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Physiological response of *Crassostrea gigas* during exposure and depuration period to dinoflagellate *Prorocentrum lima*

Respuesta fisiológica del ostión *Crassostrea gigas* durante un período de exposición y depuración al dinoflagelado *Prorocentrum lima*

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ABSTRACT

Dinoflagellate *Prorocentrum lima* blooms produce toxins causing significant health risks when present in mollusks consumed by humans; thus, short-term exposure and depuration periods were studied to assess the induced effects on *Crassostrea gigas* physiological system. Three treatments were evaluated: control diet with 2×10^6 cell/mL *Chaetoceros muelleri*; T1 oysters fed with *P. lima* (6×10^3 cell/mL); T2 oysters fed with *P. lima* (6×10^3 cell/mL) and *C. muelleri* (2×10^6 cell/mL) in day 13 exposure, followed by 15 days depuration. Differentiation and hemocyte count were examined, as well as hemolymph parameters (protein, glucose, lactate, cholesterol, and triglycerides). Accumulation of the okadaic acid in *C. gigas* digestive gland was quantified after 13 days of exposure. A significant decrease in total hemocyte, granulocyte, and hyalinocyte counts was detected in the treatments with *P. lima* after 13 days exposure, followed by a small increase during depuration; *C. gigas* physiological response against the dinoflagellate was also affected with significant differences in glucose, lactate, cholesterol, and triglyceride compared between exposure and depuration. The experimental results suggest a detriment to *C. gigas* physiological response by *P. lima* exposure with recovery in hemocyte count after 8 days with control diet (depuration phase).

Keywords: Harmful algae; *Crassostrea gigas*; *Prorocentrum lima*; hemocyte; physiological response; immune system.

RESUMEN

El dinoflagelado *Prorocentrum lima* produce toxinas dañinas para la salud humana por consumo de moluscos; la exposición y depuración de *Crassostrea gigas* fue analizada para conocer los efectos en el sistema fisiológico. Tres tratamientos fueron evaluaron: dieta control, 2 x 10^6 células/mL *Chaetoceros muelleri;* T1 alimentados con *P. lima* (6 x 10^3 células/mL); T2 alimentados con *P. lima* (6 x 10^3 células/mL) y *C. muelleri* (2 x 10^6 células/mL) por 13 días, seguido de una fase de 15 días de depuración. Se examinó diferenciación y recuento de hemocitos, así como parámetros en hemolinfa: proteína, glucosa, lactato, colesterol y triglicéridos; cuantificación de la toxina ácido okadaico en glándula digestiva de *C. gigas* después de 13 días de exposición. En los tratamientos con *P. lima* se detectó disminución significativa en hemocitos totales, granulocitos e hialinocitos. El sistema fisiológico de *C. gigas* también se vio afectado, reportando diferencias en la concentración de glucosa, lactato, colesterol y triglicéridos entre las fases de exposición y depuración. El sistema fisiológico de *C. gigas* se ve afectado como consecuencia de la exposición a *P. lima*, además se reporta una recuperación en hemocitos después de 8 días con la dieta control (fase de depuración).

Palabras clave: Alga nociva, *Crassostrea gigas*, *Prorocentrum lima*, hemocitos, respuesta fisiológica, sistema inmune.

INTRODUCTION

Microalgal bloom events, also known as harmful algal blooms (HABs), are cellular proliferation of toxic or noxious algal species potentially harmful to aquatic animals, consequentially affecting food web interaction (Neves *et al*., 2021). HABs can occur by several factors, including temperature increase, coastal eutrophication, wind, and human dispersal (Glibert and Burkholder, 2006; Gobler, 2020).

HABs development in aquatic environments results in accumulation of phycotoxins that can be captured by suspension feeders. Furthermore, the excessive accumulation of HABs could induce damage to the animal respiratory system, resulting in death, and high biomass bloom can result in oxygen depletion (Karlson *et al*., 2021). HABs consist of multiple algal species capable of producing a variety of bioactive compounds and toxins, which are responsible for human poisoning syndromes related to crustacean shellfish, mollusk, and fish consumption. Toxins are known as paralytic, neurotoxic, amnesic, azaspiracid, and diarrhetic shellfish poisoning according to their effects on human health (Anderson *et al*., 2021; Lad *et al*., 2022; Lassudrie *et al*., 2020).

Filter-feeding bivalves represent an important component of the coastal ecosystem; specifically, *Crassostrea gigas* is one of the most important shellfish produced worldwide due to adaptability, rapid growth rate, and nutritional value (Bai *et al*., 2024). However, *C. gigas* farming is frequently affected by

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toxic species produced by algae of the genus *Prorocentrum*, *Dinophysis*, *Ostreopsis*, and *Gambierdiscus* (Gaillard *et al*., 2020; Accoroni *et al*., 2024; Neves *et al*., 2019; Economou *et al*., 2007). The benthic dinoflagellate *Prorocentrum lima* is widely distributed in tropical and temperate regions (Nishimura *et al*., 2020) and produces okadaic acid and dinophysistoxins, causing gastrointestinal symptoms (Grigoriyan *et al*., 2024). *P. lima* induces diarrhetic shellfish poisoning (DSP) in humans by the consumption of bivalves contaminated with toxins, due to the accumulation of toxins produced by dinoflagellate (Ayache *et al*., 2023; Faustino *et al*., 2021). DSP toxins are lipophilic polyether compounds, including okadaic acid (OA), dinophysistoxin-1 (DTX1), DTX2, and other derivative forms (Gerssen *et al*., 2010). Lipophilic toxins are captured and digested to accumulate in the digestive gland of filter-feeding mollusks, and even concentrations as low as 5 cells/mL may accumulate toxins to induce DSP in humans (Yasumoto *et al*., 1984; Huguet *et al*., 2020).

Mollusk bivalves have an innate immune system, based on nonspecific reactions from cellular and humoral responses. The cellular part is formed by hemocytes, which are a key factor in the immune system together with the barrier tissues, synthesizing humoral factors with antimicrobial activity (Tan *et al*., 2020; Andreyeva *et al*., 2022). Hemocytes are involved in phagocytosis, encapsulation, nodule formation, cytotoxicity, and antigenic self/non-self discrimination (Alesci *et al*., 2023). Therefore, the quantification of the circulating hemocytes is a useful determination of the immune system response (Faustino *et al*., 2021; Andreyeva *et al*., 2021).

Bivalve exposure to *P. lima* induces damage to the digestive system, negative effects on feeding, respiratory rate, and alteration in the circulating hemocyte concentration (Neves *et al*., 2019; Faustino *et al*., 2021). Nonetheless, lack of information still exists of the oyster physiological response to dinoflagellate toxins, thus, hemolymph parameter concentrations and hemocyte count could serve as a valuable tool for rapid quantification of the physiological response to stress in C. *gigas*. Therefore, the present study aims to describe *C. gigas* cellular and immune physiological response to DPS toxin producer dinoflagellate *P. lima*.

MATERIAL AND METHODS

Oyster acclimatization

Healthy adults of the Japanese oyster *Crassostrea gigas* were obtained with an initial weight of 40 ± 5 g, and 50 ± 10 mm shell length, from a local oyster farm (Estero la Cruz, Kino Bay, Sonora, Mexico). Oysters were transported to the laboratory to be cleaned of epibionts. The animals were maintained in 50-L tanks with controlled salinity (34 practical salinity units, PSU), and temperature (24 ± 1°C) for a 15-day acclimation period. Oysters were fed with *Chaetoceros muelleri* daily, according to Helm *et al*. (2004).

Algal culture

The benthic dinoflagellate *Prorocentrum lima* (strain PLHV-4) was obtained from CIBNOR (Centro de Investigaciones Biológicas del Noroeste, S.C). *P. lima* was cultivated using modified F/2 medium + Se in Fernbach flasks and glass carboy, maintained at 22 °C ± 1 °C, light/dark= 12:12, and 200 µmol photons m^{-2} s^{-1} light intensity, according to Núñez-Vázquez *et al*. (2003). *P. lima* strain can produce OA = 2,041 pg/cells, DTX1 = 1.33 pg/cells, and DTX2 = 0.09 pg/cells of toxins.

The dinoflagellate *P. lima* was harvested at the late exponential growth phase at day 20 (determined before the experimental phase) to feed the oysters, whose cell density was quantified after cell fixation with Lugol's solution (Gifford and Caron, 2000), using a 1 mL Sedgwick-Rafter counting chamber (Olympus BX41, Tokyo, JP) under a microscope.

The control microalgae used for the present experiment was *Chaetoceros muelleri*, provided by the Universidad de Sonora (DICTUS), and cultured in Bubble column photobioreactors (PBRs) using F/2 medium (Guillard and Ryther, 1962), with constant temperature 22 ± 1 °C and illumination 250 µmol photons m^{-2} s^{-1}.

Experimental exposure and sampling

After the acclimation period, oysters were fed once a day for 13 days of trial in three treatments: Control or non-toxic diet with 2 x 10^6 cell/mL of *C. muelleri*; T1 oysters fed with *P. lima* cells only (6×10^3 cell/mL); and T2 oysters fed with a combination of *P. lima* (6×10^3 cell/mL) + *C. muelleri* (2×10^6 cell/mL). Dinoflagellate concentrations were based on field observation data of HAB events in the Gulf of California (Hallegraeff *et al*., 1995). The exposure effects of the toxic dinoflagellate *P. lima* on *C. gigas* were examined in triplicate samples for each treatment, using 20 oysters by replica, placed in 10-L plastic containers with constant aeration.

During the exposure phase, four oysters from each experimental unit were sampled randomly on days 1, 5, 10, and 13. For the depuration phase, four oysters from each experiment were collected on days 3, 6, and 15 after the dinoflagellate exposure. Sampled organisms of each experimental unit were replaced by the same number of organisms (previously marked, and kept under the same experimental conditions) to maintain the number of organisms constant in each unit; additionally, each experimental unit received 100% water exchange every 24 h, before feeding, and a *P. lima* count was performed to record the consumption percentage. Oyster mortality, feeding behavior, hemocyte count, and cell-free hemolymph metabolite quantification were evaluated.

To determine okadaic acid toxin accumulation in *C. gigas* after 13 days of feeding with *P. lima*, the digestive gland of four animals was removed and lyophilized. Okadaic acid extraction was performed according to Lee *et al*. (1987) with some modifications, while okadaic acid quantification was performed by high-performance liquid chromatography (HPLC) analysis using Agilent 1200 Series Liquid Chromatographer, with a diode array detector (DAD) (240 nm), fluorescence detector (FLD) (266 nm excitation and 316 nm emission), and an ultrasep C_{18} column (250×5 mm, Agilent Bonus); a mobile phase flux of 1.250 mL/min (acetonitrile: water 80:20) and 20 µL of the sample were injected (Pinto-Silva *et al*., 2005).

Hemocyte count

Hemolymph was obtained from the oyster pericardium using a 1-mL syringe; a hemolymph pool was prepared for each replicate. Immediately after sampling, 100 µL of hemolymph from 3 individual oysters were added to 900 µL of anticoagulant (sodium citrate, pH 7.5), and the rest of the samples were centrifuged at 9000*g* for 5 min (4°C) to separate plasma for analysis. Hemocytes were counted using a Neubauer chamber; the granulocyte and hyalinocyte cell classification was performed according to the morphologic parameters under a light microscope (Olympus BX41) (Faustino *et al*., 2021). Total hemocyte count was expressed as the number of hemocytes ($\times 10^6$) mL^{-1} of hemolymph.

Physiological analysis

The plasmatic parameter concentrations, such as protein, glucose, lactate, cholesterol, and triglycerides were quantified using the commercial Home Testing Kits RANDOX® (Great Britain), following the manufacturer's specifications, with some modifications. Absorbance was read in a microplate scanner (Bio Rad, iMark ™), according to Sánchez-Paz *et al*. (2007).

Statistical analysis

Data were analyzed using a normality test; homogeneity of variances was verified by Leven's test. A two-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to compare metabolite concentration and hemocyte counts among treatments and time. Differences were considered significant when $P < 0.05$. Data were expressed as mean ± standard deviation (SD). Statistical analyses were performed with JMP® Pro 16.0.0 Software.

RESULTS AND DISCUSSION

***Crassostrea gigas* feeding behavior**

Oysters filtered and ingested both microalgal cells. The control group (fed exclusively with *C. muelleri*) maintained a normal feeding behavior; no closure of valves or mantle retraction was observed, and the cilia were observed outside the valves during feeding; additionally, a high-water clarification and rich stool production was recorded during the trial. The oysters ingested 100% of *C. muelleri* cells, and up to 93% of *P. lima* (Table 1).

On the other hand, oysters fed with the dinoflagellate *P. lima*, produced fecal pellets partially degraded, indicating a partial degradation of the cells. However, a change in *C. gigas* feeding behavior with *P. lima* diets was observed at the beginning of the depuration phase with a delay in feed captured (*C. muelleri*) by the oysters; after two days of depuration, the oysters were capable of recovery and capture all feed in the first hours.

Quantification of the toxin Okadaic acid, was determined in the digestive gland of oysters after 13 days of feeding *C. gigas* with *P. lima* (T1 and T2), reporting 250 ng $OA.g^{-1}$ for the *P. lima* treatment group (T1), and 200 ng $OA.g^{-1}$ for the *C. muelleri* + *P. lima* treatment (T2).

Table 1. Consumption percentage of *Prorocentrum lima* after 24 hours feeding of *Crassostrea gigas* with T1 (6×10^3 cell/mL of *P. lima*), and T2 (*C. muelleri* + 6×10^3 cell/mL of *P. lima*).

Tabla 1. Porcentaje de consumo en *Prorocentrum lima* despues de alimentar *Crassostrea gigas* por 24 horas, con T1 (6×10^3 cel/mL de *P. lima*), y T2 (*C. muelleri* + 6×10^3 cel/mL de *P. lima*).

Day	T1 (%)		T 2 (%)	
1	98 ±	0.1	97 ±	0.7
2	95 ±	1.3	95 ±	0.6
3	97 ±	0.9	95 ±	1.3
4	97 ±	1.2	93 ±	0.7
5	98 ±	0.5	94 ±	0.2
6	98 ±	0.6	94 ±	0.2
7	96 ±	0.7	95 ±	1.0
8	97 ±	1.2	96 ±	1.3
9	94 ±	1.4	93 ±	1.1
10	96 ±	1.0	95 ±	1.2
11	97 ±	0.3	96 ±	0.5
12	96 ±	0.4	97 ±	0.6
13	95 ±	1.6	98 ±	0.1

Effects of *Prorocentrum lima* exposure in *Crassostrea gigas* hemocytes

Total hemocyte count (THC) reported significant differences between exposure time and experimental groups ($P < 0.05$). The control group showed the highest hemocyte concentration with no significant variation during the trial (Figure 1A). Nevertheless, groups fed with *P. lima* (T1) and *P. lima* + *C. muelleri* (T2), reported differences compared to the control group and during the time with a decrease in hemocyte concentration, reaching the lowest concentration on day 13 of the dinoflagellate exposure. Furthermore, a recovery in the hemocytes was detected during the depuration phase (15D) in T1 and T2.

The granulocyte concentration reported significant differences ($P < 0.05$) between treatments and during the exposure and depuration phases (Figure 1 B). The control group presented the highest granulocyte concentration compared to T1 and T2, and no differences were reported between T1 and T2 during the exposure and depuration phases. Conversely, the hyalinocyte concentration reported significant differences between the control group with the highest concentrations, and the groups exposed to *P. lima* ($P < 0.05$) (Figure 1 C); however, no differences were reported during the time among treatments. Finally, the lowest concentration of granulocytes and hyalinocytes was reported on day 13 of the exposure phase with an increase on day 15 of the depuration phase.

Physiological analysis in *Crassostrea gigas* exposed to *Prorocentrum lima*

Alterations in *C. gigas* plasma parameters were detected after *P. lima* exposure, except for protein concentration, which remained constant during the dinoflagellate exposure phase with no significant differences ($P < 0.05$), and an average of 25.8 ± 0.8 mg/mL (Figure 2A). On the other hand, glucose concentration in *C. gigas* plasma reported significant differences ($P < 0.05$) between exposure and depuration

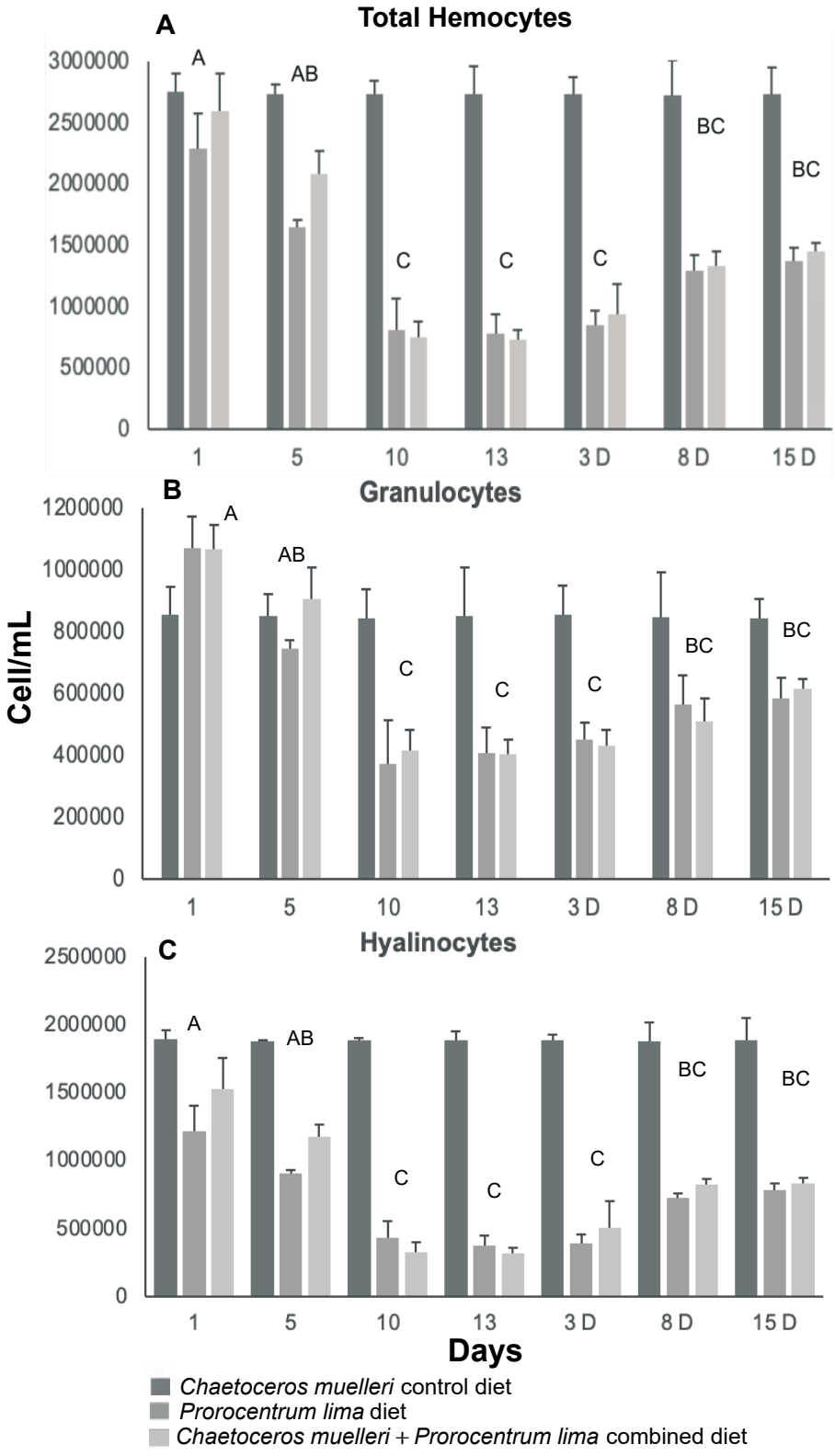

Figure 1. Total circulating hemocytes (TCH) (A), granulocytes (B) and hyalinocytes (C) counts in the hemolymph of *Crassostrea gigas* during an exposure phase with the dinoflagellate *Prorocentrum lima* (13 days) and a depuration phase (15 days). Letters indicates significant differences between exposure and depuration phases ($p < 0.05$); the mean ± standard deviation (SD) is indicated.

Figura 1. Recuento total de hemocitos circulantes (TCH) (A), Granulocitos (B) hialinocitos (C) en la hemolinfa de *Crassostrea gigas*, durante una fase de exposición al dinoflagelado *Prorocentrum lima* (13 días) y una fase de depuración (15 días). Letras indican diferencias significativas entre las fases de exposición y depuración ($p < 0.05$); se indica la media ± desviación estándar

phases; nonetheless, no differences were reported among treatments. After 5-day exposure to the dinoflagellate, glucose concentration increased in T1 and T2; the lowest glucose concentration was reported in the control group at day 15, with a concentration of 1.05 mg/mL (Figure 2B).

Lactate levels on plasma reported significant differences ($P < 0.05$) with the highest lactate concentrations on day 5 of the exposure phase for T1 (0.34 mg/mL), while, a significant decrease was reported on day 8 of the depuration phase in T2 (0.29 mg/mL) (Figure 3A). Additionally, a negative correlation of $r = -0.2$ was detected between lactate and glucose parameters during the exposure phase.

Cholesterol concentration in *C. gigas* plasma reported significant differences over time of exposure to *P. lima*, with the lowest concentration on day one in T1; after the exposure phase, a significant increase was reported in all experimental groups (Figure 3B). Similarly, a significant increase ($P < 0.05$) was reported in triglyceride concentration on day 15 of the depuration phase, while, the lowest one was reported on day 10 of the dinoflagellate exposure phase (0.32 mg/mL) (Figure 4).

DISCUSSION

Oysters filtered and ingested both microalgal cultures used in the experiment, producing fecal pellets containing partially degraded cells of the dinoflagellate *P. lima* in the form of pseudo-feces. Similar *C. gigas* feeding behavior exposed to *P. lima* and reduction of the clearance rate were reported previously (de Romero-Geraldo *et al*., 2014; de Romero-Geraldo *et al*., 2016; García-Lagunas *et al*., 2019). This phenomenon,

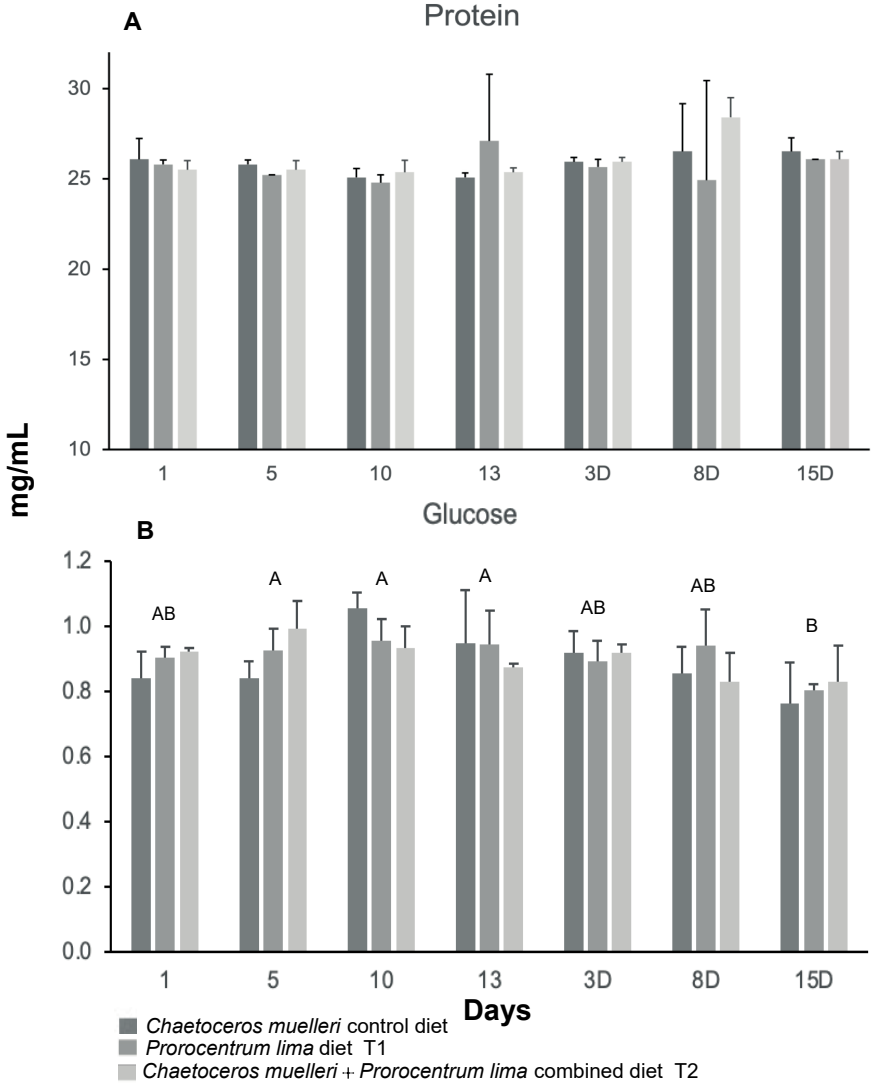

Figure 2. Protein (A) and glucose (B) values in *Crassostrea gigas* plasma, during an exposure phase with the dinoflagellate *Prorocentrum lima* (13 days) and a depuration phase (15D depuration days). Letters indicates significant differences between exposure and depuration phases ($p < 0.05$); the mean ± SD is indicated.

Figura 2. Valores de proteína (A) y glucosa (B) en plasma de *Crassostrea gigas,* durante una fase de exposición al dinoflagelado *Prorocentrum lima* (13 días) y una fase de depuración (15D días). Letras indican diferencias significativas entre las fases de exposición y depuración ($p < 0.05$); se indica la media ± desviación estándar.

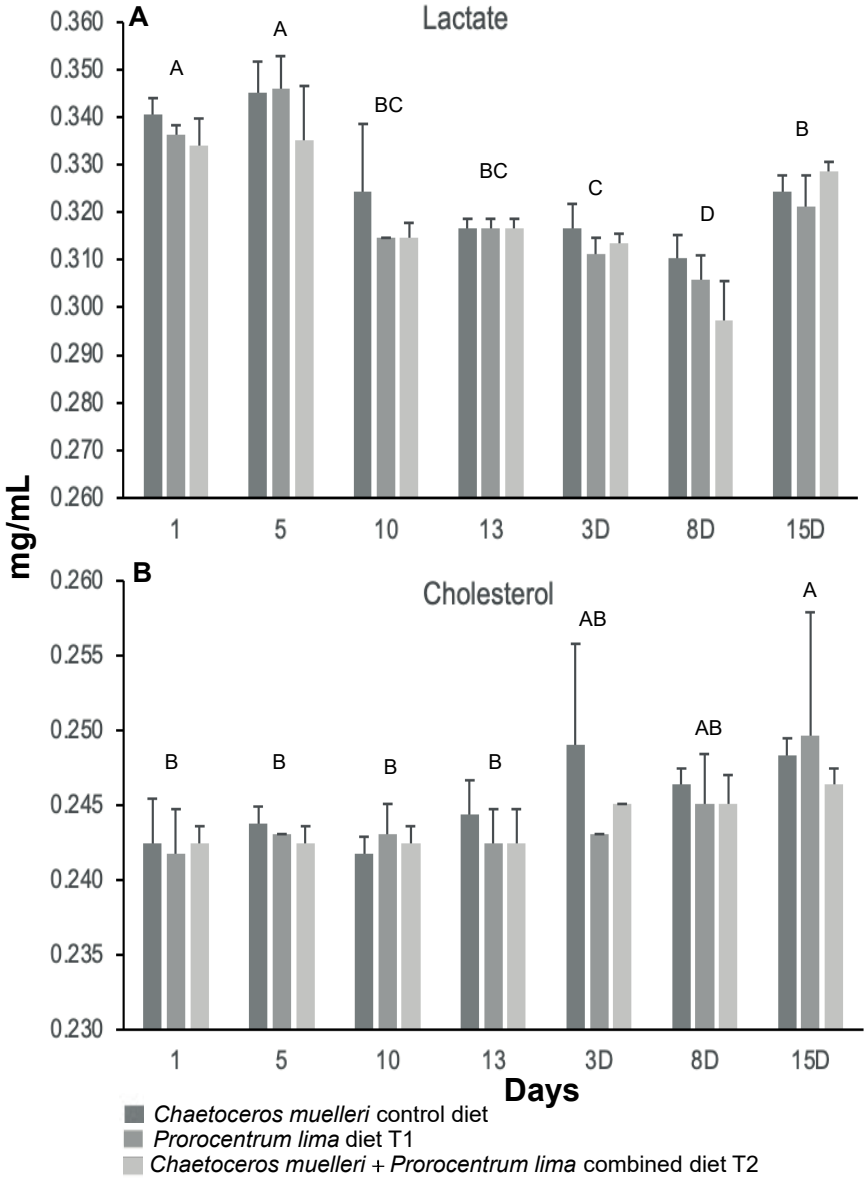

Figure 3. Values of plasma lactate (A) and cholesterol (B) in *Crassostrea gigas,* during an exposure phase with the dinoflagellate *Prorocentrum lima* (13 days) and a depuration phase (15D depuration days). Letters indicates significant differences between exposure and depuration phases ($p < 0.05$); the mean ± SD is indicated.

Figura 3. Valores de lactato (A) y colesterol (B) en plasma de *Crassostrea gigas*, durante una fase de exposición al dinoflagelado *Prorocentrum lima* (13 días) y una fase de depuración (15D días). Letras indican diferencias significativas entre las fases de exposición y depuración ($p < 0.05$); se indica la media ± desviación estándar.

where the harmful algal cell can pass as a viable cell through the digestive system and be introduced again into the environment, is a real challenge to mollusk aquaculture (Hégaret *et al*., 2007).

No mortality was observed in oysters by the toxic dinoflagellate exposure. Evidence suggests that bivalves can survive and develop during HABs by the action of the immune system responding to DSP produced by dinoflagellates, activating protection against okadaic acid (Hégaret *et al*., 2011; Mello *et al*., 2013). Similarly, previous research demonstrates that *C. gigas* exposure to 30×10^3 cell/mL of *P. lima* did not result in mortalities (de Jesus Romero-Geraldo *et al*., 2014; Tan *et al*., 2023; García-Lagunas *et al*., 2019).

Diarrhetic shellfish toxin accumulation occurs mostly in the digestive gland; the amount of toxins accumulated by mollusks depends on their ingestion rates, particle capture, and capacity for selective feeding (do Prado Leite *et al*., 2021; Bricelj and Shumway, 1998). The okadaic acid concentration reported in *C. gigas* digestive gland after 13-day exposure to *P. lima*, are above the regulatory limit for human consumption (160 ng OA per gram of meat) (Moreira-González *et al*., 2022). In the same way, okadaic acid values are similar to

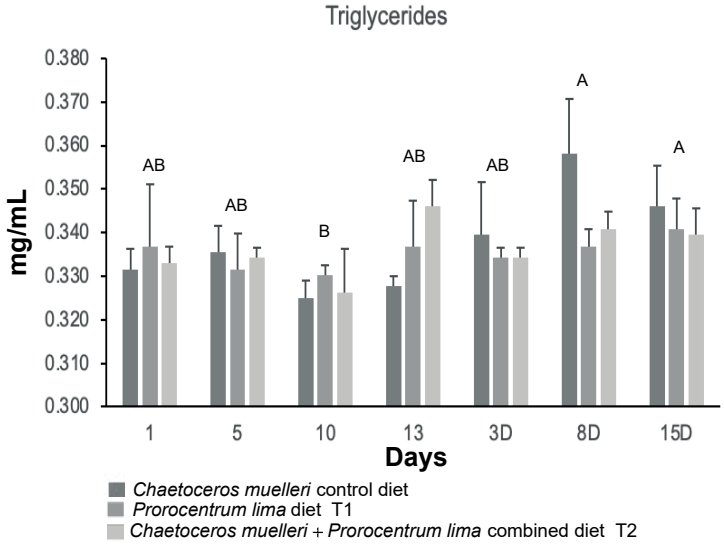

Figure 4. Values of plasma triglycerides in *Crassostrea gigas*, during an exposure phase with the dinoflagellate *Prorocentrum lima* (13 days) and a depuration phase (15D depuration days). Letters indicates significant differences between exposure and depuration phases ($p < 0.05$); the mean ± SD is indicated.

Figura 4. Valores de triglicéridos en plasma de *Crassostrea gigas,* durante una fase de exposición al dinoflagelado *Prorocentrum lima* (13 días) y una fase de depuración (15D días). Letras indican diferencias significativas entre las fases de exposición y depuración ($p < 0.05$); se indica la media ± desviación estándar).

those reported by Mafra *et al*. (2015), 183.3 ng OA.g^{-1} in *C. gigas* visceral tissue exposed to the dinoflagellate *Dinophysis acuminata* (13 750 cell/L) after two weeks.

Hemocytes modulate the invertebrate immune system as a key immune response component, responsible for phagocytosis, recognition, and cytotoxic reactions (Weng *et al*., 2022). Additionally, hemocytes participate in biological processes, such as shell production, nutrition, endocrine signals, and wound healing (Song *et al*., 2010). Hemocytes function as a rapid, and efficient response to stress (Weng *et al*., 2022; Lassudrie *et al*., 2020), and the effects of harmful algal toxins have been reported in bivalve hemocyte response (Tan *et al*., 2023).

Exposure to harmful algal toxins could induce an immunostimulant or an immunosuppressive effect, depending on the dinoflagellate toxicity and health status of the animal (Hégaret *et al*., 2011). In the present study, a significant decrease of hemocytes was recorded on the first 13-days of the exposure phase to *P. lima*. The decrease of hemocyte concentration is related to the toxins assimilated by *C. gigas*, because of the activation of the oyster immune system in response to the toxins. In the same way, a decrease in hemocyte concentration was reported for the bivalve *Perna perna* after 48 h exposure to 9×10^5 cell/mL of *P. lima* (Neves *et al*., 2019), and a 54% decrease in the mussel hemocyte count after *P. perna* and *Dinophysis acuminate* exposure (Simões *et al*., 2015). Similarly, exposure of the bivalve *Argopecten irradians* to different concentrations of okadaic acid (50, 100 and 500 nM) produced by the dinoflagellate *Alexandrium tamarense* induced a decrease in hemocyte concentration after 12 h (Chi *et al*., 2016).

Granulocytes and hyalinocytes can be distinguished by the presence or absence of cytoplasm granules. Both cells contribute to the immune system response, through different mechanisms such as phagocytosis and encapsulation. Moreover, the main distinction between these two hemocytes has been suggested to be the different life stages of the same cell type, however, they perform different functions (De la Ballina *et al*., 2022). In the present study, granulocyte and hyalinocyte were also affected by *P. lima* with a significant decrease in both cell types. Mello *et al*. (2010) reported a 12% decrease in bivalve granulocytes during the natural bloom of *Dinophysis acuminata*; the author also suggests a similar hyalinocyte and granulocyte production, but a granulocyte migration to the affected tissue (gills and intestines) could explain the decrease of this type of cell.

Plasmatic parameters in the invertebrate hemolymph can be a useful indicator of the physiological status (López-Elías *et al*., 2016). To our knowledge, this is the first report on *C. gigas* physiological status using commercial quick response kits. It is important to determine the normal rate of each parameter, as a rapid way of determining *C. gigas* physiological and health status (Medina Félix *et al*., 2017).

The molluscan hemolymph plays an important role in metabolite and oxygen transport through the open circulatory system. The main protein in mollusk hemolymph is hemocyanin; this respiratory pigment is a glycoprotein, responsible for oxygen transportation (Machałowski and Jesionowski, 2021). Additionally, some proteins in mollusk hemolymph have been related to the immune response, such as coagulation and melanization (Pascual *et al*., 2006). Hemolymph protein concentration in bivalves could be

affected by a stress situation indicating a physiological response (Simões *et al*., 2015). Protein concentration in *C. gigas* hemolymph was not significantly affected by the dinoflagellate *P. lima* exposure.

Glucose concentration in *C. gigas* hemolymph was affected by the dinoflagellate exposure, inducing an increase in glucose concentration after 5 days of exposure, and a decrease at day 10 of the exposure phase. Glucose in the mollusk hemolymph is the main source of energy regulating glycogen metabolism and other ATP-dependent physiological mechanisms (Borges *et al*., 2004; Principe *et al*., 2019). Glucose concentration in plasma is a powerful tool to estimate the health status of animals, nevertheless, it may be dependent on the diet, temperature, and immune system (Shadenko and Sidorov, 2020). During temperature stress, the concentration of cortisol and glucose increases in *C. gigas* hepatopancreas, indicating a physiological stress response, where the cortisol hormone played a significant role in glucose metabolism (Wang *et al*., 2022). In the present research, glucose concentration was maintained from 0.7-1.0 mg/mL with a significant increase as a response to the dinoflagellate exposure phase.

Lactate dehydrogenase is an indirect lactate measurement, which is the final product of anaerobic metabolism. In mollusks, the lactate dehydrogenase enzyme has been detected in the foot and adductor muscle, and is responsible for the cytosolic redox balance during anaerobiosis cellular metabolism (Lee and Lee, 2011). Therefore, lactate is a confinable indicator of stress in mollusks (O'omolo *et al*., 2003). Lactate concentration increases on day 5 after the dinoflagellate *P. lima* exposure, and decreases on day 10; in mollusks during hypoxia conditions, large concentrations of lactate are produced, mainly by muscle; in those cases, lactate is eliminated through gluconeogenesis, with a positive correlation between glucose and lactate (Medina Félix *et al*., 2019; Tripp-Valdez *et al*., 2019). The present study observed an increase in glucose levels on day 10 after *C. gigas* exposition to *P. lima*; at the same time, a decrease in the lactate concentration indicated a negative correlation between these parameters.

Lipids are important energy sources in invertebrate hemolymph; they function as an important energy source and are essential for growth, survival, and reproduction. Lipids can be stored as energy in the digestive gland and mantle of mollusks (Medina Félix *et al*., 2019; Martínez-Pita *et al*., 2012). Additionally, aquatic mollusks have a lower lipid concentration in hemolymph than terrestrial species (Machałowski and Jesionowski, 2021). For instance, cholesterol and triglyceride contents in *Achatina fulica* hemolymph were 0.016 and 0.06 mg/mL respectively (Lustrino *et al*., 2010), compared to an average of 0.24 mg/mL cholesterol and 0.33 mg/mL triglycerides in *C. gigas* hemolymph.

CONCLUSIONS

The present study evaluated *C. gigas* immune and physiological responses after exposition to the dinoflagellate *P. lima* demonstrating a significant stress situation. These data represent valuable tools for rapid physiological evaluation of oysters, providing reference parameters for *C. gigas*. A negative correlation has been reported in the present study between glucose and lactate in *C. gigas* hemolymph, with a decrease of lactate on day 5 of the dinoflagellate exposure, and an increase on the same day of glucose concentration, indicating the gluconeogenesis pathway activation. *Crassostrea gigas* exposure to the dinoflagellate *P. lima* induced accumulation in the digestive gland, and alterations in glucose, lactate, cholesterol, and triglyceride; at the same time, hemocyte composition was also affected by *P. lima*, with higher total hemocytes, granulocytes, and hyalinocytes in the control group. Moreover, *C. gigas* exhibited signs of recovery during the depuration phase. Therefore, the present study reaffirms the harmful effect that HAB-forming species possess, such as *P. lima*, on coastal benthic species as *C gigas*, and their possible recovery if exposure to toxic cells is for a short period.

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CONFLICTS OF INTEREST

No conflicts of interest to declare.

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