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

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**INTRODUCTION**

Ocean acidification is the reduction of pH in the seawater due to the increased levels of atmospheric CO₂. At a quickened rate, the CO₂ deposition into the atmosphere has been increased since the industrial revolution (beginning in the 1750s). The increases in atmospheric carbon dioxide partial pressure (pCO₂), and different greenhouse gases have been given a share in global warming. The Current average atmospheric pCO₂ of approximately 400 ppm is higher than the highest pCO₂ 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 Henry’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$_2$SYS 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$_2$SO$_4$ 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: 30x3x4=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°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 (dying) 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):

\[
OCR = (DO_0 - DO_t) \frac{V}{(DW \times T)}
\]

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):

\[
CR = \frac{V (\ln C_1 - \ln C_2)}{t}
\]

Where: CR is the clearance rate, V is the volume of water used, C₁ and C₂ 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 PCO₂ 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 PCO₂ treatments, *Ruditapes decussatus* showed no significant changes in their shell length between SL before the start and at the end of the experiment (P ≥ 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 ≥ 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 (pCO₂ = 420, 550, 750 and 1050 ppm).

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<td>Ammonia Excretion intervals (TIMES)</td>
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</table>

**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).

![Condition index](image)

Fig. 1: Mean condition index (gDW ±SD) of *Ruditapes decussatus* exposed to different ocean acidification conditions represented by different pCO₂ (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 higher 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).

![Mortality](image)

**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 amongst 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).

![Metabolic rate](image)

**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).

![Clearance Rate](image1)

Fig. 4: Clearance rate of algae 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).

![Ammonia excretion](image2)

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

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) occurred 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 unneutralized 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 (Melahtun et al. 2013). On the other hand, the clams under 550ppm CO$_2$ showed high metabolic rate associated with the highest mortality as explained previously. However, this was referred to as an increase in food absorption efficiency (Fernández-Reiriz *et al*., 2011). 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 pCO₂ (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 existence 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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