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Current decontamination challenges and potentially complementary solutions to safeguard the vulnerable seafood industry from recalcitrant human norovirus in live shellfish: Quo Vadis?

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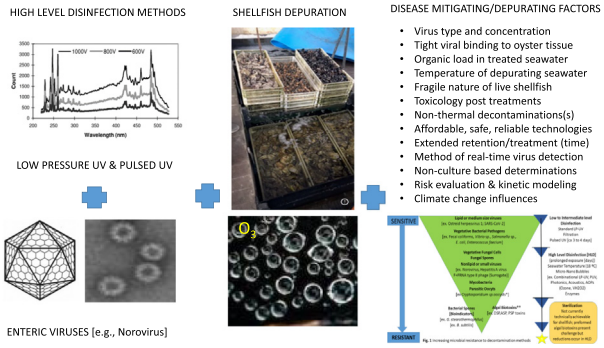
HIGHLIGHTS

- Choice of appropriate non-thermal intervention(s) is limited as shellfish are consumed raw.
- Depuration using standard UV-irradiation effectively addresses bacterial contaminants in live shellfish.
- Additional non-thermal mitigation strategies are required to completely destroy norovirus in live oysters.
- New diagnostic method(s) are required to confirm non-viable norovirus post depuration.
- Global warming of coastal growing environments may increase risks for commercial shellfish producers.

GRAPHICAL ABSTRACT



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ABSTRACT

Safeguarding the seafood industry is important given its contribution to supporting our growing global population. However, shellfish are filter feeders that bioaccumulate microbial contaminants in their tissue from wastewater discharged into the same coastal growing environments leading to significant human disease outbreaks unless appropriately mitigated. Removal or inactivation of enteric viruses is very challenging particularly as human norovirus (hNoV) binds to specific histo-blood ligands in live oyster tissue that are consumed raw or lightly cooked. The regulatory framework that sets out use of clean seawater and UV disinfection is appropriate for bacterial decontamination at the post-harvest land-based depuration (cleaning) stage. However, additional non-thermal technologies are required to eliminate hNoV in live shellfish (particularly oysters) where published genomic studies report that low-pressure UV has limited effectiveness in inactivating hNoV. The use of the standard genomic detection method (ISO 15, 216–1:2017) is not appropriate for assessing the loss of infectious hNoV in treated live shellfish. The use of surrogate viral infectivity methods appear to offer some insight into the loss of hNoV infectiousness in live shellfish during decontamination. This paper reviews the use of existing and potentially other combinational treatment approaches to enhance the removal or inactivation of enteric viruses in live shellfish. The use of alternative and complementary novel diagnostic approaches to discern viable hNoV are discussed. The effectiveness and virological safety of new affordable hNoV intervention(s) require testing and validating at commercial shellfish production in conjunction with laboratory-based research. Appropriate risk management planning should encompass key stakeholders including local government and the wastewater industry. Gaining a mechanistic understanding of the relationship between hNoV response at molecular and structural levels in individually treated oysters as a unit will inform predictive modeling and appropriate treatment technologies. Global warming of coastal growing environments may introduce additional

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contaminant challenges (such as invasive species); thus, underscoring need to develop real-time ecosystem monitoring of growing environments to alert shellfish producers to appropriately mitigate these threats.

Contents

1. Introduction

The seafood industry is an important sector of food production worldwide (Ruiz-Salmön et al., 2020). An estimated 18 million tons of marine molluscs are globally harvested each year with an estimated value of $35 billion, comprising 9 % of the value of fisheries worldwide (Sharp et al., 2021). Shellfish consumption is an increasingly important part of human diet and is an emerging area for economic growth worldwide (Ruiz-Salmön et al., 2020; Ruiz-Salmön et al., 2021; Laso et al., 2022; Cooney et al., 2023). This is particularly relevant given that our growing global population recently reached eight billion people. Shellfish filter hundreds of litres of coastal water for nutrients and can bioaccumulate human pathogens including norovirus from their growing marine environment, if contaminated with faecal material (Compos and Lees, 2014). Human norovirus is found in high concentrations in faeces (10^{11} virus/g) (La Rosa et al., 2012); consequently, this virus is recognized as a high risk for environmental transmission (McLeod et al., 2017: Sharp et al., 2021). Pilotto et al. (2019) reported concentrations of 10^{11} genome copies/per gram (cg/g) of murine norovirus (MNV1) that were achieved during 24 h bioaccumulation in the Pacific oyster *Crassostrea gigas*; whereas, Maalouf et al. (2011) noted that both human norovirus genogroups GI and GII were shown to simultaneously bioaccumulate in *C. gigas* oysters to 10^6 cg/g. Contamination of raw seafood with pathogenic microorganisms occurs through primary production and from infected food handlers (McLeod et al., 2017; Sharp et al., 2021).

In many parts of the world, microbiological pollution of coastal areas with human sewage, and agricultural run-off, can readily occur in commercial harvesting such that shellfish bioaccumulate large amounts of bacterial and/or viral pathogens (Bosch et al., 1995; Winterbourn et al., 2016); thus, requiring to be appropriately cleaned (depurated) (Rupnik et al., 2018; Razafimahefa et al., 2020; Rupnik et al., 2021). The faecal indicator organism *Escherichia coli* is frequently used as a general indicator of sewage contamination and for assessing the effectiveness of shellfish depuration processes (Sharp et al., 2021). These authors reported that non-pathogenic *E. coli,* pathogenic *E. coli* O157:H7 and hNoV GII RNA accumulate rapidly in mussels using simulated water contamination after a point-source release from a combined sewer overflow (CSO) and untreated wastewater released directly into the coastal zone. "All three microbiological indicators reached close to maximum concentration within 3 h of exposure, demonstrating that short CSO discharges pose an immediate threat to shellfish harvesting areas" (Sharp et al., 2021). Depuration in clean seawater proved partially successful at removing pathogenic and non-pathogenic *E. coli* from shellfish tissue, but failed to eradicate hNoV GII RNA. The authors concluded that current EU standards for evaluating microbiological risk in shellfish are inadequate for protecting consumers against exposure to hNoV; thus, intimating a need to improve depuration efficiencies including developing new appropriate mitigation technologies (McLeod et al., 2017; McMenemy et al., 2018).

It is notable that hNoV is the leading viral cause of human gastroenteritis, where consuming contaminated shellfish contributes as a vector in this transmission (Yu et al., 2015). In developed countries, it accounts for 95 % of non-bacterial foodborne outbreaks, and over 50 % of all microbial outbreaks (Dewey-Mattia, 2018; Wikswo et al., 2021). In the US alone, noroviruses are responsible for ~20 million cases and >70,000 hospitalizations of infected children, annually (Smith and Smith, 2019). Oysters are frequently implicated as the source of this human gastroenteritis illness posing a particular risk to young, elderly and immunocompromised (Centers for Disease Control and Prevention (CDC), 2020). While most adults recover from viral diarrhoea, such illness in young children can lead to hospitalization and life-threatening dehydration (Centers for Disease Control and Prevention (CDC), 2020). Notably, contaminated shellfish are estimated to cause between 9 and 34 % of all foodborne norovirus cases in the US (Pouillot et al., 2021), and similar etiological ranges have been reported internationally (Havelaar et al., 2008; Davidson et al., 2011; Advisory Committee on the Microbiological Safety of Food, 2015). No vaccine currently exists that can prevent hNoV infection (Centers for Disease Control and Prevention (CDC), 2020). Best published evidence suggests that oysters are a main source of foodborne hNoV-transmission due to (a) the mode of transmission (as they mainly consumed raw or lightly cooked) (Rupnik et al., 2018); (b) production of oysters in same intertidal waters where human sewage is discharged as a source of hNoV (Rupnik et al., 2018; Sharp et al., 2021); and (c) specific retention of hNoV strains in oysters through binding to ligands enabling lengthy persistence (McLeod et al., 2017).

Oysters are premium food products of economic importance that are consumed globally (McLeod et al., 2017); however; failure to appropriately depurate contaminated shellfish can lead to reputational damage to the exporting industry and a commensurate loss of confidence for importing countries (Younger et al., 2020). Currently, it is technically challenging to confidently address the effective removal or destruction of recalcitrant enteric viruses, particularly from live contaminated shellfish (Leduc et al., 2020; Younger et al., 2020; Rupnik et al., 2021). The Pacific oyster (*C. gigas*) is the most commonly produced oyster globally; but other species are also commercially harvested including *Crassostrea viginica* (the Eastern oyster) in the US, *Saccostrea glomerata* in Australia, and flat oysters (*Ostrea edulis*) that are produced in many countries including Ireland, the United Kingdom, and Croatia (McLeod et al., 2017).

Norovirus is a small (approximately 30 nm in diameter) non-enveloped, single-stranded RNA virus that belongs to the family Caliciviridae (Sharp et al., 2021). Norovirus was named after the original Norwalk strain, which caused an outbreak of gastroenteritis in a school in Norwalk, Ohio in 1968. Noroviruses are now classified into ten genogroups (GI-GX) and 48 genotypes where typically GI and GII predominate (McLeod et al., 2017; Chhabra et al., 2019; Rupnik et al., 2021). While wastewater treatment plants (WWTPs) can contribute to enteric virus removal, it appears that improvements in the effectiveness of technologies or processes are required to fully remove or inactivate enteric viruses from human sewage (Barrett et al., 2016). Data published in a recent EFSA baseline survey highlights that peak hNoV concentration and prevalence in contaminated oysters was observed in the months of January and February when almost 65 % of samples tested were positive for hNoV [mean concentration of 661 genome copies/g] (EFSA, 2019).

Hepatitis A Virus (HAV) is also a small (approximately 30 nm in size), non-enveloped, icosahedron-shaped, RNA enteric virus that contaminates shellfish in polluted seafood leading to human illness (Woods and Burkhardt III, 2010). A vaccine exists for HAV (Centers for Disease Control and Prevention (CDC), 2020). Coincidently, viruses with icosahedral-shaped capsids efficiently package their RNA (Martin-Bravo et al., 2021), which will be discussed later in this review in the context of a focus for real-time detection and as a structural target for decontaminating enteric viruses of similar geometry (such as hNoV and HAV). Notably, researchers have reported on geometric defects and icosahedral viruses that may influence assembly, dissociation, or accessibility of cellular proteins to virion components (Wang et al., 2018). Many complex shellfish pathogens, such as these enteric viruses and waterborne protozoan enteroparasites (such as *Cryptosporidium* oocysts, and *Giardia* cysts), do not grow on standard laboratory based culture media, and require use of more sophisticated enumerations methods post-treatment, such as quantitative PCR (ISO standard) (Garvey et al., 2010; Garvey et al., 2013; Hayes et al., 2013; Gerard et al., 2019; Franssen et al., 2019; Sharp et al., 2021; Rupnik et al., 2021). There are in vitro cell culture methods to study the viability of treated surrogate enteric viruses (Barrett et al., 2016) and for waterborne parasites (Garvey et al., 2014a; Garvey et al., 2014b), which can be combined with the standard qPCR method to inform decontamination. Currently, there is no appropriate *in vitro* model for studying the infectivity of hNoV strains that has hindered development, testing and standardization of treatment approaches for the shellfish industry, particularly at commercial depuration phase (McLeod et al., 2017; Rupnik et al., 2018; Rupnik et al., 2021). Sophisticated diagnostic techniques are not routinely available in standard food-testing laboratories for enteric viruses; for example, hNoV and HAV are classified as belonging to Human Pathogen Hazard Group II viral pathogens necessitating use of more specialized Cat II facilities (Centers for Disease Control and Prevention (CDC), 2020).

These enteric viruses have a low infectious dose and may remain infectious for weeks in the environment or on food surfaces (Nasheri et al., 2021). However, limited physiological or mechanistic information is available regarding viral survival, persistence and transmission in contaminated shellfish. Interestingly, Kokkinos and co-workers (2021) noted that "the vast majority of viral agents, which are transmitted via the faecal-oral route are non-enveloped, highly stable under environmental conditions, characterized by extremely small size, and include emerging and re-emerging Caliciviridae, Adenoviridae, Hepevirdae, Picornaviridae and Reoviridae. The enteric viruses of human stool and urine belong to more than 140 types (Kokkinos et al., 2011) where untreated wastewater has been identified as the most diverse viral metagenome examined thus far. Most sequence reads have little or no sequence relations to known problematic viruses, underscoring that most of the viruses have yet to be characterized", and are underestimated in prevalence (Cantalupo et al., 2011).

Previous researchers have reported that under EU law, sanitary classification of shellfish production areas is recognized based on the presence and concentration of the faecal bacterium *Escherichia coli* as designated EU Regulation 627/2019 (Rupnik et al., 2021; Hunt et al., 2023). Currently, the minimum time and water temperature used for commercial depuration are not stipulated in EU Regulation, however, Rupnik et al. (2021) had noted that such depuration should be performed for a minimum of 42 h with a water temperature of no <8 °C in Ireland. Specifically, shellfish harvesting waters are classified as A, B, or C, which is based on increasing *E. coli* concentrations measured in shellfish flesh and fluid (Hunt et al., 2023). Consequently, within each class, specific post-harvest decontamination methods such as depuration and relaying are mandated before any live product can be sold (Rupnik et al., 2018; Hunt et al., 2023). However, studies have indicated that monitoring faecal indicator bacteria in shellfish may be a poor indicator of water pollution and the risk of human exposure to pathogens from consuming shellfish (Romalde et al., 1994; Younger et al., 2018). Essentially, "bacterial species are traditionally used as indicators of faecal contamination of agricultural products, shellfish and shellfish waters" (Garcia et al., 2020). This led to the aforementioned formulation of legislation based on the measurement of faecal indicator bacteria (EU, 2020). Previous researchers have reported that there is a poor correlation between concentrations of *E. coli* and norovirus, making it less relevant as an indicator organism (Flannery et al., 2009; Lowther et al., 2019; Hunt et al., 2023). Although methods for detection and quantification of problematical pathogens (e.g., *Vibrio* spp.) in shellfish exist, these also have yet to be incorporated into EU legislation due to the lack of robust evidence and non-consensus agreements over what new standards should be incorporated (Hassard et al., 2017; EFSA, 2019; Sharp et al., 2021).

Post-harvest disease mitigation typically occurs in land-based tanks containing clean seawater. Duration of treatment is governed by several biological and environmental factors that are informed by the monitoring of enteric virus concentration (or viral load) (Rupnik et al., 2021). Thus, oysters and other bivalve molluscan shellfish harvested from class B category waters (accounting for the majority of overall oyster production from European countries) (McLeod et al., 2017), must undergo appropriate depuration before human consumption (EFSA, 2019). These regulations have informed the effective decontamination of bacterial-associated illness caused by contaminated oysters; however, despite same, there are numerous reports of enteric viral outbreaks caused by depurated oysters (Rajko-Nenow et al., 2013). For example, Doré et al. (2010) noted that illnesses have also been reported for the consumption of contaminated oysters that were harvested from category A waters where post-harvest decontamination is not mandatory. Consequently, in order to mitigate against the occurrence of enteric viral illness and to avoid the loss of consumer confidence in shellfish products, many commercial producers apply depuration treatment for oysters harvested from category A growing waters as part of their risk management procedures (Rupnik et al., 2018).

The most widely practised post-harvest treatments is depuration, whereby bivalve shellfish undergo self-purification in land-based tanks containing clean seawater (McLeod et al., 2017). However, the effect of depuration is reducing harmful norovirus in live shellfish is less well established compared with treating *E. coli*, particularly at the standard conditions of 48 h at 8 to 15 °C (McLeod et al., 2017; Hunt et al., 2023). The use of viral surrogates is a common approach to studying depuration efficacy such as using F + RNA bacteriophage type I or II (designated FRNA or FRNAPII) where there are established infectivity assays to offset this technical problem for assessing hNoV post treatments using the ISO standard detection method (Polo et al., 2014; Rupnik et al., 2018; Leduc et al., 2020; Rupnik

et al., 2021). "F-specific coliphages (F+ coliphages) are bacteriophages that infect *Escherichia coli* cells possessing F pili. F+ coliphages are classified into FDNA phages (FDNAPHs) or FRNA phages (FRNAPHs), depending on whether their genomes consist of single-stranded DNA or single-stranded RNA, respectively" (Hata et al., 2016). Oysters can bioaccumulate high concentrations of enteric viruses after a few hours (Flannery et al., 2012; Pilotto et al., 2019) which promotes rapid contaminated with potentially several hNoV strains. Optimal reduction of norovirus load was previously reported in the region of 1 $\log_{10}$ (McLeod et al., 2017). Other experiments have reported marginal improvements in norovirus reductions, particularly using elevated seawater temperature (>11 °C) during depuration (Rupnik et al., 2021).

Oysters retain smaller particles, such as hNoV that bind to these particles depending on their isoelectric point (McLeod et al., 2017). However, it is also appreciated that the recognition of hNoV persists for longer periods than bacteria where oysters are depurated due to specific hNoV-ligand mechanisms (McLeod et al., 2017) as also reported in humans (Hutson et al., 2002). This is one of the main reasons for viral persistence in oysters compared to bacteria where contaminated shellfish can retain virus copies for weeks or months after initial exposure; thus, acting as a reservoir for foodborne transmission (Maalouf et al., 2010; Mathijs et al., 2012). For example, hNoV GI.1 strain binds to the midgut and digestive diverticula of Pacific oysters, but not to other tissues (Le Guyader et al., 2012). While hNoV GII strain binds to various tissue types including digestive diverticula, midgut (intestine), gills, mantle, and labial palps, McLeod et al. (2017) has provided a comprehensive review of factors affecting hNoV binding in oysters including seasonal variations where these authors note that published findings support strain-specific variations in hNoV binding patterns. Oysters retain hNoV through specific ligand-binding in tissues that affects selective accumulation and persistence that may help explain their long retention in oysters (as observed in depuration, McLeod et al., 2017). However, there appears to be a lack of published studies on the ability of oysters to specifically bind surrogate viruses that would inform the comparative ability for infectious viral removal or deactivation at depuration. Hunt et al. (2023) advocated the need for new monitoring methods and regulatory regimens for the specific hazard of enteric viruses in oysters to better manage this risk. EFSA (2019) recommends better understanding of the actual risk associated with positive hNoV test results from contaminated production areas for all genogroups. However, Hunt et al. (2023) stated that "where to apply quantitative thresholds remains a core question in addition to what post-harvest or other interventions could be applied to further manage the risk of norovirus contamination in oysters. To determine the best methods for controlling shellfish virus risk is currently a matter of live discussion in the EU".

Consequently, this constitutes the first review to compare the effectiveness of different established and emerging technologies affecting the decontamination of enteric and surrogate viruses in live oysters. It also considers other complementary novel approaches for the real-time detection of viable and infectious hNoV strains to that of using a standard genomic method (RT-qPCR) that may potentially inform their effectiveness and advance studies underpinning the virological safety of shellfish, particularly addressing oysters.

2. Methods for human norovirus removal or destruction in contaminated live shellfish

A review of publications from PubMed and Scopus database over period 1981 to 2023 was used to address this research topic using PRISMA guiding framework. Of the combined keywords, norovirus (n = 7781) and "depuration" (n = 50), 43 publications were deemed eligible based on criteria: on the follow; (a) norovirus detection, persistence, and accumulation in shellfish, (b) decontamination technologies and operational factors affecting effectiveness for live shellfish applications; (c) use of surrogate microorganisms; (d) kinetic inactivation modeling and (e) foodborne transmission outbreaks. Seven publications were excluded for the reason that they addressed post processing of non-living shellfish; use of chlorine and heating in shellfish, food distribution chain and studies on norovirus aerosols in wastewater production plants. Combining "depuration"-based publications with "enteric viruses" (n = 10,305) over the period 1988 to 2023 revealed 22 matching publications where 6 were different to the aforementioned "norovirus" publication list. These addressed treatment of hepatitis A virus (HAV) in mussels using a closed circulatory system, treatment of the surrogates HAV, poliovirus type 1 and coliphage MS2 in shelled clambs, public health implications of viral-contaminated mussels, and development of diagnostic tests. One study was excluded for the reason that it addressed virological control of contaminated ground water that is not the subject of this review.

Decontamination of live shellfish is mainly carried out at the commercial depuration phase post-harvest using clean seawater where in-line or bolt-on solutions must be non-thermal in nature to mitigate against damage to the treated live oysters (Rupnik et al., 2021). "Acceptable post-harvest treatments available to ensure oysters meet the *E. coli* standard include self-purification in land-based tanks containing clean seawater by a process called depuration or relaying bivalve shellfish to clean marine locations for an extended period (four weeks)". Norovirus-related gastroenteritis outbreaks have occurred even when oysters have been demonstrated to be fully compliant with regulatory end-product standards. Therefore, the combination of marine harvest area controls and post-harvest treatments as currently practised does not completely protect consumers from the virological safety risk associated with norovirus-contaminated shellfish (Rupnik et al., 2018). Findings from various researchers would support the idea that shorter, less-intense solar irradiation in the winter (such as in Northern hemisphere countries) may also contribute substantially to the environmental persistence of human noroviruses in shellfish (Younger et al., 2020; Rupnik et al., 2021).

Typically, solutions for live shellfish decontamination must consider inter alia: the volume of live seafood products to be treated; the cellular and molecular mechanism(s) of action of the applied decontamination approach(es) ensuring irreversible inactivation, or removal; the microbial load or initial starting population including composition, such as the type of pathogen(s) present as these frequently differ in behaviour and level of susceptibility to decontamination methods, particularly hNoV (Fig. 1); the amount of decontaminant applied or intensity of removal/disinfection methods; the exposure or treatment time (hours, days); the appropriate inactivation kinetic performance of applied decontamination approaches (log linear, or bi-/tri-phasic adaptive microbial responses); the environmental parameters (seawater depuration temperature, salinity, pH, presence of interfering suspended solids or turbidity), nutritional factors (oysters are consumed raw; thus nutritional and organoleptic characteristics must be maintained post non-thermal treatments); scalability; affordability; the availability of subject-matter technical assistance for equipment operation; operator safety (UV-irradiation), detection and infectivity enumeration methods; and biocompatibility post treatments including environmental (ecotoxicological) compliance. The sequence of susceptibility of treated microorganisms (and surrogates) to applied disinfection technologies, as shown in Fig. 1, is indicative as it is appreciated that the mode of action of biocidal action may differ depending upon the type of treatment methods. For example, specific hNoV strains (such as G1) may generally be more tolerant to a variety of technologies as specifically bind to ligands in oyster tissues that may confer greater protection (Leduc et al., 2020; Rupnik et al., 2021) (Table 1); thus, they are potentially more recalcitrant compared to treating similar hNoV genotypes under less complex planktonic situations as often replicated in laboratories. Waterborne parasites (such as *Cryptosporidium parvum* oocysts or *Giardia lamblia* cysts) appear to exhibit greater tolerance to reactive oxygen species (ROS) associated with advanced oxidative processes (AOP) yet appear to be more susceptible to UV-irradiation (Garvey et al., 2014a; Garvey et al., 2014b). However, combined use of UV/H_2O_2 appears more effective for microbial disinfection compared to separately using the treatment technologies.

Effective post-harvest processing technologies that are recognized by regulators (such as the US FDA) have had limited acceptance in the domestic industry due to the high initial capital equipment costs and the

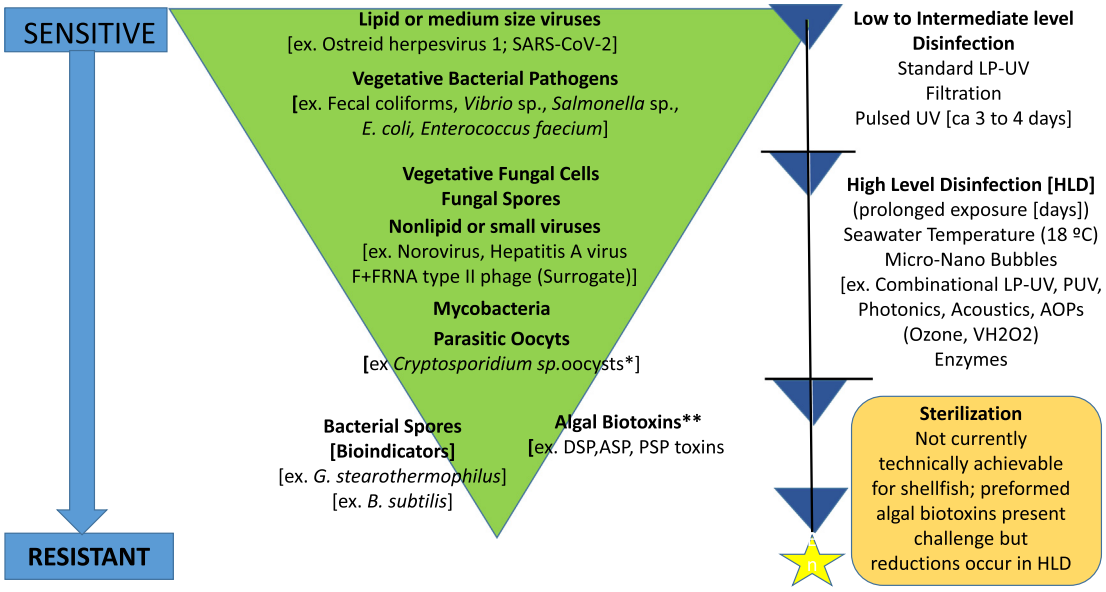

Fig. 1. Increasing microbial resistance to decontamination methods.

economics of transporting and storing shell oysters. While advances have been made to try to standardize or harmonize non-thermal disinfection technologies for food-based processes including packaging (Gómez-López et al., 2022), there is a marked knowledge gap in the development and reporting of appropriate treatment technologies (used alone or combined) for commercial-scale seafood applications, particularly, for technologies or approaches that can address complex challenges including enteric viruses, parasitic oocysts, algal toxins and other potential contaminants of concern (McLeod et al., 2017; Fehrenbach et al., 2022). However, the majority of physical and chemical disinfection technologies used in food and adjacent medical device processing industries would not be deemed appropriate for live shellfish decontamination. For example, researchers have highlighted similar challenges to effectively process and sterilize heat-sensitive medical devices containing complex materials; however, such Medtech-approved chemical disinfectants (such as glutaraldehyde), and terminal sterilization modalities (gamma, x-ray, electron beam, ethylene oxide are not appropriate for use in seafood depuration due to killing of live shellfish, cost of equipment, and general non-practicalities. However, the use of vaporized hydrogen peroxide by the medical device industry may have a future role in commercial shellfish decontamination. In addition, there is a pressing commensurate need to establish the safety of new technologies including their environmental impact. For example, Hayes et al. (2013) reported that pulsed-plasma gas-discharge (PPGD) in oxygen-sparged water generated a range of short-lived highly-oxidative biocidal properties killing enteric bacterial pathogens and recalcitrant waterborne parasitic oocysts. This PPGD technology has been applied for contact food surface decontamination studies (Rowan et al., 2007, However, despite demonstrating biocidal efficacy, this PPGD system was reported to produce unwanted toxicological endpoints post treatments making it unsuitable for the intended use (Hayes et al., 2013). This is also relevant given scalability issues surrounding the transition from early discovery (lab-based) to full commercial testing of technologies at depuration where there are limited published studies addressing shellfish decontamination. Such an innovative disease mitigation topic is underappreciated when considering the combined technological (TRL), societal (SRL) and policy readiness level (PRL) framework for evaluating new decontamination approaches ranging from discovery to full commercial deployment (as exemplified by Rowan and Casey, 2021), particularly for safely and affordably treating live shellfish. Given the amount of key governing factors, complexity of data generated and the need to achieve real time outcomes for effective decision-making (Naughton et al., 2020), potential practical disease mitigation solutions will be informed, supported and enabled by digital technologies (Rowan, 2022; Rowan et al., 2022).

2.1. Using conventional low-pressure UV irradiation and filtration methods

Reference to the use of UV has appeared in 198,506 journals since 1946 to present day (combined PubMed and Scopus), where 117 journals combine UV with norovirus. A total of 48 papers were excluded from consideration in this review as they focused on the combined use of UV with other approaches (such as heating, H_2O_2 generating wipes, peracetic acid, TiO2) for treating strawberries, onions, and lettuce or treating reclaimed water a golf course, simulating vomiting, SARS-CoV-2 (COVID-19) applications, therapeutic applications for intestinal microbiome, solar radiation, electrochemical paper-based devices, and chlorination

UV processes have been developed for food and water disinfection applications and have been divided into three main categories (a) low pressure/low intensity lamps (b) low pressure/high intensity (c) medium pressure/high intensity lamps (Fitzhenry et al., 2021). Barrett et al. (2016) noted that these UV systems vary with respect to operating pressure and output level; for example, it is low and medium pressure assets that are deployed for treating wastewater. Also, turbidity and suspended solids can affect UV disinfection efficiency by decreasing transmissivity (i.e., the transmission of UV light through the water body) (Barrett et al., 2016). Yet, these factors are not routinely considered when monitoring UV performance. UV light technologies have supported the effective treatment of drinking water for decades. Low pressure (LP) UV disinfection results in photochemical damage to viral RNA thus inhibiting viral reproduction (Fitzhenry et al., 2021). Advances have been recently made in the reduction of norovirus in contaminated oysters at the depuration phase using filtration and LP-UV (Rupnik et al., 2018; Leduc et al., 2020; Younger et al., 2020; Rupnik et al., 2021) (Table 1).

Previous researchers have reported that depuration time and seawater temperature are parameters that may influence human viral decontamination efficacy (Lees and Cen, 2010; Rupnik et al., 2018), but these parameters are not currently stipulated in EU regulation (Rupnik et al., 2021). Rupnik et al. (2021) reported that laboratory-based studies revealed up to 74 % of initial norovirus GII concentrations were achieved after three days of 17–21 °C [designated 'high' depuration temperature], and after 11 °C to 15 °C [designated 'medium' depuration temperature], compared to 44 % reductions at 7 °C to 9 °C [designated 'low' depuration temperature] where artificial seawater (mimicking water salinity of estuary) was treated

Table 1
Examples of the application of LP-UV or Pulsed UV irradiation for the decontamination of norovirus with relevance to shellfish.

Target	Source	Treatment	Method	Characteristics	Duration	Pre-depuration	Post-depuration Log10 reduction		Reference
NoV GI	WWTP effluent	LP-UV	RT-qPCR*	Flow through 60s, 120 S	24 h	10^6/100 mL	0.09 ± 0.04 to 0.52 ± 0.06 [60s]	0.15 ± 0.12 to 0.7 ± 0.6 [120 s]	Barrett et al. (2016)
NoV GII	WWTP effluent	LP-UV	RT-qPCR*	Flow through 60s, I20 S	24 h	10^6/100 mL	−0.11 ± 0.55 to 0.4 ± 0.5 [60s]	−0.11 ± 0.65 to 0.7 ± 0.1 [120 s]	Barrett et al. (2016)
FRNAPH	WWTP effluent	LP-UV	RT-qPCR	Flow through 60s; 120 s	24 h	106/100 mL	−0.43 to 1.07 ± 0.27 [60s]	−1.27 ± 2.31 to 1.1 ± 0.2 [120 s]	Barrett et al. (2016)
FRNAPH	WWTP effluent	LP-UV 117 mJ/cm^2	Plaque Assay	HRT (0.23 m^3/h	24 h	10^5 PFU/mL	Ca 2 log		Barrett et al. (2016)
FRNAPH	WWTP effluent	LP-UV 51.6 mJ/cm^2	Plaque Assay	HRT (0.52 m^3/h	24 h	10^5 PFU/mL	Ca 2 log		Barrett et al. (2016)
FRNAPH	WWTP effluent	LP-UV 31.6 mJ/cm^2	Plague Assay	HRT (0.85 m^3/h	24 h	105 PFU/mL	Ca 1.5 log		Barrett et al. (2016)
FRNAPH	WWTP effluent	PUV 3.4 J/cm^2	Plague Assay		24 h	10^7 PFU/mL	1.24 to 2.21		Barrett et al. (2016)
FRNAPH	WWTP effluent	PUV 6.9 J/cm^2	Plague Assay		24 h	10^7 PFU/mL	1.4 to 3.14 ± 0.03		Barrett et al. (2016)
FRNAPH	WWTP effluent	PUV 1.2 J/cm^2	Plague Assay	120 s [900 V, 1 pps]	24 h	10^7 PFU/mL	1.3		Barrett et al. (2016)
FRNAPH	WWTP effluent	PUV 0,55 J/cm^2	Plague Assay	120 s [600 V, 1 pps]	24 h	10^7 PFU/mL	1.0		Barrett et al. (2016)
FRNAPH	WWTP effluent	PUV 0.14 J/cm^2	Plague Assay	HRT 120 s [300 V, 1 pps]	24 h	10^7 PFU/mL	0.4		Barrett et al. (2016)
FRNAPH	WWTP effluent	PUV 3.4 J/cm2	Plague Assay	60s, SS (57.5 mg/L); ToC 8 mg/L; TIC 24 mg/L	24 h	10^7 PFU/mL	2.2 ± 0.2		Barrett et al. (2016)
FRNAPH	WWTP effluent	PUV 6.9 J/cm2	Plague Assay	120 s, SS (57.5 mg/l); ToC 8 mg/l; TIC 24 mg/L	24 h	10^7 PFU/mL	3.1 ± 0.2		Barrett et al. (2016)
FRNAPH	Unfiltered WWTP effluent	PUV 3.4 J/cm2	Plague Assay	60s	24 h	10^6 PFU/mL	2.3 (Site 1)	Barrett et al. (2016)	Barrett et al. (2016)
FRNAPH	Unfiltered WWTP effluent	PUV 6.9 J/cm2	Plague Assay	90s	24 h	10^6 PFU/mL	<1 PFU/ml (6 log) Site 1)	Barrett et al. (2016)	Barrett et al. (2016)
NoV GI	*G. gigas*	Not stated	RT-PCR [LoQ 100 gcg^{-1}]	Commercial Production Site A 12–16 °C	7 d	251 gcg^{-1}	121 (0.4 log or 67.5 % reduction)		Rupnik et al., 2021.
NoV GI	*G. gigas*	Not stated	RT-PCR [LoQ 100 gcg^{-1}]	Commercial Production Site B 18 °C	3 d	241 gcg^{-1}	129 (0.4 log or 66.4 % reduction)		Rupnik et al., 2021.
NoV GII	*C. gigas*	Not Stated	RT-PCR [LoQ 100 gcg^{-1}]	Commercial Production Site A 12–16 °C	7 d	281 gcg^{-1}	45 (0.79 log or 83.95 % reduction)		Rupnik et al., 2021.
NoV II	*C. gigas*	Not Stated	RT-PCR [LoQ 100 gcg^{-1}]	Commercial Production Site B 18 °C	3 d	526 gcg^{-1}	66 (0.90. log or 87.47 reduction)		Rupnik et al., 2021.
NoV GII	C.gigas	LP-UV (3 W) No fluence	RT-PCR [LoQ 100 gcg^{-1}]	Lab; D.O. 80 % **8 °C**, 2000 L/h	1–7 d	290 gcg^{-1} [example]	310 gcg^{-1} (d1); 284 (d2); 142 (d3); <LoQ (d4); 249 (d5); 147 (d6); 225 (d7)		Rupnik et al., 2021
NoV GII	C.gigas	LP-UV (3 W) No fluence	RT-PCR [LoQ 100 gcg^{-1}]	Lab; D.O. 80 % **12 °C**, 2000 L/h	1–7 d	452 gc/g$^-$ (example)	143 gcg^{-1} (d1); 231 (d2); 202 (d3); < LoQ (d4); <219 (d5);181 (d6); <LoQ (d7)		Rupnik et al., 2021
NoV GII	C.gigas	LP-UV (3 W) No fluence	RT-PCR	Lab; D.O. 80 % **18 °C**, 2000 L/h	1–7 d	405 gc/g$^-$	236 (d1); 147 (d2); <LoQ (d3); <LoQ d4) < LoQ (d5) < LoQ (d6); <LoQ (d7)		Rupnik et al., 2021
NoV GII	C.gigas	LP-UV (3 W) No fluence	RT-PCR [LoQ 100 gcg^{-1}]	Lab; D.O. 80 % **20 °C**, 2000 L/h	1–7 d	290 gcg^{-1} [example <1000 gc/g	165 (d1); <LoQ (d2); <LoQ (d3); <LoQ (d4); <LoQ (d5); <LoQ (d6); <LoQ (d7)		Rupnik et al., 2021
NoV GII	C.gigas	LP-UV (3 W) No fluence	RT-PCR [LoQ 100 gcg^{-1}]	Lab; D.O. 80 % **20 °C**, 2000 L/h	1–7 d	5573 gcg^{-1} [example >1000 gc/g]	4670 (d1); 5896 (d2); 2296 (d3); 2414 (d4); 1769 (d5); 1513 (d6); 2054 (d7)		Rupnik et al., 2021
NoV GI	Oyster digestive tissue	LP-UV (80 to 90 mJ/cm^2)	RT-qPCR	10 °C	1 to 43 d	293 gcg^{-1}	47.8 days (Time for 1 log reduction in genomic material)		Leduc et al., 2020
NoV GI	Oyster digestive tissue	LP-UV (80 to 90 mJ/cm^2)	RT-qPCR	10 °C	1 to 43 d	802 gcg^{-1}	26.7 days (Time for 1 log reduction in genomic material)		Leduc et al., 2020
FRNAPH	Oyster	LP-UV (80 to 90 mJ/cm^2)	RT-qPCR	10 °C	1 to 43 d	463 gcg^{-1}	43.9 days (Time for 1 log reduction in genomic material)		Leduc et al., 2020
NoV GI	Oyster	LP-UV (80 to 90 mJ/cm^2)	**ICC**-qRT-PCR	10 °C	1 to 43 d	463 gcg^{-1}	26.7 days (Time for 1 log reduction in infectious particles)		Leduc et al., 2020

Low Pressure UV (LPUV); Pulsed UV (PUV); Suspended Solids (SS); Total Organic Carbon (TOC), Total Inorganic Carbon (TIC), Waste Water Treatment Plant (WWTP).

with a 36 W UV-C lamp (fluence was not reported) (Table 1). Rupnik et al. (2021) determined norovirus GI and GII concentrations in contaminated oysters using standardized quantitative real-time reverse transcription PCR (RT-qPCR) in accordance with ISO, 2017. Seawater was circulated and maintained within 1 °C of the target depuration temperature and bar sprinklers were deployed for aeration where dissolved oxygen levels were kept in excess of 80 % in all trials. "Oysters were analysed for the presence of norovirus GI and GII before depuration where norovirus GII concentrations in environmentally contaminated oysters ranged from 178 to 16,426 norovirus gc/g (noting, the limit for quantification [LOQ] and limit of detection [LOD] for Nov GII were ≤100 genome copies/g and 20 genome copies/g respectively)" (Rupnik et al., 2021). Results showed that heavily initial norovirus GII concentrations (≥850 genome copies/g) remained above 300 genome copies/g in oysters irrespective of depuration water temperature after seven days of treatment (Rupnik et al., 2021). For example, samples were reduced from 1739 to 1248 norovirus GI concentration (genome copies/g) at 14 °C [medium temperature]; and from 5573 to 2054 norovirus GII concentration (genome copies/g) at 20 °C [high temperature], after seven days depuration. The authors noted that the ability to reduce hNoV concentration in oysters to <LOQ (100 genome copies/g, or 2

log hNoV g^{-1} potentially remaining) using this LP-UV treatment method differed when contaminated below or above 1000 genome copies/g (or 4 log hNoV g^{-1} present).

These important findings have potentially profound implications for determining the choice of disinfection technology for complementary or alternative use to LP-UV to improve human NoV removal or inactivation efficiencies in oysters that can be informed by viral kinetic inactivation modeling data (Rowan et al., 2015). In addition, the potential use of viral monitoring and screening approach for contaminated oysters ($\leq$1000 NoV genome copies/g) may inform suitability for achieving appropriate levels of disinfection treatment based on current disinfection methods at depuration, or for relaying to clean seawater for four weeks (>1000 genome copies/g) from a risk-based assessment and virological safety perspective. However, the RT-qPCR detection method was solely used by Rupnik et al. (2021) where there is no infectivity assay for NoV; therefore, it is uncertain if the genomic copies of NoV per gram detected in oyster post these LP-UV treatments were viable where the use of ISO 15216-1 standard may underestimate the level of NoV lethality or removal achieved. It is appreciated that the combinational laboratory-commercial decontamination study of Rupnik et al. (2021) has made a significant contribution to advancing this technical NoV challenge in oysters. This is timely given that the EFSA has published a baseline survey on comprehensive scientific data for norovirus prevalence in European oyster harvesting areas that will inform future safety limits on norovirus concentration in oysters.

Rupnik et al. (2021) also noted that norovirus reductions were also assessed in two Irish commercial depuration systems that are routinely used to produce oysters. The authors reported up to 68 % reduction for hNoV GI and up to 90 % for hNoV GII reduction. This finding also supports the general observation by other researchers that intimates hNoV GI exhibits greater tolerance or persistence over hNoV GII strain possibly due to the specific behaviour of ligand binding in oyster tissue (Leduc et al., 2020; Younger et al., 2020). Additionally, other researchers have also deployed standard UV decontamination for removing norovirus and FRNAPII from oysters during depuration using genome and infectivity assays (Leduc et al., 2020; Younger et al., 2020). Specifically, these studies intimate that standard UV irradiation of seawater under aerated depuration circulatory conditions either inactivates human norovirus GI and GII genogroups (Leduc et al., 2020 [UV dose, 80 to 90 mJ/cm^2], or, destroys and removes this enteric virus (Younger et al., 2020 [2 × 25 W lamps, no UV dose was reported]); thus, highlighting that greater studies are required to understand the mechanism of viral reduction and residual potential to cause infection at low concentrations (Table 1). Younger et al. (2020) found approximately 46 % removal of hNoV GII at 18 °C after two days and 60 % after five days compared with a maximum of 16 % hNoV GI removal. These researchers noted that "twice the rate of NoV GII removal was achieved at 18°C compared with 8°C after five days. Younger et al. (2020) also found that FRNAP-II was more readily removed than noroviruses. Notably, no significant difference was found between the rate of removal (as measured by RT-qPCR) and inactivation (as measured by bioassay) of FRNAPII". Younger et al. (2020) inferred from their results that the reduction in FRNAPII may be primarily due to physical removal (or destruction) rather than in situ inactivation of the virus. Also, the efficacy of RT-qPCR method to confirm the viability of the remaining norovirus post treatments remains questionable given that this molecular approach does not distinguish between live or dead viruses. Leduc et al. (2020) reported that FRNAPII infectivity bioassay (presence of viable phage) may be appropriate for informing disinfection effectiveness of viable hNoV given that a 1 log reduction in FRNAPII infectivity occurred after 20.6 days treatment using LP-UV (80 to 90 mJ/cm^2) compared to its genome (43.9 days) and NoV GI genome (47.8 days). Leduc et al. (2020) reported that "FRNAPII and NoV genomes may display similar behaviours with low kinetic removal from the oysters under all purification conditions tested". In terms of surrogate representation of hNoV, Lowther et al. (2019) reported that "both viruses were in high concentrations in outbreak-related samples and that infectious FRNAPII were detected in all outbreak samples (n = 9)". Leduc et al. (2020) and Lowther et al. (2019) suggest combining RT-qPCR testing with a test for infectious FRANPII detection in order to improve the hNoV risk assessment in shellfish. Leduc et al. (2020) also highlighted the importance of developing appropriate testing to identify new strategies for the effective elimination of hNoV, as addressed in this review. FRNAPII bacteriophages have been studied for shellfish decontamination due to their structural similarity to waterborne viruses and proof of faecal pollution coming from urban areas (Leduc et al., 2020).

Recent results underscore the importance of determining the typical range of norovirus prevalence and concentration in contaminated oysters to deploy appropriate decontamination methods. However, the best practice would be 'a multi-actor approach to strategically manage this problem by working with local government authorities and the water industry to reduce the occurrence of human enteric viruses in sewage effluent that may be discharged into the same intertidal growing areas where commercial oyster production occurs. Thus, placing greater emphasis on disease prevention, rather than relying on introducing complex removal strategies at the depuration phase for these enteric viruses in live oysters where there are currently limited appropriate solutions.

Jeong et al. (2021) also reported that after 60 h of depuration equipped with a standard UV light source *Vibrio vulnficus* cell numbers were reduced by <4.0 log MPN/g in Pacific oyster tissue from an initial population of ca. 8 log MPN/g. Lee (2020) reported that the use of standard UV for treating seawater in depuration tanks could extend the shelf life (two to three days) of raw oysters with minimal changes in food quality with faecal coliforms maintained at or below 2 log/g compared to non-depurated and generally packaged oysters. Depuration at temperatures between 7 °C and 15 °C using UV-treated seawater reduced *V. parahaemolyticus* populations in oysters by >3 log MPN/g after five days with no loss of live oysters (Phuvasate et al., 2012). The US National Shellfish Sanitation Program established time/temperature regulations that limit maximum hours of holding shellfish from harvest to refrigeration ($\leq$10 °C) to reduce the risk of infections from *Vibro* spp. associated with shellfish consumption (Reid and Durance, 2000).

2.2. Pulsed light (PL) technology

Only 6 of 4239 "Pulsed UV" and "Pulsed Light" publications [2010 to 2023] have focused on treating norovirus or enteric viruses when assessing PubMed and Scopus databases. One study was excluded for the reason that it focused on PPE decontamination. Ten publications were also included that provided supporting context to background technology. Pulsed light (PL) technology is an exciting approach that delivers ultra-short bursts of broad spectrum (200 nm to 1100 nm light) (Rowan, 2019) and is commercially deployed by companies such as Claranor (France, https://www.claranor.com/en/) for food packaging sterilization with over 500 units installed in 52 countries. The benefits of using PUV reflect the potentially ultra-short disinfection against viruses, waterborne protozoan parasite oocysts (*Cryptosporidium parvum*) (Garvey et al., 2014a, 2014b) and cysts (*Giardia lamblia*) (Garvey et al., 2014b), and biological endospore indicators using laboratory-based static or limited flow-through treatment configurations (Garvey et al., 2010; Rowan, 2011; Hayes et al., 2012; Garvey et al., 2013; Garvey and Rowan, 2015). PL has been referred to as high-intensity pulsed UV light (HIPL), pulsed UV (PUV), high-intensity broad spectrum UV light (BSPL), intense light pulsed (ILP) and pulsed white light (PWL) (Rowan et al., 1999; Rowan, 2019). PL has been approved by the US FDA in the production, processing and handling of foods since 1996 up to cumulative UV dose (or fluence) of 12 J cm^{-2} where emission spectra are to be kept between 200 and 1100 nm and pulsed duration $\leq$2 ms (Rowan, 2019). The technological principle of PL disinfection is based upon the accumulation of high discharge voltage in a capacitor where the stored energy is delivered in ultra-short pulses through a light source filled with xenon gas. The xenon-light source emits a broad spectrum light flash typically in the range of ca. 200 to 1100 nm with approximately 25 % in the UV range (Gómez-López et al., 2022). PL disinfection efficiency is higher compared with continuous-wave low-pressure UV irradiation (CW-UV) due to its high peak power along with the ability to deliver stored

energy over short durations, typically 1 to 10 pulses per second (Garvey and Rowan, 2019). The main reporting parameters governing effective PL operation for disinfection are the fluence [J cm^{-2}], exposure time [s], number of pulses applied [*n*], pulse width [τ], frequency [Hz], and the peak power [W] (Rowan et al., 2015; Gómez-López et al., 2022). Garvey et al. (2015) reported on a satisfactory ecological assessment of pulsed UV light treated water containing microbial species and *Cryptosporidium parvum* using a microbiotest test battery.

Very limited studies have been conducted on the inactivation of pulsed light for inactivating enteric viruses that have focused on bench-scale applications (Barrett et al., 2016). Barrett et al. (2016) compared the efficiency of pulsed light irradiation and low-pressure UV irradiation as a means of hNoV and FRNA bacteriophage using secondary treated wastewater effluent. While hNoV GI and GII inactivation could not be determined, it was found that a maximum UV dose of 6.9 J/cm^2 (at a hydraulic residence time of 120 s in a flow-through system) achieved 2.4 log $_{10}$ reduction of FRNA bacteriophage, which indicates the need for high pulsed UV doses to fully remove NoV (Table 1). Vimont et al. (2015) reduced murine NoV using pulsed UV by 3 log_{10} in <3 s (fluence, 3.45 J/cm^{-2}), while Jubinville et al. (2022) reported on the efficacy of pulsed UV against HAV on berries. Three studies have discussed the potential of using pulsed UV for enteric-virus decontamination on foods or for environmental applications (Jean et al., 2011; Pexara and Govaris, 2020).

All pulsed UV dosage rates related to wavelengths <300 nm and the average initial FRNAPII concentration was 10^6 PFU/mL (Barrett et al., 2016). The authors also found that increasing concentration of suspended solids impacted PUV disinfection efficiency. The use of LP-UV reduced FRNAPII phage by ca. 2 log_{10} using a significantly reduced UV dose of 31 mJ/cm^2; however, the combined use of LP-UV and pulsed light for enteric virus reductions were not considered. Results also indicate that absorption of viral particles to solids in wastewater occurs; therefore, it would be prudent to also introduce a barrier process such as a tangential flow filtration system that filters the particulate matter and the flow through when using UV-irradiation. Interestingly, PUV was capable of achieving up to 3 log_{10} reduction in FRNAPII infectivity within 24 h treatment under varying conditions (such as flow rates [120 s], total suspended solids [57.5 mg/L]) in unfiltered secondary wastewater effluent with enhanced destruction (up to 6 log_{10} PFU/ml) achieved in filtered secondary wastewater effluent (Table 1). This finding contrasts with other previously reported LP-UV studies that required up from 3 to 47 days to achieve a l log reduction in NoV and FRANPII in shellfish during depuration (Table 1). Thus, there is potential to augment enteric viral destruction in contaminated shellfish using a combinational LP-UV and PUV approach. PUV offers the benefits of delivering a broad high-intensity light spectrum that includes wavelengths in the blue light spectrum (such as 406 nm) that has been previously reported to generate hydroxyl radicals from oxygen in water via an advanced oxidation process (AOP) (Kingsley et al., 2018). Barrett et al. (2016) also noted that as RT-qPCR provided inconsistent results; therefore it was deemed not an appropriate method for assessing the inactivation of NoV and FRNAPII via pulsed light after treatments. Other researchers also observed this discrepancy (Pecson et al., 2011). Baert et al. (2009) found RT-qPCR results were unable to distinguish between infectious and non-infectious NoV using murine norovirus (MNV) as surrogate post-heat treatments. Uslu et al. (2016) employed PUV as a wastewater disinfection tool and indicated that in addition to pathogen removal/inactivation, it also reduces the organic load of municipal wastewater effluent by reducing chemical oxygen demand and total organic carbon.

However, Fitzhenry et al. (2021) recently reported that LP-UV is superior to that of using pulsed light for converting energy through the light source to UV dose for submerged wastewater-treatments. Thus, advances in PUV design is required (such as improving light source including the use of light emitting diodes, introducing more light sources such as in parallel; reflective surfaces for light scattering, and inclusion of smart materials that includes photocatalysis of TiO_2 for localized ROS generation) in order to realize the dual benefits of combining PUV (broad spectrum) and fixed-wavelength LP-UV for improved decontamination effectiveness. The authors report that a pulsed UV system output of 2052 mJ/m^{2-} (energy below 300 nm) was required for a 2 log inactivation of *Bacillus pumilus*, where low a lower LPUV system output of 12 mJ/cm^2 produced a similar level of inactivation in flow-through water systems. Complete inactivation of *B. pumilus* was achieved via LP-UV disinfection using a UV output of 30 mJ/cm^2. Fitzhenry et al. (2021) reported that "while a typical xenon gas PUV system is comprised of light emissions within the broad spectrum range of UV, and infrared light, it may be an important consideration to prioritize UV dose/output calculations in terms of 'biocidal PUV dose/out' (i.e., the energy applied from wavelengths below 300 nm ahead of the 'total PUV dose/output', which infers the total energy applied across the whole spectrum output). This has been previously demonstrated in a number of studies with the aid of spectrometer/pyroelectric detectors and in some cases the UV dose/output from PUV systems in within the same order of magnitude as LPUV dose/outputs such as 1 – 100 mJ/$m^{2"}$. However, measurements from Fitzhenry et al. (2021) indicated that only 26 % of the lamp energy reached the same sample (at 900 V and a distance of 10.75 cm), and of that, only 8 % was within the UV wavelength range.

Interestingly, the PUV and flow-through treatment configuration used by Fitzhenry et al. (2021) is the same system that was used by Barrett et al. (2016) to inactivate NoV GI and GII and FRNAPII phage with moderate success (such as 2.4 log reduction with 6.8 J/cm^2) (Table 1). This outcome could be improved by replacing the xenon source with a specific wavelength light-emitting diode (LED). For example, Wen et al. (2022) report UV-LEDs are safe algicidal technologies for inactivating the marine microalgae *Tetraselmis* sp. These authors showed that the wavelength of 265 nm exhibited maximum inactivation efficiency, whereas 285 nm achieved optimal energy efficiency. UV irradiation is also affected by turbidity where viruses can be protected on particles, which must also be considered at the depuration stage, particularly in developing countries where water quality per se may not be appropriate for depuration.

Garvey and Rowan (2015) reported a reduction of 4.23 log_{10} in *Bacillus megaterium* vegetative cells using a UV dose of 6.48 μJ/cm^2 with no significant further microbial reduction after doubling the UV dose to 12.96um in a flow-through PUV system at a retention time of 60s and flow rate of 30 L/h. However, only a 1.48 log_{10} and 1.43 log_{10} a reduction occurred of *B. megaterium* and *B. cereus* endospores respectively at 12.98 μJ/L (RT 60s), indicating reduced efficacy of PUV in treating recalcitrant pathogens in submerged flow-through treatment configuration. The presence of inorganic contaminants did not significantly reduce PUV efficacy at the concentrations used.

2.3. Potential combined use of advanced oxidative processes (AOPs)

Of the 1764 published studies focusing on advanced oxidation processes or "AOPs" appearing in PubMed and Scopus databases over period 1981 to 2023, only 3 addressed norovirus. The reason for including AOPs in this review reflected the emergence of AOPs as non-thermal approaches to improve and enable decontamination performances for food treatments and for environmental applications. One was excluded for the reason that it was COVID-19 orientated. However, there is an increased sharing of knowledge on the potential for using advanced oxidation processes (or AOPs) for enteric viral destruction. This aligns with the need for new or more efficient methods that will destroy NoV in shellfish (Gerba et al., 2018; Kokkinos et al., 2021). AOPs relay on the in situ formation of chemical oxidants to disinfect liquids and degrade diverse harmful organic contaminants (Shabat-Hadas et al., 2017). Kokkinos et al. (2021) noted that "AOPs are, in practice, redox technologies that encompass different processes such as ozonation, ozonation coupled with hydrogen peroxide, and/or UV radiation, Fenton and alike reactions, photocatalysis activated by semiconductors, sonolysis, electrochemical oxidation, and various combinations of these." They are based on the generation of highly reactive oxygen species (ROS), characterized by the non-selectivity of the target and can be deployed before or after treatment of a biological process (Galeano et al., 2019).

The principle oxidizing agent is the hydroxyl radical; however, other ROS may be produced including hydroperoxyl radicals and superoxide radical anions (Shabat-Hadas et al., 2017). Kokkinos et al. (2021) noted that photo-Fenton AOP in which hydroxyl radicals are produced from light, iron and H_2O_2 is a well-studied, environmentally-friendly, simple, low-cost process that inactivates complex or resistant microorganisms (Giannakis et al., 2017). Enteric viruses and a wide range of recalcitrant microorganisms are inactivated through the action of ROS such as singlet and triplet oxygen, anion-radical superoxide, hydroxyl and hydroperoxyl radical, and H_2O_2. ROS are recognized oxidants for inactivating a variety of molecules including proteins, lipids and nucleic acids. "When considering the lethal action of ROS on treated enteric viral nucleic acids, this AOP can change the nucleotides, break important phosphodiester bonds, enhance the formation of pyrimidine dimers, change the tri-dimensional structure, and affect RNA replication" (Kokkinos et al., 2021). Recently, photo-Fenton and alike processes have been developed as a 'green' alternative to chemical disinfection (such as the use of chlorine) for water and wastewater applications (Giannakis, 2018). Evaluation of the disinfection efficacy using light-mediated AOPs has been based on using varying methods such as computational fluid dynamics, chemical actinometry or biodosimetry (Shabat-Habas et al., 2017). An understanding of these assessment methods is critical to informing the efficacy of shellfish decontamination processes from a standardization, reliability and repeatability perspective (as per Rowan, 2019).

The utility of UV technologies has been enhanced through the combinational use of AOPs over the last few decades (Timchak and Gitis, 2012); particularly as an emerging high-efficiency technique for disinfecting enteric viruses leaving no unwanted disinfection by-products (Chu et al., 2012). Microbial inactivation is achieved through UV-induced photochemical reactions on genetic material (Rowan, 2019). Endogenous (direct) inactivation encompasses the absorbance of UVB light by the treated viral genome that causes its degradation. While the full antiviral mechanistic process has yet to be fully elucidated at molecular or structural levels, it is appreciated that UVC/UVB are strongly absorbed by enteric viral RNA with additional decontamination effects. "UVA cannot damage RNA and has no direct photochemical reactions; but it can produce reactive intermediates such as ROS (such as hydroxyl and superoxide radicals, hydrogen peroxide and so forth), which in turn can damage critical microbial targets (such as proteins, nucleic acids)" (Kokkinos et al., 2021). Thus, the role of using an optimised pulsed light technology that produces an intense broad spectrum (200 nm to 1100 nm) may potentially enhance AOP efficacy for enteric virus disinfection. Interestingly, compared to DNA, RNA is known to be more susceptible to the lethal action of UV irradiation (Galeano et al., 2019). The UVB/UVA and visible light wavelengths are absorbed by different water sensitizers through exogenous (or indirect) disinfection processes, such as organic matter, nitrate, and iron-containing complexes. Thus, the type of virus, the suspension menstruum (including suspended solids) and conditions (such as pH, temperature) are important governing factors affecting disinfection efficacy for MS-2 virus (Kosel et al., 2017).

Various AOP approaches have been studied including combined UV with H_2O_2 for treating complex wastewater such as in the meat processing industry (Yapıcıoğlu, 2018). Another technique potentially applicable for the treatment of circulating water in the depuration tanks is pulsed-plasma gas-discharge. Such an AOP produces hydrodynamic cavitation that causes viral disinfection through photochemical (production of hydroxyl radicals), and physical mechanisms (pressure gradients, shock and acoustic waves, shear forces, and very high local temperatures) (Kosel et al., 2017). The mechanisms of hydrodynamic cavitation have yet to be elucidated, but it is theorized that it causes disruption to the viral capsid (icosahedral) and destabilizes recognition receptors.

Mycoystin_LR (MC-LR) is produced by cyanobacteria that attract attention due to its high toxicity and high concentration in aquatic systems. Lu et al. (2018) showed that the combined use of UV/H_2O_2 process and O_3/H_2O_2 were effective methods to remove MC-LR from water and they performed better that UV-, O_3-, H_2O_2 alone processes under the same conditions. However, UV dosage of 1800 mJ/cm^2 was required to remove 90 % Of 100 mg/L MC-LR, where the amount significantly decreased to 500 mJ/cm^2 when 1.7 mg/L H_2O_2 was added. Murray et al. (2017) reported that the use of pulsed light reduced the toxicity of the dinoflagellate algal toxin okadaic acid where ecotoxicological assessments were also performed using a miniaturised format of the conventional in vivo freshwater crustacean *Daphnia* sp. acute toxicity test. Findings revealed a 24-h EC50 of 25.87 μg/L for PL-treated okadaic acid at a UV dose of 12.98 μJ/cm^2 compared to a 24-h EC50 of 1.68 μg/L for the untreated okadaic acid control, suggesting a 15-fold reduction in toxicity to *Daphnia pulex*.

Despite positive observations as a clean decontamination technology, it remains uncertain as to the mechanism by which cavitation generated bubbles clean, disinfection and kill microbial organisms including viruses and enhance chemistry activity (Zupanc et al., 2019). Zupanc et al., 2019 also reported that "cavitation describes the formation of small vapour bubbles (cavities) inside an initially homogeneous liquid medium. It is a rapid physical phenomenon triggered by a sudden decrease in pressure. As the pressure recovers the bubble goes through a violent collapse and possible rebounds. By bubble growth, an energy from the surrounding liquid is collected and released by bubble collapsation, where extreme conditions can be formed locally. Bubble collapse can cause pressure shocks up to several 100 MPa and if the bubble collapses asymmetrically the so-called microjets with high velocities above 100 m/s can form". These observations are also aligned with the related studies of Chahine and Hsiao (2015). In addition, the so-called hot spots with extreme temperatures in order of several 1000 K can form at the centre of the bubble at its collapse, which can cause the formation of highly reactive radicals (Koda et al., 2003). Zupanc et al. (2019) also stated that the "exact manifestation of cavitation is influenced by liquid properties (temperature, density, viscosity and surface tension) and quality (number of solid particles and amount of dissolved gasses, which can both act as a nuclei). In general, two types of cavitation are recognized, hydrodynamic and acoustic cavitation. The difference is in the mechanism, which causes the local pressure to drop, while the principles which govern the hydrodynamic bubble and the acoustic bubble are basically the same".

The effects of cavitation including mechanical and thermal effects are: (a) microsteaming that can damage microorganism – together with shockwaves generated by bubble collapse; (b) chemical effects (implosion of bubbles and formation of hot spots for homolytic cleavage of H_2O molecules and formation of highly oxidizing free radicals (*OH and *H) - *OH readily oxidize particulate matter and also form H_2O_2 – many other species can form ($*O_2H$, *N, *, $1O_2$) where different gases air/oxygen are dissolved in water; (c) oxidation of miroorganism's constituents (AC process produces a level of ROS beyond microbial antioxidant stress response capabilities) and kills a broad range of pathogens including viruses (McDonnell and Russell, 1999). Also different ROS affects polysaccharides, proteins, lipids and RNA/DNA); (c) oxidation of lipids, polysaccharides and nucleic acids by oxidative stress initiative by (ROS). High-frequency ultrasound inactives *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Legionella pneumophila, Enterobacter aerogenes, Vibrio cholera, Salmonella enterica*, coliforms and *Mycobacterium* species albeit at different rates. However, different cellular and molecular mechanisms were postulated including cell wall breakage for *Bacillus subtilis* where this was the only study to report on simultaneous and sequential mechanisms using a pulsed plasma-gas-discharge approach (Hayes et al., 2013), where a similar approach was used to explain the lethal action of pulsed light (Farrell et al., 2011). Variations in effectiveness were also evident between using cavitation treating microalgae including *Scendeesmus* sp. The majority of researchers noted that the effect of cavitation on microalgae and microorganisms depends on the ultrasound frequency and that higher intensities and longer exposures correlate to more effective lethality (Wu et al., 2012). MS2 virus appears to be very susceptible to inactivation with all types of cavitation (Su et al., 2010) regardless of the initial concentration and medium tested only that in the case of higher concentrations more time for inactivation was needed. The exact mechanism of how cavitation causes virus inactivation is not yet elucidated. Su et al. (2010) suggested that the damages

inflicted during cavitation on the outer protein capsid itself or recognition sites on the capsid surface could be the reason for virus inactivation.

2.4. Photonics

There is also a potential role for developing photonics as a combined method for destroying enteric viruses, such as using visible blue light wavelengths (380–480 nm) that have been safely deployed for the inactivation of microbial pathogens in healthcare settings; thus, enabling the simultaneous activities of healthcare workers (Tomb et al., 2018). Maclean et al., 2009 have postulated that blue (405 nm) light inactivates bacteria via its interactions with porphyrins (five and six carbon multi-ring contain alternating single and double bonds) within the membrane of bacteria. The Irish SME, Atlantic Photonic Solutions Ltd., is developing innovative photonic solutions for the aquaculture Industry (APS, 2023). This company has identified specific wavelengths that can eliminate complex parasites without harming the hosts, such as high-throughput removal of sea lice from salmon in commercial aquaculture processes.

Kingsley et al. (2018) reported on oxygen-depended laser inactivation of murine norovirus using visible light lasers. Kingsley et al. (2018) used a tuneable mode-locked Sapphire laser where the frequency was doubled to generate femtosecond pulses at wavelengths of 400, 408, 450, 465 and 510 nm. >3 log murine NoV were achieved after 3 h exposure at 408, 425 or 450 femtosecond light pulses; thus, viral destruction was not wavelength specific. The use of photosensitizers, such as riboflavin, rose Bengal or methylene blue that generates singlet oxygen substantially improved the efficiency of inactivation. Findings indicate "a photochemical mechanism of the laser-induced inactivation where the action of relatively low-power blue laser light generates singlet oxygen" (Kingsley et al., 2018).

Kingsley et al. (2018) also noted that atmospheric sunlight at sea level contains visible light (400–700 nm), but also contains a UV component that is predominately 300–400 nm (Bird et al., 1983) which does not substantially penetrate water as UV is strongly absorbed. Recent research has indicated that sunlight can interact with dissolved organic molecules in aqueous settings to catalyse the formation of O_2 ($a^1\Delta_g$), commonly referred to as singlet oxygen, as well as other reactive oxygen species (Rosado-Lausell et al., 2013). These complex organic molecules act as sensitizers that become energetically excited by UV–Vis light and then transfer electronic energy to oxygen molecules producing singlet oxygen. Ultrashort pulse laser (USPL) light treatments were previously shown to be capable of inactivating murine norovirus (MNV) and other viruses (Tsen et al., 2007; Tsen et al., 2012); where impulsive stimulated Raman scattering (ISRS) was the postulated inactivation mechanism (Tsen et al., 2014; Tsen et al., 2014). Essentially the ISRS hypothesis was that high-frequency resonance vibrations are potentially induced by the 425 nm USPL, with a bandwidth of 420–430 nm, which may be capable of causing vibrations of sufficient strength such that the icosahedron capsid is destroyed after short nonthermal treatments (Kingsley et al., 2018). However, Kingsley et al. (2018) demonstrated that the inactivation of MNV by the USPL with variable wavelengths of blue light (400–450 nm) and by 408 nm CW indicated the inactivation mechanism was not specific to the 425 nm wavelength and, although more substantial inactivation was achieved with femtosecond pulse light, was not dependent on light pulses. This suggested that inactivation may have been via a non-ISRS mechanism. The addition of sodium bisulfate, an oxygen scavenger, substantially reduced CW laser inactivation, strongly implicating singlet oxygen as the cause of visible laser light-induced virus inactivation.

Kingsley et al. (2018) noted that as a priori, one would not expect vibrational resonance induced by ISRS to necessarily be wavelength-dependent. However, due to pulse-width dependence, it is well understood that ISRS cannot be induced using CW lasers since they do not generate light pulses. The authors demonstrated that CW lasers can inactivate MNV, which potentially conflicts with the hypothesis that ISRS is the mechanism of inactivation as proposed by Tsen et al. (2014) although it remains formally conceivable that both ISRS and singlet oxygen mechanisms could both contribute to laser inactivation observed by the USP laser. Also, it is difficult to envision a scenario in which the low concentrations of sodium bisulfate that reacts with, and sequesters, dissolved oxygen molecules within the MNV sample would inhibit a laser-induced vibrational mechanism. Indeed it was noted that purified MNV and MNV from a cell lysate had roughly equivalent inactivation rates (2014) suggesting that singlet oxygen enhancers are not substantially present in virus stocks, which are derived from virus-infected cell lysate. This suggests that either the virus capsid itself may function as an endogenous enhancer molecule, or that an enhancer may not be strictly required to produce singlet oxygen when interacting with intense blue light. This finding offers a potential explanation as to why norovirus illness was originally termed the 'winter vomiting disease.' The use of photonics has significant potential for nonthermal inactivation of microbial and parasitic pathogens, including, potentially, seafood.

2.5. Use of micro- and nano-bubble technology

Increased aeration and mixing can be potentially achieved in seawater depuration tanks using bulk micro-nanobubble (NB) water using hydrodynamic cavitation (Zhou et al., 2022). Nanobubbles (NBs) show technological potential in commercial applications including wastewater treatment, floatation, agriculture, nanoscope cleaning, and biological and medical applications (Ghadimkhani et al., 2016; Sun et al., 2022). The gas mix could be adjusted for delivery of hydrogen peroxide or ozone to accelerate disinfection of NoV deep in tissue. Such applications are attributed to the unique properties of NBs including low ascending velocity, long longevity retention time, massive interfacial surface area, high internal gas pressure, and negatively charged surface characteristics. Ultrafine NBs can form at the solid-liquid interface and in solutions and have a diameter of between 10 and 100 nm (Alheshibri et al., 2016). For commercial depuration of contaminated oysters, bubble concentrations and sizes in bulk NB water under different conditions should be evaluated along with the putative relationship between dissolved oxygen concentration and cavitation behaviours of bubbles used for this purpose. Thus, NBs have received attention for their unique physicochemical characteristics, including the previously mentioned large specific surface area, long residence time, high gas-liquid mass transfer efficiency, high zeta potential, and reactive oxygen species production (Zhang et al., 2022a, 2022b). Notably, NBs have exhibited outstanding performance, particularly in food safety and quality. Jaykus and Green (2015) describe a funded pilot-scale project addressing the efficacy of ozonated microbubbles for shellfish indicator organisms, Vibrio bacteria and NoV reduction in post-harvest depuration processing of Eastern oysters. The company ScanAqua now provides advanced nanobubble oxygenated technology with 'game-changing integrated engineering services and world-wide support for improved productivity, better water quality and fish welfare in related aquaculture.

The gas composition can be adjusted for NB generation to enhance antiviral activities, such as introducing reactive oxygen species (ROS) (Hayes et al., 2013). There is also potential to combine cold plasma with the nano-bubble biocide delivery concept. Despite positive observations as a clean decontamination technology, it remains uncertain as to the mechanism by which cavitation-generated bubbles clean, disinfect and kill microbial organisms including viruses and enhance chemistry activity (Zupanc et al., 2019) (Table 2). Cavitation describes the formation of small vapour bubbles (cavities) inside an initially homogeneous liquid medium. It is a rapid physical phenomenon triggered by a sudden decrease in pressure. As the pressure recovers the bubble goes through a violent collapse and possible rebounds. By bubble growth, energy from the surrounding liquid is collected and released by bubble collapses, where extreme conditions can be formed locally. Bubble collapse can cause pressure shocks up to several 100 MPa and if the bubble collapses asymmetrically the so-called microjets with high velocities above 100 m/s can form (Chahine and Hsiao, 2015). In addition, the so-called hot spots with extreme temperatures in order of several 1000 K can form at the centre of the bubble at its collapse, which can cause the formation of highly reactive radicals (Koda et al., 2003). Exact manifestation of cavitation is influenced by liquid properties (temperature, density, viscosity and surface tension) and quality

(number of solid particles and amount of dissolved gasses, which can both act as nuclei) (Zupanc et al., 2019). In general, two types of cavitation are recognized, hydrodynamic and acoustic cavitation. The difference is in the mechanism, which causes the local pressure to drop, while the principles which govern the hydrodynamic bubble and the acoustic bubble are the same.

The effects of cavitation including mechanical and thermal effects are: (a) microsteaming that can damage microorganism – together with shockwaves generated by bubble collapse; (b) chemical effects (implosion of bubbles and formation of hot spots for homolytic cleavage of H_2O molecules and formation of highly oxidizing free radicals (*OH and *H) - *OH readily oxidize particulate matter and also form H_2O_2 – many other species can form ($*O_2H$, *N, *, $1O_2$) where different gases air/oxygen are dissolved in water; (c) oxidation of microorganism's constituents (AC process produces a level of ROS beyond microbial antioxidant stress response capabilities) and kills a broad range of pathogens including viruses (McDonnell and Russell, 1999). Also different ROS affects polysaccharides, proteins, lipids and RNA/DNA); (c) oxidation of lipids, polysaccharides and nucleic acids by oxidative stress initiative by ROS. High-frequency ultrasound inactives *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Legionella pneumophila*, *Enterobacter aerogenes*, *Vibrio cholera, Salmonella enterica*, coliforms and *Mycobacterium* spp. albeit at different rates However, different cellular and molecular mechanisms were postulated including cell wall breakage for *Bacillus subtilis* where the study my laboratory was the only one to report on simultaneous and sequential mechanisms using a pulsed plasma-gas-discharge approach (Hayes et al., 2013), where similar approach was used to explain lethal action of pulsed light (Farrell et al., 2011).

Binding behaviour of noroviruses in oyster tissue may contribute to persistence and further challenge effectiveness of appropriate decontamination.

Careful considerations should be given to applying solutions that address the bioaccumulation of different NoV strains in oysters. This potentially contributes to the lengthy persistence of NoV in depurated oysters (McLeod et al., 2017). For example, immunochemistry studies carried on using Pacific oyster tissues, along with confirmatory tests using monoclonal antibodies and NoV particles with mutated capsids of similar tissues, established that GI·I binds specifically to the midgut and digestive tissue via an A-like carbohydrate, similar to the HBGAs used for NoV attachment to human epithelial cells (Le Guyader et al., 2006). In contrast, NoV GII binds to various tissue types in oysters, including the digestive tract, diverticula, midgut (intestine), gills, mantle, and labial palps (Wang et al., 2008; McLeod et al., 2009). Collectively, McLeod et al. (2017) noted that these findings intimate strain-specific variations in NoV binding patterns in contaminated oysters that can be seasonal due to higher bioaccumulation efficiency (GI·I strain), or no variations in seasonal patterns (GII strains). There is a need to understand typical viral load levels to deploy appropriate counter-measures; for example, there have been NoV outbreaks from depurated oysters containing ca. 10^3 genome copies/g oyster tissue (far exceeding the median infectious dose [ID50]) (McLeod et al., 2017). Yet, the authors noted that "half of the published NoV reduction studies reported no decrease in NoV during depuration, and in the remaining studies it took between 9 and 45.5 d for a 1 – log reduction – significantly longer than commercial time frames".

3. Use of safe enteric surrogates to monitor and assess disinfection of shellfish

McLeod et al. (2017) noted that comparative elimination studies to date have shown that surrogate viruses (including phage, Feline calicivirus, Tulane virus, Mengovirus) are more rapidly depurated than NoV under a variety of depuration conditions, including temperatures of 8 to 25 °C, times varying between 23 h and 8 wk., and using both recirculating and flow-through systems that have UV and/or filtration disinfection. The rapid reductions noted for surrogate viruses may be partly attributable to the quantitation of infectious virions in some studies, whereas hNoV quantitation is based on genome detection; however, several studies (Ueki et al., 2007; Drouaz et al., 2015) have used genome detection for the analysis of both hNoV and surrogate viruses and large differences in reduction rates were still observed. McLeod et al. (2017) reported that "for a surrogate virus to provide useful information on hNoV infectivity, it is important that the characteristics of hNoV and the surrogate virus are similar within oyster tissues, including the way in which they interact with ligands, the stability of the virus capsid, and their persistence". Given that some researchers have reported more rapid depuration of certain surrogate viruses tested to date (such as FRNAPII), they may not be suitable for assessing the virological safety of depurated oysters when used alone without other confirmatory method. This is challenging given that there is currently no infectivity method for hNoV.

However, it is frequently challenging to use actual enteric viruses for developing and testing the efficacy of established or emerging disinfection technologies due to their infectious nature, the need to use Cat 2 facilities and the lack of a simple in vitro cell culture model. In addition, there is uncertainty surrounding the use of RT-qPCR for amplifying genomic material from treated enteric pathogens as this molecular approach does not distinguish between live or dead viruses. This is further complicated by the fact that using infectious hNoV at commercial plant level would not be appropriate for validating new technologies due to concerns over introducing pathogens into live shellfish. It is not uncommon to deploy different yet safe microorganism(s) as representative of the target pathogen of interest from a disinfection perspective; for example, *Geobacillus stearothermophilus* and *Bacillus atrophaeus* endospores are used as bioindicators for the terminal sterilization industry (McEvoy and Rowan, 2019), which exhibit a greater level of resistance to the applied lethal stress (such as UV) compared

Table 2
Examples of cavitation effects of treated microbial and algal species (adopted from Zupanc et al., 2019).

Target organism	Characteristic size and shape	Treatment medium	Pre-treatment concentration	Ultrasound frequency (kHz)	Effectiveness (% reduction)	Reference
Enterobacter aerogenes	0.7 × 3.5 μm (rod)	SS	10^6 CFU mL^{-1}	20 [LFUS]	4.4 % after 20 min	Gao et al. (2014)
Haemophilus influenza	0.5 μm diameter (spherical)	BS	10^3 CFU mL^{-1}	20 [LFUS]	99 % after 10 min	Monsen et al. (2009)
Klebsiella pneumoniae	0.7 × 1.6 μm (rod)	BS	10^3 CFU mL^{-1}	20 [LFUS]	90 % after 15 min	Al Bsoul et al. (2010)
Legionella pneumophilia	0.5 × 2 μm (rod)	BS	1.5×10^3 CFU mL^{-1}	33 [LFUS]	20 % after 60 min	Šarc et al., 2014
Vibrio cholerae	0.4 × 3.1 μm (rod)	ASW	$2–5 \times 10^6$ CFU mL^{-1}	19 [LFUS]	90 % after 0.9 min	Holm et al. (2008)
Coliforms		SUW	250 CFU mL^{-1}	20 [LFUS]	70.8 % after 15 min	Al-Juboori et al. (2015)
Escherichia coli	0.5 × 1.5 μm (rod)	ASWS	10^6 CFU mL^{-1}	19 [LFUS]	90 % after 1.4 min	Holm et al. (2008)
Enterococcus faecalis	1 μm (oval)	DW	10^6 CFU mL^{-1}	–	100 % after 10 mins	Cerecedo et al. (2018)
Microcystis sp. (Algae)	4–5 μm (Oval)	GM	10^9 cell mL^{-1}	11 [LFUS]	5 % after 155 min	Zhang et al., 2006
Chlamydomonas sp. (Algae)	3–10 μm (spherical)	GM	5.5×10^7 cells ml^1	1100 [HFUS]	85 % after 7 min	Bigelow et al. (2014)
Scendesmus sp. (Algae)	6–8 μm (bean shaped)	GM	5.2×10^4 cells ml^{-1}		85 % after 60 min	Batista et al. (2017)
MS2 bacteriophage	24–27 nm	BS	10^6 PFU mL^{-1}	20 [LFUS]	4.62 % after 30 min	Su et al. (2010)
Feline calicivirus	27–40 nm	BS	10^6 PFU mL^{-1}	20 [LFUS]		Su et al. (2010)
Murine norovirus	28–35 nm	BS	10^6 PFU mL^{-1}	20 [LFUS]	>3.8 % after 30 min	Su et al. (2010)

Artificial seawater (ASW); Surface Water (SUW); Salt Solution (SS); Buffer Solution (BS), Distilled Water (DS); Growth Medium (GM); Low Frequency ultrasound (LFUS); high frequency ultrasound (HFUS); Plague Forming Units (PFU); Colony Forming Units (CFU).

to similarly-treated microbial pathogens there is also a commensurate need to propagate high population levels of microorganisms (typically ≥ 6 $\log_{10}$ microbes/ml) in order to measure, standardize and to model the inactivation rate and kinetic shape to ensure sufficient disinfection performance. This is known as 'sterility assurance level' or SAL in the adjacent terminal sterilization industry that is used to inform appropriate modalities for the medical device industry. Several safe viral surrogates (or bioindicators [BIs]) have been used for assessment removal or disinfection performance of pathogenic hNoV in live shellfish, such as FRNAPII.

Norovirus is a fastidious virus as it does not grow on culture-based media where there is a reliance on molecular-based approaches to quantify the removal of norovirus and other enteric viruses post treatments; but, molecular approaches do not confirm if the material is alive or dead. Indeed, many existing molecular-based detection methods have great limitations and it is necessary to find alternative or complimentary methods for rapid multi-species and strain testing (Mi et al., 2021). Researchers have highlighted potential misalignment on the use of standard RT-qPCR that may be amplifying residual genetic determinants in the treatment process along with intact virion; which was informed by comparative studies that used FRNA surrogate virus (Rupnik et al., 2021). Plante et al. (2021) reported on improvements in efficiency to standard ISO 15216-1 genomic method using a Droplet-Digital PCR approach.

4. Advanced real-time diagnostics: future use of combined confocal microscopy and RAMAN spectroscopy (CRS)

Of the 53,612 publications that featured RAMAN in title, abstract or main body of the text in PubMed and Scopus during period 1995 to 2023, 12 focused on detecting norovirus and enteric viruses. The reason for including RAMAN spectroscopy reflected increasing evidence-based research that supported use of this technology for rapidly identifying and differentiating microorganisms including viruses. The development of rapid, cost-effective decontamination approaches for foodborne pathogens including viruses is of great importance for food safety, early detection of disease, and environmental monitoring (Yin et al., 2020).

There is a pressing opportunity to exploit the fact that hNoV strains and some surrogates (such as FRNAPII) have similar icosahedron-shaped capsids. Detection, and potentially differentiation between viable and inactivated (non-infectious) NoV and surrogates can be potentially achieved using Confocal Raman spectroscopy (CRS) combined with chemometrics as a novel assessment tool to inform disinfection efficacy. CRS is a fast, reliable and efficient method for detecting and identifying microorganisms without laborious pre-treatments. Raman spectroscopy is already accepted as a "powerful analytical technique for the rapid characterization of bacteria without external labels or tedious preparation. Raman spectrum, deriving from molecular vibrations, can be considered as a typical whole organism fingerprint of the biochemical composition of microorganisms where these vibrational spectra could show differences of molecular composition in various bacterial pathogens at the molecular level" (Lin et al., 2019). Thus, Raman spectra has been used to discern strain-specific physiological, metabolic and phenotypic states in microorganisms (Chisanga et al., 2020).

Huang et al. (2021) recently provided a comprehensive review addressing the use of Raman spectroscopy for fast and accurate detection of viruses; it also offers hand-held instruments for aiding practicality for point-of-use or care applicability. Mori et al. (2018) described the use of RAMAN spectroscopy for studying norovirus encapsulation. Other researchers have also exploiting use of this surface-enhanced scattering technology (SERS) for ultrasensitive and rapid identification of norovirus including; use of molybdenum trioxide nanocubes with graphene oxide (Achadu et al., 2020); molybdenum-trioxide quantum dots used with naonogels (Achadu et al., 2021), peroxidase-like graphene-gold particles (Ahmed et al., 2017), polyhedral Cu nanoshells (Kim et al., 2017) combined use of sulfur-doped carbon dots polydopamine-functionalized magnetic silver nanomaterials (Achadu et al., 2021), and plasmonic biosensing (Mauriz, 2020). Zhang et al. (2022a, 2022b) reported the use of CRS to identify and differentiate several types of bacterial pathogens (*Vibrio cholera*, *Salmonella flexneri*, *L. monocytogenes*, *S. aureus*, *S. typhimurium*, and *Clostridium botulinum*) based on characteristic peaks and peak intensity ratio. Principal component analysis (PCA), decision tree, artificial neural networks and Fisher's discrimination analysis were used to investigate differences in microbial detection. Combing Raman spectroscopy with confocal enables the identification of single bacterial cells in high spectral resolution, combining the power of 3D analysis with focused biological component in aqueous medium (Vlasov et al., 2020). The combined use of chemometrics to spectral data enables the analysis of nucleic acids, proteins, lipids, and carbohydrates for distinguishing different bacterial species (Zhang et al., 2022a, 2022b). The use of CRS may also be relevant for ensuring loss of all pathogens of concern including *Vibrio* species – it is particularly relevant to include a battery of test reference strains (*E. coli, Vibrio* sp., hNoV and surrogates) during the development of testing so as to ensure that bacterial and viral pathogens of concern are both addressed in live shellfish. For example, the use of pulsed light offers broader light spectrum targeting cellular constituents (cell membrane, lipid peroxidation and so forth) in Gram negatives bacteria; however, a focus on hNoV destruction from best-published data intimates delivering a short intensity of fixed wavelengths <300 nm that specifically targets viral RNA (and bacterial DNA) (Farrell et al., 2011; Hayes et al., 2012).

5. Advanced real-time diagnostics: future use of flow cytometry

Of the 253,560 publications cited in PubMed and Scopus on using "flow cytometry" of FCM, 26 papers described the combined use of FCM with noroviruses between period 1974 and 2023. Sixteen papers were excluded for the reason that they focused on non-enteric virus detection in shellfish or food systems, namely immune dysregulation associated with villous atrophy; NoV infection or Primary B Cell immune activation in vitro; interactions with *Cryptosporidium parvum* during infection of HCT-8 cells; identification of human NoV CD8+ T Cell restricted to HLA-A*0201 allele; experimental inoculation of juvenile rhesus macaques; and bacterial-linked analysis of geothermal bathing pools. Detection or loss of important epitopic sites on NoV capsids by exposure to treatment technologies may affect the binding of these antibodies that can be rapidly determined in real-time using flow cytometry (FCM) – this is particularly relevant given that an intact capsid retaining surface receptors are critical for enabling viral attachment to specific human histo-blood group antigen (HGBA) sites to cause infection (Esseli et al., 2019). Razafimahefa et al. (2021a, 2021b) described the development of a specific anti-capsid antibody and magnet bead-based immunoassay to detect human NoV in stool samples and spiked mussels. Ponterio et al. (2013) reported on the phenotypic analysis use of FCM to detect pattern of human antigen presenting cells by genotype GII.4 norovirus. Hamza et al. (2011) noted that not all viruses are able to produce cytopathic effects where use of FCM may help with human NoV detection that have no available cell line for propagation. Annamalai et al. (2019) used FCM to evaluate infectivity of GII.4 human norovirus where the authors showed that this approach can inform differentiation between T-B-NK+ severe combined immunodeficiency (SCID) and non-SCID gnotobiotic pigs, implicating the role of NK cells in mediation of human NoV infection.

Thus, use of flow cytometry (FCM) is already an established immunological tool (Cossarizza et al., 2021) including for use in fisheries and shellfish research, where it has been applied to bivalve molluscan shellfish to analyse haemocytes (Van Nguyen and Alfaro, 2019). Cossarizza et al. (2021) noted that "FCM is a laser-based technique that is used to analyse physical characteristics of cells or particles in heterogenous fluid mixtures as they pass through the light source. When a sample of interest is injected into the FCM, targeted components are excited by the laser which emits light in a band of wavelengths. Here, the fluorescence intensity is measured for each particular target cell/particle (virus) at a rate of thousands per second. FCM provides a fast, accurate, convenient, simple to use and affordable tool that can achieve the desired simultaneous measurements of 'multiple' cellular or viral components in real time. This includes inter

alia total cell or particle counts, cell viability including assessing sub-cell populations, quality of genomic content, phagocytosis, oxidative stress and apoptosis (Van Nguyen and Alfaro, 2019). Molecular targets of interest are typically labelled with fluorescent reagents that may include a broad range of dyes, stains, monoclonal antibodies or quantum dots (Van Nguyen and Alfaro, 2019). FCM also enables specific gating of plotted data for a more fine-grain focus on characteristic(s) of interest. For example, my research team (McEvoy et al., 2021) used flow cytometry as a real-time quantitative tool to enumerate biological indicators (*Bacillus atrophaeus* and *Geobacillus stearothermophilus*) in a commercial vaporized hydrogen peroxide (VHP) process used by the terminal sterilization industry.

FCM has been used for studying and quantifying viruses including clinical and aquatic applications for four decades (Hercher et al., 1979; McSharry, 1994; Marie et al., 1999; Kraus et al., 2007; Yang et al., 2008; Brussaard et al., 2010); for example, Marie et al. (1999) reported that FCM can successfully be used to enumerate viruses in seawater after staining and noted that the technique was first optimised by using the phaeocystis lytic virus PpV-01. Yang et al. (2008) advanced the use of microfluidic FCM for virus detection by introducing and integrating several functional micro-devices including antibody recognition and capturing of target viruses. Kraus et al. (2007) compared plaque and FCM-based methods for measuring dengue virus neutralization.

Given that this approach is based on the loss or reduction of a measured signal from fluorophore conjugated antibody that targets treated hNoV capsid – FCM can be deployed as a tool for determining the removal and inactivation modalities including assessing hNoV infectivity post treatments. FCM can be used to compare hNoV detection and quantitative efficiency of the established RT-qPCR genomic method at the laboratory bench (such as discovery phase, TRL 1–3) to compare and inform effectiveness of technologies at commercial plant level (TRL 6 to 9). Thus, and in theory, rapid detection (or loss of detection) of a cocktail of specific monoclonal antibodies matching epitopic sites on hNoV capsid may be used to directly inform quantification of inactivated hNoV by FCM (such as Van Nguyen and Alfaro, 2019). FCM can also be used to simultaneous assess the immunological status of treated live oysters in terms of vitality indicators (Van Nguyen and Alfaro, 2019). Commensurately, loss of specific spectral signals from combined use of CRS method will inform physical disruption of hNoV capsids post treatments, to be supplemented with use of scanning electron microscopy (SEM). For example, such approaches have also contributed to a generation of unique data in our laboratory that elucidates simultaneous and sequential changes at molecular and cellular level informing mechanistic of irreversible lethal action of pulsed light technology against clinical strains of *Candida albicans* (Hayes et al., 2013).

It is envisaged that this advanced suite of imaging techniques (FCM, CRS, SEM) will be centrally located in regional research and enterprise hubs linked to academic experts and industry (Rowan and Casey, 2021), where end-users can access and use these tools affordably that includes offering bespoke training to industry, as available in my laboratories. For example, this suite of advanced imaging tools will be initially used to compare effectiveness for rapid detection of hNoV methods including the provision of new real-time data to help understand the loss of hNoV infectivity during depuration treatments. This combined approach giving techniques that produce real-time spectral measurements would also be appropriate for future automation including the development of deep learning/machine learning algorithms. The Raman community needs to encourage the deposition of virus spectral findings to a central database so as to grow this technique for the food and healthcare sectors.

6. Modeling of enteric viral removal and inactivation and risk mitigation

Of the 1,973,267 papers published on mathematical “modeling” that appear in PubMed and Scopus databases [1933–2023], 26 addressed modeling for detecting and enumerating norovirus in shellfish [1996–2023]. Twelve papers were excluded for the reason that they focused on risk assessment and modeling of cooked or high-pressure-processed shellfish, and development of a model virus or model system. The majority of modeling studies estimated distribution of norovirus or enteric viruses in shellfish, particularly oysters (Hunt et al., 2020) that included sewage impact (Winterbourn et al., 2016). For example, Campos et al. (2017) deployed modeling to determine the zone of impact of norovirus contamination in shellfish production areas through microbiological monitoring and hydrographic analysis. Whereas Razafimahefa et al. (2021b) used this approach to optimize a PMAxx™ -RT-qPCR assay and the preceding extraction method to selectively detect infectious murine norovirus particles in mussels.

Kinetic modeling of viral removal or inactivation performance is important in order to understand the behaviour of treated viruses to the applied treatment technology (Polo et al., 2014; Rowan et al., 2015; Polo et al., 2015; Qin et al., 2022). Qin et al. (2022) noted that a framework is needed to describe the complex nonlinear virus-oyster interactions that included a mathematical model addressing key processes for this viral dynamics, such as oyster filtration, viral replication, the antiviral immune response, apoptosis, autophagy, and selective accumulation. This has implications for informing the non-homogenous distribution of norovirus in oysters and effectiveness of appropriate depuration or relaying. Ideally, a first-order log-linear inactivation plot is achieved over the treatment regime that eliminates or destroys the target pathogens of concern exponentially. However, depending on the efficacy of the treatment modalities, the resistance or tolerance potential of the microorganisms (Fig. 2), and other governing or interfering parameters (such as the presence of organic matter and particulates or biofilms, pH, salinity, dissolved oxygen and so forth), prior microbial stress adaption to applied lethal technologies, the shape of the inactivation plot may differ (Fig. 2). Microbial kinetic inactivation plots may exhibit an initial protective ‘shoulder’ effect followed by a linear kinetic plot, and/or a pronounced adaptive or resistant ‘tailing’ effect that may be evident after longer exposure times. These are referred to as bi-phasic if the shoulder or tail effect accompany a log linear kinetic plot, or triphasic if both the shoulder and tailing effects are present. For example, the absence of a shoulder and tailing effect in microbial inactivation plots for the adjacent sterilization industry is essential as medical devices must achieve a 12 log reduction in bacterial endospore numbers where the first 6 logs are enumerated, but the next 6 logs are predicted based on the probability of a linear plot from the first 6 log (half-cycle). If the tailing effect is evident in first 6 log half-cycle plot, then it cannot be assumed that linearity occurred; thus, introducing the possibility of microbial survivors in the latter treatment stages (McEvoy et al., 2023). For food treatments, it is desirable to have an initial starting microbial population of 5 to 6 log orders in order to standardize treatments between laboratories (Rowan, 2019; Gómez-López et al., 2022) and to achieved a discernible number of D-value reductions (a D value relates to the time taken for a 1 log reduction in microbial numbers achieved under a fixed treatment regime) (Rowan et al., 2000). US FDA also recommend using a 6 log starting population for PUV irradiation studies for food surface decontamination to include natural microbial contaminants during testing and validation (Rowan, 2019).

However, determining accurate viral kinetic modeling for a complete norovirus reduction in shellfish is challenging due to: (a) there is no infectivity assay that would allow the propagation of human NoV to artificially high levels (viral load) such as log 6 gcg^{-1} for kinetic disinfection studies; (b) contamination of shellfish such as oysters appear to be typically contaminated at significantly less viral loads (such as ≤ 5 log); (c) is its uncertain if the RT-qPCR alone will provide data on individual virus post treatments including potential for amplifying genomic artefacts in whole or disintegrated capsid; (d) the LOQ for RT-qPCR (100 gcg^{-1}; Rupnik et al., 2021); (e) use of existing surrogates such as FRNAP II may be less resistant to actual hNoV strains in contaminated live virus due in part to differences in behaviour or non-ligand binding to oysters; and (f) as yet, there is no universally accepted model for determining kinetic destruction of hNoV in shellfish with particular relevance to commercial depuration deployment.

There is a marked gap in data on hNoV inactivation modeling for shellfish. However, Rupnik et al. (2021) reported that norovirus GII exhibited bi-phasic viral reduction kinetic performance using LP-UV irradiation in

contaminated Pacific oysters. After the initial rapid linear reduction of GII, the rate of depuration decreased with a pronounced tailing effect; no further reduction was observed between days 3 and 7, or days 4 and 7 for high and medium depuration temperatures. Interestingly, the two phase viral reduction kinetics was not evident at low temperature trials, with no oyster mortalities observed during the first two weeks of depuration. This pronounced hNoV 'tailing' effect is a potential concern where there is a pressing need to test and develop complementary treatment technologies (such as possibly pulsed light, AOPs, photonics, micro- nano-bubbles) and improved hNoV detection techniques. Potential use of complementary approaches to assess effectiveness of hNoV removal or destruction below the existing LoQ or LoD for RT-qPCR method would be beneficial (as proposed and discussed in the next section). Also, for future modeling studies, it will be relevant to include a test reference hNoV strain, along other microbial reference strains (*E. coli*, *Vibrio* sp), in order to compare the broad-spectrum antimicrobial action of treatment technologies through collaborative inter-laboratory validation trials with commercial producers of oysters.

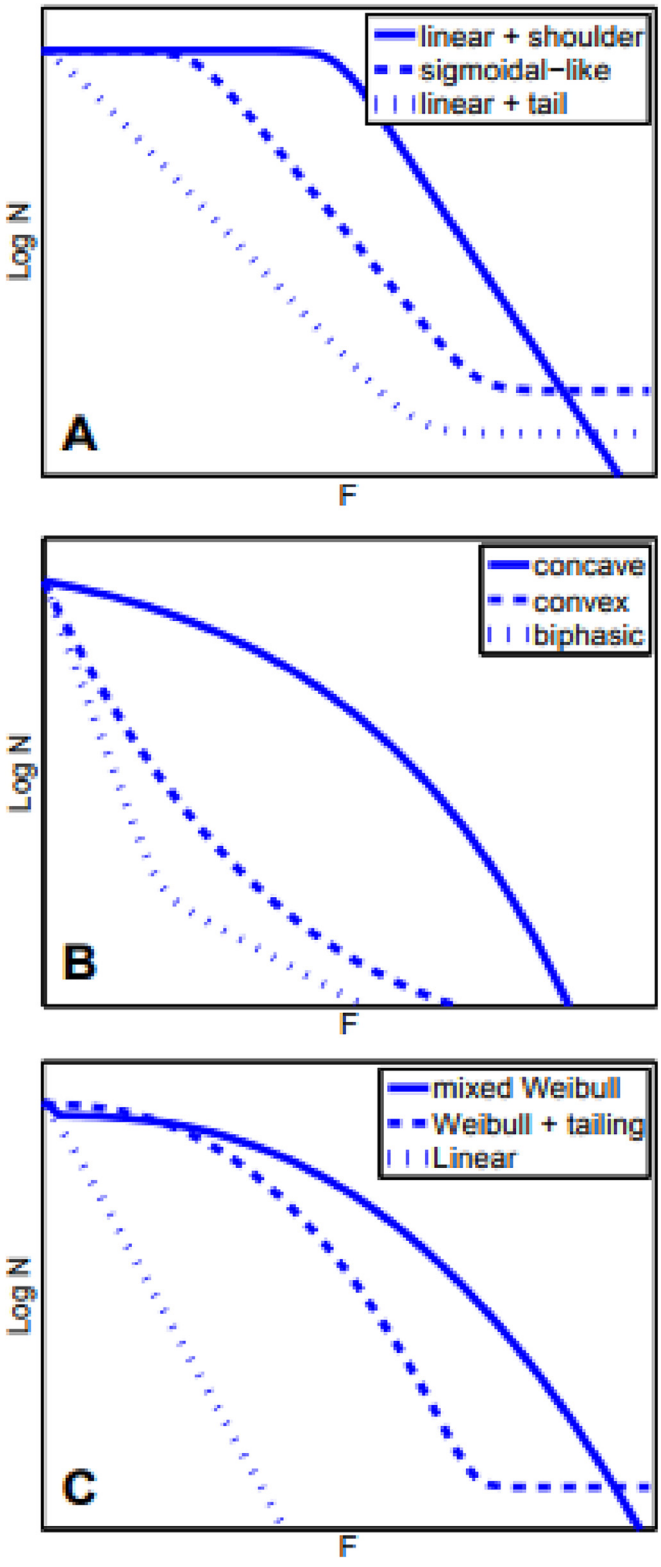

Fig. 2. Commonly observed types of inactivation curves during non-thermal UV processing expressed as $\log_{10}$ N versus F (fluence, J/cm^2). Plot A: sigmoidal-like, linear with a preceding shoulder, log-linear with a tailing. Plot B: biphasic, concave upwards or downwards. Plot C: Linear, Weibull incorporating a tailing effect, two mixed Weibullian distributions (adopted from Rowan et al., 2015).

Thus, lessons can be learnt from the adjacent Medtech domain where complex disinfection and sterilization challenges to address disinfection and sterilization are met in tandem consultation with regulators (such as the USF FDA) for sterility assurance levels. Starting viral populations of 6 $\log_{10}$ for NoV GI and GII (and surrogates such as FRNAPII) are required in order to model inactivation or removal profile to confirm efficiency of modalities where it is assumed that inactivation is exponential (or log-linear). McMenemy et al. (2018) proposed a mathematical model for estimating pathogen variability (including *E. coli*, hNoV) in shellfish and predicting minimum depuration times (including FRNAPII). This interesting model assumes a 'worst case scenario' for viability of pathogens and is then used to predict minimum depuration times to achieve hNoV levels which fall within possible risk management levels, which is a logical approach to mitigating this challenge as a tool to assist in future control strategies given its complexity. The authors state that this model is based on documented assumptions that hNoV is log-normally distributed throughout a population of oysters, and that the pathogen load decay during depuration is exponential. This model requires the "input of four parameters: i) the initial average hNoV load, ii) the depuration efficacy, iii) the desired assurance level (i.e., proportion of shellfish pathogen in depuration which must have pathogen loads less than pathogen threshold limit at the end of the depuration), and iv) the required hNoV threshold" (McMenemy et al., 2018).

However, observations from best-fit linear plots of hNoV and FRNAPII inactivation data in treated oysters appear to frequently show distinct initial shoulder and latter tailing effects that are associated with bi and triphasic viral survivor plot responses (Leduc et al., 2020; Rupnik et al., 2021); therefore, not all documented decontamination studies appear to exhibit viral data that are log-linear in performance. This is important as the pronounced tailing of the hNoV survivor plot would necessitate longer or alternative treatment approaches to ensure complete decontamination of enteric viruses in live shellfish (Rowan et al., 2015). The initial shoulder or increase in hNoV numbers after the onset of depuration (Rupnik et al., 2021) may be attributed to the release of viruses from more concentrated locations in oysters or possibly due to variance in treatment methods and conditions.

In the context of related modeling in adjacent domains, McEvoy et al. (2021) recently reported that vaporized hydrogen peroxide effectively kills recalcitrant bioindicators (*Geobacillus stearothermophilus* and *Bacilllus atrophaeus*) in a commercial process where the inactivation produces linear death rate kinetics. This provides confidence for the industry where there were prior assumptions made through the half-cycle sterilization process (6-D log10 reductions) that linear inactivation occurs in a closed box end-to-end monitoring treatment system. This is particularly important as the terminal sterilization makes inferences about the probability of linear inactivation in order to deliver treatments for 12 $\log_{10}$ reductions in *Bacillus* endospores. Modeling of kinetic data for norovirus destruction (such as longitudinal models for Covid-19, Rowan and Moral, 2021), along with gaining an understanding of the mechanistic destruction of this virus at a molecular level will also help inform reliable and repeatable destruction of this food-borne pathogen for the seafood industry. Interestingly, several publications refer to sterilization (Cong et al., 2021), when it is a 'high-level disinfection' process for human norovirus decontamination as one cannot

assume the destruction of all microbial life that is defined by 'sterilization'. There is a pressing need to develop an appropriate risk mitigation approach using a source-pathway-receptor model in order to determine the efficacy of hNoV removal or destruction from live shellfish. This is likely to be a quasi-quantitative approach given the complexity of the challenge as there is likely to be a residual risk to consumers and commercial seafood producers given their current understanding of existing treatment combinations. The question remains as to what is the acceptable risk given the uncertainly in diagnostic (RT-qPCR) for determining viable hNoV and the limited data available on the efficacy of established and emerging disinfection technologies for full removal or inactivation of enteric viruses deployed at commercial depuration for the shellfish industry. The quasi-quantitative, risk assessment-based approach of Tahar et al. (2017) may inform the aforementioned given that this study addressed the efficacy of WWTPs to remove complex contaminants of emerging concern including risks associated with discharge to receiving waters.

Modeling may also inform previous researcher observations that noted hNoV depuration to adopt a 'two-phase' response with elimination in the first few days being more rapid than subsequent days (Love et al., 2010; Polo et al., 2014, 2015). The first rapid phase of viral depuration is likely related to extracellular digestion and purging of the digestive tract; the speed of purging is governed by physiological traits of the shellfish species concerned, including filtration and clearance rates, digestion rate, and enzymatic activity. Thus, optimizing the physiological state of shellfish through adjusting different parameters such as temperature and salinity contributes to maximizing hNoV reductions in the first phase of depuration (that is, gut purging). However, the persistence of hNoV in shellfish during the second slower phase of elimination indicates that other properties are at play. Indeed, the binding of hNoV to HBGA-like ligands present on oyster gastrointestinal cells, gills, and mantle represents a major barrier to enhancing depuration and will be a key point to address in future studies (Maalouf et al., 2011; Polo et al., 2014). McLeod et al. (2017) also introduced the novel concept of enzymatic pre-treatment to help remove hNoV that could also be modelled for effectiveness.

Hunt et al. (2023) reported for the first time on a quantitative exposure assessment model to predict variations in per-serving norovirus consumption using results from the standard SIO 15216–1:2017 detection method. This model recognises that norovirus is infectious at low doses intimating that small variations in human exposure can potentially lead to significant differences in consumer health. Hunt et al. (2023) findings show "the boundaries for potential exposure following a given ISO detection result, and the relative importance of mean concentration, serving size, and oyster grade. This is directly relevant to potential regulatory thresholds being considered in the EU". However, improvements in the application of appropriate decontamination technologies would potentially impact positively on reduced risk in this model for consumers as these may remove or deactive noroviruses. Addressing norovirus reduction is a complex challenge for risk mitigation with reliance on the LoQ for the ISO method (i.e., 100 gc/g), with a LoD of 20 gc/g. Hunt et al. (2023) noted that the EFSA baseline survey (EFSA, 2019) defined LoQ of 300 gc/g, as achievable for routine monitoring EU. Findings published by Hunt et al. (2023) show that a mean of 200 g/g predicts 95 % consumption range of 57 to 338 gc in a serving of a single oyster. However, the median infectious dose attributed to oysters must be below 2020 genomic copies of hNoV; thus, implying a high probability of infection even at levels lower than the LoQ. Hunt et al. (2023) noted that the median illness-causing does is higher, with a median predicted value of 260 copies needed, as calculated from outbreak events. Additional factors need to be considered to fully progress this QMRA model given the large difference between proportion of oyster batches testing positive for hNoV (such as 76.2 %, [Lowther et al., 2012]), and the proportion of positive samples associated with illness, which is estimated at <0.28 % (Lowther et al., 2010) in the UK. Future use of this QMRA model that incorporates additional factors such as disinfection potential of technologies can also potentially address challenges for hNoV risk assessment.

7. Climate change influences

Climate change is increasing seawater temperatures that can cause disruption in aquatic microbiome enabling the emergence of toxigenic phytoplankton and invasive species, toxic algal blooms, and survival of *Vibrio parahaemolyticus* (Winder and Sommer, 2012; Duchenee-Moutiner and Netto, 2021) in marine environments (Noorian et al., 2023); thus, presenting additional shellfish depuration or relaying challenges. Moreover, 2022 was a year of climate extremes with record high temperatures and rising concentrations of greenhouse gas (Copernicus, 2023). This report highlighted that the "last eight years have been the eight warmest on record globally. La Niña conditions persisted during much of the year. The annual average temperature was 0.3°C above the reference period of 1991-2020, which equates to approximately 1.2°C higher than the period 1850-1900. Europe saw its second warmest year on record, exceeded by 2020 and only slightly warmer than 2019, 2015 and 2014. Europe experienced its hottest summer ever recorded in 2022, where autumn was the third warmest on record. The world's oceans reached temperatures in 2021 despite a La Niña event that typically has a cooling influence". In 2021, the upper 2000 m of the ocean, where most of the warming occurs, absorbed 14 more zettajoules (a unit of electrical energy equal to one sextillion joules) than it did in 2020. This amount of extra energy is 145 times greater than the world's entire electricity generation which, by comparison, is about half of a zettajoule (Milman, 2022). U.S. EPA (2021) reported that "sea surface temperature has been consistently higher during the past three decades than at any other time since reliable observations began in 1880". Seawater temperature rises have been attributed to changes in marine ecosystems such as the recent toxic algae bloom that caused significant losses at commercial salmon sites (Mowi) in Ireland (Intrafish, 2021).

Numerous reports have connected climate change impacts to influencing infectious diseases due to increased hazard, exposure and vulnerability, such as through modeling (Semenza and Paz, 2021; O'Neill et al., 2022a, 2022b). Extreme precipitation events have caused waterborne outbreaks and longer summer seasons (Semenza and Paz, 2021). Researchers have also noted that project risks from infectious disease can be reduced in the future by acting on hazard, exposure and vulnerability through appropriate mitigation and adaption, such as a described in this review (Semenza and Paz, 2021). Carlson et al. (2022) reported that climate change increases cross-species viral transmission risk where there is a mechanistic link between global environmental change and disease emergence. Notably, these authors stated that "this ecological transition may already be underway and holding warming under 2°C within the twenty-first century will not reduce future viral sharing. Our findings highlight an urgent need to pair viral surveillance and discovery efforts with biodiversity surveys tracking the range shifts of species". O'Neill et al. (2022b) reported on the role of using next-generation sequencing and bioinformatics to profile freshwater aquaculture-biome to understand the impact of climate variance of key algae and bacteria including the emergence of waterborne parasites (O'Neill and Rowan, 2023). Duchenne-Moutien and Neetoo (2021) also intimated a link between climate change and food safety issues.

Rindi (2014) reported that terrestrial algae (green algae and diatoms) are more directly affected by climate change and can therefore respond in a more immediate way. This is attributed in part to the fact that algae have short generations, fast turnovers and respond quickly to changes in environmental conditions. Sarmaja-Korjonen et al. (2006) demonstrated that algae appeared to be comparatively good indicators of environmental conditions by representing productivity disparities during changing climatic conditions. Hallegraeff (2010) has also indicated that changes in algal communities can putatively provide a sensitive early warning for climate-driven uncertainties in aquatic ecosystems. There has been increased interest in alternative uses of microalgae within aquaculture to assist with sustainability, in addition to enabling an ecotoxicological assessment and water quality control (O'Neill et al., 2019; O'Neill and Rowan, 2021). Eutrophication occurs when a water body is put under pressure with large levels of organic matter and nutrient waste that is taken in and biologically processed which in turn leads to algal blooms (Jegatheesan et al., 2011;

Martinez-Porchas et al., 2014; Sikder et al., 2016). Algal blooms in turn can lead to decreases in light and oxygen production, which can suffocate aquatic life (Jegatheesan et al., 2011; Chislock et al., 2013; O'Neill et al., 2019) and can be exacerbated by increased marine and freshwater temperatures.

Barry and Hoyne (2021) reported that changes in weather systems, such as increased precipitation, snow and ice events, heatwaves and storms, have led the European Commission to develop new policies and strategies to deal with extreme events. Moreover, Barry and Hoyne (2021) suggested seeking international agreement on indicators to inform ecological resilience, along with economic (such as a number of new SME creations, innovation, investment in training, and specialist upskilling), social enterprise and culture (such as diversity of youth initiatives to increase civil action, solidarity and engagement). Several researchers have also reported on the impact of climate change shifts on receiving waters including flooding (Blöschl et al., 2019).

8. Quadruple Helix Hub Approach

The role of Quadruple Helix HUB (academia-industry-regulators-society) to unlock challenges and to provide subject-matter inputs and shared use of complex or sophisticated equipment (such as novel treatment and diagnostic technologies) from an educational, training and innovation perspective (Rowan and Casey, 2021; Rowan, 2022) is vital. This approach can concentrate single-access supports for industry, entrepreneurs and disruptors; such as step-change in physical infrastructure and systems supports, pre-start-ups; ideation and design thinking to inform technology readiness level (TRLs); market research and enterprise support including early needs analysis and product market fit analysis; early technical validation (such as test the tech, experimentation and validation of pre-pilot and scale to commercial setting); digital transformation (including end-to-end monitoring, AI, remoting immersive training); and a conduit for grant or funding support (Rowan, 2022; Rowan et al., 2022). There is a pressing need for real-time monitoring and confirmation of disease mitigation efficacy – possibly through loss of essential housekeeping or virulence molecular determinant(s) in treated hNoV. This could be elucidated through combinational bioinformatics and next-generation sequencing (NGS). There is an enormity of potential parameters that require simplification that will inform decision making on technology adoption and deployment (Rowan and Galanakis, 2020). There is pressing need for concerted multi-actor collaborations that address the open sharing of data and knowledge that involves hurdling IP discovery phase in TRLs from the screening pilot phase studies for realistic translation to commercial depuration (O'Neill and Rowan, 2022c; O'Neill et al., 2022c). This could all be accelerated through digital transformation that can also address the sustainability of the applied monitoring and disease mitigation technology from a commensurate energy, carbon footprint and risk evaluation perspective that will also help with investment in solutions. Studies conducted in the Hub linked to industry can inform toxicology (Usuldin et al., 2021; Wan-Mohtar et al., 2012

There is a need for a new generation of workers cross-trained in different converging topics to enable sustaining, and potentially, disruptive approaches to tackling industry and societal challenges. This can be met through the Quadruple Helix Hub concept that can also help streamline top-down policy with a bottom-up user understanding at a critical interface to inform decision-making. Testing across independent laboratories and pilot/commercial plants internationally for validation and to support investment in solutions. The greatest challenge is time, as effective solutions that includes confirmation of disease mitigation efficacy are required now.

9. Conclusions and implications for future research

Shellfish are an important source of nutritious food that will contribute to feeding our increasing global populations. However, bivalve molluscan shellfish (particularly oysters) are filter feeding animals and can bioaccumulate pathogenic microorganisms in their digestive tissue that lead to outbreaks of foodborne illness and damage the reputation of the seafood industry. Existing disease mitigation measures rely upon either relaying contaminated shellfish to intertidal locations of clean water for four weeks; or more commonly, depuration using clean seawater combined with a treatment technology (such as UV-irradiation) at the commercial shellfish production site. Growing intertidal environmental locations for shellfish production are graded A to C to dictate requirements for treatment based on microbiology quality; category B growing locations require mandatory depuration. However, many European shellfish producers operate depuration from category A sites that have resulted in contaminated oysters. While bacterial pathogens can be effectively removed at the depuration phase, there is a pressing need to improve existing technologies to address complex human NoV that can persist for lengthy periods due to specific ligand-binding of NoV strains to bivalve molluscan tissues (where the type of tissue for viral attached varies depending on the strain). Assessing the effectiveness of the removal or an inactivation approach for hNoV in live oysters is further complicated by the fact that there is no infectivity assay for this enteric pathogen with reliance upon the use of genomic RT-qPCR technique that does not distinguish between live or dead hNoV. Consequently, the use of surrogate viruses (seen as representative of hNoV to the applied treatment technologies) is adopted such as using the FRNA bacteriophage. However, best-published evidence suggests that existing depuration practices remove viral surrogates faster than targeted hNoV intimating differences in behaviour in viral binding to oyster tissue, and possibly the variance in enumeration methods. Therefore, there is a pressing need to improve both hNoV removal and inactivation methods at depuration along with implementing more appropriate detection and enumeration approaches for hNoV at the commercial depuration phase. There is also uncertainty regarding the appropriateness of using standard molecular-based method for assessing hNoV post treatments compared with surrogate infectious virus values, thus, it is potentially plausible that the level of viral lethality achieved is underestimated given current diagnostic challenges.

The following recommendations are proposed to help with this challenge:

- Further investigate and potentially apply combinational decontamination and treatment technologies for hNoV elimination in live shellfish such as PUV, AOPs, photonics, nano-bubbles and potentially enzymology.
- Develop appropriate laboratory-based diagnostic methods to focus on the destruction of icosahedron-shaped viral capsid, specifically addressing enteric viruses (noroviruses) and appropriate viral surrogates (such as FRNAPII).
- Compare the effectiveness of genomic RT-qPCR (ISO standard), and infectivity assays (such as FRNPH plaque method) against the aforementioned capsid-stability diagnostic method(s) (such as immuno-phenotypic profiling using FCM, confocal-RAMAN spectroscopy) pre and post treatment.
- Enhance support and engagement with the shellfish industry such as through the Quintuple Helix HUB framework (academia-industry-government-environment-society) that addresses access to specialist equipment, subject-matter experts and contract-service provision. This can inform testing and translation of new decontamination technologies to support commercial oyster producers in addition to implementing a practical strategy for unlocking affordable effective solutions.
- Determine actual representative hNoV viral load for testing and validating decontamination technologies.
- Determine the link between detection (ISO, 2017), disinfection efficacy and consumer exposure for norovirus contamination in oysters that is particularly relevant to commercial producers and for risk managers in EU.
- Develop appropriate inactivation kinetic models that embrace circumstances where there are deviations from exponential log-linear plots (such as bi-and tri-phasic performances associated with protective shouldering and tailing) that will inform decision-making for shellfish managers.
- Develop an appropriate holistic 'multi-actor' risk mitigation strategy for preventing hNoV contamination of shellfish including local government

and wastewater industry. This can be combined with new ecological profiling of the same intertidal growing environments as commercial production shellfish production that will address early detection of established recalcitrant hNoV strains and emerging microbial or toxicological contaminations (such as algal biotoxins and invasive species that may be influenced by climate change/variance).
- Translate findings into supporting and facilitating new policies on hNoV in shellfish.
- Develop appropriate training that embraces new techniques including digital technologies.
- Given the complexity of multiple factors potentially governing reliable and repeatable hNoV decontamination of shellfish, introduce edge-computing for end-to-end monitoring and AI/machine learning and blockchain to evaluate and analyse datasets for real-time decision-making.
- Routinely include standard microbial reference strains and for stakeholders to reach consensus on an agreed harmonized method for hNoV decontamination (for example, UV/PUV dose, viral load, depuration temperature, flow rate, duration and so forth).
- Enhance social marketing to help inform stakeholders in real-time and for behavioural change.

CRediT authorship contribution statement

Neil Rowan designed and wrote this paper solely.

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Data availability

Data will be made available on request.

Declaration of competing interest

The author declares no conflict of interest.

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