Development of genomic markers for environmental resilience in mussels

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PROJECT SUMMARY

(1) Organization title: University of Washington

(2) Principal Investigator(s) (PI): Dr. Emily Carrington (PI)

(3) Address, telephone number, and email address of Principal Investigator(s):

UW Biology Department, 3747 West Stevens Way NE, Life Sciences Building (LSB),
Seattle, WA 98195, (201) 221-4676, ecarring@uw.edu

(4) Project title: Development of genomic markers for environmental resilience in mussels

(5) Project objectives for the project period: Our proposal seeks to support the sustainable expansion of the shellfish aquaculture industry by investigating the downstream impact of ocean acidification (OA) and ocean warming (OW) on the survival and successful cultivation of marine bivalves. Our research objective is to describe the response of commercially relevant species of marine mussels to current and near-future OA and OW, utilizing cutting-edge molecular technologies to identify genetic markers that confer resilience to environmental change.

(6) Summary of work to be performed within the project period: Mussel aquaculture production faces an unprecedented challenge as climate change continues to increase the environmental variability within our oceans. To effectively select and cultivate genetic stocks that are resilient to global processes such as ocean acidification (OA) and ocean warming (OW), research that describes the genomic diversity present in farmed and wild mussel populations is needed. To accomplish this goal, we have assembled an interdisciplinary team with expertise in ecological physiology, genetics, and material science to identify the downstream effects of stress-induced changes in gene expression on the ability of marine mussels to attach to aquaculture lines. We anticipate to be able identify several biological pathways whose stress-induced dysregulation result in reproducible changes in the composition, structure, and performance of byssal threads, the protein-based fibers mussels use to adhere to surfaces underwater. In collaboration with our industry partner, Penn Cove Shellfish LLC, **the measure of success for this proposal will be the identification of genetic markers that, when used as selection criteria for mussel broodstock, will produce adults with robust attachment to aquaculture lines under near-future OA and OW**. By defining these gene-environment interactions, our results stand to support commercial growers in the development of selective breeding programs to ensure the efficient, sustainable, and profitable production of mussels within the United States.

(7) Budget Information:

- Total funds requested from PSMFC: $124,980
- Current and pending support (if applicable): N/A
- Cost sharing to be provided to this project: N/A
- Total project cost: $124,980

PROJECT DESCRIPTION

Introduction

Bivalve aquaculture produces a total global economic value of 20.6 billion USD per year, of which marine mussels account for 13% (FAO, 2019). A sustainable source of protein, domestic mussel farming helps to offset the substantial US seafood trade deficit and has seen a steady expansion on both coasts since the 1970's. Today, the oldest and largest mussel farm, Penn Cove Shellfish, operates in Washington State and produces more than 2 million pounds of mussels each year using suspended raft culture. **Suspended raft culture is widely regarded as one of the most efficient, sustainable, and environmentally friendly aquaculture practices** [1].

Marine mussels attach to surfaces underwater using a network of proteinaceous fibers called **byssal threads** (Fig. 1a). Tipped with a powerful biological glue (adhesive plaque), these sticky fibers enable mussels to live along rocky shorelines without being dislodged by waves [2, 3]. To grow mussels in suspended raft culture, shellfish farmers co-opt this attachment strategy by applying post-larval mussels to rope lines made from braided plastics or natural fibers [4]. Loaded with mussels, culture lines are then hung *en masse* from floating rafts, where the animals filter microalgae from the water column at a safe distance from mobile predators that roam the seafloor. As they grow to marketable size (12-18 months), mussels remain attached by continually making new threads as older ones decay [5]. As a result, **robust attachment is important for mussel survival and a likely predictor of farm yield at the end of a growing season** [6].

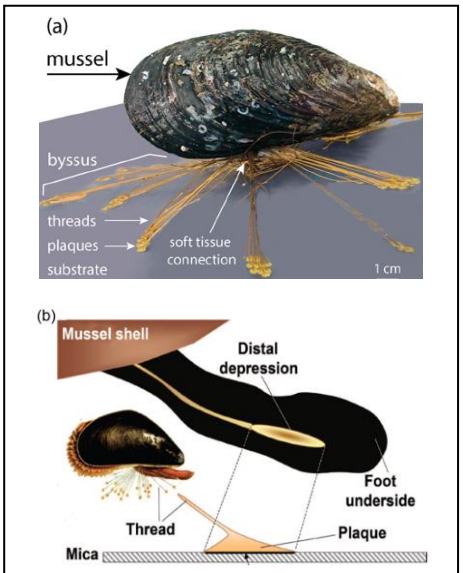

Figure 1. (a) Mussel with attached **byssal thread** network. (b) Anatomy of a mussel's foot and diagram of how a byssal adhesive plaque is made.

Despite the many advantages of suspended raft culture, mussel farming faces significant challenges on the horizon, including a reduction in the availability of permits and growing sites [7]. However, perhaps the largest threat to the industry is the looming economic damage caused by ocean acidification (OA) and ocean warming (OW), the combined effects of which are projected to reduce shellfish production in Europe alone by as much as $1 billion USD annually by 2100 [8]. OA, or the steady decrease in oceanic pH that results from the influx of atmospheric CO_2 into the worlds' oceans, is particularly relevant to mussel aquaculture, which operates in nearshore environments that already experience large oscillations in seawater chemistry as a result of riverine inputs [9], industrial pollution [10], coastal upwelling [11], and agricultural runoff [12]. Similarly, OW, or the incremental increase of sea surface temperature of approximately 0.9°C over the past 40 years (IPCC AR5, 2014), has been shown to negatively impact shellfish physiology, resulting in weakened mussel attachment [13, 14]. In this context, **OA and OW have the potential to increase the exposure frequency, magnitude, and duration of mussels to low-pH (increased acidity) and high-temperature seawater**, particularly when grown in high density aggregations with limited flow [15].

In natural and suspended raft culture settings, each mussel maintains a network of approximately fifty byssal threads that tethers itself to substrates (Fig. 1a). Threads are produced by secreting protein precursors from specialized glands that line a groove that runs along the length of a mussel's foot (Fig. 1b). Adhesive plaque production begins when a small depression at the tip of the foot is pressed against a surface, and the resulting cavity is filled with a class of proteins aptly named mussel foot proteins, or Mfp(s). To date, more than 20 Mfps have been identified, with many

containing 3,4-Dihydroxy-L-phenylalanine (DOPA) residues that display pH- and redox-sensitive interactions with surfaces and like-proteins [16-18].

There is a growing body of evidence that mussel attachment strength is impacted by OA and OW due to the downstream effects of exposure to stressful conditions on mussel physiology. Previous work from our laboratory demonstrates that mussels native to the Pacific Northwest (*Mytilus trossulus*), when acclimated to acidified conditions (high pCO_2, low pH) [19] and high temperatures [20] over the course of two weeks produce fewer byssal threads that are significantly weaker (Fig. 2). Subsequent investigations by others in 2017 [21] and this year [22] in *Mytilus coruscus* found similar results, including that OA and OW depressed thread production and altered the expression of key genes that encode Mfps with demonstrated roles in adhesion (mfp-2, mfp-3, and -5). **These results suggest that the byssal thread production pathway is disrupted by OA and OW, leading to compositional and/or structural changes within the crosslinked Mfp network that weakens attachment**.

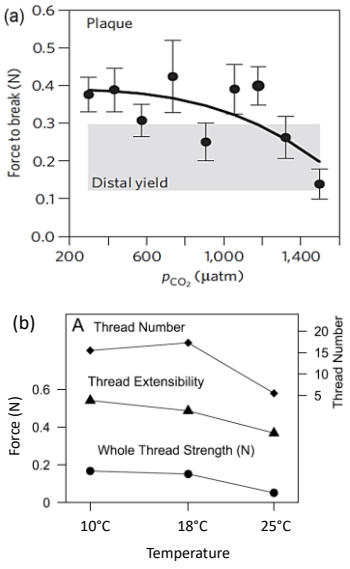

Figure 2. Byssal thread strength from mussels after exposure to (a) OA (increased pCO_2, decreased pH, increased acidity) and (b) OW (elevated ocean temperature).

While the results of Zhao et al. (2017) and Li et al. (2020) confirm that exposure to OA and OW resulted in the downregulation of key byssal proteins, they also reported the upregulation of others in some individuals. One such case was the iron-binding protein mfp-1, which acts as a coating on the exterior of the thread and plaque and increases its structural integrity [23]. This result is intriguing, as it suggests that genomic variation exists within mussel populations that may facilitate adaptive responses that minimize the adverse effect of OA and OW on mussel attachment. However, to explore this possibility, larger datasets detailing the transcriptomic landscape within a mussel's foot and adjoining secretory glands are needed, as well as additional information about any differentially expressed genes in species that are commercially relevant to the Pacific coast under stressful OA and OW conditions.

Specific Objectives

Within the last three years, transcriptomic [24] and proteomic [25] studies of mussel foot glands have greatly expanded the number of known genes associated with Mfp production and byssal thread formation. In this project, we seek to leverage these discoveries through the use of next-generation sequencing to identify byssal thread genes that are differentially expressed under OA and OW. In collaboration with our industry and academic partners, these data will support **our primary research objective of identifying genetic markers that can be used as broodstock selection criteria to ameliorate the impacts of OA and OW on mussel attachment.**

Methods

Two mussel species grown commercially within Washington State (*Mytilus trossulus* and *Mytilus galloprovincialis*) will be obtained from our industry partner, Penn Cove Shellfish LLC's (*see letter of support*) Whidbey Island mussel farm and housed within aquaria at the University of Washington. After acclimation to laboratory conditions, mussels will be exposed to ambient (pH~8.0, pCO_2~400 µatm, T~10°C) and severe OA (pH~7.0, pCO_2~5500 µatm) and elevated temperature (T~23°C) conditions that mimic observed environmental variability at growing sites [15]. Environmental conditions will be maintained using a pH-stat system that aerates each tank with

a CO_2/air mixture and dynamically tracks a pH and temperature setpoint. After 7 days, the byssal threads of mussels within each tank will be cut at the root and discarded in a way that does not hurt the animal, leaving each mussel within their respective treatment conditions. After 3 additional days, newly secreted byssal threads from each animal will be removed and undergo mechanical testing to determine their adhesive strength (maximum force to dislodge) using an Instron-5565 material testing machine [2]. Mussels will also be removed from treatments at this time, sacrificed, and a portion of the foot (phenol gland/distal depression), mantle tissue, and gill tissue will be frozen using liquid nitrogen for further transcriptomic analysis.

Differential gene expression analysis will be accomplished through the RNA sequencing (RNA-seq) of collected feet, mantle, and gill tissue, comparing both the global expression and targeted expression of Mfps through CaptureSeq. RNA will be isolated from samples using the Purelink RNA isolation kit (ThermoFisher) after homogenization with a mortar and pestle under liquid nitrogen. RNA quality will be assessed with the TapeStation 2200 system (Agilent Technologies) and quantified using a Nanodrop (ThermoFisher). RNA library preparation will be undertaken using the TruSeq Stranded mRNA Library Prep Kit (Illumina). Sequencing will be completed using a NextSeq 500 (Illumina), and bioinformatics analysis will be accomplished following established protocols that are commonly used within the Dr. Steven Roberts laboratory within the School of Aquatic & Fishery Sciences at UW (*see letter of support*) [26].

Performance Measures

The primary milestone associated with our main research objective will be the identification of genes that encode Mfps that are differentially expressed under OA and OW in two species of mussels that are commercially grown in Washington state. This outcome will identify gene markers for broodstock selection by our industry partner, Penn Cove Shellfish LLC.

Expected Significance

This proposal will generate large-scale transcriptomic datasets that will serve as an invaluable resource for state agencies, academic consortia, and the mussel aquaculture industry during the execution of future breeding programs whose goal is to select genetic lines that are resilient to OA and OW. The results of this project will also generate novel biomarkers of weakened attachment that can promote the better utilization of shellfish fisheries within the Pacific States region by enabling farmers to harvest from at risk aquaculture lines before ‘fall-off’ events occur, increasing farm yield.

Relation to longer-term goals

Our longer-term goal is to apply the information gained from this project to generate protocols that ensure the successful selection of mussel seed that is resilient to OA and OW. We plan on streamlining this protocol with the help of Penn Cove Shellfish LLC so that it can be used as a model that can be applied to other commercially-relevant mussel species.

Relation to other work planned, anticipated or underway

This project is an extension of over two decades of research within the Carrington laboratory that investigates the environmental basis of weak attachment in mussels and how it impacts the aquaculture industry (*see appendix 2*). The outcome of this project will be the first transcriptomic dataset from commercially farmed mussels within Washington State and we expect our results will lay the groundwork for an industry-academia partnership that uses next-generation sequencing to address the vulnerabilities of aquaculture species to OA, OW, and other environmental stressors.

PROJECT BUDGET

Salaries & Fringe Benefits	
PI (Carrington) Salary (0.25 months)	$3,543
Postdoctoral Scholar (George) Salary (2 months)	$9,187
Research Scientist (White) Salary (3 months)	$19,024
Fringe Benefits	$8,819
Materials and Supplies	
Transcriptomics Analysis	$30,000
General Molecular Laboratory Supplies	$5,000
Laboratory Mesocosm Study Supplies	$4,800
Total Direct Costs	$80,373
Indirect Costs (UW)	$44,807
TOTAL PROJECT COST	**$124,980**

Budget Justification

Personnel

Emily Carrington, Professor and project PI – University of Washington:
Dr. Emily Carrington is a Professor of Biology at the University of Washington whose research focuses on environment-physiology relationships within marine organisms. Over the past 25 years, her work on the 'ecomechanics' of mussels and their byssal attachment has drawn from the fields of materials science, fluid mechanics, organismal biology and environmental science to better understand how mussel aquaculture will need to adapt in the face of a changing ocean. Dr. Carrington will be responsible for overseeing the coordination of the project as a whole and experimental design and materials testing. For the completion of the objective outlined in the proposal, we are requesting 0.25 academic months (or 2.78% effort) of PI salary at $3,543. PI Carrington is on a salaried position with a current monthly base salary of $13,358. The project salary requested is based on the current monthly base salary plus an anticipated 2% increase for a projected monthly base salary of $13,625. The University of Washington's current fringe benefit rate for Instruction and Research Faculty is 24.0% and covers expenses such as Worker's Compensation, Unemployment Compensation, Health Plans, Retirement Plan, Social Security, Medicare, and Separation Leave. The total requested project salary plus fringe benefits for PI Carrington is $4,393 ($3,543*0.24 = $850 in fringe benefits).

Matthew George, Postdoctoral Scholar – University of Washington:
Dr. George is a postdoctoral scholar with joint appointments within the School of Aquatic and Fishery Sciences at UW and NOAA's Northwest Fisheries Science Center. Dr. George has significant prior research experience working with mussels and the transcriptomic analyses (RNA-

seq) outlined in this proposal. Dr. George will complete the laboratory mesocosm studies described in this proposal, assist in transcriptomic analysis, and analyze the resulting data. We are requesting 2 calendar months (or 17% effort) of salary support at $9,187. Dr. George is on a salaried position with a current monthly base salary of $4,417. The project salary requested is based on the current monthly base salary plus an anticipated 4% increase for a projected monthly base salary of $4,594. The University of Washington's current fringe benefit rate for Postdoctoral Scholars is 24% and covers expenses such as Worker's Compensation Unemployment Compensation, Health Plans, Retirement Plan, Social Security, Medicare, and Separation Leave. The total requested project salary plus fringe benefits for Dr. George is $11,392 ($9,187*0.24 = $2,205 in fringe benefits).

Sam White, Research Scientist – University of Washington:
Mr. White is a research scientist with over 13 years of experience working within molecular genetics as a member of the School of Aquatic and Fisheries Sciences at UW. Mr. White will assist with the mesocosm studies described in this proposal, as well as perform the bulk of the transcriptomic analysis, including RNA isolation and library preparation. We are requesting 4 calendar months (or 33% effort) of salary support at $19,024. Mr. White is on a salaried position with a current monthly base salary of $4,573. The project salary requested is based on the current monthly base salary plus an anticipated 4% increase for a projected monthly base salary of $4,756. The University of Washington's current fringe benefit rate for Professional Staff is 30.3% and covers expenses such as Worker's Compensation Unemployment Compensation, Health Plans, Retirement Plan, Social Security, Medicare, and Separation Leave. The total requested project salary plus fringe benefits for Mr. White is $24,788 ($19,024*0.303 = $5764 in fringe benefits).

Collaborators

Ian Jefferds, Owner and General Manager – Penn Cove Shellfish LLC
Mr. Jefferds, as the owner and general manager of Penn Cove Shellfish LLC for over 25 years, has overseen the company's expansion into the largest mussel aquaculture operation in the North America. Mr. Jefferds and Penn Cove Shellfish LLC have committed to supporting the objectives of this project by providing access to mussel seed, adult mussels grown on aquaculture lines, the use of space and equipment within their Coupeville WA warehouse, logistical and technical support, the assistance of aquaculture personnel, and full access to their shellfish hatchery operated in partnership with Coast Seafoods Co in Quilcene, Washington.

Steven Roberts, Associate Professor, School of Aquatic and Fishery Sciences – University of Washington
Dr. Robert's is an Associate Professor within the School of Aquatic and Fishery Sciences at UW whose research focuses on characterizing the physiological response of aquatic species to environmental change with a particular emphasis on the relationship of transcriptomics, genetics, and epigenetics. Dr. Roberts, as an expert in transcriptomic analysis, will provide knowledge and technical assistance, access to equipment, and the laboratory space necessary to complete the transcriptomic analyses outlined in this proposal. The Roberts lab will also curate the management of all genomic datasets collected, as outlined in the data management plan included in this proposal.

Travel

No funds are requested for travel.

Contractual

No funds are requested for contracts.

Supplies

A total of $39,800 is requested in materials and supplies. $30,000 of this amount will cover the cost of transcriptomic analysis for approximately 150 samples (per sample cost of $200, Purelink RNA isolation and library preparation kits (Illumina). $5,000 is requested for general molecular supplies to be used during sample prep (e.g. pipette tips, gloves, tubes etc.). An additional $4,800 is requested for supplies necessary to complete the outlined mesocosm experiments (e.g. tubing, pumps, algal paste etc.). All activities related to mesocosm and materials testing experiments will be carried out in PI Carrington's laboratory at the University of Washington. All transcriptomic sample prep and analysis will be performed by Dr. George and Mr. White in Dr. Roberts lab at the University of Washington.

Equipment

No funds are requested for equipment.

Indirect costs

The University of Washington′s current DHHS Rate Agreement dated September 30, 2020 uses an indirect cost rate of 55.5% for On-Campus Organized Research. Total indirect costs for this project are $44,607. We have included a copy of the UW rate agreement in Appendix 5.

APPENDIX 1: CURRICULUM VITAE

EMILY CARRINGTON
(formerly Emily Carrington Bell, 1991-1999)

Professor, Department of Biology & Friday Harbor Laboratories, University of Washington
Box 351800, Seattle WA 98195
Tel: (206) 221-1884; **e-mail:** ecarring@uw.edu; **website:** http://sites.uw.edu/ecarring

(a) Professional Preparation:

Cornell University	Ithaca, NY	Biological Sciences	B.A. 1985
Stanford University	Stanford, CA	Biological Sciences	Ph.D. 1992
University of British Columbia	Vancouver, BC Canada	Comparative Biomechanics	Postdoctoral Fellow 1992-1995

(b) Appointments:

2010-present	Professor of Biology, University of Washington Department of Biology and Friday Harbor Laboratories (FHL)
2016-2019	Program Director, National Science Foundation, Alexandria, VA (on leave from UW), BIO Directorate (IOS-PSS Cluster)
2012-2014	Director, Ocean Acidification Environmental Laboratory, Friday Harbor Laboratories, University of Washington
2005-2010	Associate Professor of Biology, University of Washington
2003-2005	Associate Professor of Biological Sciences, University of Rhode Island
1996-2003	Assistant Professor of Biological Sciences, University of Rhode Island

(c) Products/Publications:

(i) Five publications most closely related to this proposed project:

1. George, MN, J Andino, **E Carrington**, 2019. Microscale pH and dissolved oxygen excursions within mussel raft aggregations: implications for byssal thread adhesion and mussel attachment. *J. Shellfish Research* 38(3):795-809, doi:10.2983/035.038.0329.
2. **Carrington, E**, JH Waite, G Sara and K Sebens, 2015. Mussels as a model system for integrative ecomechanics. *Annual Review of Marine Science* 7: 443-469. doi:10.1146/annurev-marine-010213-135049.
3. **Carrington, E**, GM Moeser, J Dimond, JJ Mello, and ML Boller, 2009. Seasonal disturbance to mussel beds: field test of a mechanistic model predicting wave dislodgment. *Limnology and Oceanography* 54: 978-986. https://doi.org/10.4319/lo.2009.54.3.0978.
4. Sansoucy M, Tremblay R, **Carrington E**, Marcotte I, and Sleno L, Investigating byssogenesis with proteomic analysis of byssus, foot and mantle in Mytilus mussels by Lc-Ms/Ms. Proteomics, 2020: p. 2000014.

5. George, MN and **E Carrington**, 2018. Environmental post-processing increases the adhesion strength of mussel byssus adhesive. *Biofouling* 34:4: 388-397, doi:10.1080/08927014.2018.1453927.

(ii) Five other significant publications:

1. Gosline, J, M Lillie, **E Carrington**, P Guerette, C Ortlepp, and K Savage, 2002. Elastic proteins: biological roles and mechanical properties. *Phil. Trans. R. Soc. Lond.* B 357:121-132. doi: 10.1098/rstb.2001.1022.
2. Helmuth, B, JG Kingsolver, and **E Carrington**, 2005. Biophysics, physiological ecology, and climate change: does mechanism matter? *Annual Review of Physiology*, 67:177-201. https://doi.org/10.1146/annurev.physiol.67.040403.105027.
3. O'Donnell, MJ, George, MN and **E Carrington**, 2013. Ocean acidification weakens mussel byssus attachment. *Nature Climate Change* 3:587-590. https://doi.org/10.1038/nclimate1846.
4. Newcomb LA, MN George, MJ O'Donnell, and **E Carrington**, 2019. Only as strong as the weakest link: combined effects of temperature and pCO_2 on mussel attachment. *Conservation Physiology*, 7(1): coz068, doi: 10.1093/conphys/coz068.
5. George, MN, B Pedigo, **E Carrington**, 2018. Hypoxia weakens mussel attachment by interrupting DOPA cross-linking during adhesive plaque curing. J. Royal Society, Interface, doi: 10.1098/rsif.2018.0489.

(d) Synergistic Activities

- I served 3+ years as Program Director at the National Science Foundation in the Directorate for Biological Sciences, Division of Integrative Organismal Systems, Physiological Mechanisms and Biomechanics Program (2016-2019) and represented the National Science Foundation on the Interagency Working Group for Aquaculture (IWG-A, 2017-2018).
- I partner with, and disseminate information about environmental effects on wild and farmed mussels to, shellfish growers and government agencies (Penn Cove Shellfish LLC, Westcott Bay Shellfish, Puget Sound Shellfish Growers Association, Washington State Department of Natural Resources, NOAA Washington Sea Grant program, NOAA National Sea Grant).
- I perform editorial duties for scientific journals: *Functional Ecology*, Associate Editor (2015-present); *Marine Ecology Progress Series*, Associate Editor (2016-present); *Invertebrate Biology*, Editorial Board (2010-present).
- I routinely share environmental data I collect in my research and value the importance of sharing this information. In 2005 my lab launched a weather station and these data are publicly accessible in real-time (http://depts.washington.edu/fhl/fhl_wx.html), and as an asset at NANOOS (www.nanoos.org). Many students and researchers from FHL have incorporated these data into their research projects and the website is also regularly accessed by the public, especially local mariners and aviators. Over ten years of data (2006-2019) are archived at bco-dmo.org: https://www.bco-dmo.org/dataset/491262/data.
- I lead K-12 Outreach/Service Learning activities with UW students in my undergraduate Marine Biology course, in collaboration with UW Friday Harbor Laboratories (FHL) K-12 Science Outreach Program and Friday Harbor Elementary School (2009-present).

Steven B. Roberts

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ORCID: 0000-0001-8302-1138 - email: sr320@uw.edu - website: robertslab.info

Professional Preparation

PhD, University of Notre Dame, 2002, Biological Sciences

BSc, North Carolina State University, 1997, Natural Resources

Appointments

2019-present: Associate Director - SAFS, University of Washington

2013-present: Associate Professor - University of Washington

2015: Visiting Professor (2 month) - Interdisciplinary Center for Aquaculture Research (INCAR) - University of Concepcion; Chile

2006–2013: Assistant Professor - University of Washington

2013: Visiting Professor (1 month) - University of Brest - IFREMER; France

2003–2006: Assistant Research Scientist - Marine Biological Laboratory, MA

2002–2003 Post-doctoral scholar – USDA NRI Fellowship

Synergistic Activities

- USDA Aquaculture Panel Manager (2018, 2019, 2020); serving on NSF, USDA, SeaGrant grant review panels and as ad hoc reviewers for other national and international programs
- Co-Coordinator: USDA NRSP-8 National Animal Genome Research Program - Aquaculture (2016-present) managing small research grant program ($60k / year)
- Affiliate Faculty: UW eScience Institute (2015-present)
- Member UW Reproducibility and Open Science Working Group (2014-present)
- Editorial Board: Scientific Data (2013-present); Marine Biotechnology (2018-present), consistently reviewing for numerous journals.
- Advocate for open notebook science and data sharing. All students and staff maintain open access electronic lab notebooks.

Recent Publications

Emma Timmins-Schiffman, José M. Guzmán, Rhonda Elliott Thompson, Brent Vadopalas, Benoit Eudeline and Steven B. Roberts (2020) Larval Geoduck (*Panopea generosa*) Proteomic Response to Ciliates Scientific Reports 10, 6042. doi:10.1038/s41598-020-63218-x

Venkataraman Yaamini R., Downey-Wall Alan M., Ries Justin, Westfield Isaac, White Samuel J., Roberts Steven B., Lotterhos Kathleen E (2020) General DNA Methylation Patterns and Environmentally-Induced Differential Methylation in the Eastern Oyster (*Crassostrea virginica*) Frontiers in Marine Science, Volume 7, Page 225. doi:10.3389/fmars.2020.00225

Gurr SJ, Vadopalas B, Roberts SB, Putnam HM (2020) Metabolic recovery and compensatory shell growth of juvenile Pacific geoduck *Panopea generosa* following short-term exposure to acidified seawater Conservation Physiology, Volume 8, Issue 1, coaa024.

Spencer, LH, Venkataraman, YR, Crim R, Ryan S, Horwith MJ, and Roberts SB (2020) Carryover effects of temperature and pCO2 across multiple Olympia oyster populations. Ecological Applications 00(00):e02060. doi:10.1002/eap.2060

Dimond JL and Roberts SB. (2020) Convergence of DNA Methylation Profiles of the Reef Coral *Porites astreoides* in a Novel Environment Frontiers in Marine Science. vol 6. doi:10.3389/ fmars.2019.00792

Venkataraman YR, Spencer LH, Roberts SB. (2019) Larval Response to Parental Low pH Exposure in the Pacific Oyster *Crassostrea gigas* Journal of Shellfish Research, 38(3), 743-750. doi:10.2983/035.038.0325

Timmins−Schiffman E, Guzmán JM, Elliott Thompson R, Vadopalas B, Eudeline B, Roberts SB. (2019) Dynamic response in the larval geoduck (*Panopea generosa*) proteome to elevated pCO2. Ecol Evol.00: 1– 13. doi:10.1002/ece3.5885

Gallardo-Escárate C, Valenzuela-Muñoz V, Núñez-Acuña , Carrera C, Gonçalves AT, Valenzuela-Miranda D, Benavente BP, Roberts SB. (2019) Catching the complexity of salmon-louse interactions Fish & Shellfish Immunology. doi:10.1016/j.fsi.2019.04.065

Spencer LH, Horwith M, Lowe AT, Venkataraman YR, Timmins-Schiffman E, Nunn BL, Roberts SB. (2019) Pacific geoduck (*Panopea generosa*) resilience to natural pH variation Comparative Biochemistry and Physiology Part D: Genomics and Proteomics. doi:10.1016/j.cbd.2019.01.010 bioRxiv

Venkataraman YR, Timmins-Schiffman E, Horwith MJ, Lowe AT, Nunn B, Vadopalas B, Spencer LH, Roberts SB. (2019) Characterization of Pacific oyster *Crassostrea gigas* proteomic response to natural environmental differences Mar Ecol Prog Ser 610:65-81. doi:10.3354/meps12858 bioRxiv

Javier A. Rodriguez−Casariego, Mark C. Ladd, Andrew A. Shantz, Christian Lopes, Manjinder S. Cheema, Bohyun Kim, Steven B. Roberts, James W. Fourqurean, Juan Ausio, Deron E. Burkepile, and Jose M. Eirin−Lopez. (2018) Coral epigenetic responses to nutrient stress: Histone H2A.X phosphorylation dynamics and DNA methylation in the staghorn coral *Acropora cervicornis* Ecol Evol. 2018;00:1–15. doi:10.1002/ece3.4678

Roberts SB, Gavery MR. (2018) Opportunities in Functional Genomics: A Primer on Lab and Computational Aspects Journal of Shellfish Research 37(4):747-754. doi:10.2983/035.037.0406

Silliman KE, Bowyer TK, Roberts SB. (2018) Consistent differences in fitness traits across multiple generations of Olympia oysters Scientific Reports volume 8, Article number:6080. doi:

10.1038/s41598-018-24455-3

Heare JE, White SJ, Vadopalas B, Roberts SB. (2018) Differential response to stress in Ostrea lurida as measured by gene expression PeerJ 6:e4261. doi: 10.7717/peerj.4261

Goetz FW, Jasonowicz AJ, Roberts SB. (2017) What goes up must come down: Diel vertical migration in the deep-water sablefish (*Anoplopoma fimbria*) revealed by pop-up satellite archival tags Fish Oceanogr. 2017;00:1–16. doi: 10.1111/fog.12239

Gavery MR, Roberts SB. (2017) Epigenetic considerations in aquaculture PeerJ 5:e4147 doi: 10.7717/peerj.4147

Jake Emerson Heare, Brady Blake, Jonathan P Davis, Brent Vadopalas, Steven Roberts (2017) Evidence of *Ostrea lurida* Carpenter, 1864 population structure in Puget Sound, WA Marine Ecology 38:e12458 doi: 10.1111/maec.12458

Megan Hintz, Katherine Gratz, Bonnie Becker, Brent Vadopalas, and Steven Roberts (2017) A Nonlethal Anesthesia Protocol for Accessing the Mantle Cavity of Olympia Oysters in the Laboratory or Field Journal of Shellfish Research 2017 36 (2), 353-357 doi: 10.2983/035.036.0207

Samuel J. White, Brent Vadopalas, Katherine Silliman & Steven B. Roberts (2017) Genotoype-by-sequencing of three geographically distinct populations of Olympia oysters, *Ostrea lurida* Scientific Data 4, Article number: 170130 doi: 10.1038/sdata.2017.130

Dimond JL, Gamblewood SK, Roberts SB. (2017) Genetic and epigenetic insight into morphospecies in a reef coral Molecular Ecology. 00:1–12. doi: 10.1111/mec.14252

Emma B. Timmins-Schiffman, Grace A Crandall, Brent Vadopalas, Michael E. Riffle, Brook L. Nunn and Steven Roberts (2017) Integrating discovery-driven proteomics and selected reaction monitoring to develop a non-invasive assay for geoduck reproductive maturation Journal of Proteome Research doi: 10.1021/acs.jproteome.7b00288

Number of Mentees (to date): 16

Matthew N. George, Ph.D.

Post-Doctoral Scholar, School of Aquatic and Fishery Sciences, University of Washington
Post-Doctoral Scholar, National Atmospheric and Oceanic Administration (NOAA)
1122 NE Boat St Box 355020. Seattle, WA 98195-5020
Phone: +1 (425) 328-5141; email: mngeorge@uw.edu

POSITIONS HELD

Post-Doctoral Scholar, 2020 to Present
The Cooperative Institute for Climate, Ocean, and Ecosystem Studies, joint appointment in:
School of Aquatic and Fishery Sciences, University of Washington, Seattle, Washington; and
Northwest Fisheries Science Center, National Atmospheric and Oceanic Administration, Seattle, Washington

Post-Doctoral Fellow, 2019 to 2020
Children's Hospital of Philadelphia, Center for Cellular and Molecular Therapeutics, Philadelphia, Pennsylvania

Post-Doctoral Research Fellow, 2018 to 2019
Mayo Clinic, Department of Physiology and Biomedical Engineering, Rochester, Minnesota

NSF Graduate Research Fellow, 2012 to 2018
University of Washington, Department of Biology, Seattle, Washington

Research Technologist, 2011 to 2012
Friday Harbor Laboratories, Ocean Acidification Environmental Laboratory, Friday Harbor, Washington

Research Assistant, 2009
Smithsonian Tropical Research Institute, Panama City, Panama

Research Assistant, 2008 to 2010
Gonzaga University, Biology Department, Spokane, Washington

EDUCATION

Ph.D. in Biology, 2018, University of Washington, Seattle, Washington
Dissertation Title: "Mussel attachment in a dynamic ocean: an ecomechanical perspective"

B.Sc. in Biology, 2010, Gonzaga University, Spokane, Washington

PUBLICATIONS

(i) Five publications most closely related to this proposed project:

George MN, Andino J, Huie J, and Carrington E (2019). Microscale pH and dissolved oxygen fluctuations within mussel aggregations and their implications for mussel attachment and raft aquaculture. Journal of Shellfish Research 38:795-809. 10.2983/035.038.0329.

Newcomb LA, **George MN**, O'Donnell MJ, and Carrington E (2019). Only as strong as the weakest link: structural analysis of the combined effects of elevated temperature and pCO2 on mussel attachment. Conservation Physiology 7(1):coz068. 10.1093/conphys/coz068.

George MN, Pedigo B*, and Carrington E (2018). Hypoxia weakens mussel attachment by interrupting DOPA cross-linking during adhesive plaque curing. Journal of the Royal Society Interface 15(147):20180489. 10.1098/rsif.2018.0489.

George MN and Carrington E (2018). Environmental post-processing increases the adhesion strength of mussel byssus adhesive. Biofouling 34(4):388-397. 10.1080/08927014.2018.1453927.

O'Donnell MJ, **George MN**, and Carrington E (2013). Mussel byssus attachment weakened by ocean acidification. Nature Climate Change 3(6):587-590. 10.1038/nclimate1846. (+100 citations per Google Scholar)

(ii) Five over significant publications:

Liu X, **George MN**, Li L, Gamble D*, Miller II AL, Gaihre B, Waletzki BE, and Lu L (2020). Injectable two-dimensional black phosphorus and carbon nanotube hydrogel with enhanced electric conductivity and phosphate release for bone tissue engineering. ACS Biomaterials Science and Engineering 6(8):4653-4665. 10.1021/acsbiomaterials.0c00612.

Sun Y., Liu X, **George MN**, Park S, Gaihre B, Terzic A, and Lu L (2020). Enhanced nerve cell proliferation and differentiation on electrically conductive scaffolds embedded with graphene and carbon nanotubes. Journal of Biomedical Materials Research Part A. 10.1002/jbm.a.37016.

George MN, Liu X, Miller II AL, Xu H, and Lu L (2019). Phosphate functionalization and enzymatic mineralization synergistically enhance oligo[poly(ethylene glycol) fumarate] hydrogel osteoconductivity for bone tissue engineering. Journal of Biomedical Materials Research Part A 108(3):515-527. 10.1002/jbm.a.36832.

George MN and Carrington E (2014). Spine reorientation influences drift particle capture efficiency in sea urchins. Journal of Experimental Marine Biology and Ecology 461:102-106. 10.1016/j.jembe.2014.08.001.

Swanson BO, **George MN**, Anderson SJ*, and Christy J (2013). Evolutionary variation in the mechanics of fiddler crab claws. BMC Evolutionary Biology 13(1):137.

A full list of my publications can be found at https://orcid.org/0000-0003-1264-8667

APPENDIX 2: PAST, CURRENT AND PENDING SUPPORT

PI: Dr. Emily Carrington

Table 1. past, current, and pending support relevant to the proposed project

Title	Status	Start date	End date	Source	Amount
Development of genomic markers for environmental resilience in mussels (this proposal)	Pending	03/2021	04/2022	PSMFC – Marine Aquaculture Pilot Projects	$124,980
Microscale interactions of foundation species with their fluid environment: biological feedbacks alter ecological interactions of mussels	Awarded	02/2021	01/2024	National Science Foundation (NSF)	$456,411
University of Washington: Bridge Funding 2019	Current	06/2019	05/2021	University of Washington	$98,000
Impacts of ocean acidification on wild and farmed mussels in Puget Sound, WA.	Past	03/2014	05/2018	Washington Sea Grant	$257,700
Ocean Acidification-Category 1: Effects of ocean acidification on coastal organisms: an ecomaterials perspective.	Past	06/2010	04/2016	National Science Foundation (NSF)	$869,182

APPENDIX 3: LETTERS OF SUPPORT

University *of* Washington
OFFICE OF SPONSORED PROGRAMS

Office of Research
Office of Sponsored Programs

December 9, 2020

Pacific States Marine Fisheries Commission

To Whom It May Concern:

The University of Washington is pleased to submit this letter in support of the application entitled, "Development of genomic markers for environmental resilience in mussels." This application was prepared by Emily Carrington.

We present this application for your review and request support in the amount of $124,980 for the period March 15, 2021 through April 30, 2022.

The University of Washington reserves the right to negotiate the terms and conditions of the award should this application be funded.

We certify that we have in place a written and enforced financial conflict of interest policy at least as rigorous as that mandated by 42 CFR Part 50, Subpart F or 45 CR Part 94, and that our financial conflict of interest policy will apply to our project director, principal investigator, and any other individual responsible for the design, conduct, or reporting of the budgeted activities.

Thank you for your consideration.

Sincerely,

Alison Schultz
Grant and Contract Analyst
Authorized Signing Official

Please reference our **#A164335** on all correspondence concerning this application.

4333 Brooklyn Avenue NE Box 359472 Seattle, WA 98195-9472
206.543.4043 fax 206.685.1732 www.washington.edu/research/osp

Ian W. Jefferds, G.M.
P.O. Box 148, Coupeville, WA 98239
ian@penncoveshellfish.com
360.678.4803

December 11, 2020

Dear PSMFC Project Selection Committee,

It is a pleasure to write this letter in support of Dr. Emily Carrington and her teams' proposal to The Pacific States Marine Fisheries Commission's (PSMFC) Marine Aquaculture Pilot Projects program entitled "Development of genomic markers for environmental resilience in mussels." As the Owner and General Manager of Penn Cove Shellfish LLC, I have worked with Emily on a number of grant-funded research projects during her tenure at the University of Washington, and I can attest that her laboratory has the personnel and resources necessary to successfully complete the specific objectives of this proposal.

Penn Cove Shellfish was established by my family in 1975 and has grown to become the largest commercial mussel farm in North America. Although our operation has diversified over the years to include clams and oysters, suspended raft culture of mussels continues to make up the majority of our operation. Our mussel production is currently split between two locations within the greater Puget Sound of Washington State, which produce more than 2 million pounds of mussels each year. As a company that is dedicated to caring for our community, we are proud of our track record of producing high-quality, sustainable seafood that also supports the local biodiversity of our ecosystems.

I have had the opportunity to collaborate with Dr. Carrington since 2011, on projects funded by the National Science Foundation and NOAA-Washington Sea Grant. The projects investigate the link between environmental variability and so-called "fall-off" events, an increasingly frequent and disturbing trend that we (and other growers worldwide) have observed, where entire crops of mussels detach from culture lines and are lost during harvest. After several years of working together to establish an environmental monitoring network at our farms and complete a series of laboratory and field experiments, we were able to show that temperature stress, ocean acidification, and hypoxia can each weaken a mussel's ability to attach to our aquaculture lines.

Now that we have identified the stressors that weaken mussel attachment, we are excited about the fact that we are now able to seek solutions before this problem impacts our operation any further. Currently, we are using broodstock from natural mussel set and individuals that performed well the previous year. This pilot project would enable us, for the first time, to quantify the genetic diversity of our mussel seed, enabling us to begin to select for mussels that display strong attachment regardless of environmental conditions. We see this research as one of our top priorities going forward to meet the demands put upon our industry by a changing climate.

Ian W. Jefferds, G.M.
P.O. Box 148, Coupeville, WA 98239
ian@penncoveshellfish.com
360.678.4803

As a local leader that represents and distributes the products of dozens of other independent and family-owned shellfish farms, we are able and willing to take the lead and commit to provide the personnel, equipment, resources, and broodstock necessary to ensure the success of this project. As the owner of Penn Cove Shellfish, I have the upmost confidence in Dr. Carrington's team and look forward to assisting them in whatever way I can. If you should have any further questions, please do not hesitate to reach me at (360) 678-4803 or via email at ian@penncoveshellfish.com.

Sincerely,

Ian Jefferds
General Manager

SCHOOL OF AQUATIC and FISHERY SCIENCES

Dr. Steven B. Roberts
Associate Director
Graduate Program Coordinator
Associate Professor

UNIVERSITY OF WASHINGTON

December 14, 2020

PSMFC
205 SE Spokane Street,
Suite 100 Portland, OR 97202

Dear Selection Committee,

I am writing to offer my enthusiastic support for the proposal entitled "*Development of genomic markers for environmental resilience in mussels*" for consideration under the Marine Aquaculture Pilot Projects program provided by The Pacific States Marine Fisheries Commission (PSMFC). I can attest that Dr. Emily Carrington and her team are uniquely qualified to complete the objectives outlined in this proposal.

Mussels are a valuable aquaculture product that have the potential to continue to emerge as a primary product with research such as that proposed by Dr. Carrington and Dr. George. Both researchers have substantial experience studying mussels with a particular attention to how they perform under changing environmental conditions. This proposed work will provide some much needs resources to ensure this species maintains resilience into the future. While I have not worked with mussels myself, I have used the approaches proposed here and am very willing to offer my support in terms of resources including data analysis workflows and any complementary infrastructure including lab space, computational resources, and seawater facilities. This includes supporting the management of all genomic datasets generated by this project, as outlined in the data management plan.

I look forward to working with this research group and the industry partner, Penn Cove Shellfish, on this exciting project. Please do not hesitate to contact me if you have any questions.

Sincerely,

Steven Roberts

Box 355020 Seattle, WA 98195-5020 phone: 206.866.5141 web: robertslab.info email: sr320@u.washington.edu

APPENDIX 4: DATA MANAGEMENT PLAN

Data Description and Types

As part of this research effort a significant amount of sequence data will be generated from the Illumina Hi-Seq platform. This will include files in fastq format where both quality and nucleotide information are included. These files will be the basis of analysis that will primarily be carried out using Monocle, and downstream statistical packages (R, python).

Access, Timeline for Data Sharing, and Policies for Addressing Data Stewardship

Raw data from DNA sequence platforms will be transferred to the lab of our collaborator (Dr. Roberts) where an internal data stewardship policy is followed (https://github.com/RobertsLab/resources/wiki/Data-Management). Raw data is organized on a network attached storage (NAS) device with RAID redundancy. This NAS is open to the public (http://owl.fish.washington.edu/nightingales/). To make it easier for searching and discovery we also maintain a separate database including metadata (see below) and direct links to files (https://goo.gl/5uTYaU). Within one month of acquiring raw data, it will be uploaded into the appropriate repository at NCBI. Specifically, the sequence data would be deposited in the NCBI SRA. All data will be released once the results are published or no later than three months after the project end date. Raw data from secondary procedures will be available in real-time on the Roberts Lab website where all lab notebooks are open to the public (robertslab.info). All phenotype data will also be available in real-time in online lab notebooks. Data will be in nonpropriety formats such as tab-delimited text files.

Metadata

The Roberts Lab database includes information such as file name, data, taxa, tissue, molecule, platform, length, description, and file locations. Essential information including a description of the sample, library, sequencing method will be included in the SRA repository. Data tags will allow the data to be easily retrievable at NCBI.

Archiving, Backup, and Policies for Addressing Data Preservation

As described, raw data from DNA sequencing is stored on a NAS RAID server and will be uploaded to NCBI for archiving as well as providing access. Raw data will also be stored in two physical locations in the School of Aquatic Fishery Sciences at the University of Washington. Furthermore, all data on (http://owl.fish.washington.edu/nightingales) are mirrored on the Amazon Glacier service. All lab notebooks where analysis is carried out is published via RSS feed and backed up to PDF. Notebooks in the Wordpress platform are archived using the XML export option on a regular basis.

Prior Experience

Both the Carrington and Roberts Laboratories have extensive experience in Data Management and Data Management Plans (for NSF, USDA, and NOAA funded projects) and serve as a reference for other groups across the University of Washington.

APPENDIX 5: UNIVERSITY OF WASHINGTON RATE AGREEMENT

COLLEGES AND UNIVERSITIES RATE AGREEMENT

EIN: 916001537

ORGANIZATION:

University of Washington
Management Accounting and Analysis
4300 Roosevelt Way NE, Suite 300
Box 354966
Seattle, WA 98195-4966

DATE:09/30/2020

FILING REF.: The preceding agreement was dated 07/21/2017

The rates approved in this agreement are for use on grants, contracts and other agreements with the Federal Government, subject to the conditions in Section III.

SECTION I: INDIRECT COST RATES

RATE TYPES: FIXED FINAL PROV. (PROVISIONAL) PRED. (PREDETERMINED)

	EFFECTIVE PERIOD				
TYPE	**FROM**	**TO**	**RATE(%)**	**LOCATION**	**APPLICABLE TO**
FINAL	07/01/2014	06/30/2015	54.50	(1) & (A)	Organized Research
PRED.	07/01/2015	06/30/2017	54.50	(1) & (A)	Organized Research
PRED.	07/01/2017	06/30/2018	55.00	(1) & (A)	Organized Research
PRED.	07/01/2018	06/30/2020	55.50	(1) & (A)	Organized Research
FINAL	07/01/2014	06/30/2015	26.00	(1) & (B)	Organized Research
PRED.	07/01/2015	06/30/2020	26.00	(1) & (B)	Organized Research
FINAL	07/01/2014	06/30/2015	53.00	(1) & (A)	Instruction
PRED.	07/01/2015	06/30/2020	53.00	(1) & (A)	Instruction
FINAL	07/01/2014	06/30/2015	26.00	(1) & (B)	Instruction
PRED.	07/01/2015	06/30/2020	26.00	(1) & (B)	Instruction
FINAL	07/01/2014	06/30/2015	33.80	(1) & (A)	Other Sponsored Activities
PRED.	07/01/2015	06/30/2016	33.80	(1) & (A)	Other Spon Act

ORGANIZATION: University of Washington Management Accounting and Analysis

AGREEMENT DATE: 9/30/2020

TYPE	FROM	TO	RATE(%)	LOCATION	APPLICABLE TO
PRED.	07/01/2016	06/30/2020	37.00	(1) & (A)	Other Sponsored Activities
FINAL	07/01/2014	06/30/2015	26.00	(1) & (B)	Other Spon Act
PRED.	07/01/2015	06/30/2016	26.00	(1) & (B)	Other Sponsored Activities
PRED.	07/01/2016	06/30/2020	25.00	(1) & (B)	Other Spon Act
FINAL	07/01/2014	06/30/2015	42.00	(1) & (C)	Core Grant
PRED.	07/01/2015	06/30/2016	42.00	(1) & (C)	Core Grant
PRED.	07/01/2016	06/30/2020	38.10	(1) & (C)	Core Grant
FINAL	07/01/2014	06/30/2015	78.00	(1) & (C)	Non-Core Fed
PRED.	07/01/2015	06/30/2016	78.00	(1) & (C)	Non-Core Fed
PRED.	07/01/2016	06/30/2020	83.10	(1) & (C)	Non-Core Fed
FINAL	07/01/2014	06/30/2015	17.00	(1) & (D)	
PRED.	07/01/2015	06/30/2016	17.00	(1) & (D)	
PRED.	07/01/2016	06/30/2020	19.00	(1) & (D)	
FINAL	07/01/2014	06/30/2015	25.00	(2) & (E)	
PRED.	07/01/2015	06/30/2020	25.00	(2) & (E)	
FINAL	07/01/2014	06/30/2015	74.00	(1) & (F)	Organized Research
PRED.	07/01/2015	06/30/2016	74.00	(1) & (F)	Organized Research
PRED.	07/01/2016	06/30/2017	75.00	(1) & (F)	Organized Research
PRED.	07/01/2017	06/30/2019	76.00	(1) & (F)	Organized Research
PRED.	07/01/2019	06/30/2020	76.50	(1) & (F)	Organized Research
PROV.	07/01/2020	Until Amended		(G)	

*BASE

ORGANIZATION: University of Washington Management Accounting and Analysis

AGREEMENT DATE: 9/30/2020

(1) Modified total direct costs, consisting of all direct salaries and wages, applicable fringe benefits, materials and supplies, services, travel and up to the first $25,000 of each subaward (regardless of the period of performance of the subawards under the award). Modified total direct costs shall exclude equipment, capital expenditures, charges for patient care, rental costs, tuition remission, scholarships and fellowships, and the portion of each subaward in excess of $25,000.

(2) Direct salaries and wages including vacation, holiday and sick pay and other paid absences but excluding other fringe benefits.

(A) On-Campus
(B) Off-Campus
(C) Washington National Primate Research Center - see Section II Special Remarks.
(D) Applied Physics Laboratory
(E) Vessel Operations
(F) Lake Union Campus

(G) Use same rates and conditions as those cited for fiscal year ending June 30, 2020.

ORGANIZATION: University of Washington Management Accounting and Analysis

AGREEMENT DATE: 9/30/2020

ORGANIZATION: University of Washington Management Accounting and Analysis

AGREEMENT DATE: 9/30/2020

SECTION I: FRINGE BENEFIT RATES**

TYPE	FROM	TO	RATE(%)	LOCATION	APPLICABLE TO
FIXED	7/1/2018	6/30/2019	26.10	(1) & (B)	Faculty & Res. Assoc.
FIXED	7/1/2018	6/30/2019	32.50	(1) & (A)	Medical Residents & Senior Fellows
FIXED	7/1/2018	6/30/2019	17.30	(1) & (A)	Grad. Students
FIXED	7/1/2018	6/30/2019	17.00	(1) & (A)	Post Doc. Trainees
FIXED	7/1/2018	6/30/2019	40.50	(1) & (B)	Class. Staff
FIXED	7/1/2018	6/30/2019	34.10	(1) & (B)	Prof. Staff
FIXED	7/1/2018	6/30/2019	21.10	(1) & (B)	(D)
FIXED	7/1/2018	6/30/2019	21.60	(1) & (B)	(E)
FIXED	7/1/2018	6/30/2019	8.60	(1) & (B)	(F)
FIXED	7/1/2018	6/30/2019	20.90	(1) & (A)	Hourly
FIXED	7/1/2018	6/30/2019	27.80	(1) & (A)	Pre-Doctoral Trainees & Fellows
FIXED	7/1/2018	6/30/2019	60.70	(2) & (C)	Class. Staff
FIXED	7/1/2018	6/30/2019	56.50	(2) & (C)	Prof. Staff
FIXED	7/1/2018	6/30/2019	41.00	(2) & (C)	Faculty & Research Associates
FIXED	7/1/2019	6/30/2020	23.90	(1) & (B)	Faculty & Res. Assoc.
FIXED	7/1/2019	6/30/2020	31.00	(1) & (A)	Medical Residents & Senior Fellows
FIXED	7/1/2019	6/30/2020	21.20	(1) & (A)	Grad. Students
FIXED	7/1/2019	6/30/2020	22.70	(1) & (A)	Post Doc. Trainees
FIXED	7/1/2019	6/30/2020	41.20	(1) & (B)	Class. Staff
FIXED	7/1/2019	6/30/2020	32.10	(1) & (B)	Prof. Staff
FIXED	7/1/2019	6/30/2020	19.10	(1) & (B)	(D)
FIXED	7/1/2019	6/30/2020	21.90	(1) & (B)	(E)
FIXED	7/1/2019	6/30/2020	8.90	(1) & (B)	(F)

ORGANIZATION: University of Washington Management Accounting and Analysis

AGREEMENT DATE: 9/30/2020

D	7/1/2019	30/2020	20.90 (1)	&	urly
D	7/1/2019	30/2020	27.10 (1)	&	e-Doctoral Trainees & Fellows
D	7/1/2019	30/2020	64.20 (2)	&	ass. Staff
D	7/1/2019	30/2020	53.90 (2)	&	of. Staff
D	7/1/2019	30/2020	34.50 (2)	&	culty & Research Associates

** DESCRIPTION OF FRINGE BENEFITS RATE BASE:

(1) Direct salaries and wages including vacation, holiday, and sick pay but excluding other fringe benefits.

(2) Direct salaries and wages excluding vacation, sick leave, holidays, other paid absences and all other fringe benefits.

(A) Entire University
(B) All except Applied Physics Laboratory
(C) Applied Physics Laboratory
(D) Professional Staff - Global (No Health)
(E) Professional Staff - Global (No Retirement)
(F) Professional Staff - Global (No Health or Retirement)

ORGANIZATION: University of Washington Management Accounting and Analysis

AGREEMENT DATE: 9/30/2020

SECTION II: SPECIAL REMARKS

TREATMENT OF FRINGE BENEFITS:

The fringe benefits are charged using the rate(s) listed in the Fringe Benefits Section of this Agreement. The following fringe benefits are included in the fringe benefit rate(s):
HEALTH INSURANCE, SOCIAL SECURITY & MEDICARE TAXES, WORKERS COMPENSATION, MEDICAL AID & INDUSTRIAL INSURANCE, UWRP, STATE RETIREMENT, UNEMPLOYMENT COMPENSATION, SEPARATION LEAVE PAYMENTS FOR CLASSIFIED & PROFESSIONAL STAFF, AND PAID FAMILY AND MEDICAL LEAVE.

TREATMENT OF PAID ABSENCES

Vacation, holiday, sick leave pay and other paid absences are included in salaries and wages and are claimed on grants, contracts and other agreements as part of the normal cost for salaries and wages. Separate claims are not made for the cost of these paid absences. Beginning July 1, 2011, unused leave payments made upon separation of Classified and Professional Staff are included in the fringe benefit rates.

Beginning October 1, 1996 the Applied Physics Laboratory (APL) has separate fringe benefit rates from the remainder of the University of Washington. These rates include paid absences. Therefore, charges for direct salaries and wages from APL must exclude charges for paid absences, including vacation, sick leave, holidays, and other paid absences.

DEFINITION OF EQUIPMENT
Prior to 07/01/2016, equipment means tangible personal property (including information technology systems) having a useful life of more than one year and a per-unit acquisition cost which equals or exceeds $2,000. Effective 07/01/2016, equipment means tangible personal property (including information technology systems) having a useful life of more than one year and a per-unit acquisition cost which equals or exceeds $5,000.

DEFINITION OF ON-CAMPUS, OFF-CAMPUS AND SPECIAL RATES:
DEFINITION OF OFF-CAMPUS RATE
a. An off-campus program is one that is conducted (1) in leased facilities where space related costs (e.g. rent, utilities and maintenance) are charged directly to the program, or (2) in facilities made available (at no cost) to the program by a non-University organization, or (3) away from the University over an uninterrupted period of time in excess of 30 days for field work. The Off-Campus rate is not to be used as a substitute for the Vessel Operations rate or the Applied Physics Laboratory rate. Even though Pack Forest, Big Beef Creek, and Olympic Natural Resource Center are owned and operated by the University, these facilities are considered to be off campus.

b. Projects conducted at two or more locations:
There are instances where a project supported by a single grant or contract

is conducted at two or more locations, thus requiring special consideration in determining the appropriate indirect cost provision. The following should be observed in such circumstances:

(1) Where the total annual amount of the grant or contract direct costs is less than $250,000, a single indirect cost rate will be applied. This rate will be the one currently applicable to the location where the preponderance of project salaries is located.

(2) Where the total annual amount of the grant or contract direct costs is $250,000 or more, the appropriate rate for each location will be applied to the modified total direct costs specifically assigned to the respective location.In the absence of the institution's ability to specifically identify and assign costs to each location, the appropriate rate for each location will be applied to total project costs in the same ratio as direct salary costs incurred at each location during the period covered by the project billing or accounting.

PRIMATE CENTER RATES:
The Washington National Primate Research Center (WNPRC) has two Federally recognized rates for each time period. The NIH Office of the Director Primate Research Center (P51) Core Grant rate is 42.0% for 07/01/14 - 06/30/16. The NIH Office of the Director Primate Research Center (P51) Core Grant rate is 38.1% for 07/01/16 - 06/30/20. The Non-Core Federal Rate of 78.0% for 07/01/14 - 06/30/16 is the sum of the Core Grant (42.0%) and the WNPRC specific F&A expenditures (36.0%). The Non-Core Federal Rate of 83.1% for 07/01/16 - 06/30/20 is the sum of the Core Grant (38.1%) and the WNPRC specific F&A expenditures (45.0%).

This rate agreement updates the fringe benefits only.

NEXT PROPOSAL DUE DATE
A fringe benefit rates proposal based on actual costs for fiscal year ending June 30, 2019 has been received and is under review.

ORGANIZATION: University of Washington Management Accounting and Analysis

AGREEMENT DATE: 9/30/2020

SECTION III: GENERAL

A. LIMITATIONS:

The ratea in this Agreement are subject to any statutory or administrative limitations and apply to a given grant, contract or other agreement only to the extent that funds are available. Acceptance of the rates is subject to the following conditions: (1) Only coste incurred by the organization were included in its facilities and administrative cost poola ao finally accepted: such costs are legal obligations of the organization and are allowable under the governing cost principles; (2) The same costs that have been treated as facilities and administrative costs are not claimed as direct coota; (3) Similar types of costs have been accorded consistent accounting treatment; and **(4)** The information provided by the organization which was used to establish the rates is not later found to be materially incomplete or inaccurate by the Federal Government. In such situations the rate(s) would be subject to renegotiation at the discretion of the Federal Government.

B. ACCOUNTING CHANGES:

Thia Agreement is based on the accounting system purported by the organization to be in effect during the Agreement period. Changes to the method of accounting for coats which affect the amount of reimbursement resulting from the use of **th iu Agreement require prior approval of the authorized representative of the cognizant agency. Such changes i nclude , but are not limited to, changes in the charg ing of a particular type of cost from facilities and adminis rative to direct. Fai lur e to obtain approval may result in coat diaallowances.**

a. FIXED RATES:

If a fixed ruce ie in this Agreement, it is baaed on an estimate of the costs for the period covered by the rate. When the actual coats for this period *are* determined, an adjustment will be made to **a** rate of a future year(s) to compensate for the difference between the costs used to establish the fixed rate and actual costs.

D. USE BY OTHER PllPEBAJ, A.Gt:Nrrns:

The rates in this Agreement were approved in accordance with the authority in Title 2 of the Code of Federal Re gu lations , Part 200 (2 CFR 200), and should be applied to grants, contracts and other agreements covered by 2 CFR 200, subject to any **limitations in A above. The organization may provide copies of the Agreement to other Federal Agencies to give them early not.ification of the Agreeme nt.**

E. **OTJiEll...;**

If any Federal contract, grant or other agreement is reimbursing facilities and administrative costs by a means other than the approved rate(sl in this Agreement, the organization should (1l credit such costs to the affected programs, and (2) apply the approved rate(s) to the appropriate **base** to identify the proper amount of facilities and administrative costs allocable to these programs.

BY THE INSTITUTION:

University of Washington Management Accounting and Analysis

(INSTITUTION)

Brian McCartan

(SIGNATURE)

Brian McCartan

(NAME)

Finance

(TITLE)

9/30/20

(DATE)

ON BEHALF OF THE FEDERAL GOVERNMENT:

DEPARTMBNT OF HEALTH AND HUMAN SERVICES

(AGEN Y)

Arif M. Karim -5 Digitally signed by Arif M. Karim-S Dale: 2020.09.30 15:27:2 \ -05'00'

(SIGNATURE)

Arif Karim

Director , Coat Allocatio n Services

(TITLE)

9/30/2020

(DATE) 7194

!IIIS REPRESENTATIVE: Janet Turner

Telephone: (415) 437-7820

APPENDIX 6: REFERENCES CITED

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