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Simultaneous ocean acidification and warming do not alter the lipid-associated biochemistry but induce enzyme activities in an asterinid starfish

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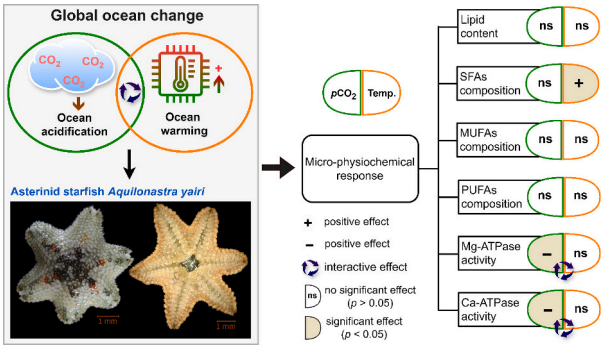
HIGHLIGHTS

- Ocean acidification and warming impacts on tropical-subtropical asterinid starfish were investigated.
- Combined stressors did not affect starfish lipid and fatty acids.
- Ocean warming increased starfish total lipid, SFAs, and PUFAs but reduced MUFAs concentration.
- Elevated temperature allowed starfish to cope with the negative effect of increased pCO_2 on enzyme activities.

GRAPHICAL ABSTRACT



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ABSTRACT

Ocean acidification and warming affect marine ecosystems from the molecular scale in organismal physiology to broad alterations of ecosystem functions. However, knowledge of their combined effects on tropical-subtropical intertidal species remains limited. Pushing the environmental range of marine species away from the optimum initiates stress impacting biochemical metabolic characteristics, with consequences on lipid-associated and enzyme biochemistry. This study investigates lipid-associated fatty acids (FAs) and enzyme activities involved in biomineralization of the tropical-subtropical starfish *Aquilonastra yairi* in response to projected near-future global change. The starfish were acclimatized to two temperature levels (27 °C, 32 °C) crossed with three pCO_2 concentrations (455 µatm, 1052 µatm, 2066 µatm). Total lipid (ΣL_C) and FAs composition were unaffected by combined elevated temperature and pCO_2, but at elevated temperature, there was an increase in ΣL_C, SFAs (saturated FAs) and PUFAs (polyunsaturated FAs), and a decrease in MUFAs (monounsaturated FAs). However, temperature was the sole factor to significantly alter SFAs composition. Positive parabolic responses of Ca-ATPase and Mg-ATPase enzyme activities were detected at 27 °C with elevated pCO_2, while stable enzyme activities were observed at 32 °C with elevated pCO_2. Our results indicate that the lipid-associated biochemistry of *A. yairi* is resilient and capable of coping with near-future ocean acidification and warming. However, the

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calcification-related enzymes Ca-ATPase and Mg-ATPase activity appear to be more sensitive to pCO_2/pH changes, leading to vulnerability concerning the skeletal structure.

1. Introduction

Approximately ≈25 % (mean uptake of -2.7 ± 0.3 Pg C per year^{-1}, 1 Pg = 10^{15} g) of the annual CO_2 emissions are directly absorbed by the oceans (Gruber et al., 2023) and significantly alter seawater chemistry (i.e., reductions in pH and re-equilibration of carbonate systems), termed ocean acidification (OA) (Zeebe, 2012). These alterations leave an imprint on oceanic and coastal environments with potential impacts on the eco-physiology of marine organisms, especially calcifying species (Feely et al., 2004; Doney et al., 2012; Leung et al., 2022). Previous studies have shown that OA impacts physio-chemical aspects (i.e., behavior, reproduction, growth, development, survival, fitness, and metabolism) and skeletal mineralogical structures (i.e., biomineralization) of marine organisms (Kroeker et al., 2013; Dubois, 2014; Byrne and Fitzer, 2019; Melzner et al., 2020; Figuerola et al., 2021; Leung et al., 2022). Furthermore, the effects of OA on marine organisms can interact, often non-intuitively, with those of other environmental stressors, including ocean warming (OW) (Gao et al., 2020). OW alone is recognized to elicit detrimental consequences on vital biological processes of a wide range of marine organisms, with cascading effects on habitat structure and ecosystem functioning (Smale et al., 2019).

Among marine ecosystems, intertidal regions are predicted to experience the most significant impacts of OA and OW due to their exposure to high variability in temperature, pH, and direct anthropogenic drivers (Harley et al., 2006). This environmental volatility could expose marine intertidal organisms (e.g., anthozoa, asteroidea, bivalvia, and gastropoda) to conditions beyond the limits of their tolerance range, which may substantially impact their biological performance metrics (Byrne, 2011; Gao et al., 2020; Melzner et al., 2020). OA and OW can affect cellular and molecular processes that are physically reflected by an organism (Pörtner, 2008). Furthermore, OA and OW have been recognized to influence the intracellular ionic balance in calcifying organisms (Ramesh et al., 2017). Consequently, major reductions in biological performance and shifts in the organismal mode of life (i.e., active vs. passive) can follow, subsequently disrupting the trophic levels and causing further shifts in the food web system (Guinotte and Fabry, 2008; Pörtner, 2008).

As slow-moving intertidal echinoderms, asteroids (common name: starfish or sea stars, class asteroidea) are highly susceptible to abiotic and biotic changes in their habitat. Owing to their capacity as ectothermic animals with narrow tolerable to temperature alterations, asteroids are considered useful model organisms for global change studies focusing on the effects of OW and OA (Nguyen and Byrne, 2014; Lang et al., 2023). OA can narrow the thermal tolerance range, resulting in a higher susceptibility to extreme temperatures and reducing an organism's performance metrics, altering morphological structures, behavioral responses, and physiological processes (Pörtner, 2008; Walther et al., 2009; Schalkhausser et al., 2012). Furthermore, the asteroid endoskeleton is composed of high Mg-calcite (>4 wt% $MgCO_3$ (Weber, 1969; Dickson, 2002)); hence, it is more susceptible to dissolution under OA conditions (Dubois, 2014; Figuerola et al., 2021). Previous research has shown that interactive effects (additive or synergistic) of OA and OW in the asteroid class elevated the metabolic rate (Khalil et al., 2023), increased larval mortality (Byrne et al., 2013), produced larval developmental delay (Hue et al., 2022), behavioral modifications (McLaren and Byrne, 2022), modified coelomic fluid and coelomocytes (Wahltinez et al., 2023), and altered skeletal mineralogy (Khalil et al., 2022). On the contrary, other studies have found that some asteroids could benefit from combined OA and OW, showing increased growth (Gooding et al., 2009), and feeding enhancement (Kamya et al., 2016). However, the biochemical and physiological mechanisms underlying the phenotypic response of asteroids to lower pH (high pCO_2) and increased temperature remain unknown.

Understanding how organisms might respond to combined OA and OW can be gained through laboratory experiments that expose organisms to manipulated levels of seawater pCO_2 and temperature. While in recent years, abundant experiments have been carried out studying the effects of future environmental changes (e.g., elevated temperature, reduced pH, hypoxia, and changes in seawater Mg^{2+}/Ca^{2+} ratio as a sole or combined stressor) on the physiological processes and skeletal production of echinoderm species (Smith et al., 2016; Byrne and Fitzer, 2019; Sampaio et al., 2021; Hu et al., 2022; Lang et al., 2023; Azcarate-Garcia et al., 2024), we here examine how a tropical-subtropical asterinid starfish regulates its physiological performance in response to environmental stressors (OA and OW) by analyzing the composition of lipid-associated fatty acids (FAs) and the activities of enzymes typically involved in the calcification process. Lipids and associated FAs play a critical role in maintaining the functions of growth, metabolism, and buoyancy control; lipid-FAs are not only an essential factor for the fluidity of the plasma membrane but also a carrier for the preservation of material and energy in marine animals and serve as indicators of an organism's dietary pattern and bio-physiological condition (Dalsgaard et al., 2003; Zhukova, 2022). Marine organisms also utilize their lipids as a defense mechanism against the influence of environmental stressors or lipid peroxidation by restructuring FAs and sterols (isoprenoid-derived lipids, e.g., cholesterol in animals) of the lipid bilayer to preserve the physical characteristics of biological membranes (Hazel and Williams, 1990; Parrish, 2013). FAs indicate biochemical alterations in response to organismal physiological stress conditions (Filimonova et al., 2016; Bennett et al., 2018), immunity (Gao et al., 2018), and inflammation (Calder, 2010). FAs contain carboxylic acids with long hydrocarbon chains of different lengths and saturation grades (classified by the number of double bonds), generally classified into saturated fatty acids (SFAs, no double bonds), monounsaturated fatty acids (MUFAs, one double bond per molecule) and polyunsaturated fatty acids (PUFAs, two or more double bonds per molecule) (IUPAC-IUB, 1977). Among these FA chains, PUFAs such as eicosapentaenoic acid (EPA; C20:5ω3) and docosahexaenoic acid (DHA; C22:6ω3) are FA compounds essential for growth, reproduction, and survival (Kattner et al., 2007), and are involved in maintaining cell structures and functions in marine organism (Filimonova et al., 2016).

It is well known from marine animals that FA compounds and membranes are influenced by environmental factors (Hazel and Williams, 1990; Hazel, 1995; Dalsgaard et al., 2003; Yoon et al., 2022), including temperature (e.g., elevated temperature significantly reduced PUFAs and increased SFAs concentration) (Valles-Regino et al., 2015; Garzke et al., 2016) and pCO_2 (e.g., high levels of pCO_2 increase SFAs and reduced PUFAs concentrations) (Rossoll et al., 2012). Increased ratios of PUFAs (ω6:ω3) in animals indicate inflammation and physiological unhealth (Calder, 2010; Safuan et al., 2021). However, ectothermic animals are recognized to have the ability to counteract the effects of environmental stressors through an organism-specific physiological response termed homeoviscous adaptation (HVA) (Sinensky, 1974; Ernst et al., 2016). This adaptation process allows organisms to strengthen their cell membrane structure (i.e., PUFAs formation alters membrane fluidity and SFAs formation increases membrane stability) to avert membrane destabilization and maintain the functional state of cell membranes (Sinensky, 1974; Hazel, 1995; Ernst et al., 2016).

Enzymes play the main role as protein catalysts in most physiological processes to accelerate and regulate biochemical reactions (Hill et al., 2012). They are fundamental in the FA synthesis pathway (e.g., desaturation and elongation of FAs carbon chain) (Zhuang et al., 2022).

Furthermore, enzymes are the principal agents of physiological transformation in that they are responses to alterations in the external environment (Hill et al., 2012). Changes in enzyme activity under OA/OW are reliable proxies to determine the resulting physiological-related process disruption (Donachy et al., 1989; Prazeres and Pandolfi, 2016), including biomineralization processes in calcifying organisms (Chave, 1984; Ivanina et al., 2020). Moreover, alterations in membrane-bound enzymes (e.g., Ca-ATPase and Mg-ATPase) are biomarker indicators of organismal stress, where the level of enzyme variation mirrors the impairment of physiological function that entails these enzyme systems (Vijayavel et al., 2007). Although studies have shown that OA or OW as sole stressors can significantly alter Ca-ATPase and Mg-ATPase activities in coral (Jiang et al., 2019) and foraminifera (Prazeres et al., 2015; Prazeres and Pandolfi, 2016), the interactive combined effects of OA and OW on asteroids remain largely unknown.

To address these knowledge gaps on FAs biochemical composition and enzyme activities (Ca-ATPase and Mg-ATPase) in asteroids, we performed a controlled laboratory experiment and investigated the long-term interactive effects of OA and OW for 90 days in the small asterinid starfish *Aquilonastra yairi* (Echinodermata: Asteroidea: Asterinidae). *A. yairi* is a nocturnal species that lives under rocks, reef structures, and in rubble areas; distributed from tropical to subtropical regions particularly in the eastern Mediterranean Sea, the Red Sea, and the Gulf of Suez (O'Loughlin and Rowe, 2006; Ebert, 2021). Our study provides insights into the physiological tolerance and resilience of *A. yairi* when exposed to near-future combined OA/OW conditions and supports our understanding of the consequences on their biological performance.

2. Methods

2.1. Experimental design and exposure conditions

Adult specimens of *A. yairi* were taken from stock cultured in the MAREE (Marine Experimental Ecology) facility of ZMT, Bremen, Germany (342 specimens: size 3–11 mm) and were cleaned from debris. They were allowed to acclimate in a communal tank with recirculating artificial seawater (Red Sea Salt, Germany) to a temperature of ≈27 °C for seven days. Then, asteroids were randomly assigned to 18 experimental tanks (19 specimens in each tank) for a further seven-day acclimation period. During the acclimation period, no visible signs of stress were observed (i.e., discoloration and erratic flipping). Following acclimation, asteroids were exposed for 90 days to one of the six combinations of two temperature levels (27 °C and 32 °C) crossed with three concentrations of pCO_2 (455 μatm, 1052 μatm, and 2066 μatm), which represent factorial combinations of ambient environments and the forthcoming levels of changes in the temperature and pCO_2 regime according to the IPCC-Representative Concentration Pathways (RCPs) 8.5 emission scenario for year 2100 (IPCC, 2014). Moreover, the ambient temperature (27 °C) represents the summer mean sea surface temperature (SST) (June to October) in natural habitats of *A. yairi* (e.g., in the Gulf of Suez). Three replicate experimental tanks were set up for each treatment. Each experimental tank was independent of the others, with isolated chillers, heaters, and CO_2 systems. To prevent physiological shock, temperature and pCO_2 concentration levels were ramped up over ten days. During experimental exposure, *A. yairi* was feeding on living diatoms that were allowed to grow on the walls of aquaria and deposited detritus flocs. The experimental conditions and design are described in detail in Khalil et al. (2022).

Seawater samples were periodically taken during experiments to assess the carbonate chemistry of the seawater. Briefly, CO_2-free air and pure CO_2 gas were mixed with solenoid-valve mass flow controllers to generate gas mixtures that were formulated to the target pCO_2 conditions, in compliance with the standard operating procedure (SOP) for ocean CO_2 measurements (Dickson et al., 2007). The resulting gas mixtures were then bubbled into each treatment group's seawater reservoirs using flexible microporous air stones and repeatedly pumped into replicate tanks. A programmed thermostat was used to regulate a closed-circle heating system that maintained treatment tank temperatures. Water parameters (i.e., temperature, salinity, $pH_{T\ (total\ scale)}$, and $pH_{NBS\ scale}$) and water carbonate chemistries (total alkalinity (A_T) and dissolved inorganic carbon (C_T)) were measured periodically, which were subsequently used to calculate the water carbonate system. Details of the seawater chemistry control and manipulation, seawater parameters, and carbonate chemistry for the experimental tanks are provided in Supplementary material (Supplementary text and Table S1) and Khalil et al. (2022).

2.2. Total lipid and fatty acids analysis

Three specimens from each of the six treatment tanks were collected after 30, 60, and 90 days. The specimens were snap-frozen by submerging them in liquid nitrogen, freeze-dried, and stored at −80 °C until further analysis. Total lipid (ΣL_C) was extracted and purified according to the methods described by Bligh and Dyer (1959). Briefly, to separate the lower chloroform phase containing lipids from the rest of the tissue, two purification cycles of a 2:1 chloroform/methanol solution and ultrapure water were performed. The upper phase of the homogenates was discarded after centrifugation (3500 rpm for 5 min). ΣL_C was determined gravimetrically by drying and weighing a subsample and expressed in mg lipid per g asteroids dry weight. The fatty acids of the asteroids were determined as fatty acid methyl esters (FAMEs) at the end of the experiment. The relative composition of 14–24 carbon chain FAs of each individual was determined by transmethylation of dry asteroids lipid samples by acid-catalyzed esterification with 1 % sulfuric acid in methanol, incubated at 50 °C for 16 h, and extracted into FAMEs in accordance with methods from Christie (1998). FAMEs were analyzed and quantified using a flame ionization detector gas chromatograph (GC) (Agilent 7890B MSD 5977 GC-FID, USA), and the output chromatograph peaks were identified using an FA standard mixture (37-component FAME, Supelco, Bellefonte PA) (Galloway et al., 2015). The FA profile of an individual was interpreted using a printed output chromatograph. To calculate the absolute concentration of each FA, the area of each FA was divided by the area of the internal FA standard. This value was multiplied by the internal FA standard concentration added at the beginning and normalized to their dry weight. Final concentrations of FA are expressed as μg mg^{-1} of dry weight (DW).

2.3. Ca-ATPase and Mg-ATPase activity assays

For Ca-ATPase and Mg-ATPase activity assays, nine *A. yairi* specimens were collected from each of the treatment groups after 30, 60, and 90 days of incubation time. As for the lipid analysis, the specimens were snap-frozen, freeze-dried, and stored at −80 °C. The samples were thawed on ice and homogenized in 200 μL Tris buffer (500 mM sucrose, 150 mM KCl, 20 mM Tris, 1 mM dithiothreitol, and 0.1 mM phenylmethylsulphonyl, pH 7.6). Homogenates were then centrifuged at 12,600*g* for 15 min at 4 °C. The supernatants were transferred to a new tube, and an aliquot of 50 μL was preserved for the determination of protein concentration determination using the Bradford assay (Bradford, 1976). Briefly, BioRad's Bradford micro-assays set on a 96-well flat bottom plate was adapted with a standard protein solution prepared using bovine γ-globulin (1 mg mL^{-1}). In each well of the microplate, 10 μL of each sample was added along with 290 μL of Bradford reagent (Sigma-Aldrich, USA). After 15 min of agitation at 150 revs min^{-1}, the absorbance was read at 600 ηm using an Absorbance Microplate Reader (Tecan, Switzerland). Protein concentrations were expressed in mg of protein mL^{-1}.

Ca-ATPase and Mg-ATPase activities were measured according to protocols initially developed by Chan et al. (1986), Busacker and Chavin (1981) and modified by Prazeres et al. (2015). The working buffer for Ca-ATPase contained 80 mM NaCl, 20 mM Tris-Base, 15 mM KCl, and 15 mM $CaCl_2$. Mg-ATPase was measured using a similar working buffer,

where $MgCl_2$ replaced $CaCl_2$ at the same concentration, while the pH was adjusted to 8.1. The sample homogenates (20 μL) were mixed with 250 μL of working buffer containing 1 mM ouabain. The reaction started with the addition of 30 μL ATP stock solution (3 mM). Subsequently, the mixture was incubated at 30 °C for 30 min. The reaction was stopped by adding Malachite Green Reagent in a Phosphate Assay Kit (Sigma-Aldrich, USA). Three technical replicates were measured for each sample. The inorganic phosphate (Pi) released by enzyme activity was determined based on a colorimetric method (Fiske and Subbarow, 1925) using the Phosphate Assay Kit (Sigma-Aldrich, USA) and calculated using a standard curve constructed with 1 mM Pi standards (Sigma-Aldrich, USA). The Pi concentration in the reaction mixture on 96-well microplates (Greiner Bio-One, Germany) was quantified at 620 ηm by using an Absorbance Microplate Reader (Tecan, Switzerland). Homogenization buffer was used as a blank control. Ca-ATPase and Mg-ATPase activities were normalized to the total protein content and are expressed as μmoles Pi mg protein^{-1} min^{-1}.

2.4. Statistical analysis

All data manipulation, visualization, and statistical analysis were performed using the R programming language v. 4.3.2 (R Core Team, 2023). The Shapiro-Wilk statistic *W* test (Shapiro and Wilk, 1965) combined with visual Q-Q plots and histograms was used to test the data for normality, while Levene's test was applied to check the homogeneity of variance (Levene, 1960), before statistical analysis was performed. When data were not normally distributed or indicated heteroskedasticity, we transformed our predictor variables to improve normality assumptions using 'bestNormalize' v.1.9.1 in the R-statistical package (Peterson, 2021). The effects of elevated temperature and pCO_2 and their interaction on ΣL_C and enzyme activities (i.e., Ca-ATPase and Mg-ATPase) were analyzed using a two-way multifactorial analysis of covariance (ANCOVA) using 'car' v.3.1–2 R-statistical package (Fox and Weisberg, 2019), with temperature and pCO_2 as fixed factors, while incubation time was treated as a continuous covariate. Subsequently, Tukey's honest significant difference (HSD) post hoc comparisons were used to detect the origin of variation for significant interactions. The magnitude of the effect sizes of the statistical models expressed as partial eta squared (ηp^2), are also specified.

OA/OW and their interaction effects on the asteroids fatty acids class (i.e., SFAs, MUFAs, and PUFAs) were analyzed using a two-way multivariate analysis of covariance (MANCOVA) in 'MASS' v.7.3-60 R-statistical package (Venables and Ripley, 2002) complemented with 'car' v.3.1-2 R-statistical package (Fox and Weisberg, 2019). Temperature levels and pCO_2 concentrations (solely and combined) were treated as fixed factors, and incubation time was a covariate in this initial MANCOVA test. Following the MANCOVA test, a series of two-way ANCOVAs were performed on each of the response variables of the FA classes and continued with post hoc comparisons of Tukey's HSD to distinguish significant differences among treatments. In addition, principal component analysis (PCA) was performed to explore differences in FA classes between treatment groups and to identify those FAs that explain most of the variability in the data set, carried out using base R functions combined with the 'ggbiplot' v.0.55 R-package (Wickham, 2016) to visualize the PCA result. The results were considered statistically significant (moderate evidence of an effect) at alpha values of $p \leq 0.05$. All functional response figures were plotted using 'ggplot2' v. 3.4.4 (Wickham, 2016).

3. Results

3.1. Total lipid

Total lipid (ΣL_C) exhibited a linear decrease with increasing levels of pCO_2 at ambient temperature (27 °C). In contrast, at high temperature (32 °C), elevated pCO_2 concentrations resulted in a parabolic trend in lipid content (Fig. 1). Under elevated pCO_2, ΣL_C in 27 °C treatments (mean ± SE; 0.739 ± 0.040 mg g^{-1} DW) was 20.08 % higher than in 32 °C treatments (0.591 ± 0.102 mg g^{-1} DW). The highest lipid content occurred at 32 °C and medium pCO_2 concentration (1052 μatm; 1.327 ± 0.562 mg g^{-1} DW), while low pCO_2 concentration (455 μatm) at 32 °C treatment produces the lowest lipid content (0.652 ± 0.096 mg g^{-1} DW). The quantities of lipid content in *A. yairi* were not significantly affected by either elevated temperature (ANCOVA, $p > 0.05$; Supplementary Table S2) or pCO_2 (ANCOVA, $p > 0.05$; Supplementary Table S2) as sole factor neither combined factors (ANCOVA, $p > 0.05$; Supplementary Table S2), but showed differences between incubation time with higher lipid contents on day 90 compared to day 30 (ANCOVA, $F_{2,43} = 4.85$, $p = 0.02$, $\eta p^2 = 0.18$; Supplementary Table S2 and Table S4). ΣL_C exhibited a linear increase with incubation time, except for the 32 °C: 2066 μatm treatment where ΣL_C changed in a negative parabolic pattern (Supplementary Fig. S1).

3.2. Fatty acids composition

Saturated FAs (% ΣSFAs = 43.50 % of total detected FAs (Σ_dFAs)) were the most abundant components in all treatment groups (Table 1, Fig. 2, and Supplementary Fig. S2), with the exception of the 27 °C: 455 μatm treatment, followed by monounsaturated FAs (% ΣMUFAs = 31.81 % of Σ_dFAs), and polyunsaturated FAs (% ΣPUFAs = 24.62 % of Σ_dFAs). Furthermore, *A. yairi* contained more omega-3 (ω3; PUFAs C18:3ω3, C20:5ω3, and C22:6ω3 = 21.24 % of Σ_dFAs) than omega-6 (ω6; PUFAs C18:2ω6, C20:2ω6, and C20:4ω6 = 3.38 % of Σ_dFAs) in all treatment groups (Table 1). Overall, SFAs were the most dominant FA class in *A. yairi*, followed by MUFAs and PUFAs under all treatment conditions.

The FA compositions of *A. yairi* exposed to an elevated temperature of 32 °C (2362.63 ± 37.41 μg mg^{-1} DW) had a higher total FA composition compared to asteroids at an ambient temperature of 27 °C (2198.67 ± 22.90 μg mg^{-1} DW), reflecting the decrease in MUFAs and PUFAs at 27 °C: 1052 μatm, and 2066 μatm treatments, including a strong decrease in MUFAs C16:1ω9 and C18:1ω7, and PUFAs C18:2ω6 and C20:5ω3 (Table 1, Fig. 2, and Supplementary Fig. S2). FAs composition at high-temperature treatments increased in a parabolic pattern in response to elevated pCO_2 (Fig. 2), with the highest intensification observed at 32 °C: 1052 μatm for all FA classes (SFAs, MUFAs, and PUFAs; Table 1). Furthermore, SFAs (mean) exhibited an increase at high-temperature and elevated pCO_2 treatments (32 °C: 1052 μatm and 2066 μatm) compared to ambient temperature and low-pCO_2 treatment (27 °C: 455 μatm), whereas MUFAs (mean) reveal a decreased with elevated pCO_2 in all temperature treatment groups (Fig. 2 and Supplementary Fig. S2). It is noticed that the concentration of PUFA C18:3ω3 (ALA) in high pCO_2 (2066 μatm) at both temperature treatments declined to close to zero (Table 1). EPA:DHA ratio decreased with increasing temperature from ca 8.61:1 to 7.47:1 (Table 1).

A PCA on the entire FAs composition data set provided a two-dimensional pattern (Fig. 3), which explained 65.1 % of the total variance. Principal component PC1 explained 52.7 % of the FAs variability, with the majority of the contribution from FSAs (C20:0, C18:0, C16:0, C14:0, C22:0), MUFAs (C16:1ω9, C18:1ω7, C18:1ω7, C20:1ω9), and PUFAs (C20:5ω3, C20:2ω6). PUFAs C18:2ω6, C18:3ω3, C20:2ω6, and C20:5ω3 contributed the most to the principal component PC2, followed by MUFA C22:1ω9 and SFA C24:0 which explained 12.4 % of the variability in the FAs composition. The 95 % confidence interval ellipses of PCA showed no separation between the treatment groups. Furthermore, the PCA showed that there were no notable variations in FAs composition between the six treatment groups.

There was no significant effect of pCO_2 as the sole factor in FA compositions or combined factor with temperature (MANCOVA, $p > 0.05$; Supplementary Table S3). Nonetheless, temperature was the only sole factor found to affect SFAs significantly (MANCOVA, $F_{1,6} = 365.84$, $p = 0.04$; Supplementary Table S3), but found not significantly to affect other FA classes (MANCOVA, $p > 0.05$; Supplementary Table S3). FA

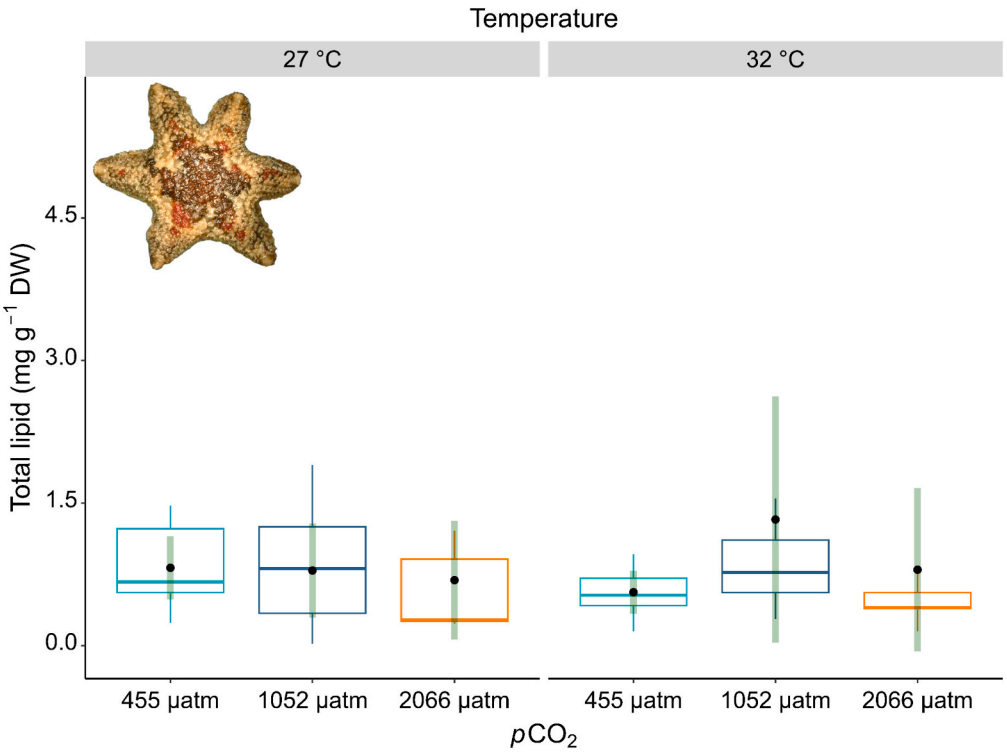

Fig. 1. Total lipid (mg g^{-1} DW) of *A. yairi* (shown in the upper left corner) for 90 days of exposure to different temperature levels (27 °C, 32 °C) and pCO_2 concentrations (455 µatm, 1052 µatm, and 2066 µatm). Boxplots display the mean effect in each treatment (black dots), median (horizontal solid bar inside the box), interquartile (upper and lower horizontal lines of the box), and 1.5× interquartile ranges (whiskers). Vertical dark-green bars denote 95 % confidence intervals of ΣL_C values; $n = 54$.

classes showed no significant alter by incubation time in all treatment groups (MANCOVA, $p > 0.05$; Supplementary Table S3). Subsequent ANCOVA analyses for each of the three FAs classes revealed statistically significant effects of incubation time on SFA C16:0 (ANCOVA, $F_{2,39} = 6.06$, $p = 0.01$, $\eta p^2 = 0.24$; Supplementary Tables S2 and S4) and PUFA C20:5ω3 (ANCOVA, $F_{2,29} = 3.45$, $p = 0.05$, $\eta p^2 = 0.19$; Supplementary Tables S2 and S4), while temperature as the sole factor observed significantly affected SFA C18:0 (ANCOVA, $F_{1,29} = 5.19$, $p = 0.03$, $\eta p^2 = 0.19$; Supplementary Tables S2 and S4) only. However, pCO_2 as the sole or combined factor with temperature did not affect any FAs profile in all treatment groups (ANCOVA, $p > 0.05$; Supplementary Table S2). Furthermore, the ω3:ω6 ratio was found not to change significantly affect by any factor (ANCOVA, $p > 0.05$; Supplementary Table S2).

3.3. Ca-ATPase and Mg-ATPase activities

Ca-ATPase and Mg-ATPase activities exhibited a positive parabolic response pattern to increasing pCO_2 at ambient temperature (27 °C) treatment, whereas enzyme activity shows a stable pattern in response to increasing pCO_2 at high temperature (32 °C) (Fig. 4). The highest Ca-ATPase activity (mean ± SE; 0.73 ± 0.03 µmoles Pi mg protein^{-1} min^{-1}) was observed under ambient temperature and low pCO_2 concentration (455 µatm), and the lowest value (0.59 ± 0.02 µmoles Pi mg protein^{-1} min^{-1}) at ambient temperature and medium pCO_2 concentration (1052 µatm) condition. Ca-ATPase activity was significantly affected by incubation time, where Ca-ATPase activity showed a significant decrease as incubation time progressed with exception of 27 °C: 1055 µatm (ANCOVA, $F_{2,151} = 27.96$, $p = 0.00$, $\eta p^2 = 0.27$; Supplementary Table S2, Table S4, and Fig. S3*a*), pCO_2 concentration (ANCOVA, $F_{2,151} = 4.02$, $p = 0.02$, $\eta p^2 = 0.05$; Fig. 4a, Supplementary Table S2 and Table S4) and by the interaction of the factors temperature and pCO_2 (ANCOVA, $F_{2,151} = 6.01$, $p = 0.00$, $\eta p^2 = 0.07$; Supplementary Table S2 and Table S4), but not by temperature (ANCOVA, $p > 0.05$; Supplementary Table S2).

Similarly, Mg-ATPase activity was also significantly affected by the combined factors of temperature and pCO_2 (ANCOVA, $F_{2,151} = 7.75$, $p = 0.00$, $\eta p^2 = 0.09$; Fig. 4b, Supplementary Table S2 and Table S4), as well as by the sole factor of pCO_2 (ANCOVA, $F_{2,151} = 3.92$, $p = 0.02$, $\eta p^2 = 0.05$; Supplementary Table S2 and Table S4) and incubation time (ANCOVA, $F_{2,151} = 25.62$, $p = 0.00$, $\eta p^2 = 0.25$; Supplementary Table S2 and Table S4). However, temperature as the sole factor was found to not significantly affect Mg-ATPase activity in *A. yairi* (ANCOVA, $p > 0.05$; Supplementary Table S2). Furthermore, the highest Mg-ATPase activity was observed in 27 °C: 456 µatm treatment, whereas the lowest was found in 27 °C: 1052 µatm. Apparently, Mg-ATPase activity decreased over incubation time, except for the 27 °C: 1052 µatm treatment with a negative parabolic pattern (Supplementary Fig. S3*b*).

4. Discussion

4.1. Lipid-associated resilience and homeoviscous adaptations

Assessing lipid and associated FAs allows defining the metabolic status, oxidative stress, potential energy provision, cell remediation, developmental potential, and reproductive capacity of an organism (Stanley-Samuelson, 1987; Murphy, 2001). Our results show that increased pCO_2 concentration and temperature level as a sole stressor or combined stressors do not significantly affect the total lipid content (ΣL_C) of *A. yairi*. They imply that this asterinid species is relatively robust against conditions predicted in future global change scenarios. A possible explanation for the absence of significant changes in asteroid ΣL_C in response to elevated temperature and pCO_2 was their retention of foraging capacity and feeding performance under stressful conditions (Gooding et al., 2009); hence, the lipid generation and conservation mechanism could remain functional. Furthermore, tropical-subtropical marine organisms are less exposed to seasonal diet pulses and have

Table 1
Total lipid and fatty acids composition of *A. yairi* reared under different temperature levels (27 °C, 32 °C) and pCO_2 concentrations (455 μatm, 1052 μatm, and 2066 μatm). Total lipid is expressed as mg g^{-1} (mean ± SE) of asteroids tissue dry weight (DW). FAs are expressed as μg mg^{-1} (mean ± SE) of asteroids tissue DW.

Lipid (mg g^{-1} DW) and fatty acids (μg mg^{-1} DW) profile	Temperature: 27 °C			Temperature: 32 °C		
	pCO_2: 455 μatm	pCO_2: 1052 μatm	pCO_2: 2066 μatm	pCO_2: 455 μatm	pCO_2: 1052 μatm	pCO_2: 2066 μatm
Lipid content						
ΣL_C (total lipid)	0.819 ± 0.144	0.790 ± 0.215	0.689 ± 0.255	0.561 ± 0.096	0.780 ± 0.146	0.431 ± 0.067
Saturated fatty acids (SFAs)						
C14:0 (myristic acid)	33.951 ± 8.349	21.664 ± 4.777	29.336 ± 7.308	22.794 ± 5.978	37.598 ± 7.772	34.765 ± 13.073
C16:0 (palmitic acid)	158.177 ± 42.055	116.436 ± 26.763	164.601 ± 58.930	126.428 ± 26.863	220.231 ± 38.858	140.754 ± 27.394
C18:0 (stearic acid)	96.067 ± 39.108	130.907 ± 24.849	89.930 ± 8.192	126.056 ± 21.900	167.943 ± 35.030	151.656 ± 28.139
C20:0 (arachidic acid)	8.166 ± 1.756	7.110 ± 1.735	9.409 ± 3.769	6.460 ± 1.201	12.793 ± 2.381	8.335 ± 1.877
C22:0 (behenic acid)	11.936 ± 2.672	9.080 ± 2.461	9.426 ± 3.768	9.077 ± 1.772	11.741 ± 3.650	11.318 ± 3.124
C24:0 (lignoceric acid)	20.086 ± 1.894	24.359 ± 3.796	19.606 ± 2.111	23.600 ± 5.575	22.408 ± 5.374	26.593 ± 8.162
Monounsaturated fatty acids (MUFAs)						
C14:1ω5 (myristoleic acid)	1.273 ± 0.350	0.410 ± 0.226	1.057 ± 0.338	1.275 ± 0.657	0.489 ± 0.235	1.372 ± 0.539
C16:1ω9 (palmitoleic acid)	147.920 ± 52.933	79.149 ± 24.489	63.579 ± 10.241	61.512 ± 21.641	120.378 ± 34.358	46.091 ± 12.424
C18:1ω9 (oleic acid)	22.467 ± 8.341	26.892 ± 12.687	43.533 ± 19.549	21.538 ± 4.703	32.295 ± 5.261	22.141 ± 4.685
C18:1ω7 (vaccenic acid)	163.476 ± 41.671	90.653 ± 17.174	110.357 ± 29.423	78.131 ± 17.304	134.293 ± 47.998	96.548 ± 28.360
C20:1ω9 (gondoic acid)	11.605 ± 2.614	13.559 ± 5.891	12.744 ± 7.125	11.294 ± 2.604	21.905 ± 8.873	16.205 ± 3.774
C22:1ω9 (erucic acid)	0.054 ± 0.054	0.195 ± 0.195	0.211 ± 0.141	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Polyunsaturated fatty acids (PUFAs)						
C18:2ω6 (linoleic acid, LA)	4.920 ± 4.657	2.863 ± 2.250	0.000 ± 0.000	15.610 ± 5.486	3.375 ± 3.375	8.124 ± 3.731
C18:3ω3 (α-linolenic acid, ALA)	0.929 ± 0.929	1.450 ± 0.950	0.000 ± 0.000	3.185 ± 1.763	0.000 ± 0.000	0.000 ± 0.000
C20:2ω6 (eicosadienoic acid)	5.529 ± 0.938	4.017 ± 0.667	9.202 ± 4.425	10.381 ± 3.112	7.191 ± 1.968	8.360 ± 1.498
C20:4ω6 (arachidonic acid, AA)	10.370 ± 3.247	9.563 ± 3.897	9.090 ± 5.129	15.184 ± 4.967	13.884 ± 4.845	16.522 ± 7.814
C20:5ω3 (eicosapentaenoic acid, EPA)	153.044 ± 54.857	143.242 ± 29.017	112.185 ± 23.199	69.901 ± 16.069	294.261 ± 24.507	83.323 ± 32.343
C22:6ω3 (docosahexaenoic acid, DHA)	8.821 ± 2.640	20.431 ± 7.753	18.139 ± 9.174	15.914 ± 7.676	27.689 ± 6.617	16.309 ± 6.696
Fatty acids (FAs) indices						
ΣSFAs	308.297 ± 28.811	285.196 ± 27.412	302.703 ± 29.911	290.815 ± 27.931	450.306 ± 43.512	346.827 ± 31.749
ΣMUFAs	346.741 ± 31.199	210.664 ± 16.309	231.270 ± 17.653	173.750 ± 13.478	309.360 ± 24.561	182.356 ± 14.898
ΣPUFAs	183.613 ± 24.525	181.567 ± 22.774	148.617 ± 17.702	130.174 ± 9.844	346.400 ± 47.474	132.638 ± 11.024
Σω3	226.409 ± 17.135	209.600 ± 10.568	157.673 ± 10.193	108.284 ± 8.284	321.950 ± 7.781	99.632 ± 9.760
Σω6	20.819 ± 2.947	16.443 ± 2.271	23.533 ± 4.932	41.175 ± 4.522	24.449 ± 3.396	33.006 ± 4.348
Σω3:Σω6	7.82:1	10.04:1	7.13:1	2.16:1	13.17:1	3.02:1
EPA:DHA	17.35:1	7.011:1	6.19:1	4.39:1	10.63:1	5.11:1

ΣSFAs: sum of SFAs, ΣMUFAs: sum of MUFAs, ΣPUFAs: sum of PUFAs, ω3: omega-3, ω6: omega-6, Σω3:Σω6: ratio of omega-3 (ω3) fatty acids to omega-6 (ω6) fatty acids, EPA:DHA: ratio of eicosapentaenoic acid (EPA) to docosahexaenoic acid (DHA).

faster metabolic rates (Brett et al., 2009), which allows them to control relative lipid levels and subsequently use it rapidly to cope with environmental stressors. Lipids and bioaccumulation of minerals obtained by consuming microalgae (e.g., diatoms and microbial mats) are required continuously throughout the diet to support physiological development and maintain health and fitness (Kainz and Fisk, 2009). Additionally, a higher ΣL_C (in mean) was observed at higher temperatures and medium pCO_2 concentration treatment (32 °C: 1052 μatm), implying that the starfish could have enhanced its biosynthetic capacity to convert non-lipid molecules into lipids in such environmental settings. Future studies should thus verify this in manipulative trials with fully controlled diets.

The lipid sensitivities to OA and OW as sole or combined stressors in marine invertebrates show different scales, varying from functional adaptability (positive effects) to specific physiological losses (negative effects). Our findings concur with earlier research demonstrating that marine invertebrate species exposed to elevated pCO_2 and temperature levels (as sole or combined factors) did not exhibit alterations in their lipids content, e.g., sea urchin *Strongylocentrotus purpuratus* (Matson et al., 2012), sponges *Carteriospongia foliascens*, *Cymbastela coralliophila*, *Rhopaloeides odorabile* and *Stylissa flabelliformis* (Bennett et al., 2018), and the corals *Porites* spp. and *Acropora millepora* (Strahl et al., 2016). In contrast, other studies have found alterations in the organismal lipid content in response to OA and OW, e.g., Caribbean coral *A. cervicornis* (ΣL_C ↑; OA*) (Towle et al., 2015), scallop *Crassadoma gigantea* (ΣL_C ↑; OA*) (Alma et al., 2020), blue mussel *Mytilus edulis* (ΣL_C ↑; OW*) (Matoo et al., 2021), Arctic pteropod *Limacina helicina* (ΣL_C ↓; OA:OW*) (Lischka et al., 2022), oyster *Crassostrea virginica* (ΣL_C ↓; OA*) (Schwaner et al., 2023). These differences in responses indicate that the sensitivity is species-specific and probably highly dependent on geographic distribution, habitat, physiological acclimation capacity, stressor intensity, and life history of species.

In addition to their significance in energy conservation, fatty acids are involved in numerous essential cellular functions, most notably through their function as building blocks of cell membranes. Essential FAs are also recognized as key determinants of ecosystem health and

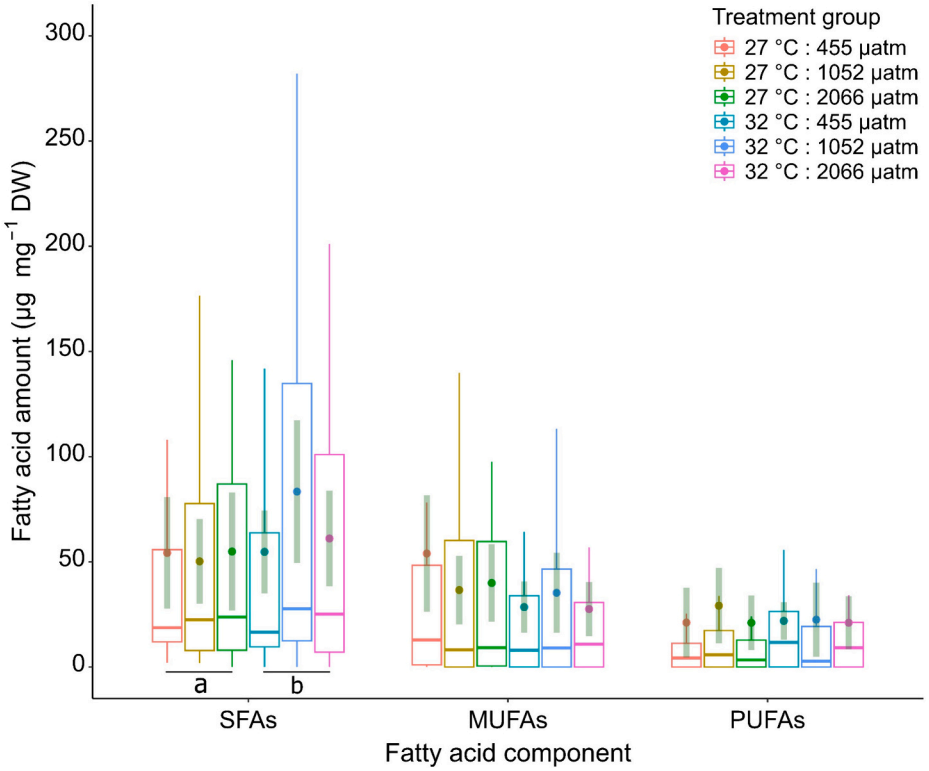

Fig. 2. Fatty acids composition of asteroid *A. yairi* exposed to different temperature levels (27 °C, 32 °C) and pCO_2 concentrations (455 µatm, 1052 µatm, and 2066 µatm) for 90 days of incubation time. Boxplots display the mean effect in each treatment (color dots), median (horizontal solid bar inside the box), interquartile (upper and lower horizontal lines of the box), and 1.5× interquartile ranges (whiskers). Vertical dark-green bars denote 95 % confidence intervals of fatty acids. Letters designate significant differences between the temperature treatment ($p < 0.05$); $n = 54$.

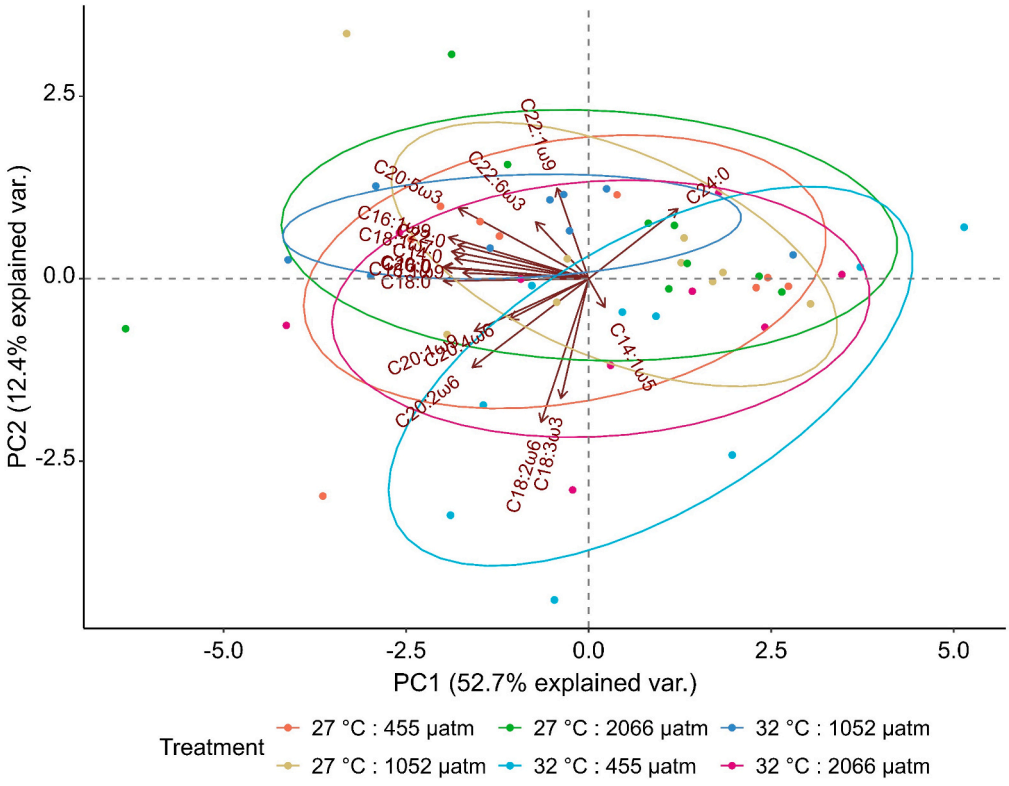

Fig. 3. Biplot of principal component analysis (PCA) based on fatty acid profiles ($n = 18$ classes) for asteroid *A. yairi* exposed to different temperature levels (27 °C, 32 °C) and pCO_2 concentrations (455 µatm, 1052 µatm, and 2066 µatm) for 90 days of incubation time; $n = 54$. Individual samples are color-coded according to the treatment level. The plot is shown with 95 % confidence ellipses.

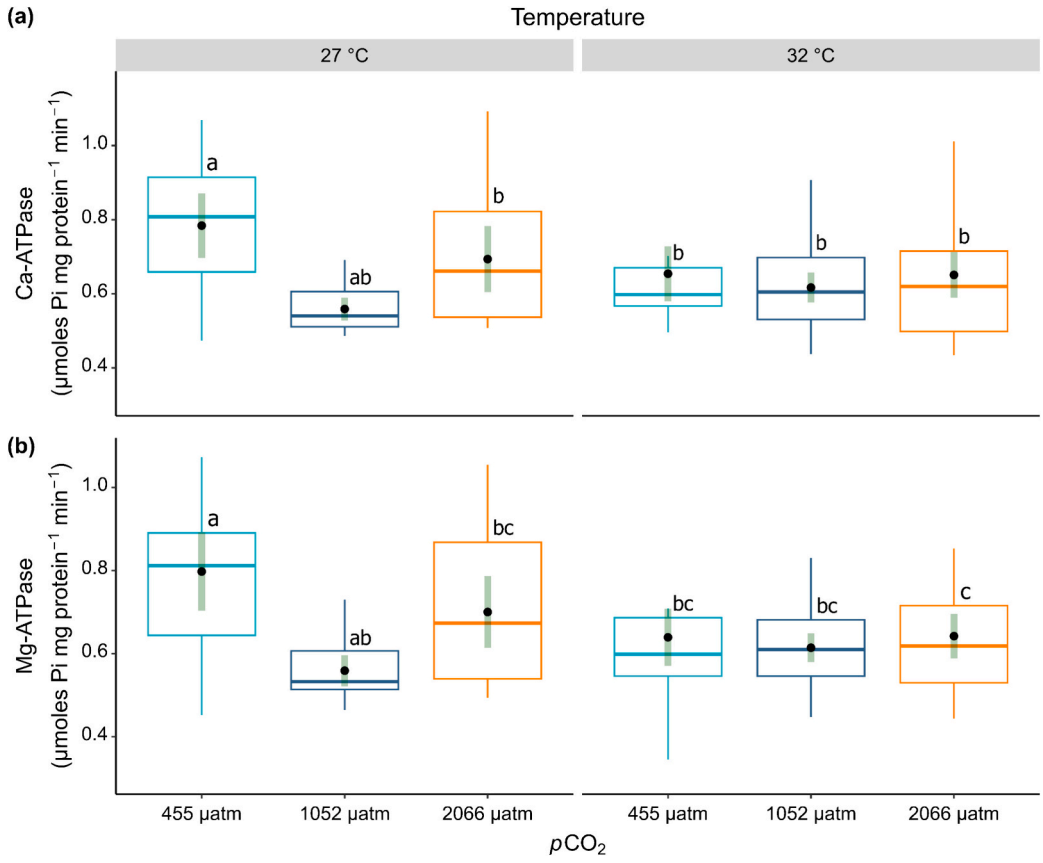

Fig. 4. Enzyme activities of asteroid *A. yairi* exposed to different temperature levels (27 °C, 32 °C) and pCO_2 concentrations (455 µatm, 1052 µatm, and 2066 µatm) for 90 days of incubation time; (a) Ca-ATPase (µmoles Pi mg protein^{-1} min^{-1}), and (b) Mg-ATPase (µmoles Pi mg protein^{-1} min^{-1}). Boxplots display the mean effect in each treatment (black dots), median (horizontal solid bar inside the box), interquartile (upper and lower horizontal lines of the box), and 1.5× interquartile ranges (whiskers). Vertical dark-green bars denote 95 % confidence intervals of enzyme activity values. Letters designate significant differences between the treatment groups (Tukey's HSD, $p < 0.05$); $n = 53$.

stability (Parrish, 2013). Overall, elevated water temperature exerted a larger effect on the FA compositions relative to acidification. Our results showed that under future ocean scenarios, the temperature (as the sole stressor) affects the composition of asteroid FAs, where the FAs composition becomes more saturated during elevated temperature acclimation (i.e., SFAs increase in the negative parabolic pattern). This result is consistent with previous studies in other marine invertebrate species, such as sea cucumber *Apostichopus japonicus* (Yu et al., 2016), copepod *Paracalanus* sp. (Garzke et al., 2016), gastropod *Dicathais orbita* (Valles-Regino et al., 2015), and shrimp *Palaemon elegans* (Maia et al., 2022).

Temperature appears to contribute to increasing the amount of all SFA classes, with SFAs C18:0 alone found to have a statistically significant increase at high temperatures (ANCOVA, $p < 0.05$). However, it seems that the effects of OA might quantitatively modify the impacts of temperature on asteroid SFAs composition. We suggest that the increased concentration of SFA under high-temperature environmental conditions is attributed to the enhanced allocation of SFA to cell membranes to preserve membrane stability and viscosity and is functional in counteracting the reduced internal cell pH due to acidification (Maia et al., 2022); where high temperature often leads to protein degeneration in the lipid bilayer of biomembranes, which generates a preponderance of membrane fluidity and increases membrane permeability (Hazel, 1995). Furthermore, SFA C16:0 is the final major by-product of FAs biosynthesis within the cell cytosol, serving as precursors for long-chain de novo biosynthesis of both saturated and unsaturated FAs (Gurr et al., 2002). C16:0 is used as a lipid mediator for cell signaling, neuroprotective, anti-neuroinflammatory, and analgesic activities (Carta et al., 2017). Increases in SFA C16:0 and C18:0 that were found in this study are suggested to represent a response mode to suppress physiological disorders resulting from elevated temperature beyond thermal optima; C16:0 and C18:0 are synthesized on-demand and exert their functions in loco during inflammations and neurodegenerative disorders to counteract inflammation and neuronal lesion. However, drastic increases in C16:0 and C18:0 could have adverse physiological consequences - apoptosis, oxidative stress, and endothelial dysfunction (Wang et al., 2007; Carta et al., 2017). Furthermore, SFAs are categorized as rapid energy resources, and their extraction could accelerate in response to OA/OW conditions to offset the higher energetic expenses associated with survival in a chronic environment (Garzke et al., 2016).

Although neither temperature nor pCO_2 as a sole or combined factor lacked a statistically significant effect on MUFAs (MANCOVA, $p > 0.05$), there was a decreasing trend in the MUFAs abundance as temperature-pCO_2 increased markedly in MUFAs C16:1ω9 and C18:1ω7. This might indicate thermal-acidity stress responses by the asteroid. MUFAs represent a highly catabolizable energetic resource (Tocher, 2010); thus, asteroids would probably metabolize them to yield energy to offset the energetic deficits that ensue due to increased energy expenditure for cell-physiological processes in parallel with thermoregulation during stressful conditions. Other physiological factors, e.g., development and reproduction under extreme environmental conditions, require immense energy expenditure (Doney et al., 2012), which might also contribute to depletion in MUFA stores. Decreased MUFA levels under warming and acidification conditions were recorded in the sponges *C. foliascens*, *C. coralliophila*, and *S. flabelliformis* (Bennett et al., 2018). Interestingly, however, MUFA palmitoleic acid (C16:1ω9) at 32 °C: 1052

μatm was increased compared to 27 °C: 455 μatm. In this context, C16:1ω9 enhancement is suggested as a potential acclimation measure to diminish the inflammatory effects caused by elevated temperature, because animal and cell culture studies indicate that C16:1ω9 acts as an anti-inflammatory and reduces the adverse effects of higher SFA (de Souza et al., 2018).

Asteroid PUFAs did not significantly alter in response to changes in temperature and/or pCO_2 (i.e., MANCOVA and ANCOVA statistical results). However, individuals reared at 27 °C contained less ΣPUFAs than those reared at 32 °C. All PUFA classes were found to enhance as temperature increased, with a particular major increase observed in C18:2ω6 (linoleic acid) and C20:4ω6 (arachidonic acid, AA) by ≈273 % and ≈68 %, respectively. pCO_2 concentrations most likely contributed to modifying the quantitative level of PUFA shifts in response to elevated temperature. These increased levels of PUFAs suggest that tropical-subtropical asteroids are predicted to become more resilient and adaptable to current and future global ocean changes, where enhancement of PUFAs could be utilized to cope with the adverse physiological effects of stressors (Yoon et al., 2022). Relative enhancement of PUFAs ω3 and ω6 with increasing temperature arguably reflects physiological differences that may promote the resistance of this species to environmental stress (i.e., resilience to physiological stress) (Tocher, 2010; Filimonova et al., 2016). Increased PUFAs could be related to acclimation mechanisms to maintain membrane fluidity and permeability and subsequent cell homeostasis, regulate ion flux and thermoregulation in the encounter of environmental changes; besides, PUFAs play important roles in the reproductive process, neurological development, immune system, and serve as essential hormone precursors (i.e., for eicosanoids) (Stanley-Samuelson, 1987; Calder and Grimble, 2002; Zhukova, 2022). Asteroids acquire PUFAs from their diets; therefore, an increase in PUFAs may be related to feeding performances that are not affected by stressors. Moreover, elevated temperatures were found to increase feeding rates in some asteroids species (Gooding et al., 2009), leading to higher nutrition intake. The sources of PUFAs in asteroids diets are unclear, but it has been associated with microorganisms living in benthic habitats, e.g., macroalgae, specific diatom species, protozoa, and microeukaryotes (Howell et al., 2003). Furthermore, some marine invertebrates have the ability to directly synthesize PUFAs (e.g., C20:4ω6, C20:5ω3, and C22:6ω3) through the FA desaturation process (Nakamura and Nara, 2004; Yoon et al., 2022). However, further research is required to identify the potential desaturation process in asteroids for generating PUFAs. On the contrary, previous studies have shown that, mostly, marine invertebrates exposed to elevated temperatures as the sole factor or combined with pCO_2 produce a reduction in ΣPUFAs, e.g., sea cucumber *A. japonicus* (Yu et al., 2016), gastropod *D. orbita* (Valles-Regino et al., 2015), scallop *C. gigantea* (Alma et al., 2020), shrimp *P. elegans* (Maia et al., 2022), and hard coral *A. digitifera* (Safuan et al., 2021).

We noted a reduction in PUFA ω-3 along with an enhancement of PUFA ω-6 as temperature increases. This alteration is related to the lipid peroxidation activation mechanism in protecting membranes against oxidation damage induced by stressors. Temperature can modulate cell membrane structure by stimulating the lipid peroxidation pathway that initiates by reactive oxygen species (ROS) (Butow et al., 1998), in which membrane phospholipids (i.e., primarily PUFAs) are exposed to oxidative reactions by ROS. Higher lipid saturation and high O_2 concentration lead to an increase in the velocity of the lipid radical chain reactions (Anacleto et al., 2014). PUFAs ω-3 and ω-6 are further employed to inhibit ROS through increasing macrophage phagocytosis capacity (Ambrozova et al., 2010). Furthermore, the PUFAs ω-3:ω-6 ratio is well-known to significantly contribute to membrane functions and cellular processes, e.g., cell survivability (Schmitz and Ecker, 2008). Although PUFAs ω3:ω6 ratio was marginally significant (ANCOVA, $F_{1,41} = 3.10$, $p = 0.09$, $\eta p^2 = 0.07$; Supplementary Table S2) affected by temperature as sole factor, PUFAs ω-3:ω-6 ratio was recoded to decrease from 8.25:1 at ambient temperature (27 °C) to 5.18:1 after being exposed to high-temperature (32 °C) treatment for 90 days. The ratio shift suggests a switch of PUFAs regulation from the anti-inflammatory mode (more PUFAs ω-3) to the pro-inflammatory mode (more PUFAs ω-6) (Schmitz and Ecker, 2008). Inflammation plays an important role in systemic mechanisms of cellular defense and helps protect against harmful stimuli, e.g., immune responses, cell-tissue repair, and warning signals of physiological anomalies; however, prolonged or excessive inflammation can lead to tissue damage, organ dysfunction, and increased risk of various health problems (Calder and Grimble, 2002; Calder, 2010).

The present study reveals a potential acclimation mechanism of cell membrane remodeling homeoviscous adaptation in response to elevated seawater temperature (Hazel and Williams, 1990; Hazel, 1995). An increase in asteroid SFAs and PUFAs followed by a reduction in MUFAs and PUFAs ω-3:ω-6 ratio implied compensatory alterations in fluidity and permeability of cell membrane lipid bilayers to preserve membrane biophysical functions and properties under chronic environmental stress. Still, it remains obscure whether this biochemical acclimation strategy is viable under more prolonged durations and higher intensities of stressors. Moreover, further research is essential to assess the impacts of future ocean scenarios on asteroids larvae (produced by sexual reproductive mode) or 'juvenile' individuals (produced by fissiparous in asexual reproductive mode) since a higher vulnerability at the early developmental stage may pose barriers to species' survival in the continuously changing oceans.

4.2. Enzyme activities under multiple stressors and their potential role in calcification

Ca-ATPase and Mg-ATPase activities show susceptibility to changes in pCO_2 and temperature. Statistical analysis indicated that the interaction of OA and OW significantly affected Ca-ATPase and Mg-ATPase activities; enzyme activities decreased with increasing temperature and pCO_2 concentration, suggesting impaired adenosine triphosphate (ATP) production and enzyme function, resulting in reduced energy supplies needed to sustain the metabolic demands of routine maintenance. In addition to serving as energy storage for various physiological cellular processes (Sokolova et al., 2012; Petersen and Verkhratsky, 2016), ATP is an organic molecule that plays a vital role as a biochemical catalyst in physiological cellular processes, ion transporters or exchangers (e.g., Ca^{2+} and Mg^{2+}) in the calcification process, and in the removal of excess protons (H^+) from the biomineralization site for the skeleton (Ivanina et al., 2020). In ectothermic animals (e.g., echinoids), those bio-cellular processes involve high-energy utilization and intensive catalysts that are provided by ATP (Pan et al., 2015; Stumpp and Hu, 2017). Studies of Ca-ATPase and Mg-ATPase activities under the influence of OA and OW as combined or single factors have yielded different and inconsistent responses. For instance, Ca-ATPase and Mg-ATPase activities of the benthic foraminifera *Amphistegina lessonii* were enhanced after exposure to OA and OW for 30 days; however, *Marginopora vertebralis* had the opposite response (Prazeres et al., 2015). A finding similar to that of the present study was reported in the razor clam *Sinonovacula constricta*, where elevated CO_2 (decreased pH) suppressed Ca—Mg ATPase activity after one week of exposure (Peng et al., 2017). Duration of exposure, stressor intensity, species-specific differences, experimental setups, and methodology appear to contribute to differences in the response of these important enzymes to OA/OW.

Our results showed different Ca-ATPase and Mg-ATPase activities between ambient (27 °C) and high temperature (32 °C) treatments in response to increasing pCO_2 concentrations. Enzyme activity at ambient temperature displayed a positive parabolic curve in response to increased pCO_2 concentrations, indicating a plasticity-physiological acclimation response to balance intracellular acid-base conditions due to changes in pH. Furthermore, lower enzyme activity at medium concentrations of pCO_2 specified stress conditions, where asteroid lost their capacity to accelerate physiological enzyme activities due to disturbances of their energy balance in low-pH environments (Bisswanger,

2017; Khalil et al., 2023). The energy balance could be adversely influenced by OA both directly through the negative effects of lower extracellular and intracellular pH on energy metabolism and indirectly through increased energy expenditure for biomineralization and acid-base regulation processes (Sokolova et al., 2012). On the contrary, at high concentrations of pCO_2 (27 °C: 2066 μatm), asteroid increased enzyme activities to balance extra- and intracellular acid-base conditions to maintain optimal physio-chemical microenvironment conditions, thus ensuring fundamental asteroid component survival (very low mortality, see Khalil et al., 2023) in these chronic stress environments. Enhanced enzyme activity may allow asteroids to actively pump H^+ to maintain pH homeostasis in the extracellular calcifying fluid (ECF, pH_{CF}). Consequently, up-regulation of the H^+ pump under high OA renders more metabolic CO_2 produced by cell organelle-mitochondria, which subsequently aggravates calicoblastic cell acidosis, making H^+ dissipation more arduous (Laurent et al., 2014; Melzner et al., 2020) and skeletons become more susceptible to dissolution (see Khalil et al. (2022) for the descriptions of skeletal dissolution of asteroids *A. yairi* under OA/OW). We thus suggest that this rapid acceleration would involve a high-energy demand that might result in a trade-off and temporal energy reallocation with other physiological functions (Sokolova et al., 2012; Khalil et al., 2023). Furthermore, increased enzyme activity may indicate increased Ca^{2+} and Mg^{2+} ion provision into the ECF to promote asteroids calcification under OA conditions (Khalil et al., 2022). However, increasing ion supply seems unable to counteract the adverse effects of OA, as evidenced by significantly decreased calcification rates (Khalil et al., 2022).

The opposite condition was observed in the high-temperature regime (32 °C), where Ca-ATPase and Mg-ATPase activities exhibited low-stable responses, although they were significantly reduced compared to the ambient temperature and low pCO_2 concentrations (27 °C: 455 μatm) treatment. Elevated temperature seems to allow *A. yairi* to cope with the negative effect of increased pCO_2 (decreased seawater pH) on enzyme activities (antagonistic interactive effects). High temperatures are likely to stimulate and stabilize enzyme activity by intensifying ATP synthesis and utilization during intracellular acid-base homeostasis. We suggest that elevated temperatures could accelerate enzyme activity by utilizing a high energy supply through optimum-efficiency metabolic pathways to counteract another stressor (i.e., elevated pCO_2) (Somero, 1978), thus alleviating physiological stress and allowing asteroids to maintain other vital physiological-related functions (e.g., biomineralization).

Furthermore, we previously showed that the high-temperature treatment combined with elevated pCO_2 resulted in a low-stable calcification rate in the asteroid *A. yairi* (see Khalil et al. (2023)), which was also reflected in enzyme activity. These identical characteristics represent a strong correlation between enzyme activity and the calcification process, indicating the role of Ca-ATPase and Mg-ATPase as crucial enzymes involved in the biomineralization process in asteroids, in addition to V-type H^+-ATPase (Tresguerres, 2016), carbonic anhydrase (CA), and Na^+/K^+-ATPase (Pan et al., 2015; Ivanina et al., 2020) in echinoderm species. Biomineralization processes (e.g., calcification) are known to be influenced not only by environmental conditions such as pH and temperature or salinity, but also closely related to organic molecules and biochemical cellular compounds (e.g., protein and enzyme) (Ivanina et al., 2020). The process of Ca^{2+} and Mg^{2+} balance in echinoderm species through intracellular modulation via trans-membrane transporters was described in prior studies (Stumpp and Hu, 2017; Kolbuk et al., 2020); however, the enzymes involved in the process have not been elucidated. The present study has reflected on the roles of enzymes, specifically Ca-ATPase and Mg-ATPase, in the calcification process of echinoids. However, the physio-chemical mechanisms underlying this process remain unknown; hence, further studies are needed in this direction.

5. Conclusions

The impacts of concurrent ocean warming and acidification on lipid content, fatty acids composition, and calcification-related enzyme activities (Ca-ATPase and Mg-ATPase) of the asteroid *A. yairi* were investigated for the first time. Examining total lipid and associated FAs provides insight into how these tropical-subtropical asteroids utilize their energy resources under current and near-future global environmental changes. Under OA/OW conditions, *A. yairi* exhibits acclimation ability to maintain energy homeostasis presumably through efficient lipid-associate regulation and concurrently remodel membrane biophysics (e.g., membrane structure, fluidity, permeability, and functional properties) by adjusting their lipid-FAs biosynthesis, thereby, giving them potential compensatory mechanisms to adapt with changes in ocean temperature and pH (i.e., homeoviscous adaptation). Furthermore, elevated temperatures seem to confer a physiological benefit in maintaining enzyme activities that minimize the effects of reduced pH, indicating that Ca-ATPase and Mg-ATPase activities in asteroid species are temperature- and pH-dependent physiological functions. Collectively, the findings of this study provide insight into potential biochemical adaptation mechanisms utilized by the asteroid species *A. yairi* to cope with ongoing and impending global changes in ocean conditions, specifically OW and OA. However, it is important to note that different asteroid species may respond differently to OW/OA, depending on the magnitude and rate of environmental change, as well as interactions with other species and ecological processes.

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CRediT authorship contribution statement

Munawar Khalil: Conceptualization, Methodology, Investigation, Resources, Sample processing and analysis, Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing, Funding acquisition. **Marleen Stuhr:** Methodology, Writing - review & editing. **Andreas Kunzmann:** Writing - review & editing. **Hildegard Westphal:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition, Supervision.

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

The data that support the findings of this study are available at PANGAEA open access repository; data of total lipid: https://doi.pangaea.de/10.1594/PANGAEA.965904 (Khalil et al., 2024c), fatty acid compositions: https://doi.pangaea.de/10.1594/PANGAEA.965902 (Khalil et al., 2024b), and enzyme activities: https://doi.pangaea.de/10.1594/PANGAEA.965905 (Khalil et al., 2024a).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.173000.

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