

Harmful Algal Blooms in Benthic Systems: recent progresses and future research

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Highlighted captions

Ciguatera Fish Poisoning is the most frequent algal toxin-disease caused by the ingestion of fish contaminated with ciguatoxins produced by Gambierdiscus and Fukuyuo species.

A major activity of the BHAB CRP has involved both the morphological and genetic characterization of the already known and new isolated species to develop species-specific detection and quantification tests for the toxic species.

We propose additional research to determine the relative importance of other dinoflagellate, diatom and cyanobacteria taxa in BHABs over the next 10 years.

The potential expansion of benthic HABs is still uncertain, but global warming could increase the burden of ciguatera in both endemic and new areas. The potential impact of ocean acidification on BHABs is also unknown.

Progresses in analytical techniques, cell culture and functional assays offer realistic hopes for the development of reliable, cheap and fast methods for toxin screening in fish. Still, a major constraint for advance is the limited availability of analytical standards for ciguatoxins, maitotoxins, palytoxins and analogues.

There is a big need to clarify the mechanisms of toxin transfer through the food webs up to humans, and the nature of the irritative compounds associated to the Ostreopsis blooms.

Abstract

Shallow, well-illuminated coastal waters from tropical to temperate latitudes are attractive environments for humans. Beaches and coral reefs have provided lodging and food to coastal communities for centuries. Unfortunately, tropical regions traditionally have been threatened by outbreaks of the toxic benthic dinoflagellate *Gambierdiscus* associated to the Ciguatera Fish Poisoning. The ciguatoxins produced by *Gambierdiscus* are bioaccumulated in reef fishes and are responsible for the most common algal toxin-related illnesses, globally affecting the greatest number of victims and often with significant long-term health effects. Recently, *Gambierdiscus* has also been documented in new areas, in subtropical and temperate latitudes. Blooms of another benthic and toxic dinoflagellate, *Ostreopsis*, have become more frequent and intense, especially in temperate waters. *Ostreopsis* produces palytoxins and analogues and some outbreaks have been associated with massive benthic faunal damage and respiratory irritations in humans exposed to aerosols.

The scenario of more frequent harmful events and increased biogeographic extension associated with benthic microalgae incentivized the launch of a GEOHAB Core Research Project on Benthic Harmful Algal Blooms in 2010. Here we summarize the main scientific advances on the knowledge on these events with the ultimate goal of managing and mitigating their impacts on human illnesses and marine resource losses.

Introduction

The BHAB CRP was the last CRP launched by GEOHAB. In the international Open Science Meeting (OSM) in Honolulu (Hawai'i, USA) on June 2010, the participants analyzed the state of the art about benthic HABs (Figures 1 and 2) and identified the main gaps in knowledge and research priorities to advance in the comprehension of the dynamics of these events, their prediction and the management of their impacts on human health and the environment. The existing problems and uncertainties concerning taxonomy, biogeography, genetic diversity, ecology and physiology of the BHAB organisms, and sampling methods were presented in the Report GEOHAB (2012). The objectives and main questions were organized following the key research Elements of the GEOHAB Science and Implementation Plans. A distinctive section on toxins and impacts on human health and the ecosystems was also included in GEOHAB (2012). The document constituted a sound basis for the implementation of the BHAB CRP and was also presented at the First International Conference of *Ostreopsis* Development held in Villefranche-sur-mer (France) in April, 2011 (Zingone et al., 2012).

Since the launch of the BHAB CRP, the international community worked intensively to address the key questions identified in 2010. With the GEOHAB programme concluded in 2014 this is the first document produced as a global product of the BHAB CRP. It is likely not a complete summary, but it aims to show the rapid and relevant progresses made while addressing the overall goals of the BHAB CRP: to achieve a better understanding of the BHAB blooms and to provide tools to manage and mitigate the impacts of these events on

human health and the environment. We also highlight research activities that require further efforts and will benefit from co-operative international research.

Threats to human health and wellbeing posed by BHABs

CFP, the most frequent algal toxin-related seafood borne disease, is caused by the ingestion of marine fish (Figure 1) contaminated with ciguatoxins (CTXs). CTXs are produced by several *Gambierdiscus* species, which grow epiphytically, mainly in macroalgae in coral reefs. The toxins enter the food web through herbivorous fish and are subsequently bioaccumulated and biomagnified in the upper trophic levels (carnivorous fish) that are consumed by humans; however, the exact food web transfer pathway needs clarification. CTXs are neurotoxins and typical CFP intoxication is characterized by gastrointestinal, cardiovascular (bradycardia with hypotension), and neurological (cold allodynia, paresthesia, dysesthesia) symptoms that can last from weeks to months (e.g. Friedman et al., 2008, and references there in). CFP is estimated to affect globally 25,000 to 50,000 people annually, but the real incidence is not known due to under-reporting and misdiagnosis of illnesses. It is estimated that people consulting a physician would account only for 0.1% or less of intoxicated persons in the Caribbean islands and 20% of the actual cases in the South Pacific (Skinner et al., 2011). Furthermore, medical professionals often lack knowledge of CFP or do participate in voluntary notification programs (e.g. Chateau-Degat et al., 2007).

Although endemic in tropical and sub-tropical areas, increased fish trade and tourism has resulted on “imported” cases of CFP in Europe, Canada and the US (e.g. Center for Disease Control and Prevention, 2013; Mattei et al., 2014). Furthermore, CFP endogenous intoxication cases have also reported in previously unaffected areas such as the West Africa Coast, Madeira or the Canary Islands (Bienfang et al., 2008; Boada et al., 2010). Recent observations suggest expansion of the bio-geographical range of *Gambierdiscus* spp. and ciguatoxic fish (see next section "*Future trends of BHABs with climate change*").

In the tropical areas, CFP affects other aspects beyond human health (e.g. Rongo and van Woesik, 2012, and references there in). It results in: increased public health system costs; lost productivity due to illness; underutilization of valuable fisheries resources and loss of tourism revenue; cultural changes from the traditional food supply to new, imported protein sources; and requires costly monitoring and management programs.

Similarly, *Ostreopsis* constitutes a threat to human health and wellbeing (Figure 2). The potent neurotoxin palytoxin (PLTX) has been isolated in several *Ostreopsis* species (e.g. Taniyama et al., 2003; Ciminiello et al., 2010). PLTX from *Ostreopsis* was first related, although not confirmed, to dramatic seafood intoxication (clupeotoxism) in tropical areas (Randall, 2005). In the last twenty years, outbreaks of *Ostreopsis* have been increasing in more temperate waters associated to macrofauna mortality (e.g. Shears and Ross, 2009). In the Mediterranean, blooms *O. cf. ovata* in certain beaches have been related to acute respiratory irritation (rhinorrhoea, pharyngeal pain, dry cough, nose irritation) and general malaise, headache, fever ($\geq 38^{\circ}\text{C}$), eye irritation and/or dermatitis (references in

Vila et al., 2016). Most of the symptoms disappear within a few hours without specific medication when people move away from the affected area in the vicinity of the *Ostreopsis* bloom, and rarely require hospitalization. The irritative compounds are still not known. In the aerosol, molecular analyses confirmed the presence of *O. cf. ovata* cells (Casabianca et al., 2013) while only ovatoxin-a (OVTX-a, a PLTX-like toxin) was only rarely detected (Ciminiello et al., 2014). A simultaneous epidemiology and ecology study in an *O. cf. ovata* hot spot recently suggested that the health symptoms seem to occur during short periods along the bloom (Vila et al., 2016). The irritative compounds could be produced during a particular physiology stage of the cells, and certain wind and water hydrodynamic conditions could facilitate dispersion, but more deep studies are required. Fortunately, until now, no seafood intoxication has been reported in the Mediterranean. However, the detection of PLTX-like compounds levels in marine macrofauna (Aligizaki et al., 2008; Biré et al., 2015) above the recommended threshold levels established by the European Food Agency (EFSA, 2009) raised concern. Although the impact on human health is minor by now, the potential expansion of the genus in temperate waters indicates worst impacts are possible (Lemée et al., 2012). Mostly affecting touristic areas, it was estimated that, for instance, in the Provence-Alpes-Côte-d'Azur region in the French Mediterranean coast, an increase in *Ostreopsis* outbreaks could have an economic cost from several hundred thousands to several million euros, depending on the severity of the bloom (Lemée et al., 2012).

For all the above, there is a need of tight multidisciplinary collaboration of scientists, managers and public health authorities to address together the challenges posed by BHABs.

A better estimation of the incidences on human health, the coordination between the medical and the environmental monitoring, and the improved evaluation of the global costs, are among the identified priorities.

Improving taxonomy to identify organisms

A consistent taxonomy of *Gambierdiscus* and *Ostreopsis* species is fundamental to efficiently monitor their distribution trends across geography and ascertain the environmental influences on population dynamics. For these reasons, great efforts have been made to develop sound molecular methods to overcome the limitations of identifications based solely on morphological characters.

In the case of *Gambierdiscus*, comparison of ribosomal sequences has allowed Litaker *et al.* (2009) to clarify the phylogenetic relationships between *G. australes*, *G. belizeanus*, *G. caribaeus*, *G. carolinianus*, *G. carpenteri*, *G. pacificus*, *G. polynesiensis*, *G. ruetzleri*, *G. toxicus*, *G. yasumotoi*, and two ribotypes (1 and 2) that could represent new species. Screening of samples from divergent locations with these assays has provided new insights on the biogeography of these species (e.g. some are endemic to the tropical Pacific or Caribbean while others are more widely distributed). Since this date, four additional species have been newly described: *G. excentricus* and *G. silvae* from the Canary Islands (Fraga *et al.*, 2011; Fraga and Rodriguez, 2014); *G. scabrosus* from Japanese costal waters (Nishimura *et al.*, 2014); and *G. balechii* discovered in the Celebes Island (Fraga *et al.*, 2016; Figure 3). The

Gambierdiscus cf. caribaeus isolate characterized by Jeong et al. (2012) likely represents a distinct species as well. Recently, globular species of *Gambierdiscus* were transferred to a new genus, *Fukuyoa*, which now includes the former species *G. ruetzleri* and *G. yasumotoi* plus a new one, *F. paulensis* (Gomez et al., 2015).

The taxonomy of the *Ostreopsis* genus requires major revision due to the high variability of the morphology of the species described by now, and the lack of genetic characterization. Several recent phylogenetic studies supplied an increasing number of molecular clades (i.e. representing groups with a common ancestor) that may include different species that were previously identified as a single one (cryptic species; e.g. see Sato et al., 2011). According to Penna et al. (2012) the *Ostreopsis* species would be grouped in four main clades: a) *Ostreopsis cf. lenticularis* / *O. cf. labens* (in the Indo-Pacific region), b) *Ostreopsis cf. siamensis* (Atlantic, Pacific and Mediterranean isolates), c) *Ostreopsis cf. ovata* (species complex including different Atlantic, Mediterranean and Pacific isolates), and d) *Ostreopsis* spp. (including new isolates from the Mediterranean Sea and East Atlantic Ocean).. Recently, strains isolated from the Cyprus Island and Lebanon coast were identified as the new species *Ostreopsis fattorussoi* (Accoroni et al., in press).

Progress in molecular taxonomy has fostered the development of species-specific PCR assays. So far, qPCR assays exist for most of the *Gambierdiscus* species (Vandersea et al., 2012, Nishimura et al., 2016) and for *O. cf. ovata* (Perini et al. 2011; Casabianca et al., 2013; Furlan et al., 2013). Among the expected achievements, the availability of such assays applied to different kind of environmental samples (i.e. macroalgae, water, aerosol and

other substrates) will be extremely helpful for timely monitoring of target cells during harmful events.

Towards a standardized sampling method for cell abundances

The interest for the benthic HABs is relatively recent, which could explain the lack of a standard quantitative method for the estimation of cell abundance to compare data across studies. Indeed, finding the ideal sampling procedure for BHABs is not an easy task. The method should be compatible with the structural complexity and intrinsic patchiness of the benthic habitats, the diversity of substrates where the benthic cells attach and proliferate (thallic and turf algae, seagrass, sand, pebbles, rocks, coral and coral rubble), the biology of the species (which alternate planktonic and benthic stages) and the associated spatial and temporal scales of variability. At small scale, variability may be quite high, linked to local habitat characteristics (e.g. wave and light exposure, topography, substrate). At larger scale, there is a need to track the possible biogeographic and global changes of BHABs.

Direct collection of benthic substrates constitutes the most commonly used method for cell abundances estimation. Macroalgae (or coral) are collected, placed in plastic containers and shaken vigorously to remove attached cells. The released cells are then successively sieved through mesh screens to remove detritus and larger organisms before cells are preserved for counting. Cell densities are expressed as cells·g⁻¹ wet (or dry) weight algae (or coral). Substrate collection has been specially questioned concerning coral destruction

for sampling. Searching for a more environmental friendly procedure, Parsons et al. (2010) proposed the vacuum collection method, which displaces cells from the diverse substrates and capture them by filtration. However, the samples tend to contain loose substrates (sand, mud) that clog the filtration apparatus and difficult cell counts.

More recently, the use of an artificial substrate has been proposed as a standard method for sampling BHAB species (Tester et al., 2014). A fiberglass screen (e.g., 10.2 cm x 15.2 cm), easy to deploy in a non-destructive fashion allows clean sample collection in the water (Figure 4). The method is based on the observation that benthic HAB species migrate into the water column and colonize new substrates over short distances and in a relatively very short time (Nakahara et al., 1996). Thus, the benthic cells recruited on the artificial substrate over 24 hours normalized to surface area of the screen, constitute a proxy of the overall density of the BHAB cells on macrophytes or corals in the surrounding habitat. The method allows comparison of results among groups, independent of the local substrates, but it requires two trips to the sampling site. The protocol proved successful in the Pacific and the Caribbean Sea (Tan et al., 2013) and in the Mediterranean (Jauzein et al., 2016) although some refining is still on going.

In the Mediterranean, new sampling, preservation and counting methods have also been developed and tested through standardization exercises among different laboratories to facilitate the establishment of a common data basis for *Ostreopsis* in that area. This work, conducted in the framework of the European Research Project M3HABs (<http://m3-habs.net>) will be published soon.

Improved and reliable detection of toxins in *Ostreopsis* and *Gambierdiscus* cells and through the food web

The accurate characterization of toxin profiles is essential to determine the interspecific and intraspecific toxicity of BHAB species and isolates. This information can also support the use of species identification to determine the environmental risk factors for CFP or any other health risk. Currently, two of the greatest impediments to progress include the lack of: i) publicly available analytical CTX, PLTX-like compounds (ovatoxins and ostreocins) standards and ii) cheap and reliable screening tests for these toxins.

In the last years, major advances have occurred simultaneously on analytical technology (e.g Caillaud et al., 2010), cell culture techniques and functional assays such as the mouse neuroblastoma cell line N2A and the receptor-binding assay (RBA). Some preliminary data about species-specific *Gambierdiscus* toxicity have been obtained by a new, rapid extraction procedure, which facilitates the simultaneous detection of the lipid-soluble CTX and water-soluble maitotoxin (MTX) fractions obtained from *Gambierdiscus* extracts, and validated by liquid chromatography mass spectrometry (LC/MS) and functional bioassay (Lewis et al., 2016). Based on the combination of these and other methods, CTX and MTX have been clearly detected in *G. polynesiensis* and *G. scabrosus* in the Pacific, and *G. excentricus* in the Caribbean/Atlantic; these species would likely pose the main concerns for toxin transfer into the marine food webs where detected (Chinain et al., 2010a; Rhodes et al., 2014; Fraga et al., 2011; Nishimura et al., 2014).

Furthermore, Hardison et al. (2016) developed a fast (<3 hours to complete), non-radioactive, cost effective fluorescence based receptor binding assay (RBA_(F)) for screening fish samples for CTXs. The assay is stable over long periods of time, is compatible with common extraction methods for CTX and most fluorescence plate readers, and it could be incorporated into routine CTX monitoring programs in the CFP endemic areas. It requires relatively little (although costly by now) purified toxin standards.

With the latest advances in liquid chromatography-high-resolution multiple-stage mass spectrometry (LC-HRMS/MS) analytical technologies, new PLTX analogues have been described. García-Altare et al. (2014) identified OVTX-g and isobaric PLTX in strains isolated from the south of Catalonia (NW Mediterranean) and Tartaglione et al. (2016) four new OVTXs (i, j₁, j₂ and k) in the *Ostreopsis fattorussoi* from Cyprus. This new species exhibited very low total toxin content (0.06-2.8 pg·cell⁻¹) compared to the values reported on *O. cf. ovata* Mediterranean strains (10 to 75 pg·cell⁻¹; e.g. Séchet et al., 2012, see more references in Accoroni and Totti, 2016). Usually, OVTX-a is the dominant form. The active research on the modulation of cell toxin production by different physiology and environmental factors is rapidly providing new data (e.g. Pezzolesi et al., 2016).

Refined analytical methods are also necessary to ascertain the toxin transfer processes through the food webs and the impacts on the macrofauna. Progresses are going slowly for CTX (e.g. Mak et al., 2013; Silva et al., 2015) and PLTX-like compounds (Biré et al., 2015; Brissard et al., 2014). Ecotoxicology tests are revealing the distinctive sensitivity of model organisms to these toxins (e.g. Giussani et al., 2015). Pathological inflammatory responses

in tissues and organs have been observed in mussels (Carella et al., 2015). In natural populations of the Mediterranean sea urchins *Paracentrotus lividus*, *Ostreopsis* causes reproductive impairments and developmental anomalies in the progeny which are visible several months after the bloom (Migliaccio et al., 2016).

Understanding habitat preferences and ecological links

BHABs occur in relatively shallow waters, where microalgae attach to different substrates by producing mucopolysaccharide filaments and mucous layers (e.g. Honsell et al., 2013). At small scale, these habitats are highly variable. Water motion may be often intense although calm conditions often favor the blooms. Usually irradiance is high, but the microalgae can also find protection within the mucous matrix or the macroalgae structure. *Gambierdiscus* blooms occur in tropical waters, but the controlling role of temperature is not clear in the case of *Ostreopsis* thriving in more temperate coasts. Efforts have been focused on understanding the physiological adaptations of these organisms to variations in physical, chemical and biological conditions, which limit or promote their growth and toxicity. So far, the research shows species-specific responses, and only some aspects are summarized here. Overall, *G. caribaeus*, *G. carpenteri* and *G. pacificus* would be more tolerant to variable environmental conditions than other species tested (*G. australes*, *G. belizeanus*, *G. carolinianus*, *F. ruetzleri* and ribotype 2; Xu et al., 2016). Such comparison among *Ostreopsis* species has not been made yet.

Concerning *Gambierdiscus* species, in the laboratory optimal growth would occur on temperatures between 21.0 to 32.5 °C and salinities between 20 and 45, with growth rates up to 0.3 (rarely higher) divisions day⁻¹ (Kibler et al., 2012; Yoshimatsu et al., 2014; Tawong et al., 2016; Xu et al., 2016). In the Caribbean, *Gambierdiscus* cell abundances were positively correlated with temperature (Tester et al., 2010).

In the case of *Ostreopsis*, optimal growth conditions in the laboratory are found between 22 °C and 30 °C, but it is not clear a direct correlation with temperature. In the northern Mediterranean (Mangialajo et al., 2011), the higher cell abundances mostly occur in mid-summer (end of July) in the NW basin and in late summer-early (September-October) fall in the Adriatic. However, data on three consecutive years (2007-2009) from 14 sites, showed a poor correlation between cell densities and seawater temperature, with maximal abundance periods appearing to be site- and year-specific. In the NW Adriatic Sea, the highest abundances of *O. cf. ovata* were always recorded when temperature was decreasing from 25 °C to 20 °C (Accoroni and Totti, 2016). Cohu et al. (2011) suggested the the existence of distinct patterns in bloom timing in the NW Mediterranean Sea corresponding to very different hydroclimatic scenarios, and especially, spring weather. Temperature windows (23 °C - 27.5 °C) allow *Ostreopsis* growth in the French Mediterranean coast where the first epiphytic cells were detected at 13.0 °C and high abundances (>10,000 cells g⁻¹ of macroalgae fresh weight) began to occur at 18 °C. Southwards, in the Catalan coast, bloom burst usually starts at the end of June (Vila et al., 2016), after a maintained increase in temperature, above a threshold of 20 °C in the air and the water and wind intensities below 3 m·s⁻¹ (on average). In the Atlantic coast of the Iberian Peninsula, absolute

temperature cannot alone explain the *Ostreopsis* distribution (David et al., 2012); three continuous months with sea surface temperature above 19.5 °C, could be necessary for *Ostreopsis* to be present in that area.

The role of salinity in *Ostreopsis* growth and bloom development is still unclear. Again, laboratory experiments report species-specific and strain-specific responses to salinity ranges. In the field, maximum cell abundances are found at salinities of 30 to 39, and very low in the river plumes (salinity values 25-35; e.g. Delgado et al., 2006; Blanfuné et al., 2015). However, because low salinities are associated with land runoff and potential nutrient supply, discrimination of the individual role of these two factors is difficult.

The limited available data do not allow drawing a clear causal relationship between inorganic nutrient supply and *Ostreopsis* sp. blooms (GEOHAB, 2012). In their conceptual model, Accoroni et al. (2015) suggested that the onset of the bloom in the northern Adriatic Sea would depend on an increased supply of PO₄ (and low N:P ratios) concurrently to temperatures above 25°C and calm waters. *Gambierdiscus* blooms do not usually occur in eutrophic environments. The nutrients required to reach bloom concentrations are likely acquired directly from the nutrient rich sediments, or directly from the macroalgal surfaces (Litaker et al., 2010 and references therein). Furthermore, it is hypothesized (but not tested) that nutrient inputs, which favor the growth of macroalgae (Lapointe et al., 2010), could also promote blooms.

The role of irradiance in *Ostreopsis* blooms dynamics is also difficult to separate from the preferential depth, the possibility of the cells to avoid photo-damage under the protection of the macroalgae host thallus, or to select relatively calm environments to avoid high sun exposure. Furthermore, the available experiments are not conclusive and often contradictory as reviewed in Accoroni and Totti (2016). In the case of *Gambierdiscus*, for all strains tested growth was higher at 110-400 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ than at 55 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$.

As an adaptation to benthic life, in addition to flattened shapes, both *Gambierdiscus* and *Ostreopsis* produce a mucopolysaccharide matrix to attach to the substrates. The internal organelles related to the mucus production and extrusion, trichocysts and mucocysts (Figure 5) were described in *Gambierdiscus* (Durand-Clément and Conté, 1991) and *Ostreopsis* (Honsell et al., 2013; Escalera et al., 2014). However, the exact role of these unique structures (e.g. attachment, defense) is not clear in these dinoflagellates.

Finally, interesting new research has been initiated to determine the allelopathic relationships between *Ostreopsis* spp. and microphytobenthos and macroalgal substrates and to characterize the related bacterial assemblages (e.g. Blanfuné et al., 2015; Accoroni and Totti, 2016 and references there in; Vanucci et al., 2016).

Investigating life cycle of benthic dinoflagellates to better understand bloom dynamics

For many dinoflagellate species, life cycle is known to play a key role in bloom formation (e.g. Díaz et al., 2014). Currently we are just in the beginning stages of understanding *Gambierdiscus* and *Ostreopsis* life cycles sufficiently to address this issue. Meiosis and gametogenesis, and the occurrence of division processes in both the sexual and asexual cycle in *Gambierdiscus* were described by Bravo et al. (2014). Still the exact occurrence of mitosis and meiosis is unclear. *Ostreopsis* species exhibit a high morphological variability that could correspond to different life cycle phases and processes, although the exact role have not been established yet). Sexual and asexual reproduction, temporary and resting cysts have been observed both in laboratory and field samples (Bravo et al., 2012; Accoroni and Totti, 2016). Big (and dark) cells would be related to less favorable proliferation conditions (e.g. nutrient limitation, excessive hydrodynamism) and/or to the stationary phases of the bloom. However, the role of the small cell forms is difficult to assign because they are found in different phases of the blooms.

Exploring new BHAB events producing organisms

GEOHAB (2012) noticed the uncertainties regarding the toxicity of other benthic genera including other dinoflagellates (*Coolia* spp., *Prorocentrum lima*), diatoms (*Nitzschia navis-varingica*, *Amphora coffeaformis*) and cyanobacteria. In the case of *Coolia*, a taxonomic

confusion led to the assumption that *Coolia monotis* is a toxic species (Holmes et al., 1995). At the time of that publication, *C. monotis* was the only described species of the genus but it was shown later that the toxic strain in that study actually belonged to *Coolia tropicalis*. Numerous strains of *C. monotis*, were morphologically genetically and toxicologically characterized, and no toxins were detected (Penna et al., 2005).

Several cyanobacteria species, *Hydrocoleum glutinosum*, *Phormidium laysanense*, *Spirulina weissii*, *Oscillatoria cf. bonnemaisonii*, *Anabaena sp.* and *Trichodesmium erythraeum* were associated to atypical CFP incidents in New Caledonia, French Polynesia and Vanuatu (Laurent et al., 2012) following the ingestion of giant clams and/or fish from lower trophic levels. Toxicological studies conducted on cyanobacterial mats and/or the molluscs collected from contaminated locations using RBA, cell-based assay and LC-MS/MS confirmatory analysis suggest the presence of complex toxin suites in these organisms including CTXs-like compounds, paralyzing toxins, PLTX, anatoxin-a and homoanatoxin-a (Kerbrat et al., 2011; Méjean et al., 2010). Recent observations from French Polynesia have also pointed out similar potential health risks linked to the consumption of the gastropode *Tectus niloticus* (Chinain et al., in prep.). A new ecotoxicological phenomenon is proposed, "Ciguatera Shellfish Poisoning" (CSP) and monitoring cyanobacteria is now recommended, concurrently with other toxic microalgae in order to effectively manage tropical seafood poisonings.

Future trends of BHABs with climate change

As noticed also for other harmful algae, benthic HAB species and events are apparently increasing in both temperate and tropical regions (Parsons et al., 2012; Kibler et al., 2015). This could be due on one side to more effective detection through improved observation and monitoring capacities; on the other, to anthropogenic forcing and global climate change.

The genus *Gambierdiscus* has a pan-tropical distribution between 35°S and 35°N (see map distribution e.g. in GEOHAB, 2012). Recent observations also indicate expansion of *Gambierdiscus* to temperate areas is now effective (Jeong et al., 2012). The possibility of range expansion in certain areas was suggested by Tester et al. (2010) who found positive correlations between water temperature and the abundance of different *Gambierdiscus* species. Also, Gingold et al. (2014), found associations between climate variability and CFP incidence and suggested that, provided that other (complex) socio-environmental factors remain, climate change could increase the burden of CFP. These findings have implications for disease prediction, surveillance, and public health preparedness in view of the ongoing climate change. The situation could be aggravated by other natural (hurricanes, coral bleaching episodes or *Acanthaster planci* outbreaks) and anthropogenic (dredging, construction or sewage discards in touristic coastal areas) disturbances (Figure 1) also fostering the increase of CFP outbreaks in the endemic areas (Chinain et al., 2010b; Rongo and van Woesik, 2012).

The *Ostreopsis* genus is present in tropical and temperate coasts of Australia, the Indian Ocean, eastern Asia, the Caribbean sea, Brazil and, in the last decade, in colder waters of the North Pacific coasts of Japan and Russia, New Zealand, Portugal, and the Atlantic coasts of France and Spain (*e.g.* see references in Parsons et al., 2012 and Accoroni and Totti, 2016). In the last 20 years *Ostreopsis cf. ovata* has been blooming recurrently in some locations in the northern and southern Mediterranean (*e.g.*, Mangialajo et al., 2011; Illoul et al., 2012).

It is unclear yet, how global warming could affect the possible expansion and intensification of *Ostreopsis* blooms. As noticed earlier neither the physiological studies in the laboratory nor the field observations allow clarification of the modulation of the blooms by this environmental factor. In addition, variations in sea temperature could have a greater impact on hydrography (*e.g.* wave intensity and currents), which may lead to indirect and hardly predictable consequences for the abundance and distribution of benthic species.

The potential effects of ocean acidification on BHAB events require investigation. The few available data by now suggest that direct effects are instead unlikely. At a site with volcanic CO₂ emissions of the Ischia Island (Mediterranean Sea) *Ostreopsis cf. ovata* was recorded at bloom concentrations at pH values similar or even lower than those predicted for the 2100 coastal ocean (Di Cioccio et al., 2014). However, ocean acidification could exert indirect effects on BHABs, *e.g.* through the demise of coral reef and their replacement by macroalgal-dominated systems, which are more prone to BHABs.

Finally, an additional concern is how the new environmental conditions of temperature, pH, hydrodynamic regimes under the climate change, could affect toxin production of BHAB organisms.

Conclusions and Next steps

Many advances have occurred in a very short period of time since the launch of the BHAB CRP of GEOHAB and have been briefly summarized here. Still many of the questions under each GEOHAB Element as formulated in the GEOHAB (2012) Report require further research, and many of them have already been noted in the former sections. In addition, future studies should address two main elements of GEOHAB: the comparative approach and modeling. The comparative approach can contribute to understand similarities and differences concerning CFP incidences and *Gambierdiscus* dynamics in the main affected areas, i.e. the Pacific and the Caribbean. Comparing the dynamics of *Ostreopsis* and *Gambierdiscus* can also shed light on poorly known ecological aspects related to the benthic way of life and facilitate the design of effective management strategies in each case. For this purpose, modelling BHABs dynamics will be a useful tool, mainly limited nowadays by the scarce parametrization of many biological and physical processes at small scale in the complex benthic ecosystems. Furthermore, long time temporal series of data on BHAB events and health impacts are necessary.

The progresses on benthic HABs research have greatly benefit from the international and multidisciplinary coordination fostered by GEOHAB. This will continue under the umbrella of the new programme GlobalHAB with the final objective to cope with harmful algal blooms.

Legends

Figure 1. Anthropogenic disturbances such as A) dredging and B) dock constructions are factors likely to favor the proliferation of the ciguatera-causing dinoflagellate *Gambierdiscus* (C, scale bar: 10 μm) within macro-algal mats colonizing dead coral substrates (D). Ciguatera represents a serious impediment to the development of coral reef fish trade in the tropics (E).

Figure 2. Optical microscopy image of *Ostreopsis* cf. *ovata* cells (A; scale: 20 μm) accumulated at surface during a bloom (B) in the summer 2014, in Sant Andreu de Llavaneres beach (NW Mediterranean). Underwater, the mucous carpet containing *Ostreopsis* cells cover the macroalgal community (C). Detail of the cell aggregates released from the benthos in Villefranche-sur-mer (South of France). Credits: E: Berdalet and M. Vila (A, B, C) and C. Jauzein (D).

Figure 3. Scanning Electron Microscopy image of *Gambierdiscus balechii* from the Celebes Sea. Scale bar: 20 μm .

Figure 4. Artificial substrate method deployed (A; credit: S. Kibler, NOAA) and detail of the attached *Gambierdiscus* cells (B; credit: M. Vandersea, NOAA).

Figure 5. Ultrastructure (longitudinal sections) of *Ostreopsis cf. ovata*. Whole cell (A) and detail (B) of the internal canal (Ca) surrounded by mucocysts (M) and trichocysts (T), located in the sulcal area of the cell (upper left corner in A). From Escalera et al. (2014), with permission.

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Figure 1

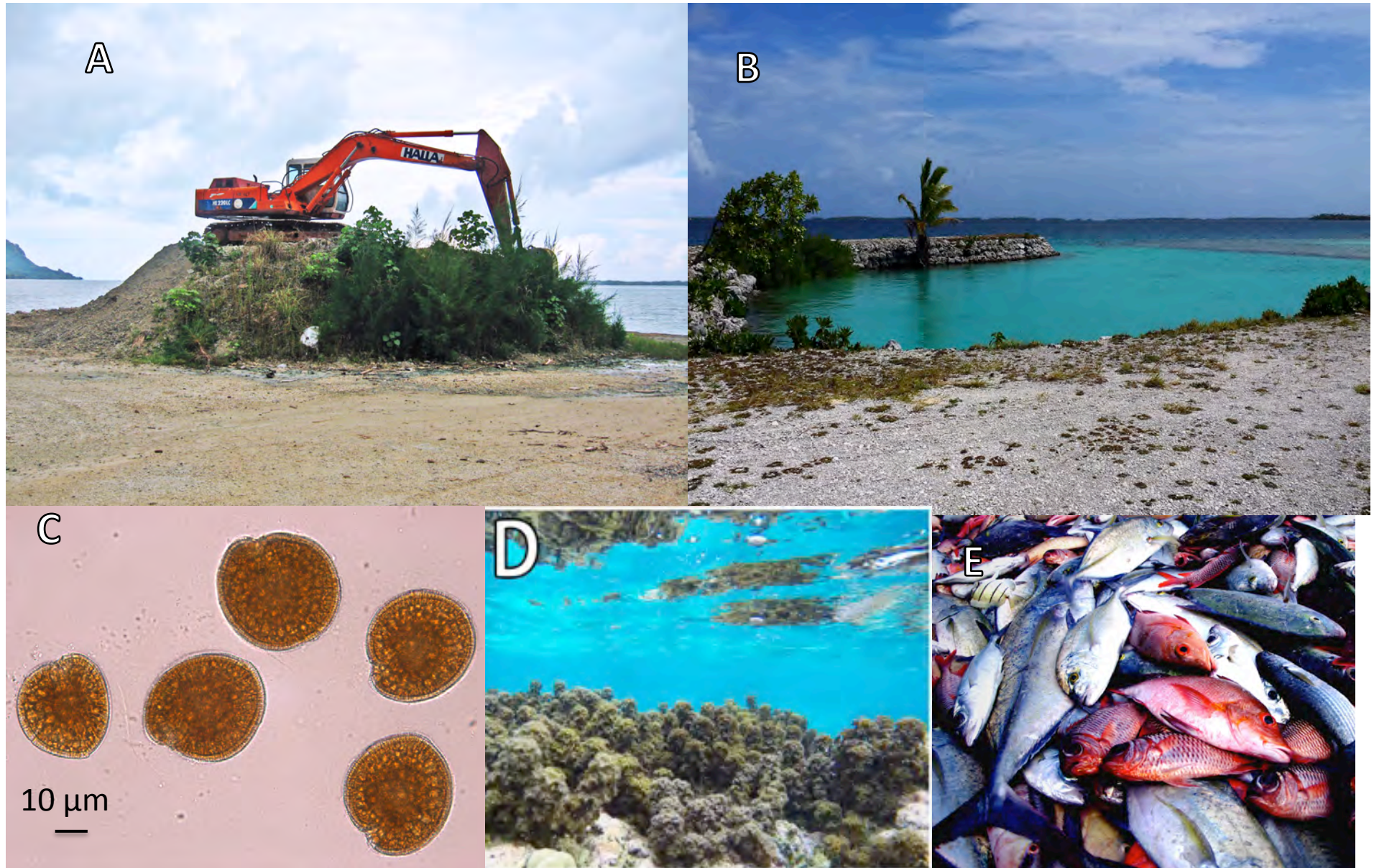


Figure 2

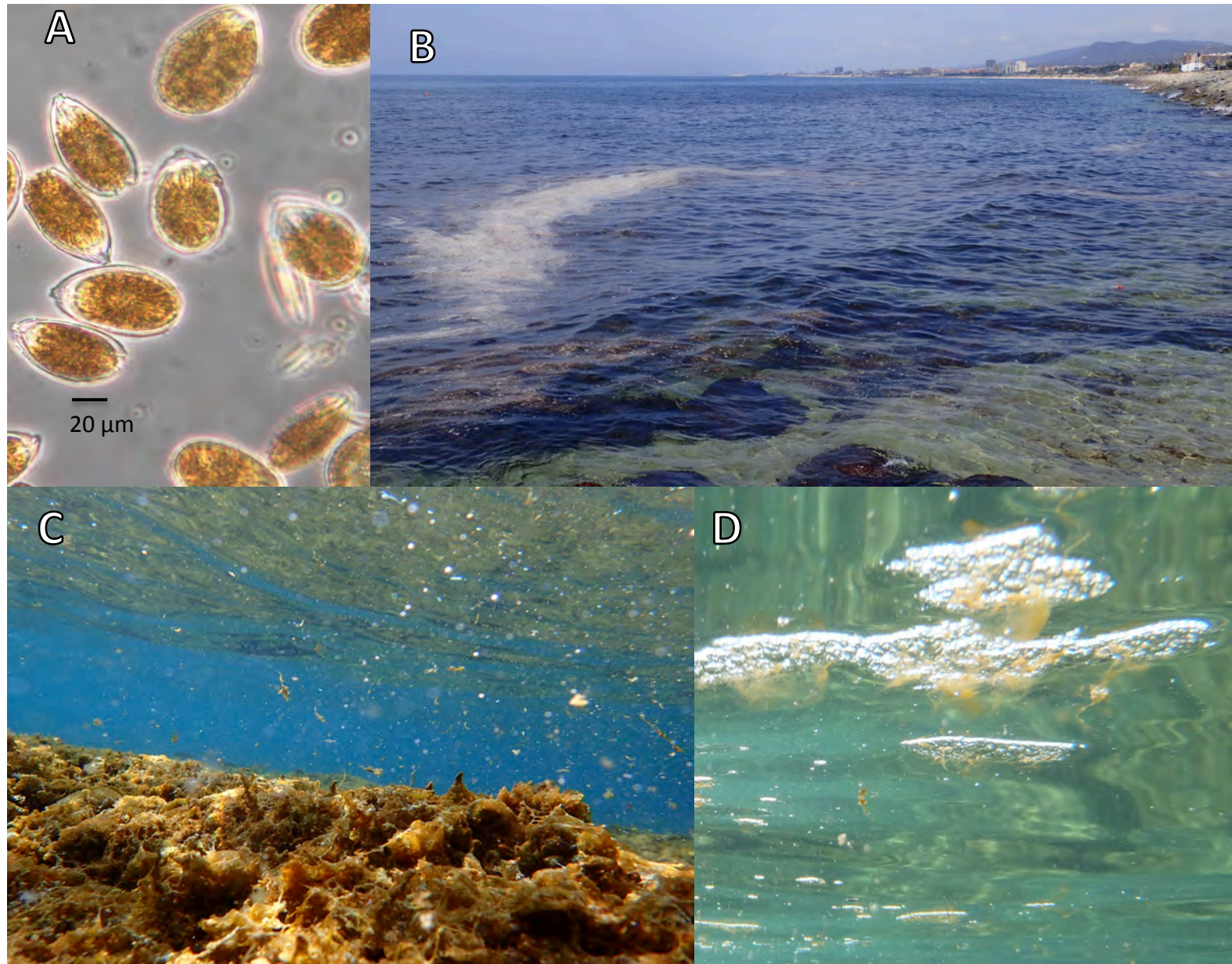


Figure 3

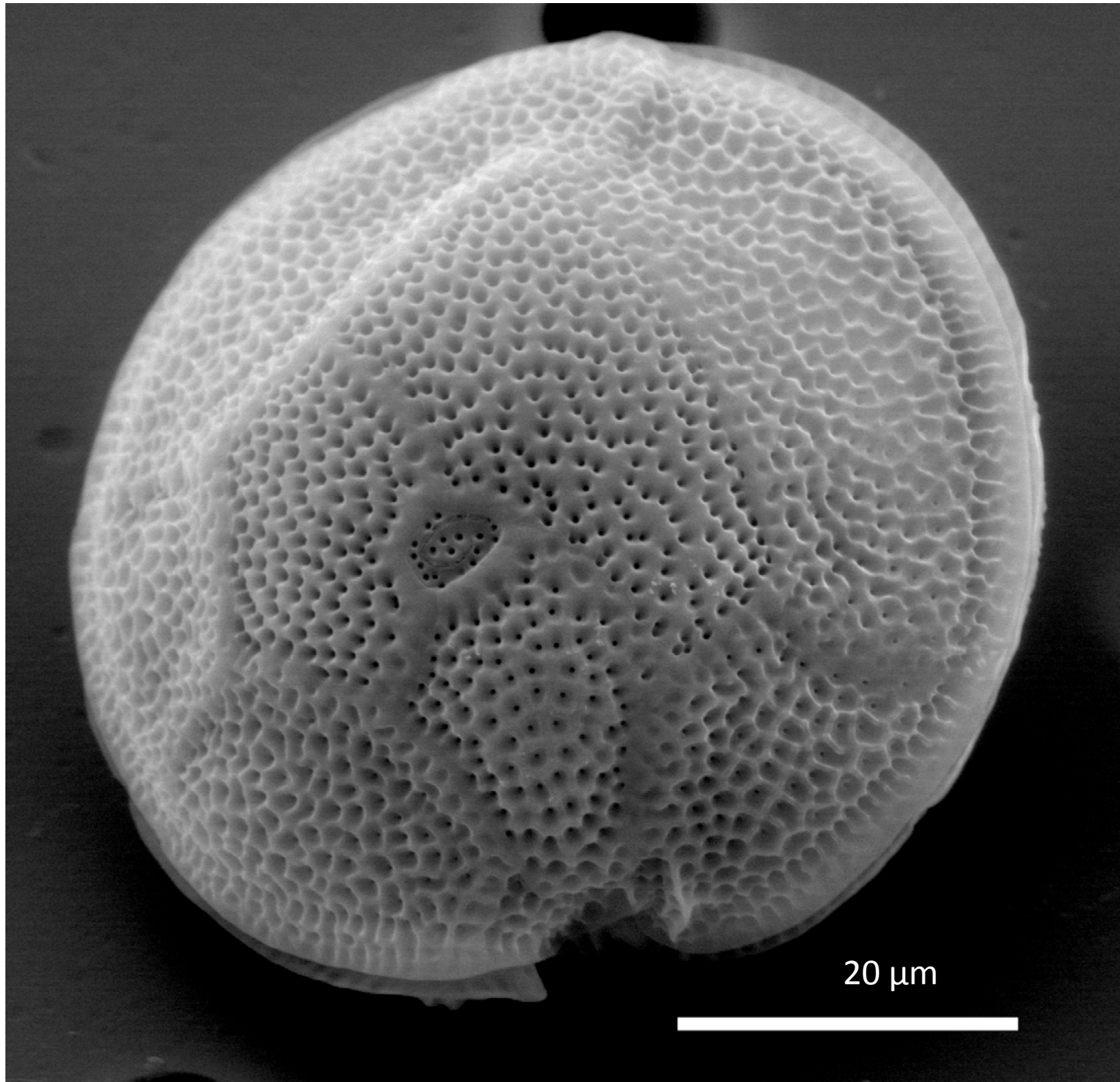




Figure 4

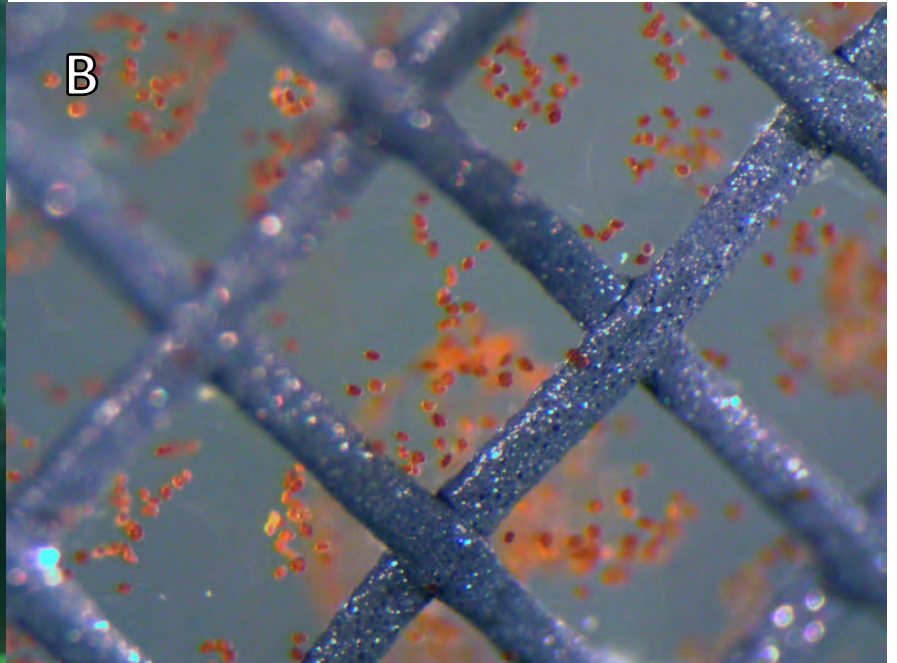


Figure 5

