Toxic Algal Blooms and their socio-economic impacts: What can nuclear techniques provide for their Management?

Beatriz Reguera, Florence Boisson, Taiana Darius, Marie-Yasmine Bottein
“harmful algal blooms” (HABs), a term coined by IOC to designate any microalgal proliferation—regardless their concentration—that is perceived as a harm for its negative effects in human health, fisheries, aquaculture, tourist industry and other resources.

Red tides ≠ Harmful Algal Blooms
Toxin producing HAB species include **planktonic** and **benthic** microalgae belonging to different classes and orders, with very diverse physiological requirements.

Diatoms (13)

Dinoflagellates: 11 Dinophysiales
23 Gonyaulacales
5 Peridiniales
13 Prorocentrales
19 Gymnodiniales

Haptophytes (9)
Raphidophyceans (7)
Dictyochophyceans (2)

[Database]

http://ioc-unesco.org/hab/
"What I tell you three times is true."
(Lewis Carroll)

Harmful Algal Blooms are increasing in frequency, intensity and geographic distribution (Smayda 1990; Anderson 1991)?

Depends which species, where and when
Distribution of living and fossil resting cysts of *Pyrodinium bahamense* (GEOHAB 2002)

Distribución de quistes de *Pyrodinium bahamense* (Zingone, 2002)
### Significant Economic Loss

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Species</th>
<th>Loss (US$ million)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1972</td>
<td>Japan</td>
<td>yellowtail</td>
<td>~47</td>
</tr>
<tr>
<td>1977</td>
<td>Japan</td>
<td>yellowtail</td>
<td>~20</td>
</tr>
<tr>
<td>1978</td>
<td>Japan</td>
<td>yellowtail</td>
<td>~22</td>
</tr>
<tr>
<td>1978</td>
<td>Republic of Korea</td>
<td>oyster</td>
<td>4.6</td>
</tr>
<tr>
<td>1979</td>
<td>Maine, US</td>
<td>many</td>
<td>2.8</td>
</tr>
<tr>
<td>1981</td>
<td>Republic of Korea</td>
<td>oyster</td>
<td>&gt;60</td>
</tr>
<tr>
<td>1985</td>
<td>Long Island, US</td>
<td>scallops</td>
<td>2</td>
</tr>
<tr>
<td>1986</td>
<td>Chile</td>
<td>red salmon</td>
<td>21</td>
</tr>
<tr>
<td>1987</td>
<td>Japan</td>
<td>yellowtail</td>
<td>15</td>
</tr>
<tr>
<td>1988</td>
<td>Norway/Sweden</td>
<td>salmon</td>
<td>5</td>
</tr>
<tr>
<td>1989</td>
<td>Norway</td>
<td>salmon, trout</td>
<td>4.5</td>
</tr>
<tr>
<td>1991</td>
<td>Washington St., US</td>
<td>oyster</td>
<td>15-20</td>
</tr>
<tr>
<td>1991-92</td>
<td>Republic of Korea</td>
<td>farmed fish</td>
<td>133</td>
</tr>
<tr>
<td>1996</td>
<td>Texas, US</td>
<td>oyster</td>
<td>24</td>
</tr>
<tr>
<td>1998</td>
<td>Hong Kong</td>
<td>farmed fish</td>
<td>32</td>
</tr>
</tbody>
</table>

Phytoplankton blooms can have major economic impacts on fisheries, aquaculture and tourism.
Raphidophyceans

- Chattonella antiqua
- Ch. globosa
- Ch. marina
- Ch. subsalsa
- Ch. verruculosa
- Fibrocapsa japonica
- Heterosigma akashiwo
Fish Aquaculture: Caged fish exposed to toxic HABs (canary in the coal mine) reveal the presence of a pre-existing problem.
First report of *Chattonella verruculosa* and fish kills in Europe, April-May 1998.

Satellite images (surface pigments) and operational oceanography models may (NOT ALWAYS) provide prediction of the patch movements.
HAB with multiple noxious effects: Blooms of *Karenia brevis* in the Gulf of Mexico

Toxic sea spray causes respiratory and skin irritations

Foams and mucilage accumulated in the beach or clogging nets

Discolorations, hypoxia or hyperoxigenation, damage to bottom fauna

Fish mortalities

Shellfish contamination with brevetoxins

(modified from GEOHAB 2003)
HBAB = Harmful Benthic Algal Blooms

Benthic HABs with multiple negative effects

The case of *Ostreopsis* spp in the Mediterranean Sea

- Skin and respiratory tract irritation;
- Production of potent toxins: palytoxins, ostreopsinas.

www.bentoxnet.it
Shellfish filter-feeds toxic phytoplankton species and transmit their toxins through the food chain causing toxic syndromes

- Paralytic shellfish poisoning PSP
- Diarrhetic shellfish poisoning DSP
- Amnesic shellfish poisoning ASP
Toxin producing microalgae

The main threat for public health and shellfish exploitations

- PSP
- DSP
- NSP
- ASP
- CFP
Each species, or even each strain, has a characteristic toxin profile. *Fingerprint*

PSP toxin profile of *Alexandrium minutum* from Ria de Vigo. Chromatogram from high performance liquid chromatography (HPLC) analysis of a culture extract.
Ciguatoxins, Maitotoxins
Ciguatera Fish Poisoning

Ostreopsins, palitoxins
Respiratory tract and skin irritation through sea spray

Okadaic acid
Diarrhetic Shellfish Poisoning
Management of CFP: Avoid eating dangerous species; identify risk areas and educate the population and health practitioners.
Polymorphic Life cycles

But most field studies only pay attention to the vegetative stages

Fossilizable resting cyst can be traced in radio-dated sediments!!

The life cycle of *Gymnodinium catenatum* (Blackburn et al. 1988)
Plate cyst-1

Cysts of Gymnodiniales

Gymnodinium catenatum

Gyrodinium instriatum

Gyrodinium impudicum

Phaeopolykrikos hartmannii

Cochlodinium sp.

Polykrikos kofoidii/schwartzii complex

Gymnodinium catenatum

microphotographs by K. Matsuoka, Y. Fukuyo & I. Imai

microphotographs by K. Matsuoka, S. Yoshimatsu & Y. Fukuyo
Plate cyst-5

Cysts of Gonyaulacales

Pyrodinium bahamense
Alexandrium
Gonyaulax verior

Pyrodinium bahamense

Gonyaulax spinifera Complex

Lingulodinium polyedrum
Protoceratium reticulatum
Pyrophacus steinii

Cyst form

Thecate form

microphotographs by Y. Fukuyo & K. Matsuoka

microphotographs by Y. Fukuyo & K. Matsuoka
Radionuclide-based techniques* may provide powerful sensitive and selective tools to address questions related to HAB research and monitoring.

**BUT GOOD RESULTS REQUIRE TO PUT DIFFERENT SPECIALISTS TOGETHER**

Micropaleontologists, paleoclimatologists......

*These techniques are transferred to IAEA Member States in Africa, Asia and Latin America through Technical Cooperation projects.
Introduction of alochtonous species may contribute to expand the geographic range of some HAB species

- Currents and storms
- Animals
- Ballast water discharge
- Shellfish seed
How can I tell if a species has been introduced in a new area??

How can I tell if my problem species is increasing in frequency and intensity or is just going through normal decadal fluctuations?

WE NEED LONG TIME SERIES (> 50 y) ONLY AVAILABLE IN A FEW PLACES!

The Alternative: Radiometric sediment core dating combined with fossil cyst abundance
Looking into the past to predict the future!

Radiometric dating of long sediment cores in HABs areas related to fossil cysts distribution

- Determine whether or not a species has been recently introduced in a new area
- Obtain decadal and secular time series related to climate variability
Reconstruct past HABs events

Radiometric dating of sediment cores in HABs areas related to vertical cysts distribution, to:

- Assess factor promoting HABs
- Map HABs risk areas
- Improve HABs monitoring
Collection of Sediment Cores

Cut into sections

Plating

Driving off excess acid

(Silver discs)

Counting

Driving (Oven, Freeze Dryer)

+Tracers Acids

Microwave Bomb Digestion

Sedimentation Rate Calculations according to established models
Example of dating of past HABs events

Core 3

Vertical distribution of P. bahamense cysts

Core 4

Computing sedimentation rates

Lead-210 (Bq/kg) with sediment core depth

Results obtained by the IAEA Collaborating Centre in the Philippines (Sombrito et al.) to understand Pyrodinium bahamense Harmful Algal Blooms in Manila Bay and Malampaya Sound (Philippines)
Since 1998, the IOC-ANCA group of experts for the Caribbean region, identified 2 HAB-related priorities:

- Paralytic Shellfish Poisoning (PSP) events
- Ciguatera Fish Poisoning (CFP)

Basic mitigation of socio-economic impacts:

**EARLY WARNING OF THE PRESENCE OF HARMFUL MICROALGAE, AND OF TOXINS IN SEAFOOD**
RBA can be used to estimate toxicity (pg. STX equiv. per cell)

Each species, or even each strain, has a characteristic toxin profile. *Fingerprint*

PSP toxin profile of *Alexandrium minutum* from Ria de Vigo. Chromatogram from high performance liquid chromatography (HPLC) analysis of a culture extract.
Monoalgal cultures

The basic raw material to undertake further investigations on:
- Taxonomy. IDENTIFICATION OF THE SPECIES
- Toxin profile and content WHAT TOXINS AND HOW
- Physiology. RESPONSE TO ENVIRONMENTAL CONDITIONS

Culture collection of toxic microalgae from IEO-Vigo (CCVIEO)
Methods to detect and quantify phycotoxins in shellfish

<table>
<thead>
<tr>
<th>Assays</th>
<th>Analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>in vivo</em></td>
<td></td>
</tr>
<tr>
<td>mouse rats</td>
<td>HPLC</td>
</tr>
<tr>
<td><em>in vitro</em></td>
<td>Fluorimetry</td>
</tr>
<tr>
<td>Receptors</td>
<td>UV, FD</td>
</tr>
<tr>
<td>Cellular</td>
<td>Mass spectrometers</td>
</tr>
<tr>
<td>Enzymatic inhibition</td>
<td></td>
</tr>
</tbody>
</table>

Most standard shellfish toxin analyses still based on mouse bioassays
EU: To end mouse bioassays by 2013!
Hong Kong
2000-03: 233 people affected
2004 live coral fish from Kiribati caused 247 cases
CFP

Manila: 50 cases in 2001

Hot spot for CFP
10,000 case/year in Kiribati
(60 kg fish/yr)
>10 per 1000 annually in Tokelau et Tuvalu

Toxic blooms and associated disease are increasing in intensity, frequency and distribution
3843 cases, 8 death
1993-2002

20,000 cases/yr
Ciguatera Fish Poisoning, Canary Islands 2005

Jose-Luis Pérez-Arellano,* Octavio P. Luzardo,† Ana Pérez Brito,§ Michele Hernández Cabrera,* † Manuel Zumbado,* Cristina Carranza,* † Alfonso Angel-Moreno,* † Robert W. Dickey,§ and Luis D. Boada

Ciguatera: the detection of neurotoxins in carnivorous reef fish from the coast of Cameroon, West Africa 2008

P Bienfang, B Oben, S DeFelice, P Moeller, K Huncik, P Oben, R Toonen, T Daly-Engel, B Bowen

Autecology of the Toxic Dinoflagellate Gambierdiscus toxicus Adachi et Fukuyo (Dinophyceae) in Central Coastal Areas of Tanzania 2006

Charles Lugomela
Department of Fisheries Science and Aquaculture, Faculty of Aquatic Sciences and Technology, University of Dar es Salaam, P.O. Box 35064 Dar es Salaam, Tanzania
RBA for CFP, NSP, PSP following similar microplate formats

1. Membrane preparation containing receptor sites

2. Incubation of $[^3\text{H}]$ligand + toxin standard or sample + membrane preparation

3. Unbound $[^3\text{H}]$ligand and toxin removed by washing and filtration

4. $[^3\text{H}]$ligand bound to receptor sites determined on scintillation counter

Microplate or conventional LSC
Receptor Binding Assay for Ciguatoxins (CTXs)

Sodium channel Na$^+$ receptor of marine toxins

Ciguatoxines, CTX
Brévétoxines, PbTx

Gambierdiscus
Karenia brevis

Receptor 5 of the $\alpha$ subunit of sodium channel
RBA advantages and limitations

LIMITATIONS
• No ID of toxin derivatives
• No absolute confirmation of toxin presence in sample (cf mouse bioassay)

ADVANTAGES
• Rapid, high throughput
• Sensitivity <nM (~300X MB)
• Function-based specificity
• Detect only toxic forms
• Estimate integrated toxic potency
• Same method as for NSP, PSP and CFP
• No use of live animals
Ciguatera risk assessment in French Polynesia

Receptor Binding Assay for CTXs
Receptor Binding Assay for Ciguatoxins (CTXs)

**Biological matrix**
- *Gambierdiscus*
- *Ostreopsis*
- Fishes
- Cyanobacteria
- Giant clams, Sea urchins

**CTXs extraction**
- Mass protocol
- Sep-Pak protocol
- Mass protocol + Sep-Pak protocol

**liposoluble fraction**
- 5,000 cells
- 5 g of flesh
- 2 mg of extract
- 2 & 5 g of flesh

Receptor Binding Assay (RBA)
Receptor Binding Assay for Ciguatoxins (CTXs)

Preparation and incubation

Filtration

Incubation

Results

Counting

3h30
1h00
1h30
4h00

IC 50
Ciguatera risk assessment programme in French Polynesia and beyond

French Polynesia

Vanuatu

New Caledonia

Lifou

Emao

Nuku-Hiva
General methodology

- Public information meetings prior to any sampling
- Questionnaire translated in French and Tahitian, to be completed by the locals, to collect historical information on:
  - fish and other seafood poisonings cases
  - infected reef areas within the lagoon
  - fish species known to be toxic
- Field sampling of dinoflagellates, cyanobacteria, fishes and marine invertebrates like giant clam or sea urchins
- *In vitro* culture from wild cells of *Gambierdiscus* and cyanobacteria
- Species identification of both wild and in vitro samples of *Gambierdiscus* and cyanobacteria
- Species identification of fishes and marine invertebrates
- Toxicity analyses using *in vivo* and/or *in vitro* tests
- Restitution of the results to the local people
Objectives of the ciguatera risk programme

- Reactivate the fish poisoning record keeping with the medical centre of a given island
- Check the existence of cases of intoxication by consumption of invertebrates like giant clams or sea urchins
- Assess the CFP risk: *Gambierdiscus* abundance and distribution, toxin levels in *Gambierdiscus*, cyanobacteria, fish and giant clam populations
- Initiate community awareness and education with a view to prevention
Raivavae - Chronology of aggressions

- Dredging work
- North Pass
- Embankment
- Raivavae airport landing strip
- Motu de la femme

### Agressions of climatic origins (according to the Atlas climatologique de Météo France)

<table>
<thead>
<tr>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 2nd-9th, 1976</td>
<td>FRAN tropical cyclone (970 hpa of intensity)</td>
</tr>
<tr>
<td>April 17th-23rd, 1977</td>
<td>ROBERT tropical cyclone (955 hpa of intensity)</td>
</tr>
<tr>
<td>March 9th-13th, 1981</td>
<td>TAHIR tropical cyclone (990 hpa of intensity)</td>
</tr>
<tr>
<td>February, 28th - March 3rd, 1988</td>
<td>Heavy tropical depression CLILLA (985 hpa of intensity)</td>
</tr>
<tr>
<td>December 5th-13th, 1991</td>
<td>WASA tropical cyclone (955 hpa of intensity)</td>
</tr>
<tr>
<td>October 30th-November 4th, 1992</td>
<td>Tail of MARTIN tropical cyclone (955 hpa of intensity)</td>
</tr>
<tr>
<td>November 21th-27th, 1997</td>
<td>Tail of ASEA tropical cyclone (954 hpa of intensity)</td>
</tr>
<tr>
<td>November 2003</td>
<td>Tail of tropical cyclone</td>
</tr>
</tbody>
</table>

### Agressions of anthropic origins (according to the informations recorded from the local population)

- The island's first lime kilns used sheets of coral gathered from the edges of the lagoon.
- **Excavations of coral with in the area of the Motu de la Femme, for the building and upkeep of the circular road.**
- Dynamite used for the first time to enlarge the North pass, in order to facilitate entry of material necessary for the NEP to build an observation centre for potential falls-out during the nuclear tests period.
- Filling-up of the lagoon for the building of Raivavae town hall and extension of the sports hall (carried out with rocks and earth, without use of coral slush).
- Second filling-up of the lagoon to accommodate the current sports ground facility and concrete works needed to build the Rairua landing quay.
- More dynamite usage for the pass at the north of the island to maintain the current access.
- More dynamite usage for the Northern pass, but of which there is no official record. In reality this would have entailed moving up 2 isolated coral blocks.
- Construction of the airport in the Southern part of the island.
- Dredging work for the construction of a marina between the landing strip and the coast. Works ceased at the end of 2005.
RBA results of ciguatera risk assessment program

RBA results confirm the link between aggressions of the lagoon and CFP
RBA results of ciguatera risk assessment programme

- stratification of the lagoon with toxic areas and safe areas
- congruence with epidemiological data
**RBA : in vitro culture of Gambierdiscus can be as toxic as natural samples**

- **Wild cells and blooms : 149 samples**

<table>
<thead>
<tr>
<th>Location</th>
<th>RBA(^{-}) (pg P-CTX-3C eq.cell(^{-1}))</th>
<th>RBA(^{-}) (105)</th>
<th>[0.36 – 6.21] (44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tahiti, Moruroa,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tahiti, Nuku-Hiva,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raivavae</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Algal collection : 78 in vitro cultures**

<table>
<thead>
<tr>
<th>Location</th>
<th>RBA(^{-}) (pg P-CTX-3C eq.cell(^{-1}))</th>
<th>RBA(^{-}) (75)</th>
<th>[3.36 – 5.8] (3)</th>
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<tbody>
<tr>
<td>French Polynesia</td>
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<tr>
<td>Réunion, St Barthélémy, Canary Island</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Tubuai, Raivavae</td>
<td></td>
<td></td>
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<tr>
<td>Rangiroa</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

- **4 endemic species :** *G. pacificus, G. australis, G. toxicus, G. polynesiensis*,

- \( \leq 0.33 = \text{RBA}^{-} = \text{atoxic} \)
- \( \text{LOQ} = 15.5 \text{ fg P-CTX-3C eq.cell}^{-1} \)
RBA : application on fishes

No correlation between RBA values and size/weight

*Scarus altipinnis*
filament-finned parrotfish

Small fish can be more toxic than bigger specimens
# RBA: application on fishes along the food chain

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Tuamotu</th>
<th>Australes</th>
<th>Marqueses</th>
<th>Vanuatu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moruroa</td>
<td>Fakarava</td>
<td>Tubuai</td>
<td>Raivavae</td>
</tr>
<tr>
<td><em>Ctenochaetus striatus</em></td>
<td>1.8 - 12.73</td>
<td>1.83 - 5.26</td>
<td>&lt;0.31 - 3.20</td>
<td>&lt;0.31 - 5.43</td>
</tr>
<tr>
<td></td>
<td>(24)</td>
<td>(4)</td>
<td>(34)</td>
<td>(34)</td>
</tr>
<tr>
<td><em>Naso unicornis</em></td>
<td>&lt;0.31 - 2.3</td>
<td>&lt;0.31 - 5.26</td>
<td>1.15 - 6.59</td>
<td>1.18 - 8.83</td>
</tr>
<tr>
<td></td>
<td>(20)</td>
<td>(1)</td>
<td>(24)</td>
<td>(3)</td>
</tr>
<tr>
<td><em>Kyphosus cinerascens</em></td>
<td>&lt;0.31 - 3.54</td>
<td>&lt;0.31 - 2.55</td>
<td>0.46 - 4.25</td>
<td>&lt;0.31 - 2.88</td>
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<tr>
<td></td>
<td>(26)</td>
<td>(20)</td>
<td>(7)</td>
<td>(29)</td>
</tr>
<tr>
<td><em>Chlorurus microrhinos</em></td>
<td>&lt;0.31 - 1.58</td>
<td>&lt;0.31 - 4.04</td>
<td>2.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(26)</td>
<td>(1)</td>
<td>(4)</td>
<td>(3)</td>
</tr>
<tr>
<td><em>Scarus altipinnis</em></td>
<td>1.91 - 3.23</td>
<td>0.36 - 4.52</td>
<td>&lt;0.31 - 5.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>(12)</td>
<td>(38)</td>
<td></td>
</tr>
<tr>
<td><em>Scarus rubroviolaceus</em></td>
<td>&lt;0.31 - 1.14</td>
<td>&lt;0.31</td>
<td>&lt;0.31 - 18.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>(1)</td>
<td>(38)</td>
<td></td>
</tr>
<tr>
<td><em>Crenimugil crenilabis</em></td>
<td>0.88 - 7.19</td>
<td>0.6</td>
<td>&lt;0.31 - 21.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(26)</td>
<td>(1)</td>
<td>(4)</td>
<td></td>
</tr>
<tr>
<td><em>Lutjanus bohar</em></td>
<td>&lt;0.31 - 8.47</td>
<td>1.26 - 5.33</td>
<td>&lt;0.33 - 3.85</td>
<td>0.39 - 0.72</td>
</tr>
<tr>
<td></td>
<td>(35)</td>
<td>(6)</td>
<td>(5)</td>
<td>(2)</td>
</tr>
<tr>
<td><em>Lethrinus olivaceus</em></td>
<td>&lt;0.31 - 5.26</td>
<td>0.26 - 2.10</td>
<td>1.06</td>
<td></td>
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<tr>
<td></td>
<td>(26)</td>
<td>(6)</td>
<td>(1)</td>
<td>(2)</td>
</tr>
<tr>
<td><em>Cephalopholis argus</em></td>
<td>&lt;0.31 - 1.34</td>
<td>&lt;0.31 - 1.34</td>
<td>&lt;0.31 - 4.22</td>
<td>&lt;0.31 - 1.34</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(10)</td>
<td>(1)</td>
<td>(3)</td>
</tr>
<tr>
<td><em>Epinephelus polyphekadion</em></td>
<td>&lt;0.31</td>
<td>&lt;0.31 - 2.38</td>
<td>1.95 - 3.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>(24)</td>
<td>(3)</td>
<td>(1)</td>
</tr>
<tr>
<td><em>Plectropomus laevis</em></td>
<td>&lt;0.33 - 4.65</td>
<td>0.19 - 3.72</td>
<td>0.36 - 3.29</td>
<td>&lt;0.31 - 0.77</td>
</tr>
<tr>
<td></td>
<td>(31)</td>
<td>(23)</td>
<td>(6)</td>
<td>(8)</td>
</tr>
</tbody>
</table>

**RBA value in ng P-CTX-3C eq. g⁻¹ of flesh**

<0.31-0.33 = RBA⁻ = atoxic

**Herbivores can be as toxic or more toxic than carnivores**
### RBA: the effective dose to human

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Area</th>
<th>Severity index</th>
<th>Delay</th>
<th>Number of anterior CFP</th>
<th>Number of people intoxicated</th>
<th>RBA ng P-CTX-3C eq.g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluefin trevally</td>
<td>Nuku-Hiva</td>
<td>3</td>
<td>ND</td>
<td>0</td>
<td>2</td>
<td>1.63</td>
</tr>
<tr>
<td>Humphead wrasse</td>
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Atoxic fish < 0.31 ng eqv P-CTX-3C/g
RBA: application on diverse biological matrix

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<td>81</td>
<td>0</td>
<td>19 Hippopus hippus</td>
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</table>

- Fish species like parrotfish, seachub, unicornfish, surgeonfish, groupers, snappers, wrasses, emperors,.....

- Toxicity (Neuroblastoma cell assay) versus Affinity to Na channel (RBA)
Conclusions

- **RBA is a suitable tool for many kind of biological matrix when matrix effect is under control.**

- **Toxin extraction protocol need to be improved when dealing with more than one toxin producer like *Gambierdiscus* + cyanobacteria.**

- Fish RBA values are not correlated with size/weight, then smaller fish can be as dangerous as bigger ones. Herbivores can be more toxic than carnivores.

- **RBA results are congruent with epidemiological data and with the knowledge of local people regarding risky and safe areas and edible fish species.**
  - RBA data of fish that have intoxicated people need to be enhanced in order to define an accurate threshold for human consumption.

- Evaluation of the overall toxicity of all biological matrix with the help of Neuroblastoma cell assay

- International standardization of RBA for seafood safety is needed.
Gracias por su atencion!