

Field effectiveness of Aquaflor® and
Terramycin 200 For Fish® to control
mortality in coolwater and warmwater
finfish due to motile Aeromonad infections.

RECORD

Terramycin 200 For Fish®

Aquaflor® Logbook

INAD # 11-902

INAD # 11-306

WAE #5
LOGBOOK

<u>Printed Name</u>	<u>Signature</u>	<u>Initial</u>	<u>Date</u>
Aaron R. Cupp	<i>Aaron R. Cupp</i>	ARC	15 JUL 11
Susan M. Schlein	<i>Susan M. Schlein</i>	SUS	18 JUL 11

Abbreviations

D.O. = dissolved oxygen

WAE = walleye

SLFH = Spirit Lake Fish Hatchery; 122 25th Ave, Spirit Lake, IA, 51360

712-336-1840

RW = raceway

Equipment Abbreviations

WC-1 Hach Water Chemistry meter (pH/D.O./temp) Model HI9142A;
S/N 081100026343

BAL-1 Sartorius Model LP34000 IP; S/N 14006043

D.O.-2 YSI D.O. Meter, MODEL 55/12FT, S/N

pH-3 HANNA pH meter, MODEL

15 JUL 11 Experimental WAE were distributed throughout 10 raceways by SLFH personnel. Water quality consisting of: dissolved oxygen, pH and temperature were taken at the bottom end of raceways by use of an externally calibrated meter and recorded on data sheets. Feed (florfenicol, oxytetracycline dihydrate and controls) was prepared and allocated to study raceways on 14 JUL 11 according to exact procedures described in AEH-09-MAS-02 #1 page 1. Presumptive diagnosis was made before Day 0 feeding by hatchery manager. Kidney samples/stabs were taken from necropsied fish by hatchery manager and quadrant streaked on TSA plates and incubated at 30.0°C. Following presumptive diagnosis and tank cleanings, pre-allocated feed was distributed to shake-feeders by SLFH personnel. Presumptive samples were transported to UMESC by study personnel. Feedings for 16 JUL 11 were measured on a calibrated balance and designated for destination tanks. Methanol was used to clean weighing area between different test chemicals. Source feed was stored in an air-conditioned room to limit excessive humidity and temperature exposure. 15 JUL 11 ARC →

16 JUL 11 Water quality (pH, D.O, and temperature) were measured and recorded from an externally calibrated Hach brand meter. Measurements were taken at the lower end of the tanks. All tanks were drained

16 JUL 11 cont

and cleaned by SLFH personnel. SLFH personnel also collected and recorded mortalities. Feed, which was pre-weighed and assigned, was added to feeders for its 24h feed cycle. Feed was assigned on 15 JUL 11 and measured on a Sartorius LP 34000 IP balance.

ARC sampled 2 mortalities from tanks 11-18 to culture MAS. Tanks 19 and 20 were not sampled due to zero mortalities in those tanks. Sampling consisted of recording weights and lengths, assessing exterior and interior conditions of mortalities, using a loop to streak a sample from the posterior kidney on TSA media plates and incubating plates at 28°C for 48h. Feed for 17 JUL 11 was weighed-out using the Sartorius LP 34000 IP balance following its internal calibration and verification. Note that during sampling, instruments and tools were disinfected between samples to assure aseptic techniques were followed. 16 JUL 11 ARC

17 JUL 11 SLFH cleaned tanks, collected and recorded mortalities, confirmed a flow rate of two tank exchanges per hour, and added feed measured out on 16 JUL 11 to 24h feeders. Tank 20 mortalities were taken for samples. Two fish from tank 20 mortalities were kidney sampled and streaked to TSA media in the same manner as on 16 JUL 11. Samples from 16 JUL 11 were assessed for colony growth. Sufficient growth for pure culturing at a later date, was present

17 JUL 11 Cont and samples were moved from incubator to refrigerator at 24h instead of the original 48h incubation period. Feed for 18 JUL 11 was weighed out and assigned to tanks using same procedure used in previous days. ARC measured and recorded pH, D.O. and temperature following the Hach meter external calibration.

17 JUL 11 ARC

18 JUL 11 Feed for 19 JUL (DAY 4) was weighed out on BAL-1, which had been calibrated and verified. Original bags were first weighed; the tank's labeled feed container was placed on the balance and tared. Feed was scooped into the appropriate container with a labeled scoop and the weight was recorded on the Feed Weight Recording Form. The container was removed from the balance, balance was tared and the original bag was weighed. Because of all the handling, the seams on the bottoms of the bag were starting to let loose resulting in some feed loss. Duct tape was used to repair leaks as needed. The labeled feed containers were transported to the hatchery for feeding tomorrow. The balance and counters were wiped down with methanol after weighing, and the floors were swept to remove feed. 18 JUL 11 SMS

18 JUL 11 Water quality (pH, D.O. and temperature) was conducted on all test tanks and recorded from the Hach brand meter following an external calibration. Mortalities were collected and recorded by SLFH. SLFH also cleaned all test tanks by sweeping all waste to the drain. Kidney samples were taken from tank 19 mortalities and streaked to TSA plates. Plates were placed in a 28°C incubator for a

18JUL11 24 h incubation period. Samples from 17JUL11 ~~we~~ showed sufficient growth for pure culture and were placed in a refrigerator to inhibit further growth. 18JUL11 ARC →

19JUL11 SLFH collected mortalities and cleaned raceways. SLFH also verified flow rates of two tank exchanges per hour. Water quality was conducted using a calibrated WC-1 and pH, N.O. and temperature was recorded. No kidney samples were taken. Kidney samples from mortality WAE will be sampled on 21JUL11, 19JUL11 ARC →

19JUL11 To decrease the loss of medicated feed from the paper storage bags, remaining feed from TM-1 and FCC-1 was transferred to labeled buckets with sealed tops. Weights are recorded in ^{wrong} ~~log book~~ ^{19JUL11} ~~SMS~~ ^{SMS} respective log books. When the feed from TM-1 and FCC-1 in the buckets was used up, TM-2 and FCC-2 were retrieved from the locked temperature/humidity controlled storage building. Each of the bags had aliquots removed into the labeled buckets to decrease bag deterioration. The weights were recorded in the log books. The buckets of feed were then used for weighing out the daily feed aliquots with weights recorded on the Feed Wt Recording form (FF 7). All weights were measured on calibrated BAL-1. Control feed continued to weighed from the original feed bag. Feed samples for analysis were also weighed out. Approximately 600-700 g of TM-2 and FCC-2 were removed from the original bags, (1 scoop top, 1 scoop middle and 1 scoop bottom). Each

bag's aliquots were weighed into a tared gal. size hip-loc, shaken to combine, and then split into 2 labeled qt. size hiplocs. One sample will be sent to Eurofin for analysis and one will be retained at UMESC from each bag (TM-2 + FCC-2). Bags will be stored @ ambient temperature until the feed aliquots weighed out today are placed in the feeders. At that time they will be placed in the freezer until shipment to Eurofin and transfer to UMESC. Additional note: 18JUL11 sample plates remain in the incubator @ 30° for an additional 24 hr. 19JUL11 SMS →

20JUL11 Mortalities were collected, assessed and recorded by SLFH. Prior to cleaning, water quality was measured using the calibrated WC-1 and pH, N.O. and temperature were recorded. No additional kidney samples will be taken on mortalities until 20JUL11. Samples taken from tank 19 mortalities from 18JUL11 were transferred from incubator into a refrigerator. All samples were then taken out of the refrigerator and boxed into shipping coolers which contained shipping packs to maintain cool temperatures. Samples were then transported to UMESC where they will be placed into another refrigerator. SLFH added feed to feeders for all tanks, prepared 19JUL11. Feed for 21JUL11 was weighed out on calibrated and verified BAL-1. Source feed came from buckets taken from Tm200-2 and FCC2. (see 19JUL11 entry for details). 20JUL11 ARC →

21JUL11 Feed aliquots for the WAE for 22JUL11 were weighed see next page 21JUL11 SMS

21JUL11 Water quality was measured prior to tank cleanings and pH, D.O. and temperature readings were recorded on data sheets. D.O. was measured using the WC-1 and recorded; however, the pH portion of the WC-1 would not calibrate. pH was measured and recorded using the calibrated HI 991001 pH meter. SLFH personnel cleaned tanks and collected mortalities. ARC sampled two mortalities from tanks 11 through 18. One sample was taken from tank 19. Zero samples were taken from tank 20 due to lack of mortality. Sampling consisted of recording mortalities length and weights. Next, physical observations, internal and external were assessed. Then, an incision was made along the abdomen to expose the kidneys. Using aseptic techniques, a loop was used to plate out a sample from the posterior kidney on TSA plates using the quadrant streaking method. Plates were then incubated at 30°C and growth checked on 22JUL11. Flow rates of test tanks were verified at two tank-exchanges per hour by SLFH. 21JUL11 ARC

21JUL11 Feed aliquots for the WAE for 22JUL11 were weighed out from the buckets containing FEC-2 and TM-2. Tanks 11 and 16, which were receiving control feed, are being switched to medicated feed. Tank 11's aliquot was from the flocculent

21JUL11 (cont.) Bucket and Tank 16 will receive TM-200. All weights were recorded on the Feed Wt Recording form 21JUL11 SMS

22JUL11 Water quality consisting of pH, D.O., and temperature was measured and recorded prior to sampling. pH was measured using an externally calibrated HI 991001 pH meter. D.O. was measured using a calibrated DO-2 meter. Mortalities were collected and tanks were cleaned by SLFH technicians. One mortality from tank 19 and two samples from tank 20 were sampled using procedure described on 21JUL11 entry (Page 6). Samples in incubator taken on 21JUL11 were assessed for growth and will be removed from incubator on 23JUL11. Feed was added to feeders by SLFH. 22JUL11 ARC

22JUL11 The feed aliquots for 23JUL11 were weighed out on BAL-1. RW11 received FEC-2 feed and RW-16 received TM-2 feed due to rising mortalities in the designated control tanks of production feed. 22JUL11 SMS

23JUL11 D.O-2 and pH-3 were calibrated and used to measure D.O., temperature and pH in tanks 11-20 at the outflow end of each tank. Hatchery personnel cleaned tanks and collected mortalities, which were assessed for disease, injury, or cannibalization by the hatchery. The feed weighed out by UMESC personnel yesterday was put into the feeders by hatchery personnel. Feed for 24JUL11

23 JUL 11 (cont.) was weighed on BAL-1 into labeled pitchers. Bag 3 of the TM-200 and ~~Aquaflor feed~~ ^{wrong 23 JUL 11} SNS medicated feed were opened and used for the WAE. 23 JUL 11 SNS →

24 JUL 11 DO-2 and pH-3 were calibrated and used to measure the temperature, DO, and pH in WAE ~~font word~~ ^{wrong 24 JUL 11} SNS raceway 11 → 20. Hatchery personnel cleaned tanks, assessed mortality, and filled feeders with the aliquots that were weighed out on 23 JUL 11. Medicated feed for tanks 11 (FFC) and 16 (TM-200) was weighed out on BAL-1 into pitchers labeled with the respective raceway number. 24 JUL 11 SNS

25 JUL 11 Mortalities were sampled in each tank on the first day post-dosing. BAL-2 was calibrated and used to record weights of all sampled fish. Lengths were also recorded. External and internal characteristics were also recorded. An incision was made along the abdomen to expose the posterior kidney. Using a loop and aseptic ~~th~~ ^{mis-spelled} techniques, samples were quadrant streaked to TSA media and incubated at 30°C for 48h. 25 JUL 11 ARC →

~~25 JUL 11 wrong date 25 JUL 11 SNS~~ →

26 JUL 11 D.O. 2 and pH 3 were calibrated and used to measure the temperature, D.O. and pH in each raceway. Hatchery personnel cleaned the raceways, counted and assessed the mortalities. Hatchery personnel weighed out unmedicated feed for all raceways except RW 11 and RW 16. The feed weighed out yesterday for those 2 raceways was placed in the feeders by hatchery personnel. Medicated feed for RW 16 and RW 11 for tomorrow was weighed out on calibrated BAL-1. 26 JUL 11 SNS →

~~25 JUL 11 SNS~~ ^{wrong word} Repeated ~~25 JUL 11~~ SNS went from Davon's 25 JUL 11 to 26 JUL 11 but it's really 25 JUL 11 SNS

26 JUL 11 SNS - Calibrated meters were used to check and record the water chemistry in RW 11 → RW 20. Feed for RW 11 (FFC) and RW 16 (TM-200) was ~~weighed out for 27~~ ^{wrong - forgot word 26 JUL 11 SNS} not weighed out as the hatchery intended to put all the fish back on medicated feed. 26 JUL 11 - SNS →

27 JUL 11 D.O. 2 and pH 3 were calibrated and used to measure the temperature, DO and pH in each raceway. Hatchery personnel cleaned tanks, counted and assessed the mortalities. The hatchery personnel weighed out all feed for the raceways; the FFC medicated feed prepared by UMESC personnel yesterday was fed to the tanks previously treated with FFC feed. RW 16 was fed TM-200 medicated feed. The other tanks ~~fed~~ ^{wrong 27 JUL 11} SNS that had previously fed on TM-200 feed were given appropriate amounts of control feed. 27 JUL 11 SNS

LATE ENTRY FOR 28 JUL 11 - due to relying equipment to UMESC personnel - Temperature, D.O., and pH readings were taken with calibrated DO-2 and pH-3. The hatchery personnel cleaned raceway, counted and assessed mortalities and weighed out medicated and unmedicated feed for each raceway. See data sheet for feed assignments and weights. 29 JUL 11 SNS →

29 JUL 11 D.O.-2 and pH-3 were used to measure temperature, D.O. and pH in each tank (raceway) after calibration. Hatchery personnel cleaned the raceways, counted and assessed mortalities. They also weighed out medicated feed for each tank, either OTC or FFC according to previous tank assignment, utilizing feed prepared by UMESC personnel on 26 JUL 11. See data sheet for weights. 29 JUL 11 SNS →

30 JUL 11 All WAE tanks were switched to TM-200 medicated feed today by hatchery personnel. They also cleaned tanks and counted masts. Calibrated DO-2 and pH-3 were used to measure temp, D.O. and pH in RW 11 only. 30 JUL 11 SMS →

31 JUL 11 Nothing to report 31 JUL 11 SMS

1 AUG 11 All WAE continue on TM-200 medicated feed. Water chemistry was done in RW 12 only. 1 AUG 11 SMS →

02 AUG 11 Two mortalities from each tank were sampled. Weights were recorded from a calibrated BAL-2. Lengths, external characteristics and internal characteristics were recorded. A loop was used to take a sample from the posterior kidney and quadrant streak it to TSA media. Plates were incubated at 30°C for 48h.

02 AUG 11 ARC →

LABORATORY NOTEBOOK



AEH-09-MAS-02

TRIAL 2

Field effectiveness of Aquaflor[®] (florterenol) and Terramycin[®] 200 For FISH (oxytetracycline) to control mortality in cool water and warm water finfish due to Motile Aeromonad Sepsisemia

Principal Investigator: Maren Tuttle-Kau

Supervisory Biologist: Mark Gaikowski

<u>Printed Name</u>	<u>Signature</u>	<u>Initials</u>	<u>Date</u>
Susan M Schleis		SMS	8 JUN 12
Michael P Wellens		MPW	6/21/12

Abbreviations

SLFH = Spirit Lake Fish Hatchery; 122 25th Ave, Spirit Lake, IA, 51360
712-336-1840

WAE = walleye

FFC = fluorinated

OTC = oxytetracycline

TL = total length

Equipment Abbreviations

BP-BAL = Sartorius BP 3100 S, S/N 12907582

LC-BAL = Sartorius LC 34000P, S/N 30303922

BAL-1 = CORE Model CPT 2601, S/N AEH75502109

DO-2 = YSI 55/12 FT, S/N 07G101523

PH-1 = Beckman Φ 250, S/N 2496

THERM-1 = AEH 027

DO-1 = YSI 55/12 FT, SN 07G101308

LABORATORY NOTEBOOK

Notebook No.: #2

Assigned to: AEH-09-MAS-02

Date: 8JUN12

Use Thermo Scientific Nalgene Cat. No. 6302-1000 to reorder.

Printed in USA

©2010 Thermo Fisher Scientific Inc. All rights reserved.

9 JUN 12 FEED PREP: BP-BAL and LC-BAL were calibrated. Aquaflo, LOT 035184, Bag 1 of 1 was removed from locked storage. 1.30g was weighed into a tared cup on BP-BAL. The labeled cup was sealed in preparation for transport to the feed mixer. Terramycin 200 For fish, LOT N1011024XF was removed from locked storage. A labeled tared cup was used on LC-BAL to weight out 8.0g of the oxytetracycline. Appropriate amounts of oil (~60g) were weighed on LC-BAL into labeled cups. Before the chemicals were weighed, the feed was weighed. There were (2) 10 kg bags of OTONIME EP-1, S/N 2012 3 451, labeled 1 of 2 and 2 of 2. Feed for use as medicated feed was taken from BAG 1 of 1. Bags (2 gal Ziploc) were labeled for OTC and FFC. LC-BAL was used to weigh out ~3 kg into each bag. The oil cups were heated for ~30 sec. Then the oil, chemical and feed aliquots were transported to the feed mixers. The original containers of Florfenicol and Terramycin were transported back to secure storage. The cleaned mixers were allowed to dry in the sun for ~2 hrs before beginning. Some of the OTONIME had been weighed out on BAL-LC to flush each mixer. This was added to the mixer, and allowed to mix for ~10 min. The flush feed was discarded. The FFC and OTC feed batches were mixed in the same manner but in separate, labeled mixers. First the aliquot of feed was placed in the mixer. The mixer was turned on, and the chemical was poured onto the feed. Small amounts of feed were scooped into the cup to shake loose all the medication. 3 times. The feed was mixed for 1-2 min and then the warm vegetable oil was sprayed onto the feed for ~2 min. The medicated feed was allowed to mix for 3-4 min. The mixer was then emptied into a large bag, and the large bag was emptied into the labeled 2 gal Zip-loc. Medicated feed was locked up, along with the Otonime BAG 1 of 2 and 2 of 2. For weights, times, see FF7. 9 JUN 12 SMS
NOTE: A small amount (<5g) of the FFC medicated feed was spilled during transfer from the mixer. Also, the entire amount of oil for each batch of feed was not used as the spray bottle doesn't empty completely. 9 JUN 12 SMS →

LATE ENTRY FOR 12 JUN 12 - recorded on 13 JUN 12 due to lateness of hour on 12 JUN 12
Tanks and equipment were transported to Spirit Lake Fish Hatchery on 12 JUN 12. Before leaving WMEC, the control feed was weighed out on calibrated LC-BAL for the contaminant analysis. (2) 2 kg aliquots were weighed into labeled 2 gal Zip-loc bags and placed in the Ultra-Cold freezer to be shipped for analysis. The remaining control

Continued on Page 2

Read and Understood By

Susan M. Schlein

Signed

13 JUN 12

Date

Signed

Date

(12 JUN 12 entry recorded on 13 JUN 12, cont.) feed was then weighed out into containers so that the weight was less than 1800 g. This was done because the balance that will be used in the field has a limit of 2100 g. A total of 5 containers were used to contain the feed. See FF 7 for actual weights. The medicated feed that was prepared on 9 JUN 12 was also broke down in the same way except that 2 containers were used for the OTC medicated feed and 2 containers were used for the FFC medicated feed, each containing < 1025 g, with ~1000 g remaining in the original bags. Feed was secured in the mobile lab for transport. ~~Upon~~ ^{wrong} ~~and~~ ^{13 JUN 12} ~~mis~~ For actual weights see respective feed log book. Upon arrival at site, the tanks were set up on cement blocks in a space close to electrical outlets, and trenches for outflow. In-flow and outflow pipes were attached, airline and air stones set up and connected to air pumps, one for each tank. The in-tank lights and feeders were all wired and connected. 13 JUL 12 SMS

13 JUL 12 The black plastic enclosure was assembled by building a frame from 1/2" PVC pipe which was hung from the rafters by means of rope. Black 6 ml plastic was cut into sheets of appropriate size to assemble the 'roof' and walls. The plastic was attached to the frame by means of clips, clothespins, and duct tape. 13 JUL 12 SMS

14 JUL 12 The WAE were held in 2 tanks (11 + 20) and were transferred to 9 additional tanks for grow-out so that each tank held ~ 3100 fish (3148 to 3222). Hatchery personnel also did a check-weigh, resulting in a mean weight of 2.8 g. The P.I. performed the pre-distribution assessment by netting indiscriminately a group of fish from the source tanks. The fish were euthanized by an overdose of MS-222. Fish were measured for TL to the nearest mm and weighed on BAL-1. Each fish was examined externally and internally. A kidney stab was performed and streaked on a TSA plate. Plates were transported back to UMESC for incubation by the P.I. Flow rates for each tank were measured and adjusted to 600-750 mL/min. 14 JUL 12 SMS

15 JUL 12 The hatchery decided to treat their fish with medicated feed and DIQUAT to counteract the stress from the transfer process yesterday so the fish needed for the study were distributed to the test tanks. 3 pieces with the net were made in one of the hatchery tanks, and the netted WAE

Continued on Page 3

Read and Understood By

Susan M Schlier

Signed

15 JUL 12

Date

Signed

Date

15 JUN 12 (cont.) were put in a 5 gal of culture water. The pail was carried over to the test tank area. A small net was used to indiscriminately scoop out a group of fish which were then counted into the tank designated by the S&S generated mass distribution sheet. Fish were counted into the tanks in groups of 10 or less, although a fish occasionally jumped into the tank from the net after it had been counted into the tank. Fish were taken from each of the 10 hatchery source tanks to maintain their similar population numbers. Distribution rounds were continued until 63 fish were in each test tank. Temperature, pH, and DO were taken in the source tanks and in 6 of the test tanks immediately after distribution. The feeder timers were set and the lights were adjusted. Control feed was weighed out on BAL-1, ~12.3 g per tank for a feeding rate of 7% BW. The distribution process was completed at 10:25, the feeders were filled and the timer was set to go off the first time at ~1300. The fish calmed down quickly and acted normally within 30 min of placement. When the feeders dropped feed into the tanks, fish were observed feeding. Control feed was returned to secure storage. 15 JUN 12 SWS

16 JUN 12 pH-1 and DO-2 were calibrated, and along with THERM-1 used to check pH, DO, and temperature in tanks A1-A9, and B1-B9. All tanks were cleaned by siphoning. Some of the feeder collars had loosened so they were adjusted as needed. The feeder's timer was adjusted down to a 2 sec vibration, as the 5 sec interval shook out too much feed, even with the single opening. No mortalities or morbidities were observed. BAL-1 was calibrated and used to weigh out 12.3-12.4 g of feed for each tank. Feed was placed in each tank's feeder. Fish were observed feeding. 16 JUN 12 SWS

17 JUNE 12 pH-1 and DO-2 were calibrated. The meters and THERM-1 were used to check water chemistry in each tank. Tanks were cleaned by drain and siphoning. One morbid fish was removed and cultured by stabilizing the kidney and inoculating a TSA plate. BAL-1 was calibrated and used to weigh out 12.3-12.4 g of feed for each tank. 17 JUNE 12 SWS

18 JUN 12 THERM-1 along with calibrated pH-1 and DO-2 was used to measure water chemistry in all tanks. Tanks were siphoned and drained to clean. BAL-1 was calibrated and used to weigh 12.3 to 12.4 g of feed. Feed was placed in each feeder. Fish were observed feeding. 18 JUN 12 SWS

Continued on Page

Read and Understood By

Susan M. Schlein

Signed

18 JUN 12

Date

Signed

Date

19 JUN 12 pH-1 and DO-2 were calibrated. Tank B6's inflow had become clogged overnight so that the flow to the tank was severely decreased. All WAE in that tank expired. No other mortalities were observed. Flow to the other tanks was increased after water quality was measured. Tanks were drained and siphoned. BAL-1 was calibrated and used to weigh out 12.3 to 12.5 g of food for each tank. Fish were observed eating. 19 JUN 12 SWS →

20 JUN 12 pH-1 and DO-2 were calibrated and used to measure water quality in each tank, along with THERM-1. 1 mortality was removed but was too decomposed to culture. Tanks were cleaned by draining and siphoning. BAL-1 was calibrated and used to weigh out 12.4-12.6 g of food for each tank. 20 JUN 12 SWS →

21 Jun 12 pH-1 and DO-2 were calibrated and used to measure water quality in each tank, as well as temp-1. Tanks were cleaned by draining and siphoning. BAL-1 was calibrated and used to weigh 12.4-12.6 g of food for each tank. 21 Jun 12 MPW

22 Jun 12 pH-1 and DO-2 were calibrated and used to measure water quality in each tank, as well as temp-1. Tanks were cleaned by siphoning. BAL-1 was calibrated and used to weigh 12.4-12.6 g of food for each tank. Walleye grower was mixed with original feed in a 50/50 combination for food. 22 Jun 12 MPW

23 Jun 12 pH-1 and DO-2 were calibrated and used to measure water quality in each tank, as well as Temp-1. Tanks were cleaned by siphoning and drainage. 12 mortalities from A6, 3 mortalities from A2, and 2 mortalities from A9 were removed and plated. BAL-1 was calibrated and used to weigh a 50/50 mixer of feed and walleye grower of 12.4g-12.6g for each tank. Filter was cleaned and siphoned. 23 Jun 12 MPW

Continued on Page

Read and Understood By

Mind 6/23/12

Signed

Date

Signed

Date

PROJECT _____

Continued From Page _____

24 Jun 12 pH-1 and DO-2 were calibrated and used to measure water quality in each tank, as well as Temp-1. Tanks were cleaned by siphoning and drainage. 4 mortalities from A9, 3 mortalities from A6, 3 mortalities from A2, 4 mortalities from B2, and one mortality from B5 were removed and plated. BAL-1 was calibrated and used to weigh a 50/50 mixture of feed and walleye grower and 12.4 - 12.6g were given to each tank. Jun 24-12 MPW

25 JUN 12 pH-1 and DO-2 were calibrated and used with THERM-1 to measure water quality in each tank. Tanks were cleaned by siphoning and draining. Mortalities were counted and removed but not plated due to the arrival of the PI who would do the disease assessment. Two fish were removed from each tank except B6 which had no fish for a total of 34 fish. BAL-1 was calibrated and used to weigh each fish and it was measured for TL. A kidney stab was performed on each fish and plated on TSA. The 34 fish were not identified as to tank - all 34 were netted into the same pail as a representative population sample. The number of fish in each tank was calculated by subtracting the total number of morts and the 2 disease assessment fish from the original 63/tank distributed on 15 JUN 12. ^{25 JUN 12 5:11 wrong} The count ^{revised} 3 fish from each tank except 4 from A2, A8, B3, B5 (chosen randomly by drawing a slip of paper) were transferred to B6 to populate it for a total of 54 fish. Other than B6, each tank's original number was reduced to 50 by netting an appropriate number of fish into a pail of culture water. For example A1 had 63 fish, no morts, and 2 removed for disease assessment for a total of 61 so 11 fish were netted into the pail. A2 had 63 - 9 morts and 2 disease assessment fish for a total of 52 fish so 2 fish were removed to the pail. Tanks A3 and A6 had less than 50 so no fish were removed. All the A tanks were placed together in a pail and all the B tanks were placed in a pail. When all fish were collected the pails were combined. Then starting with the base number (A1=50), fish were added to each tank to equal 54 (A1 receive 4), and then a second round of 2 was added to each tank. On a few occasions an extra fish jumped into a tank and remained but a note was made. 3 fish jumped

Continued on Page 6

Read and Understood By _____

Jessie M. Gable 25 Jun 12

Signed _____ Date _____ Signed _____ Date _____

into the floor and were discarded. 3 fish remained in the pooled fish and were discarded. Every effort was made to avoid as much stress to the fish as possible. The weight obtained from the disease assessment was used to determine the tank's biomass (4.0 g/fish). An error was made by using 55 (rather than 54 fish/tank) so each tank received 15.4 ± 0.1 g of its assigned treatment code (blinded data generated by SJS; only viewed by lead tech who weighed feed). FFC and OTC medicated feed aliquots were removed (~400 g ea, then split into ~200 g ea) from the feed container by weighing into a tared, labeled Ziploc on BAL-1. The A+B aliquots of each feed batch were taken back to UMESC by the PI to be placed in the ultracold freezer until shipment for concentration verification analysis. After this was completed (see respective logbooks for actual weights), the feed was weighed on BAL-1 for each tank (FF12b). Feed was placed in the feeders between 1:45 and 1:55 pm. A few mortalities were noted after feed was put into the feeders (A2=3, A5=1, B6=1) and were removed. When feed fell into the tanks, fish fed avidly. 25 JUN 12 SJS

26 JUN 12 Feed weights placed in feeders yesterday were based on 55 fish per tank, with a mean wt of 4.0 g per fish. All tanks received $15.4 \text{ g} \pm 1$ of the type of feed assigned to the tank, due to the necessity of getting the medicated feed to the fish as soon as possible after necessary staff arrived on site. Much later that evening a spreadsheet was developed to calculate the number of fish that should be in each tank using all available data sheets. Several changes appeared in the expected numbers due to inaccurate addition (A4 originally received 71 fish during the 1st distribution on 15 JUN 12), some missed morts and additional morts that occurred shortly before feed was placed in the feeders. Due to discrepancies, it was decided to physically count 3 tanks' populations, 1 from each treatment group. The tanks that had no mortalities today were written down on slips of paper, and placed in groups according to treatment assignment. The staff who is unaware of treatment assignment selected 1 slip from each group and gave them to the person who then counted the fish in each of the selected tanks. One tank had 1 more fish than the calculated #, 1 had one less, and one was correct.

Continued on Page 207

Started to write
date 26 JUN 12 SJS

Read and Understood By

Susan M Schli

26 JUN 12

Signed

Date

Signed

Date

26JUN12 (cont.) Since the numbers were close, and the need to avoid stressing the fish to avoid unintentional mortalities great, the calculated population numbers will be used for further feed calculations, other than the 2 counted tanks whose actual numbers were used in the calculations. The tank number of fish was put into the Excel calculation sheet, along with the mean wt (4.0) and estimated daily wt gain per fish (0.11g) to derive the feed weight needed to give the proper dose at a 7% BW feed rate for each tank. 26JUN12 SMS

26JUN12 DO-2 and pH-1 were calibrated. Mortalities were counted and removed to containers labeled with the appropriate tank number. Each mort was weighed on BAL-1 and measured for TC. Selected mortalities were necropsied, and a kidney stain was plated to TSA. Water quality (temp, DO, pH) was measured in each tank. Tanks were drained and siphoned to clean and uneaten feed was observed. Feed was weighed out on BAL-1 according to the Feed Wt Calculation Sheet and placed in the feeders between 1 and 2 pm. Afternoon mortalities were removed and processed. 26JUN12 SMS

27JUN12 DO-2 and pH-1 were calibrated. Temperature, DO and pH were measured in each tank. Mortalities were counted and removed. Selected mortalities were necropsied and a kidney stain was plated to TSA. Fin clips, skin scrapes, and gill arches from some mortalities were examined under 40x on the microscope. Each tank was drained and siphoned. Uneaten feed was assessed. BAL-1 was calibrated. Tanks were checked in the afternoon for mortalities. Feed aliquots for each tank were weighed on BAL-1 according to the Feed Weight Calculation Sheet, and placed in the feeders between 1 and 2 pm. 27JUN12 SMS

27JUN12 cont. Additionally, all in-flow valves were opened to flush the lines until the in-coming water ran clear. Flow rates were then measured and adjusted to 660-900 ml/min. 27JUN12 SMS

28JUN12. Mortalities were counted and removed. BAL-1 was calibrated and used to weigh each mortality, which was then measured to TC. DO-2 and pH-1 were calibrated. Temperature, DO, and pH were measured in each tank. Tanks were cleaned by draining and siphoning. Feed consumption was assessed. Tanks were checked for mortalities again in the afternoon.

Continued on Page 8

Read and Understood By

Susan M. Schlein

28JUN12

Signed

Date

Signed

Date

28 JUN 12 (cont.) Feed aliquots for each tank were weighed out for each tank and placed in the feeders between 1 and 2 pm. Fish were observed feeding in every tank. 28 JUN 12 SMS →

29 JUN 12 Mortalities were counted, removed and processed as necessary (weight and TL on all, necropsied and kidney stab cultured on TSA, or microscopy). BAL-1 was calibrated before use. DO-2 and pH-1 were calibrated. Temperature, D.O and pH were measured in each tank. Tanks were cleaned by draining and siphoning, and tanks were assessed for uneaten feed. very little was seen. Mortality numbers were recorded in the Feed Weight calculation sheet and feed aliquots were weighed out for each tank. Tanks were checked for mortalities and feed was placed in the feeders between 1 and 2 pm. 29 JUN 12 SMS →

30 JUN 12 Mortalities were counted and removed. BAL-1 was calibrated and used to weigh each fish. Fish were measured for TL. DO-2 and pH-1 were calibrated. Temperature, DO, and pH were measured in each tank. Tanks were cleaned by draining and siphoning. Tanks were assessed for uneaten feed. Feed weights were calculated and aliquots were weighed out for each tank. Light levels were checked in each tank with the Milwaukee Light meter, SN . The sensor showed 0 LUX at the center of the tank at the water level so it was held under water ~ 1 cm from the light, and the resulting reading was recorded. Tanks were checked for mortalities in the afternoon and feed was placed in each feeder between 1 and 2 pm. 30 JUN 12 SMS →

1 JUL 12 BAL-1 was calibrated. Mortalities were counted and removed. Each fish was weighed and measured for TL. DO-2 and pH-1 were calibrated and used to measure water chemistry. Tanks were drained, siphoned, and assessed for uneaten feed. Tanks were checked for mortality in the afternoon. Feed aliquots were calculated and weighed out for each tank on BAL-1. Feed was placed in the feeders between 1 and 2 pm. 1 JUL 12 SMS →

2 JUL 12 BAL-1, DO-2 and pH-1 were calibrated. Mort. were counted and removed. All fish were weighed and measured for TL. Water chemistry (temp, DO, pH) was measured in each tank. →

Continued on Page 9

Read and Understood By

Susan M. Schlei

2 JUL 12

Signed

Date

Signed

Date

2 JUL 12 (cont.) Each tank was cleaned by siphoning and draining. Feed consumption was observed and assessed. Mortalities from yesterday afternoon and this morning were entered into the Feed Calculation Sheet and today's feed amounts were calculated. Feed aliquots were weighed on BAL-1. Mortalities were checked for in the afternoon and feed aliquots were placed in the feeders between 1 and 2 pm. 2 JUL 12 SMS

3 JUL 12 BAL-1 was calibrated and used to weigh the morning mortalities. Mortals were measured for TL and processed as needed (plated or microscopic exam). DO-2 and pH-1 were calibrated and used to measure water chemistry. Each tank was siphoned and drained to clean. Feed consumption was assessed by evaluating material feed remaining in each tank; very little to none observed. Mortalities were entered into the Feed Wt Calculation Sheet and feed aliquots calculated. Each tank's feed was weighed on BAL-1. Tanks were checked for mortalities in the afternoon. Feed was placed in feeders between 1 and 2. 3 JUL 12 SMS

4 JUL 12 BAL-1 was calibrated. Mortalities were counted and removed. All mortalities were weighed and measured for TL. DO-2 and pH-1 were calibrated and used to measure water chemistry in each tank. Tanks were cleaned by siphoning and draining. Feed consumption was assessed. Flow rates were measured and adjusted as needed (660-900 mL/min). A water sample was removed from B1 and alkalinity and hardness were measured (SOP 706 + 712). Mortals were entered into the Feed Wt Calc. Sheet and required feed weights were weighed out for each tank on BAL-1. As on all previous days, each vibratory feeder was checked for unreleased feed. Any remaining was floated in the tank near its light before the feeder was refilled. Feed aliquots for the final dosing day analysis samples were weighed on BAL-1 into labeled bags, double bagged and placed in the freezer. Tanks were checked in the afternoon for mortalities. The flow to tank B1 was reduced to a few drips and 15 of the WAE had died. All were removed, weighed and measured for TL. 2 were plated to TSA. Feed was placed in the feeders between 1 and 2 pm. 4 JUL 12 SMS

Continued on Page 10

Susan M. Schlier 4 JUL 12
Signed Date Signed Date

PROJECT

5/July 12 pH-1 and DO-2 were calibrated and used to measure water quality, as well as Temp-1. Tanks were cleaned by siphoning and draining. Mortalities were observed and plated on TSA agar. BAL-1 was calibrated and used to weigh feed. Feed was distributed to each tank between 1-2 pm
 5 July 12 MPW →

6 July 12 pH-1 and DO-2 were calibrated and used to measure water quality, as well as TEMP-1. Tanks were siphoned and drained and mortalities were observed and plated on TSA agar. BAL-1 was calibrated and used weigh feed which was then distributed to each tank between 1-2 pm 6 July 12 MPW

7 July 12 pH-1 and DO-2 were calibrated and used to measure water quality, as well as Temp-1. Tanks were siphoned and drained for cleaning. Mortalities were collected and weighed using BAL-1 and plated on TSA agar as necessary. BAL-1 was used to weigh feed which was distributed to each tank between 1-2 pm. 7 July 12 MPW ←

8 July 12 pH-1 and DO-2 were calibrated and used to measure water quality, as well as Temp-1. Tanks were cleaned by siphoning and draining. BAL-1 was calibrated and used to weigh mortalities and mortalities were plated on TSA or observed under microscope as necessary. BAL-1 was used to weigh feed and feed was distributed to each tank between 1-2 pm. 8 July 12 MPW

9 July 12 pH-1 and DO-2 were calibrated and used to measure water quality, as well as Temp-1. Tanks were cleaned by siphoning and draining, and the screens from individual tanks were removed and rinsed. BAL-1 was calibrated and used to weigh mortalities and mortalities were plated on TSA as well as observed under microscope as needed. BAL-1 was used to weigh feed and feed was distributed to each tank between 1-2 PM. 9 July 12 MPW →

Continued on Page _____

Read and Understood By _____

[Signature] 7/9/12
 Signed _____ Date _____

Signed _____

Date _____

PROJECT _____

Continued From Page _____

10 Jul 12 pH-1 and DO-2 were calibrated and used to measure water quality as well as Temp-2. BAL-1 was calibrated and used to weigh mortalities. Mortalities were plated on TSA, or observed under microscope as necessary. Tanks were cleaned by siphoning and draining. BAL-1 was used to weigh feed and feed was distributed to each tank between 1-2 pm. 10 Jul 12 MPW

11 Jul 12 pH-1 and DO-2 were calibrated and used to measure water quality as well as Temp-1. BAL-1 was calibrated and used to weigh mortalities that were found. Mortalities were observed any microscope as necessary. Tanks were cleaned by siphoning and draining. BAL-1 was used to weigh feed and feed was distributed to each tank between 1-2 pm 11 Jul 12 MPW

12 Jul 12 pH-1 and DO-2 were calibrated and used to measure water quality, as well as Temp-1. BAL-1 was calibrated and used to weigh mortalities that were found. Mortalities were plated on TSA. Tanks were cleaned by siphoning and draining. BAL-1 was used to weigh feed and feed was distributed to each tank between 1-2 pm 12 Jul 12 MPW

13 Jul 12 pH-1 and DO-1 were calibrated and used to measure water quality, as well as Temp-2. DO-2 had readings that would not stabilize, so DO-1 was used. BAL-1 was calibrated and used to weigh mortalities and mortalities were plated on TSA, or observed under microscope as needed. Tanks were cleaned by siphoning and draining. BAL-1 was used to weigh feed and feed was distributed to each tank between 1-2 pm. Flow rates were also taken for each tank. Flow was increased to maximum to clear the line and then returned to 600-900 ml per minute. 13 Jul 12 MPW

14 Jul 12 pH-1 and DO-1 were calibrated and used to measure water quality as well as Temp-1. BAL-1 was calibrated and used to weigh mortalities and mortalities were plated on TSA, or observed under microscope as necessary. CONT 12 → MPW

Continued on Page _____

Read and Understood By _____

 7/14/12

Signed _____ Date _____ Signed _____ Date _____

PROJECT

Continued From Page _____

CONT) Tanks were cleaned by siphoning and draining. Due to low DOs flow rates were increased to 1000-1500 mL/min. BAL-1 was used to weigh feed and feed was distributed to each tank between 1-2 pm. 14 Jul 12 MPW

15 Jul 12 pH-1 and DO-1 were calibrated and used to measure water quality, as well as Temp-1. BAL-1 was calibrated and used to weigh mortalities and mortalities were plated on TSA as needed. Tanks were cleaned by siphoning and draining. BAL-1 was used to weigh feed and feed was distributed to each tank between 1-2 pm. 15 Jul 12 MPW

16 Jul 12 pH-1 and DO-1 were calibrated and used to measure water quality, as well as Temp-1. BAL-1 was calibrated and used to weigh mortalities, and mortalities were plated on TSA. Tanks were cleaned by siphoning and draining. BAL-1 was used to weigh feed and feed was distributed to each tank between 1-2 pm. A water sample was collected from tank A9 and used to measure Alkalinity and water hardness. 16 Jul 12 MPW

17 Jul 12 pH-1 and DO-1 were calibrated and used to measure water quality, as well as Temp-1. BAL-1 was calibrated and used to weigh mortalities, and mortalities were plated on TSA. Tanks were cleaned by drainage and siphoning. BAL-1 was used to weigh feed and feed was distributed to each tank between 1-2 pm.

17 Jul 12 MPW

18 Jul 12 pH-1 and DO-1 were calibrated and used to measure water quality, as well as Temp-1. BAL-1 was calibrated and used to weigh mortalities, and mortalities were plated on TSA, or observed under microscope as needed. Tanks were drained and remaining fish count was recorded for each tank. Remaining fish were then put into SLFH runways. Tanks were emptied and then scrubbed and rinsed. Tanks were disassembled and loaded onto trailer. Fish nets were sanitized with iodine

(13) Continued on Page _____

Read and Understood By _____

[Signature] 7/18/12

Signed

Date

Signed

Date

and remaining study equipment was loaded on the trailer 18 Jul
MPW

17SEP12 All feed samples were shipped ^{to cov} via FEDEX overnight to be analyzed for concentration verification. Sample #12 CONT was ^{17SEP12 SWS} #12 JUN12 CONTA #25 JUN #4 JUL FFCA were shipped to Eurofins Lancaster Labs in Postage MI. Sample 12 JUN12 CONTA is unmedicated feed, and is to be analyzed for the presence of Romet and florfenicol. It was stored in the Ultracold freezer in Room 21, Model ULT-2090-9-114 S/N 510F-301144-SF. The double-bagged labeled 500 was placed in an insulated shipping carton on frozen gel packs. Samples 4 JUL and 25 JUN12 FFCA are to be analyzed to verify the expected concentration of 214 of florfenicol. They were stored in the 50-LOW freezer in Rm 125, MODEL PR120-S/N 06071105. Due to the decision to have the OTC samples analyzed at Covance Labs, some of the control sample (12 JUN12 CONTA) needed to be removed and placed in a COVANCE LABS sample bag. It was removed from Ultra Cold, and weighed on LC-BAL which had been calibrated. 249.1 g of feed was weighed into a labeled COVANCE bag, and the original bag was weighed again. Then, 266.2 g of the control sample was weighed into a labeled (CONTROL-1) 2 freezer bag for use by COVANCE in their OTC analyses procedure. All bags were returned to the Rm 21 Ultracold freezer until being readied for shipment. 2 samples (25 JUN12 OTC A and 4 JUL OTC B) that are to be sent to COVANCE were removed from Rm 125 Ultra-cold freezer and taken to Rm 15. 25 JUN12 OTC A was weighed on the LC-BAL. The labeled COVANCE bag was placed on the balance & tared. The entire bag's contents (216.5 g) was emptied into COVANCE sample bag 25 JUN12 OTC and the empty bag was weighed. Sample 4 JUL OTC A was then weighed on LC-BAL. It was poured into a labeled COVANCE sample bag (259.7 g) 4 JUL OTC. The remainder in the original bag was weighed. All bags were returned to the Ultra-cold freezer until being readied for shipment. A form was filled out recording weights of withdrawals (Tracking sheet for withdrawal from original sample bags, FF 7). Approximately 1 hr before FedEx pick-up time, samples were removed from their respective freezers and placed in the appropriate cooler on frozen gel packs. Copies of all paperwork and FEDEX airbills were made and placed in the study file (FF 7). 17SEP12 SWS

9 OCT12 The samples that were sent on 17SEP12 to Lancaster Labs in Postage MI were shipped to the Metairie Lab by the Postage MI Lab on

Continued on Page 1.

Read and Understood By

Susan M. Schuler

9 OCT12

Signed

Date

Signed

Date

26 SEP 12, arriving on 27 SEP 12. The Postage Lab was asked about testing for oxytetracycline, FFC and Ronnet on 17 AUG 12. However when they received the samples on 18 SEP 12 they notified us that their lab did not do any Ronnet analyses. They suggested that all samples (FFC + Ronnet, oxytet samples were sent to Covance per request of Philre) be sent to Metairie as they could do both tests. However, after the samples were sent to LA lab, we discovered the LA lab did not perform the validated FFC method (20 OCT 12). A decision was made to have the Metairie lab send the 25 JUN 12 and JUL 12 sample back to the Postage MI lab for analysis, along with a portion of the 12 JUN 12 control feed sample, for FFC analysis. We were also informed that the 12 JUN 12 sample of control feed arrived in the same shipper with no separation, and only single bagged so there was some concern of the possibility of contamination by the FFC samples. So we shipped the reserve sample (12 JUN 12 CONT B) sample to Metairie LA lab today. It was removed from the Ultra cold in Rm 21, and shipped FEDEX standard overnight on frozen gel packs. They will remove what they need for the sulfadimethoxine and Oxytetracycline testing, and ship the remainder to the Postage MI lab, along with the 25 JUN 12 and JUL 12 samples for FFC analysis. Copies of the paperwork are in FF7. 9 OCT 12 SMS

Continued on Page

Read and Understood By

Signed

Date

Signed

Date