First Report of *Dinophysis acuta* in culture

Several species of the genus *Dinophysis* associated with production of DSP toxins bloom every year during spring-summer in coastal waters of Huelva (Southwestern Andalucía, Spain). *Dinophysis cf acuminata* and *D. acuta* are the main species responsible for harvesting closures of natural shellfish beds of *Donax trunculus* and other commercially important species in the region. Cultures of *Dinophysis* may contribute to a better knowledge of their ecophysiology, life cycle and toxin production.

Following the discovery of *Myrionecta rubra* as an optimum prey for mixotrophic species of *Dinophysis* [1], attempts to cultivate our local species started at the *Laboratorio de Control de Calidad de los Recursos Pesqueros de Andalucía*. Cultures of *D. cf acuminata* were established in 2007 after overcoming manipulation and culture problems associated with the culture of its prey, *M. rubra*, fed with cryptophytes [2]; both the ciliate and the *Teleaulax*-like cryptophyte were isolated from our local waters. Cultures of *D. fortii* [3], *D. caudata* [4] and *D. infundibulus* [5] were recently reported by other authors. Here we report, for the first time, the establishment of cultures of *Dinophysis acuta* that have been maintained in our laboratory since summer 2005.

Cultures were transferred fortnightly in groups of 6 cells/well. Of all conditions tested, only cells of *D. acuta* maintained with autoclaved aged seawater, with addition of *Chaetoceros* sp., lasted until August 2006 (9 months). In cultures with f/2-Si *Chaetoceros* sp. always overgrew *Dinophysis*. The maximum number of *D. acuta* cells/well recorded after 15 days was 16. Immediately after isolation, cells had yellow brown pigmented chloroplasts (Fig. 1a); these faded with time. After 2 months in culture, small *D. dens*-like cells (Fig. 1b) were observed. When *D. dens* cells were transferred to new medium, they were observed to divide but never lasted more than 3 transfers. Only transfers of *D. acuta* cells and of mixed groups of *D. acuta* and *D. dens* allowed higher transfer numbers. When only normal-sized cells were transferred, eventually a mixed culture would develop, and small cells would tend to outnumber *D. acuta*. It is not clear whether the increase in small cells was mainly due to small cell division or to the progressive conversion of *D. acuta* to *D. dens* as suggested by the observed (Cont’d on p. 2)

Long term maintenance of *Dinophysis acuta* in co-culture with *Chaetoceros* sp.

Here we report on a series of experimental settings aiming at culturing *Dinophysis acuta*. Cells of *D. acuta* were isolated in December 2005 from vertical net hauls (20 µm) collected from Lisbon Bay, Portugal (38º 41.34’ N; 9º 24.54’ W).

In the laboratory, single cells of *D. acuta* were isolated by micropipette under an inverted microscope, and transferred to 4 well 2-ml culture plates (NUNC ref. 176740) with the following media: 1) filtered seawater from the isolation site; 2) aged autoclaved seawater from the isolation site; 3) f/2-Si. In addition, cells of a non-chain forming *Chaetoceros* sp., abundant at the time of isolation, were added to two of the wells in the above experimental settings. Cultures of *Chaetoceros* sp. were also established for further use and were regularly added to the *Dinophysis* cultures.

All cultures were maintained in a culture cabinet at 15°C±1°C (Aralab Fitoclima 750E), with overhead illumination of 20 µmol photons · m⁻² · s⁻¹, supplied with cool white fluorescent lamps, and a light:dark cycle of 14:10h. Cells of *D. acuta* were transferred fortnightly in groups of 6 cells/well.

Of all conditions tested, only cells of *D. acuta* maintained with autoclaved aged seawater, with addition of *Chaetoceros* sp., lasted until August 2006 (9 months). In cultures with f/2-Si *Chaetoceros* sp. always overgrew *Dinophysis*. The maximum number of *D. acuta* cells/well recorded after 15 days was 16. Immediately after isolation, (Cont’d on p. 2)
Individual cells of *D. acuta* were isolated from samples collected in the course of weekly sampling of the Andalucian Monitoring Programme for the control of sanitary conditions in shellfish production areas in Huelva (Andalucía). *D. acuta* was grown in L1 medium (without silicates), at 33 psu, 18.5°C and a 14:10 L:D cycle. *Myrionecta rubra* was added as prey every 2-3 days. Both the ciliate and the depauperating divisions described by Reguera et al. [1] (Fig. 1c). No mating pairs were ever observed. Tetrads of cells aligned with their ventral and dorsal margins in parallel to each other and similar to those illustrated from cultures of *Dinophysis fortii* [2] were recorded in July 2006 (Fig. 1d). The nature of these tetrads is still unresolved.

No direct evidence of *Dinophysis* feeding on *Chaetoceros* sp. was observed. The possible ingestion of small bacteria-like particles was observed by immobile cells on the bottoms of the wells that generated trapping currents with the cingular flagellum. However, this was common in all the experimental settings, and does not account for the much longer survival of cells in aged autoclaved sea water with *Chaetoceros* sp. The same experimental setting described above was used for cells of *D. fortii*, *D. caudata* and *D. acuminata*, but the maximum time in culture was 5 months. All species eventually produced small cells and in contrast to *D. acuta* the engulfment/conjugation process was observed. Tetrads were also observed in *D. fortii*.

Since Park et al. [3] reported the cultivation of *D. acuminata* feeding on *Myrionecta rubra*, other species of the genus have been successfully established in cultures with the same prey. To our knowledge this is the first report of *D. acuta* kept in culture conditions for 9 months and of other *Dinophysis* species for more than 5 months without feeding on *M. rubra*. These results suggest *Chaetoceros* sp. may act as a possible nutritional source for *D. acuta*, but further work is needed to better clarify the nature of this interaction.

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**References:**


**Fig. 1. Different stages of D. acuta:**

- a) Normal-sized vegetative cell and b) Small *D. dens*-like cell (both in http://planktonnet.awi.de/);
- c) A dividing pair leading to smaller-sized cells;
- d) A tetrad of uncertain nature. Scale bar = 20 µm.

**Reference:**


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Chrysochromulina outbreaks, and large species in French Atlantic waters

Outbreaks of Chrysochromulina spp. are not unusual in French Atlantic waters from spring to autumn, and densities can exceed $10^7$ cells L$^{-1}$ in spring. In 2008, such a bloom was observed south of Brittany during the RAMVIL cruise (31 May - 05 June) (Fig. 1). Cells are usually small in size (only a few microns) but since 2006, large ones have been observed. They measured 14 µm to 20 µm long with a rather short haptonema. They were roundish to heart-shaped or with an irregular outline (Fig. 2). Moreover, some of them were sheathed by a meshwork pattern visible in light microscopy (Figs. 2B–C). From a sample of July 2006 collected during the HABIT 2006 cruise (Fig. 1) and containing numerous large cells, a preliminary examination of scales by TEM has been made. The major type of scale (0.8-1.2 x 0.6–0.9 µm) is round to oval in shape and patterned with radiating rectangular perforations arranged in 7-9 concentric circles (Fig. 3A). But much larger scales (3.4-3.7 x 2.7–2.9 µm) were found with a faint monomorphic surface patterning, a distinct rim (0.13 µm), and a cruciform centre (Fig. 3B). The former type resembles that of Chrysochromulina sp.7 described by LeRoi & Hallegraeff [1], while the latter, larger scale may belong to the outer meshwork, interpreted as an alternate stage of the complex life cycle [2, 3]. Further observations are needed to identify these large species that are not currently recorded by the monitoring, but are able to dominate the genus in summer or autumn waters with a potential threat of toxicity.

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Ostreopsis cf. ovata on Abruzzo coast, W Adriatic

Ostreopsis belongs to the family Ostreopsidaceae: cells are narrow, ovate in dorso-ventral view with biconvex, drop-shaped theca, scattered pores and eight sulcal plates [1, 2]. The life cycle includes a resting stage, probably a hypnozygote [3]. This species is occasionally planktonic but generally benthic on macroalgae, rocks, sediment or detritus aggregates, and forms a mucilaginous matrix within which the solitary cells can move [4]. Ostreopsis ovata produces palytoxin-like compounds [5]. The production of putative palytoxin with other palytoxin-like compounds by Ostreopsis ovata from Italy has recently been confirmed [6].

Blooms of O. ovata can cause hypoxia, anoxia and benthic invertebrate kills by forming mucilaginous layers hosting thousands of cells covering both biotic and abiotic substrates, as occurred on the Tuscany coast in 1998 [7]. The presence of O. ovata in the Mediterranean Sea is traced back to the 1970s on French coasts. It was first detected on the western coast of Italy (Tyrrenian Sea) in the 1980s [8]. In the last decade, massive blooms of this species have become more frequent, above all in the Tyrrenian and Ligurian Seas, causing death of benthic organisms and human health problems such as respiratory difficulties and skin irritation [9].

O. ovata was first detected in the southern Adriatic, and later in northern areas, off Ancona and Trieste [5], and on the Abruzzo coast last summer. Here we record the presence of O. cf. ovata in Abruzzo coastal waters.

Pump samples, 0.5 m below the surface, were taken at six stations located 500 m and 3000 m from the coasts of Pescara, Ortona and Francavilla (Fig. 1). Samples were also collected at these stations with a 10 mm mesh phytoplankton net. Monthly sampling also took place inside Pescara and Ortona harbours and along the rocky banks of Chieti Province (San Vito and Fossacesia), during June, July and August 2008. Stations inside harbour areas and on rocky banks were selected to localize epiphytic and epibenthic species like O. ovata. In the harbour areas and on the rocky banks, samples were taken only at 0.5 m below the surface, without a net. But more accuracy was obtained by taking samples in areas rich in macrophytes such as Ulva lactuca.

Observations were made by light microscopy (ausJENA Telaval 3) at 200x, 400x and 1000x magnification (Figs. 2 a & b). Epifluorescent microscopy was used for plate tabulation (Zeiss Axiophot microscope at 200x, 400x and 1000x magnification) following the Calcofluor method [10] using fluorescent brightener 28 (Sigma – Aldrich) (Figs. 3 a & b). For SEM

Fig. 1. Research Area.

Fig. 2. Light microscopy photographs
Ostreopsis cf. ovata: a) cells stuck to Petri plate, 200x, scale bar = 50 µm; b) single cell where it is possible to observe the drop-shaped theca, 1000x, scale bar = 10 µm.
the harbour areas. Cell concentrations in surface samples were $1.0 \times 10^6$ cells · L$^{-1}$ and $5.0 \times 10^5$ cells · L$^{-1}$ respectively. These two species were absent at the 500 m and 3000 m coastal sampling stations. But other toxic and potentially toxic species were observed during June, July and August 2008 (maximum concentrations in 0.5 m net samples): Proorocentrum minimum in June 2008 at Francavilla 3000 m from coast (4.8 x $10^5$ cells · L$^{-1}$ and 11.0 x $10^3$ cells · L$^{-1}$ respectively); Pseudo-nitzchia in July 2008 at Ortona 3000 m from coast (8.1 x $10^3$ cells · L$^{-1}$ and 44.0 x $10^2$ cells · L$^{-1}$ respectively); Dynophysis sacculus in August 2008 at Pescara 500 m from coast (20 cells · L$^{-1}$ and 5.0 x $10^2$ cells · L$^{-1}$ respectively)

Additional studies are needed to observe the periodical presence of Ostreopsis cf. ovata and other toxic and potentially toxic species along the coasts, inside harbours and on rocky banks of the Abruzzo Region.

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SEM observations of *Pseudo-nitzschia* from Beagle Channel: *P. seriata* in the southern hemisphere?

*Pseudo-nitzschia seriata* (Cleve) H. Peragallo is known as a potentially toxic diatom species [1, 2], and is related to domoic acid accumulation in mussels, resulting in the recurrent closure of shellfish harvesting in Prince Edward Island, Canada [3, 4]. Its strong morphological resemblance to *P. australis* Frenguelli has led in the past to common misidentifications and to confusion about the worldwide distribution of both species [5]. However, based on an extensive literature survey, it has been noted that *P. seriata* is confined to cold waters of the Northern Hemisphere while *P. australis* is found in both Northern and Southern Hemispheres [6].

Traditionally, the presence of striae composed of 3–5 rows of poroids has been the most distinctive character to discriminate *P. seriata* from *P. australis*, which has only 2 rows of poroids per striae [5, 7]. Nevertheless, this difference is not definite, since strains of *P. seriata* can also show only two rows of poroids in laboratory cultures [2]. As a consequence, the density of poroids per striae seems to be the most reliable character to discriminate between them (4–5 and 6–8 in 1 µm in *P. australis* and *P. seriata*, respectively) [8]. Unfortunately, time-consuming examination using electron microscopy is essential to observe this ultrastructural character.

Located in extreme southern South America, the Beagle Channel is a strait separating islands of the Tierra del Fuego Archipelago (Fig. 1). The biggest settlement on the channel is Ushuaia in Argentina followed by Puerto Williams in Chile, two of the southernmost settlements of the world [9]. Within the framework of a monitoring program of toxic algae carried out in the Easter sector of the Beagle Channel, we focused on the diversity of *Pseudo-nitzschia* species for the first time in this area. Phytoplankton net samples were examined by phase contrast, DIC and scanning electron microscopy (SEM) following traditional methods. Sampling points included Bay Brown (inside and outside) and Punta Paraná (Fig. 1) where both biological and oceanographic variables have been examined weekly. These areas are usually closed to harvesting each year during summer, when toxic algal blooms are a serious problem for consumer’s health and therefore to the economy of the area [10].

Based on valve shape and morphometric data (length, width, density of striae and fibulae and the lack of central interspace), light microscopy observations suggested the presence of *P. australis* in the Beagle Channel. This was not too surprising considering that this species is commonly found in coastal and shelf waters of the Argentine Sea [11–13]. However, subsequent SEM analyses revealed the fine structure of the striae, which disagreed with classical descriptions of *P. australis* [5, 7]. In contrast, it was strikingly similar to unusual specimens of *P. seriata* isolated from Scottish waters [2]. The specimens showed slightly asymmetrical lanceolate valves with rounded ends (Figs. 2a–b), 80.5–103.5 µm long and 7–8.9 µm wide. Interstriae and fibulae...
were usually distributed in equal number (14–19 in 10 µm) and without delineating a central interspace (Figs. 2a–c). The striae were composed of two rows of small poroids (7–8 in 1 µm) (Fig. 2d), but in some cases, a few single poroids (incipient striae?) were visible between them (Fig. 2b).

The observed specimens are in conflict with classical descriptions of both P. seriata and P. australis (Table 1). The presence of striae mainly perforated by two rows of poroids (typical of P. australis) but in number of 7–8 in 1 µm (as in P. seriata) was rather disconcerting. However, the results are similar to the unusual specimens of P. seriata identified in Scottish waters by means of TEM analyses and DNA sequencing [2]. Unlike the traditional description of P. seriata, which has 3–5 rows of poroids per striae [5], Scottish strains showed two rows of poroids plus a few single poroids, or lack of a third row visible between the two rows of poroids; a third complete row was only rarely observed [2]. These authors attributed this unusual morphology to the fact that the cells had been kept in culture for approximately six months before the TEM observations. Therefore, our field study could provide additional information supporting the finding of this unusual morphology in natural populations and prompt new questions regarding the morphological differentiation of P. seriata and P. australis. Moreover, if the identity of specimens collected in the Beagle Channel could be confirmed as P. seriata performing additional TEM and molecular analyses, the geographical distribution of this species would be greatly extended.

Given the increasing number of semi-cryptic species mentioned for the genus Pseudo-nitzschia [e.g. 14–17], we consider that further more detailed morphological investigations in combination with molecular data are required to elucidate the morphological variability and geographical distribution of P. seriata and P. australis. With this in mind, we are now attempting to establish cultures of these specimens and are examining other diatom samples from different regions of Argentina for this unusual morphology. It would be highly beneficial to work in collaboration with colleagues with previous experience in DNA extraction, amplification and sequencing of species from the genus Pseudo-nitzschia.

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Molecular identification of *Ostreopsis* species in Mediterranean coastal waters using fluorescent oligonucleotide probes

Epiphytic and benthic dinoflagellate *Ostreopsis* species are present in coastal areas of the Mediterranean Sea [1-4]. *Ostreopsis* cells colonize a variety of substrates, such as macroalgae, rocks, mussel shells and benthic invertebrates; cell abundance varies between areas, from a few cells to 10⁸ cells g⁻¹ fw on macroalgae samples. This genus has been detected sporadically in the Mediterranean basin since 1970 (Max Taylor, pers. comm.). However, since the late 1990s, massive blooms of the species *O. ovata* have become harmful to humans and the marine ecosystem along the Italian and Spanish coasts [5]. The impacts of such high biomass proliferation of *Ostreopsis* spp. are severe for both the marine environment, resulting in benthic fauna and fish mortality, and human health, resulting in respiratory and skin problems due to exposure to marine aerosols and seawater [6]. In the Eastern Mediterranean, natural *Ostreopsis* populations occur and cause the contamination of shellfish tissues with putative PLT (palytoxin) compounds [7].

Identification of *Ostreopsis* species is difficult due to variability in the morphological and morphometric features both in field samples and cultured material under optical, epifluorescence and scanning electron microscopes. In the Mediterranean, analyses based on ribosomal phylogenetic and morphological studies grouped *Ostreopsis* spp. isolates into two distinct species, *O. ovata* and *O. cf. siamensis*, and within each species all individuals were genetically identical; both species produce palitoxin-like compounds [8]. Furthermore, based on new ribosomal sequence data the toxic genotype *O. ovata* has a wider distribution than the toxic *O. cf. siamensis* in coastal areas [9], and is also expanding into colder northern areas, such as the northern Adriatic Sea, with massive recurrent blooms [10].

Genus- and species-specific molecular primers for PCR based assays have previously been used for the detection of the genus *Ostreopsis* and species *O. ovata* and *O. cf. siamensis*, and validated on field populations of *Ostreopsis* spp. *Ostreopsis* cells were isolated using a micropipette, and monospecific cultures were maintained in F/4 medium at 21 ± 1 °C with a 14:10 h (light:dark) photoperiod. Illumination was provided by fluorescent tubes with a photon irradiance of 100 mmol photons m⁻² s⁻¹.

Field samples were collected from different sites at different periods during blooms or occurrences of *Ostreopsis* spp. (mean abundance of 10⁵ to 10⁷ cells g⁻¹ fw): at Conero Riviera, NW Adriatic Sea in September 2008, Taormina, Ionian Sea in July 2007 (samples kindly provided by M. G. Giacobbe) and San Felice Circeo, Tyrrenian Sea, in July 2006 (samples kindly provided by R. Congestri). *Ostreopsis* spp. cells were scraped from mats and macroalgal thalli, fixed in formalin at 4% and then processed for FISH-TSA analysis and PCR-based assay using the species-specific probes and primers for toxic *O. ovata* and *O. cf. siamensis* and validated on field populations of *Ostreopsis* spp.

*Ostreopsis* cells were isolated using a micropipette, and monospecific cultures were maintained in F/4 medium at 21 ± 1 °C with a 14:10 h (light:dark) photoperiod. Illumination was provided by fluorescent tubes with a photon irradiance of 100 mmol photons m⁻² s⁻¹.

**Fig. 1.** Epi-fluorescence microscopy: hybridized cells of *Ostreopsis siamensis* CNR-T5 (A) and *O. ovata* CBAL (B) with species-specific rRNA probes.
Ostreopsis spp. When the FISH-TSA method was applied to field samples from an Ostreopsis bloom the rRNA target probe for O. ovata properly hybridized to natural population cells (Fig. 2); no fluorescence signal was detected using the O. cf. siamensis-specific probe. The finding that monospecific blooms were dominated by the genotype O. ovata was also confirmed by the species-specific PCR amplification: PCR amplification reactions were positive only for the presence of O. ovata in all examined samples. Cell enumeration based on the FISH-TSA assay of cultured and field samples was compared with the traditional light microscopy counting method of formalin-fixed cells and the efficiency of positive detection was comparable for the two methods, whereas uncertainty regarding species-specific identification remained unresolved using traditional light microscopy.

To summarize, we have developed a specific and rapid FISH-TSA method using molecular rRNA-target probes for the identification and enumeration of two toxic species of Ostreopsis that are found along the Mediterranean coasts. The data shown are preliminary results of the molecular validation in field samples and it will necessary to test the sensitivity of the whole cell fluorescence assay on a discrete number of natural samples that may contain both species of Ostreopsis and different levels of cell abundance. The FISH-TSA method may be used as an alternative to the PCR-based assay for the detection and monitoring O. ovata and O. cf. siamensis. The method does not require a high degree of experience; further, the FISH-TSA method can confirm visually whether or not a fluorescent signal is specific to the targeted species by epifluorescence and light microscopy. Thus, with this whole cell hybridization assay information on species-specific cell identity and counts may be obtained during surveys; this information may be used together with environmental parameters to understand the bloom dynamics of these toxic Ostreopsis species.

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MIDTAL is a new FP7 project entitled Microarrays for the Detection of Toxic Algae. It started on Sep 1st, 2008, is funded by the Theme 6 Environment (including climate change) of the European Commission, and will continue for 45 months. Ten partners make up the consortium and include scientists from 7 European countries and the USA. Funding for the USA partner will be applied for through NSF or NO.AA. Partners include Marine Biological Association (co-ordinator), Stazione Zoologica Anton Dohn, University of Kalmar, Instituto Español de Oceanografía, Martin Ryan Institute, National University of Ireland, University of Oslo, University of Westminster, DHI, Instituto Tecnoloxico para o control do Medio Marino de Galicia, and University of Rhode Island. Some partners are exclusively involved in probe design and testing, whereas others are responsible for making calibration curves from established cultures and others are devoted to taking field samples for two years to validate the microarray.

Microalgae in marine and brackish waters of Europe regularly cause “harmful effects or blooms”, considered from the human perspective, in that they threaten public health and cause economic damage to fisheries and tourism. For adequate management of these phenomena, monitoring of microalgae is required. However, the effectiveness of monitoring programmes is limited by the fact that it is time consuming and morphology, as determined by light microscopy, may be insufficient to give definitive species and toxin attribution. In many countries, once cell numbers reach a threshold level, then shellfish are selected for toxin analysis by mouse bioassay. In others, both are mandatory before harvesting. The mouse bioassay is used to ensure toxins do not exceed control levels and is employed more frequently when potentially toxic cells have been identified as present. Molecular and biochemical methods are now available that offer rapid means of both species and toxin detection. MIDTAL will target rapid species identification using rRNA genes. These target rRNA genes include regions that are so variable that they are species or even strain specific. These regions can be targeted for probe design to recognize species or even strains. Antibody reactions to specific toxins produced by these microalgae are also included because harmful algal populations may be patchy and escape detection, and probes against the toxins may allow an early warning in shellfish. Microarrays are state of the art technology in molecular biology for the processing of bulk samples for detection of target RNA/DNA sequences. Existing rRNA probes and immunoassays for toxic algal species/strains and their toxins will be adapted and optimized for microarray use to strengthen the EU’s ability to monitor for toxin-producing microalgae. The purpose of MIDTAL is to support the common fisheries policy and to aid national monitoring agencies by providing new rapid tools for identification of toxic algae and their toxins, so that they can comply with EC directive 91/1491/CEE potentially reducing the need for the mouse bioassay.

The methodology MIDTAL plans to develop in order to support monitoring can be summarised as follows:

- Take a water sample, add a known amount of a *Dunaliella* culture as an internal control for extraction and hybridisation efficiency
- Extract total RNA and DNA
- Extract total toxins
- Apply RNA to microarray and determine the fluorescent signal of the hybridised probe to its target RNA. If insufficient RNA is available, then PCR products of the rRNA genes will be used as an alternative
- Apply toxins to microarray and determine the fluorescent signal of the hybridised probe to its target antibody.

- Extrapolate using novel algorithms to cell counts per litre using calibration curves
- Compare manual phytoplankton counts using Light Microscopy or FISH probes, where appropriate, with signals from microarray to validate monitoring effectiveness.

The more accurate counts (e.g. distinction between toxic and non-toxic strains) of species numbers that can be achieved, the less the need for the mouse bioassay to determine if toxic algae or toxins are present. Monitoring for toxic algae using molecular techniques will allow more samples to be analysed in a shorter time period and with greater accuracy, without the need to have personnel trained in taxonomy. It will offer a near real time analysis of the ecosystem, and offer an early warning system for all European countries with aquaculture and toxic algal problems. Mitigation actions can be implemented immediately rather than to wait 3–5 days for results to be returned from the national monitoring agencies using traditional methods. Instead, results can be available in 3–4 hours. Such a development, offering the prospect of handling more detailed field sampling than previously possible will also allow us to advance knowledge of the ecology of ‘harmful’ species.

We will follow a hierarchical probe approach to the choice of probes for our microarray. Below are listed the probes/species that we plan to have on our microarray. Some have already been adapted and tested on the microarray during the EU FP6 project FISH AND CHIPS, others are being developed as part of this project. At the end of the project, a workshop is planned to train other potential users in the use of the microarray.

At our start-up meeting (Feb 16-20, Bremerhaven, Germany), all participants received training in probe design using the ARB program,
### Objectives include:

1. to test and optimise existing rRNA probes for toxic species, and immunoassays for toxins, for their application to a microarray
2. to design and test the specificity of any new probe needed
3. to construct a universal microarray from the probes tested and optimised by all partners for the detection of harmful algae and their toxins
4. to provide national monitoring agencies with a rapid molecular tool to monitor toxic algae, to validate or replace traditional monitoring methods
5. to integrate European efforts to monitor coastal waters for toxic algae

Microarray hybridisation and analysis with a GenePix microarray reader and phylolochip analyser program for the analysis of hierarchical probes on a microarray. We standardised how all the calibration curves would be made, and how the field samples would be collected. For further information on the project, please visit our website: www.midtal.com.

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**Taxonomic level** | **Probe** | **Specific for** | **On array**
--- | --- | --- | ---
**Domain** | Euk 328, 1209 | all eukaryotes | +
**Class** | DinoB, DinoE12 | all dinoflagellates | +
 | Hetero01 | Heterokonta | +
 | PRIM 01, 02, 03 | Haptophyta, Prymnesiophyceae | +
**Clade** | Dinophy GL B-12, Dinophy FL G+6 | phytoautotrophic Dinophysis | |
 | CLADE1 | Chrysochromulina & Prymnesium | |
**Genus** | Dinophy FL A-5, Dinophy FL G-8 | all Dinophysis | |
 | PSNGENUS | all Pseudo-nitzschia ex delicatissima | +
 | ALEX | all Alexandrium | +
 | PLIMA/PAQ D | benthic Proorocentrum | |
 | PCH-1, PCH-3 | Pseudolettocionella spp | |
**Species** | AMIN D+1 | Alexandrium tamarense, North America | +
 | NAI, ATNA02 | | |
 | AOST01, AOST02 | A. ostenfeldii | +
 | CPOLY01 | Chrysochromulina polylepis | |
 | GBREVE | Karenia breve | |
 | GMIKJ01 | Karenia mikimotoi species complex | |
 | PLIMA, PLIMAB | Proorocentrum lima | |
 | PMICA02 | P. micans | |
 | PMINI H, PMINIC | P. minimum | |
 | PSNAUS A-8 | Pseudo-nitzschia australis | +
 | PSNMUL A+4 | P. multiseries | +
 | PSNPUN A-12 | P. pungens | +
 | Prymparv | Prymnesium parvum | |
 | Hetaka | Heterosigma akashiwo | |
 | Dacum B | D. acuminata | |
 | Dacut B | D. acuta | |
 | Dnory B | D. norvegica | |
 | Dinophy GL H-3 | D. acut. / D. norv. clade | |

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**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

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Ciguatera and some Caribbean customs

Caribbean fishermen boast of being able to detect when a fish is “ciguato” (contaminated with ciguatera toxins) by using folk methods, such as carrying out their own “bioassay” by feeding a piece of the fish to a cat, checking if the scales fall off a freshly caught fish, or if its eyes are bulky, that they vehemently claim to be appropriate tests. In San Andrés Island (Colombia), fishermen claim they can recognize the barracuda with ciguatoxins by the colour of its blood. They make a longitudinal cut along the spinal cord of freshly caught fish: if the blood is black, the animal is supposed to be toxic and is thrown back to the sea; if the blood has a normal reddish colour, it is safe to eat it. Obviously, none these folk methods are based on scientific evidence.

Recently I heard about a ciguatera episode that occurred in the Cuban world heritage city of Trinidad (21°05’–21°15’ N, 79°45’–80°07’ W; Fig. 1). A fisherman’s family ate a carangid (Caranx latus Agassiz, 1831), known in Cuba as “jurel”, and one hour later the parents and two children showed clear symptoms of intoxication that were diagnosed and treated as ciguatera. This event was not particularly newsworthy, since Ciguatera Fish Poisoning (CFP) is endemic in the Caribbean, even in Cuba, despite the efficient epidemiological control and fisheries regulations implemented by the Ministry of Fisheries (MIP) to avoid these outbreaks. In Cuba, MIP resolution 457/96 forbids the capture or landing of fish included in a list of potentially toxic fish (determined by species and size). The specimen eaten by the family of this story was a large fish, given as a present by a fisherman friend, so beyond the reach of the commercial regulations. In Puerto Rico, large carangids and barracudas have a bad reputation as carriers of CFP toxins, and their sale is forbidden.

Epidemiological records in Cuba show there were in the past two CFP outbreaks in the same region of the country associated with the ingestion of the same kind of fish, then misidentified as Caranx fallax. In an outbreak in 1986, 26 consumers got sick after eating “jurel” [1]. Young cats were used for the “bioassays”. This practise was abandoned in toxicity studies, but juvenile cats were used in others.

The carangid misidentified as Caranx fallax in the epidemiological study of Rojas-Valladares et al. [1] and elsewhere [2, 3] is Caranx latus, called “jurel común” or “Gallego” (“Galician jurel”) (Fig. 2). There are two more described species of the same genus, the “cibi amarillo” (Caranx bartholomaei) and the “tifiosa” or “tifiosa prieta” (Caranx lugubris), easily recognized by fishermen and also included in the list forbidden for sale. The “jiguaua” (Caranx hippos) and the “cibi carbonero” (Caranx ruber) are also in the list of potentially toxic species [4, 5]. Despite regulations and CFP is still a chronic problem in Cuba. Between 2001 and 2006, there were 570 reported cases of fish intoxication, 72% of which were due to CFP [6]. The picúa (barracuda, Sphyraena barracuda) and jurel (Caranx latus) were responsible for 43% and 16% of the cases respectively.

Within the Caribbean region, CFP is a major food safety problem with a large socio-economic impact; it also affects tourism. Countries such as Puerto Rico, United States and Cuba, have implemented regulations to forbid the capture and sale of species with a high risk of containing CFP toxins. Unfortunately in many other Caribbean countries, the lack of medical awareness and public education is responsible for a high number of hidden cases of CFP, the symptoms of which are confused with other kinds of seafood intoxication.

The IOC Regional Working Group ANCA (Algas Nocivas en el CAribe) is seriously concerned by the extent of CFP in their region, and for more than 10 years has been promoting the dissemination of basic knowledge on CFP, the fish species that cause it, the risky areas, the characteristic symptoms of CFP in the Caribbean region, and with a predominance of gastrointestinal disorders, which contrasts with the syndrome in the Pacific Region where neurotoxic symptoms predominate.

There is an urgent need for economic techniques to detect different toxins of the CFP complex (which includes palytoxins and DSP toxins in the same black box) in contaminated fish. Currently available techniques are expensive and difficult to implement. Public health campaigns to educate people are also needed to alleviate the impact of CFP in the Caribbean region.
First Coordination meeting of the IAEA–ARCAL Technical Cooperation Project to address impacts of HABs in the Caribbean region

From 27th to 30th of January 2009, the Government of Cuba through the Centro de Estudios Ambientales de Cienfuegos (CEAC) hosted in La Habana (Cuba) the first coordination meeting of the International Atomic Energy Agency (IAEA)–ARCAL Technical Cooperation project on “Designing and Implementing Systems for Early Warning and Evaluation of the Toxicity of Harmful Algal Blooms (HABs) in the Caribbean region. Applying Advanced Nuclear Techniques, Radioecotoxicological Evaluations and Bioassay” in the coastal zones of Chile, Colombia, Costa Rica, Cuba, Dominican Republic, El Salvador, Haiti, Honduras, Mexico, Nicaragua, Uruguay and Venezuela.

Reports on the socio-economic impacts of Harmful Algae Blooms in Caribbean countries are increasing in parallel with the increasing exploitation of their coastal areas (tourism, aquaculture). Accumulation of Paralytic Shellfish Poisoning (PSP) toxins in shellfish associated with blooms of Pyrodinium bahamense var compressum on the Pacific coast, and of Gymnodinium catenatum on both Caribbean and Pacific coasts, has caused hundreds of human intoxications and nearly a hundred fatal cases during the last two decades. Ongoing monitoring programmes are often restricted to control of shellfish exports, and do not guarantee the health safety of the vast majority of the population that makes its living from artisanal fisheries.

Ciguatera Fish Poisoning (CFP), caused by consumption of certain tropical fish, has been reported as the main source of human intoxication in the region and represents a serious problem for tourism, the fishing industry and public health in the countries concerned. Ciguatera is endemic in the Caribbean region. Several hundred cases are recorded each year, but many more are unreported due to a lack of medical awareness and public education. The limited knowledge in the region of the toxic algal species involved and the degree of HAB toxicity is aggravating the problem.

The objective of this 5-year project, managed by the IAEA Technical Cooperation Department in Vienna, with technical support of the IAEA Marine Environment Laboratories in Monaco, is the transfer of isotopic techniques for the early detection, quantification of PSP and CFP toxins, and for the reconstruction of HABs occurrence history. The methods concerned are the Receptor Binding Assay and the dating of sediment cores with recent and fossil cysts analysis. The information generated will contribute significantly to the mitigation of the negative socio-economic impacts of PSP and CFP in Latin America.

During the meeting, a workplan was established for the whole duration of the project based on the country reports presented and on the significant contributions of experts from the National Oceanic and Atmospheric Administration (Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC, US), from the Intergovernmental Oceanographic Commission (IOC–IEO Science and Communication Centre on Harmful Algae, Vigo, Spain) and from the Vice-Chair of the IOC–ANCA (Algas Nocivas en el Caribe) regional group. Reference Laboratories will be equipped and selected demonstration sites will serve for implementation criteria, and reference guide preparation, to increase the knowledge and skills of, the local human people.

The experience gained in a similar IAEA project in Chile coordinated by Prof. Benjamín Suárez-Isla, will be used for this technology transfer to other countries of the Caribbean and Latin America region, making this project a good example of South-South collaboration.

Coordination meeting with Dr. Guillermo García-Montero, President, IOC Sub-Commission for the Caribbean and Adjacent Regions (IOCARIBE) and Manuel Fernández Rondón, President, Agencia de Energía Nuclear y Tecnologías Avanzadas (AENTA, Cuba)

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The Agency is grateful for the support provided to its Marine Environment Laboratories by the Government of the Principality of Monaco.

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New toxic benthic dinoflagellates from south central Cuba

Coastal Cienfuegos Province in south-central Cuba (Fig. 1) is very diverse, with bays, lagoons, mangroves, coral reefs, and brackish rivers. The dry season lasts from November to April, and the rainy season from May to October. This province, like other regions in Cuba, is affected by ciguatera episodes [1]. Until 2005, there had only been one study of the taxonomy and ecology of potentially toxic dinoflagellates in Cienfuegos Bay, which is estuarine. This study revealed the presence of *Prorocentrum belizeanum* and *P. lima*, the latter being dominant [2, 3]; but the typical agents of ciguatera such as *Gambierdiscus toxicus* had not been recorded.

Following the passage of Hurricane Dennis through the region in July 2005, water quality studies were initiated in the eastern coastal region of the province, *Ostreopsis* sp. (9.3 x 10^7 cells L^-1) and *Gambierdiscus* sp. (2.3 x 10^7 cells L^-1) were detected in the water column in a shallow bay where macroalgae and seagrasses abound, in 2006. The presence of these species led to a preliminary study of benthic dinoflagellates in the bay, and to a seasonal study in 2007, using a mixture of the most abundant macroalgae in the area as substrate; these were the red algae *Ceramium nitens* and *Spyridia clavata*, followed by the green *Bryopsis plumosa* and *Caulerpa sertularioides*, and the brown *Padina* sp.

During the dry season, *Ostreopsis siamensis* (101.3 cells g^-1 wet weight) and *Gambierdiscus toxicus* (53.4 cells g^-1 wet weight) were the dominants. Other species present in lower numbers were *Prorocentrum lima* (9.2 cells g^-1 wet weight) and *Ostreopsis ovata* (4.6 cells g^-1 wet weight).

In the *O. siamensis* specimens, only large pores were present on the plates; this feature and the form of the girdle distinguish this species from *O. lenticularis*. The 2007 rainy season was one of the wettest in recent years, and seems to have affected the benthic vegetation in the bay, as witnessed by lower diversity and abundance of the macroalgae and an increase in suspended particles. After the rains, the structure of the benthic dinoflagellate community changed radically, and *Ostreopsis ovata* (203.11 cells g^-1 wet weight) and *Prorocentrum lima* (93.7 cells g^-1 wet weight) were the dominants; *Ostreopsis lenticularis* was present in lower numbers (6.2 cells g^-1 wet weight).

In July 2008 in the western part of the province, *Prorocentrum concavum* (150.5 cells g^-1 wet weight) was dominant, together with *Prorocentrum emarginatum* (8.2 cells g^-1 wet weight) on *Padina* sp, in a rocky biotope influenced by waves, with browsing sea urchins and molluscs such as the cigua (*Cittarium pica*). On the same date in Cienfuegos Bay, *Prorocentrum mexicanum* (3.1 x 10^7 cells g^-1 wet weight) was recorded on the filamentous brown algae *Feldmannia irregularis* and *Hincksia mitchelliae*; these macroalgae bloom at the end of the dry season (April), and disappear when the heavy rains begin. Other dinoflagellates found at the end of the dry season in 2008 were *Ostreopsis ovata*, *Prorocentrum concavum*, *Ostreopsis lenticularis* and *Gambierdiscus toxicus*, all new records, the last two in the canal which links the bay to the Caribbean.

With the exception of *Prorocentrum lima*, the species recorded during these surveys are new records for the south central part of the island. These are the first records of *Ostreopsis siamensis*, *O. ovata* and *Prorocentrum emarginatum* in Cuba. The presence of this wide variety of toxic benthic species may be linked to outbreaks of ciguatera in the region, and refute the belief that the south of Cuba is unaffected by this syndrome.

Despite the need to augment these data, the results of this survey combined with epidemiological studies [4] provide evidence of a high incidence of ciguatera in the south central part of the island during the dry season (November–April), in contrast to the north where most episodes occur during the rainy season (May–October) [5]. Everything seems to indicate the existence of more estuarine conditions in the south, where the main river basins occur, and affect the stability of benthic communities during the rainy season. But due to climate changes occurring worldwide, there is now great variability in the frequency and intensity of the rainy periods. For example, the last years of the 20th century and the first four years of this one were a period of extreme drought in Cuba, while since 2005 several tropical storms and hurricanes have caused severe flooding in the island.

One feature which needs to be taken into account in future studies of ciguatera is the state of conservation of the coral reefs. Recent studies on the coast of La Habana, the densely populated capital of the country, have shown that the zones most affected by ciguatera coincide with the zones where the reefs are most deteriorated [6], a result of human activities. This deterioration also seems to be increasing as a result of the increased frequency...
First report of *Protoperidinium* bloom from India waters

An extensive bloom of *Protoperidinium* was observed on 8th October 2008 during an RV *Sagar Sampada* cruise (Ministry of Earth Sciences, Govt. of India) along the west coast of India. The bloom occupied an area of about 3.2 nm circumference off Mangalore (12° 51.83’N & 74° 20.00’E); it was not monospecific, but a *Protoperidinium* species was dominant, with up to 5x10^8 cells L⁻¹. Species such as *Protoperidinium oceanicum*, *Ceratium furca*, *C. fusus*, *C. trichoceros*, *Prorocentrum gracile* were abundant. This is the first report of a *Protoperidinium* bloom from Indian waters. The *Protoperidinium* is non-photosynthetic, so although the cell count was very high, the chlorophyll a concentration was (0.9 mg m⁻³) low. At the time of the bloom, water temperature was 29°C, salinity 34.64 psu, pH 8.34, and dissolved oxygen concentration 6.53 ml L⁻¹. Nitrate was below the detectable range while concentrations of phosphate and silicate were low (0.245 and 1.68 µm L⁻¹, respectively).

During the *Sagar Sampada* cruise, 53 stations were occupied on 10 transects (within 07°01.83 and 16°59.97 N latitudes) along the west coast of India. Algal blooms were observed at three stations. Besides the *Protoperidinium* bloom, a bloom of *Noctiluca miliaris* was observed off Goa at two successive stations (15° 30.50’N & 73° 36.82’E; 15° 30.28’N & 73° 40.30’E) with 2x10^4 cells L⁻¹. Surface discoloration of surface water was not conspicuous but cells appeared as loose patches. No fish kills or other mortalities were recorded in the bloom area.

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Acknowledgements
To Beatriz Reguera (Centro Oceanográfico de Vigo, Spain) and staff of the IOC–IEO Science and Communication Centre.

References:

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More than 60 marine experts from around the world attended FerryBox 08 on 29 and 30 September – a two day conference at the National Oceanography Centre, Southampton UK co-hosted by Chelsea Technologies Group. The subject of the conference is one of the fastest growing areas of marine science – where sophisticated equipment is fitted to cargo and passenger ships to provide continuous monitoring of the world’s oceans to improve our understanding of climate change.

Shipping companies are collaborating with oceanographers to measure what is happening in our oceans, seas and lakes on a day-to-day basis. Such information is essential if we are to understand and quantify both the causes and effects of our Earth’s changing climate. Innovative sensor systems installed on the ships collect the data and a satellite communications system sends the numbers ashore for assessment. The oceanographic community is enthusiastic about expanding this interaction with the shipping industry and the shipping community is equally keen to find ways to partner.

Richard Burt (Chelsea Technologies Group) said, ‘Chelsea was delighted to co-host the event as we have been supplying real time oceanographic monitoring systems for both military and commercial vessels for many years, and have built up a broad understanding of the design, installation and operation of such systems’. This knowledge has been further advanced through involvement in the European Union ‘FerryBox’ programme which led to the development of the Chelsea AquaLine FerryBox System. At the heart of the AquaLine System are a suite of sensors, proven in some of the harshest environments in the world, a passenger display system and automatic data transfer to a shore facility.

FerryBox 08 heard from users worldwide including Akira Harahishma (National Institute for Environmental Studies, Japan) about studies in the Seto Inland Sea and Herman Ridderinkhof (Royal Netherlands Institute for Sea Research), on how the shortest route to Texel has changed understanding of sediment transport into the Wadden Sea. Ute Schuster (UEA) presented the North Atlantic study by the EU’s Science Framework 6 project CarboOcean on the marine carbon cycle, and the International Ocean Carbon Coordination Project.

David Mills (CEFAS) led discussion on further development of the FerryBox concept as part of integrated observing systems, and described the new European Marine Ecosystem Observatory (Emeco) initiative. Emeco will support and make measurements over decades at coastal and regional scales to quantify variability in physical, chemical and biological properties.

The Swire Group Charitable Trust recently pledged over £300,000 to the National Oceanography Centre, Southampton to support ocean monitoring with sensors fixed to a cargo ship. With sensors on Swire’s MV Pacific Celebes, NOC scientists have been able to capture data about remote areas where ocean-atmosphere interactions are largely unknown. Dr David Hydes (NOCS Ferrybox programme) commented “the North Atlantic plays a major role in controlling the amount of carbon dioxide taken up by the oceans each year... we now have the ability to monitor how the uptake of carbon dioxide changes from year to year, with a precision that can be used to quantify the results of reduced emissions of carbon dioxide when these are achieved.”

David was pleased to report that the EuroGOOS Office in cooperation with SMHI, Sweden will be hosting next FerryBox Meeting in March 2010.

For more details contact: E.Keegan, Chelsea Technologies Group, Email: ekeegan@chelsea.co.uk.

D. Hydes, National Oceanography Centre, Southampton. Email: djh@noc.soton.ac.uk
HAB trail blazers: “Max” FJR Taylor

Education

Max was born in 1939 in Cairo, Egypt, only son of a South African-born officer serving in the Royal Air Force and an Australian mother. His parents dragged him around the world, from Dar es Salaam, Tanzania to Northwood, Middlesex and Leuchars, Scotland and then back to South Africa. Max received most of his education in a village near Sydney, Australia. He did a double major in Zoology and Botany and was awarded his PhD from the University of Cape Town in 1965. His thesis was a contribution to the International Indian Ocean Expedition and dealt with phytoplankton communities in the SW Indian Ocean, including the Agulhas Current. His thesis was rather large - as he put it, “he didn’t have time to write a shorter one!”

Honours

2000 Yasumoto Lifetime Achievement Award by 9th Int Conf Harmful Algal Blooms (Hobart); 1995 Palaeontological Society Golden Trilobite Award; 1997 elected Fellow of the Royal Society of Canada; three teaching awards while at University of British Columbia; etymology of Alexandrium taylori, Amphisolenia taylori, Blepharocysta taylori, Gonyaulax taylori, Strombidiniopsis taylori.

Professional Career

“Max” FJR Taylor joined the faculty of the University of British Columbia at the end of 1964 at the age of 24, having been offered a job even before his PhD thesis was formally approved. He was promoted to full Professor at the age of 35. Max joined the UBC Institute of Oceanography, then headed by George Pickard.

Key contributions

At UBC Max continued work for the Indian Ocean Expedition, using material collected by the R.V. “Anton Bruun”, from a much wider area encompassing the Bay of Bengal, Andaman Sea, Arabian Sea and the whole western Indian Ocean. This material formed the basis for his beautifully illustrated monumental 1976 Indian Ocean Dinoflagellate Atlas. This work also included many of the earliest scanning micrographs of dinoflagellates. Max’s students primarily explored the phytoplankton ecology of the coastal waters of British Columbia while he continued to develop his specialty: the ecology of red tides. Max has been doing research in this field longer than anyone else, having published his first paper on a mass mortality in False Bay, near Cape Town, in Nature in 1962. Shortly after arriving in British Columbia in 1965 he will get them to check the accuracy of what we write about them and seek their permission to publish their portrait photographs. The idea is to initially put up these stories on our web-site but by the time we have written up 40-50 people, we will consider publishing them in an ISSHA booklet, perhaps embellished by HAB conference summaries. We may even want to consider in future a collection of HAB cartoons or HAB poetry! Let me know if you are willing to contribute to this initiative. I hope you will find reading these stories on the lives of our HAB heroes as stimulating as I am enjoying collating them.

Gustaaaf Hallegraeff, School of Plant Science, University of Tasmania, Australia. Email: Hallegraeff@utas.edu.au.

From the ISSHA Secretary: Dear present and future ISSHA members

As newly elected secretary of ISSHA I would like to introduce myself and the on-going work on the construction of a new interactive ISSHA web-site. I am currently a professor in Limnology at Lund University, in southern Sweden. Although I am a freshwater ecologist by training, I work with both limnetic and marine systems. My research focus has been on dinoflagellate life cycles, allelopathic interactions, and lately, genetic diversity in microalgal populations. Since December 2008, I have been in charge of leading the work to create a modern web-site for ISSHA. It is our strong belief, that an attractive website is central to the functioning and development of our organization. The new web-site will include the following features: quick on-line membership applications and membership payments through PayPal, a member discussion forum, an events calendar, possibility for members to upload articles/reports, etc.

Karin Rengefors. Email: Karin.Rengefors@limnol.lu.se
investigated with Anand Prakash, a case of human death due to paralytic shellfish poisoning (PSP). It was the first such case anywhere in which the causative dinoflagellate was caught still at the scene. Dinoflagellates always held Max’s closest attention, and he subsequently gracefully withdrew in favour of the resurrection of the older name Alexandrium. Along his diverse career path, Max worked on cryptomonad endosymbionts in Mesodinium, the feeding mechanism in Protoperidinium, the motility of the dinoflagellate transverse flagellum, the causative dinoflagellate of ciguatera, Mesodinium, and bloom prediction of the fish-killing Heterosigma carterae (=akashiwo). Max always had a strong on-going interest in cell evolution. In 1974 he formalized the Serial Endosymbiosis Theory that in eukaryotic cells the mitochondria and chloroplasts had a symbiotic origin from bacteria. These were old independent proposals revitalised and combined by Lynn Margulis in the 1970s, but highly unpopular at the time even though they made complete sense to Max, who published further papers as evidence accumulated. It was largely this body of work which earned him a Fellowship in the Royal Society of Canada in 1997. Max still mourns the lack of interest that greeted what he believes was his most significant discovery which resulted from a sabbatical at Oxford University in 1986-87. Although the codons (the triplet bases that determine which amino acids will be incorporated in proteins) were claimed by Nobel prize winner Francis Crick to be randomly assigned but unchanging, Max and David Coates found that it is the most highly ordered data set they ever encountered.

Max is an intensely social person and an extraordinary raconteur with an anecdotal memory for details. These skills put him centre stage at numerous international conferences, whether as the co-founder in 1975 of the International Society for Evolutionary Protistology, the Founding President of ISSHA in 1998 or conferences on Fossil Dinoflagellates, and he produced numerous insightful state-of-the-art conference overviews.

**Notable students**

Alan Cembella, Greg Gaines, Paul Falkowski, Juan Saldarriaga.

**10 Key publications**


Based partly on: SCOR Newsletter 15, May 18, 2005; EOS Alumni newsletter 8 (2005); portrait by G.Hallegraeff.
Saxitoxins in Argentinian inland waters

Saxitoxins from cyanobacterial algae were detected in the River Salado, Chaco Province (Argentina) in summer 2006. This is the first report of saxitoxin production in a freshwater environment in Argentina. River Salado is an alternative drinking water source for Castelli, a town with 36,000 inhabitants located at 25°56’S y 60°37’W. Seven sampling stations were placed along 14km of the river (Fig. 1). The river had a maxima depth of 2m, and several closures along its course, stopping water flow.

This study was carried out from late 2005 to 2006, during a long drought. Water samples after the drought period were analyzed during January and February 2007.

Physical, chemical and biological parameters were analyzed according to the Standard Methods for the Examination of Water and Wastewater [1]. Mouse bioassays were made at the Laboratory of Legal Chemistry of the National University of La Plata (Argentina), and toxin analysis by HPLC at the Federal University of Rio Grande (Brazil) on samples from February 2006.

Potentially toxic algae were abundant, causing troubles in drinking water treatment. The average species concentrations were: *Raphidiopsis curvata* 1.83 x 10^6 cell mL^{-1}, *Raphidiopsis mediterranea* 1.49 x 10^6 cell mL^{-1}, *Planktothrix agardhii* 0.49 x 10^6 cell mL^{-1}, and *Cylindrospermopsis raciborskii* 0.20 x 10^6 cell mL^{-1}. The highest algae abundance was detected between northern closure and Mereles, quickly decreasing at Parra Bridge. In November 2005, dominance of both *Raphidiopsis mediterranea* and *Raphidiopsis curvata* were detected, and represented 42-56% and 14-18% of biomass, respectively. Total phytoplankton reached 15.0 x 10^6 cell mL^{-1} (Fig. 2).

Biomass averages showed that *R. mediterranea* decreased from Pampa Argentina to Native Colony and increased from Native Colony to Parra Bridge. *R. curvata* behaved in inverse manner, with the highest value of 92% of total biomass at Native Colony (Fig. 3). The maximum algal concentration was 6.64 x 10^6 cell mL^{-1}.

During late 2005 and 2006 total rain was less than 200 mm. Water samples taken in November 2005 showed high levels of pH, turbidity, nitrates and chlorophyll-a. During early 2007 total rain reached 250 mm. Water samples taken in February and March showed improved water quality compared with the previous period, decreased nutrients, algal biomass and other parameters (Table 1).

Mouse bioassays showed the possible presence of paralytic shellfish poison (saxitoxins). Toxin analysis by HPLC from raw water confirmed the presence of saxitoxin (STX), decarbamyl saxitoxin (dcSTX), neosaxitoxin (NeoSTX) and gonyautoxins 1 and 5 (GTX1 and GTX5), reaching a total of 105.33 µg L^{-1} STX-eq. (Table 2). The guideline value of this toxin in drinking water is 3 µg L^{-1} [2, 3]. No other toxins were detected. The water had 1.84 x 10^6 cell mL^{-1} of *Raphidiopsis mediterranea*, 0.59 x 10^6 cell mL^{-1} of *Planktothrix agardhii*, 0.53 x 10^6 cell mL^{-1} of *Raphidiopsis curvata*, and 0.42 x 10^6 cell mL^{-1} of *Cylindrospermopsis raciborskii*.

It is known that *Raphidiopsis mediterranea* can produce the neurotoxic alkaloids anatoxin-a and homoanatoxin-a [4], and that *Raphidiopsis curvata* and *Cylindrospermopsis raciborskii* can generate the hepatotoxic alkaloids cylindrospermopsin and deoxycylindrospermopsin [5]. *C. raciborskii* can also produce saxitoxins [6]. *Planktothrix agardhii* can produce hepatotoxic peptid microcytins and the alkaloid anatoxin-a [7].

Saxitoxins were the only toxins detected, and were present at high concentrations. This could belong together with the presence of *Cylindrospermopsis raciborskii* that would be the responsible for the presence of saxitoxins, just as it happens in Brazil [8, 9].

River Salado under drought conditions behaves as a lake, with a closure each end of the sector studied. The reduction of water flow together with high temperatures, lack of rains and sufficient nutrients, generated ideal conditions for optimal growth of algae.

Under these conditions, toxins removal must be assured, to do not overcome the guide values for drinkable water.

Acknowledgements

I thank Dr. D. Andrino (National University of La Plata, Argentina) for his work for mouse bioassays, and Dr. J. Sarkis Yunes (Federal University of Rio Grande, Brazil) for toxin quantification.

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9th Session of the IOC Intergovernmental Panel on HABs

The Ninth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB) was held at UNESCO Headquarters, Paris, 22–24 April 2009. The Session was attended by representatives from: Brazil, Canada, Chile, Cook Islands, Croatia, Denmark, Greece, Italy, Malaysia, Mexico, Morocco, Namibia, New Zealand, Oman, Peru, Slovenia, Spain, Sweden, Thailand, Tunisia, United Kingdom, United States of America, Uruguay, IAEA, PICES, UNEP, and the International Society for the Study of Harmful Algae (ISSHA).

The purpose of the Panel is to develop and implement by IOC and partners during 2007–2009. The Panel noted with concern the reduced financial support for the IOC HAB programme, and stressed that the IOC HAB Programme will only be able to develop and be implemented in 2010-2011 if there is additional extra-budgetary support from Member States to fund programme staff and activities. The major achievements reported include: (i) the development of the integrated IPHAB–IODE Harmful Algae Information System; (ii) developments within GEOHAB including the launch of the GEOHAB Research Plans for the Core Research Projects in Stratified Systems and the development of a plan for GEOHAB Asia; (iii) development of the regional activities ANCA, FANS, HANA; (iv) the implementation of twelve training courses and training-through-research projects; (v) the continued publication of the IOC Harmful Algae News; (vi) results from the ICES-IQC WGHABD; (vii) the IOC co-sponsorship of international HAB conferences; and (viii) provision of HAB literature to developing countries.

The Panel decided on a work plan and budget for 2010–2011 which has its main focus on regional HAB Programme development, capacity building, GEOHAB, the development of the Harmful Algal Information System, Task Teams on ‘Biotoxin Monitoring, Management and Regulations’, ‘Algal Taxonomy’ and ‘HAB Monitoring within the Global Ocean Observing System’. The Panel also adopted guiding principles for capacity enhancement with respect to mitigating the effects of harmful algae, a draft strategy for IPHAB, and a focus for activities on the transfer and introduction of HAB species by human activity such as shipping (ballast water). The Panel made recommendations to assist the IOC Ocean Science Section in the development of a plan for integrated coastal research. Dr. Leonardo Guzman (Chile) was re-elected as Chair and Phil Busby (New Zealand) was re-elected as Vice-Chair.

More details: www.ioc.unesco.org/ hab under Documents, IPHAB-IX

Future events

JUNE 2009

IOC/WESTPAC Workshop on Marine Invasive Species and Management in the Western Pacific Region

This will be held at Chulalongkorn University, Bangkok, Thailand, 4–5 June 2009. Presentations will cover the regional status of marine invasive species, their impacts, data quality, methods to confirm occurrence of bioinvasion; transport mechanisms of invasive species; factors that facilitate their establishment, and countermeasures. A group discussion will refine WESTPAC-BIODIVERSITY projects, work plans and research priorities, publish a current status report, and other actions related to invasive species in the WESTPAC region.

For further information contact Dr. S. Chavanich, Chulalongkorn Univ. at: suchana.c@chula.ac.th.

Pacific NW AOAC Meeting

This will be held June 17–18, 2009, at the Univ. of Puget Sound, Tacoma, WA, USA, with seminars and presentations on phycotoxins, and dietary supplements; and training sessions on method validation (AOAC). We now have poster presentations including student participation.

Workshops are listed at www.aoacpcnw.com, along with registration forms and more AOAC meeting details.

For additional information contact J. Hungerford, Chair of AOAC Task Force: James.Hungerford@fda.hhs.gov

OCTOBER-NOVEMBER 2009

A special course, Taxonomy of Recent Dinophyceae, will be held from 26 Oct–05 Nov 2009 at the Wattenmeerstation Sylt of the Alfred Wegener Institut, in List/Sylt, Germany. It is restricted to 10 participants to assure individual attention. Welcome are all people who already have basic knowledge of dinoflagellates.

The programme includes an introduction to the group and its nomenclature, new taxa described since 2000, microscopical work on participants, an introduction to benthic and coccal Dinophyceae, and to aberrant forms.

Accommodation will be provided in the station guest-house. No funding is available from the German side.

To register, please contact M. Elbracht (melbraechter@awi-bremerhaven.de) by 15 August 2009

Harmful Algae News

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