Recombinase Polymerase Amplification for *Gambierdiscus* and *Fukuyoa* detection: a step further in the ciguatera risk management

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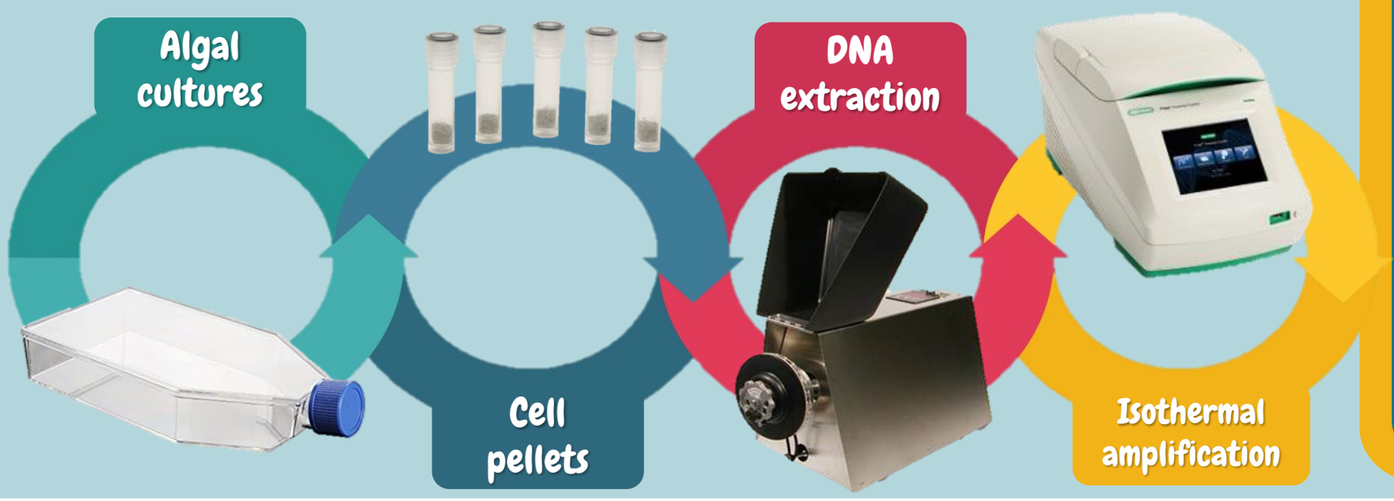
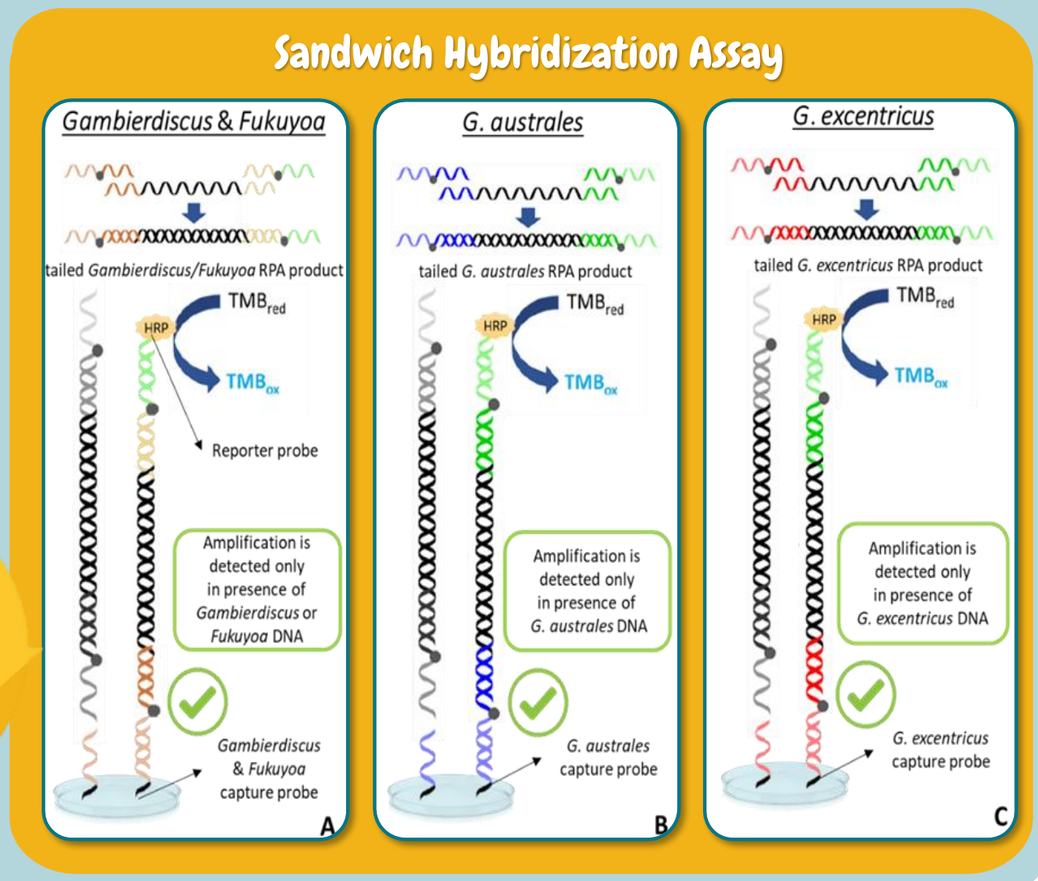
Why is it important to detect microalgae of the genera *Gambierdiscus* and *Fukuyoa*?

Ciguatera fish poisoning is one of the most relevant seafood-borne illnesses worldwide. It is caused by the ingestion of fish contaminated by ciguatoxins (CTXs). Primary producers of CTXs are dinoflagellates of the genera *Gambierdiscus* and *Fukuyoa*.

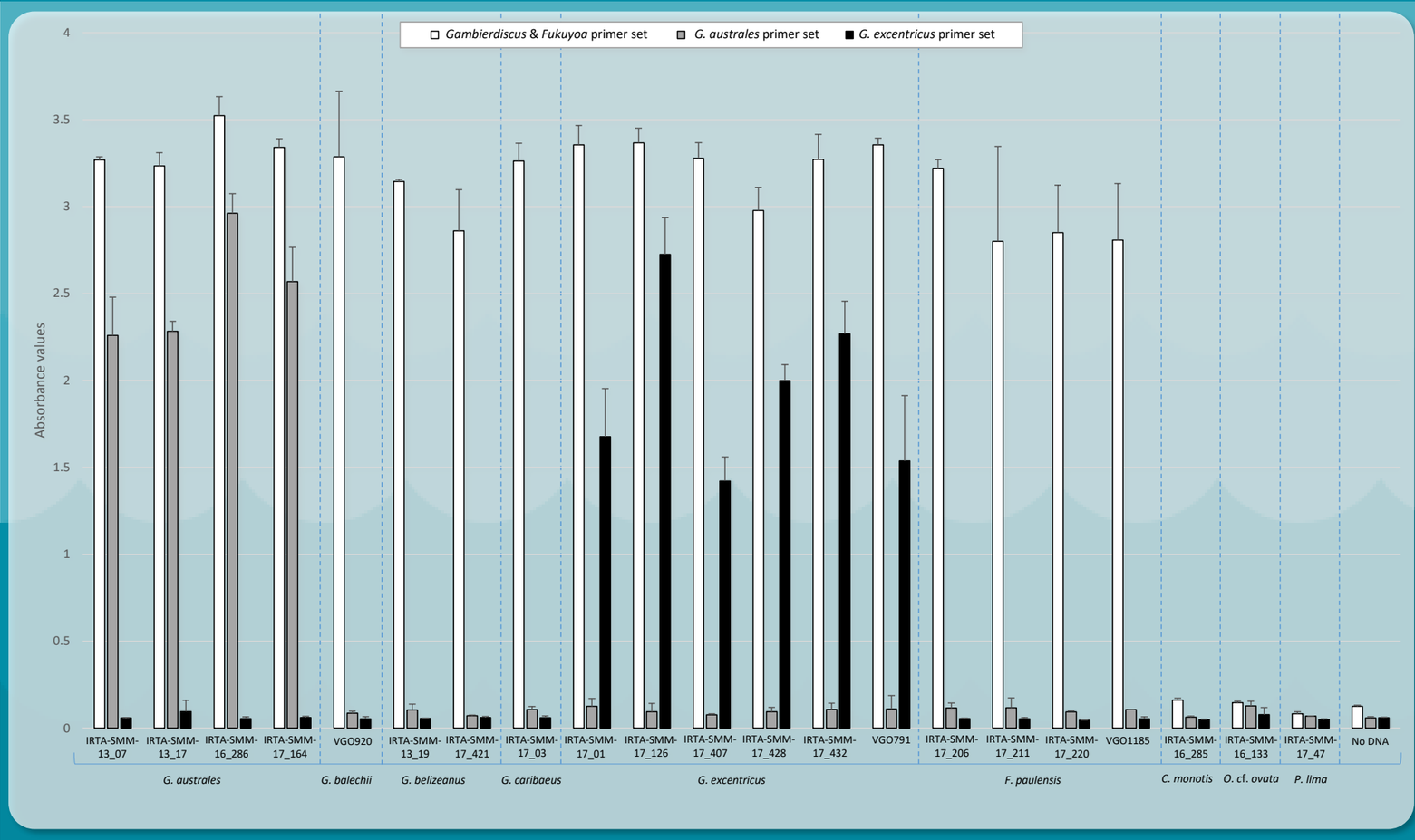
Strategy

One primer set for the genera (A) *Gambierdiscus* and *Fukuyoa* and two species-specific primers set for *Gambierdiscus* species ((B) *G. australes* and (C) *G. excentricus*) were designed within the D1-D3 and/or D8-D10 of the of the 28 S LSU ribosomal DNA. Primers were modified with tails, resulting in amplicons of dsDNA flanked by ssDNA tails. Recombinase Polymerase Amplification was performed at 37 °C for 30 minutes (TwistAmp® Kit). Samples were purified before the Sandwich Hybridization Assay.





Results



Obtained results demonstrate the ability of the system to discriminate not only the genus *Gambierdiscus* and *Fukuyoa* from other microalgae (white) , but also *G. australes* (grey) and *G. excentricus* (black) species from their congeners.

Species	Strain	*Gambierdiscus* & *Fukuyoa* primer set	*G. australes* primer set	*G. excentricus* primer set
G. australes	IRTA-SMM-13_07	+	+	-
G. australes	IRTA-SMM-16_286	+	+	-
G. balechii	VGO920	+	-	-
G. belizeanus	IRTA-SMM-13_19	+	-	-
G. belizeanus	IRTA-SMM-17_421	+	-	-
G. caribaeus	IRTA-SMM-17_03	+	-	-
G. excentricus	IRTA-SMM-17_126	+	-	+
G. excentricus	IRTA-SMM-17_407	+	-	+
F. paulensis	IRTA-SMM-17_206	+	-	-
F. paulensis	IRTA-SMM-17_211	+	-	-

Single cells were extracted from clonal cultures. Each extract was divided in 3 aliquots and tested with the primer sets (*Gambierdiscus* & *Fukuyoa, G. australes, G. excentricus).* Amplification signals were observed only in presence of target DNA.

Conclusions

These results demonstrate the potential of the system to discriminate *Gambierdiscus* and *Fukuyoa* genera and two *Gambierdiscus* species from other microalgae, and its limit of detection is as low as a single cell. This approach is more rapid, specific and user-friendly than traditional microscopy techniques, and paves the way towards the deployment of portable devices for in situ detection of microalgae.

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