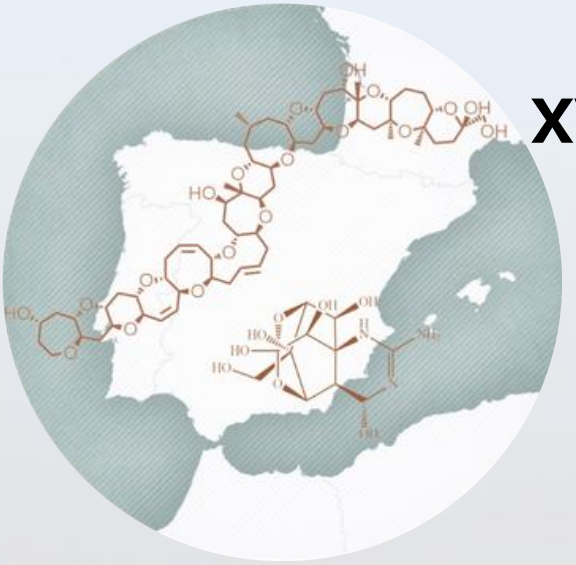

XVI Reunião Ibérica sobre Microalgas Nocivas e Biotoxinas Marinhas

XVI Reunión Ibérica sobre Microalgas Nocivas y Biotoxinas Mariñas

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LIVRO DE RESUMOS

Libro de Resúmenes

XVI Reunião Ibérica sobre Microalgas Nocivas e Biotoxinas Marinhas

CCMAR – Universidade do Algarve

Faro, 27 a 29 de Maio 2026

Organização

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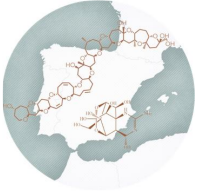

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Notas de Boas Vindas

Bem-vindos,

à XVI Reunião Ibérica sobre Microalgas Nocivas e Biotoxinas Marinhas. Há mais de 30 anos que investigadores portugueses e espanhóis se reúnem, de dois em dois anos, com o objetivo de debater a problemática da proliferação (blooms) de microalgas nocivas e os seus impactos ambientais e socioeconómicos, em particular nos setores da aquacultura, das pescas e do turismo costeiro. Ao longo das últimas décadas, estes encontros têm contribuído para a partilha de conhecimento científico, o fortalecimento das relações e colaborações entre investigadores dos dois países e a adoção concertada de boas práticas na monitorização e gestão destes fenómenos a nível ibérico.

Num contexto marcado fenómenos climatéricos extremos, por uma cada vez mais rápida evolução tecnológica, e por um período de instabilidade e conflito a nível global, emergem cada vez mais novos desafios e mais necessidade de aprofundar o conhecimento nesta área e de reforçar o apoio a produção. Sejam estes desafios tanto no estudo das componentes tradicionais, como na abordagem de toxinas e compostos emergentes. Torna-se, assim, cada vez mais importante promover encontros científicos desta natureza, onde é privilegiando o debate, a inovação e a cooperação, de modo a estreitar os laços entre as comunidades científicas ibéricas e de promover a colaboração entre investigadores.

Pela primeira vez, a Reunião Ibérica realiza-se em Faro, no Algarve, em plena Ria Formosa, uma das principais zonas de produção de bivalves em Portugal. O evento terá lugar nas instalações do CCMAR, na Universidade do Algarve, entre os dias 27 e 29 de maio de 2026, contando com cerca de 130 participantes. Conta com 64 comunicações (42 orais e 22 pósters), organizadas em cinco sessões temáticas: i) Alterações climáticas e tendências globais; ii) Novas toxinas e toxinas emergentes; iii) Impactos das microalgas tóxicas e biotoxinas; iv) Aplicações biotecnológicas de biotoxinas; v) Monitorização, mitigação e apoio à decisão.

A todos os participantes, desejamos uma excelente reunião que corresponda às vossas expectativas e promova o intercâmbio de conhecimentos.

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Financiamento

A XVI Reunião Ibérica sobre Microalgas Nocivas e Biotoxinas Marinhas realiza-se no âmbito científico de cinco projectos:

PREHAB – Predator-Prey Chemical Warfare and the Forecasting of Harmful Algal Blooms (LCF/BQ/PR23/11980049), financiado pela Fundação "la Caixa" (ID 100010434), através de uma Bolsa Junior Leader Retaining Fellowship.

PROCOAST – Producers & Toxicity Assessment of Tetrodotoxin and analogues on the Portuguese Coast (2024.04866.RESTART), financiado pela Fundação para a Ciência e Tecnologia, através do Programa RESTART – 2ª edição.

EarlyTOX – Rapid molecular strategies for early detection of toxic microalgae in bivalve production (Refª 2024.17161.PEX) financiado por fundos nacionais através da FCT – Fundação para a Ciência e a Tecnologia, I.P.

MultiALIVE – Multi observation tools for the detection of harmful algal blooms and forecasting shellfish contamination foi apoiado pelos(s) Algarve 2030, Lisboa 2030 pelo Portugal 2030 e pela União Europeia. Os Fundos Europeus Mais Próximos de Si.

Missão Interface. Este trabalho foi desenvolvido com financiamento da Missão Interface, projeto cofinanciado pelo PRR – Plano de Recuperação e Resiliência pela União Europeia (código de operação 01/C05-i02/2022.P148).

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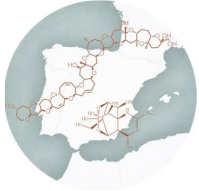

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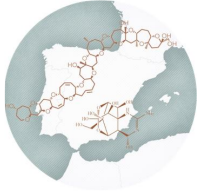

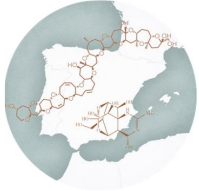

Sessão

Novas toxinas e toxinas emergentes

Apresentações Orais

Tetrodotoxinas no Algarve: distribuição temporal e potenciais vectores

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Resumo

A tetrodotoxina (TTX) é uma neurotoxina potente, identificada pela primeira vez em peixes da família Tetraodontidae, mas também detectada em invertebrados marinhos. Um episódio de intoxicação humana após o consumo de uma buzina *Charonia lampas*, provavelmente apanhada na costa sul portuguesa (Algarve), juntamente com o aumento de relatos da detecção de TTX em bivalves de águas europeias, levou a Autoridade Europeia para a Segurança dos Alimentos (EFSA) a recomendar limites máximos seguros para o consumo humano de marisco. No entanto, a TTX ainda não é monitorizada nem regulada a nível europeu.

Esta apresentação resume estudos recentes realizados no CCMAR destinados a avaliar potenciais vetores, e variabilidade sazonal da TTX e dos seus análogos (TTXs) em organismos marinhos do Algarve. As TTX e os seus análogos foram analisados por cromatografia líquida acoplada à espectrometria de massa de alta resolução (LC–HRMS) em buzinas, estrelas-do-mar (*Astropecten aranciacus*), e caranguejos intertidais (*Afruca tangeri* e *Carcinus maenas*).

A buzina representa um risco potencial para a saúde pública devido à sua capacidade de acumular TTXs em elevadas concentrações. Embora estas toxinas se concentrem principalmente nas visceras, níveis inseguros podem ser transferidos para partes comestíveis, o que enfatiza a necessidade de regulamentação europeia para monitorizar a TTXs em produtos do mar e de informar os consumidores sobre práticas seguras de preparação. A origem da TTXs nas buzinas permanece incerta, mas a deteção de TTXs nas sua principal presa, a estrela-do-mar *A. aranciacus*, apoia uma via de aquisição exógena através da teia alimentar. Além disso, a ocorrência de análogos de TTX em espécies de caranguejo sugere a presença de múltiplos vetores potenciais ao longo da costa do Algarve.

Agradecimentos

Este trabalho foi apoiado pelo programa de investigação e inovação Horizonte 2020 da União Europeia através de uma Bolsa Individual Widening Marie Skłodowska-Curie (n.º 101003376), pela Fundação la Caixa (ID 100010434) através de uma Bolsa Junior Leader Retaining (LCF/BQ/PR23/11980049), e por fundos nacionais portugueses da FCT – Fundação para a Ciência e a Tecnologia através dos projetos UIDB/04326/2020, UIDP/04326/2020 e LA/P/0101/2020; pelos programas operacionais CRESC Algarve 2020 e COMPETE 2020 através do projeto EMBRC.PT ALG-01-0145-FEDER-022121.

Tetrodotoxin trail: how prey composition and gut microbiome predicts contamination in *Charonia lampas*

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Abstract

The trumpet shell, *Charonia lampas,* is a predatory gastropod commonly found in the Western Mediterranean and the northeast Atlantic. In 2007, the first report of food poisoning was linked to the ingestion of *C. lampas* contaminated with tetrodotoxin (TTX), a powerful neurotoxin. Recently, we documented a seasonal prevalence of TTX in *C. lampas* collected from the southern Portuguese coast and identified the sea star *Astropecten aranciacus* as a potential TTX source. In this study, we aim to determine whether the diets and gut microbiome of *C. lampas* individuals with high versus low TTX levels differ, testing the hypothesis that TTX is transferred through the food web via contaminated prey. We performed metabarcoding using the broad metazoa marker COI, with the primers mlCOIintF/jgHCO2198 to analyze the diet of *C. lampas,* and the primers 341F/806R targeting the V3V4 region of the 16S rRNA for microbiome analysis. By examining 24 trumpet shell specimens, we found that *C. lampas* has a diverse diet, mainly feeding on the phyla Echinodermata, Mollusca, and Cnidaria. Our results also suggest that TTX-contaminated trumpet shells have a broader diet. Specimens with higher TTX levels ingested taxa such as *Mytilus, Antedonidae, Epizoanthus,* and *Astropecten.* Finally, no significant effect on the trumpet shell microbiome composition upon TTX contamination was observed. These findings contribute to a better understanding of how diet influences TTX presence in *C. lampas* and its implications for human food safety.

Acknowledgements

This project has received funding from the "la Caixa" Foundation (ID 100010434) through a Junior Leader Retaining Fellowship (LCF/BQ/PR23/11980049), and Portuguese national funds from FCT—Foundation for Science and Technology through projects 2024.04866.RESTART, UIDB/04326/2020, UIDP/04326/2020, LA/P/0101/2020 and the operational programs CRESC Algarve 2020; COMPETE 2020 through project EMBRC.PT ALG-01-0145-FEDER-022121.

Yessotoxins and its potential producers on the Basque Coast (southeastern Bay of Biscay) assessed by bioassays and field data

Aitor Laza-Martínez[1,2]*, Héctor Ibáñez[1,2], Oihana Solaun[3], Pilar Riobó[4], Helena David[5], Ivan Patrick Tualla[2], Beatriz Paz[6], Mónica Cid[7], Marta Revilla[3]

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Introduction

Monitoring of phycotoxins and toxic phytoplankton in a bivalve aquaculture area off the Basque coast (SE Bay of Biscay) has revealed recurrent poisoning events. This study focuses on yessotoxins (YTXs), assessing their occurrence, environmental drivers, and potential producers.

Materials and Methods

YTXs regulated in the European Union were analysed in mussels by LC-MS/MS, from 2016 to 2023. In June 2022 seven strains of *Protoceratium reticulatum* and two of *Lingulodinium polyedra* were isolated and later analysed for YTXs production. Growth and YTX production in four *P. reticulatum* strains were tested at three temperatures (15, 20, 25 °C). Finally, one strain of each species was exposed to caged and non-caged *Acartia tonsa* copepods to test whether predator presence induces toxin production.

Results and discussion

Yessotoxin (YTX) was the most frequent (54 % of samples) and abundant analogue and its oxidation product 45-hydroxy-YTX could be quantified in 37 % of the samples. Homo-YTX and its oxidation product 45-hydroxyhomo-YTX were always below their limit of quantification. During most of the study period, YTXs concentration was below the regulatory limit (3.75 mg YTX eq. kg^{-1}). However, higher peaks were found from May to October 2021 (4-6 mg YTX eq. kg^{-1}). Observed potential YTXs producers were *L. polyedra* and *P. reticulatum*. Low concentrations of *Gonyaulax* cf. *spinifera* were also found throughout the year. GAM models showed that YTXs peaked in June and July, while the maxima of the three taxa occurred between late spring and early summer. Molecular identification (rDNA regions of partial LSU and ITS) of cultured strains showed that *P. reticulatum* strains shared identical ITS sequences with Mediterranean strains in clade B (*sensu* Wang et al. 2019, *Harmful Algae,* 88: 101610). *L. polyedra* strains did not produce detectable amounts of toxins and *P. reticulatum* strains produced YTX but no Homo-YTX. Temperature response was strain-specific in *P. reticulatum*, but all showed highest growth rates

at 25 °C (0.40–0.33 d^{-1}), in contrast to the response reported by other strains from the clade B. Toxin production response was strain specific, with two strains producing more extracellular toxins at 25 °C. *L. polyedra* exposed to *A. tonsa* did not produce YTXs, whereas *P. reticulatum* produced more extracellular toxins when in direct contact with copepods.

These results suggest that clade B of *P. reticulatum* is the most probable source of YTXs on the Basque coast. On the other hand, Basque strains of this species showed warmer temperature adaptations compared to other temperate strains, and were phylogenetically closer to Mediterranean strains than to Atlantic strains.

Acknowledgements

This work was supported by the Dpt. of Education of the Basque Government through the Consolidated Research Groups (GIC21/82), the project PES20/29 of UPV/EHU, and the Fisheries and Aquaculture Directorate, Dpt. of Economic Development, Sustainability and Environment of the Basque Government, through several projects.

Toxin profile characterisation of *Gambierdiscus* strains from five different species

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Introduction

Gambierdiscus spp. are marine benthic dinoflagellates that can produce cyclic polyether neurotoxins such as ciguatoxins (CTXs), maitotoxins (MTXs), gambierones (GAMBs), gambieric acids (GAs), gambierol and gambieroxide. These toxins are widely studied in relation to their potential involvement on ciguatera poisoning.

The aim of this study was to characterise MTXs, GAMBs, GAs and CTXs in 13 *Gambierdiscus* strains from the Canary Islands (Spain), Cuba and Indonesia.

Materials and Methods:

Strains from *G. excentricus, G. australes, G. carolinianus, G. balechii* and *G. silvae* were isolated from macrophytes and maintained in the CCVIEO culture collection. Strains were grown in K/2 medium at 25 °C and approx. 75 µmol photons m^{-2} s^{-1} until abundances of 0.33-1.2×10^6 cells·L^{-1} were reached.

Cell pellets were resuspended in LC-MS grade MeOH and broken with a Biospec products bead beater. MeOH extracts were centrifuged and filtered. Toxins were analysed by LC-MS/MS (UPLC-Xevo TQXS) and HRMS (Orbitrap IDX and Revident LC-QTOF G6575A).

Results:

Gambierone was quantified in the 3 *G. silvae* strains and in *G. balechii*. 44-methylgambierone was quantified in all the *G. australes* and in a *G. silvae* strain. MTX5 and gambieric acids C and D were confirmed in all the *G. australes* strains.

MTX4 and sulfogambierone were confirmed in both *G. excentricus* strains, while dihydrosulfogambierone was only confirmed in one of them. CTX1B, CTX3C, CTX4A, C-CTX1, I-CTXs (among others), were not detected in any of the analysed strains.

Discussion and Conclusion:

Toxin profiles from the studied strains are species specific. Similarly to *G. australes* from the Mediterranean Sea, *G. australes* strains from the Canary Islands produce 44-methylgambierone, MTX5, and gambieric acids C and D.

The toxic profile of *G. excentricus* strain VGO-1473 from the Canary Islands is similar to that of the previously studied strain VGO-791, from the same origin.

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Mechanisms involved in the accumulation of okadaic acid and domoic acid in bivalve mollusks.

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Abstract

Accumulation of toxins is the product of the balance between uptake and elimination. Understanding the ways in which these processes take place is important to minimize it. However, there is little information about the mechanisms involved. We review the existing information about those mechanisms. In the case of okadaic acid, entry into the digestive gland cells occurs through simple diffusion, and once in the cytosol, it enters high-density lipoproteins, which allow its transport to the endoplasmic reticulum, where acetyl-Coenzyme A acyltransferase 2 (ACAT2) or an equivalent enzyme esterifies it with fatty acids. Once esterified, OA is excreted in the faeces by a mechanism that remains unknown, although xenobiotic resistance proteins may be involved. Additionally, there is a minor alternative vesicular transport route. In the case of domoic acid (OA), entry into the cells occurs via a membrane transporter and, once inside the cells, it mainly exists in two forms: free in the cytosol and associated with autophagosomes or organelles structurally very similar. No substantial transformations of this toxin occur. The mechanisms involved in excretion or retention are not clear, with currently two possibilities being considered. The first is mediation by membrane transporters, with candidates being transporters from the Solute Carrier (SLC) family. The second is vesicular transport via autophagosomes, although the potential role of these structures in either storing domoic acid or facilitating its removal is currently being debated.

Presencia de un isómero no identificado del ácido okadaico en los episodios de DSP de Galicia: 2014-2025.

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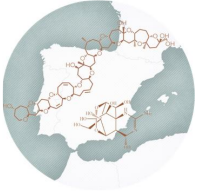
Resumen

Un isómero del ácido okadaico (AO) ha sido detectado en la mayor parte de las muestras de episodios de toxinas del grupo AO que han tenido lugar en Galicia desde 2014, año a partir del cual los análisis de estas toxinas se vienen realizando mediante LC-MS/MS. Este isómero se detecta en diferentes moluscos bivalvos (mejillones, almejas, berberechos...) desde las rías de la costa cantábrica de Galicia hasta Baiona, en la costa atlántica. Las concentraciones del isómero están relacionadas con las del AO, pero no con las de DTX2. Los niveles que se detectan son sustancialmente inferiores a los del AO (entre 1/5 y 1/50). Se detecta tanto en los episodios de primavera-verano, cuando sólo está presente el AO, como en los episodios de otoño-invierno, cuando están presentes AO y DTX2. Su tiempo de elución es posterior al de DTX2 y coincidente con el de un isómero catalogado cómo desconocido en el material de referencia certificado CRM-DSP-Mus-c y podría ser uno de los isómeros descritos en mejillón y/o fitoplancton en Irlanda. Una pequeña proporción, en el mejillón, se encuentra en forma conjugada (probablemente esterificada) ya que su concentración en muestras sometidas a hidrólisis alcalina es ligeramente superior a la de las muestras sin hidrolizar.



Toxins produced by *Ostreopsis* in the South-East Spanish coasts

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Introduction

The communities of benthic dinoflagellates in the South-East coast of Spain have been largely understudied, even though there is at least one epidemiological report indicating that blooms of *Ostreopsis* pose a risk to the local population and beachgoers.

Materials and Methods

In this work, we have analysed by LC-MS/MS and HRMS^n more than 200 field samples of benthic dinoflagellates sampled from macroalgae. The field samples were collected in 15 beaches along the coasts of Murcia and Almería during 12 months (October 2024 to September 2025), plus in 5 locations in the marine reserve "Cabo Tiñoso" during the summer of 2025. Moreover, we isolated and cultured 7 strains of *Ostreopsis* cf. *ovata* from the study area and characterized its toxin profile.

Results:

We confirmed and quantified the presence of ovatoxins -a to -g and isobaric palytoxin in field samples and in cultures, being the main toxin profile similar to the most reported Mediterranean toxin profile. In field samples, we could quantify ovatoxins (LOQ 7 ng/mL) in 49% of the samples collected, and in the summer months (June to September), 62% of samples contained ovatoxins. Toxin content varied significantly among beaches and reached concentrations over 1400 ng/g macrophyte. In cultures of *O.* cf. *ovata*, toxin profiles varied among strain, including strains with all ovatoxins, and strains only with ovatoxins with 129 carbons (ovatoxins -a, -d, -e and -g). Interestingly, one strain did not produce any ovatoxins, which is very uncommon among Mediterranean strains of *O.* cf. *ovata*.

Discussion and Conclusion:

According to these results, we identified the beaches with higher concentrations of ovatoxins (Las Palmeras, Calarreona y La Higuerica) and recommend future monitoring strategies to protect public health.

Acknowledgements:

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Presence of *Gambierdiscus* in Mediterranean Islands close to the Iberian Peninsula and toxin profile characterisation on cultures

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Introduction

Little is known about the diversity, distribution, and toxicity of *Gambierdiscus spp.* in the western Mediterranean Sea. The presence of CTX-producing species may be indicative of the risk of ciguatera poisoning. Fukuyoa paulensis had been reported in 2011 in Formentera and *Gambierdiscus* was identified in the Balearic Islands in a sample from 2016. *G. australes* and *F. paulensis* have been present in Formentera, Ibiza, Mallorca, and Menorca, and *G. excentricus* and *G. carolinianus* have been detected in Mallorca and Menorca. This study aims to assess the presence of *Gambierdiscus* in Mediterranean islands near the Iberian Peninsula and identify the species involved, given their varying toxicity.

Materials and Methods

Eight sampling stations on the western Mediterranean Sea, along the Iberian Peninsula shore were sampled from north to south: Illa de la Maça d'Oros, Illa Massina, Illes Medes, Illes Formigues, Illes Columbretes, Tabarca, Illa Grosa and Islas Hormigas in October 2024 and October 2025. The most predominant macrophytes at 0-3 m depth were sampled by snorkelling. Field samples were analysed by high-throughput sequencing (HTS) metabarcoding. Single *Gambierdiscus* and *Fukuyoa* cells were isolated under an inverted microscope and some isolates of *Gambierdiscus* were selected to evaluate their toxin profile by LC-MS/MS and LC-HRMS.

Results, Discussion and Conclusion

HTS metabarcoding results confirmed the first detection of *G. australes* in Columbretes (Castelló), Tabarca (Valencia) and Illa Grosa (Murcia) from the eight Mediterranean Islands close to the Iberian Peninsula studied. Several *Gambierdiscus* strains from the same three locations were successfully established at IRTA. Their toxin profile revealed the presence of 44-methylgambierone, gambieric acid C, gambieric acid D and maitotoxin 5, consistent with profiles previously reported for *G. australes* from the Balearic Islands.

Acknowledgements

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Iminas cíclicas na costa portuguesa: primeira deteção de pinatoxinas em bivalves portugueses

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Resumo

As iminas cíclicas são compostos tóxicos produzidos naturalmente por dinoflagelados marinhos. No diverso grupo das ICs, incluem - se famílias de compostos como as pinatoxinas e os espirolídeos, toxinas produzidas por dinoflagelados das espécies *Vulcanodinium rugosum* e *Alexandrium ostenfeldii*, respetivamente. Estas potentes neurotoxinas atuam como antagonistas dos recetores nicotínicos e muscarínicos da acetilcolina, e são conhecidas como "toxinas de ação rápida".

A ocorrência abundante de microalgas produtoras de ICs pode levar à contaminação dos moluscos bivalves, que por sua vez poderão causar intoxicação quando consumidos por humanos. Bivalves contaminados com pinatoxinas já foram detetados na Europa desde 2009, mais recentemente na Galiza em 2020. Os espirolídeos foram detetados pela primeira vez em Portugal em 2013 na costa continental em bivalves como o mexilhão (*Mytilus spp.*), e ainda em algumas espécies de gastrópodes marinhos e equinodermes. Na Ilha da Madeira foram detetados em 2020 em duas espécies de estrela do mar.

De modo a avaliar a situação em Portugal, foram analisadas amostras de bivalves de duas zonas estuarino-lagunares, a Ria de Aveiro e a Lagoa de Albufeira em Setúbal, recorrendo à cromatografia líquida com deteção por espectrometria de massa em tandem (LC-MS/MS).

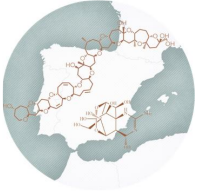
Os resultados, relativos ao período entre Junho e Dezembro de 2024, demonstram que na Lagoa de Albufeira a contaminação do mexilhão (*Mytilus spp.*) por pinatoxinas é constante, com níveis mais altos nos meses de verão. Na Ria de Aveiro o panorama é semelhante, mas nesta zona a contaminação do mexilhão e berbigão (*Cerastoderma edule*) é devida a espirolídeos. Estes resultados apontam para uma contaminação frequente por ICs na costa portuguesa e sugerem a presença de *V. rugosum* e *A. ostenfeldii* em Portugal, o que ainda não está reportado.

Blooms, ou proliferações de microalgas produtoras de toxinas, são fenómenos cada vez mais frequentes e com maior distribuição geográfica. O aquecimento das águas juntamente com o excesso de nutrietes proveniente de efluentes agrícolas favorecem a eutrofização dos ambientes marinhos, sendo os ambientes estuarinos mais vulneráveis. Embora não estejam descritos casos

de intoxicação por ICs, o seu potencial toxicológico e a ausência de vigilância ambiental levantam preocupações quanto a um possível risco para a saúde pública, que carece de investigação mais aprofundada.

Agradecimentos

Trabalho financiado pela BI 2024.02120.BDANA da Fundação para a Ciência e Tecnologia. Amostras disponibilizadas pelo Laboratório de Biotoxinas Marinhas do Instituto Português do Mar e da Atmosfera.



Sessão de Posters

Prymnesium parvum in the coast and continental waters of the Basque region

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Introduction

Prymnesium parvum is notable among toxic marine microalgae for its ability to form blooms not only in marine systems such as estuaries, but also in inland waters. In the latter case, blooms typically occur in waters that exhibit some degree of salinization due to natural and/or anthropogenic factors.

Based on ITS sequences, recent studies have identified phylogenetic structuring within this morphospecies, with three main lineages corresponding to the type of toxin produced by the cells (A-, B-, and C-type prymnesin). In the Iberian Peninsula, there are few reports on this species - and to our knowledge, none from inland waters- and the lineage affinities of its populations remain unknown.

In this study, we present observations on: (1) the diversity of *P. parvum* in both estuarine and inland environments of the Basque region; and (2) the challenges that this diversity poses for its detection using molecular techniques (qPCR).

Materials and Methods.

Three strains of *Prymnesium* spp. were isolated from the estuary of Urdaibai and two additional strains were obtained from Navarre: one from the endorheic lagoon of Pitillas, and another from a saline stream in the Bardenas area.

ITS-region (ITS1-5.8S-ITS2) sequences were obtained and strains were used to test the performance of published *P. parvum*-specific SYBR Green qPCR assays (Galluzzi et al. 2008, doi:10.1111/j.1472-765X.2007.02294.x; Zamor et al. 2012, doi:10.1016/j.hal.2011.10.028).

Results and discussion.

Unexpectedly, we found that the two continental strains belonged to different *P. parvum* lineages: one to the A-type and the other to the C-type (C1 sub-clade).

The strains from the estuary of Urdaibai also differed among themselves: two belonged to the A-type and to C-type (C3 sub-clade) of *P. parvum*, and the third represented an unidentified species.

Strains of the A-type linage and from the C3 sub-clade of C-type where successfully amplified through qPCR, whereas a polymorphism (including an indel) in the annealing site of the forward primer prevented amplification of the strains belonging to the C1 subclade of the C-type.

Acknowledgements.

Work supported by the project PES20/29 of UPV/EHU and by the Basque Government through a grant to the consolidated research groups IT1723-22 and IT1743-22.

Tetrodotoxin and analogues in bivalvia and associated bycatch from the Southern Portuguese Coast.

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Abstract

Tetrodotoxin (TTX) is a potent neurotoxin, first identified in fish from the Tetraodontidae family, but also detected in marine invertebrates. A Human poisoning episode after consumption of trumpet shell *Charonia lampas*, likely caught off the Portuguese mainland southern coast (Algarve), together with the increasing reports of TTX in bivalves from European waters, led the European Food Safety Authority (EFSA) to recommend maximum safe limits for Human consumption of shellfish meat. However, TTX is currently only monitored in the Netherlands and France. On the southern coast (Algarve) of Portugal, TTX concentrations were detected in trumpet shells exceeding the levels considered safe for human consumption throughout the year. Nevertheless, there are no data on the incidence of TTX in other species from this area. This study aims to assess the presence of TTX and its 30 chemical congeners in a broad taxonomic range of marine invertebrates including Bivalvia, Gastropoda, Malacostraca and Polychaeta species. Samples were collected within the framework of the monitoring bivalve dredge fishing survey coordinated by the Portuguese Institute for the Sea and Atmosphere (IPMA). A total of 45 species were analysed, including 20 Bivalvia, 10 Malacostraca, 9 Polychaeta, and 6 Gastropoda. TTX concentrations in the were either not detected or remained below the EFSA-recommended safety limit. However, the systematic screening of both bivalves and associated bycatch revealed a widespread presence of TTX congeners. Since the toxicity of the analogues is estimated, rather than fully characterized, their detection across multiple trophic levels increases the potential risk of intoxication and raise concerns for public health.

Acknowledgements

This work was supported by the "la Caixa" Foundation (ID 100010434) through a Junior Leader Retaining Fellowship (LCF/BQ/PR23/11980049), and Portuguese national funds from FCT—Foundation for Science and Technology through projects 2024.04866.RESTART, UIDB/04326/2020, UIDP/04326/2020, and LA/P/0101/2020; the operational programs CRESC Algarve 2020; COMPETE 2020 through project EMBRC.PT ALG-01-0145-FEDER-022121. The bivalve dredge fishing survey was supported by the "Programa de Monitorização da Pequena Pesca e Apanha (MOPPA)" funded by the Fisheries Operational Programme (MAR 2030) and cofinanced by the European Maritime, Fisheries and Aquaculture Fund (EMFAF 2021-2027).

Analytical Challenges in Ciguatoxin identification: discrepancies between Low- and High- Resolution Mass Spectrometry

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Abstract

Ciguatera poisoning (CP) is a foodborne illness caused by the consumption of fish contaminated with naturally occurring marine toxins, known as ciguatoxins (CTXs). The global spread of these toxins has increased the relevance of CP as a public health concern.

However, the identification and characterization of CTXs remain challenging due to the limited availability certified reference standards. This lack of certified reference standards limits the interpretation of data obtained using targeted techniques such as low-resolution mass spectrometry (LRMS), which rely on previously reported molecular fragmentations patterns.

The high structural complexity of CTXs, its biotransformation in fish, the large number of analogues, and the limited characterization of their molecular formulas, further complicate their accurate identification and quantification.

In this study, profiles of Caribbean ciguatoxins (CTXs) in potentially toxic amberjack samples from the eastern Atlantic coast were compared using LRMS and high-resolution mass spectrometry (HRMS). The tentative identification of CTX analogues by LRMS was not supported by HRMS analysis.

The results revealed significant discrepancies between LRMS and HRMS analyses, highlighting the limitations of low-resolution targeted methods for CTX identification. These findings emphasize the critical role of high-resolution analysis for the reliable detection and identification of CTXs in the absence of reference materials and raise concerns regarding the accuracy of results derived solely from targeted low-resolution methods.

Spatial heterogeneity of *Gambierdiscus* drivers in the Balearic Islands: implications for ciguatera monitoring and decision support

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Introduction

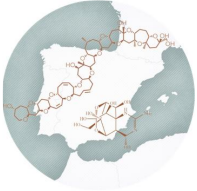
Ciguatera Fish Poisoning represents the most significant non-bacterial marine biotoxin-related foodborne illness worldwide. Climate-driven warming has facilitated the poleward expansion of these toxic dinoflagellates into the Western Mediterranean, where *G. australes* is now established in the Balearic Islands. Yet the environmental drivers of *Gambierdiscus* abundance and their transferability across monitoring sites remain unverified — a critical gap for operational decision support. This study identifies which predictors are transferable across the archipelago and which are spatially non-stationary, informing island-tailored monitoring protocols and a ciguatera early warning system.

Materials and Methods

We sampled 33 fixed stations across the four Balearic Islands annually during the *Gambierdiscus* growth season (September–October 2021–2024; n = 348). Cell abundance was quantified by Utermöhl method. Environmental predictors from Copernicus Marine Service products at multiple temporal lags (0–63 days) were combined with substrate cover and orientation from regional benthic cartography. Seven modelling approaches were validated through leave-one-site-out cross-validation (LOSO-CV), with predictor importance assessed by island-stratified SHAP analysis.

Results

Gambierdiscus abundance showed significant positive spatial autocorrelation (Moran's I = 0.346, $p < 0.001$), with Formentera as a persistent hotspot and Menorca as a persistent coldspot across years. Machine learning achieved strong in-sample fit (XGBoost $R^2 = 0.85$) but collapsed under LOSO-CV ($\Delta R^2 = -0.55$), revealing severe overfitting; the hierarchical GAMM achieved the best generalisation ($\Delta R^2 = -0.18$). Two archipelago-wide thresholds emerged: a thermal ceiling at 25.75–25.9 °C and phosphate saturation at ~0.012 mmol·m^{-3}. Island-stratified SHAP revealed substantial spatial non-stationarity, with each island showing a distinct dominant mechanism: substrate in Menorca, hydrodynamics in Mallorca, productivity in Ibiza, and refuge dynamics in Formentera.



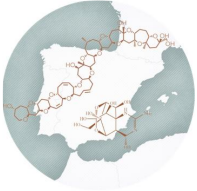
Discussion and Conclusion

Random cross-validation overestimates predictive skill in spatially structured data, leading to spurious driver identification and risking compromised operational decisions. Phosphate emerged as the only universal driver, confirming chronic phosphorus limitation as the proximate regulator of *Gambierdiscus* in the Western Mediterranean. Island-specific predictor profiles show that no single protocol can characterise ciguatera risk across heterogeneous archipelagos. We propose a multi-layer decision-support framework integrating Copernicus near-real-time alerts, island-tailored field validation, and public-health communication.

Acknowledgements

Funded by EUROCIGUA 2 (EFSA) and CIGUAADAPT (MICIN/AEI).

Sessão
Alterações climáticas e tendências globais



Apresentações Orais

21 Anos de Monitorização de Fitoplâncton Nocivo em Portugal: Tendências e Mudanças de Sazonalidade

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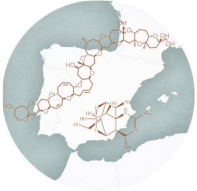
Resumo

No contexto das alterações climáticas, as modificações nas comunidades de fitoplâncton apenas podem ser estudadas com séries longas de monitorização, que podem ajudar a antecipar alterações nos padrões interanuais e sazonais de transferência de biotoxinas marinhas nas teias tróficas e no consumo humano.

O IPMA assegura a monitorização de fitoplâncton nocivo na costa Portuguesa, através de uma amostragem semanal desde 2003, na costa ocidental (litoral de Peniche e de Cascais) e na costa sul (litoral de Monte Gordo, Ria Formosa em Tavira e Faro) no âmbito do Sistema Nacional de Monitorização de Moluscos Bivalves (SNMB). Para este estudo analisaram-se *Dinophysis caudata*, *D. acuta*, *D. acuminata/ovum* complex, *Lingulaulax polyedra* e *Pseudo-nitzschia* spp..

A análise dos resultados de 21 anos (2003-2023) revelou tendências multidecadais distintas entre as espécies estudadas. Observou-se uma diminuição da abundância média de *D. acuta* nas costas sudoeste e sul, tanto ao nível da frequência de ocorrência como da magnitude dos blooms. Em contraste, verificou-se um aparente aumento da frequência de ocorrência de *Pseudo-nitzschia* spp. nos vários locais. Por outro lado, não foram detetadas tendências em *D. caudata*, *D. acuminata/ovum* e *L. polyedra*.

Ao longo da série temporal de 21 anos, além de alterações na abundância, foram também identificadas modificações na sazonalidade de várias espécies. No caso de *D. acuta*, observa-se nos anos mais recentes um aparente desfasamento temporal das maiores abundâncias de verão, passando a ocorrer duas ou 3 semanas mais tarde no ano, na costa ocidental. Relativamente ao complexo *D. acuminata/ovum*, observa-se uma aparente expansão da ocorrência na costa ocidental, passando de um período de maio-agosto, para abril-setembro em anos mais recentes; na costa sul, observa-se um aparente prolongamento da sua ocorrência até dezembro em anos mais recentes, enquanto no início da série terminava em final de setembro. Relativamente a *Pseudo-nitzschia* spp., na costa sul nos anos mais recentes, a época produtiva parece estar a começar mais tarde (2 ou mais semanas) no início de abril.



A variabilidade e tipo de modificações multidecadais encontrados são úteis para o desenvolvimento de futuros projetos de modelação numérica de previsão de blooms de fitoplâncton nocivo.

Agradecimentos

EU Fisheries Operational Program 2030, 'FEAMP - Mar2030', Projecto 'SNMB-Monitor V'.

Seasonal Biogeochemical Forcing of Phytoplankton Community Succession and Karlodinium Bloom Development in a Hypereutrophic Tropical Bay (Guanabara Bay, Brazil)

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Introduction

Guanabara Bay (Rio de Janeiro, Brazil; ~384 km²; ~16 million inhabitants in the drainage basin) is one of the most eutrophied coastal systems in the Southern Hemisphere, with documented harmful algal bloom (HAB) events involving ichthyotoxic dinoflagellates including *Karlodinium spp.* The seasonal biogeochemical conditions that trigger these events have never been characterized with an integrated dataset combining nutrient stoichiometry, carbonate chemistry and phytoplankton community data across contrasting seasons. The DINOHAB project (Instituto Serrapilheira) was designed to address this gap.

Materials and Methods

Three campaigns were conducted at 11 fixed stations spanning the bay's salinity gradient: C1 (September 2025, dry season), C2 (December 2025, wet season onset) and C3 (February 2026, wet season peak). Measurements included temperature, salinity, DO, pH, total alkalinity (AT), chlorophyll-a and accessory pigments (spectrofluorometry), dissolved inorganic nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, $Si(OH)_4$), and carbonate system parameters (pCO_2, DIC, Ω_ara) computed from paired pH–AT. Phytoplankton community composition was assessed by light microscopy and FlowCam.

Results

During C1, the inner bay was dominated by *Chaetoceros* spp. and *Tetraselmis* spp., with moderate DIN (mean 51.5 µmol L^{-1}) and residual silica ($Si(OH)_4$: 5.4 µmol L^{-1}). Extreme NH_4^+ (up to 196.3 µmol L^{-1}) at innermost stations indicated intense sewage-driven ammonification. By C2, silica was critically depleted (Si:DIN < 0.05) and DIN:DIP collapsed far below Redfield (mean 3.9 ± 3.5), with surface chlorophyll-a reaching 202.5 µg L^{-1}. By C3, *Karlodinium* spp. dominated inner bay stations (P07–P10), accompanied by extreme CO_2 drawdown (pCO_2 minimum 19.8 µatm, pH 9.0, Ω_ara 13.5).

Discussion and Conclusion

The C1–C3 sequence documents a complete mechanistic pathway consistent with the Smayda (1990) succession model: diatom blooms consumed the Si pool; the resulting Si depletion, combined with severely sub-Redfield DIN:DIP conditions driven by first-flush wet-season loading, selectively excluded diatoms and favoured mixotrophic dinoflagellates. High NH_4^+ in C1 likely reinforced this filter by suppressing nitrate-preferring taxa. This is the first dataset to mechanistically link seasonal nutrient stoichiometric shifts to *Karlodinium* bloom development in Guanabara Bay with paired biogeochemical and community evidence, providing baseline for HAB prediction and early warning.

Acknowledgements

This research was funded by Instituto Serrapilheira (Brazil) through the DINOHAB project. C.C.R. acknowledges FCT (Portugal) for support as Investigador Auxiliar (CCMAR/IAO/38/2024). D.F.G. and I.F.G. acknowledge CNPq and CAPES doctoral fellowships.

A Turn Of The Tides: Emergence and Dispersal af Non-Indigenous Harmful Microalgae in Portuguese Waters

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Abstract

Non-indigenous species (NIS) are increasingly reshaping marine ecosystems as global maritime traffic, aquaculture, and climate-driven environmental shifts facilitate their introduction and spread beyond natural dispersal ranges. Ballast water transport remains the dominant vector for NIS introductions, with an estimated 10 billion tonnes of water moved annually, leading to a new marine invasion roughly every nine weeks. Among these introductions, harmful microalga represents one more group of concern, since they are capable of disrupting marine food webs, compromising seafood safety, and generating severe ecological and socio-economic impacts.

This work brings together recent detections of potentially newly introduced microalgal species and a revisitation of historical NIS cases in Portuguese waters. It discusses current knowledge on introduction pathways, establishment processes, and ecological consequences, with a focus on species responsible for harmful algal blooms (HABs) and toxin production.

Finally, we address the challenges associated with early detection, monitoring, and management of NIS microalgae, highlighting critical research gaps and underscoring the need for harmonized surveillance frameworks to mitigate the spread and impacts of these harmful species.

Acknowledgements

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Transporte de Dinoflagelados Formadores de HABs através de Vórtices das Agulhas: Implicações para o Atlântico Sul

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Resumo

Os Vórtices de Agulhas constituem importantes vetores de transporte de microrganismos entre os oceanos Índico e Atlântico, podendo influenciar a dispersão de espécies potencialmente nocivas. Este estudo teve como objetivo investigar a presença, abundância e dispersão de dinoflagelados formadores de florações algais nocivas (HABs) em três vórtices de diferentes idades durante a expedição FORSA (Following Ocean Rings in South Atlantic). Foram identificadas três espécies potencialmente tóxicas: Dinophysis acuminata, Phalacroma rotundatum e Prorocentrum cordatum, sendo esta última a mais abundante. P. cordatum demonstrou elevada capacidade de aproveitamento das condições de turbulência e enriquecimento nutricional promovidas pelos vórtices, apresentando densidades significativamente maiores no vórtice intermediário (E3). D. acuminata, produtora de toxinas diarreicas (DSP), foi detectada em baixas densidades, mas representa risco devido à sua elevada toxicidade. A análise filogenética revelou forte sinal evolutivo na maioria das características funcionais, exceto na capacidade de produção de toxinas, sugerindo evolução independente deste traço. Os resultados indicam que os vórtices de Agulhas podem atuar como vetores de dispersão de espécies HAB ao longo do Atlântico Sul, com potenciais implicações para a costa brasileira. Este mecanismo representa um desafio emergente para a monitorização e gestão de HABs, especialmente no contexto das mudanças climáticas e do aquecimento dos oceanos.

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Predator cues trigger bioluminescence and toxin production in marine dinoflagellates

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Introduction

Copepodamides (CAs) are a class of polar lipids released by copepods into the surrounding water. Phytoplankton can detect these and respond by inducing traits such as bioluminescence, toxin production, changes in colony size and morphology. These responses may reduce grazing pressure and contribute to the ecological success of harmful algal bloom (HAB)-forming species; however, only a limited number of species have been exposed to copepodamides.

Here, we investigated bioluminescence and toxin production in response to CAs in three marine HAB-forming dinoflagellates: *Alexandrium catenella*, *Protoceratium reticulatum* and *Gymnodinium catenatum,* and assessed potential growth trade-offs.

Materials and Methods

Cultures of all three species were exposed to increasing concentrations of copepodamides (0—5 nM). Bioluminescence was measured on Days 0, 1, 3 and 5 using a luminometer, while toxin content was quantified on Days 0, 4 and 8 using liquid chromatography-high resolution mass spectrometry. Cell counts were monitored to calculate growth rates and potential costs.

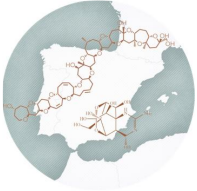
Results

Alexandrium catenella and *P. reticulatum* showed significant increases in bioluminescence in response to copepodamides, reaching up to an 83% increase after five days of exposure. Likewise, *G. catenatum* showed a strong induction of paralytic shellfish toxins, with up to a 165% increase.

This study also provides the first evidence of copepodamide-induced GC-toxin production. *A. catenella* also showed a trend toward increased toxin production. No clear trade-off between induced defenses and growth rate was found.

Discussion and conclusion

All three species responded to copepodamides by increasing toxin content and/or bioluminescence, supporting the role of CAs as a general alarm cue in marine phytoplankton. The simultaneous up-regulation of both traits in *A. catenella* suggests that coordinated defenses may enhance grazer deterrence and contribute to the success of HAB-forming species.



These findings highlight the importance of chemically mediated predator–prey interactions in structuring plankton communities and influencing HAB dynamics.

Acknowledgements

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Spatial variation in copepodamides production by Acartia spp., Centropages chierchiae and Paracalanus spp.

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Abstract

Copepodamides are polar lipids produced and released by copepods. When detected by microalgae, these chemical cues trigger defensive traits, including morphological changes, bioluminescence and the increased toxin production, in harmful algal bloom (HAB) forming species. This study aims to characterize and compare the copepodamides profile in different copepods species between two aquatic environments, Ria Formosa coastal lagoon and Guadiana River estuary. Using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS), we annotated 47 copepodamides, including 15 new copepodamides structures, and assess their variability between these two environments. Marine water copepods contain both copepodamide subgroups, the copepodamides (CA) and the dihydro-copepodamides (dhCA), whereas brackish copepods were largely dominated by dhCAs. Furthermore, focusing on Ria Formosa, we evaluated spatial variability across sampling points and species-level variability among the three dominant copepod species (i.e. *Acartia* spp., *Centropages chierchiae* and *Paracalanus* spp.). Preliminary results reveal a significant spatial variability across Ria Formosa sampling points and clear species-specific chemical signatures. *Acartia* spp. predominantly produced dhCA, while *Paracalanus* spp. produced predominantly CA. In contrast, *C. chiechiae* exhibited production of both subgroups.

Acknowledgements

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From cyanobacterial blooms to bivalve contamination in coastal areas: evidence of accumulation of microcystins in mussels from Portugal

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Abstract

Freshwater cyanobacteria bloom frequently and with great magnitude in Portugal's inland waters, with reservoirs often showing elevated toxin concentrations. Since these toxin-producing species were expected to reach coastal and estuarine bivalve farming areas, weekly water-sample screenings were carried out at nine Portuguese sites in 2021. These preliminary screenings crossed microscopical observations with the presence of cyanobacterial toxin gene. *Planktothrix* and *Microcystis* were the most frequently observed cyanobacterial toxin producers. Consistently, the *mcy*A gene was the most frequently detected toxin-related gene. The coast of Peniche in central Portugal was identified as a potential risk area for the occurrence of such contamination. Consequently, in October 2025, a field transplanted experiment was performed in this area in order to follow-up this potential risk and study the accumulation kinetics of cyanotoxins in mussels (*Mytilus* spp.). Four weeks after the cyanobacteria *Planktothrix agardhii* concentration started to increase in samples collected at Peniche coast, a set of mussels from the inlet of Ria de Aveiro was transplanted to the site in Peniche where the freshwater streams flow into the sea. Water was sampled weekly (days 0, 8, 15, 21 and 27) showing between $3.8x10^4$ and $5.5x10^5$ cells.L^{-1} of the cyanobacteria. In the same sampling dates thirty mussel specimens were sampled for the quantification of microcystin- LR (MC-LR) and microcystin-RR (MC-RR). Concentrations of MCs above quantification limits were registered on all sampling dates. The toxin profile showed similar concentrations of MC-LR and MC-RR across experimental dates, from 31.5 µg MC-RR.kg^{-1} (day 8) to 127 µg MC-LR.kg^{-1} (day 24). Mussels' exposure to a moderate cyanobacteria bloom for 24 days exceeded the existing health guideline value for MCs in mussel tissue (51 µg MCs.kg^{-1}). The *in-situ* exposure trial confirmed the risk zone and showed that even moderate *Planktothrix agardhii* blooms are capable of driving microcystin accumulation in mussels to concentrations exceeding existing health guideline values within only a few weeks of exposure, highlighting the need for monitoring freshwater cyanotoxins in marine organisms to safeguard shellfish safety and protect public health.

Acknowledgements

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Novos dados sobre a distribuição de Gambierdiscus spp. no Atlântico Oriental: do Golfo da Guiné aos Açores

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Resumo

Os dinoflagelados bentónicos do género *Gambierdiscus* são responsáveis pela produção de toxinas com elevada toxicidade, como maitotoxinas e precursores de ciguatoxinas, associadas à intoxicação por *Ciguatera* (CP). Originalmente considerados restritos a regiões tropicais, este género tem sido cada vez mais reportado, em zonas subtropicais e temperadas, incluindo as ilhas do Atlântico Oriental na região da Macaronésia. Esta aparente expansão geográfica tem sido associada ao aquecimento dos oceanos e evidencia a necessidade de monitorização e avaliação de risco em novas áreas.

Este estudo tem como objetivo caracterizar a distribuição e diversidade de espécies de *Gambierdiscus* em cinco arquipélagos do Atlântico Oriental: Açores, Madeira, Selvagens, Cabo Verde e São Tomé e Príncipe. Foram recolhidas por snorkeling amostras de diferentes macroalgas, em sete ilhas, de onde foram isolados e cultivados *Gambierdiscus*. As estirpes obtidas foram estudadas através de abordagens morfológicas (SEM) e moleculares (regiões LSU rRNA D1-D3 e D8-D10).

Gambierdiscus foi detetado em todos os arquipélagos estudados. Foram identificadas quatro espécies (*G. carolinianus*, *G. excentricus*, *G. australes* e *G. caribaeus*), com uma distribuição geográfica distinta. *Gambierdiscus carolinianus* foi isolado nos Açores, constituindo o primeiro registo deste género no arquipélago e o registo mais a norte conhecido até à data (39°40'19"N). *Gambierdiscus caribaeus* foi registado em São Tomé e Príncipe e em Cabo Verde, correspondendo, respetivamente, ao primeiro registo deste género e ao primeiro registo desta espécie nestes arquipélagos.

Em Cabo Verde e São Tomé e Príncipe, as comunidades de dinoflagelados bentónicos nocivos (BHAB) foram estudadas utilizando substratos artificiais, com o objetivo de comparar com a utilização dos substratos naturais de macroalgas. Foram avaliadas as abundâncias celulares quantitativas dos géneros *Gambierdiscus*, *Prorocentrum*, *Ostreopsis*, *Coolia* e *Amphidinium*, fornecendo os primeiros dados sobre a estrutura e a contribuição relativa destes géneros em comunidades tropicais do Atlântico Oriental. Ambos os métodos revelaram uma composição relativa semelhante, com dominância de *Ostreopsis* em São Tomé, enquanto em Cabo Verde se observou co-dominância de *Ostreopsis* e *Prorocentrum*, sendo *Amphidinium* o género menos abundante em ambos os arquipélagos.

Estes resultados expandem a distribuição biogeográfica conhecida de *Gambierdiscus* no Atlântico Oriental e evidenciam um potencial risco de CP ainda pouco avaliado, reforçando a necessidade de implementar programas de monitorização consistentes em regiões ainda pouco estudadas.

Agradecimentos

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Benthic dinoflagellates in the South-East Spanish coasts

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Introduction

Benthic harmful algal blooms (BHABs) are an increasing concern in temperate coastal ecosystems due to their ecological and human health impacts. Among benthic dinoflagellates, *Ostreopsis* and *Gambierdiscus* are particularly relevant for the Mediterranean, yet the environmental factors driving their proliferation remain poorly understood.

Materials and Methods

Monthly sampling (2024–2025) was conducted at 15 beaches in southeastern Spain. Macroalgal substrates (*Jania* and *Corallina*) were collected to quantify benthic dinoflagellates. Environmental variables, including temperature, salinity, nitrate, phosphate, and silicate, were measured.

Multivariate analyses included principal component analysis (PCA) to explore patterns, PERMANOVA to test for differences among sites, and betadisper with Tukey post-hoc tests to assess heterogeneity of dispersion.

Results:

Ostreopsis was detected at all sites, with peak abundances at Las Palmeras (8×10^6 cells·g^{-1} fw). Genetic sequencing of isolates confirmed the presence of *Ostreopsis* cf. *ovata*, *Gambierdiscus australes*, and several *Coolia* species, highlighting the presence of multiple potentially toxic genera.

PCA revealed spatial gradients in environmental conditions, and PERMANOVA indicated significant differences among sites ($p = 0.001$). Betadisper analysis confirmed that Las Palmeras exhibited significantly higher environmental variability than other beaches (Tukey post-hoc, $p < 0.001$).

Discussion and Conclusion:

Las Palmeras site could represent a hotspot for *Ostreopsis* cf. *ovata*, consistent with its proximity to other beaches in northern Almería where respiratory symptoms associated with *Ostreopsis* aerosols have been reported.

The detection of *Gambierdiscus australes* highlights the importance of monitoring multiple potentially toxic genera in the region.

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Sessão de Posters

Seasonal succession of zoosporic parasite communities: a mechanism of resilience in coastal ecosystems during the proliferation of Ostreopsis in the Bay of Biscay.

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Abstract

The climate emergency is increasing the frequency and intensity of toxic dinoflagellate blooms in regions where they were not previously present. Such is the case of the annual Ostreopsis blooms in the Bay of Biscay, which caused public health issues in 2020. This study analyzed the biological factors influencing the development of *Ostreopsis* blooms, focusing on the relationships with parasites and saprophytes. The diversity of zoosporic parasites was evaluated across five locations on the Gipuzkoa coast (Mutriku, Zumaia, Zarautz, San Sebastian, and Hondarribia) during 2023 and 2024, using a combination of metabarcoding, microscopic observation, and the cultivation of isolated strains.

The results revealed a diverse community associated with *Ostreopsis* blooms, highlighting three main groups: Syndiniales (Dino-Group-II), Perkinsids (Perkinsidae), and Chytrids (Chytridiomycota). The structure of this community varied seasonally according to host availability. Chytrids were the least abundant group of parasites and predominated in spring and summer, while Perkinsids were the second most abundant group, with a presence almost exclusively in summer during the development of massive blooms. For their part, Syndiniales showed their highest relative abundance between summer and autumn. The parasitic interaction between *Ostreopsis*, Perkinsids, and Chytrids was confirmed through isolation and identification in culture. These findings suggest a dynamic and seasonal relationship between parasites and hosts, which is key to understanding the resilience mechanisms of coastal ecosystems during microalgal blooms, thereby providing additional information for the management and monitoring of these events.

How diet shapes copepodamides profiles in Acartia spp.

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Abstract

Marine copepods, the most abundant animals in the world's oceans, release chemical cues known as copepodamides into the surrounding water. These compounds trigger defensive responses in various marine phytoplankton, including the induction of toxin synthesis in harmful algal blooms (HABs) species, bioluminescence, and changes in colony size.

Copepodamides comprise two subgroups, dihydro-copepodamides (dhCA) and copepodamides (CA), distinguished by a methyl or methylene group at position C3. The fatty acid moiety at C5 typically contains a long-chain ω-3 polyunsaturated or saturated even-numbered fatty acids. Thirty-one structural variants have been identified to date. The copepodamides profile is both species-specific and habitat-dependent, differing between marine and freshwater environments.

Therefore, in the present study we examine whether microalgal diets with different fatty acid profiles (i.e. *Tetraselmis chui*, *Nannochloropsis oceanica*, and *Tisochrysis lutea*) alter the profile and concentration of copepodamides in the copepod *Acartia* spp., and whether copepodamides can be synthesized de novo from dietary fatty acids.

This study will help to elucidate the factors that regulate copepodamides production and clarify their contribution to HABs dynamics and toxicity.

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Catch me if you can: dynamics of Gambierdiscus australes on the eastern coast of the Iberian Peninsula

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Abstract

The presence of the genus *Gambierdiscus* Adachi & Fukuyo, which produces the toxins responsible for Ciguatera Fish Poisoning, in the Mediterranean coastal waters of the Iberian Peninsula is puzzling. In the quarterly sampling conducted from March 2023 to July 2024, *Gambierdiscus australes* Chinain & MA Faust was detected in surface waters (upper 5 meters of the water column) along the Dénia-Jávea coast at concentrations ranging from 20 to 140 cells/liter. However, it was not observed in the three subsequent samplings (October 2024, February 2025, and July 2025). In this study, the relationship between the presence/absence of *Gambierdiscus australes* and several physicochemical parameters was investigated. Temperature, salinity, turbidity and dissolved oxygen were recorded on sampling days using a multiparametric data logger JFE Advantech, while nitrates, nitrites, phosphate, silica and chlorophyll-*a* were subsequently determined from water samples collected in situ. Generalized Linear Model (GLM) analysis performed in R statistical software indicated that among all parameters studied, only salinity showed a significant effect. Nevertheless, this result should be interpreted with caution, as salinity values recorded when *Gambierdiscus australes* was absent fell within the optimal growth range for this species reported under laboratory conditions by several authors. A longer time series and the inclusion of additional factors such as local currents and marine storms or extreme rainfall events (e.g. "cut-off low" phenomenon), which can induce ecosystem changes, may help to clarify the drivers of its spatiotemporal dynamics.

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Ondas de calor marinhas poderão estimular o crescimento e o potencial toxigénico de *Prorocentrum lima* e *P. hoffmannianum*

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Resumo

As ondas de calor em meio marinho (sigla inglesa MHWs) têm vindo a aumentar em frequência e intensidade no contexto das alterações climáticas, contudo os seus efeitos em dinoflagelados nocivos bentónicos não estão estudados. Considerando que a intoxicação por toxinas diarreicas – *Diarrhetic Shellfish Poisoning* (DSP) – é a intoxicação associada ao consumo de bivalves mais comum em Portugal e que algumas espécies bentónicas do género *Prorocentrum* são produtoras de toxinas desse grupo, é importante compreender os potenciais efeitos das MHWs nestes organismos.

Neste estudo, foi simulada uma MHW de Categoria IV (+0.6 °C día^{-1} de incremento; pico de 10 dias) e estudado o seu efeito no crescimento e produção de toxinas em quatro estirpes de *Prorocentrum lima* e uma estirpe de *P. hoffmannianum*, isoladas de amostras recolhidas na costa ocidental e sul de Portugal. Durante 28 dias, três réplicas de cada estirpe foram expostas a uma simulação de MHW, atingindo 24 °C (+5.5 °C acima da temperatura de referência) enquanto os controlos (n=3) foram mantidos a 18.5 °C. A dinâmica de crescimento foi monitorizada por citometria de fluxo e as concentrações celulares de toxinas foram quantificadas por LC-MS/MS ao longo da curva de crescimento em três estágios-chave da fase exponencial (inicial, intermédia, tardia) e também na fase estacionária.

A dinâmica de produção de toxinas foi marcadamente influenciada pela fase de crescimento, tendo sido verificadas concentrações mais baixas durante a fase exponencial e valores mais elevados nas fases exponencial tardia e estacionária. Destaca-se que a estirpe de *P. hoffmannianum* apresentou um aumento progressivo da produção de OA ao longo do tempo, enquanto *P. lima* exibiu maior variabilidade dependente da estirpe.

Considerando a exposição à MHW, observou-se um estímulo no crescimento em três das cinco estirpes, resultando em aumentos significativos das taxas de crescimento exponencial e em maiores abundâncias celulares. A produção de toxinas também variou, tendo-se observado

um aumento nas frações intracelulares de ácido ocadaico (OA) e dinofisistoxina-1 (DTX1) em três das cinco estirpes estudadas. *Prorocentrum hoffmannianum* apresentou as maiores concentrações de OA, mas uma produção negligenciável de DTX1, enquanto nas estirpes de *P. lima* o OA representou apenas cerca de 70% do total de toxinas.

Num contexto mais amplo, estes resultados sugerem que em zonas temperadas as MHWs poderão aumentar tanto o crescimento como o potencial tóxico de populações bentónicas de *P. lima* e *P. hoffmanianum*. Contudo, a variabilidade na resposta intraespecífica à MHW evidencia a importância de considerar a diversidade intraespecífica na avaliação do risco de proliferações de algas nocivas (HABs) em cenários futuros de alterações climáticas.

Niche breadth of harmful dinoflagellate species during two Alexandrium minutum blooms in a coastal productive system.

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Abstract

Harmful algal blooms (HABs) are natural phenomena whose frequency and intensity appear to be increasing globaly. Understanding the ecological niches of HAB-forming species provides a robust framework to disentangle the relative influence of environmental constraints and biotic interactions on bloom dynamics. While bloom formation has traditionally been linked to abiotic drivers, the role of microbial associations remains insufficiently explored. We investigated the niche breadth and associated microbiome of the toxic dinoflagellate *Alexandrium minutum* during two bloom events in the Ría de Vigo (NW Spain). A total of 104 samples were collected over a three-year period in Baiona Bay, and prokaryotic and eukaryotic communities were characterized through partial 16S and 18S rRNA gene sequencing, respectively. The niche breath of *A. minutum* was estimated using Outlying Mean Index (OMI) analysis based on twelve environmental variables and compared with those of co-occurring toxic dinoflagellates. The mean environmental conditions occupied by *A. minutum* largely overlapped with the mean environmental conditions of the study site, indicating broad environmental tolerance and suggesting that abiotic conditions do not strongly constrain its proliferation during blooms. In contrast, co-occurring species such as *Dinophysis acuminata* and *Prorocentrum rhathymum* exhibited narrower niche breadths, reflecting more restricted environmental requirements. Distance-based redundancy analysis (dbRDA) and DISTLM revealed that environmental variables explained only a limited fraction of the variance in eukaryotic community composition during *A. minutum* blooms, whereas the structure of the associated prokaryotic microbiome accounted for a substantially larger proportion. These results indicate that abiotic factors alone are insufficient to explain *A. minutum* bloom formation, and instead point to biotic interactions as key drivers of its proliferation.

Acknowledgements

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Interactive effects of warming and nitrogen enrichment on community structure in Microcystis-dominated freshwater phytoplankton

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Abstract

Climate warming and eutrophication are key drivers of the global expansion of harmful cyanobacterial blooms, frequently dominated by Microcystis aeruginosa. These blooms pose significant risks to freshwater ecosystems and human health. While temperature and nutrient enrichment are known to promote bloom formation individually, their interactive effects on natural, multi-species phytoplankton communities remain insufficiently understood. This study aims to assess how warming and nitrogen enrichment jointly influence phytoplankton community structure.

Natural phytoplankton communities collected from freshwater systems where Microcystis is present were used to establish laboratory cultures under controlled conditions. A fully factorial design combined two temperature levels (18°C and 21°C) with two nutrient regimes (N:P = 320:20 µM and 320:200 µM), each with three replicates. Cultures were maintained under semi-continuous conditions with periodic medium renewal. Phytoplankton community composition was assessed using microscopy-based taxonomic analysis, allowing evaluation of changes in species composition over time.

Warming and nitrogen enrichment are expected to promote Microcystis dominance and alter phytoplankton community structure. Elevated nutrient availability is expected to enhance the relative abundance of Microcystis within the community, while higher temperature may accelerate community shifts. The interaction between these drivers is expected to amplify these effects, leading to a stronger dominance of Microcystiscompared to single-factor treatments. This study provides a community-level perspective on how interacting global change drivers shape phytoplankton composition during bloom development. The expected outcomes highlight the risk of increased cyanobacterial dominance under future climate and eutrophication scenarios.

Sessão Impactos das microalgas tóxicas e biotoxinas

Apresentações Orais

Temporal dynamics of the genus Ostreopsis Johs. Schmidt in La Restinga (El Hierro, Canary Islands): abundance and benthic community composition

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Abstract

The temporal dynamics of the genus Ostreopsis were investigated in La Restinga (El Hierro, Canary Islands) over an 18-month period (2024–2025), aiming to characterise cell abundance and benthic community composition under bloom and non-bloom conditions. Two major Ostreopsis bloom events were identified, together with a single peak of Gambierdiscus. Maximum abundances reached ~10^6 cells·g^{-1} for Ostreopsis and ~10^4 cells·g^{-1} for Gambierdiscus, indicating pronounced temporal variability. Morphometric analyses revealed well-defined size classes (small, medium and large morphotypes). Small and medium morphotypes dominated the population, whereas the largest morphotype (>70 µm) remained a minor but persistent fraction. Size distributions showed a non-random population structure (χ^2, $p < 0.001$). Despite strong fluctuations in abundance, community composition remained stable. Relative proportions were conserved under bloom and non-bloom conditions, indicating that increases mainly reflect population growth rather than structural changes. Thus, blooms represent predominantly quantitative changes at the population level. The largest morphotype exhibited distinct traits, including larger cell size and two types of thecal pores. These features are consistent with Ostreopsis lenticularis, supporting its identification based on morphology. This species has been reported as non-toxic in strains from the type locality. Environmental variability in abundance was mainly associated with hydrodynamics, irradiance, and temperature (~84%). The increase of the largest morphotype (~2% to ~10%) suggests a differential environmental response, although its relative contribution remained stable during blooms. Overall, the results reveal strong abundance fluctuations within a structured population embedded in a stable community, highlighting the need to jointly assess abundance and composition in non-monospecific blooms for ecological interpretation and risk assessment.

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The perfect storm in a coastal pond: duckweed, botulism, bird mortalities and toxic blooms of *Euglena sanguinea*

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Introduction

In summer 2024, a freshwater eutrophic coastal pond in northwest Spain (A Congorza, Cangas) experienced its first massive mallard (*Anas platyrhynchos*) mortalities, repeated in 2025. The present study analysed the abiotic and biotic factors that could explain these episodes and proposed measures to restore the ecological equilibrium in the pond.

Materials and Methods:

Nutrient and plankton analyses (microscopy, pigment and molecular methods); toxin determination (BoNT, cyanotoxins, euglenophycin); organic pollutants.

Results:

Surface of the pond was covered by a bloom of duckweed (*Lemna minor*) and euglenophytes (*Euglena sanguinea*). Water, tissues and maggots from dead specimens were analysed to assess physico-chemical characteristics, plankton composition, clinical signs and toxins in order to explain these mortalities. Poor water quality parameters were determined during summer 2025 with suboxic dissolved oxygen and high levels of biological and chemical oxygen demand that revealed severe degradation. Clinical signs and toxin analyses confirmed avian botulism (type C and C/D toxins) as the primary cause of mallard deaths. LC-HRMS detected putative euglenophycin isomers in *E. sanguinea* pellets.

Discussion and Conclusion:

Our findings indicated that avian botulism was the primary cause of mallard mortalities in A Congorza. The dominance of L. minor and recurrent E. sanguinea red blooms evidence the eutrophication and severe habitat degradation potentially contributing to a multifactorial stress fatal to A. platyrhynchos.

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We thank collaborators (IIM-CSIC, LABOKLIN, Laboratorio Central de Veterinaria de Algete, and Xunta de Galicia (INTECMAR, ROAGA-LMAG, Laboratorio de Saúde Pública de Galicia, Natural Heritage Service and WRC of Carballedo) and funding by GPC-VGOHAB IN607B 2023/11), CCVIEO, PTA2021-020052-I, and EQC2021-007156-P.

Variations in toxicity and toxin profile of *Gambierdiscus australes* under different temperatures

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Introduction

Gambierdiscus australes are widely distributed in the Balearic Islands and represent a potential risk of ciguatera poisoning (CP). However, its toxicological characterization remains limited. The study aims to characterize its toxicity and toxin profile and their variation under different temperature conditions.

Materials and Methods

A monoclonal strain of G. australes isolated from Mallorca, Balearic Islands was cultured in ES medium under different temperatures: 20, 22, 24, 26, and 28 °C, while salinity (37) and irradiance (100 µmol. m-2.s-1) were kept constant.

Key parameters such as growth rate, maximum cell yield, cellular toxicity, and toxin profile were evaluated. Growth curves were performed, and microalgae for toxin analysis were harvested at the end of the exponential phase. Cellular toxicity was assessed with the Neuro-2a cell-based assay (CBA), while toxin profile and content were analyzed with ultraperformance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) and high-resolution mass spectrometry (LC–HRMS).

Results and Discussion

Optimal growth temperature was determined to be 24.9 °C. Interestingly, cellular toxicity increased at lower temperatures. However, this toxicity could not be associated with ciguatoxins (CTXs), since no CTX analogues were detected by UPLC-MS/MS or UPLC-HRMS analyses. Instead, it is likely attributable to other compounds produced by Gambierdiscus, such as maitotoxin-5 (MTX5).

Chemical analysis confirmed the presence of 44-methylgambierone and MTX5, as well as gambieric acids C and D.

Notably, significant variations in toxin content were observed across temperature conditions, with higher toxin levels detected at lower temperatures. In contrast, toxin content was consistently lower at 28 °C, likely due to culture decline under this condition.

Conclusion

This study provides new insights into the toxicity and toxin profile of G. australes in the BI and highlights the need to integrate complementary analytical approaches to improve CP risk monitoring and assessment.

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Removal methodologies of paralytic shellfish toxins from marine systems

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Abstract

Paralytic shellfish toxins (PST) comprise dozens of analogues sharing a common core structure, and their toxic potential varies according to their substituent groups. In marine environments, these toxins are produced by microalgae such as Gymnodinium catenatum, or by several species of Alexandrium. They may be directly ingested by organisms that inhabit these environments, particularly bivalves. Human poisoning occurs when organisms with high PST concentrations are consumed and may be fatal. Despite the severity of the problem, effective and viable solutions to eliminate these toxins from bivalves, or to convert them into less toxic analogues, remain unavailable. The methods described in the literature were discussed recently, with emphasis on their suitability, feasibility and liabilities, clearly showing the need to address.

In recent years, our group has carried out work aimed at the optimization of adsorbent materials, based on ion exchange resins and modified clays. This communication aims to present the potential uses of these adsorbents for removing PST from marine environments, discussing their advantages as well as their limitations. Our studies were conducted using standards, in aqueous solutions, as well as on G. catenatum culture and in live bivalves, using mussels Mytilus edulis. PST were quantified in the different matrices, according to the current official method, using high-performance liquid chromatography with fluorescence detector (HPLC-FLD), with pre-oxidation. The results show different efficiencies of the materials in the different matrices tested, revealing that the natural constituents of marine systems exert a strong influence on PST removal, which is explained by competition, as well as by interactions between the molecules in the systems.

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Neuro-Epithelial Toxicity of Diarrheic Shellfish Toxins in a Caco-2/SH-SY5Y Co-culture Model

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Introduction

Diarrhetic shellfish toxins (DSTs) represent a recurrent threat to seafood safety. While their gastrointestinal effects are well known, the mechanisms behind intestinal permeability and neuro-epithelial interactions remain unclear. The transient receptor potential vanilloid 1 (TRPV1) is a non-selective, ligand and voltage gated cation channel. TRPV1 is widely expressed in sensory neurons and in epithelial cells of the gastrointestinal system where it plays a critical role in many physiological functions, including permeability and nutrient uptake. This study evaluated the TRPV1 modulation and neuro-epithelial toxicity of Okadaic acid (OA), Dinophysistoxin-1 (DTX1) and Dinophysistoxin-2 (DTX2) using an in vitro Caco-2/SH-SY5Y co-culture model.

Materials and Methods:

In the coculture: Caco-2 cells, that in 21 days spontaneously differentiate in enterocytes, were seeded on 1.1 cm^2 inserts and the neuroblastoma cell line SH-SY5Y in 12-well plates. Transepithelial Electrical Resistance (TEER) was used to measure permeability. DSTs were applied apically in the presence or absence of TRPV1 agonist capsaicin or the antagonist capsazepine to explore the effect of ion channel modulation.

Results

DSTs decreased TEER, and therefore increased permeability. DTX1 exhibited the highest potency, inducing significant and persistent TEER decline for 24 hours. Regarding TRPV1 modulation, capsazepine significantly potentiated the toxic effect of OA and DTX2 when co-administered, even though the effect on DTX1 was low. The presence of capsazepine increase the permeability particularly of OA, as indicated by higher toxin detected in the basolateral compartment compared to the toxin-only treatment.

Conclusion:

Although all DSTs affects the epithelial barrier, DTX1 induces greater increase in permeability. Modulation of TRPV1 suggests that this channel may play a complex role in DST-mediated intestinal pathophysiology

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A New Player in Ciguatera Risk: Bluefish from the Canary Islands

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Abstract

The Canary Islands are a region with a strong fishery tradition and are well-known to be a hotspot of ciguatoxins (CTXs). Since 2008, a total of 28 poisonings (CP) have been recorded up to May 2025. Since 2011, an official monitoring programme has been in place to prevent at-risk species from reaching the market with CTX-like toxicity. Bluefish (Pomatomus saltatrix) is a species included in this program and it has been related to outbreaks in this area. The aim of this study is to provide data of CTX-like toxicity in bluefish captured in the Canary Islands over the last decade.

Muscle samples were sampled from 566 bluefish (0.8 - 15.4 Kg) caught in the Canary archipelago between September 2016 and March 2026 and analysed by neuro-2a MTT cell based-assay. All these samples were collected within a food safety framework, with the majority coming from the monitoring programme (n = 482) and, to a lesser extent, from recreational fishing and purchases at local markets.

Of the total, 373 samples (65.9%) showed CTX-like activity. It should be noted that the size of the specimens has decreased over time, due to the occurrence of CP outbreaks. Whilst in the early years the median weight was around 9.4 Kg, in recent years the median was 5.3 Kg. Besides, an increase in CTX-like activity has been observed, rising from 12.8% to 69.8%. The island of Fuerteventura stands out, with the highest number of samples analysed (n = 319) and percentage of toxicity presence (74%).

Bluefish has become a key focus in the monitoring of CTXs in the Canary Islands and in the risk of poisoning. Two outbreaks have been reported, affecting four people, and its weight in the monitoring programme has been reduced by 10 Kg over the last decade. This has raised serious concerns among recreational and professional fishermen, as well as within food safety management, as the number of samples to be analysed has risen from nine in 2018 to 333 in 2025, with this being the species analysed with the lowest weight limit and the highest toxicity rate. In conclusion, bluefish has acquired great relevance in the Canary Islands due to the presence of CTXs and the implications for outbreaks and further ecological studies are needed to understand the recent increase in toxicity.

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Proceso de acumulación y detoxificación de ciguatoxinas en Goldfish (*Carassius auratus*)

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Introducción

Las ciguatoxinas (CTX) son producidas por dinoflagelados bentónicos de los géneros *Gambierdiscus* y *Fukuyoa* que se asientan sobre las macroalgas (Kibler et al., 2017). Estas pueden bioacumularse a lo largo de la cadena alimentaria, pudiendo acarrear riesgos para la salud. En este trabajo se ha intentado semicuantificar la acumulación de toxinas en diferentes tejidos de los peces, y analizar el proceso de detoxificación de los mismo con el paso del tiempo.

Material y métodos

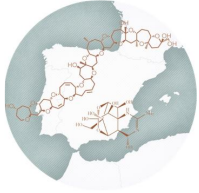
La experiencia contó con un total de 63 peces, que se dividieron en 3 grupos. Un grupo se alimentó con carne muy tóxica (n = 30), otro con carne poco tóxica (n = 27), y un grupo control que recibió carne libre de CTX (n = 6). Todos los grupos de peces fueron muestreados en 10 muestreos distintos por triplicado en diferentes momentos del estudio, en un total de 70 días. En cada uno de ellos se realizó la disección completa de cada ejemplar, extrayendo CTX de los músculos y los hígados. La extracción de CTX se realizó según el protocolo de Tudó et al. (2022). Tras obtener los extractos se realizó una purificación mediante extracción en fase sólida (SPE). Finalmente, se estima la cantidad de CTX mediante un ensayo basado en células de N2a (CBA) (Caillaud et al. 2010).

Resultados y discusión

El único grupo en el que se consiguió sintomatología a causa de la ingestión de CTX, fue el grupo que recibió la carne muy tóxica. Al noveno día de la fase de acumulación comenzaron a aparecer signos en los peces del grupo muy tóxico, consistente en letargo, alteración del brillo del color y pérdida de apetito. Algunos peces presentaron sintomatología hiperactiva, como episodios de natación errática y espasmos. Tanto el músculo como el hígado presentan un comportamiento similar en el período de la detoxificación, donde a partir del día 34 no se detectó ciguatoxina, encontrándose pico más alto en el día 18.

Discusión y conclusión

Este comportamiento se ha podido observar en otros estudios, donde los peces comenzaron a tener síntomas a los 15 días después de alimentar a los peces. Otros autores también



mencionan la reducción de velocidad o aceleración de la natación, así como la pérdida de equilibrio.

Este estudio ha permitido comprobar los procesos de acumulación y detoxificación de CTX en peces experimentales, permitiendo entender los procesos de metabolización de la CTX con el paso del tiempo en los peces.

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Dinâmica do fitoplâncton potencialmente tóxico na Baía de Luanda (Angola): Implicações para a contaminação de moluscos bivalves

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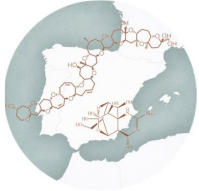
Resumo

A Baía de Luanda (BL), situada na costa centro-ocidental de Angola, é uma zona costeira de elevada importância ecológica e socioeconómica, mas sujeita a intensa pressão antropogénica devido à expansão urbana, descargas industriais e domésticas. Estas condições promovem o enriquecimento em nutrientes, criando ambiente favorável à ocorrência de proliferações de fitoplâncton potencialmente tóxico. O estudo teve como objetivos: avaliar a dinâmica das espécies de fitoplâncton potencialmente tóxicas na BL e quantificar os níveis de ficotoxinas em moluscos bivalves. As amostras de água foram recolhidas em oito estações (E1–E8) da BL, entre abril de 2023 e março de 2025. A abundância e composição específica do fitoplâncton foram determinadas por microscopia invertida (Utermöhl, 1958). Amostras de bivalves das espécies Senilia senilis e Mactra glauca (n=18) foram recolhidas no mesmo período, sendo analisadas para diferentes ficotoxinas regulamentadas na UE, usando os métodos de referência europeus: cromatografia líquida com deteção por fluorescência (saxitoxinas), deteção UV (ácido domóico) e espectrometria de massa em tandem (ácido ocadaico e restantes toxinas lipofílicas). Foram identificadas espécies potencialmente tóxicas, incluindo Alexandrium tamarense, Dinophysis acuminata, D. caudata, D. fortii, D. rotundata, Gambierdiscus cf. toxicus, Gonyaulax spinifera, Gymnodinium catenatum, Karenia cf. mikimotoi, Prorocentrum concavum, Protoceratium reticulatum e Pseudo-nitzschia spp. (grupo seriata). O ácido ocadaico (AO) foi detetado em mais de 80 % das amostras, concentrações superiores em S. senilis (máximos anuais: 1435 µg AO eq/kg em fevereiro 2024 e 618 µg AO eq/kg em fevereiro 2025), excedendo o limite da UE (160 µg AO eq/kg) em 22 % das amostras. Não foram detetadas outras toxinas lipofílicas, como iessotoxinas (YTX) ou azaspirácidos, nem toxinas hidrofílicas, como a saxitoxina e o ácido domoico. Os resultados representam uma contribuição relevante para o conhecimento da dinâmica do fitoplâncton potencialmente tóxico na BL, evidenciando um risco relevante de intoxicações alimentares na ausência de programa de monitorização eficiente. Estes dados reforçam a necessidade urgente de implementação e fortalecimento de programas de monitorização, gestão e mitigação de ficotoxinas marinhas em Angola.



Agradecimentos

Ao Laboratório de Toxinas Marinhas do IPMA pela disponibilização das instalações. Ao INIPM, Angola pelo apoio ao longo das amostragens. A Fundação para Ciência e Tecnologia (FCT) pela concessão financeira através da bolsa de doutoramento (PRT/BD/154844/2022) e aos projetos UIDP/00350/2020 (CIMA-U) e LA/P/0069/2020 (ARNET).

Sessão de Posters

Allelopathic effects of *Microcystis aeruginosa* on phytoplankton community structure in semi-natural microcosms

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Introduction:

Blooms of the toxic cyanobacterium Microcystis aeruginosa are a major concern in freshwater ecosystems due to their ecological and health impacts. Beyond resource competition, allelopathic interactions mediated by microcystins may play a key role in shaping phytoplankton community dynamics. However, these effects remain insufficiently understood in more complex, natural systems. This study aimed to investigate how the presence of M. aeruginosa influences phytoplankton community structure through allelopathic interactions.

Materials and Methods:

Semi-natural phytoplankton communities were established and maintained in laboratory microcosms under nitrogen-limited conditions. Experimental treatments consisted of communities with and without the addition of M. aeruginosa, each in triplicate. The experiment was conducted over 43 days under semi-continuous conditions. Phytoplankton community composition was assessed using light microscopy, and further analysed by 16S and 18S rRNA gene sequencing. Nutrient concentrations and microcystin levels were measured throughout the experiment.

Results:

Preliminary observations indicate that the presence of M. aeruginosa altered phytoplankton community composition. Certain taxa appeared to be inhibited, while others persisted or increased in relative abundance. These shifts were accompanied by changes in overall community diversity.

Discussion and Conclusion:

The differential response among taxa indicates that cyanobacterial blooms may restructure communities by selectively inhibiting sensitive groups while allowing more tolerant taxa to persist. These findings highlight the importance of considering species interactions and toxin-mediated effects when assessing bloom dynamics and ecological impacts in freshwater systems.

Acknowledgments:

This work was supported by contract 2024_069_IA_TENURE_1 (Portugal) and the Erasmus Mundus JMD programme (2022/23).

Resources or Predators? What Drives Toxin Production in Harmful Algae

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Abstract

Harmful algal blooms have increased in frequency and intensity in recent decades due to excessive nutrient enrichment and climate change, which influences environmental shifts. These events, driven by the rapid proliferation of toxin-producing microalgae have significant ecological, economic, and health impacts. Among the toxins produced, Phycotoxins produced by harmful algae accumulate in marine food webs and increase ecological and health risks, particularly through their transfer to higher trophic levels and their role in cases of shellfish poisoning. Despite recent advances in elucidating their biochemical pathways, the ecological mechanisms governing phycotoxin synthesis remain poorly understood. Two main hypotheses prevail: (I) bottom-up regulation, in which a nutrient imbalance promotes toxin production, and (II) top-down regulation, in which chemical cues from grazers induce phycotoxin to protect harmful algae from grazing.

This research will investigate the effect of nitrogen enrichment and copepod-derived chemical cues (copepodamides) on toxin production and bioluminescence in factorial experiments with three species of harmful dinoflagellates: *Alexandrium catenella*, *Protoceratium reticulatum* and *Gymnodinium catenatum*.

Based on current evidence, both species are expected to exhibit increased toxin production in response to nitrogen enrichment and exposure to copepodamides. *P. reticulatum spp.* is also expected to exhibit increased bioluminescence under these conditions. The project is ongoing; we expect bottom-up and top-down factors to synergistically regulate dinoflagellate inducible chemical defenses.

Acknowledgements

This work was funded by the Swedish Research Council grant to Erik Selander (VR 2025-06098), the "la Caixa" Foundation (ID 100010434) through a Junior Leader Retaining Fellowship (LCF/BQ/PR23/11980049), and Portuguese national funds from FCT—Foundation for Science and Technology through projects UIDB/04326/2020, UIDP/04326/2020, and LA/P/0101/2020; the operational programs CRESC Algarve2020 and COMPETE 2020 through project EMBRC.PT ALG-01-0145-FEDER-022121

Sessão Aplicações biotecnológicas de biotoxinas

Sessão de Posters

Screening of Marine Microalgae Biotoxins for Industrial Applications: Key Findings from the MiTOXin Project

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Introduction

Marine microalgae produce bioactive compounds, including phycotoxins relevant for food safety, environmental monitoring and biomedical research. These toxins are also essential for developing certified reference materials (CRMs). The MiTOXin project (MARINNONET) aims to identify toxin-producing strains suitable for industrial and analytical applications.

Materials and Methods

Approximately 80 strains and environmental samples from three European culture collections (CCVIEO, BEA-ULPGC and RCC) were screened. The analysed organisms included several harmful dinoflagellates (e.g. Alexandrium, Gambierdiscus, Karenia, Prorocentrum, Dinophysis, Protoceratium) and toxin-producing cyanobacteria (Microcystis, Nodularia, Anabaena). Biomass extraction was performed using optimised protocols adapted to toxin polarity, including solvent selection, multiple extraction cycles and controlled sonication. Extracts were analysed by targeted UPLC-MS/MS methods covering more than 10 toxin groups.

Results

A high diversity of toxin producers was identified. Alexandrium strains showed PSTs, Gambierdiscus produced gambierone-related compounds, Protoceratium reticulatum was confirmed as a YTX producer, and cyanobacteria showed microcystins and nodularin. Several strains were highlighted as promising candidates for toxin production.

Discussion and Conclusion

MiTOXin highlights the value of culture collections as sources of biotoxins and establishes a basis for CRM development. Selected strains are currently being cultivated for future applications in toxin monitoring and marine biotechnology.

Acknowledgements

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Cone snail peptides of two different superfamilies differentially modulate human trpv1 channels

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Introduction:

Cone snails are marine venomous gastropods characterized by possessing a potent cocktail of neurotoxic peptides, used both for hunting and self-defense, are a family of rich-cysteine short-to-medium sized peptides known as conotoxins. Among vermivorous cone snails, there is a group of species that are specialized in feeding on another group of venomous annelids of the family Amphinomidae known as fireworms.

Materials and Methods:

From the proteotranscriptomic study of two specialized fireworms eaters, *Rhombiconus imperialis* and *Rhombiconus fuscatus*, we selected two different conotoxins for further functional studies: CPFDPWCG (CON01-HMP, 1 disulfide bridge, superfamily O2-contryphan, *R. imperialis*) and YSCCPRIFSCCIIHS-NH2 (YS-15, 2 disulfide bridges, superfamily T, *R. fuscatus*). The folded peptides were obtained by solid phase peptide synthesis (SPPS) using the Fmoc/t-But approach to render the C-terminal carboxylic group either free (CON-01-HMP) or amidated (YS-15). Bioactivity was analyzed by whole cell patch clamp in human TRPV1 (hTRPV1) channels expressed in HEK293 cells.

Results:

Both peptides had opposite effects on hTRPV1 currents. CON01-HMP completely blocked hTRPV1 currents, this effect was not reverted in the presence of the endogenous hTRPV1 channel agonist anandamide. Moreover, CON01-HMP also blocked hTRPV1 currents induced by the agonist capsaicin and by the increase in intracellular ATP content. In contrast, YS-15 at 1 to 100 nM activated TRPV1 channels but this effect decreased at higher concentrations.

Discussion and conclusions:

All together, the findings presented in this work indicate that contryphans are highly potent inhibitors of hTRPV1 channels, a fact that highlights the need to pursue further biological activities of these peptides, due to the well-known implication of Transient Receptor Potential Vanilloid receptors in various chemical pathways involving, among others, temperature and pain sensation.

Acknowledgements

This research has received funding from: Conselleria de Cultura, Educación e Ordenación Universitaria, Xunta de Galicia, GRC (GI-1682-2025). From Ministerio de Ciencia, Innovación y Univ.es, number PID2022-138477NB-C21, PID2023-149618OB-I00, PID2024-159418OB-I00; ACF acknowledges a FPU scholarship. From Interreg EAPA-0032/2022 - BEAP-MAR, and EAPA_0130/2024 - REVALGAE. (cofunded by the EU), from European Union HORIZON-CL6-2023-CIRCBIO-01 COMBO - 101135438. From Univ. of Cadiz and Univ. of Cadiz project sol-202400284306-tra.

Biphasic effect of the marine toxins azaspiracids on human TRPV1 channels

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Introduction:

Polyether toxins bellowing to the azaspiracid (AZAs) group of toxins were first detected in Europe at the end of the 20th century after human poisonings reported in Ireland. Since then, several in vitro and in vivo studies were performed to characterize the mechanism of action of these compounds. Among the most intriguing effects of azaspiracids are their effects on ion channels since AZAs function as partial blockers of voltage gated ion channels and activated voltage regulated anion channels in an ATP-dependent manner. To gain more insight into the mechanism of action of azaspiracids, the possible action of these toxins on human Transient Receptor Potencial Vanilloid Receptor 1 (hTRPV1) was evaluated.

Materials and Methods:

Azaspiracid-1 analogue (AZA1, CAS number of 214899-21-5 and Azaspiracid-2 (AZA2, CAS number: 265996-92-7) with ≥98% purity were purchased from CIFGA (Lugo, Spain). The effect of both azaspiracids was analysed in HEK293 cells expressing hTRPV1 channels by whole cell voltage clamp recordings.

Results:

The results obtained indicated that both AZA-1 and AZA-2 elicited a biphasic response on TRPV1 channels, increasing the currents at the lower concentrations evaluated and lowering the effect at higher concentrations. Thus, AZA1 caused a maximum increase in TRPV1 currents at concentrations of 5 nM and 25 nM, and this effect decreased at higher concentrations (50, 100 and 200 nM). AZA-2 increased TRPV1 currents in a concentration dependent manner from 5 to 100 nM, while its effect decreased at 200 nM.

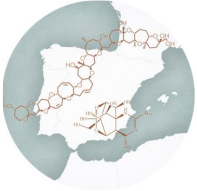
Discussion and conclusions:

TRPV1 is a nonselective ligand and voltage gated cation channel widely expressed in sensory neurons and involved in the detection of thermal, chemical, and nociceptive stimuli that can desensitize in the presence of the agonist capsaicin and other vanilloids. Although receptor desensitization cannot be excluded yet, other mechanisms triggered by azaspiracids such as calcium influx or other intracellular signaling cascades can underline the biphasic effect of azaspiracids on TRPV1 channels.

Acknowledgements:

This research was founded by Consellería de Cultura, Educación e Ordenación Universitaria, Xunta de Galicia, GRC (GI-1682-2025), Grant CPP2021-008447, MCIN/AEI/10.13039/501100011033, and by The European Union NextGenerationEU/PRT. From Ministerio de Ciencia, Innovación y Univ.es, PID2023-149618OB-I00, PID2024-159418OB-I00. From Interreg EAPA-0032/2022 – BEAP-MAR, and EAPA_0130/2024-REVALGAE (cofunded by the EU), from European Union HORIZON-CL6-2023-CIRCBIO-01 COMBO-101135438.

Sessão Monitorização, mitigação e apoio à decisão



Apresentações Orais

Seagrass meadows as a potential natural control of harmful algal blooms caused by toxic dinoflagellates

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Abstract

Biotic interactions are a key factor in the development of harmful algal blooms. A lower abundance of planktonic dinoflagellates has been recently observed in areas dominated by seagrass meadows compared to surrounding zones and several studies reported negative effects of compounds produced by seagrasses on cultures of planktonic dinoflagellates. However, the mechanisms underlying this allelopathic interaction are still poorly understood.

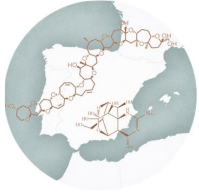
In this study, the potential role of meadows of the seagrass *Zostera marina* as modulators of the growth of toxic dinoflagellates was experimentally investigated, using cultures of *Alexandrium minutum* as a model species. The objective was to characterize the effects of seagrass exudates by examining its seasonal and spatial variability and exploring the potential allelopathic effect of the compounds produced and naturally released by the plants and the seagrass microbiome as possible mechanisms behind the negative seagrass-dinoflagellate interaction.

A. minutum growth rate was generally reduced following exposure to *Z. marina* exudates, and the inhibitory effect showed seasonal and spatial variability. While the primary effect appears to be caused by compounds released by the plant, its microbiome may modulate this interaction at some seasons of the year.

Our results suggest that seagrass meadows may play a role as natural regulators of toxic dinoflagellate proliferations, providing an ecosystem service that has so far received limited attention.

Acknowledgements

This study was supported by the State Investigation Agency of the Spanish Ministry of Science and Innovation for the BIOTOX (PID2021-125643OB-C21) and INTERES (CTM2017-83362-R) projects. Alexandra Díaz-Alonso was awarded a predoctoral fellowship from the Xunta de Galicia (ED481A-2020/197) and a predoctoral fellowship from the Spanish Ministry of Universities (FPU20/001100).



Implementation improvements for HILIC-MS/MS method for paralytic shellfish toxins detection

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Abstract

The ultra-high-performance hydrophilic interaction liquid chromatography with tandem mass spectrometry method for the determination of paralytic shellfish toxins (Turner et al. 2020) in bivalve shellfish has several problems. In particular, retention and resolution losses, as well as poor peak shapes for N-OH toxins, were observed after a limited number of injections. These problems are probably due to the degradation of the stationary phase in the Acquity UPLC BEH Amide/Glycan column due to the presence of water at concentrations up to 50% in the mobile phase, particularly under alkaline pH conditions. These deviations could be partially corrected by increasing both the organic phase percentage and the tampon concentration in the mobile phases. However, this also results in chromatographic problems and/or significant loss of sensitivity. To identify more robust alternatives, we tested other more stable columns under less aggressive chromatographic conditions. The best option was achieved using columns with a zwitterionic stationary phase and medium particle size, under acidic conditions. These circumstances also allow for work at lower pressures, which is always recommended. In addition, the optimal column and chromatographic conditions were tested for a fast and reliable method for tetrodotoxin analysis. The results are presented herein.

Acknowledgments

The present work was supported by funds from the Conselleria do Mar (Xunta de Galicia) within the framework of the MEMETOX Project CIMA 23/02.

Assessing Palytoxin and Ovatoxins Cytotoxicity through a High-Sensitivity Cell-Based Model

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Abstract

Palytoxin-like compounds like ovatoxins (OVTXs) are characterized by high cytotoxic potency and are naturally produced by dinoflagellates. Palytoxin (PLTX) and OVTXs share the same mechanism of action, which targets the Na^+/K^+-ATPase pump that commonly maintains sodium (Na^+) and potassium (K^+) ion gradients across the cell membrane of eukaryotic cells. These toxins bind to the pump and convert it into a constantly open non-selective ion channel, leading to osmotic imbalance and cell lysis. The symptoms concern excitable tissues and could be fatal, comprising paralysis and respiratory failure. The critical health risks associated with PLTX and OVTXs and their potential presence in seafood and in the environment highlight the need for relevant tools for cytotoxicity assessment.

The present study shows the setup of a cell-based assay (CBA) using Neuro-2a cells to evaluate palytoxin-like activity on eukaryotic cells and quantify the total toxin content both in culture and field samples of epiphytic benthic dinoflagellates collected from macrophytes in the South-East coast of Spain. Semi-purification of OVTX-a, OVTX-d, and OVTX-e was also performed through a fractionation of *Ostreopsis* cf. *ovata* culture extracts by HPLC to then compare their toxicity through CBA.

Results showed that CBA with ouabain treatment is a highly sensitive methodology that can be employed to assess cytotoxicity of PLTX and OVTXs paired with liquid-chromatography-mass spectrometry for confirmation. Moreover, the analysis revealed that OVTX-d exhibits the highest toxicity among the different OVTXs and PLTX, despite not being the most prevalent toxin in the commonly observed profiles.This study provides a sensitive toxicological approach to strengthen food safety and environmental monitoring strategies against potent marine neurotoxins that target the Na^+/K^+-ATPase pump.

Acknowledgements

This work relates to project LEVABENTOX 2, developed with the collaboration of the Biodiversity Foundation of the Ministry for Ecological Transition and the Demographic Challenge, through the Pleamar Programme, and is co-financed by the European Union through the European Maritime, Fisheries and Aquaculture Fund.

Predicting biotoxin contamination in shellfish through integrated modeling of satellite and in-situ data

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Introduction

Shellfish production in Europe is increasingly affected by harvesting closures caused by biotoxins associated with harmful algal blooms, leading to significant economic losses. Predictive models could improve planning and reduce the risk of unexpected closures, thereby minimizing the risk of economic losses.

Materials and methods

We propose a two-step model to predict shellfish contamination using the southern Portuguese coast as a case study. First, 2D convolutional autoencoders extract latent representations of sea surface temperature (SST) and chlorophyll-a (chl-a) variability from Sentinel-3 satellite images. These features are then combined with historical diarrhetic shellfish poisoning (DSP) records from IPMA, including time-lagged values, to predict maximum DSP toxin levels over variable forecast horizons using ensemble regression models.

Results

Combining satellite-derived features with historical DSP data improves predictive performance compared to models using historical contamination alone, capturing environmental patterns linked to toxin occurrence.

Discussion and conclusion

The framework can be applied to other coastal regions influenced by SST and chl-a dynamics and extended with additional environmental variables. This approach supports early warning systems and data-driven decision-making in shellfish harvesting and public health.

Acknowledgements

Supported by the project "Pacto da Bioeconomia Azul" (C644915664-00000026), WP2 Bivalves Vertical (Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan, Agendas for Business Innovation).

Advanced oxidation strategies for controlling harmful marine microalgae driven by emerging UV-LED sources

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Introduction

Harmful algal blooms (HABs) are increasing in frequency and intensity in coastal environments, posing significant risks to marine ecosystems, aquaculture, and public health. Effective strategies are therefore required to control harmful phytoplankton in aquaculture systems and prevent their spread through maritime transport and ballast water. This study aimed to evaluate the effectiveness of oxidation-based treatments for the control of HAB-related microalgae. Specifically, the work investigated (i) the biocidal performance of several oxidants and (ii) the potential of UV-LED irradiation combined with H_2O_2 as an advanced treatment strategy for microalgal inactivation.

Materials and Methods

Laboratory experiments were conducted using harmful marine microalgae species including *Heterosigma akashiwo, Prorocentrum lima, and Prymnesium parvum*. The effects of different oxidants (H_2O_2, peracetic acid, peroxymonosulfate, peroxydisulfate, and chlorine) were evaluated through concentration-response assays over a 14-day incubation period. Additionally, photochemical treatments using UV-LED irradiation at 265 and 275 nm were tested individually and in combination with H_2O_2, including simultaneous and sequential exposure strategies. Biological responses were assessed through growth inhibition and photosynthetic quantum yield measurements.

Results

Chemical oxidation assays showed that H_2O_2, peracetic acid, and peroxymonosulfate effectively inhibited microalgal growth, whereas peroxydisulfate exhibited negligible activity. H_2O_2 showed sustained inhibitory effects, although species exhibited different sensitivities. *P. parvum* was the most sensitive species (EC_{50} = 6.67 mg/L), followed by *H. akashiwo* (EC_{50} = 7.08 mg/L), whereas *P. lima* showed markedly higher resistance (EC_{50} = 21.4 mg/L). UV-LED irradiation produced species-dependent responses following the same sensitivity trend, causing growth inhibition and impairment of photosynthetic activity, particularly at 275 nm. The combined UV-LED/H_2O_2 treatment achieved the highest inactivation levels, with growth inhibition exceeding 80% for *H. akashiwo* and approximately 70% for *P. lima*.

Discussion and Conclusion

Combined treatments enhanced efficacy through cumulative cellular damage caused by UV-induced stress and oxidative exposure. Overall, integrating chemical oxidants with UV-LED technology represents a feasible and environmentally compatible strategy for controlling toxic microalgae and associated risks.

Acknowledgement

Project MOBILED (PCM_00015) co-funded by the Regional Government of Andalusia and by the European Union NextGenerationEU/PRTR. C.17, I1.

Building analytical capacity for marine biotoxin monitoring: integrating regulatory methods and HRMS-based approaches in Portugal

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Abstract

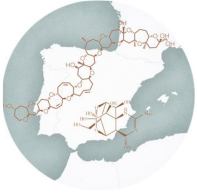
Marine biotoxins represent a significant concern for food safety, public health, and the sustainability of shellfish harvesting. In Portugal, monitoring frameworks primarily target regulated toxin groups—amnesic shellfish toxins (AST), lipophilic toxins (OA, DTX, YTX and AZA), and paralytic shellfish toxins (PST)—yet remain largely constrained to targeted analytical approaches.

In this work, we present the establishment of a marine biotoxins laboratory, boosted by a signed cooperation protocol between CIIMAR and IPMA. The laboratory is progressing towards ISO 17025 accreditation through the implementation and validation of robust LC-MS/MS methods for regulated toxins, contributing to the reinforcement of national biotoxins analytical capacity.

Targeted LC-MS/MS methods enable the simultaneous detection of multiple toxin groups, supporting monitoring activities and timely management decisions. In parallel, the integration of high-resolution mass spectrometry (HRMS) introduces untargeted analytical capabilities, enabling the systematic acquisition of high-resolution datasets for all analyzed samples and facilitating data reuse across monitoring and research activities.

These datasets support the development of spectral libraries, currently lacking at national level, enabling retrospective analysis and facilitating the identification of emerging or previously unmonitored biotoxins.

This integrated approach contributes to the evolution of marine biotoxin monitoring towards more comprehensive and proactive risk assessment frameworks, reinforcing analytical capacity and supporting public health protection in Portugal.



Monitoring and control of *Ostreopsis* blooms in Catalan beaches

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Introduction

In the Mediterranean, *Ostreopsis* blooms have been associated with upper respiratory tract irritation in people who spend several hours near affected beaches. In some cases, they have caused health problems in significant numbers of individuals (e.g. Italy, France, Spain and Algeria). Directive 2006/7/EC and Royal Decree 1341/2007 on the management of bathing water quality establish the need to control massive blooms of phytoplankton and to inform bathers of their associated risks.

As *Ostreopsis* blooms are frequent and recurrent every year in Catalonia, and can be harmful, the Regional Government (ACA) in coordination with ICM-CSIC control them during the bathing season.

Material and methods

Technicians responsible for sampling BHABs received a training session by the scientific team. A coordination system was established to share the data, observations and photographs obtained during the sampling, as well as, the results of the *Ostreopsis* abundances. These were categorized in five levels including an alert and a threshold for risks to human health arising from *Ostreopsis*-related symptoms.

Results

The microalga *Ostreopsis* has been detected at all the sampling stations during the bathing seasons analysed (2020-2025). Furthermore, the alert or risk threshold was exceeded at more than half of the stations. It is noted that although respiratory irritations are often common in Sant Andreu de Llavaneres (a hotspot for *Ostreopsis* blooms), they have only been detected sporadically at other stations.

Discussion and conclusion

Ostreopsis blooms pose a risk to human health; therefore, to manage them effectively, it is required to establish reference thresholds to warn of potential risks. This study has used values obtained from previous research carried out in various parts of the Mediterranean, but given the relatively new nature of *Ostreopsis* blooms, it is necessary to increase the observations to adjust them. It should also be noted that coordination between the ICM-CSIC and the ACA has enabled

us to identify skin reactions in bathers in areas where *Ostreopsis* blooms and to rule out this genus as the causative agent.

Acknowledgements

We thank the work carried out by the different samplers hired by the ACA. Financial support was provided by the ACA (CTN2100434, CTN2100814, CTN2400085), ECOS project (PID2022-138753OB-C21) and Severo Ochoa Centre of Excellence accreditation (CEX2024-001494-S).

The International Phytoplankton Intercomparison Exercise (IPI)

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Abstract

Participation in phytoplankton proficiency exercises is necessary to ensure the validity of phytoplankton analysis. The IPI exercise has been running since 2015 as the continuation of the previous BEQUALM and NMBAQC exercise and counts on the continued collaboration of the IOC Science and Communication Centre on Harmful Algae since 2011. The lack of accredited reference materials for phytoplankton identification and quantification highlights the importance of these intercomparisons as the only available method for the estimation of the method uncertainty in each laboratory.

The exercise consists of a phytoplankton taxonomy assessment (online HAB quiz) using the IOC online platform 'Ocean Teacher Global Academy' (OTGA) and the analysis of the species abundance and composition in marine water samples. Samples are produced using a mixture of ten microalgae cultures from different taxa belonging to harmful and non-harmful species. Statistical analysis is carried out using PROlab Plus using the methods laid out in ISO13528.

The total number of participants ranges between 75 and 122, variable depending on the year (2016-2024). The results of the taxonomic assessment in the last 10 years show that on average 66% of the participants are proficient, 23% are good, 7% are acceptable and 4% needs improvement. Analyst performance on the correct composition of species in the samples is generally quite good. On average, 93% of the participants per year, pass the identification and quantitation test that requires at least 80% of the results to be correct to pass this test with Z-scores between -2 and +2 STDev. The IPI Advisory Expert Committee resolves the queries from participants when there are issues on questions that may have different interpretations.

The IPI proficiency exercise is designed to assess analyst performance through a very comprehensive set of evidence. The results of the test also highlight the need for improvement if the description of some species as well as for the production of reference materials.

Acknowledgements

We would like to express our gratitude to the participants in the International Phytoplankton Intercomparison Exercise (IPI) as well as to the institutions hosting it.

Dinâmica Tidal de Espécies Potencialmente Tóxicas e Interações Predador-Presa em Sistema Estuarino Eutrófico: Implicações para Monitorização de Florações Algais Nocivas

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Resumo:

Sistemas estuarinos eutróficos sob forte pressão antropogênica são ambientes propícios ao desenvolvimento de florações algais nocivas (HABs), sendo a dinâmica tidal um fator-chave na distribuição de espécies potencialmente tóxicas. Este estudo teve como objetivo caracterizar a dinâmica de dinoflagelados e ciliados tintinídeos ao longo de um ciclo tidal completo, com foco nas implicações para monitorização de HABs. Foi realizada uma série temporal de 25 horas em estação fixa no Canal Estuarino de Santos (Brasil), com amostragens bihorárias de microplâncton e medições físico-químicas associadas. Foram identificadas 14 espécies de tintinídeos e 9 de dinoflagelados. Dinophysis caudata foi detectada exclusivamente durante maré alta, confirmando a advecção tidal como mecanismo de importação de espécies DSP para o estuário. A dinâmica predador-presa revelou padrões antagônicos entre dinoflagelados e tintinídeos, modulados pela maré. Abundâncias máximas de dinoflagelados ocorreram durante maré alta, correlacionadas com a altura da maré ($r = +0,80$; $p = 0,002$), enquanto tintinídeos atingiram maiores densidades durante vazante, associadas à produtividade in situ em condições hipereutróficas. A co-ocorrência entre dinoflagelados e seus predadores durante maré alta sugere potencial controle top-down da comunidade importada. Os resultados indicam que a advecção tidal é o principal mecanismo de introdução de espécies HAB no sistema, com risco de estabelecimento sob condições favoráveis. Esses achados evidenciam a necessidade de estratégias de monitorização que considerem a variabilidade tidal, recomendando amostragens pareadas entre enchente e vazante para avaliação mais precisa do risco toxigênico.

Prevalent blooms of the harmful dinoflagellate *Ostreopsis* sp. in the south-east Bay of Biscay: A multi-technical approach

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Abstract

The benthic dinoflagellate *Ostreopsis* has become a prevalent and abundant genus during the warmer months in the Bay of Biscay. In fact, the co-occurrence of both *Ostreopsis* sp. 9 and *Ostreopsis* cf. *ovata* has been confirmed in the south-east Bay of Biscay, with the latter linked to multiple health issues reported since 2020 in the area. The limitations of light microscopy highlight the necessity of molecular techniques for accurate differentiation among species of *Ostreopsis* with varying toxic potentials. The present study aimed to assess the succession of *Ostreopsis* spp. in five sites in the south-east Bay of Biscay over three annual cycles through a multi-technical approach.

The most intense blooms occurred from July to September, when water temperatures were highest, with maxima of 1.86×10^6 cells/g and 1.33×10^5 cells/L in San Sebastian. Sites featuring rocky substrates with low complexity macroalgal communities, like Mutriku, and especially San Sebastian, recorded the most intense and prevalent blooms of *Ostreopsis* spp. The eDNA metabarcoding results revealed the dominance of *Ostreopsis* as the principal photosynthetic genus at San Sebastian, with a maximum relative abundance of 12.6 % within the eukaryotic community. Both *Ostreopsis* sp. 9 and *Ostreopsis* cf. *ovata* were identified, with the latter being more prevalent. The qPCR results for *O.* cf. *ovata*, with a maximal detection of 10^4 cells/L, exhibited a consistent pattern when compared to those obtained by microscopy and metabarcoding. The toxin profiles of the *O.* cf. *ovata* strains isolated from San Sebastian were comparable to those reported for Mediterranean strains. In view of the association of these toxins with multiple health issues along the Mediterranean coasts and the south-east Bay of Biscay, this finding highlights the necessity for continuing a rigorous monitoring of this species in the area. Finally, the results of this study underscore the importance of combining the microscopic approach with molecular tools to monitor and establish early warning systems for *Ostreopsis*.

Acknowledgements

The present work was carried out in the frame of Y. Laurenns-Balparda´s PhD thesis, financed by the Basque Government. This study was supported by the Ostreobila project, financed by the Interreg POCTEFA programme, URA (Basque Water Agency), Gipuzkoa Provincial Council and San Sebastián City Council.

Risk assessment of Harmful Phytoplankton Blooms in the NW Mediterranean Sea

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Introduction

Harmful algal blooms (HABs) in coastal areas pose risks to public health and interfere with recreational coastal activities. Although HABs can be natural phenomena, their frequency and intensity may increase under intense coastal exploitation. Assessing HABs risk and determining its variability is critical for identifying risk areas and the local pressures that may favour bloom development. Here, we asses HABs risk in two different coastal environments identifying high-risk areas along the NW Mediterranean Sea integrating seasonal variability.

Materials and Methods

This study assessed HABs risk along the Catalan coast (NW Mediterranean) by analysing long-term phytoplankton monitoring data from harbours (2000–2012) and beaches (2000–2023). Specific risk thresholds were defined for each species and for each environment type. These thresholds were based on the abundance levels at which the species are known to cause adverse effects and were defined for i) toxic species (health risk), ii) species harmful to marine life, and iii) those that degrade water quality and affect tourism.

Results

All sampled harbours exhibited bloom risk in some degree, with high-risk sites distributed all along the Catalan coast and dominated by toxin-producing dinoflagellates. In contrast, risk at beaches was more spatially heterogeneous, with high-risk zones mainly in the north of the coast and associated with high-biomass blooming species. Bloom risk peaked in spring and summer, in harbours, where conditions promote water-column stability and bloom development.

Discussion and Conclusion

Although thresholds must be tailored to local species, their harmful effects, and the affected environments, this approach provides a useful tool for identifying high-risk areas. It can help prioritise sites for intensified monitoring and guide further investigation of local pressures that may explain the observed risk patterns. The development of these risk maps also enables the monitoring of potential changes over time.

Acknowledgements

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Transforming HAB Monitoring in the Azores: Early Insights from the MeTis Project Using Shotgun Metagenomics

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Introduction

Harmful algal and cyanobacterial blooms (HABs) are increasing worldwide, including in the Azores, driven by eutrophication and climate change. Regional monitoring remains limited in taxonomic resolution and analytical capacity.

The MeTis project addresses these gaps by integrating metabarcoding, high-resolution chromatography and remote sensing to develop improved biotoxin monitoring tools for Azorean aquatic systems.

Materials and Methods

Eight freshwater lakes and four coastal sites on São Miguel Island were sampled, and surface water was filtered within 24 h. eDNA was extracted using a magnetic bead-based kit and sequenced via shotgun metagenomics (Illumina, 6 Gb per sample).

Bioinformatic analysis was performed using Kraken2 and Braken, with the standard databases, for taxonomic assignment, followed by decontamination and downstream analyses in R (Phyloseq for community composition and Vegan for PERMANOVA and PCoA).

Results

Metagenomic profiles revealed clear separation between freshwater and marine communities.

Freshwater systems showed higher diversity and dominance of bloom-forming cyanobacteria (e.g., *Dolichospermum*, *Microcystis aeruginosa*) and *Polynucleobacter*. Marine samples displayed lower diversity and strong *Acinetobacter* dominance.

Discussion and Conclusion

These preliminary results highlight the ecological complexity of Azorean waters and the need for high-resolution monitoring.

Ongoing work includes toxin profiling and eukaryotic metagenomics, while upcoming campaigns will incorporate 16S/18S metabarcoding (Illumina and PacBio) and the development of remote-sensing tools to enhance HAB detection and forecasting.

Acknowledgements

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Monitoring data of marine toxins in Portugal over the last decade – improving the cost/effort ratio in the management system

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Abstract

Temporal and spatial trends of paralytic shellfish toxins (PSTs), amnesic shellfish toxins (ASTs) and lipophilic toxins of okadaic acid group (OA+DTXs) in bivalves from portuguese estuarine and coastal waters were examined using long-term data from the national biotoxin monitoring programme (2014–2025). The use of long-term data from monitoring is crucial to detect shifts in phytoplankton species composition and dynamics of toxin contamination in bivalves.

The aim of this study is to identify production areas with higher risk of toxins contaminating bivalve intended for human consumption. On the other hand, areas with lower risk may have their sampling frequency reviewed, optimizing resources. Decisions regarding prioritizing areas for sampling and analyses to be performed leads to a robust monitoring system improving the cost/effort ratio, namely the management of highly specialized human resources.

Data were chosen for indicator bivalve species harvested in the 38 (2014) – 34 (2025) production zones of the portuguese coast. The results were obtained using the official EU methodologies: liquid chromatography (LC) with mass detection (OA+DTXs); LC with ultraviolet detection (ASTs) and LC with fluorescence detection (PSTs).

From a total of 25,885 samples analysed results above the regulatory limit for each group of toxins were considered in order to establish a risk scale (high, moderate, low) for each production zone.

Peniche coastal area and Ria de Aveiro were the production zones found to be high-risk for the three groups analysed and, therefore, considered priority zones for toxin analysis in the monitoring system.

In opposition, Sado estuary, identified as a low-risk zone, had no occurring episodes of AST and PSTs in 11 years, an exception is a sample that exceeded the RL for OA+DTXs in 2024.

The sampling frequency of areas of moderate-risk for OA+DTXs (Ria Formosa – Olhão, Faro, Fuzeta; Lima, Mondego and Tagus Estuaries) eventually may be reviewed, or nearby coastal areas that act as warning zones may be identified, contributing to a reduction in sampling effort and costs. Identifying areas of higher-risk with regard to PSTs (Setúbal coastal area) and ASTs

(Matosinhos and Aveiro coastal areas) could contribute to prioritizing analyses, leading to faster results for these areas.

Acknowledgements

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End-user product design of a Bivalve Aquaculture Contamination Forecasting Tool

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Introduction

Closures of bivalve harvesting areas due to biotoxin contamination are essential to protect public health but frequently result in significant economic losses. In this context, the MultiAlive project aims to develop tools for toxin detection, modelling and forecasting in the southwestern coast of Portugal, with the goal of supporting producers in managing offshore production and mitigating economic impacts. As main end-users of the knowledge generated by the project, producers are to be engaged from the start, co-tailoring communication tools, to ensure the full exploitation of scientific developments. Given its operational needs, it is important that the message to be delivered is tailored to be promptly understood, and the selected channels are accessible and effective.

Material and Methods

First steps of the communication plan have been addressed internally, by mapping significant stakeholders, and discussing briefly several communication engagement tools. The forecasting service design is planned to be interactive, involving periodic individual meetings with producers, and a World Cafe session, where several activities will allow the incorporation of new ideas, promote a two-way engagement and discussion of the project outputs and facilitate the adoption of tools and prototypes developed as outcomes of the project. REDIBAL conference expert audience can serve as focus group for evaluating diverse communication formats also. Collection of insights will follow also an interactive way (e.g. Slido, Mentimeter), to collect real-time expert opinions regarding different distribution channels, periodicity and content of the forecast communication tool.

Results, Discussion and Conclusions

Results from the interactive engagement with REDIBAL expert forum will provide important insights regarding the design of bivalve contamination forecasting tools planned in MultiAlive project.

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Umbrales de actuación de *Alexandrium minutum* y *Gymnodinium catenatum* para la gestión del riesgo de biotoxinas paralizantes en las Rías Gallegas a partir de 30 años de monitorización

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Resumen

Las proliferaciones de dinoflagelados productores de toxinas paralizantes representan un riesgo para la salud pública y la acuicultura en las Rías Gallegas. El objetivo de este estudio es definir umbrales de concentraciones celulares de fitoplancton en la columna de agua para mejorar la gestión del riesgo en los planes de actuación de las zonas de producción de moluscos.

Se analizaron datos de concentraciones celulares (Utermöhl) de *A. minutum* y *G. catenatum*, obtenidos mediante muestreo semanal durante 30 años en 48 estaciones oceanográficas. Se validó la taxonomía de *A. minutum* por identificación de sus placas tecales en cada episodio y se diferenció *G. catenatum* de otros dinoflagelados formadores de cadenas. Se recopilaron datos del estado administrativo (abierto/cerrado) de las subzonas de producción de moluscos en relación con toxinas PSP. Se aplicaron métodos estadísticos y probabilísticos para estimar las concentraciones umbral para cada especie.

A diferencia de otras especies tóxicas, con sólo la presencia de *A. minutum* y *G. catenatum* en las muestras de red de fitoplancton se realiza la activación de las primeras alertas tempranas, debido a su rápida evolución y elevada toxicidad. Se observa una relación significativa (ρ=0,80 n=107 y ρ=0,83 n=104, respectivamente) entre la concentración celular en el agua y los cierres en las zonas de producción. Para una probabilidad conservadora del 80% de cierre en la misma semana en los polígonos de bateas próximos a la estación oceanográfica, se estimaron umbrales de 24 500 cel/L para *A. minutum* y 1 360 cel/L para *G. catenatum*. Para un nivel de alerta temprano (probabilidad de 50%), las concentraciones fueron de 19 300 y 1 040 cel/L, respectivamente.

Las diferencias observadas en los umbrales entre ambos dinoflagelados paralizantes se relacionan con su tamaño, el contenido de toxina por célula y las condiciones oceanográficas. La monitorización de fitoplancton tóxico es obligatoria en las zonas de producción en la Unión Europea y constituye una herramienta clave para la gestión del riesgo. Los umbrales probabilísticos definidos, basados en 30 años de datos, mejoran la capacidad predictiva del sistema de monitorización y refuerzan los planes de actuación en las Rías Gallegas. Su aplicación permite optimizar la toma de decisiones incluyendo el ajuste del muestreo y el establecimiento

de cierres cautelares, y contribuye a la protección de la salud pública y la seguridad alimentaria. Este enfoque es transferible, si bien los umbrales deben de ser específicos para cada tipo de biotoxina, especie de fitoplancton tóxico y ecosistema.

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Bioanalytical Solutions for the Analysis of Seafood with Co-Existing Toxins: Paralytic Shellfish Toxins and Tetrodotoxins

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Introduction

Paralytic shellfish toxins (PSTs) and tetrodotoxins (TTXs) are two highly potent toxin families. Due to the significant risk that PSTs pose to public health, they are routinely monitored in shellfish. In contrast, TTXs are not yet included in monitoring programs in most European countries. This gap is particularly concerning because both toxin groups share the same molecular target and mechanism of action; consequently, their toxic effects may be comparable and potentially additive. As a result, analysis focused on a single toxin family may not provide adequate protection in cases of toxin co-occurrence.

Materials and Methods

To address this issue, two novel bioanalytical strategies are proposed for the simultaneous detection of PSTs and TTXs. The first approach involves a multiplexed immunosensor for toxin screening, which integrate two immunoassays within an electrode array and incorporating internal controls aligned with regulatory/safety thresholds. The second approach consists of a single-cell, automated patch-clamp biosensing platform that assesses the overall toxic potency of a sample by measuring the inhibition of sodium currents in Neuro-2a cells.

Results, Discussion and Conclusion

After a proper development, optimisation, and validation through analysis of samples, both bioanalytical approaches proved to be effective for the screening and quantification of PSTs and TTXs in naturally contaminated seafood samples. The sample collection included both pufferfish and shellfish, including some with co-existence of PSTs and TTXs. These techniques represent an important step forward toward more integrative analytical strategies, ultimately enhancing food safety and public health protection.

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Sessão de Posters

Anomalias na ocorrência de *Dinophysis* e DSTs: Lagoa de Albufeira, o local mais atípico em Portugal

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Resumo

No contexto das alterações climáticas, é necessário compreender a dinâmica de eventos anómalos e/ou extremos de fitoplâncton nocivo. O programa de monitorização semanal do IPMA de fitoplâncton nocivo e biotoxinas marinhas em bivalves de Portugal desde 2013, permitiu observar um bloom atípico na Lagoa de Albufeira de *Dinophysis acuminata* complex e *Prorocentrum* spp., produtores de toxinas lipofílicas do grupo do ácido ocadáico (Diarrhetic Shellfish Toxins- DSTs). Este local estuarino/lagunar é particular, com renovação hídrica limitada, abertura sazonal ao mar e frequente estratificação, que apresenta frequentemente distribuições de *D. acuminata* complex com máximos anuais durante o Inverno, em contraste com o restante país. Durante o Outono/Inverno 2025-2026, observaram-se abundâncias muito elevadas dos Dinoflagelados estudados, especialmente *D. acuminata* complex que ultrapassou 33000 cél. L^{-1} em dezembro 2025, quando a média nesse mês para 2013-2024 é de 280 cél. L^{-1} (máximo 2500 cél. L^{-1}). Este bloom atípico prolongou-se mais de um mês, observando-se 8000 cél. L^{-1} em janeiro 2026 (quando a média nesse mês para 2013-2024 é de 100 cél. L^{-1} e máximo 1700 cél. L^{-1}). Esta ocorrência atípica de *D. acuminata* foi inesperada, pois começou no final de Novembro a seguir a uma semana com precipitação acima de 100 mm, o que geralmente induz condições de turbulência muito desfavoráveis a Dinoflagelados. Ao longo da série temporal 2013-2025 na Lagoa de Albufeira, apenas 46% dos máximos anuais de toxicidade DST na espécie de bivalve indicadora (*Mytilus*) ocorreram nos meses de Abril a Outubro, enquanto por exemplo, no sistema estuarino-lagunar da Ria de Aveiro (costa noroeste) com forte contaminação por DSTs, 100% dos máximos anuais de toxicidade DST ocorreram nesses meses. Na costa oeste de Portugal, a toxicidade DST em bivalves no período outonal é causada maioritariamente por *Dinophysis acuta*, enquanto na Lagoa de Albufeira esta espécie não é dominante. Os resultados aqui apresentado podem contribuir para antecipar potenciais eventos extremos de populações "adormecidas" e isoladas, minimizando o risco de exposição atípica de consumidores humanos a DSTs.

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Hydrodynamic connectivity of *Gambierdiscus* and *Fukuyoa* in the Mediterranean Sea: evaluating introduction pathways

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Abstract

Benthic dinoflagellates *Gambierdiscus* and *Fukuyoa*, associated with Ciguatera Fish Poisoning (CFP), are increasingly reported in the Mediterranean Sea. To understand their potential introduction pathways, we used a Lagrangian connectivity framework (OceanParcels tracking with Copernicus currents, 1998–2018) to compare natural entry through the eastern gateway (Suez Canal) versus the western gateway (Strait of Gibraltar).

Our simulations reveal that particles released from Suez efficiently reach eastern targets (Cyprus/Crete in 1–3 years) but show negligible connectivity to the western Mediterranean. Thus, long-range westward dispersal from Suez is highly unlikely under modeled circulation. Conversely, releases from Gibraltar rapidly reach the Balearic Islands and the Spanish coast (<1 year) and spread eastward to the Levantine basin within 5–7 years.

These hydrodynamic patterns support two non-exclusive scenarios: (i) a primary entry through Gibraltar followed by basin-wide eastward spreading, establishing earlier in the warmer eastern waters; or (ii) independent introductions through both gateways. While human-mediated dispersal and biological traits remain unsimulated, these findings provide a critical physical baseline for Mediterranean CFP risk assessment.

Acknowledgements.

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EarlyTOX: Rapid molecular strategies for early detection of HAB's in bivalve production

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Introduction

Bivalve aquaculture is a rapidly expanding and sustainable sector of seafood production. However, the increasing frequency of harmful algal blooms (HABs) have raised concerns regarding the accumulation of marine biotoxins in shellfish. Current monitoring programs are time-consuming, labour-intensive, and highly dependent on taxonomic expertise, which can delay risk assessment and regulatory decisions. The EarlyTOX project addresses these limitations by developing a rapid molecular detection approach based on quantitative real-time PCR (qPCR) and digital PCR (dPCR) technologies to detect and quantify toxin-producing microalgae in aquaculture environments.

Results

Within EarlyTOX, specific molecular assays are being designed to target the most relevant species associated with HAB events in Portuguese bivalve production areas. Both general and species-specific primers were developed and optimized for qPCR detection, as well as multiplex assays for simultaneous detection of several taxa in a single reaction. Successful optimization was already achieved for *Gymnodinium catenatum*, *Gamberdiscus,* and *Ostreopsis*. dPCR will then be optimized to enable highly sensitive and accurate identification and quantification of target species in more complex environmental samples and results will be compared with monitoring data issued by The Portuguese Institute for the Ocean and Atmosphere (IPMA). This will validate the molecular tool under real-world conditions and evaluate its capacity to support early detection and risk assessment during HAB events.

Discussion and Conclusions

The implementation of molecular monitoring tools represents a significant advance for HAB surveillance in aquaculture systems. qPCR offers important advantages over traditional microscopy, including higher sensitivity and specificity, while reducing reliance on specialized taxonomic expertise. By enabling rapid detection and quantification of toxin-producing microalgae, the EarlyTOX project can improve early warning capacity and support more timely management decisions in bivalve production areas. Overall, EarlyTOX aims to deliver an

innovative and reliable diagnostic tool that complements existing monitoring systems, supporting the sustainability, economic resilience, and public health protection of the bivalve aquaculture sector under changing environmental conditions.

Acknowledgments

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Monitoring of Harmful Phytoplankton Species in the Port of Bilbao

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Abstract

Port ecosystems represent strategic environments for the surveillance of harmful phytoplankton species (Harmful Algal Blooms, HABs), due to elevated anthropogenic pressures and intense maritime traffic, which enhance both species introduction and via ballast waters and subsequent dispersal. In this framework, the implementation of robust and sensitive monitoring strategies is critical for the early detection of HAB events and the mitigation of their impacts on marine ecosystems, port operations, and public health. This study assesses the applicability of an integrative monitoring approach combining light microscopy and DNA metabarcoding for the detection and characterization of harmful phytoplankton communities in the Port of Bilbao over the period 2024–2025. A total of 20 sampling campaigns were conducted at three stations (BIL-1, BIL-2, and BIL-3), selected to encompass a wide range of hydromorphological and operational conditions, with a temporal resolution of approximately one to one and a half months. Surface water samples were collected at 1 m depth, approximately 3 hours post high tide, to ensure representative and hydrodynamically comparable phytoplankton assemblages. Metabarcoding analyses across both years enabled the detection of over 60 potentially harmful phytoplankton taxa, including both toxic and bloom-forming species, distributed among 35 genera and showing a relatively homogeneous spatial distribution among sampling stations. Dinoflagellates and diatoms, with additional contributions from haptophytes, dictyochophytes, chlorophytes, raphidophytes, and pelagophytes, dominated taxonomic composition. The recurrent detection of high-risk genera such as *Pseudo-nitzschia*, *Dinophysis*, and *Alexandrium* underscores the necessity of sustained monitoring efforts in port environments. Complementarily, quantitative cell counts obtained via light microscopy allowed the identification of multiple episodes exceeding alert thresholds, indicating potential toxicity risk associated with these genera. Overall, these results demonstrate the effectiveness of a combined microscopy–metabarcoding framework for enhancing taxonomic resolution, sensitivity, and early detection capacity in HAB monitoring programs. This integrative approach constitutes a robust tool for supporting early warning systems and informed decision-making, ultimately contributing to improved risk management of HAB events in port ecosystems.

AI-BIOdiv Project – Automated Imaging for Marine Biodiversity

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Introduction

Phytoplankton is fundamental to marine ecosystems yet its monitoring in coastal areas, including potential harmful taxa, relies on time-consuming traditional microscopic analyses. This study evaluates the performance of a low-cost, open-source imaging system (PlanktoScope) combined with AI-based classification (EcoTaxa), including a comparison with advanced imaging systems and light microscopy observations.

Materials and Methods

Samples have been collected since February-March in two contrasting coastal environments in Brittany (Roscoff, France) and Galice (Ría de Vigo, Spain). Phytoplankton communities (<200 µm) have been analyzed using advanced imaging systems (IFCB, CytoSense, FlowCam), together with the PlanktoScope and Utermöhl cell counts. Calibration and validation will be performed using reference cultures from RCC and CCVIEO, and AI models will be trained and validated using the EcoTaxa platform.

Results

The preliminary analysis of phytoplankton samples shows that Planktoscope provides useful information to assess phytoplankton biodiversity despite a lower optical resolution in comparison with advanced imaging systems tested.

Discussion and Conclusion

AI-BIOdiv aims to provide a cost-effective, accessible, and scalable approach to marine biodiversity monitoring with Planktoscope.

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Harmful Algae Grazing Fear: A Tool to Predict Bivalve Biotoxin Contamination

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Abstract

Harmful Algal Blooms (HABs) threaten coastal ecosystems and aquaculture by contaminating seafood with biotoxins, leading to economic, environmental, and public health risks. Current methods for predicting biotoxin accumulation in bivalve molluscs are limited. This project proposes an innovative approach to predict biotoxin accumulation in bivalve molluscs by integrating insights from predator-prey chemical interactions, specifically through copepodamides (polar lipids produced and released by copepods) that induce defensive traits (e.g.,toxin production and bioluminescence) in harmful algae. We intend to (1) determine the copepodamides profile of copepods from the NE Atlantic and evaluate the effects of feeding and temperature on their production by copepods; (2) isolate copepodamides from natural populations; (3) assess copepodamides toxin and bioluminescence induction in dinoflagellates and if these inductions are strain- and/or temperature-dependent; (4) measure copepodamides concentrations in bivalve molluscs and passive samplers, alongside harmful algae cell counts and oceanographic data, to optimize bivalve molluscs biotoxin contamination predictive models. The present project will improve the accuracy of predictions regarding bivalve molluscs contamination by biotoxins. This will help determine optimal harvesting periods, preventing unnecessary harvesting costs and the destruction of contaminated products. The outcomes will have significant socio-economic benefits for the bivalve molluscs industry and play a crucial role in preventing shellfish poisoning. This abstract presents a granted PhD research plan.

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Machine Learning classification approaches for the early detection of DSP-producing Dinophysis blooms in the Gulf of Cádiz

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Abstract

Harmful algal blooms (HABs) caused by *Dinophysis* spp. and associated diarrhetic shellfish poisoning (DSP) represent a recurrent threat to bivalve aquaculture and public health along the southwestern Iberian coast. The development of operational early warning tools for this region remains limited, despite its ecological and socioeconomic relevance.

Phytoplankton abundance (cell/mL) data were retrieved from the *Junta de Andalucía* shellfish production areas and its monitoring programme, covering three coastal stations in the Gulf of Cádiz associated with the Guadiana (St102) and Guadalquivir (St106-107) river basins, for the period 2018–2023. Different evironmental predictors were included (e.g., satellite-derived data, global ocean reanalysis products, a coastal upwelling index transport (CSET), among others), and bloom events of DSP-producing species (*D. acuta*, *D. caudata*, *D. fortii*, *D. sacculus*, and the *D. acuminata* group) were defined following previous studies, using a threshold of 5% above the annual local median abundance, sustained over at least one consecutive week.

A preliminary comparison of logistic regression and decision tree classifiers, applied independently to each station, showed moderate discriminant capacity (AUC 0.62–0.65), with decision trees achieving substantially higher bloom detection sensitivity (0.49–0.52) than logistic regression (0.08–0.10). The most influential predictors were photosynthetically available radiation (PAR), mixed layer depth (MLD), sea surface temperature (NSST), and sea surface salinity (SSS), consistent with findings reported for adjacent southern Portuguese coastal waters. Decision tree rules revealed station-specific environmental thresholds: in the Guadiana basin, bloom occurrence was associated with high PAR, low SSS, and NSST above ≈17.6°C, reflecting the influence of riverine freshwater input; in the Guadalquivir basin, blooms were excluded at NSST ≥ 24.1°C and favoured under shallow mixed layers (MLD < 10.7 m), pointing to a well-defined thermal and stratification window for *Dinophysis* development.

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Resultados del programa de control de microalgas tóxicas y biotoxinas en la costa de Andalucía en los años 2024 y 2025.

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Introducción

Presentación de los principales eventos microalgales asociados o no a toxicidad en moluscos durante el último bienio.

Material y métodos

Identificación y recuento del fitoplancton tóxico basado en el método Utermöhl.

Determinación de ácido domoico con detector UV (CL-UV).

Análisis de toxinas paralizantes (PSP) por cromatografía líquida con detector de fluorescencia (LC-FLD, oxidación precolumna).

Análisis de toxinas lipofílicas por LC-MS/MS

Todos los métodos utilizados son los normalizados y se encuentran acreditados por ENAC.

Resultados

Durante el bienio 2024-2025, se han dado episodios de AST (max: 54.5 mg DA/Kg) asociados a *Pseudo-nitzschia cf australis* (max: 22800 cel/L), de DST (max: 798 µg AO/kg) asociados principalmente a *Dinophysis* del grupo acuminata (max: 3000 cel/L) y de PST (max: 13408 µg eq STX.2HCL/kg) asociados principalmente a *Gymnodinium catenatum* (max: 58320 cel/L).

Discusión y conclusión

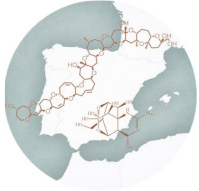
En 2024 y 2025 los episodios de AST han sido poco intensos, los de DST muestran un patrón similar al resto de años de monitoring y los de PST reaparecen después de no haberse detectado durante varios años.

Agradecimientos

Agradecemos el apoyo y colaboración de las unidades administrativas de la Junta de Andalucía con y para quién realizamos nuestra labor como laboratorio oficial para el control de las condiciones sanitarias de las zonas de producción de moluscos bivalvos y otros invertebrados marinos.

Índice Remissivo

Autores



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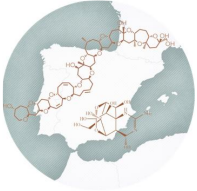

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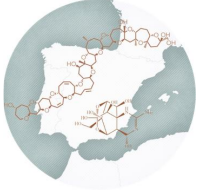

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