

**Wednesday September 5<sup>th</sup> – Tilly / Tupper**  
**Antibiotic Use / Pharmacology**  
**Moderator - Patricia Gaunt ( Mississippi State University )**

3:15 PM	<b>Antibiotic Use / Pharmacology</b>	<u>Gaunt</u> – Benefits vs.Costs of Antibiotic Medicated Feed Use vs. Unmedicated Feed Use During Bacterial Outbreaks in Pond-Reared Warmwater Fish
3:30 PM		<u>Blair</u> – USFWS Aquatic Animal Drug Approval Partnership Program
3:45 PM		<u>Geiseker</u> – Setting Epidemiological Cutoff Values for Monitoring Antibiotic Resistance Oof <i>Aeromonas hydrophila</i> Isolates Collected from Fish
4:00 PM		<u>Oyebanji</u> – Knowledge of Antibiotic Resistance Among Fish Farmers in Oyo Town, Nigeria
4:15 PM		<b><u>Pravdova</u> – Association Between Pharmaceuticals and Parasite Infection in Natural Brown Trout (<i>Salmo trutta</i>) Populations</b>
4:30 pm		<u>Sidhu</u> – Comparative Pharmacokinetics of Oxytetracycline in Tilapia <i>Oreochromis</i> spp. Maintained at Three Different Salinities
4:45 PM		<u>Karadzovska</u> – Effect of Medicated Feeding Period on the Efficacy of a Potential Sea Lousicide Containing Lufenuron
5:00 PM		<u>Zargar</u> – The Effect of <i>Echinacea purpuria</i> and <i>Cinnamomum verum</i> on Some Immune Parameters of Rainbow Trout ( <i>Oncorhynchus mykiss</i> )



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

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



## **Benefits Vs. Costs of Antibiotic Medicated Feed Use Vs. Withholding Feed During Bacterial Outbreaks in Pond-Reared Warmwater Fish**

Patricia S. Gaunt\* and Ganesh Karunakaran

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Bacterial disease outbreaks in pond-reared fish are frequently experienced during growing season when fish are being fed to achieve maximal growth rate by fall. When there is detection of disease associated with a bacteria susceptible to antibiotics, frequently farmers will treat with medicated feed. This presentation will assess the benefits vs. costs of treating with medicated feed, withholding feed, and unmedicated feed for control of mortality associated with bacteria in pond-reared fish.

Early signs of bacterial disease in fish can range from anorexia to morbidity such as erratic swimming to acute mortality. When the fish are diagnosed, the farmer must decide on treatment options. Should he use medicated feed containing an antibiotic that the bacteria are susceptible to? Should he feed unmedicated feed to the appetent fish so that he will have fewer, but larger fingerlings by fall? The advantages and disadvantages of these strategies will be explored in this presentation. A partial budget analysis will be performed to explore the economic effects of specific medicated and therapeutic treatments.

**Conference Session Designation:**

( Antibiotics )

**Presentation Format:**

( Oral )



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## **USFWS Aquatic Animal Drug Approval Partnership Program**

Marilyn “Guppy” Blair<sup>1\*</sup>, Bonnie Johnson<sup>1</sup>, Niccole Wandelaar<sup>1</sup>, Molly Bowman<sup>1</sup>

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The mission of the U.S. Fish and Wildlife’s (USFWS) Aquatic Animal Drug Approval Partnership Program (AADAP) is to obtain U.S. Food and Drug Administration (FDA) approval of safe and effective new medications for use in aquaculture and fisheries management. As the only program of its kind in the U.S., the AADAP team works with other Federal, State, Tribal, University, and private sector partners from across the country to administer the National Investigational New Animal Drug (INAD) Program, conduct research to support New Animal Drug Approvals (NADAs), and provide for drug and chemical use information dissemination. This presentation will give an overview of the drug approval process as well as an update of the current status of drugs approved by FDA or currently available under an INAD exemption for aquaculture in the U.S.

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

( Aquatic Animal Health Management )  
( Oral )



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## Setting Epidemiological Cutoff Values for Monitoring Antibiotic Resistance of *Aeromonas hydrophila* Isolates Collected from Fish

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Antimicrobial resistance is a major public health issue that has created concerns about the use of antibiotics in aquaculture. Therefore, laboratories need standard methods and criteria called epidemiological cutoff values (ECVs, a.k.a. ECOFFs) to monitor for the development of resistance. ECVs are a critical part of standard susceptibility tests as they are used to interpret if an isolate has lost susceptibility to an antibiotic. To create ECVs for the pathogen *Aeromonas hydrophila*, we gathered 286 isolates from various fish health laboratories and confirmed the isolates identity with *rpoD* and/or *gyrB* gene sequencing. One hundred four isolates were confirmed as *A. hydrophila*. Using Clinical Laboratory Standard Institute (CLSI) guidelines, we tested the susceptibility of these isolates with standard minimal inhibitory concentration (MIC) and zone of inhibition (ZOI) testing against eight antibiotics: erythromycin, florfenicol, gentamicin, oxytetracycline, enrofloxacin, oxolinic acid, ormetoprim / sulfadimethoxine, and trimethoprim / sulfamethoxazole. We then analyzed frequency distributions for each antibiotic to estimate a cutoff value which separates the wild-type isolates without resistance from the non-wild-type isolates that have developed resistance. We determined a cutoff value for six of the eight antibiotics tested. No ECV was estimated for the erythromycin ZOI due to excessive intra-laboratory variation. ECVs were not estimated for the two potentiated sulfonamides since the potentiator could mask sulfonamide resistance. The ECVs proposed from this study are being reviewed by CLSI to be included in a guideline for standard testing of aquatic bacteria. If approved, the ECVs will be included in the next revision of the guideline. Standard test methods and interpretive criteria allow for effective surveillance of antibiotic resistance, promoting judicious use of antibiotics that farmers need for managing the health of their fish.

**Conference Session Designation:**

( Antibiotic Use / Pharmacology )

**Presentation Format:**

( Oral )



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## **Knowledge of Antibiotic Resistance Among Fish Farmers in Oyo Town, Nigeria**

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Antibiotic resistance is one of the biggest threats to global health, food security, and development today. This survey was carried out to test the knowledge of antibiotics use and antibiotic resistance of fish farmers in Oyo state, Nigeria. Thirty-four fish farmers were interviewed with the aid of a structured questionnaire. The data generated were analyzed using SPSS data package software version 14 for descriptive statistics. Rates were computed and the results were tested for responses using correlation analysis. 71% of the respondents were male while 29% were females. Majority (44.12%) of the farmers were between the age range of 41-50 years, and 58.8% were married. 44.2% of the respondents had high school certificate as the highest level of education attained while 5.88% had no formal education. 64.7% were into small scale production, while 5.88% were into large scale production. 79.4% said it was easy for them to have access to antibiotics, 14.9% said it was very easy for them to have access to antibiotics, 2.99% said they find it difficult. 55.8% got their antibiotics from their farm consultant, 17.65% from mobile salesman and 26.47% from shop. Oxytetracycline was the most used antibiotics followed by streptomycin while penicillin was the least used. 76.4% of the respondents disagreed that antibiotic resistance is a problem in Nigeria while 47.1% did not agree that the issue could affect them. 64.7% did not agree resistance could spread from person to person. In conclusion, there is a need for concerted effort to educate the fish farmers in this region on use of and resistance to antibiotics. There should also be restriction on access to antibiotics and standard treatment guidelines to control antibiotics use.

**Conference Session Designation:** ( Antibiotic Use / Pharmacology )

**Presentation Format:** ( Oral )



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## Association Between Pharmaceuticals and Parasite Infection in Natural Brown Trout (*Salmo Trutta*) Populations

Markéta Pravdová<sup>1,2,\*</sup>, Markéta Ondračková<sup>1</sup>, Jitka Kolářová<sup>3</sup>, Kateřina Grabicová<sup>3</sup>, Tomáš Randák<sup>3</sup> and Pavel Jurajda<sup>1</sup>

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Contaminants in the natural environment are known to affect aquatic biota. In recent years, the role of pharmaceutical contaminants has grown in importance due to the ever increasing amounts released into the environment and their biological activity. Sewage treatment plants represent one of the most important sources of such contaminants. In addition to direct negative impacts on fish health, pollutants can also have a more indirect impact on another natural stressor, parasites. High pollutant concentrations can lead to a lowered immune response, resulting in higher parasitic infection. On the other hand, pollutants can also negatively affect free living parasite stages, thereby reducing parasite abundance. In this study, I assess the association between pharmaceuticals and parasite infection in a natural brown trout (*Salmo trutta m. fario*) population. Fish were obtained from the Zivny stream (Czech Republic) upstream (control) and downstream (polluted) of a sewage treatment plant known to release pharmaceuticals. Fish length and condition parameters (condition factor, hepatosomatic index, spleen somatic index, gonadosomatic index) did not differ between sites. Of the 79 pharmaceuticals measured, 42 were detected in fish tissue, with highest concentrations found in liver, followed by kidney and brain. Antibiotics and antidepressants dominated at both localities, with concentrations significantly higher at the downstream polluted locality (together with beta-blockers). CNS stimulants showed similar concentrations at both sites. There was a negative relationship between overall pharmaceutical load and fish condition factor and hepatosomatic index at the polluted locality, while parasite abundance increased with overall pharmaceutical load at both localities. Fish were infected by four parasite species: two monogeneans *Gyrodactylus derjavinoideus* and *Gyrodactylus truttae*, one nematode *Salmonema ephemericidarum* and one trematode *Crepidostomum metoecus*. Ectoparasite abundance was higher at the polluted site, while endoparasite abundance was higher at the control site. The higher number of gyrodactylids at the polluted site probably reflects an increased parasite reproduction rate under stressful conditions. As macrozoobenthos density (including the intermediate hosts of the target parasites) was higher at the polluted locality, we hypothesise that the decrease in endoparasites potentially resulted from as yet unidentified negative effects of pharmaceutical contaminants on the parasite's free living stages.

**Conference Session Designation:** ( Parasitology General )  
**Presentation Format:** ( Oral )  
**Student Presentation:** ( Yes )



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## Comparative Pharmacokinetics of Oxytetracycline in Tilapia (*Oreochromis spp.*) Maintained at Three Different Salinities

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The pharmacokinetics of a drug in fish can be affected by environmental factors such as temperature, pH, and water salinity in which the animals are maintained. This can affect the plasma concentrations and clearance rates of drugs used in fish, and as a result can affect the withholding times of treated fish used for human food. The purpose of this study was to generate pharmacokinetic data on oxytetracycline (OTC) in tilapia (*Oreochromis spp.*) maintained in three different salinity environments. Juvenile tilapia (mean weight 122±0.9 gm) were divided into three groups (n =138) and acclimated to and maintained in three separate recirculation systems with three different aquatic environments: freshwater (0 ppt salinity), brackish water (15 ppt salinity) and salt water (30 ppt salinity). Water quality parameters (temperature, ammonia, nitrites, nitrates, pH and salinity) were monitored on a daily basis and a standard pelleted tilapia feed was provided to the fish at a daily rate of 3% body weight. Fish were fasted 24h prior to oral gavage of OTC (Bio-Mycin 200) at a dose rate of 50mg/kg. At 0 time (control), 6 fish from each group were sedated with buffered MS-222, bled from the caudal tail vessels and then euthanized. After OTC administration to the remaining fish, blood samples from six fish in each group were collected at 23 additional time points (0.25, 0.5, 1, 2, 4, 6, 9, 12, 24 h, and 2, 4, 6, 8, 10, 12, 14, 18, 22, 26, 30, 34, 38 and 42 days). Blood samples were placed in individual plasma separator tubes containing lithium heparin (BD Microtainer), centrifuged at 3000 x g for 10 minutes and the plasma samples separated and frozen at -80°C until analysis. Oxytetracycline was extracted from fish plasma by Solid Phase Extraction (SPE) and an ultra-high-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method was used to determine OTC concentrations. Pharmacokinetic data were analyzed using a non-compartment method. The mean plasma peak levels of OTC were 1.221±0.124, 1.343±0.212 and 1.220±0.257 µg/ml in the freshwater, brackish and salt water tilapia, respectively. Plasma concentrations in salt water tilapia were lower than freshwater and brackish water fish at all time points after C<sub>max</sub>. The T<sub>max</sub> was 6h in salt water tilapia compared to 8h and 12h in freshwater and brackish water tilapia, respectively. The AUC<sub>0-∞</sub> in salt water tilapia (55.5h.µg/ml) was ~3 times lower than the values obtained for freshwater (165.3h.µg/ml) and BW (144.6h.µg/ml) tilapia. The terminal half-lives of OTC in freshwater, brackish water and salt water tilapia were 176h, 154h and 69.3h, respectively. This study suggests that in tilapia, the pharmacokinetics of OTC differs with water salinity conditions as the drug clearance rates in freshwater (0.293L/h/kg) were remarkably lower than in salt water (0.878L/h/kg). This is the first study reporting OTC pharmacokinetic differences between freshwater, brackish water and salt water in the same species of fish.

**Conference Session Designation:**

( Antibiotic Use / Pharmacology )

**Presentation Format:**

( Oral )



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## Effect of Medicated Feeding Period on the Efficacy of a Potential Sea Lousicide Containing Lufenuron

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The proposed product, code-named AH-2178, contains 10% lufenuron and is dosed *via* medicated feed to young Atlantic salmon in freshwater for a target 7 days while fish are at the hatchery. The total dose to be offered is 35 mg/kg. Field studies in several countries indicated that erratic fish feeding behavior in certain situations may require extended feeding periods (up to 14 days) to ensure the dose is accepted. A large-scale field study was therefore conducted at a commercial fish farm in Canada to confirm that drug uptake and/or efficacy were not affected by increasing the feeding period while maintaining the total dose at 35 mg/kg. Approximately 422,000 salmon were included as either treated groups with dosing regimens of 7, 10 or 14 days (noting a feeding error saw the 7-day group fed for 8 days) or as untreated controls. Once medicated feeding was finished the salmon were transferred to the marine site where regular louse counts were undertaken until loss of protection was determined by comparison of treated groups to untreated controls. Fish from each group were sacrificed and fillets analyzed for lufenuron residues at 3 days post-treatment (DPT) and the end of the study (251 DPT). Statistical differences in lufenuron concentrations in fillet between the treatment groups were analyzed using analysis of variance, using the Tukey's method of adjustment of the p-values. There were no statistically significant differences in lufenuron concentrations between the dosing regimens of AH-2178 at 3 or 251 DPT. The duration of efficacy against *Lepeophtheirus salmonis* for each regimen was determined to be 172–186 days (parasite stage dependent; efficacy, >95%, was still evident at 228 days although the result could not be used in the statistical analysis due to reduced louse burdens on controls). Duration of efficacy against *Caligus elongatus* couldn't be estimated for the same reason. The administration of 10% lufenuron in medicated feed at 35 mg/kg over 7–14 days was well tolerated and the dosing period had no impact on efficacy.

**Conference Session Designation:**

( Parasitology Sea Lice – Ectoparasites )

**Presentation Format:**

( Oral )



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## The Effect of *Echinacea Purpuria* and *Cinnamomum Verum* on Some Immune Parameters of Rainbow Trout (*Oncorhynchus Mykiss*)

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The aim of this study was to investigate the effect of purple coneflower (*Echinacea*) and cinnamon extracts alone and together on some immune parameters of rainbow trout for 60 days rearing period. Nine hundred specimens ( $39.5 \pm 0.5$  g) were randomly allocated into 18 fiber glass tank (4100 L) at a density of 50 fish per tank (150 fish per treatment). Fish in the first and second groups were fed diet supplemented with echinacea (1 and 1.5 gram per kilogram feed). The third and fourth groups were fed diet supplemented with cinnamon (1 and 1.5 gram per kilogram feed). The fifth group were fed diet supplemented with echinacea and cinnamon together (1 gram per kilogram feed from each one) and the last group fed basal diet. The leukocyte counts, serum lysozyme and complement, serum biochemical factors, total IgM were measured in fifteen days interval till the end of the experiment. The results revealed that feeding trout with 1.5 g kg<sup>-1</sup> echinacea remarkably elevated the immune parameters tested ( $P < 0.05$ ). Group fed cinnamon also showed increased immune parameters compared to control group; however, the difference was not significant ( $P > 0.05$ ). The results also indicated that there is no synergistic effect between echinacea and cinnamon extracts on immune parameters of rainbow trout.

**Conference Session Designation:**

( Pharmacology / Medicine / Immunology )

**Presentation Format:**

( Oral )



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