"Development of a free, open access, Vibrio parahaemolyticus risk portal for Atlantic coast shellfish harvesting areas, and an examination of growth rates of O4:K12 strains as determined by temperature and salinity".

EXECUTIVE SUMMARY

Whereas V. parahaemolyticus serotype O3:K6 has historically been the major world-wide strain of concern, a newly emerging strain (O4:K12) has resulted in significant outbreaks in the US in 1997, 2004, 2012 and 2013. The most recent cases occurred in 13 Northeastern states and were associated with consumption of raw shellfish. Because the O4:K12 strains appear to be more cold adapted, we expect them to exhibit a significant ability to survive in colder, Northeastern waters compared to the classic “warm water” strain. The recent emergence of “Pacific Northwest” V. parahaemolyticus strains along the Atlantic coast of the USA has widespread economic, public health, risk assessment and regulatory consequences in the region, particularly along the Northeast coastline of the USA. There are several key data gaps that the recent emergence of these strains has highlighted, and that urgently require elucidation. Firstly, new and comprehensive control measures for V. parahaemolyticus, including time/temperature controls, have not been systematically assessed. Growth data with regards to highly pathogenic O4:K12 strains are lacking, as is information on the temporal and spatial distribution of these strains in the environment based on their temperature and salinity preferences. In addition, risk assessment tools to determine the potential public health risks associated with these strains have not been exhaustively examined in the context of their recent arrival along the Northeast coastline of the USA, especially compared to other pathogenic strains. These data gaps have significant ramifications for our collective ability to assess, manage and prevent the spread of these highly pathogenic bacteria in the environment, and into clinical settings.

We wish to study both serotypes, especially the most common serotypes found in the US, to characterize their growth characteristics in response to varying temperature and salinity, and from these data to develop “risk maps” to predict when and where human outbreaks may be more likely. Coupled with and directly correlated with these lab-based studies, we will employ recently developed satellite-based technology that can view any coastal or oceanic region on the surface of the Earth to predict coastal regions of the Northeastern US where human Vibrio cases are likely to emerge over the following 5-days. Numerous stakeholders would benefit from this application, including risk managers, public health organizations and managers, the shellfish harvesting industry, and government agencies among others.

SCOPE, APPROACH, AND METHODOLOGY

Globally, waterborne infectious diseases are an increasingly significant cause of disease, affecting both tropical and temperate regions. In many instances outbreaks are strongly seasonal, however the environmental conditions driving infections are poorly understood, which limits the management and forecasting of associated risk. Many pathogens of human health relevance, such as V. parahaemolyticus, grow preferentially in warm, low salinity waters. In 2012 we developed a risk mapping approach that utilizes a combination of remote sensing and instrumental analysis of temperature and salinity. This data can be used to explain Vibrio disease emergence using retrospective bacterial outbreaks, such as those observed during heat waves in Northern Europe (Baker-Austin et. al. 2013. Nature Climate Change 3:73–77), the first of its kind to do so (Fig. 1). We have shown that this experimental prediction model is rapid, reliable and crucially, has global coverage. We would like to refine this pilot project to focus on the coastal regions of the Northeast USA. As an extension to epidemiological applications, the ability to pinpoint areas undergoing increasing waterborne risk may have huge practical
implications for clinical, regulatory and economic sectors. This is an entirely desk-based project, utilizing remote sensing and oceanographic datasets, including partners at the CDC (Centers for Disease Control, USA) and ECDC (European Centre for Disease Control, Sweden). We have subsequently developed a web-portal that utilizes temperature and salinity fields for *Vibrio* risk-assessment purposes (see https://e3geoportal.ecdc.europa.eu/SitePages/VibrioRiskMap.aspx). Surface salinity data from the satellite fields e.g. NOAA, SMOS and Aquarius/SAC-D will be used. In addition, data regarding salinity may be extrapolated from relevant NASA datasets (see www.aquarius.gsfc.nasa.gov/index.html) as well as local measurement from instrument networks. Data derived will be used to analyze, at first retrospectively, large-scale *V. parahaemolyticus* outbreaks (e.g. Alaska, 2005, Northeastern USA 2012-2013). This ‘ground-verification’ of the risk mapping algorithm will ensure its robustness for real-time applications. During this process we would try to identify key environmental signatures (e.g. temperature anomaly data) prior to reported infections and outbreaks. Several limitations to this current approach are apparent, and will be further refined and improved here. These include the use of higher resolution datasets for sea surface temperature (instead of 5km pixel, down to 250m resolution SST fields), incorporation of shellfish harvesting density data around coastlines, and exploration of growth data from Pacific Northwest *Vibrio parahaemolyticus* strains to improve risk resolution for the Northeast coastline of the USA. The current system provides views of the NE coast similar to those shown in Fig. 2.

![Fig. 2. Risk maps of (a) East coast of the USA, and (b) the Northeast coast of the USA. These screenshots demonstrate some of the advantages as well as limitations of this current tool. The website URL risk portal tool is fast, open access, and can be used to view any region on the surface of Earth. It is updated daily, providing near real-time risk mapping data. The risk mapping portal also uses a 5-day forecasting tool for sea surface temperature and salinity which can be used for short to medium-term forecasting. One apparent disadvantage of the current portal, and which will be addressed from our proposed project, is the resolution and granularity of risk data (see Chesapeake catchment, right). This project will develop and employ higher quality 250 meter rather than 5km fields, greatly increasing the risk resolution, particularly around coastal regions.](https://e3geoportal.ecdc.europa.eu/SitePages/Vibrio%20Map%20Viewer.aspx)

In a recently completed pilot (1 year) study examining the influence of temperature and salinity on the incidence of *V. vulnificus* and *V. parahaemolyticus* in commercial NC oyster beds and surrounding waters (Froelich et al., 2014. Submitted to Appl. Environ. Microbiol.). We found *V. vulnificus* in both oyster and water samples to exhibit significant positive correlations with water temperature and negative correlations with salinity. However, while we found strong, significant linear relationships between water temperature and *V. parahaemolyticus* abundance in both waters and oyster, there was no correlation with salinity. Yet there remained instances in both water and oyster samples in which there were no detectable *V. parahaemolyticus*, even when water temperatures were relatively warm. This suggests that there are yet unrevealed factors that contribute to the success of *V. parahaemolyticus*.

Whereas historically *V. parahaemolyticus* serotype O3:K6 was the major world-wide concern, *V. parahaemolyticus* O4:K12/O4:Kut, often referred to as the “cold water” or “Pacific Northwest” (PNW) strain, was first reported in Washington in 1988, with the first large outbreaks in the US occurring in 1997 and 2004. These were due to consumption of Pacific shellfish. In 2012, 28 cases from 9 states occurred in states along the Atlantic coast (Martinez-Urtaza et al., 2013. New Engl. J. Med. 369:1573-1574). Another 104 persons became ill in 2013. These cases occurred in 13 Northeastern states, and were again associated with consumption of raw shellfish (Newton et al., 2014. Morbid.
Mortal. Week. Rep. 63:335-336). That study stated “This PNW strain is possibly becoming endemic in an expanding area of the Atlantic Ocean. The mechanisms for this introduction are not known”. Clearly, there is a need for a better understanding of the environmental parameters that determine the distribution and abundance of the PNW strains. To that end, we intend to determine growth rates of the two major *V. parahaemolyticus* strains, the “classic” O3:K6 serotype and the newly emerging PNW O4:K12 serotype, as affected by temperature and salinity. Because the PNW strains appear to be cold adapted, we anticipate a significant difference in these two serotypes in their ability to survive in colder waters, compared to other pathogenic strains of *V. parahaemolyticus*. We will employ a 96 well plate format with varying temp/salinity/time to characterize the growth characteristics *V. parahaemolyticus* O3:K6 and O4:K12 strains. The method we will employ uses quantitative PCR, allowing large sets of studies to be rapidly compared.

Finally, we will attempt to establish “risk maps” for these two strains, based on their growth characteristics. Combining the satellite imaging, occurrence of human *Vibrio* infections in the studied waters, and the lab-based growth studies, we expect to be able to apply these risk maps to predict occurrences of *V. parahaemolyticus* in the water column, and hence a proxy for risk. The developed risk maps would be similar to those that resulted from our NC study, an example of which is shown in Fig. 3. Numerous stakeholders would benefit from this application, including risk managers, public health organizations and managers, the shellfish harvesting industry, and government agencies among others.

**Methods**

The graduate student will be based at the University of North Carolina Charlotte and utilize a strain bank encompassing a range of clinically-derived Pacific Northwest (PNW) O4:K12, O4:Kut, and O3:K6 strains for the growth experiments. These will be conducted on liquid cultures, varying the time, temperature, and salinity, and will take advantage of the speed and accuracy of quantitative PCR (qPCR) for enumeration. These data will be used to develop a range of downstream risk assessment tools.

The second part of the student project will be based in the UK (co-supervised between Dr. Baker-Austin (Cefas) and Dr. Jaime Martinez-Urtaza (University of Bath), and will help amend the existing free, open access risk assessment tool focusing on the coastal shellfish harvesting regions of the Northeastern USA. This tool, which is updated daily, includes a 5-day forecasting option, and uses temperature and salinity data for *Vibrio* risk prediction purposes. To improve this approach, the student will utilize new and high quality remote sensing datasets, which will increase the resolution (down to 250m), and hence accuracy and granularity of risk maps. The tool will be further refined by the incorporation of the salinity and temperature growth data from PNW strains generated at the Charlotte lab. This will subsequently improve the accuracy and relevance of this risk assessment technology for use in the Northeastern USA.

**PROJECT MANAGEMENT APPROACH**

The overall project will be overseen and managed by Dr. Oliver. He has over 40 years’ experience studying pathogenic *Vibrio* spp., publishing some 150 scientific papers during that time on numerous aspects of their biology, virulence, and relationship to shellfish. He is considered by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) to be one of foremost experts in the world on pathogenic vibrios. He will insure that all participants are remain actively involved in the studies, using routine e-mails and faxes, and including a one-time workshop/conference to which all participant will attend and present. The senior researchers on this project have collaborated in the past, and have excellent individual and collaborative successes. Dr. Oliver will be directly responsible
for the involvement and progress in the project by the Masters level graduate student. We anticipate that these studies will form the major portion of his/her MS Thesis.

Dr. Baker-Austin will supervise the graduate student while he/she is at the Cefas lab, with significant input on the satellite imaging studies by Dr. Jaime Martinez-Urtaza. Both of these senior investigators have pioneered and developed the website to be employed in the proposed studies, and are longstanding international experts in the use of remote sensing-based technologies to track, predict and assess *Vibrio* disease emergence.

The project and student will benefit directly from the availability of strains (Cefas possesses one of largest and most diverse collection of pathogenic *Vibrio* strains in Europe), and studying in this European Reference Laboratory responsible for monitoring bacterial and viral contamination of bivalve shellfish.
DETAILED ITEMIZED BUDGET (See below for budget justification)

Salaries
- Full time (12 months) MS student: $12,000

Fringe Benefits
- Graduate/Undergraduate Student, including health: $1,652
- Student Tuition: $2,308

Laboratory/Research Supplies: $5,000

Travel
- Student to UK, airfare: $1,200
- Student per diem (2 months): $2,000
- Two UK collaborators to UNC Charlotte, airfare: $2,400
- UK collaborators per diem: $1,601
- Workshop: $300

Total Direct (ISSC): $28,461

Indirect Costs: $0

Cost Sharing* (UNCC): $28,461

Total Costs: $56,922

*Cost Sharing: The Department of Biological Sciences has committed to support this proposal with a 100% in-kind cost-share contribution of $28,462. This cost sharing will take the form of one course release for the PI James Oliver each semester, which equates to a total 2.25 person months of effort (1.125 person months per course released) towards this project. A letter from the Biology Chair indicating this is attached at the end of this proposal.

Budget justification

Salaries: A standard Masters-level stipend of $1000/month is requested for the student who will carry out these studies as part of their thesis research. Tuition, which is mandated by the University for all external grants which include a graduate student, is also included for the one-year of this effort. Due to the short turn-around on this RFP, and the very rapid start time, it is conceivable that we will not be able to identify, and have admitted into the program, a new graduate student to meet the Aug 31 start time. In that (unlikely) event, we will hire an advanced undergraduate student to initiate the studies until he/she is admitted into the graduate program, or another grad student is identified.

Travel: Funds are requested to allow the student in this project to travel to Cefas (Weymouth, UK) to study under Dr. Baker-Austin in order to further develop and utilize the satellite imaging website, and to learn the qPCR methods to be employed in quantifying the various V. parahaemolyticus strains as regards their growth characteristics in response to temperature/salinity

Per diem funds, based on University/State guidelines, are requested for the two months the student will be in the UK.

Funds are requested to allow the two UK collaborators to travel to UNC Charlotte (London-Charlotte) in order that the team (Drs. Oliver, Baker-Austin, Jaime Martinez-Urtaza, graduate student) can directly interact and discuss research methods and progress. Formal presentations will be made by each team member, and a day allocated for discussion of the data obtained to date and the best ways to proceed for successful completion of the overall project. In addition to the team members, selected researchers/regulatory personnel (e.g. Patti Fowler, Ken Moore) will be invited to attend this mini-conference. $300 is also requested to defray expenses for the workshop.

Per diem, at standard University/State rates, is requested for the UK collaborators while in Charlotte for 7 days.

Supplies: a very modest amount of supplies funds are requested, primarily for the growth studies, which require not only bacterial media/supplies, but molecular reagents for the qPCR.
APPENDIX: REFERENCES


APPENDIX: PROJECT TEAM STAFFING

There are three senior researchers included in the proposed studies:

- Dr. James D. Oliver, Professor, Dept. Biological Science, UNC Charlotte, Charlotte, NC
- Dr. Craig Baker-Austin, Senior Research Scientist, Centre for Environment, Fisheries and Aquaculture Science, Weymouth Laboratory, Weymouth, Dorset DT4 8UB, United Kingdom
- Dr. Jaime Martinez-Urtaza, Reader in Infection and Immunology, Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, United Kingdom

A one-page biography of each senior investigator is appended.

APPENDIX: COMPANY OVERVIEW

University of North Carolina at Charlotte
DUNS ID 06-630-0096
SIC: 8221 – Colleges, Universities, and Professional Schools
9201 University City Blvd, Charlotte, NC 28223-0001
Tel: 704-687-8622 Fax: 704-687-0089 (College of Liberal Arts and Sciences)

Key contact name, title, direct phone and fax:
Dr. James Oliver, Professor of Biological Sciences, tel: 704-687-8516; fax: 267-327-2083

Person authorized to contractually bind the organization:
Deborah Bolick, Contracts Manager, University of North Carolina at Charlotte

Brief history:
UNC Charlotte has been a State university since 1965

Conflicts of Interest:
The University requires that all faculty annually complete a conflict of interest report; this is on file for Dr. Oliver if requested. It contains no COI relevant to this proposal. As regards the current proposal, the only person for which a COI could exist is with is Patti Fowler, Section Chief, Shellfish Sanitation & Recreational Water Quality, NC Division of Marine Fisheries. The reason is that we currently have a federally-funded research collaboration with Ms. Fowler.
Appendix – Biographical Sketches of Senior Investigators

James D. Oliver
Bonnie E. Cone Distinguished Professor
Professor of Microbiology
University of North Carolina at Charlotte
and
Adjunct Professor
Duke University
Tel: 704-687-8516    Fax: 267-327-2083    email: jdoliver@uncc.edu

Education:
BS – University of Arizona, 1968
PhD – Georgetown University, 1973
PD – University of Ottawa, Canada, 1973-74

Visiting Professorships:
University Goteborg, Sweden
Duke University Marine Lab
University of Copenhagen
National University of Ireland, Galway
University of Aberdeen, Scotland
Mary Derrickson McCurdy Scholar, Duke University

Honors and Academic Activities:
Fellow, American Academy of Microbiology
Burrows Welcome Fund Visiting Professor in the Microbiological Sciences
Member, Editorial Boards of: FEMS Microbial Ecology, Pathogens, International Journal of Microbiology
Steering Committee Member, Program Chairman, and Co-Chair, “Vibrios in the Environment 2010”, Biloxi, MS.
Member, International Organizing Committee, “Vibrio 2014”, Edinburgh
Students receiving graduate degrees from Oliver lab: 51

From the Joint FAO/WHO Expert Meeting on Risk Assessment Tools for Vibrio parahaemolyticus and Vibrio vulnificus Associated With Seafoods held in Rome in 2010:
“Dr. Oliver is a microbiologist who has studied the bacterium, Vibrio vulnificus, for 35 years and is considered by the World Health Organization to probably be the foremost expert in the world on this bacterium”.

Publications and Presentations:
Publications: 158 + 19 book chapters
Papers Presented at Conferences: 206
University Seminars and Invited/Keynote Talks: 124 in 18 countries
Recent Publications Relevant to the Proposed Studies:
Dr Craig Baker-Austin
Cefas (Centre for Environment Fisheries and Aquaculture Science)
Weymouth, Dorset, UK. DT4 8UB
Telephone: +44 (01305) 206619 Email: craig.baker-austin@cefas.co.uk

Dr Craig Baker-Austin is currently the Director of the National Reference Laboratory (NRL) for monitoring bacteriological and viral contamination of bivalve molluscs. His research interests bridge the gap between marine microbiology, molecular biology, and disease emergence. He is interested in the dynamics of pathogenic marine bacteria, in particular microbes such as vibrios that represent a global emerging disease threat. This is a highly varied and interdisciplinary research background, encompassing numerous aspects of molecular microbiology, marine microbiology, climate sciences, biological oceanography, and genomics, among others. Dr Baker-Austin has extensive experience in the field of molecular microbiology, developed during his PhD based in the UK, and postdoctoral post at the Savannah River Ecology Laboratory, and more recently as a senior research scientist at Cefas. Dr Baker-Austin has recently pioneered the use of real-time PCR for the detection and characterisation of pathogenic marine vibrios in shellfish matrices, next generation sequencing technologies to type and characterise strains of human health relevance, and the use of remote sensors to track and predict marine-borne bacterial disease emergence in the context of climate warming.

Education:
2000-2001: MRes (Hons) Environmental Microbiology, 1st Class Distinction. Univ. of Ulster, Coleraine, UK.
1997-2000: BSc (Hons) Environmental Sciences, University of East Anglia, Norwich, UK.

Professional experience:
2014-present Acting Director of the National Reference Laboratory (NRL) for monitoring bacteriological and viral contamination of bivalve molluscs, Cefas, UK.
2008-2014 Senior Government Research Scientist (Molecular Microbiology), Centre for Environment Fisheries and Aquaculture Science (Cefas), Barrack Rd, Weymouth, UK.
2005-2007: Postdoctoral Research Associate, Department of Energy, Savannah River Ecology Laboratory, Aiken, South Carolina, USA. 'The role of metal contamination in the proliferation of antibiotic resistance in marine water-borne pathogens'.
2001-2004: Graduate Teaching and Research assistant, School of Biological Sciences, University of East Anglia, Norwich, UK.

Significant/relevant publications (taken from a total of 39):


Dr. Jaime Martinez-Urtaza
Reader in Infection and Immunology
Department of Biology and Biochemistry
University of Bath
Bath BA2 7AY, United Kingdom
Email: J.L.Martinez-Urtaza@bath.ac.uk Tel: +44 (0)1225 384292

Dr. Jaime Martinez-Urtaza has more than 20 years of experience in the study of food and waterborne bacterial pathogens with an active participation in ecological and epidemiological investigations carried out in different regions of the world. An overarching theme in his research work has been the fusion of several scientific disciplines, including molecular biology, marine microbiology, population genetics, oceanography, climate sciences, and epidemiology to glean insights into biology of marine bacteria associated with diseases.

Professional Experience
2014- Reader, University of Bath, UK
2012-2014 Expert in Food and Waterborne Diseases, European Centre of Disease Prevention and Control (ECDC), Sweden.
2002-2012 Lecturer and Research Scientist, Instituto de Acuicultura, University of Santiago de Compostela, Spain.
1997-2001 Head of the Unit for Food Safety in Fishing Industries, Instituto de Acuicultura, University of Santiago de Compostela, Spain.

Education
2001 Ph.D. Biochemical and Molecular Biology, University of Santiago de Compostela, Spain.
1991 Master in Fishing and Aquiculture. Universidad Pontificia de Comillas (ICADE), Madrid, Spain.
1983-1988 Bachelor of Science, Biological Science Degree. University of Santiago de Compostela, Spain.

Other merits
FAO consultant in the area of Vibrio diseases (2010).
FAO and WHO expert on Vibrio and Salmonella.
European Food Safety Authority (EFSA) expert.
Visiting Professor at Universidad Nacional Mayor de San Marcos, Lima, Peru.
Associate Researcher and Lecture (PhD program) at the INDICASAT, Panama.
Visiting Researcher and external examiner (PhD program) at the CIAD, Culiacan, Mexico.

Recent publication
TO: Program Officer  
Program “Techniques and Practices for Vibrio Reduction”  
Interstate Shellfish Sanitation Conference

FROM: Martin G. Klotz, Ph.D.  
Professor & Chair  
Department of Biological Sciences  
http://mgkmicro.com

RE: Letter of Support / Cost-share for Dr. James D. Oliver

DATE: July 24th, 2014

I write to confirm the support of the Department of Biological Sciences at UNC Charlotte for the ISSC Program Grant proposal entitled “Development of a free, open access, *Vibrio parahaemolyticus* risk portal for Atlantic coast shellfish harvesting areas, and an examination of growth rates of O4:K12 strains as determined by temperature and salinity” submitted by Dr. James D. Oliver for your consideration. Dr. Oliver is a tenured full professor in my Department, holding a Bonnie E. Cone Distinguished Professorship. The Department of Biological Sciences will commit 100% in matching funds over one year (2 x $14,231 per semester = $28,462) - in the form of release from teaching for one course per semester, time that Dr. Oliver can devote towards the coordination of the research and the training and mentoring of students who work on this very worthy project.

As the Chair of the Department of Biological Sciences at UNC Charlotte, I am strongly committed to supporting the *Techniques and Practices for Vibrio Reduction* program initiative of the Interstate Shellfish Sanitation Conference. Should the proposal be funded, I will provide administrative assistance to ensure that the work under this grant meets its objectives. Please do not hesitate to call on me if you require further assistance.

The University of North Carolina is composed of the sixteen public senior institutions in North Carolina  
An Equal Opportunity/Affirmative Action Employer