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1 **Title : Can shellfish be used to monitor SARS-CoV-2 in the coastal**  
2 **environment?**

3

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21

22

## 23 **Abstract**

24 The emergence and worldwide spread of SARS-CoV-2 raises new concerns and challenges regarding  
25 possible environmental contamination by this virus through spillover of human sewage, where it has  
26 been detected. The coastal environment, under increasing anthropogenic pressure, is subjected to  
27 contamination by a large number of human viruses from sewage, most of them being non-enveloped  
28 viruses like norovirus. When reaching coastal waters, they can be bio-accumulated by filter-feeding  
29 shellfish species such as oysters. Methods to detect this viral contamination were set up for the  
30 detection of non-enveloped enteric viruses, and may need optimization to accommodate enveloped  
31 viruses like coronaviruses (CoV).

32 Here, we aimed at assessing methods for the detection of CoV, including SARS-CoV-2, in the coastal  
33 environment and testing the possibility that SARS-CoV-2 can contaminate oysters, to monitor the  
34 contamination of French shores by SARS-CoV-2 using both seawater and shellfish.

35 Using the porcine epidemic diarrhea virus (PEDV), a CoV, as surrogate for SARS-CoV-2, and Tulane  
36 virus, as surrogate for non-enveloped viruses such as norovirus, we assessed and selected methods to  
37 detect CoV in seawater and shellfish. Seawater-based methods showed variable and low yields for  
38 PEDV. In shellfish, the current norm for norovirus detection was applicable to CoV detection. Both  
39 PEDV and heat-inactivated SARS-CoV-2 could contaminate oysters in laboratory settings, with a lower  
40 efficiency than a calicivirus used as control. Finally, we applied our methods to seawater and shellfish  
41 samples collected from April to August 2020 in France, where we could detect the presence of human  
42 norovirus, a marker of human fecal contamination, but not SARS-CoV-2.

43 Together, our results validate methods for the detection of CoV in the coastal environment, including  
44 the use of shellfish as sentinels of the microbial quality of their environment, and suggest that SARS-  
45 CoV-2 did not contaminate the French shores during the summer season.

46

47 **Keywords**

48 SARS-CoV-2, coastal environment, seawater, shellfish, detection method, genomic detection.

49

50 **1. Introduction**

51 The emergence and global spread of Severe-Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2),  
52 responsible for the COVID-19 pandemics, poses an overwhelming challenge to health policies worldwide  
53 and has stirred many initiatives to investigate the circulation of this virus in the human population. SARS-  
54 CoV-2 belongs to the *Coronaviridae* family, which is characterized by a 30kb, positive-sense, single-  
55 stranded RNA genome and enveloped virions of around 120-nm in diameter (Gorbalenya et al., 2020).  
56 Five genera of CoV have been described, among which alpha- and beta- coronavirus (CoV) comprise  
57 coronaviruses infecting humans (HCoV). SARS-CoV-2 is grouped among the betaCoV genus with other  
58 HCoV, SARS-CoV, MERS-CoV and the seasonal HKU1 and OC43 (Gorbalenya et al., 2020). Two other HCoV,  
59 the seasonal NL63 and 229E, belong to the alphaCoV genus (Gorbalenya et al., 2020). Other known CoV  
60 infect vertebrates hosts, and some were used as surrogates for HCoV, such as the alphaCoV Porcine  
61 Epidemic Diarrhea Virus (PEDV) and Transmissible Gastroenteritis Virus in pigs ; the betaCoV Murine  
62 Hepatitis Virus in mice and Bovine coronavirus in cattle ; and gammaCoV in birds (Ahmed et al., 2020;  
63 Randazzo et al., 2020; Saif, 2004).

64 HCoV are respiratory viruses mainly transmitted from person to person, through exposure to droplets  
65 generated by coughing, sneezing or breathing, either directly in the airways, or through hand-mediated  
66 contact (Zhang et al., 2020). Yet, other transmission routes have been described for HCoV and especially  
67 SARS-CoV-2: aerosol-borne and the fecal-oral route (reviewed in (Arslan et al., 2020)). Indeed, the  
68 presence of HCoV RNA in feces of infected people has been reported several times (reviewed in (Jones et  
69 al., 2020)). SARS-CoV-2 was detected in stool samples from infected individuals, even in the absence of

70 symptoms. Viral RNA concentration in feces was lower than in saliva or sputum but could reach 10<sup>7</sup>  
71 genome copies (gc)/ml (Jones et al., 2020).

72 Following its shedding in body fluids, SARS-CoV-2 is drained into wastewaters, where its genome has been  
73 detected now in many countries (reviewed in (Kitajima et al., 2020)). Genome concentration of SARS-CoV-  
74 2 in sewage paralleled the number of human cases in the corresponding population (Peccia et al., 2020;  
75 Wurtzer et al., 2020) and could reach 10<sup>6</sup> gc/L (Jones et al., 2020). Thus, wastewater-based epidemiology  
76 (WBE) is now proposed as an efficient strategy to monitor SARS-CoV-2 dynamics in the human population  
77 (Kitajima et al., 2020). Yet this promising approach still faces many challenges, especially in areas where  
78 wastewater networks are not implemented (Arslan et al., 2020; Street et al., 2020).

79 The contamination of aquatic environments by human sewage has long been recognized as an important  
80 transmission route for enteric pathogens, such as human enteric viruses, either through direct exposure  
81 to contaminated waters, or through their use for food production and consumption of contaminated  
82 foods. (Bosch et al., 2018; Sano et al., 2016). In the case of HCoV, sewage or fecal-borne outbreaks  
83 through aerosols generation were suspected occasionally for SARS-CoV and SARS-CoV-2 (Kang et al.,  
84 2020; McKinney et al., 2006; Yuan et al., n.d.), but foodborne outbreaks were never reported (Jones et al.,  
85 2020). However, SARS-CoV-2 has been detected occasionally in treated sewage (Westhaus et al., 2020;  
86 Wurtzer et al., 2020) and in rivers (Guerrero-Latorre et al., 2020; Rimoldi et al., 2020), albeit at lower  
87 levels than in raw sewage. This re-inforces the hypothesis that SARS-CoV-2 can reach the aquatic  
88 environment, due to insufficient wastewater treatment (Guerrero-Latorre et al., 2020; Wurtzer et al.,  
89 2020) or sewage spillover before treatment (Rimoldi et al., 2020). Coastal marine waters are also  
90 submitted to anthropogenic pollution and sewage contamination, but, to our knowledge, the presence of  
91 SARS-CoV-2 in coastal water remains unstudied to date.

92 Upon contamination of these waters by sewage containing human pathogens, shellfish can become  
93 contaminated in turn and transmit these pathogens back to human hosts (Iwamoto et al., 2010). Indeed,  
94 filter-feeding bivalve molluscan shellfish are known to concentrate in their tissues pollutants or micro-

95 organisms that are present in the surrounding waters. As such, they can be used as sentinels of the  
96 seawater quality (Donia et al., 2012; Fiorito et al., 2019; Metcalf et al., 1980; Winterbourn et al., 2016). In  
97 the recent years, shellfish have been monitored mainly considering the risk for human consumption as  
98 illustrated by the recent study performed in Europe on prevalence of norovirus (NoV) in oysters (EFSA,  
99 2019). Thus, studying the microbiological contamination of shellfish has a dual purpose: monitoring the  
100 presence of micro-organisms in the aquatic environment, and assessing the sanitary risks posed to  
101 consumers.

102 Many families of human enteric viruses, such as *Astroviridae*, *Reoviridae* (human rotavirus A),  
103 *Picornaviridae* (aichivirus, enterovirus, hepatovirus) and especially *Caliciviridae* (human NoV, sapovirus)  
104 can be detected in sewage-contaminated marine shellfish, leading to human infection upon consumption  
105 (Benabbes et al., 2013; Fusco et al., 2019; Le Guyader et al., 2008). Conversely, the occurrence of  
106 *Coronaviridae* in shellfish has never been reported. This could be due to the absence of CoV in the marine  
107 environment, to the lack of studies pertaining to this question, or to the inadequacy of current detection  
108 methods which were mainly optimized for non-enveloped enteric viruses (La Rosa et al., 2020). Following  
109 the emergence and spread of SARS-CoV-2, and its detection in sewage in France, we undertook this study  
110 to validate detection methods for *Coronaviridae* in samples from the coastal environment, assess the  
111 ability of bivalve shellfish to accumulate these viruses, and monitor the presence of SARS-CoV-2 on the  
112 French shores using shellfish and seawater samples.

113

## 114 **2. Material and methods**

### 115 **2.1. Virus stocks and cell lines**

116 Tulane virus (TuV) strain M033, kindly provided by T. Farkas (Louisiana State University, Baton Rouge,  
117 USA) was produced on the LLC-mk2 cell line as described previously (Polo et al., 2018). Porcine  
118 Epidemic Diarrhea Virus (PEDV) strain CV777 was produced in vero-E6 cells as described previously

119 (Bigault et al., 2020). The heat inactivated SARS-CoV-2 was kindly provided by Dr C. Bressolette-Bodin  
120 (Nantes Université, Centre de Recherche en Transplantation et Immunologie, UMR 1064, ITUN,  
121 Nantes, France). Mengovirus (MgV) strain pMCO (kindly provided by A. Bosch, University of Barcelona,  
122 Spain) was propagated in HeLa cells as previously described (Martin et al., 1996).

123 When specified, viruses were inactivated for 15 sec. at 60°C (Abraham et al., 2020). For SARS-CoV-2,  
124 inactivation was verified by TCID50 assay.

## 125 **2.2. Artificial contamination of seawater and oysters (bioaccumulation)**

126 For protocol validation, 1 L of coastal water sampled in November 2019 and February 2020 were  
127 spiked with PEDV and TuV (Table 1). This was repeated two or three times to ensure replicate  
128 extractions for each sample and method.

129 Oysters (*Crassostrea gigas*) were either purchased live from a producer (commercial oysters), or  
130 harvested on the French shore (wild oysters), and kept overnight at 4°C. Artificial contaminations were  
131 carried out by bioaccumulation of oysters for 24 h at room temperature (18-20°C) in aerated seawater  
132 seeded with known concentrations of the viruses (Table 1). The volume of seawater was adjusted to  
133 the number of animals in the tank (Table 1), with a ratio of 1L / 6 animals for commercial oysters, and  
134 1.5L / 6 animals for wild oysters which were twice bigger based on the weight of digestive tissues (DT)  
135 recovered. For each experiment, a fraction of the viral inoculum was titrated in parallel by qRT-PCR to  
136 calculate the total amount of each virus used for bio-accumulation. After 24 h of bioaccumulation,  
137 oysters were open, shucked and dissected to collect the DT, the gills and the mantle. Tissues from all  
138 oysters were pooled by type, minced, and stored as 2g-aliquotes at -20°C before analysis.

139

## 140 **2.3. Environmental sampling**

141 Along the French coastline, 21 sites were selected based on exposure to human sewage contamination  
142 as demonstrated by *Escherichia coli* (Piquet et al., 2019) or NoV contamination (data not shown)

143 (Figure 1, black dots). The sites were selected to cover the different French coastal areas (Figure 1).  
144 From each site, one shellfish sample was collected bi-monthly, when possible, from mid-April 2020 to  
145 end of August. Only shellfish present onsite for at least 6 months or from wild populations were  
146 harvested, so that they could reflect the local viral contamination. Most collected samples were  
147 cupped oysters (*Crassostrea gigas*), two samples were mussels (*Mytilus spp.*) and one, clams  
148 (*Ruditapes philippinarum*). One sample was constituted of at least of 12 oysters, 20 mussels or 20  
149 clams. Shellfish samples were shipped on ice to the laboratory, where they were dissected and the DT  
150 from 10 animals pooled, minced, and stored at -20°C as 2 g-aliquotes.

151 Coastal water (1 L) was sampled together with shellfish from seven sites (Figure 1, red dots), sent on  
152 ice to the laboratory, where they were stored at -20°C until processing.

153

154

155 Besides this scheduled sampling, additional shellfish samples were collected on an ad-hoc basis in  
156 other coastal sites upon alerts of microbiological contamination characterized by increased *E. coli*  
157 concentrations in shellfish flesh (Piquet et al., 2019). A total of 18 shellfish samples linked to alerts  
158 were collected (eleven oyster samples, four mussel samples and three cockle samples), as well as  
159 seven water samples.

160

#### 161 **2.4. Extraction of viral nucleic acids from coastal water**

162 Samples of coastal water (1 L) were analyzed by two methods based on negative-charged membrane  
163 filtration (MF) (Katayama et al., 2002) and FeCl<sub>3</sub> flocculation (FF) (John et al., 2011). For method MF,  
164 coastal water samples were directly filtered on a negative-charged HA-type membrane with a 47 mm  
165 diameter and 0.45µm pores (Millipore, Burlington, MA, USA) placed on a vacuum sterile bottle. Filters  
166 were rinsed with 100 ml of 0.5 mM H<sub>2</sub>SO<sub>4</sub> (pH 3) prior to viral elution with 1 mM NaOH (pH 10.5). After



167 pH neutralization, 10 ml of viral suspension were concentrated using a 50 kDa Centriprep ultrafiltration  
168 device (Millipore) to obtain 2 ml of viral concentrate. In parallel, for method FF, 200 µl of 10 g/L FeCl<sub>3</sub>  
169 solution was added to the filtrate from method MF (kept at 4°C), and incubated 2 h at 10°C under  
170 gentle agitation, in the dark. A flocculate was then collected on a 0.8 µm pore-size polycarbonate filter  
171 (Whatman, Maidstone, UK). Virus resuspension was achieved with 2 ml of ascorbate-oxalate–EDTA  
172 buffer during a 30 min incubation at 4°C under agitation. Viral suspensions (method FF) and  
173 concentrates (method MF) were extracted using the NucliSens kit (bioMérieux, Lyon, France) with 10  
174 ml of lysis buffer and 140 µl of magnetic silica, and eluted in 100 µl of the kit's elution buffer.

175

## 176 **2.5. Extraction of viral nucleic acids from shellfish**

177 Three methods were tested on 2 g-aliquotes of oyster tissues. The PK-ISO method was applied as  
178 described in the norm for Hepatitis A and NoV detection in shellfish (ISO 15216-1:2017). Briefly, tissues  
179 were incubated with 2 ml of a 3000U/l solution of proteinase K (PK) for 1 h at 37°C and 15 minutes at  
180 60°C, centrifuged for 5 minutes at 3,500 x g at 4°C, and 500 µl of supernatant was used for extraction  
181 directly using the NucliSens kit (bioMérieux). The remaining supernatant (2.5-3 ml) was used for the  
182 PK-PEG extraction method, for which it was sonicated 3x1 min at full power with a Sonopuls sonicator  
183 equipped with a cup-horn (Bandelin, Berlin, Germany), with 1-min resting on ice between each  
184 sonication. Pyrophosphate (100 mM) was added 1:10 in the supernatant, which was then incubated at  
185 4°C for 40 min with agitation and further treated as described previously (Strubbia et al., 2020) until  
186 concentration by poly-ethylene-glycol (PEG)-6000 precipitation. For the chloroform:butanol/PEG  
187 method (CB-PEG), tissues were homogenized with a pestle in a potter with 2 ml glycine buffer (glycine  
188 3.75g/l, NaCl 9g/l, pH 9.5). Additional 3 ml of glycine buffer were used to rinse the pestle and potter,  
189 and added to the tissue homogenate before adding 6 ml of chloroform:butanol (50% vol:vol) solvent  
190 and mixing by 30 sec on vortex. Cat-Floc T (Calgon, Ellwood City, PA) was added (173 µl per tube), the  
191 mixture agitated for 5 minutes at room temperature, before being centrifuged for 15 min at 13500 xg

192 at 4°C (Atmar et al., 1995). The supernatant was collected, 3 ml of PEG-6000 (24%) – NaCl (7%) were  
193 added and incubated 1-2h at 4°C with agitation, before a final centrifugation for 20 min at 11000 x g at  
194 4°C. For both the PK-PEG and the CB-PEG methods, the pellet was resuspended in 1 ml ddH<sub>2</sub>O pre-  
195 heated at 56°C, by vortexing and pipetting. All viral eluates/concentrates were extracted using the  
196 NucliSens kit (bioMérieux) following the manufacturer’s instruction, with 2 ml lysis buffer and 50 µl  
197 magnetic silica, and eluted in 100 µl of the kit’s elution buffer.

198

## 199 **2.6. Process control**

200 The MgV, a murine picornavirus, was used as a process control for nucleic acid extraction from  
201 shellfish, as described in (ISO15216-1,2017). Briefly, 100 µl of MgV solution were added to each tissue  
202 aliquot just before extraction, and an extraction control was carried out with 100 µl of pure MgV  
203 solution in each series of extraction. MgV concentration in nucleic acids extracted from shellfish  
204 tissues were compared to the extraction control to calculate the efficiency of each series of extraction.  
205 For the environmental screening, samples whose extraction efficiency was below 1% were not  
206 considered for the final analysis, since any absence of virus detection could be due to extraction issues  
207 (ISO15216-1,2017). The extraction efficiency was not evaluated for water samples collected in the  
208 environmental screening.

209

## 210 **2.7. Detection of viral genomes by one-step quantitative RT-PCR**

211 The Ultrasens one step quantitative RT-PCR kit (Life technologies, Carlsbad, CA, USA) was used for all  
212 qRT-PCR reactions, following the manufacturer’s indications, using an Aria Mx or MxP3000 real-time  
213 PCR system (Agilent Technologies, Santa Clara, CA, USA). For SARS-CoV-2, two sets of primers and  
214 probes were used: IP4, targeting the polymerase gene (Etievant et al., 2020) and E, targeting the  
215 envelope gene (Corman et al., 2020). Cycling were adapted to comply with the qRT-PCR kit

216 requirements: reverse-transcription for 15 min at 55°C, first denaturation and *Taq* polymerase  
217 activation for 5 min at 95°C, and 45 cycles of denaturation (94°C, 15 s), annealing (58°C, 30 s) and  
218 extension (65°C, 30 s) followed by fluorescence acquisition. The MgV, TuV and NoV genogroup I (GI)  
219 and II (GII) qRT-PCR were carried out as described previously (Drouaz et al., 2015; Le Guyader et al.,  
220 2009). For PEDV, previously described primers (Bigault et al., 2020) and probe (Kim et al., 2007) were  
221 used based on the same cycling conditions as NoV GII.

222 For quantification, duplicate 6-points standard curves were made with TuV synthetic DNA (Drouaz et  
223 al., 2015), PEDV in-vitro transcript T171 (Bigault et al., 2020) and SARS-CoV-2 RNA transcript (CNR des  
224 virus respiratoires, Pasteur Institute), and the synthetic ssRNA-EURM-019 (European Commission Joint  
225 Research Center).

226 Considering the sensitivity of our qRT-PCR assays, the theoretical detection limit was set as 1 genome  
227 copy per 5 µl of nucleic acid that were assessed. For shellfish samples, this means 50 gc/g of tissue  
228 analyzed using the PK-ISO method, 10 gc/g for the CB-PEG method, and 13 gc/g for the PK-PEG  
229 method. For seawater, this equals to 20 gc/l for both methods.

230 For virus detection in shellfish field samples, after verification of extraction efficiency and absence of  
231 inhibitors, triplicates of undiluted nucleic acid extracts were assessed and for water samples  
232 amplifications were performed on duplicate of undiluted extracts and 1/10 dilutions in molecular  
233 grade water. For their quantification in seeded or bioaccumulated contaminated samples, duplicates  
234 of undiluted, 1/10 and 1/100-diluted extracts were used. Good laboratory practices were observed  
235 throughout the analysis process, with dedicated separate rooms for oyster bioaccumulation, shellfish  
236 dissection, viral elution from shellfish, seawater processing, nucleic acid (NA) extraction, preparation  
237 of reaction mixtures, template addition, positive controls addition, and amplification. No-template  
238 controls were included in all qRT-PCR assays and proved always negative.

239

## 240 **2.8. Statistics**

241 GraphPad Prism version 8.4.3 was used for statistical analysis of the data by 2-way ANOVA with  
242 Tukey's multiple comparisons test. In some instance, the viral concentrations in oyster tissues were  
243 below the theoretical limit of detection, or even non-detected. This was observed before with other  
244 viral targets, and may be due to the complex matrix in oyster extracts. We chose to keep these values  
245 for statistical analysis.

246

### 247 **3. Results**

248 To validate protocols for the extraction of SARS-CoV-2, we used a surrogate coronavirus, the porcine  
249 epidemic diarrhea virus (PEDV) to mimic the behavior of SARS-CoV-2 (which requires access to a BSL3  
250 facility). In addition, we used the TuV, a simian calicivirus often used as a surrogate for human NoV, as  
251 a non-enveloped control virus known to be bio-accumulated by oyster (Drouaz et al., 2015; Polo et al.,  
252 2018).

#### 253 ***3.1. Assessment of extraction methods for CoV in seawater.***

254 Several protocols were previously described allowing the concentration and extraction of viruses from  
255 environmental waters, including seawater. We selected two methods that were found efficient for the  
256 recovery of enteric viruses (John et al., 2011; Katayama et al., 2002) and applied them to coastal water  
257 samples spiked with PEDV and TuV (Table 1). The first method (MF) allowed to recover the PEDV and  
258 TuV genomes with a mean yield of 0.981% and 1.33% respectively (Table 2), but with high inhibition of  
259 RT-PCR enzymes necessitating at least 2-log dilutions of nucleic acid extracts. The second method (FF)  
260 was applied to two samples, where it allowed the recovery of 1.78% and 0.23% of PEDV and TuV,  
261 respectively (Table 2). Both methods showed a high variability of recovery on both viruses across the  
262 different samples, and statistical comparison were not significant (Table 2,  $p>0.05$ ). As they present  
263 complementary approaches, we chose to apply both methods on environmental seawater samples for

264 SARS-CoV-2 monitoring. Besides, given the low viral recovery in seawater samples, another approach  
265 was tested with the use of shellfish to concentrate the contamination.

266

### 267 **3.2. Assessment of extraction methods for CoV in shellfish.**

268 The current preconized method for the detection of NoV or hepatitis A virus in shellfish relies on a  
269 simple protocol based on proteinase K (PK) digestion to release viruses from DT (PK-ISO) (ISO 15216-1).  
270 It was compared to the original protocol set up to detect enteric viruses in shellfish, which uses  
271 chloroform-butanol to elute viruses and PEG to concentrate them (CB-PEG) (Atmar et al., 1995). A  
272 third protocol, combining PK elution and PEG concentration, able to recover a high diversity of viruses  
273 from shellfish (Strubbia et al., 2020) was also tested here (PK-PEG). We used three tissues dissected  
274 from PEDV/ TuV- bioaccumulated oysters to compare these methods: the mantle (MT), the digestive  
275 tissues (DT) and the gills (GL) (Figure 2). Three to four series of extraction were performed. Their  
276 efficiencies were calculated for each method and tissue using the MgV process control, and were  
277 comprised between 0.4 and 10% for PK-ISO, 0.03 and 4% for CB-PEG, and 0.3 and 5% for PK-PEG. The  
278 three methods allowed to recover TuV to similar levels ( $p>0.05$ , Figure 2) and this virus was more  
279 concentrated in the DT than in other tissues ( $p=0.0002$ , Figure 2). PEDV was recovered from the three  
280 shellfish tissues using PK-based methods, when the CB-PEG was poorly efficient, allowing PEDV  
281 detection only in the gills at a very low concentration (Figure 2). Although it used more PK eluate, the  
282 PK-PEG method was not significantly more efficient at recovering both viruses. The simpler PK-ISO  
283 method was the most efficient on all tissues for PEDV recovery ( $p<0.05$  or  $0.01$ ), (Figure 2). Finally, all  
284 tissues appeared equally suited for PEDV detection ( $p>0.05$ , Figure 2).

285

### 286 **3.3 Oysters bioaccumulation with inactivated SARS-CoV-2**

287 Oysters are known to bio-accumulate very efficiently some enteric viruses, such as human NoV  
288 (Maalouf et al., 2011), while other viruses may be poorly uptaken or kept in their tissues, like bovine  
289 NoV (Zakhour et al., 2010). To test the bio-accumulation of SARS-CoV-2 by oysters, and validate the PK-  
290 ISO protocol on the target virus, we used SARS-CoV-2 from cell culture, heat-inactivated (in.) for safety  
291 reasons. Three different batches of *C. gigas* oysters were incubated with in. SARS-CoV-2, and with TuV  
292 and PEDV as controls. Using the PK-ISO method, the concentration in viral genomes was then  
293 quantified in three tissues (Figure 3). TuV was highly concentrated in oyster tissues, and most  
294 concentrated in the DT ( $p < 0.0001$ , Figure 3, A), as expected, with similar levels of contamination for  
295 the three batches. In the two first batches (B1112 and B1113), PEDV and in. SARS-CoV-2 were detected  
296 mainly in the gills and the DT, respectively, at very low levels (Figure 3, A). In the third batch, higher  
297 quantities of in. SARS-CoV-2 (Table 1) were used to contaminate oysters, and CoV were detected in the  
298 three tissues at intermediate levels, with apparent highest concentration in the DT that did not reach  
299 statistical significance ( $p > 0.05$ ) (Figure 3, A). Variability of results across the three oyster batches can  
300 be explained by a slight inhibition of PCR and lower extraction efficiencies for the first batch (2-4%),  
301 while the last batch was contaminated with more inactivated SARS-CoV-2, and also showed the  
302 highest extraction efficiencies (1-21%), which may have resulted in higher amounts of CoV detected.  
303 Importantly, PEDV and in. SARS-CoV-2 displayed very similar distributions and concentrations in each  
304 oyster batch (Figure 3, A), which supports the use of PEDV as a surrogate for SARS-CoV-2 in shellfish.

305 To compare the data more easily regarding the initial amount of virus used for oyster contamination,  
306 the viral concentration in oyster tissues was divided by the initial viral concentration in seawater  
307 (Figure 3, B)(Maalouf et al., 2011). TuV bioaccumulation index reached a mean value of 10.6 in oyster  
308 DT and was highly reproducible across the three oyster batches. For PEDV and inactivated SARS-CoV-2,  
309 the mean bioaccumulation index was highest in DT (0.012 and 0.0017 respectively), and varied  
310 between oyster batches. Together, our data show that CoV can contaminate oyster tissues but are not  
311 as efficiently bio-accumulated as a calicivirus like the TuV.

312

313 For safety reasons, we could not use native, infectious SARS-CoV-2 to contaminate oysters, and had to  
314 rely on heat-inactivated SARS-CoV-2. To check that heat inactivation does not impact the  
315 bioaccumulation efficiency and tissue distribution of CoV, we contaminated oysters with TuV and  
316 native PEDV or TuV and heat-inactivated PEDV (in. PEDV), in two separate aquariums with the same  
317 batch of oysters at the same time. Two independent experiments using different batches were  
318 conducted (Figure 4). For both, TuV displayed the expected distribution and was equally concentrated  
319 in each tissue between oysters from the two aquariums (data not shown). The MgV extraction  
320 efficiencies were also similar, with respective mean values of 5.5% (range 1 – 22%) and 4,6% (1 – 11%).  
321 In the first experiment (B1110-11), inactivated PEDV appeared more concentrated than native PEDV in  
322 the oyster tissues (Figure 4, circles). In the second experiment (B1117-18), native and inactivated PEDV  
323 exhibited the same levels of concentration (Figure 4, triangles). Considering both experiments, the  
324 mean concentration of native and inactivated PEDV did not differ significantly ( $p>0.05$ , Figure 4), and  
325 their tissue distribution were similar, suggesting that heat inactivation does not impair CoV  
326 bioaccumulation by oysters, and validating our results with in. SARS-CoV-2.

327

### 328 ***3.4. Screening of environmental samples for the presence of SARS-CoV-2***

329 A total of 187 samples were collected from 37 sites, including 21 sites regularly sampled (monitoring,  
330 Figure 1) and 16 sites sampled upon alerts on microbiological contamination (alerts). All these samples  
331 were processed by the PK-ISO method. Among these, three samples (one from Normandy, and two  
332 from Brittany area) provided extraction efficiencies lower than 1% despite repeated extractions, and  
333 thus were excluded of the analysis.

334 Among the 166 samples collected during the monitoring survey, 141 were oyster samples, 17 mussel  
335 samples and 8 clam samples. None of these samples were found contaminated by SARS-CoV-2 using  
336 any of the two primer sets (Table 3). NoVs searched to confirm human sewage contamination were

337 detected in 35 samples (21%), 69% of these positive samples being detected at the beginning of the  
338 study (from mid-April to end of May). Four sampling sites (L, J, P, R) were devoid of NoV contamination  
339 and NoV were detected once in nine sites (F to I, O to U). Most of NoV-contaminated samples were  
340 detected in eight sites including three sites (A, L and N) located close to the mouth of large rivers  
341 which displayed the highest contamination frequency and highest concentrations.

342 Among the 18 shellfish samples collected following microbiological alerts suspected to be linked to  
343 sewage contaminations events, none were found contaminated by SARS-CoV-2. They were collected  
344 mainly in May and August. Three samples (two collected in May and one in June) were found  
345 contaminated by NoVs confirming the human fecal contamination.

346 None of the water samples were found contaminated by SARS-CoV-2, however NoV were detected in  
347 10 samples. Both methods gave positive results with two samples being positives for both methods,  
348 two with the MF method and 6 with the FF method. NoV were not detected in site G, while they were  
349 detected twice or three times in all the other sampling sites (concentrations ranged from 20 to 300  
350 RNaC/L). On one occasion (site F, sampled on May 5) both water and oyster samples were found  
351 positive for NoV.

352

#### 353 **4. Discussion**

354 Most existing protocols for the detection of viruses in environmental samples are optimized for non-  
355 enveloped, enteric viruses such as gastroenteritis or hepatitis viruses (Bosch et al., 2018). The  
356 emergence and possible environmental spread of the SARS-CoV-2, an enveloped virus, raised new  
357 challenges to environmental virologists (La Rosa et al., 2020). Our first aim was to select a method to  
358 detect CoV, in samples from the coastal environment, using real-time, quantitative RT-PCR, which is  
359 one of the most sensitive and robust techniques available for virus detection in environmental samples  
360 (Haramoto et al., 2018). As manipulating infectious SARS-CoV-2 required working in a biosafety level 3



361 laboratory, we first selected a surrogate virus allowing to assess detection methods without this safety  
362 considerations. Important points to select a surrogate are the genetic proximity to the target virus, the  
363 physical and chemical characteristics but also the absence of human pathogenicity, and/or easy way of  
364 production (Cromeans et al., 2014). In this study, to use this surrogate with seawater and oysters, the  
365 lack of natural contamination was another constraint. Phages are good surrogate for some eukaryotic  
366 viruses but their presence in environmental samples may complicate their use (Flannery et al., 2012).  
367 Usually a virus from the same family is preferred so that target and surrogate viruses share a similar  
368 size, structure, and other characteristics. For example, the TuV, prototype strain of the genus *Recovirus*  
369 within the *Caliciviridae* family, is used to mimic NoV behavior (Drouaz et al., 2015). Among the  
370 *Coronaviridae* family, we selected PEDV, a porcine enteric CoV which belongs to a different group of  
371 CoV than SARS-CoV-2 (alpha and beta-CoV, respectively). The first one is an enteric virus while the  
372 second is respiratory, which could imply differences in environmental stability. Nevertheless, porcine  
373 enteric CoV have been used in the past to as surrogates for HCoV, including SARS-CoV-2 (Randazzo et  
374 al., 2020), and in a recent study, all tested CoV (including PEDV) fitted in the same model regarding  
375 their sensitivity to temperature in fomites (Guillier et al., 2020). Altogether with the TuV, it allowed us  
376 to control the efficacy of our methods on a target, non-enveloped virus, and to compare with  
377 enveloped coronavirus data.

378

379 As the aim of this work was to evaluate the possible coastal contamination by SARS-CoV-2 shed by  
380 infected people, we first evaluated methods for SARS-CoV-2 detection and quantification in seawater.  
381 In human feces and in sewage, which are the sources of human viruses in the coastal environment,  
382 viruses are rarely free but adsorbed onto particles. Thus, we selected a combination of two  
383 complementary methods, one recovering large particles (membrane filtration, MF) and the other one,  
384 smaller aggregates and free viruses (FeCl<sub>3</sub> flocculation, FF). When applied on seawater samples spiked  
385 with the TuV and the PEDV, these methods allowed to detect both viruses, however at low yield and

386 with high variability between water samples. These very low yields could be explained by the use of  
387 coastal marine waters, which were turbid and contained PCR inhibitors (Hata et al., 2020). Surprisingly,  
388 results were similar for TuV and PEDV for each sample, which suggest that the yield of the methods is  
389 mostly influenced by parameters of the seawater matrix (presumably particulate material, PCR  
390 inhibitors) and not by the nature of the virus. Considering that the two methods showed similar ranges  
391 of yields, they were both applied on naturally contaminated seawater samples during environmental  
392 monitoring, where NoV, but not SARS-CoV-2, were detected. These results underline that virus  
393 detection from environmental waters is not an easy process. In the ISO15216:1-2017 norm, as low as  
394 1% recovery rate is considered an acceptable quality parameter. A recovery of 11% for PEDV and MgV  
395 in raw sewage using aluminum hydroxide adsorption-precipitation was achieved, but the recovery of  
396 PEDV was down to 3% in treated sewage (Randazzo et al., 2020). Here, the filtration of one-liter  
397 samples was difficult to achieve while still being too small for the detection of SARS-CoV-2 that is likely  
398 present at very low concentrations (if present) in the environment. Even if the detection of some NoV  
399 confirmed the efficacy of these methods in the field, a grab sample of such a small volume is also not  
400 representative of the whole water present in a site. Given these limitations for direct seawater  
401 analysis, we proposed to use shellfish, which are filter-feeding animals known to concentrate chemical  
402 and microbial contaminants, as sentinel for the detection of SARS-CoV-2 in the coastal environment.

403 Like was done for seawater, we first evaluated different methods to detect CoV in oysters  
404 contaminated with TuV and PEDV. Two methods used proteinase K (PK) for viral elution from the  
405 oyster tissues, and one used lipophilic solvents (chloroform/butanol). The latter method was  
406 inefficient on PEDV, with only traces of this CoV detected in one tissue, while the non-enveloped TuV  
407 was detected in high concentrations in all tissues. Lipophilic solvents disrupt lipid membranes like viral  
408 envelopes, and chloroform was already shown to dramatically alter the recovery of CoV (Conceição-  
409 Neto et al., 2015). Contrarily, the PK-based elution methods allowed the detection and quantification  
410 of both TuV and PEDV in three oyster tissues. We thus chose to apply the current recommended  
411 ISO15216:1-2017 method for NoV and hepatitis A virus detection in shellfish for the next experiments.

412 Indeed, using the ISO method allows for comparisons with more studied viruses (such as NoV). It is  
413 also a simple protocol, that could be easily implemented in laboratories for routine analysis if this  
414 becomes needed for SARS-CoV-2.

415 Using PEDV and inactivated SARS-CoV-2, we show that CoV can contaminate oysters. To our  
416 knowledge, this is the first demonstration that oysters can bioaccumulate a CoV. PEDV and heat-  
417 inactivated SARS-CoV-2 displayed very similar distributions and levels of contaminations in three  
418 oyster batches. In addition, we show that heat inactivation does not impair the distribution of PEDV in  
419 oyster tissues nor negatively impact its bio-accumulation by oysters. These results validate our  
420 observations with inactivated SARS-CoV-2 and reinforce our confidence that PEDV can be used as a  
421 surrogate for SARS-CoV-2 in oysters. The low impact of thermal inactivation on CoV bioaccumulation  
422 by oysters also suggest that partially degraded SARS-CoV-2 present in sewage may still be able to  
423 contaminate shellfish when reaching the coastal environment. These observations are encouraging for  
424 the use of shellfish as sentinel of human contamination. However, given the expected low levels and  
425 low stability of CoV in the environment, the persistence of CoV RNA in shellfish tissues needs to be  
426 investigated to estimate how long after contamination the virus could still be detected.

427 Both PEDV and inactivated SARS-CoV-2 were less efficiently bio-accumulated by oysters than TuV, a  
428 calicivirus, which could indeed be due to a lower stability in seawater and oysters, and/or to a lower  
429 affinity for oyster tissues. The tissue distribution pattern of CoV does not show a marked concentration  
430 in DT, contrarily to TuV, and high concentrations of viruses were needed to contaminate oysters, as  
431 previously shown for mengovirus, from the *Picornaviridae* family (Drouaz et al., 2015).  
432 Bioaccumulation efficiency may vary from one virus to another or depend on the shellfish species. If  
433 for NoV the impact of ligands and their seasonal expression has been demonstrated, this is still unclear  
434 for other human enteric viruses (Grodzki et al., 2012; Maalouf et al., 2010; Zakhour et al., 2010).

435 In the coastal environment, expected concentrations of enteric viruses are usually much lower than  
436 those used for artificial bioaccumulation (Gentry et al., 2009; Keller et al., 2019), and may be even

437 lower for SARS-CoV-2. Yet, repeated exposures to the virus in the open environment, where larger  
438 volumes of seawater are filtered by shellfish, may still lead to their contamination. *C. gigas* oysters are  
439 present on all French shores and in many countries worldwide (Europe, North Africa, China, Japan,  
440 Korea, Australia, Pacific coast of USA and Canada) as a farmed animal and/or an invasive species  
441 (Herbert et al., 2016), and is thus suitable for use as sentinel in many settings. As mentioned above,  
442 other filter-feeding shellfish species may exhibit differences in bioaccumulation efficiency and should  
443 be tested in further work, such as *Dreissena polymorpha* proposed as a biomonitoring tool in fresh  
444 water (Géba et al., 2020).

445 Considering that seawater sampling and analysis is complicated and unlikely to be positive for SARS  
446 CoV-2, and our results showing a possible bioaccumulation of SARS-CoV-2 in oysters, we set up a  
447 monitoring survey that begun at the end of the first wave of infections in France to evaluate the  
448 possible contamination of coastal areas before the summer season, using shellfish as sentinels. We  
449 used mostly oyster samples, as it was the species in which methods were tested, but some samples  
450 consisted in mussels or clams in areas where oyster were not available. Sites known for their sensitivity  
451 to human sewage contamination were sampled, hypothesizing that if SARS-CoV-2 could contaminate  
452 the coastal environment, these sites should be positive. Indeed, the observed prevalence in NoV (20.5  
453 %) was high compared to previous surveys, especially considering the low epidemic burden of NoV in  
454 summertime (EFSA, 2019; Schaeffer et al., 2013). Several water samples were also found contaminated  
455 with NoV showing that in some instance this approach can be complementary to shellfish sampling,  
456 although technical improvements are necessary to increase the recovery rate.

457 Conversely, all samples (shellfish and seawater) were negative for SARS-CoV-2. The survey period  
458 covered the end of the French lock-down (until may 11<sup>th</sup>, 2020) and the summer season when tourism  
459 results in a larger population on the French coastline. During the first wave of SARS-CoV-2 in France  
460 (March to May 2020), most cases occurred in the north-eastern part of France, and viral  
461 concentrations were likely very low in sewage from the rest of the territory, including western and

462 southern coasts. After the lock-down, although some Covid-19 clusters were reported in seaside  
463 communities, the overall prevalence of SARS-CoV-2 remained low in France throughout the survey  
464 period (“Taux d’incidence de l’épidémie de COVID-19 (SI-DEP) - data.gouv.fr,” n.d.) which was carried  
465 out between the two first waves of Covid-19 (Spaccaverri et al., 2020). Although we cannot rule out a  
466 transient contamination, or contamination outside the study sites, these results suggest that SARS-  
467 CoV-2 did not reach the French coastal environment during summer 2020 at significant levels.  
468 Environmental monitoring should be continued during the winter season, where the risk of viral  
469 spread in the environment is likely to increase due to the second wave of Covid-19 in the French  
470 population, cold temperatures stabilizing the virus and heavy rainfalls resulting in sewage spillover.

471 This pandemic raises many questions, including some technical issues regarding CoV detection in different  
472 types of environmental samples. As mentioned above, environmental virology in the past has tended to  
473 consider mainly non-enveloped viruses. After the first emergence of SARS-CoV, a study demonstrated the  
474 persistence of some strains in environmental waters (Casanova et al. 2009). Recently, if many papers have  
475 been published regarding sewage contamination by SARS-CoV-2, to our knowledge none report on its  
476 detection in seawater and/or shellfish. In developed countries with efficient sewage treatment systems,  
477 the risk of coastal contamination may be limited, and linked to accidental contamination with untreated  
478 sewage. Yet, in some settings, using shellfish as sentinels for viral diffusion in the environment may be  
479 useful, and we show here that two CoV, including SARS-CoV-2, can contaminate oysters under  
480 experimental conditions. The demonstration that a surrogate porcine CoV, PEDV, may be used to mimic  
481 SARS-CoV2 in oysters, suggest that it could be used in other matrices and, to some extent, to evaluate the  
482 stability of infectious particles. Infectious SARS-CoV-2 was isolated from several, but not all, stool or urine  
483 samples from Covid-19 patients (Jones et al., 2020; Sun et al., 2020; Xiao et al., 2020). Although in two  
484 outbreaks, sewage was suspected as a SARS-CoV-2 contamination source (Kang et al., 2020; Yuan et al.,  
485 2020), attempts at isolating infectious SARS-CoV-2 from raw or treated sewage, or freshwater, remains  
486 unsuccessful to date (Rimoldi et al., 2020; Wang et al., 2020). A recent study reports the infection of non-  
487 human primates through gastrointestinal inoculation with a high inoculum of SARS-CoV-2 (Jiao et al.,

488 2020). Yet, in humans, the fecal-oral route of transmission has never being observed for SARS-CoV-2  
489 (Zuber and Brüßow, 2020). The sanitary risk posed by potential contamination of shellfish by SARS-CoV-2  
490 is likely very low but having a method to detect this virus in a food matrix known to be at risk for virus  
491 transmission is important to anticipate questions that may raise with environmental or food  
492 contamination by this virus.

493 To conclude, we believe that surveying shellfish may help to monitor the viral diffusion in seaside  
494 communities, and may be especially suited for countries lacking centralized sewage collection  
495 infrastructures, in which environmental contamination is also more likely (Guerrero-Latorre et al.,  
496 2020). Further work is needed to evaluate and adapt existing methods for the detection of SARS-CoV-  
497 2 in the environment, that may also be suited for other emerging enveloped viruses such as Influenza,  
498 Ebola, or Nipah viruses, should we face another emerging viral pandemic.

499

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515

## 516 **Author's contributions**

517 MD: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project  
518 administration; Visualization; Roles/Writing - original draft; Writing - review & editing. JCP:  
519 Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Supervision;  
520 Visualization; Writing - review & editing. CW: Investigation; Writing - review & editing. CLM:  
521 Investigation; Writing - review & editing. SP: Formal analysis; Investigation; Methodology; Writing -  
522 review & editing. SJ: Investigation; Writing - review & editing. SR: Investigation; Methodology; Writing  
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532 Formal analysis; Funding acquisition; Methodology; Project administration; Resources; Supervision;  
533 Validation; Visualization; Roles/Writing - original draft; Writing - review & editing.

534

535 **Figure legends.**

536 **Figure 1. Localization of the sampling points for SARS-CoV-2 monitoring along the French coasts.**

537 Shellfish (black dots) and coastal seawater (red dots) were sampled bimonthly in 21 sites distributed  
538 along the French coasts and belonging to 4 geographical areas: Normandy (sites A to C), Brittany (sites  
539 D to J), Atlantic (sites K to R) and Mediterranean (sites S to U).

540 **Figure 2. Assessment of extraction methods for CoV in oysters.** Oysters (*C. gigas*) were incubated in

541 presence of TuV and PEDV for 24h, and the concentration of each virus was measured in three tissues  
542 – the mantle (MT, beige), the digestive tissues (DT, brown) and the gills (GL, grey) – by qRT-PCR  
543 following repeated extractions by three different methods – PK-ISO (plain, n=4), CB-PEG (horizontal  
544 lines, n=3), PK-PEG (dots, n=4). \*: p<0.05, \*\*: p<0.01, ns: non-significant (ANOVA). Theoretical limits of  
545 detection: PK-ISO, 50 gc/g ; CB-PEG, 10 cg/g ; PK-PEG, 13 cg/g.

546 **Figure 3. Bio-accumulation of heat-inactivated SARS-CoV-2 in oysters.** Three batches of *C. gigas*

547 oysters (B1112, B1113, B1114) were incubated for 24h in presence of TuV, PEDV and heat-inactivated  
548 (in.) SARS-CoV-2. **A.** The viral concentration was quantified in three tissues - mantle (MT), digestive  
549 tissues (DT) and gills (GL) - by duplicate extractions using the PK-ISO method. \*\*\*\*: p<0.0001, ns: non-  
550 significant (ANOVA), n= 2 series of extractions. In B1112 and B1113, PEDV or SARS-CoV-2 were not  
551 detected (ND) in some tissues. Theoretical limit of detection: 50 gc/g (dotted line). **B.** The virus  
552 concentration of in each tissue was divided by the initial virus concentration in the seawater to  
553 calculate the bio-accumulation index. Each oyster batch is plotted as a black symbol (circle, B1112 ;  
554 triangle, B1113 ; square, B1114) when the virus was detected in the corresponding tissue, missing  
555 symbols corresponding to undetected virus. The arithmetic mean values of the three experiments are  
556 plotted as columns, for the three tissues. \*\*\*\*: p<0.0001, ns: non-significant (ANOVA), n= 3  
557 experiments with different oyster batches.

558 **Figure 4. Impact of heat inactivation on CoV bioaccumulation in oysters.** Oysters (*C. gigas*) from two

559 batches (B1110-11 and B1117-18) were incubated in presence of native PEDV (plain columns) or heat-



560 inactivated (in.) PEDV (hatched columns) for 24h. The concentration of viral genome was quantified in  
561 three tissues - the mantle (MT), the digestive tissue (DT) and the gills (GL) - following duplicate  
562 extractions with the ISO-PK method and qRT-PCR. Columns represent geometrical means and error  
563 bars, geometrical standard deviations. \*\*\*\* :  $p < 0.0001$ , ns : non significant (ANOVA), n=2 experiments  
564 with different oyster batches. Theoretical limit of detection : 50 gc/g (dotted line).

565

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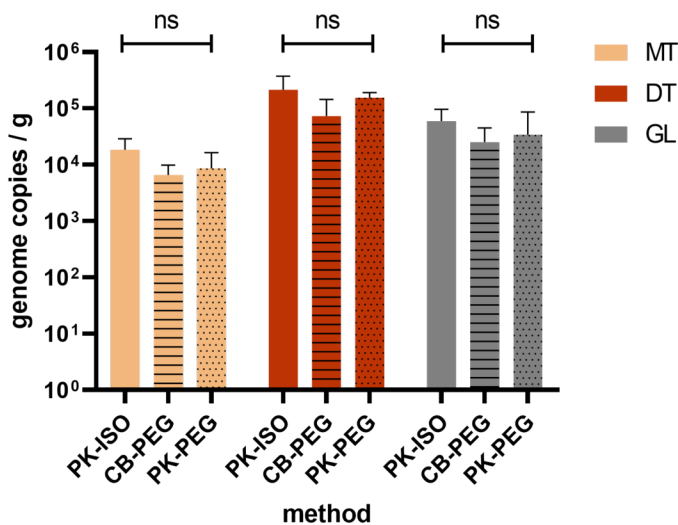
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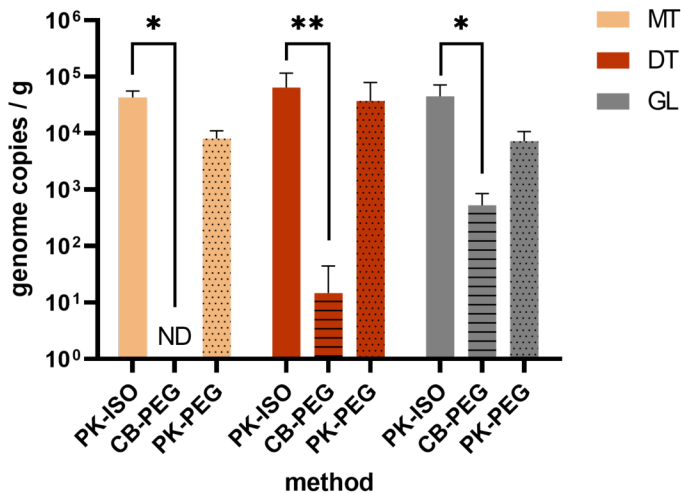
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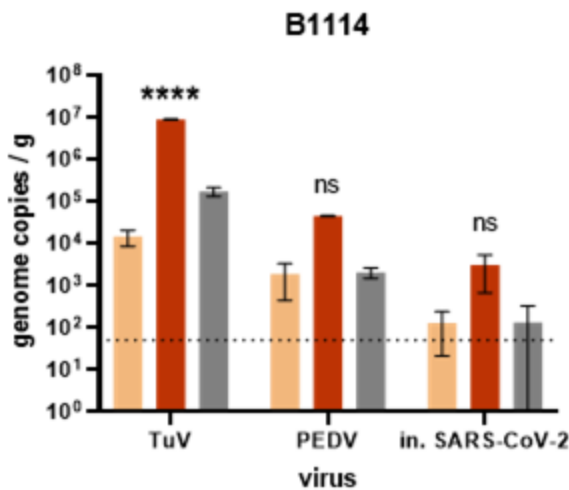
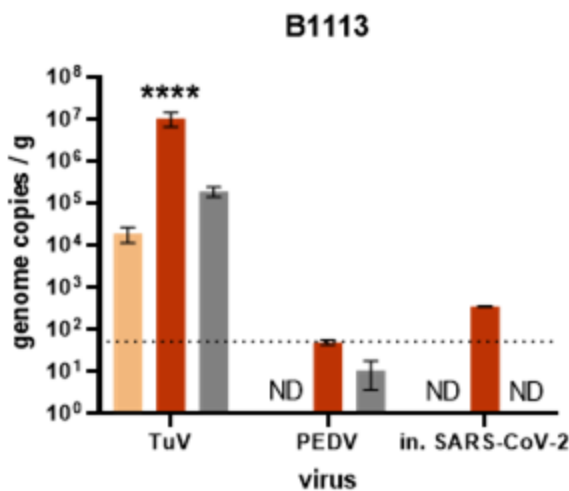
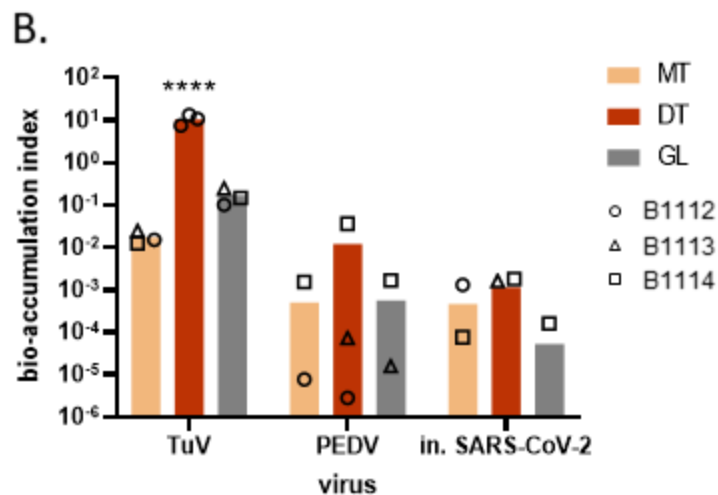
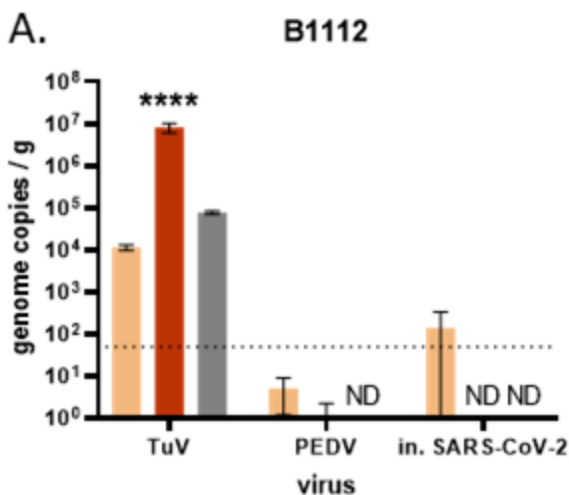


## TuV

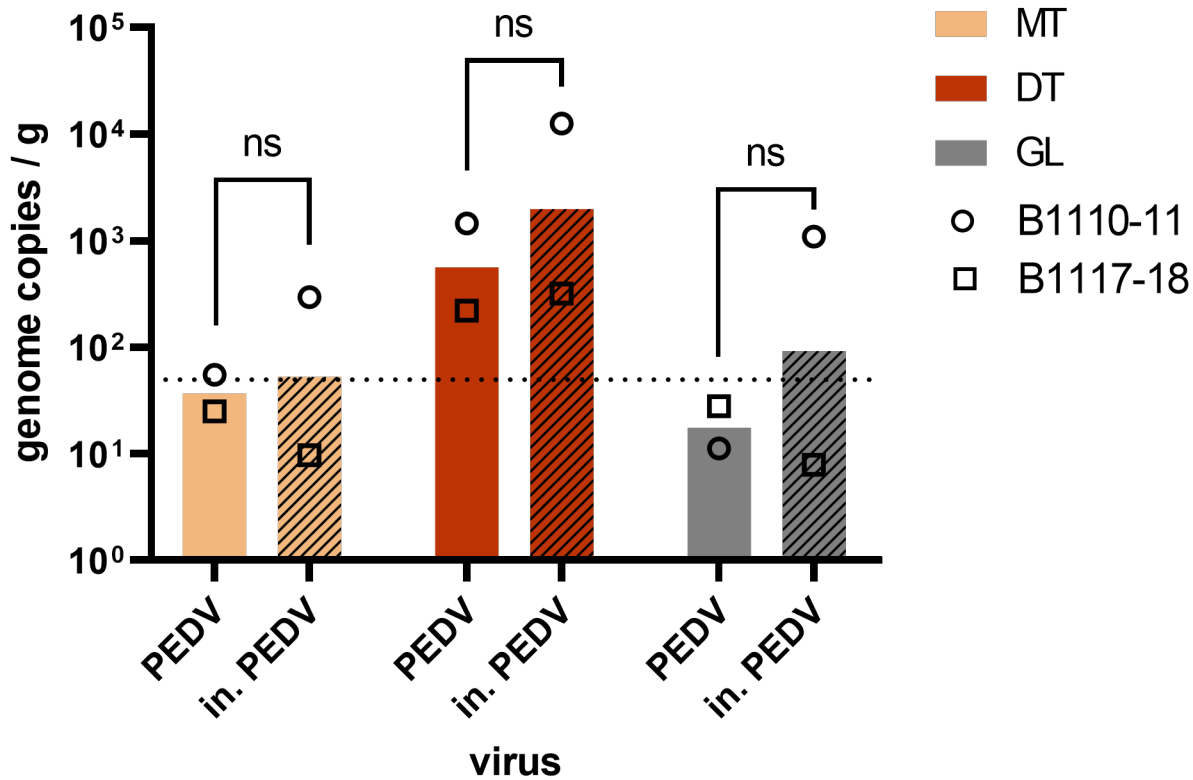


## PEDV









**Table 1. Characteristics of artificially contaminated samples**

Sample	Matrix	Collection date	Viral inoculum (genome copies)			
			TuV	PEDV	Inactivated PEDV	Inactivated SARS-CoV-2
<b>E1980</b>	Coastal seawater site O	Oct. 2019	1.8x10 <sup>9</sup>	2x10 <sup>9</sup>		
<b>E1982</b>	Coastal seawater site G	Oct. 2019	1.8x10 <sup>9</sup>	2x10 <sup>9</sup>		
<b>E1989</b>	Coastal seawater site O	Feb. 2020	2x10 <sup>8</sup>	3.7x10 <sup>10</sup>		
<b>E1990</b>	Coastal seawater site G	Feb. 2020	2x10 <sup>8</sup>	3.7x10 <sup>10</sup>		
<b>B1109</b>	36 commercial oysters	Jun. 2020	2x10 <sup>9</sup>	3.7x10 <sup>10</sup>		
<b>B1112</b>	12 wild oysters	Jul. 2020	2.3x10 <sup>9</sup>	2x10 <sup>9</sup>		6.4x10 <sup>8</sup>
<b>B1113</b>	18 commercial oysters	Jul. 2020	2.3x10 <sup>9</sup>	2x10 <sup>9</sup>		6.4x10 <sup>8</sup>
<b>B1114</b>	18 commercial oysters	Aug. 2020	3.5x10 <sup>9</sup>	3.7x10 <sup>9</sup>		5.5x10 <sup>9</sup>
<b>B1110</b>	9 commercial oysters	Jul. 2020	2.3x10 <sup>9</sup>	2x10 <sup>9</sup>		
<b>B1111</b>	9 commercial oysters	Jul. 2020	2.3x10 <sup>9</sup>		4x10 <sup>9</sup>	
<b>B1117</b>	9 commercial oysters	Sep. 2020	3.1x10 <sup>9</sup>	7.9x10 <sup>8</sup>		
<b>B1118</b>	9 commercial oysters	Sep. 2020	3.1x10 <sup>9</sup>		1.2x10 <sup>9</sup>	

**Table 2.** Yields in PEDV and TuV using two methods for virus extraction from coastal waters.

Method			Method MF		Method FF		ANOVA
Virus	Sample	N	Mean recovery (%)	SD (%)	Mean recovery (%)	SD (%)	p value
PEDV	E1980	3	0.0754	0.126	3.55	3.38	p=0.0004
	E1982	3	0.687	0.600	0.0112	0.00899	p=0.5707
	E1989	2	1.61	0.339	ND		
	E1990	2	1.55	0.979	ND		
	<b>mean</b>		<b>0.981</b>	<b>0.736</b>	<b>1.78</b>	<b>2.50</b>	<b>ns</b>
TuV	E1980	3	0.0777	0.0818	0.471	0.0750	p=0.2575
	E1982	3	0.471	0.472	0.00513	0.00449	p=0.0511
	E1989	2	0.948	0.247	ND		
	E1990	2	3.84	1.09	ND		
	<b>mean</b>		<b>1.33</b>	<b>1.71</b>	<b>0.238</b>	<b>0.329</b>	<b>ns</b>

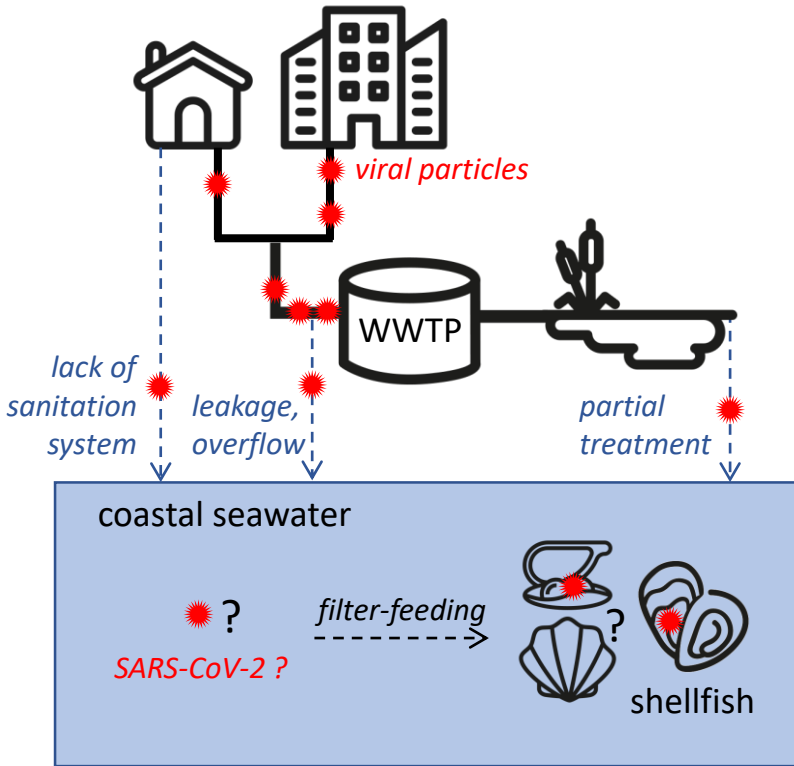
ND : not done

**Table 3.** Results obtained on water and shellfish samples collected during the monitoring study or the microbiological alerts.

Area		Shellfish			Water		
		monitor.	alert	total	monitor.	alert	total
Normandy	Nb of sampling sites	3	3	6	1	1	2
	Nb of samples collected	23	3	26	8	1	9
	<b>SARS-CoV-2 positive samples</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
	NoV positive samples	6	0	6	2	0	2
	NoV positive sites	2	0	2	1	0	1
Brittany	Nb of sampling sites	7	9	16	2	3	5
	Nb of samples collected	59	11	70	18	4	22
	<b>SARS-CoV-2 positive samples</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
	NoV positive samples	8	3	11	3	0	3
	NoV positive sites	6	3	9	1	0	1
Atlantic	Nb of sampling sites	8	3	11	3	1	4
	Nb of samples collected	57	3	60	14	2	16
	<b>SARS-CoV-2 positive samples</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
	NoV positive samples	18	0	18	3	0	3
	NoV positive sites	6	0	6	2	0	2
Mediterranea	Nb of sampling sites	3	1	4	1	0	1
	Nb of samples collected	27	1	28	9	0	9
	<b>SARS-CoV-2 positive samples</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
	NoV positive samples	3	0	3	2	0	2
	NoV positive sites	3	0	3	1	0	1
<i>Total</i>	<i>Nb of sampling sites</i>	<i>21</i>	<i>16</i>	<i>37</i>	<i>7</i>	<i>5</i>	<i>12</i>
	<i>Nb of samples collected</i>	<i>166</i>	<i>18</i>	<i>184</i>	<i>52</i>	<i>7</i>	<i>59</i>
	<b><i>SARS-CoV-2 positive samples</i></b>	<b><i>0</i></b>	<b><i>0</i></b>	<b><i>0</i></b>	<b><i>0</i></b>	<b><i>0</i></b>	<b><i>0</i></b>
	<i>NoV positive samples</i>	<i>35</i>	<i>3</i>	<i>38</i>	<i>10</i>	<i>0</i>	<i>10</i>
	<i>NoV positive sites</i>	<i>19</i>	<i>3</i>	<i>22</i>	<i>5</i>	<i>0</i>	<i>5</i>

monitor.: samples collected during regular monitoring ;

alert: samples collected following alerts of microbiological contamination in additional locations.



➡ Tools to study SARS-CoV-2 : surrogate virus, extraction methods

➡ SARS-CoV-2 bioaccumulation in oysters

➡ Field monitoring of SARS-CoV-2 in seawater and shellfish