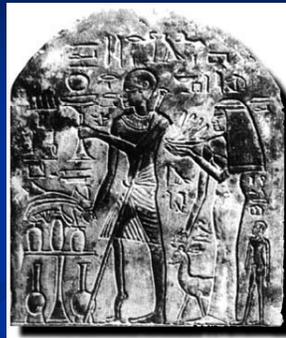


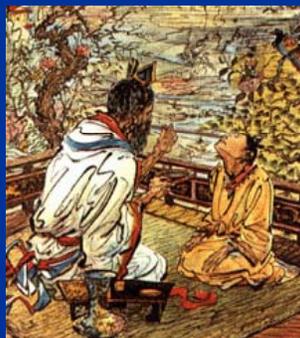


## Intro to Virology

- First record of a virus infection Ancient Egypt hieroglyphics (3700 BC)



- Smallpox was common in China (1000 BC)
- Variolation



# Intro to Virology

## ■ Small Pox Vaccine

- Edward Jenner (1796)
  - Milkmaid
    - Protected from smallpox
  - Inoculated a small boy
  - Tested his “hypothesis” by infecting the boy a month later



[www.microbiologybytes.com](http://www.microbiologybytes.com)

# Intro to Virology



[www.microbiologybytes.com](http://www.microbiologybytes.com)

## Intro to Virology

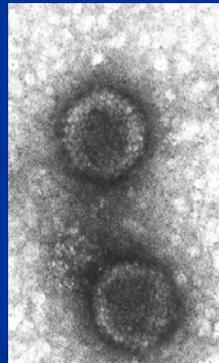
- Named Filterable Agents
- Louis Pasteur (late 1890's)
  - Named it Virus
  - Latin for Poison
- “Viruses” were studied heavily in 1900's
  - Tissue Cell Culture 1930's



[www.bdbiosciences.ca](http://www.bdbiosciences.ca)

## What is a Virus?

- Sub-microscopic obligate intracellular parasite
- Do Not Grow
- Do Not Undergo Division
- Dependant on Host Cells
  - Cannot Produce Energy
  - Cannot Produce Proteins



[www.lumc.edu](http://www.lumc.edu)

# Virus Structure

- Nucleic acids
  - DNA
  - RNA
- Proteins
  - Hemagglutinin (Influenza)
    - Cell Recognition
    - Fuses Cell Membrane to Viral Membrane
  - Neuraminidase (Influenza)
    - Release from Cell
  - Matrix
- Envelope or Unenveloped

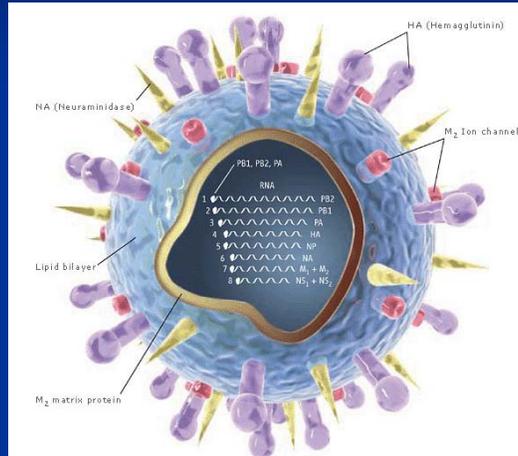
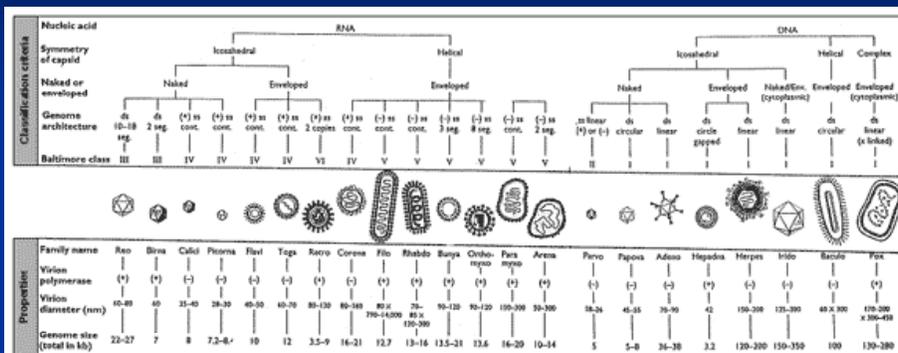


Illustration: Chris Bickle/Science. Reprinted with permission from Science Vol. 312, page 380 (21 April 2006) © 2006 by AAAS

# Virus Classification



**LEGEND : Classification schemes for animal viruses.** Summary of the major characteristics of 21 representative families of viruses that infect vertebrates and 1 that infects insects. Not all virus families are shown in the figure - the one insect virus family (*Baculoviridae*) is included because it has become an important tool in biotechnology. Adapted from G. P. Martelli et al. (ed.), *Virus Taxonomy Classification and Nomenclature of Viruses Sixth Report of the International Committee on Taxonomy of Viruses* (Springer-Verlag, Vienna, Austria, 1995).

# Are Viruses Living?

## ■ Classical Properties of Living Organisms

- Reproduction
- Nutrition
- Respiration
- Irritability
- Movement
- Growth
- Excretion



Adapted from [www.mcb.uct.ac.za/tutorial/definitions.htm](http://www.mcb.uct.ac.za/tutorial/definitions.htm)

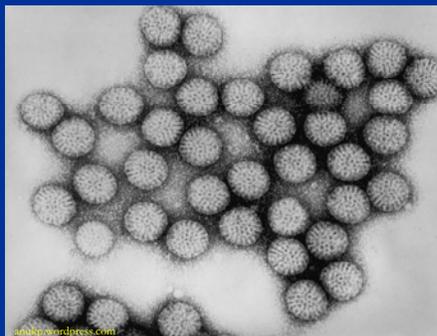
# Are Viruses Living?

## ■ Viruses are Living

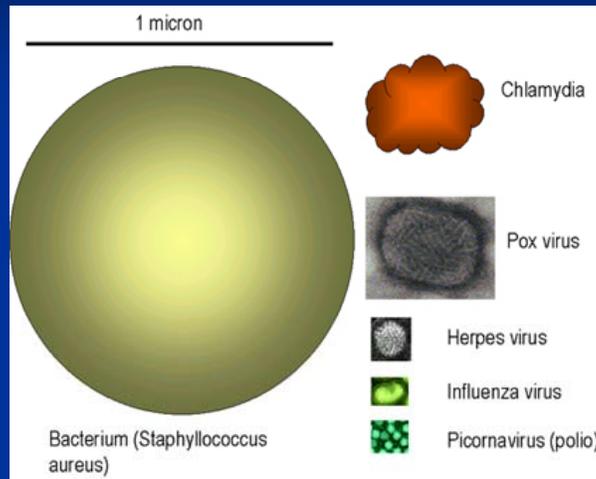
- Can Reproduce
- Have Genetic Material

## ■ Viruses are not Living

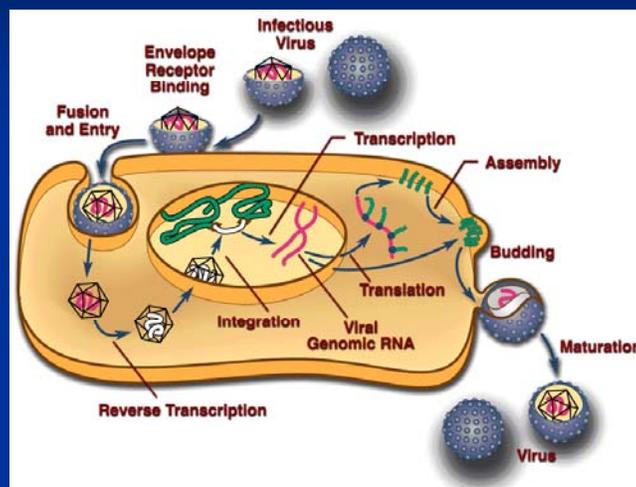
- Lack Metabolism
- Do not grow
- Cannot reproduce on their own



# Virus Size

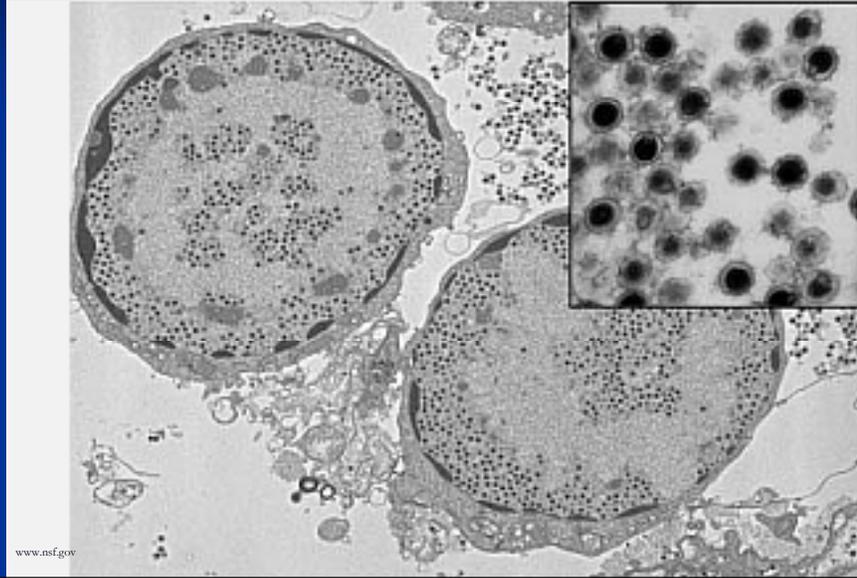


# Lifecycle

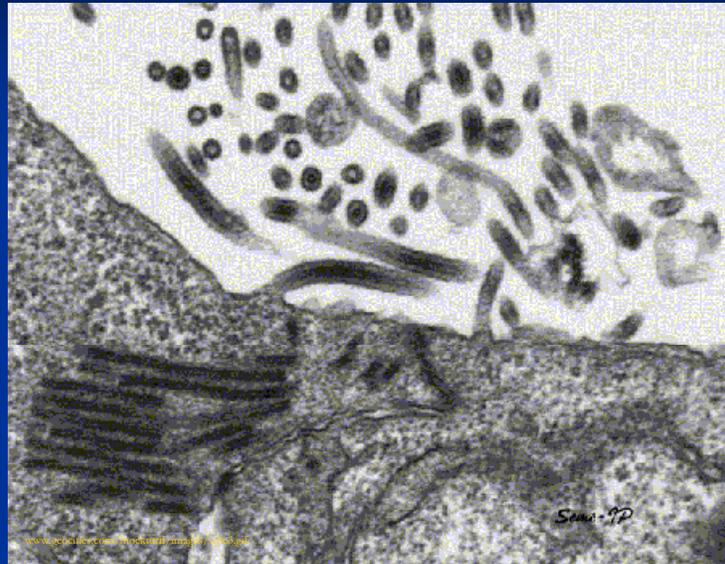


[www.retrovirus.info](http://www.retrovirus.info)

## Lifecycle



## Virus Lifecycle



## Methods

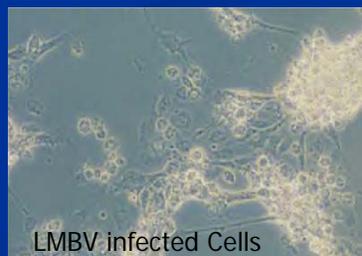
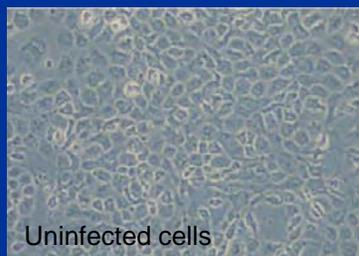
### ■ Virus Isolation

- Kidney/Spleen placed in HBSS
- Placed on Cells
  - CHSE (Chinook Salmon)
  - EPC (Carp)
  - BF-2 (Bluegill)
  - FHM (Fathead Minnow)
  - BB (Brown Bullhead)
  - KF-1 (Koi)
  - WSS (White Sturgeon)



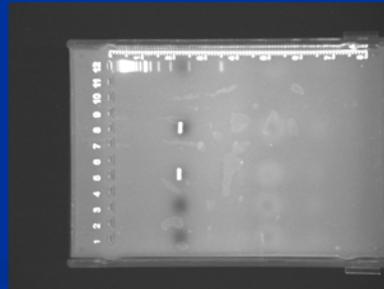
## Virus Diagnosis

- Screening-cell culture (14 Days)
- Blind Passed (14 Days)



## Confirmation of the Virus

- Extract Viral Nucleic Acids
- Polymerase Chain Reaction (PCR)



## Important Fish Viruses

- Warm Water Fishes
  - Largemouth Bass Virus
  - Spring Viremia of Carp Virus
  - Channel Catfish Virus
  - Golden Shiner Virus
  - Lymphocystis
- Cold/Cool Water Fishes
  - Epizootic Epitheliotrophic Virus Disease
  - Infectious Hematopoietic Necrosis Virus
  - Infectious Pancreatic Necrosis Virus
  - Onchorhyncus masou virus
  - Viral Hemorrhagic Septicemia virus
- “New” Viruses are found frequently



[weblogs.baltimorean.com](http://weblogs.baltimorean.com)

# Important Fish Viruses

## ■ Factors involved in Virus-Related Fish Kills

### ■ Stressors

- Handling
- Crowding
- Poor nutrition
- Spawning Stress
- Environmental Conditions
- Water Temperature



[www.pntf.org/](http://www.pntf.org/)

# Largemouth Bass

## ■ Predator

### ■ Diet:

- Insects
- Frogs
- Crawfish
- Smaller fish

### ■ Aggressive



[www.duiops.net](http://www.duiops.net)

## Largemouth Bass

- Highly sought gamefish
  - Hard fighting and acrobatic
  - 5 million caught in WI (2005)
  - 14,000 caught every day
  - More days spent pursuing bass than any other fish
- \$12.8 Billion annually
  - Lures
  - Boats



[www.fws.gov](http://www.fws.gov)

## Largemouth Bass

- Tournaments
  - Income for local economies
    - ~\$24 million for Bassmaster Classic
  - Prize money
    - Skeet Reese
    - Won 2009 Bassmaster Classic (\$500,000)
- Thousands of smaller tournaments throughout the United States



<http://sports.espn.go.com>

## Largemouth Bass

### ■ World Record Bass

- George Perry
- Montgomery Lake, GA
- 1932
- 22 lbs. 4 oz.
- Won \$75, rod and reel, new shotgun



[www.mflurebox.com](http://www.mflurebox.com)

## Largemouth Bass

### ■ \$1,000,000 bass

- Mac Weakly
- Dixon Lake (San Diego County)
- Potential new world record (25.1 lbs.)
- Foul hooked
- Released



[www.startribune.com](http://www.startribune.com)

## Largemouth Bass

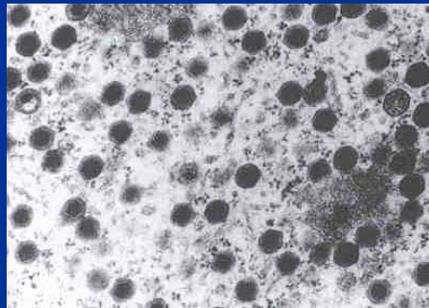
- Lake Biwa, Japan
  - Manabu Kurita
  - 29 inches
  - 22 lbs 5 oz
- A Tie?
  - IGFA
    - Under 25 lbs
    - Must weigh 2 oz more to break record



<http://sports.espn.go.com>

## LMBV Background

- Family *Iridoviridae*, Genus *Ranavirus*
- Infects Fish, Reptiles, and Amphibians
- Never reported from warm blooded animals



John L. Plumb

## LMBV Background

- Largemouth Bass – only species in which disease develops
- Other species serve as carriers:
  - Centrarchidae: smallmouth bass, spotted bass, rock bass, Suwanee bass, black crappie, white crappie, bluegill, and redbreast sunfish
  - Moronidae: white bass, striped bass
  - Esocidae: Muskellunge
  - Scianidae: freshwater drum
  - Amphibians and reptiles may also carry LMBV



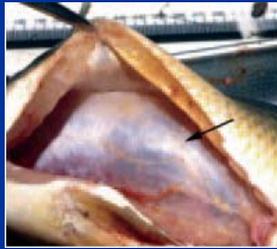
## Signs of Disease

- Few or no obvious external signs
- Fish floating at surface, swimming slowly
- Loss of equilibrium



## Signs of Disease

- Abdomens may be bloated (fluids in swim bladder)
- Yellow, waxy swim bladder



[www.fisheries.org](http://www.fisheries.org)

## Signs of Disease

- Red Lesions may occur on skin (associated with secondary bacterial infections)



## Transmission of LMBV

- Horizontal Transmission
  - Water
    - Virus particles shed in mucus
    - Remain infective for days
  - Eating infected fish
- Vertical Transmission
  - No reports in hatcheries



[www.blacksalty.com](http://www.blacksalty.com)

## Origins of LMBV

- Two Hypothesis
  - LMBV has been here
  - LMBV is an exotic species



[www.fhcrnig.com](http://www.fhcrnig.com)

## Origins of LMBV

- Supports the hypothesis that LMBV is native:
  - Different serotypes present
    - The virus had time to evolve here
  - However, the method of detection (Tissue Cell Culture) has been around



## Origins of LMBV

- Supports the hypothesis that LMBV is exotic:
  - Virus Mutation
  - Two Ornamental Fish Viruses
    - Doctorfish Virus
    - Guppy Virus
  - Virus can be spread through feeding



## LMBV-Related Fish Kills

- Viral presence does not mean there will be a fish kill
  - Healthy fish can carry virus
    - Often times infected fish show no signs of infection
    - In wild populations the number of infected fish is usually low
  - Stressors appear to “activate” the virus



## LMBV-Related Fish Kills

- Summer months
  - Warm water temperatures
    - Aids viral replication
    - Low dissolved oxygen
  - Water Quality
    - Fluctuations in water chemistry



[www.bio.brandeis.edu](http://www.bio.brandeis.edu)

## LMBV-Related Fish Kills

- Angling Pressure
  - Tournaments?
    - Handling Stress
    - Catch and release angling alone does not influence expression



[www.agfc.state.ar.us](http://www.agfc.state.ar.us)

## LMBV-Related Fish Kills

- Tournaments (continued)
  - Livewell
    - Crowding (Stress)
    - Excess Mucus (Virus)
    - Transmission likely
  - Weigh-in
    - Further handling
  - Many Questions Remain



[www.bassworldsports.com](http://www.bassworldsports.com)

## LMBV-related Fish Kills

- Large scale mortality
  - Kills many fish
  - Adult fish are more susceptible



[www.bucsfishingreport.com](http://www.bucsfishingreport.com)

## LMBV Outbreaks

- Greatly reduce population
- Angler Effort (Wheeler Reservoir, AL)
  - Before: ~200 hours to catch a 5 lb. bass
  - After: ~6,000 hours
  - 3 years later: ~1000 hours
- Brood stock
  - Large females
    - More fecund



[www.gotalgae.com](http://www.gotalgae.com)

## LMBV Outbreak

- LMBV Outbreaks
  - First LMBV-related fish kill occurred in South Carolina (1995)
  - Santee Cooper Reservoir
  - At least 1,000 Largemouth Bass Died



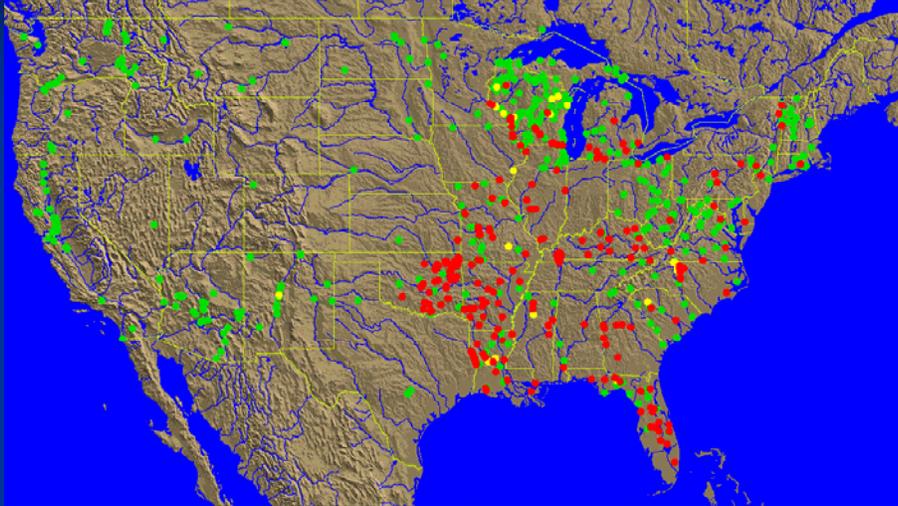
[www.livingcolor.com](http://www.livingcolor.com)

## LMBV Outbreaks

- Southeastern United States
  - Fish Kills in Florida, Mississippi, Alabama, Louisiana, Texas, Oklahoma, Arkansas, Georgia, South Carolina
- Midwest
  - Lake George (2000) on Michigan/Indiana Border
  - Since then Missouri, Iowa, Indiana
  - Mississippi River 2006

## Distribution of LMBV

Source: Wild Fish Health Survey Database



## Management Implications

- Fishery Managers
  - Test all fish before transfer
    - To a new lake
    - A hatchery for brood stock
- Many different species are known carriers



[www.uticaod.com](http://www.uticaod.com)

## Common Carp

- Native to Asia and Europe
- Brought to the United States in 1800's as a food source
- Range covers most of U.S.
- Not considered a gamefish in U.S.



[www.dlia.org](http://www.dlia.org)



[www.dlia.org](http://www.dlia.org)

## SVCV in Aquaculture

- Carp are very important in aquaculture
  - Earliest report of carp in aquaculture dates back to 400 B.C.
  - Carp account for 46% of the total weight of fish produced
  - Ornamental fish are also important
- Viral outbreaks can be very expensive
  - Loss of fish
  - Cost of disinfection



[en.wikipedia.org](http://en.wikipedia.org)

## SVCV in Aquaculture

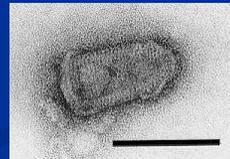
- Facilities with a controlled environment
  - Water temperatures above 20°C
- Vaccines are available
  - Intraperitoneal Injection
  - Immersion
  - Feed



apl.ning.com

## Spring Viremia of Carp Virus

- *Rhabdoviridae* Family
- *Vesiculovirus* Genus
- Enveloped
- 90nm x 180nm
- Single Stranded RNA
- Negative Sense



www2.hawaii.edu

## Animals Susceptible to SVCV (Natural & Experimental infections)

- Cypriniformes-common carp, Bighead Carp, Silver Carp, crucian carp, goldfish, grass carp, koi carp, orfe, roach, and tench
- Cyprinodontiformes-guppy
- Perciformes-pumpkinseed
- Salmoniformes-northern pike, rainbow trout
- Siluriformes-sheat fish
- Crustacea-carp louse, penaeid shrimp
- Insecta-fruit fly
- Annelida-leech



## Spring Viremia of Carp Virus

- Outbreaks Occur in Spring and Fall
  - Water Temperatures between 10°C and 17°C
  - Mortality Rates as high as 90%
  - Fish that do survive develop immunity



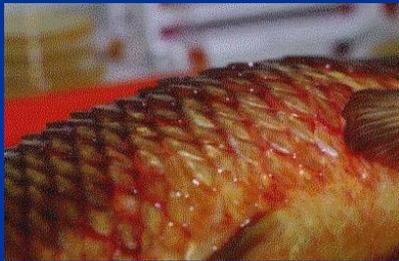
## Signs of SVCV

- Distended Abdomen
- Pop-eye
- Hemorrhaging of Skin
- Hemorrhaging of Internal Organs
- Ascites Fluid



[www.gorge.org](http://www.gorge.org)

## Signs of SVCV Infection



WI DNR



WI DNR

## Transmission of SVCV

- Virus shed in feces and urine
- Spread via parasites
  - *Argulus* (Fish Lice)
  - Leeches



## Outbreaks

- Historically a problem in Europe, Asia, and Middle East
  - First Recorded in Middle Ages
- Reported in North Carolina (Spring 2002)
- First U.S. Feral Fish Kill in Wisconsin (Spring 2002)



## Significance

- Represent first reports in North America
- SVCV is one of five fish viruses listed as notifiable by the International Aquatic Animal Health Code of the OIE (Office International des Epizooties).
- May affect trade (shipment of fish, eggs, fish by products) between countries where SVCV is present and where it is absent.
- No information on the effects of SVCV on native cyprinids and related species

## Two SVCV Epizootics in the U.S. (Spring 2002)



Farm raised koi in North Carolina

Wild carp in Cedar Lake, Wisconsin



## Blueridge Fish Farm

- Largest koi producer in U.S.
- Draws water from nearby creeks/rivers and releases effluent into same waters
- Sold fish to 45 states
- Observed 10% death rate of young koi
- Disease diagnosed as SVCV by A. Goodwin, U of Arkansas
- Wild fish in adjacent waters had serological titers indicative of exposure to the virus



## Cedar Lake Carp Kill May 2002

- Fish size - 810 to 900 mm primarily larger fish
- Estimate 20,000 + died
- Visible signs: Ascites, edematous kidney and spleen, adhesions
- No hemorrhage on swim bladder or on body wall

## Cedar Lake, Polk/St. Croix Co., WI

- 1,107 Acres
- Maximum Depth 28 feet
- Flowage
- Many connected waters
- Discharge to the Apple River; ultimately to the St. Croix and Mississippi Rivers



## SVCV in the UMR

- Isolated from the UMR in May 2007
  - Carp Kill near Dresbach Dam (Pool 8)
- Antibodies to SVCV have been previously detected from UMR carp
  - Virus has been here



## Biological Control

- Bighead Carp

- (*Hypophthalmichthys nobilis*)



[www.aouo.com](http://www.aouo.com)

- Silver Carp

- (*Hypophthalmichthys molitrix*)



[www.usgs.gov](http://www.usgs.gov)

## Bighead and Silver Carp

- Brought here in 1970's for use in aquaculture
- Have spread up the Mississippi River
- Feeding habits similar
  - Zooplankton, Blue Green Algae, and Aquatic Insects
    - Compete directly with all native fish larvae, gizzard shad, and mussels



[jakst.files.wordpress.com](http://jakst.files.wordpress.com)

## Bighead and Silver Carp

- Growth
  - May weigh 20-25 lbs. In 4 years (Bighead Carp)
- Potential to Disrupt Ecosystem
  - Illinois River
    - 90% of Biomass
  - Great Lakes?
- Danger to Boaters



media.commercialappeal.com

## Bighead and Silver Carp

- Problematic to Commercial Fisherman



fic.er.usgs.gov



illinoiscommercialfishing.com

## Use of SVCV as a Biological Control

- Characteristics of a Good Biological Control
  - Genetically Engineered Virus
  - High host specificity
    - Only infect the intended host
    - Always check possible human interaction
  - Highly Virulent
    - No Survivors
    - Survivors are immune
  - Easily Transmitted
    - At a high rate



## Biological Control

- Problems with SVCV
  - Wide host range
  - Requires certain environmental conditions
  - Needs higher virulence
- However.....
  - It may be possible to genetically engineer SVCV to make it a suitable biological control
  - Currently SVCV is not used as a biological control

## Use of Viruses as a Biological Control

- Rabbits in Australia
  - Rabbits were released in 1859
  - Fastest spread ever recorded by any mammal
  - Vegetation was eaten
    - Left land wide open to erosion



[wikipedia.org](http://wikipedia.org)

## Use of Viruses as a Biological Control

- Unable to control rabbit populations
  - A population of 600 Million Rabbits
  - Myxoma Virus Released (1950)
  - Calicivirus (aka Rabbit Hemorrhagic Disease) was released (1996)
  - Viruses have some success
    - By 1991, population down to 200-300 million
    - Hope that the viruses will be more effective together



[www.bbc.co.uk](http://www.bbc.co.uk)

## Significance of SVCV

- OIE reportable disease
  - World organization for Animal Health
- May be more carp die-offs in future
- Mutation
- Currently, not suitable for use as a Biological Control



farm1.static.flickr.com

## Channel Catfish Virus

- Popular Sportfish
  - 8 Million Anglers in U.S.
  - Popular Table fare
  - Rapid Growth in Aquaculture
    - ~1 Billion dollar/year industry



www.outdooralabama.com

## Channel Catfish Virus

- DNA Herpes Virus
- Infects fish less than 4 months old
- Sudden Mortalities
  - Up to 100%
- Reported in most Southern States
- Outbreaks from June to September



[/www.floridafishfarms.com](http://www.floridafishfarms.com)

## Channel Catfish Virus

- Virus Transmission
  - Vertical
  - Horizontal
- Other Ictalurid species have been successfully infected



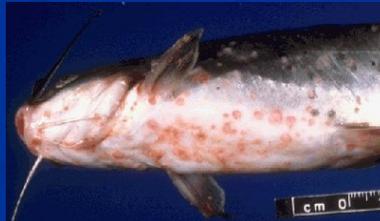
[pubs.caes.uga.edu](http://pubs.caes.uga.edu)

## Signs of Channel Catfish Virus

- Sudden increase in Mortality of young CCF
- $\geq 25^{\circ}\text{C}$
- Distended abdomen
- Exophthalmia
- Hemorrhaging
- Enlarged Spleen
- GI Tract empty
- Erratic Swimming



[aquatic.org](http://aquatic.org)



[www.cnr.vt.edu](http://www.cnr.vt.edu)

## Vaccine

- CCV Vaccine developed
- Largely ineffective
- Best Strategy is Prevention and Good Management Practices



[www.vetcare.gr](http://www.vetcare.gr)

## Golden Shiner Virus

- Common bait minnow
- Also found to infect Smallmouth Buffalo
- Isolated from pond-raised Golden Shiners in the 1970's



[img.taminc.net](http://img.taminc.net)

## Golden Shiner Virus

- Signs of Disease
  - Listless
  - Near the surface
  - Petechial Hemorrhaging
- Low Pathogenicity
  - Except when crowded
- Normal conditions
  - Chronic infections occur
  - Low mortalities



[www1.hollins.edu](http://www1.hollins.edu)

## Golden Shiner Virus

- Impact on Baitfish Industry
  - Relatively unknown
  - Massive mortalities have not been reported from baitfish owners



[www.agecon.msstate.edu](http://www.agecon.msstate.edu)

## Lymphocystis

- Chronic viral disease
- Infected cells increase by 50,000 to 100,000X
- Easily transmitted
- Lesions can remain for a year or more



[www.gffc.org](http://www.gffc.org)

## Lymphocystis

- Percids, Centrarchids most susceptible
- Can occur internally
- Virus particles released when enlarged cells burst
- Seldom Fatal



[www.zecdelanvierblanche.com](http://www.zecdelanvierblanche.com)

## VHS

- Before 2003
  - Common in hatchery reared salmonids
  - Europe and Pacific Northwest
- Four known isolates
  - 3 European
  - 1 North American (2 strains)
    - Great Lakes (IVb)
    - 2003



[ak.fishology.com](http://ak.fishology.com)

## VHS

- Bulging eyes
- Bloated abdomens
- Change in activity
- Hemorrhaging in the eyes, skin, gills, base of fins, internal organs
- Loss of osmotic function



## VHS in the Great Lakes

- First confirmed in Lake Ontario in Spring 2005 in freshwater drum
  - Fall 2005 – Confirmed in Lake St. Clair muskies
  - Confirmed as VHS Isolate IVb
- Spring 2006
  - Large scale mortalities
    - Muskies – Lake St. Clair
    - Freshwater Drum – Lake Erie
    - Yellow Perch – Lake Erie
    - Round Gobies – Lake Ontario



Huntings.com

## VHS in the Great Lakes

- Fall 2006
  - Walleye and lake whitefish from Lake Huron – Thunder Bay fish kill
  - Chinook salmon – Swan River Weir
  - Archived Fall 2005 lake whitefish sample from Lake Huron – Cheboygan
  - By Fall, found in 17 species
- Possibly arrived in 2002 via ballast water from the Maritime Provinces
  - Archived 2003 Lake St. Clair muskie sample



[www.biavic.com.au](http://www.biavic.com.au)

## Susceptible Species

- Black Crappie
- Bluegill
- Bluntnose minnow
- Brown bullhead
- Channel catfish
- Chinook salmon
- Coho salmon
- Chum salmon
- Emerald shiner
- Freshwater drum
- Gizzard shad
- Grayling
- Herring
- Largemouth bass
- Muskellunge
- Northern pike
- Pink salmon
- Pumpkinseed



[www.mfish.org](http://www.mfish.org)



[www.dnr.state.oh.us/](http://www.dnr.state.oh.us/)

# Susceptible Species

- Rainbow trout
- Pink salmon
- pumpkinseed
- Redhorse sucker
- Rock bass
- Round goby



www.fly-fishing-discounters.com

- Smallmouth bass
- Yellow perch
- Walleye
- White bass
- Whitefish



personal.bgsu.edu



www.miscgrant.umich.edu

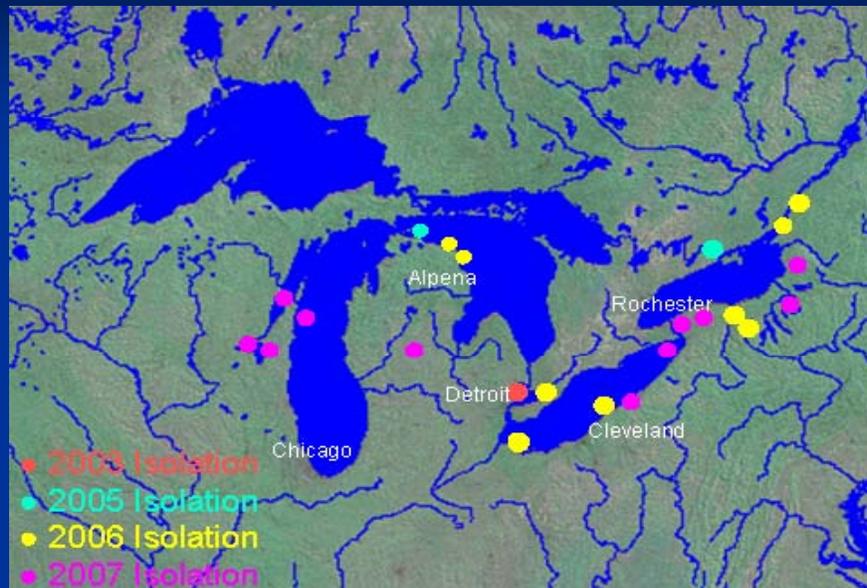


www.epa.gov/



toledotalk.com

# VHS Distribution



## Infectious Pancreatic Necrosis (IPNV)

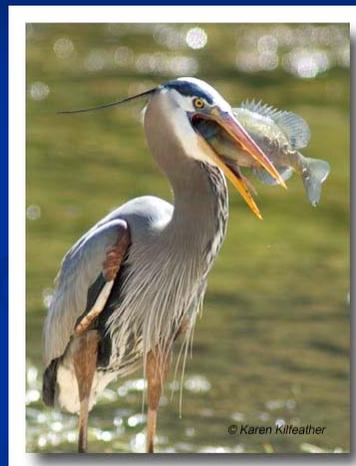
- Small icosahedral birnavirus
  - RNA
- Stable for 3 months at 10 C
- Trout and Salmon
  - All Species
- <6 months old
  - Most susceptible
  - Mortalities possible



[www.aquagen.no](http://www.aquagen.no)

## Infectious Pancreatic Necrosis (IPNV)

- >6 months
  - Minor infections
  - Low mortalities
- Vertical and horizontal transmission
- Birds can serve as vectors
- Signs of Infection
  - Sudden Mortalities
  - Largest Most Vigorous Fry



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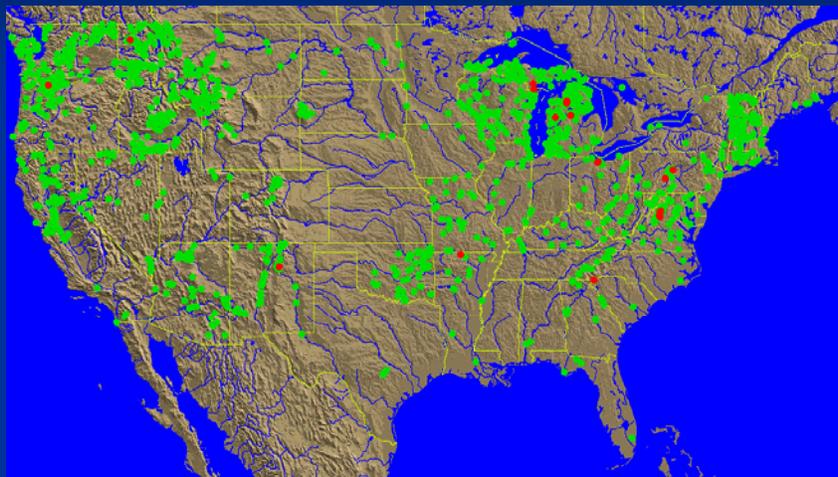
## Signs of IPNV

- Dark pigmentation
- Distended abdomen
- Petechial hemorrhaging
- Mucoïd casts-white
- Pale kidney and spleen
- Rapid mortalities



## Distribution of IPNV

Source: Wild Fish Health Survey Database



## Infectious Hematopoietic Necrosis (IHNV)

- Rhabdovirus
- Susceptibility
  - Highest in fry
  - Decreases with age
- Water temperatures <10°C
- Horizontal and Vertical transmission



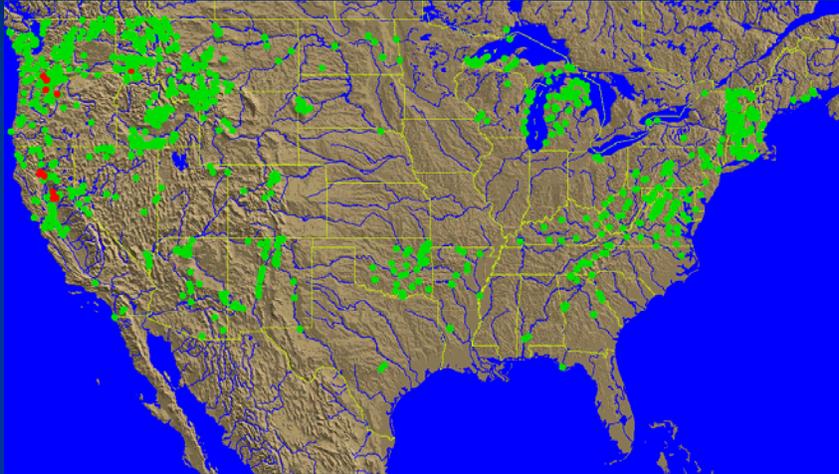
## Signs of IHNV

- Mucoïd cast – off white
- Distended abdomen
- Exophthalmia
- Pale gills
- GI tract missing food
- Hemorrhaging
- Necrosis of hematopoietic tissue



## Distribution of IHNV

Source: Wild Fish Health Survey Database



## Epizootic Epitheliotropic Virus Disease (EEVD)

- Lake trout and splake
- 1980's
  - Seven hatcheries in 3 states
- 2009
  - Hatchery Outbreak
  - Confirmed by PCR
- Horizontal transmission



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## Signs of EEVD

- High in race ways
- Hemorrhaging in bases of fins
- Hemorrhaging in eye
- Rapid opercular movements
- Secondary fungal infections



## Epizootic Epitheliotropic Virus Disease (EEVD)

- Difficult to confirm
- Cannot be grown in Tissue Cell Culture
  - EM
  - New PCR assay
- Potential Problems
  - No Screening Method
  - Extracting from Tissue



*Any*  
**Questions?**