Column E Explanation

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1. Registration Number: 54-G-0001

2. Number of animals used in this study: 1,895

3. Species (common name) of animals used in the study: rainbow trout

4. Explain the procedure producing pain and/or distress.

   Juvenile fish were infected with the Flavobacterium psychrophilum, the causative agent of bacterial cold water disease. The pathogen was introduced by intramuscular or intraperitoneal injection of the bacterial preparation in a volume of 25 µL, 50 µL or 100 µL dependent on fish size, and delivered with either a 27- or 26-guage × ½-inch needle while fish were in an anesthetized state. During the 21-day post-challenge observation period, fish experienced typical disease signs including inappetance, altered ventilation and swimming, loss of equilibrium, discoloration, injection-site lesions, and either succumbed to the disease naturally or were identified as being moribund and immediately euthanized via immersion in 250 mg/L of tricaine methanesulfonate for a minimum of 10 minutes following cessation of respiratory movements. We are reporting those fish that experienced more than momentary discomfort in Column E.

5. Attach or include an explanation with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   Few data are available regarding the use of analgesics in fish, and protocols tested to date only pertain only to alleviation of acute pain and distress (Sneddon, 2012, J. Exotic Pet Medicine, 21:32-43). Furthermore, the effects of anesthetics, analgesics, and tranquilizers on disease resistance mechanisms are unresolved and may have interfered with the normal course of disease progression and host response, and thus could not be used in these studies for long-term periods. Tricaine is currently the only anesthetic/analgesic agent approved for use in fish in the U.S., and its long term use for the duration of this study was not feasible.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

   N/A

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1. Registration Number: 54-G-0001

2. Number of animals used in this study: 35

3. Species (common name) of animals used in the study: rainbow trout

4. Explain the procedure producing pain and/or distress.

   Juvenile fish were infected with *Aeromonas salmonicida*, the causative agent of furnuculosis disease. During the 21-day post-challenge observation period, some fish experienced typical signs of disease, including decreased appetite, altered ventilation and swimming, loss of equilibrium, and discoloration. Most fish were humanely euthanized, although some died before euthanasia could be performed. We are reporting those fish that experienced more than momentary discomfort in Column E.

5. Attach or include an explanation with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   Few data are available regarding the use of analgesics in fish, and protocols tested to date only pertain only to alleviation of acute pain and distress (Sneddon, 2012, J. Exotic Pet Medicine, 21:32-43). Furthermore, the effects of anesthetics, analgesics, and tranquilizers on disease resistance mechanisms are unresolved and may have interfered with the normal course of disease progression and host response, and thus could not be used in these studies for long-term periods. Tricaine is currently the only anesthetic/analgesic agent approved for use in fish in the U.S., and its long term use for the duration of this study was not feasible.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

   N/A

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1. Registration Number: 54-G-0001

2. Number of animals used in this study: 227

3. Species (common name) of animals used in the study: rainbow trout

4. Explain the procedure producing pain and/or distress.

   Fish were infected with *L. garvieae* the causative agent of Lactococcosis. During the 28-day post-challenge observation period, some fish experienced typical signs of disease, including decreased appetite, altered ventilation and swimming, loss of equilibrium, discoloration, exophthalmia, and lesions at the injection site. Most fish were humanely euthanized prior to the development of severe disease, although some died before euthanasia could be performed. We are reporting those fish that experienced more than momentary discomfort in Column E.

5. Attach or include an explanation with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   Few data are available regarding the use of analgesics in fish, and protocols tested to date only pertain only to alleviation of acute pain and distress (Sneddon, 2012, J. Exotic Pet Medicine, 21:32-43). Furthermore, the effects of anesthetics, analgesics, and tranquilizers on disease resistance mechanisms are unresolved and may have disrupted the normal course of disease progression and host response, and thus could not be used in these studies for long-term periods. Tricaine is currently the only anesthetic/analgesic agent approved for use in fish in the U.S., and its long term use for the duration of this study was not feasible.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

   N/A

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1. Registration Number: 54-G-0001

2. Number of animals used in this study: 328

3. Species (common name) of animals used in the study: rainbow trout

4. Explain the procedure producing pain and/or distress.

   Fish were infected with *Y. ruckeri*, the causative agent of Enteric Redmouth Disease. During the 28-day post-challenge observation period, some fish experienced typical signs of disease including, decreased appetite, altered ventilation and swimming, loss of equilibrium, discoloration, exophthalmia, and lesions at the injection site. Most fish were humanely euthanized prior to the development of severe disease, although some died before euthanasia could be performed. We are reporting those fish that experienced more than momentary discomfort in Column E.

5. Attach or include an explanation with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   Few data are available regarding the use of analgesics in fish, and protocols tested to date only pertain only to alleviation of acute pain and distress (Sneddon, 2012, J. Exotic Pet Medicine, 21:32-43). Furthermore, the effects of anesthetics, analgesics, and tranquilizers on disease resistance mechanisms are unresolved and may have interfered with the normal course of disease progression and host response, and thus could not be used in these studies for long-term periods. Tricaine is currently the only anesthetic/analgesic agent approved for use in fish in the U.S., and its long term use for the duration of this study was not feasible.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

   N/A

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1. Registration Number: 54-G-0001

2. Number of animals used in this study: 3,636

3. Species (common name) of animals used in the study: rainbow trout

4. Explain the procedure producing pain and/or distress.

   Juvenile fish were infected with *Flavobacterium columnare*, the causative agent of columnaris disease. During the 21-day post-challenge observation period, some fish experienced typical signs of disease including, decreased appetite, altered ventilation and swimming, loss of equilibrium, and discoloration. Most fish were humanely euthanized prior to the development of severe disease, although some died before euthanasia could be performed. We are reporting those fish that experienced more than momentary discomfort in Column E.

5. Attach or include an explanation with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   This experiment is to determine the broad-based resistance to *F. columnare* in rainbow trout that are selectively bred for resistance to a single strain of the pathogen. Thus, accomplishment of the research goals requires the disease to progress naturally without interference, resulting in relatively high rates of mortality due to natural causes and/or euthanasia of moribund animals. Throughout the course of this study, fish will be observed at least once daily and closely monitored. Any fish that are found to be moribund will be immediately euthanized to minimize pain and discomfort. Few data are available regarding the use of analgesics in fish, and protocols tested to date only pertain to alleviation of acute pain and distress (Sneddon, 2012, J. Exotic Pet Medicine, 21:32-43). Furthermore, the effects of anesthetics, analgesics, and tranquilizers on disease resistance mechanisms are unresolved and may have interfered with the normal course of disease progression and host response, and thus could not be used in these studies for long-term periods. Tricaine is currently the only anesthetic/analgesic agent approved for use in fish in the U.S., and its long term use for the duration of this study was not feasible.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

   N/A

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1. Registration Number: 54-G-0001

2. Number of animals used in this study: 76

3. Species (common name) of animals used in the study: rainbow trout

4. Explain the procedure producing pain and/or distress.

   Fish were infected with *Edwardsiella piscicida*, delivered under anesthesia (80 mg/L of MS-222) using a 27G1/2 inch needle. During the 28-day post-challenge observation period, some fish experienced typical signs of disease including, decreased appetite, altered ventilation and swimming, loss of equilibrium, discoloration, exophthalmia, and lesions at the injection site. Most fish were humanely euthanized prior to the development of severe disease, although some died before euthanasia could be performed. We are reporting those fish that experienced more than momentary discomfort in Column E.

5. Attach or include an explanation with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   Few data are available regarding the use of analgesics in fish, and protocols tested to date only pertain only to alleviation of acute pain and distress (Sneddon, 2012, J. Exotic Pet Medicine, 21:32-43). Furthermore, the effects of anesthetics, analgesics, and tranquilizers on disease resistance mechanisms are unresolved and may have interfered with the normal course of disease progression and host response, and thus could not be used in these studies for long-term periods. Tricaine is currently the only anesthetic/analgesic agent approved for use in fish in the U.S., and its long term use for the duration of this study was not feasible.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

   N/A

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1. Registration Number: 54-G-0001

2. Number of animals used in this study: 57

3. Species (common name) of animals used in the study: rainbow trout

4. Explain the procedure producing pain and/or distress.

   Fish were infected with *Weissella ceti*, delivered under anesthesia (80 mg/L of MS-222) using a 27G1/2 inch needle. During the 28-day post-challenge observation period, some fish experienced typical signs of disease including, decreased appetite, altered ventilation and swimming, loss of equilibrium, discoloration, exophthalmia, and lesions at the injection site. Most fish were humanely euthanized prior to the development of severe disease, although some died before euthanasia could be performed. We are reporting those fish that experienced more than momentary discomfort in Column E.

5. Attach or include an explanation with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   Few data are available regarding the use of analgesics in fish, and protocols tested to date only pertain only to alleviation of acute pain and distress (Sneddon, 2012, J. Exotic Pet Medicine, 21:32-43). Furthermore, the effects of anesthetics, analgesics, and tranquilizers on disease resistance mechanisms are unresolved and may have interfered with the normal course of disease progression and host response, and thus could not be used in these studies for long-term periods. Tricaine is currently the only anesthetic/analgesic agent approved for use in fish in the U.S., and its long term use for the duration of this study was not feasible.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

   N/A