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Ocean acidification impact on the grooved carpet shell clam (*Ruditapes decussatus*)

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ABSTRACT

The grooved carpet shell clam (*Ruditapes decussatus*) is one of the most economicallyimportant mollusks inhabiting Mediterranean lagoons and sandy beaches both from fisheries and aquaculture. The present study aims to study the impact of different levels of acidification on this calcifying organism. Juvenile clams (avg. Shell Length, SL= 23.22 ± 0.84 mm) were incubated in CO_2 enriched seawater at four different CO_2 concentrations [420 ppm (ambient control), 550 ppm, 750 ppm and 1050 ppm] representing projected atmospheric CO_2 concentration scenarios for the year 2100 by IPCC. The studied biological parameters showed slight decrease with increasing pCO_2. However, differences were not significant. Standard length decreased as pCO_2 concentration increased, with a maximum average decrease of (-0.12) recorded at 750 ppm as compared to the control group. Regarding total weight, the decrease was highest (-0.10) in both 550 and 1050 ppm. Moreover, clams kept at 550 ppm showed the lowest condition index (11.40 ± 1.49) and highest mortality rate of 8%. The study of physiological response showed increase in metabolic rate and ammonia excretion in both 550 ppm and the control 420 ppm groups. Algal feed clearance rate decreased with increasing acidification with highest value in the control (420 ppm) group and lowest average value of 3.34 l/h^{-1}in the extremely high pCO_2 (1050 ppm) group. By the end of century, ocean acidification may exert additional stress on the health of *R. decussatus* and its economic value.

INTRODUCTION

Ocean acidification is the reduction of pH in the seawater due to the increased levels of atmospheric CO_2. At a quickened rate, the CO_2 deposition into the atmosphere has been increased since the industrial revolution (beginning in the 1750s). The increases in atmospheric carbon dioxide partial pressure (pCO_2), and different greenhouse gases have been given a share in global warming. The Current average atmospheric pCO_2 of approximately 400 ppm is higher than the highest pCO_2 over the past 2.1 million years (approximately 300 ppm; Hönisch *et al.* 2009). According to the Intergovernmental Panel on Climate Change's (IPCC) B1 scenario (stabilized population growth by 2050 and a more service- and information–driven economy), proposed average global CO_2 emissions will attain 650 ppm by 2100 (Caldeira and Wickett 2005). Following Henery's law, the increase in atmospheric pCO_2 will result in an increase in its concentration in an adjacent water body as a result of physical dissolution through the surface microlayer. About a quarter of the CO_2 produced daily by human activities is being absorbed by the oceans, causing ocean acidification which results from changing the seawater carbonate system chemical equilibrium. The dissolution of CO_2 in oceans causes increase in acidity (decrease in pH) and reduction in the obtainable carbonate ions (CO_3^{2-}), and consequently become increasingly shallower under saturation horizon of $CaCO_3$ (Feely *et al.*, 2004, 2012), which is projected to become more persistent and widespread by 2050 and beyond, (Gruber *et al*, 2012). If population growth occurs at a higher rate accompanied by slow economic development and limited technological changes (IPCC’s A2 scenario), emissions will likely reach 970 ppm by 2100 (Caldeira and Wickett 2005). Ocean acidification is expected to affect ecosystems at an accelerating pace over the next century (Caldeira and Wickett 2003; IPCC 2007).

$CaCO_3$ is required by marine organisms as the main building blocks of their skeletons, shells and other calcareous frames. In 250 years, Ocean acidity has increased by 30%, which is equivalent to a decreasing of surface seawater pH of 0.1 units (Orr *et al.*, 2005), therefore, the proposed scenarios by 2100 could be tripled.

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. The most heavily influenced species by their surrounding environment are the ones without these systems and can be rapidly menaced by changes in acidity. Although the research concerned with the impact of ocean acidification on marine environment is growing very fast, still poorly understood and the picture is not fully clear. However, few studies identified some marine organisms that can be threatened by changing the environment (Gazeau *et al.*, 2007; Beniash *et al.*, 2010; Michaelidis *et al.*, 2005; Thomsen and Melzner, 2010, Ibrahim *et al.*, 2014, Khairy *et al.*, 2014).

The carpet shell clam *Ruditapes decussatus* is rated as one of the most popular bivalves with a high economic value in many countries (Chessa *et al.* 2005; Prado-Alvarez *et al.*, 2009; Lucrezia *et al.* 2011). It is intended as an auspicious candidate for the emerging bivalve aquaculture progression in Egypt (El-Wazzan *et al.* 2012; Abbas *et al.* 2018). The present study aims to study the impact of different levels of acidification on this calcifying organism.

MATERIALS AND METHODS

Study organism collection

The grooved carpet shell clam *Ruditapes decussatus*was was collected in November 2017 from the Lake Timsah in Ismailia, Egypt, which is located on the Suez Canal at 30°34'N and 32°18'E. Clams of experiments were transported and placed in the receiving tank to recover from the transportation stress and acclimate to lab conditions in wet laboratories at the National Institute of Oceanography and Fisheries – Alexandria, Egypt. The mean length at the start of the study was 23.22 mm (±0.84 SD).

Experimental design

The experimental design was composed of four treatments representing different PCO_2 (420 ppm, 550 ppm, 750 ppm, 1050 ppm). After two days of acclimation, clams were housed in 3 L of 1µm filtered seawater in 4-liter tanks. The experiment was running in two sets A and B with exactly the same condition. Each set was composed of 12 experimental tanks (3 replicate tanks per each PCO_2 treatment). Each tank had an air pump for providing oxygen for healthy circulation system. Set A was used to measure metabolic rate, clearance rate and ammonia excretion, while set B had been set up for mortality recording, shell length, total weight and condition index measurements .

Clams were fed with *Isochrysis galbana* at concentration of 100,000 cell/ml once daily. Clams were incubated for a period of 36 days at the four pCO_2 concentrations mentioned above keeping alkalinity constant.

Seawater chemistry manipulation

Seawater was adjusted to the four CO_2 concentrations {420 ppm (ambient control), 550 ppm, 750 ppm and 1050 ppm} and the corresponding pH's by mixing with CO_2 saturated sea water while keeping alkalinity constant by using the seacarb package within R program. This CO_2 saturated seawater was acidified through bubbling of 1µ filtered sea water with pure CO_2 gas till saturation. This design for acidification experiment manipulation had declared the best way to manipulate the pCO_2 at constant alkalinity.

Seawater sampled for the initial pH was measured by pH meter (Jenway 3505). The pH electrode was calibrated with TRIS buffer on a total (T) scale following Dickson *et al*. (2007). Total alkalinity (TA) was measured following Sarazin *et al.* (1999). Certified reference materials (CRM batch 115) were used to calibrate and establish correction factors for TA measurements that were obtained from Professor Andrew Dickson at the Marine Physics Laboratory of the Scripps Institute of Oceanography, University of California, San Diego. Seawater temperature, salinity, silicate and phosphate were measured spectrophotometry according to Grasshoff *et al.* (1983) and used as an input for CO_2 sys calculations.

Seawater carbonate chemistry parameters in the beginning of the experiment and throughout it were determined via calculations by CO_2SYS using the two measured CO_2 parameters, pH_{sw} and TA_{sw}. Regarding the carbonate system, dissociation constants K1 and K2 (Mehrbach *et al*., 1973, refitted by Dickson and Millero, 1987) were used. The acidity constant of the H_2SO_4 in seawater was calculated using the constants of Dickson (1990).

Water chemistry in each system was replaced daily, monitored and adjusted every 2 hours using carbon dioxide saturated Seawater.

Biometrics measurements

Shell length and total weight:

Shell lengths of 360 individuals (30 individuals per replicate jar x 3 replicates for each of 4 treatments: 30×3×4=360) were measured initially and at the end of the experiment using a Vernier caliper to 0.1 mm. In clams, the length corresponds to the anterior/posterior axis and is measured perpendicularly from the height line, matching the dorsal/ventral axis. Weights of the same group were also measured using four decimals ±0.0002 ordinary laboratory balance.

Condition index (C.I):

A day before setting up the experiment, condition index (C.I) 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^{o}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].

Mortality:

Mortality was daily recorded by counting dead and gapping (diying) clams after which they were removed from the jars and their shell lengths measured and recorded by date.

Physiological measurements

Metabolic rate:

Metabolic rate was calculated by measuring oxygen consumption rate (OCR) by incubating one clam per jar from each treatment in 250 mL oxygen bottle for 3h. Clams were not fed for a period of 6h before the start of the incubations. Oxygen consumption rates were estimated as the rate of oxygen concentrations decrease over the 3h incubations as measured according to the modified Winkler method for initial and after 3h incubation time. OCR was calculated using the following equation (Cerezo Valverde *et al*. 2006):

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

The initial and final concentrations of dissolved oxygen (DO) are denoted by subscripts 0 and t time respectively, V is the volume of 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:

Clearance rate was determined through the volume of water cleared of particles per unit time, and it can be determined indirectly by monitoring the decline in algal cells in a “closed system”. After allowing 5 minutes for the algal cells to mix thoroughly. A sample of 10 ml was taken from the center of each jar with a 10-mL pipette. Samples were collected after 2 hours. The cell concentrations were counted immediately using an electronic particle counter Coulter Counter® Model ZM or D, fitted with a 140 µm aperture tube. Cell concentrations were the mean of 3 to 4 counts. Clearance rate was calculated using the equation (Coughlan, 1969):

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

Where: CR is the clearance rate, V is the volume of water used, C_1 and C_2 are the cell concentrations between two sampling times, and t is the time increment.

Ammonia excretion rate:

The experiment was conducted by placing 30 adult clams (~ 6 g dry weight) in 3 L of seawater and monitoring the ammonia concentration initially and 24 hours after feeding with *I. galbana*. This analysis was performed according to methods described by Grasshoff *et al*. (1983).

Statistical analysis:

All statistical analyses were performed using SPSS (version 22). Paired T- test has been performed to declare the differences between before and after the experiment in both shell length and total weight of the studied clams. Moreover, a one-way ANOVA was performed with treatment as a single factor to determine differences among treatments (different acidification levels) and effect of time post exposure on different indices of clam physiological responses.

RESULTS

Mean size of clams used in the present study was SL= 23.22 mm ± 0.84 SD. pH of the ambient control represented by PCO_2 of 420 was about 8.12 ± 0.06, and for those representing ocean acidification conditions of 550 ppm, 750 ppm and 1050 ppm were 7.98 ± 0.05, 7.84 ± 0.04 and 7.62 ± 0.06 respectively.

The impact of ocean acidification on shell length:

Comparing different PCO_2 treatments, *Ruditapes decussatus* showed no significant changes in their shell length between SL before the start and at the end of the experiment ($P \geq 0.05$; Paired t- test in each treatment) with no significant differences in SL among all treatments including the control after exposure to different acidification levels (ANOVA shown in Table 1).

The impact of ocean acidification on total weight:

Total weight had insignificant decrease amongst different treatments ($P \geq 0.05$; Table 1) after 36 days of acidification experiment at different pCO2 concentrations. Paired t-test showed that there were significant differences between total before and after the experiment for both treatments 550 and 1050 ppm (P= 0.024 and 0.002, respectively).

Table 1: One-way ANOVA testing the biological and physiological parameters in *Ruditapes decussates* clams exposed to different acidification conditions (pCO_2 = 420, 550, 750 and 1050 ppm).

Source	df	F	Sig.
Shell length	3	1.226	0.300
Total weight	3	3.809	0.010
Condition Index	4	23.299	0.000
Metabolic Rate (concentration)	3	0.445	0.723
Metabolic Rate intervals (TIMES)	8	7.122	0.000
Clearance Rate (concentration)	3	0.681	0.572
Clearance Rate intervals (TIMES)	7	3.818	0.006
Ammonia Excretion (concentration)	3	1.349	0.326
Ammonia Excretion intervals (TIMES)	3	4.1	0.051

The impact of ocean acidification on condition index:

Condition index values decreased in all treatments compared to the initial values of the stock (Fig. 1).

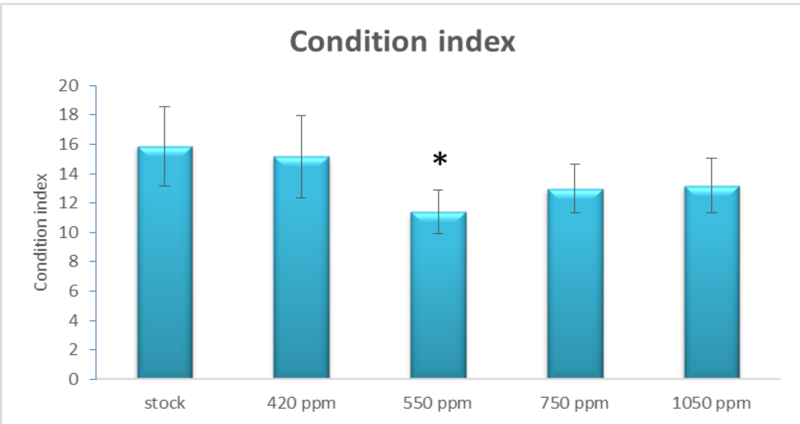

Fig. 1: Mean condition index (gDW ±SD) of *Ruditapes decussatus* exposed to different ocean acidification conditions represented by different pCO_2 (420 ppm (ambient control), 550 ppm, 750 ppm and 1050 ppm) for 36 days experiment. Asterisk (*) represent significant difference.

Result showed that the lowest condition index of 11.40 ± 1.49 was observed in the 550 ppm treatment, while the ambient control group showed C.I value of 15.17 ± 2.80. Condition index had significant differences among different treatments ($P < 0.05$; Table 1).

The impact of the ocean acidification on clam mortality:

No significant effect of ocean acidification was observed on clam mortality. Mortality of *Ruditapes decussatus* incubated clams at 550 ppm had highr mortality of 8% compared to the ambient group (3%) which is not significant in both cases. No other high mortalities occurred in any of the treatments (Fig. 2).

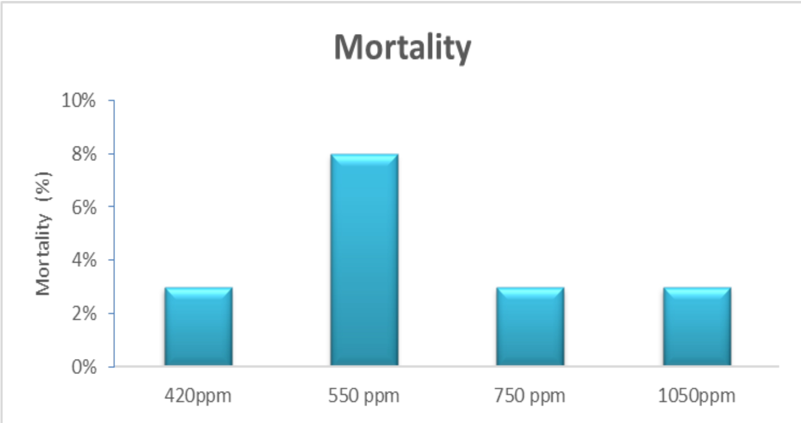

Fig. 2. Mortality (%) of *Ruditapes decussatus* exposed to different ocean acidification conditions represented by different pCO_2 (420 ppm (ambient control), 550 ppm, 750 ppm and 1050 ppm) for 36 days' experiment.

The impact of the ocean acidification on metabolic rate:

In calculating oxygen consumption rate, a high metabolic rate was observed at the beginning of the experiment in all treatments (Fig. 3). Metabolic rates fluctuated throughout the experimental duration but in general was lower than initial value. However, It did not show significant difference amongh different groups (Table 1). Briefly; following the metabolic rate over the experimental time intervals, metabolic rates of clams were lower than initial values after 5 days and 9, 31 and 36 days as compared to initial values (Fig. 3). Time post exposure to different acidification levels had significant impact on metabolic rate in clams (P=0.000).

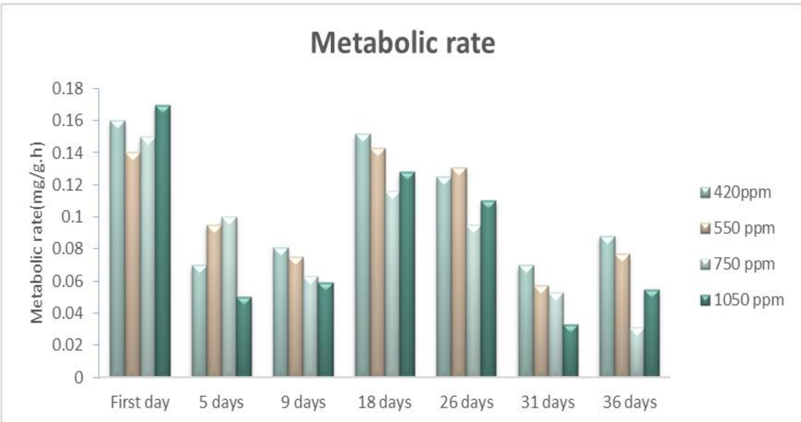

Fig. 3. Metabolic rate of clam (*Ruditapes decussatus*) batch (A) under different acidification conditions (pCO_2 = 420, 550, 750 and 1050 ppm).

The impact of the ocean acidification on clearance rate:

The algae clearance rates by *Ruditapes decussatus*, at different acidification concentration, showed no significant differences among treatments (Table 1). Increasing the time of exposure to acidification showed significant differences among time intervals (P= 0.006). Clams incubated at pCO_2 (1050 ppm) showed the lowest clearance, while the highest clearance rate was recorded by the ambient group pCO_2 (420 ppm; Fig. 4).

Fig. 4: Clearance rate ofalgae by *Ruditapes decussatus* under different acidification conditions (pCO_2 = 420, 550, 750 and 1050 ppm).

The impact of the ocean acidification on Ammonia excretion:

Ammonia excretion determination showed that the highest ammonia concentration was recorded in the groups of 420 ppm ambient control and 550 ppm, while the lowest ammonia concentration was recorded in the 1050 ppm group (Fig. 5). However, ANOVA test recorded no significant differences between different groups (Table 1). Moreover, ANOVA cleared that there was a significant effect of time of exposure to acidification (P= 0.05; Table 1).

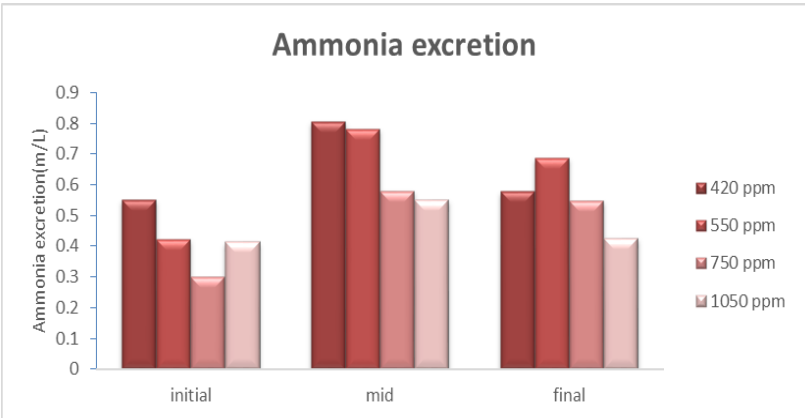

Fig. 5: Ammonia excretion of *Ruditapes decussatus* under different acidification conditions (pCO_2 = 420, 550, 750 and 1050 ppm).

DISCUSSION

The present study is focused on the effect of ocean acidification on the Mediterranean clam *Ruditapes decussatus* which is rated as one of the most commercially important and highly economic bivalve in many countries including Egypt (FAO 2018; Abbas *et al*. 2018). This is due to increasing consumer demand and significance for potential aquaculture of bivalve in Egypt.

As the assent within the ocean acidification field escalates by time, the impacts of lowered seawater pH are hugely affecting the energy distribution requirements of the clams, as more energy is needed for the conservation processes of the physiological balance (Wood *et al.* 2008, Thomsen and Melzner 2010, Stumpp *et al.* 2011, Pan *et al.* 2015).

Studies that examined bivalve's responses to ocean acidification showed that adult bivalves are mostly less sensitive to changes in oceanic pCO_2 than the larval stages, but they still display changes in growth, calcification, and physiological responses (Dupont *et al*., 2010). Bivalve shell deposition is negatively impacted by elevated pCO_2(Gazeau *et al*., 2007), and some studies have illustrated decreased shell growth in low pH-exposed juveniles and adults (Beniash *et al*., 2010; Michaelidis *et al*., 2005; Thomsen and Melzner, 2010).

The impact of the ocean acidification on biometric measurements:

Growth of marine organisms is a physiological process which becomes significantly slower under long-term exposure of increased CO_2 levels (Michaelidis *et al*., 2005).

Some studies have shown a link between pH conditions and bivalve growth rates. Researches are pointing to the inhibiting effect of ocean acidification on the growth of different species of bivalve (Michaelidis *et al*., 2005, Berge *et al*., 2006). For example, (Shirayama and Thornton, 2005) found that even very average increases in CO_2 of 200 ppm above present levels lead to reduction in the growth rate and the survival rate of echinoderms and gastropods, indicating that long-term of exposure to CO_2 changes can affect the growth of calcifying organisms.

Bressan *et al.* (2014) found that under acidified conditions, the *Chamelea gallina* did not grow, but their live weight decreased greatly and a slight reduction in shell length was even observed.

In the present study, the impact of increased CO_2 on growth is represented in terms of shell length and weight. SL changes showed a slight insignificant reduction in shell size. Similarly, experiments on juvenile *Veneridae* (Venus clam) have not shown any significant difference in growth which is measured as size, weight and net calcification (Range *et al*., 2011, Talmage and Gobler, 2011). Moreover, the studies on *Mytilus edulis* (Melzner *et al*. 2011) showed no significant CO_2 effect to shell mass and length. The studies of the impact acidification on shell length and shell weight of *Mytilus galloprovincialis* (Gazeau *et al*., 2014) did not appear to be highly sensitive to ocean acidification. Similar recorded results on the clam*Mercenaria mercenaria* were explained as a result of shell erosion (Ringwood and Keppler, 2002).

Regarding the recorded total weight during the current experiment, results showed no positive growth was recorded in terms of total live weight in the clams, with no significant differences being detected between the control and treated animals. Other studies described that growth might be significantly reduced under medium and long-term exposure to elevated pCO_2 levels in mussel *Mytilus chilensis* (Navarro *et al*., 2013; Duarte *et al*., 2014). Moreover, both shell and soft tissue growth of the oyster *C. virginica* were reduced when exposed to high pCO_2 levels (pH 7.5; Beniash *et al*., 2010).

The recorded results in the current study of the C.I. values decreased throughout all treatments compared to the ambient control group. Result showed the lowest condition index (11.40 ± 1.49) at 550 ppm, compared to the control group (15.17 ± 2.80) that was approximately unchanged from the stock. The high condition values are good indicators for nutrient reserves accumulation for total weight growth in bivalves (Filgueira *et al*. 2013). The lowest recorded condition index (C.I) occurd at 550ppm CO_2 where it is consistent with the highest mortality observation. This may explain the observed lower energy allocation to growth under pCO_2 conditions (550 and 1050ppm).

Mortality (%) of clam *Ruditapes decussatus* in the present study showed a maximum mortality of 8% recorded at 550 ppm and delayed mortality at higher pCO_2 (750 and 1050 ppm). This could be explained according to the hypothesis of Gazeau *et al*. (2014) by which exerting metabolic depression CO_2 can alleviate the level of stress and delay mortality through more efficient exploitation of energy reserved and passive tolerance as an adaptation strategy. This is achieved through more efficient imposition of energy reserves and passive tolerance. Therefore, the results of the current study showed low metabolic rate and low mortality rate recorded at both treatments (750 and 1050 ppm), whereas group 550 ppm showed higher mortality (8%) as they had high metabolic rate relative to the ambient control group.

The impact of the ocean acidification on physiological responses:

Several studies have described metabolic depression in different species of marine organisms at elevated pCO_2 concentrations as caused by the low capacity to compensate for disturbances in extracellular ion and acid–base status (Michaelidis *et al*., 2005; Pörtner, 2008). This is in agreement with the results of the present study, where the oxygen uptake was depressed at 750 and 1050 ppm CO_2 as compared to the control seawater. A depression in the metabolism caused by the un neutralized acid-base status and produce "trade-off" in energy budget in many species. This adaptation strategy is utilized to match Adenosine triphosphate supply and Adenosine triphosphate demand and thus extend their survival (Melatunan *et al* 2013). On the other hand, the clams under 550ppm CO_2 showed high metabolic rate associsated with to the highest mortality as explained previously. However, this was referred to as an increase in food absorption efficiency (Fernández-Reiriz *et al*., 2012). Fernández-Reiriz *et al*. (2011)reported similar results for the specific rate of oxygen consumption by juveniles of the clam *R. decussatus*, with lower values in individuals exposed to high levels of pCO_2 with (Δ pH -0.7).

Considering the impact of sea water acidification on clams feeding, the results showed a pronounced decrease in the clearance rate at the highest pCO_2 level (1050 ppm) corresponding to pH of (7.62). The negative effect of increasing pCO_2 on the feeding rate of *R. decussatus* observed in the present study had also been recorded for the same species (Bamber 1987; Fernandez-Reiriz *et al*. 2011). Fernandez-Reiriz *et al*. (2011) observed a reduction in the feeding rate in the clam *Ruditapes decussatus* at the highest experimental pCO_2 equivalent to pH 7.48. Similarly, according to Bamber (1987), the feeding activity of the clam *R. decussatus* is inhibited at pH < 7.0, and both, tissue and shell growths were significantly reduced. Bamber (1990) also described a negative effect of seawater acidification on the feeding activity and growth of the bivalves *Ostrea edulis*. Results of the present study may be explained by indicating possible deficiencies in the functioning of the digestive systems under conditions of seawater acidification which is synergized with metabolic decline, where the oxygen uptake decreased at elevated pCO_2 levels with high pCO_2 levels in the seawater(Navarro *et al*., 2013).

Several studies reported low ammonia excretion (AE) of marine bivalves with elevated pCO_2 levels (Bayne and Newell, 1983; Velasco and Navarro, 2005). Considering the impact of seawater acidification on clam ammonia Excretion (AE), Wang *et al*. (2015) observed low values for AE (20–45%) in the mussel *Mytilus coruscus* with no significant differences between three different pH levels (8.1, 7.7, and 7.3). Navarro *et al*. (2013) reported that elevated pCO_2 levels (pH 7.57) significantly reduced the AE of *Mytilus chilensis*, indicating possible deficiencies in the functioning of the digestive system under conditions of seawater acidification. In the present study, Ammonia excretion was affected by acidification, and a decrease was observed in clams exposed to the highest pCO_2 (1050 ppm; pH 7.62). These results are in consistence with the recorded low clearance rate and low metabolic rate in sequence.

CONCLUSION

The present study suggests that ongoing ocean acidification may not pose great threats to the exixtence of Mediterranean clam *Ruditapes decussatus* as many senarios expected. The poor, clearance rate (feeding rate), growth and condition index upon exposure to ocean acidification may lead to poor aquaculture potential and health of *R. decussatus* unless adaptation mechanisms may develop with continuous gradual exposure to increasing ocean acidification. Risk assessment is needed for the Mediterranean bivalve aquaculture in the current century. More research is needed on consequent generations to investigate the possibility of the organisms to create adaptation strategies convenient to future climate scenarios at both organismal and genetic levels.

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