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Diversity and toxic potential of algal bloom-forming species from Takaroa lagoon (Tuamotu, French Polynesia): a field and mesocosm study

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ABSTRACT: Pearl farming lagoons are economically important, yet poorly studied ecosystems in French Polynesia. This paper describes a study conducted in 2016 in Takaroa (Tuamotu Archipelago), an atoll recurrently affected by harmful algal bloom (HAB) events. The objectives were to gain insight into phytoplankton community composition, identify the main bloom-forming species and investigate their potential for toxicity. A mesocosm approach was used to assess the response of phytoplankton communities to 3 nutrient treatments: Conway with Si, Si-depleted f/2 and a commercial N–P fertilizer. In total, 87 morpho-species were described from Takaroa lagoon, with dinoflagellates as the most diverse group. Diatoms (*Extubocellulus* sp., *Cylindrotheca closterium*, *Nitzschia* spp.), dinoflagellates (*Gymnodinium* spp., *Heterocapsa* spp.) and flagellates (*Cryptomonas* sp., *Pyraminonas* spp.) were among the major bloom-forming species identified. Most markedly, *Extubocellulus* sp., a diatom never reported from French Polynesia before, was able to bloom even in Si-poor environments. Additionally, *in vitro* cultures of 12 bloom-forming strains were successfully established and tested for their toxicity. Preliminary results suggest that 9 strains, including dinoflagellates (*Prorocentrum lima*, *Amphidinium* spp., *Heterocapsa* sp.), Pyraminonadales (*Pyramimonas* sp.) and cryptophytes (*Cryptomonas* sp.), are the likely producers of cyclic imine neurotoxins and toxins acting on voltage-gated sodium channels. The contribution of these toxins to the mortality events previously reported in Takaroa lagoon is further discussed. Overall, this study highlights the relevance of a mesocosm approach which can be applied to other understudied atolls of French Polynesia recurrently threatened by HABs.

KEY WORDS: Phytoplankton · Taxonomy · Mesocosm · Bloom-forming species · Toxicity · French Polynesian lagoons

1. INTRODUCTION

French Polynesia is the world's top producer of cultured black pearls. *Pinctada margaritifera* farming represents the second highest source of income for the country and the main activity in 26 atolls and 4 islands. This pearl industry was very successful in the early 1980s but has been confronted with an important crisis over the past 10 yr, a situation partially due to massive mortalities in *P. margaritifera* livestock, along with poor management and control of black pearl distribution at the international level (Andréfouët et al.

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2012). Human-induced environmental changes as a consequence of overcrowding/overproduction, combined with the effects of climate change are believed to be one of the major causes of the decline in black pearl activity. However, their true ecological impact remains poorly understood given the large diversity of geomorphologic, geographic and climatic conditions as well as varying farm densities in atoll lagoons. Among these potential ecological impacts, one major issue is the increasing occurrence of algal blooms.

Algal bloom events, often referred to as harmful algal blooms (HABs) when their development is accompanied by adverse effects, have arguably expanded worldwide since the 2000s (Van Dolah 2000, GEOHAB 2001). It is believed that their higher frequency, duration and severity are a consequence of degradation in water quality, intense aquaculture and/or climate change (Heisler et al. 2008, Hallegraeff 2010, Anderson 2014, Paerl et al. 2016).

The Tuamotu Archipelago lagoons, renowned as a black pearl farming hotspot, are subject to periodic HAB events (called 'vaitīa' by local people), with serious societal, economic and environmental consequences (Table S1 in the Supplement at www.int-res.com/articles/suppl/a083p015_supp.pdf). For example, the 2013–2014 HAB event that affected the Takaroa lagoon was particularly intense, long and damaging, resulting in fish mortality and, most importantly, ≥60% mortality of the pearl oyster spat collection, a reduction of growth rates in surviving oysters and the closure of most farms, which had severe socio-economic consequences (Fougerouse et al. 2014). During the 2014 event, unusual climatic conditions were reported, e.g. the striking absence of wind and higher seawater temperatures for a long period of time. In addition, numerous spillways blocked by coral rubble and sand have likely limited the ocean–lagoon exchanges. Weather anomalies, such as long periods of calm weather associated with a long cyclonic period, especially during summer, could be the catalyst for algal bloom developments in the Tuamotu lagoons (Adjeroud et al. 2001). They are usually preceded by long periods of drought coupled with higher temperatures and reduced lagoon–ocean exchanges (Alberteau 1998). Closed atolls appear to be more vulnerable, but atolls with passes can also experience such events if the winds and swells are very low (Andréfouët et al. 2015) or if the pass is narrow and the lagoon is protected from the prevailing waves, as is the case in Takaroa atoll (Van Wynsberge et al. 2017). Algal blooms are natural phenomena which are not always linked to eutrophic conditions. Indeed, other factors such as hydrodynamics (e.g. vertical mixing, ocean–lagoon exchanges) and the effects of climate change are also important HAB-driving factors. In the case of Takaroa, however, the overexploitation of the lagoon for pearl farming, which may result in excessive production and release of organic matter into the water column, and consequently increased nutrient concentrations, could likely act as an aggravating factor.

Since 1990, numerous research projects have focused on the environmental, technical and socio-economic implications of pearl farming in French Polynesia, in order to ensure better sustainability and management of this highly lucrative activity (Gueguen et al. 2016 and references therein). In contrast, very few studies have actually dealt with phytoplankton communities (Ricard 1986, Delesalle 1990, 1994, Delesalle et al. 2001, Thomas et al. 2010, Fournier et al. 2012, Henry 2016), let alone the species involved in HAB events (Harris & Fichez 1995, Adjeroud et al. 2001). Indeed, except for species involved in ciguatera fish poisoning, the taxonomic composition and potential toxicity of species involved in HAB events in French Polynesian lagoons still remain poorly documented. There are several reasons for this knowledge gap, such as the episodic and stochastic nature of HABs and the lack of field infrastructure and trained staff on these often isolated atolls, but also the delay between bloom initiation and the alert sent by the local population to both local authorities and scientists.

Following the 2013–2014 dramatic algal bloom event in Takaroa, the EFFLOREX project (EFFLORescences algales dans les lagons EXploités de Polynésie) was launched in 2014 to assess the likely causal connections between nutrient inputs and HAB occurrence, and the potential impacts on black pearl farming and/or human health. Here, we present the results of a field-based mesocosm study conducted in Takaroa atoll in March 2016 with 3 main objectives: (1) to estimate the diversity of phytoplankton communities in this lagoon; (2) to follow up the emergence of algal species in nutrient-enriched mesocosms, focusing primarily on bloom-forming species; and (3) to examine the potential toxicity of several of these bloom-forming species.

2. MATERIALS AND METHODS

2.1. Study site: Takaroa lagoon

This study was conducted from 9–22 March 2016 (during the austral summer) in Takaroa atoll, located

at 14° 28′ S, 144° 59′ W in the Tuamotu Archipelago (Fig. 1A). Takaroa lagoon is 27.4 km long and 7 km wide (total surface of 85 km^2, volume of 2.2 km^3) with a mean depth of 26 m and a maximum depth of ca. 47 m (Le Gendre et al. 2010). This atoll is also characterized by a deep passage to the ocean (Teauonae Pass) located on the southwest part of the atoll, and several reef-flats (called 'hoa' by local people) along the reef rim. The average water renewal time is estimated to be 76 d (Andréfouët et al. 2001). The climate is tropical with an average rainfall of 1219 mm and an average temperature of 27.6°C. The hot and wet season takes place between November and April, and maximum precipitation occurs in December–January. Wind periods are episodic throughout the year, with prevailing easterly trade winds being stronger from April to October.

2.2. Mesocosm setup and experimental design

Three large mesocosms were deployed in the southeastern part of the Takaroa lagoon (Fig. 1B). This site was chosen for several reasons: (1) local people classed it as a 'frequent HAB area'; (2) the site contains a high diversity of phytoplankton species, as evidenced by preliminary field observations conducted in 2015 in the area (Henry 2016); (3) there is low exposure to oceanic influence; (4) it is shallow in depth (13 m), which allows proper stabilization of the mesocosm enclosures from the bottom and easy sampling; and (5) its proximity to a field laboratory which was set up for this study.

The mesocosms (Insinööritoimisto Haikonen Oy company) consisted of large transparent bags (~9 m^3) made of polyethylene strengthened by nylon meshing in between and fitted with sediment traps at the bottom (Fig. 1C). The bags were supported by a flotation frame to maintain the mesocosms in an upright position 50 cm above the sea surface to avoid dilution and potential contamination by lagoon water. Below the surface, each mesocosm bag was rigidified and kept wide open by 3 PVC rings.

The 3 mesocosms (M1, M2 and M3) were filled with lagoon seawater by sinking them into the water column and lifting them up slowly, and then left to stabilize overnight. On the following day (Day 0), each mesocosm was enriched with a different mixture of macro- and micronutrients and immediately thoroughly stirred with a paddle, to test a possible causal link between HAB events previously reported in Takaroa lagoon and increased nutrient concentrations as a result of farming activities. Three distinct nutrient enrichment regimes were tested: M1 was enriched once with Conway medium with silicates to promote diatom growth (Tompkins et al. 1995); M2 received Si-depleted f/2 medium (Guillard 1975) in order to favor the development of non-siliceous organisms; and M3 was amended twice with a commercially available N–P fertilizer solution with ammonium as the N source (Earth Juice®; Hydro-organics™), on Days 0 and 4, respectively, to avoid nutrient depletion. This third enrichment condition was tested in order to mimic episodic nutrient inputs associated with fertilizer runoff or sewage. The initial nutrient concentrations in the mesocosms following the enrichment step are given in Table 1. Each mesocosm was then monitored on a daily basis over an 11 d period.

In most mesocosm studies, the use of 3 or more replicates per tested condition is usually required in

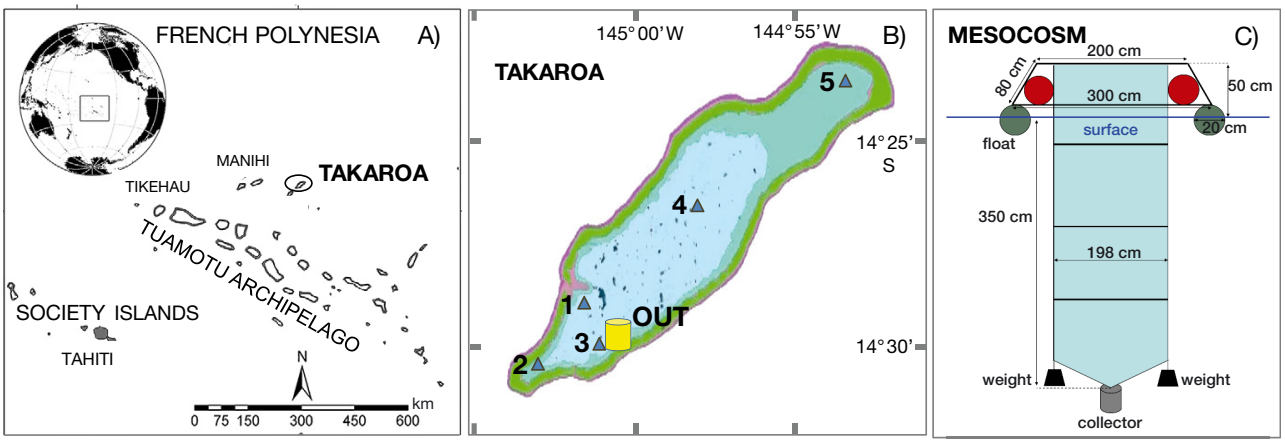

Fig. 1. (A) French Polynesia and (B) Takaroa atoll showing the location of Stn OUT and the deployment site of mesocosms (yellow cylinder) of March 2016 study. Stns 1 to 5 are the sampling locations from the 2015 study (Henry 2016); (C) schematic drawing of the mesocosm bags deployed in March 2016

Table 1. Initial concentrations of nutrients (µmol l^{-1}) and elemental ratio (mol:mol) outside (Stn OUT) and within the mesocosms (M1, M2 and M3) right after addition (Day 0). For M3, the enrichment regime consisted of 2 successive amendments at Day 0 and Day 4 (values in parentheses)

	PO_4^{3-}	NO_x	NH_4^+	$Si(OH)_4$	N:P	N:Si
Stn OUT	0.20	0.06	0.01	1.9	<1	<1
M1(Conway + Si)	4.84	35.11	0.08	249.9	7	<1
M2 (f/2 – Si)	1.10	29.91	0.06	2.2	27	13
M3 (NP fertilizer)	0.38	0.23	8.88	2.0	24	5
	(0.49)	(0.30)	(6.90)	(2.5)	(14)	(3)

the experimental design (Lalli 2012). Here, due to logistical and cost issues inherent in conducting such experiments at a location with poor accessibility, such a volume of experiments, including daily sampling in the mesocosms, would have been impossible to manage. Therefore, only one mesocosm was set up per nutrient treatment, since the primary objective of these experiments was not to conduct a comprehensive study of the effect of nutrients on phytoplankton dynamics, but rather to test different scenarios likely to trigger the growth of a variety of bloom-forming species. Likewise, no control mesocosm (i.e. a mesocosm with no nutrient addition) was set up; instead, water samples collected daily in the lagoon nearby the mesocosms (referred to as Stn OUT) were used as a reference, reflecting the ambient conditions in the lagoon. Due to this lack of replicates and control, no statistical analyses were performed on the data derived from these mesocosm experiments.

2.3. Profiling and sampling

Stn OUT and the 3 mesocosms were monitored daily for nutrients, chlorophyll and phytoplankton enumeration and identification over an 11 d period. Water subsamples were collected every morning (between 08:00 and 11:00 h) at 2 m depth with a 5 l Niskin bottle. At the end of the study period, the total amount of water removed from each mesocosm was estimated to be less than 2% of the initial volume. In addition to discrete sampling, temperature, conductivity and pH profiles were performed every day inside and outside the mesocosms using a YSI probe.

2.4. Nutrient concentrations

Ammonium (NH_4^+) was analyzed immediately by fluorometry using a Turner Trilogy fluorometer (module #7200-041) as described in Holmes et al. (1999). Nitrate + nitrite (NO_x), phosphates (PO_4^{3-} or dissolved inorganic phosphate, DIP) and silicate ($Si(OH)_4$) samples were $HgCl_2$-poisoned and analyzed within 2 months at the LAMA-IRD laboratory (New Caledonia) by standard colorimetric methods (Aminot & Kérouel 2007), using a segmented flow analyzer AA_3 (SEAL Analytical). Dissolved inorganic nitrogen (DIN) refers to the sum (NO_x + NH_4^+).

2.5. Assessment of phytoplankton biomass and taxonomy

2.5.1. Chlorophyll *a*

Chlorophyll *a* (chl *a*) concentrations were assessed daily by fluorometry after methanol extraction (Le Bouteiller et al. 1992), using a Turner Designs fluorometer equipped with module #7200-040 (chl *a* extracted-acidification) and calibrated with pure chl *a* standard (Sigma). Total chl *a* concentrations were determined from 0.25 l water samples filtered onto ~0.7 µm GF/F Whatman filters. In parallel, size-fractioned chl *a* was assessed from 0.50 l water samples filtered separately through 2 and 10 µm nucleopore filters. For the highest biomasses (>10 µg l^{-1}), the percent contribution of each fraction should be considered with caution due to the clogging of GF/F and 2 µm filters.

2.5.2. Identification and enumeration of nano- and micro-phytoplankton species

This study focused primarily on a description of the nano- and micro-phytoplankton communities and their dynamics during a bloom event. To this end, fresh phytoplankton samples were observed daily without delay to determine a quick overview of species diversity, then 1.5 l of each sample was concentrated ~10-fold by sedimentation and siphoning the overlying seawater after 48 h and then preserved in a borate-buffered formalin (4% v:v) solution. Counting and identification were carried out by microscopy according to the Utermöhl method (Utermöhl 1958). Sedimented volume was 50 ml or less, depending on the densities of organisms and the presence of detritus. Phytoplankton species were counted using a phase-contrast inverted microscope (Wild M40) and identified to the lowest possible taxa using appropri-

ate keys (Thomas 1996, WoRMS 2019). Unidentified flagellates were pooled into the broad designation 'small autotrophic nanoflagellates' (ANF) and 'monads' for pigmented coccoid cells <3 µm (Hasle 1978) categorized as ultraphytoplankton.

2.6. *In vitro* cultures

To investigate whether the mass mortalities reported during the 2013–2014 bloom event could be due to the toxic metabolites produced by HAB species, we examined the toxic potential of several bloom-forming species isolated from Takaroa. To this end, *in vitro* phytoplankton cultures were tentatively established in the laboratory to produce sufficient cell biomass for toxicity tests.

In the field, algal cells were sorted out individually and inoculated into 24-well culture plates. The selection of these species was done based on their abundance and/or ability to bloom in mesocosms.

Different culture media were used, e.g. Si-enriched Conway medium for diatoms (Tompkins et al. 1995), f10k medium for dinoflagellates and cyanobacteria (Holmes et al. 1991), and Si-depleted f/2 medium for cryptophytes (Guillard 1975). Live samples were then kept in a field incubator (Aqua Lytic) and transferred to the laboratory for further processing. After 4 wk, healthy divided cells were transferred into increasing volumes of culture medium, i.e. in 10 ml tubes, 250 and 500 ml Erlenmeyer flasks. Finally, Fernbach flasks containing 1 l of culture medium were inoculated at an initial cell density of 250–370 cells ml^{-1}, and grown at 26 ± 1°C under 100 µmol photons m^{-2} s^{-1} of light (daylight fluorescent tubes) in a 12 h light:12 h dark photoperiod and permanent aeration. Cultures were harvested by filtration onto 90 mm glass-fiber Whatman filters in their late exponential/early stationary growth phase (~28 d post-inoculation). Each filter was then stored at −20°C until extraction.

2.7. Toxicity tests

2.7.1. Extraction procedures

Each filter-bearing cell sample was freeze-dried for 29 h (Martin Christ, Beta 1-8 LDplus) and extracted 4 times in 20 ml of MeOH:H_2O (80:20). After centrifugation, the 4 supernatants were pooled and dried under vacuum. The resulting crude cellular extract was weighed, dried under vacuum and stored at 4°C until tested for its toxicity. Crude extracts for the neuroblastoma cell-based assay (CBA-N2a) were re-suspended in 4 ml of methanol and subjected to an additional purification step by liquid–liquid partition using 50 ml of dichloromethane (CH_2Cl_2) and 2 × 25 ml of 60% aqueous MeOH. The resulting dichloromethane fraction, likely to contain lipid-soluble compounds, was then dried under vacuum, weighed and stored at 4°C until tested for toxicity.

2.7.2. Torpedo test®

The Torpedo test® is a colorimetric receptor-binding assay used for detection of cyclic imine neurotoxins acting as competitive antagonists of nicotinic acetylcholine receptors (nAChRs) (Aráoz et al. 2012, Rubio et al. 2014). All steps of the assay were performed according to the manufacturer's recommendations. Crude cellular extracts were re-suspended in methanol at a final concentration of 0.1 mg of dry extract $µl^{-1}$. For each extract, 3 distinct concentrations (3, 1.5 and 0.75%) were tested in triplicate per plate. In our assay conditions, a percentage of inhibition of Alpha-bungarotoxin (α-BTX) binding to nAChRs of ≥10% indicates the putative presence of cyclic imine compounds in algal extracts.

2.7.3. Neuroblastoma cell-based assay

CBA-N2a is used for the detection of a wide range of marine neurotoxins acting on voltage-gated sodium channels (VGSCs) (Reverté et al. 2014). The procedure used in this study followed the method previously described by Roué et al. (2016). The maximum concentration of dry cellular extract (MCE) that can be tested without causing non-specific cytotoxicity in neuroblastoma cells (Neuro-2a) (i.e. matrix interferences) was estimated at 9500 pg $µl^{-1}$ in this study. The lipid-soluble fractions obtained from algal species were first tested at a unique concentration of 9500 pg $µl^{-1}$ (MCE) to quickly screen for the presence of any potential toxic activity. In our assay conditions, a loss of Neuro-2a viability ≥20% would be indicative of the presence of lipophilic toxic compounds in the extracts. Quantification of these compounds was further carried out only in extracts inducing a loss of cell viability >80% by testing them at 8 distinct concentrations ranging from 74 to 9500 pg of dry extract $µl^{-1}$, to get a full CBA curve. Each concentration was tested in OV– (untreated cells; i.e. without addition of Ouabain

and Veratridine) and OV+ (cells treated with Ouabain and Veratridine mixture) conditions, in triplicate per plate. A standard of okadaic acid (OA) (Ref. CRM-OA-d; National Research Council of Canada) was also tested under the same assay conditions, at a concentration range of 0.93 to 119 µg µl^{-1}.

Neuro-2a viability data were fitted to a sigmoidal dose-response curve (variable slope) allowing the calculation of EC_{50} values (the concentration of extract capable of inducing 50% of mortality in Neuro-2a cells, expressed in pg µl^{-1}) using Prism v.6.0.7 software (GraphPad).

3. RESULTS

The whole study period was characterized by relatively stable meteorological conditions, with moderate east/northeast winds, no rain and sunny sky conditions except between Days 4 and 7, which had numerous cloudy periods.

3.1. Core parameters, nutrients and phytoplankton biomass

In the lagoon at Stn OUT, the water column was homogenous down to 10 m (no measurements were available below 10 m) with temperature and salinity increasing slightly during the experiment from 30.3 to 30.7°C and from 34.45 to 34.58, respectively, due to sunny weather conditions. The recorded temperatures were relatively high for the season, corresponding to an anomaly of ~0.9°C, a likely consequence of the strong 2015–2016 El Niño episode (Van Wynsberge et al. 2017). pH values fluctuated between 8.18 and 8.23 on the surface, but reached 8.04 at 10 m. Nutrient conditions remained relatively constant during the whole study with extremely low N concentrations, below 0.07 µmol l^{-1} (Table 2, Fig. 2) as previously recorded (Henry 2016). Using the nutrient ratios as a criteria, N appeared to be the main limiting factor in the lagoon (DIN:DIP < 16 and DIN:Si < 1), which is a prevalent feature of most tropical coral reef lagoons despite relatively low Si concentrations (Charpy et al. 2012 and references therein). Phytoplankton biomass (i.e. chl *a*) showed no clear trend or changes over the experiment, with a mean value of 0.41 ± 0.09 µg l^{-1} and a predominance of the picoplanktonic fraction (<2 µm) representing on average 74% of total chl *a* (Table 2, Fig. 3).

In the 3 mesocosms, the seawater temperature increase over the study period was the same as in the lagoon but the increase in salinity was more pronounced, from 34.47 to 35.23, due to higher evaporation. No thermohaline stratification was observed during the whole study period. pH values averaged 8.21 at the beginning of the experiment and increased over time to reach 8.84, 8.77 and 8.33 in M1, M2 and M3, respectively, concomitant with phytoplankton development (see below) and the resulting stronger demand for CO_2. As in the lagoon, the pH values at the bottom of the mesocosms were 0.4 to 0.7 lower than on the surface, which might be associated with higher remineralization at depth and/or higher photosynthesis in the upper layer.

Phytoplankton biomass (i.e. chl *a*) varied differently over time depending on the mesocosm considered (Fig. 3). In M1, the addition of the Si-enriched Conway medium induced a bloom within 4 d, in which the algal biomass peaked at 33.7 µg chl *a* l^{-1}. Thereafter, the biomass decreased rapidly down to 4.9 µg l^{-1} and then increased again until the end of the experiment. In M2 (enriched with Si-depleted f/2 medium), a bloom also occurred on Days 4 to 5, with chl *a* values up to 22.6 µg l^{-1}, which was followed by a 2 d decline and a stabilization phase. Using chl *a* as

Table 2. Average (±SD) temperature, nutrients, chl *a* and nano-microphytoplankton abundances in the 0 to 10 m surface layer at Stn OUT during the 11 d mesocosm experiment in March 2016. Total chl *a* concentration and phytoplankton abundance are shown in **bold**. nd: not detected

Parameters	Stn OUT
Temperature (°C)	30.49 ± 0.13
Salinity	34.51 ± 0.03
pH	8.19 ± 0.04
NO_x (µmol l^{-1})	0.03 ± 0.03
NH_4 (µmol l^{-1})	0.01 ± 0.01
PO_4 (µmol l^{-1})	0.22 ± 0.04
$Si(OH)_4$ (µmol l^{-1})	1.94 ± 0.04
Total chl *a* (µg l^{-1})	**0.41 ± 0.09**
Chl *a* < 2 µm (%)	74 ± 10
Chl *a* 2–10 µm (%)	18 ± 11
Chl *a* > 10 µm (%)	7 ± 2
Nano-microphytoplankton (× 10^5 cells l^{-1})	**1.0 ± 1.5**
Diatoms (%)	2 ± 3
Dinoflagellates (%)	66 ± 17
Autotrophic nanoflagellates (ANF) (%)	29 ± 20
Pyramimonadales (%)	2 ± 4
Prymnesiophytes (%)	<1
Cryptophytes (%)	<1
Euglenophytes (%)	<1
Chlorophytes (%)	nd
Filamentous cyanobacteria (%)	<1
Monads 1–3 µm (10^5 cells l^{-1})	23.25 ± 25.11

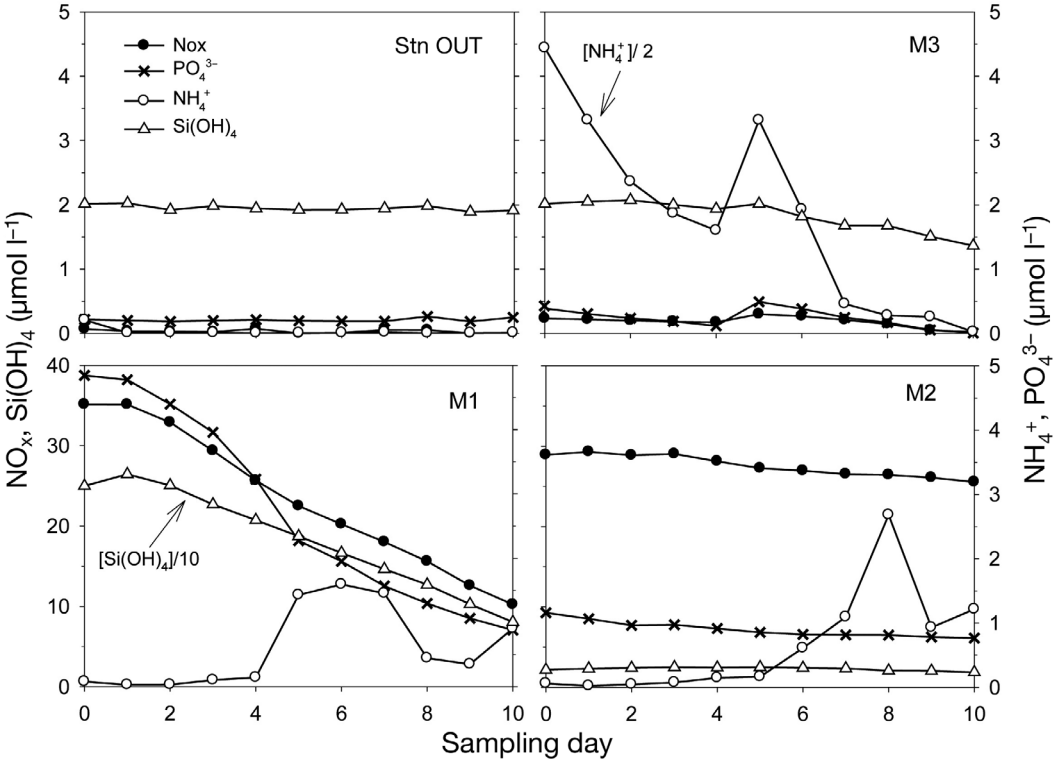

Fig. 2. Nutrient evolution in the 3 mesocosms (M1, M2 and M3) and at the reference station (Stn OUT). In M3, fertilization (N, P) was done twice at Day 0 and Day 4 to avoid nutrient depletion during the experiment. Note the change of scale on the *y*-axis between the top and bottom panels

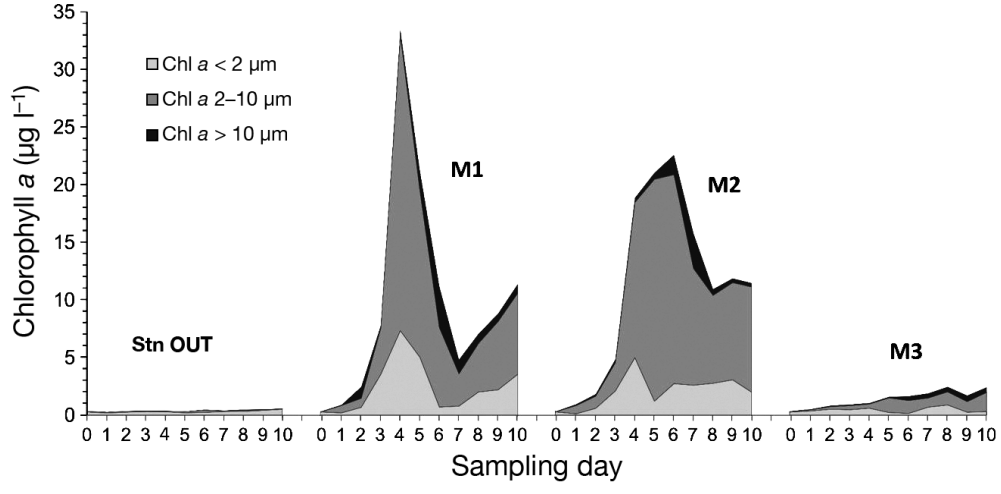

Fig. 3. Total and size-fractionated chl *a* at Stn OUT and inside the 3 mesocosms (M1, M2 and M3) during the 11 d experiment in Takaroa lagoon (March 2016)

a proxy, the net growth of the phytoplankton community during the exponential phase was 1.18 d^{-1} (doubling time of 0.58 d) and 0.99 d^{-1} (0.70 d) in M1 and M2, respectively. Of note, chl *a* biomass at the end of the experiment was similar in both enclosures, i.e. 11.3 µg chl *a* l^{-1}. In M3, the addition of fertilizer (NH_4^+ + P) did not trigger an algal bloom but induced a slow and regular net growth of the phytoplankton community. Chl *a* values (2.5 µg chl *a* l^{-1}) were 10-fold lower than in M1 and M2. In the 3 mesocosms, the chl *a* increase was largely attributable to the nanoplankton fraction (2 to 10 µm), which became rapidly predominant, representing more than 60% of the total chl *a* and up to 90% during the bloom (Fig. 3).

Phytoplankton development induced a decrease of N and P nutrients in all 3 enclosures (Fig. 2). However, the striking decrease in concentrations recorded in M1 was likely due to the partial flocculation of nutrients observed immediately following the addition of the Conway medium. After the blooms subsided in M1 and M2, NH_4^+ concentrations, which were initially low, accumulated in the enclosures (up to 1.6 and 2.7 µmol l^{-1} in M1 and M2, respectively), and were thereafter reduced by phytoplankton uptake. Silicate concentrations dropped sharply in M1 similar to the other nutrients, but they remained quite stable in M2 and M3 during the first days of the experiment before showing a smooth decrease until the end. In M2, the N:Si ratio remained very high (>11) throughout the whole experiment compared to Redfield ratio, indicating possible Si-limitation, in contrast to M1 which was always Si-replete and to M3 which was mainly P-limited (N:P ratios > 20 from Days 0 to 5).

3.2. Phytoplankton diversity and dynamics

3.2.1. At Stn OUT

The phytoplankton community remained relatively stable throughout the study period (Fig. 3). Analysis by light microscopy (Table 2) revealed a high numerical dominance of ultraphytoplankton in the range of 1 to 3 µm, mainly monads (10^6 cells l^{-1} on average), which is consistent with chl *a* size distribution. In comparison, the nano- and micro-phytoplankton were less numerous (10^4 to 10^5 cells l^{-1}) and dominated (90%) by relatively small organisms (<10 µm), mostly dinoflagellates followed by the pool of unidentified ANF (Table 2, Fig. 4). The diatoms and the prasinophytes (Pyramimonadales) were less abundant (~2% of cell counts), and the other groups could be regarded as minor. However, prymnesiophyte (coccolithophores) abundances presented in this paper may have been slightly underestimated as shown by in-field observations on fresh samples, likely due to the fixation and/or preservation of organisms (Cros 2001).

A total of 56 taxa were identified including 36 dinoflagellates, 14 diatoms, 1 prymnesiophyte, 2 prasinophytes, 1 cyanophyte, 1 euglenophyte and 1 cryptophyte (Table 3). The dinoflagellates showed the higher diversity with 5 dominant species: *Heterocapsa* spp. (25% of total nano-microphytoplankton counts), *Gymnodinium* spp. and *Prorocentrum triestinum* (13%, each), *Scrippsiella trochoidea* and *Alexandrium* sp. (4% each). Among identified diatoms, 3 pennate species were predominant: *Nitzschia* spp. and, to a lesser extent, *Cylindrotheca closterium* complex and *Pseudo-Nitzschia delicatissima*. Only 2 Pyramimonadales were identified — *Pyramimonas* (=*Polyble-*

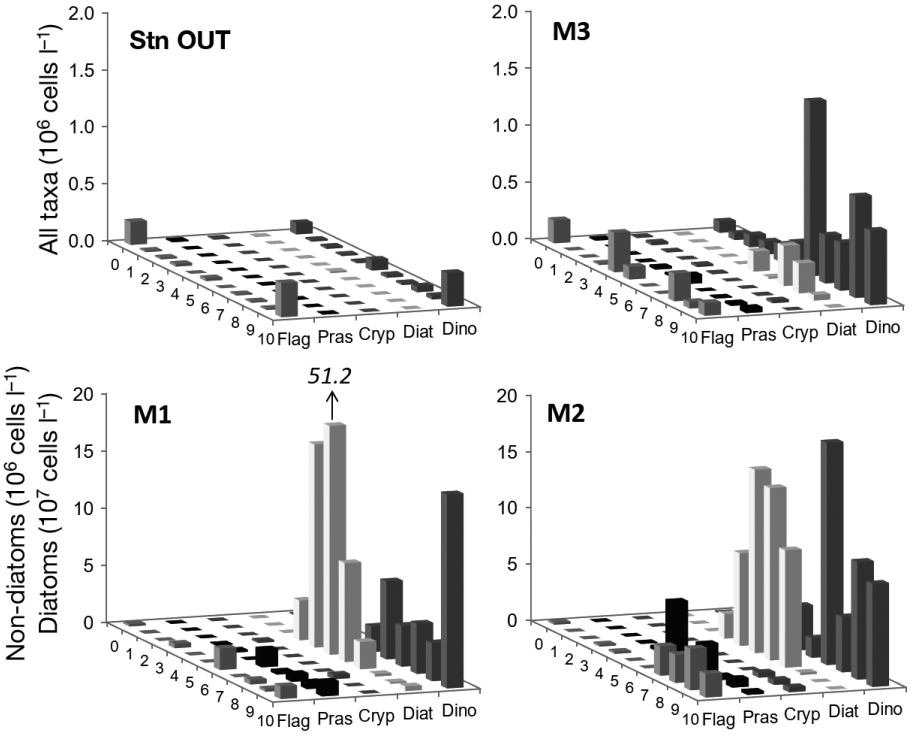

Fig. 4. Nano-microphytoplankton abundances during the 11 d experiment in Takaroa lagoon. Only the dominant classes are shown for simplification: nanoflagellates (Flag), prasinophytes (Pras), cryptophytes (Cryp), diatom (Diat), dinoflagellates (Dino). Note the difference in units between the diatom and non-diatom abundances in mesocosms M1 and M2 and the change of *y*-scale between the top and bottom panels

Table 3. Phytoplankton species and respective abundance in Takaroa lagoon, based on 2 consecutive studies conducted by the same scientific team in February 2015 (Henry 2016) and March 2016 (this study). Abundance data were obtained from counting conducted under light microscopy and represent the maximum abundance reported for each taxa. (×) Not observed; (○) <10 cells l^{-1}, (●) 10^1 cells l^{-1}, (●●) 10^2 cells l^{-1}, (●●●) 10^3 cells l^{-1}, …, (●●●●●●●●) 10^8 cells l^{-1}

Taxon	Feb 2015	Mar 2016			
	Stns 1 to 5	Stn OUT	M1	M2	M3
BACILLARIOPHYCEAE					
Amphora sp.	×	×	●●	×	×
Bleakeleya notata (Grunow) Round	×	×	●●	×	●
Chaetoceros coarctatus Lauder	●	×	×	×	×
Chaetoceros teres Cleve	×	×	×	×	●
Cylindrotheca closterium (Ehrenberg) Reimann & J.C.Lewin	×	●●	●●●●●●	●●	●●●●
Dactyliosolen fragilissimus (Bergon) Hasle	×	○	×	×	×
Extubocellulus sp.	×	×	●●●●●●●●	●●●●●●●●	×
Fragilaria sp.	●	×	●●●	×	●●●
Hemiaulus membranaceus Cleve	×	×	×	●●	×
Licmophora remulus Grunow	×	○	×	×	×
Licmophora sp.	●	○	●	○	×
Lioloma elongatum (Grunow) Hasle	●	×	×	×	×
Lioloma pacificum (Cupp) Hasle	●	●	●●	●●	●●●
Naviculoids spp.	×	●	×	×	×
Nitzschia longissima (Brébisson) Ralfs	×	●	●●●●●	●●	●●●●
Nitzschia sigma (Kützing) W.Smith	×	○	×	×	×
Nitzschia sp.1 (25 µm)	×	●●●	●●●	×	×
Nitzschia sp.2 (40 µm)	×	●●●	●●●●●	●●	●●●●
Nitzschia sp.3 (>40 µm)	×	×	●●●●	×	●●●●
Plagiotropis sp.	×	○	●●	×	×
Pseudo-nitzschia delicatissima complex	×	●●	×	○	×
Pseudo-nitzschia seriata complex	●	×	×	×	×
Pseudosolenia calcar-avis (Schultze) B.G.Sundström	●	×	×	×	×
Surirella sp.	○	×	×	×	×
Synedra ulna	○	○	○	×	×
Thalassionema cf. *bacillare* (Heiden) Kolbe	●	●	●●●●	●●	●
Thalassionema nitzschioides (Grunow) Mereschkowsky	○	×	○	×	×
Thalassiothrix sp.	×	×	●●	●	×
Toxarium hennedianum (Gregory) Pelletan	○	×	×	×	○
DINOPHYCEAE					
Alexandrium sp.	●●●	●●●	●●●	●●●●●	●●●●
Amphidinium carterae Hulburt	×	●	●●●	●●	●●●
Cochlodinium sp.	×	●	○	●●●●	×
Dinophysis sp.	×	●	×	○	●
Diplopsalis sp.	○	×	×	●●	○
Gonyaulax polygramma Stein	●	●	●	●●	●
Gonyaulax sp.	●	●●	●●	●●●	●
Gonyaulax spinifera (Claparède & Lachmann) Diesing	×	●	●●●	●●	●
Gymnodinium sp.1 (<25 µm)	●●●	●●●●	●●●●●●	●●●●●●●	●●●●●
Gymnodinium sp.2 (40 µm)	○	●●	●●●●	●●●●●	●●●●
Gyrodinium sp.	○	●	●●●●	●●●●●	●●●●
Gyrodinium cf. *fusus* (Meunier) Akselman	×	×	×	×	●●
Heterocapsa cf. *minima* A.J.Pomroy	●●●	×	×	×	×
Heterocapsa cf. *rotundata* (Lohmann) G.Hansen	×	●●●●	●●●●●●●	●●●●●●●	●●●●●
Heterocapsa sp.	●●●	●●●	●●●●●●	●●●●●●●	●●●●●
Heterocapsa triquetra (Ehrenberg) Stein	×	×	○	●●	×
Karenia brevis (C.C.Davis) Gert Hansen & Ø.Moestrup	×	○	×	×	○
Karenia papilionacea A.J.Haywood & K.A.Steidinger	×	○	×	●●●	●●
Oxytoxum laticeps Schiller	●●	●●	×	×	○
Oxytoxum sp.	●●	○	×	●	×
Oxytoxum tesselatum (Stein) F.Schütt	×	●●	●	×	●●
Oxytoxum variabile Schiller	●●	●●	○	○	×

(Table continued on next page)

Table 3. (continued)

Taxon	Feb 2015	Mar 2016			
	Stns 1 to 5	Stn OUT	M1	M2	M3
Phalacroma rotundatum (Claparéde & Lachmann) Kofoid & Michener	●	●	○	●●	●
Pronoctiluca acuta (Lohmann) Schiller	○	×	×	×	×
Pronoctiluca pelagica Fabre-Domergue	×	●	●●●	●	●
Prorocentrum dactylis (Stein) Dodge	○	×	×	×	×
Prorocentrum emarginatum Y.Fukuyo	○	×	×	×	×
Prorocentrum lima (Ehrenberg) F.Stein	×	○	×	●●	●
Prorocentrum maximum (Gourret) Schiller	×	○	○	○	○
Prorocentrum triestinum J.Schiller	●●●●	●●●●	●●●●●	●●●●●	●●●●●
Protoperidinium cf. *anguipes* (Balech, 1967) Balech	×	○	×	×	×
Protoperidinium bispinum (Schiller, 1937) Balech	○	○	×	●●●●	●●●●
Protoperidinium conicum (Gran, 1900) Balech	×	×	×	●●●	×
Protoperidinium sp.	●	●	●●●	●●●	●●●●
Scrippsiella spinifera G.Honsell & M.Cabrini	×	×	●●	×	●●
Scrippsiella trochoidea (Stein) Loeblich III	●●●	●●●●	●●●●	●●●●●	●●●
Scrippsiella trochoidea (cysts)	●●	×	×	×	×
Triadinium polyedricum (Pouchet) Dodge	×	●	×	×	×
Tripos arietinus (Cleve) F.Gómez	×	○	×	×	×
Tripos contrarius (Gourret) F.Gómez	×	×	×	○	○
Tripos furca (Ehrenberg) F.Gómez	●	○	●●	●●	●
Tripos horridus (Cleve) *F.Gómez*	×	●	×	●●	●●
Tripos kofoidii (Jörgenen) F.Gómez	×	×	○	×	×
Tripos muelleri Bory de Saint-Vincent	×	×	×	○	○
Tripos teres (Kofoid) F.Gómez	×	●	○	○	○
Tripos trichoceros (Ehrenberg) F. Gómez	○	○	●	●	●
Warnowia polyphemus (Pouchet) J.Schiller	○	○	×	×	×
Warnowia pulchra (J.Schiller) J.Schiller	×	●	×	×	×
Warnowia sp.	×	●	●●	○	○
CRYPTOPHYCEAE					
Cryptomonas sp.	●●	●●	●●●●●	●●●●●	●●●●
CYANOPHYCEAE					
Trichodesmium cf. *erythraeum* Ehrenberg ex Gomont	○	●	●	●●	●
Chroococcales (colonies) non-identified	×	●	○	×	×
Filamentous cyanophyceae sp.	●	○	×	×	○
EUGLENOPHYCEAE					
Euglena sp.	×	○	●●●	●●	●●
Eutreptia sp.	×	×	×	●	○
Phacus sp.	×	×	●●	×	×
PYRAMIMONADOPHYCEAE – CHLORODENDROPHYCEAE[a]					
Pyramimonas (*Polyblepharides*) *amylifera* (Conrad) H.Ettl	●●●●	●●●	●●●●●●	●●●●●●	●●●●
Pyramimonas cf. *grossi*	●●●	●●●	●●●●●●	●●●●●●	●●●●
Tetraselmis sp.	×	×	●●●●●	●●●●●	●●●●
PRYMNESIOPHYCEAE					
Coccolithophorids non-identified	●●	○	×	○	×
Gephyrocapsa oceanica Kamptner	●●●●●	●●	×	○	×
CHLOROPHYCEAE					
Chlamydomonas sp.	●	×	×	○	×
Volvocales non-identified	●	×	×	×	×
NANOFLAGELLATES non-identified	●●●	●●●●●	●●●●●●	●●●●●●	●●●●●
MONADS non-identified	●●●●●●	●●●●●●	●●●●●●	●●●●●●●	●●●●●●●

[a]Also referred to as prasinophytes in the text

pharides) *amylifera* and *Pyraminonas* (cf. *grossi*)—and a single cryptophyte—*Cryptomonas* sp. The main coccolithophore was *Gephyrocapsa oceanica* and filamentous cyanobacteria were represented by the genus *Trichodesmium*.

3.2.2. In nutrient-enriched mesocosms

In M1 (Fig. 4), enrichment with Conway medium generated a bloom dominated 99% by diatoms, reaching a maximum cell density of 5.1×10^8 cells l^{-1}, an increase of 4 orders of magnitude compared to their initial concentration. This drastic increase of diatoms was almost exclusively due to the growth of the nano-diatom *Extubocellulus* sp., a species undetectable at the onset of the experiment and in the lagoon (Fig. 5, Table 3). Diatoms still represented 15% of the overall plankton community after the decline of *Extubocellulus* sp. in contrast to 2% on Day 0. This post-bloom mainly consisted of large diatoms such as *C. closterium* and 3 species of *Nitzschia* including the great *N. longissima* (>400 µm). During the second half of the experiment, diatoms were substituted by dinoflagellates, which represented more than 63% of the nano-microphytoplankton community at the end of the experiment with a maximum abundance of 1.7×10^7 cells l^{-1}. Three taxa clearly

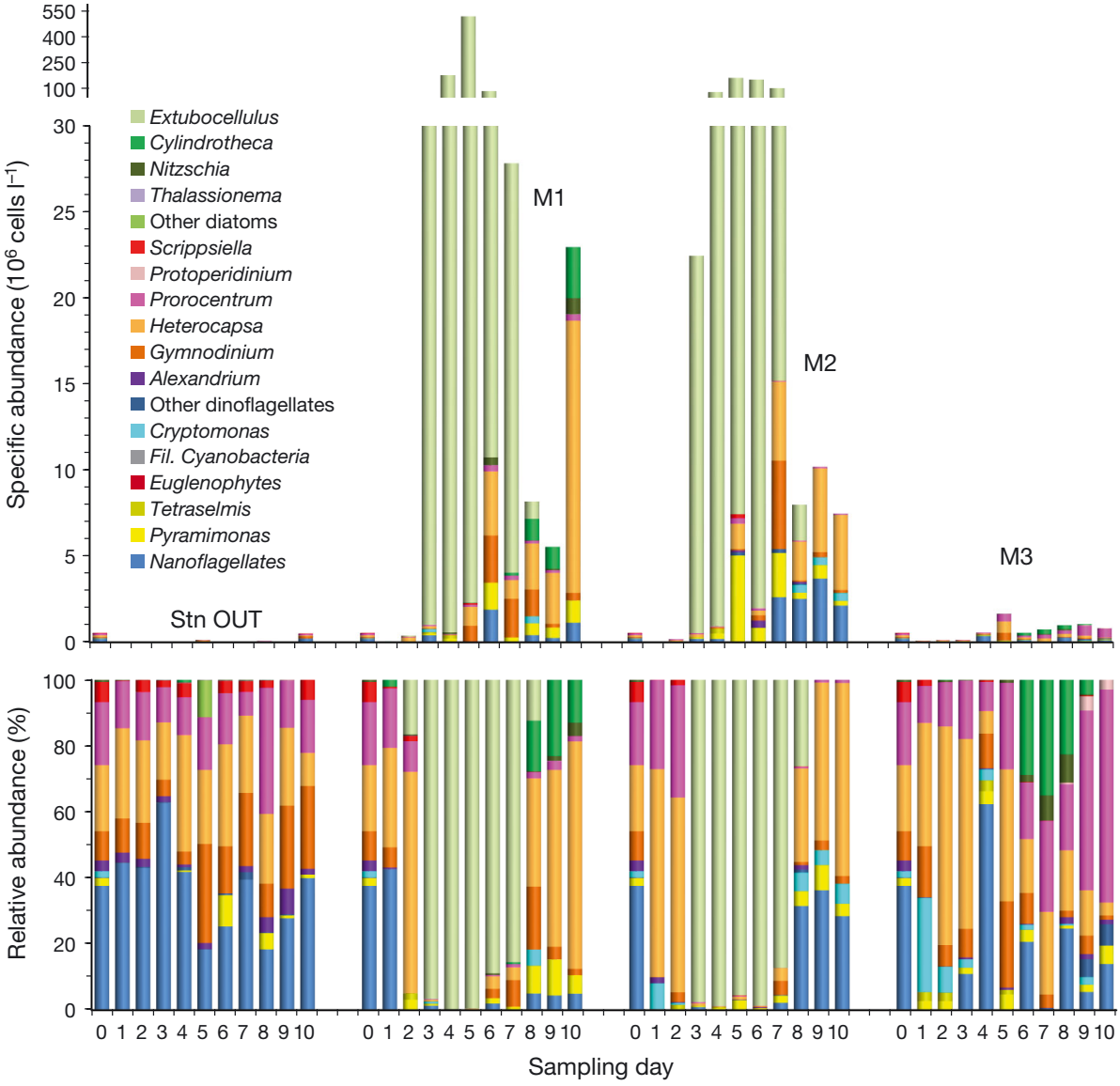

Fig. 5. Nano-microphytoplankton genera/species abundances at the reference station (Stn OUT) and in the 3 mesocosms (M1, M2 and M3): (top panel) cell counts; (bottom panel) relative abundance. Only major genera (mean abundances > 1000 cells l^{-1}) are presented

dominated in the mesocosm: *Heterocapsa* cf. *rotundata* and *Heterocapsa* sp., *Gymnodinium* sp. and, to a lesser extent, *Prorocentrum triestinum*. The prasinophytes, cryptophytes and unidentified nanoflagellates also increased after the diatom bloom but less significantly and less steadily than dinoflagellates. Of note, *Tetraselmis* sp., which was undetected in the lagoon, was also among the bloom-forming species.

Surprisingly, a monospecific bloom dominated by *Extubocellulus* sp. also developed in M2 on Day 4, although the peak cell abundance (1.7×10^8 cells l^{-1}) was 3-fold lower than in M1 (Fig. 5, Table 3). Following the decline of the *Extubocellulus* sp. bloom, diatoms remained poorly represented in M2 until the end of the study ($<2 \times 10^2$ cells l^{-1}). A gradual increase in dinoflagellate populations was visible as early as Day 4, reaching a peak on Day 7 ($\sim 20 \times 10^6$ cells l^{-1}), and remained abundant until the end of the experiment, representing 77% of cell counts. Overall, the dinoflagellate species that proliferated in M2 were the same as in M1, with a clear dominance of *Gymnodinium* sp. and *Heterocapsa* spp. (up to 90% of the dinoflagellate community). *Alexandrium* sp. also developed in M2 even though its abundance never exceeded 10^5 cells l^{-1}. The nanoflagellate fraction increased on Day 7, co-occurring with dinoflagellates, and represented the second major group. Additionally, *Pyramimonas* spp. and *Tetraselmis* sp. both briefly pulsed ($\sim 3 \times 10^6$ cells l^{-1}) during the diatom bloom while *Cryptomonas* sp. slightly increased at the end of the experiment.

In M3 (Fig. 4), the total abundance of nano- and micro-phytoplankton over the whole experiment ranged from 10^5 to 10^6 cells l^{-1}, which is 10-fold higher than in the lagoon but much lower than in M1 and M2. After a delay of 4 d, the densities of dinoflagellates increased and they remained the major group throughout the experiment. The assemblages were rich in *Prorocentrum triestinum, Heterocapsa* sp. and *Gymnodinium* sp. but *P. triestinum* became dominant at the end of the experiment, which contrasts with the 2 other mesocosms (Fig. 5). A small diatom population dominated by *Cylindrotheca closterium*, *Nitzschia* sp. and *N. longissima* developed in the second half of the experiment while the other groups remained either too low or fluctuated too much to denote a clear trend.

Considered separately, the monads did not grow in M1 and their number slightly increased ($>10^7$ cells l^{-1}) in M2 and M3. This ultraphytoplankton remained numerically dominant in M3 throughout the experiment and in the lagoon, but was rapidly surpassed in M1 and M2. Finally, microscopic observations showed an increase in protozooplankton (mainly heterotrophic ciliate) abundance during the second half of the experiment in all 3 mesocosms, which was 100- to 1000-fold higher than what was observed in the lagoon.

3.3. Toxicity results

A total of 12 algal strains showing a positive response following nutrient addition were successfully established into cultures and tested for their toxic potential (Table 4), including *Extubocellulus* sp. and several tycoplanktonic taxa, e.g. *Prorocentrum lima* and *Amphidinium* spp. Indeed, although regarded as a benthic species, *P. lima* is sometimes abundant in plankton assemblages (Maranda et al. 1999). Unfor-

Table 4. Toxicity data obtained for the 12 cultured strains originally isolated from mesocosm blooms, using the Torpedo test®. A percentage of inhibition >10% indicates the putative presence of cyclic imine compounds in the corresponding algal extracts. NT: not tested; –: no inhibition detected

Species	Cellular biomass harvested ($\times 10^6$ cells)	Weight of dry crude extract (g)	Inhibition (%)	Strains regarded as potentially toxic
Gymnodinium sp.	1.7	68.8	–	
Heterocapsa sp.1	37.5	241.6	18	✓
Heterocapsa sp.2	29.1	70.7	17	✓
Prorocentrum lima	12.7	63.7	20	✓
Amphidinium carterae	171.3	196.7	17	✓
Amphidinium cf. *steinii*	116.5	172.6	18	✓
Extubocellulus sp.	399.4	105.8	–	
Fragilaria sp.	2430.2	71	–	
Cryptomonas sp.	95.3	73.2	18	✓
Pyramimonas amylifera	143.7	51.2	20	✓
Pyramimonas cf. *grossii*	182.4	66.8	14	✓
Euglena sp.	117.4	51.5	NT	

tunately, isolates of *Nitzschia* sp., *Cylindrotheca* sp., *P. triestinum, Alexandrium* sp., etc., which were among the dominant taxa in mesocosms, did not survive in isolation wells.

Crude dry extracts obtained from these 12 strains were first tested for their toxicity using the Torpedo test®. A total of 8 strains out of 12 displayed an inhibitory activity towards α-BTX (% of inhibition >10%), indicative of the presence of compounds active on nAChRs, with *Pyramimonas amylifera* and *Prorocentrum lima* showing the highest inhibitory activity (Table 4). Additionally, the lipid-soluble fractions purified from crude extracts were also tested for the potential presence of toxic metabolites acting on VGSCs using CBA-N2a. At a concentration of 9500 pg µl^{-1} (MCE), only *P. lima*, *Amphidinium carterae* and *Extubocellulus* sp. extracts were able to induce a cytotoxic activity >20% in neuro-2a cells, i.e. 99, 29 and 22%, respectively (Fig. 6). A full CBA-N2a curve was further obtained for the *P. lima* lipid-soluble fraction which was the only strain showing a quantifiable toxic activity (loss of cell viability >80%; see Section 2.7). Fig. 7A shows the sigmoidal dose-response curve displayed by neuro-2a cells when exposed to *P. lima* extract in both OV– and OV+ conditions. This response was very similar to the one obtained with a standard of OA tested in the same assay conditions (Fig. 7B), although EC_{50} values for *P. lima* extract were 100-fold higher compared to the OA standard.

4. DISCUSSION

Large-scale mesocosm experiments are widely used in marine and freshwater environments; they provide greater complexity and realism over a longer period of time compared to smaller microcosm experi-

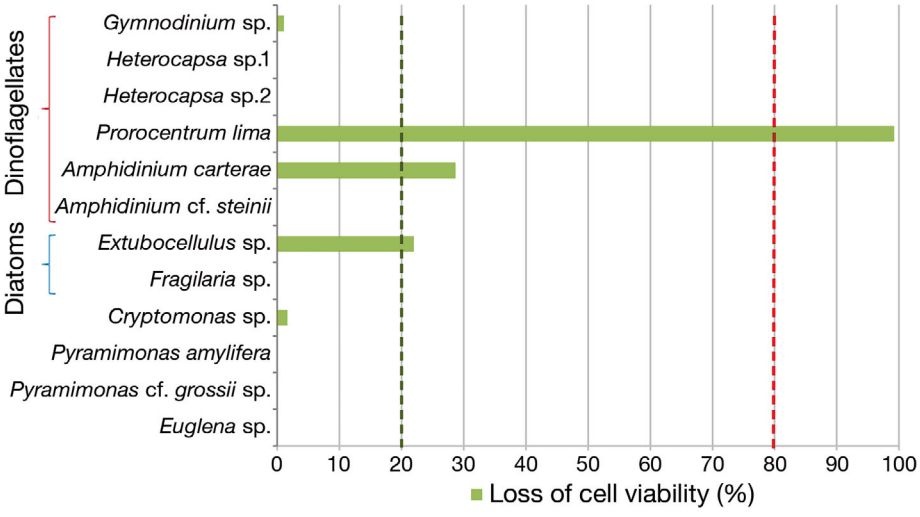

Fig. 6. Effects of lipid-soluble fractions prepared from cultured strains on Neuro-2a cell viability. A loss of cell viability ≥20% indicates the presence of toxic compounds active on voltage-gated sodium channels (VGSCs). The green and red vertical dashed lines correspond to a loss of cell viability of 20 and 80%, respectively

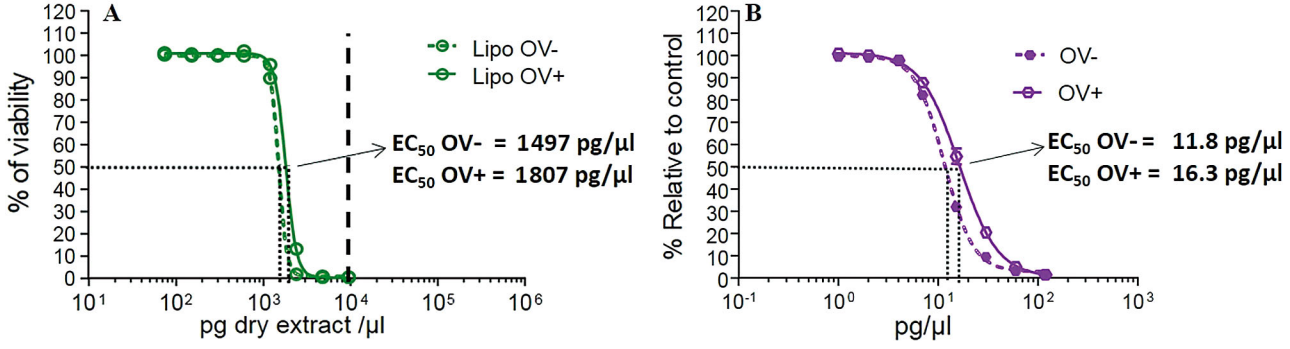

Fig. 7. Dose–response curves of Neuro-2a cells without addition of Ouabain and Veratridine (OV–) and with Ouabain and Veratridine mixture (OV+), when exposed to (A) increasing concentrations of liposoluble fraction of *Prorocentrum lima* and (B) a standard of okadaic acid, following the CBA-N2a protocol described in Roué et al. (2016). Each concentration was run in triplicate. The dashed vertical line corresponds to the maximum concentration of dry extract (MCE = 9500 pg µl^{-1}) for matrix interferences and the dotted vertical lines indicate the EC_{50} value determined under (OV–) and (OV+) conditions

ments (Lalli 2012, Bonnet et al. 2016). Furthermore, when using this approach, replicates and proper control are usually required to statistically validate the experiment (Gamble & Davies 1982, Lalli 2012). In the present study, this approach was used to gain insights into the diversity of phytoplankton communities in a farming lagoon recurrently affected by HABs, and to describe the response of these communities to nutrient addition. In this regard, the mesocosm approach proved to be a very useful and efficient tool, despite several difficulties encountered in setting up large-volume mesocosms in an isolated area. Daily samples drawn from the mesocosm enclosures provided very good temporal resolution and allowed identification of rapid changes in plankton community composition and nutrient concentrations and highlighted differences in community composition between treatments. However, since we chose to test only one mesocosm per nutrient treatment, our results are somewhat subject to an unknown variability due to the lack of replicates. This being said, it is unlikely that the observed differences between treatments could be solely the result of an overwhelming 'mesocosm effect'. Moreover, the lack of clear differences in the environmental variables (temperature, salinity, pH, etc.) between mesocosms and Stn OUT also points to the nutrient addition as a likely cause of algal development in the mesocosm enclosures.

4.1. Algal bloom development and bloom-forming species following nutrient addition

Addition of high concentrations of $N\text{-}NO_3^-$, P and micronutrients triggered a major diatom bloom in both M1 and M2. Diatoms are known to be NO_3 opportunistic (Glibert et al. 2016) and to respond quickly to favorable conditions due to their higher growth potential (Egge & Aksnes 1992). When diatom populations subsided, they were replaced by dinoflagellates and various flagellates, a typical pattern of phytoplankton population dynamics (Glibert 2016) and likely favored by enclosure and stratification (Heisler et al. 2008). In contrast, no bloom was observed in M3 following the addition of the high NH_4^+/low P concentration mixture, except for the moderate development of dinoflagellates and nanoflagellates known to be better competitors for NH_4^+ and P (Glibert 2016). These results are consistent with previous observations from Tuamotu lagoons on the synergistic limiting effect on algal growth of N–P or N–P–micronutrients (Dufour & Berland 1999, Sakka et al. 1999).

One of the major blooming species identified from Takaroa was a tiny chain-forming nano-diatom, namely *Extubocellulus* sp. Originally defined by Hasle et al. (1983), this genus of the family Cymatosiraceae currently comprises 3 distinct species: *E. spinifer*, *E. cribriger*, and *E. brasiliensis* (Garcia 2013). It is known for its widespread distribution from cold to subtropical waters, and from costal to offshore areas (Martín-Cereceda et al. 2007 and references therein). In the tropical south Pacific, *E. spinifer* has been reported only off Chile (Rivera et al. 2010) but, to the best of our knowledge, it has never been observed in French Polynesia or in tropical lagoons. One possible explanation is that this very delicate and tiny microorganism can easily be overlooked, especially if present in low abundance and/or sampled with a net (Riaux-Gobin & Chrétiennot-Dinet 2000). It is also possible that this nano-diatom is rapidly grazed, as it represents a direct trophic link to nanoplanktonic protists in the same size range (Martín-Cereceda et al. 2007). *Extubocellulus* sp. was also found to develop under low Si conditions, as observed in M2. A possible explanation to this somewhat unexpected result is that it could be merely an artefact; but as already discussed, the lack of replicates and a proper control in our experimental design makes it difficult to resolve this issue. In contrast, this finding is consistent with previous observations that Si is not a limiting factor in Tuamotu lagoons and that diatoms are able to adapt and thrive at low Si concentrations (Dufour & Berland 1999, Sakka et al. 1999). Although the specimens described from Takaroa (5–6 µm and <4 cells $chain^{-1}$) stand in the upper range of dimensions reported in the literature (Table 1 in Martín-Cereceda et al. 2007, Rivera et al. 2010), the small size that characterizes the genus *Extubocellulus* may confer an ecological advantage of this organism over larger forms in natural environments. Indeed, small organisms often show higher growth rates over larger forms, which is consistent with observations that *Cylindrotheca closterium* and *Nitzschia longissima* were able to develop in M1 only after the demise of *Extubocellulus* sp. bloom. Moreover, small size in diatoms or specific morphology such as thin and lightly silicified frustules is likely an adaptive response to low Si concentrations. As an example, in Takapoto atoll (Tuamotu), Sakka et al. (1999) reported that *Proboscia alata*, a diatom characterized by extreme weakly silicified frustules, was able to grow at low Si concentrations. Similarly, reports exist in the oligotrophic south Pacific (Gómez et al. 2007). Since the present study used light microscopy, it was not possible to assess the thickness and rigidity of *Extubocel-*

lulus sp. frustules. However, additional analyses carried out with a FEI Quanta 200 scanning electron microscope (SEM) (N. Gayet pers. comm.) revealed that the Si:C ratio in *Extubocellulus* sp. isolated from M2 was around 0.02 (atom:atom), which is lower than the average Si:C ratio of 0.09 ± 0.03 reported for marine nano-diatoms (<20 µm) by Brzezinski (1985). All these observations tend to confirm that *Extubocellulus* from Takaroa has actually developed an adaptative response to Si-limited conditions. In all cases, further studies are required to better understand its ecophysiology and ecological niche.

Four tycoplanktonic diatoms, namely *Cylindrotheca closterium* and 3 species of *Nitzschia*, also responded positively to nutrient addition in M1 whereas populations of 2 strictly pelagic diatoms, *Pseudo-nitzschia* and *Thalassionema*, remained low. This dominance of tycoplanktonic forms over pelagic ones may be the result of a positive effect of enclosures (e.g. presence of walls and reduction of photosynthetically active radiation), most notably during bloom and post-bloom phases. Of note, these tycoplanktonic forms did not grow in M2, suggesting a higher Si requirement and, therefore, lower potential as blooming species in atoll lagoons. Inversely, under low P and/or micronutrient conditions such as in M3, these diatoms appeared more competitive than *Extubocellulus*, which remained undetectable.

Dinoflagellates were also well-represented among the bloom-forming species in Takaroa. In particular, 4 species were able to develop in mesocosms: *Gymnodinium* spp., *Heterocapsa* spp., *Prorocentrum triestinum* and *Alexandrium* sp. These taxa are all known to be frequent bloom-forming species in estuaries, nearshore and coastal areas worldwide (Lassus et al. 2016), and are also often involved in HAB events. In contrast, *Scrippsiella trochoidea*, another potential bloom-forming species also present in Takaroa lagoon, did not grow in the mesocosms.

Several taxa belonging to prasinophytes (mainly the Pyramimonadales) also showed blooming capacities, most notably *Tetraselmis* sp., a genus which is not frequently seen in French Polynesian lagoons. This genus is known for its high nutritional properties for bivalve molluscs and crustacean larvae and is commonly used as forage algae by *Pinctada margaritifera* larvae (Southgate et al. 1998), which highlights its potential interest for aquaculture activities in farming atolls.

These findings have revealed different species/genera from Takaroa lagoon having the potential to bloom when exposed to favorable conditions. Surprisingly, some species — such as the diatom *Proboscia alata* which appears as a major component of HABs in Tuamotu atolls, including Takaroa (Table S1) — were not recorded at all in the present study. Such observations suggest that other bloom-forming species from Takaroa lagoon may have been overlooked. They also point to the necessity for local populations to be highly reactive in sending alerts when HABs occur, to allow proper identification of the species truly responsible for the initiation of the bloom.

4.2. Phytoplankton community composition in Takaroa lagoon

Detailed light microscopy analysis of the Stn OUT samples provided insights into the present phytoplankton community in Takaroa lagoon, prior to nutrient addition. The nano- and micro-phytoplankton communities were dominated primarily by small organisms (<10 µm), reflecting an adaptive strategy to low-nutrient conditions commonly observed in plankton communities from the oligotrophic South Pacific Ocean and French Polynesian atoll lagoons (Charpy & Blanchot 1998, Gomez et al. 2007, Thomas et al. 2010).

In terms of abundance, data fluctuated around 10^5 cells l^{-1}, which stands in the lower range of values reported from Tuamotu atolls (Delesalle 1990, Sakka et al. 1999, Delesalle et al. 2001, Fournier et al. 2012). The phytoplankton community composition (e.g. dinoflagellates, nanoflagellates, Pyramimonadales, prymnesiophytes) characterizes oligotrophic and relatively calm ecosystems (Margalef 1978, Glibert et al. 2016). Moreover, although most of these species are obligate phototrophs, they are also able to feed on dissolved organic matter and/or are mixotrophic (Stoecker 1999, Collos et al. 2009), which gives them an obvious ecological advantage over strictly autotrophic species, most notably in nutrient-limited but organic matter-rich lagoons (Charpy-Roubaud et al. 1990).

In terms of diversity, data from this study and those from a previous survey conducted in February 2015 (Henry 2016, Table 3) indicate that at least 87 species are found in Takaroa lagoon, with dinoflagellates standing out as not only the most abundant but also the most diverse group (49 species). Diatoms represented the second most diverse group (29 species), while the remaining groups were composed of very few identified species. The plankton community in Takaroa comprised both pelagic and tycoplanktonic forms, an observation typical of shallow environments. Interestingly, both the taxonomic composition of nano-microphytoplankton and major taxa de-

scribed from Takaroa lagoon were consistent with previous observations made by Delesalle (1990) and Delesalle et al. (2001) in 2 other Tuamotu lagoons, Mataiva and Takapoto.

Light microscopy analyses were used to capture the phytoplankton composition in Takaroa. However, this approach along with the sampling methodology (e.g. 'conventional' volume) may lead to potential biases in data. In particular, some species go under-reported or are simply overlooked due to their low abundance, small size and/or high fragility, such as *Extubocellulus* sp. and *Tetraselmis* sp. in this study. In this regard, the metabarcoding study conducted in parallel in March 2016 in Takaroa (Sorrodjé 2017) should provide additional information on phytoplankton diversity. Moreover, one should keep in mind that the data presented here only represent a snap-shot of the phytoplankton composition which is subject to temporal variability, as evidenced by the changes observed in Takaroa plankton composition between 2015 (Henry 2016) and 2016 (this study). Short-term variability of planktonic community is known to be high and dependent on weather conditions in Tuamotu lagoons (Thomas et al. 2010), but other factors (either hydrographic, anthropogenic or climatic) may also be involved (Delesalle et al. 2001, Thomas et al. 2010).

Finally, one may wonder about whether the over-exploitation of Takaroa lagoon for *P. margaritifera* farming may have drastically impacted the current phytoplankton composition in Takaroa. Indeed, *P. margaritifera* oysters, but also *P. maculata* (an abundant non-cultured oyster; Adessi 1999), have been shown to feed selectively on cryptophytes because of their higher nutritional value and digestibility compared to prymnesiophytes and chlorophytes of similar size (Loret et al. 2000) and, hence, may influence the prevalence of certain taxa over other species. Similarly, the overabundance of oysters whose diet is based on slightly larger prey items (Fournier et al. 2012) could also favor the predominance of smaller forms in phytoplankton communities.

4.3. Potential toxicity of bloom-forming species

The potential for toxicity of several bloom-forming species isolated from Takaroa lagoon was also investigated in the present study. Preliminary data indicate that at least 8 of these strains are the potential source of 2 groups of bioactive metabolites: (1) *Prorocentrum lima*, *Pyramimonas amylifera*, *Heterocapsa* sp.1 and sp.2, *Cryptomonas* sp., *Amphidinium* cf. *steinii* and *Amphidinium carterae*, which are potential producers of cyclic imine toxins, and (2) *P. lima*, *A. carterae* and *Extubocellulus* sp., whose lipid-soluble extracts contained metabolites active on VGSCs. However, more thorough investigations, using for instance a bio-guided fractionation approach, are needed to confirm these preliminary results and elucidate the chemical nature of the compounds involved.

Regarding the 8 strains that are likely producers of cyclic imine neurotoxins, including *P. lima*, the percentage of inhibition obtained in the present study remained relatively low (<30%). However, assays conducted in parallel with another strain of *P. lima* isolated from Moorea island (Society Archipelago, French Polynesia) indicate that when *P. lima* extract is concentrated 2-fold prior to analysis by the Torpedo test®, the resulting inhibition rate is increased by 8-fold (data not shown). Cyclic imine neurotoxins include azaspiracids (AZAs), gymnodimines (GYMs), pinnatoxins (PnTXs) and spirolides (SPXs), which are currently regarded as emerging toxins in Europe, where they have been under close surveillance since 2010 (Aráoz et al. 2012). Indeed, PnTxs are known to be potential contributors to Alzheimer's and Parkinson's neurodegenerative diseases (Andjelkovic 2012), while the presence of AZAs in bivalves has been directly linked to diarrhetic poisoning syndromes in consumers (Amzil et al. 2001). So far, the only genera unambiguously identified as the producers of cyclic imine neurotoxins are the dinoflagellates *Karenia selliformis* (GYMs), *Vulcanodinium rugosum* (PnTXs), *Alexandrium ostenfeldii* and *A. peruvianum* (SPXs) (Aráoz et al. 2012). Findings in the present study suggest that additional taxa found in Takaroa lagoon, including *Prorocentrum* sp., some Pyramimonadales and cryptophytes, may also be potentially involved in the production of compounds acting on nAChRs.

Based on CBA-N2a results, 3 strains were also identified as potential producers of toxins acting on VGSCs, especially one strain of *P. lima* whose lipid-soluble fraction yielded a response very similar to OA, a toxic compound implicated in diarrhetic shellfish poisoning (DSP) syndrome (Amzil et al. 2001). However, the 100-fold difference in EC_{50} values obtained for the OA standard versus the *P. lima* toxic extract suggests a low toxic potential in this strain. While the production of OA is a well-known phenomenon in *P. lima* (Billard et al. 2001), the potential detection of active compounds on nAChRs in cultures of this dinoflagellate constitutes a novel finding. In tropical marine ecosystems, *P. lima* is often found in close association with the genus *Gambierdiscus*, a benthic dinoflagellate currently under close surveil-

lance in French Polynesia as it is the cause of frequent ciguatera outbreaks (Chinain et al. 2016, Darius et al. 2018). The potential production of OA and cyclic imine toxins by *P. lima* revealed by the present study suggests the potential emergence of other phycotoxin risks in French Polynesian lagoons, such as those associated with the proliferation of *P. lima*, thus highlighting the need to also include the survey of this taxa in current monitoring programs.

Algal proliferation events often result in accompanying mass mortalities and/or growth reduction in surrounding marine fauna (Nagai et al. 1996, Adjeroud et al. 2001, Tang & Gobler 2011). The major bloom event that occurred in Takaroa in 2015 was no exception to this rule. Dinoflagellates in the genus *Alexandrium*, *Gymnodinium* and *Heterocapsa*, identified as bloom-forming species in Takaroa lagoon, are believed to be the potential source of ichthyotoxins (Lassus et al. 2016) while *Gymnodinium* and *Heterocapsa* blooms have been shown to cause important mortalities in cultured pearl oyster juveniles in Japan (Nagai et al. 1996). Although attempts to establish *in vitro* cultures failed for several algal bloom-forming species observed in Takaroa lagoon, e.g. *P. triestinum*, *C. closterium*, *N. longissima* and another *Nitzschia* sp., some of these species have been associated with mass mortality events: for instance, the diatom *Nitzschia* is recognized as an important producer of domoic acid exudates and has been implicated in plankton and fish kills (Bates 2000). Moreover, even if not commonly regarded as a HAB species, *C. closterium* has recently been reported as responsible for bloom events in southern Australia (Leterme et al. 2014), with foam and mucilage production which may exert deleterious effects on marine organisms (Edwards & Hardy 2004). There is also the case of *Scrippsiella*, whose cultures are known to exert detrimental effects on oyster larvae (Tang & Gobler 2011). Another possible explanation for the 2015 mortalities observed in pearl oysters in Takaroa may be a mere lack of food: indeed, previous samplings conducted in Takaroa lagoon have revealed the episodic predominance of a non-toxic diatom, *P. alata*, and a coccolithophore, *Gephyrocapsa oceanica* (Fougerouse et al. 2014), whose siliceous and calcareous theca, respectively, make them unattractive or inedible for pearl oysters. Moreover, due to their cell shape, certain species can also permanently damage the gill tissues of various marine organisms by simple mechanical effects. Finally, although non-toxic, certain HABs can attain high biomass resulting in substantial serious hypoxia or anoxic conditions following the decomposition of senescent blooms.

5. CONCLUSIONS

The data presented herein provide novel insights into the composition of the nano-microphytoplankton communities found in Takaroa lagoon, an economically important yet understudied pearl farming lagoon of the Tuamotu Archipelago (French Polynesia). In total, 87 species were identified and enumerated by means of light microcopy analyses, a majority of which belonged to dinoflagellates and diatoms, so far the 2 most diverse groups. The major taxa described from Takaroa were also similar to those reported from other Tuamotu atolls. The response of the plankton community to nutrient addition was also assessed through mesocosm-based experiments, an approach particularly relevant for French Polynesia atolls in locations with poor accessibility. Depending on the nutrient treatments, several bloom-forming species were identified, most notably a nano-diatom, *Extubocellulus* sp., which exhibited an unexpected capacity to bloom even under low Si conditions, suggesting some adaptive responses to nutrient status in atoll lagoons. This morpho-based data set obtained by light microscopy analyses will be compared to metabarcoding (16S DNA sequencing) data obtained from the same experiment, which will increase the reliability of our results and provide a deeper assessment of algal diversity.

Moreover, cultures of several blooming species isolated from Takaroa lagoon were successfully established in the laboratory, and 9 out of the 12 strains were identified with potential for toxicity. This novel finding sheds interesting light on some of the factors likely responsible for the mortality events previously reported in Takaroa marine fauna following HAB episodes.

Overall, the present study allowed us to validate the relevance of a mesocosm approach which can be applied in many other economically important atolls in French Polynesia in the future, in particular those recurrently threatened by HABs. This work also underlines the importance of continuous field surveys in lagoons that are threatened by blooms and/or important for the Polynesian economy. This could be achieved through the implementation of a network of local observers trained in user-friendly sampling procedures in locations with poor accessibility.

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