Detection of the oyster parasites *Bonamia* and *Marteilia* in gill and seawater through qPCR and dPCR

Delphine Tanghe

Bachelor of Biomedical Laboratory Technology, HOWEST, Rijselstraat 5, 8000 Brugge, Belgium
E-mail: delphine.tanghe15@gmail.com

The flat oyster Ostrea edulis is a bivalve species native to Europe that used to be very prominent in the North Sea. As they grow, they merge together and form banks, so-called oyster reefs, which provide important habitats for various species, harbouring a high biodiversity. Because of its significance for the marine ecosystem, protection and restoration of flat oyster populations are being encouraged. These banks however, are being plagued by the protozoan parasites Bonamia ostreae and Marteilia refringens, causing mass mortality events of flat oysters in Europe. The resulting diseases, bonamiosis and marteiliosis, lead to a massive decrease of flat oyster production and a decline of wild populations in Europe.

The aim of this study is to define the most sensitive method for the detection of the two shellfish regulated parasites in both oysters and seawater. This way, the prevalence of the parasites in the North Sea can be monitored and where relevant, absence of infection can be demonstrated. To detect the parasites, the following DNA-based methods were being used: quantitative Polymerase Chain Reaction (qPCR) and digital PCR (dPCR). Both methods needed to be validated and optimized, prior to collecting samples from the field. To verify the sensitivity of both methods, a serial dilution series of plasmid DNA, containing the PCR targeted sequences of Marteilia refringens and Bonamia sp., was used as a positive control. Based on these plasmids, the most sensitive method could not be determined. Afterwards, 96 oysters from two different locations and water samples from three locations were collected from the Spuikom in Ostend to investigate the prevalence of both parasites and to determine the most feasible method for this purpose. No oysters or water samples were found to be infected with B. ostreae or M. refringens, indicating the absence of the parasites in the Spuikom. Thereafter, a ringtest was conducted with 20 oyster samples from the Netherlands using qPCR and dPCR to investigate the efficiency of both methods. All results, except for one sample, obtained with the dPCR were corresponding to those of the Netherlands, showing that this method is suitable for detection of the oyster parasite. The dPCR was able to detect more infected oysters with lower concentrations of parasite DNA than the qPCR, as the qPCR could only detect positive oysters with a high infection rate. Based on a serial dilution series of an oyster infected with Bonamia, the detection limit of both methods could be determined. The lowest dilution producing consistent positive results was 5 c / µl for both methods, with an average concentration of 2,02 c / µl for the dPCR and an average Ct-value of 36,29 for the qPCR. In a future study, this could be examined for Marteilia in the same way. To investigate whether it is possible to detect the parasites in seawater using dPCR, 78 flat oysters infected with Bonamia were placed in a tank with 300 L seawater to obtain a suspension with freshly released parasites. The presence of the oysters themselves in the water samples was also verified with an additional optimized dPCR. To do so, the detection of oyster DNA was first verified in oyster samples, finding that the dynamic range of the dPCR is smaller than the qPCR. All water samples collected from the tank with infected oysters were positive for both the oysters and parasites, which indicates that the dPCR appears to be sensitive enough for detection of the parasites in seawater.

It can be concluded that the dPCR is the most sensitive method for the detection of the parasites in both oysters with a low infection rate and water samples, while the qPCR is more suitable for screening oysters with very high infection rates, since its dynamic range is larger. The eDNA detection offers a non-invasive sampling technique that can be applied when oysters are kept in closed tanks, but as it cannot be guaranteed that this method can detect infections in a larger environment, e.g. the Spuikom, further research is required.