R/CONT-216 Sea grant project completion report

Noroviruses in coastal waters: Implications for seafood cultivation and human health

Project Hypothesis

We tested coastal California surface waters and shellfish for human noroviruses (NoVs) to understand their propensity to associate with marine macroaggregates and to estimate their impact on human health using Quantitative Microbial Risk Assessment (QMRA). It was hypothesized that NoVs are present in freshwater (FW) discharges; are preferentially attached to particles in estuarine and marine waters; are correlated with the presence of other human pathogens; and that they pose a significant risk to consumers of raw shellfish.

Project Goal and Objectives

The overall project goal was to evaluate the distribution of NoVs in coastal California surface waters and shellfish, to understand their propensity to associate with marine macroaggregates, and to estimate their impact on human health using QMRA. The specific project objectives were (1) to determine the fate and transport of NoVs in estuarine and coastal waters; (2) to correlate NoVs in estuarine and coastal waters with the prevalence of zoonotic pathogens (*Toxoplasma*, *Cryptosporidium, Giardia,* and *Salmonella*) and fecal indicator bacteria (FIB); and (3) to perform QMRA on NoV prevalence in ambient waters and shellfish.

Briefly Describe Project Methodology

The prevalence of known human NoVs (genotypes I and II) in freshwater (FW) discharges, seawater (SW), marine macroaggregates and mussel hemolymph was established over the course of a two-year sampling campaign using water filtration and quantitative PCR. The potential for NoVs to associate with macroaggregates as a function of salinity was tested in controlled laboratory experiments using two different viral surrogates: the bacteriophage PP7 and murine norovirus (MNV). Lastly, the risk that NoVs pose to raw shellfish consumers was estimated by developing a quantitative microbial risk assessment model.

Describe progress and accomplishments toward meeting goals and objectives

Objective 1: Determine the fate and transport of NoVs in estuarine and coastal waters.

Hypothesis 1: NoVs are preferentially attached to particles in estuarine and marine waters.

In Year 1 of the project, the potential for viruses to associate with aquatic macroaggregates was evaluated by conducting experiments wherein unfiltered fresh, estuarine and marine waters were spiked with PP7, a bacteriophage that has been used extensively as a viral surrogate in water filtration studies. To further address the need to specifically investigate norovirus' association with aquatic macroaggregates, a second laboratory experiment was performed in Year 2 using murine norovirus (MNV) as the viral surrogate. Following preliminary trials to optimize experimental conditions using MNV, a full-scale aggregation experiment was conducted in September 2013 to test the hypothesis that an increase in salinity will enhance the magnitude of MNV association with aggregates. Salinity was isolated as the test variable by

adding increasing concentrations of artificial sea salt to unfiltered freshwater samples (450 mL), which resulted in a range of salinities tested (0, 8, 16, 24 and 33 ppt). Quantification of MNV was performed in the top aggregate-poor water fraction and the settled aggregates using a validated RT-qPCR assay (Baert et al. 2008). Filtered samples of each salinity concentration were tested as a control for assessing MNV distribution in the top and bottom water fractions in samples that lacked suspended particulates that could form aggregates. Data analysis included a Kruskal-Wallis one-way analysis of variance (ANOVA) to compare the median total number of MNV gene copies recovered in aggregates across the five salinities. A Mann-Whitney test was used to compare the concentration of MNV (gc/mL) between top and bottom fraction of each unfiltered sample and between the bottom fraction in the unfiltered and filtered control. Overall, it was found that MNV concentrations were consistently higher in aggregates than in aggregate-poor water and that aggregation was highest at estuarine salinities (8, 16 and 24 ppt).

Hypothesis 2: NoVs are present in FW discharges into coastal waters in California, and are preferentially detected in aggregates and mussels.

Surveillance of Central CA coastal rivers and bays for presence of NoV:

To determine the presence and quantify the abundance of NoVs that are discharged into California coastal waters, freshwater outflow sites and marine waters near shellfish cultivation regions were monitored for NoVs. In total, twelve sampling efforts were conducted with a total of 42 freshwater and 13 seawater samples collected. All sites were sampled at least twice during both wet and dry weather, with wet weather defined by connectivity between freshwater discharges and the ocean during the months of December and May Samples were concentrated using a validated hollow-fiber ultrafiltration method (Rajal et al. 2007a, Rajal et al. 2007b) and retentates were analyzed for the presence of known human NoVs genogroups I and II using a sensitive and validated reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay (Wolf et al. 2007). An additional 375 mL was collected at each sampling location and analyzed for traditional fecal indicator bacteria (FIB) using standard methods, including total and fecal coliforms, *E. coli*, and *Enterococcus*. Noroviruses were detected in 24% of surface water samples. Genogroups I and II were equally prevalent during wet season events (14%), whereas NoV GI was more prevalent in dry events (10%) compared to NoV GII (5%).

Surveillance of NoVs in aggregates and mussels:

To further evaluate the prevalence of NoVs in coastal ecosystems, field samples were collected to evaluate whether NoVs are more likely to be detected in marine aggregates and mussel tissues compared to the surrounding bulk water. Bulk water, aggregates, and in-situ mussels were sampled at two sampling locations in the Carmel Region during seven sampling events, and at two sampling locations in the Cambria Region during eight sampling events . These trips included 4 dry season and 3-4 wet season events, with the wet season defined as events during the months of December through May. At each site, ten 1-liter replicates of bulk seawater samples were collected. Aggregate-rich fractions were separated from each bulk water sample in the field employing published methods that utilize settling cones (Lyons et al., 2005). The remaining bulk water from each replicate was combined, spiked with PP7, and concentrated using HFF, as previously described, yielding an “aggregate-poor feed” and “aggregate-poor retentate” sample. RNA was extracted from all aggregate-rich, aggregate-poor feed and aggregate-poor retentate samples and analyzed for NoV GI and GII via RT-qPCR, as previously described. Aggregate-rich and aggregate-poor water samples were also tested for zoonotic protozoa by microscopic techniques and *Salmonella* by cultivation (Miller et al. 2005, Miller et al.

2006a, Miller et al. 2006b). Bulk water and aggregate-rich samples were also processed for FIB (total and fecal coliforms, *E. coli,* and *Enterococcus*).

Alongside aggregate-rich and -poor seawater samples, 60 mussels were collected. Thirty mussels were processed per batch by homogenizing tissue and testing for fecal coliforms as well as *Salmonella.* The hemolymph from the remaining 30 mussels was removed and nucleic acids were extracted and tested for NoV GI and GII via RT-qPCR, as well as for *T. gondii*, *Giardia,* and *Cryptosporidium* via conventional PCR.

Objective 2: Correlate NoVs in estuarine and coastal waters with the prevalence of zoonotic pathogens (*Toxoplasma, Cryptosporidium, Giardia,* and *Salmonella*) and fecal indicator bacteria (FIB).

Hypothesis 3: NoVs are correlated with Toxoplasma, Cryptosporidium, Giardia, Salmonella, and FIB in water, aggregates and shellfish.

The association between the detection of NoV and the presence of FIBs or zoonotic pathogens was evaluated using two approaches: First, logistic regression was used to evaluate the relationship between presence of NoV and FIB concentrations in mussel tissues or surrounding seawater as a continuous predictor variable. Second, a Chi square statistic and logistic regression were both employed to evaluate for a statistical association between presence of NoV in mussels and binary predictor variables including presence/absence of other zoonotic agents (*Toxoplasma, Giardia* and *Salmonella*), and FIB concentrations that were categorized as below or above threshold values set by regulatory agencies as limits for safe consumption of shellfish or recreation in marine water bodies.

Objective 3: Perform quantitative microbial risk assessment on NoV prevalence in ambient waters and shellfish.

Hypothesis 4: Human NoVs pose a significant risk to consumers of shellfish.

The QMRA model for this project is concerned with the risk of gastroenteritis through the exposure of consumption of raw shellfish contaminated with NoVs. This model specifically considers the consumption of oysters in California since these shellfish are most commonly consumed raw and NoVs were measured in mussels native to the California coast. A dose-response relationship has been developed for norovirus (Teunis et al. 2008) based on a clinical trial in which doses of norovirus were administered to 108 volunteers. Viral aggregation was not accounted for in this project's QMRA. It is noteworthy that the clinical trial method for the NoV dose-response curve was based on RT-qPCR data. However, the RT-qPCR assay used in the clinical trial was different than the assay used in this project.

Input parameters for pathogen dose calculations were obtained and these parameters were estimated either as point estimate values or as distributions, so as to capture the variability surrounding that parameter. Thereafter, the final pathogen dose distribution was used as input into the dose-response model, in order to estimate the probability of the risk of infection. For this QMRA model, the exposure pathway began at the point of oyster harvesting. This approach considers the initial concentration of NoVs in the shellfish at the moment of harvest and at the subsequent steps of refrigeration, retail, and ultimately at consumption. It was assumed that a depuration step after oyster harvesting removes 50% of NoVs. The exponential decay of NoVs in oysters was considered with respect to time (Tu et al. 2008). Due to the fact that NoVs can

survive freezing and heating up to 60°C, the decay of NoVs with respect to temperature was not considered. The number of raw oysters consumed per serving was estimated as a lognormal distribution with a mean of 13.83 and a standard deviation of 10.44, based on data from a regional telephone survey conducted by the Florida Agricultural Market Research Center, University of Florida (Degner and Petrone, 1994). We assumed that the Florida survey data applies nationwide, which may have introduced some bias. The mean number of servings of raw oysters consumed per person per annum in California was estimated by a consumption survey performed by the Interstate Shellfish Sanitation Conference (ISSC, 2004). Harvest duration in California was estimated to be 3 hours (Paul Olin, personal communication) and the mean time spent between first refrigeration and retail was estimated to be 7.7 days (FDA, 2005). Norovirus infectivity was assumed to be 10% at consumption and an individual's susceptibility to infection was assumed to be homogenous among the population. Measured NoV concentrations in mussel hemolymph samples were used as the initial concentration of NoVs in oysters at harvest. These data were fitted to an exponential distribution with a beta parameter of 871.89 using the distribution fitting function of the Excel-compatible software @RISK (Palisade Corporation 2008). This distribution was chosen because it is commonly used in environmental data. Non-detects in this data set were replaced with half of the Sample Limit of Detection (SLOD). The distribution was bounded including and excluding outliers. Outliers were determined using Grubbs' test.

Norovirus dose distributions at each step of the exposure pathway were calculated by random sampling of the input variables many times using the Monte Carlo statistical modelling method (Haas et al., 1999). The final NoV dose distribution at consumption was used as an input distribution into the dose-response model, wherein a risk profile was then obtained. The model was run for 100 individuals exposed 1000 times. Once the risk distribution was obtained, the individual's illness risk (IIR) was calculated, which is defined as the total number of individual illnesses predicted divided by the total number of potential exposures.

Sensitivity Analysis:

A sensitivity analysis was performed to determine the relative importance of the variables that were considered in this model. Refrigeration time of oysters after harvesting was determined to be the variable that drives the majority of the risk of norovirus infection upon raw oyster consumption.

QMRA was performed using @RISK. We employed a static model, meaning that all exposed individuals are in the same epidemiological state, an assumption that under low risk conditions has been found to provide satisfactory risk estimates (Soller and Eisenberg 2008).

Project Modifications:

Viral surrogates and aggregation experiments

It is possible that human noroviruses (NoVs) display unique transport characteristics based on their size, surface properties, specific gravity, and environmental resistance. Compared with other *Caliciviruses,* murine norovirus (MNV) is emerging as a promising surrogate for human NoVs due to its genetic similarities to NoVs as well as resistance to inactivation and stability in the environment. In addition, the ability to propagate MNV in cell culture enables its application in transport experiments (Cannon et al. 2006, Bae and Schwab 2008). Therefore, in addition to studying the aggregation of PP7, an experiment was performed to evaluate the propensity for

NoVs to attach to particles using MNV as surrogate virus particles (as described under Objective 1, Hypothesis 1).

Field sampling scheme modifications

After an initial sampling event at Estero Bay in May 2012, the project team elected to remove this site from future field collections (due primarily to logistical challenges associated with the large distance from other sites and UC Davis) and to instead prioritize collection of seawater in the shellfish harvesting area in Tomales Bay, where freshwater samples were also collected. Due to study time constraints, five sampling efforts were conducted at each of the sampling sites, as opposed to the originally proposed six trips.

Ancillary research projects

A pre-proposal was submitted to Sea grant (2014) for continued research on the transport and fate of NoV in coastal ecosystems, focusing on the potential impact of aquatic polymers on concentration and acquisition of NoV by shellfish. However the project was not recommended for submission as a full proposal.
An externally funded One Health workshop (PIs Wuertz and Miller) entitled 'Pathogen Pollution and Risk Assessment' was held as part of the National Shellfisheries Association 104th annual meeting in Seattle from March 25-29th, 2012.

Project Outcomes:

Objective 1: Determine the fate and transport of NoVs in estuarine and coastal waters.

Hypothesis 1: NoVs are preferentially attached to particles in estuarine and marine waters.

Results of the PP7 aggregation study indicate that viruses display a pattern of enhanced aggregation in estuarine and marine waters, as described in our published manuscript (Shapiro et al. 2013).

The mean total numbers of MNV gene copies recovered in the aggregate fraction were observed to be highest in estuarine salinities (8, 16 and 24 ppt). Results from a Kruskal-Wallis test indicate that there was a significant difference in the medians of the total number of gene copies of MNV recovered in aggregates across the five salinities ($p<0.05$). As expected, there was no difference in MNV concentrations in the aggregate-poor water and the aggregates in freshwater (0 ppt). MNV gene copies were found to be 8-112 times more enriched in aggregates as compared to aggregate-poor water, with maximum enrichment occurring at a salinity of 8 ppt. In all saline waters, the concentration of MNV appeared to be higher in aggregates as compared to MNV concentrations in the equivalent bottom water fraction in filtered controls. However, the only significant (Mann Whitney $p<0.05$) differences occurred in water groups spiked with sea salt at the 8 and 24 ppt levels. Overall, results support our initial hypothesis that NoV particles can concentrate in sinking marine aggregates, and that the degree of aggregate-association is enhanced in saline waters.

Hypothesis 2: NoVs are present in FW discharges into coastal waters in California, and are preferentially detected in aggregates and mussels.

Norovirus prevalence in mussel hemolymph

One-way ANOVA was performed to compare the mean volume (mL) of hemolymph extracted per mussel across the four sites monitored. A significance difference ($p<0.01$) was observed for the means of the volume of hemolymph per mussel across the four sites and, therefore, a site-specific mean hemolymph volume per mussel was calculated to convert detected genomes of NoVs per volume to genomes of NoVs per mussel.

A total of 870 mussels were collected, wherein the hemolymph from five or six mussels was pooled to create 145 batched hemolymph samples. Of the 145 batched samples analyzed, NoVs were detected in 19% of samples, ranging in concentration from 91 to 37,837 genomes per mussel. NoV G1 was detected in 13% of samples and was more prevalent in mussel hemolymph than NoV GII, which was detected in only 6% of samples. Viruses were often detected in the Carmel sampling sites (Carmel River Beach and Point Lobos). NoV GI was detected consistently in this region during the 2011 - 2012 wet season. A subset of the positive hemolymph samples with the highest concentrations of NoV GI and GII are currently in preparation for confirmation via sequencing.

Norovirus prevalence in freshwater and seawater

Noroviruses were found in 13 (24%) of the 55 surface water samples analyzed Genogroups I and II were equally prevalent during wet season events (14%), whereas NoV GI was more prevalent in dry events (10%) as compared to NoV GII (5%). In the shellfish harvesting region of Tomales Bay, NoVs were only detected at the freshwater site Walker Creek (40%), located at the mouth of the bay. Prevalence was high at freshwater sites Waddell Creek (40%), Soquel Creek (40%), Watsonville Slough (100%, but note that only a single sample was collected from this site), and Carmel River (50%), which all discharge to the Pacific Ocean. Noroviruses were also found in 33% of samples taken at Elkhorn Slough, a tidal salt marsh which provides habitat for hundreds of animal species.

Norovirus prevalence in aggregates

Noroviruses were detected in 50% of aggregate-rich water samples and in 58% of aggregate poor retentate samples. NoV GI was more commonly detected in aggregate-rich (39%) and aggregate-poor retentate samples (43%) as compared to NoV GII (11% and 14%, respectively). Viruses were often found at the Point Lobos site in the Carmel Region for both aggregate-rich (66%) and aggregate-poor retentate (66%) water types. However, 86% of aggregate-poor retentate samples tested at White Rock in Cambria were positive for NoV. NoV GI prevalence in dry events was equal for both water types (66%) and was higher than wet weather prevalence (19% for aggregate-rich and 25% for aggregate-poor retentate samples). NoV GII prevalence was highest in aggregate-poor retentate samples taken during dry weather events (25%), but was still consistently detected at a lower rate than NoV GI for these water types.

Objective 2: Correlate NoVs in estuarine and coastal waters with the prevalence of zoonotic pathogens (*Cryptosporidium, Giardia,* and *Salmonella*) and fecal indicator bacteria (FIB).

Hypothesis 3: NoVs are correlated with Toxoplasma, Cryptosporidium, Giardia, Salmonella, and FIB in water, aggregates and shellfish.

Toxoplasma gondii was detected in six hemolymph samples from the Carmel region and 7 hemolymph samples from the Cambria region. *Giardia* was detected in 3 hemolymph samples

collected from Cambria. *Cryptosporidium* was not detected in this study. *Salmonella* was detected in two mussel homogenate sample collected from Carmel and one sample collected from Cambria.

FIB were present at higher numbers attached to aquatic aggregates as compared with numbers in surrounding seawater. Protozoal pathogens were rarely present in water samples. *Cryptosporidium* oocysts were visualized in one aggregate-poor seawater sample from Cambria, and one aggregate-poor and two aggregate-rich water fractions from Carmel. However, these microscopical findings could not be confirmed molecularly, likely due to the low number of oocysts and associated DNA present. *Toxoplasma*, *Giardia* and *Salmonella* were not detected in any of the water samples.

Presence of NoV in mussels was not associated with season (wet vs. dry) or proximity to freshwater runoff. Sampling location was a significant predictor variable, with White Rock (Cambria) having significantly fewer mussels with detectable NoV RNA. An inverse relationship was detected between the likelihood of detecting NoV in mussels and other zoonotic agents ($p < 0.001$), which was a surprising finding. To evaluate relationships between NoV in mussels and water quality standards set by seafood and/or recreation safety agencies, FIB concentrations were transformed into a binary variable with 'exposure' indicated as any concentration above set limits. A positive and significant relationship was detected between NoV presence in mussels and seawater concentrations of fecal coliforms, with a 3.7 increase in odds of detecting NoV (Odds Ratio = OR) when fecal coliforms exceeded 14 MPN/100 mL (a limit established by the National Shellfish Safety Program (NSSP)). Surprisingly, a negative relationship was detected between NoV presence in mussels and fecal coliform concentrations in mussel tissue (OR=0.7), as well as with levels of *Enterococcus* in seawater.

Objective 3: Perform quantitative microbial risk assessment on NoV prevalence in ambient waters and shellfish.

Hypothesis 4: Human NoVs pose a significant risk to consumers of shellfish.

From the QMRA analysis, a risk profile of the cumulative number of cases of norovirus illness for 100 people exposed on any random occasion over 1000 independent occasions was produced, resulting in 10^5 iterations. The resulting cumulative frequency distribution is summarized via the IIR (as a percentage), which is numerically identical to the mean value of the short-term predicted illness risk. The individuals' illness risk (IIR) of norovirus infection was predicted to be 46.16% when assuming 50% removal by depuration, 10% NoV infectivity and replacing non-detects with 50% of the SLOD. This number reduced to 44.84% when outliers were removed from the NoV hemolymph concentrations and a new exponential distribution was fit. If NoV infectivity at consumption was reduced to 1%, the IIR was calculated to be 37.27% and 34.6% when outliers were included and excluded from the data set, respectively.

A sensitivity analysis was performed wherein the values of input parameters were changed so as to evaluate the variables that drive the risk of NoV infection. Keeping NoV removal at depuration at 50%, non-detects at 50% SLOD, NoV infectivity at consumption at 1% and removing outliers, the IIR dramatically increases to 51% if refrigeration time is reduced to 24 hours. If depuration was assumed to remove up to 99% of NoVs, IIR was reduced to 20.2% and 5.32% when infectivity was assumed to be 10% and 1%, respectively. Harvest duration and the mean number of oysters consumed per serving and per serving per person per annum did not appear to have a marked effect on the IIR. In conclusion, both refrigeration time and depuration

are important factors that are can reduce the illness risk and are amenable to control measures by industry.

Impacts of Project:

Overall, this project has advanced the current understanding of the behavior of NoVs in the coastal environment and in shellfish. Studies on NoV association with aquatic aggregates provide novel data demonstrating significant association of virus particles with sinking aggregates, which serve as a source of food for shellfish that are often consumed as seafood by people. Further research that aims to characterize temporal or spatial factors that impact the degree of NoV association with macroaggregates may therefor provide insight into best management strategies for optimal selection of shellfish growing sites and harvesting times.

The risk information generated by this project has set the stage for risk communication to shellfish producers, retailers and consumers. The project supports QMRA as an important tool for the seafood industry in order to protect consumers from emerging pathogens like NoV, and provides important data for research, as well as tools related to the development and implementation of seafood management strategies.

In addition to advancing scientific discovery relevant to shellfish food safety, our project included a successful outreach program that was accomplished in collaboration with the Gulf of the Farallones National Marine Sanctuary educational center (located in San Francisco). Our team developed a 60-90 minute educational program targeted for delivery to K-12 children, and included a participatory power point presentation, artifact showcase, and interactive games. Overall, the goal of the outreach program was to bring 'real scientists' into the classroom and convey how our actions on land can impact water quality of our watersheds, that ultimately drain into our coasts where pollution can impact food sources that are critical to both wildlife and people. In total, our team reached over 1,500 children in both affluent and underserved communities during our project timeline.

Many undergraduate and graduate students were instrumental in the completion of this project and in turn were trained in the field and laboratory methods necessary to complete this project. These students include graduate students Kaitlyn Hanley, Katja Fricke, Claudia Llerandi and Ezequiel Santillan and undergraduate students Desirae Costello, Mirann Tsumara, Roberto Castaneda and Brittany Leung.

Benefits, Commercialization, and Application of Project Results:

The study provided new insights into the fate and transport of noroviruses in the coastal environment, including the importance of viral attachment to aggregates in saline waters. There is tremendous potential for the development of Best Management Practices to help shellfish growers improve food safety. Quantitative microbial risk assessment (QMRA) was shown as a useful risk evaluation tool that can be tailored for future public health questions and industry-related research or policy decisions. We have not yet transmitted our results to shellfish growers because outcomes of QMRA have only become available very recently, but plan to do so in the coming months.

Economic Benefits generated by discovery, exploration and development of new, sustainable coastal, ocean and aquatic resources (i.e., aquaculture, marine natural products, foods, pharmaceuticals).

We worked closely with the California Sea Grant Extension Program to network with California-based seafood growers. Key strategies for follow up activities are 1) to reach as many shellfish growers as possible, 2) to provide shellfish growers with relevant science-based information and 3) to facilitate the exchange of ideas between shellfish growers and researchers.

Issue-based forecast capabilities to predict the impacts of a single ecosystem stressor, developed and used for management (i.e., climate change, extreme natural events, pollution, invasive species, and land resource use).

Based on the scenarios tested in QMRA, our recommendation would be to take steps to reduce the risk of norovirus infection before retailing oysters for consumption. From this project, it was determined that a depuration of oysters after harvesting and adequate refrigeration time before retail are key steps that harvesters can take to reduce the risk of norovirus infection.

Tools, technologies and information services developed (i.e., land cover data, benthic habitat maps, environmental sensitivity index maps, remove sensing, biosensors, AUVs, genetic markers, technical assistance, educational materials, curricula, training).

No new tools were developed but the utility of QMRA as a risk management tool was shown.

Publications

Technical Reports

Shapiro, K., Miller, W.A. Silver, M.W. Odagiri, O. Largier, J.L., Conrad P.A., and J.A.K. Mazet (2013). Association of zoonotic pathogens with fresh, estuarine, and marine macroaggregates. Microbial Ecology. 65(4): 928-33

Conference Papers, Proceedings, Symposia

1. Schriewer, A., Kim, M., Wuertz, S. (2012) Microbial Source Tracking in Coastal Waters. 104th Meeting of the National Shellfisheries Association, Seattle, USA, 25-29 March, 2012.
2. Odagiri, M., Schriewer, A., Shapiro, K., Miller, W., Wuertz, S. (2012) A Current Understanding of Correlations Between FIB and Pathogens in Coastal Water. 104th Meeting of the National Shellfisheries Association, Seattle, USA, 25-29 March, 2012.
3. Miller, W., Wuertz, S., Shapiro, K., Schriewer, A., Odagiri, M. (2012) Stakeholder-Driven One Health Research. 104th Meeting of the National Shellfisheries Association, Seattle, USA, 25-29 March, 2012.
4. Shapiro, K., Silver, M., Largier, J., Mazet, J., Miller, W., Odagiri, M., Schriewer, A. (2012) Pathogen Aggregation: Understanding When, Where, and Why Seafood Contamination Occurs. 104th Meeting of the National Shellfisheries Association, Seattle, USA, 25-29 March, 2012.

5. Stefan Wuertz, Kaitlyn T. Hanley, Alexander Schriewer, Claudia Llerandi, Katja Fricke, Woutrina Miller, and Karen Shapiro. Detection and Behavior of Noroviruses in Coastal Waters. II Simposio Latinoamericano de Virologia Ambiental. Salto, Uruguay, 8-10 April, 2013. Oral presentation
6. Kaitlyn T. Hanley, Alexander Schriewer, Claudia Llerandi, Katja Fricke, Woutrina Miller, Stefan Wuertz and Karen Shapiro. Noroviruses in coastal waters: Implications for seafood cultivation and human health. International Water Association (IWA) 17th Symposium on Health-Related Water Microbiology, Health-Related Water Microbiology Meeting, Florianopolis, Brazil, 13-20 September 2013. Poster presentation
7. Alexander Schriewer, Kaitlyn T. Hanley, Claudia Llerandi, Katja Fricke, Woutrina Miller, Stefan Wuertz and Karen Shapiro. Behavior of Noroviruses in Coastal Environments and Implications for Seafood Cultivation and Human Health. 1st Water Microbiology Conference, The University of North Carolina, Chapel Hill 5-7 May, 2014. Oral presentation.
8. Karen Shapiro, Alexander Schriewer, Kaitlyn Hanley, Claudia Llerandi, Katja Fricke, Woutrina Miller, and Stefan Wuertz "Noroviruses in Coastal Waters: Implications for Seafood Cultivation and Human Health" PacRim Shellfish Sanitation Program, March 18-19, 2014, Oakland, California.
9. Kaitlyn T. Hanley, Alexander Schriewer, Claudia Llerandi, Katja Fricke, Woutrina Miller, Stefan Wuertz and Karen Shapiro. Accumulation of noroviruses in EPS-rich aggregate fraction in coastal environments. IWA conference: The perfect slime - Nature, Properties, Regulation and Dynamics of EPS. 10-12 September 2014, Essen, Germany.

Cooperating Organizations

The National Shellfisheries Association, founded in 1908, is one of the oldest international organizations of scientists, management officials and members of industry, and hence holding a workshop at the beginning of the project as part of the annual meeting in 2012 allowed for close interactions with stakeholders. Furthermore, we invited two guest speakers from Japan and New Zealand to the workshop who have extensive experience with multi-year NoVs monitoring studies in Japan, and with applications of quantitative microbial risk assessment (QMRA) in water quality management worldwide, respectively. The workshop included an interactive panel discussion to exchange ideas between shellfish growers and researchers to develop strategies for future collaborative work. The panel discussion was moderated by Paul Olin, a Sea Grant Extension Advisor who has worked extensively in aquaculture policy and in the shellfish industry. We interacted closely with the California Sea Grant Extension Program throughout the project period.

International Implications

None

Awards

None

Keywords

Norvirus, food safety, marine snow, aggregation, QMRA

References

Bae, J. and Schwab, K.J. (2008) Evaluation of murine norovirus, feline calicivirus, poliovirus, and MS2 as surrogates for human norovirus in a model of viral persistence in surface water and groundwater. Applied and Environmental Microbiology 74(2), 477-484.

Baert, L., Wobus, C.E., Van Coillie, E., Thackray, L.B., Debevere, J. and Uyttendaele, M. (2008) Detection of murine norovirus 1 by using plaque assay, transfection assay, and real-time reverse transcription-PCR before and after heat exposure. Applied and Environmental Microbiology 74(2), 543-546.

CDC, 2006: Vital Signs: Foodborne Norovirus Outbreaks — United States, 2009–2012. Morbidity and Mortality Weekly Report. June 6, 2014 / 63(22);491-495.

Cannon, J.L., Papafragkou, E., Park, G.W., Osborne, J., Jaykus, L.-A., Vinj and Jan (2006) Surrogates for the study of Norovirus stability and inactivation in the environment: A comparison of murine norovirus and feline calicivirus. Journal of Food Protection 69(11), 2761-2765.

Degner and Petrone, 1994 Consumer and restaurant manager reaction to depurated oysters and clams. Report to the Levy County Board of County Commissioners by the Florida Agricultural Market Research Center, U FL.

FDA, 2005. Quantitative risk assessment on the public health impact of pathogenic *Vibrio parahaemolyticus* in raw oysters. Federal Register Notice. 70:41772-41773.

Haas, C.N., Rose, J.B., and Gerba, C.P. 1999. Quantitative Microbial Risk Assessment. John Wiley, New York.

ISSC, 2004. Raw Oyster Consumer Follow-Up Survey: 2004 Technical Report

Lyons, M.M., Ward, J.E., Smolowitz, R., Uhlinger, K.R., Gast, R.J., 2005, Lethal marine snow: Pathogen of bivalve mollusc concealed in marine aggregates. Limnol Oceanogr 50, 1983-1988.

Miller, W.A., Atwill, E.R., Gardner, I.A., Miller, M.A., Fritz, H.M., Hedrick, R.P., Melli, A.C., Barnes, N.M. and Conrad, P.A. (2005) Clams (*Corbicula fluminea*) as bioindicators of fecal contamination with *Cryptosporidium* and *Giardia* spp. in freshwater ecosystems in California. Int J Parasitol 35(6), 673-684.

Miller, W.A., Gardner, I.A., Atwill, E.R., Leutenegger, C.M., Miller, M.A., Hedrick, R.P., Melli, A.C., Barnes, N.M. and Conrad, P.A. (2006a) Evaluation of methods for improved detection of *Cryptosporidium* spp. in mussels (*Mytilus californianus*). J Microbiol Methods 65(3), 367-379.

Miller, W.A., Miller, M.A., Gardner, I.A., Atwill, E.R., Byrne, B.A., Jang, S., Harris, M., Ames, J., Jessup, D., Paradies, D., Worcester, K., Melli, A. and Conrad, P.A. (2006b) *Salmonella* spp., *Vibrio* spp., *Clostridium perfringens*, and *Plesiomonas shigelloides* in marine and freshwater invertebrates from coastal California ecosystems. Microb Ecol 52(2), 198-206.

Rajal, V.B., McSwain, B.S., Thompson, D.E., Leutenegger, C.M. and Wuertz, S. (2007a) Molecular quantitative analysis of human viruses in California stormwater. Water Research 41(19), 4287-4298.

Rajal, V.B., McSwain, B.S., Thompson, D.E., Leutenegger, C.M., Kildare, B.J. and Wuertz, S. (2007b) Validation of hollow fiber ultrafiltration and real-time PCR using bacteriophage PP7 as surrogate for the quantification of viruses from water samples. Water Research 41(7), 1411-1422.

Shapiro, K., Miller, W.A., Silver, M.W., Odagiri, M., Largier, J.L., Conrad, P.A. and Mazet, J.A. (2013) Research commentary: Association of zoonotic pathogens with fresh, estuarine, and marine macroaggregates. Microb Ecol 65(4), 928-933.

Soller, J.A. and Eisenberg, J.N.S. (2008) An evaluation of parsimony for microbial risk assessment models. Environmetrics 19(1), 61-78.

Teunis, P.F.M., Moe, C.L., Liu, P., E. Miller, S., Lindesmith, L., Baric, R.S., Le Pendu, J. and Calderon, R.L. (2008) Norwalk virus: How infectious is it? Journal of Medical Virology 80(8), 1468-1476.

Tu, E.T.-V., Bull, R.A., Kim, M.-J., McIver, C.J., Heron, L., Rawlinson, W.D. and White, P.A. (2008) Norovirus excretion in an aged-care setting. Journal of Clinical Microbiology 46(6), 2119-2121.

Wolf, S., Williamson, W.M., Hewitt, J., Rivera-Aban, M., Lin, S., Ball, A., Scholes, P. and Greening, G.E. (2007) Sensitive multiplex real-time reverse transcription-PCR assay for the detection of human and animal noroviruses in clinical and environmental samples. Applied and Environmental Microbiology 73(17), 5464-5470.