

**12th INTERNATIONAL CONFERENCE ON MODERN
AND FOSSIL DINOFLAGELLATES**

The logo for DIN 12 features a stylized 'DIN' in black, followed by a circular emblem composed of concentric lines in a rainbow gradient (red, orange, yellow, green, blue, purple). To the right of the emblem is the number '12' in black. Below the emblem is a red, stylized, swirling graphic element.

DIN 12

**Las Palmas de Gran Canaria
Canary Islands, SPAIN
July, 4th - 8th 2022**

Book of Abstracts

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12th INTERNATIONAL CONFERENCE ON MODERN AND FOSSIL DINOFLAGELLATES

Conference Centre of the Canary Islands
Auditorio Alfredo Kraus
Las Palmas de Gran Canaria
July, 4th – 8th 2022

Organised by



Observatorio Canario de algas nocivas

WELCOME

On behalf of the Scientific and Organizing Committee, we cordially welcome you to the 12th edition of the International Conference on Modern and Fossil Dinoflagellates (DINO12) organized by the Canarian Observatory of Harmful Algae of The Canarian Science and Technology Park Foundation of the University of Las Palmas de Gran Canaria.

DINO12 is intended to provide a place for the presentation and discussion of current research and recent advances in the field of Modern and Fossil Dinoflagellates. In this edition, DINO12 will be framed within the common topic of Global warming while preserving the essence of the meeting, in which the dinoflagellates and their cysts are the major protagonists. It provides a broad forum for basic and applied research on this issue including Climate Change, Ecology, Toxic Dinoflagellates and Biotoxins, Palaeoecology and Palaeoceanography, Mesozoic and Cenozoic Dynocyst Stratigraphies, Phanerozoic and deep time scales, Dinocyst Systematics, Life Cycles and Nutritional Strategies, Biodiversity and Systematics, Stratigraphy, Evolution, and Taxonomy.

DINO12 follows a balanced combination of plenary lectures, demonstrations, oral presentations, poster sessions, workshops, and social events that will offer you great opportunities to meet other colleagues in a formal and informal way.

We expect that the DINO12 will improve the exchange of knowledge and future necessities among the participants who come from Asia, Africa, America, and Europe.

We would like to thank all the collaboration of public organizations that have sponsored the organization, as well as colleagues that believe in the necessity and the significance of dealing with this issue in the Canary islands by hosting the 12th International Conference on Modern and Fossil Dinoflagellates.

We wish you will find plenty of scientifically and socially rewarding experiences during your attendance at the DINO12 and stay in Gran Canaria.

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Canarian Observatory of Harmful Algae (OC-HABs)
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The 12th edition of the International Conference on Modern and Fossil Dinoflagellates (DINO12) was held in the scientific framework of MIMAR+ (MAC2/4.6d/249) “Seguimiento, control y mitigación de proliferaciones de organismos marinos asociadas a perturbaciones humanas y cambio climático en la Región Macaronésica” INTERREG V-A Spain-Portugal Cooperation Programme MAC (Madeira-Azores-Canary Islands) 2014-2020



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INDEX

PLENARY SESSIONS

P.01	Dinoflagellates and Global Climate Change: what should we expect?	2
P.02	<i>Ostreopsis</i> blooms: an indicator of the need to act in the climate and environmental emergency.	4
P.03	Paleontological evidence of dinoflagellates as early eukaryotes.	6
P.04	Review of current trends in dinoflagellate taxonomy.	7

ORAL COMMUNICATIONS AND POSTERS SESSIONS

SESSION 1. ECOLOGY AND CLIMATE CHANGE

1.01	During droughts and temperature extremes, don't assume a patch of brown cloudy water in a karst spring run is suspended. Sediment: hypoxia and hyperthermia increased within dinoflagellate blooms of rarely reported yet regularly occurring <i>Thompsodinium intermedium</i> in Comal Springs, Texas.	11
1.02	Ecological properties of the green seawater discoloration forming dinoflagellate <i>Lepidodinium chlorophorum</i> : from cellular ecophysiology to ecosystem impacts.	12
1.03	The effect of temperature and light on the growth dynamics of the harmful dinoflagellate <i>Margalefidinium polykrikoides</i> .	14
1.04	Playing hide and seek: distribution with depth of potentially harmful epibenthic dinoflagellates of southern El Hierro island, Canary Islands (NE Atlantic).	18
1.05	Fifty shades of red tide: assessing the remote-sensing reflectance fingerprint of highly-concentrated coastal harmful algal blooms.	20
1.06	Tracing the origin of <i>Gymnodinium catenatum</i> blooms in central west Iberian shelf.	22
1.07	Phagotrophy of bloom-forming dinoflagellates under different light and nutrient conditions.	24

1.08	HOBO light/temperature probes shed light on simultaneous stratification patterns and diel cycling dynamic of dinoflagellate benthic to planktonic life stage transitions not previously reported in the scientific literature on dinoflagellates.	26
1.09	Dinoflagellate cyst production, excystment, vertical transport and lateral dispersion in surface waters and the deeper water column off Cape Blanc (NW Africa).	27
1.10	Seasonal, annual and multi-annual variability of dinoflagellate cyst export production in the upwelling area off Cap Blanc based on a 18 years sediment trap time series.	30
1.11	Spatial distribution of dinoflagellate cysts along an inshore-offshore transect off Cape Blanc (NW Africa).	34
1.12	Dinoflagellate cysts in the benthic nephroid layer along a land-sea transect off Figueira da Foz (NW Iberia).	39
1.13	Dinoflagellate indicators of Arctic Ocean multiyear ice: filling the knowledge gaps.	46

SESSION 2. TOXIC DINOFLAGELLATES AND BIOTOXINS I

2.01	First observation of <i>Fukuyoa paulensis</i> Gómez, Qiu, Lopes & Lin and <i>Coolia canariensis</i> Fraga along the Italian coasts (Sardinia island).	51
2.02	BHAB ecology in the oceanic Madeira island (subtropical eastern Atlantic).	53
2.03	In-depth biochemical mapping of <i>Pyrodinium bahamense</i> reveals insights on its molecular machineries.	55
2.04	Detection of palytoxin-like toxicity in <i>Ostreopsis</i> cf. <i>siamensis</i> from the Cantabrian coast.	57
2.05	Spatiotemporal patterns and implications of <i>Alexandrium catenella</i> cyst suspension in the Alaskan Arctic.	59
2.06	Tricky dinoflagellate toxins, how to get a good grasp of them!	61

SESSION 3. TOXIC DINOFLAGELLATES AND BIOTOXINS II

3.01	First exceptional record of <i>Ostreopsis</i> cf. <i>siamensis</i> in the Cantabrian plankton after 20 years of harmful algal blooms monitoring.	63
3.02	From laboratory to pilot scale: increasing culture productivity of a <i>Gambierdiscus excentricus</i> strain native to Canary Islands.	65
3.03	The incidence of ciguatoxin in the Canary archipelago in recent years recorded in the MIMAR Project.	67
3.04	Ciguatoxins and small-scale fisheries: thirteen years of experience in the Canary Islands.	69
3.05	An unprecedented bloom of <i>Lingulodinium polyedra</i> on the French Atlantic coast during summer 2021.	71
3.06	Interannual and spatial variability of <i>Dinophysis acuminata</i> in north-western Iberian shelf over a 25-year time serie.	72
3.07	Persistence of ciguatoxin producers in the Balearic Islands.	77
3.08	Assessment of the growth and toxicity of different strains of <i>Gambierdiscus</i> sp.	79
3.09	A 10 year gap on blooms of <i>Dinophysis sacculus</i> and DSP closures in Alfacs Bay.	81
3.10	Biodiversity of benthic dinoflagellates in the northern Red Sea.	82
3.11	Morphology of four marine planktonic dinophysoid (Dinophysales) dinoflagellates from the Mexican central pacific: <i>Dinofurcula ultima</i> , <i>D. ventralis</i> , <i>Latifascia inaequale</i> and <i>Tripodosolenia longicornis</i> .	83
3.12	<i>Matsuokaea loeblichii</i> , rare but everywhere?	84
3.13	Natural sampling of phytoplankton and parasitoid abundances in Cala Santanyí.	86
3.14	The role of physical forcings on the daily variability of near shore phytoplankton accumulation in the Mediterranean Sea.	87
3.15	Assessment of dinoflagellate cyst assemblages and sedimentary concentrations in the northern Bering Sea.	91

- | | | |
|-------------|--|-----------|
| 3.16 | New ecological insight of calcareous dinoflagellate cysts export production based on an 18 years sediment trap study in the Cap Blanc upwelling area (NW. Africa). | 93 |
| 3.17 | Marine synthetic peptides interact with SARS-COV-2 replication protein complex. | 97 |

SESSION 4. PALEOECOLOGY AND PALEOCEANOGRAPHY I

- | | | |
|-------------|---|------------|
| 4.01 | Dinoflagellate cyst assemblages from the Gulf of Corinth (eastern Mediterranean Sea) reveal environmental and climatic shifts since the Middle Pleistocene. | 100 |
| 4.02 | Distributions of dinoflagellate cysts and diatoms in surface sediments of the Chukchi Sea in relation to the upper water masses. | 102 |
| 4.03 | Using the chemistry of dinoflagellate cyst walls; Infrared analyses of individual specimens. | 103 |
| 4.04 | High-resolution reconstructions of Holocene sea-surface conditions from dinoflagellate cysts in the northern south China Sea. | 105 |
| 4.05 | Calibration of dinoflagellate cysts as paleo-environmental indicators in the Kitimat Fjord system (northern BC, Canada): preliminary results. | 107 |
| 4.06 | Late Holocene changes in oceanography and regional export primary production in northwestern Baffin Bay. | 108 |

SESSION 5. BIODIVERSITY & SIYSTEMATICS

- | | | |
|-------------|--|------------|
| 5.01 | Planktonic athecate dinoflagellates from western coasts of Baja California, Mexico, with a redescription of <i>Gymnodinium hamulus</i> . | 111 |
| 5.02 | International Phytoplankton Intercomparison (IPI) exercises in the abundance and composition of marine microalgae: a blueprint for biological capacity building in community analysis. | 112 |
| 5.03 | Species of the planktonic dinoflagellate genus <i>Alexandrium</i> (Dinophyta) from the tropical and subtropical Mexican Pacific Ocean. | 113 |
| 5.04 | Temporal changes of dinoflagellate communities in inner Frobisher Bay, Nunavut, Canada. | 114 |

SESSION 6. DINOCYST SYSTEMATICS, MESOZOIC AND CENOZOIC DYNOCYST

6.01	Dual nomenclature in organic-walled dinoflagellate cysts: concepts, methods and applications.	117
6.02	<i>Pentaplacodinium lapazense</i> sp. nov. from southwestern Gulf of California, a new non-toxic gonyaulacalean resembling <i>Protoceratium reticulatum</i> .	118
6.03	The dinoflagellate cyst correlation across the Campanian-Maastrichtian boundary between the Tethyan and boreal Europe.	119
6.04	<i>Cannosphaeropsis franciscana</i> morphological variance across the K/Pg boundary; the case for vesiculation as an ecophenotypic character within spiniferate gonyaulacaceans.	121
6.05	Macromolecules analyses of simple-shape cysts using ATR-FTIR and Raman spectroscopy.	123
6.06	Calcareous dinoflagellate cyst variance across the K/Pg boundary at DSDP site 577, Shatsky Rise, Western North Pacific.	126
6.07	Marine phytoplankton turnover and record of paleoenvironmental change across the K/Pg boundary in the Eastern North Pacific.	128

SESSION 7. PALEOCOLOGY AND PALEOCEANOGRAPHY II, EVOLUTION, TAXONOMY AND BIOSTRATIGRAPHY

7.01	Hydrographic development of the sea of Japan during the Pliocene-Pleistocene transition: the dinoflagellate cyst record at IODP site U1424.	132
7.02	Dinoflagellate cysts in coastal surface sediments from NW Iberia related to environmental gradients: new supporting data for palaeoenvironmental studies.	134
7.03	Dinoflagellate cysts and acritarchs from the Pliocene-Pleistocene transition in the Southern Bering Sea: IODP site U1341.	139
7.04	Novel nuclear proteins in dinoflagellate mediate the highly compacted liquid-like state of dinoflagellate chromosomes.	140
7.05	Recent advances in infrared spectroscopy applied to single specimen dinoflagellate cyst: methodological framework, acquired insights and future perspectives.	141
7.06	Ecological signals in dinoflagellate biostratigraphy.	143



PLENARY SESSIONS

P.01 Dinoflagellates and Global Climate Change: what should we expect?

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Abstract: Climate changes affect the physics and chemistry of the world's oceans and have the potential to alter every functional relationship in the marine food web either directly or indirectly. These effects will be noticed first in the lower trophic levels and portend significant effects on phytoplankton physiology, productivity, biomass, species shifts or dominance, range extensions, and myriad other populations level changes. Chief among our concerns is the effects climate change will have on harmful algal blooms, and for the purposes of this DINO12 Conference, we will narrow the focus to dinoflagellate genera. The newly released (28 February 2022) Intergovernmental Panel on Climate Change (IPCC) report on Impacts, Adaptation and Vulnerability released on 28 February 2022, recognized six major issues. One related to food security, specifically points to the compounded risks of seafood safety due to increasing contamination from harmful algal blooms. With sea surface temperatures in the world's oceans well on the way to the projected 1.5 °C or more increase by 2050, many temperate (*Alexandrium*) and sub-tropical/tropical (*Karenia*, *Gambierdiscus*) dinoflagellates will exhibit higher growth rates over much of their current geographic range, resulting in higher population densities. The primary exception to this trend may be in the tropics where temperatures could exceed species-specific upper thermal tolerances (30–31 °C) beyond which growth slows significantly. As surface waters warm, migration to deeper habitats is expected to provide refuge for some genera. Range extensions of several degrees of latitude also are anticipated, but only where species-specific habitat requirements can be met (e.g., temperature, suitable substrate, low turbulence, light, salinity, pH). The current understanding of habitat requirements that determine species distributions are reviewed to provide fuller understanding of how individual species will respond to climate change from the present to 2050 while addressing the paucity of information on environmental factors controlling small-scale distribution in localized habitats. Based on the available information, we hypothesized how complex environmental interactions can influence abundance and potential range extensions of dinoflagellate species in different biogeographic regions and identify sentinel sites appropriate for long-term monitoring programs to detect range extensions and reduce human health risks from harmful algal blooms.

Key words: Harmful algal blooms, habitat requirements, biogeography, range extension

Acknowledgments: Sincere thanks to Prof. Emilio Soler Onís and the Organizing Committee for their kind invitation to speak at DINO12.

References: IPCC, 2022: *Climate Change 2022: Impacts, Adaptation, and Vulnerability*. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press. In Press.

P.02 *Ostreopsis* blooms: an indicator of the need to act in the climate and environmental emergency

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Abstract: The life in our planet is a history of continuous extinction and survival of organisms adapting to progressive or abrupt changes in the ecosystems. There is no doubt that we are facing a period of rapid changes in our planet caused by human pressures since the industrial revolution, with global warming as a major driving force. The argument of this presentation is that, through the genus *Ostreopsis* (Figure 1), nature is talking about the ongoing planetary changes.

The biogeographic distribution of the identified *Ostreopsis* species, first identified in tropical areas and present in warm temperate coasts, has expanded from 20°S - 28°N in 1995, to 35°S-45°N in 2020 (Tester et al. 2020). This increased trend can be explained by a better and targeted monitoring, as well as by improved detection and species characterization by molecular tools. But the interest on *Ostreopsis* since the beginning of the 21st century is mainly due to the occurrence of high biomass blooms of certain species in temperate beaches during the summer vacation period. These proliferations have been associated to bathing water quality deterioration, respiratory and cutaneous irritations on humans exposed to marine aerosols, massive mortalities of some benthic macrofauna and the potential risk of food borne poisonings, because some *Ostreopsis* species synthesize analogues of the potent palytoxin related to fatalities (although not completely proved yet) in the tropics.

Which are the main drivers of *Ostreopsis* blooms at present and which could be the expected trends in the projected scenarios of global warming? Focusing on *O. cf. ovata*, that proliferates recurrently in some Mediterranean beaches, seawater temperature is a major factor modulating its bloom phenology (Drouet et al. 2022). In the 13-years dataset in Larvotto beach (Monaco), the highest *in situ* net growth rates were estimated in the 21°C - 25°C temperature range, while maximal values reached 27.5°C. Furthermore, blooms occurred earlier in the season when spring Sea Surface Temperatures were warmer than usual. The potential expansion of another species, *O. cf. siamensis*, towards warming waters of the Bay of Biscay (NE Atlantic) was also documented using *in situ* cells sampling, eDNA and connectivity modeling in the area (Drouet et al. 2021).

Ostreopsis blooms occur in shallow, illuminated and relatively calm coastal waters, where cells thrive mainly attached to biotic (Figure 2) and abiotic surfaces. Being photosynthetic, inorganic nutrients should be available to allow high biomass blooms, although the direct link has not been clearly established. Often, *Ostreopsis* cells thrive as epiphyte of dense macroalgal turf carpets indicating that the microalga tolerates eutrophic and deteriorated habitats.

With the available knowledge we can design strategies to mitigate the impacts of *Ostreopsis* blooms, diminish their occurrence while also acting in the present climate and environmental emergency.

Keywords: *Ostreopsis* cf. *ovata*, seawater warming, habitat destruction, respiratory irritation

Acknowledgments: Sincere thanks to Prof. Emilio Soler Onís and the Organizing Committee for their kind invitation to speak at DINO12.

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Drouet, K., Jauzein, C., Gasparini, S., Pavaux, A-S., Berdalet, E., Marro, S., Davenet-Sbirrazuoli, V., Siano, R. and Lemée, R. (2022). The benthic toxic dinoflagellate *Ostreopsis* cf. *ovata* in the NW Mediterranean Sea: Relationship between sea surface temperature and bloom phenology. *Harmful Algae* 112, 102184.

Drouet, K., Jauzein, C., Herviot-Heath, C., Hariri, S., Laza-Martinez, A., Lecadet, C., Plus, M., Seoane, S., Sourisseau, M., Lemée, R. and Siano, R. (2021). Current distribution and potential expansion of the harmful benthic dinoflagellate *Ostreopsis* cf. *siamensis* towards the warming waters of the Bay of Biscay, North-East Atlantic. *Environmental Microbiology*, 23(9), 4956-4979.

Tester, P. A., Litaker, W. R. and Berdalet, E. (2020). Climate Change and Benthic Harmful Algae. *Harmful Algae* 91, 101655.

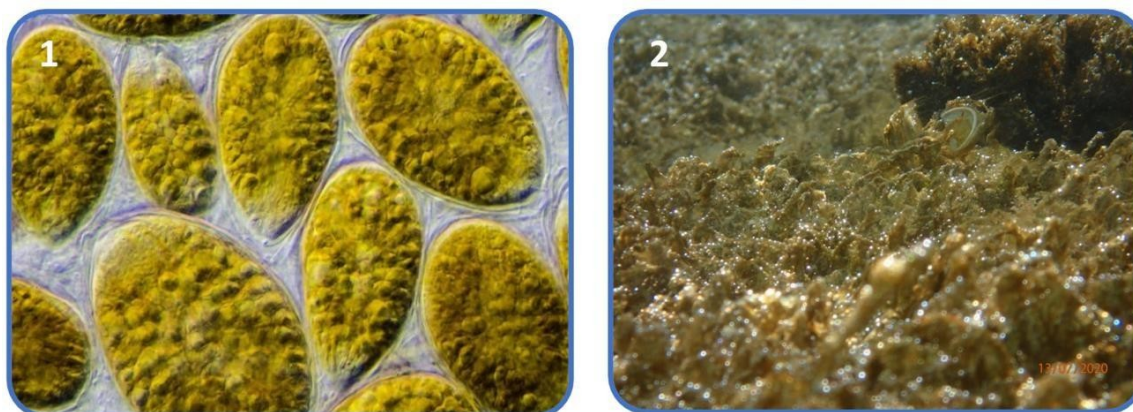


Fig.1. *Ostreopsis* cf. *ovata* cells. Ruben Duro©. Fig.2. Proliferation of *O. cf. ovata* epiphyte of *Padina pavonica*, *Jania* spp., *Elisolandia* spp.

P.03 Paleontological evidence of dinoflagellates as early eukaryotes

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Abstract: Molecular phylogeny is challenging paleontology to show evidence of early eukaryotes. Phylogenetic trees identify early groups, but almost all lack a fossil record. Dinoflagellates are the only group identified that have an extensive established fossil record, but this extends only back to the Middle Triassic (around 240 Myr), which is around one Byr after the prokaryotic/eukaryotic transition. Living dinoflagellate biology and geochemical biomarkers support the group being early eukaryotes, and it has been suggested that a major group of microfossils, the acritarchs, may include as yet unidentified early dinoflagellates.

Evitt (1963) introduced the morphologic criteria formally used to separate acritarchs and fossil dinoflagellates. This presentation critically reviews these criteria based on the wealth of information documented for both acritarchs and fossil dinoflagellates since Evitt (1963). Modifying the criteria allows the re-identification of some early acritarchs as early dinoflagellate.

P.04 Review of current trends in dinoflagellate taxonomy

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Abstract: Current trends in dinoflagellate taxonomy are reviewed, with special emphasis on publications with taxonomic innovations. Molecular biology is the main technological advance for taxonomy in the 21st century. However, the availability of DNA sequences is often not always accompanied by progress in taxonomy. For example, thousands of environmental sequences identified as *Amoebophrya* are available, but there is no contribution on the species-level taxonomy, or *Gonyaulax spinifera* sequences cluster into at least six distinct clades without a clear definition of the identity of that species. The requirement of molecular phylogeny in the publications has induced a bias towards the description of new species of autotrophic taxa, while groups such as open ocean and/or heterotrophic species are less represented. Descriptions of new species based on cultures or easy accessible coastal species allow detailed studies (including ultrastructure, multigene phylogenies). However, requiring the same protocols for oceanic species makes progress difficult because these techniques are difficult to apply to the few individuals available from open ocean samples.

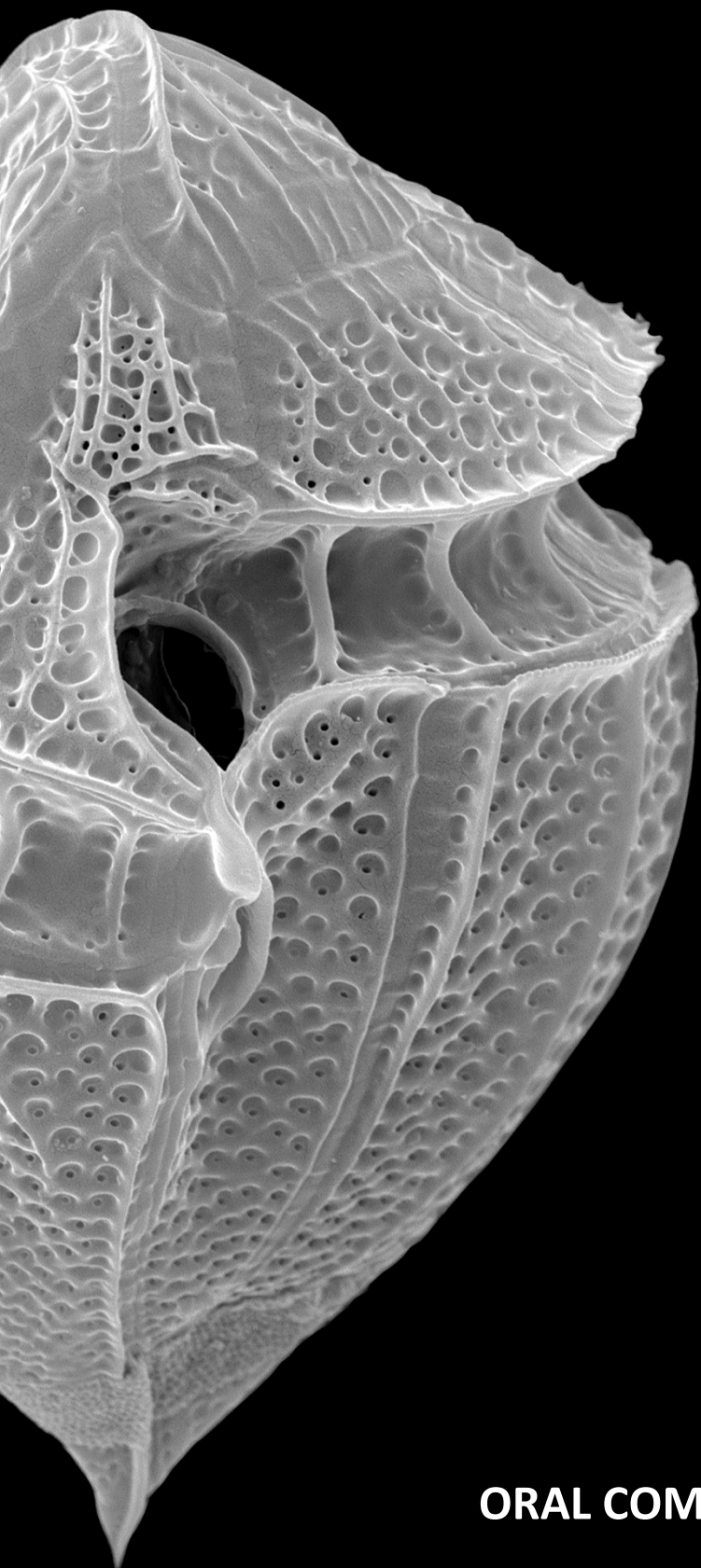
The DNA sequences of morphologically identified isolates or strains make it possible to determine the morphological diagnostic characters that are genetically supported for classification. The species of classic macrogenera such as *Gymnodinium*, *Gyrodinium*, *Amphidinium* or *Cochlodinium* have been reclassified in distinct genera establishing new generic diagnostic characters such as the shape of the apical groove. For example, the initial division of *Gymnodinium sensu lato* into four genera (*Gymnodinium sensu stricto*, *Karenia*, *Karlodinium*, *Akashiwo*) was well supported. More doubtful, however, is the further division of *Gymnodinium sensu stricto* into numerous new derived genera. For example, there is no molecular support for considering *Pellucidodinium* as distinct from *Nusuttodinium*. The main diagnostic morphological character of *Nusuttodinium* species is the presence of chloroplasts derived from Cryptophyta. A few months after the genus description, the same taxonomic authorities described a species of *Nusuttodinium* without chloroplasts. A similar example for thecate dinoflagellates is the case of *Pentaplecodinium*, which main diagnostic character is the presence of five precingular thecal plates. Months after the genus description, the same taxonomic authorities described a second species with six precingular plates. The genus *Takayama* differs from *Karlodinium* in the presence of a sigmoidal apical groove that is not evident in some species. Molecular phylogenies do not support this character because the DNA sequences of some *Karlodinium* species are closer to the type species of *Takayama*. The DNA sequences of the type species of *Brachidinium* F.J.R. Taylor 1963 and *Asterodinium* Sournia 1972 cluster with species of *Karenia* Gert Hansen & Moestrup 2000. This implies that all or at least some species of *Karenia* must be transferred into *Brachidinium*, and the family name Brachidiniaceae Sournia 1972 has the priority over Kareniaceae Bergholtz et al. 2006. Molecular phylogenies do not support diagnostic characters used for the classical generic division of unarmored dinoflagellates such as the cingular displacement or number of cingulum around the cell.

Although the proposal of *Torquentidium* as distinct from *Pseliodinium* or the placement of *Polykrikos geminatum* in a different genus are not supported in molecular phylogenies, some authors continue using the number of turns of the cingulum as a generic diagnostic character. In the case of *P. geminatum*, it is replaced by *Pseudocochlodinium profundisulcus* without a comparative study of both taxa. Other examples are the replacement of *Takayama acrotricha* by *T. xiamenensis*, the description of *Ceratocorys malayensis* ignoring *Melanodinium nigricans*, or the establishment of *Prorocentrum obtusidens* as the senior synonym of *P. shikokuense*. Almost all of these questionable taxonomic studies are published the same two journals. There is an excessive proliferation of new genera, without molecular support and/or based on unstable diagnostic characters. This contrasts with the existence of a single genus to encompass the species of *Prorocentrum* or *Alexandrium* Halim *nom. inval.* that are not monophyletic genera.

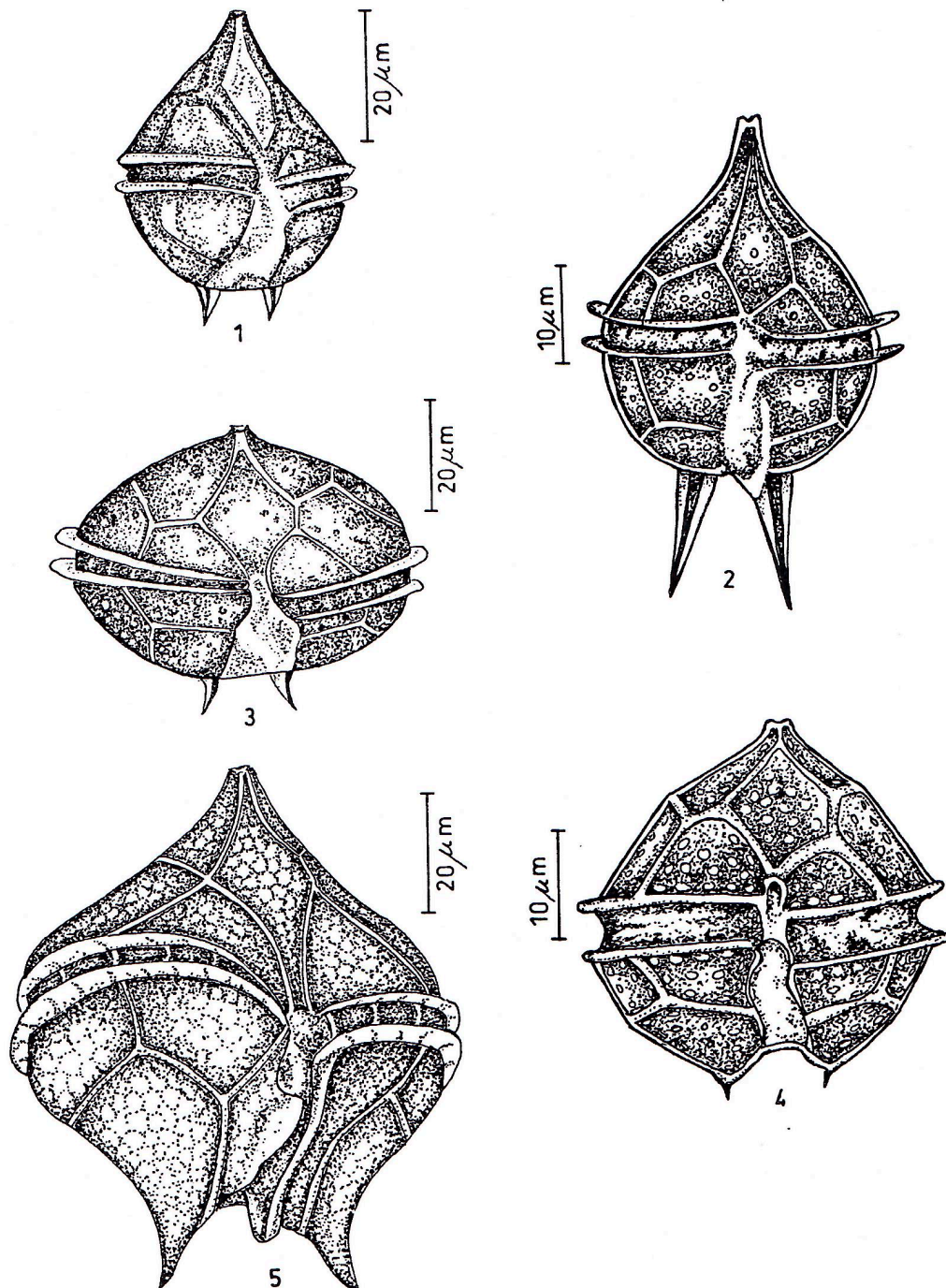
New taxonomic innovations appear recently due to questionable reinterpretations of old descriptions of common taxa. *Peridinium acuminatum* is reinterpreted as a species of the peridinioid genus *Scrippsiella*, rather than the basionym of the gonyaulacoid genus *Goniodoma*. This resulted in the reclassification of common taxa such as *Scrippsiella trochoidea* as *S. acuminata* or *Goniodoma polyedricum* as *Pyrrhotriadinium polyedricum*. The highly questionable interpretation of the identity of *Blepharocysta splendor-maris* as an earlier description of the species *Alexandrium balechii* is a source of taxonomic instability for the podolampadacean genus *Blepharocysta* and for *Alexandrium* Halim *nom. inval.*

One of the advances of the last decade is free online access to scientific literature, especially old taxonomic publications in Biodiversity Heritage Library or JSTOR. This is expected to facilitate the development of the dinoflagellate taxonomy in regions with scarce tradition such as Africa, Middle-East, India or Latin America where the diversity of dinoflagellates receives less attention than the temperate waters of the northern hemisphere. However, free access to the literature is not enough if there are no financial resources, especially for expensive tools such as molecular biology, and the knowledge of the morphological techniques. The Western Pacific region, from Indonesia to Korea, and especially China, has experienced a large increase in the publications with taxonomical innovations in the last decade. However, as reported in the previous examples, the taxonomic innovations of these multi-authored publications with an excessive over-split of the results reveal the lack of scarce scientific rigor of some journals. There is a clear decline in taxonomic expertise in North America, and partially in Europe, due in part to that the academic and research positions recruit profiles in molecular biology or bioinformatics for the study of biodiversity.

Keywords: Dinophyceae, Dinophyta, molecular phylogeny, nomenclature, taxonomy, systematics



ORAL COMMUNICATIONS
and
POSTERS SESSIONS



SESSION 1

ECOLOGY and CLIMATE CHANGE

1.01 During droughts and temperature extremes, don't assume a patch of brown cloudy water in a karst spring run is suspended. Sediment: hypoxia and hyperthermia increased within dinoflagellate blooms of rarely reported yet regularly occurring *Thompsodinium intermedium* in Comal Springs, Texas.

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Abstract: The purpose of this investigation was to determine if hypoxia (dissolved oxygen levels less than 4 mg L⁻¹) and hyperthermia (temperature levels greater than or equal to 27 degrees C) were reached during 24-hour periods more often where the planktonic form of the dinoflagellate, *Thompsonidium intermedium*, recurred at some sites daily during its 2011 summer bloom event in karst Comal Springs, Texas. Between June and October was a period of climatic extremes with high heat and lacking flow. Daily recurring stratification co-occurred in patches with daily recurring cycles of planktonic dinoflagellates. Discrete early morning water samples from 6:30 am to 9:00 am and late afternoon samples from 2:00 pm and 6:00 pm were taken 3 times a week to measure cell counts, temperature, and dissolved oxygen at 0.3 m below the surface. Temperature measurements were taken with a hand-held thermometer (standard method #2550-B APHA 2005) in the morning and evening and were used to calibrate the HOBO probes. Discrete dissolved oxygen was measured using a La Motte D.O. Kit, an azide modification of the Winkler Method (Standard Method #4500-O C APHA 2005). Where the water was turbid, a bloom was determined using a Palmer-Maloney counting chamber (standard method #10200F, APHA 2005). A planktonic dinoflagellate bloom was recorded if the cell concentration was greater than 10,000 cells mL⁻¹. Planktonic dinoflagellate cells were considered absent if less than 0.01 cells mL⁻¹. In order to verify the absence of dinoflagellate cells, a liter of water was reduced to 10 mL (Zooplankton volume reduction method: standard method # 10200G APHA 2005). T-test with a confidence level of 99% and p-value of 2.58 showed significant afternoon to morning differences in dissolved oxygen and temperature between data sets obtained inside and outside blooms. Morning hypoxia was found in 41% of bloom vs 1% of non-bloom sites. Afternoon Hyperthermia was found in 68% of bloom vs 11% of non-bloom sites.

Key words: *Thompsodinium intermedium*, climate extremes, hypoxia, hyperthermia

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1.02 Ecological properties of the green seawater discoloration forming dinoflagellate *Lepidodinium chlorophorum*: from cellular ecophysiology to ecosystem impacts.

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Abstract: Blooms of the dinoflagellate *Lepidodinium chlorophorum*, known to produce Green Seawater Discolorations (GSD) in southern Brittany (NE Atlantic, France), have been associated with mass mortalities of cultivated bivalves. Despite this important effect of the ecosystem, few information is available about the biology and ecology of this species. This work allows the elaboration of a first ecological conceptual model of the species, which connects the cellular to the ecosystem scale analyses. *L. chlorophorum* is the main producer of Transparent Exo-polymeric Particles (TEP) within the cultivated dinoflagellate-bacteria consortium. Bacteria have a negative effect on the growth of *L. chlorophorum* and induce an overproduction of TEP by this dinoflagellate. This production of viscous and carbon enriched material is a main biological characteristic of the species. Bloom phenology was studied *in situ* during two phases: the initiation and advanced (GSD) conditions. Bloom initiation was studied during the non-productive (autumn-winter) period using the metabarcoding of the environmental DNA (eDNA). In the water column, the genus *Lepidodinium* was detected at very low abundances and increases in abundance were correlated with ammonium inputs after sediment resuspension. Rare *Lepidodinium* eDNA was detected in the sediment and any benthic cysts were observed by microscopy and germination experiments, questioning the existence of resting stages in the sediments. The advanced bloom phase was studied in July 2019 during a GSD that occurred in Vilaine Bay. Freshwater inputs and thermal stratification were favorable to the establishment of a GSD. Remote sensing highlighted the importance of tidal currents in the surface spatial dynamics of the GSD. Bacteria, through the remineralization of TEP present within the GSD, is supposed to regenerate nutrients, sustaining bloom development for more than one month. Therefore, TEP might correspond to an ecological benefit for this dinoflagellate. However, such a high amount of organic matter could have negative environmental impacts, causing for instance negative effects on cultivated bivalves. To investigate this aspect, the ecophysiological recovery of the oyster *Crassostrea gigas* exposed to *L. chlorophorum* GSD concentrations was studied under controlled experimental conditions. The ingestion of dinoflagellate cells by oysters was shown, but the production of acid glycoconjugates by

digestive tubules, an impaired filtration and reduced assimilation were suggested in the animals analyzed previously exposed to *L. chlorophorum*. These results suggest that a

high concentration of *L. chlorophorum* and probably TEP might have a deleterious effect on oysters during long-term exposure to bloom conditions. These first insights into the ecological properties of *L. chlorophorum* in southern Brittany and the impacts generated on bivalves constitute the baseline for further studies in other ecosystems impacted by blooms of this species.

Keywords: *Lepidodinium chlorophorum*, Harmful algal bloom, Ecophysiology, Transparent exo- polymeric particles

Acknowledgments: The authors wish to thank the different laboratories implicated on the project for their technical and scientific support.

Project: This work was carried out in the frame of the PhD of Pauline Roux (financed by Ifremer and the Region Pays de la Loire). It was financed by Ifremer (project CLOCCLO), the Region Pays de la Loire (project LEPIDO-PEN [06582 2019]) and the Agence de l'Eau Loire-Bretagne (project EPICE [1804088001]) and was supported by the GDR PHYCOTOX, a CNRS/IFREMER network on Harmful Algal Blooms (<https://www.phycotox.fr/>) (project PICCOLO).

1.03 The effect of temperature and light on the growth dynamics of the harmful dinoflagellate *Margalefidinium polykrikoides*

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Abstract: *Margalefidinium polykrikoides* (formerly *Cochlodinium polykrikoides*) is a mixotrophic harmful dinoflagellate that has bloomed in the York River for over 50 years and in the lower James River and its tributaries for over 25 years. *M. polykrikoides* is known for causing mortality to juvenile fish such as *Cyprinodon variegatus* and juvenile oysters (*Crassostrea virginica*), an iconic Chesapeake Bay species around which restoration efforts have been focused. *M. polykrikoides* blooms usually initiate in the lower Chesapeake Bay when water temperatures reach around 26° C. However, in other parts of the world blooms occur when water temperatures are as low as 20 C. The lower Chesapeake Bay is warming at a rate twice that of the upper Bay due to climate change and warming could potentially alter the timing of *M. polykrikoides* blooms. Because *M. polykrikoides* is mixotrophic and the estuary eutrophic, the availability of light may be key to the ability of this organism to bloom in the Chesapeake Bay. Here we present results from culture experiments examining temperature growth kinetics of *M. polykrikoides* at two irradiances. Also, we examine the photosynthesis rates, respiration rates, and pigment concentrations of cultures acclimated to two irradiance and five temperature treatments. Temperature treatments include a range of temperatures where *M. polykrikoides* blooms have been observed, and lower and upper boundaries where they are thought to survive. Light treatments ranged from irradiances observed from the bottom to the top of the euphotic layer during summer, when blooms occur, during both cloudy and sunny conditions. Growth rates were calculated using the logistic growth equation. Pigments were identified using a spectrophotometer and then concentrations calculated using spectrophotometric equations. Photosynthesis and respiration rates were calculated during the exponential phase of growth using an oxygen electrode. Results show that *M. polykrikoides* grow optimally over a broad range of temperatures (20 - 32 °C) and two irradiances (140 and 720 $\mu\text{E m}^{-2} \text{s}^{-1}$). Cultures acclimated to high irradiance were photo inhibited, resulting in a decrease of the photosynthesis maximum rate relative to cultures acclimated to low irradiance. Cultures acclimated to low irradiance contained higher chlorophyll "a" and "c" per cell ratios than cultures acclimated to high irradiance. However, there was not a significant difference between carotenoid per cell ratios of cultures acclimated to high and low irradiance. Mycosporine-like amino acids were identified in the UV side of the absorbance spectrum and their absorbance was higher in cultures acclimated to high irradiance. The respiration rates of the cultures increased with temperature. This study shows that *M. polykrikoides* is a versatile harmful dinoflagellate capable of acclimating to a broad range of environmental conditions, which allows them to conquer the Chesapeake Bay during the summer and fall.

Keywords: *Margalefidinium polykrikoides*, Harmful algal blooms, Temperature growth kinetics, Photosynthesis, Respiration, Chesapeake Bay

Acknowledgments: This work was supported by grants from NOAA ECOHAB [grant number NA18NOS4780176]

Project: “ECOHAB 2018: Toward a predictive understanding of *Cochlodinium* and *Alexandrium* blooms in lower Chesapeake Bay.”

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1.04 Playing hide and seek: distribution with depth of potentially harmful epibenthic dinoflagellates of southern El Hierro island, Canary Islands (NE Atlantic)

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Abstract: The study of epibenthic assemblages of harmful dinoflagellates (BHABs) is commonly conducted in shallow infralittoral zones (0 – 5 m) and is seldom investigated at deeper waters. In this study, the distribution with depth of five BHAB genera (*Gambierdiscus*, *Ostreopsis*, *Prorocentrum*, *Coolia* and *Amphidinium*) was investigated in the south of El Hierro Island (Canary Islands, Spain). Sampling involved the use of a standardized artificial substrate deployed at three depth levels (5, 10 and 20 m) that were visited at three different times throughout one year. The influence of three depth-correlated abiotic parameters, i.e. light, water motion and water temperature, on the vertical and seasonal distribution of the BHAB assemblage was also assessed. Two vertical distribution patterns were observed consistently through time: cell abundances of *Ostreopsis* and *Coolia* decreased from 5 to 20 m while those of *Gambierdiscus*, *Prorocentrum* and *Amphidinium* showed the reverse pattern, although significant differences were only observed between 5 and 10 - 20 m depth. In April, two members of the latter group, *Gambierdiscus* and *Amphidinium*, were even absent at 5 m depth. The recorded environmental parameters explained a high percentage of the observed distribution. In particular, model selection statistical approaches indicated that water motion was the most significant parameter. An analysis of *Gambierdiscus* at species level revealed the co-occurrence of four species in the study area: *G. australes*, *G. belizeanus*, *G. caribaeus* and *G. excentricus*. The species *G. excentricus*, reported here for the first time in El Hierro, showed a more restricted vertical and seasonal distribution than the other species, which may explain not being detected in previous studies in the area. The results obtained in this study highlight the importance of considering a wider

depth range and different seasons of the year when investigating the ecology of BHAB sand assessing their risk and impacts on human health and the environment. Only then, efficient monitoring programs will be implemented in the Canary Islands and globally in areas affected by these events.

Keywords: Vertical distribution, BHAB dinoflagellates, Canary Islands, Artificial substrate

Acknowledgments: This work was funded by MIMAR Project (MAC/4.6d/066) under the Interreg MAC 2014-2020 Programme.

Projects: MIMAR Project (MAC/4.6d/066) and (MIMAR+) (MAC2/4.6d/249) Interreg MAC 2014-2020 Programme.

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1.05 Fifty shades of red tide: assessing the remote-sensing reflectance fingerprint of highly-concentrated coastal harmful algal blooms

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Abstract: Harmful algal blooms (HABs) caused by dinoflagellates are frequent in the coastal ocean and can have severe environmental and economic impacts either by the production of toxins or by causing anoxia. Monitoring HABs is a major concern; it is however challenging because phytoplankton diversity and biomass can change over short time scales and display patchy distribution. Complementary to in situ phytoplankton measurements, remote sensing makes it possible to observe blooms worldwide over a wide range of spatial and temporal scales. While satellite maps of chlorophyll-a concentration are operationally produced and distributed by space agencies, the discrimination of the dominant bloom-forming phytoplankton species is still a challenge. The majority of HAB remote sensing studies generally had a regional focus, and only few bloom-forming species were separately documented so far. Here, we provide a broader perspective for the satellite assessment of phytoplankton blooms, in order to better resolve HAB optical and taxonomical diversity. Our main objective was to assess, in the case of highly concentrated blooms, the ability of remote sensing to discriminate dinoflagellates from other causative species, as well as to identify which groups of dinoflagellates could be distinguished from satellite data.

For that purpose, a worldwide database of high-biomass and quasi mono-specific blooms both documented in situ and using synchronous satellite observation has been compiled. By searching bloom events in the HAEDAT and REPHY records, about 120 Sentinel-2 (S2) images were selected in 22 countries, covering 29 different bloom-forming species. The optical characteristics of each record were analyzed in order to evaluate S2 ability to distinguish bloom optical types. A hierarchical clustering analysis suggested that at least six bloom types could be identified. One optical type corresponded to blooms dominated by cyanobacteria such as *Trichodesmium*,

Nodularia, *Aphanizomenon*, or *Microcystis*. Four other optical clusters corresponded to blooms dominated by dinoflagellates, with possible discrimination between the red and green forms of *Noctiluca scintillans*, as well as between species of distinct pigments type (i.e. typical peridinin-bearing species such as *Alexandrium minutum* or *Prorocentrum micans* could be distinguished from chlorophyll-b bearing *Lepidodinium chlorophorum*). Interestingly, the ciliate *Mesodinium rubrum*, an important prey for *Dinophysis*, could be discriminated from other types of HABs. While the current database is inherently limited to the available concomitant in situ and satellite datasets, it aims at shedding light on the visible tip of coastal eutrophication amenable to satellite detection. Improving remote sensing capability to detect HABs is crucial because coastal blooms are likely to increase in both frequency and amplitude in the next decades due to escalating nutrient runoffs derived from human activities.

Keywords: satellite, remote sensing, red tides, harmful algal blooms

1.06 Tracing the origin of *Gymnodinium catenatum* blooms in central west Iberian shelf

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Abstract: In West Iberian coastal waters, the detection of Paralytic Shellfish Toxins (PST) is related to the occurrence of blooms of *Gymnodinium catenatum*. The life cycle of this species includes a benthic resting cyst that needs no pre-conditioning for maturation and is characterized by having a very short mandatory dormancy period (a few days). Possibly because of these characteristics live cysts are usually found at very low concentrations in surface sediments and their role as a seed source for planktonic blooms is frequently questioned. In this study, Lagrangian experiments were carried out to investigate the possible contribution of shelf cyst beds for the initiation of *G. catenatum* blooms observed in central W coast of Iberia (Aveiro) in late-spring to early-autumn of 2008, 2009 and 2010. Results from laboratory experiments on the influence of temperature and light on cyst germination and germling viability, and published data on the exponential growth rate of the vegetative stage as a function of temperature, were used to parameterize a computationally simulated particle model.

A state-of-the-art hydrodynamic model, ROMS-CROCO, and an offline Lagrangian model (ROFF) were used to obtain the hydrodynamic fields, consisting in temperatures and velocities and: a) forward in time offline integration of the trajectory of the particles from the nepheloid region (fine and muddy sand patches with depths shallower than 125m) to the euphotic region, on the West coast of the Iberian Peninsula; b) backward in time integration, from a target region in order to determine the origin of the particles. Throughout the forward trajectory, the life cycle transitions of *G. catenatum* were simulated considering the model temperatures and particle depth. The results were

compared with weekly time series data (2008-2010) on harmful phytoplankton (HAB) from the national harmful algae monitoring program (IPMA).

The model captured well the timing of several of the blooms observed in Aveiro supporting the hypothesis of cysts as a potential seed source for planktonic blooms. However, in September 2009, a significant mismatch between observations and model results occurred.

This was further investigated running backward trajectories to unveil the origin of the observed blooms. The results were compared with time series observations (2008-2010) from the Portuguese HAB monitoring program, and from the Galician Rias INTECMAR monitoring program.

The model results qualitatively explained part of the observed blooms and unravel the role of the physical mechanisms in the initiation and transport of *G. catenatum* blooms. Comparison between model results and observations for the three-year period indicate no single source can be considered when investigating the origin of *G. catenatum* blooms in the Aveiro region. Both cyst beds and planktonic populations must be considered to fully capture the intra-annual variability.

Keywords: bloom initiation, cysts, Lagrangian model, physical-biological coupling, HAB

Acknowledgments: HABWAVE project LISBOA-01-0145-FEDER-031265, co-funded by EU ERDF funds, within the PT2020 Partnership Agreement and Compete 2020, and national funds through Fundação para a Ciência e Tecnologia, I.P. (FCT, I.P.). Funding by FCT, I.P. through the strategic projects UIDB/04292/2020, UIDB/04326/2020 and UIDP/50017/2020 + UIDB/50017/2020.

Project: HABWAVE, From benthic resting stages to HAB events: understanding the role of physical-biological coupling off NW Portugal (LISBOA-01-0145-FEDER-031265).

1.07 Phagotrophy of bloom-forming dinoflagellates under different light and nutrient conditions

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Abstract: Mixotrophic phytoplankton are organisms that combine the uptake of nutrients through photosynthesis, osmotrophy and phagotrophy (i.e., ingestion and digestion of living prey and/or particles) in the same cell. These flexible organisms, ubiquitous in marine environments, can increase the carbon transfer efficiency to higher trophic levels and the oceanic carbon storage, resulting in major effects on biogeochemical cycling (Mittra et al., 2014; Ward and Follows, 2016). The strict line between autotrophic and heterotrophic organisms has been blurred since the study of mixotrophy, which has caused a paradigm shift in our understanding of the marine foodweb in the last decades (Hartmann et al., 2012). Although several studies showed that phagotrophy can sustain growth or survival of mixotrophs under nutrient and/or light limitations (Anderson et al., 2018; Carvalho and Granéli, 2010), our knowledge on the relevance of phototrophy vs. phagotrophy in the wide range of mixotrophic phytoplankton is still scarce, and the influence of environmental factors on their mode of nutrition remains unclear.

In this study we compared, for the first time, the growth and phagotrophy of three common bloom-forming dinoflagellates under contrasting light and nutrient conditions. We acclimated *Alexandrium minutum*, *Heterocapsa triquetra* and *Prorocentrum micans* to high (150 μ E) and low (20 μ E) light intensities and to replete (medium K) and phosphate-limited (medium K/3) nutrient conditions. Phagotrophy was investigated for the different light and nutrient treatments using three different sizes of fluorescently labelled preys: bacteria (~0.7 μ m; FLB), a cyanobacteria, *Synechococcus* (~1 μ m; FLS), and a small algae, *Isochrysis galbana* (~6 μ m; FLA). We quantified ingested preys and/or digestive vacuoles at different times after prey addition using a combination of epifluorescence microscopy and flow cytometry.

In general, the three dinoflagellates showed higher phagotrophy in the low-light nutrient-replete treatment, with higher ingestion rates on FLS (10-20% of *H. triquetra* and *P. micans* cells feeding, and up to 6% of *A. minutum* cells feeding). In phosphate-limited conditions, dinoflagellates had higher ingestion rates under the high-light treatment. Low light intensity alone clearly induced phagotrophy in the three species, whereas nutrient limitation influenced phagotrophy differently in the three dinoflagellates showing different prey size

preferences. Light and nutrient co-limitation did not imply higher decrease of growth rate or higher ingestion rates compared to the other treatments in none of the dinoflagellates. Overall, our results suggest that phagotrophy can be highly variable depending on the type of environmental limitation but also on the available prey. Although the three species are capable of phagotrophy, *A. minutum* showed more dependence on photosynthesis for growth, whereas *H. triquetra* and *P. micans* would have greater nutritional flexibility.

Altogether, our results reveal contrasting mixotrophic lifestyles for the three dinoflagellates, suggesting different light and nutrient requirements. These findings highlight the importance of continuing our efforts to define the mixotrophic lifestyles of different groups, genus or species of phytoplankton in order to improve our understanding and prediction of oceanic carbon storage.

Keywords: mixotrophy, phagotrophy, light limitation, nutrient limitation, dinoflagellates, nutritional strategies

Project: EXAM - Importance of phagotrophy for *Alexandrium minutum* under different environmental conditions (P302-0070-01-MS). Funded by IFREMER and Région Bretagne. PI: A. Regaudie de Gioux (IFREMER, France).

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1.08 HOBO light/temperature probes shed light on simultaneous stratification patterns and diel cycling dynamic of dinoflagellate benthic to planktonic life stage transitions not previously reported in the scientific literature on dinoflagellates

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Abstract: Relatively few physical ecology impacts and environmental associations outside of nutrient availability and uptake and toxin production have been reported in literature regarding monitoring during bloom events of most dinoflagellate species. However most dinoflagellate blooms are triggered instantaneously while nutrient triggered blooms of other types of phytoplankton have been shown to be dependent upon time and rates. Stratification of water also occurs instantaneously.

Much about the general bloom dynamics of the dinoflagellate group of phytoplankton remains a mystery. Logistically, dinoflagellate blooms are difficult to monitor on large oceanic and limnological spatial and temporal scales. However, a dinoflagellate bloom daily recurring in a karst spring run in Texas provided an opportunity for documenting the close relationship between daily stratification and mixing patterns and diel patterns of cycling between planktonic and benthic stages of a dinoflagellate *Thompsodinium Intermedium* on a much smaller scale in the field for the first time. These observed close relationships between dinoflagellate blooms with stratification or boundary layer formations in the spring run may apply at larger scales in lakes and oceans.

Continuous monitoring with HOBO light intensity and temperature probes at two depths below the surface were used to determine diel dinoflagellate bloom dynamics in relation to stratification patterns in a Karst Texas spring run during periods of combined extremes of atmospheric temperature and lack of flow.

Keywords: Dinoflagellate life stage transitions, climate extremes, HOBO light temperature probes, stratification, *Thompsodinium intermedium*

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1.09 Dinoflagellate cyst production, excystment, vertical transport and lateral dispersion in surface waters and the deeper water column off Cape Blanc (NW Africa)

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Abstract: Dinoflagellate cysts play an important role in the life cycle, dispersal and long-term survival of many dinoflagellate cyst species. Several cyst-producing species can be associated with harmful algal blooms (HAB) and their dispersal forms a major economic and health threat (Figuerola et al., 2018). It is therefore important to obtain detailed insight into the transport/dispersal of dinoflagellate cysts from their production in the upper water column to their embedding in the sediment. Apart from being of major importance for HAB studies, information about production and vertical/lateral transport of dinoflagellate cysts in the water column is of major importance for dinoflagellate cyst reconstructions of palaeoclimate, -oceanography and -environment as well (e.g. García -Moreiras et al., 2018). Although over the last years, information increased about cyst dispersal due to human activities (e.g. Rodríguez-Villegas et al., 2020) as well as natural dispersal in coastal settings (e.g. Brosnahan et al., 2017) extremely limited information exists on cyst dispersal in the open ocean.

Here we extend the understanding of dinoflagellate cysts production, succession, excystment, vertical transport and lateral dispersal, by presenting the results of drifting trap- and in-situ pump studies performed in the upwelling region off Cape Blanc (NW Africa) at times of maximal and minimal upwelling intensity in November 2018 and August 2020 respectively.

We performed three drifting trap surveys of several days' duration in an active upwelling cell (DTS1), at the outer rim of an active upwelling cell (DTS2) and in an offshore drifting upwelling filament (DTS3). Cyst associations and concentrations have been compared to particles collected from in subsurface- and bottom nepheloid layers as well as the deeper waters collected by in-situ pump systems. These samples have been collected along a transect perpendicular to the coast from the region of active upwelling near the shelf break, via the region where filaments of upwelled surface waters drift offshore towards the open ocean (Fig. 1).

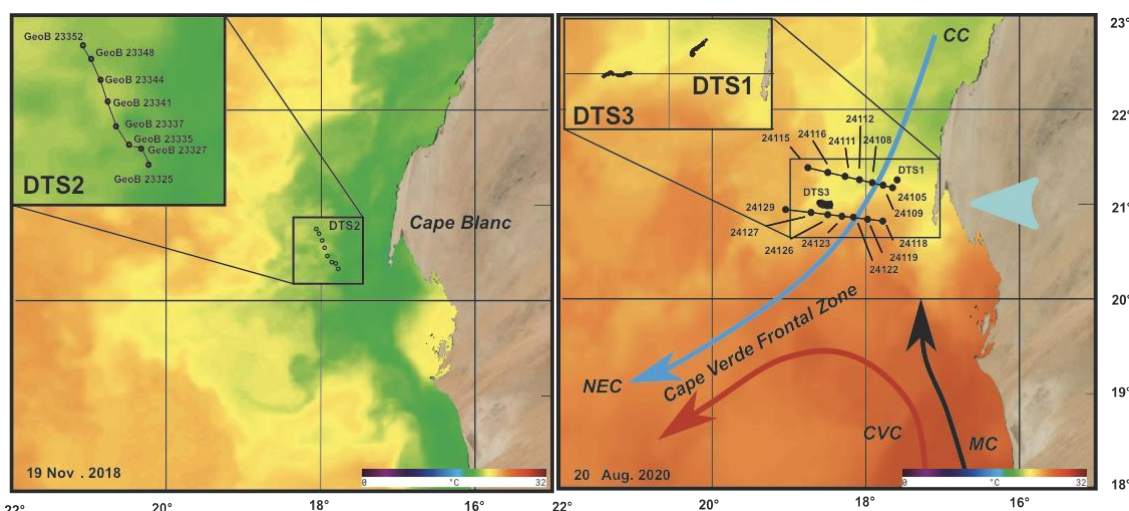


Fig.1. Satellite derived surface water temperature in the research area with major upper water current systems and station positions (satellite image with courtesy of State of the Ocean: <https://podaac-tools.jpl.nasa.gov/soto/>). CC = Canary Current, NEC = North Equatorial Current, CVC = Cape Verde Current, MC = Mauritania Current. Blue arrow represents the direction of the Sahara air layer. Redrawn from Zonneveld et al., 2021.

We will show that during DTS1 light limitation hampered cyst production in the center of active upwelling cells where up- and downward movement of water masses occurred. Cyst (export) production increased when the trap drifted from the central part of the upwelling cell, towards the stratified waters at the rim of the upwelling cell. When waters started to drift offshore in the form of an upwelling filament with even more stratified waters (DTS2) cyst (export) production decreased (Zonneveld et al., 2021). Cyst export production in the offshore upwelling filament (DTS3) was up to a factor 3 lower than that in the active upwelling.

Resuspension of cysts from shelf sediments was only observed in November 2018 but could not be registered in August 2020 indicating that resuspension of shelf sediments is not a permanent process. Cyst association composition in the upwelling cells were dominated by *Echinidinium zonneveldiae* in 2018 and *Echinidinium delicatum* in 2020. In 2020 relative abundances of *Echinidinium zonneveldiae* and *Bitetadodinium spongium* were high in the upwelling filament. In both years and seasons *Impagidinium* spp. and cysts of *Gymnodinium microreticulatum/nolleri* had highest abundances at the most distal stations of the transect.

Comparing concentrations of cysts with and without cell contents showed that in both years and seasons the majority of cysts hatched before reaching deeper waters, displaying a dormancy period of less than 6 days. About 5% of the living cysts reached deeper waters and/or the ocean floor where they may have functioned as a seed bank. Living cysts in the surface waters might form a major factor in the dispersal of cysts in upper waters as we observed major offshore transport of cysts within surface water upwelling filaments (Fig.2). This observation might have economic implications. In caseships exchange ballast waters in the studied region, there is a high risk they take up laterally transported living cysts that originate from the coast. When released in the port of arrival, they are potential “invader species” that can threaten the economy and/or health.

Lateral cyst transport was also observed in the bottom nepheloid layers and in deeper waters between about 800 m - 1200 m water depth to a maximal extension of about 130km off the shelf break. This implies that downcore sediments in the region will contain a regional signal rather than a local signal. This insight contributes to the improvement environmental reconstructions of the Cape blanc upwelling system based on downcore cyst associations.

Keywords: dinoflagellate cysts, fluxes, succession, encystment, dispersal, ecology, upwelling

Acknowledgments: We thank captain Detlef Korte and the crew of the R.V. METEOR for the professional support and excellent cooperation during cruise M165. We especially thank Götz Ruhland, Marco Klann, Hendrik Grotheer and Kristina Kotzem for their support during sampling. We greatly appreciate the support, excellent cooperation and flexibility of the head office Gutachterpanel Forschungsschiffe (GPF), the German Research Fleet Control Centre (LDF) and Bries Research Forschungsschiffahrt that provided us this unique possibility to execute the research cruise M165 in Corona Pandemic times. The expedition was funded by the Deutsche Forschungsgemeinschaft GPF 18-1_18. The financial support in the form of scholarships and positions of participants of the cruise was provided by the Deutsche Forschungsgemeinschaft GPF 18-1_18 and the MARUM Cluster of Excellence "The Ocean Floor – Earth's Uncharted Interface".

Project: PostDoc Project of Gerard Versteegh (2020-2022) GPF 18-1_18 and the MARUM Cluster of Excellence "The Ocean Floor – Earth's Uncharted Interface" Receiver Unit 3.

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1.10 Seasonal, annual and multi-annual variability of dinoflagellate cyst export production in the upwelling area off Cap Blanc based on a 18 years sediment trap time series

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Abstract: Dinoflagellate resting cysts in marine sediments archives form a useful tool to reconstruct past changes in marine environments (e.g. de Vernal et al., 2020). Embedded in the sediments they can be vital for time intervals up to a century (e.g. Ellegaard and Ribeiro, 2018). Especially sedimentary “seed banks” of cysts of harmful algal bloom species form a potential risk for future hazards (Figueroa et al., 2018). An adequate paleo-environmental interpretation of fossil assemblages depends on our knowledge of their ecology and factors that influence their production on seasonal to multi-annual resolution. The same holds for studies on the production and bloom dynamics of harmful cyst producing dinoflagellates. Here, we aim to enhance the ecological knowledge of organic-walled cyst forming dinoflagellates and provide information of factors that influence the cyst export production on seasonal, annual and multi-annual scales based on a study of 18 years sediment trap record obtained from the Cape Blanc upwelling system. This upwelling system is one of the Eastern Boundary Upwelling Ecosystems (EBUEs), known as one of the most productive regions in the world. Surface waters are characterized by year-round upwelling of intermediate waters into the photic zone (Fig. 1b), as well as frequent input of Sahara dust, both fertilizing the surface ocean (Lathuilière et al., 2008; Cropper et al., 2014). The upwelled waters can be laterally transported offshore in the form of eddies and filaments.

The organic cysts were studied in material collected by a sediment trap located under the filament track of the main upwelling cell at the depth around 1300 m (Fig. 1). The trap collected sinking particles since 2003 until 2020, with the range of 1-3 weeks sampling intervals. A 1/125th split of each sample was sieved over 20 µm high precision sieve (Storck-Verco) with tap water. A known aliquot of every sample was embedded in glycerin gelatin and sealed with a cover slip on a palynological slide. The quantification and identification of dinoflagellate organic-walled cysts was performed under light microscopy and referred to the determination of Zonneveld and Pospelova (2015), Mertens et al. (2020), and van Nieuwenhoven et al. (2020). Both variability in export flux (cysts/m²/day) and relative abundances of cysts were compared with seasonal, annual, and multi-annual variability in local environmental parameters that influence the Cap

Blanc oceanographic environments. The parameters include: sea surface temperature (SST) difference between the trap site and an open ocean location 200nm west of the trap location (SSTa), Chlorophyll-a concentrations at the trap site, local wind speed, local wind direction and dust-storm events. Information about the local wind system and dust events were obtained from the meteorological report of Nouadibhou airport (Cap Blanc). Data of SST and Chlorophyll-a were extracted from ERDAPP satellite observation (<https://coastwatch.pfeg.noaa.gov/erddap/griddap/ncdcOisst2Agg.html>). Correlation has been performed using the multivariate ordination method of Canonical Correspondence Analysis (Jongman et al., 1995).

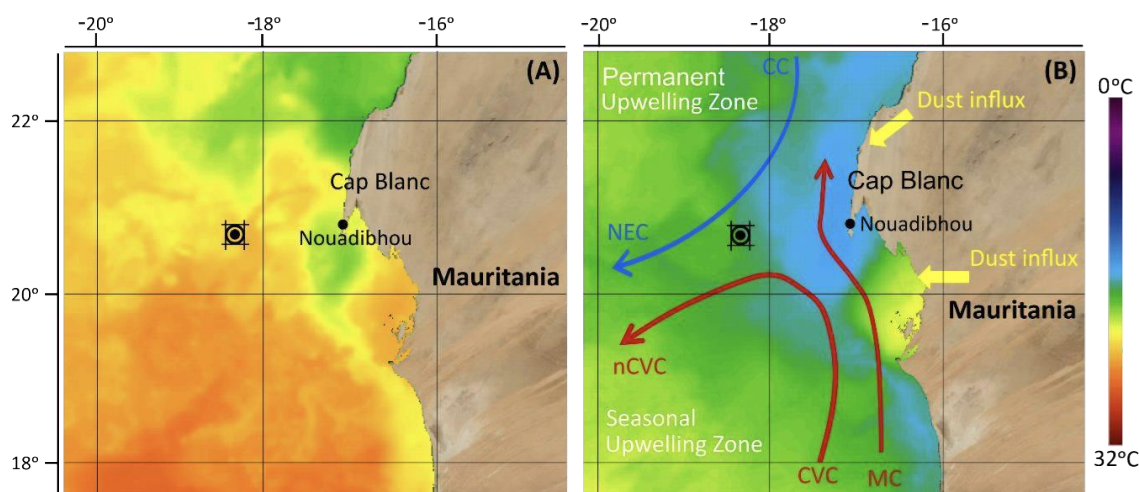


Fig. 1. Location of CBeu sediment trap (double black circle) and oceanography of studied area. Blue arrow indicates cold water of Canary Current (CC) and North equator Current (NEC); red arrows indicate warm water of polarward Mauritanian Current (MC), Cabo Verde Current (CVC), and north Cabo Verde Current (nCVC). Satellite image depicted from NASA "State Of The Ocean (SOTO)" of sea surface temperature (SST); (a) low upwelling intensity on November 24th, 2020, (b) high upwelling intensity on March 24th, 2021.

The organic-walled cysts association is dominated by the heterotrophic dinoflagellates with photo-/mixotrophic species forming only around 6% of the total cyst. Maximum cyst export production was observed at times of high upwelling intensity in spring- summer. During these intervals, the cyst association was dominated by cysts of *Brigantidium* spp. and *Echinidium* species. Both cyst export production and relative abundance of cysts of the photo-/mixotrophic species slightly increased at times of upwelling relaxation (weak upwelling intensity). *Lingulodinium machaerophorum* and cysts of *Gymnodinium* species are specifically observed at times of weakening upwelling. Our long-time record shows large signal of annual and inter-annual variability. We observed that the cysts associations composition of individual upwelling episode was variable. Although the occurrence of several species was bound to intensive upwelling and can be characterized as typical upwelling species, they were not present in every upwelling event. Additionally, *Lingulodinium machaerophorum* and cysts of *Gymnodinium* were typically observed at times of upwelling relaxation, they were not present at the end of every upwelling event and only sporadically showed largely enhanced absolute and relative abundances. We assume this to be the result of interspecies competition. These results also clearly show that the studied system is

highly variable and information about the ecology of cyst forming species can only be achieved when long time series are being studied.

On a multi-annual scale, we observe a consisting association change around 2009. Upwelling events prior to 2009 were dominated by *Brigantidinium* spp. with a varying number of cysts of *Protoperidinium americanum*, *P. monospinum*, and *Stelladinium stellatum*. After 2009 the cyst associating to upwelling events was characterized by *Echnidinium* species, notably the species *Echinidinium aculeatum*, *E. delicatum*, *E. granulatum*, *E. transparentum*, and *E. zonnaveeldiae*. This change in association is contemporaneous with an increase of dust input into the coastal region. Whereas dust input in the region occurred seasonally during winter - spring, it became a more permanent character with maxima both in winter and summer from 2011. From this year an increase in Chlorophyll-a in surface waters at the trap site can be observed. Within the dinoflagellate cyst assemblage, we observed higher export production and relative abundances of species that in several regions of the world could be linked to the presence of anthropogenic induced pollution (e.g., Krepskeovich and Pospelov, 2010; Pospelova and Kim, 2010; Zonneveld et al. 2013). We assume that these species; *Polykrikos kofoidii*, *P. schwartzii*, *Quinquecuspis concretum*, and *Votadinium calvum* reflect a eutrophication of the Cape Blanc upwelling ecosystem.

Keywords: dinoflagellate cyst, ecology, upwelling seasonality, Saharan dust, ecosystem changes.

Acknowledgments: We would like to thank the captains and crew members of RV Poseidon, RV METEOR, and RV Maria S. Merian, as well as The MARUM sediment trap team for deploying and recovering the sediment trap. We are also thankful of the support from German, Moroccan, and Mauritanian authorities. This extensive research is supported by the funding from German Research Foundation (DFG) through MARUM Excellence Cluster "The Ocean in the Earth System". Lastly, we thank all institutions and individuals who have participated and contributed throughout this research.

Project: Doctoral Project of Surya Eldo V. Roza (2020-2023): Natural and Anthropogenic forcing on marine pelagic ecosystems.

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1.11 Spatial distribution of dinoflagellate cysts along an inshore-offshore transect off Cape Blanc (NW Africa)

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Abstract: Resting stages (cysts) produced by dinoflagellates act as passive particles that sink to the bottom sediments of the ocean. Benthic cysts can play an important role in the species' dispersal and bloom dynamics, as well as in their long-term survival (Pilska et al., 2014). The sediment cyst record constitutes a good palaeo archive of the history of the species (Ellegaard et al., 2017). However, oceanographic processes such as transport, resuspension and selective degradation can bias the interpretations of the fossil cyst record (Zonneveld et al., 2008). Studying the factors that affect the distribution and accumulation patterns of dinoflagellate cysts in the water column and in the sediments can help to better interpret the signal left by cysts in the sediments. Off Cape Blanc (NW Africa) upwelled waters near the coast can be transported offshore in the form of filaments, which enhance the productivity in the open ocean as they transport nutrient-rich surface waters to the oligotrophic offshore regions. Within the region nepheloid layers occur, water layers that show enhanced particle concentrations. Whereas subsurface nepheloid layers (SNL) located just below the offshore drifting upwelling filaments and bottom nepheloid layers (BNL) above the ocean floor are permanently present, nepheloid layers at intermediate water depths (INL) have a more sporadic character. It is assumed that particles forming the INL and BNL originate at the shelf/shelf edge in the active upwelling region. Furthermore, it is assumed that they form key mechanisms for the transport of organic particles into the deeper ocean (Oliveira et al., 2002; Zonneveld et al., 2018a). To what extent dinoflagellate cysts are being transported offshore within these layers is so far largely unknown.

The main motivation of this study was to contribute to filling the knowledge gaps that still exist in relation to lateral and vertical transport and accumulation patterns of dinoflagellate cysts (and other organic particles) in the ocean and the factors influencing them. The composition and abundances of dinoflagellate cysts were analysed in 36 samples collected within and between nepheloid layers at 10 stations along an inshore-offshore transect off Cape Blanc (Fig. 1). Samples were collected at different depths using in-situ pumps (ISP).

The cyst records from the water column were compared to 9 samples from the underlying sediments collected along the same transect. The position and thickness of NL have been investigated along the same transect from CTD profiles.

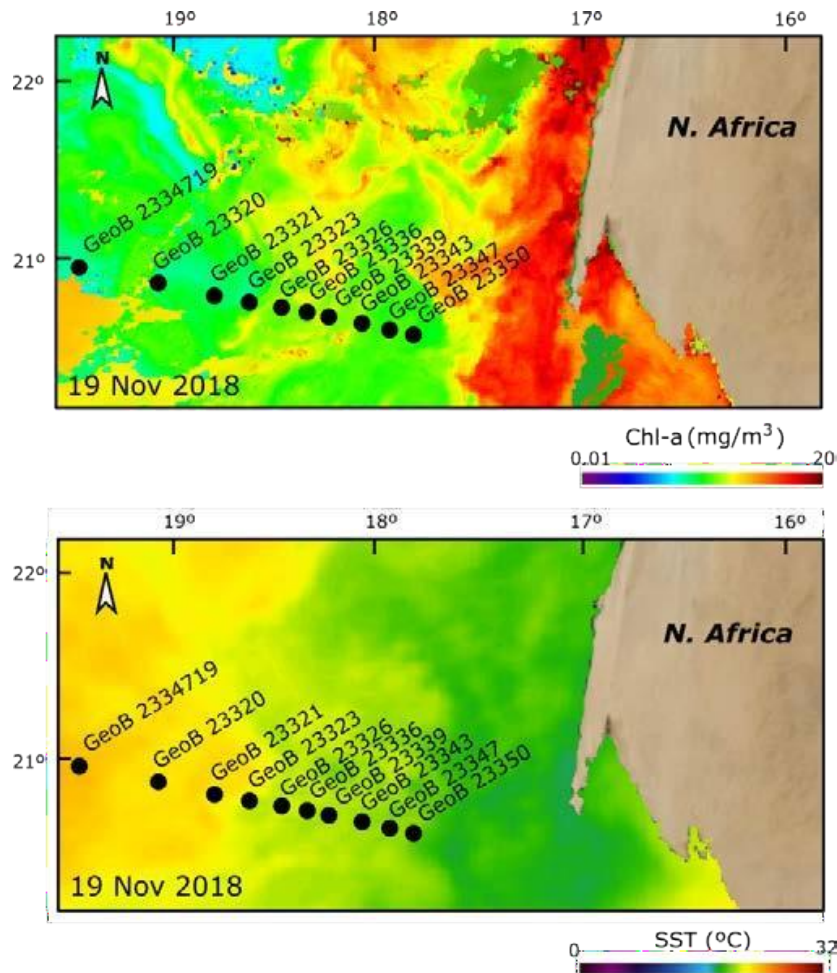


Fig. 1 Satellite-derived chlorophyll-a and surface water temperatures (SST) off Cape Blanc (NW Africa) ('State of the Ocean' website: <https://podaac-tools.jpl.nasa.gov/soto/>) and the position of the sampling stations for NL and surface sediment samples.

In the study area, CTD profiles showed a well-stratified water-column with warm temperatures in the upper water column ($\sim 20^{\circ}\text{C}$) (Zonneveld et al., 2018b; Zonneveld et al., 2022). Turbidity increased in the surface and the near-bottom layers, additionally, several intermediate layers of enhanced numbers of particles can be recognized (Fig. 2). In this study, a total of ~ 40 types of dinoflagellate cysts were identified. In the water column, the great majority of cysts were empty. Cysts with cell contents were observed in a few samples (only 1-2 full cysts per sample) until about 85 km from the shelf break (station #26, Fig 3). Results indicate that most cysts probably come from the resuspension of seabed sediments rather than from the recent production in the upperwater layers.

Heterotrophic cysts were always dominant in the water column (>87%), *Brigantedinium* spp. (incl. *Brigantedinium* sp., *B. cariacense*, *B. simplex* and *Diplopsalis*-type) being the most abundant type. Spinny brown cysts (SBC, incl. several species of *Archaeoperidinium* and *Echinidinium*) were also notably abundant, being *Archaeoperidinium minutum*, *A. monospinum* and *E. zonneveldiae* de most abundant species. *Impaginium* spp. (incl. *Impaginium* sp., *I. aculeatum*, *I. striatum* and *I. plicatum*) and *Gymnodinium* spp. (incl. *Gymnodinium* sp., *G. catenatum* and *G. microreticulatum*) the most abundant genera among autotrophic species.

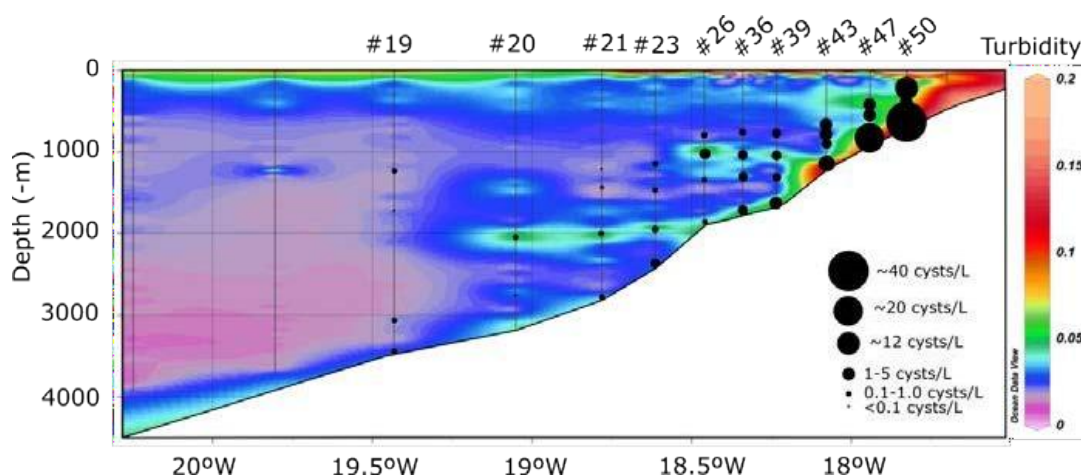


Fig. 2 Distribution of total dinoflagellate cyst concentrations in the water column along the inshore-offshore transect studied off Cape Blanc (NW Africa). Samples overlap the turbidity profile.

Total cyst concentrations generally decreased offshore and in between the nepheloid layers (Fig. 2). All heterotrophic species showed maximal abundances closer to the shelf and decreased offshore, whereas the distribution of autotrophic cysts was variable between cyst types. Clustering grouped the samples into 4 groups according to their dinoflagellate cyst composition (Fig. 3). Some samples that had very few cyst counts (<10 cysts) were excluded from the statistical analysis (grey circles, Fig. 2b). Similarities between cyst associations from the intermediate and bottom nepheloid layers (at similar depths but different stations) suggest that organic particles may have been transported offshore by currents (Fig. 3). The study of the dinoflagellate cysts in the water column and their comparison with the cyst records from surface sediments provided new insights on the origin of the organic particles in the nepheloid layers and on their vertical and horizontal transport off Cape Blanc (NW Africa).

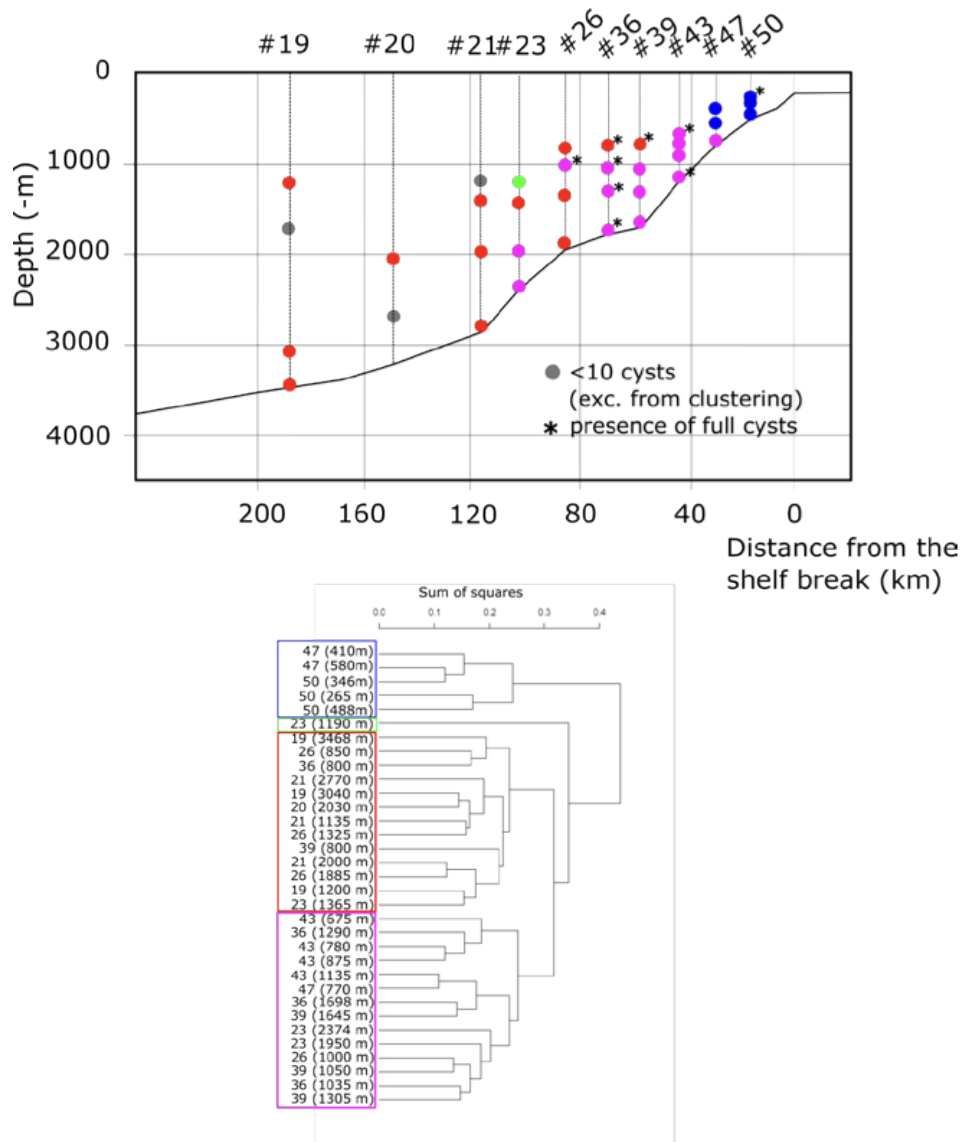


Fig. 3 Clustering results. The same colours represent samples with similar cyst associations. Some samples (grey circles) were excluded from the statistics since their counts were very low.

Keywords: Dinoflagellate cysts, Nepheloid layers, Particle transport, Upwelling, NW Africa.

Acknowledgments: This work was supported by the Deutsche Forschungsgemeinschaft [Mer/Met: 17-87]. Iria García-Moreiras was supported by a postdoctoral fellowship from Xunta de Galicia (ED481B-2019-074, 2019). A. Amorim was supported by FCT, I.P. under project UIDB/04292/2020.

Project: This work was a contribution to the project: Marine carbon production, export, relocation and degradation under varying ocean redox conditions off NW Africa (MACPEI).

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1.12 Dinoflagellate cysts in the benthic nepheloid layer along a land-sea transect off Figueira de Foz (NW Iberia)

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Abstract: Life-cycle transitions have long been recognized as cornerstones for the understanding of the ecology and phenology of phytoplankton species. In particular, the production of long-lived resting stages, such as benthic resting cysts produced by dinoflagellates, is known to influence species' dispersal, bloom initiation and decay, and long-term survival (Fig. 1). To better understand species dynamics, it is very important to quantify and map the distribution of resting cysts in surface sediments, but also in upper water layers (Nehring, 1996; Pilskan *et al.*, 2014). Bottom sediments are constantly being resuspended and dispersed by several oceanographic processes occurring at the water-sediment interface, generating near-bottom particle resuspension layers or benthic nepheloid layers (BNLs). The BNL may also be enriched in resting cysts and could act as a reservoir of viable cysts (Pilskaln *et al.*, 2014). Nepheloid layers are known to be a key mechanism for the transport of organic particles in the ocean (*e.g.* Oliveira *et al.*, 2002; Zonneveld *et al.*, 2018; Villaciers-Robineau *et al.*, 2019). However, very little is known about the relevance of these NL on the geographical redistribution of cysts and their role as reservoirs for bloom initiation.

The development of the present work was motivated by the little knowledge available on dinoflagellate cysts and bottom sediment dynamics on the Atlantic Iberian margin. Our main objectives were: 1) to study the main physical factors affecting (temporally and spatially) BNL development and the associated cyst populations, 2) to study the

role of benthic and suspended cysts in the BNL in the initiation and decline of planktonic blooms and 3) to contribute to a better understanding of cyst transport and accumulation patterns that may be useful to interpret how well the sediment cyst records reflect water column characteristics and therefore being relevant for palaeoecological studies.

To achieve these objectives we studied the dinoflagellate cyst distribution in the near-bottom nepheloid layer (BNL) along a land-sea transect off Figueira da Foz (NW Iberian margin) (Fig. 2) in relation to oceanographic conditions. BNL cyst concentrations were compared with the distribution of cysts and vegetative cells in the upper water column and the cyst distribution in the underlying sediments.

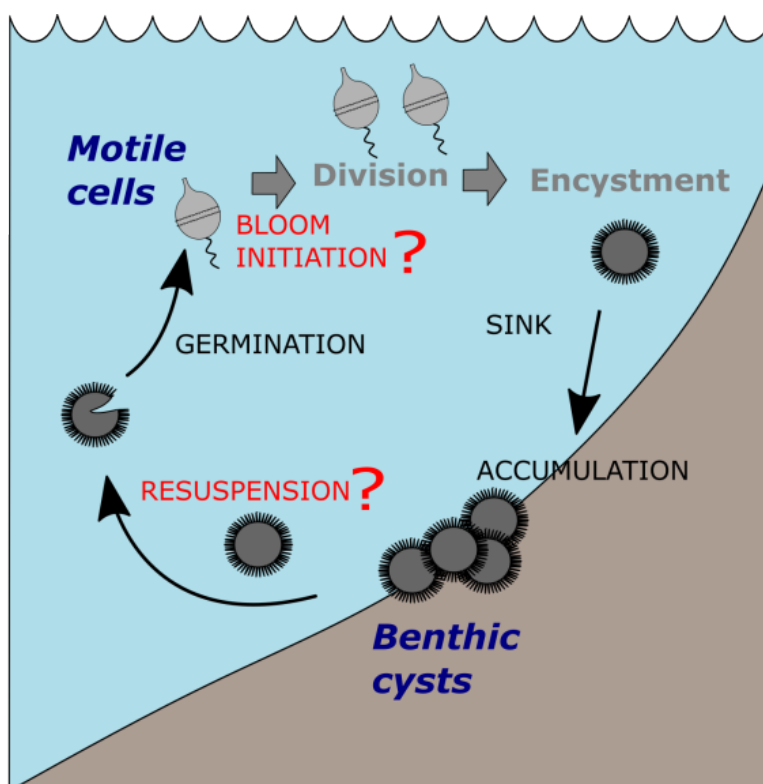


Fig. 1. Simplification of the life cycle of cyst-producing dinoflagellates and relationship between benthic (resting) and planktonic (motile) stages. The hypothesis that resuspension of benthic cysts favours their germination and initiation of planktonic populations is represented. Adapted from Nehring (1993, 1996).

On 14th and 19th September 2019, during the Hydrographic Institute-HABWAVE multidisciplinary cruise, 14 water samples for particle analyses were obtained in the BNL (Fig. 2) using a rosette firing system associated with an Idronaut 320 plus CTD and a Sea point turbidity meter. Water column samples were also collected for the study of phytoplankton communities. At one of the sampling stations, a mooring with a sediment trap was deployed at -90 m depth to record the export production of dinoflagellate cysts from 7th to 19th September 2019. Three sediment samples were collected with a Smith McIntyre grab during the same campaign in September 2019. One of these samples was located at the mouth of the Mondego river (POR) and the

remaining two (F1 and F3) at the sediment trap and 07 sampling station (Fig. 2). Cysts in the BNL, upper water column and sediment trap were compared to cyst records of surface sediments by clustering. In addition, cyst records from 6 surface sediment samples obtained in March 2019 (García-Moreiras *et al.*, 2021) were also used in the statistical analysis (B82-B63; Fig. 2). Furthermore, oceanographic data — temperature, salinity, density and suspended sediment concentration (SSC)— were obtained from CTD casts along a 40.2°N cross-shelf section (Fig. 3).

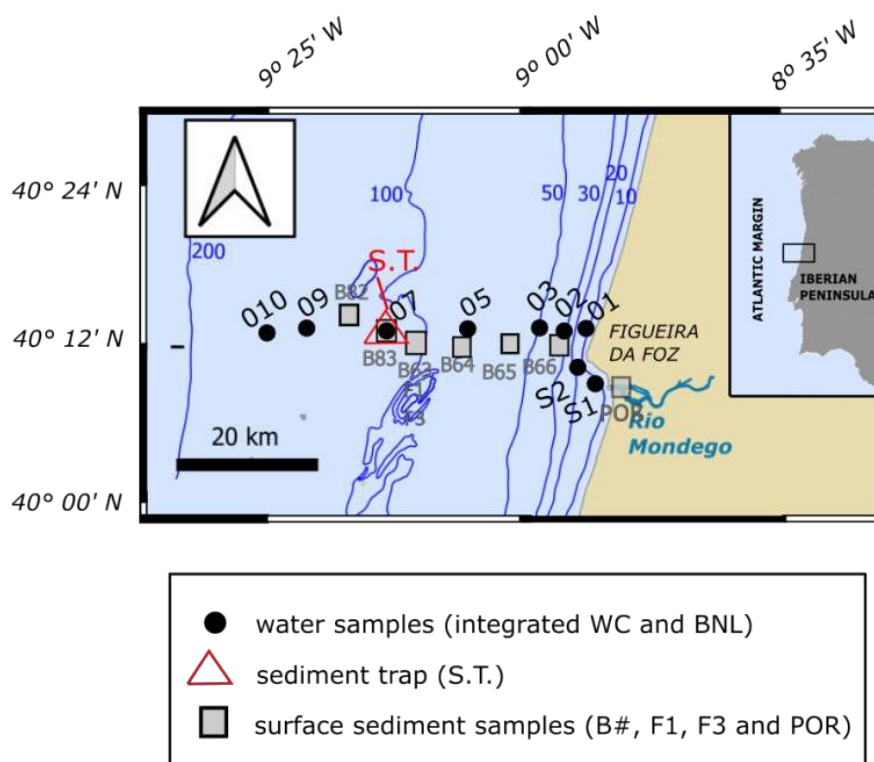


Fig. 2. Study site and location of the sampling stations. The mooring is located at the location of station 07, and surface sediment samples F1, F3 and B3 were collected at the same coordinates.

CTD profiles indicated the presence of a BNL along the studied transect during the survey. It was more evident and increased its thickness below 80 m, particularly between 100-130 m depth (Fig. 3). The survey took place after a series of upwelling events (30th August-12th September 2019) under upwelling relaxation conditions (Moita *et al.* in press). On 14th September, colder SST close to the coast with warmer waters displaced offshore still reflected the influence of upwelled waters (Fig. 3A). On 19th September, the warmer waters had converged to the coast reflecting the progression of upwelling relaxation conditions. The water column was stratified during the entire study period (Fig. 3B).

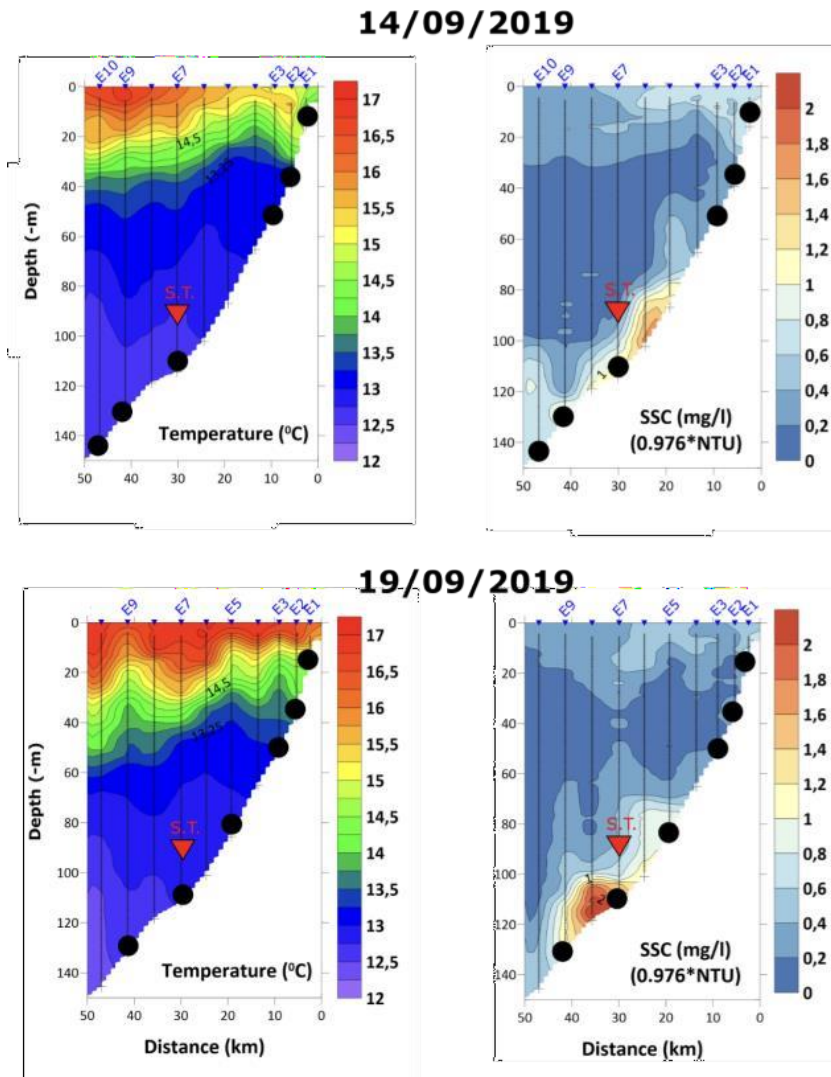


Fig. 3. Hydrographic section, temperature and suspended sediment concentration (calibrated turbidity) along a cross-shelf section at 40.2°N during 14th (top) and 19th (bottom) September. Vertical lines: position of the CTD stations; black dots: location of BNL samples; red triangle: sediment trap. Note that on day 19th two additional BNL samples were collected South of Cape Mondego (S1 and S2) that are not represented here.

In only 5 days, the dinoflagellate cyst assemblages in the BNL changed substantially. Heterotrophic cyst taxa were dominant in most samples. Autotrophic cysts generally decreased inshore and with time. As upwelling relaxed, an outstanding increase in concentrations of heterotrophic small spiny brown cysts with cell contents (SSB, incl. *Protoperidinium monospinum*) in the BNL was detected. SSB/*P. monospinum* was also very abundant in the cyst rain collected by the sediment trap. Accordingly, in the watercolumn, concentrations of SSB increased and extended offshore during the survey.

Several lines of evidence suggested that the origin of the dinoflagellate cysts in the BNL was mainly the biological productivity in the upper waters and not the resuspension of cysts from the bottom sediments. Firstly, the generally high proportions of cysts with cell contents (=full cysts) in BNL samples suggests a recent production rather than resuspension. Second, similar patterns in the distribution of some species (*e.g. Protoceratium reticulatum*) in the BNL and the water column were observed. Finally, cluster analysis detected notable differences between the cyst records of the BNL and those of surface sediments (Fig. 4), not supporting sediment resuspension.

Understanding the relationships between benthic cyst assemblages, the upper water environmental conditions, and planktonic populations is key for the interpretation of the cyst signal left in the sediments (*e.g. Zonneveld et al.*, 2008, 2018). The comparison between the abundances of cells in the planktonic populations and their cysts in the BNL and the upper water column off Figueira da Foz suggested possible over- or under-representations of some dinoflagellate species in the benthic (cyst) record. Moreover, some discrepancies between surface sediment and BNL cyst assemblages were observed. For instance, total cyst concentrations in surface sediments increased offshore, while in BNL they increased inshore. This suggests the occurrence of lateral transport (offshore) in the BNL that could affect the fingerprint of local planktonic populations in the sediments. Transport and deposition dynamics of cysts and other fine particles in the seabed off Figueira da Foz are notably affected by coastal hydrodynamics and topography (*e.g. Oliveira et al.*, 2007; García-Moreiras *et al.*, 2021). Other processes may also contribute to some of these discrepancies. For instance, it is well established that cysts of different groups/species have different sensitivity to post-depositional degradation, being cysts of heterotrophs usually less resistant to oxidation (*e.g. Zonneveld et al.* 2008).

New data obtained in this study may be useful to better understand the role of resting stages in maintaining phytoplanktonic populations. A recent cyst survey in shelf sediments covering the study area showed that very few viable cysts (with cell contents) are present in the bottom sediments (García-Moreiras *et al.*, 2021), thus suggesting that there are no major cyst beds in the surface sediments of this area. In the present study, we document the presence in high proportions of viable cysts in the BNL. This raises the possibility that sediment cyst surveys may underestimate the potential regional cyst bed size. Viable dinoflagellate cysts in suspension in the BNL may act as a reservoir of cysts that would serve as inoculum to seed phytoplanktonic populations. Suspended cysts could be transported to upper waters more easily than bottom cysts, where they would find the necessary conditions to germinate.

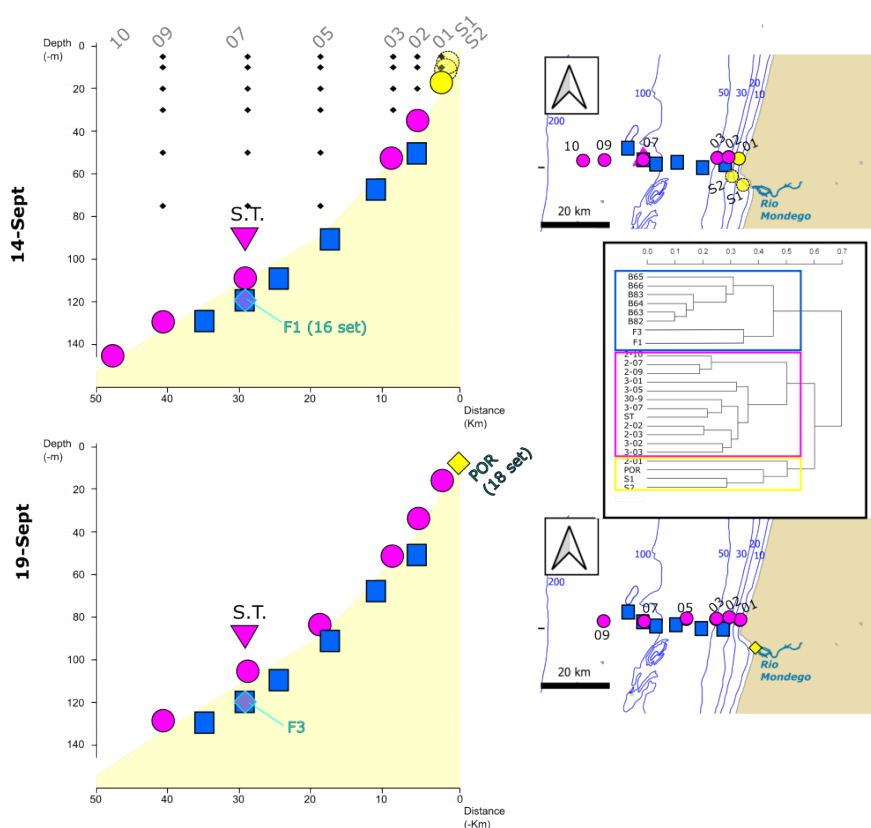


Fig. 4. Transect studied off Figueira da Foz depicting sample positions of the sediment trap (inverted triangle, S.T.), the BNL (circles) and surface sediment (squares) samples, and the bathymetry (right) (bathymetric contours are in meters and were taken from the General Bathymetric Chart of the Oceans (<https://www.gebco.net/>)). Different colours represent different clusters according to similarities in dinoflagellate cyst composition (results of clustering performed on relative abundance data are shown on the right).

Keywords: Dinoflagellate cysts, Benthic nepheloid layer, Suspended cysts, *Protoperidinium monospinum*, Atlantic Iberian Margin.

Acknowledgments: This research was supported by EU ERDF funds, within the PT2020 Partnership Agreement and Compete 2020, and national funds through Fundação para a Ciência e Tecnologia, I.P. (FCT, I.P.) (project HABWAVE (LISBOA- 01-0145-FEDER- 031265 and FCT, I.P. through project UIDB/04292/2020). Iria García-Moreiras was supported by postdoctoral fellowship from Xunta de Galicia (ED481B-2019-074, 2019).

Project: This work was a contribution to the LISBOA-01-0145-FEDER-031265 (*HabWAVE – Relevância da conjugação de processos biológicos e físicos na iniciação de blooms de algas nocivas na costa NW de Portugal*).

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1.13 Dinoflagellate indicators of Arctic Ocean multiyear ice: filling the knowledge gaps

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Abstract: The semi-enclosed Arctic Ocean north of Greenland, America and Eurasia is unique in being the smallest, coldest and freshest of the global oceans. This polar biogeographic province located north of 79° N differs from the Antarctic circumpolar ocean in having multiyear sea- ice >2 m thick year-round, increasing from 2 m at the North Pole to 4 - 7 m on Canada's polar margin. Because ships cannot access thick perennial sea-ice for sampling, the dinoflagellate flora in and under multiyear sea ice is the least well-known (Bluhm et al., 2017; de Vernal et al. 2020). Spatial variability of Arctic dinoflagellate species diversity poses another problem. For example, the most recent 2017 biodiversity census reports 14 sympagic dinoflagellate taxa from five records for the subregion north of 80° N compared to 22 taxa from >10 sources in the Beaufort Sea subregion near 79° N, and 112 taxa for an Atlantic Arctic subregion comprising the Greenland and Barents seas 80°- 60° N. Understanding of the ecology and taxonomy of the dinoflagellate multiyear ice flora before it disappears has important implications for interpretation of the geological record of climate change, particularly in Arctic deep ocean basins where preservation of calcareous and siliceous microfossils is often poor (de Vernal et al., 2019; De Schepper et al., 2019).

The main purpose of this paper is to present new morpho-taxonomic, spatial and seasonal distribution data for dinoflagellates from 1) a unique collection of multiyear ice samples collected on the Canadian Polar Margin (ca. 80° N) from 1985 to 1988, and 2) from a smaller area over Gakkel Ridge at ca. 86° N, collected from the PFVS Polarstern during Arctic Expedition ARK IV/3 in summer 1987. These years coincide with the start of the accelerating global warming that today melts 55% more of the first-year ice in summer, and has irreversibly thinned the multiyear sea ice by 58% (Jeffries et al., 2013). These 1980's data can be compared with a collection made by Meunier (1910) during summer eighty years earlier in the Kara Sea at ca. 72°N.

Ice algae are adapted to dark survival under thick ice. Therefore, their ability to persist in a warming world of rapidly thinning ice is now considered an important unsolved problem. Past studies have considered two survival methods: heterotrophy and cyst production. Heterotrophy could be an adaptive advantage to ice algae but early field experiments in ice floes did not fully support that idea. In contrast, the main dinoflagellate cyst (dinocyst) sea- ice proxies found in seabed sediments below the ice seem to have heterotrophic thecate-stage sources. These well-known cryophilic dinocysts, *Islandinium minutum* ssp. *minutum* and ssp. *barbatum*, *Islandinium? cezare*, *Echinidinium karaense*, and Arctic morphotypes of *Polykrikos? sp.* and brown

protoperidinioid cysts, mostly *Brigantedinium* spp., are believed to be produced by heterotrophic dinoflagellates. At present; however, most do not have a confirmed thecate-cyst relationship beyond the Protoperidiniaceae (Potvin et al., 2018). The cyst production of cryophilic and sympagic dinoflagellates also needs closer study. Earlier reports indicate that most ice algae, including the dominant Bacillariophyceae (diatoms), do not produce resting cysts, and de Vernal et al. (2019) found no dinocysts in sediments below central Arctic multiyear ice. Notably, however, the dinocyst database of de Vernal et al., (2020) excludes many Arctic Ocean samples because cyst abundance cannot meet the criterion of 60 cysts necessary for valid statistical analysis. This 2020 database also misses dinoflagellate taxa such as *Polarella glacialis* producing cysts that do not preserve well but are traceable in sediments using aDNA (De Schepper et al., 2019). Other dinoflagellates may be found by processing sediments to allow the recovery of calcareous and siliceous dinoflagellates.

Data from 8 sea-ice samples, 10 plankton tows and 3 sediment traps obtained over five years from a Canadian ice island camp 1985–1989 reveal a seasonal succession of sympagic and cryophilic algae in one of the last remaining areas of multiyear sea-ice. Here, at 79 – 81°N, the Polar Year begins with solid freeze-up of new ice in September and the descent of darkness from October to April. The interval September through March therefore could only be sampled by sediment traps. The winter trap samples contained abundant Chrysophyte statocysts (3 taxa) and diatoms, diverse zooplankton, including juvenile foraminifers and ice nematodes, radiolaria and silicoflagellates, but dinoflagellates were not found. During the Arctic spring (April - June), ice samples contained common cysts of *Polarella (Echinum) glacialis* (two morphotypes), other *Echinum* species, and cysts of *Becheleria cincta* and cf. *B. hangoei* although thecate stages of these taxa were not recognized. The peak abundance of an enigmatic black cyst provisionally referred to *Boreadinium breve* also marked early spring ice. Snow algae were common in April ice, mainly as red-brown cysts of the common snow chlorophyte, *Chlorella nivalis*, but possibly including the red-pigmented cysts of the dinoflagellate *Borghiellia pascheri*. The May ice dinocysts flora was similar but also included rare cysts and a few cells of *Peridiniella catenata* and *Dinophysis arctica*; the latter can form a cyst with mucilaginous outer wall. In June, there was a large decline in *Polarella* cysts, and *Peridiniella catenata* cells were found to be very common at the base of ice cores. By early July, the start of Arctic summer, most snow had melted and large meltwater ponds formed that drained through the ice by cryoconite holes, releasing snow alga cysts into the water column and to the seabed. From July through August there was a species succession from a predominance of suessoid taxa, *Polarella (Echinum) glacialis*, cysts of *Becheleria cincta* and *B. cf. hangoei*, occasional gymnodinioids, including cf. *Diplopsalisphaerica* Meunier, 1910 in early July to a large bloom of *Peridiniella catenata* between August 8 and 28. Other dinoflagellates in the summer samples were common *Echinum* spp., rare thecae or pentasters of *Actiniscus pentasterias*, and *Caracomia arctica* vegetative “cysts” in both ice and water column.

The sympagic flora sampled in summer (early August) from the Polarstern over Gakkel Ridge at ca. 86°N was similar to that of the spring flora on the Canadian margin at ca. 80°N. There was a predominance of cf. *Boreadinium breve*, common *Polarella glacialis*, *Echinum* spp., *Becheleria* and *Chlorella nivalis* cysts. This northernmost flora also

included cysts of *Diplopsalis*, *Polykrikos*, *Caracomia*, rare pentasters of *Actiniscus pentasterias* and several acritarchs not found in the Canadian samples. Most of these cysts were also illustrated by Meunier in his samples of yellow snow and ice in the Kara Sea. Meunier described *Polarella glacialis* under the name of *Echinum majus*, along with a spinose spherical species *Echinum sphericum* and other morphotypes, probably *Biecheleria* species or chrysophyte statocysts.

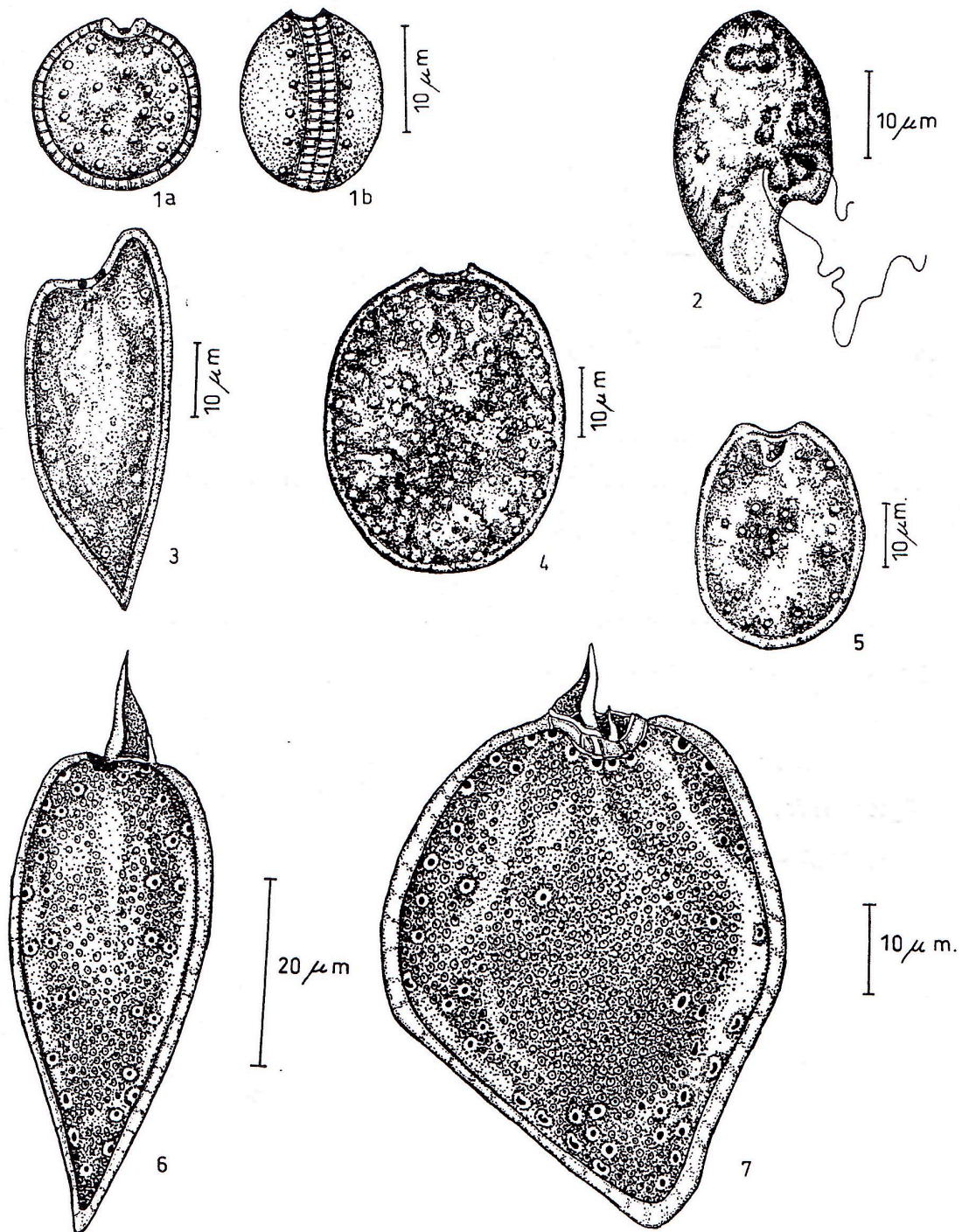
In total contrast to the sympagic flora and plankton flora of the Canadian polar margin, the surface sediments beneath the pack ice contained a disjunct organic-walled dinoflagellate cyst flora, primarily including protoperidinioid taxa *Islandinium minutum*, *Brigantedinium* spp., *Echinidinium* spp., *Multispinula* cf. *M. quanta* and rare *Impagidinium pallidum*. The enigmatic spiny dark brown or black cyst of *Boreadinium breve* is one of very few dinoflagellate species recognised in both ice samples and the sediment. More work is needed to verify the identity of *Biecheleria* and some spiny brown cysts, as well as other small round brown cysts that may be organic linings of stomatocysts. At the Polarstern ARK IV/3 stations 371 - 376, large numbers of a small unicellular alga identified as an *Archeomonas* species were found together with cysts of *Chlamydomonas nivalis* and a non-pigmented organic-walled cyst resembling *Cyclopsiella*. In contrast, sediment at ca. 82° N (Stn. 287 - 15) contained a palynoflora similar to that of the Canadian Polar margin, but with greater cyst diversity that includes *Nematosphaeropsis labyrinths*, *Multispinula quanta* and two other undescribed acritarchs.

Initial conclusions from the Arctic Ocean multiyear ice studies are that much can be learned about dinoflagellate biodiversity by broadening the focus of research to include taxonomic studies of ice algae, their transport to and their preservation in surface sediments, whether in small or large numbers. Other conclusions are that there appears to have been little change in the multiyear ice flora biodiversity between 1910 and 1989 but new studies are needed to determine the reality of the idea that nowadays, only *Peridiniella catenata* and *Polarella glacialis* are true multiyear ice algae. It is also now clear that mixotrophy can allow adaptation to Arctic species in addition to heterotrophy, as illustrated by the chain-forming species *Peridiniella catenata* which is remarkably well-adapted to polar ice environments. Within the spring ice, thecal cells of *P. catenata* are usually unicellular, dorso-ventrally flattened, lacking pigments, and there are flattened, discoidal cysts. During the summer bloom, however, the thecate stage of this mixotrophic species forms colonial cell chains, mostly (74%) with 2 or 4 cells and up to 8 divisions, interconnected by cytoplasm and interlocked at posterior sulcal and apical plates. Each interlocked cell would be propelled by two flagella, thus facilitating the search for prey during the planktonic summer phase. Chloroplasts are peripherally located and together with luminescence emission could further aid movement toward light or prey in the dimly lighted polar ice environment. Two sizes of trichocysts could also facilitate prey capture as an alternative to photosynthesis. The colourless dinoflagellate *Actiniscus pentasterias* is also reported to be phagotrophic but there is uncertainty about its production of resting cysts as described for *Actiniscus canadensis*.

Keywords: Arctic sea-ice biodiversity, sympagic ecology, cryophilic dinocysts

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SESSION 2

TOXIC DINOFLAGELLATES AND BIOTOXINS I

2.01 First observation of *Fukuyoa paulensis* Gómez, Qiu, Lopes & Lin and *Coolia canariensis* Fraga along the Italian coasts (Sardinia island)

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Abstract: The genera *Fukuyoa* and *Coolia* include marine benthic/epiphytic dinoflagellates both comprising species potentially producers of ciguatera toxins (CTX). The first detections of *Fukuyoa* species in the Mediterranean were reported in the southeast area first (Greece and Cyprus) and in the northwest (Balearic Islands) then (Laza-Martínez et al. 2016, Tudó et al. 2020). Instead, *Coolia* genus is represented by the widespread species *C. monotis* Meunier and by the recently observed *C. malayensis* Leaw, Lim, Usup (Abdennadher et al. 2021). In this study, macroalgae samples (prevalently *Padina pavonica* (Linnaeus) Thivy) were collected from three sites along the Sardinian coast in summer 2020 and 2021. Each sample was intensively shaken, sieved and subsamples were observed under an inverted light microscope. The benthic dinoflagellate community from the collected samples was mostly composed by *Prorocentrum* species, *Ostreopsis cf ovata*, *Coolia* and *Fukuyoa* species, and very few cells attributable to *Gambierdiscus* genus (Fig 1A). Single cells were isolated and cultured and the established strains were morphologically and molecularly characterized, resulting in the first detection of *C. canariensis* in the Mediterranean and the first report of *F. paulensis* along the Italian Coasts (Sardinia Island, NW Mediterranean). Two strains of *F. paulensis* (Fpau_Cip and Fpau_TrG; Fig. 1B-C) were obtained both showing globular laterally compressed cells with plate formula Po, 4', 6'', 6c, ?s, 5''', 2'''''. Cells showed a mean length and depth of 59.0±5.4 and 56.9±5.7 µm (Fpau_Cip) and of 60.1±2.7 and 58.3±2.7 µm (Fpau_TrG). The length: width ratio was about 1.3 µm for both strains.

The *C. canariensis* strain (Ccan_Cip; Fig 1D) showed almost spherical cells with plate formula Po, 3', 7'', 6c, ?s, 5''', 2'''''. Cells showed a mean length and width of 36.4±4.5 and 38.0±4.6 µm. Fpau_Cip and Ccan_Cip strains were analysed for toxicity determination with the neuroblastoma cell-based assay showing a weak signal of CTX-like toxicity that should be further confirmed, only for the strain Fpau_Cip. These new reports show that the distribution range of these species is far to be known. The toxicity expressed by the *F. paulensis* strain although weak, together with the observation of *Gambierdiscus* cells in some samples, strengthen the potential for ciguatera poisoning in the area.

Keywords: epiphytic, Mediterranean Sea, LSU, *Fukuyoa*, *Coolia*.

Acknowledgments: Authors thank Mr. M. Meridda and Mr. J. Culurgioni for his precious help during samplings.

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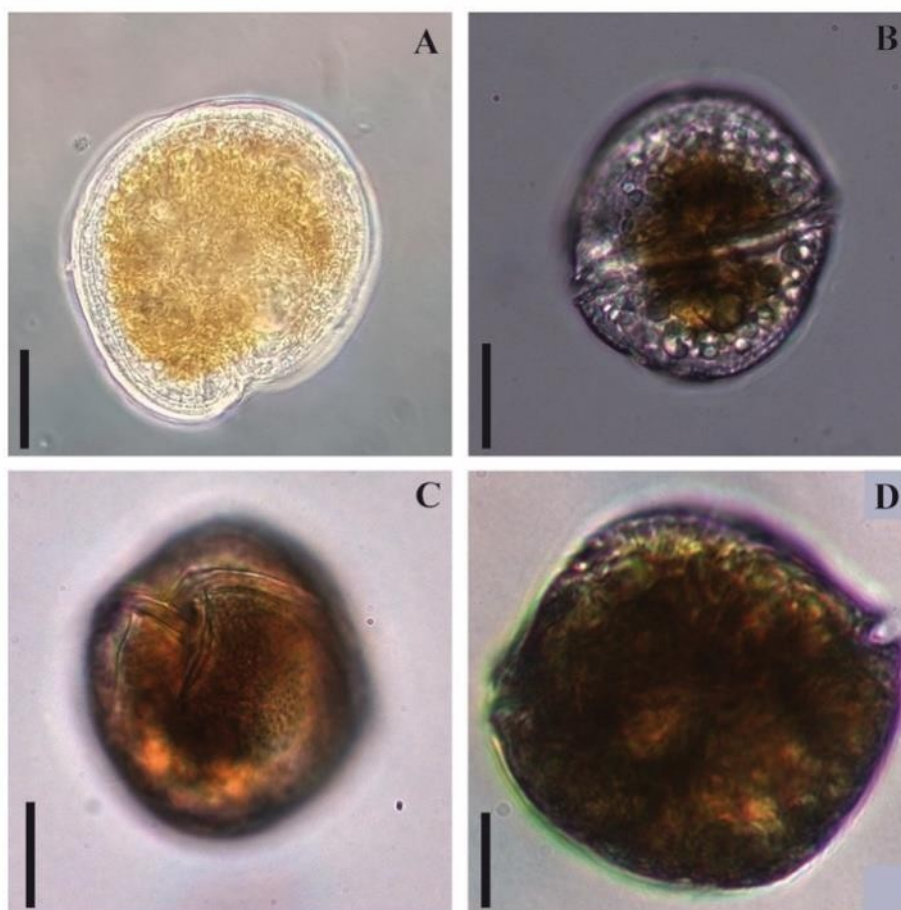


Fig. 1. Light micrographs of a *Gambierdiscus* cell from field Sardinian samples (A), *Fukuyoa paulensis* strain Fpau_Cip (B), *F. paulensis* strain Fpau_TrG (C) and *Coolia canariensis* strain Ccan_Cip (D). Scale bars of micrographs A-C: 20 μ m; scale bar of micrograph D: 10 μ m.

2.02 BHAB ecology in the oceanic Madeira island (subtropical eastern Atlantic)

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Abstract: Ciguatera fish poisoning (CFP) syndrome is caused by the human consumption of ciguatera fish. Ciguatera toxins are produced by species of the benthic dinoflagellate genera *Gambierdiscus* and *Fukuyoa*, often associated with the palytoxin-producing genus *Ostreopsis*. *Gambierdiscus* and *Ostreopsis* have been recorded in the Madeira Archipelago and CFP episodes were reported from the Selvagens Islands. Still, very little is known on the diversity and ecology of benthic harmful algal bloom (BHAB) species in Madeira. The aim of this study was to 1) identify BHAB genera present in Madeira, 2) characterize their abundance, frequency and seasonality, and 3) investigate the influence of abiotic environmental factors on the community dynamics. Sampling was mainly performed during summer (between June and October), from 2018 to 2021 at Funchal, a touristic city located in southern Madeira. Samples were collected using standardized artificial substrates. Investigated abiotic factors included: (1) *in situ* temperature, light intensity and dissolved nutrients, and (2) regional data records of sea surface temperature (SST), wave height and meteorological data (precipitation, solar radiation and wind conditions). The dynamics of the BHAB community and its relation to the different environmental variables was investigated using Correspondence Analysis (CA) Principal Component Analysis (PCA) and Redundancy analysis (RDA). Statistical significance of the correlations was analysed by Spearman's rank correlation coefficient. The BHAB community included: *Gambierdiscus*, *Ostreopsis*, *Coolia*, *Amphidinium* and *Prorocentrum*. Maximum cell densities were reached by *Ostreopsis* while *Gambierdiscus* presented the lowest value.

Coolia, *Prorocentrum* and *Amphidinium* showed a significant positive correlation suggesting they occupy a similar niche space. Conversely, *Ostreopsis* and *Gambierdiscus* did not correlate with other genera, indicating they each occupy a unique ecological niche.

For the four-year study period, from mid-June onwards, *Ostreopsis* was the dominant genus, reaching values several orders of magnitude higher than the other genera. It exceeded in several sampling dates the level of concern referred in the literature for respiratory syndrome outbreaks.

Gambierdiscus was found to be always a minor component of the dinoflagellate assemblage. Both genera showed low interannual variability while *Coolia*, *Prorocentrum* and *Amphidinium*, presented significant interannual variability. *Prorocentrum* and *Amphidinium* always occurred in low abundances, but *Coolia* was observed to dominate the assemblage in June 2018. The most relevant abiotic variables driving the community were *in situ* temperature, radiation, wave height, and the silicate/nitrate+nitrite (Si/N) ratio. The only environmental variable that significantly correlated with *Gambierdiscus* was *in situ* temperature which also significantly influenced *Ostreopsis* dynamics. By contrast, *in situ* temperature did not show a significant influence on *Coolia* and *Prorocentrum* which were mainly influenced by the Si/N ratio. Other environmental variables influencing *Ostreopsis* dynamics were radiation and wave height. Given the confirmed occurrence of CFP episodes in the Archipelago, the study area is possibly not an important source for CFP. On the other hand, the high abundances recorded for *Ostreopsis* indicate this genus as the main potential harmful-causing genus in Funchal.

Keywords: *Gambierdiscus*, *Ostreopsis*, Time-series, temperature

Acknowledgments: We thank APRAM (Madeira Port Authority) for providing temperature and wave height data, Jesus Reis and Ricardo Faria from Oceanic Observatory of Madeira for providing oceanographic and meteorologic data used in this study. This study was funded by the Interreg MAC 2014-2020 projects; MIMAR (MAC/4.6.d/066) and MIMAR+ (MAC2/4.6d/249). TS was supported by a PhD grant (SFRH/BD/ 04292 /2019) funded by ARDITI through Project M1420-09-5369-FSE-000002 and the Program Madeira 14-20 - Fundo Social. Funding by FCT, I.P. under project UIDB /04292/2021 is also acknowledged.

2.03 In-depth biochemical mapping of *Pyrodinium bahamense* reveals insights on its molecular machineries

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Abstract: The biochemical components of the toxic harmful algal bloom (HAB)-causing organism *Pyrodinium bahamense* were mapped to reveal insights on its molecular machineries and biochemical processes (Subong, et al. 2021). Total sugar and fatty acid content were $20.31 \pm 2.60\%$ w/w and $18.12 \pm 3.19\%$ w/w of the total biomass, respectively. The average moisture and ash contents were $2.392 \pm 0.004\%$ w/w and $18.47 \pm 0.09\%$ w/w, respectively. Protein and other components were estimated to be $\sim 40.71\%$ w/w of the total biomass.

To further understand the biochemistry behind the organism, we performed a shotgun proteomics using label-free quantitation followed by protein identification using the *P. bahamense* transcriptome and translated protein databases of various organisms which include *Marinovum algicola*, *Alexandrium* sp., *Cylindrospermopsis raciborskii*, and *Symbiodinium kawagutii* for annotation enabled the characterization of the proteins in *P. bahamense*. Most of the annotated proteins were involved in amino acid biosynthesis and carbohydrate degradation and metabolism, indicating the active roles of these molecules in the vegetative stage of *P. bahamense*.

The highest number of annotated hits were obtained from *M. algicola*, a bacterial endosymbiont, and highlighted the contribution of microorganisms associated with *P. bahamense*. Further, proteins mapped in dimethylsulfoniopropionate (DMSP) degradation such as propionyl CoA synthetase and acryloyl-CoA reductase were identified, suggesting the DMSP cleavage pathway as the preferred route in *P. bahamense*.

To understand the toxin production of this organism, we mapped proteins that are directly and indirectly involved in saxitoxin biosynthesis, arginine biosynthesis, and polyketide synthesis pathway. Despite the depth of coverage using five reference genomes for annotation, we only identified 20 proteins that are potentially relevant to organismal toxicity. Despite this wealth of information, mapping the entire toxin biosynthesis route remains a challenge comparable with previous proteomic studies of other toxic dinoflagellates. The characterization of the proteins, carbohydrates, and lipids of *P. bahamense* based on mass spectrometry and chromatographic analyses provided groundwork information on the biochemical characteristics and molecular machineries of *P. bahamense* at the vegetative stage. These gave new insights into the cellular machinery behind this HAB-causing organism.

Keywords: proteomics, *Pyrodinium bahamense*, paralytic shellfish toxins, biomolecules

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2.04 Detection of palytoxin-like toxicity in *Ostreopsis cf. siamensis* from the Cantabrian coast

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Abstract: Palytoxins (PLTXs) are among the most toxic compounds produced by biological organisms. They were initially discovered in zoanthids of the *Palythoa* genus and later in other marine organisms, including fish, crustaceans and mollusks (Riobó et al., 2011). The first detection of Palytoxin analogs in dinoflagellates was in *Ostreopsis siamensis* (ostreocins) and later in other species such as *O. ovata* (ovatoxins), *O. mascarenensis* (mascarenotoxins) and *O. lenticularis* (ostreotoxins) (Terajima et al., (2019), García-Altares et al., (2015), Lenoir et al., (2004), Meunier et al., (1997)).

In the last decade, the presence of *Ostreopsis* has been frequently recorded on the Northern Iberian Peninsula coast between Cantabria and the Basque Country (both Spanish and French), identified as *Ostreopsis cf. siamensis*. During 2021, poisonings associated with the presence of this benthic microalgae occurred on beaches in the Basque Country and the coast of the Basque Country in France, including news about surfers affected by vomiting and respiratory distress after exposure to marine aerosols on the Iparralde beach or skin irritations of bathers in San Sebastián.

A water sample was collected during an occasional sampling carried out on September 14, 2021 in San Vicente de la Barquera (43° 25'57''N, 4°23'36''W). Once in the laboratory, the careful observation of this sample under light microscopy allowed the identification of *Ostreopsis* cells, that were individually isolated establishing monoclonal cultures.

The genetic identification of the cultures (3 strains) was performed by sequencing the LSU rRNA gene (region D1-D3). The molecular analyses results showed correspondence to *Ostreopsis cf. siamensis* (sp. 9 sensu Nguyen-Ngoc et al 2021), accordingly to previous sequences from nearby areas, and different to the true clade of *O. siamensis* established by these authors.

Toxicity was evaluated by a specific hemolytic assay developed for PLTX (Riobó et al., 2008). In order to achieve enough biomass to carry out toxin analyses, one clonal strain (F3) was chosen to scale the culture up to a volume of 1315 mL, implying that 1.516.195 cells were harvested. Toxin extraction was performed with 3 mL of 80% methanol. As result, PLTX-like activity was detected in this extract, causing delayed hemolysis that was inhibited in the presence of ouabain, a PLTX antagonist compound. This assay allowed to estimate the toxicity of *Ostreopsis cf. siamensis* (sp. 9) strain F3, as 1.4 pg equivalents of PLTX cell⁻¹, being the first time that toxicity has been determined in that molecular ribotype of *Ostreopsis*.

Keywords: *Ostreopsis cf. siamensis*, Palytoxin-like compound, Cantabrian coast, Hemolytic assay

Acknowledgments: Acknowledgments are due to VGOHAB staff (IEO-CSIC, Vigo), involved inculturing facilities, Isabel Ramilo and Nuria Lluch.

Project: This research was funded by a national project from the Spanish Ministry of Science and Innovation and the European Community (FEDER) (Project DIANAS- CTM2017-86066-R) and a grant for Galician Networks of Excellence (GRC-VGOHAB IN607A-2019/04) from the Innovation Agency of the Xunta de Galicia (GAIN).

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2.05 Spatiotemporal patterns and implications of *Alexandrium catenella* cyst suspension in the Alaskan Arctic

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Abstract: Rapidly changing conditions in the Alaskan Arctic are driving widespread concern about harmful algal blooms (HABs) and their potential environmental and health impacts. Recent research has revealed the presence of a massive accumulation of living cysts of the toxin-producing dinoflagellate *Alexandrium catenella* in the region and indicates that warming temperatures have the potential to significantly increase the rate at which resting cysts of this species can germinate and form blooms. While the majority of cysts are found in bottom sediments, *Alexandrium* cysts can also be suspended throughout the water column, with the highest densities generally occurring in and above the nepheloid layer. Suspended cysts experience elevated light, temperature, and oxygenation, leading to the potential for enhanced germination under appropriate seasonal conditions. To further characterize the Arctic cyst bed and its connectivity with the water column, we analyzed water, sediment, and sediment trap samples for the presence of *Alexandrium* cysts. During a shipboard expedition aboard the R/V Sikuliaq in October-November 2020 (SKQ202014S), paired nepheloid layer and sediment samples were collected from across the study region for a comparison of *A. catenella* cyst abundance between benthic sediments and bottom waters. Along the Ledyard Bay transect, a known high-density cyst region, additional samples were obtained from the upper water column in order to determine vertical cyst distribution. Suspended cyst concentrations correlated with particle resuspension (measured by transmissivity) and were highest near-bottom (max 15,800 cysts m⁻³), but cysts also persisted at lower concentrations into surface waters. Analysis of sediment trap time-series samples collected at three DBO sites from summer 2017 to summer 2019 allowed observation of seasonal patterns of cyst flux; this revealed evidence of delivery of new cysts (max 235,200 cysts m⁻³ day⁻¹) during the late summer, as well as strong seasonal signs of resuspension in the fall and winter. While germination is not possible in the winter months, these results indicate that a resuspension event in the spring or early summer could place cysts higher in the water column, where favorable temperature, light, and oxygen conditions may promote germination. As the severity and frequency of storm events in the Arctic increase across all seasons, large-scale resuspension events are becoming a more common occurrence. Application of a temperature and light-driven germination model to the observed concentrations demonstrates that suspended cysts could contribute a significant fraction of germination relative to the benthic cyst reservoir.

Keywords: *Alexandrium catenella*, resting cyst, resuspension, Arctic, HABs

Acknowledgments: We acknowledge the contributions of the crew and science party of the R/V Sikuliaq SKQ202014S. The following funding sources supported this work: NSF Office of Polar Programs OPP-1823002, NOAA Arctic Research Program NA14OAR4320158 and NA19OAR4320074. NSF Graduate Research Fellowship Program.

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2.06 Tricky dinoflagellate toxins, how to get a good grasp of them!

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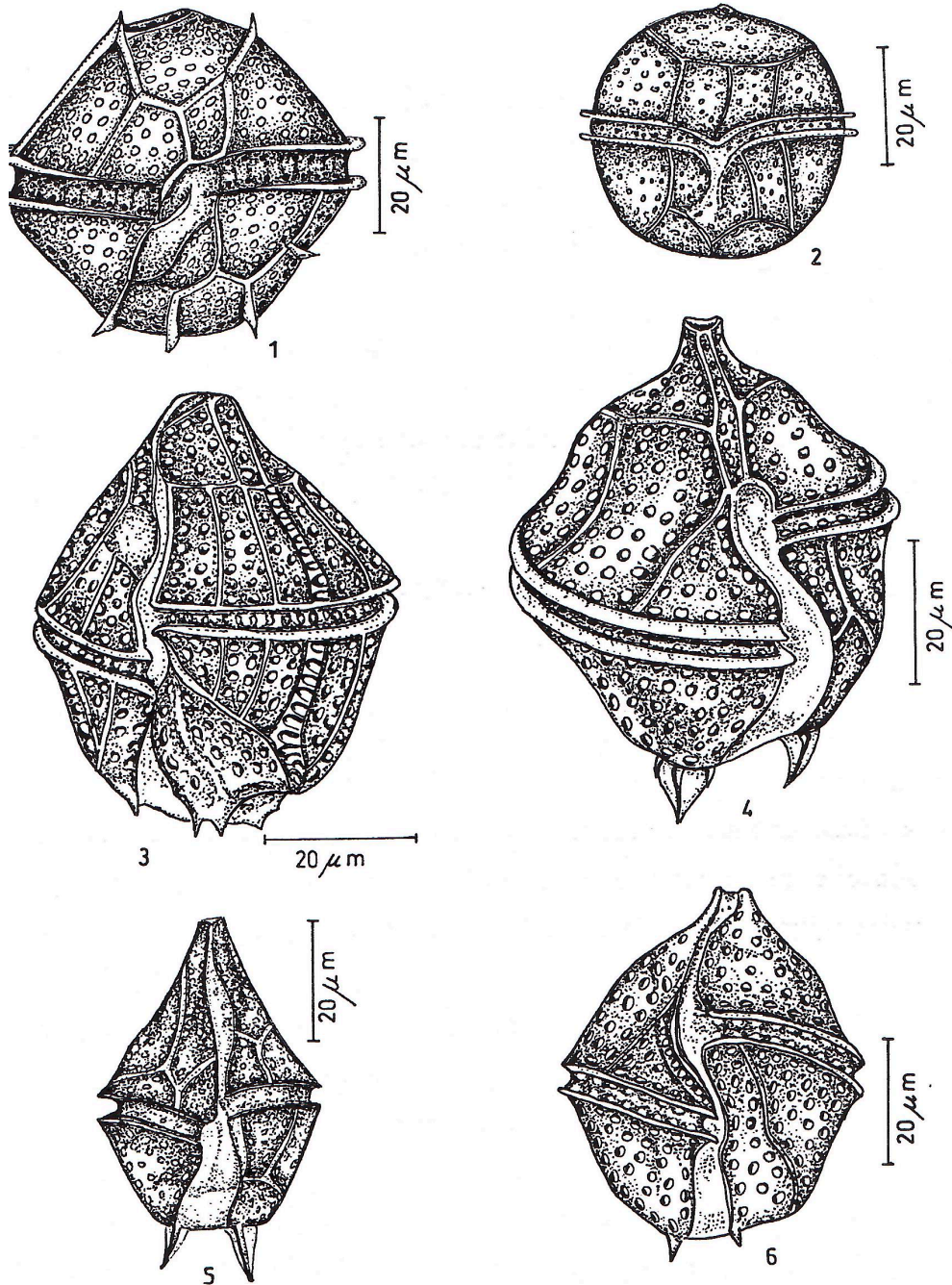
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Abstract: Marine dinoflagellates include several species of toxin producers. A high variety of very potent dinoflagellate toxins and their metabolites, structurally and functionally different, is responsible in our oceans for several acute and chronic seafood poisonings (e.g., PSP, DSP, ASP, NSP, AZP, Ciguatera). The detection, identification, and quantification of toxins that dinoflagellates produce, often in very low amounts, is a major challenge when characterizing toxic species. This is also crucial to understand their ecology, and correctly assess the toxicological impact they may cause to the environment or to food safety.

Strategies employed over the last 15 years for the detection, identification and characterization of dinoflagellate toxins based on functional, toxicological, and structural recognition will be presented. Methods focusing on the toxicity or mechanism of action will include i) cell-based assays and ii) enzyme inhibition assays and biosensors. The structural recognition of toxins will be described with iii) immunoassays and immunosensors, iv) instrumental analysis including LC-MS/MS and LC-HRMS. Finally, strategies for the capture or purification of these toxins will include the use of v) SPATT bags and vi) cyclodextrins.

As technological methods evolve, these continuously offer new tools that allow us to go deeper into the understanding of marine toxins. The complementarity approach among different methodologies, when assessing marine dinoflagellate toxins, is proposed as the most efficient strategy.

Keywords: Toxin detection, cytotoxicity, immunoassays, biosensors, instrumental analysis, LC-MS/MS, LC-HRMS, SPATT.



SESSION 3
TOXIC DINOFLAGELLATES
and
BIOTOXINS II

3.01 First exceptional record of *Ostreopsis cf. siamensis* in the Cantabrian plankton after 20 years of harmful algal blooms monitoring

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Abstract: *Ostreopsis c.f. siamensis* (Schmidt 1901, Nguyen-Ngoc 2021) was first detected by INTECMAR (Xunta de Galicia) in San Vicente de la Barquera (Cantabrian Sea; Bay of Biscay, N. Spain) in the oceanographic station S3 (43°25'57''N, 4°23'36''W). This species was recorded in the autumn of 2021, during the weekly sampling performed by the Cantabria government since 2002 in order to monitor toxic phytoplankton species in the shellfish production areas. In two dates, the 14th (10:30 a.m, 17.7°C, salinity 35) and the 28th (time 11:00 am, 18.2°C, salinity 34.3) of September, *O. cf. siamensis* was detected in samples collected using plankton nets (10 µm) and fixed with formaldehyde, although quantification was not possible because of the low cell concentration (<40 cell.L⁻¹). Quantification (Utermöhl) was possible the 5th and 19th of October (10:30 am, 18.2°C, salinity 33.1 and 9:30 a.m, 16.2°C, 34.5 salinity respectively) in samples collected with a bucket and fixed with Lugol, achieving a concentration of 360 cell. L⁻¹ and 200 cell.L⁻¹. Species identification followed COI-UNESCO taxonomical criteria. *O. cf. siamensis* is an armoured, marine and benthic dinoflagellate species, antero-posteriorly compressed, ovate and tear shaped, ranging from 16.5-31.5 µm in Length and 16.5-31.5 µm in Width. Meteorological conditions during this period included water turbidity and heavy rainfall and flooding. *O. cf. siamensis* was not detected in any other plankton sample from the Santander stations, neither in Santoña (Cantabria) nor in Asturias (Ribadeo). It was not detected either in any other plankton sample of the Galician coast, which includes 18 coastal stations and 48 inner -inside the Rias- stations monitored weekly since 1992. Given that *O. cf. siamensis* is a epiphytic species, its detection in plankton samples suggests that a much higher concentrations could have been reached in benthic environments. Accordingly, during the same dates of 2021, a bloom of *O. cf. siamensis* was reported, which caused the adoption of swimming prevention measures in several beaches in San Sebastián (yellow flags) and a total ban on swimming (red flag) in Santa Clara island (Basque Country).

Ostreopsis species are known producers of palytoxin-like compounds, which are among the most potent non-protein marine biotoxins in existence. These toxins have been detected in various marine organisms such as fish, mollusks, crabs, corals and sponges. Several cases of palytoxin poisoning through seafood consumption in humans have been documented, some of them involving human fatalities. But the most negative impact for humans is originated through palytoxin exposure by inhalation that causes serious human respiratory distress after exposure to marine aerosols and also by dermal contact.

Although *O. cf. siamensis* was previously detected in benthic samplings in close areas (Santoña), its detection during the monitoring sampling (plankton) in a new location (San Vicente de la Barquera) pose new and important risk warnings, as this is the most western position in which this species was detected in north Spain, an important area regarding both tourism and the production of mollusks

Keywords: *Ostreopsis*, Palytoxin, Cantabrian Sea, HAB

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3.02 From laboratory to pilot scale: increasing culture productivity of a *Gambierdiscus excentricus* strain native to Canary Islands

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Abstract: The marine dinoflagellates belonging to genus *Gambierdiscus* are responsible of global Ciguatera Fish Poisoning (CFP). Cultivation is required to achieve a sufficient number of cells for toxins structure characterization and potential biotechnological purposes. However the typical benthic behavior and slow cell growth rate make these microalgae challenging to grow. To the best of our knowledge, cultivation of *Gambierdiscus* spp. is carried out at laboratory scale, whereas pilot-scale cultivation has not been performed so far. Currently traditional microalgal cultivation systems (i.e., industrial scale photobioreactors) result to be inadequate. Homogenous sampling of pilot scale benthic culture represents an additional bottleneck preventing traditional approaches for growth monitoring.

In this study, cultivation from laboratory to pilot scale of a *Gambierdiscus excentricus* strain native to Canary Islands (OCH45) was optimized to increase biomass productivity. Cells were cultured in polystyrene flasks using f/2 medium (-Si; -Cu; +Se) with a N:P ratio of 24 (control) and 120 (treatment). Although maximum cell densities were not different, productivity in the treatment was 1.7 times higher than in the control due to a faster growth ($\mu = 0.107 \pm 0.002$ vs 0.071 ± 0.004 day⁻¹). Based on the results, cultures in f/2 medium N:P 120 were scaled up from flasks to polypropylene trays with 7 times higher surface area and productivity was calculated at 20, 40 and 50 days. The maximum productivity value corresponding to 84 cell ml⁻¹ day⁻¹ was reached at 40 days with a significant decrease up to 60 cell ml⁻¹ day⁻¹ at 50 days, indicating that 40 days of cultivation are sufficient to achieve maximum cell densities.

In order to maximize areal productivity, a polymethylmethacrylate (PMMA) prototype for *G. excentricus* pilot scale cultivation was designed. Preliminary results indicate that while the best volumetric productivity in the prototype (34 ± 3 cell ml⁻¹ day⁻¹; obtained at 50 days) was significantly lower than the best volumetric productivity in polypropylene tray and cell flask (60 ± 8.3 , 75 ± 7.2 cell ml⁻¹ day⁻¹ respectively; obtained at 40 days), its areal productivity was higher due to the possibility to stack multiple vessels.

All the results obtained in this study seem to confirm that a pilot scale cultivation of *G. excentricus* is achievable.

Keywords: *Gambierdiscus*, benthic, cultivation, productivity, culture scale-up

Project: MIMAR+ (MAC2/4.6d/249) INTERREG MAC 2014-2020

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3.03 The incidence of ciguatoxin in the Canary archipelago in recent years recorded in the MIMAR Project

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Abstract: Ciguatoxins are toxins resulting from the metabolism in herbivorous fish of their gambiertoxin precursors, produced by some benthic dinoflagellates, giving rise to more toxic forms throughout the food chain and consumed by humans, and producing what is known as intoxication by ciguatera (CP) (Otero and Silva, 2022). Since 2004, cases of CP have been reported in tropical-temperature zones, such as in the Canary archipelago, and since 2016 in the MIMAR project (Monitoring, control and mitigation of the proliferation of marine organisms associated with human disturbances and climate change in the Macaronesia Region) are studying different species likely to be carriers of the toxin throughout the food chain. Among other species that have been studied are Ornate wrasse (*Thalassoma pavo*), parrotfish (*Sparisoma cretense*), Canary damsel (*Similiparma lurida*), Blacktail comber (*Serranus atricauda*), amberjack (*Seriola* spp.), Island grouper (*Mycteroperca fusca*), Dusky grouper (*Epinephelus marginatus*), Macaronesian sharpnose-puffer (*Canthigaster capistrata*), Red porgy (*Pagrus pagrus*), etc. Although top predators are more likely to be highly toxic, ciguatoxin has been detected in fish species that belong to the intermediate links of the food chain and these biotoxins have even been detected in detritivores invertebrates (Otero and Silva, 2022). After collecting samples of fish and invertebrates from, among others, the species mentioned above, throughout the entire Canary archipelago and from 2016 to 2022, toxicity was analyzed by screening CTX-like toxicity with the neuroblastoma cell-based assay (neuro-2a CBA) (Caillaud *et al.* 2010, 2012).

The results indicate a higher incidence of ciguatoxin in fish samples on the island of El Hierro in 2016, following a natural phenomenon, a toxic bloom of dinoflagellates (Soler-Onís *et al.* 2016). This incidence decreases in general throughout the archipelago until 2019, except on the island of Gran Canaria, where there is an increase. As of this year, the incidence is gradually increasing on the island of El Hierro and decreasing on the island of Gran Canaria, which is undergoing fluctuations over the years. In no case have the levels of El Hierro in 2016 been reached.

With the results we have obtained over the years, although the incidence has decreased, it cannot be concluded that there is no risk of finding a ciguateric animal, since the results fluctuate over the years, over the seasons within the same year and even between islands.

Keywords: CTX, Canary archipelago, MIMAR project, *Gambierdiscus*

Acknowledgments: The present work was supported by funds from the Project “Seguimiento, control y mitigación de proliferaciones de organismos marinos asociadas a perturbaciones humanas y cambio climático en la región Macaronésica-MIMAR”, of the call for Cooperation Projects INTERREG MAC 2014-2020 (Ref. MAC/4.6d/066). We would like to thank the Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, S. G. de Protección de los Recursos Pesqueros D. G. de Recursos Pesqueros y Acuicultura Secretaría General de Pesca for its help in carrying out the samplings in La Restinga Marine Reserve.

Project: Seguimiento, control y mitigación de proliferaciones de organismos marinos asociadas a perturbaciones humanas y cambio climático en la Región Macaronésica (MIMAR+) (MAC2/4.6d/249).

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3.04 Ciguatoxins and small-scale fisheries: thirteen years of experience in the Canary Islands

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Abstract: The presence of certain marine biotoxins in food is regulated by European Union (EU). With regard to bivalve mollusks toxins - for example, related to Amnesic Shellfish Poison (ASP) or Paralytic Shellfish Poison (PSP) - there are maximum concentrations that allow the sale for human consumption (see Regulation (EC) No 853/2004). In the case of ciguatoxins (CTX), which may cause Ciguatera Fish Poisoning (CFP), concentrations are not established, but their mere presence precludes their sale. This is likely due to the fact that, historically, CFP cases in the EU did not originate from catches in EU waters (scarce investigation), not to mention the difficulty of setting toxin thresholds [1].

However, after the outbreaks in 2008 and 2009 [2], there was a turning point for both the health and fishing authorities of the Canary Islands. Currently, more than 100 cases of CFP have been reported. The control and monitoring of landings, in coordination with other competent authorities, are part of the competencies of the Directorate General for Fisheries (DGP) of the Canary Islands Government. Approximately 750 boats and 1,300 people on board make up the small-scale fisheries (SSF) that supply fresh fish to restaurants, hotels, and individuals. There are dozens of species of interest for the SSF, but only a few might have CTX. Moreover, species related to the CFP outbreaks in the archipelago can be counted on the fingers of one hand. So, how to deal with this (dynamic) scenario?

In 2009, DGP implemented an innovative protocol [3] for the professional fishing sector based on the analysis of the presence of CTX in certain species and sizes [4], [5]. Its implementation has eliminated the occurrence of CFP outbreaks caused by fish from the authorized points of the first sale in the Canary Islands. Moreover, this protocol has improved food security, undermining the economic impact that the ban on catches would have had. In this sense, according to official data, 89% of the specimens analyzed in the last five years (5,595 large fish) have been sold with a total guarantee of the absence of CTX. The recent protocol update (January 2022) has resulted in significant changes in how information is managed between the stakeholders involved: professional fishermen, the laboratory, and the regional fishery administration. The efficiency, traceability, and safety of the entire process have been increased.

Also, the DGP, through GMR Canarias, and the professional fishing sector aim to evaluate areas where the likelihood of capturing certain individuals with CTX could be significant. Moreover, in these times of climate change, species whose presence or abundance varies are being considered for monitoring purposes. The ocean literacy is another key element, so training for the fishing sector and the general population, in addition to several educational materials, are being produced.

The aim of this communication is to present the evolution of the fisheries and CTX presence in the Canary Islands.

Keywords: Ciguatoxins (CTX), Ciguatera Fish Poisoning (CFP), Small-Scale Fisheries (SSF), Canary Islands.

Acknowledgments: This work was developed within the framework of the MIMAR+ Project (MAC2/4.6d/249), co-financed by the European Regional Development Fund (EDRF) and INTERREG V-A Spain-Portugal MAC 2014-2020.

Project: MIMAR+

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3.05 An unprecedented bloom of *Lingulodinium polyedra* on the French Atlantic coast during summer 2021

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Abstract: At the end of July 2021, a bloom of *Lingulodinium polyedra* developed along the French Atlantic coast and lasted six weeks. The REPHY monitoring network and the citizen participation project PHENOMER contributed to its observation. A maximum concentration of 3,600,000 cells/litre was attained on September 6 at Doëlan-sur-mer, concentrations never recorded on French coastlines. Unusually high precipitation during mid July led to high river discharges by the Loire and Vilaine rivers which likely triggered the bloom, which was accompanied by increased sea surface temperatures. Satellite observations of estimated Chl a concentrations, confirmed that the bloom reached its highest abundance and spatial extension early September, covering about 2500 km² on September 4. The winds drifted the bloom westward. The bloom was preceded by *Leptocylindrus* sp. and was succeeded by high *Noctiluca* concentrations. Cultures were established and ITS-LSU sequencing identified the species as *L. polyedra*. The pigment composition of the bloom was similar to that of cultured *L. polyedra*, suggesting that a biomass dominated by this species. The thecae displayed the characteristic tabulation and sometimes a ventral pore. Cysts were observed in the plankton, towards the end of the bloom, with concentrations up to 30,000 cells/litre and relative abundances up to 99%. The bloom deposited a seed bank, with cyst concentrations up to 100,000 cysts/gram dried sediment, particularly in fine-grained sediments. Concentrations of yessotoxins up to 747 µg/kg were recorded in mussels, below the safety threshold of 3,750 µg/kg. Oysters, clams and whelks also were contaminated but at lower concentrations. The established cultures did not produce yessotoxins at detectable levels, although yessotoxins were detected in the sediment. Blooms of other dinoflagellates such as *Alexandrium minutum* occurred in nearby embayments. The unusual environmental conditions that triggered the bloom provide an important model to understand future harmful algal blooms along the French coastline.

Keywords: Seed bank, yessotoxins, Vilaine Bay, southern Brittany, harmful algal blooms

3.06 Interannual and spatial variability of *Dinophysis acuminata* in north-western Iberian shelf over a 25-year time serie

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Abstract: Harmful Algal Blooms (HABs) are extreme biological events that affect upwelling systems in all aquatic environments. One of the causative specie is the dinoflagellate *Dinophysis acuminata*, which is associated with lipophilic toxin outbreak happening yearly, from spring to autumn, at the Galician Rias Baixas, causing shellfish harvesting bans. Blooms of this species are designated when the presence of cells per liter, at the Galician Rias Baixas, is equal to or higher than 200 cells/L. A 25-year time serie, from 1996 to 2020, recorded by Instituto Tecnológico para o Control do Medio Mariño de Galicia (INTECMAR), was first used to study the temporal and spatial variability of *D. acuminata* in the Rias Baixas. This dataset [cells/L] consists in counting cells weekly sampled at 29 stations (Figure 1), at Ría de Arousa (10 stations), Pontevedra (11 stations), and Vigo (8 stations), between 0 m and 15 m depth. To get a finer temporal and spatial frequency, we applied a linear temporal and spatial interpolation to the *D. acuminata* [log₁₀ cells/L] densities at the three Rias, obtaining a daily frequency and covering the whole of each of the three Rias. Empirical Orthogonal Function (EOF) analysis was performed on this dataset through singular value decomposition (SVD).

The seasonal cycle of *D. acuminata* cells [cells/L] estimated by monthly average at eacg Ría (Figure 2a) depicted a maximum in April at Ría de Pontevedra, and in May at Ría de Vigo and Arousa, after what values decreased, increasing again in August at the three Rias to reach lowest values in December. A synoptical view of average data at each Ría (Figure 2b) showed a gradient towards the inner shelf, where lower densities were reached. This average also remarked the predominance of cells densities at Ría de Pontevedra over the other two Rias (Figure 2b). This Ría had the first seasonal maximum and the highest one in April at the inner part (P0; Figure 2c), which coincided with an increase at outer part of the same Ría (P4) and at the outer part of Ría de Arousa (A0). The inner part of Ría de Vigo (V3) has a monthly maximum in May, during which maximum at outer Ría de Arousa remained. After these firts maximum, *D. acuminata* cells decrease, increasing again in August for outer part of three Rias (P4, V5, and A0 by descendent order), in September at inner Ría de Pontevedra

(P0), and in October at inner Ría de Vigo (V3). The lowest values of the seasonal average are found at inner Ría de Arousa (A3), where bathymetry is shallower. Regarding blooms of *Dinophysis acuminata* [cells/L_≥200], these are present along all the 25-year time serie

(Figure 3). The years with the longest days duration of blooms (Figure 3a,b) and higher numbers of blooms (Figure 4c,d) are from 1998 to 2005 and from 2011 to 2016. Comparing the three Rias, Ría de Pontevedra presents a higher number of blooms (Figure 3c) with a longer duration (Figure 3a) than the other Rias.

Empirical Orthogonal Function (EOF) analysis was applied to temporal and spatially interpolated *D. acuminata* cells count [log₁₀ cells/L]. Three first EOF modes (Figure 4) explain 68.35%, 6.18%, and 4.74% of the total variance respectively, summing up to 79.27%. The first spatial EOF (Figure 4a) has a similar pattern to the spatial average depicted in Figure 2a. The second spatial EOF (Figure 4b) shows a latitudinal pattern with a southerly gradient from Ría de Arousa to Ría de Vigo and the third spatial EOF (Figure 4c) is alike to first spatial EOF. Linear correlations coefficients (*r*) were done between temporal PC and the mean of different forcings for the previous 15, 30, and 45 days (Table 1). Mode 1 shows higher and positive correlations with surface downward short-wave radiation flux, SST and zonal wind component, and a negative one with precipitation. The second mode has a positive correlation with the meridional wind component, around 0.2, and the third mode shows higher correlations with radiation, and meridional wind component, being positive and negative, respectively. Following these correlations, we can hypothesize that the first mode can be more influenced by meteorological variables and zonal wind components, the second mode by the occurrence of upwelling/downwelling events, and the third mode by a combination of what affects the first and second modes. Future works might be aimed at the computation of more complex statistical tools, such as techniques of machine learning. It will help to explore how, and which environmental factors play a role in the spatial and interannual distribution of *D. acuminata* cells.

Keywords: HAB, *D. acuminata*, NW Iberian shelf, EOF, interannual.

Acknowledgments: The authors gratefully acknowledge the financial support of “Fundação para a Ciência e Tecnologia” (FCT–Portugal), through the research scholarship PD/BD/143085/2018.

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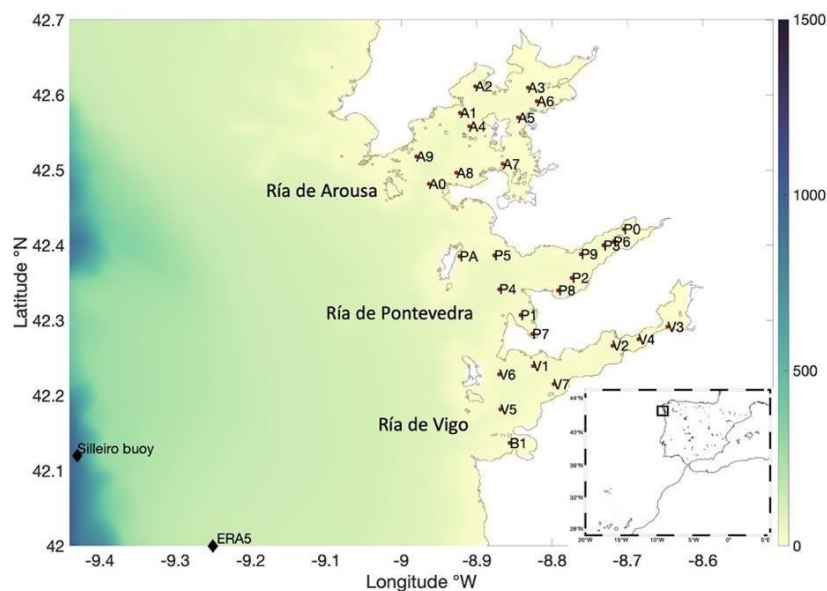


Fig. 1. Study area of Ría de Arousa, Pontevedra and Vigo with locations of INTECMAR stations, Silleiro buoy and point where data from ERA-5 was taken. Bathymetry is depicted by colorbar.

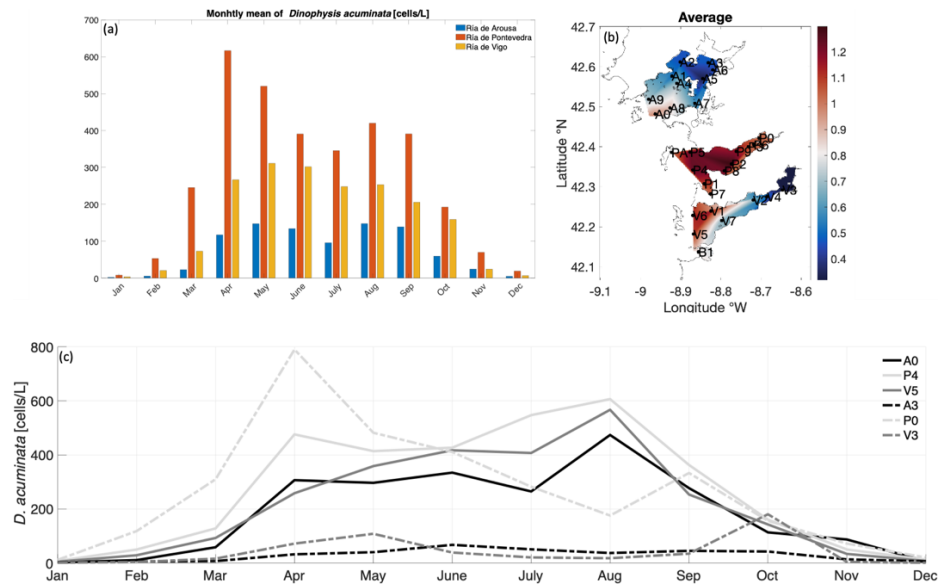


Fig. 2. (a) Monthly average of *Dinophysis acuminata* [cells/L] from 1996 to 2020 at Ria de Arousa (blue), Pontevedra (orange) and Vigo (yellow). (b) Average of *Dinophysis acuminata* [log10 cells/L] from 1996 to 2020 at each Ria with locations of INTECMAR stations. (c) Monthly average of *Dinophysis acuminata* [cells/L] from 1996 to 2020 at outsiders (solid line; A0, P4 and V5) and insiders (dashed line; A3, P0 and V3) stations of Ria de Arousa, Pontevedra and Vigo.

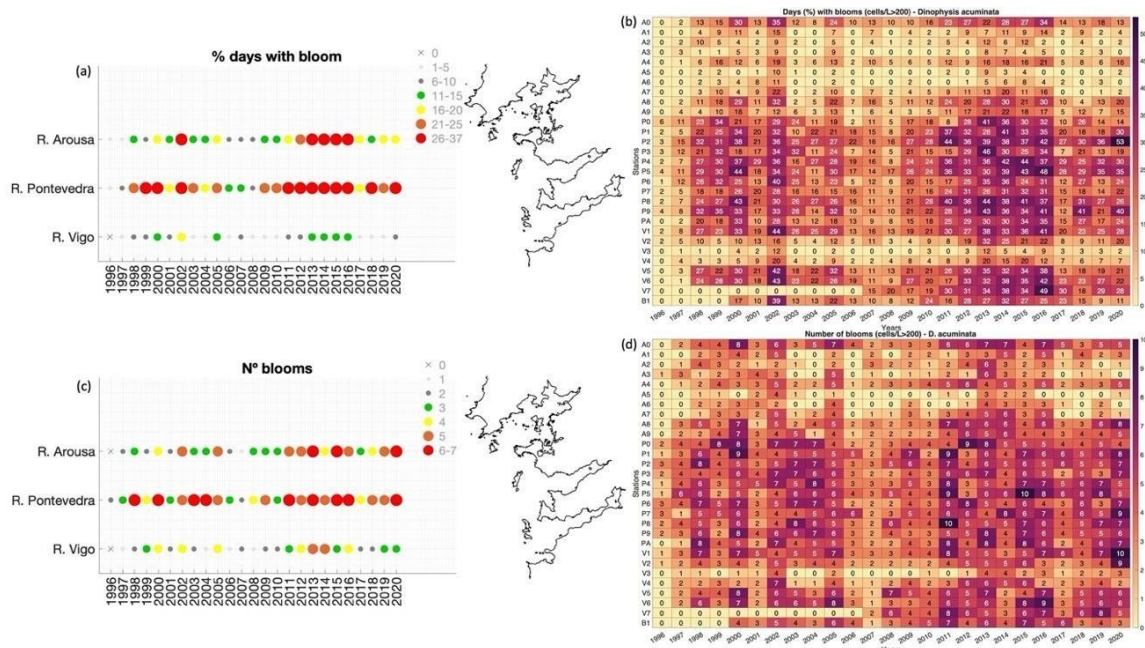


Fig. 3. (a) Average of percentage of days that each bloom of *Dinophysis acuminata* lasts at Ria de Arousa, Pontevedra and Vigo from 1996 to 2020. (b) Percentage of days that each bloom of *Dinophysis acuminata* lasts per station and year. (c) Same as (a) for average of number of blooms. (d) Same as (b) for number of blooms.

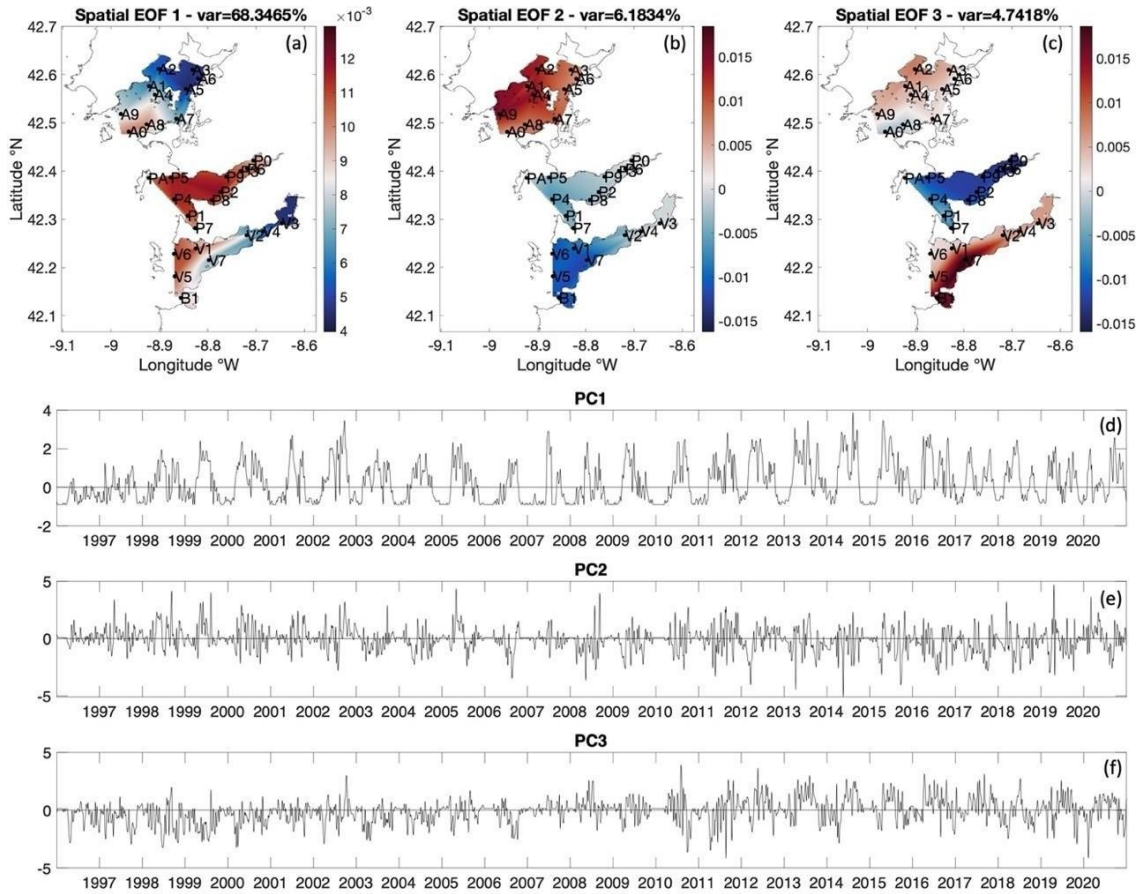


Fig. 4. Three first (a,b,c) spatial and (d,e,f) normalized temporal EOF modes (by subtracting the median and dividing by the standard deviation) of *Dinophysis acuminata* [log10 cells/L] from 1996 to 2020 at Riá de Arousa, Pontevedra and Vigo.

Table 1. Linear correlation between three first PC modes and zonal (u) and meridional (v) wind velocity components (m/s), mean surface downward short-wave radiation flux (Rad; W/m²), Sea Surface Temperature (SST; oC), mean total precipitation (Prec; kg/m² s) for the mean at the previous 15, 30 and 45 days.

	u ₁₅	v ₁₅	Rad ₁₅	SST ₁₅	Prec ₁₅	u ₃₀	v ₃₀	Rad ₃₀	SST ₃₀	Prec ₃₀	u ₄₅	v ₄₅	Rad ₄₅	SST ₄₅	Prec ₄₅
Mode 1	0.16	-0.08	0.53	0.27	-0.23	0.19	-0.20	0.56	0.18	-0.31	0.20	-0.30	0.56	0.10	-0.35
Mode 2	0.02	0.20	-0.10	0.04	0.11	0.03	0.19	-0.09	0.03	0.13	0.03	0.18	-0.07	0.02	0.12
Mode 3	-0.01	-0.10	0.11	0.07	-0.07	0.02	-0.10	0.11	0.07	-0.08	0.06	-0.08	0.12	0.07	-0.05

3.07 Persistence of ciguatoxin producers in the Balearic Islands

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Abstract: *Gambierdiscus* spp., and *Fukuyoa* spp., are ciguatoxin (CTX) producing dinoflagellates, reported in the Balearic Islands in recent years (Laza-Martínez et al. 2016; Tudó et al. 2018). This study confirms their persistence in the Balearic archipelago over the last 6 years. Macrophytes were sampled by scuba diving in a non-continuous way from 2016 to 2021, in October, in Mallorca, Menorca, Ibiza and Formentera. The identification at the genus level was carried out under an inverted light microscope while molecular identification was performed for achieving the species level identification of the clonal cultures isolated from the samples. *Gambierdiscus* spp. were found in all the islands. In 2020 and 2021 a total number of 31 points were sampled each year where the 5 macrophytes most frequently sampled were *Cladostephus spongiosus*, *Halopteris scoparia*, *Padina pavonica*, *Digenea simplex* and *Posidonia oceanica*. Among all macrophytes from the 31 sampled points, the only species in which *Gambierdiscus* spp. was not found in all samples was *Posidonia oceanica* (absent in 39.3% of the samples). Toxicity was evaluated with Neuro2a cell based-assay from clonal cultures obtained from isolated *Gambierdiscus* cells. The CTX-like toxicity ranged between 1.38

⁻¹ (Tudó et al. 2020) and 395.05 fg CTX1B equivalents (equiv.)·cell (present study). Although no ciguatera cases have been reported in the Balearic Islands, this study provides very valuable ecological data that will contribute to assessing ciguatera risk.

Keywords: *Gambierdiscus*; *Fukuyoa*, Ciguatera; ciguatoxins (CTXs); Balearic Islands

Project: The financial support was received from the European Food Safety Authority (EFSA) through the EUROCIGUA project (GP/EFSA/AFSCO/2015/03) and from the Ministerio de Ciencia e Innovación (MICIN) and the Agencia Estatal de Investigación (AEI) through the CIGUARISK (PID2019-108781RR-C21) and MICIN fellowship (PRE2020- 096800) awarded to Sandra Gimeno-Monforte.

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3.08 Assessment of the growth and toxicity of different strains of *Gambierdiscus* sp.

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Abstract: Microalgae of the genus *Gambierdiscus* are producers of precursors of ciguatoxins (CTXs), which bioaccumulate in the marine trophic chain and are responsible for ciguatera fish poisoning in humans. Ciguatera is a foodborne illness typically known as a tropical disease, but ciguatera cases are being increasingly reported in the North-Eastern Atlantic. The appearance of fish contaminated with CTXs has become more and more common in areas such as the Canary Islands, but the mechanisms underlying the bioaccumulation and biotransformation of the CTXs in this region are still poorly discerned (Soliño & Costa, 2020).

The study presented here is part of the European project “Ciguarisk” and has the objective to assess cell growth and toxin production of two *Gambierdiscus* strains from the Atlantic region selected for fish feeding experiments, i.e., *G. excentricus* IRTA-SMM-17-429 and *G. carolineanus* BEA1923. Cell growth was performed in culture flasks under the same culture conditions, using a semi-continuous batch approach. The culture method is aimed to harvest the highest biomass production possible for use in dietary exposure experiments in fish. Two culture media (L1 and F2) were evaluated for their influence on the growth of the strains. Both media were suitable for culturing *Gambierdiscus*, although some slight differences were observed in growth rates and cell behaviour. *G. excentricus* IRTA-SMM-17-429 presented slower growth with respect to *G. carolineanus* BEA1923 in all conditions tested. Ciguatoxins were extracted from fresh biomass samples and pre-purified using liquid-liquid partitioning. CTX-like toxicity of the strains was screened using the neuroblastoma neuro-2a assay (Caillaud *et al.*, 2010). Preliminary data suggest that *G. excentricus* IRTA-SMM-17-429 is more toxic than *G. carolineanus* BEA1923.

These results presented in this study are in accordance with previous studies on other strains of *G. carolinianus* and *G. excentricus* (Pisapia *et al.*, 2017). Further studies will focus on culture optimization and scale-up, quantitation of the toxic content, and micro-encapsulation of the biomass for the feeding experiments. The results obtained from this project will ultimately help to a better understanding of the bioaccumulation and biotransformation of CTXs in the food chain in the Atlantic region.

Keywords: Ciguatoxins, *Gambierdiscus*, toxicity, bioaccumulation, food chain

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3.09 A 10 year gap on blooms of *Dinophysis sacculus* and DSP closures in Alfacs Bay

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Abstract: Blooms of *Dinophysis sacculus* were frequent in Alfacs Bay (Western Mediterranean) during the last decade of the twentieth century and the first decade of the twenty-first century (Fernández-Tejedor et al., 2010). These blooms were associated to DSP (Diarrhetic Shellfish Poisoning) closures due to toxin accumulation over regulatory levels in shellfish. The last DSP closure occurred in 2012 (García-Altares et al., 2016) and since then no closures had to be enforced until January 2022 when *D. sacculus* reached abundances over alert level (> 1000 cells/L) and okadaic acid was detected over regulatory level. Changes in toxin analytical methodologies, from the mouse bioassay to the LC-MS/MS, could have been involved in the reduction of shellfish closures to a certain extent. Despite the reduction of false positives due to the implementation of LC-MS/MS analysis for lipophilic toxins, a real reduction in the abundance of *D. sacculus* during 10 years in this coastal embayment is the main explanation for the gap in DSP closures.

Keywords: *Dinophysis sacculus*, Mediterranean Sea, okadaic acid, marine toxins

Acknowledgments: The authors wish to acknowledge the technical staff from IRTA and the funding from the Fisheries Directorate.

Project: Monitoring program of water quality, mollusks and toxic phytoplankton at the shellfish growing areas along the Catalan coast (PSQAM).

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3.10 Biodiversity of benthic dinoflagellates in the northern Red Sea

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Abstract: Benthic dinoflagellate assemblages in sub-tropical and tropical areas have been a subject of great attention during the last decades in response to their capability to produce toxins and cause toxic blooms in the coastal areas. However, the diversity and occurrence of benthic dinoflagellates in the Red Sea are less documented so far. Pilot taxonomic surveys of the benthic dinoflagellates in the northern Red Sea were performed for the first time along the Jordanian coast in the Gulf of Aqaba (October 2009 and 2011) and the Egyptian Red Sea coast at Hurghada (July 2018 and January 2022). The bottom sandy sediments, macroalgae and near-bottom water were collected from the shallow slope of the inshore coral reefs by snorkeling at depths of 1.5-2 m. Identification and taxonomic observations of the benthic dinoflagellate samples were made using light, epifluorescence, and scanning electron microscopy. The coastal area of the northern Red Sea was inhabited by taxonomically diverse and abundant assemblages of sand-dwelling and epiphytic dinoflagellates. A total of 62 dinoflagellate taxa belonging to six orders were recorded in this study, including Amphidinales: *Amphidinium*; Gymnodinales: *Ankistrodinium*, *Apicoporus*, *Bindifendia*, *Gymnodinium*, *Moestrupia*, *Nusuttodinium*, *Testudodinium*, *Togula*; Dinophysiales: *Sinophysis*; Gonyaulacales: *Coolia*, *Fukuyoa*, *Gambierdiscus*, *Ostreopsis*; Peridinales: *Amphidiniopsis*, *Bysmatrum*, *Durinskia*, and Prorocentrales: *Prorocentrum*. The records of Dinophyceae *insertae sedis* included rarely encountered taxa belonging to *Amphidiniella*, *Cabra*, *Laciniporus*, *Madanidinium*, *Plagiodinium*, and *Pileidinium*. More than half of the identified benthic dinoflagellates were recorded for the first time in the Red Sea. Based on the obtained material, a new sand-dwelling dinoflagellate *Ailadinium reticulatum* gen et sp. nov. has been described from the Jordanian coast. The high diversity of sand-dwelling dinoflagellates in the northern Red Sea was supported mainly by gymnodinioid, amphidinioid, and prorocentroid taxa. The epiphytic dinoflagellate assemblages were dominated by *Coolia*, *Fukuyoa*, *Gambierdiscus*, *Ostreopsis*, and *Prorocentrum* species. The benthic dinoflagellates of the genus *Prorocentrum* with 15 taxa identified and *Amphidinium* with 8 taxa were among the most diverse and abundant. A wide range of known toxin producers were observed including the ichthyotoxic dinoflagellates of the genus *Amphidinium*, okadaic acid producing *Prorocentrum* species, cooliatxin producing *Coolia*, palytoxin producer *Ostreopsis*, and ciguatera-related *Fukuyoa* and *Gambierdiscus* species. The presence of known toxic dinoflagellate species indicates a potential risk to marine food webs and human health in the northern Red Sea, and underscores the need for further studies on taxonomy, ecology and toxicology of benthic dinoflagellates in this region.

Keywords: Benthic dinoflagellates, New records, Harmful species, Red Sea, Jordan, Egypt

**3.11 Morphology of four marine planktonic dinophysoid (Dinophysales) dinoflagellates from the Mexican central pacific:
Dinofurcula ultima, *D. ventralis*, *Latifascia inaequale*
and *Triposolenia longicornis***

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Abstract: Dinoflagellates are an important component of marine phytoplankton due to their high diversity and considerable contribution to biomass, but additionally for their role as primary and secondary producers. Dinophysoids are very particular thecate dinoflagellates with solitary cells divided in two halves, divided by a sagittal suture, reduced epitheca, and prominent cingular and sulcal lists. During routine analyses of phytoplankton net samples from the central Mexican Pacific, collected in various oceanographic cruises, some specimens of four rare and little-known dinophysoid species were found: *Dinofurcula ultima*, *D. ventralis*, *Latifascia inaequale* and *Triposolenia longicornis*, which were studied using light microscopy. Apparently, the four species are heterotrophic forms, with no chloroplasts but with several vacuoles in the two species of *Dinofurcula* and *T. longicornis*. As in a previous study of *Dinofurcula ultima*, which included observations by scanning electron microscopy, both cell shape and measurements were slightly different from those of the original description, whereas *D. ventralis* was almost identical (shape and size) as the original illustration and description. Detailed observations of *Latifascia inaequale* were made, including a dissection which aimed to show many of the plates that characterize this species, particularly plate H1 (first hypothecal). *Triposolenia longicornis* had a very similar shape and size of the original description; it was surprisingly found in one coastal station. *Dinofurcula ventralis*, *Latifascia inaequale* and *Triposolenia longicornis* represent new records in the Mexican Pacific.

Keywords: Dinoflagellates, Dinophysoids, Morphology, New records, Mexican central Pacific, Phytoplankton.

3.12 *Matsuokaea loeblichii*, rare but everywhere?

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Abstract: A deep analysis into the Peridinales with the objective of clarifying the phylogenetic position of the clade comprising the genera *Ensiculifera* Balech and *Pentapharsodinium* Indelicato & Loeblich III in family Thoracosphaeraceae, was recently performed by Li et al. (2020). This study erected family Ensiculiferaceae Li, Gottschling, Mertens, Gu & Shin with three different clades corresponding to *Ensiculifera*, *Pentapharsodinium* and the new monospecific genus *Matsuokaea* Li, Mertens, Gu & Shin. The three genera share the same tabulation pattern of the motile stage and can only be resolved based on cyst morphology and phylogeny.

Matsuokaea loeblichii, the only species currently assigned to this genus, was first described as *Ensiculifera loeblichii* Cox & Arnott in 1971 from Salton Sea (California, USA) an artificial continental high salinity lake (synonyms: *Peridinium loeblichii* (Cox & Arnott) Dale and *Pentapharsodinium trachodium* Indelicato & Loeblich III). Other records for this species are from off Namibia in the Southeast Atlantic, and from Jinhae Bay (Korea) in the Northwest Pacific.

The present work reports the presence of *M. loeblichii* from the Northeast Atlantic. Cells were identified from a cultured strain established from the isolation of a resting cyst collected from surface sediment samples in Lisbon Bay (Portugal). The cyst was almost spherical with a smooth organic wall and cell contents was characterized by a marked pigment body. The morphology, thecal tabulation and surface ornamentation of motile cells coincided with the description of motile cells of *M. loeblichii*. Phylogenetic analysis of the ITS-5.8S and LSU rDNA revealed that the studied strain grouped in a well-supported clade with three sequences identified as *M. loeblichii* from other geographic locations.

So far, reports of *M. loeblichii* suggest a discrete but wide geographical range, despite the rare occasions it has been cited in the literature. Motile cells of *M. loeblichii* are small (<32µm) and indistinguishable in routine phytoplankton analysis from other small autotrophic peridinioids such as species of *Ensiculifera*, *Pentapharsodinium* and *Scrippsiella* Balech. Likewise, the absence of distinctive features on the morphology of the cyst does not allow unequivocal species identification without germination experiments to establish the cyst-motile stage relationship. In addition, cysts do not resist palynological treatment and are thus disregarded in many cyst surveys. All the above suggest *M. loeblichii* may be underreported in the literature. It is likely that *M. loeblichii* has a wider geographical distribution and its presence in the plankton is more frequent than currently known.

Keywords: Cyst-theca relation, Ensiculiferaceae, Phylogeny, Taxonomy

Acknowledgments: HABWAVE project LISBOA-01-0145-FEDER-031265, co-funded by EU ERDF funds, within the PT2020 Partnership Agreement and Compete 2020, and national funds through Fundação para a Ciência e Tecnologia, I.P.(FCT, I.P.). Funding by FCT, I.P. through the strategic projects UIDB/04292/2020, UIDP/50017/2020 + UIDB/50017/2020 and UIDB/00329/2020. H. David acknowledges FCT funding (SFRH/BPD/121365/2016).

Project: HABWAVE, From benthic resting stages to HAB events: understanding the role of physical-biological coupling off NW Portugal (LISBOA-01-0145-FEDER-031265).

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3.13 Natural sampling of phytoplankton and parasitoid abundances in Cala Santanyí

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Abstract: Harmful algal blooms (HABs) are algal blooms which can have negative impacts on the environment, human activities and human health. In the Mediterranean, HABs frequently occur during the touristic summer months and are usually caused by dinoflagellates, some species of which produce toxins. While many studies have looked into the causes of HABs, fewer have looked into the reasons why HABs terminate. One possible reason for their termination is due to parasitoid infections in dinoflagellates. As part of a larger project for understanding the biological and biophysical mechanisms of parasitoid-dinoflagellate interactions, we are conducting long-term observations of the microplankton community (5 - 100 μm) and physical parameters at Cala Santanyí. Cala Santanyí is a tourist beach on the Southeastern side of Mallorca, Spain, where both HAB-causing dinoflagellates and parasitoids have been detected. The purpose of these observations is to measure year-round abundance, composition, and prevalence of dinoflagellates and parasitoids, as well as the physical and/or biological factors which may influence them. These observations will be combined with future microfluidic experiments exploring individual parasitoid-dinoflagellate interactions to develop a model to predict parasitoid infection rates in dinoflagellate communities. Here we present preliminary observations from data collected from June 2021 to April 2022.

Keywords: Parasitoids, Harmful Algal Blooms, Mediterranean Sea, Infections

Acknowledgments: This project is funded by the European Commission as a Horizon 2020 Marie Skłodowska-Curie Individual Fellowship (Grant No. 896043).

Project: Towards HAB-Controlling Technology: Studying Parasitoid-Dinoflagellate Interactions on Individual- and Population-Scales

3.14 The role of physical forcings on the daily variability of near shore phytoplankton accumulation in the Mediterranean Sea

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Abstract: Harmful algal blooms (HABs) frequently affect the coastal waters of the Mediterranean basin and other world areas, representing a threat to wildlife, humans and to socio-economic activities developed in coastal zones. In the last decades, extensive urbanization on the shore, recreational use of the coastline and intensive agricultural activity have favored HAB occurrences. In Mallorca, groundwater seeps from coastal aquifers provide the necessary nutrients to sustain high phytoplankton biomass in semi-enclosed nearshore areas^[1]. During summer, conditions become optimal for the initiation of algal blooms.

Previous studies have suggested that physical factors, such as wind forcing, may determine the short term dynamics of biomass accumulation, therefore, regulating the occurrence of high biomass episodes. For example, the sea-breeze cycle, characterized by onshore winds during the day and offshore weaker winds at night, may be crucial in explaining the biomass accumulation patterns observed at many locations of the Balearic and Catalan coasts ^[1, 2]. In fact, over short timescales (< 1 day), biotic factors alone cannot explain the observed variations in phytoplankton biomass, as the growth rate of the dominant phytoplankton species are typically low ($0.3-0.7 \text{ d}^{-1}$)^[3].

In this work, we investigate the short-term nearshore dynamics of biomass accumulation during a dinoflagellate algal bloom at Palma Beach (Mallorca, Spain). Continuous records of temperature (T), salinity (S), fluorescence and current velocity were obtained at fixed moorings deployed at depths of 1.2 m, 2 m, and 3 m. Cross-shore continuous near surface Chl-a, T and S data were acquired at 3 hour intervals with a flow-through sampling. Results show that the nearshore biomass accumulation is in phase with local wind forcing. As shown in the figures, the chlorophyll accumulated in the first 100 meters from the shoreline peaks every 24 h around midday, as do the cross-shore winds. Calculating the cross-correlation of the two time-series, we find that the maximum correlation follows a periodic pattern, the periodicity being roughly 24 hour. The two signals result to be positively correlated, the maximum value of correlation corresponding to a zero lag between them.

To describe the temporal variability of the observed accumulation pattern we suggest a simple 1D advection-diffusion model. In this approximation, biological processes are neglected and the biomass is passively advected by wind, salinity and temperature- driven currents.

We propose that the observed temporal and spatial variability of the biomass accumulation is dominated by an interplay between different physical forcings. The already discussed submarine water discharge represents a freshwater input to the system, lowering the salinity of the coastal waters and initiating a salinity-gradient driven circulation. Likewise, the bathymetric gradient and the stability of the water column, lead, under constant surface heating from the Sun radiation to a differential heating of the coastal waters and the initiation of a thermally driven circulation. These two forcings, which tend to advect the biomass off-shore, enter in competition with the on-shore wind driven advection during the day. The concomitant effect of these forcings can control either the retention of plankton biomass near the coastline or its spread over a broader area.

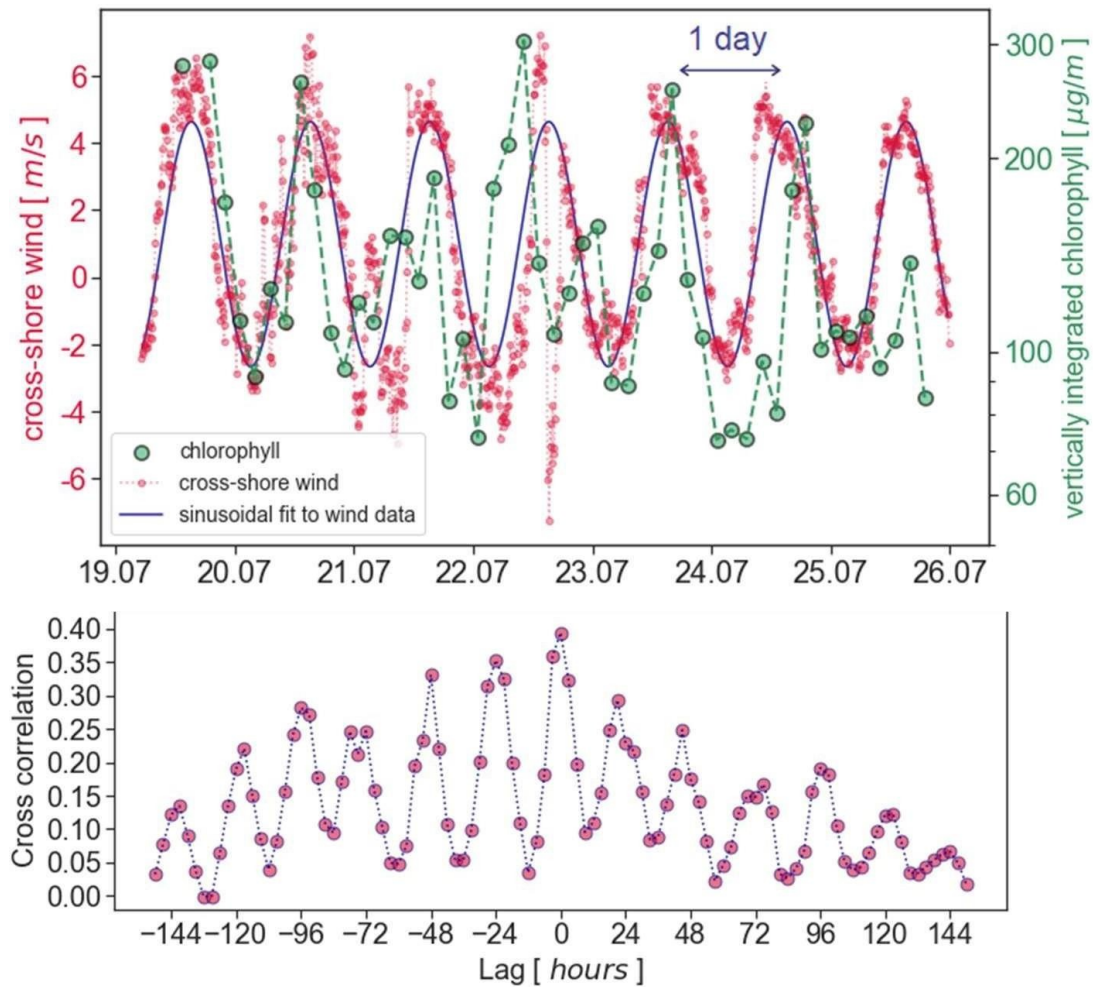
Our result suggests that the physical forcings alone can explain to good extent the observed biomass accumulation pattern.

Keywords: phytoplankton, algal bloom, wind, nutrients

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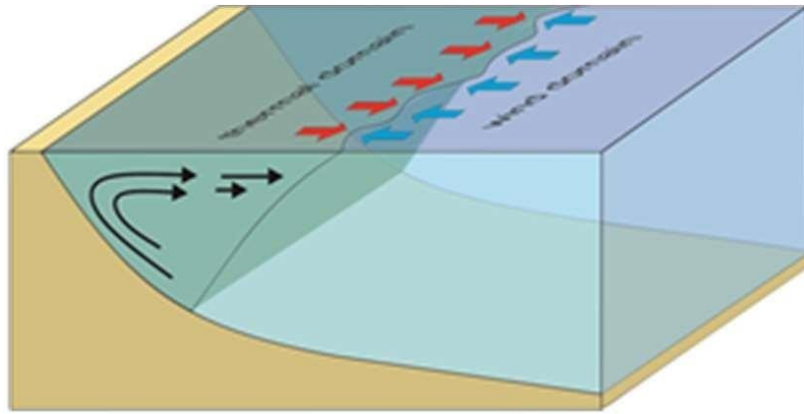
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Figures:



Up: Daily chlorophyll accumulation pattern vs cross-shore wind. The green dots represent the observed nearshore biomass accumulation at Palma Beach during a week of measurements. Biomass accumulation is calculated as the integral of the measured chlorophyll concentration up to 100 meters from the coastline. The pink dots represent the cross-shore wind at the time and location of the observations. The best sinusoidal fit to the wind data is shown by the continuous blue line.

Down: Cross correlation between the chlorophyll accumulation pattern vs cross-shore wind. The cross correlation between the two measurements timeseries in the upper left figure show that the maximum correlation follows a periodic pattern, the periodicity being 24 hours. The accumulation pattern thus results in phase with the periodic wind forcing.



Schematic representation of the physical forcings and nearshore circulation. Water masses near the shore are subject to two opposite concurring forcings during the day hours: an on-shore wind forcing and an off shore forcing resulting from temperature and salinity gradients. The wind forcing, stronger in the early afternoon hours, pushes the water masses towards the coast, creating a nearshore region where the SGD low salinity waters, rich in nutrients, are retained together with the advected biomass. When the system relaxes, the water masses spread out off-shore.

3.15 Assessment of dinoflagellate cyst assemblages and sedimentary concentrations in the northern Bering Sea

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Abstract: A subset of 12 surface sediment samples from the Northern Bering Sea was studied for dinoflagellate cyst assemblage composition, diversity and concentrations. This pilot study is part of ongoing collaborative research on dinoflagellate cysts in the coastal waters of Alaska, and it is currently focused only on the understudied section of this marginal sea in the Pacific Ocean. All samples were collected with a Van Veen grab during the 2019 Distributed Biological Observatory-Northern Chukchi Integrated Survey cruise and processed using a standardized palynological preparation technique (e.g., Pospelova et al., 2010). It includes drying sediments ~40°C, sieving through 120 and 15 µm nylon Nitex meshes, and treating with room-temperature (50% HF and 10% HCl) acids, as recommended by Mertens et al. (2012) and Price et al. (2016). No oxidizing reagents were used to avoid the loss of delicate dinoflagellate cysts (e.g., Dale, 1976; Marret, 1993; Zonneveld et al., 2008). Our preliminary results revealed diverse dinoflagellate cyst assemblages represented by taxa commonly found in nutrient-rich coastal waters in the North Pacific, including *Operculodinium centrocarpum* sensu Wall and Dale 1966, *Spiniferites* spp., *S. elongatus*, *Brigantedinium* spp., and *Islandinium minutum*. All dinoflagellate cysts appear to be well-preserved, and fragile cysts of toxic *Alexandrium catenella* were identified and counted. By comparing sedimentary concentrations of *A. catenella* cysts in our palynologically treated samples with those reported by Anderson et al. (2021) from the same sediment samples, when no sieving or acid treatment was used (Anderson et al., 2014), we estimated potential losses of cysts of *A. catenella* due to palynological sample preparation.

Keywords: Dinoflagellate cyst, cysts of *Alexandrium catenella*, extraction methods, sedimentary concentrations

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3.16 New ecological insight of calcareous dinoflagellate cysts export production based on an 18 years sediment trap study in the Cap Blanc upwelling area (NW. Africa)

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Abstract: Some phototrophic species of dinoflagellates are known to form calcareous cysts. Downcore calcareous dinoflagellate cyst associations are useful to reconstruct changes in past ecosystems, notably in the open ocean (e.g., Heinrich and Zonneveld, 2013). For an adequate establishment of such reconstructions, detailed ecological information with respect to factors that influence cyst production is essential (Zonneveld et al., 2005). Compared to organic-walled dinoflagellate cysts the ecology of calcareous cysts is much less studied. Here, we enrich the ecological information of calcareous dinoflagellate cysts by providing results from an 18 years sediment trap study executed in the Mauritanian upwelling area. For this we compared the cyst export flux with changes in oceanographic conditions at times of deposition. Cape Blanc region is one of the most productive regions in the world, as a result of upwelling of deep nutrient rich waters into the photic zone and mineral dust input from the Sahara (Cropper et al., 2014). Permanent upwelling occurs along a small band near the shelf edge (Fig. 1). Nutrient rich upwelled waters are being transported offshore in the form of large filaments. Although upwelling is present throughout the year, maximal intensity is observed in winter and spring.

Calcareous dinocysts were collected by a sediment trap at about 1300m from 2003 to 2020 with sampling intervals of 1 to 3 weeks. From every sample, 1/125th split material was sieved over 20 µm precession sieve (Storck-Verco) with tap water, concentrated to 0,5 ml solution, and transferred to an eppendorf tube. A known aliquot of the material was embedded in glycerin gelatin and sealed with a cover slip on a palynological slide. The calcareous cysts were counted and identified under polarized-light microscopy. Cysts accumulation rates and relative abundances have been compared with environmental conditions at times of deposition notably sea surface temperature (SST), sea surface temperature difference between the trap site and an open ocean location 200km west of the trap location (SSTA), Chlorophyll-a concentrations at the trap site, local wind speed, local wind direction and dust input. Correlation has been performed using the multivariate ordination method Redundancy Correspondence Analysis (RDA)

(Jongman et al., 1995). Information about the local wind system and dust events are derived from the meteorological report of Nouadibhou airport (Cape Blanc). Data of SST, SSTA and Chlor-a are obtained from ERDAPP satellite observation (<https://coastwatch.pfeg.noaa.gov/erddap/griddap/ncdc/Oisst2Agg.html>).

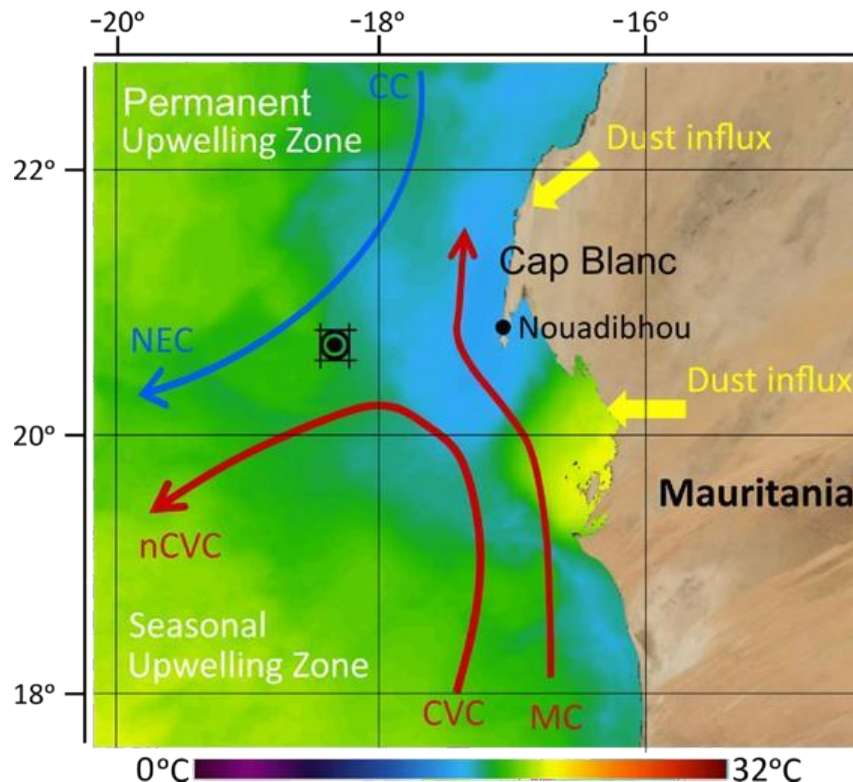


Fig. 1. Location of CBeu sediment trap (double black circle) and oceanography of studied area. Blue arrow indicates cold water of Canary Current (CC) and North equatorial Current (NEC); red arrows indicate warm water of polarward Mauritanian Current (MC), Cabo Verde Current (CVC), and north Cabo Verde Current (nCVC). Satellite image depicted from NASA "State of The Ocean (SOTO)" of sea surface temperature (SST) on March 24th, 2021.

The calcareous-walled cyst association is dominated by *Thoracosphaera heimii*, followed by *Calciadinellum albatrosianum*, and *Leonella granifera*. Total cysts flux appeared to be increased at times of low upwelling intensity (upwelling relaxation). This is in line with previous findings that generally documented enhanced cyst concentrations in oligotrophic environments with stratified upper water column (e.g., Höll et al., 1998; Esper et al., 2000; Vink et al., 2000). Based on visual examination of the data and the RDA ordination (see fig. 2), we could arrange 4 groups of species with comparable relationship to environmental parameters. Group 1 consist of one species; *T. heimii* that is ordinated in the negative side of the first RDA axis and positive side of the second RDA axis. The axis of this species is heading to the same direction of the wind direction but opposite to the upwelling indices parameters (wind velocity and SSTa). The abundance of *T. heimii* increases at the time of stratified upper water, linked to the result of mentioned publications above. Group 2 consists of *C. albatrosianum*, *C. operosum*, and *Pernambugia tuberosa*, and other rarer species. These species are ordinated at the negative side of the first RDA axis. They show high relative abundances at times of low wind speed with winds blowing from the northeast, slow wind speed, and relative high surface water temperatures. This suggest that these species have highest relative abundances at times of minimal upwelling intensity. Group 3 consist of *L. granifera*, *Lebbessphaera urania*, *Scripsiella trifida*, and *S. trochoidea* ordinated at the

positive side of the first RDA axis and negative side of the second RDA axis. It is positively related to one of upwelling indices (SSTa) and dust-storm events. but negatively to SST and wind direction. In line with previous findings, *L. granifera* species is most abundant

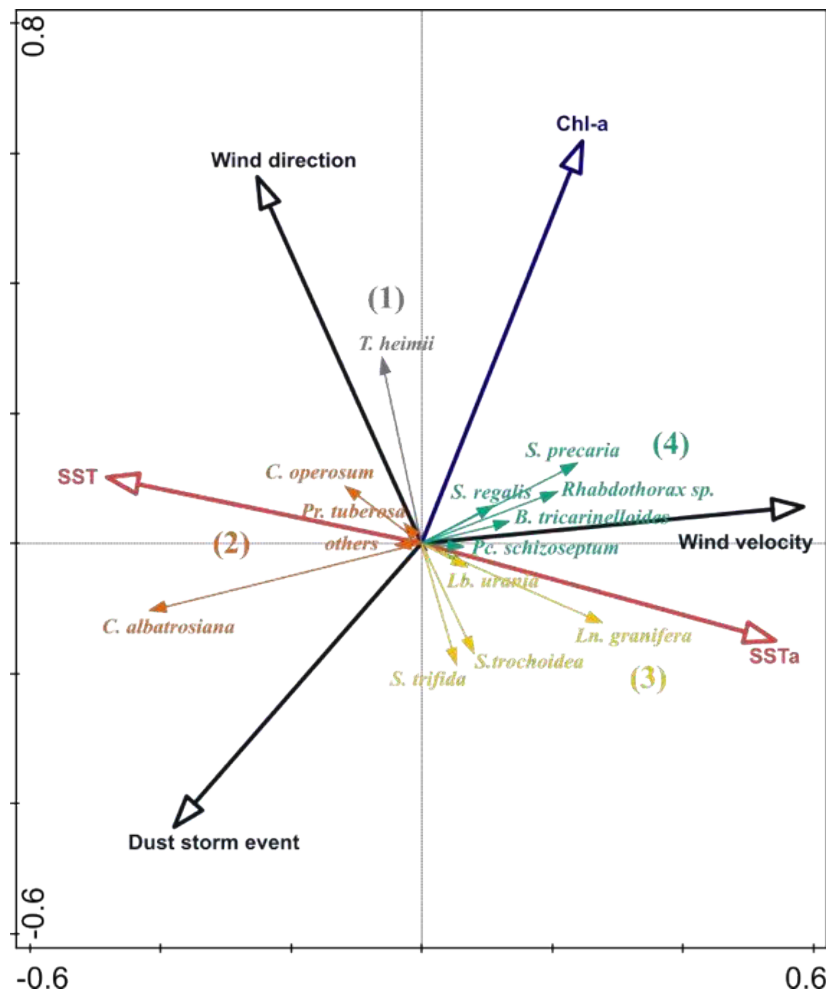


Fig. 2. Result of ecological groups based on RDA of 18 years sediment trap observation. Name of the genus are given as follows; *Bicarinellum* (B.), *Calciadinellum* (C.), *Leonella* (Ln.), *Lebbessphaera* (Lb.), *Praecalacigonellum* (Pc.), *Pernambugia* (Pr.), *Scripsiella* (S.), *Thoracosphaera* (T.). Ecological groups are symbolized with number as follows; (1) cosmopolite, (2) warm temperature, (3) eutrophic-1, (4) eutrophic-2.

at times of enhanced dust input in the region (e.g., Siggelkow et al., 2002; Zonneveld et al., 2005). Group 4 consist of *Bicarinellum tricarinelloides*, *Praecalacigonellum schizoseptum*, *Rhabdothorax sp.*, *S. precaria*, and *S. regalis*. These species are ordinated at the positive side of the first and second RDA axes in the same direction as wind speed (the main driven-factor of upwelling in the region), SSTa, and Chl-a. The ordination of group 3 and 4 (except for *L. granifera*) suggest that these species are more abundant at times of enhanced upwelling intensity. So far, only high abundance of *S. trochoidea* have been reported to be linked to nutrient rich environments (Zonneveld et al., 2000; Wendler et al., 2002). Therefore, we assume species of group 3 and 4 are more tolerable to the upwelling conditions and eutrophic surface waters compared to the other calcareous dinoflagellate cysts species. This finding can improve the knowledge of calcareous cyst ecology, since previous studies often relate the enhanced production of calcareous cysts as indication of limited nutrient environment.

Keywords: calcareous-walled cysts, dinoflagellates, ecology, Cap Blanc, upwelling, upwelling relaxation.

Acknowledgments: We would like to thank the captains and crew members of RV Poseidon, RV METEOR, and RV Maria S. Merian, as well as The MARUM sediment trap team for deploying and recovering the sediment trap. We are also thankful of the support from German, Moroccan, and Mauritanian authorities. This extensive research is supported by the funding from German Research Foundation (DFG) through MARUM Excellence Cluster “The Ocean in the Earth System”. Lastly, we thank all institutions and individuals who have participated and contributed throughout this research.

Project: Doctoral Project of Surya Eldo V. Roza (2020-2023): Natural and Anthropogenic forcing on marine pelagic ecosystems.

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3.17 Marine synthetic peptides interact with SARS-COV- 2 replication protein complex

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Abstract: Dinoflagellates and marine organisms are great sources of natural products with potential biomedical applications. Potential biomedical compounds include neosaxitoxin (Rodriguez-Navarro, et al. 2007) and gonyautoxin (Garrido, et al. 2005) which are currently being explored as anti-pain medicines. Dinoflagellates are mostly composed of 40.71% proteins, 18.12% fatty acids and 20.31% sugars (Subong, et al. 2021). The diverse macromolecules and secondary metabolites are potential sources of new synthetic compounds that can be used to probe disease mechanisms or treat new diseases.

In this study, we synthesized peptides which include a natural marine natural product template we named PSL-169, molecular weight of 1085.3 amu and a marine natural product inspired peptide from template peptide, ILL-170, molecular weight of 1,194.2. Peptides were synthesized using a solid-phase peptide synthesizer, purified using high-performance liquid chromatography and molecular masses were confirmed using liquid chromatography mass spectrometry- electrospray ionization (LCMS-ESI).

The SARS-CoV-2 proteins namely nonstructural proteins 7 and 8 (nsp7-8), viral replication proteins, were fused to 8x histidine tag and FLAG tag and were then transfected to Flp-In T-Rex 293 cells. Upon induction of doxycycline, the expression of the said protein complex was expressed. Western blot analyses using anti-FLAG antibody confirmed expression of the proteins upon induction. Immunostaining showed localization of the proteins at the cytoplasm. Purification was performed using histidine tag purification followed by FLAG-tag purification.

Nano-isothermal calorimetry showed that PSL-169 has shown binding affinity towards nsp7-8 at the micromolar quantity whereas ILL-170 has binding affinity at sub-micromolar quantity. Moreover, MTT assay showed both peptides are non-toxic even at 100 μ M concentrations. We determined potential interaction binding sites of the ILL-170 peptide and nsp7-8 using *in silico* analyses. The best model indicated a root-mean-square deviation of atomic positions at 0.77 Å. The peptide can interact with 34 positions in the nsp7-8 protein complex (with 4.5 Å estimated distance). This highlights good binding capacity and binding positions for the peptide-protein interaction.

The development of this peptide can be further utilized as a potential tool to probe viral replication mechanism and can be further explored for potential anti-viral replication activity. The study highlights marine natural products as potential sources of new compounds in the study of SARS-CoV-2.

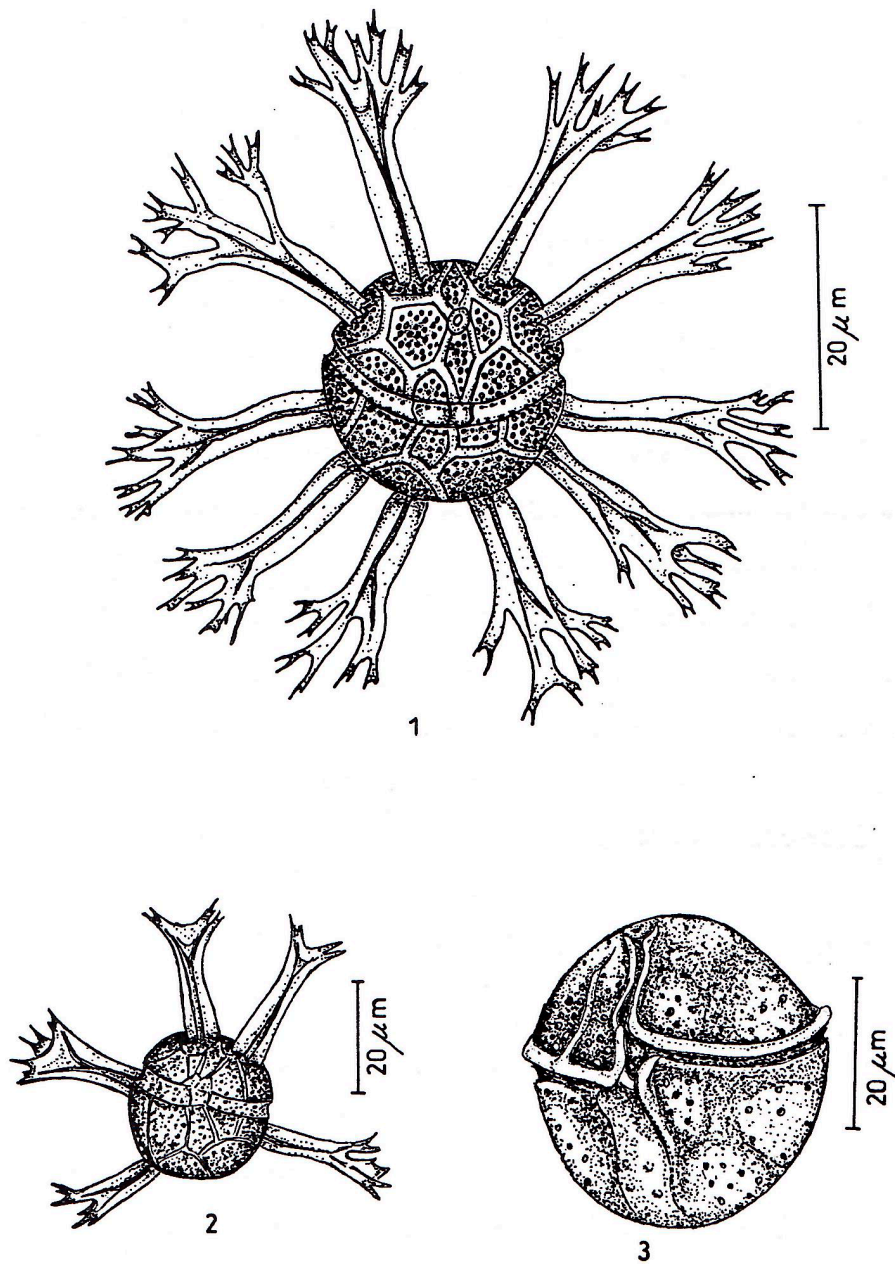
Keywords: peptides, natural products, toxins, SARS-CoV-2

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SESSION 4
PALEOECOLOGY
AND
PALEOCEANOGRAPHY I

4.01 Dinoflagellate cyst assemblages from the Gulf of Corinth (eastern Mediterranean Sea) reveal environmental and climatic shifts since the Middle Pleistocene

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Abstract: Site M0078, drilled during IODP Exp. 381, is located at the central part of the Gulf of Corinth in central Greece. The Gulf is connected, at its western part, with the open Mediterranean Sea through the 60m shallow Rion sill. The area holds a long record of syn-rift sedimentation, with the earlier rifting phase being exposed onshore, while the younger phase deposits are found within the Gulf. As a result of its geomorphology, this semi-enclosed basin is sensitive to Quaternary climatic changes and sea level fluctuations. The Corinth Gulf was repeatedly isolated from and reconnected to the Mediterranean Sea during glacial/interglacial cycles (McNeil et al., 2019), resulting in the amplification of paleoenvironmental gradients. Alternation of marine and (semi-)isolated intervals and the impact of those shifts on local aquatic ecosystems were investigated through the palynological analysis of the site M0078 deposits, focusing on organic-walled dinoflagellate cysts. These palynomorphs are an excellent proxy for palaeoenvironmental reconstructions, as they are sensitive to shifts in the environmental conditions and thrive in a wide range of climatic conditions and habitats, from freshwater to the open ocean.

In the present study, the M0078 core was regularly sampled at a mean 2m interval, spanning several climatic cycles since the Middle Pleistocene. The samples originate from both marine, isolated and transitional intervals. An average of 200 dinoflagellate cysts were counted, while samples containing fewer than 50 cysts were not included in the analysis. The dataset was subjected to non-metric multidimensional scaling (nMDS) and to Principal Component Analysis (PCA).

The microscopic analysis recorded the presence of more than 35 dinocyst species. Dinoflagellate cysts are sorted in two major ecogroups presenting alternations between marine and isolated/brackish conditions. The marine intervals are characterized by high dinocyst diversity, with several representatives of the *Spiniferites* group, as well as *N. labyrinthus*, *O. centrocarpum*, *L. machaerophorum*, *P. zoharyi*, *T. pellitum*, *B. tepikiense* and representatives of the *Impagidinium* group.

The brackish intervals are characterized by the presence of *S. cruciformis*, *P. psilata*, *C. rugosum*, *I. caspiense* and *L. machaerophorum* with short processes (<10µm).

The most characteristic species were the low-salinity indicators *S. cruciformis* and *P. psilata*. In several samples, *S. cruciformis* is dominant, characterizing the assemblage as monospecific. The comparison of our dinocyst assemblages with existing sediment core records and surface sediment samples shows affinity to the Black-, Caspian- and Marmara-Seas.

The alternations between marine and brackish conditions recorded in the Corinth Gulf reveal changes in surface water salinity and temperature, in response to the Quaternaryglacial-interglacial cycles. These seem to be in good agreement with global sea-level changes and trace orbital driven climate shifts as shown in the global Marine Isotope Record.

Keywords: Dinoflagellate cysts, salinity, Corinth Gulf, Mediterranean Sea, Quaternary

Acknowledgments: The QECCoRA project is supported by the Hellenic Foundation of Research and Innovation (H.F.R.I., Project Number: 1026)

Project: Quaternary Environmental Changes in the Corinth Rift Area: the IODP 381 palynological record (QECCoRA).

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4.02 Distributions of dinoflagellate cysts and diatoms in surface sediments of the Chukchi Sea in relation to the upper water masses

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Abstract: Dinoflagellate cyst and diatom analyses were performed on 22 surface sediment samples from the Chukchi Sea in order to document their geographical distributions in one of the most understudied sections of the Arctic Ocean and to examine the influence of upper water masses on these two major groups of phytoplankton. Total concentrations vary from 0.9 to 5.9 x 10⁶ valves g⁻¹ for diatoms and from 0.8 to 12.5 x 10³ cysts g⁻¹ for organic-walled dinoflagellate cysts, with the highest values for both groups observed in the southern part of the Chukchi Sea and away from the Bering Strait. Diatom valve concentrations were three orders of magnitude higher than those of dinoflagellate cysts. Well-preserved microfossils were recovered, with a total of 35 and 88 taxa of dinoflagellate cysts and diatom, respectively. The most abundant diatoms are *Paralia sulcata*, *Thalassiosira antarctica*, *Thalassiosira nordenskioeldii*, and *Chaetoceros* spp., whereas cysts of autotrophic *Alexandrium* spp., *Operculodinium centrocarpum*, and heterotrophic *Islandinium minutum* and *Brigantedinium* spp. were most common in dinoflagellate cyst assemblages. Cysts of HAB's causing *Alexandrium* spp. were found in most of the samples, with the highest abundances in the Herald Canyon where it contributes ~56.6 % to the cyst assemblage. As expected, cysts produced by heterotrophic dinoflagellate were more abundant where sedimentary diatom concentrations were the highest.

Statistical analysis identified three major dinoflagellate cyst and diatom clusters: 1. Sites influenced by the Alaska Coastal Current in the eastern part of the Chukchi Sea are characterized by high abundances of *P. sulcata* and *O. centrocarpum*; 2. The western part and Herald Canyon in the northwestern part of the Chukchi Sea are distinguished by diatoms *Chaetoceros* spp., *T. antarctica* and dinoflagellate cysts of *Alexandrium* spp. and affected by the Siberian Coastal Current and Bering Shelf Water; and 3. Assemblages in the southern part of the Chukchi Sea are recognized by noticeable abundances of *T. nordenskioeldii*, cryophilic group of diatoms and heterotrophic *Islandinium minutum*, as well as by overall lower percentages of cysts of *Pentaparsodinium dalei* and *Brigantedinium* spp. This work revealed the potential applicability of the combined use of diatoms and dinoflagellate cysts for reconstructions of past dynamic water mass changes in the Chukchi Sea.

Keywords: Arctic Ocean, diatoms, coastal waters, *Alexandrium* cysts.

4.03 Using the chemistry of dinoflagellate cyst walls; Infrared analyses of individual specimens

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Abstract: Dinoflagellate resting cysts are a useful source of information on present and past environments. Traditionally, this information lies in combining cyst morphology with temporal and spatial cyst occurrence. Different cyst wall chemistries may relate to differences in cyst genesis, biosynthetic pathways which may differ between taxa or as a result of the current environment. Different cyst wall chemistries may also be induced post-mortem, reflecting different preservation environments and diagenetic processes (e.g. Bogus et al., 2012, 2014; Meyvisch et al., 2021; Versteegh et al., 2007, 2010, 2020).

Through a combination of chemical analytical techniques including, infrared spectroscopy, and gas-chromatography / mass spectroscopy after pyrolysis and chemolysis different cyst wall chemistries have been documented and these have been ascribed to differences between species, related to feeding strategy, or attributed to diagenetic processes.

We present a further exploration of what information can be obtained from infrared analysis of individual cysts. We concentrate on different *Impagidinium* species from water samples, oxic and anoxic Recent to Pleistocene sediments from the eastern Mediterranean and off Cap Blanc (NW Africa). We will present the extent to which we can ascribe the differences between individual cysts to differences between species, between regions and between preservation environments.

Keywords: dinocyst, wall-chemistry, infrared spectroscopy, environment, preservation, phylogeny.

Acknowledgments: We thank the captains, crew and scientists on expeditions of the RV Poseidon, RV METEOR, and RV Maria S. Merian for enabling sample recovery. We also acknowledge support from German, Italian, Greek, Moroccan, and Mauritanian authorities. Finally, financial support was obtained from the German Science Foundation (DFG) through grant GPF 18-1_18 and through MARUM Cluster of Excellence "The oceanfloor - Earth's uncharted interface / Receiver unit 3". Lastly, we thank all institutions and individuals who have participated and contributed throughout this research.

Projects: MERMED (German Science Foundation (DFG) grant GPF 18-1_18); Receiver unit 3 (DFG funded MARUM Cluster of Excellence “The ocean floor - Earth's uncharted interface”).

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4.04 High-resolution reconstructions of Holocene sea-surface conditions from dinoflagellate cysts in the northern south China Sea

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Abstract: A high-resolution dinoflagellate cyst analysis on a sediment core GLW31D from the northern South China Sea (SCS) was performed to reconstruct paleoceanographic conditions over the last 12,500 years through qualitative, semi-quantitative, and quantitative methods. A modern dataset with 398 reference sites in the northern Pacific was assembled and used to identify the relationship between dinoflagellate cyst assemblages and sea-surface temperature (SST), sea-surface salinity (SSS) and primary productivity (PP). Modern analog technique (MAT) was applied to offer first dinoflagellate-cyst-based quantitative estimates of Holocene sea-surface conditions in the western North Pacific. The downcore reconstructions show that SST,

SSS and PP were predominantly controlled by the changes in coastal and oceanic currents due to the changes in sea level and monsoon systems. Our results indicate that SST increased while SSS and PP decreased from 12,500 to ~6800

cal yr BP, reaching the maximum SST and the minimum SSS and PP during ~6800–5000 cal yr BP, and followed by a slight decline in SST with minor increases in SSS and PP. The three intervals correspond to the regional onshore sea-level stages of rising, stabilization in a highstand and slight drop, respectively. The Kuroshio Current strongly influenced the core site before ~9900 cal yr BP, reflected by the highest abundances of oceanic *Impagidinium* spp. and high reconstructed SSS values. This can be explained by a lack of water input from the East China Sea before the opening of the Taiwan Strait. The warmest period, from ~6800 cal yr BP to ~5500 cal yr BP, is recorded by the highest *Dapsilidinium pastielsii* abundances. Two short-term high-PP events of ~2700–2400 cal yr BP and ~1000–600 cal yr BP, which were characterized by opposite climatic conditions, coincided with two notable societal (dynasty) collapses of China. Enhanced anthropogenic activities since the Late Bronze Age most likely partially affected the high PP through influencing river inputs to the northern SCS.

Keywords: Paleoceanography, Dinoflagellate cysts, the South China Sea, Holocene, Anthropogenic impacts

4.05 Calibration of dinoflagellate cysts as paleo-environmental indicators in the Kitimat Fjord system (northern BC, Canada): preliminary results

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Abstract: The Kitimat Fjord System (KFS, British Columbia's North coast, Canada) is a large, complex water body that connects the active port of Kitimat to the open Pacific Ocean. Anthropogenic pressures on this pristine coastal ecosystem include logging, tanker traffic (mostly exporting liquefied natural gas) and industrial activities in Kitimat (e.g., aluminum smelter) as well as the global trends toward a warmer, more acidic and less oxygenated ocean. This study is part of a project that aims at (1) establishing a robust baseline of natural environmental variability (Holocene to seasonal scales) in the KFS using sedimentary records, and (2) estimating the system's resilience to oil spills over the decades to come.

The modern oceanography of the KFS is now quite well understood, thanks to extensive surveys led by Fisheries and Oceans Canada (DFO); similarly, the post-glacial history of sediment accumulation and submarine landslides have been extensively studied under programs led by Natural Resources Canada (NRCan). Building on these efforts, we now study dinoflagellate cysts as paleo-indicators in the KFS, in order to inform on past environmental change and provide context on natural variability and recent human impacts in the system. Dinoflagellate cysts are sensitive and reliable indicators of conditions that prevailed at the time of their deposition. They have successfully been used for paleoreconstructions from sedimentary records worldwide, including in the NE Pacific where cyst concentrations can be particularly high, and assemblages quite diverse. This presentation will focus on preliminary results from the calibration of dinoflagellate cysts as proxies in the KFS. The calibration is based on (1) the spatial distribution of dinoflagellate cysts in surface sediments (n = 37 samples) in relation to "modern" environmental variables, and (2) seasonal variations in dinoflagellate cyst production from a 3-year long sediment trap time series. In turn, our findings will guide interpretations of dinoflagellate cyst assemblages from three box cores covering the last ~ 120 years of sedimentation, and two piston cores spanning the Holocene.

Keywords: Paleoceanography, surface sediment, sediment trap, estuaries, human impacts.

Project: This study is part of project MOSS (Marine Oil Spill Studies), funded by the Environmental Science Program of the Geological Survey of Canada (NRCan). Sampling was conducted by DFO with funding from the Government of Canada's World Class Prevention, Preparedness and Response for Oil Spills from Ships Initiative.

4.06 Late Holocene changes in oceanography and regional export primary production in northwestern Baffin Bay

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Abstract: The 21 century warming has rapidly changed the state of the Arctic cryosphere with increased freshwater export to lower latitudes. Northern Baffin Bay is a major gateway for this freshwater export via Nares Strait and the Canadian Arctic Archipelago (Moore et al., 2021) and is host to the North Water (NOW) polynya, an area of open water in a region characterized by high sea-ice. These open water conditions allow for an earlier and longer phytoplankton bloom that helps sustain a diverse food web (Heide-Jørgensen et al., 2013) which is important for the surrounding communities in Greenland and Nunavut (Egeesias et al., 2017). The formation and maintenance of the NOW relies on three factors. The first is ice bridges that form north of the polynya in Nares Strait that prevents sea ice from flowing into the NOW region (Moore et al., 2021). The second is strong northerly winds that push newly formed sea ice towards southern Baffin Bay. Finally, due in part to the strong northerly winds, the upwelling of the relatively warm West Greenland Current in the eastern portion of the polynya helps inhibit sea ice formation (Rysgaard et al., 2020). In recent decades, the formation of ice bridges in Nares Strait has become more variable, sometimes failing to form completely (Moore & McNeil, 2018). In these cases, there is increased freshwater and ice flowing along the Canadian coast, into the northern Baffin Bay.

Our objective is to assess the impact of increased freshwater input to northwestern Baffin Bay on the NOW's export primary production and water column conditions. A multi-proxy (geochemical and microfossil) approach is applied on two sediment cores collected onboard the CCGS Amundsen in the summer of 2019. Here we will present preliminary results from the analysis of organic-walled dinoflagellate cysts (dinocysts) on one of the sediment cores (AMD19-2.7BC) as well as the age model from ²¹⁰Pb analyses. Based on cluster analysis, the core can be divided into 4 dinocyst assemblage zones. The first, from 1660-1720 AD is largely dominated by heterotrophic cold-water taxa (*Islandinium minutum*, *Islandinium? cezare*, and *Echinidinium karaense*; Head et al., 2001) with only low contribution of autotrophic taxa, suggesting reduced formation of the polynya. From 1720- 1960 AD the transition to a more open and productive polynya is evidenced by an increase in the abundance of autotrophic taxa (*Operculodinium centrocarpum* and the cyst of *Polarella glacialis*) and total dinocyst fluxes. From 1960- 2012 AD, maximum values of the autotrophic to heterotrophic (A/H) ratio, and total dinocyst fluxes, suggest a long open water season associated with peak export primary production. From 2012-2019 AD, our record is marked by a decline in total dinocyst fluxes and A/H values, which we associate with increased freshwater and ice input into the NOW potentially due to the aforementioned Nares Strait ice bridge failures. These preliminary results indicate that current climate warming and associated freshwater input into the northwestern Baffin Bay do have a considerable influence on dinoflagellate assemblage composition and production.

Keywords: northern Baffin Bay, paleoceanography, climate change, primary production, dinoflagellate cysts, polynya

Project: ArcticNet Arctic Seafloor Mapping Data Processing and Dissemination

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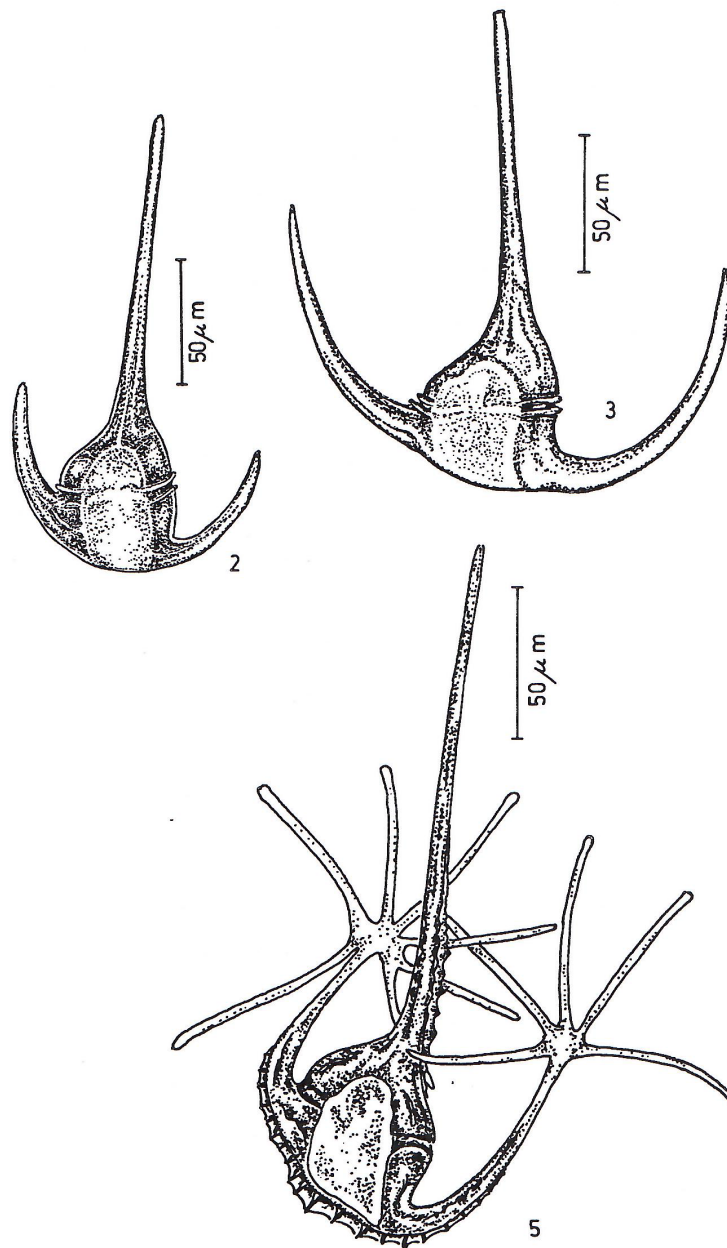
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SESSION 5
BIODIVERSITY
and
SYSTEMATICS

5.01 Planktonic athecate dinoflagellates from western coasts of Baja California, Mexico, with a redescription of *Gymnodinium hamulus*

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Abstract: Dinoflagellates may dominate the protozoan community in the planktonic realm, considering environmental sequences. They play an important role in the Carbon transfer and in the regeneration of nutrients in the microbial plankton. Many photosynthetic dinoflagellates practice mixotrophy: they are able to exploit dissolved organic Carbon and heterotrophic bacteria or photosynthetic cyanobacteria, or other phytoplankton elements. Athecate (or naked) dinoflagellates, devoid of rigid thecae or plates, have been little studied in Mexican seas. During an oceanographic cruise along western coasts of Baja California and Baja California Sur, by September-October, 2020, water samples (500 mL) were collected at depths of the chlorophyll *a* maxima, and then fixed with Lugol's solution. Light (LM) and Scanning Electron Microscopy (SEM) were used for species identification, whereas cell countings were made with the inverted microscope method. Four stations have been analyzed and 35 species of athecate dinoflagellates have been identified, including photosynthetic and truly heterotrophic forms, from which some remarkable, frequent, and representative species were: *Amphidinium acutissimum*, *A. sphenoides*, *Gyrodinium fusiforme*, *Kapelodinium vestifici*, *Protodinium simplex*, *Torquentidium helix*, *Warnowia* sp. The potentially harmful species *Karenia selliformis* was also recognized. Most of the species found belong to the nanoplankton fraction and have been rarely reported. The species *Gymnodinium hamulus*, a very tiny form, described more than 100 years ago but recorded only once again, was studied with certain detail and is here redescribed. Although their densities are relatively low, their ecological relevance in the plankton, fulfilling various functions as primary producers and consumers.

Keywords: Dinoflagellates, Diversity, Heterotrophic forms, Mexican Pacific, Nanoplankton, Phytoplankton.

5.02 International Phytoplankton Intercomparison (IPI) exercises in the abundance and composition of marine microalgae: a blueprint for biological capacity building in community analysis

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Abstract: The International Phytoplankton Intercomparison (IPI) programme is a Proficiency testing scheme in marine microplankton identification and quantification. This scheme was developed in direct response to the requirements of the Oslo-Paris Commission (OSPAR) to establish a European infrastructure for biological effects quality assurance and quality controls (QA/QC) so that laboratories can attain defined quality standards and contributes to national and international marine monitoring programmes of harmful algae. The purpose of this exercise is to evaluate and compare the performance of testing laboratories and to monitor the laboratories ongoing competency and performance over time.

This programme was initiated in 1998 under the BEQUALM (Biological Effects Quality Assurance in Monitoring Programmes) umbrella, funded by the European Union through the Standards, Measurements and Testing programme of the European Commission and changed its name to the IPI programme in 2015. This programme has been running continuously since 2004 and it is recognised internationally. From 2021 to 2025, the IPI programme is hosted by the Canary Islands HAB Observatory (OCHABS) in Las Palmas, Gran Canaria, Spain with the continued collaboration of the IOC of UNESCO Science and Communication Centre on Harmful Algae, the Ocean Teacher Global Academy (OTGA) and in association with NMBAQC in the UK.

5.03 Species of the planktonic dinoflagellate genus *Alexandrium* (Dinophyta) from the tropical and subtropical Mexican Pacific Ocean

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Abstract: All species of the dinoflagellate genus *Alexandrium* Halim (Dinophyta) are marine and truly planktonic forms, many species are widely distributed, and some are recognized to form “red tides” and Harmful Algal Blooms (HAB) and to produce saxitoxins causing Paralytic Shellfish Poisoning (PSP) in humans. The purpose of this work was to recognize the species of *Alexandrium* from coasts of the tropical and subtropical Mexican Pacific Ocean. Based on samples obtained following several methods and from various localities of the tropical and subtropical Mexican Pacific, from coasts of Baja California Sur (Gulf of California) to Chiapas (Gulf of Tehuantepec), we have found and identified nine species (morphospecies) of *Alexandrium*: *Alexandrium affine*, *A. concavum*, *A. globosum*, *A. margalefii*, *A. minutum*, *A. monilatum*, *A. pseudogonyaulax*, *A. tamarense* and *A. tamiyavanichii*. These species are described and illustrated by light microscopy, after thecal dissociation, and epifluorescence microscopy, using calcofluor. Additional observations of certain species were made by scanning electron microscopy. Molecular phylogenies (using the D1/D2 LSU rDNA regions) including 7 *Alexandrium* strains belonging to 3 species, isolated from the same study area, are also provided. The species *Alexandrium affine*, *A. minutum*, *A. monilatum*, *A. pseudogonyaulax*, *A. tamarense* and *A. tamiyavanichii* have been flagged as potentially toxic. This is the first record of *Alexandrium globosum*, and the first morphological documentation of *A. pseudogonyaulax* from the study area (Mexican Pacific Ocean). Currently, there are 16 species of *Alexandrium* listed for the Mexican Pacific, but we believe that this number will increase when more studies dedicated to the genus can be undertaken, including cultures and use of molecular tools.

Keywords: Dinoflagellates, Mexican Pacific, Morphology, Phytoplankton, Red tides, Taxonomy.

5.04 Temporal changes of dinoflagellate communities in inner Frobisher Bay, Nunavut, Canada

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Abstract: The Arctic Ocean is experiencing radical modifications due to rapid climate change. The ongoing sea ice decline, and the increase of water temperature and freshwater runoff will affect the current distribution and dynamics of phytoplankton communities and may enhance probability and frequency of harmful algal blooms (HABs). In addition, the increase of shipping activities may increase potential introductions of non-indigenous species (NIS), including dinoflagellates. Some dinoflagellates produce toxins that can be transferred through food chain and can be lethal to marine fauna and humans. In the Canadian Arctic, human populations are highly dependent on marine resources for their livelihoods and HABs could impact their health through consumption of infested animals. The lack of information about native species composition in the Canadian Arctic may act as a barrier to NIS and HABs detection. In this context, the main objective of this study is to survey the diversity and distribution of dinoflagellate communities over three years of sampling (2015, 2019 and 2020) in inner Frobisher Bay near Iqaluit (Nunavut), which is one of the busiest ports in the Canadian Arctic. Dinoflagellate samples were collected using a 20 µm plankton net and identified using an inverted microscope. Preliminary results showed the presence of 44 dinoflagellate taxa during August 2015, 2019, and 2020, among which 32 taxa newly observed in Frobisher Bay and 6 potentially toxin-producing taxa: *Alexandrium cf. catenella*, *Dinophysis acuminata*, *Dinophysis norvegica*, *Gonyaulax spinifera*, *Phalacroma rotundatum* and *Protoceratium reticulatum*. Dinoflagellate communities differed between years. Days between sea ice melt and sampling, surface and bottom water temperature, bottom salinity, stratification index, bottom depth and surface mixed layer depth explained 49% of the communities' variability. A comparison between sampling years showed that richness was 5 times greater in 2019 than 2015, and 7.2 times greater in 2020 than in 2015. In fact, early sea ice melt in August 2019 and 2020 induced water column stratification, providing favorable conditions for dinoflagellate growth. Early sampling relative to the sea ice melt in 2015 occurred during a diatom bloom, and *Protooperidinium bipes* was the most dominant dinoflagellate taxa (85%). In fact, this species is the most successful and abundant due to its swimming speed and growth rate. Our results indicate that this region is a suitable ecosystem for potentially toxin-producing taxa. Temporal variability in dinoflagellate communities was observed between years and was strongly linked to the timing of the sea ice melt. Overall, this study helps building a database to detect future changes in harmful algae, HABs and NIS in Frobisher Bay. Future sampling in summer 2022 will improve our knowledge on dinoflagellate communities and may reveal additional toxin producers and patterns of their annual variation.

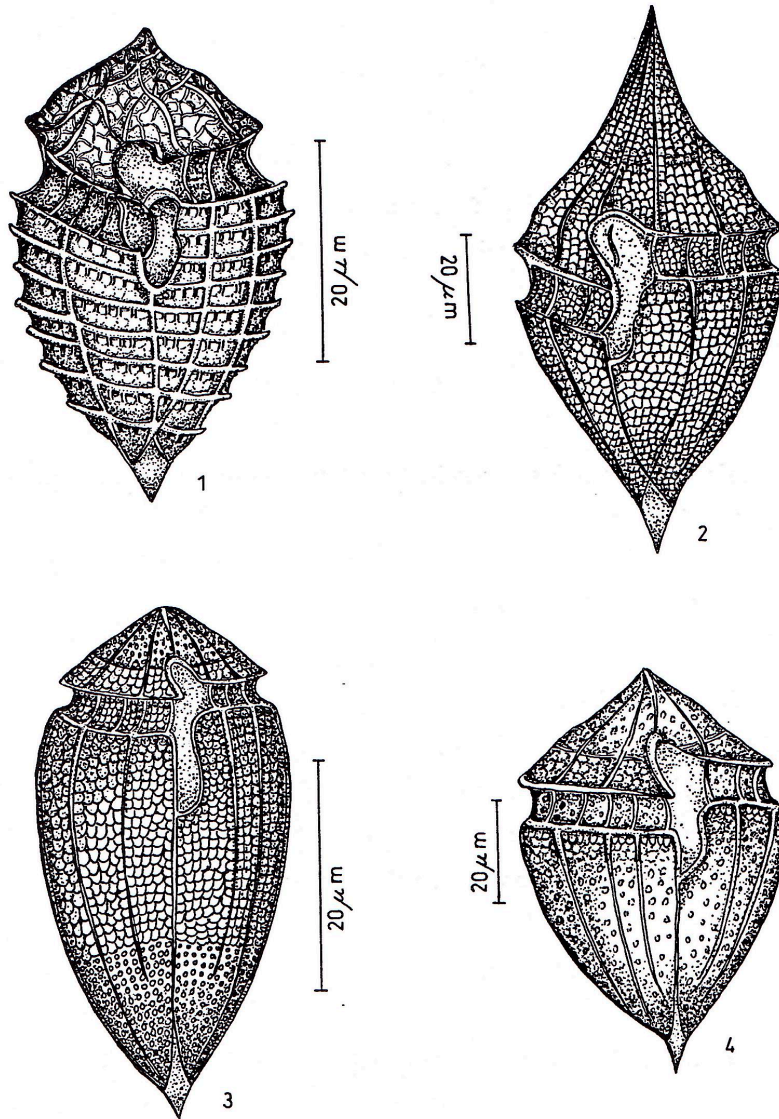
Keywords: Dinoflagellates, Canadian Arctic, Frobisher Bay, Non-indigenous species, Harmful algae, indigenous species

Acknowledgments: This work was supported by Fisheries and Oceans Canada (DFO) (Ocean and Freshwater Science Contribution Program (OFSCP)) and Natural Sciences and Engineering Research Council of Canada (NSERC) (Gosselin and Rochon Discovery Grant). This is a contribution to the research programs of Québec-Océan (Theme 2.4: Knowing marine species and their population and community dynamics) and Institut des sciences de la mer de Rimouski (ISMER). Special thanks to people who helped with fieldwork preparation and data/sample management.

Project: Diversity of pelagic primary producers in coastal habitats and the potential for harmful blooms in Eastern Canadian Arctic, with a focus near Iqaluit, Nunavut

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SESSION 6
DINOCYST SYSTEMATICS, MESOZOIC
and
CENOZOIC DYNOCYST

6.01 Dual nomenclature in organic-walled dinoflagellate cysts: concepts, methods and applications

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Abstract: Dual nomenclature in dinoflagellates allows a fossil-defined (usually cyst) species to bear a name other than that of its equivalent non-fossil species as established for example by incubation experiments. Two names can then apply to the same cyst morphotype, reflecting the separate concepts and criteria used for fossil and non-fossil taxa. The current nomenclatural code sanctions both dual and unified approaches to nomenclature for dinoflagellates, permitting choice with respect to the method used. Fossil-species are normally and logically assigned to fossil-genera and non-fossil species to non-fossil genera, a practice that facilitates dual nomenclature. Inconsistencies and ambiguities arise when binomials combine fossil- with non-fossil names, or non-fossil cyst-based names are used to typify a genus. Examples of this hybrid nomenclature include:

- 1) A non-fossil cyst-typified species assigned to a fossil-genus (e.g., *Nematosphaeropsis labyrinthus* (Ostenfeld, 1903) Reid, 1974),
- 2) A non-fossil cyst-typified species defining a non-fossil genus that includes fossil-species (e.g., the cyst-defined genus *Echinidinium* Zonneveld, 1997 ex Head et al., 2001, and its type species *E. granulatum* Zonneveld, 1997 ex Head et al., 2001),
- 3) A fossil-genus emended to incorporate non-fossil species (e.g., the fossil-genus *Lingulodinium* Wall, 1967, emend. Dodge, 1989),
- 4) A fossil-species assigned or transferred to a non-fossil genus (e.g., *Peridinium ponticum* Wall and Dale in Wall et al. 1973),
- 5) A non-fossil theca-defined species assigned to a fossil-defined genus (e.g., "*Stelladinium stellatum*" (Wall in Wall and Dale, 1968) Reid, 1977).

The consequences of these hybrid nomenclatural constructions are examined, and solutions are offered.

Keywords: dual nomenclature, taxonomy, fossil, non-fossil

Acknowledgments: MJH acknowledges support from a Natural Sciences and Engineering Research Council of Canada Discovery Grant.

6.02 *Pentaplacodinium lapazense* sp. nov. from southwestern Gulf of California, a new non-toxic gonyaulacalean resembling *Protoceratium reticulatum*

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Abstract: A new *Pentaplacodinium* species with six precingular plates is described from Bahía Concepción and Bahía de la Paz, Gulf of California. The non-fossil motile stage is described as *Pentaplacodinium lapazense*, whilst the fossil stage is described as *Operculodinium lapazense*. The cyst morphology is compared to topotype material of *Operculodinium israelianum*, which is larger, has longer processes and has a difference wall structure. The motile cells display a plate formula of Po, Pt, X, 2'+*2', 6'', 6c, 6s, 5''', 2p, 1'''. A typical gonyaulacalean fission line and plate overlap are observed. SSU-ITS- LSU ribosomal DNA sequences demonstrate that *P. saltonense* is its closest relative. The species is homothallic. This species occurred in shallow and restricted coastal areas, and has a preference for higher temperatures and salinities. MicroFTIR spectra of the cysts are compared to spectra of cysts of other gonyaulacaleans. No yessotoxins were detected in all analyzed strains, and this species can thus not be responsible for elevated yessotoxin concentration in shellfish on the southwestern coastal region of the Gulf of California.

Keywords: *Operculodinium*, Yessotoxins, Bahía Concepción, Bahía de La Paz, microFTIR spectroscopy

6.03 The dinoflagellate cyst correlation across the Campanian-Maastrichtian boundary between the Tethyan and boreal Europe

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Abstract: The Global Boundary Stratotype Section and Point (GSSP) for the base of the Maastrichtian Stage was formally defined in Tercis les Bains (a Tethyan section located in SW France) as an arithmetic mean of the twelve stratigraphic levels at which selected macro- (ammonites, inoceramid bivalves) and microfossil key taxa (benthic and planktonic foraminifera, dinoflagellate cysts, calcareous nannofossils) have their first (FOs) or last (LOs) occurrences (Odin and Lamaurelle, 2001). Such definition causes serious problems when trying to locate the boundary outside the GSSP, since some of the biostratigraphic boundary markers may be missing and some others (FOs of *Pachydiscus neubergicus* and *Diplomoceras cylindraceum*) were proved to be diachronous (e.g. Christensen et al., 2000; Remin et al., 2015). This claim is further supported by a high-resolution organic-walled dinoflagellate cyst (dinocyst) biostratigraphy, recently developed for the upper Campanian–lowermost Maastrichtian of the Middle Vistula River composite section, Poland (Niechwedowicz and Walaszczyk, 2022). When compared with coeval dinocyst assemblages documented from other regions, the data from the Vistula section revealed that of the dinocyst boundary markers, used in formal definition of the base of the Maastrichtian, only the LOs of *Raetiaedinium evittigratia* and *R. truncigerum* are chronostratigraphically reliable proxies; the LOs of *Samlandia carnarvonensis* and *S. mayi* are distinctly younger and the stratigraphic utility of *Corradinisphaeridium horridum* is mostly limited to southern Europe. Instead, it was found that the LOs of *Apteodinium deflandrei*, *Areoligera microreticulata*, *Callaiosphaeridium asymmetricum*, *C. bicornatum* (LO of the genus), *Coronifera oceanica*, *Heterosphaeridium bellii*, *Odontochitina costata*, *O. dilatata*, *O. streelii*, *Xenascus ceratioides*, *X. gochtii*, *X. sarjeantii* (LO of the genus), and the FOs of *Cladopyxidium saeptum*, *C. velatum*, *C. verrucosum*, *Florentinia mayi*, *Glaphyrocysta expansa*, *G. pala*, and *Pervosphaeridium tubuloaculeatum* are stratigraphically significant dinocyst events for approximating the position of the base of the Maastrichtian. Some of these bioevents enabled correlation of the Polish section with Tercis (supporting the results based on inoceramid bivalves and ammonites), and with other selected Tethyan (southern Germany, northern Apennines) and Boreal successions (Belgium and the Netherlands).

Keywords: dinoflagellate cysts, biostratigraphy, correlation, Campanian–Maastrichtian boundary, Middle Vistula River section, Europe.

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6.04 *Cannosphaeropsis franciscana* morphological variance across the K/Pg boundary; the case for vesiculation as an ecophenotypic character within spiniferate gonyaulacaceans

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Abstract: The discovery of an Upper Cretaceous–lower Paleogene (upper Maastrichtian–lower Selandian) succession within the Oyster Bay Formation on eastern Vancouver Island, British Columbia, Canada, has been made based on dinoflagellate cyst biostratigraphic controls (McLachlan and Pospelova 2021). This recent work has presented the opportunity to analyse an unprecedented range of variation within an enigmatic spiniferate gonyaulacacean genus and species, *Cannosphaeropsis franciscana* (McLachlan et al. 2021). Using both transmitted light and scanning electron microscopy, we observe forms that range across a broad morphological plexus which encompasses differences in process type, autophragm geometry and most significantly, vesiculation. The presence and extent of cyst vesiculation is established as a key diagnostic character resulting in the erection of three subspecies: *Cannosphaeropsis franciscana franciscana* (autonym), *Cannosphaeropsis franciscana vacuoseptata* subsp. nov., and *Cannosphaeropsis franciscana vesiculata* subsp. nov. The onset and decline of vesiculate forms within the lowest Danian interval forms the basis for the interpretation that these subspecies reflect an ecophenotypic response to changing paleoenvironmental factors above the K/Pg boundary, specifically those of unstable, stratified marine conditions associated with the post-Cretaceous transgressive phase. Assemblage data throughout the formation also reveal members of the vesiculate genus *Hafniasphaera* increase their abundances in nutrient-rich and likely stratified coastal waters when *Spiniferites* species are also abundant. *Hafniasphaera* species saw their greatest abundance during Late Cretaceous–early Paleogene time (e.g. Hansen 1977; Fensome et al. 2009) and similar vesiculate morphologies persist among extant spiniferate assemblages (e.g. Price and Pospelova 2014). The development of vesicles is herein suggested as an adaptation to prolong cyst buoyancy and slow descent in the water column. An extensive literature review of over 240 published records considering all illustrated reports of formally recognized species belonging to the genera *Cannosphaeropsis* and *Hafniasphaera* is also presented alongside taxa chronostratigraphic ranges.

Keywords: Cretaceous, Paleogene, North Pacific, Nanaimo Group, Dinocysts, Paleoecology

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6.05 Macromolecules analyses of simple-shape cysts using ATR-FTIR and Raman spectroscopy

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Abstract: The formation of dinocysts and their decomposition/preservation processes in the sediments are essential for understanding the bloom-forming mechanisms of dinoflagellates. *Alexandrium catenella/pacificum* is one of the toxic species (paralytic shellfish poisoning) that produce elliptical transparent cysts. These simple-shaped (spherical, ellipsoidal and ovoidal) cysts are deposited in the sediments. Light microscopic examination of the sediments is a common method to determine if they are infested by this toxic species. Unfortunately, several non-toxic dinoflagellate cysts and other organisms produce very similar resting stages. Although the cysts may be morphologically indistinguishable, their walls may be chemically different. Fourier Transform Infra-Red (micro-FTIR) spectroscopy can detect these differences and, therefore, has been used to separate cyst species on the basis of cyst wall chemistry. (e.g., Bogus et al., 2014; Gurdebeke et al., 2020; Meyvisch et al., 2022).

The simple-shaped cysts of *A. catenella/pacificum* are mostly rather labile and after hatching, generally do not preserve in the sediments. This contrasts with many other dinoflagellate cysts. These differences in preservation potential may also result from macromolecularly different cyst walls (see also Versteegh et al., 2022).

In order to see if we can reliably separate *A. catenella/pacificum* from similar morphotypes chemically, and to shed more light on the dynamics of cyst wall preservation, we investigated cyst wall using both micro-FTIR and micro-Raman microscopy. The latter enables a much higher (micron- or submicron) resolution. Although Zimmermann et al. (2015) succeeded to analyze macromolecules of pollen using micro-Raman spectroscopy, it is difficult to get good spectra from dinocysts due to the higher emission of autofluorescence using light with short wavelengths (e.g., 532 nm laser).

We present the results of both micro-FTIR and micro-Raman analysis of dinocysts, especially the cysts of *A. catenella/pacificum*.

Individual dinocysts were isolated from the sediment samples using a micromanipulator with a micropipette. For micro-FTIR analysis using attenuated total reflection (ATR), individual cysts were washed with distilled water, placed on a gold-mirror and dried. For Raman analysis, individual cysts were placed on a quartz-glass bottom dish using distilled water.

Micro-FTIR-ATR spectra of *A. catenella/pacificum* cysts are characterized by a dominant absorption band between 1200-1000 cm^{-1} and weak bands between 1450-1200 cm^{-1} and 1800-1600 cm^{-1} . Cluster analysis of the IR spectra for wavelengths between 800- 1800 cm^{-1} suggests that the macromolecular structure of *A. catenella/pacificum* cysts is very similar to cellulose. However, the highest peak in the spectra of *A. catenella/pacificum* cysts in the 1200-1000 cm^{-1} window is at $\sim 1033 \text{cm}^{-1}$ which is lower than for cellulose ($\sim 1059 \text{cm}^{-1}$). The Raman spectra carried out with a 785 nm laser, revealed main peaks around 1050 - 1170 cm^{-1} which overlap with those of cellulose. The Raman spectra of *A. catenella/pacificum* cyst also displayed higher peaks around the 1500-1200 cm^{-1} region and show a moderate peak at 898 cm^{-1} . This spectral pattern is characteristic for polysaccharides with 1,6- and 1,4- β -glucoside bonds, respectively. Therefore, we suggest that the macromolecules in the cyst of *A. catenella/pacificum* are composed of polysaccharides with β -1,4-glucoside bonds including smaller numbers of β -1,6-glucoside bonds. These data suggest the cyst of *A. catenella/pacificum* is susceptible to decomposition by cellulase. This may explain the difference in preservation potential compared to cysts of other gonyaulacoid species.

Keywords: macromolecule, ATR-FTIR, micro-Raman, polysaccharide

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6.06 Calcareous dinoflagellate cyst variance across the K/Pg boundary at DSDP site 577, Shatsky Rise, Western North Pacific

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Abstract: An analysis of calcareous dinoflagellate cysts was carried out on sixteen samples from across the K/Pg boundary interval from Deep Sea Drilling Project Site 577 situated on Shatsky Rise in the western North Pacific. This is the first time that a detailed study of this microfossil group has been undertaken from deep sea sediments of Late Cretaceous–early Paleocene age in the North Pacific realm. Prior to this investigation (McLachlan and Pospelova 2021a), deep time work on dinoflagellate cysts has been limited to studies of organic-walled taxa in the North Pacific region, all derived from coastal to neritic settings in California (e.g. Drugg 1967), Japan (e.g. Kurita 2004), and British Columbia (e.g. McLachlan et al. 2018, 2019, McLachlan and Pospelova 2021b). From within the Shatsky Rise core sediments, eight species of calcareous dinoflagellate cysts were found to comprise low diversity assemblages of which half are representative of extant taxa. The most pronounced bioevent is that of *Cervisiella operculata* reaching its highest proportions at the K/Pg boundary transition underscoring the utility and cosmopolitan distribution of the species with its acme as a marker for the earliest Danian. We also identify an indirect sea surface paleotemperature signal as represented by variance in wall thickness in two distinct morphotypes of *Thoracosphaera heimii* (morphotypes A and B). In light of modern cyst culturing (Karwath et al. 2000) and field studies of this species (Zonneveld et al., 2005), the cyst concentrations and relative abundances of these *T. heimii* morphotypes are interpreted to reflect the late Maastrichtian warming event and subsequent late Maastrichtian cooling phase prior to the interval of gradual warming in the early Danian marked by the opportunistic capitalization of *C. operculata* during a time of ocean recovery following the terminal Cretaceous.

Keywords: Cretaceous, Paleogene, K/Pg boundary, Pacific Ocean, Shatsky Rise, DSDP

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6.07 Marine phytoplankton turnover and record of paleoenvironmental change across the K/Pg boundary in the Eastern North Pacific

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Abstract: Sedimentary rocks of the Oyster Bay Formation exposed along the eastern coastline of Vancouver Island have been the subject of paleontological research since the 1990s with a focus on resolving the age of the strata (e.g. Haggart et al. 1997; Cockburn and Haggart 2007; Haggart et al. 2018). These rocks are represented at three locations, among them the beds of the Appian Way locality, which are renowned for their exquisite preservation of an extensive paleobotanical (e.g. Mindell et al. 2006) and marine invertebrate (e.g. Schweitzer et al. 2009) fossil record. Prior to this investigation, deep time work in marine palynology of the North Pacific was largely limited to California (e.g. Drugg 1967), Japan (e.g. Kurita 2004), and only recently, British Columbia (McLachlan et al. 2018, 2019). The present study (McLachlan and Pospelova 2021) has established the Oyster Bay Formation as comprising a ~93.5 m thick succession spanning ~4.5 to 5 myr of geological time ranging from the late Maastrichtian to early Selandian across stratigraphic sections from each location using dinoflagellate cyst relative dating. Furthermore, approximate placement of the K/Pg boundary was made possible by a suite of temporally constrained dinoflagellate cyst K/Pg interval indicator taxa. This result places eastern Vancouver Island as the location of the first conformable K/Pg boundary section in North America west of the Rocky Mountains and in the eastern North Pacific realm based on biostratigraphic controls. In addition to having developed an age framework of the formation, the exceptionally well-preserved dinoflagellate cyst assemblages allow for reconstruction of the marine phytoplankton community in the lead up to the terminal Cretaceous event and its subsequent response in the early Paleocene in tandem with signals reflecting localized shifts in nutrient input. From a global standpoint, the palynological assemblages mirror environmental changes across the K/Pg boundary consistent with observations from numerous localities across both hemispheres, following a synthesis of over 650 species reports. These findings also carry broader morphological implications for dinoflagellate cyst taxonomy and ecophenotype utility in paleoenvironmental reconstructions (McLachlan 2021; McLachlan and Pospelova 2021; McLachlan et al. 2021).

Keywords: Cretaceous, Paleogene, K/Pg boundary, North Pacific, Dinocysts, Primary productivity

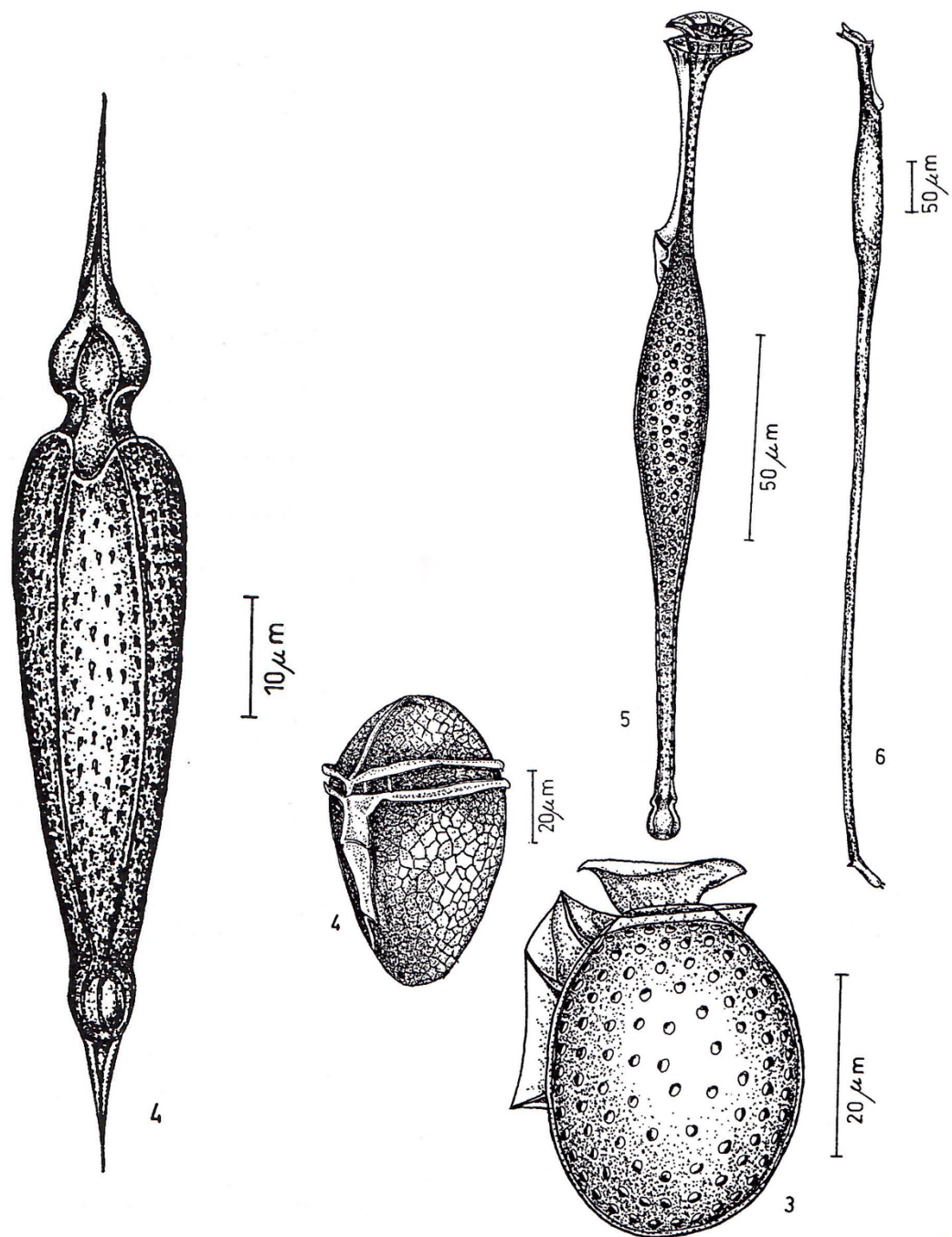
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SESSION 7
PALEOCOLOGY and
PALEOCEANOGRAPHY II, EVOLUTION, TAXONOMY
AND BIOSTRATIGRAPHY

7.01 Hydrographic development of the sea of Japan during the Pliocene-Pleistocene transition: the dinoflagellate cyst record at IODP site U1424

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Abstract: The Pliocene-Pleistocene transition marks a significant intensification of Northern Hemisphere glaciation (iNHG) and the beginning of high-amplitude glacial-interglacial cyclicity that characterises the Quaternary. While many mechanisms have been proposed for this change in climate state, including the closure of the Isthmus of Panama and the onset of ocean stratification in the Pacific at ~2.7 Ma, the role of the Pacific Ocean remains poorly understood.

The largely enclosed Sea of Japan has been a sensitive recorder of climate change since its formation, owing to its: shallow connections to the Pacific and adjacent basins which prevent bottom water exchange; reliance on the Tsushima Warm Current as its major source of nutrients, salt and heat; proximity to the Westerly Jet; and position relative to the East Asian Summer and Winter Monsoon systems. This makes it ideally suited for understanding the role and response of East Asia and the Pacific Ocean across this interval of crucial climatic change. Dinoflagellate cysts, acritarchs, and other palynomorphs are here used to elucidate hydrographic changes during the Pliocene–Pleistocene transition at Integrated Ocean Drilling Project (IODP) Site U1424, east-central Sea of Japan. The study covers 2.85 Ma to 2.41 Ma with an average sample spacing of ~4 kyr, allowing paleoenvironmental reconstructions at suborbital resolution. This is the first high-resolution study using dinoflagellate cysts for the Sea of Japan.

A total of 125 samples were analysed, and results reveal more than 60 marine palynomorph taxa and moderate to excellent preservation. Changes in assemblages correspond to shifts in marine isotope stages (MIS). In particular, strong responses coincide with glacial MIS 100 (2.52 Ma) and 102 (2.57 Ma), these being characterized by higher numbers of *Corrudinium harlandii*, *Habibacysta tectata*, *Spiniferites elongatus*, and cysts of *Protoceratium reticulatum*. Although initially rare, the first appearance of the warm water species *Tuberculodinium vancampoe* at ~2.814 Ma suggests the influence of warm-water inflow to the Sea of Japan, probably from the southern strait. Conversely, *Cymatiosphaera? invaginata* is abundant in some intervals, seemingly tied to specific glacial climate cycles. Although the paleoecology of this acritarch is not fully known, its prevalence elsewhere in the high- to mid-latitudes likely implies the flow of subarctic water masses into the Sea of Japan from its northern straits. The marine palynomorph assemblages reflect the fluctuating influence of these contrasting water masses during the iNHG. The shallow sills connecting the Sea of Japan to the North Pacific impose further controls on inflow during these times of strongly fluctuating global sea level.

Results from IODP Site U1424 will be compared with a dinoflagellate cyst analysis of Ocean Drilling Program Site 1148 in the northern South China Sea to obtain a more complete picture of paleoceanographic changes in the western Pacific across the Pliocene-Pleistocene transition.

Keywords: Pliocene, Pleistocene, Sea of Japan, Pacific, palynology.

Acknowledgments: Support from a Natural Sciences and Engineering Research Council of Canada Discovery Grant to MJH is acknowledged.

7.02 Dinoflagellate cysts in coastal surface sediments from NW Iberia related to environmental gradients: new supporting data for palaeoenvironmental studies

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Abstract: Studies on the relationships between dinoflagellate cyst assemblages in surface sediments and present-day environmental gradients may provide supporting evidence for the interpretation of environmental signals from fossil cyst records and may help to obtain more accurate palaeoecological reconstructions (Ellegaard et al., 2017). In this work, we analysed spatial distributions of organic-walled dinoflagellate cysts in surface sediments from two coastal sites in the Atlantic Iberian margin. Cyst composition and abundances (percentages and concentrations) were analysed in a total of 83 sediment samples: 31 collected in the Riá de Vigo (NW Spain) and 51 off Figueira da Foz (NW Portugal) (Fig. 1AB). At both study sites, cyst assemblages were compared to major environmental gradients by multivariate statistics (clustering and RDA), namely: water depth (WD), sea-surface temperature (SST), sea-surface salinity (SSS) and chlorophyll-a concentration (CHL). Additionally, in the Riá de Vigo (RdV), influences of sea-surface nitrate concentration (SSN), bottom oxygen saturation percentage (OSP), and distance to the main river (DTM) on cyst distributions were also investigated. Off Figueira da Foz (FF), dinoflagellate cyst distributions in surface sediments were also compared to bottom temperature (BTT) and grain-size (GRAN) distributions.

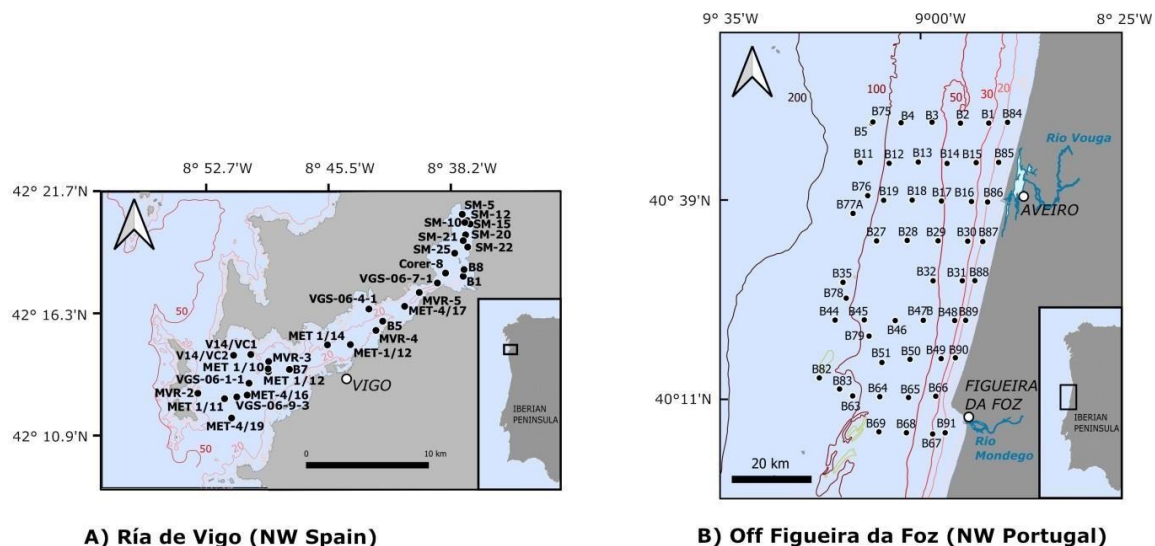
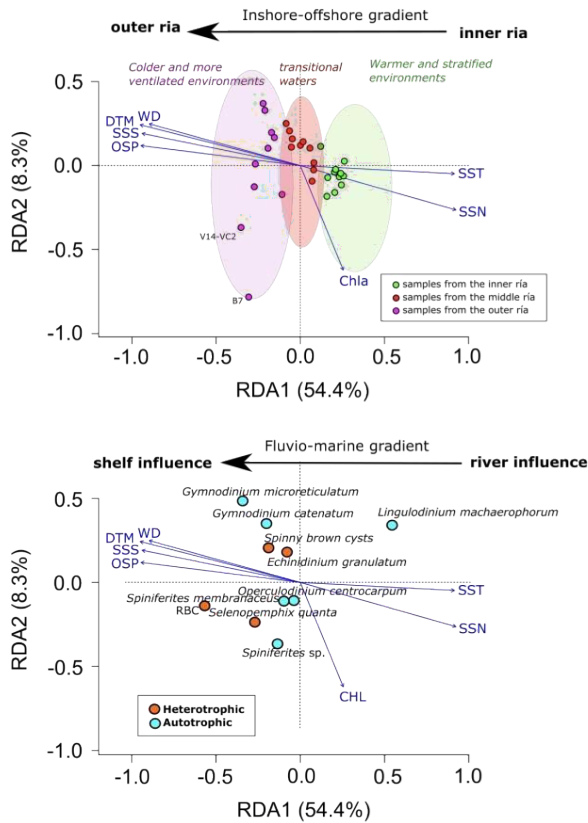


Fig. 1 Location of the study sites and sampling stations of surface sediments in the RdV (A) and off FF (B) on the Atlantic Iberian margin. Bathymetric contour is in meters. Cartographic data are from Xunta (<http://mapas.xunta.gal/productos-cartograficos/capassix/hidrografia>) and the General Bathymetric Chart of the Oceans (<https://www.gebco.net/>).

RDA revealed a marked inshore-offshore gradient in the distribution of dinoflagellate cysts at both sites (Fig. 2AB). Cyst distributions in the RdV were very much related to the fluvio-marine environmental gradient (Fig. 2A). Here, three distinct environmental regimes were recognised (Fig. 2A): 1) the inner ria, including warmer and more stratified environments with stronger river influences; 2) the middle ria, with transitional environments between the warmer and shallower waters from the inner ria to the colder and deeper environments from the outer ria; and 3) the outer ria with more ventilated and colder waters. The autotrophic species *Lingulodinium machaerophorum* dominated the inner and middle sections of the ria. Towards the ria's mouth, with increasing influence of shelf waters, *L. machaerophorum* abundances significantly decreased while cyst diversity, heterotrophic taxa and *Gymnodinium* spp. (including *G. microreticulatum*, *G. catenatum* and unknown *Gymnodinium* sp.) gradually increased.

Off FF, the environmental gradient that seemed to most influence the cyst distribution patterns was summer upwelling (reflected by SST variability) (Fig. 2B). Cyst distributions were also much related to grain size, with highest values (>500 cyst/ml) generally found in deeper stations with finer sediments (García -Moreiras et al., 2021). Furthermore, in addition to an inshore-offshore gradient, a latitudinal gradient was also detected (Fig. 2B). Here, two main environmental regimes could be identified: 1) the southern and offshore sites characterized by warmer and stratified environments (higher SST, lower BTT and lower CHL), and 2) the northern and shallower sites characterized by colder and more productive waters, with stronger influence of upwelling (lower SST and higher BTT and CHL). These two main environmental regimes were reflected on the cyst assemblages by a clear trophic segregation. In the first case, autotrophic taxa, such as *P. reticulatum*, *L. polyedra*, and *G. catenatum* dominated the assemblage, while in the second case heterotrophs were the dominant group (Fig. 2B, bottom).

A) Ría de Vigo



B) Figueira da Foz

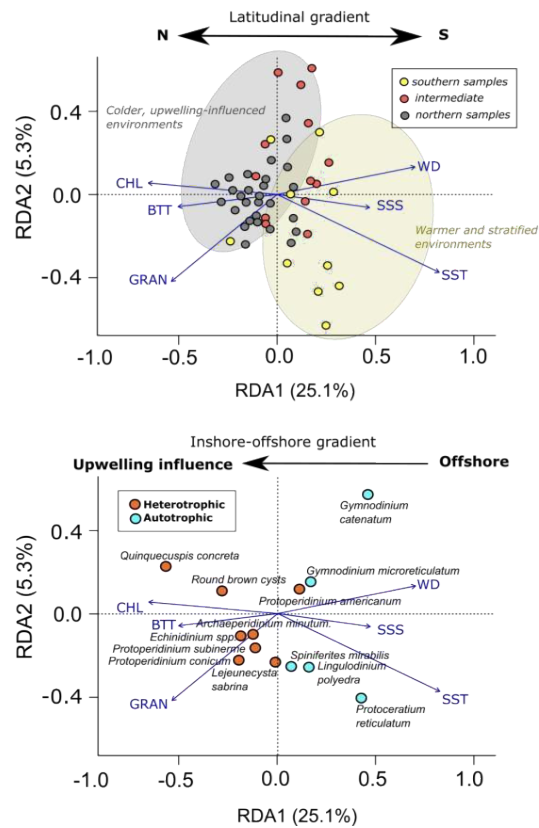


Fig. 2 RDA of samples and environmental parameters (top), and dinoflagellate cyst taxa (only the most significant) and environmental parameters (bottom) in the Ría de Vigo (A), and off Figueira da Foz (B) (García -Moreiras et al., 2021).

Dinoflagellate cyst distributions between the two studied sites showed notable differences, which would reflect the different environmental and deposition conditions. First, surface samples in the RdV showed very high total cyst concentrations, with values usually between $5 \cdot 10^3$ and $133 \cdot 10^3$ cysts·ml⁻¹. In contrast, maximum cyst concentrations in surface sediments off FF were lower ($\sim 1.5 \cdot 10^3$ cysts·ml⁻¹). Cysts of *Lingulodinium machaerophorum* dominated the assemblages from the RdV (12-99%), whereas off FF, *Gymnodinium catenatum* (0-51%) and *Operculodinium centrocarpum* (0-36%) were the most abundant single species. Our results suggested that *G. catenatum* species may reflect a different ecological niche from other autotrophs (Figs. 2AB). Heterotrophic cysts were also an important component of cyst assemblages in the samples from off FF (11-76%), among which Peridinoid brown cysts (*Quinquecupis concreta*, *Lejeunecysta sabrina* and *Selenopemphix nephroides*) and Round brown cysts (*Brigantedinium* spp. and *Dubridinium* sp.) were the most prominent groups (García -Moreiras et al., 2021). In contrast, in the RdV autotrophic taxa are generally dominant and heterotrophic taxa only reached notable abundances (18-58%) in the deepest samples from the outer ria. Moreover, some species occurring in the RdV were not observed in FF, such as *Bitectatodinium tepikiense* and *Peridinium ponticum*. Finally, dinoflagellate cyst morphotype richness was generally lower in RdV, but it gradually increased towards the outer area of the ria, with values (10-22 types) getting closer to those obtained in the samples from the shelf, off FF (10-33 types).

Cysts of heterotrophic taxa, particularly *Brigantedinium* spp, are very sensitive to degradation under oxygenated conditions (Zonneveld et al., 2008). This could affect cyst distribution patterns and bias the interpretation of the environmental signals. The fact that abundances of heterotrophic cysts increased in the outer ria with increasing OSP values (Fig. 2A, bottom), suggests that post-depositional degradation is probably not an issue here.

Overall, three main ecological signals were identified in the modern dinoflagellate cyst assemblages of the RdV and FF: (1) the heterotroph signal as the main upwelling signal; (2) the *Lingulodinium machaerophorum* signal, indicative of warm and stratified conditions; and (3) the *Gymnodinium catenatum* signal for the presence of mid-shelf upwelling fronts off FF. In coastal embayments like rias, increasing abundances of *Gymnodinium* spp. could indicate the entrance of shelf-waters during upwelling events.

In this study we contribute with new data on modern dinoflagellate cyst assemblages in relation to modern environments that may be useful to interpret historical records and reconstruct past marine ecosystems from the Atlantic Iberian Margin. Despite the uncertainties related to sediment and cyst transport and the possible disparity between the time scale of the cyst record and that of the environmental data used, a good correspondence between dinoflagellate cyst assemblages and the main environmental gradients in both studied sites supports that dinoflagellate cyst assemblages well represent the water-column environmental features and therefore could be used as supporting evidence for the interpretation of the fossil cyst records.

Keywords: Dinoflagellate cysts, Surface sediments, Environmental gradients, Palaeocology, Atlantic Iberian margin

Acknowledgments: This work was developed during the postdoctoral project of Iria García - Moreiras funded by Xunta de Galicia (Fellowship: ED481B-2019-074, 2019). The study in the Ria de Vigo was supported by the Spanish Ministry of Education and Science CGL2012-33584 (co-financed with ERDF funds) and the Xunta de Galicia GRC 2015/020 projects. The research off Figueira da Foz was supported by EU ERDF funds, within the PT2020 Partnership Agreement and Compete 2020, and national funds through Fundação para a Ciência e Tecnologia, I.P. (FCT, I.P.) and FCT, I.P. under the projects HABWAVE (LISBOA- 01-0145-FEDER-031265 UIDB/04292/2020) and AQUIMAR (MAR2020 No. MAR-02.01.01- FEAMP-017).

This study has been conducted using E.U. Copernicus Marine Service Information, and environmental data obtained from the Physical Oceanography Distributed Active Archive Centre (PO.DAAC) at JPL (Jet Propulsion Laboratory), and Instituto Tecnológico para o Control do Meio Mariño de Galicia (INTECMAR).

Project: This work was a contribution to the CGL2012-33584 (*Evaluación y contextualización de reservorios de metano de las rias gallegas con relación a las variaciones del nivel del mar y los cambios ambientales durante el postglacial*) and GRC2015/020 projects. It is also a contribution to the LISBOA-01-0145-FEDER-031265 (*HabWAVE – Relevância da conjugação de processos biológicos e físicos na iniciação de blooms de algas nocivas na costa NW de Portugal*) and MAR2020 No. MAR-02.01.01-

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7.03 Dinoflagellate cysts and acritarchs from the Pliocene-Pleistocene transition in the Southern Bering Sea: IODP site U1341

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Abstract: The latest Pliocene–earliest Pleistocene (2.83 to 2.40 Ma) interval from the southern Bering Sea Integrated Ocean Drilling Program Site U1341 at Bowers Ridge has been analyzed for dinoflagellate cysts, acritarchs, and other palynomorphs. Based on 86 samples, with a spacing equivalent to between 500 and 6000 years, this is the most stratigraphically detailed late Cenozoic marine palynological study yet undertaken in the Bering Sea. The dinoflagellate cyst assemblages are characterized by low taxonomic richness: round brown cysts including *Brigantedinium simplex* dominate assemblages which also include *Lejeunecysta cinctoria*, *L. fallax*, *Selenopemphix nephroides*, *Trinovantedinium variabile*, and *Trinovantedinium* cf. *harpagonium* (heterotrophic taxa); and *Cerebrocysta?* sp., *Impagidinium detroitense*, *Impagidinium* spp. indet., and *Nematosphaeropsis labyrinthus* (phototrophic taxa). Marine acritarchs are represented by *Cymatiosphaera?* *invaginata* which is often abundant. Two informal assemblage biozones are proposed: biozone MP-A (~2.828 to 2.497 Ma) and biozone MP-B (~2.477 to 2.401 Ma). The co-dominance of round brown cysts and the extinct high-latitude acritarch *Cymatiosphaera?* *invaginata* in biozone MP-A reflects predominantly cold and reduced-salinity surface waters with intermittent incursions of warm and higher salinity waters from the Alaskan Stream. The absence of pronounced changes in the dinoflagellate cyst assemblages within biozone MP-A reflects the enclosed nature of the Bering Sea and the low taxonomic richness of the marine palynomorph record. However, the MP-A/MP-B biozone boundary at 2.47 Ma marks a major change in the hydrography of the Bering Sea, as expressed by an abrupt decline in *Cymatiosphaera?* *invaginata* and increased proportions of terrestrial plant matter, suggesting an important increase in the influence of river discharge. Sporadic incursions of the Alaska Stream nonetheless continued after 2.47 Ma.

Keywords: Bering Sea, Pliocene-Pleistocene, paleoceanography

Acknowledgments: MJH acknowledges support from a Natural Sciences and Engineering Research Council of Canada Discovery Grant.

7.04 Novel nuclear proteins in dinoflagellate mediate the highly compacted liquid-like state of dinoflagellate chromosomes

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Abstract: DNA in most eukaryotic nuclei is packaged using histone-based nucleosomes to enable DNA condensation and regulation. Dinoflagellates are an exception to this universal feature as they have apparently abandoned canonical nucleosomes. They re-engineered their chromatin biology coincident with the gain of novel nuclear proteins through multiple lateral gene transfer events. First dinoflagellates acquired genes for a family of DNA-binding proteins of putative viral origin called ‘dinoflagellate/viral nucleoproteins’ (DVNPs). DVNPs are found in all extant dinoflagellates including the early-diverging Syndiniales. Subsequently dinoflagellates gained bacterial proteins for ‘histone-like proteins’ (type-I and type-II HLPs) and these genes were acquired on multiple occasions independently but after Syndiniales diverged. The outcome of these changes in the protein biochemistry of dinoflagellate nuclei is the enormous expansion of nuclear DNA content and the formation of liquid crystalline permanently condensed chromosomes, a highly unusual nuclear state. To understand the role of DVNPs and HLPs in dinoflagellate nuclei, we expressed and purified these proteins and examined their interaction with DNA. We observed both DVNP and HLPs rapidly bind to DNA; however, whereas DVNP forms phased separated coacervates with DNA, HLPs form solid DNA aggregates. Using optical tweezers to measure DVNP’s effects on single DNA molecules, we observe changes to the physical properties of DNA and extremely rapid DNA contraction although through DNA tensions less than those produced by canonical nucleosomes. DVNP structural data and co-evolutionary analysis identifies a disordered C-terminal domain as playing an important role in DNA compaction. Together, our results suggest that DVNPs and HLPs interact differently with DNA implying different contributions of these two proteins to the highly unusual dinoflagellate nuclear state.

Keywords: Syndiniales, nucleosomes, DVNP, condensation, chromatin

7.05 Recent advances in infrared spectroscopy applied to single specimen dinoflagellate cyst: methodological framework, acquired insights and future perspectives

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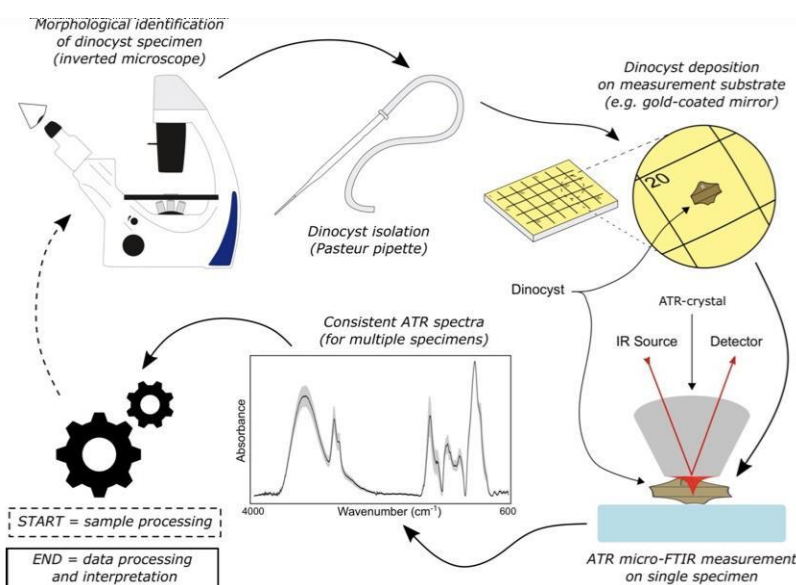
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Abstract: Fourier-transform infrared (FTIR) spectroscopy is a spectrochemical technique able to retrieve macromolecular information from organic materials. When combined with a microscope (micro-FTIR), the bulk (geo)chemical composition of single specimen of dinoflagellate cysts (dinocysts) can be determined. Micro-FTIR studies on dinocysts are sparse, partially due to the complexity of the method in terms of possible disruptive variables and the interpretability of the data it provides. Nevertheless, it can be a powerful, rapid, and relatively low-cost tool for retrieving macromolecular information from small organic particles. That is, if a certain degree of methodological insights and control can be ensured. This recently led to the development of a methodological framework (Meyvisch et al. 2022) wherein a wide array of variables are discussed. The result of this study is a method based on attenuated total reflection (ATR) micro-FTIR spectroscopy which allows for the collection of robust spectrochemical datasets, and which will be presented here.



Earlier micro-FTIR studies on dinocysts have explored the potential of the method to be used as a chemotaxonomical tool (Bogus et al. 2012), a proxy for trophic affinity (Bogus et al. 2014) and to assess preservation of organic matter in oxic and sulphidic depositional environments (Versteegh et al. 2020). Within the light of the later developed methodological framework and by applying chemometrics, some of these authors' findings are re-evaluated and supplemented with more elaborate and robust data sets.

The overview provided on the current state of this – relatively underexplored – research domain perfectly sets the stage to discuss its future perspectives, with a focus on providing answers to major standing questions regarding the (geo)chemical nature and variability of dinoflagellate cyst walls. Finally, other useful and complementary analytical methods which can contribute to elucidating these problems will be briefly discussed.

Keywords: Fourier-transform infrared spectroscopy, organic-walled dinoflagellate cysts, cyst wall composition, methodology, chemometrics

Acknowledgments: This work was supported by the Hercules Foundation (FWO, Flanders) (FT-IMAGER project-AUGE/13/16).

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7.06 Ecological signals in dinoflagellate biostratigraphy

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Abstract: During the past fifty years, we and co-workers have pursued a research program to explore how recent and living dinoflagellate cysts reflect their ecology. One of the main goals is to suggest how paleoecological influence may affect the biostratigraphical record. We first produced our own global database of recent cyst distributions, funded mainly by BP, an AASP consortium of companies, and Statoil (now Equinor). Comparing cyst distributions with environmental parameters, we identified ecological signals and developed statistical methods for modeling these (SMES). We showed that ecologic models based on living ecology could help pick flooding surfaces in Paleocene sequences in offshore Norway (Dale et al. 2005). As follow-up to this, we carried out projects for Statoil identifying water-depth signals in both offshore and shallower-water Cretaceous sequences. Here, we present illustrated examples of how the main ecological signals, water temperature, salinity, nutrients and distance from shore (as a water depth proxy) may affect biostratigraphy. Possible water depth signals may provide particularly useful input to basin analysis and sequence stratigraphy. This is an example of biological research contributing to the development of applications in geology. Other research has shown that these methods can also be applied to the environmental sciences, for example in studies of eutrophication, pollution and climate change.

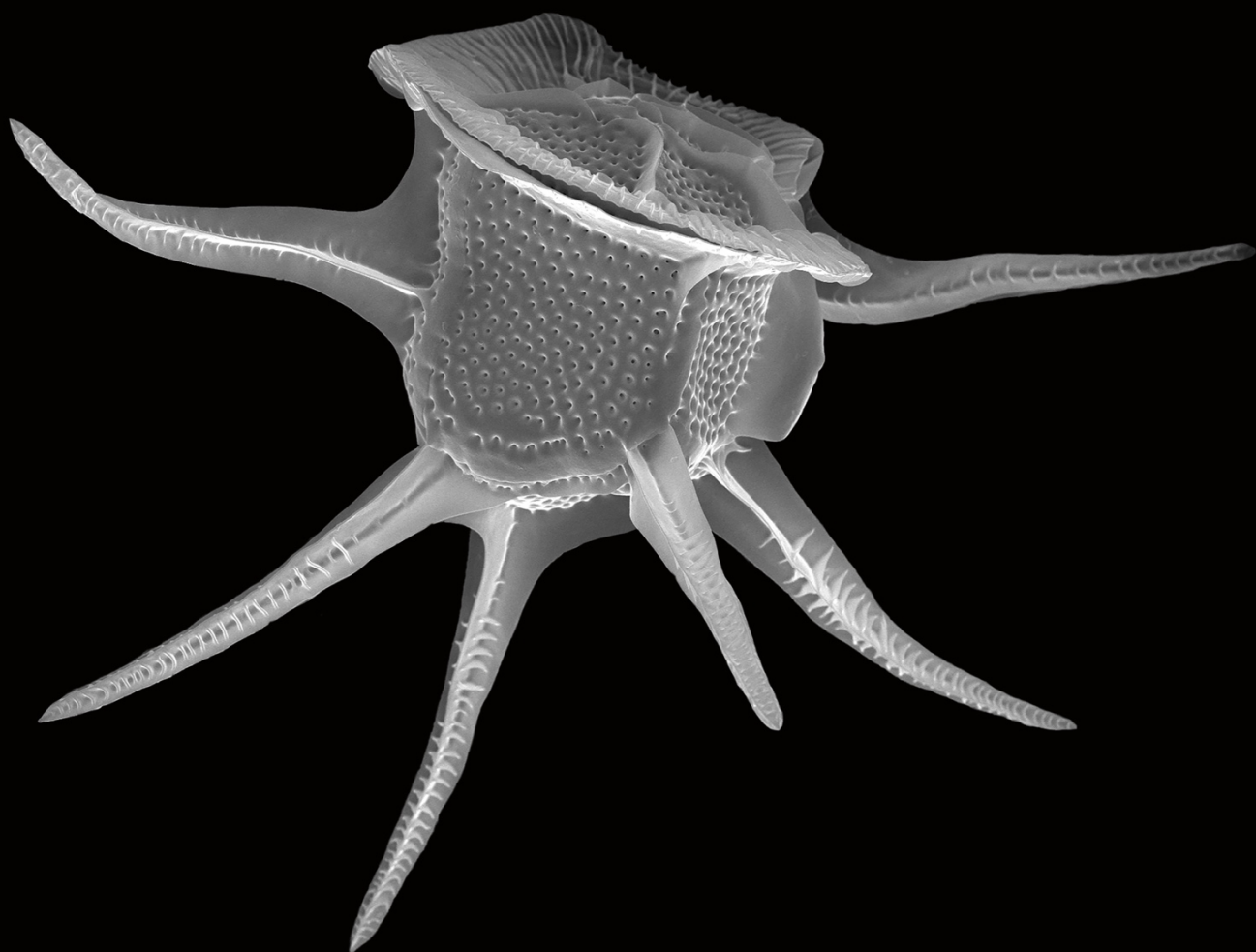
Keywords: Ecology, Paleoecology, Biostratigraphical-applications, statistical-modelling

Acknowledgments: We wish to thank the companies named above for their support for this work.

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AUTHOR INDEX

Acosta, F.....	3.03
Alonso-Rodríguez, R.	5.03
Al-Silwadi, S.	7.01
Amorim, A.....	1.04, 1.06, 1.11, 1.12, 2.02, 3.12, 7.02
Anderson, D. M.	2.05, 3.15
Ando, T.	6.05
Andree, K.	3.07
Arana-García, J.	5.01
Azanza, R. V.	2.03
Barón-Campis, S. A.	5.03
Basterretxea, G.	3.14
Beedessee, G.....	7.04
Benítez-Santana, M. I.....	3.04
Berdalet, E.	P.02
Bilbao-Sieyro, A.....	3.04
Bilien, G.	3.05, 6.02
Bordón-Rodríguez, E.	3.04
Bravo, J.	3.03
Bringué, M.	4.05
Caballero, I.....	1.05
Cabrera-Suárez, F.	3.04
Caeiro, M. F.	3.12
Campàs, M.	2.01, 2.06
Carbonell-Moore, C.....	6.02
Costas, S. V.	7.02
Cruz, E. R.	1.06, 3.06
Cucchi, F.	3.08
Dale, A. L.	7.06
Dale, B.	P.03, 7.06
Darias-Dágfeel, Y.....	3.08
David, H.....	3.12
De Schepper, S.	7.03
Derrien, A.....	3.05, 6.02
Dhifallah, F.....	5.04
Diogène, J.....	2.01, 2.06, 3.07, 3.09
Doner, A.	3.05
Dubert, J.....	1.06, 3.06

Duval, A.	3.05, 6.02
Enevoldsen, H.	5.02
Esqueda-Lara, K.	5.03
Fachon, E.	2.05, 3.15
Fatourou, E.	4.01
Fensome, R. A.	6.01
Fernández-Tejedor, M.	3.09
Fernández-Zabala, J.	1.04
Figuerola, R. I.	2.04, 3.01
Fleury, E.	1.02
Flores, C.	2.06
Font-Muñoz, J. S.	3.13
Francois, R.	4.04
Gárate-Lizárraga, I.	6.02
Garcés, E.	2.01
García- Gil, S.	7.02
García-Álvarez, N.	3.08
García-Anaya, T. M.	3.11
Gernez, P.	1.05, 3.05
Gilpin, C.	1.01, 1.08
Gimeno-Monforte, S.	3.07
Gómez, F.	P.04
Gómez-Pinchetti, J. L.	3.08
Gosselin, M.	5.04
Gu, H.	6.02
Gueroun, S. K. M.	2.02
HajSoltan, F.	7.03
Harmel, T.	1.05
Hatherly, M.	1.06, 1.12
Head, M. J.	6.01, 7.01, 7.03
Henk, V.	7.05
Hernández-Becerril, D. U.	3.11, 5.01, 5.03
Hernández-Fariñas, T.	1.05
Herrera, R.	1.04
Hoppenrath, M.	2.01
Hu, I.	7.04

Humphrey, E. C.	6.04
Ishigaki, M.	6.05
Johannessen, S. C.	4.05
Kafetzidou, A.	4.01
Kane, M. K.	3.13
Kaufmann, M.	2.02
Koerner, K. A.	4.06
Kolesnik, A. N.	4.02
Kouli, K.	4.01
Lacour, T.	1.05, 3.05
Lalande, C.	2.05
Larsen, J.	5.02
Li, Z.	4.04
Limoges, A.	4.06
Liu, L.	4.04
Lluisma, A. O.	2.03
Long, M.	1.07
Lorand, O.	1.07
Loures, P.	2.04, 3.01
Lugliè, A.	2.01
Maciel-Baltazar, E.	5.03
Malestroit, P.	1.07
Malto, Z. B. O.	2.03
Manach, S.	3.05
Marrero, C.	3.02
Marret, F.	4.01
Martel, A.	3.08
Matsuoka, K.	6.05
McLachlan, S. M. S.	6.04, 6.06, 6.07
Mena, C.	1.07
Mertens, K. N.	3.05, 3.15, 4.04, 6.01, 6.02, 7.05
Meyvisch, P.	6.02
Meza-García, P.	3.11
Moita, M.T.	1.06, 1.12
Monzón-Atienza, L.	3.03

Moreiras, I. G.....	1.11, 1.12, 7.02
Morquecho-Escamilla, L.	5.03, 6.02
Mudie, P. J.	1.13
Mulholland, M.R.	1.03
Navarro, G.	1.05
Niechwedowicz, M.	6.03
Nolasco, R.	1.06, 3.06
Obrezkova, M. S.....	4.02
Oliveira, A.	1.06, 1.12, 7.02
Oliveira, P. B.	1.06, 1.12, 7.02
Ozawa, T.	2.03, 3.17
Padedda, B. M.....	2.01
Padín, X. A.....	3.06
Panagiotopoulos, K.....	4.01
Pavón-Salas, N.....	3.04
Pazos, Y.	2.04, 3.01
Perez-Cruz, B.	6.02
Pérez-González, Y.	3.04
Pérez-Mora, M.....	3.01
Pérez-Vega, E.	1.03
Pichardo-Velarde, J. G.....	5.03
Pieter, G.	7.05
Pisapia, F.	3.08
Pjotr, M.	7.05
Portillo, E.....	3.02
Pospelova, V.	3.15, 4.02, 4.04, 4.05, 6.02, 6.04, 6.06, 6.07
Prado, P.....	3.07
Principe, T.	3.02
Pulina, S.....	2.01
Queiroga, H.	1.06
Rambla-Alegre, M.....	2.06, 3.09
Real, F.	3.08
Regaudie de Gioux, A.....	1.07
Reñé, A.....	2.01

Reth, M.....	3.05
Rey, M.	3.07
Riobó, P.	2.04, 3.01
Robert, E.	3.05
Rochon, A.....	4.06, 5.04
Rodríguez, F.....	2.04
Roux, P.	1.02
Roza, S. E. V.	1.10, 3.16
Saburova, M.	3.10
Saito, Y.....	4.04
Salas, R.	5.02
Salvador-Reyes, L. A.	2.03
Sánchez-Henao, A.....	3.08
Santos, A. I.	1.12, 7.02
Satta, C. T.	2.01
Schapira, M.	3.05, 1.02
Schiffrine, N.	5.04
Séchet, V.	3.05
Sergeant, F. S.....	3.08
Siano, R.....	1.02
Silva, T.	2.02
Sobrino, C. M.....	7.02
Soler-Onís, E.	1.04
Song, B.....	4.04
Sousa, A.....	2.02
Stacey, C. D.	4.05
Stephen, L.	7.05
Stott, K.....	7.04
Subong, B. J. J.	2.03, 3.17
Terre-Terrillon, A.	3.05
Tester, P.	P.01
Tudó, A.....	3.07
Tuval, I.	3.13, 3.14
Tuya, F.	1.04
Vázquez-Montes, E. D.....	3.11
Venuleo, M.	3.02

Versteegh, G. J.M.	1.09, 1.10, 3.16, 4.03, 6.05, 7.05
Villarreal-Martínez, A. M.	3.11
Walachczyk, I.	6.03
Waller, R. F.	7.04
Watson, M.	7.04
Winifred, V. Y.	3.15
Wright, C. A.	4.05
Wu, Y.	4.04
Xie, X.	4.04
Yamamoto, T.	6.05
Zanoli, M.	3.14
Zhou, R.	4.04
Zilhão, R.	3.12
Zimmerman, R. C.	1.03
Zoffoli, L.	1.05, 3.05
Zonneveld, K. A. F.	1.09, 1.10, 1.11, 3.16, 4.03, 6.05



