3.3 *Yersinia ruckeri* (Enteric Redmouth Disease, ERM)

Yersinia ruckeri affects fingerlings of potentially all salmonids. In salmonids, mortality increases dramatically following periods of stress due to environmental conditions and handling. Survivors of enteric redmouth disease outbreaks become carriers, after which the bacteria are shed from the intestinal tract in large numbers during a regular 36 to 40 day cycle. Severity of infection decreases at water temperatures below 10°C. *Yersinia ruckeri* has been isolated from the kidney of some non-salmonids as well. It is generally accepted that, although not ubiquitous in the environment, any freshwater fish can carry the bacteria with or without exhibiting signs of the disease (Bullock 1984; Thoesen 1994).

A. Summary of Screening Test

1. Bacterial Culture and Biochemical Analysis

a. Aseptically inoculate samples onto tubes or plates as described in Section 2, 2.2 Sampling.

b. Incubate for 24 to 48 hours at 20 to 24°C, (room temperature). If no growth occurs at either 24 or 48 hours, record this information on the data sheet. **If no growth occurs after 96 hours, samples are discarded and reported as negative for *Y. ruckeri***.

c. When primary culture occurs on tubes or plates use a sterile loop or needle to select a single isolated colony to subculture onto fresh TSA or BHIA plates. If colonies are not well isolated on the original media, a new plate will have to be streaked over the entire plate surface to achieve isolation of bacteria.

d. Incubate at 20 to 24°C for 24 hours to allow bacterial growth; all tests should be performed on 24 to 48 hour cultures.

e. Using a sterile needle or small loop, pick individual distinct bacterial colonies to represent each colony type. Use of a dissecting microscope can aid in distinguishing between differing colony types. Assign an isolate number to each pure colony and record all colony characteristics on the data sheet.

f. Begin initial identification of pure strain bacterial cultures (Section 2, 3.A1 Laboratory Reference Flow Chart Appendix 1).

   i. Gram Determination (Section 2, 3.8.A “Gram Reaction”)
   Yersinia ruckeri is Gram-negative. **Gram-positive isolates may be reported as negative for *Y. ruckeri*.**

   ii. Presence of Cytochrome Oxidase (CO) (Section 2, 3.8.B “Cytochrome Oxidase”)

Yersinia ruckeri is CO negative. **CO positive isolates may be reported negative for *Y. ruckeri***.

iii. Motility (Section 2, 3.8.C "Motility")
Most isolates of *Yersinia ruckeri are* motile, but non-motile isolates have been reported (Wheeler et al. 2009 and Welch et al. 2011).

g. Perform biochemical testing on each isolate (Section 2, 3.A1 Laboratory Reference Flow Chart Appendix 1)

i. Tube Method (Section 2, 3.8.D.1 "Tube Method")

1. Triple Sugar Iron (TSI) (Section 2, 3.8.D.1.b "Triple Sugar Iron (TSI)")
Yersinia ruckeri will yield an alkaline over acid (K/A) or alkaline over acid with gas (K/AG) result. **Any isolate with a result other than this may be reported as negative for *Y. ruckeri*.**

2. Carbohydrate Utilization (Section 2, 3.8.D.1.e "Carbohydrate Utilization")

a. Arabinose cannot be utilized (fermented) by *Y. ruckeri*.

b. Rhamnose cannot be utilized (fermented) by *Y. ruckeri*.

c. Sucrose cannot be utilized (fermented) by *Y. ruckeri*.

i. Salicin cannot be utilized (fermented) by *Y. ruckeri*.

ii. **Isolates yielding positive results for any of these tests may be reported as negative for *Y. ruckeri***.

3. Malonate Test (Section 2, 3.8.D.1.g "Malonate Test")
Isolates yielding positive results for this test may be reported as negative for *Y. ruckeri*.

4. Indole Test (Section 2, 3.8.D.1.d "Indole Test")
Isolates yielding positive results for this test may be reported as negative for *Y. ruckeri*.

5. Esculin Test (Section 2, 3.8.D.1.h "Esculin Test")
Isolates yielding positive results for this test may be reported as negative for *Y. ruckeri*.

6. Decarboxylase Test (Lysine) (Section 2, 3.8.D.1.f "Decarboxylase Test (Lysine)")
Isolates yielding negative results for this test may be reported as negative for *Y. ruckeri*.

7. Sorbitol (Section 2, 3.8.D.1.e "Carbohydrate Utilization")

a. Isolates that satisfy all previous conditions in this section are **PRESUMPTIVELY positive** for *Y. ruckeri*.

b. Sorbitol is used to differentiate between Type I and Type II *Y. ruckeri*.

i. Isolates yielding negative results for this test are **PRESUMPTIVELY positive** for *Y. ruckeri* Type I.

ii. Isolates yielding positive results for this test are **PRESUMPTIVELY positive** for *Y. ruckeri* Type II.

ii. Commercial Identification Systems (Section 2, 3.8.D.2 "Commercial Identification Systems")

1. Biolog (Section 2, 3.8.D.2.b "Biolog")

2. API
If isolates are tested with the commercial system API described in Section 2, 3.8.D.2.a "API-20E," it is recommended that the reference profiles be consulted in Section 2, 3.A2 Profiles Obtained with API-20E for Known Fish Pathogens."

3. When testing is complete, either cryopreserve isolates of interest or discard bacterial plates and biochemical tubes in a biohazard bag and autoclave before proper disposal.

4. Positive control isolates of *Yersinia ruckeri* can be obtained from the American Type Culture collection (ATCC). The Internet location for ATCC is http://www.atcc.org. Below are suggested isolates to use for positive control cultures:

Yersinia ruckeri serovar Type I – ATCC # 29473.

Yersinia ruckeri Type II – ATCC # 29908.

B. Confirmatory Test

1. Fluorescent Antibody Test (FAT) (Section 2, 3.8.E "Fluorescent Antibody Test (FAT)")
FAT is performed on at least one representative isolate from each lot inspected and found positive during screening.

a. A separate antibody must be utilized for confirmation of either Type I or II strains.

b. Positive bacterial isolates will fluoresce strongly and have the same morphology as the positive control.

c. A list of sources from which antibodies may be obtained is provided in Section 2, 3.8.E.6 "Commercial Sources for Antibodies."