Wednesday September 5th – Archibald / Campbell
Emergent Disease 1
Moderator - Al Camus (University of Georgia)

3:15 PM	**Emergent Disease I**	Camus - Pathology of Emergent Infectious Diseases in the Ornamental Fish Industry
3:30 PM		Becker - Risks to Australia's Biosecurity from the trade of Ornamental Fish
3:45 PM		Forwood - ~~Border Control – Stopping the Spread of Emerging Diseases~~ **(WITHDRAWN)**
4:00 PM		Surachetpong - Implementation of Biosecurity to Limit the Spreading of Emerging Diseases in Tilapia Farms
4:15 PM		Soto - *Erysipelothrix rhusiopathiae* spaB, an Emerging Pathogen of Cultured Barramundi, *Lates calcarifer*
4:30 pm		**Stilwell - First Detection of *Erysipelothrix* sp. Infection in Western Mosquitofish, *Gambusia affinis*, from Channel Catifsh, *Ictalurus punctatus*, Ponds in Mississippi**
4:45 PM		**Armwood - Molecular Characterization and Histopathology of *Edwardsiella anguillarum* Infections in Nile Tilapia (*Oreochromis niloticus*)**
5:00 PM		Griffin - *Edwardsiella piscicida*, an Emergent Pathogen in Farmed Channel ♀, *Ictalurus punctatus* x Blue ♂, *Ictalurus furcatus* Hybrid Catfish Cultured in Mississippi

Pathology of Emegent Infectious Diseases in the Ornamental Fish Industry

Alvin C. Camus[1*], Abigail R. Armwood

Department of Pathology, College of Veterinary Meicine, University of Georgia, 501 D.W. Brooks Drive, Athens, GA USA 30602 camus@uga.edu, adbigail.armwood@uga.edu

International trade in ornamental fish, is a multibillion dollar industry that contributes significantly to the local economies of several countries. The United States and the European Union represent the world's two largest import markets. Involving both capture and culture fisheries, less than 10% of marine ornamental fish are captive bred, while a larger proportion of freshwater fish are cultured. The industry is multifaceted, involving local collectors, producers, and dealers, exporters and importers, wholesalers, retailers, and shippers. Although import/export regulations have increased at various international, national, and state levels, health surveillance and biosecurity measures are often limited. Lack of disease monitoring coupled with the inherently stressful nature of capture, holding, and shipment favor the development and dissemination of infectious disease. This report characterizes disease outbreaks diagnosed since 2010 by the University of Georgia's Aquatic Pathology Service, involving four pathogens emerging in the ornamental fish trade. The agents include *Francisella noatunenis* subsp. *orientalis*, *spa*C-type *Erysipelothrix* sp., megalocytivirus, and betanodavirus. Franscisellosis was diagnosed in four groups of damselfish (*Chromis viridis*, *Chrysiptera springeri*) or fairy wrasse (*Cirrhilabrus* spp.) species. Characterized by disseminated granulomas and granulomatous inflammation, lesions are typified by macrophages containing the small, gram-negative coccobacilli within intracytoplasmic vacuoles. An *Erysipelothrix* sp., distinct from *Erysipelothrix rhusiopathiae*, was isolated in several diseased tetra (Family *Characidae*) species with necrotizing dermatitis and myositis. Acute lesions contained massive numbers of gram-positive bacterial rods that have distinct tropism for connective tissues. Six cases involving freshwater (*Trichopodus leeri*, *Mesonauta festivus*, Family *Cichlidae*), brackish water (*Toxotes chatareus*) and saltwater (*Pomacanthus xanthometopon*) fish species had histologic features of megalocytivirus, Family *Iridoviridae*, infection characterized by cytomegalic inclusion bodies, typically adjacent to vascular lumens, in multiple organs. Betanodavirus infections caused two outbreaks in species of anthias (*Pseudoanthias* spp.) fish. Histologically, vacuolar degeneration was present in retinal lesions and brains. The ongoing emergence of pathogens in the ornamental fish trade emphasizes the need for routine surveillance and trained professionals to recognize their diagnostic features, as well as pursue appropriate confirmatory testing.

Conference Session Designation: (Ornamental Diseases)
Presentation Format: (Oral)

Risks to Australia's Biosecurity from the Trade of Ornamental Fish

Joy Becker[1*], Paul Hick[2], Alejandro Trujillo Gonzalez[3], Terrence Miller[3] and Kate Hutson[3]

[1] 425 Werombi Road, School of Life and Environmental Sciences, Faculty of Science, University of Sydney, Camden, NSW, 2570, Australia joy.becker@sydney.edu.au
[2] 425 Werombi Road, Sydney School of Veterinary Sciences, Faculty of Science, University of Sydney, Camden, NSW, 2570, Australia paul.hick@sydney.edu.au
[3] College of Marine and Environmental Sciences, James Cook University, Townsville, 4811, Australia kate.hutson@jcu.edu.au

The ornamental fish industry presents a high risk to Australia for introducing exotic aquatic pathogens of international significance with several documented occurrences. Notably, these include the megalocytivirus, *Infectious spleen, and kidney necrosis virus* (ISKNV), cyprinid herpesvirus 2 (CyHV-2) and *Edwardsiella ictaluri*, with the latter two now considered endemic in some wild fish populations. Nearly 18 million ornamental fish are imported annually to Australia under a policy based on an Import Risk Analysis published in 1999. Recently, there has been particular interest in the risk associated with imported ornamental fish infected with the megalocytiviruses, ISKNV and red sea bream iridoviurs (RSIV). The objective of this project was to determine if aquatic pathogens of potential biosecurity concern are entering Australia through the trade in ornamental fish.

Repeated cross sectional surveys were undertaken in imported freshwater and marine ornamental fish under quarantine prior to entry into Australia. They were tested for the presence of nationally listed aquatic viral and bacterial pathogens and to identify external and internal parasite assemblages. A design prevalence of 2% to 10% was used depending on specific pathogen and diagnostic test. Fish hosts were prioritized based on prior knowledge of infection with the listed pathogens, volumes of importation to Australia and current import conditions.

Testing was completed on 62 populations of fish representing 12 consignments received from five different countries. We detected viruses of biosecurity concern, including ISKNV-like megalocytiviruses and viral nervous necrosis viruses (NNV). About 52% (24/46) of the populations tested for ISKNV were positive, which included five species of marine fish. NNV was detected in 13% (3/23) of marine fish, with all positive populations received from Indonesia. There was no evidence of koi herpesvirus (CyHV-3), spring viremia of carp virus (SVCV), viral hemorrhagic septicemia virus (VHSV), *Aeromonas salmonicida* or *Edwardsiella ictaluri*. The parasite assemblages found on pre-import ornamental fish were diverse and abundant. Despite the import conditions requiring freedom, many fish, in particular goldfish (*Carassius auratus*) from several countries were heavily infected with freshwater dactylogyrid gill trematodes.

The risk imported ornamental fish present to Australian aquatic animal industries and natural resources was high with respect to megalocytiviruses and parasitic agents. Recommendations to support revision to Australia's national biosecurity policy were made so that appropriate regulations can be put in place to manage the risk.

Conference session: (Emergent Diseases)
Presentation format: **(** Oral)

Border Control – Stopping the Spread of Emerging Diseases (WITHDRAWN)

James M Forwood

Department of Agriculture and Water Resources, 7 London Circuit, Canberra, ACT, Australia james.forwood@agriculture.gov.au

Biosecurity has played a critical role in reducing risk and placing Australia as one of the few countries in the world to remain free from the world's most severe agricultural diseases. While our geographical isolation has played a key role in maintaining this status, the objective is now to maintain freedom from these diseases in an environment of increasing global trade while meeting our international trade agreement obligations.

To reduce the risk of introducing an exotic disease with imported goods, countries often undertake formal risk assessments as prescribed by the OIE, from which appropriate risk management measures are developed and applied by the importing country, to reduce the biosecurity risk with the trade in aquatic animals and their products to an acceptable level. Risk assessments are complex and resource intensive and can only account for the diseases known at the time of the assessment. Unless new scientific evidence is acquired these risk assessments are often not revised due to the resources required to undertake such reviews.

Emerging animal diseases are a considerable risk for regulators, because a risk assessment is a snapshot of the perceived risks at a point in time, based on available knowledge and research and emerging diseases are often unknown and not considered, or there is limited scientific information available when developing the assessment. Therefore, the development of suitable risk management measures may over time lose their efficacy for maintaining the appropriate level of protection for the safe trade of susceptible species. The development of suitable risk management measures requires vigilance in the pursuit of new and emerging scientific evidence, and the regulated review of risk assessments as appropriate.

One challenge in developing suitable risk management measures for an emerging disease is the variability among trading partners of their willingness or capacity to conduct the necessary research and effectively communicate findings with other trading partners. Effective communication between trading partners on emerging diseases will facilitate importing countries to better evaluate the efficacy of their risk assessments, and if necessary, undertake the formal review and revise risk management measures accordingly. This can also be mutually beneficial for all parties with the potential for collaborative research between trading partners and the sharing of intellect acquired.

Improved communication between trading partners can not only strengthen the disease status of the importing country, viability of susceptible domestic industries and protect the environment, but also limit the geographical spread of the emerging disease and reduce the global spread and impact of the disease. Australia's approach to managing the emergence of dwarf gourami iridovirus and related viruses in freshwater ornamental finfish will be discussed.

Conference Session Designation: (Emergent Disease)
Presentation Format: (Oral)

Implementation of Biosecurity to Limit the Spreading of Emerging Diseases in Tilapia Farms

Win Surachetpong

Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand fvetwsp@ku.ac.th

Biosecurity is a standard practice that aims to limit the introduction and spread of pathogen in the production environment**.** For tilapia production, recent emerging of viruses and bacterial diseases such as Tilapia Lake Virus **(**TiLV**)** and *Streptococcus agalactiae* have been reported in Nile tilapia and red hybrid tilapia in different parts of the world**.** These emerging pathogens associate with high mortality of 80-90**%** within 1-2 weeks after the disease has been observed**.** Importantly, the pathogens may spread horizontal and/or vertical with infected fish**.** For example, a recent study by our laboratory suggested that TiLV could be detected in the mucus of moribund tilapia and that infected virus could spread through fish mucus until 12 days post infection**.** Therefore, removing of moribund and dead fish will reduce the risk of disease transmission and prevent the spread of pathogens in the farm and region**.** To limit the catastrophic loss of infectious diseases, implementation of biosecurity and control measures should be applied at the farm, regional, national and international levels**.** Such concepts that could be employed including the screen of live fish and broodstock using PCR or real-time PCR, restricting fish movement, applying disinfectants as a standard practice, fry vaccination, and eliminating potential vectors**.** Overall, applying a standard of biosecurity plan and control measures at the farm and national level should limit the sources of disease outbreak**.**

Conference Session Designation: (Emergent Disease)
Presentation format: (Oral)

Erysipelothrix rhusiopathiae spaB, an emerging pathogen of cultured barramundi, *Lates calcarifer*

Esteban Soto[1], Eric K. Pomaranski[1], Cem Giray[2], Bill Keleher[2], Matt Griffin[3], Al Camus[4]

[1] Department of Medicine & Epidemiology, School of Veterinary Medicine, University of California, Davis, 2108 Tupper Hall, Davis, CA 95616-5270, ekpomaranski@ucdavis.edu sotomartinez@ucdavis.edu
[2] Kennebec River Biosciences, Richmond, ME 04357 USA, cgiray@kennebecbio.com; wkeleher@kennebecbio.com
[3] Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, PO Box 197, 127 Experiment Station Road, Stoneville, MS 38776, USA, matt.griffin@msstate.edu
[4] Department of Pathology, College of Veterinary Medicine, University of Georgia Athens, Athens, GA, USA camus@uga.edu

Members of the genus *Erysipelothrix* have recently been described as emergent pathogens of cultured Australian eels, *Anguilla reinhardtii* (Steindachner, 1867) and *A. australis* (Richardson, 1841) in Australia, as well as several ornamental centrarchid and cyprinid species in the USA. Since 2013 *E. rhusiopathiae* has been reported from outbreaks of disease in barramundi, *Lates calcarifer*, cultured in North America. Eight *E. rhusiopathiae* isolates were recovered from diseased fish during different outbreaks. The *E. rhusiopathiae* isolates from barramundi were compared phenotypically and genetically to *Erysipelothrix* sp. *spaC* isolates recently characterized from ornamental fish and *E. rhusiopathiae* recovered from aquatic and terrestrial animals from multiple facilities. All barramundi isolates were PCR positive for surface protective antigen type B (*spaB*). Additionally, isolates from clinically affected barramundi had ≥ 99.7% sequence similarity among concatenated MLST gene sequences, indicating a high degree of genetic homogeneity. These isolates were > 99% similar to other *spaB* positive isolates, consistent with findings for other *spa* types. While concatenated MLST sequences demonstrated >99% similarity within *spa* groups, *spaA* and *spaB* isolates shared <98% similarity between them, and <90% similarity to *spaC* isolates. Experimental immersion challenges in tiger barbs, *Puntigrus tetrazona* were attempted in efforts to fulfill Koch's postulates. Tiger barbs were exposed to 5 x 10^7 CFU/mL *E. rhusiopathiae spaB* for 1 hour at 26 C. Within 5 days of challenge, 85% of the exposed tiger barbs died, with the first mortality observed 3 d post-challenge. *Erysipelothrix rhusiopathiae spaB* was re-isolated from moribund fish. This study supports previous work citing the genetic variability of *Erysipelothrix* spp. spa types and the emergence of members of the genus *Erysipelothrix* as nascent fish pathogens.

Conference session designation: (Emerging Diseases)
Presentation format: (Oral)

First Detection of *Erysipelothrix* sp. Infection in Western Mosquitofish, *Gambusia affinis*, from Channel Catfish, *Ictalurus punctatus*, ponds in Mississippi

Justin M. Stilwell[1*], Alvin C. Camus[1], Esteban Soto[2], Thomas G. Rosser[3], John Leary[1], Charles C. Mischke[4], and Matt Griffin[3,]

[1] Department of Pathology, University of Georgia, 501 D.W. Brooks Drive, Athens, GA,30606 stilwellj@uga.edu
[2] Department of Medicine & Epidemiology, University of California-Davis, Tupper Hall, Davis, CA 95616;
[3] College of Veterinary Medicine, Mississippi State University, 240 Wise Center Drive, Starkville, MS 39762;
[4] Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, 127 Experiment Station Road, Stoneville, MS 38776

Native and introduced fish species can serve as reservoirs for multiple pathogens in cultured fishes, such as the channel catfish. Three hundred and fifty-one western mosquitofish collected in commercial catfish ponds in the Mississippi Delta were surveyed histologically for pathogens using light microscopy. Following routine processing, sectioning, and H&E staining, a number of disease agents were detected, including five myxozoan species in various tissues, intestinal acanthocephalans, and branchial epitheliocystis inclusions. In eight fish, numerous, ill-defined, basophilic colonies of short, slender, Gram-positive, rods lined connective tissues and basement membranes of the skin, skeletal muscle, bone, pharynx, intestines, bile ducts, kidneys, and nasal mucosa. Lesions consistent with descriptions of *Erysipelothrix* sp. infection in tropical fish species. The diagnosis was confirmed molecularly by excising bacterial colonies from formalin fixed paraffin embedded tissue sections using laser capture microdissection (LCM). Sequencing of the 16s, gyrase B (gyrB), and surface protective antigen (spa) genes identified the bacteria as an *Erysipelothrix* sp. Spa C type, with 91% and 99% sequence identity to *E. rhusiopathiae* at the gyrB and 16s gene sites, respectively. The bacteria groups phylogenetically with other recently characterized *Erysipelothrix* sp. isolates believed to represent a novel species within the genus *Erysipelothrix*. *Erysipelothrix* sp. has caused significant mortality in cultured characin and cyprinid species and represents an emerging disease in the ornamental fish industry. To the authors' knowledge, this represents the first report of *Erysipelothrix* sp. infection in a poeciliid fish. Susceptibility of other fish species, including the channel catfish, is largely unknown. Due to the significance of commercial catfish aquaculture in the southeastern United States and the presence of mosquitofish in ponds, experimental immersion and injection challenges were initiated. Results were not available at the time of submission, but will be presented during the meeting presentation.

Conference Session Designation: (Emergent Disease)
Presentation Format: (Oral)
Student Presentation: (Yes)

Molecular Characterization and Histopathology of *Edwardsiella anguillarm* Infections in Nile Tilapia (*Oreochromis niloticus*)

Abigail Armwood[1*], Alvin Camus[1], Adrian Lopez-Porras[2], Matt Griffin[2], and Esteban Soto[3].

[1] Department of Pathology, College of Veterinary Medicine, University of Georgia, 501 D.W. Brooks Drive, Athens, GA 30602 USA abigail.armwood@uga.edu
[2] Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, 127 Experiment Station Road, Stoneville, MS 38776 USA matt.griffin@msstate.edu
[3] Medicine and Epidemiology, School of Veterinary Medicine, University of California Davis, Tupper Hall, One Shields Avenue, Davis, CA 95616 USA sotomartinez@ucdavis.edu

The genus *Edwardsiella*, family Enterobacteriaceae, contains the well-known fish pathogens, *E. ictaluri* and *E. tarda*, as well as two recently described species, *E. piscicida* and *E. anguillarum*, which affect various cultured fish species worldwide. Distinction between species is complicated by similar phenotypic characteristics and similarities in the 16S rRNA gene sequence, highlighting the need for more resolute methods of identification. *Edwardsiella anguillarum* was originally described in 2015 from cultured eel species in China. In 2017, mortalities of 10 to 30% occurred for two months in Nile tilapia (*Oreochromis niloticus*) fry and fingerlings in an aquaculture facility in Central America. Clinical signs were limited to erratic swimming, exophthalmia, and progressive lethargy. Bacteria cultured from affected fish were consistently identified as *E. anguillarum* using an *Edwardsiella* spp. quantitative multiplex PCR, *E. anguillarum* specific end-point PCR, as well as ~1800 bp of the *gyrB* and ~500 bp of the *sodB* gene sequences. The isolates were found to be largely homogenous by repetitive sequence mediated (rep) PCR using the ERIC and BOX primer sets. Additional ancillary diagnostics, including testing for tilapia lake virus and *Francisella noatunensis* subsp. *orientalis*, were negative. Microscopic examination of whole tilapia fingerlings revealed disseminated, mixed, multifocal to coalescing sheets of granulomatous inflammation, dominated by epithelioid macrophages, and discrete granulomas. Lesions often contained large central regions of necrotic debris and numerous 3 to 5 µm, intra- and extracellular Gram-negative bacilli. The most severely affected tissues included the spleen, anterior kidney, and posterior kidney. In multiple fish, additional lesions were present in the ocular choroid rete, gill, pseudobranch, heart, swim bladder, liver, gastrointestinal wall, and skeletal muscle. Severe involvement of the spinal cord and lateral ventricles of the brain were present in several fish and may account for signs of erratic swimming. Findings suggest that *E. anguillarum* may be an emerging pathogen in the aquaculture industry with an expanding host range. Additional work is required to identify overall prevalence of infection and susceptible species.

Conference Session Designation: (Emergent Disease)
Presentation Format: (Oral)
Student Presentation: (Yes)

Edwardsiella Piscicida, an Emergent Pathogen in Farmed Channel ♀, *Ictalurus Punctatus* X Blue ♂, *Ictalurus Furcatus* Hybrid Catfish Cultured in Mississippi

Matt Griffin[1,2*], Stephen Reichley[3], Wes Baumgartner[2], Suja Aarattuthodiyil[4], Cyndi Ware[1,2], James Steadman[1,2], Marsha Lewis[1,2], Pat Gaunt[1,2], Lester Khoo[1,2] and David Wise[4]

[1] Thad Cochran National Warmwater Aquaculture Center, Aquatic Research and Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University, Stoneville, MS, 38776 matt.griffin@msstate.edu bsa122@msstate.edu cware@cvm.msstate.edu steadman@cvm.msstate.edu mlewis@cvm.msstate.edu Gaunt@cvm.msstate.edu khoo@cvm.msstate.edu Dwise@drec.msstate.edu

[2] Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762 baumgartner@cvm.msstate.edu

[3] Clear Springs Foods, Inc., Buhl, ID stephen.reichley@clearsprings.com

[4] Thad Cochran National Warmwater Aquaculture Center, Mississippi Agriculture and Forestry Experiment Station, Mississippi State University, Stoneville, MS 38776

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, hybrid utilization is estimated to comprise 40%-50% of total catfish production. There is a trend towards increased incidence and prevalence of *Edwardsiella piscicida*-septicemia in US catfish aquaculture, particularly in hybrid catfish. From 2013-2017, a total of 3,242 disease case submissions were submitted to the Aquatic Research and Diagnostic Laboratory (ARDL) at the Thad Cochran National Warmwater Aquaculture Center in Stoneville, MS. Of these, 1,400 (43.2%) were hybrids. *Edwardsiella piscicida* was suspected in 138 (4.3%) cases, the majority of which (89.1%) were from hybrid catfish. A molecular survey of these isolates confirmed the majority (92.0%) to be *E. piscicida*. Furthermore, cases of *E. piscicida* from hybrids submitted to the ARDL, and the Aquatic Diagnostic Laboratory of the Mississippi State University College of Veterinary Medicine in Starkville, MS, were documented for gross lesions and histological analysis. Grossly, *E. piscicida* presents with small dermal ulcerations, a raised fluid-filled cranial mid-line lesion that is frequently ulcerated, hemorrhage in the gills, exophthalmia, and abdominal distension. Internally, lesions include splenomegaly, straw-colored ascites, renomegaly and occasionally hemorrhagic intestines. Histopathological examination is in agreement with gross observations and infected fish repeatedly demonstrate a mononuclear meningoencephalitis, hemorrhagic branchitis, splenitis, ulcerative dermatitis, granulomatous interstitial nephritis and hepatitis coupled with a hemorrhagic enteritis.

Conference Session Designation: **(** Emergent Disease)
Presentation Format: **(** Oral)