Supplementary Material 4. Level of validation (as presented by Thalinger et al., 2021) for each qPCR assay.

Validation level definition	UIBK	INRAE	UCC	IMR
Level 1: *In silico* analysis, target tissue testing	✔	✔	✔	✔
Level 2: Comprehensive reporting of PCR conditions, testing of closely related nontarget species	✔	✔	✔	✔
Level 3: Extraction method defined and performed on eDNA samples, concentration of eDNA from environmental samples reported, positive detection obtained from environmental samples	✔	✔	✔	✔
Level 4: Limit of Detection (LOD) determined, extensive field testing of environmental samples, in vitro testing on co-occurring nontarget species	✔ *	✔ *	✔ *	✔ *
Level 5: Comprehensive specificity testing, detection probability estimation from statistical modeling, understanding ecological and physical factors influencing eDNA in the environment	X	X	X	X

* Extensive field testing not conducted for this study.