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XIII REUNIÓN
IBÉRICA DE
ALGASTÓXICAS Y
BIOTOXINAS
MARINAS



VI INTERNATIONAL SYMPOSIUM
ON MARINE SCIENCES 2018

Universidade de Vigo



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Editors:

Figuerola, R., Rodríguez, F., Fraga, S., Bravo, I.

Abstract Volume XIII Iberian Toxic Algae and Marine Biotoxins Meeting, June 2018

ISBN: 978-84-8158-791-3

Universidade de Vigo

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**DYNAMICS OF PARTICLE FLUXES OFF NORTHWESTERN
AFRICA:
INFLUENCE OF ATMOSPHERIC DUST AND EFFECT OF
LATERAL TRANSPORT**

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Abstract

Eastern Boundary Upwelling Ecosystems (EBUEs) cover only about 1% of the total ocean area but contribute with about 15% of the total marine primary production (Carr, 2002). Roughly, 20% of the marine global fish catch is provided by the four major EBUEs: the Canary, the Benguela, the Californian and the Humboldt Current Systems (Freon et al., 2009). EBUEs may be responsible for >40% of the carbon sequestration in the ocean (Muller-Karger et al., 2005) and are thus relevant for the global carbon cycle. The Canary EBUE along the Northwestern African margin extends approximately between 12° and 35°N and is affected by diverse factors (trade winds, chlorophyll filament, nutrient availability, lateral advection). These factors influence the productivity in surface waters in different ways, depending on the intensity of each factor and each particular area along margin.

This conference will address the seasonal and multiannual variability of particles fluxes dynamics, with particular focus on the Mauritanian upwelling. In this coastal area, the University of Bremen maintains a continuous monitoring program of particle dynamics using moorings since 1988. Our observations reveal strong interannual fluctuations, superimposed on a decadal fluctuation pattern. Distinct dust outbreaks and deposition in winter enhances particle sedimentation and carbon export on short timescales via the ballasting effect. Episodic perturbations of the marine carbon cycle by dust outbreaks might have weakened the relationships between fluxes and large-scale climatic oscillations. A significant amount of diatoms, biogenic silica (opal) and organic carbon produced in waters overlying the Mauritanian shelf is effectively transported in intermediate waters at the outer slope. The impact of the intermediate and bottom-near nepheloid layers-driven transport in the transfer of valves and bulk particulates and its potential contribution to the export of biogenic materials from the shelf and uppermost slope might play a significant role in hemipelagial fluxes off Mauritania.

Key words: coastal upwelling, diatoms, nepheloid layers, NW Africa, particle fluxes.

Acknowledgments: This long-time research work has benefited from the contribution from several colleagues at Marum. The German Research Foundation (DFG) has provided financial support.

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MONITORING COASTAL WATER QUALITY

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Abstract: Coastal environments are vulnerable ecosystems providing a variety of unique services. However, coastal water bodies, including transition waters and the coastal ocean, also face increasing pressure due to worldwide demographic trends and industrial development. Nutrient and contaminant inputs from continental sources may undergo rapid changes and their superposition with other environmental stressors (e.g. temperature, pH, parasites, etc.) may cause adverse effects on coastal ecosystem health and productivity. Water quality monitoring is part of the ecological status assessment of transition waters and the coastal ocean, supported by regulations such as the European Water Framework Directive and the Marine Strategy Framework Directive. Monitoring efficiency, related understanding of biogeochemical cycles and risk assessment depends on various factors, such as analytical limitations, observation strategies, regulation, etc., leaving room for improvement and challenging perspectives, not only for emerging contaminants. Perspectives of using examples of traditional monitoring tools and such recently under development, as well their combination with different approaches, including exposure experiments, are addressed.

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OCEAN REANALYSIS FOR THE STUDY OF THE EVOLUTION OF THE STATE OF THE OCEAN OVER THE LAST DECADES

Aida Alvera Azcarate

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Abstract: Assessing the role of the ocean and sea ice on climate variability is critical for understanding global climate change. The oceans have the largest heat capacity in the climate system, and ocean circulation redistributes this heat. Moreover, variability in ocean circulation determines seasonal to decadal variability in climate. Ocean synthesis products are extensively used for this purpose. Ocean syntheses are comprehensive estimations of the ocean state over the last decades (mainly temperature, salinity, sea level and currents) calculated by merging hydrodynamic ocean models and all available observations using data assimilation. Ocean syntheses are used as initial conditions for operational estimations of the ocean state, for short-term predictions aiming at studying specific processes, for seasonal and decadal predictions, and for climate-related activities. They can be also used to estimate the interaction between the ocean and the atmosphere, needed to assess the role of the ocean in the heat balance of the Earth and its influence in the global water cycle. Ocean syntheses are necessary to compute transports across ocean basins and key straits, in order to understand how heat is transported across the ocean.

The COST Action “Evaluation of Ocean Syntheses” (EOS) is an initiative aiming at promoting the use of ocean syntheses and reanalyses among the scientific community, by raising awareness of these products and promoting their use. A series of networks have been created to inter-compare several ocean syntheses in different regions, with the aim of providing a well-documented quality assessment on the various products that will allow the end user to choose the ocean synthesis that is more appropriate for their intended use. After an introduction to ocean syntheses, the main advancements achieved through the COST Action EOS and the main conclusions reached will be presented to the audience.

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**"THE SECRET LIFE OF DIATOMS: FASCINATING QUESTIONS
FROM UNICELLULAR MICROALGAE"**

Marina Montresor

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Abstract: Diatoms, as many unicellular eukaryotes, have life histories that include different phases: a growth phase in which they divide and increase their numbers, a resting phase in which they can withstand adverse environmental conditions, a sexual phase in which genetic recombination occurs. Diatom cells progressively decrease in size following cell division and large cells are produced during the sexual cycle, thus counteracting the miniaturization process.

I will illustrate examples of research on diatom life histories including observations at the Long Term Ecological Research site in the Gulf of Naples, experimental investigations carried out in the laboratory and molecular studies aimed at detecting the molecular mechanisms regulating diatom sexual phase. I will outline the implications that different aspects of life histories have for population dynamics and micro-evolution of this group of microalgae.

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**RIA DE VIGO: WHEN THE ATLANTIC KNOCKS ON YOUR
DOOR**

Ricardo Torres

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Abstract: The Ria de Vigo is one of the four semi-enclosed and elongated bays of the Rias Baixas system situated at the northern limit of the Iberian-Canary Current Eastern Boundary Upwelling Ecosystem. The Ria de Vigo has one of the most important mariculture industries in Europe and a rich history of oceanographic research spanning the last seven decades. In this talk I will review how our understanding of the circulation of the Ria de Vigo has evolved, from the pioneering work in the 50s through to the 80s from such scientists like Fernando Fraga and Ramon Margalef to the subsequent substantial contributions of Ricardo Prego, Francisco Figueiras, Xose Alvarez-Salgado, Fiz Perez and today's efforts led by Gabriel Roson, Miguel Gilcoto and Eric Desmond Barton and of course many others. The talk will trace the evolution over the years of our understanding of how Atlantic wind patterns and the circulation on the adjacent shelf (namely upwelling and downwelling events) shape the circulation inside the Ria.

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LA ISLA DE COCO Y LA IMPORTANCIA DE SER PATRIMONIO NATURAL DE LA HUMANIDAD

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Abstract: La Isla del Coco, Costa Rica, está ubicada a 500 km de la costa en el Pacífico Tropical Oriental (PTO). Es una isla oceánica con un alto endemismo terrestre (~35%) y una alta diversidad marina (más de 1700 especies). Se diferencia de otras islas oceánicas del PTO por ser muy húmeda y estar cubierta de vegetación, es por eso que ha sido visitada y estudiada desde el Siglo XVI. A finales del Siglo XIX un gran número de expediciones empiezan a desarrollar investigaciones en el Isla y se ha generado gran cantidad de información, sin embargo, se continúa descubriendo nuevas especies. Geopolíticamente es muy importante para Costa Rica ya que le genera más del 50% de su mar patrimonial. La Isla del Coco fue declarada Parque Nacional en 1978, Patrimonio Natural de la Humanidad de la UNESCO en 1997 y Humedal de Importancia Internacional Ramsar en 1998. La Isla del Coco se ha llamado la Isla del Tesoro, porque hay leyendas de varios tesoros enterrados allí, siendo el más grande el Tesoro de Lima. El Parque Nacional Isla del Coco (PNIC) es de los sitios mejor protegidos, sin embargo, sufre el embate de la pesca ilegal y anteriormente el de la búsqueda de tesoros. Con la declaratoria del PNIC como Patrimonio de la Humanidad se detuvo totalmente todo intento de búsqueda del tesoro. Esta declaratoria también ha servido internacionalmente para resaltar la importancia del PNIC en el PTO y a nivel mundial como uno de los últimos refugios de grandes pelágicos (tiburones, atunes, picudos, entre otros). Además, la destaca como un sitio especial, principalmente para el turismo de buceo. Localmente, la declaratoria de Patrimonio de la Humanidad, ha servido para aumentar la conciencia sobre la importancia de ambientes naturales bien protegidos y ha generado interés nacional por su conservación, principalmente marina.

Key words: Isla de Coco, Patrimonio Natural, UNESCO

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CLIMATE CHANGE DRIVERS IMPACTS ON MARINE BIOTOXINS CONTAMINATION AND GENOTOXICITY IN *MYTILUS GALLOPROVINCIALIS*: COMBINING THE EFFECTS OF WARMING, ACIDIFICATION AND HARMFUL ALGAL BLOOMS.

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Abstract: *Mytilus galloprovincialis* under current environmental conditions and simulated climate change scenarios (warming, acidification and warming with acidification) were exposed to PSP toxins-producer *Gymnodinium catenatum*. Shellfish toxicity and DNA damage (comet assay) were assessed in mussels from all treatments. Mussels were acclimated (21 days), exposed to *G. catenatum* (5 days, uptake), and feed with a non-toxic diet (10 days, elimination). PSP analyses were carried out by Liquid Chromatography with Fluorescence Detection. The highest PSP content was observed under actual conditions, day 5 ($1493.8 \pm 202.4 \mu\text{gSTXeq.kg}^{-1}$), exceeding safety limits ($800 \mu\text{gSTXeq.kg}^{-1}$). Significantly lower contents were observed under climate change scenarios. The lowest, $661.9 \pm 22.8 \mu\text{gSTXeq.kg}^{-1}$ was found in warming, followed by acidification ($761.2 \pm 62.3 \mu\text{gSTXeq.kg}^{-1}$). However, warming and acidification interaction did not reveal an additive effect. After shifting to non-toxic diet, lower toxins elimination was observed in warm-acclimated mussels. Genotoxicity was assessed after each period. In non-exposed mussels, the highest damage was observed under warming and acidification interaction after 36 days. In *G. catenatum* exposed mussels, DNA damage significantly increased earlier, just after uptake. The treatments acidification and interaction of warming with acidification, revealed higher damage than actual conditions, highlighting synergistic impacts. DNA damage decreased during elimination, although subtly under warming and acidification interaction. This is the first study assessing the impact of combined warming, acidification

and biotoxins in mussels. In conclusion, it was provided evidence that climate change may lead to lower PSP contents, but also to slower elimination rates and to synergistic effects on DNA damage implying possible consequences for the mussels populations.

Key words: Harmful algal blooms, Climate change, *Gymnodinium catenatum*, Seafood safety, Genotoxicity

Acknowledgments: This study was supported by CERES project (European Union's Seventh Framework Programme - FP7 /2007-2013). Ana C. Braga has a Doctoral Grant (PD/BD/113484/2015) from the Portuguese Science and Technology Foundation (FCT). António Marques and Pedro R. Costa are supported through the FCT Investigator program (IF). This work contributes to project UID/Multi/04326/2013 from the Portuguese Foundation for Science and Technology (FCT). Thanks are due for the financial support to CESAM (UID/AMB/50017 - POCI-01-0145-FEDER-007638), to FCT/MCTES through national funds (PIDDAC), and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020.

METABARCODING OF PROTIST COMMUNITY FROM MARINE COASTAL SEDIMENTS: EVALUATION OF TWO DIFFERENT SAMPLE TREATMENTS

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Abstract: High-throughput metabarcoding techniques have become widely applied to characterize microbial communities. Studies on the community composition of marine coastal sediments are still scarce, although they represent a large reservoir of marine protists and the community composition is distinct to that of the water column. In this study, we characterized it using Illumina MiSeq sequencing of the V4 region of 18S rDNA on samples obtained in three different locations from the Mediterranean Sea during six months. All samples were subjected to two different treatments (DNA extraction directly from sediments, or previously eluting the organisms from the sediment using the seawater-ice Uhlig method). Our results show that despite being the methodology most commonly used in these studies, the low amount of sediments used in direct DNA extraction from sediments results in a larger dispersion of samples and replicates, in contrast to eluted samples, which allow the use of much larger amounts of sediments. As expected, the community composition and relative abundance of major taxonomic groups varied among treatments, with ciliates and dinoflagellates being predominant in eluted samples (>60% of reads). However, most abundant representatives were shared among treatments, representing an 80% of the total number of reads. Thus, both methods allowed the characterization of the community of protists. While the larger amount of sediment used in eluted samples resulted in higher richness values and a better capture of less abundant organisms, direct DNA extraction from low amount of sediments resulted in a better characterization of all present taxonomic groups but a larger variability among samples.

Key words: protists, sediments, coast, metabarcoding

EVALUACIÓN DEL SUSTRATO ARTIFICIAL PARA EL MUESTREO DE DINOFLAGELADOS BENTÓNICOS EN LA REGIÓN MACARONÉSICA

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Abstract: El diseño de nuevas metodologías para el muestreo de dinoflagelados bentónicos potencialmente tóxicos, basadas en el uso de sustratos artificiales, está experimentando en los últimos años una lenta pero gradual aceptación por parte de la comunidad científica. Los sustratos artificiales se fundamentan en la capacidad que tienen algunas microalgas de colonizar nuevas superficies, y suponen una alternativa a la recogida tradicional de macrófitos que, debido a su morfología variable, pueden dificultar la comparación de resultados obtenidos entre muestreos realizados a diferentes escalas temporales y espaciales.

El seguimiento y control de floraciones algales nocivas (FAN) en la Región Macaronésica se ha convertido en un objetivo importante dado que, desde los últimos 15 años, se ha producido un aumento en la frecuencia e intensidad de estos eventos, en muchos casos producidas por dinoflagelados bentónicos. En esta región, con más de 3000 km de costa, es metodológica y técnicamente complicado realizar estos trabajos ya que reúne unas características bioclimáticas y oceanográficas diversas, por lo que el uso de métodos estandarizados de muestreo es fundamental. Este estudio analiza la relación existente entre la abundancia de dinoflagelados bentónicos asociados a macroalgas (sustratos naturales) y los que se fijan a superficies de red de fibra plástica (sustratos artificiales). Además se establece un rango óptimo de número de replicas para el muestreo de estos organismos en diferentes ambientes presentes en la región. Para ello, se estudiaron 4 localidades de las Islas Canarias y Cabo Verde a lo largo de un año. Los resultados obtenidos muestran la eficacia de esta metodología para el seguimiento y control de eventos FAN en un área tan extensa y avalan el uso de un sustrato artificial para la estandarización de los métodos de muestreo de dinoflagelados bentónicos tales como *Gambierdiscus*, *Ostreopsis*, *Prorocentrum*, *Coolia* y *Amphidinium*.

Key words: Sustrato artificial, Dinoflagelados bentónicos, Macaronesia

Acknowledgments: Proyecto MIMAR INTERREG V-A MAC 2014-2020, Viceconsejería de Medio Ambiente, Gobierno de Canarias

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CIGUATERA IN THE CANARY ISLANDS: SCIENTIFIC, LEGAL AND DIVULGATIVE OVERVIEW

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Abstract: The 2004 year was an inflection point for the ciguatera in the Canary Islands since: it happened the first outbreak in 5 humans for the consumption of a fish caught in Fuerteventura, at the same time a dinoflagellate of the genus *Gambierdiscus* was isolated in the waters of Tenerife and La Gomera and, in addition, it came in to force an European regulation that prohibited selling fishery products with ciguatoxins. One hundred and thirteen affected humans have been recorded from 2004 to 2016. The purpose of this work is to know the trends in terms of scientific publications and legal and divulgative outputs to contribute to the future management of strategies in this matter. Between 1993 and 2017 there were 45 references (67% scientific, 20% legal and 13% divulgative) all but one between 2004 and 2017. Scientific production was relevant in 2010 (5 refs.) - after the episodes of human intoxications (34 cases) two years before, and also between 2014 and 2017 (13 refs.) in the framework of research projects. Several legal rules showed up after the outbreaks of 2008 and 2009 and also during the last three years as a result of a better knowledge and also of a warning that temporarily affected El Hierro. However, the divulgative activity has been quite poor (2 articles in generic journals and 4 webs) and without specific trend. This last question is a very important issue because intoxications were, in most of the cases, due to consumption of species whose origin was recreational fishing. In this fishing modality ciguatoxin analyses are not required (unlike fish that come from professional fishing). This is why it is recommended to carry out social diffusion actions, and in particular, to certain risk groups such as recreative fishermen and restaurants.

Key words: Ciguatera, knowledge review, Canary Islands

Acknowledgments: This work was developed within the framework of the MIMAR Project (MAC/4.6d/066), with the support of the European Union (EU) and co-financed by the European Regional Development Fund (EDRF) and the INTERREG V-A Spain-Portugal MAC 2014-2020 (Madeira-Azores-Canarias).



XIII Reunión Ibérica de Algas Tóxicas y biotoxinas marinas 2018
Vigo (Spain), 20-22th June 2018

OFFICIAL CONTROL PROGRAMME FOR CFP IN THE CANARY ISLANDS: RESULTS OF CTX ANALYSIS IN 2017

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Abstract: The epidemiological Surveillance System for Ciguatera Fish Poisoning (CFP) in the Canary Islands established following the first autochthonous cases (Boada et al., 2010; Friedman et al., 2017), has recorded 18 outbreaks between 2008 and 2017, affecting 113 people. These cases occurred following the consumption of not officially controlled fish which came from local market and sport fishing. Thus, the action protocol implemented by the DG Fisheries of the Canary Government for the detection of ciguatoxin (CTX) in extractive fish at the authorized first sale points, seeks to protect consumer health against CFP. Through this programme, fish samples from professional fishermen associations are sent to the laboratory of the University Institute of Animal Health and Food Safety (IUSA) of the University of Las Palmas de Gran Canaria (ULPGC) in order to determine the CTX presence in fish flesh.

From January to December 2017, 1058 fish samples were analysed through the official control of CFP by a Cellular Bioassay Analysis (CBA) (Caillaud et al., 2012). The study species included Amberjack spp. (*Seriola spp.*) (n=537), Grouper (*Epinephelus marginatus*) (n=82) and Wahoo (*Acantocybium solandri*) (n=23). The grouper species showed the highest percentage of CTX positivity (25.6 %), followed by the amberjack

(11.4 %) and the wahoo, with only one positive result out of 23 samples (4.3 %). According to the island of capture, Lanzarote and Fuerteventura accounts for a slightly higher percentage of CTX positivity (13.1%) than Tenerife (12.7 %), followed very closely by Gran Canaria (10.7 %) and El Hierro (9.9%). However, La Palma and La Gomera showed much lower number of CTX positive fish (2.7 % and 2.3 %, respectively) within the Canary Archipelago.

Key words: Canary Islands, *Gambierdiscus*, Ciguatoxin, CFP, Cellular Bioassay Analysis.

Acknowledgments: The authors acknowledge financial support from the Canary Government and the technical support thanks to the European Food Safety Authority (EFSA) through the EUROCIQUA project (GP/EFSA/AFSCO/2015/03).

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DIVERSITY AND DISTRIBUTION OF *GAMBIERDISCUS* IN CANARY ISLANDS: CURRENT KNOWLEDGE AND PROBLEMS

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Abstract: The wide distribution and the diversity of *Gambierdiscus* recently reported in Canary Islands by Rodriguez et al 2017 point out these ciguatoxin-producer dinoflagellates to be more common and diverse than previously thought. Five species have been detected up to now in the region: *G. australes*, *G. caribaeus*, *G. carolinianus*, *G. excentricus* and *G. silvae*. Although *Gambierdiscus* was found in all the islands studied so far (5 out of 7), remarkable differences were observed in the composition of species between the eastern and western islands. In the present work, we describe the spatial distribution of *Gambierdiscus* in the islands of La Gomera and La Palma, from which there was still no data. While *G. australes*, *G. caribaeus*, *G. excentricus* and *G. silvae* were found in La Gomera; only *G. excentricus* was detected in La Palma. Cell concentrations reached up to 292 cells/gr⁻¹ wet weight of macrophytes and 21 cells/gr⁻¹ wet weight of macrophytes in those islands respectively, which is consistent with the lower concentrations in western islands reported by Rodriguez et al 2017. Nevertheless, there is a problem in performing field studies on *Gambierdiscus* since the morphologically similar species are difficult to distinguish using morphological features. In order to know to what extent we can discern species or groups of species by means of thecal parameters, a morphometry study were carried out on cells from cultures which had been identified by DNA sequencing. And the discriminant functions were afterwards applied to *Gambierdiscus* cells from natural environment. In addition, the possibility of alternative methodologies based on molecular techniques for rapid discrimination of *Gambierdiscus* species is discussed.

Key words: Gambierdiscus, ciguatera, Canary Island

Acknowledgments: To the proyect “Tropicalización y ciguatera en Canarias” funded by “Fundación Biodiversidad (MAPAMA)” and “Instituto Español de Oceanografía (MINEICO)”.

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MONITORING OF CIGUATOXIN CARRIER FISHES IN EL HIERRO ISLAND AFTER A TOXIC DINOFLAGELLATED BLOOM

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Abstract: Ciguatera fish poisoning (CFP) is the most common non-bacterial food intoxication in the world caused by ingestion of fish with bioaccumulated ciguatoxins (CTXs) (Darius et al., 2013). Historically, CFP was associated with isolated island communities that relied heavily on subsistence fishing for their food supply (Skinner et al., 2011), but the geographic reach of CFP has expanded due to international seafood trade and travel (Epelboin et al., 2014). In addition, people consuming fish from areas previously free of CFP may be at risk when such areas become prone to CFP (Villareal et al., 2007, Robertson et al., 2014, CDC, 2013, Bravo et al., 2015) due to changes in the aquatic environment. In October 2016 a toxic microalgae bloom was detected by researchers of the Spanish Bank of Algae (Soler et al., 2017), and a taxonomic study of them was carried out. The study revealed the presence of toxic dinoflagellates belonging to the genus *Gambierdiscus* and to the specie of *G. caribaeus*. A total of 34 fish species have been sampled, with a total number of 200 specimens, in three different samples from December 2016, April 2017 and December 2017, showing a total of 62,5%, 19% and 10% of the positive fish respectively. An increase is shown up to 40% after the bloom (December 2016), and then it is smoothed to values between 9 and 12% in april and decembre 2017 respectively.

Key words: Ciguatoxin, El Hierro island, *Gambierdiscus*.

Acknowledgments: The present work was supported by funds from the Projeet “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 the La Restinga Marine Reserve

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ANÁLISIS DE TOXICIDAD DE *GAMBIERDISCUS AUSTRALES* Y *FUKUYOA PAULENSIS* DE LAS ISLAS BALEARES

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Abstract: *Gambierdiscus* spp. y *Fukuyoa* spp. son los géneros de microalgas epibentónicas que producen unas de las más potentes neurotoxinas, conocidas como las ciguatoxinas (CTX). Estas neurotoxinas se bioacumulan en peces y mariscos y están relacionadas con la ciguatera, una intoxicación alimentaria endémica de zonas tropicales y subtropicales que es provocada por el consumo de pescado o marisco contaminado con ciguatoxinas (Bagnis et al., 1980, 1985). No hay evidencias de casos de ciguatera en el Mediterráneo, aunque desde el año 2004 se han citado 4 especies de *Gambierdiscus* (*G. belizeanus*, *G. carolinianus*, *G. silvae*, *Gambierdiscus* sp.) y un *Fukuyoa* sp. en las costas de Grecia (Aligizaki & Nikolaidis, 2009, Holland et al., 2013) y *Fukuyoa paulensis* en el archipiélago Balear (Laza-Martínez et al., 2016), aunque poco se conoce de las toxicidades de estas cepas.

De cara a estudiar la posible presencia de *Gambierdiscus* y *Fukuyoa* en las Islas Baleares, se muestreó el litoral de las islas de Mallorca, Menorca y Formentera del archipiélago Balear. Se recogieron muestras de sustrato de rocas y de macroalgas, y se registraron los parámetros ambientales como la salinidad, el pH, profundidad y nivel de oxígeno. Una vez en el laboratorio se aislaron las microalgas y se hicieron crecer 20 cepas monoclonales de *Gambierdiscus* y 3 de *Fukuyoa* para obtener suficiente biomasa para la identificación morfológica mediante la tinción con calcoflúor o microscopio electrónico de barrido, biología molecular mediante la amplificación de la región (D8-D10 LSU rRNA) y para analizar la producción de ciguatoxinas durante la fase exponencial mediante un ensayo de citotoxicidad de la línea celular Neuro2A.

Como resultado del muestreo se confirmó la presencia de *G. australes* siendo así la primera cita de *Gambierdiscus* en las Baleares y de *G. australes* en el Mediterráneo (Tudó et al., 2018). Mediante el análisis de toxicidad de las 23 cepas ampliaremos la información de las toxicidades de *Gambierdiscus* y *Fukuyoa* y contribuiremos a la evaluación del riesgo alimentario de la Ciguatera en el Mediterráneo.

Key words: *Gambierdiscus*, *Fukuyoa*, neurotoxinas, ciguatoxinas, toxicidad, neuroblastoma.

Acknowledgments: Los autores agradecen el soporte económico a la European Food Safety Authority (EFSA) que financia el proyecto EUROCIQUA

(GP/EFSA/AFSCO/2015/03) y al Ministerio de Economía, Industria y Competitividad (MINECO) que financió el proyecto SEASENSING (BIO2014-56024-C2-2-R) y financia el proyecto CIGUASENSING (BIO2017-87946-C2-2-R). También se agradece el apoyo del programa CERCA de la Generalitat de Catalunya y a el IRTA-URV-Banco Santander por promover la investigación con las becas predoctorales a A. Tudó and A. Toldrà (2016 PMF-PIPF-74 and 2015 PMF-PIPF-67).

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THIN LAYERS AND HARMFUL PHYTOPLANKTON IN THE RÍA DE PONTEVEDRA (NW IBERIA)

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Abstract: Thin layers of phytoplankton (TLP) are a particular case of blooms where high-cell densities are located within a narrow depth interval. Our understanding about the role that these features have in the growth of toxic phytoplankton in the Galician Rías (upwelling region off NW Iberia) is still limited. Aiming to investigate the relationship between the occurrence of TLP and toxic phytoplankton densities, weekly data obtained by INTECMAR (Instituto Tecnológico para o Control do Medio Mariño) at eleven stations in Ría de Pontevedra, between 2012-2015, were analyzed. Amongst the eight phytoplankton species quantified, *Pseudo-nitzschia* spp. and *Dinophysis acuminata* were the most frequently (>70 % of cases) present in TLP. In order to investigate the seasonal variations in the densities of these species, as well as to establish the threshold values used to identify density increases linked to thin layers, a longer dataset was analyzed (1992-2015). The seasonal cycles of the target species exhibited higher cell densities during the upwelling season. *Dinophysis acuminata* showed two maxima, in April and August-September, whereas higher densities of *Pseudo-nitzschia* spp. were quantified in July-August. Despite the lack of statistically significant differences in the seasonal cycles computed for *Pseudo-nitzschia* spp and *D. acuminata* at the different sampling stations, P2-Bueu was characterized by slightly higher mean densities of *Dinophysis acuminata* (496 [254-658] cell L⁻¹), whereas for *Pseudo-nitzschia* spp. higher values were computed at station P6-Tambo (70516 [39956-108160] cell L⁻¹). Around 43% of TLP were associated with cell densities of *D. acuminata* above the median, 19% above the third quartile and 15% with outliers. For *Pseudo-nitzschia* spp. these values were 28, 13 and 9%, respectively. These results suggest that, at least in the case of Ría de Pontevedra, the occurrence of TLP could be related either with growth or accumulation of toxic phytoplankton species.

Key words: Thin layers of phytoplankton, harmful phytoplankton, *Dinophysis acuminata*, *Pseudo-*

nitzschia spp., Ría de Pontevedra.

Acknowledgments: This is a contribution to the REMEDIOS project (CTM 2016-75451-C2-1-R) funded by Ministerio de Economía y Competitividad to Beatriz Mouriño-Carballido.



FINE SCALE PHYSICAL BIOLOGICAL INTERACTIONS IN A *DINOPHYSIS ACUMINATA* POPULATION DURING A RELAXATION-UPWELLING TRANSITION

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Abstract: Increased input of energy, shoaling of isoclines and shear stress inflicted to planktonic cells may lead to important changes in the rate of physiological processes (Juhl and Last 2002). The cruise “ASIMUTH-Rías” 2013, 17-21 June 2013, in the Galician Rías and shelf, aimed to study small-scale processes associated with spring blooms of *Dinophysis acuminata*. The cruise coincided with the initiation of an upwelling pulse following relaxation. A 36-h cell cycle study carried out on 18-20 June showed a brusque decline (0.51 to 0.25 d⁻¹) in division rates (μ), associated with increased vertical diffusivity (K_z). These negative effects were not observed at a mid shelf station ($\mu_{\min} = 0.5$ d⁻¹) used as reference. Here we describe microscale changes in water column structure and in the distribution and physiological status of the *D. acuminata* population and co-occurring ciliates as a result of increased mixing. Special attention is given to the distribution of the ciliate prey (*Mesodinium* spp) in relation to *Dinophysis*. Upwelling pulses, in addition to a direct physical effect of advective dispersion, appeared associated with a physiological disturbance to the cells probably through shear stress. The short-term impact of upwelling pulses in this population and the role of mid shelf populations as a relatively undisturbed reservoir of *Dinophysis* cells for subsequent blooms inoculation is discussed.

Key words: *Dinophysis*, population dynamics, physical-biological interactions

Acknowledgments: P.A. Díaz had a CONICYT/BECAS-CHILE grant. I. Ramilo, P. Rial and G. Fernández provided technical assistance. Funded by EU project ASIMUTH (EC FP7-SPACE-2010-1 # 261860) and Spanish DINOMA (CGL2013-48861-R).

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THE TURBULENT LIFE OF *DINOPHYSIS*

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Abstract: Dinoflagellates species of *Dinophysis*, in particular *D. acuminata* and *D. acuta*, produce lipophilic toxins and jeopardize shellfisheries in Western Europe (Reguera et al. 2014). In northwestern Iberia, *D. acuminata* has a long (spring-summer) growing season and populations start growing as soon as a mild stratification develops in the upwelling season. In contrast, *D. acuta* blooms in late summer when stratification, combined with moderate upwelling, is maximal. Project DINOMA aimed to compare growth conditions of both species to describe their niches and interpret their spatio-temporal distributions. Based on field observations, our starting hypothesis was that *D. acuta* is more sensitive to turbulence than *D. acuminata*.

We tested the response of well-fed cells of *Dinophysis* to three different turbulence levels—low (LT), $10^{-6} \text{ m}^2 \text{ s}^{-1}$; medium (MT), $10^{-5} \text{ m}^2 \text{ s}^{-1}$ and high (HT), $10^{-3} \text{ m}^2 \text{ s}^{-1}$ —generated by *Turbogen*, a sophisticated, computer-controlled system (Amato et al. 2016). These levels can be found in the Galician Rías during the upwelling season (Villamaña et al. 2017). Patches of *Dinophysis* in the bottom of the containers were observed in all treatments and controls (with no turbulence). Cells of both species exposed to LT and MT showed similar growth to the controls although *D. acuminata* exposed to MT grew faster and reached an earlier plateau phase followed by a sharp decline not observed in the control. Marked differences between species were found in the response to HT: *D. acuminata* showed a moderate growth after an initial lag phase, whereas *D. acuta* declined and remained stationary. Results from this study confirmed our initial hypothesis and the importance of turbulence to shape the species-specific spatio-temporal distribution of *Dinophysis*.

Key words: *Dinophysis*, turbulence, growth rate, ϵ

Acknowledgments: We thank Alessandro Manfredonia for material cleaning and medium culture preparation and Cosimo Vestito and Gianluca Zazo for the LED panels installation. This study was funded by Spanish projects DINOMA (RETOS Programme, CGL2013-48861-R) and MARBioFEED (ERANET Marine Biotechnology, PCIN-2015-252).

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**PARVILUCIFERA COROLLA (ALVEOLATA, PERKINSOZOA): A
GENERALIST PARASITOID OF DINOFLAGELLATES THAT
INFECTS PYRAMIMONAS (CHLOROPHYTA,
PRASINOPHYCEAE)**

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Abstract: Marine parasitoids of the genus *Parvilucifera* are considered specific endoparasites of dinoflagellates (Garcés et al. 2013). Recently *P. corolla* was described in the Catalan coast (NW Mediterranean) and Canary Islands (NE Atlantic), but its host range was not examined (Reñé et al. 2017). In the present study the host selectivity of *P. corolla* isolated from tidal ponds in Lanzarote (Canary Islands) was screened, including dinoflagellates and other groups. 106 strains of dinoflagellates belonging to 23 genera were checked as potential hosts for *P. corolla*. The parasitoid was capable to infect some or every *Akashiwo*, *Alexandrium*, *Amphidinium*, *Barrufeta*, *Coolia*, *Dinophysis*, *Gymnodinium*, *Heterocapsa*, *Lepidodinium*, *Levanderina*, *Ostreopsis*, *Prorocentrum*, *Protodinium*, *Scrippsiella*, *Takayama* and *Tripos* strains tested. On the contrary, species of the genera *Fragilidium*, *Fukuyoa*, *Gambierdiscus*, *Karenia*, *Karlodinium*, *Lingulodinium* and most *Prorocentrum* strains were resistant. Encystment was stimulated in the dinoflagellate *Symbiodinium natans* above a certain threshold of parasite:host ratio. *P. corolla* did not infect other algal groups (chlorophytes, cryptophytes, chrysophytes, diatoms, haptophytes and raphidophytes) but one strain of the prasinophycean *Pyramimonas*, although infections and sporangia were not viable.

Key words: *Parvilucifera*, parasitoid, dinoflagellates.

Acknowledgments: the authors acknowledge funding from CICAN, CCVIEO and CIGUATROP, and assistance with cultures to P. Rial and A. Fdez.-Villamarín.

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TEMPORAL AND SPATIAL DISTRIBUTION OF MARINE PHYCOTOXINS IN BIVALVE MOLLUSCS IN THE SOUTH ATLANTIC COAST OF MOROCCO

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Abstract: The accumulation of marine phycotoxins by bivalve molluscs poses a health risk and creates serious disruptions for the aquaculture industry. This study utilized geographic information system (GIS) techniques to analyze the primary data on Lipophilic Shellfish Poisoning (LSP), as well as Paralytic Shellfish Poisoning (PSP) data, in the South Atlantic coast of Morocco since 2003. The frequency of phycotoxins was mapped by kernel density estimation, and the relative risk posed by phycotoxins was assessed based on their physical–chemical characteristics. In addition, the spatial patterns and the trend of phycotoxins events were examined by nearest neighbor analysis and time series analysis, respectively. The results revealed that phycotoxins events not only had an increasing trend and significant seasonality, but also were clustered in space and time. Phycotoxins events displayed a higher frequency and a higher risk in the South Atlantic coast of Morocco. Shellfish toxins were detected in areas with high harmful algal blooms (HAB) risk, but were not correlated with the risk. This contribution provides a novel method to assess the relative risk caused by phycotoxins and some useful information for phycotoxins monitoring and management and aquaculture development.

Key words: Harmful algal bloom, Risk assessment, Geographic information system, Kernel density estimation, Nearest neighbor analysis

META-ANALYSIS OF PHYSIOLOGICAL EFFECTS OF TOXIC *ALEXANDRIUM* ON *ACARTIA*: NAÏVE VERSUS ADAPTED POPULATIONS

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Abstract: Dinoflagellates of the genus *Alexandrium* are typical causal agents of blooms with production of paralytic shellfish toxins (PST). Mesozooplankton can heavily graze on dinoflagellates (Calbet et al 2003). This group consumes in average ~12% of primary productivity (Calbet 2001). Calanoid copepods of the genus *Acartia* usually contribute to the major fraction of pelagic mesozooplankton biomass.

For the above reasons, the study of the interaction between *Acartia* and *Alexandrium* was perceived as a key step for understanding the fate of PST in the trophic chain. A number of laboratory and field works were published, in which researchers focused in food selection behavior and physiological effects of PST on copepods.

Recently, some authors explored the role of adaptive evolution in the response of copepod populations to their interaction with toxic dinoflagellates (Colin and Dam 2007; Zheng et al. 2011). These works suggested that populations of copepods that have experienced toxic dinoflagellates for several generations would develop enhanced resistance to the effects of PST.

The present work aims to unveil, with a Meta-analytic approach, the effect of this latter adaptive mechanism in the *Alexandrium* – *Acartia* interaction. Data from published studies was gathered, and the physiological effects of PST on copepods were analyzed considering, as a new aspect, the effect of the degree of adaptation of the copepod population to *Alexandrium*.

The results showed a positive effect of adaptation for total ingestion rate, ingestion of non-toxic food and egg hatching, but not for ingestion of *Alexandrium* and egg production. Among the adapted populations there were no differences based on their relative degree of adaptation. These results extend Colin and Dam conclusions to other populations of copepods. However, it is suggested that adaptation is manifested only by an increased resistance to the dose of toxins ingested, without changes in feeding behavior.

Key words: *Alexandrium*, *Acartia*, PST, adaptation, Meta-analysis.

Acknowledgments: This work was supported by a FCT fellowship SFRH/BPD/73286/2010

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THE IMPACT OF SEXUALITY UPON PARALYTIC SHELLFISH PRODUCTION IN ALEXANDRIUM MINUTUM

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Abstract: Paralytic shellfish toxins (PST) are a group of small neurotoxic alkaloids that are among the most potent natural toxins known. PST-producing plankton species include *Alexandrium* species, and the two dinoflagellates *Gymnodinium catenatum* Graham and *Pyrodinium bahamense* Plate. On a worldwide basis, *Alexandrium* species are the most abundant and widespread and recently, it was shown that there is a strong positive correlation between the total amount of PST produced in actively growing *A. minutum* cells and the copy number of the 4th domain of the starting gene of paralytic shellfish toxin (PST) synthesis, stxA4 (Stüken et al., 2015). As consequence, sexual reproduction could increase PST production due to the additional load of stxA4 copies in the diploid zygotes; depending the PST production in the offspring on the pattern of stxA4 gene recombination and inheritance during meiosis. However, the control of the genetic expression in mobile zygotes and the patterns of sexual recombination have been scarcely studied in dinoflagellates, and specifically, are totally unknown in *Alexandrium*. Therefore, how sexuality could affect toxicity is still an enigma. In this study, we followed by flow cytometry a sexual compatible cross between a strong PST producer strain (6.4±0.5 sxtA4 copy number per cell) and a weak PST producer strain (2.4±0.9 sxtA4 copy number per cell) in order to study PST production regarding two aspects: i) During zygote formation and resting cyst formation; ii) In the F1 offspring derived from the germination of the produced resting cysts (30 clones). Our results indicate, first, that toxicity was drastically reduced during sexuality and second, that the total production of PST followed a linear correlation in the haploid F1 offspring, implying that PST is inherited as a quantitative trait locus (QTL).

Key words: *Alexandrium*, PSP, sexuality, stxA, zygotes, resting cysts

Acknowledgments: The present work was funded by a FORMAS (Sweden) project (Formas 215-2010-824) to R.I.F.

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POLYKETIDES FROM *AMPHIDINIUM CARTERAE*

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Polyketides are a significant biosynthetic group in the Natural Product's world due to their potential for drugs development. Among marine dinoflagellates, *Amphidinium carterae* possesses one of the most prolific biosynthetic machineries for the production of several clades of polyketides characterized by a vast range of sizes and complexity (Kubota et al., 2006).

Despite these organisms are believed to be endosymbionts, they grow easily in cultures and produce a large diversity of bioactive compounds (Fürstner, A. 2011; Molina-Miras, A. et al. 2018). In this work, we report on the isolation and structure determination of several metabolites belonging to the interesting group of amphidinols, a series of long chain polyketides that usually show antifungal, cytotoxic and hemolytic activities (Kobayashi, J. and Kubota, T. 2007).

Key words: Microalgae, dinoflagellate, *Amphidinium carterae*, amphidinols.

Acknowledgments: This work was funded by Grant CTQ2014-55888-C03-01/R from MINECO. A.M.A. acknowledges to MINECO-FPI program for a PhD contract. We thank to S. Fraga for *Amphidinium* strains.

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NEW mELISA FOR THE RAPID SCREENING OF TETRODOTOXINS IN SHELLFISH, PUFFER FISH AND HUMAN BODY FLUID SAMPLES

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Abstract: The recent detection of tetrodotoxins (TTXs) in puffer fish and shellfish in Europe highlights the necessity to monitor the levels of TTXs by rapid and cost-effective methods to protect human consumers. An immunoassay for TTX detection based on the use of self-assembled monolayers (SAMs) for the immobilisation of TTX on maleimide plates (mELISA) (Reverté et al. 2015) was modified and adapted to the analysis of shellfish, puffer fish and human body fluids samples derived from a food poisoning incident. The replacement of carboxylate-dithiols by cysteamine for the SAM formation allowed decreasing the required time and cost, while maintaining high enough sensitivity (LOD ~2 µg/L) (Reverté et al. 2018). Matrix effects for the different type of samples were studied, demonstrating to be strongly dependent on the sample type and the sample treatment. The determination and application of correction factors to TTX contents provided by the immunoassay overcame the matrix effects in the analysis of oyster and mussel samples (Reverté et al. 2018), providing effective LODs in good agreement with the level of 44 µg TTX equiv./kg shellfish meat, considered not to result in adverse effects in humans by the European Food Safety Authority (EFSA, 2017). The mELISA was also applied to the analysis of two juvenile *Lagocephalus sceleratus* from Greece, demonstrating the early presence of TTX in these individuals from the Mediterranean Sea. Furthermore, the analysis of urine human samples derived from a puffer fish poisoning incident in New Caledonia contributed to the confirmation of TTXs as the causative agent of the intoxication (Rambla-Alegre et al. 2018). Highly analogous results were determined in the comparison of the analysis of naturally contaminated samples by mELISA with LC-MS/MS analysis. Work focused on improving and simplifying toxin extraction protocols is in progress to really foster the use of TTX immunoassays as powerful screening tools.

Key words: tetrodotoxin, immunoassay, shellfish, puffer fish, urine.

Acknowledgments: The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) through the ECsafeSEAFOOD project (grant agreement no. 311820), from the HORIZON 2020 Framework Programme (H2020/2014-2020) through the SEAFOODTOMORROW project (grant agreement no. 773400), from the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) through the PROMAQUA project (RTA2013-00096-00-00) and from CERCA/Generalitat de Catalunya program.

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PRODUCCIÓN METABÓLICA DE *Prorocentrum hoffmannianum*, UN CASO DE GRAN DIVERSIDAD ESTRUCTURAL

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Abstract: Durante los últimos años nuestro grupo de investigación ha venido desarrollando una línea de trabajo orientada al estudio químico-estructural y biosintético de las toxinas marinas responsables del síndrome diarreico. Disponer de una fuente continua y no depender del fenómeno natural es fundamental a la hora de abordar estos estudios. Con este motivo, se han llevado a cabo cultivos del dinoflagelado *Prorocentrum hoffmannianum* (*P. belizeanum* PBMAO1) a gran escala estudiando tanto la producción metabólica intracelular como los metabolitos de excreción. Como resultado se han identificado y determinado las estructuras de metabolitos de alta complejidad sin precedentes en la naturaleza los cuales han mostrado interesantes actividades farmacológicas. En la comunicación oral se comentarán los principales metabolitos aislados, su implicación desde el punto de vista biogénético y la metodología para la resolución estructural.

Key words: Toxinas DSP, Policéticos, *Prorocentrum*

Acknowledgments: CTQ2014-55888-C03-01 (MINECO).

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BIOSÍNTESIS DEL BELIZEANOLIDO, UN MACRÓLIDO CITOTÓXICO DE GRAN TAMAÑO PRODUCIDO POR *Prorocentrum hoffmannianum*,

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Abstract: A lo largo de los últimos 25 años, en el grupo de productos naturales marinos en La Laguna hemos llevado a cabo el estudio químico-estructural de diferentes organismos marinos. En este sentido hemos centrado gran parte de ese trabajo en el estudio del grupo de microalgas tóxicas implicadas en el síndrome diarreico (DSP) con el fin de identificar la naturaleza química de nuevas moléculas bioactivas. Igualmente, hemos llevado a cabo el estudio del origen biosintético de varias toxinas de tipo DSP producidas por dinoflagelados del género *Prorocentrum*, ya sea a partir de la realización de experimentos de incorporación de isótopos estables (¹³C) mediante la adición de precursores enriquecidos o a partir del aislamiento de metabolitos que puedan ser considerados un intermedio en la biogénesis de aquellos que son objeto del estudio principal. En esta comunicación oral se comentarán los principales resultados obtenidos en el estudio biosintético del belizeanolido, a partir de cultivos del dinoflagelado *Prorocentrum hoffmannianum* (*P. belizeanum* PBMAO1). El origen biogénético de este macrociclo, que incluye una lactona de 54 miembros, podría ser de gran interés para comprender la biosíntesis de cadenas carbonadas de gran tamaño por parte de los dinoflagelados marinos.

Key words: Biosíntesis, Policéticos, *Prorocentrum*

Acknowledgments: CTQ2014-55888-C03-01 (MINECO).

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ALEXANDRIUM OSTENFELDII EN LA BAHÍA DE PARACAS

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El género *Alexandrium* incluye especies fitoplanctónicas que producen toxinas y síndromes tóxicos como intoxicación paralizante por mariscos. *Alexandrium ostenfeldii* es considerada una especie mixotrófica de agua fría (Gribble et al., 2005) y puede producir neurotoxinas, tales como toxinas paralizantes de moluscos y espiróolidos (Cembella et al., 2000). Las toxinas paralizantes de moluscos son motivo de preocupación, porque se pueden transferir a través de la cadena alimentaria. El presente estudio tiene por objetivo analizar la variación de las densidades de *Alexandrium ostenfeldii*, en el área Atenas de la bahía de Paracas, región Ica, Perú, para el periodo marzo - mayo 2017.

Se realizó el seguimiento de los resultados de análisis de las muestras tomadas en el área Atenas 006-PAR-01. Para muestras de agua de mar, se determinó cuantitativamente la densidad fitoplanctónica por microscopio invertido según APHA-AWWA-WEF y para muestras de concha de abanico, se determinó los valores de la toxina paralizante de molusco (PSP) por medio del bioensayo en ratón según AOAC 959.08. El 21 de marzo de 2017, se identificó la especie *Alexandrium ostenfeldii* en concentraciones superiores 10^5 cel/L y el 24 del mismo mes se detectó PSP en concha de abanico, con un valor de 1065 $\mu\text{g eq STX 2HCL/Kg}$, resultado superior al límite establecido en normativa nacional (800 $\mu\text{g eq STX 2HCL/Kg}$). Se realizó el seguimiento intensivo de las densidades de las especies precursoras de la toxina paralizante y de los valores de PSP hasta el 3 de mayo 2017, cuando la concentración de *Alexandrium ostenfeldii* disminuyó a 400 cel/L y el valor de PSP a 480 $\mu\text{g eq STX 2HCL/Kg}$. Las altas concentraciones celulares de *Alexandrium ostenfeldii* estuvieron asociadas a temperaturas mayores de 20°C, salinidad de 34.82 ppm y un pH de 8.01.

Key words: *Alexandrium*, toxina paralizante de moluscos, bahía Paracas, densidad,

concha de abanico.

Acknowledgments: Agradecemos al Ing. Rodolfo Vargas por su colaboración en la elaboración de mapas y al personal de los laboratorios de Fitoplancton y Biotoxinas Marinas de SANIPES, por su apoyo en la realización de los ensayos requeridos en este trabajo.

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ATYPICAL OKADAIC ACID DERIVATIVES IN COCKLE

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Abstract: In the transition from mouse bioassay to LC-MS/MS as reference method for the quantification of bivalve toxicity by lipophylic toxins, it was observed that the depuration time for some infaunal species increased substantially in some occasions. It was hypothesized that, in those cases, some compounds not detected by the bioassay but that could yield okadaic acid after alkaline hydrolysis, could be present. A cockle extract for which the toxicity estimate by LC-MS/MS was higher than that by mouse bioassay was subjected to a fractionation by Sephadex LH-20 and each fraction obtained was analysed by LC-MS/MS both, before and after alkaline hydrolysis. Some fractions contained free okadaic acid and 7-O-acyl derivatives as it could be expected. Some other fractions that eluted from the Sephadex column earlier than the 7-O-acyl derivatives, and that consequently should be expected to have a higher molecular weight were also found to yield okadaic acid after alkaline hydrolysis.

Key words: Okadaic acid, Derivatives, Cockle

Acknowledgments: This work has been funded by the Consellería do Mar of the Xunta de Galicia through the research action “Efecto de las biotransformaciones de toxinas de tipo diarreico (DSP) en organismos del intermareal sobre las diferencias de toxicidad estimada por LC-MS/MS y bioensayo de ratón y sus repercusiones para los cierres de explotación (ESTINFA)”

CHALLENGES OF THE ANALYSIS OF EMERGING MARINE BIOTOXINS IN THE EU

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Abstract: The increased distribution of marine biotoxins worldwide is being widely reported and mostly associated to climate change, although other factors should be also taken into account in this global distribution. Ciguatera Fish Poisoning (CFP) is being considered one of the most relevant emerging risk nowadays in the marine biotoxin field in the EU, due to its impact in certain European coastal areas, such as Canary Islands and Madeira, other toxins such as cyclic imines, palytoxins, brevetoxins have been also reported and included among the emergent risks in seafood in the EU. Recent reports on the occurrence of Tetrodotoxin (TTX), a marine biotoxin of bacterial origin and a well known impact in fish from certain areas worldwide, in bivalves from certain coastal areas in the EU, prompted the interest on the evaluation of its occurrence in bivalves from the different production areas in the EU. The characterization of both CFP and TTX risk is being quite challenging, in particular because of the lack or limited amount of reference materials available which also make challenging not only the method development for the risk characterization, but also the evaluation of the risk. This work is focused on providing an update about the work carried out on the risk characterization of both CFP toxins and TTXs in seafood samples from the EU and to accomplish this aim, the activities carried out for the development and optimization of screening methods based on cell assays (N2a) and the further confirmation by LC-MS/MS are going presented. The implementations carried out in the N2a assays used for screening of TTX and CTXs will be discussed, while the results obtained for the optimizations of LC-MS/MS for the confirmation of CFP toxins in fish samples from Madeira and Canary Islands and the evaluation of the occurrence of TTX in bivalves from production areas from Galician Rias, are also going to be presented to accomplish the main objectives of this work.

Key words: TTX, dinoflagelados bentónicos, Macaronesia, ciguatera.

Acknowledgments: Prof. Yasumoto, Dr. Dickey and Dr. Manger for their contribution to the work carried out on the method development for the characterization of Ciguatera fish poisoning (CFP) in Europe, not only by providing pure standards of CTXs, but also providing scientific advice and support. The financial support through EUROCIQUA Project: "Risk Characterization of Ciguatera Fish Poisoning in Europe" GP/EFSA/AFSCO/2015/03, co-funded by the European Food Safety Authority (EFSA). ASMECRUZ Association of mussel

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SOMELIKE IT HOT: DIVERSIDAD DEL GÉNERO *OSTREOPSIS* EN LA REGIÓN MACARONÉSICA

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Resumen: Uno de los objetivos del proyecto INTERREG-MAC “Seguimiento, control y mitigación de proliferaciones de organismos marinos asociadas a perturbaciones humanas y cambio climático en la región Macaronésica-MIMAR” es conocer la biodiversidad de dinoflagelados potencialmente tóxicos presentes en esta región biogeográfica. Para ello se han muestreado 64 localidades en las islas Canarias y Cabo Verde con una periodicidad estacional. Estos muestreos han revelado la diversidad y distribución del género *Ostreopsis* para estas latitudes, hasta ahora sólo representado por *O. ovata*. Este género está ampliamente distribuido por todo el mundo, desde las zonas tropicales hasta las templadas y está formado por 11 especies epífitas o ticoplanctónicas, que crecen asociadas a una gran variedad de sustratos como macroalgas, fanerógamas, rocas y arena entre otros. De las 11 especies descritas, 7 son capaces de producir potentes neurotoxinas del tipo palytoxina que pueden afectar la salud humana.

En este trabajo se citan por primera vez 3 especies de *Ostreopsis* para Canarias y Cabo Verde: *O. lenticularis*, *O. fattorussoi* y *Ostreopsis* sp.; se presenta su distribución en la región Macaronésica y se describen las floraciones registradas durante el año 2017, entre los meses de junio a noviembre, en 5 islas del archipiélago canario y 1 de Cabo Verde. El seguimiento y análisis de los datos climáticos y oceanográficos registrados en este año, sugieren que el aumento de la temperatura del agua de mar y las intrusiones recurrentes de polvo sahariano (calimas) juegan un papel decisivo en el desarrollo y permanencia en el tiempo de estos eventos, que hasta ahora no habían sido registrados en estas latitudes con anterioridad.

Palabras claves: *Ostreopsis*, calentamiento global, Macaronesia

ROLE OF ENVIRONMENTAL CONDITIONS AND PHYSIOLOGY ON THE HARMFUL IMPACTS OF *Ostreopsis cf. ovata* BLOOMS

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Abstract: Temperature increases and man-assisted activities are favouring geographical expansion of tropical and subtropical marine species to higher latitudes. In the Mediterranean, one of the world places receiving most invasive species, the tropical genus *Ostreopsis* is detected, since the late 90s, in many beaches and in some of them it reaches huge abundances. *Ostreopsis cf. ovata* blooms have been related to different impacts on ecosystems and human health and wellbeing including massive mortalities of benthic fauna, mucilages covering benthic seaweeds, foams at surface, and respiratory irritations in people exposed to marine aerosols. However, the relationship between *Ostreopsis* blooms and respiratory irritations in humans is far to be direct because these symptoms are only detected in small periods of time during the proliferation. This paradox could be due to a multiple factors such as meteorological, environmental and *Ostreopsis* physiology. This led us to follow the blooms with an integrative approach, combining ecology, epidemiology and experiments along the bloom period. Surprisingly, first data analyses suggested that wind intensity and direction would play a minor role on the aerosol impacts. Thus, we studied how water motion could affect toxin synthesis. *Ostreopsis* cultures and natural communities from the hot spot were exposed during 1-3 weeks to either to calm or turbulence (generated by an orbital shaker simulating the wave motion). *O. cf. ovata* toxin production was four times higher in the stationary phase than in the exponential one, and populations exposed to turbulent conditions had 30% lower toxin content than the still cultures. The interplay between ecology and epidemiology has been crucial in determining this particular harmful pattern. Future citizen science activities will let us the opportunity to explore the potential respiratory symptoms in humans linked to *Ostreopsis* presence and/or blooms in new areas.

Key words: *Ostreopsis*, respiratory irritations, turbulence, toxins

Acknowledgments: Authors acknowledge the Spanish Government (MINECO) funded project OstreoRisk (CTM2014-53818-R) and the ERA-NET CoCliME project (CSIC Ref. OPE01531) funded by FORMAS (Sweden).



FIRST *OSTREOPSIS* BLOOM DETECTED IN THE BAY OF CASCAIS, PORTUGAL

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Abstract: During late September 2017 samples of macroalgae and surface waters were collected from rocky pools in the bay of Cascais (Western Iberian Peninsula) along a geographical gradient (E-W). The macroalgae communities were characterised by small, mostly finely branched and foliose thalli, forming a diverse cespitose assemblage. Analysis of benthic harmful algal species (BHABs) revealed high concentrations of potentially toxic microalgae of the genus *Ostreopsis* to be present, not only in the benthos but also in the water column. The geographical distribution of samples allowed the identification of a concentration gradient of *Ostreopsis* spp. with a well-defined hotspot (concentrations up to 1.04×10^5 cells g⁻¹ dry weight of macroalgae turf). Several cultures were established from single-cell isolation and molecular identification of the species was achieved using the ITS rDNA region. Although *Ostreopsis* has been previously reported from the bay of Cascais, this is the first time a bloom has been recorded.

Key words: Bay of Cascais, Benthic HAB, Dinoflagellates, *Ostreopsis*, Portugal

Acknowledgments: Fundação para a Ciência e Tecnologia (FCT) for funding through strategic projects UID/MAR/04292/2013 and UID/AMB/50017 - POCI-01-0145-FEDER-007638. H. David also acknowledges FCT funding SFRH/BPD/121365/2016. The authors also acknowledge students from the Biologia Fitoplâncton course for help during sampling.

COLORIMETRIC AND ELECTROCHEMICAL DNA-BASED ASSAYS FOR THE RAPID DETECTION AND QUANTIFICATION OF MICROALGAE OF THE GENERA *OSTREOPSIS*

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Abstract: *Ostreopsis* is a benthic dinoflagellate involved in human allergies and intoxications as well as marine fauna mortalities. Over the last decade, *O. ovata* and *O. siamensis* blooms have been repeatedly reported along the Spanish Mediterranean coast, negatively affecting human health, tourism, fishery and aquaculture. Given that optical microscopy is time consuming and not able to differentiate among *Ostreopsis* species, rapid and accurate species-specific DNA-based assays are highly needed.

In this work, we report the development of two innovative DNA-based assays: a colorimetric assay for *O. ovata* and *O. siamensis* and an electrochemical assay for *O. ovata*. Both approaches involved an isothermal recombinase polymerase amplification (RPA) step using species-specific primers designed within the ribosomal DNA. These primers were elongated with appropriate ssDNA tails for the subsequent colorimetric and electrochemical detection of the RPA amplicon via a sandwich hybridization assay. For the colorimetric assay, the thiolated capture probes were immobilized on maleimide-modified microtitre plates, whereas for the electrochemical assay the thiolated capture probe was immobilized on maleimide-modified magnetic beads, which were subsequently placed on a magnetised electrode. After hybridization of the RPA amplicon, colorimetric and electrochemical detection was achieved via addition of an HRP-reporter probe.

Both systems showed high specificity for the target *Ostreopsis* species and no interferences from the other non-target *Ostreopsis* species. Calibration curves were successfully constructed using genomic DNA. Finally, both assays were applied to naturally contaminated seawater and macrophyte samples from different sites of the Catalan coast. The two developed DNA-based assays are more rapid and specific than traditional microscopy techniques and show strong potential for application in the monitoring and management of harmful *Ostreopsis* blooms.

Key words: *Ostreopsis ovata*, *Ostreopsis siamensis*, RPA, magnetic beads, colorimetric and electrochemical assays

Acknowledgments: The authors acknowledge financial support from the Ministerio de Economía y Competitividad (MINECO) through the BIO2014-56024-C2-2-R project. Anna Toldrà acknowledges scholarship from IRTA-URV-BANCO SANTANDER. We thank Vanessa Castan, María Rey, Josep Fumadó and Maria Curto for the technical help.

PREDICTING *PSEUDO-NITZSCHIA* SPP. BLOOMS IN COASTAL WATERS USING ARTIFICIAL INTELLIGENCE METHODS (GALICIAN RIAS, NW SPAIN)

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Abstract: Phytoplanktonic blooms in the coastal embayments (rias) at the NW part of Spain were mentioned for the first time in 1918 and since then they have been associated numerous times with negatives impacts to a very important economic activity in the area, mussel production. In this study, eight years of *Pseudo-nitzschia* spp. abundance and associated meteorological and oceanographic data were used to develop and validate support vector machine (SVM) and AdaBoost/RandomForest models for the prediction of these diatoms one week in advance. SVM were used to identify presence/below low detection limit, bloom/no bloom conditions of *Pseudo-nitzschia* spp. And SVM and AdaBoost/RandomForest models finally to predict blooms due to these diatoms in the coastal systems of the Galician rias. The best models were selected on the basis of parameters and their performance was evaluated in terms of accuracy and kappa statistics. Regarding the presence/below low detection limit, bloom/no bloom models the best results in the validation dataset were achieved using all the variables: ria code, day of the year, temperature, salinity, upwelling indices and bloom occurrence in previous weeks. In these models the bloom occurrence in previous weeks was identified as a key parameter to the prediction performance. In this paper, toxic *Pseudo-nitzschia* spp. blooms could not be predicted due to limited information on toxin concentration and species composition. Nevertheless, this study demonstrates that the approach followed here is capable for high predictive performance which could be of great aid in the monitoring of algal blooms and offer valuable information to the local shellfish industry.

Key words: *Pseudo-nitzschia* spp. abundance, SVM, AdaBoost/RandomForest models

Acknowledgments: This work was funded by European Project H2020 called CoastObs. We thank the ‘‘Technological Institute for the Control of the Marine Environment of Galicia (INTECMAR)’’, for providing us with the in-situ data.

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MECHANISM OF ORIENTATION IN MARINE PENNATE DIATOMS

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Abstract: Diatoms are an ancestral phytoplankton group playing a fundamental role in the ocean's ecology and global biogeochemical cycles. They form extensive blooms and are responsible of ~40% of the net primary production in the ocean and up to 50% of the organic carbon exported to the ocean interior. Unlike other phytoplankton groups, diatom cells mostly lack motility and therefore experience rapid mass sinking when growth environmental conditions become unfavorable, importantly contributing to the vertical fluxes in the ocean. Diatoms present different morphologies that range from centric to elongated pennate shapes. However, despite their ecological relevance, little is known about their reorientation capacities, a mandatory requisite for fundamental functions such as sexual reproduction in pennate cells. It has been speculated that pennate diatoms could reorient in the water column due to gravity, favoring encounter rates between cells, but field and experimental evidence is weak. In this study, we present field measurements using laser diffractometry revealing that pennate diatoms often exhibit orientations that are far from randomly distributed, increasing their vertical alinement with depth. We also provide a series of laboratory experiments using laser scattering and video-microscopy showing reorientation patterns of *Phaeodactylum tricornutum* cells as they sink. Our results show differences in the reorientation of dead and living cells suggesting some biologically active process maintaining vertical alignment. We provide mechanistic explanation to these patterns depicted from numerical modeling. Our results enlighten a process driving the dynamics of a key group of phytoplankton and the vertical mass transfer in the sea.

Key words: diatoms, pennate, orientation, sedimentation

Acknowledgments: This study is funded by the Ministry of Economy and Competitiveness (MINECO) projects GRADIENTS (CTM2012-39476) and SIFOMED (CTM2017-83774-P).

A NEW *GONYAULAX* SPECIES CAUSING A RED TIDE IN THE TROPICAL MEXICAN PACIFIC, *GONYAULAX UNDISTORTATA* NOV. SP.

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Abstract: Blooms of marine planktonic dinoflagellates are usual in coasts of the Mexican Pacific Ocean, sometimes producing toxic red tides. We report here a red tide event, initially observed from satellite imagery, during an oceanographic cruise, which was produced by high densities of an unidentified species of the thecate dinoflagellate genus *Gonyaulax*. Morphological and phylogenetic studies revealed that the red tide causative organism was, in fact, a new species, here proposed as *Gonyaulax undistortata* nov. sp. The morphology of *Gonyaulax undistortata* was studied by LM and SEM, and showed cells relatively small in size (32-37 μm length, 26-28 μm width), polyhedral in shape, with a short apical protrusion and shoulders in the epitheca, and usually two short, asymmetrical antapical spines. Cells are strongly ornamented with striae and pores, cingulum is nearly median, fairly displaced (1-1.5 the cingulum width), cavozone, and with no (or slight) overhang. Specimens have the typical formula plate of the genus: Po, 4', 0a, 6'', 4-5s, 6c, 5''', 0p, 2'''''. Po is large, conspicuous and well defined, 1' is narrow and elongate, and 4' has a conspicuous ventral pore. The first posterior sulcal plate (1''''') has a poorly developed list and the second one (2''''') is large. *Gonyaulax undistortata* is phylogenetically related to *Gonyaulax spinifera*. The maximum density of *Gonyaulax undistortata* reached 1.74×10^6 cells L^{-1} , and the planktonic flora included other dinoflagellates and diatoms in low numbers. Environmental conditions were: temperature 23° C, salinity 34.7, dissolved Oxygen 10.8 mg L^{-1} , and Chla 14 μg L^{-1} .

Key words: Dinoflagellates, Marine phytoplankton, *Gonyaulax*, Red tides, Tropical Mexican Pacific.

¿INCREMENTAN LAS PROLIFERACIONES ALGALES NOCIVAS? EL CASO DE LA COSTA CATALANA

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Abstract: Se acostumbra a decir que las ocurrencias de Proliferaciones Algales Nocivas (PANs) están aumentando a nivel mundial debido a factores antropogénicos como la eutrofización y el calentamiento global. Otra razón citada que puede explicar esta tendencia sería el incremento de los lugares y la frecuencia de muestreo, por lo que existe cierta incertidumbre sobre el aumento real de estos eventos. Para resolver esta cuestión se requieren estudios de la dinámica de estas especies algales en lugares con un muestreo de frecuencia similar a lo largo de un periodo largo de tiempo.

Utilizando series temporales de fitoplancton de diversas estaciones de la costa catalana (NO del Mediterráneo), analizamos las tendencias de especies potencialmente tóxicas, así como las de algunas variables ambientales. El objetivo fue determinar si las proliferaciones de especies tóxicas aumentaron durante el periodo de estudio en la costa catalana y en caso positivo si el aumento se podría relacionar con la eutrofización y/o un aumento de temperatura.

Mientras las proliferaciones de especies potencialmente productoras de toxicidad PSP, aumentaron en general en muchas de las estaciones estudiadas, debido al aumento de *A. minutum*, las proliferaciones de especies productoras de toxicidad DSP no aumentaron. Se observó también un claro aumento del género *Pseudo-nitzschia* (género con especies potencialmente productoras de toxicidad ASP) en la mayoría de las estaciones.

Los resultados mostraron diferentes dinámicas de los taxones dependiendo la longitud de la serie, la especie y la localidad y, no mostraron ninguna evidencia que el incremento de algunos taxones en algunas localidades fuera debido a un aumento de nutrientes inorgánicos o de temperatura.

Key words: series temporales, Proliferaciones Algales Nocivas, Aguas costeras, NO del Mediterráneo, Eutrofización

Acknowledgments: Los autores quieren agradecer a R. Ventosa y M. Abad los análisis de nutrientes, y a J. Riba y A. Mourello la recolección de muestras. La financiación fue proporcionada por la Agència Catalana de l'Aigua (Departament de Medi Ambient, Generalitat de Catalunya) y el CSIC a través del contrato "Plà de Vigilància de fitoplancton tòxic i nociu a la costa catalana".

25 YEARS MONITORING TOXIC PHYTOPLANKTON AND OCEANOGRAPHY IN GALICIA, NW SPAIN (1992-2018)

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Abstract: The main toxic phytoplankton species in Galicia is *Dinophysis acuminata*. With a 41% presence of the 66625 samples weekly analyzed in the almost 60 oceanographic stations, is the responsible for most of the closures of mussel rafts and natural banks. It blooms in spring, associated with thermohaline stratification of the water column by *in situ* growth, and does another autumn bloom by advection. Almost every year *D. acuminata* appears with *D. acuta* during October downwelling in very fast episodes affecting also the infaunal molluscs. The paralyzing toxins producer dinoflagellate, *Gymnodinium catenatum* is detected almost every year in very low concentrations, but it produces episodes only in warmer years. Its detection follows a temporal pattern from the outside to the interior of the Rias and from south to north of the Galician coast and is associated with a northward surface Portuguese coastal current, before entering the Rias. The startup of *G. catenatum* bloom was, in some years, from the inner part of Pontevedra. The PSP producer *Alexandrium minutum* proliferates in summer in Baiona, Ares and Camariñas related to *in situ* growth during haline stratification and a local resuspension of cysts. *Pseudo-nitzschia* spp. blooms associated with domoic acid accumulation in the mussel rafts are short time duration and they affect the entire coast being the most frequent toxic species *P. australis* and *P. pungens*. Reddish red tides occur almost each year due to *Lingulodinium polyedra* in the Ria of Ares; brownish summer discoloration by *Heterosigma akashiwo* in Pontevedra; the very frequent *Myrionecta rubra* red tides which indicate an imminent proliferation of *Dinophysis* since it is its prey and the bioluminescent waters due to *Noctiluca scintillans*, a voracious predator of toxic plankton.

Key words: *Dinophysis*, *Gymnodinium catenatum*, *Alexandrium*, *Pseudo-nitzschia*

MONITORING OF TOXIC PHYTOPLANKTON AND BIOTOXINS IN SHELLFISH GROWING AREAS OF THE CATALAN COAST. YEARS 2016-2017.

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Abstract: Along the Catalan coast, there has been a decrease in HAB events during the last years. The last important closure due to blooms of *Dinophysis sacculus* in Alfac Bay was in 2012 (García-Altarets et al. 2016). A reduction of closures of shellfish harvesting areas, to some extent, is due to the application of new methodologies for the analysis of toxins (LC-MS for lipophilic toxins), changes in the regulated limits of toxin contents in shellfish and a decrease in the phytoplankton cell abundance for some toxic species. During the years 2016 and 2017 a total of 2608 phytoplankton samples were analysed. In the same period, samples of molluscs and echinoderms were collected for the analysis of toxins: 643 for lipophilic toxins including DSP (Diarrhetic Shellfish Poisoning) toxins, 620 for PSP (Paralytic Shellfish Poisoning) and 421 for ASP (Amnesic Shellfish Poisoning) toxins. Two preventive closures were enforced due to the presence of *Alexandrium minutum* over alert levels in the area of Vilanova. There were no closures due to detection of toxins over regulatory levels. The presence of *Pseudo-nitzschia* in high abundances is frequent in the Ebro delta embayments where blooms of *Pseudo-nitzschia* last over months in the area (Andree et al., 2011) while domoic acid in shellfish has never been detected over regulatory levels. The phytoplankton alert level for *Pseudo-nitzschia* in embayments and enclosed areas was increased 2015 from 200,000 to $2 \cdot 10^6$ cells/L in order to avoid false alerts which were frequent in the past, while in the open shore is maintained in 200,000 cells/L.

Key words: Mediterranean Sea, HABs, Biotoxins, Shellfish growing areas, Catalonia.

Acknowledgments: The authors acknowledge the General Directorate of Fisheries for providing the funds to conduct the monitoring program as well as the support in providing some of the samples. The authors acknowledge the work conducted by the IRTA technical staff.

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SEGUIMIENTO DE FITOPLANCTON TÓXICO Y BIOTOXINAS EN LAS ZONAS DE PRODUCCIÓN DE MOLUSCOS BIVALVOS DE ANDALUCÍA (2011-2017).

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Resumen:

En el trabajo se describen las principales proliferaciones de microalgas tóxicas y episodios detectados en moluscos en el periodo entre 2011 y 2017. La información procede de los análisis que se llevan a cabo dentro del Programa de Control Oficial de las zonas de producción de moluscos bivalvos en Andalucía. Para estudiar la existencia de patrones y tendencias temporales y espaciales se evalúan las series históricas de las principales especies tóxicas que originan cierres (*Dinophysis acuminata*, *Dinophysis acuta*, *Gymnodinium catenatum* y *Pseudo-nitzschia cf australis*) y se introducen especies detectadas en estos años cuyo riesgo se considera potencial y/o emergente. El significativo aumento en la frecuencia de análisis de biotoxinas que tuvo lugar a partir de 2013 para dar cumplimiento a la normativa europea, ha permitido disponer de un importante banco de datos, aumentando el conocimiento en detalle del desarrollo de cada episodio tóxico: inicio, desarrollo y final de proliferaciones, sensibilidad de las distintas especies de moluscos a las toxicidades, entre otros.

Abstract: the abstract should be 300 words at most in one page. The abstract should indicate the subject and scope of the paper and also summarize the conclusion. Structured abstract must be a brief, comprehensive summary of the contents of the scientific work. It allows readers to survey the contents as fast as possible. An abstract summarizes the major aspects of a paper. It should succinctly summarize the purpose of the paper, the methods used, the major results, and conclusions.

Key words: Key word 1, Key word 2, Key word 3 (3 to 5 key words)

Acknowledgments: If authors wish to acknowledge funding bodies and other parties, the acknowledgments may be placed in a separate section at the end of the text, before references.

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TOXIC MICROALGAE FROM THE BASQUE COAST AND ESTUARIES: A 15 YEAR COMPILATION

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Abstract: Observations of toxic eukaryotic microalgae over 15 years from the Basque coast and estuaries are reviewed and contextualized through recent toxin analyses in mussels from a local pilot-scale farm. The IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (the checklist hereafter) has been used as a checklist. Two haptophytes, twelve diatoms, four ochrophytes and twenty four dinoflagellates from the checklist were identified. Some microalgae potentially included in the checklist were not identified to species level (*Azadinium* spp. and *Pfiesteria*-like dinoflagellates). Five *Prymnesium* species isolated from the Basque coast showed haemolytic activity (Seoane et al. 2017) although only *P. parvum* is currently included in the checklist. Additionally, *Phaeocystis globosa* is common in the area. Thirteen species of *Pseudo-nitzschia* were identified: twelve already reported in Orive et al. (2010, 2013), and *P. cf. brasiliiana*. Domoic acid was detected once, at low concentrations. Among ochrophytes, raphidophyceans *Heterosigma akashiwo* and *Fibrocapsa japonica* and dictyochophyceans *Vicicitus globosus* and *Pseudochattonella cf. farcimen* were identified. Only *H. akashiwo* appeared in bloom proportions, although not associated with fish mortalities. Six species of *Alexandrium* were identified, three of which are included in the checklist (*A. ostenfeldii*, *A. pseudogonyaulax*, *A. minutum*). Very low concentrations of PSP toxins were detected in a few samples, but could not be attributed to any species. Yessotoxins levels, which never exceeded legal limits, correlated with *Lingulodinium polyedra*. *Protoceratium reticulatum* and *Gonyaulax spinifera* were also observed. Seven Dinophysiales from the checklist were identified. DSP toxins were found related to *Dinophysis acuminata* and were measured on some occasions exceeding legal limits. Seven Kareniaceae species (four from the

checklist) and *Prorocentrum cordatum* were the rest of the planktonic dinoflagellates that were identified. Among potentially toxic benthic dinoflagellates, three *Prorocentrum* species and *Ostreopsis* cf. *siamensis* were reported (Laza-Martínez et al. 2011) and different *Amphidinium* species are being identified.

Key words: Basque coast, microphytobenthos, phytoplankton, toxic microalgae.

Acknowledgments: Many of the reported observations are related to monitoring programs funded by the Bilbao-Bizkaia Water Consortium (monitoring program of the estuary of Bilbao), the Basque Water Agency URA (Program for the monitoring and assessment of the ecological status of transitional and coastal waters of the Basque Country) through a convention with AZTI-Tecnalia, and the Bizkaia Provincial Council (study of microalgae from selected beaches). Projects IT-1040-16 of the Basque Government and UPV/EHU-PPG17/67 of the University of the Basque Country are also acknowledged. O. Muñiz was funded by a grant from the Basque Government (BOPV núm. 201; 2013/4467), which also supported the projects “IM16SIMMA” and “IM17MUSSELS”.

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DETECTION AND QUANTIFICATION OF TOXIC MICROALGAE KARLODINIUM VENEFICUM AND KARLODINIUM ARMIGER COMBINING ISOTHERMAL DNA AMPLIFICATION AND COLORIMETRIC SANDWICH HYBRIDIZATION ASSAY

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Abstract: *Karlodinium* is a dinoflagellate responsible for fish-killing events worldwide. In Alfacs Bay (NW Mediterranean Sea), the presence of two *Karlodinium* species (*K. veneficum* and *K. armiger*) with different toxicity has been reported. Current toxic microalgae monitoring is performed by optical microscopy, which is time-consuming and hinders rigorous microalgae identification. Therefore, rapid, accurate and *in situ* platforms are highly needed to provide timely warnings.

In this work, the combination of recombinase polymerase amplification (RPA) with enzyme-linked oligonucleotide assay (ELONA) to identify, discriminate and quantify *K. veneficum* and *K. armiger* is presented. For the RPA, a set of primers (two species-specific primers and one genus-specific primer) were designed within the ribosomal DNA and then modified by adding probe-complementary ssDNA tails at the ends. For the ELONA, two distinct thiolated-capture probes were immobilized on maleimide microtitre plates, which specifically hybridize to their corresponding RPA amplicon. Finally, colorimetric detection was achieved via the addition of HRP-reporter probe.

The system was characterized using synthetic DNA and genomic DNA, and the specificity was confirmed by cross-reactivity experiments. Calibration curves were constructed using 10-fold dilutions of cultured cells, attaining a limit of detection of around 50,000 cells/L, much below the *Karlodinium* spp. alert threshold (200,000 cells/L). Finally, the assay was applied to spiked seawater samples, showing a good correlation with the spiking levels and light microscopy counts. This approach is more rapid, specific and user-friendly than tradition microscopy techniques, and shows great promise for the surveillance and management of harmful algal blooms.

Key words: *Karlodinium veneficum*, *Karlodinium armiger*, RPA, ELONA, algal blooms.

Acknowledgments: The authors acknowledge financial support from the Ministerio de Economía y Competitividad (MINECO) through the BIO2014-56024-C2-2-R project. Anna Toldrà acknowledges scholarship from IRTA-URV-BANCO SANTANDER. We thank María Rey, José Luis Costa, Josep Fumadó and Maria Curto for the technical help.

ELECTROCHEMICAL IMMUNOASSAYS AND IMMUNOSENSORS FOR THE DETECTION OF AZASPIRACIDS IN MUSSELS

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Abstract: Given the widespread occurrence of azaspiracids (AZAs), it is clearly necessary to advance in simple and low-cost method for the rapid detection of these marine toxins in order to protect seafood consumers. To address this need, electrochemical immunoassays and immunosensors for the detection of AZAs based on a competitive direct immunoassay using peroxidase-labelled AZA as a tracer are developed (Leonardo et al. 2017, 2018).

Bioaffinity interactions are used to provide a controlled and stable immobilisation of the antibody, either directly on the electrode surface (Leonardo et al., 2018) or using magnetic beads (MBs) as immobilisation supports (Leonardo et al., 2017). The use of MBs as immobilisation supports enables to perform the assay in suspension and improves assay kinetics, allowing the entire assay to be completed in only 15 minutes. On the other hand, sensitivities are slightly improved in the case of immunosensors achieved by the immobilisation of the antibody directly on the transducer surface through protein G or avidin-biotin interactions. The lower immunoreagent concentrations needed and the avoidance of MBs provides economic savings compared to the MB-based immunoassay.

All immunoapproaches enable the detection of all regulated AZAs below the regulatory limit (160 µg AZA-1 equiv./kg) (European Commission 2004), as well as a broad range of other toxic analogues. The immunoassays and immunosensors are applied to the analysis of naturally contaminated mussel samples from the Irish monitoring program and results are compared with those obtained by LC-MS/MS analysis. The excellent correlation obtained with the reference method demonstrates the potential implementation of electrochemical immunoapproaches as powerful screening tools for routine analysis of AZAs in food safety monitoring programs.

Key words: azaspiracid, mussel, immunoassay, electrochemical, magnetic bead.

Acknowledgments: The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no 311820 and under the *Sea Change* strategy (PBA/AF/08/001(01) with the support of the Marine Institute and the Marine Research Sub-Programme of the National Development Plan 2007–2013, co-financed under the European Regional Development Fund. The publication reflects the views only of the author, and the European Union cannot be held responsible for any use that may be made of the information contained therein. The authors acknowledge financial support from CERCA Programme / Generalitat de Catalunya. Sandra Leonardo acknowledges scholarship from IRTA-Universitat Rovira i Virgili-Banco Santander (2013PIPF URV-IRTA-BS-01).

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EVOLUTION OF THE CIGUATERA IN THE CANARY ISLANDS (2011-2017)

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Abstract: Ciguatera fish poisoning (CFP) is the most common non-bacterial food intoxication in the world caused by ingestion of fish with bioaccumulated ciguatoxins (CTXs) (Darius et al., 2013). The origin of CTXs is due to a biotransformation of other protoxins, gambiertoxins, which are produced by a microalga, a benthic dinoflagellate of the genus *Gambierdiscus*. These toxins are bioaccumulated in the food web when toxic dinoflagellates attached to surfaces, for example macroalgae, are consumed by herbivorous fish, which are then preyed on by carnivorous fish (Darius et al., 2013). CFP is characterized by gastrointestinal, neurological, and cardiovascular symptoms. In addition, after the initial or acute illness, neuropsychological symptoms may be reported. Clinical features can vary depending on elapsed time since eating the toxic meal. In the Canary Islands Archipelago since 2008 have been detected cases of ciguatoxin poisoning. In this period, 16 cases have been detected in 4 different islands, affecting a total of 108 people. Since 2011, analyzes have been carried out that show the evolution of the presence of ciguatoxin in fish destined for human consumption (Bravo et al., 2015). Thus we find that since 2011 the numbers of positive fish are increasing from 1 to 19%.

Key words: Ciguatoxin, Canary island, 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).

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FLAGELLATES BIODIVERSITY UNDER LAND FAST ICE IN THE ROSS SEA (ANTARCTICA).

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Abstract: Antarctic pack ice may cover a surface of $2 \cdot 10^7$ km² at its peak extension, represents a large and very dynamic ecosystem. Ice melting plays a pivotal role in microplankton bloom development. Studies conducted in the Ross Sea are mainly focused on the two taxa that dominate this environment: diatoms and prymnesiophytes. However, some microplankton taxa, such as small flagellates (<10 µm) are, in several cases, underestimated because of the difficulties in preserving and identifying them with traditional methods. Here, we focused on their diversity by including not only prymnesiophytes but also chlorophytes and chrysophytes, Formalin fixed samples, collected using a Niskin bottles during austral summers (2015-2016), were observed under light and scanning and transmission electron microscopes. Flagellates were an important fraction of the microplankton community and reached concentrations up to $28 \cdot 10^6$ cell/l and, in some cases, represented the 81% of the total phytoplankton community. Single, colonial, autotrophic, mixotrophic, superficial and deeper species were found under land fast ice in the Ross Sea. The Chlorophyte *Chlamydomonas papilla* was observed during the ice melting at surface and the Chrysophyte *Pentalamina corona* in deeper waters at the same time. The most abundant flagellate species was the Prymnesiophyte *Phaeocystis* cf. *antarctica*, that was observed both in single and colonial forms. This haptophyte co-occurred in high concentrations with the Chrysophyte *Ochromonas* spp. within the water column. The presence and abundances of small flagellate species, which can be very high, vary at different temporal scales and their role within this environment should be studied more in detail.

Key words: Antarctica, Flagellates, Pentalamina, Phaeocystis, Ochromonas.

Acknowledgments: This project was funded by the “Programma Nazionale per la Ricerca Antartica (PRNA)” of Italy.

SENTINEL-3 OBSERVATIONS OF *PSEUDO-NITZSCHIA* BLOOMS IN THE GALICIAN RIAS (NW SPAIN)

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Abstract: A combined analysis of satellite imagery data, measurements of physical and biological parameters and mixed effects modelling was used to study *Pseudo-nitzschia* spp. blooms in the optically complex surface waters of Galician (NW Spain) rias (embayments), sites of extensive mussel culture. A regionally/cluster-specific chlorophyll a (chl-a) algorithm for Ocean and Land Colour Instrument (OLCI) from Sentinel 3 satellite will be applied to a series of images where in-situ data were available. The application of a regional algorithm in combination with measurements of physical and biological parameters allowed us to obtain an accurate mapping of chl-a, detect different clusters of optical types and relate them with the presence of *Pseudo-nitzschia* spp. in the rias. The optimal model for the *Pseudo-nitzschia* spp. abundance and DA concentration suggested the significant effect of some macronutrients as well as other abiotic and biotic parameters, approximating in that way the potential environmental causes and effects of the harmful *Pseudo-nitzschia* spp. blooms in the area. Moreover, regionally specific algorithms for Sentinel-3 (OLCI) and Sentinel-2 (MSI) data were explored as valuable sources of information information about the blooms and their integration to monitoring programs was discussed.

Key words: *Pseudo-nitzschia* spp, Sentinel 3, regionally specific algorithms.

Acknowledgments: This work was funded by European Project H2020 called CoastObs. We thank the “Technological Institute for the Control of the Marine Environment of Galicia (INTECMAR)”, for providing us with the in-situ data.

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CITOTOXICIDAD DE DIFERENTES ESPECIES DEL GÉNERO GAMBIERDISCUS AISLADAS EN LAS ISLAS CANARIAS

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Las microalgas epi bentónicas del género *Gambierdiscus* incluyen especies responsables del síndrome de la ciguatera, una intoxicación alimentaria endémica de algunas zonas tropicales y subtropicales que se adquiere por el consumo de peces contaminados con ciguatoxinas, que se han acumulado a través de las redes tróficas (Parsons et al., 2012).

En los últimos años la tropicalización producida por el cambio climático está provocando una posible distribución de *Gambierdiscus* hacia latitudes más altas pudiendo constituir un problema emergente en el Archipiélago Canario donde ya se han registrado más de 100 casos de ciguatera en la última década (Rodríguez et al., 2017; Bravo et al., 2015).

El grupo de Microalgas Nocivas del IEO de Vigo estudia estas microalgas en el marco del Proyecto CIGUATROP con el objetivo de caracterizar su hábitat, la diversidad de especies y el potencial tóxico de las mismas, aspectos esenciales para valorar el impacto del cambio climático en una posible expansión del riesgo de Ciguatera en las costas españolas. El IRTA participa en el marco del proyecto EUROCIQUA.

En estudios previos encontramos diferencias en la potencia tóxica de las distintas especies de *Gambierdiscus* identificadas en Canarias (Fraga et al., 2011; Pisapia et al., 2017). Los resultados que se presentan aquí son fruto de la continuación de estos estudios.

Se han cultivado varias cepas de cada una de las especies de *Gambierdiscus* identificadas hasta la fecha en las Islas Canarias (*G. australes*, *G. caribaeus*, *G. carolinianus*, *G. excentricus* y *G. silvae*) con el fin de estimar su toxicidad y la variabilidad intra y/o interespecifica mediante ensayos celulares de Neuroblastoma.

Palabras Clave: Ciguatoxinas, *Gambierdiscus*, tropicalización, toxicidad, neuroblastoma.

Agradecimientos: Proyecto CCVIEO y Fundación Biodiversidad por la financiación del proyecto CIGUATROP. Agradecemos igualmente a la European Food Safety Authority (EFSA) a través del proyecto EUROCIQUA (GP/EFSA/AFSCO/2015/03). Este trabajo es una contribución a la Unidad Asociada CSIC-IEO y del IRTA.

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EVALUACIÓN DE LAS IMINAS CÍCLICAS EN MOLUSCOS DEL DELTA DEL EBRO Y CARACTERIZACIÓN DEL RIESGO DE INTOXICACIÓN

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Abstract: Las iminas cíclicas (ICs) constituyen un grupo recientemente descubierto de biotoxinas marinas que actúan sobre los receptores neurales y que se bioacumulan en los mariscos. En 2010, la European Food Safety Authority (EFSA) evaluó los riesgos para la salud humana relacionados con el consumo de espirólios (SPXs), giminodiminas (GYMs), pinnatoxinas (PnTXs) y pteriatoxinas (PtTXs) en mariscos. Estas toxinas son compuestos macrocíclicos y se agrupan debido al funcionamiento del grupo imino como su núcleo farmacóforo común, responsable de la neurotoxicidad aguda en ratones. Las iminas cíclicas no se han relacionado aún con intoxicaciones humanas y no están reguladas en Europa, aunque la EFSA requiere más datos para realizar una evaluación de riesgo concluyente para los consumidores.

En este trabajo, se evaluaron varias muestras de moluscos durante el 2015 y 2016 para identificar las ICs emergentes como la gymnodimina A (GYM A), 13-desmetil espirólido-C (SPX-1) y pinnatoxina G (PnTX-G) en las zonas de mayor producción de la costa Catalana, que corresponden a la bahía de Alfacs y la bahía del Fangar situadas en el Delta del Ebro.

Como resultado del muestreo se confirmó la presencia de ICs por LC-MS/MS. El 2015 se analizaron 394 muestras donde se detectaron ICs en concentraciones superiores al LOQ en porcentajes de 0.3%, 1% y 18.8% para SPX-1, GYM-A y PnTX-G, respectivamente. Durante el 2016 se analizaron 344 muestras de moluscos bivalvos encontrando ICs en porcentajes de 13% (> LOQ) y 10% (nivel de trazas) para PnTX-G, 22% trazas de SPX-1 y 12% trazas de GYM-A. Para realizar una correcta caracterización del riesgo de intoxicación por ICs y de acuerdo con las estadísticas de consumo de marisco publicado por el Ministerio de Agricultura, alimentación y medio ambiente del 2015 y 2016, se realizó un cálculo aproximado de las cantidades de ICs consumidas durante el 2015 y 2016.

Key words: Iminas cíclicas, cromatografía, espectrometría de masas, moluscos.

Acknowledgments: Los autores agradecen el soporte económico al Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) que financió el proyecto PROMAQUA (RTA2013-00096-00-00). También se agradece el apoyo del programa CERCA de la Generalitat de Catalunya.

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SCRIPPSIELLA ACUMINATA VERSUS SCRIPPSIELLA RAMONII: A CELL AND LIFE CYCLE COMPARISON

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Abstract: *Scrippsiella* is a cosmopolitan dinoflagellate genus known by their ability to form Harmful Algal Blooms (HABs) in coastal waters (Hallegraeff, 1992, Wang et al., 2007). The large physiological, morphological and genetic variability observed in this genus suggests the existence of cryptic species (Montresor et al., 2003).

In this study, flow cytometric analyses were carried out to compare two species of this complex genus, *S. ramonii* and *S. acuminata*. In addition, LSUrDNA sequencing and HPLC pigment analyses were performed in both species

Cell cycle light-controlled dissimilarities were observed, the S phase was detected during the light period, though at different hours between species, whereas the G2/M phase occurs during the light in *S. ramonii* and during dark conditions in *S. acuminata*. The detection of 4C stages, planozygotes and resting cysts in the unclonal *S. ramonii* strain, undoubtedly point out to the existence of sexuality. A sexual process was not found in *S. acuminata* strain likely due to have used a clonal strain, suggesting, for this species, a hetherothallic trait. Differences in genome size, pigment profiles and lipid content were also reported.

Key words: *Scrippsiella*; cell cycle; life cycle; flow cytometry; genome size

Acknowledgments: we thank Pilar Rial and Isabel Ramilo for technical support. This study was funded by the CCVIEO project.

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OPTIMIZATION OF LABORATORY CULTURES OF TWO *DINOPHYSIS* SPECIES

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Abstract: The main objective of the MARBioFEED project is the mass cultivation of microalgae to obtain bioactive compounds with enhanced biorefining methods. Diarrhetic shellfish poisoning (DSP) toxins are produced by some benthic species of *Prorocentrum*, but mainly by mixotrophic species of *Dinophysis* which also produce pectenotoxins (PTX). Although PTXs have a wide spectrum of applications, their supply was hindered due to our inability to grow *Dinophysis* in laboratory cultures. The first successful culture of *D. acuminata* was established using the ciliate *Mesodinium rubrum* as prey, which in turn feeds on cryptophytes (Park et al., 2006). However, conditions for mass culturing of *D. acuminata* and *D. acuta* are not optimized yet. Small scale experiments performed to compare survival of prey-limited *D. acuminata* and *D. acuta* grown with different (*L-Si*, *f2-Si* and *K-Si*) culture media, showed best results were obtained with *K-Si* medium (Keller et al., 1987), especially with *D. acuminata*, where a 6 fold yield was obtained compared with the other media. Then, *K-Si* medium was selected to produce larger volumes (4L) of the two species. Maximal growth rates reached were 0.33 d⁻¹ by *D. acuminata* and 0.26 d⁻¹ by *D. acuta*. Next, four cryptophytes species (*Teleaulax amphioxeia*, *T. minuta*, *T. gracilis* and *Plagioselmis prolunga*) were given to *Mesodinium* grown with *K-Si* medium, to identify the optimal prey for the ciliate. Maximal ciliate yield (32.34 x 10³ cells mL⁻¹) was obtained using *T. amphioxeia* while the highest growth rate was attained with *T. minuta* (0.22 d⁻¹). Establishment of optimal conditions for small-volume cultures of *D. acuminata* and *D. acuta* is a key step before scaling up to large volumes in photobioreactors.

Key words: *Dinophysis*, *Mesodinium*, Pectenotoxin, culture conditions, photobioreactor

Acknowledgments: This research was seconded by EU-ERANET Biotechnology (MARBioFEED project) and funded by Spanish MINEICO Programme for International Cooperation (PCIN-2015-252).

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RAPID AND NOVEL DETECTION OF TETRODOTOXIN BY HPLC-MS/MS

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Abstract: Tetrodotoxin (TTX) is one of the most potent neurotoxins and it was initially expected to occur only in puffer fish. Recently, TTX has been found in northern European bivalves, therefore, it is important to analyze this emergent compound in shellfish matrices. A procedure has been developed for the identification and determination of TTX and its analogue 4,9-anhydro tetrodotoxin (4,9-anhTTX) by liquid chromatography coupled to mass spectrometry (LC-MS/MS) in four shellfish matrices: mussel (*Mytillus galloprovincialis*), clam (*Ruditapes decussatus*), scallop (*Pecten maximus*) and oyster (*Ostrae edulis*). The clean-up procedure used in this work allowed us to obtain good results regarding both identification and quantification of TTXs, as well as for the rest of the validation parameters of the method.

Key words: Tetrodotoxin, emergent toxins, shellfish, LC-MS/MS

Acknowledgments: The research leading to these results has received funding from the following FEDER cofunded-grants. From CDTI and Technological Funds, supported by Ministerio de Economía, Industria y Competitividad, AGL2014-58210-R, AGL2016-78728-R (AEI/FEDER, UE), ISCIII/PI16/01830 and RTC-2016-5507-2. From CDTI under ISIP Programme, Spain, IDI-20130304 APTAFOOD and under the FEDER ININTERCONNECTA Programme 2016, ITC-20161072. From European Union POCTEP 0161-Nanoeaters -1-E-1, and Interreg Atlantic Area Transnational Cooperation Programme 2014-2020: EAPA_317/2016, ALERTOX-NET, and by ENISA funding SME 2016.

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