Tuesday September 4th – Tilly / Tupper
World Aquatic Veterinary Medical Association
Moderator – A. David Scarfe (University of Pretoria)

1:15 PM	WAVMA	**Parker-Graham - Pharmacokinetics of a Single Dose of Danofloxacin Administered Intramuscularly in Koi *Cyprinus carpio***
1:45 PM		Pulver - Past, Present, and Future Perspectives on Fish Drug Development
2:15 PM		**Parker-Graham - Effect of Anesthetic Time and Concentration on Blood Gasses, Acid-Base Status and Electrolytes in Koi (*Cyprinus carpio*) Anesthetized with Buffered Tricaine Methanosulfonate (MS-222)**
2:45 PM		Refreshments
3:15 PM	WAVMA	**Parker-Graham - Treatment of Severe Fishing Line Entanglement Injuries in a Free-Ranging Canada Goose, *Branta canadensis***
3:45 PM		Hickey - Size of Release? Time of release? What About Health Status at Release?
4:15 PM		Spark - Sector Specific Biosecurity Plans: Development and Implementation
4:45 PM		Nietlisbach - Tilapia Health on Wisconsin's Aquaponics Farms

Pharmacokinetics of a Single Dose of Danofloxacin Administered Intramuscularly in Koi (*Cyprinus Carpio*)

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Despite the frequency of antimicrobial use in pet fish there remains a paucity of pharmacokinetic studies critically evaluating commonly-employed drugs. Danofloxacin, a synthetic fluoroquinolone antimicrobial, is frequently employed as a first line treatment against infectious skin disease and septicemia in fish. For this study we evaluated culture and minimum inhibitory concentration (MIC) data (n=204) from ornamental fish presented to the Veterinary Medical Teaching Hospital at UC Davis and found that Aeromonas spp, Vibrio spp., and Pseudomonas spp. were the most commonly isolated pathogens from skin and posterior kidney. The MIC90 of danofloxacin for these pathogens was 1 µg/ml, which was equivalent or lower than the MIC90 of oxytetracycline, ceftiofur, and enrofloxacin on the same sensitivity panels.

A single 10 mg/kg dose of danofloxacin (Advocin, 180 mg/ml injectable solution, Zoetis Laboratories, Parsippany, New Jersey, USA) was administered to adult koi intramuscularly (IM). Fish were sampled at each time point: 0.25, 0.5, 0.75, 1, 4, 12, 24, 72, 96, 120, and 144 hours post-injection. Whole blood was drawn antemortem and heparinized plasma was obtained from these samples; fish were euthanized and samples of liver, spleen, gill, anterior kidney, posterior kidney, skin and muscle, and scales were collected. Plasma and tissue concentrations were determined by a validated liquid chromatography- mass spectrometry method and non-compartmental pharmacokinetic analysis was performed for plasma. Subsamples of examined tissues of each fish sampled at 144 hours were evaluated via histopathology.

Peak plasma danofloxacin concentration of 8.31 µg/ml was reached 0.75 hours post-injection. The plasma elimination half-life was 15 hours and danofloxacin was detected in some tissues for at least 144 hours. Danofloxacin sustained concentrations greater than 1 µg/ml in all examined tissues except scales for at least 96 hours post-injection. Histopathology at 144 hours was unremarkable. This study shows that danofloxacin at a dose of 10 mg/kg administered IM reaches therapeutic concentrations rapidly in plasma and tissues and maintains concentrations greater than the observed MIC90 for commonly isolated aquatic bacterial pathogens for at least 96 hours in koi housed at 18°C.

Conference session designation: (American Association of Fish Veterinarians)
Presentation format: (Oral)
Student presentation: (Yes)

Past, Present, and Future Perspectives on Fish Drug Development

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The Food and Drugs Administration (FDA) is the government agency responsible for approving aquaculture drugs in the United States. The first drug application specifically for fish was approved in 1967 (sulfamerazine). Over fifty years later, the current products approved for fish include only nine active ingredients. There are 15 different products approved and marketed for 26 different indications. The number of drugs approved for fish is significantly lower than the number approved for the major animal species. This is largely due to the relatively small market for aquaculture drugs in the U.S. and the corresponding lack of financial incentive for companies to invest in aquaculture products. Steps were taken to address the issue of incentives with the passage of the Minor Use and Minor Species Animal Health Act of 2004; however, some challenges remain.

CVM is supportive of drug development for minor species, including fish. For example, CVM has conducted studies to support drug approval projects, provides a FDA liaison to The Minor Use Animal Drug Program (formerly USDA National Research Support Project #7), and has utilized alternative approaches to meeting the drug approval requirements. CVM also supports the aquaculture industries by meeting regularly with public partners and impacted groups, continuing to attend and present at meetings where fish drug development is discussed, and conducting outreach to better understand regulations and policies.

While there has been significant progress over the past fifteen years in making more drugs legally available for aquaculture, there is still a need to complete drug approval projects to provide for additional approved drugs and indications, and to identify where else drugs are needed. For example, only one drug compound (formalin) is approved to treat parasitic diseases, yet parasite infestations are a major cause of fish disease. The development of marine aquaculture in the U.S. will require new approved drugs for the treatment of sea lice and other diseases of marine fish. Looking forward, drug development efforts will face additional challenges in responding to industry transformations (e.g. new species, the use of recirculating aquaculture systems), emerging diseases, and the need for new antimicrobial products.

Opportunities are abundant for pharmaceutical companies, researchers, veterinarians, and hatcheries to contribute to the development of new animal drugs to meet these needs. Aquaculture drug development is challenging for a myriad of reasons; however, CVM continues to encourage the industry to communicate with us to find solutions to protect human and animal health.

Conference Session Designation: (American Association of Fish Veterinarians)
Presentation Format: (Oral)

Effect of Anesthetic Time and Concentration on Blood Gases, Acid-Base Status, and Electrolytes in Koi (*Cyprinus Carpio*) Anesthetized with Buffered Tricaine Methanesulfonate (MS-222)

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Anesthesia is commonly employed in aquatic medicine to reduce the stress of handling on fish and to facilitate physical exams, diagnostics, and surgical interventions. Tricaine methanesulfonate (MS-222) is the most commonly used anesthetic for fish and is currently the only anesthetic approved by the United States Food and Drug Administration, Center for Veterinary Medicine (FDA-CVM) for food-producing fish. Despite the frequency of anesthetic procedures in fish, anesthetic monitoring remains rudimentary in many facilities. This study evaluated the impact on blood gases, acid-base balance, and electrolytes in koi (*Cyprinus carpio*) anesthetized with two different concentrations of buffered MS-222: 100 mg/L and 150 mg/L. Blood samples from 25 fish per anesthetic treatment group were collected after five and twenty minutes of anesthetic immersion and analyzed on the pHOx Ultra table-top blood gas analyzer (Nova Biomedical, Waltham, Massachusetts 02454-9141, USA).

All but one fish from the 150 mg/L group recovered uneventfully from anesthesia and all koi were sufficiently anesthetized to facilitate handling and venipuncture. Results showed significant increases in pCO2 ($p=0.006$) and hyperglycemia ($p=<0.0001$) with both increasing anesthetic concentration and increasing time under anesthesia. There was a significant decrease in pO2 with increased anesthetic time ($p=0.021$), independent of anesthetic concentration. There were several electrolyte changes observed as well with both increasing anesthetic time and concentration. Despite the changes all electrolytes except potassium remained within published reference ranges for koi; potassium showed a significant decrease in concentration associated with increasing anesthetic time and increasing anesthetic concentration. Plasma lactate concentrations were not significantly different across the study groups, suggesting that koi maintained adequate perfusion throughout the duration of the study period. The results of this study indicate that buffered MS- 222 at 100 mg/L and 150 mg/L are safe anesthetic concentrations for koi undergoing minimally- invasive diagnostics; however, koi anesthetized with MS-222 at a concentration of 150 mg/L experienced more significant changes in blood gases, acid-base balance, and electrolyte concentrations, including hypercapnia, hypoxemia, and hyperglycemia and may require more careful monitoring to avoid physiologic imbalances while under anesthesia.

Conference session designation: (WAVMA)
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Student presentation: **(** Yes)

Treatment of Severe Fishing Line Entanglement Injuries in a Free-Ranging Canada Goose (*Branta Canadensis*)

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Injuries caused by discarded fishing gear are commonly encountered in wildlife medicine; monofilament lines, discarded nets, lead sinkers, and hooks are pervasive environmental threats to aquatic birds. Cellulitis, ischemia, osteomyelitis, and loss of limb function are common sequelae to fishing line entanglement injuries. This case describes a Canada goose (*Branta canadensis*) that was presented for fishing line entanglement; several feet of monofilament fishing line were tangled around the bird's feet and legs, causing severe constrictive injuries to both pelvic limbs. On physical exam deep soft tissue wounds with purulent discharge and exposure of the tarsometatarsi were appreciated bilaterally. Both feet appeared to have deep pain sensation but the goose demonstrated profound proprioceptive deficits in both feet and was ventrally recumbent. Radiographs were notable for cortical bone erosion on the lateral aspects of both tarsometatarsi and severe soft tissue swelling proximal and distal to the constrictive lesions.

Supportive care was initiated including subcutaneous fluids with B complex vitamins, meloxicam, tramadol, and ceftiofur. Surgical debridement of the wounds revealed that the combined flexor and extensor tendons were intact; the wounds were dressed with topical Manuka honey and any constrictive scabs that formed in the wound bed were debrided for the next week. The legs were treated globally with cold laser therapy. After a week of intensive wound care the soft tissue trauma was healing well but the goose continued to be ventrally recumbent and had profound proprioceptive deficits in both feet. A pair of orthopedic splints were created for the goose to hold the feet in a normal digitigrade stance and physical therapy was initiated, including walking the goose with the orthopedic boots in the place and swimming the goose daily. After three days of exercise with the boots in place the goose began to place the right foot normally. Nineteen days after intake the goose had sufficient proprioceptive placement of both feet and was able to walk normally without the corrective splints and was discharged to a wildlife rehabilitator for pre-release conditioning. The goose was released back to the wild with apparently normal proprioception and musculoskeletal function of both legs twenty-nine days after initial intake. This case highlights a constellation of possibilities for treatment of fishing line entanglement wounds that are low in cost and readily accessible to practitioners.

Conference session designation: **(** World Aquatic Veterinary Medical Association)
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Number Released? Size at Release? Time of Release? What About Health Status At Release?

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For many fish hatchery enhancement programs, there are three major metrics to measure production success: number released, size at release, and time of release. One metric that is often overlooked and undervalued is fish health status at release. Pre-release examinations should always include review of mortality records and inspection of all containers of fish to be released. Other aspects of a pre-release examination may include necropsy and disease surveillance testing, including testing lots of fish to determine pathogen prevalence.

Even when pre-release health checks are incorporated into a program, they may be viewed as an impediment as opposed to an important indicator of the overall viability and performance of an enhancement program. First and foremost, pre-release health checks protect both hatchery fish and wild fish from serious diseases. Second, hatchery programs that release unhealthy fish or fish experiencing an ongoing epizootic is a practice that may have significant, and often massively underestimated, negative impacts on an enhancement program. Finally, repeated fish health problems apparent at a pre-release fish health examination may suggest that other metrics of success--number released, size at release, and time of release--are unreasonable expectations for that hatchery program. For example, fish that are experiencing mortality from furunculosis secondary to the stress of being held past smolting might benefit from being released earlier; or for fish experiencing a bacterial coldwater disease outbreak due to high densities, reduction in number of fish produced at a facility may result in improved health and ultimately release numbers.

Just performing pre-release examinations is not enough. It is critical for fish veterinarians to get involved in management meetings where the other metrics of success--number released, size at release, and time of release--are decided, because they have critical information to help inform the decision making process.

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Sector Specific Biosecurity Plans: Development and Implementation

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Worldwide, there is increasing risk of significant known and unknown aquatic animal diseases emerging and spreading. Although Australia has a relatively favorable aquatic animal health status, two of Australia's highest priority aquatic diseases, abalone viral ganglioneuritis (AVG) and Pacific Oyster Mortality Syndrome (POMS), have caused substantial economic impacts in Australia's seafood industries and now present trade barriers for movement of livestock. Despite the abalone and oyster industries and relevant jurisdictions have implemented a range of measures to mitigate these significant disease risks, both industry sectors were lacking a nationally consistent, agreed approach to auditable biosecurity to facilitate trade.

The Commonwealth Department of Agriculture and Water Resources (DAWR) and the Fisheries Research and Development Corporation (FRDC) consequently jointly funded a national project to deliver sector-specific biosecurity plans (templates and guidance documents) for the abalone and oyster industries. These will become an essential component of health accreditation programs and import protocols to facilitate safe trade in aquatic animals.

The effective implementation and operation of good on-farm biosecurity provides improved business security through reducing risks to production additional to enhanced market access. Unnecessary costs and production losses can be avoided by good biosecurity especially when disease can be prevented from entering the farm. The ultimate aim is a more profitable, secure and resilient aquaculture industry.

Biosecurity plans describe the systems necessary to protect a farm from diseases. The development of farm biosecurity plans involves the identification of relevant risks (or routes of disease introduction and spread); implementation of appropriate risk mitigation measures; and the development and maintenance of supporting documentation. Although every farm is unique, there are common risk pathways (e.g. animals, people, equipment, water, feed and waste) and effective risk mitigation measures are shared by farms within a sector. These common risks and mitigation measures are the basis on which these plans have been developed in consultation with industry.

This project was completed in 2017 and as a result all South Australian land based abalone farms have audited biosecurity plans in place as of early 2018 with significant progress being made with oyster hatcheries.

Conference Session Designation: **(** Aquatic Animal Health Management)
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Tilapia Health *Oreochromis niloticus* on Wisconsin's Aquaponics Farms

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Aquaponics is a sustainable method of farming that merges recirculating aquaculture systems and hydroponics by feeding plants with nitrogenous waste products produced by fish. Its purported attributes include significantly less water usage, highly efficient space utilization, and reduced pollutant-rich effluent compared to traditional aquaculture and agriculture systems. Aquaponics also allows local produce to be grown year-round, even in harsh climates.

Aquaponics has become increasingly popular across the country in recent years, and especially in Wisconsin. Despite the number of aquaponics farms in the state, very little is known about the health status of fish in these systems. This is possibly due to the fact that, despite being integral to the system, fish only account for about 10% the profits. The goal of this project was to investigate disease issues present on Wisconsin aquaponics farms, specifically focusing on Nile Tilapia (*Oreochromis niloticus)*. Tilapia are hardy, productive, and are often mistakenly billed as a "disease-resistant fish," making them the most popular choice for new aquaponics farmers in Wisconsin.

We found that aquaponics farms do indeed struggle with fish health problems. We found *Trichodina sp.* and monogenean trematodes (*Gyrodactylus sp.* and *Dactylogyrus sp.)* on every farm we visited. Some farms had particularly high numbers of external parasites, yet few fish exhibited clinical signs of infestation. But, these parasites could easily become a health problem if the fish experience a stressful and immunosuppressive event. Some farms also experienced bacterial infection outbreaks. Piscine francisellosis (*Francisella noatunensis*), one of the major emerging bacterial diseases of cultured tilapia, was diagnosed on one farm. We also investigated a severe, acute mortality event, which was diagnosed as a particularly virulent strain of *Aeromonas veronii,* serovar *sobria.*

A better understanding of the health status of fish on aquaponics farms needs to be studied further. Doing so will give the veterinary community a better idea of how we can be of service to the growing aquaponics industry.

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