

# HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

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No. 40

## • France

### *Alexandrium catenella* in Thau lagoon (France) is not a recent introduction from Asia?

In Thau Lagoon (France), cells belonging to the *Alexandrium tamarense* species complex were observed first in July 1995 by the French national monitoring network of phytoplankton and phycotoxins (REPHY) [1]. The first major bloom responsible for a paralytic shellfish-poisoning (PSP) outbreak occurred in 1998 [2]. Since then, recurrent toxic blooms have been observed over spring and autumn [3]. Studies using genetic markers revealed that the species associated with toxic events recorded during autumns 1998, 2004 and 2007 was the toxic dinoflagellate *Alexandrium catenella* belonging to the Temperate Asian clade within the *A. tamarense* species complex [4–6].

The sudden occurrence of *A.*

*catenella* in Thau lagoon, its geographical disconnection from other Mediterranean populations [7], and relationships established with Temperate Asian populations had supported the hypothesis of introduction by human-mediated transport, e.g. the transfer of resting cysts from Temperate Asia through ships ballast waters [4] or the export of living molluscs [5].

Within the framework of the first study based on microsatellites [6], we aimed at resolving the origin of *A. catenella* from Thau, using hypervariable nuclear markers able to describe its genetic diversity in Temperate Asia. Samples were collected: (a) in Thau lagoon (n=74 samples); (b) along Japanese coasts (n=411), i.e. Pacific coasts, Seto Inland

Sea and Sea of Japan; and (c) along the Zhejiang coast in the East China Sea (n=166).

Monoclonal strains were established by single cell isolation based on seawater or sediment sampled between 2003 and 2009. Seven polymorphic microsatellites were used to score the genotypes of 651 monoclonal strains. Primers and PCR conditions are given in [8] for the primer pair *ACAT14*, *ACAT16*, *ACAT49* and *ACAT50* and in [9] for *FMAC16*, *FMAC54* and *FMAC55*. Allele sizes were determined from electrophoresis patterns using the real-time system Gel-Scan 3000 (Corbett Robotics) according to a size marker (GeneScan-350, Applied Biosystems). Factorial correspondence analysis (FCA) and

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## • Mexico

### Raphidophytes in Bahía de La Paz, Gulf of California

Raphidophyceae, golden-brown algae, are named for their extrusive organelles, or trichocysts, that under light microscopy, resemble the raphides (thin crystals of calcium oxalate) of plants. Raphidophytes are mainly marine, single-celled, flagellated, and typically range in size from 30–90 µm, and are considered naked flagellates because they lack cell walls and scales. Raphidophytes are associated with fish kills in coastal waters, and some produce toxins that pose a threat to human health. The mechanism causing fish kills is still debated. Brevetoxins, hemolysins, hemagglutinins, reactive oxygen species (ROS), and free fatty acids are present

in raphidophyte species.

Five species of bloom-forming raphidophytes have been reported in the Gulf of California [1–5]. They are *Chattonella marina*, *C. ovata*, *C. subsalsa*, *Fibrocapsa japonica*, and *Heterosigma akashiwo*. A bloom of *Chattonella marina* occurred in Bahía de La Paz, but fish mortalities were not reported [2]; since then, raphidophytes have been monitored in the bay. Samples were taken from December 2008 through May 2009 every two weeks at the petroleum port north of the city of La Paz (24°13' 19" N; 110°19'4" W).

Seawater samples were collected at the surface, and surface and vertical net phytoplankton hauls (20-µm mesh) made.

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Caption on p. 4

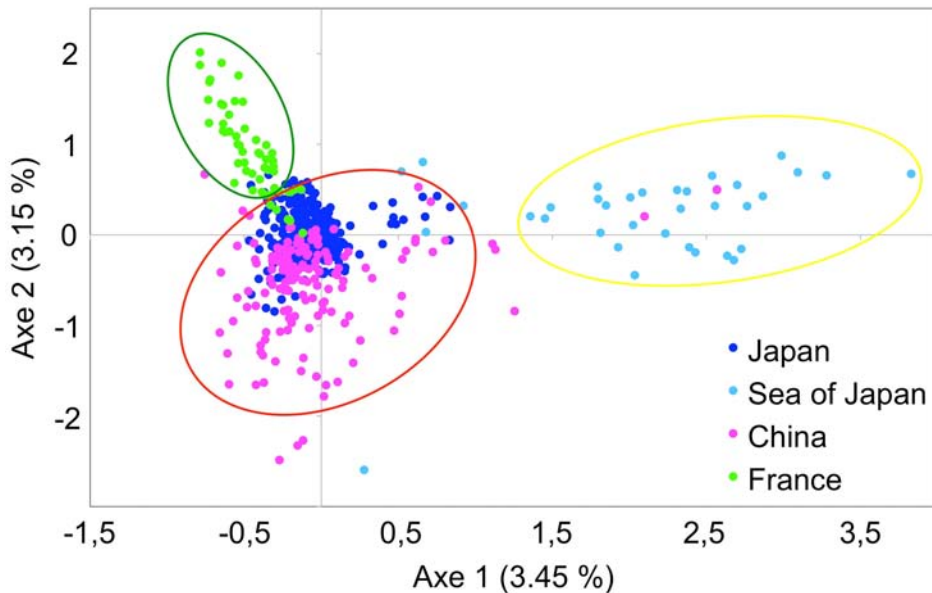


Fig. 1. Factorial correspondence analysis based on 651 *Alexandrium catenella* samples and 7 microsatellite loci. Here, samples were represented according to axes 1 and 2. Green circle: France; red circle: China and most of Japan samples (including Pacific coasts and Seto Inland Sea samples); yellow circle: Sea of Japan. (For a full color version see the online issue).

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pairwise  $F_{ST}$  estimate (significance tested through 10000 permutations) were conducted using the software GENETIX® 4.05 [10]. Assignment analyses were performed using the software STRUCTURE® 2.2 [11].

According to the FCA, the majority of the 651 samples (= individuals) could be divided into three distinct clusters (Fig. 1): i) French; ii) Chinese and most of the Japanese samples (i.e. samples from Pacific coasts and Seto Inland Sea); iii) Sea of Japan. Pairwise  $F_{ST}$  estimations evidenced highly significant differentiation among French, Chinese and Japanese populations; showing a clear genetic structuring (Table 1). Pairwise  $F_{ST}$  values varied widely and allowed us to define a range of values. The lowest values were observed among sites composing Japanese (including Pacific coasts and Seto Inland Sea samples) and among Chinese clusters, suggesting the possible existence of limited gene flows. However, as described above, the Sea of Japan population emerged as genetically differentiated from the Japanese group. This isolation may be due to some ecological barriers. Finally, highest and significant values were observed between Thau and all other Temperate Asian populations, suggesting the absence of gene flow, either through natural or artificial migrations.

Cluster analysis by STRUCTURE indicated that the four clusters best explained the uppermost hierarchical level of genetic structuring in *A. catenella* samples. When  $K=4$ , individuals were divided into populations from the Pacific coasts and Seto Inland Sea of Japan as Cluster 1, the Zhejiang coast populations as Cluster 2, the Sea of Japan population as Cluster 3 and Thau lagoon population as Cluster 4 (Fig. 2). In the Sea of Japan and Thau populations, most individuals were assigned with high probability to one of the clusters. On the other hand, admixtures of clusters were detected in other Japanese and Zhejiang coast populations, suggesting a mix of populations from different origins or the limited capacity of the software to discriminate recently diverging populations. More loci should be involved to overcome this problem.

*Alexandrium catenella* population blooming in Thau lagoon strongly diverges from the current Temperate Asian populations (according to microsatellite analyses), whereas both origins are gathered together according to the analyses of ITS1-5.8SrRNA gene-ITS2 sequences [4–6]. Despite the limited number of Asian samples

analysed in this study, the genotypic data of *A. catenella* by microsatellites were globally collected in the area between latitudes 20–45°. Possibly the microsatellite data disprove the hypothesis of a recent introduction through human-mediated transport from Temperate Asia.

However, we have still some concerns and questions about: (1) *Is our sampling effort sufficient in Temperate Asia?* It does not provide a thorough description of the genetic diversity in Temperate Asia. However, it permits us to describe a large range of diversity, and to ascertain if the Thau lagoon population diversity overlapped those of Temperate Asia populations. (2) *Is our genotyping effort sufficient?* Yes for a large structure description, but more loci should be tested for an accurate description of close Asian populations; (3) *Did *A. catenella* originate from Japan-China area?* Our results clearly exclude an Asian origin from 20–45° latitude. More northern and southern regions should be investigated; (4) *Is *A. catenella* from Thau an indigenous population, which has been distributed as part of a hidden flora?* The existence of a fossil gene flow has been suggested to explain the current distribution of the *A. tamarense* species complex in the Mediterranean Sea [12]. This hypothesis assumes a shared origin between Mediterranean and Asian populations, which could explain genetic links between Thau and Temperate Asian populations observed through analysis of the ribosomal operon.

In conclusion, the recent expansion of HABs species could be explained not only by human-mediated transport, but also by the long-term presence of populations. If we accept this hypothesis, which needs to be investigated further, these possibly

Table 1. Pairwise  $F_{ST}$  estimated (10000 permutations) for 6 comparisons between 3 populations of *Alexandrium catenella* (total of 651 samples) based on 7 microsatellite loci. Significant  $F_{ST}$  values, obtained after sequential Bonferroni correction [13].

	Japan	Sea of Japan	China	France
Japan	---			
Sea of Japan	0.14305***	---		
China	0.12181***	0.18086***	---	
France	0.17160***	0.31127***	0.24444***	---

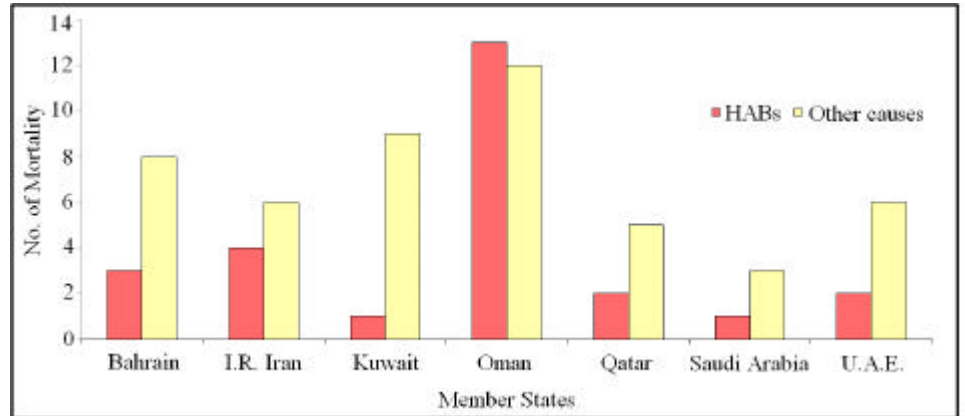
\*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ ; \*  $P < 0.05$ , significant after sequential Bonferroni corrections for 6 multiple tests.

• Kuwait

## ROPME-IOC Regional Symposium on HABs

The Gulf region has in recent years been severely affected by harmful algal events. HABs have affected fisheries, aquaculture, operation of desalination plants, and tourism, and caused significant economic losses. The first of these events took place in 1999, as a result of a bloom of *Karenia selliformis*, and caused death to at least 130 types of fish, as reported Dr. Salah Al-Mudh'hi, Chairman of the Board and Director General of the Kuwait Environment Public Authority (EPA). More recently, blooms of *Cochlodinium polykrikoides* in particular have caused problems.

Nutrients enrichment from land-based human activities is thought to be one factor which plays a major role in triggering HAB outbreaks in the region. Dr. Abdul Rehman Al-Awadi, Executive Secretary of the Regional Organisation for the Protection of the Marine Environment (ROPME) commented that a fundamental challenge is that



Number of marine mortality events during 1976-March 2009 in the ROPME Sea area.

people tend not to understand the relationship between human behaviour and its effect on the environment.

To strengthen regional cooperation in observing and mitigation these events, the Kuwait Environment Public Authority (EPA) hosted a Regional Symposium on Harmful Algal Blooms from 12 to 14 October 2009. The Symposium was organised as a joint effort of the ROPME and the IOC of UNESCO.

About 50 experts from ROPME member states attended the symposium. The presentations by representatives of research and monitoring agencies in the region provided an overview of HAB events and of the needs for capacity building in their institutions.

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ancient settlements could have recently become visible (bloom) because of the modification of their environment (anthropogenic pressures, global warmth) or increased monitoring efforts.

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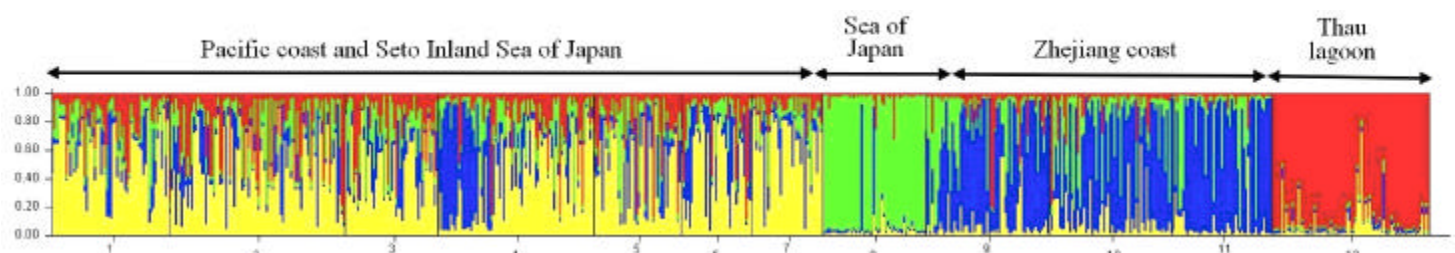


Fig. 2. Population structure based on 7 microsatellite loci of *Alexandrium catenella* estimated by genotype clustering by STRUCTURE. Assignment of 651 individuals to  $K=4$  genetically distinguished groups; 3 runs. For each run, we used a burn-in period of 50,000 generations and 200,000 iterations.

(Cont'd from p. 1)

Live cells of *Chattonella* and *Fibrocapsa* species were identified in unfixed samples. For cell counts, water samples were fixed using non-acid Lugol and analyzed by the Utermöhl technique. Fixed cells were deformed and shrunken, making it difficult to identify species without studying live cells. Manipulation of live cells provoked them to become round-shaped. We observed that light passing through the microscope also deforms the cells and chloroplasts migrate to the center of the cell.

SST from December through May ranged from 18.5 °C to 22.5 °C. Four raphidophyte taxa were observed from Dec 2008 through May 2009. *Chattonella marina* was found in net hauls collected in Dec 2008 and Feb-Mar 2009. Cells of *C. marina* vary in size and form (Plate 1; Figs. 6, 7), from 40–55 µm × 24–28 µm (n = 10). *Fibrocapsa japonica* was found in May 2009 (Plate 1; Figs. 8, 9), for the first time in Bahía de La Paz. The most distinctive characteristic of this species is the group of mucocysts in the posterior part of the cell; *F. japonica* cells ranged from 22–31 µm × 14–18 µm (n = 22).

Two cells of an unidentified raphidophyte were also found in samples collected in January 2009 (Plate 1, Fig. 5). *Chattonella ovata* was found from early winter (Jan 2009) to the end of May; abundance ranged from 3000 in February to 37000 cells L<sup>-1</sup> in March 2009 in surface samples; cells vary greatly in size and shape (Plate 1; Figs. 1–4), from 30–85 µm × 30–47 µm (n = 45). Cells of *C. cf ovata* have been observed in Bahía Kun Kaak, Sonora (28° 52' N, ~112° 04' W) and in Bahía de Mazatlán [1, 3]. In Bahía de La Paz, no dead fish or shellfish were observed during the first bloom of *C. marina* [2]. The low densities of *Chattonella* and *Fibrocapsa* species found during this study may explain the absence of mortality.

Identification of live plankton was an opportunity to observe several heterotroph dinoflagellates (such as *Noctiluca scintillans*) and ciliates [Plate 1, Fig. 10] feeding on *C. ovata*. *N. scintillans* is one of the most

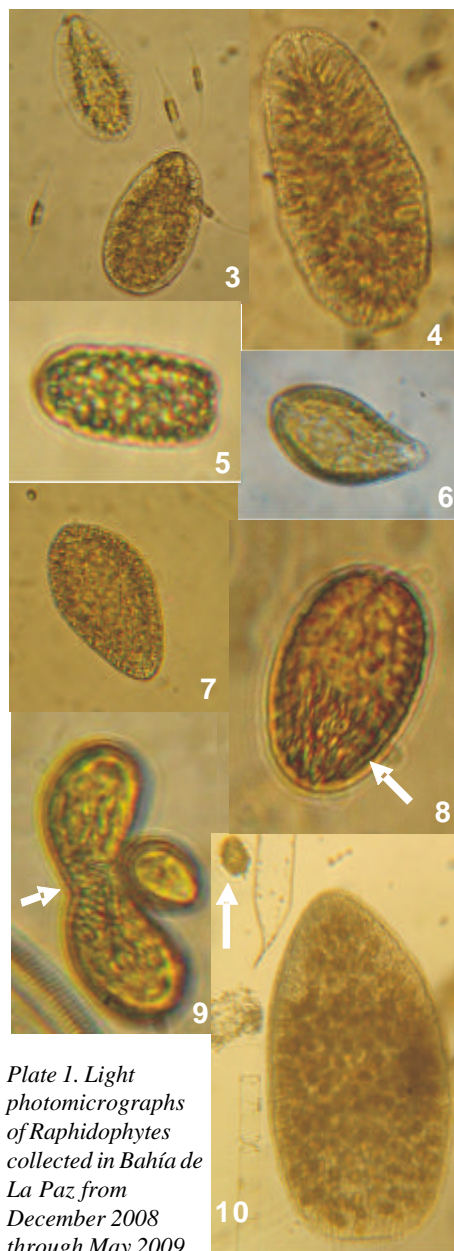


Plate 1. Light photomicrographs of Raphidophytes collected in Bahía de La Paz from December 2008 through May 2009.

Live cells *Chattonella ovata* (1–4). Unidentified raphidophyte cell (5). *C. marina* (6–7). *Fibrocapsa japonica*; arrow indicates presence of mucocysts in the posterior of the cells (8–9). Ciliate full of *Chattonella* cells; arrow indicates a *Chattonella ovata* cell (10).

common dinoflagellate predators in the Gulf of California. A bloom of *Cylindrotheca closterium* (1.24 × 10<sup>6</sup> cells L<sup>-1</sup>) occurred on 19 Mar 2009 (Plate 1, Figs. 3) together with *Chattonella ovata*. Dividing cells of *C. ovata* and *F. japonica* indicate conditions were favorable (Plate 1; Figs. 2, 9, respectively).

Raphidophytes, like other flagellates, are very delicate and deform or disintegrate in formalin; samples preserved with lugol change morphology, but can be recognized by a well-trained eye. Studies of live phytoplankton lead to identification of species otherwise omitted in traditional

phytoplankton monitoring. We suspect that raphidophytes, as well as naked dinoflagellates, have not been detected in Mexican coastal waters because traditional methods used for sampling and preserving phytoplankton destroy identifying characteristics. For more accurate monitoring of harmful algae, unpreserved samples should be used to observe delicate phytoplankton forms. Some raphidophytes appear to have differences in morphology, but are genetically the same, as demonstrated with *C. marina*, *C. antiqua*, and *C. ovata* [6]. Cloned cultures of *C. ovata* from the bloom here sampled were isolated to evaluate toxicity and confirm their identification with molecular tools.

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•Uruguay

# First report of *Pseudo-nitzschia multistriata* in Uruguay – January 2009



Fig. 1. Map showing the study area with location of samples in Punta del Este and La Paloma (Uruguay), where *P. multistriata* and *P. multiseries* were detected.

A long-term phytoplankton monitoring program has been conducted in Uruguay since 1980 by the National Management of Aquatic Resources (DINARA, Dirección Nacional de Recursos Acuáticos). This programme extends over about 250km of shoreline (Fig. 1). It includes weekly samples of phytoplankton, biotoxins and environmental factors measurements. Several toxic phytoplankton blooms have affected these estuarine and coastal waters almost every year, mainly with Paralytic Shellfish Toxin (PST) above the regulatory limits and the presence of Lipophylic Shellfish Poisoning (LSP). The species associated with PST outbreaks are *Gymnodinium catenatum* and *Alexandrium tamarense*. LSP producing species reported in the area are *Dinophysis acuminata*, *D. caudata*, *D. acuta*, and *D. rotundata*. The only Amnesic Shellfish Toxin (AST) event reported in mussels from Uruguay was in December 2001, below the regulatory level, caused by *Pseudo-nitzschia multiseries*. PST and LSP in mussels were determined by official bioassay and Domoic Acid (DA) by the standard HPLC method [1–3].

Qualitative and quantitative phytoplankton samples were analyzed under Light Microscope (LM), Leitz Diaplan and inverted microscope (Leitz Labovert FS), using the Uthermöhl method, respectively. In later studies at the University of La Plata, samples

were analyzed using Leica DM 2500 LM and scanning electron microscope (SEM) Jeol JSM-6360 LV. Cells were washed with distilled water and treated using conventional methods, material not cleaned and cleaned of organic matter was mounted on permanent glass slides using Naphrax medium, and examined with phase contrast LM, equipped with Leica DFC420 digital camera.

Examination of natural water samples (January 2009) fixed with Lugol, from Punta del Este and La Paloma (Fig. 1), showed blooms of potentially toxic diatom *P. multistriata*, and another *Pseudo-nitzschia* species [4, 5]. There was no associated toxicity in local mussels. This is the first report of *P. multistriata* in shelf waters of Uruguay.

In field samples from Punta del Este and La Paloma (Fig. 1), a *P. multistriata*

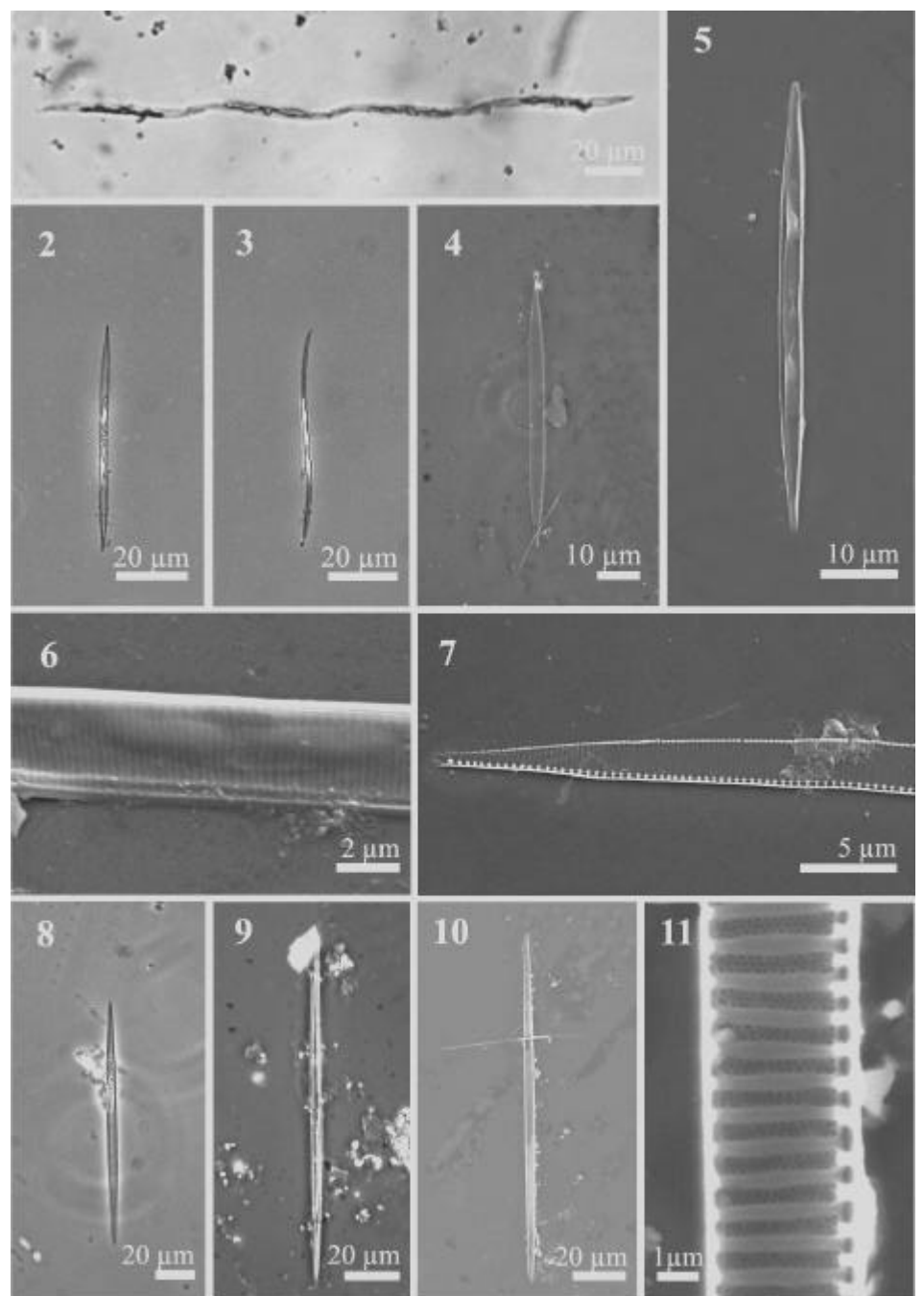


Fig. 2. Images of *P. multistriata* 1-7, (1-4 LM, 5-7 SEM) and *P. multiseries* 8-11, (8-9 LM, 10-11 SEM), from Uruguayan samples, January 2009.

• Mexico

# *Pseudo-nitzschia subcurvata* (Bacillariophyceae) in the Gulf of Mexico?

Species of the genus *Pseudo-nitzschia* are commonly characterized by linear to spindle-shaped narrow valves; only one species with curved valve outlines has been described, *Pseudo-nitzschia subcurvata* (Hasle) G. Fryxell. This species has been traditionally considered to be restricted to the cold waters of Antarctica [1] but

there are now records from the Northern Hemisphere as well as tropical and subtropical waters (Table 1). This species may represent one of the most important fractions of the phytoplankton assemblage in the Antarctic Ocean [2, 3].

In routine observations (using light microscopy) of phytoplankton samples

collected during a one-year period (2007–2008) in the National Park Sistema Arrecifal Veracruzano, a coral reef system in the southern Gulf of Mexico facing the state of Veracruz, Mexico (19°02'–19°16'N, 95°46'–96°12'W), a diatom species resembling *Pseudo-nitzschia*, with valves with  
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bloom was observed simultaneously with *P. multiseriata*. *P. multistriata* formed chains of sigmoid shape in girdle view (Fig. 2, 1). The distinctive shape of the colonies in girdle view is a character that can be easily observed under LM, however it was identified under SEM (Fig. 2, 5–7).

Cells of *P. multistriata* overlap at their ends for about one-eleventh the frustule length and the valve are slightly asymmetric, with one side straight and the other convex, tapering rapidly towards rounded ends (Fig. 2, 1–5). Valve width is 2.5–3.3 µm and length 52–63 µm. With SEM, valve surface, show 25–28 fibulae in 10 µm and 35–40 striae in 10 µm, each striae biseriate, rarely with one or three rows of circular small poroids. The poroids number 11–13 in 1 µm. There is no central interspace between the two central fibulae (Fig. 2, 5–7).

The number of poroids is the same as material from the Atlantic coast of France [6], the Gulf of Naples, Italy [4] and Peter the Great Bay, Russia [7]. Although this differs from the original description [8] of 5–6 poroids per 1 µm, Larsen & Nguyen [9] noted that this number is probably an error, since Takano's indication of 5–6 poroids is in conflict with his published photographs.

*P. multistriata* is recorded from Japan [8], New Zealand [10], Italy [4], Spain [11], Vietnam [9] and France [6]. This is the first report of *P. multistriata* from Uruguay and the second report for South America since it has only been found in Brazil before [12, 13].

*P. multiseriata* was analyzed with LM and SEM, and is characterized by

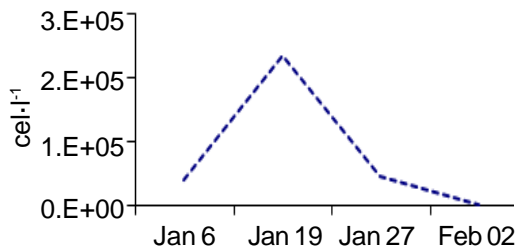


Fig. 3. *Pseudo-nitzschia multistriata* in Punta del Este, Uruguay, January 2009.

symmetrical frustules, linear to lanceolate in girdle as well as in valve view, with rounded ends. The length of the valve is 90–140 µm and width 3.5–4 µm. (Fig. 2, 8–9). With SEM the number of interstriae is 12–15 in 10 µm and fibulae 13–15 in 10 µm. Each striae has three rows of poroids. The number of poroids per 1 µm is 4–5.5. The valve shows no central interspace (Fig. 2, 10–11).

Field samples showed that the bloom started in early January, reached the maximum concentration of 236 × 10<sup>3</sup> cells L<sup>-1</sup> in January 19 at Punta del Este (Fig. 3); lower concentrations of 200 cells L<sup>-1</sup> were observed at La Paloma in early February.

Environmental conditions during the bloom were characterized by salinity between 32.5 and 34.4 and water temperature 19–23°C. The reported salinity is considered high for this region. Summer environmental data from this monitoring programme were studied and the average salinity and temperature (January–February 1991–2009) was 26.7 and 22.1°C respectively.

The presence of *P. multistriata* in Uruguayan waters may be related to high salinity values reported during the bloom after several months of drought.

We think this environmental condition could be a limiting factor in accordance with studies by Villac [12] who found salinity to be a growth factor for this species under experimental conditions.

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Table 1. Morphometric data of *Pseudo-nitzschia* spp. with curved cells.

Species	Valve shape	Apical axis ( $\mu\text{m}$ )	Transapical axis ( $\mu\text{m}$ )	Stria in $10\ \mu\text{m}$	Fibulae in $10\ \mu\text{m}$	Distribution	Comments	Reference
<i>Pseudo-nitzschia granii</i> var. <i>curvata</i> Hasle	Rhomboid valves, curved cells probably in girdle view	25-79	1.5-2.5	44-49	12-18	Northern cold waters to temperate? Subarctic Pacific Ocean	Solitary, associated with <i>Phaeocystis</i>	Hasle 1964 [4] Hasle & Syvertsen 1997 [1] Taylor and Water 1982 [8]
<i>Pseudo-nitzschia subcurvata</i> (Hasle) G. Fryxell in Fryxell <i>et al.</i>	One side straight or slightly concave, the other convex	47-113	1.5-2.5	44-49	12-18	Southern cold water regions  Bering Sea  Central and southern Great Barrier Reef  Arctic sea ice	Solitary, abundant in Antarctic zone	Hasle 1964, [4] Hasle & Syvertsen 1997 [1]  Flint <i>et al</i> 2001 [3]  Crosbie & Furnas 2001 [9]  Ikävalko 2003 [10]
	One margin straight, the other convex	47-113	1.5-2.5	44-49	12-18	Antarctic	Solitary or in chains	Scott & Thomas 2005 [11] Almandoz <i>et al</i> 2008 [5]
	One side sharp or slightly concave, the other convex	48-86	1.3-1.8	43-55	12-22	Weddell Sea	Mostly solitary, rarely cells in pairs	
<i>Pseudo-nitzschia</i> cf. <i>subcurvata</i>	Both sides of valves curved (one concave, the other convex)	28-36	1.9-2.5	?	?	Veracruz Reef System, Gulf of Mexico	Cells in pairs, rarely forming chains 3-6 cells	This report

markedly curved outlines was detected (Fig. 1). This species was rare, and few cells were observed in net hauls (mesh 30  $\mu\text{m}$ ). The cells were encountered commonly in pairs and rarely in chains of 3 to 6 cells. Cells were joined by their valve surfaces overlapping 1/7 to 1/8 of the cell length. The valves were narrow, attenuated towards the tapering apices, and both sides of the valves were parallel curved. No valve structures were resolved under the light microscope. General morphology, i.e. valve outline, was consistent with that described for *P. subcurvata*, but the length of the apical axis was less than the range recorded for *P. subcurvata*. According to Hasle 1964 [4], the fibula can be observed with a light microscope; we were unable to resolve it in our specimens using an oil-immersion 100x/1.25 Ph objective. Almandoz *et al.* 2008 [5] in their study of *Pseudo-nitzschia* species in the Weddell Sea gave a

description of *P. subcurvata* and showed a picture of two cells. This picture resembles the paired specimens we observed in our samples from the Gulf of Mexico. We are aware of the morphological similarities between various species of the genus [6], and complementary techniques (electron microscopy, cultures and molecular studies) must be used for accurate species identification. However, the conspicuous curved outline of the valve, the records in the Northern Hemisphere, and the record of *P. subcurvata* in tropical areas make us believe that we may be observing a *P. subcurvata* morphological variation or a closely related species. *Pseudo-nitzschia* species have shown to be potential producers of domoic acid, and even when they may not be detected in routine phytoplankton counts, they can be "hidden" in the environment [7]. Our future efforts must be directed to the

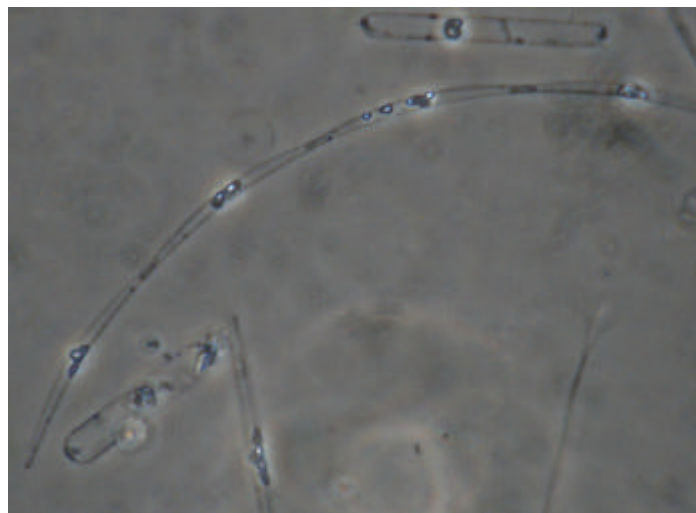


Fig. 1. Light micrograph of *Pseudo-nitzschia* cf. *subcurvata* found in the southern Gulf of Mexico.

detection of this morphotype during the monitoring programs of harmful algal blooms that we (Unidad de Investigación de Ecología de Pesquerías in collaboration with the Acuario de Veracruz) are implementing in the Veracruz coastal waters to initiate cultures of local species and to

collaborate with the investigators working with the molecular biology of the genus.

### Acknowledgements

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• Mexico

# *Cochlodinium polykrikoides* and *Gymnodinium catenatum* in Bahía de Acapulco, Mexico (2005–2008)

Microalgae blooms are frequent and periodic throughout the year along the Pacific coast of Mexico. A recent bibliographic study reveals the occurrence of more than 175 blooms along the coast [1]. Species with the greatest increase are the ciliate *Myrionecta rubra* and the dinoflagellates *Noctiluca scintillans* and *Gymnodinium catenatum* [1]. One of the most studied areas in the Pacific region is the Gulf of California [1, 2]. HABs are common events along the coasts of Guerrero, particularly in Bahía de Acapulco. The predominant species in this bay are: *Akashiwo sanguinea*, *Ceratium balechii*, *Hemiaulus hauckii*, *Noctiluca scintillans*, *Prorocentrum gracile*, *Pyrodinium bahamense* var. *compressum*, and *Takayama* sp. [1, 3–4]. In the last four years, naked, chain-forming, ichthyotoxic dinoflagellate *Cochlodinium polykrikoides* and the paralytic shellfish toxin producer *Gymnodinium catenatum* are two of the most recurring species. *C. polykrikoides* is characterized by a conical epitheca and a bi-lobed hypotheca with single cells being ellipsoidal with a displaced cingulum. Although the first blooms of *C. polykrikoides* in Bahía de La Paz were found in 2000 and 2001 [5], blooms of this species were reported in Bahía de Mazatlán since 1979 as *Cochlodinium* sp. [6] and later as *C. catenatum* [7] in Bahía Banderas. *C. polykrikoides* was originally described

by Margalef and commonly occurs in warm-temperate and tropical waters, and is broadly distributed along the Pacific coast of Mexico [8] and also *G. catenatum* are prominent in blooms [1, 9–10]. Sometimes, these two species seem to occur simultaneously in our coasts [5, this study].

This report describes the blooms of *C. polykrikoides* and *G. catenatum* that were documented at sampling stations inside Bahía de Acapulco from 2005 through 2008 (Fig. 1). Samples were taken with a plastic bucket and preserved with Lugol's solution. Temperature was recorded with a bucket thermometer. In some cases, a sub-sample was taken for live phytoplankton analysis. Live samples of the red tides were used for identification of *C. polykrikoides* (Figs. 2 and 3) and *G. catenatum* (Figs. 4 and 5). Abundance was estimated using a Sedgwick chamber under an inverted microscope [2].

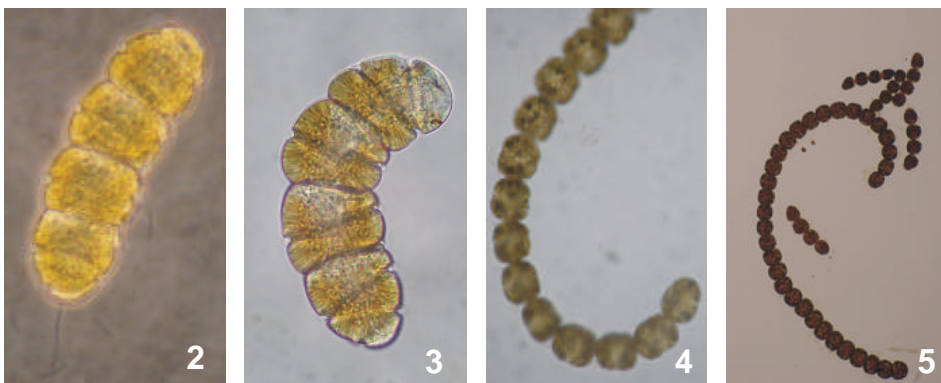
The first record of *G. catenatum* in Bahía de Acapulco had a low density ( $7\text{--}78 \times 10^3$  cells  $L^{-1}$ ) in March 1999 [11, 12]. The first bloom of *C. polykrikoides* was observed with a bloom of *G. catenatum* in December 2005, through February 2006. The highest density of *C. polykrikoides* ranged from  $1264 \times 10^3$  cells  $L^{-1}$  at Punta Bruja to  $8228 \times 10^3$  cells  $L^{-1}$  at Playa



Fig 1. Sampling stations in the shore of Bahía de Acapulco, Acapulco, Guerrero, Mexico.

Icacos. Highest density of *G. catenatum* ranged from  $141 \times 10^3$  cells  $L^{-1}$  at Punta Bruja to  $1604 \times 10^3$  cells  $L^{-1}$  at Playa Icacos. Estimated density of *C. polykrikoides* during January and February 2006 ranged between  $980\text{--}1600 \times 10^3$  cells  $L^{-1}$ . Cells counts of *G. catenatum* ranged between  $4200\text{--}10,000 \times 10^3$  cells  $L^{-1}$  (Table 1). Blooms of *C. polykrikoides* but not *G. catenatum* occurred in June 2007. Density of *C. polykrikoides* varied from  $44.8 \times 10^3$  cell  $L^{-1}$  in the entrance of the bay to  $2088 \times 10^3$  cells  $L^{-1}$  at the Muelle Base Naval. In December 2007, the two species flourished together, with *C. polykrikoides* reaching  $532 \times 10^3$  cells  $L^{-1}$  and *G. catenatum* reaching  $1942 \times 10^3$  cells  $L^{-1}$ . Temperatures were within a narrow range of 26 to 27 °C during this bloom. During 2008, several blooms of *C. polykrikoides* occurred. Lowest counts were ( $39\text{--}58 \times 10^3$  cells  $L^{-1}$ ) in early January and the highest counts were ( $1100\text{--}1250 \times 10^3$  cells  $L^{-1}$ ) in late January. Temperatures were 23–27 °C. Another bloom occurred in April, with *C. polykrikoides* at  $1160\text{--}6000 \times 10^3$  cells  $L^{-1}$  at several sampling stations. Temperatures were 25–29 °C. In October of the same year, a bloom of *C. polykrikoides* occurred at Playa de Icacos with highest density of  $2680 \times 10^3$  cells  $L^{-1}$  (Table 1).

Although a monitoring program identifies and quantifies toxic and potentially toxic species, such as *C.*



Figs. 2–5. Light photomicrographs of live four cells chains *Cochlodinium polykrikoides* (1, 2) and *Gymnodinium catenatum* (3). Lugol's fixed cells of *Gymnodinium catenatum* (4) from Bahía de Acapulco, Acapulco Guerrero.



Table 1. Abundance ( $\times 10^3$  cells  $L^{-1}$ ) of *Cochlodinium polykrikoides* and *Gymnodinium catenatum* at five sampling stations in Bahía de Acapulco, Acapulco, Guerrero, Mexico

Date	Species	Punta Bruja	Casa Díaz Ordaz	La Bocana	Muelle Base Naval	Playa de Icacos
06/12/2005	<i>G. catenatum</i>	0	0	0	1104	141
	<i>C. polykrikoides</i>	2	6	0	6432	1692
15/12/2005	<i>G. catenatum</i>	6	4	0	16	1604
	<i>C. polykrikoides</i>	86	8	0	241	8288
22/12/2005	<i>G. catenatum</i>	48	0	0	8	32
	<i>C. polykrikoides</i>	1264	94	0	4	54
		0	0	0	0	0
10/01/2006	<i>C. polykrikoides</i>	0	0	0	1100	1600
	<i>G. catenatum</i>	0	0	0	10000	7600
14/02/2006	<i>C. polykrikoides</i>	0	0	0	980	1400
	<i>G. catenatum</i>	0	0	0	5000	4200
28/06/2006	<i>C. polykrikoides</i>	0	0	0	232	246
	<i>G. catenatum</i>	0	0	0	130	136
		0	0	0	0	0
12/06/2007	<i>G. catenatum</i>	0	0	0	0	0
	<i>C. polykrikoides</i>	1792	44.8	1292	2088	110
06/12/2007	<i>C. polykrikoides</i>	17	62	192	30	130
	<i>G. catenatum</i>	27	257	1942	120	3
18/12/2007	<i>C. polykrikoides</i>	0	0	0	532	0
	<i>G. catenatum</i>	458	487	0	8	1083
		0	0	0	0	0
09/01/2008	<i>C. polykrikoides</i>	0	0	0	39	58
31/01/2008	<i>C. polykrikoides</i>	0	0	0	1100	1250
02/04/2008	<i>C. polykrikoides</i>	0	1370	0	2070	6000
08/04/2008	<i>C. polykrikoides</i>	0	0	0	1160	3150
29/10/2008	<i>C. polykrikoides</i>	0	0	0	36	2680

*polykrikoides* and *G. catenatum*, new programs, including hydrological and phytoplankton monitoring must be improved. These programs are necessary to prevent future mass kills of marine animals, particularly caged species of economic value. No fish mortalities were observed in Bahía de Acapulco during the blooms of *C. polykrikoides*. Toxicological studies of blooms revealed high toxicity levels. In March 1999, toxicity was 120–209  $\mu\text{g STXeq } 100 \text{ g}^{-1}$  [11, 12] and similar levels occurred in December 2005 (25–217  $\mu\text{g STXeq } 100 \text{ g}^{-1}$ ) [1]. During the blooms of *G. catenatum* here reported, catches of marine mollusks were prohibited to avoid health public problems. Monitoring of other PSP toxin-producing species, such as *Alexandrium tamarense*, which was recorded in the bay [3] and *Pyrodinium bahamense* var. *compressum* must be addressed because this last species have generated public health problems in the past [4]. Adequate water quality is

necessary to achieve sustainability of coastal ecosystems. Insights into the mechanisms that determine lethal species composition are needed to predict the effect of water quality. On the other hand, examination of live phytoplankton has identified other bloom-forming dinoflagellates, such as *Gyrodinium*, *Karenia*, and *Takayama* [1].

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#### SCOR Visiting Scholars for 2010

In 2009, SCOR initiated a program of SCOR Visiting Scholars and named one scholar, Paulo Relvas (Portugal), to teach a physical oceanography course in Guatemala. SCOR's program covers all areas of ocean science, but not observations or modeling, which are included in a POGO program (see: [www.oceanpartners.org/index.php?option=com\\_content](http://www.oceanpartners.org/index.php?option=com_content)). SCOR is looking for both individuals interested in serving as Visiting Scholars in 2010 and institutions in developing countries who are interested in hosting a SCOR Visiting Scholar. If you or your institution are interested in participating in the program, please visit: [www.scor-int.org/capacity](http://www.scor-int.org/capacity). Requests are accepted by 31 January 2010.

• India

## *Microcystis aeruginosa* bloom in a high altitude lake in the Western Ghats of India



Fig. 1. Map showing the location of the Lake in the Western Ghats.

Toxin producing Cyanobacteria in lakes and reservoirs form a threat to humans, bird and fish as well as various other forms of aquatic life. Increased detection of cyanotoxins in water bodies generates a complex challenge for water resource managers all over the world; among these toxins, microcystins are potent liver toxins and tumor promoters produced by several common freshwater Cyanobacteria including *Microcystis* [1].

An algal bloom was first observed in 2005 in Mullaperiyar Lake in the Western Ghats of India (Fig. 1), coincident with a mass death of tadpoles. Itching of the skin among fishermen and forest officials was also reported. A study to determine the presence of toxic algae in the bloom was carried out. The Mullaperiyar Dam was

constructed across the Periyar River in 1895, and has a surface area of 26 km<sup>2</sup>.

Water samples were collected from the Lake between 15<sup>th</sup> and 20<sup>th</sup> of every month of 2005 in sterilized polythene bottles, from different locations in the Lake. The samples were analyzed in the laboratory for the presence of toxic algae; the blooms were dominated by *Microcystis aeruginosa* (Fig. 3). During the pre-monsoon (February to March) and northeast monsoon (October to January) of 2005, *M. aeruginosa* flourished at different locations in the Lake. *Microcystis aeruginosa* is an S-strategist, i.e. stress tolerant, with low growth rate, high nutrient storage capacity, and enhanced resistance to sinking and grazing losses, and apparently characteristic of waters with phosphate fluctuations, is a specialist in phosphate storage and efficient in regulating its density [2]. *Microcystis* is widely distributed, dominates the phytoplankton community in nutrient rich lakes and is the main microcystin producer of lakes [3–4]. The gut analysis of the tadpoles revealed the presence of *Microcystis* cells. Thus the cause of the tadpole deaths could have been “microcystin”. Hence presence of this alga in the lake poses a serious threat also to wildlife in this sanctuary (especially to the endangered tiger population of the area); the degree to

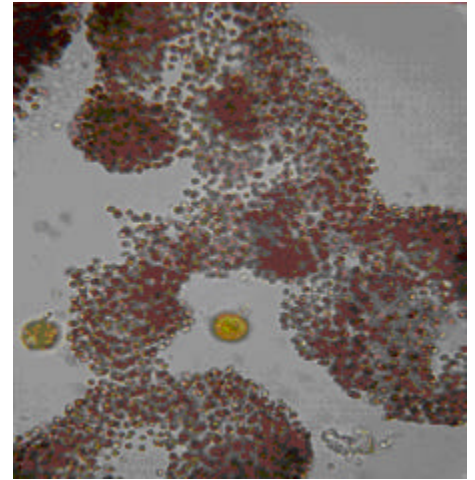


Fig. 3. Microcystis cells x 45.

which they may be affected by this alga should therefore be monitored. ‘Cyanotoxins’ in general are hepatotoxins, known tumor promoters, and have been associated with high rates of primary liver cancer in people drinking water with high concentrations of microcystins. The presence of this toxic bloom was reported during 2008 by one of the authors in Chalakudy River originating from the southwestern Ghats. This should be seriously considered since the water is the sole source for drinking in five districts of the neighboring state of Tamil Nadu.

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Fig. 2. Mullaperiyar Lake.

# 14<sup>th</sup> International Conference on Harmful Algae

## First Announcement - June 2009

The International Society for the Study of Harmful Algae (ISSHA) is pleased to announce that the 14<sup>th</sup> International Conference on Harmful Algae will take place in Hersonissos, Crete, on 1–5 November 2010, with the support of the Hellenic Centre for Marine Research (HCMR).

## Aims & Topics

The Conference will address the following topics:

- Population dynamics of HABs
- Time series of HAB events: Climatic and anthropogenic induced impacts
- Impact of HABs on marine food webs and ecosystem structure and function
- Biological interactions: allelopathy, mixotrophy, parasitism, symbiosis, bacteria and viruses
- New regional HAB events
- Inland Seas and HABs
- Introduction of alien species and HABs
- Cyanobacterial ecology, physiology and bioactive compounds
- Genomics and genetic diversity of HABs
- Toxins: chemical structure and synthesis, detection and analytical methods
- Novel sensor technologies for bio-sensing applications in HAB research and monitoring
- Harmful Algae culture collections
- Management, mitigation and public outreach for HABs

The official language of the Conference is English.

The Conference proceedings will be published under the auspices of the International Society for the Study of

Harmful Algae (ISSHA) and the Intergovernmental Oceanographic Commission (IOC).

## Abstract submission

You may submit your abstract(s) online, by 1 June 2010. Further details on abstracts and topics to be covered by the Conference will be available soon. If you have any questions please contact: [info@hab2010.gr](mailto:info@hab2010.gr)

of Heraklion. It offers many accommodation options, two sandy beaches, traditional and international cuisine restaurants, souvenir shops and a famous nightlife.

## Transportation

Direct flights from several European cities to the N. Kazantzakis international airport in Heraklion, Crete or connecting international flights via the Athens El.



## Important dates

Deadlines, for Early Registration 1 June 2010, for Late Registration 15 Sep, for Full Paper Submission 30 Nov.

## About Crete

Crete is the largest Greek island, one of the thirteen regions of Greece and the fifth largest island in the Mediterranean. Heraklion, the capital, is a dynamic city, the fifth largest in Greece. Crete is renowned for its natural beauty and diverse landscape. Spectacular mountains are sliced by impressive gorges that spill out to the sea, while infinite plateaus and fertile plains form its interior, while stunning endless beaches and isolated coves comprise its 1046 km coastline. Crete's traditional villages, cultural heritage and significant ancient history; as the centre of Europe's most ancient civilization; the Minoan; are some of the reasons why Crete is one of the most popular tourist destinations in Europe. Hersonissos, a traditional and picturesque town in northern Crete, is situated 26 km east

Venizelos international airport operate daily. Alternatively, ferries operate both ways between the Heraklion and Piraeus.

## Participation in social events

During the Conference, participants may choose from a selection of tours in the area. These include tours of the Knossos Palace —the largest Bronze Age archaeological site on Crete and what was probably the ceremonial and political centre of the Minoan civilization —and the Cretaquarium— a site of marine science and aquaculture, a meeting point where science, discovery and recreation are equally provided.

More info will be posted on the 14<sup>th</sup> ICHA website at: [www.hab2010.gr](http://www.hab2010.gr)

## ICHA2010 Secretariat

Hellenic Centre for Marine Research (HCMR), Institute of Oceanography, 47 km Athinon-Souniou, 19013 Anavyssos, Attiki, Greece. Tel: +30 229 10 76466, Fax: +30 229 10 76323. Email: [info@hab2010.gr](mailto:info@hab2010.gr)

## Tentative registration fees

All payments must be in EUR.

Please visit the ISSHA website ([www.issha.org](http://www.issha.org)) and/or [www.hab2010.gr](http://www.hab2010.gr) to keep up to date with conference information.

Early, until 1 June 2010		Late, from 1 June to 15 Sep 2010		After 15 Sep 2010 and On-site	
Participant	Fee	Participant	Cost	Participant	Cost
ISSHA Member	330 €	ISSHA Member	400 €	ISSHA Member	450 €
Non ISSHA Member	430 €	Non ISSHA Member	500 €	Non ISSHA Member	550 €
Full Delegate		Full Delegate		Full Delegate	
Students	165 €	Students	200 €	Students	225 €
Accompanying	165 €	Accompanying	200 €	Accompanying	225 €
Day Card	100 €	Day Card	125 €	Day Card	150 €

• Mexico

## *Gambierdiscus toxicus* in the southeastern Gulf of Mexico

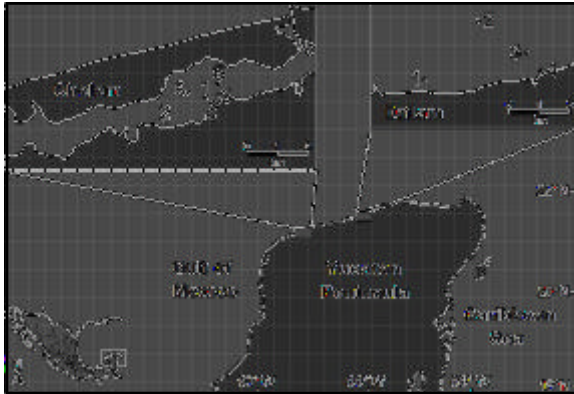


Fig. 1. Sampling sites at the northern coast of Yucatan Peninsula, Mexico, in 2008-2009.

*Gambierdiscus toxicus* Adachi et Fukuyo is known in Gulf of Mexico waters: e.g., the Florida Keys [1; M. Parsons, unpublished data]; Mexican coastal waters [2], and the northwestern Gulf [3]. This species most likely causes ciguatera, and affects fisheries and human health in the gulf. The first written report of ciguatera fish poisoning in Florida was in 1918 [4]. It is estimated that 1300 cases of ciguatera occur annually in just four counties in Florida (Dade, Broward, Palm Beach, and Monroe) [5].

Ciguatera is also common in Mexico, where 291 incidents were reported in the 1980-1990s from Baja California Sur in the Pacific, and from the Mexican Gulf states, Quintana Roo and Yucatan [6-7]. It is still unknown if *G. toxicus* is endemic or a recent invader to the Gulf of Mexico. *G. toxicus* was rare in the southern region of the Gulf of Mexico, where we recorded it in Veracruz reef zone waters in biweekly samples in 2005 [2].

Epiphytic microalgae were monitored at 10 stations in two localities near the northern Yucatan Peninsula, Mexico, between May 2008 and May 2009, (2008-May 19, 21; August 20, 26; November 25, 28; 2009-March 3, 6; May 19, 21). About 250 samples of macrophytes with associated microalgae were taken at Dzilam de Bravo (6 sites) and in the lagoon El Ojo de Agua near Chelem (4 sites) to obtain data on annual dynamics of epiphytic dinoflagellates (Fig. 1). Samples were

collected manually during free diving to a 4-m depth. Water temperature (20.5-32.7°C), salinity (26.5-38.6 psu), dissolved oxygen (2.27-12.6 mg/L), turbidity (36.73-63.8 ntu), nitrates (0.15-26.56  $\mu$ M), nitrites (0.01-0.73  $\mu$ M), ammonium (0.02-6.96  $\mu$ M), phosphates (0.13-1.76  $\mu$ M) and silicates (1.65-139.96  $\mu$ M) were measured. Dinoflagellates were identified and counted using Olympus BX51 and CKX41 microscopes and a JEOL JSM-5310LV scanning electron microscope (Fig. 2-F).

Macrophyte beds were dominated

by the seagrasses *Thalassia testudinum*, *Syringodium filiforme*, *Halodule wrightii* and *Rupia maritima* and by algae of the genera *Acetabularia*, *Batophora*, *Caulerpa*, *Chaetomorpha*, *Chondria*, *Euclima*, *Gracilaria*, *Halimeda*, *Laurencia*, *Penicillus* and *Udotea*.

While pennate diatoms usually prevailed as epiphytes, dinoflagellates were common and present in almost all samples. *Prorocentrum* spp. (at least 6 species of which are potentially toxic), *Coolia monotis*, *Gambierdiscus toxicus*, *Amphidinium* cf. *carterae*, *Bysmatrum caponii*, *Pileidinium* sp., *Sinophysis microcephala*, *S. ebriolum*, *S. stenosoma*, *Ostreopsis* cf.

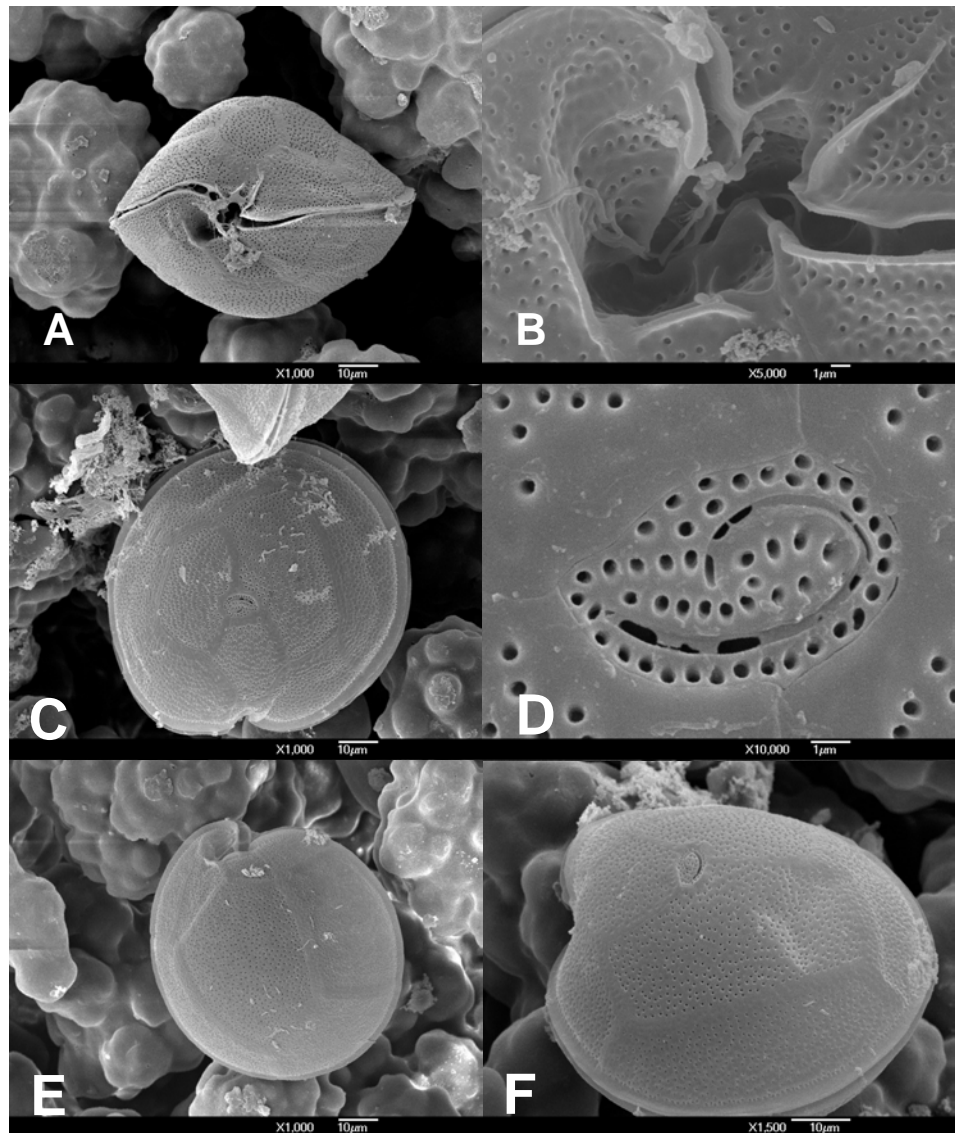


Fig. 2. *Gambierdiscus toxicus* from Yucatan: a) ventral view, b) sulcul area, c) apical view (epitheca), d) the pore plate, e) antapical view (hypotheca), f) apical-left-side view.

*heptagona* and some dubious taxa preliminarily identified as *Durinskia* sp., *Cabra matta* and *Togula*-like species contributed to the epiphytic associations. Frequently, *Prorocentrum mexicanum* was the dominant dinoflagellate.

In comparison with the Veracruz epiphytic associations, the almost complete absence of *Ostreopsis* spp. and the relative abundance of *G. toxicus* were characteristics of the Yucatan samples. Yucatan specimens of *Gambierdiscus* correspond well with the original description and figures of *G. toxicus*, although showing a rather narrow range of size variability: 52.5–70  $\mu\text{m}$  long ( $58.59 \pm 4.34$ ), 65–95  $\mu\text{m}$  wide ( $78.52 \pm 6.83$ ) and 63–87.5  $\mu\text{m}$

angulated, especially in its narrower part, which is directed to the left side of the cell, with a fishhook-shaped apical pore; the pore plate is 6.23–8.61 ( $7.32 \pm 0.75$ )  $\mu\text{m}$  long and 4.56–5.38 ( $5.03 \pm 0.24$ )  $\mu\text{m}$  wide, length/width ratio 1.27–1.63 ( $1.47 \pm 0.14$ ) ( $n = 8$ ). The 2' plate is about 1.5 times larger than the 3' plate. Precingulars 1'' and 7'' are extremely small and do not contact with the apicals 2' and 4'. The 4'' plate is almost symmetrical in relation to the longitudinal axis of the cell. The 1''' plate is about 1.5–2 times smaller than the 2''' plate. Compared to the original illustrations [9], the cells from Yucatan have a relatively wider 1' plate. Nevertheless, we identify them as *G. toxicus*.

At Dzilam (Fig. 3A, 4A) *G. toxicus* was sporadically found only in May and November 2008, in low numbers at stations 1 and 4 close to the coast (Fig. 1). Near Chelem (Fig. 3B, 4B) it was present year round, but only in one sample in August 2008. The highest numbers of *G. toxicus* were encountered in May and November 2008 (Table 1). The absence of *G. toxicus* in early March 2009 at Dzilam, and very low concentrations (maximum 17 cells/g wet weight) at Chelem, may have been related to comparatively low water temperatures (20.5–22.7°C). Curiously, the total absence of this species in early March samples at Dzilam coincided with the minimal registered nitrate, nitrite and ammonium concentrations. In general, the distribution of abundance of *G. toxicus* in the two areas was very heterogeneous (Fig. 3A–B).

*G. toxicus* can easily be overlooked when present in low densities. We recommend sampling with a hand net in shallow waters, because *G. toxicus* is sometimes rather abundant in horizontal net hauls; besides, net sampling provides a cleaner material for scanning electron microscopy. It is obvious that *Gambierdiscus* cells

Table 1. Maximal abundances of *Gambierdiscus toxicus* found at the northern Yucatan coast in 08–09 (in cel/g substrate wet weight).

Substrate	Month	Site	St.	Cells/g
Hydroid	May-09	Chelem	5	9988
<i>Halodule wrightii</i>	Nov-08	Chelem	5	3962
<i>Halodule wrightii</i>	May-08	Chelem	5	1733
<i>Batophora oerstedii</i> , <i>B. occidentalis</i>	Nov-08	Chelem	6	1255
<i>Enteromorpha</i> spp.	May-08	Chelem	5	647
<i>Halodule wrightii</i>	Nov-08	Dzilam	4	532
Rhodophyta gen. spp.	Nov-08	Chelem	4	425
<i>Gracilaria</i> sp.	May-08	Chelem	6	318
<i>Caulerpa paspaloides</i>	Nov-08	Chelem	1	224

can be transported along the coast to new sites.

There is no commercial fishery in the areas studied. Most of fish are carnivorous. The omnivorous pinfish *Lagodon rhomboides* (Sparidae), typical in shallow subtropical estuaries, is the dominant species. In Florida waters, older pinfish consume increasing amounts of plant material in addition to animal prey, and their foraging activities are capable of altering the composition of estuarine epifaunal seagrass communities [10]. Although the pinfish is not consumed by local Yucatan communities, the consumption of the great barracuda *Sphyraena barracuda* (Walbaum) should be considered.

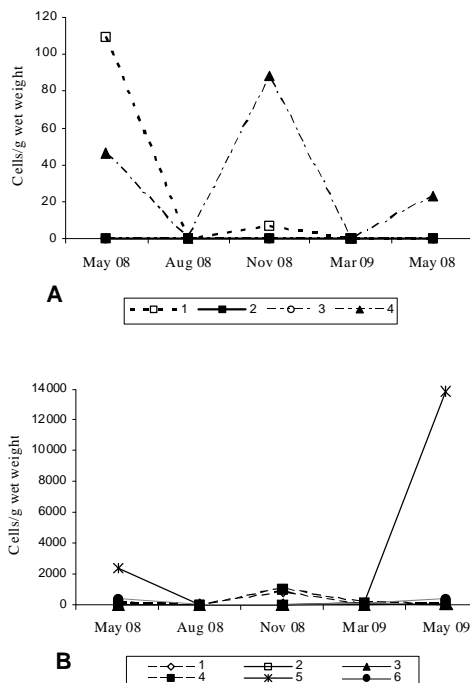


Fig. 3. Annual cycle of *Gambierdiscus toxicus* at Dzilam at 4 stations (A) and Chelem at 6 stations (D), Gulf of Mexico. Mean values averaged from all the samples for each station are shown.

deep ( $72.96 \pm 6.75$ ), width/length ratio 1.28–1.62 ( $1.39 \pm 0.06$ ) and width/depth ratio 0.71–1.21 ( $1.03 \pm 0.16$ ) ( $n = 100$ ). All measured cells are smaller than those reported from the Mexican Caribbean, 97–102  $\mu\text{m}$  wide [8]. Cells are lenticular, considerably anterior-posteriorly compressed and slightly compressed ventrally, with smooth and porous theca. Cingulum is narrow and deep, in general circular but ascending on ventral side, forming a sigmoid line around the sulcal area. Sulcus is deep, bordered posteriorly with a V-shaped rim. Apical pore complex is ovoidal and somewhat

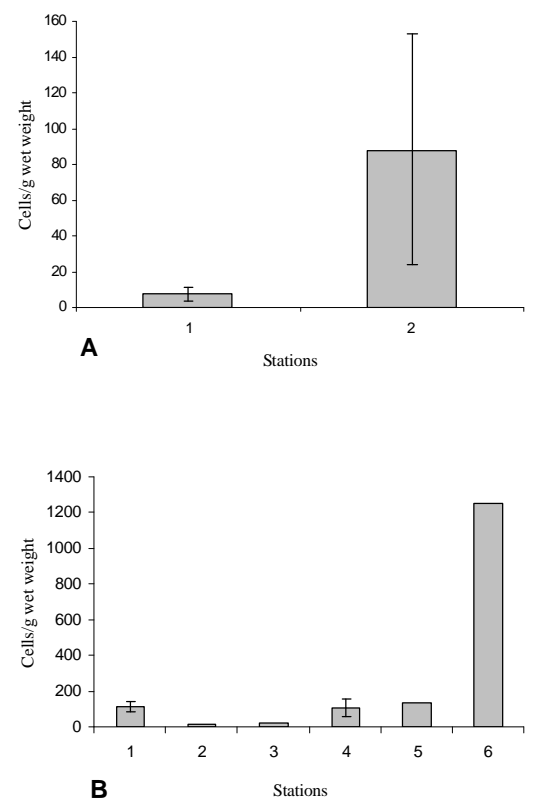


Fig. 4. Abundance of *Gambierdiscus toxicus* in November 2008 at Dzilam (A) and Chelem (B), Gulf of Mexico (rectangulars present mean values per station and a standard error bars are shown in case of more than one sample per station); at sts. 3 and 4 at Dzilam the species was not detected.

# First record of *Gymnodinium* cf. *catenatum* and other potentially toxic planktonic dinoflagellates in southern Cuba



Fig. 1. Sampling stations in Bahía de Cienfuegos.

Cienfuegos Bay (southern Cuba) is a semienclosed embayment of 90 km<sup>2</sup> and a mean depth of 14 m. The bay is divided in two parts by a 1.5 m-deep submerged bank. Four rivers, Arimao, Caonao, Damují y Salado (Fig. 1) enter this bay subject to two distinct seasons: a dry season from November to April—average salinity of 33.8 psu and temperature of 27.5 °C—and a rainy season, from May to October—mean salinity of 23.7 psu and temperature of 29.8 °C [1]. The northern part, with sewage input from the city of

Cienfuegos (106 504 inhabitants), as well as industrial waste and the inflow from rivers Damují y Salado, is under stronger anthropogenic impacts than the southern part, that receives the inflow of rivers Caonao and Arimao and has a natural park (Laguna Guanaroca) for the protection of migratory birds.

Figure 1 shows the location of the 16 stations that are sampled every 3 months as part of a monitoring programme for water quality control that includes estimation of physico-chemical parameters and phytoplankton composition. In June 2009, at the beginning of the rainy,

species diversity and abundance, mainly of diatoms (*Rhizosolenia hebetata* f. *semispina*, *Dactyliosolen fragilissimus*) and dinoflagellates (*Prorocentrum compressum* and *P. micans*) usually increase. Some potentially toxic

dinoflagellates were found in these communities, including *Gymnodinium* cf. *catenatum*, potential producer of PSP toxins and first time reported in southern Cuba.

*Gymnodinium* cf. *catenatum* was mainly found in the southern cleaner waters of the Bay. The maximum concentration was detected at stations 14 ( $3.3 \times 10^4$  cel. L<sup>-1</sup>) and lower levels at station 13 ( $8.3 \times 10^3$  cel. L<sup>-1</sup>), 16 ( $7.2 \times 10^3$  cel. L<sup>-1</sup>) and 12 ( $9.0 \times 10^2$  cel. L<sup>-1</sup>). Four-celled chains were predominant, but pairs, triplets and 6 to 8 celled chains were also observed (Fig. 2). Cells suffered deformation with the fixatives but still their characteristics incline us

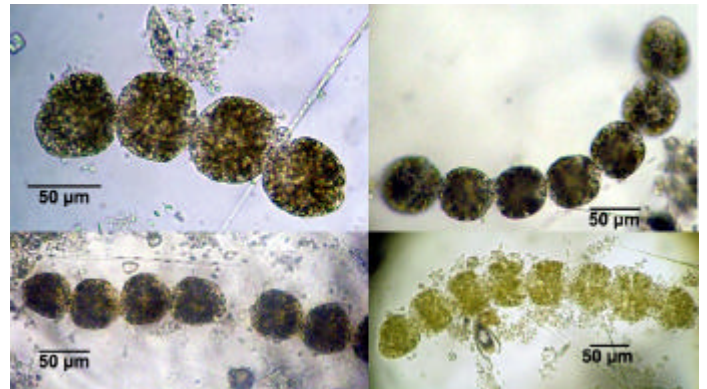


Fig. 2. *Gymnodinium* cf. *catenatum* (lugol-preserved), chains of 4, 7 and 8 cells.

(Cont'd from p. 13)

The relative abundance of *G. toxicus* near Chelem, especially in May (both 2008 and 2009) and November, and the presence of a ciguateric food web, including rich seaweed beds in an extensive shallow zone around the Yucatan Peninsula with fine bottom sediments, indicate a potential risk for a future ciguatera outbreak. Monthly monitoring of epiphytic and benthic dinoflagellates coupled with routine toxicity analyses are urgently needed to study seasonal changes and to minimize or prevent toxicity to humans.

## Acknowledgements

We thank A. Aguilar-Trujillo for technical support, J.M. Hernández de Santillana for the data on fish and fishing (both from CINVESTAV, Merida), and K.A. Steidinger for her help. Financial

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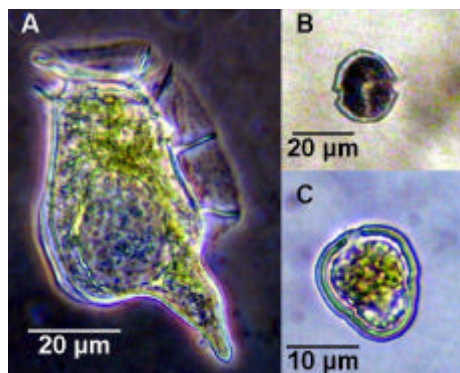


Fig. 3. A. *Dinophysis caudata*. B. *Alexandrium cf. minutum*. C. *Prorocentrum minimum*.

to identify it as *Gymnodinium catenatum*, a species with larger and more elongated cells (53 x 39 µm) in the extremes of the chain than the morphologically close species *Gymnodinium impudicum*. Some of the inner cells were shorter (49 x 44 µm) and most of them isodiametrical (43 x 43 µm), and were full of plastids. The conditions observed in the area where *Gymnodinium cf. catenatum* bloomed were: temperature 30.6 °C, salinity 31.4 psu, dissolved oxygen 7.06 mg · L<sup>-1</sup> and chlorophyll *a* 3.12 µg · L<sup>-1</sup>.

In the Caribbean Sea, *Gymnodinium catenatum* associated with PSP outbreaks was first reported in Venezuela [2] and low concentrations of the species (4-8 x 10<sup>2</sup> cel L<sup>-1</sup>) were reported by Leal *et al.* [3], who considered them as part of the cryptic flora, in the archipelago Sabana-Camaguey in northeast Cuba.

A small dinoflagellate species of *Alexandrium*, *Alexandrium cf. minutum* (Fig. 3A), potential producer of PSP toxins, was observed in moderate concentrations (10<sup>2</sup>-10<sup>3</sup> cel L<sup>-1</sup>). In comparison with *Gymnodinium catenatum*, this species was more scarce but with a wider spatial distribution (present at most sampling



Fig. 6. Green mussels (*Perna viridis*). Photo by Raul Fernández.

stations). Maximum levels of *Alexandrium cf. minutum* were observed in shallow areas by the city.

The potential PSP producer *Pyrodinium bahamense* was first reported in the Cuba. Cells were loose (Fig. 4) and not in chains, the latter a characteristic of the non-toxic *Pyrodinium var. bahamense* that occurs in the neighbour island of Puerto Rico (tropical western Atlantic). *Pyrodinium bahamense* was observed off the Bay (concentrations up to 6.3 x 10<sup>3</sup> cel L<sup>-1</sup>) in the eastern littoral region under estuarine influence, in the beginning of the dry season (Nov 2008).

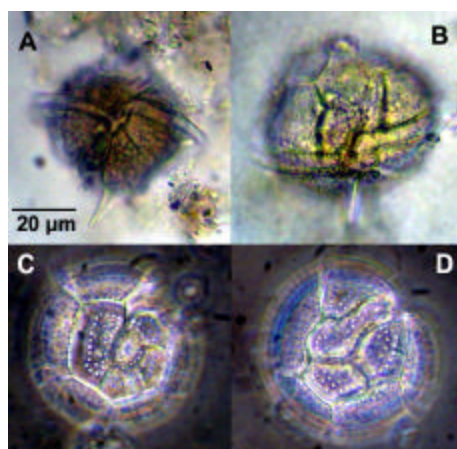


Fig. 4. *Pyrodinium bahamense*. A-B. Ventral view; C. Apical view. D. Antapical view.

Within the potential DSP-toxin producers, the most frequently observed species (concentrations up to 10<sup>2</sup> cell L<sup>-1</sup>) was *Dinophysis caudata* (Fig. 3A), that was most abundant in areas subject to anthropogenic and riverine inputs. *Dinophysis rotundata* is a very rare species. *Dinophysis tripos* has been detected in previous samplings by the mouth of the Damují river, an area with high concentrations of electrolites [4]. In May 2009, *Dinophysis cf. ovum* (Fig. 5) was detected in periphyton samples at the entrance of the Bay. This is the first report for Cuban waters of this species that was recently associated with a toxic outbreak in Texas (Gulf of Mexico).

*Prorocentrum minimum* (Fig. 3C) is a species the toxicity of which needs to be confirmed. Its

concentration increases in the beginning of the rainy season (10<sup>2</sup>-10<sup>3</sup> cel L<sup>-1</sup>) at the stations under the influence of sewage and industrial waste and river outflow.

It is more abundant in the southern part, where maximum concentrations of 10<sup>4</sup> cel L<sup>-1</sup> in previous years. It has been observed, together with *Dinophysis caudata*, in multispecific patches (red tides) during the dry season (i.e. January 2005) when water stability is highest.

There is no shellfish cultivation in the Bay of Cienfuegos and harvesting from natural banks is very limited. Nevertheless green mussels (*Perna viridis*) (Fig. 6), an invasive species from southeast Asia, was first detected in the area in May 2004 [5]. Initially settled in the northern part of the Bay (stn. 10), where it caused damage in the cooling system of the electric plant, is currently expanded through the whole area and has displaced other autochthonous species, such as the “osti6n” (*Crassostrea virginica*). The possible recollection of green mussels by local consumers with a potential contamination with PSP and DSP toxins pose a serious threat for human health in the Bay of Cienfuego that should be taken into account in future monitoring efforts.

#### Acknowledgements

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Fig. 5. *Dinophysis cf. ovum*.

# ISSHA's Corner



## HAB trail blazers

# Ramon Margalef (1919-2004)

### Education

Margalef was born in Barcelona in 1919. His education was interrupted by the Spanish civil war in 1938, when he was recruited by the Republican army. After Franco's victory he was forced to do three more years of military service. He then worked as a messenger in Barcelona's Botanical Institute, and as an insurance clerk until, thanks to the help of several scientific personalities who appreciated his intellectual potential, he got a scholarship and managed to obtain his BSc from the Universitat de Barcelona (1949). He finished his PhD only two years later in 1951.

### Honours

2002 CSIC Gold Medal, Spain; 1995 Excellence in Ecology Prize, Germany; 1990 Alexander von Humboldt Award, Germany; 1984 Ramon y Cajal Award, Spain; 1980 Huntsman Prize, Canada; 1972 Prince Albert medal, France. Margalef became the most cited Spanish researcher, sharing with Santiago Ramón y Cajal and Severo Ochoa the fact of being the three most relevant Spanish scientific workers in the life sciences out of 95 researchers in the world. The book *Perspectives in Ecological Theory* (1968) and the articles "*On certain unifying principles in ecology*" (1963), "*Life-forms of phytoplankton as survival alternatives in an unstable environment*" (1978) and "*From hydrodynamic processes to structure (information) and from information to process*" (1985) are citation classics, and the first is considered to be one of the two classical articles in the life sciences of the 20<sup>th</sup> Century.

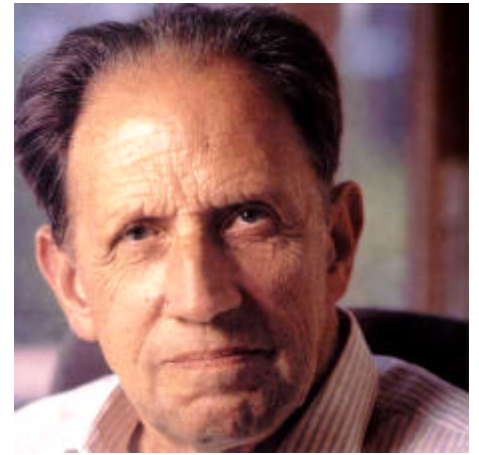
### Professional Career

After finishing his PhD, Margalef

started to work in the newly created Institute for Fisheries Investigation in Barcelona, an institution which he later presided over from 1965 to 1967. In 1967 he became Spain's first professor of ecology, a position he held at the Universitat de Barcelona until his retirement 20 years later. As is to be expected in a man gifted with a prodigious curiosity, his involvement in active research never vanished, and he continued to visit his tiny office in the Ecology Department of the Universitat de Barcelona until a few weeks before his death in 2004.

### Key HAB contributions

Margalef was one of the founding fathers of modern ecology and one of the most distinguished Spanish scientists of the twentieth century. His contributions have been extremely fertile in fields as diverse as limnology, oceanography, and theoretical ecology. Margalef was a prolific writer and he authored over 400 scientific papers and books. His first studies, published mostly in Spanish in the 1940s and 1950s, focused on the organization of planktonic communities in continental and oceanic waters. It was not until the late 1950s, with the translation into English of his inaugural lecture as a member of the Barcelona Royal Academy of Arts and Sciences "*Information Theory in Ecology*", that he gained a worldwide audience. Another groundbreaking article, "*On certain unifying principles in ecology*", published in *American Naturalist* in 1963, and his book "*Perspectives in Ecological Theory*" (1968), based on his guest lectures at the University of Chicago, consolidated him as one of the leading thinkers of modern ecology. His studies have greatly contributed to our understanding of the spatio-temporal structure of



ecosystems, the relationship between diversity, biodiversity, stability and connectivity, the role of external energy in biological productivity, and the interplay between ecological succession and evolution. He proposed a conceptual model for the ordination of major phytoplankton life-forms as a function of availability of nutrients and external energy, later called "the phytoplankton mandala", that provided a valuable framework for the ecological understanding of red tides. In his lectures at the Universitat de Barcelona, or in the numerous invited courses and seminars elsewhere, he always promoted creative thinking and transmitted in a fresh and challenging fashion his views on how nature works, prompting students to "get out and discover nature" for themselves. His views were summarized in two monumental textbooks: "*Ecología*" (1974) and "*Limnología*" (1983). Ramon Margalef exemplified one of those rare cases in which an outstanding intellect coexists with equally exceptional personal qualities. The scope of his knowledge, his humane nature, his modesty, his honesty and his sense of humour gave him a human dimension well beyond his scientific qualities. Much is to be learned from his approach to science and to life in general, in which an insatiable, childlike curiosity sprang from the intimate pleasure he found in observing the world around him. Some of his views have proven to be wrong, but they have been always original and inspiring and often truly revolutionary. In a scientific world dominated by reductionism he always was one of the few minds capable of seeing forests where most saw only the trees. He was also very much interested



in the public appreciation of science and always advocated for the engagement of scientific rigour in environmental policy. “If God has put us on Earth, we have the right to make use of it but we might as well do so with a modicum of intelligence”. We owe to him the introduction of information theory to the study of ecological diversity, arguably one of the major inflection points in the history of ecological thinking.

#### Mentored

Martha Estrada, Dolores Blasco, Rosa Miracle.

#### 10 Key HAB publications

**Margalef R.**, 1997. Red Tides and ciguatera as successful ways in the evolution and survival of an admirable old phylum. In B. Reguera, J. Blanco, M.L. Fernandez and T. Wyatt (eds). *Harmful Algae*, Proc. of the VIII

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*Adapted from Margalef biography by Jordi Martínez Vilalta (2004) and comments by M. Estrada.*

*One of a series of articles prepared by Professor Gustaaf Hallegraeff, ISSHA's Vice-President, for the ISSHA Website (www.isssha.org).*



## An early *Karenia* bloom off southwest Ireland?

*The Skibbereen & West Carbery Eagle* newspaper began publication in 1857, the year before the foundation of the Irish Republican Brotherhood and the Fenians. The year 1865 marked the end of the American Civil War ... Skibbereen was the epicentre of the Great Famine which had sent 2 million Irish refugees to emigrate, most of them to USA. Some returned in 1867 to participate in the first Fenian uprising, one of the many precursors of Irish independence.

#### From the *Skibbereen & West Carbery Eagle*, Saturday, 6th May, 1865:

Many reports have reached us that a streak of coloured matter has shown itself in the sea water along this coast for the last two weeks. These reports vary as to the length, breadth, appearance and nature of this very extraordinary phenomenon. Putting them all in a collected form and adding what we have ourselves seen, the facts seem to be these: - On last Wednesday week we visited Castletownsend, and the evening being a lovely one took a boat to enjoy a row along that picturesque harbour. The sea was smooth as glass, and we were tempted beyond our usual limits and boldly pulled outside the harbour. Scarcely had we reached the open ocean when we at once noticed

that the usually clear, transparent waters of the great deep were now muddy and forbidding to look upon. Rowing along we saw that all the ocean waters were not so tinged but that the colour was confined to a streak or river, as it were, in the sea, and along the rocks where no tide swept for about 20 to 30 feet the water was clear as ever. We happened to be fishing for pollock, and found that no fish took where the colour was great; but whether this was owing to the fact that the fish could not see the bait or that the colouring matter was poisonous, we are not prepared to say. We went down two or three times since and saw the same appearance, but the colour seemed more diffused, and less obnoxious to the fish, as they took the bait when it showed itself. Several

people have told us about a similar appearance all along Lough-Ine, Baltimore, Cape Clear, and Crookhaven. Some say it is the blood of American patriots that has been borne down her mighty rivers into the Gulf of Mexico, and has thence been carried by the great Gulf Stream to these our sympathising shores. Others (and with more probability) ascribe it to some volcanic eruption or upheaval which has taken place beneath our waters, and has thrown up mud, scoria, and ashes, thus to show us that we are not beyond the reach of this mighty scourge of some of the fairest portions of our earth. We do not attempt to decide between these rival theories. We leave our readers to visit the coast, and having seen for themselves, judge for themselves.

# GEOHAB

Global Ecology and Oceanography of  
Harmful Algal Blooms

## HABs and Eutrophication: A Core GEOHAB Project

The relationship between HABs and eutrophication is complex and of global concern. The complexity arises from many factors. Both high-biomass forming HABs and those that cause harm even when representing a small proportion of the total plankton assemblage occur in eutrophic environments. Additionally, the physiology of these diverse organisms not only varies between species (and even strains) but may include both autotrophy and heterotrophy, the degree of which may change with nutrient cell status or availability of nutrient form. Although there is growing awareness of mixotrophy in the nutrition of many HABs, our overall knowledge of the flux of organics, their bioavailability and the propensity for individual species, which may include prey for HABs, to be promoted under changing organic flux conditions is very poorly known.

Adding to the above is the problem of determining how nutrients may affect biological processes such as motility, release of mucus, and allelopathy, among other processes. The relationship between HABs and eutrophication is also complex because although eutrophication is occurring

globally, nutrient export from coastal watersheds is not evenly distributed, and in many regions of the world is changing rapidly. Changes in nutrient loads are occurring due to population growth, changes in sewage treatment - both overall increases and in some cases improvements to treatments - as well as other changes in land use. Animal husbandry operations are resulting in more animal wastes reaching some coastal areas. Changing compositions of fertilizer in many agricultural areas is also altering the composition of nutrient loads.

Also, without doubt, the expanding aquaculture industry in many parts of the world is altering localized nutrient loads and concentrations. Nutrient loads in one form are also rapidly transformed through biochemical process and trophic interactions in the water column and in the benthos, so the form of nutrient actually "seen" by a HAB community may be different from those that were discharged from land. Lastly, changes in nutrient loads to coastal watersheds are also changing in many areas coincident with other changes related to climate, so that differentiating the effects due to eutrophication from those due to

climate is difficult; in some cases these changes are synergistic. Thus, it is a formidable challenge to relate nutrient loads to HAB occurrences.

Promoting research that addresses this complexity is the objective of the Core Research Project of the GEOHAB Programme on HABs and Eutrophication. GEOHAB, The Global Ecology and Oceanography of Harmful Algal Blooms Programme, has as its central mission the fostering of international cooperative research on HABs in ecosystem types with common key species or common processes (including anthropogenic) that affect their population dynamics. The goal of GEOHAB is to improve our predictive capability of HABs and their impacts by integrating biological, chemical, and physical studies supported by enhanced observational and modeling techniques.

The goal of promoting international cooperation and collaboration on the topic of HABs and eutrophication was recently advanced through the Second GEOHAB Open Science Meeting on HABs and Eutrophication, held in Beijing from October 18-21, 2009. Over 130 participants from 23 countries attended this event. It was particularly

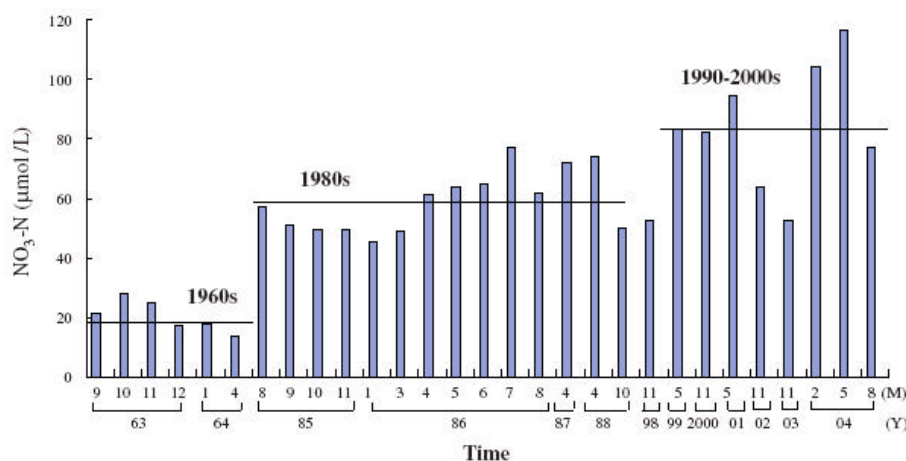


appropriate that this meeting was held in China, as the rate of nutrient loading to Chinese coastal waters has increased rapidly over the past several decades with the rapid industrialization of this country, and eutrophication-related HAB events are now common along the Chinese coast. As noted by Mingjiang Zhou, co-convener of the conference, "The international gathering of scientists in Beijing for the GEOHAB HABs and Eutrophication conference will help to inform local managers of the importance of this issue for China."

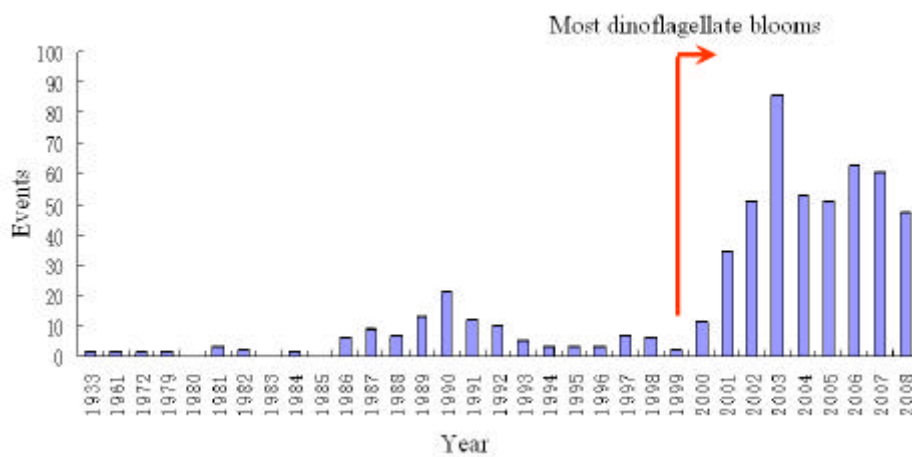
The conference featured seven keynote presentations, with reviews of the rapidly expanding HAB events and their impacts throughout Chinese waters in the past decade (Mingyuan Zhu), global estimates of the expanding distribution and impacts of *Nocticula scintillans* (Paul Harrison), and nutrient discharges from expanding aquaculture operations and their impacts (Lex Bouwman and Sandor Mulow). Participants also heard reviews of eutrophication related HAB events in the Yucatan Peninsula, Mexico (Jorge Herrera Silveira), and the Gulf of Oman (Adnan Al-Azri). Bioengineering approaches to the problems of cyanobacterial blooms in the Baltic Sea were also addressed (Wilhelm Graneli). Thus, the goal of global networking was achieved without question.

Contributed talks described a broad range of topics, underscoring many aspects of the complexity of the relationships of HABs with nutrients described above. Among the common themes were the impacts of sewage discharge in the US and in Hong Kong, the effects of varying stoichiometric ratios in many regions and with respect to the growth of numerous species, factors promoting the expansion of cyanobacterial blooms, and interactions of anthropogenic nutrients and regional and global climate change. Many new approaches and technologies were also highlighted.

The local organizers did an outstanding job of preparing for this conference, with no detail left unattended. All attendees were able to enjoy wonderful meals and tremendous hospitality in beautiful Beijing along with excellent science. The city of Beijing has



Increase of nitrate concentration at the Changjiang River estuary in 40 years, (From Zhou et al 2008)



Red tides increase at the Changjiang River estuary and its adjacent sea areas (Zhou redrawn from SOA data)

been transformed in the past few years, in large part because of the 2008 Olympics. During the entire conference blue skies prevailed, demonstrating the reduction in air pollution in this region.

To further the dialogue and the sharing of information on this important topic, a special issue of the *Chinese Journal of Oceanology and Limnology* is being prepared to capture the highlights of this meeting. Two types of papers will be accepted: regular manuscripts and notes. Please contact Pat Glibert (glibert@umces.edu) if you wish to contribute to this special issue.

Information will also be available on the conference website ([www.geohab-osm-bj.ac.cn](http://www.geohab-osm-bj.ac.cn)) as will the any photos of the conference that document so well what an excellent symposium this was. The Core Research Project on HABs and Eutrophication aims to continue the tradition of Open Science Meetings, first conducted in Baltimore, MD, USA, in 2005, through a Third Open Science Meeting in several years- most likely in Europe. Follow the GEOHAB website

([www.geohab.info](http://www.geohab.info)) for information about this meeting as it develops in the coming years. Given the rapidly of advances in knowledge and application of new techniques and models, and the expanding impact of eutrophication globally, it can be projected that there will be much to report in a few years' time.

### Harmful Algae News only exists if the Editor gets input from YOU!

Write the Editor NOW with news on your work, HAB events in your country or region, or any other matter you wish to share with HAB scientists and managers worldwide. Harmful Algae News has more than 2000 subscribers.

# GEOHAB

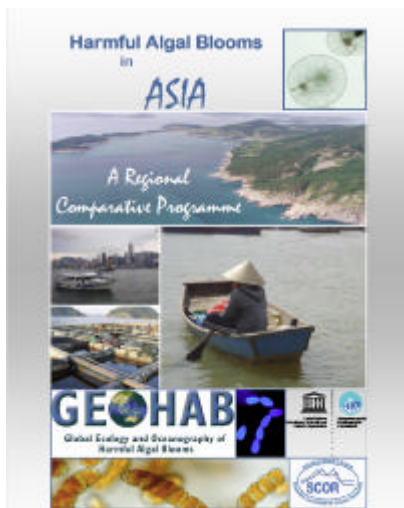
Global Ecology and Oceanography of  
Harmful Algal Blooms

## Open Science Meeting on Benthic Harmful Algal Blooms

Tentative dates 21-23 June 2010,  
Hawaii, USA

The Programme will focus on Intoxications, Biodiversity and Biogeography including biogeographical species distribution, global analyses/data sets, methodology for sampling to abundance and distribution, methodology for taxonomic identification; Ecological Factors such as methodology for evaluation/comparison of habitats, anthropogenic forces and/or stimuli, bottom-up and/or top-down control, controls such as nutrients and grazing, relationships with macroalgae and/or substrates, and effects of toxins on marine foodwebs; Adaptive Strategies including physiological characteristics and life cycle traits; Phylogenetic Characteristics; and Modelling.

Detailed info will appear at [www.geohab.info](http://www.geohab.info). Contact: P. Bienfang ([bienfang@soest.hawaii.edu](mailto:bienfang@soest.hawaii.edu))



The Science Plan for GEOHAB in Asia will be launched in December 2009 and will be available on line at: [www.geohab.info](http://www.geohab.info).

# ISSHA



International Society for the Study of Harmful Algae

NEW ISSHA website to be launched by  
mid-December: [www.isssha.org](http://www.isssha.org)

## Future events

### Yasumoto to be Honored in Spain

Vigo, Spain, January 28, 2010

Professor Takeshi Yasumoto will be awarded with the distinction of "Doctor Honoris Causa" by the University of Vigo, Spain, in an academic ceremony. The award recognizes his many achievements and leadership in the marine toxins field. The following day, on January 29<sup>th</sup>, a one-day seminar entitled "Marine Biotoxins Analysis and Toxicology" will be held, followed by a round table discussion: "Are New Limits on Seafood Biotoxins Necessary?". Invited speakers will include: J. Lawrence, M. Quilliam, R. Dickey, J. Hungerford, Y. Oshima, P. McNabb, P. Burdaspal, S. Franca, A. Tubaro, etc.

The aim of these events, besides presenting gratitude and respects to Prof. Yasumoto, is to gain new insights into this exciting and crucial field of research by joining together world experts on both analysis and toxicology.

The venue for all events is the campus of the University of Vigo and the organizers are Ana Gago Martínez and Dr. José A. Rodríguez Vázquez. In case you are interested in participating, please contact Ana Gago ([anagago@uvigo.es](mailto:anagago@uvigo.es)) by 1<sup>st</sup> January 2010.

### VI International CONyMA'2010 Workshop on Pollution and Environmental Protection

Havana, Cuba, 1–5 March 2010

The workshop, focused on ciguatera, a potential risk in the Caribbean, will cover the following topics: ciguatera: ecotoxicology & socioeconomic aspects; marine pollution; biodiversity; environmental management; preservation and protection of mangroves; toxic and harmful algae; environmental education; marine biotechnology and pharmacology; database use and implementation; management of coastal ecosystems; trophic dynamics; and process modelling. Papers are accepted until Jan 15, 2010.

Further info: [conyama@cip.telemar.cu](mailto:conyama@cip.telemar.cu).

### Harmful Algae News

Previous issues of HAN and newsletters of the IOC HAB Programme can be downloaded at <http://ioc.unesco.org/hab/news.htm>

### Requests for subscription

Subscription to HAN is made by sending a request with a complete address to Ms V. Bonnet: [v.bonnet@unesco.org](mailto:v.bonnet@unesco.org).

## HARMFUL ALGAE NEWS

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