FINAL REPORT (AQUATIC ANIMAL HEALTH TRAINING SCHEME)

AWARD CODE and TITLE

2009/315.21 People Development Program: Aquatic Animal Health Training Scheme – Dr Jo Bannister

AWARD RECIPIENT: Dr Jo Bannister

ADDRESS: 29 Hadley Gardens, Kardinya, WA 6163

DATE: 24/06/2013

ACTIVITY UNDERTAKEN

Attendance at AQUAVET II: Roger Williams University, Bristol, Rhode Island in The United States of America between 26th May until 8th June 2013 (flew home to Perth WA Wednesday 12th June 2013). AQUAVET is a specialist and unique aquatic veterinary medicine program presented by the School of Veterinary Medicine University of Pennsylvania and the College of Veterinary Medicine Cornell University. AQUAVET II is a two week intensive training course in Comparative Pathology of Aquatic Animals.

OUTCOMES ACHIEVED TO DATE

AQUAVET II provides a detailed overview of aquatic pathology, pathogenesis of disease and histopathology and covers topics such as diseases/histopathology of marine and freshwater fish, tumour biology of aquatic animals, diseases of ornamental and laboratory aquatic animals and dissection practicals allowing further examination of aquatic animal anatomy and physiology. I learnt so much on this two week course. I do feel that I learnt more in two weeks on AQUAVET than I did during my five years of Vet School! The level and quality of the education that was provided was exceptional and I believe that I now understand certain pathological processes and concepts more clearly and in much better detail. I also feel more confident in composing morphological diagnoses and written descriptions. This is very important for my field of work as I am required to write concise pathology reports every day that detail the cause of death or disease. I feel much more confident with my report writing and am very excited to put this into practice at work. I felt that I was also able to teach and help my fellow colleagues when it came to identifying normal aquatic animal tissues. Many of my fellow AQUAVET II participants work solely in mammalian pathology, and had not been exposed to much aquatic animal histopathology prior to the course. One skill that I feel I needed further advice and assistance with was how to successfully identify and classify parasites. This is a very difficult area of expertise, however I now feel that I can successfully identify protozoan and metazoan parasites and if I am not certain what I am looking at down the microscope, I know how to research and discover what family of parasites I am viewing. I also dissected a species of elasmobranch (Skate). This was a great experience as I had never had the opportunity to explore the anatomy and physiology of these amazing creatures. I helped my dissection group successfully identify an organ that we were uncertain about and discovered that it was the nidamental (shell) gland. I particularly enjoyed the tumour histopathology slide session and lectures. I really enjoy the challenge that tumour cases present, and I was able to identify numerous different types of neoplasms in the slides that were provided for our viewing. Before I left to attend the course, I diagnosed a nephroblastoma tumour in the kidney of a Siamese fighting fish. During the tumour slide sessions, I viewed many other nephroblastoma cases and was very pleased to find numerous similarities between these cases and the one I recently diagnosed.

Acknowledgments

I would like to thank the FRDC for providing me with this amazing opportunity to attend this sensational learning experience. I am very grateful.

I would also like to thank and acknowledge The Department of Fisheries for allowing me to participate in this unique and specialised training course overseas and for supporting and encouraging me to attend.

I would also like to thank the following professionals who wrote me letters of support to accompany my FRDC grant application: Dr Brian Jones, Dr Fran Stephens, Dr Richmond Loh, Dr Mike Laurence, Dr Alistair Brown, Dr Weng Yan Nash, Dr Rick Fletcher, Dr Steeg Hoeksema and Rod Hughes.

Background

All AQUAVET courses bring together vets who believe that the profession is in a position to contribute to the wellbeing of aquatic ecosystems and inhabitants. AQUAVET is well-renowned and accepted within the veterinary profession and many of the previous participants have pursued long, meaningful careers in aquatic animal health.

Course information from the AQUAVET website: *'Members of the veterinary medical profession are increasingly expressing a desire to contribute to the welfare of the inhabitants of the aquatic world. Furthermore, excessive harvesting, ever more significant pollution problems, and disease have had devastating effects on many sea animal populations, once taken for granted. In a protein-hungry world, control and prevention of disease among aquatic animals, especially those cultured for human consumption, is crucial. The biomedical skills used so effectively by veterinarians to deal with disease and increase productivity among terrestrial animals can be applied to aquatic animals as well. But this will be possible only if schools of veterinary medicine develop programs to train students and stimulate research in aquatic animal medicine.*

One major effort toward accomplishing these goals is AQUAVET® sponsored by the School of Veterinary Medicine at the University of Pennsylvania and the College of Veterinary Medicine at Cornell University, and presented in collaboration with three marine science institutions at Woods Hole, Massachusetts: the Marine Biological Laboratory, the Northeast Fisheries Science Center of the National Marine Fisheries Service, and the Woods Hole Oceanographic Institution.

AQUAVET® began formally in the spring of 1977 as the result of efforts, conducted in the summer of 1976, to identify and bring together persons and institutions sharing the belief that the veterinary profession is in a position to contribute to the well-being of the aquatic environment and its inhabitants, and is poised to carve out an important niche in the burgeoning aquaculture industry, as well. During the formative months, it became clear that many shared this belief and that students in veterinary medicine were eager to learn more about aquatic animals than is offered in the traditional curricula.

The positive responses received in 1976 led to a successful application to the New York Sea Grant Institute for funding to launch AQUAVET® in the spring of 1977. The grant provided the necessary support for a four-week, intensive course, during which the potential of the field of aquatic veterinary medicine was explored with sixteen students from the veterinary schools at Cornell and Pennsylvania. Following the course, which was presented by more than fifty faculty members from eighteen institutions, eight of the students remained with the program for eight weeks of research at the laboratories of several cooperating institutions at Woods Hole and elsewhere in the country.

Drawing from the lessons learned during AQUAVET® '77, the program directors decided to increase the number of students in AQUAVET® '78, and more importantly, to extend the opportunity to participate to students at all schools and colleges of veterinary medicine in the country. In addition, a limited number of openings were made available to persons who had already received the veterinary degree. At that juncture, it was felt that this was the best way to achieve one of the major goals of the program -- to identify, stimulate, and encourage as many potential leaders of this emerging branch of veterinary medicine as possible.

In 1983, the first AQUAVET® II course was offered. Attended by six individuals, most of whom were alumni of AQUAVET® I, the course lasted four weeks and served to augment the introductory course by covering, in greater detail, subjects in mariculture, toxicology, nutrition, and diseases of cultured fish.

Over time, AQUAVET® has become well established and accepted within the profession, and many of its alumni are presently pursuing careers in aquatic animal medicine. Today, the goal of AQUAVET® is to provide for the orderly progression of students into positions of leadership, from which the real contributions of the profession can be made to society. The generation and application of new knowledge must be the ultimate mission.

AQUAVET® was held at the Marine Biological Laboratory (MBL), Woods Hole, Massachusetts from 1977 to 2009. A major renovation project of MBL's Loeb Building, the primary "home" for the AQUAVET® Program, led the Directors of AQUAVET® to identify new facilities for the presentation of the 2010 Program. In 2010 the AQUAVET® Program was held at the Stony Brook Southampton campus in Southampton, New York on the eastern end of Long Island. The Southampton campus was closed by Stony Brook University in 2010 and is not available. In 2011, the Directors found an excellent location to present the 2011 AQUAVET® Program. Roger Williams University, in Bristol Rhode Island will again be the hosts for the upcoming summer schedule.'

Attending AQUAVET II has allowed me to refine the many useful skills I learnt at vet school and transfer these over to working with aquatic animals. It has also provided me with the opportunity to foster networking connections, has given me an insight into the latest and greatest medical/diagnostic technology and given me a more detailed knowledge of aquatic animal diseases and husbandry requirements. This scholarship has accelerated my pathology learning and I feel that I am now better educated to impart knowledge to the entire aquatic animal health community and members of the public.

Need

There are very few aquatic health veterinarians Australia wide, in particular, there are only three veterinarians (excluding myself) that are seriously dedicated to aquatic animal health, welfare and medicine in Western Australia. There is a need for passionate veterinarians in this country to specialise in aquatic animal health to ensure that these animals receive first class health care and the full range of services
(diagnostic workup, husbandry recommendations, treatment options, surgical opinions and management advice) expected from a veterinarian in any field of practice. I am a unique veterinarian as I possess a strong and passionate interest in all aspects of aquatic animal health, medicine and pathology and am prepared to dedicate my career to these wonderful creatures to ensure they are healthy, respected and protected.

Objectives

1. To network and foster a strong relationships with the international network of fish veterinarians for future collaborations nationally and internationally.

My fellow AQUAVET II participants came from all corners of the globe. I met a fellow Australian veterinarian who had completed her residency in Anatomic Pathology at Cornell University and was working at a cancer research institute in New York City. She had been living and working in the USA for four years. I met two other participants from Europe (Spain and Norway) and was very interested to hear about the different and unique challenges their aquaculture industries faced. In particular I was fascinated to learn that Norway has numerous research projects looking at the use of biological cleaner fish such as Wrasse species to help control sea lice! I also met a New Zealander who currently works with an old colleague (and boss) Dr Brain Jones! It was a pleasure to meet this participant as we had a lot in common working with Dr Jones. I also met and made friends with the other local American participants that came from all over the United States. These American participants had many different interests ranging from being professional 'fish show judges' to working with zebra fish on research projects and with laboratory animals at human health institutes. I believe that I was able to provide an insight regarding Western Australia's Fish Kill Program and advise my fellow participants how we deal with fish kill incidents, teach staff how to collect samples and submit samples that are of excellent diagnostic value. I came to realise that WA has an excellent fish kill incident response program and was able to share knowledge of such incident management with my fellow classmates.

I made some fantastic new friends on AQUAVET II, many of which I will have for life! I would definitely like to meet up with my fellow AQUAVET II colleagues at other conferences in the future.

2. To expand the skill set and accelerate the learning of the principal investigator for the provision of aquatic animal health veterinary services to all types of aquatic animal producers and wildlife and aid in ensuring Australia's aquatic animals are healthy and protected.

During the two week intensive pathology training course I learnt how to better identify normal aquatic animal tissue and recognise pathological lesions and abnormalities. I already had approximately six months to one year training as a junior fish pathology intern, however the intensive learning style of this course, really helped to reinforce the existing knowledge I had and gave me more confidence to describe lesions or abnormalities that I could identify

and articulate this in report format. We examined so many different types of aquatic animals ranging from molluscs including cephalopods and bivalves, crustaceans, fin-fish, corals and prawns. I now feel confident and comfortable to examine such species histologically and provide succinct and accurate diagnoses and my opinion as to what I believe is the causative agent or reason for disease. Lectures and slide sessions occupied at least 12 hours of our days, which was fantastic for me, as I was able to consolidate my knowledge, practice slide interpretation, gain help when I needed it to identify lesions or parasites and clarify issues that I was not 100% certain about. I feel that my skill set has greatly expanded as I am better informed about the health issues these particular aquatic animal industries face, not just in Australia, but worldwide. In particular, I learnt about the many OIE listed diseases and now know how to access this resource and apply the information to cases and diagnostic testing we conduct here in Western Australia. I feel confident now that my colleague and mentor, Dr Fran Stephens could rely on me to examine a case and write a histology report for her to check before distribution.

3. To share information, skills, knowledge and impart advice to all members of the Australian aquatic animal health community.

As previously mentioned, I learnt about many different Aquatic animals ranging from Octopi, to Scallops to fin-fish, prawns or shrimp and crustaceans. I even learnt about how to recognise coral internal anatomy! Below in the 'Benefits and Adoption' section on this report, I comment on the many diseases of aquatic animals I learnt about and how these relate to our Australian aquaculture and production systems.

On return from my fabulous AQUAVET II experience, I feel much more confident in offering my professional opinion regarding aquatic diagnostic pathology cases and now know that there are alternative resources, references and opinions that I can seek outside of Australia. I also have new aquatic animal colleagues from all around the world, and will endeavour to maintain these relationships throughout my career. I also feel that some of my more senior AQUAVET II colleagues that were completing their American Anatomic Pathology residencies or had just completed their exams are friends that I could call upon for advice and assistance regarding cases. I feel in particular that three of these colleagues are mentors that I admire and respect! After completing AQUAVET II, I feel that I am a much more 'rounded' aquatic animal pathologist with better knowledge of the health issues facing different aquatic animal industries and I have refined my report writing skills and feel confident composing histopathological descriptions and morphologic diagnoses.

Methods

AQUAVET II ran for two weeks from arrival at Roger Williams University, Bristol Rhode Island on Sunday 26th May, checking out Saturday 8th June 2013. We had one day off for recreational activities (whale watching and visiting the local towns) on Sunday 2nd June. I spent three full days in New York city after the course from the afternoon of Saturday 8th June until I flew out of the USA on Wednesday 12th June 2013.

Each day consisted of a series of lectures and slide sessions that ran from 8am until approximately 8-9pm at night. Lecturers usually presented background information on case topics, aquaculture systems and diseases for approximately two hours and then gave us a series of slide sets to examine. During the slide session we identified parasites, specific

disease lesions, learnt to recognise normal tissues features of various different aquatic animals including octopi, bivalves (scallops, mussels and oysters), various species of finifish including Chanel catfish, Salmon, Trout, Bass, and research animals such as Zebra fish and Killifish and various different species of ornamental and other production fish, prawns, corals and elasmobranchs. The evening sessions usually involved case studies, where we were given a mystery slide and some case history, and then asked to comment on the histological lesions we could identify, attempt written descriptions and morphological diagnoses and provide some examples of diseases that might cause such lesions. I thoroughly enjoyed the evening sessions, as we were able to put into practice the skills and knowledge that we had learnt earlier in the day and were able to verbalise our descriptions and morphological diagnoses.

Below is an outline of the lecture material and slide sessions that were covered on each day. I have also included my typed lecture notes as appendix 4.

Sunday, 26. May 2013

14:00	Arrival and orientation at Roger Williams University
15:00	RWU Required Safety Lecture
16:00	Fish Haematology = *cancelled due to lecturer illness. This session was replaced by Dr 'Skip' Jack: The Chanel Catfish Industry.*

Monday, 27. May 2013

8:00	Comparative Anatomy of Shellfish - Introduction to Pathology of Molluscan Diseases = Dr. Roxanna Smolowitz – Roger Williams University
13:00	Invertebrate Pathology - Diseases of Bivalves = Dr. Roxanna Smolowitz
18:30	Normal Anatomy and Diseases of Cephalopods and Opistobranchs = Dr. Roxanna Smolowitz

Tuesday, 28. May 2013

8:00	Using Animals in Research = Dr. Amy Hancock-Ronemus – Marine Biological Laboratory
9:30	Introduction to Diseases of Aquaculture Species – Warmwater Catfish = Dr. Sherman Jack – Mississippi State University
13:00	Pathology of Catfish Diseases = Dr. Sherman Jack
18:30	Diagnostic Case Studies and Practicum - Aquacultured Species = Drs. Sherman Jack and Rod Getchell

Wednesday, 29. May 2013

8:00	Toxicologic Pathology of Fishes = Dr. Jeff Wolf - Experimental Pathology Laboratories, Inc
13:00	Toxicologic Pathology of Fishes (continued) = Dr. Jeff Wolf
13:00	Toxicologic Pathology of Fishes (continued) – slide session = Drs. Jeff Wolf and Rod Getchell

Thursday, 30. May 2013

8:00 Toxicologic Pathology of Fishes (continued) = Dr. Jeff Wolf
13:00 Toxicologic Pathology of Fishes (continued) = Dr. Jeff Wolf
18:30 Normal Anatomy of Echinoderms and Jellies = Dr. Roxanna Smolowitz

Friday, 31. May 2013

8:00 Normal Anatomy of Crustaceans and Limulus = Dr. Roxanna Smolowitz
13:00 Diseases of Crustaceans and Limulus = Dr. Roxanna Smolowitz
18:00 AQUAVET® Joint Class Picnic

Saturday, 1. June 2013

8:00 Parasites in Aquatic Animals = Dr. Sarah Poynton - John Hopkins Medical Institutions

Sunday, 2. June 2013

8:30 Leave campus for Whale Watch – Barnstable, MA
10:30 Check in for Whale Watch

Monday, 3. June 2013

8:00 Invertebrate Pathology – Dissection practical = Dr. Roxanna Smolowitz
13:00 Invertebrate Pathology – Dissection practical = Dr. Roxanna Smolowitz
18:30 Aquaculture Vaccine Reactions Histologically Speaking = Dr. Rod Getchell

Tuesday, 4. June 2013

8:00 Diagnostic Case Studies and Practicum - Aquacultured Species = Dr. Mark Fast, Dr. Sal Frasca – University of Connecticut and Dr. Rod Getchell
11:00 Diseases of Coldwater Aquaculture Species - Infectious and Non-Infectious = Drs. Mark Fast, Sal Frasca, and Rod Getchell
13:00 Diseases of Coldwater Aquaculture Species - Infectious and Non-Infectious (continued) = Drs. Mark Fast, Sal Frasca, and Rod Getchell
18:30 Diseases of Coldwater Aquaculture Species - Infectious and Non-Infectious (continued) = Drs. Mark Fast and Rod Getchell

Wednesday, 5. June 2013

8:00 Conundrums = Drs. Mark Fast, Rod Getchell and/or Paul Bowser
10:00 Fish as Lab Animals = Dr. Paul Bowser – Cornell University, AQUAVET® Associate Director Emeritus
13:00 Corals = Dr. Ilze Berzins – Aquatic Health Consultant
18:30 Corals (continued) = Dr. Ilze Berzins

Thursday, 6. June 2013

8:00 Neoplasia of Fish = Dr. Renate Reimschuessel – US FDA

13:00 Fish Diagnostics and Techniques – practical session = Dr. Rod Getchell
18:30 Emerging Viral Fish Diseases in the US – SVCV, SHSV, KHV, LMBV = Dr. Rod Getchell

Friday, 7. June 2013
8:00 Overview of the Principal Infectious Diseases Found in Farmed Penaeid Shrimp = Dr. Arun Dhar – Shrimp Disease Research and Dr. Robert Bullis – Florida Keys Community College
13:00 Overview of the Principal Infectious Diseases Found in Farmed Penaeid Shrimp = Drs. Arun Dhar and Robert Bullis
18:30 AQUAVET®II Clambake group dinner

Saturday, 8. June 2013
By 12:00 checked out and train back to New York City.

Results/Discussion

- **What did I learn? =**
  - So much! Its incredible how much information I absorbed, retained and have used already within one week back at work!
  - I learnt normal histological features of various different aquatic animals, learnt how to interpret disease and identify lesions, how to describe these lesions correctly, how to dissect an elasmobranch, what are the new emerging diseases to look out for on a world-wide scale, how to examine tissues of coral species, how to identify parasites and distinguish cestodes, nematodes, flukes and trematodes and about the American shrimp industry and how volatile this can be! My lectures notes (Appendix 4) are good representation of the information that I learnt.

- **What skills did I develop? =**
  - More concise histopathology reporting skills.
  - I feel more confident to make histological descriptions and attempt morphological diagnoses.
  - More confidence in examining histology slides and confidence in my interpretation of the lesions/abnormalities.
  - I can better identify neoplastic lesions and classify these using tumour terminology.

- **How has this activity developed helped your business or career development?**
  - The entire experience has given me the confidence to travel comfortably on my own, meet new friends and colleagues, tackle any difficulties I came across on my journey (which were actually few and far between luckily!), write a grant application (I had never done this prior to applying for the FRDC

Aquatic Animal Health Training Scheme), write a 'trip budget' and contemplate the finances involved in such an experience.

- This experience also gave me the opportunity to improve my general veterinary pathology knowledge and allowed me to understand certain concepts that I feel I did not grasp at vet school.
- I am very grateful for the new friendships and colleagues that I have as a result of this experience. I know that I will have some of these new friends for life and am already planning on catching up with these people in the near future!
- I was also able to witness the respect and pride the American vet students had for their peers and qualified veterinarians/professors. This was quite 'eye-opening' to me, as I feel along with many others in the veterinary profession here in Australia, that respect is lacking at educational institutions where many highly qualified and prestigious individuals are practicing veterinary medicine or teaching it to others. For example, I noticed that all vet students called the professor or vet lecturing us 'Dr' which is unheard of here in Australia!
- I also learnt how to access the American Veterinary Pathology Journal. This is a very well respected veterinary journal and I would like to subscribe to receive this.

Benefits and Adoption

Many different Australian Aquaculture industries will benefit from the education I received at AQUAVET II 2013. I not only learnt about the various diseases that plague each of these industries and how to recognise and describe them histologically, but I also learnt how these industries function, what management processes are undertaken including vaccination strategies, environmental factors that influence growth and the various costs of production. These Australian industries include:

- Certain fin-fish disease topics that we discussed during the two week course included: Largemouth bass virus, Viral Haemorrhagic Septicaemia Virus, Columnaris disease, various protozoal organisms, Rickettsia like organisms, ecto and endoparasites and diseases of Chanel catfish such as Enteric Septicaemia of Catfish and *Edwardisella ictaluri* The information regarding this particular bacterial pathogen (*E.ictaluri*) was very relevant to me, as last year in 2012, my diagnostic fish health laboratory diagnosed the presence of this bacterial agent in a species of ornamental fish imported from overseas. This was a very significant finding and learning about the implications of this disease in America helped me understand why we do not want this bacteria in Australia!

- We also learnt about various neoplastic conditions with retroviral aetiologies that can affect such fin-fish species. For example, we were very lucky to be lectured by Dr Paul Bowser. Dr Bowser is a world renown fish pathologist working at Cornell University, who has discovered the causative agent of walleye dermal sarcoma. Dr Bowser's laboratory group identified tumours in Salmon and found them to be of viral aetiology and studied how they regress on a seasonal basis.

- Different types of fin-fish industries (many of which are grown in WA and/or Australia wide) include:
    - Barramundi (Bass)
    - Tuna
    - Trout
    - Salmon:
        - We had lectures and slide sessions on the various bacterial pathogens found in Salmon including: *Aeromonas salmonicida, Yersinia Ruckeri,* filamentous bacteria causing bacterial gill disease, *Flavobacterium psychrophila* and *Renibacterium salmoninarum* (BKD). This lecture was of great significance to me, as whilst I was an undergraduate student studying veterinary science, I completed two weeks practical on salmon farms in Australia. I saw first-hand the gross lesions caused by some of these bacterial pathogens, and was very thrilled to see the microscopic histopathological lesions they cause on numerous slides at AQUAVET II.
    - Mulloway
    - And Kingfish.

- Mollusc industries including:
    - Pearl Oysters:
        - Learnt about Oyster general anatomy and physiology
        - Lifecycle events
        - Reproductive strategies:
            - This particular lecture series was also very relevant to me as I was recently asked to comment on the stage of spawning of some mussels. Prior to this initial experience, I had very little knowledge regarding the reproductive anatomy of molluscs and now feel more confident and certain that I could identify male Vs female specimens and provide some opinion regarding stage of spawning.
        - Certain diseases including:
            - Bacillary necrosis, Larval mycosis, Hinge ligament disease of juvenile bivalves, Oyster Velar Virus Disease, Oyster herpes virus, Dermo disease caused by *Perkinsus marinus* (which is exotic and notifiable in Australia), Amber disease, Bonamiasis (also exotic/notifiable in Australia), QPX disease, digenean trematodes, Coccidia, Rickettsia and the many different types of tumours affecting bivalves.
    - Octopus research:
        - Learnt about Octopus general anatomy and physiology:
            - Once again, this lecture and slide series was of great importance and relevance to me. We have a group of researchers who are working with a native octopus species and my fish health group have been in consultation with them to try and understand why production is not occurring to full capacity. I was able to learn that octopi have a 'cyclical turning

over' of gut epithelial cells. This information was very significant to me, as it allowed me to understand that some lesions we had identified in these particular larvae, may in fact be normal digestive gland epithelial turn over cells.

- Lifecycle events
- Reproductive strategies
- And diseases such as:
    - Ichthyobodo, Cestodes, Dicyemennea, bacterial pathogens such as *Vibrio sp*. and Aggregatta.

- There has been talk of a <u>new prawn</u> industry to be developed in WA:
    - We received one full day of lectures and slide sessions on paenid prawns (or shrimp as they are known in the USA!) and these lectures covered topics such as:
        - Viral diseases including: Gill Associated Virus (GAV), Whitespot diseases (WSSV) and Taura Syndrome. All of these viral diseases are exotic to Australia, but endemic in the USA. I also learnt that the Americans think very highly of Australia for not allowing ANY prawn material to enter the country! This has protected our native stocks and shows how effective our biosecurity policies and procedures really are.

- My Department also conducts disease investigation/testing for the Australian Quarantine and Inspection Services (AQIS) by examining tanks of imported <u>ornamental fish</u> (usually grown in South East Asia) when there are 25% mortalities in tanks over a given quarantine period. I learnt about numerous ornamental fish diseases including iridovirus, gold fish herpes virus (cyprinid herpes virus-2), mycobacteriosis, Koi herpes virus and parasites such as monogenean trematodes including Dactylogyrus and Gyrodactylus.

- I also dissected and studied the internal anatomy of an elasmobranch species (Skate). My work colleagues back in Australia have never dissected an elasmobranch before, so this was a very exciting experience for me! During the dissection, we collected tissues from this specimen and fixed them in formalin for further examination. A fellow AQUAVET II colleague processed the tissues at her laboratory and we identified that there were some abnormalities in the kidney of this animal. We identified tubular degeneration/atrophy with possible regeneration causing the hypercellularity which may be due to fibrosis and inflammation. We also identified many epicardial granulomas caused by the presence of parasites in a 'toad-fish' specimen we dissected.

Further Development

In regards to the AQUAVET training program itself, I have nothing but exceptional feedback to provide! The course was very well organised, executed and delivered. I was very impressed at the level and quality of lecture material and content passed on to the participants. The course has been run for over 35 years now and I truly believe this to be the

best aquatic animal medicine and pathology course offered around the world. I would highly recommend it to any fellow Australian fish veterinarians and encourage more people in this field of veterinary science to attend. I would be more than happy for any future AQUAVET participant to contact me to find out more about the course, travel options, what to take/what not to take and give my overall impression/experience to help them design their AQUAVET experience.

In regards to the professional award I was granted, I also have nothing but exceptional feedback to provide. I found the entire application process very straight forward and easy to follow and received prompt and useful advice from the program co-ordinator in regards to any difficulties I experienced with the application. I was extremely impressed at how quickly the whole application process was carried out and would definitely recommend fellow fish industry colleagues to considered seeking out and applying for this professional award.

References

- AQUAVET Website: http://www.vet.cornell.edu/aquavet/
- Various lecturers that presented information/slide sessions: see course schedule for lecturer information.

Intellectual property

The lecture notes I took during class and slide sessions contain many images that were provided to us by the associated lecturer or institution. We were asked to use these images with respect and not to re-distribute lecture notes to wider audiences.

Appendices

1. Course outline
2. Itinerary
3. Photos
4. Course notes I took during lectures/slide sessions
5. Certificate of continuing education (5a) and completion (5b).

AQUAVET®

A Program in Aquatic Veterinary Medicine
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2013 Schedule for AQUAVET®II

Locations;

Marine and Natural Sciences Building = MNS
Lecture rooms: MNS 200, MNS 210, MNS 212
Lab: MNS 103, MNS 106

Sunday, 26. May 2013

14:00 Orientation
MNS 200 Directors

15:00 RWU Required Safety Lecture
MNS 200 Caitlin Conley

16:00 Fish Hematology
MNS 212 Dr. Diane Brown – Harvard Medical School

18:30 Fish Hematology (con't)
MNS 212 Dr. Diane Brown

Monday, 27. May 2013

8:00 Comparative Anatomy of Shellfish – Introduction to Pathology of Molluscan Diseases
MNS 212 Dr. Roxanna Smolowitz – Roger Williams University

13:00 Invertebrate Pathology – Diseases of Bivalves
MNS 212 Dr. Roxanna Smolowitz

18:30 Normal Anatomy and Diseases of Cephalopods and Opistobranchs
MNS 212 Dr. Roxanna Smolowitz

Tuesday, 28. May 2013

8:00 Using Animals in Research
MNS 200 Dr. Amy Hancock-Ronemus – Marine Biological Laboratory

9:30 Introduction to Diseases of Aquaculture Species – Warmwater – Catfish
MNS 212 Dr. Sherman Jack – Mississippi State University

13:00 Pathology of Catfish Diseases
MNS 212 Dr. Sherman Jack

2013 Schedule for AQUAVET®II

18:30 Diagnostic Case Studies and Practicum – Aquacultured Species
MNS 212 Drs. Sherman Jack and Rod Getchell

Wednesday, 29. May 2013

8:00 Toxicologic Pathology of Fishes
MNS 212 Dr. Jeff Wolf – Experimental Pathology Laboratories, Inc

13:00 Toxicologic Pathology of Fishes (continued)
MNS 212 Dr. Jeff Wolf

13:00 Toxicologic Pathology of Fishes (continued) – Lab Open to View Slides
MNS 212 Drs. Jeff Wolf and Rod Getchell

Thursday, 30. May 2013

8:00 Toxicologic Pathology of Fishes (continued)
MNS 212 Dr. Jeff Wolf

13:00 Toxicologic Pathology of Fishes (continued)
MNS 212 Dr. Jeff Wolf

18:30 Normal Anatomy of Echinoderms and Jellies
MNS 212 Dr. Roxanna Smolowitz

Friday, 31. May 2013

8:00 Normal Anatomy of Crustaceans and Limulus
MNS 212 Dr. Roxanna Smolowitz

13:00 Diseases of Crustaceans and Limulus
MNS 212 Dr. Roxanna Smolowitz

18:00 **AQUAVET® Joint Class Picnic**
courtyard

Saturday, 1. June 2013

8:00 Parasites in Aquatic Animals
MNS 212 Dr. Sarah Poynton – John Hopkins Medical Institutions

Sunday, 2. June 2013 – OFF

8:30 leave campus for OPTIONAL Whale Watch – Barnstable, MA
10:30 check in for Whale Watch
11:30 OPTIONAL Whale Watch $35per person
Barnstable, MA

2013 Schedule for AQUAVET®II

Monday, 3. June 2013

8:00 **Invertebrate Pathology – WET LAB**
MNS 103 **(need dissection kit, lab coats)**
Dr. Roxanna Smolowitz

13:00 **Invertebrate Pathology – WET LAB**
MNS 103 **(need dissection kit, lab coats)**
Dr. Roxanna Smolowitz

18:30 Aquaculture Vaccine Reactions Histologically Speaking
MNS 212 Dr. Rod Getchell

Tuesday, 4. June 2013

8:00 Diagnostic Case Studies and Practicum – Aquacultured Species
MNS 212 Dr. Mark Fast
Dr. Sal Frasca – University of Connecticut
Dr. Rod Getchell

11:00 Diseases of Coldwater Aquaculture Species – Infectious and
MNS 212 Non-Infectious
Drs. Mark Fast, Sal Frasca, and Rod Getchell

13:00 Diseases of Coldwater Aquaculture Species – Infectious and
MNS 212 Non-Infectious (continued)
Drs. Mark Fast, Sal Frasca, and Rod Getchell

18:30 Diseases of Coldwater Aquaculture Species – Infectious and
MNS 212 Non-Infectious (continued)
Drs. Mark Fast and Rod Getchell

Wednesday, 5. June 2013

8:00 Conundrums
MNS 212 Drs. Mark Fast, Rod Getchell and/or Paul Bowser

10:00 Fish as Lab Animals
MNS 212 Dr. Paul Bowser – Cornell University
AQUAVET® Associate Director Emeritus

13:00 Corals
MNS 212 Dr. Ilze Berzins – Aquatic Health Consultant

18:30 Corals (continued)
MNS 212 Dr. Ilze Berzins

2013 Schedule for AQUAVET®II

Thursday, 6. June 2013

8:00 Neoplasia of Fish
MNS 212 Dr. Renate Reimschuessel – US FDA

13:00 **Fish Diagnostics and Techniques WET LAB**
MNS 103 **(need dissection kit, lab coats)**
Dr. Rod Getchell

18:30 Emerging Viral Fish Diseases in the US – SVCV, SHSV, KHV, LMBV
MNS 212 Dr. Rod Getchell

Friday, 7. June 2013

8:00 Overview of the Principal Infectious Diseases Found in Farmed Penaeid
MNS 212 Shrimp
Dr. Arun Dhar – Shrimp Disease Research
Dr. Robert Bullis – Florida Keys Community College

13:00 Overview of the Principal Infectious Diseases Found in Farmed Penaeid
MNS 212 Shrimp
Drs. Arun Dhar and Robert Bullis

18:30 **AQUAVET®II Clambake**

Saturday, 8. June 2013

By 12:00 check out – back to reality

APPENDIX 2

People Development Program: Aquatic Animal Health Training Scheme

Dr Jo Bannister

Attendance at AQUAVET II: Roger Williams University, Bristol, Rhode Island, USA 26th May-8th June 2013

ITINERARY

- Depart Perth, WA: 23/05/13 = 23.10pm for Sydney
- Leave Sydney: 24/05/13 = 10:00am for New York, JFK
- Arrive New York, JFK: 24/05/13 = 5:25pm
- Stay in New York, Manhattan two nights (24-26th May)
- On morning of 26/05/13 = catch Amtrax train to Providence and then bus/lift to Roger Williams University Campus: Course begins at 2pm 26/05/13
- Course goes for two weeks: 26/05/13-08/06/13
- On 08/06/13 = travel back to New York on bus and train
- Staying 08/06/13 – 12/06/13 in New York Manhattan (four nights)
- Depart New York: 12/06/13 = 6:55pm
- Arrive in Sydney: 14/06/13 = 07.40am
- Depart Sydney for Perth: 14/06/13 = 10.10am
- Arrive home in Perth: 14/06/13 = 1.20pm

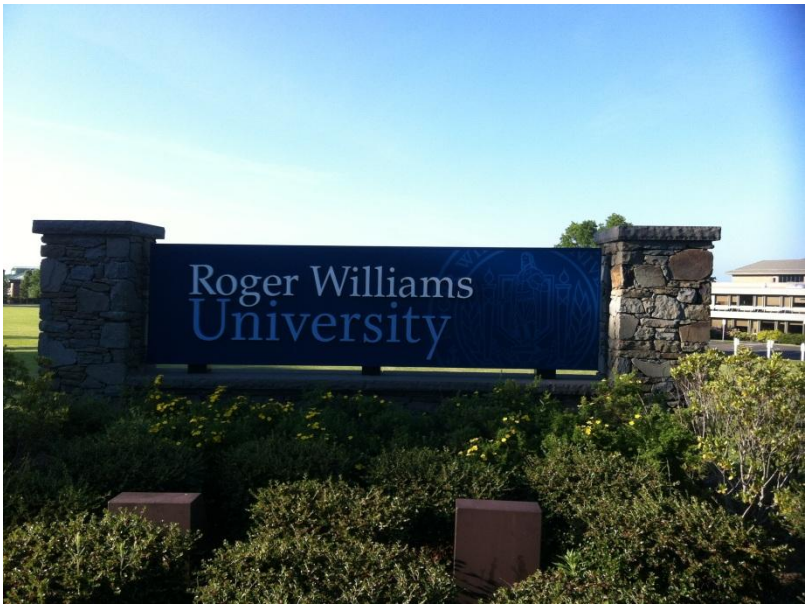
APPENDIX 3

People Development Program: Aquatic Animal Health Training Scheme

Dr Jo Bannister
Attendance at AQUAVET II: Roger Williams University, Bristol, Rhode Island, USA
26th May - 8th June 2013

PHOTOS

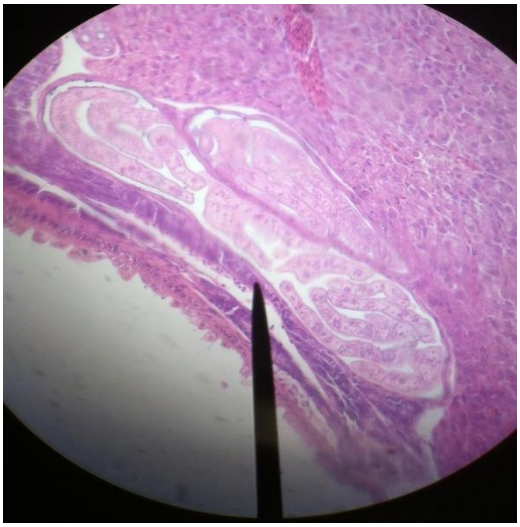
The Marine & Natural Sciences Building at Roger Williams University where lectures, slide sessions and dissection practicals were held. We spent the majority of our days here listening to and participating in lectures and looking at histology sections.

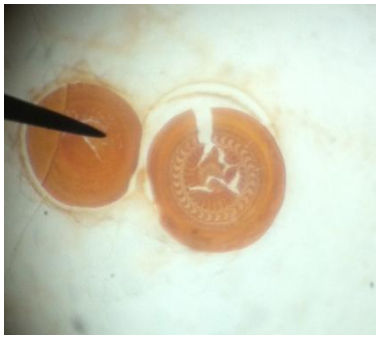


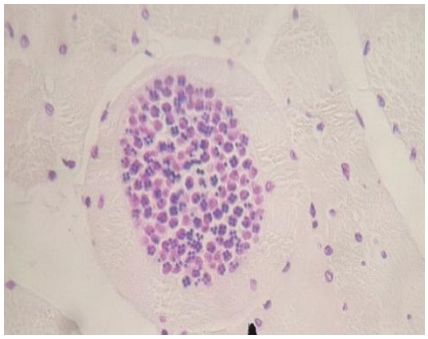
Roger Williams University; where the two week AQUAVET course was held.

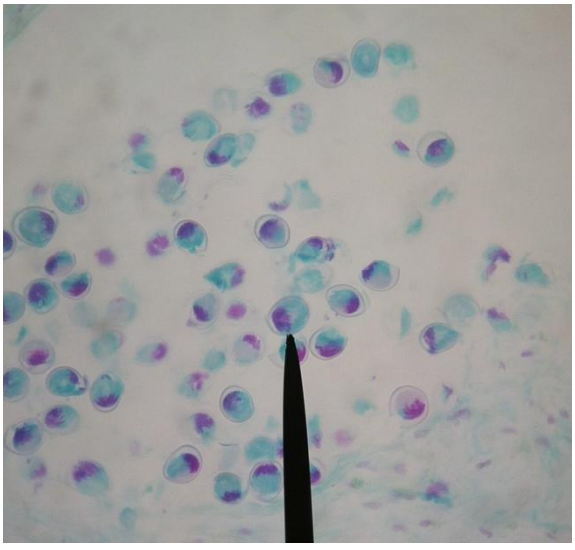
Our class room where we had lectures/slide session from 8am until approximately 8.30-9pm each day. Dr Smolowitz (Mollusc specialist) is pictured in the background giving us our morning lecture session. Dr Smolowitz also lectured us in Cephalopod anatomy and disease. I found this lecture and slide set particularly interesting as I was able to learn that the digestive gland in Cephalopods undergoes a cyclic change in the epithelial appearance as digestion occurs. This is significant to me as at work, we have an octopus client and have been studying their digestive gland anatomy and trying to work out what is normal for this creature.

Dr Jeff Wolf is a Veterinary Toxicologist from the Experimental Pathology Laboratories in VA and he lectured us for two days in the first week. I particularly liked Dr Wolf's lecturing style and believe that I learnt the most in the time he taught us. In a slide session with Dr Wolf I found a parasite (pictured to the right) that he had not identified in this cut section of Blue Gill liver before. Dr Wolf set me the challenge of identifying this parasite and after some research (and time!) I concluded that this parasite was a Myxozoan species. I found a reference by Azevedo *et al.* (2011) that described this particular parasite in detail and how it has a predilection to parasitise the gall bladder of teleost fish.

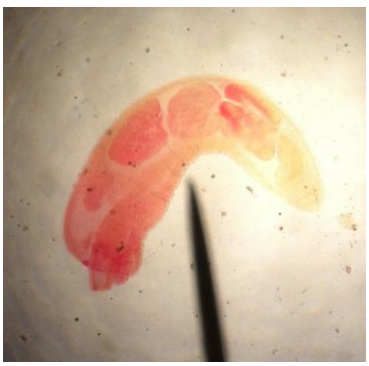


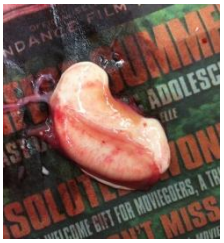




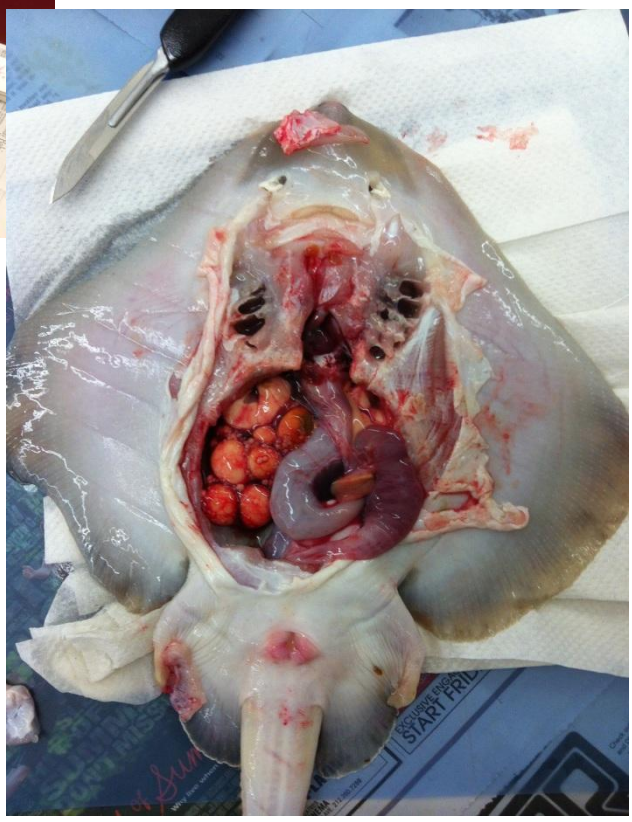


Another lecturer who I learnt so much from was Dr Sarah Poynton. I was absolutely thrilled to meet Dr Poynton as I use her book 'An Atlas of Metazoan Parasites in Animal Tissues' frequently at work. Sarah was inspirational and I thoroughly enjoyed the entire parasitology session with her. I learnt so much about protozoal organisms. Above left is a Trichodina parasite. This is my favourite aquatic animal parasite and I believe it to be very beautiful! Above in the middle are Myxosporidia (Kudoa) spores. Above on the right are a coccidian species stained with 'Feulgen' stain. This particular stain helps to identify chromosomal material and DNA and is very useful in the identification of parasites. We also learnt how to classify metazoan parasites and now I understand how to do this correctly. Below right is a Spiney headed worm known as *Acanthocephalus lucii*.

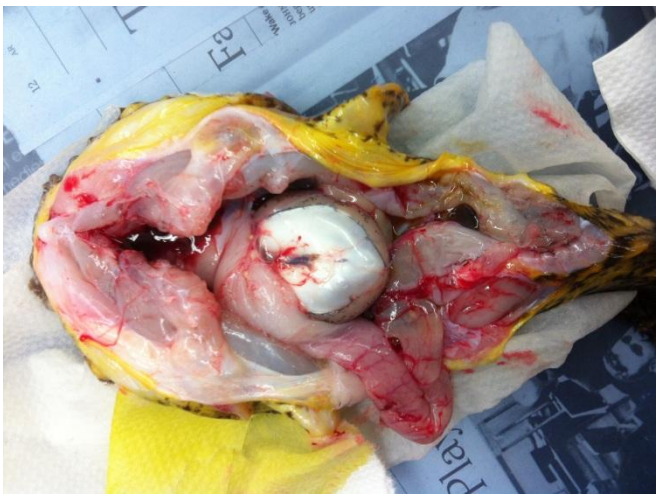




Shell/nidamental gland from the Skate below.

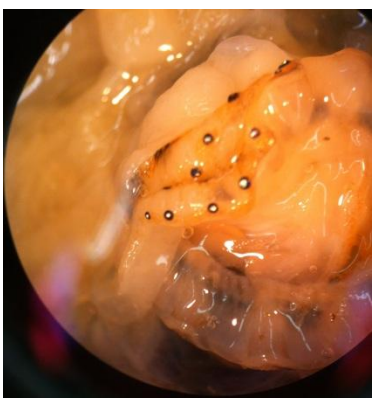


One of two dissection practical classes we had in the two weeks: we learnt how to dissect a Skate and recognise the internal anatomy. I had never dissected an elasmobranch before and was fascinated how this creature has such different and unique reproductive anatomy. My group and I were unable to identify one particular organ (pictured above right). After some research we agreed that this organ was the 'shell-gland'. The shell gland or as it is correctly known: nidamental gland, is a specialised organ that is part of the anterior oviduct of elasmobranchs where 1) fertilization of the ovum occurs and 2) the fertilized ovum is encapsulated in an egg case. Egg capsules range from thin and membranous (in the case of rays) to structurally complex and extremely durable (in the case of skates). In some species of batoid, the



A local Frog Mouth fish that we dissected on the same day.

My favourite bivalve! A Scallop! Scallops have numerous eyes located all around their mantle; you can see them here!



My AQUAVET II 2013 class mates!

My AQUAVET II t-shirt design (above) that won its place on the front of our class t-shirts (below)! I won and received a lovely book called '*Beautiful Swimmers: Watermen, Crabs and the Chesapeake Bay* (1976)'. This is a Pulitzer Prize winning non-fiction book by William W. Warner about the Chesapeake Bay, blue crabs and watermen.

Whale watching excursion; Cape Cod. We saw two whales on this trip. The whale in this photograph is a 'Fin-back'. This is the second largest mammal on the planet after the Blue whale. We also saw a Minke whale. This was such a fantastic and unforgettable experience.

Beautiful Bristol (the town where Roger Williams University is located). Bristol is located in Rhode Island. By AMTRAK TRAIN, it took approximately 3.5 hours to travel from New York City to this very picturesque coastal town.

Some of my new AQUAVET friends and I caught a bus out to 'Newport'. Newport is quite a large tourist town one hours bus ride away and is famous for its beautiful coast line and 'coastal walk' where you can view the huge and magnificent mansions!

Statue of Liberty

Out enjoying the sites of this fabulous city (New York) after the course had ended: 9th- 12th June 2013.

Manhattan from Staten Island Ferry

Hell's Kitchen Fire Department!

Central Park

Empire State Building

AQUAVET 2013
My notes

Sunday 26/05/13

Channel Catfish Industry

Dr Sherman Jack: 'Skip'

- *Ictalurus punctatus*
- Industry located in deep south of USA
- Most farms in 'delta' region of Mississippi = very flat region
- Catfish water:
    - pH = 6.5-8.5
    - Chloride > 100ppm = helps reduce stress in fish, can tolerate nitrites up to 10ppm
    - TAN = higher temp and pH affects
    - Alkalinity = >50ppm
    - DO = survive at > 5ppm, turn on aerators at < 5ppm
    - NO_2 = if you keep Cl high (10 times higher than NO_2), then reduce risk of brown blood disease
- CO_2 highest at 6pm = just before sunrise
- O_2 highest between noon and 6pm
- High CO_2 = low pH
- NH_3 = ammonia = toxic
- Charged species like NH_4+ don't cross lipid membranes
- pH highest middle of day
- low alkalinity = big swings in pH
- Catfish are long day breeders
    - Usually 15th April
    - Sexually dimorphic
    - Two openings:
        - Holes further apart in males
        - Males = penile protuberance.
    - Cavity spawners.
    - Spawn in cans
    - Only spawn when water gets above 70F
    - Emulate 'males tail' with plastic paddles
    - Hatch with yolk sac attached = 'sac-fry'
    - Feed ground salmon chow to 5 day old fry to get them used to eating
    - After week or so, move out into fry ponds; relatively shallow (10-15 inches deep), no thermocline
    - 100,000 stocking density per acre fry ponds
    - Vaccinated on way to fry ponds
    - Thin fry ponds out when growing to reduce stress
- Feeding = blown into water = feed floating feed = feed at 30% protein

- Most farms take 2-3 years to grow fish out
- Catfish are worth about 95cents per pound at the moment
- Market at 1-1.5 pounds = haven't reached sexually maturity, so less fat = main reason is that you want 5-8 inch fillet
- Fish autolyse (self digestion) quickly as fish have a body temperature that doesn't drop when they die = enzymes are still functioning
- Septicaemia = gram negative rods in blood

- PBL Case study:
    - Petechiation = *sepsis*
    - Enlarged spleen = *sepsis*
    - Haemorrhagic enteritis = *sepsis*
    - 'Hay-stacks- filamentous bacteria = flavobacteria (columnaris disease = stress related disease: secondary)
    - Enlarged spleen = sepsis
    - Sero-sanguinous peritoneal fluid = sepsis
    - = DIAGNOSIS = ENTERIC SEPTICAEMIA OF CATFISH = seasonal, maybe related to overstocking.
        - Edwardsiella
    - Aquaflor antibiotics will treat both diseases.

Monday 27/06/13

Invertebrate Anatomy, Physiology and Diseases

Dr Smolowitz

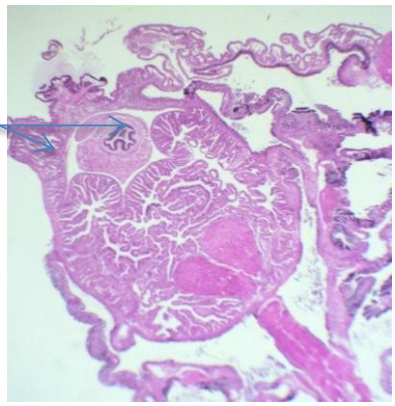
- *Phylum Molluscs*
    - *Class Bivalva*
    - *Class Cephalopoda*
    - *Class Opistobranchia*
- *Phylum Echinodermata*
- *Phylum Arhtropoda*
    - *Subphylum Chelicerata*
        - *Class Merostomata*
    - *Subphylum Hexapoda*
        - *Class Crustacea*

- **Bivalve (clam) anatomy and physiology**
    - 13,000 species (85% are marine
    - Most live in shallow shores
    - Suspension feeders = look for algae, where there is lots of sunlight hitting water
    - Laterally compressed
    - Two valves hinged at the dorsal midline
    - Adductor muscle results in valve closure = takes energy to close

- ‘Gapping’ = when valves don’t stay together
- Elastic dorsal hinge
- **Life cycle:**
  - Start of with eggs/sperm = external fertilization
  - Trochophore = last for short time = 12-14 hours = changes into Veliger (velum = two layers of cilia to collect food and swim through water column) = then forms calcified shell (very thin) = then develop into Pedoveliger (foot) = at this point under metamorphosis = then develop into adult.
  - After metamorphosis = development depends on type of species
- **General anatomy:**
  - IS = incurrent siphon = circulates through gills
  - ES = excurrent siphon
  - Mouth is anterior = usually at opposite end of siphon
  - Anterior and posterior adductor muscles
  - Hinge keeps shells together at one end = but doesn’t keep closed = need muscles for this
  - Pallial line
  - Outer surface of shells = rings = some are very distinct = can usually tell how old animal is = rings get bigger in spring/summer when start growing again
  - Ligament is dorsal
  - Siphon is posterior
  - Oyster = hinge usually dorsal = have right and left valve = but this depends on where the animal grew, as to how this develops
  - Loose pediveliger and anterior adductor muscle at metamorphosis = total rearrangement of inner organs of animal
  - For histo = just take cross section through to see organs
  - Scallop =
    - At metamorphosis = don’t loose foot, but it becomes very tiny = gill becomes very larger around whole outer edge of animal
  - Most bivalves have separate sexes, but there are a few that are hermaphroditic = eg: Bay scallop =
    - Usually male sperms before the female to avoid self fertilization
- **Water flow through bivalves:**
  - Collect particulate matter from water
  - Gills are more feeding apparatus
  - Water comes through incurrent siphon and comes into mantle cavity (this is external to the animal’s body)
  - Cillia on gills = lots of mucus on cilia
  - Water goes through gills to dorsal chamber, then directed back through excurrent siphon
  - Directed flow = more likely to catch feed = getting most organisms from the water this way
  - Pores = ostium

        - ***Photomicrograph of histological section***
        - Water flow in oyster:
            - Water just expelled on other side of septum
            - Still has directed water flow, just different
        - Water flow in scallop:
            - Most water directed because of way shell directed and where cilia beating
            - Scallops can't close down totally at edges of wing
    - **Palps:**
        - Found near mouth, extend far down over gills and help to move particulate matter and help to sort this and move it to the mouth
        - Oyster palps = bilateral = two on each side
        - Scallop = have sensory organs, not sure of function
        - Palps sense what food is
        - Bivalves pick what they want to eat, and discard (as pseudofaeces) other things that they decide are no appropriate food
    - **Feeding:**
        - Rate of digestion:
            - Altered depending on food eaten
            - Increased residence time for algae that is more digestible
            - More debris in water = less efficient at feeding themselves
        - Clearance rate:
            - Decreased at high food concentrations
            - Sediment concentration in the water column at high levels interfere with clearance rate
        - Bivalves are reason we used to have clean water = we no longer have the same number of feeding bivalves we used to have
        - Style and digestive gland = DG is basically liver, pancreas, gall bladder and small intestine = particulate matter is broken up in the stomach = waste is removed via the same duct
        - Head of style moves food up to stomach = which breaks down food = by chemical and enzymatic action = constantly being produced
        - Gastric shield = thicker chitin surface
        - Digestive gland = tubules, that move into blinded ends (where absorption occurs) = whatever is not absorbed moves back up tubules
        - INTERTIDAL = at least sometimes out of water
            - Often see changes in digestive gland epithelial height
            - If after tide in for a while = able to collect food = absorptive epithelium more columnar
            - As tide goes out = digesting things and getting rid of stuff and epithelium tends to be more cuboidal
        - Subtidal = never out of water = always below tide
        - Intestine loops down in the foot
        - Gonadal tissue mostly in foot
        - Intestines mainly for absorption, but nothing like digestive gland

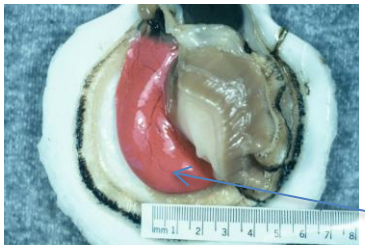
        - Most clams live of plankton they filter from water column:
            - But some bivalves often have zooanthellae (in giant clams) live in mantle cavity and produce energy/sugars that are used by tridacna (genus of large water clams) for food
    - **Mantle:**
        - External
        - Also produces shell = outer rings and inner surface of shell = produces rings
        - Epithelium lines mantle cavity
        - Three layers to shell:
            - Nacreous = biggest and most important part of the shell = layed continuously throughout life of the animal
            - Prismatic
            - And periostracum = chitinous/flakey layer = wears away quickly
        - Mantle is 3 lobed
        - A lot of muscle and connective tissue here. And lots of nerves/ganglia here too
        - Tries to maintain pH about ~8
        - Scallop:
            - Middle lobe contains 'eyes' and other sensory apparatus
            - Inner and outer lobes
        - Nacreous layer = pearls from here
            - Natural pearl formation = Some kind of bug/parasite gets between shell epithelium and causes the nacreous epithelium to start laying down more nacre, which forms pearl.
            - Freshwater pearls = tend to stay associated with inner surface = get flatter pearls
            - Oysters can disconnect pearls from epithelium = get rounder pearls
    - **Heart:**
        - Found posterior (towards siphon) to animal
        - In pericardial sac
        - Made up of two atria leading to one ventricle = has aortas that go anterior and posterior
        - Intestines run through middle of heart
        - Hypobranchial chamber
        - Colon runs through centre of heart
        - Haemolymph = open circulatory system = consisting of arteries, sinusoids and veins
            - Hydraulic system for quick movement
            - Sinusoids instead of capillaries



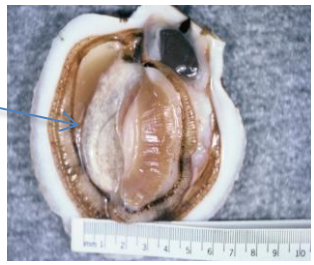
    - **Blood cells:**
        - Invertes don't have immune systems:
            - Cant vaccinate any invertebrate
        - Innate immune system

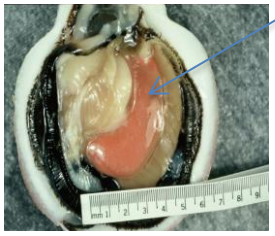
        - No memory = no lymphocytes
        - **Haemocytes:**
            - Two types:
                - Granulocyte (has lots of granules, kind of mix between macrophage and neut) = and these granules are imptx because they have digestive enzymes (like neutrophils)
                    - Tend to be larger = 10-12 μm
                - Hyalinocyte:
                    - Agranulocytes
                    - Small = 8-10 μm
                    - Nearly clear cytoplasm
        - **Humoral factors:**
            - Lectins = opsinins: diverse protein group with one or more CHO-recognition domains
            - On cells or free in haemolymph
            - Promotes phagocytosis
    - **Connective tissue:**
        - Vesicular cells
        - Fat cells filled with lipid and glycogen
    - **Renal anatomy:**
        - Pericardial gland:
        - Two part kidney
        - Don't know heaps about the kidney
    - **Neural system:**
        - Bivalves don't have brains = infact invertes don't have brains (apart from cephalopods)
        - Have series of paired ganglia
        - Bivalves: cerebral, visceral and pedal ganglia = three pairs of major ganglia connected by major nerves
        - Tough to anaesthetize/euth = but can paralyze them
    - **Gonads:**
        - In hard clams = in the foot
        - Towards dorsum of foot at base of gills
        - Most bivalves are broadcast spawners
        - Millions of fertilized eggs = but only a few hundred survive
        - Adult clams produce gametes throughout their life; no decrease in fecundity with age
        - Larger females produce greater numbers of ova
        - Individual females require several spawning events overall several weeks to release all ova
        - Spawning depends on temperature and quality of food = usually get one big spring spawn
        - Bivalves have different spawning patterns:
        - Oysters tend to spawn in spring; but if lots of food and good temp, can spawn again

- Usually male part of hermaphrodite spawns first, which triggers the rest of them = mass spawning
- Must be some soluble hormone in water to cause mass spawning events??
- Can cause spawning with temp changes:
    - If ripe (lots of eggs and sperm ready) = and change temp = spawn immediately
    - Can inject them with .. to cause them to spawn
    - Temperature shock = temp can go either way = usually up to shock them
    - Or strip spawn
    - Might take multiple weeks to get all spawning done
    - If in area with high temps/good food = might get second spawn
    - Takes very little stress to cause spawning to occur
- Predominately separate sexes
- Some are hermaphrodites
- A lot of bivalves change sex: go either way
- Clams after metamorphosis = are usually male
- Oyster undergo sex change ~ 2 years
- Spawning in hatchery =
    - Artificially manipulated by food, temp…
- Sea scallops = can tell sex as colour coded = females = orangey



- Male that has spawned
- Female that hasn't spawned
- Female that has spawned, but not completely finished
- you can do squash preps to see sperm/eggs





- **Foot:**
    - Foot produces bissel = protein threads
    - Glands produce protein
    - Not sure why scallop has bissel = because they don't use it
    - Mussel uses bissel to attach to a rock:
        - If in an area with lots of crabs = produces thicker bissel
- **Salinity effects of hard clams:**
    - <15ppt produce a negative effect on:
        - feeding rate
        - burrowing
        - growth
        - long term survival of juveniles and adults
    - For clams: sal > 32ppt = detrimental to eggs and larvae
        - Larvae grow best at 26-26ppt
- **Ectothermic:** so all metabolic processes depend on enviro they live:
    - Summer = met going crazy fast
    - Winter = slow down
    - This puts whole different influence on disease!
- **Sediment characteristics:**
    - Lots of sediment = makes it hard for animals to feed properly

- **O_2 consumption:**
  - Depends on temp
  - <5ml/L = suffer from lack of oxygen

- **NORMAL BIVALVE SLIDES:**
  - Green/grey granules in enterocytes = residual material in epithelium = normal = glycogen/lipid vacuoles = very common in scallops
  - Scallop has both male and female gonads

- **DISEASED SLIDES:**
  - Haemocytes in intestinal epithelium = too many = could indicate 'dermo' disease

- **Diseases of bivalves**
  - Bivalve aquaculture = form of agriculture
  - Broodstock collected = identified as male/female = unless hermaphroditic
  - Broodstock is conditioned: food/water temp
  - Conditioned animals are spawned by a cold shock
  - Fertilized eggs hatch in 10 hours
  - Larve are raised in static water and fed algae till metamorphosis
  - 'D' stage = 'umbo' stage = veliger = late umbo veliger = pediveliger stage
  - At metamorphosis = the larvae become juvenilles
  - Before metamorphosis = all bivalves pretty much look the same
  - Post-set juvenilles are grown in upwellers
  - At approx. 12mm in diameter = they are moved/sold to grow out area/lease
  - Maine to Florida = family farms and all along California
  - OIE standards = 150 animals
  - Here in USA = no certified standard methods = for juvenilles = 60. Test many more for larvae
  - Composite testing = same as pooled
  - Disease = outcome of infectious agent = however, their metabolism depends on temperature and salinity = greatly affects disease occurrence = also affects metabolism of agent
  - Sources of pathogenicity in a culture facility:
    - Broodstock
    - Algal stocks
    - Seawater source
    - Food source
    - Seawater system
    - Larval cultures
    - Juvenile cultures
    - Vectors: gear, fomites and other animals
    - Air sources: filters, airborne bacteria…
  - 'Setting' or 'Set' = same thing as metamorphosis

- **Diseases of larval and juvenile species:**
  - Bacillary necrosis (Larval and Juvenille Vibriosis)
    - Causes = various species of Vibrio
    - Vibrio likes warmer water temps
    - Gross appearance = sudden necrosis of body tissues
    - Initial location of infection varies = mantle, velum and digestive tract = bacteria attach to all these places
    - Can do squash prep of animals, can culture (light alcohol wash, then crush animals and plate on Vibrio specific plate)
    - Histo = see abundant bacteria destroying tissues
    - Very common
    - Tell hatchery = check for sources of contamination/entry
    - Management = clean/sterilize

  - Larval Mycosis
    - Fungal infection of larvae
    - Certain species of fungus = proliferate in larvae = usually happens over 2-3 days = kills larvae = relatively quick demise of larval animals
    - Cause = *Sirolpidium sp.*
    - Can do squash prep and see larval hyphae
    - Can also stain animal with 'Neutral red dye' = and can see red stained mycelia
    - Management = reduce density, clean/sterilize and provide quality food

  - Hinge ligament disease of juvenile bivalves
    - Host = all bivalves, especially juvenilles <1cm
    - Cause = cytophaga-like gliding bacteria
    - Gross appearance = rupture of the ligament
    - Animal is still alive and contracts muscle to close shells
    - Epidem = opportunistic
    - Often see in response to poor quality handling = too many in trays, didn't clean try and general poor care

  - Isonema-like flagellate infections
    - Common in freshwater and marine habitats = associated with sediments and marine plants
    - Usually in winter months
    - Invasive protists

- **Diseases of larval and juvenile species:**
  - Oyster Velar Virus Disease (OVVD, Blister disease)
    - Iridovirus
    - Occurs in larval pacific oysters = usually in 'D' stage
    - Affects epithelial cells of larvae

        - Basophilic viral inclusions
        - Variable mortality, but can be severe

    - Oyster herpes virus
        - Usually seen in juvenilles
        - Hypertrophic nuclei in haemocytes, connective tissue and epithelium
        - Intranuclear inclusions

    - Juvenille Oyster Disease (JOD)
        - Alpha-proteobacteria = *Roseovarius crassostrae*
        - Gross appearance = mantle retraction, deep cupping of the left valve, conchiolon deposition on the inner surfaces of valves and reduced condition
        - Get this disease when temp hits 21-22^{o}C, when salinities are 25-32ppt, larger animals (25-40mm) show signs of infection but do not die
        - High mortalities (100%) four to six weeks after first signs
        - Pathogenesis: progressive necrosis/ulceration of shell epithelium with focally severe inflammatory reaction
        - Dx = grossly, histo and PCR
        - Management =
            - Spawn broodstock early to allow for seed >5mm in erly summer (before it hits 21 ^{o}C)
            - Decrease density
            - Increase water flow rates
            - Develop resistant animals = breed survivors and F1 generation were able to be resistant
        - More of an opportunistic organism

    - Overwintering disease of seed clams
        - Gross signs = empty shells
        - Cold winter, poor spring food quality or quantity

- **Diseases of Adult bivalves:**
    - Dermo disease
        - Host = Crassostrea virginica (Eastern Oyster)
        - Cause = *Perkinsus marinus*
        - Dermo = Dinoflagellate/protozoan
        - Gross appearance = watery, poor condition
        - Forms in tissues = Signet rings with vacuole containing a refringent body (mature meronts) = matures into a schizont/sporangium
        - Form in seawater = zoosporangia containing zoospores
        - Tissue infected: digestive tract = causing ulceration of the gastric and intestinal epithelium
        - Haemocytes engulf but cannot destroy
        - Directly infective disease

- But can be transferred via snails
- Agent proliferates >18^{o}C
- Dermo can tolerate low salinities
- More of estuarine organism
- Dx: Ray/Mackin Fluid Thioglycollate media (RFTM) assay:
    - Culture tissue in media for 4-7 days
    - Stain with Lugol's iodine = stains hypnocell walls
    - Examine under microscope for black spheres
    - Rank Mackin Index 0-5
    - Histo not best method of identification
    - PCR/qPCR
    - Can draw haemolymph and do PCR
- Management:
    - Spawn and raise seed at low salinities till late in first year

- Perkinsus sp. Infection in clams
    - Gross appearance = whitish nodules or cysts on surface of gill, mantle and body
    - Forms little granulomas

- SSO (Seaside organism)
    - Haplosporidium costale
    - Protozoan
    - Believe it has indirect lifecycle
    - Invades into digestive system
    - Dx: insitu staining, PCR and histo

- Amber disease (Digestive gland disease)
    - Cause:
        - *Martella refringens*
        - Protozoan
        - Import risk into the USA

- Bonamiasis (Microcell)
    - *Bonamia ostrea*
    - Protozoan
    - Gross appearance:
        - Gaper
        - Poor condition
        - Non-specific gill leisons
    - Initial lesions: haemocytes
    - Potential haemocytic anaemia and granulomas plus tissue necrosis
    - Directly infective
    - Appears that as animals get older, may develop resistance

- Denman Island Disease
  - Microcystos mackini
  - Protozoan
  - Directly infective disease
  - Pathogenesis = connective tissue necrosis, intense inflammation resulting in pustules and granulomas

- QPX (Quahog Parasite Unknown) ~ Dr Smolowitz has done a lot of work on this disease
  - Host = hard clams
  - Can cause up to 90% mortality in some aquaculture sites
  - Found in Cape Cod in 1995
  - Causes swellings and nodules, irregular swelling in the mantle edges, inability to close the shells to form the mantle cavity
  - Protozoan
  - Forms thalli, sporangia, endospores in tissues and in tissue culture
  - Organism produces very thick mucus
  - Opportunistic
  - Diff strains produce diff amounts of mucus
  - Haemocytes try hard to phagocytose organisms
  - Organism grows best at 24^{o}C
  - Infection most often occurs at the base of the siphon and in the adjacent mantle
  - Disease is worst in spring and fall

- Nematopsis (Gregarian parasitism)
  - Phagocytosis of gymnospores by haemocytes through body wall
  - In connective tissue of body wall

- Digene trematodes
  - Bivalves are great intermediate hosts
  - Primarily *Bucephalus sp*, but others also
  - Affect gonadal tubules causing parasitic castration
  - Can do squash prep as well
  - Mollusk is intermediate host

- Xenomas
  - Ciliates: Thigmotrix
  - Form large tumours in the water tubules = infect cytoplasm of epithelial cells of the water tubule causing hyperplasia and sloughing to the tubule

- Ovocystis
  - Pappillomavirus-like papovirus
  - No gross appearance
  - Epidem = incidental finding

        - Pathogen = causes massive hypertrophy of individual gametes and gametogenic epithelium

- Coccidia
    - Coccidia (Klossia and Pseudoklossia)
    - No gross appearance
    - Pathogen = meronts, gamont in epithelial cells of the kidney = cause kidney damage, but no apparent to low levels of mortality
    - Usually incidental finding

- Rickettsia/Chlamydia
    - Affect: digestive gland, siphon, mantle and gill

- Tumours of bivalves
    - Gonadal tumours:
        - Many bivalves
        - Dysgerminoma
    - Haemocytic sarcoma (Leukemia):
        - Been around for quite a long time
        - Occurs in all types of bivalves
        - High mitotic index, abnormal nuclei
    - Pollution can be related to neoplasia

- Hyperplastic conditions
    - See often in animals in highly polluted areas
    - See in gill and kidney

- Effects of harmful algal blooms (HABS)
    - Red (dinoflagellates) and brown (algae) tides
    - *Prorocentru minimum*
    - Toxic affects, physical affects and starvation
    - Can get digestive adenitis = bacteria build up

- Concretions
    - Common in animals in higher organic load areas
    - Not considered significant
    - Most calcium phosphorus

- Fouling organisms
    - Trichodina = don't seem to cause a problem in sea scallops
    - Mud blisters = Polychaetes (*Polydora sp.)* = like to embed through shell and develop tunnels = bivalves responds by producing more nacre = can't sell this product to raw bars
    - *Cliona sp.* = burrowing sponge = creates hole that go down through the shell = cause shell to become very fragile

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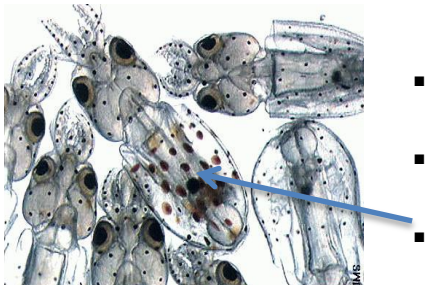
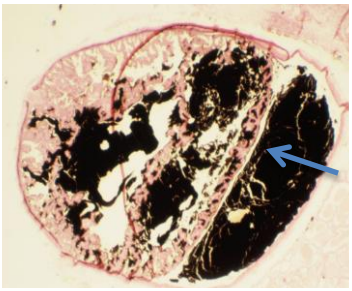
Monday 27/06/13

Cephalopod molluscs

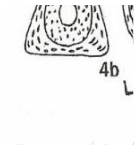
Dr Smolowitz

- Two major groups:
  - *A.colleoidea* =
    - Contain an inner shell or no shell
    - Cuttlebone/Pen
    - Eg:
      - Squid
      - Octopus
      - Cuttlefish
  - *B.ectococoleate* =
    - Calcified chambered shells
    - Eg:
      - Nautilus = only 6 species remain
- Normal anatomy:
  - Have flexible arms with suckers or hooks
  - Octopi have 8 arms
  - Sepia and squid = 8 arms and 2 tentacles
  - Have specialized arms/tentacles = Hectcocotyli = used to put sperm packet into female = still external fertilization but occurs inside cavity of female
  - Exclusively marine
  - Enormous differences in size
  - Extremely poor osmoregulatory ability
  - Gills are for oxygenation/CO_2 removal
  - Digestive system =
    - Carnivorous =
      - Nautilius = scavengers
      - Rest are macrophagic predators
  - Buccal mass =
    - Horny beak
    - Radula
  - Paired salivary glands
  - SEPIA GI TRACT = Oesophagus, crop (depending on species), stomach (lined by chitin) and caecum (has spiral leaflets to increase surface area for absorption) right beside each other (exchange between these two organs), digestive gland (more like the liver), pancreas (separate organ that adds enzymes) and rectum
  - SQUID = have cartilaginous pin in dorsal cavity
  - CUTTLEFISH = have cuttlebone = very highly calcified = lots of mineral matrix
  - OCTOPUS = have a crop off oesophagus, mantle cavity is still ventrally and gills suspended here

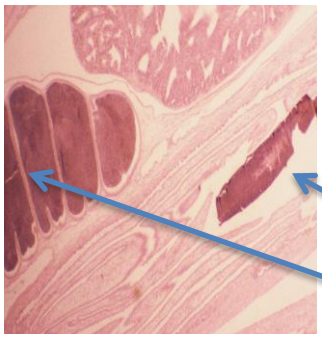




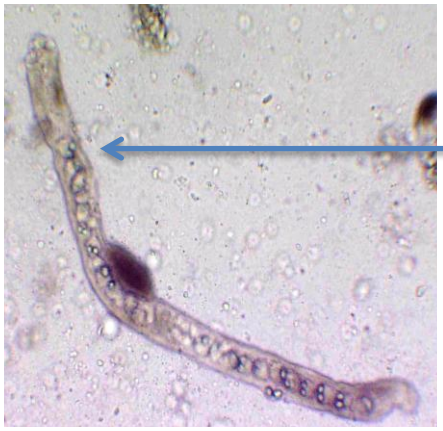
- Skin =
  - Simple columnar epithelium = very thin = easy to ulcerate = so thin because so much going on in the dermis = need thin skin so you can see whats going on underneath = this is how they communicate with each other
  - Dermal layer = fibroblasts, collagenous fibres and ground substance
  - Chromatophores (system of cells: red, yellow, brown, black colour spots), iridophores (brown dots)
  - Hatched squid iridophores = plates reflecting light (blue/green colours) = innervated and co-ordinated by neural system
  - Each chromatophore system has many muscles supplying it
  - Leucophores = produce white patch underneath epithelium
  - Reflector cells = only seen in octopi = little stacked discs in extensions = each cell is innervated separated
- Ink gland = overlays rectum and empties beside it = this photo shows ink gland and storage of ink
- Muscle =
  - Little to no skeleton
  - Muscular hydrostat = twisted muscle = spiraled = so when it lengths, un-twirls (this is how the arms can go so far out and zap back) = obliquely striated
- Jet propulsion =
  - Cavity enclosed by mantle fills with water
  - Water is forced out through siphon = animal moves
- Vascular system =
  - High pressure circulatory system
  - Almost a closed system
  - Haemolymph volume = ~6% of body weight
  - Some peripheral sinuses = networks
  - Haemtopoetic organs = white body = right behind the eye = multilobed glands within blood sinuses behind the eyes
  - Produced haemocytes = one granulated type
  - Have two kinds of hearts =
    - Systemic heart = has multiple aortas coming in
    - Brachial heart = pumps blood through gills = brachial heart appendage (like an atria) = also see stored ferritin and haemocyanin here = use copper pigment to move oxygen around
- Reproduction =
  - Separate sexes
  - Female =
    - Ovary
    - Oviductal gland
    - Nidamental gland = long white structures which underlay heart and ink sac =



- Accessory nidamental gland = orangey yellow glands = carry vibrio species that add fluorescence
- Seminal receptacle
- Atritic egg
- Can have multiple males fertilizing females eggs



- Male =
  - Testes
  - Spermatophoric glands = where sperm matures and packages sperm into spermatophores
  - Copulatory organ = hectocotylus
  - Sperm packet = going out as white needles
  - Efferent vessel = with immature sperm
- Nervous system =
  - Brain = series of ganglia
  - Brain enclosed in a hyaline cartilaginous cranium
  - Eyes can be composed of multiple lobes
  - Large nerve chords in tentacles
  - Nervous system is largest and most complex
  - In mantle = in squid = stellate ganglia = lower motor centres for movement of mantle muscle = this is from where all nerve work has been done
- Sensory organs =
  - Do not believe they see colours
  - They see contrast
  - Have rods and cones
- Diseases of cephalopods =
  - Senescence = short life spans
    - Usually die at end of reproductive season = most only make it through one season
  - Ichthyobodo =
    - Common in octopi
    - Comma shaped protozoa organisms
    - Due to poor water quality = opportunistic
    - Can considerable affect respiration
  - Cestodes =
    - Pleurocercoids present in the caecum
    - Incidental but can be numerous
  - Final form of tape work found in colon of tape fish
  - Don't believe they cause too much damage



- Dicyemennea =
  - Live in renal sac
  - Start to destroy renal epithelium = cause secondary infections

- Bacteremia =
  - Usually water quality issue
  - Most commonly *Vibrio alginolyticus*
  - Bacterial thrombi and associated tissue necrosis
  - Inflammation in several organs
  - Can culture from the brachial appendage
- Aggregatta =
  - Coccidian infection
  - Usually in octopi
  - White nodules in submucosa
  - Interfere with digestive function
  - Two host life cycle = merogony occurs in another animal = crabs
- Traumatic lesions =
  - Apex of the mantle suffers most damage
  - Fighting = ripe for secondary bacterial infections
  - Sucker lesions = can use dilute antiseptics and abx
  - Can also get exfoliative dermatitis

Tuesday 28/05/13

Lab animal medicine

Dr Hancock-Ronemus ~ MBL (Marine Laboratory)

Tuesday 28/05/13

Histopathology of Catfish

Dr Jack: jack@cvm.msstate.edu

- Pathos = suffering
- -ology = study of
- lesions = evidence or manifestation of disease
- disease dx = name of disease = eg; Rabies
- etiologic diagnosis = diagnosed based upon causative agent = eg; bacterial meningitis or viral encephalitis
- morphological diagnosis = organ, process, modifiers = eg; suppurative meningitis

- eg:
  - *Organ = Brain = encephalo*
  - *Process =*
    - *Inflammation = itis*
    - *or –osis (disease of)*
    - *or –opathy (non descript damage to)*
    - *or –oma (benign)*
    - *or –carcinoma (epithelial)*
    - *or glandular epithelial (adenocarcinoma)*
    - *or sarcoma (malignant mesenchymal: connective tissue: bone, cartilage, fibrous connective tissue, blood) NOTE: lymphoma = lymosarcoma (no such things as benign lymphoma)*
  - *Modifiers =*
    - *Character (age: peracute, acute, subacute, severe)*
    - *Severity (mild, mod, severe)*
    - *Distribution (focal, focally extensive, multifocal, multifocally coalescing, diffuse and disseminated).*
- Insult = neuts (acute = PMNs), lymphocytes (sub-acute inflame), macrophages (clean up guys), fibroblasts (scarring = chronic inflammation)
- PMNs = heterophils in fish are generally neuts
- Suppurative = neuts and necrosis
- Perivascular cuffing = viral encephalitis = lymphocytes around blood vessels
- Brain = non-suppurative: Rabies = mutilfocal, subacute, non-suppurative (lymphoplasmacytic) encephalitis
- Causes of disease:
  - D = egeneration
  - A = nomaly
  - M= etabolic
  - N = eoplastic
  - I = nfectious
  - T = raumatic
- Necrosis: coagulative, suppurative,

- BACTERIAL DISEASES OF CHANNEL CATFISH
  - ESC
  - Columnaris disease
  - Motile Aeromonad Septicaemia
  - Emphysematous Putrifactive Disease

- **ESC (Enteric Septicaemia of Catfish)**
  - *Edwardsiella ictaluri*
  - Sepsis = gram negative bacteria/toxins in blood
  - Gram negative rod
  - In chronic form, can produce hole in head = bacteria might gain access to CNS through choroid plexus
  - Bacterial has thermal window it likes: 22-28^{o}C
  - Clinical Signs:
    - Erratic swimming: cork screw
    - Circling
    - Lethargy
    - Anorexia
    - Death
    - Ascites
    - Cutaneous petechiation
  - Culture: brain and posterior kidney (drainage goes to posterior kidney)
  - Lesions:
    - All features of gram negative sepsis
    - Big liver, spleen, kidney (gets drainage back from caudal GIT and is where blood cells produced) and haemorrhagic (serosanguinous) peritoneal fluid
    - Necrosis = loss of cellular detail
  - Morph dx: Severe, multifocal, necrohaemorrhagic hepatitis
  - Eitological dx = bacterial hepatitis
  - Disease name = Edwardisella ictaluri
  - Bacteria are intracellular
  - Offer farmer Aquaflor on spot with these clinical signs
  - Pathogenesis:
    - Overcrowding
    - Faecal/oral
    - Spring time/disease
    - Why is fall peak higher than spring peak?? = these are the young fish that get affected as they hatch in April
  - Not a rule out if not in season
  - Treatment and control:
    - Withdraw feed
    - Medicated feed =
      - Romet (potentiated sulphonamide) =
        - was historical drug of choice, but had a lot of feed refusal, tastes bad.
        - Was a 3 day withdrawl in catfish and 21 day in trout = this is due to skin issue; this drug is cleared through skin
        - Available over counter

        - Terramycin =
            - Available over counter
        - AquaFlor =
            - (Relatively new on market 6-7 years
            - Florfenicol)
            - Expensive
            - Feed for 10 days withdraw for 15 days
            - Also combats Columnaris
    - Reduce stocking density =
        - This depends on management style
        - Around 6000/acre
        - Varies depends on pond depth aswell
    - Vaccination?

- **Columnaris disease**
    - *Flavobacterium columnaris*
    - Aka: Flexibacter, Cytophagia
    - Synonyms = Saddleback
    - Gram negative flexing or gliding rod
    - Yellow pigmented (Latin: Flavo = yellow)
    - Bacteria present in the water
    - Worldwide
    - Preferential water temp = 15-25^{o}C
    - Brought on by stressful events, handling, seining, poor water quality, high organic loads
    - Clinical signs =
        - Lethargy
        - Dyspnea
        - Mortality
        - Cutaneous erosion to ulceration
        - Depigmentation
        - Loss of sheen
        - 'Saddle back'
        - 'Cigar smoker' = lesions on lips: necro-ulcerative stomatitis
    - Lesions =
        - Necrosis everywhere
        - Missing epithelium on surface
        - Matts of blue/grey filamentous bacteria on surface
        - Necrotizing branchopathy with intralesional bacteria
    - Dx =
        - Wet mounts = see bacteria flexing and 'haystacks'
        - Can isolate on Ordal's media (low nutrient, soft)
    - Tx=
        - Superficial disinfection = for mild aquarium type situations
        - Systemic antibiotics:
            - Terramycin
            - AquaFlor

- Vaccination? =
  - Efficacy is questionable

- Slide Session =
  - Liver does have central veins
  - Hard to see bile cannuliculi
  - Erythrocyte size = 7-10 microns (in all species)
  - Count tissues on slide = and report on that number of slides
  - 250 microns in diameter = ich
  - size, shape, colour, consistency (bone, fat, soft, flat, cartilaginous)
  - MMCs = wear and tear pigments
  - Hepatocyte 1-2 times size of red blood cell = 15-20 microns
  - Spleen = look for fibromuscular stroma, a lot of smooth muscle in spleen and lymphocytes
  - Stable cells = hepatocytes = life span = months-year
  - Gill epithelium turns over labile cells = cells than turn over all the time
  - Pillar cells = support cells
  - Inter-lamellar trough
  - Congestion = more blood cells within vessels
  - Haemorrhage = red blood cells outside the vessel
  - PCR is detection of agent, not disease

- **Motile Aeromonad Septicaemia (MAS)**
  - *Aeromonas hydrophila, A. sobria, A.veronii*
  - Gram negative rods
  - Affect many poikilotherms
  - Common secondary invader
  - Stress-related opportunist
  - Overfeeding, handling, overcrowding
  - Aeromonas strain variations
  - Signs/lesions:
    - Acute V chronic
    - Frayed haemorrhagic fins
    - Cutaneous ulcers
    - Serosanguinous ascites
    - Petechial, ecchymoses
    - Haemorrhagic to mucoid enteritis
  - Dx =
    - Culture
  - Tx =
    - Control V treatment
    - Resolve primary insult

- **Emphysematous Putrefactive Disease (EPDC)**
  - *Eitology:*
    - *Edwardsiella tarda*
    - Gram negative rod
    - Normal water inhabitant
    - Abscesses in a catfish
    - ZOONOTIC!
    - Chronic disease
    - Less than 5% mortality
    - Worse in warmer months (>30^{o}C)
    - Signs/lesions =
      - Low to moderate mortality
      - Small shallow ulcers to deep fistulous cavities
      - Malodorous lesions
      - Fish smell like they have been dead for a week
    - Tx =
      - Improve water quality
      - Antibiotics?

- VIRAL DISEASES OF CHANNEL CATFISH

- **(CCVD) Channel Catfish Virus**
  - enveloped virus
  - alpha herpes virus
  - dead fry and fingerlings (small fish)
  - takes out kidney = can lay dormant in
  - summer time disease = young fish and in very warm water
  - DNA enveloped
  - CS =
    - Exopthalmia
    - Ascites
    - Ventral petechiation
    - Congestion/haemorrhage in liver and kidneys
  - Dx =
    - On CS
    - Virus isolation
    - Microbiologist and Virology
  - Tx =
    - None
    - Prevention
    - Destroy that cohort of fish
  - Epizootiology =
    - Highest mortality at >28^{o}C, low DO
    - Incubation 2-3 days at 30 ^{o}C
    - Assymptomatic carriers

- PARASITES

- **Proliferative Gill Disease (PGD)**
  - 'Hamburger gill'
  - Proliferative branchitis
  - *Henneguya ictaluri* aka Aurantioactinomyxon
  - Myxosporean
  - Spring/fall 14-26^{o}C
  - *Dero digitata* (annelid)
  - Thought there is another intermediate host??
  - CS =
    - Anorexia, lethargy
    - Hypoxaemia = act hypoxic in face of normal oxygen
    - Pale or haemorrhagic gills
    - Cartilaginous fractures
  - Dx =
    - Branchial wet mounts = See thickened epithelium and missing cartilage in middle
    - Histopath
  - Tx =
    - Mostly supportive care
    - Maintain water quality
    - Salt
    - Maintain DO and ammonia
    - Potassium permanganate ($KMnO_4$)
    - Formalin?

- **Ichthyophthirius (Ich)**
  - White spot disease
  - >250 microns (20 times size of red blood cell)
  - 'C' macronucleus
  - holotrichous ciliate
  - multiple tx of formalin
  - for aquarium fish = can move to freshwater every day for seven days
  - can look round on histo section

- **Ichthyoboda (Costia)**
  - Very small parasite
  - Atrophy of secondary lamellae
  - 10 microns
  - flagellate

- **Trichodina**
- **Trichophyra**

- **Ambiphyra**
- **Henneguya**
- **Flukes (monogenes)**
    - Not that pathogenetic
    - *Bulbophorus sp*
    - Complex lifecycle = snail, bird, fish (thinking is that pelican is primary host)
    - See measle like lesions
    - Haemorrhage lesions under skin
    - Treatment is to get rid of the snails
    - Do see tape worms = but incidental finding

- **Saprolegnia (Winter Mortality Syndrome)**
    - Water mould
    - Secondary invader = same as Flavobacteria
    - Due to low water temps <15^{o}C
    - Immunosuppressive
    - Tx = salt and limestone and wait for warmer weather. Formalin also used. Tincture of time!
        - Can harvest fish = as catfish skin comes off.
    - CS = thin, dry skin, see fungus on surface, sometimes deep ulcers, deep dermatomyositis
    - Dx = histo (see fungal hyphae), fungal culture, wet mount
    - Formalin = very bioreactive = don't there is a withdrawal time

- **Channel Catfish Anaemia**
    - 'White lip' or 'no blood'
    - profound anaemia PCV <10^{o}C
    - pond problem
    - seasonal
    - idiopathic
    - Histo = kidney = cells are big, increased immature cells, occasional MMC
    - Tx = symptomatic tx, maintain water Q, minimize stress

- **Visceral toxicosis**
    - Emerging disease = ~ 10 years
    - Neuro signs = tremors = deaths
    - Intussusception
    - Middle to large fish
    - Botulism!! = Clostridium

- **Trematodes**
  - Platyhelminthes
  - Acoelomate = no internal body cavity
  - Bilateral symmetry
  - No body cavity
  - Flat worm
  - Soft, unsegmented bodies
  - No respiratory/circulatory organsdorsoventrally flattened
  - Triploblastic = possess three main cell layers =

- **Slide session**
  - *Cauliflower like mass on nose of a goldfish size of a goldball =*
    - Benign: respect basement membrane…
    - Firbropapilloma = combination/dual tumor: fibrous proliferation of connective tissue
    - Papilloma = benign epithelial tumour
    - Multi-locculated = many cystics structures = probably are interconnected
    - Papillary fronds

  - *Catfish =*
    - Eye = lens = protein
    - Haematoxylin = Blue = nucleic acid (DNA/RNA) or mineral
    - Eosin = Red = protein
    - Lipid/CHO = extracted from tissue = so fat/water goes away
    - CCV = extensive necrosis in anterior and posterior kidney

  - *Bream =*
    - Metazoan parasite
    - Look for GIT, muscle to help identify
    - Phylometra??

  - *Catfish =*
    - Gill:
      - Partial filling of the interlamellar troughs, lesser numbers of lymphocytes with secondary epithelial hyperplasia and hyptertrophy.
      - There are tear shaped, curved 7 micron basophilic structures consistent with Ichthyobodo.
      - Morph dx: Severe, sub-acute to chronic, diffuse, hypertrophic branchitis with intralesional protozoa.

  - *TB =*
    - Necrosis, wall of macrophages, with ring of lymphocytes around
    - Granuloma ddx: parasites, fungal, bacteria
    - MMCS pick up lipofuchin

- *Metazoan parasite =*
    - Features of this round nematode worm:
        - Has a body cavity
        - Gut lumen = *multiple cells (metazoan)*
        - Mature worms have ovaries
        - Has a cuticle
        - Has internal structures

- *Trematode parasite =*
    - Features of this flatworm:
        - Has a solid body = loose connective tissue
        - Has no body cavity

- *Cestode parasite =*
    - Features of this flatworm
        - Calcareous corpuscle
        - Has no body cavity

Wednesday 29/05/13

Toxicology Pathology of Fishes

Dr Jeff Wolf

Reference = Perry et al.

Zebrafish: Anatomy Histology and Anatomy

- *Danio rerio* = prototypical fish species
- Jeff desn't like formalin as first fixative = creates a lot of artefacts
- Prefers Davidson's or Bouins for 24 hours, then rinse in acetic acid and switch to 10% NBF
- Don't use formalin for journals when you want to take histomicrographs
- Glycerol is difference between modified and normal Davidson's
- Davidson's is not so toxic; becoming more popular
- Get most out of parasagittal plane of cutting = but can't compare bilaterally symmetrical lesions
- Longitundinal sectioning good for bilateral lesions
- Serial sections: 5 sagittal/parrasagittal sections = good but can be destructive
- Fish tissues decompose very quickly = can do small batches to avoid autolysis

- **INTEGUMENT =**
    - Very little keratinized tissue in a fish
    - Dermis contains scale pocket

- Hypodermis = subcutis = minimal
- Scales are membranous bones embedded in pockets within the epidermis and dermis
- Zebrafish has elasmoid ctenoid scales
- Alarm cells = 'club cells' = dermal injury causes cells to release hypoxanthine-3N-oxide (Schreckstoff) pheromone
- Chromatophores:
  - Iridophores = silver
  - Melanophores = brown
  - Xanthophores = yellow
  - Brown + yellow = blue
- Integument = barrier system
- Fish mucus contains antimicrobial substances
- Freshwater fish = hypertonic relative to their surroundings = so breaches can lead to generalized oedema

- **SKELETON =**
  - Axial
  - Appendicular
  - Most bones are endochondral = most bones
  - Skull/scales = mebranous bones
  - Vary from poorly cellular to acellular
  - Skeletal muscle =
    - Myomeres = functional unit of trunk musculature
    - Zig-zag = contract as needed for motion
    - White muscle = majority of trunk muscle
      - Poor vascular supply
      - Useful for short bursts
      - Rapidly fatigues
    - Red muscle =
      - Narrow, superficial band
      - Slow twitch
      - Aerobic
      - Extensive vascular supply with little myoglobin

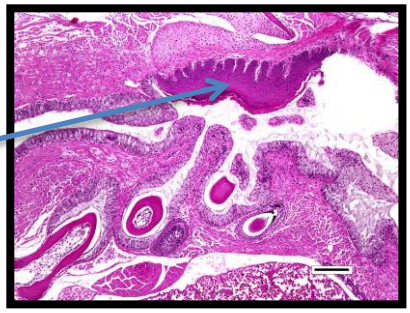
- **HEART =**
  - Fish don't have true diaphrapm = but do have transverse septum
  - *Has two chambers, but four compartments*
  - Sinus venosus = like cranial vena cava
  - Atrium = thin walled
  - Ventricle =
    - Spongy outer compact layer
    - Coronary arteries
    - Bulboventricular valve
  - Bulbus arteriosus =
    - Analogous to ascending aorta
    - Fibroelastic, smooth muscle

- Have ventral and dorsal aortas
- Have hepatic portal system =
    - Enterohepatic cicurlation
    - Venous blood enters from intestines to liver
- Have renal portal system =
    - Venous blood from caudal musculature enters kidney = affects drug distribution
        - This is why we usually don't inject IM in tail = better to inject IM closer to head end
- Lymphatics =
    - Fish don't have lymph nodes
- Haematopoesis =
    - Primarily occurs in renal interstitium
    - No bone marrow
    - Thymus = T-lymphocyte maturation
    - Erythrocytes = nucleated = rely on aerobic metabolism (diff to humans, we have anaerobic)
    - Thrombocytes = nucleated
    - Leukocytes =
        - Monocyte = macrophage (tissue version)
        - Neutrophils = only about 5-7% = so don't see pus so much in fish
        - Eosinophilic granule cells = all of literature about this = their function tends to be a bit more ambiguous = need to know species of fish, cause sometimes they are just there/normal, don't want to over diagnose
    - Renal haematopoetic tissue =
        - Sinusoidal reticuloendothelial tissue = loose network of tissue, tissue is more permeable and endothelial cells don't form solid walls
        - Zebra fish have urinary kidney all throughout
        - Systemic inflammation = if you see tubules being pushed apart by cells inbetween, maybe because there is some infectious/inflame process going on in animal
    - **Spleen =**
        - Left mid abdo below swim bladder
        - Capsule thin in Zebrafish
        - Ellipsoids = periarterioral macrophages sheaths
        - Red pulp >> white pulp
        - Lymphoid follicles rare
        - Look for tiny arterioles to help you find spleen
        - Spleen congestion can be artifact of anaesthesia
        - Can't really diagnose lymphoid depletion in spleen as there is not that much tissue
        - 'bag of blood'
    - **Thymus =**
        - Paired = in dorsomedial branchial cavity

        - No obvious cortex and medulla in adult zebrafish
        - Doesn't usually involute
        - Lymphocytes, macrophages and apithelial cells
        - Consists mostly of small lymphocytes

- **GILLS =**
    - Bilateral branchial chambers
    - Each chamber has four sets of cartilaginous gill arches
    - **Lamellar cells =**
        - Pavement (epithelia cells) = single layer
        - Endothelial cells = line capillaries
        - Pillar cells = support walls of capillaries
        - Chloride cells = maintain ionic balance = usually pink or clear
        - Mucous cells = increase with irritation
            - Mucus = the actual thing
            - Cells = Mucous cells
    - **Pseudobranch =**
        - Countercurrent system for supplying and regulating oxygenated blood to eyes
    - Gill function =
        - Oxygenation
        - Removal of 80% of total nitrogen elimination
        - Osmoregulation
        - Acid-base balance

- **DIGESTIVE SYSTEM =**
    - Herbivorous fish lack stomach
    - Have pharyngeal teeth = no oral teeth
    - Pharyngeal pad has keratine surface

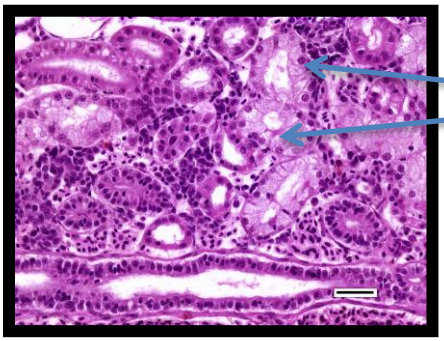


    - **Oesophagus =**
        - Abundant mucous cells
        - Highly distensible
        - Lots of folds
    - **Intestines =**
        - Morphology changes very little along length
        - No glandular stomach
        - Lumen and folds become smaller caudally
        - Relatively thin muscularis
        - Submucosa barely evident
        - Few relatively leukocytes in healthy gut
    - There are certain fish that do have glandular stomachs =
        - Eg: Winter Flounder = predator
            - Has gastric glands
    - **Liver =**
        - Cyprinids =

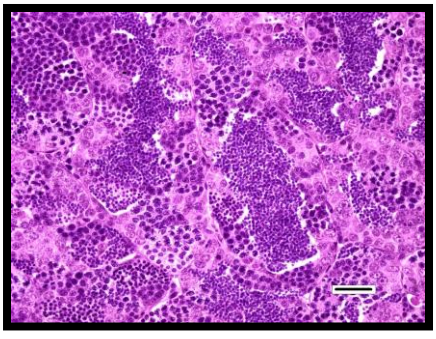
        - Multi-lobed liver = almost scattered throughout abdo cavity
            - 3 lobes in Zebrafish
    - Fish livers lack defined lobular architecture
    - Very few hepatic arterioles
    - Indistinct portal triads
    - No lymph vessels
    - No Kupffer cells = catfish might
        - Do have resident macrophages??
    - Fish livers thought to have tubules rather than hepatic plates
    - Appearance of liver can vary by sex
        - Reproductively active females = have basophilic livers due to increased production of vitellogenin
            - A protein used for egg yolk production
    - Same liver functions as mammals
    - Xenobiotics = chemicals that are not normally there
- **Gall bladder =**
    - In Zebrafish = non descript sac in caudal abdo
    - Common bile duct is extrahepatic
    - Lined by transitional epithelium
- **Exocrine pancreas =**
    - Not a dicrete organ in fish
        - Islands of acinar tissue scattered throughout the abdo mesentery
        - Red zymogen granules inside
        - Jeff doesn't like 'hepatopancreas': this is more in invertes = better to call 'intrahepatic pancreas'
- **Swimbladder =**
    - Located dorsal to abdominal viscera and ventral to spine and kidneys
    - Zebrafish = two chambers = Cyprinids also have this
    - Medaka = one chamber
    - *Physoclist =*
        - Kleisein = to close
        - No connection between oesophagus and swim bladder
        - Eg: Cichlids, killifish, seahoreses and most marines
    - *Physostome =*
        - Retained pneumatic duct
        - Eg: catfishes, salmonids, cyprinids
    - Pneumatic duct =
        - Tortuous tube-like structure that connects oesophagus and swim bladder
    - Gas gland = in fish that don't have these ducts = inflation through vascular system
    - Functions = (species dependent)

- Neutral buoyancy
- Generation of sounds
- Oxygen respiration
- Detection of sound waves or pressure changes
- Zebrafish =
    - Have Weberian Ossicles
        - Series of four tiny bones facilitate transmission of sound waves from swim bladder to the inner ear
        - This apparatus = otophyseans

- **URINARY SYSTEM =**
    - Pronephros
    - Mesonephros = fish stop here = Opisthonephros
    - Metanephros
    - **Kidneys =** retroperitoneal
    - Seahorses = no glomeruli
    - Anterior kidney in Zebrafish = has urinary components unlike other fish
    - Kidneys =
        - Glomeruli are variably sized and sparse
        - Zebrafish thing = to have these proximal tubules that hav mucoid appearance to epithelial cells = this is normal = especially prominent in male zebra fish = NORMAL
        - Opisthonephric duct = ureter = ciliated columnar epithelium
        - Freshwater fish = hypertonic to surrounds = excrete dilute urine to avoid systemic oedema

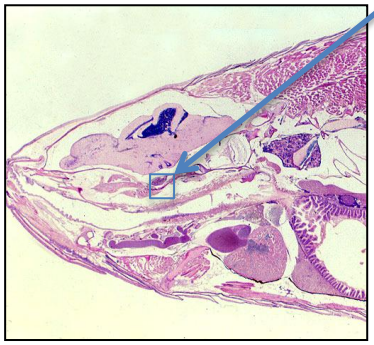


- **REPRODUCTIVE =**
    - **Ovary =**
        - Functional unit = follicle
        - Follicle = oocyte + granulosa and theca cells
        - Ovulation occurs into an ovarian cavity
        - Ovarian wall contiguous with oviduct
        - Phase of follicles =
            - Perinucleolar = least mature
            - Cortical alveolar
            - Vitellogenic = most mature
    - **Testis =**
        - Functional unit = spermatocyst
        - Two types of seminiferous tubule organization
        - Testis = paired structures = mid to caudal abdo
        - Sperm go from larger to small = whereas ovaries are opposite
        - Leydig cells = hormone producing cells = in interstitium

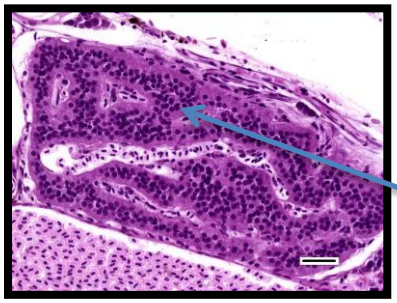


        - Ducts deferens = sperm exits fish
        - Zebrafish = gonochoristic = maintain same gender throughout
            - However all ZF are phenotypically female until ~3wks post-fert
        - Females are usually bigger grossly
    - HPGL axis =
        - Hormonal control similar in mammals

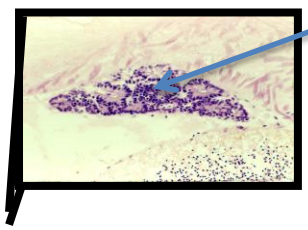
- **ENDOCRINE =**
    - **Pituitary =**



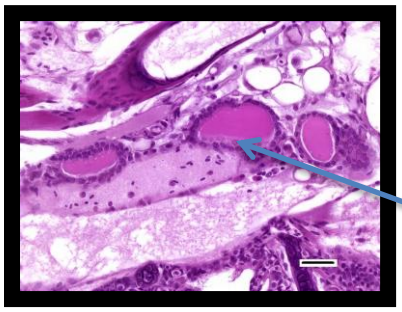
        - Floor of diencephalon, beneath hypothalamus
        - 'The master gland'
        - controls HP axes
        - +ve and –ve feedback loops
        - major hormones produced =
            - Thyroid Sysnthesis Hormone (TSH)
            - FSH/LH gonadal hormones
            - Growth hormones = controls somatic growth
            - Prolactin = osmoregulatory
    - **Pineal gland =**
        - Epithalamus
        - Pineal complex = pineal gland plus parapineal organ
        - Neurosensory component
        - Hormonal component = Melatonin = indole amine = secreted mainly during dark phase of day
    - **Urophysis =**



        - Organ uniwue to jawed fishes
        - Ventral swwlling at the posterior end of the spinal cord
        - Caudal neurosecretory system =
            - Urotensin I
            - Urotensin II
    - **Corpuscle of Stannius =**
        - Organ unique to teleost and holostean fishes
        - Embedded in tail region of posterior kidneys



    - **Ultimobranchial body**
    - Seminomas = germ cell tumors of testis = ZF can produce spontaneous tumours
    - **Interrenal and chromaffin tissues =**
        - Inconnected tissues
        - Chromaffin = paler cells
            - Produces catecholamines
                - Epinephrine (adrenaline)
                - Noradreneline
        - Interrenal = darker cells
            - Produce corticosteroids



- **Pancreatic islets of Langerhans =**
  - Roles in amino acid metabolism and possible CHO metabolism
- **Thyroid glands =**
  - Loosely distributed follicles
  - T4 produced converte to T3
  - Thyroid follicles
  - Cells with potential to to form thyroid follicles can be found almost anywhere in fish

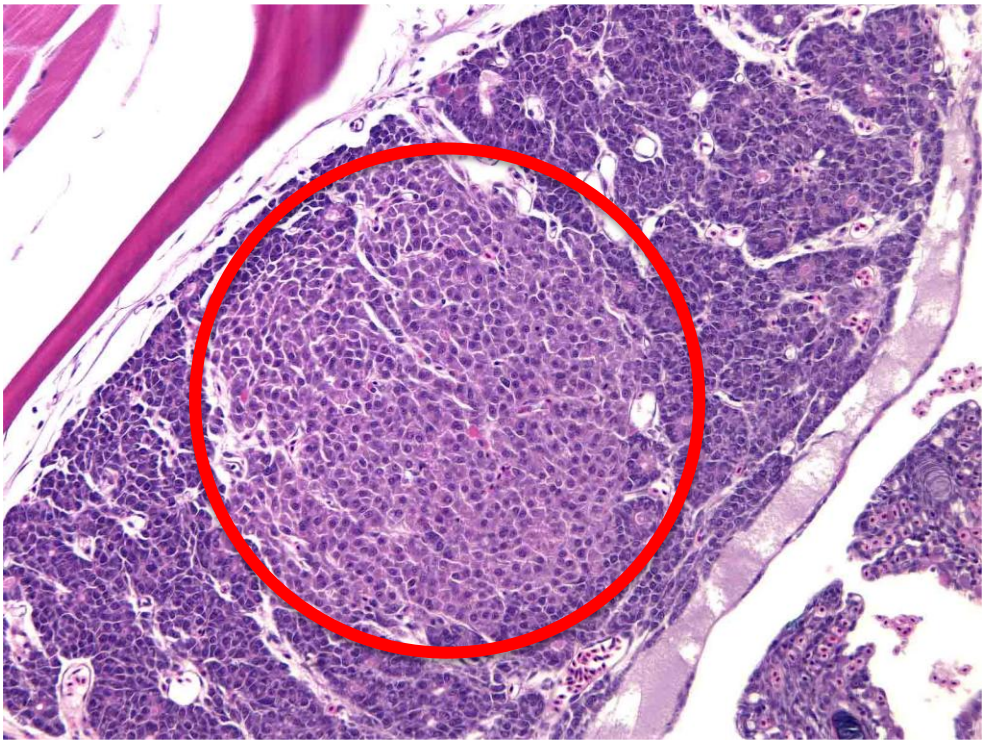
- **NERVOUS =**
  - **Brain =**
    - 5 regions
  - **Spinal cord**
  - **Eyes =**
    - Cornea thicker in fish
    - Spherical lens
    - May need to decalcify lens
    - Retina = colour and UV vision
  - **Statoacoustic organ =**
    - Inner ear = rely on anterior, posterior and horizontal/lateral canals for hearing
    - Fish don't have external ear
    - Don't have cochlear
    - No middle ear
    - Otoliths disturbs hair cells, which sends signals
  - **Olfactory tissue =**
    - Nares = usually always looked inflamed = cautious about calling this inflamed
    - Olfactory organ =
      - Neurosensory component
  - **Lateral line system =**
    - Linear dermal canals
    - Neurosensory cells are called neuromasts = sensory to water movement

Fundamental concepts of Veterinary Toxicological Pathology

- *Tox path = examines tissues or bodily fluids to determine potential effects of a test article or toxicant by comparing exposed to unexposed animals*
- More concerned with difference between treated and untreated animals
- Control groups =
  - Negative controls most common =
    - Untreated controls
    - Vehicle controls = put substance in to something

        - Placebo controls
    - Positive may be used
    - Need more replicates in fish studies due to tank effects =
        - Need to prove treatment groups are the same = replicates
- **Prevalence =** proportion of a defined test population affected by a certain disease or condition
- **Severity =** degree that an organism or tissue is affected by a certain disease or condition
- **Don't confuse dose-responsive and treatment related affects**
- Fisher Exact Test = looks at prevalence
- Need to know ahead of experiment how many animals you actually need
- **Carcinogenicity terms:**
    - Hyperplasia =
        - non neoplastic
        - reversibility
        - similarity to normal tissue from which cell derived
        - often accompanied by hypertrophy
    - Benign neoplasia =
        - Tumours that are not anticipated to invade locally or spread distantly
        - Moderate similarity to normal tissue
        - Relatively slow growth
        - Compression of adjacent tissues
        - Genomic changes
    - Malignant
    - Cells of origin = mesenchymal, epithelial and round

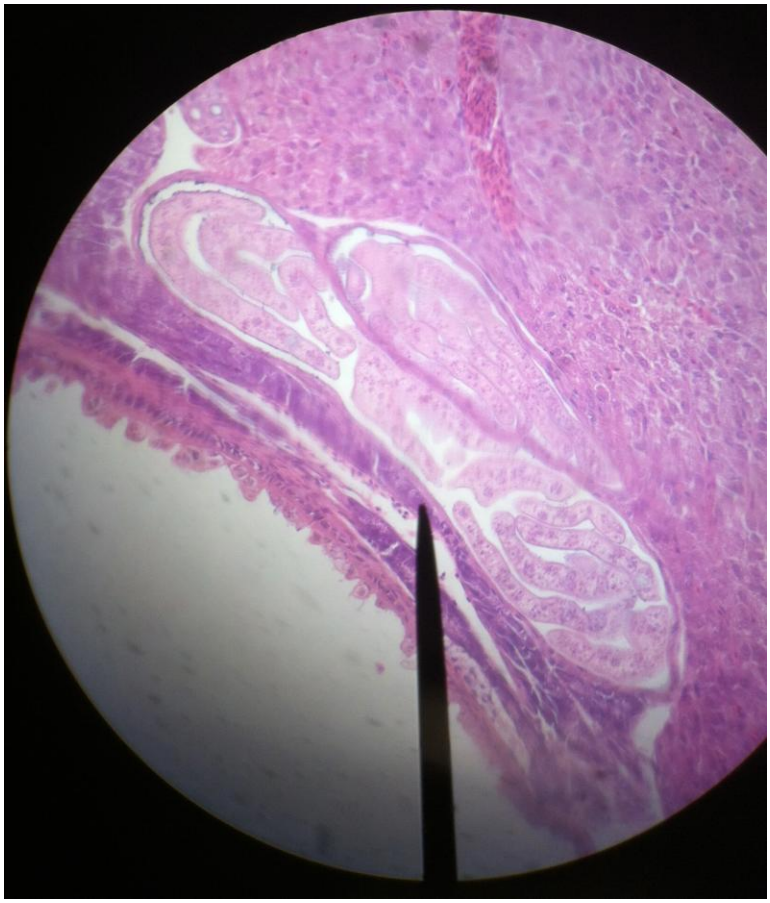
- EXAMPLES
    - Thyroid follicular cell Adenoma = discrete mass pushing tissue away, not forming follicles, poor tinctorial quality (diff colour)



- Look at powerpoint for other images

Slide reading

- *Myxozoa (Myxozoan) parasite in liver of Bluegill (male)*
  - Parasitize the gall bladder of teleost fish
  - *Reference I found: Myxidium volitans sp. nov., a parasite of the gallbladder of the fish, Dactylopterus volitans (Teleostei: Triglidae) from the Brazilian Atlantic coast - Morphology and pathology by = Carlos Azevedo[I, II, +]; Graça Casal[I, III]; Sérgio Carmona São Clemente[IV]; Leila Maria Silva Lopes[IV]; Patrícia Matos[V]; Abdel Azeem Abdel-Baki[II]; Elsa Oliveira[I]; Edilson Matos[VI]*



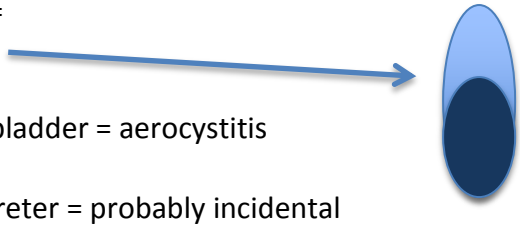
The changing face of endocrine disruption research

- Endocrine disruptor = is an exogenous substance or mixture that alters function

The mystery of Epizootic Oral Tumors in Spring Chinook Salmon

- Tumors of lower animals paper!!!
- Tumours of enamel origin = ameloblastomas
- Virions were seen on TEM
- Tumours starting showing up on fins, gill arch =
  - Metastatic
  - Not odontogenic
- No conclusion yet??
  - Jeff guesses its viral

Slides

- Acid-fast stained slide = Microsporidia = thought to be fungi = fungal infection of swim bladder =
  - Microsporidia look like 
  - Also gram positive
  - Inflammation of swim bladder = aerocystitis

- Myxozoans (protozoa) in ureter = probably incidental
  - More round in shape with spores
  - Often found in this organ

- Features of worms =
  - Digestive tract
  - Smooth muscle
  - Suckers or appendages

- Trematode in lens =
  - Smooth cuticle on outside, complex structure on inside (so rules out cestode), solid (helps rule out nematodes)

- Cataract = lighter pink globules = what degenerated lens fibres look like

- Mudpuppy =
  - Like a salamander
  - Amphibian
  - Pigmented fungal infection = Phaeohypomycosis
    - Opportunistic
    - Systemic

- Think =
  - Normal/abnormal
  - Neoplastic/non-neoplastic

- Neoplasm attached to normal ovary comprised of neural tissue and cartilage = (two different types of tissue in abnormal location) =
  - Different tissue types arising from ovary = Teratoma
  - Teratomas can arise anywhere, just need two or more abnormal tissue types in one location

- Gligal cells around degenerating neuron = satellitosis

- Replace 'blob' with structure or organism, micro-organism, multiple foci of…

- Clear space = vacuole

- Poorly demarcated mass elevating the epithelium

- Tumors that try to produce bone, but originally produce scales = *lepidocytoma*
  - Equivalent of dermal sarcoma/osteosarcoma
    - Sarcoma producing matrix trying to be bone = osteosarcoma

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Thursday 30/05/13

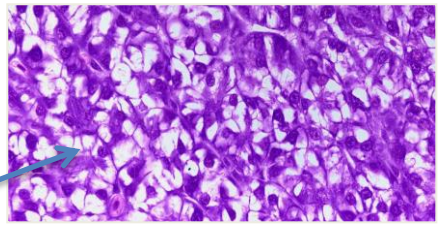
Liver Histopathology

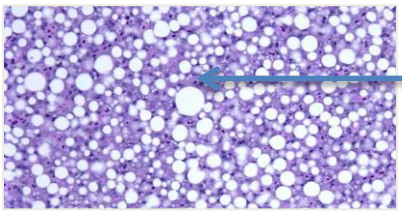
Dr Jeff Wolf

Liver

- Medaka = reproductively active female = produces lots of vitellogenin = lots of RER, RNA, very active cells, basophilic = BLUE LIVER
- Intrahepatic pancreas = not hepatopancreas
- No Kuppfler cells = except for some catfish
  - Kuppfler function = scavenger cells, breakdown products, may have antigen presenting function
- Pigmented macrophage aggregates =
  - MMCs
  - Resident macrophage aggregates
  - Antigen presenting centres
  - Help breakdown products
  - Ger larger with exposure to toxin compounds and when fish ages
    - Lipofuscin

        - Ceroid = another wear and tear pigment
        - Melanin
        - And haemosidderin
    - Must differentiate from granuloma
        - Nuclei are smaller, pushed to one side and granular material = MMC
        - These structures are relatively benign
        - Better to call PMCs = pigmented macrophage centres
    - Sturgeon liver = usually have overwhelming melanin pigment
    - Gall bladder present in most fishes = but not cod
        - Epithelial lining = cuboidal epithelium
    - MFO = mixed function oxidase
- Assessing liver =
    - Size
    - Colour
    - Shape = want nice sharp margins, not rounded
    - Look at capsule = look for peritoneal inflammation
        - But watch out for not mistaking IP injection peritoneal inflammation as real
    - Look at bile ducts and gall bladder
    - Look at parenchyma of hepatocytes themselves
- Anomalies of fish liver =
    - Liver with ectopic ovary and ViceVersa
- Metabolic changes =
    - Changes in hepatocyte size and vacuolation
        - **Energy storage** =
            - Glycogen type (starch) = fuzzy
            - Lipid = very round in shape (spherical, not a fuzzy: very sharp defining outline, nuclei are smaller and pushed over to the side of the cell and more angular looking, lipid distends cytoplasm more.

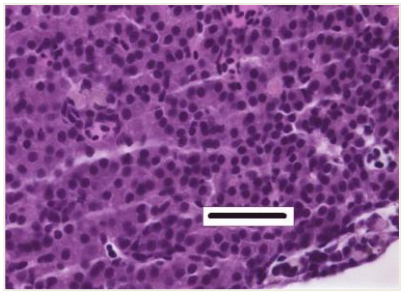




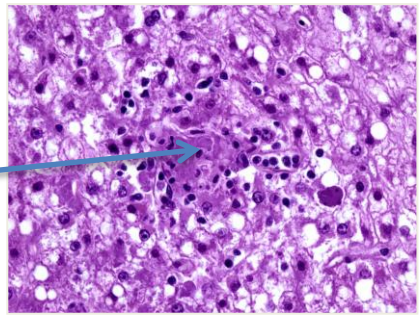
        - Period schift reaction (PAS) to stain to see if glycogen is actually there = areas light up red
        - To better establish glycogen is there = digestion process = can pretreat liver sections with amylase which digests starch = then apply PAS = once treated with amylase, hardly see any red at all
        - Fattier livers in hatchery situation = very energy rich livers compared to those of wild animals
        - Sharks don't have swimbladders, might use liver as buoyancy
        - Tend to see less vacuolated livers in wild fish or sick/stressed/starving fish
        - See more vacuolated livers in fish = captive reared, overfed and toxicant induced
        - Female livers are more often basophilic
            - Under influence of oestrogen
            - Liver cells become basophilic due to increased metabolic activity as hepatocytes gear up for vitellogenin production

- Normal for females, abnormal for males

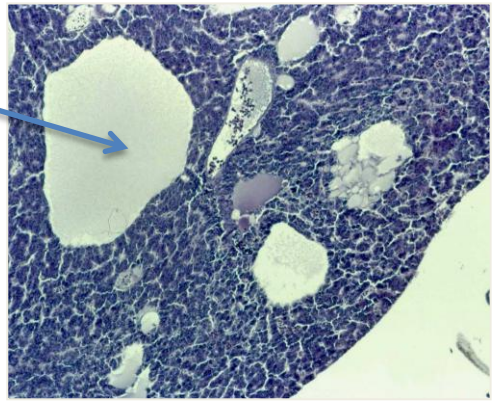
- Atrophy of liver =
  - Results from –ve energy balance
  - See grossly small, dark livers
  - Severe atrophic liver = starving fish
  - Nuclei are very condensed looking
  - So little cytoplasm

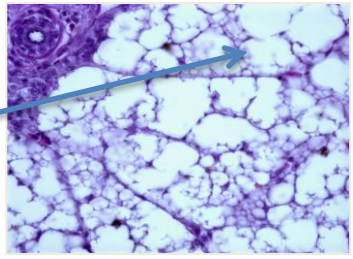


- Infectious agents =
  - Cestodes = segmented, no body cavity, has suckers, no digestive cavity or repro organs
  - Viral, bacterial, fungal = tend to affect kidney, spleen and pancreas
  - Eosinophilic type inflame can cause green livers
- Inflammatory lesions =
  - Granulomatous =
    - Primarily macrophages
      - Patterns =
        - Discrete granulomas
        - Diffuse inflammation
  - Mononuclear cells =
    - Primarily lymphos and plasma cells
  - Can have mixed cell inflame =
    - Neuts, eosin granular cells
  - Focal granuloma =
    - Little foci of cell necrosis, some mononuclear cells =
      - Jeff usually monitors and records these
      - Granulomata
      - Very common, don't necessarily indicate disease problem
      - See these in other animals = rodents, dogs

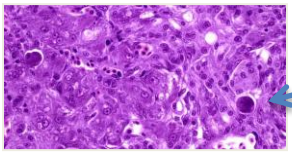


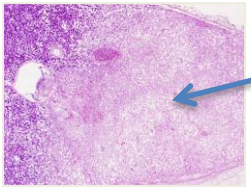
  - Multinucleated giant cells =
    - Syncytia
    - Common conditions where we see giant cells =
      - Foreign bodies = eg: mineral
      - Fungi = stain with PAS/Silver stain = cell walls light up
      - Acid fast bacteria = bacteria persist
      - Decript = granulomatous inflammation with multinucleated giant cells
- Cystic degeneration of the liver =
  - Females seem more predisposed
  - Spongiosis hepatis and hepatic cysts
  - Can see increased incidence with toxicity
  - Can look like they have fluid in or empty
  - How do you distinguish from blood vessel =
    - Should see endothelial cells in vein
      - Little flattened cells
    - Cysts have no endothelial lining

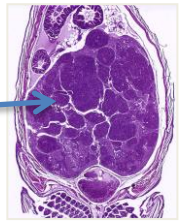




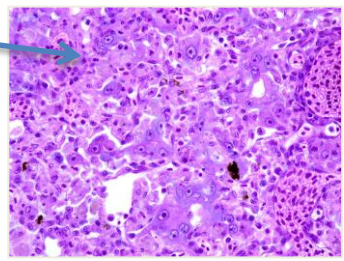
- Degeneration =
  - Lipidosis =
    - When walls of hepatocytes break down, this implies lipidosis
- Necrosis =
  - Patterns =
    - Individual hepatocyte degeneration = cells get very dense, halo where it separates from other cells, rounds up = individual cell necrosis
    - Massive necrosis
    - Infarcts = acute infarcts often have a lot of haemorrhage, but as they age become depressed and white
      - eg: systemic fungal infection = some are vasculotrophic and love to follow blood vessels







- Hyperplasia =
  - Can have benign or malignant
  - Tumour like hyperplasia = due to heptoxocosis
  - Oval cell proliferation =
    - Regenerating cells that can become bile duct cells or hepatocytes
    - Proliferation of streaming oval to polygonal cells
  - Bile duct hyperplasia =
    - Increased epithelial cells and or increased ducts
    - Can be response to chronic inflammation
    - Can be accompanied by peribiliary fibrosis
  - Foci of hepatocellular alteration
    - Some of these are considered to be neoplastic
    - Definitely record these changes
    - See changes in cell size, cytoplasmic density and/or colouration
    - Eosinophilic focus
    - Basophilic focus
    - Clear cell focus =
    - Foci in general are a step down for adenoma
      -
  - Regenerative hepatocellular hyperplasia =
    - Response to severe injury
    - Have a lot of necrosis and inflam cells here
  - Ectopic thyroid follicular cell hyperplasia



- Neoplasia =
  - Common site for spontaneous and induced tumours
  - Eg: aflatoxin induced liver tumour in trout
  - Primary liver neoplasms=
    - Hepatocellular adenoma and carcinoma
    - Bile duct origin = choleangioma/carcinoma
  - Also common site for tumour metastasis
  - Seminoma in male medaka liver =
    - Testicular tumour that met to liver

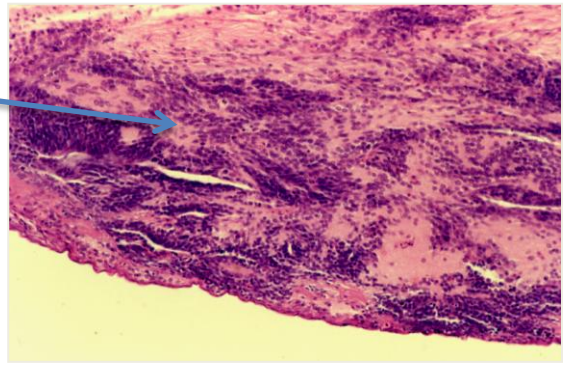
- (apoptosis = programmed cell deaths= where cell wants to intentionally die)

Unusual Findings in Medaka

Dr Jeff Wolf

Head of Medaka with cellular, expansile lesion on mandible

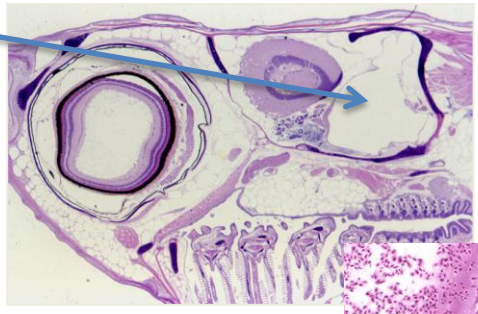
- On the ventral aspect of the mandible, there is some haphazardly, disorganised arranged tissue with some spaces (clefts), channels and streaming… expanding the ventral region of the lesion
- Looks neoplastic with inflammation
- At higher mag = looks like neuro tissue
  - neurons with neuropil
  - looking a bit less like neoplasia =
    - looks like normal brain cell nuclei
  - DDx:
    - tumour of neural cell origin
    - or displaced tissue in wrong location
- Always worth noting artefacts =
  - Just say you suspect it…
- Choristoma =
  - Normal tissue in abnormal location



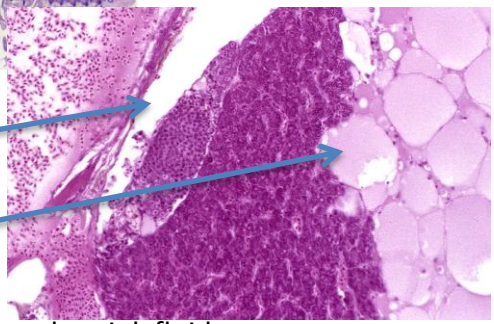
ECTOPIC TISSUES = Neural tissue in nose region and behind eye, in the eye

- This is not retinoblastoma, but retinoblastoma looks like =
  - Rosettes
  - Neurosensory tissue trying to form tumour

Statoacoustic organ



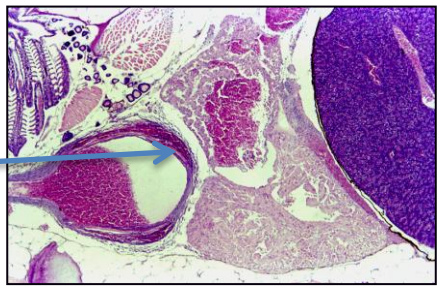
Transverse septum



- Not a true diaphragm
- Liver with cystic dengeration
- not lipid change as fat cells don't get this bit and do not have the pink fluid, more clear. Fat cells also have nuclei. These cysts don't have nuclei

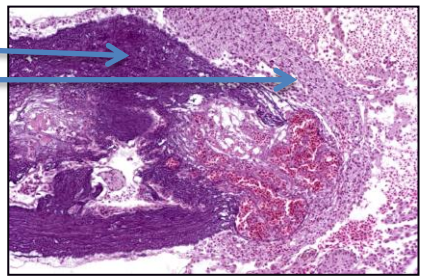
Heart

- enlargement of bulbus arteriosus
- wall of BA is thinner
- wall looks haemorrhagic
- dx = dilatation and aneurysms



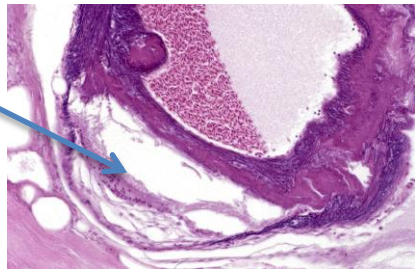
Heart

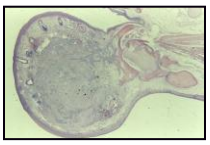
- purple stuff = mineral looks purple on histo section
- mineralization
- with macrophages
    - granulomatous inflammation
- Von kossa stain = stains for mineral = phosphate



Heart

- More mineralization
- macrophages trying to eat mineral



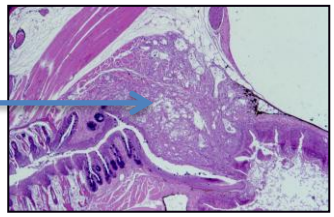


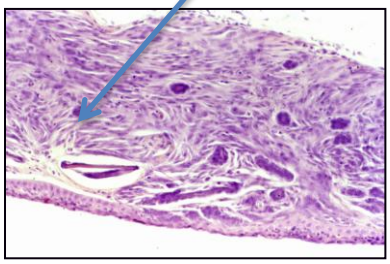
Abnormal Medaka mandible

- expansile, well demarcated mass
- looks like it is arising from mandible bone
- there are teeth
- some bone formation
- jaw masses
- this was seen as an incidental problem

Uncommon/rare neoplasms

- Oesophageal carcinoma
- Blastoma = means primitive looking tissue
- Lepidocytoma (scale tumour) =
    - streaming spindle cells
    - little nests of osteoid = clusters of cells trying to lay down osteoid = osteoblasts



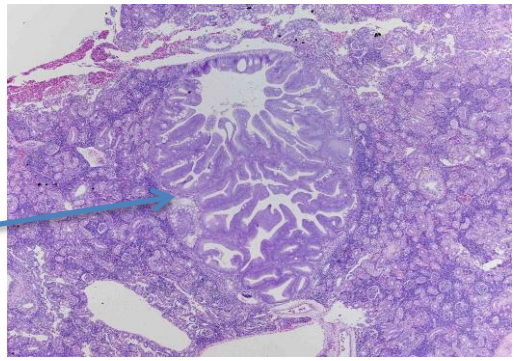


Renal Lesions in Fish

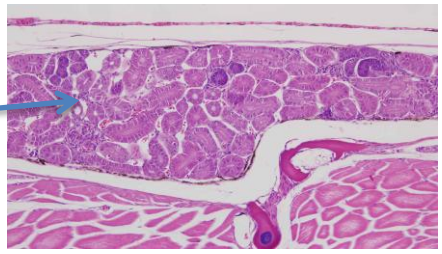
Dr Jeff Wolf

Kidney Physiology

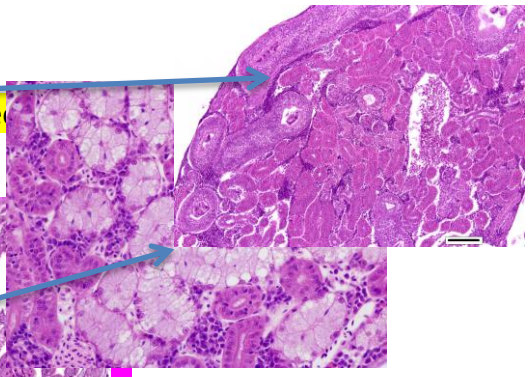
- Ca regulation = Corpuscle of stannous
- Electrolyte balance
- Haemtopoiesis
- Lymphopoiesis
- Antigen processing
- Horomone production
    - Adrenergic = chromaffin
    - Corticosteroid = interrenal
- Fish only get mesonephros = opisthonephros
- Many different kidney configurations
- Head, anterior, cranial kidney = just be consistent
- Anterior and posterior sections are attached
- Left and right sides usually conjoined
- Bilaterally symmetrical
- No loop of henle
- Can't concentrate urine
- Aglomerular kidney = eg: seahorse
- Renal portal system



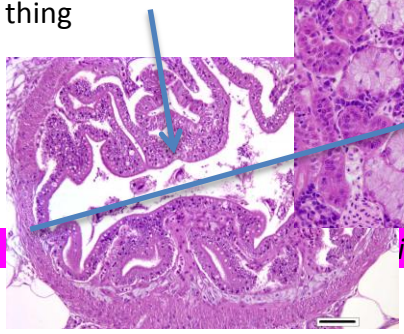
- Case one = Brown Bull Head
    - Tubular cell adenoma
        - Well encapsulated
        - No mitotic figures
        - Not hyperplastic, because it should retain some normal tissue
        - Benign tumor of glandular origin
        - Architecture is tubular



- Case two = Bluegill
    - Kidney not remarkable
    - Does have some features of hyperplasia
    - Dark areas are newly regenerating tubules
    - Fish have ability (unlike mammals) to regenerate nephrons following nephrons
    - This would resemble hyperplasia in mammal



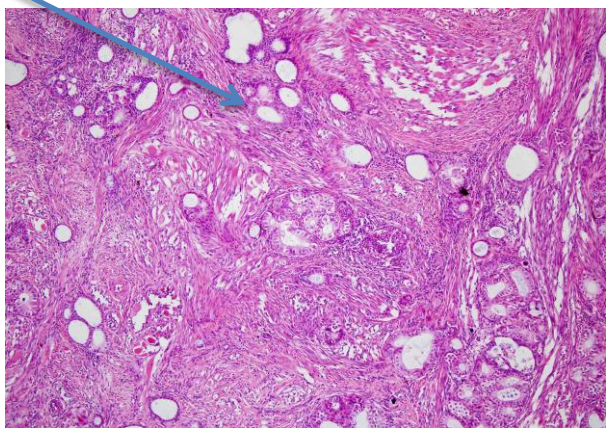
- Case three = Japanese Medaka
    - Tubular cell hyperplasia due to Trichodina in colle
    - This seems to be a Medaka thing
    - And in urinary bladder



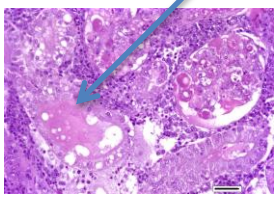
- Case four = Zebra danio
    - Kidney not remarkable
    - *Males can have vacuolated* *ic*

- Case five = Japanese Eel

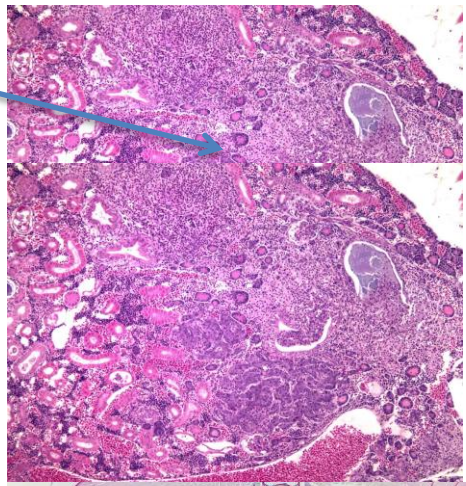
- Nephroblastomas can have mesenchymal cell origin as well
- Skeletal muscle cells are present here as well, so Rhabdomyosarcoma could be ddx
- NEPHROBLASTOMA = Primitive tumour of renal forming tissue origin. How to differentiate this Vs tubular carcinoma = nephron has immature blast cells, mesenchymal tissues
- Wilem's tumour in children



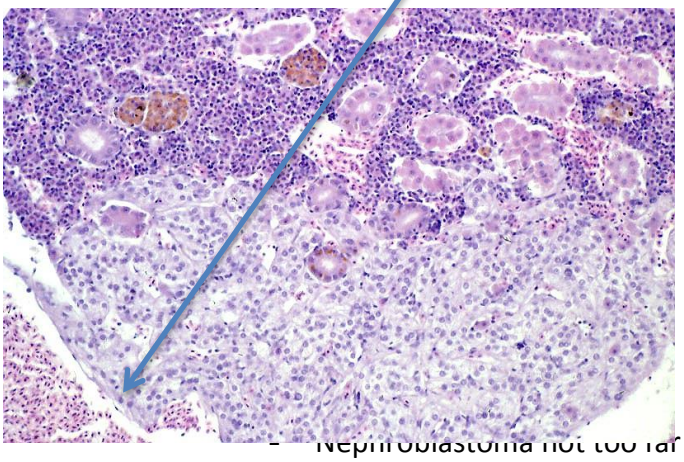
- Case six = Fathead minnow
  - Oestrogen toxicosis
  - Protein-like material in tubules
    - Amorphous eosinophilic fluid
    - Pink fluid = usually protein
  - Protein accumulation in kidneys
  - signs of injury
  - protein goes along with glomerular injury
  - lining of tubule has some vacuoles
  - some condensed nuclei = evidence of injury
  - enlarged glomeruli = massively compared to normal size
  - in males, oestradiol goes into liver, converted into vitellogenin = appears as proteinaceous fluid
  - hydrophic changes = one of earliest changes = loose ability to osmoregulate on individual basis

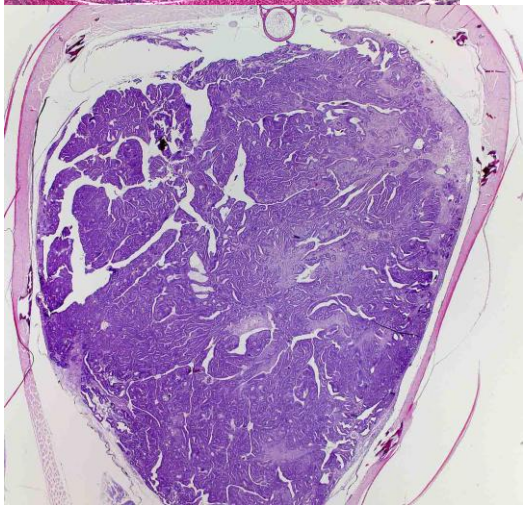


- Case seven = Japanese Medaka
  - Ectopic thyroid follicular cell hyperplasia
    - Follicle cell carcinoma of thyroid



- Case eight = Guppy
  - Chromaffin cell hyperplasia





- Nephroblastoma not too far off
  - Carcinoma because =
    - Disorganized
    - Well differentiated

            - There are tubules
        - Malignant
        - Mitotic figures
        - Some areas of necrosis, so growly fast
            - Sometimes they outgrow blood supply
            - Secrete necrosis factors that cause them to die
        - Multiple mutations = cell cycle disrupted, different repair/lost repair mechanisms
            - Invade blood vessels
            - Break apart or go through basement membrane

- Rodlet cells =

- MMNG slides
    - Classify:
        - Neoplastic OR
            - Neoplastic generally one cell type
        - Non-neoplastic =
            - Congential
            - Inflammatory
            - Traumatic
            - Degenerative

    - Gill slide =
        - Nodular mass, not really inflammatory, more neoplastic as one cell type present, endothelial in origin
        - Tumour of blood vessel origin = haemangioma
            - If benign = no mitoses
            - Mitotic figures are imptx for haemagiosarcomas…
        - Population of endothelial cells trying to replicate what they need to do, but proliferated too much

    - Ovaries typically have cavity into which they ovulate, but testis doesn't
        - Interpret whole thing as ovary, with seminiferous tubules
        - Intersex = both sexes
        - Liver seems very dark = female
        - Theory this animal was initially female
        - Neoplastic process initially = as part of this process, intersex occurs = gonadal tumour with teratoma
        - Intersex condition going on = teratoma can form any sex they want, so often have both male and female components in them

    - Head area =
        - Fish generally tend to get granulomatous inflammation

    - Mass near gills =

        - Infiltrative = tissue trying to make blood vessels = vascular channels with blood cells arranged in streams/whorls, not obeying many laws = haemangiosarcoma
        -

THINK!!!

- *Abnormal or not*
- *Neoplastic or not*
- *Which cell type: round, epithelial, mesenchymal*
- *What is the tissue trying to be?*
    - Fibrosarcoma tend to be more robust, collagenous appearance
    - Haemangiopericytoma =
        - Nerve sheath tumors

- Squamous cells line epithelium in gills
    - Epithelial tumour in gills = squamous cell carcinoma

- Gas gland = gland =
    - Nerve sheath tumour in gas gland = neurofibrosarcoma

- Seminoma = tumour of male cell origin
    - More epithelial

- Lamellar fusion =
    - Infectious agent
    - Bad water quality
    - Irritant

- Cystic degeneration =
    - Indicates something happening more in between hepatocytes, more macroscopic change

- Primitive looking cells with rosette structures (cells typically palisade around a vessel: pseudorosette is around blood vessel) = suggestive of neural origin or embryological origin
    - Jeff thinks category of neural when see rosettes = neurosensory tissue is very common in

- Degenerating follicles = atresia/atretic

- Granulosa cells = peripheral to developing egg

- Xenomas = microsporida

- GMS stain =
    - Fungi stain black and become a lot more apparent
    - Grocott's Methenamine silver stain
    - Also stains bacteria

        - A lot of fungi don't have pigment, so stain very lightly with H&E

- Dark staining = highly cellular, interspersed with areas of pallor

- GOLDFISH ABDOMEN = Fragments of cellular debris, rim of mixed inflame cells, faint connective tissue capsule, variably sized granulomas with necrotic centre (coagulative necrosis), some fibroblasts and large cells are activated macrophages…
    - Granulomas = ddx
        - Bacteria
        - Fungus = intracellular like myco
        - Foreign body
        - Parasitic
            - Metazoan
            - Protozoan
    - If you can't find a parasite, scrutinize macrophages as they are there to clean up something
    - Infectious agent =
        - Cytoplasmic round basophilic, 1-2micron structure, have some tiny pink cytoplasm in eccentric nucleus = amoeba = goldfish amoebiasis

- Necrosis causes =
    - Infectious agent = direct acting affects
    - Toxins
    - Inflammatory mediators

- Acute cell death and necrosis without attracting lots of neuts and granulocytes = bump bacterial off ddx list

- Intraviral nuclei pushed aside and clear cytoplasm = virus =
    - Goldfish herpes virus = attacks haemtopoietic cells
        - Herpes virus 2

Friday 31/05/13

Anatomy and diseases of Crustaceans

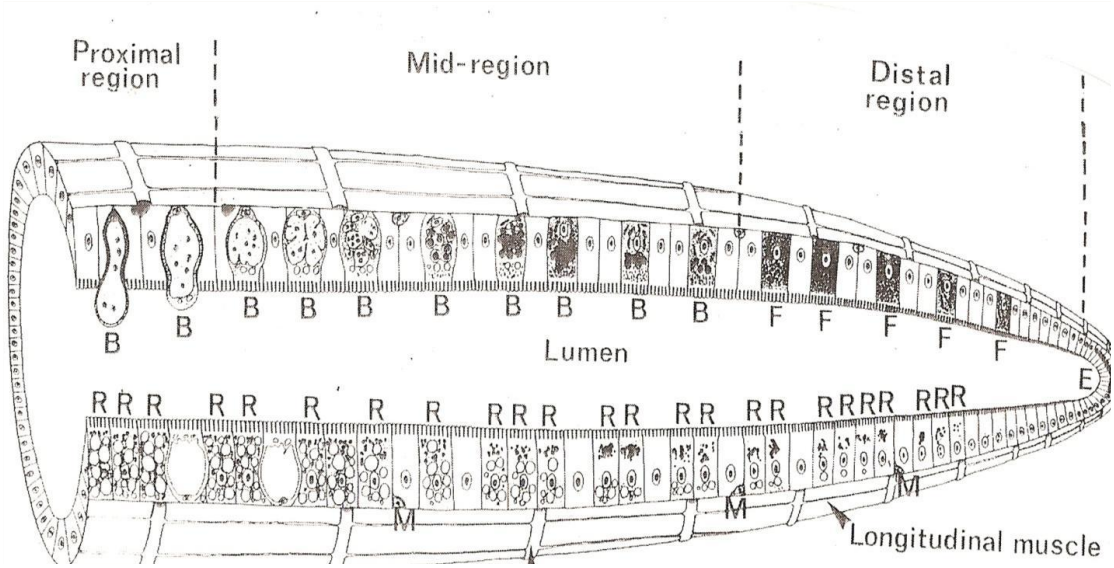
Dr Smolowitz

Decopod crustaceans

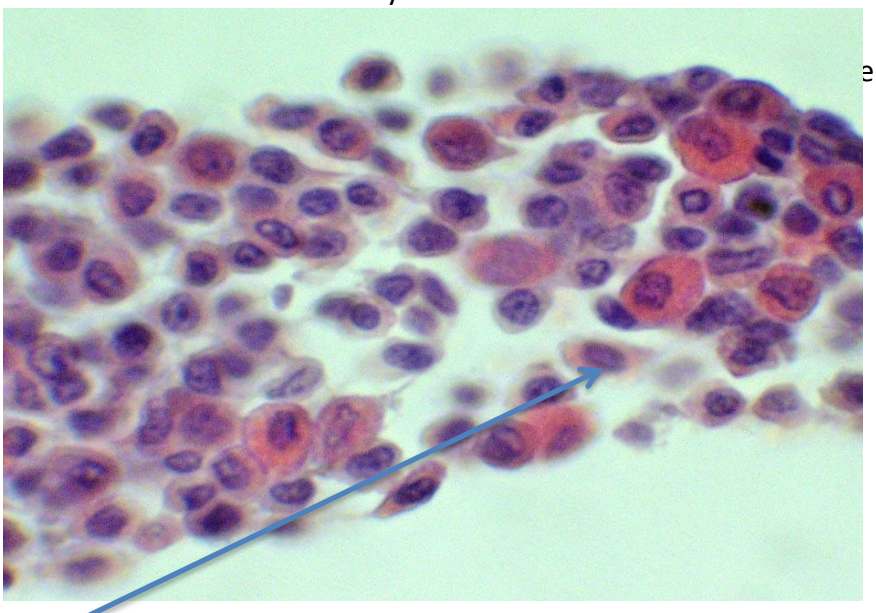
- Shrimp:
    - Penaeid Shrimp
- Crayfish
- Lobsters:
    - Primary decopod crustacean model
    - First walking legs have ben modified to form crusher and pincher claws
    - Athrodial membrane = on ventral surface and allows for movement
    - Can regenerate
- Crabs:
    - Abdomen becomes like a little flap
    - Hold egss between abdomen flap and ventral portion of cephalothorax
    - 5^{th} walking leg = modified swimmerettes = only on some crabs
    - soft shell crabs = recently moulted
    - crabs can regenerate
- Prawns

- Internal anatomy =
    - Cerebral ganglion near mouth = dose not mean they have a brain there
    - Cardiac stomach = cardiac and pyloric stomach
    - Tamale = hepatopancreas = bit people like to eat = probably not safe to eat = these animals live in sediment = so pick up parasites
    - Heart just underneath posterior portion of cephalothorax
        - Cardiac groove = is where heart is underneath
    - As eggs mature = get darker, deeper green
    - Digestive system =
        - Cardiac stomach = gastrolith = occurs bilaterally = repository of calcium = right before moult, these gastroliths get very hard, calcified plates
    - Spicules in cardiac stomach = help break food down = gastric mill = grinds food/grinds teeth
    - Sieve = ampullary filter = food goes out to digestive gland
    - Cardiac stomach = lined by thin keratin carapace = this comes out at moulting
    - Rectum also has thin layer of carapace = when moulting, this comes out too
    - Hepatopancreas has serosa around = sinusoidal area around tubules = thin connective tissue = haemolymph floating around arms = sinusoidal not capillary
    - Each tube in hep-pancreas has 5 different cells inside =
        - Very sophisticated organ =
            - E = REGENERATIVE CELLS
            - F = ENZYMATIC CELL (VACUOLE OF ENZYMES IN THE CYTOPLASM)

- B = PINOCYTOTIC CELLS (SMALL VACUOLES IN YOUNG CELLS AND LARGE VACUOLES IN OLD CELLS
- R = LIPID AND METAL STORAGE CELLS
- M = ENDOCRINE CELLS

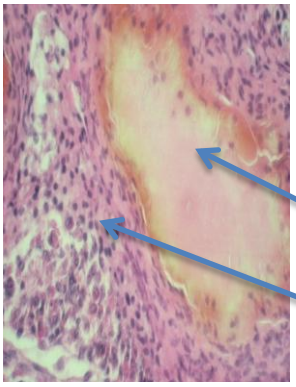


- Open vascular system =
  - One large ventricular beneath cephalothorax
  - Osteia in heart =
    - One pair in crabs
    - Two in lobsters
  - Heart sits within pericardial sac
  - No atria
  - Haemolymph comes back to pericardial sac, when heart relaxes, holes open, haemolymph goes through, haem gets pushed out through aortas, used pericardial sac like atria.
  - Thick lipid glycogen connective tissue on outer surface of heart
- Haemtopoeitic system =
  - Area above cardiac stomach that produced haemocytes
  - Three kinds of blood cells =
    - Granulocytes

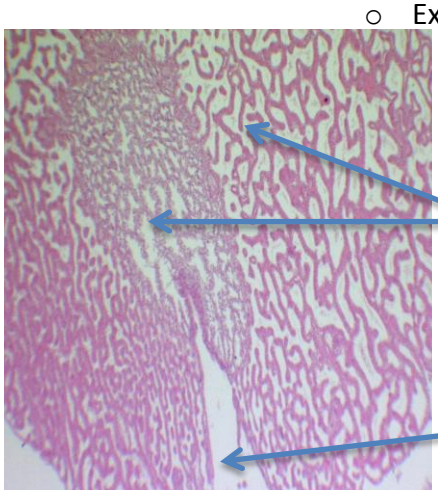


e how

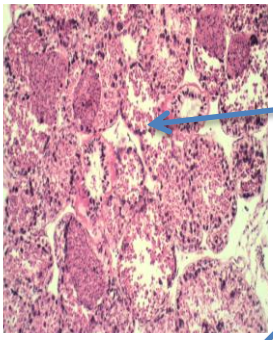
Semi
Granulocyte
Agranulocyte

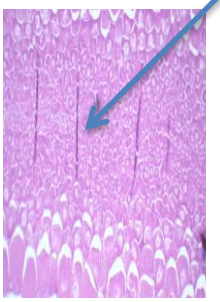


- Inflam system =
  - Prophenoloxidase present in granules of hemocyte
  - Recognition of non-self causes release of the granules and transformation to phenoloxidase (tyrosinase)
  - Phenoloxidases catalyze the hydroxylation of monophenols to diphenols (dopamine and dopa)
  - Phenoloxidase then oxidizes the diphenol to an ortho-quinone - Highly reactive molecule
  - *Melanization process* (cross-linking/destruction of proteins)
  - *Encapsulation response*
  - **End up with areas of haemocytes (thick cellular wall) surrounding homogenous material = this turns pinky when decalcified = otherwise it is black/brown**
- Lobster gills =
  - Must work well = exchange oxygen, not used for food/particulate collection
  - Found in cephalothorax
  - Consist of groupings of gills separated by walls = branchial chambers
  - Water travels up legs, up gills to surface of branchial chamber = this is reason you can take animal out of water = chamber keeps filaments moist
  - Two major vessels in gills = haemolymph circulates through filaments laterally
    - Water has major contact all along surface of filaments = increases ability of gas exchange over epithelium
- Excretory systems =
  - Green gland or kidney = found at base of antennae
  - Hard to find in crabs = browny and small
  - Easy to find in lobsters = green colour = easier to see
  - Kidney divided into 2 portions =
    - Coelmosac
    - Labyrinth
    - Nephridal canal
    - Bladder = never really see this = heads out through underneath of antenna = nephridiopore
      - arrow = heading out to bladder

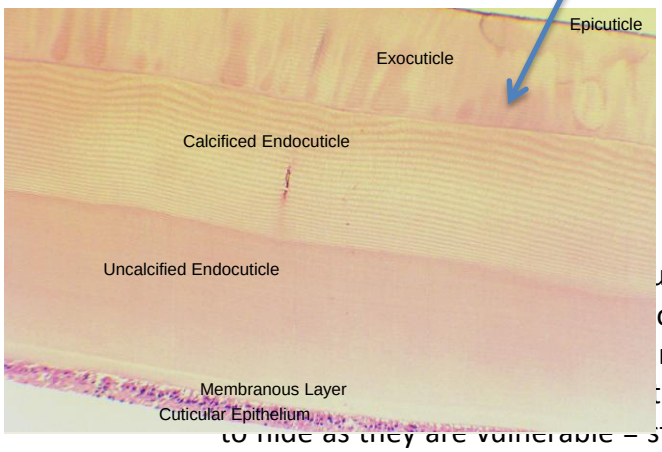


- Repro system =
  - Separate sexes
    - Males = gonads with convoluted tubules
      - Have egg packets at start = diff stage egg packets
    - Sperm come out base of 5th leg
  - Actually mate belly to belly = male injects sperm in
  - Females =
    - Gonads similar location =
      - Eggs come out of base of 3rd leg
    - Most females have seminal receptacle = can hold sperm and do not have to mate = can hold for several months
  - Lobsters only mate when they have just moulted
  - Can tell diff in crabs =
    - Abdo flap =
      - Female = larger and wider
      - Male = more pyramidal in shape
      - Differences in crab species





- Tegumental system =
  - Skeleton grows so have to moult
  - Major part = C4 and DO = portion of cycle in which carapace has been formed =
    - *arrow = C4 DO stage*
    - *produces cells in layers = goes down from epicuticle through to cuticular epithelium = these can be cuboidal*
  - Most of moult is in stasis form
  - Some giant lobsters only moult every 8-10 years
  - Discards old moult and comes out as soft new carapace = wrinkly with no calcium = this is when they mate = then start to put Ca into new shell



ulting = then secrete some material that dissolved of shell = usually back out of cephalothorax first = moulting = covered by thin cuticle = very soft and te = then mineralized shell = then find nock/cranny to hide as they are vulnerable – stay here till layers develop = weeks = till they have enough cuticle for protection = then come out

- Tegumental gland =

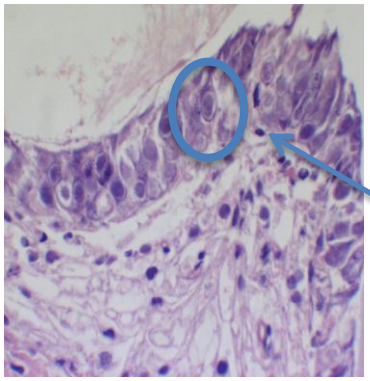
- Really active right at moult
- But once animal hits C4DO time period = these guys regress
- One location in animal where really imptx = ventral abdo dermis of female in dermis here = because way she holds eggs on pleiopods = very sticky glue holds eggs for 9 months in some species until hatched

- Nervous system =
  - Only on very ventral portion of animal = need to separate muscles to get here
  - Paired ganglia
    - Cerebral ganglia in head region = but not brain
    - Probably has more sensory role than other ganglia
    - But still a lot going on in other ganglia

- Neuroendocrine system =
  - Y organ =
    - Found in cephalothorax = won't find grossly = only microscopically
      - Produced *ecdysone* = hormone that tells epithelial cells that animal is ready to moult = causes hypertrophy of epithelial to produce next layer
    - Moulting hormone
    - Underlies the epidermis in the maxillary portion of the head
  - Mandibular organ =
    - Produces methylfarnesoate = causes ovarian maturation
  - Sinus gland or eyestalk gland =
    - Inhibits moulting hormone
    - Tells body to when in C4DO = not to do anything = no to moulting /mating
    - Controlling enzymes that keeps methylfarnesoate levels from rising
      - Cut of eyestalk when want animal to mate = gets rid of inhibition
      - Can try in animals that are in bad shape or have shell disease = but not that easy, things need to be just right/primed and ready = sometimes have to take two stalks off = do bleed = eye stalks will regenerate = takes awhile
        - this is done routinely on small shrimp
        - hard to do on bigger guys = can use tissue glue, but hard
      - not sure if they feel pain

Diseases of crustaceans

- **OIE Notifiable diseases**
  - **SHRIMP =**
    - **Taura (TSV) =**
      - Post larvae disease
      - Picornavirus
      - Gun shot like like lesions on cuticular epithelium that produces cuticle = melanisation
      - Necrotic cuticular epithelium
      - First happened in central America =
        - People thought that was a pesticide initially
    - **White spot syndrome =**



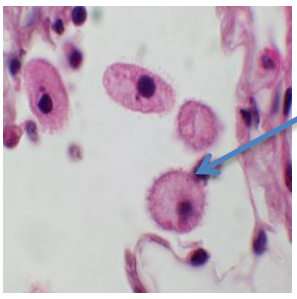
      - Seen in crayfish in Southern Louisiana in 2007
        - Then started to see going into wild pops = now endemic in South
          - Cause is actually infectious agent =
            - 3 or more closely relacted Baculovirus sp.
      - Affects many tissues causing acute, severe necrosis =
        - Epithelial and mesothelial
        - Cuticle epithelium = grossly causing white spot
      - can see inclusion bodies
    - Yellowhead
    - **Tetrahedral baculovirosis =**
      - Hosts = several Penaeus sp.
      - Baculovirus penaei
      - Post larval shrimp
      - Necrosis and loss of hepatopancreas
      - Triangular inclusions in necrotic hp cells = can be seen on squash preps
      - Also in wild American stock
    - Spherical baculovirosis
    - Infectious hypodermal and haematopoietic necrosis (IHHN)
  - **CRAYFISH =**
    - Crayfish plague = fungus
  - **LOBSTER =**
    - **Panulirus argus virus =**
      - Caribbean spiny lobsters
      - PaV1 = unenveloped nonoccluded icsohedral virus
      - Affects haemocytes

- Get haemolytic anaemia = milky or chalky = changes throughout haemolymph that prevents movement of gases throughout body
- Direct transmission

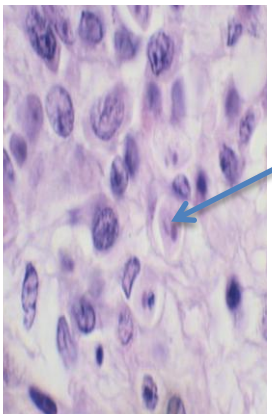
- Shell disease

- **Gaffkemia =**
  - Red tail
  - Bacterial =
    - Tetrads of Cocci (gram +ve) surrounded by a polysaccharide capsule = secrete mucus capsules
    - *Aerococcus viridans*
    - invade vascular system and proliferate there, fill sup vascular spaces, obstructs, don't get blood flow
    - eventually, lobster not get oxygen and die
    - turns haemolymph red/pink = 'Red Tail' = draw blood and look for these = do bacterial stain
    - Tx = Oxytet = approved for tx in USA = one of only drugs approved for inverts
      - Withholding time = 30 days
      - http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM252410.pdf
    - Not seen in wild
  - Big problem in American lobsters kept in holding systems
  - Wasting disease
  - Animals slowly go down hill = not getting nutrients and can't get rid of waste
  - Opportunisitic = ubiquitous
  - Pretty easy to culture
  - Seen in areas >15oC in recirc systems = 'impoundment disease'

- **Bumper Car Disease =**
  - Holotrich ciliate
  - Cause = Mugardia sp. In lobsters = but there are many other species that affect other Decapods = Anophryoides haemophillia
  - Cooler weather disease

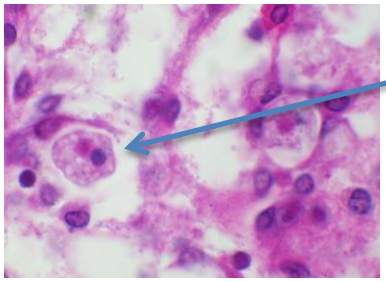


- Id in wild stock = can cause morts in wild
- Proliferates in vascular system
- Sometimes hang around hep-pancreas = can do touch prep to see them
- If you see disease = see animals with issues in haemolymph

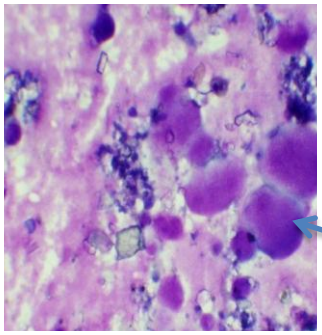


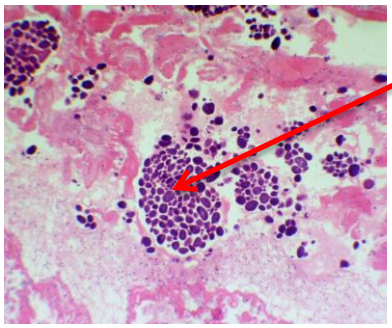
- **Paramoeba =**
  - American lobsters
  - Found in neurochords
    - Cause swelling in neurochord = grey swellings
  - causes granulomas = may see a few = not huge deal, until something causing problem = then they proliferate

- **CRABS =**

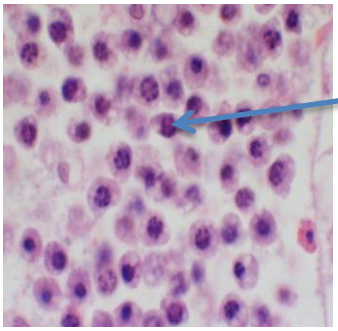


- **Grey Crab Disease =**
  - Paramoeba =
  - Few of theses
  - One imptx on NE coast = Blue Crab disease
  - *Paramoeba perniciosa*
  - Haemocytes can't seem to phagocytose and kill these organisms
  - See tiny granulomas in tissue
  - And then granulomas become apparent all over body
  - Haemolymph looks cloudy white and ventral surface is grey coloured
  - Early summer disease





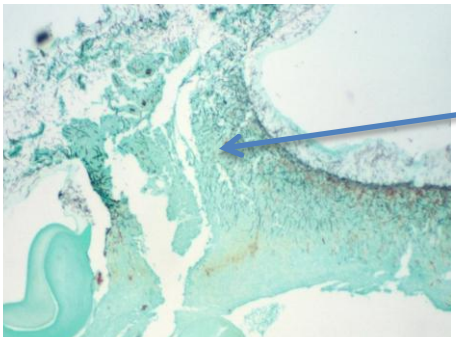
- **Chlamydiosis =**
  - Forms elementary intermediate and reticulate bodies through out tissues
    - Blue arrow = elementary
    - Red arrow = reticulate
  - Highly fatal, transmissible
  - not much money to study this
  - can do smear and geimsa stain
  - *** I saw haemocytes phagocytosing debris from chlamydia



- **Hematodinium sp. =**
  - Affects Blue Crab = so lots of money to study
  - Dinoflagellate
  - Prolifs in vascular spaces

- Haemocytes are destroyed = milky white
- Organs covered with frosty covering
- Bitter taste to tissues
- Hard to tell apart from haemocytes in early stages
- Most mortalities in fall
- Problem in shedding operations

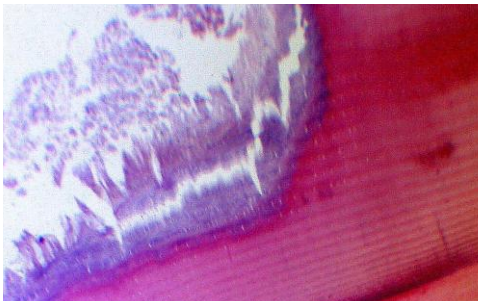
- **Hermit crab disease =**
  - Fusarium solani branchitis
  - Infects gills and arthrodial membranes of branchial attachments to the body
  - GMS stain to help find fungs = looks black = affects arthrodial membrane side, not hard carapace side
  - Don't always assume its fungus
  - This fungus really needs oxygen
  - Encapsulation/granulomas
  - Hermit crabs are intermediate host for tape worms too



- Crustaceans are often intermediate hosts for worms

Shell disease of American Lobsters

- 4 groups =
  - **Enzootic** =
    - low levels all the time
    - looks same as epizootic dz grossly
    - severe trauma can also look like very bad shell dz
    - very low incidence
    - 1 in several thousand animals
    - not sure of cause
  - **Epizootic =**
    - Occurred in long island sound
    - Continuous to occur = 30-50% of lobsters in this area occurs
    - Decreases commercial worth
    - Lesions usually start up near head
    - Doesn't seem to have a pattern like impoundment shell dz does
    - **ESD =**
      - Gross appearance of severe dz =
        - Dorsal surfaces of cephalothorax and abdo
        - Almost never on ventral surface
        - Coming from outside in = something coming in and eating it away
        - 4 stages



        - stage 1 = no cells here, but inflame response
        - stage 2 = deeper erosions into calcified endocuticle, see material sticking up into lesion = cuticle formed in diff methods = diff proteins, some produce spirals very crystal like
            - arrow = *inflammatory cuticle* = only occurs in reaction to erosion in C4DO animal = adding more layers of protection to stop things getting further into layers
            - has to be in C4DO to do this = static period of shell formation
            - melanization
        - stage 3 =
            - severe erosions into uncalcified endocuticle
        - stage 4 =
            - frank ulceration
            - ulceration of cuticular epithelium
            - enormous number of haemocytes
            - severe and true ulceration at this point
    - Causes of ESD =
        - nematodes
        - protistans
        - bacteria = *Aquamarina homaria* = flavor/flexibacter group = bad bacteria for surface lesions in fish
            - is present on normal carapace, but very rarely
            - found high levels in these lesions
            - this was primarily a bacterial infection with secondary parasite infection
        - other causes = molecular levels changes in the carapace
        - global warming decreasing pH of the oceans
            - CO_2 pollution, not pesticides

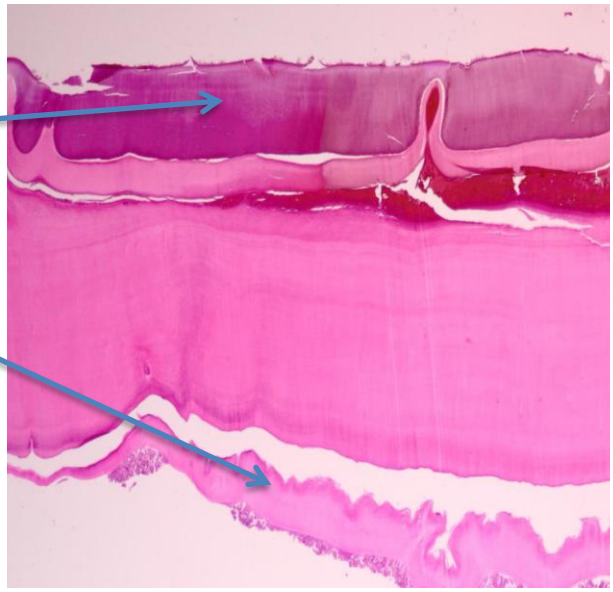
- **Impoundment** =
    - centred around tegmental gland duct opening and sensory hairs on the cuticle
    - bilaterally symmetrical = at least early in the disease
    - most bacteria are surface loving
    - bacteria and algae start to settle around tegmental duct openings

        - most are erosions = …
        - ulcers go through epithelium

    - **Blackspot =**
        - Ddx = trauma
        - Black = phenyloxidases cause region to turn black

Shell disease in other crustaceans

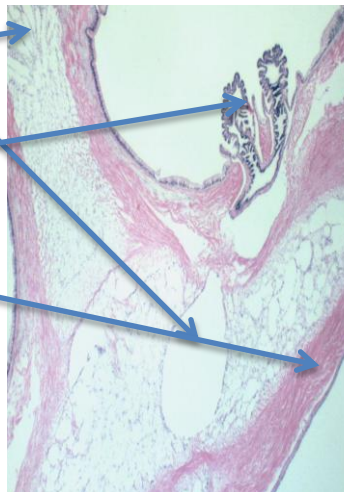
- **Rock Crab =**
    - Melanisation
    - Inflamm cuticle
- **Spider crab =**
    - Fungi

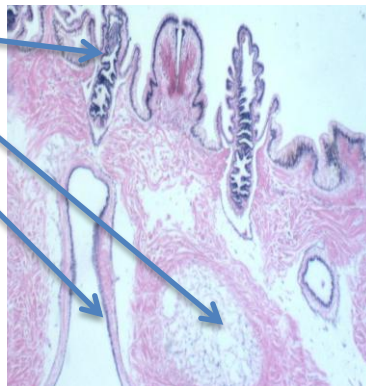


Phylum Echinodermata

- Pemtamerous
- Radial symmetry
- Central disk
- 5 arms
- mouth is ventrally
- ventrum or arms = ambulacral groove
    - innervates tube feet = tube feet help them move

- mouth = goes into two part stomach = cardiac/pyloric
- branches of tubes = two on each arm = depending on time of day, either hepatopancreas or digestive gland
- anus on surface
- clear areas filled with Ca and P = give strength to outside of animal
- muscle
- Spine
- Pinchers = keep things from attacking surface
- perivisceral coelom
- calcified disks = plaques
- papillar = like gills
- kidney and gill are same thing
- surface epithelium = simple columnar
    - one cell layer thick
- Very thin cuticle over microvilli
- Digestive system =
    - Extends out in arms
    - Carnivores
    - Love to eat bivalves
    - Put arms around bivalves =
        - Open them
        - Prolapse cardiac portion of stomach out of mouth and extend into bivalve = digests it = and pulls it back into pyloric stomach and distribution out tubes to body where more digestion/absorption





- Have incredible water vascular system = seen only in inverts
    - Madreporite = like a sieve
        - Central ring that supplies tube feet
        - Water goes back and forth through this system = and there is pressure release valve, feels little bulb tubes up = if muscles around bulb contrac, push water in and expand = if comes into contact with surface, muscles relax, water goes back up and holds foot to surface that it expanded on before water contracted.
            - One alone, not so powerful
            - So have thousands co-ordinating = extremely powerful for their size
            - Suction is passive
            - Found only in echinoderms
        - There really is a blood vascular system =
            - Microscopic
            - hardly see on histo
            - think haemocytes produced or storage channels for pigements, or lipids/glycogen
            - so there is a hemal system
- In each arm, there are two gonads
- Haemolymph cells =
    - Sea star =

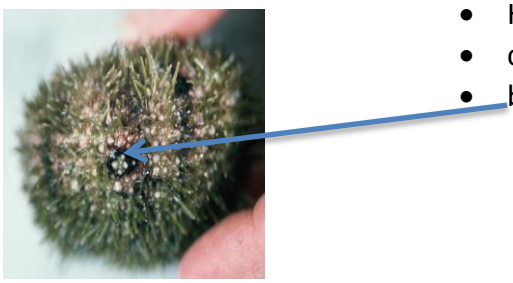
        - coelomocyte
    - Urchins =
        - Get from perivisceral coelom
        - Phagocytes =
            - Most numerous
            - Large cells
            - giant
        - Red spherule cells
            - Contain a lot of red pigment
        - Colourless spherule cells
        - Vibratile cells
            - Second most common
            - Long flagellum
            - Cytoplasmic granules
- Have separate sexes =
    - Sea stars = Bilateral symmetrical larve
    - Some sea stars brood their young = Leptasterias = keep babies around mouth, let them out, then gather them up again
    - Most are broadcast spawners

- Sea stars =
    - Regenerate = even more than crustaceans
    - Can take everything apart from central disk and will grow back
    - If you take leg off, closes hole down in 2 hours
        - This is perivisceral coelom = not vascular = as long as they don't get anything in, not a problem

- Sea urchins =
    - Have interambulacral plates
    - Herbivores, not carnivores
    - Eat macroalgae
    - Can actually destroy all macroalgae in an area
    - 5 beaked mouth
    - two gonads for every ambulacral plate

- **DISEASES =**
    - **Starfish =**
        - **Ulcerative dermatitis of seas stars =**
            - Happening right now
                - Dorsal ulceration of the arm and disks
                - With deep erosion
                - White part = calcified plates
                - Can get prolapse of digestive gland through holes
                - Every kind of echinoderms gets this

- No cause found to date
- All around the world



- **Sea urchin melting syndrome =**
  - Happening in touch tanks
  - can repair and regenerate spines in non-severe lesions
  - black spot disease
    - reddish black = because rubrocytes = are like neutrophil and move to areas of inflame = red quinone pigment = cause red/black spots = inflam cells

- **Black sea urchin plague =**
  - 1983-84 = loss of these in Carribean
  - never came up with a cause
  - did identify Clostridium perfringens and C. sordelli = not so sure about this
  - is this pollution related

- **Sea star protozoal castration =**
  - Holotrich ciliates
  - Ciliates eats sperm and spermatogonia
  - Can be severe in some populations = Japan

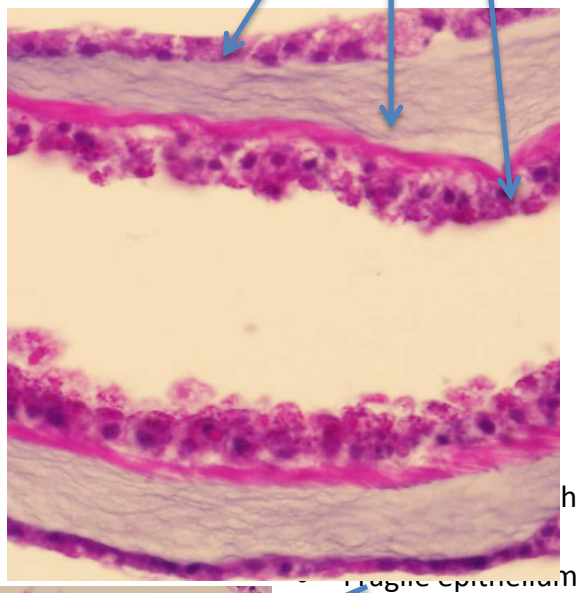
- **Paramoeba infection of sea urchins =**
  - See in Maine
  - Paramoeba invadens
  - Muscle degeneration of tube feet, spines and mouth parts
  - Hurricane occurences =
    - The hurricanes swirl things up, pick things up, move up coast of eastern USA, paramoeba = like sea urchins = invade and destroy internal tissues
    - Get outbreaks after a few weeks of the hurricane

- **Nematode parasites =**
  - Echinomermella sp.
  - Larvae in perivisceral coelom
  - Final host = sharks and rays = these each sea urchins

- **Tubellarian parasitism =**
  - Another flat worm
  - Don’t have segments
  - Do encyst
  - Can infect sea urchins
  - Reside in intestinal tract

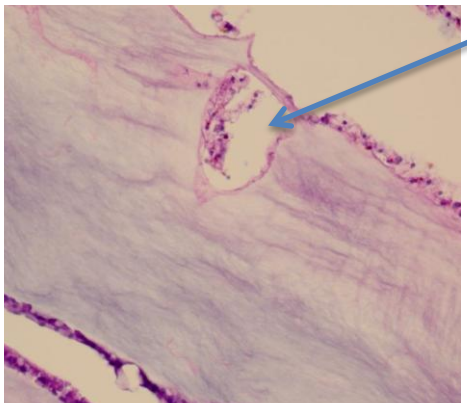
- **Sand dollars: chronic Ulcerative Algal Dermatitis =**
    - Algae invaded epithelium
    - Problem in large collections of these guys
    - Dr Smolowitz has not been able to tx these

- **JELLY FISH (Cnidaria) =**
    - **Medusozoa =**
        - Jellyfish
        - Imptx in aquarium settings
        - Anything with a bell
        - Think that box jellies are unrelated to the others
        - Radial symmetry
        - Have epidermis
        - Mesogloea = jelly part filling bell up and often arms =
            - Some cells in here
            - Few and far between
        - Gonad often within or just underneath bell
        - This time of year (May) = gonads look thick and yellow = producing sperm/eggs
        - Neural nets = nerves connect all over body =
            - No central location
            - One side is only aware of something, the other is not
            - Epidermis
            - Mesogloea
            - gastrodermis



hin epithelium

m =



- ulcer and erosion =
    - Once destroyed, can't control osmoregulation = swells up, gets bacteria and fall apart
    - Can't get them to heal

        - Put these in the tank!!!
    - Mesogloea doesn't regenerate at all
    -

- Erosion =
    - Basement membrane is intact
    - Surface of epithelium is lost
- Ulceration =
    - Goes through epidermis and can be deeper

AQUAVET 2013
My notes

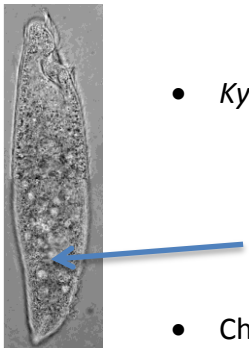
Saturday 01/06/13

Parasites

Dr Poynton
Sarah has a book coming out 2014!

- Parasitos = one who eats at anothers table

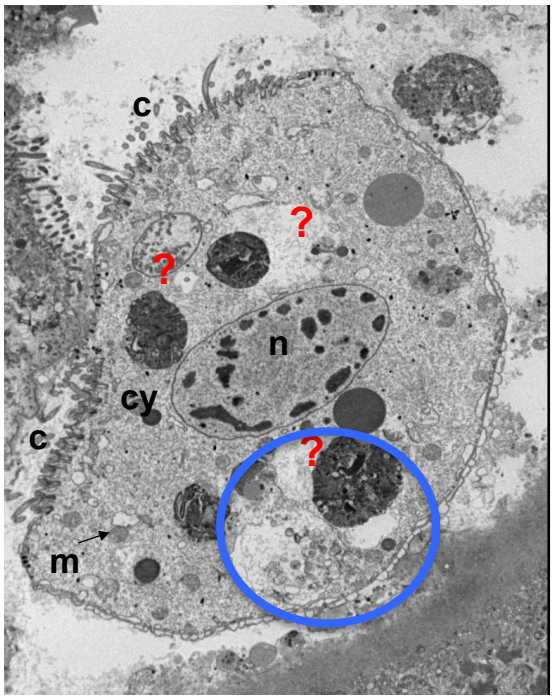
Dolphin powerpoint =



- Look at skin scrapes ASAP =
  - Parasites are very sensitive

- *Kyaroikeus cetarius* dysteriid
  - Often find in resp tract in cetaceans
  - But these were from skin
  - Protargol = silver protein stain = shows every individual cilia
  - Live specimen

- Chilodonella in cetaceans too =
  - Have rows of cilia
  - These parasites were actively dividing in the skin lesions
  - Divide by transverse binary fission
  - ? = digestive vacuole (in blue) had white blood cells in side



?

***Chilodonella* sp.**

Spironucleus sp. powerpoint

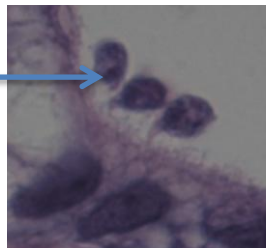
- In intensive aquaculture = typically see direct life cycle parasites
- Diplomonads = name tells us that organism is double = has two sets of nuclei and everything
    - Found in wild, farmed and aquarium fish
    - Flagellate =
        - At anterior end have two sets of flagella for locomotion
        -
    - 10 micron long, swim really rapidly
    - Imptx pathogen in fish
    - Was called Hexamita
    - Most commonly find swimming in lumen of intestine
    - Common in angel fish, trout, discus
- *S. salmonis* =
    - Associated with chronic disease
- *S. salmonicida* =
    - Atlantic salmon
    - Systemic infection in marine phase of life
    - Exopthalmia
    - Parasites travel through muscle = turn it to red/pink jello = might produced proteolytic enzymes
    - Causes acute losses of stock
- Minimum infective dose = need to prime system before it takes hold
- These parasites have posterior flagella that are stuck together
- Parasites typically transfer when there is a particular trigger/enviro change =
    - In intestine to rectum = these conditions might trigger this
    - And when fish dies = this is biggest trigger of change in enviro
    - Clusters of spironucleus =
        - Adds buoyancy = more likely to stay up in water column for fish to come along and eat it
        - Protective
- At the moment, tx with Metronidazole = but some resistance is developing

Cell length (µm)	L1	27	12.8	1.2	10.9
Cell width (µm)	L2	27	8.3	1.0	7.0
Cell shape index (L1/L2)	Ci	27	1.57	0.17	1.24
Cell area (μm^2)	Ac	26	72	13	55
Nucleus area (μm^2)	An	23	12	3	5
Area index (An/Ac)	Ai	23	0.17	0.04	0.10
Position of nucleus at L1 (µm)	N1	27	5.9	0.9	4.6
Position of nucleus at L2 (µm)	N2	27	4.3	0.7	3.3
Nucleus position index X (N1/L1)	Xi	27	0.46	0.04	0.39
Nucleus position index Y (N2/L2)	Yi	27	0.52	0.05	0.45
Nucleus dimensions at L1 (µm)	L3	23	4.0	0.7	2.5
Nucleus dimensions at L2 (µm)	L4	23	3.6	0.5	2.6
Extent of flagellar pocket at L1 (µm)	L5	21	9.5	1.0	8.2
Pocket index (L5/L1)	Pi	21	0.74	0.06	0.64
Number of kinetoplasts	K	12	19.8	9.3	7

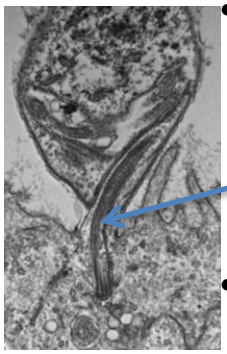
Octopus and Ichthyobodo. powerpoint

- Occy with resp symptoms
- Molecular approach to id ichthyobodo is best
- Swimming stage with two flagella
- Then attached stage = puts root like structure down into cells = flagella are held there
- A = swimming stage
- B = attached stage

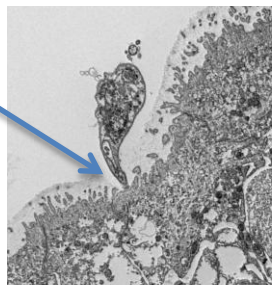
- Adult occy affected = died at Baltimore Aquarium
    - Did SEM and TEM
    - Fix immediately in glutaldehyde quickly = want small tissue
        - Can post fixed formalin fixed tissues in paraformaldehyde
    - And molecular taxonomy approach (fix in ethanol)
- Histo sections = pear shaped parasites



    - Diffuse severe acute haemocytic bronchitis
- SEM =
    - Had round structures all over gills
    - Puncture like lesions where parasite attaching
- TEM =



    - Parasite affecting mucus covering = by point of attachment of parasite
    - and caused damage by feeding on host =
        - produce a feeding tube (Cytostome) that goes into host cell
        - takes nutrients out of host cell
        - this host cell dies



- Costia may be able to transmit between fish and cephalopods =
    - But need more sequences to be sure
    - rRNA genes

- This one was most closely related to freshwater fish
    - Dwarf cichlid, hybrid striped bass and swordtail

Identification of parasites

- An atlas of protozoan parasites in animals tissues
- Journal of diseases in Aquatic organisms =
    - 2006 Bruno, Nowak, Elliot
    - Guide to id of fish protozoan and metazoan parasites
- Sept 2012 = major review of classification of eukaryotes
    - Adl. Journal of Eukaryotic Microbiology
        - The most up to date review of single cell organisms

- Sarah likes some sections cut at 2-3 microns to see protozoa

- Diff stages of parasites can look very diff

- ONLY REPORT TO YOUR LEVEL OF CERTAINTY!!!!
    - Only report what you actually know
    - If you don't know, say that
    - 'consistent with' or 'similar to'….

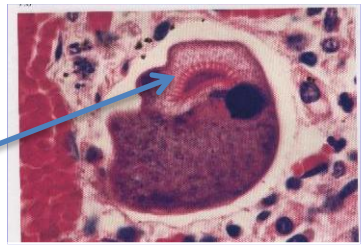
- Special stains very imptx for protozoa and myxo =
    - H&E ok
        - But doesn't give as much info for some parasite groups

- Spore forming parasites =
    - Apicomplexa
    - Microsporidia = now been shown to be a fungi
    - Myxosporidia = not protozoans, these are multi cellular, so a metazoan

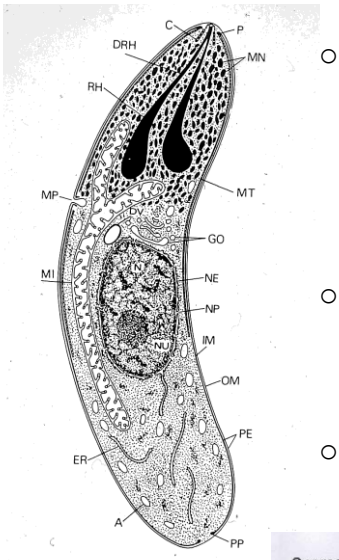
PROTOZOANS

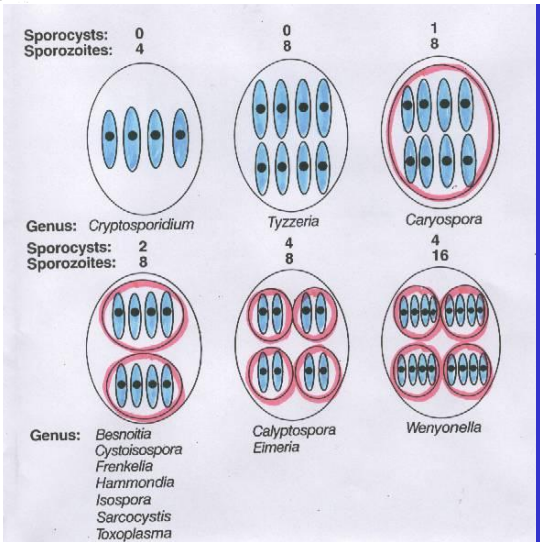
- **FLAGELLATES =**
    - +/- flagella
        - number diff to determine
    - some loose flagella = Histomonas
    - +/- undulating membrane
    - number of nuclei
    - +/- axostyle
    - Stains =
        - Normarski
        - Protargol = silver protein stain = super useful for flag and ciliates
            - Can see paired nuclei
            - Flagella
        - Geimsa
        - Feulgen = nucleus stains up magenta and everything else turquoise
        - DAPI = shows paired nuclei
    - Spironucleus = nuclei are spiral

- **CILIATES =**
  - Bigger = some can get up to 1mm across
  - Has cilia around outside of body = pink fringe
  - Cytoplasms are typically foamy
  - Might se digestive granules inside
  - Two types of nuclei =
    - Macro = can see on sections
    - Micro
  - Have a cell mouth = highly specialized with lots of cilia/bristle like structures = can recognize in sections



- **APICOMPLEXA =**
  - Spore forming parasites
    - Includes = Coccida
  - At some point of lifecycle = have an organelle called apical complex = can't see with light microscope
  - have three diff stages of life cycle =
    - 3 diff stages of repro =
      - merogony = vegetative reproduction
      - gametonuy = production of gametes
      - sporogony = production of spores
        - easiest stage to recognise
  - sporozoites =
    - need to be protected = so they are packages in sporocyst
    - sporocysts are packaged in oocyst
    - packaging is very characteristic
  - packaging arrangement helps determine genus





black = oocyst

pink = sporocyst

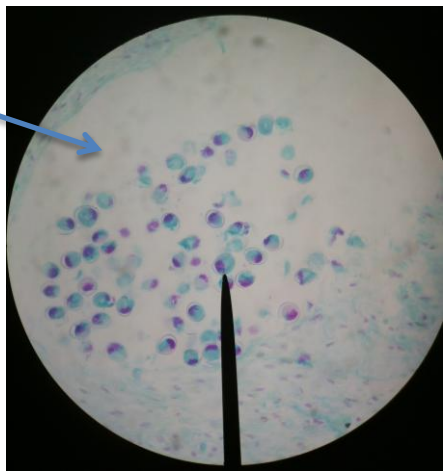
blue = sporozoite

- **MICROSPORIDIA**
  - Very tiny = 5-20 microns long
  - Fungi
  - Spore is typically paired shaped
    - Inside has highly coiled tube
  - Have direct lifecycle = just need one host
  - Can induced xenoma = greatly enlarged host cell full of developing parasites
  - TEM very useful to determine genera
  - Special stains good =
    - Gram stains
    - Acid-fast stain = only stains up fully mature spore
    - PAS = stains up little structure at tip of spore = little red dots

- **MYXOSPORIDIA**
  - Some have indirect life cycles
  - Actinosporean stage in inverts
  - And myxosporean stage in fish
  - Multicellular = metazoans
  - Have polar capsules =
    - Pear shape structures at pole of cell = hatching apparatus
  - Stains imptx =
    - Geimsa really good = stains up polar capsules
    - Sometimes Acid-fast = shows up mature spores
    -

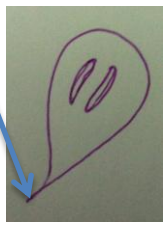
SLIDES

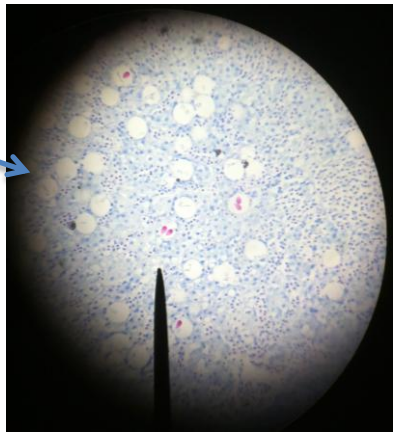
1. Coccidian Aggregata = Feulgen stain



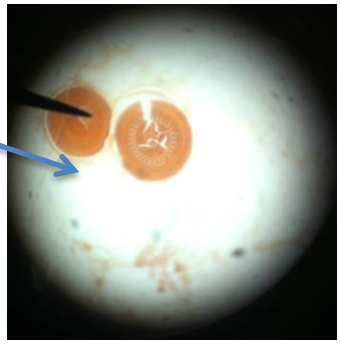
2. Coccidian calyptospora =

- Has a projection = that helps distinguish it from others
- Tear shaped organism = sporocyst with sporozoites inside

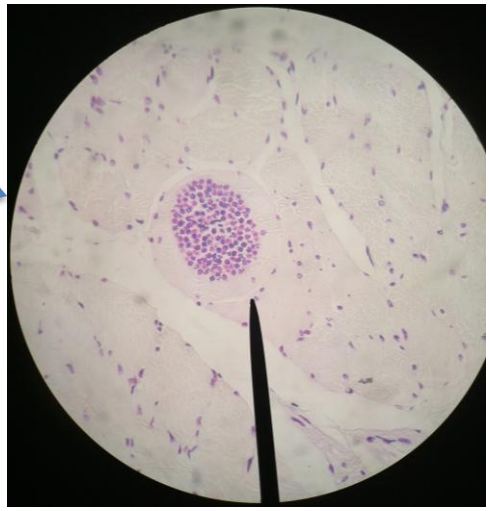




3. Trichodina



4. Myxosporidia = Kudoa spores = Multivalvulida



METAZOANS

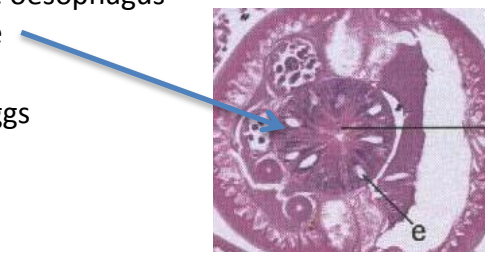
- Bigger than protozoans

- Major groups =
    - Helminths (parasitic worms)
        - Playhelminths (flat worms)
            - Monogenean trematodes (monogeneans) =
                - *Monopisthocotylea*
                - Have specialized attachment structure at posterior end = haptor = opisthahaptor = very imptx from identifying monogeneans =
                    - Might have pair of hooks around edge = gyrodactylus
                    - Some have suckers = large muscular discs
                    - Refractile yellow hook on section
                - Body dorsally flattened
                - Bifurcate gut = Y shapes
                - Hermaphoriditic

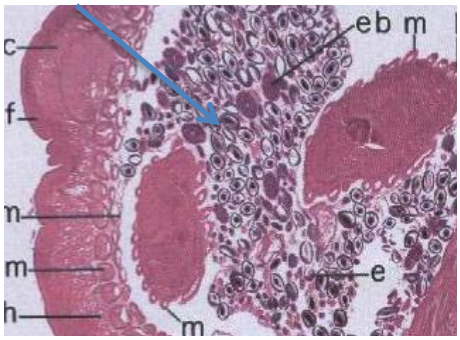
            - Digenean trematodes (flukes) =
                - Some have spiney surface= spine integument = good for traction and attachement
                - Ventral sucker
                - Body filled with spongey material = parenchyma

- Cestodes (tapeworms) =
  - *Scolex = head*
  - Neck
  - Strobili = body
  - *No digestive tract*
  - *No body cavity, but filled with parenchyma*
  - *Don't have a mouth* = don't take in large amounts of food = take in nutrients through body surface
  - Hermaphrodite
  - Perenchyma has calcareous corpuscles = don't really know what they do = granular/refractile purple/grey structures
  - Scolex has difffeatures/diff kinds of holdfast organs
  - See segments = each one has complete suite of male and female repo structures

- Nematodes (round worms) =
  - Easy to recognize
    - Contain most taxonomic info in section
  - No digestive tract posterior
  - No repro structures anteriorly
  - Work for outside in to classify nematode
  - Cuticle = outermost surface, then hypodermis
  - Then move in and look at musculature and structures of body wall, intestine, digestive and repro
  - Ornamentations =
    - Outside of worm (cuticle) can be diff
    - Might have bulb, collar
  - Hypodermis
  - Digestive tract =
    - Longitudinal section = can see oesophagus =
    - If transverse = looks triradiate
  - Female =
    - might have larvae inside or eggs

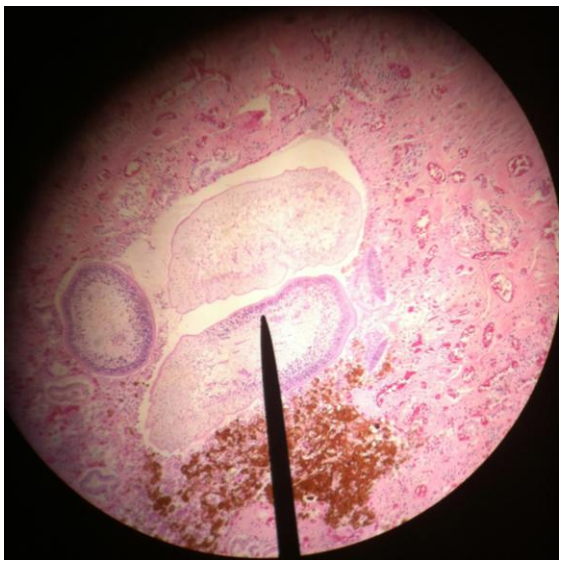


- Acanthocephalans (spiny-headed worms) =
  - Separate male and female
  - Have proboscis = has spines on it
  - In female, most of body packed full of eggs

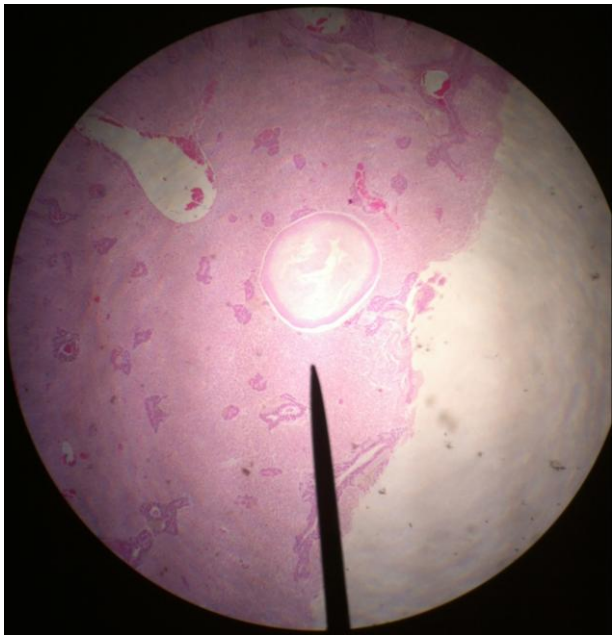


SLIDES

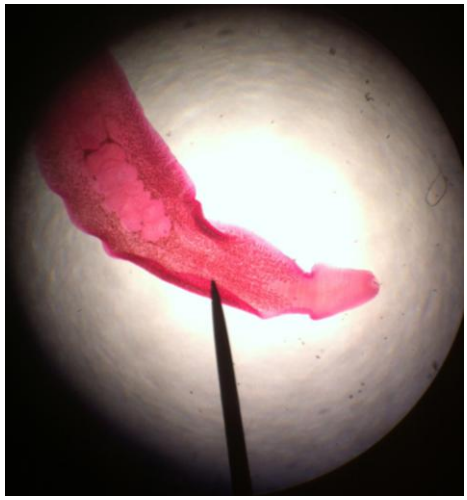
1. Cestode = adult from Pygmy Sperm Whale rectum



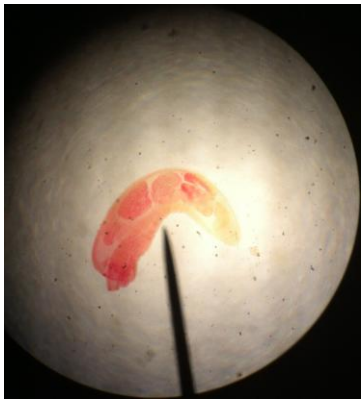
2. Encysted larval cestode from catfish



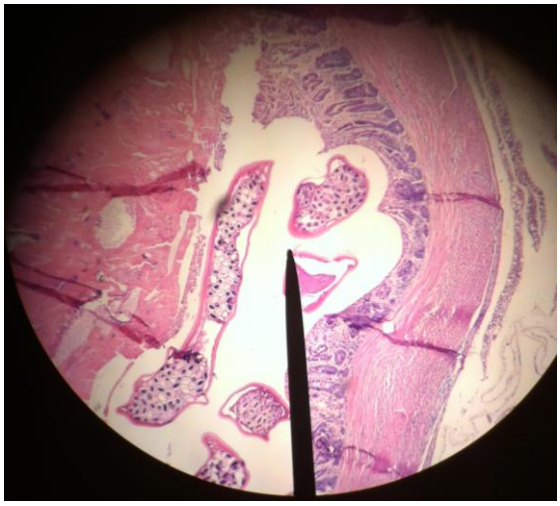
3. Cestode



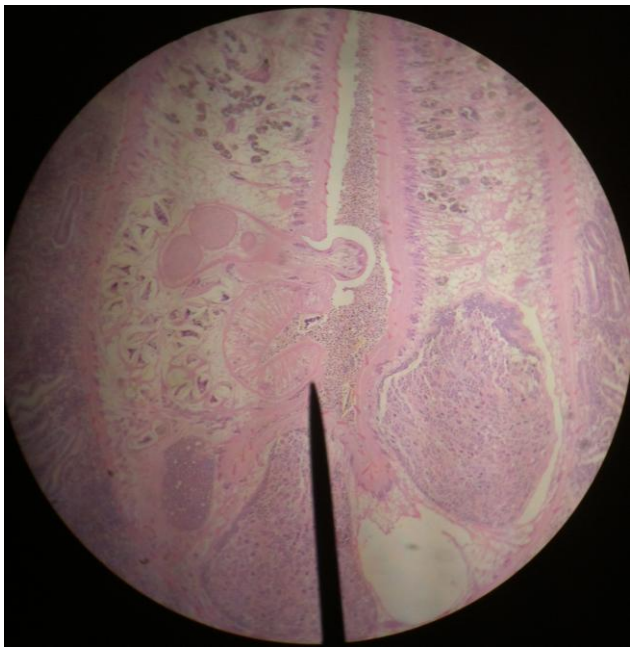
4. Acanthocephalus lucii



5. Adult nematode with eggs



6. Digean = adult trematode and eggs



AQUAVET 2013
My notes

Monday 03/06/13

Aquaculture Vaccines

Dr Rod Getchell

Prezi.com software!

- Annual growth rate for aquaculture is approx. 9% annually
- Wild fisheries have plateaued
- A lot of aquaculture coming out of SEA = family business
- 1 year salmon smoult fry in hatchery = ready to move to saltwater
- catfish dominates as aquaculture species in USA
- Viruses =
    - ISA
    - IPNV
    - Spring Viraemia of Carp
- Fish under 3-4 grams = won't develop immunity to a vaccine, if you vaccinate them then
- IgT = new immunoglobulin in fish
- Asian Seabass vaccination strategy =
    - Can do immersion vaccination
    - but better to do parental vaccinate >20g
- Vaccines imptx in large scale fish farming
- Trial and error to get vaccines to work
    - Getting better as immunology improved
- IHNV = Canada working on this vaccine a lot
- Pharmaceutical companies invest loads of money to get products in market
- Need to pick right antigen to attack when designing vaccines – need to develop challenge model
- Also have oral vaccines
    - Hardest part to manage is getting vaccine past acid part of stomach
- Vaccine side effects =
    - Adverse effects from **oil-based** adjuvants include =
        - Slower growth rate = can affect growth class
        - Surface scarring
        - Spinal deformities
        - And adhesions in peritoneum
        - Can get melanin deposits = MMCs = seem to target injection site
        - Adjuvant usually stays at site of inflammation
- Incidence of these side effects = is approx: 5-10%
- Muscle tells take up plasmid and incorporate antigenic fragment to MHC

- The quantity of DNA in vaccines is so small, but can be effective

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Tuesday 04/06/13

Viral diseases of coldwater fish

Dr Rod Getchell

RNA VIRUSES

RHABDOVIRIDAE = Single stranded

- **IHN = Infectious Hematopoeitic Necrosis**
  - Disease of salmonids
  - CS =
    - Lethargy, anorexia
    - Many CS are similar to other dieases
  - Histo =
    - Attacks kidney
    - Causes necrosis
    - Loss of tissue architecture (loss of tubules), many necrotic cells, loss of nuclei, some fibrin dispersed throughout

- **VHS = Viral Haemorrhagic Septicaemia**
  - Similar CS and IHN
    - Pale liver and kidney = from haemorrhaging
    - Necrosis in hematopoietic necrosis
    - Huge spleen
    - Nervous system involvement = brain tissue that is affected
  - Found in round gobies, rainbow trout
  - Fin clips are good for picking up virus
  - Histo =
    - Lots of MMCs
    - Decreased lymphoid cells

BIRNAVIRIDAE = Double stranded RNA virus

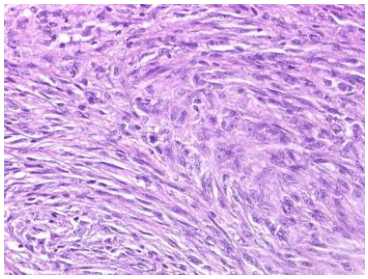
- **IPN = Infectious Pancreatic Necrosis**
  - Multiple hosts
  - Affects really young like IHN
  - Classic look of fish = fish are spiraling
  - Abdo distention
  - Pop eye
  - For larger fish = not as many morts as young fish
  - Haemorrhaging in abdo fat
  - Pale liver, haemorrhagic omentum
  - Necrosis of pancreas, but also see in kidney and spleen

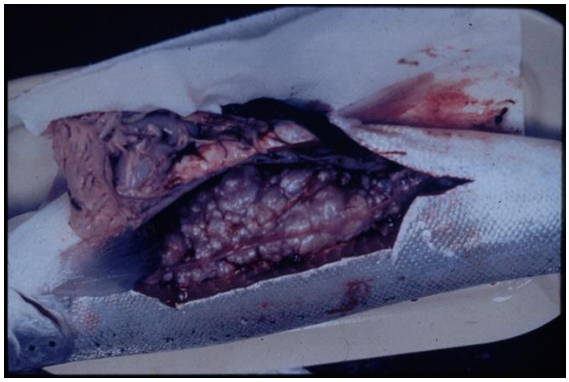
ISAV = Infectious Salmon Anaemic Virus

- Highly infectious for Atlantic Salmon
- Orthomyxoviridae
- Gross signs similar to others
- Anaemia
- Transmission =
    - occurs during salt water rearing
    - horizontal
    - coprophagy
    - sea lice as vector
    - transfer by contaminated equipment, people, boats…
- Histo =
    - See sinusoidal congestion
    - Renal tubular necrosis
    - Hepatocellular necrosis

Atlantic Salmon Swimbladder Sarcoma = Dr Bowser is the world expert

- First seen in Scotland
- Older fish affected
- Chronic morts
- Lumpy abdomens
- Want morts <1%



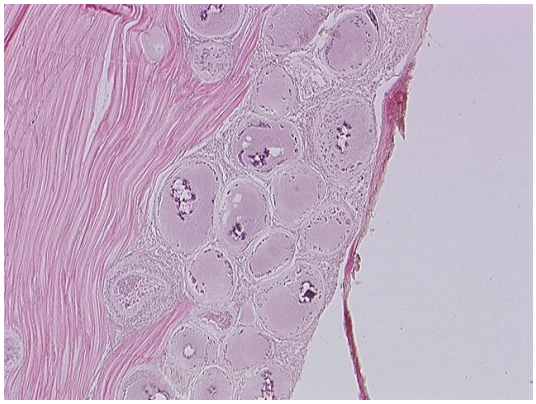


DNA VIRUSES

HERPES VIRIDAE

- **Lake Trout Herpesvirus**
    - Severe epizootics of fingerlings/yearlings
    - Also called EED = epizootic epitheliotrophic diease
    - See corckscrew diving in fish
    - See flashing in fish
    - Infiltrate of lymphocytes and macrophages
    - See in Maine state

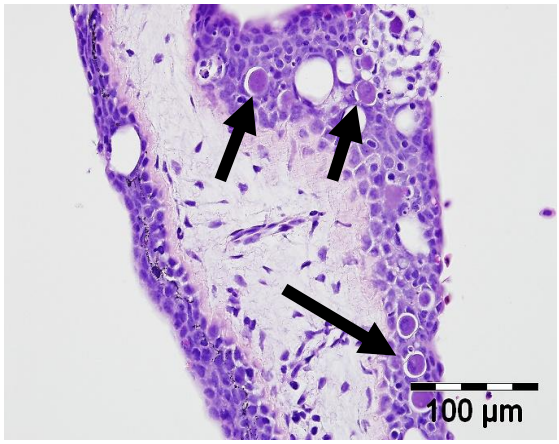
- **White Sturgeon Herpesvirus**
    - Occurs in California
    - Morts up to 50% of juvenile fish
    - Occurs in rapidly growing, healthy appearing fish
    - Usually no gross external signs
    - Ron Hedrick = Davies Uni California = did lot of work on these viruses
    - Get hyperplasia and hypertrophy of epithelial cells



IRIDOVIRUS

- **Lymphocystis virus**
  - Very common virus
  - Hypertrophied cells
  - Like sand paper = nodules
  - Can prove with histo – this is diagnostic
  - Lesions can be really severe or just little nodules
  - Single cells become so big, you can actually see them grossly

- **White Sturgeon iridovirus**
  - Original source believed to be wild fish
  - Pale liver
  - Emaciated fish
  - Hypertrophied cells
  - Sometimes you can see inclusions on histo



Blue book = diagnostic techniques
American Fisheries Society
Every 2-3 years = come out with new DVD
Has dx techniques and sections that describe dz
AFS web page = can order that DVD

Tuesday 04/06/13

Diseases of Coldwater Fish = Protozoa, fungi, mesomycetozoea and non-infectious

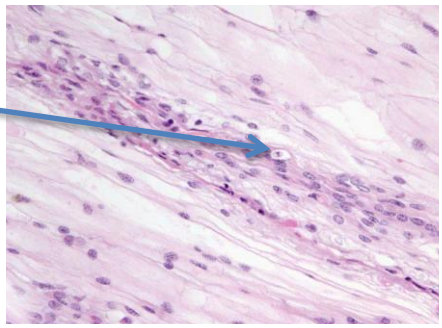
Dr Sal Frasca ~ University of Connecticut, Dept of Pathobiology

- Emerging disease = increasing in incidence in an area or a new disease that has not been seen in an area before
- Understand how diff organs respond to disease and how their components respond to injury

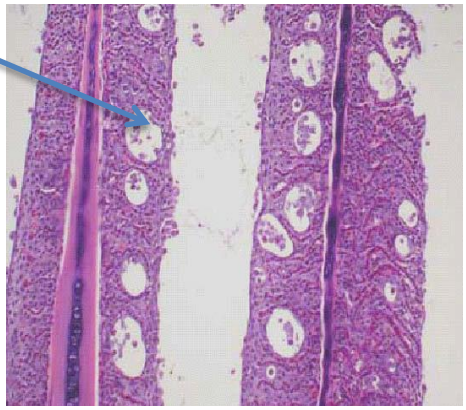
PROTOZOAL DISEASES

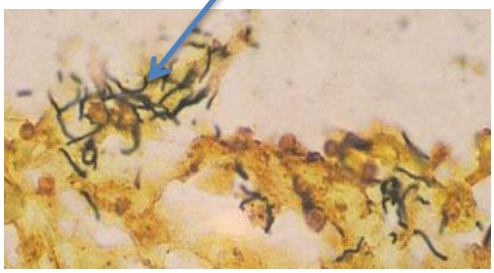
- **Amoebic Gill Disease (amoebic bronchitis)**
  - Atlantic salmon
  - *Neoparamoeba pemaquidensis*
    - Paramoeba
  - Intracytoplasmic endosymbiotic organism
  - The same agent found in lobsters
  - Destruction of gill =
    - Elevated sodium levels due to obstruction of electrolyte exchange across branchial epithelium which preceeds behavioural change
    - Lesions =
      - Occur during increases in temp =
        - Agent is more active

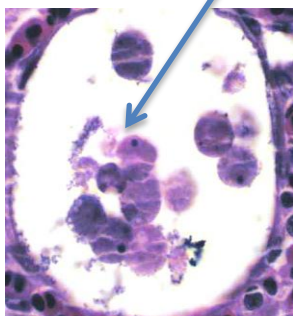
        - And host is more metabolically/physiological stressed
    - Histo =
        - Interlamellar filling by hyperplastic filling and bridging of lamellae, organisms that look like cobblestones on surface of gills = flattened on surface of gills
            - *Picture in Noga*
- Paramoeba
    - Parasome always has kidney bean shape
- There is an infiltrate of inflammatory cells in nerve here



- **Nodular Gill disease**
    - Focal nature of lesion
    - Hyperplastic response of epithelium
        - Synechiae form on tips of lamellae
            - Pseudovacuolar space = tunnels that form as a consequence of synechiae that form subsequent to epithelial hyperplasia
            - attached cells in vacuoles and amoeba
    - Associated with poor water quality
    - Bacteria are common in this scenario



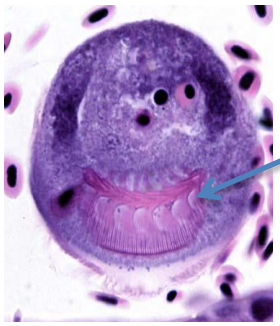




- **Ichthyobodosis**
    - Was called 'Blue slim disease'/'Costiasis'
    - *Ichthyobodo necator* = was called *Costia necatrix*
    - Histo = irritation to gill epithelium =
        - Spongiotic change
            - Intercellular oedema in epithelium
        - Also hydrophic degeneration as well
    - Lifecycle = freeswimming parasitic stage =
        - Long and short flagellum
        - Parasitic stage = has attachment disc
        - Presumed encystment in environment
    - Crowding, increased organic loads, increased ammonia and decreased pH, temperature between 10-25^{o}C favours parasite repro = predispose
    - Always look for vascular changes in lamellae = to help you work out what is going on
    - Gill histo changes are typically of irritation =

        - Synechiae
        - Hyperplasia
        - Pseudovacuolar formation
    - flagellum = tethers organism to epithelium = followed by attachment

- **Trichodiniosis**
    - Row of denticles
    - All cultured fishes are susceptible
    - Agent attaches to surface of the fish
    - Attachment disc = screws into host epithelium
    - Eosinophilic arrangement of fibres on **ventral** surface = denticle
    - Protozoan has a mouth oriented away from attachment disc = uses fish to move itself around, then eats bacteria and organic debris from environment = uses cilia to beat organisms into mouth = feed on what is in enviro, rather than the fish



    - Dz often seen in conditions of poor water Q
    - Tx of protozoan dz =
        - Topical agents =
            - Formaling
            - Copper sulphate
            - Designed to kill parasite whilst minimizing damage to host
        - Improve water quality

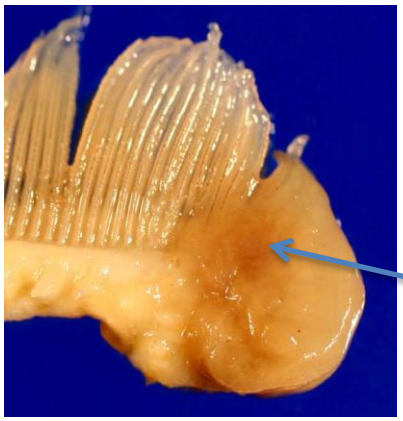
FUNGAL DISEASES

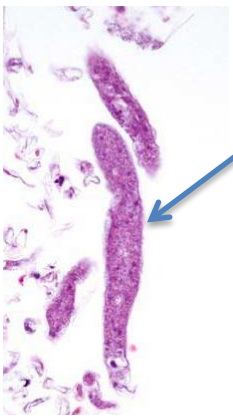
- Emerging diseases are the world
- Either see a dz that hasn't occurred in a location, or see a dz that has occurred before, but occurring at bigger frequency than expected.
- MICROSPORIDIA are now in the fungi class = as a result of molecular studies = used to think these were protozoa
- *AN ASIDE NOTE = Myxozoa = used to think these were protozoa = actually metazoan parasites = closely related to jellyfish*

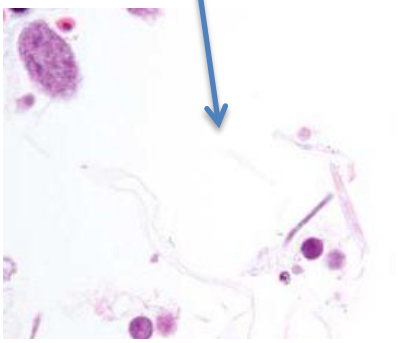
- **ASCOMYCOTA**
    -

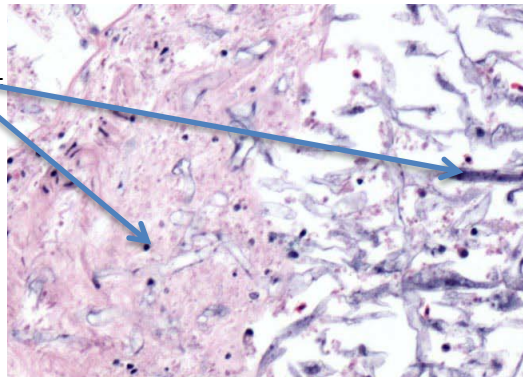
- **OOMYCETES**
    - Mastigomycotins =
        - ***Saprolegniales* = Saprolegnia** = *Fungus related or like organism*
            - 'Water mould' or 'cotton mouth'
            - Winter kill in catfish industry
            - Blossoms as fish are stressed
            - Multifocal raised white cotton like plaques on surface of skin and on gills

- Histo = begins with erosion that progresses to ulceration, superficial necrosis, begins from outside and moves inwards
- **Aseptate** = don't have true walls or cross striations
    - Thick-walled hyphae
- All oomycetes =
    - have motile biflagellate spore
    - asexual repro occurs by production of zoospores
- **IMPTX = determine in all fungal cases if hyphal form is septate or non-septate**
- Typically thought to be secondary pathogens = but under certain situations, may be primary =
    - Lesions associated with heavy bacterial growth or where bacteria advance injury = probably situation where bacto played more significant role
    - Primary sapro infections = tend not to have bacterial involvement
- Zoospore – is infective stage
- Temp has huge affect = when declined to low physio range of host = many sapro species are more active = some of hosts are stressed
- Skin and gill major sites of infection
    - Matt of thick yellow slime on gills
    - Blue dots = pyknotic nuclei
    - hyphae here
- look at structure of zoosporangia =
    - take on diff shapes
    - can see zoospores inside
    - transverse zoosporangia





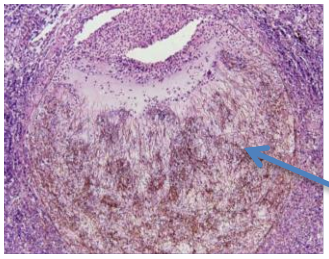


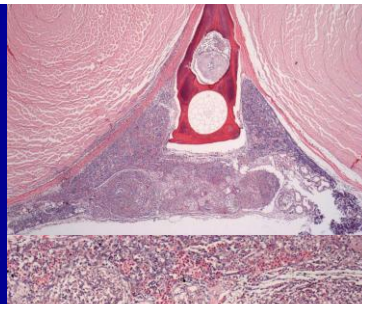


- **Branchiomyces** =
  - Gill rot
  - American and European eels very susceptible
  - Also occurs in pond species
  - Often acute and high morts
  - Because lesions occur in the gill
  - Fish display increased respiratory effort
  - Infarcted necrosis of gill
  - Non-septate hyphae
  - Alanospores
  - Another water mould
  - Infection thought to be transmitted by spores released into enviro

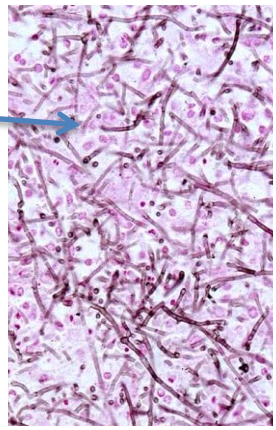
- **Phoma**=
  - Lesions located in swimbladder
  - Affected fingerlings
  - Organism travels up pneumatic duct into swimbladder
  - Occurs in physostomous fishes rather than physoclistic
  - Organisms = Phoma herbarum = Coelomycete = asexual state of an ascomycete

- **Phaeohyphomycosis** =
  - Black mould or black yeast infection
  - Seen in fish and humans
  - *Fungi can be hyalin (clear hyphae that are non-melanised) or phaeloid (melanised) = imptx to discern this difference*
    - *Not all hyaline stages are not melanised*
    - *Production of melanin is imptx = virulence factor*
    - Like calling a bacteria gram +ve or –ve
  - Salmonids, ornamentals, seahorse, sea dragons
  - Cutaneous and systemic forms =
    - Cutaneous = ulcers, cavitation with black ulcers
    - Systemic = multiple foci in multiple organs
  - Histo =
    - Hypo and dermal invasion
    - Blood vessel invasion = *angioinvasion*
    - Ulceration
  - Organisms is slender, filamentous and septate
  - Organism is brown
  - Diff types of organisms causing phaeo..
  - All = mycelial
  - Common environmental organisms
  - Dr Sal has experience with Exophiala



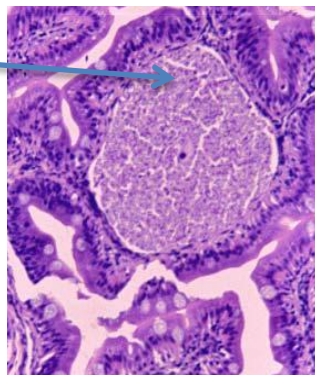


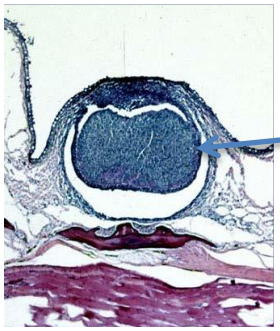
- Basically large areas of necrosis
- example of angioinvasion
- proteinaceous oedema leaks out
- lesions grossly =
    - sharply demarcated
- can culture organism
- slender, brown, parallel walls, septae
- always stain with **Fontana masson**
- contaminant fungus = non melanised

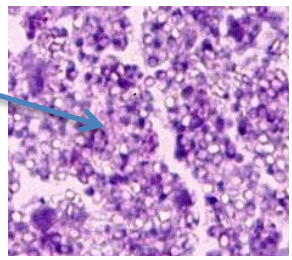


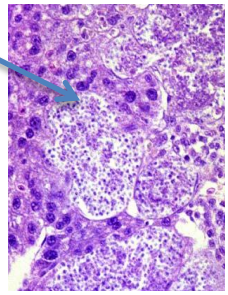
* Notes are shifting from organisms that's have hyphae to **microsporidia = don't form hyphae, obligate intracelluar pathogens**

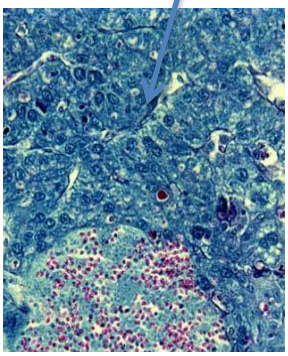
- **MICROSPORIDIA** =
    - Multiple in cytoplasm of host cell = cause host cell to get larger and larger until it become membrane bound structure = xenoma = thin walled cystic structure
    - Xenoma might cause damage to wall
    - Excrusion of antigen of spore stage organisms = incites host inflammatory reaction = granulomatous
    - Freshwater and saltwater fish
    - Can be incidental
    - Get lesions in muscle = decreases value of fish
    - Gross = multi-centric 1-3mm raised white nodules usually in skin
    - Host tissue reaction is largely towards antigens of spore stage
    - Typically there can be no infam reaction with xenoma
    - Has a direct lifecycle =
        - In closed systems = possibility for amplification
    - Host cell nucleus = remnant
    - Mature stages of spores =
        - Can be acid fast =
            - organism = Glugea
    - xenoma in liver
    - Can use PAS stain

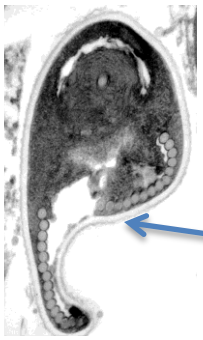




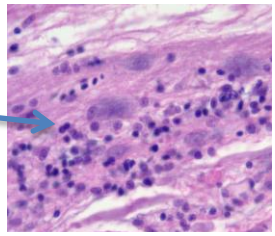


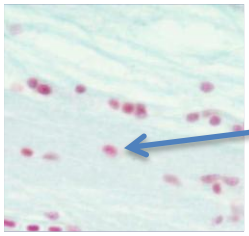






- Microsporidial spores contain no mitochondria
- Thought they have evolved to be obligate intracellular parasites =
- Organisms has exo and endo cuticle
- Zebrafish can get this in spinal cord =
    - Pseudoloma neurophila
- Microsporidia always look like single cell =
    - Look like head of eraser!
    - Whereas myxozoa are multicellular
- Can be stained with gram stain
- always have polar vacuole

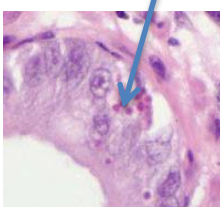


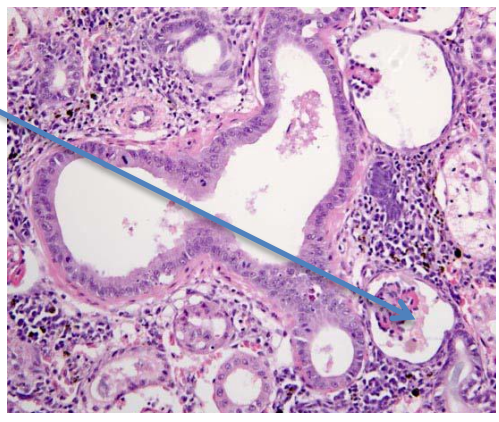


MESOMYCETOZOEAL DISEASES

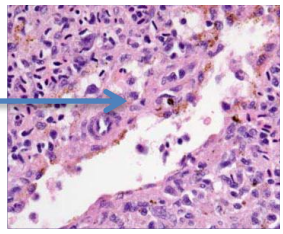
- In between animals and fungi
- Molecular evidence shows they lie between fungi and animals

- **ROSETTE AGENT =**
    - Pathogen of salmonids = particularly Chinock = typically freshwater situations = post-smoults coming back in
    - Have id in cyprinids
    - Experimental transmission in other salmonids
    - Natural infection occurs in pacific northwest
    - Sub-adult and adult fish are hosts of this = morts can be severe = 90%
        - Usually highest in autumn and summer
    - Organomegally typically observed
    - Two patterns of host response =
        - Disseminated = systemic distribution of parasite = liver, kidney, spleen
            - Minimal host inflam reaction
        - Multi-focal or nodular pattern = kidney, spleen, liver
            - Organism restricted to granulomas
            - Tubular necrosis is kidney
            - Long lived lesions
            - Membraneous glomerulopathy
    - New name = *Sphaerothecum destruens*
    - Within family Mesomycetozoea
        - Dermocystium
        - And Rhinosporidium seeberi
    - Rosette agent produced unique uniflagellated zoospore
    - Agent also released from bile, urine, repro fluids during spawning
    - This organism is hard to manage
    - Dilation of tubules and glomeruli spaces
    - closeup = clusters of rosette agents

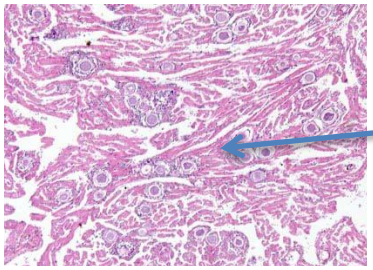




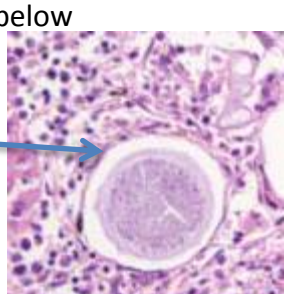
- organisms present in cytoplasm of cells
- organism in mononuclear phagocytic cells of spleen

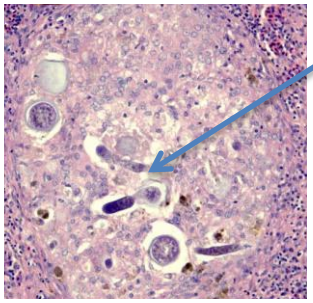


- **ICHTHYOPHONUS =**
  - Used to be thought of as a fungus
  - In salmon = called 'swinging' disease
  - Endemic infectious in feral cold water marine fish populations
  - Gross = shallow skin ulcers, emaciation, sand-paper like texture
    - Have multiple granulomas in dermis and hypodermia
    - Raised white nodule lessions on serosal serfaces of organs and parenchyma of viscera
    - Occur in highly vacularised tissues = kidney, heart, liver, spleen, hypodermis
    - Granulomas centred around spores
      - Spores have double contoured wall
    - Organism = *Ichythyophonus hoferi*
    - Member of DRIP CLADE organisms = Ichthyosporea
    - There are diff life cycle forms of this organism
      - Produce plasmodia
      - Multi-cellular spores
      - Even has amoebic motile stage like a protozoa at high pH = thought to be infective stage
      - Produces hyphae under conditions of low pH



    - Sometimes CNS infection occurs
    - Heart with thin walled granulomas centred on spores just below endothelium and proliferating
      - Endothelium of heart = is phagocytic
    - double contoured wall of granuloma
    - granulomas form around spores
    - ***granulomatous inflam around double contoured spore***
    - granuloma in spleen with hyphae formation
    - Dr Sal rarely sees amoebic form =
      - This form has been elusive in animals
    - This is only mesomycetum that can be cultured

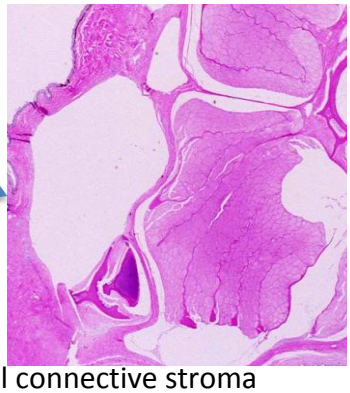




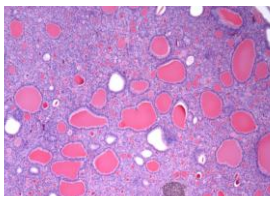
NON-INFECTIOUS DISEASES

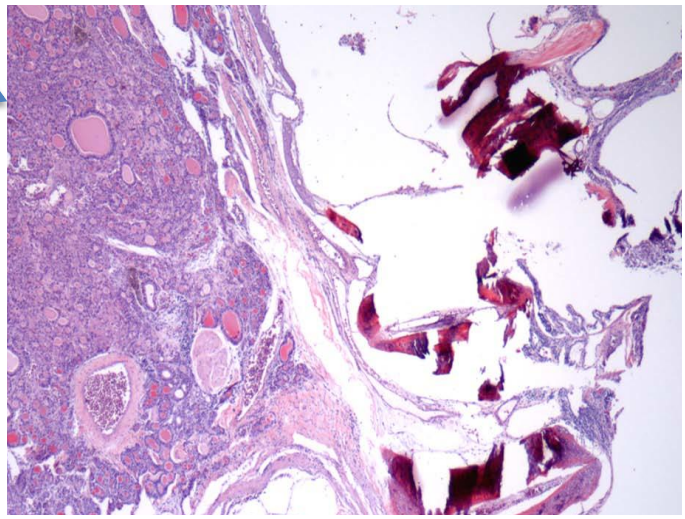
- **GAS BUBBLE DISEASE =**
  - Choroid rete of eye
  - Supersaturation of water colun from nitrogen or oxygen
  - In subadults = see gas biubbles in eye, skin, mouth and swimbladder
  - Increased temps = release gas bubbles as DO increases
  - Leaks in plumbing

- Water can be drawn from below
- Typically lesions in skin and blood vessels
- intraocular emphysematous bullae



- **IODINE DEFICIENCY =**
  - Goiter
  - Culture salmon can have endemic goiter
  - Clinically = anorexia, lethargy, dysphagia
  - Thyroids tissue = Disseminated along hypobranchial connective stroma along margins of ventral aorta
  - Gills get pushed back and out = gills start to protrude outside posterior margin of operculum = this gets persistent flaring = incapable of feeding from bulge of goiter that has formed
  - Histo = follicular hyperplasia
  - Grossly = swelling that occurs at gill arches fro base of tongue to heart
  - Reduced thyroxine synthesis = increased TSH secretion = leads to follicular hyperplasia
  - Size of goiter = depends on severity and duration of iodine deficiency
  - Mets do appear in these cases
  - Systemic effect of TSH = where there were small areas of ectopic thyroid, now there are hyperplastic areas of thyroid
  - These are generally cases of hyperplasia rather than carcinoma
  - Iodised salt can help tx condition in fish food
  - Similar problem occurs in sharks in aquaria
  - Mass is all thyroid with follicles (homogenous material – colloid)
  - typically see in aquaria fish =
    - individual or groups
  - most are saltwater fish
  - majority are tropicals



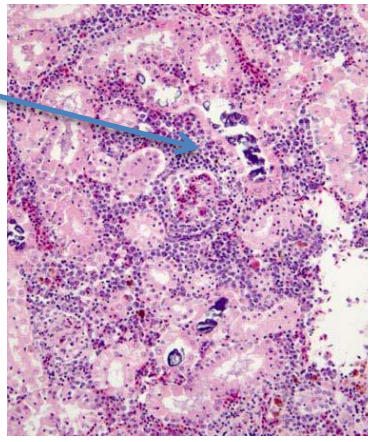


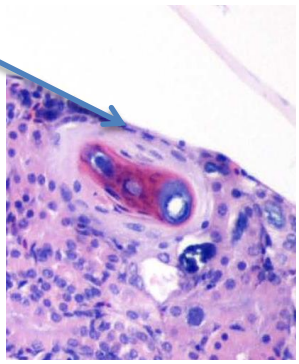
- **VIT C DEFICIENCY =**
  - Broken back syndrome
  - Scoleosis, lordosis
  - Vertebral fractures
  - Dysplasia and deformity of bone
    - And gills rays
  - Callous formation
  - When no Vit C = weak collagen
  - Weak organic matrix to bone =
    - Type 1 collagen = vertebral abnormalities

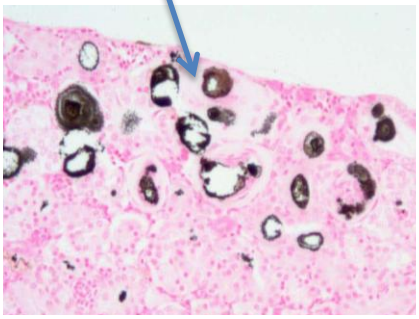
- **PANTOTHENIC ACID DEFICIENCY =**
  - Nutritional gill disease
  - Thickened and club gill filaments
  - Dr Sal never seen this lesion
  - Characteristic = hyperplasia of gill epithelium on 2/3 of gill lamellae

- **AFLATOXICOSIS =**
  - *Aspergillis flavus* and *Aspergillus parasiticus*
  - Seen commonly in rainbow trout
  - Chronic morbidity
  - Usually hepatomegaly
  - Toxin induced neoplasia
  - Initiating factor = switch from wet feed to dry pelleted ration
  - Some fish species highly susceptible = Rbow trout = seems to be most susceptible of salmonids
  - Chanel catfish are far less susceptible
  - Agent = aflatoxicol = carcinogenic metabolite of AFB1 molecule
    - Major metabolite that is formed in Rbow trout
    - They make the toxic carcinogenic metabolite

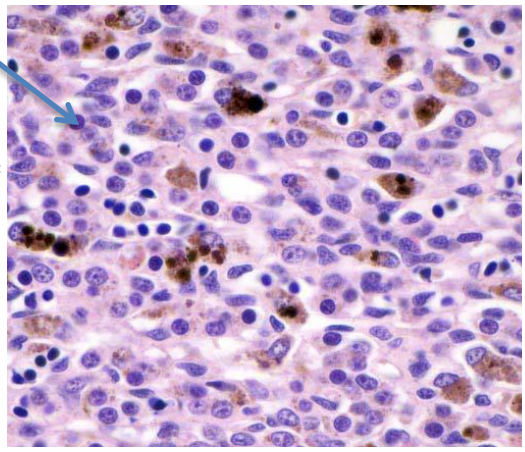
- **NEPHROCALCINOSIS =**
  - Urolithiasis
  - Renomegally
  - Low morts
  - But at end of dz = when loss of calculi formed = coelomic distension in severe cases
  - Grey white chalky deposits in renal parenchyma
  - Ductular ectasia and granuloma formation
  - There can be fibrosis that occurs
  - Can see mineral calculi in gastric lamina propria
  - High levels of CO_2 thought to predispose or inappropriate levels of Ca and P
  - Usually dz of intensive aquaculture
  - Posterior tubules with mineral calculi with some degeneration and necrosis of tubular epithelium
  - granulomas can form around tubules
  - Von kossa stain =
    - stains cation salts = appear black
    - Highlights mineral



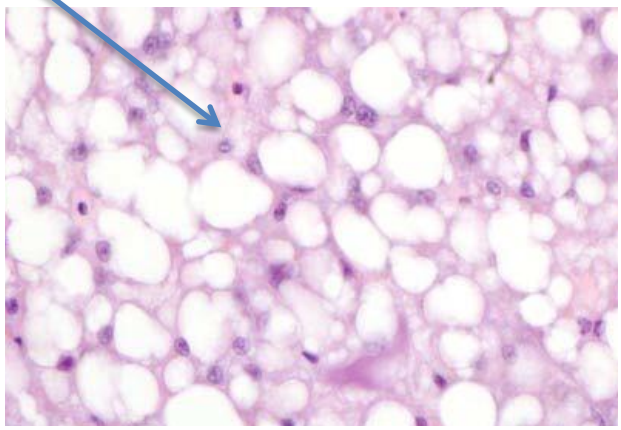




- **HAEMOSIDEROSIS =**
  - Marine and freshwater
  - No specific clin signs
  - Unknown whether a result of iron storage or metabolic defect in iron processing
  - Haemosiderosis = Stainable iron in absence of architectural changes
  - Haemachromatosis = architecture changes as a result of iron toxicity
  - Brown granularity in hepatocytes
  - Prussian blue stain = stains Fe blue
  - Sometimes use stains to help detect parenchymal collapse = trichrome stain and reticulum stain
  - don't really see in wild fish



- **HEPATIC LIPIDOSIS =**
  - Difficult to make general statement of lipid in fish =
    - Can vary depending on species
    - Repro status
    - Dietary intake
    - Behavior of fish
    - Gender
    - Age of fish
    - Whether exposure to toxins
  - Clear sharply demarcated clear vacuoles in cytoplasm, nucleus pushed to periphery
    - However this is normal in the shark
    - Worry about little lipid in sharks



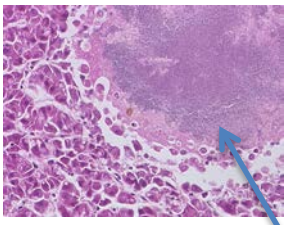
AQUAVET 2013
My notes

Tuesday 04/06/13

Bacterial Pathogens of Salmonids

Dr Mark Fast

- **Gram negative bacteria =**
  - **Furunculosis = Aeromonas salmonicida subsp. Salmonicida =**
    - Intracellular bacteria =
      - Gets taken up by macrophages and survives well in those
    - Non motile rod = differs from goldfish = goldfish is typical
    - Don't see lumps in chronic form of disease
    - A lot of abx resistance
      - But Abx use has actually declined
    - There are numerous vaccines = effective
    - Signs =
      - Fish darkened, lethargic, wide spread haemorrhages
      - Can get soft kidney = friable
      - Swollen spleen
      - Get fluid filled furuncles in chronic form = dead neuts
      - Affects wild and hatchery salmonids
      - A number of strains
      - Both acute and chronic forms
      - Can result in quite high morts
      - Carrier fish
      - Can get exopthalmia



    - Enviro modulators =
      - Changes in water quality, temp, salinity, transport stress
    - Histo =
      - Foci of bacterial colonies surrounded by necrotic areas
    - Gives off brown pigment when grown on TSA
    - Haematopoietic tissues affected
    - Also causes oedema and necrosis

  - **Enteric Red Mouth = Yersinia ruckeri**
    - Exopthalmia
    - Hyperpigmentation
    - Anorexia, lethargy
    - Reddening around mouth, lower jaw
    - Additional haemorrhaging = eye, fin bases and body surface
    - Petechial haemorrhaging throughout coelomic cavity

- Free fluid accumulation
- Enlarged spleen/kidney
- Histo =
    - Generalized bacteraemia
    - Congestion, haemorrhage, inflam, necrosis

- **Coldwater Disease (BCWD)**
    - Cytophagia – Flexibacter – Flavobacterium
    - Rainbow trout fry syndrome
    - Associated with colder water temps
    - Peduncle or saddleback disease = older fish
    - Younger fish = systemic disease and higher morts
    - Generally passed horizontally
    - Strains vary in pathogenicity
    - Oytet baths for small fish and oral t as they get bigger
    - Similar signs to F.columnare

- **Flavobacterium branchiophilum**
    - Causative agent of bacterial gill disease
    - Clear signs of resp distress
    - Feed refusal and lack of a flight response
    - Can see filamentous bacteria associated with gills
        - On wet prep and histo

- **Gram positive =**
    - **Bacterial kidney disease**
        - Renibacterium salmoninarum
        - Obligate intracellular bacteria
        - One of few g+ve pathogens
        - Cull broodstock if they have this infection
        - Commonly see greyish lesions in the lesions with a lot of granuloma formation
        - Infects many species of salmonids
        - Bacteria is difficult to culture, to dx and tx
            - Take a long time to grow up on media
        - Can do IFAT test = P57 antibodies on squash prep
        - Often fish appear clinically normal
        - Very severe abdo distension
        - Nodules within kidney
        - Solation of reni on agar plate = definitive method for confirming BKD
        - SKDM (Selective Kidney Disease Medium) media is good
        - Also ELISA
        - In terms of host response = can get diffuse granulomatous response
        - May have fibrous tissue capsule

- Intracellular = phagocytosis of bacteria
- Agent resists TNF-a mediated killing by macrophages
- Enhanced MH class II responses = this should be good for an intracellular pathogen
    - Immunomodulatory implications
- Constant inflammatory influx

- **Mycobacterium =**
    - *M.marinum* (zoonotic), *M.Shotsii, M.pseudoshotti, M.fortuitum*
    - Acid-fast, non motile
    - On necropsy = gross lesions of viscera consist of grayish white, necrotic foci that sometimes coalesce to form tumour like masses
    - See in striped bass
    - When you cut intestines open in a striped bass = see Pomphorhynchus laevis infection = allows bacteria to get in

Tuesday 04/06/13

OIE Reportable diseases in finfish ~ Mostly viruses
*** Except Gyrodactylus and Alphanomyces**

Dr Mark Fast

- **Gyrodactylys salaris =**
    - Monogean trematode
    - Freshwater parasites
    - Common ectoparasites
    - Economically/ecologically imptx
    - Affects Atlantic salmon in Norway
    - Size range = 0.3-20mm
        - G.slaris = 1mm
    - Feed on blood, mucus and epithelial cells of host fish
    - Single host life cycle
        - Skin and gills = mainly skin
    - Can use PCR and sequencing to id species
        - rDNA
    - often quite host specific
    - ventral opisthaptor
    - viviparous = reproduce quickly =
        - can have sexual ad asexual repro
        - can sometimes see three generations when looking under scope
    - cannot survive in SW for long
    - horizontal transmission

- cause severe stress and osmoregulatory difficulties/impairment
- untreated fish can harbor parasites

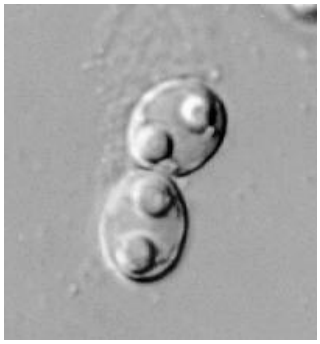
- **Digenes =**
  - 2 lifecycle
  - can be intermediate or definitive hosts
  - usually, these are incidental findings
  - *Centrocestus formosanus* = gill parasite eliciting cartilage proliferation
    - Fish is 2nd intermediate host
    - Larvae released, picked up by snail, infection of darta with metacercariae and then get nasty infections in gill of darta, then taken up by final host
  - Gross signs =
    - Filaments are shortened
    - Gill epithelial hyperplasia
    - Gill cartilage deformed
    - Flaring of operculum
  - Histo =
    - Cercariae migrate outside gill cartilage = and distort architecture = loose effective gas/ion exchange

- **Parasitic Copepods =**
  - Imptx economically
  - Family Caligidae = commonly known as sealice
    - *L. salmonis*
  - > 1400 siphonostomatoid parasites
  - look for paired egg sacs off parasite
  - cost for effective tx is high
    - SLICE treatment stopped working = avermectin
      - Was a one week in feed tx
      - Was about 90-95% effective
      - Protected fish for several months
  - Was called sealice infection = as lice congregate around head and anal fin = parasite feeds down to the skull
  - Dx = diagnostics are much easier
  - Tx =
    - Organophosphates
    - Formalin
    - FW/SW baths
    - Pyrethroids
    - Ivermectins
    - Avermectins
    - Moulting inhibitors = Teflubenzuron
      - These organisms are crustacean, so need to moult
    - Resistance is major issue
  - In some cases, only female is parasitic

- Host can react to feeding site or attachment site =
  - Parasite can use this to its advantage = feeds on epithelial cells when there is hyperplasia
- Often sexual dimorphic and size dimorphism
- Not all ectoparasites
- Mytilicola = affects digestive gland of bivalves
- Order: Poecilostomatoida = gill maggot
- Ergasilids
- Ommatokoita elongata = infection of cornea resulting in thickened regions = usually the left eye = 99% of the time
- An differentiate via frontal filament
- In resistant hosts = see extensive inflam and hyperplasia around parasite = host encapsulates parasites and kills it in a few days
- Coho (*Oncorhynchus kisutch*) salmon are resistant host
- Try and tx in April and May
- Parasite dies off over winter
- Tx = (these are not legal in every country) =
  - Hydrogen peroxide = can only use a certain temps, environmentally sound, used in Canada
  - Emacetin benzoate
  - Pyrethroids = used in UK and Norway
  - Chitin synthesis inhibitors
  - Organophosphates
  - Biological control = Wrasse
  - Functional feed = immunostimulants

- **Protozoa =**
  - Oodinium = FW
  - Amyloodinium = SW
  - Velvet/Rust disease
  - Have chloroplasts
  - Can go dormant
  - 10-14 day lifecycle
    - similar to ich

- **Myxozoa =**



  - Bivalvulids = Myxobolus = has intermediate host = develops through tubifex worms
  - Multivalvulids = Kudoa = parasites releases proteases = fillet turns to liquid = difficult to assess = muscle trophism
  - Whirling disease is best described = *Myxobolus cerebralis*
    - Requires immediate host = tubifex tubifex = grows in muddy conditions

AQUAVET 2013
My notes

Wednesday 05/06/13

Conundrums

Dr Paul Bowser

- **Mississippi catfish pond slide =**
  - Cystic structures = with about 200-300 henagya inside
  - Objectives give you different magnifications
  - Cellular
  - Henneguya

    - Metazoan parasite
    - Myxosporean
    - Not motile
    - Has two tails with polar filaments
    - Caused = secondary lamellar fusion, epithelial hyperplasia, interlamellar filling, epithelial necrosis
    - Approx. 12 micron in length
    - Most myxosporeans have complex lifecycle
    - Lifecycle = parasite gets into fish, kills fish, then goes into Dero digitata worm, then another life form emerges
      - Similar life cycle to whirling disease
      - Most myxosporeans have a lifecycle that involves aquatic inverte

- **Large mouth bass raised at technical high school in closed circ aquaculture system =**
  - Bowmans space is dilated, as are tubules
  - Sloughing of epithelial cells into the lumen
  - Strange looking glomerular tuffs
  - Some sort of metazoan parasites in longitudinal section
  - Ectatic tubules = markedly dilated tubules
  - Some eggs with refractile brown cell
  - Does not have a pseudo coelom = so most likely to be trematode = digenean =
    - This parasite in section, looks quite complicated
    - To identify digenean =
      - Worm has ventral sucker
      - With gonads going down the side
      - Monogenean parasites are moslt external parasites in fish:
        - Same size as digean
        - Has haptor on end = might have one or two pairs of anchors or no anchors
        - Some can be live bearers or egg layers

        - Monogenean have no coelom = same a digenean
    - This worm has spikes when put finger under microscope to make refractile =
        - This worm is actually a monogenean where the haptor has hooklets = *Acolpenteron*
            - One of the few monogenean that is internal = has predilection for kidney
            - Has been found in large mouth bass several times within closed recirc systems
    - Spiney headed worm has proboscis = acanthocephalan = has pseudocoelom = so this is not this worm

- **Case involved lots of species of trout at game reserve, owner declined to do fsh health inspection =**
    - Gills
    - Geimsa stained slide
        - Helps stain microsporidia
        - Parasites
    - Incoming state has legislative authority/responsibility for health status on any animals being brought into the state
        - But an aquarium or garden pond with rubber is not considered waters of new york state
    - Pathogens that you are typically required to inspect trout for moving across states in USA =
        - **Bacteria =**
            - Aeromonas salmonicida
            - Bacterial kidney disease = Renibacter salmoninarum
            - Yersinia ruckeri
        - **Viruses =**
            - VHSV
            - (ISA) only if fish came from Maine
            - IPN
            - IHN
        - **Parasites =**
            - C.shasta
            - M.cerebralis = can see in cartilage = Whirling disease
                - Look like 'deer hoof prints'
    - IPN and Whirling disease were found in this instance

- **Wild carp submitted by New York State DEC: massive carp mortality event =**
    - Faced lots of VHS in these before and Koi Herpes virus (hallmark lesions is necrosis and proliferation)
    - Gills
    - These animals were negative for PCR Koi herpes virus
    - This case occurred during summer time
    - Some secondary lamellar fusion, hyperplasia, cellular debris, might be an inflam infiltrate

- Diseases causing extensive epithelial hyperplasia
  - Ichthyobodo is attached to outside of gills
- This is a very severe COSTIA infection
  - Tear drop shaped parasites with flagella
  - Flicker like a candle flame
- Costia is non-host specific
  - Size = about same size as RBC
- Use 25 or 40X objective to find them
- Common name = blue slime disease

- **Zebra fish =**
  - Mycobacteria
  - Pseudoloma = microsporidia = has predilection for spinal cord
  - Pseudocapillaria = nematode
  - ZN = stains these well
  - Always check for these in spinal cord

Wednesday 05/06/13

Fish as Lab Animals

Dr Paul Bowser

LOOK THIS UP = USFWS, NCTC = resources, search histology, a manual of … histology

- Zebrafish are relatively new on scene since 1960s as lab animal =
  - Efficient way of conducting research

- Rainbow trout hepatocellular carcinoma =
  - Came about as a result of feed contaminant
    - Aflatoxin contamination

- Lamprey =
  - Good for spinal cord research

- Playt X swordtail hybrid =
  - First fish to demonstrate melanoma
  - Laboratory created neoplasia

- Medaka =
  - Good for toxpath studies and developmental studies

- Fathead minnow and bluegill =
  - Used for toxicity testing
  - Good book = now at 17th edition

- **Fish facility management =**
    - **Temp =**
        - Ideal for a particular species
        - Below 55 is too cold for Tilapia = true tropical fish
        - High temps = fish running so fast, but cant carry enough oxygen

    - **Water Q =**
        - Ammonia =
            - High levels = lifting of epithelium of gill structure
        - Nitrite =
            - Nitrite is a Monovalent anion =
                - If you add chloride in water, you can slow down uptake of nitrite = methaemaglobinreductase = makes reaction go in reverse
                - If fish exposed a second time = much more efficient at combating this problem
            - Methaemaglobin = can't carry oxygen
                - Brown blood disease
                    - Methaemaglobin cant carry O_2 as it is carrying nitrite
        - Process of N cycle is aerobic = don't want it to become anaerobic
        - Nitrosomonas – Ammonia to nitrite
        - Nitrobacter – Nitrite to nitrate
        - At higher pH and/or higher temps = get more NH_3
            - But pH is big player
            - Table numbers = percentages of total ammonia in unionized toxic form
                - Eg = at temp of 24 and pH = 7.0 = half of ammonia in toxic form
            - Need to measure alkalinity = keep around to 100-120 ppm
        - Nitrate =
            - Need lots to cause problem
            - Get rid of by adding in new water

    - **Biological filter =**
        - Bacteria detoxify waste products
        - Create a big surface area =
            - Larger the SA = greater number of bacteria that will be there = more efficient filter

    - **pH =**
        - ammonia in saltwater system = ammonia is ten times headaches in this system
            - put fewer fish in saltwater aquaria = less feed = and biofilter can take care of it = don't load system wth Nitrogen

- **Hardness =**
  - Want around 100-120 ppm
    - Primarily Ca and Mg

- **Alkalinity around same =**
  - Primarily carbonate, bicarbonate and silicate

- **Dissolved oxygen =**
  - As temp increases, dissolved O_2 carrying capacity decreases
  - In a closed fish system = primarily putting O_2 into system by moving that water
  - Biofilter is aerobic = have to have oxygen there

- **Fish density =**
  - How many fish??
    - There is a real physiological response when you have right numbers of fish

- **Water Q monitoring =**
  - New tank syndrome

- Don't use compressors = gives you low volume of high pressure air
  - Better to use blower

- Want isolated areas

- Put lids on tanks =
  - Fish jump out
  - Pathogens move

- **Diseases of Zebra fish and other fish =**
  - **Identification of diseased fish =**
    - Obvious lesions
    - Reduced feeding
    - Unusual behavior
    - Animal located apart from the group
    - Unexpected dark colour change

  - **Big three =**
    - **Mycobacteria =**
      - Skinny fish = but lots of things can cause fish to be skinny
    - **Pseudoloma neurophilia =**
      - Skinny fish
      - See little xenomas in spinal cord
      - Fungi-Flour stain
    - **Pseudocapillaria tomentosa =**
      - Nematode

- **Viral =**
  - Don't have a pathogenic one in Zebra fish yet! But give it time
  - Can cool zebra fish down to use as model for VHSV
  - Be careful of source fish
  - Zebra fish = ideally only get eggs from another facility =
    - If get fish = quarantine fish, hatch eggs, steralise eggs…
    - Lysol is best agent to clean up from Mycobacteria

- **Bacterial =**
  - **Obligate** = can only live inside parasite = don't hang around in the environment = only stay in a fish
    - Want to avoid these
  - **Opportunisitic =**
    - **Pseudomonas fluorescens**
    - **Columnaris =**
      - Haystacking bacteria
    - **Aeromonas hydrophila**
    - **Mycobacteria**
  - Myco =
    - Opportunistic
    - Everywhere
    - Rare in flow through systems
    - Found commonly in closed recirc
      - Home aquari fish = myco until proven otherwise
    - Best to manage it =
      - Rotate out old fish = bring new brood fish through system
      - Clean tanks = this bacteria tends to reside in biofilm
      - Put new tanks in system

- **Protozoal =**
  - If you get protozoa in system = wonder why you get fish from
    - Don't get fish from aquarium stores
  - **Ciliates =**
    - Whitespot =
      - Spots are inside the fish, not on the fish
      - Under the epidermis of the fish
      - Has implications for tx = tx every 3-4 days for 3-4 treatments formalin at 25ppm (1ml of formalin per 10 gallons)
        - Must add oxygenation at same time
      - Adult parasites burrows out through fish, encysts, this breaks open and get infectious tomites, these must get in fish within 48 hours, other wise dead =
        - You are treating adult form when swimming down or tomites when swimming up

        - Life cycle is 7-10 days = so 3-4 treatments kills adult forms
    - Chilodonella =
        - Cilia in rows
    - Trichodina =
        - tx formalin 25ppm for one tx as this parasite has direct lifecycle
- **Flagellates =**
    - Costia
        - tx formalin 25ppm for one tx as this parasite has direct lifecycle
        - small tear shaped flagellate

- **Metazoan =**
    - Myxosporeans
    - Monogenes =
        - Gyro = live bearer
        - Dactylogyrus = egg layer
            - 2 pairs of eye spots
            - one pair of hooklets
    - Cestodes =
        - Larval tissue forms are more dangerous = actively burrowing through tissues and leave scar tissue behind
        - Identified based on scolex structure
    - Nematodes =
        - Can find these anywhere = can burrow in the skin/fins
    - Digeneans =
        - 2 host lifecycle
        - fish eating bird as definitive host
        - shed eggs in faeces
        - goes into clam or snail
        - comes out as freeswimming cercarae
        - then burrow into the fish
            - eg = diplostomum
    - Acanthocephalans =
        - Spiny headed worms
        - Typically found in lumen in gut
        - Push proboscis into gut and can perforate out to the other side
        - Can have larval stages that inhabit the fish = burrowing forms that move around in the tissue = cause scar tissue

- **Non-infectious =**
  - Nephrocalcinosis
    - Imbalance of Ca and Mg in water
    - High CO_2
    - Von kossa stain for calcium = stains deposits black
    - H&E = mineralized appearing structures that block of kidneys
    - Buffer system with sodium bicarbonate, no calcium bicarbonate = as Cabicarb causes this

- Cu toxicity =
  - Epithelial hyperplasia
  - Excessive mucus
  - Gill function compromised
  - Avoid copper/brass at all costs
  - Filaments become very thick

- Chloride toxicity =
  - Looks very similar to Cu tox if you have low level tox
  - Diffuse gill epithelial hyperplasia

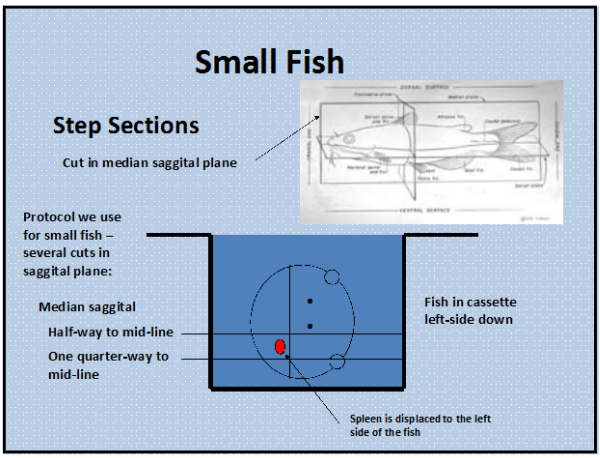
- Ammonia =
  - Looks similar to Cl and Cu tox =
  - Low enough level = diffuse gill epithelial hyperplasia

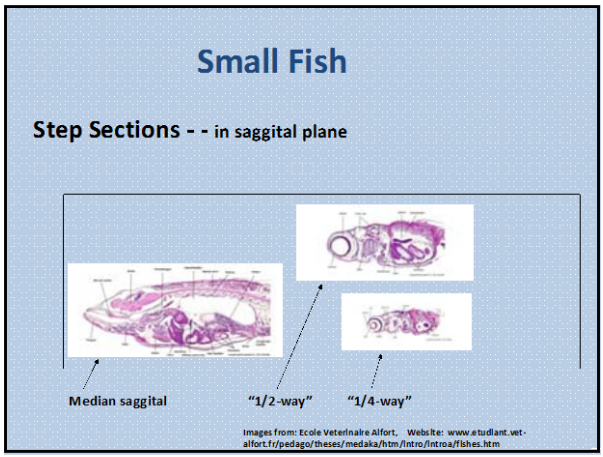
- Nitrite toxicity =
  - Brown blood dz

- Gas bubble dz =
  - Gas filled pustules all over fish =
    - Supersaturation of oxygen
  - Gas emboli that come out in capillaries
  - Eg: 80 gallon aquarium, water too green, too much algae, what is day/night cycle in lab?? = lights were on 24 hours a day. Better to turn off 12 on/12 off.
    - Tx =
      - Massive water change to dilute algae out
      - Put tarp over tank to reduce light
  - Eg: catfish floating upside down with abdo distended with gas = water full of nitrogen.
  - Where can gas come from =
    - Rapid increase in water temp = create supersaturated condtion = water can't hold that oxygen
    - Imperfections in plumbing
    - Prolonged overproduction of oxygen by algae
    - Bringing water to the surface from deep wells = pressure release

- Releasing water from the base of a dam of a deep impoundment = water under pressure on upstream side = if release from pipe at bottom of dam =
    - Can cause swim bladder to evert

- **Heath management =**
    - Source of fish =
        - Make sure broodstock fish never come into contact with main room fish
        - Need to have breaks in the lifecycle
    - Source of water
    - Access to facility
    - Routine diagnostics
    - Surveillance = early detection =
        - Sentinel program
            - Where do you select fish from?
    - Testing =
        - Sagittal step-sections of small fish





- **Prevalence tables - Might be there at 5% or less (0.05)**
    - Magic number for health inspections of fish = 60 fish from a cultured situation = controlled finite population
        - If you don't find any select pathogens, can be at least 5% sure no pathogens there
        - Cant really say 100% sure not there
        - Stats based test

AQUAVET 2013
My notes

Wednesday 05/06/13

Coral Biology and Histology

Dr Ilze Berzins

- **Ctenophores**
  - Comb Jellies
  - 90-150 sp.
  - 8 rows of fused cilia
  - colloblasts = sticky cells to gather prey
  - problems in captivity =
    - very sensitivity to water quality
    - degeneration secondary to traumatic abrasions in tank?

- **Cnidarians**
  - 9000+ species
  - radial symmetry
  - hydrostatic skeleton
  - many have nematocysts (stinging cells)
  - Polyp vs medusa stage
  - Classes =
    - Hydrozoa = hydra and fire corals
    - Scypozoa = Jellyfish
    - Anthozoa = sea anemones and corals
    - Cubozoa = box jellies
  - Most jellies are predators
  - Sexual and asexual repro
  - Only have two cells layers =
    - Epidermal
    - Gastrodermal layer
  - Zooxanthellae = give sea anemone the colour and this gets lost when bleached
  - Different clades of zooaxnthellae =
    - Each clade shows adaptability to diff conditions
    - A or B clade = found in more stable environments =
      - Stress coming in = heat or cold = these zooxanthellae get kicked out
        - No one knows how or why they get kicked out
  - Energetic cost to getting zooaxthellae out of gastrodermis
  - Zooxanthellae is a plant
  - Some of the diseases we are seeing are actually plant component
  - Specific triggers cause them to spawn =
    - Broadcast spawners

        - Some are brooders
    - Defense mechanisms =
        - Mucus
        - Cellular immunity
        - Bioactive compounds with antibiotic efficacy
            - In non stressed corals = there are certain levels of these abx, but when then get stressed = they down regulate these abx
                - Eg: like getting sick after exams

- **Corals**
    - Two cell layers
    - Different corals secrete diff densities of skeletons
    - radial symmetry
    - mesoglea
        - acellular material inside
        - gastrodermis is part in the middle
    - Types =
        - hermatypic
        - with zooxanthellae
        - Soft Vs. hard/stony
            - **Soft corals =**
                - Sea fans, sea whips
                - Non reef building
                - Majority without zooxanthellae
                - No hard skeleton
            - **Hard corals =**
                - Most involved in reef building
                - Skeleton of CaCarbonate
                - All hermatypic corals contain zooxanthellae

- **Zooxanthellae**
    - In most species of hard coral
    - Unicellular algae
    - Live in gastrodermal cells = symbiosis
    - Photosynthetic
    - Provide colour to coral
    - If removed = get kicked out, but if doesn't get enough nutrition = dies
    - clades

- **CORAL DISEASES = trying to classify these diseases is hard!**
  - **Predation by =**
    - Fish
    - Inverts
  - **Non-predation =**
    - **Tissue loss =**
      - **Back band disease =**
        - Dark or fuzzy band
      - **Aspergillosis =**
        - Purple coloured areas around dead tissue
      - **Red band disease =**
        - Finding Cyanobacteria = but is this truly whats causing this problem?
    - **White diseases =**
      - **White band =**
        - Found in Acroporids
        - Vibrios are characterisitic here
      - **White plague**
      - **White patch =**
        - Serratia marcenses = human pathogen
    - **Tissue discolouration =**
      - **Bleaching**
        - Loss of zooxanthellae
        - High or low temps, UV, high sediment, high turbidity
        - Can recover
      - **Dark spot disease**
      - **Caribbean Yellow Band disease**
    - **Growth abnomalies**

ABOVE ARE DISEASES YOU MIGHT SEE IN THE FIELD

- **CAPTIVE CORAL DISEASES =**
  - Rapid tissue necrosis

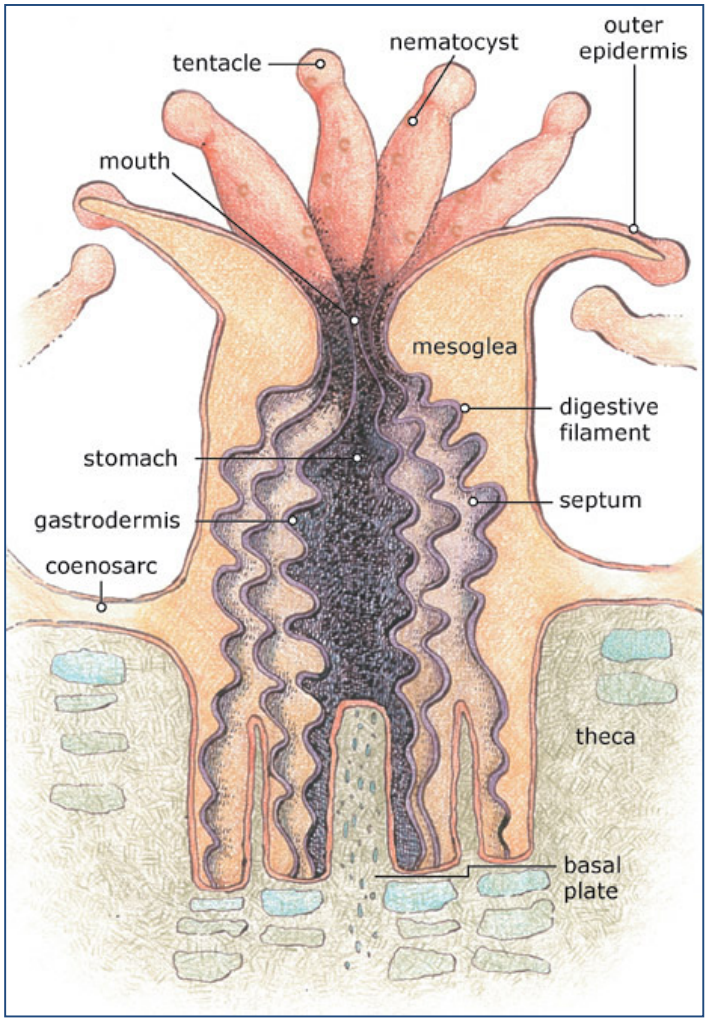
- **CORAL HEALTH ISSUES =**
  - Corals need Ca for skeleton = so can add coral drips to system
  - Need water flow =
    - Dump buckets
  - Need light for zooxanthellae =
    - What bulbs are they using?
    - What light spectrum are they exposed to?
  - Community balance =
    - Competition for space
  - How do you know if coral is sick =
    - Abnormal discolouration

        - Polyp shrinkage >1 day
        - Polyp recession, eversion or extrusion
        - Irregular colouration
        - Not opening mouth or keeping it constantly closed
        - Necrosis
    - Normal =
        - Brown oral strings/pellets
        - Mucus capture webs
        - Extended, responsive polyps
    - Immunology =
        - Innate immune system
        - Mucus
        - Immune receptors
    - Tx =
        - Sealants
        - Debridement
        - Antiseptics = Revive Coral Cleaner
        - Abx = topical or immersion baths
        - Antiparasitics
        - Euthanasia = relaxants = magnesium chloride or alcohol, then put in formalin
        - Probiotics
        - Antioxidants
        - Bacteriophages
        - Focal irradiation therapy

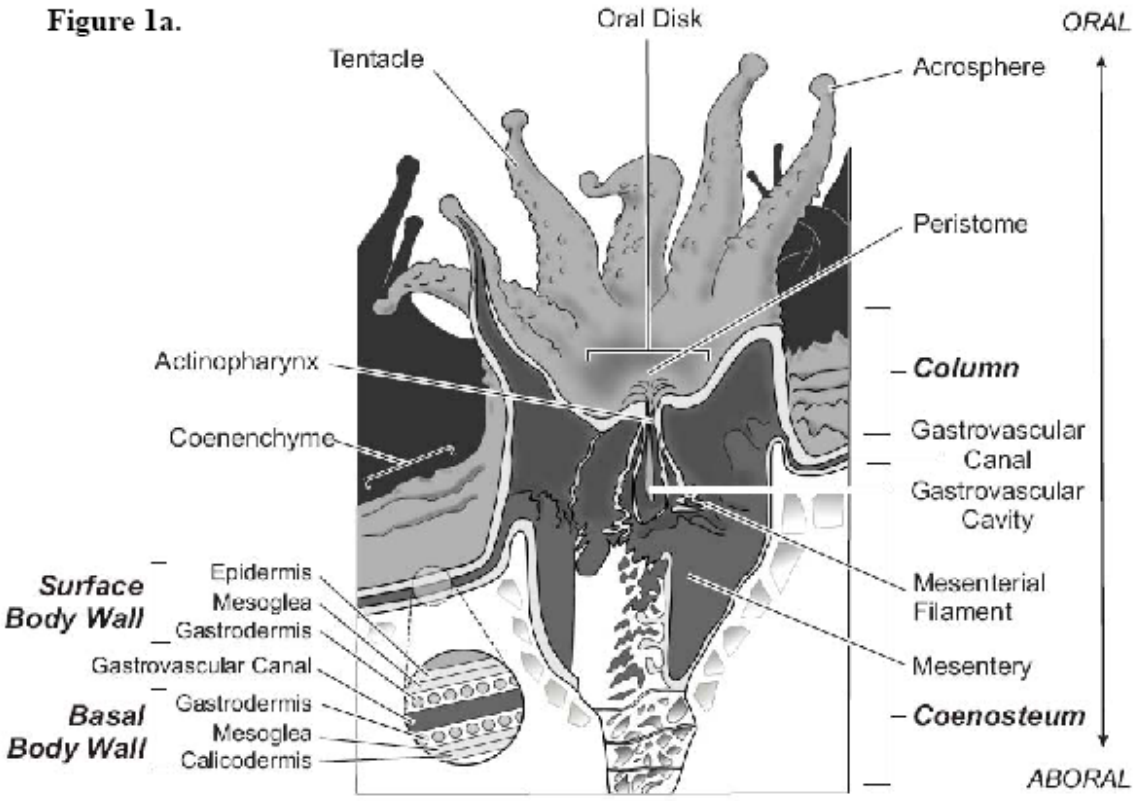
- **ANATOMY AND HISTOLOGY =**
    - Fixatives =
        - What are you looking for?
        - Usual is 10%NBF
        - Z-fix concentrate = better than formalin =
            - Zinc formaldehyde
            - Buffer with seawater = 1 part solution, 4 parts sewater
        - Then agar enrobement of fixed samples
            - SeaKem Agar is liquid, put samples in, then put in vacuum oven (25mm Hg)
            - Cut hole in agar to expose skeleton, then decalcify with Neutral EDTA
            - Then just process

- **Terminology =**
    - Numerous interconnected polyps
    - Base = aboral
    - CaCarbonate =
        - Cup = caolyx = polyp secretes this
        - Theca = walls of the cup
        - Floor = basal plate

        - Looking down on coral = septae = different arrnagements helps depicts species
- Epidermal tissue lines cup = floor and walls = and secretes CaCarbonate base
- Some corals only grow 10cm a year!
- Space between cups = coenosteum
- Tissue between polyps = coenosarc
- **3 layers =**
    - **epidermis (outside) =**
        - find nematocysts = stinging cells
        - mucocytes, pigment cells
        - epitheliomuscular cells
        - sensory receptors and primitive nerve cells
        - Calicoblastic cells = secrete CaCarbonate (aragonite) skeleton
    - **Mesoglea (acellular) =** between epidermis and gastrodermis
    - **Gastrodermis (inside) =**
        - Zooxanthellae = in membrane bound vacuoles
        - Amoebocytes = like a phagocytic cell = often need stains to pick up
        - Myoneme = is the base of an epitheliomuscular cells which attaches the cell to the mesoglea
    - **Mesentaries =**
        - Spermaries/Oocytes can hang out here
        - Armeries



- Tentacles surround the oral disc
- Bulbous tip = acrosphere
- If mouth elevated = not just a little opening = peristome = mouths leads into pharynx (actinopharynx) which leads into gastrovascular cavity = this is connected throughout all other appendages
- Polyps connected via ceonosarc
- Have in-foldings from cup
    - Like mesentery margins
    - Packed full pneumatocysts
- Within gastrodermal tissue = have zooxanthellae
- Calicodermis = secretes CaCarbonate skeleton
- Basal body wall = everything that is touching the skeleton
- Trichrome stain = stains mesoglea blue
- Don't really know function of granular cells = are they immune?



AQUAVET 2013
My notes

Thursday 06/06/13

Neoplasia

Dr Renate Reimschuessel

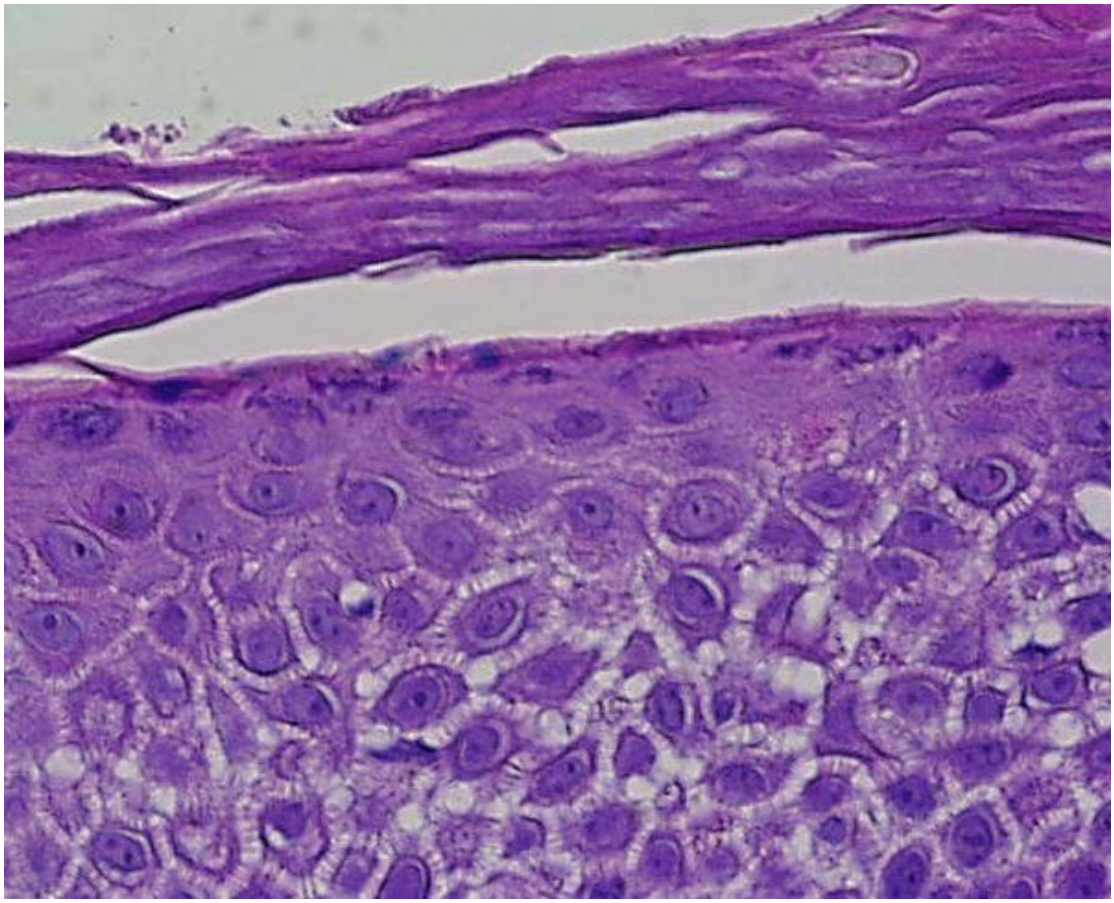
- Invasion = hallmark of malignancy

- Causes of neoplasm =
    - Genes that turn on division= stimulate or deregulated
    - Genes that turn off division are lost or mutated
    - Micro-environmental feedback

- *Look at lecture notes for more*

- *Seabream case =*
    - Neural tumours often lumps on skin
    - Used immunohistochemistry
    - Schwanomma

- Example of spongiosis = *below*



Thursday 06/06/13

Chylamidia and Rickettsial like organisms

Dr Rod

Eagle Ray paper: Epitheliocystis hyperinfection in captive spotted eagle rays Aetobatus narinari associated with a novel Chlamydiales 16S rDNA signature sequence. Alvin Camus, Esteban Soto, Aimee Berliner, Tonya Clauss, Susan Sanchez Department of Pathology and 4Athens Veterinary Diagnostic Laboratory and Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, USA. Diseases of Aquatic Organisms (impact factor: 2.2). 04/2013; 104(1):13-21. DOI:10.3354/dao02586 Source: PubMed

Thursday 06/06/13

Emerging Diseases of Fish in the USA

Dr Rod

- **VHS =**
    - 2005 = fish dying, but no good specimens to look at
    - 2006, die offs
    - millions of fish dead = acute epidemic
    - causes haemorrhagic disease
    - water temps were not ideal for VHS
    - live bait transferred virus
    - late 2006, DEC getting worried, Rod and team went out and sampled
    - bait fish did not look haemorrhagic but had highest levels of VHSV
    - THIS IS AN OIE REPORTABLE DISEASE =
        - If it is found in an unusual site or in new species
    - This is devastating pathogen
    - But does not hit Salmon as much
    - Hadn't developed quantative PCR methods yet = takes a while to validate
    - 2007 = first salmonid implicated (single fish)
    - paper published
    - saw deaths from 2005-2009

- **Large Mouth Bass Virus =**
    - 2004 = virus isolated from Lake Champlain
    - Ranavirus = closely related to frog virus three
    - Saw overinflated, hyperaemic swimbladder
    - Seemed to kill prized sized fish
        - Competitions = boats go from lake to lake to lake
    - Took five fish from sources
    - qPCR performed

- 2005 mass mortality

- **Koi Herpes Virus =**
  - Discovered in late 90s
  - Very important dz
  - Path = gill hyperplasia and necrosis, fusion of gill lamellae
    - Looks like columnaris dz too
  - Intranuclear viral inclusion
  - Sygnet ring = causes chromatin to marginate
  - Dx in koi in several small ponds in NY in 2003-04
  - 2005 = approx. 25,000 adult carp dead

- **Spring Viraemia of Carp Virus =**
  - CEFAS = OIE reference lab

- **Cyprinid herpes virus 2**
  - Necrosis and inclusions
  - Fran's paper!

Why is carp not on VHS list?

- *Found in lampreys in NYState*
- *Not on list of regulated species!!*
- *OIE method to detect VHS = isolate in cell culture, then RTPCR*

AQUAVET 2013
My notes

Friday 07/06/13

Shrimp Diseases

Dr Bissel

- Rate of autolysis is incredible
- Need good fixation
- Pick moribund animals, fix at pond site
- Don't have cell culture
- But have really good molecular diagnostics
- Few therapeutics
- Variety of immune stimulators
- Biosecurity is most imptx part of this industry
- *P.vannamei* (Pacific White) = >99% of production
    - $3.50 per pound commodity shrimp
- Now have 25 generations of domestic shrimp
- $100/lb = *P.japonicus* = Japanese shrimp = highest value agricultural product
- need salt water for shrimp ponds
- need proximity to coastal resources
- Some countries have lost product due to virus
- Shrimp industry and greed has created disease pressure
- In USA, freshwater shrimp are prawns
- Hard to make a lot of money in Shrimp farming these days = as there are so many producers
- When shrimp farmers can no longer produce shrimp =
    - Hybrid stripped bass, tilapia, channel catfish, red drum, cobia
- Shrimp is imptx as bait
    - Everyone used to buy frozen shrimp that was diseased!!
- Lifecycle =
    - 25 days lifecycle
    - most people are only broodstock doing genetic improvement (doing experiments challenging against virus…) OR produce PLs
        - we now have domesticated shrimp that are completely diff from wild shrimp
    - PL to subadult = 4-6 months farm pond
    - Spawning adult = 2-3yr lifespan = hatchery maturation = egg
    - Thaliand egg to harvest = in 63 days!! One month in PLs and one month in growth out
- Natural lifecycle =
    - Ocean =
        - Eggs > naupilus > Zoea > Mysis then move to estuary (freshwater) > postlarva > back to beach > juvenile > adults

- In good years, might spawn twice
- But in marginal times, only once
- Factors affecting shrimp health =
    - Enviro =
        - DO = >4ppm
        - Water temp = 28-30^{o}C
        - Salinity = variable
        - pH = 7.3-9
        - ammonia = 0.1-0.5ppm
        - Algal blooms/organic load =
            - Good in small larval shrimp – loads of things to eat here
                - Why do they have so many metamorphic stages?? =
                    - Each time they moult, their oral mouth diameter changes
                    - In hatchery, need several species of algae/rotifers
    - Husbandry
    - Nutrition
    - Genetics
    - Infectious disease
- Semi-intensive farming =
    - Most everyone does this
    - But super-intensive is the future =
        - High stocking densities
        - High yield = 20,000-100,000kg/ha
        - Water recirc
        - Aeration/emergency O2
        - Automated feeders
        - Biosecurity
        - Unrestricted location
    - Don't put production unit after production unit!

DISEASES

- Most worried about VIRAL dz
- Bacterial =
    - Vibrio
- Rickettsia =
    - NHP
- Fungal =
    - Fusarium
    - Lagenidium
- Parasitic =
    - Protozoa
    - Cestodes
    - Nematodes
    - Trematodes

- Microsporidia
- Gregarines

- **Anatomy =**
  - Exoskeleton
  - 6 abdo segments, 7 if you coutn telson
  - swimmerets = pleopods = each abdo segment has pair
    - take these for diagnostics
  - craw on pereopods
  - swim backwards
  - dorsal sulcus runs all the way down = can draw haemolymph from here
  - ridge marks space where haeme can be drawn from
  - top half of segments = turgum
  - and bottom half called pleuron
  - gut runs on dorsal side
  - and artery runs on ventral side
  - deveining = pulling gut off top
  - value adding processing = charge extra for preparing shrimp
  - haemtopoeitic =
    - agranulocytes = phagocytes
    - semigranulocytes = sort of like neutrophil, 1st at site of infection
    - granulocytes = packed with granules that contain antimicrobial material
    - highly active, mitotic tissue
  - antennal glands =
    - imptx to repro
    - and moulting
    - have to grow to moult
    - filling up carapace till rigid case preventing weight gain = the pressure stimulates hormonal cycle to induce moulting
      - swell up with water, begin to harden, once harden and maintains shape, gets rid of water, meat shrinks down to normal size, but now there is a growth space, then weight gain to occupy space till there is no more room = so have to growth before you moult
    - lymphoid organ =
      - where you dx TSV
      - imptx organ
      - caudal to mouth
    - hepatopancreas = liver, pancreas and SI
      - digestion, detoxification, blood glucose regulated here
    - lymphoid organ and heptatopancras = VERY IMPORTANT diagnostically
    - heart is right up dorsally
      - insert needle right behind carapace = draw haeme here = heart stick
        - semi-open circ system

        - open chamber where haem flows around
        - left side pumps = pumps to open circulation
    - super-oesophageal ganglion = some viruses affect this
    - neuro-hormonal organ = goes to eyes
        - ratio of hormones produced here control moutling =
            - ablate one of eye stalks = this dramatically reduces level of hormone, induces ratio change and causes them to moult
            - animal welfare hate this!
                - So people looking for alternatives
    - Farmers can't wait a year for them to breed naturally

- **Dx methods =**
    - Histo essential =
        - Need decal for lrage shrimp due to thick carapace
        - Can do whole shrimp
        - Stain with H&E
        - Routine health monitoring
        - Lab confirmation of results
        - Davidsons fixative = shrimp turns orange
            - Bob prefers Davidsons for shrimp
                - Acid hydrolysis =
                    - pH low = cause hydrolysis of ribosugar molecules
                    - if pH = RNA more labile, degrades faster = don't get good in situ hybrid reaction
                    - Davidsons relatively neutral
        - PL15-20 = is when you sell larvae
        - Most of what we look at today (Fri 7th) is PL 15-30s
    - As is molecular techniques =
        - PCR and DNA probes insitu
    - TSV/YH = RNA virus
        - Taura Syndrome Virus =
            - Acute =
                - When you add ISH = lights up blue = heavily infected
                - and chronic phases
        - WSSV/IMNV = DNA virus
    - Transmission EM = imptx for dz morph
    - Viral accommodation = has something to do with apoptosis
        - One degree in high temps does something to the virus and alters proteins, so shrimp virus held stable
    - To summarise diagnostics =
        - Do not rely on single dx method

- Confirm initial findings using a second dx method before reporting notifiable dz

- **Sampling =**
  - Need to determine prevalence
  - Don't select dead shrimp
  - Don't select ones about to die right now
  - Select moribund ones
  - Want 5-10 abnormal shrimp
  - Screening health check =
    - Needs to be stat significant = no. of norma appearing shrimp based on population size
    - Animal size =
      - PL5-15 = 60-100 for histo and 60-100 PCR
      - >PL30 = 10-30 for histo and 60-150 PCR

- **signs of dz =**
  - abdnormal colour
  - cuticular deformities
  - lethargic, immobile, erratic swimming behavior
  - empty midguts (anorexia)
    - these animsl should eat 24 hours a day
  - cuticular lesions = black spots = melanisation
  - gill/appendage/exoskel fouling =
    - immunosuppression
  - moribund/dying
  - slow haemolymph coagulation

- **Davidson's =**
  - 1 part tissue:10 parts Davidson's
  - 95% ethanol
  - 115ml glacial acetic acid
  - 220ml 100% formalin
  - 335ml water
    - infiltrate both sides of carapace
    - inject into hepatopancreas
    - infiltrate = 4^{th}, $5^{th}$and 6^{th} abdo segments = then submerse in a vial = cut cuticle down carapace before immersion

- **PCR =** remove pleiopods for PCR analysis
  - In two moults later pleiopods regenerate
  - Submerge in 95% ethanol
  - Want some of the muscle of the top of the pleiopods as well = muscular base = don't cut too low
    - Cut closer to the tail region

- **Dr Arun = Molecular techniques**
  - Why imptx to use molecular dx =
    - Rapid and accurate id of causative agent
    - Use conventional methods = still play a critical role
      - But also need latest techniques
  - Memoirs of a shrimp farmer by John R.Cheshire!!!
  - Landmarks in shrimp dz =
    - Collapse of maritech (1st shrimp industry company in florida) =
      - Unavailability of adequate number of PLs
    - Establishment of captive breeding programs in early 80s
      - Growth improvements and dz resistance
  - Major footprints =
    - First viral dz =
      - Baculovirus penae in 1978
      - Early 80s/90s = emergence of no. of viral dz
      - 1982 = IHHNV outbreak in mexico
      - mid 80s = MBV in Taiwan
      - 1992 = 1st outbreak of TSV in Ecuador, spread to Americas and Asia
      - Mid 90s = YHV, major losses in Thailand
      - Early 90s = WSSV outbreaks in Asia, spread to the Americas, Europe and Middle East =
        - This was when a lot of money was poured into research
      - 2002,06 = IMNV outbreak in Brazil and Asia
  - How are viruses names??
    - Bases on location isolated
    - Name of host
  - Positive sense = can immediate translate into protein
  - **Mechanisms of virus intro =**
    - **Intermediate to long range transmission**
      - Infected live shrimp
      - Infected frozen imported shrimp
      - Migratory sea birds
      - Ship ballast water
    - **Short to intermediate range transmission =**
      - Pond intake water
    - 3 ways genome editing happens = genome editing systems =
      - bacteria use this!!
        - Zinc fingler nucleus (ZF)
        - TALE
        - CRISPR = edits genome/modifies to give protection again cognate virus

- **DNA viruses =**
  - **IHHNV = Infectious Hypodermal and Hematopoeitic Necrosis Virus**
    - OIE notifiable
    - ssDNA virus
    - Parvoviridae
    - All penaeids hosts = P.stylirostris and P.vannamei
    - Vectors = infected shrimp, vertical transmission, sea birds
    - Distribution = 1981 (why in 81?? = people brought tiger shrimp from Philippines to countries producing shrimp and spread) HI, throughout eastern Europe and western hemisphere
    - CS =
      - Cuticular deformities
      - Stunted growth
      - Bent rostrum = bad = as this is used to fight
    - Intranuclear inclusions bodies
      - Cowdry type A **intranuclear** (means it is in the nucleus) in the hypertrophied nuclei of cells in tissues of ectodermal and mesodermal origin
        - Where as with RNA virus = look in the cyoplasm
    - Effect =
      - Runting in P.vannamei, mortality in P.styrlirostris
    - Now, most shrimp are resistant
    - Virus morph =
      - Virions = icosahedral 22nm diameter
      - ssDNA 4.1kb
      - capsid protein can self assemble = if some of the virions look darker
        - means you can make virus like particles for this virus
      - family = parvoviridiae
    - 3 diff genotypes =
      - Type I = infectious, prevalent in Americas, SEA (Thailand, Philippines, Vietnam)
      - Type II = prevalent in South Asia and the Americas
      - Type III = Non-infectious = prev East Africa, Madagascar, Mauritius and Australia
    - IHHNV is integrated into host genome

- **HPV =**
  - Infects cells of endodermal origin
  - OIE notifiable
  - Reduce growth of juvenilles
  - Not present in tail muscle
    - Need hepatopancreas
  - Icosahedral 22-24nm
  - ssDNA
- **WSSV =**
  - dsDNA
    - ds = double stranded
  - enveloped
  - bacilliform virus
  - belonging to the family Nimaviridae
  - first reported in 1992 in china and Taiwan
  - OIE notifiable
  - Vectors =
    - Infected live shrimp
    - Frozen shrimp
    - Numerous secondary hosts
  - Distribution =
    - Throughout Asia, middle east, North, south and central Americas
  - CS =
    - Lethargy, anorexia, rapid death, white spots on skeleton, deposition of calcium salts, dark colour, red antennae and uropods
  - Histo =
    - Large intranuclear inclusion bodies in cuticular epithelium (how do you diff from IHHNV = only see halo around in earlier stages, but not later, lymphoid organ, antennal gland and connective tissues
  - Effect = mortality reaching 100% within 3-10 days of onset of clinical signs, severe economic loss
  - > 90 hosts =
    - some insects also hosts
    - polychaetes
    - artemia
    - krill
    - copepods
    - rotifers
  - circular genome
  - family Nimaviridae
  - genus Whispovirus
  - WSSV = expressed in cascade =

        - Immediate, early, early and late
            - Helps target treatment
            - Early genes = not expressed in abundant quantities =
                - So for therapy = best to target early genes
            - Later = expressed in larger amounts
    - Temp > 32°C (hyperthermia) and IHHNV pre-exposure reduces WSSV replication
- Only 6% of Open Reading Frames show similarity to other viruses
- Very unique virus
- Microsatellite =
    - Repeating nucleotides = unique =
        - If you amplify this region, one from father and one from mother = very unique for particular individuals

- **RNA virus = this is very error prone**
    - **TSV = Taura Syndrome virus =**
        - *P.vannnamei* is primary host
        - ssRNA
        - non-enveloped
        - icosahedral particle
        - first emerged in 92 in Ecuador
        - horizontal transmission = cannibalism and contaminated water, vertical suspected
        - vectors = infected shrimp, marine birds, water boatmen
        - distrib = Americas and SEA
        - see 30-40d post stocking
        - today at least 4 genetically defined isolates
        - CS =
            - Lethargic, anorexic, difficulty swimming, dark colour, flaccid, opaque musculature, survivors with black spots
        - Histo =
            - Acute phase =
                - Necrotic cuticular epithelium
            - Transition phase =
                - Hemocytic infiltrates
                - Black spots = melanized lesions
            - Chronic phase =
                - Lymphoid organ spheroids
        - Morts =
            - 60-95%
        - icosahedral 32nm in diamtre
        - +ve ssRNA, 10.2kb, two ORFs
        - different isolates of the virus

- pyknosis and karyorrhexis
- dark spots = TSV +ve

- **Yellow head disease =**
  - ssRNA virus
  - Ronaviridae
  - Thailand 1991
  - 6 strains id
  - vertical transmission possible
  - very similar to Gill Associated Virus (GAV) and lymphoid organ virus (LOV)
  - OIE notifiable
  - CS =
    - Lethargy
    - Anorexia
    - Opaque musculature
    - Mass mortality
  - Virions = 70nm X 180nm
  - Enveloped virus
  - 26.6kb
  - 4 ORFs

- **IMNV = Infectious myonecrosis disease**
  - Affects muscle
  - Affects all size shrimp
  - dsRNA
  - spherical
  - non enveloped
  - trans = infected shrimp
  - distrib = Brazil 2002, estimated loss since emergence $100-200 million (20,000T)
  - spread to Indonesia in 2006
  - CS =
    - Stress triggers acute onset
    - Tail muscle opacity
    - Lethargy, death
  - Histo =
    - Widespread muscle degen, necrosis together with fibrosis and hemocytic infiltrates, numerous ectopic and lymphoid organ spheroid, extensive necrosis of the muscle
  - 40nm diameter
  - 2 ORFs
    - one encodes capsid protein
    - 7.65kb

- **Conventional PCR reaction =**
    - Involves series of amplification steps to produce detectable levels of segment of pathogen-specific DNA or RNA. The PCR products are then visualized by gel electrophoresis.
    - Pathogen-specific
    - High detection sensitivity and specificity
    - Used for routine monitoring or confirmation of historical findings

- **Nested PCR =**
    - Do a PCR first time with two primers, but didn't get a signal
    - Nested is really two rounds
        - First try to amplify
        - Then second round is to further amplify first round
        - More sensitive technique

- Thicker band = ?

- **Limitiations of conventional PCR =**
    - Lower sensitivity than real time PCR =
        - About 100-1000 less sensitive
        - 1000 time probability of missing an animal with very low load
    - short dynamic range <2 logs
    - low resolution
    - non-automated
    - size-based discrimination only
    - most qualitative
    - ethidium bromide for staining is not very quantitative
    - requires post-PCR processing

- **Real time PCR =**
    - Quantitative method to measure viral copy number
    - Highly sensitive, virus-/isolate-/genotype specific amplification, wide dynamic range of detection
    - High throughout automated = all you need is shrimp tissue and reagent = machine does the rest
    - Different chemistries used: TaqMan probe, SYBR Green Chemistry (this one you add a dye, fluorescence)

- Can't culture hep C virus
- Don't have any cell lines with shrimp

- **Dr Bissel = Histo**
- **Baculovirus Penae (BP)**
    - Often been used as a carrier
    - Polyhedral inclusion bodies (PIBS) = can see with light micro
    - Triangle shaped inclusions

- Around tubules of hepatopancreas = pass through into faeces to re-establish infections
- Inclusion bodies actually in lumen

- **Monodon baculovirus (MBV)**
  - Big enough to see with light microscope
  - Early stage infected nuclei in hep-pancreas
  - Bodies being sloughed into lumen

- **Infectious Hematopoietic Necrosis virus (IHHN)**
  - Co-infection with whitespot virus helps ….
  - CAI = round inclusions with epithelial predilection

- **White spot (WSSV)**
  - See intranuclear inclusions = epitheliotrophic
  - Antennae gland contains X and Y organs = have hormones
  - See in epith cells
  - See in lymphoid organ = looks like a donut
  - See in haem tissue
  - Late stage = see whole cowdry body
  - Cuticular epith
  - Vas deferens = see inclusions

- **Taura Syndrome Virus (TSV)**
  - RNA virus
  - Reddish necrosis = acute phase and can recover =
    - See recovery phase is white with black pigmentation = melanisation
  - See cuticle pulling back
  - Chromatophore injection
  - Can have carriers of the virus
  - There are survivors = means that this is an RNA virus
  - A,B,C = virus is constantly mutating
    - Selected survivors and bred resistance stock = no vaccines for shrimp!!!
  - Buckshot nuclei = pyknotic nuclei
  - Detachment from cell matrix
  - Cytoplasmic eosinophilia
  - They do emergency harvest in shrimp
  - Seems that dz resistance is inversely proportional to growth rates
  - Multiple eosinophilic to slightly basophilic inclusions
  - Looks like black hole
  - ISH good
  - Once past acute phase = transition phase =
    - Some reddish pleopods
    - Some melanisation

        - See normal histo muscle, epithelium = have clinical carriers without signs of dz
            - These animals are still a hazard to risk moving
        - RtPCR would be nice here, can detect down to 1-2 copy numbers =
            - can detect low grade infection
        - transition phase terminates with moulting
    - lymphoid organ is pathopneumonically affected in TSV
    - chronic phase =
        - TSV has induced spheroids in lymphoid organs
        - Round balls of TSV
    - Moulting =
        - A = paper stage
        - B = tanning = phenyloxidase = used to tan or toughen up exocuticle = turns brown and gets tough like leather
        - C = intermoult = most crustaceans spend their life in this lifecycle
            - Crabs stay in this stage for rest of their life
        - Moult cycle could be a month in a shrimp
            - Fast growth = faster moult cycle goes around
            - Happens to a lobster in a year
            - Stops spinning entirely in a crab!
    - Can see the development of resistance and can select for resistant animals

- **Infectious Myonecrosis Virus (IMNV)**
    - Misdiagnosed as Taura Virus initially, but it didn't have all the other signs
    - Spheroids

- **Yellow head Virus (YHV)**
    - Gill infections
    - Pyknotic and karyorrhectic nuclei
    - Primary clin sign = yellowing of head
    - Worry about avirulent forms = due to false positives or negatives
    - Affects lymphoid organ = no spheroids

Slides =

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