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ORIGINAL PAPER

Mortality on zoea stage of the Pacific white shrimp *Litopenaeus vannamei* caused by *Cochlodinium polykrikoides* (Dinophyceae) and *Chattonella* spp. (Raphidophyceae)

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Abstract *Cochlodinium polykrikoides* and *Chattonella* spp. are responsible for harmful algal blooms along the Mexican coasts. These microalgae have the ability to produce toxic compounds such as reactive oxygen species, brevetoxin-like compounds, nitric oxide, and free polyunsaturated fatty acids, which can be harmful to marine fauna. However, scarce information exists about the effect of these harmful phytoplankton species on potential zooplankton grazers. In this study, the effect of microalgae *Cochlodinium polykrikoides* and *Chattonella* spp. isolates from the Gulf of California were evaluated on larval stages of the shrimp *Litopenaeus vannamei*. Several bioassays were performed from nauplii to zoea stages. Nauplii of *Litopenaeus vannamei* were placed (1 well^{-1}) in microdilution plates, and in each well 1 mL of different cell concentrations (0.5, 3, and 6×10^3 cell mL^{-1}) of *Cochlodinium polykrikoides, Chattonella subsalsa, C. marina* var. *marina*, and *C. marina* var. *ovata* was added. Nontoxic *Chaetoceros calcitrans* and *Tetraselmis suecica* were used as controls. Higher mortalities were observed when *L. vannamei* larvae reached the zoea stage. Sudden increase in mortality was caused by *C. polykrikoides* at the beginning of the zoea stage, followed by *C. marina* var. *ovata* (LT_{50} ~ 1 day), *C. subsalsa* (LT_{50} 1 day 19 h), and *C. marina* var. *marina* (LT_{50} ~ 2 days). This study showed that *L. vannamei* larvae could be affected by *C. polykrikoides* and *Chattonella* spp. causing mortalities close to 100% during the zoea stage, when they start feeding on phytoplankton.

Keywords Harmful algal blooms · LT_{50} · Mortality rate · Shrimp larvae · Toxicity

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Introduction

Several harmful microalgae species have been detected along the Mexican coasts, some of which form recurrent harmful algal blooms (HAB). The most studied coastal zone with multiple records of HAB is the Gulf of California, followed by the Mexican Pacific, and to a lesser extent the coast of the Gulf of Mexico (Hernández-Becerril et al. 2007; Band-Schmidt et al. 2011; Pérez-Morales et al. 2015). Dinoflagellates and diatoms are the main groups implicated in HAB that have caused problems to public health, economic impact (fisheries and tourism), and environmental damages in Mexican coastal waters (Hernández-Becerril et al. 2007; Pérez-Morales and Band-Schmidt 2011). However, other phytoplankton groups have been identified as potentially harmful with high cell concentrations such as cyanobacteria, haptophytes, raphidophyceans, and silicoflagellates (Band-Schmidt et al. 2011; Pérez-Morales et al. 2015).

The dinoflagellate *Cochlodinium polykrikoides* and the raphidophyceans of the genus *Chattonella* have been reported off the coasts of Mexico (Fig. 1) (Cortés-Lara et al. 2004; Band-Schmidt et al. 2011; López-Cortés et al.

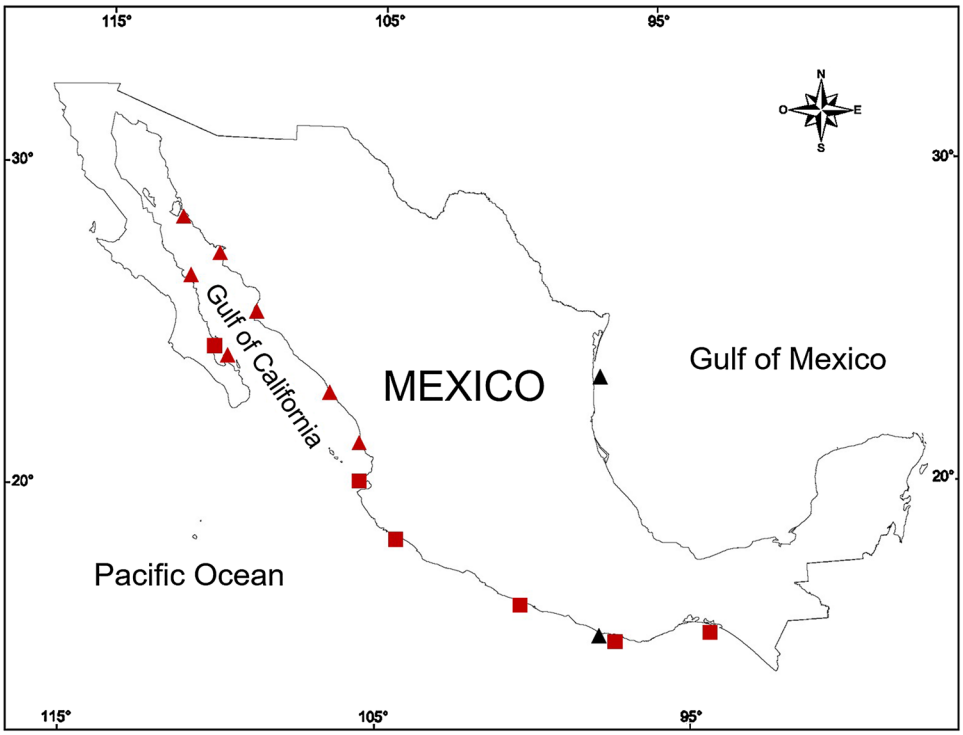

Fig. 1 Geographical distribution of *filled squares Cochlodinium polykrikoides* and *filled triangles Chattonella* spp. off the coasts of Mexico. *Red figures* indicate reports of blooms, *black figures* indicate presence (Cortés-Lara et al. 2004; Band-Schmidt et al. 2011; Gárate-Lizárraga et al. 2011; López-Cortés et al. 2011, 2014; Pérez-Morales and Band-Schmidt 2011)

2011, 2014; Pérez-Morales and Band-Schmidt 2011), with blooms in the Gulf of California, and in the coastal waters of the states of Nayarit, Colima, Guerrero, and Oaxaca on the Mexican Pacific. In some cases, the blooms of these species have been associated with high mortalities of marine fauna such as fish (*Apterchtus equatorialis, Astroscopus zephyreus, Balistes polylepis, Canthigaster punctatissima, Chaetodon humeralis, Cirrithus rivulatus, Citarichthys gilberti, Congriperla estriada, Diodon holocantus, Gnathypops snyderi, Haemulopsis nitidus, Holocantus passer, Letharchus rosenblatii, Muraena argus, Ophichthus triserialis, Ophiodon galaeoides*, and *Trachinotus paitensis*), shellfish (*Atrina maura, A. tuberculosa, Chione gnidia, Dosinia ponderosa, Hexaplex erythrotomus, Laevycardium elatum, Megapitaria aurantiaca, M. squalida*, and *Pinna rugosa*), and mollusks (*Octopus* spp.) causing great economic losses (Barraza-Guardado et al. 2004; Cortés-Lara et al. 2004; Cortés-Altamirano et al. 2006).

These marine microalgae have the ability to produce different toxic compounds, mainly reactive oxygen species (ROS) (Shimada et al. 1989, 1991, 1993; Tanaka et al. 1992, 1994; Oda et al. 1992, 1994, 1995, 1997, 1998; Kim et al. 1999, 2009; Tang and Gobler 2010; Band-Schmidt et al. 2012) brevetoxin-like compounds (not always found in toxic strains) (Onoue et al. 1990; Khan et al. 1995, 1996; Bourdelais et al. 2002; Marshall et al. 2003; Band-Schmidt et al. 2012), nitric oxide (Kim et al. 2006, 2008), free polyunsaturated fatty acids (Marshall et al. 2003; Dorantes-Aranda et al. 2009; Band-Schmidt et al. 2012), and hemagglutinin and hemolysin compounds (Fu et al. 2004; Kuroda et al. 2005; de Boer et al. 2009; Dorantes-Aranda et al. 2009). Moreover, some authors have reported that these are not the only toxic compounds produced by *Cochlodinium* and *Chattonella* species, since they may also produce different unidentified unstable molecular compounds (e.g., labile toxins) that could also be implicated in their toxicity (Kim et al. 2002, 2009; Marshall et al. 2003; Shen et al. 2010).

Besides, the shrimp fishery is a profitable economic activity in Mexico recognized by the national and international markets, with a national production of 161,852 tons occupying the 10th place in world production in 2012 (SAGARPA 2014). In the Mexican Pacific, shrimp catch is represented by four species: *Litopenaeus vannamei* "Pacific white shrimp", *L. stylirostris* "Blue shrimp", *Farfantepenaeus californiensis* "Brown shrimp" and *F. brevirostris* "Crystal shrimp". The National Fisheries Institute (INP 2012) indicates that this fishery generates the largest number of jobs in Mexico, with 57 shrimp processing plants, mainly in the states of Sinaloa, Sonora and Nayarit. In relation to shrimp aquaculture, the main farm raised species in these regions is *L. vannamei*, for which specialized techniques for intensive breeding and rearing have been developed with great success, reaching a national production of 100,321 tons in 2012 (SAGARPA 2013).

Although information exists about the negative effects of harmful phytoplankton on embryonic and larval development of several marine invertebrate organisms and

potential zooplankton grazers (Jiang et al. 2009; Almeda et al. 2011; Aylagas et al. 2014; Chang 2015; Lin et al. 2016), little is known about the impact on early stages of development of decapod crustacean species of commercial importance. In the Gulf of California, the Pacific white shrimp *L. vannamei* has a reproductive season from March to October with a gonad maturity from June to July, and *L. vannamei* larvae become dominant in the coastal zooplankton during these months (Garduño-Argueta and Calderón-Pérez 1994). Thus, harmful phytoplankton and shrimp larvae cohabit in the same coastal waters where HAB have been reported. In this sense, the consequences of the mortalities in *L. vannamei* larvae may significantly affect the local economy, since it is not known if the presence of these harmful microalgae can affect their survival in natural populations. Therefore, in this study the toxic effect of *C. polykrikoides* and *Chattonella* spp. isolates from the Gulf of California was evaluated on early life stages of the Pacific white shrimp *L. vannamei*.

Methods

Maintenance and growth of algal strains

Strains of the dinoflagellate *Cochlodinium polykrikoides* (COPAZ-8) and the raphidophyceans *Chattonella subsalsa* (CSNAV-1), *C. marina* var. *marina* (CSCV-1, CSPV-3 and CSJV-2), and *C. marina* var. *ovata* (CMOPAZ-1, 2 and 3) were isolated from different regions of the Gulf of California, Mexico. Isolation details of *Chattonella* strains are described in Band-Schmidt et al. (2012). The strain of *Cochlodinium polykrikoides* was isolated in October 2012 from a bloom in Ensenada de La Paz by C. Band-Schmidt. Strains of the diatom *Chaetoceros calcitrans* and the chlorophyte *Tetraselmis suecica* were used as controls. All microalgae strains were maintained in 50-mL culture tubes with 25 mL of medium, under controlled conditions at 24 ± 1 °C, salinity of 34 ± 1, photoperiod of 12:12 h light–dark cycle, and 150 µmol m^{-2} s^{-1} of illumination. The seawater was filtered (Whatman GF/F, VWR, Sweden, Ø 25 mm and pore size of 0.47 µm) with a vacuum pump to 380 mmHg (15 inches Hg) of pressure, autoclaved, and enriched with modified f/2 medium (Guillard and Ryther 1962) by the addition of selenium (H_2SeO_3 to 10^{-8} M), and the reduction of copper concentration ($CuSO_4$ to 10^{-8} M). Cultures of *C. polykrikoides, Chattonella* spp., *Chaetoceros calcitrans* and *Tetraselmis suecica* were doubled stepwise from 25 to 1000 mL, inoculating 10% of culture under the same conditions as described previously.

Growth curves

Each strain was grown in triplicate in 250-mL glass flasks in batch cultures (100 mL), and maintained under the same conditions. A sample of 2 mL of each culture was taken every second day until cultures reached the stationary phase of growth. Each sample was fixed in Lugol's iodine and counted in 1-mL Sedgwick Rafter counting slide (for *Cochlodinium polykrikoides* and *Chattonella* spp.) or Neubauer haemocytometer (for *Chaetoceros calcitrans* and *Tetraselmis suecica*) under an optical microscope (Carl Zeiss). Growth rates were calculated using cell concentration of each counting according to Guillard (1973).

Experimental design

Nauplii (N II) of the Pacific white shrimp *Litopenaeus vannamei* were placed in darkness. Nauplii were taken from container and placed in a Petri dish with a light source on one side. Nauplii that swam vigorously towards the light source were considered viable for microalgae experiments and isolated using sterile Pasteur pipettes, following the technique proposed by Pérez-Morales et al. (2016). Nauplii were acclimated at room temperature for 2 h before starting the exposure to the microalgae.

The sensitivity of *L. vannamei* larvae to *C. polykrikoides* and *Chattonella* spp. strains was determined. For this, one nauplii per well was incubated in 48 polystyrene microdilution well plates using sterile Pasteur pipettes. In each well, 1 mL of different cell concentrations of *C. polykrikoides* (COPAZ-8) (0.5, 3 and 6×10^3 cell mL^{-1}), *C. subsalsa* (CSNAV-1) (3 and 6×10^3 cell mL^{-1}), *C. marina* var. *marina* (CSCV-1) (0.5, 3 and 6×10^3 cell mL^{-1}), (CSPV-3) (3 and 6×10^3 cell mL^{-1}), (CSJV-2) (3×10^3 cell mL^{-1}), and *C. marina* var. *ovata* (CMOPAZ-1) (0.5, 3 and 6×10^3 cell mL^{-1}), (CMOPAZ-2) (3×10^3 cell mL^{-1}), (CMOPAZ-3) (3×10^3 cell mL^{-1}) was added. Cell concentrations tested (0.5, 3 and 6×10^3 cell mL^{-1}) correspond to initial log phase, middle log phase and stationary phase of growth. Nontoxic *Chaetoceros calcitrans* (0.5 and 1×10^6 cell mL^{-1}) and *Tetraselmis suecica* (0.5 and 1×10^5 cell mL^{-1}) were used as controls. Cell concentrations for controls were based on a previous work done by Pérez-Morales et al. (2016). It is worth noting that only cell concentrations of harmful microalgae that were available were used for the experiments. The plates with shrimp nauplii were incubated in triplicate with continuous light, and kept in an acrylic incubator at 23 ± 1 °C. Survival was evaluated every 24 h (mortality was considered when no movements were registered within 5 s). To consider the test as valid, the limit of mortality in the control was fixed to 25%. Experimental exposure of larvae lasted 168 h, starting from nauplii (N II) until they reached zoea (Z III) (Fig. 2).

Fig. 2 Initial development stages **a** nauplii II, **b** zoea I, **c** zoea II, and **d** zoea III of *Litopenaeus vannamei* exposed to several strains of *Cochlodinium polykrikoides* and *Chattonella* spp. from the Gulf of California

After this stage the carnivore feeding of the shrimp started. Descriptions of initial ontogeny of *L. vannamei* reported by Kitani (1986) were used to identify each developmental stage by direct observation under an optical microscope (Carl Zeiss).

Statistical analysis

Data set of *L. vannamei* mortality for each treatment was statistically tested. Differences among treatments were determined using one-way statistical analyses of variance (ANOVA). The minimum level of statistical significance was set at $p < 0.05$. For multiple comparisons, Tukey post hoc tests were carried out (SigmaPlot ver. 11, Systat Software, San Jose, CA).

The LT_{50} (median lethal time) was calculated by Probit analysis, which is a model that transforms sigmoid responses to linear data (Song and Lee 2005). LT_{50} was calculated to determine the necessary time that a specific cell concentration of a harmful microalgae strain causes a lethal response in 50% of individuals.

Results

Shrimp larvae toxicity test

Zoea larvae of *L. vannamei* exposed to *C. polykrikoides* (COPAZ-8) showed a sudden increase in mortality, with significant differences compared to control treatments. A 100% of larvae mortality was observed at 120 h of exposure (Fig. 3), showing a direct relationship between cell concentration and larval mortality rate at 96 h post-exposure. The strain of *C. subsalsa* (CSNAV-1) caused mortality in

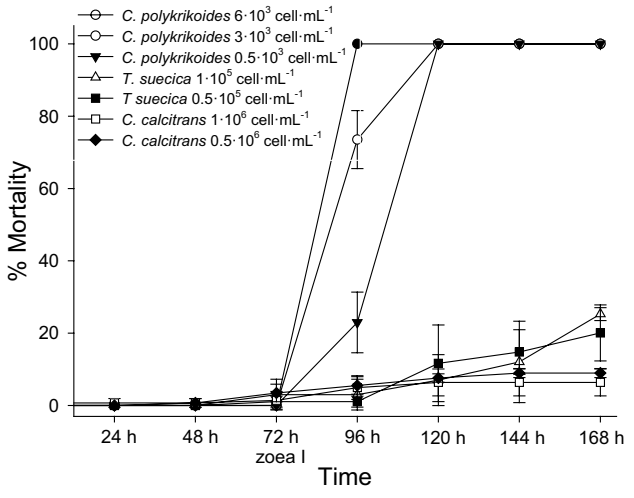

Fig. 3 Mortality (%) of *Litopenaeus vannamei* exposed to *Cochlodinium polykrikoides* (0.5, 3 and 6×10^3 cell mL^{-1}), *Tetraselmis suecica* (0.5 and 1×10^5 cell mL^{-1}), and *Chaetoceros calcitrans* (0.5 and 1×10^6 cell mL^{-1})

larvae only at 3×10^3 cell mL^{-1}, showing significant differences with the treatment at 6×10^3 cell mL^{-1} and the control (Fig. 4a), where a linear correlation was observed between 96 and 144 h of exposure with ~12 to 100% mortality, respectively. Treatments with *C. marina* var. *marina* (CSJV-2) caused higher mortality in a shorter exposure time than CSPV-3 strain at the same cell concentration (3×10^3 cell mL^{-1}) (Fig. 4b), which showed a linear correlation from 96 to 144 h of exposure. No statistical differences ($p>0.05$) were detected at the highest cell concentration (6×10^3 cell mL^{-1}) of *C. marina* var. *marina* (CSPV-3) compared to control treatments. All concentrations tested of *C. marina* var. *marina* (CSCV-1) increased larvae mortality from 72 to 168 h post-exposure (Fig. 5a). Additionally, the lowest cell concentration (0.5×10^3 cell mL^{-1}) caused 100% larvae mortality at 144 h, which was significantly higher than those observed at higher algal concentrations (i.e., 3–6×10^3 cell mL^{-1}). Treatments of *C. marina* var. *ovata* (CMOPAZ-1, 2 and 3) caused higher mortalities in larvae (~100%), especially strains CMOPAZ-2 and CMOPAZ-3, which caused ~100% mortality in a shorter time (120 h) compared to strain CMOPAZ-1 (Fig. 5b). All cell concentrations tested on *C. marina* var. *ovata* were statistically different from controls. For all treatments, mortality increased markedly when larvae reached the zoea stage (at 72 h) contrary to control treatments of *T. suecica* and *C. calcitrans*, which showed mortalities below 25%.

Probit analysis

The LT_{50} values were calculated only for zoea stage, although for *C. polykrikoides* (COPAZ-8), LT_{50} values

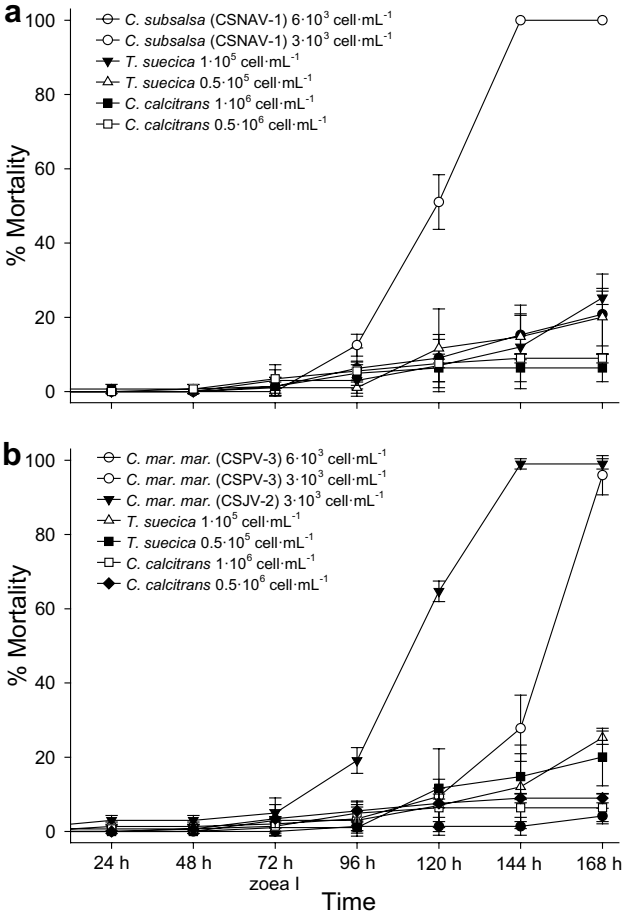

Fig. 4 Mortality (%) of *Litopenaeus vannamei* exposed to **a** *Chattonella subsalsa* (CSNAV-1) (3 and 6×10^3 cell mL^{-1}), and **b** *C. marina* var. *marina* (CSPV-3 and CSJV-2) (3 and 6×10^3 cell mL^{-1}). In both **a, b**, *Tetraselmis suecica* (0.5 and 1×10^5 cell mL^{-1}), and *Chaetoceros calcitrans* (0.5 and 1×10^6 cell mL^{-1})

could not be calculated since a sudden increase in mortality rate occurred at the beginning of the zoea stage. Therefore, LT_{50} values for the zoea stages exposed to harmful algae were only determined for *Chattonella* strains. Larvae exposed to *C. marina* var. *ovata* showed LT_{50} values between 20 h, and 1 day 14 h (Table 1); for larvae exposed to *C. subsalsa* (CSNAV-1) the LT_{50} values were of 1 day 19 h; for *C. marina* var. *marina* (CSCV-1, CSPV-3, and CSJV-2) the LT_{50} values were between 1 day 11 h, and 2 days 2 h.

Discussion

In the present study, there was no effect of *C. polykrikoides* and *Chattonella* spp. on the nauplii stage. However, regardless of the cell concentration of microalgae, a marked effect was observed at the beginning of the zoea stage in phase

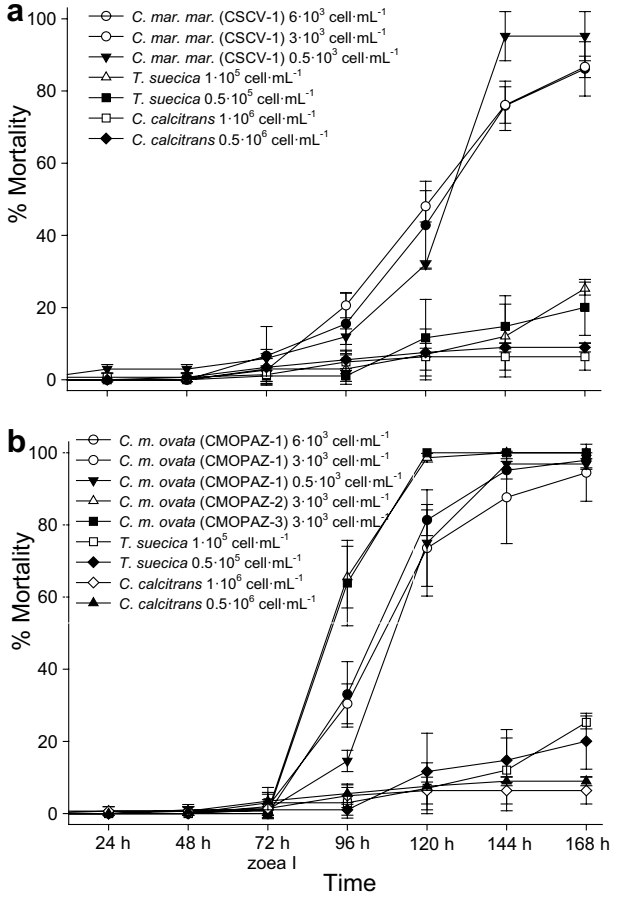

Fig. 5 Mortality (%) of *Litopenaeus vannamei* exposed to **a** *Chattonella marina* var. marina (CSCV-1) (0.5, 3 and 6×10^3 cell mL^{-1}), and **b** *C. marina* var. *ovata* (CMOPAZ-1, 2 and 3) (0.5, 3 and 6×10^3 cell mL^{-1}). In both **a, b**, *Tetraselmis suecica* (0.5 and 1×10^5 cell mL^{-1}), and *Chaetoceros calcitrans* (0.5 and 1×10^6 cell mL^{-1})

Table 1 Lethal time 50 (LT_{50}) calculated for *Litopenaeus vannamei*, exposed to different cell concentrations of *Chattonella* spp. strains

Species (strain code)	Concentration ($\times10^3$ cell mL^{-1})	LT_{50}
C. marina var *marina* (CSCV-1)	6	2 days 02 h
C. marina var *marina* (CSCV-1)	3	2 days 01 h
C. marina var *marina* (CSPV-3)	3	1 day 23 h
C. marina var *marina* (CSCV-1)	0.5	1 day 23 h
C. subsalsa (CSNAV-1)	3	1 day 19 h
C. marina var. *ovata* (CMOPAZ-1)	0.5	1 day 14 h
C. marina var *marina* (CSJV-2)	3	1 day 11 h
C. marina var. *ovata* (CMOPAZ-1)	3	1 day 11 h
C. marina var. *ovata* (CMOPAZ-1)	6	1 day 08 h
C. marina var. *ovata* (CMOPAZ-3)	3	0 day 20 h
C. marina var. *ovata* (CMOPAZ-2)	3	0 day 20 h

I, when larvae started to depend on exogenous feeding. During the three phases of the zoea stage, cells of harmful microalgae were consumed (not completely) in all treatments (unquantified data), which were verified by direct observation under an optical microscope. Microalgae used as controls (*Tetraselmis suecica* and *Chaetoceros calcitrans*) were also consumed, with mortality rates lower than 25%, which is common under these experimental conditions (Pérez-Morales et al. 2016). Hence, the effect of *C. polykrikoides* and *Chattonella* spp. on *L. vannamei* larvae was caused by microalgae cells ingested by the zoea stage and not by cell contact during nauplii stage. Thus, it is possible that differences in mortality rates among nauplii and the zoea stages could be related to the ontogeny of osmoregulation in crustaceans. It has been documented that embryos develop temporary osmoregulatory organs for ion transporting, and for enzymes like Na^+–K^+ ATPase, allowing hatchlings to osmoregulate the surrounding salinity level. In crustacean decapods, this osmoregulation ability is present at the time of hatching, but may decrease at higher larval stages when metamorphosis occurs (Charmantier and Charmantier-Daures 2001). In turn, this osmoregulatory ability in nauplii may be permeable for certain flow of molecules, but probably impermeable for toxic compounds such as those released by *C. polykrikoides* and *Chattonella* spp.

All cell concentrations of *C. polykrikoides* and *Chattonella* spp. tested in this study are found in nature (Lu and Göbel 2000; Jugnu and Kripa 2009; López-Cortés et al. 2014). Nevertheless, the strain of *C. polykrikoides* was the most harmful, with a remarkable sudden increase in mortality rate at the beginning of the zoea stage (for this reason, the LT_{50} value could not be calculated), causing 100% mortality of *L. vannamei* larvae in a short period of time. This dinoflagellate is well known for its high toxicity in nature because it can induce high mortalities in a short time in several marine fauna species, mainly fish (Bourdelais et al. 2002; Jiang et al. 2009; Jugnu and Kripa 2009). Different mechanisms of fish killing by *C. polykrikoides* have been proposed, and their toxicity has been associated with the combined effect of at least two toxic compounds such as labile toxins, paralytic shellfish poisoning (PSP)-like compounds, cytotoxic agents, extracellular polysaccharides (mucus), ROS, and neurotoxic, hemolytic and hemolysin toxic fractions (Kim et al. 1999, 2002, 2006; Dorantes-Aranda et al. 2009). These multiple biologically active metabolites may produce excessive mucus-like secretions and cause a series of alterations in respiratory function by disturbance of gill lamella integrity, causing suffocation in fish. At the cellular level, these compounds may damage the epithelium of gills and cause degeneration of their chloride cells (Kim et al. 1999, 2008, 2009; Tang and Gobler 2009a, b, 2010).

The toxicity of *C. polykrikoides* in fish and shellfish larvae has been previously reported. Rountos et al. (2014) documented that exposure to several concentrations of *C. polykrikoides* increased the mortality rate in both embryos and eleutheroembryos of different fish larvae, and mentioned that the sensitivity to toxic compounds differed among fish species (*Menidia beryllina* > *M. menidia* > *Cyprinodon variegates*). Tang and Gobler (2009b) observed higher mortality rates (close to 100%) in shellfish larvae of several bivalve species (*Crassostrea virginica, Argopecten irradians*, and *Mercenaria mercenaria*) exposed to *C. polykrikoides*, as compared to other HAB species, such as *Karenia brevis, Karlodinium veneficum, Alexandrium tamarense*, and *Prorocentrum minimum* (mortality rate <80%).

Bioassays with *Chattonella* strains (*Chattonella subsalsa* CSNAV-1, *C. marina* var. *marina* CSPV-3, CSJV-2, and CSCV-1, and *C. marina* var. *ovata* CMOPAZ-1, -2, and -3) caused different mortality rates in *L. vannamei* larvae. Concentrations of 3×10^3 cell mL^{-1} for all *Chattonella* strains evaluated caused the highest mortalities close to 100%. In addition, *Chattonella* strains could be considered to have an inadequate nutritional content for the development and survival of *L. vannamei* larvae. However, in the bioassays with *C. subsalsa* (CSNAV-1) and *C. marina* var. *marina* (CSPV-3) at concentrations of 6×10^3 cell mL^{-1}, low mortality rates (20.8 and 4.2%, respectively) were observed, which were similar to microalgae controls.

The most harmful strain was *C. marina* var. *ovata*, followed by *C. subsalsa*, and *C. marina* var. *marina* with LT_{50} values of 20 h; 1 day 19 h; and ~2 days, respectively. These values are similar to LT_{50} values observed in other zooplankton or meroplankton species, such as rotifers (LT_{50} 20 h), and fish embryo (LT_{50} 19 h to 2 days 19 h) exposed (direct contact with cells or extracts) to *Chattonella* spp. strains (Pérez-Morales et al. 2014; Chang 2015). In a previous study, Pérez-Morales et al. (2014) reported that embryos of the spotted sand bass (*Paralabrax maculatofasciatus*) exposed to different cell concentrations of *Chattonella* strains did not have the same mortality rates, i.e., *C. subsalsa* (CSNAV-1) at 6, 8, and 10×10^3 cell mL^{-1} caused higher mortality rates than 2 or 4×10^3 cell mL^{-1}, and *C. marina* var. *ovata* (CMOPAZ-1) at 2, 4, 6, and 8×10^3 cell mL^{-1} caused higher mortality rates than 10×10^3 cell mL^{-1}, which suggest that the nature of toxin production in *Chattonella* is strain specific and not dependent on the cellular increase.

According to several studies, the toxicity of *Chattonella* strains does not show a positive correlation between the increase of the cellular concentration and its toxicity, which has been demonstrated evaluating the mortality of damselfish (*Acanthochromis polyacanthus*), marine medaka (*Oryzias melastigma*), and juvenile red sea bream (*Pagrus major*) (Khan et al. 1995; Marshall et al. 2003; Shen et. 2010). These authors indicate that the highest toxicity of *Chattonella* strains are found during logarithmic phase and the lowest toxicity are found during the stationary phase of growth, thus they suggest that there is no positive correlation with the increase in cellular concentration and its toxicity, which may be determined by metabolism of each *Chattonella* strain.

In post-embryonic stages of the Japanese pearl oyster, *Pinctada fucata martensii*, lethal effects were also observed when exposed to *C. marina, C. antiqua* and *Heterosigma akashiwo*, only fertilized eggs and developing embryos were not affected; however, the three algal species tested affected the survival of trocophores and D-larvae, early-stage D-larvae and late-stage pre-settling larvae (Basti et al. 2016). The diverse anomalies and changes in larval behavior observed in the Japanese pearl oyster were cell density dependent, and the strongest negative effects were also observed with *C. marina*.

Particularly, it has been documented that raphidophyte species may produce a higher amount of toxic compounds (such as ROS) during exponential growth phase (Twiner and Trick 2000; Liu et al. 2007). Nevertheless, the production of distinct toxic compounds in Raphidophyceae species such as brevetoxin-like compounds, nitric oxide, free polyunsaturated fatty acids, hemagglutinin and hemolysin compounds, is highly variable and strain specific; also, their toxicity is considered to be a synergistic effect between two compounds or more, which can be produced at distinct phases of growth (Marshall et al. 2003; Fu et al. 2004; Kuroda et al. 2005; Kim et al. 2006, 2008; de Boer et al. 2009; Shen et al. 2010; Pérez-Morales et al. 2014). Therefore, the results observed in this study indicate that at least one toxic compound or more produced by *Chattonella* could be implicated as the main cause of mortality in *L. vannamei* larvae.

Available literature also suggests that raphidophyte species possess ejectosomes (*Heterosigma*), trichocysts and mucocysts (*Chattonella*) surrounding the cell surface, which under stress conditions are easily released (Hallegraeff and Hara 1995). Basti et al. (2016) observed that trocophores and larvae of *Pincatada fucata martensii* were trapped in a conglomerate of glycocalyx and mucus, which most probably was a mixture of larval mucous and raphidophyte mucocysts and tricocysts. Scarce information exists about the main function of these structures as defense mechanisms against resource competitors or predators.

Harmful effects by blooms of *Chattonella* on *L. vannamei* larvae have been previously reported. In Kun Kaak Bay, Sonora (Mexico), during a bloom in April 2003, numerous laboratories of post-larvae production of shrimp were affected by the contamination of their recirculating systems with seawater containing *Chattonella*

cells (Barraza-Guardado et al. 2004). This HAB was dominated by *Chattonella* and was associated with mortalities of 40% of *L. vannamei* post-larvae production. In nature, meroplanktonic larvae are unable to escape during a HAB, affecting the incorporation of new recruits to stocks with repercussion on fisheries (Almeda et al. 2011; Pérez-Morales et al. 2014).

The nature of bioactive metabolites produced by harmful microalgae has been associated with allelopathy, mainly for resource competition, and for protection against predators such as zooplankton grazers (Jiang et al. 2009; Tang and Gobler 2010). Therefore, it is important to perform studies with grazers, such as decapod crustacean species of commercial importance. Moreover, for a long time it has been considered that toxic bioactive metabolites produced by harmful microalgae affect only adult fishes, but in recent studies it has been demonstrated that they may affect a wider number of organisms in the marine fauna, including zooplankton, which has been verified through experimental bioassays (Jiang et al. 2009; Almeda et al. 2011; Aylagas et al. 2014; Pérez-Morales et al. 2014; Chang 2015; Basti et al. 2016; Lin et al. 2016).

In summary, this study showed that *L. vannamei* larvae are negatively affected by *C. polykrikoides* and *Chattonella* spp., causing mortalities close to 100% during the zoea stage. A point to conclude in this study is that direct physical contact with raphidophyte cells did not harm *L. vannamei* nauplii. Future research should try to identify the mechanisms of action by which these microalgae cause mortalities in shrimp larvae at the zoea stage when they start to feed on live cells.

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Compliance with ethical standards

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