

Wednesday September 5th – Gray / Palmer / Pope Ballroom
Myxozoa 3 & 4

Moderators - Graham Rosser (Mississippi State University) Sascha Hallett (Oregon State University)

1:45 PM	Myxozoa 3	<u>Alexander</u> - Predicting Myxozoan Disease Dynamics in The Context of Climate Change using a Model Ensemble
2:00 PM		<u>Hallett</u> - Mitigating Enteronecrosis in Klamath River Salmon with Managed Flow Events
2:15 PM		<u>Griffin</u> - Monoculture of Hybrid Catfish Can Limit Proliferative Gill Disease Caused by <i>Henneguya ictaluri</i> (Myxozoa: Myxobolidae) in Catfish Aquaculture Ponds
2:30 PM		<u>Barrett</u> - RNA-seq Analysis of the Early Immune Response to the Parasite <i>Ceratonova shasta</i> in Resistant and Susceptible Lines of Steelhead
2:45 PM		<u>Marshall</u> - Measures of Prevalence and Genetic Diversity of <i>Kudoa thyrsites</i> Infections Within Farmed Atlantic Salmon (<i>Salmo Salar</i> L.) Suggest That New Infections After 2000 Degree Days Are an Exception
3:00 PM		Refreshments
3:15 PM	Myxozoa 4	<u>Freeman</u> - The Role of Proliferative Kidney Disease (PKD) in the Severe Decline of Arctic Charr, <i>Salvelinus alpinus</i> , in Lake Ellidavatn, Iceland
3:30 PM		<u>Bailey</u> - Black and White With Shades of Grey: Exploring Tolerance and Resistance Concepts and the Immune Response of Brown Trout During Proliferative Kidney Disease (PKD) Infection
3:45 PM		<u>Nelson</u> - Renal Myxozoanosis in Salmonids in the Western United States
4:00 PM		<u>Lovy</u> - Myxozoan Parasites as They Correlate with Life History in Anadromous River Herring



8th International Symposium on Aquatic Animal Health

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada



Predicting Myxozoan Disease Dynamics in the Context of Climate Change Using a Model Ensemble

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Abstract Climate change has been linked with changes in the dynamics of infectious diseases in aquatic systems. Climate related shifts in water temperatures and precipitation patterns will have significant effects on the myxozoan disease dynamics, but predicting the magnitude and direction of specific responses is challenging. We present an overview of myxozoan disease dynamics illustrated with data from salmonid ceratomyxosis in the Klamath River CA, USA. Using a model ensemble we predicted host and parasite dynamics under future climate scenarios (hot/dry-cold/wet). We used data from hydraulic and water temperature models, predictive statistical models, and empirical data to parameterize an epidemiological model. Epidemiological model outputs were compared to observations from the Klamath River collected from 2006 to 2017. The majority of climate scenario predictions were similar to values measured in years having high disease risk for salmonids. This result suggests *C. shasta*-induced mortality will likely remain high and could increase in Klamath River salmonids, making the recovery and management of salmon even more challenging.

Conference Session Designation: (Aquatic Epidemiology or Climate Change)
Presentation Format: (Oral)



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Mitigating Enteronecrosis in Klamath River Salmon with Managed Flow Events

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Ceratonova shasta causes enteronecrosis in juvenile salmon in the Pacific Northwest of North America, and is associated with population-level impacts in the Klamath River. Transmission occurs through waterborne stages: actinospores released from polychaete worms develop into myxospores in salmonid fishes. To better understand host/parasite dynamics in this regulated system and inform model development and management actions, we contribute to a parasite monitoring program that includes molecular quantification (qPCR) of parasite DNA in both river water samples and outmigrating juvenile salmonids. During salmonid outmigration (April through June), sampling occurs weekly and data are shared semi-real-time to inform management actions, such as modification of the river flow through water release from the lowermost dam. Two different managed flow events have occurred, which we monitored to evaluate their impact on disease in juvenile salmonids.

The 2013 Biological Opinion (NMFS & USFWS) includes the management action that if waterborne levels of *C. shasta* exceed 5 spores/L of genotype II and water temperature exceeds 16°C, a 'pulse flow' would be released. In May 2014, our data showed that parasite levels were over this threshold, and thus triggered a pulse flow: dam release ramped up from 1140 cfs to 1910 cfs, then back down after 12 hours. Before, during and after the flow event, we monitored parasite levels in river water samples, infection in sentinel fish and sampled polychaetes. We found that parasite levels decreased during the flow event, and mortality was lower in one of four groups of fish exposed during the flow; polychaetes were absent from fine substrate after the flow.

In 2017, the trigger conditions for a managed flow were modified by a US Court Order. An 'emergency dilution flow' is now required if either 5 spores/L is detected (of any *C. shasta* genotype at any index site) or prevalence of infection in juvenile salmonids exceeds 20%. In May 2018, the infection threshold was surpassed and thus the flow was triggered: dam releases were ramped up from 1300 cfs to 3130 cfs, and remained at 3000 cfs for 12 days before ramping back down to base flow levels of ~1100 cfs. Again, we measured effects of the flow event on parasite levels, infection in sentinel fish and polychaete abundance. We will present results from the 2018 flow event at the meeting.

Conference Session Designation:

(Aquatic Animal Health Management)

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Monoculture of Hybrid Catfish Can Limit Proliferative Gill Disease Caused by *Henneguya ictaluri* (Myxozoa: Myxobolidae) in Catfish Aquaculture Ponds

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Catfish aquaculture is the largest foodfish aquaculture industry in the United States and a vital economic component of several southern states. Recent industry trends have led to increased production of channel ♀ (*Ictalurus punctatus*) x ♂ blue (*I. furcatus*) hybrid catfish to take advantage of more favorable production characteristics. As a result, hybrids are estimated to comprise 40%-50% of total catfish production. Proliferative gill disease (PGD) caused by the myxozoan parasite *Henneguya ictaluri* is the most prevalent parasitic disease in Mississippi catfish aquaculture. Known colloquially as “Hamburger gill,” PGD accounts for 10-30% of annual disease case submissions to the Aquatic Research and Diagnostic Laboratory of the Thad Cochran National Warmwater Aquaculture Center in Stoneville, MS. In channel and hybrid catfish, continuous exposure to the actinospore stage of the parasite life cycle triggers a severe inflammatory response at the gills, leading to impaired osmoregulatory and respiratory function, resulting in reduced feeding activity and in severe outbreaks mortality can reach 100%. The static, earthen bottom ponds of catfish aquaculture, and the close proximity of fish and oligochaete hosts, provides an ideal environment for the propagation of myxozoan life cycles. Control measures to reduce incidence of PGD have been largely unsuccessful, however, several controlled studies have revealed an arrested development of *H. ictaluri* in hybrid catfish. While hybrids demonstrate an inflammatory response comparable to channels during acute stages of infection, research indicates significantly less *H. ictaluri* DNA present in hybrid tissues across the developmental timeline and mature *Henneguya* spp. myxospores in these studies, while abundant in channel catfish, are almost non-existent in hybrids. To evaluate the impacts of these findings at the pond level, 18, 1-acre ponds were stocked with channels (n=9) or hybrids (n=9) and maintained as monoculture systems for 3 years, with harvest and understocking performed as warranted. Water samples were collected monthly for eDNA analysis, with additional samples collected in April and May when PGD is most prevalent. In addition, sentinel fish exposures were performed in April and May to estimate PGD severity in naïve fish stocked into these systems. While no differences were observed in the first year, by the second year of the study, *H. ictaluri* DNA and lesion scores in hybrid systems were significantly reduced, and PGD lesions in sentinel fish placed in hybrid ponds were negligible. These results suggest hybrid monoculture can significantly reduce the burden of PGD on catfish aquaculture in the southeastern US.

Conference Session Designation:

(Myxozoa)

Presentation Format:

(Oral)



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RNA-Seq Analysis of The Early Immune Response to the Parasite *Ceratonova Shasta* in Resistant and Susceptible Lines of Steelhead

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Ceratonova shasta is virulent myxozoan parasite of salmonid fish in the Pacific Northwest of North America. It is a significant cause of mortality in out-migrating juveniles and has been associated with pre-spawn mortality of returning adults. *C. shasta* attaches to the gills and replicates in the gill blood vessels before migrating to the intestine. Successful establishment in the intestine results in hemorrhaging and necrosis of the tissue leading to mortality of the host and release of myxospores. Fish resistant to *C. shasta* appear to limit parasite establishment in the intestine via an effective immune response. However, the mechanisms at work in the early stages of infection remain unclear. Here, we investigated this early immune response by exposing resistant and susceptible stocks of steelhead (*Oncorhynchus mykiss*) to *C. shasta* and collected gill tissue at 1 day post exposure (dpe) and intestine at 7, 14 and 21 dpe, along with additional tissue samples for histology. qPCR results indicate that the resistant fish prevent parasite establishment in the intestine while the susceptible fish fail to limit establishment or proliferation. RNA-seq was conducted on gill tissue from 1 dpe and intestinal tissue from 7 dpe for both susceptible and resistant fish, along with their respective controls. The results indicate a more severe reaction to the initial infection in susceptible fish and a failure to recognize parasite establishment in the intestine. This is the first study to employ RNA-seq in order to profile the transcriptional response to this important parasite. Our results support the hypothesis that resistance to *C. shasta* occurs before the parasite reaches the intestine and will inform future studies investigating the specific mechanism of resistance.

Conference Session Designation: (Genomic Applications in Aquatic Animal Health)
Presentation Format: (Oral)
Student Presentation: (Yes)



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Measures of Prevalence and Genetic Diversity of *Kudoa thyrsites* (Myxozoa) Infections Within Farmed Atlantic Salmon (*Salmo Salar* L.) Suggest That New Infections After 2000 Degree Days Exposure are an Exception.

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Kudoa thyrsites causes post-harvest myoliquefaction or ‘soft flesh’ in a variety of marine fishes. In farmed Atlantic salmon, intracellular cysts form within skeletal muscle 1500-2000 degree days (DD) post exposure with highest prevalence usually occurring between 2000-3000 DD. In some regions of British Columbia (BC), Canada, infection intensities can negatively affect up to 4-7% of fillets, thus impacting the competitiveness of BC’s farms. Mitigation strategies for farms located in areas known to have higher infection risk involve rearing smolts at nursery farms located in low risk regions prior to transfer to the higher risk recipient grow out farms. Under these conditions, soft flesh after harvest is reduced to near that expected of fish reared entirely under low risk conditions, suggesting that fish may be only be vulnerable to infection during the beginning of their seawater exposure.

We measured the genetic diversity of *K. thyrsites* from populations of infected fish to look for evidence of new infections after 2000 DD chronic exposure. Within fish haplotype diversity was also measured through comparisons of discrete tissue samples. Under conditions of chronic high risk exposure, we tested 12 fish each at 2000, 3000, and 4000 DD, assuming that under continuous infection haplotype diversity would increase. To identify the origin of infection in transferred fish, we compared haplotype diversity of *K. thyrsites* in three populations of fish: 1) 20 fish reared entirely at a low risk farm; 2) 20 fish reared entirely at a high risk farm; and 3) 10 fish transferred from the low risk donor farm to a high risk recipient farm after 2000 DD. Haplotype counts and frequencies were calculated from 39 alleles from 891 sequences amplified from clone libraries produced from infected flesh. We also used PCR and histology to measure change in infection prevalence in four unrelated populations transferred after 2000 DD.

Under chronic high exposure, diversity and haplotype counts did not increase with time and low haplotype counts of one to five within individual fish suggested that new parasites were unlikely to invade the flesh of previously infected fish. In transferred fish, high levels of genetic variability precluded identity of the origin of infections; however, prevalence monitoring indicated that increases in infection prevalence following transfer varied between 0 and 50%, depending on the population. Matching haplotype identities between different tissue samples within the same fish support a model of mixed infection but with few individual parasites successfully clonally amplifying within the infected fish --thus emphasizing the importance of understanding host parasite dynamics during early stages of infection. Such information could be applied to current mitigation practices in order to improve the efficiency or perhaps mimic the effects of fish transfers.

Conference Session Designation:

(Parasitology Myxozoa)

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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.

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Presentation Format:

(Parasitology Myxozoa)
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Black and White With Shades of Grey: Exploring Tolerance and Resistance Concepts and the Immune Response of Brown Trout During Proliferative Kidney Disease (PKD) Infection

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In Europe proliferative kidney disease (PKD) of salmonids is an emerging disease of economic and environmental concern. PKD is caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*. In evolutionary ecology once, infected organisms may protect themselves against parasitic infection either by reducing parasite burden (resistance) or the damage caused by parasites in spite of high pathogen burdens (tolerance). However, little is understood concerning resistance and tolerance concepts and their application in fish host-parasite interactions. While the main aim of the present study is to describe the brown trout host immune response after exposure to the parasite *T. bryosalmonae*, we also took an evolutionary ecology perspective and explored salmonid patterns of tolerance and resistance during infection, comparing them in multiple species (the native brown trout and the farmed non-native rainbow trout). Regarding, the brown trout host immune response at day 40 and 50 post-exposure IgM sec and Blimp1 were strongly upregulated, whereas Pax5 was downregulated. Hence, the combinatorial signature of the B cell molecules at these time points indicates plasma blast/plasma cell phenotype. In addition, expression of Blimp1 was also strongly correlated with parasite development. While all Th1-like transcripts measured in the study were elevated at day 50 post-exposure. Concerning tolerance / resistance patterns species-specific differences were seen in resistance with brown trout conferring relative resistance but only rainbow trout were able to confer absolute resistance resulting in a different evolutionary outcome for each salmonid species, in that rainbow trout can clear the infection and in brown trout the parasite persists i.e. the stable co-evolved host-parasite system. Further exploration of tolerance/resistance and an association of immune mechanisms with such concepts opens an additional gateway for interpreting fish host-parasite interactions.

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(Immunology General)
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Renal Myxozoanosis in Salmonids in the Western United States

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Renal myxozoanosis occurs in many farmed and wild salmonids in the Western United States, and several species are implicated with varying pathogenicity and clinical significance. This retrospective study of diagnostic cases seen at the Washington Animal Disease Diagnostic Laboratory (WADDL) during the last 10 years includes mountain whitefish, rainbow trout, and salmon species. Myxozoan genera diagnosed include *Tetracapsuloides*, *Parvicapsula*, and a species newly identified at WADDL. Infections range from subclinical with primarily intratubular involvement to clinically significant with significant epithelial necrosis and/or interstitial inflammation. While *Tetracapsuloides* has only non-sporogonic stages within lesions, most renal myxozoan infections have intraluminal sporogonic stages.

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Myxozoan Parasites as They Correlate with Life History in Anadromous River Herring

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River herring are made up of two species including the alewife *Alosa pseudoharengus* and the blueback herring *Alosa aestivalis*. Both are considered threatened and declining species due to a number of anthropogenic factors including habitat fragmentation from dams, historical overfishing, an increase in predation, and other habitat alterations resulting from coastal development. Though these factors have directly impacted populations throughout their range, less is known of the infectious agents and potential disease impacts in these threatened populations. In New Jersey, sampling for river herring has been done annually to survey young-of-the-year (YOY) and adult fish to better understand their population levels. From sampling conducted between 2014-2018, we describe three myxozoan parasites in river herring which correlate to particular life history stages of the fish. In YOY fish two myxozoan species were detected, including *Myxobolus mauriensis* and a *Kudoa* spp. According to sequences of the 18S rDNA, *M. mauriensis* was most closely related to other marine myxobolids with tropism for cartilage, with closest identity of 83% to *Myxobolus groenlandicus*. Histology suggested this species to be pathogenic in young fish, causing lysis and breaks in the rib bones associated with chondritis and myositis. Lesions extended to the skin causing dermatitis and extracorporeal release of spores. The *Kudoa* spp. also occurred in YOY fish indicating that infection with this parasite occurred within the river environment prior to out-migration into the ocean. Little to no host response was associated with *Kudoa* in the muscle. Genetic analysis was conducted to understand the relationship of this *Kudoa* spp. to others found in the marine environment. Adult river herring returning to spawn in their natal rivers were infected with myxozoan developmental stages located beneath the renal tubular epithelium. Sequence analysis of the 18S rDNA indicated this to be a species closely related to the genus *Ortholinea*. Though this myxozoan was found at a high prevalence in river herring, the lack of host changes associated with infection suggested this to be a non-pathogenic species. It is believed that this myxozoan is associated with fish during their spawning migration, since when adult non-spawning river herring were examined from the ocean, this myxozoan was not detected. The association of these infections with specific stages in the fish's life history provides insights into environments in which the intermediate hosts for these myxozoan parasites likely exist. Further research is required to identify the intermediate hosts for these myxozoans and to fully understand risk factors for infection.

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