

Meeting Minutes
9th Meeting of the
National Aquaculture Drug Research Forum
Friday, June 12, 2009
8:00 – 10:15 am

Held in conjunction with the
15th Annual Aquaculture Drug Approval Coordination Workshop
Little Rock, Arkansas

Attendees of the 9th meeting of the National Aquaculture Drug Research Forum (Forum) included aquaculture drug researchers, research coordinators, chemical and pharmaceutical sponsors, and representatives from CVM.

The focus of the meeting was to discuss issues/challenges about conducting field trials to evaluate the effectiveness of chemotherapeutants to control ectoparasite infestations in finfish.

Before we launched into discussions relative to the focus of the meeting, Dr. Steve Yan brought a joint message (of Drs. Christine Moffitt and Steve Yan) from the “Antimicrobial Group” of the NADRF. He commented that for addressing pre-approval requirements pertaining to antimicrobial resistance (GFI #152) and impact on the human gut flora (GFI #159), this forum could provide a unique, informal opportunity for different sectors to discuss general and non-proprietary issues of those requirements and facilitate scientific and technical needs, if any. He also stated that CVM’s Microbial Food Safety Team supports the group and welcomes inquiries regarding specific submission needs in relation to both GFIs #152 and #159. Dr. Jeff Gilbert is the Team Leader overseeing both requirements.

Results from a Survey of Parasite-Related Aquatic Animal Health Issues indicated that *Ichthyobodo* (costia), *Trichodina*, and *Chilodonella* were the protozoans of primary concern that impacted health and survival of infested fish, and that *Epistylis* and *Ambiphyra* were protozoans of secondary concern. Similarly, it was found that *Gyrodactylus* and *Dactylogyrus* were the monogeneans of primary concern, and that *Cleidodiscus* was a monogenean of secondary concern.

1. Describe similarities and differences between Regulatory and Academic Science - Jim Bowker (USFWS-AADAP) pointed to the document attached to the agenda (http://sciencepolicy.colorado.edu/klamathbasin/science_policy/regulatory_vs_academic.html) and commented that the overall regulatory science goal is to provide information needed to meet regulatory requirements and to provide reliable information for decision makers (i.e., CVM), whereas the primary goal of academic science is that of original research framed by scientists and driven by rational analysis and expert judgment.
2. Terms commonly used to describe parasite populations - Dan Carty (USFWS-AADAP) and Mark Gaikowski (USGS-UMESC) both defined terms commonly used in parasite studies (e.g., prevalence, mean abundance, intensity, incidence, and density) in their presentations during the Workshop. Bowker commented that in all likelihood, density will be the term used to describe parasite populations in studies conducted to evaluate the effectiveness of chemotherapeutants to control parasite infestations.

3. Label claims – Bowker stated that in all likelihood, parasiticides will be used to control parasite infestations rather than to control mortality caused by parasites. Gaikowski stated that in their study (control of *Gyrodactylus* on coaster brook trout), they did not detect a significant difference in mortality between treated and control groups, but did detect a significant difference in the number of parasites between the groups.
4. Treatment efficacy – Bowker stated that in all likelihood, a statistical difference between parasite density in treated and control fish, and a reduction of parasite load to a pre-defined threshold, will be needed to demonstrate treatment efficacy. Discussion of this topic included: What is a biological threshold for a given parasite (i.e., how much of a load can be tolerated before fish health is compromised or clinical signs (e.g., flashing, elevated mucus production))? Nobody had an answer to this question – and it will likely be a case-by-case situation; Dick Endris (Intervet/Schering-Plough Animal Health Corp.) asked if the pathology associated with the presence of a specific parasite was known. According to terrestrial guidance documents, a reduction of 90% + a statistical difference is required to demonstrate treatment efficacy. However, for fish it might depend on the drug used and the parasite in question.

Jen Matysczak (CVM Aquaculture Team) suggested looking at INAD data to see if anybody is reporting fish parasite numbers. Bonnie Johnson (USFWS AADAP) reported that an open ocean hatchery is the only facility using 35% PEROX-AID[®] under the USFWS INAD as a parasiticide, and they are providing parasite density data pre- and post-treatment. Maren Tuttle-Lau (USGS-UMESC) suggested contacting the USFWS La Crosse Fish Health Center (LFHC) because they were doing parasite counts on samples sent to them via the wild fish health survey (WFHS) program.

For clarification, Becky Lasee (LFHC Project Leader) said during the meeting that she has utilized University of Wisconsin – La Crosse grad students to count parasites on fish submitted to the LFHC as part of the WFHS program, but counts are done as part of a broader project on parasite ecology (e.g., identifying populations of fish based on parasite communities, host switching) but not in an effort to correlate parasite density and fish health – Lasee offered to help in any way she could.

It was suggested that we should reach out to fish health professionals to get them involved to collect count data (i.e., density). Drew Mitchell (USDA-SNARC) commented that diagnosticians do not routinely count parasites, but rather make an assessment whether the infestation warrants treatment or not. It was also noted that sick fish typically are infested with multiple parasites; when assessing parasite loads, the diagnostician may simply note whether the infestation is mild, moderate, or severe. Moribund fish often have heavy parasite loads, especially if they are reared in outdoor ponds. Bowker commented that *Trichodina* were found on moribund fish sampled during a field trial to evaluate the effectiveness of AQUAFLO[®] to control mortality in coho salmon due to furunculosis. At the end of the study, Dr. Joy Evered (USFWS Olympia Fish Health Center) pulled moribund and healthy-appearing fish, examined them for parasites, and found that the infestation levels were similar between groups of moribund and healthy fish. Mitchell stated that fish-to-fish variability with respect to parasite densities may confound the decision of whether to treat or not.

Fish tolerate some parasites (e.g., *Trichodina*) better than they tolerate other parasites (*Ichthyoptherius*, *Ichthyobodo* (*Costia*), *Chilodonella*); therefore, the treatment threshold would be dependant upon the parasite – not can not be standardized for all ectoparasites. For example, if *Ichthyoptherius* is detected at very low levels, a decision may be made to treat the population, whereas such a decision would be made not to treat if low levels of *Trichodina* were detected in some fish in a population.

Steve Sharon (WYG&F) stated that, based on his experience, most fish culturists will observe fish behavior as an indicator of whether they should treat or not (not worry about counting parasites). Bowker indicated that counting parasites will likely only apply to pivotal field effectiveness trials. Jim Brackett (Aquatic Life Sciences) suggested developing challenge models for parasites and indicated that he is aware of challenge models used for sea lice infestations. CVM reminded the group that challenge models should mimic natural infections/infestations. There was also a comment that much effort is required to maintain parasite cultures. Bowker commented that such models may be useful for pilot studies but may not be appropriate for pivotal efficacy trials.

Doug Munson (IDF&G) and Mike Mason (IA DNR) both think that, on the ground, parasiticides should reduce mortality, morbidity, and abnormal behavior. There was general agreement that pivotal studies will need to capture parasite numbers and qualitatively describe clinical signs, whereas supportive studies need only to capture reduction in clinical signs; and that potential label claims may be for reduction in parasite density but not a reduction in clinical signs. Bowker asked for a clarification from Susan Storey (CVM Aquaculture Team) about mixed infestations (infestations in which more than one parasite genus or genus species is present). Storey indicated that studies done on populations of fish with mixed infestations have greater utility if you can show a reduction in more than one of the parasite genus. In such cases, CVM will infer that a similar reduction will also be observed if the parasite genus was present by itself. Bowker commented that testing fish populations with mixed infestations may result in effectiveness technical section completion more quickly than if studies are conducted in populations of fish infested with one parasite species. The question was asked if a label claim could be for “all freshwater parasites” if researchers could determine which was the most resistant parasite species to a particular parasiticide, and effectiveness was demonstrated against that particular species. Matyszczak/Storey said that drug effectiveness is evaluated on the basis of each parasite species (or genus) included in the proposed label indication.

Bowker asked about parasite infestations mixed with bacterial infections (i.e., disease complex), commenting that it's not uncommon to see a disease complex associated with bacteria such as *Flavobacterium columnare* on fish. He asked if studies can be conducted on such a fish population. Gaikowski asked if it might be more acceptable if the bacterial infection was “low grade” and that the parasite was the primary pathogen causing mortality/morbidity/abnormal behavior. Storey indicated that this might be acceptable; however, consideration should be given to sampling a few sick fish to determine that the bacterial infection is there, and then evaluate the healthy-appearing fish to determine parasite densities and evaluate parasite density reduction following treatment. Bowker indicated that situations like the one at Iron River – where parasite infestation outbreaks are predictable based on spawning stressors - would be ideal, but such situations are either uncommon or the public data generating partners need to “beat the bushes” to find other such situations.

5. Statistical power in parasite studies – brief discussion about the need for data from pilot studies to help determine how many replicate test tanks and # fish/tank will be needed to detect treatment efficacy.
6. Parasite identification to species – Bowker posed the question about identifying parasites to genus (field diagnostics rarely ID parasites beyond genus or family) or species (likely a requirement for pivotal field effectiveness trials). Mitchell commented that there are five genera in the family *Trichodinidae* that are found in the U.S., and some species have yet to be identified (that it might be easiest to key to genus or even family); whereas there is only one major *Ichthyobodo* (*Costia*) species in N. America and three *Chilodonella* species (he indicated that it would be easy to key both parasites in either genera to species – Mitchell provided further clarification that in his diagnostic work, they have dealt with three species (*C. hexasticha*, *C. piscicola* or *cyprini*, and *C. cucullulus*). There may possibly be one or two other species (proper ID issues) in N. America and *C. cucullulus* may not be pathogenic. It is likely that differentiation between the two other species may not be necessary). Mitchell further commented that there are few people with expertise to key major parasites down to species, and that we may need to send parasite specimens to “experts” for reliable identification. Roz Schnick (National Coordinator for Aquaculture NADA) suggested we write a White Paper to describe which parasites can and should be ID’d to species and which ones can’t (and therefore shouldn’t) be keyed to species. Mitchell indicated that *Epistylis* might only be keyed to genus because a key to ID species is not available. Bowker brought up the concept of lumping genus based on similar characteristics (e.g., life cycle, mode of action, etc.). Schnick suggested a White Paper to request that similar genera be lumped. As another potential way to support lumping parasite genera, Endris suggested that it should be determined if there is a common chemical mode of action or a common receptor site affected by a chemical treatment. There was also discussion on PCR identification and whether there could be a session of the World Aquaculture Society meeting in 2010 on parasite identification (CVM commented that if parasite(s) can not be identified to species based on current techniques, then this needs to be explained in either study protocols or final study reports).
7. How many studies need to be conducted and accepted for a claim – Bowker posed the question about how many opportunities “we’ll” have to conduct studies – and nobody knows the answer to that question. There is concern that opportunities such as the one presented at Iron River and Marquette State Fish Hatchery (salmonids infested with *Gyrodactylus*) may be few and far between. Storey suggested that, depending upon drug marketing, products such as 35% PEROX-AID® can be used extra-label by a licensed veterinarian following the provisions of 21 CFR 530. There was also a comment that it might be best to conduct pivotal efficacy studies first (and ID parasites to species if possible) and then develop “lumping” white papers and other white papers. Storey also indicated that the terrestrial model for lumping parasites might not transfer well to aquaculture. It was suggested that to present an argument to reduce the number of efficacy studies required to complete a claim for a particular parasite for all freshwater finfish, we should use the argument that ectoparasites utilize the fish body surface as a substrate and if a drug is effective in reducing parasite densities on one or more finfish species, it should be equally effective in reducing densities of the same parasite in all finfish species. Gaikowski commented that it may be best to get a claim for a few parasites, and then let veterinarians prescribe extra-label. Schnick agreed – just need to get one approval. Bowker asked how much a veterinarian might charge to prescribe extra-label use, and indicated that the cost of the veterinarian would likely exceed the

cost of treatment (implying that a hatchery might not go the extra-label route due to cost). Pat Gaunt (MSU-CVM) said that for her to prescribe extra-label use for a product like 35% PEROX-AID[®], she would charge for an office visit.

8. Enumeration – Bowker presented the issue of quantifying parasites and the concept of Too Numerous To Count (TNTC), and Todd Blessinger (CVM Biometrics Team 2) lead most of the discussion. Quantitative (count every parasite) will be easiest to analyze. However, the concept of TNTC needs to be addressed (and defined). TNTC needs to be a large enough number so that significant differences can be detected. Blessinger hadn't had much experience with, and therefore wasn't comfortable with, TNTC - until he got more involved in the Iron River study, where 2,000 – 3,000 *Gyrodactylus* were counted on one slide. He understands that a concept such as TNTC needs to be looked into. Can TNTC be utilized in a semi-quantitative approach? Such an approach will need 3 – 5 categories that include TNTC and we will need to establish the TNTC threshold. Blessinger would like "TNTC" to occur infrequently. Mitchell stated that, based on his experience, utilizing a concept such as TNTC is not a rare occurrence.

Mitchell brought up a proposed 60:60 method for parasite enumeration - in which counts are made until 60 parasites are found or 60 seconds has elapsed. The process requires two participants – an evaluator and a timer/data recorder. Gaikowski asked Blessinger if time to count 60 parasites could be used as a covariate (time it takes to count 60 may be different depending upon density and could be used to more accurately reflect parasite density). Mitchell indicated that for parasites such as *Costia*, their movement is critical, and that *Costia* begin to die within about 5 minutes of preparing a wet mount. Bowker asked if different counting methods should be used for different parasites. He also asked Mitchell to write up the 60:60 procedure and provide it to CVM for review. Mitchell also suggested that other diagnosticians "field validate" this method. CVM is open to the 60:60 procedure, but good justification needs to be provided to support the use of the procedure.

9. Distribution of parasites on host – Bowker commented that the group will need to conduct pilot studies to generate baseline data to establish standard sampling procedures. Gaikowski commented that the procedures will likely be parasite specific. Standard sampling procedures should be representative of the parasite distribution on fish.
10. Sampling schemes – Bowker briefly discussed some of the issues relative to sampling schemes, such as sampling live fish vs. dead fish, sampling live fish w/ and w/o replacement, effect of fish density in a tank if fish are sampled w/o replacement (a sufficient number of fish need to be placed in each experimental unit so that sampling fish w/o replacement does not become a variable), sampling different areas of fish at each sampling event, how to deal with mortalities. Consider utilizing a similar sampling schedule as that used by Gaikowski and Tuttle-Lau – sample on posttreatment day 1 and 14.
11. Utilizing vet (or pre-vet) students to help conduct pilot studies – There was mention that there are probably such students who would be willing to help generate some of the pilot data to enhance their resume as they near completing their degree. Bowker will follow up with Storey, Gaunt, and Lester Khoo (MSU-CVM; AVMA) to further investigate the feasibility of utilizing vet students.

The meeting was adjourned at 10:15 am

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