U.S. Fish & Wildlife Service

The Aquatic Animal Drug Approval Partnership Program

"Working with our partners to conserve, protect and enhance the Nation's fishery resources by coordinating activities to obtain U.S. Food and Drug Administration approval for drugs, chemicals and therapeutants needed in aquaculture"

AADAP Aquatic Animal Drug Approval Partnership Program

Volume 9-2

AADAP NEWSLETTER

September 2013

Lower Falls of the Grand Canyon of the Yellowstone River, Yellowstone National Park, Wyoming

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WHAT'S SHAKIN'

Recap: 19th Annual USFWS Aquaculture Drug Approval Coordination Workshop

The 19th Annual U.S. Fish and Wildlife Service (USFWS) Aquaculture Drug Approval Coordination Workshop (Workshop) was held July 30 - August 1, 2013, in Bozeman, Montana. The Workshop was attended by approximately 55 aquaculture professionals from federal, state, tribal, and private entities. During the Workshop, the status of recently completed and ongoing aquaculture drug approval work [pivotal research and Investigational New Animal Drug (INAD) activities] was presented and discussed. Also, there was a session on the status and use of vaccines in aquaculture. After-hours activities included the Welcome Social at Bridger Brewing, a local craft beer and pizza establishment; Ice-Breaker BBQ at scenic Hyalite Reservoir; Trout Trot Fun Run (1, 3, and 5 mile routes) with Bozeman's Big Sky Wind Drinkers running club; and Decompression Raft Trip on the Yellowstone River.

The Workshop's first day included presentations on topics such as the (1) economic value and importance of fisheries and aquaculture in the U.S.; (2) shared needs and responsibilities of fisheries in the U.S.; (3) future of global and U.S. aquaculture and the importance of legal, effective, and safe fish drugs; (4) roles of the USFWS and its Aquatic Animal Drug Approval Partnership (AADAP) Program; (5) importance of the overall aquaculture drug approval effort to U.S. fisheries; (6) selected federal-, state-, tribal-, and private-entity perspectives on the status of U.S. aquaculture drug approvals and the U.S. aquaculture drug-approval process; and (7) incentives provided by the U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) Office of Minor Use and Minor Species for pursuing and obtaining aquaculture drug approvals in the U.S.

The Workshop's second day comprised many presentations related to obtaining initial or expanded approvals of drugs needed in U.S. aquaculture, fishery management, or both. An update from the FDA CVM Aquaculture Drugs Team addressed FDA's recent and upcoming aquaculture drug-approval outreach efforts, the reauthorization of the Animal Drug User Fee Act, and the need for drug sponsors and public data-generating partners to communicate early and often with FDA during the aquaculture drug-approval process. An update from the FDA CVM Office of Research touched on (1) standardized testing methods for aquatic bacteria as such methods relate to disease modeling and (2) aquaculture pathogen identification as it relates to diagnosing diseases. Along with the FDA updates were presentations from public data-generating partners and aquaculture drug sponsors related to ongoing research and INAD use of experimental antibacterials, antiparasitics, antifungals, skeletal (fluorescent) marking agents, masculinizing agents, immediate-release and transport sedatives, and spawning aids. Also, three presentations were given on the development, and use, of bacterial vaccines.

The final morning of the Workshop comprised a (1) meeting of The American Fisheries Society's Fish Culture Section Working Group on Aquaculture Drugs, Chemicals, and Biologics and (2) general discussion about "branding" the U.S. aquaculture drug approval effort so that fisheries professionals have a better understanding of the U.S. aquaculture drug-approval process—as well as the value and importance of using FDA-approved fish drugs.

The Workshop could not have been held this year without the generous support of sponsors. This year's sponsors (listed in alphabetical order) included Aqua Pharma US; AquaTactics Fish Health Services; Aquatic Life Sciences, Inc.; AQUI-S New Zealand; Euorpharma USA; Fish Vet Group, Inc.; Hybrid Catfish Company; Merck Animal Health, Inc.; PennField Animal Health; and Solvay Chemicals, Inc.

The 20th Annual USFWS Aquaculture Drug Approval Coordination Workshop is tentatively scheduled to be held in late July or early August 2014—once again in Bozeman, Montana. That said, we hope to see many familiar faces—as well as many new faces—here in Bozeman next year.

Romet® 30 and Romet® TC Now Available from AquaTactics Fish Health Services

Effective August 1, 2013, the fish antibiotic products Romet® 30 and Romet® TC (active ingredients, sulfadimethoxine and ormetoprim) are back on the U.S. market and available for immediate distribution and sale from AquaTactics Fish Health Services, Kirkland, Washington. AquaTactics recently acquired the U.S. market distribution rights from Aquatic Health Resources (Minnetonka, Minnesota), which for the past 9 years had distributed Romet® 30 and Romet® TC in the U.S. under an agreement with Pharmaq AS (Overhalla, Norway).

For the remainder of 2013, Romet® 30 and Romet® TC pricing will remain at the levels implemented in January 2012. However, with the transition to new contract manufacturing and analytical testing facilities, a modest price increase will likely occur in 2014.

AquaTactics hopes to provide a seamless transition from Aquatic Health Resources and encourages customer comments and questions via their toll free phone number (1-866-676-5975) or email (info@aquatactics.com). More details about AquaTactics, Romet® 30, and Romet® TC are available at http://www.aquatactics.com.

Aquaflor®: Environmental Assessment for Freshwater-Reared Finfish in Recirculating Aquaculture Systems

Merck Animal Health has been notified by the FDA that the Environmental Assessment (EA) technical section is now "complete" for the use of Aquaflor® (50% florfenicol) Type A Medicated Article in freshwater-reared finfish in recirculating aquaculture systems. The completion of this EA technical section is a major step toward FDA approval for the use of Aquaflor® in these types of aquaculture systems. More details will be available from Merck Animal Health later this fall. For more information about Aquaflor® and its FDA-approved uses in U.S. aquaculture, please visit http://www.aquaflor-usa.com/.

FDA Answers Your Questions About Fish Drugs

The American Fisheries Society's (AFS) Fish Culture Section (FCS) Working Group on Aquaculture Drugs, Chemicals, and Biologics (WGADCB) has announced that the much anticipated article, *FDA Answers Your Questions About Fish Drugs*, will be published this fall in the AFS monthly publication, *Fisheries*. The article, which was written by the U.S. Food and Drug Administration (FDA), addresses many important issues related to the U.S. aquaculture drug-approval process. Jim Bowker (AADAP) and Dr. Jesse Trushenski (Southern Illinois University, Carbondale, Illinois) have written a preface, which will appear with and provide context for the article.

If you are not an AFS member or are just in a hurry, an advance copy of the article can be downloaded from the AFS FCS WGADCB webpage: https://sites.google.com/site/fishculturesection/working-group-on-aquaculture-drugs-chemicals-and-biologics/wgadcb-resources-and-tools.

AADAP DRUG UPDATES

We're wrapping up a couple of big projects while waiting for FDA responses to our research study protocol submissions and technical section complete requests. Here's what we've been up to:

AQUI-S®20E (10% eugenol)

Effectiveness—We submitted a letter to FDA on November 20, 2012, requesting that the effectiveness technical section be considered complete for the use of AQUI-S®20E to sedate all freshwater finfish to handleable. On May 16, 2013, we heard back from FDA that the effectiveness technical section has been considered complete to sedate all freshwater finfish, including ornamental finfish, to handleable. Ta-da!

In addition, FDA provided us with comments on the draft AQUI-S®20E product label, which we had submitted to them for review. We're addressing FDA's comments while making a few minor revisions of our own. We'll turn the revised draft label over to the sponsor (AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand), who will ultimately submit it to FDA when applying for an initial approval.

Target animal safety—As you may recall, three separate and independent studies were conducted to evaluate the safety of AQUI-S®20E to fingerling rainbow trout, yellow perch, and channel catfish. All three studies have been completed. The rainbow trout final study report (FSR) was submitted to FDA on June 12, 2013. The yellow perch FSR has been reviewed by our Quality Assurance Officer (QAO) and is ready to be submitted to FDA for review. And last, we're putting the finishing touches on the channel catfish FSR and will be handing it over to our QAO for inspection. We anticipate submitting the yellow perch and channel catfish FSRs to FDA this fall, along with a request that the target animal safety technical section be considered complete for sedating all freshwater finfish to handleable.

Channel Catfish Pituitary

Effectiveness—We received an efficacy protocol End Review Amendment on July 23, 2013, requesting a number of changes to the protocol. We were under a bit of a time crunch (i.e., the 19th Annual USFWS Aquaculture Drug Approval Coordination Workshop was starting the next week) and knew that we had to submit the amended protocol by July 26. We jumped on it, made the necessary changes, and submitted the protocol right on time. The protocol has now been accepted.

SLICE (0.2% emamectin benzoate)

Target animal safety—Recently, we received a letter from FDA informing us that the SLICE® target animal safety study that we conducted on rainbow trout has been accepted. In this study, we found that SLICE® could be administered in feed to fingerling rainbow trout at a dose 2-3 times higher than the proposed efficacious dose (50 µg emamectin benzoate/kg fish/day) for 2 times the proposed treatment period (7 days) with no adverse effects (i.e., no mortality and no gross or microscopic lesions). This definitely puts us one step closer to an approval. Ta-da!

Recent Publications

In previous newsletters, we've reported on trials conducted to evaluate the safety or effectiveness of various drugs. Since then, we've worked with a number of collaborators to publish the following articles:

Bowker, J. D., D. Carty, and M. P. Bowman. *In press*. The safety of Aquaflor (50% florfenicol) administered in feed to fingerling yellow perch. *North American Journal of Aquaculture*.

Bowker, J. D., D. Carty, and M. P. Bowman. 2013. The safety of SLICE (0.2% emamectin benzoate) administered in feed to rainbow trout. *North American Journal of Aquaculture* **75:455-462**.

Bowker, J. D., D. Carty, J. T. Trushenski, M. P. Bowman, N. Wandelear, and M. D. Matthews. 2013. Controlling mortality caused by external columnaris in largemouth bass and bluegill with chloramine-T or hydrogen peroxide. *North American Journal of Aquaculture* **75:342-351**.

Matthews, M. D., J. D. Bowker, D. G. Carty, N. Wandelear, M. P. Bowman, J. C. Sakmar, and K. Childress. 2013. Efficacy of Aquaflor (50% florfenicol) to control mortality associated with *Flavobacterium columnare* infection in largemouth bass and bluegill. *North American Journal of Aquaculture* **75: 385 -392**.

Text provided by Jim Bowker (jim_bowker@fws.gov), Research Program Manager; USFWS AADAP; Bozeman, Montana USA.

FINS & TAILS, BITS & BOBBERS

AQUI-S®20E (10% eugenol) Withdrawal Periods

There have been questions about the withdrawal periods required for fish sedated with AQUI-S®20E under INAD 11-741. These withdrawal periods are as follows:

(1) Hatchery and marine fish: a 72-hour withdrawal time is required; and

(2) Freshwater fish sedated in the field : Immediate release (i.e., 0-hour withdrawal time) approved.

AQUI-S®20E (10% eugenol) Data Collection and Data Reporting Reductions

The AADAP Program has recently received some very good news from FDA about the need to collect and report time-to-handleable and time-to-recovery data after sedating fish with AQUI-S®20E under INAD 11-741. Effective immediately, these two types of data will

not need to be collected and reported if your study meets the treatment criteria listed below (please note that all other data collection and reporting, including documentation of any adverse events, are still required):

Freshwater salmonids—sedation to handleable

(1) fish sedated at 25-40 mg/L eugenol

(2) time to sedation does not exceed 5 minutes

(3) time to recovery does not exceed 20 minutes

Freshwater nonsalmonids—sedation to handleable

(1) fish sedated at 40-100 mg/L eugenol

(2) time to sedation does not exceed 5 minutes

(3) time to recovery does not exceed 20 minutes

However, if the treatment parameters are outside of those listed immediately above, then you must collect and report time-to-handleable and time-to-recovery data as has been the norm in the past.

Aquaflor® (50% florfenicol) Withdrawal Period for Warmwater Finfish

The AADAP Program has received from FDA an amended authorization for Aquaflor® INAD 10-697. We are now allowed a 15-day withdrawal period (instead of a 28-day period) for warmwater finfish treated under this INAD. This authorization is effective immediately.

Text provided by Bonnie Johnson (bonnie_johnson@fws.gov), National INAD Program Administer, USFWS AADAP; Bozeman, Montana USA

DRUG CALCULATION UPDATES

AQUI-S®20E (10% eugenol)

In the U.S., AQUI-S®20E (10% eugenol) can be used to sedate fish to handleable under USFWS INAD 11-741 (http://www.fws.gov/fisheries/aadap/AQUIS-E.HTM) while efforts are ongoing to obtain FDA approval. As INAD 11-741 participants know, AQUI-S®20E is administered via static immersion bath; hence, the product is mixed with "source water" (e.g., hatchery water, stream water, lake water) to achieve a desired concentration of the active ingredient, eugenol.

Calculating the amount (g or mL) of AQUI-S®20E to add to a known volume (gal) of water to achieve a target eugenol concentration (mg/L) is not intuitive, in part because AQUI-S®20E is 10% eugenol and in part because AQUI-S®20E (specific gravity = 1.124) is slightly heavier than water. Consequently, we offer the following guidelines for preparing AQUI-S®20E solutions for use to sedate fish to handleable under INAD 11-741:

Calculate weight (g) of AQUI-S®20E to add to a known volume of water

Equation:

AQUI-S®20E (g) = (A × B × C) ÷ D

A = target eugenol concentration (mg/L)

B = treatment water volume (gal)

C = 0.00378 (conversion factor for g per gal)

D = 0.1 (to account for the fact that AQUI-S®20E is 10% eugenol)

Example:

To prepare a 10-gal solution of AQUI-S®20E in which the target eugenol concentration is 25 mg/L, the amount (g) of AQUI-S®20E needed is:

AQUI-S®20E (g) = (25 × 10 × 0.00378) ÷ 0.1

AQUI-S®20E = 9.45 g

Calculate volume (mL) of AQUI-S®20E to add to a known volume of water

Equation:

AQUI-S®20E (mL) = [(A × B × C) ÷ D] ÷ E

A = target eugenol concentration (mg/L)

B = treatment water volume (gal)

C = 0.00378 (conversion factor for g per gal)

D = 0.1 (to account for the fact that AQUI-S®20E is 10% eugenol)

E = 1.124 (specific gravity of AQUI-S®20E)

Example:

To prepare a 10-gal solution of AQUI-S®20E in which the target eugenol concentration is 25 mg/L, the amount (mL) of AQUI-S®20E needed is:

AQUI-S®20E (mL) = [(25 × 10 × 0.00378) ÷ 0.1] ÷ 1.124

AQUI-S®20E = 8.41 mL

Note: The amount (g or mL) of AQUI-S®20E needed should be added directly—while constantly mixing—to the full volume of treatment water. Be sure to rinse the weighing or measuring container with the treatment water to ensure that all of the product is dispensed. Do not make stock solutions or other dilute solutions of AQUI-S®20E before use.

Terramycin® 200 For Fish (44.09% oxytetracycline dihydrate)

In the U.S., Terramycin® 200 For Fish Type A Medicated Article (TM200) is FDA-approved as a feed additive for several therapeutic uses in aquaculture and for marking the skeletal tissue of Pacific salmon. The *Quick Desk*

Reference Guide to Approved Drugs for Use in Aquaculture (*Desk Reference*; http://www.fws.gov/fisheries/aadap/PDF/Flip-Book_FINAL%20for%20web%2023may2011l.pdf) describes the FDA-approved therapeutic and marking uses of TM200 (pages 7-1 through 7-3) and provides instructions for calculating the percent body weight at which to feed to deliver a desired daily dose of the active ingredient, oxytetracycline dihydrate (OTC), as measured in g OTC/100 lbs fish/d. As the *Desk Reference* states (pages 7-4 and 9-1), the percent body weight at which to feed is, in part, based on the percentage of TM200 in the feed.

In the current edition of the *Desk Reference*, the TM200 calculation instructions are based on the fact that most feed mills will sell TM200-medicated labeled at 1%, 2%, or 3% TM200 in the feed. However, some feed mills will sell TM200-medicated feed labeled at 2 g, 4 g, or 6 g OTC/lb feed. To our knowledge, there is no easy way to convert g OTC/lb feed to % TM200 in the feed for use with the TM200 equations provided in the *Desk Reference*. Consequently, we provide the following conversions between TM200-medicated feed labeled as g OTC/lb feed and TM200-medicated feed labeled as % TM200 in feed:

2 g OTC/lb feed = 1% TM200 in feed

4 g OTC/lb feed = 2% TM200 in feed

6 g OTC/lb feed = 3% TM200 in feed

Example:

(1) Given that TM200 is 200 g OTC/lb premix, then…
(200 g OTC/454 g premix) × 100% = 44.1% OTC (0.441 OTC)

(2) When TM200-medicated feed is labeled at 2 g OTC/lb feed, that equates to 1% TM200 in the feed (as follows):

(a) (0.01 TM200)(454 g feed) = 4.54 g TM200/lb feed.

(b) (4.54 g TM200/lb feed)(0.441 OTC) = 2.00 g OTC/lb feed.

Text provided by Dan Carty (dan_carty@fws.gov), Fish Biologist, USFWS AADAP, Bozeman, Montana USA.

19th Annual Aquaculture Drug Approval Coordination Workshop participants celebrate after completing the 4th Annual Trout Trot in Bozeman, Montana

EDITORIAL

Fundamentals of Aquaculture Drug Efficacy and Target Animal Safety Studies

by

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Note: The views expressed in this editorial are not necessarily those of the U.S. Fish and Wildlife Service or the Aquatic Animal Drug Approval Partnership Program.

On June 4 and 6, 2013, the U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine hosted an online Data Quality Webinar (Webinar), which provided information about the many factors that can affect the quality of effectiveness (EFF) and target animal safety (TAS) data submitted to FDA. The Webinar covered the lifespan of EFF and TAS data—from the conceptualization of the need for these two types of data, to data collection and analysis, and finally to the submission of final study reports (FSRs) to FDA. One underlying theme of the Webinar was that the generation and submission of "high quality" EFF and TAS data can contribute to a significant decrease in the time required to obtain a new or expanded use of an animal drug.

While I was listening to the Webinar, I was thinking that researchers—as well as potential end-users of fish drugs—might be interested in reading about some of our (AADAP's) experiences related to developing, conducting, and reporting EFF and TAS studies. Consequently, the objective of this editorial is to briefly describe how AADAP typically develops, conducts, and reports EFF and TAS studies designed to contribute to the body evidence required to obtain FDA approvals of new or expanded uses of drugs needed in U.S aquaculture, fisheries management, or both. Along the way, I hope to provide information that will benefit other researchers and end-users should they decide to become involved in these types of studies.

Research Study Protocols

All of AADAP's high quality EFF studies and all of AADAP's TAS studies are conducted under FDA-reviewed-and-accepted research study protocols (RSPs). An RSP is built around a draft label claim, which proposes what a drug will be used for and how it will be used. A draft label claim can be specific, e.g., *Use Product A to control mortality in Fish Species B due to Disease C associated with Fish Pathogen D—Administer Product A in feed at X mg active ingredient per kg fish per day for Y consecutive days*. Alternatively, a draft label claim can be general, e.g.,

Use Product A to sedate all freshwater-reared finfish to handleable—Administer Product A as a static bath treatment at between X and Y mg of active ingredient per L of water. A draft label claim is always subject to modification, depending in part on the outcomes of the EFF and TAS studies conducted.

An RSP must include what I refer to as the 13 most important elements of an EFF or TAS study. An RSP specifies the (1) drug, including the product name and active ingredient, (2) treatment and control regimens, and (3) test animals (e.g., fish species and life stage). An RSP also describes a study's (4) null and alternative hypotheses, (5) experimental design, (6) experimental procedures, (7) statistical and nonstatistical analyses, and (8) how the statistical and nonstatistical analyses will be used to draw conclusions about a product's efficacy or target animal safety. Finally, an RSP details a study's (9) randomization, (10) masking (blinding), and (11) dose-verification procedures, along with the criteria that will be used for (12) initiating the study and (13) including or excluding experimental units (e.g., individual fish or tanks of fish). The rationale for including these 13 elements in an RSP is this: Given a *perfectly* written RSP and a *perfectly* designed and conducted study, it should be relatively simple to infer whether a product is efficacious for its proposed use or safe for use on the target animals.

Conducting High Quality EFF and TAS Studies

The time required for conducting an EFF or TAS study is highly variable and depends on the drug and hypothesis or hypotheses being tested. For example, a study evaluating the efficacy of a proposed sedative could take as little as 1-2 days, whereas a study evaluating the efficacy of an experimental therapeutant could take from a few weeks to nearly 2 months. Similarly, a study evaluating the safety of an experimental drug to a target animal can take 1-4 weeks. All TAS studies must be conducted under Federal Good Laboratory Practice (GLP) guidelines, a fact that limits the locations at which these studies can be conducted and limits who can be involved. In addition, all TAS studies must be inspected by a qualified Quality Assurance Officer (QAO).

Of course, years of experience have taught us that there is no *perfectly* written RSP and no *perfectly* designed and conducted EFF or TAS study; hence, there are almost always "deviations" from the RSP. A deviation is defined as an event that occurs during a study that was not anticipated and does not conform to what is written in the RSP. For example, a study participant might fail to collect water chemistry data when scheduled or inadvertently administer treated feed to a nontreated control tank. We are required to document each deviation when it occurs and to evaluate the potential effect of each deviation on the outcome of a study.

A deviation is usually judged to have had "no effect" on the outcome of a study if it corrected for a minor ambiguity or mistake in the RSP or adjusted for minor differences among study sites. However, a deviation is usually judged to have had a "negative effect" on the outcome of a study if it resulted in (1) major changes to the experimental design, (2) violation of study initiation or inclusion-exclusion criteria, (3) nonrandom allocation of treatments to experimental units, (4) nonrandom allocation of fish to tanks, (5) violation of masking, or (6) invalidation of dose-verification results. A study in which negative-effect deviations occur might be terminated before being completed or, if completed, could be downgraded in evidentiary value or not considered to be a valid test of the study's null hypothesis. Obviously, study participants must understand the RSP well enough to minimize the chances of making a deviation that could negatively affect the outcome of a study.

Writing Final Study Reports

After an EFF or TAS study has been conducted, AADAP writes an FSR. An FSR must address all sections of its corresponding RSP, including the 13 most important study elements previously described. Every piece of raw data generated during a study must be included in an FSR, and the raw data must be identified, verified, and tracked in all statistical and nonstatistical analyses. All deviations from the RSP must be described, and these deviations—individually and collectively—must be assessed for their overall effect on the outcome of a study. Finally, based on the statistical and nonstatistical analyses performed, a conclusion is drawn about the efficacy or target animal safety of the product tested.

An FSR is submitted to FDA for review only after it has been proofed. All EFF and TAS FSRs are proofed "in-house," and all TAS FSRs are also inspected by a QAO for completeness and compliance with the GLPs. Ultimately, it is the FDA who decides whether a given study has adequately demonstrated the efficacy or target animal safety of the product tested.

Conclusion

Many researchers and end-users hesitate to become involved in aquaculture drug EFF or TAS testing because it can seem like "too much work." However, the writing (AADAP's job) and reviewing (FDA's job) processes take up all but a few days to a few weeks of the time required to shepherd one of these studies through the RSP, in-life testing, and FSR phases. Much professional satisfaction can be derived from having FDA accept a study that you conducted and from knowing that you contributed to a new or expanded use of a drug needed for use in U.S. aquaculture, fishery, management, or both. Consequently, if you are thinking about becoming involved in EFF and TAS testing, we can always use your help!

USGS's UMESC CORNER

Eugenol

The U.S. Geological Survey's (USGS) Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, Wisconsin, completed a definitive study to characterize the depletion, distribution, and identification of eugenol residues in the fillet tissue from exposed fish (a total residue depletion study). Rainbow trout were exposed to 100 mg 14C labeled AQUI-S®20E/L for 1 h. Data indicated that (1) maximum eugenol and 14C-eugenol equivalent residue concentrations in the fillet tissue were measured immediately after the exposure (44.5 and 38.8 µg/g, respectively); (2) eugenol was the primary 14C-residue (>90% of all 14C-residues) in extracts from fillet tissue taken from fish sampled immediately after the exposure (0 min) and from fish sampled at 30 and 60 min after the exposure; and (3) the depletion of 14C-eugenol residues from the fillet tissue was rapid (t1/2 = 26.25 min) after transferring the exposed fish to fresh flowing water. The final report was submitted to the FDA Center for Veterinary Medicine (CVM) April 19, 2013. Contact Jeff Meinertz, jmeinertz@usgs.gov, for more information.

UMESC completed work to assess a method as the determinative method for eugenol in the edible fish fillet tissue, where eugenol is the marker residue for AQUI-S®20E. The following method characteristics were assessed: selectivity, sensitivity, precision with tissue containing biologically incurred eugenol, accuracy and precision with eugenol fortified tissue, eugenol stability, and method ruggedness. With regard to selectivity, there were no compounds in fillet tissue extracts from 7 fish species that would interfere with eugenol analyses. In addition, select aquaculture drugs incorporated into fish fillet tissue did not interfere with eugenol analyses. Method sensitivity (~0.01 µg/g) was more than adequate relative to the working tolerance of 11 µg/g established by FDA CVM. In nearly all cases, method precision with fillet tissue containing biologically incurred eugenol satisfied FDA CVM criteria of <10% CV. Method accuracy and precision with eugenol fortified fillet tissue were within FDA CVM acceptance criteria, i.e. <10% CV for precision and a recovery of 80 - 110% for accuracy. Eugenol was stable for at least 14 d in solutions of acetonitrile and water, in tissue extracts for 4 d, in frozen fillet tissue for more than 12 weeks, and in tissue undergoing freeze thaw cycles. In most cases the method was rugged, i.e., small changes in the method procedures did not impact method performance. Additional analyses are planned to further assess method precision with biologically incurred tissue. Concomitantly, the comprehensive final report for this work is undergoing development.

UMESC conducted a series of studies to assess the utility of using AQUI-S®20E as a sedative to reduce the activity of yellow perch and tilapia during live transport. A portion of the research assessed exposure parameters (concentration and duration) that would safely sedate fish while maximizing fish loading density during transport. Both species were exposed to 0, 100, 200 and 300 mg AQUI-S®20E/L at 3 loading densities; yellow perch, 120, 240, and 360 g/L; tilapia, 240, 360, and 480 g/L. There was greater than 95% survival with yellow perch and greater than 90% survival with tilapia after exposure to all concentrations and loading densities and for exposure times as long as 10-h. At the AQUI-S®20E concentrations that will be used during live transport (100 – 200 mg/L), recovery times from sedation were < 10 min for each species. A comprehensive final report is progressing through the final stages of the USGS review process. The final report is expected to be submitted to the FDA CVM by October, 2013. Contact Aaron Cupp, acupp@usgs.gov, for more information.

Hydrogen Peroxide

UMESC is conducting research to expand the label for 35% PEROX-AID® to include the reduction of *Gyrodactylus* sp. infestation density on cool and warmwater fish species. Two trials have been completed, one with fathead minnows with a natural infestation of *G. hoffmani* and a second trial with yellow perch with a natural infestation of *G. freemani*. The study objective was to assess the efficacy of 35% PEROX-AID® to reduce *Gyrodactylus* sp. infestation density and included the evaluation of parasite density on fish following assignment to 1 of 3 treatment regimens, (1) a nontreated control group; (2) a group treated at 50 mg/L for 60 min; and (3) a group treated at 75 mg/L for 60 min. The 35% PEROX-AID® treatments were applied once daily on alternate days for a total of 3 treatments. Following treatment, both fathead minnows and yellow perch experienced a reduction of >97% in parasite density on fish in the treated groups. The reports are expected to be submitted to FDA CVM by September, 2013. Contact Sue Schleis, sschleis@usgs.gov, for more information.

Text provided by Jeff Meinertz (jmeinertz@usgs.gov), Research Physiologist; USGS UMESC; La Crosse, Wisconsin USA.

19th Annual Aquaculture Drug Approval Coordination Workshop participants "decompress" while rafting on the Yellowstone River near Livingston, Montana

USDA's ARS CORNER

Aquaculture America 2014

The Aquaculture Drug Research and Drug Approval Status special session, which is moderated by Jim Bowker and Dave Straus, has decided to take a break after 11 years. We will return for the Aquaculture America 2015 meeting in New Orleans. Keep us in mind as we hope to have a good turn-out in 2015!

Copper Sulfate ($CuSO_4$)

Chemistry, Manufacturing and Control (CMC) technical section—The Biotherapeutics Team of the Division of Manufacturing Technologies in the Office of New Animal Drug Evaluation at FDA CVM has asked the sponsor to update the CMC Technical Section. We are in the process of assisting them with this task.

Saprolegniasis label—Effectiveness dose-confirmation Final Study Reports (lab and field) for the study on $CuSO_4$ to control fungus on channel catfish eggs were completed at the U.S. Department of Agriculture's (USDA) Agricultural Research Service (ARS) Stuttgart National Aquaculture Research Center (SNARC) in Stuttgart, Arkansas, and submitted by the sponsor to FDA CVM in November 2012 and March 2013. The sponsor received a Technical Section Complete letter dated June 12, 2013. As you know, this is a significant accomplishment toward gaining approval of $CuSO_4$ to treat fungus on eggs.

This completes all major technical sections, except for Environmental Safety under a hatchery scenario.

Ichthyophthiriasis label—The Environmental Safety technical section for the indication "… to control mortality associated with ichthyophthiriasis on channel catfish cultured in earthen ponds" has been prepared at SNARC and is being completed by the sponsor's environmental consultant. A draft label has been prepared, and "all other information" is being compiled.

Peracetic Acid

Acute toxicity and effectiveness studies are in progress at SNARC on a variety of fish species and diseases.

Text provided by Dr. Dave Straus, (*dave.straus@ars.usda.gov*) Research Toxicologist; U.S. Department of Agriculture, Agricultural Research Service; Harry K. Dupree – Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas USA.

AFS's WGADCB CORNER

The American Fisheries Society's (AFS) Fish Culture Section (FCS) Working Group on Aquaculture Drugs, Chemicals, and Biologics (WGADCB) met August 1, 2013, in Bozeman, Montana, in conjunction with the 19th Annual Aquaculture Drug Approval Coordination Workshop. The following issues were discussed:

Revising the AFS Guide to Using Drugs, Biologics, and Other Chemicals in Aquaculture

The WGADCB plans to revise the *AFS Guide to Using Drugs, Biologics, and Other Chemicals in Aquaculture* (*Guide*) in late fall 2013. The revision will include any new or expanded aquaculture drug approvals that have occurred since the *Guide* was last revised in 2012.

Opportunities to Conduct Field Effectiveness Trials and Target Animal Safety Studies

A "wish-list" of studies that need to be conducted to help get certain drugs approved for use in U.S. aquaculture has been posted on the AFS FCS WGADCB website (*https://sites.google.com/site/fishculturesection/working-group-on-aquaculture-drugs-chemicals-and-biologics/wgadcb-resources-and-tools*). If you are interested in becoming involved in these types of studies, please contact WGADCB co-chair Jim Bowker today at *jim_bowker@fws.gov*.

Support for the Fish Drug Approval Effort in the U.S.

There was considerable discussion about how to foster greater understanding of and support for the fish drug approval effort in the U.S. Listed below are some of the issues discussed:

- How can milestones and successes in the drug approval process be better defined, promoted, and disseminated? Although completion of an FDA-required technical section (e.g., efficacy, target animal safety) is an important milestone in the approval process, it is not fully understood or appreciated by those not intimately involved. Drug approvals/label expansions are huge successes; however, it can take years to obtain an initial approval—so how can a new approval be promoted so as to reach a broader audience?
- What is the long term solution to sustainable funding for the drug approval effort? The roles of the public data-generating partners are too important to lose; however, base funding is shrinking. A group will convene at the upcoming Association of Fish and Wildlife Agencies (AFWA) meeting to develop a strategy for long-term sustainable funding for the drug approval effort.
- The Catfish Farmers of America developed a crisis management plan that dealt with every foreseeable crisis in their industry—a plan that included contact names, which were incorporated into the plan horizontally and vertically in case a crisis arose. Perhaps something similar can be done for the U.S. fish drug approval effort by establishing a core group (include someone with crisis management

experience) to develop a list of potential issues and a list of individuals who can help resolve crises.

- There is a need to network with groups that communicate regularly with public agency decision makers. Perhaps AFWA might be one entity to effectively "get the message out." We would need to go through appropriate AFWA channels that have already been established for communicating with Directorate(s) of selected U.S. agencies.
- Funding Issues: Short term crises (STC) versus Long Term Strategies (LTS). An STC suggestion: put together press releases (very short and to the point) and send to groups like the Sierra Club, Trout Unlimited, fishing groups (e.g., Michigan Muskie Alliance). Write articles for specific magazines and newsletters and include the message "how you can help." An LTS should include how to elevate the importance of aquaculture drug approval work within a public agency and the importance of public agencies operating within the legal framework established through the Federal Food, Drug, and Cosmetic Act. A drug use policy document is a good idea for an LTS but not for an STC.
- How can the Directorates of the U.S. Fish and Wildlife Service, U.S. Geological Survey, U.S. Department of Agriculture, and National Marine Fisheries Service be informed and educated about how important the fish drug approval effort is to these agencies?
- A core group of 5-7 individuals was established and will convene weekly telephone conferences to discuss strategies and progress.

Updates to FDA Guidance Document #61

A brief update was provided on *FDA Guidance Document #61: Guidance for Industry, FDA Approval of New Animal Drugs for Minor Uses and for Minor Species*. The original document was outdated and included much redundancy. The revision will be guidance for minor species only (no reference to major species) and will not include information on Index Drugs (which will be in a separate document). It is FDA's goal to have a draft revision "cleared" in-house by the end of this summer, after which there will be a public comment period.

Update from the WGADCB Pathogen Grouping Task Force

Bacterial gill disease (BGD) is caused by the pathogen *Flavobacterium branchiophilum*; however, diagnosticians in the field believe other pathogens may be involved in the disease process as well. A study is being conducted to determine what other bacterial genera and/or species are involved in BGD. A first step has been to assess the feasibility of using technologies available at the FDA CVM Office of Research (OR) to accurately identify *Flavobacteria* spp., as well as other common aquatic pathogens. The systems currently under assessment include the Biolog, the MIDI Microbial Identification System (MIDI MIS), and 16S sequencing. The FDA CVM OR is also evaluating the appropriateness of various media (broth and agar) and incubation temperatures for initial culture from field samples so as to maximize putative pathogen recovery yet minimize contamination.

For more information on current WGADCB activities, please see past meeting minutes available at the AFS Fish Culture Section website https://sites.google.com/site/fishculturesection/working-group-on-aquaculture-drugs-chemicals-and-biologics/wgadcb-resources-and-tools, contact one of the co-chairs, or better yet, *come to our next meeting*!

AFS FCS WGADCB updates provided by Jim Bowker (jim_bowker@fws.gov), Research Program Manager; USFWS AADAP; Bozeman, Montana USA, and condensed for this newsletter by Dan Carty.

RELEVANT LITERATURE

Listed below are journal citations with particular relevance to the broad topic of drugs and aquaculture species. With some exceptions, this list includes citations not previously included in our newsletter. Our most recent Relevant Literature Master list, which dates back to 2009, can be viewed or downloaded at: http://www.fws.gov/fisheries/aadap/PDF/Relv%20Lit_Master%20List_12-20-12.pdf.

Inclusion of a citation in our newsletter does not imply (1) acceptance by the U.S. Food and Drug Administration's Center for Veterinary Medicine of a drug's safety or effectiveness, (2) endorsement of a drug or product by the U.S. Fish and Wildlife Service, (3) recommendation of the technique to any particular situation, or (4) concurrence with a treatment procedure/drug.

Note: The pro– and pre-biotics literature sections will return in the next issue. In the meantime, please send citations of interest to Dan Carty (dan_carty@fws.gov).

Antibiotic and Bacterial

Antony, JJ, et al. 2013. Antimicrobial activity of *Leucas aspera* engineered silver nanoparticles against *Aeromonas hydrophila* in infected *Catla catla*. *Colloids and Surfaces B: Biointerfaces* **109:20-24**.

Balasundaram, A, et al. 2013. A study on genetic variability of pathogenic *Aeromonas hydrophila* strains and the varied responses of the strains towards phyto-extracts. *Pakistan Journal of Biological Sciences* **16(21):1303-1310**.

Boran, H, et al. 2013. Bacterial diseases of cultured Mediterranean horse mackerel (*Trachurus mediterraneus*) in sea cages. *Aquaculture* **396-399:8-13**.

Bowker, JD, et al. 2013. Controlling mortality caused by external columnaris in largemouth bass and bluegill with chloramine-T or hydrogen peroxide. *North American Journal of Aquaculture* **75(3):342-351**.

Cabello, FC, et al. 2013. Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. *Environmental Microbiology* **15 (7):1917-1942**.

Cavallo, RA, et al. 2013. Antibacterial activity of marine macroalgae against fish pathogenic *Vibrio* species. *Central European Journal of Biology* **8(7):646-653**.

Cesare, AD, et al. 2013. Aquaculture can promote the presence and spread of antibiotic-resistant enterococci in marine sediments. *PLoS ONE* **8(4):e62838**.

de Orte, MR, et al. 2013. Assessing the toxicity of chemical compounds associated with marine land-based fish farms: the use of mini-scale microalgal toxicity tests. *Chemistry and Ecology* **29(6):554-563**.

Di Salvo, A, et al. 2013. Florfenicol depletion in edible tissue of rainbow trout, *Oncorhynchus mykiss* (Walbaum), and sea bream, *Sparus aurata* L. *Journal of Fish Diseases* **36 (8):685-693**.

Gou, X-I, and Wang, L. 2013. Pathogenicity and detection of virulence genes of bacterium *Yersinia enterocolitica* isolated from yellow catfish (*Pelteobagrus fulvidraco*). *Fisheries Science* **32(5):293-296**. (in Chinese with English abstract).

Haines, AN, et al. 2013. First report of *Streptococcus parauberis* in wild finfish from North America. **Veterinary Microbiology 166(1-2):270-275**.

Han, SM, et al. 2013. Activity of honey bee venom against select infectious fish pathogens. *North American Journal of Aquaculture* **75(3):445-448**.

Henriksen, M, et al. 2013. Effect of hydrogen peroxide on immersion challenge of rainbow trout fry with *Flavobacterium psychrophilum*. *PLoS ONE* **8(4):e62590**.

John, N, and Abdulla, MH. 2013. Distribution, extracellular virulence factors and drug resistance of motile aeromonads in fresh water ornamental fishes and associated carriage water. *International Journal of Aquaculture* **3(17):92-100.**

Kang, Y-J, et al. 2013. Bioassay-guided isolation and identification of active compounds from *Macleaya microcarpa* (Maxim) Fedde against fish pathogenic bacteria. *Aquaculture Research* **44(8):1221-1228**.

Kim, D-H, et al. 2013. Low-value fish used as feed in aquaculture were a source of furunculosis caused by atypical *Aeromonas salmonicida*. *Aquaculture* **408-409:113-117**.

Labella, A, et al. 2013. High incidence of antibiotic multi-resistant bacteria in coastal areas dedicated to fish farming. *Marine Pollution Bulletin* **70(1-2):197-203**.

Matthews, MD, et al. 2013. Efficacy of Aquaflor (50% florfenicol)-medicated feed to control mortality associated with *Flavobacterium columnare* infection in Florida largemouth bass and bluegill. *North American Journal of Aquaculture* **75(3):385-392**.

Okolie, C, and Chenia, HY. 2013. Assessment of aquatic *Aeromonas* spp. isolates' susceptibility to cinnamaldehyde, vanillin, and crude *Kigelia africana* fruit extracts. *Journal of the World Aquaculture Society* **44(4):486-498**.

Rose, S, et al. 2013. Imported ornamental fish are colonized with antibiotic-resistant bacteria. *Journal of Fish Diseases* **36(6):533-542**.

Sadeghi, S, and Jahani, M. 2013. Selective solid-phase extraction using molecular imprinted polymer sorbent for the analysis of florfenicol in food samples. *Food Chemistry* **141 (2):1242-1251**.

Sharafati-Chaleshtori, R, et al. 2013. Residues of oxytetracycline in cultured rainbow trout. *Pakistan Journal of Biological Sciences* **16(21):1419-1422**.

Shehata, SA, et al. 2013. Antibacterial activity of essential oils and their effects on Nile tilapia fingerlings performance. *Journal of Medical Sciences* **13(5):367-372**.

Tanaka, T, et al. 2013. Electrochemical disinfection of fish pathogens in seawater without the production of a lethal concentration of chlorine using a flow reactor. *Journal of Bioscience and Bioengineering* **116(4):480-484**.

Tkachenko, H, et al. 2013. Effects of chloramine-T exposure on oxidative stress biomarkers and liver biochemistry of rainbow trout, *Oncorhynchus mykiss* (Walbaum), brown trout, *Salmo trutta* (L.), and grayling, *Thymallus thymallus* (L.). *Archives of Polish Fisheries* **21(1):41-51**.

Tuševljak, N, et al. 2013. Antimicrobial use and resistance in aquaculture: findings of a globally administered survey of aquaculture-allied professionals. *Zoonoses and Public Health* **60(6):426-436**.

Parasite and Fungus Control

Banfield, MJ, and Kamoun, S. 2013. Hooked and cooked: a fish killer genome exposed. *PLoS Genetics* **9(6):e1003590**. (*Saprolegnia parasitica*)

Carmichael, SN. 2013. Salmon lice (*Lepeophtheirus salmonis*) showing varying emamectin benzoate susceptibilities differ in neuronal acetylcholine receptor and GABA-gated chloride channel mRNA expression. *BMC Genomics* **14(1):408 (16 pages)**.

Cone, DK, et al. 2013. A new gyrodactylid (Monogenea) parasitizing bay pipefish (*Syngnathus leptorhynchus*) from the Pacific Coast of North America. *The Journal of Parasitology* **99(2):183-188**.

Farmer, BD, et al. 2013. Pretreating cannel catfish with copper sulfate affects susceptibility to columnaris disease. *North American Journal of Aquaculture* **75(2):205-211**.

Farmer, BD, et al. 2013. Effectiveness of copper sulphate, potassium permanganate and peracetic acid to reduce mortality and infestation of *Ichthyobodo necato*r in channel catfish *Ictalurus punctatus* (Rafinesque 1818). *Aquaculture Research* **44(7):1103-1109**.

Forwood, JM, et al. 2013. Validation of a rapid counting method for assessing treatment efficacy against *Lepidotrema bidyana* infecting silver perch *Bidyanus bidyanus*. *Diseases of Aquatic Organisms* **105(3):253-257**.

González, Á, et al. 2013. Effects of different bronopol treatments on final survival rates in the artificial incubation of crayfish eggs (*Pacifastacus leniusculus*, Astacidae). *Aquaculture Research* **44(3):354-358**.

Hirazawa, N, et al. 2013. Differences in sensitivity to the anthelmintic praziquantel by the skin-parasitic monogeneans *Benedenia seriolae* and *Neobenedenia girellae*. *Aquaculture* **404-405:59-64**.

Hoseini, SM, and Tarkhani, R. 2013. Effect of short-term treatment with potassium permanganate on stress markers and blood biochemistry in goldfish *Carassius auratus*. *Aquaculture Research* **44(6):869-875**.

Hu, X-G, et al. 2013. *In vitro s*creening of fungicidal chemicals for antifungal activity against *Saprolegnia. Journal of the World Aquaculture Society* **44(4):528-535**.

Ishimaru, K, et al. 2013. Praziquantel treatment against *Cardicola* blood flukes: determination of the minimal effective dose and pharmacokinetics in juvenile Pacific bluefin tuna. *Aquaculture* 402-403 :24-27.

Jaafar, RM, et al. 2013. Comparative efficacies of sodium percarbonate, peracetic acid, and formaldehyde for control of *Ichthyobodo necator*—an ectoparasite flagellate from rainbow trout. *Acta Ichthyologica et Piscatoria* **43(2):139-143**.

Kong, X, et al. 2013. Effects of copper exposure on the hatching status and antioxidant defense at different developmental stages of embryos and larvae of goldfish *Carassius auratus*. *Chemosphere* **92(11):1458-1464**.

Kouba, A, et al. 2012. Ultraviolet light and semi-recirculating systems in artificial incubation of noble crayfish (*Astacus astacus*) eggs: opportunities and limitations. *Aquaculture Research* **44(1):67-74**.

Marchand, P-A, et al. 2012. Reduction of *in vitro* growth in *Flavobacterium columnare* and *Saprolegnia parasitica* by products containing peracetic acid. *Aquaculture Research* **43(12):1861-1866**.

Marchand, P-A, et al. 2013. Effect of water hardness on peracetic acid toxicity to zebrafish, *Danio rerio*, embryos. *Aquaculture International* **21(30:679-686**.

Marchiori, N, et al. 2013. New technique for collecting eggs from monogenean parasites. *Experimental Parasitology* **134(2):138-140**.

Paixao, LF, et al. 2013. Evaluation of formalin and copper sulfate on monogenetic parasite (*Urodeidoides* sp.) of *Hemigrammus* sp. (Osteichthyes: Characidae) juveniles. *Acta Amazonica* **43(2):211-216**. (in Portuguese with English abstract)

Pedersen, L-F, et al. 2013. Peracetic acid degradation in freshwater aquaculture systems and possible practical implications. *Aquacultural Engineering* **March 2013: 65-71**.

Rigos, G, et al. 2013. Tissue distribution and field evaluation of caprylic acid against natural infections of *Sparicotyle chrysophrii* in cage-reared gilthead sea bream *Sparus aurata*. *Aquaculture* **408-409:15-19**.

Rodrigues, N, et al. 2013. Notes on the husbandry and long-term transportation of Bull ray (*Pteromylaeus bovinus*) and Dolphinfish (*Coryphaena hippurus* and *Coryphaena equiselis*). *Zoo Biology* **32(2):222-229**.

Rogers, LA, et al. 2013. Modeling parasite dynamics on farmed salmon for precautionary conservation management of wild salmon. *PLoS One* **8(4):e60096**.

Sáez, MI, et al. 2013. Effect of sublethal concentrations of waterborne copper on lipid peroxidation and enzymatic antioxidant response in *Gambusia holbrooki*. *Environmental Toxicology and Pharmacology* **36(1):125-134**.

Wei, JZ. Et al. 2013. Ichthyophthiriasis: emphases on the epizootiology. *Letters in Applied Microbiology* **57(2):91-101**.

Xu, H, et al. 2013. Monoclonal antibody-based enzyme-linked immunosorbent assay for detection of total malachite green and crystal violet residues in fishery products. *International Journal of Environmental Analytical Chemistry* **93(9):959-969**.

Sedation or Anesthesia

Bauquier, SH, et al. 2013. Evaluation of the sedative and anaesthetic effects of five different concentrations of alfaxalone in goldfish, *Carassius auratus*. *Aquaculture* **396-399:119-123**.

Bjørlykke, GA, et al. 2013. Slaughter of Atlantic salmon (*Salmo salar* L.) in the presence of carbon monoxide. *Fish Physiology and Biochemistry* **39(4):871-879**.

Crossman, JA, et al. 2013. Experimental examination of surgical procedures for implanting sonic transmitters in juvenile shortnose sturgeon and Atlantic sturgeon. *North American Journal of Fisheries Management* **33(3):549-556**.

Iversen, MH, et al. 2013. The efficacy of Aqui-S vet. (iso-eugenol) and metomidate as anaesthetics in European eel (*Anguilla anguilla* L.), and their effects on animal welfare and primary and secondary stress responses. *Aquaculture Research* **44(8):307-1316**.

Jahanbakhshi, A, et al. 2013. Effects of different concentrations of 2-phenoxyethanol on primary and secondary stress responses in Persian sturgeon *Acipenser persicus*. *Journal of Applied Ichthyology* **29(3): 499-502**.

Kramer, MJ. 2013. The trophic importance of algal turfs for coral reef fishes: the crustacean link. *Coral Reefs* **32(2):575-583**.

Matsche, MA. 2013. Relative physiological effects of laparoscopic surgery and anesthesia with tricaine methanesulfonate (MS-222) in Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus*. *Journal of Applied Ichthyology* **29 (3):510-519**.

Posner, LP, et al. 2013. Repeated exposure of goldfish (*Carassius auratus*) to tricaine methanesulfonate (MS-222). *Journal of Zoo and Wildlife Medicine* **44(2):340-347**.

Uçar, A, et al. 2013. Effects of anesthetic substances on some antioxidant enzyme activities of trouts. *Journal of Fisheries Sciences* **7(2):152-160**.

Skeletal (Fluorescent) Marking

Farley, JH, et al. 2013. Age estimation and validation for South Pacific albacore *Thunnus alalunga*. *Journal of Fish Biology* **82(5):1523-1544**. (oxytetracycline).

Ibáñez, AL, et al. 2013. Evaluation of marking efficiency of different alizarin red S concentrations on body fish structures in *Oreochromis niloticus* (Perciformes: Cichlidae) juveniles. *Revista de Biología Tropical* **61(1):193-201**.

Wells, RJD, et al. 2013. Age validation of juvenile shortfin mako (*Isurus oxyrinchus*) tagged and marked with oxytetracycline off southern California. *Fishery Bulletin* **111 (2):147-160**.

Spawning Hormones and Sex Manipulation

Arski, D, et al. 2013. Effect of different commercial spawning agents and thermal regime on the effectiveness of pikeperch, *Sander lucioperca* (L.), reproduction under controlled conditions. *Aquaculture International* **21(4):819-828**.

Cejko, BI, et al. 2012. The effectiveness of hormonal preparations (Ovopel, Ovaprim, LHRHa, hCG and CPE) in stimulating spermiation in dace *Leuciscus leuciscus* (L.). *Journal of Applied Ichthyology* **28(6):873-877**.

Hill, H, et al. 2013. Influence of lineage, broodstock conditioning, and hormone injection on Gila trout reproductive success and egg fatty acid composition. *North American Journal of Aquaculture* **75(3):393-403**.

Kanamori, A, and Toyama, K. 2013. A transgenic medaka line with visible markers for genotypic and phenotypic sex. *Environmental Science & Technology* **47(12):6640-6645**.

Kausar, N, et al. 2013. Status of whole body cortisol and total protein content in eggs, embryos and larvae of silver carp (*Hypophthalmichthys molitrix*). *International Journal of Agriculture and Biology* **15(2):252-258**. (Ovaprim)

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Li, M, et al. 2013. Molecular cloning and characterization of amh, dax1 and cyp19a1a genes and their response to 17α-methyltestosterone in Pengze crucian carp. *Comparative Biochemistry and Physiology—Toxicology & Pharmacology* **157(4):372-381**.

Liñán-Cabello, MA, et al. 2013. Somatic growth effects of intramuscular injection of growth hormone in androgen-treated juvenile Nile tilapia, *Oreochromis niloticus* (Perciformes: Cichlidae). *Revista de Biología Tropical* **61 (1):203-212**.

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Owodeinde, FG, et al. 2013. Survival, growth and feed utilization of the reciprocal hybrids of *Clarias gariepinus* (Burchell, 1822) and *Heterobranchus bidorsalis* (Geoffroy, 1809) in concrete tanks. *Journal of Fisheries and Aquatic Science* **8(1):122-128**.

Palaiokostas, C, et al. 2013. Mapping and validation of the major sex-determining region in Nile Tilapia (*Oreochromis niloticus* L.) using RAD sequencing. *PLoS One* **8(7):e68389**.

Park, C-b, et al. 2013. Transient effects of methyltestosterone injection on different reproductive parameters of the hermaphrodite fish *Kryptolebias marmoratus*. *Ecotoxicology* **22(7):1145-54**.

Pompini, M, et al. 2013. Temperature-induced sex reversal is not responsible for sex-ratio distortions in grayling *Thymallus thymallus* or brown trout *Salmo trutta*. *Journal of Fish Biology* **83(2):404-411**.

Sahu, DK, et al. 2013. Identification of reproduction-related genes and SSR-markers through expressed sequence tags analysis of a monsoon breeding carp rohu, *Labeo rohita* (Hamilton). *Gene* **524(1):1-14**.

Saoshiro, S, et al. 2013. Sexual bipotentiality of behavior in male and female goldfish. *General and Comparative Endocrinology* **181:265-270**.

Singh, AK. 2013. Introduction of modern endocrine techniques for the production of monosex population of fishes. *General and Comparative Endocrinology* **181:146-155**.

Svinger, VW, et al. 2013. Synchronization of ovulation in brook char (*Salvelinus fontinalis*, Mitchill 1814) using emulsified D-Arg "ProNEt" sGnRHa. *Aquaculture International* **21(4):783-799**.

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Vernetti, CHMM, et al. 2013. Genes involved in sex determination and the influence of temperature during the sexual differentiation process in fish: a review. *African Journal of Biotechnology* **12(17):2129-2146**.

Zheng, Y, et al. 2013. Molecular characterization of five steroid receptors from Pengze crucian carp and their expression profiles of juveniles in response to 17α-ethinylestradiol and 17α-methyltestosterone. *General and Comparative Endocrinology* **191:113-122**.

Vaccines/Biologics—Salmonids

Ballesteros, NA, et al. 2013. The pyloric caeca area Is a major site for IgM^+ and IgT^+ B cell recruitment in response to oral vaccination in rainbow trout. *PLoS ONE* **8(6):e66118**.

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Chettri, JK, et al. 2013. Comparative evaluation of administration methods for a vaccine protecting rainbow trout against *Yersinia ruckeri* O1 biotype 2 infections. *Veterinary Immunology and Immunopathology* **154(1-2):42-47**.

Fredriksen, BN, et al. 2013. Intramuscular challenge of rainbow trout (*Oncorhynchus mykiss*) with two Norwegian field strains of *Flavobacterium psychrophilum*. *Fish & Shellfish Immunology* **35(2):595-598**.

Harkness, JE, et al. 2013. Demonstrated efficacy of a pilot heterologous whole-spore vaccine against microsporidial gill disease in rainbow trout. *Clinical and Vaccine Immunology* **20(9):1483-1484**.

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Wolf, A, et al. 2013. A hemagglutinin-esterase-expressing salmonid alphavirus replicon protects Atlantic salmon (*Salmo salar*) against infectious salmon anemia (ISA). *Vaccine* **31 (4):661-669**.

Vaccines/Biologics—Catfish

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Pridgeon, JW, et al. 2013. Biochemical and molecular characterization of the novobiocin and rifampicin resistant *Aeromonas hydrophila* vaccine strain AL09-71N+R compared to its virulent parent strain AL09-71. *Veterinary Microbiology* **165(3-4):349-357**.

Pridgeon, JW, et al. 2013. Identification of gyrB and rpoB gene mutations and differentially expressed proteins between a novobiocin-resistant *Aeromonas hydrophila* catfish vaccine strain and its virulent parent strain. *Veterinary Microbiology* **166(3-4):624-630**.

Santander, J, et al. 2013. Mechanisms of intrinsic resistance to antimicrobial peptides of *Edwardsiella ictaluri* and its influence on fish gut inflammation and virulence. *Microbiology* **159(7):1471-1486**.

Vaccines/Biologics—Tilapia

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UPCOMING MEETINGS

34th Fish Feed and Nutrition Workshop (September 22-24, 2013, Carbondale, Illinois USA)

The 34th Fish Feed and Nutrition Workshop will be hosted by the Southern Illinois University (SIU) Center for Fisheries, Aquaculture, and Aquatic Sciences in Carbondale, Illinois USA on September 22-24. The preliminary agenda includes a welcome social (Sep 22); technical presentations, a tour of the SIU aquaculture facilities, and a catfish dinner (Sep 23); and

tours of local fish farms (Sep 24). Please see the workshop brochure at http://fishdata.siu.edu/ffnw13.pdf.

Indiana Aquaculture Association Workshop (October 5, 2013, Scottsburg, Indiana USA)

The Indiana Aquaculture Association will hold an aquaculture workshop on Saturday, October 5, 2013, from 9 am to 4 pm at Mid-America Science Park, Scottsburg, Indiana USA.

Some of the topics covered during the 1-day workshop will include: Aquaculture is growing, is it for you? Who can I talk to? What do I need to do before start up? Can I use my pond or my barn? What marketing practices really work? What are some of the financial/risk considerations? How about aquaponics?

Reservations for this workshop are requested because seating is limited. Register online or by phone (317-417-0090). Please visit http://www.indianaaquaculture.com/ for complete details.

Virginia Aquaculture Conference (November 15-16, 2013, Newport News, Virginia USA)

The 2013 Virginia Aquaculture Conference (VAC) will be held November 15-16 at the Newport News Marriott at City Center in Newport News, Virginia USA.

Whether you are an active shellfish or finfish culturist or thinking about starting an aquaculture business—this is the place to be! The VAC provides opportunities to learn about current and upcoming industry issues, explore new developments in culture technology, and interact with others with similar interests. The format includes a day and a half of shellfish and finfish/prawn content in concurrent sessions with an on-going trade show. The trade show consists of a wide variety of exhibitors including the latest in gear and technology, insurance, loan information, agency support and much more. The VAC also provides the opportunity to connect with the aquaculture trade organizations. For registration and agenda information, please visit http://www.vaaquacultureconference.com/.

Texas Aquaculture 44th Annual Conference and Trade Show (January 29-31, 2014, Fredericksburg, Texas)

The Texas Aquaculture Association will hold its 44th Annual Conference and Trade Show on January 29-31, 2014, in Fredericksburg, Texas USA. Details will be available later this fall at http://www.texasaquaculture.org/.

Midcontinent Warmwater Fish Culture Workshop (February 3-5, 2014, Council Bluffs, Iowa USA)

The 36th Annual Midcontinent Warmwater Fish Culture Workshop will be held February 3-5, 2014, in Council Bluffs, Iowa USA.

The workshop will begin with a Welcome Social on the evening of February 3, and technical sessions will be held February 4-5. Participants will share and learn what many public fish culture facilities, researchers, universities, and aquaculture-related businesses have been working on during the past year. For more information, please visit http://www.wisconsinaquaculture.com/Events_Details.cfm?RID=72.

Aquaculture America 2014 (February 9-12, 2014, Seattle, Washington USA)

Aquaculture America 2014 will be held February 9-12, 2014, at the Washington State Convention Center in Seattle, Washington USA.

In 2014, the U.S. Aquaculture Society (formerly the U.S. Chapter of the World Aquaculture Society) will be joined by the National Aquaculture Association and the U.S. Aquaculture Suppliers Association to produce this conference. In addition, the annual meetings of the (1) Aquacultural Engineering Society, (2) American Tilapia Association, (3) Striped Bass Growers Association, (4) U.S. Trout Farmers Association, (5) U.S. Shrimp Farming Association, and many more associations will be held, which will make Aquaculture America 2014 the one meeting in the U.S. that you don't want to miss! Please visit https://www.was.org/meetings/default.aspx?Code=AA2014 for complete details.

World Aquaculture 2014 (June 7-11, 2014, Adelaide, South Australia)

World Aquaculture 2014 will be held June 7-11, 2014, in Adelaide, South Australia.

Australia is proud to be hosting World Aquaculture for the first time since 1999. This annual event will incorporate the biennial Australasian Aquaculture conference and trade show and will see several thousand attendees from around the world converge on the city of Adelaide and tour the central hub of Australian aquaculture in Port Lincoln. Contributions to the progress of developing new and existing ideas to stimulate this vital industry are welcome. With almost half of the world's consumption of seafood coming from farms, aquaculture is playing an increasingly important role in meeting the challenge of global food security. World Aquaculture 2014 will be an opportunity for the international aquaculture community—academics, industry researchers, market and industry analysts, government officials, policy makers and industry representatives to present their work and exchange ideas and develop a vision for the future of the aquaculture industry as we focus on the theme of *Create, Nurture, Grow*. An event not to be missed, World Aquaculture 2014 will offer a chance to gauge the sector's progress, whilst we discuss and debate the issues, ideas, mechanisms and hands-on practical approaches towards building a better industry. In addition, there will be ample opportunity to network during both structured and free-flowing events. We look forward to seeing you in Adelaide. For complete details, please visit: https://www.was.org/meetings/default.aspx?code=WA2014.

Note: Information about many other aquaculture-related meetings being held in the U.S. and around the world during 2013 and 2014 can be found via the following links:

https://www.was.org/EventCalendar.aspx

http://aquaculturedirectory.co.uk/aquaculture-events-2/

http://www.thefishsite.com/events/

Cover Photo:

The Yellowstone River is the longest undammed river in the continental United States. The river originates south of Yellowstone National Park on the slopes of Younts Peak, Wyoming, and travels more than 600 miles north-northeast to its terminus in North Dakota, where it empties into the Missouri River. The Grand Canyon of the Yellowstone River and the river's Upper and Lower Falls lie wholly within Yellowstone National Park (YNP). The Grand Canyon of the Yellowstone is 10,000-14,000 years old, with a length of 20 miles, a depth ranging from 800 to 1,200 feet, and a width ranging from 1,500 to 4,000 feet. The Lower Falls, which is near Canyon Junction, is 308 feet high, and the volume of water flowing over it can range from a high of 63,500 gal/

second in spring to a low of 5,000 gal/second in fall and winter. A photo-tour of the canyon can be accessed via the following U.S. National Park Service webpage: *http://mms.nps.gov/yell/features/canyontour/index.htm*.

19th Annual Aquaculture Drug Approval Coordination Workshop participants gather for a group photo during the Ice-Breaker BBQ at scenic Hyalite Reservoir near Bozeman, Montana