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SHIOHIGARI and PSP toxins in Japan: Initiatives to save traditional recreational clam picking

Shiohigari has been enjoyed by people in Japan for centuries, as depicted in the Japanese art style Ukiyo-e by the artist Hiroshige in 1852 (Fig. 1). Although much time has passed since Hiroshige's day, today *Shiohigari* remains a popular marine recreational activity. It is a traditional as well as recreational springtime event, when crowds of people flock to tidelands to gather shells during low tide. Nowadays, *Shiohigari* is a rare opportunity for not only families but also schools to teach children the importance of conservation of marine resources and the environment.

In spite of the cultural importance of *Shiohigari*, it is at risk due to the occurrence of toxic algal blooms. Paralytic shellfish poisoning (PSP) toxins in bivalves (PST) exceeding regulatory limits have been detected in the seas surrounding Japan [1] and almost every year in shellfish collected from the seas along Osaka prefecture since 2002 [2]. The main causative species has been identified as *Alexandrium pacificum*, (formerly *A. tamarense*). Operators of recreational clamming parks in Osaka prefecture (Fig. 2) have taken the initiative with innovative measures to keep recreational clamming parks open for *Shiohigari* even when paralytic shellfish toxins levels exceed the regulatory limit.

In Japan, people can enjoy clamming in two different locations: coastal areas where anyone can take clams from their natural habitat free of charge and clamming parks, where an entrance fee is charged and the clams are under the control of the operators of the clamming park. In most cases, the operators of these clamming parks are fisheries cooperatives. These fisheries cooperatives monitor growth of wild juvenile clams and ensure enough are available in the clamming areas so that people can dig them up and take them home. They even purchase clams to ensure that stocks are sufficient.

In general, operators of clamming parks voluntarily refrain from opening parks when PST exceeding regulatory limit is detected to prevent shellfish poisoning cases (Fig. 3). At clamming parks in Osaka, however, they keep parks open by purchasing non-toxic clams and exchanging the non-toxic ones for those collected by clammers. This is extraordinary.

The exchange of the non-toxic clams for those collected by clammers started at Tannowa clamming park run by Tannowa Fisheries Cooperative. Surpris-

Fig. 1 Ukiyo-e drawing of "Shiohigari" at Shinagawa seashore by Hiroshige Utagawa (from Digital Collection of National Diet Library, Japan)

Content

exchanging a fixed quantity of clams for those collected by visitors to prevent visitors from taking away too many.

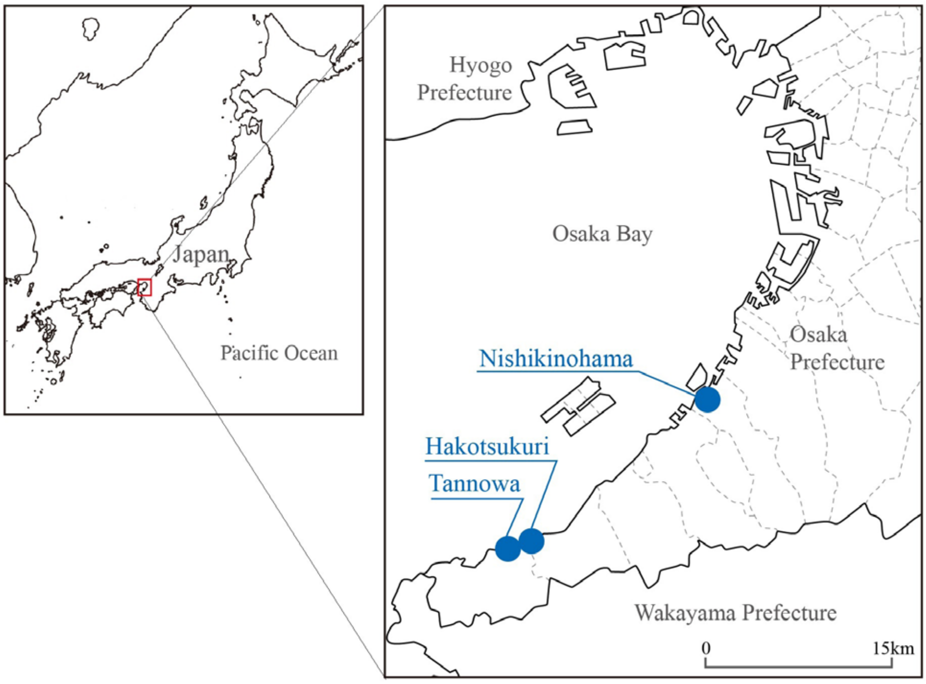

Fig. 2 Clamming parks in Osaka Prefecture (Modified from a digital map of The Geospatial Information Authority of Japan)

ingly, they had developed the exchange system to secure viability of their operations before 2002 when PST exceeding the regulatory limit were first detected in clams from seas along Osaka prefecture. .

One of the species most targeted at clamming parks is *Ruditapes philippinarum*. Commercial catch of *R. philippinarum* in Osaka prefecture had its peak at 556 tons in 1953, then decreased sharply in late 1950's, 30 years before its decrease throughout Japan [3]. The major reasons for the rapid decrease and lack of recovery of commercial catch of *R. philippinarum* are considered to be the extensive reclamation of tidal flats and severe deterioration of the environment of Osaka Bay. Because of this, by the 1960s at the latest, operators of clamming parks in Osaka prefecture had to purchase clams to meet the requirements of people visiting for recreational clamming.

Tannowa clamming park is not a natural but an artificial beach. Tannowa Fisheries Cooperative originally operated recreational clamming in natural tidal flats in different coastal areas. However, these tidal flats were reclaimed to build a harbour for yachts which led to a subsequent shift of focus to the present clamming park. Operations were restarted where clams cannot grow naturally due to the nature of bottom sediments. Due to these circumstances, the Cooperative can only operate the clamming park by purchasing clams from outside areas. Through operation of clamming parks with purchased clams, fishermen of Tannowa Fisheries Cooperative developed the system of exchanging a fixed quantity of clams for those collected by visitors to prevent visitors from taking away too many.

Fortunately, this exchange system worked well addressing the new challenge, i.e., how to keep the clamming parks open despite the unexpected detection of shellfish toxins exceeding the regulatory limit. The fishermen of Tannowa Fisheries Cooperative kept the park open by ensuring the safety of the externally purchased clams by independently getting toxin tests (Fig. 4). The clam exchange system then spread to other clamming parks, such as Nishikinohama and Hakotsukuri (Fig. 2). When Nishikinohama Tourism Association, operator of Nishikinohama clamming park self-closed the park when shellfish toxins exceeded the regulatory limit in 2002, they received numerous phone calls from citizens requesting the opening of the park and enquiring the reason for its closing. In order to meet the needs of people for clamming, they introduced the system of exchanging clams in 2007 after the third incidence of PSP exceeding the regulatory limit detected in Osaka prefecture.

At first, the Fisheries Division of Osaka prefectural government requested operators of clamming parks not to open the parks until a safety declaration was issued. In the last few years however, self-closure of the parks on detection of shellfish toxins surpassing the regulatory limit is no longer requested. Thorough management of both clams and visitors by the operators resulted in no record of poisoning cases from the clamming parks, which led to no requests for self-closure of the parks by the Osaka prefectural government [4]. The once decreasing number

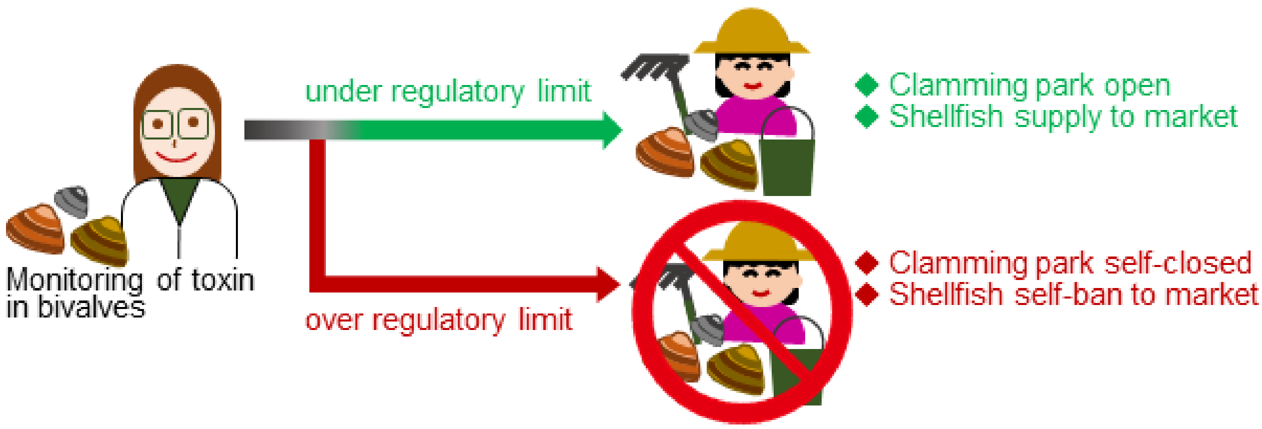

Fig. 3 General framework of risk management of shellfish poisoning in Japan

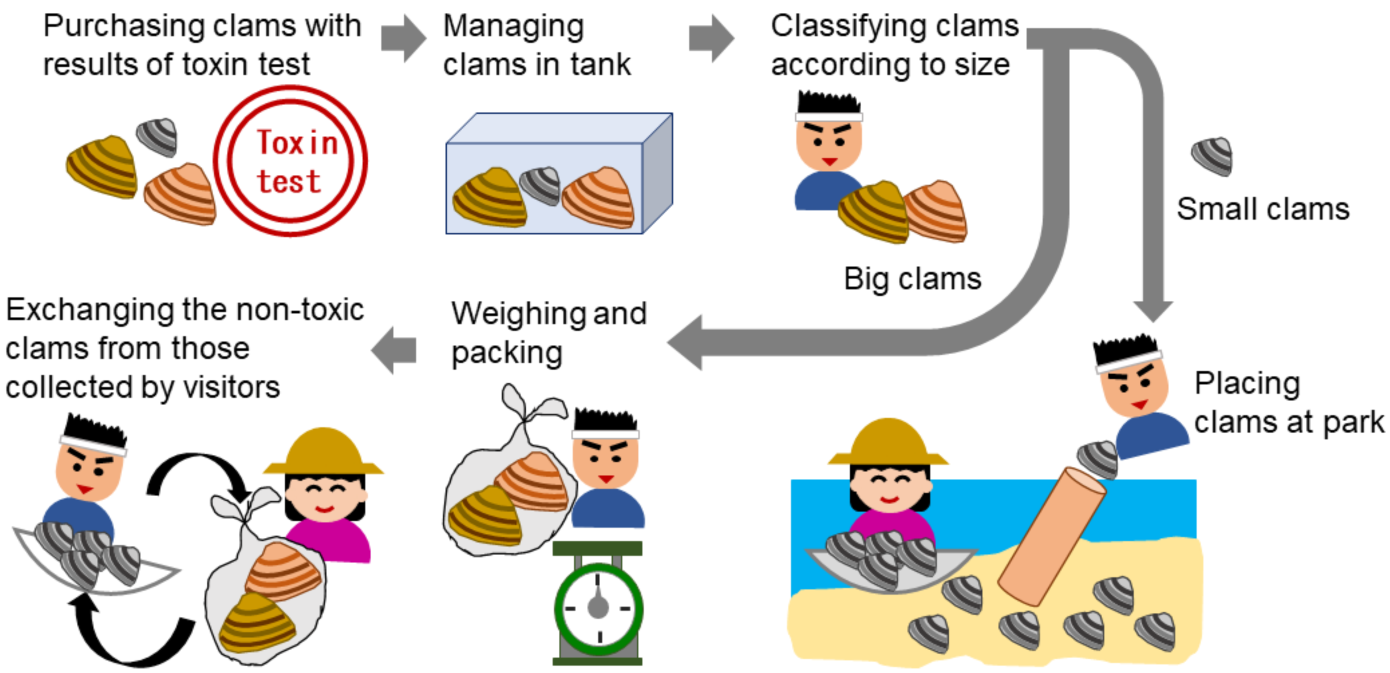

Fig. 4 System of exchanging clams which secure food safety at Tannowa clamming park (Modified from [3]}

of visitors to clamming parks has now recovered.

This is a success story of how to mitigate socio-economic impact on recreational clamming in Osaka prefecture due to PST. Thanks to the initiatives and careful measures to ensure safety by the operators and the understanding of the Osaka prefectural government, opportunities for recreational clamming have continued to be provided even when shellfish toxin exceeding the regulatory limit is detected. The complete absence of any poisoning cases from clamming parks is what has made this mechanism possible. *Shiohigari* has recreational, cultural, and educational values for citizens in Japan. How can we pass on the traditional culture of *Shiohigari* to the next generation given the spread of shellfish toxins as well as the critical status of clam resources? This is one of the most urgent issues facing Japan if we are to prevent a further decrease in the number of people enjoying and being interested in the sea.

Marine recreation such as *Shiohigari* has a strong influence on enhancing people's behaviours around marine conservation [5]. Thus, the exchange system of clams at clamming parks will have an effect not only on mitigating the socio-economic impact PST but also on promoting people's willingness to conserve the marine environment by continuously providing opportunities for *Shiohigari*. Although introduction of the exchange system at clamming parks is still limited, park operators in other prefectures might become interested if PST over the regulatory limit becomes more widely spread and more frequently detected. For *Shiohigari* to succeed, further study on social acceptance of the clam-exchange measures is necessary, as is a better understanding of the challenges for securing food safety and the continued operation of clamming parks.

Acknowledgements

I am grateful to interviewees from Tannnowa Fisheries Cooperative and Nishikinohama Tourism Association for their invaluable talk about the history of operations at the clamming parks. Interviews were supported by a research grant from the Institute for Food and Health Science, Yazuya Co., Ltd. I would also like to thank Yasuwo Fukuyo for his guidance on my research on socio-economic aspects of harmful algal blooms. Special thanks are extended to John Dolan for his kind proofreading.

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HABs and the Mixoplankton Paradigm

Mixotrophs are defined as organisms that are "*able to use photo-autotrophy and phagotrophy or osmotrophy to obtain organic nutrients*" [1]. It is notable that all phototrophic protists are potentially mixotrophic if only through expression of osmotrophy enabled by the uptake of amino acids and sugars, processes that were much studied back in the 1970's and 1980's. Over the last decade, however, there has been an increasing awareness of the importance of the activity of plankton combining phototrophy and phagotrophy [2]. And that includes many HABs, which are not just "algae", but also predators.

That awareness of the importance of phagotrophy in so-called algae prompted a reclassification of protist plankton functional groups [3] according to their different modes of photo+phago 'trophic nutrition. The most basic division was between those species with a constitutive (innate) ability to photosynthesise, and the non-constitutive species that acquired phototrophy from their prey. Subsequently, to remove the ambiguity between mixotrophy enabled by {photo' + osmo' trophy} versus {photo' + osmo' + phago' trophy}, the use of the term "mixoplankton" was proposed [4] for those capable of phagotrophy. "Phytoplankton" was then proposed to describe protists capable of phototrophy but not phagotrophy. Planktonic cyanobacteria, including HAB species, would by default also be phytoplankton.

The role of mixotrophic plankton in HABs has long been recognised [5]. With the newly proposed mixoplankton term and its allied paradigm [4] that sees the potential for bacteria-mixoplankton and mixoplanktonic predator-prey interactions, the question arises as to how HAB species align with this new take on marine science?

In order to identify and hence differentiate between the mixotrophic and mixoplanktonic HABs, we have adapted the new protist functional group classification [3] specifically for Harmful Algal Bloom species (Fig. 1). In Fig. 1 example species names have been obtained from the IOC-UNESCO HABs list [6] and assigned to functional groups according to the mixoplankton database [7]. The primary difference between the HAB functional group classification presented here and the protist functional group classification [3] is the inclusion prokaryotic plankton as there are various known HAB species within this group.

As before [3], this HAB functional group classification chart starts with the question of whether the HAB species is capable of carbon fixation. A negative response to this leads us to one end of the spectrum – the pure heterotroph describing the traditional protozooplankton (or, microzooplankton) functional group. Within the current IOC-UNESCO HABs list [5] there is one only species, *Phalacroma rotundatum* which falls within this group. However, if the HAB species has the ability to photosynthesize, the next question is whether the species can also engage in osmo-heterotrophy. A null response results in what would be a purely photo-autotrophic organism; there are no known HAB species which would satisfy this description – indeed there is no known microbe that aligns with this state. A *yes* response gives us a mixotroph capable of photo-autotrophy and osmo-heterotrophy. Once we have a mixotroph, the next criteria to test is whether the mixotroph is also capable of phagotrophy. A negative leads to the traditional "phytoplankton" which include the HAB species within the Bacillariophyceae (diatom) and cyanobacteria groups. A positive response results

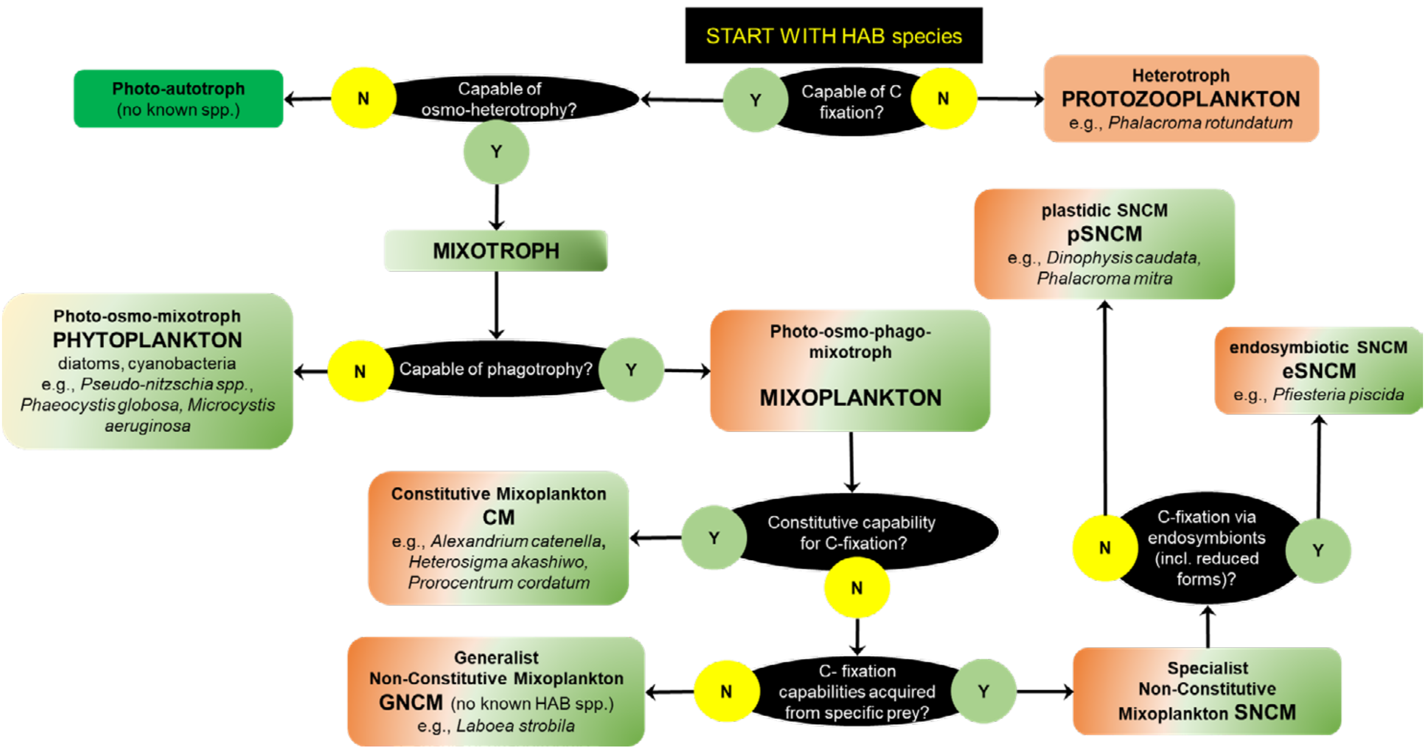

Fig. 1. Functional group classification key for Harmful Algal Bloom Species. N, no; Y, yes. Key developed from the protist functional group key [3] with example species from the IOC-UNESCO HABs list [6] aligned to functional groups according to the Mixoplankton Database [7]

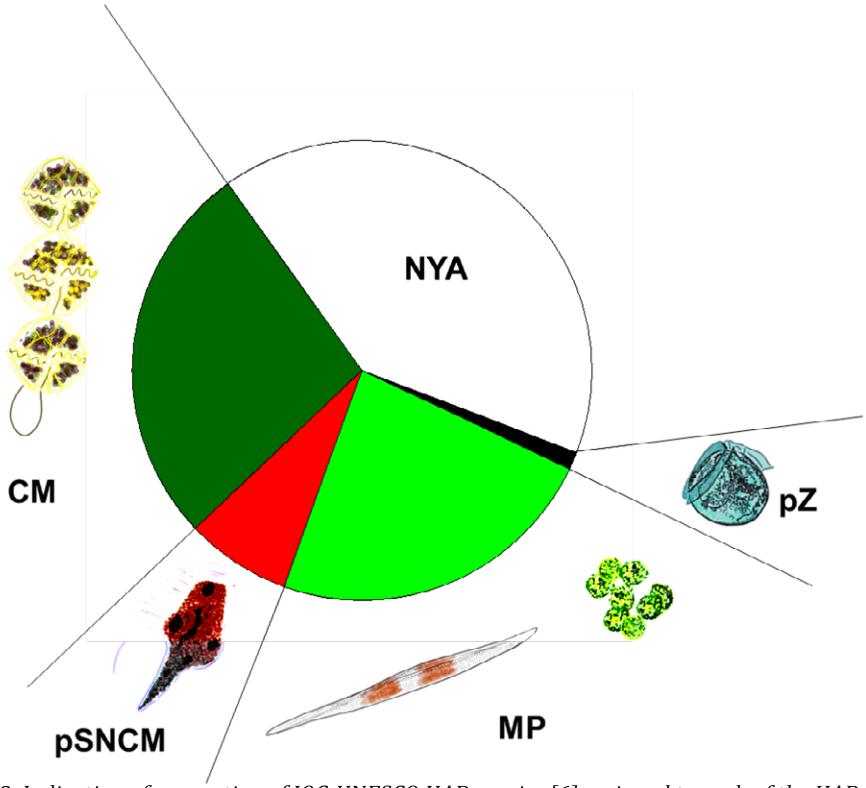

Fig. 2. Indication of proportion of IOC-UNESCO HAB species [6] assigned to each of the HAB plankton functional groups according to key in Fig.1 compiled by cross-reference to a database on mixoplankton species. CM, constitutive mixoplankton; pSNCM, plastidic specialist non-constitutive mixoplankton; MP, mixotrophic phytoplankton including eukaryotic diatoms and prokaryotic cyanobacteria; pZ, protozooplankton; NYA, not yet assigned. See text and Fig. 1 for further explanations.

in a photo-osmo-phago-mixotroph – the mixoplankton - functional group; these are primarily planktonic protists.

The mixoplankton HABs are then broadly classified into constitutive and non-constitutive mixoplankton. Here, the constitutive mixoplankton (CM) are HAB species which have innate photosynthetic capabilities; these include various species from the Dinophyceae (e.g., *Alexandrium minutum*, *Karenia brevis*, *Karlodinium veneficum*), Raphidophyceae (e.g., *Chattonella marina*, *Heterosigma akashiwo*) and Haptophyta (e.g., *Chrysochromulina leadbeateri*, *Prymnesium parvum*) groups.

The non-constitutive mixoplankton (NCM) are those that need to acquire their obligate phototrophic capabilities from either generic species (generalist non-constitutive mixoplankton, GNCM) or specific species (specialist non-constitutive mixoplankton, SNCM). There are no known HAB GNCMs; GNCMs comprise various ciliates, such as *Strombidium* and *Laboea* species [8]. The SNCMs are further divided into those that retain plastids and parts of the prey (plastidic SNCM, pSNCM) and those that maintain endosymbionts (endosymbiotic mixoplankton). The well-known HAB *Dinophysis* fall within the pSNCM group while the HAB *Pfiesteria piscida* with their reduced endosymbiont fall within the r-eSNCM group. *Dinophysis* acquires its plastids from the SNCM ciliate, *Mesodinium*, which in turn acquires its plastids from CM cryptophytes such as *Teleaulax*. Thus, SNCM species always depend on the availability of other species for acquired phototrophy while CMs have no such restriction on their growth. Furthermore, the conditions of growth effects the longevity of acquired plastids in NCMs and thus the components of the food chain are closely coupled.

Fig. 2 presents preliminary results from assigning this functional group classification to the 190 HAB species currently recorded in the IOC-UNESCO list. The “not yet assigned” (NYA) group represents HABs within the Dinophyceae group which are known to be mixotrophic by virtue of being photo-osmo-heterotrophic but we have not found any published records evidencing their capability (or, otherwise) to engage in phagotrophy. The primary reason behind allocating “NYA” to these groups rather than “mixotrophs” is the presence of various well-known mixoplanktonic species within the same genus (e.g., the CM *Alexandrium catenella* vs the NYA *A. hiranoi*; the CM *Karenia brevis* vs the NYA *K. cristata*).

Phagotrophy has a direct consequence for trophic dynamics, with the removal of competitors and potentially also predators by the collective action of high abundance blooms of HAB species. Phagotrophy also provides nutrients which, according to traditional inorganic nutrient analysis, may otherwise be considered as limiting. The consumption of bacteria, for example, appears common. The traditional food web considers bacteria as a competitor for nutrients with phytoplankton; indeed, nutrient stressed phytoplankton release increased amounts of organics that promote bacteria growth in a positive feedback loop [10]. Protozooplankton then control the dynamics of the interaction (Fig. 3). According to the mixoplankton paradigm [3-4], the protists are not in competition with the bacteria, but *de facto* farm them to acquire nutrients (Fig. 3). The consequential dynamics of the growth of the phototrophic protist (‘phytoplankton’ in the traditional scenario; ‘mixoplankton’ under the mixoplankton paradigm) differs greatly (Fig. 3),

Labelling HAB species as mixoplankton, or otherwise, has obvious important consequences for how we consider factors affecting the growth and demise of these organisms. It also then affects how those responsible for monitoring water quality and ecosystem services may view the ecosystem. In short, assuming the activity of HAB species aligns with ‘phytoplankton’ needing light and inorganic nutrients, can be considered for many if not most species as inappropriate. That *Chrysochromulina* is a mixoplankton, and grows to such perfusion around salmon farms [11] is perhaps no coincidence.

For more about mixoplankton, please see the article about the recent Mixoplankton International Conference in this edition of HAN, and also visit www.mixotroph.org

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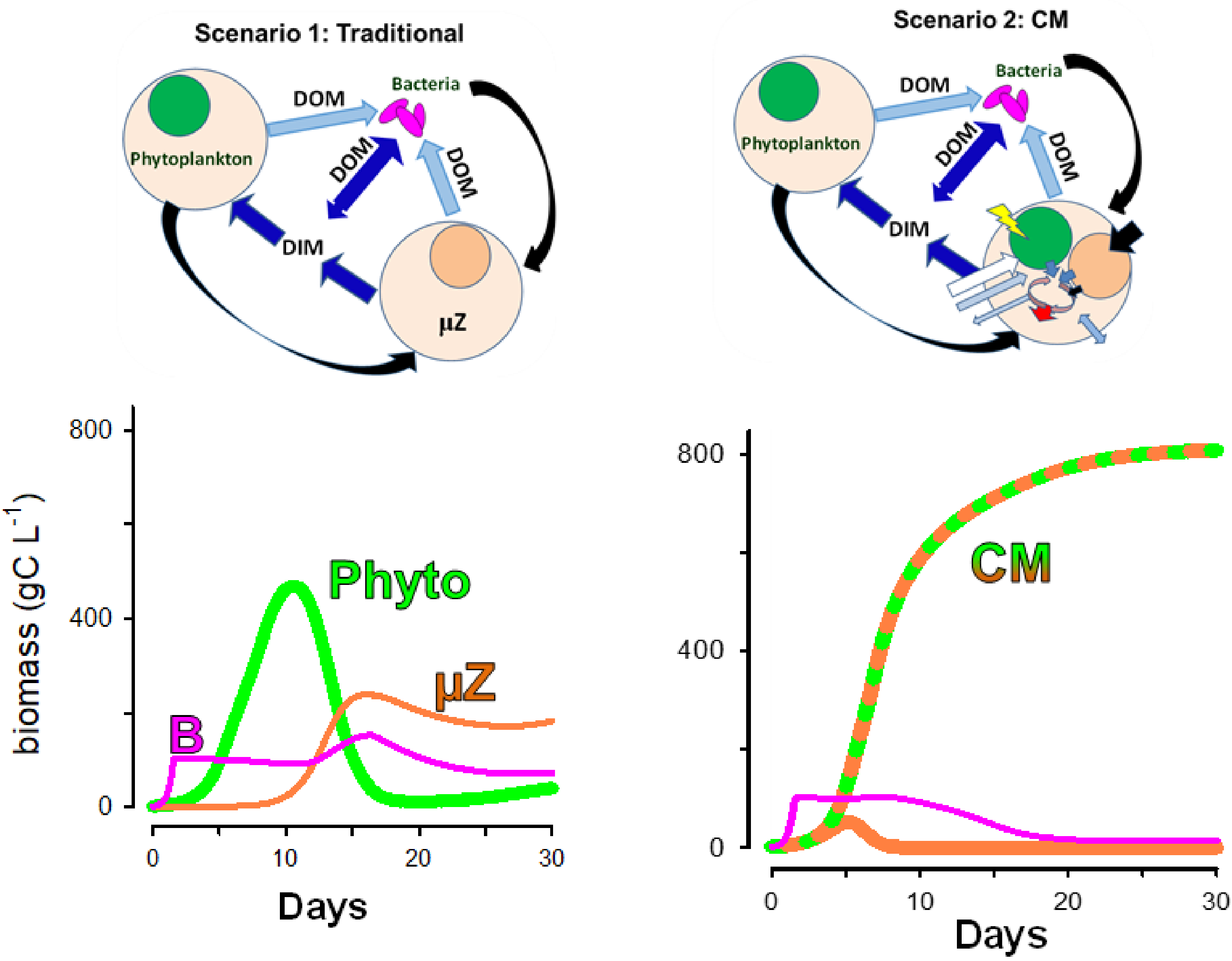

Fig. 3. Schematics and model simulation outputs run under the traditional paradigm (left) versus the mixoplankton paradigm (right). See text for explanation. B – bacteria; Phyto – phytoplankton (non-phagotrophic phototroph); µZ – protozooplankton; CM – constitutive mixoplankton (photo-phago-trophic); DIM – dissolved inorganic matter (nutrients); DOM – dissolved organic matter. Adapted from [3]

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Tiny cells with a big impact: An unexpected bloom in the mid-Atlantic

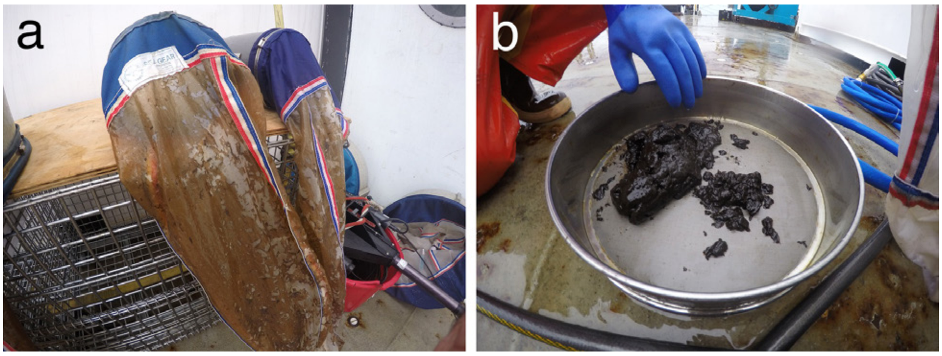

Fig. 1. a) Bongo nets fouled with the brown mucilaginous plankton. b) Dark and gelatinous content of the plankton nets scraped into a sample tray.

Since 1992, the US NOAA Ecosystem Monitoring (EcoMon) cruises survey the Northeast U.S. Continental Shelf between 36˚ N and 41˚ N three to four times a year. Plankton and larval fish are sampled along with physical and chemical oceanographic parameters. During November 2018, the cruise encountered an unusual massive bloom of mucilaginous plankton that completely clogged 165-µm and 335-µm zooplankton nets with a puzzling dark brown slimy substance (Fig. 1). The plankton nets were extensively fouled at 16 of the 60 stations, predominantly north of 40˚ N (Fig. 2). The fouled double-oblique Bongo plankton tows ranged in depth from the surface to ~ 5 m from the bottom, i.e. between 26 and 57 m, depending on the station (CTD depths). With no microscope on board to observe fresh material, some of the brown slime was scraped from the nets into 95% ethanol, which changed it to a dark green color.

Upon return to onshore laboratories, identification of the material by light microscopy remained somewhat elusive, although the super abundant ~7.5-µm cells hinted at the presence of either a diatom or the prymnesiophyte *Phaeocystis*. Aliquots of the preserved material from station 60 were digested in H_2O_2 and prepared for scanning electron microscopy (SEM).

We identified the tiny cells enmeshed in chitan threads as *Thalassiosira mala* [1]: a single strutted process (fultoportula) offset from the center of the valve; a ring of marginal strutted processes 1 to 1.5 µm apart from each other; a single labiate process (rimoportula) located within the ring of marginal strutted processes; and 25 to 30 areolae in 10 µm (Fig. 3). Copious chitan threads emanate from the ring of marginal strutted processes and are responsible for creating the mucilaginous consistency of the colonies; the threads were markedly obvious on micrographs (Fig. 4). Additional morphological features of this taxon have been previously described [2-3]. Subsequent review of data at 3 m from the Imaging FlowCytobot (IFCB) onboard the EcoMon vessel indeed revealed the presence of numerous colonies as well as single cells of what we can now identify as *T. mala* (Fig. 5) [4]

Because of its small size, this diatom is often misidentified or goes unnoticed by light microscopy; it is however easily detected when intense blooms form massive gelatinous colonies, such as during the widespread event of the EcoMon 2018 cruise. The species is considered cosmopolitan within temperate and tropical waters [3,5] its presence in the mid-Atlantic region is not unusual [5]. It is observed in waters closer to the coast, such as in Narragansett Bay (41˚ 34' N, 71˚ 23' W), so far at non-bloom densities [6] (Figure 6).

Given that the EcoMon crew did not visually notice any discoloration of the surface water at the time, one can surmise either that a bloom occurred at depth or that the colonies were abundant and somewhat dispersed through the water column. Vertical profiles of temperatures and salinities indicated generally well-mixed water columns, thus may suggest colony dispersal at most stations rather than thin layers of concentrated materials at depths. For example, at station 56, chlorophyll concentrations were nearly constant at 2.4, 2.4 and 2.1 µg L^{-1} at 2, 20 and 30 m, respectively (in situ fluorometry) over a very slight sigma-t difference of 0.001 unit. However, at station 60 where the heaviest fouling occurred, the chlorophyll concentrations were

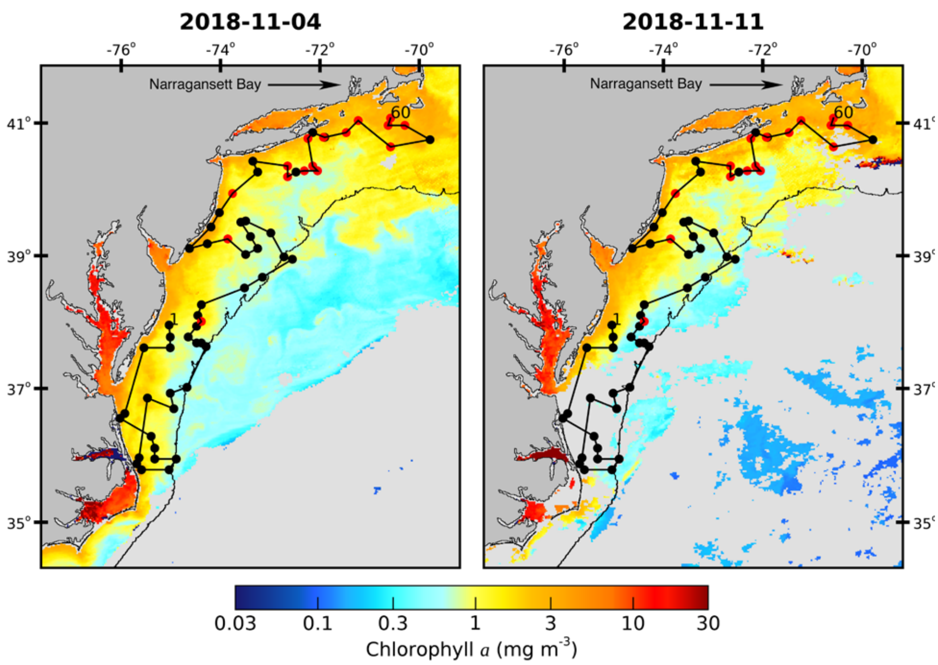

Fig 2. Composite maps of chlorophyll a concentration over a range of 0.03 to 30 µg L^{-1} from the MODIS-Aqua, Suomi-NPP-VIIRS and NOAA20-VIIRS satellite sensors: Nov 3-5 (left panel) and Nov 10-12 (right panel) 2018 during the time of the EcoMon cruise. Sampling on the R/V Sharp took place November 2-12, 2018. Dots on top of the cruise track indicate sampling stations; red dots specify stations with extensive fouling of the nets. The cruise ended at station 60. The thin topographic grey line represents the 100-m depth contour.

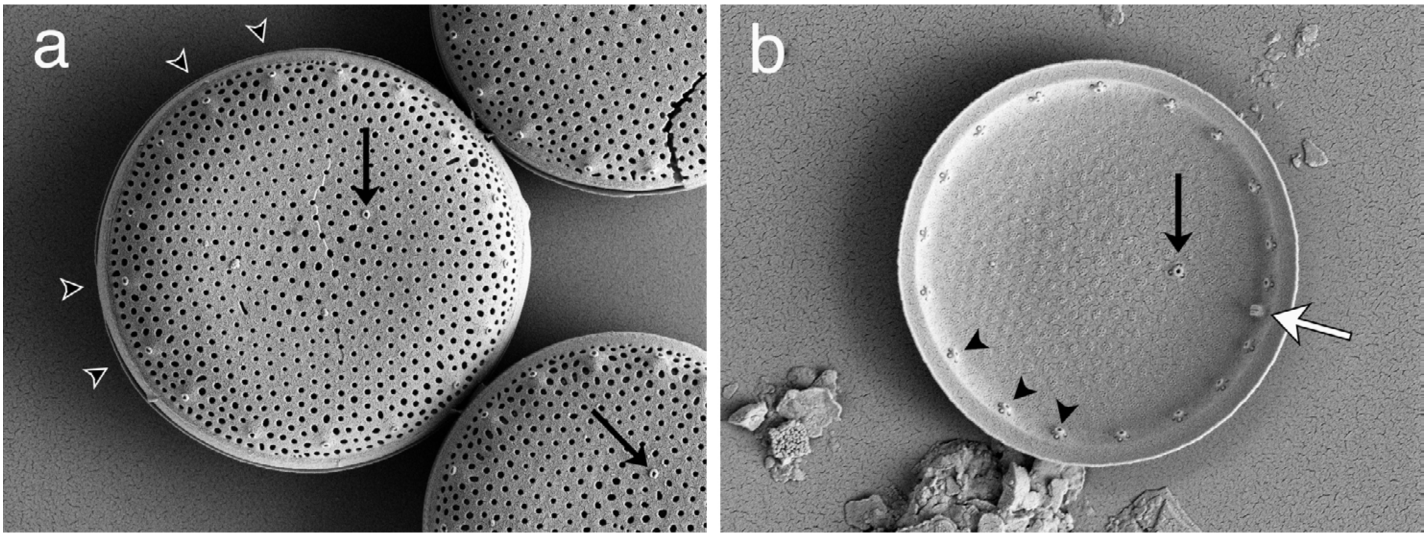

Fig. 3. Scanning electron micrographs of frustules in valve view of Thalassiosira mala. Note the single eccentric strutted process (black arrow), the ring of marginal strutted processes (arrowheads) and the single labiate process (white arrow) located within the ring of marginal strutted processes seen on the outside (a) and inside (b) of the frustule. 3a EcoMon Station 60, November 12, 2018; 3b Narragansett Bay December 10, 2018.

5.2, 4.9, and 9.3 µg L^{-1} at 2, 20 and 30 m (over a 0.0175 sigma-t unit), respectively, possibly suggesting a deep somewhat denser bloom. These November observations may thus document a settling or settled later stage of a bloom. We compared ship-based observations to remote sensing data for this time (Fig. 2). Satellite imagery shows coastal chlorophyll-*a* concentrations of up to 3 mg m^{-3} in the area of the survey where the heaviest fouling of nets and instruments occurred.

This *T. mala* bloom may have been more widespread, and of longer duration than the EcoMon data can document. Independently, and a few weeks earlier, an observer on a pelagic monitoring vessel noted the daily presence of conspicuous 'brown clouds' on the surface of the sea off the coast of Delaware (~39° N), sometimes in large patches (Fig. 7, September 29, 2018) (S. McConnell, pers. comm.). Unfortunately, no samples were taken, thus we must stress that although it seems likely, we can only speculate that this may have been an earlier surface expression of the widespread regional *T. mala* bloom, which by the time of the EcoMon cruise had settled to depth or been dispersed.

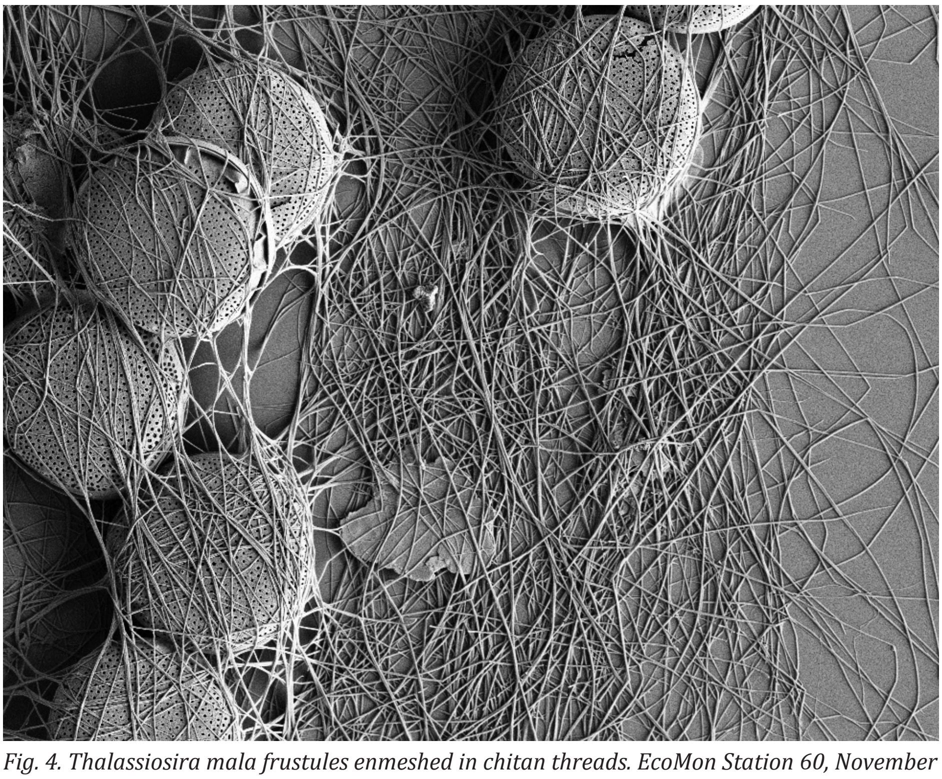

Fig. 4. Thalassiosira mala frustules enmeshed in chitan threads. EcoMon Station 60, November 12, 2018.

Returning to Narragansett Bay, observations on local plankton help to document the extent of the presence of *T. mala* in Northeast U.S. coastal waters. The taxon was occasionally reported from various locations from September to December 2018, but always at low densities: water samples and plankton tows (our own samples and Dr. D. Borkman, RI Department of Environmental Management, pers. comm.), and the local IFCB ([7]). The morphology of living cells and colonies from Narragansett Bay was documented with light microscopy (Figure 6) and identification was confirmed with SEM (Fig. 3b).

Intense blooms of *T. mala* mucilaginous colonies can impair the growth of filter-feeding shellfish, primarily documented in bivalves and suspected of affecting efficient gill function [8]. The deleterious mode of action on shellfish is thus likely physical in nature rather than chemical, this tiny harmful alga is not believed to produce a toxin. We know of no reports of harmful consequences outside of coastal events; specific intense offshore blooms are not likely to be investigated, unless by accident, such as during the EcoMon cruise. No adverse effects on cultured or wild shellfish were reported for Narragansett Bay.

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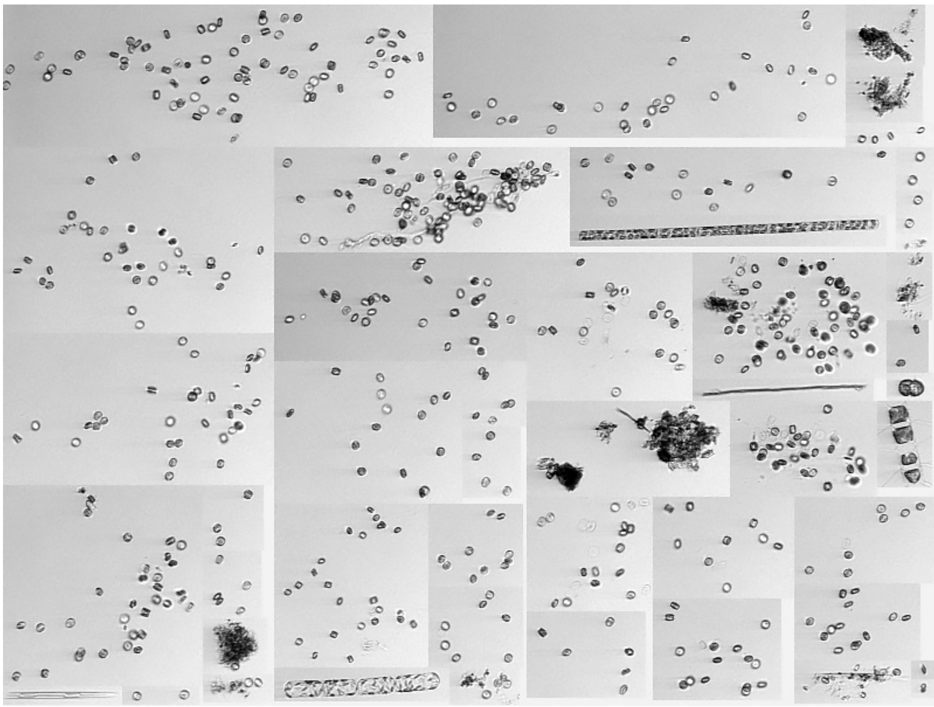

Fig. 5. Live Thalassiosira mala as viewed with the continuous Imaging Flow Cytobot aboard the R/V Sharp during the EcoMon 2018 cruise, near station 45 at 3 m, November 11, 2018 (https://ifcb-data.whoi.edu/timeline?dataset=NESLTER_broadscale&bin=D20181111T094425_IFCB127)

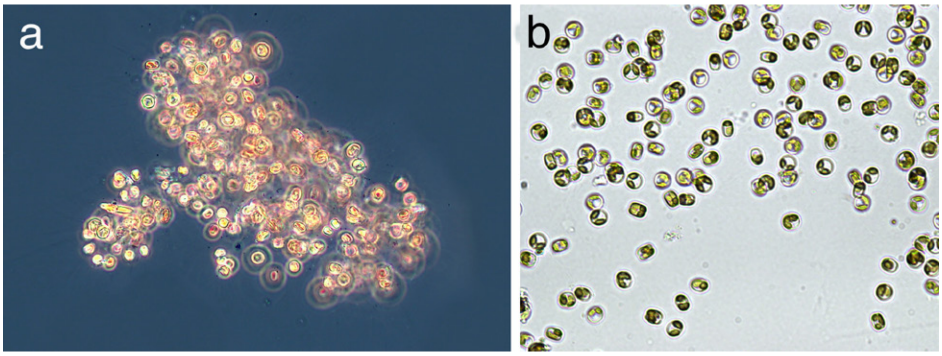

Fig. 6. Live Thalassiosira mala a) in dense mucilaginous colonies (phase contrast), and b) as loosely associated cells where two chloroplasts per cell are clearly seen (bright field). Photographs from Narragansett Bay; identification confirmed by SEM (Fig. 3b). Note that as a diatom, T. mala appears round in valve view and rectangular in girdle view, which is clearly seen here, indicating that these images could not depict Phaeocystis, whose cells appear irregularly spherical, with no rectangular profiles.

Fig. 7. Surface patches of a dense phytoplankton bloom several miles off the coast of Delaware on September 29, 2018. Photo credit S. McConnell.

First report of an *Ansanella granifera* bloom in Cuban waters, Caribbean region

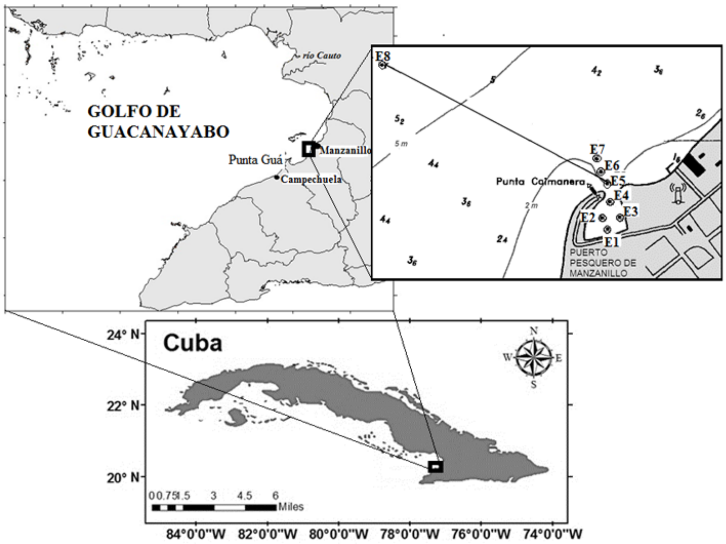

Fig. 1. Map of the study area showing the location where the dinoflagellate bloom occurred in southeastern Cuba.

Harmful Algal Blooms (HABs) have been associated with fish and shellfish kills, ecosystem damage, human health impacts, and significant economic losses to the aquaculture and tourist industries throughout the world. The increase of HABs in recent years is generally related to direct and indirect anthropogenic activities (e.g. eutrophication, climate change)[1].

In August 2018, an intense red water discoloration was observed in the Fishing Port of Manzanillo city, southeastern Cuba (20°19.891'N and 077°09.350'W) (Fig. 1). The discoloration was caused by a small dinoflagellate later identified by genetic sequencing as *Ansanella granifera*. The bloom, located near the coast, occupied an area of about 1.85 km^2 inside the Port and its adjacent waters, and was large enough to be detected by satellite imagery (Fig. 2).

Surface water samples (0.30 m depth) were collected with a Van Dorn bottle for phytoplankton and nutrient analysis (N-NO_3^-, N-NH_4^+, P-PO_4^{3-}). Water temperature, salinity and dissolved oxygen were measured in-situ with a multi-parameter sonde (HI 9828, Hanna Instruments, Inc., USA) at eight sampling stations (Fig. 2). Phytoplankton samples were preserved with acidic Lugol's solution and analyzed with a Zeiss Axiovert 40 inverted microscope (Zeiss, Oberkochen, Germany) using a Sedgwick–Rafter counting chamber. Micrographs were taken with a digital camera (Canon ELH 135) and cell length and width measurements collected from 20 individuals. For Scanning Electron Microscopy (SEM) examination of the bloom sample, cells were filtered on a 3 µm polycarbonate filter, desalted with a 10% step gradient of seawater to freshwater and dehydrated using a 10% step gradient of freshwater to ethanol followed by 100% hexamethyldisilazane (HMDS). The resulting dehydrated sample was placed on an aluminum stub using double stick tape and sputter coated with gold-platinum using a Denton Vacuum Desk II Sputter Unit prior to examination with a JEOL 5600LV SEM.

Individual cells were isolated with microcapillary pipettes, and placed into 25 µL of Chelex solution (InstaGene™ Matrix; Bio-Rad, Hercules, California, USA). Single cell DNA extractions were performed following protocols adapted from Richlen & Barber (2005) [2], as outlined in Gómez et al (2017) [3]. The D1-D3 domains of the LSU rRNA gene were amplified using primers D1R and D2C [4], and PCR products were visualized, cloned, and sequenced as described in Gómez et al (2017) [3]. DNA sequences were analyzed using Basic Local Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi) against databases in GenBank. The most closely related sequences (99.08-99.85% identity), as determined using BLAST searches, were those of *Ansanella granifera* (GenBank Accession no. HG792066.1). The newly generated consensus sequences were deposited in DDBJ/EMBL/GenBank under accession numbers MW698929-MW698931.

Ansanella granifera occurred in a nearly monospecific bloom at a maximum concentration of 2.16 × 10^8 cells L^{-1}. Other dinoflagellates (*Alexandrium* sp., *Blixaea quinquecornis*, *Scrippsiella trochoidea*) and diatoms such as *Cylindrotheca closterium*, *Hemiaulus hauckii, Nitzschia longissima*, *Skeletonema* sp. were found at very low cell densities.

Ansanella granifera cells were pentagonal to oval in shape; the episome was conical with a round apex, and larger than the trapezoidal hyposome. The nucleus was oval and located in the anterior to central part of the cell. A bright red eyespot was located near the sulcus. Cell length 9.70–13.60 µm (average 10.75 ± 1.11, n=20); cell width 8.0–12.5 µm (average 9.11±1.22, n=20) (Figs. 3,

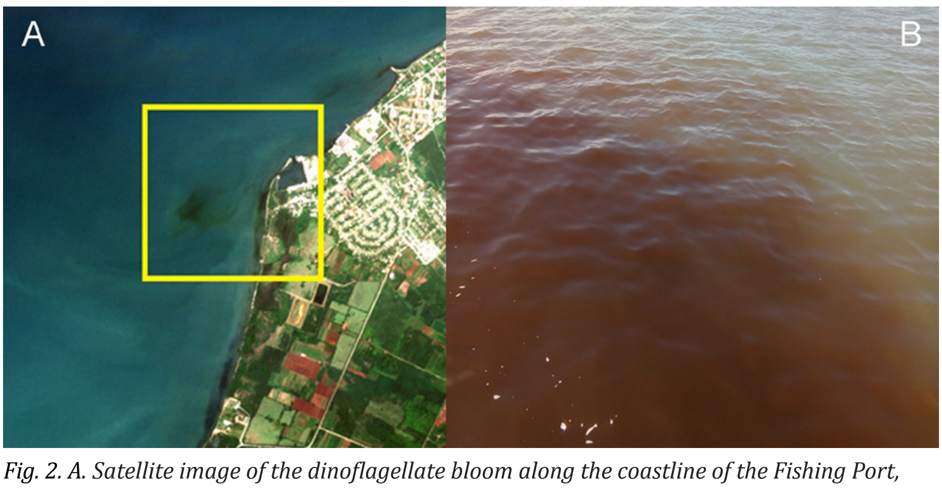

Fig. 2. A. Satellite image of the dinoflagellate bloom along the coastline of the Fishing Port, Manzanillo city, Cuba. B. Red water discoloration due to the bloom.

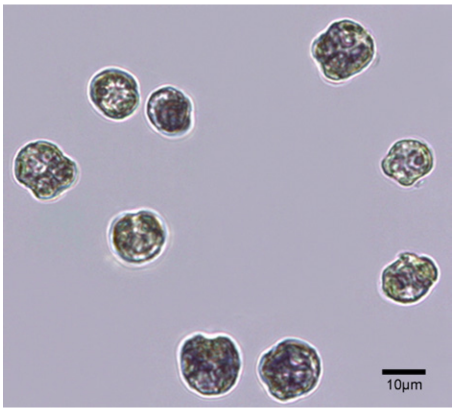

Fig. 3. Light microscopy images of fixed cells of Ansanella granifera.

4). *Ansanella granifera* is a dinoflagellate belonging to the family Suessiaceae (order Suessiales) that was recently described from Korea [5]. To our knowledge, the occurrence in waters from southeastern Cuba represents the first record outside its type-locality, including the first report of the taxon for the Caribbean region, for the entire Western Atlantic, as well as the first record as bloom forming species worldwide.

The application of molecular methods is particularly valuable for small dinoflagellates (e.g. "gymnodinioid forms") of ambiguous taxonomy like *Ansanella granifera* (very similar to *Gymnodinium* species). They are nanoplankton sized forms that cannot be properly identified in optical microscopy examinations. Recently, a *Gymnodinium* species (*G. natalense*) has been transferred to the genus *Ansanella* (*A. natalensis*) by combining morphological and genetic studies [6].

When the event occurred, weather conditions were favorable for bloom formation in southeastern Cuba, with high water temperature (30.66 ºC) and salinity (37.44 psu); and ammonium concentration ($NH4^{+}$) was also moderately high (0.20 mg/L). This level of ammonium could be due to organic nutrient input linked to discharges in the coastal zone from the wastewater treatment plant of the nearby local fishery industry. In spite of its location in the open sea, symptoms of eutrophication such as high values of chemical oxygen demand (COD) (average of 5.31 mg/L) and concentrations of oxygen below 5 mg/L (average of 4.76 mg/L) were recorded along the coastline of the study area (Fishing Port and adjacent waters), an area that is characterized by organic rich sediments. Further work is needed to determine the composition and distribution of dinoflagellate cysts from sediments of this coastal area to determine the historical occurrence of bloom-forming species.

Many dinoflagellates are better adapted to use ammonium and other organic nitrogen forms like urea, in comparison with diatoms which are nitrate specialists [7, 8]. *Ansanella granifera*, similar to other red tide dinoflagellates, is also a mixotrophic species that is capable of photosynthesis and acquiring nutrients in pre-packaged or particulate form (including heterotrophic bacteria and other small photosynthetic microalgae) together [9]. Mixotrophy is a competitive advantage for many dinoflagellate species that allows them to dominate in the ocean. Mixotrophic species are responsible for ~ 40% of the species forming red tides globally [10, 11]. *Ansanella granifera* is one of the fastest growing mixotrophic dinoflagellates reported to date. Mixotrophic dinoflagellates can increase their populations by migrating between well-lit surface and eutrophic bottom waters [6, 12].

Eutrophication processes associated with nitrogen loading in the coastal zone have resulted in algal blooms, fish kills, altered trophic interactions, and oxygen depletion, and have caused other environmental problems in different coastal regions around the world [1, 13]. Dinoflagellate blooms including toxic species have also been reported near areas of sewage in semi-enclosed bays from Cuba [14, 15].

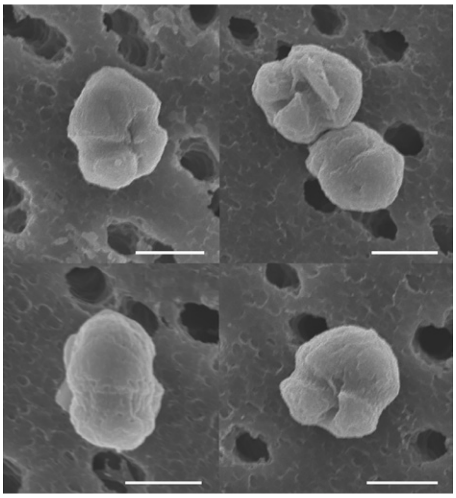

Fig. 4. Scanning electron micrographs of Ansanella granifera cells from field samples. Scale bar = 5 µm.

Acknowledgements

This work was supported by CiguaPire (NSF OISE Award # 1743802), which facilitated collaboration among researchers from USA and Cuba, leading to studies and training on HABs. Additional support was provided by the Greater Caribbean Center for Ciguatera Research (NIH 1P01ES028949- 01 and NSF 1841811). We thank Evie Fachon for her valuable technical and laboratory assistance.

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Dolichospermum spiroides blooms in a man-made lake in Sarawak, Borneo

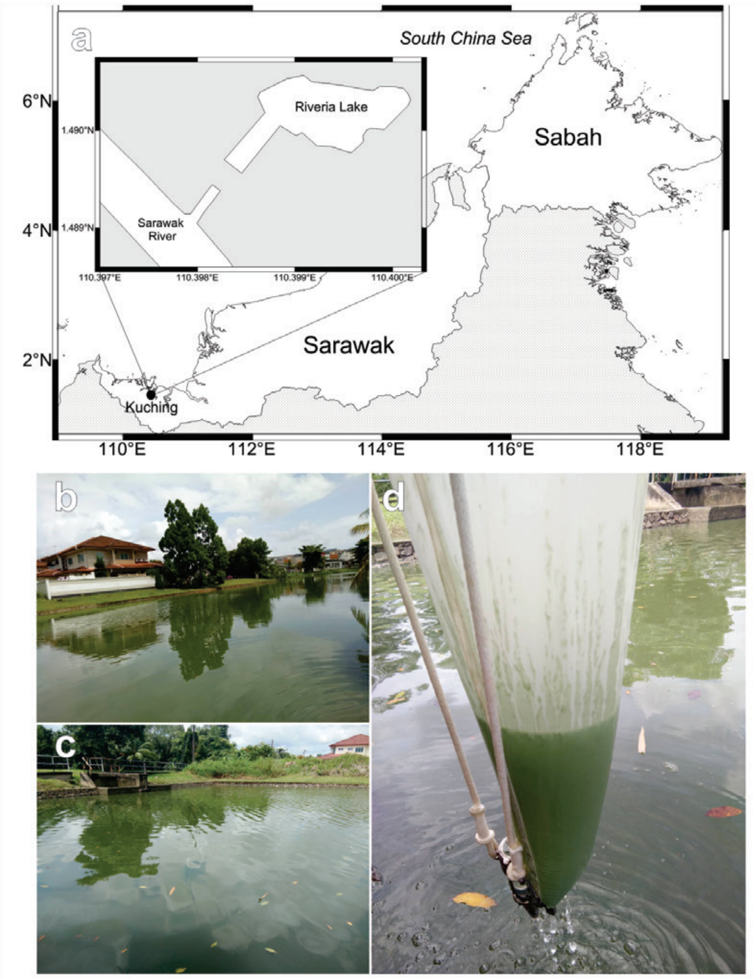

Fig. 1. (a) Map of Malaysia Borneo showing the bloom location at the man-made lake in Kota Samarahan, Sarawak. (b, c) Green water discoloration detected at the study site. (d) Concentrated plankton net haul samples.

Dolichospermum (formerly *Anabaena*) is a cyanobacterial genus that includes toxic bloom forming species [1]. Species of *Dolichospermum* are free-living cyanobacteria found in lentic water bodies such as freshwater lakes, ponds, reservoirs as well as brackish waters [2].

In this study, we report a water discoloration caused by a massive bloom of a filamentous heterocyst-forming cyanobacteria, between February and March 2020. The discoloration, with an extension of approximately 2.22 acres, was formed in a lake at the center of a residential area and connected to the Sarawak River in Borneo (Fig. 1). The cyanobacteria was identified as *Dolichopermum spiroides* using light microscopy (Fig. 2) and reached densities of 1.21 to 1.25×10^6 cells L^{-1}. Cells were filamentous and often curly. Trichomes were surrounded by a mucilaginous envelope.

To date there are a very limited number of studies of cyanobacteria [3,4,5] in Sarawak. In Malaysia, a bloom of *Dolichospermum* (reported as *Anabaena*) was recorded in a freshwater fish pond in Serian, Sarawak, and co-existed with a *Microcystis* bloom. However, the species and cell density for both genera were not recorded [3].

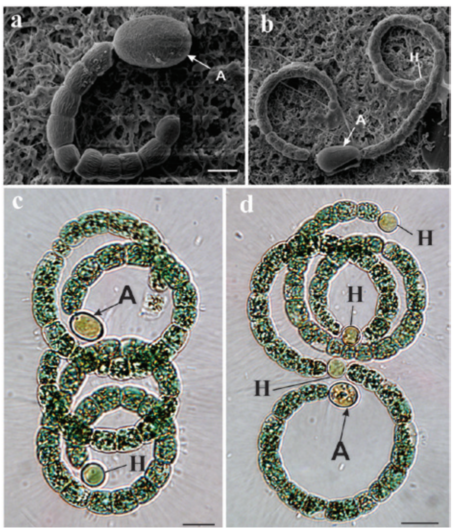

Fig. 2. SEM (a, b) and LM (c, d). A: Akinete, H: Heterocyst of Dolichospermum spiroides

This is the first documented report of *D. spiroides* in Sarawak waters. The occurrence of *Dolichospermum* blooms encouraged the implementation of monitoring the freshwater system to avoid any intoxications by cyanotoxins. Fortunately, there were no human health problems as the lake water is not used for drinking, however there are some recreational activities, such as fishing and kayaking which may be affected in the future.

Acknowledgements

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An online platform (GEE App) for Trophic State Index monitoring of inland waters in Latin America

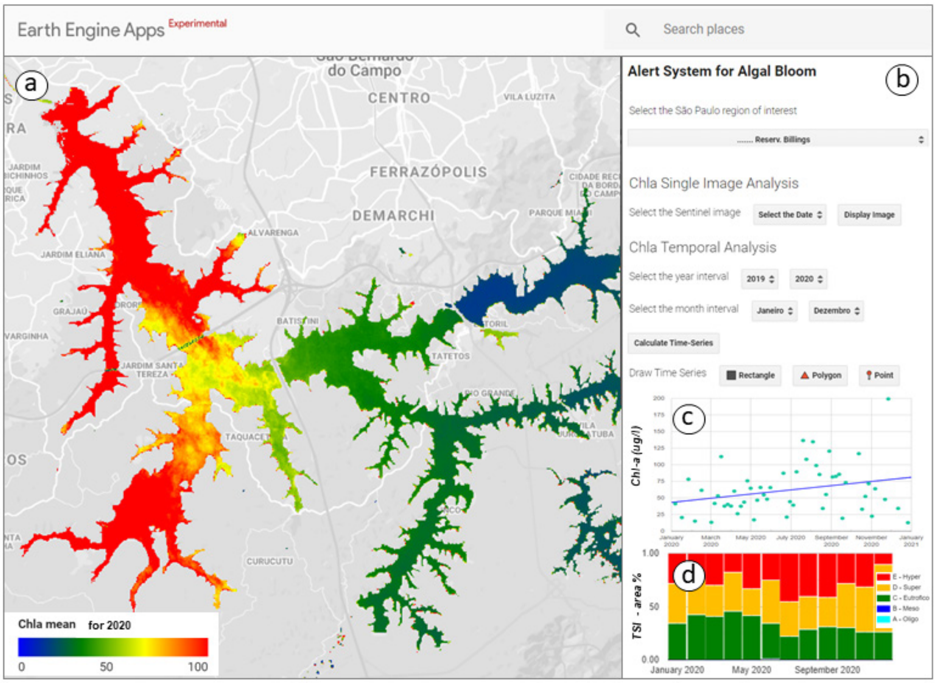

Fig. 1. a) The dark gray region shows the Paraná River Basin in Brazil; b) Water masses within Paraná River Basin palette according to the Chl-a concentration average for 2020. The red rectangle indicates the Billings Reservoir taken as an example for the GEE App (see Fig. 2).

Human activities on a global scale have significantly contributed to the change in quality of water bodies caused by increasing nutrients levels. In these circumstances, algae have proliferated rapidly and their concentrations are above normal values. The lack of *in situ* data in Latin American countries limits the capacity of water quality management due to the paucity of information about the current status of surface waters. In this scenario, remote sensing and cloud computing techniques are fundamental tools to deliver precise and quick indicators of algal bloom (AB) occurrence and Trophic Levels over large areas, supporting decision-makers and management actions.

Last year, in collaboration with several researchers, a project to develop a tool that maps ABs over the main water bodies and reservoirs in Latin America was approved by Google Earth Engine (GEE) [1] and Earth Observation Data Science (EO) [2]. So far, the proposed methodology uses Sentinel-2 images corrected for atmospheric and sun-glint effects to generate an image collection of the Normalized Difference Chlorophyll-a Index (NDCI) [3] for the entire time-series (August 2015 to present) of a given area. The NDCI retrieved from the imagery are compared with chl-a measured *in situ* with a time window of ± 2 days for match-ups. NDCI is used to estimate chl-a concentration by applying a non-linear fitting model, and is also used to classify every pixel into 5 classes of Trophic State Index (Oligo, Meso, Eutrophic, Super and Hypereutrophic) [4] based on a tree-decision model. Once the approach is developed and validated using pilot sites, the method will be transferred to stakeholders at several levels to provide AB information for the main water bodies/reservoirs in Latin America.

In this article, we report the first results for the Tiete River Basin, one of the Paraná River Basin's main tributaries, located in the State of São Paulo, Brazil (Fig. 1). Water in large urban areas in São Paulo is supplied by a variety of reservoirs. The rapid urbanization and industrialization process led to high nitrogen and phosphorus concentrations in surface waters and consequently degradation of water quality and eutrophication. For example, the Billings reservoir, which is located in the upstream Tietê basin, has faced serious water pollution problems due to the expansion of urban slums with no sewage or solid waste collection system. As a result, high chl-a and TSI levels were often observed throughout the past year (Fig. 2).

The use of cloud computing (GEE) to process all Sentinel-2 imagery and generate the NDCI collection, allows for quick access to the chl-a and TSI (Trophic State Index) levels for any water body within the study area (Paraná River Basin, Fig. 1), which includes several reservoirs and dams, such as Itaipú, Três Marias, Cantareira System and others.

More importantly, all of this information will be freely accessible for the general public via an Earth Engine APP (Fig. 2) that allows personalized evaluation of a given water mass that can either be chosen from a list or drawn by the user on the map canvas. The APP is currently in development and soon will be released in the GEE APP gallery. The user will be able to define date range, ROI (Region of Interest), time-series charts (either NDCI or Chl-a) and TSI area charts (% class area), and save these plots as texts or image formats.

For the project's following activities, we plan to improve the APP and release it on-line within the following weeks. We will also extend the NDCI collection to other important South America regions, such as Uruguay, Argentina, and North-eastern Brazil. However, this extention depends on the availability

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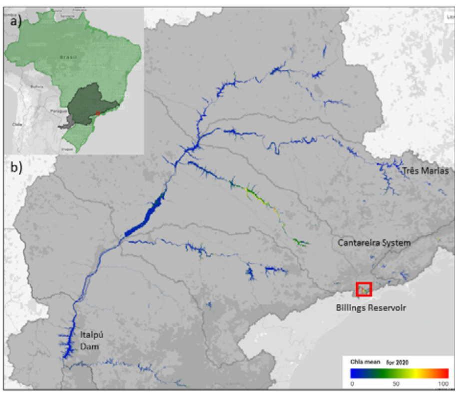

Fig. 2. Screen grab of the current version of the experimental GEE App. a) Display of maps and classifications; b) User interface, where the user can pick date range, ROI and select the products (NDCI, Chl-a and/or TSI) to be displayed as charts (c and d).

Remote sensing of recurrent cyano-HABs in Patos Lagoon, Brazil

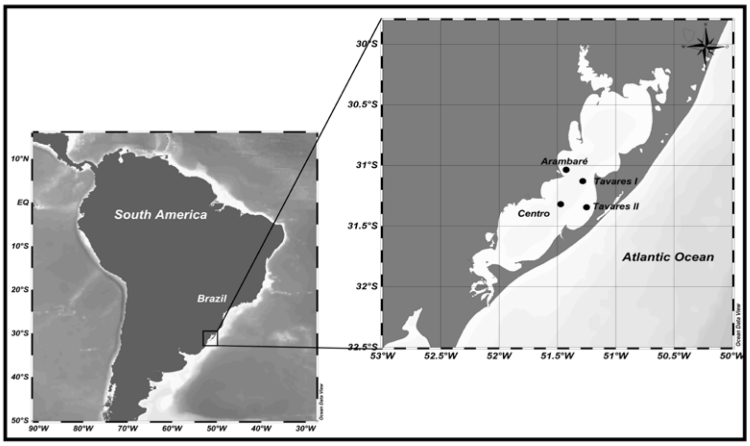

Fig. 1. Map of Patos Lagoon (southernmost part of Brazil) taken from [7]. Black circles indicate the four sites chosen forNDCI values retrieval [4].

Every austral summer, dense surface growth and accumulations of cyanobacteria threaten public health and local economic and leisure activities in the Patos Lagoon system (PL) in the southernmost part of Brazil (Fig. 1) [1]. *Microcystis* and *Dolichospermum* species can be the main components of these cyanobacterial harmful blooms (cyanoHABs). *Microcystis* species produce microcystins and *Dolichospermum* species, saxitoxins [2,3]. Due to the great spatial coverage of PL by these blooms, *in situ* studies and monitoring programs for cyanoHABs are major tasks that require resources that are not always available.

We are conducting studies using relatively new tools, remote sensing and modeling, that can jointly address two important questions: 1) To what extent can the spatial and temporal distribution pattern of cyanoHABs be better understood? 2) Are distribution patterns being modulated by climate change? We used an index of phytoplankton biomass derived from Sentinel-2 images, i.e., the Normalized Difference Chl-*a* Index (NDCI) [4], for the central part of PL spanning July 2019 to June 2020 (Fig. 1; see all four sites chosen for retrieving NDCI and water temperature values). NDCI, where values greater than 0 imply bloom occurrence, is based on the use of the 708 nm and 655 nm bands combined to evaluate bloom status in the water. We observed two cyanoHAB events during the summer of 2019–2020: one on the east (Tavares town) on December 30^{th} 2019 (Fig. 2) and the other on the west margin (Arambaré town) on January 10^{th} 2020 (Fig. 3). To correlate meteorological data with the NDCI index, two meteorological stations were considered: Mostarda's for the Tavares and Camaquãfor the Arambaré bloom events, both for the same time period. In addition, a 30-year meteorological time series of data assembled from the literature was retrieved with a closer grid point for the two locations [5]. The available information included rainfall (1980–2015), air temperature (1980–2013) as well as wind speed and direction for the summer 2019–2020. This approach helped us to classify the years 2019 and 2020 within a changing climate scenario. Lastly, water temperature values for 2019-2020 were obtained using MODIS (Moderate Resolution Imaging Spectroradiometer)-Terra imagery. Preliminary findings showed strong winds (>6 ms^{-1}) were recorded before the observed blooms, followed by relatively weak winds (<<6 ms^{-1}) during the bloom days. Thus, calm winds would have allowed the growth and surface accumulation of phytoplankton cells (Fig. 4). NDCI peaks coincided with the highest water temperature values, confirming an expected correlation between high temperatures and exponential cyanobacterial growth, especially near the margin of Tavares town (Fig. 5) [3,6]. We concluded that in a climate change context, summer 2019–2020 was drier in comparison to the historical mean, in particular during November-December 2019 and February 2020. Meanwhile, Tavares' region showed a negative (-100-mm) anomaly in cumulative rainfall during the summer. Currently we can state that relatively weak winds and low rainfall, especially in Tavares (highest NDCI values) contribute to bloom formation. This is possibly due to the increase of water residence time within PL, a situ-

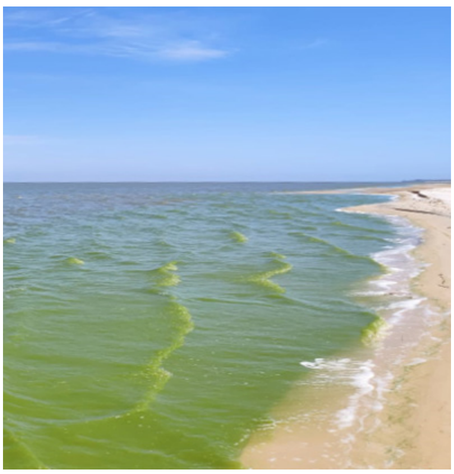

Fig. 2. Bloom on the Tavares' margin, on December 30^{th} 2019 (Source: a local, anonymous inhabitant).

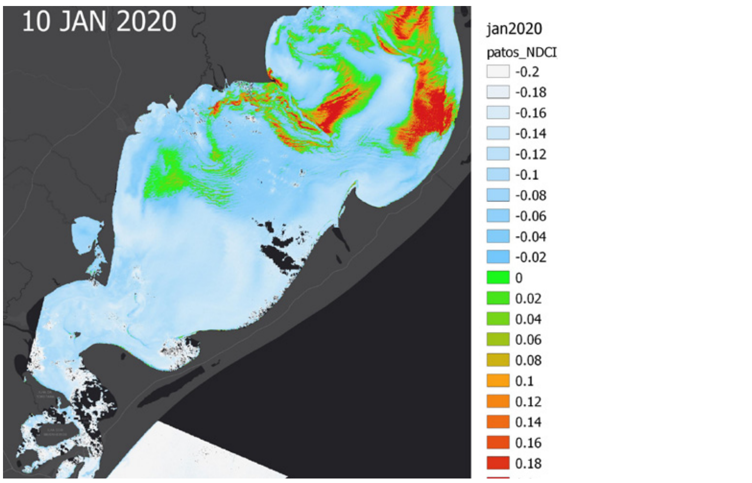

Fig. 3. Sentinel-2 image on January 10^{th} 2020 depicting NDCI features across the central part of the PL system, particularly near the west margin, including Arambaré town (see Fig. 1). Unfortunately, there was no image available for December 30^{th} 2019.

ation promoting the prevalence and duration of cyanoHABs.

More detailed information will be published soon adding modeling tools to locate dominant cyanoHAB accumulation sites within the PL, and their potential exportation to the ocean. Future studies are needed to discriminate between local effects from agricultural inputs near the PL's margins over the recurring appearance of cyanobacterial blooms, transport and advection processes within the PL and the modulation associated with climate change effects.

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Table 1. 30-year average mean and standard deviation (1980–2015; retrieved from [5]) and monthly rainfall. The monthly values were retrieved from the Camaquã's and Mostardas' meteorological stations for 2019 and up to June 2020. Note the monthly values highlighted in italics and bold denoting that almost all months since November 2019 were drier than their respective historical average.

	30-year average rainfall		Camaquã's station		Mostardas' station	
Month	**Mean**	**Standard deviation**	**2019**	**2020**	**2019**	**2020**
January	76.5	52.2	139.2	155.6	83.6	85.4
February	88.1	49.0	***70.8***	***35.4***	***50.4***	***19.8***
March	90.9	59.0	***81.2***	***44.8***	-	***27.4***
April	98.9	62.3	***78.6***	***48.2***	-	***28.6***
May	115.5	69.1	278.4	117.6	-	0.00
June	123.1	65.4	41.2	165.2	47.6	25.2
July	137.8	80.4	179.8		137	
August	114.5	70.1	155		131	
September	133.8	65.1	108.8		103.8	
October	120.5	79.0	127.8		238.2	
November	80.6	56.4	***44.6***		***59.2***	
December	85.8	64.5	***76.6***		***19.2***	

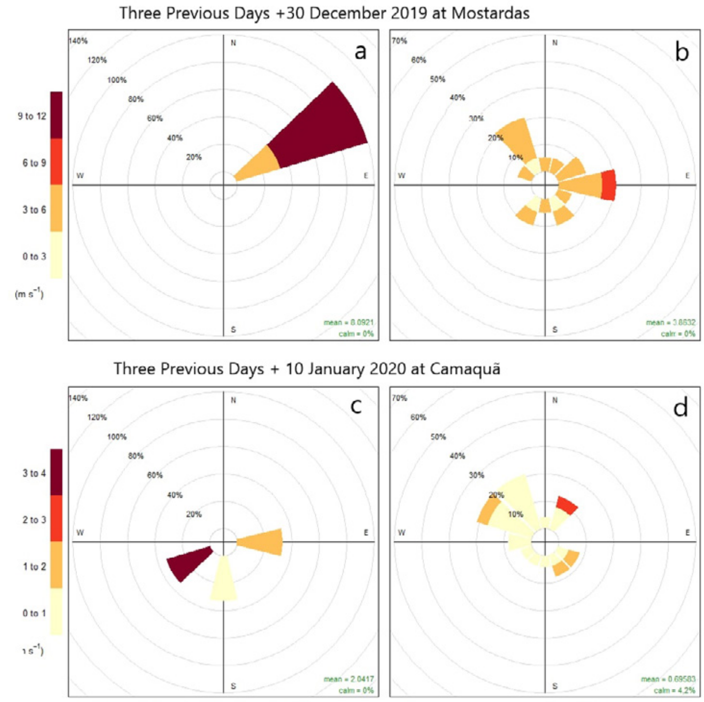

Fig. 4. Frequency of occurrence of wind direction and intensity by wind roses for the three previous days (a,c) and highest NDCI value days (b,d) collected from the Mostardas and Camaquã meteorological stations, respectively. Note the different scales adopted for each corresponding meteorological data, with higher values obtained at Mostardas (>6 ms^{-1}) whereas Camaquã gave values lower than 6 ms^{-1}.

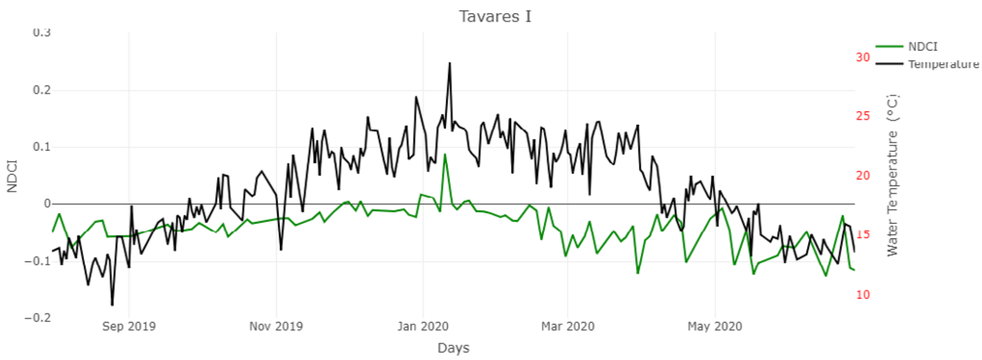

Fig. 5. Water temperature (black line) and NDCI values (green line) for one of four sites, Tavares I (see also Fig. 1), spanning July 2019 to June 2020.

Blooms of *Akashiwo sanguinea* (Dinophyceae) in a tropical estuary in northeastern Brazil

We report an inter-annual bloom of the unarmored dinoflagellate *Akashiwo sanguinea* in a pristine estuary (Figure 1F) in Brazil. The estuarine section of the Serinhaém River, Camamu Bay is a species-rich ecosystem located in the state of Bahia (Northeastern Brazil, Figure 1G), comprising preserved mangrove vegetation and remaining fragments of Atlantic Forest. It is an important regional center of economic activities based on coastal tourism and fishing. We sampled ten areas along a 30 km transect starting downstream in March 2013 and April 2014 during the rainy period (260 mm), and October 2013 and November 2014, during the dry period (235 mm). Water and surface sediment samples were collected along a salinity gradient, stored in plastic bottles and preserved in Lugol's solution and formaldehyde. Cell counts were made following the Utermöhl method with 5 ml of material observed using an inverted microscope Olympus CKX 41. Linear dimensions, such as length, width, and height were measured under 400x magnification prior to calculating biovolume by geometric shape.

Based on discontinuities in spatial variability, the estuary was divided into three sections (Figure 1E) according to Ward agglomerative clustering of log transformed biovolume: (I) the four downstream sites (SE1–SE4) with high values of salinity, mainly in the dry seasons, high pH, and where *A. sanguinea* did not occur; (II) the three intermediate sites (SE5–SE7) where salinity varied from ~17 to ~28 and *A. sanguinea* biovolume varied from intermediate values in 2013 to high values in the rainy season of 2014, along with local extinction during the dry season in this same year; and (III) the three upstream sites (SE8–SE10), which formed a group due to remarkable enhanced abundances of *A. sanguinea*, reaching up to 2 million cells L^{-1}, as well as enhanced environmental heterogeneity (variability in nutrient and hydrological conditions). The highest biovolumes observed in the recent study were during the first dry season, specifically at the warm, stratified upstream conditions occurred during October/November (Figure 1C).

Bloom events occur in response to specific environmental conditions, such as high nutrient concentrations and low turbulence [1]. These constraints partly explain the high accumulation of *A. sanguinea* during the first dry season, when stratification from reduced turbulence created vertical gradients of nutrients and light [2]. Under these conditions, *A. sanguinea* is able to vertically migrate in spiral movements and overcome the vertical displacement of nutrients, optimizing its resource exploitation and generating advantages over non-motile taxa. The local source of nutrients or disturbance also explains high accumulations upstream, since SE10 is the site nearest to the town of Ituberá (68 meters), where the river receives an average effluent discharge of 18.08 m^3 s^{-1}. It has been demonstrated that potentially harmful algae blooms (among them *A. sanguinea*) can be spatially correlated with effluent discharge gradients [3]. On another level, a disturbance source

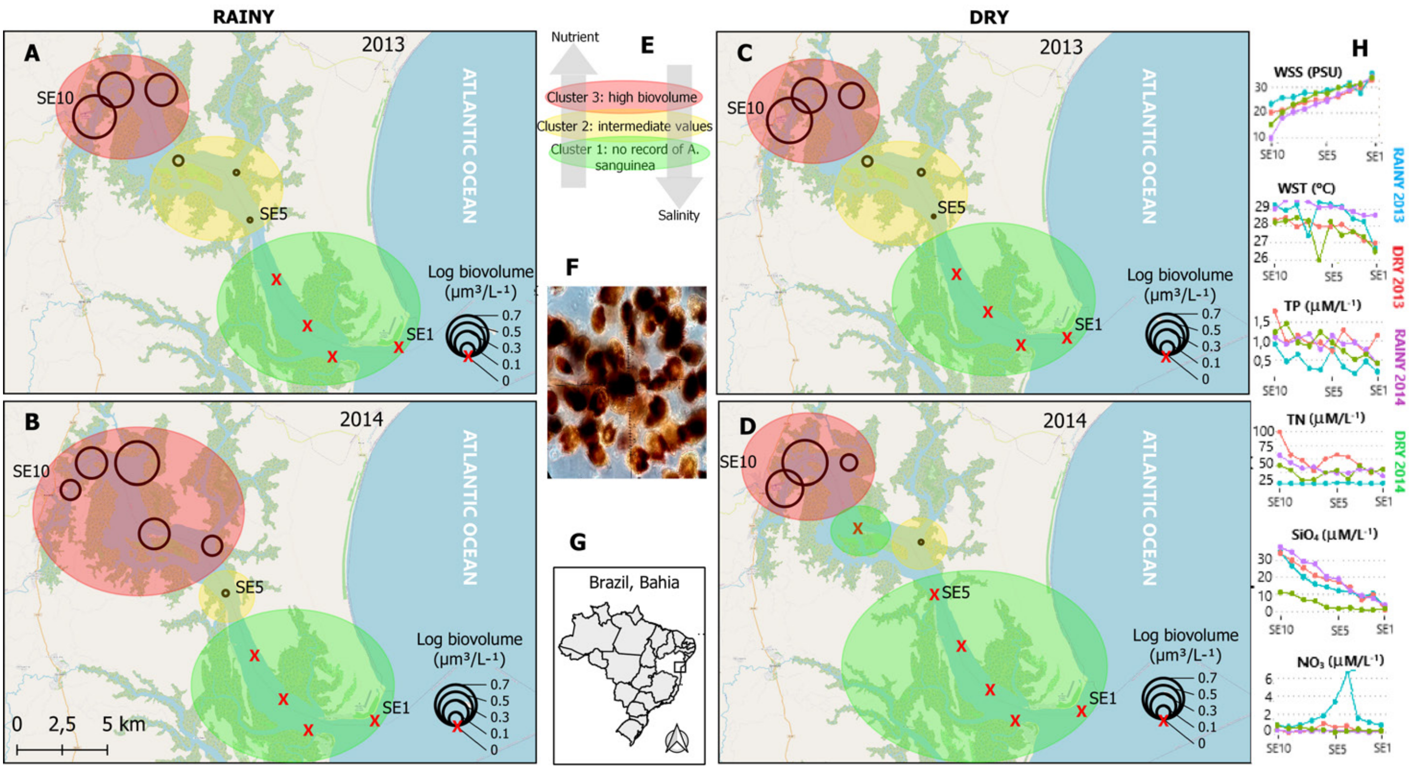

Figure 1. Map showing (G) the geographic location of the study area in Brazil, (A-D) the sampled sites along the Serinhaém River, (E) biovolume (log transformed) of A. sanguinea along the Serinhaém River (point density) and clusters based on spatial discontinuities of biovolume values along sites, (F) light micrograph of sample from site SE10 during the dry season of 2013 (highest accumulation) showing several cells at different side views (200x magnification) and (H) temporal and spatial variability of the main nutrient and environmental descriptors of the Serinhaém River. The colors represent each season (blue and purple lines for rainy seasons, red and green lines for dry seasons), the figure is plotted downward in order to match the map visualization.

can affect the entire structure of a community due to changes in composition due to outcomes of biotic interactions with one species being benefited while another one is harmed. The spatial location of SE10 within an area of potential disturbance driven by urban tributaries alters ecological stoichiometry locally and regionally, since the sites are connected by dispersal [4].

In Camamu Bay the main significant variables explaining the biovolume site-based typologies according to discriminant analysis were salinity and silicate. However, Si:DIN ratio and dissolved oxygen were significant only in 2013, while pH, nitrate and Si:TP were significant only in 2014. While total nitrogen peaked in the first rainy season (6.7µM L^{-1}), it reached higher values in the dry seasons, coincidentally when the highest biovolume of *A. sanguinea* was detected, contradicting most of the research elsewhere. There was a remarkable decrease in biovolume during the rainy seasons, especially in 2014. Although there was no phosphorus limitation at the sites where high biovolumes predominated (Figure 1, density plots), the decrease in phosphate from 0.7 µM to 0.3 µM L^{-1} represents an important stoichiometric consequence that could lead to changes in maximum abundance. Nitrogen, on the other hand, was a limiting resource at several sites throughout the second rainy season indicating a strong bottom-up effect, given that DIN:TP and Si:DIN ratios, used to determine nitrogen limitation, were significant variables in explaining biovolume variability in both rainy seasons.

The environmental variables (Figure 1H) clearly demonstrated the estuarine characteristics of the downstream section of the Serinhaém River, wherein well marked gradients were observed with salinity increasing seaward and nutrient concentrations increasing towards the warm, nutrient-rich upstream sites. The annual mean temperature ranged from 21ºC to 25ºC and significantly explained biovolume during the second rainy season (discriminant analysis, p = 0.02). Conversely, temperature differed remarkably between the two rainy seasons at SE7 (where biovolume increased inter-annually) with ~27ºC in 2013 and ~29ºC in 2014. These gradients are important ecological features for understanding both the distribution and development of *A. sanguinea* blooms in tropical waters, especially due to the lack of causal explanations for them in regions with rainy and dry periods and temperatures above 20ºC the entire year, instead of four well-marked seasons as in temperate regions, where most studies have been concentrated. The role of temperature is then more important when considering the current scenario of global climate change, which leads to the hypothesis, through supporting experimental studies, that *A. sanguinea* may benefit from ongoing climate change or global warming due to increased acidification, temperature and irradiance [5].

The formation of harmful algae blooms by the dinoflagellate *Akashiwo sanguinea* in estuarine waters is considered to be ecosystem disruptive [6,7], demanding conservative attention and management efforts.

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of *in-situ* data for algorithm validation, which could be accomplished with more *in-situ* chlorophyll data provided by the users and collaborators. Finally, this project represents the state-of-art application of Remote Sensing and Cloud Computing to provide near-real-time algal bloom alerts in the Latin America region, helping governments, institutions, and decision-makers protect and mitigate environmental impacts derived from algae bloom events.

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Can artisan fishermen help to prevent HABs intoxication? A science communication project in Rio de Janeiro, Brazil

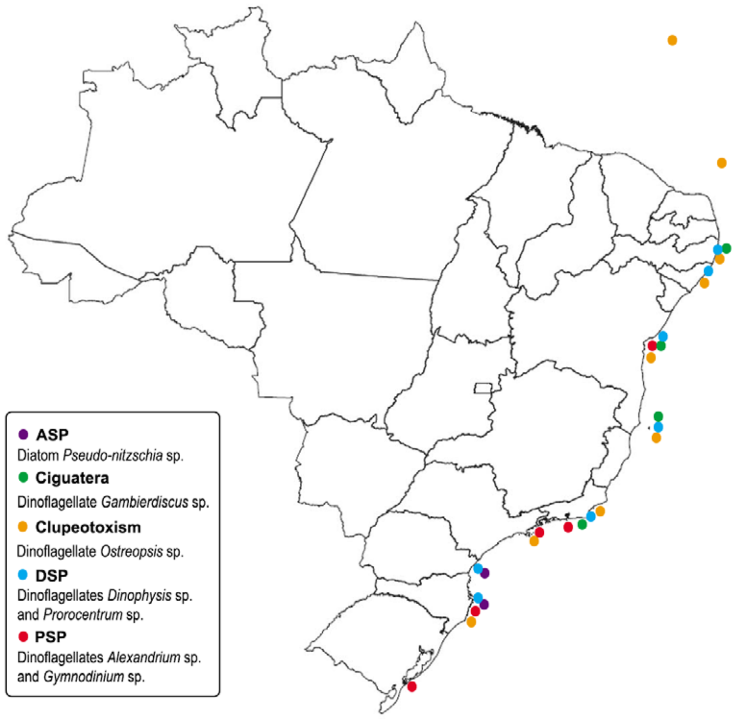

Fig. 1. Geographic distribution of the main harmful microalgae genera in Brazilian coastal waters. These genera may induce different poisoning syndromes: amnesic shellfish poisoning (ASP, purple circle), ciguatera (green circle), clupeotoxism (also called as Ostreopsis poisoning, yellow circle), diarrhetic shellfish poisoning (DSP, blue circle), and paralytic shellfish poisoning (PSP, red circle).

The occurrence and expansion in the distribution of toxic microalgae is a global concern, considering their toxicity to marine ecosystems and human health [1]. Thirty nine species from the main toxic genera of marine microalgae (e.g., *Alexandrium*, *Dinophysis*, *Gambierdiscus*, *Gymnodinium*, *Ostreopsis*, *Prorocentrum*, *Pseudo-nitzschia*) have been reported along the Brazilian coast (Fig. 1). Given the geographic distribution of these microalgae species, five different poisoning syndromes could be expected during bloom events depending on the region of the country (Fig. 1) [2-4]. Brazilian society, shellfish farmers and fishing communities are barely aware of the existence of toxic microalgae and poisoning syndromes induced by contaminated seafood and/or direct contact with toxic cells during HAB events. In the United States and European countries, commercial and recreational fisheries closures are put into place when toxin levels in seafood exceed regulatory limits for human consumption [5,6]. In contrast, Santa Catarina is the single state over the Brazilian territory to periodically monitor microalgal densities in coastal waters to control shellfish production in marine farms. Despite all the related effects to vulnerable fishing communities [5,7], fisheries closures are not only an effective management response for preventing poisonings due to the consumption of contaminated seafood, but also a way to elucidate the occurrence of HABs on coastal areas and their toxicity to human health.

In light of the ecological, economical and human health impacts of HABs, in 2019 we have started a science communication project in Rio de Janeiro coast (Brazil), entitled "Knowing the HABs" (in Portuguese, Conhecendo as HABs). This project has been developed in collaboration with other colleagues and laboratories from the Federal University of the State of Rio de Janeiro (UNIRIO). Artisan fishermen are daily in contact with seafood and effectively working in aquatic systems; thus, we believe that local fishing communities can serve as a starting point for the recognition of HABs in coastal areas and, consequently, for the prevention of human poisonings induced by the consumption of contaminated seafood. We have started creating a process for frequent contact with artisanal fishermen. Within the "Knowing the HABs" project, science communication activities have been planned and developed for the artisan fishermen organized in the main fishing association of Rio de Janeiro city (Colônia Z-13). Colônia Z-13 includes more than 950 artisan fishermen whose daily activities are undertaken from Urca Beach (22°57'07"S; 43°09'49"W) to the Pontal do Recreio (23°02'10"S; 43°29'31"W). This area includes the Rodrigo de Freitas coastal lagoon (22°58'05"S; 43°11'57"W), which comprises more than 50 km along the coast of Rio de Janeiro city. Science communication activities consist of small meetings, photo and video presentations to the fishermen to "introduce" them to the most common HABs clues in marine ecosystems (e.g., water color changes, changes in fish behavior), as well as ichthyotoxic mechanisms of noxious microalgae and poisoning syndrome symptoms.

Until the beginning of the Covid-19 pandemic and social restrictions, science communication activities started in two units of the fishing association – Z-13 Copacabana (Fig. 2) and Z-13 Rodrigo de Freitas Lagoon (Fig. 3). After their training in the recognition of HABs in marine systems, most of the artisan fishermen related that they had never heard about HABs or their impacts on fishing, ecology and human health. However, they have already noted changes in water coloration, mainly a brown color, when fishing in the coastal areas of Rio de Janeiro. Some of them have also related symptoms that could be indicative of phycotoxin poisoning. This project, based on personal contact to promote collaboration between the research conducted in the university and the reality of fishermen, had to stop during the Covid-19 pandemic. After social restrictions, we expect to restart the science communication activities in areas we have previously visited, as well as to visit the other units (e.g., Z-13 Ipanema, Z-13 Urca, Z-13 Barra) in order to extend the training for more artisan fishermen. Our insights from the

Fig. 2. Photos of the meeting, held in the fishing association Z-13 Copacabana on 9th May 2019, with the team of the science communication project "Knowing the HABs" and the artisanal fishermen from the Rio de Janeiro, Brazil.

Fig. 3. Photos of the meeting, held in the fishing association Z-13 Lagoa Rodrigo de Freitas on 2nd October 2019, with the team of the science communication project "Knowing the HABs" and the artisan fishermen from the Rio de Janeiro, Brazil.

first contacts with artisan fishermen led us to believe that the fishing communities of Rio de Janeiro city are extremely vulnerable to HABs impacts, particularly economic and human health effects, and that efforts to inform the artisan fishermen may provide future records of HABs on Rio de Janeiro coast and prevent the capture of contaminated shellfish and fish and, consequently, human poisonings.

Acknowledgements

We are grateful to the fishermen from the fishing association (Colônia Z-13) that received us and dedicated their time during our meetings and presentations, and Mariana G. Tavares from the Project "Ilhas do Rio" that introduced us to the fishing association.

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8 Meeting HAB-ANCA IOCARIBE Group

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

The VIII Workshop of the Group Harmful Algal Blooms of the Caribbean (ANCA) of IOCARIBE (Intergovernmental Oceanographic Commission for the Caribbean and adjacent waters of UNESCO) was held virtually from March 3rd – 5th 2021. Experts from different countries of the Caribbean region such as Colombia, Costa Rica, Cuba, El Salvador, Guatemala, Honduras, Jamaica, Mexico, Panama and Venezuela participated in the workshop. The event was broadcast on social networks and was attended by 82 people from 16 countries in America, Europe and Asia. *https://unesco-org.zoom.us/webinar/register/WN_Ourz98shSdqNp993LnuMEQ*

After the opening, Elisa Berdalet, presented the GlobalHAB program and discussed possible mechanisms for the inclusion and promotion of HAB-ANCA-IOCARIBE in this initiative. Cesar Toro, Executive Secretary of IOCARIBE, spoke about the United Nations Decade of Ocean Sciences for Sustainable Development (2021-2030). Ernesto Mancera presented the contribution of ANCA-IOCARIBE to the HAB status report for Latin America and the Caribbean based on OBIS and HAEDAT.

Carlos Seixas and Adriana Santos-Martínez, paid a posthumous tribute to María Esther Meave (Mexico) and Luis Alfonso Vidal (Colombia). Their dedicated work contributed significantly to improve knowledge on phytoplankton taxonomy and training of scientists.

On the second day of the meeting, Henrik Enevoldsen presented new features of the IOC HAIS/HEADAT tool, widely used for the documentation of HAB events around the world.

Each country presented a complete report on the HAB events in recent years, as well as the actions, achievements and results related to HAB issues. In some of the Caribbean countries there are specific government entities to deal with HAB matters, but only a few have a national HAB monitoring program. Universities and research institutions are important allies, but it is necessary to improve cooperation mechanisms between government entities and those allies. Country delegates recognized the importance of including HAB events in HAEDAT but expressed the difficulty of doing so in many cases. There is an important group of experts in the region but it is necessary to strengthen capacity development, mainly on issues such as cyanotoxins and mass accumulations of *Sargassum* on beaches. Although local institutions and international cooperation projects provide financial support in some cases, in general the delegates consider it urgent to finalize a regional proposal to integrate HAB research in the Caribbean region.

On the third day, the participation of the HAB-ANCA-IOCARIBE group in the XIX International Conference on Harmful Algae (ICHA) was discussed. The XIX ICHA will be held in October 2021 and organized by Mexico (Dr. Christine Band Smith). Erick Nuñez also provided a complete report on Mexico's progress with issues associated with ciguatera.

During the development of the workshop, commitments were also made for a management of the HAIS / HEADAT webinar; ANCA-IOCARIBE participation in ICHA; design of a virtual HAB course; elaboration of a macro-regional project and design of a web page- ANCA-IOCARIBE. Finally, Gustavo Arencibia Carballo, representative of Cuba, was elected as president of HAB-ANCA-IOCARIBE.

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Aotearoa/New Zealand – Japan collaboration strengthened through HAB research at Cawthron Institute

The collaboration between New Zealand and Japan has been continued and strengthened since Dr Tomohiro Nishimura (Fig. 1) has been working at Cawthron Institute, Nelson, New Zealand. He arrived in 2018 on a two year *Japan Society for the Promotion of Science (JSPS)* Overseas Research Fellowship. During that time, Tomohiro's research focused on the diversity of, and diarrhetic shellfish toxin production by, the benthic dinoflagellate genus *Prorocentrum* in some regions of Oceania [1–3]. He also reported the first instance of DTX1 production for multiple New Zealand *P. lima* complex strains [T. Nishimura, unpublished data]. Several new records of benthic *Prorocentrum* species and other benthic/planktonic dinoflagellate genera/species were also recorded during that research period [T. Nishimura, unpublished data] and will be added to the New Zealand checklists of benthic and planktonic dinoflagellates [4,5]. He was also involved in other HAB research, relating to New Zealand or Japan, published between 2018 and 2020 [6–14].

Cawthron Institute then offered a scholarship for a further two years, beginning in April 2020. Despite the difficulties encountered during COVID-19 lockdowns, Tomohiro focused on the amnesic shellfish poisoning toxin producing planktonic diatom genus *Pseudo-nitzschia* and cultured more than 100 isolates from New Zealand's coastal waters for investigating species diversity and toxin production. The presence of fourteen species, several of which were new records for New Zealand's coastal waters were confirmed. Preliminary toxin analyses suggested that multiple strains of these newly reported species did not produce domoic acid and epi-domoic acid [15]. The results from this research were presented to the New Zealand seafood industry and regulatory representatives at the Seafood Advisory Group meeting, held by the Cawthron Institute in Nelson in December 2020. The upside of the research was that the new species reported did not alter the current monitoring regime and may even allow for some relaxing of the risk alerts for some *Pseudo-nitzschia* species.

Fig. 1. Tomohiro Nishimura, sampling at Te Uenga Bay, Northland, New Zealand, 2019

A further project has recently been funded through the Catalyst: Seeding New Zealand - Japan Joint Research Project Programme, collaborating with Kochi University, Japan. This will lead to the mapping of the predicted distribution of toxic benthic microalgae given the forecasted global warming of Japan's and New Zealand's coastal waters.

In 2020 Tomohiro was awarded the '19th Young Investigators Award' from the Plankton Society of Japan and the '16th Young Researchers Award' from the Japanese Society of Phycology for his research on harmful algae in Japan.

Acknowledgements

The technical help of Lucy Thompson and Jacqui Stuart was appreciated. Diarrhetic shellfish toxin and amnesic shellfish poisoning toxin analyses were carried out by J. Sam Murray, Joshua Fitzgerald and Michael J. Boundy. The research was partly supported by the NZ government-funded Seafood Safety programme (Contract No. CAWX1080).

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ANNOUNCEMENT:
The International Phytoplankton Intercalibration

The International Phytoplankton Intercalibration (IPI) Proficiency Testing scheme in abundance and composition of marine microalgae programme 2021 is now open for registration for 2021 through www.iphyi.org. The schedule for 2021 and all other information relating to the programme can be found at the website. The IPI is open for participation to all relevant laboratories globally. The purpose of IPI is to compare and evaluate the performance of testing laboratories and to monitor the laboratories continuing performance over time on the composition and abundance of marine microalgae in preserved marine samples. We work mainly with laboratories engaged in national official/non-official phytoplankton monitoring programmes, water framework directive, marine strategy framework directive and others (environmental agencies, consultancies, private companies) working in the area of analysis of water samples for marine phytoplankton abundance and composition.

From 2021 to 2025, the IPI moves from being hosted by the Marine Institute Ireland to the Canary Islands Harmful Algal Observatory (OCHAB) at the University of Las Palmas de Gran Canaria (ULPGC), Spain. The programme continues under the auspices of IOC UNESCO (http://hab.ioc-unesco.org) through its Science and Communication Centre on Harmful Algae in Denmark.

The IOC collaboration involves the provision of algal cultures, the elaboration of a marine phytoplankton taxonomy assessment (online HAB quiz) using the IOC online platform 'Ocean Teacher Global Academy' (OTGA), and the organisation of a training workshop which is held annually to discuss the results of the intercomparison exercise and to provide training on phytoplankton taxonomy. This workshop has become an important forum for phytoplankton taxonomists working on phytoplankton monitoring programmes from around the world to convene and be able to discuss taxonomical matters related to monitoring, new advances and finds, taxonomical nomenclature changes, as well as looking at samples from different geographical areas and listening to relevant stories from other laboratories about harmful algal events in their regions of relevant ecological importance. This workshop has taken the format of a full 3 days training workshop with at least 2 days dedicated to lectures on algal groups in rooms equipped with microscopes and using live cultures and preserved samples from participants and locations across the globe.

OCHABS recognises that regular quality control assessments are crucial to ensure a high quality output of phytoplankton data. OCHAB is planning to apply for the accreditation of this proficiency testing scheme under ISO 17043 in due course. All OCHAB work is carried out following the technical and managerial requirements for PT schemes (ISO17043) and the data is statistically analysed using the statistical methods as laid out in ISO13528. OCHAB use the statistical database software ProLab Plus from QuoData to do the statistical evaluation of the participant's data.

The IPI is divided into two main parts: The first part includes the analysis of water samples sent to the participating laboratories comprising a number of unknown Phytoplankton species which analysts must identify and enumerate. The second part is an online taxonomic assessment where participants must show theoretical proficiency. At the end of the exercise participants are invited to attend a full 3 day annual workshop.

The calendar for 2021 is as follows:
Registration: Open from **Monday, 8th March 2021**
Samples sent to analysts + Oceanteacher quiz from July 30th to September 30th 2021
Deadline for submission of samples and online test results to lead laboratory: Tuesday, 30th November 2021
Intercalibration Report (January 2022) and Workshop: From 5th January 2022 to 1st March 2022

If you have any queries about registration or the programme, please contact

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The 19th International Conference on Harmful Algae 2021 (ICHA2021) is going virtual! We appreciate the responses that many of you provided in the recent survey which indicated that >85% of respondents will participate in a virtual meeting. The abstract submission deadline is 9 April 2021. Details on *abstract submission can be found here*. The International Society for the Study of Harmful Algae (ISSHA) Council is busy preparing the meeting with the local steering committee and is excited to announce that we will provide options for live participation in several time zones. We ask for your patience as we continue to update the *conference website*. The meeting will feature:

1. Pre-recorded presentations
2. Live Q&A sessions with oral presenters
3. Interactive poster sessions
4. Exciting side sessions on Early Warning Systems, Fish Farming, New HAB Technologies, and more!
5. Opportunities for informal discussion and "coffee breaks"
6. Online fun (inter)activities!

I am sure you will be pleased to see the greatly reduced registration rates for the virtual conference found *here*. Please register by 11 June 2021 to enjoy the early bird rates of 100 USD (student, retired, Yasumoto awardee, Lifetime achievement awardee), 200 USD (regular participant). You also have an option to register as a "full participant" to help support a student or a colleague from an under-represented nation.

Please also provide your nominations for the 2021 Yasumoto Lifetime Achievement Award and the Patrick Gentien Young Scientist Award *(both nominator and nominee need to be current ISSHA members)*

ISSHA members are invited to submit nominations for the Yasumoto Lifetime Achievement Award and the Patrick Gentien Young Scientist Award. The nominator and the nominee both need to be current ISSHA members. Further information on the appropriate profile of the nominees can be found *here*.

Any ISSHA member in good standing may submit nominations for either achievement award, **using the nomination forms that can be found on the website *here*.** The nominator and the nominee both need to be current ISSHA members. Nominations should include a description of the nominee's contribution (not more than one page). Please, make sure that you and your nominee have renewed your membership for the period 2020-2021.

Nominations for the 2021 Yasumoto Lifetime Achievement Award and the Patrick Gentien Young Scientist Award should be sent by e-mail to Dr. Marta Estrada (*marta@icm.csic.es*), Chair of the Committee on Achievement Awards. **Deadline: May 15, 2021.**

Please write "ISSHA Achievement Awards 2021" in the message subject. Nominations will be considered by the ISSHA Council, and eventual awards will be presented at the virtual 19th ICHA Conference, October 11-15, 2021. La Paz, B.C.S. Mexico.

Student Registration Fee Award for ICHA 2021 Mexico

In order to encourage student participants and young investigators to participate in our biennial conference, ISSHA is glad to announce student registration fee awards to cover registration fee for ICHA2021 Mexico.

Selection criteria

Membership of ISSHA (If you are not a member, please *join the society* prior to the submitting of your application;

1. application of non-member will not be considered).
2. Quality or nature of science of the paper to be presented in an oral or a poster presentation. Students who are first authors will be given higher priority for funding.
3. Priority will be given to first time applicants and from least developed or developing nations over award recipients of the past ICHA conferences.

Please indicate your wish to apply for the Student Registration Fee Award during the registration, and submit your application letter as an e-mail attachment to Dr Po Teen LIM, Email: *poteenlim@gmail.com*, Chair of Student Travel Award Committee.

The letter should contain the following information:

1. Name, address, telephone, FAX, e-mail
2. Institutional affiliation and educational status
3. Name(s) of advisor(s)
4. Research interest (please use keywords)
5. Activity at HAB 2021: poster or oral presentation
6. Copy of the abstract

Please state "**ISSHA Student Registration Fee Awards 2021**" in the subject of your e-mail. Applications not submitted correctly may be rejected. The deadline for receipt of applications will be **15th May 2021**. Applications will be reviewed by the ISSHA Travel Award Committee and classified according to merits presented. Decisions will be announced by 1 June 2021.

More details will be provided soon on conference side sessions, the schedule to accommodate different time zones, and the popular auction. Please check the *ICHA2021 website* frequently for updates. Please take some time to submit your abstract and nominate your colleague for an award.

Vera Trainer (ISSHA President)

In memoriam

Maria Esther Angélica Meave del Castillo (1960-2020)

María Esther Meave (who also received the nickname "Teté" or "Tey" by many of her friends) was born in Mexico city, Mexico (September 5th, 1960) and passed away on December 6th, 2020, after contracting COVID-19. She earned her Master and Doctorate degrees in Biology from the Universidad Nacional Autónoma de México (UNAM). In her early professional career she worked on the biology and taxonomy of freshwater Chlorophyta, in particular with *Cladophora* from waterfalls and lotic environments. A topic that was reflected in her graduate and postgraduate theses

Teté began her study of marine phytoplankton in 1989 when she was appointed by the Universidad Autónoma Metropolitana-Unidad Iztapalapa (Mexico). There she increased her scientific knowledge about marine phytoplankton through participation on a number of national and international courses. These courses were an opportunity to meet and interact with academics and researchers from different parts of the world and through these she established not only academic links, but also a number of friendships.

Teté developed her original research on marine phytoplankton with a focus on the taxonomy and ecology of phytoplankton. She successfully led a number of external funded and institutional scientific projects, and was an author and coauthor of 24 scientific papers in national and international journals, some of which focused on the study of toxic and non-native species in Mexican coasts (Mexican Pacific and Gulf of Mexico). She was also an author/coauthor on 23 book chapters and coeditor of two books.

Teté was very enthusiastic about educational issues and participated in many courses at graduate and postgraduate levels. She was an advisor of several students in graduate and postgraduate levels, and was director of 7 Master and 4 Doctorate theses.

Teté also contributed to numerous national and international scientific meetings (including various HA meetings), and organized three academic congresses. She was part of the Sistema Nacional de Investigadores (National Researchers System) (Level I), where she had an important coordinating role (e.g. Chief of Area, Coordinator, part of Committees). She was the Editor-in-chief of the scientific journal Hidrobiológica, from UAM-I.

Teté made a number of important contributions to the taxonomy and description of new marine phytoplankton genera/species including;

(1) *Calyptrella* Hernández-Becerril *et* Meave
(2) *Calyptrella robusta* (Norman) Hernández-Becerril *et* Meave
(3) *Neocalyptrella* Hernández-Becerril *et* Meave
(4) *Neocalyptrella robusta* (Norman) Hernández-Becerril *et* Meave
(5) *Triposolenia fallax* Hernández-Becerril *et* Meave
(6) *Ceratium balechii* Meave del Castillo, Okolodkov *et* Zamudio (present basyonym of *Tripos balechii* (Meave del Castillo, Okolodkov *et* Zamudio) Gómez)
(7) *Ceratium balechii* f. *longum* Meave del Castillo, Okolodkov *et* Zamudio (present basyonym of *Tripos balechii* f. *longus* (Meave del Castillo, Okolodkov *et* Zamudio) Gómez)
(8) *Pleurosigma gracilitatis* Sterrenburg, Meave *et* Tiffany
(9) *Fryxelliella sepulvedana* Meave, Zamudio *et* Fernandes (present synonym of *Fryxelliella pacifica* Hernández-Becerril *et* Barón-Campis)

We regret her passing away and will certainly miss Teté very much. We extend our deep condolences to her family (her father, two sisters and two brothers, and her son) and friends.

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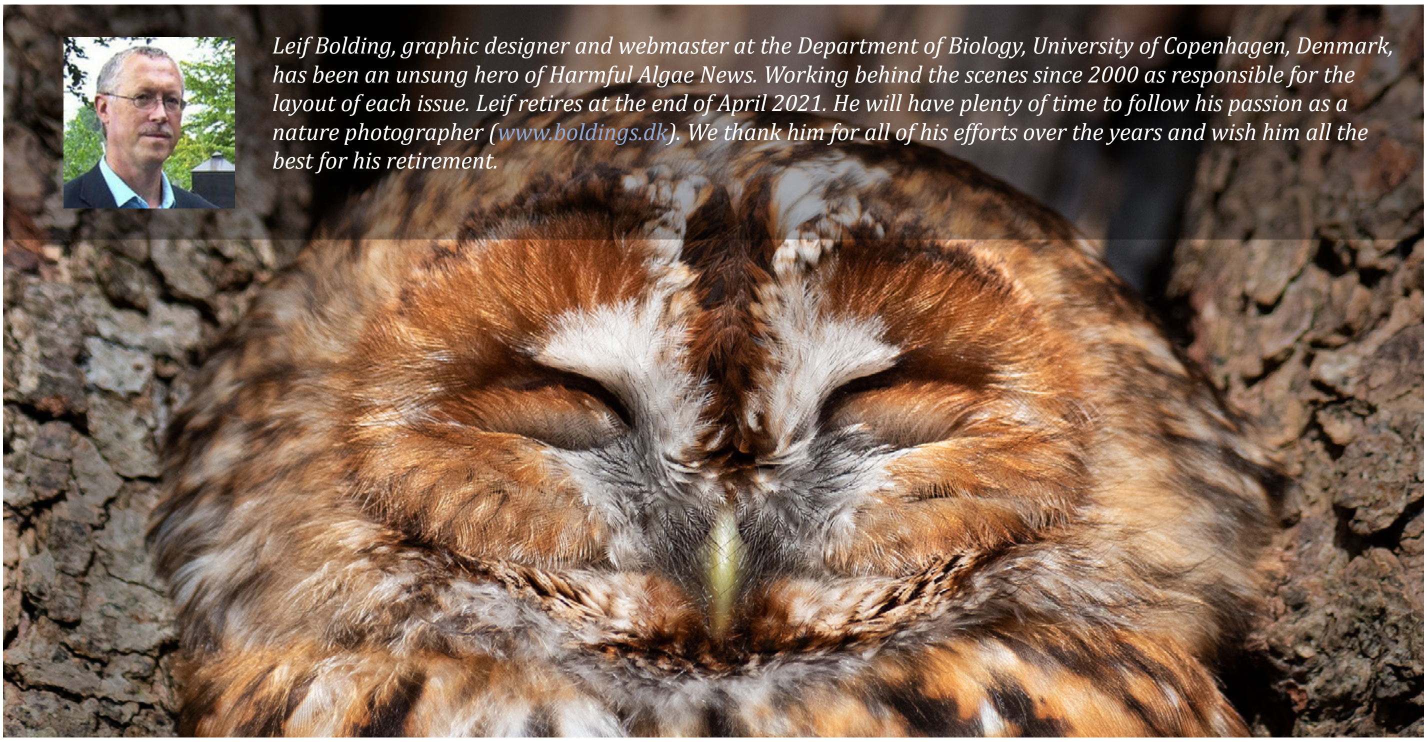

Leif Bolding, graphic designer and webmaster at the Department of Biology, University of Copenhagen, Denmark, has been an unsung hero of Harmful Algae News. Working behind the scenes since 2000 as responsible for the layout of each issue. Leif retires at the end of April 2021. He will have plenty of time to follow his passion as a nature photographer (www.boldings.dk). We thank him for all of his efforts over the years and wish him all the best for his retirement.

Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

Deadline
Deadline to submit material for HAN 68:
June 30th, 2021

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Lay-out
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