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# Germination of *Pyrodinium bahamense* Cysts from a Pristine Lagoon in San José Island, Gulf of California: Implications of Long-Term Survival

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**Abstract:** The production of cysts by dinoflagellates can be part of the life cycle of some species, improving their survival under adverse environmental conditions; cyst germination may explain the recurrence of algal blooms in some cases. In order to evaluate the germination rates of *Pyrodinium bahamense*, its cysts were retrieved from surface sediments collected in San José Lagoon, SW Gulf of California, and germination assays were carried out through the cysts incubation under two contrasting light and nutrient concentration conditions. Also, to evaluate cysts viability, we isolated *P. bahamense* cysts and other dinoflagellate species from different depth layers of a $^{210}$Pb-dated sediment core (~100 years) to examine their germination for 20 days. Germination rates were higher under light (28–56%) than in darkness (23–34%); there were indications that the nutrient-enriched media was more effective in promoting germination than seawater. Furthermore, germination was observed in cysts isolated from all selected core depths, even those corresponding to ~100 years. These results demonstrate that cysts remain viable for long periods, and *P. bahamense* cysts germinate in any light and nutrient conditions. The results of this research provide relevant information to understand its physiology and complex population dynamics. This species should be closely monitored in the area in the context of climate change, as current natural conditions are likely to change.

**Keywords:** coastal lagoons; dinoflagellate cysts; germination; harmful algal blooms; recent sediments; Mexico; Gulf of California; Baja California Sur



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## 1. Introduction

Dinoflagellates are second only to diatoms as primary producers in the ocean. However, they are even more important as secondary producers since most dinoflagellates are mixotrophs or heterotrophs [1] and are the main group forming harmful algal blooms (HABs), which produce toxins affecting aquatic organisms and human health [2]. To date, it has been described that approximately 15% of the 2300 species of dinoflagellates produce resistant cysts [3–5] during sexual or asexual reproduction as part of their life cycle [6]. A cyst is a resting stage that remains in sediments when conditions are unfavorable for vegetative growth. They can be reintroduced to the water column and germinate if favorable conditions are restored, especially at the time of bloom formation [7].

Different ecological roles have been attributed to the formation of resting cysts, such as genetic recombination when cysts are formed by sexual reproduction, geographic dispersal, and regulation of the seasonal succession of dinoflagellates [8,9]; they are resistant to unfavorable environmental conditions for the species, its wall protects them against viruses, parasites and as a strategy to survive predation [10–12]. Cyst germination is regulated by

internal and external factors [13]. Internal factors include dormancy cycling, involving two different states of dormancy: mandatory dormancy (maturation period for cysts to germinate), which occurs immediately after cyst formation and can last from 2 weeks to 5 months, depending on the species [9]. Dormancy can also be mediated by an annual internal biological clock [14,15]. Secondary dormancy is a reversible state mainly regulated by temperature [16–18].

Temperature and oxygen are the major external factors regulating the germination of non-dormant or quiescent cysts (quiescence is a state in which mandatory dormancy has been achieved, and cysts germinate on exposure to favorable conditions [19]. Induction of cyst germination has been linked to temperature variations around the optimal range (window), which can vary as a result of the geographic origin of the species [19]. Cysts generally cannot germinate in anoxic sediments, not even under favorable temperature conditions [20–23]. Dinoflagellate cysts may respond differentially to light. Germination might be inhibited in the dark [20,21,24], although cysts of some species can germinate at lower rates (delayed germination) in darkness than in light conditions [20–22,25–28]. On the other hand, dinoflagellates such as *Gonyaulax rugosa* Wailes 1928, *Alexandrium tamarense* (Lebour) Balech 1995, *A. affine* (H.Inoue & Y.Fukuyo) Balech 1995, and *Levanderina fissa* (Levander) Moestrup, Hakanen, Gert Hansen, Daugbjerg & M.Ellegaard 2014 have shown a similar germination pattern under light and dark conditions [20,29–31].

Nutrient availability (nitrates and phosphates) has no significant influence on cyst germination in the *A. tamarense* species complex [27], *A. minutum* Halim 1960 [25], and *Gymnodinium catenatum* H.W. Graham 1943 [26]. In *A. catenella*, the amount of cyst germination in natural (i.e., non-enriched) seawater was higher and faster than in a culture media enriched with nutrients and trace metals. However, it has not been discerned whether the germination of *A. catenella* cysts in culture media was inhibited by macronutrients or any metal or vitamin [32]. Conversely, in *Scrippsiella acuminata* (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S.Soehner, Kirsch, Kusber, & Gottschling 2015, cyst germination was higher in a standard culture media than in non-enriched seawater [33].

*Pyrodinium bahamense* L. Plate 1906 has caused important adverse effects on human health in Mexico. The Gulf of Tehuantepec in southeastern Mexico has been the most affected area, with at least 200 cases of paralytic shellfish poisoning and 15 deaths from 1989 to 2007 [34,35]. The vegetative and resting stages of this dinoflagellate are widely distributed along the Pacific and Atlantic Mexican coasts [36]. However, abundance, seasonality, and species distribution tend to decrease from tropical to subtropical areas, and blooms typically occur inside restricted shallow mangrove lagoons during rainy summers [36], influenced by seawater temperature, salinity, and high ammonium and phosphate concentrations [37]. In the Gulf of California, the occurrence of *P. bahamense* vegetative stages has been documented, with moderate blooms (63–151 $\times$ $10^3$ cell $L^{-1}$) at its southern end [36,37], while the resting stage has been reported in Holocene and Miocene sediments collected in the central [38] and upper gulf [39,40].

The effect of some variables on the germination of resting *P. bahamense* cysts has been studied, and cysts from San José Lagoon exhibit thermophilic and euryhaline affinities. The highest germination rate occurred from 20 to 35 °C, with a peak between 25 and 30 °C, at absolute salinities from 20 to 35 g $kg^{-1}$. Germination occurred in natural seawater and enriched culture media but was highest at the optimal temperature range in GSe supplemented with terrestrial soil extract and selenium [41]. In *P. bahamense* from Florida, USA, the release from dormancy can be mediated by extended exposure to low temperatures (15 °C), and extended exposure to high temperatures (30 °C) induces secondary dormancy in non-dormant cysts [17].

Another study of dinoflagellates cyst assemblages in sediments from San José Lagoon in the last 100 years showed that *P. bahamense* was the dominant species over the past 50 years [42] and an increase in cyst fluxes (cysts $cm^{-2}$ $yr^{-1}$) of *P. bahamense*, *Lingulodinium polyedra* (F.Stein) J.D. Dodge 1989, and *Gonyaulax* spp. in the past ~20 years were related to warmer conditions and higher nutrient supply [42].

In the Gulf of California, El Niño Southern Oscillation (ENSO) contributes 50% to climatic variability and dominates the interannual scale [43–45]. Oceanic decadal-interdecadal climate variations also are represented by the North Pacific Decadal Oscillation (PDO) with periodicities of 60–11 years [45]. Surface waters warmed 2 °C from the early 18th century to the 1950s, followed by an apparent rapid cooling of 1 °C between the 1950s and 1980s [46]. Since the 1970s, sea surface temperature (SST) in Mazatlán Bay has increased by ~0.5 °C per decade [47]. According to recent estimates, the mean atmospheric temperature in Mexico has risen by nearly 0.2 °C from 1970 to 2000 [48]. In the period 1940−2014, air temperature in the Baja California Peninsula showed a trend toward a significant rise in maximum temperatures, and a similar trend of minimum temperatures has been recorded in some areas of the peninsula [49]. The optimum growth of *P. bahamense* occurs at temperatures between 25 and 30 °C [41,50]. Under a climate-warming scenario and considering the thermophilic characteristics of *P. bahamense*, a temperature rise may affect bloom seasonality and recurrence; therefore, studying the physiology and dynamics of this and other potentially toxic dinoflagellate species is highly relevant.

This study aimed to explore the effect of light and nutrients on the germination rates of *P. bahamense* cysts found in surface sediments collected in San José Lagoon. The hypothesis was that light and nutrient enrichment would promote a higher germination rate than observed under darkness and nutrient limitation. Considering that the viability of resting cysts to long-term has been evidenced for other authors [51–54], we evaluated the long-term viability of cysts of different dinoflagellate species, including *P. bahamense*, isolated from selected sections of a $^{210}$Pb-dated sediment core (~100 years); we expected cysts germination in all selected core sections. The results from this study provide elements to advance our knowledge of the population dynamics of *P. bahamense* inhabiting restricted shallow mangrove lagoons in the Gulf of California and elsewhere.

## 2. Materials and Methods

### *2.1. Study Area*

San José Lagoon is located in the southern part of San José Island, southwest Gulf of California (24°52′–25°06′ N; 110°43′–110°35′ W; Figure 1). It is a semi-enclosed, shallow (5–10 m depth), small (~86 ha) mangrove lagoon connected to the open sea through a 1.5 km-long channel in the north-northwest section and an intermittent outlet in the southwest section [37]. The local climate is dry; between 1961 and 2015, the annual rainfall in the area ranged between 156 and 608 mm, with mean monthly air temperatures from 13 to 36 °C [55]. San José Lagoon was declared a Protected Natural Area in 1978 [56].

### *2.2. Sampling*

Two different cyst samplings were conducted in San José Lagoon (Figure 1): (i) sampling of surface sediments in 3 locations that will be used for testing light and nutrients effect on cyst germination, and (ii) sampling of core sediment for testing cyst abundance and survival of long-term sediments. Surface sediments were collected in October 2008 by scuba diving [37] at 7 m depth. The divers collected the top 1–2 cm of surface sediments until a 50 mL conical plastic tube was completely filled. The tubes were wrapped in aluminum foil and stored in dark conditions at 20 ± 2 °C until the germination assay, performed in October 2015 following the recommendations of Anderson [57] and Morquecho et al. [41].

A push core (San José core, 42 cm long, 7 cm inner diameter) was collected by scuba divers with an acrylic tube in February 2015 from the inner zone of San José Lagoon at 8 m depth (24°52′29.7″ N–110°33′3.3″ W; Figure 1). Sediments were extruded, and 1 cm sections were obtained under conditions of low light intensity to prevent cyst germination. Except for the 0−1 cm section due to insufficient material, 2 sets of subsamples were obtained from each section. Set-A samples were freeze-dried (FreeZone 12 Liter Console Freeze Dry System, Labconco) at −50 °C and 0.11 mbar for 72 h, stored in plastic bags, and used for geochemical analyses and 210Pb dating (results reported in Cuellar-Martinez et al. [58]). Set-B samples were placed in 15 mL plastic tubes, carefully filled with filtered seawater

from San José Lagoon, covered with aluminum foil, and kept in the dark at 23 ± 1 °C until the viability test was performed in May 2016.

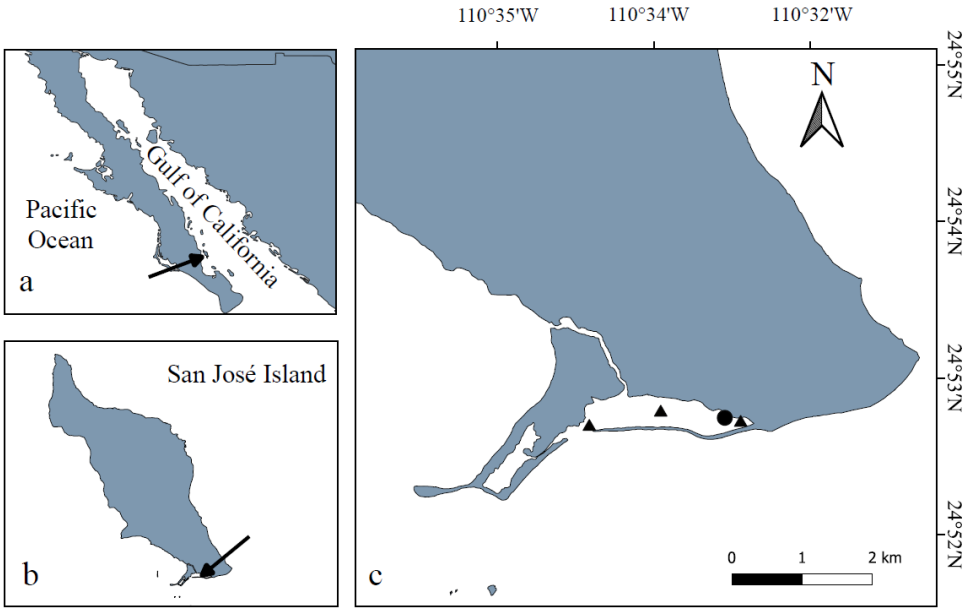



**Figure 1.** Map of the study site. (**a**) Location of San José Island in the SW Gulf of California (arrow); (**b**) location of San José Lagoon (arrow); (**c**) collection site of San José core (•) and surface (▲) sediment samples.

### *2.3. Laboratory Analysis*

#### 2.3.1. Core Dating

The core chronology (Figure S1) was obtained using the $^{210}$Pb-dated method, as detailed in Cuellar-Martinez et al. [58]. Briefly, $^{210}$Pb was analyzed through its descendant radionuclide $^{210}$Po by alpha spectrometry, following the methodology described by Ruiz-Fernández and Hillaire-Marcel [59]. The sediment chronology was obtained using the Constant Flux (CF) model [60], and uncertainties were computed by Monte Carlo simulation with 30,000 iterations [61]. To corroborate the $^{210}$Pb-dating, $^{239+240}$Pu was analyzed following the method described by Ruiz-Fernández et al. [62] and the references therein.

#### 2.3.2. Germination Experiments with Cysts from Surface Sediments: Effects of Light and Nutrients Conditions

The 3 surface sediment samples collected (Figure 1) were processed in fractions of 2 g. In total, 20 g of sediment was extracted as described by Matsuoka and Fukuyo [9] to obtain a cyst stock solution. Each sediment subsample was resuspended in filtered seawater (0.45 Whatman$^{TM}$ cellulose filter), treated with an ultrasonic cleaner (CD-4800; KENDAL, Practical Systems, Armidale, Australia) for 5 min, and filtered through 100 µm and 20 µm sieves. The fraction concentrated in the 20 µm sieve was repeatedly washed with filtered seawater until the wash water remained clear, then transferred to a 100 mL amber flask. Cyst density in this solution was determined using a 2.5 mL Utermöhl sedimentation chamber adapted to an inverted microscope (ECLIPSE TS 100; Nikon Instruments Inc., Melville, NY, USA). *P. bahamense* cysts were identified according to their morphological characteristics described in detail by Wall and Dale [63] and Morquecho et al. [41]. Living cysts were quantified in triplicate; the cyst density recorded was 9 ± 4 cysts mL$^{-1}$ (mean ± 2$\sigma$).

Cyst germination was tested under 2 nutrient conditions: natural (i.e., non-enriched) seawater and selenium-enriched f/2 media ($1 \times 10^{-5}$ M $H_2SeO_3 \cdot 9H_2O$; [64,65]. The natural surface seawater used in our experiment was collected from the San José Lagoon in February 2015. In the laboratory, seawater was filtered (0.45 and 0.22 µm WhatmanTM cellulose filter, Whatman plc, Maidstone, Kent, United Kingdom) and stored at 5 °C; this water was

also used to prepare the selenium-enriched f/2 culture media. Prior to the experiment, natural seawater was tested for nutrient concentrations. The concentration of nitrates and orthophosphates was determined with a continuous flow analyzer (San++ system, Skalar Analytical B.V., Breda, The Netherlands). Nitrites were analyzed following the procedure by Strickland and Parsons [66]. Nutrient concentrations were 0.8 µM of nitrates, 0.1 µM of nitrites, and 5.3 µM of orthophosphates.

One milliliter of the concentrated cyst solution (9 ± 4 cysts $mL^{-1}$) was inoculated into a 70 mL glass tube containing 2 grams of sterilized red clay [67] and 50 mL of culture media (natural seawater or selenium-enriched f/2 media). The experiment was carried out with five replicates. The incubation conditions were a 12:12 h light-dark photoperiod (hereafter light conditions), 23 ± 2 °C, and 53 µmol photons $m^{-2}$ $s^{-1}$ [41]. The germination rate was evaluated after 10 and 20 days of incubation. To determine the effect of darkness on cyst germination, a series of tubes with natural seawater and f/2 media were kept in the dark for 20 days. A red-light bulb was used during the processing and inoculation of cysts. In summary, the treatments were: (a) light–f/2 media incubated for 10 days (light–f/2 media–10 days), (b) light–f/2 media–20 days, (c) light–seawater–10 days, (d) light–seawater–20 days, (e) dark–f/2 media–20 days, and (f) dark–seawater–20 days.

After the incubation period, tube contents were concentrated by filtering through a 20 µm sieve, then washed with filtered seawater (0.45 WhatmanTM cellulose filter, Whatman plc, Maidstone, Kent, United Kingdom), and finally transferred to a 10 mL vial. Living and empty cysts were counted in triplicate using a 2.5 mL Utermöhl sedimentation chamber fitted to an inverted microscope (ECLIPSE TS 100; Nikon Instruments Inc., Melville, NY, USA). The germination rate [68] was calculated as follows:

$$Germination\ (\%) = \frac{\left(C_f - C_i\right)}{C_i} \times 100$$

where $C_f$ = the average number of living cysts at the end of the incubation period, and $C_i$ = the average number of living cysts at the beginning of the incubation period.

#### 2.3.3. Abundance and Viability of Dinoflagellate Cysts from the Sediment Core

Except for sections 10–11 cm, 13–14 cm, and 22–23 cm (due to scarcity of sediment sample), living and empty *P. bahamense* cysts were counted using an Utermöhl chamber under an inverted microscope (Axiovert 100, Carl Zeiss A.G., Jena, Germany). The wet weight of samples was registered, and the water content was determined from samples selected for geochemical and dating analyses (Section 2.2). The abundance of living and empty cysts was expressed as cysts $g^{-1}$ dry-weight sediment [69] using the following formula:

$$C = \frac{N}{W(1-R)}$$

where $C$ = the abundance of living/empty cysts $g^{-1}$, $N$ = the number of cysts, $W$ = the weight of the sediments analyzed, and $R$ = the proportion of water content in sediments.

To assess the viability of dinoflagellate cysts across the San José core, 9 samples between surface and 26 cm depth were selected since these contained sediments accumulated within the past 100 years (1–2, 4–5, 7–8, 10–11, 13–14, 16–17, 19–20, 22–23, and 25–26 cm). These subsamples were suspended in filtered seawater, sonicated for five minutes, and sieved following Matsuoka and Fukuyo [9]. Immediately, living cysts (i.e., those containing protoplasm and fully developed morphologically) of both *P. bahamense* and other Gonyaulacales and Peridiniales morphotypes (*Alexandrium* spp., *Gonyaulax* spp., *Lingulodinium polyedra*, *Protoperidinium* spp., *Protoceratium reticulatum*, *Pyrophacus steinii*, and *Scrippsiella* spp.) were collected with a capillary pipette under an inverted microscope and inoculated separately into 48-well culture plates previously filled with filtered seawater (0.45 and 0.22 µm WhatmanTM cellulose filter, Whatman plc, Maidstone, Kent, United Kingdom). Cysts were incubated at 25 ± 2 °C under 53 µmol photons $m^{-2}$ $s^{-1}$ and a

12:12 h light/dark cycle [41]. Cyst germination was monitored at two-day intervals for twenty days.

### *2.4. Statistical Analysis*

According to the Shapiro–Wilk and Levene tests [70], the dataset was normally distributed and homoscedastic ($p < 0.05$). Thus, to evaluate statistical differences in the germination rate of *Pyrodinium bahamense* cysts in natural seawater and f/2 media after 10 and 20 days of incubation under light/dark conditions, two-way ANOVA tests were performed with a significance level of $\alpha = 0.05$.

## 3. Results

### *3.1. Germination of Pyrodinium bahamense Cysts from Surface Sediments*

The germination rate of *Pyrodinium bahamense* cysts (Figure 2) incubated under light conditions ranged from 6% to 72% (Table S1).

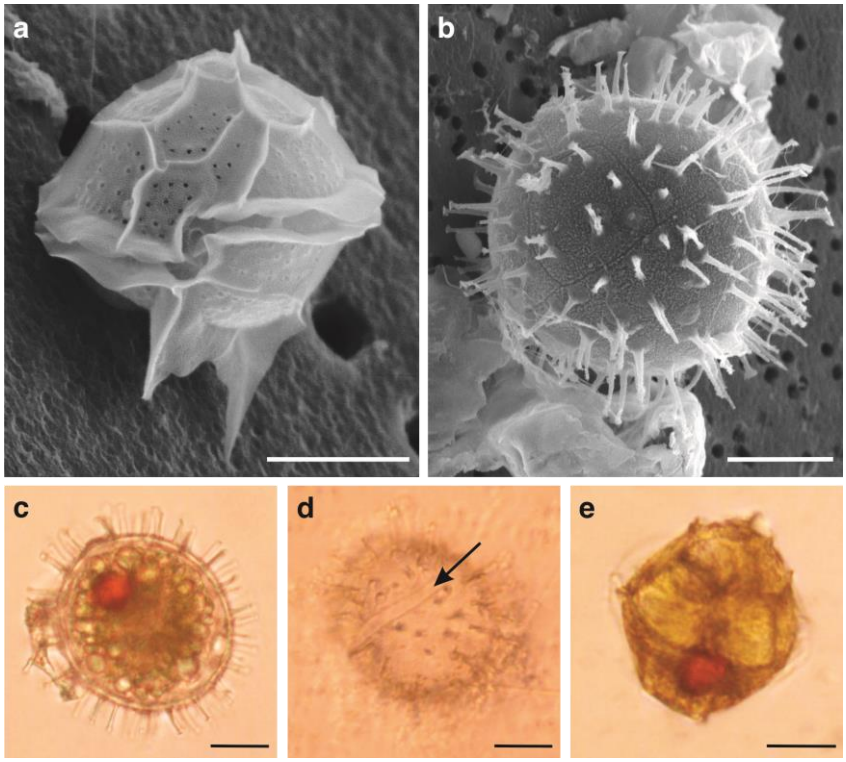
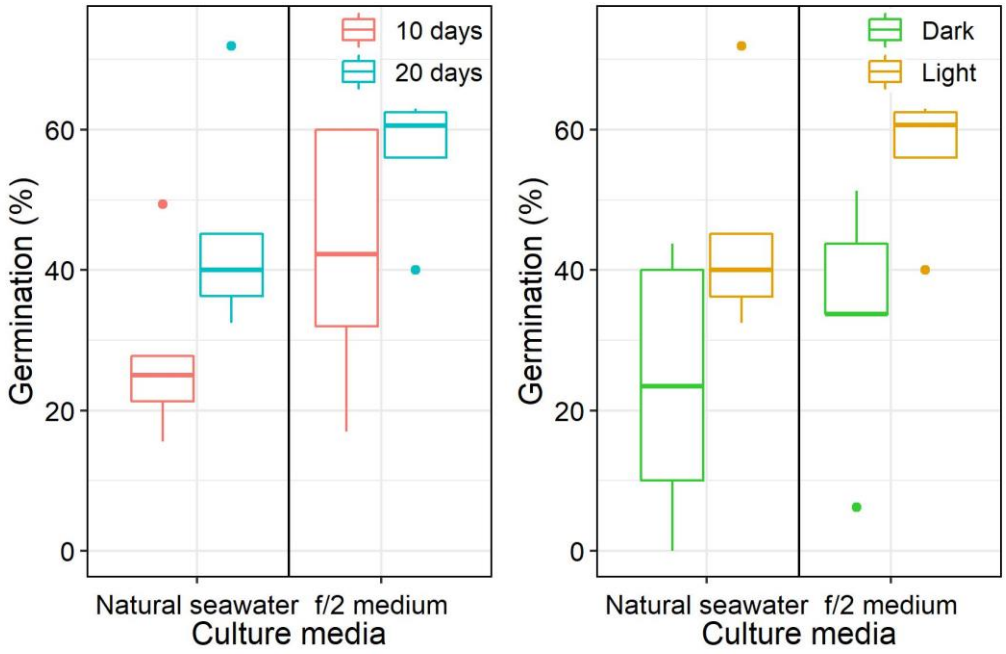



**Figure 2.** *Pyrodinium bahamense* under scanning electron (**a**,**b**) and light microscopy (**c**–**e**). (**a**) Single vegetative cell in ventral view; (**b**) dorsal view of cyst showing apical and precingular plates; (**c**) living whole cyst showing the red accumulation body; (**d**) empty cyst showing an archeopyle (arrow); (**e**) motile cell with theca in development showing the red accumulation body. Scale bars = 20 µm.

In light conditions, cyst germination was higher ($p = 0.023$) after 20 days (32–72%) than after 10 days of incubation (17–60%; Figure 3). Germination was not completely inhibited in the dark (0–51%) but was lower ($p = 0.004$) than in light conditions. Regarding the nutrient concentrations (Figure 3), there were indications that the selenium-enriched f/2 media was more effective in promoting cyst germination since the *p*-value (0.058) indicates marginal significance [71].




(**a**) (**b**)

**Figure 3.** Germination of recent *Pyrodinium bahamense* cysts from San José Lagoon, SW Gulf of California: (**a**) in natural seawater and f/2 media for 10 and 20 days, and (**b**) under light and dark conditions for 20 days. Box plots show the median, lower (Q1) and upper (Q3) quartiles, and outliers (dots).

### *3.2. Abundance of Living Pyrodinium bahamense Cysts in the Sediment Core*

The number of living and empty *P. bahamense* cysts is shown in Figure 4. Due to scarce sediment samples, the cysts were not quantified in sections 10–11 cm, 13–14 cm, and 22–23 cm. The highest abundances of total cysts were observed in sections 1–2 cm (4804 cysts $g^{-1}$) and 4–5 cm (4459 cysts $g^{-1}$) depth. Most *P. bahamense* cysts from the San José core were empty (610 ± 291–4787 ± 815 cysts $g^{-1}$). Living cysts ranged from 2 ± 1 to 23 ± 10 cysts $g^{-1}$ (Figure 4), representing < 1% of the total cyst abundance. The highest abundances of living cysts were observed in sediments deposited in 1944 ± 5.0 yr (19–20 cm) with 23 ± 10 cysts $g^{-1}$, and in 1965 ± 2.8 yr (16–17 cm) with 19 ± 4 cysts $g^{-1}$ (Figure 4).

### *3.3. Long-term Viability of Dinoflagellate Cysts from San José Core Sections*

Ninety-four dinoflagellate cysts belong to *Pyrodinium bahamense*, *Alexandrium* spp., *Gonyaulax* spp., *Lingulodinium polyedra*, *Protoceratium reticulatum*, *Protoperidinium* spp., *Pyrophacus steinnii*, and *Scrippsiella* spp. were isolated from the San José core. The most abundant cysts were autotrophic species (*P. bahamense* and *Gonyaulax spinifera* (Claparède & Lachmann) Diesing 1866; Table S2), whereas the cysts of *Protoperidinium* represented heterotrophic species.

Germination of *P. bahamense* cysts occurred from the fifth day of incubation. In the 19–20 cm section, two cysts from the six isolated germinated, but cell division did not occur in this section. In general, 39 percent of the cysts germinated (38 cysts), and 63% of these were able to divide (24 cysts). However, *P. bahamense* cells did not continue dividing further, and strain establishment did not occur. Living cysts of *P. bahamense* and *G. spinifera* found in the 25.5 cm section (beyond the 210Pb-derived chronology, i.e., older than 1918 ± 9 yr) were able to germinate (Table S2).

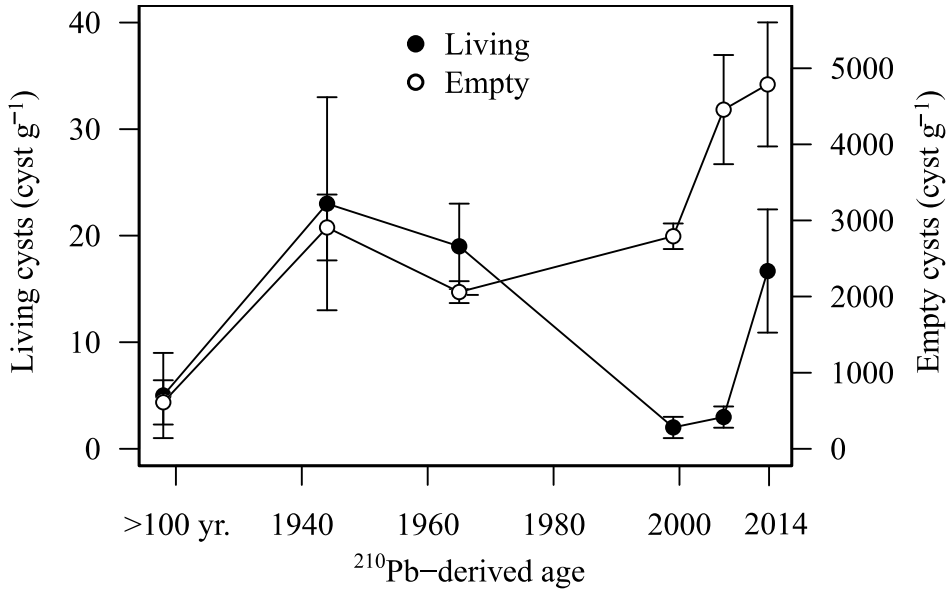



**Figure 4.** The abundance of living and empty cysts of *Pyrodinium bahamense* in the San José core (San José Lagoon, SW Gulf of California).

## 4. Discussion

### *4.1. Effects of Light and Nutrients on Pyrodinium bahamense Cyst Germination*

Although the highest germination of *P. bahamense* cysts occurred in light conditions, darkness did not completely inhibit it. These results are similar to those reported for *G. rugosum*, *A. tamarense*, *A. affine*, *Gyrodinium instriatum* Freudenthal & J.J.Lee 1963, and *Peridiniella catenata* (Levander) Balech 1977, i.e., cyst germination occurred in both light and dark conditions [14,29–31,68]. However, Vahtera et al. [28] found that 90% of *A. fundyense* Balech 1985 cysts germinated after 30 days when incubated in light conditions but after 50 days in dark conditions. Nonetheless, the shorter duration of our experiment (20 days) did not allow us to determine whether the light/darkness cycle affected the excystment frequency in *P. bahamense*.

Our results on the effect of nutrients indicate that selenium-enriched f/2 media promote cyst germination, while other authors have concluded that nutrients did not influence the germination of freshwater [23] and marine [25–27] meroplanktonic dinoflagellate species. Morquecho et al. [41] observed more prolific germination using GSe media. This may be related to the fact that the coastal lagoons surrounded by mangroves have been the environments with the highest record of algal blooms of *P. bahamense*. It is hypothesized that mangroves provide some compound that favors its growth [36].

### *4.2. Long-Term Viability of Living Pyrodinium bahamense Cysts*

The high abundance of cysts in the first core centimeters is related to warm conditions and high concentrations of nutrients. According to Cuellar-Martinez et al. [42], this result indicates an increase in the abundance of vegetative cells in the water column in recent years. Although we cannot rule out the cyst germination during sediment storage, the low number of living *P. bahamense* cysts found in the San José core was consistent with previous observations [37] that reported a greater abundance of empty cysts in surface sediments from July to October 2008. Indeed, we observe a high number of empty cysts in the core depth of 4.5 cm, corresponding to that year.

Morquecho et al. [37] observed an increase in the abundance of living cysts only after a moderate bloom occurred in summer that declined in October 2008. This common pattern was also observed in *P. bahamense* blooms in Manila Bay [72,73]. The optimal environmental window that favors the formation of blooms of this species is associated with high summer temperatures and higher ammonium and phosphate levels from rainfall

and runoff [37]. The steady increase in the flux of *P. bahamense* in the San José core from the mid-1960s [42], with a predominance of empty cysts (~99%), may indicate the prevalence of favorable conditions for a recurrent germination process with the development or not of bloom events. Starting in 2005, the vegetative stage of *P. bahamense* was observed in phytoplankton samples collected in several coastal sites of the southern Gulf of California, first in low abundances (100–240 cells $L^{-1}$ [74,75]) and later as moderate blooms (maximum abundances: 151 $\times$ $10^3$ cell $L^{-1}$ [37,76]).

Although the intervals of temperature and salinity that favor germination of *P. bahamense* cysts from San José Lagoon are known, in this study and the one by Morquecho et al. [41], it was not possible to achieve long-term maintenance of the strains established from cyst germination, suggesting that other biotic and abiotic factors may influence vegetative growth and bloom development. Therefore, inter-annual in-situ studies should be conducted to understand further the population dynamics of *P. bahamense* in the Gulf of California.

Cysts germinated from sediments accumulated over ~37 to ~100 years (Table S2). This is the first study in which the long-term viability of *P. bahamense* resting cyst was tested since the viability of temporary cysts had only been evaluated, the pellicle formation, and its viability is influenced by low temperature (i.e., 13 °C; [77]).

The viability recorded in our study for *P. bahamense* was previously observed for other dinoflagellate species (Table S3), such as *L. polyedra* [51], *Pentapharsodinium dalei* Indelicato & A.R. Loeblich 1986 [52], *A. tamarense* [53], *S. acuminata* [54], and *Apocalathium malmogiense* (G.Sjöstedt) Craveiro, Daugbjerg, Moestrup & Calado 2016 [78]. Although long-term viability in phytoplankton resting stages, including dinoflagellate cysts, is related to the presence of thick and multi-layered walls, accumulation vesicles of starch, lipids or other materials (i.e., pigments or unidentified granular materials), the use of alternatives sources of respiration, mechanism of shut-down or anoxibiosis require to be investigated. Also, hormones such as abscisic acid and melatonin could be implicated in life-cycle transitions and persistence [79]. Delebecq et al. [80] used biostimulants (melatonin and gibberellic acid) to promote germination in dinoflagellate cysts; in *A. minutum* and *Heterocapsa minima* A.J. Pomroy 1989, cysts isolated from sediments of up to 117 $\pm$ 21 yr germinated only after the application of a biostimulant.

The long-term viability of dinoflagellate cysts has been associated with low oxygen concentrations in sediments [52,54,81], and there is evidence that cysts do not germinate under anoxic conditions [20,22]. Although we did not measure oxygen concentration in sediments, high concentrations of Mn and Fe (diagenetically mobile elements, indicators of redox conditions; ref. [82]) were detected in the upper core segment [58], which may indicate oxidizing conditions near the sediment-water interface [83]; thus, most cysts in the sediment core remained buried under low oxygen conditions.

The natural stressors of coastal lagoons are storms and hurricanes and the impacts of climate change, including increased temperatures [84]. In a global warming scenario, it is predicted that temporal windows of warm seawater will expand [85], favoring thermophilic species. Exposure to high temperatures affects *P. bahamense* dormancy, which could influence its bloom dynamics [17,18]. However, the response of this species to climate change will be much more complex than cell and cyst responses to higher temperatures. Changes in ecosystems associated with climate change (oceanic coastal circulation, precipitation, winds, water stratification, incidence of hypoxia/anoxia) could affect the dynamics of harmful algal blooms in different ways, such as an increase in the incidence of more resilient harmful species; bloom seasonality; and changes in HAB species distribution, physiology, toxicity, and photosynthetic efficiency, among others [18,86]. Therefore, studies to understand the dynamics of *P. bahamense* in the Gulf of California are needed. Resting stages deposited in historical records or seed banks can provide an inoculum that may influence present populations through "dispersal from the past"; due to their standing genetic diversity, cysts are important for the adaptation of dinoflagellate species to future environments [79].

Changes in SST can lead to variations in water column stratification and the supply of nutrients, which influence phytoplankton dynamics. Nutrient concentrations and their ratios affect phytoplankton growth. Some studies that conducted long-term revisions using in-situ data and satellite imagery analysis in the Gulf of California (1960–2016) indicate an increasing south-to-north gradient in nitrate, phosphate, and silicate concentrations in surface waters [87,88]. Cuellar-Martinez et al. [42] used the terrigenous index (Iterr) as an indicator of nutrient inputs, and cyst fluxes were positively correlated with Iterr values. Our results indicated that nutrient concentrations were not a limiting factor for the germination of *P. bahamense* cysts in-vitro. However, there were indications that the selenium-enriched f/2 media was more effective in promoting cyst germination.

The results of this and other studies in subtropical mangrove lagoons [36,37] indicate that in the Gulf of California: the vegetative stage of P. *bahamense* is uncommon, recent blooms of this species have occurred with moderate abundances, and the species is restricted to shallow mangrove lagoons over a short-term environmental window (rainy summers). Although we observe a dominance of empty cysts in recent years, which may imply a high germination rate, habitat limitations and environmental conditions would determine the vegetative growth. Despite the factors mentioned before, considering the potential risk associated with the production of toxins, it is important to continue monitoring *P. bahamense* populations in the coastal lagoons of the Gulf of California.

## 5. Conclusions

Sediments collected in San José Lagoon, southwest Gulf of California, were used to assess the germination potential and viability of dinoflagellate cysts, mainly belonging to *Pyrodinium bahamense*. Germination of cysts retrieved from surface sediments was examined through cyst incubation in contrasting light and nutrient conditions, and the viability of dinoflagellate cysts was evaluated using cysts isolated from different depth layers of a $^{210}$Pb-dated sediment core. The highest germination rate of *P. bahamense* cysts was observed in light conditions; nutrient enrichment was not mandatory for germination, and cysts germinated from all selected core depths, even in those corresponding to ~100 years. Further studies are needed to clarify the mechanism involved in the long-term survival of the cysts, as well as establish cultures from the germination of *P. bahamense* cysts to develop physiological studies that allow understanding of the dynamics of this species, as has been done with *Alexandrium* [89,90].

Since *P. bahamense* is a thermophilic species and SST has increased since the second half of the 20th century in the Gulf of California, global warming conditions would be expected to promote its proliferation. However, in this species, vegetative growth and bloom development are seasonally limited (rainy summer), while light and nutrients are not limiting factors for the recurrent germination of cysts. Therefore, *P. bahamense* should be closely monitored in the area in the context of climate change, as current natural conditions are likely to change.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/phycology3010005/s1, Figure S1: $^{210}$Pb-derived chronology of the San José core (San José Island, SW Gulf of California); Table S1: Germination rate of *Pyrodinium bahamense* cysts isolated from San José Lagoon SW Gulf of California surface sediments; Table S2: Number of dinoflagellate cysts isolated (germinated) from selected sections of the San José core. Table S3: Maximum age at which germination of dinoflagellate cysts occurred in sediments from $^{210}$Pb-dated cores.

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