

Tuesday September 4th – Langeve / Cartier
Invertebrate and Shellfish Diseases 1
Moderator – Roxanna Smolowitz (Roger Williams University)

10:45 AM	Invert/Shellfish 1	<u>Atherley</u> - Microsporidiosis in the Caribbean Spiny Lobster: A Rare Infection
11:00 AM		<u>Elliott</u> - First Detection of <i>Panulirus argus</i> Virus 1 (PAV1) by PCR in Spotted Spiny Lobsters (<i>Panulirus guttatus</i>) Held in Captivity
11:15 AM		<u>Hawke</u> - An Update on White Spot Syndrome Virus Disease in Louisiana Red Swamp Crawfish <i>Procambarus clarkii</i>
11:30 AM		<u>Battison</u> - Observation of Epithelial and Endothelial Intracytoplasmic and Hepatopancreatic Intranuclear Viral Particles in Snow Crab <i>Chionoectes opilio</i> From the Gulf of St. Lawrence and the Scotian Shelf
11:45 AM		<u>Mahadevan</u> - Previously Undescribed Histopathology Findings From Research Investigating Poor Post-Capture Survivability in Australian Southern Rock Lobster (<i>Jasus edwardsii</i>)
12:00 PM		Lunch



8th International Symposium on Aquatic Animal Health

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



Microsporidiosis in the Caribbean Spiny Lobster: a Rare Infection

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The Caribbean spiny lobster, *Panulirus argus*, is important to the economy of several countries in the Caribbean region, including Saint Kitts and Nevis. According to the IUCN red list, the *P. argus* population is currently decreasing and is believed to be exploited throughout its geographical range. As a result, it is important to investigate the factors that would affect population health and consequently a safe commercial yield. Although few lobster diseases and parasites have been reported, surveillance is important since existing diseases may affect yield and marketability of this species. Microsporidians comprise a group of spore-forming, unicellular organisms, related to the fungi, which are obligate intracellular parasites. These parasites have been known to infect a range of organisms from commercial insect colonies to fish and numerous mammals. This includes several species causing disease in crustaceans such as crabs, shrimp and lobster. Clinical signs of microsporidiosis in Crustacea are generally characterized by a change in the muscle from translucent to white/opaque, resembling cooked meat.

Lobsters were collected from fishermen during the period July 2017 to January 2018 as part of a PhD project entitled 'The biology, ecology and diseases of *Panulirus argus*'. This project seeks to examine the population dynamics and health together with novel ageing studies of lobsters from St Kitts. During necropsies, abnormal skeletal muscle and myoliquefaction was observed in one lobster. Wet-mounts confirmed the presence of microsporidian spores. Samples of muscle, eyestalk, ovary, gill and heart tissue were placed in Davidson's fixative for histopathological analysis. DNA was extracted from muscle samples and subsequent PCR reactions allowed for the amplification of partial sequences for the ribosomal RNA gene. Thereafter, database searches were used to identify the closest known microsporidian sequences.

Based on necropsies and the histopathological analysis of one hundred and fifteen lobsters, one lobster was infected with microsporidian spores; therefore, the prevalence of this parasite in Saint Kitts is only 0.87%. Although this parasite is seemingly not prevalent in wild lobster populations, there is a concern this could change with the potential development for aquaculture of the spiny lobster in the Eastern Caribbean region. The intensive systems generally used in lobster aquaculture may lead to higher levels of infection in the cages or tanks, which will likely reduce commercial yields and possibly impact local wild populations, as high numbers of resilient spores would be produced.

Screening of the lobster population in Saint Kitts is ongoing and will continue for the next two years as part of this PhD study. A literature review of previously published work revealed that the incidence of microsporidiosis in spiny lobster populations is rare and has up to now been restricted to regions around Florida. However, our DNA data indicates that this is the same microsporidian parasite, which clearly has a more widespread distribution than previously thought. These results highlight its possible geographical expansion and this is the first report of this microsporidian in the Eastern Caribbean region.

Conference Session Designation: (Shellfish)
Presentation Format: (Oral)
Student Presentation: (Yes)



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First Detection of *Panulirus Argus* Virus 1 (PaV1) By PCR in Spotted Spiny Lobsters (*Panulirus Guttatus*) Held in Captivity

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Panulirus argus Virus 1 (PaV1) is the first naturally occurring pathogenic virus described in Caribbean spiny lobsters (*Panulirus argus*). The virus has been reported throughout the Caribbean sea and is typically lethal to infected juvenile lobsters within weeks to months. Previously, PaV1 infection has not been detected in other decapods that co-occur with *P. argus*, including the spotted spiny lobster (*Panulirus guttatus*); however, these studies primarily used histological evaluation, and PCR was not performed. In 2016, 14 Caribbean spiny lobsters (*P. argus*) and 5 spotted spiny lobsters (*P. guttatus*) were collected off of Summerland Key, Florida to supplement the resident population at the Aquarium of the Americas in New Orleans, Louisiana. The lobsters were transported and placed in quarantine tanks at Audubon's Aquatic Center housed at the Freeport McMoran Audubon Species Survival Center. After 5 months lobsters began to show clinical signs of lethargy and dying in the molt. Tissues were submitted to the Louisiana Animal Disease Diagnostic Laboratory (LADDL) at the Louisiana State University School of Veterinary Medicine for necropsy. Initial histopathological investigation revealed intranuclear inclusion bodies in cells of the exoskeletal membrane, indicating a viral infection. Differential diagnoses included White Spot Syndrome Virus (WSSV), a devastating disease of shrimp and crawfish also known to infect lobsters, and PaV1. Real time qPCR was performed on samples of haemolymph and tissues from both species of lobster for WSSV at LADDL and for PaV1 at the University of Florida; samples were negative for WSSV, but positive for PaV1. Tissues from the deceased spotted spiny lobsters were too severely autolyzed for critical histologic diagnostic evaluation. Remaining Caribbean spiny lobsters were euthanized in 10% MgCl in 100% ETOH and tissues were fixed in Davidson's fixative, gluteraldehyde, and 80% ETOH. Transmission electron microscopy (TEM) was performed to further characterize the viral infection. The most significant lesions were found in the hepatopancreas, where the virus is known to infect fixed phagocytes and circulating hemocytes including hyalinocytes and semi-granulocytes. TEM revealed that viral inclusions were localized exclusively in the cells underlying the hepatopancreatic epithelium of the hepatopancreatic tubules. This case demonstrates that PaV1 is a continuing threat to populations of Caribbean spiny lobsters held in captivity, as well as their co-habitant, the spotted spiny lobster (*P. guttatus*).

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



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An Update on White Spot Syndrome Virus Disease in Louisiana Red Swamp Crayfish *Procambarus clarkia*

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The red swamp crawfish *Procambarus clarkii* is the most economically important aquaculture species grown in Louisiana. In 2016 the farm gate value of crawfish aquaculture was approximately \$196 million, with an additional added value of \$128 million from processing and marketing. There are 1500 farm operations and approximately 220,000 acres of ponds many of which are in rotation with rice production. The crawfish harvesting season extends from November to June most years. White spot syndrome virus WSSV was discovered in the Louisiana crawfish industry in 2007 from specimens submitted to the Aquatic Disease Section of the Louisiana Animal Disease Diagnostic Laboratory at the LSU School of Veterinary Medicine. A surveillance project conducted by USDA/APHIS followed with detection of the virus by real time PCR in 60% of 184 sites sampled from 18 parishes across the state. Although its occurrence was widespread, losses over the next nine years were apparently not significant to the industry. However, mortalities in 2017 and 2018 have been much more severe causing many farmers to suspend production early. Clinical signs of WSSV in crawfish are lethargy, high mortality rates of large crawfish in the ponds, dead and dying large crawfish in traps with young active crawfish in the pond, and drastic drops in catch rate. White spots are typically not seen in the carapace. There are many unanswered questions and unidentified predisposing factors that trigger high mortality rates in crawfish ponds. For this reason we propose to engage in future research to evaluate effects of size, age, dissolved oxygen concentrations, and water temperature on susceptibility of red swamp crawfish to WSSV. We will utilize methods of viral quantification and experimental infection developed previously in our labs at LSU.

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



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Observation of Epithelial and Endothelial Intracytoplasmic and Hepatopancreatic Intranuclear Viral Particles in Snow Crab *Chionoecetes Opilio* from the Gulf of St. Lawrence and the Scotian Shelf

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Viral inclusions were observed during review of haematoxylin and eosin stained histologic sections of gill and hepatopancreas tissues from snow crab (*Chionoecetes opilio*) as part of a study to collect biological baseline information on the species. Tissues were collected from crabs at three sampling stations in the southern Gulf of St. Lawrence (Margaree NS, Cheticamp NS and Grand Riviere QC) and one on the Scotian Shelf (Louisbourg NS). Intracytoplasmic coarsely granular eosinophilic to amphophilic inclusions were observed in 18/446 crabs where gill tissue was examined. Inclusions were most conspicuous in the gill epithelium but were also noted in endothelium of the gill and hepatopancreas. Transmission electron microscopy revealed numerous hexagonal viral particles (~ 70 – 80 nm in diameter) often packed in honeycomb-like arrangements in the cytoplasm of affected cells. The intracytoplasmic particles were observed more often in samples from the Cheticamp NS and Margaree NS sampling stations. Large basophilic to amphophilic homogenous intranuclear inclusions were observed nearly completely filling the nuclei of hepatopancreas B-cells in 6/1113 crabs – four from the Louisbourg NS, one from the Margaree NS, and one from the Grande Rivière QC sampling station. Transmission electron microscopy images suggested closely packed linear aggregates of viral particles in the nucleus. Co-infections were not detected.

Conference Session Designation:
Presentation Format:

(Invertebrate and Shellfish Disease)
(Oral)

Previously Undescribed Histopathology Findings from Research Investigating Poor Post - Capture Survivability in Australian Southern Rock Lobster (*Jasus Edwardsii*).

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During 2017 the wild catch Southern Rock Lobster (SRL) industry initiated research into improving post-capture survivability and in particular the possible effects of Australia's changing environment on lobster health. The research was divided into three areas of investigations; SRL physiology, epidemiology of reduced survival and pathology associated with mortality events. As part of the pathology component multiple SRL samples from mortality, reduced survival events and health surveillance sampling were submitted in by processors across Australia for diagnostic evaluation. Histopathology findings were detailed for organs systems that showed consistent pathology from all accessions (ie: gills, hepatopancreas, nerves, reserve cell tissue and antennal glands). A range of degenerative and inflammatory lesions were noted in antennal glands with some extensive destruction of the gland tissue associated with high levels of intra-lesionary bacteria. Three other significant histological changes were observed. Two of which have not previously been noted for SRL (ie: mineralised inclusions, nuclear basophilic inclusions). Additionally there was repeat observations of cytoplasmic eosinophilic inclusions which have been previously described. These inclusion changes were noted in 66% of the animals examined and in the majority with apparently normal antennal gland tissue. This presentation will discuss the general histopathological findings noted in 2017 and focus particularly on changes observed in the antennal gland of SRL that prior to this study appear to have not been described or published.

Conference Session Designation:

(Invertebrate and Shellfish Disease)

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