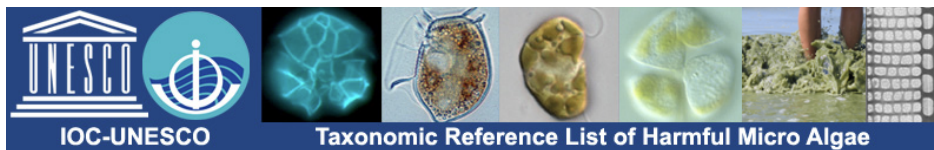


# Harmful Algae News

AN IOC NEWSLETTER ON TOXIC ALGAE AND ALGAL BLOOMS

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Intergovernmental  
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## New toxic species – and what about their names?

### News from the IOC-UNESCO Task Team on Algal Taxonomy

The IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (available via the [HAB Index](#)) is an actively maintained and comprehensive list of all microalgae known to produce toxins.

- ◆ It may serve as a starting point for assessing toxigenic microalgae.
- ◆ It provides up-to-date and accurate nomenclature.

The list presently includes 116 dinoflagellates, 43 cyanobacteria, 31 diatoms, eight haptophytes, seven raphidophytes, and three dictyochophytes, and the number is steadily increasing.

A group of editors (listed below) continuously updates the list and welcomes suggestions for modifications.

Before reviewing the changes to the list over the past few years, the editorial team would like to extend a heartfelt thank you to Santi Fraga for his invaluable contributions as the editor of the *Alexandrium* group!

We also welcome new editors: Shauna Murray (responsible for the Amphidinales), Urban Tillman (responsible for the Amphidomataceae, Peridinales) and Rafael Salas (responsible for the Thoracosphaerales) – thank you for joining the team!!

Recently, we have begun updating information on each species by adding details on [morphology](#), particularly features important for accurate identification, including micrographs. Information on resting stages ([cysts](#), [akinetes](#), [etc.](#)) has been included, as well as references to selected [GenBank sequences](#), preferably from or near the type locality. Furthermore, we are working on including more cyanobacteria, this section of the list remains incomplete, particularly concerning freshwater species. Additionally, the list has been updated

to reflect that several species have been confirmed to be toxic (Table 1).

### Additions to the list

(in red: recently described species, in black: species not recently described but newly identified as toxic):

#### Dinoflagellates

- *Alexandrium fragae*, *A. limii*, *A. ogatae*, *A. taylorii*
- *Centrodinium punctatum*
- *Gambierdiscus caribaeus* and *G. silvae* (new algal CTX-toxin: CTX5), *G. cheloniae*, *G. holmesii*, *G. honu*, *G. lewisii*
- *Gonyaulax bohaiensis*, *G. taylorii*
- *Prorocentrum caipirignum*, *P. fukuyoi*, *P. porosum*, *P. steidingerae*
- *Coolia malayensis*
- *Amphidinium magnum*, *A. pseudo-massartii*, *A. tomasii*

#### Diatoms

- *Pseudo-nitzschia bipertita*, *P. punctationis*, *P. simulans*, *P. subcurvata*

#### Raphidophytes

- *Chattonella malayana*

Additional modifications to the list are outlined below:

### Some species have been renamed

- *Karenia digitata* has been transferred to *Karlodinium digitatum*.
- *Karenia umbella* is a junior synonym of *Karenia longicanalis*.
- *Lingulodinium polyedra* is now renamed as *Lingulaulax polyedra*. *Lingulaulax polyedra* is a new name for *Lingulodinium polyedra*; as such, the genus *Lingulodinium* Wall 1967 is retained in its exclusively fossil status [1].

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Table 1. Newly described or known species, which have recently been shown to produce toxins with indications of toxin types as well as reference.

Species name (new species in bold)	Identified toxins	References
<i>Alexandrium fragae</i>	GTX2, GTX3, and STX	Branco et al. 2020 10.1016/j.hal.2020.101793
<i>A. limii</i>	GDs, with GDA as major variants or demethyl variant	Abdullah et al. 2023 10.1016/j.hal.2023.102475
<i>A. ogatae</i>	GDs, with GDA as major variants	Abdullah et al. 2023 10.1016/j.hal.2023.102475
<i>A. taylorii</i>	GDA and lytic compounds but no PSTs	Tillmann et al. 2020 10.3390/toxins12090564
<i>Centrodinium punctatum</i>	STX, GTX1-4, neoSTX, deoxy-STX	Shin et al. 2020 10.1016/j.hal.2020.101923
<i>Gambierdiscus caribaeus</i>	44-Methylgambierone; C-CTX5 in the Caribbean	Murray et al. 2019 10.1016/j.tetlet.2019.01.043 Murray et al. 2021 10.3390/toxins13050333 Mudge et al. 2023 10.1016/j.jchromb.2021.123014
<i>G. chelonii</i>	MTX-3, MTX-3 analogue,	Smith et al. 2016 10.1016/j.hal.2016.10.006
<i>G. holmesii</i>	MTX-(44-Methylgambierone)	Kretzschmar et al. 2019 10.1016/j.protis.2019.125699
<i>G. honu</i>	MTX	Munday et al. 2017 10.3390/md15070208
<i>G. lewisii</i>	MTX-(44-Methylgambierone)	Kretzschmar et al. 2019 10.1016/j.protis.2019.125699
<i>G. silvae</i>	44-Methylgambierone and gambierone; C-CTX-5 in the Caribbean	Mudge et al. 2022 10.1016/j.jchromb.2021.123014. Mudge et al. 2023 10.1016/j.chemosphere.2023.138659
<i>Gonyaulax bohaiensis</i>	YTXs	Gu et al. 2022 10.1111/jpy.13245
<i>G. taylorii</i>	YTX and homoYTX	Álvarez et al. 2016 10.1016/j.hal.2016.07.006
<i>Prorocentrum caipirignum</i>	OA and prorocentrolide	Nishimura et al. 2020 10.1016/j.hal.2019.101687
<i>P. cf. fukuyoi</i>	A strain belonging to the <i>P. fukuyoi</i> complex produces OA	Nishimura et al. 2020 10.1016/j.hal.2019.101687
<b><i>P. porosum</i></b>	OA	Arteaga-Sogamoso et al. 2023 10.1016/j.hal.2022.102356
<i>P. steidingerae</i>	OA	Steidinger KA & ME Meave del Castillo (Eds) 2018. Free download at [2].
<i>Coolia malayensis</i>	YTX analogue C <sub>56</sub> H <sub>78</sub> O <sub>18</sub> S <sub>2</sub> and other analogues, C <sub>57</sub> H <sub>80</sub> O <sub>18</sub> S <sub>2</sub> and C <sub>58</sub> H <sub>86</sub> O <sub>18</sub> S <sub>2</sub>	Phua et al. 2021 10.1016/j.hal.2021.102120
<i>Amphidinium magnum</i>	Brine shrimp bio-assay – 63% decrease compared to controls, toxin not identified.	Karafas et al. 2017 10.1016/j.hal.2017.08.001
<i>A. pseudomassartii</i>	Brine shrimp bio-assay – 95% decrease compared to controls, toxin not identified.	Karafas et al. 2017 10.1016/j.hal.2017.08.001
<i>A. tomasii</i>	Brine shrimp bio-assay – 90% decrease compared to controls, toxin not identified	Karafas et al. 2017 10.1016/j.hal.2017.08.001
<i>Pseudo-nitzschia bipertita</i>	DA	Dong et al. 2020 10.1016/j.hal.2020.101899
<b><i>P. punctationis</i></b>	DA	Niu et al. 2023 10.1111/jse.13016
<b><i>P. simulans</i></b>	DA	Li et al. 2017 10.1016/j.hal.2017.06.008
<i>P. subcurvata</i>	DA, DA- isomer C	Olesen et al. 2021 10.3390/toxins13020093
<b><i>Chattonella malayana</i></b>	Not known	Lum et al. 2022 10.1016/j.hal.2022.102322

Abbreviations: CTX (Ciguatoxin), deSTX (decarbomoyls), DA (Domoic acid), GDs (Goniodomins), GDA (Goniodomin A), GTX (Gonyautoxin), homoYTX (homoyessotoxin), OA (Okadaic Acid), STX (Saxitoxin), MTX (Maitotoxin), neoSTX (Neosaxitoxin), YTX (Yessotoxin).

**Others have been removed from the list and added to the Grey List of species** for which toxicity is doubtful, as the presence of toxins has not been demonstrated.

- The two pelagophytes (*Aureococcus anophagefferens* and *Aureoumbra lagunensis*).
- The diatom *Halamphora coffeaeformis*.
- The dinoflagellate *Prorocentrum micans*.

### **Taxonomic issues concerning *Alexandrium***

A notable taxonomic issue that has been discussed is the fact that the genus *Alexandrium* is not a valid name according to the Botanical Code for Nomenclature because it lacked a Latin description and did not indicate a type when Halim described it in 1960. This issue has been known for some time, but it was generally accepted that *Alexandrium* was valid according to the Zoological Code, as this code requires neither a Latin description nor a reference to a type. However, it is only valid under the zoological code if it was clear that Halim considered *Alexandrium* as an animal. Recent phylogenetic analyses reveal that *Centrodinium punctatum* is nested within *Alexandrium*, and since *Centrodinium* (described in 1907) predates *Alexandrium* (described in 1960), it has priority.

To make a short story long, several solutions were considered to avoid changing the name *Alexandrium*, which has been used in thousands of publications; therefore, preserving its name is essential for nomenclatural stability. A solution has hopefully now been found, as a paper is being published supporting that *Alexandrium* should be treated as an animal according to Halim (1960) and thus agreeing with the Zoological Code. The final acceptance depends on a vote in the Commission of the Zoological Code (Gottschling, M. & Elbrächter, M. (in press) Case 3886 — *Alexandrium* Halim, 1960 (Dinoflagellata, GONYAULACIDAE): confirmation of treatment as an animal taxon. – Bulletin of Zoological Nomenclature 81).

### **A new list of harmful but non-toxic species**

The scientific and managerial communities have for a long time requested

a list of non-toxic harmful microalgal species. These microalgae can be responsible of fish kills or other animal mortalities, seawater discoloration, mucilage, and foam formation, among other issues, thus negatively impacting marine life and human activities such as fisheries, aquaculture, tourism, and recreational use of the marine environment.

Listing non-toxic harmful species may be complex and even misleading, as these species are generally beneficial and should not be the focus of management practices. Negative effects occur only in some cases or specific locations, while any species reaching excessive abundance can be harmful to marine life or the ecosystem. With this in mind, it was decided to consider only cases documented either in the Harmful Algae Event Database (HAEDAT, <https://haedat.iode.org/>) or in the peer-reviewed literature. The compilation is divided in two parts, the first addresses species associated with impacts on the health of marine fish and other animals, causing harm due to e.g. cell barbs and spines, anoxia, or other mechanisms not involving toxins. This first list, now completed, comprises 55 species (23 diatoms, 25 dinoflagellates and seven from other groups), and covers 106 documented events or cases. The second list, currently in preparation, will include species responsible for seawater discoloration, mucilage,

foam formation, and other events that impact water quality and human activities.

### **Challenges we see**

In the group, we foresee a challenge in having enough individuals in the next generation of taxonomists. In many countries, the number of phytoplankton taxonomists is declining or is expected to decline soon, as many are nearing retirement.

**We need to be aware to encourage and train the next generation of taxonomists!!**

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Øjvind Moestrup (past Chair) and Nina Lundholm, Chair of the Task Team on Taxonomy, enjoying Japanese food during a break at the 20<sup>th</sup> ICHA, Hiroshima, November 2023.



# Akashiwo sanguinea blooms, anoxia and mass mortality of fish and invertebrates during the 2023–2024 spring–summer “El Niño” in Peru

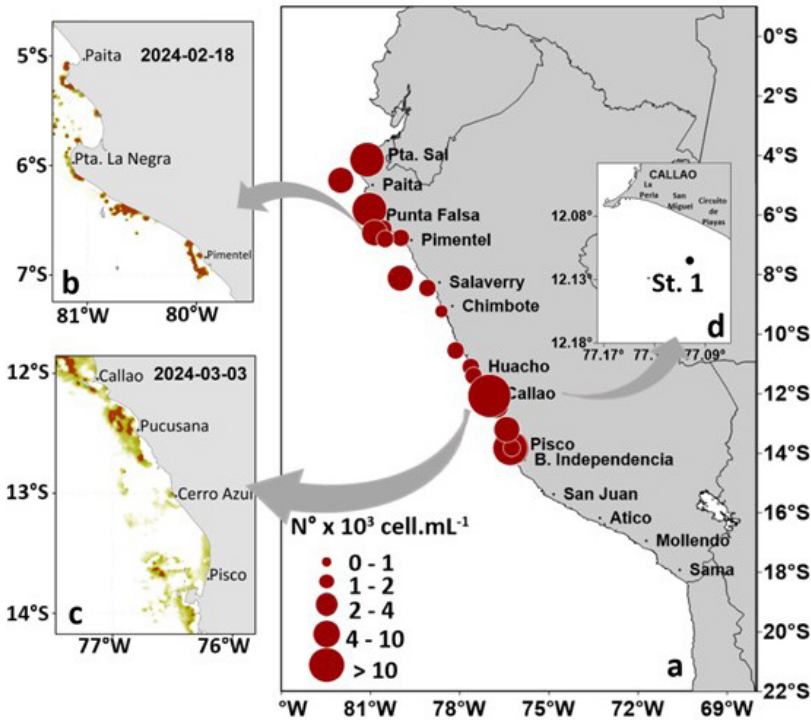


Fig. 1. (A). Distribution of *Akashiwo sanguinea* (cells · mL<sup>-1</sup>) in Peruvian coastal water. (B–C). Remote sensing detection of the discolorations. Remote sensing (IOPifa, MODIS- AQUA) satellite detection of the bloom. (D). Callao - Station 1. February - April 2024.

In early 2023, the advection of warm equatorial surface waters to the Peruvian coast, with positive anomalies of 6–8°C in sea surface temperature (SST) (related to the 1991–2020 mean, [1]), signalled the onset of the Western Pacific 2023–2024 El Niño Southern Oscillation (ENSO) event. In February–March 2024, still under the influence of a moderate-to-weak El Niño (El Niño Coastal Index – ICEN), a bloom of *Akashiwo sanguinea* (K.Hirasaka) Gert Hansen & Moestrup 2000 developed. Dark brown discolorations visible to the naked eye and extending along 1,272 km of coastline (04°S to 14°S), persisted for 2 months (February to early April 2024) and caused mass mortalities of marine fauna and heavy losses to the shellfish industry (Fig. 1). This event was monitored by personnel from the Instituto del Mar del Perú (IMARPE) and other regional laboratories.

*Akashiwo sanguinea* is a cosmopolitan dinoflagellate widely distributed in coastal waters, including the Humboldt Current Upwelling System, where it causes major socioeconomic impacts [2, 3]. Since the 1980s, recur-

ring summer and autumn blooms of *A. sanguinea* have become the most frequent high-biomass HAB in the region. The duration of these blooms has progressively increased from a few days to weeks, and in some cases, up to 3 months, as seen in summer 1986 [4], 2004 [3], and 2017 [5].

In 2024, surface water samples were collected from various geograph-

ic locations exhibiting discolorations. Additional sampling was conducted at a fixed station in Miraflores Bay, Callao (12°S). There, CTD (0–15m) casts measured physical properties and water column structure, while water samples were collected with Niskin bottles at 0 m, 5 m, 10 m and 15 m for chemical and microphytoplankton analyses. Cell counts were carried out using 1-mL Sedgewick-Rafter chambers.

Maximal cell densities of *Akashiwo sanguinea* (ranging from  $1.3 \times 10^6$  to  $63 \times 10^6$  cells L<sup>-1</sup>) and Chl *a* concentrations up to 241.7 µg L<sup>-1</sup> were recorded within the top 5 m, corresponding to a SST range of 19.4°–27.3 °C and sea surface salinity (SSS) between 34.32–35.39. Vertical profiles of environmental conditions (Fig. 2) revealed moderate-to-low levels of inorganic nutrients in the top 10 m layer and anoxic conditions from 10 to 15 m, with dissolved oxygen (DO) depleting at 10 m. Compared to abiotic conditions during similar events (SST: 18°–25°C; SSS: 34.372–35.224) from 2000 and 2018, the 2024 event occurred within a broader temperature (+2°C) and salinity (+0.1) range (Fig. 3).

*A. sanguinea* is a constitutive mixotroph, with a capacity to perform photosynthesis with its own plastids in addition to feed on live prey. *Akashiwo* benefits from different inorganic and organic nitrogen sources (including ni-

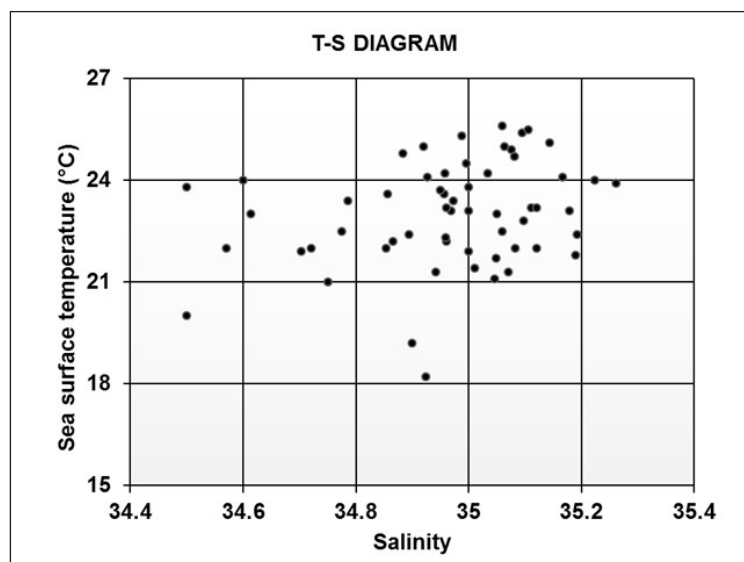


Fig. 2. Temperature–Salinity (T-S) diagram during *Akashiwo sanguinea* (2000–2018) events.



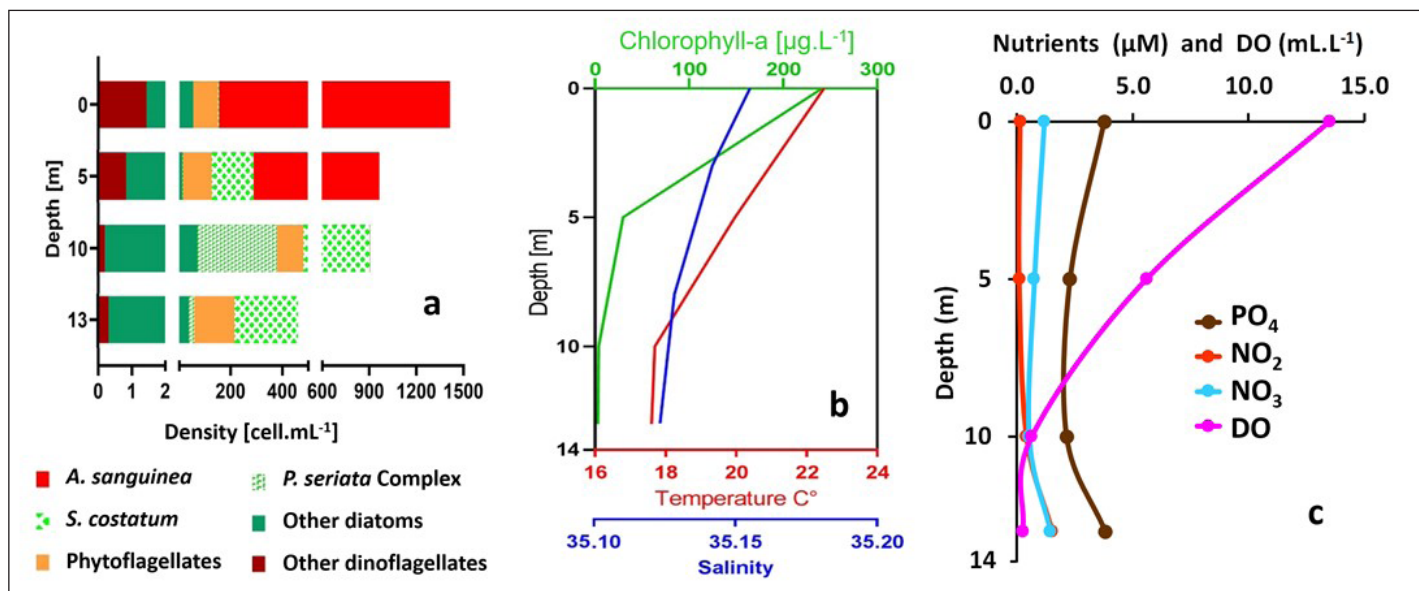


Fig. 3. (A). Vertical distribution of cell densities (cells·mL<sup>-1</sup>) of *Akashiwo sanguinea* and accompanying phytoplankton groups. (B). Temperature, salinity and Chl a. (C). Nutrients and dissolved oxygen levels in March 2024.

trate, ammonia, and urea) and eat cyanobacteria and nanoflagellates. This nutritional versatility allows the species to persist and proliferate for extended periods when resources are limiting for other competitors [6].

To estimate the extent and spatio-temporal dynamics of the discolorations, remote sensing monitoring utilised a satellite index (IOPifa) generated with daily high-resolution satellite data on phytoplankton absorption (aphy, GIOP) and non-algal detrital material plus coloured dissolved organic matter, CDOM (adCDOM, GIOP) from the Generalized Inherent Optical Properties (GIOP) model of MODIS-Aqua [7]. Despite cloudy weather, the estimated IOPifa index exceeded 2, indicating a good physiological status (fitness) of the offshore *A. sanguinea* populations. Discolourations extended offshore, ranging from about 4 km off Punta La Negra to 43 km off Pisco (Fig. 1 A, B).

During the last week of March, local

fishermen reported mass mortalities of marine fauna in Balneario San Bartolo - Lima (12°25'00"S) and Paracas Bay - Pisco (13°49'20,5"S), leading to significant economic losses over an area equivalent to 900 m<sup>2</sup>. Various marine fish and invertebrate species were washed ashore (Table 1) under SSTs of 27.4 °C, a pH of 7.3, and depleted oxygen levels. Anoxia was particularly harmful to the "concha abanico" (Peruvian calico scallop, *Argopecten purpuratus*), the primary commercially exploited species in the region. Losses exceeded USD 12 million in Paracas Bay when SST ranged from 22.3 to 26.5 °C and DO levels ranged from 0.71 to 3.80 mL L<sup>-1</sup>. Values below 1 mL L<sup>-1</sup> were measured in the areas where mass mortalities were reported.

Mass mortality of invertebrates and fish may have been triggered by a combination of gill clogging and oxygen depletion during bloom decay. However, the precise mechanism of fish mortality caused by *Akashiwo* remains

unknown. Laboratory culture experiments have shown that *A. sanguinea*'s toxicity changes across population growth phases. Lithic substances, which are lethal to zooplankton and inhibit the growth of other phytoplankton species, were most prominent during the exponential to declining growth phases [8]. Mucilage and resting cyst formation observed towards the end of the blooms may indicate unfavourable conditions or the conclusion of the growth season [9].

Observations during the 2024 *A. sanguinea* bloom in Peru suggest populations displayed good physiological fitness and tolerance to broader than usual temperature ranges. Future blooms are likely, even in a climate change scenario, as the species appears resilient to increased temperature, acidification or irradiance [10].

Increased duration and geographic extent of recurring *A. sanguinea* blooms in Peruvian coastal waters will exacerbate their negative impacts on artisanal fisheries and shellfish resources. Supporting ongoing monitoring activities is crucial. Enhanced research is needed to i) identify the drivers and origins of these blooms and ii) unveil the mechanisms underlying the intense and long-lasting blooms in the Humboldt Upwelling system, Peru.

## Acknowledgements

Thanks are given to the staff of the Coastal Laboratories-IMARPE, HAB observation network for their help with sample collection and to staff of the

Table 1. List of fish and invertebrate taxa included in the mass mortality of marine fauna.

Sample type	Scientific Name	Common names Spanish	Common names English - FAO
Fish	<i>Eucinostomus sp.</i>	Mojarra	Pacific flagfin mojarra
	<i>Fistularia corneta</i>	Pez corneta	Pacific cornetfish
	<i>Gymnura marmorata</i>	Raya mariposa	California butterfly ray
	<i>Mugil cephalus</i>	Lisa	Flathead grey mullet
	<i>Paralichthys adspersus</i>	Lenguado	Fine flounder
	<i>Pseudobatos planiceps</i>	Guitarra	Pacific guitarfish
	<i>Scomber japonicus</i>	Caballa	Pacific chub mackerel
	<i>Scartichthys gigas</i>	Borracho	Giant blenny
	<i>Stellifer minor</i>	Mojarrilla	Minor stardrum
Invertebrate	<i>Argopecten purpuratus</i>	Concha de abanico	Peruvian calico scallop
	<i>Farfantepenaeus californiensis</i>	Langostino café	Yellowleg shrimp
	<i>Octopus mimus</i>	Pulpo	Head-footed octopus
	<i>Pattalus mollis</i>	Pepino de mar	Sea cucumber
	<i>Platyxanthus orbigny</i>	Cangrejo violáceo	Crab



Fig. 4. Mass mortality of fan shell from Paracas Bay – Pisco (13°49'20,5" S), March 2024.

<https://diariocorreo.pe/edicion/ica/pisco-maricultores-de-la-zona-de-atenas-denuncian-mortandad-de-conchas-de-abanico-noticia/>

Phytoplankton Laboratory at Callao for sample analyses and figures. This work was supported by Goal 137 of the Coastal Margin Project– DGIOCC/IMARPE.

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Fig. 5. Different species of marine organisms washed on the beaches (March 2024).



# Outbreak of an unknown flagellate and a massive European conger eel mortality event in late summer 2024 in La Forêt Bay (Brittany France)

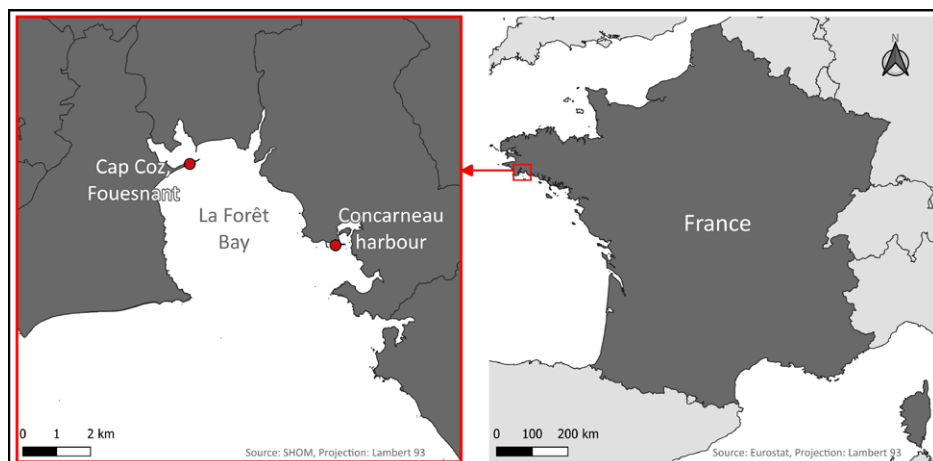


Fig. 1. Location of La Forêt Bay (47.878764 N, -3.961087 W), Atlantic coast, France.

Launched in 2013 by Ifremer (French Research Institute for Exploitation of the Sea), PHENOMER is a French citizen science programme designed to improve the detection and understanding of microalgal blooms by inviting citizens to report water discolourations along the French coast through a web application (<https://www.phenomer.org/>) [1]. PHENOMER has demonstrated its complementary value to the routine French phytoplankton monitoring network (REPHY) [2]. This programme leverages public participation to collect valuable data on the frequency, duration, and spread of blooms across the French coast.

On 18<sup>th</sup> and 21<sup>st</sup> August 2024, two reports of brown water discolourations at Cap Coz Beach and Concarneau Harbour in La Forêt Bay, Southern Brittany (Fig. 1) were submitted via the PHENOMER application. Water samples examined with an inverted microscope revealed a high concentration (several million cells per litre) of an unknown green-yellow flagellate species. Concomitant with this bloom, mass strandings of dead European conger eel (*Conger conger*) were reported along the beaches of La Forêt Bay and the surrounding area, mostly in Concarneau and Fouesnant (Fig. 2). More than 1,000 fish, mainly large specimens that had already started to decompose, were collected or counted along the coastline of the bay between 23<sup>rd</sup> and 28<sup>th</sup> August. Due to the public health risks associ-

ated with the degradation of dead fish, the Concarneau town council decided to ban access to its beaches from 23<sup>rd</sup> to 28<sup>th</sup> August 2024. The two phenomena — water discolourations and conger eel strandings — lasted for approximately twelve days.

## Environmental conditions

Data on environmental conditions were collected as part of the French phytoplankton monitoring network (REPHY) in La Forêt Bay. Measurements of physicochemical parameters (CTD profiles) (Fig. 3), and surface water samples (1 m) and bottom water samples (23 m depth) were carried out off fortnightly in La Forêt Bay (47.789633°N, -3.954117°W). During August 2024, significant peaks of fluorescence, uncommon for this location and time of the year, were observed.

On 13<sup>th</sup> and 27<sup>th</sup> August, vertical profiles of temperature and salinity indicated slight stratification, with the

base of the thermocline located at a water depth of 10 m. Below the thermocline, a fluorescent layer with an estimated chlorophyll a (Chl *a*) maximum of 36 µg·L<sup>-1</sup> on the 13<sup>th</sup> August (Fig. 3A) and 60 µg·L<sup>-1</sup> on the 27<sup>th</sup> August (Fig. 3B) was located between 15 m and the bottom water (23 m). The surface water temperature was between 18 and 19°C, while the bottom water temperature was around 15°C. Dissolved oxygen concentrations were normal for the season, at approximately 9 mg·L<sup>-1</sup> at the surface and around 8 mg·L<sup>-1</sup> at the bottom water. Salinity profiles (data not shown) were normal and homogeneous, around 35. A water sample collected at the depth of the fluorescent peak of 27<sup>th</sup> August revealed a bloom of the same unknown flagellate as that observed by the PHENOMER programme at Cap Coz beach and Concarneau harbour.

## The unknown flagellate

The microalgal flagellate which bloomed in August 2024 in La Forêt Bay exhibited a distinct morphology. The naked, biflagellated specimens examined under the inverted light microscope exhibited some affinities with some raphidophycean species (Stramenopiles). Within the same sample, a variety of forms were observed, ranging from spherical to ovoidal (Fig. 4B) and bilobed, dividing cells (Fig. 4A). Their surface displayed many vesicle-like structures (verrucae), particularly in the bilobed dividing cells. Cell body diameters measured approximately 15 µm in length and 25 µm in width. Nu-



Fig. 2. Dead European conger eels stranded in Concarneau harbour in August 2024.



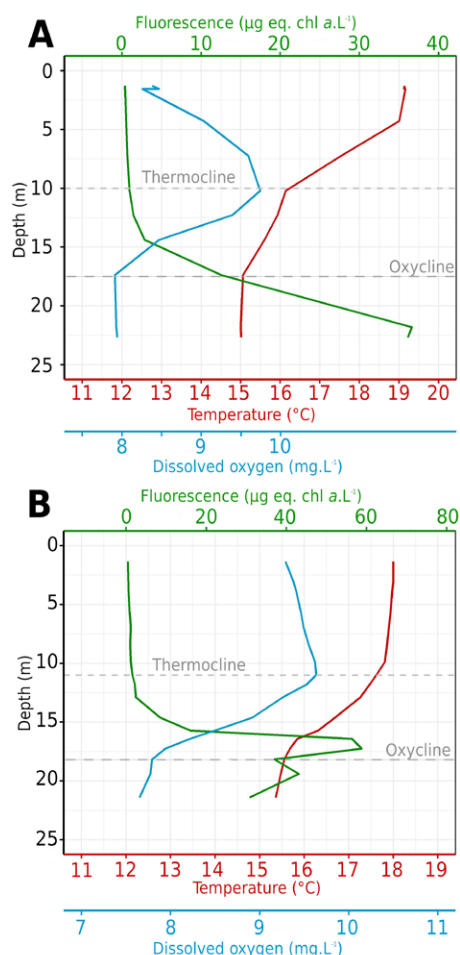


Fig. 3. Vertical profiles of Chl a fluorescence of Chlorophyll a, dissolved oxygen concentration and temperature profiles in La Forêt bay (REPHY data) obtained on (A) 13<sup>th</sup> August 2024, and (B) 27<sup>th</sup> August 2024.

merous green or green-yellow ovoid-shaped chloroplasts were located at the periphery of the cell. Swimming, controlled by two heterodynamic flagella — an anterior flagellum describing a forward sinusoidal trajectory and a posterior trailing flagellum — was relatively slow (Fig. 4). Preliminary sequences of ribosomal genes confirmed that the unknown flagellate belongs to the Stra-

menopiles (heterokonts) but it cannot be classified either within the Raphidophyceae or any other known taxonomic rank. Further studies are in progress to clarify its taxonomic position.

Several cultures of this organism have been established, enabling subsequent morphological, ultrastructural, and molecular analyses. Given its similarity to flagellates belonging to the class Raphidophyceae, which includes ichthyotoxic species, and its coincident occurrence with the conger eel mortality event, toxicity studies using available bioassays, such as the fish-gill cell line [3], should be conducted to assess the toxic potential of the unidentified flagellate.

### Hypothetical link between the unknown flagellate bloom and the conger eel mortality

At present, and considering the limited amount of environmental data available from the fish mortality period, it is not possible to confirm the link between the conger eel mortality and the unknown flagellate bloom. However, Chl a fluorescence profiles indicated that the cell maximum occurred near the sea floor. Therefore, the primary hypothesis is that the fish mortality was caused by hypoxia associated with microalgal respiration at night or by the biological oxygen demand during the bloom decay. Indeed, the affected conger eels were primarily large individuals (up to 2.5 m in length and 40 kg), which have higher oxygen demands [4]. Additionally, European conger eels are demersal, nocturnal, and territorial fish, with sedentary behaviour [5]. Therefore, conger eels would have been particularly exposed to a bloom located near the seafloor

and the putative hypoxia, explaining why this species was more affected than other organisms. Only a small number of dead specimens from a few other fish species, including sea bass (*Dicentrarchus labrax*) and ballan wrasse (*Labrus bergylta*), were reported.

There is only one previous report of a similar massive fish stranding event in summer during a microalgal bloom associated with hypoxia, dating back to July 1982 in Vilaine Bay (Southern Brittany, France) [6]. During that historic event, 30–50 tonnes of dead fish, mostly conger eels, were reported [6].

The remarkable similarities between the July 1982 and August 2024 fish mortalities events in Vilaine Bay and La Forêt Bay, Brittany, France, present an opportunity to compare environmental conditions associated with both events and to unveil the identity of a putative new harmful microalgal species and its impacts on the marine fauna.

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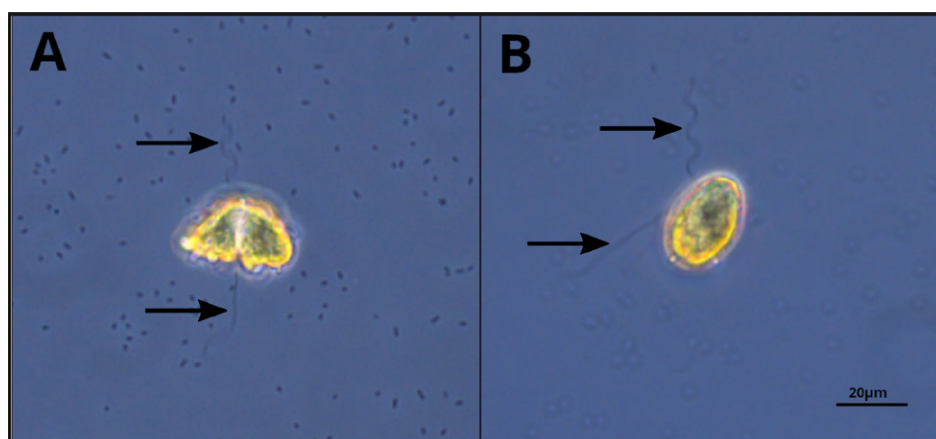


Fig. 4. Morphological variability of the unknown flagellate under the light microscope: (A) bilobed- and (B) ovoid-shaped specimen with two opposite flagella (arrows).



# Mucilage appearance in the Adriatic Sea during Summer 2024



Fig. 1. Floating macroaggregates at Passetto (N Adriatic Sea).

During the summer 2024, the Adriatic Sea experienced the ‘dirty sea’ phenomenon, characterized by the formation of large mucilage aggregates in the water column (Fig. 1). This event, which has occurred periodically since 1729, has been particularly extensive during recent decades, especially between 1988–1990 and 1999–2002. Beginning in June 2024 near the Croatian coast of Rovinj, the mucilage aggregates spread following the typical counterclockwise circulation, moving through the Gulf of Trieste, the Venice Lagoon, the Emilia Romagna and Marche coasts, and eventually reaching as far south as Apulia.

Similar to other regions (Marmara Sea, Aegean Sea, NW Mediterranean, and New Zealand), this phenomenon began with a bloom of the dinoflagellate *Gonyaulax fragilis* (formerly reported as *G. hyalina* [1]) (Fig. 2). As

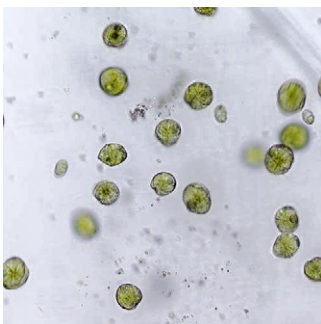


Fig. 2. *Gonyaulax fragilis* in mucilage samples (LM micrographs).

the bloom declined, the rupture of the thecae released sticky polysaccharide-rich cytoplasmic content [2]. This material formed aggregates, embedding the marine snow already abundant in the water column. The aggregation process, facilitated by a stable, stratified water column and reduced circulation, led to the formation of larger aggregates such as macroflocs (Fig. 3), stringers (Fig. 4), clouds (Fig. 5), etc. [3]. When these ag-

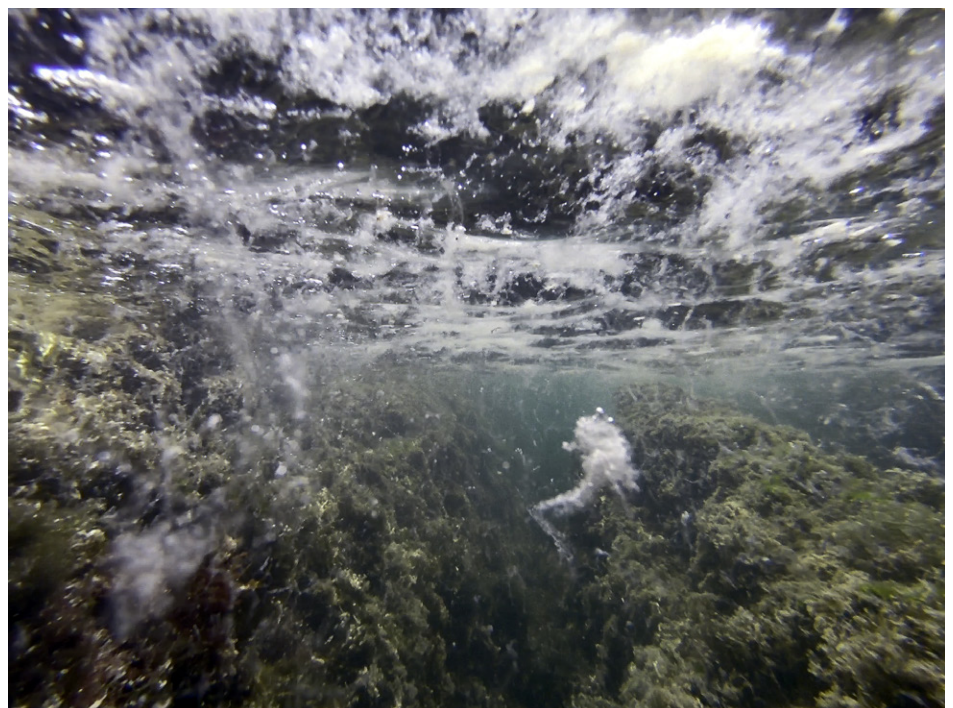


Fig. 3. Mucilage macroflocs in the upper water column and at surface.

gregates settled onto the seafloor, they potentially caused harm to benthic organisms.

After the initial phase, *G. fragilis* was no longer detectable in the mucus, except for the presence of its empty thecae. Instead, the aggregates became colonized by a diatom-dominated microalgal community, primarily *Nitzschia gobbii* and *Thalassionema nitzschioides*. This community was similar to the surrounding water, but exhibited abundances up to three orders of magnitude higher [4]. Mucus provides an ideal microenvironment for microbial growth, where efficient nutrient recycling by bacteria supports the proliferation of microalgae [5].

As usual, this mucilage event was highly dynamic. Aggregates shifted in shape, consistency, and location throughout the day due to wind and currents, moving up and down the water column depending on its turbulence and stability. The phenomenon persisted for approximately three months. Although classified as a non-toxic harmful algal bloom (HAB), the mucilage had significant impacts. It disrupted tourism and hindered fishing activities by clogging nets and other gear. Moreover, an unprecedented mortality of wild mussels (*Mytilus galloprovincialis*) along the Conero Riviera was observed toward the end of the mucilage event (Fig. 6). This mortality was likely linked to the



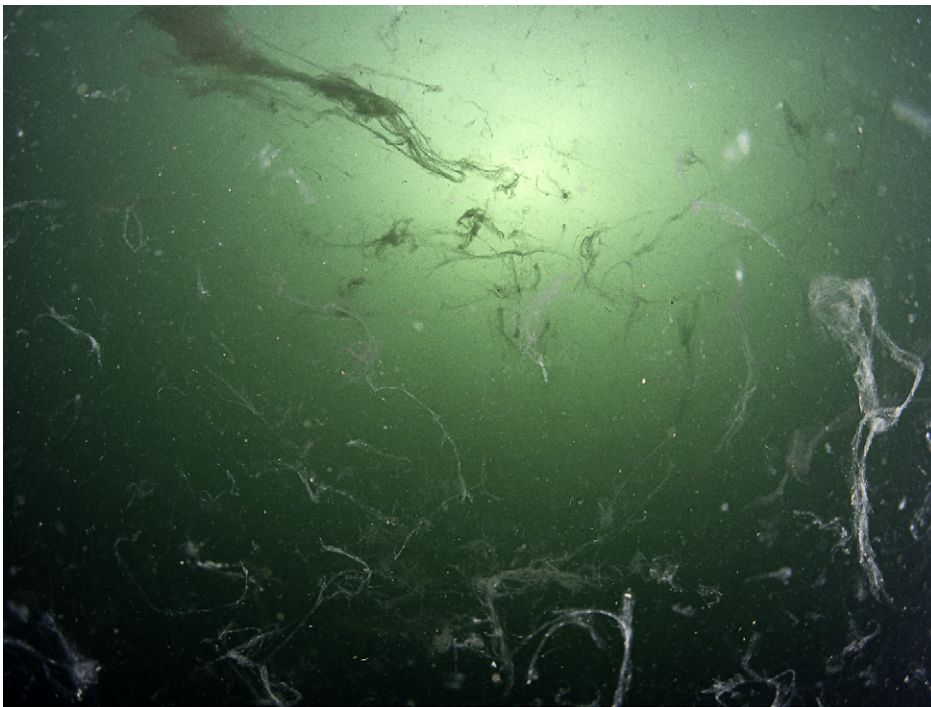


Fig. 4. Stringers in the water column.



Fig. 5. Clouds of mucilage floating in the mixed layer.



Fig. 6. Mortality of wild mussels (*Mytilus galloprovincialis*) on the rocky bottom.

combination of exceptionally high temperatures and the suffocating effect of the mucilage on filter feeders.

Although mucilage aggregates were observed throughout the entire water column (in the mixed layer, at the thermocline, and in the bottom layer), mucilage events in the Adriatic Sea are of pelagic origin. They should not be confused with benthic mucilage, such as observed e.g. in the Tyrrhenian Sea, where mucus covering benthic organisms and substrata is produced locally by benthic chrysophytes and/or ectocarpalean macroalgae.

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# Harmful Dinoflagellates in Odessa Bay (Black Sea)

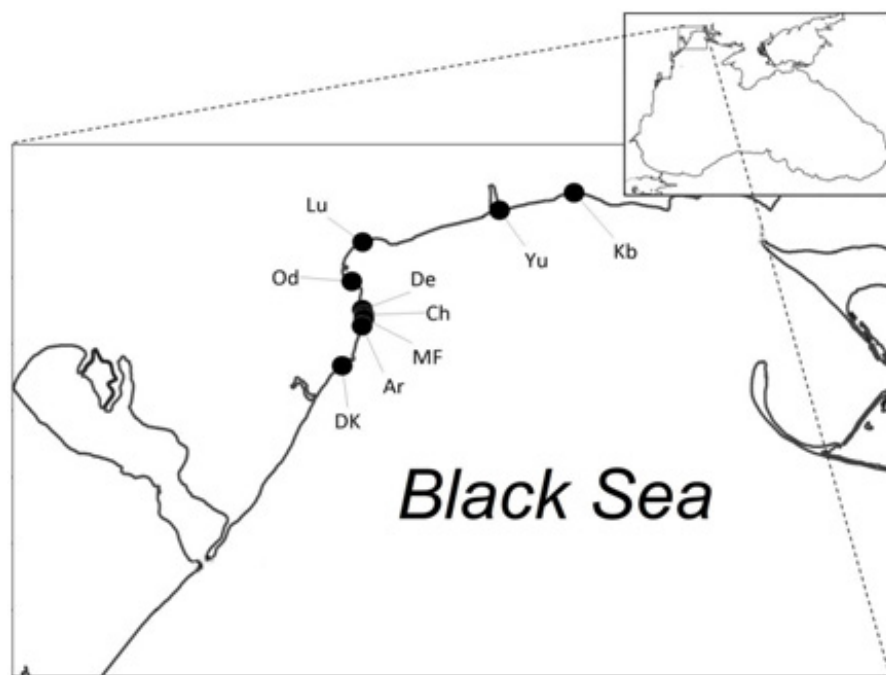


Fig. 1. Map of sampling stations: Koblevo (Kb); Yuzhnoe (Yu); Luzanovka Lu; Od, Port (Od); Delphin (De); Chkalovskiy (Ch); Maliy Fontan (MF); Arcadia (Ar); Dacha Kovalevskogo (DK).

In the 1960s, anthropogenic eutrophication in the Black Sea greatly intensified, with an increased influx of biogenic elements leading to mass phytoplankton development [1]. By the early 1970s, the first cases of harmful algal blooms (HABs) caused by the dinoflagellate *Prorocentrum cordatum* (Ostenfeld) Dodge (= *P. minimum*; synonymy according to [2, 3]) were recorded in the northwestern part of the sea [4]. Currently, *P. cordatum* remains one of the most abundant species of harmful dinoflagellates in Odessa Bay and the Black Sea, in general [5, 6].

By the 2000s, HAB episodes in the bay were induced by other dinoflagellates: *Akashiwo sanguinea* (Hirasaka) Hansen & Moestrup, *Protodinium simplex* Lohmann, *Gyrodinium cornutum* (Pouchet) Kofoid & Swezy, *Levanderina fissa* (Levander) Moestrup, Hakanen, Gert Hansen, Daugbjerg & M.Ellegaard 2014, *Lingulaulax polyedra* (F.Stein) M.J.Head, K.N.Mertens & R.A.Fensome 2024, *Kryptoperidinium triquetrum* (Ehrenberg) Tillmann, Gottschling, Elbrächter, Kusber & Hoppenrath 2019 (Ehrenberg) Stein, *Scrippsiella trochoidea* (Stein) Loeblich, and *Prorocentrum micans* Ehrenberg [7].

In this study, harmful algal species Dinoflagellata of Odessa Bay were sampled monthly between 2020 and 2024 at eight stations located in the coastal zone (Fig. 1). Salinity at the sampling sites ranged from 12 to 17. In summer 2023, salinity values as low as 3.9 were recorded following the destruction of the Kakhovka Reservoir during military actions. A very intense cyanobacterial bloom developed during the most acute phase of the impact, lasting about three months [8].

Phytoplankton samples were examined with Mikmed-2 and Olympus BX51 light microscopes equipped with differential interference contrast (DIC, Nomarski), and epifluorescence, with preliminary staining of the cells with Calcofluor White M2R [9]. Some samples were also examined using a scanning electron microscope (SEM) JEOL JSM-35C and a field emission scanning electron microscope (FE-SEM) JEOL JAMP 9500F, following the protocol specified by [10].

The analyses of samples revealed the presence of 18 harmful species [11] in Odessa Bay associated with the following syndromes:

1. Shellfish toxin producers:
  - Paralytic shellfish poisoning (PSP): *Alexandrium minutum* Halim.
  - DSP and PTX: *Dinophysis acuminata* Claparède & Lachmann, *D. acuta* Ehrenberg, *D. caudata* Kent, *D. fortii* Pavillard, *D. hastata* Stein, *D. ovum* Schütt, *D. sacculus* Stein, *Phalacroma rotundatum* (Claparède & Lachmann) Kofoid & J.R.Michener 1911 and benthic *P. lima* (Ehrenberg) Stein.
  - Yessotoxins YTX: *Gonyaulax spinifera* (Claparède & Lachmann) Bütschli, *Lingulaulax polyedra* (F.Stein) M.J.Head, K.N.Mertens & R.A.Fensome 2024 and *Protocera-tium reticulatum* (Claparède & Lachmann) Bütschli (Fig. 2).
2. Fish and invertebrate mass mortalities: *Margalefidinium polykrikoides* (Margalef) Gómez, Richlen & Anderson: High biomass HABs.
3. Anoxic events: *Akashiwo sanguinea* (Hirasaka) Hansen & Moestrup.
4. Mucilage and other physical-mechanical problems: *Kryptoperidinium triquetrum* (Ehrenberg) Tillmann, Gottschling, Elbrächter, Kusber & Hoppenrath 2019 (Ehrenberg) Stein; *Prorocentrum cordatum* (Ostenfeld) Dodge.

Currently, *Lingulaulax polyedra* has become the dominant HAB species in terms of abundance in Odessa Bay. In 2020 and 2024, this dinoflagellate caused water discolorations near Odessa seaport. On October 6, 2020, a cell maximum of  $56.1 \times 10^6$  cells·L<sup>-1</sup> was recorded at a temperature 19.7°C and salinity 14.3 [12]. In late September, 2024, the cell maximum was  $11.5 \times 10^6$  cells·L<sup>-1</sup>, under very similar conditions of temperature (19.7–20.0°C) and salinity (13.9–15.9) as in the 2020 bloom.

Such high cell densities have been associated with high nutrient levels in this area of Odessa Bay. Outbreaks of *L. polyedra* occurred following heavy rains and runoff from adjacent territories. Different abiotic and biotic drivers have been suggested as potential causes of *L. polyedra* red tides. These include observed trends of increasing air and sea-water temperatures, the resuspension of *L. polyedra* cysts from the seabed to the surface layers of the sea and strong wind-driven advection of offshore populations of *L. polyedra*, leading to high

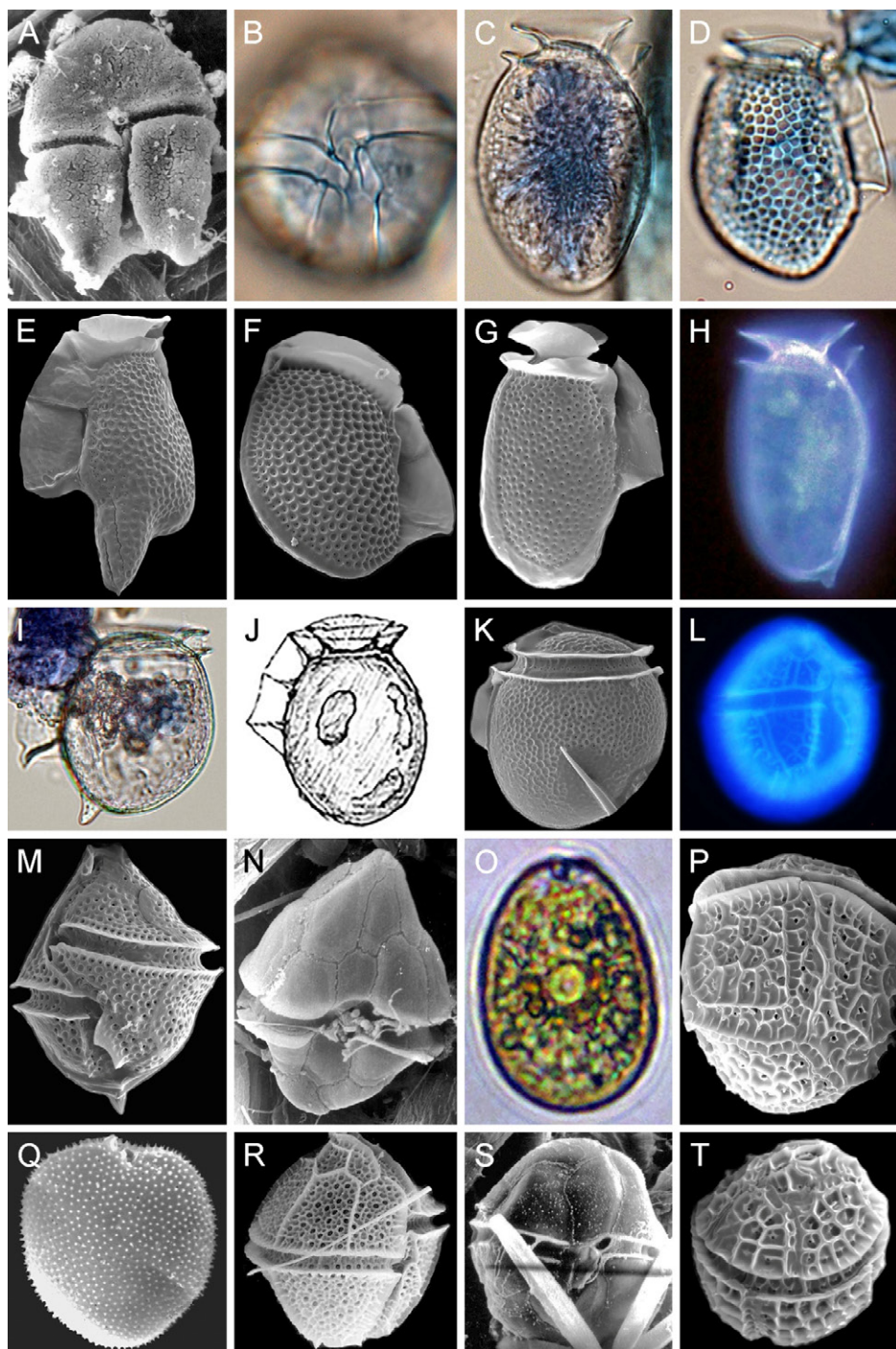


Fig. 2. Light microscopy and scanning electron micrographs of potentially toxic and harmful dinoflagellates of Odessa Bay: (A). *Akashiwo sanguinea*. (B). *Alexandrium minutum*. (C). *Dinophysis acuminata*. (D). *D. acuta*. (E). *D. caudata*. (F). *D. fortii*. (G–H). *D. sacculus*. (I). *D. hastata*. (J). *D. ovum*. (K). *Phalacroma rotundatum*. (L, P, T). *Protoceratium reticulatum*. (M). *Gonyaulax spinifera*. (N). *Kryptoperidinium triquetra*. (O). *Prorocentrum lima*. (Q). *P. cordatum*. (R). *Lingulaulax polyedra*. (S). *Durinskia baltica*. Original photos. Images not to scale.

cell numbers in the surface layer of Odessa Bay. During the discolorations, an intense glow of the seawater was observed in the evening and at night.

In conclusion, the ongoing monitoring and understanding of harmful dinoflagellate species in Odessa Bay are crucial for managing the ecological impacts of nutrient enrichment, climate change, and human activities, ensuring the protection of marine biodiversity and the health of local ecosystems.

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# Not so toxic: Antarctic *Pseudo-nitzschia* isolates give domoic acid the cold shoulder

The genus *Pseudo-nitzschia* is monitored worldwide because it can produce toxins, such as domoic acid (DA), which cause amnesic shellfish poisoning (ASP) in humans when accumulated in sea food [1]. Additionally, *Pseudo-nitzschia* species are associated with numerous large-scale harmful algal bloom (HAB) events that have led to mortality in marine organisms [2-3]. Approximately 27 species in this genus are known to potentially produce the neurotoxin DA along with up to six DA isomers (DA-IA, DA-IB, DA-IC, DA-ID, DA-IE and epi-DA) [4,5 and references therein]. These toxins can bioaccumulate in various organisms across different levels of the food web, including krill, bivalves, and fish, and have been detected in marine mammals and seabirds [4 and references therein]. Exposure to DA can result in seizures, reduced fitness, acute or chronic poisoning, and even death, particularly in marine mammals [3]. In humans, symptoms of ASP from DA exposure range from gastrointestinal, neurological, and cardiovascular to death in severe cases ([1]; Fig. 1).

Despite global monitoring efforts, Antarctica has not been a primary focus for studies on the occurrence and toxicity of the *Pseudo-nitzschia* genus. Several species have been detected in Antarctic waters, including the endemic *P. prolongatoides*, *P. subcurvata*, and *P. turgiduloides* [6-8]. Most Antarctic *Pseudo-nitzschia* strains tested for DA have shown no evidence of toxin production [7-8]; however, DA and the DA-IC isomer were detected in three *P. subcurvata* isolates from the Southern Ocean [9]. There is growing interest in HAB species detected in Antarctica, driven by the potential impacts of climate change on microalgal communities [9,10]. Investigating the presence of DA-producing *Pseudo-nitzschia* in this region is critical for understanding whether DA could impact the Antarctic food web and ecosystem dynamics.

Here we report the detection of *Pseudo-nitzschia* species at multiple sites in the Ross Sea Region, and over multiple years at Cape Evans (McMur-

do Sound, Antarctica). Environmental DNA (eDNA) metabarcoding targeting the 18S ribosomal DNA (rDNA) V9 region was employed to identify these species in seawater samples. Additionally, domoic acid and its isomers were screened in 17 *Pseudo-nitzschia* cultures isolated from Cape Evans in 2021, using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for quantification.

## Methods and Materials

Samples for eDNA analyses were collected across multiple years (Fig. 2) via plankton net tows (15–20 µm mesh),

sea ice, and platelet ice cores (Fig. 3). Lower-latitude samples from Cape Adare to Coulman Island were gathered as part of the 2023 National Institute of Water and Atmospheric Research (NIWA) RV *Tangaroa* voyage. Additional samples were collected across various science seasons as part of on-continent field deployments through the Antarctic Science Platform and Antarctica New Zealand (Projects K892 and K043).

Samples for eDNA metabarcoding were preserved in RNAlater, ethanol, or 10% glycerol and stored at -80 °C until DNA extraction. These different preservation solutions were used due to the opportunistic re-use of samples from multiple projects spanning several Antarctic field seasons. From this point, all samples were processed, sequenced, and analysed following the protocol in Stuart et al. [11]. Although the 18S

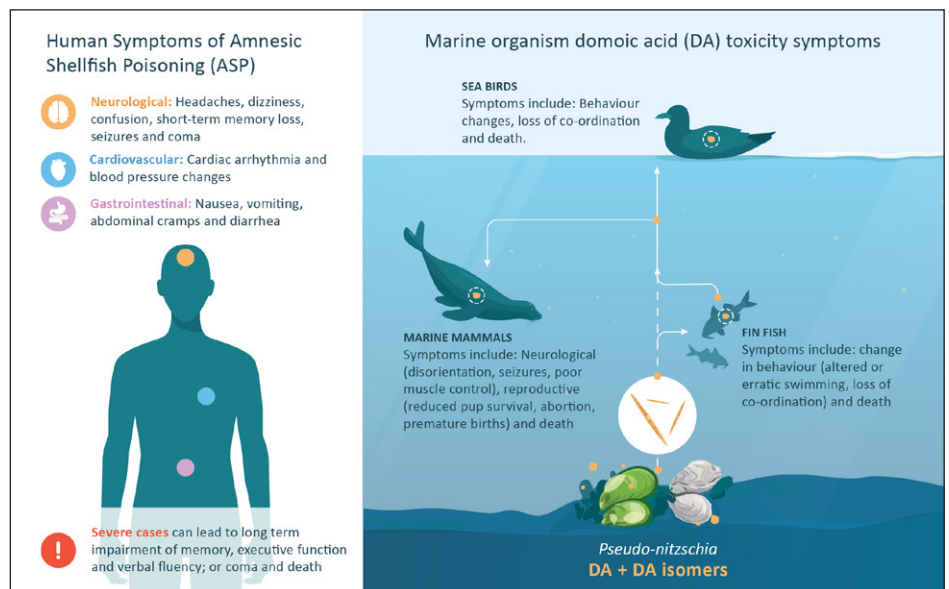


Fig. 1. Symptoms and signs of Amnesic Shellfish Poisoning (ASP) caused by domoic acid (DA) toxicity in humans and DA toxicity symptoms in marine organisms. Information collated from [1-4].

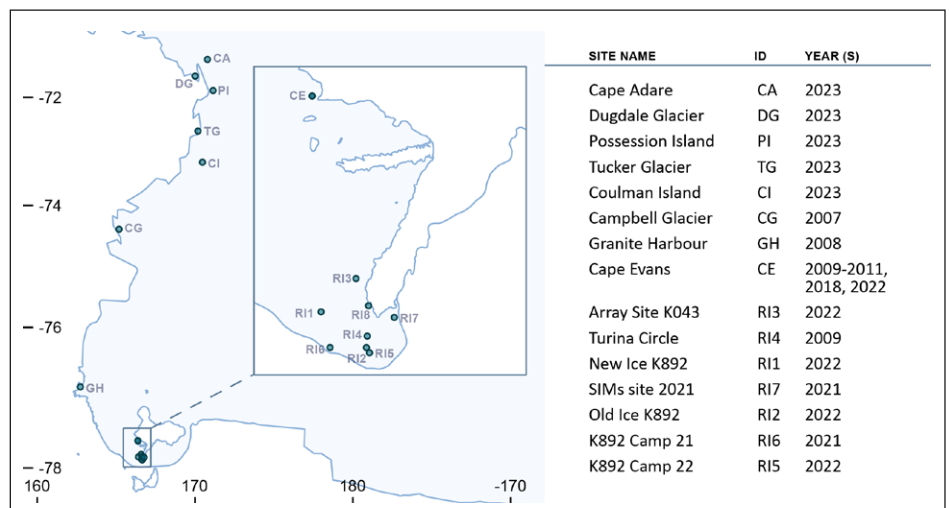


Fig. 2. Details of sampling sites used in this study from the Ross Sea Region, Antarctica.





Fig. 3. Sampling for eDNA and live mixed samples in Antarctica, including (A) sea ice auger to collect brine samples, (B) plankton net deployed below ice, (C) SIPRE core drill for sea ice core samples and (D) sub-ice platelet layer (SIPL) sampler. Image credits: Jacqui Stuart.

rDNA V9 gene region is not the most common or effective marker for *Pseudo-nitzschia* species identification [12], it was chosen for a broader assessment of coastal marine microalgal communities (Stuart et al., submitted). The data presented here is a subset of that broader community assessment. Amplicon sequence variants (ASVs) identified as *Pseudo-nitzschia* spp. by the PR2 database were further confirmed via BLAST (Basic Local Alignment Search Tool) at the National centre for Biotechnology Information (NCBI). The data analysis pipeline is available on GitHub <https://github.com/JustJaxz/AntarcticPseudos>. Phylogenetic reconstruction of ASVs was complete using Geneious Prime 2019.0.4 (<https://www.geneious.com>).

Microalgal cultures were isolated from unpreserved samples collected at Cape Evans in 2021, with cells isolated between December 2021 and January 2022 (Fig. 2). Cultures of *P. cf. subcurvata*, *P. subcurvata*, and *P. turgiduloides* were maintained at the Cawthron Institute Culture Collection of Microalgae (CICCM, Nelson, New Zealand) at  $4 \pm 1$  °C and  $80 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$  photon irradiance (12:12 h L:D photoperiod) in f/2:K (1:1) media [20]. Species-level identification of the cultures was confirmed via Sanger sequencing of the ITS region (Table 1) following protocols previously described [5]. For LC-MS/MS analysis, cultures were transferred

to fresh f2 medium, harvested after 17 days of growth, and replicate cell counts were performed. Isolates were harvested in the early stationary growth phase by centrifugation (50 min,  $3180 \times g$ ), and the resulting pellets were stored at  $-20$  °C until further extraction.

Toxin extractions were completed using the protocol previously described [5]. Quantitative analysis of the culture extracts was conducted on a Waters Xevo TQ-S triple quadrupole mass spectrometer coupled with a Waters Acquity UPLC i-Class, equipped with a flow-through needle sample manager.

## Results

Across all sites and years, two unique ASVs identified as *Pseudo-nitzschia* spp. were detected via eDNA metabarcoding. ASVs identified as *Pseudo-nitzschia subcurvata* (ASV1) were detected at 12 of the 15 sites sampled within McMurdo Sound, with ASV abundance ranging between 226 – 16,597 (<1 – 33% of total eukaryotic microalgae ASVs) at individual sites (Fig. 4a). ASVs identi-

fied as *P. subcurvata* were detected for four of the five years sampling occurred at Cape Evans, showing the highest ASV abundance in 2010 and the lowest in 2022 (Figure 4b). ASVs identified as *Pseudo-nitzschia seriata* (ASV2) were only detected at site RI1, with an ASV abundance of 72.

Phylogenetic reconstruction of the ASVs resolved ASV1 with moderate support in a clade with *P. subcurvata* (Fig. 4c). ASV2 did not resolve within a specific clade but was identified via NCBI BLAST as *P. seriata* (BLAST results: Query cover: 100%; E-value:  $3e-62$ ; % identity: 100%). No ASVs identified as *P. turgiduloides* were detected; however, only a single partial 18S sequence is available on GenBank for this species (Accession no. AY257839). The region overlap between the reference sequence and the ASVs from this study was only 68%, making reliable identification unlikely. No DA or its isomers were detected in any of the 17 cultures of *P. cf. subcurvata*, *P. subcurvata* and *P. turgiduloides* isolated from Cape Evans. All levels were below the limit of detection (Table 2).

## Discussion

The detection of *Pseudo-nitzschia subcurvata* at multiple sites over several years in McMurdo Sound demonstrates the presence and persistence of this diatom species in Antarctic waters. This finding contributes to the growing evidence that HAB species, such as those belonging to *Pseudo-nitzschia*, are not limited to temperate or tropical regions [4, 14, 21]. Our results highlight that Antarctic waters, particularly in the Ross Sea Region, are home to potentially harmful microalgal species. This aligns with studies identifying HAB species in other areas around Antarctica, including the Antarctic Peninsula [17], Southern Ocean [16] and Weddell Sea [15].

Metabarcoding analysis revealed that *P. subcurvata* was prevalent across multiple years at the Cape Evans site,

Table 1. Basic Local Alignment Search Tool (BLAST) results of representative *Pseudo-nitzschia* sp. isolates from Cape Evans.

Strain code	GenBank accession number	Gene	BLAST match	Percent identity	Coverage	E-value
CAWB176	PQ276752	Internal transcribed spacer region	<i>P. cf. subcurvata</i>	99.58%	100%	0
CAWB179	PQ276753		<i>P. subcurvata</i>	100%	100%	0
CAWB178	PQ276754		<i>P. turgiduloides</i>	99.86%	100%	0

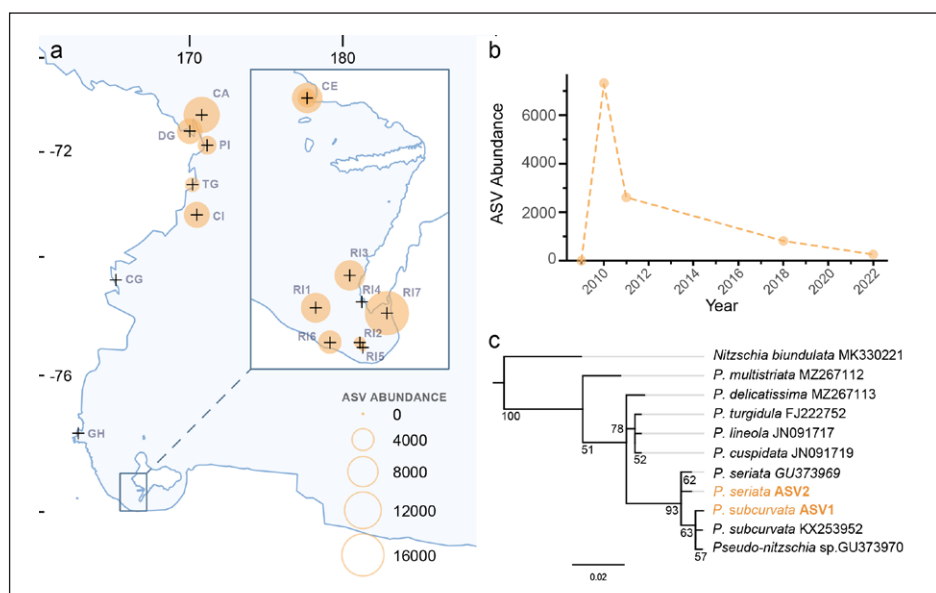


Fig. 4. Amplicon sequence variant (ASV) abundance identified as *Pseudo-nitzschia subcurvata* (ASV1) detected in samples from (A) sites in the Ross Sea Region. Sampling sites include Cape Adare (CA), Dugdale Glacier (DG), Possession Island (PI), Tucker Glacier (TG), Coulman Island (CI), Campbell Glacier (CG), Granite Harbour (GH), Cape Evans (CE), Array Site K043 (RI3), Turina Circle (RI4), New Ice K892 (RI1), SIMs Site (RI7), Old Ice K892 (RI2), K892 camp 2021 (RI6), K892 camp 2022 (RI5). (B) Abundance of *P. subcurvata* (ASV1) over time at Cape Evans, McMurdo Sound (CE); and (C) Maximum likelihood phylogenetic tree of detected *Pseudo-nitzschia* 18S ribosomal DNA V9 ASVs. Bootstrap support values are reported on the nodes. Evolutionary analyses were conducted using 10,000 bootstrap replications. The scale bar represents the number of substitutions per site.

Table 2. Summary of domoic acid (DA) and DA isomer production of 17 *Pseudo-nitzschia* isolates from Cape Evans, Antarctica, including species name, isolate or Cawthron Institute Culture Collection of Microalgae (CICCM) identifier and ranges of DA and isomers A-E. Zero values indicate that no toxins were detected at the limit of detection (LoD)

Species	Isolate or CICCM ID	Range of DA and DA Isomers Cell Quota (pg cell <sup>-1</sup> )						
		DA	epi-DA	Iso-DA A	Iso-DA B	Iso-DA C	Iso-DA D	Iso-DA E
<i>P. cf. subcurvata</i>	K1Ps01	0	0	0	0	0	0	0
<i>P. cf. subcurvata</i>	CAWB176	0	0	0	0	0	0	0
<i>P. cf. subcurvata</i>	CAWB177	0	0	0	0	0	0	0
<i>P. cf. subcurvata</i>	K1Ps06	0	0	0	0	0	0	0
<i>P. cf. subcurvata</i>	K2Ps04	0	0	0	0	0	0	0
<i>P. cf. subcurvata</i>	L4Ps01	0	0	0	0	0	0	0
<i>P. cf. subcurvata</i>	L4Ps03	0	0	0	0	0	0	0
<i>P. cf. subcurvata</i>	L4Ps04	0	0	0	0	0	0	0
<i>P. turgiduloides</i>	CAWB178	0	0	0	0	0	0	0
<i>P. turgiduloides</i>	K2Ps01	0	0	0	0	0	0	0
<i>P. turgiduloides</i>	K2Ps02	0	0	0	0	0	0	0
<i>P. turgiduloides</i>	K2Ps03	0	0	0	0	0	0	0
<i>P. turgiduloides</i>	CAWB181	0	0	0	0	0	0	0
<i>P. subcurvata</i>	K3Ps01	0	0	0	0	0	0	0
<i>P. subcurvata</i>	CAWB179	0	0	0	0	0	0	0
<i>P. subcurvata</i>	CAWB180	0	0	0	0	0	0	0
<i>P. subcurvata</i>	L2Ps03	0	0	0	0	0	0	0

but no *P. turgiduloides* was detected, despite multiple isolates being established from samples at this location. This may be due to the limited availability of 18S rDNA V9 reference sequences for this species in both GenBank and the PR2 database. *P. seriata* was detected at one of the 15 sites, a species not previously reported in Antarctic waters. BLAST results had high coverage and confidence in the species identification; however, the length of the amplicon and the specificity of the selected gene region may limit accurate species-level assignment. This could also be due to the limited availability of reference sequences for Antarctic *Pseudo-nitzschia* species.

The lack of DA, epi-DA, or DA isomers A-E in our results is consistent with previous research indicating that most Antarctic *Pseudo-nitzschia* strains assessed do not show evidence of toxin production [7–8]. However, this contrasts with studies reporting DA production in some isolates of *P. subcurvata* from the Southern Ocean [9]. The 18S rDNA V9 region is not the most effective region for species-level identification of *Pseudo-nitzschia* but can provide valuable insights into the broader eukaryotic microalgal community [11]. It is important to note that other gene regions, such as the Internal Transcribed Spacer (ITS) regions, are more commonly used for precise species identification in phylogenetic studies of *Pseudo-nitzschia* [e.g. 5]. This limitation should be considered when interpreting our results, as the 18S rDNA V9 region may not provide the same level of taxonomic resolution as other markers, potentially underestimating the diversity of *Pseudo-nitzschia* species present in Antarctica.

This study highlights the presence of *P. subcurvata* in the Ross Sea Region of Antarctica and contributes to the growing understanding of the distribution of HAB species in polar ecosystems. Although no DA was detected in the cultured isolates, ongoing monitoring and research are essential to assess the potential risks posed by *Pseudo-nitzschia* in Antarctica. Future studies should consider using additional gene regions for more accurate species identification and include broader geographic and temporal sampling to better understand the potential impacts of HAB species on the food web in McMurdo Sound.

## Acknowledgements

We extend our thanks to Sarah Challenger and Lucy Thomson at the Cawthron Institute for assistance with lab work and microalgal culture. We also thank the Scott Base staff and all members of the K892 and K043 field teams for logistics support and assistance in sampling. We thank Svenja Halfter, Ollie Twigge and Stacy Deppler (NIWA) for sample collection and logistics as part of the 2023 NIWA RV Tangaroa voyage. We thank Antarctica New Zealand for their financial and logistic support via the Sir Robert Irvine Doctoral Scholarship (J.S.). New Zealand's National Institute for Water and Atmospheric Research [NIWA; Contract COAO404] and Antarctic Science Platform [Contract ANTA1801] supported multiple field sampling seasons, as well as time for NR, KR. The FORST Foundation of Research Science and technology also supported earlier field seasons and time for KR. This work was funded by the Ministry of Business, Innovation and Employment Strategic Science Investment Fund (SSIF) Seafood Safety research,

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# First report of *Ostreopsis cf. siamensis* exceeding alert thresholds in Bay of Plentzia (South-East Bay of Biscay)

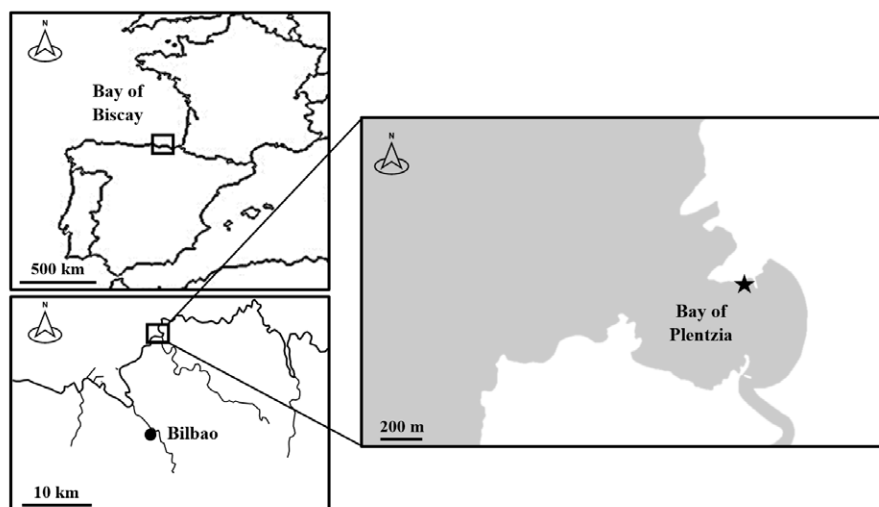


Fig. 1. Location of the sampling site.

Several species of benthic dinoflagellates belonging to the genus *Ostreopsis* are potentially toxic and were originally distributed mostly in warm and tropical

waters. Their presence has been associated with several toxic episodes affecting humans, with symptoms including dermatological and respiratory issues

[1]. Palytoxin analogues, including ova-toxins, ostreocins and liguriatoxins, have been proposed as the toxins responsible for these health issues, although no direct evidence has yet been established [2, 3].

Since *Ostreopsis* was first reported in the Basque Country in 2007 [4], there has been a notable increase in its abundance and distribution along the Basque coast, and it is now common during the summer months [5, 6]. Notably, during summer 2021, *Ostreopsis* cell densities exceeded the alert threshold for the first time in the Bay of Biscay, resulting in the closure of several beaches. Chomérat *et al.* [7] determined that the blooms observed in the French Basque coast during summer 2021 were not monospecific, containing both *Ostreopsis cf. siamensis* and *Ostreopsis cf. ovata*, with the latter confirmed as the toxin producer. Concurrently, high densities of *Ostreopsis* spp. were also observed in San Sebastián on the Spanish Basque coast, where respiratory problems were reported among sunbathers. This



prompted the development of a monitoring programme to gain insight into the seasonal dynamics of this genus in coastal waters of the Basque Country.

One of the selected areas for intensified summer sampling was the sheltered Bay of Plentzia (Fig. 1), a popular summer destination in Biscay. The sampling site, located in the northernmost part of the bay in a rocky intertidal area beyond the breakwater protecting Astondo beach, is semi-exposed to the open sea. The rocky seabed is rich in macroalgae growing in intertidal pools. Between June and early September, six samplings were conducted in both summer 2023 (20 June–1 August) and summer 2024 (26 June–4 September). *Ostreopsis* spp. cells were quantified through light microscope counts of water column samples (cells·L<sup>-1</sup>) and epiphytic samples (cells·g<sup>-1</sup>). For the latter samples, five macroalgal species were sampled due to changes in macroalgal community dominance: *Centroceras clavulatum*, *Cladostephus spongiosus*, *Dictyota* sp., *Ellisolandia elongata*, and *Halopteris scoparia*. Alert thresholds that have

been established for the north-western Mediterranean were taken into consideration [2]. Environmental variables were also characterised and molecular analyses were conducted to identify the different *Ostreopsis* species.

Blooms of *Ostreopsis* spp. occurred during the summer months of both 2023 and 2024 in the Bay of Plentzia. Abundances (Fig. 2) remained below 250 cells·L<sup>-1</sup> and 1600 cells·g<sup>-1</sup> in June and July of both years. However, from August towards the end of the summer period there was a marked increase in both the planktonic and the epiphytic abundances of *Ostreopsis* spp., with 1.59 × 10<sup>4</sup> cells·L<sup>-1</sup> and 3.07 × 10<sup>4</sup> cells·g<sup>-1</sup> on 18 August 2023, or 1.05 × 10<sup>4</sup> cells·L<sup>-1</sup> and 5.45 × 10<sup>4</sup> cells·g<sup>-1</sup> on August 20 2024. On 4 September 2024, the maximum *Ostreopsis* spp. abundances of the study were recorded in both the planktonic and the epiphytic samples (3.55 × 10<sup>4</sup> cells·L<sup>-1</sup> and 9.54 × 10<sup>4</sup> cells·g<sup>-1</sup>), even exceeding the alert threshold established for the water column in the Mediterranean region [2]. These blooms coincided with consist-

ently warm waters exceeding 20.5 °C, peaking at 23.6 °C on 18 August 2023. This is consistent with findings of other studies [1], which associate *Ostreopsis* spp. blooms in temperate areas with water temperatures between 20°C and 29 °C. Elevated oxygen saturation levels (>109%) accompanied the highest abundances of *Ostreopsis*, reflecting the extensive macroalgal coverage of the site's rocky sea floor. Salinity and pH showed minimal variation, with ranges of 32.9–34.4 and 8.13–8.39, respectively, making their impact on blooms unclear.

Eight *Ostreopsis* strains isolated from the water column are now deposited at the Basque Microalgae Culture Collection (BMCC) at the University of the Basque Country. The amplification of the ITSA and ITSB rDNA regions confirmed that the eight strains belonged to *O. cf. siamensis*. Further studies, including qPCR and metabarcoding, will enable the detection of other potentially present *Ostreopsis* species, such as *O. cf. ovata*.

## Acknowledgements

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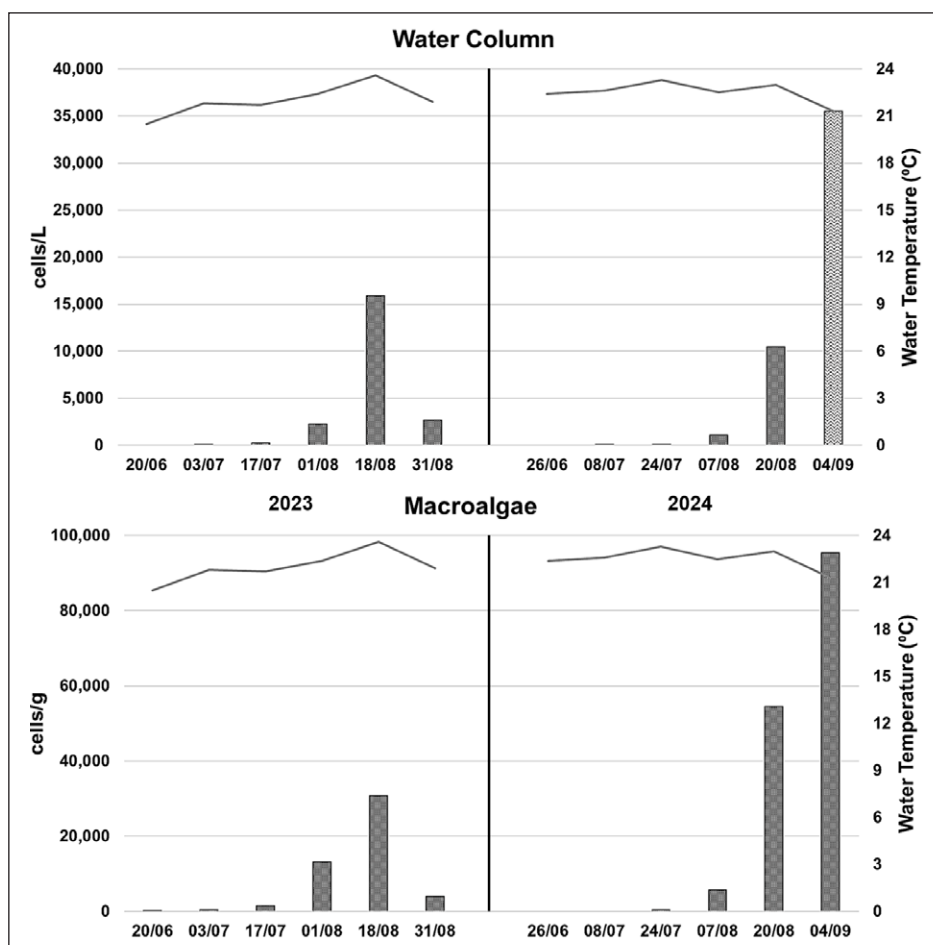


Fig. 2. Planktonic (top) and epiphytic (down) abundances of *Ostreopsis* spp. (bars) and water temperature (line) in summer 2023 and summer 2024. The distinctive bar pattern indicates that the alert threshold established by [2] for planktonic samples, 3 × 10<sup>4</sup> cells·L<sup>-1</sup>, was exceeded. The alert threshold established for epiphytic samples, 2 × 10<sup>5</sup> cells·g<sup>-1</sup> was not exceeded.

# Species behaviour affects recommended expose times of artificial substrates to sample benthic dinoflagellates

Harmful algal blooms caused by benthic microalgae (Benthic HABs) are far less studied than those caused by planktonic species. However, in recent years, the study of potentially harmful benthic dinoflagellates has garnered significant interest among HAB researchers [1]. In the southern Caribbean, notable observations include occurrence and persistence of benthic dinoflagellates belonging to the genera *Gambierdiscus*, *Prorocentrum* and *Ostreopsis*, alongside a long history of harmful events attributed to their presence [2, 3]. Species of *Gambierdiscus* are associated with the production of ciguatoxins and maitotoxins; benthic *Prorocentrum* species with okadaic acid and *Ostreopsis* species with palytoxins, ovatoxins, maccarenotoxins, ostreocins and other toxins that accumulate in shellfish and fish. These toxins can pose risks to public health and adversely impact economic activities such as tourism, fishing, and aquaculture.

The complex life styles of benthic dinoflagellates render their study somewhat difficult. While they are generally found in shallow waters and occasionally in the water column, these benthic microalgae primarily live in association with different types of substrates, such as macroalgae, seagrass, corals, rocks, and sediments [4].

Methods for quantifying benthic dinoflagellate populations face several limitations. Approaches based on natural substrates such as macrophytes (macroalgae and marine phanerogams) [5] must account for the morphological diversity and spatial and temporal variability of these substrates. Such variability complicates methodological standardization, hindering comparisons between studies. To overcome these challenges, artificial substrates have been increasingly employed [6]. Results are typically expressed as the number of cells per unit area, rather than per substrate weight, making quantitative comparisons across studies more practical and independent of natural substrate availability.

Artificial substrates offer several advantages. Unlike some natural macrophytes, they do not exude substances that attract or repel benthic dinoflagellates. Moreover, artificial substrates are less affected by grazing due to their non-palatability [7]. In addition to simplifying the estimation of benthic microalgal abundance, artificial substrates enable experimental manipulation to investigate the dynamics of substrate colonization. This can be achieved by varying exposure times and spatial arrangements. To minimize the effects of biotic processes such as competition and predation, short exposure times, not exceeding 24 hours, are generally recommended. However, given the diversity of life-cycle traits among benthic dinoflagellate species, optimal exposure times may vary depending on the target species and environmental conditions. This study presents findings (as part of a doctoral thesis) from experiments conducted to determine the appropriate exposure times of artificial substrates for representative species of the genera *Gambierdiscus* and *Prorocentrum* in the coastal waters of the southern Caribbean [8]. To evaluate the attachment and maintenance behaviour of dinoflagellates across different climatic periods (dry, transitional, and rainy seasons), artificial substrates made of fiberglass mesh (Fig. 1) were deployed in two bays within Tayrona

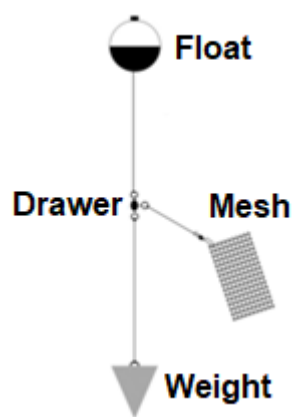


Fig. 1. Structure of the artificial fiberglass mesh substrates used to sample benthic microalgae (modified from [7]).

National Natural Park, Colombia. Population abundance was monitored after 24, 72, and 144 hours of exposure over six consecutive days. The assays revealed behavioural differences between *Gambierdiscus* sp. and *Prorocentrum* cf. *lima*. For *Gambierdiscus* sp. no significant changes in population abundance were observed between substrates exposed for 24, 72, and 144 hours, indicating that this species does not show a sustained increase or decrease in abundance with longer exposure times. Abundance estimates ranged from absent to 2,601 cells per 100 cm<sup>2</sup>. These findings align with Nakahara et al. [9], who reported that *Gambierdiscus* cells do not attach permanently to substrates but detach in response to disturbances. Similarly, Parsons et al. [10] described *Gambierdiscus* as a non-obligate epiphyte, capable of both attachment and detachment over time.

In contrast, *Prorocentrum* cf. *lima* exhibited increases in average daily abundances during the dry season, with maximal densities of up to 118,931 cells per 100 cm<sup>2</sup> observed on substrates exposed for 72 and 144 hours (Fig. 2). This species demonstrated a stronger association with substrates, indicating longer attachment times. Consequently, using prolonged exposure times (>24 hours) may introduce biases in population assessments for *Prorocentrum*.

The study highlights the need to tailor substrate exposure times to the specific behaviours and habitat conditions of the target species. For *Gambierdiscus* sp., substrates can be exposed for 24 to 144 hours without significant changes in population abundance, whereas for *Prorocentrum* cf. *lima*, shorter exposure times (<24 hours) are advisable to avoid overestimating population densities.

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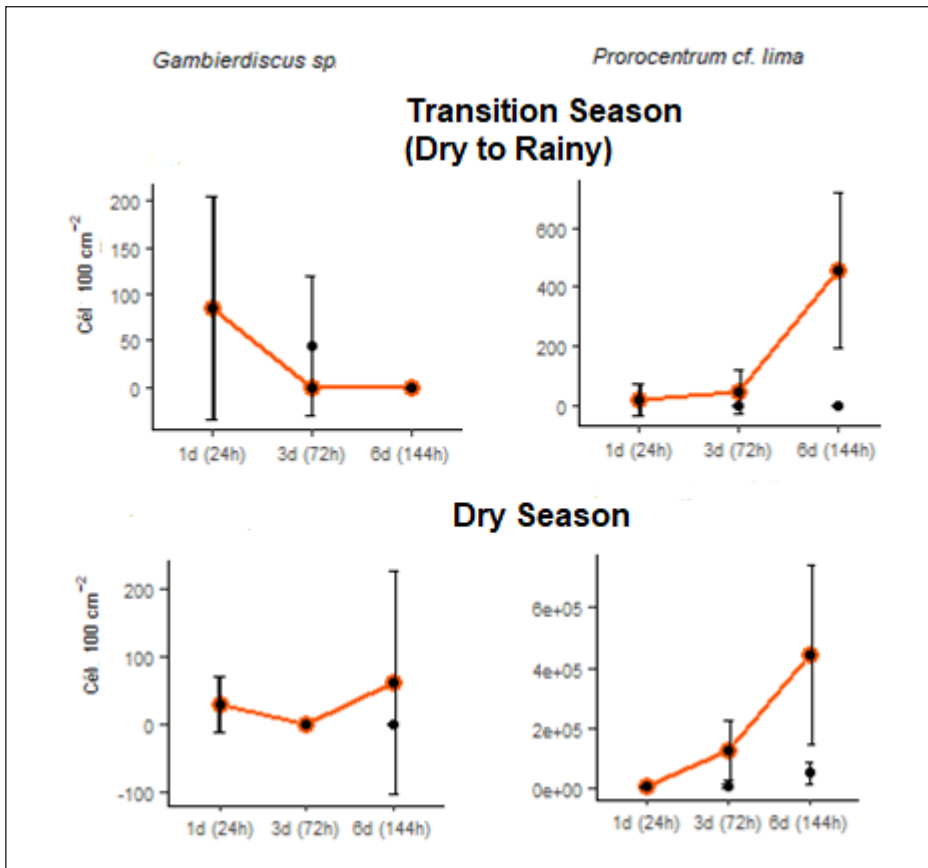


Fig. 2. Average abundance (cells per 100 cm<sup>2</sup>) and standard deviation of *Gambierdiscus* sp. and *Prorocentrum* cf. *lima*, in fiberglass meshes exposed for 24 h, 72 h, and 144 h. The orange line represents the data obtained for each exposure period, while the black dots indicate the average abundance recorded during 24 h of exposure on the same day as the meshes exposed for the longer periods.

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# New!

## IOC Manual and Guides on Sargassum

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Paris: UNESCO-IOC-GE-SAMP, 61 pp. (IOC Manuals and Guides, 96). *Doi: 10.5281/zenodo.13935854*



# First observation of *Raphidiopsis* (*Cylindrospermopsis*) *raciborskii* (Nostocales) during a heatwave in Lake Comabbio (Northern Italy)

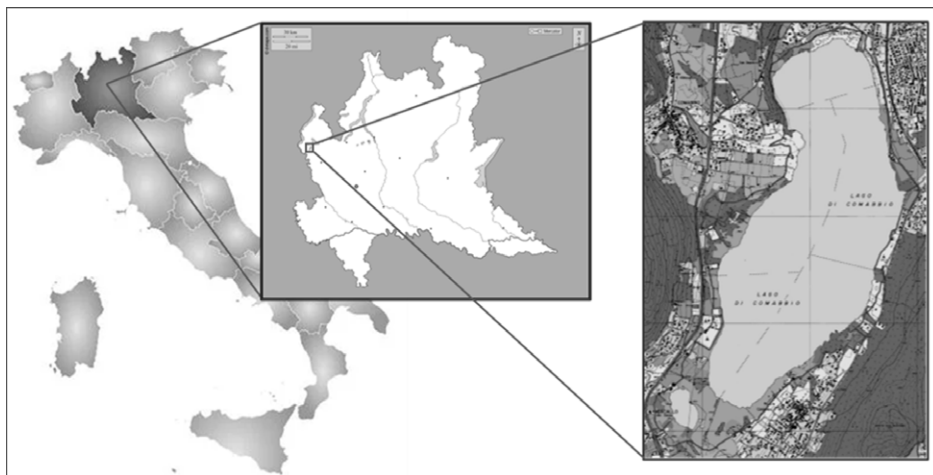


Fig. 1. Collection site showing the location of the *Raphidiopsis* (*Cylindrospermopsis*) *raciborskii* bloom.

We report the first documented observation of the freshwater filamentous cyanobacteria *Raphidiopsis raciborskii* (synonyms *Anabaena raciborskii*, *Cylindrospermopsis raciborskii*) in Northern Italy, specifically in Lake Comabbio during July 2015. Previously, *C. raciborskii* blooms were observed in Central Italy and Sardinia. This species is considered an invasive and potentially toxic filamentous cyanobacterium, originally described as a tropical species, now spreading into temperate regions. It can produce toxins like cylindrospermopsin and saxitoxins, which pose a significant health risk to animals and humans. These toxins have been demonstrated to bioaccumulate in aquatic invertebrates, and they can lead to mass mortalities of fish and birds. The increasing intensity and duration of heatwaves, predicted to rise this century, create favorable conditions for harmful, bloom-forming freshwater cyanobacteria, necessitating heightened monitoring of these evolving risks.

In recent years, *R. raciborskii* has expanded globally, particularly in temperate regions [1], due to its tolerance to a wide range of climatic conditions. This bloom-forming species, typically found in eutrophic waters, usually does not generate surface scums but shows a relatively uniform distribution throughout the euphotic zone [2, 3]. Factors such as global, eutrophication, and hydrological changes [4–6] are driving the expansion

of cyanobacteria, often occurring simultaneously [7]. In Italy, blooms of *R. raciborskii* were first observed in Central Italy in 1995 at Trasimeno Lake, followed by Albano Lake in 2002 and Cedrino Lake in Sardinia in 2003. A further study in 2004 revealed the presence of cylindrospermopsin in two of these lakes [8].

In July 2015, during a heat wave, a bloom dominated by *R. raciborskii* occurred in Lake Comabbio, a small, eutrophic, shallow polymictic lake in Northwest Italy, which is also a Natura 2000 Site and a Special Area of Conservation (ZSC IT2010008) (Fig. 1). The lake has a large watershed relative to its surface area. The inflow is provided by rainfall, groundwater and small tributaries which consist exclusively of minor torrential watercourses except for a small stream [10] and its naturally high trophic level is exacerbated by slow hydrological renewal and nutrient loading from domestic effluents [11].

Phytoplankton composition and

abundance were assessed using samples preserved in acetic Lugol's solution, and the abundance was quantified according to Uthermöhl method using an inverted microscope Leica DMI4000b at 20x, 40x and 100x magnification. Saxitoxin concentrations were quantified using enzyme-linked immunosorbent assay (ELISA). The analysis was conducted on frozen samples. Saxitoxin total content (intracellular plus dissolved toxin) was measured on the whole water samples, after freeze-thawing twice. For immunochemical detection (ELISA), 5 mL water samples were filtered by membrane filters (0.22 mm, Millex-GV), and analyzed directly with a commercially available ABRAXIS® Saxitoxin (PSP) ELISA Microtiter Plate (Abraxis, Los Angeles, California, USA), by measuring the absorbance at 450 nm (Wallac Victor2 spectrofluorometer, Perkin Elmer Inc, USA). The detection limit was 0.015 ng/ml. No cylindrospermopsin analysis were performed because this toxin standard was not available.

Chl-a concentration maps were derived from satellite data by applying a bio-optical model previously corrected for atmospheric effects. Chl-a products were obtained from images of the MSI sensor on board of the European Space Agency's SENTINEL-2 satellite with a

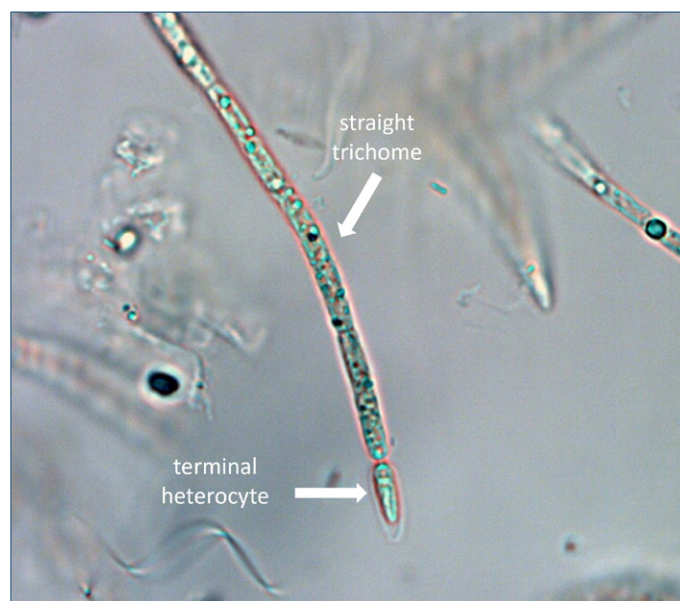


Fig. 2. *Raphidiopsis* (*Cylindrospermopsis*) *raciborskii*: trichome collected from Lake Comabbio (100x).



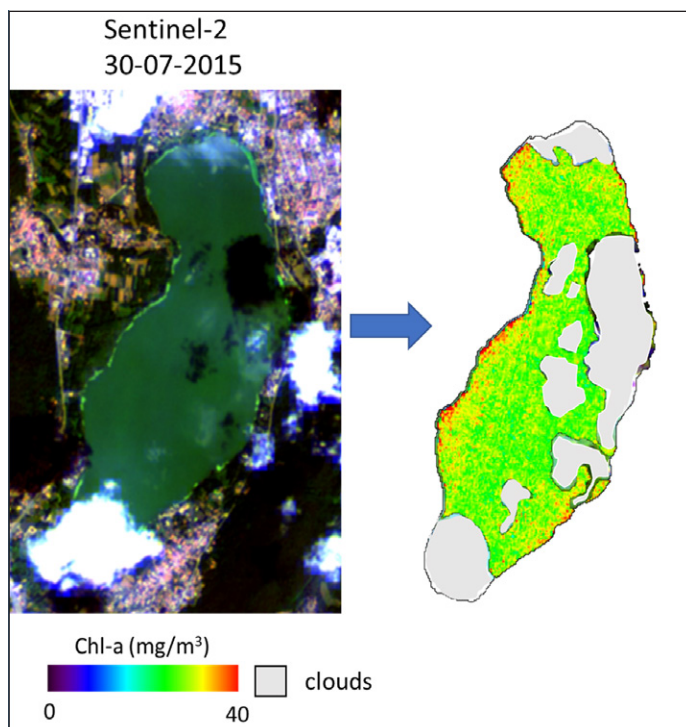


Fig. 3. Chl-a derived from Sentinel-2A imagery in Lake Comabbio.

spatial resolution of 10 meters, while for surface water temperature two products were obtained from the TIRS sensor aboard NASA's LANDSAT-8 satellite. For meteorological data, a dataset for the year 2015 based on daily average temperature (in °C) was provided by Centro Geofisico Prealpino <https://www.astrogeo.va.it/meteo/>

*R. raciborskii* in Lake Comabbio was primarily observed as straight fila-

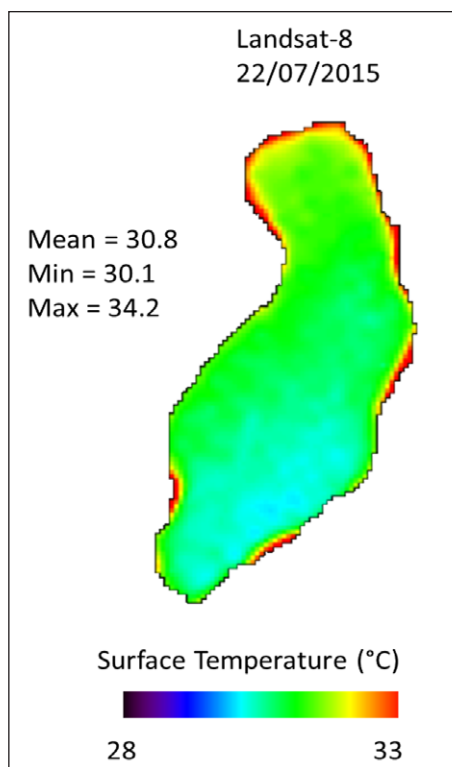


Fig. 4. Surface temperature maps derived from Landsat-8 imagery in Lake Comabbio.

ments with terminal drop-shaped heterocytes with pointed ends (Fig. 2). Coiled trichomes and akinetes were not observed, and heterocytes were observed generally at one end of the trichome. The average density of *R. raciborskii* on July 24<sup>th</sup> was  $2.5 \times 10^9$  cells·L<sup>-1</sup>, representing almost the total phytoplankton community. The highest density was  $2.6 \times 10^{10}$  cells·L<sup>-1</sup>, while the lowest was  $1.9 \times 10^8$  cells·L<sup>-1</sup>.

Chemical analysis of lake samples revealed saxitoxin concentrations of 0,27 to 0,36  $\mu\text{g}\cdot\text{L}^{-1}$ , investigated through ELISA immunoassay analysis, and were correlated with *R. raciborskii* presence.

For chlorophyll estimation, satellite images were atmospherically corrected with the 6S radiative transfer code and the obtained reflectances were converted into Chl-a concentrations by means of a semi-empirical algorithm based on the ratios between the reflectances in the regions between 700 and 650 nm. Satellite-derived Chl-a concentrations ranged from  $30 \text{ mg}\cdot\text{m}^{-3}$  to  $40 \text{ mg}\cdot\text{m}^{-3}$  in the littoral zones (Fig. 3). Two satellite-derived temperature maps were available for the study period (20<sup>th</sup> July and 6<sup>th</sup> August), and we focused on the image from 20<sup>th</sup> July. Superficial temperatures showed a mean value of 30.8°C with a maximum of 34.2°C (Fig. 4). The heat wave in July 2015 was the longest on record, surpassing previous records of 2003 and 2014. Maximum temperatures exceeded 30°C for 26 consecutive days, from June 30<sup>th</sup> to July 25<sup>th</sup>.

Saxitoxins were detected at low concentrations, but further analysis via high-performance liquid chromatography (HPLC) is necessary. Landsat-8 and Sentinel-2A satellite data were instrumental in confirming the high Chl-a concentrations associated with this bloom, which occurred alongside high surface temperatures. Satellite and mi-

croscopic data were consistent, reinforcing the challenge in detecting this type of bloom, which does not form visible surface scums and only slightly alters water color. Due to the presence of cyanotoxins, this emerging threat to public health underscores the need for effective monitoring systems. Additionally, raising public awareness and informing stakeholders about the risks associated with these toxins is critical for ensuring community safety and well-being.

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# Addressing the increasing risk of Paralytic Shellfish Toxins in Ireland

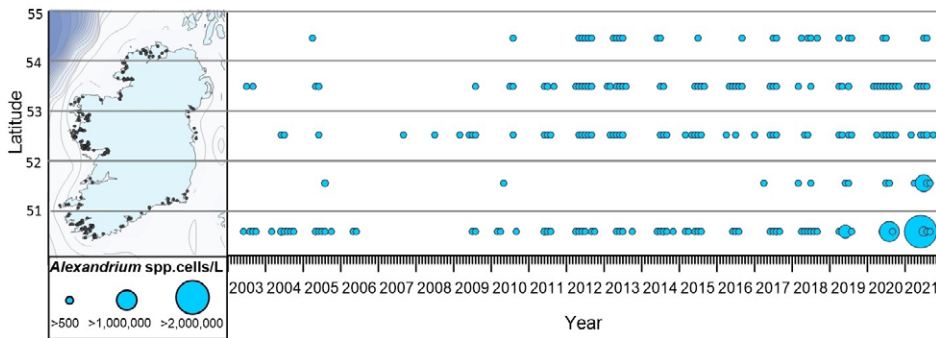


Fig. 1. Temporal and spatial distribution of *Alexandrium* species cell counts (>500 cells L<sup>-1</sup>) from 2003 to 2021. The X-axis represents month/year, while the Y-axis represents latitude. The bubble-plot shows average monthly *Alexandrium* species cell counts (cells L<sup>-1</sup>).

Over recent decades, Irish inshore and coastal waters have experienced noticeable changes in the occurrence, abundance, and geographical distribution of *Alexandrium* species (Fig. 1). These changes are associated with an increased frequency of Paralytic Shellfish Toxin (PST) events and accumulation of PST concentrations in several marine bivalve and mollusc shellfish species.

Since PST surveillance and monitoring began in Ireland in the mid-1990s, PST concentrations exceeding the permitted EU regulatory threshold were initially confined to a single classified production area on the south coast, Cork Harbour. However over the last decade (2011–2020), there have been significant changes in the geographic distribution, bloom frequency, and

peak cell abundance (>2 million cells L<sup>-1</sup> observed) of both toxin and non-toxin-producing *Alexandrium* species, most notable off the southwest coast of Ireland (Fig. 2). In 2019, PST concentrations exceeded regulatory levels for the first time in mussels from a classified production area, Castlemaine Harbour, in the southwest.

Castlemaine Harbour (County Kerry, southwest Ireland; Fig. 3) is an important classified shellfish production area, producing Pacific oysters and blue mussels. Since 2019, this area has experienced near-annual closures, typically lasting several weeks, due to both mussels and oysters exceeding EU regulatory limits for PSTs. Notably, in 2020, the highest PST concentrations ever recorded in Ireland — approximately

three times the regulatory limit— were observed since the commencement of national PST monitoring in the mid-1990s (Fig. 4).

The public health risks, along with the significant economic impact on the shellfish industry, underscore the need for rapid, efficient, and reliable monitoring tools. To address these challenges, the PSPSafe Project (Ref. 2021R578, [www.pspsafe.ie](http://www.pspsafe.ie)) was established, funded by the Irish government (Department of Agriculture, Food, and the Marine, DAFM). PSPSafe is a multi-disciplinary collaboration involving the Marine Institute, Atlantic Technological University Galway, and University College Dublin. The project commenced in November 2021 and will continue until May 2026, aiming to address knowledge gaps regarding the causes, timing, environmental factors, and mechanistic pathways of PST occurrences in Castlemaine Harbour. The project workflow includes establishing a comprehensive sampling programme in the area (Work Package 1), developing novel molecular methods to target toxin-producing *Alexandrium* strains (Work Package 2), and implementing predictive models and risk assessment tools to support the regulatory authorities and the shellfish industry (Work Package 3). By achiev-

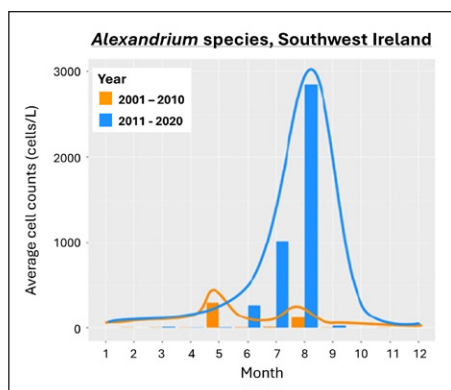


Fig. 2. Average monthly *Alexandrium* species cell counts (cells L<sup>-1</sup>) southwest coast of Ireland; for two time periods, 2001–2010 (orange) and 2011–2020 (blue). The X-axis represents months, and the Y-axis represents average monthly *Alexandrium* species cell counts (cells L<sup>-1</sup>). The orange/blue distribution lines are hand-drawn for visual purposes only, illustrating seasonal changes in abundance between the two time periods; they are not statistically derived.



Fig. 3. Pacific oyster trestles in the PSPSafe project study area, Castlemaine Harbour. Reproduced from [1].



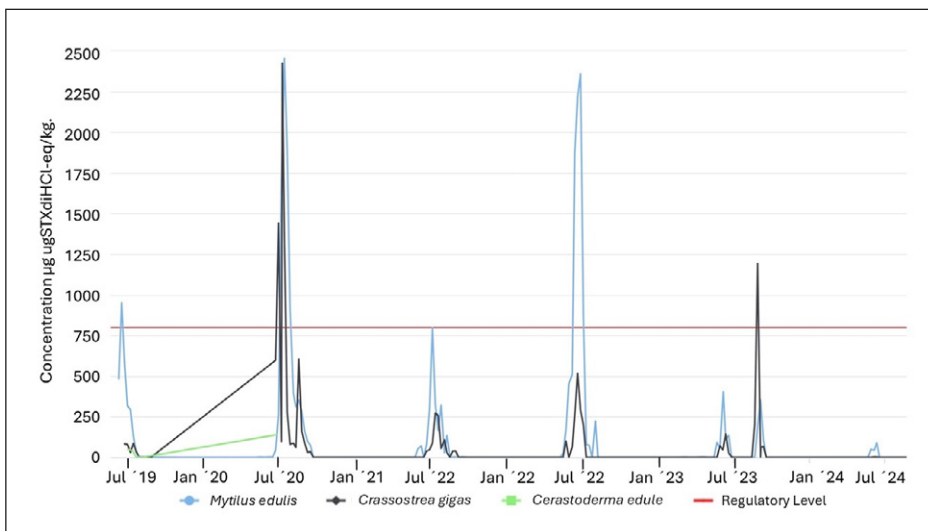


Fig. 4. Results of the National Monitoring program for PST analysis in mussels and oysters in Castlemaine Harbour from 2019 to 2024. Source: HABs Website (<https://webapps.marine.ie/habs>), Marine Institute

ing these objectives, the project aims to support the shellfish industry while ensuring consumer food safety.

**Work Package 1** involves an annual, intensive sampling campaign in Castlemaine Harbour, focusing on the onset and occurrence of PST events. During the predicted bloom period, samples are collected weekly from four sampling stations (Fig. 5). These include water, shellfish (Pacific oysters and mussels), and sediment. Both shellfish and water samples are analysed to identify and quantify the PST analogues present. Further analysis are conducted to morphologically and taxonomically identifying and distinguish *Alexandrium* species in water samples and resting cysts in sediments, using light, epifluorescence, and scanning electron microscopy. To study the biology and toxicology of the observed *Alexandrium* species, live monocultures are established either from isolated motile cells in water samples or from the germination of cysts in the sediment samples.

*Alexandrium minutum* has been identified as the primary PST-producing species in Castlemaine Harbour. Further analysis will aim to identify any possible variation within the species complex.

**Work Package 2** focuses on developing and implementing molecular tools to identify and characterize *Alexandrium* species potentially responsible for PST events in Castlemaine Harbour. Molecular tools offer a promising solution for improving *Alexandrium* detection, surveillance, management, and early warning strategies due to their

sensitivity, accuracy at both intra- and inter-specific levels, quantitative capability, and rapid execution. This work package aims to establish a reference tissue bank and an associated DNA sequence database of *Alexandrium* species relevant to Irish waters, and to validate a panel of key genetic markers targeting potential PST-producing *Alexandrium* strains. Furthermore, gene expression analysis will be conducted to characterize inter- and intra-specific variations in genes associated with PST production.

**Work Package 3** aims to develop a model to estimate the occurrence of PST in shellfish in Castlemaine Harbour. This will use phytoplankton and toxin monitoring data collected in Work Package 1, alongside in-situ datasets from the national monitoring programme,

and environmental, oceanographic, and meteorological data. Additionally, it seeks to develop a quantitative risk assessment of PSTs and the potential food safety issues associated with consumption of contaminated shellfish. Environmental data to date indicate that bloom distribution is primarily driven by temperature, tide fluctuations, and current patterns, while bloom abundance is influenced by turbidity and competition from other blooming species.

The outputs of the PSPSafe Project will include a trial of a new early warning system for PSTs and *Alexandrium minutum* events for the industry, regulators, and the public, thanks to the development, validation, and implementation of novel genetic markers to complement traditional methods for detecting PST-producing *Alexandrium*.

Additionally, the project envisages the development of predictive modelling and risk assessment tools to forecast the occurrence and onset of PST events. These tools will be adaptable and applicable to other aquaculture production areas that may be at risk of PST events. The ultimate goal is to address key questions regarding the recent increase in PST concentrations and the large-scale blooms of both toxic and non-toxic *Alexandrium* species in aquaculture areas.

A crucial component of the PSPSafe Project deliverables is knowledge transfer through annual workshops, webinars, and national and international conferences, as well as reports on biotoxin, phytoplankton, and molecular

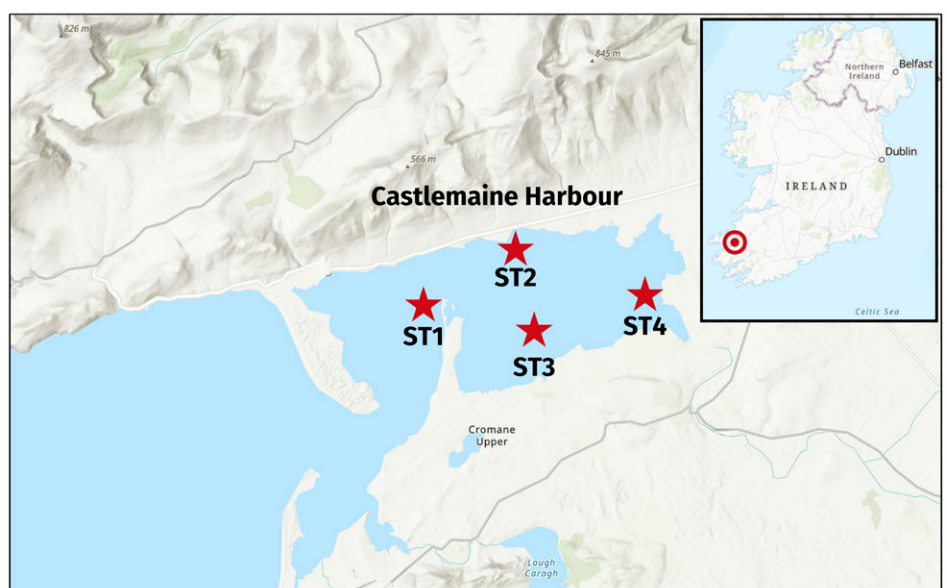


Fig. 5. Map of Castlemaine Harbour showing PSPSafe Project sampling stations (St.1, St. 2, St. 3, and St. 4).

analysis results. The final project reports will cover the spatio-temporal occurrence and abundance of *Alexandrium minutum* in Castlemaine Harbour, discoveries from the genetic analysis of PST-producing *Alexandrium* species in Irish waters, and recommendations for early-warning protocols for detecting Harmful Algal Blooms (HABs) in shellfish production areas.

The outputs and deliverables of the project will enable the implementation of risk management strategies and tools for the aquaculture industry and regulatory authorities. This will help ensure consumer safety and preserve the integrity and commercial reputation of Irish shellfish.

### Acknowledgements

The PSPSafe Project is supported and funded by the Irish Department of Agriculture, Food and the Marine (DAFM, Project Ref 2021R578). We are grateful to the shellfish producers and industry within Castlemaine Harbour for their support with sampling campaigns, advice, and local knowledge. Our thanks also to the local representative offices from Sea Fisheries Protection Authority and Bord Iascaigh Mhara.

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## Marine fungi in HABs: Revealing new parasite species and their role in dinoflagellate blooms

Harmful blooms of marine dinoflagellates harbor a remarkable diversity of specific fungal parasites, which have been little studied until now. Fungal parasites have been shown to play an important role in bloom dynamics in freshwater ecosystems. For this reason, and given the current lack of knowledge about marine fungi—particularly regarding their diversity, spatial patterns, and ecological functions in marine ecosystems—a doctoral thesis was conducted to deliver insights into the diversity and ecology of marine chytrids.

The main objective of the thesis was to investigate the fungal community composition in a Mediterranean coastal ecosystem, with a specific focus on the basal fungal parasite group Chytridiomycota (chytrids), capable of infecting dinoflagellates. To achieve this, the following specific objectives were proposed: i) to evaluate the spatial distribution of Chytridiomycota diversity in various coastal areas during dinoflagellate proliferations [1]; ii) to examine

the temporal dynamics of the fungal community in epiphytic communities during benthic dinoflagellate proliferations and investigate their interaction with hosts [2]; and iii) to identify and

describe new parasitic Chytridiomycota species associated with their host proliferations [3].

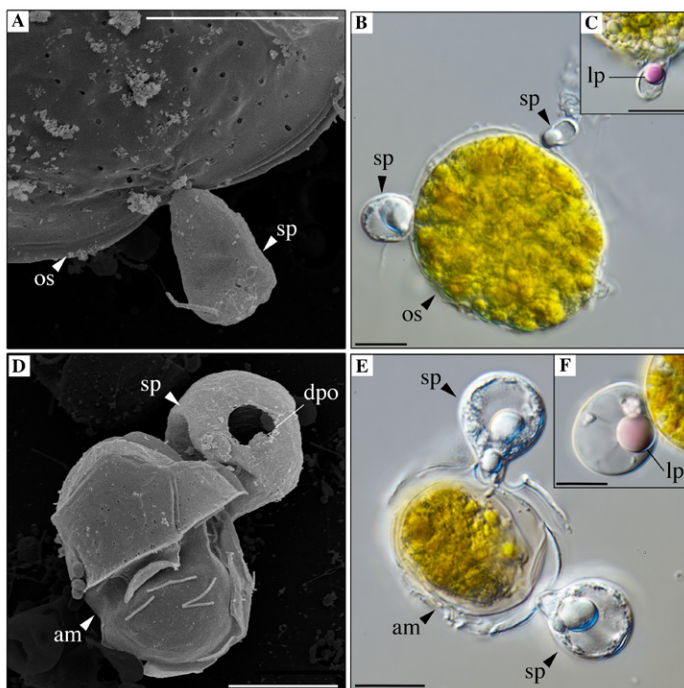
The chytrid community in coastal environments is particularly dynamic and diverse, with a marked specificity for dinoflagellate hosts such as *Alexandrium minutum* and *Ostreopsis* spp. These fungal parasite communities are more abundant and diverse in coastal sediments and macroalgal surfaces (biofilms) than in the water column. During dinoflagellate blooms, the relative abundance of chytrids in the water column increases, suggesting opportunistic parasitic behavior in situations of high host abundance [1].

In the laboratory, strains of four chytrid species, including the most abundant ones detected through metabarcoding sequencing, were cultured and identified [1]. Three of these strains correspond to previously undescribed species, and the cultures have enabled the morphological and molecular characterization of two species, *Dinomyces gilberthii* and *Paradinomyces evelyniae*, which were formally described [3] (Figs. 2–3). In terms of geographic distribution, *Dinomyces gilberthii* is widely distributed in Mediterranean coastal habitats and is particularly prevalent during summer blooms of *Alexandrium*

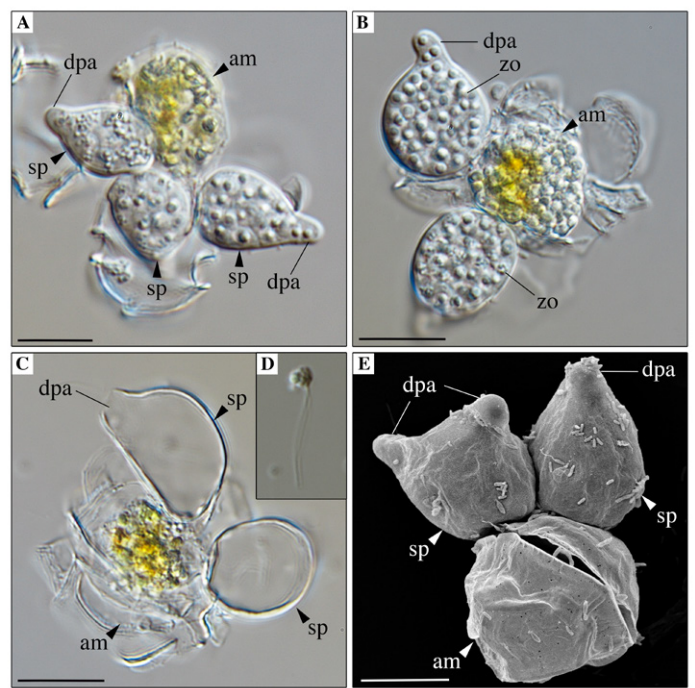


Fig. 1. AD Fernández-Valero doctoral thesis.





**Fig. 2.** Life-cycle stages of *Dinomyces gilberthii* sp. nov. in the light microscope (B–C) and scanning electron microscopy (D). (A) Sporangia showing the lipid globule compartmentalization during zoospore formation. (B) Mature sporangium with a discharge papilla. (C) Empty sporangium with a discharge papilla. (D) Two mature sporangia with one and two discharge papillae. Abbreviations: am, *A. minutum*; ap, apophysis; dpa, discharge papillae; sp, sporangium; zo, zoospores. Scale bars = 10  $\mu$ m. Adapted from [3].



**Fig. 3.** Life-cycle stages of *Paradinomyces evelyniae* sp. nov. in the light microscope (B, C, E, F) and scanning electron microscopy (A, D). (A) Early sporangium. (B) Two young sporangia infecting an *Ostreopsis* sp. cell. (C) Nile Red staining of a lipid globule from a young sporangium. (D) Empty sporangium with a discharge pore. (E) Development of the infection of two sporangia in a cell of *A. minutum*. (F) Growth of the lipid droplet in a Nile Red-stained sporangium. Abbreviations: am, *A. minutum*; dpa, discharge papilla; dpo, discharge pore; lp, lipid globule; rh, rhizoid; sp, sporangium; os, *Ostreopsis* sp. Scales bars = 10  $\mu$ m. Adapted from [3].

*minutum*, representing up to 15.5% of the eukaryotic community during these blooms events. In contrast, species such as *Paradinomyces evelyniae* exhibit a more restricted distribution in these habitats [1, 3].

The study of the temporal dynamics of fungal communities in less explored niches, such as macroalgal biofilms, has revealed an alternation in the abundance of different fungal groups, significantly influenced by the activity of species of the epiphytic dinoflagellate genus *Ostreopsis* [2]. After the peak in *Ostreopsis* abundance, the species *Dinomyces arenysensis* dominated the community, with chytrids constituting up to 30% of the eukaryotic community. Additionally, experiments conducted to evaluate the impact of these parasites on *Ostreopsis* growth showed that *D. arenysensis* had a remarkable adaptability in its survival strategies (from harmless to lethal parasitism), including trophic versatility (ability to infect different dinoflagellate species and even pollen) [2].

The results obtained show that chytrids play an important role in the

dynamics of marine dinoflagellate blooms and in structuring microbial communities, both planktonic and benthic, in coastal ecosystems.

### Acknowledgments

This research was conducted as part of the doctoral thesis “Delivering insights into the diversity and ecology of marine chytrids” [4], with the support of the research team from the Littoral Biological Processes (PBL) group at the Institute of Marine Sciences (ICM) in Barcelona, Spain.

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# Ifremer-Cawthron Blue Chair initiative: A joint scientific endeavour to advance the Blue Economy

In 2023, Ifremer and the Cawthron Institute signed a Memorandum of Understanding (MoU) to formalize and expand their long-standing collaboration. This partnership took a significant step forward in May 2024, when a delegation of experts from Ifremer visited the Cawthron Institute in Nelson, New Zealand (Fig. 1), to co-develop plans for a landmark initiative known as the “Blue Chair.”

The Blue Chair is an ambitious scientific collaboration designed to harness the combined expertise of both institutions, accelerating research in critical areas for the blue economy across France, the Pacific, and Aotearoa New Zealand. The initiative seeks to

provide innovative science and technology solutions to address emerging challenges associated with climate change, environmental stressors, and sustainable food production, with a particular focus in aquaculture.

Key objectives of the Blue Chair include:

1. Developing advanced surveillance and monitoring tools to better understand marine environments and detect potential threats, such as harmful algal blooms (HABs).
2. Designing holistic approaches to explore the complex interactions between marine species and their environments.

3. Innovating sustainable breeding, husbandry, and disease management strategies to minimize environmental impacts and increase the resilience of blue food production systems.

By focusing on sustainability and adaptability, the Blue Chair aims to secure a robust future for the global blue economy, contributing to food security while reducing environmental degradation. This initiative underscores the shared commitment of Ifremer and Cawthron to pioneer transformative solutions for aquaculture and ocean health in a rapidly changing world.

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Fig. 1. Ifremer scientists with Cawthron colleagues at the Cawthron Aquaculture Park, Nelson, New Zealand, May 2024.





# The 13<sup>th</sup> Advanced Phytoplankton Course: Holding back the taxonomy crisis for three weeks

Taxonomy forms the foundation of all research fields concerning phytoplankton, encompassing ecology, physiology, harmful algal blooms, and biotechnology. Species names act as anchors, linking information on morphology, life cycles, distribution and biochemistry of a given organism, thereby constructing the edifice of human knowledge. Traditionally, the taxonomy of unicellular microalgae relied on morphological characters visible through light microscopy. The advent of electron microscopy in the last century unveiled a plethora of ultrastructural features and their organic or inorganic walls, producing a leap forward in taxonomic knowledge. More recently, DNA sequencing has revolutionized the field, validating or challenging traditional identification methods and phylogenetic theories.

Despite its significance, phytoplankton taxonomy has faced a profound crisis over recent decades in many countries, as fewer researchers have been trained in this discipline, at times considered 'old fashioned'. However, the increasing use of DNA (meta)-barcoding methods and the burgeoning interest in biotechnological applications have given a new impetus to taxonomic research. Many unicellular microalgae remain undiscovered, undescribed, or misclassified due to the high occurrence of cryptic species. Without detailed taxonomic studies, we cannot fully comprehend phytoplankton diversity, ecological roles, responses to environmental change, or their potential applications in human health and other biotechnological applications.

The Advanced Phytoplankton Courses (APC) series, focusing on Taxonomy and Systematics was initiated in 1976 upon recommendation of the SCOR-Working Group on Phytoplankton Methods (WG 33, 1969). The first APC, led by Grethe R. Hasle, was hosted at the Marine Botany Section of the University of Oslo. After APC1, two other advanced Courses were held at the Biological Station in Drøbak (Norway), in 1980 and

1983. In the following years, eight APCs were organized at the Stazione Zoologica Anton Dohrn in 1985, 1990, 1995, 1998, 2005, 2008, 2015 and 2024, while APC10 was held at the University of Copenhagen in 2012 and APC12 at the Station Biologique de Roscoff (SBR, France) in 2019.

The APC series aims to provide already experienced participants with up-to-date knowledge on the identification and taxonomy of marine phytoplankton, including diatoms, dinoflagellates, coccolithophores and other phytoflagellate groups. The interest in the Course is testified by the high number of applications (often around 100) received at every call. Over the years, distinguished colleagues in the field of phytoplankton ecology and taxonomy have been trained at one of the phytoplankton courses. Because selection is

based on expertise, the course is also a forum for in-depth taxonomic discussions where participants can share their experience and knowledge.

The 13<sup>th</sup> Advanced Phytoplankton Course (APC13) was hosted at the Stazione Zoologica Anton Dohrn in Naples (6–26 October 2024), and was organised together with the Station Biologique de Roscoff and the IOC Science and Communication Centre on Harmful Algae (IOC UNESCO/ SCCHA), with support of the Ocean Teacher Global Academy (IOC UNESCO/ OTGA). The Museum-Darwin Dohrn of the SZN provided the venue, in a room equipped with 23 microscopes graciously provided by ZEISS. The course faculty consisted of 13 experts of the different phytoplankton groups (Fig. 1) engaging in a three-weeks-long taxonomic marathon with 21 researchers from 15 different countries – from Europe, but also from China, United States, South America, Australia and New Zealand (Fig. 2).

The intensive three-weeks programme consisted of lectures, demonstrations and practical sessions dur-



**13<sup>th</sup> Advanced Phytoplankton Course**  
Taxonomy and Systematics  
Naples, 2-26 October 2024

**Faculty**

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Fig. 1. The APC13 faculty. APC13 logo by Leonilde Roselli.



Fig. 2. APC13 group picture outside of the Darwin-Dohrn Museum of the SZN.

ing which participants were trained in classical techniques integrated by new approaches of automated imaging and molecular taxonomy. The presentations were made available to the students through the IOC platform OTGA. Participants had the opportunity to examine a diverse collection of preserved and live materials (Fig. 3), which were identified based on up-to-date taxonomic literature, and learned how sequence data and web resources can be used for iden-

tification purposes. More than 100 different microalgal strains were offered by the Roscoff Culture Collection (RCC) and the Norwegian Culture Collection of Algae (NORCCA). There were demonstrations of strain isolation and maintenance, observations of samples through scanning electron microscopy, and an introduction to automated imaging tools. In addition to criteria and methods for species identification, course topics included principles of phyloge-

netics and DNA metabarcoding, while the potential to be harmful or beneficial to human activities was highlighted whenever appropriate.

Despite the tight schedule of lessons and observations, along the three weeks there were also some opportunities for breaks and social activities. A full immersion in the lively Sanità district of Naples in a splendid sunny day started with a visit to the ancient catacombs of San Gennaro (Fig. 4) and to other churches of the area. During one day of the second week, a guided tour explored the ancient Roman streets, houses and shops of the amazing archaeological site of Pompeii, the town buried under the ashes of the Mount Vesuvius's eruption in 79 AD. Rain did not disturb the visit too much, while grey clouds around the summit of the Vesuvius somehow evoked a volcanic plume.

A full recognition of the utility of the three-week course has always been the dominant feeling in each APC, which constitutes a reward for the considerable efforts of the organisers, teachers and participants. On the last day, an open discussion brought to light the overall enthusiastic appreciation by all participants but also several sugges-



Fig. 3. Studying plankton samples under the microscope.





Fig. 4. APC13 visiting the catacombs of San Gennaro.

tions for future improvements. Since knowledge on phytoplankton taxonomy has significantly expanded over ca. 50 years since the first APC, the Course should last at least a few more days to accommodate properly all the taxonomic novelties and allow a better assimilation of the great amount of information offered to the students. Yet, APC

can only disclose tools and directions to the phytoplankton taxonomy knowledge, which the participants will have to explore and deepen in their future career.

Plans for the next APC are already underway, with the conviction that experts should keep on offering a broad training and a discussion forum to new

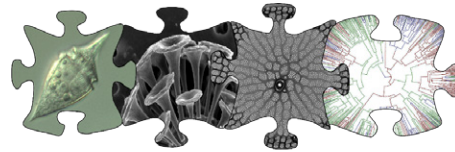
generations of phytoplankton taxonomists. The challenge is to keep on raising awareness on the need of taxonomy courses, engage young taxonomists in the faculty and open more positions in the field. Funding for the organization, materials and travels is also a growing issue. APC has so far relied on the volunteer initiative of a number of researchers, whereas in the future it should be consolidated and fully supported with the help of relevant international organizations concerned with biodiversity and marine ecosystem health issues.

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Fig. 5. The APC13 participants. APC13 logo by Leonilde Roselli.

# ICES-IOC Working Group on Harmful Algal Bloom Dynamics 2024 Meeting

The joint ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD) serves as an important forum for ICES (International Council for the Exploration of the Seas) and the Intergovernmental Oceanographic Commission of UNESCO (FAO-IOC-UNESCO) to review and discuss HAB events, and to provide annual advice and updates on the state of HABs in the North Atlantic region. It also facilitates interaction between scientists working in diverse areas of HAB science and monitoring and provides a forum for the interchange of various approaches to HAB research. The group reports to both the ICES Science Committee (SCICOM) through the Ecosystems Processes and Dynamics Steering Group and FAO-IOC UNESCO Intergovernmental Panel on Harmful Algal Blooms (IPHAB).

The group's annual meeting, (hybrid format), was held over three days, 16<sup>th</sup>–18<sup>th</sup> April, 2024 at Ifremer, Nantes, France. It brought together 35 representatives from 14 countries, including Denmark, Estonia, USA, UK, Ireland, France, Spain, Portugal, Netherlands, Germany, Poland, Sweden, Norway, and Canada (Fig.1). The meeting was official-

ly opened by Dr. Philipp Hess, director of research unit PHYTOX, IFREMER, and current chair of IPHAB.

For the new three-year cycle (2024–2026), WGHABD is co-chaired by Dave Clarke, Ireland and Lars Johan Naustvoll, Norway. This meeting and its participants focused its discussions, presentations and progress which are directly linked to the previously agreed current cycle's Terms of Reference (ToR) descriptors which detail the ToR descriptor background, its expected duration, and expected Year 1 deliverables as detailed and approved in the ICES document WGHABD Terms of Resolutions 2024–2026 and also in accordance with the described Year 1 summary of the identified work plan. New topic areas for this cycle include HAB distribution and frequency in a changing climate, Early Warning Systems for HAB forecasting, bloom mitigation and control, and interactions of HAB dynamics with multidimensional environmental stressors on marine mortalities.

There was a number of additional presentations from chair invited guests to this meeting which covered a range of relevant topic areas to the Working

Group including *Lingulodinium polyedra* blooms along the French Atlantic coast; update on remote sensing tools and applications; new tools for investigating relationships between HAB and mixotrophy; AI for HABs in an early warning system; and updates from our joint co-sponsor IOC-UNESCO on Toxins database; HAB-Solutions (UN Decade of Ocean Science approved project) and update on GlobalHAB Scientific Steering Committee activities and outputs. A newly funded project on *Ostreopsis* – *Ostreabilla* was also presented.

The next WGHABD annual meeting is scheduled for April 2025, and will be hosted by the Swedish Meteorological & Hydrological Institute (SMHI), Gothenburg, Sweden.

Information on HAB event occurrence in the North East Atlantic margin area is reported annually by the Working Group members and is publicly available in the Harmful Algal Event database (HAEDAT <http://haedat.iode.org/>) and also through Harmful Algal Information System (HAIS <https://data.hais.ioc-unesco.org/>).

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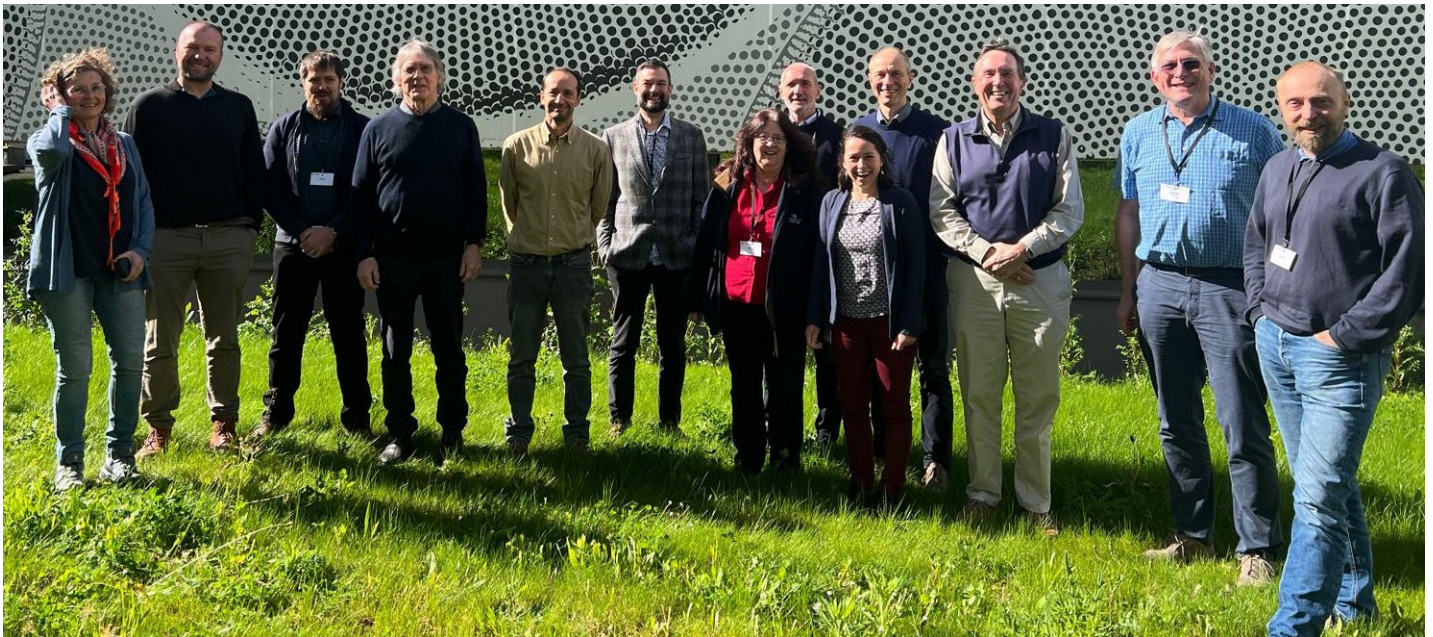


Fig. 1. WGHABD participants, from left to right: Morgan Le Moigne, Marnix Poelman, Adam Lewis, Allan Cembella, André Sobrinho Gonçalves, Dave Clarke, Cynthia McKenzie, Per Andersen, Maud Lemoine, Henrik Enevoldsen, Don Anderson, Bengt Karlson, and Lars-Johan Naustvoll.



# Forthcoming

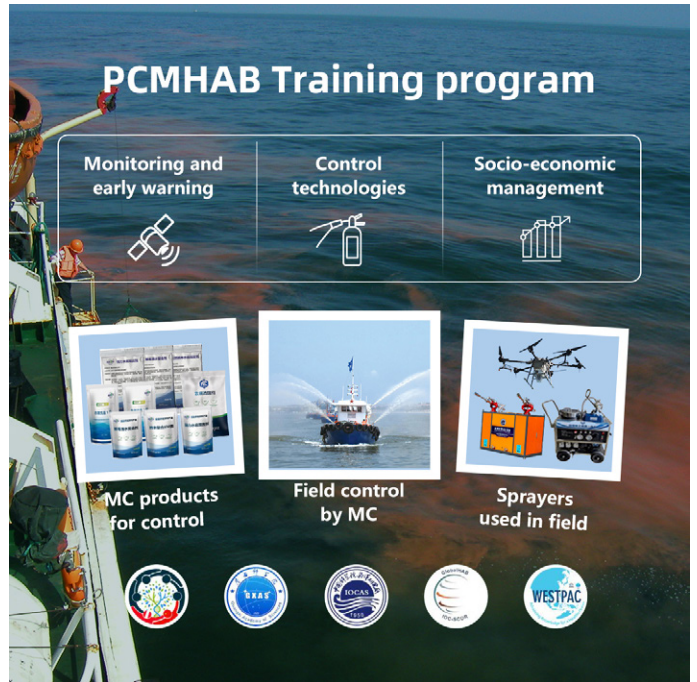
## The International Training Program on Prevention, Control, and Mitigation of Harmful Algal Blooms (PCMHABs)

Dear HAB community, we are excited to announce an international training programme, aimed at introducing the Modified Clay (MC) approach for the prevention, control, and mitigation of harmful algal blooms (PCMHABs). The training programme is organized by the Institute of Oceanology, Chinese Academy of Sciences (IOCAS). Scheduled from December 1 to 11, 2024, in Guangxi province, China. The programme will cover key topic lectures, laboratory operations, and field practical applications.

Details of the programme can be found on the GLOBAL-HAB website:

<https://www.globalhab.info/activities/globalhab-endorsed-projects/17-globalhab-endorsed-projects/149-training-programs-on-pcm-habs-for-mariculture-stakeholders-of-southeast-asian-countries>

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## ISSHA 21<sup>st</sup> International Conference on Harmful Algae (ICHA)

The 21<sup>st</sup> edition of ICHA will be held in Punta Arenas (Magallanes, Chile) from October 19 to 24, 2025.

### More information

<https://issha.org/icha-conference/>  
and <https://icha2025.org/>

### Important dates

*Registration opens:* January 13<sup>th</sup> 2025  
*Early Bird ends:* May 16<sup>th</sup> 2025  
*Late Registration ends:* Sept. 19<sup>th</sup> 2025

### Abstract submission

*Opens:* January 20<sup>th</sup> 2025  
*Deadline:* June 1<sup>st</sup> 2025

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# New HAN co-Editor-in-Chief

Kenneth Neil Mertens, originally from Belgium, has been a researcher at Ifremer since 2016. He completed his PhD on dinoflagellate cysts, which laid the foundation for his continued research on dinoflagellate taxonomy and paleoecological applications. Kenneth's work primarily focuses on understanding the ecological roles of dinoflagellates, their identification through molecular techniques, and their use in reconstructing past environmental conditions. Currently

based at Concarneau, France, he contributes to various international projects examining the impacts of harmful algal blooms and the use of dinoflagellates as bioindicators of environmental change. Now serving as the new co-editor in chief of *Harmful Algae News*, Kenneth takes over the role from Eileen Bresnan, whose dedicated service has been invaluable. He looks forward to continuing the journal's legacy, advancing research in harmful algal blooms, and fostering

collaboration among scientists worldwide.



Kenneth Neil Mertens

## Editors-in-chief

Beatriz Reguera  
Kenneth N. Mertens

## IPHAB

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**Vice-Chair** Maggie Broadwater

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**Biotoxins** Philip Hess  
**HAIS/GHSR** Eileen Bresnan  
**Early Warning** Alejandra Silva  
**Fish Kills** Allan Cembella  
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## Deadline to submit material for next issue

**1 February 2025**

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