Risk Assessment of Norovirus Illness from Consumption of Raw Oysters in the United States and in Canada

Régis Pouillot,1 Mark Smith,2 Jane M. Van Doren,1,∗ Angela Catford,2 Jennifer Holtzman,2 Kevin R. Calci,3 Robyn Edwards,4 Gregory Goblick,3 Christopher Roberts,5 Jeffrey Stobo,5 John White,6 Jacquelyn Woods,3 Angelo DePaola, Jr.,3 Enrico Buenaventura,2 and William Burkhardt, III3

Human norovirus (NoV) is the leading cause of foodborne illness in the United States and Canada. Bivalve molluscan shellfish is one commodity commonly identified as being a vector of NoV. Bivalve molluscan shellfish are grown in waters that may be affected by contamination events, tend to bioaccumulate viruses, and are frequently eaten raw. In an effort to better assess the elements that contribute to potential risk of NoV infection and illness from consumption of bivalve molluscan shellfish, the U.S. Department of Health and Human Services/Food and Drug Administration (FDA), Health Canada (HC), the Canadian Food Inspection Agency (CFIA), and Environment and Climate Change Canada (ECCC) collaborated to conduct a quantitative risk assessment for NoV in bivalve molluscan shellfish, notably oysters. This study describes the model and scenarios developed and results obtained to assess the risk of NoV infection and illness from consumption of raw oysters harvested from a quasi-steady-state situation. Among the many factors that influence the risk of NoV illness for raw oyster consumers, the concentrations of NoV in the influent (raw, untreated) and effluent (treated) of wastewater treatment plants (WWTP) were identified to be the most important. Thus, mitigation and control strategies that limit the influence from human waste (WWTP outfalls) in oyster growing areas have a major influence on the risk of illness from consumption of those oysters.

KEY WORDS: Food safety; microbial risk assessment; Monte Carlo model; norovirus; shellfish

1. INTRODUCTION

Norovirus (NoV) is a leading cause of foodborne illnesses in the United States and Canada with an estimated 5,500,000 (90% credible interval: 3,200,000–8,300,000) and 1,050,000 (90% credible interval: 680,000–1,400,000) foodborne illnesses per year, respectively (Scallan et al., 2011; Thomas et al., 2013). NoV illness is rarely fatal; however, it can present heightened risks to vulnerable populations, including children, the elderly, and other immunocompromised individuals (Bok & Green, 2012; Hall et al., 2013). Bivalve molluscan shellfish (e.g.,
clams, oysters, and mussels), is one commodity commonly identified as being a vector of NoV. Indeed, the proportions of NoV illness caused by seafood is estimated to be between 9–34% of the total number of foodborne NoV illnesses in the United States (see Supporting Information Appendix for further discussion) (Batz, Hoffman, & Morris, 2011; Davidson, Ravel, Nguyen, Fazil, & Ruzante, 2011).

Human NoV only replicates in the human host and is spread by direct exposure to human excretions (e.g., feces and vomitus) or foods contaminated with these wastes. Bivalve molluscan shellfish grow in and are harvested from coastal estuaries where they can potentially be exposed to human fecal waste derived via discharges from wastewater treatment plants (WWTP), combined sewer overflows, or discharges from marine vessels. As filter feeders, bivalve molluscan shellfish can then accumulate viruses, which may be bound to particulate matter, if these hazards are in the surrounding waters. Virus concentrations in the shellfish can then be greater than those in the surrounding water due to this process of bioaccumulation (Burkhardt & Calci, 2000; Flannery, Keaveney, Rajko-Nenow, O’Flaherty, & Doré, 2012; Maalouf et al., 2011; Shieh, Baric, Woods, & Calci, 2003). Risk of illness from bivalve molluscan shellfish consumption can then be exacerbated if the shellfish, primarily oysters and clams, are consumed raw or lightly cooked.

In the United States and Canada, regulations, recommended measures, policies, and guidelines, to manage bivalve molluscan shellfish safety are collaboratively developed between State, Provincial, and Federal entities and, additionally, in the US, with industry partners. Policies and guidelines for the United States and Canada are found in the National Shellfish Sanitation Program (NSSP) (NSSP, 2017) and the Canadian Shellfish Sanitation Program (CSSP) (CSSP, 2018), respectively. CSSP regulatory requirements can be found in the Management of Contaminated Fishery Regulations (Government of Canada, 2019a) and the Safe Food for Canadians Regulations (Government of Canada, 2019b). The recommended measures to ensure sanitary conditions for shellfish cultivation and handling include sanitary surveys of the harvest area, which assess known and potential sources of pollution that can impact the sanitary quality of the water in which shellfish grow and are harvested, and water quality monitoring for total or fecal coliforms, which verify sanitary quality of the water. Interventions considered effective at controlling bacterial foodborne pathogens are less effective for controlling viruses in many settings (Hoelzer, Fansaselle, Pouillot, Van Doren, & S., 2013; Said, Perl, & Sears, 2008; Tung, Macinga, Arbogast, & Jaykus, 2013), but this has not been previously assessed quantitatively, in the context of existing bivalve molluscan shellfish programs.

In an effort to better understand the elements that contribute to potential risk of NoV infection and illness from consumption of bivalve molluscan shellfish, the U.S. Department of Health and Human Services/Food and Drug Administration (FDA), Health Canada (HC), the Canadian Food Inspection Agency (CFIA), and Environment and Climate Change Canada (ECCC) collaborated to conduct this quantitative risk assessment addressing the potential risk to consumers posed by NoV in bivalve molluscan shellfish, using the example of oysters. This work provides a framework for describing the complex interaction of the factors that impact the potential risk and estimating said risk across a range of situations (i.e., season, region, water temperature, dilution, type of WWTP) expressed through different perspectives (i.e., growing area water contamination, shellfish contamination, risk of human infection, and risk of illness per serving and per annum). Additionally, the risk assessment model allows exploration of the effectiveness of different intervention strategies aimed at reducing this potential risk.

Male-specific coliphage (MSC) is a well-studied marker of viral contamination, and is also included in the risk assessment model, for two reasons. First, MSC has long served as a surrogate for understanding the behavior of a virus resulting from steps in oyster production since actual data for NoV are sparse. Second, MSC is a candidate for future water and shellfish surveillance and is being considered as the basis for setting performance objectives, for example for contamination in shellfish growing waters.

The concentration of viruses in estuaries observed in usual situations, that is when the contamination flux is approximately constant and virus concentration in the estuary is modulated by typical tidal and solar cycles, can be thought of as a kind of quasi-steady state. This quasi-steady state can be disrupted by short-term exceptional events, defined here as short duration periods (typically an hour to a few days) when higher than usual amounts of human waste contamination, untreated or partially treated, enter the estuary distally to or directly in the shellfish harvest area. Examples of infrequent exceptional events include a WWTP malfunction, heavy rainfall resulting in incomplete wastewater treatment, and a
nonpoint source discharge from a marine vessel in contravention of legal prohibitions. This first study will study and evaluate the risk in the quasi-steady state. A second study will describe the use of the quantitative assessment model to investigate risk of NoV illness during exceptional events and potential mitigations and controls, including delayed harvesting, relay, depuration, and postharvest processing.

This study introduces and describes a quantitative assessment of the risk of NoV illness linked to consumption of raw oysters harvested from a quasi-steady-state situation. The focus of this risk assessment is to estimate how influencing factors interact and to characterize the relative order of magnitude of the impact of each parameter.

2. MATERIALS AND METHODS

A Monte-Carlo model to simulate the concentration of infectious and noninfectious NoV Genotype I (NoV GI), NoV Genotype II (NoV GII), and the concentration of MSC along the oyster product pathway was developed. MSC is a cultivable virus that is commonly used as a NoV surrogate because of its similar size and typical presence in human sewage at easily measured concentrations. Some references used and discussed in this study referred to NoV and MSC by other names, such as Norwalk virus and MSB, respectively. For clarity, we will simply use NoV and MSC in all cases.

The product pathway steps included: raw sewage entering a WWTP, the WWTP output, the growing area water, the oysters at harvest, and distribution to consumption, possibly after implementation of mitigation strategies including relay and depuration which may occur in the United States and in Canada if the growing area water does not meet approved NSSP/CSSP water quality standards (exposure assessment). A dose–response model (hazard characterization) was then applied to predict the average risk of NoV infection and illness following the ingestion of one meal of oysters (risk characterization). The Monte-Carlo simulation integrates variabilities along this chain, where each iteration represents one meal of oysters consumed from one estuary at random. The model considers region and season. Regions for the United States include Gulf of Mexico (i.e., Gulf), South Atlantic, Middle Atlantic, New England, and U.S. Pacific; and for Canada include North Gulf of St. Lawrence (i.e., St. Lawrence), Canadian Maritimes, and Canadian Pacific. With respect to seasons, winter, spring, summer, and fall are defined in three-month intervals, winter being defined as December, January, and February, and the remainder following as expected. Oyster meal origins are sampled proportionally to landings for that region and month. The oyster landing volumes by origin and a summary of the regional and seasonal data that are used in the model are provided in the Supporting Information Appendix.

Table I and Fig. 1 together describe the process steps considered in the model development, as well as factors thought to impact the overall risk of illness following a meal of oysters. Until recently (Ettayebi et al., 2016), NoV was not culturable, so it was not possible to measure the number of potentially infectious particles in environmental or oyster samples. As such, studies on NoV were developed either (i) using RT-qPCR or any method able to enumerate the number of genome copies (gc), including infectious particles and noninfectious particles with measurable RNA or; (ii) using cultivable virus, as surrogates, such as MSC or other viruses. For data originating from surrogates, the assumption used in this model is that the impact of the physical or chemical treatment on the surrogate is similar to the impact on NoV.

2.1. Raw Sewage and WWTP Effects

Pouillot et al. (2015) quantified WWTP influent concentration and WWTP log-reduction in this concentration from a meta-analysis of the joint concentration of NoV GI, NoV GII, and MSC in published data sets and unpublished raw data that U.S. and Canadian federal agencies provided. That analysis evaluated the concentration and seasonal variation of the viruses in WWTP influent, the WWTP efficiency in reducing the virus concentration, the correlation between influent concentration and WWTP efficiency, the WWTP to WWTP variation, and parameters that influence virus concentrations in WWTP effluent (type of WWTP and type of disinfection). The mean of the log$_{10}$ concentration of infectious NoV GI (log$_{10}$ gc/L), NoV GII (log$_{10}$ gc/L), and MSC (log$_{10}$ plaque forming units (pfu)/L) for the WWTP influent in a given month is predicted using the mean of the log$_{10}$ concentration of NoV GI, NoV GII, and MSC, with the addition of a month effect (log$_{10}$ gc/L or log$_{10}$ pfu/L) and the addition of the log$_{10}$ of the proportion of infectious particles (for NoV only).

The mean influent concentration and log$_{10}$ reductions are evaluated for NoV GI, NoV GII, and MSC using a multivariate normal distribution with
Fig 1. Risk assessment model pathway. Numbers in circles indicate the factors considered for baseline (or nominal) operations. (1) influent raw sewage from a community may contain NoV at a steady-state range that varies by month; (2) wastewater treatment can reduce the level of microbes, including viral pathogens; (3) treated effluent is released into an estuary and becomes diluted; (4) the action of tide cycles contributes to reduction of microbe levels; (5)(a) sunlight and (b) water temperature impact the persistence or decay of infectious virus particles in the water column; (6) oysters filter feed and may take up virus in the water column prior to harvest; (7) harvested oysters are cleaned, packaged, and labelled at a commercial facility; (8) oysters are distributed for sale and prepared either raw or cooked before serving, either in restaurants or domestic kitchens (only raw oyster consumption is considered in this study). In addition to the nominal structure, exceptional events (EE) could be considered, namely wastewater treatment plant failures or bypasses (EE1), severe weather events (EE2), and overboard discharges of waste from marine vessels (EE3). Different mitigation controls (M) could also be considered, including conditional management planning (M1), relay (M2), depuration (M3), and cooking (M4).

Parameters

\[
M = \begin{pmatrix}
1.48 \\
3.92 \\
6.23 \\
-1.66 \\
-1.97 \\
-2.79
\end{pmatrix}
\]

for mean influent concentration of NoV GI (log\textsubscript{10} gc/L; Credible Interval 95%: [0.412; 2.412]), NoV GII (log\textsubscript{10} gc/L; CI 95%: [3.504; 4.348]), MSC (log\textsubscript{10} pfu/L; CI 95%: [6.056; 6.402]), and mean log\textsubscript{10} reduction (LR) for NoV GI (log\textsubscript{10} gc/L; CI 95%: [−3.28; −0.195]), NoV GII (log\textsubscript{10} gc/L; CI 95%: [−2.931; −1.017]), and MSC (log\textsubscript{10} pfu/L; CI 95%: [−3.458; −2.147]), respectively, with a variance-covariance matrix V as provided in Table II.

The month effect, which is the variation in viral mean concentrations around the annual mean concentration, is evaluated using values from Table III. The WWTP effect and disinfection is evaluated using values from Table IV.
Table I. Considered Steps, Major Parameters, and Factors that May Impact the risk of NoV Illness Following the Ingestion of a Meal of Raw Oysters

| Step                                      | Major Parameter                                      | Influencing Factor included in Modeling this Step |
|-------------------------------------------|------------------------------------------------------|--------------------------------------------------|
| Wastewater treatment plant (WWTP) influent| Mean concentration of infectious and noninfectious NoV GI, NoV GII, and concentration of MSC in influent | MonthProportion of infectious NoV                 |
| WWTP treatment                            | Treatment efficiency                                  | WWTP type (mechanical, lagoon, none)              |
| Harvest Water                              | Virus inactivation                                    | Water temperature (function: region, day)         |
|                                           |                                                      | Light energy (function: region, month)            |
|                                           |                                                      | Time to reach the estuary (function: tide type)   |
|                                           |                                                      | Dilution at mean tide (function: estuary/Tide (function: region, hour) |
| Oysters at harvest                         | Bioaccumulation                                       | Concentration of NoV GI, NoV GII, and MSC in the water |
|                                           |                                                      | Season (high bioaccumulation season: October–February; low bioaccumulation season: March–September) |
|                                           |                                                      | Water temperature (function: region, day)         |
| Consumption                                | Meal size                                             | Number of oysters eaten                           |

Table II. Variance-Covariance Matrix [95% Credible Interval] of the Parameters of the Multinormal Distribution for Influent and Mean Log Reduction Simulation (from Pouillot et al., 2015)

|                        | Influent NoV GI (log$_{10}$ gc/L) | Influent NoV GII (log$_{10}$ gc/L) | Influent MSC (log$_{10}$ pfu/L) | Log Reduction NoV GI (log$_{10}$ gc/L) | Log Reduction NoV GII (log$_{10}$ gc/L) | Log Reduction MSC (log$_{10}$ pfu/L) |
|------------------------|-----------------------------------|------------------------------------|---------------------------------|----------------------------------------|----------------------------------------|-------------------------------------|
| Influent NoV GI (log$_{10}$ gc/L) | 4.899 [2.13; 9.926]               | --                                 | --                              | --                                     | --                                     | --                                  |
| Influent NoV GII (log$_{10}$ gc/L) | 1.997 [0.659; 3.815]              | 1.682 [1.038; 2.626]               | 0.304 [0.051; 0.593]            | 0.347 [0.226; 0.518]                   | 1.797 [0.628; 4.146]                   | --                                  |
| Influent MSC (log$_{10}$ pfu/L)     | 0.386 [−0.215; 1.061]             | 0.034 [−0.128; 0.513]              | 0.347 [0.226; 0.518]            | 1.797 [0.628; 4.146]                   | --                                     | --                                  |
| Log Reduction NoV GI (log$_{10}$ gc/L) | −0.199 [−2.858; 2.283]           | −0.294 [−1.728; 1.164]             | −0.041 [−0.461; 0.396]          | 1.797 [0.628; 4.146]                   | --                                     | --                                  |
| Log Reduction NoV GII (log$_{10}$ gc/L) | −1.051 [−2.865; 0.533]           | −1.189 [−2.3; −0.375]              | −0.252 [−0.67; 0.129]           | 1.064 [−0.415; 2.834]                  | 3.143 [1.642; 5.571]                   | --                                  |
| Log Reduction MSC (log$_{10}$ pfu/L) | −0.861 [−2.764; 0.842]           | −0.983 [−1.922; −0.161]            | −0.224 [−0.573; 0.075]          | 0.997 [−0.401; 2.476]                  | 2.311 [1.242; 3.8]                    | 2.613 [1.619; 4.085]                |
The model simulated, in parallel, the concentration of total (infectious and noninfectious) and infectious NoV particles. At the WWTP influent step, it was assumed that 1/40 NoV particles (Burkhardt, Blackstone, Skilling, & Smith, 2002; Guix et al., 2020; Jansen, Newbold, & Lemon, 1985; Rotbart, 1990; Teunis et al., 2008; Thebault, Teunis, Le Pendu, Le Guyader, & Denis, 2013), when measured as gc, were infectious in the WWTP influent. A sensitivity analysis was developed to evaluate the impact of this parameter on the risk at consumption. All MSC, being measured in pfu, are considered “infectious.” The estimated log reduction in gc from NoV GI and NoV GII was used to evaluate the actual removal of virus particles. The estimated log reduction (LR) for MSC, if greater than the log reduction in gc, was used as a surrogate of the additional reduction in infectious particles, that is:

log10 [NoV GI\textsubscript{infectious, effluent}] = log10 [NoV GI\textsubscript{total, influent}] + max (LR NoV GI, LR MSC)

and, similarly, for NoV GII. Note, then, that along the pathway, the proportion of 1/40 infectious particles will vary according to the differential losses of particles and losses of infectivity.

### 2.2. NoV Concentration in the Estuary and Harvest Water

As the water current carries NoV and MSC from the pollution source to the harvest area, the concentration of total particles is further reduced from that in WWTP effluent through mixing with receiving waters (dilution), and tidal flushing; and the number of infectious particles is reduced further from inactivation within the water column arising from time, temperature, and sunlight.

---

**Table III.** Month Effect (i.e., Differences in Viral Monthly Mean Concentration and the Annual Mean Concentration) [95% Credible Interval] for the NoV GI (log10 gc/L), the NoV GII (log10 gc/L), and the MSC (log10 pfu/L) Concentration in Influent (from Pouillot et al., 2015)

| Month     | NoV GI       | NoV GII      | MSC          | Log Reduction MSC |
|-----------|--------------|--------------|--------------|-------------------|
| January   | 0.206 [-0.364; 0.875] | 0.767 [0.51; 1.029] | -0.017 [-0.073; 0.036] | 0.017 [-0.156; 0.211] |
| February  | 0.771 [-0.028; 1.491] | 1.249 [0.97; 1.527] | -0.081 [-0.187; 0.021] | 0.415 [0.151; 0.683] |
| March     | 1.076 [0.454; 1.664] | 0.954 [0.703; 1.208] | -0.157 [-0.242; -0.066] | 0.362 [0.177; 0.549] |
| April     | 0.912 [0.161; 1.67] | 0.36 [0.068; 0.662] | -0.246 [-0.348; -0.14] | 0.347 [0.121; 0.571] |
| May       | 0.342 [-0.254; 0.985] | -0.022 [-0.29; 0.247] | -0.292 [-0.391; -0.186] | 0.694 [0.471; 0.918] |
| June      | -0.104 [-0.593; 0.452] | -0.179 [-0.355; 0.017] | -0.194 [-0.336; -0.051] | 0.717 [0.423; 1.017] |
| July      | -0.068 [-0.654; 0.128] | -0.275 [-0.485; -0.087] | 0.04 [-0.066; 0.143] | -0.027 [-0.313; 0.244] |
| August    | -0.145 [-1.101; 0.631] | -0.395 [-0.696; -0.086] | 0.253 [0.124; 0.38] | -0.747 [-1.07; -0.418] |
| September | -0.582 [-1.222; 0.229] | -0.59 [-0.811; -0.363] | 0.306 [0.209; 0.4] | -0.684 [-0.913; -0.452] |
| October   | -1.003 [-1.696; -0.345] | -0.85 [-1.093; -0.624] | 0.224 [0.119; 0.329] | -0.338 [-0.59; -0.083] |
| November  | -0.957 [-1.603; -0.255] | -0.834 [-1.082; -0.581] | 0.119 [0.014; 0.22] | -0.364 [-0.558; -0.175] |
| December  | -0.411 [-1.099; 0.252] | -0.187 [-0.452; 0.067] | 0.041 [-0.068; 0.168] | -0.395 [-0.634; -0.171] |

*The month effects for the NoV log reduction were globally not significant (Pouillot et al., 2015).*

**Table IV.** Effect of the WWTP Characteristics on the NoV (log10 gc/L) and the MSC (log10 pfu/L) log Reduction [95% Credible Interval] (from Pouillot et al., 2015)

| Effect                  | NoV       | MSC       |
|-------------------------|-----------|-----------|
| Type of WWTP            |           |           |
| Mechanical              | -0.498 [-1.28; 0.175] | 0.361 [-0.247; 0.978] |
| Lagoon                  | 0.498 [0.175; 1.28]   | -0.361 [-0.978; -0.27] |
| Disinfection            |           |           |
| None (reference)        | 0         | 0         |
| Chlorine                | -0.237 [-1.106; 0.61] | -0.421 [-0.703; -0.139] |
| UV                      | -0.825 [-1.89; 0.245] | -1.88 [-2.208; -1.565] |

The model simulated, in parallel, the concentration of total (infectious and noninfectious) and infectious NoV particles. At the WWTP influent step, it was assumed that 1/40 NoV particles (Burkhardt, Blackstone, Skilling, & Smith, 2002; Guix et al., 2020; Jansen, Newbold, & Lemon, 1985; Rotbart, 1990; Teunis et al., 2008; Thebault, Teunis, Le Pendu, Le Guyader, & Denis, 2013), when measured as gc, were infectious in the WWTP influent. A sensitivity analysis was developed to evaluate the impact of this parameter on the risk at consumption. All MSC, being measured in pfu, are considered “infectious.” The estimated log reduction in gc from NoV GI and NoV GII was used to evaluate the actual removal of virus particles. The estimated log reduction (LR) for MSC, if greater than the log reduction in gc, was used as a surrogate of the additional reduction in infectious particles, that is:

\[
\log_{10} \left[ \text{NoV GI}_{\text{infectious, effluent}} \right] = \log_{10} \left[ \text{NoV GI}_{\text{total, influent}} \right] + \text{LR NoV GI}
\]

\[
\log_{10} \left[ \text{NoV GII}_{\text{infectious, effluent}} \right] = \log_{10} \left[ \text{NoV GII}_{\text{total, influent}} \right] + \text{LR NoV GII}
\]

and, similarly, for NoV GII. Note, then, that along the pathway, the proportion of 1/40 infectious particles will vary according to the differential losses of particles and losses of infectivity.
2.2.1. Dilution

A simple dilution model was used to quantify how virus concentrations decrease by dilution from the WWTP discharge pipe to the harvest water. We define the dilution $D$ as the ratio of the virus concentration associated with human fecal waste at the source to the concentration of virus at a location downstream (harvest area).

$$D = \frac{[N]_{s}}{[N]_{h}} = \frac{N_{s} W_{h}}{N_{h} W_{s}} > 1$$

Here, the concentration of particles is denoted $[N]$, $N$ is the number of particles, and $W$ is the volume of water. These variables are indexed as $s$ for the source or $h$ for the harvest site. The dilution factor ($<1$) is defined as $D_{f} = 1/D$. The dilution is independent of the measured contaminant (e.g., virus, bacteria, dispersible compound, etc.) but influenced by changes in either the WWTP, tidal, and/or freshwater flow and can be measured experimentally, for example using dye studies (Campos, Goblick, Lee, Wittamore, & Lees, 2017; Goblick, Anbarchian, Woods, Burkhart, & Calci, 2011; Goblick, Ao, Anbarchian, & Calci, 2017).

Rhodamine dye release studies performed in various estuaries (Anbarchian, Ao, Calci, & Goblick, 2014; Ao & Goblick, 2016; Campos et al., 2017; Goblick et al., 2011; Goblick, Anbarchian, & Calci, 2013) showed a linear relationship between the log dilution range (difference of the log of the high dilution and the log of the low dilution) and the tidal range (see Supporting Information Appendix). A model simulating this behavior was developed. Tides data were obtained for U.S. reference stations using the rtide R package (Thorley, Fleishman, & Miller, 2017) and obtained from Fisheries and Oceans Canada reference stations for Canada (see Supporting Information Appendix). The estimated tides $T_{r,h}$ (m) for region $r$ and hour $h$ were standardized by removing their estimated means for the considered region: $T_{r,h}' = T_{r,h} - T_{r}$. Using “New England” as a reference without loss of generality, the tides data were standardized by dividing the data by the inverse of the minimum standardized data for New England $T_{r,h}' = \frac{T_{r,h} - T_{r}}{T_{min} (T_{r,h})} = \frac{T_{r,h} - T_{r}}{T_{r} + 0.81}$. At that step, the minimal value $T_{r, min}$ for New England is, by construction, $-1$. For other regions, it is scaled to New England, for example: the minimum value is $-0.43$ for the Gulf; $-2.4$ for U.S. Pacific and $-5.03$ for Canadian Maritimes. For a given dilution of mean tide $D_{fm} = 1.000$ (dilution factor of 1/1,000), the minimum dilution (maximum dilution factor) for New England would be set to $D_{fl} = \alpha \times D_{fm}$. $\alpha < 1$ being a scaling parameter, to be estimated from observed data. The dilution factor at standardized tide $T_{r,h}'$ is then evaluated as

$$\log_{10} (D_{f,r,h}) = \log_{10} (D_{fm}) + T_{r,h}' (\log_{10} (D_{fm}) - \log_{10} (D_{fl}))$$

That simplifies to

$$\log_{10} (D_{f,r,h}) = \log_{10} (D_{fm}) - T_{r,h}' (\log_{10} (\alpha))$$

The properties of this model are that (i) for New England, the dilution factor varies with a minimum dilution factor of $D_{fl}$ and a dilution factor at mean tide ($T_{r,h}' = 0$) of $D_{fm} = D_{fl} / \alpha$, and (ii) for other regions, the dilution factor varies with a dilution factor at mean tide of ($T_{r,h}' = 0$) of $D_{fm}$. The $\log_{10}$ of the minimum dilution factor will be $\log_{10} (D_{fm}) - T_{r, min} (\log_{10} (\alpha))$. The amplitude of the log dilution will be linearly linked to the amplitude of the tides, as observed in the data; (iii) the model is scalable from a dilution at mean tide of 1.000 to any dilution at mean tide, variable from estuary to estuary. Using dye dilution data (Supporting Information Appendix) established in Yarmouth (ME), Blaine (WA), Mobile (AL), and Chichester (UK), a parameter $\alpha$ of 0.4 was derived.

The main inputs for this component of the model thus include the tides, which are based on the choice of region and month (informed by hourly tide data), and the dilution at mean tide, which is assumed to vary, from nominal harvest, as Uniform (1,000; 100,000), that is in the order of magnitude of the dilution at mean tide expected in North America. Dilution is identified as a decrease in the concentration of total viral particles.

2.2.2. Tidal Flushing

Tidal flushing impacts the proportion of virus particles that are still present at a given site after one tide period. If little tidal flushing occurs, a relatively small amount of the contaminant is removed with the tide and the contaminant persists in the estuary. For the sake of simplification, the effect of the tidal flushing on virus concentration is captured in the model through a single parameter, $\tau$, between 0 and 1, representing the proportion of virus particles that are still present at a given site after one tide period $T$. If $\tau$ is high, little tidal flushing occurs. We assume that particles do not travel out of and back into the estuary, but are slowly flushed away,
following a simple exponential decay model \([N]_t = [N]_0 \exp(-\lambda t)\) where \([N]_0\) is the concentration of particles at time \(t\), given that the initial concentration was \([N]_0 = 0\), and \(\lambda\) is the exponential decay constant. After a tide period, \(T\), we have \([N]_t = \tau [N]_{t-\tau} = [N]_0 \exp(-\lambda T)\) leading to \(\lambda = -\ln(\tau) / T\). Discretizing the model by periods of one hour, we have \([N]_{t+1} = [N]_t \tau^{1/T}\). A baseline \(\tau\) value of 0.25 was chosen as a typical value and we tested a range of values to evaluate the impact of this parameter on the main outputs.

In a dynamic model that combines dilution and tidal flushing, the concentration of particles at the harvest site is expressed as \([N]_{h,t+1} = \tau^{1/T} [N]_{h,t} (\frac{D_{t+1}}{D_t}) + [N]_{t+1} D_{t+1}\). Here, \(\tau^{1/T} [N]_{h,t} (\frac{D_{t+1}}{D_t})\) is the concentration of virus particles in the harvest area remaining after flushing at time \(t + 1\), corrected for the change in dilution with time, and \([N]_{t+1} D_{t+1}\) is the additional contribution of virus particles from the source at \(t + 1\). In this recursive formula, the concentration of virus at a given time point \((t + 1)\) depends on the state of the system, specifically the concentration and dilution factor at the time point immediately prior \((t)\).

2.3. Viral Inactivation

NoV and MSC inactivation is modeled from data inferred from Burkhardt, Calci, Watkins, Rippey, and Chirtel (2000) in which the impact of time, water temperature, and accumulated sunlight energy on MSC concentration were measured. In our model, inactivation of the viruses was considered allowing for variations per region (different water temperature and sunlight energy), month, and hour. This inactivation is considered as a decrease in the proportion of infectious particles.

Virus inactivation by exposure to sunlight and temperature within an estuary is considered to proceed as a first-order rate process. Log-linear models considering time, temperature, sunlight energy, and their interactions were tested using raw data from Burkhardt et al. (2000). We tested the more flexible Weibull model and various potential interactions of the impact of time, temperature, and energy, as fixed or random effects. Based on the principle of parsimony, the decrease in the viable virus concentration in water over time was best represented by the log-linear model:

\[
\log ([\text{NoV Gx}_{\text{infective}, t+1}]) = \log ([\text{NoV Gx}_{\text{infective}, t}]) + \beta_1 (t_{t+1} - t_t) + \beta_2 T_{t+1} (t_{t+1} - t_t) + \beta_3 E_{t+1}
\]

where \([\text{NoV Gx}_{\text{infective}, t+1}]\) is the concentration of infectious NoV \(\text{Gx}\) (GI or GII) at time \(t\), \(T_{t+1} (\circ\text{F})\) is the temperature measured at time \(t + 1\), and \(E_{t+1} (\text{J/cm}^2)\) is the accumulated sunlight energy the viruses were exposed to during the interval \(\Delta t\). \(\beta_1, \beta_2,\) and \(\beta_3\) are parameters, to be estimated from Burkhardt et al. (2000) data (Table V). Time \(\Delta t\) and the interaction of time and temperature \((\beta_2 T \Delta t)\) are fixed effects that accelerate the inactivation of infectious virus concentration. The sunlight energy term \(\beta_3 E\) is a random effect, where \(\beta_3 \sim \text{Normal}(\mu_{\beta_3}, \sigma_{\beta_3}^2)\). Conceptually, this parameter captures how the rate of decrease changes among harvest occasions capturing all of NoV, water, and tide at the same temperature and sunlight energy.

Daily water temperatures were obtained for the United States and Canada from the National Oceanic and Atmospheric Administration (NOAA) from the same reference station (or the closest one) as the one used for tides (National Centers for Environmental Information/National Oceanic and Atmospheric Administration, 2019).

In order to evaluate \(E\), the sunlight energy \((\text{J/cm}^2)\) received in the estuary according to the region and the month, we evaluated solar irradiances for each different region using the SMARTS radiative transfer model (see Supporting Information Appendix) (Gueymard, 2001, 2005).

2.4. Oyster Uptake and Elimination of NoV in Harvest Waters

2.4.1. Bioaccumulation

Filter feeding oysters take up viruses and, in short order, the virus concentration in the oyster can exceed the virus concentration in water through the process of bioaccumulation (Burkhardt & Calci, 2000). Virus concentrations in oysters decline more slowly when virus concentrations in water decline, even under optimal conditions (Burkhardt, Rippey, & Watkins, 1992). We used published literature in our risk assessment model to quantify how the virus concentration in oysters compares to the virus concentration in water, thus defining an overall or net bioaccumulation factor (BAF). The BAF is defined as the ratio of the concentration of virus in the animal to the concentration in the water. It is known that this factor changes with feeding activity, which in turn changes with season and temperature (Burkhardt & Calci, 2000). The BAF is highest at an optimum temperature, and then decreases at higher or lower...
Table V. Parameter of Virus Inactivation by Exposure to Sunlight Energy and Temperature as Estimated from Burkhardt et al. (2000)’s Raw Data

| Variable                        | Parameter | Estimate [95% Wald-type confidence interval] |
|---------------------------------|-----------|-------------------------------------------|
| Time (h)                        | $\beta_1$ | $-0.005028 [-0.06046; 0.05024]$           |
| Time $\times$ Temperature(h$\times$°F) | $\beta_2$ | $-0.0009300 [-0.001745; -0.0001142]$      |
| Sunlight Energy (J/cm$^2$)      | $\mu_\beta$ | $-0.01257 [-0.01905; -0.009045]$          |
|                                 | $\sigma_\beta$ | $0.00354 [.00172; .007289]$              |

Fig 2. Mean bioaccumulation factor as a function of the season and the temperature. Data points are values observed in oysters by Burkhardt and Calci (2000). Fit lines ($\alpha\nu(T)$) to the observed data also include cutoffs (i.e., minimum and a maximum temperature of bioaccumulation set at 4.8 °C and 32 °C) to account for physiological limits.

temperatures (Burkhardt et al., 1992; Comeau, 2014; Comeau, Mayrand, & Mallet, 2012; Fiandrino, Martin, Got, Bonnefont, & Troussellier, 2003). There exist minimum and maximum temperatures at which oysters will not feed or accumulate particles, at which the BAF is defined as 1 (equal concentration in oyster and in water). Burkhardt and Calci (2000)’s raw data provided observations on MSC concentration in several oyster pools collected on each of several sampling occasions in a high oyster accumulation and low oyster accumulation season (hereinafter referred as “High bioaccumulation season” and “Low bioaccumulation season”), and the sampling occasions’ mean of MSC in water concentrations taken within one week of harvest (Fig. 2). Note that for this model, minimal and a maximal temperature of bioaccumulation were set at 4.8 °C and 32 °C, to account for the physiological limitations of the oyster species represented (Burkhardt & Calci, 2000; Glastoff, 1926).

In Burkhardt and Calci (2000) net BAF data, functions of temperature that scale the mean over oysters, have roughly the exponential form (Fig. 2). We used the following model for single oyster, single harvest occasion BAF

$$B(T | T_{\text{min}} < T < T_{\text{max}}) \sim \text{Gamma}(\alpha, \nu(T)),$$

with

$$\nu(T) = \lambda \times \begin{cases} 
    e^{-\gamma l |T - T_{\text{opt}}|}, & T_{\text{min}} < T \leq T_{\text{opt}} \\
    e^{-\gamma u |T - T_{\text{opt}}|}, & T_{\text{max}} > T \geq T_{\text{opt}}
\end{cases},$$

where Gamma($\alpha, \lambda(T)$) is the gamma distribution with shape parameter $\alpha$ and scale parameter $\lambda(T)$. The parameters, ($\alpha, \lambda, \gamma_l, \gamma_u, T_{\text{opt}}$), are different for the “high bioaccumulation season” and the “low bioaccumulation season” harvest occasions. Parameters were estimated from raw data (Burkhardt & Calci, 2000) using maximum likelihood methods (Table VI).

In the model, the two seasons were defined as “high bioaccumulation season,” from October to February and “low bioaccumulation season” from March to September (Burkhardt & Calci, 2000). We note that for some sections of North America, water temperatures in oyster harvest areas are sometimes below 4.8 °C and thus no bioaccumulation is expected to occur during those periods regardless of whether the period is nominally in the “high bioaccumulation season” or “low bioaccumulation season.” Variation around the mean BAF from oyster to oyster within a harvest occasion are specified using gamma distributions. The BAF were considered the same for a given oyster for infectious and non-
Table VI. Estimates for Fitted Gamma Distribution Parameters, Burkhardt and Calci (2000) MSB Bioaccumulation Factor Data

| Parameter | Point Estimate [95% Wald-Type Confidence Interval] |
|-----------|---------------------------------------------------|
|           | High Bioaccumulation Season                      | Low Bioaccumulation Season |
| $\alpha$  | 0.200 [0.127, 0.314]                              | 0.143 [0.100, 0.205]       |
| $\theta$  | 340 [136, 852]                                    | 92.8 [37.6, 228.9]         |
| $\nu(T)$  | $T_{opt}$ 20.9 [20.0, 21.7]                      | 25.6 [23.5, 27.9]          |
| $\gamma_l$| 2.51 [1.675, 9.37]                               | 9.11 [6.90, 12.03]         |
| $\gamma_u$| 23.7 [16.2, 34.7]                                | 11.2 [2.97, 42.3]          |

Infectious particles, and for NoV GI, NoV GII, and MSC.

2.4.2. Viral Elimination

Data in the literature documents that the concentration of viruses in shellfish can be reduced over time, when the contaminated shellfish are placed in water containing no viruses or a lower concentration of viruses than the water in which the shellfish became contaminated. The risk assessment used a meta-analysis of published sets of experimental MSC depuration trials to quantify elimination in terms of its rate, and variability among shellfish at the same temperature and in response to temperature changes. Data from nine studies, including 39 MSC depuration trials on oysters with single time point data (Doré, 2003; Dore & Lees, 1995; Doré, Henshilwood, & Lees, 1998, 2000; Henshilwood, Doré, Anderson, & Lees, 2003; Kator & Rhodes, 2001; Lees & Doré, 1992; Loisy et al., 2005) and single time point data with replicates (Burkhardt et al., 1992; Kator & Rhodes, 2001; Mcleod, Hay, Grant, Greening, & Day, 2009; Neish, 2013; Ueki et al., 2007) were used. This information was used to model the impact of depuration or relay processes, as well as the dynamics of uptake and elimination in estuaries where the effect of the tides must also be considered. Kinetic data describing the time evolution of viral contamination levels in oysters during depuration have typically been modeled with a simple exponential decay (Doré, 2003; Henshilwood et al., 2003; Loisy et al., 2005). We tested a more general and flexible Weibull model that depends on depuration trial temperature. We used:

$$\log_{10}(E(Y(t))) = \log_{10}(E(Y(0))) - \left(\frac{t}{\delta(T)}\right)^p,$$

where $Y(t)$ is single oyster concentration, which simplifies to an exponential model when $p = 1$. $p$ was found to be significantly different from 1 when the model was applied to the data, and thus the Weibull model was retained. To account for how the mean, over independent depurations, time to first log$_{10}$ reduction, $E(\delta(T))$, declines with increasing temperature, we used

$$E(\delta(T)) = \mu \times e^{-\beta T}$$

with $\mu > 0$ and $\beta > 0$. The single depuration time to first log$_{10}$ reduction is modelled as Gaussian distributed, that is

$$\delta(T) \sim \text{Normal}(E[\delta(T)], E[\delta(T)]\sigma^2).$$

Parameters were estimated from raw data, when available, or from data extracted from the publications (Fig. 3) using maximum likelihood methods (Table VII). Viral elimination was considered independently for each oyster of a meal, and each elimination process, and was considered equal for infectious and noninfectious NoV as well as for MSC.
Table VII. Depuration Rate Model Parameters as Estimated from a Meta-Analysis of Elimination Data

| Parameter | Point Estimate [95% Wald-Type Confidence Interval] |
|-----------|---------------------------------------------------|
| $\rho$    | 0.653 [0.587, 0.719]                             |
| $\mu$     | 31.8 [24.0, 39.5]                                 |
| $\beta$   | 0.109 [0.094, 0.123]                              |
| $\sigma^2$| 0.959 [0.908, 1.01]                               |

Fig 4. Hypothetical concentration of virus in water (red line) varying in time and consequent concentration in oyster (blue line) designed to illustrate how the dynamic modeling works. At time 0, both concentrations are at 0 particles/unit (A). When the concentration in the water increases (B), the concentration in the oyster rapidly reaches the level of the concentration in water $\times$ BAF (dashed line). After a decrease of the concentration in water, the concentration in the oyster decreases slowly (C). This dynamic process leads to cycles of elimination and bioaccumulation.

2.4.3. Resulting Dynamic Model of the Concentration of Viruses in Oysters

The concentration of viruses in the oyster was then modeled dynamically as a function of the concentration in water: each oyster accumulates viruses, generally quite quickly (Burkhardt & Calci, 2000), if their internal concentration is lower than the product of their BAF and the current concentration in the surrounding water. An oyster will release viruses, albeit at a slower elimination rate, when its internal concentration is higher than this product. An illustration of this dynamic process is in Fig. 4. In the absence of an exceptional event, the concentration in oyster reaches a steady state.

Concentration of particles in oysters per gram were converted to the number of particles per oyster using a meat weight distribution. This distribution was estimated from data from measured weights of pooled oysters in a 2007–2008 market survey (DePaola et al., 2010; Supporting Information Appendix). Specifically, average oyster weight, $Z$, varies from harvest to harvest as

$$\ln(Z) \sim \text{Normal}(2.379, 0.2943^2),$$

and, among oysters from the same harvest, single oyster weight, $W$, varies as

$$\ln(W|Z = z) \sim \text{Normal}(\ln(z) - 0.5 \times 0.133^2, 0.133^2),$$

leading to a mean weight of 11.3g (5th and 95th percentiles: 6.26g and 18.2g, respectively).

2.5. Oyster Consumption

In this study, oysters are assumed to be consumed raw without any additional treatment or process applied. The impact of relay, depuration, cooking, and other treatments on the concentration of NoV in oysters and the risk will be reported in the abovementioned second study.

Ideally, risk linked to NoV in raw oysters among regions should consider differences in how meal sizes from oysters harvested in the different regions are different. In the absence of such detailed data, we decided to base comparisons on a standardized meal size distribution, to use in common for all regions’ harvested oysters.

Twenty-four-hour recall nutrition surveys like the U.S. and Canadian national consumption surveys What We Eat in America (WWEIA), the dietary survey portion of the National Health and Nutrition Examination Survey (NHANES), and Canadian Community Health Survey (CCHS 2.2) are designed to provide information about intakes on a day at random. However, even national survey designs with large sample sizes do not capture specific consumption characteristics of infrequently consumed foods like raw oysters, (e.g., 1999–2008 survey, i.e., five cycles of NHANES/WWEIA studies, report raw oyster consumption data for 10 consumers only). As a result, these data sources could not be used to accurately determine meal size distribution. Targeted surveys are thought to provide more precise information about such characteristics as shellfish meal sizes. Following precedents (Food and Agriculture Organization/World Health Organization, 2005, 2011; Food and Drug Administration, 2005), we used meal size
data from a regional telephone survey that included 1,012 adults in seven metropolitan areas in north and central Florida (Degner & Petrone, 1994). The survey was conducted by The Florida Agricultural Market Research Center, University of Florida, during April and May of 1994 and determined, among other information, the typical number of raw oysters consumed in a meal (Degner & Petrone, 1994). Three hundred and six of the respondents reporting raw oyster consumption at least once in the previous year and provided self-reported or recall information as to the number of oysters that they typically consumed per serving (Table VIII. The typical serving sizes most frequently reported by the respondents were 6, 12, and 24 oysters, with 12 being the most frequent.

We constructed a single raw oyster meal size distribution as Multinomial(ϕ), where {ϕi} = {1/306 + 306yi} for i = 1, …, 72, using {yi} from Degner and Petrone (1994) reported typical meal size frequencies. This meal size distribution has a mean of 13.8 oysters per oyster meal.

2.6. Dose–Response

Multiple dose–response models have been developed for NoV infection, and NoV illness. In these models, infection is usually defined as confirmed fecal excretion of virus and seroconversion, while illness is usually defined as the observation of related symptoms (e.g., “diarrhea and/or vomiting combined with other symptoms as abdominal pain, myalgia, fatigue, chills and headache more than 8 hours after challenge” in Teunis et al. (2008)). Each of the dose–response models has limitations and they all are specific to particular situations (Schmidt, 2015; Van Abel, Schoen, Kissel, & Meschke, 2017). During model development, we tested four dose-infection models, relating the dose and the probability of infection (Messner, Berger, & Nappier, 2014; Schmidt, 2015; Teunis et al., 2008; Thebault et al., 2013), paired with three illness-given-infection models, relating the probability of illness once the infection is confirmed as a function, or not, of the dose (Lindesmith et al., 2003; Teunis et al., 2008; Thebault et al., 2013). Our baseline choice was the simpler Messner et al. (2014)’s fractional Poisson model. It describes that the probability to be infected for a susceptible individual is 0.72, regardless of dose if >0 infectious particle. We associated this model with a constant, user defined, dose-illness given infection model with probability 0.3, that is the observed illness/infection rates in Lindesmith et al. (2003).

A proportion of consumers are susceptible to NoV infection as per their histo-blood group antigen status, (i.e., Se+ status indicates a susceptible individual; Hutson, Airaud, LePendu, Estes, & Atmar, 2005; Kindberg et al., 2007; Lindesmith et al., 2003; Thorven et al., 2005). For this model, we chose an estimate of 74% for the proportion of the U.S. and Canadian populations that are Se+ from the study by Smyth et al. (2011) which examined a British 1958 birth cohort. The estimate from this study was selected because of its large sample size (10,008, which was at least an order of magnitude larger than that in the other 34 studies identified). A more specific estimate, for the North American population in this case, should be used when available, considering for example the lower proportion of Se+ observed in the Hispanic population (Payne et al., 2015). Note, however, that the results obtained are directly proportional to the value chosen for the fraction of Se+ individuals in the consuming population, and the a priori comparability of populations with our populations of interest.

2.7. Development of the Model

The Monte-Carlo model was integrated over 10,000 iterations. The model was written in R (R Development Core Team, 2019) with a web interface developed with the Shiny R framework (Chang, Cheng, Allaire, Xie, & McPherson, 2019). It is available at https://fda-riskmodels.foodrisk.org/NSRA/. The code syntax was validated through a translation of the original code in Python.

We tested the impact of some of the parameter estimates or models for which data were limited, by running the model using various values/models and estimated the output under these alternative values/models and these results are presented in Section 3.2.

A global importance analysis was developed using a random forest model (Liaw & Wiener, 2002). For quantitative outputs (for this model the output was the log risk of illness per serving), the measure of importance was the percent increase in Mean Standard Error (MSE) of predictions, estimated with out-of-bag error, as a result of predictive variables being systematically permuted while all others are left unchanged (Liaw & Wiener, 2002). For qualitative output (for this model, we used risk ≤ 0.1/100 servings vs. risk > 0.1/100 servings) the same was done using rate for classification and measured by the mean
Table VIII. Three Hundred and Six Respondents’ Typical Number of Oysters Eaten per Occasion (Degner & Petrone, 1994; Table 12, pg. 19) and Frequency Considered in the Model

| Oysters | Observed Frequency | Considered Frequency | Oysters | Observed Frequency | Considered Frequency | Oysters | Observed Frequency | Considered Frequency |
|---------|--------------------|----------------------|---------|--------------------|----------------------|---------|--------------------|----------------------|
| 1       | 1                  | 0.3%                 | 25      | 5                  | 1.6%                 | 49      | 0                  | 0.0%                 | 0.3%                 |
| 2       | 1                  | 0.3%                 | 26      | 0                  | 0.0%                 | 50      | 3                  | 1.0%                 | 1.1%                 |
| 3       | 9                  | 2.9%                 | 27      | 0                  | 0.0%                 | 51      | 3                  | 0.0%                 | 0.3%                 |
| 4       | 10                 | 3.3%                 | 28      | 0                  | 0.0%                 | 52      | 0                  | 0.0%                 | 0.3%                 |
| 5       | 15                 | 4.9%                 | 29      | 0                  | 0.0%                 | 53      | 0                  | 0.0%                 | 0.3%                 |
| 6       | 61                 | 19.9%                | 30      | 0                  | 0.0%                 | 54      | 0                  | 0.0%                 | 0.3%                 |
| 7       | 1                  | 0.3%                 | 31      | 0                  | 0.0%                 | 55      | 0                  | 0.0%                 | 0.3%                 |
| 8       | 11                 | 3.6%                 | 32      | 0                  | 0.0%                 | 56      | 0                  | 0.0%                 | 0.3%                 |
| 9       | 0                  | 0.0%                 | 33      | 0                  | 0.0%                 | 57      | 0                  | 0.0%                 | 0.3%                 |
| 10      | 15                 | 4.9%                 | 34      | 0                  | 0.0%                 | 58      | 0                  | 0.0%                 | 0.3%                 |
| 11      | 0                  | 0.0%                 | 35      | 0                  | 0.0%                 | 59      | 0                  | 0.0%                 | 0.3%                 |
| 12      | 95                 | 31.0%                | 36      | 0                  | 0.0%                 | 60      | 1                  | 0.3%                 | 0.5%                 |
| 13      | 1                  | 0.3%                 | 37      | 0                  | 0.0%                 | 61      | 0                  | 0.0%                 | 0.3%                 |
| 14      | 0                  | 0.0%                 | 38      | 0                  | 0.0%                 | 62      | 0                  | 0.0%                 | 0.3%                 |
| 15      | 5                  | 1.6%                 | 39      | 0                  | 0.0%                 | 63      | 0                  | 0.0%                 | 0.3%                 |
| 16      | 0                  | 0.0%                 | 40      | 0                  | 0.0%                 | 64      | 0                  | 0.0%                 | 0.3%                 |
| 17      | 1                  | 0.3%                 | 41      | 0                  | 0.0%                 | 65      | 0                  | 0.0%                 | 0.3%                 |
| 18      | 8                  | 2.6%                 | 42      | 0                  | 0.0%                 | 66      | 0                  | 0.0%                 | 0.3%                 |
| 19      | 0                  | 0.0%                 | 43      | 0                  | 0.0%                 | 67      | 0                  | 0.0%                 | 0.3%                 |
| 20      | 8                  | 2.6%                 | 44      | 0                  | 0.0%                 | 68      | 0                  | 0.0%                 | 0.3%                 |
| 21      | 0                  | 0.0%                 | 45      | 0                  | 0.0%                 | 69      | 0                  | 0.0%                 | 0.3%                 |
| 22      | 0                  | 0.0%                 | 46      | 0                  | 0.0%                 | 70      | 0                  | 0.0%                 | 0.3%                 |
| 23      | 0                  | 0.0%                 | 47      | 0                  | 0.0%                 | 71      | 0                  | 0.0%                 | 0.3%                 |
| 24      | 37                 | 12.1%                | 48      | 4                  | 1.3%                 | 72      | 0                  | 0.0%                 | 0.3%                 |
Table IX. Scenarios Evaluated. Within a Given Line, Scenarios Involving All Combinations of Variables were Evaluated

| Region                | Season     | WWTP - Disinfection       | Dilution          |
|-----------------------|------------|---------------------------|-------------------|
| 1 US                  | Full Year  | All WWTP                  | Mean tide: [1,000:1–100,000:1] |
| 2 Canada              | Full Year  | All WWTP                  | Mean tide: [1,000:1–100,000:1] |
| 3-42 Gulf             | Spring     | All WWTP                  | Mean tide: [1,000:1–100,000:1] |
| South Atlantic        | Summer     | All WWTP                  |                   |
| Middle Atlantic       | Winter     | All WWTP                  |                   |
| New England           |            | All WWTP                  |                   |
| US Pacific            |            | All WWTP                  |                   |
| North Gulf of St. Lawrence |        | All WWTP                  |                   |
| Canada Maritimes      |            | All WWTP                  |                   |
| Canada Pacific        |            | All WWTP                  |                   |
| 43-106 Gulf           | Winter     | All WWTP                  | Low tide: [1000:1] |
| South Atlantic        |            | Mechanical-UV             |                   |
| Middle Atlantic       |            | Mechanical-Chlorine       |                   |
| New England           |            | Mechanical-none           |                   |
| US Pacific            |            | Lagoon-UV                 |                   |
| North Gulf of St. Lawrence |        | Lagoon-Chlorine          |                   |
| Canada Maritimes      |            | Lagoon-none               |                   |
| Canada Pacific        |            | None                      |                   |

decrease in prediction accuracy. In summary, for both importance values, the higher the reported statistic percent increase in MSE or mean decrease in accuracy, the more important the variable. For comparability, this specific analysis was implemented using an equal number of iterations for each combination of region-season.

2.8. Scenarios

Baseline scenarios, evaluating risk of NoV illness for consumers, were developed for the United States and Canada. Each baseline scenario evaluates risk across all months and oyster harvest regions within each country. Additional scenarios were developed to evaluate risk by region across all months and by season. Scenarios were also developed within each region to explore the impact of WWTP type, water temperature, and dilution (Table IX). A final set of scenarios were developed to explore the relationship between the concentration of MSC predicted in water and, separately, in oysters and the predicted risk.

3. RESULTS

3.1. Baseline risk

Under the baseline scenario (all months, all regions), the expected number of NoV illnesses for 100 meals of raw oysters are predicted as 0.108 and 0.188 for the United States and Canada, respectively, when the model integrates month and regions based on oyster landings data. This translates to annual predictions of 60,255 cases in the United States and 6,860 cases in Canada or 183 and 181 cases per million in the populations of the United States and Canada, respectively, based on total annual oyster landings, total country populations (as of January 1, 2020), and assuming 50% of oysters are consumed raw (Food and Drug Administration (FDA), 2005; Statistics Canada, 2020; United States Census Bureau, 2020).

Fig. 5 details the predicted number of illnesses per 100 meals of raw oysters according to the region and the season. These scenarios were developed using a variability range of dilution factor at mean tide of 1:1,000 to 1:100,000. The risk of illness per meal is higher with oysters harvested in the South Atlantic coast and the Canadian and U.S. Pacific coasts, and during the winter months. It is lower during summer months (Fig. 5; Table A.7 in Supporting Information Appendix).

3.2. Uncertainty Analysis

3.2.1. Impact of the Proportion of Infectious Particles in WWTP Influent

In the baseline, the proportion of infectious particles to total NoV particles in WWTP influent was set to a constant of 1/40. This parameter was chosen based on a consideration of estimates derived from data collected for NoV and NoV surrogates San Miguel Sea Lion Virus-17, Hepatitis A virus, Rotavirus, and Murine NoV in a number of settings
Fig 5. Predicted number of NoV illnesses per 100 meals of raw oysters as a function of region and season (actual values in Supporting Information Appendix).

Table X. Impact of the Choice of the Proportion of Infectious Particles in WWTP Influent for Two Selected Regions on the Predicted Number of Illnesses for 100 Meals (Dilution at Mean Tide: 1,000—100,000, All Year)

| Region           | Proportion of Infectious Particles |
|------------------|-----------------------------------|
|                  | 1/4 | 1/40 | 1/400 | 1/4000 |
| Gulf             | 0.32| 0.11 | 0.025 | 0.0046 |
| Canada Pacific   | 0.78| 0.27 | 0.075 | 0.017  |

\(^a\)model default.

(Burkhardt et al., 2002; Guix et al., 2020; Jansen et al., 1985; Rotbart, 1990; Teunis et al., 2008; Thebault et al., 2013). As a sensitivity analysis, we tested the impact of changes of this parameter on the prediction for all regions. In Table X, we provide the results for the two regions which differ most in terms of tides, temperature and energy (Gulf and Canada Pacific). The low impact of the ratio of infectious particles to total particles on the predicted number of illnesses for these two contrasting regions suggests that the parameter is not strongly region dependent.

3.2.2. Impact of Tidal Flushing

In the baseline, the tidal flushing parameter was chosen based on author knowledge of relevant estuaries. As a sensitivity analysis, we tested the impact of changes of this parameter on the prediction for all regions, and provide the results for two regions with contrasting levels of tides (Gulf and Canada Pacific). The risk prediction increases, albeit slightly, when tidal return is larger, and no other parameters change (Table XI). This is because a larger tidal return (smaller flushing rate) results in a larger quasi-steady-state concentration of NoV in the harvest area. Overall, these results indicate that this parameter has little impact on the risk of illness within the typical range encountered.

3.2.3. Impact of the Dose–Response Model

Table XII provides an example of the impact of the considered dose–response models (all regions, all seasons for a mean tide dilution varying from 1,000:1 to 100,000:1). The Messner et al. (2014) model was used as a default in this study, given limitations of the available data (Schmidt, 2015). This dose–response model provides illness predictions in the
Table XI. Impact of the Choice of the Tidal Return Parameter for Two Selected Regions on the Predicted Number of Illnesses for 100 Meals (Dilution at Mean Tide: 1,000-100,000, All Year)

| Region           | Tidal return $\tau$ | 0.125 | 0.25$^a$ | 0.50 | 0.75 |
|------------------|---------------------|-------|----------|------|------|
| Gulf             | 0.10                | 0.10  | 0.11     | 0.12 |
| Canada Pacific   | 0.20                | 0.21  | 0.26     | 0.30 |

$^a$model default.

Table XII. Predicted Number of Infection and Illness for 100 meals of Raw Oysters (Dilution at Mean Tide Varying from: 1,000:1 to 100,000:1, All Regions, All Seasons)

| Reference of the Dose-Infection Model | Model or Reference for the Illness Given Infection Model | Infections/100 Meals | Illnesses/100 Meals |
|--------------------------------------|----------------------------------------------------------|----------------------|---------------------|
| Messner et al. (2014)$^a$            | Constant (0.3)                                           | 0.38                 | 0.11                |
| Thebault et al. (2013)                | Constant (0.3)                                           | 0.28                 | 0.084               |
| Teunis et al. (2008)                  | Constant (0.3)                                           | 0.24                 | 0.071               |
| Schmidt (2015)$^b$                    | Constant (0.3)                                           | 0.0014               | 0.00043             |
| Thebault et al. (2013)                | Thebault et al. (2013)                                    | 0.28                 | 0.17                |
| Teunis et al. (2008)                  | Teunis et al. (2008)                                      | 0.24                 | 0.00027             |

$^a$default model.

$^b$The model from Schmidt (2015) tested in this risk assessment is one version of “exact Beta-Poisson”

order of magnitude to those generated using the models by Thebault et al. (2013) and Teunis et al. (2008), when considering a constant illness-given-infection model. The closeness of the Thebault et al. (2013), the Teunis et al. (2008), and the Messner et al. (2014) dose–response models for infection was previously described (Messner et al., 2014; Schmidt, 2015; Thebault et al., 2013). Notwithstanding, the Schmidt (2015) model provides a much smaller predicted infections per 100 meals, and the illnesses-given-infection models from Teunis et al. (2008) provide much smaller illness per 100 meals than using a constant illness-given-infection model. Those illness-given-infection models are based on little data. Note, moreover, that the results obtained are directly proportional to the value chosen for the fraction of Se+ individuals in the consuming population.

3.3. Comparison of the Predictions with Epidemiological Data

While the focus of this risk assessment is to quantitatively describe the complex interaction of factors that impact the potential risk associated with raw oyster consumption and to use this description to reveal how risk is impacted by different situations, mitigations and controls, this model also provides predictions of the annual number of NoV cases associated with raw oyster consumption in the United States and Canada. These (“bottom-up”) predictions can be compared with estimates derived from epidemiology data (“top down”) as one type of validation.

The number of NoV illnesses linked to consumption of oysters is difficult to estimate using existing epidemiological data, and thus the values are characterized by and associated with a large amount of uncertainty. We estimated from epidemiological data, a “lower plausible bound” of 35,000 and an “upper plausible bound” of 715,000 NoV illnesses annually associated with raw oyster consumption in the United States. We estimated similarly, a lower bound of 10,900 and an upper bound of 552,000 NoV illnesses annually associated with raw oyster consumption in Canada (see Supporting Information Appendix).

Our quantitative risk assessment model (including all the factors described above) leads to a prediction of 60,255 annual cases in the United States and 6,860 annual cases in Canada. The annual case predictions provided in this risk assessment for quasi-steady-state situations (with no exceptional events) is lower but within the uncertainty interval (United States), or lower and slightly lower than the lower uncertainty bound (Canada) estimated using available epidemiological studies and are of the same order of magnitude. The model predictions above consider
### Table XIII. The Most Important Factors Influencing the Log of the risk of NoV Infection Following an Oyster Meal. Results Produced Using a Random Forest method. The 18 (Over 49 Tested) Most Important Factors are Presented, in Decreasing Order of Importance

| Factor                                                                 | % Increase Mean Standard Error |
|-----------------------------------------------------------------------|-------------------------------|
| Concentration of NoV GII in the WWTP influent                         | 89                            |
| Mean Dilution in the estuary                                          | 76                            |
| Log Reduction of NoV GI (Physical removal in the WWTP)                | 61                            |
| Log Reduction NoV GII (Physical removal in the WWTP)                  | 61                            |
| Log Reduction NoV GI (Inactivation in the WWTP)                       | 45                            |
| Meal Size                                                             | 43                            |
| Log Reduction NoV GII (Inactivation in the WWTP)                      | 41                            |
| Maximum Bioaccumulation Factor (BAF) of NoV GII over the oysters in the meal | 36                            |
| Concentration of NoV GI in the WWTP influent                          | 36                            |
| Mean BAF of NoV GII over the oysters of in the meal                   | 36                            |
| Sunlight Energy in the estuary                                        | 35                            |
| Maximum BAF of NoV GI over the oysters in the meal                    | 35                            |
| Mean BAF of NoV GI over the oysters in the meal                       | 35                            |
| Mean Inactivation of NoV in the estuary                               | 31                            |
| Water Temperature in the estuary                                      | 30                            |
| Time for the Water to reach the harvest site                          | 25                            |
| Cold Month                                                            | 15                            |
| WWTP UV Disinfection                                                  | 15                            |

Only the “baseline” scenario, that is, cases estimated in the absence of exceptional events of contamination situations during which the risk would be higher. The predictions from the model described herein, ignoring exceptional events, are logically lower than the actual mean risk of NoV associated with the consumption of raw oysters in these countries, which include steady-state situations as well as exceptional situations.

### 3.4. Critical Factors that Contribute to the Risk of NoV Infection from Consuming Raw Oysters

The sole source of NoV in the oyster harvest water is human waste. A combination of physiological and environmental phenomena, which are not directly controllable, as well as control strategies set forth in sanitation program documentation (e.g., NSSP and CSSP), can affect the level of virus within oysters during production.

The Random Forest analysis was developed for a quantitative output (the log risk of NoV infection per serving, Table XIII) and on a binary output (risk of NoV infection for 100 meals greater than 0.1, Table XIV). The latter can predict the impact of parameters on risky situations that could lead to outbreaks. The results suggest that the concentration of virus in the influent of the WWTP and its treatment have the greatest impact on the outcome of illness. This suggests that efforts to control the amount of virus that enters (through reduction of illness from all causes in the population) and exits a WWTP (through treatment) and those that limit entry of contaminated waste into estuaries from other sources (e.g., combined sewer overflows) will have the greatest impact on the load of virus in oysters.

Of interest, beyond the efficiency of the WWTP to reduce the concentration of infectious NoV in the effluent, the level of dilution of NoV concentration from the effluent of the WWTP to the harvest area is highlighted as a factor impacting the risk of NoV infection following the ingestion of a meal of oysters.

### 3.5. Impact of the WWTP Type

As illustrated in the sensitivity analysis Tables XII and XIII the log reduction of NoV achieved by the WWTP strongly influences the NoV illnesses per 100 raw oyster meals. Fig. 6 compares the risk predictions associated with different WWTP types and disinfection strategies, as a function of the region for an example situation of winter season (December–February, i.e., during the high bioaccumulation season), and a dilution at low tide of 1000:1. Lagoons and mechanical WWTPs with UV disinfection are the most efficient means to reduce the risk. This efficiency can be traced to the particularly large reductions afforded by UV disinfection, including biological inactivation of NoV particles, as estimated here by the excess reduction of MSC evaluated by
Table XIV. The Most Important Factors Influencing the Presence of a Risk of NoV Infection Following an Oyster Meal Serving >0.1%. Results Produced using a Random Forest Method. The 18 (Over 49 Tested) Most Important Factors are Presented, in Decreasing Order of Importance

| Factor                                                                 | Mean Decrease in Prediction Accuracy |
|------------------------------------------------------------------------|-------------------------------------|
| Concentration of NoV GII in the WWTP influent                          | 103                                 |
| Concentration of NoV GI in the WWTP influent                          | 86                                  |
| Log Reduction NoV GII (Physical removal in the WWTP)                   | 70                                  |
| Log Reduction of NoV GI (Physical removal in the WWTP)                 | 67                                  |
| Log Reduction of NoV GI (Inactivation in the WWTP)                     | 66                                  |
| Log Reduction NoV GII (Inactivation in the WWTP)                       | 63                                  |
| Mean Dilution in the estuary                                          | 43                                  |
| Mean Inactivation of NoV in the estuary                                | 40                                  |
| Water Temperature in the estuary                                       | 40                                  |
| Sunlight Energy in the estuary                                         | 35                                  |
| Mean Bioaccumulation Factor (BAF) of NoV GI over the oysters in the meal| 34                                  |
| Mean BAF of NoV GII over the oysters in the meal                       | 34                                  |
| Maximum BAF of NoV GI over the oysters in the meal                     | 33                                  |
| Maximum BAF of NoV GII over the oysters in the meal                    | 33                                  |
| WWTP UV Disinfection                                                  | 24                                  |
| Meal size                                                             | 23                                  |

Fig 6. Number of illnesses for 100 meals of raw oysters (logarithmic scale) as a function of WWTP type and region (Dilution at low tide: 1000:1, Season: Winter (i.e., December-February, high bioaccumulation season)).

culture methods as compared with RT-qPCR enumerated NoV particles.

3.6. Water Temperature Influence

Coastal Canada and the continental United States, where shellfish are harvested, cover many degrees of latitude, with a wide range of temperatures. The concentration of NoV in the WWTP influent is associated with temperature and season (Pouillot et al., 2015). Temperature and season also impact the persistence of NoV particles suspended in the environment (Burkhardt et al., 2000), and the uptake and elimination of NoV particles by oysters through the overall adaptation of oysters in the harvest water (Burkhardt & Calci, 2000). These factors influence...
the resulting concentration of NoV in oysters at the time of harvest.

Trends for the impact of water temperature on NoV levels in oysters are made evident by considering the season and the region separately (Fig. 7). Overall, the evolution of risk as a function of temperature is complex, notably because of the complicated BAF-temperature and season relationship. Fig. 7 illustrates the general behavior of the model as a function of season. The impact of temperature on the overall risk is strongest during the winter and, to a lesser extent, spring seasons, with higher risk at higher temperature and lower risk at lower temperature during those seasons.

3.7. Dilution of WWTP Effluent Influence

Dilution of WWTP effluent prior to entering a shellfish harvest area is a complex phenomenon that can influence the level of risk of NoV infection. Allowing shellfish harvesting only after sufficient dilution is achieved is also a major management technique to reduce the risk of NoV illness. For a given mean tide, the model considers the hour-to-hour variation, as well as the region-to-region variation of dilution in the harvest area because of tides. For a given dilution at mean tide, tides of high amplitudes would lead to periods with low dilution (at low tides), that could impact the concentration of NoV in oysters. Fig. 8 illustrates the variation in the impact of dilution with region and season. WWTP effluent dilution is always associated with reduction in the risk of illness but is not necessarily a proportional factor.

3.8. Link between MSC Concentration in Water or Oysters and Risk

Figs. 9 and 10 illustrate the relationship between the concentration of MSC predicted in water and in oysters, respectively, and the predicted risk of NoV per serving. A significant increase in risk is observed when the concentration of MSC increases. However, a large variability of the prediction of the risk as a function of the MSC concentration is observed, as illustrated by the width of the cloud of points around the modeled trends. While a higher MSC concentration usually signs the presence of a higher risk, some situation of high concentration of MSC with low risk might be observed, and vice versa.

4. DISCUSSION

The quantitative risk assessment model developed evaluates the risk of NoV illness from consumption of raw oysters considering various influencing factors. The model created also explicitly
evaluates the prevalence and levels of NoV along the different steps of the process, from WWTP influent to oysters at the point of consumption. Development of this model required consideration of diverse and complex physical and biological phenomena. Most of these phenomena are influenced by environmental parameters including water temperature and tides, whose variations must then also be considered. As a result, we developed a risk assessment model that is able to provide region- and season-specific predictions of NoV concentrations throughout the process and consequent NoV cases in the United States and
Canadian populations associated with consumption of raw oysters. Overall, the risk assessment model was developed with the best available data and resulted in a baseline prediction for annual cases that was not inconsistent with epidemiological data in the absence of exceptional events. Our predictive process model also enables users to evaluate a wide diversity of situations (scenarios) to quantify impacts of and interactions among individual risk factors and to quantify the impacts on risk of currently used or potentially new mitigations and controls. This risk assessment was developed specifically for oysters. However, most of the conclusions regarding relationships between risk and influencing factors area applicable to other bivalve molluscan shellfish grown in such estuaries and consumed raw or lightly cooked because the relevant biology is similar (e.g., bioaccumulation dynamics). Note that contamination of oysters via symptomatic or asymptomatic food handlers was out of the scope of this model and some outbreaks may arise from this route of transmission (Hardstaff et al., 2018).

A significant limitation of the model is the use of surrogate data (mostly MSC) to develop some of the underlying mathematical models. As examples, the BAF and elimination process models are based on data obtained using MSC data. The ability to propagate NoV (Ettayebi et al., 2016) should help to refine the models in the future, should this culture method be found to be applicable to shellfish concentrates (Woods, Calci, Marchant, & Burkhardt, 2016) and is used to collect data needed to inform this risk assessment. Other identified limitations are (i) use of the same BAF and elimination models for NoV GI and NoV GII in spite of literature that suggests some differences (Maalouf et al., 2011); (ii) the use of a simple dilution model that captures overall dilution for all estuaries while more complex far-field models could be developed or used instead for each estuary that better account for their unique features. Our choices for our integrated baseline model were made considering the availability of sufficient high-quality data that is amenable to developing refined mathematical models.

The focus of this risk assessment is to illustrate how influencing factors interact and to characterize the relative order of magnitude of the impact of each parameter on the predicted risk. Another subsequent study will describe how exceptional events and mitigations can alter this risk. Among the many factors that influence the risk of NoV illness for raw oyster consumers, the concentrations of NoV in the influent (raw, untreated) and effluent (treated) of WWTPs were identified to be the most important. Thus, mitigation and control strategies that reduce or limit the level of NoV in untreated and/or treated wastew-
water can have a major influence on the risk of illness from consumption of those oysters. As an example, Schaeffer et al. (2018) documented reductions in prevalence and levels of NoV in oysters close to the outfall of a WWTP when a poorly performing sewage lagoon was replaced with a membrane bioreactor sewage treatment plant.

The large influence of the concentration of NoV in both WWTP influent and effluent is the main contributor to the higher risk associated with oyster harvest and consumption in winter predicted by our model, reflecting the observation that the incidence of NoV illness in the population (and thus human waste) is highest in winter (Hall et al., 2013). While NoV illness in the population has seasonal influences and our model results by season reflect this influence, we also find that there is generally no season without risk to raw oyster consumers. This is because the virus is generally found in WWTP influent year-round (Pouillot et al., 2015). The presence of the virus in WWTP influent regardless of season arises in part from the fact that a single individual shedding NoV can release high numbers of NoV particles with estimated concentrations as high as $10^{10} \, \text{gc/g feces}$ (Atmar et al., 2008; Chan et al., 2006). When human populations contributing to municipal wastewater are very small, NoV concentrations are expected to be highly variable and may also be sporadic. Specific data on estuaries influenced by very small communities would be needed to accurately predict the risk in these situations.

Our model results quantify differences in risk arising from differences in WWTP types and disinfection strategies. These differences in risk result from differences in efficiency in reducing the level of NoV, where the WWTP efficiency has a strong inverse influence on the predicted risk of illness from the consumption of oysters in growing areas impacted by that WWTP. From our previously published meta-analysis developed to inform the WWTP model in this risk assessment, lagoons are generally more efficient than mechanical WWTP in reducing the level of infectious NoV (Pouillot et al., 2015). Chlorine disinfection is less effective than UV-disinfection in reducing NoV (Pouillot et al., 2015), and is notably ineffective in inactivating NoV, unless chlorine levels are above those routinely used for sewage treatment (Kingsley et al., 2017). Typical assessments of sewage treatment efficiency are based on reductions of coliform bacteria rather than enteric viruses, which likely overestimates the microbiological safety of the water from a virologic perspective (Campos & Lees, 2014).

Oysters uptake NoV when present in the surrounding water rapidly, except when the water is very cold (Burkhardt & Calci, 2000). Consequently, any spike in NoV concentration in water may have important consequences in terms of overall risk of NoV illness from consumption of oysters. Similarly, our model results indicate that virus dilution at low tide is better correlated with risk than is dilution at mean tide. Elimination rates of NoV from bivalves are slow (Burkhardt et al., 1992), on the order of weeks, based on our meta-analysis of viral elimination kinetic data available, compared to bacteria which can be reduced in the matter of hours or days at most temperatures. Removal of contaminants from bivalves has generally relied on natural elimination by the animal. Elimination rates for NoV from bivalves are faster at higher water temperatures (but still slow when compared with other contaminants such as fecal coliforms and Escherichia coli). Overall, the impact of water temperature on the risk of NoV illness from consumption of raw oysters is complex, as it has different impacts on each step of the overall baseline process. Generally, our model predicts that an increase in water temperature during one of the winter months increases the risk for regions where oysters are still active, primarily because of the increased ability of shellfish to bioaccumulate NoV.

Our model results indicate that prevalence, rather than levels, of NoV in oysters drives the risk of illness for raw oyster consumers. This is because the probability of infection and illness at low dose is relatively large and levels of NoV in oysters in approved and conditionally approved growing areas, when present, are generally low. In our study, using the Messner et al. (2014) dose–response model reinforces this fact. Other dose–response models, notably the model of illness-given-infection of Teunis et al. (2008) are more sensitive to the quantity of ingested NoV. Consequently, while there is a relationship between reduction in NoV levels (or MSC levels—see below) and risk of NoV illness per serving, it is closer to a log-linear relationship (log reduction in concentration is approximately proportional to risk reduction) than a linear relationship. This is primarily because increased log reduction also reduces prevalence of NoV in oysters.

MSC is recognized as a marker of viral contamination from human sources as it is generally present in WWTP effluent and in oysters impacted
by WWTPs at detectable levels (Calci, Burkhardt, Watkins, & Rippey, 1998). Our model results identify and quantify the relationship between the expected concentration of MSC in WWTP effluent and in oysters, and the risk of NoV illness. A correlation between MSC and NoV was also observed in WWTP influent with some situation-to-situation variability. These results support the potential use of MSC as a surrogate for NoV contamination of oysters for surveillance or performance objectives.

In summary, we have developed a stochastic predictive risk assessment model that includes the key processes involved in and impacting potential contamination of oysters in United States and Canadian harvest areas. We have used the model to quantitatively evaluate the impact on risk associated with environmental parameters such as season, water temperature and tidal range, as well WWTP types and disinfection strategies influencing oyster harvest areas, and dilution from WWTP outfall to harvest area. We have characterized the relationship between levels of the human viral marker MSC in WWTP effluent or in oysters, and risk of NoV illness; and included in the model the ability to predict MSC levels together with NoV levels in WWTP influent, WWTP effluent, harvest water, and oysters. The region- and season-specific predictions of the model provide risk managers with a better characterization of the risk and the elements that contribute to potential risk of NoV infection and provides them with a powerful new tool to evaluate the impact of current and potential new risk mitigation and control strategies.

Acknowledgements

This work was carried out under official FDA, HC, CFIA, and ECCC duties and by contract (HHFS223201710033I with Goldbelt C6, LLC (RP, since 2017; FDA before 2017). We would like to thank and acknowledge the contributions of S.B. Dennis (FDA) and W. Ross (HC), who initiated this work and provided advice during the project, and W. Hajen (CFIA), A. Locas (CFIA); D. Plante (CFIA), T. Edge (ECCC), and Y. Ao (FDA) who contributed to early stages of model development. We thank G. Coulombe and R. Rutley for advice during model development. We acknowledge FDA-University of Maryland/JIFSAN student interns L. Wei, S. Kreshpanji, and S. Zilko for their help running scenarios of the early model. We thank and acknowledge J. Bowers (FDA) for providing a technical review of an early draft of this manuscript. We also thank IF-SAC, for setting up a special working group and the special workgroup members M. Batz, M. Bazaco, A. Hall, R.M. Hoekstra, C. Aston, and L. Richardson, for providing epidemiological data, references, and review related to the model results comparison (described in manuscript’s Supporting Information Appendix).

References

Ambarchian, J. M., Ao, Y., Calci, K. R., & Goblick, G. N. (2014). Evaluating the dilution of wastewater treatment plant effluent, treatment efficiency, and viral impacts on shellfish growing areas in Blaine, Washington. U.S. Report. Montgomery, MD: Food and Drug Administration.

Ao, Y., & Goblick, G. N. (2016). Application of hydrodynamic modeling to predict viral impacts from wastewater treatment plant discharges adjacent to shellfish growing areas. Paper presented at the U.S. EPA 2016 Recreational Waters Conference, New Orleans, LA. Retrieved from https://archive.epa.gov/epa/beach-tech/2016-recreational-waters-conference-poster-presentations.html

Atmar, R. L., Opekun, A. R., Gilger, M. A., Estes, M. K., Crawford, S. E., Neil, F. H., & Graham, D. Y. (2008). Norwalk virus shedding after experimental human infection. Emerging Infectious Diseases, 14(10), 1553–1557.

Batz, M. B., Hoffman, S., & Morris, G. Jr. (2011). Ranking the risks: The 10 pathogen-food combinations with the greatest burden on public health. Gainesville, FL: University of Florida, Emerging Pathogens Institute. Retrieved from https://fol.io.iupui.edu/bitstream/handle/10244/1022/72267report.pdf

Bok, K., & Green, K. Y. (2012). Norovirus gastroenteritis in immunocompromised patients. New England Journal of Medicine, 367(22), 2126–2132. https://doi.org/10.1056/NEJMr1207742

Burkhardt, W., III, Blackstone, G. M., Skilling, D., & Smith, A. W. (2002). Applied technique for increasing calicivirus detection in shellfish extracts. Journal of Applied Microbiology, 93(2), 235–240. https://doi.org/10.1046/j.1365-2672.2002.01681.x

Burkhardt, W., III, & Calci, K. R. (2000). Selective accumulation may account for shellfish-associated viral illness. Applied Environmental Microbiology, 66(4), 1375–1378.

Burkhardt, W., III, Calci, K. R., Watkins, W. D., Rippey, S. R., & Chirtel, S. J. (2000). Inactivation of indicator microorganisms in estuarine waters. Water Research, 34(8), 2207–2214. https://doi.org/10.1016/S0043-1354(99)00399-1

Burkhardt, W., III, Rippey, S. R., & Watkins, W. D. (1992). Depuration rates of Northern Quahogs, Mercenaria mercenaria (Linnaeus, 1758) and Eastern Oysters Crassostrea virginica (GMelin, 1791) in ozone- and ultralight light-disinfected seawater systems. Journal of Shellfish Research, 11(1), 105–109.

Calci, K. R., Burkhardt, W., III, Watkins, D., & Rippey, S. R. (1999). Occurrence of male-specific bacteriophage in feral and domestic animal wastes, human feces, and human-associated wastewaters. Applied Environmental Microbiology, 65(12), 5027–5029. https://doi.org/10.1128/AEM.65.12.5027-5029.1998

Campos, C. J., & Lee, D. N. (2014). Environmental transmission of human noroviruses in shellfish waters. Applied Environmental Microbiology, 80(12), 3552–3561. https://doi.org/10.1128/AEM.04188-13

Campos, C. J. A., Goblick, G., Lee, R., Wittamore, K., & Lees, D. N. (2017). Determining the zone of impact of norovirus contamination in shellfish production areas through microbiological monitoring and hydrographic analysis. Water Research, 124, 556–565. https://doi.org/10.1016/j.watres.2017.08.021
Canadian Shellfish Sanitation Program (CSSP). (2018). Manual of operations. Ottawa, Ontario: Canadian Food Inspection Agency. Retrieved from https://inspection.gc.ca/food-safety-for-industry/food-specific-requirements-and-guidance/fish/canadian-shellfish-sanitation-program/eng/1527251566006/1527251566942

Chan, M. C., Sung, J. J., Lam, R. K., Chan, P. K., Lee, N. L., Lai, R. W., & Leung, W. K. (2006). Fecal viral load and norovirus-associated gastroenteritis. Emerging Infectious Diseases, 12(8), 1278–1280.

Chang, W., Cheng, J., Allaire, J. J., Xie, Y., & McPherson, J. (2019). shiny: Web Application Framework for R. Retrieved from https://CRAN.R-project.org/package=shiny

Comeau, L. A. (2014). Spring awakening temperature and survival of sediment-covered eastern oysters Crassostrea virginica. Aquaculture, 430, 188–194. Retrieved from http://doi.org/10.1016/j.aquaculture.2014.04.009

Comeau, L. A., Mayrand, É., & Mallet, A. (2012). Winter quiescence and spring awakening of the Eastern oyster Crassostrea virginica at its northernmost distribution limit. Marine Biology, 159(10), 2269–2279. https://doi.org/10.1007/s00227-012-2012-8

Davidson, V. J., Ravel, A., Nguyen, T. N., Fazil, A., & Ruzante, J. (2007). Food-specific attribution of selected gastrointestinal illnesses: Estimates from a Canadian expert elicitation survey. Foodborne Pathogens and Disease, 8(9), 983–995. https://doi.org/10.1089/fpd.2010.0786

Degner, R. L., & Petrone, C. (1994). Consumer and restaurants manager reaction to depurated oysters and clams. Gainesville, FL: Florida Agricultural Market Research Center.

DePaola, A., Jones, J. L., Woods, J., Burkhardt, W., III, Calci, K. R., Krantz, J. A., ... Nabe, K. (2010). Bacterial and viral pathogens in live oysters: 2007 United States market survey. Applied Environmental Microbiology, 76(9), 2754–2768. https://doi.org/10.1128/AEM.02909-09

Doré, W. J. (2003). Development of procedures for improved viral reduction in oysters during commercial depuration. Retrieved from http://www.foodbase.org.uk/admintools/reportdocuments/491-1-877_B04002_viral_depuration_final_report_with_reviewers_comment.pdf

Doré, W. J., Henshilwood, K., & Lees, D. N. (1998). The development of management strategies for control of virulological quality in oysters. Water Science and Technology, 28(12), 29–35. https://doi.org/10.1016/S0273-1227(98)00796-3

Doré, W. J., Henshilwood, K., & Lees, D. N. (2000). Evaluation of F-specific RNA bacteriophage as a candidate human enteric virus indicator for bivalve molluscan shellfish. Applied and Environmental Microbiology, 66(4), 1280–1285. https://doi.org/10.1128/AEM.66.4.1280-1285.2000

Doré, W. J., & Lees, D. N. (1995). Behavior of enterochiria-coli and male-specific bacteriophage in environmentally contaminated bivalve mussels before and after depuration. Applied and Environmental Microbiology, 61(8), 2830–2834.

Ettayebi, K., Crawford, S. E., Murakami, K., Broughman, J. R., Karandikar, U., Tenge, V. R., ... Estes, M. K. (2016). Replication of human noroviruses in stem cell-derived human enteroids. Science, 353(6306), 1387–1393. https://doi.org/10.1126/science.aaf5211

Fiandrino, A., Martin, Y., Got, P., Bonnefont, J. L., & Troussonellier, M. (2003). Bacterial contamination of Mediterranean coastal seawater as affected by riverine inputs: Simulation approach applied to a shellfish breeding area (Thau lagoon, France). Water Research, 37(8), 1711–1722. https://doi.org/10.1016/S0043-1354(02)00573-0

Flannery, J., Keaveney, S., Rajko-Nowick, P., O’Flaherty, V., & Doré, W. (2012). Concentration of norovirus during wastewater treatment and its impact on oyster contamination. Applied Environmental Microbiology, 78(9), 3400–3406. https://doi.org/10.1128/aem.07560-11

Food and Agriculture Organization/World Health Organization. (2005). Risk assessment of Vibrio vulnificus in raw oysters. Interpretable summary and technical report. Retrieved from http://www.fda.gov/downloads/Food/FoodScienceResearch/UCM196915.pdf

Food and Agriculture Organization/World Health Organization. (2011). Risk assessment of Vibrio parahaemolyticus in seafood. Interpretable summary and technical report. Retrieved from http://www.who.int/foodsafety/publications/micro/MRA_16_JEMRA.pdf

Food and Drug Administration. (2005). Quantitative risk assessment on the public health impact of pathogenic vibrio parahaemolyticus in raw oysters. Retrieved from http://www.fda.gov/downloads/Food/FoodScienceResearch/UCM196915.pdf

Glastooff, P. S. (1926). New methods to measure the rate of flow produced by the gills of oyster and other mollusces. Science, 63(1626), 233–234. https://doi.org/10.1126/science.63.1626.233

Goblick, G. N., Anbarchian, J. M., & Calci, K. R. (2015). Hydrographic study of wastewater treatment plant effluent in the royal and Cousins Rivers of Yarmouth, Maine. U. S. College Park, MD: Food and Drug Administration.

Goblick, G. N., Anbarchian, J. M., Woods, J., Burkhardt, W., & Calci, K. (2011). Evaluating the dilution of wastewater treatment plant effluent and viral impacts on shellfish growing areas in mobile bay, Alabama. Journal of Shellfish Research, 30(3), 979–987. https://doi.org/10.2983/030.030.0341

Goblick, G. N., Ao, Y., Anbarchian, J. M., & Calci, K. R. (2017). Determination of buildup and dilution of wastewater effluent in shellfish growing waters through a modified application of super-position. Marine Pollution Bulletin, 115(1–2), 164–171. https://doi.org/10.1016/j.marpolbul.2016.12.011

Government of Canada. (2019a). Justice Laws Website: Management of Contaminated Fisheries Regulations (SOR/90-351). Available at: https://laws-lois.justice.gc.ca/eng/regulations/sor-90-351/index.html

Government of Canada. (2019b). Justice Laws Website: Safe Food for Canadians Regulations (SOR/2018-108). Available at: https://laws-lois.justice.gc.ca/eng/regulations/SOR-2018-108/index.html

Gueymond, C. A. (2001). Parameterized transmittance model for direct beam and circumsolar spectral irradiance. Solar Energy, 71(5), 325–346.

Gueymond, C. A. (2005). Interdisciplinary applications of a versatile spectral solar irradiance model: A review. Energy, 30(9), 1551–1576.

Guix, S., Fuentes, C., Pinto, R. M., Blanco, A., Sabria, A., Anfruns-Estrada, E., ... Bosch, A. (2020). Infectivity of norovirus GI and GII from bottled mineral water during a waterborne outbreak, Spain. Emerging Infectious Diseases, 26(1), 134–137. https://doi.org/10.3201/eid2601.190778

Hall, A. J., Lopman, B. A., Payne, D. C., Patel, M. G., Gastanaduy, P. A., Vinje, J., & Parashar, U. D. (2013). Norovirus disease in the United States. Emerging Infectious Diseases, 19(8), 1198–1205. https://doi.org/10.3201/eid1908.130465

Hardstaff, J. L., Clough, H. E., Lutje, V., McIntyre, K. M., Harris, J. P., Garner, P., & O’Brien, S. J. (2018). Foodborne and food-handler norovirus outbreaks: A systematic review. Foodborne Pathogens and Disease, 15(10), 589–597. https://doi.org/10.1089/fpd.2018.2452

Henshilwood, K., Doré, W., Anderson, S., & Lees, D. (2003). The development of a quantitative assay for the detection of Norwalk-like virus and its application to depuration. Molluscan Shellfish Safety, 451–465. Retrieved from https://www.who.int/foodsafety/publications/micro/MRA_16_JEMRA.pdf

Hoelzer, K., Fanselase, W., Pouillot, R., Doren, J. M., & Dennis, S. (2013). Virus inactivation on hard surfaces or in suspension by chemical disinfectants: Systematic review and meta-analysis of norovirus surrogates. Journal of Food Protection, 76(7), 1006–1016. https://doi.org/10.4315/0362-028X.JFP-12-438

Hutson, A. M., Aird, F., LePendu, J., Estes, M. K., & Atmar, R. L. (2005). Norwalk virus infection associates with secretor
Norovirus in shellfish risk assessment

status genotyped from sera. Journal of Medical Virology, 77(1), 116–120. doi:10.1002/jmv.20423

Jansen, R. W., Newbold, J. E., & Lemon, S. M. (1985). Combined immunofluorescence-DNA-RNA hybridization assay for detection of hepatitis A virus in clinical specimens. Journal of Clinical Microbiology, 22(6), 984–989.

Kator, H., & Rhodes, M. (2001). Elimination of fecal coliforms and F-specific RNA coliphage from oysters (Crassostrea virginica) reared in floating containers. Journal of Food Protection, 64(6), 796–801.

Kindberg, E., Akerlund, B., Johnsen, C., Knudsen, J. D., Heltberg, O., Larson, G., … Svensson, L. (2007). Host genetic resistance to symptomatic norovirus (GGI/4) infections in Denmark. Journal of Clinical Microbiology, 45(8), 2720–2722. https://doi.org/10.1128/jcm.00162-07

Kingsley, D. H., Fay, J. P., Calci, K., Pouillot, R., Woods, J., Chen, H., … Van Doren, J. M. (2017). Evaluation of chlorine treatment levels for inactivation of human norovirus and MS2 bacteriophage during sewage treatment. Applied Environmental Microbiology, 83(23). https://doi.org/10.1128/AEM.01270-17

Lee, D., & Doré, W. (1992). The behaviour of F specific bacteriophage in depurifying shellfish with reference to their use as pollution indicator organisms. Paper presented at the Conference Internationale sur la Purification des Coquillages, 6–8 April, Rennes, France.

Liaw, A., & Wiener, M. (2002). Classification and Regression by randomForest. R News, 2(3), 18–22.

Lindesmith, L., Moe, C., Marionneau, S., Ruvoen, N., Jiang, X., Lindblad, L., … Baric, R. (2005). Human susceptibility and resistance to Norwalk virus infection. Nature Medicine, 9(5), 548–553. https://doi.org/10.1038/nm860

Loisy, F., Atmar, R. L., Le Saux, J. C., Cohen, J., Caprais, M. P., Pommepuy, M., & Le Guyader, F. S. (2005). Use of rotavirus virus-like particles as surrogates to evaluate virus persistence in shellfish. Applied and Environmental Microbiology, 71(10), 6049–6053. https://doi.org/10.1128/Aem.71.10.6049-6053.2005

Maatoul, H., Schaeffer, J., Parnaudeau, S., Le Pendu, J., Atmar, R. L., Crawford, S. E., & Le Guyader, F. S. (2011). Strain-Dependent Norovirus Bioaccumulation in Oysters. Applied and Environmental Microbiology, 77(10), 3189–3196. https://doi.org/10.1128/AEM.03010-10

McLeod, C., Hay, B., Grant, C., Greening, G., & Day, D. (2009). Inactivation and elimination of human enteric viruses by Pacific oysters. Journal of Applied Microbiology, 107(6), 1809–1818. https://doi.org/10.1111/j.1365-2672.2009.04373.x

Messner, M. J., Berger, P., & Napper, S. P. (2014). Fractional poisson—a simple dose-response model for human norovirus. Risk Analysis, 34(10), 1820–1829. https://doi.org/10.1111/risa.12207

National Centers for Environmental Information/National Oceanic and Atmospheric Administration. (2019). NOAA Satellite and information service. Retrieved from https://www.nco.ncep.noaa.gov/

National Shellfish Sanitation Program (NSSP). (2017). Guide for the control of mussels shellfish. College Park, MD: Food and Drug Administration (FDA), and the Interstate Shellfish Sanitation Conference (ISSC) Retrieved from https://www.fda.gov/media/117080/download

Neish, A. (2013). Investigative trials on the purification of oysters to identify ways of reducing norovirus. Retrieved from https://www.cefas.co.uk/media/52851/2013-cefas-contract-report-c5224.pdf

Payne, D. C., Currier, R. L., Staats, M. A., Sahni, L. C., Selvarangan, R., Halasa, N. B., … Morrow, A. L. Parashar, U. D. (2015). Epidemiologic association between FUT2 secretor status and severe rotavirus gastroenteritis in children in the United States. JAMA Pediatrics, 169(11), 1040–1045. https://doi.org/10.1001/jamapediatrics.2015.2002

Pouillot, R., Van Doren, J. M., Woods, J., Plante, D., Smith, M., Goblick, G. N., … Calci, K. R. (2015). Meta-analysis of the reduction of norovirus and male-specific coliphage concentrations in wastewater treatment plants. Applied Environmental Microbiology, 81(14), 4669–4681. https://doi.org/10.1128/Aem.00509-15

R Development Core Team. (2019). R: A language and environment for statistical computing. Retrieved from http://www.R-project.org

Rothart, H. A. (1990). Enzymatic RNA amplification of the enteroviruses. Journal of Clinical Microbiology, 28(3), 438–442.

Said, M. A., Perl, T. M., & Sears, C. L. (2008). Healthcare epidemiology: Gastrointestinal flu: Norovirus in health care and long-term care facilities. Clinical Infectious Diseases, 47(9), 1202–1208. https://doi.org/10.1086/592299

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., … Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. Emerging Infectious Disease, 17(1), 7–12.

Schaeffer, J., Treguier, A., Piquet, J. C., Gachelin, S., Cochenec-Laurereau, N., Le Saux, J. C., … Le Guyader, F. S. (2018). Improving the efficacy of sewage treatment decreases norovirus contamination in oysters. International Journal of Food Microbiology, 286, 1–5. https://doi.org/10.1016/j.ijfoodmicro.2018.07.016

Schmidt, P. J. (2015). Norovirus dose-response: Are currently available data informative enough to determine how susceptible humans are to infection from a single virus? Risk Analysis, 35(7), 1364–1383. https://doi.org/10.1111/risa.12523

Shieh, Y. C., Baric, R. S., Woods, J. W., & Calci, K. R. (2003). Molecular surveillance of enterovirus and norwalk-like virus in oysters relocated to a municipal-sewage-impacted gulf estuary. Applied Environmental Microbiology, 69(12), 7130–7136. https://doi.org/10.1128/ae69.12.7130-7136.2003

Smyth, D. J., Cooper, J. D., Howson, J. M., Clarke, P., Downes, K., Mistry, T., … Todd, J. A. (2011). FUT2 Nonsecretor status links type 1 diabetes susceptibility and resistance to infection. Diabetes, 60(11), 3081–3084. https://doi.org/10.2337/db11-0638

Statistics Canada. (2020). Quarterly demographic estimates October to December 2019. Retrieved from https://www150.statcan.gc.ca/n1/pub/91-002-x/91-002-x2019004-eng.htm

Tennis, P. F., Moe, C. L., Liu, P., Miller, S. E., Lindemith, L., Baric, R. S., … Calderon, R. L. (2008). Norwalk virus: How infectious is it? Journal of Medical Virology, 80(8), 1468–1476. https://doi.org/10.1002/jmv.21237

Thebault, A., Tennis, P. F., Le Pendu, J., Le Guyader, F. S., & Denis, J. B. (2013). Infectivity of GI and GII noroviruses established from oyster related outbreaks. Epidemics, 5(2), 98–110. https://doi.org/10.1016/j.epidem.2012.12.004

Thomas, M. K., Murray, R., Flochhart, L., Pinar, K., Pollari, F., Fazil, A., … Marshall, B. (2013). Estimates of the burden of foodborne illness in Canada for 30 specified pathogens and unspecified agents, circa 2006. Foodborne Pathogens and Disease, 10(7), 639–648. https://doi.org/10.1089/fpd.2012.1389

Thorley, J., Fleishman, A., & Miller, L. (2017). ride: Tide heights. Retrieved from https://CRAN.R-project.org/package=ride

Thorven, M., Grahn, A., Hedlund, K. O., Johansson, H., Wahlinrd, C., Larson, G., & Svensson, L. (2005). A homozygous nonsense mutation (428G→A) in the human secretor (FUT2) gene provides resistance to symptomatic norovirus (GGIIIH) infections. Journal Virology, 79(24), 15351–15355. https://doi.org/10.1128/jvi.79.24.15351-15355.2005

Tung, G., Macinga, D., Arbogast, J., & Jaykus, L. -A. (2013). Efficacy of commonly used disinfectants for inactivation of human noroviruses and their surrogates. Journal of Food Protection, 76, 1210–1217. https://doi.org/10.4315/0362-028X.JFP-12-532

Ueki, Y., Shoji, M., Suto, A., Tanabe, T., Okimura, Y., Kikuchi, Y., … Omura, T. (2007). Persistence of caliciviruses in artificially contaminated oysters during depuration. Applied
**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table A.1: Oyster annual landings used in the study in pounds of meat.

Table A.2: Distribution of average oyster meat plus mantle fluid weight derived from the quotients of pooled oyster meat plus mantle fluid weights and numbers of oysters in each pool collected as part of the DePaola et al. (2010) market survey.

Table A.3: Beta distribution parameter $\text{Beta}(\alpha, \beta, \theta, 1)$ estimates fit to oyster meat to oyster meat plus mantle fluid ratio data collected in the Kaufman et al. (2003) study.

Table A.4: Reference stations for the Tides and overall amplitude (m).

Fig. A.1: Relationship between dilution range and tidal range.

Table A.5: Reference sunlight energy (J/cm$^2$) for each month based on data from 1995, as used in the risk assessment. Model from Gueymard (2005), results are expressed as in Nguyen et al. (2014) (Equation 3, page 3893).

Table A.6: WWTP characteristics according to the region.

Table A.7: Expected number of NoV illnesses per 100 meals of raw oysters as a function of region and season.

Table A.8: Number of cases for 100 meals of raw oysters as a function of WWTP type and region (Dilution at low tide: 1000:1, Season: Winter).