GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

OPEN SCIENCE MEETING
on the
CORE RESEARCH PROJECT: HABS IN BENTHIC SYSTEMS

Hilton Waikiki Prince Kuhio
Honolulu, Hawaii, USA
21-23 June 2010

International Council for Science
SCOR
Scientific Committee on Oceanic Research

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

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21-23 JUNE 2010

PROGRAMME AND ABSTRACTS

The GEOHAB Scientific Steering Committee (SSC) is grateful for the support for this meeting supplied by the Intergovernmental Oceanographic Commission (IOC), Scientific Committee on Oceanic Research (SCOR), and U.S. National Science Foundation (Division of Ocean Sciences).

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ORGANIZATION OF THE OPEN SCIENCE MEETING

Convener
Paul Bienfang, USA

Planning Committee
Mireille Chinain, French Polynesia
Jacqueline Evans, Cook Islands
Jacob Larsen, Denmark
Dominique Laurent, France
Patricia Tester, USA
Adriana Zingone, Italy

Assistance with Meeting Preparation
Lora Carter, SCOR
Henrik Envoldsen, IOC
Elizabeth Gross, SCOR
Ed Urban, SCOR

AN INTRODUCTION TO THE MEETING

Welcome to the GEOHAB Open Science Meeting on HABs in Benthic Systems.

The GEOHAB Programme, sponsored by the Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission (IOC) of UNESCO, is an international programme aimed at fostering and promoting co-operative research directed toward improving the prediction of harmful algal bloom events.

Core Research Project: HABs in Benthic Systems
The GEOHAB Implementation Plan describes plans for Open Science Meetings designed to stimulate international input to focused research projects. This Open Science Meeting is the fifth in the series.

The GEOHAB Core Research Project on Harmful Algal Blooms (HABs) in Benthic Systems must be comparative, interdisciplinary, and international. It will directly address the goal of GEOHAB of improved prediction of HABs by determining the ecological and oceanographic mechanisms underlying their population dynamics, integrating biological, chemical, and physical studies supported by enhanced observation and modelling techniques. The overall objective is to understand and quantify the critical processes underlying benthic HAB population and community dynamics.

The purpose of this meeting is to obtain community input for the development of a detailed research plan for the GEOHAB Core Research Project on HABs in Benthic Systems. The first draft of this plan will be brought together on Thursday, 24 June, by the OSM Planning Committee. The plan will be emailed to meeting participants for comment and will be augmented as additional planning occurs.

The GEOHAB SSC and OSM Planning Committee thank you for your participation in developing and implementing GEOHAB research.

Raphael Kudela
GEOHAB SSC Chair

Paul Bienfang
Convenor

ABOUT THIS BOOK

We hope you will find this book to be helpful, both as a reference during the Open Science Meeting and afterward. The list of participants includes all those who completed registration for the meeting before this book went to print on 14 June 2010. Similarly, the abstracts, both for speakers and the poster sessions, reflect the status of the program for the conference on that date. Changes to the program will be announced and posted at the conference and you are advised to look for these.

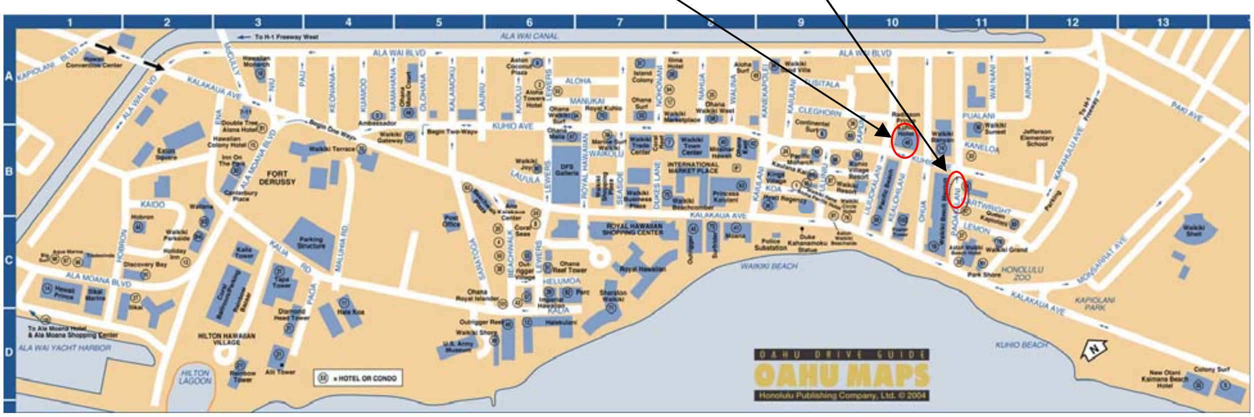
LOGISTICAL INFORMATION

Transportation

From Honolulu Airport to Waikiki Hotels:
The least expensive option to reach your hotel from the airport is to take a shuttle bus, such as . Ilima Airport Shuttle (800-713-0146). It should cost around US$15 roundtrip. Taxis are quicker, but more expensive.

Hotels

The meeting will be held in the Hilton Waikiki/Prince Kuhio, at 2500 Kuhio Ave. Many participants are staying in the Ocean Resort Hotel Waikiki, which is located about 0.2 miles from meeting hotel, at 175 Paoakalani Ave. Shuttles or taxis from the airport should go to any of the Waikiki hotels.



(From http://www.waikiki.us/waikikimap.html)

Meals

Continental breakfasts, lunches, and breaks are included in the registration fee. A group dinner will be held at the end of the meeting, on June 23; other dinners will be on your own. There are many dining options in the Waikiki area.

PROGRAMME SUMMARY

Monday 21 June	Tuesday 22 June	Wednesday 23 June	Thursday 24 June
Registration Poster Set-up 8:00 – 9:00 a.m. Continental Breakfast	Continental Breakfast	Continental Breakfast	Planning Committee Meets in Closed Session to Write Meeting Report
9:00 a.m. Plenary Session	9:00 a.m. Plenary Session	9:00 a.m. Pleanary Session	
10:30 a.m. COFFEE	10:30 a.m. COFFEE	10:30 a.m. COFFEE	
11:00 a.m. Plenary Session	11:00 a.m. Plenary Session	11:00 a.m. Plenary Session	
1:15 p.m. LUNCH	12:30 p.m. LUNCH	12:30 p.m. LUNCH	
2:15 p.m. Breakout Discussion Sessions	1:30 p.m. Breakout Discussion Groups	1:30 p.m. Plenary Session	
3:30 p.m. COFFEE	4:00 p.m. COFFEE	3:00 p.m. COFFEE	
4:00 p.m. Poster Session	4:30 p.m. Breakout Dicussion Groups	3:30 p.m. Concluding Plenary Session	
		6:00 p.m. Group Dinner	

DETAILED PROGRAMME

Notes for Participants

Talks: Invited speakers will be expected to adhere to the allocated times for their talks. The chairs of the plenary sessions will be strict about the timing, in order to keep the conference running smoothly.

Posters: Posters can be set up from 8:00 to 9:00 a.m. on Monday, 21 June. Supplies will be provided for mounting the posters. Posters will be left up for the entire meeting to make it possible for participants to view them during coffee breaks and lunch times. Posters should be taken down in the afternoon of Wedmesday, 23 June.

Program

June 21 (Monday) (Hawaii 1 and 2)
Session Chair: Mona Hoppenrath, Senckenberg Research Institute, Wilhelmshaven, Germany
(30 minute presentations and 15 minutes for questions and answers)

8:00 a.m.	Poster Set up and Continental Breakfast
9:00 a.m.	Introduction to GEOHAB – Raphe Kudela, University of California at Santa Cruz
9:30 a.m.	Introduction to Open Science Meeting – Paul Bienfang, Convener, University of Hawaii
9:45 a.m.	*Gambierdiscus* taxonomy --morphology & DNA methods – Wayne Litaker, NOAA Beaufort Lab, USA
10:30 a.m.	Break
11:00 a.m.	Morphological Identification and Taxonomy of Potentially Toxic Benthic Dinoflagellates – Katarina Aligizaki, Aristotle University of Thessaloniki, Greece
11:45 a.m.	Genetic Diversity of Three Harmful Benthic Genera of *Ostreopsis*, *Coolia* and *Prorocentrum* – Antonella Penna, University of Urbino, Italy
12:30 p.m.	Sampling Methodology for Benthic Dinoflagellates – Patricia A. Tester and Steve Kibler – NOAA Beaufort Lab, USA
1:15 p.m.	Lunch (Kauai Room)
2:15 p.m.	Breakout Session

1. Review of current national and regional projects/programs to identify elements of research that could contribute to core research & identification of interested participants and designated regions for comparative research - Pacific/Asia Region (Hawaii I)
   Discussion Leader: TBD
   Rapporteur: TBD

2. Review of current national and regional projects/programs to identify elements of research that could contribute to core research & identification of interested participants and designated regions for comparative research - Mediterranean and Caribbean Seas (Hawaii II)
   Discussion Leader: TBD
   Rapporteur: TBD

3:30 p.m. Break

4:00 p.m. Poster Session (Hawaii 1 and 2)

6:00 p.m. Adjourn for the Day

June 22 (Tuesday)
Session Chair: Pat Tester, National Oceanic and Atmospheric Administration

8:00 a.m. Continental Breakfast

9:00 a.m. A simple model capable of simulating the population dynamics of *Gambierdiscus*, the benthic dinoflagellate responsible for ciguatera fish poisoning – Michael Parsons, Florida Gulf Coast University

9:45 a.m. A Review of the Ecological Factors Driving Biogeography of *Gambierdiscus* Species – Lesley Rhodes, Cawthron Institute, New Zealand

10:30 a.m. Break

11:00 p.m. Overview of the Distribution of *Ostreopsis* and Benthic *Prorocentrum* Species Around the World Seas – Adriana Zingone, Stazione Zoologica Anton Dohrn, Italy

11:45 p.m. Reports Back from Monday Breakout Sessions

12:30 p.m. Lunch

1:30 p.m. Breakout Sessions
1. Research priorities and approaches for *Gambierdiscus* (Hawaii I)
Discussion Leader: Lesley Rhodes
Rapporteur: Jacqui Evans

2. Research priorities and approaches for *Ostreopsis*, *Prorocentrum* and *Coolia* (Hawaii II)
   Discussion Leader: TBD
   Rapporteur: Adriana Zingone

4:00 p.m. Break

4:30 p.m. Resume Breakout Sessions

6:00 p.m. Adjourn for the Day

June 23 (Wednesday)
Session Chair: Jacqui Evans, Te Ipukarea Society Inc.

8:00 a.m. Continental Breakfast

9:00 a.m. Ecophysiology of *Gambierdiscus* **–** Steve Morton, NOAA Charleston Lab, USA

9:45 a.m. Foodweb effects and/or controls of *Gambierdiscus* (i.e., top-down-bottom-up controls, grazing controls, foodweb toxicity, etc.) **–** Brian Lapointe, Harbor Branch Oceanographic Institution, USA

10:30 a.m. Break

11:00 a.m. Ecophysiology of *Ostreopsis & Prorocentrum* species (i.e., adaptive strategies, physiological characteristics, life cycle traits, temperature preferences & tolerances, growth rates) – Lam Nguyen, Institute of Oceanography, Vietnam

11:45 a.m. What is Different in Benthic vs. Planktic HABs? – Santiago Fraga, Francisco Rodríguez, Isabel Bravo, and Manuel Zapata, Instituto Español de Oceanografía, Spain

12:30 p.m. Lunch

1:30 p.m. Report Back from Breakout Sessions

The ciguatera fish poisoning “plague” in the Pacific Island Countries & Territories, 1999 to 2008: results of a recent questionnaire. – Mark Skinner, University of Queensland

1-slide presentations of priority research topics by any participant

Identification of cross-cutting research issues and new topics following break-out group reports

3:00 p.m. Break

3:30 p.m. Next Steps and Timeline

4:00 p.m. Adjourn

6:00 p.m. Meeting Dinner at Tiki's Bar and Grill (2570 Kalakaua Ave.)

June 24 (Thursday)
Writing Session - Planning Committee only (University of Hawaii)

GAMBIERDISCUS TAXONOMY - MORPHOLOGY AND DNA METHODS

Litaker, R. Wayne, Mark W. Vandersea, Steven R. Kibler, Mireille Chinain, William C. Holland, Michael J. Holmes, Maria A. Faust and Patricia A. Tester

Gambierdiscus species identification is important because there is a 100 to 250 fold difference in toxicity among species according to literature reports. In contrast, the within species toxicity, varies only 2-9 fold. This suggests species composition is a major determinant of ciguatera fish poisoning (CFP) events. Accurate identification using light microscopy, however, is difficult even though *Gambierdiscus* cells are relatively large. A review of the major morphological characteristics, such as the size and shape of the cells and various plates such as the 2', 4'' and, 1P, will be presented to illustrate the challenges associated with species identification. In most cases, unambiguous identification requires some type of molecular confirmation. There is a close concordance between the distinct genetic clusters found in SSU, ITS and LSU rDNA phylogenies and the currently described species. To date, phylogenetic analyses indicate the existence of at least 12 different *Gamberidiscus* species, ten of which have been formally described based on morphological criteria. It is likely that additional species will be identified. By noting where the source materials used in the phylogenetic analyses originated, it is possible to develop global distribution maps for *Gambierdiscus* species. Our data indicate five species are endemic to the Atlantic, five are endemic to the tropical Pacific, and that two species, *G. carpenteri* and *G. caribaeus* are globally distributed. Future efforts will be directed at documenting the relative toxicity of the various species, and in understanding their relative abundance and habitat preferences in an effort to better predict CFP events.

[1]NOS/NOAA, 101 Pivers Island Road, Beaufort, North Carolina 28516, USA. (Wayne.Litaker@noaa.gov)
[2]Laboratoire Des Micro-Algues Toxiques, Institut Louis Malardé, BP 30 98713 Papeete, Tahiti
[3]Tropical Marine Science Institute, 14 Kent Ridge Road, National University of Singapore, Singapore 119223
[4]Department of Botany, Smithsonian Institution, 4210 Silver Hill Road, Suitland, Maryland, 20746, USA

MORPHOLOGICAL IDENTIFICATION AND TAXONOMY OF POTENTIALLY TOXIC BENTHIC DINOFLAGELLATES

Aligizaki, Katerina[1]

Accurate taxonomy is the prerequisite for every ecological and biogeographical study especially in HAB species. Morphological identification of potentially toxic benthic dinoflagellates constitutes a difficult task, as in almost every genus there are specific ambiguities regarding the reliable morphological criteria that should be used.

The lack of compliance among more recent SEM and older LM descriptions, as in the case of some *Prorocentrum* and *Ostreopsis* taxa, or the variability of morphotypes included to a species, seem to have hampered exact taxonomy; *Prorocentrum lima* morphotypes exhibit cell shapes from oblong to round, with round to kidney-shaped valve pores, leading to the characterization as "*P. lima* complex" until the taxonomy of the species/genus is clarified. The erroneous synonymy in some cases (*P. rhathymum* vs. *P. mexicanum*) or the erection of too many species (*P. concavum* vs. *P. arabianum*) in some others seems to be also related to the perpetuation of the taxonomical confusion in the genus *Prorocentrum*.

Species description only from field samples, with no capability for observations repeatability or molecular and toxicity analyses may have also resulted in taxonomical inconsistencies; some *Ostreopsis* species have not been detected following their original description, while a characteristic (and currently clarified) example is the case of *Gambierdiscus toxicus* description that actually included more than one species. The use of culture material certainly eliminates such problems and gives the possibility for manifold approaches (life-cycle, molecular & toxicity analyses); however, shape distortion in culture conditions, which has been observed for several *Ostreopsis* strains, may cause remarkable difficulties.

[1] Department of Botany, School of Biology, Faculty of Sciences, Aristotle University, 54124 Thessaloniki, GREECE (aligiza@bio.auth.gr)

GENETIC DIVERSITY OF THREE HARMFUL BENTHIC GENERA OF *OSTREOPSIS, COOLIA* AND *PROROCENTRUM*

Penna, Antonella[1]

The molecular approaches permitted to explore the genetic complexity within each genus. *Ostreopsis* spp. shows a high level of morphological variability and most of the *Ostreopsis* morphotypes described were not sequenced for the genotype assignment. Few identified genetic species, as *O.* cf. *ovata*, *O.* cf. *siamensis*, *O. lenticularis* and *O. labens*, were analyzed based on the phylogeny and nucleotide diversity at inter- and intra- species level especially at the Mediterranean area. In the *O.* cf. *ovata* different genetic lineages correlated with macrogeographical distribution are present; they are represented by the Mediterranean/Atlantic and Indo-Pacific clades. *O.* cf. *ovata* is found to be widely dispersed, while the other species turn out restricted to just one of the two main warm-water oceanic basins. Molecular phylogenetic, morphological and other phenotipical parameters are strongly suggested to be applied together for the reconstruction of the taxonomy of this genus. Few species of *Coolia* are genetically identified, as *C. monotis*, *C. canariensis* and *C. malayense*, and it is still remaining to clarify the phylogenetic position of not sequenced *C. tropicalis* and *C. areolata,* as well as other underscribed strains worldwide. In the phylogenetic analyses of Prorocentrales, a controversy at genus level arises on consisting in the polyphyletic or monophyletic origin of the *Prorocentrum* based on different target genes, outgroups and number of taxa included in the phylogenetic analyses; further, unresolved grouping of planktonic and benthic *Prorocentrum* species need for additional investigation to determine whether these lineages should be assigned to two separate genera and to solve ambiguities within assigned taxonomic taxa.

[1]Dep. of Biomolecular Sciences, University of Urbino, 61121 Pesaro (PU), Italy (antonella.penna@uniurb.it)

SAMPLING METHODOLOGY FOR BENTHIC DINOFLAGELLATES

Tester, P.A.[1], Kibler, S.R.[2], Vandersea, M.W., Holland, W.C. and R.W. Litaker

Benthic dinoflagellates in the genera *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* are generally associated with a variety of benthic substrates. Quantifying their abundance presents methodological challenges, not the least of which includes relatively low cell abundance and a high degree of spatial/temporal variability. Historically, the most common method for quantifying the abundance of benthic dinoflagellates is by enumerating cells associated with benthic macrophytes, where cell abundance is reported as cells g^{-1} wet weight macrophyte. Data collected in this manner is difficult to normalize because of large variability in surface area among different macrophytes and limited distribution of particular macrophytes in different tropical habitats. We review the historical sampling methodologies and advocate the use of an artificial substrate (fiberglass screen). This method is inexpensive, easily replicated, non-destructive and a reliable substitute for benthic macrophytes. We have used the screen method to examine distribution and abundance of benthic dinoflagellates in a number of different tropical systems in the Caribbean region. Field testing of this method showed a significant correlation between abundance of benthic dinoflagellates on screens and those on adjacent macrophytes for *Gambierdiscus* ($r = 0.82$), *Ostreopsis* ($r = 0.82$) and *Prorocentrum* ($r = 0.86$). Cell abundance data from screens had an equal or smaller coefficient of variation than did comparable data from macrophytes.

[1]Pat.Tester@noaa.gov
[2]Steve.Kibler@noaa.gov

A SIMPLE MODEL CAPABLE OF SIMULATING THE POPULATION DYNAMICS OF *GAMBIERDISCUS*, THE BENTHIC DINOFLAGELLATE RESPONSIBLE FOR CIGUATERA FISH POISONING

Michael L. Parsons, Chelsie J. Settlemier, and Paul K. Bienfang

Ciguatera fish poisoning (CFP) is the most prevalent form of phycotoxin-borne seafood poisoning in the world, afflicting tens of thousands of individuals annually. Efforts to monitor for and limit exposure to CFP are hindered by the spatially and temporally sporadic nature of CFP outbreaks. This presentation describes a simple, one-dimensional model that was developed using physico-chemical parameters to simulate the population dynamics of the causative organism behind CFP events, the benthic dinoflagellate, *Gambierdiscus* spp. The data used for this model includes *Gambierdiscus* cell density and associated nutrient and physico-chemical data that were collected monthly over 3.5 years at two windward and two leeward sites on the island of Hawaii. Data from the windward sites were used to construct and calibrate the model, which was then verified against actual *Gambierdiscus* cell density values that were collected at the leeward sites. Additional modeling scenarios indicated that bloom scenarios resulted from subtle (yet complementary) changes in environmental conditions, and that nutrient enrichment and warming sea surface temperatures stimulated *Gambierdiscus* growth and resulted in higher cell densities. The results from this proof-of-concept effort to simulate the population densities of this benthic dinoflagellate suggests promise for more elaborate modeling efforts, and their potential value to protect against human impacts from future CFP outbreaks.

A REVIEW OF THE ECOLOGICAL FACTORS DRIVING BIOGEOGRAPHY OF *GAMBIERDISCUS* SPECIES

Rhodes, Lesley

Global mapping of the genus *Gambierdiscus*, both temporal and spatial, is a challenging exercise. The genus continues to expand its geographic range, probably due to both increased research and to an increased presence. The expansion may well be due to both natural and anthropogenic changes. There are many and varied drivers for its establishment in a new area, including natural events (for example, cyclones and earthquakes leading to tsunamis), anthropogenic occurrences (e.g. dredging, building of boat ramps, etc.), and altered nutrient and trace metal inputs into coastal waters. Pinpointing a particular driver is fraught with difficulties as *Gambierdiscus* can proliferate on a range of substrates, within relatively wide temperature and salinity ranges, and its distribution is often linked more closely to substrate disturbance than to season and climate. Reports of ciguatoxin poisoning are increasing in some areas. For example, in the Cook Islands serious ciguatera illnesses have been reported in local communities, and an increased number of tourists returning to New Zealand have exhibited symptoms on arrival home. This could be due to a greater recognition of the illness and to better record keeping, but it could equally be due to more toxic species blooming and entering the food web. The potential causes for the apparent spread of *Gambierdiscus* globally will be reviewed.

Cawthron Institute, Nelson, New Zealand (lesley.rhodes@cawthron.org.nz)

OVERVIEW OF THE DISTRIBUTION OF *OSTREOSPIS* AND BENTHIC *PROROCENTRUM* SPECIES AROUND THE WORLD SEAS

Zingone, Adriana

The first benthic *Prorocentrum* species, *P. lima*, was described from the Mediterranean Sea. However, most records of benthic *Prorocentrum* and *Ostreopsis* species were initially restricted to tropical and subtropical seas. In the last decade, increasing attention has been paid to potentially toxic benthic dinoflagellates, due to a higher frequency of harmful events associated with these species in temperate areas. *Ostreopsis* species have been recently found at high concentrations (up to 10^6cells g^{-1} fw of macroalga) in New Zealand, Brazil and the Mediterranean Sea. Most of the time *Ostreopsis* species are found along rocky shores, where they grow either as epiphytic on macroalgae or directly on the rocks or in the sand. The colonization of these substrates appears to be favored by the production of mucous layers, although high abundance in absence of mucous layers is also recorded. Peaks of *Ostreopsis* density are generally associated with the warm season, but a relationship with high temperatures is not always verified. A relationship with high nutrient concentrations is also not supported by the available data. Several *Prorocentrum* species show either an epiphytic or a benthic habit, but they are also found frequently in the water column. The most widespread and abundant species, *P. lima*, reaches concentrations of 10^5 cells g^{-1} fw of macroalga. After the first toxic episodes that drew more attention to the benthic environment, records of *Ostreopsis* and *Prorocentrum* species have increased, indicating that these species may have a wide distribution in coastal areas around the world.

Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy (zingone@szn.it)

ECOPHYSIOLOGY OF GAMBIERDISCUS

Morton, Steve L.[1]

Gambierdiscus is a slow growing non-planktonic dinoflagellates usually associated with macroalgae in subtropical and tropical locations. In culture, *Gambierdiscus* produces copious amount of mucus which allows this species to attach to macrophytes and other substrates. Growth rates of this dinoflagllate are generally less than 0.5 divisions/day. *Gambierdiscus* produces toxins throughout its entire growth phase. To date there has been no reports of sexual reproduction and/or cyst formation in this species. Early reports of "lumpy" or abnormal cells were an artifact of culture conditions. Toxin production appears to originate from this genus and not bacteria since xenic and axenic cultures produce similar profiles. In *Gambierdiscus* and other benthic dinoflagllates, toxin production within a given strain grown under the same acclimated conditions has been shown by a number of studies to be a stable trait. Factors governing toxin production and growth in *Gambierdiscus* have been shown to vary with the environmental factors of light intensity, light quality, temperature, salinity, algal extracts, nutrient ratios and nutrient form. Studies have shown the manipulation of environmental variables can result in the almost complete suppression of toxin production even in the most toxic strains. These previous studies will be reviewed and put in perspective because of the advances in toxin identification utilizing mass spectroscopy and the newly described species within this genus.

[1]NOAA/NOS/NCCOS, Marine Biotoxins Program, 331 Fort Johnson Rd., Charleston, SC 29412

BENTHIC MACROALGAE AS SUBSTRATA FOR *GAMBIERDISCUS*: BOTTOM-UP AND TOP-DOWN CONTROLS

Brian Lapointe[1], Brad Bedford, and Phil McGillivary

Gambierdiscus is a benthic dinoflagellate that has been associated with a wide variety of tropical benthic macroalgae. Previous studies have suggested that this association provides important benefits to *Gambierdiscus*, which include protection from herbivory (top-down controls) and increased availability of dissolved nutrients (bottom-up controls). High cell densities of *Gambierdiscus* reported in the literature have occurred on red, green and brown macroalgae (*Chaetomorpha*, *Dictyota*, *Jania*, *Acanthophora*, *Heterosiphonia*, *Spyridia*) that are generally palatable and consumed by herbivores. Many of these filamentous and/or branched genera grow abundantly in nutrient-enriched tropical reef environments, such as protected back-reef habitats, and have high surface areas for epiphytic growth of *Gambierdiscus*. These observations support the hypothesis that benthic macroalgae provide nutrient-enriched microhabitats that enhance growth of *Gambierdiscus.* Recent studies showing increased toxicity of *Gambierdiscus* with elevated N:P ratios helps explain ciguatera outbreaks in offshore, low-lying, carbonate-rich environments where high N:P ratios occur. In addition, land-based runoff from limestone islands can increase N:P ratios, contributing to greater toxicity of *Gambierdiscus* following storm events. Different dynamics of macroalgae and ciguatera toxicity could occur on high volcanic islands due to geologic differences that decrease N:P ratios and nutrient limitation. Considering current models of global climate change, ocean acidification and nutrient enrichment, conditions favoring *Gambierdiscus* growth and toxicity could increase or decrease in the future, depending upon the complexities and characteristics of different environments.

[1]Harbor Branch Oceanographic Institute at Florida Atlantic University, 5600 US 1 North, Ft. Pierce, FL 34946; belapointe@gmail.com

ECOPHYSIOLOGY OF *OSTREOPSIS & PROROCENTRUM* SPECIES

Nguyen-Ngoc, Lam[1], The Ho-Van[1], and Jacob Larsen[2]

Species of *Ostreopsis* and *Prorocentrum* do not in general tolerate low salinity or low temperature. Thus, optimal growth of *O. siamensis* has been shown to be at temperatures of 14-25ºC and salinities of 30-45 psu. At 10ºC, it produces a vegetative resting cyst. Preferred substrates of *Ostreopsis* spp. seem to be macroalgae, e.g. species of *Amphiroa*, *Dictyota, Galaxaura,* and *Padina*,; but with very erratic population dynamics perhaps related to physical parameters such as wave action. Experiments indicate that the macroalgae may produce exudates which favour the growth of the benthic dinoflagllates. Observations from Vietnamse coastal indicate that some species of macroalgae have much denser populations of benthic dinoflagelates than others.

Among *Prorocentrum* species, *P. lima* appears to be cosmopolitan in temperate-tropical waters. It has been shown to have optimal growth, together with *P. concavum* and *P. P. mexicanum* (*P. rhathymum*?), at 25-30^{o}C; and at 30 psu for *P. concavum*, and *ca.* 28 psu for *P. lima* and *P. mexicanum*, but both *P. lima* and *P. mexicanum* may grow at temperatures as low as 5ºC.

[1] Institute of Oceanography, Cau Da 01, Vinh Nguyen, Nha Trang City, Viet Nam
[2] IOC Science and Communication Centre on Harmful Algae, University of Copenhagen
Øster Farimagsgade 2D, DK-1353, Copenhagen K, Denmark

WHAT IS DIFFERENT IN BENTHIC vs. PLANKTIC HABS?

Fraga, Santiago [1], Francisco Rodríguez [1], Isabel Bravo [1] and Manuel Zapata [2]

Algal blooms dynamics depend on the balance between gains and losses of cells. If gains, due to replication or advection of cells are higher than losses from grazing, mortality and dispersion, a bloom will occur. While these terms have been well studied for phytoplankton, this is not the case for benthic microalgae like *Ostreopsis* and *Prorocentrum*. Phytoplankton communities are associated to a particular water body that can deplete nutrients limiting their growth, but benthic dinoflagellates are associated with a substrate where water is changing continuously and where they have a limited role in its nutrient dynamics. Most benthic dinoflagellates live in shallow shores and need an adaptation to this light regime. Light curves obtained by PAM fluorescence on benthic dinoflagellates include characteristics more typical of shade-adapted organisms that can be reflected in the pigment composition showing a high peridinin:chlorophyll c_2 ratio compared to planktic dinoflagellates. While plankton is grazed mainly by filter feeders like copepods or tunicates, benthic dinoflagellates are grazed by different groups like herbivorous fishes, gastropods or amphipods. Mucilage production, in addition of being an attachment mechanism to the sediment avoiding losses by dispersion, it can reduce grazing pressure as well.

The role of parasites is being increasingly recognized in planktic species but they also affect benthic species and may play an important role ending blooms. Although benthic dinoflagellate toxins are present in the foodweb, the role that toxins may play to reduce grazing pressure has to be evaluated. Encystment could also relax grazing pressure under adverse conditions.

[1] Centro Oceanográfico de Vigo, IEO (Instituto Español de Oceanografía), Subida a Radio Faro 50, 36390 Vigo, Spain. (santi.fraga@vi.ieo.es)
[2] Instituto de Investigacións Mariñas de Vigo, CSIC, Eduardo Cabello 6, 36208 Vigo, Spain.

THE CIGUATERA FISH POISONING "PLAGUE" IN THE PACIFIC ISLAND COUNTRIES & TERRITORIES, 1999 TO 2008: RESULTS OF A RECENT QUESTIONNAIRE.

Skinner, Mark P.[1], Tom Brewer[2], Ron Johnstone[3], Glen Shaw[4] and Richard J. Lewis[5]

Ciguatera fish poisoning (CFP) occurs throughout the tropics. People that consume contaminated fish may exhibit a number of both short and long term debilitating gastrointestinal and neurological symptoms. Such debilitation can place increased burden on coastal communities and national health services. Unfortunately, the true extent of illness and impact on human communities and ecosystem health is still poorly understood. A questionnaire was sent by email to the Health and Fisheries authorities of the Pacific Island Countries and Territories (PICTS) in order to obtain the latest records of CFP for the Oceanica region. The following countries participated: Cook Islands, Fiji, French Polynesia, Guam, State of Hawaii, Kiribati, Marshall Islands, Nauru, New Caledonia, Niue, Northern Mariana Islands, Palau, Samoa, Tokelau, Tonga, Tuvalu, Vanuatu and Wallis & Futuna. The reported cases (33,284) from these 17 PICTs showed that the mean annual incidence of ciguatera across the region for the 11 years from 1998 to 2008, was 229 people per 100,000 population. This proportion is more than twice the reported annual incidence of ciguatera for the 11 years from 1973 to 1983, which was 97/100,000. If the official reported CFP represents 20 percent of actual incidence (conservative estimate for food-borne illnesses) then the actual number of cases for this period could be as high as 166,420. We estimate that, since 1973, approximately half a million PICT inhabitants have had CFP. Given the current population of the region surveyed, not including the State of Hawaii, is approximately 2,084,000, this means that approximately one in every four persons, in the Oceanica region, has had the disease. This level of incidence exceeds many definitions of a plague as used for other diseases. CFP has largely been ignored by PICT national governments and international agencies. This new data emphasise that ciguatera remains a poorly managed disease, despite research advances in our understanding of detection, treatment and the culprit organisms, genera *Gambierdiscus* and other microalgae. Adequate, urgent action is required to address this important health problem that is expected to grow in significance in parallel with environmental degradation. Multidisciplinary research teams, effectively funded through an international agency, is needed to translate research advances into practical management solutions.

[1]Entox (National Research Centre for Environmental Toxicology), University of Queensland, 39 Kessels Road, Coopers Plains, QLD, 4108, Australia
(mark_skinner59@yahoo.com.au)

[2]ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD. 4811;
[3]Coastal Ecosystems & Resource Management, School of Geography, Planning & Environmental Management and Centre for Marine Studies, University of Queensland, St.Lucia, QLD, 4113;
[4]School of Public Health, Griffith University, Meadowbrook, QLD, 4131;
[5]Institute of Molecular Bioscience, University of Queensland, St. Lucia, QLD, 4720, Australia.

POSTER ABSTRACTS

FIRST REPORT OF CIGUATERA-ASSOCIATED DINOFLAGELLATES IN KUWAIT'S MARINE ENVIRONMENT

Al-Yamani, Faiza [1] and Maria Saburova [2]

The spatial and temporal abundance of dinoflagellates associated with ciguatera was studied in the northwestern part of Arabian (Persian) Gulf along Kuwait's shore during 2008-2009. Six potentially toxic dinoflagellate species were isolated from selected macroalgae (Phaeophyta) and intertidal sediments: *Prorocentrum concavum*, *P. emarginatum*, *P. lima*, *P. rhathymum*, *Coolia monotis*, and *Ostreopsis ovata*; four of them are new reports for Kuwait's marine environment.

P. lima and *P. rhathymum* were previously observed in Kuwait's waters; in the summer of 1999, a bloom of *P. rhathymum* (reported as *P. mexicanum*) caused a massive fish kill in Kuwait.

All the species of *Prorocentrum* and *O. ovata* were associated mainly with floating *Sargassum* sp. as well as were found sporadically in adjacent sediments in low numbers. *C. monotis* was found as associated with attached to hard substrate *Padina* sp. Bloom of *C. monotis* was recorded in May 2009 on the coast south of Kuwait Bay. The dinoflagellate epiphyte assemblage on macroalgae was sometimes accompanied by other potentially toxic species such as *Amphidinium carterae* and *A. operculatum*.

[1]Kuwait Institute for Scientific Research, P.O. Box 1638, 22017 Salmiya, Kuwait
[2]Institute of Biology of the Southern Seas, Pr. Nakhimova, 2, 99011, Sevastopol, Ukraine (msaburova@gmail.com)

DIVERSITY OF BENTHIC DINOFLAGELLATES IN SOUTH–BRITTANY (NW FRANCE) WITH EMPHASIS ON THE GENUS *PROROCENTRUM*

Chomérat, Nicolas[1] and Elisabeth Nézan[1]

In tropical areas, benthic dinoflagellates have been well studied since they are often associated with toxin production and are identified as causative agents of ciguatera. However, although their presence is known for a long time in temperate areas, they are comparatively little studied. A taxonomical study of benthic thecate taxa has been undertaken in South Brittany, Northwestern France, using scanning electron microscopy and when possible, a molecular approach to ensure the identification. It revealed a high diversity and several new taxa have been described in the genera *Cabra*, *Prorocentrum* and *Sinophysis*. *Prorocentrum* is the most abundant genus among sand-dwelling dinoflagellates and eight species have been identified in the samples. Among them, the most abundant species posseses the characters of *P. tsawwassenense* that has been recently described from South Canada. Some other species were not identified as existing taxa and were thus described. The phylogenetic analysis of *P. consutum*, one of the recently described species, showed that this species groups together with species with a symmetric shape which comprise mainly tropical and okadaic-acid producing species. Thus, the toxin production by *P. consutum* in temperate area remains a question and this risk has not been assessed yet.

[1]Ifremer LER FBN, 13 rue de Kérose, 29187 Concarneau Cedex (FRANCE)
(nicolas.chomerat@ifremer.fr)

BENTHIC HABS ALONG THE MIDDLE TYRRHENIAN SEA (MEDITERRANEAN SEA, ITALY): MICROCOSM EXPERIMENTS TO STUDY *OSTREOPSIS* (DINOFLAGELLATES) OUTBREAKS

Congestri, Roberta

The requirement of intervention for benthic Harmful Algal Blooms (HABs) by public officers and scientists has increased alarmingly in Italian coastal areas since 1998 when blooms of the toxic dinoflagellate *Ostreopsis* were first recorded in Tuscany (Middle Tyrrhenian Sea). More recently benthic HAB impacts on marine ecosystem and human intoxication have been serious in Italy.

In the framework of a national research program aimed to improve the knowledge of *Ostreopsis* HABs in Italy, a laboratory microcosm approach was used to grow microphytobenthic communities, sampled during bloom incidents, under controlled conditions. HABs occur in nature as thick mucilaginous biofilms covering both biotic and abiotic substrates during summer along the middle Tyrrhenian Sea coast. Within the biofilm, *Ostreopsis* is associated with other toxic dinoflagellates along with a variety of benthic diatoms, mostly pennates, and filamentous cyanobacteria, all embedded in a common mucilage matrix (ExoPolymeric Substances, EPS).

A flow lane incubator prototype, designed to study microphytobenthic growth on artificial substrata, was used to perform experiments at 25°C, 110 µmol photons m^{-2} s^{-1} and 50 L h^{-1} flow velocity of K/2 medium. Three biofilm inocula were washed off bryozoans and macroalgae collected at two stations in the Lazio region in July and August 2009. Up to $15x10^6$ *Ostreopsis* cells were present in the inocula. Qualitative data, from a variety of microscopical techniques and quantitative results on cell composition and matrix EPS will be presented in order to enhance understanding of *Ostreopsis* bloom formation, development and eventual dispersion in relation to environmental controls and substrata.

Department of Biology, University of Rome "Tor Vergata", Via della Ricerca Scientifica 1, 00173 Rome, Italy (roberta.congestri@uniroma2.it)

COOLIA, RECENT TAXONOMY AND BIOLOGY CONSIDERATION

Doan Nhu Hai, Ho Van The, and Nguyen Ngoc Lam

Dinoflagellate genus *Coolia* has been introduced since it first type description in 1919 with *Coolia monotis* Mieuner 1919. The genus now comprises of 5 species with 4 species are newly described within the last two decades. Most species are identical with morphology and identifiable with fluorescent microscopy. However, recent studies reveal a need to carefully examination of morphology and taxonomy of two species *C. monotis* and *C. malayanse* and toxin profile of *C. monotis*. Previously, biological features of *C. monotis* have been well described including life cycles and growth rate under different salinity, temperature and light intensity. This present study includes results of experiments on autecology and toxicity of *C. tropicalis* isolated from Vietnamese waters. *C. tropicalis* growth bester at temperature between 26-29 ^{o}C and salinity at 30-35 ‰, the highest growth rate is 0.38 d^{-1} at 26 ^{o}C, 30 ‰ and light intensity of 3600 lux. *Coolia tropicalis* isolated from Vietnamese coastal waters shows highly toxic to mice.

Department of Marine Plankton, Institute of Oceanography, 01 Cau Da, Vinh Nguyen, Nha Trang, Viet Nam (habsea@dng.vnn.vn)

PROJECT "EBITOX"

Franco, José M.[1], Santiago Fraga [2], Francisco Rodríguez [2], Isabel Bravo [2], Pilar Riobó[1], Magda Vila [3], M. Montserrat Sala [3] and Emiliano Cacho [4]

Blooms of *Ostreopsis* in the Mediterranean Sea are being related to respiratory problems and/or allergic reactions in some coastal areas in summer and they pose also a potential risk of food poisoning. Ciguatera was registered in the Canary Islands (NE Atlantic Ocean) for the first time in 2004, the same year that *Gambierdiscus* was detected and isolated from those waters. As a consequence, a research project started in 2009 to study morphological, genetic, toxicological and environmental aspects of these genera. The main objectives are: 1) To characterize morphologically and genetically different strains of *Ostreopsis* and *Gambierdiscus* from the Mediterranean Sea and from Canary Islands. 2) To describe temporal and spatial distributions of both genera and dynamics of associated epiphytic communities. 3) To progress in the identification of environmental conditions which determine the proliferation of target species. 4) To describe life cycles of the two genera and to identify how they overwinter. 5) To determine toxin profiles of strains cultured in the laboratory and to compare them with field samples. 6) To determine if toxins produced by *Ostreopsis* are present in aerosols in the sea shore. 7) To characterize the toxic potency of different toxins produced by selected cultured strains, to use them in toxicological studies. 8) To detect the potential presence of toxins in mollusks and fish. 9) To describe the benthic dinoflagellates community associated to toxic species in the target areas. This project is coordinated with others dealing with chemistry and biosynthesis of bioactive polyiketides from benthic dinoflagellates, toxicology and engineering for producing bioactive substances.

[1] Instituto de Investigacións Mariñas de Vigo, CSIC, Vigo, Spain.
[2] Centro Oceanográfico de Vigo, IEO (Instituto Español de Oceanografía), Vigo, Spain. (santi.fraga@vi.ieo.es)
[3] Institut de Ciències del Mar, CSIC, Barcelona, Spain.
[4] Dependencia del Area de Sanidad en Vigo, Spain.

CENTRE OF EXCELLENCE FOR DINOPHYTE TAXONOMY (CEDiT) AT THE GERMAN CENTRE FOR MARINE BIODIVERSITY RESEARCH (DZMB)

Hoppenrath, M. and Elbrächter M.

Living dinoflagellates are the focus of diverse research topics. They contribute significantly to the biodiversity of marine ecosystems as primary producers and secondary consumers. Many toxic or harmful flagellates in the plankton are dinoflagellates. The taxonomic knowledge of living dinoflagellates (comprising about 2500 species) is scanty and restricted to few specialists worldwide. Nevertheless, many new dinoflagellate taxa have been described over the last decades. Any taxonomic work starts with the original descriptions of the taxa that are often scattered in various European journals from the nineteenth century – not available to many scientists.

The centre will provide a complete check-list of all described living dinoflagellate genera and species with exact bibliographic details online. In addition, we intend to provide all original descriptions electronically. Next to this core information we want to make available information of general interest for the dinoflagellate research community, e.g. important links, literature lists and images. Type material can be deposited in the centre. In the future one main task will be to archive reference material from cultures or field samples of dinoflagellate blooms. A library is in the stage of development. The centre was founded in 2005 and visible online since 2008: http://www.dinophyta.org/ .

Forschungsinstitut Senckenberg, DZMB, Wilhelmshaven, Germany,
mhoppenrath@senckenberg.de

FIVE TROPICAL BENTHIC DINOFLAGELLATES NEW TO THE EGYPTIAN MEDITERRANEAN WATERS

Ismael, Amany A. and Y. Halim

A survey of benthic microalgae was carried out along Alexandria coastal waters (June 2005 to December 2007) following the mass mortality of a bottom feeding fish, *Siganus rivulatus*. Although the mortality of fish occurred due to the proliferation of *Oscillatoria acutissima*, five tropical benthic dinoflagellates were reported for the first time in the Egyptian Mediterranean waters; *Coolia monotis*, *Ostreopsis* sp., *Ostreopsis ovata*, *Prorocentrum lima* and *Gambierdiscus* spp.

The statistical analysis between the standing crop of the epiphytic harmful microalgae and environmental parameters showed a significant correlation between the total standing crop and temperature. The five species increased in abundance with high temperature. The maximum standing crop of *Ostreopsis* sp. was recorded during summer at temperature 28.7˚C. The other species were recorded in low abundance not exceeding 4 $x10^2$ cell fwg^{-1}.

The epiphytic harmful microalgae to host association were investigated. The dinoflagellate species showed a preference for some macroalgal species. Generally, preferences were not consistent among the harmful species, indicating that it is a matter of epiphyte-specific requirements. The increase in abundance of *Coolia monotis* and *Prorocentrum lima* and the heavy benthic blooms of *Ostreopsis* sp and *O. ovata* are statistically associated with brown and red algae (*Hypnea*, *Laurencia*, *Jania*, *Corallina* and *Sargassum*) at p=0.002 but absent or insignificant elsewhere where *Ulva* sp. is dominant. *Gambierdiscus* spp. statistically did not exhibit a preference for any of the host species tested. As a result *Coolia monotis*, *Ostreopsis* sp. and *Ostreopsis ovata* are restricted to the eastern side of Alexandria where phaeophyta and rhodophyta are more diversified and abundant most of the year. While *Prorocentrum lima* occurred along Alexandria coast both on macroalgal species and sediment.

Although there is no harmful effect associated with the five species, their increase in abundance may form a threat in the near future.

Oceanography Dept., Faculty of Science, Alexandria University, Alexandria 21511, Egypt (amany_3@yahoo.com)

DIVERSITY OF BENTHIC DINOFLAGELLATES FROM PAPUA NEW GUINEA

Kohli, Gurjeet Singh[1], Shauna A. Murray[2] and Brett A. Neilan[3]

The objective of this study was to conduct the first ever diversity survey of benthic dinoflagellates in the coral reefs of Papua New Guinea, using a molecular clone library approach. Samples were collected from three different locations i.e. 1) Lion Island, Port Moresby, 2) Lolata Island, Port Moresby and 3) Tavali, Alotau, Milne Bay, Papua New Guinea. As dinoflagellates are known to live epibenthically with numerous macroalgae, specimens of *Amphiroa* sp., *Turbinaria* sp., *Sargassum* sp., *Halimeda* sp. and *Padina* sp. were collected and their associated dinoflagellate populations were investigated. Dinoflagellate identification was achieved through DNA barcoding of a 0.45 kb region of the mitochondrial cytochrome *b* (*cob*) gene. Clone libraries were constructed and DNA sequences obtained for phylogenetic analysis. Phylogenetic analysis revealed the presence of *Amphidinium* spp, *Prorocentrum* spp, and other *Gonyaulacalean* spp, which were confirmed by microscopic examination. Additionally, microscopic analysis revealed the presence of *Ostreopsis* spp as well. This is the first study to apply the *cob* barcoding technique to identify dinoflagellate lineages in benthic environmental samples, and shows that this is a promising tool for rapid identification of potentially toxic dinoflagellates.

[1]School of Biotechnology & Biomolecular Sciences, University Of New south Wales, Sydney, NSW 2052, Australia (gurjeet@unsw.edu.au)
[2]School of Biotechnology & Biomolecular Sciences, University Of New south Wales, Sydney, NSW 2052, Australia (s.murray@unsw.edu.au)
[3]School of Biotechnology & Biomolecular Sciences, University Of New south Wales, Sydney, NSW 2052, Australia (b.neilan@unsw.edu.au)

SPATIAL AND TEMPORAL VARIATIONS OF PLANKTONIC AND EPIBENTHIC CELLS OF *OSTREOPSIS* CF. *OVATA* IN NW MEDITERREAN (LIGURIAN SEA)

Lemée, Rodolphe [1], Thierry Thibaut[2], Stéphanie Cohu[1&2], Aurélie Blanfuné[1&2] and Luisa Mangialajo[2]

This work was done within the framework of the research program MediOs 2 (Mediteranean *Ostreopsis*). The goal of this national French project was to acquire and analyze pertinent scientific knowledge on *Ostreopsis* occurrence in areas as diverse as ecology, biology, chemistry, epidemiology or socio-economic issues in the Mediterranean Sea, in order to provide a decision-making support for administrative action.

Variability in concentrations of planktonic and epibenthic cells of the toxic dinoflagellates *Ostreopsis* cf. *ovata* was studied in 2008 and 2009 in NW Mediterranean Sea (from Cassis, France to Genoa, Italy). Different spatial and temporal scales were analyzed, from meter to hundreds of km and from hours to years.

Spatial patterns indicated that temperature, nutrients and local hydrodynamics could be linked to the severity and the length of a bloom in a specific area, even if great variances were observed, not always clearly relatable to biological substrate characteristics or depth.

Temporal patterns showed the occurrence of a large first bloom in June, July or August, according the area and a second less important bloom can occur in autumn. A high-frequency sampling indicated a daily rhythm, with higher quantities of benthic cells in the morning and higher concentrations of planktonic cells before night.

[1] Laboratoire d'Océanographie de Villefranche, CNRS UMR 7093, Université Pierre et Marie Curie, 06234 Villefranche-sur-mer, France (lemee@obs-vlfr.fr).
[2] Université de Nice-Sophia Antipolis, Ecology of Coastal Marine Ecosystems and Responses to Stress, EA 4228, Parc Valrose, 06108 Nice Cedex 2, France.

INFLUENCE OF TEMPERATURE ON GROWTH PARAMETERS OF *OSTREOPSIS OVATA, PROROCENTRUM* CF. *MACULOSUM, COOLIA MONOTIS* AND *AMPHIDINIUM* SP. STRAINS FROM RIO DE JANEIRO, BRAZIL

Nascimento, Silvia M.[1], Eliliane V. Corrêa[2]

Epi-benthic dinoflagellate species are common along the Rio de Janeiro coastline where *O. ovata* blooms are associated with periods of warmer temperatures. In order to study some aspects of cell physiology, laboratory cultures were established from strains locally isolated. Cells were kept in L2 medium (or L2/2 for *O. ovata*), 60 µmol photon flux m^{-2} s^{-1} irradiance and 12h light:12 h dark cycles. The growth rate, doubling time, culture yield and chlorophyll-a content of four dinoflagellate species, *Ostreopsis ovata*, *Prorocentrum* cf. *maculosum*, *Coolia monotis* and *Amphidinium* sp. were determined under three temperatures, 16, 20 and 24 ^{o}C to evaluate the influence of temperature on growth parameters. *O. ovata* was cultivated under 16, 20 and 26 ^{o}C. The Tukey-Kramer test (GraphPad InStat) was used to test differences between treatments. Growth rates of all species were significantly lower at 16 ^{o}C and *O. ovata* cultures at this temperature showed lower chlorophyll-a values and chlorosis aspect. Culture yield were also significantly lower at 16 ^{o}C, except for *C. monotis*. However, *Ostreopsis ovata* and *Amphidinium* sp. growth rates at 20 ^{o}C & 26 ^{o}C and at 20 ^{o}C & 24 ^{o}C were not significantly different. In the study area aquaculture and tourism activities are important for the local economy and may be susceptible to the impacts of harmful algae bloom events. Culture studies may give support to broaden the knowledge of species physiology and better understand species responses to a changing environment.

[1]Laboratório de Ecologia e Recursos Marinhos, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Av. Pasteur, 458, 22.290-240, Brazil (silvia.nascimento@gmail.com).
[2]Programa de Pós-graduação em Ecologia e Recursos Naturais, Universidade Estadual do Norte Fluminense, Av. Alberto Lamego, 2000, 28.013-602, Brazil.

EPI-BENTHIC DINOFLAGELLATES FROM THE RIO DE JANEIRO COASTLINE, BRAZIL

Nascimento, Silvia M. [1], Priscila O. Monteiro[2], Alice G. Alencar[2], Annaliza C. Meneguelli[2]

There are sporadic reports of epi-benthic dinoflagellates along the Brazilian coastline. *Ostreopsis ovata* blooms have been reported since 1998 in the Arraial do Cabo (AC) region associated with sea-urchin (*Echinometra lucunter*) die-off. The aim of this study was to identify and quantify epi-benthic dinoflagellate species in the dominant macroalgae of AC and Búzios (Bz) and relate abundance data to abiotic factors. Macroalgae samples were collected from June 2006 to November 2007 and the epiphytic microalgae were dislodged. The epiphyte suspension was preserved with neutral lugol iodine for microscopic identification and enumeration. Water temperature and salinity were measured during sampling. *Ostreopsis ovata* and *Prorocentrum lima* were the dominant species. Other species found as minor constituents include *Prorocentrum emarginatum*, *Prorocentrum rhathymum*, *Prorocentrum fukuyoi*, *Prorocentrum* cf. *maculosum*, *Gambierdiscus* cf. *toxicus*, *Coolia* sp. and *Amphidinium* sp. Many species were reported for the first time in the Brazilian coast. Three *O. ovata* blooms were reported and coincided with periods of warmer temperatures (air temperature higher than 24 ^{o}C). Monthly mean *O. ovata* densities in Bz reached 3 x 10^4 cells.g w.w.$^{-1}$ in *Sargassum vulgare*, while lower mean numbers were found at AC. *P. lima* followed *O. ovata* in cell numbers and peak values of 1,2 x 10^4 cells.g w.w.$^{-1}$ were recorded at AC in February 2007. The genus *Gambierdiscus* was reported for the first time in the region and showed low cell abundances throughout the period (maximum values of 74 cells.g w.w.$^{-1}$). Other species were minor constituents of the community.

[1]Laboratório de Ecologia e Recursos Marinhos, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Av. Pasteur, 458, 22.290-240, Brazil (silvia.nascimento@gmail.com).
[2]Laboratório de Ciências Ambientais, Universidade Estadual do Norte Fluminense, Av. Alberto Lamego, 2000, 28013-602, Brazil.

CIGUATERA CAUSATIVE SPECIES FOUND IN MAIN LAND OF JAPAN

Omura, Takuo, Yukio Nagahama and Yasuwo Fukuyo

Ciguatera fish poisoning (CFP) has been known for a long time only in Okinawa, the most southern islands having subtropical climate in Japan. However, several food poisoning cases suspected as CFP occurred in recent years after eating stone flounder (*Oplegnathus punctatus*) caught by surf fishing at several cities facing the Pacific Ocean in the temperate area of Japan. To clarify the distribution of *Gambierdiscus toxicus,* causative organism of CFP, field observation studies were conducted in the Pacific coast region (Wakayama Prefecture, Shizuoka Prefecture and Hachijo Island). *G. toxicus* was observed at all the research areas, though cell numbers were not high, rather rare. It was clarified that the distribution of the species is not limited only in tropical and subtropical regions, but also in temperate region. Culture strains were established from Wakayama Prefecture and Hachijo Island for comparison of their physiological characters. The strain from Wakayama was able to survive for one month or more at shortest at low temperature (15°C). At that condition a culture strain from Tahiti could not survive. On the other hand, salinity tolerance range showed little difference among culture strains (Wakayama, Hachijo and Tahiti).

Asian Natural Environmental Science Center, the University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan (aomura@mail.ecc.u-tokyo.ac.jp)

BIODIVERSITY OF KUWAIT'S BENTHIC DINOFLAGELLATES WITH EMPHASIS ON POTENTIALLY TOXIC SPECIES

Polikarpov, Igor[1], Maria Saburova[2] and Faiza Al-Yamani[1]

The diversity of benthic flagellates and their distribution on different substrates were studied in the northwestern part of Arabian (Persian) Gulf along the Kuwait's coastline during 2007-2009. The diversity of the flagellated group was mainly due to sand-dwelling and epiphytic dinoflagellates (56 taxa); most of them being reported from Kuwait from the first time. *Amphidinium*, with 17 species, was among the most abundant and diverse sand-dwelling dinoflagellate genera.

Among taxa of benthic dinoflagellates recorded in Kuwait were nine species that must be considered as potentially toxic: *Prorocentrum concavum*, *P. emarginatum*, *P. lima*, *P. rhathymum*, *Coolia monotis*, *Ostreopsis ovata*, *Amphidinium carterae*, *A. gibbosum* and *A. operculatum*. Potentially toxic species of *Amphidinium* genus were common among sand-dwelling dinoflagellates, whereas all *Prorocentrum* species and *O. ovata* were associated with macroalgae *Sargassum* sp. *C. monotis* was found mainly on *Padina* sp. Most of potentially toxic dinoflagellates were present in Kuwait's benthic microalgal community in low numbers; however, bloom of *C. monotis* was recorded in May 2009 in Al-Salmiya beach of Kuwait.

[1] Kuwait Institute for Scientific Research, P.O. Box 1638, 22017 Salmiya, Kuwait (igor.polikarpov@gmail.com)
[2] Institute of Biology of the Southern Seas, Pr. Nakhimova, 2, 99011, Sevastopol, Ukraine

CAN THE CASES OF AIRBORNE INTOXICATION OF BEACH USERS IN SOUTH COAST OF BAHIA (16^{O}.24´ S – 39^{O}.02´ W) BE RELATED TO MICROALGAE?

Proenca[1], LAO, GL Boemer[2], JP Dias[3], MM Hatherly[4], IL Mendes[5], LAM Mendes[5], MCQ Mendes[4], W.C. Rossi[6], MS Tamanaha[1], DR Tenenbaum[4], MA Schramm[1]

The south coast of Bahia at Porto Seguro and Santa Cruz Cabrália is a tropical environment and a major touristy destination in Brazil. In the last 10 years several episodes of intoxication of beach users along this 50km long coast have being registered. The episodes occur from December to April and are very punctual in time and space. It is estimated that more than 10.000 people have experienced the intoxication. Symptoms prevalence are sore throat(59%), headache(57%), myalgy(53%), fever sensation(53%), cough(50%), rhinorrea(46%) and others. In January/2008 a study was carried out to investigate the hypothesis that these intoxications could be related to microalgae. Plankton, neuston and periphyton samples were collected from water column along coral reefs, tidal pools and macrophyte. Aerosol was also sampled. During the sampling period no toxic episodes were observed but several potentially harmful species were found: *Pseudo-nitzschia* complex *delicatissima*, *Prorocentrum concavum*, *P. emarginatum*, *P. lima*, *Dinophysis acuminata*, *D. caudata, Alexandrium tamiyavanichi, Ostreopsis ovata*, *Lyngbia* spp, *Oscillatoria* spp, *Phormidium* spp and *Anabaena* sp. Extracts from scum and microperiphyton were toxic to sea-urchin larvae and produced haemolisys. Extracts from of *O.ovata* and from aerosol were analyzed by LCMS/MS. *O. ovata* presented a palytoxin-like peak with mass spectrum characteristic for ovatoxin-a. No evidences of palytoxin were found in aerosol. These results do not confirm our hypothesis that microalgae are the source of intoxications. On the other hand, circumstances of how they occur are quite similar to those reported for Mediterranean coast, which have being associated to *Ostreopsis* blooms.

[1]Universidade do Vale do Itajaí lao.proenca@hotmail.com
[2]Instituto Brasileiro do meio Ambiente dos Recursos Naturais Renováveis
[3]Diretoria de Vigilância Sanitária e Ambiental, Bahia
[4]Universidade Federal do Rio de Janeiro
[5]Prefeitura Municipal de Santa Cruz Cabrália, Secretaria Municipal de Saúde
[6]Centro de Recursos Ambientais, Bahia

POTENTIALLY TOXIC BENTHIC DINOFLAGELLATES IN THE BAY OF TULÉAR, MADAGASCAR

Rasoanandrasana, Rafalimanana [1*], Christian Ralijaona[1], Mawai Rabenevanana[1], Roberto Komeno[1], Lalaina Ravaloson[1]

Works report on the potentially toxic dinoflagellates in the bay of Tuléar have shown, at least the presence of 30 species, divided into 8 genuses (*Prorocentrum, Ostreopsis, Gambierdiscus, Coolia, Amphidinium, Gymnodinium, Gyrodinium, Sinophysis*).
Ostreopsis and *Gambierdiscus* are observed on the substrates from external zone of the reef, with rather clear water and strong hydrodynamism. *Prorocentrum* and others species prefer the internal parts of the coral reef, between the slope of the lagoon and seagrass bed with turbide water and the sediment. The benthic dinoflagellates proliferate especially on the grass algal of

the dead coral, thus constitutes a good indicator of the degradation state of the reef. The higher cells number of benthic dinoflagellates is observed at the end of the dry and cold season (September and November), particularly for *Ostreopsis* and *Gambierdiscus* and during the hot and rainy season for *Prorocentrum* (March and January). The lowest densities of cells are recorded during the cold and dry season (May –July). The general degradation of the coral reef contributes to develop toxic benthic dinoflagellates. Sometimes, the peaks of dinoflagellates cells number synchronise with the cases of icthtyosarcotoxisms observed in Tuléar.

For public health progress in Tuléar, it would be necessary,

- to improve human competency to recognize with precision the unidentified cells sources of the intoxications in Madagascar.
- to identify and to count cells present within the digestive system of the suspected fishes.

[1]Marine Science and Fishery Institute, University of Tuléar, P.O. Box 141, Tuléar (601), Madagascar (nandrasanarf@gmail.com)

EFFECTS OF DEPTH, HABITAT, AND WATER MOTION ON THE ABUNDANCE AND DISTRIBUTION OF CIGUATERA DINOFLAGELLATES AT JOHNSTON ATOLL, PACIFIC OCEAN

Richlen, Mindy L. [1] and Phillip S. Lobel[2]

A major impediment to understanding the seemingly random occurrence of ciguatera toxicity is uncertainty regarding the field ecology of benthic dinoflagellates that introduce toxins into the coral reef food web. This study employed a statistically rigorous approach, including standardized methodology, to investigate the distribution and abundance of toxigenic benthic dinoflagellates from the genera *Gambierdiscus, Prorocentrum, Ostreopsis*, and *Amphidinium* at Johnston Atoll, Pacific Ocean, to determine how water flow, depth and habitat type influence patterns of biodiversity. Sampling stations located in lagoon and channel habitats in the Atoll supported the highest total dinoflagellate abundance, while dinoflagellate numbers were lower at sampling stations in back reef and reef crest habitats. Total dinoflagellate abundance was primarily determined by the degree of water motion; however, this effect varied among genera. Three of the four genera surveyed (*Gambierdiscus*, *Prorocentrum*, *Amphidinium*) were negatively correlated with water motion; conversely, *Ostreopsis* was positively correlated with water motion. Habitat separation was observed between *Ostreopsis* and *Prorocentrum* spp., which were negatively correlated. *Gambierdiscus* was present at all sampling stations and at all habitats; however, was rarely the dominant genus. This study describes the field ecology of ciguatera dinoflagellates across a variety of coral reef habitats, and greatly contributes to an accurate and coherent characterization of the population dynamics of this important dinoflagellate community.

[1] Biology Department, Woods Hole Oceanographic Institution, MS 32, Woods Hole, MA, 02543, USA (mrichen@whoi.edu)
[2] Department of Biology, Boston University, 5 Cummington Street, Boston, Massachusetts 02215, USA

PHOTOSYNTHESIS-LIGHT RESPONSES OF BENTHIC DINOFLAGELLATES MEASURED BY PAM FLUOROMETRY AND PIGMENT ANALYSES

Rodríguez, Francisco[1], Santiago Fraga[1], Manuel Zapata[2] and José Luis Garrido[2]

The rapid light curves (RLC's) obtained by PAM fluorescence on benthic dinoflagellates (e.g. *Gambierdiscus* spp., *Ostreopsis* spp. and *Prorocentrum levis*) are more typical of "shade-adapted" organisms. They develop fast photoprotective responses (increment of non-photochemical quenching (NPQ) at low light intensities) and lower relative electron transport rates (rETR) in comparison with planktonic species. This "shade" behavior is more accentuated in the case of *Gambierdiscus* and *P. levis*, while *Ostreopsis* would represent an intermediate model.

These results have been explained by the fact that benthic epiphytes growing on macroalgae can minimize their light exposure, as it would be the case of *Gambierdiscus* (Villareal and Morton 2002). This would agree with the apparent inability of *Gambierdiscus* to grow in high-light in the laboratory. However, this shouldn't be extrapolated to all benthic species which can display diverse life strategies. For example, during the incubation of an *Ostreopsis* culture exposed to full sunlight we observed healthy cells and intense growth, as expected for an organism "blooming" on the rocky shore.

Regarding pigment composition in benthic dinoflagellates, peridinin-containing organisms represent the dominant pigment pattern in this environment, in contrast with different plastid types found in planktonic species. Benthic dinoflagellates also display higher pigment ratios to chl a than most planktonic species. However, benthic organisms can't be simplified to a single model species and they exhibit distinct photoacclimation responses depending on their ecological characteristics. These adaptations, mainly thanks to an efficient mechanism of photoprotection, enable them to thrive in high light environments, either in steady or rapid fluctuating conditions.

[1]Centro Oceanográfico de Vigo, IEO (Instituto Español de Oceanografía), Subida a Radio Faro 50, 36390 Vigo, Spain. (santi.fraga@vi.ieo.es)
[2]Instituto de Investigacións Mariñas de Vigo, CSIC, Eduardo Cabello 6, 36208 Vigo, Spain.

A PROBABLE NEW *PROROCENTRUM* SPECIES FROM COLD TEMPERATE SOUTH AMERICA

Uribe, Juan Carlos[1]; Sylvia Oyarzún[2]; Carlos García[3] and Nestor Lagos[3]

Benthic species of *Prorocentrum* are widely distributed in tropical and warm waters with only a small number ocurring in temperate zones. Here we describe a (probable) new toxic *Prorocentrum* found in the Straits of Magellan in Southernmost Chile. We also provide some ecological information for this species. Toxin analysis was performed with a HPLC-MS system in extracts of *Prorocentrum sp*. cells collected from the field. The analysis showed the presence of DSP toxins like okadaic acid and DTX1. The taxonomic study was carried out using light and SEM microscopy. *Prorocentrum sp*. is a medium size species with oblong cells. Thecal surface is smooth in the center and the rest of the valve has several radially arranged rows of pores that reach the valve margin. The periflagellar area is made up of 10 platelets. The species seems to differ from all the *Prorocentrum* until now described. The species is found as a sand-dwelling organism in tidal plains, altough probably extends its distribution to subtidal reaches. It occurs from spring to autumn, mostly as minor component of the microphytobenthic community. Its highest abundance has been observed from mid-summer to autumn. The temperature in this particular habitat varies from 7 ºC in spring to 10 ºC in summer, whereas salinity fluctuates around 30.5 psu. To the authors´ knowledge this is the first sand-dwelling *Prorocentrum* described in the cold temperate South America.

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[1]Instituto de la Patagonia, Universidad de Magallanes, P. O. BOX 113-D, Punta Arenas, Chile (juan.uribe@umag.cl).
[2]Departamento de Ciencias y Recurso Naturales, Facultad de Ciencias, Universidad de Magallanes, Punta Arenas, Chile.
[3]Laboratorio Bioquímica de Membrana, Programa de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile Santiago, Chile.

IMPACT OF TEMPERATURE AND NUTRIENTS ON THE GROWTH AND TOXICITY OF *OSTREOPSIS OVATA*

Vidyarathna, Nayani and Edna Granéli

Ostreopsis ovata Fukuyo is an epiphytic, toxic dinoflagellate, inhabiting tropical and sub-tropical waters worldwide. Toxic blooms of *O. ovata* have recently been reported in SE Brazil in 1998/99 and 2001/02 and the French-Italian Riviera in 2005 and 2006. *O. ovata* cells produce palytoxin, a very strong toxin, only second in toxicity to botulism. Laboratory experiments were performed with *O. ovata,* isolated from the Ligurian coast, Italy to determine the effects of water temperature and how the cells growing under N and P deficiency versus sufficiency, have on the growth and toxicity of the cells. The cultures were grown at 20 °C and 30 °C and N and P deficient conditions (N:P ratios of 3.2:1 and 80:1 respectively) and also under NP sufficiency (N:P ratio of 16:1). Cell toxicities were measured on two occasions during the stationary phase. Irrespective of N:P ratios, the toxicities in the cells grown at 20 °C were significantly higher than that of 30 °C. However, at 20 °C under N deficiency cell toxicity was significantly higher than in the P deficient and NP sufficient treatments. Contrarily, cells densities were higher at 30 °C in all nutrient treatments. The lowest cell densities were found under N deficient condition at 20 °C. Our results show that low temperature (20 °C) and N deficiency, increase *O. ovata* toxicity although temperature has a greater impact than nutrient conditions. Thus changes in sea surface temperatures and N and P unbalanced conditions might have a crucial role in *O. ovata* bloom formation.

Marine Ecology Department, Linnaeus University, 391 82 Kalmar, Sweden.
(nayani.vidyarathna@lnu.se)

GROWTH RESPONSE OF *OSTREOPSIS OVATA* OVER A RANGE OF TEMPERATURE AND PHOTOPERIOD COMBINATIONS

Zingone, Adriana, Eleonora Scalco, Francesca Marino and Marina Montresor

In the last years, blooms of *Ostreopsis ovata* repeatedly occurred along the Mediterranean coasts during summer and/or autumn, and they caused problems to human health and economic activities. In order to gain insights on the factors that regulate the bloom dynamics of *Ostreopsis ovata*, we tested its growth response over a multifactorial combination of temperature (14, 18, 22, 26, 30 and 34°C), photoperiod (9L:15D, 12L:12D, 15L:9D) and irradiance (50 and 200 μmol photons m^{-2} s^{-1}) conditions simulating those recorded in the natural environment over the annual cycle. We tested duplicates of 3 monoclonal cultures isolated from different locations along the Italian coasts: Trieste, Ancona and Napoli.

Ostreopsis ovata can grow over a range of temperature varying between 18 and 30°C. At 14°C the species can survive but does not grow; at 34°C cells die. The maximum growth rates, spanning from 0.56 to 0.82 divisions·day^{-1}, were recorded at the two longer daylength conditions and at temperature values comprised between 18 and 26°C. A peak of 1.2 divisions·day^{-1} was observed at 26°C, 12L:12D). At low irradiance, no significant differences were recorded between growth rates at 12L:12D and 15L:9D, whereas at higher irradiance, longer daylength conditions often caused a decrease in the growth capability.

Our results show that the ‘theoretical growth niche’ of *O. ovata* matches the conditions at which the species is recorded in the natural environment and suggest that the species is adapted at the relatively low irradiances recorded within the macroalgal vegetation.

Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy (zingone@szn.it)

LIST OF PARTICIPANTS

Katerina Aligizaki
Aristotle University of Thessaloniki
Dept. of Botany, School of Biology,
P.O. Box 109
Thessaloniki 54124
GREECE
Phone: +302310998376
E-mail: aligiza@bio.auth.gr

Icarus Allen
Plymouth Marine Laboratory
Prospect Place
Plymouth, PL1 5LT
UNITED KINGDOM
Phone: +44 1752 633100
E-mail: jia@pml.ac.uk

Karen Arthur
University of Hawaii
Dept Geology & Geophysics, POST 701
1680 East West Road
Honolulu, HI 96822
UNITED STATES
Phone: +1-808-220-0627
E-mail: arthur4@hawaii.edu

Cecilia Battocchi
University of Urbino "Carlo Bo"
Viale Trieste 296
Pesaro 61121
ITALY
Phone: +39-721423526
E-Mail: c.battocchi@campus.uniurb.it

Elisa Berdalet
Institut de Ciencies del Mar (CSIC)
Passeig Marítim, 37-49
Barcelona, Catalunya 8003
SPAIN
Phone: +34932309595
E-mail: elisa.berdalet@icm.cat

Paul Bienfang
University of Hawaii-Manoa
Oceanography Dept. MSB#205
Honolulu, HI 96822
UNITED STATES
Phone: +1-808-956-7402
E-mail: bienfang@soest.hawaii.edu

Nicolas Chomerat
Ifremer
13 rue de Kerose
Concarneau Cedex 29187
FRANCE
Phone: +33 2 98 97 43 38
E-Mail: nicolas.chomerat@ifremer.fr

William Cochlan
San Francisco State University
Roomberg Tiburon Center, SFSU
3152 Paradise Drive
Tiburon, CA 94920
UNITED STATES
Phone: +1-415 338-3541
E-mail: cochlan@sfsu.edu

Roberta Congestri
University of Rome "Tor Vergata"
Via della Ricerca scientifica snc
ROME 133
ITALY
Phone: +39 0672594332
E-Mail: roberta.congestri@uniroma2.it

Hai Doan
Institute of Oceanography
01 Cau Da
Nha Trang, Khanh Hoa 8458
VIETNAM
Phone: +84 58 3590 476
E-mail: habsea@dng.vnn.vn

JK Patterson Edward
Suganthi Devadason Marine Research Institute
44-Beach Road
Tuticorin Tamil Nadu 628001
INDIA
E-mail: edwardjkpatterson@yahoo.co.in

Jacqui Evans
Te Ipukarea Society, Inc.
Cook Islands
E-mail: TepaeruEvans@gmail.com

Liam Fernand
Centre for Environment, Fisheries & Aquaculture
Science (Cefas)
Lowestoft Laboratory
Pakefield Road
Lowestoft, Suffolk NR33 0HT
UNITED KINGDOM
Tel. +44(0) 1502-524538
E-mail: liam.fernand@cefas.co.uk

Santiago Fraga
Instituto Español de Oceanografía
Subida a Radio Faro 50
Vigo E-36390
SPAIN
Phone: +34986492111
E-mail: santi.fraga@vi.ieo.es

Leonardo Guzman
Instituto de Fomento Pesquero
Balmaceda 252
Puerto Montt, Region de Los Lagos
CHILE
Phone: +56 65 342309
E-mail: lguzman@ifop.cl

William Holland
NOAA
101 Pivers Island Road
Beaufort, NC 28516
UNITED STATES
Phone: +1-252-728-8799
E-mail: Chris.Holland@noaa.gov

Mona Hoppenrath
Senckenberg Research Institute
Suedstand 44
Wilhelmshaven D-26382
GERMANY
Phone: +49 421 9475116
E-mail: mhoppenrath@senckenberg.de

Amany Ismael
Faculty of Science, Alexandria University
Alexandria University
21511 EGYPT
Phone: +20-0127929135
E-mail: amany_3@yahoo.com

Steve Kibler
NOAA National Ocean Service
101 Pivers Island Road
Beaufort, NC 28516
UNITED STATES
Phone: +1-252-728-8737
E-mail: steve.kibler@noaa.gov

Raphael Kudela
University of California Santa Cruz
Ocean Sciences Department
1156 High Street
Santa Cruz, CA 95064
UNITED STATES
Phone: +1-831-459-3290
E-mail: kudela@ucsc.edu

Brian Lapointe
Harbor Branch Oceanographic Inst.
5600 U.S. 1 North
Ft. Pierce, FL 34946
UNITED STATES
Phone: +1-772-465-2400 ext 276
E-mail: belapoint@gmail.com

Jacob Larsen
IOC Science and Communication Centre on Harmful Algae
Oester Farimagsgade 2D
Copenhagen DK-1353 K
DENMARK
Phone: +45 3313446
E-mail: jacobl@bio.ku.dk

Wayne Litaker
NOAA
Beaufort, NC 28516
Phone: +252-728-8791
E-mail: wayne.litaker@noaa.gov

Francesca Marino
Zoological Station "Anton Dohrn"
Via Arenella 12
Naples 80129
ITALY
Phone: 393335911169
E-mail: francescamarino@inwind.it

Jonathan Molina
University of Hawaii-Manoa
MSB #205 1000 Pope Rd
Honolulu, HI 96822
UNITED STATES
Phone: +1-808 343 9947
E-mail: jomolina@hawaii.edu

Steve Morton
NOAA/National Ocean Service
331 Fort Johnson Road
Charleston, SC 29412
UNITED STATES
Phone: +1-843-762-8857
E-mail: steve.morton@noaa.gov

Silvia Nascimento
Universidade Federal do Estado do Rio de Janeiro (UNIRIO)
Av. Pasteur, 458, sala 314, Urca
Rio de Janeiro 22.290-240
BRAZIL
Phone: +55 21 2244 5483
E-mail: silvia.nascimento@gmail.com

Lam Nguyen
Institute of Oceanography
01 Cau Da, Vinh Nguyen
Nha Trang Khanh Hoa 58
VIETNAM
Phone: +84905111824
E-mail: habviet@dng.vnn.vn

Henrik Oksfeldt Enevoldsen
Intergovernmental Oceanographic Commission of UNESCO
University of Copenhagen, Øster Farimagsgade 2D
Copenhagen K 1353
DENMARK
Phone: +45 33134446
E-mail: h.enevoldsen@unesco.org

Takuo Omura
The University of Tokyo
1-1-1 Yayoi, Bunkyo-ku
Tokyo, 113-8657
JAPAN
E-mail: aomura@mail.ecc.u.tokyo.ac.jp

Michael Parsons
Florida Gulf Coast University
10501 FGCU Blvd South
Fort Myers, FL 33965
UNITED STATES
Phone: +1-239-590-7526
E-mail: mparsons@fgcu.edu

Antonella Penna
UNIVERSITY OF URBINO
vilae Trieste 296
Pesaro 61121
ITALY
Phone: +390721423526
E-mail: antonella.penna@uniurb.it

Karl Petermann
Senckenberg Research Institute
Suedstrand 44
Wilhelmshaven D-26382
GERMANY
Phone: +49 4421 9475 116
E-mail: mhoppenrath@senckenberg.de

Kathleen Pitz
Woods Hole Oceanographic Institution
266 Woods Hole Road
Woods Hole, MA 02543
UNITED STATES
Phone: +1-508-289-3815
E-mail: kpitz@whoi.edu

Igor Polikarpov
Kuwait Institute for Scientific Research
PO Box 1638
Salmiya 22017
KUWAIT
Phone: +96597642088
E-mail: igor.polikarpov@gmail.com

Luis Proenca
Universidade do Vale do Itajai
Itajai Santa Catarina 88306806
BRAZIL
Phone: +55 (47) 33417713
E-mail: luis.proenca@univali.br

Lesley Rhodes
Cawthron Institute
98 Halifax St East
Private Bag 2
Nelson 7042
NEW ZEALAND
Phone: +64 3 548 2319
E-mail: lesley.rhodes@cawthron.org.nz

Mindy Richlen
Woods Hole Oceanographic Institution
266 Woods Hole Road
MS 32, Redfield 332
Woods Hole, MA 02543
UNITED STATES
Phone: +1-508-289-2552
E-mail: mrichlen@whoi.edu

Lemée Rodolphe
CNRS and University Pierre and Marie Curie
LOV, UMR 7093
BP 28
Villefranche-sur-mer 6234
FRANCE
Phone: +33 4 93 76 38 39
E-mail: lemee@obs-vlfr.fr

Suzanne Roy
Universite du Quebec a Rimouski
310 Allee des Ursulines
Rimouski Quebec G5L 3A1
CANADA
Phone: +1-418-724-1650 x1748
E-mail: suzanne_roy@uqar.qc.ca

Mariya Saburova
Institute of Biology of the Southern Seas
Prospekt Nakhimova 2
Sevastopol 99011
UKRAINE
Phone: +380692546629
E-mail: msaburova@gmail.com

Mark Skinner
Entox, University of Queensland
67A kiora Street, Canley heights
Sydney NSW 2166
AUSTRALIA
Phone: +61 421466511
E-mail: mark_skinner59@yahoo.com.au

Patricia Tester
NOAA
101 Pivers Island Road
Beaufort, NC 28516
UNITED STATES
Phone: +1-252 728 8792
E-mail: pat.tester@moaa.gov

Ho Van The
Institute of Oceanography
01 Cau Da
Nha Trang Khanh Hoa 84 58
VIETNAM
Phone: +84 0983 883 559; +84 58 3590 476
E-mail: hvthe-ion@vnn.vn

Charles Trick
PICES - HABS
Room 402 NCB, University of Western Ontario
London Ontario N6A5B7
CANADA
Phone: +1-519-661-3899
E-mail: trick@uwo.ca

Ed Urban
Scientific Committee on Oceanic Research
College of Earth, Ocean and Environment
Robinson Hall, University of Delaware
Newark, DE 19716
UNITED STATES
Phone: +1-302-831-7011
E-mail: ed.urban@scor-int.org

Juan Uribe
Universidad de Magallanes
Avenida Bulnes 01890
Punta Arenas 62000
CHILE
Phone: +56 61 209 991
E-mail: juan.uribe@umag.cl

Gires Usup
Universiti Kebangsaan Malaysia
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
Bangi Selangor 43600
MALAYSIA
Phone: +60-6359388
E-mail: giresusup@gmail.com

Natalie Wallgrove
University of Hawaii
1000 Pope Road MSB 205
Honolulu, HI 96822
UNITED STATES
Phone: +1-808-351-3166
E-mail: nw@hawaii.edu

Being Yeeting
Secretariat of the Pacific Community
B.P. DS
Noumea Cedex 98848
NEW CALEDONIA
Phone: +687 260187
E-mail: beingy@spc.int

Adriana Zingone
Stazione Zoologica Anton Dohrn
Villa Comunale
Naples 80121
ITALY
Phone: +39 0815833295
E-mail: zingone@szn.it