Title: Energetic response of Atlantic surfclam *Spisula solidissima* to ocean acidification

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Abstract

In this study, we assessed the Atlantic Surfclam (*Spisula solidissima*) energy budget under different ocean acidification conditions (OA). During 12 weeks, 126 individuals were maintained at three different ρCO_2 concentrations. Every two weeks, individuals were sampled for physiological measurements and scope for growth (SFG). In the high ρCO_2 treatment, clearance rate decreased and excretion rate increased relative to the low ρCO_2 treatment, resulting in reduced SFG. Moreover, oxygen:nitrogen (O:N) excretion ratio dropped, suggesting that a switch in metabolic strategy occurred. The medium ρCO_2 treatment had no significant effects upon SFG; however, metabolic loss increased, suggesting a rise in energy expenditure. In addition, a significant increase in food selection efficiency was observed in the medium treatment, which could be a compensatory reaction to the metabolic over-costs. Results showed that surfclams are particularly sensitive to OA; however, the different compensatory mechanisms observed indicate that they are capable of some temporary resilience.

Introduction

Increasing CO_2 in the atmosphere is being absorbed by the oceans, resulting in what has been termed ocean acidification (OA) (Caldeira & Wickett, 2003; Doney et al., 2009; Raven et al., 2005). OA is a shift in the carbonate system that results in increased ρCO_2 and HCO_3^- along with decreases in CO_3^{2-} and the saturation states (Ω) of calcite and aragonite (Duarte et al., 2013; Feely et al., 2004; Hönisch et al., 2012). The buffering ability of the ocean that historically occurs on longer geological time scales (10,000-100,000 years) has been decoupled from rate of CO_2 change, resulting in decreasing pH and Ω (Hönisch et al., 2012; Zeebe, 2012; Zeebe et al.,

2016). In North America's Mid-Atlantic Bight and Gulf of Maine region, seawater has historically high dissolved inorganic carbon (DIC), low *in situ* pH, Ω aragonite, and buffering capacity as a result of numerous inputs of fresh and low-alkalinity waters, along with the subsurface accumulation of respiratory products from high primary productivity (Biao et al., 2004; Goldsmith et al. 2019; Wang et al., 2017; Wang et al., 2013). This habitat is experiencing rapid change, with time-series data from 1981 to 2014 showing a drop in pH of 0.018 and Ω of 0.065 (Salisbury & Jönsson, 2018). On shorter time scales, the east coast of the United States also is characterized by strong seasonal variability in surface water pH (high in winter, low in summer) and Ω (low in winter, high in summer; Cai et al., 2020; Xu et al., 2020). It is predicted that some areas along the Northeast US coast will experience significant acidification as soon as 2030 (upper Maine), which is earlier than the global projection of 2100 (Ekstrom et al., 2015).

Among marine organisms, bivalves are thought to be highly susceptible to OA as their shell is formed with different calcium carbonate polymorphs (i.e., calcite or aragonite). Several bivalves (i.e., Atlantic sea scallops, surfclams, oysters, hard clams NOAA Landings 2018) support valuable fisheries, with shellfisheries in the Mid-Atlantic and New England region in 2018 accounting for 86% of the value of all marine bivalves harvested ($852.8 million US dollars) in the United States. Published studies of OA effects upon bivalves have been dominated by studies of estuaries species (*Crassostrea virginica,* Gobler & Talmage, 2014; Keppel et al., 2016; Young & Gobler, 2018, *Mytilus edulis,* Dickey et al., 2018; Griffith & Gobler, 2017; Gu et al., 2019; Stapp et al., 2017; Young & Gobler, 2018*, Argopecten irradians,* Griffith & Gobler, 2017; Talmage & Gobler, 2009, *Mya arenaria,* Clements & Hunt, 2018; Clements et al., 2016; Network et al., 2019, *Mercenaria mercenaria,* Griffith & Gobler, 2017; Miller & Waldbusser,

2016; Young & Gobler, 2018). Inhabitants of an estuarine environment are exposed to hourly, daily, weekly, and seasonal variability in carbonate chemistry (Feely et al., 2010; Waldbusser & Salisbury, 2014). Many studies have provided useful information about how estuarine species respond to OA, but less research on coastal/oceanic bivalve species has been done. To date, there are no published studies on the effects of OA upon Atlantic surfclams, *Spisula solidissima*, a federally-managed species in the US; although a vulnerability assessment for the Northeast United States categorized the Atlantic surfclam as highly susceptible to OA (Hare et al., 2016).

Atlantic surfclams are located along the continental shelf of the North American east coast from the Gulf of St. Lawrence to Cape Hatteras, NC (Marzec et al., 2010), primarily in water 10-50 m in depth, but moving to deeper waters in recent years (Center, 2017; Ropes, 1980). Individuals can be long-lived (>20 years, Jones et al., 1978). In 2018, the Atlantic surfclam commercial fishery was valued at $29.5 million, with surfclam harvests being considered well managed (NOAA Fisheries, 2019).

To date, little is known about the energetic responses of surfclams to OA. Surfclam habitat currently is experiencing the changes in carbonate chemistry described above (Cai et al., 2020; Xu et al., 2017, 2020). Off the New Jersey coast, pH and Ω_{arag} ranges observed in subsurface waters were 7.906–8.205 and 1.48–2.22, respectively (Saba et al. 2019). Models project mean surface seawaters in the Gulf of Maine to reach a threshold affecting bivalve larvae (Ω_{arag}=1.5) in 2050 (Ekstrom et al., 2015). For the MAB, this threshold may not be reached before 2100; however, bottom waters are known to be less basic than surface waters. Accordingly, 10-50-m deep waters Ω_{arag} range from 1-2.5 and 1-2 in the MAB and Southern New England/Gulf of

Maine areas, respectively (Wanninkhof et al., 2015). For sediments in which surfclams live burrowed, pH and Ω_{arag} of the pore water can be particularly low (pH from 6.18 to 8.34, Ω_{arag} from 0.3 to 3.52 in Long Island Sound, Meseck et al., 2018; Ω_{arag} from 0.1 to 1 in Gulf of Maine, Green et al., 2009), despite supersaturated overlying waters (Ω_{arag}=3.5-4, Green et al., 2009). These levels of alkalinity/saturation have been shown to cause death by dissolution in juvenile northern quahogs *M. mercenaria* (Green et al., 2009).

The measurement of physiological rates, (e.g., feeding, assimilation, respiration, and excretion) under experimentally-varied conditions provides insight into energetic responses of a species to environmental changes such as OA (Bayne & Newell, 1983; Widdows & Johnson, 1988). Physiological responses and energy expenditure, rather than a 'direct' measurement of growth, has been used in bivalves for decades (Bayne & Newell, 1983; Vargas et al., 2015; Vargas et al., 2013; Widdows & Johnson, 1988). 'Direct' measurements provide limited information because: (1) total production can be lost to gamete formation, and (2) there is inconsistent coupling between shell growth and tissue growth (Hilbish, 1986). Scope for growth (SFG) rates, based upon physiological energy budgets, are linked holistically to growth, reproduction, and the physiological plasticity of organism response to environmental changes and are considered to be the most sensitive measurement for determining fitness of an organism (Navarro et al., 2013; Sarà et al., 2008; Vargas et al., 2015; Widdows & Johnson, 1988). SFG is a balance between energy acquisition and energy expenditure and has been used as an index of OA effects upon several marine organisms (Navarro et al., 2013; Vargas et al., 2015; Vargas et al., 2013).

In addition to allowing the calculation of SFG, direct measurements of feeding, respiration, and excretion rates contribute to the understanding of organism metabolic responses to OA conditions. For example, bivalve feeding measurements such as clearance rates (CR) generally are depressed by elevated dissolved ρCO_2 concentrations (*Ruditapes decussatus* Fernàndez-Reiriz et al., 2011*, M. chilensis* Navarro et al., 2013, 2016*, M. coruscus* Wang et al., 2015, Siu et al., 2016, *Perumytilus purpuratus* Vargas et al., 2015). Under OA conditions, increases in respiration and excretion rates, which are tracers for metabolic loss (Brett & Groves, 1979), would suggest increased energy expenditures to maintain homeostasis or a switch in metabolic strategy. Excretion was shown to be stimulated by increased CO_2 conditions in *M. galloprovincialis* (Michaelidis et al., 2005; Fernàndez-Reiriz et al., 2012), *M. edulis* (Thomsen and Melzner; 2010), and *R. decussatus* (Fernàndez-Reiriz et al., 2011) as a result of changes in N-metabolism for acid-base regulation needs (Langenbuch & Pörtner, 2002). Despite some consistency, bivalves show conflicting results in respiratory response to elevated ρCO_2 (increase: *C. gigas* Lannig et al. 2010; curved response: *M. edulis* Thomsen and Melzner; 2010 ; decrease: *Pecten maximus* Schalkhausser et al. 2013, *M. chilensis* Navarro et al., 2016, *M. chilensis* Navarro et al., 2013, *M. coruscus* Wang et al., 2015, Sui et al., 2016; no effect: *M. galloprovincialis* Fernández-Reiriz et al. 2012). Calculation of excreted oxygen:nitrogen ratio (O:N) provides additional information about changes in substrate used to fuel metabolism (Mayzaud & Conover, 1988). The proportion of the three metabolic substrates (carbohydrates, lipids, and proteins) is indicated by O:N ratio, with pure protein metabolism occurring at a ratio between 3 and 16, amino acid catabolism being near 20, and balanced degradation of lipids and protein indicated by O:N of 50-60. Thomsen & Melzner (2013) reported a decrease in O:N from

17.9 under control conditions to 12.3 for OA conditions in *M. edulis*, suggesting that OA resulted in a switch in substrates used to fuel metabolic processes. Research on bivalve physiology provides useful information about how these organisms may adapt metabolic responses to cope with increased dissolved environmental ρCO_2.

To maintain the sustainability of the regional surfclam resource and its exploitation, it is important to describe physiological responses and understand metabolic adaptation under elevated CO_2 conditions as environmental change proceeds. Thus, this study investigated the effects of experimentally elevated CO_2 upon juvenile Atlantic surfclam physiological rates (food intake, assimilation, respiration and excretion). Further, physiological rate data were compiled to determine if the surfclam bioenergetic budget, as measured by SFG, changed as a result of OA and, if so, how the level of CO_2 concentration affects energy allocation and the metabolic strategy adopted by surfclams.

Materials and Methods

Experimental design

Nine-month-old, hatchery-reared surfclams from the Downeast Institute (Maine, USA) were acclimated in a flow-through system at the NMFS Laboratory in Milford, Connecticut (41°12’42.82 N, 73°3’7.82 W) for 4 weeks prior to the start of the experiment. Juveniles were used in this study as they do not express high mortality rates as larvae facing OA and also do not invest energy into reproduction, which adds complexity to energy budget assessment. After

acclimation, 126 surfclams were divided in 9 groups of equal biomass and placed into 9 bins (n=72, 43.2 cm x 36.8 cm x 25.1 cm, total volume = 38L) with a holding rack (40.6 cm x 30.5 cm x 12.7 cm). Surfclams were exposed for 12 weeks to one of three levels of ρCO_2 (low, medium, or high, Table 1) with unfiltered seawater that was supplemented with cultured phytoplankton, 2 mL min^{-1} of a 50/50 mixture of *Chaetoceros neogracile* (Chaet-B) and *Tetraselmis chui* (PLY429) at 50 000 cell/L. Initial surfclam flesh mass (0.0049 ± 0.0004 g, S.E.) and shell length (length=10.00 ± 0.33 mm) were measured on an additional 66 individuals. Daily water variables (temperature, salinity) were measured with a YSI probe (Model 556, Yellowspring, OH USA). Temperature was held constant at 16 °C (Table 1). During the 12-week experiment, no mortality was observed in any of the three CO_2 treatments.

A schematic representation of the CO_2 delivery system used has been described in Perry et al. (2015). Briefly, compressed air was passed through a carbon-dioxide absorber (Puregas, Broomfield, CO, USA) to produce CO_2-stripped air. The CO_2-stripped air was combined with research grade CO_2 at different mixing ratios using mass-flow controllers (Aalborg Instruments and Controls, Orangeburg, NY, USA). The resulting CO_2-enriched air was bubbled in seawater flowing through PVC columns to produce CO_2-enriched water that continuously flowed into the experimental tanks. In addition, CO_2-enriched air was bubbled into each individual tank to maintain the CO_2 concentration desired. All tanks had an outflow rate of 973.43 ± 24.57 ml min^{-1}. To avoid a potential CO_2 column effect, CO_2 inputs to the columns were rotated three times within two weeks; the experimental tanks and input air also were rotated to maintain the appropriate CO_2 treatment. This ensured that each treatment was exposed to all the columns before physiological measurements were taken. During this rotation, tanks were cleaned.

Carbonate chemistry measurements

Seawater samples were taken twice per week (n=24) during the experiment. Seawater samples were collected in dark, polypropylene bottles (500 mL) from each tank outflow and analyzed immediately for dissolved inorganic carbon (DIC) and pH. DIC samples were analyzed on an Apollo SciTech DIC analyzer (Apollo SciTech, LLC, Newark, DE). Precision of the DIC instrument was confirmed by an international inter-laboratory comparison exercise that consisted of low and high CO_2 test seawater samples, with the laboratory being within 0.5% of assigned values (Bockmon & Dickson, 2015). During analysis, replicate measurements (n=3) on a certified reference material were within ± 2.7 μmol kg^{-1} of the reported value. Total pH was determined colorimetrically at 20 ^oC using a metacresol purple indicator dye (Sigma-Aldrich, St. Louise, MS, Dickson & Goyet, 1994) with a UV-VIS spectrophotometer (Cary100, Agilent, Santa Clara, CA, USA). Tris-buffer was used to ensure accuracy of the measurements (± 0.0014, n=12). DIC and pH were used in CO2SYS (Pierrot et al., 2006) for the calculation of ρCO_2 (μatm), $\Omega_{Calcite}$, and $\Omega_{aragonite}$ using the following constants: K1, K2 from Mehrbach et al. (1973) refit by Dickson & Millero (1987); K hydrogen sulfate from Dickson (1990); and total Boron from Uppström (1974).

Physiological rates

Physiological measurements -- feeding, excretion (ER) and respiration (RR) rates -- were done every two weeks on seven individuals sampled randomly from each CO_2 treatment. Feeding measurements were done after RR and ER measurements. Shell length, width, height, and volume were recorded, and clams were shucked for dry tissue weight and dry shell weight

determinations. Physiological feeding rates (Table 2), including clearance rate (CR), selection efficiency (SE), organic ingestion rate (OIR), assimilation rate (AR), and assimilation efficiency (AE) were measured using the biodeposition method (Iglesias et al., 1998; Galimany et al., 2013; Galimany et al., 2017; Galimany et al., 2018). Briefly, this method relies upon the use of seston inorganic matter as a tracer in the food source, feces, and pseudofeces to track organic matter through filtration, ingestion, assimilation, and egestion steps (Table 2). Three identical biodeposition apparatuses, corresponding to the one described in Galimany et al., (2018), were used. Each one processed simultaneously 7 individual clams from one of the CO_2 treatment. Accordingly, the water inflow in each header tank was CO_2-treated with the surfclams exposure corresponding to the longer-term treatment. Surfclams were acclimated to the system for 12 hours prior to the collection of water samples and feces/pseudofeces. The gut transit time (GTT) was measured by feeding surfclams with a green algae *Nannochloropsis* sp. (UTEX-2341) and timing production of green feces. Water from the header tank inflow and control chamber outflows were sampled for each treatment every 20 minutes for approximately 2 hours and filtered on pre-weighted, pre-combusted (450 ºC for 4 hours) GF/C filters and rinsed with isotonic ammonium formate. The collection of feces and pseudofeces was offset by the GTT. Feces and pseudofeces were collected continuously and separately for each individual for 2 hours, and then filtered and rinsed with isotonic ammonium formate. Separation of the feces and pseudofeces allows for the computation of total, organic, and inorganic rates of ingestion, egestion, and rejection. All filters were frozen until processing. Total weight and organic/inorganic fraction of samples (chamber inflows/outflows, surfclam feces and pseudofeces) were calculated by drying the filters in an oven at 60 ºC to constant weight and then

combusting at 450 ºC for 4 hours and weighing again. All feeding measurements started at week 5 as the surfclams prior to that time were too small to properly distinguish feces from pseudofeces, which was necessary for the biodeposition method. Twice a week, seawater samples were taken and preserved (Lugol 3%) for microalgal identification to determine if harmful phytoplankton were present during biodeposition experiments and throughout the 12-week experiment. Species were identified with a Zeiss Observer Z1 inverted microscope (Jena, Germany). All treatments had the same natural phytoplankton community (mainly diatoms) and no toxic algae were observed at high concentration.

After 24h of depuration, RR and ER for 7 individuals were determined using closed chambers filled with 0.35-µm filtered seawater at appropriate CO_2 concentrations. Oxygen concentration was measured for 1 hour with a Loligo® respirometry system (Viborg, Denmark) in 8 closed chambers (1 control with empty shell and 7 experimental with live surfclams). RR was calculated from the linear regression applied to oxygen depletion while surfclams were active. Background noise measured in the control chamber was subtracted from experimental chambers. Oxygen saturation was maintained above 80% in the chambers to ensure that clams were not stressed.

At the end of each RR measurement, a 5-mL seawater sample was collected from each chamber for ammonia-N determination. Ammonia-N was determined with salicylate based ammonia HACH TNT-830 test kits (equivalent EPA 350.1, EPA 351.1, and EPA351.2) with increased reaction time; validation tests on seawater samples spiked with ammonia indicated that 3.5 hours was needed for full color development rather than 15 minutes. The detection limit was

0.015 mg l^{-1}. After color development, absorbance was determined at 690 nm using a Perkin Elmer Lambda 35 UV/Vis spectrometer (Perkin Elmer, Brandford, CT). A low-nutrient seawater reference standard was used to validate each run with an average recovery of 95%. Individual ER was based upon the effective active time observed for each individual (i.e. period when the clam was respiring) and was determined with the following equation:

$$ER\ (\mu g\ N\ h^{-1}) = C_{NH4\text{-}N} * (V_c - V_{sc}) / t_{ea}$$

where $C_{NH4\text{-}N}$ was the concentration of ammonia-N in the physiological chamber ($\mu g\ N\ mL^{-1}$), V_c was the volume of the physiological chamber (mL), V_{sc} was the volume of surfclam (mL) and t_{ea} was the effective active time (h). O:N ratios were calculated as the atomic ratio of oxygen consumed to nitrogen excreted.

Standardization of physiological rates

All physiological rates were standardized for a 0.11 g of dried tissue weight individual. This weight, corresponding to the median weight of all individuals monitored during this experiment, was chosen to keep standardized rates consistent with one weight and minimize error. Physiological rates were standardized from the following equation:

$$Y_w = (W_s/W_{sc})^b\ Y_e$$

where, Y_w was the weight-standardized physiological rate, Y_e was the experimentally measured rate, W_{sc} was the dry body mass measured for each surfclam, W_s was the standard weight (i.e.

0.11g) and b was the power value that scales physiological rates to body weight for a species (Hawkins & Bayne, 1984; Hawkins et al., 1998; Hawkins et al., 1990). To date there are few data with which to calculate the b value for surfclams for any physiological processes. The allometric coefficients "b" were estimated from surfclam dry tissue weight and the physiological processes (CR, RR, ER) following the equation $Y_e=aW_{sc}^{b}$. To fit this relationship, the data obtained from surfclams exposed to the control treatment only were used (Appendix A). Results gave 0.76, 0.62 and 1.06 as b factors for CR, RR and ER, respectively, with reliable r squared (0.62, 0.74 and 0.76, respectively); therefore, these b values were used for physiological standardization.

To account for potential variations in temperature during the recording of physiological rates (± 0.5 °C), temperature standardization also was applied to the different physiological rates. As detailed in Galasso et al. (2018), the rates (already weight-standardized) were standardized for a temperature of 16 ˚C following the Arrhenius relationship:

$$Y_{(w,T)} = Y_w \, exp\left(\frac{T_A}{T_1} - \frac{T_A}{T}\right)$$

With the weight and temperature standardized rate at the temperature T_1 (289.15 K), T_A the Arrhenius temperature (7000, obtained from the Dynamic Energy Budget parameter collection) for *S. solidissima,* the weight-standardized rate, and T the temperature (in K) measured experimentally.

Scope for Growth

Scope for growth was calculated using the formula: SFG= (C*A)-(R+U) where SFG was the scope for growth (J h^{-1}), C was the rate of energy ingestion (J h^{-1}), A was the assimilation

efficiency (without units), R the rate of energy catabolized through respiration (J h^{-1}), and U ammonia excretion (J h^{-1}; Din & Ahamad, 1995; Hornstein et al., 2018; Sanders et al., 2014; Stuart, 1982; Stumpp et al., 2011). The energetic content of the food supply (Δ_{food}, J mg dry wt^{-1}) was determined using the following equation: Δ_{food}= 0.632+0.086 (%C) (Platt & Irwin, 1973), "%C" being the organic ratio of the trophic environment while surfclam feeding rates were monitored. The conversion for ER into energy expenditure was 24.83 J mg^{-1} NH_4-N, and the conversion for oxygen was 14.0 J mg^{-1} O_2 (Elliott & Davison, 1975). The rate of energy ingested (C, J h^{-1}) was calculated with the following: CR the clearance rate (L h^{-1}), POM the particular organic matter measured in the water (mg L^{-1}), and the energetic content for food (Δ_{food}, J mg dry wt^{-1}) detailed above.

Statistics

The physiological responses of surfclams exposed to three ρCO_2 treatments were analyzed using the program Statgraphics Centurion (Statgraphics Technologies, Inc. The Plains, Virginia, USA). Data were first checked for normality (Shapiro Test). All ratios and % data were arcsin(sqrt) transformed before checking for normality. Differences in all rates and SFG were compared using a one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls test. Means and standard errors were reported in the tables and graphed.

Results

Chemical measurements

Measured *in situ* pH (n=66) means for the three treatments were 7.86 ± 0.01, 7.51 ± 0.01, and 7.32 ± 0.01 for low, medium, and high CO_2 treatments, respectively (Table 1). DIC values (n=66) were 1737.28 ± 2.25 μmol kg^{-1} for low CO_2, 1832.08 ± 2.25 μmol kg^{-1} for medium CO_2, and 1887.64 ± 2.25 μmol kg^{-1} (n=66) for high CO_2. The rest of the carbonate system variables (ρCO_2, $\Omega_{Calcite}$, $\Omega_{aragonite}$, $pH_{in\ situ}$) were calculated at the *in situ* temperature and are reported in Table 1. The mean calculated levels of ρCO_2 were 566.14 ± 12.73 μatm, 1380.05 ± 51.40 μatm, and 2163.97 ± 62.72 μatm. There were significant differences ($p<0.01$) in all measured and calculated values between low, medium, and high CO_2 additions.

Physiological measurements

Surfclam standardized CR was significantly lower ($p=0.011$) in the high CO_2 treatment when compared to the two other treatments, which were statistically similar (Fig. 1A). Figure 1B shows that surfclams expressed significant differences in SE ($p<0.001$). The trend is not linearly related to CO_2 concentration as clams in the medium treatment (0.291 ± 0.018) showed better selection efficiency compared to those in the low (0.159 ± 0.029) and high (0.121 ± 0.039) treatments. As with CR, OIR (Fig. 1C) and AR (Fig. 1D) were significantly affected by treatment ($p<0.001$ and $p=0.029$, respectively), with the high CO_2 treatment being lower than the other two treatments. Considering AE data (Fig. 1E), no significant differences (p-value=0.131) between the CO_2 treatments were observed. AE values were 0.453 ± 0.024, 0.500 ± 0.021, and 0.433 ± 0.026 for the low, medium and high CO_2 treatments, respectively.

Standardized RR was highly variable during the experiment, with a significant difference between treatments ($p=0.047$, Fig. 2A). RR had a curved response to CO_2 concentrations: there were two homogeneous groups with the medium treatment having the highest RR (0.131 ± 0.006 mg O_2 h^{-1}) and being different from the low (0.108 ± 0.003 mg O_2 h^{-1}) and the high treatments (0.118 ± 0.005 mg O_2 h^{-1}).

Standardized ER (Fig. 2B) showed a significant difference ($p<0.001$), with the 566-µatm and 1380-µatm ρCO_2 treatments behaving similar to each other (3.308 ± 0.246 µg N h^{-1} and 4.037 ± 0.265 µg N h^{-1}, respectively), but the 2164-µatm (5.619 ± 0.382 µg N h^{-1}) was different from the other two treatments. Results showed that ER increased with increase in CO_2.

The excreted O:N ratio (Fig. 2C) was significantly different ($p<0.001$) between treatments, with two homogeneous groups. The low (18.635 ± 1.159) and medium (20.106 ± 1.1395) treatments were similar to each other and the high treatment was lower (11.940 ± 1.683).

Calculated SFG values, computed by subtracting the catabolized energy of respiration and excretion from assimilated energy, are presented in figure 2D. A significant difference can be observed between CO_2 concentration exposures, with low and medium treatments (4.693 ± 0.631 J h^{-1} and 4.243 ± 0.592 J h^{-1}, respectively) showing higher SFG compared to the high treatment (2.778 ± 0.472 J h^{-1}).

For each treatment, surfclam energy budget (energy gain, energy loss through respiration and excretion, SFG) is summarized in Table 3. Energy gain (C*A, J h^{-1}) shows a comparable

value between low and medium CO_2 concentrations, but the high treatment is significantly lower (p-value=0.024). Concerning the energy catabolized (R+U, J h^{-1}), the low CO_2 treatment is significantly lower than medium and high treatments (p=0.029, 1.596 ± 0.062 J h^{-1}, 1.929 ± 0.102 J h^{-1}, 1.787 ± 0.126 J h^{-1}, respectively). In terms of overall energy use, R calculated to 24.1%, 29.6%, 36.1% for low, medium, and high CO_2 treatments, respectively. Mean U amounted to 1.3%, 1.6%, and 3.1% for low, medium, and high CO_2 treatments, respectively.

Discussion

This study contributes new insights into surfclam response to OA. When exposed to environmental conditions that may not be favorable, marine organisms can modify metabolic strategies to cope with changes (Guppy, 2004; Pörtner et al., 2000; Langenbuch & Pörtner, 2002). In this study, the high CO_2 concentration reduced feeding, and energy loss was increased (ER increased and RR remained unchanged), resulting in less energy available for growth (decreased SFG). At medium treatment conditions, there was no difference in assimilated energy and ER, but RR increased as did the total catabolized energy (R+U) in comparison to the control treatment. These results suggest that surfclams adopted two metabolic strategies (Fig. 3) to respond to elevated CO_2 concentrations: (1) an increase in energy allocated to metabolic processes under medium ρCO_2 treatment and (2) a metabolic depression associated with a change in substrate catabolized and a decrease in clearance rate under high ρCO_2 treatment. Nevertheless, the magnitude of response to relatively modest differences in ρCO_2 indicates that surfclams are highly sensitive to OA relative to other bivalve species tested.

For clams in the highest CO_2 treatment (2163 µatm), less energy was available for overall growth (SFG) than with lower ρCO_2. In comparison with other bivalve species, surfclams appear to be strongly affected by OA. No SFG decrease was observed in *M. galloprovincialis* (Fernandez-Reiriz et al., 2012), *Pinctada margaritifera* (Le Moullac et al., 2016) or *M. coruscus* (Wang et al., 2015; Sui et al., 2016) under CO_2 concentrations of 3790, 2485, 4485 and 2579 µatm respectively. The same lack of response has been shown for *Chamelea gallina* , *R. decussatus* and *M. galloprovincialis* (contrasting 2615, 4024 and 2992 µatm respectively, Range et al., 2013). ρCO_2 of 990 and 1000 µatm, however, induced significant decreases in *Ruditapes philippinarum* and *M. chilensis* SFG, respectively (Xu et al., 2016, Navarro et al., 2016).

Reduction in SFG resulted both from increased metabolic losses and decreased energy gain which was mainly attributable to a decrease in CR. This response has been described in several bivalve species exposed to elevated CO_2 concentration (*Chlamys nobilis, Perna viridis* and *Pinctada fucata,* Liu & He, 2012; *M. chilensis,* Navarro et al., 2013, 2016; *M. coruscus,* Wang et al., 2015, Sui et al., 2016; *R. decussatus* Fernàndez-Reiriz et al., 2011; *Perumytilus purpuratus,* Vargas et al., 2015). Most of these reports are in agreement with our results and showed a linear and negative trend in CR with increasing CO_2 concentration. CR decrease is a key factor in the effect OA has on bivalve energy budgets. One likely hypothesis would be that cilia beat frequency, which is the mechanical basis of CR in bivalves, is negatively affected by CO_2 (Waldbusser et al. 2015). Recently, Meseck et al. (2020) observed decreases in CR and reduction in cilia beat frequency in *M. edulis* associated with increased CO_2 concentration. Authors suggested that CR is reduced under OA because of the activation of an inhibitory G

protein, for example dopamine or gamma-amino butyric acid (GABA), that has been shown to reduce cilia beat frequency in bivalves (Cochran et al., 2012, Mathieu et al., 2014). Change in gill mucus viscosity may also contribute to CR changes in mussels (Meseck et al., 2020). It is possible that the reduced CR observed here in surfclams exposed to CO_2-enriched seawater was because of reduced cilia beat frequency, but it is beyond the scope of this study to determine if inhibitory proteins were activated. Further research on the mechanisms of inhibitory G proteins roles on reduced feeding, as well as inhibition/activation pathways under OA, needs to be conducted.

The second effect of CO_2 on SFG is a consequence of increased metabolic loss. For the high CO_2 treatment, an increase in total metabolic loss (R+U) was attributed mostly to increased ER. Higher in osmoregulatory demand under OA conditions induced a significant increase in surfclam ER, as has been reported for other bivalves, including *M. edulis* (Thomsen & Melzner 2010), *M. galloprovincialis* (Fernàndez-Reiriz et al., 2012), and *Ruditapes decussatus* (Fernàndez-Reiriz et al., 2011). Two mechanisms could explain the observed increase in metabolic loss with high CO_2: "channel arrest" and protein degradation. Both mechanisms will be described; we cannot determine if both were occurring concurrently.

Respiration under high CO_2 remained unchanged relative to the low treatment; however, it was significantly lower than the medium treatment. First, this explains why no differences in metabolic loss were observed between the medium (high R, low U) and the high treatments (low R, high U). Secondly, it raises questions concerning how, at higher CO_2 concentrations, surfclams would be able to regulate the same metabolic losses as in the medium treatment. It can

be hypothesized that different reactions associated with a metabolic depression strategy occurred at the high CO_2 treatment, which was not observed with the medium treatment. Under normal, aerobic metabolism, bivalves balance H^+ ions in the intracellular space by accumulating and transporting HCO_3^- ions across the cell membrane through Na^+/K^+ and H^+-ATPases protein carriers, which is energetically expensive (Seibel & Walsh, 2003; Pörtner et al., 2004; Parker et al., 2013). When energy needs to be conserved, "channel arrest" can reduce the costs of maintaining H^+ ion balance by using $Na^+/Cl^-/HCO_3^-$-dependent carriers (Pörtner et al., 2000). This process allows acid-base regulation for a lower energy cost but with a slower speed (Pörtner et al., 2000). The Cl^-/HCO_3^- ionic channel is open under extracellular acidosis by GABA, allowing the inflow of HCO_3^- into the cell to buffer H^+ ions (Nilsson et al. 2012). Under OA conditions, the accumulation of HCO_3^- ions used for buffering arises mainly from the dissolution of $CaCO_3$ shells (Lindinger et al., 1984). The switch (or preferential use) toward this exchanger represents a highly-efficient energy saving as the ratio of ATP hydrolyzed per acid-base equivalent transported is divided by a factor of 3 compared to the primary exchanger, which also reduces oxygen consumption (Pörtner et al., 2000). The lack of change in R at the high treatment suggests that 'channel arrest' might be occurring; however, hemolymph analysis and GABA analysis would have to be done to confirm this hypothesis.

Protein degradation is another possible explanation for the observed change in metabolic loss. Under aerobic metabolism, bivalves catabolize energy through the breakdown of a balanced amount of lipids and proteins, yielding an O:N ratio around 20 (Mayzaud & Conover, 1988). Under OA conditions, this metabolism appears to have switched to a higher proportion of protein catabolism. With protein degradation, the decarboxylation of amino acids from α-carboxylic

group (i.e. glutamic acid and after deamination, asparagine or glutamine) produces ammonia and bicarbonate ions that can be used to buffer intracellular pH (Langenbuch & Pörtner, 2002). The degradation of the protein pool increases the production of ammonia and, as a consequence, reduces the O:N ratio as observed in our results. Low O:N ratio, however, is a less favorable metabolism; thus, even if it fulfills the minimal energy requirements and balances the intracellular pH, it often results in declining protein available for growth and reproduction (Bayne et al., 1979; Sprung et al., 1991). The mechanism enhancing this switch is not well described, but one hypothesis would be the activation of glutaminase. This enzyme, which catalyzes glutamine with a very low O:N ratio (1.5), to form glutamate and ammonia, has been shown to be up-regulated under metabolic acidosis (Labow et al., 2001, Curthoys & Lowry, 1973). An incomplete acid-base balance in the extracellular space could, thus, trigger the switch in substrate catabolized. The up-regulation of glutaminase could have other physiological consequences. Glutamate is an excitatory neurotransmitter; however, at high concentration it is considered neurotoxic (Bak et al., 2006). As a result, the glutamate concentration is constantly regulated by decarboxylation (anaerobic reaction) into GABA, that is, unlike glutamate, an inhibitory neurotransmitter. Under OA conditions, it has been suggested that GABA decreases physiological responses of bivalves, such as burrowing behavior (Clements et al., 2017), predator-escape response (Watson et al., 2014), and cilia beat (Meseck et al., submitted; Cochran et al., 2012, Mathieu et al., 2014). Glutamate homeostasis also could play a role in decreasing feeding rates observed (Jones et al., 1987). Further studies on “channel arrest” and protein degradation with respect to glutamine catabolism, glutamate concentration, and GABA inhibition activity, have to be explored under increased CO_2.

Metabolic depression is associated with degradation of structural and reserve compounds (Wood et al., 2008; Lannig et al., 2010) to cover energy requirements, which leads to slower growth rates (Michaelidis et al., 2005; Berge et al., 2006). Bivalve response to ocean acidification is species-dependent, and the CO_2 threshold that induces metabolic depression could be an important criterion to compare species sensitivity. In *M. edulis,* Thomsen and Melzner (2010) found that the CO_2 threshold inducing metabolic depression was in the pH range of 7.38 - 7.14. In *C. gigas*, a pH of 7.1 did not induce metabolic depression (Lannig et al., 2010). For Atlantic surfclams, our results indicated metabolic depression in the pH range of 7.46 - 7.28, making the surfclam more sensitive than estuarine species previously studied. Data on extracellular pH of body fluids (e.g., hemolymph) could be helpful to accurately determine this threshold in surfclams. Ecologically, the sensitivity of surfclams to low pH and elevated CO2 is meaningful as estuarine species have evolved by adapting to changing environmental conditions in fluctuating habitats (e.g. temperature, salinity, food resource, oxygen concentration; Bunn and Harthington, 2002). In contrast, coastal species live in more stable conditions. As a consequence, estuarine species could display greater plasticity in response to OA conditions. It has been demonstrated (Rivera-Ingraham and Lignot, 2017) that, within a given species (*R. philippinarum*), individuals can exhibit different redox strategies, depending upon whether they came from an estuarine (Velez et al., 2016b) or a coastal area (Velez et al., 2016a). Finally, it can be hypothesized that, unlike sessile species (e.g. *C. gigas* or *M. edulis*), Atlantic surfclams have the ability to avoid OA conditions by moving/burrowing, and thus did not evolve physiological compensation mechanisms to respond to acute OA exposure. A global study comparing bivalve

species on the basis of ecological habitat and life traits could be helpful to identify groups more or less likely to be affected by OA.

Even though the medium CO_2 treatment in the present study showed no difference in SFG relative to the low treatment, there remained significant differences in how food was selected. At the medium CO_2 concentration, CR and energy gain were the same as in the low treatment; however, a significant increase in SE was observed. SE characterizes how bivalves are able to select and ingest organic matter in food (i.e., reject pseudofeces enriched in inorganic matter) to maximize energy gain (Rosa et al., 2018). Increased CO_2 does change the conductivity of seawater (Bradshaw 1973, Millero 1995), likely affecting seston surface charge and/or wettability which govern food selection in bivalves (Rosa et al., 2013, 2017). Vargas et al. (2013) reported that under OA, *C. concholepas* switched from feeding on large diatoms to small nanoflagellates and cyanobacteria. From the present data, it can be hypothesized that the decrease in ρCO_2 of the medium treatment induced a change in electrostatic charge or wettability of particles, imparting better affinity to particles enriched in organic matter. An alternative hypothesis would be that, as a compensatory response to balance metabolic costs associated with OA, labial palp activity was stimulated, increasing the SE, to improve OIR and produce a rise in AE (Parker et al., 2013; Thomsen et al., 2013). We did not observe a significant change in OIR or AE; however, we cannot rule out that the labial palps were stimulated under increased CO_2. Investigating how OA changes seston wettability, surface charge, and selection in general will be important in understanding how surfclams and other bivalves respond to OA.

In comparison with the low CO_2 treatment, clams in the medium treatment had significantly higher total catabolized energy (U+R), a difference that was driven by increased respiration. This dose-dependent response, showing an increase at moderated CO_2, has been described in echinoderms (*Amphiura filiformis*, Wood et al., 2008), fish species (*Ostorhinchus doederleini* and *O. cyanosoma*, Munday et al., 2009), and the bivalve *M. edulis* (Thomsen and Melzner, 2010). Focusing on *M. edulis,* a noticeable hormetic response (Constantini et al., 2010) in RR, identical to the one described in the present study, was reported and attributed to maintenance of acid-base balance. It can be hypothesized that, until a threshold of acidosis is achieved, marine organisms will continue to use the energetically more-costly H^+-ATPase transport (Heisler & Evans, 1993; Reipschläger & Pörtner, 1996, Fabry et al., 2008; Michaelidis et al., 2005, Lannig et al., 2010). Continuing to use the H^+-ATPase transport at the medium treatment, with elevated CO_2 conditions, tends to increase catabolic energy requirements and therefore oxygen consumption (Pörtner et al., 2000). Moreover, our calculated O:N excretion ratios suggest that there is no change in the N-metabolism pathway between the low and medium treatments, with respiration and excretion increasing at the same scales. These results support the hypothesis that oxygen metabolism increased at the medium treatment, but overall metabolic function did not change. Our medium treatment physiological data indicate that surfclam metabolic pathways remained unchanged during a 12-week exposure to a 1380 μatm ρCO_2 concentration. Even though we did not observe a significant change in SFG, the long-term effects of increased catabolic energy requirements should not be disregarded. Long-term effects upon growth and reproduction may occur, and dynamic energy budget modeling would provide insight on this.

This study, presenting the first results of surfclam physiological response to OA, demonstrates the complex mechanistic responses of *S. solidissima*. Surfclams can adapt responses by adopting two different metabolic strategies as a function of the CO_2 concentration. Our results focused on the effect of OA upon surfclam energy budget and raised concerns, in view of SFG measurements, about fisheries production at projected future levels of CO_2. OA effects upon reproduction (gametes, larvae, broodstock) of this species remains to be described. In addition, comparison of the surfclam response with estuarine species points to the sensitivity of coastal species to OA. Regarding life traits (long-lived, coastal species), economic importance (most valuable U.S. Northeast fisheries after lobster), and the threats to habitat (Gulf of Maine, Ekstrom et al., 2015), the Atlantic sea scallop (*Placopecten magellanicus*) would be a relevant case for further study. In the future, such studies should emphasize on the pathways governing physiological responses to OA using genetic and epigenetic tools (i.e. gene, enzyme or protein expression) to illuminate a better mechanistic understanding of bivalve responses to OA. Likewise, testing if energetic effects that are observable in the field at populational scale would provide relevant context on this issue. Critically, any such study should be coupled with accurate measurements of ρCO_2, pH and Ω_{arag} in the habitats occupied by the species of interest. Finally, to have a global view and respond adequately to fisheries management and conservation problems, carbonate projections in bottom waters and sediment pore water are needed.

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Author contribution:

E.P., S.L.M., M.E.P. contributed to article redaction, experiment elaboration, set-up and measurements and data treatment.

D.H.R. and G.S. contributed to experiment elaboration and set-up.

J.M.L and L.E.W. contributed to experiment measurements and data treatment

M.S.D. contributed to algal production and experiment measurements.

G.H.W. contributed to experimental design and article preparation

S.L.M. obtained the funding for the project

D.M, Deborah H., and D.H. were co-principal investigators of funded research

Y.L. did the phytoplankton analysis

Competing interests:

The authors declare no competing interests.

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Figures:

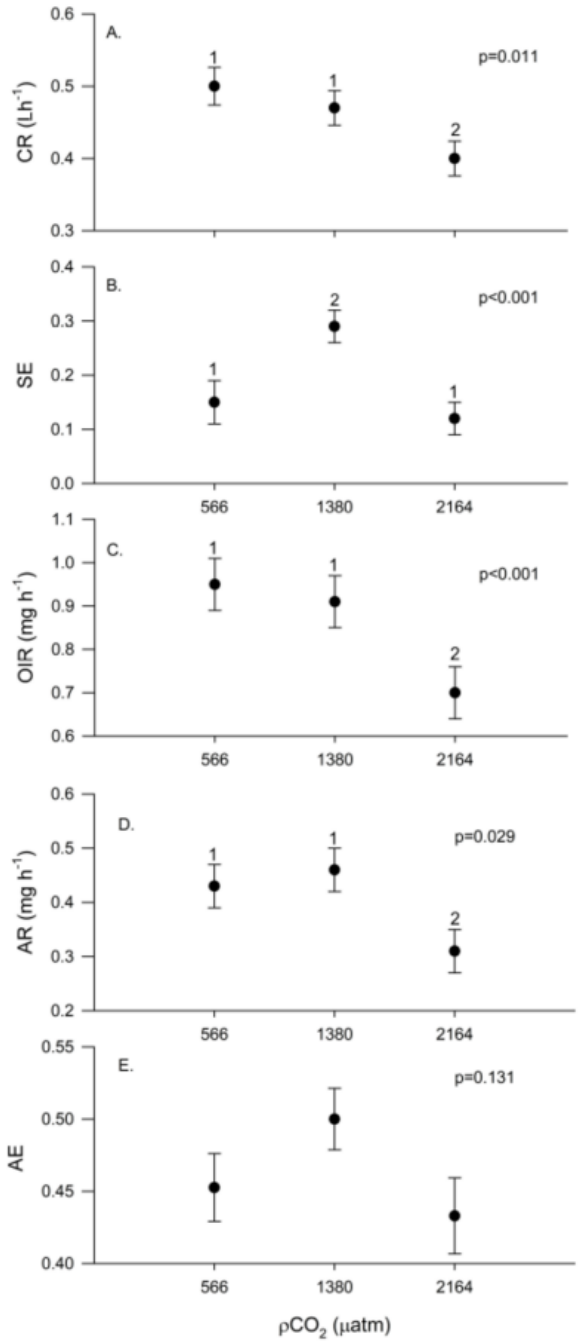
A.
p=0.011
CR (Lh^{-1})
B.
p<0.001
SE
C.
p<0.001
OIR (mg h^{-1})
D.
p=0.029
AR (mg h^{-1})
E.
p=0.131
AE
566
1380
2164
ρCO$_2$ (μatm)

Fig. 1: Mean standardized clearance rate (CR, A, L h^{-1}), selection efficiency (SE, B, -), organic ingestion rate (OIR, C, mg h^{-1}), assimilation rate (AR, D, mg h^{-1}) and assimilation efficiency (AE, E, -) in surfclams, *Spisula solidissima,* exposed to three different levels of ρCO_2. Clearance rates were standardized for a 0.11g individual (i.e. median individual) and for 16°C. Data were measured every two weeks on 7 individuals per treatment. Bars correspond to standard errors. Numbers indicate statistical differences between treatments. P-values are obtained from a one-way ANOVA.

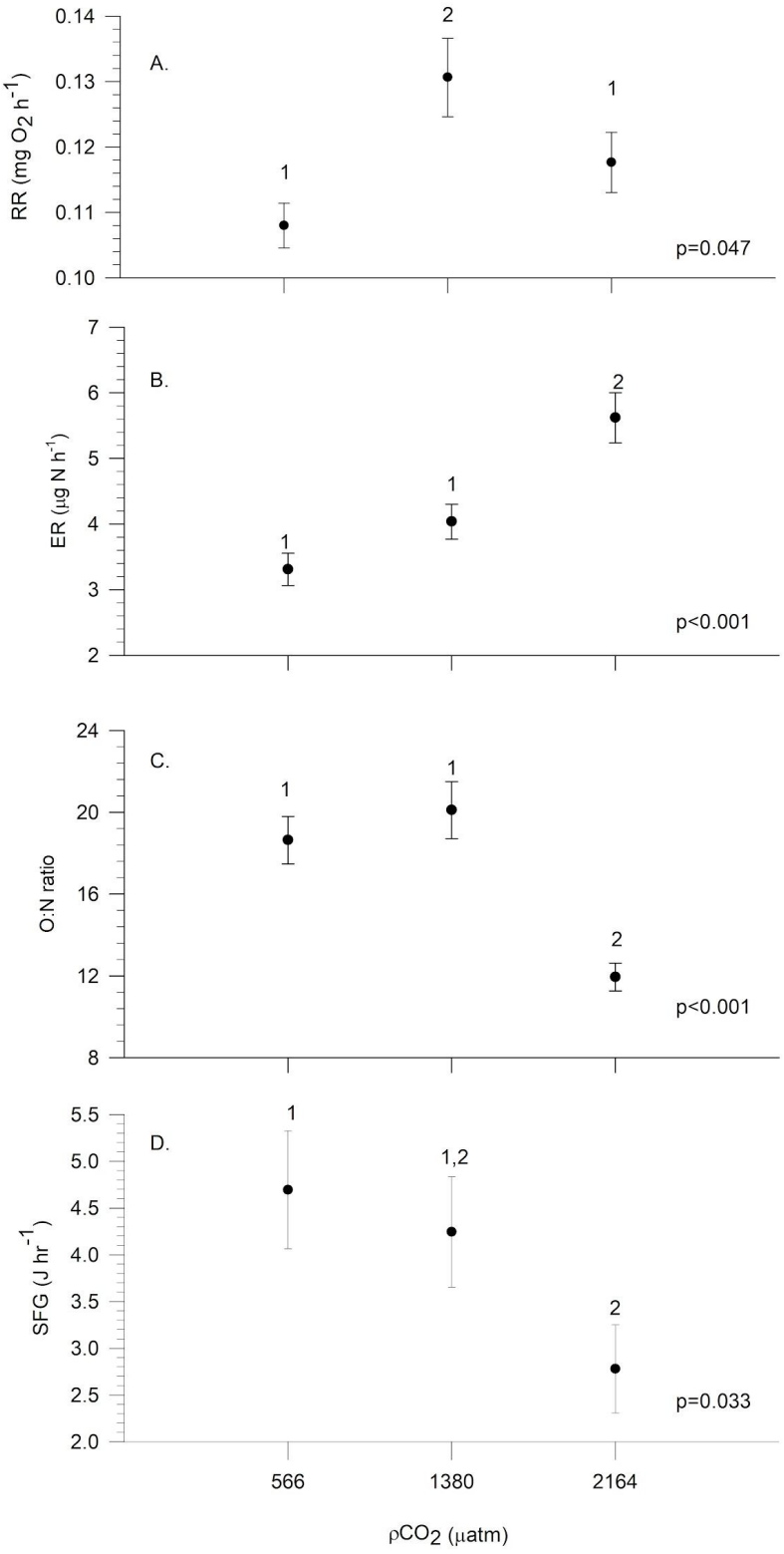
A.
RR (mg O_2 h^{-1})
0.14
0.13
0.12
0.11
0.10
1
2
1
p=0.047
B.
ER (µg N h^{-1})
7
6
5
4
3
2
1
1
2
p<0.001
C.
O:N ratio
24
20
16
12
8
1
1
2
p<0.001
D.
SFG (J hr^{-1})
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1
1,2
2
p=0.033
566
1380
2164
ρCO_2 (µatm)

Fig. 2: Mean standardized respiration rates (RR, A, mg O_2 h^{-1}), excretion rates (ER, B, µg N h^{-1}) and O:N ratio obtained from those two rates in surfclams, *Spisula solidissima,* exposed to three different levels of ρCO_2. Respiration and excretion rates were standardized for a 0.11g individual (i.e. median individual) and for 16 °C. Rates were measured every two weeks on 7 individuals per treatment. Bars correspond to standard errors. Numbers indicate statistical differences between treatments. P-values are obtained from a one-way ANOVA.

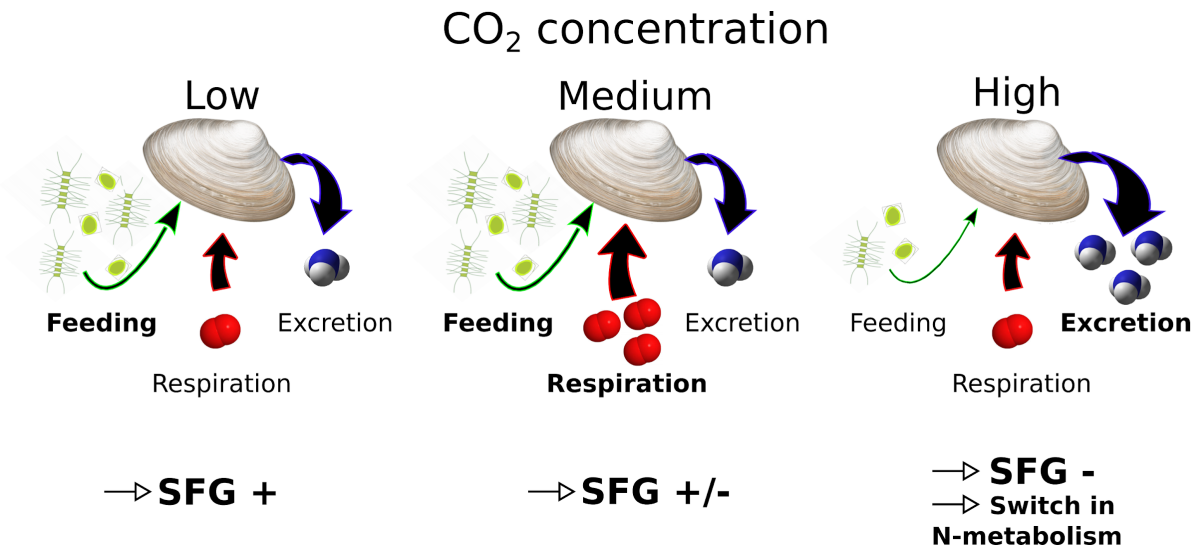

Figure 3: Synthesis scheme of the different physiological and scope for growth responses induced by the three ρCO2 treatments (566, 1380 and 2163 µatm) in Atlantic surfclams (*Spisula solidissima*).

Tables:

	Low	Medium	High
pH at 20°C (seawater scale)*	7.80 ± 0.01	7.46 ± 0.01	7.28 ± 0.01

pH *in situ* (seawater scale)	7.86 ± 0.01	7.51± 0.01	7.32± 0.01
Temperature (°C)*	16.12 ± 0.05	16.15 ± 0.05	16.14 ± 0.05
Salinity*	25.55 ± 0.45	25.60 ± 0.45	25.73 ± 0.45
ρCO_2 (µatm)	566.14 ± 12.73	1380.05 ± 51.40	2163.97 ± 62.72
DIC* (µmol kg^{-1})	1737.28 ± 2.55	1832.08 ± 2.55	1887.64 ± 2.55
$\Omega_{Calcite}$	1.86 ± 0.02	0.91 ± 0.02	0.60 ± 0.02
$\Omega_{aragonite}$	1.16 ± 0.01	0.57 ± 0.01	0.37 ± 0.01

Table 2. Physiological variables from biodeposition method for surfclams, *Spisula solidissima*.

Parameter	Acronym	Unit	Calculation
Clearance rate	CR	L h^{-1}	Inorganic matter rejected in both feces and pseudofeces per hour (mg h^{-1})/inorganic matter in water (mg L^{-1})
Organic ingestion rate	OIR	mg h^{-1}	CR * Particulate organic matter in water (mg L^{-1}) – organic matter rejected in feces and pseudofeces per hour (mg h^{-1})
Assimilation rate	AR	mg h^{-1}	OIR - organic matter rejected in feces per hour (mg h^{-1})
Assimilation efficiency	AE	-	AR/OIR
Selection efficiency	SE	-	1- organic fraction within pseudofeces/organic fraction within total particles available in water

Table 3. Mean and standard errors obtained for low, medium and high treatment for energy assimilation rate (C*A), rate of energy lost through respiration (R), excretion (U) or cumulated (R+U) and scope for growth (SFG). Letters ‘1’ or ‘2’ given after the standard errors indicate homogeneous groups. P-values obtained from one-way ANOVA are also reported.

	Low	Medium	High	p-value
C*A (J h^{-1})	6.289 ± 0.515 [1]	6.172 ± 0.438 [1]	4.565 ± 0.411 [2]	0.024
R (J h^{-1})	1.514 ± 0.081[1]	1.829± 0.091 [2]	1.647 ± 0.115 [1]	0.047
U (J h^{-1})	0.082 ± 0.006 [1]	0.100 ± 0.007 [1]	0.140 ± 0.009 [2]	<0.001
R+U (J h^{-1})	1.596 ± 0.062 [1]	1.929 ± 0.102 [2]	1.787 ± 0.126 [2]	0.029
SFG (J h^{-1})	4.693 ± 0.631 [1]	4.243± 0.592 [1,2]	2.778 ± 0.472 [2]	0.033

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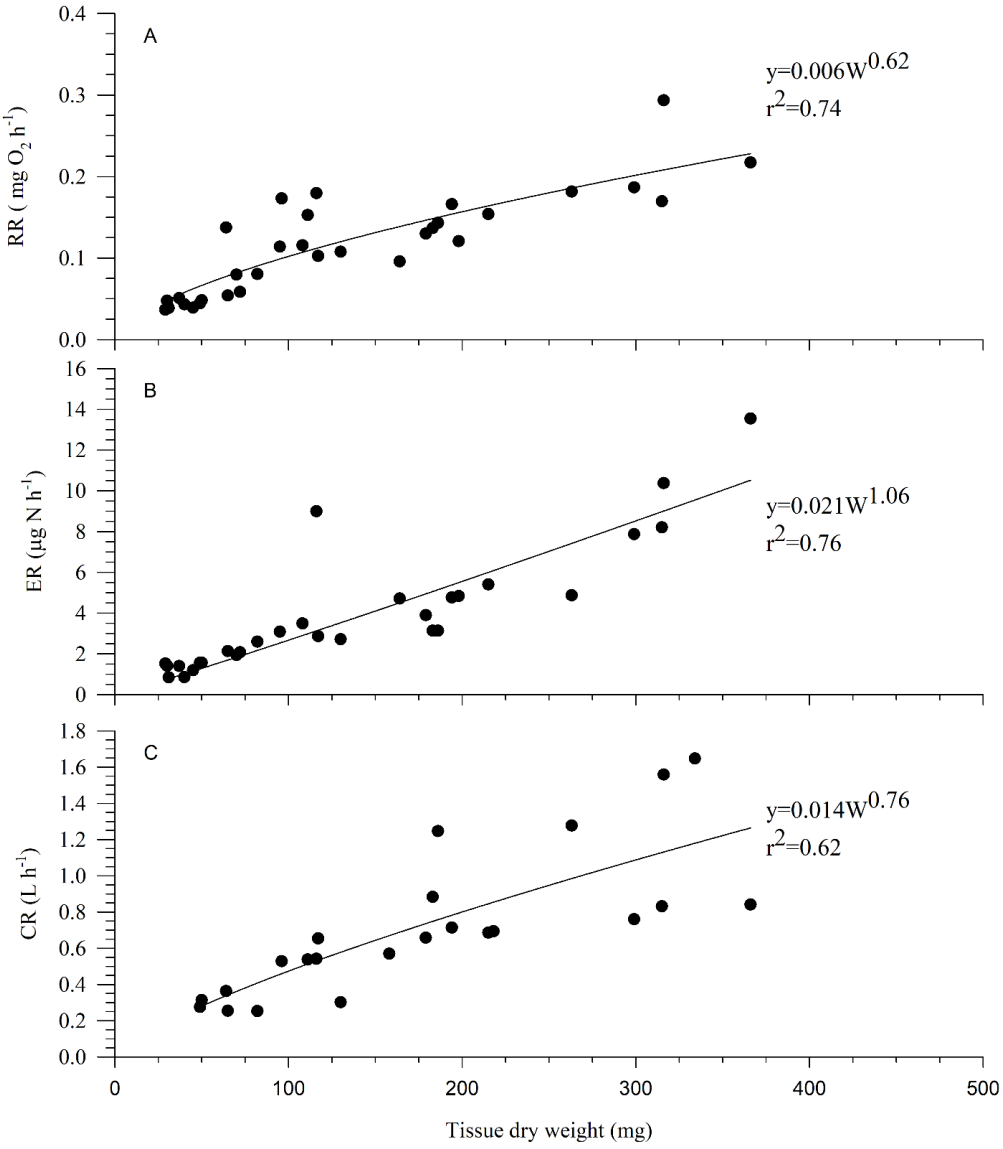
Appendix:



Appendix A. Equation fitting of physiological rates: respiration rate (RR, A, N=31), excretion rate (ER, B, N=29), and clearance rate (CR, C, N=23) for surfclams, *Spisula solidissima*. Lines were fitted from $Y_e=aW_{sc}^b$, with Y_e the physiological rate and W_{sc} the surfclam dry flesh mass. The equations obtained and r-squared are given.