Solid Phase Adsorption Toxin Tracking (SPATT) technology for field monitoring of *Gambierdiscus* toxins with passive samplers

Ciguatera poisoning is a seafood intoxication classically associated with the consumption of tropical coral reef fish contaminated with ciguatoxins (CTXs), although some marine invertebrates such as bivalves, gastropods or echinoids are also potential vectors of ciguatera [1-3]. CTXs are polyether neurotoxins produced by dinoflagellates of the genera *Gambierdiscus* and *Fukuyoa*, and are responsible for severe acute digestive, neurological, and cardiovascular symptoms in humans [4]. Ciguatera has major health and economic impacts on vulnerable island communities whose subsistence strongly relies on the sustainable exploitation of marine resources, such as in the Pacific island countries and territories (PICTs) where high incidence rates have been consistently reported in the past two decades [5].

Most surveillance programmes currently rely on the survey of cell densities and species composition of *Gambierdiscus* populations, as well as on the evaluation of CTXs concentrations in marine products. However, such methods are time consuming and expensive, thus emphasizing the need for supplementary tools, based on the spatio-temporally integrated sampling of dissolved algal toxins directly in marine environments. The SPATT (solid phase adsorption toxin tracking) technology, first introduced in 2004 [6], uses porous synthetic resins capable of adsorbing dissolved toxins directly from the water column (Fig. 1). Numerous laboratory and field studies, testing different adsorbent substrates of which Diaion® HP20 resin appears to be the most versatile substrate, have been carried out worldwide to assess the applicability of these passive monitoring devices to the detection of lipophilic and hydrophilic toxins produced by a variety of marine and freshwater microorganisms [7]. Regarding the monitoring of toxins associated with ciguatera, one laboratory study has demonstrated the efficacy of HP20 resin for the detection of dissolved CTXs and maitotoxins (MTXs) in *Gambierdiscus* cultures [8].

The efficacy of SPATT technology to detect *Gambierdiscus* toxins in the field in ciguateric biotopes was recently confirmed by the deployment of SPATT devices filled with 10 g of HP20 resin for 48 h in two French Polynesian sites: main village of Kaukura Island (Tuamotu archipelago) and Anaho Bay in Nuku Hiva Island (Marquesas archipelago), characterized by a moderate *vs.* high risk of ciguatera [9]. The presence of CTXs in SPATT devices was assessed using the neuroblastoma cell-based assay (CBA-N2a) and results showed that SPATT devices deployed in Anaho Bay were able to retain 1.30 ± 0.41 ng P-CTX3C equiv. g^{-1} HP20 resin. Liquid chromatography - tandem mass spectrometry (LC-MS/MS) analyses confirmed that P-CTX3B and P-CTX3C were primarily retained on SPATT devices. These results are coherent with environmental observations that confirmed the presence of significant amounts of *Gambierdiscus* cells in Anaho Bay, most notably in the species *G. polynesiensis* known for its very high toxic potential, and with toxicological analyses that demonstrated the presence of CTXs in all marine products tested [2,3]. In contrast, no CTXs were detected in SPATT devices deployed in Kaukura Island. However, as with SPATT devices deployed in Anaho Bay, LC-MS/MS analyses revealed the adsorption of a putative MTX analogue, known as MTX3, on SPATT devices deployed in Kaukura Island. Recent studies indicate that putative MTX3 is ubiquitous within *Gambierdiscus* genus [10], suggesting its potential use as a biomarker of the occurrence of *Gambierdiscus* cells in the natural environment. Since a very low density of *Gambierdiscus* cells was observed in Kaukura Island, the detection of putative MTX3 but not of CTXs on SPATT devices suggest either the presence of non-toxic *Gambierdiscus*

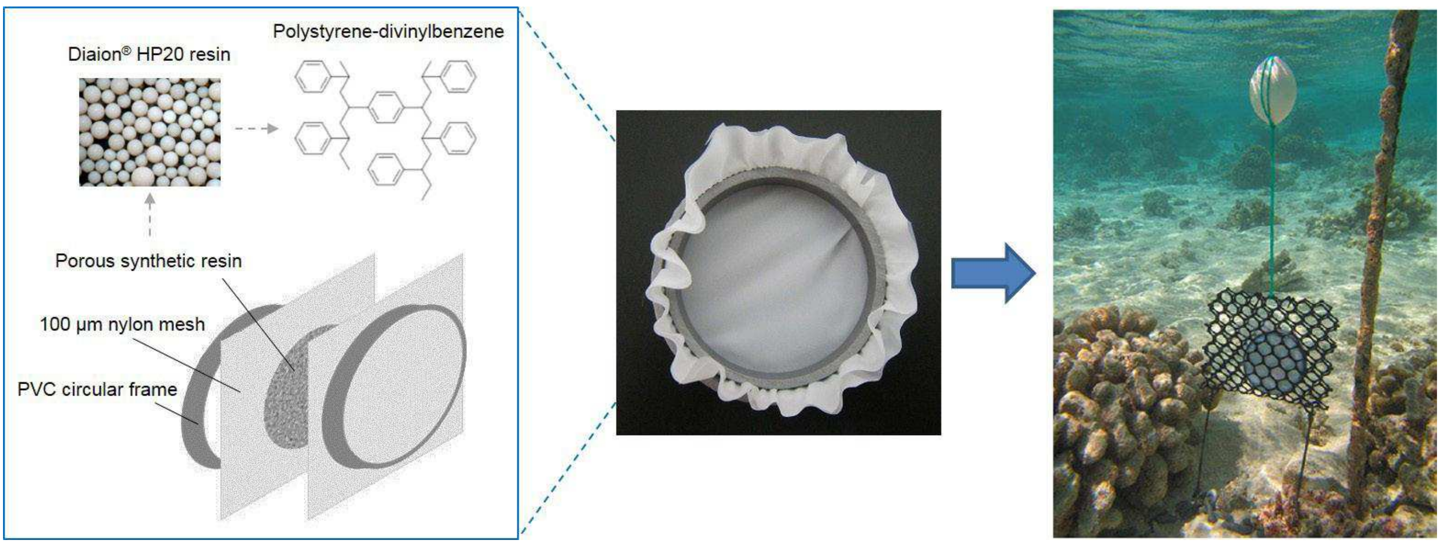

Fig. 1. Example of a design of SPATT device assembly and field deployment. Left and middle: the SPATT device is made of two nylon mesh layers filled with a porous synthetic resin (Diaion® HP20, polystyrene-divinylbenzene matrix, is the most used resin), and fixed between two PVC circular frames. Right: in the field, the SPATT device is inserted in plastic grids to prevent its damage and grazing by fish, and maintained in a vertical position in the water column using weights and floats. © M. Roué (modified from [7] and [9])

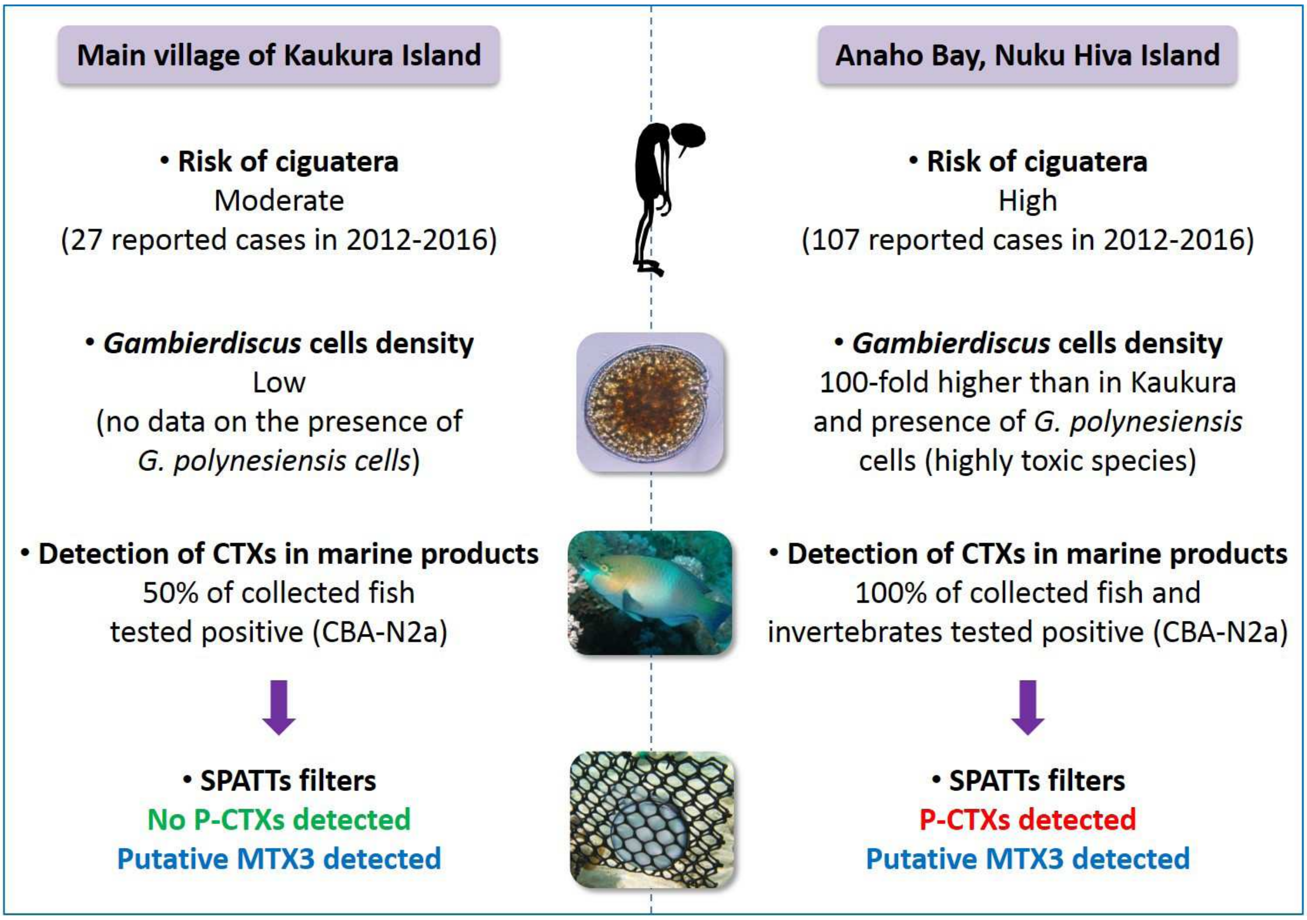

Fig. 2. Summary of the results demonstrating that SPATT *passive sampling could advantageously contribute to the reinforcement of ciguateric risk assessment and management programmes as a supplementary tool.*

or of very low densities of toxic *Gambierdiscus* cells. However, CTXs were detected in numerous fish collected in Kaukura Island (CBA-N2a), suggesting that blooms of toxic *Gambierdiscus* had previously occurred in this area, leading to the bioaccumulation of CTXs in marine products, but had disappeared at the time of the SPATT devices deployment. Another explanation could be that SPATT devices allow for the detection of CTXs in the field only when high levels of CTXs are dissolved in the seawater and are thus relevant only in highly ciguateric locations.

In all cases, these results (summarized in Fig. 2) confirmed the ability of SPATT technology to detect CTXs in the field and thus emphasize the relevance of this toxin-based technique as a useful supplementary tool when assessing the risks associated with *Gambierdiscus* proliferation in locations highly prone to ciguatera. It should be highlighted that SPATT devices are only able to give indications of the presence of *Gambierdiscus* cells (detection of MTX3) and their toxicity (detection of CTXs) in an area at a given time, but they are not effective to guarantee the edibility of marine products that could have bio-accumulated toxins in their tissues. The simplicity, low-cost and logistical advantages (e.g. storage and transport) offered by these easy-to-use devices make SPATT technology a valuable tool well adapted to the survey of CFP risk, most notably in widely dispersed and remote sampling locations in PICTs. Moreover, given the risk of simultaneous accumulation of multiple biotoxins in seafood products in tropical environments, the use of SPATT technology, when combined with downstream analyses such as LC–MS/MS multi-toxin screenings, also offers promising prospects in the framework of emerging toxins surveillance.

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References

1. *Dickey RW & Plakas SM 2010. Toxicon 56: 123-136*
2. *Roué M et al 2016. Harmful Algae 57: 78-87*
3. *Darius et al 2018. Toxins 10(1): 2*
4. *Darius et al 2018. Mar Drugs 16: 122*
5. *Skinner MP et al 2011. PLoS Negl Trop Dis 5(12): e1416*
6. *MacKenzie LA et al 2004. Toxicon 44: 901-918*
7. *Roué et al 2018. Toxins 10: 167*
8. *Caillaud A et al 2011. Harmful Algae 10: 433-446*
9. *Roué et al 2018. Harmful Algae 71: 40-49*
10. *Pisapia et al 2017. Mar Drugs 15: 220*

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