Red waters in Ría de Vigo (NW Spain)

On June 28, a call to the Oceanographic Center of Vigo (IEO) alerted us that Samil Beach waters were discolored, probably due to a red tide. The authorities sought to identify the causative agent and were evaluating to restrain bathing until the nature of the phenomenon was clarified.

Samil, the most popular urban beach in Vigo, is only a few kilometers away from IEO, so we were able to collect shore samples immediately. The patches, most likely advected by the onset of very mild northwesterly winds, were reddish-brown, forming a strip parallel to the shore and visible from the middle part of the beach to its southern limit, at the mouth of the Lagoares River (Fig. 1). Water temperature (18.7 °C) was relatively high for the upwelling-influenced Ría de Vigo in late June. Beachgoers were numerous although Samil was not as crowded as it usually is in the peak season, from mid July to the end of August.

The discolored seawater bands did not resemble the oily orange surface patches of Noctiluca, frequently observed in the rías by the end of summer [1]. Based on the previous weekly reports from INTECMAR [2] on increasing densities of Alexandrium sp, this became the main suspect, but the responsible species had not been officially reported. First of all, we visited the Samil emergency centre to inform Carlos Vales, coordinator of Vigo beach Lifeguard and Rescue Services that the patches were a natural phenomenon and did not pose any serious risk to beachgoers.

Back in the laboratory, cell counts gave density values ranging from 30 to 48 x10^6 cells L^-1 in shore samples, which looked like a monospecific culture (Fig. 2), accompanied by a few cells of other dinoflagellates species. Densities were a bit lower (10 x10^6 cells L^-1) in a sample taken 200 m offshore by a rescue boat. Light microscopy confirmed it was a dinoflagellate of the genus Alexandrium. Observations of calcofluor-stained samples under the epifluorescence microscope soon identified the species as Alexandrium minutum, a mild PSP agent usually confined to Baiona Bay, a small embayment within the Ria de Vigo. Cytogenetic analyses of the cells confirmed the characteristic low DNA cell quota of A. minutum [3]. Additionally, molecular analyses (ITS rDNA phylogeny) of single cells picked from the bloom the next day corroborated the identification. A few days later, laboratory incubations of the patch samples

![Fig. 1. PhD student María García-Portela (IEO Vigo) sampling the Alexandrium minutum red tide in Samil Beach, June 28, 2018.](image)

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Content

**Exceptional events**
- Red waters in Ria de Vigo .......... 1
- Mass mortality of sea turtles in El Salvador .................. 5
- Microcystis spp in a Chilean oligotrophic lake .............. 7
- Devastating Karenia and Cyanobacteria HABs in Florida ......... 26

**Benthic HABs**
- 1st report of Gambierdiscus in Guatemala .................. 9
- Sampling benthic HABs in the Solomon Islands ............... 11

**HAB Taxonomy**
- New nomenclature for the Alexandrium tamarense species complex ........... 13

**HAB Training and Networking**
- 13th REDIBAL highlights ............. 16
- ICES-IOC WG HAB Dynamics 2018 .......... 18
- IOC/WESTPAC WS sampling BHABs .......................... 19
- SEAFDEC-MFRD Regional training course ..................... 22

**The 18th International Conference on Harmful Algae** .... 24

**New books** ............................... 25

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**Coming soon in HAN 62**

**Highlights of the 18th International Conference on Harmful Algae**
showed the development of parasitoids identified by light microscopy as *Parviglucifera sinerae* [4].

The next day, the red tide was no longer visible by the shoreline, but was entering Ría de Vigo as indicated by aerial photos taken on June 29 afternoon. On Wednesday morning, July 4, we saw the red patches again in the Vigo yacht marina. A passer-by told us these had been visible for three days. The red discoloration seemed more intense off the pier, but it was gradually aggregated in the inner part at low tide, staining the sea surface around the sailing boats. Samples collected that day inside and outside the marina showed densities of $\sim 120 \times 10^6$ and $20 \times 10^6$ cells L$^{-1}$ respectively. In the following days, the red tide became more patchy and widespread in several areas of Vigo harbour, reaching A Lagoa (Teis) at the innermost part of the ría, but patches were also seen in Aldán and Bueu, two embayments of Ría de Pontevedra, and in the Rías Altas (Camarinas) (Fig. 3).

Red tide reports in the Rías Baixas of Vigo and Pontevedra date from as far back as 1918 in the pioneer descriptions of Sobrino [5] concerning *Gonyaulax polyedra* (= *Lingulodinium polyedrum*). Nevertheless, the 2018 red tide of *A. minutum* must be considered exceptional for two reasons.

First, its toxic nature, because nowadays summer red tides in the Galician Rías Baixas are typically formed by non-toxic organisms, such as *Mesodinium rubrum* and *Noctiluca scintillans* [1,6,7]. Short term (1-2 weeks) blooms of *Pseudo-nitzchia* (ASP) usually aggregate in cryptic thin layers (toxic carpets) at the pycnocline [8], whereas the main toxic events causing long harvesting bans are low biomass ($10^2 - 10^4$ cells L$^{-1}$) HABs of *Dinophysis* species (DSP) and occasionally of *Gymnodinium catenatum* (PSP). To our knowledge, the only red tide of *Alexandrium minutum* (in those days called *Gonyaulax tamarensis*) in Galicia was reported in the spring of 1984 in Ría de Ares-Betanzos, Galician Rías Altas [9]. These rías, north of Cape Finisterre have a hydrodynamic regime quite different from the Rías Baixas. Furthermore, *A. minutum* was not included in the list of red tide-forming dinoflagellates – *Gonyaulax diacantha*, *G. polyedra*, *G. spinifera* – frequent in the days of Margalef’s work in Ría de Vigo in the 1950’s [10].

Second, its persistence, because red tides in the Rías Baixas are ephemeral events due to the hydrodynamics: high flushing rates in these “estuaries invaded by the sea” prevent these phenomena from lasting more than a few hours. But in June-July 2018, *A. minutum* patches were observed for a month in different parts of the Ría.

What do we know about *Alexandrium minutum* and red tides in the Galician Rías Baixas?

In the last four decades, many scientific studies have focused on the characterization of HAB species present on the Galician coasts, as well as their ecology, life cycles and population dynamics. It is well established that *A. minutum* and *Gymnodinium catenatum* are the main PSP toxin producers in the Galician Rías.
Baixas, but they have contrasting “environmental windows of opportunity” and bloom strategies. *G. catenatum* is a late-summer, mid-shelf species and its population dynamics is tightly coupled to the upwelling regime. In contrast, *A. minutum* is a late spring species that thrives in “upwelling shadows”, i.e. in semi-enclosed areas protected from the highly dynamic ría-shelf water exchanges. A review of the population dynamics and life cycle of *Alexandrium minutum* concluded that their proliferations occur in sheltered coastal areas in spring-early summer associated with stability and stratification in the water column [11]. On the Galician coast, these relatively calm areas are in estuaries like Baiona Bay (Ría de Vigo) and Ría de Ares (Northern Rías), both with important freshwater inputs. *Alexandrium minutum* blooms there under heavy rain in late spring leading to haline stratification. The same species became a “winner” under the changing, anthropogenically driven, conditions on the Catalan coast, where it forms conspicuous red patches. Multiple marinas there seem to have created the perfect niche for *Alexandrium* to grow, deposit its cysts and bloom again the next year [12].

**What were the environmental conditions behind this exceptional bloom?**

In the first 11 days of June, extremely heavy rainfall was recorded by the Galician Meteorological service (Meteogalicia, Xunta de Galicia, [13]). In the second half of June, maximum air temperatures in Vigo surpassed by up to 9 °C the average maximum at this time of the year. At the same time, salinity anomalies, weak variable winds and upwelling relaxation favored a strong thermohaline stratification and the maintenance of the bloom (Fig. 4).

The temperature of the water remained high, and reached 20.7 °C on Samil Beach on July 9. It is important to mention here that in the previous autumn 2017, the countryside around Ría de Vigo experienced the most devastating forest fires, with a three times more hectares burnt than the previous record in 2011 [14], and the subsequent tons of eroded soil, rich in nutrients and chelators reaching the ría [15]. All these conditions could have fostered stability and higher nutrient levels in the surface layer, and promoted the outburst of *A. minutum*.

Low salinity values were recorded in surface samples in the Ría de Vigo, but also on the adjacent shelf (due to the river plumes from the Miño and Douro rivers) during the oceanographic cruise of the REMEDIOS project [16], carried out these days on board the R/V Ramón Margalef. Researchers from the Universidad de Vigo, IEO, CSIC and IFREMER participated in the cruise, and took advantage of the opportunity to study the proliferation of *A. minutum* in the Vigo and Pontevedra rías. The brownish patches of *Alexandrium minutum* came and went with the tidal currents and

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*Fig. 4. A: Upwelling indices before and during the long lasting red tide of *Alexandrium minutum* in Ría de Vigo (http://www.indicedeafloramiento.ieo.es). B: Vertical profile of temperature, salinity and density from a station in the outer reaches of Ría de Vigo. Alexandrium cell maxima were associated with the warmer and fresher top 3m (adapted from www.intecmar.gal/ctd)*
Concentrations inside Vigo harbour were typically much higher than outer samples, exceeding 100 x 10^6 cells per liter. The bloom did not smell bad and no respiratory or skin problems were reported. The red tide did not entail risks to the public health. But it is as well to be aware of possible skin reactions in some sensitive people. We bathed in the red tide during sampling without any effect, but in general, discolored seawater should be treated with caution (see video links #2 - 4).

Impact on fauna and shellfish exploitation

Harvesting of raft mussels in Ría de Vigo was banned for several weeks during and just after the bloom due to PSP (GTxs 1-4) toxins above the regulatory limits (800 µg equiv STX kg^-1). The event also affected infaunal shellfish (clams, razor-clams) exploitation. In contrast, only a few raft-mussel areas were closed for lipophilic toxins, the most frequent cause of harvesting bans in Galicia, in particular in the neighboring ría of Pontevedra.

Dinoflagellates of the globally distributed genus *Alexandrium* include at least 12 species confirmed as PSP-toxin producers. *Alexandrium catenella*, which causes PSP outbreaks in the antipodes (Alaska, Patagonia) is one of the most lethal representatives. Human poisoning by PSP is linked to the consumption of shellfish, but its effects in the case of massive proliferation can reach the rest of the food chain, affecting marine mammals, fish and birds. Luckily, as mentioned in the Marine Biotoxins FAO report about PSP: *From the point of view of the human being, it is very positive that herring, cod, salmon and other commercial fish species are sensitive to PSP toxins and that, unlike shellfish, they die before the concentrations of toxins in the meat reach dangerous levels. However, some toxins accumulate in the liver and other organs of the fish and endanger other fish, marine mammals and birds, which ingest the whole fish including the viscera* [17].

Based on the effects found in other areas affected by *Alexandrium* PSP events, harmful effects on the marine fauna would have been expected. Nevertheless, to our knowledge, these were apparently limited. An unusual (but not alarming) number of dead mullets (*Mugil cephalus*) were found in Vigo harbour and local fishermen told us “dead fishes are not common here, it must have been that thing in the water”. Several of these dead specimens, together with other fish and invertebrates caught in Vigo during the bloom were collected by our group in collaboration with fishermen, and brought to the laboratory for further microscopic and toxicological analyses. In addition, an otter apparently agonizing was found with signs of paralysis in its hind legs (Manuel E. Garci, pers. comm.). PSP intoxication was suspected, but the next day the animal left and the link with *A. minutum* could not be verified (Faro de Vigo, 14 July 2018). *Alexandrium minutum* strains from Galicia are “mildly toxic. During the 1984 outbreak in the Rías Altas, densities up to 10^6 cells L^-1 were observed, but maximal toxicity in shellfish was ~400 µg equiv STX 100 g^-1 [10]. Characterization of *A. minutum* isolates from Ria de Vigo showed a large variability of toxin per cell ranging from a maximum of 18 fmol cell^-1 to some strains with only traces [18]. On-going examination of field samples, *A. minutum* cultures, oceanographic and environmental conditions will help to better characterize this episode and the reasons behind this exceptional bloom.

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Mass mortality of sea turtles *Chelonia mydas* in El Salvador

During late October and early November 2017, hundreds of sea turtles, mainly green turtles (*Chelonia mydas*) were found dead floating off the coast of Jiquilisco, in western El Salvador coastal waters (Figs. 1,2). Paralytic shellfish toxins (PST) were suspected to be the cause of the mortality since similar events had occurred in El Salvador in 2005 [1], 2010 [2] and 2013 [3], and the mortality was attributed to high levels of PST (associated with blooms of *Pyrodinium bahamense* var *compressa*) found in several turtle tissues.

A total of 25 tissue samples from green turtles (*Chelonia mydas*) from Jiquilisco Bay and Los Cóbanos beach was analyzed (Table 1). The samples were delivered on November 6 and 7, 2017 at the Laboratorio de Toxinas Marinas from the University of El Salvador (LABTOX-UES) by technical staff of the Wildlife Unit, Ministry of Environment and Natural Resources. All samples belonged to dead sea turtles, except blood samples from a living turtle and a dying turtle. PST analyses by the Receptor Binding Assay (RBA), an official AOAC 2011.27 method [4,5], were carried out on November 8, 2017.

Toxic and harmful microalgae were sampled in Los Cóbanos, with the support of technicians from the Ministry of the Environment on November 7, 2017 (Fig. 2). Water samples for quantitative analyses were collected at two depths, along a 15 nm transect perpendicular to the coast; net-haul (20 µm mesh) samples were collected for qualitative analysis. Quantitative analyses were carried out under a inverted microscope (Carl Zeiss, Germany) applying the Utermöhl method. In addition, optical microscopy was performed on the intestinal contents of one specimen of *Chelonia mydas* found 15 nautical miles from Los Cóbanos beach, and on the stomach content of a dead olive ridley turtle (*Lepidochelys olivacea*) that stranded on El Sunzal Beach, department of La Libertad. Table 2 shows the average density of the most abundant microalgae detected in the samples. The diatoms *Dactyliosolen fragilissimus* (110,235 cell L⁻¹) and *Pseudo-nitzschia* spp. (4,033 cells L⁻¹) were the most abundant. All Pseudo-nitzschia species were counted together, given the difficulty in their identification and the existence of both toxic and harmless species within the genus. *Dactyliosolen*...
fragilissimus was classified as harmless according to the Taxonomic Reference List of Harmful Microalgae of UNESCO. Potentially toxic and noxious species belonging to *Alexandrium* and *Gonyaulax* were also detected, but at cell concentrations far below those associated with noxious events.

Fragments of diatoms and dinoflagellates and whole cells of species such as *Planktoniella sol*, *Scrippsiella cf trochoidea*, *Prorocentrum cf compressum* and *Prorocentrum sp.*, but no PST-producing species, were found in the gastrointestinal content of dead sea turtles (Fig. 3). Paralytic shellfish toxins were found in the liver and intestinal content in samples from dead specimens (Table 1). The highest cell concentrations were of species classified as harmless; while potentially toxic and noxious species were not found in cell concentrations high enough to categorise as an episode of a harmful microalgae bloom.

There is no information available in the scientific literature about saxitoxin levels in marine turtles, because most of them are endangered species under protection. The PST levels found in the sea turtle tissues suggest they died from a PSP intoxication, but the causative organisms bloomed elsewhere and escaped detection.

**Table 1.** Toxicity (µg STX eq./kg) in different tissues from dead sea turtles estimated using the Receptor Binding Assay AOAC 2011.27. <LD = Below the detection limit of the analysis (100 µg STX eq/kg).

<table>
<thead>
<tr>
<th>Location</th>
<th>Tissue</th>
<th>Number of samples</th>
<th>PST Concentration (µg STX eq./kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jiquilisco Bay</td>
<td>Blood</td>
<td>2</td>
<td>&lt;LD</td>
</tr>
<tr>
<td></td>
<td>Flipper</td>
<td>20</td>
<td>&lt;LD</td>
</tr>
<tr>
<td>Los Cóbanos</td>
<td>Intestinal content</td>
<td>1</td>
<td>294.51</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>2</td>
<td>252.87</td>
</tr>
</tbody>
</table>

**Table 2.** Most abundant phytoplankton species detected in Los Cóbanos on November 7, 2017. 1According to the Taxonomic Reference List of Harmful Microalgae of UNESCO. 2Some species are classified as toxic and others as harmless.

<table>
<thead>
<tr>
<th>Species</th>
<th>Density (cells L⁻¹)</th>
<th>Category¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dactyliosolen fragilissimus</em></td>
<td>110,235</td>
<td>Innocuous</td>
</tr>
<tr>
<td><em>Pseudo-nitzschia spp.</em></td>
<td>4,033</td>
<td>Potentially toxic/innocuous</td>
</tr>
<tr>
<td><em>Guinardia striata</em></td>
<td>3,361</td>
<td>Innocuous</td>
</tr>
<tr>
<td><em>Chaetoceros affinis</em></td>
<td>2,689</td>
<td>Innocuous</td>
</tr>
<tr>
<td><em>Oxytoxum sp</em></td>
<td>1,344</td>
<td>Innocuous</td>
</tr>
</tbody>
</table>

**Acknowledgements**

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Unusual winter *Microcystis* spp. bloom in a Chilean oligotrophic lake

Cyanobacteria are oxygenic prokaryotic microorganisms with a wide distribution, in freshwater and saltwater around the world. Blooms are frequently detected in eutrophic water bodies during spring and summer [1]. Recent studies have linked cyanobacterial blooms with global warming and concomitant changes in hydrological conditions [2]. In more than 50% of the cases, the cyanobacterial blooms are toxic [3], containing powerful toxins such as neurotoxins and hepatotoxins [4]. These toxic events have resulted in severe ecosystem impacts, such as massive animal mortalities through ingestion of cyanotoxins, and pose a threat to human health, because they have been associated with liver cancer [5-8].

In Chile, blooms of cyanobacteria have been reported in lakes with high concentrations of nitrogen and phosphorus [9]. In these lakes – located close to the urban centres with high population densities – cyanobacterial blooms have been detected during summer associated with an increase in lake surface temperature (LST). In 1986 a bloom (2.6×10⁵ cells L⁻¹) of the cyanobacteria *Gomphosphaeria lacustris* was detected in Chapo Lake, a eutrophic ecosystem located in Southern Chile [10]. Likewise, a toxic bloom of *Microcystis aeruginosa* (3.1×10⁶ cells L⁻¹) — a cosmopolitan cyanobacterial species — was observed in summer 2013 in an urban lake in Central Chile [9].

It has been established that high concentrations of nitrogen and phosphorus favour the growth of toxic over non-toxic strains of *Microcystis* [11]. Waters enriched with these nutrients has led to eutrophication and drastic changes in the structure and function of continental aquatic ecosystems [2]. Thus, intense blooms of cyanobacteria are characteristic of eutrophic water bodies [12]. Nevertheless, in the last decade cyanobacterial blooms have also been reported in oligotrophic (nitrogen-limited) lakes of northern Chilean Patagonia during summer.

To evaluate temporary changes of the microalgal communities in Rupanco Lake, an oligotrophic lake in Southern Chile (Fig. 1), monthly samples from March 2016 to February 2018 at five fixed depths (2, 5, 10, 15 and 30 m) were collected at a sampling station in the deepest zone of the lake. This North-Patagonian reservoir is considered a large and deep (maximum depth, 274 m) warm monomictic (summer temperature: 16–22°C) and oligotrophic (dissolved inorganic nitrogen: <0.1–3 mmol L⁻¹; soluble reactive phosphorous: <0.1–0.4 mmol L⁻¹) lake [13].

Phytoplankton analysis recorded a total of 116 microalgae species in 2017. The most abundant species were the diatoms *Synedra acus* (49.6%) and *Tabellaria fenestrata* (7.0%) and the cyanophyceae *Microcystis* spp. (4.9%) and *Nostoc* spp. (4.7%). Intense blooms of *Nostoc* spp. were detected in April and September 2017, with cell maxima of 3.1×10⁵ cells L⁻¹ and 3.8×10⁵ cells L⁻¹, respectively. This last event was associated with a marked water column stratification. At the end of June 2017, an
unusual bloom of Microcystis spp. (Fig. 2) —maximum cells density $6.7 \times 10^5$ cells L$^{-1}$— was found at 30 m. (Fig. 3). There were other accompanying Cyanophyceae (Nostoc spp., Limnococcus limneticus and Limnothrix redekei) but the bloom was overwhelmingly dominated by Microcystis spp. (98.3%). The diatom Tabellaria fenestrata was present on the top water layer (2m: 77.3%; 5m: 68.4%; 10m: 61.6% y 15m: 89.0%). This event took place when the water column was mixed (higher turbulence), promoted by the high wind velocity (16.9 km/h, daily maxima), intense daily precipitations (51.1 mm) and homogeneous vertical distribution of temperature (11 ± 0.1 °C).

Intense blooms of Microcystis spp. ($7.3 \times 10^6$ cells L$^{-1}$) comprising 89% of the population have been detected on the surface layers (2m) of this oligotrophic lake during summer [14]. Nevertheless, this is the first record of winter bloom of Microcystis spp in a Chilean oligotrophic lake. Thus, the more persistent and intense rainfall mobilized nutrients in the soil and increased the nutrient enrichment of the receiving waters [15, 16], a fact which may have promoted development of this bloom. Considering that the Rupanco Lake is nitrogen and phosphorus limited, this bloom is probably associated with global warming and changes in local hydrological conditions.

Finally, the results here show the need to consider the monitoring of potentially toxic species of cyanophyceae (e.g. Microcystis and Nostoc) when designing and implementing harmful algal bloom monitoring programmes, and not only in eutrophic continental waters.

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First report of the genus *Gambierdiscus* from the Atlantic coast of Guatemala

*Gambierdiscus* species (Gonyaulacales: Dinophyceae) are toxin-producing marine dinoflagellates causing Ciguatera Fish Poisoning (CFP), known as ciguatera, the most frequently reported seafood illness worldwide [1]. CFP is caused by consuming fish that have accumulated ciguatoxins (CTX), a group of lipid-soluble polyether neurotoxins, in their body, via the food chain [2,3]. Dinoflagellate species of *Gambierdiscus* are usually benthic microalgae associated with coral reefs and macroalgae, and several morphospecies have been reported worldwide.

Recently, Litaker et al. [4] presented the global distribution of *Gambierdiscus*, indicating the presence of five species endemic to the Atlantic (*G. belizeanus*, *G. ruetzleri*, *G. carolinianus* and ribotype 1 and 2) with six more endemic to the tropical Pacific (*G. australis*, *G. pacificus*, *G. toxicus*, *G. polynesiensis*, *G. yasumotoy*) and a unique ribotype. Only two species, *G. caribaeus* and *G. carpenteri*, are globally distributed.

To assess the presence of dinoflagellates responsible for CFP, three selected areas off the Atlantic coast of Guatemala (Fig. 1) were monitored during 2016. The first site, Estero Lagarto (15°55.545 / 88°35.576), is a shallow system fully covered by sea grass (*Thalassia testudinum*, and *Acetabularia* sp). The second and third, both coral reef areas, were Cabo Tres Puntas (15°58.128 / 88°33.241), with a high coverage of corals (70%) and a lower proportion (30%) of macroalgae (*Sargassum* sp, *Codium* sp, *Dyctiopteris* sp, and *Padina* sp), and King Fish (15°57.429 / 88°48.320) with a 40% coverage of coral and 60% of macroalgae (the same species as in Cabo Tres Puntas with the additional *Halymenia* sp present).

Dinoflagellates were collected using the macroalgae substrate method [5]. Temperature values ranged between 28.3 and 36.1°C and salinity, between 22.5 and 36.0. Samples were kept in a cooler (~5°C) and brought back to the laboratory without any preservative added. In the laboratory, samples were shaken vigorously to separate the dinoflagellates from the macroalgae. The suspension was passed through mesh sieves ranging between 250 µm and 20 µm. All the material retained on the 20 µm mesh sieve was resuspended in filtered sea water and preserved in acidic Lugol’s iodine solution. Samples observed under the light microscope revealed the presence of specimens belonging to the genus *Gambierdiscus*. Morphological features were observed from Scanning Electron Microscopy (SEM) micrographs of the samples at the Centro de Investigación en Estructuras Microscópicas from Universidad de Costa Rica.

**Fig. 1:** Benthic dinoflagellate sampling areas on the Atlantic coast of Guatemala: A: Map of Guatemala and location of sampling sites; B: Estero Lagarto (Site 1); C: Cabo Tres Puntas (Site 2); D: King Fish (Site 3).
Gambierdiscus cells were found at all of the sampling sites, even though patterns of abundance varied significantly. In general, the genus Gambierdiscus was observed in low abundance at Estero Lagarto in comparison with the other sampling sites. Maximal abundance was observed at King Fish. In addition to Gambierdiscus, other epiphytic dinoflagellate genera, such as Coolia, Ostreopsis, and Prorocentrum were recorded.

Examination by light and SEM microscopy allowed detailed observation of cell morphology and tabulation (Fig. 2). Using light microscopy, several globular golden and brownish chloroplasts could be observed in cells in antapical view. Specimens measured under SEM microscopy had an average width of 68.69 ± 0.76 µm and length of 69.75 ± 5.83 µm. The apical pore was elongated and centrally located, with a length of 6.69 µm and width of 4.46 µm (Fig. 2). Using only morphological characteristics of the Gambierdiscus cells in SEM micrographs, it was not possible to determine the precise species. This was mostly due to a limited number of samples examined using SEM, and the incorrect position of the cells when the pictures were taken, a fact which made it difficult to analyse the apical pore complex. We need to continue with further plate morphological analyses and perform molecular sequencing of the Gambierdiscus genus specimens to identify them at species level.

This work provides the first report of Gambierdiscus species in Guatemala, and improves knowledge on the distribution of this genus in Central America and the Caribbean.

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Sampling for benthic dinoflagellates in the Solomon Islands

The distribution of the ciguatera fish poisoning causing dinoflagellate, *Gambierdiscus*, has been documented throughout the Pacific Ocean [1], although very little has been published about Melanesia (western Pacific). While ciguatera is not considered a problem in the Solomon Islands, the indigenous population avoids catching large reef predators, such as parrot fish and coral trout, which could be indicative of an underlying problem. To determine if *Gambierdiscus* was present in the Solomon Islands, sampling was undertaken at four sites across two of the nine provinces between the 15th April and 5th May 2018. The four sites were Lola Island Lagoon (Zipola Habu; -8.308221, 157.163998; Western Province; Figure 1), the Hirokawa Maru (-9.385544, 159.874570; World War II ship wreck; Guadalcanal Province; Figure 2), Kinugawa Maru (-9.378672, 159.870754; World War II ship wreck; Guadalcanal Province), and the coastline and estuarine pond near Honiara (-9.430440, 159.991568; Guadalcanal Province). Samples were collected by shaking macroalgae or disturbing the benthos and trapping the disturbed microalgae in 50 mL Falcon tubes. Germanium dioxide and f/2 medium were added (0.5 mL of each per tube) to suppress the growth of diatoms and encourage dinoflagellate growth respectively.

The analysis included samples collected from a variety of macroalgae (for example, *Actinotrichia* sp. and *Padina* sp.; Figure 3), eel grasses and sediments. An array of marine life was encountered during sampling (Figure 4), although no *Gambierdiscus* was detected at any of the sites by light microscopy. No live cells of the commonly co-habiting dinoflagellate genera *Coolia* or *Ostreopsis* were detected, although dead cells of the latter were noted in the Lola Island sample. However, *Prorocentrum cf. lima*, was identified microscopically in the samples from Kinugawa Maru (*Actinotrichia* sp.) and an estuarine pond site near Honiara (*Padina* sp.) and single cells were isolated for culturing. Of the original eight individual cells isolated, four were successfully established and subjected to DNA sequencing. The maximum likelihood (ML) and neighbor-joining (NJ) phylogenetic analyses based on the LSU rDNA D1/D2 revealed that all eight Solomon Islands isolates were *Prorocentrum lima* (Figure 5). The acquired sequences were identical to those of previously reported strains, i.e., Chinese strains 2S1F7 and TIO124, a Mexican strain PL6 and a strain DNS-7 (Figure 5).

Second generation cultures of the *Prorocentrum lima* isolates will be tested for diarrhetic shellfish toxin production (okadaic acid, dinophysistoxin-1 and dinophysistoxin-2) using liquid chromatography-tandem mass spectrometry. A small (~25 µm), unidentified dark brown gymnodinoid dinoflagellate was observed on an *Actinotrichia* sp. at the Kinugawa Maru site, and on a *Padina* sp. in the Honiara pond sites. Also, a cell of *Togula* sp. was isolated from a *Padina* sp. growing in the ponds and is being cultured for further characterisation and toxin analysis.

Cyanobacteria were present at all sites and dominated the samples from the Honiara pond site. Isolates of the genera *Oscillatoria*, *Phormidium* and *Spirulina* were identified morphologically. Pennate diatoms, in particular, *Naviculoid* sp., were also present and often dominant in the samples.

Duplicate samples were treated with...
either a preservation solution for nucleic acids [2] to enable next generation sequencing using metabarcoding [3], or Lugol’s Iodine for microscopic quantification of the microalgal species present. These analyses will be performed and results published in the future.

Further sampling efforts are planned and will focus on the southern province where anecdotal reports have been received of ciguatera-like intoxication occurring. However, on this occasion no indication of the ciguatera-causing Gambierdiscus was found.

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A practical guide to new nomenclature for species within the “Alexandrium tamarensis species complex”

For several decades, the “Alexandrium tamarensis species complex” included three morphologically defined species, A. catenella, A. fundyense, and A. tamarensis [1]. Worldwide, the group is one of several responsible for paralytic shellfish poisoning, a potentially life-threatening syndrome that occurs following the consumption of shellfish contaminated with paralytic shellfish toxins (saxitoxin and analogs). Morphologically, Alexandrium catenella was distinguished by rounded, slightly anterior-posteriorly compressed cells, chain formation, and a 1’ plate lacking a ventral pore [1, 2]. Alexandrium fundyense and A. tamarensis shared the same Kofoidian plate tabulation as A. catenella, but typically exhibited less compressed cellular morphologies [1, 3]. Alexandrium tamarensis and A. fundyense were distinguished based on the presence of a ventral pore on the 1’ plate in A. tamarensis and its absence in A. fundyense [1].

Field and culture studies over the past 20 years often described “A. tamarensis species complex” cells exhibiting intermediate morphologies when compared to the original descriptions of all three species (see John et al. [4] for a summary). Phylogenetic studies of rDNA gene sequences obtained from “A. tamarensis complex” isolates fall into one of five distinct ribotype groups. These do not correlate with the original morphologically defined species and were initially designated as Groups I-V [5-8]. The genetic distances among the ribotypes are typical of those separating other dinoflagellate species. Together, these observations indicate that the original species descriptions depicted a series of “morphotypes” shared by various species in the A. tamarensis complex rather than actual species. Consequently, researchers set out to confirm that the five ribotypes represented separate species (e.g., [5, 8-11]). The most comprehensive of these efforts by John et al. [8] assembled diverse lines of evidence, including detailed morphological analyses, rDNA phylogenies, mating incompatibility assessments, ITS1/5.8S/ITS2 rDNA uncorrected genetic distances, ITS2 complementary base pair changes, saxitoxin production and the presence or absence of a key gene involved in saxitoxin synthesis. These combined data fully support the conclusion that the Group I-V ribotypes are distinct species.

Equally important, the morphological analysis by John et al. [8] showed no single morphological trait, or suite of traits, corroborated the original morphospecies descriptions, nor could any combination of morphological traits distinguish the ribotype groups from one another. There was simply too much overlap in the morphologies expressed by each of the ribotype groups for morphological characters to prove useful. For example, Group I populations in the entire north Atlantic and north Pacific including Alaska exhibit both “A. fundyense” and “A. tamarensis” morphology. In contrast, those in the eastern Pacific from San Francisco Bay south to Chile, the southeast Atlantic coast of South Africa and many areas in the western Pacific from Australia to Japan, frequently form multi-cell chains, a signature of the original “A. catenella” morphological description. These latter Group I populations also display the typical “A. catenella” morphology and are indistinguishable from the Group IV populations found in the Western Pacific (South Japan, Korea and China) and the Mediterranean Sea [5, 9, 10, 12].

Based on their analyses, John et al. [8] assigned the following species designations to each ribotype group: A. fundyense (Group I), A. mediterraneum (Group II), A. tamarensis (Group III), A. pacificum (Group IV) and A. australiensis (Group V). John et al. [8] took further steps to provide exhaustive morphological and genetic descriptions and submitted holotype and epitype material, as appropriate, to the Herbarium Senckenbergianum (FR) in the Centre of Excellence for Dinofyte Taxonomy (Wilhelmsaven, Germany). Most of these nomenclatural reassignments raised minimal concerns among taxonomists. The primary exception was the designation of the Group I ribotype as A. fundyense.

The controversy regarding the Group I designation centers on whether the cells used for the original A. catenella description were from Group I or IV, given that populations of both species in the Pacific are known to exhibit the classic “A. catenella” morphotype. Molecular analyses of “A. catenella” cells collected from the coast of California at Redondo Beach [13] and Monterey Bay [14], south of the type location, indicated that these cells belonged to Group I (Fig. 1). Further, there is no evidence that any species except Group I occurs in all of the Americas [5, 13-17]. Use of species-specific molecular assays in other parts of the world, however, have shown Group I and IV cells matching the A. catenella morphotype co-occur (see Fig. 1 and associated references). The lack of any extant type material for molecular testing and the overlapping distributions of Group I and IV cells exhibiting the “A. catenella” morphology in other regions creates uncertainty about which ribotype was described originally. Based partially on this uncertainty, John et al. [4] submitted a formal proposal to Taxon for rejection of the name Alexandrium catenella in favor of A. fundyense.

A second reason for using the A. fundyense name in lieu of A. catenella is that a large volume of literature regarding Group I blooms, especially from the Gulf of Maine, has been published using the name A. fundyense. Continued use of A. fundyense would cause less disruption to the existing documentation and serve the International Code of Nomenclature (ICN) Article 14.2 goal that states: “Conservation aims at retention of those names that best serve stability of nomenclature”. A third reason for rejecting A. catenella, and assigning Group I to A. fundyense and Group IV to A. pacificum, was to provide the scientific community a means of unambiguously distinguishing the Group I and IV cells exhibiting the same A. catenella morphotype, especially in regions where they are sympatric.

Based on the known distribution of Group I cells along the coast of North and South America, Fraga et al. [18] submitted a counterproposal to Taxon against rejecting the name A. catenella. The authors argued that the Group I
distribution in the type locale was sufficiently established to conclude only Group I cells were used for the original A. catenella description (Fig. 1). If true, the ICN rules of priority, in cases where the type material is not in question, clearly dictates retention of the name “A. catenella” for Group I because it was published prior to the “A. fundyense” Group I description [1, 2].

Nomenclature change proposals such as those of John et al. [4] and Fraga et al. [18] are adjudicated by the ICN Nomenclature Committee for Algae. This committee met to consider the proposals and ruled the name Gonyaulax catenella (Alexandrium catenella) should not be rejected and that A. fundyense and A. catenella are conspecific with nomenclatural priority being given to A. catenella [19]. For the valid names and synonyms one can refer to the following checklist: https://www.dinophyta.org/checklists/of-species/alexandrium/.

The decision of the Nomenclature Committee has the following implications:

1. All publications involving Group I cells published using the name A. fundyense should now be considered A. catenella.
2. This nomenclatural revision affects the two most widespread toxic species (Group I and IV) within the “A. tamarense complex”. Resource managers in much of the world need to be aware of this nomenclatural change as it will affect reporting requirements, communication with the public, and use of past literature when developing strategies for dealing with paralytic shellfish poisoning events and regulations.
3. This decision also places significant demands on future researchers as they investigate the literature. For example, with the exception of Chilean and South African research, most of the papers published on “A. catenella” before 2015 represent reports of the morphologically indistinguishable species A. pacificum. Similarly, future workers will need to recognize the extensive literature published between 1985 and 2017 regarding Group I A. fundyense actually refers to A. catenella.
4. The formal assignment of the name A. catenella to Group I also means this name cannot apply to Group IV (A. pacificum) despite Group IV exhibiting morphologies exactly matching the original A. catenella description.
5. Ribotype groups II, III and V species should be referred to as A. mediterraneum, A. tamarense and A. australiense, respectively [8].

Example scenarios clarifying how to identify and name “Alexandrium tamarense complex” species:

1. You work in a region where co-occurring members of the “Alexandrium tamarense species complex” are present. Based on their morphology, you have assigned them the names A. catenella and A. tamarense. What do you do?
   - You need to sequence barcoding marker regions from the strains, specifically ribosomal RNA genes or use species-specific molecular assays. Only then can you truly determine the species present, i.e. A. australiense, A. catenella, A. mediterraneum, A. pacificum, or A. tamarense.
   - A particular case is that from Japan, where species typically identified as A. catenella should be called A. pacificum, and the species recognized as A. tamarense should be designated as A. catenella.
2. You are working on an isolate from a culture collection identified as Alexandrium catenella. Cells have the typical morphology of A. catenella as described by Whedon and Kofoid [2]. When you sequence its ribosomal RNA genes, you discover that it is the A. pacificum (Group IV) genotype. What do you do?
   - The isolate should be reclassified as A. pacificum.
3. The typical toxic bloom-forming organism that occurs in your region...
is well known and has always been known as *Alexandrium fundyense* or *A. tamarense*. It has the Group I genotype. What do you do?

- You refer to the species as *A. catenella*.

4. Toxic and non-toxic strains with the morphology of *Alexandrium tamarense* coexist in your region. What do you do?

- Molecular barcoding or sequencing is mandatory.
- In the case of Scotland, the non-toxic strains correspond to *A. tamarense*, while the toxic ones are *A. catenella*.
- In the case of the Mediterranean Sea, the non-toxic species *A. tamarense* and *A. mediterraneum* may coexist. The toxic *A. pacificum* also occurs in this region but typically forms chains.

5. Chain forming cells of the “*Alexandrium tamarense* complex” are observed. What do you do?

- Start with the hypothesis that if it is a high latitude, cold-water area, the species present is *A. catenella*, but if it is a warm temperate area, it is *A. pacificum*, then confirm using molecular assays.

6. Special attention should be given to the non-toxic *A. affine*. Initially, this species was not considered part of the “*Alexandrium tamarense* species complex” because it could be morphologically identified by the position of the anterior attachment pore. However, the anterior attachment pore can be absent in old cells, making it an unreliable morphological character leaving *A. affine* morphologically indistinguishable from the “*Alexandrium tamarense* species complex”.

In conclusion, the “*Alexandrium tamarense* complex” currently includes the following five species: *A. catenella* (Group I), *A. mediterraneum* (Group II), *A. tamarense* (Group III), *A. pacificum* (Group IV) and *A. australiense* (Group V). Multiple analyses clearly show these species cannot be distinguished based on morphology. Instead, species-specific molecular assays are required for reliable identification. Vandersea et al. [17] provided an overview of the various assays that can be used for this purpose. It is further recommended that the harmful algae research community work to identify and standardize a set of molecular assays to be used when screening samples for these species. All publications regarding these species should include both the Group I–V ribotype designations and species names. This will ensure unambiguous species identifications and reduce further confusion in the literature. The routine use of multiple, standardized, species-specific molecular assays will also allow better definition of species ranges, avoid any biases regarding which species are present in a region and identify when new introductions have occurred.

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XIII Iberian meeting on harmful algae and biotoxins (REDIBAL)

The XIII Iberian Toxic Algae and Marine Biotoxins Meeting (REDIBAL 2018) was held in Vigo, Spain, from 20 to 22 June, at the Mar de Vigo Auditorium. For the first time, REDIBAL was held simultaneously with other marine science conferences (EOF, SIQUIMAR, ISC CDM, Workshop Cíes), seizing the opportunity of the VI International Symposium on Marine Sciences (ISMS) (Fig. 1). The ISMS event is held every two years as a cooperation between the six Spanish universities with Marine Science Degrees (Alicante, Barcelona, Cádiz, Las Palmas de Gran Canaria, Valencia and Vigo) and its celebration in 2018 in Vigo was indeed a great opportunity to recommence REDIBAL meetings, after a five year gap since the XII edition held in Palma de Mallorca in 2013. The local organizing committee was composed by researchers from the IEO-VGOHAB group, and the scientific committee included colleagues from Spanish and Portuguese institutions involved in harmful algae research and/or monitoring programmes (Fig. 2). Full information on the meeting, programme and abstracts can be found at https://isms.gal/redibal/

ISMS and the associated events, including REDIBAL, represented an excellent meeting point for students, researchers and companies (e.g. CIFGA, OCEOMIC) involved with marine sciences. All conferences were celebrated in the same facility and ISMS participants were allowed to attend any lecture and activity. Overall, nearly 500 participants registered for the four meetings and 45 communications (oral and poster-type) were submitted to REDIBAL. Plenary speakers were selected for each conference. The invited speaker in REDIBAL, M. Montresor, presented “The secret life of diatoms: fascinating questions from unicellular microalgae”. That was a review on the ecology and life cycle studies of Pseudo-nitzschia multistriata from the Gulf of Naples, with emphasis on the relevance of life-histories in phytoplankton ecology (Fig. 3).

Other activities during the ISMS included a panel discussion about “women and marine science” organized by the Galician Society for the Dissemination of Science (DivulgACIÓN-AGCC-CT). The REDIBAL meeting was closed with a screening of “Red Tides”, a documentary financially supported by FE-CYT (Government of Spain), which reviews the nature of red tides and HABs in Galician waters.

One of the fundamental objectives of REDIBAL meetings is the transfer of knowledge between researchers and students, with the purpose of identifying new HAB-related goals and future challenges. Furthermore, it provides an opportunity to present ongoing work and results conclusions and a suitable platform for students to share their PhD- or Master’s Degree theses. With this in mind, specialists coming from different institutions from Spain, Portugal, México and Perú presented their recent studies and debated questions in REDIBAL addressing a number of HAB-related issues. These included toxin detection, harmful algae monitoring, environmental effects on HAB organisms, innovative methodologies related to HABs and biotoxin detection, identification of new organisms, life cycles, etc.

Ciguatera Fish Poisoning (CFP)

Participants at the round table on A practical approach for the assessment and management of ciguatera risk in Europe (N. García-Álvarez, ULPGC; A. Gago...
Mediterranean Sea was reported for the first time (À. Tudó), although CFP cases have not been observed in the area yet. In addition, studies on the genus *Ostreopsis* in the Catalan and Portuguese coasts were presented by M. Vila and H. I. David.

**Biotoxin detection methods**

The challenges posed by the analysis of emerging biotoxins in the EU (D. Castro, A. Pequeño) introduced this session. The possible methods that can be used for determination of marine toxins were analyzed also in a second theme panel (P. Reis-Costa, IPMA; M. Campás, IRTA; À. Tudó, INTECMAR; J. Blanco, CIMA and P. Riobó, IIM-CSIC). Three groups of methods were considered depending on the objectives.

Methods used for monitoring toxicity in shellfish are restricted to those validated and officially accredited and should avoid the use of animals. Currently, in most countries, ASP toxins are quantified by HPLC-DAD, with no noticeable problems. In Spain and Portugal, the regulated lipophilic toxins are monitored by LC-MS/MS, based on the SOP of the EURLMB, in general with satisfactory results. Some drawbacks are derived mostly from the need of intense equipment cleaning and maintenance, which frequently halts the activity of some LC-MSs and consequently, there is a requirement to have one or several spare devices if a high throughput is expected. Matrix effects are also a problem, but it is frequently associated with the maintenance of the LC-MS's. Sample preparation methods, reliable for multi-toxin analysis are required in order to solve, or at least mitigate these problems. No issues have been detected with the validation and accreditation of these methods.

The mouse bioassay is used to quantify PSP toxicity in Spain, but in 2019 it should be replaced by the AOAC Official Method. This method uses pre-column oxidation of the toxins and HPLC analysis with fluorescence detection. The method has been in use in Portugal for some years and it is found to be useful but time consuming and labor intensive. The original method has two parts: 1) the first includes the clean-up of the extracts by solid phase extraction with a C18 column and the analysis after the oxidation with periodate, and 2) the second is performed on any toxin peak detected and requires oxidation of the C18 extract with hydrogen peroxide, fractionation of the extracts through a cationic exchange COOH-SPE column (3 fractions) and the analysis of the C18 extracts and COOH fractions and analysis. In Portugal, this process has been modified to gain effectiveness by adapting it to the known toxin profiles mostly originated by *Gymnodinium catenatum*. During blooms of that species, most toxins could be detected after C18 clean-up and peroxide oxidation, so when the concentration of the main toxins is higher that the legal limit, only a single chromatographic injection is required.

When this is not the case but some toxins are present, fractionation through cation exchange (COOH) is required. Additionally, the extracts obtained after fractionation should be subjected to an acid hydrolysis and reanalyzed in order to indirectly quantify C3 and C4 toxins, for which reference solutions currently do not exist. Obtaining final results in those cases takes a long time and requires a considerable effort, which makes it difficult and inadequate for its use in intensive monitoring systems. Some concerns about this method have been expressed. The main one, that in order to avoid the full process of quantification it is necessary to dynamically adapt the method to the toxin profile in the bivalves determined by the phytoplankton species, and this seems to be a problem for accreditation of the method (which is required for the official monitoring systems). There was a general agreement on the need for
developing an LC-MS/MS or validating an existing one with high throughput, to replace in a near future the current reference method.

Other methods not requiring complex instruments, such as immune-, aptamer- or functional assays, coupled or not to different sensing devices (biosensors), could also be employed, but their generalized use in monitoring systems seems to be difficult as multitoxin reference methods would be required and the running costs (staff and instruments) would not be substantially reduced. When chemical analyses are too complex or/and have a low sensitivity, as is the case for ciguatoxins analysis, some functional assays (neuroblastoma) are being used for monitoring. Their use by other agents, e.g. producers is promising. It would enable them to check some groups of toxins on particular batches of bivalves with a reduced cost in equipment and highly trained personnel. A critical step to foster the use of those methods would be a simplification of the extraction processes which currently are time consuming and difficult to carry out in situ.

For research purposes, different methods are being used and developed, with those based on liquid chromatography coupled to high resolution mass spectrometry probably the most widely used. The methods developed with this technology have an important drawback, which is that they are much less sensitive than those using triple quadrupole mass spectrometers. Biotoxins represented a prominent subject in Rupole mass spectrometers. Biotoxins sensitive than those using triple quad back, which is that they are much less used. The methods developed with this spectrometry probably the most widely explored were the ones developed with those based on liquid chromatography and mass spectrometry coupled to high resolution mass spectrometry.

Harmful Algae Monitoring and Research

There were presentations on historic data series and recent findings (N. Sampedro, M. Fernández, L. Mamán, A. LazartMartínez and A. del Rocio). The main topic discussed on the theme panel (chaired by M. Fernández, IRTA and L. Mamán, LCRFP) was the application of the new EU regulations on echinoderms and gastropods and how to deal with these changes in relevant harvesting areas. Progress of the EURLMB Working Group on Phytoplankton control on the generation of a “Guide to Monitoring of Toxin-producing Phytoplankton in Production Areas for Bivalve Molluscs” and the need for guidelines for risk assessment of toxins and toxic phytoplankton occurrence were highlighted.

Although harmful dinoflagellate species were the main focus in REDIBAL, other groups (i.e. diatoms) were also dealt with including a couple of studies on the detection of Pseudo-nitzschia blooms using artificial intelligence methods (F. Bellas), and mechanisms of orientation in pennate diatoms (G. Bas- terretxea). Molecular studies included DNA-based assays to detect Ostreopsis (A. Toldrá) and the evaluation of different sample treatments for metabarcoding analyses to characterize diversity in coastal sediments (Albert Reñé).

Other topics included the identification of a new non-toxic red tide forming dinoflagellate species (Gonyaulax undistortata sp. nov., D. Hernández-Beccerrl) in México, the relationship between phytoplankton thin layers formation and toxic outbreaks in the Galician Rias (E. Broullón), microscale physiological interactions in Dinophysis populations (B. Reguera), the effects of turbulence on Dinophysis (M. García-Portela), and the host range of a recently described marine parasitoid species (Parvilucifera corolla, F. Rodríguez).

Overall, REDIBAL was a fruitful and friendly meeting where colleagues from the Iberian Peninsula (Spain and Portugal) and South America (México and Perú) exchanged their knowledge and debated during three days the current status and perspectives of HAB studies, both at local and global scales. The organizers wish to express their most sincere appreciation to the scientific committee for their help before the meeting (handling abstracts and preparing the scientific agenda), and in general to all participants for their interesting contributions and fruitful discussions arose during the event.

The next edition of REDIBAL, hosted by IPMA, will be held in Lisbon, in 2021.

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ICES-IOC Working Group on Harmful Algal Bloom Dynamics 2018 meeting

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD) meeting was hosted by Dr. Margarita Fernandez-Tejedor at IRTA, Sant Carles de la Ràpita, Spain from 24-28th April 2018. The 2018 WGHABD report will be available at http://www.ices.dk/community/groups/Pages/WGHABD.aspx. WGHABD has nine ToRs in its current programme of work. The main focus of the 2018 meeting was around the WGHABD data in the IOC-ICES-PICES Harmful Algal Event (HAEDAT) database and progress towards generating an ICES Harmful Algal Event Status report. This report will form the ICES contribution to Global HAB Status Report (GHSR) which is currently being produced by the Intergovernmental Oceanographic Commission of UNESCO (IOC).

During the 2017 – 2018, progress with HAEDAT data from the ICES area was presented at a HAB data training week at the IOC office for IODE, Belgium, Sept 2017 and ICES WG Phytoplankton and Microbial Ecology, Aberdeen, UK, 2018. An abstract for an oral presentation on Regional Changes in HAB Distribution in the Atlantic Ocean using HAEDAT data was submitted to the Effects of Climate Change on the World’s Oceans in Washington DC conference, June 2018. WGHABD also participated in the Symposium for High Throughput Methods in Marine Time Series, Germany and provided input into a number of activities of the IOC GlobalHAB Scientific Steering Committee.

National reports showed that a variety of HABs continue to cause problems in the ICES area. *Pseudo-nitzschia* and Amnesic Shellfish Toxins continue cause widespread problems on the west coast of the USA and Canada and was once again recorded on the east coast. *Karenia brevis*, brown tides and *Cyclodinium* blooms also caused problems in USA. Shellfish toxins again caused problems in Europe; however in some areas (Ireland) closures were of a shorter duration. There were a number of canine fatalities in the UK resulting from dogs eating starfish and fish that contained high concentrations of PSP toxins that had been washed ashore after a storm. Ciguatoxins were once again detected in fish from the Canary Islands. *Pseudo- dochattonella* were observed in Danish, Swedish and Norwegian waters and cyanobacterial blooms were recorded in the Baltic. Tetrodotoxin (TTX) was again recorded in shellfish from Dutch waters but below the threshold level. Testing of historic shellfish samples in Ireland did not reveal the presence of TTX.

New findings included a review of ongoing projects looking at satellite imagery and generating early warning of HAB events, dynamics of HAB species along the Catalan coast, molecular methodologies, bioanalytical devices for the detection of HAB species and oomycete parasites of *Pseudo-nitzschia*. An update about the OSPAR intermediate assessment and the Marine Strategy Framework Directive (MSFD) was presented. An overview of projects underway to address the emerging risk from Ciguatera Fish Poisoning in Europe, and to investigate analytical methodologies and toxicity of *Gambierdiscus* strains from European waters were presented.

The next meeting of WGHABD will be hosted by Wenche Eikrem at the Natural History Museum in Oslo, Norway from the 2-4 April 2019. WGHABD will continue to work with HAB event data, review how physical, chemical and biological interactions control the dynamics of selected harmful micro-algae; discuss Ciguatera Fish Poisoning (CFP) as an emerging issue in the ICES area and provide an update of CFP incidences, present national reports of harmful algal events, and finally review species specific HAB detection methods and other cutting edge technologies that are now moving from research towards operational use.

WGHABD is open to experts from all IOC Member States. Experts from outside the ICES region interested in joining the WG (at own expense) please contact the WGHABD chair or the IOC.

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Fig. 1. Participants on the ICES-IOC Working Group on Harmful Algal Bloom Dynamics, Sant Carles de la Ràpita, Tarragona, 24-28th April 2018
IOC/WESTPAC training workshop on introductory scientific diving for benthic dinoflagellate sampling and processing

A week-long IOC/WESTPAC training workshop was held in Phuket, Thailand from 17-21 September 2018, with the participation of more than 30 young researchers, fishery officers and graduate students from six countries in the WESTPAC region (Fig. 1).

In view of the lack of scientific diving training and limited research capacity for benthic dinoflagellates in the WESTPAC countries, this training workshop aimed to train young scientists and the government authorities in the region with the standard protocols for introductory scientific diving, underwater sampling, sample processing, culturing and identification of marine benthic dinoflagellates.

This workshop was an intensive high-level training held in the WESTPAC region on carefully defined scientific subjects, presented by several tutors of international standing. Trainees and invited lecturers were grouped into two concurrent training programmes, respectively on: (1) introductory scientific diving, and (2) sample processing, identification and culturing techniques for benthic dinoflagellates.

The scientific diving programme was led by Dr Leo Lai CHAN (City University of Hong Kong), Lawrence Long CHAN and their scientific diving team from the Sea Dweller Underwater Academy: Ting HAN, Wing Kin FU, Ki Chun YIP and Walter Ernesto DELLISANTI. This programme was conducted through lectures and both pool and open water training. Dr Leo CHAN delivered lectures on the background and history of scientific diving. He also explained the importance of scientific diving training and its applications in different underwater scientific research. HAN and FU trained the students not only with basic scientific diving techniques such as advance kicking, buoyancy control etc., but also methodologies for collecting biologically relevant data including the use of transects, quadrats, sample bottles and bags, etc. They also trained the students on how to manage risks encountered and perform multitasking missions. YIP and DELLISANTI demonstrated how to use various scientific equipment including ‘Coral Watch’, ‘Coral Finder’, Benthic and Epiphytic Toxic Algae (BETA) sampler (Fig. 2), and Coral in Situ Metabolism and Energetics (CISME) (Fig. 3). All students obtained hands on experience with this equipment in both confined and open water environments. At the end of the training workshop, all students were aware that scientific diving systems serve a two-fold purpose: (1) a research support function that assists the diving scientist with specialized underwater equipment, advice, and diver support, and most importantly (2) a risk management function that protects the safety and health of the individual scientist, and the employing organization from excess liability exposure.

“The scientific diving training surely provided me with profound insights on what skills we should have and how to achieve scientific missions effectively,” said Lalita Putchim, a researcher from Phuket Marine Biological Center, Thailand.

Another group of trainees focused mainly on marine benthic dinoflagellates, its sampling methods, identification techniques, culture establishment and maintenance. A team of invited lecturers consisting of Drs Po Teen LIM (University of Malaya), Pengbin WANG and Douding LU (Second Institute of Oceanography, State Oceanic Administration of China). This session was conducted through a series of lectures, and hands-on exercises about the application of the single-cell isolation by micropipetting technique, culture media selection and culture preparations.

Lim delivered lectures about HABs in the WESTPAC region, recent emerging HAB related issues and current knowledge gaps in HABs, focusing on ciguatera fish poisoning and the ben-
thic dinoflagellates. He also shared the sampling methods using artificial substrates as well as his research findings in Malaysian waters. Wang demonstrated the techniques of cell isolation, culture establishment, medium preparation and culture maintenance. Lu, while lecturing on taxonomy of benthic dinoflagellates, emphasized the need to obtain not only morphological data but also molecular evidence to support species delineation.

All participants practiced observing and isolating dinoflagellate cells from the natural substrate samples collected from the nearby shore and coral reefs (Fig. 4). Several genera of harmful dinoflagellates were identified and isolated for culture establishment, including *Ostreopsis*, *Prorocentrum*, *Coo"lia* and *Amphidinium*. Participants also learned the techniques of medium preparation, setting up culture collection, maintenance and management of culture collection during the practical sessions.

The ensuing plenary discussions culminated in an agreement that participants from China, Indonesia, Malaysia, Thailand, Philippines, and Vietnam would adopt the Benthic HAB artificial substrate sampling method (Tester et al. 2014, Yong et al. 2018) and committed to carrying out comparative studies at the coral reefs and seaweed beds of the respective countries. Participants expect more workshops on ciguatera fish poisoning and benthic dinoflagellates will be organized in the near future in this region to address the emerging issues in seafood safety.

This event was co-organized by the UNESCO/IOC Sub-Commission for the Western Pacific (WESTPAC) and the Phuket Marine Biological Center (PMBC), with generous financial support of the Thai National Commission for UNESCO, and great technical assistance of the City University of Hong Kong (CityU), Sea Dweller Underwater Academy (SDUA), University of Malaya (UM), and the Second Institute of Oceanography (SIO) of the State Oceanic Administration of China.

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*Fig. 2. BETA sampler training in open water environment.*

*Fig. 3. CISME training in a confine pool.*

*Fig. 4. Microscopic observation of benthic dinoflagellate cells from samples extracted from seaweed and sediments.*
A regional training course (TC) on HAB Monitoring and Studies for the Southeast Asian participants was successfully completed from July 8-14, 2018. The TC took place at Bachok Marine Research Station (BMRS), Institute of Ocean and Earth Sciences (IOES), University of Malaya (UM), Kelantan, Malaysia, jointly organized by SEAFDEC-Marine Fisheries Research Department (MFRD), Agri-Food and Veterinary Authority of Singapore (AVA), IOES, UM and IOC/WESTPAC HAB Program. This is part of the continual efforts of SEAFDEC/MFRD on capacity building for HAB monitoring and research in the region. Twenty-one officers from 10 Southeast Asian countries attended the course: Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Thailand, Singapore and Vietnam (Fig. 1). This marked the second TC of a series of training courses by SEAFDEC-MFRD.

The training course was structured with the main goal to equip the relevant Southeast Asian fisheries personnel with the knowledge required to implement a systematic HAB monitoring program and to enhance expert capacity in HAB research and development.

In the welcome address of Dr. Po Teen Lim, Head of Bachok Marine Research Station, IOES, UM and the Chair of this TC, he highlighted the seriousness of the impact from HABs and urgency to address the HAB issues in the region. He further emphasized the importance of enhancing capacity building in the effort to minimize the impact of HABs on seafood safety and sustainability of rapid growing mariculture industries. Mr. Soon Eong Yap, Chief of SEAFDEC/MFRD in his opening comments, read by the representative from SEAFDEC/MFRD extended their appreciation to IOES for hosting the event. He stressed that the agency will continue to implement programs to enhance capacity building, in particular relating to post-harvesting technology.

The 7-days training course has been designed to expose participants to several aspects of harmful algae monitoring and investigations, these included experience of microalgae culturing, maintenance, species identification and detection. The TC was divided into two sessions: "Techniques in Culturing and Maintenance of HAB species", and "Basic Techniques in Molecular Characterization and Detection of HAB Species". The course provided extensive hands-on components and in-depth lectures. Participants had the opportunity to work in the laboratory, handling light and fluorescence microscopes, flow cytometry, and molecular instrumentation such as PCR and real-time qPCR.

An introductory lecture was given by Dr. Po Teen Lim on the overview of HABs and increasing trend of HAB events in the Southeast Asian region. He stressed the importance to have precise identification, especially in HABs monitoring, in order to advise mariculture operators on the correct preventive measures.

In this TC, Dr. Mitsunori Iwataki (Asian Natural Environmental Science Center, University of Tokyo) gave comprehensive lectures on the identification and taxonomy of unarmored dinoflagellates and raphidophytes. Prof. Dr. Haifeng Gu (Third Institute of Oceanography, China) presented two lectures on Introduction to Cysts and Azaspiracid producers in the region. The importance of cysts in the life cycles of dinoflagellates and their roles in bloom dynamics was discussed in his first lecture (Fig. 2). Lecture on harmful diatoms, including *Pseudo-nitzschia*, was given by Dr. Hong Chang Lim (Tunku Abdul Rahman University College). He explained the taxonomy requirement on a detailed assessment of the frustule structure under advanced electron microscopy in *Pseudo-nitzschia* identification. The use of the online tool “3i Interactive key and database on *Pseudo-nitzschia*” was demonstrated by Dr. Sing Tung Teng (University Malaysia...
Sarawak), who has developed several web-based interactive keys of harmful microalgal species [http://dmitriev.speciesfile.org/key.asp?key=Bacillariales&lng=En&i=1&keyN=2].

Following the lectures on specific topics, a series of extensive hands-on and lab activities were conducted. The participants learned various culturing techniques such as culture medium preparation, single-cell isolation (Fig. 3), cell counts for growth rate estimation. Species identification using light and fluorescence microscopy has been demonstrated and hands-on by the participants (Fig. 4). Later the application of flow-cytometry in assessing the changes in population dynamics during a bloom event was introduced by Dr. CP Leaw. Throughout the lab activities, participants managed to identify species from cultured specimens using both Imamura-Fukuyo (IF) and calco-flour white staining.

Molecular detection techniques including genomic DNA isolation, gene amplification by PCR were taught in this TC by Dr. Kieng Soon Hii (UM) and others. Dr. Leaw later demonstrated real-time quantitative PCR (qPCR) for rapid detection and quantification of harmful species. In the following session, a basic tutorial on phylogenetic analyses was delivered by Dr. HC Lim.

A lecture on PSP toxin and the detection methods was delivered by Dr. PT Lim. In this session, an ELISA kit for PSP toxin detection developed by Nis sui Pharmaceutical was introduced and demonstrated to participants during the TC.

The TC was ended with a discussion session, where Dr. Iwataki, the Project Leader of IOC/WESTPAC-HAB briefed the participants on the regional activities in the Western Pacific region. He also encouraged participants to strengthen the existing networking and collaboration. He pledged to continue supporting HABs activities (seminars, training courses) through expertise and reference materials in the region. The TC was finally closed by the representative from SEAFDEC, Mr. Yihang Ong, expressing his sincere thanks to IOES, IOC WESTPAC for making the TC a success.

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Fig. 2. Question and answer session with the facilitators, Prof. Dr. Haifeng Gu and Dr. Chui Pin Leaw.

Fig. 3. Participants actively involved in single-cell isolation.

Fig. 4. Participants were exposed to various microscopic techniques in species identification; a participant from Thailand using a fluorescence microscope.
Dear participants,
As chairman of ICHA 2018, and on behalf of the scientific and international advisory committees, I am pleased to present you the abstract book for ICHA 2018 (www.icha2018.com).

We are delighted to have a rich set of 621 contributions from 64 countries for which I thank all authors. This edition should be an interesting event with over 700 participants. The program contains 9 plenary and 254 oral presentations in 45 parallel sessions, as well as 45 ignite and 358 poster presentations. While the large interest is probably partly due to the two previous ICHA conferences taking place in the Southern hemisphere, we believe that it is also a testimony to a persistent research effort on harmful algae, a continuing and, in many places, growing problem for society. In line with the design of our French research network GdR PHYCOTOX and other international efforts, e.g. GlobalHAB, we have attempted to include all aspects from the causes of micro-algae and cyanobacteria to their impacts on both society and ecosystems; hence our subtitle FROM ECOSYSTEMS TO SOCIO-ECOSYSTEMS.

While the choice of topics is a continuation from the previous conferences, I would like to take the opportunity to thank all the scientific committee members who accompanied me with a lot of enthusiasm in designing the conference, especially those studying impacts. The scientific committee consisted of 45 scientists from the GdR PHYCOTOX and the GIS CYANO research networks and supported the organisation by formulating the large set of topics and by suggesting plenary speakers and international session chairs. The international session chairs, as well as members of the ISSHA Council conference-subcommittee, also helped the scientific committee in the evaluation of submissions. We went through two rounds of evaluation (one blind and one with affiliations known). Criteria for selection as oral or poster presentation were based on scientific novelty, societal interest of the contribution, gender and geographical balance and publication status. We also made every effort to balance contributions from experienced and young scientists as much as possible in the oral and ignite sessions. I would also like to very heartily thank our 30 sponsors whose participation also testifies toward the societal importance of HAB-research. In particular, the support of ISSHA, NOAA, SCOR, IAEA and GdR PHYCOTOX has helped many (ca. 60 young) scientists to attend, and 137 student contributions will be evaluated for the Maureen Keller Best Student awards. I hope you all draw inspiration from this compilation – enjoy the read!

Philipp Hess,
Chair of the ICHA 2018, Nantes, France
New books

Harmful Algal Blooms: A Compendium Desk Reference

Shumway, Burkholder & Morton (Eds)
This book provides basic information on harmful algal blooms (HAB) and references for individuals in need of technical information when faced with unexpected or unknown harmful algal events. Chapters in this volume will provide readers with information on causes of HAB, successful management and monitoring programs, control, prevention, and mitigation strategies, economic consequences of HAB, associated risks to human health, impacts of HAB on food webs and ecosystems, and detailed information on the most common HAB species.

A valuable resource to managers, newcomers to the field, those who do not have easy or affordable access to scientific literature, and individuals who simply do not know where to begin searching for the information needed, especially when faced with novel and unexpected HAB events.


Guide to the Identification of Harmful Microalgae in the Gulf of Mexico

Steidinger, Landsberg, Furnas & Burns
This guide was developed for analysts and managers world-wide involved in marine HAB monitoring, assessment and forecasting. The Guide is the result of two initial grants from the Environmental Protection Agency’s Gulf of Mexico Program and with cooperation from the University of South Florida, Florida Institute of Oceanography, Florida Fish and Wildlife Conservation Commission’s Fish and Wildlife Research Institute, Universidad Autónoma Metropolitana, Unidad Iztapalapa Ciudad de México and private funds.

It is available via a free download from: http://myfwc.com/research/redtide/research/scientific-products/.
Devasting *Karenia* and cyanobacterial blooms in Florida, USA

On the Gulf Coast of Florida, USA, there is an ongoing HAB attributed to *Karenia brevis*. Depending on the concentration of cells, the water can look red or brown, particularly from the air. Waves crashing on shore may look reddish or muddy. Brevetoxin, produced by *K. brevis*, has killed ocean wildlife, including fish and sea turtles. Brevetoxin has become incorporated into sea breezes and caused respiratory irritation in beach visitors.

Florida is also experiencing a large freshwater cyanobacteria bloom in Lake Okeechobee that is affecting the St. Lucie and Caloosahatchee Rivers. Exposure to gases, such as hydrogen sulfide and methane, emitted as the blooms senescence have been associated with adverse health effects in people. There have been reports of animal poisonings from exposure to cyanobacterial toxins.

Cyanobacterial blooms in Florida: 
http://myfwc.com/research/redtide/general/cyanobacteria/


*Photo from the Miami Herald, 2 July 2018*