# **Supplementary material**

**Table S1.** PCR methods and target regions and gene used for the amplification of potentially harmful microorganisms.

|  |  |  |  |
| --- | --- | --- | --- |
| **Potentially harmful microorganims** | **PCR methods** | **Target regions/gene** | **Reference** |
| Dinophyceae | Conventional PCR | 18S rRNA (V4 region) | See supplementary  material Table S2 |
| *Alexandrium minutum* | rt PCR, SYBR Green | ITS1 rDNA | [1] |
| *Ostreopsis* spp. | rt PCR, SYBR Green | 5.8S rRNA | [2,3] |
| *Bonamia* sp*.* /  *Marteilia refringens* | rt PCR, TAQMAN, duplex | 18S rRNA | [4] |
| *Vibrio* spp. (total vibrios) | rt PCR, SYBR Green | 16S rARN | [5] |
| *Vibrio parahaemolyticus* | rt PCR, TAQMAN | *toxR* gene | [6] |
| Ostreid herpesvirus-1(OsHV-1) | rt PCR, SYBR Green | DNA polymerase (OsHV-1) | [7] |

[1] Klouch, K.Z., Schmidt, S., Andrieux-Loyer, F., Le Gac, M., Hervio-Heath, D., Qui-Minet, Z.N., [Quéré, J.](https://annuaire.ifremer.fr/cv/16996/en/), Bigeard, E., Guillou, L., [Siano, R.](https://annuaire.ifremer.fr/cv/17456/en/), 2016. Historical records from dated sediment cores reveal the multidecadal dynamic of the toxic dinoflagellate *Alexandrium minutum* in the Bay of Brest (France). FEMS Microbiol. Ecol.92(7), fiw10. [doi: 10.1093/femsec/fiw101](https://doi.org/10.1093/femsec/fiw101)

[2] Penna, A., Bertozzini, E., Battocchi, C., Galluzzi, L., Giacobbe, M.G., Vila, M., Garces, E., Lugliè A., Magnani M., 2007. Monitoring of HAB species in the Mediterranean Sea through molecular methods. J. Plankton Res. 29(1), 19-38. Doi: 10.1093/plankt/fbl053

[3] Battocchi, C, Totti, C, Vila, M, Masó, M, Capellacci, S, Accoroni, S, Reñé, A, Scardi, M, Penna, A. 2010. Monitoring toxic microalgae Ostreopsis (dinoflagellate) species in coastal waters of the Mediterranean Sea using molecular PCR-based assay combined with light microscopy. Mar. Pollut. Bull. 60, 1074-1084. doi: 10.1016/j.marpolbul.2010.01.017

[4] European Union Reference & Laboratory for Molluscs Diseases, 2017. Standard operating procedures, Marteilia refringens and Bonamia sp.. Detection by Real time PCR reaction. 1st edition, [www.eurl-mollusc.eu/SOPs](http://www.eurl-mollusc.eu/SOPs)

[5] Thompson, J.R., Randa, M.A., Marcelino, L.A., Tomita-Mitchell, A., Lim, E., Polz, M.F., 2004. Diversity and dynamics of a North Atlantic coastal Vibrio community. Appl. Environ. Microbiol. 70, 4103-4110. doi: 10.1128/AEM.70.7.4103-4110.2004

[6] Hervio-Heath, D., Legeay, E., Evrard, J., Deshoulles, Q., Morga, B., Lecadet, C., Arzul, I.,Siano, R., Dréanno, C., 2018. A protocol to successfully extract DNA from different polymers to investigate microbial diversity and the capacity of polymers to spread harmful marine microorganisms. Conference Proceedings: MICRO 2018, Fate and Impact of Microplastics: Knowledge, Actions and Solutions. Baztan, J., Bergmann, M. (Eds), ISBN 9788409064779, pp. 397-398.

[7] Webb, S.C., Fidler, A., Renault, T., 2007. Primers for PCR-based detection of ostreid herpes virus-1 (OsHV-1): Application in a survey of New Zealand molluscs. Aquaculture 272(1-4), 126-139. doi: 10.1016/j.aquaculture.2007.07.224

**Table S2**. Composition of the PCR reaction mixtures for each target microorganism.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **in µL** | **Dinophyceae**  **(18S rDNA V4)** | ***Alexandrium minutum*** | ***Ostreopsis* spp.** | ***M. refringens / Bonamia* sp.** | ***Vibrio* spp.** | ***Vibrio parahaemolyticus*** | **OsHV-1** |
| Target ADN | 1 | 2 | 2 | 5 | 2 | 2 | 5 |
| Forward primer\* (20µM) | 0.5 | 0.3 | 0.5 | 0.5 / 0.38 | 0.63 | 0.375 | 0.625 |
| Reverse primer\* (20µM) | 0.5 | 0.2 | 0.5 | 0.5 / 0.63 | 0.63 | 0.375 | 0.625 |
| H2O | 9.75 | 7.5 | 8.5 | 4 | 8.25 | 5.625 | 2.5 |
| DMSO | 0.75 | - | - | - | - | - | - |
| TAQMAN probe\* | - | - | - | 0.75 / 0.75 | 0.25 | - | - |
| 50X exo IPC DNA | - | - | - | - | - | 0.5 | - |
| MgCl2 (50 mM) | - | - | 1 | 1 | 1 | 1 | - |
| 10 X Exo IPC mix | - | - | - | - | - | 2.5 | - |
| Mix Phusion MM 2X | 12.5 | - | - | - | - | - | - |
| iTaq Universal SYBRGreen Supermix 2X | - | 10 | - | - | - | - | - |
| Platinium quantitative supermix 2X | - | - | - | - | - | 12.5 | - |
| Platinium SYBR Green qPCR master mix 2X | - | - | 12.5 | - | 12.5 | - | - |
| Brillant III Ultra-Fast 2X | - | - | - | 12.5 | - | - | - |
| Brillant III SYBR Green 2X | - | - | - | - | - | - | 12.5 |
| Volume | 25 | 20 | 25 | 24.25 | 25.25 | 25 | 21.25 |

**Table S3.** PCR cycling conditions for each target microorganism.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **PCR cycling conditions** | **Dinophyceae (18S rDNA V4)** | ***Alexandrium***  ***minutum*** | ***Ostreopsis***  **spp** | ***M. refringens* / *Bonamia* sp.** | ***Vibrio***  **spp** | ***Vibrio parahaemolyticus*** | **OsHV-1** |
| **Denaturation** | 98°C, 30 sec | 95°C, 5 min | 95°C, 10 min | 95°C, 10 min | 95°C, 10 min | 95°C, 10 min | 95°C 10 min |
| **Number of cycles** | 25 | 40 | 40 | 40 | 40 | 40 | 40 |
| **Cycle** | 98°C, 10 sec | 95°C, 5 sec | 95°C, 15 sec | 95°C, 15 sec | 95°C, 5 sec | 95°C, 15 sec | 95°C, 30sec |
| 46°C, 30 sec | 62°C, 15 sec | 62°C 15 sec | 60°C, 1 min | 58°C, 5 sec | 60°C, 1 min | 60°C, 15 sec |
| 72°C, 30 sec | / | 72°C, 15 sec | / | 72°C, 7 sec | / | 72°C, 15 sec |
| **Final cycle** | 72°C, 10 min | / | 95°C, 1 min | / | 95°C, 1 min | / | 95°C, 1 min |
| / | / | 62°C, 30 sec | / | 58°C, 30 sec | / | 60°C, 30 sec |
| / | / | 95°C, 30 sec | / | 95°C, 30 sec | / | 95°C, 30 sec |

**Table S4.** Environmental conditions measured at each sampling date during the colonization experiment implemented in the Biguglia lagoon in 2018.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sampling date** | **Temperature (°C)** | **Salinity**  **(PSU)** | **Dissolved Oxygen (mg.L-1)** | **Turbidity (NTU)** |
| **t0/February** | 14.1 | 14.4 | 14.1 | 12.3 |
| **t0.5/February** | 5.2 | 5.6 | 11.7 | 15.3 |
| **t2/April** | 24.1 | 6.1 | 18.1 | 6.0 |
| **t7/September** | 26.8 | 20.6 | 6.1 | 8.6 |
| **t10/December** | 9.9 | 11.2 | 11.9 | 9.4 |

**Fig. S1.** Seasonal variations of environmental parameters (water temperature, salinity, dissolved oxygen concentration and turbidity) and cumulative seasonal rainfall during 2016 for the three lagoons (Prevost, Biguglia and Diana). The error bars correspond to the standard deviation obtained on triplicate field measurements.

