Tuesday September 4th – Langeve / Cartier
Invertebrate and Shellfish Diseases 2
Moderator – Roxanna Smolowitz (Roger Williams University)

1:15 PM	Invert / Shellfish 2	Smolowitz - Hemocytic Neoplasia of Hard Clams (*Mercenaria mercenaria*), an Emerging Neoplastic Disease?
1:30 PM		Ferguson - A Case Report and Statewide Surveillance of "Weak Meat" Condition of Alaska Weathervane Scallops *Patinopecten* caurinus Associated With a Recently Identified Pathogenic Apicomplexan Parasite
1:45 PM		Waller - The Unknown State of Freshwater Mussel Health and Disease
2:00 PM		Gustafson - A Birds-Eye View of Shellfish Health: Advances in Regional Management in the Eastern USA
2:15 AM		Kane - Visual Keys Support Oyster Health Monitoring and Oyster Reef Restoration Efforts
2:30 AM		**Kantzow - Conditioning Pacific Oyster *Crassostrea gigas* Spat for Improved Survival of Ostreid Herpesvirus – 1 (Oshv-1) by Controlled Infection**
2:45 AM		Corbeil - Differentially Expressed Genes in Haliotis Iris (*Paua abalone*) Associated With Haliotid Herpesvirus Challenge.

Hemocytic Neoplasia of Hard Clams (*Mercenaria mercenaria*), An Emerging Neoplastic Disease?

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In 2009, a new lethal disease was identified by Smolowitz and Murphy in hard clams (*Mercenaria mercenaria*), cultured in Wellfleet, Massachusetts, U.S., that caused high mortality. Annual monitoring of hard clams since 2009 has shown a continued high prevalence of the disease associated with significant mortality in aquacultured hard clams. Hard clams originating from multiple hatcheries are affected by the neoplastic disease indicating it is not genetic in origin and is not associated with a specific broodstock but instead provides strong evidence it is a contagious disease. Histologically, large neoplastic cells circulate in the open vascular system of the hard clams causing restriction of the hemolymph circulation in the vascular system with obturation of vessels, eventual loss of normal hemocytes, and loss of the tissue functions. The similarity of the disease to disseminated neoplasia in soft shell clams is striking. Recently evidence has strongly suggest a neoplastic cell is the infective agent in the soft shell clam disease. Identification of the cause of disease in hard clams is ongoing. The disease in hard clams has become a major concern for Wellfleet aquaculturists and the potential for spread to hard clams in other water bodies is a strong possibility.

Conference Session Designation: (Invertebrate and Shellfish Disease)
Presentation Format: (Oral)

A Case Report and Statewide Surveillance of "Weak Meat" Condition of Alaska Weathervane Scallops, *Patinopecten Caurinus*, Associated with a Recently Identified Pathogenic Apicomplexan Parasite

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Weathervane scallops, *Patinopecten caurinus*, are the largest scallop species in the world and they are distributed from Northern California to the Pribilof Islands of Alaska. They are only commercially fished in Alaska and the fishery is considered to be well managed, but there has been a recent decline in scallop catches in some management areas. In the Kamishak Bay fishing District during the 2002 season there was a dramatic decline in Catch Per Unit Effort and an unprecedented incidence of detected "clappers", dead scallops with valves connected but lacking soft tissues. Additionally, fishermen and other stakeholders have encountered scallops with abnormal adductor muscles that have been colloquially named "weak meat". The muscle of affected scallops have brownish coloration, stringy texture and will occasionally either slip off the shell with the viscera or tear apart during the shucking process. Brenner et al. (2012) examined the quality of scallop weak meat using chemical and physical parameters and concluded that nutritional stress was likely involved. A somewhat similar syndrome in sea scallops, *Placopecten magellanicus*, described as "gray meat", has been documented in the eastern U.S. and Canada, which was recently linked to an apicomplexan parasite (Inglis et al., 2016). This is the same parasite that was responsible for the collapse of Icelandic scallops, *Chlamys islandica*, and initial phylogenetic studies placed it within the family Aggregatidae (Kristmundsson et al., 2015). More recently it was identified as *Merocystis kathae* that sexually matures in common whelks, *Buccinum undatum* (unpublished data). In January 2015, fishermen reported high numbers of weak meat in their catch from the Bering Sea and samples were subsequently submitted to the Alaska Department of Fish and Game for diagnostic examination. Histopathology revealed that the poor muscle integrity was likely due to a severe apicomplexan parasite infection. Due to the reduced quality and marketability of scallops with this condition and the potential association with poor survival, we conducted a statewide surveillance study of 180 scallops from subareas within each of the three major geographically broad scallop beds in Alaska. All beds were infected and the highest infection intensities occurred near Dutch Harbor and Southwest Kodiak. A representative set of samples tested by PCR indicated this to be the same parasite as the one that infects and causes disease in other scallop species in the Atlantic Ocean. Currently there is no clear management approach for mitigating this disease.

Conference Session Designation: **(** Invertebrate and Shellfish Disease)
Presentation Format: **(** Oral)

The Unknown State of Freshwater Mussel Health and Disease

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Freshwater mussels (Unionacea) are in decline worldwide, with causes attributed to factors such as habitat degradation, pollution, and invasive species, among others. However, these purported causes cannot fully explain the enigmatic decline and large-scale die-offs of mollusks that have occurred in relatively “healthy” streams across a wide geographic region–from the Southeast to the Pacific Northwest in the U.S. and Sweden to France in Europe. The role of the microbiota and pathogens in the health of freshwater mussels has been understudied and as a result, there are few reference data to compare the “normal” microbiota of healthy to “stressed” or dying mussels. Captive propagation and stocking programs for freshwater mollusk restoration have expanded across the globe. There are no standard diagnostic protocols to assess the health and disease status of cultured, stocked or wild mussels. Continued introduction of nonindigenous species, changing climate, and high-density propagation present risks for outbreaks of opportunistic and new emergent diseases in freshwater mussel populations.

A workshop on Freshwater Mollusk Health and Disease, sponsored by the Freshwater Mollusk Conservation Society, was held in March 2018 as a first step towards advancing an initiative on this topic. The Workshop advocated for inclusion of freshwater mollusks in the One Health concept (recognition that the health of people is connected to the health of animals and the environment) and for integrating multidisciplinary technologies with traditional diagnostic tools to assess the health status of mollusk populations. This strategy is being demonstrated in response to a chronic mussel-die off occurring in the Clinch River in the southeastern U.S. The Clinch River contains one of the premier freshwater mussel communities in the country and includes 29 threatened and endangered species. Samples of mussels were collected from affected and unaffected reaches of the river in 2017. A metagenomic approach is being used to characterize and compare viral and bacterial communities among mussel populations and will be combined with histopathologic evaluation and other approaches. The project is one of the first comprehensive assessments of an on-going mortality event and will provide information not only on potential causal factors, but also on “best practices” for addressing such events. Given changing environments around the world, there is a global need for such a framework.

Conference Session Designation: (Invertebrate and Shellfish Disease)
Presentation Format: (Oral)

A Birds-Eye View of Shellfish Health: Advances in Regional Management in the Eastern USA

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Shellfish health management is typically structured along jurisdictional boundaries. Fourteen states, many reliant on inter-state trade of larvae or spat, rear molluscan shellfish in the East Coast region alone. While growth has been rapid to keep pace with public demand, pathogens - and, by association, their management - have the potential to derail both population health and industry vitality. In fact, both movement of pathogens with trade, as well as redundancy or delays in regulatory permitting of trade, can have unintended consequences. Typically, trade (or movement) decisions are made at the state, or sometimes even more local, level. Regulators are often constrained to operate on local (fragmented) knowledge, and, because mollusc disease risk and status do not adhere to jurisdictional boundaries, the industry may face redundant testing, conflicting demands, or delayed decisions. As a response to this conundrum, a consortium of academic, regulatory and industry representatives have been working to advance shellfish health management along the East Coast of the United States through partnerships in information and decision support. Central to this effort are mechanisms to enhance information exchange. Recent focus has been on the design of a shared, interactive, database for the storage and retrieval of regional health information that crosses jurisdictional and organizational bounds. We describe the importance of rapid and accurate information exchange for this growing industry, and the strategy underway to meet that goal.

Conference Session Designation: (Bivlave or Shellfish Diseases)
Presentation Format: (Oral)

Visual Keys Support Oyster Health Monitoring and Oyster Reef Restoration Efforts

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Environmental and health observations are an integral component of monitoring oyster reefs restoration projects. We developed and applied visual keys to facilitate consistency in ranking severity scores for stained *Perkinsus marinu*s hypnospores, empirical meat ranks (volume within the left shell, plumpness, translucency), and severity of boring shell parasites affecting Eastern oyster, *Crassostrea virginica*. Measurement of shell parasite prevalence and severity for *Polydora websteri* (polychaete), *Diplothyra smithii* (clam) and *Cliona celata* (sponge) is important since these organisms excavate shell matrix throughout the life of the oyster (and in the case of *Cliona*, throughout the life of the shell). Further, these parasites reduce shell density and dramatically increase surface area of the shell exposed to the environment. Higher salinity conditions foster the severity of these shell parasites, and shells with extensive excavation and high surface area may dissolve more rapidly in saltwater, break down into shell fragments, and destabilize reef structure in the long term. Visual keys that support these metrics will be demonstrated and their utility for training new investigators and providing intra- and inter-laboratory quality control for diagnostic health assessments will be discussed. Consistency in reporting health metrics as part of oyster resource monitoring has become increasingly important as restoration efforts with limited resources focus more on sustainable, measureable outcomes. This project was supported in part through the National Fish and Wildlife Foundation Gulf Environmental Benefit Fund, Florida Fish and Wildlife Conservation Commission, the University of Florida Institute for Food and Agricultural Science (IFAS), and the Florida Sea Grant Program.

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Conditioning Pacific Oyster (*Crassostrea Gigas*) Spat for Improved Survival of *Ostreid Herpesvirus – 1* (Oshv-1) by Controlled Infection

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The high mortality and economic loss in farmed Pacific oysters (*Crassostrea gigas*) caused by the virulent genotypes of *Ostreid herpesvirus - 1* (OsHV-1) has spurred research into strategies to mitigate the impact of the disease. Both elevated water temperature and the absence of previous exposure to OsHV-1 increase the level of mortality from infection with OsHV-1. Infection at 18°C can result in infection, but occurrence of mortality is dose dependent. The pathogenesis at 22°C is more rapid and results in greater mortality. The present study evaluated the effect of water temperature and pre-exposure to OsHV-1 on oyster survival following a second exposure to OsHV-1 in a controlled laboratory experiment. It was conducted with 6 month old commercial triploid Pacific oyster spat in a physical containment level 2 aquatic animal facility. Oysters were first exposed at a water temperature of either 18°C or 22°C with either OsHV-1 or a negative control inoculum. Surviving oysters were then maintained at 18°C or 22°C, or the water temperature was increased from 18°C to 22°C for the second exposure to OsHV-1. Mortality in the 10 days following the second exposure at 22°C was 10% and 24% for those pre-exposed at 18°C with OsHV-1 and the negative inoculum respectively with a hazard ratio (HR) of 0.22 (95%CI: 0.1 – 0.8). Mortality was 34% and 40% for oysters pre-exposed at 22°C with OsHV-1 and the negative inoculum respectively, HR: 0.7 (95%CI: 0.3 – 1.9). The concentration of OsHV-1 DNA at the time of death or end of the trial was assessed using a linear mixed model which indicated a significant interaction between the pre-challenge water temperature and the inoculum ($P<0.001$). This study determined that pre-exposure to OsHV- 1 can infer greater survival on re-exposure to OsHV-1. This confirms field observations that oysters which have survived an OsHV-1 outbreak are more likely to survive further outbreaks and controls for confounding factors reflecting changes in the host and environment which can impact disease outcome. A pre-challenge with OsHV-1 presents a possible method of reducing mortality from OsHV-1 epizootics in farmed Pacific oyster populations.

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Student Presentation: (Yes)

Differentially Expressed Genes in *Haliotis Iris* (Paua Abalone) Associated with Haliotid Herpesvirus Challenge.

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The natural resistance of New Zealand paua abalone (*Haliotis iris*) to infection by Haliotid herpesvirus 1 (HaHV) and to the disease abalone viral ganglioneuritis (AVG) was investigated using high throughput RNA-Seq. HaHV-infected paua up-regulated broad classes of genes that contained chitin-binding peritrophin-A domains, which may indicate the production of a defensive "peritrophic matrix", as seen in other molluscs and insects. The paua also up-regulated VAP-1, an important adhesion molecule for lymphocytes in mammals, and CHIT-1, an immunologically important gene in mammalian immune systems. Moreover, several blood coagulation pathways were dysregulated in the paua, possibly indicating viral modulation. We also saw several indications that neurological tissues were affected by HaHV, including the dysregulation of B4GALNT, GM2 ganglioside, neuroligin-4 and the Notch signalling pathway. This research may support the development of an AVG resistant breeding program in disease susceptible Australian abalone or the development of molecular therapeutics useful to control and/or manage virus outbreaks in Australian abalone culture.

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