

The Laboratory Zebrafish

Workshop in Lab Animal Medicine

May 19, 2011

David M. Kurtz, DVM, PhD



The Laboratory Zebrafish



Disclaimers

- This is **not** an ACLAM sanctioned presentation
- All information is deemed reliable and correct
 - No warranty for accuracy
- No information presented is known to be specifically included in ACLAM Board examinations

The Laboratory Zebrafish



Genus & Species?

Acknowledgments

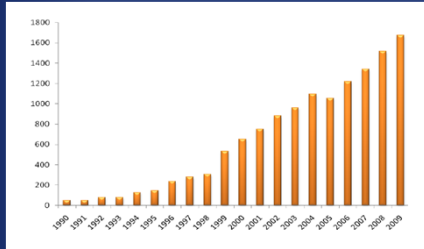
- Julia Whitaker - UNC
- Diane Forsythe & Mary Grant – NIEHS
- Mary Ann Vasbinder - GSK

The Laboratory Zebrafish

Kingdom	Animalia
Phylum	Chordata
Order	Cypriniformes
Family	Cyprinidae
Genus	Danio
Species	rerio



The Laboratory Zebrafish



Guidance on the housing and care of Zebrafish - Danio rerio

Nutrition

- Omnivorous
- Natural diet – zooplankton, insects, algae, invertebrate eggs
- Begin feeding 5 days post fertilization (dpf)
 - Yolk sac – depleted around 7-8 dpf
 - Mouth opens
 - Swim bladder inflates
 - Development of digestive tract complete



Zebrafish Characteristics

- Freshwater – Ganges River (India)
- Teleost (vertebrate)
- Sexually dimorphic (not always easy)
 - Females larger, more silver, rounded
 - Males- more brightly colored, streamlined



<http://dir.nichd.nih.gov/img/uvr/WEINSLAB.html>

Nutrition

- Laboratory diet
 - Larvae 5-14 dpf – Paramecia or rotifers
 - > 14 dpf to adult – flake or pellets
 - Do not feed more than can be consumed in 3-5 minutes. Excess = nitrogenous waste
- Artemia – Brine shrimp
 - Start at 28 dpf
 - Optimal for breeding colonies



Zebrafish Characteristics

- Adults 3-4 cm long
- Lifespan (laboratory): ~ 3.5 years
- Sexual maturity: 6-8 months of age
- Optimal breeding: 6 – 18 months
- Like most fish – possess a lateral line – series of mechanoreceptors used to sense environmental conditions
- Lack teeth – instead have pharyngeal jaw associated with posterior gill arch used to grind food

Housing

- Holding tanks
 - Static – aquarium – OK for small populations
 - Flow through – lowest levels of nitrogenous waste but uses a lot of water
 - Recirculating – most common
- Housing density ~ 5 adults/liter

Housing

Recirculating Systems



Water Quality

- What is the name of the system component indicated by the red arrow?
- What process primarily takes place here?



Water Quality

Most important aspect of research aquaculture systems

Water Quality

- Biological Filter
- Nitrogen of Nitrification Cycle



Water Source

- Municipal water – chemically treated to remove chlorine, chloramines, copper
 - Sodium thiosulfate
 - Activated charcoal
- Wells
 - few infectious agents or chemicals
 - Small volumes
- Ponds
 - not recommended
 - Lots of pathogens
 - Chemical contamination from runoff
- Distilled
 - Must add back salts
 - expensive for large volumes
- Reverse osmosis filtration
 - Must add back salts
 - most common in research aquaculture

Nitrogen Cycle



Water Quality

- Biological Filter
- Nitrogen of Nitrification Cycle

Other types of filtration?

- Mechanical
- Chemical



Zebrafish Reproduction

- Oviparous – egg layers
- Broadcast spawners
- Females produce 100-200 eggs every 1-3 days
- Females prefer to release eggs over a substrate (e.g., plants, rocks, marbles)
- Adults will eat eggs if easily accessible

Environmental Conditions

- Temperature
 - 21° – 32°C (70° – 90°F)
 - 28°C (83°F) – optimal
 - Limit rapid changes $\pm 1.5^\circ\text{C}/\text{day}$
- pH
 - Range of 7-8 – preferred 7.0
 - $\text{pH} > 8.0$ favors $\text{NH}_4^+ \rightarrow \text{NH}_3$ – more toxic
 - $\text{pH} < 5.0$ inhibits nitrifying bacteria – ammonia accumulation

Zebrafish Reproduction

Egg Collection



<http://www.tecnoplast.it/aquatic/zebtcc.php>



http://www.aquatichabitats.com/breeder_tank_small.htm

Environmental Conditions

- Salinity \approx Conductivity – total dissolved solids (ions)
 - Hardness [Ca^{++} or Mg^{++}] – 80-200 ppm
 - Important when using distilled or RO source water
- Dissolved Oxygen (DO) – 6.0 ppm
 - Too low – hypoxia, fish gather at surface
 - **Too high?**
- Light cycles – 14 light: 10 dark most common
 - Gradual brightening and dimming over 30 minutes recommended

Zebrafish Reproduction

- Large Scale egg production
- Holds up to 2000 breeding adults



MEPS - Aquatic Habitats

Research Uses

- Vertebrate embryonic development
- Gene function analysis
- Mutagenesis
- Toxicology

Embryonic development



Zebra embryo development

Movie by Tyler and Kozlowski- 2.64 MB

<http://www.devbio.uga.edu/movies/files/FishTylerKozlowski%20fish2.mov>

Normal zebrafish heart beat

http://www.exploratorium.edu/imaging_station/gallery.php?As set=Normal%20zebrafish%20heartbeat&Group=&Category=Z ebrafish&Section=Introduction

Zebrafish Models

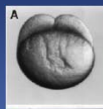
- Small size, little maintenance
 - 1/1000th the cost of mice (debatable)
- Synchronized embryonic development
- External development- view all stages of development (transparent eggs)
- Rapidly develop - hatch in ~ 72 hours
- Genome half the size of mammals'
- Genome sequenced

Genetic Models

- Over 4000 reported genetically modified lines
- Spontaneous mutations
- Mutagenized models
 - ENU
 - Radiation
- Transgenics

Embryonic development

- 45 min- first cleavage
- Gastrulation (5.5 – 10 hpf)
 - Endoderm, mesoderm, ectoderm
- 24 hpf- heart beat, blood circulation, responsive to touch



http://zfin.org/zf_info/zfbook/stages/index.html

See-through Zebrafish



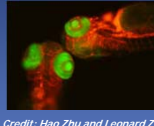
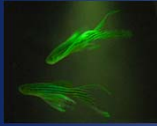
Richard White, MD, PhD

Watch heartbeat:

<http://www.vanderbilt.edu/med/medschool/research/Site2029/mainpage52029P22sublevel57.html>

Transgenic Zebrafish

- Transgenic Reporter lines
 - Green Fluorescent Protein (GFP)
 - Red Fluorescent Protein (RFP)



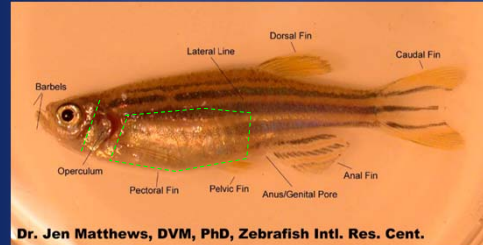
Credit: David Traver and Ken Poss

Credit: Hao Zhu and Leonard Zon

<http://ame2.asu.edu/sites/eighthday/documentation/gfpecology/>

<http://stemcells.ucsd.edu/imagegallery.asp>

Zebrafish Necropsy



Dr. Jen Matthews, DVM, PhD, Zebrafish Intl. Res. Cent.

Clinical Exam

- **Water Quality**
 - Present and historical – importance of good records
- Behavior
 - Swimming, respiration, clustering
- Clinical signs
 - Opercular flaring
 - Petechia or hemorrhage
 - Change in body color
 - Scale loss
 - Improper buoyancy
 - Lethargy/decreased swimming
 - Surface breathing

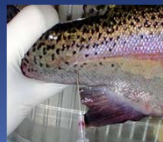
Zebrafish Necropsy



Dr. Jen Matthews, DVM, PhD, Zebrafish Intl. Res. Cent.

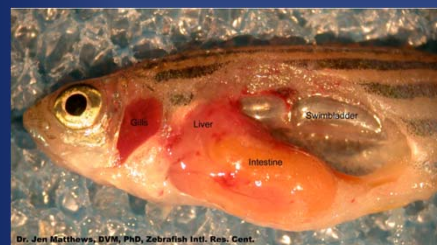
Clinical Exam

- Skin/mucus scrape
- Gill biopsy
- Fin clip
- GI wet mount (fecal)
- Blood sample



Pictures from "Clinical Diagnostic Techniques in Fish" by Carol Poll, C.I. Davis Lab Animal seminar in Chicago, April 2005

Zebrafish Necropsy



Dr. Jen Matthews, DVM, PhD, Zebrafish Intl. Res. Cent.

Zebrafish Necropsy



Diseases Associated with Water Quality

- Chlorine
- Copper
- Ammonia
- Nitrite
- Dissolved Gases

Zebrafish Necropsy



Chlorine Toxicity

- Municipal water contains 0.5-1.0 ppm
- Failure of water purification system
- All fish are very sensitive
- Acute gill necrosis
 - Opercular flaring
 - Hypoxia
 - Cherry red gills
 - Petechial hemorrhage around head

Diseases

- Water Quality – chemical toxicity
- Infectious disease

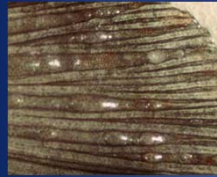
Copper Toxicity

- Leaching from copper piping (especially new systems)
- Gill damage
- Affects reproductive development
- Increased susceptibility to infectious disease

Ammonia Toxicity

- Waste product (fish and feed)
- Highly toxic
- Elevations usually due to abrupt change to system especially affecting biological filter (biofilter “crash”)
- Effects observed at levels 0.002 ppm. Should avoid > 1.0 ppm
- Primary effects on CNS (seizures)
- Hyperexcitable, anorexia, reduced growth
- Histo – gill hyperlasia (chronic exposure)

Name the Disease



<http://oregonstate.edu/dept/salmon/projects/salmon-sdipathoc.html>



http://zebrafish.org/zrc/health/Disease_images



<http://www.hf.org/TMDL2print/TMDL03print.gif>

Nitrite Toxicity

- Usually in conjunction with Ammonia toxicity
- Another name for nitrite toxicity in fish?

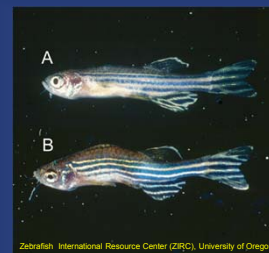
Gas Bubble Disease (GBD)

- Supersaturation of dissolved gases (O_2 or N_2)
- “Bends” in people
- Gas forms bubbles in tissues resulting in damage
- May not be apparent grossly – requires histology
 - May dissipate within 24 hours post-mortem
- Most often caused by leak in recirculating system resulting in air sucked into supply by high pressure pumps

Nitrite Toxicity

- “Brown blood disease” – Methemoglobinemia
- Reported in catfish – rare in research setting
- Nitrite crosses the gills and oxidizes hemoglobin
 - Hypoxia

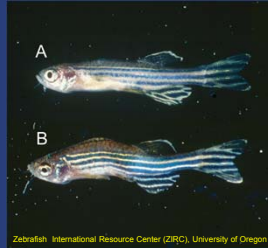
Skinny Zebrafish with curved spine



Zebrafish International Resource Center (ZIRC), University of Oregon

Most likely etiologic diagnosis?

Skinny Zebrafish with curved spine



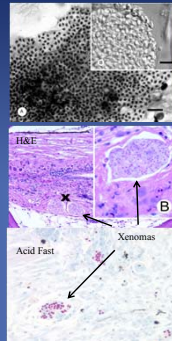
Pseudoloma neurophilia

Mycobacteriosis

- *Mycobacterium marinum*
- *M. fortuitum*
- *M. chelonae* (*chelonae*)

Pseudoloma neurophilia

- Neuronal microsporidia
- Obligate intracellular parasite
- High prevalence in research colonies (>50% tested by ZIRC)
- Severe emaciation
- Curvature of spine in older adults
- Reduced growth and fecundity
- Can cause high mortality in fry
- Possible vertical transmission
 - Cannot be eliminated via bleaching eggs
- No treatment – test (PCR) and cull



Zebrafish International Resource Center (ZIRC), University of Oregon

Mycobacteriosis

- Rod-shaped bacteria
 - Multi-organ granulomas - Liver, spleen, kidney, repro organs
- High prevalence in research colonies (> 30% tested by ZIRC)
- Long incubation, chronic, subclinical
- Clinical signs:
 - Wasting, emaciation
 - Acutely: "dropsy"
 - Petechia
 - Ulceration of scales, fin erosion

Mycobacteriosis

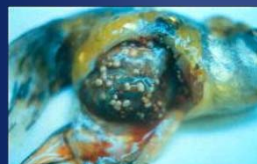
- What are the 3 most common species of atypical *Mycobacterium* in Zebrafish?



M. Kent, University of Oregon

Mycobacteriosis

Granulomas



<http://edis.ifas.ufl.edu/VM055>



<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1695495>

Dropsy

- General term for abdominal distension and scale edema
- Usually due to kidney or gill failure and loss of osmoregulation and hypoproteinemia

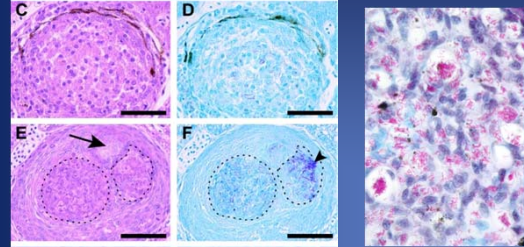


Joerg Mayer, DVM, (c) 2002
<http://www.mycops.org/bwocw/tufc/courses/5/content/D215706/C43031.jpg>



<http://www.uic.edu/louisiana.edu/~jlm2431/dropsy.jpg>

Mycobacteriosis- acid fast



<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1695491>

Dr. Virginia Godfrey, UNC

Mycobacteriosis

Special stain for Mycobacterium?

Mycobacteriosis

- No effective treatment
- Eradication of infected stocks
- Disinfect tank and filter system
 - With bleach for 3 days, rinse for 3 days
 - Restock with fish, test for reinfection

Mycobacteriosis

- Acid-fast
- Ex. Fite-Faraco, Ziehl-Neelsen

Mycobacteriosis- Zoonotic

- Cutaneous infections in humans
- “Fish handler’s granuloma” or “swimmer’s granuloma”
- Usually local and self-limiting, life-threatening if immunocompromised
- Wear gloves!!



<http://www.reefkeeping.com/issues/2003-07/sp/feature/index.php>



<http://www.aquascope.com/images/Granuloma.jpg>

Pseudocapillaria tormentosa

- Intestinal Nematode
- Common in research colonies (>24% of facilities tested by ZIRC)
- Most infections are subclinical
- Wasting and emaciation
- Associated with penetrating GI infection and neoplasia
- Dx: GI wet mount - eggs or adult
- Tx: trichlorfonmebendazole, Panacur, ivermectin



http://zebrafish.org/zirc/health/Disease_images/CapillariaWetMounted.jpg

Anesthesia/Euthanasia

- Benzocaine hydrochloride
 - 25-100 mg/L for anesthesia
 - Often used in field studies
 - Small margin of safety
- Clove Oil
 - Eugenol or Isoeugenol (90-95%)
 - Reduced cost
 - Often used in field studies
 - Not FDA approved for use in fish

Anesthesia/Euthanasia

What is the anesthetic/euthanasia agent most commonly used in Zebrafish?

Euthanasia

- Rapid Cooling
 - Currently not recommended in 2007 AVMA Guidelines on Euthanasia
 - Recent publications advocate euthanasia via rapid cooling for Zebrafish (warm water species only) over MS-222
 - Wilson JM et al., *Comp Med* 48(6), November 2009
 - Blessing JJ et al., *J Fish Biol* 76, 2010
- Current NIH Guidelines
 - Submersion in ice water (5 parts ice/1 part water; 0-4°C) for at least 10 minutes (20 minutes if unable to visualize opercular movement and for fry 4-7 dpf)
- Deviation from Guide standards?? – IACUC approval

Anesthesia/Euthanasia

- MS- 222 (tricaine methanesulfonate)
 - 0.05 - 0.15 mg/ml - anesthesia
 - 2.0 - 2.5 mg/ml – euthanasia
 - 10-20 minute emersion for euthanasia
 - Must buffer with sodium bicarbonate b/c low pH



Pictures from "Clinical Diagnostic Techniques in Fish" by Carol Post, U.I. Davis Lab Animal seminar in Chicago, April 2005

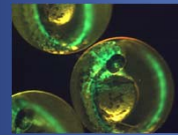
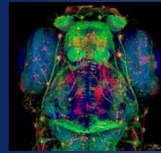
Regulatory and IACUC Issues

- Specific aquatic animal section in 2010 Guide (CH 3 - Environment, Housing and Management)
- Field studies
- Animal tracking & records
- Facility inspections – especially outside main vivarium
- Euthanasia



Zebrafish International Resource Center (ZIRC)
 5274 University of Oregon
 Eugene, OR 97403-5274, USA
 Phone: 541-346-6028
 Email: zirc@zebrafish.org
 Web: <http://zebrafish.org>

Questions

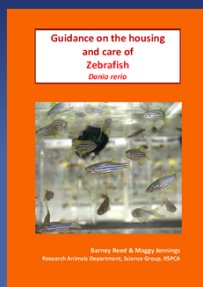
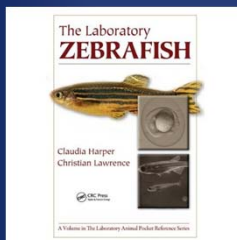


Zebrafish Husbandry Association
<http://www.zhaonline.org/>

Additional Fish Disease Slides

From J. Whitaker talk 2008
 and
 ZIRC

Zebrafish References



http://www.zhaonline.org/Guidance_on_the_housing_and_care_of_zebrafish_2010_.pdf

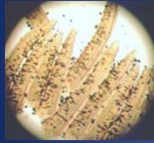
Velvet Disease

- *Piscinoodinium pillulare*
- Parasitic dinoflagellate
- Infects the skin and gills – may see grey or rust colored sheen
- Clinically - lethargy, labored breathing, stays near water surface
- Wet mount of skin scraping or fin clip
- Histological sections

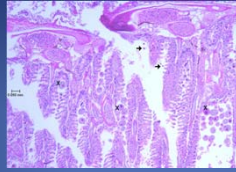
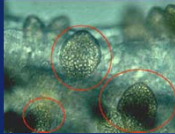


<http://www.pertfish.net/articles/pics/arn3/velvet.jpg>

Piscinoodinium pillulare



<http://fishweb.ifaa.ufl.edu/AquaticAnimalHealth/ExamplesFishDiseases.htm>



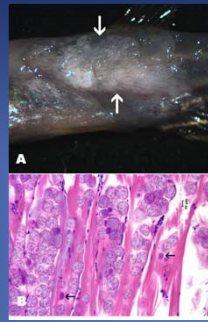
http://www.oxfs.org/library/aquarium_net0597/images/img0077.jpg

Bacterial Septicemia

- *Streptococcus iniae*
- *Edwardsiella tarda*
- *Aeromonas hydrophila*
- *Pseudomonas* spp.
- *Flavobacterium columnare*
 - Previously *Flexibacter columnaris*
- *Flavobacterium* spp.
- Which are zoonotic?

Pleistophora hyphessobryconis

- Microsporidia
- Neon Tetra disease
- Seen in multiples aquarium species – and some research institutions
- Primarily affect skeletal muscle
- Diagnosis only via histology (no PCR yet)



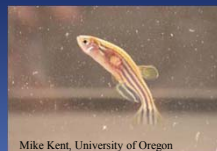
Zebrafish International Resource Center

Bacterial Septicemia

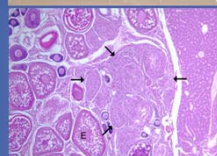
- *Streptococcus iniae* (zoonotic)
- *Edwardsiella tarda* (zoonotic)
- *Aeromonas hydrophila* (zoonotic)
- *Pseudomonas* spp. (zoonotic)
- *Flavobacterium columnare*
 - Previously *Flexibacter columnaris*
- *Flavobacterium* spp.

Egg Associated Inflammation and Fibroplasia

- Females with extended abdomens
- Common – especially aging females
- Cause unknown – egg retention and degeneration
- Grossly – enlarged tumor-like ovaries often with adhesions
- Histo – severe inflammation (arrows) +/- fibrosis



Mike Kent, University of Oregon



Zebrafish International Resource Center

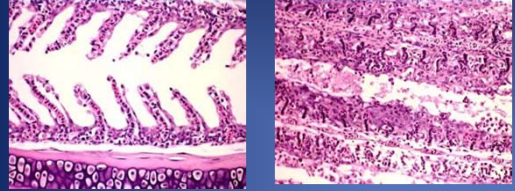
Clinical signs

- Exophthalmos
- increased respiratory effort
- Dropsy/abdominal distension
- Petechial hemorrhages- on body, around eyes, mouth, anus, opercula, or fin base
- Necropsy: Congested, hemorrhagic organs, pale liver, dark red spleen, blood-tinged peritoneal fluid

Diagnosis & treatment

- Isolation in broth or on blood agar
 - Grow at 20- 25°C 24 – 48 hr
- Antibiotics- usually bath immersion
 - Sulfadiazine-trimethoprin
 - Enrofloxacin
 - Oxytetracycline

Flavobacterium columnare

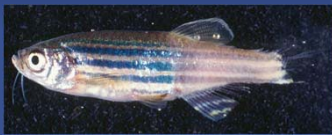


<http://www.vet.uga.edu/VPP/Undergrad/Siegel/index.php>

Flavobacterium columnare



http://www.klamathwaterquality.com/fish_disease_close_up_gill_shot.jpg

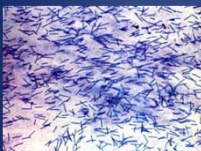


http://zebrafish.org/zirc/health/Disease_images/TailRot4.jpg

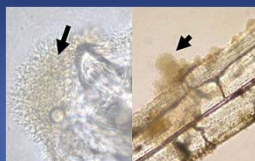
Aquatic Mycosis

- *Saprolegnia* spp.
- Oomycetes
- Opportunistic- damaged skin, poor husbandry, concurrent infection
- Signs: thin, white filaments building up to white “cottony” mattes

Flavobacterium columnare



<http://www.vet.uga.edu/VPP/Undergrad/Siegel/index.php>



http://www.umesc.usgs.gov/aquatic/drug_research/disease_model/images/haystacks_columnaris.jpg

Saprolegnia

- Dx: skin wet mounts
- Broad aseptate hyphae ± sporangia on tips
- Hyphae visible on H & E
- Pronounced with silver stain

Saprolegnia

- Ubiquitous
- Spread rapidly
- Debilitation: secondary bacterial sepsis & disrupted osmotic balance
- Tx: saltwater bath, formalin bath
- Best Tx- malachite green- (but teratogen & mutagen)

White Spot Disease

- A.k.a.?
- Caused by?

Saprolegnia



<http://www.americaaquariumproducts.com/images/graphics/saprolegniafungus.jpg>



<http://www.uib.no/bot/kurs/B8220/Algae/Figures/fish.jpg>



http://www.aquarium.it/files/articles/itgos/saprolegnia_1.jpg



http://www.ncwildlife.org/pg03_Fishing/images/img_3g1_sapro.jpg

White Spot Disease

- Ich
- *Ichthyophthirius multifiliis*

Saprolegnia



<http://www.fishlife.nl/saprolegnia%202.jpg>

http://kentsimmons.uwinnipeg.ca/16cm05/16labman05/lb2pg3_files/saprolegniazoosporangium.gif



White Spot Disease

- Ciliated protozoan
- Trophont colonizes epithelium
 - Fins, skin, gills- feeds on tissue & fluids
- Trophont ruptures overlying epithelium → attaches to substrate
- Develop into free-swimming theronts- must colonize host within 48 hrs

Ich

- Multifocal, raised, white mucoid nodules on skin and gills
- Flashing & increased respiratory effort
- Dx: skin wet mounts
 - Trophont with horseshoe-shaped nucleus
- Tx: Raise temp 1.5°C every 24-36 hr for 3 days to speed up life cycle **plus** salt bath, formalin bath

Trichodinosis

- *Trichodina* spp. (+ 5 other genera)
- Ciliated protozoan
- Skin-associated- broad host range, gill-associated- more host-specific
- Direct life cycle
- Don't persist in environment (>48 hr)

Ich



<http://www.fishpalace.org/Disease.html#Septicemia>



<http://www.vet.cornell.edu/PU/BLIC/FishDisease/AquaticProg/highlights/Ich/Ich.htm>



http://www.guppyendler.com/upload/enfermedades/ichthyophthirius_multifiliis_1.jpg

Trichodina

- Heavy infection: poor body condition, low reproductive rates, mortality
- Signs: respiratory distress, skin erosion, excess mucus, sloughed scales, frayed fins
- Histo: hyperplastic gill epithelium

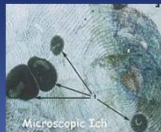
Ich



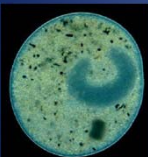
<http://www.koidream.com/ZiektenWstip4.jpg>



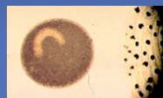
<http://www.cascadekoi.com/images/body/Koi/parasites/WhiteSpot%20Image.jpg>



<http://www.fish-helpline.co.uk/photos/Micro03.jpg>



http://www.reefs.org/library/aquarium_net/0197/images/mg0006.jpg



<http://fishweb.ifas.ufl.edu/AquaticAnimalHealth/AnimalHealthPix/Ichthyophthirius.jpg>

Trichodina

- Dx: wet mount- skin scrape or gill clip
- Circular, dome-shaped
- Concentric rows of cilia ± denticles
- Rotating motion
- Usually secondary: poor water quality, high stocking density, concurrent infection, gill damage
- Tx: salt bath, formalin

Trichodina



"Clinical Diagnostic Techniques in Fish" by Carol Poll, C.L.
Davis Lab Animal seminar in Chicago, April 2005



http://www.cnr.vt.edu/fisheries/afs/fisheries_techniques/Chapter14/Skin%20wet%20mount%20of%20catfish%20with%20trichodina.jpg



<http://home.infostations.net/elttiashen/Fish/trichodina3.jpg>

Useful References

- Fish Medicine by Stoskopf
- Fish Disease (Diagnosis and Treatment) by Edward Noga
- March 2002 Lab Animal journal (fish)
- ALN Magazine vol 7, No 2 March 2008- Aquatic Facilities

Viruses

- Infectious Pancreatic Necrosis Virus
 - Salmonids (trout & salmon)
 - Birnavirus- ds RNA
 - Subclinical in zebrafish (us. wild-type)
- Iridoviruses (goldfish)
- Rhabdoviruses (carp, cichlids)

Helpful links

- http://zfin.org/cgi-bin/webdriver?Mval=aa-ZDB_home.appg
- <http://www.fishbase.org/Doc/TheHealthDiseasesManual.php>
- Fish disease presentation:
 - http://web.sdsstate.edu/whdept/courses/WL423_523/DISEASE2.PPT
- <http://www.exploratorium.edu/>
- Goldfish & koi diseases:
 - <http://www.mv.edu/~buxton/puregold/disease/symptom/byna.htm>
- <http://www.aquaworldnet.com/awmap/diseases.htm>
- Great pictures:
 - http://www.dpi.nsw.gov.au/_data/assets/pdf_file/0005/209309/SilverfishDiseasesManual.pdf
- Video of necropsy:
 - <http://aquanec.org/real/necropsy/>