# Effects of salinity and transparent exopolymer particles on formation of aquatic aggregates and their association with norovirus

*Kaitlyn T. Hanley*[a,1], *Stefan Wuertz*[a,b,c], *Alexander Schriewer*[a,2], *Uta Passow*[d], *Woutrina Smith*[e], *Paul Olin*[f], *Karen Shapiro*[e, g *]

[a] Department of Civil and Environmental Engineering, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA

[b] Singapore Centre for Environmental Life Sciences Engineering (SCELSE), Nanyang Technological University (NTU), 60 Nanyang Drive, Singapore 637551, Singapore

[c] School of Civil and Environmental Engineering, NTU, 50 Nanyang Avenue, Singapore 639798, Singapore

[d] UCSB Marine Science Institute, University of California, Bldg 520 Rm 4002 Fl 4L, Santa Barbara, CA 93106, USA

[e] One Health Institute, University of California, Davis, One Shields Avenue, Davis, California 95616, USA

[f] California Sea Grant, UCSD Scripps Institution of Oceanography, 9500 Gilman Drive, Dept. 0232. La Jolla, California 92093-0232

[g] Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

*Corresponding Author, Phone: +1 519-546-0324, Fax: +1 530-752-3144, Email: kshapiro@ucdavis.edu; 4206 VM3A, SVM: PMI, University of California, Davis, CA 95616, USAA

[1] Current affiliation: Amec Foster Wheeler Environment and Infrastructure, 9177 Sky Park Court, San Diego, CA, 92123-4341, USA.

[2] Current affiliation: Weston Solutions, Inc. 5817 Dryden Place, Suite 101, Carlsbad, CA 92008, USA.

## ABSTRACT

Human noroviruses (NoVs) are responsible for 50% of food-related disease outbreaks and are notably associated with shellfish consumption. Despite the detrimental health impacts of human NoV-contaminated seafood to public health, there is a lack of knowledge on the physicochemical conditions that govern NoV transmission in aquatic ecosystems. In the present study, we investigated the propensity for NoVs to associate with aquatic aggregates, which have been shown to efficiently deliver nano-sized particles to shellfish. Specific physicochemical conditions characteristic of shellfish cultivation waters, specifically salinity and transparent exopolymer particles (TEP), were targeted in this study for investigating aggregate formation and NoV association dynamics. Murine norovirus (MNV) was used in aggregation experiments as a model surrogate for NoVs. Results demonstrate increased aggregate formation as a function of increasing salinity and TEP concentrations, as well as greater numbers of MNV genomes incorporated into aggregates under conditions that favor aggregation. As aggregate formation was enhanced in waters representing optimal conditions for shellfish production, specifically saline and high TEP waters, the implications to virus transport and shellfish food safety are profound: more aggregates implies increased scavenging of virus particles from surrounding waters and therefor greater risk for bivalve contamination with nano-sized pathogens. These

novel data provide insight into where and when NoVs are most likely to be ingested by shellfish via contaminated aggregates, thereby informing best management and water quality monitoring practices aimed at providing safe seafood to consumers.

**KEYWORDS**

Pathogen, transport, transmission, seafood safety, water quality, shellfish

**HIGHLIGHTS**

- Noroviruses (NoVs) are associated with aquatic aggregates.
- Enhanced aggregate formation occurred in optimal shellfish cultivation conditions.
- Enhanced aggregate formation facilitated virus-aggregate association.
- NoV transmission to seafood consumers likely enhanced by presence of aggregates.

## 1 INTRODUCTION

Noroviruses (NoVs) are small (27 – 40 nm in diameter), single-stranded RNA viruses and members of the *Caliciviridae* family. Human NoVs are the most commonly identified cause of acute gastroenteritis amongst both sporadic community cases and outbreaks (de Wit et al. 2001, Wheeler et al. 1999), and are the leading cause of viral gastroenteritis (GI) worldwide (Patel et al. 2009), responsible for 23 million cases of GI per year (Mead et al. 1999). Clams, mussels, oysters, and scallops are filter feeders that can accumulate toxins and pathogens present in cultivation waters, and have consistently proven to be effective vehicles for the transmission of

viral diseases (Le Guyader et al. 2012, Lees 2000). About 50% of food-related illness outbreaks are attributed to human NoVs (Hall et al. 2014), with shellfish as the most commonly implicated commodity of a human NoV disease outbreak (Hall et al. 2011).

Despite the detrimental health impacts of human NoV-contaminated seafood to public health, the physical mechanisms that govern NoV transmission to shellfish are largely unknown. Aquatic macroaggregates (composite particles ≥ 0.5 mm, herein referred to as "aggregates") are ubiquitous in natural waters, and form by a variety of physicochemical pathways (Silver et al. 1978). They are heavily colonized by bacteria and other heterotrophic microorganisms, and are important components of the sinking flux of organic and inorganic matter in marine ecosystems (Alldredge and Silver 1988, Grossart and Simon 1998, Kiørboe et al. 2003, Simon et al. 2002, Smith et al. 1992).

Aggregates can have a significant effect on the waterborne transmission of pathogens, as previous studies have demonstrated the propensity of pathogens like *Toxoplasma gondii*, *Cryptosporidium parvum, Giardia lamblia*, *Salmonella*, and the viral surrogate bacteriophage PP7 to associate with aggregates, particularly in estuarine and marine waters (Shapiro et al. 2013, Shapiro et al. 2012). Benthic invertebrates such as shellfish consume organic aggregates as a source of food, and can subsequently concentrate microorganisms present in these flocs (Lyons et al. 2005). Compared with freely suspended virus-sized particles, aggregates have been shown to facilitate the ability of bivalves to capture and retain viral particles in their tissues (Ward and Kach 2009). However, there is a lack of knowledge of the physicochemical conditions that govern the association of NoVs with aggregates, specifically in shellfish cultivation waters. This information is essential for predicting and monitoring high-risk zones of human NoV-contaminated waters.

88 Exopolymers such as transparent exopolymer particles (TEP) promote the formation of
89 aggregates from suspended matter (Alldredge et al. 1993, Passow 2002). TEP can act as a glue
90 that aids in the binding of small organic (e.g., detritus, phytoplankton, fecal pellets) and
91 inorganic material (e.g., clay minerals), increasing the aggregation rate, resulting in the
92 formation of more and larger aggregates that sink rapidly to the benthos (Alldredge et al. 1993,
93 Alldredge and Silver 1988, Passow and Alldredge 1995a, Passow and Alldredge 1995b).
94 Previous studies have shown that phytoplankton, bacteria, and oysters contribute to the
95 production of TEP through the natural release of exopolymers, implying that benthic suspension
96 feeders produce TEP which could lead to enhanced formation of aggregates in near-shore waters
97 (Heinonen et al. 2007). Furthermore, changes in salinity, such as in an estuarine or marine
98 environment, have been found to enhance TEP formation, and therefore also contribute to an
99 increased aggregation rate (Wetz et al. 2009) . Despite the theoretical link among shellfish
100 cultivation waters, TEP production, aggregate formation and pathogen transport, no work to date
101 has described the effect of these physicochemical conditions on the association of NoVs with
102 aggregates.

103 Predicting and mitigating human NoV contamination of seafood requires an
104 understanding of the basic mechanisms that govern virus entry into shellfish. In this study,
105 laboratory aggregation experiments utilized murine norovirus (MNV) to test three hypotheses:
106 (1) Changes in salinity and TEP concentrations cause an increase in aggregate formation; (2)
107 NoVs associate with aquatic aggregates; and (3) An increase in aggregate formation promotes an
108 increased association of NoVs with aggregates. MNV has been previously described as the most
109 appropriate viral surrogate for investigating the physical transport of human NoVs due to its
110 phylogenetic relationship, size (28-35 nm), shape (icosahedral), buoyancy, and environmental

fate (Bae and Schwab 2008, Cannon et al. 2006). Therefore, compared with previous studies on virus association with aquatic aggregates, the present study provides more robust data on NoV transport behavior in aquatic systems. Furthermore, the results of the present study provide novel insight into the conditions under which human NoV association with aggregates may be enhanced, leading to more efficient uptake by shellfish and subsequent risk of exposure to shellfish consumers.

## 2 MATERIALS AND METHODS

### 2.1 Source of murine norovirus

Strain MNV-1, considered the MNV prototype, was donated by Jackson Laboratories in Bar Harbor, Maine. This strain was passed numerous times through cell culture and was obtained at a concentration of $10^{6.8}$ 50% Tissue culture Infective Dose ($TCID_{50}$)/mL. $TCID_{50}$/mL values were converted to MNV RNA genomes/mL using RNA concentration (ng/µL) measurements made by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Multiple aliquots of the stock virus were stored at -80°C, and specific aliquots were thawed immediately prior to spiking experiments as detailed below.

### 2.2 Aggregation experiments

#### 2.2.1 Water samples

Two separate experiments were conducted to test the effects of changes in salinity (Experiment 1) and TEP concentration (Experiment 2) on the rate of aggregations and association of MNV with aquatic aggregates (Figure S1). For Experiment 1, a single surface freshwater sample (30-L) was collected from Putah Creek in Davis, CA (38°29'36.2"N, 122°01'42.7"W). Freshwater was

134 spiked with increasing concentrations of artificial sea salt (Bio-Sea true seawater formula, Aqua
135 Craft) to yield an identical water matrix that differed only in ionic strength across five salinity
136 samples ranging from fresh to marine (0, 8, 16, 24 and 33 ppt). Non-spiked freshwater served as
137 the baseline salinity condition and as the "0 ppt" water type.

138 In Experiment 2, a single surface seawater sample (30-L) was collected near the mouth of
139 Tomales Bay in Marshall, CA (38°13'01.2"N, 122°57'34.8"W). Tomales Bay is a major
140 shellfish-harvesting region in California and would therefore be representative of the baseline
141 amount of native TEP present in shellfish growing waters. Seawater was spiked with increasing
142 concentrations of alginic acid to yield seawater samples that were identical across all
143 physicochemical properties except for increasing concentrations of TEP. Unspiked seawater
144 served as the baseline measure of TEP in the water. Spiked TEP concentrations represent
145 realistic concentrations measured in natural seawaters, which range from 50 to 1042 µg Gum
146 Xanthan equivalents per liter (µg Xeq/L) along the California coast (Passow 2002). Alginic acid
147 is a commercially available anionic polysaccharide that is available in a purified form, is
148 produced by a common California macroalgae (*Macrocystis pyrifera*) (McKee et al. 1992), and
149 has been previously used as a TEP surrogate (Passow 2012, Shapiro et al. 2014). The
150 composition of TEP is largely unexplored and variable, however, TEP are operationally defined
151 as being rich in acidic polysaccharides (Passow 2002). Alginic acid was selected as a model for
152 TEP in this study due to its classic TEP characteristics, including rich-acidic-polysaccharide
153 composition and gelling behavior.

154 Alginic acid, in addition to xanthan gum, is routinely employed as a reference value for
155 the calibration of TEP (Thornton et al. 2007). A working stock solution of alginic acid was
156 prepared using 60 mg of alginic acid from *Macrocystis pyrifera* (Sigma-Aldrich CAS 9005-32-7)

and 100 mL of collected seawater. The stock solution was mixed three times using a tissue homogenizer and was then allowed to settle for 20 minutes. This mixing process was repeated twice, after which the homogenized solution was then refrigerated (4°C) in the dark until used. To prepare seawater samples of increasing TEP concentrations, the 0.6 mg/mL alginic acid stock solution was added at increasing volumes to 4-6 L of collected seawater, targeting a final concentration of 100, 300, 500, and 700 µg Xeq/L. Final TEP concentrations were 0, 169, 225, 327 and 484 µg Xeq/L.

### 2.2.2 TEP quantification

In Experiment 2, one-liter aliquots of each water type were preserved in 2% formaldehyde and stored in the dark at 4˚C for later analysis. TEP concentrations were measured by filtering up to three replicates of 100–250-mL aliquots onto 0.4-mm polycarbonate filters. Concentrations were determined using a standard semi-quantitative colorimetric assay and a UV-1201 UV-VIS spectrophotometer (SLM-Aminco DW/20) set at 787 nm (Passow and Alldredge 1995b). A calibration of the Alcian Blue dye was constructed using xanthan gum as the standard, and TEP concentrations are expressed in Gum Xanthan equivalents (Passow and Alldredge 1995b).

### 2.2.3 Aggregate production

In each experiment, water samples (450-mL) were placed in cylindrical glass bottles filled to the rim to minimize air space. Each jar was spiked with $10^8$ and $10^{10}$ MNV genomes in Experiments 1 and 2, respectively. Jars were placed on a rolling apparatus rotating at an average speed of 11 rpm for 24 h to induce aquatic aggregate formation under laboratory conditions. Aggregation control bottles (hereinafter referred to as “controls”) consisted of an equivalent-filtered sample corresponding to each water type tested. Control samples were filtered through 0.22 µm Stericup-GP, radio-sterilized polyethersulfone filters (EMD Millipore, Billerica, MA) after the

addition of artificial sea salt or alginic acid, but prior to MNV spiking. Controls were subsequently processed in an identical manner as non-filtered samples to evaluate MNV distribution in the absence of particles available to aggregate. In both experiments, five replicates of each treatment (salinity or TEP concentration) were tested. In Experiment 1, three replicates of control bottles were tested for each water type, while in Experiment 2, three replicates of control bottles were included for the lowest and highest TEP concentration samples.

### 2.2.4 Aggregate analysis and quantification

After 24 h, jars were placed upright for 20 min to allow readily visible aggregates to settle. A 2-mL sample was then taken from the top 400 mL of the jar (hereafter referred to as the "aggregate-poor" water). A preliminary experiment performed by the authors indicated a greater recovery of spiked MNV in the direct pipetting of visible aggregates, as compared to collecting a well-mixed, bottom "aggregate-rich" sample. Therefore, visible and settled aggregates were harvested via direct pipetting from the remaining 50 mL of aggregate-rich bottom fraction. The volume of all aggregates in the settled pellet was visually estimated using a calibrated microcentrifuge tube. Aggregate-poor water and aggregate samples were placed in 2-mL microcentrifuge tubes and stored at -80˚C until further analysis.

### 2.2.5 MNV quantification

Genomic RNA was extracted from 500 µL of aggregate-poor water and aggregate samples using Invitrogen PureLink™ Viral RNA/DNA Mini Kit (Invitrogen, Carlsbad, CA), following the manufacturer's protocol, yielding 100 µL of eluate, which was then stored at -80˚C until further analysis. MNV was quantified in aggregate-poor water and aggregates using a sensitive and validated RT-qPCR assay (Baert et al. 2009). Each 25-µL RT-qPCR mixture contained 2.5 µL of commercially available Multiplex Enzyme Mix with Arrayscript™ reverse transcriptase and

AmpliTaq Gold® DNA Polymerase enzyme from the Path-ID Multiplex One-Step RT-PCR Kit (Life Technologies, Grand Island, NY), 12.5 µL of buffer mix, and 200 nM (each) of forward and reverse primers and probe. For all TaqMan reactions, 8 µL of RNA was assayed in a final reaction volume of 25 µL. To assess inhibitory factors, three serial dilutions were performed in duplicate (Experiment 1) and triplicate (Experiment 2) for each sample on a 96-well plate. Reactions were run using a single-tube, one-step procedure to reverse-transcribe the MNV RNA to cDNA and subsequently amplify MNV DNA. Using the Applied Biosystems® StepOnePlus™ Real-Time PCR System, cycling conditions included one cycle at 50°C for 30 min to allow for reverse transcription to occur, followed by one cycle at 95°C for 10 min, followed by 40 cycles of 15 s at 95°C and 60 s at 60°C to allow for amplification of cDNA.

Resulting fluorescence signals were assigned a cycle quantification threshold (Ct) value when amplification greater than a threshold of 0.04 $\Delta R_n$ was detected within 40 thermal cycles. Ct values were converted to MNV genomes using a standard curve. A standard curve ($y = -3.42x + 40.18$, $R^2 = 1$) was generated (Figure S2) using 12 replicates each of a 10-fold serial dilution ($10^7$ genomic copies to 1 genomic copy) of a purified plasmid containing a full-length cDNA clone of MNV-1.CW1 (Sosnovtsev et al. 2006). Regression analyses were used to identify outliers as those with standardized residuals $\geq |3|$ (Thompson 1935). Outliers were iteratively removed until the adjusted standard curve data set no longer contained values with standardized residuals $\geq |3|$. Data analysis resulted in a final measurement of MNV genomes per mL (genomes/mL) of aggregate-poor water or aggregate sample.

The total recovery of MNV genomes for each jar was calculated by comparing the sum of the genomes quantified in the aggregate-poor water and aggregates to the total number of MNV genomes spiked into each jar. For Experiment 1, the total MNV spiked into each jar was

226 determined by RT-qPCR of the MNV stock solution placed at room temperature for the duration
227 of the rolling experiment, to account for natural degradation of MNV RNA that may occur. For
228 Experiment 2, the initial number of MNV genomes in each jar was quantified via a homogenized
229 sample taken from each jar immediately after spiking and prior to rolling. In an effort to
230 standardize measured MNV genomes for comparison across samples, the total number of MNV
231 genomes detected in aggregate-poor water and in aggregates were corrected using recovery
232 coefficients and harvest volumes, which is defined as the aggregate volume for aggregate
233 samples and 450 mL for aggregate-poor water. The number of MNV genomes recovered in the
234 aggregates and aggregate-poor water fractions were not adjusted for those samples with
235 recoveries greater than 100% (n = 2 for Experiment 1, n = 1 for Experiment 2).
236
237 **2.3 Statistical Analysis**
238 A Kruskal-Wallis ANOVA test followed by a nonparametric post-hoc pairwise
239 comparison (Siegel 1956) was performed to compare the median MNV concentrations in the
240 aggregate-poor water and in harvested aggregates across the different water types, and the total
241 median number of MNV genomes recovered in aggregates across the different water types. A
242 Mann-Whitney U test was used to test whether the concentration of MNV differed between
243 aggregate-poor water and aggregates within each water type, as well as to test whether the total
244 number of MNV genomes detected in the non-filtered bottles differed from the respective
245 filtered-control bottles.
246

## 3 RESULTS

Two separate experiments were designed to test the effects of 1) salinity and 2) transparent exopolymer particles on aggregate formation and association of NoV with aquatic aggregates. The physicochemical properties of the two water samples used in the experiments are detailed in Table 1.

### 3.1 Experiment 1: MNV aggregation as a function of salinity

Macroaggregates were observed in all water samples after the 24 h rolling period and were not visible in any controls. The total volume of aggregates formed ranged from 10 to 180 µL, with a positive exponential relationship observed between increasing salinity concentration and increasing mean aggregate volume ($R^2 = 0.98$, Fig. 1a). A significant association was further observed between increasing salinity and increasing mean number of MNV genomes detected in aggregates ($R^2$=0.94, Fig. 2a). MNV concentrations (in genomes/mL) were consistently higher in aggregates than in aggregate poor water for all salinities, and significantly higher in aggregates occurring at estuarine and marine salinities (8, 24 and 33 ppt, Fig. S2a). Conversely, within each salinity control jar, there was no significant difference in the MNV concentration between the top and bottom water fractions (equivalent to aggregate-poor water and the aggregates in corresponding water types).

Total recovery of MNV genomes ranged from 11 – 224%, with > 100% recoveries likely due to potential underestimation of total MNV RNA spiked into each jar caused by the natural degradation of MNV RNA in the spike control tube. Significantly more MNV genomes were recovered in the higher salinity water aggregates (24 and 33 ppt) as compared to freshwater aggregates ($P \leq 0.01$). Interestingly, higher numbers of MNV genomes ($P \leq 0.05$) were

recovered in controls as compared to samples for all salinity groups, except for group 8 ppt. This observation suggests that NoV did not survive as well in water samples as it did in the filtered controls. Reduced survival of MNV in unfiltered seawater may be due to predatory microorganisms and substances that could degrade the virus over the duration of the experiment.

### 3.2 Experiment 2: MNV aggregation as a function of TEP

Measured TEP concentrations in seawater samples ranged from 524 to 1305 µg Xeq/L and concentrations in filtered controls were lower than the assay detection threshold. As with Experiment 1, macroaggregates were observed in all water samples after the 24 h rolling period and were not visible in any controls. The volume of aggregates formed ranged from 10 to 90 µL, with a positive exponential relationship observed between increasing TEP concentration and increasing mean aggregate volume ($R^2$ = 0.95, Fig. 1b). Similarly, a significant association was observed between increasing TEP concentration and increasing mean number of MNV genomes recovered ($R^2$=0.85, Fig. 2b). Mean MNV concentrations (in genomes/mL) were higher in aggregates as compared to aggregate- poor water for all treatments with spiked alginic acid (TEP 2 – TEP 5, Figure S2b) with a maximum MNV concentration in aggregates at TEP 2 (757 µg Xeq/L).

Total recovery of MNV genomes ranged from 16 – 107%. Significantly more MNV genomes were recovered in aggregates from treatments with higher TEP concentrations (TEP 4 and TEP 5) as compared to the seawater control group with no TEP added ($P \leq 0.01$). Similar to Experiment 1, the total number of MNV genomes detected in the filtered controls was greater compared to their corresponding unfiltered samples.

## 4 DISCUSSION

This is the first study to conclusively demonstrate the association of NoVs with aquatic aggregates. The significant relationships between increasing salinity and TEP concentrations and aggregate formation provide a link between these physicochemical conditions and rising numbers of NoVs associated with aquatic aggregates: Increasing quantities of sinking organic aggregates can effectively scavenge viral particles from the water column, enriching them. The likelihood of NoV incorporation into aggregates is thus dependent on the amount of aggregates present. As changes in salinity and increasing TEP concentrations that are commonly present in environments that support shellfish favor the formation of organic aggregates, these findings provide a fundamentally noteworthy discovery in understanding the mechanistic entry of viral pathogens into benthic invertebrates commonly consumed as seafood.

Temperature and salinity of inter-tidal and estuarine environments closely influence shellfish physiology. These parameters have been found to alter oyster feeding, growth, respiration, oxygen consumption, and excretion rates (Chávez-Villalba et al. 2005, Dame 2011, Dunphy et al. 2006, Galtsoff 1964, Heilmayer et al. 2008) and are, therefore, closely monitored or controlled during the aquaculture farming process to optimize growing conditions. The ideal salinity range for the Pacific oyster (*Crassostrea gigas*), common along the western seaboard of the United States, is between 20 and 25 ppt (Helm 2005). Similarly, the optimal salinity range for the Eastern oyster (*Crassostrea virginica*) is between 15 and 18 ppt (Wallace 2001). Thus, shellfish that are preferentially consumed raw by seafood consumers are also coincidentally farmed and harvested from waters in which pathogen-aggregate association is enhanced.

While the data presented in this study demonstrate for the first time that NoV association with aggregates is promoted due to enhanced aggregate formation in estuarine and marine

315 waters, the observation that organic aggregates form more readily in saline waters is not new.
316 The physical mechanism that enhances aggregate formation in higher salinity waters has been
317 attributed to an alteration in particle surface charge. Specifically, the loss of surface charge that
318 occurs as particles move from low to high salinity waters has been observed in several studies
319 (Beckett and Le 1990, Je and Chang 2004, Kaya et al. 2006, Sondi and Pravdic 1998) and is
320 hypothesized to occur due to specific adsorption of positive cations onto the surface of particles,
321 as well as direct compression of the double layer (zeta potential) of counter ions (Gregory 2006).
322 These effects are especially pronounced in the presence of divalent cations such as $Mg^{2+}$ and
323 $Ca^{2+}$ (Beckett and Le 1990, Edzwald et al. 1974, Sondi and Pravdic 1998), both of which are
324 abundant in sea and estuarine waters. Human NoV entry into coastal water is likely due to
325 freshwater discharges contaminated with human waste. Therefore, it is probable that these
326 harmful viruses become scavenged by organic aggregates at locations where their formation is
327 enhanced, namely estuarine and marine habitats (Gebhardt et al. 2005, Karbassi and Nadjafpour
328 1996, Sholkovitz 1976, 1978). Data from previous studies have indicated that mussels and
329 oysters more efficiently capture, ingest and retain nanoparticles that are incorporated into visible
330 aggregates (0.1 mm to several mm) as compared to nanoparticles that are freely suspended
331 (Ward and Kach 2009). As aggregates provide a mechanism for nano-sized pathogens such as
332 viruses to be preferentially ingested by bivalves, the ramifications for shellfish quality are
333 profound: optimal water salinities for shellfish growth and harvest further accelerate the
334 association of NoVs with aggregates, thus facilitating bivalve contamination with pathogenic
335 viruses.

336 Changes in salinity, such as in estuarine and marine environments, have been found to
337 enhance the formation of TEP (Passow 2002). Therefore, the second experiment in this study

338 further investigated the role of these gel-like polymers in mediating aggregate formation, and
339 subsequent NoV incorporation into these aggregates. As increasing volumes of aggregates
340 formed in higher TEP waters relative to the baseline seawater sample, significantly greater
341 numbers of NoV particles were also recovered in aggregates from higher TEP waters. Similar to
342 the findings from Experiment 1, waters that enhanced the production of aggregates subsequently
343 facilitated larger numbers of virus particles associating with these aggregates. The impact of TEP
344 on pathogen association with aggregates is fundamentally important for shellfish food safety, as
345 higher TEP concentrations have been found to be characteristics of shellfish-growing waters
346 (Heinonen et al. 2007). In a previous study, significantly more aggregates formed in waters pre-
347 conditioned with mussels than in control waters, which was attributable to TEP concentrations as
348 opposed to total or dissolved organic carbon (Li et al. 2008). Therefore, shellfish growing waters
349 naturally boost TEP production, further perpetuating the increase in aggregate formation and
350 potential for pathogen incorporation within these organic flocs.

351 TEP facilitate aggregation due to their sticky nature; TEP are generally 1-2 orders of
352 magnitude stickier than other marine particles (Centers for Disease Control National Outbreak
353 Reporting System (NORS), Xavier et al. 2017) and serve as the glue that hold particles together
354 following their collisions. Aggregation theory predicts that the rate of aggregation is a function
355 of the rate of collision and stickiness, e.g. the probability that particles remain attached upon
356 collision (Burd and Jackson 2009, Passow 2002). In the presence of high concentrations of
357 particles, TEP thus promote aggregate formation. High TEP concentrations may be expected in
358 the presence of shellfish that excrete TEP, during episodes of phytoplankton blooms or in kelp
359 forests, as both phytoplankton and kelp effectively generate TEP (Passow and Alldredge 1994,
360 Ramaiah et al. 2001). TEP formation has also been found to be enhanced as a function of salinity

or cations, possibly due to the stabilizing effects of cations for TEP (Najdek et al. 2011, Thornton 2009, Wetz et al. 2009). Acquisition of NoVs by shellfish is likely to be further strengthened as a function of their regional density: A greater number of shellfish are likely to result in higher concentrations of TEP in surrounding waters, further promoting aggregate production.

The findings presented here should be further expanded upon in future investigations into how NoV association with aggregates may affect their (Teunis et al. 2008)viability and/or persistence in environmental waters. Previous research has suggested that microorganisms, including pathogens, may benefit from their association with organic aggregates due to a sheltering effect from UV radiation, heat and ozone (Tang et al. 2011). Investigating NoV viability has only recently become a feasible research endeavor with the description of a human enteric cell system for cultivating the virus (Ettayebi et al. 2016).

The combined results demonstrating enhanced aggregate formation and NoV association with aggregates in estuarine and saline waters that contain higher TEP concentrations may explain why shellfish, particularly species that consumers prefer to eat raw, such as oysters, are such effective vehicles of human NoV transmission. Raising shellfish in freshwater is not a suitable solution due to bivalve physiology. Therefore, a potentially feasible virus risk management strategy that the shellfish industry could implement is the postharvest practice of depuration. Depuration mitigates the risk of exposing raw seafood consumers to human NoV through the expulsion of viruses and other pathogens from shellfish prior to distribution to the human food system, while also allowing for bivalve shellfish to be marketed as live, fresh product. This technique has been found to reduce the burden of NoVs from oyster tissue, however a recent review estimates that a wide range of 9 - 45.5 days of depuration would be

384 required to achieve a 1-log reduction of NoV genomes (McLeod et al. 2017). This time period is
385 not economically feasible, as depuration is generally conducted for relatively short periods of
386 time, usually ranging from 24-96 hours. Furthermore, this review identified studies of reported
387 NoV illness outbreaks due to the consumption of oysters that were subjected to depuration. Post-
388 depuration NoV concentrations were reported at $10^2$ – $10^3$ genome copies/gram oyster tissue,
389 which exceeds the infectious does, estimated to be as low as 18 viral particles (Teunis et al.
390 2008). While depuration is a regulated practice by the FDA in the United States, it is not a
391 requirement for shellfish growing areas classified as 'approved' or 'conditionally approved'
392 when indicator bacteria (fecal coliforms) meet the 'approved' threshold (FDA 2015). However,
393 these indicators are known to not adequately reflect the occurrence of pathogens (Harwood et al.
394 2005), and do not correlate with the presence of NoVs (Skraber et al. 2004). Meanwhile, the
395 number of NoV foodborne outbreaks associated with raw oysters have been on the rise since
396 2013 (Centers for Disease Control National Outbreak Reporting System (NORS)) highlighting
397 the need for feasible and cost effective post-harvest depuration practices to be more widely
398 adopted. Relaying of contaminated shellfish to waters with low TEP and/or aggregate
399 concentrations followed by depuration may offer a more appropriate virus risk management
400 strategy. Additional strategies should focus on employing watershed and/or shellfish production
401 management measures aimed at reducing the pollutant load of fecal contamination containing
402 harmful microorganisms from upstream waters to coastal waters. Current monitoring practices
403 for determining closure of shellfish beds for harvest often rely on rainfall parameters, levels of
404 fecal coliforms, or the presence of harmful algal blooms that can lead to an accumulation of
405 toxins in bivalves (Sekiguchi et al. 2001). However other oceanography parameters not often
406 evaluated, such as non-toxic phytoplankton abundance or type, can also greatly contribute to

TEP concentrations – which may in turn enhance pathogen entry into shellfish via organic aggregates. A holistic management and monitoring approach must therefore take into account the basic mechanisms that influence pathogen transport and fate, to accurately predict when, where, and how shellfish become contaminated with harmful microorganisms.

## 5 CONCLUSIONS

- This is the first study to conclusively demonstrate the association of NoVs with aquatic aggregates. Significant associations were measured between increasing salinity and TEP concentrations and increasing numbers of NoVs recovered in aggregates. The number of NoVs incorporated into aggregates appeared dependent upon the amount of aggregates present: higher salinity and higher TEP waters set the stage for enhanced aggregation formation, providing more flocs that effectively scavenge virus particles from the water columns.
- Optimal physicochemical characteristics of shellfish cultivation waters, which include estuarine-marine salinity and high TEP concentrations, increase the risk for NoVs to be ingested by bivalves, most notably those consumed raw by humans.
- Nearshore waters with high shellfish densities are likely to have higher levels of TEP in surrounding waters, further promoting enhanced aggregate formation in shellfish cultivation waters. These findings may explain why raw shellfish is often implicated as the primary cause of NoV-borne illness in humans.
- The first approach to protecting the human population and shellfish from norovirus exposure is to prevent contamination of surface waters with viral pathogens by improving sewage treatment facilities. When sewage or insufficiently treated wastewater reach

shellfish growing waters, insight into NoV transport and fate is imperative, and the results included here indicate conditions where NoVs are most likely to be detected in shellfish. This information can inform best management and monitoring strategies to aid shellfish growers and regulators in providing safe seafood to consumers.


## ACKNOWLEDGEMENTS

Sample collection was facilitated by Mr. Colin Krusor of UC Davis and Mr. David Dann, Ms. Katherine Hewett, Mr. Chris Kwan and Ms. Catherine Davis of the UC Davis Bodega Bay Marine Laboratory. Ms. Beatriz Aguilar (UC Davis) assisted laboratory experiments. Physiochemical data was measured by Timothy "Tad" Doane of the UC Davis Department of Land, Air and Water Resources. TEP measurements were performed by Julia Sweet of the University of California, Santa Barbara. Culture strains of murine norovirus were kindly provided by Jim Fahey and Haiyan Olekszak of Jackson Laboratories in Bar Harbor, ME.

## FUNDING SOURCES

This work was supported by the National Oceanic and Atmospheric Administration California Sea Grant Program (NOAA Grant #R/CONT-216). The statements, findings, conclusions and recommendations are those of the authors and do not necessarily reflect the views of California Sea Grant, NOAA or the U.S. Department of Commerce. This work was also supported by the Innovative Developmental Award from the University of California, Davis (UC Davis) Academic Federation Research Grant Program.

## WEB REFERENCES

(Centers for Disease Control National Outbreak Reporting System (NORS)) https://wwwn.cdc.gov/foodborneoutbreaks. Access date: January 16, 2018.

## REFERENCES

Alldredge, A.L., Passow, U. and Logan, B.E. (1993) The abundance and significance of a class of large, transparent organic particles in the ocean. Deep-Sea Research Part I-Oceanographic Research Papers 40(6), 1131-1140.

Alldredge, A.L. and Silver, M.W. (1988) Characteristics, dynamics and significance of marine snow. Progress in Oceanography 20(1), 41-82.

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., Uyttendaele, M., Stals, A., E, V.A.N.C., Dierick, K., Debevere, J. and Botteldoorn, N. (2009) Reported foodborne outbreaks due to noroviruses in Belgium: the link between food and patient investigations in an international context. Epidemiol Infect 137(3), 316-325.

Beckett, R. and Le, N.P. (1990) The Role Of Organic-Matter And Ionic Composition In Determining The Surface-Charge Of Suspended Particles In Natural-Waters. Colloids And Surfaces 44, 35-49.

Burd, A.B. and Jackson, G.A. (2009) Particle Aggregation. Annual Review of Marine Science 1, 65-90.

Cannon, J.L., Papafragkou, E., Park, G.W., Osborne, J., Jaykus, L.A. and Vinje, J. (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.

Chávez‐Villalba, J., López‐Tapia, M., Mazón‐Suástegui, J. and Robles‐Mungaray, M. (2005) Growth of the oyster Crassostrea corteziensis (Hertlein, 1951) in Sonora, Mexico. Aquaculture research 36(14), 1337-1344.

Dame, R.F. (2011) Ecology of marine bivalves: an ecosystem approach, CRC Press.

de Wit, M.A.S., Koopmans, M.P.G., Kortbeek, L.M., Wannet, W.J.B., Vinje, J., van Leusden, F., Bartelds, A.I.M. and van Duynhoven, Y. (2001) Sensor, a population-based cohort study on gastroenteritis in the Netherlands: Incidence and etiology. American Journal of Epidemiology 154(7), 666-674.

481 Dunphy, B.J., Wells, R.M. and Jeffs, A.G. (2006) Oxygen consumption and enzyme activity of
482 the subtidal flat oyster (Ostrea chilensis) and intertidal Pacific oyster (Crassostrea gigas):
483 responses to temperature and starvation. New Zealand Journal of Marine and Freshwater
484 Research 40(1), 149-158.

485 Edzwald, J.K., Upchurch, J.B. and Omelia, C.R. (1974) Coagulation In Estuaries. Environmental
486 Science & Technology 8(1), 58-63.

487 Ettayebi, K., Crawford, S.E., Murakami, K., Broughman, J.R., Karandikar, U., Tenge, V.R.,
488 Neill, F.H., Blutt, S.E., Zeng, X.L., Qu, L., Kou, B., Opekun, A.R., Burrin, D., Graham, D.Y.,
489 Ramani, S., Atmar, R.L. and Estes, M.K. (2016) Replication of human noroviruses in stem cell-
490 derived human enteroids. Science 353(6306), 1387-1393.

491 FDA (2015) National shellfish sanitation program (NSSP), Guide for the control of molluscan
492 shellfish. Revision 2015, 464.

493 Galtsoff, P.S. (1964) The America Oyster Crassostrea virginica Gmelin. . Fishery Bulletin of the
494 Fish and Wildlife Service 64, 15.

495 Gebhardt, A.C., Schoster, F., Gaye-Haake, B., Beeskow, B., Rachold, V., Unger, D. and Ittekkot,
496 V. (2005) The turbidity maximum zone of the Yenisei River (Siberia) and its impact on organic
497 and inorganic proxies. ESTUARINE COASTAL AND SHELF SCIENCE 65(1-2), 61-73.

498 Gregory, J. (2006) Particles in Water: Properties and Processes, Taylor & Francis Group, Boca
499 Raton.

500 Grossart, H.-P. and Simon, M. (1998) Bacterial colonization and microbial decomposition of
501 limnetic organic aggregates (lake snow). Aquatic Microbial Ecology 15(2), 127-140.

502 Hall, A.J., Vinjé, J., Lopman, B., Park, G.W., Yen, C., Gregoricus, N. and Parashar, U. (2011)
503 Updated norovirus outbreak management and disease prevention guidelines, US Department of
504 Health and Human Services, Centers for Disease Control and Prevention.

505 Hall, A.J., Wikswo, M.E., Pringle, K., Gould, L.H. and Parashar, U.D. (2014) Vital signs:
506 foodborne norovirus outbreaks—United States, 2009–2012. MMWR Morb Mortal Wkly Rep
507 63(22), 491-495.

508 Harwood, V.J., Levine, A.D., Scott, T.M., Chivukula, V., Lukasik, J., Farrah, S.R. and Rose, J.B.
509 (2005) Validity of the indicator organism paradigm for pathogen reduction in reclaimed water
510 and public health protection. Appl Environ Microbiol 71(6), 3163-3170.

511 Heilmayer, O., Digialleonardo, J., Qian, L. and Roesijadi, G. (2008) Stress tolerance of a
512 subtropical Crassostrea virginica population to the combined effects of temperature and salinity.
513 Estuarine, Coastal and Shelf Science 79(1), 179-185.

514 Heinonen, K.B., Ward, J.E. and Holohan, B.A. (2007) Production of transparent exopolymer
515 particles (TEP) by benthic suspension feeders in coastal systems. Journal of Experimental
516 Marine Biology and Ecology 341(2), 184-195.

517 Helm, M. (2005) Cultured Aquatic Species Information Programme–Crassostrea gigas. Cultured
518 Aquatic Species Fact Sheets. FAO Inland Water Resources and Aquaculture Service. Available:
519 Accessed.

520 Je, C.-H. and Chang, S. (2004) Simple approach to estimate flocculent settling velocity in a
521 dilute suspension. Environmental Geology 45(7), 1002-1009.

522 Karbassi, A.R. and Nadjafpour, S. (1996) Flocculation of dissolved Pb, Cu, Zn and Mn during
523 estuarine mixing of river water with the Caspian Sea. Environmental Pollution 93(3), 257-260.

524 Kaya, A., Oren, A.H. and Yukselen, Y. (2006) Settling of kaolinite in different aqueous
525 environment. Marine Georesources & Geotechnology 24(3), 203-218.

526 Kiørboe, T., Tang, K., Grossart, H.-P. and Ploug, H. (2003) Dynamics of Microbial
527 Communities on Marine Snow Aggregates: Colonization, Growth, Detachment, and Grazing
528 Mortality of Attached Bacteria. Applied and Environmental Microbiology 69(6), 3036-3047.

529 Le Guyader, F.S., Atmar, R.L. and Le Pendu, J. (2012) Transmission of viruses through
530 shellfish: when specific ligands come into play. Current Opinion in Virology 2(1), 103-110.

531 Lees, D. (2000) Viruses and bivalve shellfish. International Journal of Food Microbiology 59(1–
532 2), 81-116.

533 Li, B., Ward, J.E. and Holohan, B.A. (2008) Transparent exopolymer particles (TEP) from
534 marine suspension feeders enhance particle aggregation. MARINE ECOLOGY-PROGRESS
535 SERIES- 357, 67.

536 Lyons, M.M., Ward, J.E., Uhlinger, K.R., Gast, R.J. and Smolowitz, R. (2005) Lethal marine
537 snow: Pathogen of bivalve mollusc concealed in marine aggregates. Limnology and
538 Oceanography 50(6), 1983-1988.

539 McKee, J.W.A., Kavalieris, L., Brasch, D.J., Brown, M.T. and Melton, L.D. (1992) Alginate
540 content and composition ofMacrocystis pyrifera from New Zealand. Journal of Applied
541 Phycology 4(4), 357-369.

McLeod, C., Polo, D., Le Saux, J.-C. and Le Guyader, F.S. (2017) Depuration and Relaying: A Review on Potential Removal of Norovirus from Oysters. Comprehensive Reviews in Food Science and Food Safety 16(4), 692-706.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M. and Tauxe, R.V. (1999) Food-related illness and death in the United States. Emerg Infect Dis 5(5), 607-625.

Najdek, M., Blažina, M., Fuks, D., Ivančić, I. and Šilović, T. (2011) Intrusion of high-salinity water causes accumulation of transparent exopolymer particles (TEP) in the northern Adriatic Sea. Aquatic Microbial Ecology, 63(1), 69-74.

Passow, U. (2002) Transparent exopolymer particles (TEP) in aquatic environments. Progress in Oceanography 55(3–4), 287-333.

Passow, U. (2012) The abiotic formation of TEP under different ocean acidification scenarios. Marine Chemistry 128–129, 72-80.

Passow, U. and Alldredge, A.L. (1994) Distribution, size and bacterial colonization of transparent exoploymer particles (TEP) in the ocean. Marine Ecology Progress Series 113(1-2), 185-198.

Passow, U. and Alldredge, A.L. (1995a) Aggregation of a diatom bloom in a mesocosm: The role of transparent exopolymer particles (TEP). Deep Sea Research Part II: Topical Studies in Oceanography 42(1), 99-109.

Passow, U. and Alldredge, A.L. (1995b) A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). Limnology and Oceanography 40(7), 1326-1335.

Patel, M.M., Hall, A.J., Vinjé, J. and Parashar, U.D. (2009) Noroviruses: A comprehensive review. Journal of Clinical Virology 44(1), 1-8.

Ramaiah, N., Yoshikawa, T. and Furuya, K. (2001) Temporal variations in transparent exopolymer particles (TEP) associated with a diatom spring bloom in a subarctic ria in Japan. Marine Ecology Progress Series 212, 79-88.

Sekiguchi, K., Ogata, T., Kaga, S., Yoshida, M., Fukuyo, Y. and Kodama, M. (2001) Accumulation of paralytic shellfish toxins in the scallop Patinopecten yessoensis caused by the dinoflagellate Alexandrium catenella in Otsuchi Bay, Iwate Prefecture, northern Pacific coast of Japan. Fisheries Science 67(6), 1157-1162.

573 Shapiro, K., Krusor, C., Mazzillo, F.F., Conrad, P.A., Largier, J.L., Mazet, J.A. and Silver, M.W.
574 (2014) Aquatic polymers can drive pathogen transmission in coastal ecosystems. Proceedings of
575 the Royal Society of London B: Biological Sciences 281(1795), 20141287.

576 Shapiro, K., Miller, W.A., Silver, M.W., Odagiri, M., Largier, J.L., Conrad, P.A. and Mazet, J.A.
577 (2013) Research commentary: Association of zoonotic pathogens with fresh, estuarine, and
578 marine macroaggregates. Microb Ecol 65(4), 928-933.

579 Shapiro, K., Silver, M.W., Largier, J.L., Conrad, P.A. and Mazet, J.A. (2012) Association of
580 Toxoplasma gondii oocysts with fresh, estuarine, and marine macroaggregates. Limnology and
581 Oceanography 57(2), 449-456.

582 Sholkovitz, E.R. (1976) Flocculation of dissolved organic and inorganic matter during mixing of
583 river water and seawater. Geochimica Et Cosmochimica Acta 40(7), 831-845.

584 Sholkovitz, E.R. (1978) Flocculation Of Dissolved Fe, Mn, Al, Cu, Ni, Co And Cd During
585 Estuarine Mixing. Earth And Planetary Science Letters 41(1), 77-86.

586 Siegel, S. (1956) Nonparametric statistics for the behavioral sciences.

587 Silver, M.W., Shanks, A.L. and Trent, J.D. (1978) Marine Snow: Microplankton Habitat and
588 Source of Small-Scale Patchiness in Pelagic Populations. Science 201(4353), 371-373.

589 Simon, M., Grossart, H.-P., Schweitzer, B. and Ploug, H. (2002) Microbial ecology of organic
590 aggregates in aquatic ecosystems. Aquatic Microbial Ecology 28(2), 175-211.

591 Skraber, S., Gassilloud, B. and Gantzer, C. (2004) Comparison of coliforms and coliphages as
592 tools for assessment of viral contamination in river water. Applied and Environmental
593 Microbiology 70(6), 3644-3649.

594 Smith, D., Simon, M., Alldredge, A. and Azam, F. (1992) Intensive hydrolytic activity on marine
595 aggregates and implications for rapid particle dissolution. Nature 359, 139-141.

596 Sondi, I. and Pravdic, V. (1998) The colloid and surface chemistry of clays in natural waters.
597 Croatica Chemica Acta 71(4), 1061-1074.

598 Sosnovtsev, S.V., Belliot, G., Chang, K.-O., Prikhodko, V.G., Thackray, L.B., Wobus, C.E.,
599 Karst, S.M., Virgin, H.W. and Green, K.Y. (2006) Cleavage map and proteolytic processing of
600 the murine norovirus nonstructural polyprotein in infected cells. Journal of Virology 80(16),
601 7816-7831.

602 Tang, K.W., Dziallas, C. and Grossart, H.P. (2011) Zooplankton and aggregates as refuge for
603 aquatic bacteria: protection from UV, heat and ozone stresses used for water treatment. Environ
604 Microbiol 13(2), 378-390.

605 Teunis, P.F., Moe, C.L., Liu, P., Miller, S.E., Lindesmith, L., Baric, R.S., Le Pendu, J. and
606 Calderon, R.L. (2008) Norwalk virus: how infectious is it? J Med Virol 80(8), 1468-1476.

607 Thompson, W.R. (1935) On a Criterion for the Rejection of Observations and the Distribution of
608 the Ratio of Deviation to Sample Standard Deviation. 214-219.

609 Thornton, D.C.O. (2009) Spatiotemporal distribution of dissolved acid polysaccharides (dAPS)
610 in a tidal estuary. Limnolgy and Oceanography 54(5), 1449-1460.

611 Thornton, D.C.O., Fejes, E.M., Dimarco, S.F. and Clancy, K.M. (2007) Measurement of acid
612 polysaccharides in marine and freshwater samples using alcian blue. Limnology and
613 Oceanography: Methods 5(FEB), 73-87.

614 Wallace, R.K. (2001) Cultivating the eastern oyster, *Crassostrea virginica*, Southern Regional
615 Aquaculture Center.

616 Ward, J.E. and Kach, D.J. (2009) Marine aggregates facilitate ingestion of nanoparticles by
617 suspension-feeding bivalves. Marine Environmental Research 68(3), 137-142.

618 Wetz, M.S., Robbins, M.C. and Paerl, H.W. (2009) Transparent Exopolymer Particles (TEP) in a
619 River-Dominated Estuary: Spatial-Temporal Distributions and an Assessment of Controls upon
620 TEP Formation. Estuaries and Coasts 32(3), 447-455.

621 Wheeler, J.G., Sethi, D., Cowden, J.M., Wall, P.G., Rodrigues, L.C., Tompkins, D.S., Hudson,
622 M.J. and Roderick, P.J. (1999) Study of infectious intestinal disease in England: rates in the
623 community, presenting to general practice, and reported to national surveillance. The Infectious
624 Intestinal Disease Study Executive. Bmj 318(7190), 1046-1050.

625 Xavier, M., Passow, U., Migon, C., Burd, A.B. and Legendre, L. (2017) Transparent
626 Exopolymer Particles: Effects on carbon cycling in the ocean. Progress in Oceanography 151,
627 13-37.

628

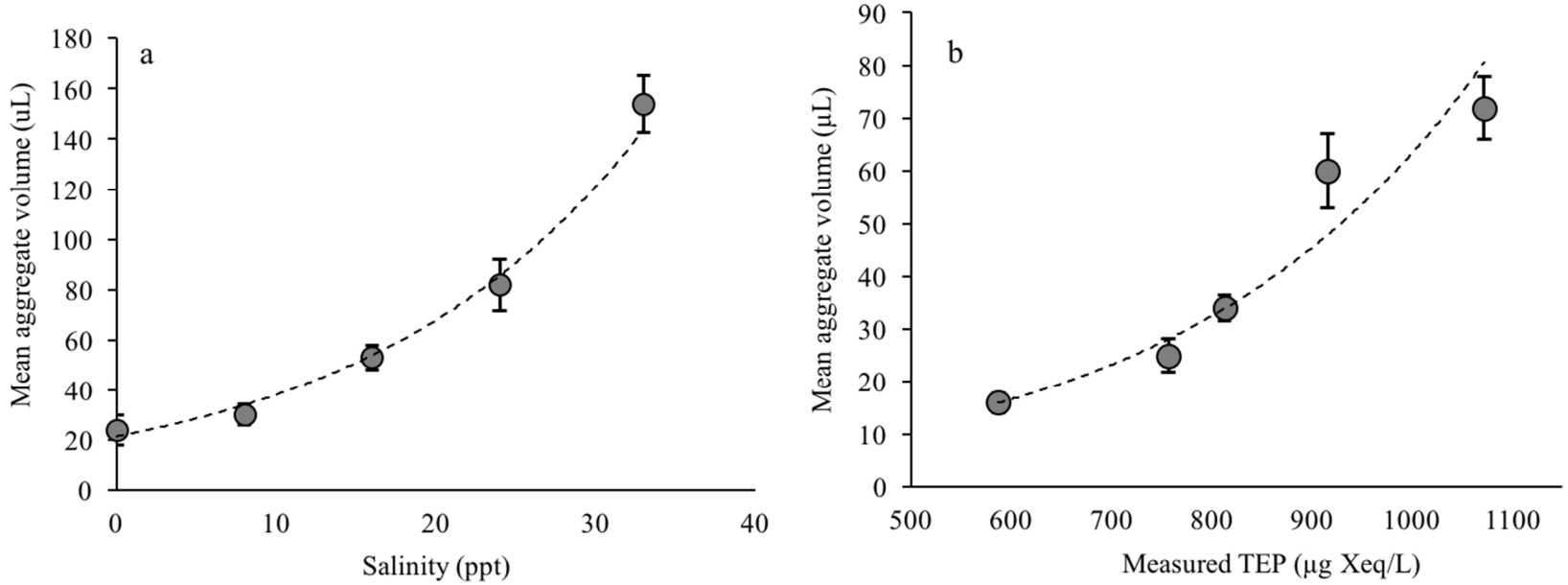


**Figure 1**. Total aggregate volume (uL) as a function of salinity (ppt) in Experiment 1 (a) or TEP concentration (µg Xeq/L) in Experiment 2 (b). Error bars indicate the standard error of the mean. Mean aggregate volume increased exponentially (dotted line) with an increase in salinity ($R^2 = 0.98$, $P \leq 0.01$) or TEP ($R^2 = 0.95$, $P \leq 0.01$).

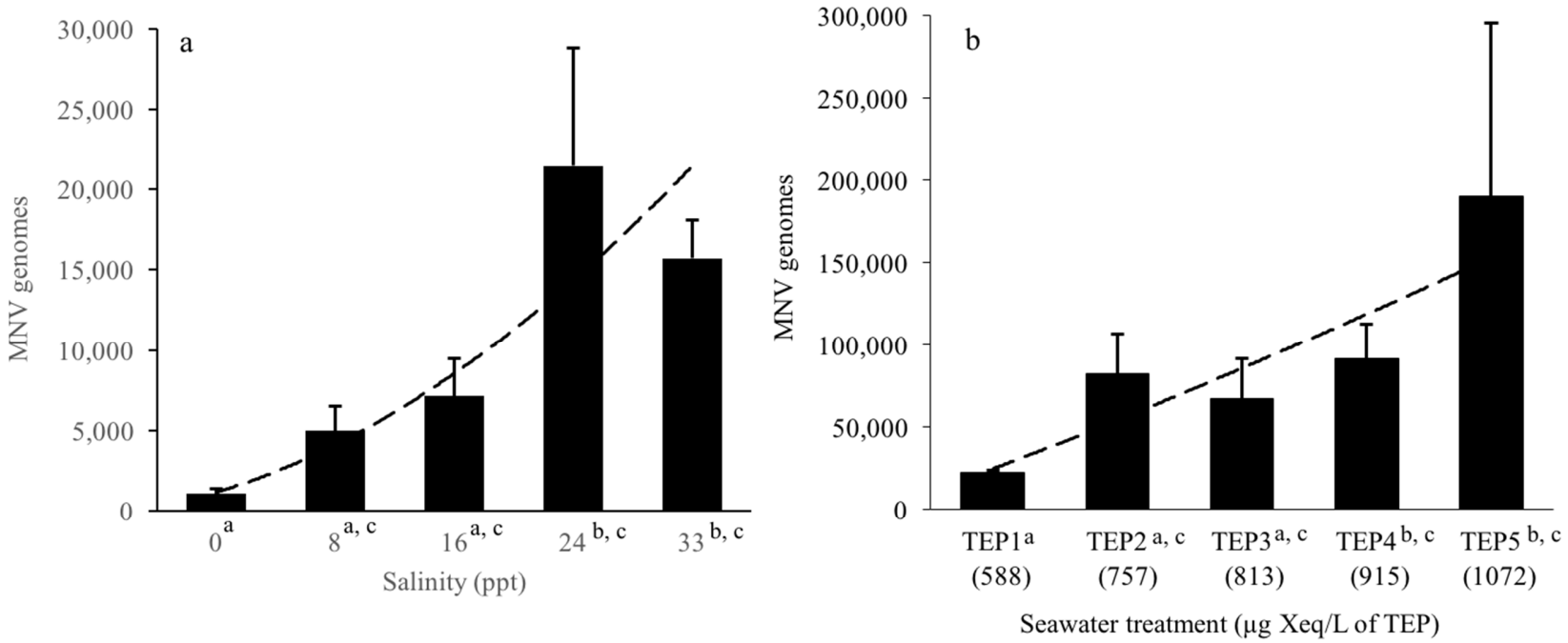


**Figure 2**. Mean number of MNV genomes recovered in aggregates of treatment samples with increasing salinity in Experiment 1 (a) or increasing concentrations of alginic acid (AA; source of TEP) in Experiment 2 (b). TEP concentrations are indicated in parentheses as µg Xanthan gum equivalence (µg Xeq/L) per liter. Error bars indicate the standard error of the mean. A significant difference in the total number of MNV genomes recovered in aggregates was present as salinity ($R^2 = 0.94$) or TEP ($R^2 = 0.85$) increased (dotted line, $P \leq 0.01$). Water types that do not share a superscript in common had significantly different proportions of organisms recovered from aggregates.

**Table 1. Physicochemical characteristics of water samples collected for aggregation experiments**

| Experiment | Matrix | Date Collected | Turbidity NTU[f] | TDS[a] mg/L | TSS[b] mg/L | TSS-N[c] mg/L | TSS-C[d] mg/L | DOC[e] mg/L |
|---|---|---|---|---|---|---|---|---|
| 1 | Freshwater | Aug 2013 | 0.427 | 220 | 0.1 | 0.02 | 0.15 | 2.7 |
| 2 | Seawater | Jan 2015 | 3.30 | 41 | 2.0 | 0.2 | 0.03 | NA[g] |

[a] Total dissolved solids

[b] Total suspended solids

[c] Total suspended solids – nitrogen component

[d] Total suspended solids – carbon component

[e] Dissolved organic carbon

[f] Nephelometric turbidity unit

[g] Not assessed