ISSC Request for Proposals

Project Title: A treatment strategy against *Vibrio parahaemolyticus* and other *Vibrio sp.* in harvested oysters

Total ISSC Funding Request: $12,957
Total Applicant Matching Funds: $12,477

Project Coordinator:
Christopher V. Davis, Executive Director
Maine Aquaculture Innovation Center
5717 Corbett Hall, Rm 436
Orono, ME 04469-
5717 cdavis@midcoast.co
m
207-832-1075

Principal Investigators and key personnel:
Cem Giray, Ph.D.
Kennebec River Biosciences, Inc.
41 Main Street, Richmond, ME 04357
Tel: 207-737-2637
Fax: 207-737-4504
E-mail: cgiray@kennebecbio.com

Christopher V. Davis, Ph.D,
Maine Aquaculture Innovation Center
5717 Corbett Hall, Rm 436
Orono, ME 04469-
5717 Tel: 207-832-1075
Fax: 207-832-6812
E-mail: cdavis@midcoast.com

Jeffrey McKeen
Pemaquid Oyster Company, Inc.
PO Box 302
Waldoboro, ME 04572
Tel: 207-446-8923
E-mail: jmckeen1@earthlink.net

Bill Mook
Mook Sea Farm
321 State Route 129
Walpole, ME 04573
Tel: 207-563-1456
E-mail: bill@mookseafarm.net

Kohl Kanwit
Maine Department of Marine Resources
PO Box 8
West Boothbay Harbor, ME 04575
Tel: 207-633-9500
E-mail: kohl.kanwit@maine.gov

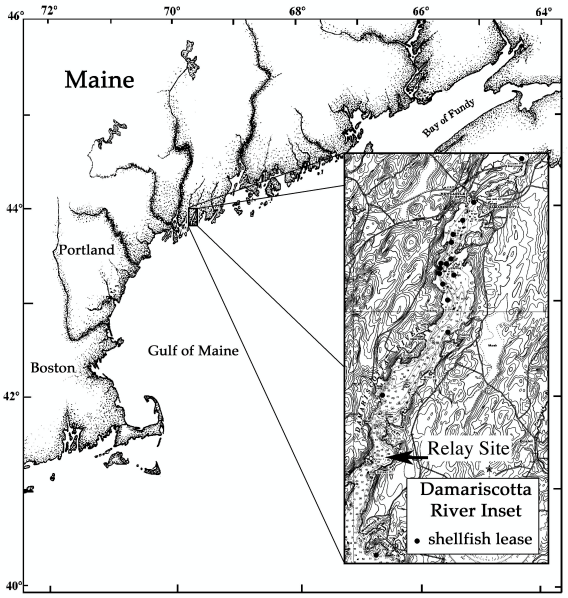
Project Coordinator's Signature _____ Date: 7/31/2014

Executive Summary

To-date, *Vibrio parahaemolyticus* has shown rare occurrence in Maine and the majority of isolates have been composed of non-pathogenic strains. Preliminary work conducted in 2013 and 2014 by Kennebec River Biosciences, Inc. and several Maine bivalve shellfish aquaculture companies has shown presence of this organism in grow- out oysters from specific regions of the Damariscotta River. The goals of this project are to gain a better understanding of the prevalence of *V. parahaemolyticus* and other *Vibrio sp.* such as *V. vulnificus* in the Damariscotta River and establish protocols for treatment of harvested oysters in order to minimize the human health risk imposed by these pathogens.

Project Approach

The past few years has shown a gradual expansion into New England waters of *Vibrio parahaemolyticus* occurrence in bivalve shellfish and associated human illness. The increase in prevalence and illness caused by this agent has shown good correlation with reported rise in coastal water temperatures (C. Davis, unpublished data). Maine has generally been assumed-free of this pathogen, inferred mainly by the lack of past detection of pathogenic strains of *V. parahaemolyticus* and *V. vulnificus* and lack of any reported illness from consumption of Maine-grown oysters. Low water temperatures is believed to have provided the Maine shellfish industry protection from other agents such as *Haplosporidium nelsoni* and *H. costale*, which although sporadically detected, have in the past rarely caused any significant disease and mortalities. In recent years however, these agents have been able to gain hold, survive winters and cause significant disease among aquaculture and wild American (eastern) oyster (*Crassostreae virginica*) populations first in the Damariscotta River and now in several other Maine estuarine systems (in prep. Giray et al.). Similarly, *V. parahaemolyticus* screening being conducted for private growers since last year has resulted in its detection in Damariscotta River oyster samples collected in late June this year (KRB client report). Considering the trend already experienced with other agents for which temperature is a determining factor, it is likely a matter of time before issues related to *V. parahaemolyticus* are also experienced in Maine. In addition, colder-water strains of the agent have also been suggested as causing new cases of disease in New England states (Seafood Source News Feb 2014).



In order to minimize the potential danger imposed by *V. parahaemolyticus*, it is necessary to have a better understanding of its distribution and put into place practices which will reduce its prevalence and prevent any associated illness. Standard handling practices during harvest which maintain oysters at low temperatures and minimize multiplication of any *Vibrios* present have voluntarily been put into place by a group of growers in the Damariscotta River. The detection of *V. parahaemolyticus* in harvested oysters despite these provisions has made it imperative that other safeguards also be put into place. This project aims to deploy and test one such approach in the Damariscotta River.

A treatment strategy against *Vibrio parahaemolyticus* and other *Vibrio sp.* in harvested oysters

To-date, a limited number of harvested oyster samples have been screened in 2013 and 2014 for *Vibrio* species from the Damariscotta River, all by cost-sharing among several growers and Kennebec River Biosciences, Inc. (KRB). This small set of samples has already shown the presence of *V. parahaemolyticus* via culture and real time PCR (using method of Jones et al. 2007) in oysters harvested from the upper Damariscotta River estuary. Data obtained showed higher *V. parahaemolyticus* prevalence in oysters collected from the surface than those located on the bottom (30 feet depth) at the upper estuary site and no detectable presence in oysters sampled on the same date from a relay site located in the lower estuary (Table 1). Results suggest that holding oysters at the relay site can diminish any presence of *V. parahaemolyticus* as a result of its water parameter characteristics (temperature, salinity, flow, etc.). The testing

has also provided preliminary insight on the efficacy of harvest and post-harvest biosecurity measures established by these growers.

Through this project we propose to test and validate a strategy that would consist of temporarily holding before shipment all oysters harvested from the upper Damariscotta estuary, and assumed *V. parahaemolyticus* positive, at a relay site in the lower estuary in order to reduce any *Vibrio* presence. This relay site in essence would act as a preventive depuration site. Through the testing conducted, we will be able to compare the efficacy of standard harvest and post-harvest temperature control procedures being used to minimize any *Vibrio* proliferation versus the use of a depuration strategy.

Table 1. *Vibrio parahemolyticus* real time PCR testing results for Damariscotta River sites (from client report KRB accessions M14062608, M14062609, M14062610)

Site	Sample	Result	C_t Value	
			Rep 1	Rep 2
SURFACE	Pool 1	Positive	18.5	18.4
	Pool 2	Positive	23.8	23.8
	Pool 3	Positive	32.9	32.9
BOTTOM	Pool 1	Weak Positive	35.4	35.6
	Pool 2	Suspect result	no value	36.8
	Pool 3	Suspect result	no value	36.2
RELAY	Pool 1	Negative	no value	no value
	Pool 2	Negative	no value	no value
	Pool 3	Negative	no value	no value

The objectives of this project are:
1) to determine whether a depuration strategy can be used as a preventive and remediation strategy in the Damariscotta River,
2) to gain a better understanding of the prevalence of *V. parahaemolyticus* and other *Vibrio sp.* such as *V. vulnificus* in upper and lower estuary water bodies and relate that to the respective water parameter characteristics,
3) to determine the minimum length of time required for holding oysters at a relay or depuration site before shipment to market,
4) to devise a model that can be applied in other grow-out locations,
5) to disseminate findings to the industry, regulatory and research community for use in their efforts and decision-making.

Project Methodology / Work Plan
The project team has already been conducting sampling for *V. parahaemolyticus* screening and is ready to embark on project efforts as soon as funding is available. Although a major part of the 2014 summer season will be over before funding can potentially be available, water

temperatures will still remain high into the Fall and we will plan on conducting analyses starting in mid-August and continuing until the end of September. Hence we will be able to collect enough data to still address the majority of the objectives and provide a preliminary report of findings in Fall 2014. Additional work will be conducted in June-July 2015 to test the strategy when air temperature is at its peak.

Work conducted will consist of the following approaches: a) analysis of oysters from the upper and lower estuary sites to compare efficacy of routine appropriate harvest / post-harvest temperature control procedures and holding at the relay or depuration site, b) same as 'a' but with deliberate mis-handling at harvest / post-harvest to assist proliferation of any potential *V. parahaemolyticus* or *V. vulnificus* present, c) since both 'a' and 'b' rely on natural presence of the bacterial agents, if agents have not been detected after completion of samplings through the end of September, a single run involving deployment of oysters laboratory infected with the June 2014 Damariscotta River *V. parahaemolyticus* isolate in the relay / depuration site will be conducted. Work conducted will be as follows:

1) Identify a site in the lower estuary with characteristics distinct from those of the upper estuary water body.
Through consultation with scientists who have been conducting research on the Damariscotta River for several decades (e.g. Larry Mayer, Univ. Maine Darling Marine Center, Walpole, ME) and Maine Department of Marine Resources, a relay site that provides characteristics distinct from the water body in the upper Damariscotta River estuary will be identified. This site will serve as the relay / depuration site during the study.

2) Monitor naturally occurring levels of *Vibrio* presence in oysters harvested from the upper Damariscotta River harvest site and lower estuary relay / harvest site.
Sample collection for the project will, in coincidence with harvest for market, be carried out by collaborating growers (W. Mook, Mook Sea Farm, Walpole ME & Jeff McKeen, Pemaquid Oyster Company, Waldoboro, ME). Samples will be collected from the surface and bottom harvest sites in the upper Damariscotta River and lower estuary relay site every two weeks from mid-August until the end of September 2014 and again in June-July 2015. This will provide four data sets in 2014 and another 4 in 2015 to compare *V. parahaemolyticus* and *V. vulnificus* presence/prevalence in the three collection sites. Water salinity and temperature will be monitored by the use of data recorders deployed at the sites throughout the study period in order to record parameter differences and effects of storm events.

The technical aspects of the pathogen screening will be conducted in KRB's laboratories with coordination by C. Giray and technical expertise from senior laboratory staff. KRB has been servicing the finfish and shellfish aquaculture industry in the US and Canada for nearly two decades and possesses the facilities and experience required for detection, culture and identification of a variety of bacterial pathogens. KRB staff to be involved in the project are well-versed in specimen handling, necropsy, bacterial culture and identification, MPN assays, and molecular protocols including nucleic acid extraction, PCR (end-point, real time and quantitative) and DNA sequence analysis. Specifically, MPN procedures as described in the Food and Drug Administration's Bacteriological Analytical Manual Chapter 9 (FDA 2004), a species-specific real time multiplex PCR assay targeting *Vibrio parahaemolyticus* tlh, tdh, trh genes (Nordstrom et al. 2007) and a species-specific PCR assay for *Vibrio vulnificus* (Campbell & Wright 2003) will be utilized for sample analyses. Additionally, routine bacterial culture and biochemical assays will be used to isolate, identify and archive any *V. parahaemolyticus* and *V. vulnificus* isolates detected.

At each round of sampling, replicates each consisting of 12 oysters will be collected in triplicate from each site. Oysters will be subject to routine harvest / post-harvest practices and then transported to the laboratory, held at 4-8^{o}C until arrival at the laboratory the same day. Once received at the laboratory, oysters will be surface disinfected, shucked and whole tissues removed. Each replicate consisting of 12 oysters will be pooled together for processing according to protocols outlined in BAM Ch. 9 and MPN assays. The resulting enrichment cultures will be sampled for further bacterial culture and identification, and evaluated by real time multiplex PCR for *V. parahaemolyticus* (Nordstrom et al. 2007) and PCR for *V. vulnificus* (Campbell & Wright 2003). Following initial characterization of any isolates detected by molecular and bacteriologic tools, further evaluation, such as by serotyping, can be pursued as needed in consultation with experts using additional molecular and serotyping assays (Jones et al. 2012). Any *V. parahaemolyticus, V. vulnificus* as well as other isolates of significance obtained through the project will be archived at -80^{o}C for potential future work and sharing with other researchers.

In order to better match *V. parahaemolyticus* real time PCR results to number of viable cells in enrichment cultures tested and to MPN results, a semi-quantitative approach will be developed. Viable cell densities and corresponding C/T values will be determined by concurrently performing real time assays and plate counts on dilution series prepared using the *V. parahaemolyticus* positive control organism.

3) Determine efficacy of relay / depuration site in purging *V. parahaemolyticus* from oysters mis-handled during harvest / post-harvest procedures.

This will be conducted as a single trial after the monitoring rounds have been completed at the end of September. Eighteen replicates of 12 market-size oysters each will be harvested from the surface site in the upper Damariscotta River, which based on previous results (see Table 1) are most likely to contain *V. parahaemolyticus*. Two replicates will be held at 4-8C and processed for determination of initial *V. parahaemolyticus* and potential *V. vulnificus* levels in the oysters. The remaining replicates will be subjected to harvest / post-harvest mis-handling by incubation at 28-30C for 2 hours to increase *Vibrio* levels. Following incubation two replicates will be processed for determination of increase in *Vibrio* presence following the mis-handling process. One half of the remaining replicates will be placed in a holding site in the upper Damariscotta River while the other half will be placed at the relay / depuration site in the lower Damariscotta River. Two replicates will be collected from each site after 24, 48 and 96 hours and processed as described above.

If testing results of post-high-temperature incubation samples are determined to be negative for *V. parahaemolyticus*, 48 and 96 hour samplings will be cancelled. Pending MDMR approval, the trial can be repeated as described above, but instead using oysters experimentally exposed to the Damariscotta River June 2014 *V. parahaemolyticus* isolate. The isolate, presently archived at -80C at KRB, would be grown overnight in tryptone salt broth at 30C. Approximate cell density would be determined via counts of formalin-killed aliquots. One 250-gallon tank containing filtered seawater with aeration would be set up in a quarantine area (KRB wet lab) cooled to 15C and a total of 204 oysters placed in the tank. Once oysters are acclimated, the bacterial culture would be added so as to produce a final concentration of 10-100 cells per ml. Oysters would be held in the tank for 1 hour. Two replicates of 12 oysters each would then be removed and processed to determine post *V. parahaemolyticus* exposure levels in oysters. The remainder would be incubated at 28-30C for 2 hours, two replicates removed and processed to determine post-incubation *V. parahaemolyticus* levels and the remainder split between the two sites as above or maintained in the quarantine area.

Project Deliverables

The aim of this project is to provide industry, public, research and regulatory bodies with data on *V. parahaemolyticus* and *V. vulnificus* prevalence in oysters from the major production region in Maine and the efficacy of routine harvest practices and a depuration strategy to minimize risk. Findings will help industry, and regulatory and public health agencies to respond appropriately to any related emerging issues and make long-term policy decisions.

Sample analysis results of presence/absence of *V. parahaemolyticus* and *V. vulnificus* will be made available to growers and MDMR as early as within 48 hours from sampling. The initial sampling, testing and concurrent reporting of findings is scheduled to be carried out between August-October 2014. Further sampling is planned for June July 2015, with evaluation of any isolates, additional data analysis and final reporting of findings to be completed by August 2015.

Results will be disseminated to the bivalve shellfish industry, and regulatory and research community via presentations at scientific and trade events (e.g. National Shellfish Association, shellfish growers' and municipal committees, trade associations), and publication in scientific and industry literature.

Project Management Approach

Christopher Davis, Executive Director of the Maine Aquaculture Innovation Center will serve as the grant administrator and assist Dr. Giray with sampling design as well as some of the collection of samples. Davis will also manage the collection of environmental data (principally temperature and salinity).

Budget

Personnel	ISSC funds	Matching funds	Total cost
C. Giray (80 hrs @ $35/hr)	$1,200	$1,600	$2,800
C. Davis (60 hrs @ $35/hr)	$0	$2,100	$2,100
Sample collection			
Mileage (450 @ $0.52)	$117	$117	$234
Oysters (1,440 @ $0.75)	$0	$1080	$1080
Laboratory analyses			
Necropsy, processing (120 @ $15)	$1,440	$360	$1,800
Molecular assays (320 @ $40)	$6,400	$6,400	$12,800
Vibrio screen & MPN assays (120 @ $30)	$3,000	$600	$3,600
Bacterial identification (20 @ $51)	$800	$220	$1,020
Reporting & Publications			
TOTAL	**$12,957**	**$12,477**	**$25,434**

APPENDIX: REFERENCES

Kennebec River Biosciences, Inc. (KRB) has fulfilled a large number of similar projects. These include an ongoing contract since 2001 with the US Dept. of Agriculture Animal & Plant Health Inspection Service (USDA APHIS) for Infectious Salmon Anemia Virus (ISAV) surveillance in Maine and shorter term contracts for the development of environmental detection methods for ISAV. For the past 7 years, KRB has performed necropsy, processing and pathogen screening for the oyster pathogen surveillance program conducted by the Cape Cod Cooperative Extension Service and Woods Hole Sea Grant. KRB has been providing bacterial culture and identification, including *Vibrio* screening and identification from finfish and bivalve shellfish, to clients for the past 15+ years. Molecular techniques for the detection of over 40 finfish, crustacean and bivalve shellfish pathogens of regulatory import/export concern are conducted routinely.

APPENDIX: PROJECT TEAM STAFFING

Project management:
Christopher Davis, PhD

Sample collection:
William Mook, Mook Sea Farm, Walpole, Maine

Jeff McKeen, Pemaquid Oyster Company, Waldoboro, Maine

Laboratory testing:
Cem Giray, PhD
BSc & MSc Microbiology, PhD Oceanography. Over 30 years of experience with aquatic animal pathogens. Broad experience pathogen detection and identification methods, including bacteriology, virology, molecular techniques, histology. Responsible for management of commercial laboratory and staff, coordinating analysis and reporting of over 450,000 finfish and shellfish samples annually.

Victoria Bowie
BSc Animal Science. Over 20 years' experience in animal health. Well-versed in sample processing and molecular techniques to be used in this project.

No employees planned to work on this project have ever been convicted of a felony.

APPENDIX: COMPANY OVERVIEW

Maine Aquaculture Innovation Center

The Maine Aquaculture Innovation Center was established in 1988 by the Maine Legislature with a mission to assist in developing economically and environmentally sustainable aquaculture opportunities in Maine. MAIC sponsors and facilitates innovative research and development projects involving food, pharmaceuticals, and other products from sustainable aquatic systems; invests in the enhancement of aquaculture capacity in

Maine; serves as a source of educational information to enhance public visibility and acceptance of aquaculture; and encourages strategic alliances tasked with promoting research, technology transfer, and the commercialization of aquaculture research.

Kennebec River Biosciences
Founded in 1996, Kennebec River Biosciences is the only commercial laboratory providing comprehensive testing and health services to farms, businesses, government agencies, and scientific research institutions involved with aquatic species. Initially focused on the needs of the salmon aquaculture industry, capabilities and services have expanded to other species and to areas such as veterinary services, bivalve shellfish testing, and contract research.

Pemaquid Oyster Company
Pemaquid Oyster Company, Inc. (POC) was founded in 1986 with the goal of producing the highest quality cultivated, cultchless American oysters (*Crassostrea virginica*) for the half-shell market. Oysters are spawned in late winter at our Bremen, Maine hatchery and reared at our summer nurseries in the plankton-rich waters of the Damariscotta River. In late fall, oyster seeds are bottom planted on our leased growing beds. Harvested from March through December, Pemaquid® oysters are held in the cold, briney Class A waters of Clarks Cove near the river's mouth prior to sale.

Mook Sea Farms, Inc.
Mook Sea Farm is an oyster farm founded in 1985 on the Damariscotta River in Midcoast Maine. We rear the American oyster (*Crassotsrea virginica)* from egg to adult size. Our hatchery produces 80 to 100 million juvenile oysters (seed) annually for sale to other oyster growers throughout the Mid-Atlantic and the Northeast, and for our own cultivation of oysters for the half-shell market.

State of Maine Department of Marine Resources
The Maine DMR is the principal state agency in charge of managing the state's marine resources, including shellfish and finfish resources.

CEM GIRAY, Ph.D.
Kennebec River Biosciences, Inc.
41 Main Street, Richmond, Maine 04357
Tel: (207) 737-2637 (ext. 207) Fax: (207) 737-4504 E-mail: cgiray@kennebecbio.com

Education

Ph.D. Oceanography, 1996. University of Maine, Orono, Maine.
M.Sc. & B.Sc. Microbiology, 1987 / 1984. University of Maine, Orono, Maine.

Positions

Vice President, Chief Science Officer, Micro Technologies, Inc. dba Kennebec River Biosciences, Richmond, Maine, June 2005-present. Responsibilities include oversight of research and development activities, supervision of service activities and laboratory personnel, providing in-house training, international business development, incorporation/review of laboratory protocols, reporting to and communication with clients, and various administrative duties.

Laboratory Director, Micro Technologies, Inc., Richmond, Maine, October 1999-June 2005. Conducted laboratory assays in all areas of laboratory including molecular biology, virology, cell culture, bacteriology and parasitology, responsible for oversight of various service- and research-oriented activities, supervised laboratory personnel, performed laboratory QA/QC, prepared proposals for outside grants.

Scientific Director, BioSpectives, Ltd., Scientific Consulting, North Cyprus, September 1997-October 1999. Director and owner of scientific consulting company, providing services internationally in the fields of marine and environmental sciences and biotechnology.

Post-doctoral Research Assistant, Dept. of Biochemistry, Microbiology and Molecular Biology, University of Maine, Darling Marine Center, Walpole, Maine, November 1996-July 1997. Analysis of biogeochemical processes within burrows of intertidal marine invertebrates, GC/MS examination of hydrocarbon degradation in marine sediments and animal burrows.

Selected Publications

(in press) Diagnostic evaluation of two real-time RT-PCR protocols for the detection and surveillance of viral hemorrhagic septicemia virus in the framework of a USA laboratory network. Dis Aquat Org

USDA APHIS VS (2010) Viral hemorrhagic septicemia virus (VHSV IVb) risk factors and association measures derived by expert panel. Preventive Veterinary Medicine 94:128-139.

Gustafson, L., S. Ellis, T. Robinson, J. Warg & **C. Giray** (2008) Estimating diagnostic test accuracy for infectious salmon anemia virus (ISAV) in Maine, USA. J Fish Dis 31:117-125

Gustafson, L., S. Ellis, T. Robinson, F. Marenghi, P. Merrill, L. Hawkins, **C. Giray,** and B. Wagner. (2007) Spatial and non-spatial risk factors associated with cage-level distribution of infectious salmon anemia at three Atlantic salmon, *Salmo salar* L., farms in Maine, USA. J Fish Dis 30:101-109.

Giray, C., H.M. Opitz, S. MacLean and D. Bouchard. (2005) Comparison of lethal versus non-lethal sample sources for the detection of infectious salmon anemia virus. Dis Aquat Org 66: 181-185.

Ellis, S., L. Gustafson, **C. Giray**, T. Robinson, F. Marenghi, P. Merrill. (2005) Hydrographics and the epidemiology of ISA: findings from a high-risk region in Maine and New Brunswick. Bull Aquaculture Assoc Canada 105:38-45.

Bouchard, D.A., K. Brockway, **C. Giray**, W. Keleher and P.L. Merrill. 2001. First report of infectious salmon anemia (ISA) in the United States. Bull Euro Assoc Fish Pathol 21: 86.

Christopher V. Davis

P.O. Box 302, 1957 Friendship Road, Waldoboro, Maine 04572
cdavis@midcoast.com, (207) 832-1075

EDUCATION

University of Maine, Orono, Maine. Ph. D. May, 2000 in marine bio-resources
Colby College, Waterville, Maine. BA May, 1978 with majors in biology and geology

RESEARCH AND PROFESSIONAL EXPERIENCE

2004 - present	Executive Director of the Maine Aquaculture Innovation Center
2003 - present	Adjunct Assistant Professor, School of Marine Sciences, University of Maine, Orono, Maine
1990 - 2003	Instructor at the University of Maine
1986 - present	Co-founder and general partner of the Pemaquid Oyster Company, Inc., a commercial oyster nursery and growout farm located in Damariscotta, Maine

PROFESSIONAL ACTIVITIES

2013 - present	University of Maine Aquaculture Advisory Board, vice-chair
2013 - present	President of the National Shellfisheries Assocaition 2013-2014 term
2010 - present	Appointed to the Maine Technology Institute Board of Directors
2009 - present	Board of Directors of the East Coast Shellfish Growers Association
2008 - 2013	Appointed member of the Maine Department of Marine Resources Shellfish Advisory Council
2008 – present	Board of Trustees of the Watershed School (Treasurer)
2007 - present	Appointed member of the Maine Innovation Economy Advisory Board
2005 - 2011	Elected as Treasurer of the National Shellfisheries Association
2004 - present	Appointed member of the Maine Department of Marine Resources Aquaculture Advisory Council
2004 - 2009	Appointed to the Maine Technology Institute Aquaculture Advisory Board
2003 - present	Founder and Treasurer of the Edward A. Myers Marine Conservation Fund
2003 - 2007	Appointed member of the Maine Science and Technology Advisory Council
2002 - present	Editorial Board of the *Journal of Shellfish Research*
2001 - 2012	Appointed to the Northeast Regional Aquaculture Center Technical Advisory Council, Chair
2001 - 2005	Editor of the *National Shellfisheries Association Quarterly Newsletter*
2001 - present	Board of Directors of the Maine Aquaculture Association
2001 - 2004	Executive Committee of the Knox-Lincoln Cooperative Extension Association
1997 - 2003	Board member of the Research Capacity Committee, Maine Science and Technology Foundation

RECENT PUBLICATIONS

Newell, Carter R., Anthony J. S. Hawkins, Kevin Morris, John Richardson, Chris Davis and Tessa Getchis. 2013. ShellGIS: a Dynamic Tool for Shellfish Farm Site Selection. *World Aquaculture* 44(3):50-53.

Bullard, Stephan G., C.V. Davis and S.E. Shumway. 2013. Seasonal Patterns of Ascidian Settlement at an Aquaculture Facility in the Damariscotta River, Maine (USA). *J. Shellfish Res.* 32:255-264.

Bullard, Stephan G., S.E. Shumway and C.V. Davis. 2010. The use of aeration as a simple and environmentally sound means to prevent biofouling. *Biofouling*, 26(5): 587-593.

Thompson, B., M.J. Perry and C.V. Davis. 2006. Phytoplankton in the Damariscotta River Estuary. Maine Office of Sea Grant Marine Research in Focus, vol.3.

Shumway, S.E., C.V. Davis, R. Downey, R. Karney, J. Kraeutter, J. Parsons, R. Rheault and G. Wikfors. 2003. Shellfish Aquaculture – In praise of sustainable economies and environments. World Aquaculture 34 (4):15-18.

Davis, C.V. 2000. Response to selection and estimation of heritabilities for rapid growth in eastern oysters (*Crassostrea virginica*, Gmelin 1791) and the use of fast growing lines to reduce susceptibility to juvenile oyster disease. Ph.D. dissertation, University of Maine, Orono, Maine. 143 pp.

Davis, C.V. and B.J. Barber. 1999. Growth and survival of genetically selected lines of eastern oysters, *Crassostrea virginica* (Gmelin 1791) affected by Juvenile Oyster Disease. *Aquaculture* 178:253-271.

Barber, B.J., C.V. Davis and M.A. Crosby. 1998. Cultured oysters, *Crassostrea virginica*, genetically selected for fast growth also exhibit increased tolerance of Juvenile Oyster Disease (JOD). *J. Shellfish Res.* 17:1171-1175.

Barber, B.J. and C.V. Davis. 1997. Growth and mortality of cultured bay scallops in the Damariscotta River, Maine (USA). *Aquaculture International* 5: 451-460.

Davis, C.V., K.C. Scully and S.E. Shumway. 1997. Juvenile and yearling growth of Atlantic surfclams (*Spisula solidissima*, Dillwyn 1817) in Maine, USA. *J. Shellfish Res.* 16:161-168.