Tables S1. Primers and probes used for both end-point PCR (ep PCR) and quantitative PCR (qPCR) and Real-Time PCR to test the reliability of the methods proposed [87,94,101,104,105,133].

Methods	Target	Primer	Annealing temperature	Amplicon	References
Ep PCR	Fungi	ITS 1F / ITS 4R	54 °C	713 bp	[101]
Ep PCR	Aquatic Bacteria	16SBAq F / 16SBAq R	60 °C	1060 bp	[133]
Ep PCR	*A. astaci*	42F / 640R	59 °C	569 bp	[87]
qPCR	*A. astaci*	AphAstITS-39F / AphAstITS-97R AphAstITS-60T	62 °C	59 bp	[94]
Real-Time PCR	*A. pallipes*	Apal_F / Apaltor_R Apal_probe	64 °C	136 bp	[105]
Real-Time PCR	*P. clarkii*	SPY_ProCla_F / SPY_ProCla_R SPY_ProCla_Probe	56 °C	65 bp	[104]

End-point PCRs

End-point PCRs were carried out with the following thermal protocol: (i) plate preheat at 94 °C for 2 min; (ii) 40 cycles of 94 °C for 30 s, 58 °C ± 4 °C for 30-60 s (according to primers) and 72 °C for 60-90 s; and (iii) final extension at 72 °C for 7 min. According to the manufacturer, each PCR contained the following reagents at their final concentration in a volume of 25 µl: 1X Buffer, 1.5 mM $MgCl_2$, 0.8 mM dNTPs mix, 0.25 µM forward primer, 0.25 µM reverse primer and 0.02 U/µl Platinum Taq Polymerase (Thermo Fisher Scientific). Template did not exceed 500 ng per reaction.

Quantitative PCR

Quantitative PCR (qPCR) were carried out to detect *A. astaci* with the following fast thermal protocol (Rusch et al. 2020): (i) plate preheat at 95°C for 5 min; (ii) 50 cycles of 95 °C for 15 s, 61 °C ± 3 °C for 30 s (according to primers) and plate read to properly collect fluorescence data. PCR were performed with QuantiNova Probe PCR Kit (Qiagen) and the final volumes were modified according to the manufacturer. Final concentration: 1X Master-mix, 0.5 µM of each primer and 0.2 µM of probe. Each amplification was performed using 4 µl of template without exceeding 200 ng of total DNA/reaction.

Real-Time PCRs

Real-Time PCRs to detect *P. clarkii* and *A. pallipes* were carried out with adapted thermal protocol, according to Manfrin et al. (2022) and Tréguier et al. (2014), respectively. Protocols were summarized as follows: (i) plate preheat at 95°C for 5 min; (ii) 50 cycles of 95 °C for 15 s, 56/64 °C for 30 s (according to primers) and plate read to properly collect fluorescence data. PCR were performed with QuantiNova Probe PCR Kit (Qiagen) and the final volumes were modified according to the manufacturer. Final concentration: 1X Master-mix, 0.2 μM of each primer and 0.1 μM of probe. Each amplification was performed using 4 μl of template without exceeding 200 ng of total DNA/reaction.