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Impacto de la acidificación aguda del océano en la supervivencia y el índice de condición de *Pinctada mazatlanica*, *Megapitaria squalida* y *Modiolus capax*.

Impact of acute ocean acidification on survival and condition index in *Pinctada mazatlanica*, *Megapitaria squalida* and *Modiolus capax*.

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RESUMEN

El cambio climático y la acidificación del mar son temas de preocupación mundial, señalando a los moluscos como especies altamente susceptibles a estos fenómenos. El método inicial para evaluar las respuestas de los animales al estrés agudo implica evaluar su supervivencia y su índice de condición (IC). En este estudio, evaluamos estas respuestas en tres especies de moluscos con importancia económica: madre perla *Pinctada mazatlanica* (Pteriidae), almeja chocolate *Megapitaria squalida* (Veneridae), y mejillón *Modiolus capax* (Mytilidae), sometidos a tres distintos pH (8.1, 7.5 y 6.9). La supervivencia en madre perla no se vio afectada por la acidificación del agua, en almeja chocolata disminuyó al 91.7% y en mejillón hasta 75%, en el pH más bajo. Se encontraron diferencias significativas en el IC entre especies, sin embargo, no las hubo entre tratamientos de pH, lo que sugiere que estos bivalvos son capaces de tolerar la acidificación del mar, al menos en corto tiempo.

Palabras clave: Acidificación del mar, bivalvos, LC_{50}, supervivencia, índice de condición.

ABSTRACT

Climate change and ocean acidification have emerged as global concerns, with mollusks identified as particularly susceptible to their impacts. The initial method to assess animal responses to acute stress involves evaluating their survival and Condition Index (CI). In this study, we investigated these responses in three high-value shellfish species: Pacific pearl oyster *Pinctada mazatlanica* (Pteriidae), Chocolate clam *Megapitaria squalida* (Veneridae), and Fat horse mussel *Modiolus capax* (Mytilidae) across three seawater pH levels (8.1, 7.5, and 6.9). Survival rates in the pearl oyster remained unaffected by acute exposure to acidified seawater, whereas survival in the chocolate clam decreased to 91.7% at the lowest pH level, and in the mussel, it further declined to 75%. Although significant differences in CI were observed between species, no differences were noted across pH treatments. This suggests that, at least in the short term, these bivalve species appear capable of tolerating ocean acidification.

Key words: ocean acidification, bivalves, LC_{50}, survival, condition index

INTRODUCTION

In the past two decades, ocean acidification and climate change have emerged as significant global concerns (Doney, Busch, Cooley and Kroeker, 2020). Climate change results in shifts in carbon cycle processes, notably marked by a substantial increase in atmospheric CO_2 levels (Doney *et al*., 2020). The rise in atmospheric CO_2 is moderated by its absorption by the oceans through simple diffusion (Doney, Fabry, Feely and Kleypas, 2009), leading to reductions in pH and lower saturation levels of calcium carbonate ($CaCO_3$) in surface waters (Doney *et al*., 2009; IPCC, 2013).

To model potential future climates up to 2100, four greenhouse gas concentration scenarios (Representative Concentration Pathways, or RCPs) have been developed. Under in surface ocean pH ranging from 0.06 to 0.07 in the most conservative scenario to 0.30 to 0.32 in the most extreme (IPCC, 2013). These changes in CO_2 levels and pH are anticipated to significantly impact organisms with calcium carbonate exoskeletons (Busch and McElhany, 2017), likely reducing their survival and calcification rates (Doney *et al*., 2009; Busch and McElhany, 2017), but also influencing the production and maintenance of calcium carbonate structures in some adult species (Busch and McElhany, 2017). Ocean acidification is expected to adversely affect bivalve survival, recruitment, and reproduction, including delaying adult maturation (Cooley, Rheuban, Hart, Luu, Glover, Hare and Doney, 2015). Consequently, this could lead to a reduction in the harvestable biomass, maximum sustainable yield, and economic value of shellfish fisheries (Cooley *et al*., 2015).

Research on the impact of water acidification on marine bivalves is crucial, predominantly focusing on the long-term effects of ocean acidification. Equally important, however, is the evaluation of the immediate impacts these changes may have on such organisms. To enhance our understanding of the response to acute pH fluctuations, this study aimed to assess the effects on the median lethal concentration (LC_{50}) and condition index of three economically significant marine bivalves from distinct families, abundant in the region. The selected species include the Pacific pearl oyster *Pinctada mazatlanica* (Hanley, 1856) (Pteriidae), notable for its significant role in the economic and political history over more than four centuries, including extensive cultivation projects (Monteforte and García-Gasca, 1994); the Chocolate clam *Megapitaria squalida* (Sowerby, 1835) (Veneridae), underpinning one of the most vital bivalve fisheries in Baja California Sur (López-Rocha, Ceballos-Vázquez, García-Domínguez, Arellano-Martínez, Villalejo-Fuerte and Romo-Piñera, 2010); and the Fat horse mussel *Modiolus capax* (Conrad, 1837) (Mytilidae), with potential for developing a sustainable aquaculture industry (López-Carvallo, Saucedo, Rodríguez-Jaramillo, Campa-Córdova, García-Corona and Mazón-Suástegui, 2017).

MATERIAL AND METHODS

Fifty commercially sized organisms of each species were collected via scuba diving in Bahía de La Paz, Mexico, in June 2012 (Fig. 1). The organisms were Pearl oysters (*P. mazatlanica*), measuring 11.9 ± 1.3 cm in length and 11.6 ± 1.1 cm in width; Chocolate clams (*M. squalida*), at 6 ± 0.3 cm long and 7.3 ± 0.4 cm wide; and Fat horse mussels (*M. capax*), with 6.5 ± 0.9 cm by 3.6 ± 0.4 cm. They were transported to CIBNOR facilities and placed in a 2,000 L oval tank with a natural seawater flow-through system (40 PSU; 24°C; pH 8.1), where they were allowed a day to acclimate under a 12:12 light/dark photoperiod. Subsequently, under the same conditions, they were fed rotifers (*Brachionus plicatilis)* and microalgae (*Nannochloropsis oculata*) for five days before the experiment.

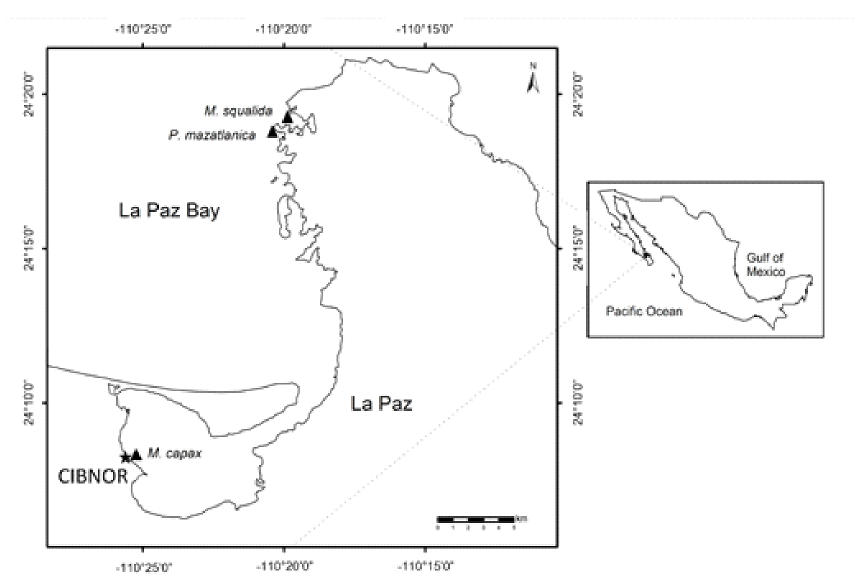

Figure 1. Sampling area at Bahía de La Paz, Baja California Sur, Mexico. Research facilities (CIBNOR), ▲ Sampling sites,
1) *Pinctada mazatlanica* (24°18'49" N, 110°20´21" W),
2) *Megapitaria squalida* (24°19'01" N, 110°20´09" W),
3) *Modiolus capax* (24°08'15" N, 110°25'25" W).

The experimental animals were fasted for 24 hours prior to the bioassay, after which they were weighed and measured (length and width). Twelve organisms of each species were then placed into individual plastic aquariums, each filled with water at predetermined pH levels (8.1, 7.5, and 6.9) within a flow-through, pH-controlled system (Fig. 2). This system utilized an Aqua Controller Apex with software (Neptune Systems®), temperature, O_2, and pH probes, and three pH-controlled tanks holding 400 L of filtered (1 µm) seawater each. The experimental pH levels (7.5 and 6.9) were maintained by adjusting CO_2 levels through electric valves. The control pH (8.1) received only filtered seawater without CO_2 bubbling. Oxygen saturation and temperature were regulated at 24 °C using probes, valves, room temperature control, and water heat pumps, while salinity was kept constant at 40 PSU. Flow rates to the experimental aquariums were set based on the species' size and the volume of their containers: 300 ml/min for a 24 L container for *P. mazatlanica*, and 100 ml/min for 8 L aquariums for *M. capax* and *M. squalida* (Fig. 2).

Organisms remained unfed throughout the experiment and were kept under their respective pH treatments for 96 hours, with daily mortality records and survival percentages calculated for each species under each pH condition. Five organisms from each species were measured before and another five from each pH treatment after the 96-hour experiment. The adductor muscle was cut to open the valves, the biomass was extracted from the shells and weighed, and then the dry shells were also weighed. The Condition Index (CI) was calculated using the formula: CI = (fresh flesh weight (g) x 100) / shell weight (g) (Duquesne, Liess and Bird, 2004).

CI means were analyzed using two-way ANOVA, employing Statistica for Windows, with "species" (*P. mazatlanica, M. squalida* and *M. capax*) and "pH" (8.1, 7.5 and 6.9) as fixed factors.

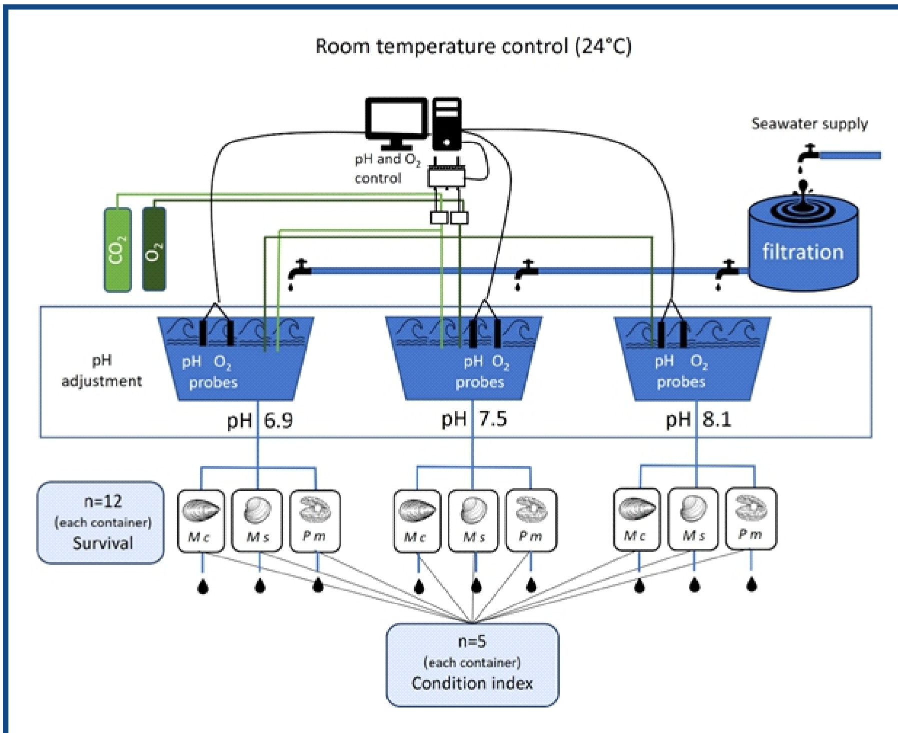

Figure 2. Diagram of the experimental pH-controlled-system.

RESULTS

The survival rate of the pearl oyster remained unaffected, with a 100% survival rate in acute acidified seawater conditions. The survival of chocolate clams decreased to 91.7% only in the most acidified conditions. However, mussels demonstrated the most sensitivity among the bivalves studied, showing the lowest survival rate (75%) in the most acidic conditions, yet the LC_{50} threshold was not attained (Fig. 3a).

The Condition Index (CI) varied significantly among species ($F_{(2, 44)}$ = 217.66, p < 0.0001), with *P. mazatlanica* and *M. squalida* showing similar values (27.3 – 29.6 and 30 - 31.5, respectively) but significantly different for *M. capax* (71.4 – 76.2) (Fig. 3b). No significant pH-related differences were observed ($F_{(3, 44)}$ = 1.1126, p = 0.35419). In *M. capax* the initial CI was slightly higher than that observed in the pH treatments after 96 hours (Fig. 3b).

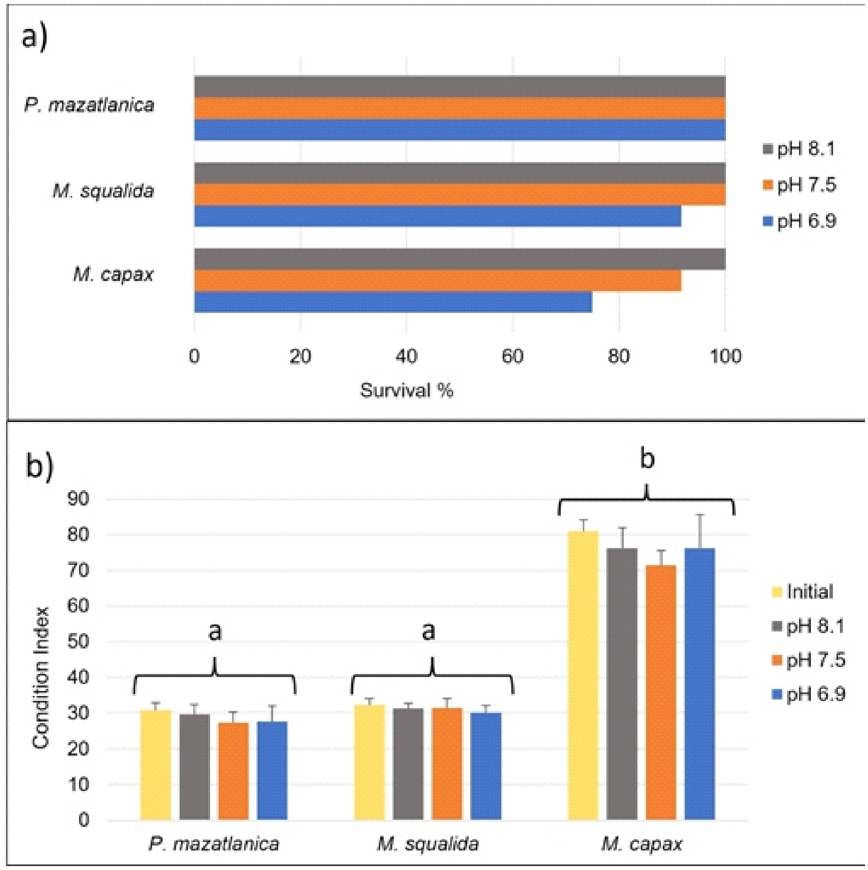

Figure 3. a) Survival of *Pinctada mazatlanica*, *Megapitaria squalida* and *Modiolus capax* and b) condition index prior the experiment (Initial) and after 96 hours of exposition to each pH treatment.

DISCUSSION

Although the most severe scenario predicted by the Intergovernmental Panel on Climate Change (IPCC) forecasts an ocean pH of 7.78 by the century's end, coastal waters exhibit much broader chemical fluctuations over shorter temporal and spatial scales (Waldbusser and Salisbury, 2014). Therefore, it's critical to grasp the immediate biological responses to acute changes such as extreme weather events, pH drops to as low as 7.0 in areas experiencing organic decomposition, pH range variation of 6.8 - 9.25 in unpolluted estuaries with freshwater contributions, or water discharges from power stations with pH levels below 6.5 (Bamber, 1987). Mollusks are among the groups most

susceptible to the impacts of ocean acidification (Busch and McElhany, 2017). An initial method to assess their response to acute pH stress involves evaluating their survival and condition index (CI).
The 96-hour LC_{50} (Lethal Concentration 50) test, which identifies the concentration of a toxicant —in this case, pH level— that results in 50% mortality of the test subjects over a 96-hour exposure, is a standard measure of toxicity (Gosling, 2004). However, due to the high survival rates of bivalves subjected to acute pH treatments in our study, it was not possible to determine the 96-hour LC_{50} for acidified water. The survival rate of *P. mazatlanica* remained unaffected, and in *M. squalida* decreased only to 91.7% at the most acidified condition, suggesting that under laboratory-simulated acidification, there are no significant lethal effects on these bivalve species, indicating their capacity to adjust their physiological homeostasis, at least in the short term. However, longer-term studies have shown that ocean acidification may impact the survival of many species (Jahnsen-Guzmán, Lagos, Quijón, Manríquez, Lardies, Fernández, Reyes, Zapata, García-Huidobro, Labra and Duarte, 2022). For instance, Bamber (1990) reported significant mortalities in oysters *Crassostrea gigas* and *Ostrea edulis* and the mussel *Mytilus edulis* exposed to acidic water at pH levels below 6.9, with the effect intensifying over time.

Given that most mussel populations are intertidal, experiencing a broad range of seawater parameter variations naturally, it was presumed they might be inherently adapted to fluctuations in pH levels (Navarro, Duarte, Manríquez, Lardies, Torres, Acuña, Vargas and Lagos, 2016), thus expecting high survival rates. However, mussels *M. capax,* demonstrated the most sensitivity among the bivalves studied, showing the lowest survival rate (75%) in the most acidic conditions (Fig. 3a), yet the LC_{50} threshold was not attained. It is noteworthy that the mussels under study were harvested from particularly turbid waters. Some researchers have found that mussels filter and feed at low algae concentrations, while high concentrations —and possibly significant water turbidity— trigger shell closure (Gosling, 2004). Prolonged periods of shell closure prevent feeding, leading bivalves to metabolize glycogen from their energy reserves for immediate survival needs (Pogoda, Buck, Saborowski and Hagen, 2013), suggesting that turbidity might impact their feeding, metabolism, growth, and overall health. Nutritional status or population origin might also play a crucial role in the variability of responses among organisms (Kroeker, Kordas, Crim, Hendriks, Ramajo, Singh, Duarte and Gattuso, 2013). Factors like suspended sediment (turbidity) or pollutants can influence oxygen consumption in bivalves (Gosling, 2004), suggesting that *M. capax* might have been in suboptimal conditions before collection, possibly impairing their resilience to acidic waters.

The Condition Index (CI), a measure of bivalve well-being calculated as the ratio of biomass to shell mass, varied significantly across species, with *P. mazatlanica* and *M. squalida* showing similar values but significantly different for *M. capax*. This variation stems from natural differences in body composition and the species-specific biomass-to-shell ratio. CI also fluctuates with seasonal changes and varies by location, influenced by environmental conditions, food availability (Thomsen, Casties, Pansch, Körtzinger and Melzner, 2013), parasitism (Gray, Lucas, Seed and Richardson, 1999), and pollution levels (Pridmore, Roper and Hewitt, 1990). Although a significant decrease in CI under acidic conditions was anticipated, no significant pH-related differences were observed. Nonetheless, a slight reduction in CI was noted in acidified water, possibly indicating that four days were insufficient to manifest a notable change in condition.

Another observation was that the initial CI was higher than that observed in the control pH (8.1) after 96 hours. During the experiment, the organisms were not fed, highlighting the critical role of starvation in the reduction of CI over a short period. Previous studies have shown that oysters with adequate food quality exhibit enhanced immune responses, accumulating energy reserves that lower disease mortality risk (Pernet, Lagarde, Jeannée, Daigle, Barret, Le Gall, Quere and Roque D'orbcastel, 2014). A similar protective mechanism could mitigate the adverse effects of ocean acidification in mussels when they are well-fed (Melzner, Stange, Trübenbach, Thomsen, Casties, Panknin, Gorb and

Gutowska., 2011). If the bivalves in this study had been fed during the experiment, it's conceivable that mortality or changes in CI would not have occurred.

Exposure to pH levels below 7 for 30 days has been associated with growth suppression, tissue weight loss, reduced shell size, shell dissolution, and diminished feeding activity in oysters and mussels (Bamber, 1990). However, other studies involving longer exposure periods (75 and 126 days) to varying pH levels have not found significant impacts on the CI of clams *Ruditapes decussatus* (Range, Chícharo, Ben-Hamadou, Piló, Matias, Joaquim, Oliveira and Chícharo, 2011), or the CI of mussel *M. edulis,* in relation to pCO_2, provided that food supply was sufficient (Thomsen *et al*., 2013).

Currently, the impact of pH levels on bivalve survival and health remains ambiguous. Both biotic and abiotic features of their habitats must be meticulously evaluated to forecast species' reactions to ocean acidification. In our study, the 96-hour LC_{50} was not achieved, nor were significant differences observed between pH levels and the condition index. Consequently, we infer that these species possess the physiological capacity to withstand acute acidification in the short term. This finding aligns with a published meta-analysis of marine biota (Kroeker *et al*, 2013), which suggest that some bivalve species may be more resilient to ocean acidification than previously assumed.

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