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Study the effect of low pH on *Ruditapes decussatus* (Mollusca:Bivalvia)

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Abstract

The grooved carpet shell clam (*Ruditapes decussatus*) is one of the most economically essential mollusks in Mediterranean lagoons and sandy beaches, with fisheries and aquaculture both contributing to its abundance. The goal of this research is to see how varying amounts of acidity affect this calcifying organism. 420 ppm (ambient control), 550 ppm, 750 ppm, and 1050 ppm were used to incubate juvenile clams in CO2 enriched saltwater. With increasing pCO2 , the biological parameters evaluated revealed a small decline. Differences, however, were not substantial. In terms of overall weight, the reduction was greatest at 550 and 1050 ppm. Furthermore, clams kept at 550 parts per million had the lowest condition index and the greatest mortality rate of 8%. Both the 550 ppm and the control 420 ppm groups demonstrated an increase in metabolic rate and ammonia excretion in the physiological response testing. With increasing acidification, the algal feed clearance rate declined, with the highest average value in the control (420 ppm) group and the lowest average value in the extremely high pCO2 (1050 ppm) group. Ocean acidification may put further strain on *R. decussatus* health and economic value by the end of the century.

Keywords

Ruditapes decussatus, low pH, Mollusca, Bivalvia

Introduction

The decrease in pH in saltwater caused by higher CO_2 levels in the atmosphere is known as ocean acidification. Since the industrial revolution, CO_2 deposition into the atmosphere has increased at a faster rate (Dupont and Portner, 2013). Because of the increasing cost of creating or maintaining shell material under lower pH, ocean acidification is projected to put energetic limitations on marine calcifying creatures like molluscs and echinoderms (Carey and Sigwart, 2016). Global warming is attributed to increases in atmospheric carbon dioxide partial pressure and several greenhouse gases. The average worldwide CO_2 emissions will reach 650 parts per million by 2100, according to the Intergovernmental Panel on Climate Change's (IPCC) B1 scenario (Caldeira and Wickett 2005). As a result of physical dissolution through the surface microlayer, a rise in atmospheric pCO_2 concentration will result in an increase in its concentration in an adjacent water body, according to Henery's law. About a quarter of the CO_2 produced daily by human activities is absorbed by the seas, resulting in ocean acidification due to a change in the chemical balance of the seawater carbonate system (Joint and Karl, 2011). The integrity of calcium carbonate structures has a direct impact on survival in many invertebrates, as they provide physical shelter from predators and environmental stressors like wave movement (Ellis *et al.,* 2017).pH has a vital role in all marine organisms, and the change in internal pH can affect an organism's health or even lead to death. Marine organisms have to preserve their internal pH prorated to that of the surrounding seawater. Some have complicated systems that can regulate internal pH (Hassellov *et al.,*2013). Ocean acidification, or changes in seawater carbonate chemistry caused by anthropogenic CO2 absorption, is a major threat to marine biodiversity (Hester *et al.,*2008). The most heavily influenced species by their surrounding environment are the ones without

these systems and can be rapidly menaced by changes in acidity (Cao and Caldeira, 2008). Although the research concerned with the impact of ocean acidification on the marine environment is growing very fast, it is still poorly understood and the picture is not fully clear. The carpet shell clam, *Ruditapes decussatus*, is regarded as one of the most common bivalves with a high economic value in numerous countries (Parker *et al.,*2013). Bivalves are commonly utilized as bioindicator species because they are sessile organisms that are susceptible to chemical pollution and environmental changes in the aquatic compartment (De Marchi *et al.,*2020).The present study aims to study the effect of different levels of acidification on this calcifying *Ruditapes decussatus*.

Material and methods

Individuals of grooved carpet shell clam *Ruditapes decussatus* were collected in January 2020 from the Lake Al-Razzaza , which is located between Karbala and Anbar provinces. At the beginning clams of experiments were transported and put in the receiving tank to recuperate from the transportation stress and acclimate to lab conditions in laboratories.The mean length at the start of the study was 22.27 mm (±0.74 SD).

The experimental design included four treatments, each reflecting a different PCO_2 concentration (420, 550, 750, and 1050 PPM). Clams were housed in 3 L of 1m filtered seawater in 4-liter tanks after two days of acclimatization. The experiment was split into two groups, A and B, each with the same conditions. There were 12 experimental tanks in each set (For each PCO_2 treatment, there are 3 replicate tanks). Each tank has an air pump that supplied oxygen to the circulation system to keep it healthy. Set A was used to record mortality, shell length, total weight, and condition index data, whereas Set B was used to record mortality, clearance rate, and ammonia excretion.

Clams were fed *Isochrysis galbana* once a day at a concentration of 100,000 cell/ml. Clams were cultured for 36 days at the four pCO_2 concentrations listed above while maintaining consistent alkalinity.

A pH meter was used to measure the original pH of seawater (Jenway 3505). Grasshoff *et al.,* (1983) employed spectrophotometry to quantify seawater temperature, salinity, silicate, and phosphate, which were then used as inputs for CO_2 sys calculations.

CO_2SYS calculations using the two measured CO_2 parameters, pHsw and TAsw, were used to determine seawater carbonate chemistry parameters at the start and throughout the experiment. The dissociation constants K1 and K2 (Mehrbach *et al.,* 1973, Dickson and Millero, 1987) were employed in the carbonate system. Dickson (1990) constants were used to derive the acidity constant of H_2SO_2 in saltwater .Every two hours, the water chemistry in each system was swapped, monitored, and corrected using carbon dioxide saturated Seawater.

Index of condition (C.I):

A day before setting up the experiment, condition index was determined for 50 individuals in pre-weighted pans. The measurements of C.I. were repeated at the end of the experiment for 30 individuals per treatment and its triplicate, the clams were dissected after their shell length and total weight had been recorded, they were opened by cutting the adductor muscle, the shell and viscera were entirely separated in their own weighed pans, both were dried in separate pans at 90 ^{0}C for 24 h, then they were weighed again. C.I was calculated according to (Walne ,1976) as follows:

C.I = [(dry flesh weight/ dry shell weight) x 100].

Metabolic rate:

Metabolic rate was estimated by incubating one clam per jar from each treatment in a 250 mL oxygen bottle for 3 hours to measure oxygen consumption rate (OCR). Clams were not fed for 6 hours prior to the start of the incubation phase. Oxygen consumption rates were calculated as the

rate at which oxygen concentrations decreased over the course of three hours of incubation, as determined using the modified Winkler method for the initial and final three hours of incubation. Cerezo *et al.,* (2006) used the following equation to compute OCR:

$OCR = (DO_0\text{-}DO_t)\ V/\ (DW\ T)$

Subscripts 0 and t time signify the initial and final concentrations of dissolved oxygen (DO), V is the volume of the respiration chamber (l), DW is the dry weight of R. decussates, and T is the time between the initial and final measurements (h).

Clearance rate:

Coughlan, (1969) used the following equation to calculate clearance rate:

$CR = V\ (\ln C_1 - \ln C_2)\ /\ t$

Where CR represents the clearance rate, V represents the volume of water consumed, C_1 and C_2 represent the cell concentrations between two sampling intervals, and t is the time increment.

Rate of ammonia excretion

The experiment was carried out by immersing 30 adult clams in 3 L of seawater and measuring the ammonia content before and after feeding with *Isochrysis galbana* for 24 hours. This analysis was carried out in accordance with the methodology described by Grasshoff *et al.*, (1983).

Statistical analysis:

SPSS was used for all statistical analyses (version 22). The paired T-test was used to determine the differences in shell length and total weight of the investigated clams before and after the experiment. Furthermore, a one-way ANOVA with treatment as a single factor was used to detect differences between treatments and the effect of time after exposure on several indicators of clam physiological responses.

Results

Acidification's effect on shell length

Ruditapes decussatus showed no significant changes in shell length between the start and end of the trial when given varied PCO2 treatments.After exposure to various amounts of acidification, there were no significant variations in SL among all treatments, including the control (Table 1).

Acidification's effect on total weight

After 36 days of acidification at various pCO_2 concentrations, total weight decreased insignificantly among different treatments($P \geq 0.05$: Table 1).

Table 1. The biological and physiological parameters of Ruditapes decussates clams exposed to varied acidification conditions were tested using one-way ANOVA.

Source	df	F	Sig.
Ammonia Excretion	3	1.351	0.289
Ammonia Excretion intervals	3	3.7	0.046
Clearance Rate	3	0.703	0.612
Clearance Rate intervals	8	3.922	0.007
Condition Index	4	21.679	0.001
Metabolic Rate	3	0.452	0.679
Metabolic Rate intervals	7	6.632	0.002
Shell length	3	1.302	0.207
Total weight	3	3.702	0.011

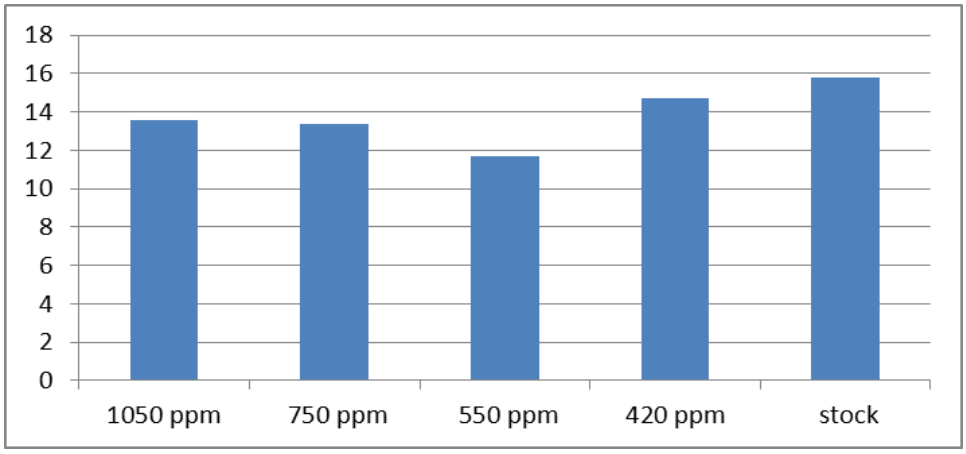

Figure 1. *Ruditapes decussatus's* mean condition index when exposed to various levels of ocean acidification.

When compared to the stock's original levels, condition index values decreased in all treatments (Fig. 1) The 550 ppm treatment had the lowest condition index of 11.7 whereas the ambient control group had the highest condition index of 15. 8. The condition index differed significantly between treatments ($P<0.05$: Table 1).

Acidification of the oceans' impact on clam mortality : The Clam mortality was not significantly affected by ocean acidification. mortality of *Ruditapes decussatus* incubated clams at 550 ppm was 7.62% compared to the ambient group 3.21%. The other treatments did not have any high mortality rates (Fig. 2).

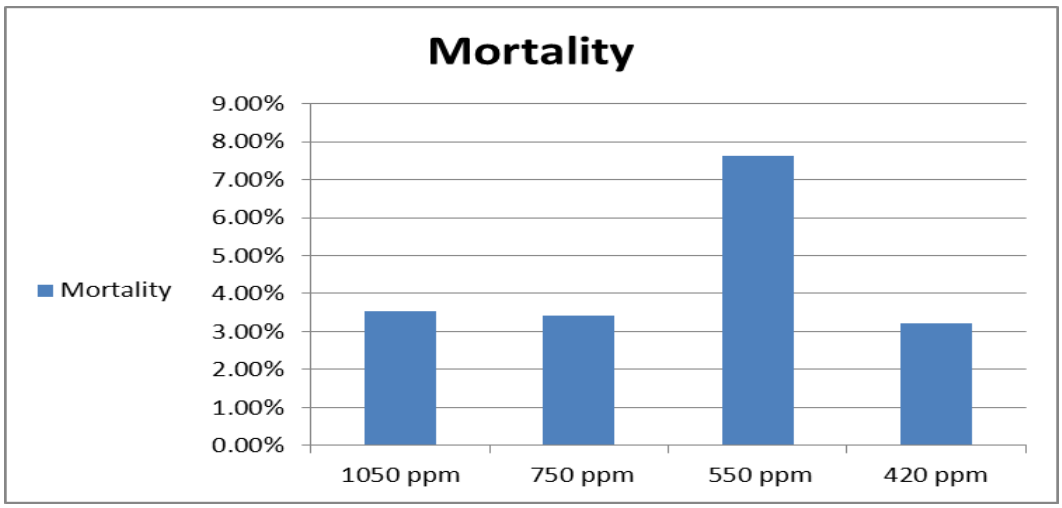

Figure 2. *Ruditapes decussatus* mortality (%) after 36 days of exposure to different ocean acidification conditions indicated by varied pCO2.

Ocean acidification and metabolic rate

In all treatments, a high metabolic rate was seen at the start of the experiment when estimating oxygen consumption rate (Fig. 3). The metabolic rate fluctuated over the course of the trial, but it was often lower than the initial number.

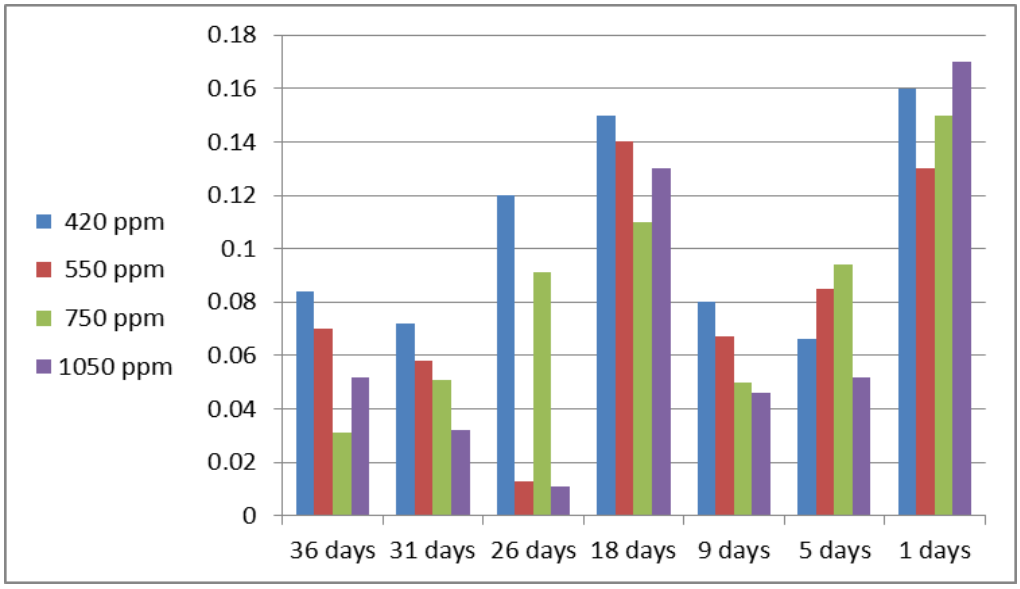

Figure 3. Metabolic rate of clam *(Ruditapes decussatus)* under different acidification

Ocean acidification and clearance rate . Figure 4 shows clams incubated at pCO2 (1050 ppm) had the lowest clearance rate, while the ambient group pCO2 had the highest clearance rate (420 ppm).

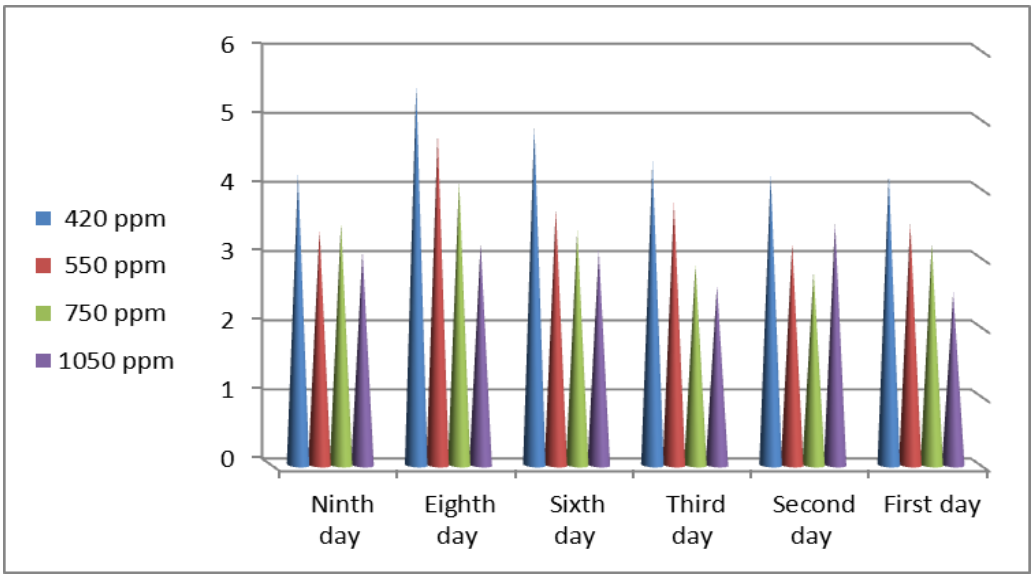

Figure 4. Algae clearance rate by *Ruditapes decussatus* under various acidity conditions.

Ocean acidification and Ammonia excretion:

The highest ammonia concentrations were found in the 420 ppm ambient control and 550 ppm groups, whereas the lowest ammonia concentrations were found in the 1050 ppm group (Fig. 5). The ANOVA test, on the other hand, revealed no significant differences between the groups (Table 1). Furthermore, ANOVA revealed a significant effect of time of acidification exposure (P= 0.05) .

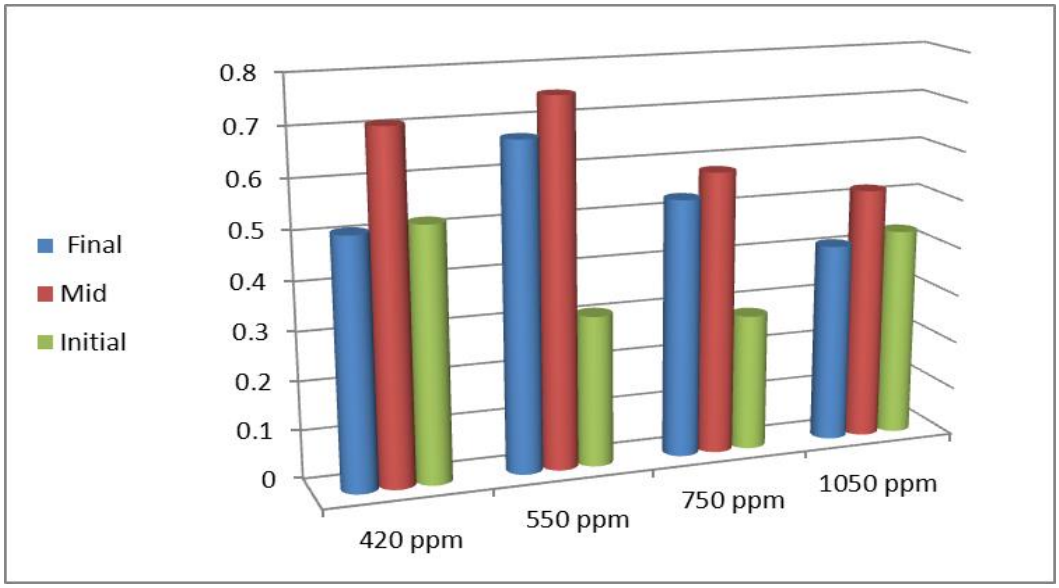

Figure 5. Ruditapes decussatus excretes ammonia for different levels of acidification.

Discussion

The The current research focuses on the impact of ocean acidification on the Mediterranean clam Ruditapes decussatus, which is considered one of the most commercially important and economically valuable bivalve species in several countries, including Iraq (El Khodary et al.,2018). This is owing to rising consumer demand as well as the potential for bivalve aquaculture in Iraq . As the rate of change in the ocean acidification field accelerates, the effects of decreased seawater pH are having a significant impact on the energy distribution requirements of clams, as more energy is required for the physiological balance conservation mechanisms (Almeida et al.,2018).

Adult bivalves are less sensitive to variations in marine pCO2 than larval bivalves, according to studies, although they nevertheless demonstrate growth, calcification, and physiological responses (Maynou et al.,2021). Increased pCO2 has a deleterious impact on bivalve shell deposition (De Marchi et al.,2017). Increased pCO2 has a deleterious impact on bivalve shell deposition (Beniash et al., 2010)

Marine creatures' growth is a physiological process that slows dramatically when they are exposed to high CO2 levels for an extended period of time (Pousse et al.,2020). Some research has found a relationship between bivalve growth rates and pH levels.Ocean acidification appears to be affecting the growth of certain bivalve species, according to studies (Jebali et al.,2007). Shirayama and Thornton (2005) discovered that even very small increases in CO2 of 200 ppm over current levels reduce the growth rate and survival rate of echinoderms and gastropods, showing that long-term exposure to CO2 fluctuations can impair calcifying species' growth.

According to Bressan et al., (2014), the Chamelea gallina did not grow under acidified circumstances, although their live weight reduced dramatically and a minor loss in shell length was noted. The effect of increased CO2 on growth is depicted in terms of shell length and weight in this study. The shell size was reduced slightly insignificantly as a result of the SL adjustments. Experiments on young Veneridae have also revealed no significant differences in growth, as evaluated by size, weight, and net calcification (Sanders et al.,2013). In terms of total live weight in the clams, no positive growth was observed during the current trial, with no significant differences found between the control and treated animals. In other research,

the growth of the mussel Mytilus chilensis was found to be considerably reduced after medium and long-term exposure to increased pCO2 levels. (Duarte et al., 2014).

In the current investigation, the clam Ruditapes decussatus showed maximum mortality at 550 ppm and delayed mortality at increasing pCO2 (750 and 1050 ppm). This could be explained by Gazeau et al. (2014), who hypothesized that exerting metabolic depression CO2 can reduce stress and delay mortality by allowing for more efficient use of energy reserves and passive tolerance as an adaptive strategy.

Several studies have found metabolic depression in several marine organisms when pCO2 levels are high, owing to a reduced capacity to correct for changes in extracellular ion and acid–base status (Michaelidis et al., 2005). This is consistent with the findings of the current investigation, which found that at 750 and 1050 ppm CO2, oxygen absorption was lower than in control seawater. In many species, a decline in metabolism is induced by an unneutralized acid-base state, which results in a change in energy budget. This adaptation approach is used to match Adenosine triphosphate supply with demand, hence increasing their chances of survival (Gazeau et al.,2013). On the other hand, clams exposed to 550ppm CO2 had a high metabolic rate, which was linked to the highest mortality, as previously stated. This was, however, referred to as a boost in meal absorption efficiency (Vargas et al.,2015).

Several studies have found that marine bivalves with high pCO2 levels have minimal ammonia excretion Wang et al. (2015) found low AE values (20–45 percent) in the mussel Mytilus coruscus, with no significant variations across three different pH levels, while considering the impact of seawater acidification on clam ammonia excretion (AE).

Conclusion

The According to the findings, continued ocean acidification may not represent as serious a threat to the survival of the Mediterranean clam *Ruditapes decussatus* as many experts had predicted. If adaption mechanisms do not occur with continuing progressive exposure to increasing ocean acidification, the low clearance rate (feeding rate), growth, and condition index may lead to poor aquaculture potential and health of R. *decussatus*. In the twenty-first century, risk assessment is required for Mediterranean bivalve aquaculture.

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