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Effect of seawater acidification on physiological and energy metabolism responses of the common Cockle (*Anadara antiquata*) of Gazi Bay, Kenya

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ABSTRACT

Ocean acidification (OA) is becoming a potential threat to marine organisms, especially in calcifying marine invertebrates. So far, along the Kenya Coast, there has been little research on the impact of OA on cockle (*Anadara antiquata*), particularly on their physiological impacts induced by exposure to acidified seawater. Hence, this study aimed to investigate the physiological and biochemical responses of *Anadara antiquata* under present and future predicted seawater pH. In this study, the *Anadara antiquata* was exposed to three pH treatments (pH 7.90, 7.60, and 7.30) for 8 weeks to mimic future OA and to understand the physiological and biochemical effects on the organisms. Condition index, energy reserves (glycogen and protein), and cellular damage (e.g., lipid peroxidation level) were measured. Condition index (CI) showed no significant difference at different pH treatments (pH 7.90, 7.60, and 7.30), whereas the survival *Anadara antiquata* was slightly reduced after 8 weeks of exposure to pH 7.30. Glycogen and protein content were not affected at reduced pH (7.60 and 7.30). However, after 8 weeks of exposure to pH 7.60 and 7.30, *Anadara antiquata* showed a slight decrease in lipid peroxidation, an indication of cellular damage. The physiological and biochemical parameters analyzed (glycogen and protein content; lipid peroxidation levels) showed useful biomarkers to assess ocean acidification impacts in cockle.

1. Introduction

Coastal areas are among the most productive and dynamic marine ecosystems, supporting a variety of essential habitats and species (Velez et al., 2016a). However, the absorption of atmospheric CO_2 by the ocean since industrial times has progressively changed seawater chemistry through a process known as ocean acidification (OA) (Small et al., 2010; Guamán-Guevara et al., 2019). These nearshore areas are characterized by small-scale interactions among physical, biological, and anthropogenic factors, which contribute to a relatively large variability (spatial and temporal) of seawater carbonate chemistry relative to the open ocean (Range et al., 2012). OA may threaten nearshore marine ecosystems by affecting the physiology and ecology of many marine calcifying organisms that produce calcium carbonate exoskeletons and shells (Kroeker et al., 2010; Tate et al., 2017; Fitzer et al., 2014).

Invertebrates exhibit a low capacity for acid-base regulation making changes in acid-base and ion status to directly interfere with their performance (Lannig et al., 2010; Melzner et al., 2009; Pörtner et al., 2004). Future increase in CO_2 concentrations is therefore expected to strongly affect invertebrates with weak acid-base regulators and unable to compensate for the OA-induced shift in extracellular pH (Lannig et al., 2010). Benthic calcifying organisms, such as marine bivalves (oysters, mussels, clams, and scallops), are among the most susceptible species to pH reduction caused by CO_2-induced shifts in the carbonate system speciation towards a decreased concentration of carbonate ions (Melzner et al., 2011; Kroeker et al., 2013; Velez et al., 2016a). This decrease in seawater pH has been recognized as a key factor that affects organisms' physiological morphology and biochemical processes required in response to environmental changes (Lannig et al., 2010; Liao et al., 2019; Dupont et al., 2008). OA may also increase metabolic energy demands exceeding the energy supply from food and accrued energy resources in the aquatic organism, leading to lack of adenosine triphosphate (ATP) to sustain routine metabolism (Berge et al., 2006; Chan et al., 2016).

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Recent studies have shown that the vulnerability of bivalves to OA differs between species. For example, the clams (*Ruditapes philippinarum*) of Southern European, Portugal, were reported to be able to maintain/regulate their physiological status and biochemical performance under reduced pH (7.30) (Velez et al., 2016a). Freitas et al. (2016) also observed that long-term exposure of polychaete (*Hediste diversicolor*) from Ria de Aveiro lagoon (Portugal) to increased OA conditions did not have adverse effects on the biochemical and physiological. Juvenile mussels (*Mytilus coruscus*) of Shengsi island, China, survived on exposure to reduced seawater pH (Wang et al., 2015). However, the juvenile mussels (*Mytilus edulis*) from Loch Fyne, Argyll, Scotland, could not produce aragonite when exposed to increased OA conditions (Fitzer et al., 2014). Furthermore, Auzoux-Bordenave et al. (2020) reported a significant reduction in juvenile abalone (*Haliotis tuberculata*) shell length, weight, and strength on exposure to reduced seawater pH. Similarly, increased OA conditions influenced gastropod (*Littorina littorea*) of Wembury Bay, South Devon, on induced morphological defenses in the form of shell thickness (Bibby et al., 2007). Whereas, exposure of bivalve *Mytilus edulis* L. of Oslofjord, Norway, to seawater with elevated pCO_2 negatively affected their growth (Berge et al., 2006). Seawater with high pCO_2 was also reported to interfere with the energy metabolism and antioxidant responses of Yesso Scallop (*Patinopecten yessoensis*) in Shandong province, China (Liao et al., 2019). While low simulated seawater pH resulted in the decrease in shell weight and growth but increase in soft tissue growth of Baltic clam (*Limecola balthica*), and did not result in any fatal outcomes after 56 days of exposure (Sokołowski et al., 2018).

Marine bivalve, cockle (*Anadara antiquata*), are sediment-dwelling filter-feeding found in the intertidal or marginally subtidal distribution and easily collected during low tide by gleaning (Pattikawa and Ferdinandus, 2009; Alati et al., 2020). These organisms have the potential to mediate benthic primary productivity through their roles in the recycling of nutrients and linking primary productivity with upper trophic levels (Range et al., 2012). *Anadara antiquata* provides a source of protein and income to the local communities' fishing families and has been investigated for their aquaculture potential along the Kenya coast (Alati et al., 2020; Magondu et al., 2022). The shell of the cockle's innermost layer (*hypostracum*) and middle layer (*ostracum*) consists of aragonite crystals which are more soluble calcium carbonate polymorph than calcite (Cubillas et al., 2005; Ong et al., 2017). Hence decreasing seawater pH decrease may lead to a decrease in the concentration of carbonate ions (CO_3^{2-}), one of the building blocks of calcium carbonate ($CaCO_3$), and likely alter the ability of tropical calcifying organisms to precipitate $CaCO_3$ (Gazeau et al., 2014). Hence, the need to understand the resilience of organisms and ecosystems exposed to permanent or periodical extreme OA conditions is vital in managing marine ecosystems. It's worth mentioning that some bivalves will adapt to the future acidified marine environments through genetic responses, whereas some of these organisms' biochemical performance will be affected by the decrease in seawater pH (Wang et al., 2016; Freitas et al., 2017).

Studies on the impact of ocean acidification on cockles (*Cerastoderma edule*) from Oosterschelde estuary, Netherlands (Ong et al., 2017), and from Ria de Aveiro coastal lagoon, Portugal (Magalhães et al., 2018), as well as cockle (*Tegillarca granosa*) of Kuala Juru, Penang, Malaysia (Nithiyaa et al., 2021), have been conducted. However, there is limited information on how OA will impact the cockles (*Anadara antiquata*) of Gazi Bay, Kenya. Hence the present study aimed to investigate the physiological and biochemical responses of *Anadara antiquata* under present day conditions and near-future ocean acidification projection scenarios (reductions of 0.3 and 0.6 units, relative to natural pH).

2. Materials and methods

2.1. Organisms collection

Adult cockles (*Anadara antiquata*) in Fig. 1 were collected during low tide in the lower intertidal zone at the Gazi Bay (4°25′19″S, 39°31′01″E), Kenya and transported in plastic buckets to KMFRI laboratories. Upon arrival in the laboratory, the organisms were measured for shell length and width (4.04 ± 0.29 cm of length and 3.25 ± 0.334 cm of width). Thereafter organisms were randomly placed in nine 50 L plastic tanks, each containing 24 individuals. The tanks had been filled with sediment to a height of 10 cm to mimic the natural habitat and the organisms were acclimatized for one week before use in the experiment. A flow-through system was used to avoid the accumulation of metabolic waste products, which might interfere with the pH treatments. Since cockles are suspension-feeding bivalves that consume minute particulate matter suspended in the water column. This matter includes both living organisms (e.g., plankton) and non-living material (such as plant debris or suspended soil particles) (Carss et al., 2020). Hence, the organisms were fed with 10 mL microalgae (*Chaetocerotaceae gracillis*) every 12 h at a concentration of 1×10^6 cells/mL to supplement organic matter resuspended from the sediment.

2.2. Experimental design and seawater carbonate chemistry

Cockles were exposed to 3 different pH (randomized design): nominal pH 7.90 (control pH), 7.6, and 7.3 in triplicate (n = 3) for 8 weeks. Pure CO_2 was bubbled into each tank to reach the target pH, and the pH set levels were controlled using a pH-stat system (Aqua Medic, Bissendorf, Germany). The pH systems were calibrated weekly using two-point calibration in standard pH buffers (Aqua medic buffer 4 and 7 solutions) at pH 4.01 and pH 7.00. Seawater pH was checked twice a day using an independent probe (Orion Star A211 Instrument) for one week before exposure and during the first week of the exposure experiment. This procedure was repeated daily during the remaining experimental period, and the pH Stat computer reset to match the pH value given by the independent probe whenever necessary. pH was measured on the

Fig. 1. Picture showing sediment-dwelling filter-feeding Adult cockles (*Anadara antiquata*) found in the intertidal zone in Gazi Bay.

total scale (pH_T) after calibration using an equimolal buffer that consists of 2-amino-2-hydroxymethyl-1,3-propanediol('Tris') and Tris hydrochloride; TRIS (Tris/HCl) provided by A.G. Dickson, Scripps Institution of Oceanography, USA. Dissolved oxygen concentrations were monitored daily, and water samples were collected from each tank weekly to quantify total alkalinity (TA) by open potentiometric titration using an automatic titrator (888 Titrando, Metrohom), using a method reported in the literature (Gran, 1952). The accuracy and precision of TA were checked against certified reference seawater samples (reference material, Batch #173, provided by A.G. Dickson, Scripps Institution of Oceanography, USA). All the physicochemical water parameters for each condition are presented in Table 1. During the experimental period, seawater was renewed every week, which was similar to temperature in Gazi Bay (ranging between 26.9 and 30.4 °C) from October to December, and pH levels re-established. Organisms were collected and stored at −80 °C for further analysis after every 2 weeks.

2.3. Laboratory analysis

2.3.1. Sample collection

The *Anadara antiquata* were collected after every 2 weeks during the 8 weeks of exposure. The whole soft tissue samples were stored at −80 °C until further analyses. Frozen tissues were homogenized (IKA T50 digital Homogenizer) under ice and separated into 0.5 g aliquots biochemical analysis. For extraction, each sample (0.5 g of homogenized soft tissues) was homogenized for 15 s at 4 °C and centrifuged for 10 min at 10,000*g* and 4 °C with specific buffers for each physiological and biochemical analysis.

2.3.2. Biochemical parameters

2.3.2.1. Energy-reserves. Total protein content was determined following Biuret spectrophotometric method described by Robinson and Hogden (1940), using bovine serum albumin (BSA) as standards (0–40 mg/mL). Absorbance was read at 540 nm and obtained concentration expressed in mg/g fresh weight (FW).

The glycogen content was quantified following the anthrone colorimetric method (Hedge and Hofreiter, 1962). Glucose standards were used to obtain a calibration curve (0–1 μg/mL). Samples were incubated at room temperature for 30 min, and absorbance was measured at 630 nm. Results were expressed in mg/g FW.

2.3.2.2. Non-enzymatic markers of oxidative stress. Lipid Peroxidation was determined using a similar method reported in the open literature (Buege and Aust, 1978). The amount of TBARS (thiobarbituric acid reactive substances) was measured based on the reaction of lipid peroxidation by- products, such as malondialdehyde (MDA), with 2-thiobarbituric acid (TBA), forming TBARS. The concentration of lipid peroxidation was measured by the quantification of malondialdehyde (MDA) equivalents, a by-product of lipid peroxidation. Absorbance was read at 535 nm, and concentration in the sample was calculated using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and expressed in mg/g FW.

Table 1
Carbonate system physicochemical parameters for each condition (mean ± SD). Measured pH and determined total alkalinity (TA) from weekly water sampling. Partial CO_2 pressure (pCO_2), bicarbonate (HCO_3^-) and carbonate ion concentrations (CO_3^{2-}), and saturation states of calcite (Ω_{Cal}) and aragonite (Ω_{Arg}), calculated with CO2SYS software.

Parameter	7.90	7.60	7.30
pH_T (measured)	7.90 ± 0.10	7.56 ± 0.11	7.30 ± 0.07
Temperature (°C)	27.8 ± 0.57	27.8 ± 0.66	27.9 ± 0.74
TA (μmol/kg)	2281 ± 49	2269 ± 36	236 ± 65
Dissolved oxygen (mg/L)	8.22 ± 0.16	8.16 ± 0.25	8.18 ± 0.27
pCO_2 (μatm)	677 ± 218	1598 ± 531	3032 ± 595
HCO_3^- (μmol/kg)	1935 ± 97	2097 ± 49	2234 ± 70
CO_3^{2-} (μmol/Kg)	147 ± 29	73 ± 15	42 ± 7.0
DIC (μmol/Kg)	2100 ± 132	2214 ± 79	2361 ± 94
(Ω_{Cal})	3.83 ± 0.82	1.91 ± 0.38	1.10 ± 0.20
Ω_{Arg}	2.47 ± 0.53	1.23 ± 0.25	0.71 ± 0.13

2.3.3. Data and statistical analysis

The other carbonate system parameters (seawater CO_2 partial pressure (pCO_2), dissolved inorganic carbon (DIC), aragonite saturation state (Ω_{Arg}) and calcite saturation state (Ω_{cal}), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) ions concentrations) for each tank were calculated using CO2SYS software from the measured parameters pH, TA, temperature, and salinity data as described in the reference Robbins et al. (2010) and Mehrbach et al. (1973) refitted by Dickson and Millero (1987), K_1 and K_2 carbonate dissociation constants, and KSO_4 from Dickson et al. (1990).

The mortality of *Anadara antiquata* was checked daily, and the dead organisms lying on top of the sediment were removed immediately during the daily observations to avoid water pollution in the tanks and risk the health of other *Anadara antiquata*. The physiology of *Anadara antiquata* was measured after 4 and 8 weeks of exposure. After the measurements, the soft tissue of at least three organisms were per condition carefully separated from shells and put in an oven at 50 °C for 48 h. Dry soft tissue and shells were weighed, and the condition index (CI) was calculated using Eq. (1) (Velez et al., 2016a).

$$CI = \frac{Dry\ flesh\ weight\ (g)}{dry\ shell\ weight\ (g)} \times 100 \quad (1)$$

All experimental data were analyzed for mean ± standard deviation with three replicates. One-way analysis of variance (one-way ANOVA) and *t*-test was performed on data, and the significance level was set as $p < 0.05$ between treatments. Principal component analysis (PCA) was performed with XLSTAT Version 2014.5.03 software to assess the variability associated with energy metabolism responses in *Anadara antiquata* exposed to varied pH treatments (pH 7.90, 7.60, and 7.30).

3. Results

3.1. Survival and condition index

After 8 weeks of exposure, the survival of *Anadara antiquata* (Fig. 2b) on exposure to pH 7.90 and 7.60 was 92 % and 86 %, with the lowest percentage recorded at the lowest pH 7.30 (76 %). A significant difference in survival between pH 7.90 and pH 7.30 (paired t-test, $t = 6.313$, $p < 0.05$) was observed after 8 weeks of exposure. The condition index (CI) of *Anadara antiquata* (Fig. 1a) showed no significant difference ($F = 0.802$, $p = 0.510$) at different pH treatments (pH 7.90, 7.60, and 7.30) after 8 weeks of exposure even though a slight decrease in CI was observed on organisms exposed to pH 7.30 treatment.

3.2. Energy-related parameters

3.2.1. Protein content

Protein has an important physiological role in the supply of structural elements and the catalysis of metabolic reactions (Anacleto et al., 2014). The protein content in *Anadara antiquate* upon collection was 67.8 mg/g, F.W. However, exposure of *Anadara antiquata* to pH 7.30 presented lower protein content from week 2 to week 8 compared to organisms exposed to pH 7.90 and 7.60 (Fig. 3a). After 8 weeks of exposure, the protein content in *Anadara antiquata* at pH 7.90, 7.60, and 7.30 showed no significant differences ($F = 2.9025$, $p > 0.05$). A slight decrease in protein content was observed in organisms exposed to pH 7.60 (mean 61.2 mg/g, F.W.) and 7.30 (mean 53.7 mg/g, F.W.) after 8 weeks, and an increase in protein content in organisms exposed to pH 7.90 (mean 83.3 mg/g, F.W.).

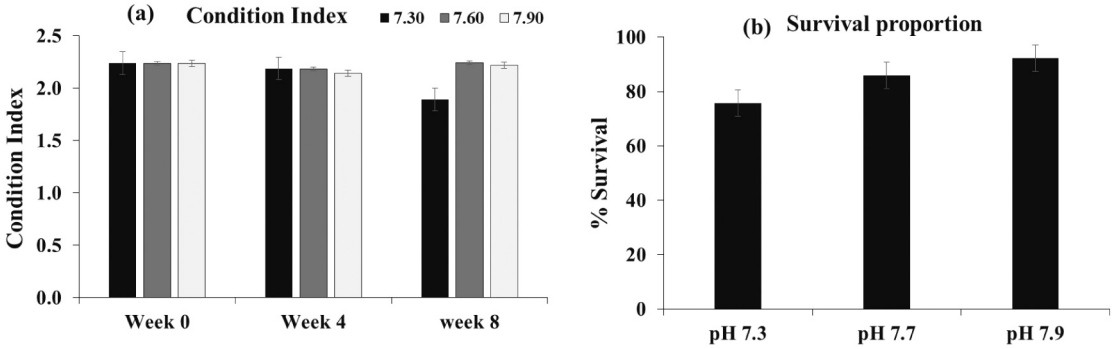

Fig. 2. Effects of (a) pH on condition index and (b) survival rate after 8 weeks exposure of *Anadara antiquata* to pH 7.90, 7.60, and 7.30 treatments.

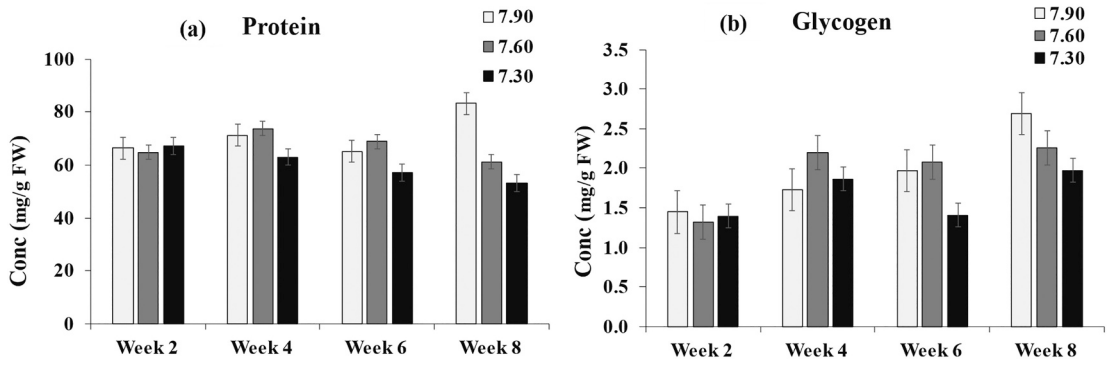

Fig. 3. Energy related parameters: Protein content (a), and glycogen content (b), in *Anadara antiquata* exposed to different pH levels: 7.90, 7.60, and 7.30.

3.2.2. *Glycogen content*

The glycogen content before the experiment showed a concentration of 2.12 mg/g, F.W. which decreased after 2 weeks of exposure to pH (7.90, 7.60, and 7.30) (Fig. 3b). High glycogen content in the organism exposed to pH 7.60 was high in week 4 and week 6 with a mean of 2.29 mg/g, F.W., and 2.08 mg/g, F.W. respectively, as compared to pH 7.90 (week 4; 1.73 mg/g, F.W. and week 6; 1.97 mg/g, F.W.). After 8 weeks of exposure, all organisms exposed to pH (7.90, 7.60, and 7.30) reported high glycogen content. No significant differences existed between organisms exposed to pH 7.90, 7.60, and 7.30 ($F = 0.636$, $p > 0.05$).

3.3. *Seawater chemistry parameters*

The mean values of measured and calculated seawater carbonate chemistry parameters for the three treatments (pH 7.90, 7.60, and 7.30) are given in Table 1. During the 8 weeks of the exposure experiment, dissolved oxygen in the exposure tanks consistently exceeded 8 mg/L during the experiment. Throughout the experiment, the mean total alkalinity ranged from 2269 ± 36 to 2361 ± 94 and presented only slight differences between treatments. Whereas, a significant correlation between DIC and pCO_2 ($p < 0.05$) was observed between the treatments. The pH treatment had a significant effect on all carbonate chemistry parameters ($F = 12.7$; $p < 0.05$), except on TA, where there was no difference ($p > 0.05$). Saturation states of calcite (Ω_{Cal}) and aragonite (Ω_{Arg}) at pH 7.90 (Ω_{Cal}; 3.83 ± 0.82 and Ω_{Arg}; 2.47 ± 0.53) and 7.60 (Ω_{Cal}; 1.91 ± 0.38 and Ω_{Arg}; 1.23 ± 0.25) treatments. However, the lowest pH treatment (7.30) yielded Ω_{Arg} values ranged between 0.53 and 0.99.

3.4. *Non-enzymatic markers of oxidative stress*

The lipid peroxide levels (Fig. 4) were high in organisms exposed to pH 7.60 and 7.30 in comparison to values found in organisms exposed to pH 7.90 ($F = 6.544$, $p < 0.05$).Prior to the experiment, the organisms

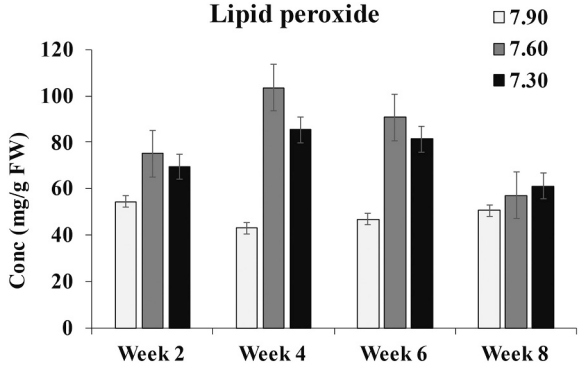

Fig. 4. Lipid Peroxidation content in *Anadara antiquata* exposed to different pH levels: 7.90, 7.60, and 7.30.

exhibited a concentration of 34.9 mg/g, F.W., which was lower than the values detected in organisms subjected to the experiment. It's worth noting that the lipid peroxide levels values obtained in *Anadara antiquata* exposed to pH 7.90 (control) and pH 7.30 showed a significantly different after 8 weeks of exposure (paired t-test, $t = -2.353$, $p < 0.05$).

3.5. *Principal component analysis*

The results from the PCA (Fig. 5) revealed that the first principal component (PC 1) explained 85.44 % of the total variation among pH conditions (7.9, 7.6, and 7.3), clearly separating organisms under control (pH 7.9) at the positive side, from organisms exposed to low pH levels (pH 7.6 and 7.3) at the negative side. PC2 accounted for 14.56 % of the total variation, with organisms exposed to pH 7.6 on the positive side and organisms at the remaining conditions (pH 7.9 and 7.3) on the negative side. The water carbonate chemistry shows that the organism

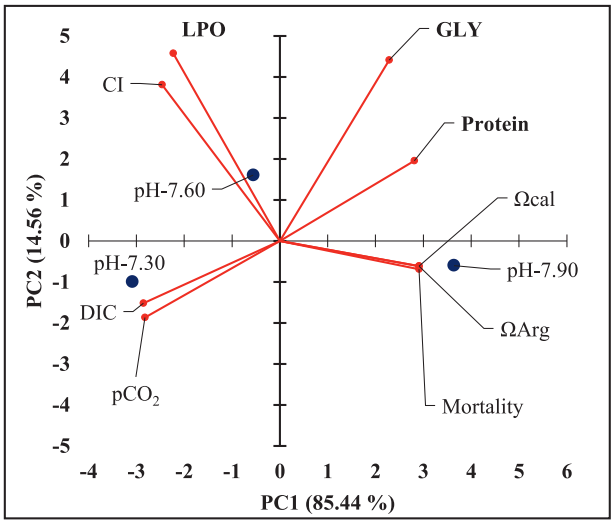

Fig. 5. Principal component analysis (PCA) ordination biplot to assess biochemical responses of *Anadara antiquata* exposed to different pH levels: 7.90, 7.60, and 7.30 for 8 weeks: GLY: glycogen content, PROT: protein content and LPO: lipid peroxidation.

exposed to pH 7.90 were significantly affected by calcite (Ω_{Cal}) and aragonite (Ω_{Arg}) variation. Whereas organisms exposed at pH 7.30 were significantly affected by variations of DIC and pCO_2. The physiological and biochemical superimposed on the PCA showed that glycogen and protein content were associated with organisms exposed to control pH (7.90). In this condition, *Anadara antiquata* presented the highest content for both energy reserves. Whereas organisms exposed to pH 7.60 were associated with LPO since the highest values for both biomarkers were found in individuals under this condition.

4. Discussion

As a direct result of human activities globally, the frequency and intensity of OA have an impact on marine ecosystems, which is expected to increase in the near future (Caldeira and Wickett, 2005; Velez et al., 2016b). Ecosystem responses to OA could have the most severe implications for marine species, ocean biological communities and ecosystems, and the risks to human communities that depend on marine resources and ecosystem services (Melzner et al., 2013; Doney et al., 2020). To the best of our knowledge, no research has studied the physiological and biochemical responses of *Anadara antiquata* under present-day conditions and near-future ocean acidification projection scenarios (reductions of 0.3 and 0.6 units relative to natural pH). Saturation states of seawater with respect calcite (Ω_{Cal}) and aragonite (Ω_{Arg}) at pH 7.90, treatments was >1 which are favorable for calcification. However, at pH 7.30, Ω_{Arg} values were less than <1.0, indicating possible dissolution of aragonite, and limited synthesis of aragonite (Talmage and Gobler, 2009).

The survival of *Anadara antiquata* decreased significantly as the pH decreased and exposure time increased (8 weeks) indicating that the predicted OA levels in the world's oceans later this century will significantly affect the survival of *Anadara antiquata*. Similar results were observed by Priya et al. (2016) on *Donax cuneatus* where the mortality rate increased on exposure to reduced pH for 15 days. Range et al. (2014) also observed an increase in mortality of mussel *Mytilus galloprovincialis*, clams *Chamelea gallina* and *Ruditapes decussatus* on long-term exposure time (75–202 days) to increased CO_2 and reduced pH. On the contrary, Auzoux-Bordenave et al. (2020) observed low mortality in juvenile abalone (*Haliotis tuberculata*) on exposure to reduced pH for 3 months. On the other hand, the condition index of *Anadara antiquata* was not impacted by elevated pCO_2. An indication that the organisms health or condition were unaffected by the change in the environmental condition and may reflect the adaptation of *Anadara antiquata* to different pH levels in intertidal and shallow water habitats. Similar results were reported by Lemasson et al. (2019) where oysters (*Ostrea edulis*) were unaffected after 12 weeks of exposure, as well as adult bivalve (*Mytilus edulis*) after six months of exposure (Mackenzie et al., 2014). Cummings et al. (2011) also observed that adult bivalve (*Laternula elliptica*) was unaffected after 120 days. This was contrary to the cockle (*Cerastoderma edule*) of Oosterschelde estuary, Netherlands, whose condition index was affected after 6 weeks of exposure (Ong et al., 2017).

The main energy storage compounds in bivalves are protein and carbohydrates (in the form of glycogen), which all have important functions in physiological processes, for instance, in gametogenesis and reproduction (Dridi et al., 2007; Lemasson et al., 2019). Higher concentrations of glycogen were observed in *Anadara antiquata* before the experiment, thereafter a decrease after exposure to three pH treatments (pH 7.90, 7.60, and 7.30) for 2 weeks, indicating physiological stress. Subsequently, there was an increase in glycogen content from week 4 to week 8, demonstrating that when organisms are exposed to stressful environmental conditions, there is an increase in energy expenditure. This increase acts as a mechanism of cellular protection to maintain organism conditions by diverting energy from growth and reproduction, allowing successful acclimation to stress conditions (Velez et al., 2016a; Sokolova et al., 2011; Anacleto et al., 2014; Patrick et al., 2006). In addition, glycogen supplies energy quickly through glycolysis and oxidative phosphorylation, and its adequate storage in muscles are important in supporting the optimal muscle movement (Jensen and Richter, 2012). This may reduce energy for other functions in the organism like reproduction and growth which in turn may have repercussions on populations of the organisms as well as their roles in ecosystem functioning (Ong et al., 2017; Zhao et al., 2020). Cao et al. (2018) observed that glycogen and protein were not altered in Pacific oysters (*Crassostrea gigas*) after being exposed to pH 7.60 for 28 days, suggesting the maintenance of energy homeostasis in this treatment. The current study also shows that *Anadara antiquata* presented a slight decrease in glycogen and protein content at low pH (7.60 and 7.30) as compared control conditions (pH 7.90) after 8 weeks of exposure. The decrease in energy reserves may be related to the increase in the electron transport system activity which provides information about the potential metabolic activity (Freitas et al., 2016). A similar observation has been reported in Yesso scallops, where the glycogen content in muscle tissue decreased after 45 days of exposure to low pH seawater (Liao et al., 2019). Lannig et al. (2010) also showed that oyster (*Crassostrea gigas*) individuals exposed to low pH 7.68 significantly reduced their glycogen levels. Studies have shown that organisms may allocate energy from energy storage pools to cover the increasing maintenance demand like tissue repair and maintenance (Ong et al., 2017). The absence of significant differences between protein concentrations after exposure of *Anadara antiquata* to three pH treatments (pH 7.90, 7.60, and 7.30) indicates that protein is more stable than other reserve molecules (Schwaner et al., 2023).

Lipid peroxidation is a well-established mechanism used as a marker of cellular damage, and its measurement is used as an indicator of oxidative damage in cells and tissues (Moreira et al., 2016; Matozzo et al., 2012). Low lipid peroxide content was observed in *Anadara antiquata* before exposure, followed by an increase after two weeks of exposure to three pH treatments (pH 7.90, 7.60, and 7.30). This suggests that under these conditions, excessive reactive oxygen species production may lead to oxidative damage and a loss of compensatory mechanisms due to insufficient antioxidant activity, contributing to higher lipid peroxide content (De Marchi et al., 2019). The concentration of lipid peroxide did not change significantly between weeks 4 to 6 of exposure to reduced pH (pH 7.60 and 7.30). This shows that reduced-pH treatment has little effect on oxidative damage in cells and tissues of the organism due to the development of defense mechanisms that prevent

cellular damage caused by reactive oxygen species (Freitas et al., 2016; Freitas et al., 2017). Hence, the ability of *Anadara antiquata* to withstand low pH exposure. A similar response was reported in the oysters (*Crassostrea angulata* and *Crassostrea gigas*) (Moreira et al., 2016; Velez et al., 2016b) revealed that clams (*Ruditapes philippinarum*) presented high lipid peroxidation content when exposed to low pH levels (7.60 and 7.30) in comparison to organisms under control pH (7.90). Liao et al. (2019) also observed that the lipid peroxidation level in yesso scallop (*Patinopecten yessoensis*) did not change significantly in the reduced pH (7.50). However, after 8 weeks of exposure to pH 7.60 and 7.30, *Anadara antiquata* showed a slight decrease in lipid peroxidation, an indication of cellular damage due to increased production of reactive oxygen species, leading to the oxidation of the lipid membranes (Velez et al., 2016a). Tomanek et al. (2011) observed that an elevation of CO_2 levels might cause oxidative stress by increasing the production of reactive oxygen species either indirectly by lowering organismal pH, which may enhance the Fenton reaction, and/or directly by CO_2 interacting with other reactive oxygen species to form more free radicals.

5. Conclusion

The sessile nature and filter-feeding habits of *Anadara antiquata* render them susceptible to biochemical changes and vulnerable to climate change, such as ocean acidification. This study provided an insight on how *Anadara antiquata* may respond to near-future seawater acidification conditions. The results indicate that condition index, mortality, energy, and oxidative status were not affected when *Anadara antiquata* were exposed for 8 weeks to different reduced pH (7.60 and 7.30). However, the change in the organism's energetic metabolism and antioxidant responses may have consequences on the reproductive output and growth performance on long term exposure to reduced pH (7.60 and 7.30), thus affecting species resilience in a changing environment. The climate change projections suggest that coastal systems will experience acidification in the coming decades. Future studies should aim to understand the long-term effects of ocean acidification and other factors that drive changes in marine ecosystems (e.g., temperature, dissolved oxygen, and salinity). These factors may produce complex interactions on the marine organism as this can reflect their adaptations in coastal areas.

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CRediT authorship contribution statement

Veronica Wayayi Ogolla Wanjeri: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft. **Eric Okuku:** Conceptualization, Formal analysis, Resources, Writing – review & editing, Supervision. **Jane Catherine Ngila:** Writing – review & editing, Supervision. **Patrick Gathura Ndungu:** Formal analysis, Resources, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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