Depuration-based Strategies adding Chitosan Micro-particles to Reduce *Vibrio* parahaemolyticus in American Oysters using the Multi-Phasic Media Method for Process Verification.

Spinney Creek Shellfish, Inc. July 31, 2014

Executive Summary

The Multi-Phasic Media (MPM) method for total *Vibrio parahaemolyticus* (Vp) offers an opportunity for NSSP laboratories to easily enumerate total Vp in oyster meats inexpensively, rapidly, and without specialized equipment and training. Recently, the Canadian Food Inspection Agency (CFIA) added total Vp end-product guidelines for raw oysters, limiting Vp counts to no more than 1 sample in 5 exceeding 100 total Vp/gram and no single sample exceeding 10,000 total Vp/gm. Studies conducted by Anita Wright and K. C. Jeong at the University of Florida demonstrate the successful use of Chitosan micro-particles (CM) as a GRAS list anti-microbial additive for use in depuration to reduce Vp. These three developments make progressive innovation of depuration-based strategies to reduce Vp in American oysters approachable. If studies show that total Vp can be successfully reduced to CFIA end point guidelines, this may represent a new live oyster processing strategy and may offer an alternative approach to meeting a state Vibrio Control Plan. Additional breakthroughs in detection methods at UNH will help the proposed research verify reductions of the most virulent Vp strains.

Scope, Approach, and Methodology

For a depuration-based strategy to be practical and effective at reducing indicator organisms to established levels, an inexpensive, rapid, and simple method that can be performed on site in a NSSP evaluated laboratory is paramount. This is the case for conventional FC-based depuration as well as MSC-based depuration and container relay. The MPM total Vp method shows promise as the primary assessment tool for process verification of depuration-based strategies to reduce Vp. CFIA guidelines create a reasonable and vetted target end-point for depuration-based strategies to reduce total Vp. In combination, the MPM method and the CFIA guidelines give the depuration plant operator the practical ability to monitor incoming Vp levels as needed, verify that the process is working, and to meet end-point standards; an approach similar to FC and MSC depuration processes.

Although temperature and salinity modification are key factors for optimizing any depuration-based Vp reduction strategy, based on collaborative work with Steve Jones at the University of New Hampshire and the results of other studies, it is likely that additional factors beyond these are needed to form the basis of a consistently effective 2-day commercial process. Consequently, effective Vp depuration may need an antimicrobial agent that is effective on Vibrio species and Vp in particular. Chitosan microparticles (CM) show the greatest promise in the search for an effective GRAS-listed, bio-

degradable, anti-microbial compound that is inexpensive and effective on Vibrio species. Anita Wright and K. C. Jeong at the University of Florida are actively developing CM for this purpose and have publications under review that show encouraging results. Building upon the work currently being conducted at the University of Florida Laboratory, this study plans to further develop the use of CM in a commercial depuration facility. Complimenting this effort, researchers Whistler and Jones at the UNH Northeast Center for Vibrio Disease and Ecology will verify changes in total and pathogenic Vp levels through newly developed molecular detection procedures for the most prevalent pathogenic regional strains. These are key collaborations bringing together the scientists who have the expertise with CM and pathogenic vibrio detection and assessment, and a commercial operator with expertise in depuration processes.

An experimental-scale depuration tank (recirculating design) will be set up at the Spinney Creek Shellfish facility. Existing full-scale depuration tanks (recirculating design) will be used as a control, when needed, while the experimental-scale depuration tank is used to isolate the effects of tempering process water, salinity modification, and the addition of CM. By this approach, critical control parameters, CM, and other anti-microbial additives, if needed, may be identified. Once identified, operational protocols for delivering significant log reductions of total Vp may then be developed and optimized in the experimental-scale tank. Once optimized, modification of full-scale production tanks and operating protocols can be considered.

To accomplish this, total Vp reduction trials will be conducted using naturally contaminated market oysters originating from Long Island Sound, Connecticut, during the Vp season (June through September). 12-oyster homogenates will be collected in triplicate at 0-day and 2-day end-point from each tank as necessary. Processing times greater than 2-days are less commercially viable and will not be considered in this study. In the SCS laboratory, the MPM method will be used to enumerate total Vp in those samples. Results from these trials will be kept in an Excel database. Further data analysis will be performed using the Prism 4 bio-statistic program. These analyses will include plotting the 0-day levels of total Vp in oysters received weekly from Long Island Sound over the study period as well as plotting of individual trials. Further analyses of successful vibrio reduction trials meeting CFIA guidelines will use log-linear regression to describe the rate of reduction under certain conditions. In this way operational protocols will be developed, evaluated, and optimized.

Many questions still remain about pathogenicity and answering those questions is not the primary purpose of this study. However, to further determine the feasibility of these protocols to reduce risk of exposure to pathogenic Vp, collaboration with researchers at the UNH Northeast Center for Vibrio Disease and Ecology will bring additional expertise and resources to this research. The ability to reduce both total and pathogenic variants will be examined through sensitive, molecular detection protocols. During July and August (2015), the months most likely to show pathogenic strains, 0 and 2-day samples will be tested using molecular methods. Analyses will be run on 12 batches/sample runs at 0 and 2 days of processing, for a total of 24 analyses. The change in concentration of total and, importantly, pathogenic Vp will be determined by the application of modified

MPN quantitative multiplex PCR detection protocol (Nordstrom). The assay involves the brief enrichment of homogenized oysters, and a three-step detection/enumeration: 1) total Vp is determined by detection of a species-specific genetic marker (tlh); 2) for samples positive for *V. parahaemolyticus*, pathogenic variants are detected and quantified by the presence of two standard genetic markers (*tdh* and *trh*), and a novel genetic marker diagnostic for the invasive O3:K6/ sequence type 36 strain which caused the majority of regional outbreaks in 2012-2013 (*prp*; Xu and Whistler; unpublished); and 3) pathogenic isolates are recovered by selective plating, and the identity confirmed by genotyping using a suite of additional informative genetic loci related to virulence (Mahoney et al, 2010).

Work funded by this grant and conducted from late August 2014 through September 2014 will focus on implementation of the MPM method and preliminary assessment of Long Island Sound oysters before and after an on-going conventional depuration process. Work conducted in May 2015 through July 2015 will focus on modification of the depuration process using CM in collaboration with Wright and Jeong in the experimental tank set-up. Full development of a processing strategy sufficiently robust to qualify as an alternative approach to meeting a state's Vibrio Control Plan will likely require additional work. Applying the MPM total vibrio method as an indicator of pathogenic Vp and using the CFIA guidelines is an appropriate approach to the development of a Vp depuration process. Verification of Vp reductions, and of the MPM assay using PCR methods by Jones and Whistler adds to the robustness of the approach.

Project Management Approach

Thomas Howell will serve as the project's Principal Investigator. His responsibilities will include working with the developers of the MPM method to get the assay up and running in the SCS laboratory and to run preliminary samples this August through October 2014. He will oversee experimental tank design and set up and will be responsible for the experimental design, real time modifications, and operation of the vibrio reduction trials. He is also principally responsible for data analysis and technical report writing. Lori Howell will serve as a Co-PI and will provide lab technical support, be responsible for project administration, communication, overall project management and client correspondence. Laura Stadig, SCS Laboratory Manager will be responsible for the laboratory component of this project. She will be the primary laboratory technician, will train other technicians and perform the required laboratory quality assurance tasks that assure reproducible data and results.

Detailed and Itemized Budget

Salaries: Thomas Howell will work on this project for a minimum of 12 hours a week for about 30 weeks or a total of 360 hours of direct PI time. Lori Howell is expected to spend a minimum of 8 hours per week over the same period or 240 hours of Co-PI time. Both Tom and Lori's time will be contributed as match. (\$30,750 and includes fringe benefits at 25%) Laura Stadig, Lab Manager, is expected to devote upwards of 8 hours per week over 25 weeks to the project (\$7,000, including fringe benefits). An additional laboratory technician is expected to spend upwards of 12 hours per week, for 20 weeks processing samples and prepping test kits. Lab Manager and technician time will be funded by the grant (\$5100, including fringe benefits).

Depuration Equipment: Setting up experimental recirculating depuration tanks is an \$8,000 capital investment. This item includes a tank (\$800), foundation or platform (\$1000), PVC plumbing and wiring (\$600), chiller, controls and aeration (\$2600) and outside labor in the amount of (\$3000), all of which will be contributed by SCS as match.

Expendable Supplies and Equipment: 96-well plates (\$2200), cups, pipet tips, cups, microbiological reagents, parafilm and other expendables, blender and jars, other incidental equipment (\$4900), Chitosan microparticles and other GRAS additives (\$3,000), oyster purchases, 2000, @.55- includes shipping and transfers (\$1100).

Twenty (25) Vibrio reduction trials are budgeted over the study period. We estimate (25) 0-day total Vp samples and (25) 2-day end-point total Vp samples all run in triplicate. This is a total of 50 total Vp tests run in triplicate. FDA will supply the kits for testing. FDA, working with Mississippi State has developed the method and the components are confidential. FDA recently made test kits available to SCS and others for field implementation.

Budget Summary Table

		ISSC	In-Kind
Personnel			
Thomas L. Howell, PI	360 hours @ \$45/hr		\$16,200
	Fringe Benefits @ 25%		\$4,050
Lori A. Howell, Co-PI	240 hours @ \$35/hr		\$8,400
	Fringe Benefits	0 00 0	\$2,100
Laura Stadig, Lab Manager	200 hours @ \$28/hr	\$5,600	
	Fringe Benefits	\$1,400	
Technician	240 hours @ \$17/hr	\$4,080	1
	Fringe benefits	\$1,020	
Personnel Total	100 000	\$12,100	\$30,750
Materials and Supplies	111		
MPM Total Vibrio Test Kits	60 test kits to be provided by FDA (estimated value \$50/each)		\$3,000
Lab Materials, supplies, incidental equipment	96-well plates, pipette tips, pipettes, cups, parafilm, chemicals, blender, jars,	\$7,100	
Oyster Purchases	2000 minimum @ .55 (includes transfers and shipping)	\$1,100	
GRAS list additives	Salt, Chitosan microparticles and others	\$3,000	
Materials and Supplies		\$11,200	
Depuration Equipment		400	
Experimental Depuration tank, set up, materials and controls			\$8,000
Depuration Equipment Total			\$8,000
Subcontract with UNH	24 PCR Analyses	\$2,400	\$2,400
TOTAL		\$25,700	\$33,150

References

Canadian Food Inspection Agency, Facilities Inspection Manual, Appendix K- HACCP Validation of Controls for *Vibrio parahaemolyticus*, Fish Products Standards and Methods Manual, Appendix 2: Bacteriological Guidelines for Fish and Fish Product (end product), April 25, 2014.

Kim, T. J., and Jones, J., 2014. Vibrio Multi-Phasic Media (MPM) Beta Testing.

Nordstrom JL, Vickery MC, Blackstone GM, Murray SL, DePaola A. 2007. Development of a multiplex real-time PCR assay with an internal amplification control for the detection of total and pathogenic Vibrio parahaemolyticus bacteria in oysters. Appl Environ Microbiol. 73(18):5840-7.

Mahoney, J.C., M.J. Gerding, S.H. Jones, and C.A. Whistler. 2010. Comparison of the pathogenic potentials of environmental and clinical *Vibrio parahaemolyticus* strains indicates a role for temperature regulation in virulence. Applied and Environmental Microbiology **76**(22): 7459-65. DOI:10.1128/AEM.01450-10

APPENDIX: REFERENCES

Research references:

Phone:

Stephen H. Jones, Ph.D. Associate Director -NH Sea Grant Program, Jackson Estuarine Laboratory, University of New Hampshire, Durham, NH 03824. Tel: (603) 862-5124 shj@unh.edu

William Burkhardt III, Ph.D., Director, Gulf Coast Seafood Laboratory, US Food & Drug Administration, Dauphin Island, AL 36528 Tel: (251) 690-3361. william.burkhardt@fda.hhs.gov

Jeff Kennedy, Environmental Analyst, Massachusetts Division of Marine Fisheries, Newburyport, MA 01950. Tel: (978) 282-0308 x165. Jeff.Kennedy@state.ma.us

APPENDIX: PROJECT TEAM STAFFING

Principal Investigator, Thomas L. Howell, M.S., President

Address: Spinney Creek Shellfish, Inc.

27 Howell Drive

Eliot, Maine 03903 207-439-2719, Ext. 4

E-mail: tlhowell@spinneycreek.com

Tom has been involved in the shellfish depuration industry for nearly 30 years; all of that time as owner/operator of Spinney Creek Shellfish. He has an undergraduate degree in General Science from Union College, Schenectady, NY and a graduate degree in Physical Oceanography from the University of New Hampshire. He has served on numerous committees of the Interstate Shellfish Sanitation Conference and chaired the depuration committee during the re-writing of Chapter XV. Although he has completed many research projects during his career; for the past 6 years his focus has been on viral reduction through depuration and he has conducted several long-term contaminant reduction studies.

Co- Principal Investigator, Lori A. Howell, M.S., J.D., Vice President

Address: Spinney Creek Shellfish, Inc.

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Eliot, Maine 03903

Phone: 207-439-2719, Ext. 4

E-mail: <u>lahowell@spinneycreek.com</u>

Before attending law school, Lori Howell's education and experience focused on human health and education issues. She has a graduate degree in Health Science and Physiology. She and her husband, Tom, started Spinney Creek Shellfish in Eliot, Maine, in 1983. After 10 years practicing law she turned to full time work in the shellfish

business. She has been involved with the Interstate Shellfish Sanitation Conference since 1993 and has chaired many committees, including the *Vibrio* Management Committee, and many subcommittees and task forces. At Spinney Creek Shellfish Lori wears many hats, including that of Laboratory Quality Assurance Officer and is also a laboratory technician. In addition, she serves on many local, state and national boards and committees relating to shellfish, business regulations, and the marine environment.

Laura Stadig, Laboratory Manager

Address: Spinney Creek Shellfish, Inc.

27 Howell Drive Eliot, Maine 03903

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Laura has been in the shellfish industry since 1994. She has worked a variety of positions from packer, to aquaculture technician, to depuration plant manager and laboratory manager. Laura has been the Lab Manager and primary Spinney Creek Shellfish lab technician for 15 years. In addition to the day to day activities of administering and maintaining an NSSP evaluated lab, Laura does the majority of the testing and has done the heavy lifting involved in 3 Single Lab Validations; Male Specific Coliphage (MSC) for Steamers (*Mya arenaria*), MSC for American oysters (*Crassostrea virginica*) and MSC for Quahogs (*Mercenaria mercenaria*). She has participated in numerous multi-year contaminant reductions studies.

Partner, Stephen H. Jones, Ph.D., Associate Director -NH Sea Grant Program

Address: Jackson Estuarine Laboratory

University of New Hampshire

Durham, NH 03824

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Steve has conducted research on shellfish sanitation, pollution source tracking, Vibrio ecology and post-harvest treatment strategies to reduce Vibrios and fecal-borne pathogens and indicator microbes for three decades at the University of New Hampshire. He has a PhD in Bacteriology from the University of Wisconsin, Madison, and has conducted post-doctoral research at Cornell University and Syracuse University. He has been a key participant in all 11 International Conferences for Molluscan Shellfish Safety and has been a long-term presenter and member at the Interstate Shellfish Sanitation Conference, the Northeast Shellfish Sanitation Association, and other microbiology and shellfish meetings. He is also the Associate Director of the NH Sea Grant Program where he manages the program's research enterprise.

Partner, Cheryl A. Whistler, Ph.D.

Address: Department of Molecular, Cellular and Biomedical Sciences Box 110370, University of New Hampshire

46 College Road Durham, NH 03824

Phone: (603) 862-2359 E-mail: <u>cac36@unh.edu</u>

Cheryl Whistler has conducted research in ecology, molecular typing, host-association mechanisms, and strain evolution of Vibrio species since 2001, and had previously developed expertise in host-microbe interactions and biological control of pathogens on agricultural crops. She has developed specific expertise in pathogenicity and ecology of *Vibiro parahaemolytius* over the last 6 years, with several publications in this area of research, and has given presentations at regional and international meetings on this work. She has a Ph.D. in Molecular and Cellular Biology from Oregon State University, and conducted post-doctoral research at University of Arizona, University of Hawaii, and University of California Davis.

Collaborator, Anita Wright, Ph.D., Associate Professor

Address: Food Science and Human Nutrition Department, University of Florida, PO

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Phone: 352-392-1991 ext311

E-mail: acw@ufl.edu

Collaborator, Dr. K. C. Jeong, Ph.D., Assistant Professor

Address: Animal Science, University of Florida, Emerging Pathogens Institute, P.O.

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Phone: 352-294-5376/352-392-3889

E-mail: kcjeong@ufl.edu

Project team members are not bonded. We are unaware of any requirement for bonding of employees for this project. Please advise if we have overlooked this. We affirm that no employees working on this project have ever been convicted of a felony.

APPENDIX: COMPANY OVERVIEW

1. Official registered name: Spinney Creek Shellfish, Inc.

Dun and Bradstreet number: 093625077

Primary SIC number: 0913

Secondary SIC number: Not Applicable

Address: 27 Howell Drive, Eliot, Maine 03903

Main telephone number: 207-439-2719 Facsimile number: 207-439-7643

2. Key contact: Thomas L. Howell, M.S., President & Research Director

Direct telephone number: 207-439-2719, Ext. 4

Fax number: 207-439-7643

3. Lori A. Howell, Vice President, M.S., J.D., is authorized to contractually bind the organization for any proposal against this RFP. She can be reached at 207-439-2719, Ext. 2.

- 4. Brief history of the company: Spinney Creek Shellfish, Inc. was founded in 1983 as a shellfish aquaculture and depuration firm. The company is a Maine licensed shellfish dealer and has been assigned certification number ME 271 DP. The company has an on-site laboratory that is evaluated and is in conformance with the National Shellfish Sanitation Program. The company has been involved with research since approximately 1986 and has completed many projects in the areas of shellfish safety, depuration, shellfish aquaculture, and Codes of Practice. The company has actively participated in Vibrio research throughout this time, primarily, but not exclusively, in collaboration with Steve Jones, Ph.D. and other researchers at the University of New Hampshire. Information security testing requirements are not applicable.
- 5. Conflicts of interest: Due to the nature of the RFP, Spinney Creek Shellfish, Inc. does not have a conflict of interest.

Thomas L. Howell

Spinney Creek Shellfish, Inc.

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EDUCATION: M.S. in Earth Science, Physical Oceanography. 1983, University

of New Hampshire, Durham, NH

B.S. in General Science 1980, Union College, Schenectady, NY

EXPERIENCE:

1983 to Present. Spinney Creek Shellfish, Inc., Eliot, ME, Pres., Gen. Man.,

Research Director

1980-1983 Research Assistant, Physical Oceanographic Research Team, UNH

1977-1978 Geophysical Exploration Observer, Seiscom Delta, Rocky

Mtn/Canadian Div.

CURRENT RESEARCH EMPHASIS:

Validation of Male Specific Coliphage laboratory method; depuration of pathogens in shellfish; seasonal and spatial variability of virus/viral indicators; development of seasonal viral depuration relay strategies concurrent with FDA research partners; collaborations regarding effect of depuration and relay on *vibrios*.

PUBLICATIONS:

Bushek, D., and T.L. Howell, 2000. The effect of UV irradiation on Perkinsus marinus and its potential use to reduce transmission via shellfish effluents., NRAC Tech. Bull., NRAC pub. No. 00-008.

B.J. Barber, Langan, R., and Howell, T.L. 1997. Haplosporidium nelsoni (MSX) Epizootic in the Piscataqua River estuary (Maine/New Hampshire, U.S.A.), J. Parisitology, vol. 83(1), p. 148-150.

Howell, T.L., Jones, S.H., and G.C. Nardi. 1995. Development of a HACCP-based program for a private shellfish purification facility, p. 207-213. in R. Poggi and J.-Y. Le Gall (ed.), Shellfish Depuration: 2nd Int'l Conference on Shellfish Depuration. IFREMER, Brest, France.

Jones, S.H., T.L. Howell, K.R. O'Neill, and R. Langan. 1995. Strategies for removal of indicator & pathogenic bacteria from commercially-harvested shellfish, p. 69-77. in R. Poggi and J.-Y. Le Gall (ed.), Shellfish Depuration: 2nd Int'l Conference on Shellfish Dep. IFREMER, Brest, France.

O'Neill, K.R., S.H. Jones, T.L. Howell, and D.J. Grimes. 1991. Occurrence of Vibrio vulnificus in water and shellfish from Maine and New Hampshire, p. 189-193. In W.S. Otwell, G.E. Roderick, and R.E. Martin (ed.), Molluscan Shellfish Depuration. CRC Press, Inc., Boca Raton, FL.

Jones, S.H., T.L. Howell, and K.R. O'Neill. 1991. Bacterial evaluation of a commercial controlled purification plant in Maine, p. 181-187. in W.S. Otwell, G.E. Roderick, and R.E. Martin (ed.), Molluscan Shellfish Depuration. CRC Press, Inc., Boca Raton, FL.

- Jones, S.H., T.L. Howell and K.R. O'Neill. 1991. Differential elimination of indicator bacteria and pathogenic vibrios sp. from Eastern Oysters in a commercial controlled purification facility in Maine. J. Shell. Res., Vol. 10, No. 1, 105-112, 1991
- Howell, T.L. and L.R. Howell. 1989. Design of a controlled purification facility, with an evaluation of the biological and economic parameters. New England Fisheries Dev. Association.
- Irish, J.D., T.L. Howell and J.M. Joy. 1988. Evaluation of a control gate for a salt pond estuary. Sea Grant UNHMP-T/DR-SG-88-1.
- Howell, T.L. and W.S. Brown. 1984. Internal waves on the California continental shelf. J.Geophys. Res., 90: 7256-7264.
- Irish, J.D., W.S. Brown and T.L. Howell. 1984. The use of microprocessor technology for the conditional sampling of intermittent ocean processes. J Atmos. Oceanic Technol., 1:58-68.
- Howell, T.L. 1983. Internal wave events on the California Shelf. M.S. 42 pp., Dept. of Earth Sci., Univ. of NH, Durham, NH.

Lori Armbrust Howell Spinney Creek Shellfish, Inc. 27 Howell Drive, Eliot, Maine 03903 207-439-2719, Ext. 2 lahowell@spinneycreek.com

EDUCATION

J.D., Franklin Pierce Law Center, Concord, NH 1985

M.S., University of New Hampshire, Durham, NH., 1981, Health Science/Physiology NATA, West Chester University, West Chester, PA, 1980, Athletic Training Intensive B.S., Ursinus College, Collegeville, PA, 1980, English, Health and Physical Education

EXPERIENCE

SPINNEY CREEK SHELLFISH, INC., 1988-Present. Vice President, Treas. & Gen. Counsel. ATTORNEY AT LAW, Practiced with Mulvey, Noucas & Cornell, P.A., Portsmouth, New Hampshire 1985-1992. Sole Practitioner, 1992 to present.

UNIVERSITY OF NEW HAMPSHIRE, 1980-1981. Instructed various health science courses.

PROFESSIONAL and CIVIC ACTIVITIES

- Interstate Shellfish Sanitation Conference: Member Executive Board; Task Force II & III, Chair, Committee Chair Assignments: Vibrio Management, Illness Review; Unresolved Issues; Labeling; Processing & Handling, Audit; Shipping & Receiving.
- Maine Shellfish Advisory Council, 2008 to present, current Chair.
- Maine Aquaculture Association: Pres. 1994-1997, 1998-2004. Vice Pres., 1992-1994. Member Board of Directors. 1990-2004.
- US Delegate to Global Symposium- Women in Fisheries, Kaohsiung, Taiwan, Nov. 2001
- Maine Aquaculture Innovation Center, Vice Chair, Board of Directors 1995 to 2005
- Water Farming Initiative Steering Committee, Oldways Exchange Preservation Trust 2000-2003
- Atlantic States Marine Fisheries Council, Committee to Develop Codes of Conduct for Aquaculture, 2000 - 2001
- Marine Resources Advisory Council: 2000-2001 Chair, member 1998-2004 and 2013
- State of Maine Aquaculture Strategic Planning Committee
- Maine Clam Advisory Council: 1995-present. Current chair, past newsletter editor
- US-Japan Natural Resources Consortium, Shellfish Delegation, Ise, Japan Nov. 2000
- Maine Science & Technology Action Plan, Steering Committee 2000-2002
- Eliot Clam Management Com: 1998-present, Chair, 1998-2004. Current Vice Chair
- Maine Legislative Task Force to Study Current Regulations on Small Business, 1999
- MEMIC: Member of Natural Resources Governing Bd. 1995- present
- Eliot Planning Board Member 1999-2000
- Eliot Harbor Committee 2000
- MSAD 35 Reaccreditation Steering Committee, 1999-2001.
- Kennebunk Savings Bank Board of Corporators, 1999-present
- Katahdin Counsel Recognition, 2012 and 2013 for substantial pro bono legal service

Stephen H. Jones

Research Associate Professor, Department of Natural Resources & the Environment Jackson Estuarine Laboratory, University of New Hampshire, Durham, NH 03824

(A) PROFESSIONAL PREPARATION

University of Maine-Orono	B.S., Soil Science	1976
University of Wisconsin-Madison	M.S., Soil Science	1980
University of Wisconsin-Madison	Ph.D., Bacteriology	1983
Cornell Univ., Inst. for Comparative & Environmental	Toxicology Postdoctoral Fellow	1984-86

(B) APPOINTMENTS

1993-present Research Associate Professor of Natural Resources and Marine Science, Department of Natural Resources and the Environment, University of New Hampshire.

2011-present Faculty Fellow in Sustainability Science, University of New Hampshire

2006-present Assistant Director for Research, New Hampshire Sea Grant College Program

2002-2006 Director, University of New Hampshire Center for Marine Biology

1989-1992 Research Assistant Professor of Natural Resources and Marine Science, Department of Natural Resources, University of New Hampshire.

Research Assistant Professor, Department of Microbiology, University of New Hampshire Research Fellow & Adjunct Professor, Department of Civil Engineering, Syracuse Univ.,

(C) CLOSELY-RELATED PUBLICATIONS

Ellis, C.N. B.M. Schuster, M.J. Striplin, S.H. Jones, C.A. Whistler and V.S. Cooper. 2012. Influence of seasonality on the genetic diversity of *Vibrio parahaemolyticus* in New Hampshire shellfish waters as determined by multi-locus sequence analysis. Appl. Environ. Microbiol. 78:3778-82.

Jones, S.H. 2011. Microbial Pathogens and Biotoxins: State of the Gulf of Maine Report. Gulf of Maine Council on the Marine Environment. http://www.gulfofmaine.org/stateofthegulf. 21 pp.

B.M. Schuster, A. Tyzik, R. Donner, S.H. Jones, V.S. Cooper, C.A. Whistler. 2011. Population structure and ecological correlations of an endemic northern temperate population of *Vibrio cholerae* with close relatives to toxigenic isolates. Appl. Envrion. Microbiol. 77: 7568-7575.

Mahoney J.C., M. J. Gerding, S. H. Jones & C.A. Whistler. 2010. Characterization of the pathogenic potential of environmental *Vibrio parahaemolyticus* compared to clinical strains indicates a role for temperature regulation in virulence. Appl. Environ. Microbiol. 76: 7459-7465.

Jones, S., M. Striplin, J. Mahoney, V. Cooper and C. Whistler. 2010. Incidence and abundance of pathogenic *Vibrio* species in the Great Bay Estuary, New Hampshire, pp. 127-134, <u>In</u>, Proceedings of the Seventh International Conference on Molluscan Shellfish Safety. Lassus, P. (Ed.). Nantes, France, June 14-19, 2009. Quae Publishing, Versailles, France.

LaValley, K. J., S. Jones, M. Gomez-Chiarri, J. Dealteris and M. Rice. 2009. Bacterial community profiling of the Eastern oyster (*Crassostrea virginica*): Comparison of culture-dependent and culture-independent outcomes. J. Shellfish Res. 28: 827-835.

(C-ii) OTHER RELATED PUBLICATIONS

Jones SH. 2009. Microbial Contamination and Shellfish Safety, Ch 1, pp. 3-42, *In* Shellfish Safety and Quality, Shumway, S and G Rodrick (Eds). Woodhead Publishing, Ltd., Cambridge, UK.

Jones, SH, N Landry, C Edwards. 2009. Enhanced Use of *Escherichia coli* Ribotyping for Tracking Bacterial Pollution Sources in Coastal New Hampshire, USA, pp. 269-274, *In*, Proceedings of the 6th International Conference in Molluscan Shellfish Safety. Busby, P (Ed.). 18 to 23 March, 2007, Blenheim, New Zealand. Miscellaneous Series 71. The Royal Society of New Zealand, Wellington.

Nelson M, SH Jones, C Edwards, JC Ellis. 2008. Characterization of *Escherichia coli* Populations from Gulls, Landfill Trash, and Wastewater Using Ribotyping. Diseases of Aquatic Organisms 81: 53-63.

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PROFESSIONAL PREPARATION

<u>Institution</u>	<u>Department</u>	<u>Years</u>
University of California, San Diego	Biology	B.A. 1991
Oregon State University	Molecular and Cellular Biology	Ph.D. 2000
University of Arizona	Plant Pathology	2000-2001
University of Hawaii, Manoa	Microbiology	2001-2003
University of California, Davis	Plant Pathology	2003-2004

APPOINTMENTS

Associate Professor, Department of Molecular, Cellular and Biomedical Sciences, University of New Hampshire, Nov. 2004-Present
Visiting Postdoctoral Scholar, University of California, Davis, 2003-2004
Junior Researcher, University of Hawaii, 2002-2003
Postdoctoral Fellow, University of Hawaii, 2001-2002
Research Associate, University of Arizona, 2000-2001
Graduate Research Assistant, Oregon State University, 1994-2000
Research Assistant, Genentech Inc., 1991-1994

AWARDS AND HONORS

United States Department of Agriculture National Needs Fellow, 1994-1997 Environmental Protection Agency Science to Achieve Results (STAR) Fellow 1997-2000 National Science Foundation Postdoctoral Fellowship in Microbial Biology, 2001-2003

CURRENT SUPPORT

NHAES Hatch: Application of alternative host models to assess virulence potential of the foodborne pathogen *V. parahaemolyticus* from native New Hampshire Oysters Role: PI \$12,000annually 10/1/2011-9/30/2014

Sea Grant: Interactions between salinity and the resident microbial community in

excluding pathogenic Vibrios from oysters

Role: Co-PI \$145,000 2/1/2012-1/31/2014

NSF IOS: The molecular bases of host adaptation and the origin of new mutualisms.

Role: PI \$716,000 9/15/2013-9/15/16

REFEREED PUBLICATIONS

- 1. E.A Heath-Heckman, Peyer SM, **Whistler CA**, Apicella MA, Goldman WE, McFall-Ngai MJ. 2013. Bacterial bioluminescence regulates expression of a host cryptochrome gene in the squid-Vibrio symbiosis. M. Bio. 4(2):_e00167-13. doi: 10.1128/mBio.00167-13.
- 2. C.N. Ellis, <u>B.M. Schuster</u>, <u>M.J. Striplin</u>, S.H. Jones, **C.A. Whistler**, and V.S. Cooper. 2012. Influence of seasonality on the genetic diversity of *Vibrio parahaemolyticus* in New Hampshire shellfish waters as determined by multi-locus sequence analysis. Applied and Environmental Microbiology, 78(10):3778-82.
- 3. Fidopiastis, P.M., B.A. Rader, D.G. Gerling, N.A. Gutierrez, K.H. Watkins, M.W. Frey, S.V. Nyholm and **C.A. Whistler**. 2012. Characterization of a *Vibrio fischeri* aminopeptidase and evidence for its influence on an early stage of squid colonization. Journal of Bacteriology. 194(15):3995-4002.
- 4. <u>B.M Schuster</u>, A. Tyzik**, R. Donner**, M. Striplin, S. Almagro-Moreno, S.H. Jones, V.S. cooper and **C.A. Whistler*** 2011. Ecology and genetic structure of a northern temperate *Vibrio cholerae* population with relatives to toxigenic isolates. Applied and Environmental Microbiology 77:7568-7575.
- 5. S.H. Jones, M.J. Striplin, <u>J.C. Mahoney</u>, V.S. Cooper, **C.A. Whistler**, 2011. Incidence and Abundance of Pathogenic Vibrio Species in the Great Bay Estuary, New Hampshire. Proceedings of the International Conference on Moluscan Shellfish Safety
- 6. <u>J. C. Mahoney</u>, M. J. Gerding**, S. H. Jones, **C. A. Whistler***. 2010. Characterization of the pathogenic potential of environmental *Vibrio parahaemolyticus* compared to clinical strains indicates a role for temperature regulation in virulence, Appl. Env. Microbiol. **76**:7459-65.
- 7. <u>B. M. Schuster</u>, <u>L. A. Perry</u>, V. S. Cooper, **C. A. Whistler***, 2010. Breaking the language barrier: Experimental evolution of non-native *Vibrio fischeri* in squid tailors luminescence to the host. Symbiosis **51**:85-96. DOI 10, 1007/s13199-010-0074-2
- 8. M.J. Hagen, V.O. Stockwell, **C.A. Whistler**, K.B. Johnson, J.E. Loper. 2009. Stress tolerance and environmental fitness of *Pseudomonas fluorescens* A506, which has a mutation in RpoS. Phytopathology **99**(6):679-688.
- 9. **C. A. Whistler***, T. A. Koropatnick, A. Pollack**, M. J. McFall-Ngai, and E. G. Ruby. 2007. The GacA global regulator of *Vibrio fischeri* is required for normal host tissue responses that limit subsequent bacterial colonization. Cellular Microbiology. **9**:766-778
- E.G. Ruby, M. Urbanowski, J. Campbell, A. Dunn, M. Faini, R. Gunsalus, P. Lostoh, C. Lupp, J. McCann, D. Millikan, A. Schaefer, E. Stabb, A. Stevens, K. Visick, C. Whistler, and E.P. Greenberg. 2005. Complete genome sequence of *Vibrio fischeri*: A symbiotic bacterium with pathogenic congeners. PNAS 102:3004-3009.

- 11. H. Lee, J.L. Humann, JS Pitrak, J.T. Cuperus, T.D. Parks, C.A. Whistler, M.C. Mok, and L.W. Ream. 2003. Translation start sequences affect the efficiency of silencing of *Agrobacterium tumefaciens* T-DNA oncogenes. Plant Physiology. **133**:966-977.
- 12. **C.A. Whistler** and E. G. Ruby. 2003. GacA regulates symbiotic colonization traits of *Vibrio fischeri* and facilitates its beneficial association with an animal host. Journal of Bacteriology. **185**:7202-7212.
- 13. **C.A. Whistler** and L.S. Pierson III. 2003. Repression of phenazine antibiotic production in *Pseudomonas aureofaciens* strain 30-84 by RpeA. Journal of Bacteriology. 185:3718-3725.
- 14. **C.A. Whistler**, V.O. Stockwell, and J.E. Loper, 2000. Protease Lon regulates antibiotic production and ultraviolet sensitivity of *Pseudomonas fluorescens* Pf-5. Applied and Environmental Microbiology **66**:2718-2725.
- 15. **C.A. Whistler**, N.A. Corbell, A. Sarniguet, W. Ream, J.E. Loper, 1998. The two-component regulators GacS and GacA influence accumulation of the stationary-phase sigma factor σ^s and the stress response in *Pseudomonas fluorescens* Pf-5. Journal of Bacteriology **180**:6635-6641.
- 16. J.E. Loper, B. Nowak-Thompson, **C.A. Whistler**, M.J. Hagen, N.A. Corbell, M.D. Henkels, and V.O. Stockwell, 1997. Biological control mediated by antifungal metabolite production and resource competition. *In* Proceedings of the Fourth International Workshop on Plant Growth-Promoting Rhizobacteria. A. Ogoshi, K. Kobayashi, Y. Homma, F. Kodama, N. Kondo and S. Akino eds., p. 73-79.

ANITA C. WRIGHT

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PO Box 110370 Gainesville, FL 32611

EDUCATION

B.S.	Florida State University, Tallahassee, FL	1974	Experimental Psychology
M.S	University of North Carolina at Charlotte, NC	1983	Biology
Ph.D.	University of Maryland, Baltimore, MD	1997	Molecular Microbiology

POSITIONS

2005-present	Associate Professor, University of Florida, Gainesville, Florida.
1999-2005	Assistant Professor, University of Florida, Gainesville, Florida
1997-1999	Post Doctoral Associate, Center for Marine Biotechnology,
	Baltimore, MD
1984-1997	Research Associate, University of MD Medical School, Baltimore,

MD

Regional Center (2012-present), Steering Committee for Vibrios (2011) and Vibrios in the Environment Conferences (2010) Conferences, ISSC Methods Committee (2005-2010), Advisory Board UF Emerging Infectious Disease Institute (2009-present), Governor's Task Force on Oyster Health in Apalachicola Bay, FL (2012), President SE Branch ASM (2011), Wall Street Journal Technology Innovation Award (2010), UF Innovation Award (2009), U MD "Best Poster" Award (1999), Sigma Xi Graduate Research Award (1983), NSF Undergraduate Research Fellowship (1974). Grant PI for USDA (2000-2004, 2004-2007, 2008-2012) and Fl Sea Grant (2003-2005, 2004-2006, 2006-2008, 2011-2013) Center for Produce Safety (2010-2012, 2013).

RESEARCH INTERESTS:

Dr. Wight is a food microbiologist who focuses on the ecology and evolution of foodborne pathogens in environmental reservoirs. Her research experience includes investigations on the virulence, survival, and environmental distribution of *Vibrio* and *Salmonella* species. Outcomes have resulted in the development of rapid methods for the enumeration and molecular characterization of these pathogens in aquatic reservoirs and food products. Collaborations with the Emerging Pathogens Institute explored the role of aquaculture in the evolution of virulence of *V. vulnificus* in Bangladesh. She directs the Southeastern component of the U.S. FDA Next Generation Sequencing Project and will access these resources for phylodynamic evaluation of pathogens. She has over 40 peerreviewed papers and has received more than \$2 million in grants support. She has supervised five PhD and 8 MS students during her tenure at UF.

SELECTED PUBLICATIONS (Out of 45 total):

- 1. Luo, Z., G. Gu, C. G. Mihai, P. Adams, G. Vellidis, A. H. C. van Bruggen, A. C. Wright*. Development of a novel cross-streaking method for isolation, confirmation, and enumeration of *Salmonella* from irrigation ponds. *J. Microbiol. Methods*. In Press.
- 2. Li, B. Vellidis, G, H., Liu, M. Jay-Russell, S. Zhao, Z Hu, A.C Wright, and C. Elkins. Improved Detection and Isolation Scheme Reveals Diversity and

- Persistence of Salmonella enterica Subtypes in Surface Water in Southeastern U.S. (under review by Appl Environ. Microbiol. The manuscript has been assigned the control number AEM00954-14.)
- 3. Gu, G., Luo, Z., Cevallos-Cevallos, J., Adams, P., Vellidis, G., Wright, A., and van Bruggen, A. 2013a. Factors affecting the occurrence of Escherichia coli O157 contamination in irrigation ponds on produce farms in the Suwannee River Watershed. *Canadian Journal of Microbiology*, 59(3): 175-82.
- 4. Gu, G., Luo, Z., Cevallos-Cevallos, J., Adams, P., Vellidis, G., Wright, A., and van Bruggen, A. 2013. Occurrence and population density of Campylobacter jejuni in irrigation ponds on produce farms in the Suwannee River Watershed. *Canadian Journal of Microbiology*, 59(5): 339-46.
- 5. Wright, A.C. and J. Harwood. (2013) "Vibrios" in Foodborne Infections and Intoxications, Fourth Edition. J. G. Morris, Jr, Editor. Academic Press. (ISBN-10: 0124160417; ISBN-13: 978-0124160415)
- 6. Tao, Z. A. Larsen, S.A. Bullard, A.C. Wright, and C. R. Arias. 2012. Prevalence and population structure of *Vibrio vulnificus* on recreational fishes from the northern Gulf of Mexico. *Appl. Environ. Microbiol.*
- 7. Staley, C., Jones, M.K., Wright, A. C., Harwood, V. J. 2011. Genetic and quantitative assessment of *V. vulnificus* populations in oyster (*Crassostrea virginica*) tissues. *Envirol Microbiol Reports*
- 8. Thiaville, P.C., Bourdage, K.L., Wright, A. C. et al. 2011. Genotype is correlated with but does not predict virulence of *V. vulnificus. Infect. Immun.* 9 (3): 1194-7.
- 9. Gulig, P. A., V. de Crecy-Lagard, V., A. C. Wright, et al., 2010. SOLiD sequencing of four *Vibrio vulnificus* genomes enables comparative genomic analysis. *BMC Genomics* 11, 512.
- 10. Gauthier, J. D., Jones, M. K., Thiaville, P., Joseph, J. L., Swain, R. A., Krediet, C. J., Gulig, P. A., Teplitski, M. A., Wright, A. C. 2010. Role of GacA in virulence of *V. vulnificus. Microbiology*.156:3722-3733.
- 11. Mahmud, Z. H., Wright, A.C. et al., 2010. Genetic characterization of *Vibrio vulnificus* strains from tilapia aquaculture in Bangladesh. *Appl Environ Microbiol* 76, 4890-4895.
- 12. Wright, A.C., Danyluk, M., Otwell, W.S. 2009. Pathogen analysis in raw foods: What the salad bar can learn from the raw bar. *Current Opin Biotech*. 20:172-177.
- 13. Srivastava, M., M. S. Tucker, P. A. Gulig, and A. C. Wright. 2009. Phase variation, capsular polysaccharide, pilin, and flagella contribute to uptake of the Eastern oyster (*Crassostrea virginica*) by *Vibrio vulnificus*. *Environ*. *Microbiol*. 11:1933-34.
- 14. Wright A.C., et al. 2007. Evaluation of post-harvest processed oysters by using PCR-based MPB enumeration of *Vibrio vulnificus* bacteria. *Appl Env Microbiol* 73:7477-7781
- **D.** Synergistic Activities: Dr. Wright is director of the SE Regional Center for the FDA Next Generation Sequencing Project. This project is aimed at expanding the whole genome sequence database in order to provide more rapid and accurate analysis and trace back of foodborne outbreaks. She served on the Governor's Task Force on Oyster Health in Apalachicola Bay, FL (2012-present). She was a member of steering committees for Vibrios 2011 Conference, Santiago DeCompastella, Spain (2011);

Vibrios in the Environment Conference (2010); Florida Marine Biotechnology Summit (2002-2007). She organized a Vibrio session for the 2014 National Shellfisheries Association in Jacksonville, FL. She is a past member of the ISSC Methods Committee (2005-2009) and for Advisory Boards for University of Florida Emerging Infectious Disease Institute (2009-2013) and for BioFlorida (2004-2009). She chaired the Graduate Committee Food Science and Human Nutrition Department (2004-2010) and was a member University of Florida Curriculum Committee (2004-2009). She currently serves on the UF Graduate Scholarship and the Faculty Enhancement Committees. She also served on review panels for USDA Food Safety NRI (2002, 2004, 2010). She was past president of the Southeastern Branch of the American Society for Microbiology (2010).

Kwang Cheol (K.C.) Jeong, Ph.D.

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EDUCATION

- Ph. D. University of Wisconsin-Madison, Food Microbiology and Toxicology; and Department of Bacteriology, Madison, Wisconsin (2004)
- M. S. Chonnam National University, Food Science and Technology, Korea (1998)
- B. S. Chonnam National University, Food Science and Technology, Korea (1996)

APPOINTMENTS

- Assistant professor, University of Florida (2011 present)
- Faculty of Animal Molecular and Cellular Biology, University of Florida (2011 present)
- Postdoctoral Research Associate, Department of Molecular Microbiology, Washington University in St. Louis, School of Medicine (2005 – 2010)

RESEARCH INTERESTS

Dr. K. C. Jeong's ultimate research goal is to intervene pathogens for the benefit of animals and humans. Developing intervention technologies to fight against pathogens using micro and nanoparticles is another key part of his research program.

PROFESSIONAL MEMBERSHIPS AND HONORS

- Associate Faculty Member in Faculty of 1000 (2010 present), member of American Society for Microbiology, IAFP, FAFP, IFT, Sigma Xi, and R&D planning/evaluation board of Ministry of Trade, Industry and Energy (MOTIE) in Korea (2013 – present), Member of
- First place, Poster competition, Annual meeting of Food Research Institute, University of Wisconsin, Madison, WI (2003). Berg/Morse Fellowship Award, Washington University in St. Louis, School of Medicine (2007). The labarotorian of the year-2012, Florida Association of Food Protection (2013). Career development award, IFAS, University of Florida (2013).

<u>PUBLICATIONS</u> (Most relevant to the current application-selected from 26 publications)

- 1. Jeon, S., W. Yeo, K. Galvao, and **K.C. Jeong**. 2014. Underlying Mechanism of antimicrobial activity of chitosan microparticles and implications for the treatment of infectious diseases (accepted by PLOS ONE)
- 2. Mir, R., T. A. Weppelmann, N.D. DiLorenzo, and **K.C. Jeong**. 2014. Age-specific prevalence of Shiga-toxin producing *Escherichia coli* in a cohort of beef cattle (in review, PLoS One).
- 3. Jeon, S., W. Yeo, K. Galvao, and **K.C. Jeong**. 2014. Underlying Mechanism of antimicrobial activity of chitosan microparticles and implications for the treatment of infectious diseases. PLoS One. 10.1371/journal.pone.0092723.
- 4. Aydin, M., G. Herzig, **K.C. Jeong**, S. Dunigan, P. Shah, and S. Ahn. 2014. Rapid and Sensitive Detection of *Escherichia coli* O157:H7 in Milk and Ground Beef Using Magnetic Bead-based Immunoassay Coupled with Tyramide Signal Amplification. J. Food Protection. 77:100-105.

- 5. Jeon, S., M. Elzo, N. DiLorenzo, C. Lamb, **K.C. Jeong**. 2013. Evaluation of animal genetic and physiological factors that affect the prevalence of *Escherichia coli* O157 in cattle. PLoS One. 10.1371/journal.pone.0055728.
- 6. **Jeong, K.C.**, O. Hiki, M.Y. Kang, D. Park, C.W. Kaspar. 2013. Prevalent and persistent *Escherichia coli* O157 strains on farms are selected by bovine passage. J. Vet. Microbiol. doi:10.1016/j.vetmic.2012.11.034.
- 7. Lim, M.S., J. Kim, J.G. Lim, B.S. Kim, **K.C. Jeong**, K.H. Lee, and S.H. Choi. 2011. Identification and characterization of a novel serine protease VvpS containing two functional domains and essential for autolysis of *Vibrio vulnificus*. J. Bacteriol. 193:3722-32
- 8. **Jeong, K.C.** and J. Yu. 2012. Investigation of *in vivo* protein interaction in Aspergillus spores, Methods Mol. Bio. 944:251-7.
- 9. **Jeong, K.C.,** M.Y. Kang, J.H. Kang, D.J. Baumler, and C.W. Kaspar. 2011. Reduction of *Escherichia coli* O157:H7 shedding in cattle by addition of chitosan microparticles to feed. Appl. Environ. Microbiol. 77:2611-2616.
- 10. Jeong, H.S., **K.C. Jeong**, H.K. Choi, K.-J. Park, K.-H. Lee, J.H. Rhee, and S.H. Choi. 2001. Differential expression of *Vibrio vulnificus* elastase gene in a growth phase-dependent manner by two different types of promoters. J. Biol. Chem. 276:13875-13880.
- 11. **Jeong, K.C.**, H.S. Jeong, S.E. Lee, S.S. Chung, J.H. Rhee, A.M. Starks, G.M. Escudero, P.A. Gulig, and S.H. Choi. 2000. Construction and phenotypic evaluation of a *Vibrio vulnificus vvpE* mutant for elastolytic protease. Infect. Immun. 68:5096-5106.
- 12. Lee, S.E., Shin, S.Y. Kim, Y.R. Kim, D.H. Shin, S.S. Chung, Z.H. Lee, J.Y. Lee, K.C. Jeong, S.H. Choi, and J.H. Rhee. 2000. *Vibrio vulnificus* has the transmembrane transcription activator ToxRS stimulating the expression of the hemolysin gene *vvh*. J. Bacteriol. 182:3405-3415.

B. Book chapters

- 1. **K.C. Jeong**, C.D. Vincent, E. Buford, and J.P. Vogel. Subcellular Localization of the Dot/Icm Type IV Secretion Proteins. *Legionella*: State of the art 30 years after its recognition. Nicholas P. Cianciotto [et al.]. Washington, D.C. ASM Press, 2006.
- 2. C.D. Vincent, **K.C. Jeong**, J. Sexton, E. Buford, and J.P. Vogel. The *Legionella pneumophila* Dot/Icm Type IV Secretion System. *Legionella*: State of the art 30 years after its recognition. Nicholas P. Cianciotto [et al.]. Washington, D.C. ASM Press, 2006.

Research interests

Dr. Jeong's ultimate research goal is to intervene pathogens in animals for the benefit of animals and humans. To achieve this goal, his research areas are not only in basic sciences but also in applied sciences. The primary goal of basic science research is to understand molecular mechanisms of antimicrobial resistance, colonization, host-microbe interactions, and survival of pathogens in hosts. Identification of genetic traits responsible for the survival of pathogens in hosts and characterization of genes and proteins will provide insights for the development of intervention technologies. Dr. Jeong's repertoire of knowledge in molecular biology, biochemistry, cell biology, genomics, metagenomics, and genetic techniques will be applicable to various aspects of researches. Developing intervention technologies to fight against pathogens using micro and nanoparticles is

another key part of his research program. Chitosan microparticles have been developed as an alternative antimicrobial agent, and his research has focused on the increment of efficacy in chitosan microparticles targeting a broad spectrum of pathogens, including antimicrobial resistant microorganisms. Furthermore, development of nanoparticles with high specificity against pathogens is ongoing interest, which has been funded by the USDA.





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July 30, 2014

To Whom It May Concern:

I am writing to express my support of the research proposed by Spinney Creek Shellfish, Inc. and entitled "Depuration-based Strategies adding Chitosan Micro-particles to Reduce Vibrio parahaemolyticus in American Oysters using the Multi-Phasic Media Method for Process Verification. Research at the University of Florida has revealed a novel approach to oyster processing that was extremely effective in the reduction of *Vibrio* species in live oysters, namely the application of chitosan to oyster depuration. Their proposal will scale up and validate this approach for commercial application and will greatly facilitate its development and implementation.

Spinney Creek Shellfish, Inc. is committed to improving the safety of molluscan shellfish and has the facilities to enable the development of practical protocol that could be widely used by growers and processors for reduction and possible elimination of the risks imposed by exposure to pathogenic Vibrios in shellfish. Dr. Jeong and I have also submitted a proposal that will optimize the chitosan processing specifically for *V. parahaemoltyicus* and are committed to collaboration on this project.

Sincerely,

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