

# Disease Surveillance in Wild and Cultured Stocks of White Seabass (*Atractoscion nobilis*)

Mark S. Okihiro

California Department of Fish and Game

4065 Oceanside Blvd, suite G

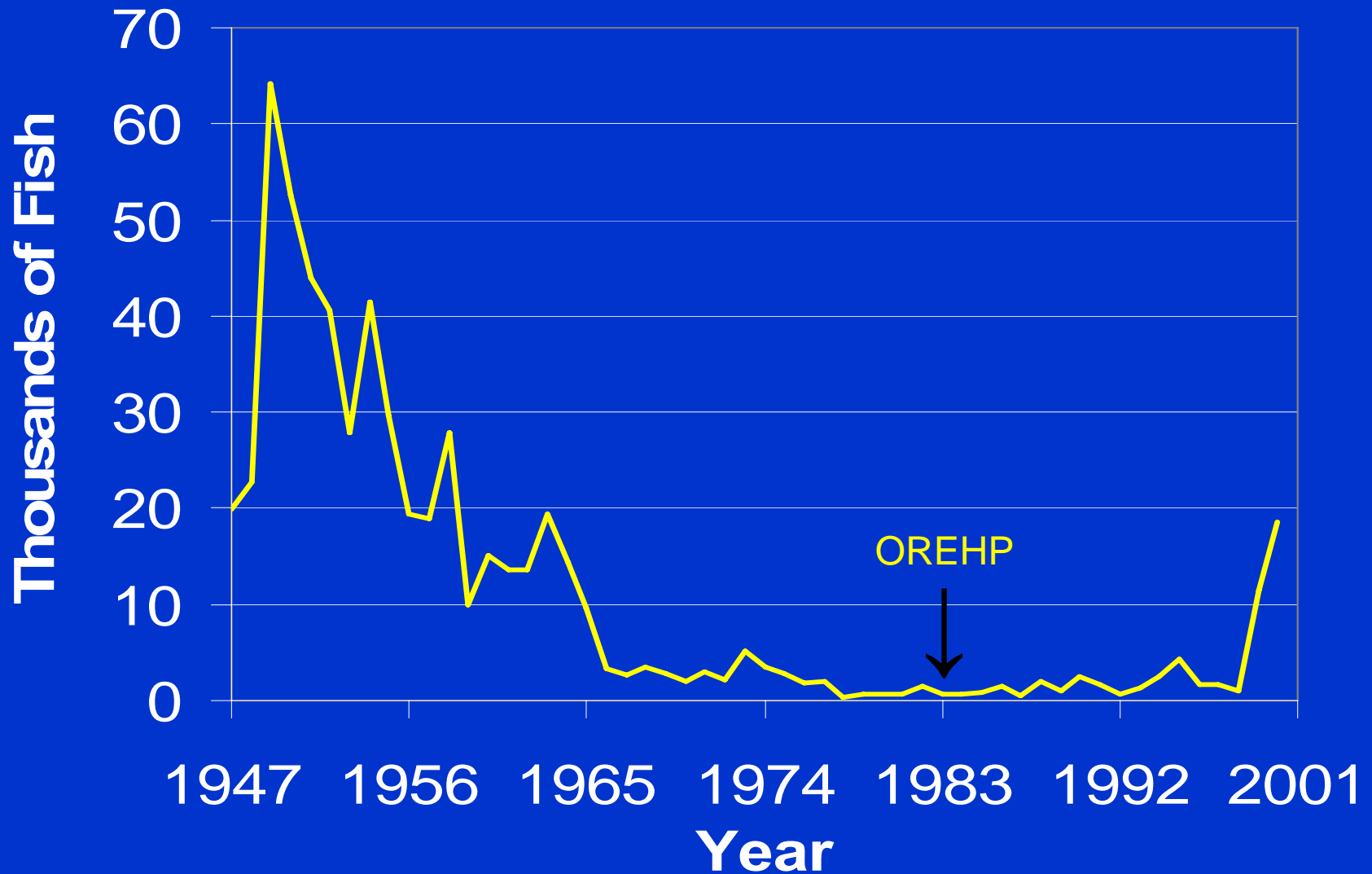
Oceanside, California, 92056

# White Seabass (*Atractoscion nobilis*)

- Family *Sciaenidae*
- one of the largest croaker species in the Pacific
  - 40 kg upper size limit
- Primary Range
  - Point Conception (central California)
  - Baja Mexico



# White Seabass Landings (Sportfishing - California waters)



# Leon Raymond Hubbard Jr.

## Marine Fish Hatchery

- Constructed 1995
- Owner/operator: HSWRI
- Production:
  - 2001: 100,000
  - 2002: 124,000
  - 2003: 142,000
  - 2004: 270,000
  - 2005: 150,000 ?



# 2005 Disease Outbreaks

- King Harbor – *P. salmonis*, *Flexibacter*
- SWYC – *P. salmonis* (?), gill flukes
- Grape St. (SDB) – *Cryptobia*, *Ichthyobodo*
- Quivira Basin (MB) – disseminated *Vibrio*
- Huntington Harbor – diss. *Vibrio*, gill flukes
- Catalina Island – gill flukes
- Dana Point – gill flukes
- Santa Barbara – *Flexibacter*
- Carlsbad Hatchery – disseminated *Vibrio*, *Flexibacter*, and Herpesvirus

# Health Checks for Cultured WSB

- Two Routine Inspections:
  - once at the hatchery  
(prior to transport to net pens)
  - second inspection at the net pen  
(prior to release)
- Any time there is unexplained mortality

# Diagnostic Approach

- History
- Clinical Signs
- Necropsy
- Sample selection
- Sample analyses

# Selecting Fish for Necropsy

- Live moribund fish are the best fish to sample
- If moribund fish are not obvious, then catch fish from areas where sick fish congregate
- Number of fish to necropsy is variable

# Necropsy

- Length / weight measurements
  - TL, SL, BW (+/-)
- Blood sample (+/-)
- Gross external exam
- Skin and gill scraping
- Gross internal exam
- Sampling internal organs

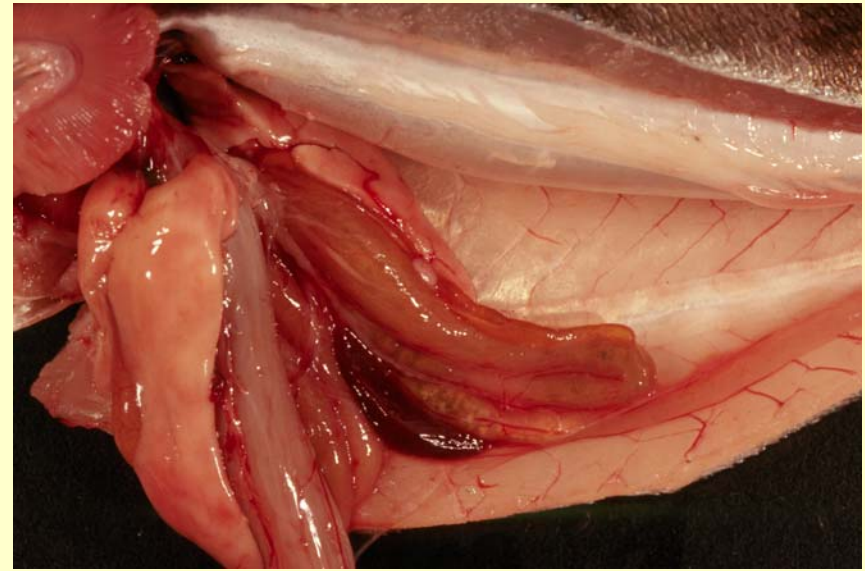
# Gross External Exam

- Skin and fins
- Eyes
- Gills
- Oral cavity

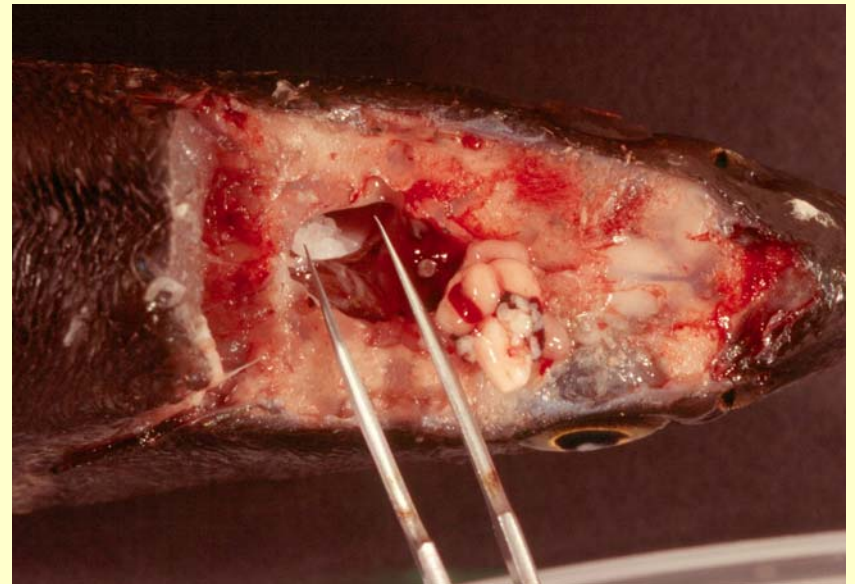
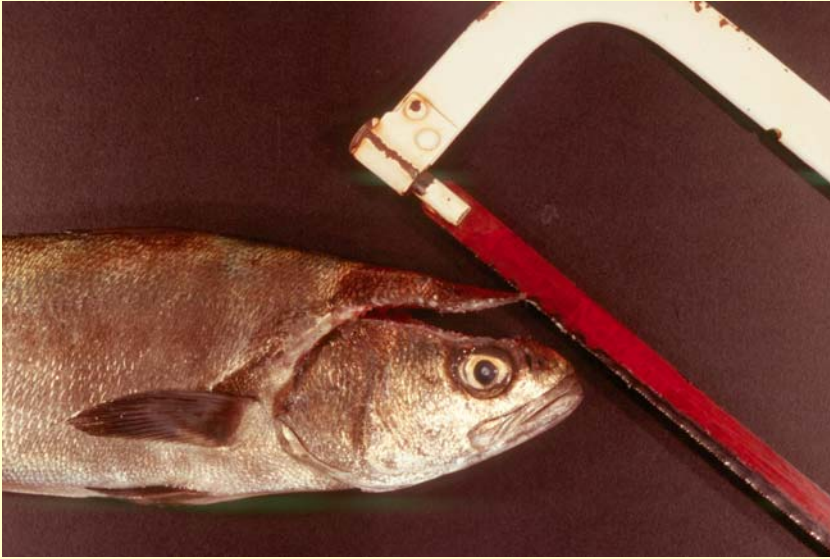


# Gross Internal Exam

- Heart
- Liver and gall bladder
- Stomach, PC, intestine
- Spleen, pancreas (endocrine)
- Swim bladder, gonad
- Kidney – head and trunk



# Brain and Otolith Dissection



# Diagnostic Techniques and Assays

- Necropsy – assessment of gross lesions
- Cytology – dark field light microscopy
- Histology – paraffin processing, HE slides
- Microbiology – virology, bacteriology
- Electron microscopy – TEM, direct EM
- Polymerase Chain Reaction (PCR assays)
- Enzyme-linked immunosorbent assay (ELISA)

# Cytology

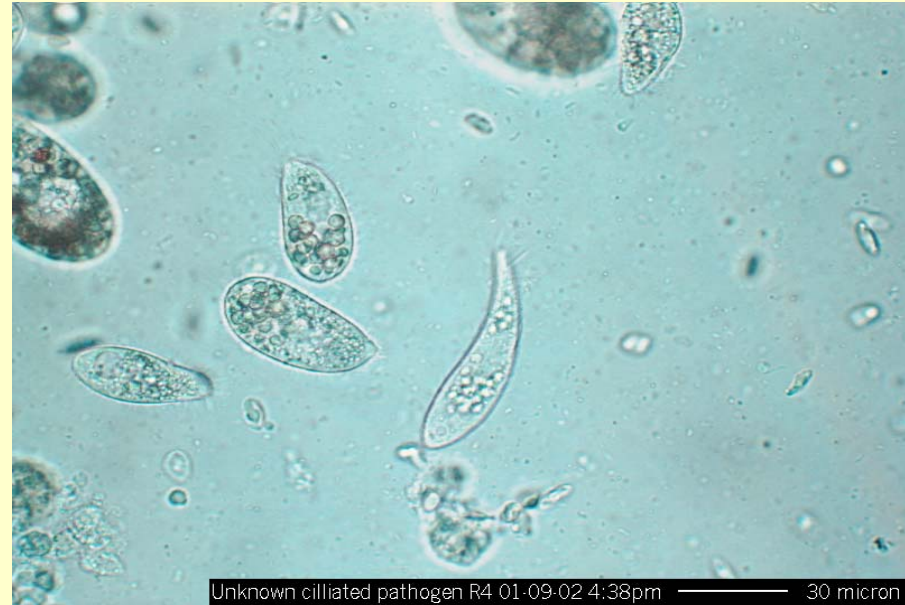
- Wet mount cytology
  - parasite ID and some microbial pathogens
- Routine – gill and skin scrapings
- Occasional – squash preps of kidney and nodular lesions
- Dark field microscopy



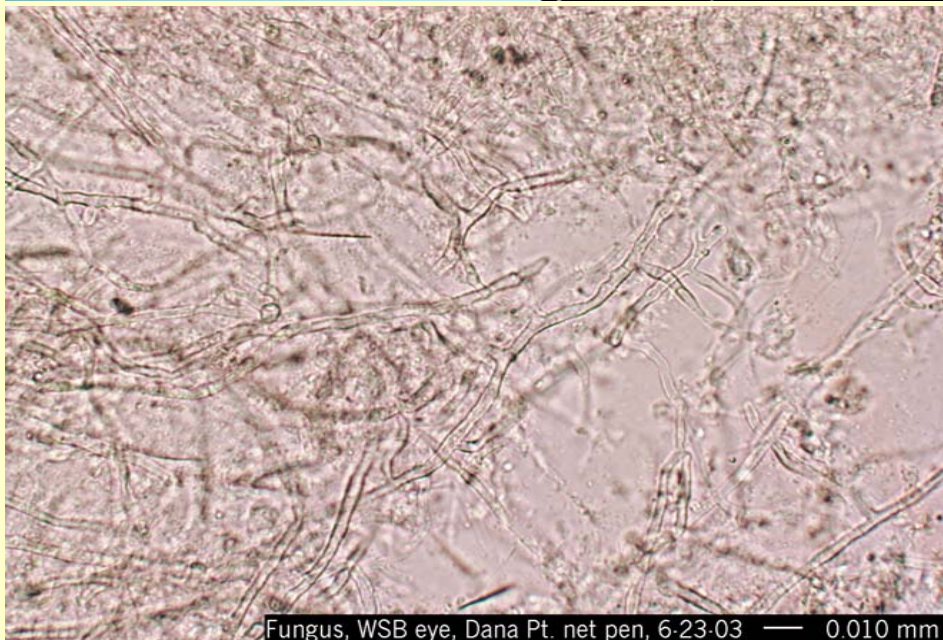
# Dark Field Light Microscopy



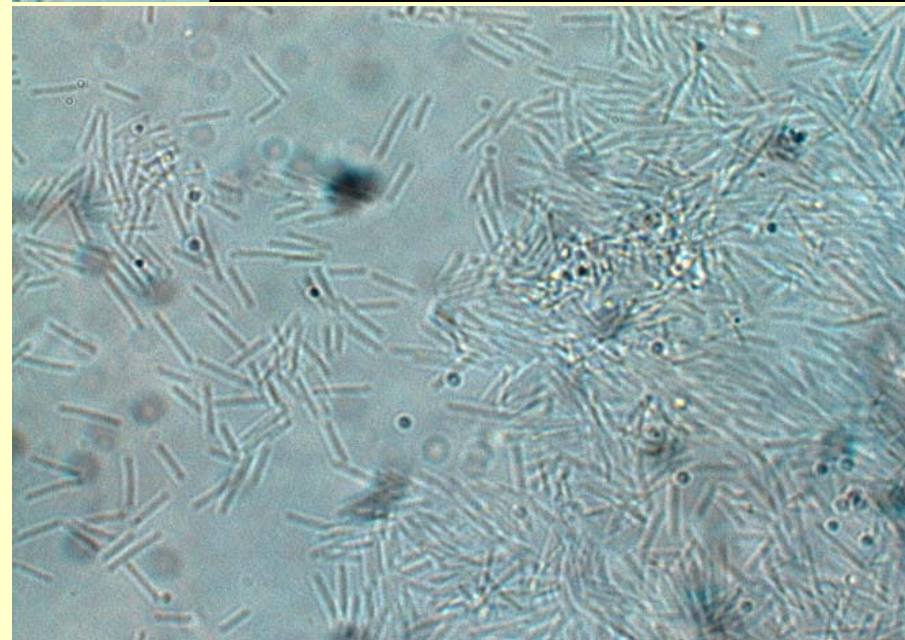
*Cynoscionicola pseudoheterocantha*



Unknown ciliated pathogen R4 01-09-02 4:38pm — 30 micron

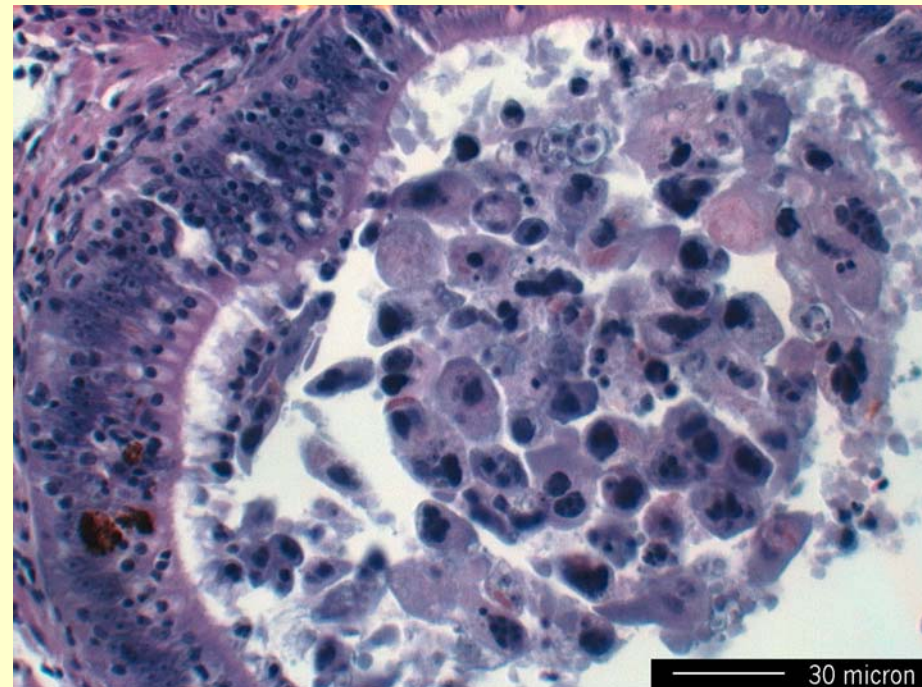
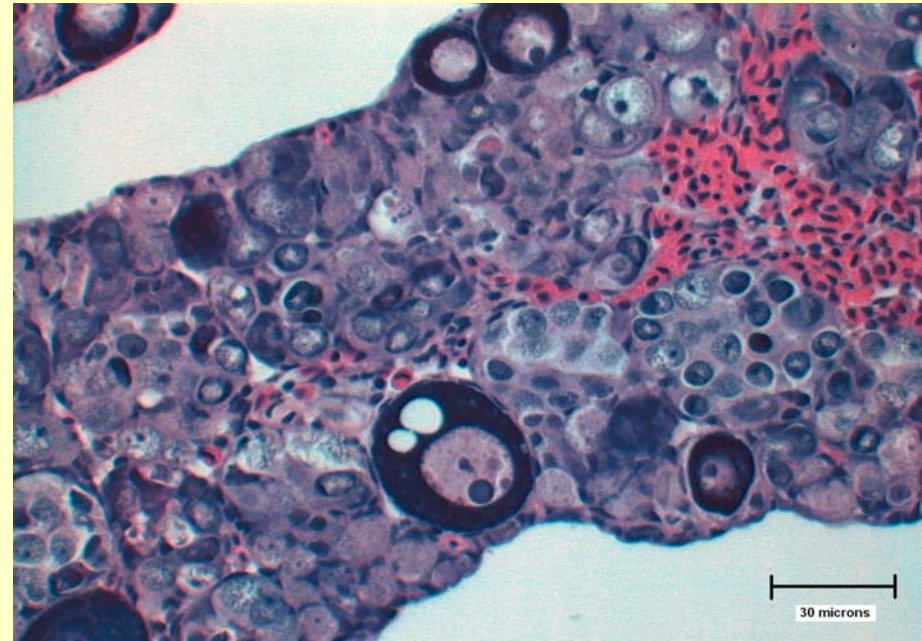


Fungus, WSB eye, Dana Pt. net pen, 6-23-03 — 0.010 mm

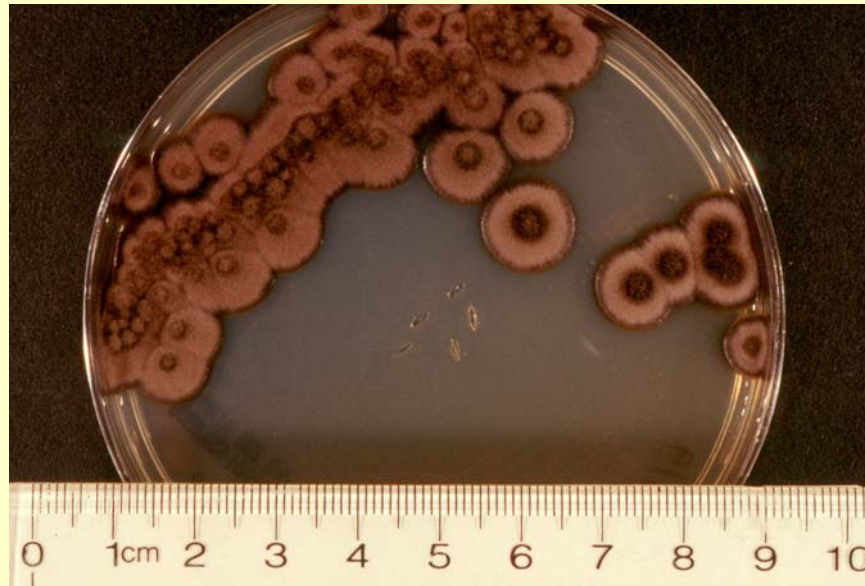
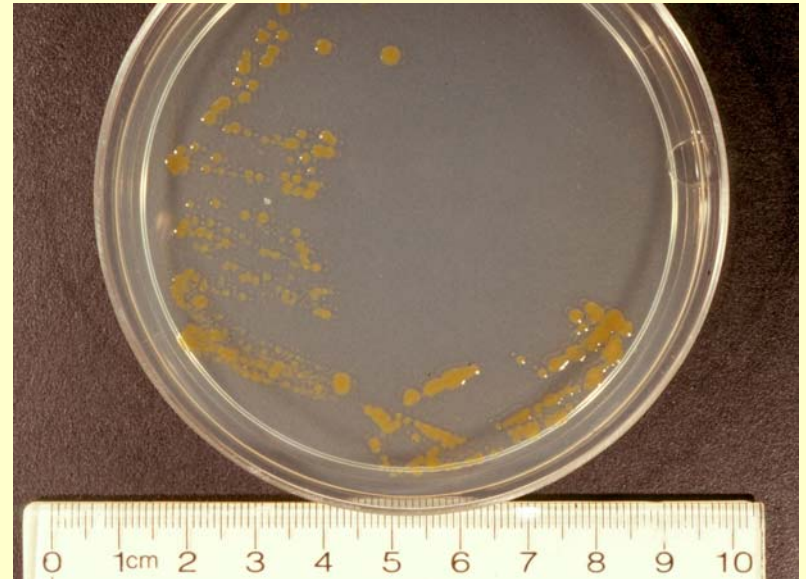
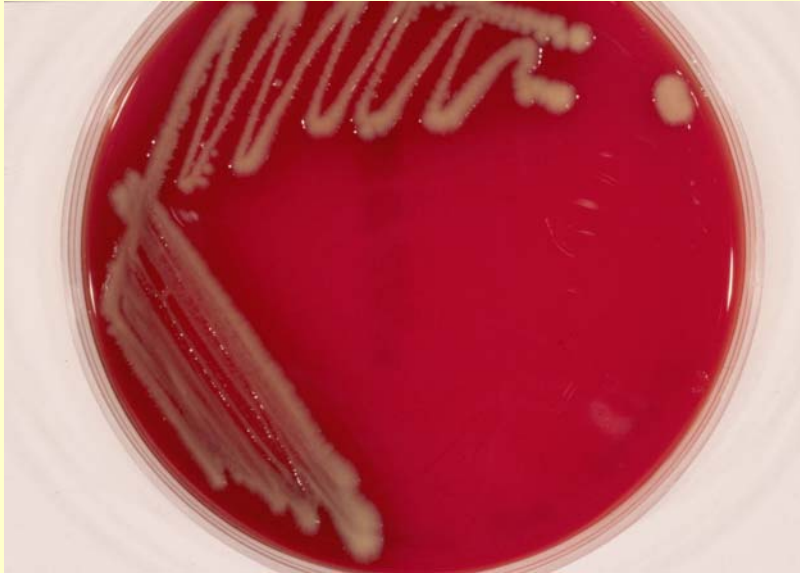


# Histology

- Used to characterize lesions and to identify pathogens
- Good application for larval fish
- 10% formalin fixation
- Routine paraffin processing; HE stain
- Turn around time as fast as 24 hrs

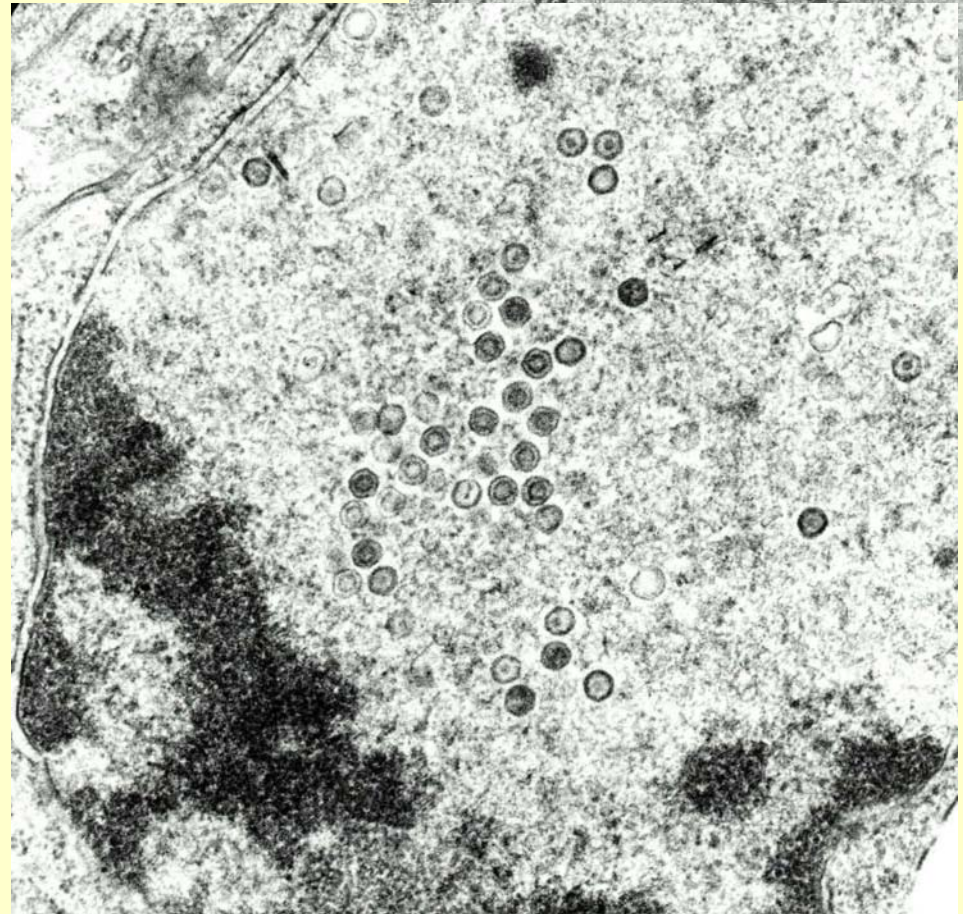
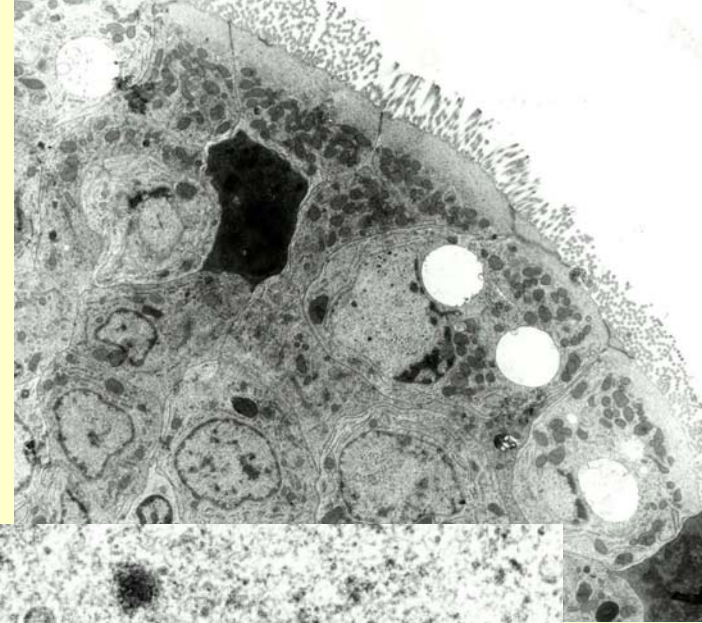


# Microbiology



# Electron Microscopy

- Transmission EM and Direct EM
- Application - viruses, sporozoans, subcellular lesions
- Slow turn-around time (wks-mos)
- Relatively expensive
- CAHFS lab (Davis, CA)



# Polymerase Chain Reaction (PCR)

- PCR assays developed for VNNV, VHSV, and *P. salmonis* (Dr. Ron Hedrick's lab at UC Davis)
- Molecular assay based on the detection of pathogen genetic material by specific primers, followed by gene amplification
- **Advantages:** highly sensitive and accurate
- **Disadvantages:**
  - cannot determine level of infection
  - cannot determine degree of host injury
  - slow turn around time

# Enzyme-Linked Immunosorbant Assay

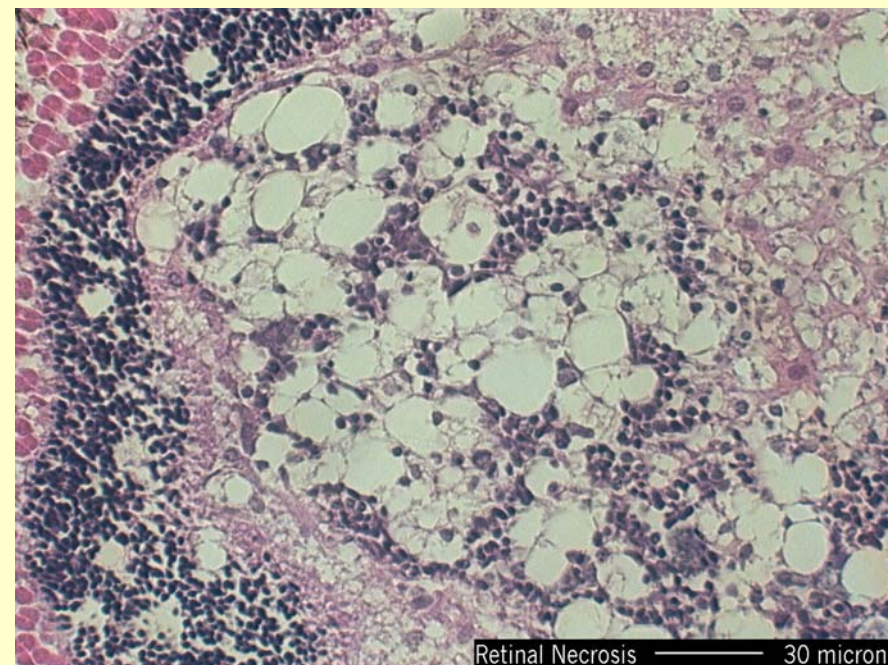
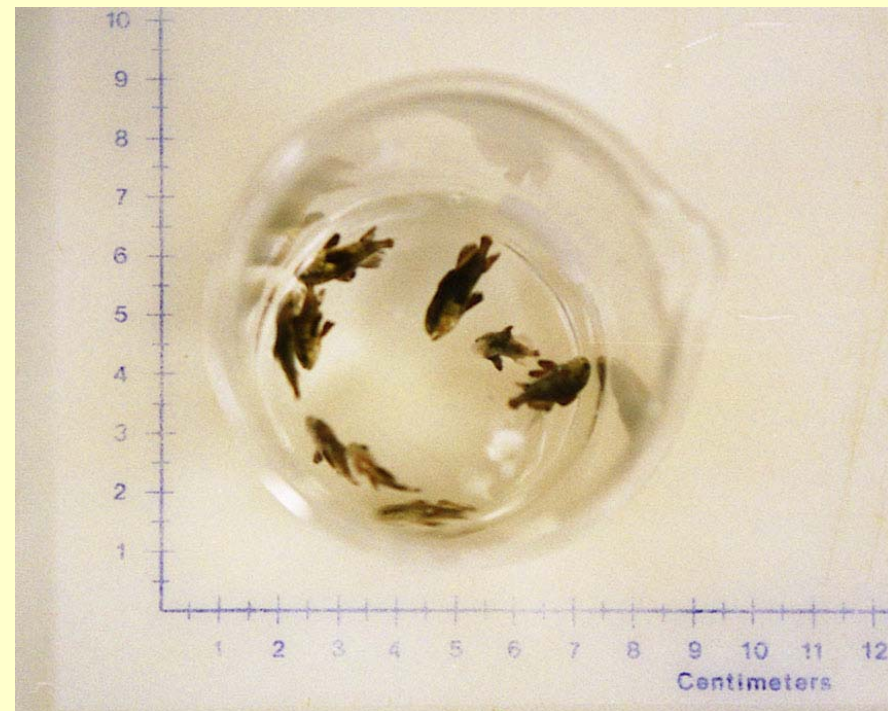
- ELISAs assess serum antibody levels to specific WSB pathogens
- ELISAs exist for VNNV, VHSV, and *P. salmonis*
- ELISAs developed/run by Dr. Hedrick's lab at UC Davis
- **Advantage:** antibodies remain in circulation for long periods of time, chances of detecting pathogen exposure are much higher compared to finding infected fish
- **Disadvantages:**
  - assays can be difficult to setup (false positives)
  - no definitive endpoints (OD given as a % of positive-controls)
  - the pathogen has to be cultured to provide enough antigen to start the assay

# Diseases of Cultured WSB

- Viral Diseases
  - VNNV, Herpesvirus, VHSV (?)
- Rickettsial Diseases
  - *Piscirickettsia salmonis*, *Epitheliocystis*
- Bacterial Diseases
  - *Flexibacter*, *Vibrio*
- Fungal Diseases
- Parasitic Diseases
  - Flukes (3 species), protozoa (8+ species)

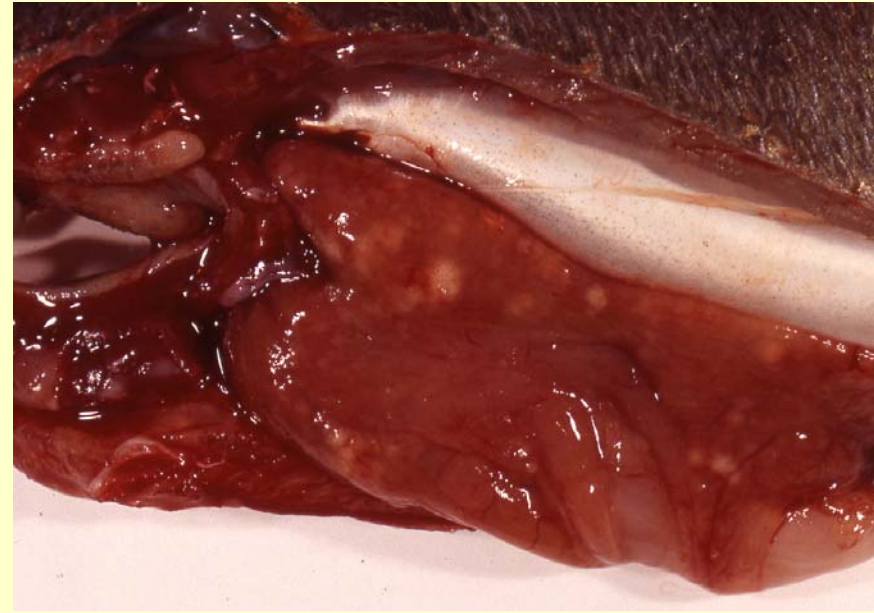
# Viral Nervous Necrosis (VNN)

- Lethal nodaviral pathogen
- Primarily affects larval WSB 20-40 dph
- Frequent epizootics prior to installation of ozone treatment system in spring 2003
- Clinical – paralyzed fish floating on side at surface
- Target organs – retina, brain, spinal cord
- Dx: histo, culture, TEM, PCR
- **No treatment available**



# *Piscirickettsia salmonis*

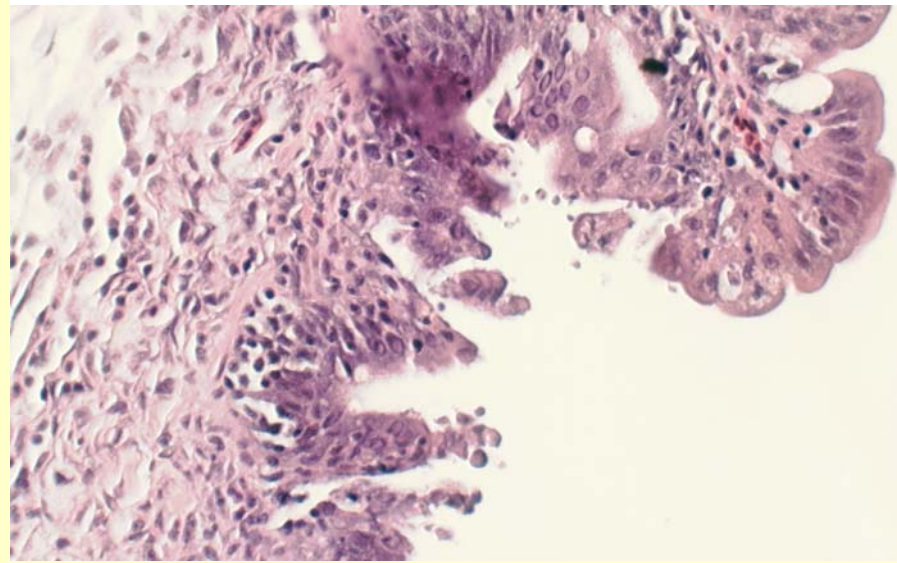
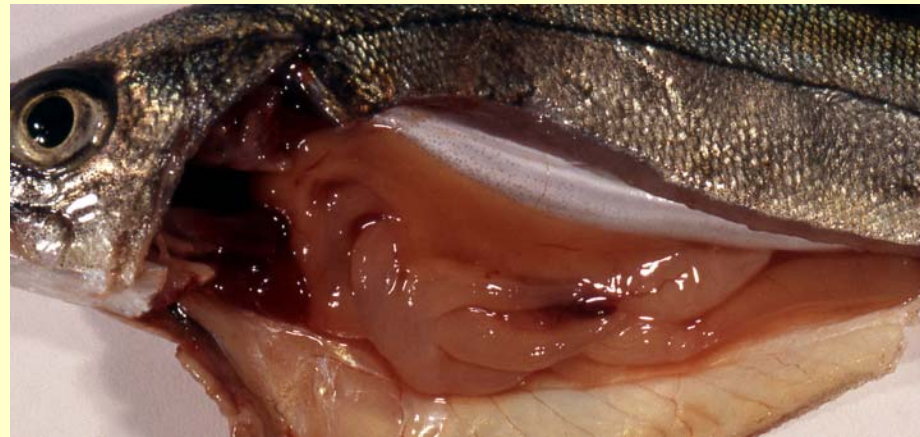
- Lethal rickettsial pathogen
- Rare disease of juvenile WSB (two epizootics: 1998, 2005)
- Clinical – moderate to high mortality; moribund fish with focal white skin lesions
- Target organs: liver, gill, heart, intestine, skin
- Dx: gross findings, histo, culture, PCR, TEM
- **Treatment ?** – possibly oxytetracycline in feed



# Herpesvirus

## Gastroenteritis

- Lethal disease of juvenile WSB
- First confirmed epizootic – fall 2002
- Currently (Nov. 2005) - ongoing epizootic at the Carlsbad Hatchery
- Presumptive etiology: herpesvirus
- Clinical - high mortality, spiraling prior to death
- Dx: gross lesions, histo, TEM
- Necropsy: moderate to severe dilation of GI tract
- **No treatment available**



# How does OREHP manage outbreaks of lethal, highly contagious disease in cultured WSB?

- Losses are never 100% even with the most virulent pathogens of WSB
- Should recovered healthy fish be released if they have been exposed to VNNV, VHS, herpes, or *P. salmonis* ?
- Management decisions with an “enhancement” program need to take into consideration disease risk to wild fish stocks

# Disease Surveillance in Wild WSB

- **Goal:** survey wild WSB to determine which diseases are naturally-occurring
- **Rationale:** if a disease is naturally-occurring, then the risk from infected or exposed cultured WSB is small
- **Plan:**
  - sample a large number of wild WSB
  - assess pathogen exposure and infection levels with a variety of diagnostic assays
  - use survey data to make informed decisions regarding release of cultured WSB

# Collection of Wild Juvenile and Subadult WSB



# Collection of Wild Adult WSB



# Wild WSB Samples Collected from 2002-2005

- 272 adult, subadult, and juvenile WSB
- 195 ELISA serum samples
- 200+ brain and eye samples
  - VNNV isolation / PCR
- 160+ spleen and kidney samples
  - VHSV isolation / PCR
- 80+ liver samples
  - *P. salmonis* isolation / PCR
- 100+ intestinal content samples
  - direct EM for herpesvirus

# Preliminary ELISA Results from Wild WSB Disease Survey

- 18% (14/78) wild juvenile WSB were ELISA positive for VNNV exposure
- 53% (9/17) wild adult WSB were ELISA positive for VNNV exposure
- 0% (0/94) wild juvenile and adult WSB were ELISA positive for *P. salmonis* exposure
- ELISA results for VHSV exposure pending
- No ELISA assay available for WSB herpesvirus (currently unable to grow herpesvirus in culture)

# Preliminary Pathogen Isolation and PCR Results from Wild WSB Disease Survey

- Virus Isolation samples – all negative
- Rickettsial Isolation samples – all negative
- PCR samples – results pending
- Detection of Herpesvirus via Direct EM
  - 50 intestinal content samples analyzed
  - One suspicious, but not definitively positive, sample

# Conclusions from Wild Fish Disease Survey

- VNNV is a naturally-occurring disease among wild WSB
- *P. salmonis* is an exotic disease
- Wild fish disease status for VHSV and herpesvirus is unknown

# Impact of Wild Fish Disease Survey on Hatchery Operations

- Since VNNV exposure is widespread among wild WSB, exposed but healthy cultured WSB can be released
- *P. salmonis* is an exotic disease, so infected cultured stocks must be destroyed
- Herpesvirus and VHSV status of wild fish are unknown; both must be assumed to be exotic diseases and infected hatchery fish cannot be released

