frontiers | Frontiers in Protistology

TYPE Original Research
PUBLISHED 07 February 2024
DOI 10.3389/frpro.2024.1302560

OPEN ACCESS

EDITED BY
Shauna Ann Murray,
University of Technology Sydney, Australia

REVIEWED BY
Gustaaf Marinus Hallegraeff,
University of Tasmania, Australia
Marilou P. Sison-Mangus,
University of California, United States

*CORRESPONDENCE
Martín Fernando Encinas-Yánez
mfencinasy@gmail.com
Christine Johanna Band-Schmidt
cbands@ipn.mx

RECEIVED 26 September 2023
ACCEPTED 15 January 2024
PUBLISHED 07 February 2024

CITATION
Encinas-Yánez MF, Band-Schmidt CJ, Zenteno-Savín T, Leyva-Valencia I, Fernández Herrera LJ and Palacios-Mechetnov E (2024) Deleterious effects of free fatty acids and hydrogen peroxide towards the dinoflagellate *Gymnodinium catenatum*. *Front. Protistol.* 2:1302560. doi: 10.3389/frpro.2024.1302560

Deleterious effects of free fatty acids and hydrogen peroxide towards the dinoflagellate *Gymnodinium catenatum*

Martín Fernando Encinas-Yánez[1]*, Christine Johanna Band-Schmidt[1]*, Tania Zenteno-Savín[2], Ignacio Leyva-Valencia[3], Leyberth José Fernández Herrera[2] and Elena Palacios-Mechetnov[2]

[1]Departamento de Plancton y Ecología Marina, Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional, La Paz, Mexico, [2]Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, Mexico, [3]Centro Interdisciplinario de Ciencias Marinas, Consejo Nacional de Ciencia, Humanidades y Tecnología (CONAHCyT)-Instituto Politécnico Nacional, La Paz, Mexico

Allelopathy refers to biochemical interactions among competing microalgae, it involves a donor species that produces metabolites which can cause inhibitory effects on susceptible species. This phenomenon can participate in the regulation of harmful algal blooms. The dinoflagellate *Gymnodinium catenatum* is negatively affected by allelopathic interactions with co-occurring microalgae species, like *Chattonella marina* var. *marina*, which has been suggested to produce reactive oxygen species (ROS) and free fatty acids (FFA) as nocive and allelopathic agents. This study explored the effect of hydrogen peroxide (H_2O_2) and the main fatty acids produced by *C. marina*. An analysis of fatty acids content of *C. marina* in exponential phase detected 16:0 (12.5 ± 0.01%), 18:4ω-3 (15.4 ± 0.36%) and 20:5ω-3 (35.4 ± 0.71%) as the most abundant. These fatty acids along with H_2O_2 were used in dose-response bioassays with cultures of *G. catenatum* in exponential phase. Results suggest that these substances affect cell morphology, including the loss of motility and signs of chlorosis, as well as the chain forming qualities of *G. catenatum*. Toxicity among these substances varied, suggesting that the polyunsaturated fatty acid 18:4ω-3 can potentially act as a more effective allelochemical (LD_{50} = 1.7 ± 0.19 mg L^{-1} at 24 h), followed by 20:5ω-3 (LD_{50} = 3.6 ± 0.17 mg L^{-1} at 24 h) and the saturated fatty acid 16:0 (LD_{50} = 6.2 ± 1.05 at 48 h). Our results suggest these substances can act, at least partially, as allelochemicals, with PUFA being the most effective metabolites. These results contribute in elucidating the potential role of ROS and FFA in allelopathy in marine phytoplankton communities.

KEYWORDS

allelopathy, dinoflagellate, phytoplankton, reactive oxygen species, fatty acids

1 Introduction

Harmful algae blooms (HABs) are natural phenomena regulated by diverse factors, including biological interactions, such as predation, competition, and parasitism (Solé et al., 2006; Mazzillo et al., 2011; Gleason et al., 2015). Allelopathy is a biochemical interaction in which metabolites are released to the surrounding environment by donor species, in this context called allelochemicals, that influence the growth and development of target species (Rizvi et al., 1992). This phenomenon has been described as concerning only chemical interactions among competing microalgae species in which one or various allelochemicals can cause inhibitory effects on susceptible species; it has been hypothesized that allelopathy encompasses adaptations that confer competitive advantages to some species over others (Keating, 1977; Legrand et al., 2003). In microalgae, the injurious effects of allelopathy on target species include the promotion of oxidative stress, by raising the endogenous production of reactive oxygen species (ROS) and inducing changes in the activity of antioxidant enzymes (Campos et al., 2013; Lu et al., 2017), photosystem II interference, electron transport chain blockage and subsequent photosynthesis inhibition (Weir et al., 2004; Qian et al., 2009; Zhu et al., 2010), disruption of cell membranes and nucleic acids which lead to high mortality in susceptible cells (Granéli et al., 2008).

The allelopathic potential of marine toxins has been explored. It has been reported that okadaic acid produced by *Prorocentrum lima* can contribute partially to allelopathy with co-occurring species (Windust et al., 1996; Sugg and VanDolah, 1999). Also, karlotoxins produced by dinoflagellates of the genus *Karlodinium* have been reported as allelochemicals capable of negatively affecting competitors and prey (Adolf et al., 2006; Wang et al., 2020). Continuous efforts to describe the mechanisms of these interactions has demonstrated that allelopathy is mediated by a great diversity of metabolites (Chaïb et al., 2021). The mechanisms of allelopathy are diverse and remain largely unknown (Śliwińska-Wilczewska et al., 2021). Nonetheless, research efforts have demonstrated that various types of secondary metabolites such as alkaloids like fischerellin (Hagmann and Jüttner, 1996; Gantar et al., 2008) and cyclical peptides like microcystins and portoamide (Kearns and Hunter, 2001; Leão et al., 2010) are associated with allelopathy in microalgae. Particularly, fatty acids in their pure form, also known as free fatty acids (FFA) have been suggested as allelochemicals produced by freshwater phytoplankton species like *Botryococcus braunii*, *Chlorella vulgaris* and *Peridinium bipes* (Uchida et al., 1988; Chiang et al., 2004; Song et al., 2017). In marine microalgae, the potential herbicidal qualities of fatty acids produced by the cyanophyte *Lyngbya aestuarii* have been explored (Entzeroth et al., 1985). Wang et al. (2023) studied the inhibitory effects of linoleic acid (18:2ω-3, LA) on the marine dinoflagellate *Karenia mikimotoi* which caused the reduction of photosynthetic pigments and an increase in ROS in the organism.

Laboratory studies have suggested that the chain-forming paralytic shellfish toxin (PST) producing dinoflagellate *Gymnodinium catenatum* can be negatively affected and dominated by the dinoflagellates *Gymnodinium impudicum*, *Margalefidinium polykrikoides*, and the raphidophyte *Chattonella marina* var. *marina* (Fernández-Herrera et al., 2016; Band-Schmidt et al., 2020; Fernández-Herrera et al., 2021; Fernández-Herrera et al., 2022). The co-occurrence and the dominance of *C. marina* over *G. catenatum* was previously reported in the Gulf of California during a HAB event (López-Cortés et al., 2011). Although the identity of the allelochemicals involved in these interactions remains unknown, *C. marina* and *M. polykrikoides*, both competitors of *G. catenatum* via allelopathy, have been hypothesized to produce compounds, including FFA and ROS that can act as allelochemicals (Marshall et al., 2003; Marshall et al., 2005; Tang and Gobler, 2010). The toxicity of FFA and ROS has been previously explored in microalgae, suggesting that susceptibility can vary among species, type of substance and laboratory conditions (Wu et al., 2006; Vale, 2018).

With the aim to document the effects of FFA and ROS towards *G. catenatum*, a strain of *C. marina* from Bahía de La Paz, Gulf of California, was isolated and its fatty acid profile was determined to pinpoint the FFA that can potentially act as allelochemicals towards *G. catenatum*. The response to the most abundant fatty acids in *C. marina* in their pure form was evaluated, as well as H_2O_2 under laboratory conditions. Changes in cell density, chain length and morphology were evaluated.

2 Materials and methods

2.1 Strains and culture conditions

Monoalgal strains of *G. catenatum* and *C. marina* isolated from Bahía de La Paz, Mexico, were cultured in modified GSe medium (Bustillos-Guzmán et al., 2015). Strain details are shown in Table 1. Culture conditions remained constant throughout this study at 24 ± 1°C, salinity of 34, 12/12 h light/dark cycle and an irradiance of ~150 µmol photons m^{-1} s^{-1}.

For fatty acid analysis, strains were grown in triplicate 1 L cultures in 2 L Erlenmeyer flasks. Each strain was harvested by centrifugation (1000 rpm, 5 min, 4°C) during mid exponential growth phase. For the dose-response bioassays, sterile, polystyrene flat bottom six welled (5 mL) culture plates were employed.

TABLE 1 Origin and isolation of strains.

Species	Strain designation	Origin	Isolation date	Isolated by
Gymnodinium catenatum	GCBAPAZ-10	Bahía de La Paz, Gulf of California, Mexico	June 2017	C. J. Band Schmidt
Chattonella marina	CMBAPAZ-2	El Mogote, Bahía de La Paz, Gulf of California, Mexico	June 2019	L. J. Fernández-Herrera

2.2 Growth curves

To determine the exponential growth rate, strains were grown in triplicate in batch cultures in 150 mL of modified GSe medium (Bustillos-Guzmán et al., 2015) within 300 mL Erlenmeyer flasks. Samples of each strain were taken every 48 h. Samples of *G. catenatum* were fixed in Lugol's iodine solution and samples of *C. marina* were fixed with hepes buffered paraformaldehyde (Katano et al., 2009). Cell densities were obtained by counting in a 1 mL Sedgewick-Rafter chamber with an optic microscope (Primo Star, Carl Zeiss). Specific growth rates were calculated using the following equation: $k = \ln (N_2/N_1)/(t_2 - t_1)$; considering the ratio of the cell counts (N_2/N_1) at times t_2 and t_1 (Guillard and Ryther, 1962).

For fatty acid analysis, strains were grown in triplicate 1 L cultures in 2 L Erlenmeyer flasks. Each strain was harvested by centrifugation (1000 rpm, 5 min, 4°C) in the mid exponential growth phase. For the dose-response bioassays, sterile, polystyrene flat bottom six welled (5 mL) culture plates were employed.

2.3 PCR, DNA sequencing and phylogenetic analysis

A 2 mL sample of the raphidophyte *C. marina* was harvested in exponential growth phase. The supernatant was removed by gentle aspiration. DNA extraction was carried out using the Quick-DNA Miniprep Plus Kit (Zymo Research). The genomic DNA samples were amplified by polymerase chain reaction (PCR) using a thermal cycler (MJ Mini, Bio-Rad, Hercules, CA). The reaction was carried out in 50 µL, employing PCR master mix 2X solution (ThermoFisher) and primers 28S F (5′-TATGCTTAAATTC AGCGGGT-3′) and 28S R (5′-GTGAACCTGCAGAAGGATCA-3′) (Hosoi-Tanabe et al., 2006). PCR conditions are the following: denaturation at 94°C for 1 min, 35 cycles at 94°C for 30 s, annealing at 55°C for 2 min, extension at 72°C for 3 min, and final extension at 72°C for 10 min.

The PCR products were purified using the Zymoclean™ Gel DNA Recovery Kit (Thermo Fisher). Sequencing was carried out by MCLAB (San Francisco, CA) using an automated DNA sequencer ABI 3730XL (Thermo Fisher). The sequence was analyzed using the software BioEdit (ver. 7.2.) and the software Molecular Evolutionary Genetics Analysis (MEGA, ver. 10.1.7.). Existing *Chattonella* sequences (Supplementary Table S1) were obtained using NCBI Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence alignment was performed with the MUSCLE algorithm. To select the best nucleotide substitution model, a comparison between 24 models was carried out using the Bayesian Information Criterion. Consequently, a phylogenetic analysis with Maximum Likelihood Algorithm was carried out. A sequence of *Heterosigma akashiwo* was used as an outgroup. The sequence of the strain of *Chattonella* from Bahía de La Paz was submitted to Genbank (Accession number: OR642744).

2.4 Fatty acids extraction and analysis

Lipid extraction and analysis was carried out using ~100 mg of biomass of each strain, samples were placed in thick-walled glass tubes, 6 mL of Folch's solution (chloroform/methanol, 2:1 v/v), 10 µL of the antioxidant butylated hydroxytoluene (BHT) and 10 µL of the internal standard fatty acid 23:0 were added to each sample. Samples were sonicated during 15 min and kept at -20°C for 24 h.

Samples were dried under nitrogen gas (N_2), 1000 µL of Boron trifluoride-methanol (BF_3 10%) were added to the samples and were incubated at 85-95°C during 5 min. After cooling for 5 min at room temperature, 1000 µL of hexane was added to each sample to extract fatty acid methyl esters (FAME). Samples were centrifuged at 2000 rpm for 5 min at 5°C. The hexane phase was washed with deionized water.

Sample analysis was performed in a gas chromatograph (6890 N, Agilent Technologies, Santa Clara, CA) employing a DB-23 silica column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness, 50% Cyanopropyl; 50% Methylpolysiloxane, Agilent J&W, USA), using helium as the carrier gas and a temperature ramp from 110 to 220°C. Identification of FAME was carried out by comparing retention times from the samples and the known standards (47885-U, Supelco, Bellefonte, USA). The concentration of each fatty acid was corrected by the response of the corresponding known standard and the internal standard (23:0). Results were presented as the proportion of each fatty acid out of the total fatty acids.

2.5 Dose-response bioassays

The three most abundant fatty acids from *C. marina* were acquired as pure analytic standards (Sigma Aldrich). Preliminary bioassays were carried out to determine the range of final concentrations where the inhibitory effect could be observed for each fatty acid. A range of final concentrations was prepared for each fatty acid analyzed by diluting with dimethyl sulfoxide (DMSO): from 0 to 32 mg L^{-1} for palmitic acid (PA), from 0 to 10 mg L^{-1} for stearidonic acid (SDA), and from 0 to 15 mg L^{-1} for eicosapentaenoic acid (EPA). For H_2O_2, a range of concentrations from 0 to 200 µM was prepared by diluting a 30% w/v solution. For bioassays with fatty acids, DMSO was used as carrier solvent (0.4% of the total volume). Treatments were done in triplicate with an initial cell density of 500 cells mL^{-1} of *G. catenatum*. Cultures of *G. catenatum* in GSe medium and of *G. catenatum* in GSe medium with the addition of DMSO (0.4%) were used as negative controls. In addition, mixed cultures of *G. catenatum* and *C. marina* in a 1:2 cell density proportion were used to compare the effects on *G. catenatum*.

2.6 Statistical analyses

All statistical analyses were carried out in R software (ver. 4.2.1, R Core Team, 2021). Normality and homogeneity of the data was

tested by Shapiro-Wilk and Levene tests, respectively. When parametric conditions were met, Welch's t test was used in the comparison of two treatments and one-way ANOVA was used for comparisons between three or more treatments followed by a *post-hoc* Tukey's test. Data on the proportions of chain lengths did not meet the parametric criteria; therefore, non-parametric Kruskal-Wallis tests followed by Conover-Iman tests were performed. For lethal dose estimation, the drm function from the "dose response curve" package (Ritz et al., 2015) was employed to model the effect of the potential allelochemicals on the cell density of *G. catenatum*. Several fitted models were compared and the most adequate were chosen based on the Akaike's information criterion.

3 Results

3.1 Phylogenetic analysis of the raphidophyte strain

The amplification of a partial sequence of the 28S region in *Chattonella* sp. strain resulted in a product of approximately 625 bp. A phylogenetic tree was constructed from sequences of 28S region of rDNA from 11 isolates of the *Chattonella* genus (Figure 1). Phylogenetic analysis placed *Chattonella* sp. strain within the *C. marina* cluster with a bootstrap support of 100%. Sequences of *C. subsalsa* were placed in a separated clade at bootstrap support of 97%, both clades were separated from the *H. akashiwo* sequence used as an outgroup.

3.2 Growth

The maximum cell density for *G. catenatum* in monoalgal cultures was 6,289 ± 375 cells mL^{-1} which was reached at day 14, while for *C. marina* 39,209 ± 2,489 cells mL^{-1} were reached at day 16 (Figure 2). The specific growth rates were 0.26 and 0.15 for *G. catenatum* and *C. marina*, respectively; doubling times were 0.21 div day^{-1} and 0.38 div day^{-1} for *G. catenatum* and *C. marina*, respectively.

3.3 Fatty acids

The fatty acid composition obtained for *G. catenatum* and *C. marina* is shown in Table 2. The most abundant saturated fatty acid was PA (16:0) in both species; *C. marina* in a proportion of 12.5 ± 0.01%, and in *G. catenatum* of 27.4 ± 0.93%. The proportion of total saturated fatty acids was 21.9 ± 1.02% for *C. marina* and 36.2 ± 2.64% for *G. catenatum*. The most abundant monounsaturated fatty acids were palmitoleic acid (16:1ω-7, 4.2 ± 0.13) in *C. marina* and oleic acid (18:1ω-9, 2.3 ± 0.45%) in *G. catenatum*. Both strains had a low percentage of monounsaturated fatty acids (12.4 ± 1.4% in *C. marina* and 7.1 ± 0.28% in *G. catenatum*).

The most abundant polyunsaturated fatty acids (PUFA) in *C. marina* were EPA (20:5ω-3, 35.4 ± 0.71%) and SDA (18:4ω-3, 15.4 ± 0.36%). In contrast, the most abundant PUFA in *G. catenatum* were docosahexaenoic acid (22:6ω-3, 23.5 ± 1.72%) and EPA (19.22 ± 0.51%). Additionally, octadecapentaenoic acid (18:5ω-3) was detected in *G. catenatum* as the third most abundant PUFA (9.0 ± 0.18%). The total proportion of PUFA was 65.62 ± 1.46% for *C. marina* and 56.6 ± 2.50% for *G. catenatum*.

3.4 Effect of free fatty acids on the cell density and chain length of *G. catenatum*

Addition of FFA in the culture medium caused mortality in *G. catenatum* (Figure 3A). A mortality higher than 90% was observed

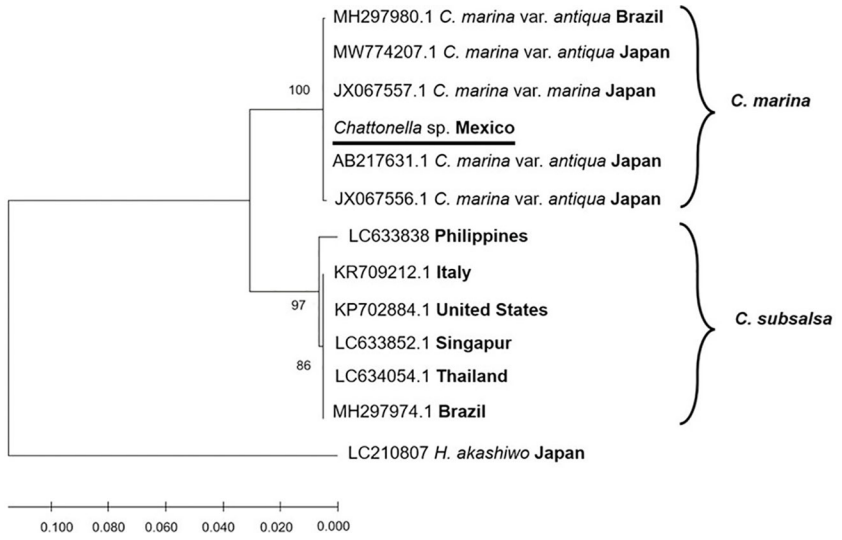

FIGURE 1
Phylogenetic tree of 28S rDNA sequences of *Chattonella* strains, using maximum likelihood method with the Kimura two-parameter (K2P) model. The strain used in the present study is underlined.

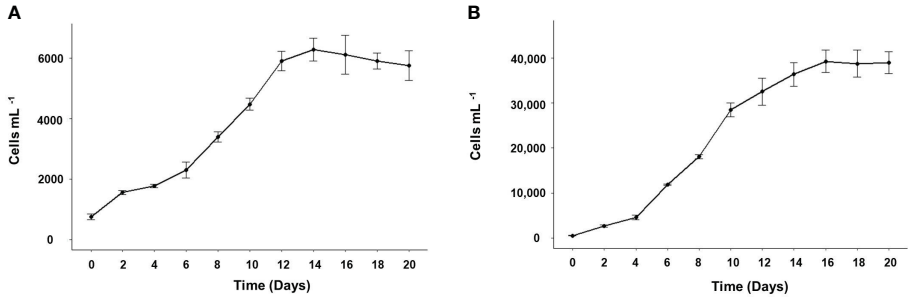

FIGURE 2
Growth curves of *Gymnodinium catenatum* **(A)** and *Chattonella marina* **(B)** in modified GSe media at 24°C and salinity of 34. Data are shown as mean ± standard deviation (n = 3).

with 32 mg L^{-1} of PA starting from 48 h and continued increasing at 72 h (> 95%). The lowest PA concentration (1 mg L^{-1}) at 48 h showed a cell density of *G. catenatum* higher than the mixed culture with *C. marina* (ANOVA $F_{3,8}$ = 5.32, $p < 0.05$). In contrast, there were no significant differences in cell density between the control, the control with DMSO, and the mixed culture with *C. marina*.

Exposure to the free polyunsaturated fatty acids SDA and EPA caused mortalities above 90% at 24 h (Figures 3B, C) with the highest concentrations analyzed (15 mg L^{-1} and 5 mg L^{-1}, respectively). Exposure to SDA at 24 h caused a decrease in cell density at concentrations above 1 mg L^{-1}; an apparent increase in cell density was observed at concentrations from 3 to 5 mg L^{-1} SDA at 48 h and 72 h. Mortalities were also higher than 90% at 48 h and 72 h in experiments with EPA with the highest concentrations used (9 to 15 mg L^{-1}).

Changes in chain length of *G. catenatum* after exposure to the fatty acids is shown in Figure 4. When exposed to the highest concentration of PA (32 mg L^{-1}) at 48 h, the proportion of individual cells (91.9 ± 10.80%) was significantly higher than in the control (56.7 ± 3.16%), the control with DMSO (62.9 ± 1.00%), and the mixed culture with *C. marina* (58.7 ± 1.58%, Kruskal-Wallis χ^2 = 22.54, $p < 0.005$). The proportion of individual cells of *G. catenatum* at 72 h was also higher with 32 mg L^{-1} of PA (91.9 ± 10.80%) than in the control (44.4 ± 6.10%), the control with DMSO (47.1 ± 3.86%), and the mixed culture with *C. marina* (39.2 ± 3.06%, Kruskal-Wallis χ^2 = 22.99, $p < 0.005$).

Exposure to 2 mg L^{-1} of SDA at 24 h (Figure 4) resulted in a higher proportion of individual cells of *G. catenatum* (55.7 ± 5.10%) than in the control (36.1 ± 4.82%), the control with DMSO (42.6 ± 3.01%), and the mixed culture with *C. marina* (32.7 ± 2.78%), as well as with treatments with the highest concentrations of SDA (3 mg L^{-1}, 40.1 ± 2.50%; 4 mg L^{-1}, 41.9 ± 3.04%; 5 mg L^{-1}, 35.2 ± 2.34%; (Kruskal-Wallis χ^2 = 22.81, $p < 0.005$). In contrast, at 72 h after exposure to SDA, individual cells of *G. catenatum* were higher in the control (50.7 ± 7.87%) than in the treatments with the highest concentrations of SDA (3 mg L^{-1}, 27.4 ± 1.48%; 4 mg L^{-1}, 28.2 ± 1.57%; 5 mg L^{-1}, 28.4 ± 2.74%), (Kruskal-Wallis χ^2 = 20.37, $p < 0.01$). At 48 h of exposure, the proportion of two-cell chains was higher in the treatment with 5 mg L^{-1} SDA (50.06 ± 0.38%) than in the control with DMSO (37.28 ± 1.53), the mixed culture (35.61 ± 6.45) and the treatment with 1 mg L^{-1} (30.44 ± 4.5, Kruskal-Wallis χ^2 = 19.926, $p < 0.05$).

When *G. catenatum* was exposed to the highest concentration of EPA (15 mg L^{-1}) at 24 h the proportion of individual cells (67.2 ± 24.88%) was significantly higher than the control (31.8 ± 2.30%), the control with DMSO (38.0 ± 3.59%) and the mixed culture (41.0 ± 1.27%) with *C. marina* (Kruskal-Wallis χ^2 = 32.87, $p < 0.05$). Furthermore, single cells of *G. catenatum* were also higher with 15 mg L^{-1} EPA at 72 h (41.7 ± 5.01%) than in the control with DMSO (16.8 ± 0.64%, Kruskal-Wallis χ^2 = 28.78, $p < 0.01$). At 72 h the proportion of 2-cell chains was significantly higher in treatments with 9 to 15 mg L^{-1} (> 30%) than in the control (22.06 ± 2.01%), the control with DMSO (21.80 ± 2.49%) and the mixed culture (20.28 ± 1.36%, Kruskal-Wallis χ^2 = 33.662, $p < 0.05$). chains of 5 or more cells disappeared from the treatment with 15 mg L^{-1} EPA from 24 to 72 h.

The toxicity of FFA to *G. catenatum* is shown in Table 3. The toxicity of the three FFA tested varied throughout the sampling times. The LD_{50} of the free saturated fatty acid PA was calculated after 48 h to be 6.2 ± 1.05 mg L^{-1}. The LD_{50} of the two free PUFA tested, EPA and SDA, was calculated to be 3.6 ± 0.17 mg L^{-1} and 1.7 ± 0.19 mg L^{-1}, respectively at 24 h. In addition, the Cedergreen-Ritz-Streibig model (Supplementary Figure S1) suggests a stimulatory response in the surviving cells of *G. catenatum* exposed to concentrations between 3 to 5 mg L^{-1} SDA at 48 h ($p < 0.05$).

3.5 Effect of hydrogen peroxide on the cell density and chain length of *G. catenatum*

Exposure to H_2O_2 in the culture medium caused mortality in *G. catenatum* (Figure 5A). A mortality higher than 80% was observed with the highest concentrations of H_2O_2 used (180 µM and 200 µM) at 12 h of exposure. At 24 h of exposure to 180 µM and 200 µM of H_2O_2 a mortality higher than 90% was observed. Moreover, at 12 h of exposure, the cell density of *G. catenatum* was significantly lower in the mixed culture with *C. marina* than in the control (ANOVA, $F_{(7,16)}$ = 29.95, $p < 0.05$). However, at 24 h of exposure no differences between cultures were observed. Additionally, a higher cell density of *G. catenatum* compared to the control was observed at 6 h of exposure to 60 µM H_2O_2 ($t_{(2.44)}$ = -4.8654, $p < 0.05$).

10.3389/frpro.2024.1302560

TABLE 2 Fatty acid composition of *Chattonella marina* and *Gymnodinium catenatum* strains from Bahía de La Paz, Gulf of California, Mexico.

Fatty acid	*C. marina* (%)	*G. catenatum* (%)
12:0	0.2 ± 0.06	0.0 ± 0.00
13:0	0.5 ± 0.04	0.3 ± 0.02
14:0	7.1 ± 0.26	1.9 ± 0.06
15:0	0.3 ± 0.01	0.05 ± 0.02
16:0	12.5 ± 0.01	27.4 ± 0.93
18:0	1.1 ± 0.76	4.14 ± 1.70
20:0	0.0 ± 0.00	1.8 ± 0.13
22:0	0.1 ± 0.05	0.0 ± 0.00
24:0	0.1 ± 0.02	0.2 ± 0.04
Σ Saturated	**21.9 ± 1.02**	**36.2 ± 2.64**
14:1 ω-8	0.5 ± 0.02	0.3 ± 0.56
15:1 ω-8	0.1 ± 0.05	0.0 ± 0.00
16:1 ω-9	2.2 ± 0.23	1.3 ± 0.50
16:1 ω-7	4.2 ± 0.13	1.3 ± 0.30
16:1 ω-5	2.4 ± 0.09	0.4 ± 0.01
18:1 ω-9	1.9 ± 0.05	2.3 ± 0.45
18:1 ω-7	0.8 ± 0.29	0.7 ± 0.01
Σ Monounsaturated	**12.4 ± 1.4**	**7.1 ± 0.28**
18:2 ω-6	1.7 ± 0.05	2.8 ± 0.00
18:3 ω-6	0.9 ± 0.03	0.0 ± 0.00
18:3 ω-3	3.7 ± 0.15	0.8 ± 0.09
18:4 ω-3	15.4 ± 0.36	0.6 ± 0.01
18:5 ω-3	0.0 ± 0.00	9.0 ± 0.18
20:2 ω-6	0.1 ± 0.01	0.0 ± 0.00
20:3 ω-3	0.1 ± 0.01	0.0 ± 0.00
20:4 ω-6	3.1 ± 0.07	0.0 ± 0.00
20:4 ω-3	0.0 ± 0.00	0.0 ± 0.00
20:5 ω-3	35.4 ± 0.71	19.2 ± 0.51
21:4 ω-6	0.2 ± 0.01	0.03 ± 0.01
22:5 ω-6	0.0 ± 0.00	0.08 ± 0.14
22:6 ω-3	5.1 ± 0.37	23.5 ± 1.72
Σ Polyunsaturated	**65.6 ± 1.46**	**56.6 ± 2.50**

Data are shown as mean ± standard deviation (n = 3).

Changes in chain length of *G. catenatum* after exposure to H_2O_2 is shown in Figure 5B.When *G. catenatum* was exposed to H_2O_2 for 6 h, the proportion of individual cells was significantly higher in the treatments with 20 µM (55.6 ± 10.65%), 120 µM (39.4 ± 13.48%), 180 µM (71.9 ± 0.56%), and 200 µM H_2O_2 (61.6 ± 2.24%) than in the control (27.7 ± 0.91%, Kruskal-Wallis χ^2 = 29.565, $p < 0.05$). After 24 h, the proportion of individual cells was higher in the treatments with 180 µM (57.0 ± 7.43%) and 200 µM H_2O_2 (48.0 ± 14.72%) than in the treatment with 20 µM H_2O_2 (17.9 ± 5.06%), the control (17.0 ± 3.17%) and the mixed culture (17.2 ± 6.49%, Kruskal-Wallis χ^2 = 26.482, $p < 0.05$). The toxicity of H_2O_2 to *G. catenatum* is shown in Table 3. The LD_{50} of H_2O_2 decreased from 161.2 ± 4.69 µM at 6 h to 103.1 ± 8.14 µM at 24 h.

3.6 Changes in the morphology of *G. catenatum* after exposure to FFA and H_2O_2

Morphological changes were observed in *G. catenatum* after exposure to the FFA and H_2O_2 (Figures 6–9). Evident changes were observed at 48 and 72 h exposure at concentrations above 4 mg L^{-1} PA, which included swelling, loss of motility and flagella, detachment of the external membrane, lysis, and apparent chlorosis at the highest concentrations (32 mg L^{-1} PA, Figure 6).

Exposure to concentrations above 2 mg L^{-1} EPA also caused evident changes in cell morphology at 24 h including swelling and vacuolization, the loss of motility and increased signs of cell lysis (Figure 7). Similar changes were observed in *G. catenatum* after exposure to SDA at 24 h, where the general loss of motility, the appearance of orange-brown accumulation bodies and swelling were also observed. Signs of lysis were more apparent at concentrations above 2 mg L^{-1} SDA; the presence of cells forming chains was also observed (Figure 8).

Cells of *G. catenatum* exposed to H_2O_2 showed similar effects on cell morphology as FFA, including the loss of motility, swelling, membrane detachment, lysis and chlorosis when exposed to the highest concentrations (180 – 200 µM H_2O_2, Figure 9). Few *G. catenatum* cells showing a similar deformation pattern were also found in mixed cultures with *C. marina*. These changes in cell morphology included swelling and vacuolization, loss of motility, presence of internal accumulation bodies and detachment of the external membrane.

4 Discussion

The results of this study show that PA, EPA, and SDA were the most abundant fatty acids in the strain of *C. marina* in exponential growth phase. Previous studies suggested these fatty acids are among the most abundant in raphidopyte strains of the species *H. akashiwo*, *Fibrocapsa japonica*, and *C. marina* from different geographic regions (Nichols et al., 1987; Marshall et al., 2002; Giner et al., 2008; Band-Schmidt et al., 2012; Dorantes-Aranda et al., 2013). The *C. marina* strain employed in the present study showed EPA as the most abundant fatty acid. This is in agreement with Band-Schmidt et al. (2012) who also suggested that EPA was the most abundant fatty acid (19.8 – 34.9%) in four strains of *Chattonella* and *F. japonica* from the Gulf of California, Mexico in exponential growth phase. Furthermore, Dorantes-Aranda et al. (2013) suggested the relative abundance of FFA in *C. marina* can vary; while some strains showed a higher abundance of FFA in the stationary phase, the strain CMCV-1 of *C. marina* from Bahía de La Paz showed a higher abundance in the exponential growth phase.

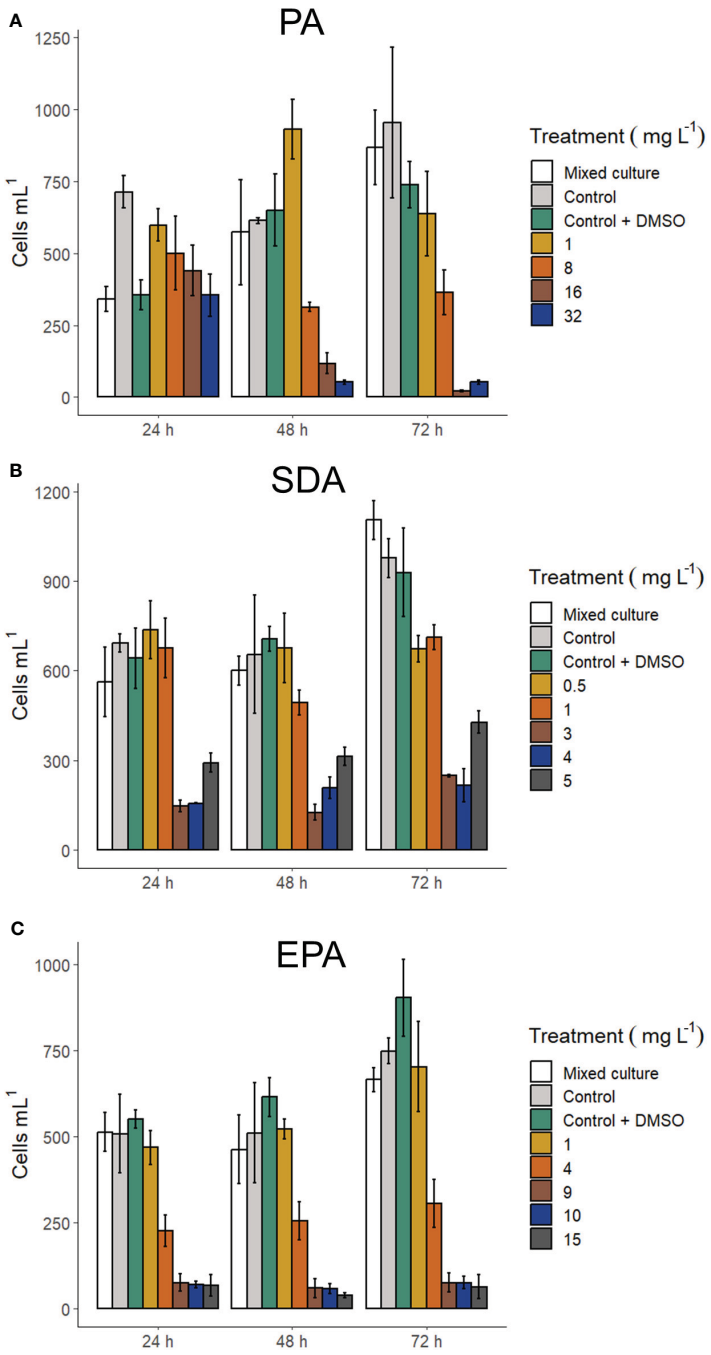

FIGURE 3
Cell density of *Gymnodinium catenatum* exposed to fatty acids: Palmitic acid **(A)**, stearidonic acid **(B)**, and eicosapentaenoic acid **(C)**. Mixed culture (*G. catenatum* and *Chattonella marina*, 1:2), control (*G. catenatum* culture in GSe medium), and control with DMSO (0.4%). Data are shown as mean ± standard deviation (n = 3).

The dinoflagellate *G. catenatum* contained a high proportion of PA (27.4 ± 0.93%), EPA (19.2 ± 0.51%), 22:6 ω-3 (DHA, 23.5 ± 1.72%) and 18:5 ω-3(9.0 ± 0.18%). A similar profile was reported by Hallegraeff et al. (1991) for laboratory cultures of *G. catenatum* and field samples, although the fatty acid 18:5 ω-3 was reported in low proportions (from 1.2 to 2.3%). This fatty acid has been reported to be quite variable among dinoflagellates (Mansour et al., 1999).

Negative effects of FFA and H_2O_2 on *G. catenatum* were observed in this study. Changes in cell density and signs of lysis in the experiments suggest the toxicity of these substances varies with the concentration and exposure time. Also, exposure to the highest concentrations of PA and H_2O_2 caused an apparent chlorosis in *G. catenatum* cells. It has been suggested that FFA are capable of interfering with the electron transport chain in chloroplasts (Venediktov and Krivoshejeva, 1983). It has also

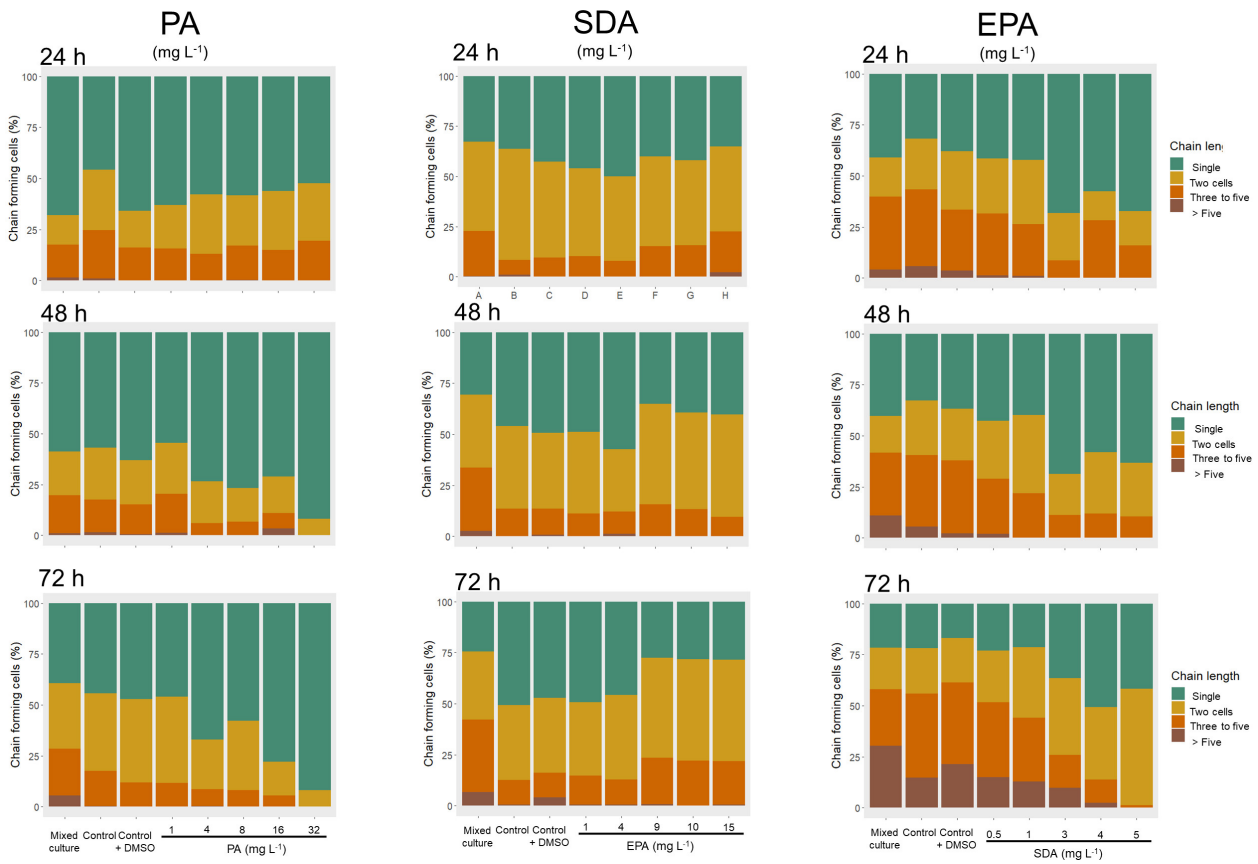

FIGURE 4
Chain length of *Gymnodinium catenatum* exposed to free fatty acids: Palmitic acid (16:0, PA), stearidonic acid (18:4ω-3, SDA), and eicosapentaenoic acid (20:5ω-3, EPA). Mixed culture (*G. catenatum* and *Chattonella marina*, 1:2), control (*G. catenatum* culture in GSe medium), and control with DMSO (0.4%). Data are shown as mean ± standard deviation (n = 3).

been suggested that FFA can cause membrane disruption and the detachment of pigments in freshwater microalgae (Wu et al., 1998; Wu et al., 2006). However, further studies are needed to elucidate the mechanisms involved.

TABLE 3 Toxicity of fatty acids and hydrogen peroxide towards *Gymnodinium catenatum* in modified GSe medium.

Fatty Acid	24 h		48 h		72 h	
	LD_{50}	LD_{20}	LD_{50}	LD_{20}	LD_{50}	LD_{20}
PA	>32	>32	6.2 ± 1.05	3.1 ± 0.54	8.0 ± 0.59	5.8 ± 0.79
EPA	3.6 ± 0.17	2.9 ± 0.18	3.8 ± 0.17	3.0 ± 0.23	3.5 ± 0.13	1.2 ± 0.53
SDA	1.7 ± 0.19	1.3 ± 0.15	–	–	–	–
	6 h		12 h		24 h	
H_2O_2	LD_{50}	LD_{20}	LD_{50}	LD_{20}	LD_{50}	LD_{20}
	161.2 ± 4.69	159.2 ± 3.62	112.8 ± 7.51	90.1 ± 5.94	103.1 ± 8.14	73.4 ± 8.59

LD_{50} and LD_{20} indicate the concentration that caused a decrease of 50% and 20% in cell abundance, respectively.
Data are shown as mean ± standard deviation (n = 3).

Chattonella marina and *M. polykrikoides* have been suggested to produce ROS and FFA as metabolites involved in their ichthyotoxicity (Marshall et al., 2003; Dorantes-Aranda et al., 2009; Dorantes-Aranda et al., 2013). The role of ROS and FFA as potential allelochemicals produced by raphidophytes has been previously suggested by Marshall et al. (2003). Their results showed that ROS production is density dependent in raphidophytes. Additionally, the behavior of different strains of raphidophytes towards ROS production varied, including observations of autolysis in a strain of *C. marina* due to its ROS production, while other strains were resistant to higher concentrations of ROS. Their results also showed that EPA in its free form can be an allelochemical towards bacteria, an effect that is enhanced by the presence of superoxide. It has been reported that H_2O_2 production in *C. marina* takes place in the intracellular medium, while the production of $O_2^{\bullet-}$ takes place at the cell surface (Kim et al., 2007). The intracellular accumulation of H_2O_2 in raphidophyte cells has been suggested, as the measured H_2O_2 is higher in ruptured *C. marina* cell suspensions compared to intact cell suspensions (Oda et al., 1994; Oda et al., 1995). A mechanism for the exogenous release of FFA to the marine environment has not been suggested for *C. marina*. Cell rupture has been identified as a key factor in *C. marina* ichthyotoxicity, and an increase in FFA proportion has been observed in the profile of ruptured cells,

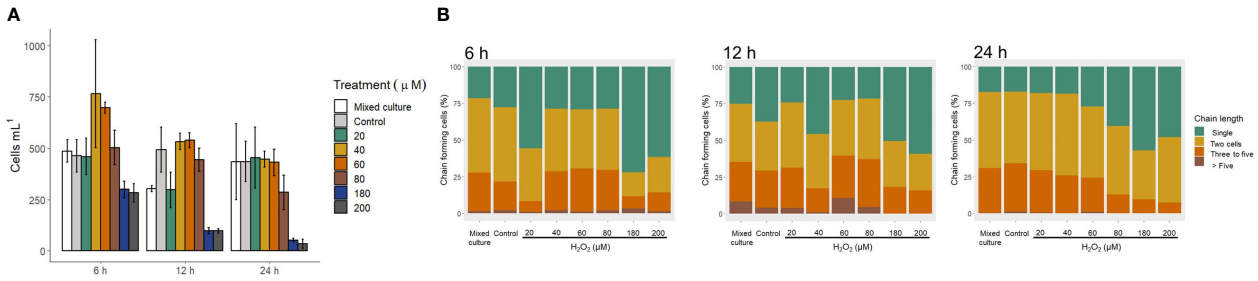

FIGURE 5
Gymnodinium catenatum exposed to hydrogen peroxide (H_2O_2). Changes in cell density **(A)** and chain length **(B)**. Mixed culture (*G. catenatum* and *Chattonella marina*, 1:2), and control (*G. catenatum* culture in modified GSe medium).

suggesting that dying cells are more toxic due to the increased release of FFA and ROS (Dorantes-Aranda et al., 2013; Dorantes-Aranda et al., 2015). It is, therefore, possible that *C. marina* populations can benefit from their dying conspecifics upon releasing putative allelochemicals from ruptured cells. This hypothesis can be explored in future studies.

Supplementary Table S2 shows differences in measurements of H_2O_2 in *Chattonella* reported in the literature. *Chattonella marina* strains show substantial variability in H_2O_2 production. Laboratory culture conditions are suggested to play a significant role in ROS production in raphidophytes; particularly, different light intensities have been reported to cause differences in H_2O_2 production among *C. marina* strains (Dorantes-Aranda et al., 2013; Li et al., 2015). According to these measurements, an exceedingly high cell density of *C. marina* cells would be needed to reach the LD_{50} H_2O_2 concentrations estimated for *G. catenatum* in this study. Indicating that the release of H_2O_2 alone cannot be responsible for the allelopathic effects towards *G. catenatum*. Previous studies have suggested that the role of ROS on the ichthyotoxicity of harmful algae is not a direct one, and experiments that involve the chemical addition of ROS substances do not mimic the toxicity of live isolates (Marshall et al., 2003; Tang et al., 2005).

The effects of the FFA on *G. catenatum* in this study, suggest that the PUFA, EPA and SDA cause mortality at lower concentrations and in a shorter time than the saturated fatty acid PA. Particularly, SDA showed the most effective inhibitory capacity at 24 h of exposure. It has been previously suggested that fatty acid carbon chain length is related to the algicidal potential of these substances (Zhu et al., 2021). These observations are also in accordance with previous studies on the effect of FFA on

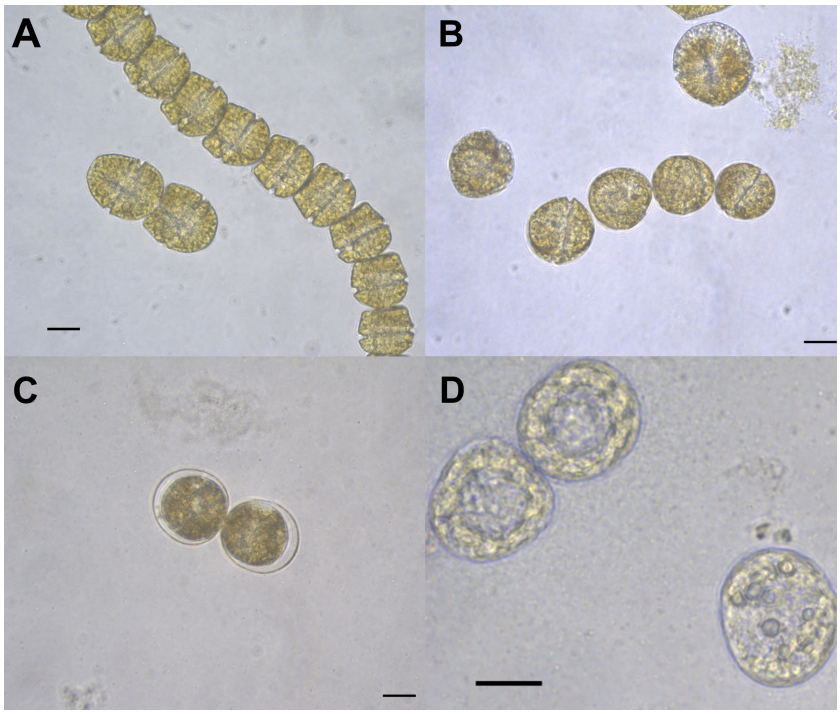

FIGURE 6
Gymnodinium catenatum cells exposed to palmitic acid (PA) at 72 h. Cell swelling **(A, B)**, cells surrounded by detached external membrane **(C)**, dead cells **(D)**. Scale bar = 20 µm.

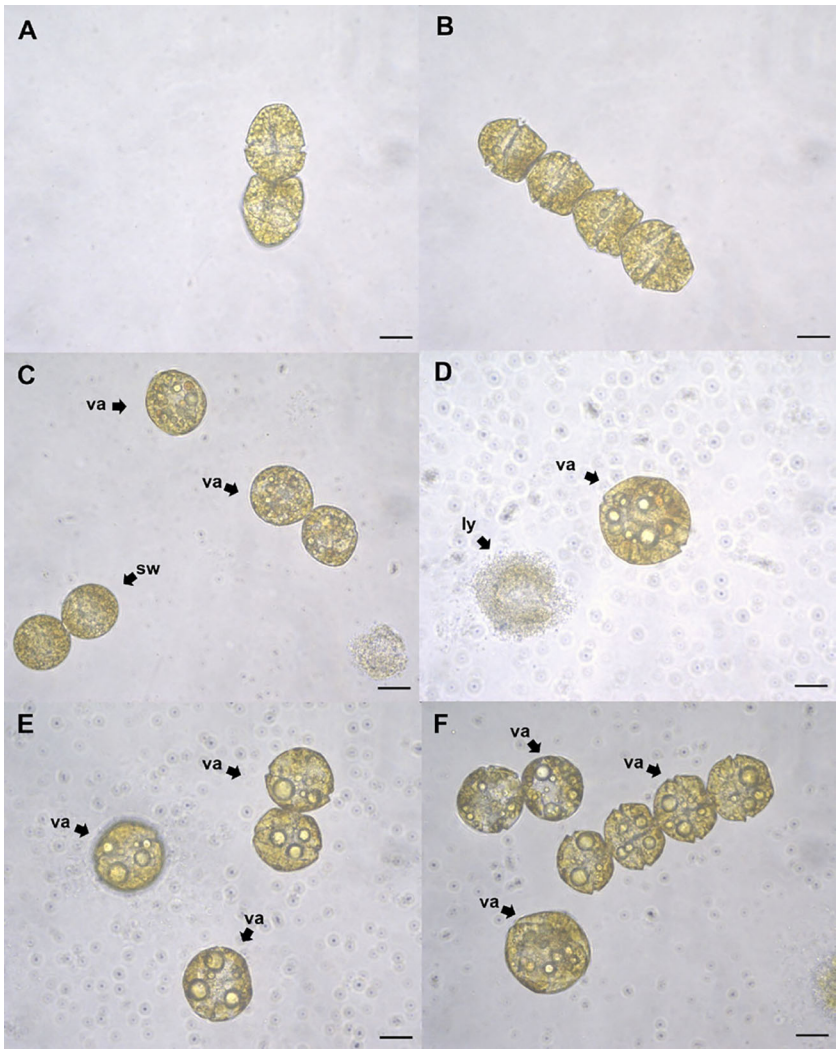

FIGURE 7
Gymnodinium catenatum cells exposed to eicosapentaenoic acid (EPA) at 24 (h) Control with dimethyl sulfoxide (DMSO) **(A)**, cells exposed to 1 mg L^{-1} **(B)**, 3 mg L^{-1} **(C)**, 6 mg L^{-1} **(D)**, 8 mg L^{-1} **(E)**, and 10 mg L^{-1} **(F)**. Swollen cells (sw), vacuolization (va), lysis (ly). Scale bar = 20 µm.

freshwater microalgae. Chiang et al. (2004) reported that *B. braunii* can produce and release a mixture of FFA as allelochemicals, including PA which showed lower toxicity than the PUFA 18:3ω-3, 18:2ω-7 and 18:1ω-9. Moreover, Wu et al. (2006) reported that the toxicity of PA and EPA can vary among different species of microalgae, suggesting cells that lack an external covering structure beyond a thin mucilage layer are more susceptible to the effects of FFA. While it is suggested that *G. catenatum* has amphiesmal vesicles, it is generally recognized as a naked planktonic dinoflagellate that lacks cellulose plates (Morey-Gaines, 1982). This characteristic could explain its susceptibility to the effects of FFA.

The observed toxicity of FFA could suggest a relevant participation of the oxidized products from these substances (Aliotta et al., 1990). Previous experiments on the ichthyotoxicity of *C. marina* and other microalgae reported that the negative effects of fatty acids, particularly PUFA, can be enhanced by the presence of ROS (Marshall et al., 2003; Marshall et al., 2005; Mardones et al., 2015). Also, it has been suggested that the ichthyotoxicity among strains of *C. marina* is influenced mainly by differences in ROS production rather than by differences in fatty acid composition (Shikata et al., 2021). A synergistic role of different allelochemicals has also been reported in experiments with *Cochlodinium geminatum* (Wu et al., 2021). In this study, the strain of *C. marina* (CMBAPAZ-2) employed in mixed cultures with *G. catenatum* did not cause a significant decrease in cell density at the end of the experiments, as opposed to the *C. marina* var. *marina* strain previously reported to induce high mortalities of *G. catenatum* (Fernández-Herrera et al., 2016; Fernández-Herrera et al., 2021; Fernández-Herrera et al., 2022) which suggests that the allelopathic capabilities of *C. marina* can vary between strains or taxonomical varieties.

Our results show that low concentrations of PA (1 mg L^{-1}) and H_2O_2 (60 µM) caused an increase in cell density of *G. catenatum* at punctual sampling times (48 h for PA; 6 h for H_2O_2). The presence of an initial stimulating phase has been reported in experiments

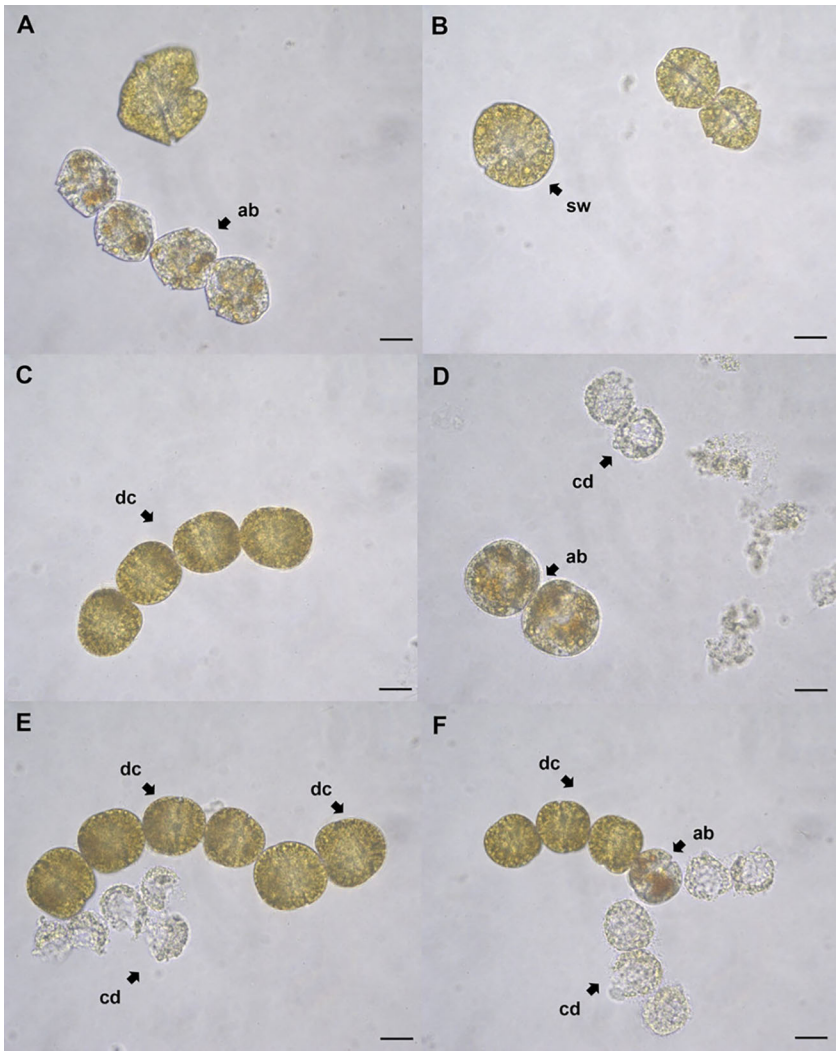

FIGURE 8
Gymnodinium catenatum cells exposed to stearidonic acid (SDA) at 48 h. Affected cells exposed to 0.5 mg L^{-1} **(A)**, 1 mg L^{-1} **(B)**, 2 mg L^{-1} **(C)** 3 mg L^{-1} **(D)**, 4 mg L^{-1} **(E)**, and 5 mg L^{-1} **(F)**. Cell with orange-brown accumulation bodies (ab); cell swelling (sw); deformed chain-forming cells (dc); dead cells (cd). Scale bar = 20 µm.

with chlorellin exposure on freshwater species (DellaGreca et al., 2010). Vale (2018) also reported the presence of an initial stimulating phase in the cell density of *G. catenatum* exposed to low concentrations of H_2O_2. It has been suggested that ROS can have cell signaling capabilities related to growth processes at low concentrations (Holmström and Finkel, 2014). Additionally, Wang et al. (2023) reported that at low concentrations of the PUFA linoleic acid (0.1 mg L^{-1}) promotes the growth of *K. mikimotoi* while higher concentrations (0.5 mg L^{-1}) cause inhibitory effects on growth, carotenoid and polysaccharide biosynthesis, porphyrin, and sphingolipid metabolism, as well as an increase in ROS production.

Exposure to FFA and H_2O_2 in our experiments also produced changes in chain formation of *G. catenatum*, in which the decrease in cell density often coincided with an increase in single cell proportions. A similar response was also reported in the experiments by Vale (2018), in which concentrations above 245 µM of H_2O_2 under halogen light caused single cells to dominate in cultures of *G. catenatum*. It has been suggested that high irradiance conditions can enhance the toxicity of H_2O_2 in consequence to an increase in its transformation into hydroxyl radical (OH) (Drábková et al., 2007).

It has been reported that a low number of *G. catenatum* cells can survive the allelopathic effect of *C. marina*, among these, chain forming cells show a higher growth rate, suggesting the survival strategy of *G. catenatum* involves chain formation (Fernández-Herrera et al., 2022). Our results also show that a low number of *G. catenatum* cells can survive exposure to the highest concentrations used of fatty acids and H_2O_2. In addition, treatments with the highest concentrations of SDA in our experiments showed an apparent growth stimulation at 48 h and 72 h. This was observed after a significant decrease in cell density at 24 h. Additionally, a decrease in single cells concomitant with an increase in chain formation was observed in treatments with concentrations of SDA above 2 mg L^{-1} at 48 h and 72 h, which suggests growing cells favor chain formation. An apparent stimulatory phase in a

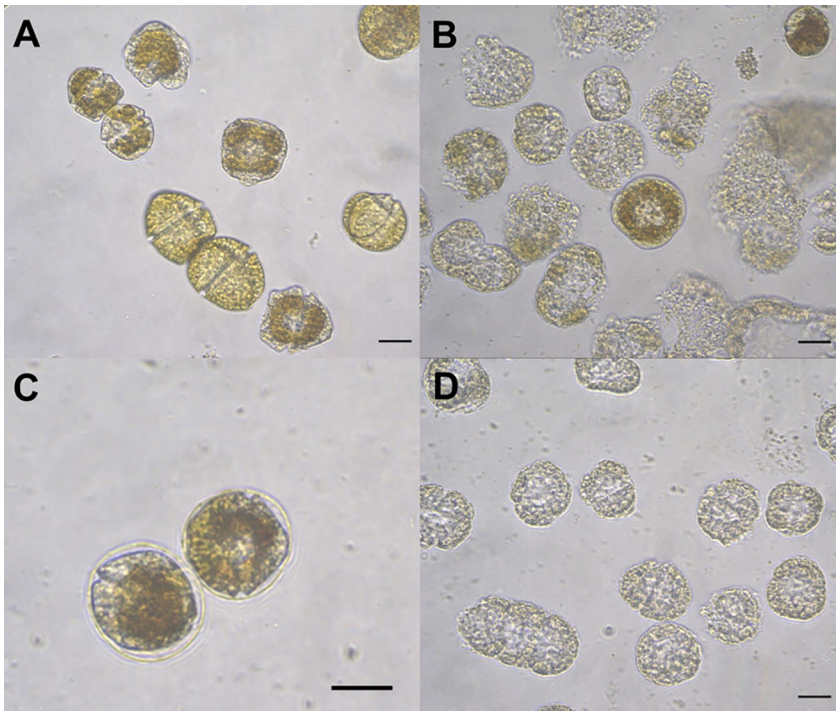

FIGURE 9
Gymnodinium catenatum cells exposed to hydrogen peroxide H_2O_2 at 24 h. Affected cells exposed to 100 µM showing deformation and membrane detachment **(A)**, affected cells exposed to 200 µM showing deformation, chlorosis and cell death **(B–D)**. Scale bar = 20 µm.

dose response curve may suggest that the surviving cells, after exposure to toxic agents, increase their growth rate in response to the inhibitory potential of the substances (Calabrese and Baldwin, 2001). The surviving cells exposed to the highest concentrations of SDA may be showing a compensatory response related to growth and chain formation which is observable at 48 h and 72 h after exposure.

The allelopathic effect of *C. marina* var. *marina* towards *G. catenatum* was reported by Fernández-Herrera et al. (2016). Their results showed that inhibition of *G. catenatum* was higher in mixed co-cultures of both species than in co-cultures with no direct contact and exposure to cell filtrates of *C. marina*. This suggested that *C. marina* can inhibit the growth of *G. catenatum* through the release of allelochemicals, although cell contact or proximity between the cells of both species can cause a higher inhibition towards *G. catenatum*. The changes observed in *G. catenatum* cells after exposure to FFA and H_2O_2 are similar, coinciding mainly in the loss of motility, swelling, detachment of the external membrane, and alterations in the length of chain formation. These effects are in accordance with the previous allelopathy experiments with *C. marina* var. *marina*, and the dinoflagellates *M. polykrikoides* and *G. impudicum* (Fernández-Herrera et al., 2016; Band-Schmidt et al., 2020; Fernández-Herrera et al., 2021; Fernández-Herrera et al., 2022).

Previous studies suggested that *Chattonella marina* var. *antiqua* can affect *Akashiwo sanguinea* via allelopathy (Qiu et al., 2011). Similar negative effects of allelopathy have been reported between other species; cells of *Heterocapsa triquetra* and *Scrippsiella trochoidea* exposed to cell-free filtrates of *Alexandrium ostenfeldii*, showed loss of motility (Tillmann et al., 2007; Hakanen et al., 2014). Studies in different species exposed to allelochemicals from *Alexandrium leei* reported that *M. polykrikoides* can be negatively affected showing alterations in chain formation, cell deformations and lysis, while the naked dinoflagellates *G. catenatum*, *Karlodinium veneficum* along with the haptophyte *Isochrysis galbana* were not dominated by *A. leii* (Shang et al., 2021). The precise identity of the allelochemicals produced by *Alexandrium* species remains unknown. However, *Alexandrium tamarense* has been suggested to produce lytic compounds targeting membrane related sterols (Ma et al., 2011).

Chain formation in dinoflagellates such as *G. catenatum* may constitute a survival strategy by enhancing motility and allowing displacement of cells (Doblin et al., 2006) out of the reach of allelochemicals in the marine environment; however, this strategy may be limited by the spatial restrictions of laboratory cultures. While being stressed, naked dinoflagellates could employ alternative defense mechanisms facing allelopathy, including chain formation or separation (Vale, 2018; Shang et al., 2021; Fernández-Herrera et al., 2022). Due to the spatial constraints of the experimental setup, the strategy of chain cell formation of *G. catenatum* as a means of escape to allelochemicals was not clearly observed in the presence of FFA and H_2O_2. Therefore, the effects on chain cell formation were not conclusive.

In conclusion, results from this study suggest that the fatty acids PA, EPA, and SDA along with H_2O_2 can individually cause deleterious effects on *G. catenatum*. Alterations in chain formation and cell morphology are similar to the expected effects of allelopathy towards *G. catenatum* by competing species, and

suggest that these substances can be, at least partially, involved with allelopathic interactions in marine environments. Toxicity among the fatty acids varies, suggesting that PUFA with shorter carbon chains can potentially act as more effective allelochemical substances, causing a decrease in cell density at lower concentrations and within a shorter time than saturated fatty acids. However, the overall allelopathic effect of *C. marina* towards *G. catenatum* may be caused by a mixture of various compounds including ROS and FFA. It remains to be seen if other uncharacterized metabolites participate in this effect.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://www.ncbi.nlm.nih.gov/; OR642744.

Author contributions

ME-Y: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. CB-S: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. TZ-S: Conceptualization, Data curation, Methodology, Resources, Supervision, Validation, Writing – review & editing. IL-V: Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – review & editing. LFH: Methodology, Visualization, Writing – review & editing. EP-M: Data curation, Methodology, Resources, Validation, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. ME-Y was a recipient of a student fellowship CONACyT-1106788 and BEIFI. CB-S is a COFFA-IPN and EDI-IPN fellow. This project was funded by institutional projects (SIP 20231019) and by the Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCyT, Ciencia básica A1-S-14968).

Acknowledgments

We are grateful for the technical support of O. Arjona-López for the analysis of fatty acids, O. Lugo-Lugo for assistance in analyses for oxidative stress indicators, and D.I. Rojas-Posadas for the molecular taxonomy methods.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/frpro.2024.1302560/full#supplementary-material

References

Adolf, J. E., Bachvaroff, T. R., Krupatkina, D. N., Nonogaki, H., Brown, P. J. P., Lewitus, A. J., et al. (2006). Species specificity and potential roles of *Karlodinium micrum* toxin. *Afr. J. Mar. Sci.* 28, 415–419. doi: 10.2989/18142320609504189

Aliotta, G., Greca, M. D., Monaco, P., Pinto, G., Pollio, A., and Previtera, L. (1990). *In vitro* algal growth inhibition by phytotoxins of *Typha latifolia* L. *J. Chem. Ecol.* 16, 2637–2646. doi: 10.1007/BF00988075

Band-Schmidt, C. J., Martínez-López, A., Bustillos-Guzmán, J. J., Carreón-Palau, L., Morquecho, L., Olguín-Monroy, N. O., et al. (2012). Morphology, biochemistry, and growth of raphidophyte strains from the Gulf of California. *Hydrobiologia* 693, 81–97. doi: 10.1007/s10750-012-1088-y

Band-Schmidt, C. J., Zumaya-Higuera, M. G., López-Cortés, D. J., Leyva-Valencia, I., Quijano-Scheggia, S. I., and Hernández-Guerrero, C. J. (2020). Allelopathic effects of *Margalefidinium polykrikoides* and *Gymnodinium impudicum* in the growth of *Gymnodinium catenatum*. *Harmful Algae* 96, 101846. doi: 10.1016/j.hal.2020.101846

Bustillos-Guzmán, J. J., Band-Schmidt, C. J., Durán-Riveroll, L. M., Hernández-Sandoval, F. E., López-Cortés, D. J., Núñez-Vázquez, E. J., et al. (2015). Paralytic toxin profile of the marine dinoflagellate *Gymnodinium catenatum* Graham from the Mexican Pacific as revealed by LC-MS/MS. *Food Addit. Contam. Part. A* 32, 381–394. doi: 10.1080/19440049.2014.1000978

Calabrese, E. J., and Baldwin, L. A. (2001). U-shaped dose-responses in biology, toxicology, and public health. *Annu. Rev. Public Health* 22, 15–33. doi: 10.1146/annurev.publhealth.22.1.15

Campos, A., Araújo, P., Pinheiro, C., Azevedo, J., Osório, H., and Vasconcelos, V. (2013). Effects on growth, antioxidant enzyme activity and levels of extracellular proteins in the green alga *Chlorella vulgaris* exposed to crude cyanobacterial extracts and pure microcystin and cylindrospermopsin. *Ecotoxicol. Environ. Saf.* 94, 45–53. doi: 10.1016/j.ecoenv.2013.04.019

Chaïb, S., Pistevos, J. C. A., Bertrand, C., and Bonnard, I. (2021). Allelopathy and allelochemicals from microalgae: An innovative source for bio-herbicidal compounds and biocontrol research. *Algal Res.* 54, 102213. doi: 10.1016/j.algal.2021.102213

Chiang, I. Z., Huang, W. Y., and Wu, J. T. (2004). Allelochemicals of *Botryococcus braunii* (Chlorophyceae). *J. Phycol.* 40, 474–480. doi: 10.1111/j.1529-8817.2004.03096.x

DellaGreca, M., Zarrelli, A., Fergola, P., Cerasuolo, M., Pollio, A., and Pinto, G. (2010). Fatty acids released by *Chlorella vulgaris* and their role in interference with *Pseudokirchneriella subcapitata*: Experiments and Modelling. *J. Chem. Ecol.* 36, 339–349. doi: 10.1007/s10886-010-9753-y

Doblin, M. A., Thompson, P. A., Revill, A. T., Butler, E. C. V., Blackburn, S. I., and Hallegraeff, G. M. (2006). Vertical migration of the toxic dinoflagellate *Gymnodinium catenatum* under different concentrations of nutrients and humic substances in culture. *Harmful Algae* 5, 665–677. doi: 10.1016/j.hal.2006.02.002

Dorantes-Aranda, J. J., García-de la Parra, L. M. G., Alonso-Rodríguez, R., and Morquecho, L. (2009). Hemolytic activity and fatty acids composition in the ichthyotoxic dinoflagellate *Cochlodinium polykrikoides* isolated from Bahía de la Paz, Gulf of California. *Mar. pollut. Bull.* 58, 1401–1405. doi: 10.1016/j.marpolbul.2009.06.007

Dorantes-Aranda, J. J., Nichols, P. D., David Waite, T., and Hallegraeff, G. M. (2013). Strain variability in fatty acid composition of *Chattonella marina* (Raphidophyceae) and its relation to differing ichthyotoxicity toward rainbow trout gill cells. *J. Phycol.* 49, 427–438. doi: 10.1111/jpy.12053

Dorantes-Aranda, J. J., Seger, A., Mardones, J. I., Nichols, P. D., and Hallegraeff, G. M. (2015). Progress in understanding algal bloom-mediated fish kills: The role of superoxide radicals, phycotoxins and fatty acids. *PloS One* 10, e0133549. doi: 10.1371/journal.pone.0133549

Drábková, M., Admiraal, W., and Maršálek, B. (2007). Combined exposure to hydrogen peroxide and light-selective effects on cyanobacteria, green algae, and diatoms. *Environ. Sci. Technol.* 41, 309–314. doi: 10.1021/es060746i

Entzeroth, M., Mead, D. J., Patterson, G. M. L., and Moore, R. E. (1985). A herbicidal fatty acid produced by *Lyngbya aestuarii*. *Phytochemistry* 24, 2875–2876. doi: 10.1016/0031-9422(85)80017-0

Fernández-Herrera, L. J., Band-Schmidt, C. J., López-Cortés, D. J., Hernández-Guerrero, C. J., Bustillos-Guzmán, J. J., and Núñez-Vázquez, E. (2016). Allelopathic effect of *Chattonella marina* var. *marina* (Raphidophyceae) on *Gymnodinium catenatum* (Dinophycea). *Harmful Algae* 51, 1–9. doi: 10.1016/j.hal.2015.10.009

Fernández-Herrera, L. J., Band-Schmidt, C. J., Zenteno-Savín, T., Leyva-Valencia, I., Hernández-Guerrero, C. J., Hernández-Sandoval, F. E., et al. (2022). Changes in Toxin Production, Morphology and Viability of *Gymnodinium catenatum* Associated with Allelopathy of *Chattonella marina* var. *marina* and *Gymnodinium impudicum*. *Toxins (Basel)* 14, 616. doi: 10.3390/toxins14090616

Fernández-Herrera, L. J., Band-Schmidt, C. J., Zenteno-Savín, T., Leyva-Valencia, I., Hernández-Guerrero, C. J., and Muñoz-Ochoa, M. (2021). Cell death and metabolic stress in *Gymnodinium catenatum* induced by allelopathy. *Toxins (Basel)* 13, 506. doi: 10.3390/toxins13070506

Gantar, M., Berry, J. P., Thomas, S., Wang, M., Perez, R., and Rein, K. S. (2008). Allelopathic activity among Cyanobacteria and microalgae isolated from Florida freshwater habitats. *FEMS Microbiol. Ecol.* 64, 55–64. doi: 10.1111/j.1574-6941.2008.00439.x

Giner, J. L., Zhao, H., and Tomas, C. (2008). Sterols and fatty acids of three harmful algae previously assigned as *Chattonella*. *Phytochemistry* 69, 2167–2171. doi: 10.1016/j.phytochem.2008.05.013

Gleason, F. H., Jephcott, T. G., Küpper, F. C., Gerphagnon, M., Sime-Ngando, T., Karpov, S. A., et al. (2015). Potential roles for recently discovered chytrid parasites in the dynamics of harmful algal blooms. *Fungal. Biol. Rev.* 29, 20–33. doi: 10.1016/j.fbr.2015.03.002

Granéli, E., Weberg, M., and Salomon, P. S. (2008). Harmful algal blooms of allelopathic microalgal species: The role of eutrophication. *Harmful Algae* 8, 94–102. doi: 10.1016/j.hal.2008.08.011

Guillard, R. R. L., and Ryther, J. H. (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* 1962 (8), 229–239. doi: 10.1139/m62-029

Hagmann, L., and Jüttner, F. (1996). Fischerellin A, a novel photosystem-II-inhibiting allelochemical of the cyanobacterium *Fischerella muscicola* with antifungal and herbicidal activity. *Tetrahedron Lett.* 37, 6539–6542. doi: 10.1016/0040-4039(96)01445-1

Hakanen, P., Suikkanen, S., and Kremp, A. (2014). Allelopathic activity of the toxic dinoflagellate *Alexandrium ostenfeldii*: Intra-population variability and response of co-occurring dinoflagellates. *Harmful Algae* 39, 287–294. doi: 10.1016/j.hal.2014.08.005

Hallegraeff, G. M., Nichols, P. D., Volkman, J. K., Blackburn, S. I., and Everitt, D. A. (1991). Pigments, fatty acids, and sterols of the toxic dinoflagellate *Gymnodinium catenatum*. *J. Phycol.* 27 (5), 591–599. doi: 10.1111/j.0022-3646.1991.00591.x

Holmström, K. M., and Finkel, T. (2014). Cellular mechanisms and physiological consequences of redox-dependent signalling. *Nat. Rev. Mol. Cell. Biol.* 15, 411–421. doi: 10.1038/nrm3801

Hosoi-Tanabe, S., Otake, I., and Sako, Y. (2006). Phylogenetic analysis of noxious red tide flagellates *Chattonella antiqua*, *C. marina*, *C. ovata*, and *C. verruculosa* (Raphidophyceae) based on the rRNA gene family. *Fish. Sci.* 72, 1200–1208. doi: 10.1111/j.1444-2906.2006.01277.x

Katano, T., Yoshida, M., Lee, J., Han, M. S., and Hayami, Y. (2009). Fixation of *Chattonella antiqua* and *C. marina* (Raphidophyceae) using Hepes-buffered paraformaldehyde and glutaraldehyde for flow cytometry and light microscopy. *Phycologia* 48, 473–479. doi: 10.2216/08-102.1

Kearns, K. D., and Hunter, M. D. (2001). Toxin-producing Anabaena flos-aquae induces settling of *Chlamydomonas reinhardtii*, a competing motile alga. *Microb. Ecol.* 42, 80–86. doi: 10.1007/s002480000086

Keating, K. I. (1977). Allelopathic influence on blue-green bloom sequence in a eutrophic lake. *Science* 196 (4292), 885–887. doi: 10.1126/science.196.4292.885

Kim, D., Nakashima, T., Matsuyama, Y., Niwano, Y., Yamaguchi, K., and Oda, T. (2007). Presence of the distinct systems responsible for superoxide anion and hydrogen peroxide generation in red tide phytoplankton *Chattonella marina* and *Chattonella ovata*. *J. Plankton Res.* 29, 241–247. doi: 10.1093/plankt/fbm011

Leão, P. N., Pereira, A. R., Liu, W. T., Ng, J., Pevzner, P. A., Dorrestein, P. C., et al. (2010). Synergistic allelochemicals from a freshwater cyanobacterium. *Proc. Natl. Acad. Sci. U.S.A.* 107, 11183–11188. doi: 10.1073/pnas.0914343107

Legrand, C., Rengefors, K., Fistarol, G. O., and Granéli, E. (2003). Allelopathy in phytoplankton - Biochemical, ecological and evolutionary aspects. *Phycologia* 42, 406–419. doi: 10.2216/i0031-8884-42-4-406.1

Li, X., Liu, T., Wang, K., and Waite, T. D. (2015). Light-induced extracellular electron transport by the marine raphidophyte *Chattonella marina*. *Environ. Sci. Technol.* 49, 1392–1399. doi: 10.1021/es503511m

López-Cortés, D. J., Band-Schmidt, C. J., Gárate-Lizárraga, I., Bustillos-Guzmán, J. J., Hernández-Sandoval, F. E., and Núñez-Vázquez, E. J. (2011). Co-ocurrencia de *Chattonella marina* y *Gymnodinium catenatum* en la Bahía de la Paz, Golfo de California (primavera 2009). *Hidrobiologica* 21, 185–196.

Lu, Z., Sha, J., Tian, Y., Zhang, X., Liu, B., and Wu, Z. (2017). Polyphenolic allelochemical pyrogallic acid induces caspase-3(like)-dependent programmed cell death in the cyanobacterium *Microcystis aeruginosa*. *Algal. Res.* 21, 148–155. doi: 10.1016/j.algal.2016.11.007

Ma, H., Krock, B., Tillmann, U., Bickmeyer, U., Graeve, M., and Cembella, A. (2011). Mode of action of membrane-disruptive lytic compounds from the marine dinoflagellate *Alexandrium tamarense*. *Toxicon* 58, 247–258. doi: 10.1016/j.toxicon.2011.06.004

Mansour, M. P., Volkman, J. K., Jackson, A. E., and Blackburn, S. I. (1999). The fatty acid and sterol composition of five marine dinoflagellates. *J. Phycol.* 35, 710–720. doi: 10.1046/j.1529-8817.1999.3540710.x

Mardones, J. I., Dorantes-Aranda, J. J., Nichols, P. D., and Hallegraeff, G. M. (2015). Fish gill damage by the dinoflagellate *Alexandrium catenella* from Chilean fjords: Synergistic action of ROS and PUFA. *Harmful Algae* 49, 40–49. doi: 10.1016/j.hal.2015.09.001

Marshall, J. A., Nichols, P. D., and Hallegraeff, G. M. (2002). Chemotaxonomic survey of sterols and fatty acids in six marine raphidophyte algae. *J. Appl. Phycol.* 14, 255–265. doi: 10.1023/A:1021101203543

Marshall, J. A., Nichols, P. D., Hamilton, B., Lewis, R. J., and Hallegraeff, G. M. (2003). Ichthyotoxicity of *Chattonella marina* (Raphidophyceae) to damselfish (*Acanthochromis polycanthus*): The synergistic role of reactive oxygen species and free fatty acids. *Harmful Algae* 2, 273–281. doi: 10.1016/S1568-9883(03)00046-5

Marshall, J. A., Ross, T., Pyecroft, S., and Hallegraeff, G. (2005). Superoxide production by marine microalgae: II. Towards understanding ecological consequences and possible functions. *Mar. Biol.* 147, 541–549. doi: 10.1007/s00227-005-1597-6

Mazzillo, F. F. M., Ryan, J. P., and Silver, M. W. (2011). Parasitism as a biological control agent of dinoflagellate blooms in the California Current System. *Harmful Algae* 10, 763–773. doi: 10.1016/j.hal.2011.06.009

Morey-Gaines, G. (1982). *Gymnodinium catenatum* Graham (Dinophyceae): morphology and affinities with armoured forms. *Phycologia* 21, 154–163. doi: 10.2216/i0031-8884-21-2-154.1

Nichols, P. D., Volkman, J. K., Hallegraeff, G. M., and Blackburn, S. I. (1987). Sterols and fatty acids of the red tide flagellates *Heterosigma akashiwo* and *Chattonella antiqua* (Raphidophyceae). *Phytochemistry* 26, 2537–2541. doi: 10.1016/S0031-9422(00)83871-6

Oda, T., Moritomi, J., Kawano, I., Hamaguchi, S., Muramatsu, T., and Ishimatsu, A. (1995). Catalase- and superoxide dismutase-induced morphological changes and growth inhibition in the red tide phytoplankton *Chattonella marina*. *Biosci. Biotechnol. Biochem.* 59, 2044–2048. doi: 10.1271/bbb.59.2044

Oda, T., Takeshita, S., Muramatsu, T., and Ishimatsu, A. (1994). Hydrogen peroxide production by the red-tide flagellate *Chattonella marina*. *Biosci. Biotechnol. Biochem.* 58, 957–958. doi: 10.1271/bbb.58.957

Qian, H., Xu, X., Chen, W., Jiang, H., Jin, Y., Liu, W., et al. (2009). Allelochemical stress causes oxidative damage and inhibition of photosynthesis in *Chlorella vulgaris*. *Chemosphere* 75, 368–375. doi: 10.1016/j.chemosphere.2008.12.040

Qiu, X., Yamasaki, Y., Shimasaki, Y., Gunjikake, H., Matsubara, T., Nagasoe, S., et al. (2011). Growth interactions between the raphidophyte *Chattonella antiqua* and the dinoflagellate *Akashiwo sanguinea*. *Harmful Algae* 11, 81–87. doi: 10.1016/j.hal.2011.08.001

R. Core Team (2021). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. (Vienna, Austria) v4.2.1. https://www.r-project.org/.

Ritz, C., Baty, F., Streibig, J. C., and Gerhard, D. (2015). Dose-response analysis using R. *PloS One* 10, e0146021. doi: 10.1371/journal.pone.0146021

Rizvi, S. J. H., Haque, H., Singh, V. K., and Rizvi, V. (1992). "A discipline called allelopathy," in *Allelopathy*. Eds. S. J. H. Rizvi and V. Rizvi (Dordrecht: Springer), 1–10.

Shang, L., Xu, Y., Leaw, C. P., Lim, P. T., Wang, J., Chen, J., et al. (2021). Potent allelopathy and non-PSTs, non-spirolides toxicity of the dinoflagellate *Alexandrium leei* to phytoplankton, finfish and zooplankton observed from laboratory bioassays. *Sci. Total Environ.* 780, 146484. doi: 10.1016/j.scitotenv.2021.146484

Shikata, T., Yuasa, K., Kitatsuji, S., Sakamoto, S., Akita, K., Fujinami, Y., et al. (2021). Superoxide production by the red tide-producing *Chattonella marina* complex (Raphidophyceae) correlates with toxicity to aquacultured fishes. *Antioxidants* 10, 1635. doi: 10.3390/antiox10101635

Śliwińska-Wilczewska, S., Wiśniewska, K., Konarzewska, Z., Cieszyńska, A., Barreiro Felpeto, A., Lewandowska, A. U., et al. (2021). The current state of knowledge on taxonomy, modulating factors, ecological roles, and mode of action of phytoplankton allelochemicals. *Sci. Total Environ.* 773, 145681. doi: 10.1016/j.scitotenv.2021.145681

Solé, J., Garcia-Ladona, E., and Estrada, M. (2006). The role of selective predation in harmful algal blooms. *J. Mar. Syst.* 62, 46–54. doi: 10.1016/j.jmarsys.2006.04.002

Song, H., Lavoie, M., Fan, X., Tan, H., Liu, G., Xu, P., et al. (2017). Allelopathic interactions of linoleic acid and nitric oxide increase the competitive ability of *Microcystis aeruginosa*. *ISME J.* 11, 1865–1876. doi: 10.1038/ismej.2017.45

Sugg, L. M., and VanDolah, F. M. (1999). No evidence for an allelopathic role of okadaic acid among ciguatera-associated dinoflagellates. *J. Phycol.* 35, 93–103. doi: 10.1046/j.1529-8817.1999.3510093.x

Tang, J. Y., Anderson, D. M., and Au, D. W. (2005). Hydrogen peroxide is not the cause of fish kills associated with *Chattonella marina*: cytological and physiological evidence. *Aquat Toxicol.* 72, 351–360. doi: 10.1016/j.aquatox.2005.01.007

Tang, Y. Z., and Gobler, C. J. (2010). Allelopathic effects of *Cochlodinium polykrikoides* isolates and blooms from the estuaries of Long Island, New York, on co-occurring phytoplankton. *Mar. Ecol. Prog. Ser.* 406, 19–31. doi: 10.3354/meps08537

Tillmann, U., John, U., and Cembella, A. (2007). On the allelochemical potency of the marine dinoflagellate *Alexandrium ostenfeldii* against heterotrophic and autotrophic protists. *J. Plankton. Res.* 29, 527–543. doi: 10.1093/plankt/fbm034

Uchida, A., Shimada, A., and Ishida, Y. (1988). Antibacterial and antialgal substances produced by the dinoflagellate *Peridinium bipes*. *Nippon Suisan Gakkaishi (Japanese Edition)* 54, 1941–1945. doi: 10.2331/suisan.54.1941

Vale, P. (2018). Resistance to hydrogen peroxide highlights *Gymnodinium catenatum* (Dinophyceae) sensitivity to geomagnetic activity. *Photochem. Photobiol.* 94, 95–104. doi: 10.1111/php.12815

Venediktov, P. S., and Krivoshejeva, A. A. (1983). The mechanisms of fatty-acid inhibition of electron transport in chloroplasts. *Planta* 159, 411–414. doi: 10.1007/BF00392076

Wang, C., Wang, R., Hu, L., Xi, M., Wang, M., Ma, Y., et al. (2023). Metabolites and metabolic pathways associated with allelochemical effects of linoleic acid on *Karenia mikimotoi*. *J. Hazard. Mater.* 447, 130815. doi: 10.1016/j.jhazmat.2023.130815

Wang, R., Wu, J., Zhou, S., Cao, R., and Chan, L. L. (2020). A preliminary study on the allelopathy and toxicity of the dinoflagellate *Karlodinium veneficum*. *Mar. pollut. Bull.* 158, 111400. doi: 10.1016/j.marpolbul.2020.111400

Weir, T. L., Park, S. W., and Vivanco, J. M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Curr. Opin. Plant Biol.* 7, 472–479. doi: 10.1016/j.pbi.2004.05.007

Windust, A. J., Wright, J. L. C., and McLachlan, J. L. (1996). The effects of the diarrhetic shellfish poisoning toxins, okadaic acid and dinophysistoxin-1, on the growth of microalgae. *Mar. Biol.* 126, 19–25. doi: 10.1007/BF00571373

Wu, X., Cai, Y., Shi, F., Duan, L., Zhang, Q., and Xu, N. (2021). Characterization of allelopathic compounds from the harmful dinoflagellate, *Cochlodinium geminatum*. *Harmful Algae* 107, 102069. doi: 10.1016/j.hal.2021.102069

Wu, J. T., Chiang, Y. R., Huang, W. Y., and Jane, W. N. (2006). Cytotoxic effects of free fatty acids on phytoplankton algae and cyanobacteria. *Aquat. Toxicol.* 80, 338–345. doi: 10.1016/j.aquatox.2006.09.011

Wu, J. T., Kuo-Huang, L. L., and Lee, J. (1998). Algicidal effect of *Peridinium bipes* on *Microcystis aeruginosa*. *Curr. Microbiol.* 37, 257–261. doi: 10.1007/s002849900375

Zhu, X., Dao, G., Tao, Y., Zhan, X., and Hu, H. (2021). A review on control of harmful algal blooms by plant-derived allelochemicals. *J. Hazard Mater.* 401, 123403. doi: 10.1016/j.jhazmat.2020.123403

Zhu, J., Liu, B., Wang, J., Gao, Y., and Wu, Z. (2010). Study on the mechanism of allelopathic influence on cyanobacteria and chlorophytes by submerged macrophyte (*Myriophyllum spicatum*) and its secretion. *Aquat. Tox.* 98, 196–203. doi: 10.1016/j.aquatox.2010.02.011