



# R & E Grant Application 17-19 Biennium

Project #: 17-055

## *Hatchery rearing density and steelhead genetics*

### ***Project Information***

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**Requested Cycle:** 17-6  
**R&E Project Request:** \$18,948  
**Other Funding:** \$14,662  
**Total Project:** \$33,610  
**Spending Start Date:** 3/1/2019  
**Spending End Date:** 12/31/2019  
**Project Start Date:** 5/18/2018  
**Project End Date:** 12/31/2019  
**Organization:** Oregon State University

### ***Fiscal Officer***

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### ***Applicant Information***

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### ***Past Recommended or Completed Projects***

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This applicant has no previous projects that match criteria.

## **Location Information**

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### **Where is it?**

The project will occur Statewide

## **Project Summary**

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### **Project Summary**

*Please provide a couple sentence summary of the proposal.*

The goal is to determine if hatchery rearing density alters the genetics of juvenile steelhead and ultimately their fitness in the wild. An experiment has been setup at the Oregon Hatchery Research Center using offspring from 12 steelhead families to test for genetic differences between high and low density treatments.

### **Overall Project Goals**

*Describe the primary goals or outcomes of the entire project, including elements not requesting funding from R&E.*

The goal is to determine if hatchery rearing density alters the genetic makeup of juvenile steelhead and thereby potentially impacts their ability to survive and reproduce as adults in the wild.

### **Primary objectives of R&E funding**

*Please describe the measurable objectives for the R&E portion of the funding request.*

Test whether juveniles reared at high density have shorter telomeres compared to juveniles reared at low density. Telomeres cap the ends of chromosomes. A relatively short telomere is indicative of poor biological state and reduced adult performance.

### **Current Situation/Justification**

*Please describe the current situation and explain why this funding is needed.*

Hatchery-reared steelhead have a lower reproductive success than natural-origin fish when spawning in the wild. The mechanism(s) underlying this fitness difference are unknown. Our hypothesis is that hatchery juvenile fish reared at high densities undergo oxidative stress that accelerates telomere shortening. These short telomeres could be an indicator of poor health that ultimately leads to decreased reproductive success as adults in the wild.

By identifying the mechanism(s) underlying fitness differences, hatchery practices can be modified to ensure that the genetic makeup of hatchery-reared fish is not altered which, in turn, will likely result in fish that perform better in the wild. This action will also minimize negative impacts on natural-origin fish. While the cost to setup and run the experiment at the Oregon Hatchery Research Center is covered, funds are requested to process (i.e. determine telomere length) the tissue samples collected from juvenile fish at the end of the experiment.

### **Recreation and Commercial Benefit**

*This project will provide benefits to:*

Recreational fisheries  
Commercial fisheries

*Explain how this project will contribute to current (and/or potential) fishing opportunities, access, or fisheries management.*

ODFW operates 33 hatcheries throughout the state. The agency's hatchery management policy has four goals: 1) foster opportunities for fishers consistent with the conservation of naturally produced native fish, 2) contribute toward the sustainability of naturally produced native fish populations through the responsible use of hatcheries and hatchery-produced fish, 3) maintain genetic resources of native fish populations spawned or reared in captivity, and 4) minimize adverse ecological impacts to watersheds caused by hatchery facilities and operations.

Research has demonstrated that hatchery-origin salmonids (e.g. Chinook, coho, Atlantic salmon, and steelhead) have lower reproductive success than natural-origin fish when spawning in the wild (reviewed in Christie et al. 2014). It is critically important to determine why and implement realistic changes to hatchery practices to improve the fitness of hatchery-origin fish and ultimately contribute to the four goals listed above.

This project could contribute to a better understanding of hatchery techniques that will lead to better fish culture with a potential for improved hatchery fish returns, an increase in harvest opportunity, a favorable view of fishery management from the public, and increased participation in the fishery.

*Percent benefit split between Commercial and Recreational anglers:*

20 % Commercial

80 % Recreational

*Please explain, or justify, how the percentage split was determined:*

While this study focuses on hatchery-reared steelhead, the findings may be applicable to Chinook and coho salmon as well. Therefore, the percentage split was determined to be 20% benefits to commercial anglers and 80% benefits to recreational anglers.

*This project has been identified as an ODFW priority for:*

Statewide

*Does this project directly support implementation of the ODFW Strategic Plan and/or current Fish Division priorities?*

*Please briefly explain when this was identified as a priority and what process or workgroup was used to identify this as an ODFW priority.*

*Identify any plan or other document that identifies this priority.*

Oregon Hatchery Research Plan

([https://www.dfw.state.or.us/fish/OHRC/docs/2016/OHRC\\_Research\\_Plan.pdf](https://www.dfw.state.or.us/fish/OHRC/docs/2016/OHRC_Research_Plan.pdf))

1. Understand mechanisms that may create differences between hatchery and wild fish

2. Develop approaches to manage hatchery fish that conserve and protect native fish

*Is this project part of an approved Salmon-Trout Enhancement Program (STEP) activity?*

No

*This project is intended to benefit the following species:*

Fall Chinook Salmon

Spring Chinook Salmon

Coho Salmon

Winter Steelhead  
Summer Steelhead

*This project will benefit anglers or fishery by providing:*

Monitoring/Research  
Hatcheries/Propagation/Liberation

#### Monitoring/Research

*This project will be used to evaluate:*

Population composition (i.e age, species, survival, size, or genetics)  
Hatchery production methods

*Has this project been reviewed or developed by an individual with appropriate qualifications (i.e ODFW biometrician, research professor)?*

Yes

This project was developed by Dr. Kathleen O'Malley, an Associate Professor at Oregon State University and the State Fisheries Geneticist

*Is this study critical to fishery management decisions?*

Yes

Can smolt production be reduced and still provide similar fishing opportunities?  
Alternatively, is it possible to maintain smolt production and improve fishing opportunities as a result of increased survival and fitness of hatchery-origin fish?

Yes

If the results indicate that hatchery rearing density affects the genetic make-up of fish, then hatchery practices could be modified to minimize this effect.

*Is there a plan to repeat this monitoring or research in the future?*

Yes

We will use previously collected tissue samples from spring Chinook salmon in the South Fork McKenzie River to test for significant differences in telomere length between hatchery-origin and natural-origin salmon. Given that the samples are part of a large-scale genetic pedigree, we will be able to evaluate telomere length of individuals with low total lifetime fitness (# adult returning offspring = 0) and high total lifetime fitness (# of adult returning offspring > 10).

*Will the data be reported or published?*

Yes

The data collected will be analyzed by an Oregon State University M.S. graduate student, Stanley Piotrowski. Stanley will summarize the findings from this study in his M.S. thesis and a peer-review manuscript.

#### Hatcheries/Propagation/Liberation

*Hatchery Name:*

Oregon Hatchery Research Center

*This is a:*

State hatchery

*As a result of this request hatchery production will:*

Maintain

*This project will:*

Improve effectiveness of hatchery operations (i.e. improve survival or return to angler)

*Fish produced at this facility are for:*

## **Project Description**

### **Schedule**

Activity	Date	RE Funding
6 male and 6 female steelhead from the Siletz River were spawned (1 x 1 crosses). Tissue samples were collected from each individual.	March 2018	No
6 male and 6 female steelhead from the Siletz River were spawned (1x1 crosses). Tissue samples were collected from each individual.	March 2018	No
Fish from the 12 families were ponded	May 2018	No
Muscle tissue samples collected from 240 offspring per tank (N = 2,160)	August 2018	No
Offspring assigned to parents using genetic pedigree analysis	December 2018	Yes
Telomere length determined for 10 fish per family/treatment (N = 1,080)	July 2019	Yes
Data analyses completed	September 2019	No
Project report completed	December 2019	No

### **Permits**

Permit	Secured?	Date Expected
No permits are required.	No	

### **Project Design and Description**

*Please describe in detail the methods or approach that will be used to achieve the project objectives.*

Dr. Michael Blouin (Oregon State University) is conducting an experiment at the Oregon Hatchery Research Center to investigate the effects of high rearing density on hatchery-reared steelhead. On March 7, 2018, six male and six female steelhead from the Siletz River were spawned (1 x 1 crosses). On March 21, six additional males and six additional females from the Siletz River were spawned (1 x 1 crosses). Offspring from these 12 Siletz River steelhead families were ponded into nine tanks on May 18, 2018 (Figure 1). There are three treatments: High Density (HD), Low Density #1 (LD1), and Low Density #2 (LD2). The HD treatment consists of three tanks each with 200 fish/family (N = 2,400/tank). The LD1 treatment consists of three tanks each with 20 fish/family (N = 240/tank)(Figure 1). The LD2 treatment also consists of three tanks each with 20 fish/family (N = 240/tank)(Figure 1). The feeding regime for the three treatments is as follows: 1) HD feeding amount (per fish) based on average of HD tanks, production hatchery size goals determining amount of feed based on grab samples of length and weight. Feeding between HD adjustment calculations will not change based on mortalities, 2) LD1 feeding amount (per fish) based on average of HD tanks, equal amount of food per fish between HD and LD tanks. Feeding between HD adjustment calculations will change in LD tanks per capita  $[(\text{amt feed to HD})/(\# \text{HD} - \text{morts})] \times (\# \text{LD} - \text{morts})$ , and 3) LD2 feeding amount (per fish) based on average of LD2 tanks, production hatchery size goals determining amount to feed based on grab samples of length and weight.

In August 2018, the experiment will conclude and M. Blouin's research group will subsample the offspring and measure fork length, weight, and take a piece of tissue for genetic analysis. In addition to M. Blouin's sampling, my research group will collect a piece of muscle tissue from 240 individuals within each of the nine tanks (N = 2,160). DNA will be extracted from each tissue sample and all individuals will be genotyped at 192 genetic markers (i.e. Single Nucleotide Polymorphisms, SNPs). Genetic parentage analysis will be performed to assign offspring to their respective parents. From these assignments, we will identify 10

offspring/family from each of the nine tanks (N = 1, 080). Using the previously extracted DNA for each sample, we will determine telomere length using the methodology of McLennan et al. (2018).

To test for differences in telomere length, we will use the statistical approach described in Thompson and Blouin (2015) but substitute telomere length for body size. First, we will calculate the intraclass correlation (ICC) for final telomere length in each tank to assess if high-density rearing increased variation in among-family telomere length (Kempthorne 1957). The ICC is a ratio of variance in telomere length among families to total variance in telomere length within each tank (sum of variance among and within families). ICCest in R (version 2.15.1) will be used to calculate ICC values and variance components (Wolak et al. 2012; R Core Development Team 2012). Next, a Welch's t test will be used to determine if the ICC values differed statistically between low- and high-density tanks. A linear mixed effects model will also be used to determine if a significant family-by-environment (family-by-density) interaction occurred. Our response is mean family telomere length. The model will include fixed terms for family, density, and the interaction between family and density. A random tank term will be included to account for the correlation between families within a tank, tank-to-tank variation, and replication. All mixed modeling will be performed following protocols of Zuur et al. (2009) using the nlme package in R version 2.15.1 (Pinheiro et al. 2012; R Development Core Team 2012).

## References

- Christie MR, Ford MJ, Blouin MS (2014) On the reproductive success of early-generation hatchery fish in the wild. *Evolutionary Applications* 7: 883-896.
- Kempthorne O (1957) An introduction to genetic statistics. John Wiley and Sons Inc., New York.
- McLennan D, Armstrong JD, Stewart DC, McKelvey, Boner W, Monaghan P, Metcalfe NB (2018) Links between parental life histories of wild salmon and the telomere lengths of their offspring. *Molecular Ecology* 27: 804-814.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, and R Development Core Team (2012) nlme: linear and nonlinear mixed effects models. R package version 3.1-108.
- R Development Core Team (2012) R: a language and environment for statistical computing [online]. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Available from <http://www.R-project.org/>.
- Thompson NF, Blouin MS (2015) The effects of high rearing density on the potential for domestication selection in hatchery culture of steelhead (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* 72: 1829-1834.
- Wolak ME, Fairbairn DJ, and Paulsen YR (2012) Guidelines for estimating repeatability. *Methods Ecology and Evolution* 3: 129–137. doi:10.1111/j.2041-210X.2011.00125.x.

## Engineering

*Does the project involve capital improvement, engineering, site grading or other construction?*

No

## Project Management and Maintenance

*What is the life expectancy of R&E funded construction, structures, equipment, supplies, data or fishery?*

*Who is responsible for long term management, maintenance, and oversight of the project beyond*

what is funded by R&E.

Will the project require ongoing maintenance?

No

Is there a plan to collect baseline data and to conduct monitoring efforts to measure the effectiveness of the project?

Not necessary

## Project Funding

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### Funding

Have you applied for OWEB funding for this project?

No

Has this proposal, or similar proposal for this project location, previously been denied by OWEB or other funding source?

[{"source":"Dr. Michael Blouin","type":"In-Kind","secured":"Secured","dollarValue":14662,"comments":"Dr. Michael Blouin has covered the cost to set up and run the experiment at the Oregon Hatchery Research Center."}]

Other Funding Source	Type	Secured	Dollar Value	Comments
Dr. Michael Blouin	In-Kind	Secured	14662	Dr. Michael Blouin has covered the cost to set up and run the experiment at the Oregon Hatchery Research Center.
		Total	14662	

## Budget

Item	Unit Number	Unit Cost	In-kind or non-cash contributions	Funding from other sources	R&E Funds	Total Costs
PROJECT MANAGEMENT						
			0	0	0	0
		SUBTOTAL	0	0	0	0
IN-HOUSE PERSONNEL						
			0	0	0	0
		SUBTOTAL	0	0	0	0
CONTRACTED SERVICES						
			0	0	0	0
		SUBTOTAL	0	0	0	0
TRAVEL						
			0	0	0	0
		SUBTOTAL	0	0	0	0
SUPPLIES/MATERIALS						
Genotyping offspring	0	0.00	0	0	8190	8190
Sequencing	0	0.00	0	0	2212	2212
Telomere length measurement	0	0.00	0	0	6823	6823
Stack space	0	0.00	600	0	0	600
Heater	0	0.00	2000	0	0	2000
Tank space	0	0.00	1800	0	0	1800
Feed	0	0.00	2737	0	0	2737
Personnel	0	0.00	7525	0	0	7525
		SUBTOTAL	14662	0	17225	31887
EDUCATION/OUTREACH						
			0	0	0	0
		SUBTOTAL	0	0	0	0
EQUIPMENT						
			0	0	0	0
		SUBTOTAL	0	0	0	0
FISCAL ADMINISTRATION						
10%	0	0.00	0	0	1723	1723
		SUBTOTAL	0	0	1723	1723
		BUDGET TOTAL	14662	0	18948	33610



## ***Internal Review Results***

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**Review Score:** 0.5 out of 3

(0 = Do Not Fund, 1 = Strengthen Proposal, 2 = Recommend, 3 = Strongly Recommend)

### **Summary of Review Team Comments**

The review team was generally not in support of this project as written. While the data may help fill a gap in knowledge it does not seem to add value to anglers or hatchery operations in the near term. OHRC funding would be a more appropriate source of funding and the applicant should clearly demonstrate why use of existing holdover funds is not a viable option, as this is the type of project those funds are for. Scores included four 0s, and four 1s.

### **Specific Review Team Comments**

Rearing density is a quantifiable measure that fish culturist use for loading ponds. Low density verse high density is subjective. The proposal should be strengthened to calculate the actual density (lbs of fish/cubic rearing space) for the low and high groups.

The application states this will help ODFW to make "realistic" changes but it seems like the way the design is set up this will only inform ODFW to "not overcrowd" which we don't do or significantly reduce rearing density which we do not have the infrastructure to meet or it will mean significant reductions in releases thus opportunity. Why is there no assessment of rearing densities around what are normally seen in hatcheries that would provide a "realistic" option for adjusting production.

Seems like a possible outcome is identifying a difference, but not knowing what that difference actually is. Another outcome is no difference between rearing densities.

Not likely to result in a change in policy either- even "better" hatchery fish would still be treated as hatchery fish. This is looking at rearing densities- how does past practices of brood stock selection or other hatchery practices play into changes in genetics. Seems like this is only one small piece of the puzzle that may just lead to more questions.

No angling benefit likely, especially in the short term. Unclear in the long term, but does not seem likely based solely on this project.

Unclear how the density findings will be valuable information for our propagation program. For example, if the research determines that rearing density does directly impact success rate of hatchery raised fish, will that be a reasonable solution for our agency. From reading the experiment description, to achieve "healthier" fish, ODFW apparently will need increase the available pond space by a factor of 10. If this is the case, ODFW may never be able to meet that ratio. If the conclusion of the research projects determines a suggested outcome that will never be achievable by ODFW, then we should not spend R&E money on this project.

This project is obviously well along the path to completion and this request seems to fund finishing off the analysis portion of the project.

Information provided does not state if brood parents were from hatchery or wild fish. Should provide a little more detail.

The project seems straightforward, but there are loose ends that also would need to be resolved in order to evaluate whether changes to rearing strategies could or should be made.

### **Specific Review Team Questions**

*Why is this not covered by OHRC budget or holdover funds? Author needs to justify why OHRC*

*holdover funds are not appropriate. The project should be funded under OHRC.*

I appreciate the feedback and I will follow up with the OHRC and the Advisory Board.

*The proposal states that "A relatively short telomere is indicative of poor biological state and reduced adult performance" and the project is designed to evaluate whether rearing density relates to telomere length. However, even if the high rearing density corresponds to shorter telomere length it seems like a necessary next step would need to be an actual evaluation of performance to returning adults relative to telomere length. Will that be a second phase of this project? If so, what would that look like?*

Yes, we plan to evaluate the performance of returning adults relative to telomere length. We will use previously collected tissue samples to determine if hatchery-origin adult (HOR) spring Chinook have shorter telomeres than natural-origin (NOR) adult Chinook returning to the South Fork McKenzie River. Since genetic pedigrees have already been reconstructed for spring Chinook salmon in this system (Sard et al. 2015), we know the total lifetime fitness (i.e. number of adult offspring) for each individual. So not only can we test for significant differences in telomere length between HOR and NOR Chinook salmon, we can evaluate telomere lengths among HOR and NOR spring Chinook salmon with high (e.g. >10) and low (e.g. 0) total lifetime fitness.

*Barring expansion of rearing capacity needed to maintain the same level of smolt production, is it reasonable to assume that the increased productivity of low-density reared fish would offset smolt reductions needed if hatchery capacity were not reduced? Will you be evaluating the rate of survival to adulthood?*

Yes, it is possible that increased productivity of low-density reared fish would offset smolt reductions needed if hatchery capacity were not reduced. Assuming we find significant differences between fish reared at high and low densities and significant differences between HOR and NOR adults with high and low total lifetime fitness, then the next phase of the project would be to evaluate the rate of survival to adulthood.

Below are responses to comments made above by the team:

The average size for the low density groups (235 fish per 3' tank) was around 270 fish per pound and each pond had about 0.07 lbs of fish per cubic foot. The high density fish (2275 fish per 3' tank) were smaller, around 350 fish per pound, so each pond had about 0.55 lbs of fish per cubic foot. The density index (i.e. the weight of the fish in the pond divided by (the volume of the pond multiplied by the length of the fish)) was around 0.03 for the low density ponds and 0.28 for the high density ponds. Since 0.3 is the number commonly used for a maximum in production hatcheries, we were following standard hatchery practice as far as density was concerned.

Again, we appreciate the comments from the Review Team and will consider them as we move forward with this research. Unfortunately, the fish in the high density rearing group were diagnosed with bacterial cold water disease and gill amoeba. As a result, the experiment was terminated. We hope to repeat the experiment next year. In light of this, I am withdrawing my proposal. Thank you for your time and reviews.

## ***Additional Files***

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Budget Information

[Budget Justification](#)

*Budget Justification*

Maps

Photos

Design Information

[Figure 1](#)

*Experimental design of the density experiment*

Management Plans and Supporting Documents

[OHRC Research Plan](#)

*OHRC Research Plan*

Permits and Reviews

Partnerships

Public Comment

Administrative Documents

## ***Completion Report***

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A completion report has not been submitted for this project.