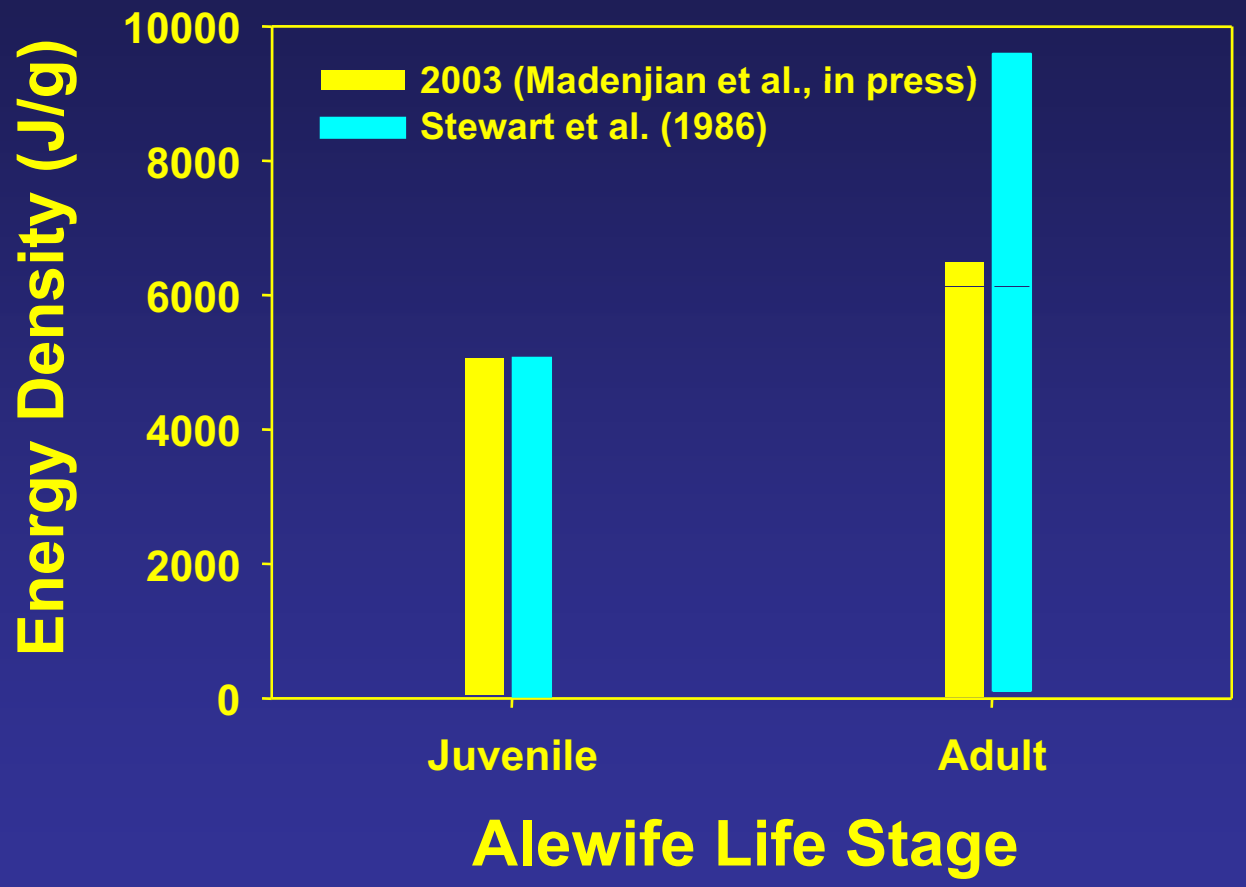


Food Web Shifts

- ***Diporeia* decline**



Nalepa et al., (in press)


Energy Density (J/g)
10000
8000
6000
4000
2000
0
2003 (Madenjian et al., in press)
Stewart et al. (1986)
Juvenile
Adult
Alewife Life Stage

Outline

I. Food-web changes, past 20 yr

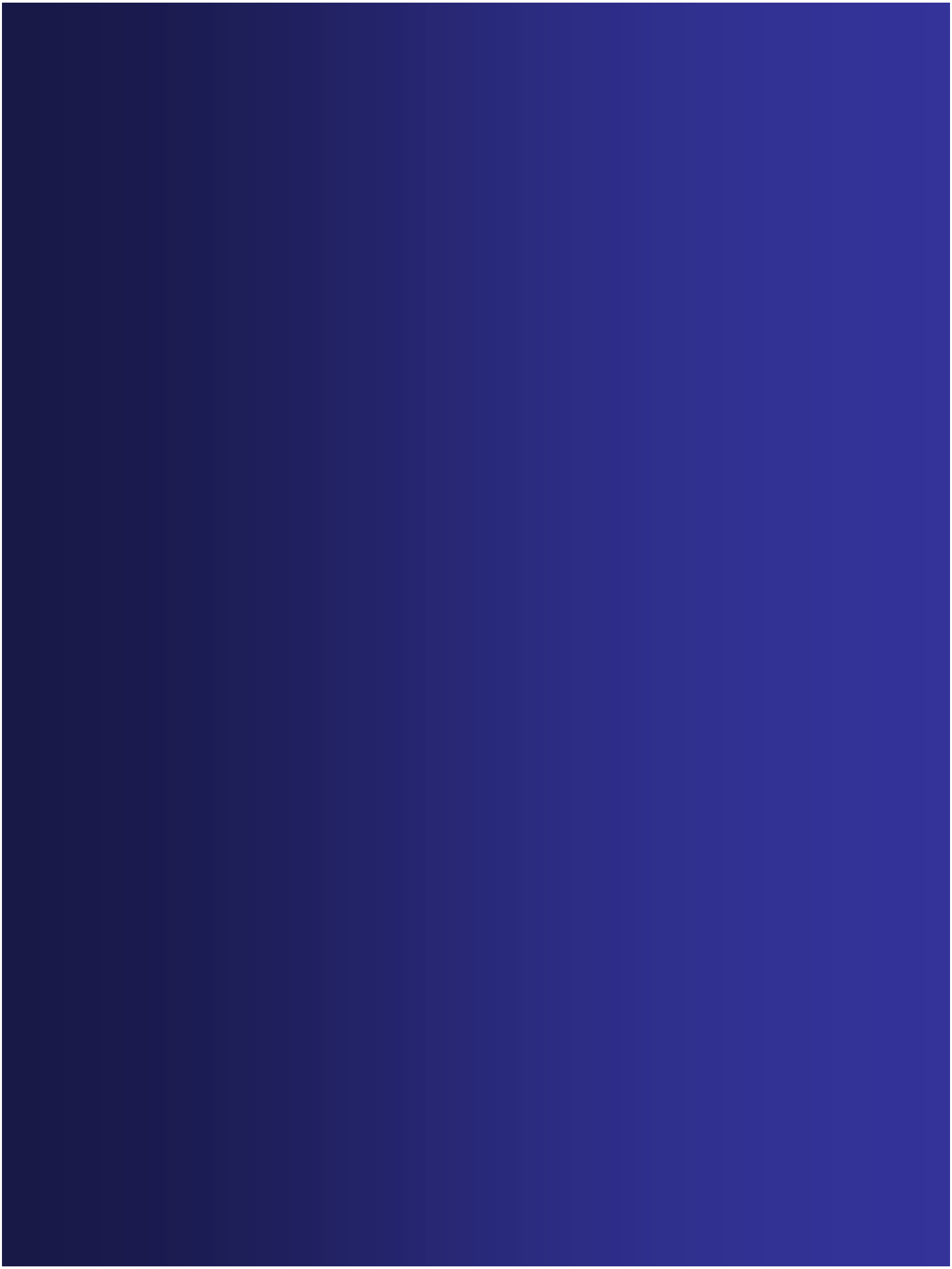
II. Direct impacts

III. Indirect impacts

IV. Fish health implications

Direct Impacts

- Changes in fish condition
  - Why? Impacts on the population?
- Elimination of intermediate hosts
  - Does parasite go away?
- Prey replacement
  - Implications for TDC
- Community shifts
  - Altered pathways of transmission?
  - Complex effects on fish health



Development of a National Aquatic Animal Health Plan

Great Lakes Fish Health Committee

February 22, 2006

Kevin Amos, Guppy Blair, Jill Rolland, & Gary Egrie

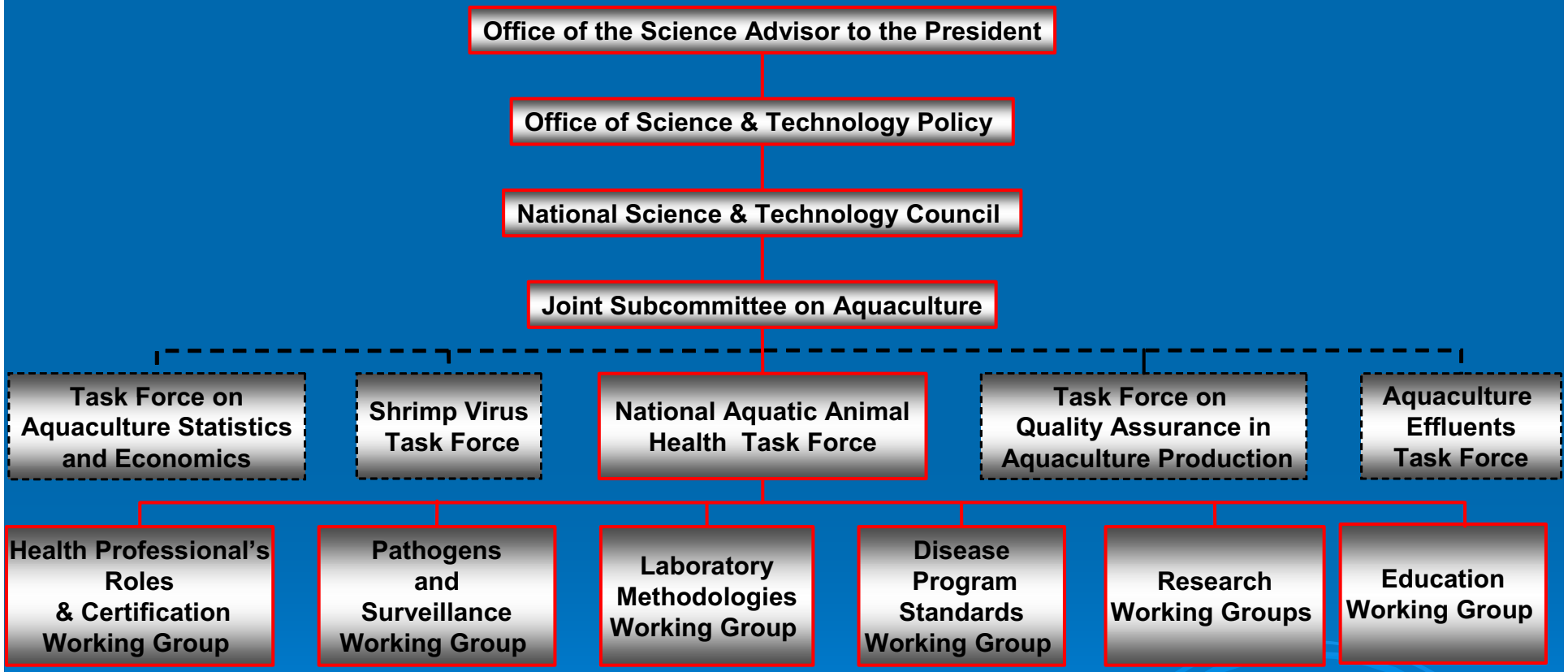
Presentation Topics

- The Joint Subcommittee on Aquaculture (JSA)
- The National Aquatic Animal Health Task Force on Aquaculture (NAAHTF)
- The National Aquatic Animal Health Plan (NAAHP)
- NAAHP Working Groups and Progress to Date

Joint Subcommittee on Aquaculture

- Mission:
  - Increase the effectiveness and productivity of Federal aquaculture research, technology transfer, and assistance programs
  - Authorized by National Aquaculture Acts of 1980, 1985
- Reporting Authorities:
  - To the National Science & Technology Council, Office of Science & Technology Policy in the Office of the Science Advisor to the President

Joint Subcommittee on Aquaculture Overview



JSA-National Aquatic Animal Health Task Force (NAAHTF)

- Mission:
  - To develop and implement a National Aquatic Animal Health Plan (NAAHP) for aquaculture in partnership with industry, State, local, tribal governments, and other stakeholders

NAAHTF

Guiding principles:

- Based on science;
- Transparent and collaborative process;
- Consistent with OIE and WTO standards.

RATIONALE FOR NAAHP

- Support effective and efficient aquaculture;
- Protect health of wild and cultured resources, especially from foreign pathogens;
- Meet our international obligations;
- Facilitate safe and efficient commerce.

Membership

USDA	DOC	DOI
T.J. Myers Chair (APHIS)	Spencer Garrett Deputy Chair (NOAA Fisheries)	Rob Bakal Deputy Chair (USFWS)
Meryl Broussard Chair of JSA (CSREES)	Kevin Amos (NOAA Fisheries)	Marilyn Blair (USFWS)
Gary Egrie (APHIS)		
Jill Rolland (APHIS)		

NAAHP Development

- Task Force identifies elements of NAAHP
- Stakeholder input received through work group meetings
- Task Force writes chapters; reviewed by JSA and stakeholders
- Federal agencies implement the Plan
- NAAHP is not a regulation!

NAAHP Working Groups

Formation:

- Invitations sent from Task Force
- Broadest representation possible

Activity

- Meet in person at least once

Purpose:

- Input to Task Force from all perspectives represented
- Task Force incorporates Working Group information as appropriate

Current and Future Working Groups

1)	Health professional roles and certification	Jan 2004
2)	Pathogens and Surveillance	Jan 2004
3)	Laboratory Methodologies	April 2004
4-9)	Species – Specific Disease Program Standards	Summer ’04 - Spring ’05
10)	State Resource Agencies	July 2005
11)	Research – Federal collaborative efforts	Oct. 2005
12)	Education (focus on health professionals)	Fall, 2006

Working Group 1
Aquatic Animal Health Professionals

Goals:

- Discuss types of professionals providing service to public/private aquaculture.
- Discuss education, skills, and training needed by health professionals.
- Discuss foreseeable needs of aquaculture industries.

Working Group 2

Diseases/Pathogens of Regulatory Significance and Their Surveillance

Goals:

- Discuss diseases of concern (notifiable)
- Discuss zonation
- Discuss surveillance

Working Group 3

Laboratory Methodologies

Goals:

- Identify a system for laboratory and personnel approval
- Discuss QA/QC programs in context of national plan
- Discuss standardization of reagents, media, cell lines, etc.
- Identify laboratory protocols

Work Groups 4-9. Disease Program Standards, Commodity Groups

- Salmonids – Twin Falls, ID. September, 2004.
- Warmwater finfish/foodfish – Biloxi, MS. November, 2004
- Ornamental/tropical fish – Tampa, FL. December, 2004
- Mollusks – Seattle, WA. March, 2005
- Baitfish – Memphis, TN. March, 2005
- Crustaceans – Tucson, AZ. May, 2005

Working Groups 4-9

Species-Specific Working Groups

(Salmonids, Warmwater food fish, Tropical Aquarium and Ornamental Fishes, Baitfish, Mollusks, Crustaceans)

Goals:

- Validate list of diseases from work group 2 by commodity;
- Discuss need/desire for programs (certification, control, eradication, etc.)
- Identify logical pathogen zones;

Working Groups 4-9

Species-Specific Working Groups

(Salmonids, Warmwater food fish, Tropical Aquarium and Ornamental Fishes, Baitfish, Mollusks, Crustaceans)

Goals (continued):

- Discuss surveillance schemes;
- Discuss methods to facilitate interstate and international commerce
- Discuss methods to prevent introduction of foreign pathogens/diseases;

Working Groups 4-9

Goals (continued):

- Address appropriate quarantine or eradication measures;
- Discuss indemnification schemes, bio-security, and emergency planning and training.

Next Steps

- Convene working groups on Education & Native Tribes
- Build partnerships with State Depts. of Ag and Wildlife
- Task Force continues drafting chapters; 1-5 drafts done
- Task Force work products submitted to JSA and stakeholders for review/comment;
- NAAHP to be completed by spring 2007;
- Federal agencies to implement plan.

Information and Contacts

Web site: www.aphis.usda.gov/vs/aqua/index.html

Look under “Hot Topics”

Task Force Technical Representatives:

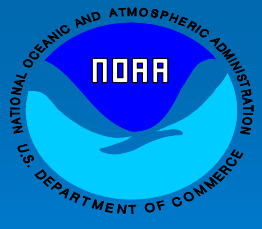
Jill.B.Rolland@aphis.usda.gov

Paul.G.Egrie@aphis.usda.gov

Marilyn_J_Blair@fws.gov

Kevin.Amos@noaa.gov

QUESTIONS ?



National Aquatic Animal Health Plan
Joint Subcommittee on Aquaculture - National Aquatic Animal Health Task Force

Government of Canada / Gouvernement du Canada

National Aquatic Animal Health Program Update

For

GLFHC 2006 - Madison, WI

Prepared by: Rod Penney and Brian Jamieson

Aquatic Animal Health Division

Canadian Food Inspection Agency

Canada

Introduction

- The National Aquatic Animal Health Program (NAAHP) focuses on the **federal government's** role in Aquatic Animal Health
  - Maintain trade and exports
  - Protect wild and farmed stocks from OIE-reportable and other pathogens and diseases
- CFIA lead agency in partnership with DFO
- Provinces, industry and academia all play an important role
- Background and current status (CFIA and DFO)

Why Cabinet funded a NAAHP (Budget 2005)

- The need to maintain export markets – over 75% of Canadian seafood is exported to over 140 countries
  - $4.5 Billion (2004)
- Canada's seafood industry provides livelihood to approximately 130,000 Canadians
  - Commercial fishing, processing and aquaculture
- Need to protect cultured aquatic animals and wild stock from the introduction and spread of disease
  - Recent significant disease-related losses to salmon production (East and West) (ISAv and IHNv respectively)

…concerns over non-tariff trade challenges

- Closure of EU market for live bivalve shellfish not for direct human consumption
- Trade partner requests for audits of Canada's official health certification program
- Requirement to meet OIE standards for aquatic animal disease management

… and other factors

- Progress made by other countries in developing national aquatic animal health programs (e.g. USA, EU, Australia)
- Key decision taken by previous President of CFIA to amend the *Health of Animals Regulations* (under the HAA) to include fish

Federal activities in AAH pre-2005

DFO

- disease research at 3 main labs
- *de facto* responsibility for import/export, although clear legislative authority is lacking
- New support in 2004 of $1.2M to establish an AAH Office within Science Branch

CFIA

- Office of CVO, vet biologics, animal feeds
- Generally ad hoc, uncoordinated

Federal Budget 2005

To support implementation of urgent federal gaps in national aquatic animal health management standards:
$59M incremental over first 5 years

CFIA: $32M incremental to provide regulatory authority under the HAA; program lead; QA/QC oversight; import controls; trade certification; disease response; surveillance of cultured animals.

DFO: $27M incremental to build up diagnostic and regulatory research support capability; surveillance of wild stocks.

A Collaborative Approach for Fish Diseases

National diseases: - Federally-led responsibility:
targeted surveillance for diseases that pose a significant threat to international and inter-provincial trade status and/or aquatic resources. Notifiable list, subject to regular review.

Regional diseases: - Provincially-led responsibility:
monitoring for infections that pose a significant risk of losses if not actively controlled

Production diseases: - Industry-led responsibility:
farm-level monitoring for infections that can be managed using husbandry, therapy, circumvention

CFIA - DFO Partnership

DFO

- **Science**
  - FHPR management *pending implementation of new HAA regs*
  - National Web Database
  - Advice to National I&T Code (health)
  - Trade & scientific advice
- **Aquaculture Management Directorate**
  - National I&T Code
  - Aquaculture Framework Agreement discussions

MOU

CFIA

- **Aquatic Animal Health Division (AP Directorate)**
  - Import Controls
  - Trade Certification
  - International Standards
  - Risk Analysis
  - Disease Control/Response
  - Chair AAHC
  - Quality Assurance/Quality Control Oversight
  - Zonation Controls
  - Regulations

Canada

Current Status of NAAHP

CFIA

- Create functional AAH Division (17 staff) (4 sections)
- Staffing process underway - NHQ and Network positions (2 BI, 8 VM positions)
- Governance structure established and operating:
  - AAH Steering Committee
  - Aquatic Animal Health Committee
    - meeting Oct 13-14, 2005 to review main program design elements – good support and engagement
    - Next scheduled meeting – April 24-25, 2006
- Canada/USA Aquatic Animal Health Technical Committee established and operating.

Aquatic Animal Health Committee *

- Currently 18 members representing:
  - Canadian Food Inspection Agency (CFIA) (Chair)
  - Fisheries and Oceans Canada (DFO)
  - Canadian Veterinary Medical Association (CVMA)
  - Canadian Aquaculture Industry Alliance (CAIA)
  - Fisheries Council of Canada (FCC)
  - Aboriginal Aquaculture Association (AAA)
  - Provincial representatives (5)

* Membership under review

Current Status of NAAHP

CFIA Program Activities

- **Surveillance and Reportable Disease List (N. Bruneau)**
  - Program planning / design
  - Draft list of reportable and notifiable diseases
  - Data gathering for decision trees - design of surveys
  - Surveillance Workshops – west / east (March)
  - Participation in international training
  - Shellfish surveillance workshop – pilot project in B.C.

Current Status of NAAHP

CFIA Program Activities cont'd:

- **Governance / Regulatory authority / Planning (A. Stewart)**
  - Review of regulatory authorities (October '05)
  - Clarifications of regulatory issues – DFO & Justice Canada Lawyers
  - Regulatory amendments
  - Staffing - Addition of regulatory specialist (B. Peart)
- **Disease Control**
  - Staffing of National Manager position is ongoing (VM)
  - General program provisions (October 2005)
  - Hazard specific response plans – generic model with (i) IHN and (ii) ISA as example diseases

Current Status of NAAHP
CFIA Program Activities cont'd:

- **Import / Export (B. Jamieson)**
  - **Imports** (Disease)
    - Program planning/Review of regulatory authorities
    - Liaison with DFO – Participation in import policies
    - Participation on development of international standards (OIE)
  - **NOTE:** Control of imports to remain with DFO until CFIA has regulatory authority
  - **Exports**
    - Working with DFO on export certification issues
    - Bilateral discussions with trading partners
    - Assume lead role in exports – June 2006?

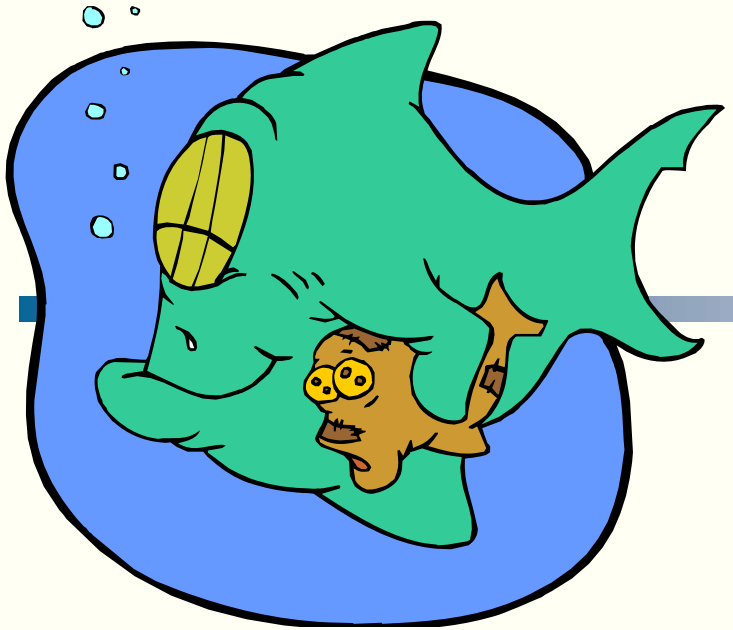
Current Status of NAAHP

DFO Program Activities:

- National Aquatic Animal Health-Science is being given Branch status
- Staffing actions underway &/or completed:
  - National Manager (S. McGladdery acting)
  - National Diagnostic Laboratory System Coordinator (P. Wright)
  - QA/QC Coordinator (S. Richardson)
  - 'Program Implementation Manager' (Apr '06)
  - National Research Coordinator
- DFO laboratories being upgraded to ISO 17025 for diagnostic testing for program diseases
- Gulf Fisheries Centre – National Centre of Expertise – coordination of research activities

Work Plan for Remainder of 2006-2007

- Complete staffing processes
- Accelerate communications activities (fact sheets and website)
- Develop & implement surveillance plans (by disease and species)
- Develop disease control program and response plans for specific disease outbreaks
- Develop import/export component within CFIA to take over from DFO
- Amend regulations / legislation
- Complete laboratory upgrades and acquire certification
- Continue to work with provinces to clarify roles and responsibilities



Thank-you!

Questions?

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