

**Monday September 3<sup>rd</sup> – Langeve / Cartier**  
**Parasitology Life Cycle**  
**Moderator – Isaure de Buron** ( College of Charleston )

1:15 PM	<b>Parasitology: Life Cycle</b>	<u>Cole</u> - Life Cycle of <i>Truttaedacnitist truttae</i> ( Nematoda: Cucullanidae ) in Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) in the Colorado River Drainage in Grand Canyon, Az
1:30 PM		<u>De Buron</u> - First Evidence of Polychaete Intermediate Hosts for Marine Turtle Blood Flukes ( Trematoda: Spirorchiidae )
1:45 PM		<u>Geist</u> - Host-Parasite Interaction Between Salmonids and Larvae of the Freshwater Pearl Mussel
2:00 PM		<u>Alberson</u> - Experimental Elucidation of the Life Cycle of <i>Drepanoscephalus auritus</i> (Digenea: Echinostomatidae) in the Double-Crested Cormorant ( <i>Phalacrocorax auritus</i> ), the Marsh Rams-Horn Snail ( <i>Planorbella trivolvis</i> ), and the Channel Catfish ( <i>Ictalurus punctatus</i> )
2:15 PM		<u>Nowak</u> - Understanding Parasitic Life Cycles Contributes to an Improved Health Management of Ranched and Farmed Bluefin Tuna
2:30 PM		<u>Rosser</u> - Investigations into the Life Cycles of Trematodes in Catfish Aquaculture Systems in Mississippi, USA
2:45 PM		<b><u>Woodyard</u> - Morphological and Molecular Data Linking the Life Stages of <i>Sebekia mississippiensis</i> ( Pentastomida: Sebekidae ) from the American Alligator <i>Alligator mississippiensis</i> and the Spotted Gar <i>Lepisosteus oculatus</i>, with Notes on Pathology in the Intermediate and Definitive Hosts</b>



**8<sup>th</sup> International Symposium on Aquatic Animal Health**

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



**Life Cycle of *Truttaedacnitis truttae* (Nematoda: Cucullanidae) in Rainbow Trout (*Oncorhynchus mykiss*) in the Colorado River Drainage in Grand Canyon Arizona.**

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*Truttaedacnitis truttae* is a cucullanid nematode of primarily salmonine fishes. Brown trout (*Salmo trutta*) in Europe reportedly become parasitized by ingesting lampreys (*Lampetra planeri*) carrying infective larvae. However, our field and laboratory observations suggested that North American *T. truttae* has an alternative life cycle. High abundance and potential impact of *T. truttae* in rainbow trout, *Oncorhynchus mykiss*, in the Colorado River drainage in Grand Canyon prompted a study on the transmission dynamics of this nematode. Eggs of *T. truttae*, collected from live gravid females, were incubated in the laboratory, and snails (*Physa gyrina* and *Lymnaea* sp.) were exposed to *T. truttae* larvae three to four weeks later. Active larvae of *T. truttae* were observed penetrating the intestinal wall of exposed snails, and worm larvae were found in the visceral tissues one week after exposure. Larvae in snails showed little growth and development two weeks later and corresponded to L3 larvae. Infected snails were fed to hatchery-reared juvenile rainbow trout in which developing stages were subsequently found in the mucosal lining and lumen of trout intestines. Adult male and female (gravid) worms were found in the ceca of trout examined five to six months after consuming infected snails. Larvae found in pepsin/trypsin digests and mucosal scrapings from wild, naturally infected trout corroborated laboratory findings. Screening of *Physa* sp. and gammarids collected from the Colorado River, Grand Canyon for natural infections with *T. truttae* using ITS-1 primers gave positive results. *Truttaedacnitis truttae* is the second species, after *T. clitellarius* of lake sturgeon, capable of using a snail as a first intermediate/paratenic host, and is similar to several other cucullanids in having a histotropic phase of development in the definitive fish host.

**Conference Session Designation:**  
**Presentation Format:**

( General Parasitology )  
( Oral )



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## First Evidence of Polychaete Intermediate Hosts for Marine Turtle Blood Flukes (Trematoda: Spirorchiidae)

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Spirorchiids that infect the vascular system of turtles are not well studied. Few life cycles of these blood flukes have been elucidated and all intermediate hosts reported are mollusks, regardless of whether the definitive host is a freshwater or a marine turtle. During a recent survey of blood fluke larvae in polychaetes on the coast of South Carolina USA, sporocysts and spirorchiid-like cercariae were found to infect the terebellid *Amphitrite ornata* and polycirrid *Enoplobranchus sanguineus*. Cercariae were furcate and had a ventral acetabulum, but no ocelli were observed. Partial sequencing of D1-D2 domains of the large ribosomal subunit (LSR), ITS2, and *cox1* genes allowed the identification of two *Neospororchis* species reported from green turtle, *Chelonia mydas* in Florida USA. *Neospororchis* sp. (Neogen 13) and *Neospororchis* sp. (Neogen 14) infected individuals of *A. amphitrite* and *E. sanguineus*, respectively. Phylogenetic analysis showed that infection of annelids by blood flukes evolved separately in aporocotylids and spirorchiids. This finding demonstrates that specificity of spirorchiid for their intermediate hosts is broader than it was thus far assumed and that survey of annelids in turtle habitats is necessary to further our understanding of the life history of these parasites.

**Conference Session Designation:** ( Parasitology General or WAVMA )  
**Presentation Format:** ( Oral )



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## Host-Parasite Interaction Between Salmonids and Larvae of The Freshwater Pearl Mussel

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Endangered freshwater pearl mussels (*Margaritifera margaritifera*) have a complex life cycle which involves a larval glochidium stage at which the mussel larvae need to attach to the gills of a suitable salmonid host fish that develops immunity after first infestation. In this study, the suitability of different salmonid species and strains as hosts for the freshwater pearl mussel was investigated using standardized infestation procedures. Also, the temperature-dependency of glochidia metamorphosis on the gills, the transfer of nutrients from the host to the mussel larvae based on stable isotope analyses, and the impacts of different infestation intensities on the performance of brown trout (*Salmo trutta*), were analyzed. In addition, genetic co-evolutionary patterns between host fish and mussels were investigated. The results suggest that freshwater pearl mussel larvae can clearly be considered parasites of their salmonid hosts as evident from the observed mortality and reduced swimming performance of hosts at high infestation densities, as well as the unidirectional nutrient transfer from the host to the growing glochidium. On the other hand, salmonids may also benefit from the presence of mussels due to their filtration activity and effects on water clarity. Timing of metamorphosis and transformation success was strongly temperature-dependent, with pronounced differences among host strains within the same species. The genetic population structure from pearl mussel and their main host fish, brown trout, suggest co-evolutionary colonization patterns revealing stronger differentiation among mussel populations and more pronounced genetic drift effects in the parasite compared to the more mobile host fish. The better understanding of this unique host-parasite relationship in which the maximum age of the mussel exceeds the maximum age of the host by a factor of 30 is useful for the conservation of both host fishes and mussel.

**Conference Session Designation:** ( Parasitology General )

**Presentation Format:** ( Oral )



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# Experimental Elucidation of the Life Cycle of *Drepanocephalus auritus* ( Digenea: Echinostomatidae ) in the Double-crested Cormorant ( *Phalacrocorax auritus* ), the Marsh Rams-horn Snail ( *Planorbella trivolvis* ), and the Channel Catfish ( *Ictalurus punctatus* )

Neely R. Alberson<sup>1</sup>, Thomas G. Rosser<sup>1</sup>, D. Tommy King<sup>2</sup>, Lester H. Khoo<sup>3</sup>, David J. Wise<sup>3</sup>, Ethan T. Woodyard<sup>1</sup>, Fred L. Cunningham<sup>2</sup> and Matt J. Griffin<sup>3\*</sup>

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*Drepanocephalus auritus* is a trematode parasite of the double-crested cormorant *Phalacrocorax auritus*, and in North America, the marsh rams-horn snail *Planorbella trivolvis*, and ghost rams-horn snail *Biomphalaria havanensis*, both commonly found to inhabit catfish aquaculture ponds in east Mississippi. Previous infectivity challenges demonstrated *D. auritus* is infective to channel catfish *Ictalurus punctatus*, although infection begins to resolve quickly. A 2-year study was undertaken to experimentally elucidate the life cycle of *D. auritus*. In both studies, *P. trivolvis* were collected from a commercial catfish operation in east Mississippi, of which several were releasing *D. auritus* cercariae. Juvenile channel catfish were exposed individually to ~150 cercariae/fish. Double-crested cormorants (DCCO) were live captured, housed individually, and given praziquantel to clear gastrointestinal helminth infections. During the first study, 3 experimental DCCO were fed *D. auritus* infected fish. Fecal samples were collected daily and observed for trematode ova. At 18 days post-exposure (dpe) birds were sacrificed, and gravid, adult trematodes morphologically and molecularly consistent with *D. auritus* were recovered from experimental DCCO. During the second study, 2 experimental DCCO were fed infected fish. Daily fecal sampling continued as in the previous year's study, and trematode ova was observed in experimental birds beginning 8 dpe. Once ova were observed, birds were allowed to defecate into clean water tanks containing naïve *P. trivolvis*. Birds were then removed and snails transferred to a laboratory aquarium for holding. Eggs from experimental DCCO feces were recovered by sedimentation and placed into a second aquarium housing 15 naïve *P. trivolvis*. In addition, eggs were placed individually in 24 well cell-culture plates and checked daily for miracidia, which were observed, on average, after 16 days at 25C. All birds were sacrificed on study day 60 and adult trematodes were again recovered from experimental birds. Recovered adults were morphologically and molecularly confirmed as *D. auritus*. One snail from the DCCO tanks shed *D. auritus* cercariae 97 dpe, while another snail, directly exposed to trematode eggs, began shedding *D. auritus* 89 dpe. This work is the first experimental confirmation of the *D. auritus* life cycle and sheds new light on the complex biology of this parasite. Moreover, this work resolves unknown developmental timelines and provides critical baseline information for the development of management practices to minimize the effects of digenetic trematodes on US catfish aquaculture.

**Conference Session Designation:**  
**Presentation Format:**

( Parasitology –Life Cycle )  
( Oral )



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## Understanding Parasite Life Cycles Contributes to an Improved Health Management of Farmed and Ranched Bluefin Tunas

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Bluefin tunas, including Atlantic Bluefin Tuna (ABT), *Thunnus thynnus*, Pacific Bluefin Tuna (PBT), *Thunnus orientalis* and Southern Bluefin Tuna (SBT), *Thunnus maccoyii* are commercially important species. Bluefin tunas are either ranched (wild fish fattened in sea pens – ABT, PBT and SBT) and farmed (life cycle closed, commercial hatchery production – PBT) in a number of countries. Parasites, in particular blood flukes *Cardicola orientalis*, *Cardicola opisthorchis* and *Cardicola forsteri* are the main health problems for ranching and farming of bluefin tunas. Husbandry is the key in bluefin tuna health management. Our knowledge of blood fluke life cycles, in particular the intermediate hosts and their habitats has contributed to the minimisation of the risk of infection with blood flukes. This is also true for other parasitic infections of SBT. For example, adult *Caligus chistos* but few chalimi were observed on SBT. Proper feed management reduces interactions between SBT and wild Degen's leatherjacket *Thamnaconus degeni* which carry chalimi and adults *Caligus chistos* and as a result decreases infections of SBT with sealice and minimises the risk of eye damage. Moving of SBT further off shore resulted in the reduction of parasitic loads. Another parasitic disease, swimmer syndrome is caused by scuticociliate *Miamensis avidus* but this infection is now rare. Improved husbandry and management of SBT health based on our understanding of the biology of parasites, including their life cycles, have increased sustainability of SBT production

**Conference Session Designation:** ( Parasitology General or Tuna Diseases )  
**Presentation Format:** ( Oral )



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## Investigations into the Life Cycles of Trematodes in Catfish Aquaculture Systems in Mississippi, USA

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In the southeastern United States, farm-raised catfish is the most extensively cultured freshwater food fish. These earthen ponds are open to the external environment and provide an ideal system for the propagation of parasite life cycles. The American white pelican *Pelecanus erythrorhynchos*, double-crested cormorant *Phalacrocorax auritus*, great egret *Ardea alba*, and great blue heron *Ardea herodias* are the primary piscivorous bird hosts plaguing the industry through depredation and while doing so also introduce trematode parasites into the ponds. These complex trematode life cycles involve avian definitive hosts, aquatic snail first intermediate hosts and fish second intermediate hosts. *Bolbophorus damnificus* is the most damaging trematode and is responsible for production deficits due to mortality and parasite induced inappetence. However, in more recent years the true diversity of the trematode species occurring in these aquaculture systems has grown considerably. Surveys of snail first intermediate hosts, fish, and piscivorous birds utilizing these ponds have uncovered previously unknown life cycles. Using classical morphological, experimental, and molecular techniques we have elucidated complete or partial life cycles of >5 trematode species in catfish ponds and have evaluated impacts on the fish hosts through experimental infection studies. Two *Austrodiplostomum* sp. are known to infect catfish and other forage fish species found in catfish ponds and are found in the eyes and brain. One of these species (*A. ostrowskiae*) utilizes the double-crested cormorant as a definitive host and *Biomphalaria havanensis* as a first intermediate host. In addition to diplostomids, catfish ponds also play a role in the propagation of at least two *Clinostomum* spp. The “yellow grubs” *Clinostomum marginatum* and *Clinostomum album* are parasites of the great egret and infect catfish, silversides, and minnows in catfish ponds. The great egrets are also hosts to a multiple species of diplostomid and strigeid trematodes, many with unknown life cycles. In addition to trematodes with fish second intermediate hosts we have identified species infectious to native amphibians and have begun exploring their impacts on the host. Discussions on methods of elucidation of life cycles will be discussed.

**Conference Session Designation:**

( Parasitology General )

**Presentation Format:**

( Oral )



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# **Morphological and molecular data linking the life stages of *Sebekia mississippiensis* (Pentastomida: Sebekidae) from the American alligator *Alligator mississippiensis* and the spotted gar *Lepisosteus oculatus*, with notes on pathology in the intermediate and definitive hosts**

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Pentastomes are an enigmatic group of parasitic crustaceans found mostly as internal parasites in reptilian and fish hosts. In the southeastern United States, *Sebekia mississippiensis* parasitizes the lungs of the American alligator *Alligator mississippiensis* as an adult. While many freshwater fish have been reported as intermediate hosts, few of these accounts have provided morphological descriptions of the nymphs and to date no molecular data have been generated for *S. mississippiensis*. During Mississippi's 2016 and 2017 alligator hunting seasons, *S. mississippiensis* were recovered from American alligators collected from a commercial alligator processor in Mississippi. Concurrently, nymphs were collected from spotted gar *Lepisosteus oculatus* from Louisiana. Recovered adult and nymphal pentastomes were morphologically identified as *S. mississippiensis* and molecular data of ribosomal (18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA) and mitochondrial (cytochrome c oxidase subunit 1) DNA were generated. Using these molecular data, the nymphal stages from fish hosts and adults from alligators were confirmed to be conspecific. In the future, these data will allow further investigations into the molecular systematics of *S. mississippiensis* and its placement within the Pentastomida as well as more robust life cycle studies of the species. Histopathological analysis of lungs from alligators and alligator gar tissues was performed and the impacts on the hosts will be discussed. The presented data will help resolve many ambiguities in the literature regarding this species and its life cycle.

**Conference Session Designation:**

( Parasitology General )

**Presentation Format:**

( Oral )

**Student Presentation:**

( Yes )



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