# Wednesday September 5th – Gray / Palmer / Pope Ballroom
## Myxozoa 1 & 2
## Moderators - Jerri Bartholomew, Stephen Atkinson ( Oregon State University )

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| 9:30 AM | **Myxozoa 1** | **Stillwell - Myxozoan Parasites of the Western Mosquitofish, *Gambusia affinis*** |
| 9:45 AM | | **Naldoni - Ultrastructure, Histology and Phylogeny of *Henneguya* spp. Parasites of *Pseudoplatystoma* spp. Taken from the Brazilian Amazon** |
| 10:00 AM | | Kaur - Evidence of Species Complexes in the Genus *Thelohanellus* (Cnidaria: Myxosporea) Infecting Cyprinid Carps from the Indian Subcontinent |
| 10:15 AM | | Freeman - Studies of Systemic *Myxidium giardi* Infections in Icelandic Eels Identifies an Overlooked Clade of Myxosporeans |
| 10:30 AM | | **Refreshments** |
| 10:45 AM | **Myxozoa 2** | Picard-Sánchez - Immune Players in Acquired Protection to *Enteromyxum leei* ( Myxozoa ) in Gilthead Sea Bream, *Sparus aurata* (Teleostei: Perciformes) |
| 11:00 AM | | Saleh - Kinetics of Local and Systemic Immune Cell Responses in Whirling Disease Infection and Resistance in Rainbow Trout |
| 11:15 AM | | Kumar - Identification of Differentially Expressed Kidney Genes During Proliferative Kidney Disease and *In Vivo* Induced Genes of *Tetracapsuloides bryosalmonae* (Myxozoa) in Brown Trout |
| 11:30 AM | | **Guo - *Multum In Parvo*: A Complex Organelle-Based Evolutionary Perspective on Cnidarian** |
| 11:45 AM | | Atkinson - Incapacitated by Salt? *In Vitro* Discharge Tests of Myxozoan Parasite Infection Mechanisms |

**Myxozoan Parasites of the Western Mosquitofish, *Gambusia affinis***

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The objective of this study was to provide updated histologic and molecular characterization of infections by the myxozoan parasites *Henneguya gambusi* and *Myxobolus pharyngeus* in western mosquitofish from experimental channel catfish, *Ictalurus punctatus*, ponds in the Mississippi Delta. Three hundred and fifty-one fish were processed routinely, stained with hematoxylin and eosin, and examined by light microscopy for the presence of myxozoans. PCR was performed on DNA extracted from fresh, formalin-fixed, and/or ethanol-fixed, paraffin-embedded tissues to facilitate sequencing of the 18S ribosomal RNA gene of each myxozoan. Myxozoal stages were isolated from formalin and ethanol fixed paraffin embedded tissue sections using laser capture microdissection (LCM). Histologic examination revealed scale pockets throughout the dermis distended by plasmodia of *H. gambusi* that contained various developmental stages of the parasite. Plasmodia of *M. pharyngeus* were embedded within the epithelium lining the branchial cavity. Three additional myxozoans were identified including a coelozoic species within the gall bladder, a *Myxobilatus*-type myxozoan in the renal tubules, and a *Myxobolus*-type myxozoan within cartilage of the head and multiple fins. Pathologic changes caused by the various parasites were minimal, with the exception of the *Myxobolus*-type, which caused cartilage lysis and necrosis. Sequencing of the 18S rRNA gene revealed that *H. gambusi* and the cartilage *Myxobolus*-type were less than 90% similar at the nucleotide level to any other myxozoan. The nucleotide sequence from the renal *Myxobilatus*-type was 100% similar to a triactinomyxon-type actinospore isolated from the oligochaete worm *Dero digitata*, which molecularly confirms the life cycle for this novel *Myxobilatus* species. At present, the complete life cycle of only one other *Myxobilatus* sp., *M. gasterostei*, reported from the ureters of the three-spine stickleback is known. This species also utilizes an oligochaete (*Nais communis)* as its definitive host. In all, we report the histological and molecular characterization of five different myxozoan parasites in the western mosquitofish and the life cycle of a novel *Myxobilatus* sp.

**Conference Session Designation:** ( Parasitology Myxozoa )
**Presentation Format:** ( Oral )
**Student Presentation:** ( Yes

**Ultrastructure, Histology and Phylogeny Of *Henneguya* Spp. Parasite of *Pseudoplatystoma* Spp. Taken From the Brazilian Amazon**

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The Amazon basin drains about 6.5 million km$^2$ and is a complex ecosystem consisting of highly diverse environments, providing a favorable habitat for exceptional fish biodiversity, with approximately 5000 fish species. Many of these fish species are economically important for commercial, sport fishing and aquarism, and other species have fish farming potential. Among the fish that are economically important for extractive fishing and farming are species of the Pimelodidae family. The present study provides ultrastructural, histological and phylogenetic analyses of *Henneguya* spp., parasites of *Pseudoplatystoma tigrinum,* popularly known as "caparari" (English name - Tiger surubim) and *Pseudoplatystoma punctifer,* popularly known as "surubim" (English name - Spotted tiger shovelnose catfish), caught in the Tapajós river, Pará state, Brazilian Amazon. These fish species were parasitised by distinct *Henneguya* spp. and the histological analysis revealed that the plasmodia of both *Henneguya* species developed in the sub-epithelial connective tissue of the gill filaments. Ultrastructural analysis showed that the plasmodial wall had some pinocytotic canals connecting the outside of the plasmodia to the ectoplasm zone and delicate projections towards the host tissue. A layer of fibrous material was found in the periphery of the plasmodia. Some mitochondrias were observed in the ectoplasm, while generative cells and several developmental stages of sporogenesis were seen in the inner layers. Both species had the plasmodial wall surrounded by a fibroblast layer. Molecular analysis based on ssrDNA from the spores of *Henneguya* sp. parasite of *P. tigrinum* resulted in a 1936 bp sequence that did not match any myxosporean species sequences available in GenBank, and the blast analysis showed *H. maculosus* Carriero et al. 2013, as the closest relative, with 93% of similarity. The sequencing of the ssrDNA from the spores of *Henneguya* sp. parasite of *P. punctifer* resulted in a 1922 bp sequence that showed 99% of similarity to *H. eirase* Naldoni et al. 2011, and were here considered co-specifics. In the phylogenetic analysis, using only myxosporean species parasites of siluriforms, *Henneguya* spp. focus of this study clustered in a sub-clade composed by other *Henneguya* species parasites of gills of South American pimelodid fish. This is the first report of myxozoans infecting *P. punctifer* and *P. tigrinum.*

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**Conference Session Designation:** ( Parasitology Myxozoa )
**Presentation Format**: ( Oral )
**Student Presentation:** ( Yes )

**Evidence of Species Complexes in the Genus Thelohanellus ( Cnidaria: Myxosporea ) Infecting Cyprinid Carps from Indian Subcontinent**

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Out of the total 2600 myxozoan species reported so far, Thelohanellus Kudo, 1933 is the second most prevalent genus comprising of about 150 species after the genus Myxobolus Butschli, 1882 infecting both freshwater and marine fishes. In general, these are mostly histozoic (within the tissues) and sometimes coelozoic (in body cavities). The present paper deals with the study of species complex among the member species of Thelohanellus genus from the Indian subcontinent infecting gills, fins and muscles of cyprinid carps. The species forming species complex are T. rohitae, T. catlae, T. jiroveci, T. seni, T. bifurcata, T. dykovae, T. neocyprini, T. filli, T. muscularis and T. theinensis. The phylogenetic analysis was done on the basis of 18S rDNA which showed close similarity between the species. The homogeneity was found to be between 90 to 99%. The factors responsible for the species complex could be phylogeography, host reluctant, organ and tissue specificity of these myxozoan parasites. Study of more genetic markers facilitated with morphotaxonomy can be used to sort out the occurrence of species complexes among the morphologically different species having similar genetic makeup and vice-versa.

**Conference Session Designation:** ( Myxozoa )
**Presentation Format:** ( Oral )

**Studies of Systemic *Myxidium giardi* Infections in Icelandic Eels Identifies an Overlooked Clade of Myxosporeans**

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The myxosporean *Myxidium giardi* was described in 1906 infecting the kidney of the European eel, having spindle-shaped myxospores and terminal sub-spherical polar capsules. Since then, numerous anguillid eels globally have been documented to have similar *Myxidium* infections. Many of these have been identified using the morphological features of myxospores or by the location of infection in the host, and some have been subsequently synonymized with *M. giardi*. Therefore, it is not clear whether *M. giardi* is a widely distributed parasite, infecting numerous species of eels, in multiple organs, or whether some infections represent other, morphologically similar but different species of myxosporeans. The aim of the present study was to assess the status of *M. giardi*-like infections in Icelandic eels, and identify any similar myxosporeans infecting the related Pacific tarpon, *Megalops cyprinoides*, from Southeast Asia. Myxosporeans were identified using spore morphology and molecular techniques in order to evaluate the diversity present.

The morphological measurements of the myxospores from Icelandic eels was not significantly different between sites of infection in the host fish, but the spores from the Pacific tarpon were noticeably smaller. However, the SSU rDNA sequences from the different tissues locations in eels, were all very distinct, with percentage similarities ranging from 92.93% to as low as 89.8%, with the sequence from tarpon being even more dissimilar. Molecular phylogenies consistently placed these sequences together in a clade that is strongly associated with the *Myxidium* clade *sensu stricto*.

Our results demonstrate that there is not a single species of *Myxidium* causing systemic infections in eels from Iceland; there are three confirmed species, one of which probably represents *M. giardi*, as it infects the kidney. Additional species probably exist that infect different tissues and the site of infection in the host fish appears to be an important diagnostic feature for members of this clade. Myxospore morphology is generally conserved in the clade, although actual spore dimensions can vary between some species. Myxosporeans from this clade are currently only known to infect fishes from the Elopomorpha.

**Conference Session Designation:** ( Parasitology Myxozoa )
**Presentation Format:** ( Oral )

**Immune Players in Acquired Protection to *Enteromyxum Leei* ( Myxozoa ) in Gilthead Sea Bream, *Sparus Aurata* ( Teleostei: Perciformes )**

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The intestinal myxosporean parasite *Enteromyxum leei* causes chronic catarrhal enteritis in gilthead sea bream (GSB, *Sparus aurata*) leading to intestinal dysfunction, poor growth, and higher susceptibility to handling and stress. It entails severe economic losses to the aquaculture sector. Previous observations in our lab showed that fish that recovered from an *E. leei* infection did not get infected upon re-exposure, hinting towards the possibility of protective immunization of GSB against *E. leei*. To study this in more detail, we re-challenged putative "resistant" (R) GSB that recovered from this myxozoan infection, by exposure to *E. leei* effluent infected water. Another group of naïve (N) GSB (never exposed to the parasite) was also challenged. Both fish groups were sampled at 0, 61 and 86 days post-exposure (p.e.) and different specific and non-specific humoral factors were measured in serum, such as the total peroxidase activity, total IgM and IgT (by ELISA), and the presence and quantity of specific antibodies against the parasite by immunohistochemistry (IHC). The expression of a panel of immune-related genes was analysed in head kidney, anterior and posterior intestines of N and R fish after 86 days p.e. At this final sampling point, 83.3% of N fish *vs* 0% of R fish tested positive for *E. leei* by histology. The total peroxidase activity decreased with the progression of the infection in both groups, but only significantly in R fish, probably due to a higher consumption of this enzyme to fight the parasite. Total IgT and IgM levels did not significantly differ between groups. However, IHC evidenced that R fish had a higher initial level of specific anti-*E. leei* IgM than N fish. The PCR array showed a differential response between N and R fish and among tissues. R fish had significant up-regulation of *IgM*, *IgT*, *il10* and *gzmA* and down-regulation of *il1β* in anterior intestine. Complement (*c3* and *fcl*) and the anti-protease *α2m* were significantly higher expressed in R posterior intestine and head kidney. However, the anti-protease *lcpI* was down-regulated in all tissues of R fish when compared to N. The higher initial pool of specific circulating antibodies against the parasite, together with the higher expression of Igs in anterior intestine, the higher cytotoxic activity in the whole target organ and the systemic increase in complement (lectin pathway) seem to be crucial to resist *E. leei* re-infection. Hence, this acquired protective immunity sets the grounds for the development of a vaccine or the production of recombinant antibodies against *E. leei*.

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**Conference Session Designation:** ( Parasitology Myxozoa )
**Presentation Format:** **(** Oral )

**Kinetics of Local And Systemic Immune Cell Responses in Whirling Disease Infection and Resistance in Rainbow Trout**

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Whirling disease caused by the myxozoan parasite *Myxobolus cerebralis* infects several salmonid fish. The disease is responsible for high mortalities in rainbow trout (*Oncorhynchus mykiss*) hatcheries and natural populations. While substantial investigations has provided insight into disease pathology and host invasion, the cellular responses and the leukocyte kinetics underlying fish resistance or susceptibility remain elusive. To elucidate how resistant and susceptible rainbow trout strains respond to early invasion, we used a well-established model of whirling disease infection to demonstrate the kinetics of the mucosal and systemic immune responses in two rainbow trout strains, a susceptible American (T) and a more resistant German (H). In the course of three weeks following exposure, leukocyte kinetics was monitored by flow cytometry in caudal fin, head kidney and spleen. For the analysis of the leukocyte composition, cells were stained with the monoclonal antibodies with known specificity for distinct subpopulations of rainbow trout leukocytes. Experiments indicated increases of T cells B cells and myeloid cells in the caudal fin of the susceptible strain at 2, 4, 8, 12 or 24 h followed by subsequent time-dependent percent suppressions after 2, 4, 8, 14 or 21 d of exposure compared to non-infected control fish. In spleen and head kidney, decreases of myeloid cells, B cells, and T cells were observed at almost all time points. On the other hand, in the resistant strain, except for percent suppressions of B cells, T cells and myeloid cells at early time point after 2, 4, 8 or 12 h as well as after 1d for T cells in caudal fin, time-dependent percent increases were observed in caudal fin, spleen and head kidney after 1, 2, 4, 8, 14 or 21 d of exposure compared to non-infected control fish. These findings highlight the significance of effective local and systemic immune reaction and indicate proper activation of B cells, T cells and myeloid cells with less T cell responses at earlz time point at the site of infection is critical for host resistance during *M. cerebralis* infection. Alteration of the leukocyte populations with early augmented local cellular responses followed by later declines leads to host immune suppression and supports parasite invasion and survival. The present study provides an initial view into the cellular basis underlying immune response and resistance of rainbow trout to the whirling disease parasite and helps us to elucidate the mechanisms that underlie the variation in resistance to whirling disease infection.

**Conference Session Designation:** ( Parasitology Myxozoa )
**Presentation Format:** **(** Oral )

**Identification of Differentially Expressed Kidney Genes During Proliferative Kidney Disease And *In Vivo* Induced Genes of *Tetracapsuloides Bryosalmonae* ( Myxozoa ) in Brown Trout**

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*Tetracapsuloides bryosalmonae* is a myxozoan parasite and the causative agent of proliferative kidney disease (PKD) in salmonids. PKD significantly affects both farmed and wild salmonids in Europe and North America, causing significant economic losses and endangers wild fish population. The life cycle of *T. bryosalmonae* requires two hosts: an invertebrate freshwater bryozoan, *Fredericella sultana* and a vertebrate salmonid, brown trout. Here, we identify differentially expressed kidney genes of brown trout during proliferative kidney disease and discuss the research plan to identify *in vivo* induced genes of *T. bryosalmonae* during the course of infection. Fish were exposed to the spores of *T. bryosalmonae*, released from infected *F. sultana* colonies. Fish were sampled, including blood at different time points. Infection in individual kidney and blood was confirmed by quantitative real-time PCR. The cDNA of kidneys of infected and non-infected brown trout were hybridized and compared by suppressive subtractive hybridization technique. Afterward, transcripts were transformed, validated and functionally analyzed. The identified transcripts function in various processes, including cell stress, cell growth, signal transduction, antigen processing and presentation in the kidney of brown trout during proliferative kidney disease. The results from this study contribute to the understanding of kidney response during *T. bryosalmonae* development. Additionally, further work is in progress to identify *in vivo* induced genes of *T. bryosalmonae* in infected brown trout using *in vivo* induced antigen technology. The identification of the molecules related to the pathogenic mechanisms would improve our understanding of *T. bryosalmonae* infection and support the development of therapeutic and vaccine applications to prevent myxozoan infection in salmonids.

**Conference Session Designation**: ( Parasitology / Myxozoa )
**Presentation Format**: ( Oral )

**_Multum-In-Parvo_: A Complex-Organelle-Based Evolutionary Perspective on Cnidarian**

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Extrusion apparatus, which has been observed in microsporidians, dinoflagellates, ciliates, cnidarians and myxozoans, is a primary example of a complex apparatus whose origins and evolutionary history have proven difficult to reconstruct. Among the extrusion-apparatus-bearing organisms, myxozoans represent a major lineage of metazoan parasites with extremely simplified morphology and structure. Recent phylogenomic evidence suggests an evolutionary origin of myxozoan within cnidaria and for a long time the homology between cnidarian nematocysts and myxozoan polar capsules is the key to indicating the close relationship of the two lineages. However, in contrast to extensively studied nematocysts, the myxozoan polar capsules still remains poorly characterized. To gain better understanding of the structure, function of this myxozoan-specific organelle, to straightforwardly re-evaluate the relationship between myxozoans and cnidarians, and to explore the origin and evolution of extrusion apparatus, here we present the first proteome map of polar capsules from myxozoans. Major components of polar capsules isolated from three myxobolids, *Myxobolus honghuensis, Myxobolus wulii* and *Thelohanellus kitauei*, were characterized by using our newly developed reference species-specific proteome called Myxozoan Comprehensive Proteomic Identification Datasets (MCPID), which are derived from deep transcriptome profile and partial genome sequencing data. The MCPID facilitated the proteomic analysis by identifying 19.1%-43.8% more proteins with a maximum 84.6% of database size reduction, finally enabling the identification of 1111, 490 and 597 polar capsule proteins (PCPs) in *M. honghuensis, M. wulii and T. kitauei* respectively. Comparative proteomics show that the polar capsule proteomes are highly elaborate and include novel structural proteins and venom proteins. Extending the comparison wihtin all available nematocyst proteomes enables us to inspect key questions in the assumptions about evolutionary scenario of nematocyst: how it evolved within the cnidarians and its relationship with organism evolution.

**Conference Session Designation:** ( Parasitology Myxozoa )
**Presentation Format:** ( Oral )

**Incapacitated by Salt ? *In-Vitro* Discharge Tests of Myxozoan Parasite Infection Mechanisms**

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Myxozans are widespread, speciose parasites of freshwater and marine fishes. They are now categorized as Cnidarians, alongside free-living species of corals, anemones and jellyfish. All Cnidarians have nematocysts: complex stinging organelles that discharge a tubule for defense, prey capture or, in the case of myxozoans, for initiating infection. Myxozoan spores detect the host, then fire their tubules to bring host epidermis into close contact, so the motile parasite sporoplasm can enter and begin to replicate. Given that nematocyst firing, sporoplasm activation and movement are essential first steps of the myxozoan infection process, can different compounds interfere with these mechanisms? We aimed to identify ions or molecules that either promote or block nematocyst firing and sporoplasm motility, and thereby gain insight into the sensory and mechanistic functions of myxozoan spores, and reveal paths to therapeutants.

We designed an *in-vitro* assay using spores from two myxozoans, *Myxobolus cerebralis* and *Ceratonova shasta*. We hypothesized that changes in external ion concentrations may block sensory receptors, and enhance or diminish the osmotic gradient which drives tubule discharge and sporoplasm release. Fresh spores were examined under a microscope to determine the proportion of 'intact' spores (nematocysts not discharged, sporoplasms present at the apex of the spore) at the start of the test, then how this changed both after addition of the test compound, then after addition of rainbow trout mucus as a proxy for host contact. We subsampled spore aliquots after each stage of the testing, and fixed these in 5% buffered formalin, to facilitate counting after the tests.. We tested chlorides of $Na^{+}$, $Ca^{2+}$, $Mg^{2+}$, $Fe^{2+}$, $Gd^{3+}$, all of which can affect nematocysts of free-living cnidarians. We determined fixation of tested spores in formalin was an excellent way to stabilize test subsamples without additional firing or migration. We found that $Ca^{2+}$ and $Mg^{2+}$ promoted tubule pre-firing. $Na^{+}$ promoted premature sporoplasm migration, but increased tubule firing sensitivity to fish mucus. $Ca^{2+}$ and $Gd^{3+}$ both reduced sporoplasm migration. We will use these findings both for *in-vivo* infection experiments with host fish, and for searches of the parasite transcriptomes for gene pathways that may be linked to sensory or nematocyst functions.

**Conference Session Designation:** ( Parasitology Myxozoa )
**Presentation Format:** ( Oral )