Fish Health Section

FHS NEWS – February 2023

Fish Health Section website: https://units.fisheries.org/fhs/

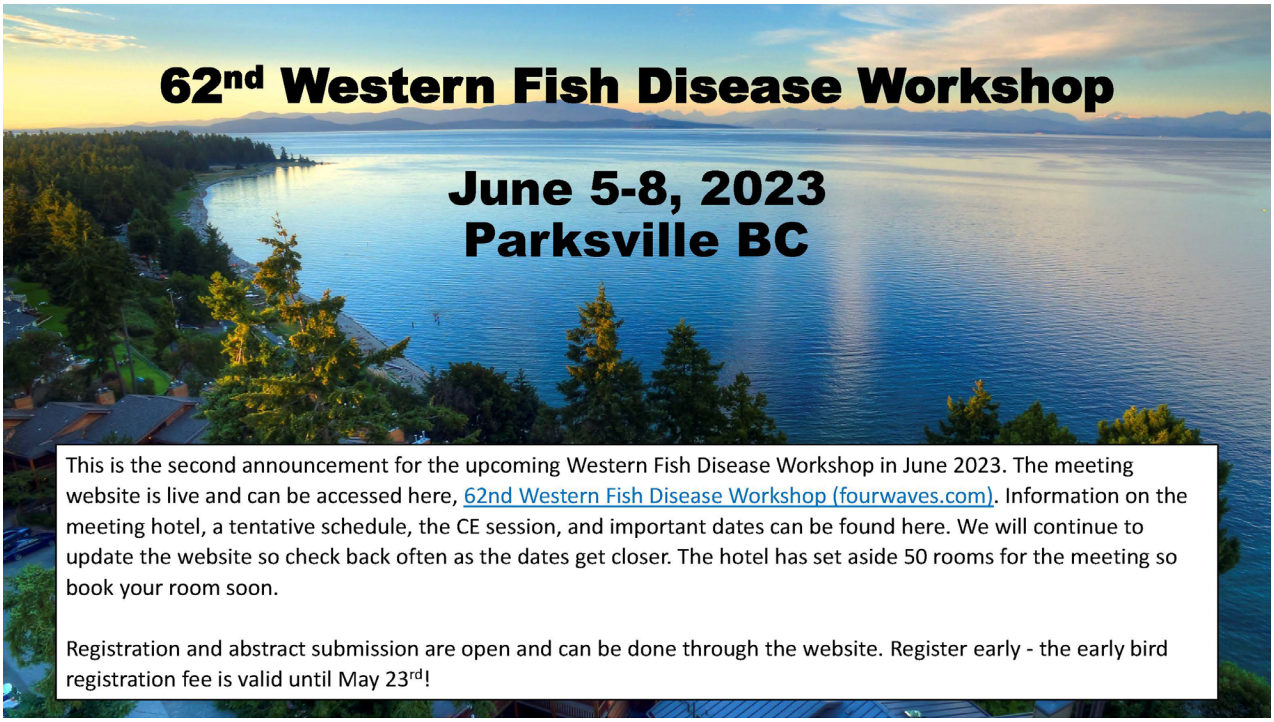
Fish Health Section Facebook Site: https://facebook.com/FishHealthSectionAFS

Fish Health Section Twitter feed: @AFSFishHealth
Would you like your recent open-access publication featured on our Twitter feed? We would like to share one publication per week. Just fill out the form at: https://forms.gle/NWVXEFoGcdYME6gh8.

Membership notice: Starting in March 2023, only paid FHS members will receive newsletters and communications from the section. We have given you several months notice to get your AFS & FHS membership up to date. We are currently around 100 fewer members than last year. Please join us and don't miss out on the connection to your peers https://fisheries.org/membership/. We have transitioned to a new listserv service so emails are coming from fhs@afsmembers.simplelists.com. Make sure to add this address to your safe list to continue to receive communications from the section!

MEETINGS, WORKSHOPS AND COURSES

Western Fish Disease Workshop
June 6-8, 2023
Parksville, B.C.
https://event.fourwaves.com/cbd19bf6-2dec-4ea7-bfd4-6762a939ace5/pages



46th Eastern Fish Health Workshop
March 27- 31, 2023
Atlantic Beach, NC
https://units.fisheries.org/fhs/eastern-fish-health-workshop/

We are happy to announce that the 46th Annual Eastern Fish Health Workshop will return to the DoubleTree by Hilton Atlantic Beach Oceanfront in picturesque Atlantic Beach, North Carolina. The meeting will begin with a welcome reception on Monday, March 27, followed by an evening of interesting, bewildering, and bemusing case reports. There will be three full-day sessions (March 28-30), followed by a full-day continuing education course on Friday, March 31. Returning again this year, we invite all of you talented (and not so talented) singers and observers to enjoy Karaoke night! We will also hold a special networking event and dinner at the North Carolina Aquarium at Pine Knoll Shores—the perfect opportunity to catch up with friends and colleagues. The banquet and Best Student Presentation award will take place on Thursday night, when there is never a shortage of dancing—not to be missed.

REGISTRATION (LINK TO COME)
The $450 registration fee (U.S. dollars) includes a reception on Monday evening with heavy hors d'oeuvres, electronic workshop proceedings, refreshments at breaks, breakfasts and luncheons on each of the three full days of the workshop (Tuesday, Wednesday, Thursday), an evening event/dinner at the North Carolina Aquarium at Pine Knoll Shores, and a banquet dinner on Thursday night. Late registration will incur an additional fee of $50 (U.S. dollars), for a total late registration cost of $500.

CONTINUING EDUCATION OPPORTUNITY
On Friday, March 31, 2023, please join us for an exciting continuing education opportunity. This year's topic will be "Fish and Aquatic Invertebrate Welfare." The cost for CE is $85 (U.S. dollars). Final CE credits are to be determined.

29th Aquaculture Drug Approval Coordination Workshop
March 27, 2023
Atlantic Beach, NC
Registration is now open for the 29th Annual Aquaculture Drug Approval Coordination Workshop! Hosted by the United States Fish & Wildlife Service - Aquatic Animal Drug Approval Partnership Program, the workshop will take place in Atlantic Beach, NC on March 27th, 2023. We also have a weather-dependent welcome social planned for March 26th, 2023 for in-person attendees. For those that are unable to join us in-person this year, we are providing the option to tune in to the workshop virtually. The deadline for registration is Wednesday, March 22nd, 2023, and if you register for the in-person option before February 28th, 2023, we'll do our best to ensure you receive a workshop t-shirt! Please visit our 29th Annual Aquaculture Drug Approval Coordination Workshop Registration Webpage to get signed up!

The 29th Annual Aquaculture Drug Approval Coordination Workshop will be hosted back to back with the 46th Annual Eastern Fish Health Workshop (EFHW), which will take place from March 27th-31st, 2023. For more information about workshop lodging, travel, and presentations, please visit our 29th Annual Aquaculture Drug Approval Coordination Workshop Webpage.

JOBS/GRADUATE ASSISTANTSHIPS

Diagnostic Fish Pathologist
California Department of Fish and Wildlife
Gold River, CA
Closes March 14, 2023
Link: https://www.calcareers.ca.gov/CalHrPublic/Jobs/JobPosting.aspx?JobControlId=357103

Are you a fish pathologist/research scientist with a passion for fish and aquatic animals and looking for a new opportunity?

We have a Fish Pathologist position (Research Scientist II job classification) available in our Fish Health team, based in Gold River, California. Our laboratory is located on the beautiful American River with tons of hiking and biking trails, kayaking, paddle boarding and swimming right outside. While this position is based in Gold River, this position travels statewide investigating fish health issues in hatcheries and wild fish populations. Our hatcheries are located in a wide variety of scenic locations from the high mountains of the Sierra Nevada and Cascade ranges, to world class wine country, the Redwood forests and sandy beaches of California's Pacific coast. Take advantage of all we have to offer in California.

Professionally, you will have the opportunity to work with trout and salmon in the state hatchery system, sport and food fish produced by private aquaculture and wild fish in waters across the state. For more information about the position, minimum qualifications, salary, and how to apply please see the following link: CalCareers

For questions regarding the position or the application process contact Dr. Mark Adkison at (916) 952-5361

Aquatic Science Technician V
Fisheries and Oceans Canada
Nanaimo, British Columbia
Closes March 13, 2023
Link: https://emploisfp-psjobs.cfp-psc.gc.ca/psrs-srfp/applicant/page1800?poster=1913713

Biological Science Research Technician 2
Oregon State University – Aquatic Animal Health Laboratory
Corvallis, OR
Closes March 15, 2023
Link: https://jobs.oregonstate.edu/postings/132680/print_preview

The purpose of this position is to provide weekend support as a fish technician and to be an emergency responder (on-call) for an Oregon State University freshwater fish research facility, the Aquatic Animal Health Laboratory (AAHL). The AAHL is located approximately 2 miles east of the OSU campus, Corvallis, Oregon.

The schedule for this position would be Friday through Sunday (or Saturday through Monday) with a somewhat flexible schedule of approximately 1 to 4 hours/day (approx. 10 hours/week) and to be on-call to respond to welfare related emergencies between the hours of Friday 5:00 pm through Monday 8:00 am.

The daily tasks when on site Saturdays and Sundays will include routine daily fish feeding, health monitoring and caring for animals as well as fish tank and equipment cleaning, equipment and

building monitoring and maintenance. On the weekends the incumbent in this position will work independently and may be on site alone.

If called out to an emergency, the immediate goal is to ensure that the fish have life sustaining support such as flowing water, aeration and temperature control specific to their current needs. The expectation will be to trouble-shoot the problem, which may include equipment failure, balancing system parameters, power interruption or plumbing issues.

Fridays and/or Mondays will be used as an opportunity to cross over with the facility manager, train, debrief and receive direction for tasks. Other work in support of the running of the lab may include equipment fabrication, minor plumbing/carpentry. Some computer use and manipulation of digital controls will be required. This position supports the overall functions of the AAHL as determined by the AAHL manager.

The Aquatic Animal Health Lab (AAHL) is a freshwater fish and invertebrate research facility within the Department of Microbiology at Oregon State University (OSU). Research studies are conducted by a wide variety of research groups including OSU and other university professors, students, state departments and private entities. The lab focusses on fish health and disease research, but also includes a range of behavioral, ecological, genetic, temperature, environmental and aquaculture related studies. The 9000 sq ft lab has over 300 tanks of various sizes housing salmonids and other freshwater species including zebrafish, koi and tilapia. The lab is supplied with continuous flow-through, UV sterilized well water, aeration, temperature control and effluent treatment. For animal welfare, the mechanical life-support system is controlled digitally and alarmed at multiple points.

Shellfish Health Scientific Aide
California Department of Fish and Wildlife
UC Davis Bodega Marine Lab, CA
Closes March 15, 2023
Link: https://www.calcareers.ca.gov/CalHrPublic/Jobs/JobPosting.aspx?JobControlId=357726

We are seeking a CDFW Shellfish Health Lab Scientific Aide for the Shellfish Health Lab located at the UC Davis Bodega Marine Laboratory. This is a perfect position for someone looking to gain research and practical experience in shellfish health. I am more than happy to answer any questions from interested applicants. The final filing date is 3/15/2023. Please see below for information about the position and the link to apply:

Are you interested in protecting the health of shellfish (i.e. oysters, abalone, crabs, sea stars etc) in the state of California? Do you hope to gain experience in shellfish research and culture techniques? Come join us at the CDFW Shellfish Health Laboratory located at the University of California Davis Bodega Marine Laboratory. In this position, you will master key skills used to assess shellfish health and detect invasive species including: examinations and disease diagnosis including qPCR, microbiology, and related techniques. Learn to conduct animal necropsies independently with appropriate sample preservation and data acquisition and analysis. Assist with the development of diagnostic protocols for novel aquatic animal pathogens and invasive species to aid management efforts. Participate in field inspections of shellfish aquaculture facilities and aquaria throughout the state. Assist permanent staff on a broad range of duties involving health and disease management of cultured and wild shellfish populations, management of research and public exotic species holding systems, and support for federal ESA listed white abalone and black abalone recovery efforts. Maintain animal life support systems for abalone and other shellfish including filter maintenance, siphoning tanks and collecting and feeding algae. Conduct laboratory maintenance such as washing glassware, autoclaving trash, and ordering supplies. Collaborate on research focused on shellfish health and disease ecology.

Natural Resources Biologist III: Aquatic Animal Health Biomolecular and eDNA Specialist

Maryland Department of Natural Resources
Oxford, MD
Originally closed 2/20/23 but reposted
Link:
https://www.jobapscloud.com/MD/sup/BulPreview.asp?R1=22&R2=001000&R3=0007&Viewer=Admin&Test=Y

The Department of Natural Resources, Fishing and Boating Services in Oxford Maryland is currently accepting applications for a Natural Resources Biologist II – Aquatic Animal Health Laboratory Biomolecular and eDNA Specialist. The purpose of this position is to conduct biological research and monitoring tasks as part of the Cooperative Oxford Laboratory (COL) Aquatic Animal Health Laboratory Project in Oxford, Maryland. This position serves as a lead biologist for the laboratory's biomolecular and eDNA laboratories. The position increases knowledge on diseases affecting the natural aquatic living resources of Maryland and the Chesapeake Bay by developing and performing polymerase chain reaction (PCR) assays for qualitative and quantitative detection of pathogen DNAs in shellfish and finfish tissues, cultured cells, and environmental samples. This position develops and performs immunoassays and in situ DNA probe hybridization (ISH) assays for the detection and identification of pathogens of shellfish and finfish of economic and ecological importance in histological and other samples. The position assists in field collection of samples and other lab projects as needed. This includes drawing blood samples from live shellfish, and dissections or biopsies of both live and dead shellfish and finfish to obtain solid tissue samples. The position extracts, preserves, and analyzes nucleic acids from tissue and cell samples. The position preserves, processes, and stains histological and cytological samples for microscopic analyses, using histochemical stains, antibodies, and DNA probes. The position isolates, propagates, identifies, experimentally manipulates, and cryopreserves pathogen cell cultures in vitro. The position performs microbiological assays on tissue samples and analyzes results. The position maintains and operates sophisticated laboratory equipment and follows strict safety procedures during common procedures that use hazardous laboratory chemicals and equipment. The position participates as an essential member of a team that acquires diagnostic tissue samples for disease assays from live oysters during an annual survey of Maryland oyster populations as well as from finfish for fish health inspections of fish located on Maryland state hatcheries.

Aquatic Animal Health Fellowship

University of California Davis
Davis, CA

This is a one-year program designed to train post-graduate veterinarians in the field of aquatic animal health. Specifically, this program will train candidates to integrate expertise in aquatic animal medicine into the broader fields of animal, human, and ecosystem health, through experiences from aquaculture, fisheries, public aquaria, companion animal medicine, academia and research. An additional goal of the fellowship program is to increase the diversity of veterinarians involved in aquatic animal medicine. Candidates are encouraged to address this and how their selection will help achieve enhanced diversity.

See attached .pdf for more details.

Experienced Histopathologist

Patogen
Oban, UK, Galway, Ireland, or Norway

We continue to grow in the field of diagnostics consisting of histology and bacteriology, and with new owners behind us, we are investing even more heavily to enable us to offer the very best diagnostic solution to our customers. We want to strengthen our histology team with more skilled histopathologists. We are looking for you if you are an experienced diagnostician who would like to join our team.

See attached .pdf for more details.

PhD Position

Atlantic Veterinary College – Department of Pathology & Microbiology
Prince Edward Island

The project focuses on characterizing the nature of skin diseases affecting wild salmonid species in Prince Edward Island (PEI) rivers, and will involve field work, laboratory-based techniques, and pathologic investigations. Over the last five years, anecdotal observations have been made of unusually high numbers of salmonid fish (primarily brook and rainbow trout) with skin diseases in certain rivers on PEI. Affected fish have been reported during the summer and fall seasons by a variety of stakeholders including provincial biologists, watershed volunteers, and residents. Occasionally diseased trout have been submitted to the Canadian Wildlife Health Cooperative (CWHC) at the Atlantic Veterinary College (AVC) for necropsy investigation which invariably revealed extensive patches of grey cottony growths on the skin consistent with infection with an Oomycete species (sometimes referred to as “water mold”). These anecdotal reports of increased incidence of oomycosis in PEI salmonid species is concerning to fisherman and provincial stake holders. An increase in oomycosis in PEI salmonids could suggest exposure to environmental contaminants, increased physiological stressors, or changes in hydrological factors such as reduced flow rate and water temperature. To our knowledge, studies on the prevalence of oomycosis and factors impacting its occurrence in PEI rivers are lacking and will be essential to determine the significance of this disease to salmonid populations as well as identifying possible risk factors leading to increased incidence in some rivers as compared to others.

For further information, see .pdf.

Research Associate – Fish Immunology

Mississippi State University
Starkville, MS
Link: https://explore.msujobs.msstate.edu/cw/en-us/job/505256/research-associate-iii-or-senior-research-associate

The College of Veterinary Medicine is seeking to hire a Research Associate III or Senior Research Associate to manage the fish immunology laboratory.

This individual will manage a fish immunology laboratory, assist in on-going grant projects, assist graduate students and maintain inventory and order supplies. Skills needed include the ability to perform immunology protocols including leukocyte isolation and enrichment, flow cytometry assays and cell culture and immunohistochemistry and statistical analyses. Also required are RNA and DNA isolation and purification, chromatin immunoprecipitation, genomic analyses and common bacteriology protocols. This position also requires rearing and maintaining catfish and zebrafish needed for research, feeding fish and clean tanks as needed.

For more information, see .pdf.

Zebrafish Related Job Announcements

https://wiki.zfin.org/display/jobs/Zebrafish-Related+Job+Announcements

RESOURCES/NEWS

Aquatic Animal Drug Approval Partnership (AADAP) Updates are now available online (new link): https://www.fws.gov/library/collections/aquatic-animal-drug-approval-partnership-update

Member Publication

I am very pleased to announce the first description of thiamine deficiency in steelhead in Oregon! See attached .pdf for manuscript in Veterinary Sciences and here is a link to the journal. https://www.mdpi.com/2306-7381/10/2/156

Thank you to my co-authors, my staff, the staff at OHRC and Alsea Hatchery for the hard work put into this important contribution.

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Simple Summary: Steelhead fry reared in hatcheries in Oregon have a high mortality rate while exhibiting some signs of a vitamin B1 (thiamine) deficiency. This study investigates if thiamine supplementation could improve the health and survival of the fry. To do this, adult, female steelhead were injected with thiamine three weeks before spawning; some of the eggs were alternatively treated with a thiamine bath at the time of spawn, and some were treated both ways. The survival and growth efficiency of the thiamine-treated fry were significantly improved compared to fry that were not supplemented with any thiamine. Fry that came from females that were injected with thiamine had greater growth and survival rates than eggs that received thiamine as a bath only; however, any thiamine supplementation improved survival compared to no thiamine supplementation. This is the first description of thiamine deficiency in Oregon's steelhead.

Editor's Random Pics

Extreme lesion caused by *Flavobacterium psychrophilum.* Rainbow trout, Wizard Falls Hatchery, Camp Sherman, OR, February 2023.

UNIVERSITY OF CALIFORNIA, DAVIS

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Aquatic Animal Health Fellowship at UC Davis

This is a one-year program designed to train post-graduate veterinarians in the field of aquatic animal health. Specifically, this program will train candidates to integrate expertise in aquatic animal medicine into the broader fields of animal, human, and ecosystem health, through experiences from aquaculture, fisheries, public aquaria, companion animal medicine, academia and research. An additional goal of the fellowship program is to increase the diversity of veterinarians involved in aquatic animal medicine. Candidates are encouraged to address this and how their selection will help achieve enhanced diversity.

This position is based in Davis, CA, but will require travel throughout California for specialized training. As directed by on-site collaborators, the fellow shares clinical, research and teaching responsibility for UC Davis aquatic animal health program, CA Academy of Sciences, Monterey Bay Aquarium and Aquarium of the Pacific. Although most of the work will focus on teleost fish medicine, the fellow will gain experience from the diverse caseload consisting of invertebrates, elasmobranchs, amphibians, reptiles, birds and marine mammals that are housed or cultured in the different institutions or in collaborating laboratories or aquaculture farms.

Salary is based on the University of California Academic Salary Scale, in accordance with current Intern Salary scale. A full-benefits package consisting of medical/dental and vision plans will also be offered. The fellow is required to relocate during the different rotations. Some funds are available to support some of the travel and relocation expenses.

The candidate must have a demonstrated ability to work in a team atmosphere. Evidence of recent research, scholarly productivity, and experience in aquatic animal, zoo, exotic and/or laboratory animal medicine are preferred. Applicants for the fellowship should be graduates of an AVMA accredited veterinary college or be ECFVG certified and be eligible for California veterinary licensure. National and international applicants are welcome. Applicants whose native language or language of instruction is not English must take the TOEFL or IELTS. The minimum scores required for admission to graduate study at UC Davis are: 550 on the TOEFL paper-based test (PBT), or 104 on the TOEFL internet-based test (iBT). TOEFL scores expire after two years. Scores that are older than two years will not be accepted by UC Davis. The minimum overall band score required for admission to graduate study at UC Davis is 7.0 on a 9-point scale. IELTS scores expire after two years. Scores that are older than two years will not be accepted by UC Davis.

Candidate must be eligible for a TN (Mexico and Canada) or J-1 visa with no bars or home country requirement. J-1 scholars must have adequate health insurance coverage for the duration of their appointment.

SPECIAL NOTE: The California Veterinary Medical Board requires all veterinarians working at the University of California, Davis with primary patient care duties to hold a full California license or a University veterinary license. To obtain a University veterinary license, veterinarians who are not licensed in the state of California will be required to take a short open-book jurisprudence test, in addition to being background checked and fingerprinted. The cost of obtaining the University veterinary license will be the responsibility of the trainee. The limited license only permits individuals to work in California as veterinarians for University-related practice.

This position is a critical position and subject to a background check. Employment is contingent upon successful completion of background investigation including criminal history and identity checks.

The University of California, California Academy of Sciences, Aquarium of the Pacific, and Monterey Bay Aquarium are affirmative action/equal opportunity employer, and we welcome diversity in science.

How To Apply:

To receive fullest consideration, applications must be received by April 1st 2023. Interested applicants should submit 1) a letter of intent outlining special interest in the position, overall related qualifications and experiences and career goals; 2) curriculum vitae; and 3) the names and addresses of three professional references to:

sotomartinez@ucdavis.edu

PatoGen is the leading provider of information and expertise in the areas of fish diagnostics and preventive fish health in Norway and Scotland. Since its startup in 2005, the company has been a significant contributor to growth and development in the aquaculture industry. Expertise, quality, customer focus and curiosity to develop the fish health area characterize the company. With the vision "We inspire actions for healthier fish", we will continue to be a strong driving force for fish farmers, fish health personnel and suppliers to the aquaculture industry to succeed with their biological goals.
PatoGen is owned by Fürst Medical Laboratory, which is the largest laboratory in the Nordic region and a leader in human diagnostics and analysis. PatoGen has close to 60 employees, head office in Ålesund, and branches in Bodø, Oslo and Oban (Scotland). PatoGen is accredited according to international standard ISO-17025.

www.patogen.com

EXPERIENCED HISTOPATHOLOGIST

We continue to grow in the field of diagnostics consisting of histology and bacteriology, and with new owners behind us, we are investing even more heavily to enable us to offer the very best diagnostic solution to our customers. We want to strengthen our histology team with more skilled histopathologists. We are looking for you if you are an experienced diagnostician who would like to join our team.

Accession: as soon as possible

Tasks:

- Interpret digital histopathology of samples from fish, make diagnoses and write reports
- Involvement in both internal projects, customer assignments and the company's R&D activity
- Contribute expertise internally and in meetings with customers

Qualifications:

- You have formal training as a fish health biologist or veterinarian
- You are passionate about your profession
- You have experience with histopathology
- You have a good understanding of fish health and the challenges of our customers

- You have broad experience of the Norwegian and Scottish farmed aquaculture species

Personal characteristics:

- You stand behind our company values as competent, engaged and reliable
- You are a team player who will help in the working and development of the dedicated histopathology group

We offer:

- Working on the best quality digital histopathology with automated histology production
- A laboratory data system that allows your findings to be quantified and structured
- Opportunities to influence the further development of PatoGen's diagnostic services
- Belonging to a positive team with a good learning climate and excellent academic integrity
- Framework to enable you to develop as a professional
- Competitive salaries
- Equipment for the home office

If you live close to either Oban (UK), Galway (Ireland) or one of our Norwegian laboratories we can provide options to combine home office with physical presence at one of our premises.

Please send your application to Chief Laboratory Officer in PatoGen: birgit@patogen.no

If you have questions about the position, please contact Chief Laboratory Officer Birgit M. Myklebust birgit@patogen.no /mobile +4791573286 or Diagnostics Manager / Histopathologist Stefanie Wüstner stefanie.wustner@patogen.no /mobile +47 476 97 074

PhD Program in Fish Health, 2023

Department of Pathology & Microbiology, Atlantic Veterinary College

The Department of Pathology & Microbiology at the Atlantic Veterinary College (AVC) is currently seeking a candidate to complete a four-year PhD research project in fish health. The position will provide a minimum stipend of $40,000 Canadian /year with the possibility of supplementation through both internal and external awards and scholarships. Pending project funding, the successful candidate will ideally begin work in May 2023.

The project focuses on characterizing the nature of skin diseases affecting wild salmonid species in Prince Edward Island (PEI) rivers, and will involve field work, laboratory-based techniques, and pathologic investigations. Over the last five years, anecdotal observations have been made of unusually high numbers of salmonid fish (primarily brook and rainbow trout) with skin diseases in certain rivers on PEI. Affected fish have been reported during the summer and fall seasons by a variety of stakeholders including provincial biologists, watershed volunteers, and residents. Occasionally diseased trout have been submitted to the Canadian Wildlife Health Cooperative (CWHC) at the Atlantic Veterinary College (AVC) for necropsy investigation which invariably revealed extensive patches of grey cottony growths on the skin consistent with infection with an Oomycete species (sometimes referred to as "water mold"). These anecdotal reports of increased incidence of oomycosis in PEI salmonid species is concerning to fisherman and provincial stake holders. An increase in oomycosis in PEI salmonids could suggest exposure to environmental contaminants, increased physiological stressors, or changes in hydrological factors such as reduced flow rate and water temperature. To our knowledge, studies on the prevalence of oomycosis and factors impacting its occurrence in PEI rivers are lacking and will be essential to determine the significance of this disease to salmonid populations as well as identifying possible risk factors leading to increased incidence in some rivers as compared to others.

Minimum requirements for Canadian and International students include holding a MSc or DVM (or equivalent) credentials with excellent academic records. Candidates with a keen interest and / or experience in wildlife health and aquatic diseases, environmental conservation and fish biology are encouraged to apply. Prior research experience using riparian field techniques (ex: electrofishing, water sampling) and molecular and diagnostic procedures (ex: PCR, microbial culture) would be considered an asset. The successful candidate must meet the AVC graduate admission requirements.

Prior to submitting their application, applicants are encouraged to contact the faculty member listed below to gauge project compatibility and interest. Interested candidates should submit a letter of interest, curriculum vitae, official transcripts of university grades, and the names, titles, institutions, email addresses, and telephone numbers of three referees.

Faculty contact information and email for submissions are listed below:

Dr. Laura Bourque, Wildlife Pathologist
Canadian Wildlife Health Cooperative – Atlantic Region
Department of Pathology & Microbiology
Atlantic Veterinary College
University of Prince Edward Island
Charlottetown, PE, C1A 4P3 Canada

e-mail: lbourque@cwhc-rcsf.ca

Research Associate (Fish Immunology)

The College of Veterinary Medicine is seeking to hire a Research Associate III or Senior Research Associate to manage the fish immunology laboratory.

This individual will manage a fish immunology laboratory, assist in on-going grant projects, assist graduate students and maintain inventory and order supplies. Skills needed include the ability to perform immunology protocols including leukocyte isolation and enrichment, flow cytometry assays and cell culture and immunohistochemistry and statistical analyses. Also required are RNA and DNA isolation and purification, chromatin immunoprecipitation, genomic analyses and common bacteriology protocols. This position also requires rearing and maintaining catfish and zebrafish needed for research, feeding fish and clean tanks as needed.

1. Beginning with a bachelor's degree:
a. To begin at the Research Associate III level, one must have a bachelor's degree and a minimum of 6 years of relevant experience and the equivalent of a master's degree.
2. Beginning with a master's degree:
a. To begin at the Research/Extension Associate III level, one must have a master's degree and a minimum of 3 years of experience.
b. To begin at the Senior Research Associate level, one must have a master's degree and a minimum of 6 years of experience.
3. Beginning with a doctoral degree:
a. A professional employee with a doctoral degree with 2 years of relevant experience and demonstrated competence can begin work at the Research Associate III level.
b. To begin at the Senior Research Associate level, one must have a doctoral degree and a minimum of 3 years of relevant experience.

Rank and Salary will be commensurate with education and experience.

Applicants must apply online at msujobs.msstate.edu. Please include cover letter and resume along with names and addresses of three references.

MSU is an equal opportunity employer, and all qualified applicants will receive consideration for employment without regard to race, color, religion, ethnicity, sex (including pregnancy and gender identity), national origin, disability status, age, sexual orientation, genetic information, protected veteran status, or any other characteristic protected by law. We always welcome nominations and applications from women, members of any minority group, and others who share our passion for building a diverse community that reflects the diversity in our student population.

veterinary sciences

MDPI

Article

Thiamine Supplementation Improves Survival and Body Condition of Hatchery-Reared Steelhead (*Oncorhynchus mykiss)* in Oregon

Aimee N. Reed [1,*], Freya E. Rowland [2], Jennifer A. Krajcik [3] and Donald E. Tillitt [2]

[1] Oregon Department of Fish and Wildlife, Fish Health Services. OSU 226 Nash Hall, Corvallis, OR 97331, USA
[2] U.S. Geological Survey, Columbia Environmental Research Center, 4200 New Haven Rd., Columbia, MO 65201, USA
[3] Oregon Department of Fish and Wildlife, Oregon Hatchery Research Center, 2457 E. Fall Creek Rd., Alsea, OR 97324, USA
* Correspondence: aimee.n.reed@odfw.oregon.gov

Simple Summary: Steelhead fry reared in hatcheries in Oregon have a high mortality rate while exhibiting some signs of a vitamin B1 (thiamine) deficiency. This study investigates if thiamine supplementation could improve the health and survival of the fry. To do this, adult, female steelhead were injected with thiamine three weeks before spawning; some of the eggs were alternatively treated with a thiamine bath at the time of spawn, and some were treated both ways. The survival and growth efficiency of the thiamine-treated fry were significantly improved compared to fry that were not supplemented with any thiamine. Fry that came from females that were injected with thiamine had greater growth and survival rates than eggs that received thiamine as a bath only; however, any thiamine supplementation improved survival compared to no thiamine supplementation. This is the first description of thiamine deficiency in Oregon's steelhead.

Abstract: Early rearing of steelhead (*Oncorhynchus mykiss)* in Oregon hatcheries is often problematic; fry can become emaciated and die during the period between hatch and first feed. Thiamine (vitamin B1) deficiency has caused early mortality in salmonids; however, the thiamine status of Oregon's steelhead populations is unknown, to date. Of the 26 egg samples from three Oregon hatcheries in 2019, 20 (77%) had thiamine levels < 10 nmol/g, and 13 of those samples (50%) had levels <6.5 nmol/g, suggesting the thiamine deficiency of adult, female steelhead. To investigate if thiamine deficiency was causally related to fry survival, females were injected with buffered thiamine HCl 50 mg/kg prior to spawning; additionally, a subset of eggs were supplemented via bath treatment with thiamine mononitrate (1000 ppm) at spawning. Cumulative fry mortality at 8 weeks post-hatch from thiamine-injected females was 2.9% compared to 13.8% mortality of fry without thiamine supplementation. Fry treated only with the thiamine via bath as eggs had a mortality rate of 6.9%. There were no additional improvements for the survival of fry from injected females that also received a thiamine bath. Furthermore, condition factors were greater in thiamine-supplemented fry than in those that received no thiamine. These data identify thiamine deficiency in Oregon steelhead and suggest supplementation with thiamine can mitigate early rearing mortality.

Keywords: aquaculture; steelhead; thiamine deficiency; fish health; hatchery

Citation: Reed, A.N.; Rowland, F.E.; Krajcik, J.A.; Tillitt, D.E. Thiamine Supplementation Improves Survival and Body Condition of Hatchery-Reared Steelhead (*Oncorhynchus mykiss)* in Oregon. *Vet. Sci.* **2023**, *10*, 156. https://doi.org/10.3390/vetsci10020156

Academic Editors: Anita M. Kelly and Timothy J. Bruce

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Published: 14 February 2023

1. Introduction

Thiamine, or vitamin B1, is an essential vitamin required in all living organisms for metabolism, growth, immunity, and neurological development and function [1]. Thiamine is an important cofactor necessary for the enzymatic activity of carbohydrate and lipid metabolism [2]. Thiamine deficiency can occur when an animal either does not uptake enough exogenous thiamine, or if the diet contains organisms that produce thiaminase,

an enzyme that can be found within tissues of certain fishes and aquatic invertebrates that degrades thiamine [3–5]. Thiamine deficiency has been observed across many taxa, including reptiles [6], birds, mussels and mammals [7], and humans [1]. Thiamine deficiency in fishes is a global problem and is responsible for declines in fish populations in the Laurentian Great Lakes [8–10], Baltic Sea [11,12], Bulgaria [13], New York Finger Lakes [14], and most recently in California in anadromous Chinook salmon (*Oncorhynchus tshawytscha*) [15]. Thiamine deficiency-related pathologies in fishes are most often observed during the early life stages of development and referred to as the thiamine deficiency complex (TDC) or early mortality syndrome. Adult life stages of fishes can also be affected by TDC, leading to neurological deficiencies, reduced swimming ability, reduced migration, and increased pre-spawn mortality [8,16]. Clinical signs of TDC in juvenile fish include edema, hemorrhage and vascular congestion, hydrocephalus, and enlarged yolk sacs with opacities and residual unabsorbed yolk [17,18]. Histologically, TDC causes the necrosis of brain cells, hepatocellular necrosis with glycogen depletion of liver, and degeneration and glycogen depletion of muscle cells leading to immobilization [19]. More overtly, behavioral aspects of TDC in early life stages are neurological deficiencies observed as an erratic or corkscrew swimming pattern, weakness as observed by an inability to swim up in the water column, underdeveloped vision, and an inability to forage [16,20]. Because yolk thiamine is dependent on the maternal transfer of thiamine into the yolk, the mortality of subsequent fry is often highest (up to 100%) during the first few weeks after hatch while fry rely on their yolk sacs as the primary thiamine source [4].

Thiamine supplementation of fishes can lead to the immediate reversal of clinical signs of TDC, eliminate abnormal behavior, and stop mortality of fry due to TDC [16,17,21]. For these reasons, thiamine supplementation has been used extensively with managed fish populations where TDC has adversely affected fish survival, such as those in the Great Lakes [21]. The most common routes for the administration of thiamine to fish are the injection of gravid females prior to spawning, or the immersion of eggs around the time of fertilization while the shell is still permeable prior to water hardening [16,21,22]. These methods have effectively provided thiamine to the developing embryonic fish to replenish thiamine stores and prevent TDC.

Steelhead trout (*O. mykiss*) are an important fish species in the Pacific Northwest. They support commercial and tribal treaty fisheries and are an iconic prized fish for recreational anglers. Steelhead express an anadromous life history, spending their first 1–3 years in freshwater, then migrating to the ocean for 1–3 years before returning to freshwater tributaries to spawn. However, unlike most of their anadromous congeners, which invariably die after spawning, some steelhead return to sea after spawning, then return in subsequent years to spawn again (i.e., iteroparity) [23]. In recent years, the Oregon Department of Fish and Wildlife released between 4.5 and 5.5 million steelhead annually from hatcheries into Oregon waters [24]. Hatchery propagation of steelhead augments recreational fisheries and alleviates harvest pressure on wild populations, provides benefits to uphold tribal cultural practices and fishing rights, and contributes to commercial fisheries. At present, 11 steelhead distinct population segments (DPSs) are listed as threatened or endangered under the federal Endangered Species Act (ESA), including four DPSs in Oregon [25].

Rearing steelhead in hatcheries is challenging—fry are easily stressed and hard to start, with a high proportion of "pin-heading" and "drop-out disease" (Reed, pers. observation). These colloquial terms refer to a high mortality rate in the first few weeks after hatching and just after the introduction of feed, with no clear cause of death. This is often diagnosed as a "failure to thrive" and can be associated with 15–30% early rearing mortality (Reed, pers. observation); however, sometimes mortality can be as high as 50%. Affected fry are found on the bottom of rearing tanks near the outflow, weak and emaciated with darkened skin; they may show head swelling or a coagulated yolk remnant in the coelomic cavity. They may also become infected with opportunistic pathogens, such as *Flavobacterium*

psychrophilum bacteria, *Saprolegnia* sp. water mold, and *Ichthyobodo sp.* parasites, which contribute to additional fry morbidity and mortality [26].

Despite the elevated mortality of fry with symptoms similar to what has been observed in other species with documented TDC [15,27], potential TDC in steelhead fry from Oregon hatcheries has never been explored. Therefore, our objectives are to investigate the thiamine levels in eggs of returning, mature, female steelhead, and to determine whether thiamine supplementation can affect the mortality or growth of steelhead fry in an Oregon hatchery.

2. Materials and Methods

Egg collection and thiamine supplementation. In the winter of 2019 (31 January–14 February), samples were collected from three steelhead hatcheries of the Oregon coast: a north-coast facility, Big Creek Hatchery in Astoria (46.1464, −123.5811); a central-coast facility, Alsea Hatchery in Alsea (44.4225, −123.5648); and a south-coast facility, Elk River Hatchery in Port Orford (42.8180, −124.3907). Unfertilized eggs from spawning hatchery steelhead were collected from nine fish at Big Creek Hatchery, ten fish at Alsea Hatchery, and seven fish at Elk River Hatchery. A 10 g egg sample from each female was collected, placed into whirl-pak bags (Nasco Company, Madison, WI, USA), immediately frozen on dry ice, and then maintained at −80 °C until thiamine analysis.

Mature female steelhead were collected and held at Alsea Hatchery in Oregon prior to spawning in January 2022. The estimated average weight of the females in the study was 4 kg and average total length was 62 cm. Each female was placed in either a thiamine treatment group (n = 20), which received an injection of thiamine, or a control treatment group (n = 14) which received no thiamine. For thiamine injections, thiamine HCl 500 mg/mL (VetOne cat no. 501057, AmerisourceBergen, Boise, ID, USA) was buffered by adding 1.5 mL 10 M NaOH into 10 mL thiamine HCl to achieve a pH of 7.0–7.5. The buffered thiamine solution was used immediately, and any extra buffered thiamine solution was discarded that same day. Adult female steelhead in the treated group were manually restrained, then injected intramuscularly with 1.0 mL buffered thiamine solution (500 mg/female) in the dorsal epaxial musculature using a 23 g needle, tagged with a garment tag in the dorsal fin, and then replaced in the holding pond. Adult female steelhead in the control group were manually restrained, then tagged with a garment tag in the dorsal fin and replaced in their holding pond. All fish were held for 21 days at the hatchery until they were spawned; fish were immobilized via electroshock, and eggs were non-lethally collected via coelomic inflation with manual palpation. A 10 g sample of eggs from 10 control fish and 10 treatment fish was collected into whirl-pak bags and immediately flash-frozen by placement on dry ice, then transferred to a −80 °C freezer for storage until processing for thiamine analysis. The remainder of the eggs from each female were brought to the Oregon Hatchery Research Center in Alsea, Oregon, and fertilized individually in clean 4 L buckets by using milt collected from individual males earlier that morning.

For the immersion treatments, half of the fertilized eggs from each female in the treatment and control groups were treated in an immersion of thiamine mononitrate (PureBulk cat no. 11459, PureBulk, Roseburg, OR, USA) and ambient river water at 1000 ppm for 1 h. Eggs from multiple females in the same treatment groups were pooled and placed into heath trays and rinsed with ambient water for 5 min before being treated with iodophor solution for 30 min, as is the standard protocol for egg-hardening and disinfection. Each group was reared at the Oregon Hatchery Research Center on ambient river water at approximately 19 l/min (5 gal/min) and treated 3 times weekly with formalin at 400 ppm for 15 min to control for external fungal growth. This yielded 4 treatment groups with an estimated 6000–7000 eggs each: eggs from injected females that also received a thiamine bath, eggs from injected females that did not receive a thiamine bath, eggs from non-injected females that received a thiamine bath, and eggs from non-injected females that did not receive a thiamine bath (control group).

Mortality monitoring and growth measurements. Daily mortality of fry in each treatment group was monitored, from when the alevins were released (post-hatch) from

the heath trays into flow-through fiberglass tanks and recorded for 8 weeks (56 days) as individual deaths per day per treatment group. Additionally, at 8 weeks post-hatch, 75 fish from each group were individually weighed and measured for fork length [28]. The condition factor (K; Equation (1)) was calculated:

$$K = \frac{10^5 * W}{FL^3} \quad (1)$$

where W is the weight in grams and FL is the fork length in mm, which determines the efficiency of the growth and development of fish [29].

Thiamine Analysis. Thiamine level in eggs was estimated by a rapid solid phase extraction (SPE) fluorometric method [30] for the 2019 survey of adult steelhead. Briefly, egg samples were preserved by freezing on dry ice and stored in a −80 °C freezer until analysis. Subsamples of 0.5–1.0 g were weighed, homogenized in 2% (w/v) TCA (trichloroacetic acid), boiled for 10 min, centrifuged at 14,000× gravity for 25 min, and the supernatant applied to a reversed-phase SPE column (Phenomenex, Torrance, CA, USA). The SPE was eluted with methanolic pH 2.05 PO_4 buffer into two fractions. The thiamine compounds in the two fractions (fraction 1: phosphorylated thiamine vitamers, and fraction 2: non-phosphorylated thiamine) were oxidized to the corresponding thiochromes using alkaline potassium ferricyanide (0.1% K_3FeCN_6), and their concentrations were determined fluorometrically (excitation 360 nm, emission 460 nm) on a 96-well plate reader (Biotek Synergy 4, Agilent Technologies, Inc. Santa Clara, CA, USA). Concentrations of phosphorylated (fraction 1) and non-phosphorylated (fraction 2) thiamine were estimated by comparison of the sample fluorescence in each fraction to that of a series of thiamine standards (phosphorylated and non-phosphorylated) taken through the same extraction procedure. Total thiamine was estimated by adding the phosphorylated and non-phosphorylated concentrations of the thiamine vitamers from each sample, normalized to the initial sample weight extracted and reported as nmol/g-egg.

For the 2022 egg analysis, thiamine determination in eggs was conducted by the HPLC method previously described [21,22], with methanol instead of N,N-dimethylformamide (DMF) as the mobile phase. Eggs were preserved and extracted as described above for the SPE method. Following the extraction, thiamine levels were determined using a high-performance liquid chromatograph (HPLC) system (Agilent Technologies 1100 series; Agilent Technologies, Inc. Santa Clara, CA, USA). The HPLC included a delivery pump, automatic sample injector, RP-HPLC column (Agilent Technologies, Inc. Santa Clara, CA, USA) with attached guard column (25 × 2.3 mm; 12 to 20 mm mesh size), and a fluorometric detector (375 nm excitation wavelength and 442 nm emission wavelength for thiochrome detection). The column thermostat was set to 30 °C, and sample injection volume was 80 µL per injection with a total run time of 25 min per sample at a flow rate of 0.6 mL/min. The mobile phase comprised of 6.25 mM potassium phosphate buffer (pH 8.4) with 1% methanol (solvent A) and 100% methanol (solvent B) [31]. A seven-point standard curve with known concentrations of thiamine was generated at the start of each group of samples and interspersed throughout the run.

Statistical analysis. All statistics and plots were generated in R v4.1.2 [32]. To compare the thiamine concentrations of steelhead eggs from hatcheries, control vs. injected egg concentrations, we used the permutation test function 'oneway_test' in the *coin* package [33] to fit asymptotic K-sample Fisher–Pitman permutation tests. This function is analogous to a one-way ANOVA but permuted 10,000 times so that the p-value is the proportion of tests with a value at least as extreme as the 'true' test. Permutation tests are useful with small sample sizes because they are insensitive to non-normality and heteroscedasticity. As a post hoc pairwise test across groups, we used the 'pairwisePermutationTest' function in the *rcompanion* package [34].

To explore how mortality differed among treatments, we fit non-linear curves to the data to estimate the initial mortality, rate of mortality, and maximum mortality (Equation (2))

using the 'nls' and 'SSasymp' self-starting asymptotic regression model functions in the *stats* package of base R:

$$f(t) = y_f + \left(y_0 - y_f\right) * e^{-e^{\ln(r)*t}} \quad (2)$$

where y_f = maximum mortality estimated as the asymptote, y_0 = estimated mortality at day 0 (should be close to 0), r = mortality rate or steepness of the curve, and t = time since hatch in days.

We compared fish condition (K) using linear models and 'aov' in the *stats* package in base R. A test for outliers using the 'outlierTest' function in the *car* package [35] indicated 2 of the 300 points in the model may be outliers. We refit the model eliminating these observations, and then tested for differences among groups with Tukey HSD tests using the 'TukeyHSD' function in the *agricolae* package [36].

3. Results

2019 egg thiamine. Eggs from the northern-coast (Big Creek) hatchery had total thiamine values that ranged from 3.9 to 18.5 nmol/g with a mean of 11.2 nmol/g (n = 9). Eggs from the central-coast (Alsea) hatchery had total thiamine values that ranged from 2.7 to 9.8 nmol/g with a mean of 4.9 nmol/g (n = 10). Eggs from the south-coast (Elk River) hatchery had total thiamine values that ranged from 3.5 to 9.2 nmol/g with a mean of 5.8 nmol/g (n = 7; Figure 1a). Permutation tests indicated that there were differences in egg thiamine among hatcheries (chi-squared = 10.623, d.f. = 2, p-value = 0.005). Pairwise tests indicated the egg samples from fish at the northern-most facility, Big Creek Hatchery, had statistically higher total egg thiamine than eggs from the other two hatcheries (Figure 1a).

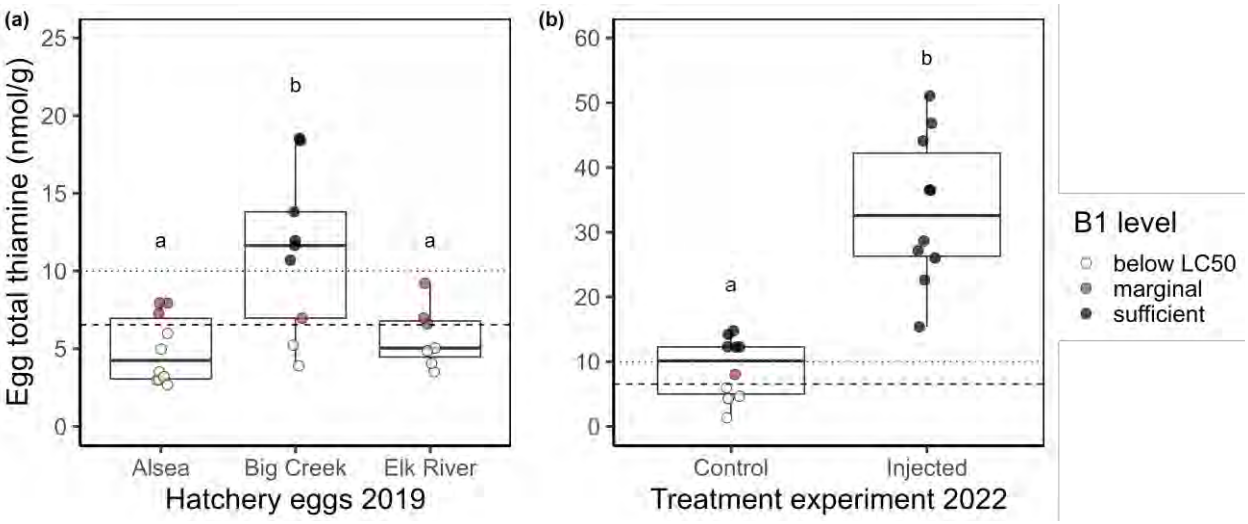

Figure 1. Boxplots (25%, 50%, and 75% quantiles comprise the box) with overlaid raw data points of steelhead egg total thiamine measurements from (**a**) three coastal Oregon hatcheries in 2019, and (**b**) control and injected female eggs from 2022. Egg concentrations are colored based on thiamine concentration thresholds. Yellow points are below LC50 for steelhead of 6.54 nmol/g (dashed line [37]), red points lie between LC50 and the value considered sufficient (10 nmol/g, dotted line [38]), and black points represent egg thiamine concentrations that are likely sufficient for early growth and development as determined from individual-based models for recruitment to lake trout populations [38]. Different letters (a or b) indicate post hoc permutation tests suggest group differences at $p < 0.05$. Note the y-axes have different scales.

2022 egg thiamine. Female, adult steelhead tolerated the injections well with no mortality and no apparent adverse effects from the thiamine injections. Eggs from the control group had an average total thiamine level of 9.0 nmol/g (range 1.3–14.8), whereas eggs from the injected group had an average total thiamine level of 33.5 nmol/g (range 15.4–51.1; Figure 1b). The statistical difference between these groups (Z = −3.60, p-value = 0.0003)

strongly suggests that the injection of thiamine effectively transferred thiamine into the egg after 21 days.

Mortality. The highest survival rate was observed in the groups that came from injected females; fry from thiamine-injected females or fry from thiamine-injected females that also received thiamine as a bath immersion at fertilization had an overall cumulative mortality 4× lower than the control group. Fry in the control group had a cumulative mortality rate of 13.8% during the first 8 weeks after hatch (Figure 2a, Table 1). Fry from non-injected females that received thiamine as a bath treatment at fertilization had a cumulative mortality rate of 6.9%. Fry from injected females and fry from injected females that also received thiamine as a bath had similar cumulative mortality rate of 2.9% (Figure 2a; Table 1).

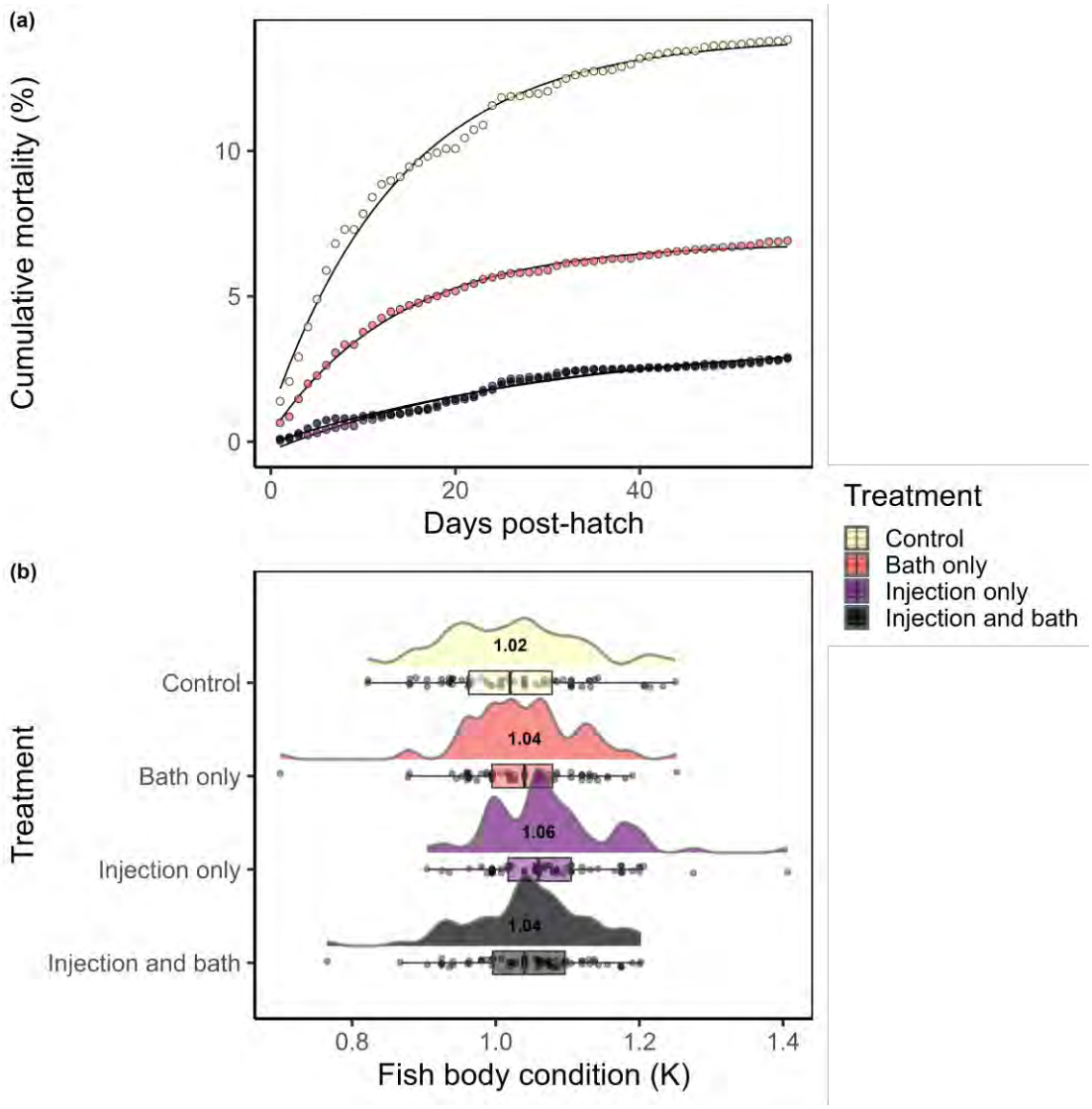

Figure 2. (**a**) Cumulative mortality was assessed daily (points) for 56 days post-hatch in the four treatments with model included as a line; endpoints are control 13.8%, bath only 6.9%, and injection only and injection and bath 2.9%; (**b**) fish body condition, K, of fry 8 weeks post-hatch (n = 75 each treatment). All data are included overlaid as points on the horizontal boxplot to show the distribution of the data (25%, 50%, and 75% quantiles are included in the box). Overlaid on top of each boxplot is a probability density function to show the density of points at each condition factor with the median value expressed under each curve.

Table 1. Model parameters from function to estimate mortality (%) within each treatment. y_0 is the initial mortality on day 0; all treatments were very close to zero initial mortality. Ln(rate) and rate describe the steepness of the mortality curve in our models, expressed both as the model uses it (natural log of rate) and back-transformed to simply a rate for ease of interpretation (rate); y_f is the asymptote of cumulative mortality. Each model was fit to the daily mortality from that treatment (n = 56, model df = 53), and total residual standard error of the model is in the last column.

Parameter	Estimate	SE	t-Value	*p*-Value	Residual Standard Error
Control					0.0031
y_f	13.907	0.105	132.59	<0.00001	
y_0	0.935	0.198	4.72	0.00002	
ln(rate)	−2.653	0.034	−77.73	<0.00001	
rate	0.070				
Bath only					0.0011
y_f	6.814	0.036	188.87	<0.00001	
y_0	0.269	0.072	3.76	0.00042	
ln(rate)	−2.622	0.024	−109.71	<0.00001	
rate	0.073				
Injection only					0.0013
y_f	3.434	0.140	24.49	<0.00001	
y_0	−0.305	0.070	−4.36	0.00006	
ln(rate)	−3.360	0.088	−38.01	<0.00001	
rate	0.035				
Injection and bath					0.0013
y_f	3.604	0.190	18.93	<0.00001	
y_0	−0.046	0.068	−0.68	0.502	
ln(rate)	−3.528	0.110	−32.16	<0.00001	
rate	0.029				

The non-linear mortality model (Equation (2)) showed that fry that had received thiamine supplementation had lower early life stage mortality than control fish (Table 1). Estimated and measured cumulative mortality rates were very similar (Figure 2a, Table 1). Fry groups from fish that received injections had half the mortality rate parameter estimate (rate ≈ 0.03) than fish that did not receive any thiamine supplementation or had a bath treatment only (rate ≈ 0.07; Table 1). Overall, thiamine supplementation improved mortality in steelhead fry, and an injection of thiamine to the gravid females had a greater positive effect on survival than a bath immersion treatment of eggs at fertilization.

Condition. ANOVA tests indicated there were no statistical differences between treatment groups for average body mass (F-statistic$_{3, 296}$ = 0.92, p = 0.43) or average fork length ($F_{3, 296}$ = 0.50, p = 0.68; data available [39]). However, fry from thiamine-injected females had a higher mean condition factor (K) compared to the other groups ($F_{3, 296}$ = 5.49, p = 0.001; Figure 2b). Average condition factor (K) of fry from the injection-only group was 1.07. Post hoc tests suggested that the injection-only fry had significantly higher body condition (K) rates than the fish from the control (mean K = 1.02, p = 0.001) and bath-only (mean K = 1.04, p = 0.041) groups, but were statistically equivalent to the group from injected females that also received a bath (mean K = 1.05, p = 0.164; Figure 2b). These data suggest that at 75 days post-hatch, fry from thiamine-injected females were growing at a more efficient rate than fry that did not receive thiamine.

4. Discussion

These data revealed that thiamine concentrations in steelhead eggs from three Oregon hatcheries were frequently below levels associated with thiamine-related mortality in this species. When the egg thiamine LC50 for steelhead proposed by Futia and Rinchard [37] was applied (dashed line in Figure 1), 50% (13/26) of the eggs sampled had total thiamine concentrations below the LC50 of 6.54 nmol/g. Sublethal effects of TDC have been observed

in lake trout when egg thiamine levels were as high as 10 nmol/g [38]; extrapolating this value of 10 nmol/g (dotted line in Figure 1) to these results suggests that 70% of the eggs sampled could experience mortality or sublethal effects associated with TDC. Although the egg thiamine levels were higher in the northern-most hatchery as compared to the central and southern hatcheries (Figure 1a), the surveillance of egg thiamine levels at additional locations should be investigated to further elucidate geographic patterns of egg thiamine levels in steelhead populations.

The improved condition factor for thiamine-supplemented fry found in this study further suggests thiamine deficiency affects Oregon hatchery steelhead performance. Fitzsimons and colleagues [40] found low egg thiamine concentrations resulted in decreased fry growth rates, reduced predator avoidance, increased vulnerability to prey, and consequently reduced feeding and foraging behavior. The results of this study are consistent with previous work suggesting growth can be the most evident and a sensitive metric in developing salmonid fry experiencing thiamine deficiency [4,40]. The differences in condition factors for thiamine-supplemented compared to thiamine-deficient fish would undoubtedly become more apparent as the fish continue to grow.

The dominant paradigm for causes of TDC is that high amounts of thiaminase-containing prey in the diets causes the depletion of thiamine in predatory fishes [5,41]; yet, the etiology of thiamine deficiency in Oregon's steelhead populations is unclear. In early 2020, Chinook salmon off the coast of California suffered elevated mortality caused by TDC [15], which was proposed to be caused by the availability of prey and increased ingestion of thiaminases from clupeids, such as Northern anchovy (*Engraulis mordax*). Oregon steelhead, however, express different life histories and ocean migration patterns than Chinook salmon; there is a considerable variation in the migration patterns and timing of Oregon Coastal and Lower Columbia River steelhead populations, and they have a rapid off-shore migration into open oceans as juvenile fish [42]. Steelhead are opportunistic and selective predators with a varied diet that consists of cephalopods, fish, and invertebrates; diets have been reported to consist of less than 5% clupeids during their marine life stage with adjustments to diet observed during warm ocean years [43,44]. However, juvenile fish and kelts (that is, fish that have already spawned) have been shown to consume high proportions of anchovy in their diet while offshore [45]. The roles of thiamine, thiaminases, and the marine environment are poorly understood as they relate to the life history of steelhead trout and are a rich area for future investigations.

Low thiamine in steelhead is an additional stressor on already struggling populations. A major factor that affects fish survival in hatcheries is infectious disease [26,46]. Thiamine deficiency contributes to T-cell-dependent and -independent immune dysfunction [47], as well as lowered WBC bactericidal activity and the mitogenesis of fish cells in vitro [48]. Whether the thiamine supplementation of steelhead adults and fry affects their immune status and ability to combat infectious disease agents in the hatchery remains unresolved. Another major threat to steelhead populations in Oregon, and elsewhere, is habitat degradation and loss [49,50]. Climate change is already influencing the success of steelhead in Oregon, as steelhead have been shown to be highly sensitive to the risks of climate change [50]. Changing ocean temperatures and prey availability will continue to affect steelhead ocean distributions [49], but also likely influence disease dynamics and thiamine status [15]. Whether these multiple stressors are additive or synergistic is an important consideration in evaluating the long-term viability of steelhead in Oregon. If the hatchery steelhead sampled in this study are representative of wild steelhead populations in Oregon, thiamine deficiency may be an important and underappreciated limiting factor for their survival.

5. Conclusions

These results show steelhead returning to the coastal hatcheries in Oregon have low thiamine levels that affects the survival and body condition of their progeny. Thiamine levels were higher in eggs from females returning to the northern-most hatchery sampled.

Supplementing progeny with thiamine improved survival rates during the first few weeks of life. Thiamine injection of adults prior to spawning had the greatest benefit to the survival and body condition of offspring; however, egg thiamine supplementation in a bath also increased survival. Providing supplemental thiamine can profoundly increase the survival, growth, and health of steelhead fry in Oregon hatcheries. This is the first description of the thiamine deficiency of steelhead populations in Oregon.

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Institutional Review Board Statement: Samples, treatments, and data were collected by ODFW employees as part of routine animal handling in accordance with the ODFW Fish Health Policy (OAR 635-007-0965) and Hatchery Management Policy (OAR 635-007-0544); therefore, an institutional ethics review was not required. However, all animal treatments and rearing were performed with considerations presented by Soulsbury et al. 2020 [51].

Informed Consent Statement: Not applicable.

Data Availability Statement: The data are available via U.S. Geological Survey data release [39] https://doi.org/10.5066/P9KDEUB, accessed on 1 December 2022.

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