Wednesday September 5th – Archibald / Campbell
Ornamental and Aquarium Medicine 1 & 2
Moderator - Johnny Shelley (USDA – Agricultural Research Service)

9:30 AM	**Ornamentals 1**	Shelley - Edwardsiellosis in Ornamental Fish
9:45 AM		Smith - An Outbreak of *Cryptobia iubilans* in a Captive Population of Mayan Cichlids *Cichlasoma urophthalmus*
10:00 AM		Kim - Use of the Microalgae *Phaeodactylum tricornutum* for the Remedy of Monogenean Infections: Treatment of *Gyrodactylus turnulli* in guppies
10:15 AM		Miller-Morgan - Ensuring Health Throughout the Supply Chain: Developing a Supply-Chain Health Management Training Program for a Wild-Caught Aquarium Fishery in Brazil – A Case Study
10:30 AM		**Refreshments**
10:45 AM	**Ornamentals 2**	**Scherbatskoy - Finding Nemo's Picornavirus**
11:00 AM		Adamek - Is there a Difference in Virulence Between Carp Edema Virus from Different Genogroups?
11:15 AM		**Koda - Phylogenomic Characterization of Megalocytiviruses in Archived Ornamental Fish Samples**
11:30 AM		**Munday - The Effects of Venting, Transport, and Holding Methods on Yellow Tang (*Zebrasoma flavescens*) Health in the Marine Ornamental Aquarium Fish Trade**

Edwardsiellosis in Ornamental Fish

John P Shelley[1*], Roy P E Yanong[2], John P Hawke[3] and Matt Griffin[4]

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Historically *Edwardsiella ictaluri* has been primarily associated with Channel Catfish, *Ictalurus punctatus*, aquaculture and the food fish industry. It is the causative agent of enteric septicemia of catfish (ESC) and in the United States is the most economically important infectious disease in farm-raised catfish. Once considered a host-specific pathogen of catfish species in the US, it has since been isolated from non-ictalurid species in natural or experimental infections from the US and internationally. *Edwardsiella ictaluri* appears to have a history within the ornamental fish industry as well, with sporadic reports in the 1980s. In 2013, Hawke *et al.* described a new strain of *E. ictaluri* as an emerging pathogen of zebrafish based on eight cases from five states that were submitted to the Zebrafish International Resource Center (ZIRC) and the Aquatic Disease Section of the Louisiana Animal Disease Diagnostic Laboratory (LADDL) between 2011 and 2012. The fish exhibited exophthalmia along with hemorrhage in the skin around the eyes, operculum, base of fins and the abdomen. Additionally, they had swollen abdomens due to ascites and they displayed neurologic swimming behaviors such as spinning, spiraling and lethargy. The zebrafish strain of *E. ictaluri* is believed to be unique compared to the catfish strain and the tilapia strain described by Soto *et al.* in 2013. Since 2011, the species that the zebrafish strain has been known to infect has expanded to include all the varieties of *D. rerio*, as well as the Blue Danio (*Danio kerri*), the Leopard Danio (*Danio frankei*) and the Giant Danio (*Devario aequipinnatus*). With the expansion of susceptible species, *E. ictaluri* is now recognized as an important pathogen of zebrafish and control methods are under investigation. Future works with the zebrafish strain of *E. ictaluri* will focus on comparing it against the channel catfish strain at the molecular level and the serological level for common and unique features related to virulence and infection. Additionally, the effectiveness of different disease management strategies will be investigated to help the ornamental fish industry combat the spread of the zebrafish strain of *E. ictaluri*.

Conference Session Designation: **(** Ornamentals and Aquarium Medicine)
Presentation Format: **(** Oral)

An Outbreak of *Cryptobia Iubilans* in a Captive Population of Mayan Cichlids (*Cichlasoma Urophthalmus*)

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Significant mortality was observed in a captive population of juvenile (~3 month old) Mayan cichlids (*Cichlasoma urophthalmus*) over a two-week period of time. The tanks with the Mayan cichlids were part of the multi-tank recirculation system (50 tanks x 15 gal) with mechanical and biological filters which also had a 10-20% water exchange per day with municipal water that was treated with carbon filters for dechlorination. Clinical signs prior to death included a distended abdomen, loss of equilibrium and erratic swimming behavior. During this time the fish were treated with chlortetracycline (50-100 mg/L) bath for 10 days and then a penicillin/streptomycin bath (0.5 mg/L) for several days. Despite these treatments, an increasing number of fish continued to demonstrate clinical signs and experience mortality. Other adult cichlid (e.g. red head, *Cichlasoma synspilum* and midas, *Amphilophus citrinellum*) and non-cichlid fish (e.g. zebrafish, pacu, and arowana) in separate tanks of the same recirculation system did not experience any clinical signs or mortality. Four morbid fish were humanely euthanized with buffered MS-222 and a complete necropsy of each fish performed. Samples of representative tissues were preserved in 10% neutral buffered formalin, trimmed and submitted to the Virginia-Maryland College of Veterinary Medicine for histopathology. In all four fish, there were multiple discrete granulomas within the wall of the stomach. In addition, there were a number of extraluminal granulomas surrounding the stomach that extended into the coelomic cavity of several fish. A small number of flagellates were observed in the lumen of the stomach. All granulomas were acid-fast negative suggesting they were not caused by *Mycobacterium* spp. There was also diffuse degeneration and necrosis of both the tubules and glomeruli of the kidney from which *Citrobacter freundii* was isolated. All other tissues examined from these fish appeared normal. Based on the limited location (i.e. primarily stomach) of the granulomas, lack of acid-fast staining of the granulomas, the presence of flagellates within the lumen of the stomach and the limited pathology observed in other organs, a diagnosis of cryptobiosis caused by the protozoan parasite, *Cryptobia iubilans* was made. The Mayan cichlid (*C. urophthalmus*) represents a new host species for *Cryptobia iubilans*.

Conference Session Designation: (Ornamental / Aquarium)
Presentation Format: (Oral)

Use of the Microalgae *Phaeodactylum Tricornutum* for the Remedy of Monogenean Infections: Treatment of *Gyrodactylus Turnbulli* in Guppies

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Parasitic diseases are a major constraint to sustainable aquaculture production and product trade. Monogenean infestations in an intensive aquaculture system reach epizootic levels due to their direct life cycle and unnaturally high densities of the host fish. Traditional chemical treatments pose associated problems, mainly human health-related risks, but also environmental concerns and resistance development. The common treatment against monogenean parasites, organophosphates, pose a risk of neural disorders in humans and this treatment was therefore recently banned in many countries.

In recent years regulations on the use of chemicals in aquaculture is becoming more stringent thus there is a growing trend and need on developing natural, therapeutants. *Phaeodactylum tricornutum* is a diatom microalga for which anti-bacterial effect against numerous bacterial species had been demonstrated. The current work aimed to test the potential of *P. tricornutum* as a treatment against a monogenean parasite of fish. *Gyrodactylus turnbulli*, monogenean infecting guppies were selected as a model for this study. An extract was prepared from *P. tricornutum* biomass using different solvents, including ethanol at different concentrations and ethyl acetate, with and without subsequent sonication and addition of a 1% of a food-grade detergent.

For *in vitro* analysis of the extracts, tail clips with up to 30 parasites were collected from heavily infected guppies and distributed between wells of 24 well plates. The effect of the extracts on *G. turnbulli* detachment from the tail fin and mortality of the parasite were analyzed by direct microscopic observation. Parasites on the tail fin clip of infected guppies were continuously observed after the addition of microalgal extracts, and time to detachment and death were recorded.

We present our findings on the efficiency of using the ethanolic extract of *P. tricornutum* against *G. turnbulli* at both *in vitro* and *in vivo* trails. At a concentration of 5 ppt, complete detachment and 97% mortality were achieved within 240 min, as analyzed *in vitro*. In a preliminary immersion treatment trial with infected guppies, the extract was effective at a concentration of 2.5 ppt, eliminating any evident parasites from the guppy tail fin within 24 h.

Results suggest that *P. tricornutum* - based preparations are potential natural therapeutants against monogenean infection in fish. Further research is focused on increasing the efficacy of the extract and evaluating its potential as a therapeutant against monogenean infection in guppies as well as additional monogenean species parasitizing fish.

Conference Session Designation: (Parasitology or Ornamentals & Aquarium Medicine)
Presentation Format: (Oral)

Ensuring Health Throughout the Supply Chain: Developing a Supply-Chain Health Management Training Program for a Wild-Caught Aquarium Fishery in Brazil – A Case Study.

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The aquarium fishery is the principal subsistence activity for the riverine communities in the municipality of Barcelos (Amazonas state, Brazil). For more than 25 years, Project Piaba has been working with the aquarium fishery of the Rio Negro. Very early on it was discovered that the capture of many of these species was not only sustainable, but it was the principal driver for creating value for the environment. Every year a small group of international fish health specialists, trade stakeholders, public aquarium biologists, and fish enthusiasts participate in an annual expedition to Barcelos and the fishing grounds. The outcomes of this program have led to a much better understanding of the role of this fishery and project members are helping the fishery adapt to changes in global markets.

The industry and the business climate in which the fishery operates have changed significantly in recent years and this fishery is increasingly in competition from native Brazilian species being farm-raised in Asian countries. In the past, customers in the import countries have been willing to expend resources to acclimate, manage minor health issues and condition these wild-caught fish in preparation for sale to customer. Today customers expect a high quality and healthy wild-caught fish that requires little in the way of post-shipment health management and conditioning. In order to stay competitive one key area in which the Brazilian industry must focus is improved health management of these fish throughout the chain of custody, from collection to export.

Project Piaba partnered with the Aquatic Animal Health Program (AAHP) to initiate a project to identify the key factors impacting fish health throughout the chain-of–custody for the Rio Negro aquarium fishery. Utilizing this information, the AAHP team developed a train-the-trainer program that would train and provide local biologists to act as trainers and consultants for the Rio Negro fishers, transit station managers and exporters as they worked to improve the health and quality of their collected fish. The trainers are fisheries biologists and aquaculture specialists selected based upon their relationships with the local communities and their knowledge of the fishery and local environment. Once trained these trainers began offering training and consultation to local fishers and facility managers throughout the supply chain addressing health management techniques that would lead to improved fish health and quality. We will discuss the development and implementation of this program and some of the early outcomes.

Conference Session Designation: (Ornamental and Aquarium Medicine)
Presentation Format: (Oral)

Finding Nemo's Picornavirus

Elizabeth C. Scherbatskoy[1*], Kuttichantran Subramaniam[1], Lowia Al-Hussinee[1], Kamonchai Imnoi[1], Samantha A. Koda[1], Patrick M. Thompson[2], Deborah B. Pouder[3], Roy P.E. Yanong[3], Jeffrey C. Wolf[4], Vsevolod L. Popov[5], Terry Fei Fan Ng[6] and Thomas B. Waltzek[1]

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Over the last decade, a number of aquaculture facilities have suffered significant mortality events in their clownfish (*Amphiprion ocellaris* and *A. percula*). Clinical signs of disease include darkened body coloration, increased gilling, reduced body condition, and abnormal positioning in the water column. Diseased clownfish were processed for parasitologic, bacteriologic, histopathologic, and virologic diagnostic testing. No significant parasite burdens were detected, and while bacteria were isolated from some of the fish, they appeared to be more consistent with a secondary infection. Histopathological examination revealed prominent single cell necrosis and mild inflammation of the mucosal epithelium within the branchial cavity, pharynx, esophagus, and/or stomach. Homogenates from pooled external and internal tissues were inoculated onto striped snakehead (SSN-1) cells, resulting in complete lysis in the initial infection and upon subsequent passages. Transmission electron microscopy of infected SSN-1 cells revealed small (28-30 nm), naked, icosahedral particles within the cytoplasm, occasionally arranged in paracrystalline arrays, consistent with the ultrastructure of a picornavirus. The virus was concentrated by ultracentrifugation prior to RNA extraction, cDNA library generation, and sequencing using an Illumina MiSeq sequencer. Sequencing recovered the full genome of a novel picornavirus most closely related to those recently described from other fish hosts including common carp (*Cyprinus carpio*), eel (*Anguilla anguilla*), bluegill (*Lepomis macrochirus*), and fathead minnow (*Pimephales promelas*). Future challenge studies are planned to elucidate the clinical significance of this picornavirus in clownfish. Disease progression will be assessed by regularly sampling fish over the study period to assess gross and microscopic lesions (histopathology and *in situ* hybridization) as well as viral load (virus isolation and RT-qPCR) within external and internal tissues.

Conference Session Designation: **(** Ornamentals and Aquarium Medicine)
Presentation Format: **(** Oral)
Student Presentation: **(** Yes)

Is There a Difference in Virulence Between Carp Edema Virus from Different Genogroups ?

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Koi sleepy disease (KSD) caused by infections with the carp edema virus (CEV) seems to pose a potential risk to carp aquaculture and koi trade. Sequence comparisons of virus isolates infecting fish in several European countries revealed the existence of two or three distinct genogroups. The genogroup I is predominantly found in cultured common carp while genogroup IIa is mostly associated with infections in koi. Our studies on imported koi suggest that genogroup IIa is constantly spread by koi trade form Japan. Viruses from genogroup IIa can be present in common carp and viruses from genogroup I in koi, but when detected, then in very low copy numbers. Therefore infection experiments with virus from these two genogroups of CEV were used to evaluate possible differences in infection biology of the virus while several carp strains were used to study influences of the genetic background of carp on their susceptibility to the infection. In an infection experiment with CEV from genogroup I Amur wild carp (AS) was less susceptible to the infection than Prerov scale carp (PS) or koi. When CEV from genogroup IIa was used all common carps (AS, PS, Rop) were far more resistant to the infection than koi. Analyses of behavioural, histopathological and molecular indicators of infection revealed differences in the virulence of the two CEV genogroups. Viruses showed higher virulence towards the same fish group as the donor fish (koi or in common carp) inducing rapid onset of KSD. The results from the study show that resistance to CEV infection is largely dependent on the genetic background of the carp. Furthermore significant differences in virulence and genetics of CEV genogroups rise questions about the geographical distribution of the genogroups and a better separation of these viruses by nomenclature.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Phylogenomic Characterization of Megalocytiviruses in Archived Ornamental Fish Samples

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The subfamily *Alphairidovirinae*, within the family *Iridoviridae*, includes three genera of double-stranded DNA viruses that infect poikilothermic vertebrates (e.g. fish, amphibians, and reptiles). The *Alphairidovirnae* genus *Megalocytivirus* (MCV) have been reported to infect >125 ornamental and food fish species in both freshwater and marine environments around the globe. Phylogenetic characterization of MCVs based on the conserved major capsid protein (MCP) supports three major genotypes: infectious spleen and kidney necrosis virus (ISKNV), red seabream iridovirus (RSIV), and turbot reddish body iridovirus (TRBIV). Recently, these three MCV genotypes have each been further subdivided into two clades based on phylogenetic analyses of the MCP. Using a pan-MCV primer set that targets the MCV myristylated membrane protein, archived samples of South American cichlids (keyhole cichlid *Cleithracara maronii* and oscar *Astronotus ocellatus*), three spot gourami *Trichopodus trichopterus*, blue chromis *Chromis cyanea*, and clownfish *Amphiprion ocellaris* from various facilities in the United States tested positive. Sanger sequencing of the purified PCR products revealed that the South American cichlid samples were infected with a TRBIV Clade 2, while the blue chromis and clownfish were both found to be infected with RSIV Clade 1. Histopathological examination of these cases revealed cytoplasmic basophilic inclusions compatible with those induced by MCVs within a variety of tissues, including hematopoietic tissues such as the spleen and anterior kidney. Transmission electron microscopy for the three spot gourami and blue chromis cases allowed identification of unenveloped, hexagonal virus particles with electron dense cores within the cytoplasm of infected cells, consistent with previous reports of the ultrastructure of MCV virus particles. Detection and molecular characterization of MCVs in freshwater and marine ornamental fishes traded in North America expands the known host range and genetic diversity of MCVs circulating in the region.

Conference Session Designation: (Ornamentals and Aquarium Medicine)
Presentation Format: (Oral)
Student Presentation: (Yes)

The Effects of Venting, Transport, and Holding Methods on Yellow Tang (*Zebrasoma Flavescens*) Health in the Marine Ornamental Aquarium Fish Trade

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Each year, 14-30 million fish are removed from coral reefs and enter the aquarium trade supply chain. Our objective was to examine this supply chain and determine how capture, transport, and holding methods affect fish health and mortality. To simulate the supply chain this, yellow tang fish (*Zebrasoma flavescens*) were captured from the wild offshore of the west coast of the island of Hawaii, USA, held in an active aquarium fish export facility for 4 days, transported by air to Portland, OR, and finally to the Hatfield Marine Science Center in Newport, OR, USA and held for 6 months. Plasma cortisol concentrations were used as a proxy for stress, and were determined when fish were first brought to the export facility, immediately before air transport, and after 6 months in captivity. Baseline cortisol was determined by collecting blood from fish on SCUBA. Reflex Action Mortality Predictors were also used to determine if reflex impairment could predict later mortality or health problems in fish. Water quality was monitored and compared between 3 active export facilities. Plasma cortisol decreased while fish were in the export facility (from day 0 to 4), and decreased to baseline concentrations after 6 months in captivity. Because all fish survived, we were unable to predict mortality using RAMP scoring. Our work demonstrates that fish plasma cortisol returns to baseline after fish are held in captivity, suggesting that captive fish held in an appropriate environment are able to acclimate to a captivity and are not stressed.

Conference Session Designation: (Ornamental Diseases)
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Student Presentation: (Yes)