ANCA IOCARIBE GROUP APRIL 2021-OCTOBER 2023 SUMMARY OF COSTA RICA

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Background

Since 1999 to date, it has been confirmed that the main problem associated with harmful algal blooms in Costa Rica is the intoxication episodes caused by PSP mainly associated with the dinoflagellates *Pyrodinium bahamense var. compressum* and to a lesser but equally important extent with *Gymnodinium catenatum*. Available data on poisoning by ingestion of contaminated mollusks in recent years indicate that to date there are no official reports of fatalities and epidemiological pictures have only been observed in some areas of the Pacific of Costa Rican.

Since 2008, we have observed episodes of mortality-producing HABs in corals on the north coast of the Pacific of Costa Rican, produced mainly by the dinoflagellate *Margalefidinium polikrykoides* as well as by cyanobacteria. In this same area, we have observed an increase in blooms of the dinoflagellate *Lingulodinium polyedrum*, various species of *Dinophysis* spp. and recently we have observed many algal blooms produced by different species of the dinoflagellate *Tripus*, however, we have not been able to corroborate the toxicity of these species due to lack of support for toxin analysis. Likewise, episodes of mass fish mortality are observed every year in the Pacific coast of the country, where the main causal species is *Margalefidinium polikrykoides*.

The Pacific Ocean of our country, is the main fishing area of the country, but despite this, the monitoring of microalgae is very limited and restricted to specific areas of oyster farms or areas of artisanal mollusk extraction, These areas of exploitation of the Japanese oyster *Magallana gigas* are more concentrated in the Gulf of Nicoya (central Pacific) because monitoring depends on the operational capacities of the public universities involved in the issue, mainly the Phytoplankton Laboratory of the National University, which carries out weekly sampling of water samples in various sites where oyster farms are located. The socioeconomic impact of the effects of HABs on the Pacific coast has been adverse because the government must subsidize with economic support the families that are affected when toxic algal blooms occur, and the extraction or commercialization of bivalve mollusks is prohibited.

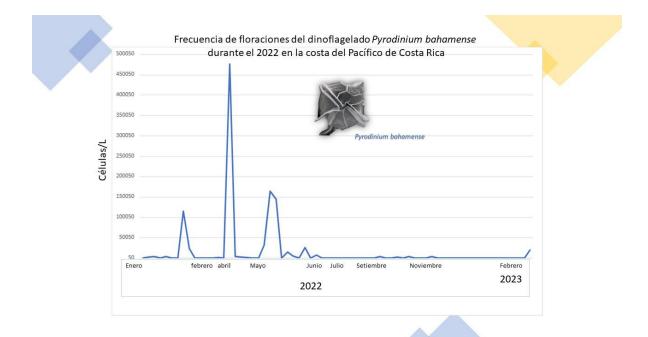


Some species of bivalve mollusks that are cultured and naturally harvested in Costa Rica

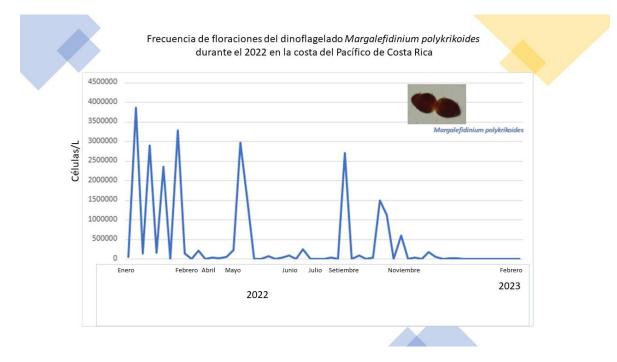
Costa Rica created by Executive Decree No 29328-MAG-S of December 20, 2000, the Inter-institutional Commission for the Prevention and Control of Red Tide since December 2021, which was completed by Executive Decree No 39669-MAG-S of June 8, 2016, and is integrated by the General Director of SENASA (NATIONAL ANIMAL HEALTH SERVICE), who presides, the Executive President of INCOPESCA (Costa Rican Institute of Fisheries and Aquaculture), the Director of Safety of Animal Products of SENASA (National Animal Health Service), the Director of the National Laboratory of Veterinary Services (LANASEVE) of SENASA, a representative of the Health Surveillance Directorate of the Ministry of Health, a representative of the Marine Phytoplankton Laboratory of the National University, a representative of the University of Costa Rica with affinity in the subject, a representative of the Ministry of Environment and Energy with affinity in the subject. All these members meet every month, where they analyze the reports sent by the LANASEVE, both water and meat samples.

This commission has decreed partial and total bans for the extraction and commercialization of bivalve mollusks, whether naturally extracted or cultured, due to the lack of controls necessary to guarantee a toxin-free product, and the sanitary measure is extended until the results of the analysis carried out by the National Laboratory of Veterinary Services of SENASA (LANASEVE), confirm that the products are fit for human consumption. The mollusks involved in the ban have been, among others, the *Anadara tuberculosa* known as piangua, mussels and the Japanese oyster *Magallana gigas*. This sanitary measure of Prohibition of Marketing and Sale of bivalve mollusks has been extended from the Central Pacific to the North Pacific of Costa Rica. By April 2022 the ban on shellfish extraction was lifted in

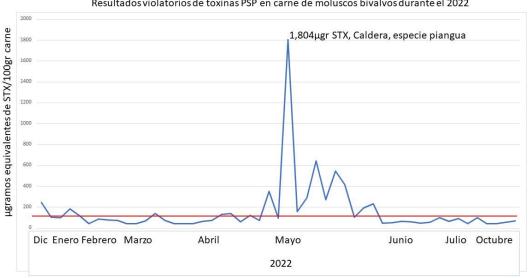
several areas of the Pacific, but in other areas the ban was maintained due to the high levels of toxins in shellfish meat, being the months of February, April and May when the highest levels of saxitoxin were detected, especially between April and May where the highest peak was detected, with a value of 1804 μ g SXT/100gr of meat.



Distribution of the dinoflagellate *Pyrodinium bahamense* in the Pacific coast of our country during the year 2022.



Distribution of the dinoflagellate Margalefidinium polykrikoides in the Pacific coast of our country during the year 2022.



Resultados violatorios de toxinas PSP en carne de moluscos bivalvos durante el 2022

— La línea roja es el límite permitido de 80μg equivalentes de STX/100 gramos de carne de molusco

Distribution of saxitoxin levels reported in mouse bioassay samples for mollusks (Data taken from SENASA reports for the Interinstitutional Commission for the Prevention and Control of Red Tide).

Servicio Nacional de Salud Animal MAG · COSTA RICA	Ministerio de Agricultura y Ganadería Servicio Nacional de Salud Animal Laboratolo Nacional de Servicios Veterinarios Departamento de Inocuidad de Alimentos Unidad de Residuos y Contaminantes en Alimentos de Origen Acuático Campus Universitario Pixo. Benjamin Núñez Lagunilla, Heredia, Costa Rica Tel (506) 25871622 REPORTE DE RESULTADOS					
				Versión 07	Påg. 1 de 2	
					Folio Nº: 03885-2	
		DATOS DE LA MU	ESTRA			
Protocolo: RA0237-22 Tipo de Muestra: Molusco Biv	alvo	Fecha de Recepción: 11/05/2022 Matriz: Plangua				
		DATOS DEL SOLIC	ITANTE			
Empresa/Establecimiento: SENASA Nº Establecimiento: Barreal, Heredia		Teléfono: 25871600	Correo electrónico:			
		DATOS DEL MUE	STREO			
Sitio de recolección : Mata Lir	nón, Caldera		Nº Lote	ə: -		
Fecha Recolección: 9/5/2022		Nº Análisis: -				
Muestreado por: Luis Alberto	Matarrita Chaverri		Tamaño del Lote: -			
Muestreo realizado por perso	nal de: Particular	N° Marchamo: -				
Fecha de Sacrificio: -		Nº Contenedor: -				
País de Origen: Costa Rica		Nº Constancia: -				
Pais de Destino: -		N° FRS: -				
			N° FAD: -			
		ORIGEN DE LA MU	ESTRA			
Finca/Establecimiento (F/E):						
Ubicación F/E: -		Código F/E: -				
Propietario: -						
		RESULTADO DE A	NÁLISIS			
Fecha de Inicio: 12/05/2022 Fecha		a de Conclusión: 12/05/2022		Fecha de Reporte: 12/5/2022		
ANALITO		RESULTADO		MÉTODO		
Toxinas PSP (UR/100 g; µg STX/100 g)	equivalentes	9250; 1804 (Violato	erio)	IA-RECAA-PT-005 Determinación de Toxinas PSP en moluscos bivalvos por Bioensayo en Ratón		

PSP toxin determination analysis performed weekly by the National Animal Health Service SENASA, of the Ministry of Agriculture.



Areas affected by the ban on the commercialization of bivalve mollusks due to high concentrations of PSP toxins (Gulf of Nicoya, central Pacific of Costa Rica).

SENASA's resolutions are always supported by the recommendations of the Commission for the Epidemiological Surveillance of Red Tide, to which several state institutions and public universities belong. In these resolutions, the prohibition of extraction and commercialization of bivalve mollusks from Pacific areas is ordered, as shown in the map.

Given the need to organize the areas of extraction and commercialization of the product, the commission proceeded to take, transport and process samples of bivalve mollusks and water, with the collaboration of different actors involved in the process, Therefore, it was determined that for the routine sampling established in the chronogram "Marine Mollusks-Phytoplankton Sampling 2023", the personnel of the offices of the SENASA and INCOPESCA Regional Directorates would collect the samples in their region in coordination with the extractors and oyster farmers and transfer them to LANASEVE (this last action being exclusive to SENASA).

Water samples for the analysis of harmful/toxic phytoplankton from the oyster farms are collected by previously trained personnel of the farms and are delivered to the personnel of the National University for prior morphological analysis and species identification, which is why a constant sampling schedule of bivalve mollusks and water samples is maintained.

In the Caribbean coast of our country, the presence of species potentially producing ciguatoxins has been detected, however, there is no information on ciguatera events in our Caribbean sector, a possible cause could be the lack of knowledge of the

disease by the health sector. In this zone, monitoring is very limited and restricted to specific areas.

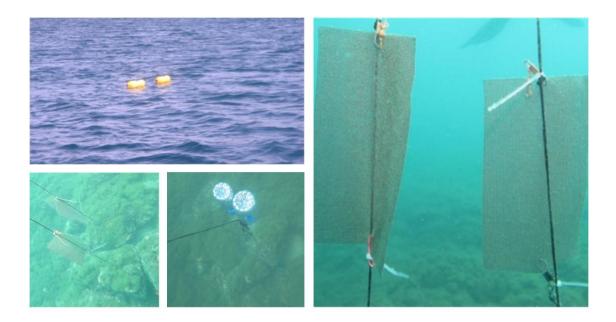
In surveys conducted since 2022, more than six species of dinoflagellates potentially producing ciguatera have been found, among these of the genus *Gambierdiscus*, *Collia* and *Prorocentrum*, this year the species *Ostreopsis* sp. was found in intertidal zones of the central Pacific coast and on Cocos Island, located in the Eastern Tropical Pacific.

During the tours conducted by the University of Costa Rica, samples were collected directly from reef areas to collect benthic dinoflagellate species, of which cultures were generated in the laboratory, but these did not grow well and died.



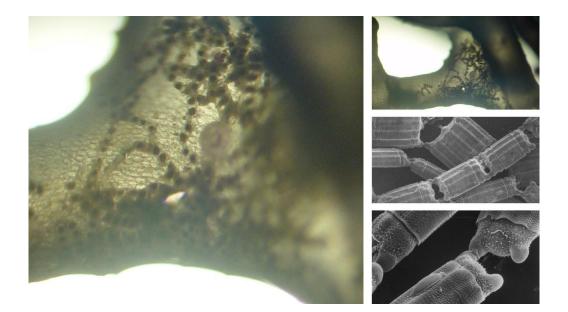
The samples collected on the coast were transported to the laboratory where several separation protocols were carried out to obtain microorganisms suitable for generating unialgal cultures.

On the other hand, an attempt was made to collect benthic dinoflagellates in submerged nets, left in the water for 24 hours, to wait for the microorganisms to approach and use the nets as protection. These nets were placed in Santa Elena Bay, at a depth of three meters.

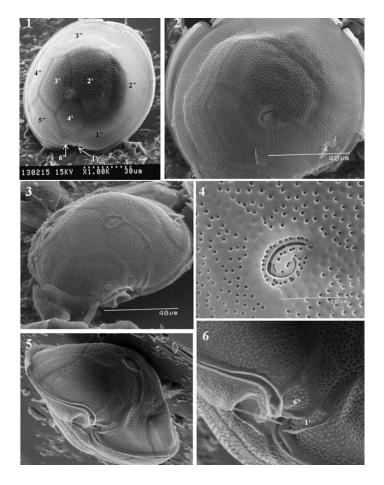


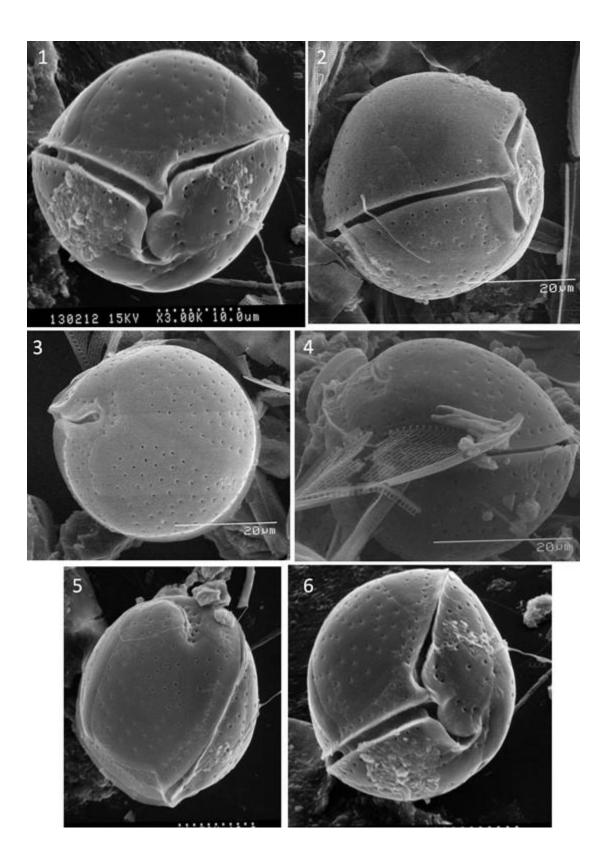
On the other hand, macroalgae were collected near reef areas in the Caribbean, in various locations:





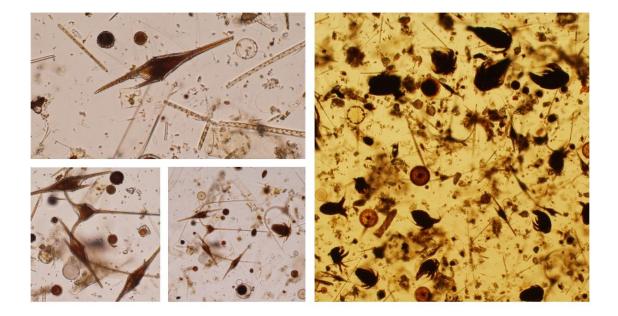
En la Isla del Coco hemos encontrado especies bentónicas de los géneros *Gambierdiscus* y *Collia*, publicados en la Revista Biología Tropical.





Recently, in the Caribbean area of Costa Rica, we have observed an increase in the concentration of dinoflagellates of the genus *Tripus*.





Costa Rica has made many efforts to support research projects in this area of study. In recent years, the International Atomic Energy Agency (IAEA), through ARCAL projects, has collaborated with several regional courses and international internships for experts from our country and other Caribbean countries, to learn and improve techniques in the study of the taxonomy and toxicology of phytoplankton species, through the following research projects:

- 1. Project RLA 7020 Establishing the Caribbean Observing Network for Ocean Acidification and its impact on Harmful Algal Blooms, using nuclear and isotopic techniques.
- 2. Project RAS 7026 Supporting the use of receptor binding assay (RBA) to Project the adverse impacts of harmful algal toxins on seafood safety
- 3. Project RLA 7014 Latin American regional proficiency test on the determination of trace elements and radionuclides in algae, soil and spiked water

In recent years, we have presented our research at two international congresses:

- Marine phytoplankton species producing B'HABs on the Caribbean coast of Costa Rica M. Vargas. 18Th International Conference on Harmful Algae, 21-26 October 2018, Nantes, France.
- Assessment of ciguatera benthic dinoflagellates from the Atlantic coast of Guatemala E. Blanda, J. Garcia-Perez, M. Vargas-Montero, L. Carrillo-Ovalle. 18Th International Conference on Harmful Algae, 21-26 October 2018, Nantes, France.
- 3. Potentially toxic species of the genus *Dinophysis* reported from the Pacific coast of Costa Rica. Maribelle Vargas Montero. 19Th International Conference on Harmful Algae, 10-15 October 2021, Baja California, México.

Costa Rica has the installed capacity to generate high-level research, for example the University of Costa Rica has electron microscopy, installed capacity for analysis of PSP toxins, high resolution liquid chromatograph coupled to a single quadrupole mass spectrometer, high resolution liquid chromatograph with flourescence and ultraviolet detector, capacity for analysis and sampling of sediments, analytical capacity for sampling chlorophyll, nutrients, total inorganic carbon in seawater, alkalinity and acidity,

Analytical capacity for analysis and sampling of sediments, analytical capacity for sampling chlorophyll, nutrients, total inorganic carbon in seawater, alkalinity and acidity, and the IAEA has trained researchers from the University of Costa Rica in sampling and handling of microplastics.

In the case of Costa Rica, sanitary controls are very limited; the Ministry of Agriculture and Livestock is the only agency in charge of performing mouse biensays for PSP-type toxin analysis, so there is a need to increase the number of certified laboratories to provide a more complete service to all mariculture producing areas of the country.

Costa Rica has very specific needs, such as the importance of training and equipment for the early detection of toxic events, the manipulation of cultures to extract toxins (PSP, ASP, DSP, NSP, Ciguatera...) capable of being identified by chemical methods, support to carry out molecular analysis for species identification,

regional support for monitoring the coast at a regional level, regional support for the implementation of endemic mollusk cultures for the identification of endemic species.) capable of being identified by chemical methods, support to achieve molecular analysis for species identification; regional support for coastal monitoring at regional level, regional support for the implementation of endemic mollusk cultures for the area and the possibility of support to small oyster associations, such as training, improvement of controls for red tide alerts.

Support is also needed to implement a regional project to study the incidence of ciguatera associated with coral reef areas, identifying the producing species and their toxins, and thus provide support to national institutions responsible for importing and exporting fish.