Harmful Algae News

AN IOC NEWSLETTER ON TOXIC ALGAE AND ALGAL BLOOMS

No. 64 - June 2020 · www.ioc-unesco.org/hab

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

The IOC Taxonomic Reference List of Harmful Microalgae

The creation of the *IOC Taxonomic Reference List of Harmful Microalgae* was first discussed 23 years ago at the Fourth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms in Vigo, Spain, 30 June-2 July 1997. It was decided to establish a *Task Team on Algal Taxonomy* in order to create an agreed reference list of harmful algal species as well as the correct citation of the author(s), date of valid publication, and a list of synonyms. The focus of the list was to be on toxin producing HAB species. Further suggestions for the list were made during the HAB 2000 Conference in Hobart, Tasmania, February 2000, and the list has subsequently been discussed at the biannual meetings of the IOC Intergovernmental Panel on Harmful Algal Blooms (IOC IPHAB) in Paris.

At recent IOC IPHAB meetings it was suggested that information about the list should be presented at regular intervals in *Harmful Algae News* and at the international HAB conferences. This is currently under consideration. The background to the taxonomic list is given here. The list was created because of the confusion that existed at the time about the proper names for HAB species and also the correct author citations. The years following the creation of the list have not resulted in a general agreement regarding the names to be used for some HAB species (nor for many other plant or animal species), and with the wisdom of hindsight this was probably a somewhat naïve aim. Research on HAB species has proceeded rapidly in many parts of the world, but the use of more and more sophisticated research tools has not always resulted in more clarity. Presently molecular methods are used as a tool for phylogenetic information and for classification. However, this has not led to a universally accepted taxonomy. Some journals have accepted articles in which the evidence presented did not stand up to closer scrutiny. Also, the old problem of splitters and lumpers remains, with little resolution in sight. Splitters prefer to divide taxa into smaller units, from the highest level (class or phylum or even higher) to the lowest (species level or below). Each taxonomic unit therefore contains only closely or very closely related taxa. The number of taxa therefore increases, sometimes drastically, and the picture becomes more complex. Lumpers prefer to work with larger taxonomic units and aim to create a simpler, but still correct system. In fact the term 'correct' is also under debate. Must all taxonomic units be monophyletic or are paraphyletic units sometimes to be preferred? There is no simple answer. Classification is a subjective exercise, and has always been. It is therefore not always easy for the members of the Task Team to decide on 'correct' names. Before going into more specific problems, a brief overview is presented.

The list presently contains 178 species. Dinoflagellates are the most numerous with 93 species, more than half the total number of species in the list. The cyanobacteria follow in second place with 37 species and the diatoms rank third, with 29 species. The remaining groups are small and contain less than 10 species: haptophytes (8 species), raphidophyceans (6 species), dictyocho- and pelagophyceans (5 species in total). While the list initially contained only marine species, problems associated with cyanobacteria (blue-green algae) continue to grow in many parts of the world and it was therefore decided some time ago to also include this group in the list.

At the IOC IPHAB meeting in Paris in 2019 more emphasis on cyanobacteria was requested and Catarina Churro from Portugal was suggested as a new thematic editor, a post she subsequently accepted. The list of cyanobacteria can be expected to grow further, how-

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ever the information presented in publications is not always easy to interpret. Toxic blooms often contain several species and studies on individual species are therefore required to determine the species responsible for the toxin production. In addition, species are sometimes difficult to identify and the species concept in cyanobacteria is a challenge.

Nomenclatural Problems

There are four specific problems facing the Task Team:

Problem 1. The *Alexandrium* problem

Alexandrium is one of the commonest used generic names for toxin-producing HAB dinoflagellate species, but there was initial disagreement about the name to be used for 'the *tamarense* group' and its allies: *Alexandrium* Halim, *Protogonyaulax* F.J.R. Taylor, *Gessnerium* Halim or *Gonyaulax* Diesing. An important step was made towards avoiding further confusion at the HAB Conference in Lund, Sweden, in 1988 when the taxonomic expert, Prof. Enrique Balech from Argentina, was invited to discuss and give his opinion on the taxonomy of the group. After a detailed presentation, Balech concluded that all species should be included in the genus *Alexandrium* Halim. This resulted in heated discussion from/in the audience but objections raised were rejected by Prof. Balech. The chairman of the session, Prof. Karen Steidinger, finally concluded the discussion by asking all persons in the audience to agree on using only *Alexandrium* in the future. This proposal was adopted and the name *Alexandrium* has subsequently been used by scientists working on HABs.

However in 2019 it was discovered that a species of the poorly known genus *Centrodinium* nested within the genus *Alexandrium* in the molecular trees provided by Li et al [1]. Species belonging to both genera formed a monophyletic group. As *Centrodinium* was described by Kofoid in 1907 [2] but *Alexandrium* by Halim only in 1960, the old name *Centrodinium* takes priority if the two genera are to be merged.

As changes of well-known names are a source of confusion, the 'botanical' code establishes that well-known names of particular importance may be conserved: 'Conservation aims at retention of those names that best serve stability of nomenclature' (International Code of Nomenclature for Algae, Fungi and Plants 2018, Art. 14, 2). The case of *Alexandrium* versus *Centrodinium* is such a case. A formal proposal to conserve the name *Alexandrium* instead of *Centrodinium* is now being prepared to be submitted to the *General Committee of the International Code of Nomenclature for Algae, Fungi and Plants*. Subsequently splitting of *Alexandrium* was suggested [3]. An article containing arguments against this move has been submitted by Mertens et al. (pers. comm.). Until the problems have been settled, the IOC List of Harmful Algae will continue to use the name *Alexandrium* in the context of the 1988 decision.

Problem 2. The *Cochlodinium polykrikoides* problem

One of the most harmful fish killers has been referred to as *Cochlodinium polykrikoides* since its description from Puerto Rico in 1961. Huge fish kills have occurred in East Asia as a result of blooms of this species and South Korea has been particularly badly impacted. The species name was recently changed to *Margalefidinium polykrikoides* (Margalef) F. Gómez, Richlen & Anderson 2017 [4]. To change the name of well-known species always causes confusion and misunderstandings, and the problem in this case is as follows. A genus is nomenclaturally anchored to its type species and the type species of *Cochlodinium* is *Cochlodinium strangulatum* (F. Schütt) F. Schütt 1896. Like many other unarmoured (naked) dinoflagellates, *Cochlodinium* is and has always been a heterogenous genus. It comprises both chloroplast-containing and chloroplast-lacking species, and molecular data have shown, not surprisingly, that the chloroplast-containing species *Cochlodinium polykrikoides* Margalef is not closely related to the heterotrophic, chloroplast-lacking type species *C. strangulatum*. They should therefore not be retained in the same genus, and the transfer of *C. polykrikoides* to the new genus *Margalefidinium* is a logical proposal. Due to the economic importance of this species, conservation of the name *Cochlodinium polykrikoides* would cause much less confusion. Conservation can be attained by formally proposing to the *General Committee of the International Code of Nomenclature for Algae, Fungi and Plants* that the genus *Cochlodinium* be conserved with *C. polykrikoides* as type species, to replace *C. strangulatum*. A proposal to that effect is now being prepared and, if accepted, will conserve the name *Cochlodinium polykrikoides*. The species *C. strangulatum* will then need to be given a new generic name but given the fact that it is a little-known species, a new generic name for this species will cause little confusion. A species from the Far East related to *Cochlodinium polykrikoides* is *Cochlodinium catenatum* Okamura described from Tokyo Bay [5]. If the proposal to conserve the genus *Cochlodinium* with *Cochlodinium polykrikoides* as type is accepted, *C. catenatum* will again be the correct name of that species.

Problem 3. *Prorocentrum rhathymum* and *P. mexicanum*

Gómez et al [4] have recently claimed that the species *Prorocentrum rhathymum* Osorio-Tafall and *P. mexicanum* A.R. Loeblich, Sherley & R.J. Schmidt are synonyms. The question of possible synonymy goes back to Steidinger in 1983 [6] who claimed, four years after *P. mexicanum* was described by Loeblich et al [7], that the two taxa were synonyms. Faust [8] went on to state, incorrectly, that Loeblich et al, when describing the new species *P. rhathymum*, considered this to be identical to the previously described *P. mexicanum* [9]. This statement makes little sense, and Loeblich et al in fact do not mention *P. mexicanum* in their article. Faust [8] illustrated material which resembled *P. rhathymum* using the radical arrangement of some of the trichocyst pores on the two large amphiesma plates, while no such arrangement was described in *P. mexicanum* by Osorio-Tafall [9]. The amphiesma surface in Faust's [8] beautiful SEMs is rugose, in contrast to both *P. mexicanum* and *P. rhathymum*, and Faust's material was subsequently given the name *Prorocentrum steidingerae* by Gómez et al. [4]. However, Faust [8] mentions that her material was sometimes smooth (as *P. mexicanum*), sometimes rugose, thus removing a main distinguishing character of *P. steidingerae*. Faust's rugose cells resemble *P. rhathymum* in the radial arrangement of some of the trichocyst pores (as in another benthic species, *P. lima*). If we accept Faust's statement that cells are sometimes rugose, sometimes smooth, then *P. steidingerae* is a synonym of *P. rhathymum*. Osorio-Tafall's *Prorocentrum mexicanum* is a separate species, which lacks the radiating arrangement of trichocyst pores.

Present evidence indicates that *P. rhathymum* is a benthic species which occurs in sediments or attached to macroalgae and floating detritus ([8] and references herein) while *P. mexicanum* appears to be planktonic. Cells of *Prorocentrum rhathymum* from Malaysia produce okadaic acid (cells documented by an excellent micrograph in Caillaud et al. [10]) as does material from Florida (not illustrated but genetically identical to the Malaysian cells: [11]). There appears to be no reliable information on the toxic potential of *P. mexicanum*.

Problem 4. The *Ceratium* question: *Ceratium*, *Neoceratium* or *Tripos*?

One of the editors asked me some time ago about which of the names *Ceratium*, *Neoceratium* and *Tripos*, should be used in monitoring programmes? *Ceratium* species are only harmful in large numbers when they may cause low levels of oxygen in the water. However since many HAB people are involved in monitoring of marine phytoplankton in general, I will address this question, which is relatively straightforward and which may serve as an example of the splitting concept.

It has been known for a long time that freshwater and marine species of *Ceratium* fall into two different groups which differ in the number of cingular plates: 4 cingular plates in the marine species [2], 5 plates in the freshwater species [12]. It was therefore no great surprise when molecular sequencing found the species to be distributed into the same two groups. Gómez et al [13] accordingly suggested splitting the genus into two, one comprising the freshwater species and the other the marine species. As the type species is from freshwater the name *Ceratium* was retained for the freshwater species while the marine species were given the name *Neoceratium* gen. nov. [13]. However this last name was against the rules of the international code of nomenclature and therefore illegitimate [14]. New generic names must not be created if a name for the same group of species exists already, and this is the case for *Ceratium*. Thus the many new combinations of names based on *Neoceratium* created by Gómez et al [13] are not to be used. Among the generic names applied to species of the *Ceratium* group, the oldest available name for the marine species is the soon 200-year-old *Tripos* Bory 1823. When this had been established, the marine species, varieties and forms of *Ceratium* were transferred to *Tripos* [15].

Then to the question: should the name *Tripos* from now on be applied to all marine species of *Ceratium*? The answer is that it depends on whether one prefers to split the genus *Ceratium* into two genera, or to keep the species in one genus. Both solutions are acceptable. *Ceratium sensu lato* containing both the freshwater and the marine species is monophyletic. *Ceratium sensu stricto*, containing only the freshwater species, and *Tripos* containing the marine species only, are also (both) monophyletic. A simple solution is therefore to retain the name *Ceratium* for all the species, freshwater and marine, but to group them into subgenera, one comprising the freshwater species, the other the marine species. This solution has been applied to other large groups of algae (e.g. *Chaetoceros*) and to many vascular plants. All species then retain the old generic name *Ceratium* Schrank 1793.

Acknowledgements

I thank António Calado for his always valuable comments.

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Massive salmon mortalities during a *Chrysochromulina leadbeateri* bloom in Northern Norway

From mid May to mid June 2019, fish farmers along the coast of Nordland and Troms, northern Norway, experienced sudden mortalities of caged salmon [1] (Fig. 1). These mortalities were assumed to be due to a bloom of the haptophyte *Chrysochromulina leadbeateri*. Fish death was relatively sudden with gill damage frequently observed. Little or no changes were seen in the pathology of the internal organs.

Around seven and a half million farmed salmon died valued at 800 million Norwegian kroner – NOK (80 million €). This is the most extensive fish mortality event associated with a HAB recorded in Norway to date.

The first reports of fish mortalities came from Astafjorden in Troms and Ofotfjorden in Nordland. The *C. leadbeateri* bloom then moved further north to Vestfjorden and Troms during the following weeks (Fig. 2). A maximum cell density of 45 x 10^6 cells L^{-1} was recorded. *Chyrsochromulina leadbeateri*, was reported to have caused fish-kills in the same area before. In the second half of May 1991, *C. leadbeateri* bloomed in Vestfjorden and surrounding areas [3,4]. The total loss then was 742 tons of salmon with an estimated value of 22.5 million NOK (3.5 mill US dollars), a substantial loss considering the size of the fish farming industry at the time. A smaller bloom of *C. leadbeateri* caused fish-kills in the same area in 2008.

One of the first extensive fish kill events in Norway was caused by a bloom of *Chrysochromulina polylepis* (now *Prymnesium polylepis*) in 1988. That bloom occurred in Skagerrak and Kattegat along the coast of Norway, Sweden and Denmark in May and June. Both farmed and wild fish were killed as well as a wide range of benthic fauna. Since then more than 30 different species of *Chrysochromulina* have been recorded in Norwegian coastal waters, some new to science. A result of these events was the formation of the Norwegian algal monitoring program (*www.imr.algeinfo.no*).

Questions remain as to why this alga bloomed again in this area, and why it became so toxic. It is still unknown which toxins are produced by *C. leadbeateri* and the conditions which promote toxicity. However, previous research on *Prymnesium polylepis* indicates that the toxicity increases during nitrogen-sufficient but phosphorus- limiting conditions. The toxicity may reduce grazing by protozoans and zooplankton, allowing rapid growth and bloom formation of these potentially toxic haptophytes [4]. To fully understand what caused these blooms and promoted toxicity of *C. leadbeateri* in 2019 more research is needed. Monoalgal strains of *C. leadbeateri* have been established at the University of Oslo from the blooms in 1991 and in 2019 and are currently being used in experiments to improve our knowledge on bloom drivers to implement mitigation practises for fish kills in the future [5] (Fig. 3).

Fig. 1. Boxes of dead salmon are transported to the company Northern Lights Salmon land base in Sør-Troms. Photo Northern Light Salmon.

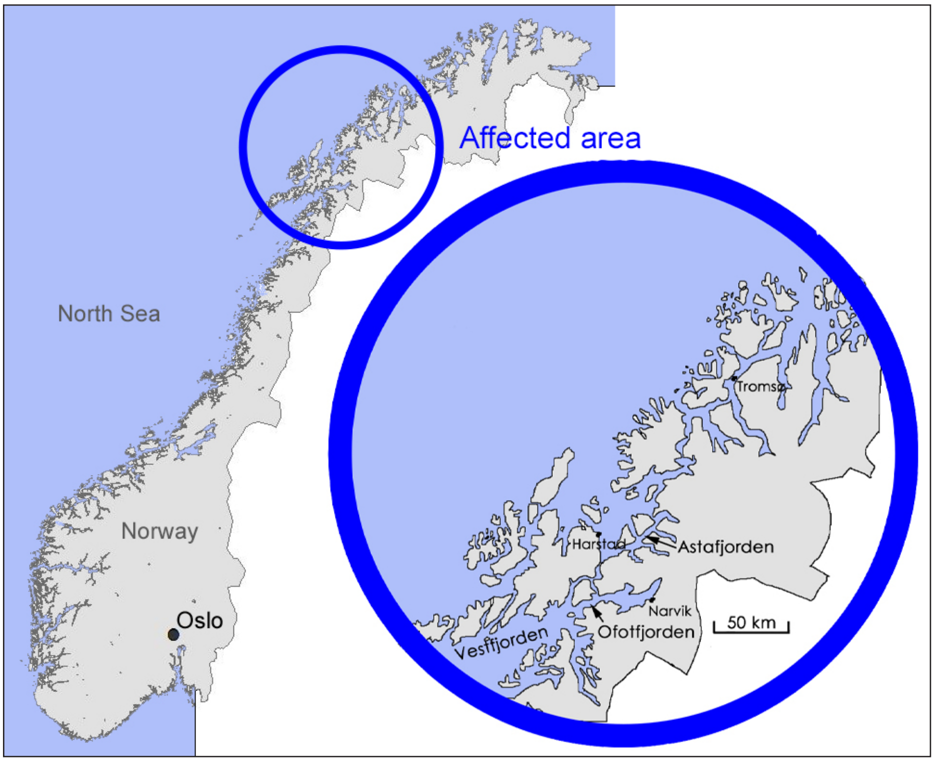

Fig. 2. Map of Norway showing the area where the Chrysochromulina leadbeateri bloom occurred causing massive fish kills in May-June 2019

Fish kill in numbers [2]

- 13 000 ton fish
- 7.5 mill salmon
- 80 mill EUR
- 14 companies

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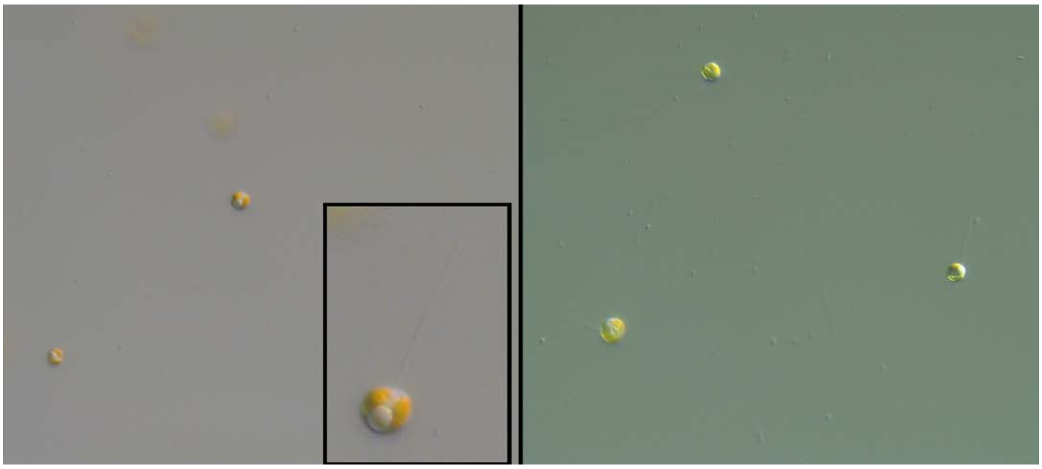

Fig. 3. Chrysochromulina leadbeatericells seen under differential interference contrast (DIC) (left) and phase contrast (right) light microscopy. They are tiny microflagellates, 5-8 µm in diameter, with two flagella, a haptonema and two chloroplasts. Photo: Bente Edvardsen

Mass mortality of marine invertebrates associated with the presence of yessotoxins in northern Chile

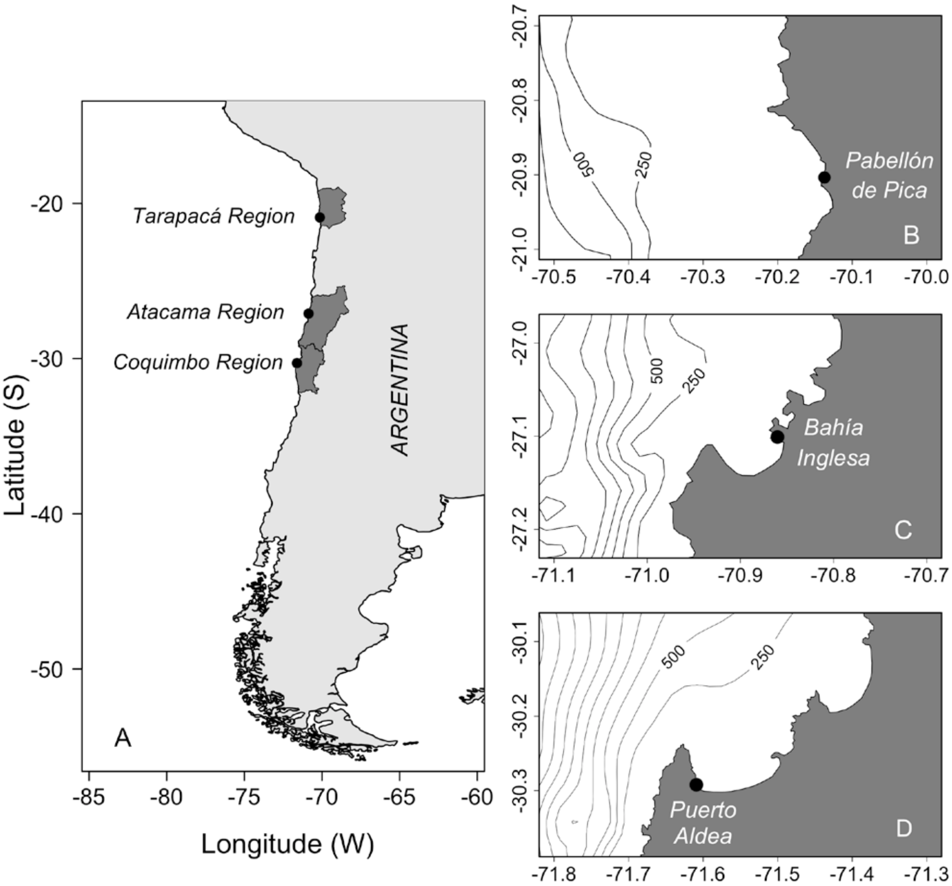

Fig. 1. Map of the study area showing A) Chilean coast; B) Pabellón de Pica, Tarapacá Region C) Bahía Inglesa, Atacama Region; D) Puerto Aldea, Coquimbo Region

During the austral summer of 2019, mass mortalities of marine invertebrates were detected at different locations throughout an extensive geographic area (up to 1000 km coastline) along the northern Chilean coast (Fig. 1). On January 24th, massive strandings of starfish (*Stichaster striatus*), red sea urchins (*Loxechinus albus*), and clams (*Ameghinomya antiqua*) were detected in Pabellón de Pica, Tarapacá Region (Fig. 2A, B). Unfortunately, accurate information about the total stranded biomass of each species was not obtained. The estimated density of sea urchins was between 15 and 20 individuals m^2. Toxin analyses of digestive tissue samples of the stranded specimens carried out by Liquid Chromatography- High Resolution Mass Spectrometry (LC-HRMS) revealed the presence of yessotoxins (YTX) with concentrations ranging between 0.1 and 0.4 mg YTX kg^{-1} (Table 1).

Two weeks later, on February 10-11th, a mass mortality of Humboldt squid (*Dosidicus gigas*) was detected in Bahía Inglesa, Atacama Region (Fig. 2C, D). During this episode, thousands of dying or dead squid were washed ashore or appeared floating near the shore of one of the most visited beaches in northern Chile. At the end of this event, the density of beached squid on the sand was estimated to be between 45 and 50 individual m^{-2} (approximately 15 tons). The health risk posed by the contaminated dead animals led the Ministry of Health to carry out a social media campaign to prevent their consumption. In addition, the City Council organized the beaches to be cleaned, to mitigate the negative impact on tourism caused by the decaying animals. LC-HRMS analyses of pooled samples of the viscera from individuals collected on February 11th revealed the presence of YTX with a concentration of 0.42 mg YTX kg^{-1}.

By the end of summer, on March 31th, a new mass mortality event of squid *D. gigas* was detected at the artisanal fishing harbor of Puerto Aldea, Coquimbo (Fig. 2E, F). During this event which took place during the night and early morning, thousands of individuals were found dead or dying, mainly lying on the surface of harbor facilities and, to a lesser extent, on nearby beaches. A total of 130 tons of stranded biomass was quickly collected and sold by fishermen to fish processing plants and the local market. However, in order to guarantee seafood safety the health authorities demanded an analysis of the squid in all of the fishing plants involved and withdrawal of the squid being sold in the local market. LC-HRMS analyses of the viscera from pooled samples revealed the presence of YTX with a concentration of 0.12 mg YTX kg^{-1}.

Table 1. Concentration of yessotoxin detected in different marine invertebrate species during the mass mortality events detected along the northern Chilean coast.

Date	Species	Location	Type of sample	mg YTX kg^{-1}
01/24/19	*Stichaster striatus*	Pabellón de Pica	Digestive tissues	0.10
01/24/19	*Loxechinus albus*	Pabellón de Pica	Digestive tissues	0.11
01/24/19	*Ameghinomya antiqua*	Pabellón de Pica	Digestive gland	0.45
02/11/19	*Dosidicus gigas*	Bahía Inglesa	Visceral mass	0.42
31/03/19	*Dosidicus gigas*	Puerto Aldea	Visceral mass	0.12

Fig. 2. A-B) Stranded specimens of the red sea urchin Loxechinus albus on the beach at Pabellón de Pica, Tarapacá Region; C) Specimens of the Humboldt squid Dosidicus gigas on the beach at Bahía Inglesa, Atacama Region; D) Control of the Chilean army to avoid commercialization of stranded organisms; E-F) Squid Dosidicus gigas on the beach at Puerto Aldea, Coquimbo Region.

The relationship between YTX and mass mortality events of invertebrates has been reported in other geographical areas around the world. In 2011, a mortality event associated with the presence of the dinoflagellate *Gonyaulax spinifera* and low levels of YTX (<0.1 mg kg^{-1}) was reported in Sonoma County, California affecting different marine invertebrates such as the red sea urchin (*Strongylocentrotus franciscanus*), the purple sea urchin (*S. purpuratus*), the starfish (*Pisaster ochraceous*) and the abalone (*Haliotis rufescens*) [1-2]. Recently, in 2017 Pitcher et al [3] reported a mass mortality of 250 tons of the abalone (*H. midae*) in different aquaculture farms along the South African coast associated with a bloom of *Gonyaulax spinifera*. During this episode, the toxin profile in the digestive gland was dominated by homo-YTX, 45-hydroxy-YTX, and a minor contribution of YTX, with average concentrations of 0.73; 0.21 and 0.09 mg kg^{-1}, respectively. The gill tissues, with an average concentration of 1.1 mg kg^{-1} (homo-YTX), 0.33 mg kg^{-1} (45-hydroxy-YTX) and 0.11 mg kg^{-1} (YTX), was the most contaminated organ. It is worth noting that the YTX concentrations found during these episodes were very similar to those reported from Northern Chile. These coincidences suggest that yessotoxins may have been the main cause of marine invertebrate mortalities. More research is needed to determine the mechanism of action and the toxin effects on tissues and cells of the main affected species. Finally, there is a need to establish an educational plan to protect the public and avoid the consumption and commercialization of potentially toxic marine invertebrates.

Acknowledgements

This work was developed within the framework of a monitoring program (Programa Nacional de Vigilancia y Control de las Intoxicaciones por Floraciones Algales Nocivas) of the Ministry of Health. Research funded by the “CONICYT + FONDEF/Primer Concurso de Investigación Tecnológica Temático en Sistemas Pesquero-Acuícolas frente a Florecimientos Algales Nocivos (FANs) Idea del Fondo de Fomento al Desarrollo Científico y Tecnológico, FONDEF/CONICYT 2017, IT17F10002”.

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First Report of *Heterocapsa minima* (Dinophyceae) from Aotearoa/ New Zealand

Small planktonic armoured dinoflagellates within the genus *Heterocapsa* are currently represented by 20 species with some having a world-wide distribution [1-2]. Blooms of some *Heterocapsa* species have been associated with fish kills due to oxygen deficiency or oversaturation. Toxicity by *H. circularisquama* has also been reported, with blooms causing tremendous damage to bivalve species [3-4]). *Heterocapsa triquetra* is one of the most common red tide species around the world [1]. Recently, Tillmann et al. [2] proposed a new species, *H. steinii*, for a taxon that has been known as *H. triquetra* [5]. It is therefore important to elucidate the diversity and distribution of *Heterocapsa* species and to understand their potential adverse impacts in coastal areas.

In Aotearoa/New Zealand, a near-shore red-tide bloom of a *Heterocapsa* species at Pukehina Beach, Bay of Plenty on 10 December 2003 was associated with swimmers complaining of skin irritations [6] (Site 3 in Fig. 1). Previous studies identified and reported four *Heterocapsa* species from New Zealand based on the results of morphological characterisations of clonal strains (Sites 1, 2, and 3 in Fig. 1) [7]: *H.* cf. *circularisquama, H. illdefina*, and *H. niei* from the subtropical zone [6,8] and *H. steinii* (previously *H. triquetra*) from the temperate zone [7]. However, the diversity of *Heterocapsa* species in the temperate zone of New Zealand has not been well elucidated.

In the present study, seawater samples were collected from two sampling sites from the South Island and three sampling sites from Stewart Island of New Zealand which are within the temperate zone (Fig. 1): Site 4 (Wedge Point, Marlborough; -41.2578, 174.0116) on 12 December 2018, Site 5 (Akaroa, Canterbury; -43.8048, 172.9653) on 18 October 2018, Sites 6–8 [Site 6 (Halfmoon Bay; -46.8968, 168.1305), Site 7 (Golden Bay Wharf; -46.9041, 168.1216), and Site 8 (Big Glory Bay; -46.9793, 168.1099), Stewart Island, Southland] on 02 September 2018. The samples were collected by hose sampling at

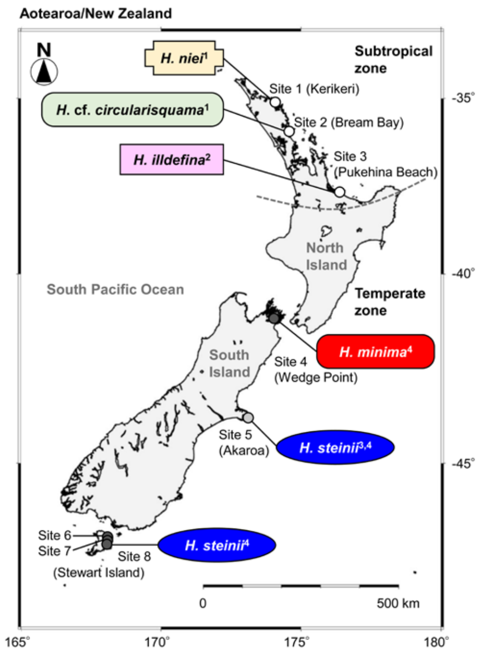

Fig. 1. Summary of the geographic distribution of Heterocapsa species in coastal areas of New Zealand. Circles represent the sampling sites of clonal strains (white: previous studies, grey: previous and present studies, dark grey: the present study). [1][8], [2][6], [3][7], [4]The present study. [1,2] Strains of H. niei and H. illdefina were identified by electron microscope examination of thecal plate structure and scale morphology. A strain of H. cf. circularisquama was identified by electron microscope examination of thecal plate structure alone. [3]A strain of H. steinii was identified by light microscope examination alone. [4]Strains of H. minima and H. steinii were identified by ribosomal RNA gene sequencing. Site 1: Kerikeri, Northland. Site 2: Bream Bay, Northland. Site 3: Pukehina Beach, Bay of Plenty. Site 4: Wedge Point, Marlborough. Site 5: Akaroa, Canterbury. Sites 6–8: Site 6, Halfmoon Bay; Site 7, Golden Bay Wharf; Site 8, Big Glory Bay; Stewart Island, Southland. The potential boundary between the subtropical and temperate zones is reported by National Institute of Water and Atmospheric Research [15].

0–15 m depths at Site 4 and from surface seawater at Sites 5–8. *Heterocapsa* cells were observed under an inverted light microscope (LM), isolated in f/2 medium to establish clonal strains, and incubated at 18 °C and 90 µmol photons $m^{-2}s^{-1}$ under a 12:12 L/D cycle. A total of 16 clonal strains were established: one from Site 4, four from Site 5, and three from Site 6 (Halfmoon Bay), three from Site 7 (Golden Bay Wharf), and five from Site 8 (Big Glory Bay) in Stewart Island. The cells of the strain from Site 4 were smaller than those from Sites 5–8 (data not shown). The cells of strains from Sites 5–8 showed the same morphology under LM (data not shown). Therefore, three representative strains were selected for molecular phylogenetic identifications: strains CAWD302 (Site 4), CAk01H (Site 5), and SSB03H (Site 8). Genomic DNA of the three strains was extracted, and the D1/D3 region of the large subunit ribosomal RNA gene (LSU rDNA) was amplified and sequenced as previously described [9]. Molecular phylogenetic analyses were conducted using maximum likelihood (ML) and neighbour-joining (NJ) methods.

The phylogenetic analyses revealed that two strains, CAk01H and SSB03H, belonged to the species *H. steinii* (Fig. 2). The sequences of these two strains were identical to those of strain UTKG7 (GenBank accession numbers: MF423357 and MF423361) that corresponds to the type material of this species. Strain CAWD302 belonged to species *H. minima* (Fig. 2). The sequence of CAWD302 was identical to those of strains JK2 and HMMJ1604 (GenBank accession numbers: KF031312 and MK483261, respectively).

Previous studies in New Zealand have reported three *Heterocapsa* species (*H.* cf. *circularisquama, H. illdefina*, and *H. niei*) from the subtropical zone [6,8]. The present study has revealed presence of two additional species: *H. minima* from Site 4 and *H. steinii* from Sites 5 and 8 in the temperate zone. Using molecular phylogenetic methods, the present study supports the previous finding of *H. steinii* (previously *H. triquetra*), identified by morphology as described above, from Site 5 [7] (Fig. 2). The present study is the first report of *H. minima* from New Zealand. Furthermore, it indicates that *Heterocapsa* species composition may be different in the subtropical and temperate zones of New Zealand (Fig. 2).

Regarding the distribution of *H. minima*, this species was first described by morphological characterization without molecular phylogenetic data from the Celtic Sea in the temperate North Atlantic Ocean in 1989 [10]. In 2001, abundance data of *H. minima* were reported in the fixed samples collected from the Bay of Biscay, North Spain in

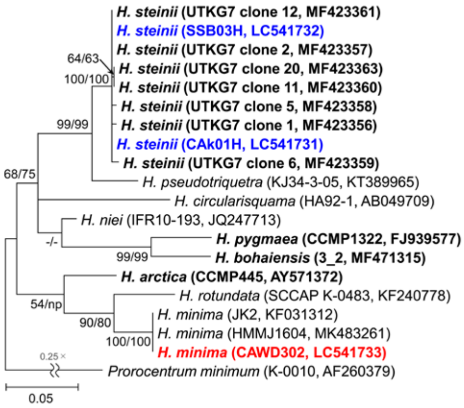

Fig. 2. Maximum likelihood (ML) molecular phylogenetic tree of Heterocapsa minima and H. steinii strains isolated from coastal areas of New Zealand based on the LSU rDNA D1/D2 sequences (20 sequences, 700 positions). The alignment site corresponded to the 69–768 bp site of a sequence from H. steinii strain UTKG7 (clone 1, MF423356). The ML and neighbour joining (NJ) phylogenies were analysed using the best-fit models, GTR+G+I and TN93+G models, respectivelywith bootstrap analyses performed with 1,000 replicates. Sequences obtained in the present study are shown in blue and red font. Sequences corresponding to the type material of each species are shown in bold font. Nodal support represents ML/NJ bootstrap values. Nodal supports that were under 50 are shown as hyphens. Nodes that were not present in the NJ tree are labelled as np.

the temperate North Atlantic. However, details of morphological and molecular characterisations were not reported [11]. In 2009, *H. minima* strain JK2 was established from Bantry Bay, Southwest Ireland in the temperate North Atlantic, potentially representing the same water mass as the type locality of *H. minima* [10], and identified by a combination of morphological and molecular phylogenetic analyses [12]. Recently, in 2016, a *H. minima* strain HMMJ1604, was established and identified by molecular and morphological analyses from Mijo Port, South Sea of Korea in the temperate North Pacific. This finding was the first report of this species from the Pacific [13]. Therefore, the present study is the second record of *H. minima* from the Pacific region and suggests that this species is widely distributed in the temperate zone of the Atlantic and Pacific. To the best of our knowledge, the present study is the first record of this species from the Southern Hemisphere.

In previous studies reporting the occurrence and abundance of *H. minima* cells [10-11,13], it was stated that this species occurred throughout the year and was widely distributed in the Celtic Sea and Korean waters [10,13]. Because the cell morphologies of *Heterocapsa* spp. are superficially similar in size, shape and swimming mode to other small dinoflagellates (e.g., *Azadinium* and *Biecheleria*) under LM observation, it is difficult to differentiate them and high magnification microscopy or molecular tools are required [14,7]. Therefore, the determination of accurate cell concentrations of these taxa from routine samples used for phytoplankton monitoring is difficult. For example, the New Zealand Marine Phytoplankton Monitoring Programme reports these cell types as "cf. *Azadinium* spp." (= *Azadinium*-like species) to provide a conservative report of potentially toxic species [9]. In the present study, the *H. minima* strain CAWD302 was isolated from a seawater sample collected at Site 4 on 12 December 2018, from which a cell density of 6,000 cells/L of "cf. *Azadinium* spp." was reported. In addition to *H. minima*, two clonal strains of *Biecheleria* spp. strains MB11 and CAWD304 (GenBank accession numbers: LC542926 and LC542925, respectively) were also isolated from the samples collected at Site 4 on 5 and 12, December 2018, respectively. To resolve this issue for *H. minima*, Lee et al. [13] developed a species-specific quantitative PCR (qPCR) method to detect and quantify cells from the Korean seawater samples. To reveal the accurate distribution of *H. minima*, applying rDNA sequencing and/or qPCR to isolated strains or seawater samples collected from the other climate zones (e.g., tropical, subtropical, and/or subboreal zones), as well as other temperate areas is required. Additionally, toxin and/or toxicity assessments are also needed to further elucidate the potential impacts of this species.

Acknowledgements

We are grateful to the Ministry of Primary Industries for allowing the use of samples for research purposes, and to Tony Bui and Catherine Moisan (Cawthron Institute Natural Toxins laboratory) for phytoplankton notifications and access to samples. Funding for this research was from the Ministry for Business, Innovation and Employment (Seafood Safety programme, Contract No. CAWX1801; National Databases contract No. CAWX0902). M. Balci was supported by the Scientific and Technological Research Council of Turkey under a TUBITAK-BIDEB-2219-International Postdoctoral Research Fellowship (Grant no: 1059B191800400) during the present study at the Cawthron Institute. T. Nishimura was supported by the Japan Society for the Promotion of Science Overseas Research Fellowship.

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Yellow-green tides could become a recurrent event along the Ligurian coast (Italy)

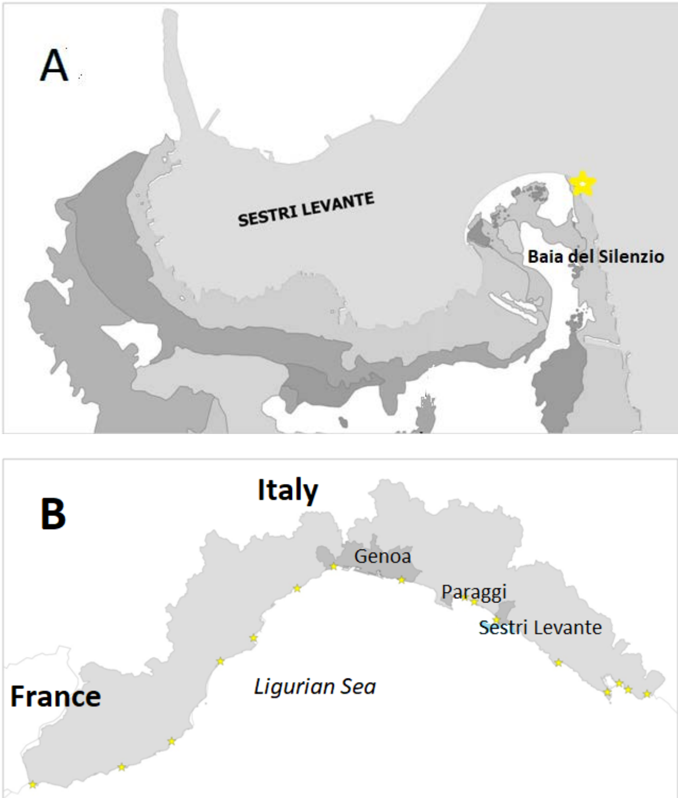

Fig. 1. A) Sestri Levante and Baia del Silenzio bay, characterized by shallow water and mixed rocky-sandy seabed containing the macrophyte, Posidonia oceanica (dark grey) and carpet-like "matte" (light grey) habitat. B) Liguria Region and the sites (yellow stars) included in the harmful algal bloom monitoring program

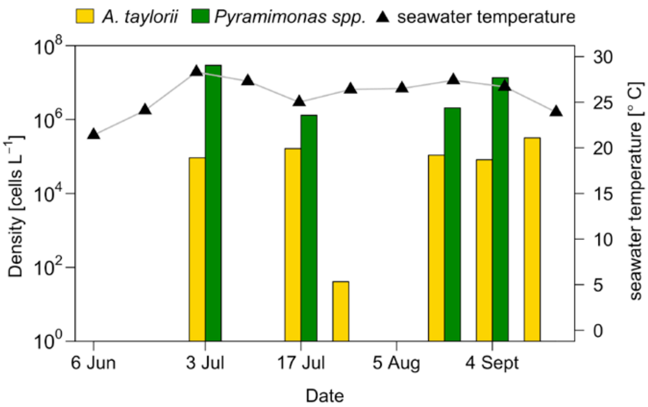

Fig. 2. Cell densities of Alexandrium taylorii and Pyramimonas spp. and seawater temperature (°C) from Sestri Levante Bay (Genoa, Italy), summer 2019.

Alexandrium blooms are becoming an increasing phenomenon in the Mediterranean Sea. These blooms mainly occur in semi-enclosed areas exposed to anthropogenic pressures from aquaculture, tourism and nutrient enrichment [1]. *Alexandrium taylorii* Balech is a potentially toxic dinoflagellate that causes a yellow-green water discoloration. Some published studies suggest that four western Mediterranean areas are affected by blooms of this species: the Catalan Coast (NE Spain), the Balearic Islands, northern Sardinia and Sicily (Vulcano Island, Italy) [2].

During the summer of 2019, blooms of *A. taylorii* impacted the eastern Ligurian coast (north-western Italy) causing significant long lasting yellow-green water discolorations. These were especially noticeable along the Sestri Levante area (Fig. 1) causing concern from both the tourism industry and local authorities.

The first bloom of *A. taylorii* in Ligurian coastal waters, with a maximum density of 1.4 x10^6 cells L^{-1}, was reported by the Regional Environmental Protection Agency of Liguria (ARPAL) in the summer of 2010. Although samples from this bloom did not test positive for algal toxins, since then ARPAL have increased monitoring efforts in compliance with the European Bathing Water Directive. Every year, from June to September, ARPAL collects and analyses samples of both seawater and macrophytes according to the Italian monitoring protocols for harmful algal blooms [3].

Significant *A. taylorii* blooms have been reported during the summers of 2012 (up to 6.0 x 10^4 cells L^{-1}), 2013 (> 3.0 x 10^6 cells L^{-1}), 2016 (5.0 x 10^4 cells L^{-1}) and 2019 (> 3.0 x 10^5 cells L^{-1}) (Fig.2). This suggests an increased frequency in the occurrence of *A. taylorii* blooms, especially on the eastern Ligurian coast. These blooms often co-occur with high cell densities of *Pyramimonas* spp., a nano-phytoplankton taxon belonging to the *Chlorophyta* group that may cause "bright green tides".

During the summer of 2019, additional water discoloration events at different sites along the eastern Ligurian coast (e.g. Paraggi, S. Margherita Ligure) with more than 6.5 x 10^4 cells L^{-1} were recorded by the local sanitary agency and Genoa University. A study was performed isolating that causative organisms into laboratory culture to confirm species identification by molecular analysis, thecal plate morphology using epifluorescence microscopy, and toxin analysis. Unfortunately, it was not possible to maintain the algal culture for the toxin characterization of the strain.

In general, *A. taylorii* is considered a high biomass HAB species that negatively impacts the use of coastal waters for recreational purposes. This species also has the potential to produce Paralytic Shellfish Toxins (PSTs) and other unknown harmful compounds [4]. To date, there have never

Continued on page 12

First report of a high biomass bloom of *Peridinium quadridentatum* (F. Stein) Gert Hansen from the tropical Cochin estuary – SW coast of India

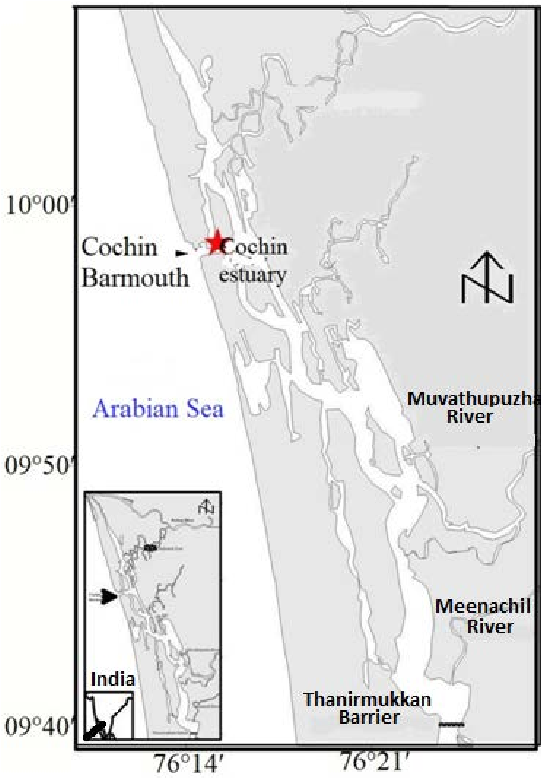

Fig. 1. Map showing the area of P. quadridentatum bloom in Cochin estuary, southwest coast of India

Peridinium quadridentatum (F. Stein) Gert Hansen is a cosmopolitan dinoflagellate which can bloom under eutrophic conditions [1-3]. It was first reported as *Heterocapsa quadridentata* from Fiji in the tropical South Pacific [4] and later renamed as *Peridinium quinquecorne*, based on morphological features such as the presence of one complete cell and one incomplete cell (hypotheca), both bearing four spines emerging from the hypotheca [5]. Only recently, Hansen [6] suggested these two species names as synonyms and proposed a new name *Peridinium quadridentatum*; but still it is commonly known under the name of *P. quinquecorne*.

P. quadridentatum is considered as non-toxic harmful dinoflagellate as it leads to reduced dissolved oxygen, higher turbidity, lower planktonic species richness and discolouration of water [7-9]. Even though *P. quadridentatum* has been reported from different parts of the world, unil now there have been no records of blooms or harmful effects of this species in Indian waters. The study reports the first bloom of *P. quadridentatum* along the tropical waters of Cochin estuary (Southwest coast of India).

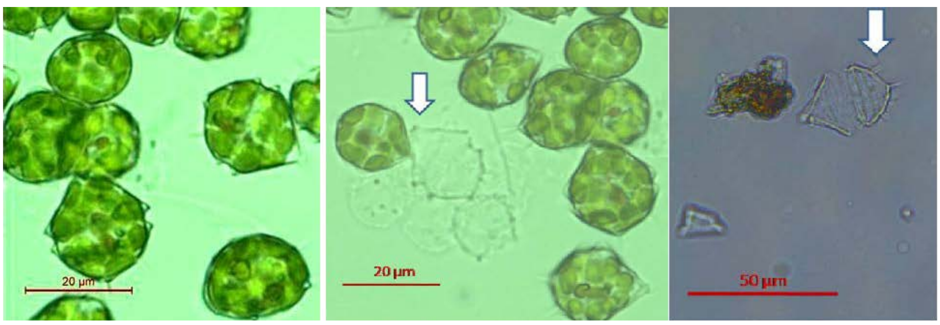

Fig. 2. Microphotographs of Peridinium quadridentatum bloom

Cochin backwater is a productive estuarine system with a high plankton diversity. It is highly influenced by the monsoon run off and inputs from anthropogenic activities which results in a dynamic change in the overall functioning of the system between seasons [10,11]. In November 2018, a sudden discolouration of water was observed near the Marine Science Jetty (Lat. 09°57'51''N; Long. 76°16'56'' E) in Cochin estuary (Fig.1). Phytoplankton samples were collected from the bloom area by filtering ~50 liters of surface water through 20µm mesh bolting silk and analysed using a Leica DM2000 microscope. Hydrobiological parameters such as temperature, salinity and dissolved oxygen were measured immediately and dissolved nutrients (nitrate and silicate) were estimated according to standard protocols. Chlorophyll a was measured spectrophotometrically using a Hitachi U-2900 UV/Visible spectrophotometer following the acetone extraction method.

Microscopic examination of samples collected from the bloom event revealed that the discoloration was caused by the dinoflagellate *P. quadridentatum* (Fig. 2). The cells were solitary, small in size and little longer than wide. The cell was overall ovoid in shape and divided by a cingulum in the middle. The epitheca was conical with pointed apex and the hypotheca was round in shape with four antapical spines, which var *Peridinium quadridentatum* (F. Stein) Gert Hansen is a cosmopolitan dinoflagellate which can bloom under eutrophic conditions [1-3]. It was first reported as *Heterocapsa quadridentata* from Fiji in the tropical South Pacific [4] and later renamed as *Peridinium quinquecorne*, based on morphological features such as the presence of one complete cell and one incomplete cell (hypotheca), both bearing four spines emerging from the hypotheca [5]. Only recently, Hansen [6] suggested these two species names as synonyms and proposed a new name *Peridinium quadridentatum*; but still it is commonly known under the name of *P. quinquecorne*.

P. quadridentatum is considered as non-toxic harmful dinoflagellate as it leads to reduced dissolved oxygen,

Table 1. Physico-chemical parameters of P. quadridentatum bloom area in Cochin Estuary

Parameters	
Water Temperature (°C)	28
Salinity (psu)	20
Dissolved Oxygen (ml L^{-1})	2.01
Nitrate (µmol L^{-1})	10.6
Silicate (µmol L^{-1})	23.3
Phosphate (µmol L^{-1})	1.3
Chlorophyll a (mg m^{-3})	27.5

higher turbidity, lower planktonic species richness and discolouration of water [7-9]. Even though *P. quadridentatum* has been reported from different parts of the world, unil now there have been no records of blooms or harmful effects of this species in Indian waters. The study reports the first bloom of *P. quadridentatum* along the tropical waters of Cochin estuary (Southwest coast of India).

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During the bloom event, the surface chlorophyll a concentration was relatively high (27.5 mg m^{-3}) and *P. quadridentatum* contributed more than 70% of the overall phytoplankton population with a cell density 6.2 x 10^4 cells L^{-1}. As well as *P. quadridentatum*, the dinoflagellate community included *Noctiluca scintillans, Tripos muelleri* and *Protoperidinium* sp. *Skeletonema costatum* and *Nitzschia sigma* were the dominant diatom species.

The *P. quadridentatum* bloom occurred when physical and chemical conditions during the bloom revealed the surface water temperature was 28°C, salinity 20 psu, and nitrate and silicate concentrations were 10.6 µmol L^{-1} and 23.3 µmol L^{-1} respectively. A low dissolved oxygen concentration (2.01 mg L^{-1}) was recorded during the bloom event, but there were no reports of fish mortalities from the bloom area.

This observation is the first bloom report of *P. quadridentatum* along Cochin estuary and further investigation on the bloom dynamics is required. This information will be useful in planning for potential HAB events along Cochin estuary.

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Continued from page 10

been any reports of toxicity associated with Mediterranean strains of *A. taylorii* [5]. For these reasons, further studies will be planned to better understand the potential risks associated with this microalga and its co-occurrence with other phytoplankton species along the Ligurian coast.

Acknowledgements

We are grateful to the ARPAL colleagues involved in the bathing water monitoring program and to Susanna Alloisio (ETT S.p.A., Genoa) for her support. This work was funded by EU project "@lgaWarning" (EuroTransBio-Program ID: ETB-2017-028).

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Are mesophotic seamounts reservoirs for potentially toxic dinoflagellates associated with Ciguatera poisoning? A case study from the SW Indian ocean (expédition La Pérouse, 2019)

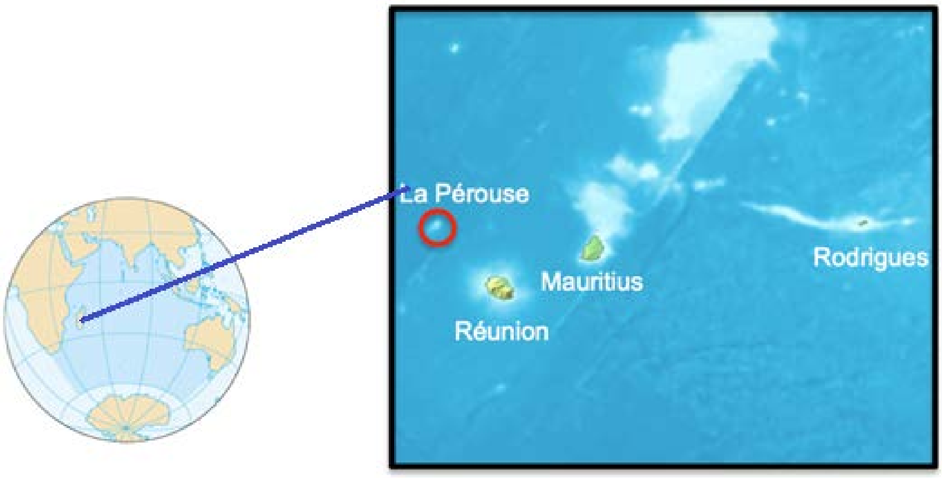

Fig. 1. Location map of La Pérouse seamount off La Réunion.

In the western Indian Ocean, a Ciguatera Fish Poisoning (CFP) hotspot is located in the coastal waters of the Mascarene Islands (Mauritius, Rodrigues, Réunion) and the offshore fishing banks of the Mauritius-Seychelles ridge, seamounts near Réunion and Mauritius (Fig. 1).

The size of the La Pérouse seamount, 160 km northwest of Réunion Island on the oceanic floor, is comparable to that of Mont Blanc in the Alps. La Pérouse is one of the rare shallow (-60m) seamounts located in the region. The plateau comprises mainly fossil limestone plaques covered with macrophytes, sponges, corals (few), and sand and rubble zones (see back page photo).

In 2005, an expedition organized by ARVAM at La Pérouse seamount (also known by local fishermen as 90 miles bank) collected carnivorous fish containing I-CTXs for analysis, as well as samples of sediment and macroalgae which subsequently facilitated the identification of *Gambierdiscus* spp in the area.

During October-November 2019, a diving and scientific expedition (La Pérouse 2019) was organized to generate a first description of the habitats and associated fauna and flora on the seamount based on a High Quality (HQ) photographic inventory. Although few samples for geological and biological analyses were taken, the focus on macroalgal biodiversity and sediments meant it was possible to isolate the microalgae assemblage from these samples.

A preliminary inventory from these samples collected at depths between -60 & -120m, revealed the presence of the main genera observed in typical CFP habitats: *Gambierdiscus, Prorocentrum, Coolia* but also athecate taxa such as *Amphidinium* and gymnodinoid dinoflagellates (Fig. 2), and thecate taxa such as *Cabra, Sinophysis, Bysmatrum* and scrippsielloids. Interestingly, among potentially toxic taxa, *Gambierdiscus* spp were clearly predominant in the samples (approx. 95% of isolated cells) while *Ostreopsis* spp and *Fukuyoa* spp were very rare. In order to give a precise identification and better evaluation of the diversity, preserved cells from approx. 35-40 morphotaxa have been isolated individually in order to allow SEM observations and molecular sequencing of LSU rDNA from single-cells in the coming months.

This study will provide a significant contribution for better knowledge of habitat preferences and vertical distribution of CFP sources. While CFP is traditionally associated with coral reefs near the ocean surface where sunlight conditions are optimal for growth and toxin production, in this case only a low concentration of light penetrates to the depths encountered at La Pérouse seamount, considered in the mesophotic zone. Only blue light reaches the benthic community here and literature about the influence of these conditions on toxin production by primary progenitors involved in seafood poisonings is scarce.

This study will also inform results of "source & sink" mechanisms that allow harmful species to spread at regional scales from these seamounts to coastal ecosystems.

Acknowledgements

To Sophie Durville and Thierry Mulochau for their enthusiasm and to Laurent Ballesta, Florian Holon and Thibault Rauby for diving deep and bringing samples to the surface.

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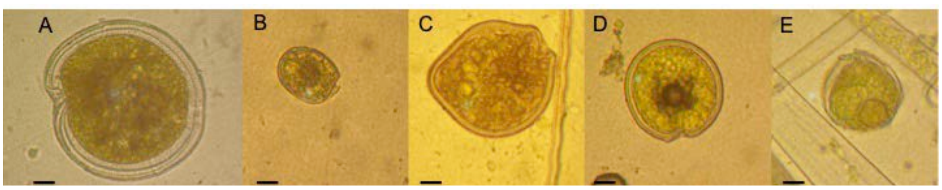

Fig. 2. Benthic dinoflagellates from La Pérouse seamount. (A) Gambierdiscus sp. (B) Amphidinium sp. (C) Prorocentrum sp. (D) Prorocentrum sp. (E) Coolia sp. Scale bars, 10 µm.

Using Machine Learning to Observe Abundance Patterns of the Dinoflagellate *Noctiluca scintillans* in the Western English Channel

Noctiluca scintillans is a Harmful Algal Bloom (HAB) species with a wide geographic distribution. It frequently blooms and causes negative impacts on marine ecosystems [1], although this species is not thought to be a toxin producer. As a result of their high biomass bloom-forming abilities, *N. scintillans* can potentially cause anoxic conditions and the generation of high levels of ammonia leading to localised die-offs of higher trophic levels [2].

We aim to develop a rapid detection of this species from flow cell-sorted images of plankton off the south-west coast of the UK. To that end, we have trained a Random Forest (RF) algorithm to i) rapidly and accurately quantify the abundance of *Noctiluca* in seawater samples of mixed plankton communities, ii) aid the examination of temporal patterns in *Noctiluca* abundance in the long-term, and iii) create a monitoring tool to show when cell counts approach bloom levels.

Seawater samples taken between March and December 2018 at the L4 autonomous buoy in the Western English Channel were processed using dynamic imaging particle analysis (DIPA) performed using FlowCam (Fluid Imaging Technologies, USA). All particles ranging in size from 380 to 1500 µm were automatically imaged and enumerated by VisualSpreadsheet (the software accompanying FlowCam).

A RF algorithm was then trained and tested on 32 particle features (for example, length, width, area, transparency etc. as measured by VisualSpreadsheet) in a mixed plankton community of 246,000 total particles; 167,000 of these particles had been manually identified as *Noctiluca* cells to give a "True Count" against which to test the model. One of the challenges of training the RF model was to encompass the ontogenetic changes seen in *Noctiluca* cells over their development and so training sets were compiled from cell samples taken on random dates throughout the year. Fig. 1 shows examples of some of the morphological and textural variety of *Noctiluca* that our RF model is tasked to classify.

The resulting model was then deployed on the 2018 dataset to estimate *Noctiluca* abundance. RF model abundance and True Count were compared using linear regression with a significance level = 0.05. A strong positive relationship was found between the RF abundance and True Count, with the RF model almost exactly matching the True Count ($p < 0.001$, $R2 = 1.000$). It must be noted that the model is limited by FlowCam's ability to identify individual particles in a sample which is likely not as accurate as manual counting with microscopy [3]

A total of 33 plankton samples were collected between March and December 2018 comprising 1,008,084 particles in total, 540,499 of which were manually identified as *Noctiluca* from the FlowCam images. To observe the temporal pattern of *Noctiluca* abundance, the RF model was deployed on the 2018 datasets and compared with total particle counts (Fig. 2).

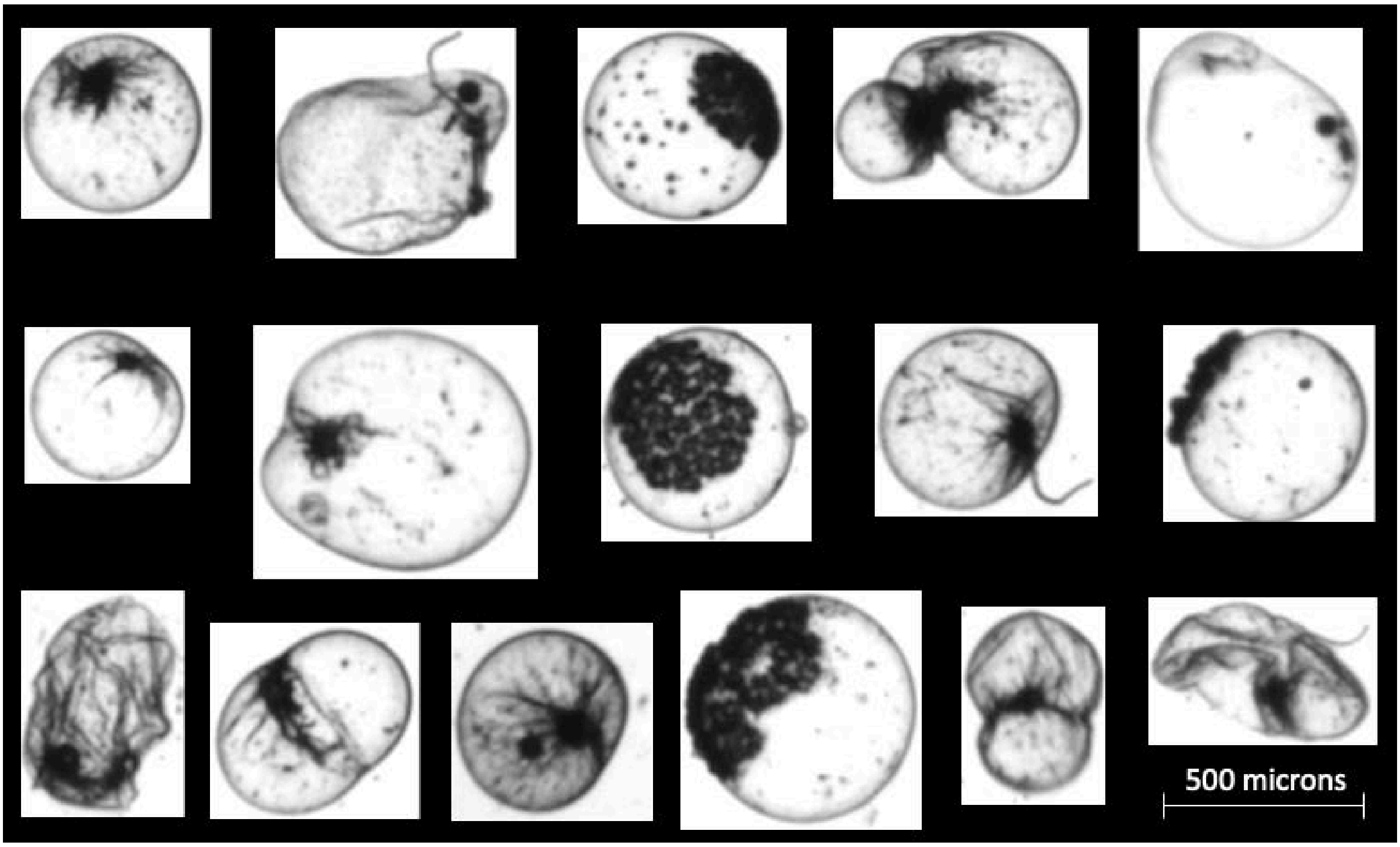

Fig. 1. Examples of morphological and textural variety of Noctiluca scintillans

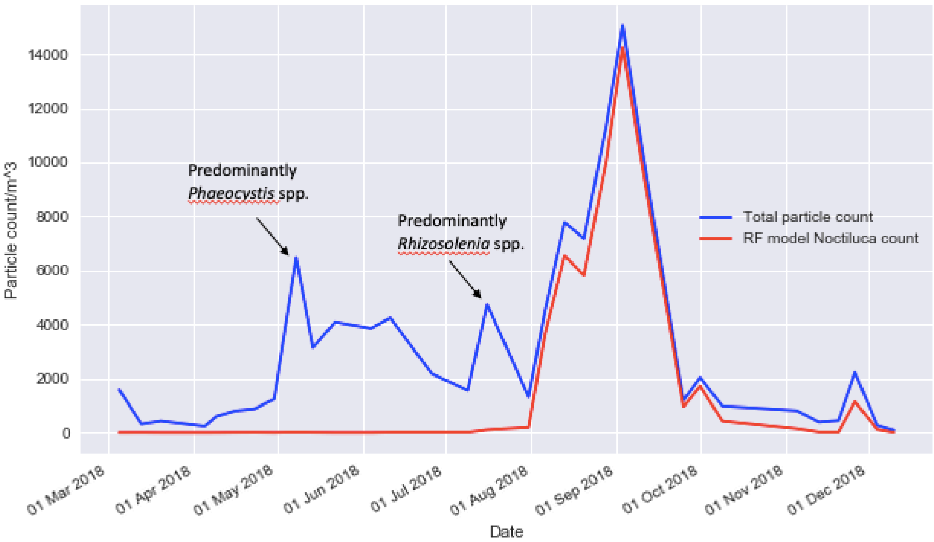

Fig. 2. Temporal abundance pattern of Noctiluca compared with total particle counts for 2018

The advantage of the RF model is its rapid sample processing time, minimal computing power requirement and excellent relative accuracy in classifying *Noctiluca* within mixed plankton communities. This lends itself as a useful tool in monitoring *Noctiluca* abundance and alerting the observer to particle numbers approaching bloom levels. In future work, we will use the model to explore spatio-temporal relationships of *Noctiluca* over longer time periods and with other physico-chemical parameters in an effort to predict bloom occurrence.

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Postponed HAB events

Due to the coronavirus (COVID-19)outbreak, the **19th International Conference on Harmful Algae (ICHA 2020)**,planned to be held in La Paz, Mexico in October 2020 will be delayed until **fall 2021**.Keep posted for the new dates.

Information will be disseminated through Harmful Algae News, and to the list of ISSHA members in good standing.

HAB experts wishing to receive the ISSHA member rate for the conference registration or to participate in the nomination/voting activities of the ISSHA Society must join ISSHA or renew their memberships. More information at: *www.issha.org* and *icha2020.com/Secciones/inicio*

Due to the coronavirus (COVID-19) crisis, the **12th International Conference on Modern and Fossil Dinoflagellates (DINO12)**, hosted by The Canarian HABs Observatory (OCH), was postponed. The new dates are from 5th to 9th July 2021. The conference will be held at the Alfredo Kraus Auditorium in Las Canteras beach, Las Palmas de Gran Canaria, Spain. For further information visit: *www.dino12conference.com*

Given the International Health situation and the confinement enforced in many country around the world, the organizers will be working from home on a temporary basis. If you needed to contact the technical secretariat, please phone between 9am and 2pm (BST or GMT+1) to **+34 676 887 596**, or write your enquiry to *info@dino12conference.com*

International validation and recognition of method for paralytic shellfish toxins in bivalve molluscs

Food safety scientists from Cefas (UK) and Cawthron Institute (New Zealand) have led an international study over the past four years to gain international recognition for a new method to quantify paralytic shellfish toxins in shellfish. It was a truly global study, incorporating 21 participating laboratories from five continents. An extensive process took place involving three pre-trials, and an enormous amount of work sourcing, testing, preparing, stabilising, and characterising study materials and shellfish samples from all around the world.

The collaboration between the two science organisations began in 2014 when Cawthron scientist Mike Boundy spent 3 months in Dr Turner's laboratory at the Weymouth Cefas facility on a Queen Elizabeth II technician's award. It had been known for some time that it was possible to chromatographically separate paralytic shellfish toxin analogues found in contaminated shellfish using HILIC chromatography. However, enhancement/suppression effects observed when monitoring the toxins in shellfish extracts were substantial and needed to be mitigated to allow development of a robust method. This technical challenge was overcome using graphitised carbon solid phase extraction (SPE) cartridges during sample preparation prior to analysis. This procedure could be performed manually or using a liquid handling robot.

Following the development and single laboratory validation of the method [1,2] it was included within the routine regulatory monitoring programme within New Zealand, with ISO17025 accreditation since 2016. During this time, it has had strong support from both regulators and industry. The method is well suited for the high throughput routine testing demands with the workflow allowing more samples to be analysed each day with faster, easier and more accurate results.

Results from the collaborative study demonstrated not just excellent performance across a variety of shellfish species, but also the ability of laboratories that use a wide range of instrument models to successfully run the method for both regulatory testing and research. The method provides accurate quantitation with higher throughput and greater specificity than existing methods of analysis with reduced complexity for the analysts. In addition, the method includes additional paralytic shellfish toxin analogues as well as tetrodotoxin (TTX), which to date have not been incorporated into any other hydrophilic marine toxin official method of analysis. The full results of the interlaboratory validation study have recently been published [3]. It is recommended that the method is used as an official alternative method for the quantitative determination of PSTs and TTX in mussels, oysters, clams, scallops, and cockles. It has been approved for regulatory testing in New Zealand and Australia, with other countries now considering its use.

Acknowledgments

We wish to sincerely thank all study participants for their time and considerable efforts in setting up the method, running the samples, and reporting the results. Thanks also to all those who supplied shellfish tissue materials for use in the study, including those who shipped materials but did not enter into the final study samples.

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Cawthron marine toxin analysts Emilie Burger, Michael Boundy, Dr Tim Harwood (L to R).

CEFAS Aquatic Toxins Team. Study co-ordinator Dr Andy Turner is located front middle.

Aotearoa/New Zealand's nationally significant Cawthron Institute Culture Collection of Microalgae (CICCM)

The CICCM is critical to international research projects and the >500 isolates of microalgae and cyanobacteria in the collection have been sourced from oceans, lakes and rivers in New Zealand, the tropics and even Antarctica. A current research focus is the likely impact of climate change on the dinoflagellate microflora of temperate regions, particularly as the temperatures of the coastal waters around New Zealand have been increasing in recent years (Rhodes et al. 2020).

The CICCM continues to expand, with the freshwater cyanobacteria collection mainly cryopreserved. The marine collection is mainly kept live and regularly sub-cultured. Curator (Fig. 1) hours have increased this year to allow for maintenance of new isolates (an 8% increase in isolates since 2018). In order to reduce the amount of labour involved, a Cawthron Institute PhD Scholarship has been offered to Joseph Kanyi, from Kenya (Fig. 2). Joseph will undertake his PhD through Victoria University of Wellington but will be based in Nelson at the Cawthron Institute. He will endeavor to cryopreserve the more recalcitrant toxic dinoflagellates in the collection and will also investigate any molecular changes associated with the cryopreservation process.

Of concern for New Zealand's marine farmers are the recurrent blooms of *Alexandrium pacificum*, which produces paralytic shellfish toxins (PSTs). Strains of the PST-producer are held in the CICCM to underpin vital research. Recently *A. pacificum* spread from Queen Charlotte Sound to Pelorus Sound in the South Island of New Zealand with a major bloom leading to large-scale closures of Greenshell™ mussel harvesting. Rapid and accurate molecular assays have been developed and tested on CICCM *Alexandrium* cultures and these assays are speeding up critical risk assessments for New Zealand's shellfish industry. The characterisation of benthic cyst beds using the molecular assays enables predications of future blooms and their risk to seafood consumers.

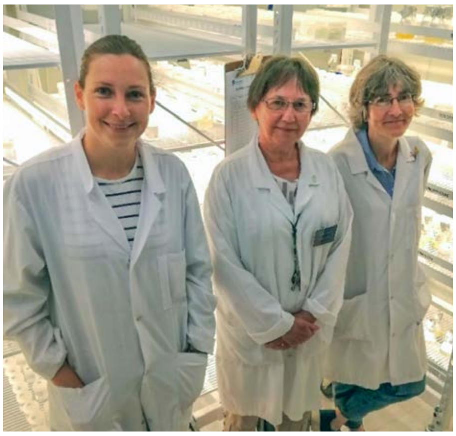

Fig. 1. Cawthron Institute Culture Collection of Microalgae curators. From left, Juliette Butler, Krystyna Ponikla, and Sarah Challenger.

As well as underpinning research into issues of immediate importance to the seafood industry, CICCM isolates are being assessed for compounds of therapeutic benefit. A Cawthron Capability Investment Fund project is underway to screen extracts from the collection in bioassays for therapeutic potential. The project, led by Dr Jonathan Puddick, (Fig. 2) will establish a library of extracts that will be assessed in-house and in partnership with collaborating organisations. This will be an exciting step-change for the collection as the isolation of new microalgal and cyanobacterial strains that show beneficial bioactivities will follow, further growing this national taonga ('treasure' in te reo Māori).

The CICCM's on-line website can be accessed at: *http://cultures.cawthron.org.nz/*

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Fig. 2. Left, Cawthron Institute PhD scholarship recipient, Joseph Kanyi Kihika. Right, Jonathan Puddick, Cawthron algal biochemist investigating the therapeutic potential to be unlocked from the CICCM

The 11th EASTHAB Symposium and 4th Philippine HAB Conference

The 11th EASTHAB Symposium and 4th Philippine HAB Conference were held back-to-back from December 11 – 13, 2019 at the Microtel Hotel, Puerto Princesa, Palawan, the Philippines. The theme was "Advances in Harmful Algal Bloom Research, Monitoring and Management". This served as a transdisciplinary platform for researchers, practitioners, educators, managers, policy makers and fisherfolk to present and discuss the recent trends, innovations and challenges in relation to HABs. This event brought together not just scientists from East and Southeast Asia, but also resource managers, policy-makers and communities in the Philippines to exchange and share their experiences on HAB occurrences, monitoring and management. There was a total number of 134 participants with 10 coming from Japan, 18 from Korea, 11 from China, 3 from Malaysia, 3 from Indonesia, 2 from Vietnam, 1 each from Thailand and Singapore, and 85 from the host country, the Philippines (Fig. 1). The conference was opened by the local government and the head of the Philippine science agency, acknowledging the significant impacts of HABs and the importance of research, monitoring and management efforts. This was followed by Dr. Rhodora Azanza setting the context for the conference and Dr. Yasuwo Fukuyo giving a historical perspective on HAB research in the country and the region as well as the outcomes of strong collaborations across countries and disciplines. Throughout the three days, there were 58 oral presentations and 24 poster presentations spread through 8 sessions: i) Recent HAB Research and Development Initiatives in the Philippines; ii) Managing HABs; iii) HAB Dynamics; iv) HAB Monitoring Tools and Approaches; v) New HAB Species from East Asia; vi) Country Reports; vii) Current HAB Monitoring and Research; and viii) the Community for Alliance for the Sustainability of our Threatened Seas (COASTS) special session.

On the first day, the Director for the Marine Resources Research Division of the Department of Science and Technology- Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST-PCAARRD), Dr. Mari-Ann Acedera shared the Philippine accomplishments and roadmap for HAB research in her plenary talk. Dr. Ma. Lourdes San Diego-McGlone highlighted the link between HABs and eutrophication in relation to potential management measures particularly for Philippine mariculture areas. This session on "Managing Harmful Algal Blooms" also showcased a diversity of topics from use of advanced technologies such as drones to community participation in monitoring and management. The COASTS special session uniquely allowed fisherfolk from three HAB-affected sites to present their own perspective on the impact of HABs and their capacity to participate in studies and monitoring.

On the second day, the HAB Dynamics session was opened by Dr. Ichiro Imai where he shared potential preventive strategies against fish-killing raphidophytes, while Dr. Aletta Yñiguez provided a meta-analysis of what is known in relation to knowledge gaps in *Pyrodinium bahamense* bloom dynamics. There were two parallel sessions on HAB dynamics with one focused on molecular studies and the other on larger ecosystem aspects. The last session for the day was on HAB Monitoring Tools and Approaches. Dr. Rhodora Azanza reviewed the monitoring tools and approaches from the common HAB-causative organisms in East Asia. Within this session, studies using traditional microscopy, metagenomics, biochemical profiling, remote sensing and modeling were presented.

On the last day, Dr. Douding Lu discussed different HABs from China and the seas nearby – from pelagic and benthic sources, as well as observations on range expansions by some species such as *Margalefidinium polykrikoides*. He was followed by Dr. Mitsunori Iwataki illustrating the morphology and phylogeny of *Azadinium* and *Amphidoma* from Japan in the session on New HAB Species from East Asia. Studies on benthic and unarmored dinoflagellates were highlighted in this session. Dr. Goh Onitsuka started off the Country Reports on HABs, presenting the case of *Karenia mikimotoi*, the species with the highest number of blooms in the past 3 years in Japan. Together with *Chattonella* spp., this species has caused severe damage to farmed fish. Japan has also experienced an increase in shellfish bans due to Paralytic Shellfish Poisoning (PSP). For South Korea, Dr. Weol-ae Lim explained the new HAB monitoring system and forecast model which included voluntary participation by aquaculture farmers. She also reported results about the *Margalefidinium* blooms in 2019, and the monitoring activities for *Alexandrium* including the use of qPCR.

Dr. Ren-Cheng Yu (China) showed HAB distribution patterns in mainland China, ranging from brown tides in the Bohai Sea, green tides in the Yellow Sea, red tides of dinoflagellates in the East China Sea, red tides of *Phaeocystis* in the South China Sea, and various toxic algal blooms along the coast. Ms. Elsa Furio reported a decline in PSP cases in the Philippines, although a few cases were recorded, from newly affected areas. She also discussed HABs due to *Alexandrium* spp., *Prorocentrum minimum, Noctiluca scintillans* and *Chattonella subsalsa*. The last session was on Current HAB Monitoring and Research. Plenary speaker Dr. Songhui Lu talked about mechanisms of brown tides occurrence in China and their effects. This session was split into two: one dealing with molecular studies and the other with ecosystem scale processes.

The IOC WESTPAC HAB group also had the opportunity to meet on the 12th of December, while the EASTHAB Scientific Steering committee met on the 13th of December. The next EASTHAB Symposium will be held in the Republic of South Korea tentatively in late November 2021.

The Book of Abstracts for this event can be downloaded from: *https://www.philhabs.net/*

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Fig. 1. Participants in the 11th EASTHAB Symposium/4th Philippine HAB Conference

Fig. 2. Department of Science and Technology secretary Fortunato dela Peña with the keynote, plenary speakers and local organizing committee leads. Left to right: Dr. Ichiro Imai, representative of the Vice Mayor of Puerto Princesa City, Dr. Mari-ann Acedera, Dr. Rhodora Azanza, Science Secretary Fortunato dela Peña, Dr. Yasuwo Fukuyo, Dr. Douding Lu, Dr. Aletta Yñiguez, Dr. Lilibeth Salvador-Reyes, Dr. Ma. Lourdes San Diego-McGlone, Dr. Deo Onda, Dr. Mitsunori Iwataki

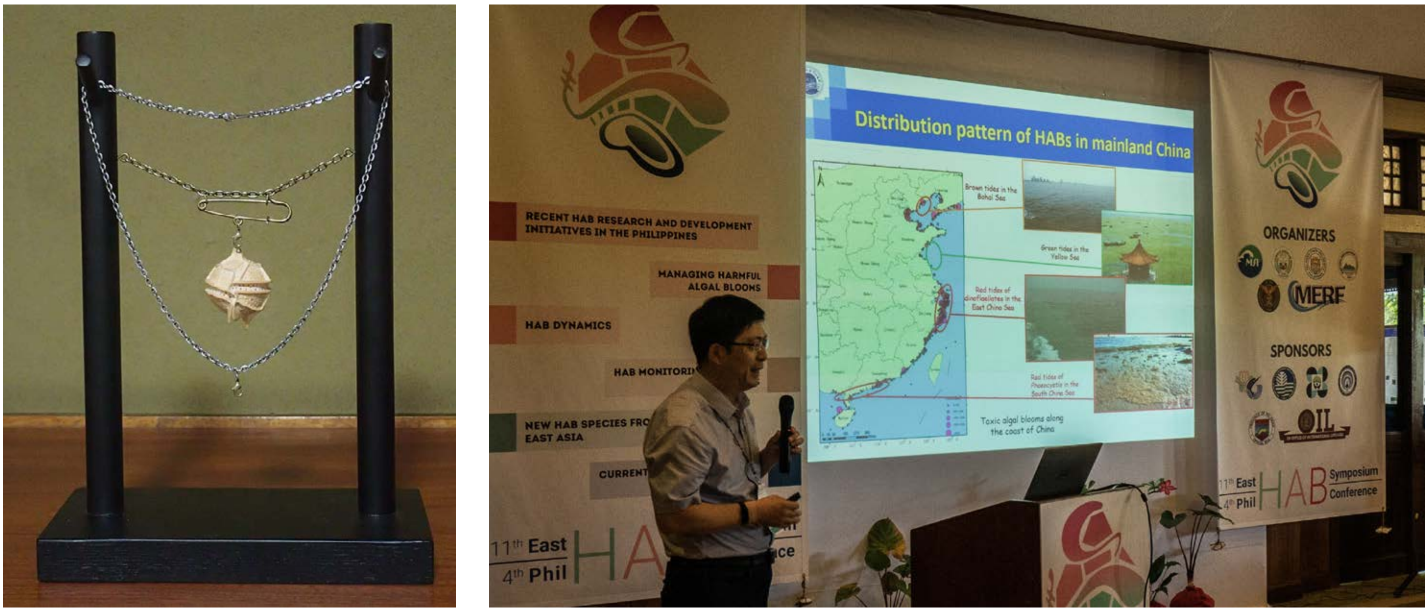

Fig. 3. A wooden Pyrodinium bahamense pendant (left), crafted by Dr. Takayama, was given to Rhodora Azanza.
Dr. Ren-Cheng Yu presenting the country report for China (right).

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics 2020 Meeting

The International Council for the Exploration of the Sea (ICES) and the Intergovernmental Oceanographic Commission of UNESCO (IOC) have collaborated closely for 26 years, stimulating research on HAB population dynamics and monitoring through the joint Working Group on Harmful Algal Bloom Dynamics (WGHABD). This year the WGHABD meeting was hosted by Hanna Mazur-Marzec and Justyna Kobos from the Institute of Oceanography, University of Gdansk in Sopot, Poland from the 3rd – 6th March 2020.

Every year working group members update the IOC-ICES-PICES Harmful Algal Event Database (HAEDAT) with HAB event data from the previous year. These data provide the basis for an ICES Harmful Algal Event Status Report which will be completed during 2020. This ICES report as well as a number of peer reviewed papers from WGHABD members will form the ICES contribution to the forthcoming IOC Global HAB Status Report.

National reports showed that HABs continued to cause a variety of problems in the ICES area during 2019. Paralytic Shellfish Toxins (PSTs) and Amnesic Shellfish Toxins (ASTs) continued to impact the east coast and west coasts of the USA, with Diarrhetic Shellfish Toxins (DSTs) also causing problems in Washington State. *Karenia brevis* continued to cause issues in the Gulf of Mexico. A bloom of *Levanderina fissa* resulted in fish and crab mortalities in the Potomac river in Virginia. Fish and marine mammal mortalities recorded on Mississippi beaches were caused by a cyanobacteria bloom resulting from river discharge from the Mississippi. PSTs, DSTs and ASTs continued to cause problems in Europe. Unusual events included blooms of *Lingulodinium polyedra* in Portugal which resulted in preventative beach closures. Fish kills in Murcia, Spain due to anoxia/mucilage formation after heavy rainfall events. In France, the majority of shellfish closures were recorded in Southern Brittany and associated with DSTs. PSTs were recorded above the regulatory limit in a new region in Ireland for the first time. Human illness was recorded in the UK after consumption of shellfish containing DSTs. An unusual bloom of *Pseudochattonella* resulted in mortalities of farmed Rainbow Trout in Denmark in November 2019. In Sweden, an extensive cyanobacteria bloom was recorded in the Baltic Sea. Beach closures were again enforced in Poland but these were not as extensive as in 2018. No impacts associated with harmful algae were recorded in the Netherlands, Belgium and Germany.

Fig. 1. ICES-IOC WGHABD 2020 meeting

New findings reported on the distribution of *Ostreopsis* in the Bay of Biscay, the extensive fish mortality event in Norway in 2019 caused by *Chrysochromulina leadbeateri*, the importance of understanding monitoring frequency in interpreting shellfish toxin monitoring data in Scotland and a study of the impacts of viruses on cyanobacteria. Investigations into the causative organism of tetrodotoxin (TTX) in the ICES area are still underway. A summary of the successful Co-Clime qPCR workshop held in Germany during October 2019 was presented.

Highlights from the EuroCigua project "Risk Characterisation of Ciguatera Fish Poisoning (CFP) in Europe" were reported. EuroCigua started in 2012 and has had a number of successful deliverables which has improved management and increased awareness of CFP issues in Europe. Epidemiological surveillance protocols were developed and 23 ciguatera outbreaks from Spain, Portugal, Germany and France between 2012 and 2018 have been identified. These involved both endemic and imported fish. *Gambierdiscus* and/or *Fukuyoa* species were identified from the Canary Islands, Madeira, Selvagem, Crete and Cyprus and *Gambierdiscus* was identified in the Balearic Islands for the first time. The majority of strains from the Canary and Balearic Islands were found to produce CTX like toxins. The N2a Assay to identify CTX toxins was standardized and validated by LC-MS-MS and successfully used to test fish in the Canary and Madeira Islands. Currently methods are being optimised to produce reference materials from fish contaminated with CTX.

For the first time WGHABD shared a day with ICES-IOC WG Ballast and Other Ship Vectors (WGBOSV) and ICES WG Introduction and Transfer of Marine Organisms (WGITMO). The Arctic is a region of interest for both ICES and the scientific community. Climate change is envisaged to alter this region in terms of biological impacts as well as increasing potential for shipping. Members from the different WGs exchanged experi-

Fig. 2. Shared day between ICES-IOC WGHABD, ICES-IOC WGBOSV and ICES WGITMO

ences using molecular methods as well as results from studies in Arctic areas. These studies presented data on HAB and invasive non-native species present in Arctic waters.

A USA study focused on the distribution, community structure, and dynamics of *Alexandrium catenella* and *Pseudo-nitzschia* in the Northern Bering, Chukchi and Beaufort Seas. Two extensive *Alexandrium catenella* cyst seedbeds were documented in the region during cruises in 2018 and 2019. This Alaskan Arctic cyst seedbed is approximately five times larger than the equivalent feature in the Gulf of Maine. Bottom temperatures recorded during the cruises were in the range to support relatively rapid germination of cysts. High concentrations of *A. catenella* vegetative cells were also detected during cruises in these regions. The data suggests that blooms in the region may originate locally and are likely self-seeding and recurrent. Established populations transported from the south through the Bering Strait may be a second source of blooms north of the Strait. Multiple species of *Pseudo-nitzschia* were also detected from Bering Strait to Chukchi Sea including *Pseudo-nitzschia australis/seriata, P. pungens, P. delicatissima*, and *P. obtusa*. A review of Canadian data revealed the widespread presence of potential toxin producing species throughout Canadian Arctic waters; Beaufort Sea, Baffin Bay, North West Passages as well as Hudson Bay and the Labrador Sea. Records include species from the genus *Pseudo-nitzschia* (*P. pseudodelicatissima, P. seriata, P. calliantha, P. granii, P. delicatissima, P. obtusa*), *Alexandrium* (*A. catenella, A. ostenfeldii*), *Dinophysis* (*D. norvegica, D. acuminata, D. acuta*), *Phalachroma rotundatum* and *Prorocentrum lima*. Reports from cruises to the west coast of Iceland and Greenland recorded *Phaeocystis* and *Pseudo-nitzschia* species. Measurement of algal toxins from the water was also a useful tool to identify the presence of HAB species. Presentations by all three WGs highlighted similar issues with the use of molecular methods. These included the standardisation of methods between laboratories and over time to allow data to be compared, primer bias and the requirement for bioinformatics expertise and computing and data power.

This was the final year in the three year ICES reporting cycle and the meeting opened with a discussion about the UN Sustainable Development Goals and the UN Decade of Ocean Science for Sustainable Development (2021 – 2030) https://www.oceandecade.org/. The UN decade is an opportunity to make HAB science more relevant to society. Discussions within WGHABD when setting terms of reference (ToRs) for the coming three year reporting cycle were framed within the context of how they would contribute to progress within the UN decade. For the next three year cycle current WGHABD ToRs focused on national reports, new findings and HAEDAT will continue. New ToRs will address increased visibility of the WG, advances in automated imaging and molecular methods for detection of HAB species, emerging toxins, sub lethal impacts of HABs on farmed fish, and the impacts of climate change on HABs. Dave Clarke from the Marine Institute, Ireland was elected as the new chair of ICES-IOC WGHABD.

The next meeting is planned for the 20 -23 April 2021 in Lowestoft, England. WGHABD welcomes new members at any time and is open to experts from all IOC Member States. HABs are a global problem and experts from outside the ICES region, interested in joining the WG (at their own expense) are particularly welcome. Now is your chance to get involved. For further information please contact WGHABD or the IOC.

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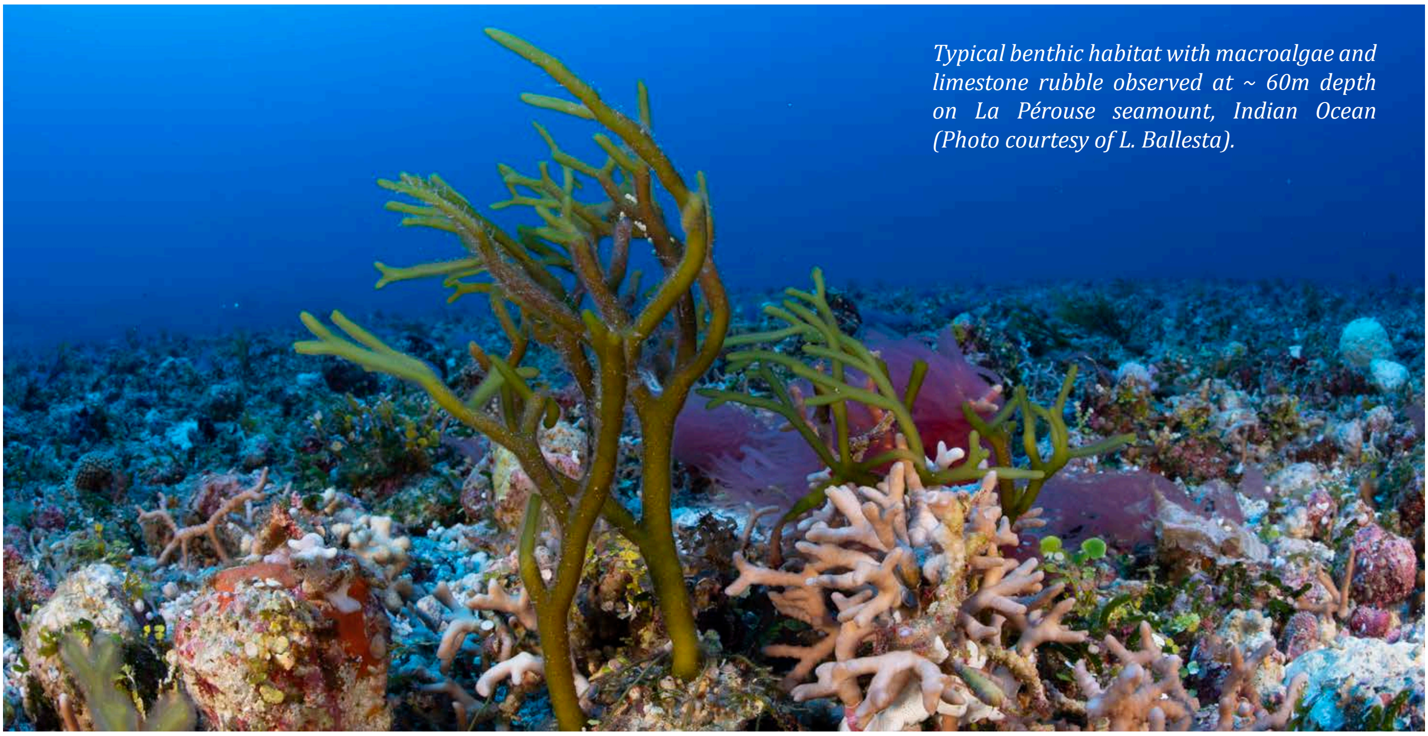

Typical benthic habitat with macroalgae and limestone rubble observed at ~ 60m depth on La Pérouse seamount, Indian Ocean (Photo courtesy of L. Ballesta).

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 65: September 1st 2020

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Lay-out
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The publication of Harmful Algae News is sponsored by the Department of Biology, University of Copenhagen

ISSN 0020-7918