

Prepared for
Kentucky Utilities Company

Document type
Quality Assurance Project Plan (QAPP) Addendum

Date
May 2018

QAPP ADDENDUM: HERRINGTON LAKE YOUNG-OF-THE-YEAR (YOY) BASS ASSESSMENT



Quality Assurance Project Plan (QAPP) Addendum
for Young-Of-The-Year (YOY) Bass Assessment
E.W. Brown Generating Station
Herrington Lake, Kentucky

Prepared By: Ramboll US Corporation
1600 Parkwood Circle, Suite 310
Atlanta, GA 30339, USA
(678) 388-1643
msorensen@ramboll.com

Prepared For: Kentucky Utilities Company
1 Quality St, Lexington, KY 40507
(800) 981-0600

Effective Date: TBD

Revision Date: May 2, 2018

Revision No. 1.0

DOCUMENT DEVELOPMENT AND APPROVAL

Title and Approval Sheet

Action By	Signature	Date
Prepared by: Richard Lockwood, Ramboll		5/02/2018
Reviewed by: Mary Sorensen, Ramboll		5/02/2018
Approved by: Mark Nielsen, Ramboll		5/02/2018

REVISION HISTORY

This table documents the revisions over time to the QAPP. The most recent iteration should be listed in the first space, with consecutive versions following. Signatures may be required for revised documents.

Date of Revision	Page(s)/Section(s) Revised	Revision Explanation
May 02, 2018		First Draft

CONTENTS

Document Development and Approval	ii
Revision History	iii
1 Distribution List	1
2 Introduction	2
3 Project/Task Organization Addendum	3
4 YOY Fish Assessment	4
5 References	6

LIST OF HERRINGTON LAKE FISH HEALTH ASSESSMENT FORMS

Herrington Lake Young-Of-The-Year (YOY) Fish Health Examination Form

Herrington Lake Young-Of-The-Year (YOY) Individual Fish Examples Inspection Form

LIST OF APPENDICES

Appendix A YOY Fish Assessment Curricula Vitae

A.1 Richard Lockwood, Ramboll Ecotoxicology Laboratory

A.2 John Hawke, Louisiana State University, School of Veterinary Medicine

Appendix B Ramboll Ecotoxicology Laboratory Overview

ACRONYMS AND ABBREVIATIONS

AMEC	Amec Foster Wheeler
ALS	Analytical Laboratory Services
Cabinet	Kentucky Energy and Environment Cabinet
CAP	Corrective Action Plan
COPEC	Constituent of Potential Ecological Concern
DQO	Data Quality Objective
ERA	Ecological Risk Assessment
HSC	Health and Safety Coordinator
ID	Identifier
KAR	Kentucky Administrative Regulation
KDOW	Kentucky Department for Environmental Protection Division of Water
KU	Kentucky Utilities
MS/MSD	Matrix Spike/Matrix Spike Duplicate
NOV	Notice of Violation
PBRCC	Precision, Bias, Representativeness, Comparability, Completeness, and Sensitivity
QA/QC	Quality assurance/Quality control
QAPP	Quality Assurance Project Plan
SOP	Standard Operating Procedure
USEPA	United States Environmental Protection Agency
YOY	Young-of-the-Year

1 DISTRIBUTION LIST

Name: Gary Revlett

Director, Environmental Affairs

Organization: LG&E and KU Energy LLC

815 Dix Dam Rd, Harrodsburg, KY

Name: Tim Hubbard

Organization: Kentucky Department for
Environmental Protection (DEP)

300 Sower Blvd, Frankfort, KY 40601

Name: Peter Goodmann

Organization: Kentucky DEP

300 Sower Blvd, Frankfort, KY 40601

Name: Lisa Hicks

Organization: Kentucky DEP

300 Sower Blvd, Frankfort, KY 40601

Name: Mark Nielsen

Title: Principal

Organization: Ramboll

101 Carnegie Center #200, Princeton, NJ 08540

(609) 243-9859

Mark Harris

ALS 1317 S. 13th Avenue

Kelso Washington 98626

Name: Mary Sorensen

Title: Senior Managing Consultant

Organization: Ramboll

1600 Parkwood Circle, Suite 310, Atlanta, GA
30339, USA

(678) 388-1647

Name: Katrina Leigh

Title: Field Manager

Organization: Ramboll

13801 E Center St, Burton, OH 44021

(440) 834-1460

Name: Jack Bender

Dinsmore & Shohl LLP

250 West Main St. Lexington, Kentucky 40507

(859) 425-1000

Name: Brent Rosser

Hunton Andrews Kurth, LLP

Bank of America Plaza, St. 3500

101 South Tryon St.

Charlotte, NC 28280

(704) 378-4707

2 INTRODUCTION

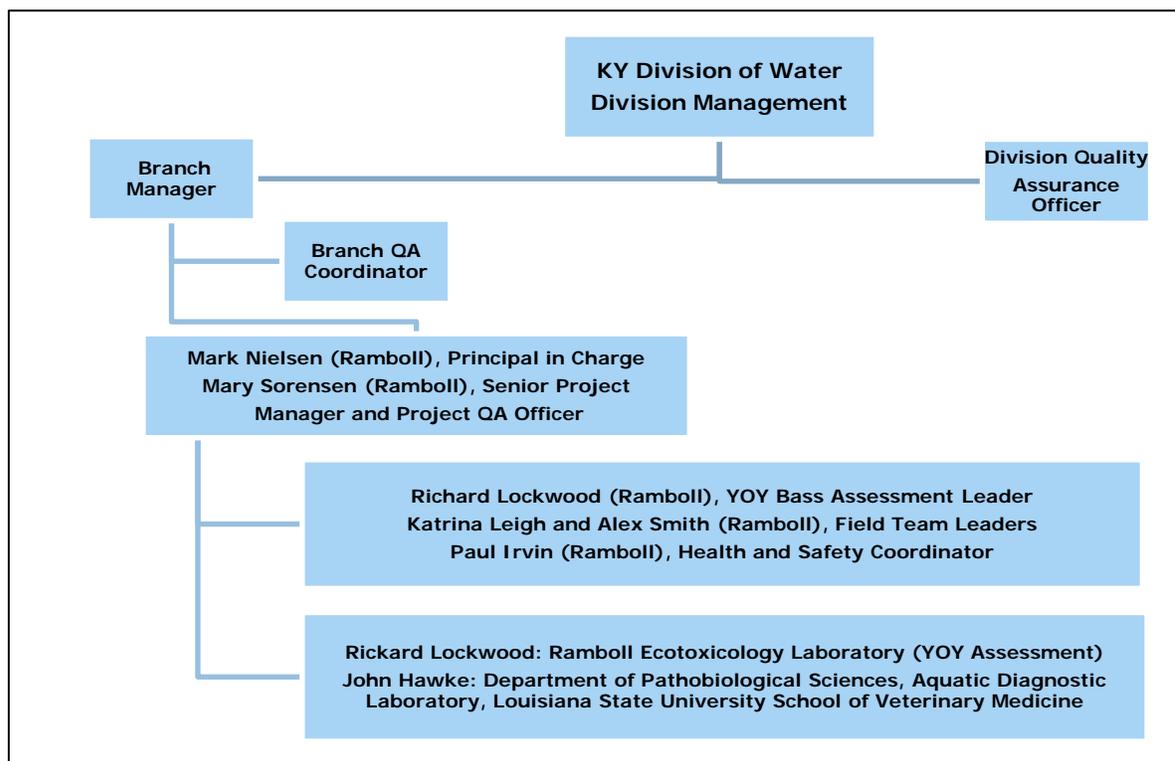
This *Quality Assurance Project Plan (QAPP) Addendum* is provided for the Young-of-the-Year (YOY) bass assessment planned for Herrington Lake. This QAPP Addendum is provided to the Kentucky Energy and Environment Cabinet (Cabinet) in accordance with the August 2017 Corrective Action Plan (CAP) for Herrington Lake (Ramboll 2017a). The CAP for Herrington Lake was developed and submitted to the Cabinet as part of efforts to resolve the January 11, 2017 Notice of Violation (NOV) received by Kentucky Utilities Company (KU) due to detections of selenium in whole-body fish-tissue from Herrington Lake at concentrations above Kentucky's water quality standard for protection of aquatic life. To resolve the NOV, KU entered into an Agreed Order with the Cabinet on January 30, 2017 that required an investigation of Herrington Lake for selenium and other constituents that can be associated with Coal Combustion Residue (CCR). The Phase I field investigation for the CAP was conducted in October through December of 2017. The Phase Technical Memorandum and Phase II Plan (hereafter referred to as the Phase II Plan) was submitted to the Cabinet on May 2 (Ramboll 2018a).

This QAPP Addendum only addresses the laboratory portion of the YOY assessment, which is a new task identified in the Phase II Plan that was not previously identified in the CAP or QAPP. As such, it supplements the initial QAPP (Ramboll 2017b). This QAPP Addendum discusses the quality assurance protocols associated with the YOY task only. To the extent practical, this QAPP Addendum is streamlined and does not duplicate information already presented in the 2017 QAPP. A Standard Operating Procedures (SOP) for collection of YOY bass in Herrington Lake was prepared under separate cover from this QAPP Addendum (Ramboll 2018b).

The Phase II Plan also proposes additional sampling and analysis of adult fish, surface water, sediment pore water, and sediment for the measurement of constituents via laboratory analysis (Ramboll 2018a). The Phase II QAPP elements for those sample media are covered in the 2017 QAPP, which was reviewed and approved by the Cabinet (Ramboll 2017b). The sample design (i.e., number of samples, location of samples, and analyses planned for Phase II samples) is described in the Phase II Plan (Ramboll 2018a).

3 PROJECT/TASK ORGANIZATION ADDENDUM

This section lists the individuals and organizations participating in the Herrington Lake project, and their specific roles and responsibilities. The majority of the project team members for this QAPP Addendum are the same as those identified in the 2017 QAPP. There are three new roles for Ramboll staff on this project in comparison to the Herrington Lake QAPP (Ramboll 2017b), as described below:



Richard Lockwood (YOY Bass Assessment and Adult Fish Sampling Leader)

Richard Lockwood will serve as the YOY bass assessment leader for both the field and laboratory portions of the assessment. Mr. Lockwood will follow the Standard Operating Procedures (SOP) for YOY bass Assessment and Fish Tissue Sampling (Ramboll 2018b) and will have overall responsibility for all YOY field collection, preservation, and sample labels, in coordination with Mary Sorensen. Mr. Lockwood will also direct the YOY and adult fish sample collection for laboratory analysis, in accordance with the Phase II Plan. Following the field effort and in the Ramboll laboratory, Mr. Lockwood will also conduct the YOY bass health assessment, as described in this QAPP. Mr. Lockwood will coordinate with the third-party validator for additional assessment needed.

Alex Smith and Katrina Leigh (Field Team Leaders)

Alex Smith will share the field team leadership role with Katrina Leigh for collection of surface water, sediment pore water, and sediment in accordance with the Phase II Plan. They will assist Mr. Lockwood as needed. Ms. Leigh and Mr. Smith have responsibility for field activities in coordination with Mary Sorensen. Field Personnel led by Ms. Leigh and Mr. Smith will be responsible for carrying out all field activities, and assisting the field sampling lead in assigned tasks. Paul Irvin (Health and Safety Coordinator)

Paul Irvin will serve as the Health and Safety Coordinator for the 2018 field efforts.

4 YOY FISH ASSESSMENT

The results of a study of 2016 YOY bass collected from Curds Inlet became publically available during the implementation of the Phase I field program. A YOY study was not conducted in the Phase I sampling. To further assess the findings reported by the 2016 study, a YOY study is proposed for Phase II for fish collected at multiple locations in Herrington Lake, including Curds Inlet and at locations away from Curds Inlet. The study will target bass species at locations in Curds Inlet and other locations in Herrington Lake. YOY fish collections will be conducted in six areas that provide opportunity to measure a gradient of potential differences away from Curds Inlet, if such a gradient exists. Two YOY areas are planned for Curds Inlet and four additional areas are planned as indicated in the Phase II Plan.

4.1 YOY Bass Health Assessment Approach

The objective of the YOY bass health assessment approach is to observe Herrington Lake YOY fish for anatomical deformities (i.e., anomalies) that could be related to selenium or other constituents potentially related to Coal Combustion Residue (CCR).

The YOY sample collection and assessment will be conducted consistent with:

- Biological Criteria for the Protection of Aquatic Life: Volume III. Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Macroinvertebrate Communities, Ohio Environmental Protection Agency (Ohio EPA 2015).
- Illustrated Field Guide for Assessing External and Internal Anomalies in Fish. United States Geological Survey (USGS), Information and Technology Report (USGS 2002).
- United States Environmental Protection Agency (USEPA) Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers (Flotemersch et al. 2006).
- Previous YOY bass sampling and assessment methodology for fish collected in Curds Inlet (Downstream Strategies 2016, Lemly 2018).

The YOY bass health assessment will be conducted at the Ramboll, Nashville TN Aquatic Toxicity Lab. Analysis will be conducted via un-aided visual inspection of the anatomy or the preserved specimens. No magnification or other optical enhancement is necessary. Each YOY health assessment sample will be comprised of up to 500 bass. The following deformities will be assessed and recorded using the Herrington Lake YOY Fish Health Assessment Form(s) provided with this QAPP Addendum for YOY Fish Assessment:

- Spinal curvature (kyphosis, lordosis, and scoliosis);
- Craniofacial defects (including mouth, jaw, and gill cover);
- Fin irregularities (missing, misshaped, vestigial);
- Eye abnormalities (including lens cataracts and exophthalmos); and
- Edema (fluid accumulation).

Specimens with observed anomalies (if any) will be counted and photographed in detail to record the deformity noted. Specimens will be placed in a separate, internally-labeled plastic bag, inside of the original one-gallon bottle used for each station sample. All 500 YOY bass from each of the 6 samples will be photographed. The YOY bass identified as “normal” will be photographed in groups. Any YOY bass having identified abnormalities will be photographed individually. All fish sample photos will

include a ruler in the image, for scale. The assessment will also be documented with written notes or findings regarding any observed deformities using the Herrington Lake Young-Of-The-Year (YOY) Fish Health Examination Form and Individual Fish Examples Inspection Form provided in this Appendix. Specimens for deformity analysis will be archived for up to three years after collection.

The YOY bass health assessment will be conducted by Richard Lockwood (Ramboll fisheries biologist and Quality Control Officer for the Ramboll Ecotoxicology Laboratory). In addition, a third-party quality assurance deformity assessment will be conducted on a randomly selected twenty-five percent subset of the YOY bass having no identified deformities and on 100 percent of any YOY bass having identified deformities. This third-party review will be conducted by Dr. John Hawke (Department of Pathobiological Sciences, Aquatic Diagnostic Laboratory, Louisiana State University School of Veterinary Medicine). After Dr. Hawke's assessment is complete, those YOY specimens will be shipped back to, and archived at, the Ramboll, Nashville TN laboratory.

4.2 Professionals Performing the Herrington Lake YOY Fish Health Assessment

Richard Lockwood, and Dr. John Hawke will conduct the YOY fish health assessment. Their experience is briefly described here and Curricula Vitae are provided in Appendix A.

Richard Lockwood

Richard Lockwood has more than 35 years of aquatic biology experience, including extensive knowledge and experience conducting aquatic bio-assessments, often related to, or in accordance with, federal regulations. Mr. Lockwood has experience as aquarium curator at the Oklahoma Henry Doorly Zoo and as Herpetarium and Aquarium Supervisor. Mr. Lockwood is experienced in the culture and husbandry requirements of plants and animals. Rick also has extensive experience coordinating biological assessment and toxicological assessments with wastewater treatability and engineering projects as part of the Ramboll Ecotoxicology Laboratory in Nashville, Tennessee. He is the Quality Control Officer and supervises the maintenance and performance of cultured test organisms in a variety of toxicity tests. He routinely conducts studies related to fish pathogens, bioassessment of fish (including fish abnormalities and deformities), effluent toxicity tests, thermal discharge impact studies, and other effluent-related bio-assessments.

Dr. John Hawke

Dr. John Hawke is a Professor of Pathobiological Sciences (PBS) at Louisiana State University. Dr. Hawke is a native of Birmingham, Alabama and has been involved in teaching, research, and diagnostics pertaining to aquatic animal health for over 40 years. His teaching interests include aquatic animal diseases, bacteriology, pathogenic mechanisms of bacteria. His research interests include identification of new pathogens in aquatic animals, the study of the pathogenic mechanisms of fish bacteria, and the development of vaccines for bacterial pathogens of fish for use in aquaculture.

4.3 Supporting Information

Additional information about the Ramboll Ecotoxicology Laboratory is provided in Appendix B.

5 REFERENCES

- Downstream Strategies, 2016. Herrington Lake Phase II Sampling Report, June 6–7, 2016. Downstream Strategies, Morgantown, WV.
- Flotemersch, J. E., J. B. Stribling, and M. J. Paul. 2006. Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers. EPA 600-R-06-127. US Environmental Protection Agency, Cincinnati, Ohio.
- Kentucky Department for Environmental Protection Division of Water. 2016. Standard Operating Procedure for Collection of Fish in Large Wadeable and Non-Wadeable Streams and Rivers. Commonwealth of Kentucky Energy and Environment Cabinet. Revision No. 1.0. Document Control No: DOWSOP03041.
- Kentucky Department for Environmental Protection Division of Water. 2017. Standard Operating Procedure for Preparation and Homogenization of Fish Tissue Samples, Commonwealth of Kentucky, Energy and Environment Cabinet, Department for Environmental Protection, Division of Water Effective Date: May 11, 2017.
- Lemly, A.D. 2018. Selenium poisoning of fish by coal ash wastewater in Herrington Lake, Kentucky. *Ecotoxicology and Environmental Safety*. 150, 49–53.
- Ohio EPA. 2015. Biological Criteria for the Protection of Aquatic Life: Volume III. Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Macroinvertebrate Communities, Ohio Environmental Protection Agency. EAS/2015-06-01.
- Ramboll 2017a. Herrington Lake E.W. Brown Corrective Action Plan. Submitted to the Kentucky Division of Water Agreed Order No. DOW-17001. August.
- Ramboll. 2017b. Quality Assurance Project Plan. Herrington Lake, E.W. Brown Station. Prepared For: Kentucky Utilities Company for Submittal to KDOW Agreed Order No. DOW - 17001. October.
- Ramboll. 2017c. Standard Operating Procedures: Fish Sampling and Analysis. Herrington Lake, E.W. Brown Station. Prepared For: Kentucky Utilities Company for Submittal to KDOW Agreed Order No. DOW - 17001. October.
- USGS. 2002. Illustrated Field Guide for Assessing External and Internal Anomalies in Fish. US Geological Survey. Information and Technology Report, USGS/BDR/ITR-2002-0007.

LIST OF HERRINGTON LAKE FISH HEALTH ASSESSMENT FORMS

Herrington Lake Young-Of-The-Year (YOY) Fish Health Examination Form

Herrington Lake Young-Of-The-Year (YOY) Individual Fish Examples Inspection Form

LIST OF APPENDICES

Appendix A	YOY Fish Assessment Curricula Vitae
A.1	Richard Lockwood, Ramboll Ecotoxicology Laboratory
A.2	John Hawke, Louisiana State University, School of Veterinary Medicine
Appendix B	Ramboll Ecotoxicology Laboratory Overview

LIST OF HERRINGTON LAKE FISH HEALTH ASSESSMENT FORMS

Herrington Lake Young-Of-The-Year (YOY) Fish Health Examination Form

Herrington Lake Young-Of-The-Year (YOY) Individual Fish Examples Inspection Form

Herrington Lake Young-Of-The-Year (YOY)
FISH HEALTH EXAMINATION FORM



Recorder:	Notes:
Primary Fish Health Assessor:	
Third-Party Assessor:	
Assessment Date(s):	# of YOY in sample:

YOY Fish Sampling Location:	Species distribution in sample (e.g. 80% percent largemouth bass):
-----------------------------	---

of Normal Fish (no visible deformities): _____

Fish Anatomy	Anatomical Anomaly	# of fish	Anatomical Anomaly	# of fish
Eyes	Eyes both normal:			
	Left		Right	
	normal:		normal:	
	exophthalmic:		exophthalmic:	
	opaque:		opaque:	
	missing:		missing:	
Other (list):			Other (list):	

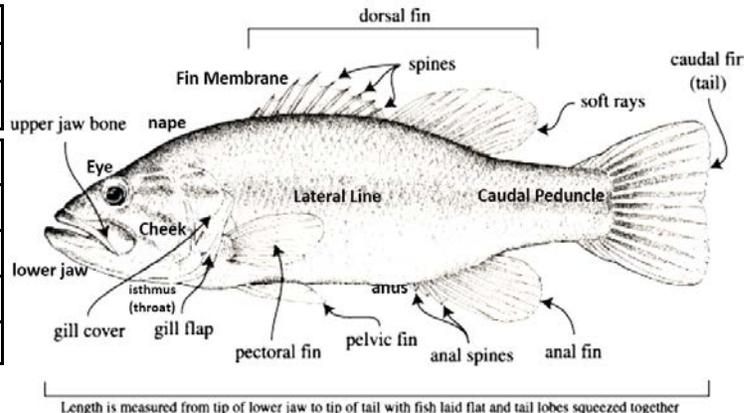
Assessment Guide for YOY Centrarchidae
Eye abnormalities - including lens cataracts and exophthalmos
Fin irregularities - missing, misshaped, partly missing (aka vestigial)
Spinal curvature - kyphosis, lordosis, and scoliosis
Craniofacial defects - mouth, jaw, and gill cover
Edema - fluid accumulation
Note: To avoid multiple-counting of fish with multiple deformities, this form counts the prominent deformity and digital images and details of specific fish will also be recorded.

Fins	Fins all normal:		Caudal fin (tail)		Anatomical Anomaly	# of fish
	Left pectoral		partly missing:			
	partly missing:		missing:			
	missing:		twisted:			
	twisted:		Dorsal fin			
	Right pectoral		Anal fin		partly missing:	
	partly missing:		missing:			
	missing:		twisted:			
twisted:		fins other (list):				

Spine	Spine normal:		Craniofacial	Head Normal:	
	kyphosis:			mouth:	
	lordosis:			jaw:	
	scoliosis:			gill cover:	
	Other (list):			Other (list):	

Edema	normal:	
	Edema:	
	Other (list):	

Other (List)			



Herrington Lake Young-Of-The-Year (YOY)
INDIVIDUAL FISH EXAMPLES - INSPECTION FORM



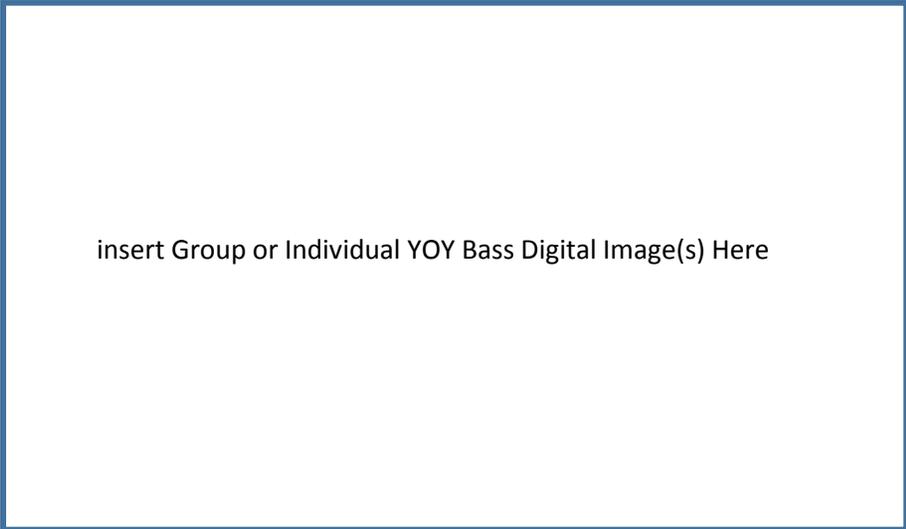
Recorder:	Notes:
Primary Fish Health Assessor:	
Third-Party Assessor:	
Assessment Date(s):	

Anatomical Category (e.g. spine, fin(s)):

Examples From YOY Bass Sampling Region (circle one):
Curds Inlet HQ Inlet LHL1(Rocky Arm) LHL3 Cove LHL 6 Cove

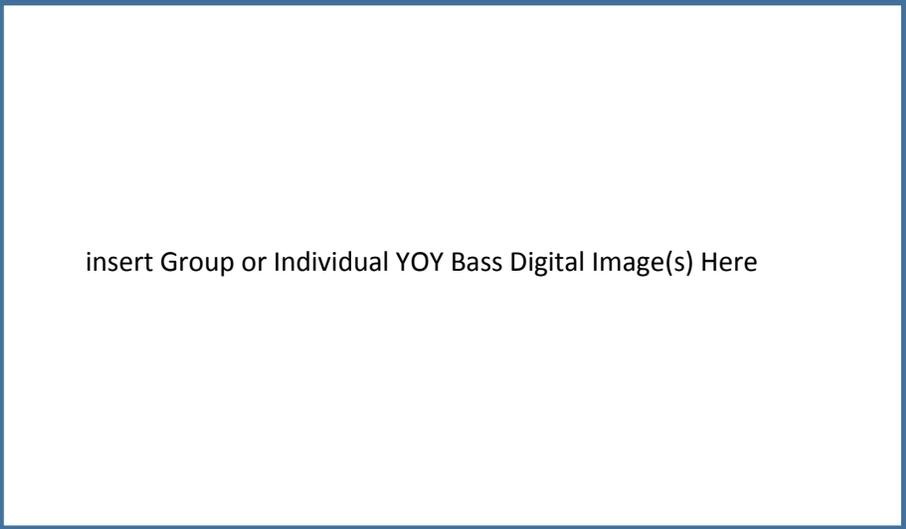
Sample ID:

Abnormality Description(s) (e.g. Top image displays right side tail deformity(ies)):



insert Group or Individual YOY Bass Digital Image(s) Here

Abnormality Description(s) (e.g. Bottom image displays left side fin deformity(ies)):



insert Group or Individual YOY Bass Digital Image(s) Here

APPENDIX A - YOY Fish Assessment Curricula Vitae

- A.1 Richard Lockwood, Ramboll Ecotoxicology Laboratory
- A.2 John Hawke, Louisiana State University, School of Veterinary Medicine

APPENDIX A - YOY Fish Assessment Curricula Vitae

A.1 Richard Lockwood, Ramboll Ecotoxicology Laboratory

RICHARD E LOCKWOOD

Managing Consultant

Rick Lockwood is the senior scientist and laboratory QC officer of Ramboll aquatic toxicology laboratory located in Nashville Tennessee. Rick's experience is diverse, including development and monitoring of testing programs conducted in support of USEPA National Pollutant Discharge Elimination System (NPDES) permits. This experience also includes conducting Whole Effluent Toxicity (WET) tests, Toxicity Reduction Evaluation (TRE) and Toxicity Identification Evaluation (TIE) programs, 316(b)-intake entrainment/impingement studies, 316(a)-thermal discharge impact studies, and *in situ* bioassessments in support NPDES and 303d – impaired waterbody assessments. Rick also is involved with developing and implementing chemical specific and product toxicity testing protocols under Good Laboratory Practice (GLP) guidelines for product stewardship campaigns.

Rick has a wide range of knowledge and experience in the culture and husbandry requirements of plants (including pelagic algae and periphyton), invertebrate, and vertebrate species. Rick also has extensive experience coordinating biological/toxicological work with wastewater treatability/engineering projects. Over the years, he has led many projects specifically evaluating water, sediment, and effluent toxicity, including consideration of developmental abnormalities in the organisms cultured and tested in the laboratory. He leads the Quality Control (QC) program of Ramboll's aquatic toxicology laboratory, and supervises the maintenance and performance of cultured test organisms. He has conducted research into fish pathogens and select chemicals in effluent toxicity tests.

CAREER

1995 – Present

Ramboll Brentwood, Tennessee. Ecotoxicologist.

1994-1995

Omaha Henry Doorly Zoo, Omaha, Nebraska, Aquarium Curator

1981-1994

Oklahoma City Zoo, Oklahoma City, Oklahoma, Herpetarium and Aquarium Supervisor. Zookeeper /Aquarist

– Zoo experience included the development of breeding/husbandry protocols for reptile species, including snake, lizard, and turtle species indigenous to the USA.

EDUCATION

1980

BS, Biology

University of California at Irvine with emphasis on behavioral ecology. Received Excellence in Research award for work in behavioral ecology.



CONTACT INFORMATION

Richard E Lockwood

rlockwood@ramboll.com

+1 (615) 2777523

Ramboll

201 Summit View Drive

Suite 300

Brentwood, TN 37027

United States of America

REPRESENTATIVE PROJECTS

316 A AND B

Section 316 a and b of the Clean Water Act refers to thermal impacts from effluent discharges (316a), and entrainment/impingement impacts of water intake structures (316b). 316a and multiyear 316b projects were conducted in support of NPDES permits for a steel manufacturer on the Grand Calumet River (IN). A 316a survey was conducted for a plastics manufacturer on tributaries to Sangamon River, near Illiopolis, IL.

316b activities include devising collection methods to intercept impinged fish on intake screens. Identify and enumerate species and age group, enumerate fish deformities, lesions, or tumors (DELT's) and develop body condition factor index. 316a activities are comparable to development of a fish Index of Biotic Integrity (IBI) with emphasis on species limitations based on thermal tolerance. A partial list of the twelve metrics required for IN 316a efforts included:

- Total Number of Fish Species
- Proportion of Sunfish Species
- Number of Minnow Species
- Number of Sensitive Species
- Percent Abundance of Tolerant Species
- Number of Individuals in a Sample - Catch per Unit Effort (fish/reach length)
- Proportion of Individuals with DELT's

The above are commonly shared metrics in other states.

REPRESENTATIVE FISH COMMUNITY HEALTH ASSESMENTS

Rick has extensive experience conducting fisheries assessments, including evaluation of fish deformities, lesions, or tumors (DELTs) and fish collections for data relevant to developing fish index of biological integrity (IBI) metrics. Examples of projects where Rick led field efforts for fish assessments include following locations:

- Buffalo River, NY Area of Concern managed under the USEPA Great Lakes Legacy Act in support of risk assessment and remediation for PAH contaminated sediments. Rick provided multiple years of fisheries assessment in the river, including evaluation of DELTs, as part of ongoing monitoring of remedy effectiveness. Fisheries assessment information conducted by Ramboll is being used by USEPA and NY State Department of Environmental Conservation for ongoing consideration of delisting biological impairments in the river. This project also included liver histopathology to evaluate potential PAH impacts on the liver of catfish.
- Grand Calumet River, in support of PAH risk assessment and remediation dredging efforts. Fish IBI metrics were developed including DELT analysis.
- Busseron Creek, IN in support of post coal mining remediation. Fish IBI metrics were developed including DELT analysis.
- Red Boiling Springs, TN monitoring support for aquatic resource alteration permit (ARAP) requirements. Fish IBI metrics were developed including DELT analysis.
- First Creek, Obion River watershed, TN in support of NPDES WET method verification and pathogen assessments. Fish IBI metrics were developed including DELT analysis.
- Hatchie River Watershed near Bolivar, TN fish assessments in support of human health and ecological risk assessments. Fish IBI metrics were developed including DELT analysis.
- Wood's Reservoir, TN in support of PCB fish advisory work. Sample fish were assessed for DELTs
- Griffin, GA in support of NPDES toxicity reduction evaluation (TRE) of a high TDS effluent discharge. Fish IBI metrics were developed including DELT analysis.

- Opossum and Valley Creeks, Fairfield AL in support of fish mercury advisory. Sampled fish were assessed for DELTs

REPRESENTATIVE LABORATORY PROJECTS

Rick trains staff and performs WET tests and TRE/TIE studies in support of various aspects of municipal and industrial wastewater effluent testing. He also performs toxicity testing in support of Ramboll engineers involved with wastewater design technologies during development or testing of wastewater treatment systems. These tests often include testing of larval fish among species evaluated. Rick evaluates fish condition at the completion of tests. Toxicity tests primarily focus on survival and growth, however, in addition, consideration is also given to potential fish teratogenic abnormalities that would include deformities such as spinal curvature, edema, fin deformities, and tumors.

COURSES/CERTIFICATIONS

Tennessee Qualified Hydrologic Professional Certification, 2015

PUBLICATIONS

2014

Application of a Unique Test Design to Determine the Chronic Toxicity of Boron to the Aquatic Worm

Lumbriculus variegatus and Fatmucket Mussel *Lampsilis siliquoidea*

Archives of Environmental Contamination and Toxicology, 66:58-68

Authors: Hall, W.S., R. Lockwood, M. Harrass

2009

Assessing the Relationship between Laboratory Whole Effluent Toxicity Test Data and In-Stream Communities

Bulletin of Environmental Contamination and Toxicology, (2009) 82:270-274

Authors: Hall, W.S., D. Beeson, M. Kinsey, L. Heise, R. Lockwood

1990

A case for fry mimicry in the Tanganyikan cichlid, *Neolamprologus lelupi*

Journal of the American Cichlid Association. 139: 18 - 23

Authors: Lockwood, R.E., Jr.

1989

A mass production method for rearing poison dart frog larvae

Vivarium Vol.2, No. 1: 24 - 27

Authors: Lockwood, R.E., Jr.

1987

Improving the efficiency of rearing Dendrobatid larvae

Proceedings of the 11th International Herpetological Symposium on Captive Propagation and Husbandry

Authors: Lockwood, R.E., Jr.

1980

Temporal use of the contest displays of the Lake Tanganyikan cichlids, *Julidochromis marlieri*, *J. transcriptus*, and *Telmatochromis bifrenatus*

Journal of Undergraduate Research of the Biological Sciences. 10:130-147

Authors: Lockwood, R.E., Jr.

PRESENTATIONS

2009

Application of ASTM Mussel Toxicity Testing Guidance to Conducting a US EPA Toxicity Identification or Reduction Evaluation

Freshwater Mollusk Conservation Society (FMCS)

2008

Total Dissolved Solids Toxicity: Challenges and Future Directions

Ecological Society of America (ESA)

2006

Cause of Toxicity of a Reverse Osmosis Reject Waste Stream with High Alkalinity

TN/KY Water Environment Federation

2006

Uptake of perchlorate by garden crops from a perchlorate-impacted soil and risk via produce consumption

The 16th Symposium in the Series on Groundwater Contaminants "Perchlorate: Progress Toward Understanding and Cleanup," Groundwater Resources Association of California

2005

Protocols for the Identification of Fish Pathogens in Industrial Effluents

WEFTEC

2002

Variables Effecting Nitrite and Nitrate Toxicity in Effluent Toxicity Tests

TN/KY Water Environment Federation

1999

Methods to Identify Fish Pathogens in Effluent Toxicity Tests

Society of Environmental Toxicology and Chemistry (SETAC)

1998

Identification of Major Ions, Calcium and Chloride as Causative Effluent Toxicants

Tennessee section of the American Water Resources Association Symposium. Poster (AWRA)

1996

Bioavailability and Site-Specific Permitting of Cyanide in a Coke Plant Effluent

SETAC Poster, TN/KY WEF

APPENDIX A - YOY Fish Assessment Curricula Vitae

A.2 John Hawke, Louisiana State University, School of Veterinary Medicine

School of Veterinary Medicine

School of Veterinary Medicine

[About](#) [Apply](#) [Departments](#) [Alumni](#) [Hospital/Services](#) [News/Events](#) [Research](#)
[Resources](#) [Jobs](#) [Give](#)

Dr. John Hawke



Professor
Pathobiological Sciences (PBS)

[✉ jhawke1@lsu.edu](mailto:jhawke1@lsu.edu)
[✆ \(225\) 578-9684](tel:(225)578-9684)

Biography:

John P. Hawke is a native of Birmingham, Alabama and has been involved in teaching, research, and diagnostics pertaining to aquatic animal health for over 40 years.

Teaching Interests:

Aquatic animal diseases, bacteriology, pathogenic mechanisms of bacteria.

Research Interests:

Identification of new pathogens of aquatic animals, study of the pathogenic mechanisms of fish pathogenic bacteria, development of vaccines for bacterial pathogens of fish for use in aquaculture.

□ Awards & Honors:

- 2012 Special Achievement Award
- 2010 S.F. Snieszko Distinguished Service Award
- 2010 LSU IBRDSC Recognition Award
- 2007 Crystal Globe Award

□ Education:

- PhD Louisiana State University
- MS Auburn University
- BS Auburn University

□ Grants:

- Antimicrobial susceptibility testing of bacteria isolated from aquatic animals to generate epidemiological breakpoints., FDA, CVM Office of Research, \$8,000.00
- Utility of wild type and mutant strains of *Edwardsiella ictaluri* from zebrafish as live attenuated vaccines in channel catfish *Ictalurus punctatus*, USDA 1433, \$7,000.00
- Comparative pathogenesis of *ureG* and *esrC* mutants of *Edwardsiella ictaluri* and their utility as live attenuated vaccines in laboratory zebrafish *Danio rerio*, USDA 1433, \$7,000.00
- Louisiana Cooperative Fish Disease Project, Louisiana Department of Wildlife and Fisheries, \$120,000.00

□ Publications:

Journal Article, Academic Journal:

- Outbreaks of edwardsiellosis caused by *Edwardsiella piscicida* and *Edwardsiella tarda* in barramundi (*Lates calcarifer*), Loch, Tom; Hawke, John; Reichley, Stephen; Faisal, Mohamed; Del Piero, Fabio; Griffin, Matt, 2017, *Aquaculture/Elsevier* , Volume: 481, Pages: 202-210
- Whole genome sequence of *Photobacterium damsela* subsp. *piscicida* strain 91-197 isolated from hybrid striped bass (*Morone* sp.) in the USA., Teru, Yuki; Hikima, Jun-chi; Kono, Tomoya; Sakai, Masahiro; Takano, Tomokazu; Hawke, John; Takeyama, Haruko; Aoki, Takashi, 2017, *Genome Announcements/ASM* , Volume: 5, Number: 29, Pages: 2
- *Perezia nelsoni* (Microsporidia) in *Agmasoma penaei*-infected Atlantic white shrimp *Litopenaeus setiferus* (Penaeidae, Decapoda) and phylogenetic analysis of *Perezia* spp. complex. , Sokolova, Yuliya; Hawke, John, 2016, *Protistology* , Volume: 10, Number: (3), Pages: 67-78
- Improved broth microdilution method for antimicrobial susceptibility testing of *Francisella noatunensis* ssp. *orientalis*, Soto, Esteban; Halliday-Simmonds, Iona; Francis, Stewart; Fraites, Trellor; Martinez-Lopez, Beatriz; Wiles, Judy; Hawke, John; Endris, Richard, 2016, *Journal of Aquatic Animal Health/Taylor and Francis* , Volume: 28, Pages: 199-207.

- Experimental Inoculation of Louisiana red swamp crayfish *Procambarus clarkii* with white spot syndrome virus (WSSV) , Pace, Barclay; Hawke, John; Subramanian, Ramesh; Green, Christopher, 2016, Diseases of Aquatic Organisms/Inter-research , Volume: 120, Pages: 143-150
- Draft genome sequence of fish-pathogen, *Mycobacterium pseudoshottsii* strain JCM15466, closely related species to *M. marinum* , Hikima, Jun-chi; Sakai, Masahiro; Aoki, Takashi; Takeyama, Haruko; Hawke, John; Mori, Kazuki; Tashiro, Kosuke; Kuhara, Satoru, 2016, Genome Announcements/ASM , Volume: 4, Number: 1, Pages: 1-2
- Draft Genome Sequences of *Edwardsiella ictaluri* Strains LADL11-100 and LADL11-194 Isolated from Zebrafish *Danio rerio.*, Wang, R; Tekedar, H; Lawrence, M; Chouljenko, Vladimir; Kim, J; Kim, N; Kousoulas, Konstantin; Hawke, John, 2015, Genome announcements , Volume: 3, Number: 6
- Comparison of *Edwardsiella ictaluri* isolates from different hosts and geographic locations. , Griffin, Matt ; Reichley, Stephen; Greenway, Terrence; Quiniou, Sylvie ; Ware, Cynthia ; Gao, Dana; Gaunt, Patricia; Yanong, Roy; Poudel, Deborah; Hawke, John; Soto, Esteban, 2015, Journal of Fish Diseases , Pages: 23
- Draft genome sequences of *Edwardsiella ictaluri* strains LADL11-100 and LADL11-194, Wang, Rui; Tekedar, Hasan; Lawrence, Mark; Chouljenko, Vladimir; Kim, Joohyun ; Kim, Nayong; Kousoulas, Konstantin; Hawke, John, 2015, Genome Announcements/ASM , Volume: 3, Number: 6, Pages: 1-2
- Characterization of isolates of *Streptococcus agalactiae* from diseased farmed and wild marine fish from the U.S. Gulf Coast, Latin America, and Thailand. , Soto, Esteban; Wang, Rui ; Wiles, Judy; Baumgartner, Wes; Green, Christopher; Plumb, John; Hawke, John, 2015, Journal of Aquatic Animal Health/Taylor and Francis , Volume: 27, Pages: 123-134
- Prevalence and distribution of three protozoan symbionts in blue crab (*Callinectes sapidus*) populations across Louisiana, USA., Rogers, Holly; Taylor, Sabrina; Hawke, John; Anderson-Lively, Julie, 2015, Diseases of Aquatic Organisms/Inter-research , Volume: 114, Number: 1, Pages: 1-10
- Variations in prevalence of viral, bacterial, and rhyzocephalan diseases and parasites of the blue crab (*Callinectes sapidus*), Rogers, Holly; Taylor, Sabrina; Hawke, John; Anderson-Lively, Julie, 2015, Journal of Invertebrate Pathology , Volume: 127, Pages: 54-62
- Morphology and phylogeny of *Agmasoma penaei* (Microsporidia) from the type host, *Litopenaeus setiferus*, and the type locality, Louisiana, USA. , Sokolova, Yuliya; Pelin, Adrian ; Hawke, John; Corradi, Nicolas, 2015,

International Journal for Parasitology/Elsevier , Volume: 45,
Number: 1, Pages: 1-16

- Disease, parasite, and commensal prevalences for blue crab *Callinectes sapidus* at shedding facilities in Louisiana, USA., Rogers, Holly; Taylor, Sabrina; Hawke, John; Schott, Eric; Anderson-Lively, Julie, 2015, Diseases of Aquatic Organisms/Inter-research , Volume: 112, Number: 3, Pages: 207-217
- Phenotypic and genotypic heterogeneity among *Streptococcus iniae* isolates recovered from cultured and wild fish in North America, Central America and the Caribbean Islands, Chou, Lucy; Griffin, Matt; Fraites, Trelor ; Ware, Cynthia; Ferguson, Hugh ; Keirstad, Natalie; Brake, John; Wiles, Judy; Hawke, John; Kearney, Michael ; Getchell, Rodman; Gaunt, Patricia; Soto, Esteban , 2014, Journal of Aquatic Animal Health , Volume: 26, Number: 4, Pages: 263-271
- Edwardsiellosis caused by *Edwardsiella ictaluri* in laboratory populations of zebrafish *Danio rerio*. , Hawke, John; Kent, Michael; Rogge, Matt ; Baumgartner, Wes; Wiles, Judy ; Shelley, Johnny ; Savolainen, Christine; Wagner, Robert; Murray, Katy; Peterson, Tracy, 2013, Journal of Aquatic Animal Health , Volume: 25, Number: (3), Pages: 171-183
- Molecular and immunohistochemical diagnosis of *Francisella noatunensis* subsp. *orientalis* from formalin-fixed, paraffin embedded tissues. , Hawke, John; Soto, Esteban ; Illannes, Oscar; Hilchie, David; Morales, Juan; Sunyakumthorn, Piyanate; Goodwin, Andrew; Riggs, Allen; Yanong, Roy; Poudel, Deborah; Francis-Floyd, Ruth; Arauz, Maziel; Bogdanovich, Lewis; Castillo-Alcala, Fernanda, 2012, Journal of Veterinary Diagnostic Evaluation/SAGE , Volume: 24, Number: (5), Pages: 840-845
- Genetic analysis and antimicrobial susceptibility of *Francisella noatunensis* subsp. *orientalis* (Syn. *F. asiatica*) isolates from fish. , Hawke, John; Soto, Esteban; Griffin, Matt; Wiles, Judy, 2011, Veterinary Microbiology , Volume: 154, Pages: 407-412
- *Francisella asiatica* as the causative agent of piscine francisellosis in the USA, Hawke, John; Soto, Esteban; Baumgartner, Wes; Wiles, Judy, 2011, Journal of Veterinary Diagnostic Investigation , Volume: 23, Number: 4, Pages: 821-825
- Attenuated *Francisella asiatica* igIC mutant induces protective immunity to francisellosis in tilapia , Hawke, John; Soto, Esteban ; Judy, Wiles; Elzer, Philip; Macaluso, Kevin, 2011, Vaccine , Volume: 29, Pages: 5
- Development of a real-time PCR assay for identification and quantification of the fish pathogen *Francisella noatunensis* subsp. *orientalis*, Hawke, John; Soto, Esteban; Bowles, Kimberly ; Fernandez, Denise, 2010, Diseases of Aquatic

Organisms/Inter-Research , Volume: 89, Pages: 199-207

- In Vitro and In Vivo Efficacy of Florfenicol for Treatment of Francisella asiatica Infection in Tilapia, Hawke, John; Soto, Esteban; Endris, Richard, 2010, Antimicrobial Agents and Chemotherapy/ASM , Volume: 54, Number: 11, Pages: 4664-4670
- Interaction of Francisella asiatica with Tilapia (Oreochromis niloticus), Hawke, John; Soto, Esteban ; Thune, Ronald; Fernandez, Denise, 2010, Infection and Immunity/ASM , Volume: 78, Number: 5, Pages: 2070-2078

Book, Textbook Chapter:

- Chapter 19. Photobacterium damsela, Hawke, John, 2017, Publisher: CABI, Pages: 258-271
- Chapter 17. Francisella noatunensis., Soto, Esteban; Hawke, John, 2017, Publisher: CABI, Pages: 233-245
- Chapter 20. The biology and management of the zebrafish, Esmail, Michael; Astrofsky, Keith ; Lawrence, Christian; Serluca, Fabrizio; Hawke, John; Baumgartner, Wes, 2015, Publisher: Elsevier-Academic Press, Pages: 1015-1045

Monograph:

- Enteric Septicemia of Catfish, Hawke, John, 2015



Louisiana State University
Baton Rouge, Louisiana 70803
webmaster@lsu.edu



Provide Website Feedback
Accessibility

School of Veterinary
Medicine
Skip Bertman Drive
Baton Rouge, LA 70803

Veterinary Teaching
Hospital
Pets and small exotics
225-578-9600
Horses and farm animals
225-578-9500
Dean's Office 225-578-
9900
Fax: 225-578-9916
vetmed@lsu.edu

APPENDIX B - Ramboll Ecotoxicology Laboratory Overview

ECOTOXICOLOGY CAPABILITIES & SERVICES



Ramboll provides high-quality technical and integrated consulting and engineering services in the field of Industrial Wastewater Management. We are a highly responsive organization, dedicated to adding value through a problem-solving approach to the environmental issues we and our primarily industrial clients face. Specific service areas include:

- Industrial Wastewater Management
- Detailed Design Engineering / Construction Services
- Innovative Technology Development
- Ecotoxicological Services
- Regulatory Management Services
- Water Resources Engineering
- Operations Services and Support

Our **Ecotoxicology Group** provides services in the areas of laboratory toxicity testing and identification, field biological and water quality assessment, and related consulting services. The following services are provided as stand-alone services or in support of interdisciplinary projects:

Laboratory Services	Whole Effluent Toxicity (WET) Testing (freshwater & saltwater) Terrestrial Toxicity Tests Product Toxicity Tests (aquatic, sediment, terrestrial) Toxicity Identification Evaluation (TIE) Microtox® Testing	Site-specific Translators and Criteria Derivation Great Lakes Initiative (GLI) Criteria Derivation Sediment and Sediment Elutriate Toxicity Tests Toxicity Reduction Evaluation (TRE)
Field Services	Biological Assessment (Fish and Macroinvertebrates) Water Quality and Habitat Assessments	Endangered Species Assessments Wetlands Delineation Fish Health Assessment (including Anomalies)
Consulting Services	Aquatic Resource Alteration Permitting (ARAP) NPDES Permitting Support Erosion Control Plans Ecological Risk/Impact Assessment	Stormwater Endangered Species Impact Assessment Site-specific Permit Limit Derivation Water Effects Ratios (WERS)

For additional information on Ecotoxicology Services, call Rick Lockwood at (615) 277-7523 / e-mail rlockwood@ramboll.com, or Liza Heise at (615) 277-7517 / e-mail lheise@ramboll.com

These services are tailored to meet client needs on a case-by-case basis; they are offered as stand-alone services or as part of an engineering and/or regulatory compliance study designed to address and meet regulatory requirements in the most cost-effective and beneficial manner to the client.

Lab Certifications or formal data approval in: AR, CA, FL, IA, GA, KY, LA, MI, NC, OK, SC, VA, WI, WV. NELAP Accredited Data Accepted in Numerous Other States.

RAMBOLL FIELD ASSESSMENT AND RELATED EXPERIENCE OVERVIEW

Ramboll provides various water quality, habitat, and biological and ecological assessment services. Most studies have been conducted in freshwater systems, although some have been in estuarine and open-ocean environments. These include:

- Water quality assessment (in situ and sample collection)
- Sediment sampling (for chemical and biological analyses)
- Mixing zone and diffuser/dye studies
- Habitat quality assessment
- Fish sampling (small streams, rivers, reservoirs)
- Fish pathogen assessment, fish kill response
- Wetlands assessments
- Endangered species assessments
- Aquatic Resource Alteration Permits (ARAP)
- Ecological hazard/risk assessment
- Chemical-specific bioavailability assessments and permitting
- Macroinvertebrate surveys

Various fish and macroinvertebrate collection equipment, water quality meters, sediment and water sampling devices, and related equipment are supplied as needed.

Project Summaries

Many biological assessments necessarily incorporate bioassessment, habitat quality assessment, and water quality assessment in the environmental compliance and other decision-making processes. Others are very specific to a question (e.g., presence/absence of fish pathogens) or incident (e.g., fish kill) at hand. Briefly summarized below are selected projects integrating at least one biological and one or more abiotic components (i.e., sediment, habitat, or water quality) of an ecosystem, and projects focusing on threatened and endangered species. Other project summaries include site-specific chemical bioavailability studies, and fish kill investigations. Finally, a summary of senior biologists' qualifications is presented.

Fish Impingement and Entrainment Studies (316b)

Ramboll conducted a multiyear assessment of fish impingement in intake structures at a steel mill on Lake Michigan in IN. Fish collected off of intake screens were identified to species, age class, and health status (including identification of disease or deformity). Fish collected live off of screens were held for determination of irreversible traumatic effects. Data analysis was used to determine the efficacy of the (live) fish return system, and the potential for engineering or process changes to decrease impingement rates to healthy fish.

Integrated Assessments

Benthos, Habitat and Water Quality (TN). In support of site reclamation efforts by Glenn Springs Holdings Company and Barge Waggoner Sumner & Cannon, Inc., Ramboll has conducted water quality (in situ and sample collection), habitat quality, and macroinvertebrate community assessments at 10 or more locations annually (including a "reference stream") at the inactive Copper Hill mining site in Polk County Tennessee. Efforts are ongoing, and have occurred for the six three years. Efforts also included site-wide habitat quality assessments of major streams and their tributaries.

This established baseline habitat quality conditions for all reaches of most perennial streams on site. Supporting efforts included statistical evaluations of benthic macroinvertebrate data, derivation of "biological integrity criteria", stressor identification support, biological and water quality characterization and baseline development pre-construction for wetlands treatment systems, and report preparation and full field support of all water quality, habitat quality, and biological community assessments. Many activities included interfacing with the clients and regulatory agencies. At present, the bioassessment results indicate success of selected remediation efforts and help in the prioritization of remediation activities.

Lake Michigan Benthos, Algae, Habitat and Sediment Quality (IN). Ramboll conducted an assessment of near-shore Southern Basin of Lake Michigan aquatic habitats, resident

organisms, water quality, and sediment characteristics in support of contaminants assessments related to an NPDES discharge permit. A literature review of resident biota potentially impacted, including endangered species, was also conducted. Field efforts included sediment collection and particle size distribution to assist in interpretation of benthic community data. In addition, benthos, chlorophyll a, and the phytoplankton (algae) community was characterized to serve as a potentially highly sensitive suite of receptor communities. In addition, diatom phytoplankton was specifically evaluated for indications of effects from increases in chloride and suspended solids discharges. The monitoring efforts were implemented to establish regional baseline conditions prior to installation of an alternative discharge configuration that included a multi-port diffuser so that lake-wide and site-specific impacts could be discerned.

Fisheries Assessments, Temperature Effects (IL). Ramboll performed seasonal (winter and summer) water quality, habitat quality, thermal regime, and fish community assessments of two central-Illinois streams to determine the possible effects of increased ambient temperatures downstream of an industrial discharge. Daily water temperature monitoring was conducted year-round by plant personnel. Index of Biotic Integrity (IBI) values, specific to Illinois, were used to make the determination that the nominal temperature increases observed were not detrimental to local fish communities. Specific IBI metrics included: species diversity, species sensitivity, and disease and deformity indices. Site-specific evaluation of the discharge site indicated habitat quality was more of a determinant than the temperature regime to fish community composition and diversity.

Fish Communities, Pathogens, Toxics, Water Quality (TN). Ramboll personnel conducted assessments of the near-discharge and far-field fish communities downstream of a manufacturing site and an aerospace testing center, as well as pertinent reference streams. Although supported by water quality and habitat assessment, the focus of the study was to assess potential pathogen effects on receiving stream fish. Fish collections, primarily via seine and gill net, were conducted to collect fish representative of various trophic

(feeding) levels. Field assessments for deformities, eroded fins, lesions, and tumors (DELTs) laboratory assessments by a veterinary fish pathologist indicated a lack of pathogen impacts to resident fish. This was contrary to what laboratory effluent toxicity tests had indicated as a potential problem, and allowances for alternative effluent toxicity testing procedures were obtained. Toxicity testing support was also provided by Ramboll.

Fish, Benthos, Crayfish, Periphyton, Water Quality (GA). Ramboll personnel conducted water quality and habitat quality, and fish, benthic macroinvertebrate, and periphyton (attached algae) community assessments at several locations in a stream receiving an effluent discharge, and in a "reference stream" near a textile site in Georgia. Fish community assessment include diversity and sensitivity indices as well as identification of disease or deformity. Special emphasis was placed on assessment of the crayfish community, given the possible presence of a threatened and endangered crayfish species. The preponderance of the biological data, especially the fish, crayfish, and benthic community data, indicated the potential for near-field effects that could be separated from regional impacts that were associated with the extensive urban land use in the area,

Fish, Bioaccumulative Chemicals (TN). Ramboll personnel collected various game fish and non-game species in support of assessing tissue levels of pesticides and PCBs. Multiple trophic (feeding) levels for the fish assemblage were assessed to better define the extent of bioaccumulative organic chemicals in the ecosystem, and potential hazards with respect to fish consumption by local anglers. Gill-netting and electroshocking methods were utilized in fish collection. Supporting water quality and habitat quality assessments were conducted as was fish collection for deformity and pathogen assessment. Toxicity testing was also conducted in support of site-specific alternative toxicity test methods for the NPDES discharge permit.

Environmental Assessments (US Army, KY and TN). Ramboll personnel served as the prime contractor for Environmental Assessments (EAs) for nine site-development projects at Ft. Campbell. Almost all projects included end-products that would be used by military

personnel and/or the public. Our primary role was project oversight, obtaining and interpreting data related to biological/ecological and archaeological resources, and data interpretation with respect to water quality issues. Project management included task coordination of specialists such as biologists and toxicologists, archaeologists, and land-planning personnel, and confirmation of presence/absence of endangered species. Various liaison between Ft. Campbell project managers and environmental personnel and Army Corps of Engineers (Nashville District) was provided.

T&E AND UNIQUE SPECIES

Threatened and Endangered (T&E) species and other unique species or ecosystems are routinely a part of all permit criteria and regulatory associated projects. In most cases, habitat limitation is the key factor for presence or absence of T&E species. When T&E species may be present, the approach is often to adopt engineering or management solutions that protect the ecological niche or habitat rather than the individual organism. An example includes design of effluent diffusers with mixing zones above the substrate so that benthic organisms such as mussels and clams are completely removed from effluent contact. This approach allows for the protection of resident and T&E species, and potential T&E species rather than protection of a single organism. Selected T&E and unique species/ecosystem attribute projects are detailed below.

Wetlands, Endangered Species (MS). Ramboll provided wetlands delineation and endangered species assessment services for a site in Mississippi on which a hotel and golf course were planned. Key activities included documenting wetlands areas with development restrictions and a focus on extensive evaluation of potential endangered species concerns that may limit continued development of the planned project. Upper Santa Clara River (CA). Ramboll recently completed database searches for chloride-sensitive species, and threatened and endangered species in the Upper Santa Clara River near Los Angeles, California. Site habitat assessment will soon be conducted to

determine the role of habitat, as opposed to elevated chloride levels, in possible inhibition of threatened and endangered species populations. A special emphasis is being placed on understanding the role in various plants, especially cottonwoods, in supporting T&E species. Toxicological data for chlorides and a recently-released program to estimate toxicity to T&E species are also being used determine the likelihood that chlorides inhibit T&E species communities.

Additionally, state and/or federal database reviews for T&E species have been conducted for the following projects:

- Potential stormwater impacts (Industrial site, TN);
- Unionid mussel community in Deep Fork River, MO
- NEPA-related Environmental Assessments (9 sites), Ft. Campbell (KY);
- Stream impact study and erosion control plan for a construction site (TN);
- Site-specific chloride limits, Pennsylvania streams.
- Dredging operations (Grand Calumet River) and NPDES permitting, industrial site (IN).
- West slope cutthroat trout, Montana.

Chemical, Effluent Toxicity to Resident Organisms

City of Franklin, TN. Ramboll conducts annual water quality, habitat quality, and benthic macroinvertebrate community assessment of the Harpeth River for the City of Franklin, Tennessee. Monitoring efforts are conducted in support of the client's NPDES discharge permit. Monitoring of the river fish and benthic communities is augmented by toxicity testing of fish and invertebrates.

Chromite Ore Bioavailability (New Jersey Estuary). Ramboll personnel evaluated chromium (total and hexavalent) concentrations in sediment, water, and biota (fish, plant roots and shoots, and blue crabs) in an enclosed estuarine bay which had received historical chromite ore deposits. Sediment analyses included sulfides and mercaptans to characterize reducing conditions in the sediments. Other characterizations included particle size and organic carbon assessments, and measurements of non-chromite ore associated metals to document that metals accumulations were largely a function of sediment characteristics promoting

metals accumulation. Documentation of the highly reduced sediment characteristics, which maintained metals in non-mobile and hence biologically unavailable forms, supported findings of low concentrations of dissolved metals in the water column and low metals concentrations in all three biological components (fish, plants, crabs).

Fluoride Toxicity Assessments (Laboratory).

Ramboll personnel evaluated the toxicity of sodium fluoride to water column and benthic organisms using static-renewal, and flow-through toxicity tests. Benthic organism testing included amphipods (*Hyalella azteca*), snails (*Physa*), aquatic worms (*Lumbriculus variegatus*), fathead minnow (*Pimephales promelas*) and midges (*Chironomus tentans*). Flow through / full life stage toxicity testing of fathead minnow included analysis of teratogenic effects. Supporting water quality and fluoride analyses were conducted. Quality Assurance/Quality Control (QA\QC) criteria for precision and accuracy of fluoride test concentrations were consistently met. The data developed were used in calculation of Great Lakes Initiative (GLI) criteria for fluoride.

Whole Sediment and Sediment Elutriate, Water Testing (Laboratory, numerous clients).

Static, static-renewal, and flow-through testing of numerous effluents, surface waters, sediments and sediment elutriates have been conducted by Ramboll personnel. Static-renewal and flow-through testing of whole sediments utilized amphipods, sediment elutriate tests utilized *Ceriodaphnia dubia*, fathead minnows (*Pimephales promelas*), and bluegill sunfish (*Lepomis macrochirus*). Effluent and surface water testing has included evaluations of survival, growth, reproduction, and disease or teratogenic anomalies with *Ceriodaphnia dubia*, *Daphnia magna* and *D. pulex*, fathead minnow, rainbow trout, mysid shrimp, killifish, sheepshead minnows, and inland silversides, among others.

Chloride and Sulfate Impact Assessments (PA).

Ramboll implanted a project for a client in Pennsylvania to assess the impact on a receiving stream's aquatic ecosystem with in-stream concentrations of total dissolved solids (TDS) above 500 mg/L. Assessment of Impacts were based upon the site-specific concentration ratios between TDS and chloride and sulfate and each constituents contribution

to a toxicity response. The discharge location was in the Delaware River Basin and the client was operating under a TDS maximum daily limit of 500 mg/L. However, due to drought, the client temporarily received relief from the limit but chose to investigate whether a higher TDS concentration adversely affects the stream biota. The efforts included a literature review, a T&E species review, and biological assessment at the receiving stream. In conducting the literature review, a discrepancies between the sodium sulfate values in the original reports and the values presented in the USEPA AQUIRE database was found. Communication with the USEPA contractor maintaining the AQUIRE database confirmed the errors. The literature review showed that an increase in TDS above 500 mg/L to the stream would not adversely harm the ecosystem, and that chloride concentrations of 1,050 mg/L were protective of aquatic life under 7Q10 flow conditions. The biological assessment supported the results of literature review and stream bioassessment results indicated an in-stream threshold of 1,723 mg/L chloride should not be exceeded for the protection of resident species. These results were accepted by the PADEP.

Salt Toxicant Confirmation (MI).

Ramboll personnel conducted a review of toxicity tests, MSDS, and Toxicity Identification Evaluation (TIE) data for a food processing facility in Michigan. The client sought additional counsel on the role of salts (primarily NaCl) in causing effluent toxicity, and a determination of whether an alternative test species was more suitable for effluent monitoring. Ramboll conducted additional chemical-specific and WET testing that included conventional chronic *Ceriodaphnia* and fathead minnow (7 day tests) and *Daphnia magna* 28 day tests, and preparation and testing of synthetic effluent to demonstrate NaCl as the primary effluent toxicant. Ramboll assisted the client with successful negotiations with Michigan DEQ regarding alternative test species selection, and use of a copper translator to address other potential effluent toxicants.

Revised Sulfate Water Quality Criteria (IL).

Ramboll participated in an IEPA-directed effort to revise the sulfate water quality industry-specific standard for the state of Illinois. The project included compiling aquatic toxicity data, determining data validity, and deriving

criteria using the recalculation procedure. Due to a lack of data, a standard was not developed. Efforts were redirected to evaluate field data to validate a proposed value.

Salt/Toxicant Identification (KY). Ramboll conducted effluent toxicity tests of key wastewaters at their percent contribution to the final effluent at an industrial site in Kentucky. Statistical evaluations of effluent toxicity levels versus various flow and in-plant waste stream chemical parameters were used to identify TDS as the key effluent toxicant to *Ceriodaphnia*. Literature reviews on the toxicity of key TDS constituents (sodium, magnesium, calcium, chloride, and sulfate) and toxicity testing of key salts (e.g., sodium chloride) were used to confirm the role of salts in causing toxicity.

Salt, Nitrite, Nitrate Toxicity Interactions (IN). Ramboll completed a Toxicity Identification Evaluation and Toxicity Reduction Evaluation (TIE/TRE) study for a steel production effluent that exhibited acute toxicity to the fathead minnow. Studies included review of process chemical additions, toxicity testing of internal source waters, effluent fractionation testing, bench simulations of internal process waters, chemical analyses, toxicity testing of synthetic effluents, and chemical additions to the effluent to reproduce effluent toxicity via suspected constituents. The study determined that nitrite was the primary toxicant. Nitrate and TDS (primarily due to calcium, chloride, and sulfate) played secondary roles in toxicity that interfered with nitrite toxicity. Key findings were that *Ceriodaphnia* were more sensitive to nitrite than expected and increased hardness or TDS decreased nitrite toxicity.

CN Recalculation Procedure, NPDES Permitting (TN). Ramboll derived a site-specific water quality limit for cyanide after application of the recently modified EPA recalculation procedure. A site-specific list of species known to “occur at the site” was developed using historical databases. The species deletion process was then applied by comparing the toxicological data in the Ambient Water Quality Criteria Document for Cyanide to the taxonomic diversity at the site. When species acceptable for deletion were identified, the water quality criteria were re-derived.

Copper Water Effects Ratio (CA). Ramboll conducted a *Ceriodaphnia* water effects ratio (WER) study using the most recent USEPA “streamlined” Water Effects Ratio (WER) procedures for copper. Acute (48 hour) WER values for a municipal effluent (Yucaipa Valley WWTP) in California were derived. WER values were derived for the “total” and “dissolved” (0.45 μ m filtered) phases of copper. All calculations included corrections for water hardness, and determined appropriate Species Mean Acute Values (SMAVs) for WER calculations as specified by USEPA. Effluent chemistry was sufficient to decrease copper bioavailability (acute toxicity). For example, the “total” copper WER for the effluent was 4.39. These data were ultimately accepted by the regulatory agency for modification of the WWTP copper discharge limit.

Dissolved Metals Translator (DMT) Studies (Various States). Ramboll has assisted clients in preparing and conducting sampling and analyses plans followed by data interpretation and presentation to regulatory agencies in support of using DMT to revise Water Quality Based Effluent Limits (WQBELs). Some of the work plans were conducted prior to state regulation and were based on draft guidance and negotiations with agencies while others were developed using the USEPA interim guidance and state regulations (normally derived from the federal GLI regulations). Metals evaluated were arsenic, cadmium, chromium, copper, nickel, lead, selenium, and zinc. Waters tested were effluents from POTW, locomotive manufacturing, petroleum refinery, OCPSF facilities, and food processors with discharges into small streams, large rivers, and large lakes.

Zinc Site-Specific Aquatic Life Criteria (OH). Ramboll performed a desktop assessment of deriving site-specific aquatic life criteria for zinc for American Ditch and a portion of Alum Creek in Ohio. The procedure utilized to derive site-specific criteria was USEPA’s Recalculation Procedure. The objective of this project was to determine if the recalculation procedure would provide relief from NPDES effluent limitations for the client.

Copper Water Effects Ratio (GA). Ramboll designed studies to evaluate the toxicity and bioavailability of copper in a textile effluent in Georgia. Studies evaluated the toxicity of free

and complex copper in dyes. The fate and toxicity of copper given various travel times and downstream water chemistry conditions were also being assessed to assure safety after discharge. TOC and alkalinity appeared to be the key parameters dictating copper toxicity in the effluent and receiving stream. WER values for two test species and three seasonal conditions were developed for use in deriving site-specific permit conditions which reflect the less toxic forms of copper in the effluent.

Site-specific Ammonia Criteria (AL). The Ramboll Toxicity Testing Facility (Nashville TN) participated in a study to determine whether water quality conditions in a utility effluent were such that ammonia effects were different than could be predicted by conventional pH/toxicity modeling of ammonia toxicity and subsequent criteria derivation. Effluent pH was held constant at 7.5, 8.0 and 8.5 s.u. using "biological buffers" (MOPS and POPSO). Effluent samples were spiked with ammonium sulfate. Fathead minnow (*Pimephales promelas*) and water flea (*Ceriodaphnia dubia*) toxicity tests were conducted to establish effluent-specific acute lethal (LC50) and chronic (seven day) sub-lethal total ammonia concentrations as a function of pH. Results of acute toxicity testing indicated that predicted and observed effects were virtually the same for both test species. Chronic testing with the fathead minnow indicated similar results. However, chronic testing with *Ceriodaphnia* indicated confounding effects, likely due to aluminum and changes in its toxicity as a function of pH and resultant solubility. Additional testing may be required to determine whether other species such as clam or mussel larvae are likely to drive ammonia criteria derivation.

Cyanide Bioavailability, Criteria, Permitting (IN). Ramboll assessed the toxicity of the free and metallic forms of cyanide in a coke plant effluent. A semi-permeable membrane technique was used to assess only the freely dissociated (toxic) forms of cyanide for comparison to the Weak Acid Dissociable (WAD) and "total" cyanide concentrations as well as effluent toxicity data. Acute and chronic toxicity testing of reagent-grade HCN (free cyanide) and ferrous cyanides (complexed forms) was conducted. Additionally, effluent and chemical tests with various organisms, including trout, were

conducted to recalculate more applicable criteria for the receiving stream. Demonstrations that the semi-permeable membrane method accurately reflects the toxic forms of effluent cyanide, and demonstrating the virtual lack of toxicity of metalocyanides provided the client with discharge limits based on free cyanide for the specific receiving stream organisms.

GLI Tier II Derivation of Benzo(a)pyrene and Benzo(a)anthracene. Ramboll personnel were contracted to review the Great Lakes Initiative (GLI) Tier II derivation of Benzo(a)pyrene and Benzo(a)anthracene for a U.S Steel (USS) permit renewal in the state of Indiana. It was determined that the criteria were not valid, based on data quality.

Rederive Water Quality Criteria for 2- and 4-chloroaniline (IL). Ramboll participated in a study to rederive Illinois water quality criteria for 2- and 4-chloroaniline. Project included review and compilation of aquatic toxicity data and data validity determination. Also included was development of a work plan for conducting additional toxicity testing to develop a valid database for criteria development according to Illinois regulations (35 IAC 302 Subpart F).

FISH KILL INVESTIGATIONS

Manufacturing Site (TN). Ramboll investigated a fish kill at a small lake on an industrial site after employees noticed numerous dead game fish and some rough fish. The incident occurred following an employee fishing event and picnic the previous weekend. Depth profiles of temperature and Dissolved Oxygen (DO), coupled with the observations of the types of fish affected and their low DO tolerance relative to the other fish indicated that low DO and high temperatures were the likely cause of the fish kill. Algae blooms were evident during the site investigation. Nutrient sampling indicated that nutrient loading and the resultant algae blooms and decay likely contributed to oxygen depletion, and the recent fish stocking in support of the fishing event may have triggered the fish kill. A review of Material Safety Data Sheet (MSDS) data for lawn management chemicals used in the area surrounding the lake indicated that these materials were likely not contributors to the fish kill. Maintenance personnel have decreased fertilizer application rates, and fish

stocking has been discontinued prior to high-use events on the lake.

Chemical Processing Site (SC). Ramboll investigated a small lake near an industrial site in response to a fish kill. The lake was a high-intensity public fishing facility downhill of the chemical processing site. Although the site investigation indicated possible routes of chemical contact, if there were a contaminated stormwater discharge, no such discharges were known to have occurred, and the fish kill was not coincidental with recent rainfall. Based on visual observations of high turbidity and knowledge of the lake's primary use, it was suspected that high Biochemical Oxygen Demand (BOD) wastes and high fish density caused low DO conditions. DO monitoring was conducted and it was confirmed that during pre-dawn hours when DO should be lowest (due to minimum sunlight and the longest period without algal photosynthesis), almost non-detectable levels of DO were present. These conditions confirmed DO depletion, as opposed to chemical toxicity, as the cause of the fish kill.

Steel Manufacturing Site (IN). Ramboll investigated the possible role of effluent toxicity in a fish kill in a large river. Citizen complaints associated with the fish kill seemed to implicate this discharge as the cause of the fish kill. Because no waters other than the NPDES-permitted effluent discharged into the river, efforts focused on the effluent. Chronic (long-term, 7-day) effluent toxicity tests were conducted with a fish (fathead minnow) and invertebrate (*Ceriodaphnia dubia*). No acute (short-term) toxicity such as that needed to induce a fish kill was observed for either organism, and chronic toxicity was minimal. Although a short-term, toxic discharge could have been missed in this assessment, this testing did not implicate the steel manufacturer as a source of waters impacting fish or other aquatic life. Recent sharp changes in ambient air temperature and any associated fast changes in water temperature were implicated as the likely cause of the fish kill.

Confidential POTW, Midwest. Ramboll investigated a Publicly Owned Treatment Works (POTW) discharge that resulted in a kill of more than 100 tons of fish. Ramboll investigated the POTW operations for possible cause. The influent and effluent were sampled

to determine potential toxicants and their fate and effect in the treatment plant and the river. Although nitrification ceased around the time of the discharge, the increase in ammonia concentrations (and increased detection of carbon disulfide) were not sufficient to cause the scale of the observed fish kill.

Ramboll investigated the Industrial Users (IUs) that were discharging to the POTW and one IU was going through special shutdown activities coincident with the fish kill timeline. The IU had routinely operated a metals wastewater pretreatment process using alkaline precipitation followed by effluent polishing with sodium methyldithiocarbamate (DTC). DTC is known to be toxic and as such is handled carefully when used for wastewater treatment. During the shutdown operations, the normal treatment steps were altered resulting in an unusually large increase in the use of DTC. Furthermore the changed treatment process resulted in DTC being broken down into tetramethylthiuram (thiram). Thiram is an agricultural fungicide known to be highly toxic to fish. High levels of thiram were detected in dead fish tissue samples and implicated as the cause of a fish kill of this magnitude.

Confidential Industry, Midwest. Ramboll investigated a fish kill in the vicinity of an industrial discharge in the Midwest that primarily affected Chinook salmon. The working hypothesis by the regulatory agency was that temperature shock caused the fish kill. Literature data on temperature preferences and tolerance for Chinook salmon were used to determine the likelihood that the client's discharge caused the event. A worst-case scenario based on discharge temperature was compared to the chinook Habitat Suitability Index (HSI) model; the Chinook instantaneous thermal effects curve exposure model; and the cumulative effects thermal exposure model. Results indicated the magnitude of temperatures and duration of exposure, whether instantaneous or cumulative over an 8-day period, was insufficient to cause the fish kill, or pose a risk for chinook mortality. Field observations did, however, suggest that something other than a natural post-spawning die off may have taken place. Infection from a number of warm-water pathogens was implicated as a possible cause given other site-specific factors prior to the fish kill event.