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RESEARCH ARTICLE

Juvenile Atlantic sea scallop, *Placopecten magellanicus*, energetic response to increased carbon dioxide and temperature changes

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Abstract

This study assessed the energy budget for juvenile Atlantic Sea Scallop, *Placopecten magellanicus*, during a natural drop in temperature (15.6˚C to 5.8˚C) over an 8-week time period during the fall at three different enrichment levels of carbon dioxide (CO_2). Every 2 weeks, individuals were sampled for ecophysiological measurements of feeding activity, respiration rate (RR) and excretion rate (ER) to enable the calculation of scope for growth (SFG) and atomic oxygen:nitrogen ratios (O:N). In addition, 36 individuals per treatment were removed for shell height, dry tissue weight (DTW) and dry shell weight (DSW). We found a significant decrease in feeding rates as CO_2 increased. Those rates also were significantly affected by temperature, with highest feeding at 9.4˚C. No significant CO_2 effect was observed for catabolic energy processes (RR and ER); however, these rates did increase significantly with temperature. The O:N ratio was not significantly affected by CO_2, but was significantly affected by temperature. There was a significant interaction between CO_2 and temperature for ER and the O:N ratio, with low CO_2 levels resulting in a U-shaped response that was not sustained as CO_2 levels increased. This suggests that the independent effects of CO_2 and temperature observed at low levels are different once a CO_2 threshold is reached. Additionally, there were significant differences in growth estimators (shell height and DSW), with the best growth occurring at the lowest CO_2 level. In contrast to temperature variations that induced a trade-off response in energy acquisition and expenditure, results from this research support the hypothesis that sea scallops have a limited ability to alter physiological processes to compensate for increasing CO_2.

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Introduction

The Atlantic Sea Scallop, *Placopecten magellanicus*, with a habitat ranging from the Gulf of St. Lawrence, Canada to Cape Hatteras, North Carolina [1], sustains the largest wild scallop fishery in the world valued at $484 million dollars in 2020 (US dollars; NMFS, 2022 https://www.fisheries.noaa.gov/foss/f?p=215:200:2854050186896:Mail:NO:::). Sea scallops are long-lived (up to 20 years), and growth rate and distribution are highly sensitive to changes in oceanic conditions [2,3]. For this reason, ocean acidification (OA) and warming, which are occurring in sea scallop habitat [2,4,5], are being monitored.

From 2007 to 2015, dissolved CO_2 increased by 2.5% in waters of the Northeast US Continental Shelf [6]. Sea scallop habitat on the continental shelf from Virginia to Maine has high variability in ρCO_2, both spatially (increased acidification northward) and seasonally (lowest values during winter and highest values in summer [7,8]). Gulf of Maine waters, including Georges Bank, a highly productive sea scallop habitat, have seasonal ρCO_2 concentrations as high as ~500–800 μatm [8]; whereas, the southern portion of the Northeast Continental Shelf ranges from 400 to 700 μatm [8,9]. Higher-latitude and colder waters have a higher ρCO_2 baseline. Discrete measurements and model projections indicate that some portions of the Northeast US Continental Shelf region already have reached global ocean projections for 2100 (720–1000 ppm), and the rest of the region should reach it by 2100 [10,11]. In addition to seawater acidification, ocean temperatures are increasing for this area, with long-term (1968–2018) analyses reporting that bottom water temperatures increased at a rate of 0.306˚C decade^{-1}, while the short-term (2004–2018) temperature increase was 1.269˚C decade^{-1} [12]. This increase in temperature for sea scallop habitat in this region is 3 times faster than the global average [12–14]. These potential changes to Atlantic sea scallop habitat have resulted in classification of this species as highly vulnerable to projected OA and warming conditions [5].

The bioenergetics status of a bivalve can be assessed by calculating the "scope for growth" (SFG, [15,16]). This estimator is based upon mathematical expressions that incorporate feeding functions such as clearance rate (CR, the volume of water totally filtered per time unit) and assimilation efficiency (AE, the fraction of organic matter consumed that is assimilated), and food availability to calculate the energy intake. Energy expenditures are estimated through respiration (RR) and excretion rates (ER) that are used to determine the catabolized energy. The subtraction of this catabolized energy from the energy intake is the SFG. Following the assumption that overall energy fluxes can be summarized through this energy intake and those two energy expenditures, positive SFG values are obtained under favorable conditions, but negative values prohibit growth and result in utilization of body reserves to maintain homeostasis. SFG provides instantaneous and quantitative responses about how organisms may respond to environment changes [17–19]. For example, if SFG values decrease but catabolized energy inputs stay the same, feeding functions would be altered, suggesting that either less phytoplankton may be available or that clearance rates and/or assimilation may be affected by a given stressor. SFG has previously been used in bivalve research to provide an instantaneous physiological indicator of response to current environmental conditions and provides insight into the underlying mechanisms of growth [16,20,21].

The physiological measurements used to calculate SFG can contribute to the parametrization of models supporting short- and long-term forecasting for expected responses of marine bivalves to OA and warming. Recently, three models have been generated to project the effects of OA upon the sea scallop [2,4,22]. All three of these models project that sea scallop biomass could be reduced by 50–80% under future RCP8.5 scenarios; however, these simulations are qualified by acknowledgements that current data on sea scallop biology are insufficient. For

example, Cooley et al. [4] projected growth rate under ocean acidification parameterized from data in 13 different experiments with 12 different species of bivalve. Although these assessments and models provide valuable insights into potential effects, research on the effects of OA and warming on US federally-managed species is warranted to anticipate future stocks and fishery production.

To understand the physiological plasticity of a bivalve and its ability to acclimate, measuring physiological responses (feeding, respiration, and excretion) of individuals to changing environmental conditions provides information needed to assess tolerance [23] and population health [3]. A century of research has focused on the physiological responses of marine bivalves to increased temperature [24]. Attention to understanding the physiological responses of marine bivalves to increased CO_2 has been increasing, however, in only the last 20 years [20,25]. Findings suggest that not all bivalve species respond in the same ways to increased CO_2 (see review [26]). For example, in the Chilean mussel, *Mytilus chilensis*, increased CO_2 resulted in no significant change in CR or ER, but a significant decrease in RR occurred [27]. In contrast, the Atlantic surfclam (*Spisula solidissima*) exhibited a decrease in CR, a U shape response for RR, and an increase in ER with elevated CO_2 [20]. More recently, shellfish research has focused on increasing CO_2 along with warming. For the northern quahog, *Mercenaria mercenaria*, the combined effects of warming and increased CO_2 resulted in increased RR, but no effect was observed in the Eastern oyster, *Crassostrea virginica* [28]. Conversely, the oyster *Ostrea chilensis* showed no effects of high temperature and CO_2 upon physiological processes, indicating that this species may show high tolerance to these changes [29]. The above findings demonstrate that CO_2 and warming should be studied concomitantly as the interaction of these two factors may change the response of a species to future environmental conditions.

To date most exposure experiments with elevated OA have applied a constant carbonate chemistry level [30,31]; however, many of the vulnerable species identified in these studies are found in environments that exhibit natural variability on diurnal or seasonal time scales [32,33]. McElhany and Busch [33] suggested that biological experiments that have fluctuations in CO_2 may provide more ecologically-relevant information on species responses. Reum et al. [34] identified the need for laboratory experiments to incorporate naturally varying carbonate chemistry systems with temperature. Knowledge about the co-variability of OA with temperature is essential to understand biological responses of organisms to future conditions [35]; however these studies are scarce in the literature. Atlantic sea scallops are exposed to seasonal and daily variability in CO_2 [7–9] and temperature [3,36] in the natural habitat. Given the natural variability to which sea scallops are already exposed, understanding physiological responses in laboratory experiments that incorporate naturally-varying temperature along with carbonate chemistry will provide important information on how they are responding to changes already occurring in their habitat.

The aim of this study was to add CO_2 at three different levels (low, medium, and high) to ambient seawater that was not thermally controlled, to allow natural variability in the carbonate chemistry and temperature, while measuring physiological responses of sea scallops to these conditions. Every two weeks sea scallops were assessed by: (1) measuring physiological rates (CR, AE, RR, ER), (2) calculating from the physiological rates measured, the amount of energy remaining for growth (SFG), and (3) determining if net growth was affected by interactive variation in CO_2. It was hypothesized that scallops exposed to increased CO_2 will show lower growth performance and that the interaction between high temperatures and CO_2 will affect how scallops adapt physiological rates to cope with those conditions, resulting in reduced energy available for growth.

Material and methods

Scallops and experimental design

Wild juvenile scallops (<1 year old) were obtained from Nate Perry (Pine Point Oyster Company), who acquired them from multiple spat collectors located offshore of Cape Elizabeth, Maine, USA (~43˚ 35' 36.8772"N, 70˚ 10' 54.7998"W) on October 16, 2019 and transported to Massachusetts Maritime Academy (MMA, 41˚ 44'15.1728"N, 70˚ 37'30.5508"W). Scallops acclimated for 2 weeks, after which 540 sea scallops were divided into 9 equal groups (n = 60) of similar total biomass. Scallops were exposed for 8 weeks (from October 23 to December 19, 2019) to seawater that was supplemented with CO_2 at low, medium, or high enrichment. During this exposure, temperature was not controlled and declined naturally from a high of 15.6˚C at the beginning of the experiment to a low of 5.8˚C at the end. Every two weeks, physiological measurements were recorded on 7 individuals from each treatment for a total of 4 time periods at which temperatures were 13.1˚C, 9.4˚C, 7.4˚C and 6.1˚C.

In addition to physiological measurements, 12 individuals (n = 36 for each treatment) representative of the total tank biomass were removed every 2 weeks to determine shell height (mm), dry tissue weight (DTW), and dry shell weight (DSW). Shell height (mm) was measured from the umbo to the outer shell margin. DTW (g) was determined by shucking the soft tissues and drying in an oven at 60˚C to constant weight. For DSW (g), all fouling organisms and particulate material were removed with a freshwater rinse, then shells were dried to constant weight at 60˚C. A subset of 63 scallops were sampled for initial measurements.

Experimental system

A mobile CO_2 delivery system similar to the stationary system described in Pousse et al. [20] was constructed and transported to MMA for the experiment. The mobile system had 4 columns (9.5 cm x 140 cm) for each treatment and could simultaneously expose bivalves to three different CO_2 enrichment levels by mixing different ratios of air and CO_2 using mass-flow controllers (Aalborg Instruments and Controls, Orangeburg, NY, USA). Mass-flow controllers were set for three different CO_2 levels (low, medium, and high CO_2 enrichment) prior to scallops being added to the system; however, because the system enriched ambient seawater, there was some natural variability in the carbonate chemistry.

The CO_2 enriched water from each of the columns flowed continuously into three replicate experimental tanks (43.2 cm x 36.8 cm x 25.1 cm, total volume = 38L) with racks (40.6 cm x 30.5 cm x 12.7cm) inserted into the tanks to keep the scallops off the bottom. Each tank was bubbled with CO_2 enriched air to maintain a set CO_2 enrichment. All tanks had an outflow rate of ~ 1,500 ml min^{-1}. As described in Pousse et al. [20], a complete column rotation of CO_2 levels occurred between each sampling point to avoid a potential column effect. During this procedure, tanks were cleaned. The system also included the ability to supplement natural phytoplankton to the containers with cultured microalgae using a variable-speed peristaltic pump Golander BT100F-1, with head DG10-12 (Goldander, Norcross, GA). A 50/50 (volume:volume) mixture of *Chaetoceros neogracile* (Chaet-B) and *Tetraselmis chui* (PLY429), grown in local seawater enriched with Guillard's F/2 nutrients, was delivered to each tank at 2ml min^{-1}. The supplementation of algal food ensured that they received at least 4% of mean dry weight and were not food limited [37].

Physico-chemical measurements

Daily water variables (temperature, salinity) were measured with a YSI probe (Model 556, Yellowspring, OH USA). Chlorophyll-a (chl-a) was monitored every 15 minutes in incoming

water with a YSI EXO2. In addition to chl-a, to ensure the effects of harmful phytoplankton were not complicating the experiment, twice a week throughout the experiment, a 100-mL sample of incoming water was collected and preserved (3% Lugol's solution) to determine the dominant phytoplankton species/taxa in the seston with special attention to harmful phytoplankton. Phytoplankton were identified with a Zeiss Observer Z1 inverted microscope (Jena, Germany).

For carbonate chemistry analysis, seawater samples were taken weekly to biweekly in dark, polypropylene bottles (500 ml) from each tank outflow (n = 12) for dissolved inorganic carbon (DIC) and pH measurements. An Apollo SciTech DIC analyzer (Apollo SciTech, LLC, Newark, DE) was used to analyze samples for DIC, which has a precision of 0.5% of assigned values in an interlaboratory comparison [38]. During analysis, certified reference material was within ±2.7 μmol kg^{-1} (n = 32) of the reported value. A UV-VIS spectrophotometer (Cary100, Agilent, Santa Clara, CA, USA) was used to determine pH (seawater scale) colorimetrically at 20˚C with m-cresol purple indicator dye (Sigma-Aldrich, St. Louise, MS, [28]. To ensure accuracy, a tris-buffer was used ±0.0014 (n = 12). DIC and pH were used in CO2SYS [39] for the calculation of $pH_{in\ situ}$, ρCO_2 (μatm), calcite, and aragonite using the following constants: K1, K2 [40]; potassium sulfate [41]; and boron [42].

Physiological rates

All physiological rates measured or calculated for this experiment are summarized in Table 1. The biodeposition method was used to determine sea scallop feeding rates [20,43]. This method uses the inorganic matter in seston as a tracer in the food source, feces, and pseudofeces to calculate: CR, organic ingestion rate (OIR), (assimilation rate) AR, and AE (Table 1). In the biodeposition apparatus, each of the three, separate CO_2 enriched header tanks dispensed water to 9 individual chambers (460 mm^2) at a flow rate of 200 mL min^{-1}. Of those 9 chambers, 2 were used as controls (empty shells), and the remaining 7 each held a sea scallop from the corresponding CO_2 enrichment. Scallops acclimated to the system for 12 hours prior to the collection of water samples and feces/pseudofeces. Water samples for total suspended matter (TSM), particulate inorganic matter (PIM), and particulate organic matter (POM) were

Table 1. List of acronyms, meanings and units of all measurements or calculations on Atlantic sea scallop, *Placopecten magellanicus*, during an 8-week exposure to CO_2 enrichment.

Acronym	Meaning	Unit	Acquisition
CR	Clearance rate	l h^{-1}	Measured
OIR	Organic ingestion rate	mg h^{-1}	Measured
AR	Assimilation rate	mg h^{-1}	Measured
AE	Assimilation efficiency	-	Measured
RR	Respiration rate	mg O_2 h^{-1}	Measured
ER	Excretion rate	μg N h^{-1}	Measured
O:N	Atomic oxygen to nitrogen ratio	-	Calculated
A	Assimilated energy	J h^{-1}	Calculated
U+R	Energy used for catabolic processes	J h^{-1}	Calculated
SFG	Scope for growth	J h^{-1}	Calculated
DTW	Dry tissue weight	g	Measured
DSW	Dry shell weight	g	Measured
TSM	Total suspended matter	mg l^{-1}	Measured
PIM	Particulate inorganic matter	mg l^{-1}	Measured
POM	Particulate organic matter	mg l^{-1}	Measured

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collected every 20 minutes for approximately 3 hours and filtered on pre-weighed, pre-combusted (450˚C for 4 hours) GF/C filters which were rinsed with isotonic ammonium formate after filtration. Data of TSM, PIM, and POM for each sampling date were similar to each other, except for 11/19, which showed lower POM, and 12/2 that had higher TSM (S1 Fig). Feces and pseudofeces were collected separately until enough material was collected to weigh (~4 to 5 h for sea scallops) and offset from water collection by the gut transit time (~60 minutes, calculated during all biodeposits sampling days). These samples were filtered on pre-weighed, pre-combusted (450˚C for 4 hours) GF/C filters and rinsed with isotonic ammonium formate. All filters were frozen until processing. Total weight and organic/inorganic fraction of each filter were calculated by drying the filters (60˚C) until constant weight (~ 5 days) and then combusting at 450˚C for 4 hours and weighing. Shell height (mm) was recorded, and scallops were shucked immediately for dry tissue weight and dry shell weight. Only a part of the December 18 samples (9 of 21) for CR were available for analysis because the rest of those samples were compromised (storage malfunction) during the COVID-19 pandemic.

The same individuals used in biodeposition were used to calculate RR and ER. Scallops first were starved in 0.35-μm-filtered seawater at appropriate CO_2 enrichment for 24 hours so that basal metabolic rates for respiration (RR) and excretion (ER) could be measured; then placed in individual, closed chambers (68 ml minus biovolume of animal) filled with 0.35-μm-filtered seawater treated at appropriate CO_2 enrichment. An eight-channel Loligo respirometry system (Viborg, Denmark) was used to measure RR on 7 individuals with 1 chamber as the control (empty shell). RR was calculated from a linear regression applied to oxygen depletion with the background noise from the control chamber subtracted out. To ensure that scallops were not stressed from low oxygen concentrations in the chamber, recordings were stopped once oxygen saturation reached 80%.

At the end of RR recordings, ER samples were taken by removing 5 mL from each chamber for ammonia-N determination. HACH TNT-830 test kits (equivalent EPA 350.1, EPA 351.1, and EPA 351.2) were used to determine ammonia-N with a modified protocol detailed in Pousse et al. (15). Briefly, absorbance was measured at 690 nm after 3.5 h to allow for color development. The method had a detection limit of 0.02 mg L^{-1}, and a low-nutrient seawater reference standard (Sigma- Simple Nutrients in Seawater) was within ± 0.01 mg L^{-1} of the reported value. Individual ER was calculated using the active time observed for each individual (i.e., period when the scallop was respiring) and was determined with the following equation:

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

where $C_{NH4\text{-}N}$ was the concentration of ammonia-N in the respiration chamber (μg N mL^{-1}), V_c was the volume of respiration chamber (mL), V_{sc} was the volume of individual (mL), t_{ea} was the effective active time (h).

Standardization of physiological rates. All physiological rates were weight-standardized to a 0.10-g individual in dried tissue weight (W_s; mean weight of the experiment) to allow for comparison of rates for different-sized individuals. The following equation was used to standardize all physiological rates

$$\mathrm{Y_w} = (\mathrm{W_s}/\mathrm{W_e})^{\mathrm{b}}\ \mathrm{Y_e} \quad (1)$$

where, Y_w was the weight-standardized physiological rate, Y_e was the experimentally measured rate, W_e was the dry tissue mass, and b was the calculated power value that scales physiological rates to body weight [44].

The b for CR, RR, and ER was calculated with the following equation $Y_e = aW^b$ applied on temperature-corrected physiological rates as detailed in Pousse et al. [20] with Arrhenius

parameters obtained from the "add-my-pet" collection https://www.bio.vu.nl/thb/deb/deblab/add_my_pet/. For sea scallops, the b value was 0.68, 0.71 and 0.40 for CR, RR, and ER respectively (S2 Fig).

Scope for growth

Rates measured during biodeposition (CR and AE), RR, and ER were used to calculate SFG–the energy available for growth. All measurements were converted to energy equivalents (Joules, J,) and calculated from the equation:

$$SFG = (A) - (R + U) \quad (2)$$

with A assimilated energy (J h^{-1}), R energy catabolized through respiration (J h^{-1}), and U excreted energy (J h^{-1}, [19]). RR was converted to oxygen-catabolized energy (R) using 14.0 J mg^{-1} O_2, ER converted to U using 24.83 J mg^{-1} NH_4-N [45]. The absorbed energy (A, J h^{-1}) calculated as $A = CR^{*}AE^{*}\Delta_{food}$, with CR (L h^{-1}) and AE (no unit) obtained from the biodeposition method and Δ_{food} the energetic content of the food (J mg^{-1}) obtained from Δ_{food} = (0.632+0.086 (% C))×4.184, where %C was the organic matter of the seston [46]. The O:N ratio was calculated to measure the balance between the breakdown of proteins and the catabolism of carbohydrate and lipids as the atomic ratio of RR to ER.

Statistical analysis

Statistical analysis was performed using the statistical software R version 4.0.3 (R Foundation for Statistical Computing, Vienna; http://www.r-project.org). The data did not meet the assumptions of normality and homoscedasticity, so non-parametric, robust methods described by Wilcox [47] were employed using R source library version 39 (downloaded from https://dornsife.usc.edu/labs/rwilcox/fotware/). Before any analyses of the physiological values were made, one-factor, non-parametric analysis on the physico-chemical measurements was performed to ensure the following: 1.) there was no significant difference in salinity between CO_2 enrichment treatments; 2.) that the change in temperature and algae concentration were the same between the three CO_2 enrichments; and 3.) that each of the three CO_2 enrichment treatments were different.

Physiological measurements were analyzed using two factors, CO_2 level and temperature, with robust statistical methods. Statistical analysis based on 20%-trimmed means was used for all data analysis to overcome some of the Type I errors that can occur when statistical assumptions (normality and homoscedasticity) are not met [47,48]. Trimmed means do not have to meet the standard assumption of normality or homoscedasticity and will minimize influence of outliers without bias by excluding a predetermined percentage of the most extreme observations. Trimmed mean comparisons have higher power relative to traditional mean comparisons [47,49]. If there was an interaction between CO_2 level and temperature, pairwise comparisons were also run.

Robust ANCOVA methods based upon parametric regression models were used to fit the growth data (shell height, DTW, DSW) for the 8-week experiment at each CO_2 level. In contrast to classic ANCOVA, using robust ANCOVA methods does not assume that the regression lines were parallel, homoscedastic, and normally distributed [47,50]. The slope of each growth variable relative to CO_2 level was compared using a percentile bootstrap method, with the Theil-sen regression estimator to generate a predictive equation. This method compares well to traditional ANCOVA without creating Type I errors [47,50]. In addition, the tests can compare between each time point to determine if there was a significant difference in size between each CO_2 level. P-values ≤ 0.05 were considered statistically significant for all analyses.

Results

Experimental conditions

Mortality was low during the 8-week experiment with only one individual in the low CO_2 enrichment dying within the first 2 weeks of the experiment, which may have been related to moving the animals.

Salinity varied from 30.17 to 31.58 (Fig 1A), while temperature varied from a high of 15.6˚C (start of the experiment) to a low of 5.8˚C (end of the experiment, Fig 1B). There was no significant difference in salinity ($p = 0.81$) or the drop in temperature ($p = 0.15$) between each of the three CO_2 levels. The system delivered a constant level of CO_2 enrichment with the mass flow controllers (see above); however, because ambient seawater CO_2 varies naturally, there was variation in the carbonate variables during the experiment. Measured pH for the experiment ranged from 7.7545 to 7.8199, 7.5661 to 7.7283, and 7.3954 to 7.6039 for the low, medium, and high CO_2 enrichment levels, respectively (Fig 1C). There was a significant difference in measured pH ($p<0.01$); therefore, the means for the carbonate chemistry of the 3 enrichment treatments were reported (Table 2). The DIC concentration for the low-CO_2 enrichment ranged from 1933.26 μmol kg^{-1} to 2058.46 μmol kg^{-1}, while the medium-CO_2 enrichment ranged from 1982.70 μmol kg^{-1} to 2138.59 μmol kg^{-1}, and the high-CO_2 enrichment ranged from 2045.18 μmol kg^{-1} to 2173.13 μmol kg^{-1} (Fig 1D). As with pH, there was a significant difference in DIC between the three CO_2 enrichment levels ($p< 0.01$). The rest of the carbonate system variables (pH^*, ρCO_2, calcite, and aragonite) were calculated at the *in situ* temperature. The calculated pH^* varied from 7.8703 to 7.9730 for the low-, 7.6657 to 7.9170 for the medium-, and 7.4700 to 7.7377 for the high-CO_2 enrichment (Fig 1E), with a significant difference between treatments (Table 2). Calculated CO_2 (μatm) ranged from a low of 435 μatm to a high of 583 μatm for the low-CO_2 enrichment, while the medium ranged from 499 μatm to 1,001 μatm and the high ranged from 813 μatm to 1,161 μatm, with a significant difference between treatments (Fig 1F, Table 2). With respect to calcite, the low ranged from 2.38 to 2.00, the medium from 1.29 to 1.88, and the high from 0.91 to 1.44 for each CO_2 enrichment level (Fig 1G). There was a significant difference ($p<0.01$) in calcite between the three enrichment levels (Table 2). The aragonite levels varied from 1.26 to 1.52 for the low treatment, 0.81 to 1.17 for the medium treatment, and 0.57 to 0.91 for the high treatment of CO_2 (Fig 1H), with a significant difference between the three treatment levels ($p<0.01$, Table 2).

During the 8-week sampling period, there was no significant difference in plankton concentration between each CO_2 treatment ($p = 0.08$). In addition to the supplemented algae, the following diatoms were found in the incoming seawater: *Guinardia delicatula*, *Licmophora* spp., *Navicula* spp., *Rhizosolenia setigera*, *Skeletonema costatum*, *Thalassionema nitzschioides*, and *Thalassiosira* spp. No harmful algae were identified throughout the experiment. Incoming seawater had a mean chl-a of 4.44 ± 0.02 μg/L (standard error), while the supplemented algae concentration varied between 31,087 to 48,043 cell/mL.

Physiological measurements

CR was significantly different between experimental treatments of CO_2 ($p = 0.02$, Fig 2A) and temperature ($p<0.01$, Fig 2E), with no interaction between temperature and CO_2 (Table 3 $p = 0.13$). CR ranged from a low of 0.18 l h^{-1} (high-CO_2 enrichment, 7.4˚C) to a high of 0.64 l h^{-1} (low-CO_2 enrichment, 9.4˚C). For the main effect of CO_2, the low-CO_2 enrichment was significantly higher than CR at high-CO_2 enrichment. The medium-CO_2 enrichment was similar to the other two CO_2 enrichments. For temperature, CR showed two distinct homogenous groups with higher CR at 9.4˚C and 13.1˚C compared to 6.1˚C and 7.4˚C.

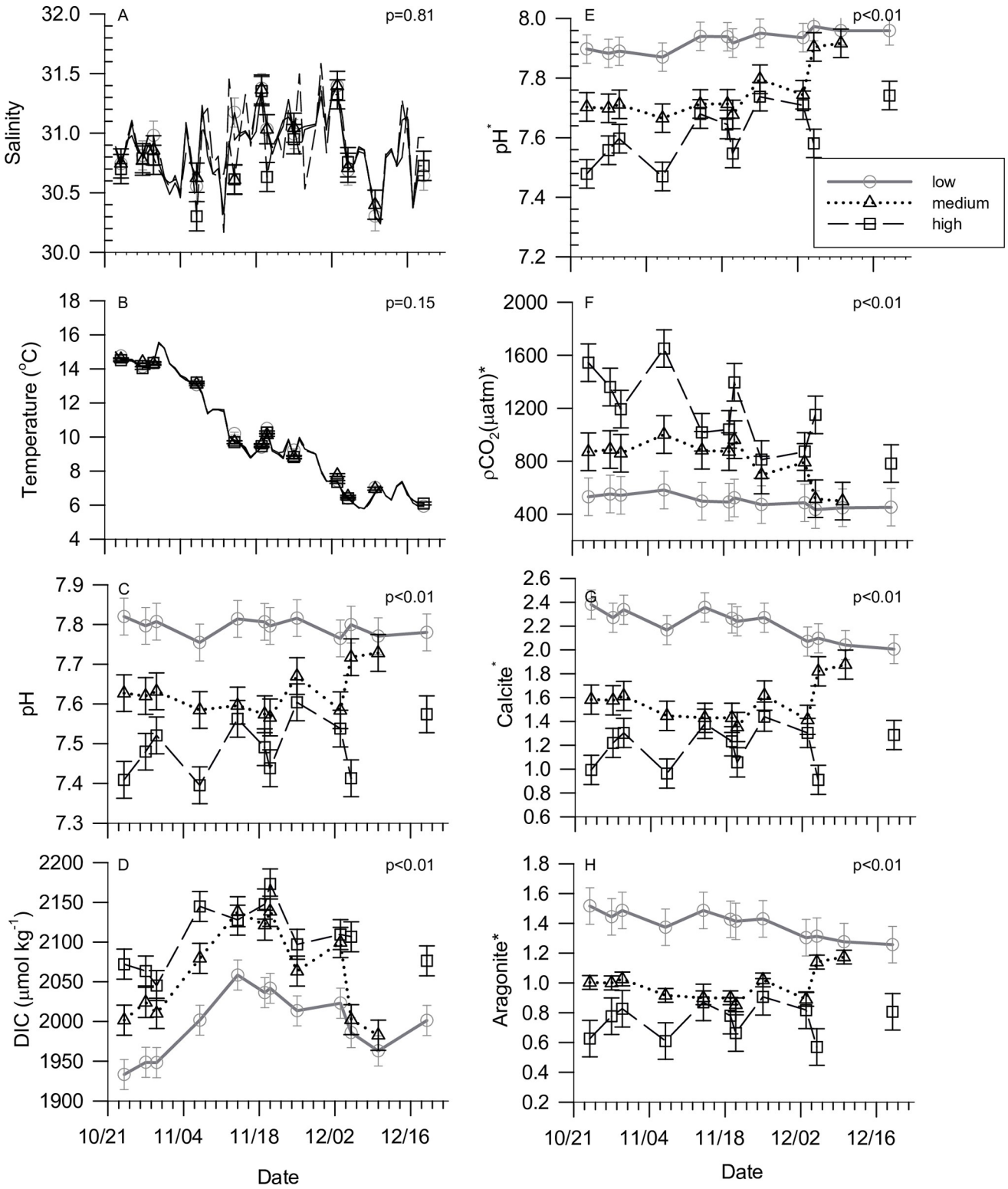

Fig 1. Experimental carbonate chemistry for Atlantic sea scallops, *Placopecten magellanicus*, during an 8-week experiment where seawater was enriched with CO_2 (low, medium, or high). Reported means ± standard error of measured (A) salinity, (B) temperature (˚C), (C) dissolved inorganic carbon (DIC, µmol kg^{-1}) and (D) pH (at 20˚C, seawater scale) in the experimental tanks (N = 3 per CO_2 enrichment). The carbonate variables (E) pH^* (*in situ* pH), (F) ρCO_2^* (µatm), (G) calcite*, and (H) aragonite* were calculated at measured temperature using CO2SYS with the constants set as Lueker et al. [40], Dickson [41], and boron value from Lee et al. [32]. The circle solid line was low-CO_2 enrichment, the triangle dotted line was medium-CO_2 enrichment, and the square dash line was high-CO_2 enrichment.

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Table 2. Mean value and standard error of carbonate chemistry for Atlantic sea scallops, *Placopecten magellanicus*, for the entire 8-week experiment for each CO_2 enrichment (low, medium, or high). Measured values were pH (at 20˚C, seawater scale) and dissolved inorganic carbon (DIC, μmol kg^{-1}). Parameters that were calculated using CO2SYS include pH^* at *in situ* temperature (seawater scale), ρCO_2, calcite and aragonite. The following constants were used in CO2SYS: K1, K2 [40], potassium sulfate [41]; and boron [42]. Superscript numbers indicate significantly different groups (p-values <0.05).

Treatment	pH	DIC (μmol kg^{-1})	pH^*	ρCO_2 (μatm)	Calcite	Aragonite
Low	7.7939 ± 0.0036^1	1996.25 ± 6.69^1	7.9262 ± 0.0055^1	501 ± 7^1	2.21 ± 0.02^1	1.39 ± 0.01^1
Medium	7.6229 ± 0.0109^2	2061.47 ± 9.30^2	7.7491 ± 0.0147^2	802 ± 29^2	1.54 ± 0.03^2	0.97 ± 0.02^2
high	7.4842 ± 0.0212^3	2104.95 ± 9.80^3	7.6125 ± 0.0236^3	1164 ± 72^3	1.18 ± 0.05^3	0.74 ± 0.03^3

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Mean OIR was significantly different between CO_2 enrichment ($p = 0.04$) and temperature ($p<0.01$) conditions (Fig 2B and Fig 2F, respectively), with no interaction between the two ($p = 0.78$, Table 3). OIR varied from a low of 0.11 mg h^{-1} (high-CO_2 enrichment, 6.1˚C) to a high of 0.29 mg h^{-1} (low-CO_2 enrichment, 7.4˚C). As with CR, the OIR at low-CO_2 enrichment was significantly higher than OIR at high-CO_2 enrichment, with the OIR at medium-CO_2 enrichment having a similar rate as both the low and high-CO_2 levels. For temperature, OIR increased with temperature with the highest at 13.1˚C and the lowest at 6.1˚C.

As with CR and OIR, AR was significantly different between CO_2 enrichment levels ($p = 0.02$) and temperature ($p<0.01$) treatments (Fig 2C and Fig 2G, respectively), with no interaction between the two ($p = 0.14$, Table 3). AR ranged from a low of 0.01 mg h^{-1} (high-CO_2 enrichment, 6.1˚C) to a high of 0.13 mg h^{-1} (low-CO_2 enrichment, 7.4˚C). For the main effect of CO_2, AR was higher at low-CO_2 enrichment compared to medium- and high-CO_2 enrichment. For temperature, the lowest AR was at 6.1˚C, but there was no difference in AR at the other three temperatures.

AE ranged from 0.07 (medium-CO_2 enrichment, 6.1˚C) to a high of 0.49 (low-CO_2 enrichment, 9.4˚C Fig 2D). For AE there was a significant effect of CO_2 enrichment ($p = 0.01$, Fig 2D), with AE having a U shaped response. At low-CO_2 enrichment there was higher efficiency than at medium-CO_2 enrichment, while the high-CO_2 enrichment behaved similarly to the other two enrichment levels for AE. There also was an effect of temperature ($p<0.01$, Fig 2H), with AE having an inverted U response with the maximum at 9.4˚C. There was a significant interaction between CO_2 and temperature ($p<0.01$, Table 3). At the low-CO_2 treatment, AE had an inverse U-shaped response with the highest AE for 7.4˚C and 9.4˚C. For the medium and high-CO_2 enrichment, the increase in AE was delayed until the temperature reached 9.4˚C.

Concerning metabolic activities, RR varied from 0.020 mg O_2 h^{-1} (high-CO_2 enrichment, 6.1˚C) to a high of 0.056 mg O_2 h^{-1} (low-CO_2 enrichment 13.1˚C). There was no significant CO_2 effect upon RR ($p = 0.07$, Fig 3A) but a significant effect of temperature ($p<0.01$, Fig 3D), with an increase in RR as temperature increased. Interaction between CO_2 and temperature for RR showed no significance ($p = 0.64$, Table 3). ER ranged from 1.31 μg N h^{-1} (low-CO_2 enrichment, 7.4˚C) to 5.52 μg N h^{-1} (low-CO_2 enrichment, 13.1˚C) with no significant effect induced by the CO_2 enrichment (Fig 3B). There was a significant effect of temperature upon ER ($p<0.01$, Fig 3E), which showed a J-shape response with ER the lowest at 7.4˚C. Unlike RR, there was a significant interaction between CO_2 enrichment and temperature on ER ($p<0.01$, Table 3). For low-CO_2 enrichment, ER decreased at 7.4˚C, but then it was consistent between the other 3 temperatures. For medium-CO_2 and high-CO_2 enrichment, ER increased with temperature (Table 3). As with RR and ER, there was no significant effect of CO_2 upon the O:N ratio ($p = 0.41$, Fig 3C); however, temperature had a significant effect upon O:N ($p<0.01$, Fig 3F), showing an inverted U-shape response, with 7.4˚C for the highest O:N ratio. There was an interactive effect of CO_2 and temperature upon O:N ($p = 0.04$, Table 3). At medium

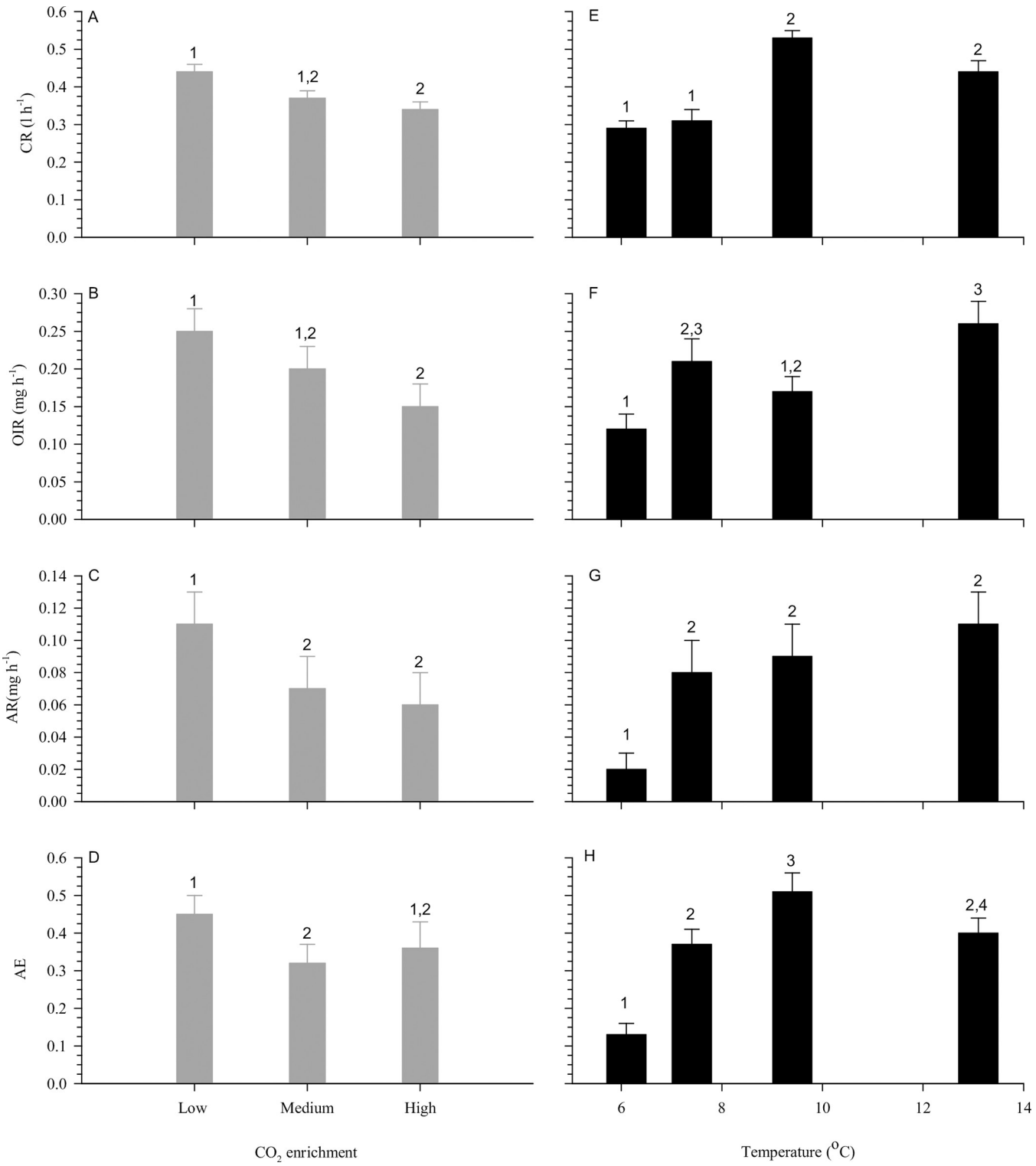

Fig 2. Feeding rates for Atlantic sea scallops, Atlantic sea scallops, *Placopecten magellanicus*. The main effect with respect to CO_2 for (A) clearance rate (CR, l hr^{-1}), (B) organic ingestion rate (OIR, mg h^{-1}), (C) assimilation rate (AR, mg h^{-1}), and (D) assimilation efficiency (AE), while the main effects of temperature were (E) CR, (F) OIR, (G) AR, and (H) AE. The numbers indicate significant differences in the measured values between each treatment level ($p<0.05$).

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Table 3. Physiological measurements for Atlantic sea scallops, Atlantic sea scallops, *Placopecten magellanicus*. Clearance rate (CR, l h^{-1}), organic ingestion rate (OIR, mg h^{-1}), assimilation rate (AR, mg h^{-1}), and assimilation efficiency (AE), respiration rate (RR, mg O_2 h^{-1}), excretion rate (ER, μg N h^{-1}), O:N ratio, assimilated energy (A, J h^{-1}), total catabolic energy (R+U, J h^{-1}), and scope for growth (SFG, J h^{-1}) measured on Atlantic sea scallops every other week during an eight-week exposure to different CO_2 treatments (low, medium, and high). If the p-value was <0.05, an interaction was detected between temperature and CO_2 enrichment. Superscripts indicate different groups within the CO_2 enrichment level when there was a significant interaction.

Biological Rates	Temperature (˚C)				Interaction p value
	6.1	7.4	9.4	13.1	
CR (l h^{-1})					0.13
low	0.42 ± 0.02	0.39±0.03	0.64 ± 0.03	0.46 ± 0.03	
medium	0.24 ± 0.02	0.30±0.03	0.58 ± 0.03	0.42 ± 0.03	
high	0.24 ± 0.02	0.18 ±0.03	0.46 ± 0.03	0.47 ± 0.03	
OIR (mg h^{-1})					0.78
low	0.16 ± 0.05	0.29±0.06	0.22± 0.06	0.28 ± 0.06	
medium	0.12± 0.05	0.20±0.06	0.16 ± 0.06	0.25 ± 0.06	
high	0.11 ± 0.05	0.13 ±0.06	0.14 ± 0.06	0.27 ± 0.06	
AR (mg h^{-1})					
low	0.05 ± 0.03	0.13±0.04	0.11 ± 0.04	0.12 ± 0.04	0.14
medium	0.01 ± 0.03	0.06±0.04	0.10 ± 0.04	0.10 ± 0.04	
high	0.01 ± 0.03	0.04 ±0.04	0.13 ± 0.04	0.13 ± 0.04	
AE					p< 0.01
low	0.33 ± 0.02[1]	0.49 ± 0.02[2]	0.49 ± 0.02[2]	0.46 ± 0.021[1,3]	
medium	0.07 ± 0.02[1]	0.28 ± 0.02[1,2]	0.45 ± 0.02[2]	0.37 ± 0.02[2]	
high	0.08 ± 0.02[1]	0.33 ± 0.02[1]	0.48 ± 0.02[2]	0.39 ± 0.02[2]	
RR (mg O_2 h^{-1})					0.64
low	0.022 ± 0.006	0.026±0.009	0.036 ± 0.008	0.056 ± 0.008	
medium	0.022 ± 0.007	0.025±0.008	0.024 ± 0.009	0.040 ± 0.009	
high	0.020 ± 0.007	0.024 ±0.008	0.026 ± 0.009	0.046 ± 0.009	
ER (μg N h^{-1})					<0.01
low	2.86 ± 0.55[1]	1.31±0.73[2]	2.61 ± 0.72[1]	5.52 ± 1.29[1]	
medium	2.99 ± 0.30[1]	1.46±0.54[2]	2.28 ± 0.87[1,2]	3.79 ± 0.73[1,3]	
high	1.65 ± 0.58[1]	1.82 ±0.72[1,2]	3.72 ± 0.42[3]	4.64 ± 0.49[2,3]	
O:N					0.04
low	6.83 ± 2.80[1]	17.42±3.18[2]	11.05± 3.14[3]	7.44 ± 3.37[1]	
medium	6.47 ± 4.37	14.82±4.06	10.03 ± 2.57	8.11 ± 2.84	
high	9.89 ± 3.95	9.94 ±3.61	8.30 ± 2.78	8.95 ± 4.94	
A (J h^{-1})					0.12
low	2.03 ± 0.05	2.44±0.06	4.34 ± 0.08	3.21 ± 0.09	
medium	0.31 ± 0.04	1.23±0.06	3.14 ± 0.06	2.72 ± 0.03	
high	0.31 ± 0.03	0.68 ±0.08	2.45 ± 0.09	3.86 ± 0.09	
R+U (J h^{-1})					0.43
low	0.39 ± 0.17	0.38±0.11	0.49 ± 0.17	0.91 ± 0.19	
medium	0.38 ± 0.13	0.39±0.15	0.47 ± 0.00	0.64 ± 0.12	
high	0.26 ± 0.10	0.38 ±0.12	0.53 ± 0.12	0.69 ± 0.13	
SFG (J h^{-1})					0.12
low	1.65 ± 0.03	2.04±0.43	3.75 ± 0.29	2.29 ± 0.34	
medium	0.00 ± 0.02	0.65±0.40	2.75 ± 0.28	1.79 ± 0.20	
high	-0.01 ± 0.03	0.47 ±0.40	1.36 ± 0.27	3.16 ± 0.33	

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and high CO_2 enrichment, the O:N was the same at each of the temperatures, but at the low CO_2 enrichment the inverted U-shaped response was still observed.

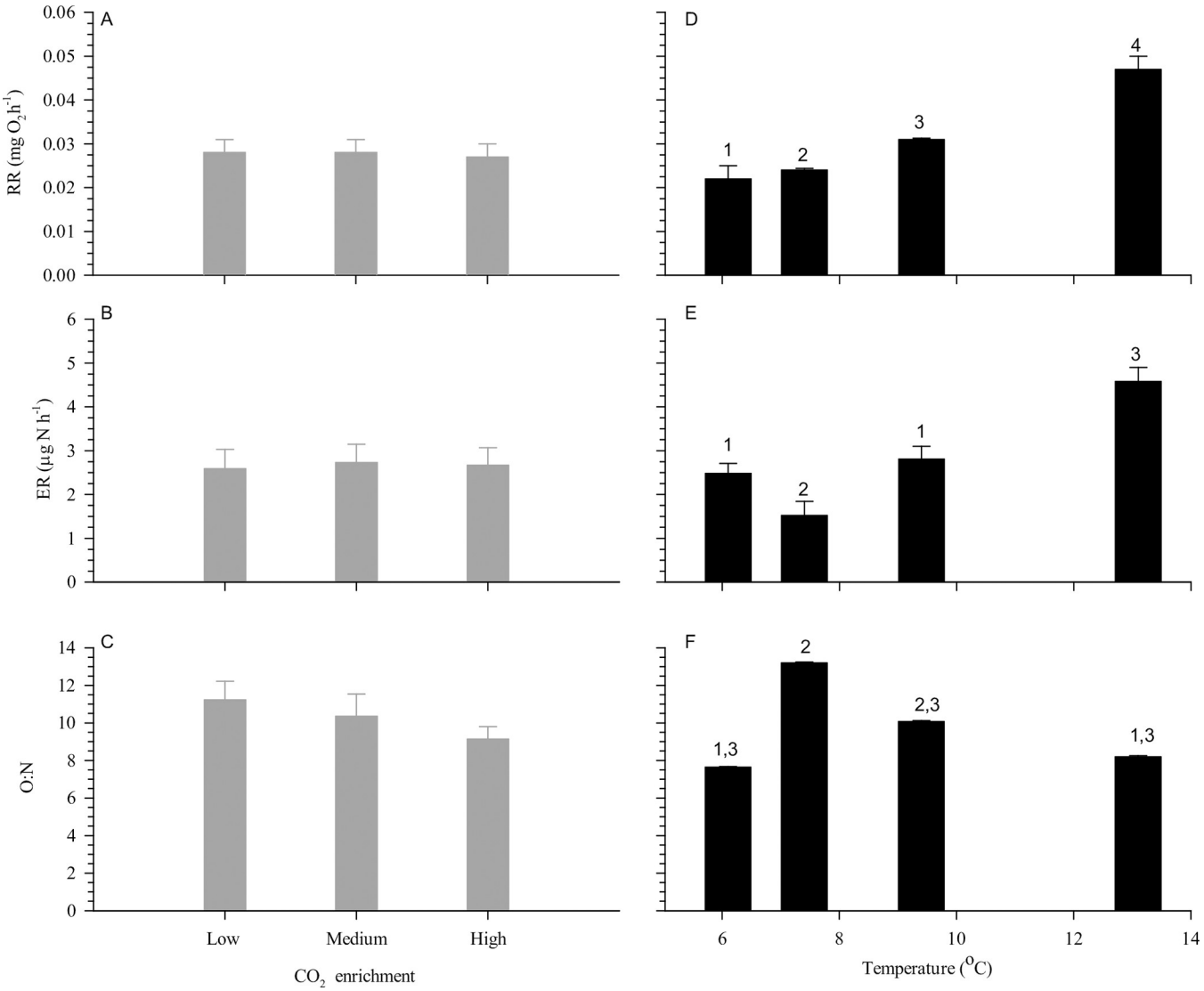

Fig 3. Respiration rate (RR, mg O_2 h^{-1}), excretion rate (ER µg N h^{-1}), and oxygen:nitrogen ratio (O:N) for Atlantic sea scallops, *Placopecten magellanicus*. The main effect with respect to CO_2 for (A) RR, (B) ER, (C) O:N, while the main effects of temperature for (D) RR, (E) ER, and (F) O:N. The numbers indicate significant differences in the measured values between each treatment level ($p<0.05$).

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The A energy for sea scallops ranged from a high of 4.34 J h^{-1} (low-CO_2 enrichment, 9.4˚C) to a low of 0.31 J h^{-1} (high-CO_2 enrichment, 6.1˚C). A significant effect of CO_2 enrichment ($p = 0.04$, Fig 4A) as well as temperature ($p<0.01$, Fig 4D) was observed on A. As CO_2 increased, the amount of A decreased. For temperature, A was double at temperatures 9.4˚C and 13.1˚C, relative to the values at 6.1˚C and 7.5˚C. There was no interaction between CO_2 and temperature ($p = 0.12$, Table 3).

The amount of energy for catabolic processes (R+U) ranged from a high of 0.91 J h^{-1} (low-CO_2 enrichment, 13.1˚C) to a low of 0.26 J h^{-1} (high-CO_2 enrichment, 6.1˚C). The amount of catabolic energy did not change with CO_2 ($p = 0.37$, Fig 4B), but increased significantly with warming ($p<0.01$, Fig 4E). Those two factors did not show interactive effects ($p = 0.43$, Table 3).

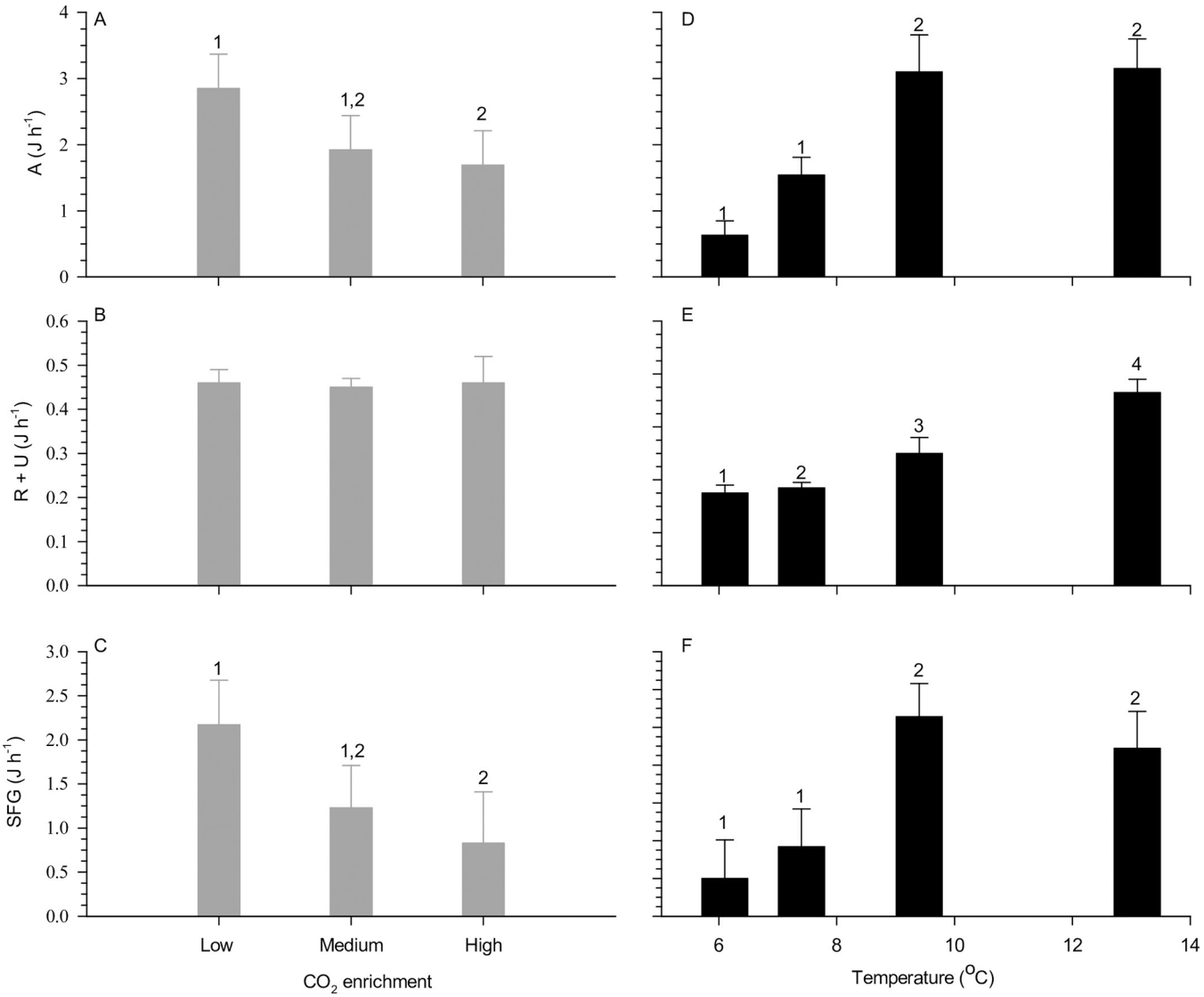

Fig 4. Energetic energy for Atlantic sea scallops *Placopecten magellanicus*. The main effect with respect to CO_2 for (A) assimilated energy (A, J h^{-1}), (B) total catabolic energy (R+U, J h^{-1}), and (C) scope for growth (SFG, J h^{-1}), while the main effects of temperature for (D) A, (E) R+U, and (F) SFG. The numbers indicate significant differences in the measured values between each treatment level ($p<0.05$).

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SFG varied from a high of 3.75 J h^{-1} (low- CO_2 enrichment, 9.4˚C) to a low of -0.01 J h^{-1} (high-CO_2 enrichment, 6.1˚C Table 3). As CO_2 increased, the SFG decreased ($p = 0.03$, Fig 4C), with the medium-CO_2 enrichment behaving similarly to low-and high-CO_2 enrichments. Temperature significantly affected SFG ($p<0.01$, Fig 4F), with higher SFG at 9.4˚C and 13.1˚C relative to the lower temperatures. There was no interaction between CO_2 and temperature ($p = 0.12$, Table 3).

Growth

At the experiment start, sea scallops were 20.81 ± 0.09 mm in shell height, 0.3764± 0.006 g in DSW and 0.0532 ± 0.005 g in DTW. Shell size increased during the 8-week exposure at all three CO_2 enrichment levels (Table 4). There was a significant difference in shell height

Table 4. Means and standard error of daily growth rates of Atlantic sea scallops, *Placopecten magellanicus* over an 8-week exposure to different CO_2 enrichment levels (low, medium, and high). Shell height (mm) was measured from the umbo to the outer shell margin, dried tissue weight (DTW, g) and dried shell weight (DSW, g) were determined by rinsing, shucking the animal, and drying tissues and shells in an oven at 60˚C until constant weight was achieved. The superscript numbers indicate significant differences in the measured values between each treatment level ($p<0.05$).

	Treatment			
	low	**medium**	**high**	**P value**
Height (mm d^{-1})	0.096± 0.030[1,2]	0.099± 0.030[1]	0.088±0.030[2]	p = 0.03
DTW (g d^{-1})	0.0010±0.0001	0.0010±0.0001	0.0009±0.0001	p = 0.10
DSW (g d^{-1})	0.0047±0.0001[1]	0.0048±0.0002[1]	0.0042±0.0002[2]	p = 0.02

https://doi.org/10.1371/journal.pclm.0000142.t004

increase between CO_2 treatments, with the medium-CO_2 enrichment having the largest net growth of 0.099± 0.030 mm d^{-1} ($p = 0.03$). The low- and high-CO_2 enrichments resulted in similar growth rates of 0.096 ± 0.030 mm d^{-1} and 0.088 ± 0.020 mm d^{-1}, respectively. Net DTW growth throughout the experiment revealed no significant difference between the CO_2 enrichments ($p = 0.10$). On the contrary, there was a significant difference in net DSW daily growth rates ($p = 0.02$), with the low-CO_2 enrichment and medium-CO_2 enrichments having higher rates of 0.0047 ± 0.001 g d^{-1} and 0.0048 ± 0.002 g d^{-1}, respectively. The high-CO_2 enrichment had the lowest DSW growth (0.0042 ± 0.0002 g d^{-1}).

Discussion

Investigating physiological rates during OA experiments at CO_2 enrichments levels with natural temperature changes can provide information about how sea scallops are coping with changing environments. The salinities and temperatures experienced during the experiment for all three CO_2 enrichment levels were well within the tolerances of sea scallops [1]. With respect to CO_2, the low and medium levels were representative of concentrations that scallops currently experience in current habitat [7–9]; whereas, the high level was more representative of levels predicted between 2050–2100 [10,11]. Increased CO_2 enrichment resulted in less energy available for growth (lower SFG), which was reflected in shell height and DSW growth rates. The laboratory growth rates of the present study were comparable to growth rates measured in the field for Atlantic sea scallops [51,52], indicating that experimental conditions were comparable to the natural environment. At the levels investigated in this study, increased CO_2 affected only feeding variables (CR, OIR, AR, and AE), but warming affected all biological processes measured (CR, OIR, AR, AE, RR, and ER) for sea scallops. The ability of scallops to vary CR, OIR, AR, AE, RR, and ER with temperature changes has been well documented [53,54]; however, the effect of OA on feeding is still poorly understood but the significant differences in CR and OIR between low- and high-CO_2 enrichments suggest that feeding suppression may be occurring within this range. The lack of interaction between CO_2 and temperature levels relative to most of the energetic rates measured suggests that those physiological responses rely upon independent metabolic pathways. The data presented above highlights that sea scallops may have limited ability to regulate physiology to compensate for OA and that the physiological strategies to optimize energy when temperature varies may change with OA.

The metabolic costs of feeding are small [55]; however, the ability to alter rates related to feeding can greatly change energy intake. In this study, increased CO_2 and temperature caused Atlantic sea scallops to vary multiple feeding variables (CR, OIR, AR, and AE). Recent research has demonstrated that increased CO_2 may decrease CR of different bivalve species. For blue mussels, *M. edulis*, a decrease in CR was observed at 976 μatm [56], but in the mussel, *Perumytilus purpuratus*, CR decreased between 15 to 70% at concentrations > 900 μatm relative to

current conditions [57]. The results presented here are consistent with these findings, with a 22% decrease in CR at the high CO_2 level (1164 μatm). With respect to temperature, CR typically will increase until the optimal temperature of the organism is reached, above which filtration is expected to decrease sharply and cease as temperature approaches the organism's upper temperature limit [58]. The change in CR with temperature presented here was consistent with previously measured CR in sea scallops [53,54]. Furthermore, Frenette [53] recorded the highest CR at 8.0˚C, which was comparable to our results with the highest CR recorded at 9.4˚C. The movement of water enabling CR relies upon gill ciliary activity, suggesting that the neuroendocrine system, which controls cilia movement, might be physiologically shifting ciliary activity to respond to environmental changes in CO_2 and temperature. The lack of interaction between CO_2 and temperature also suggests that the biochemical pathways responding to ρCO_2 and temperature might be different. Further experiments would be needed to test this hypothesis at wider ranges of CO_2 and temperatures to explore if this lack of interaction is maintained throughout the thermal tolerance range of sea scallops and at other CO_2 levels.

Catecholamines have roles associated with neurotransmitters, neuromodulators, and hormones involved in maintaining the allostasis of an organism [59] and responding to stress conditions [60]. Evidence suggests that these compounds play important roles in responding to abiotic (e.g., high temperature, salinity, oxygen variability) and biotic (e.g., bacterial) changes in the environment [61]. Specifically, both serotonin (5-HT) and dopamine (DA) receptors located in the gills of bivalves are thought to regulate ciliary activity [61,62]. Serotonin has been linked to increased ciliary movement [63], but dopamine has been linked to decreased ciliary movement [64,65]. The observed changes in CR suggest that the pumping rate–volume of water moving through the sea scallop–decreases under high CO_2 but increases with temperature. Dopamine receptors in bivalves inhibit the production of adenylyl cyclase (AC) [66,67] which, once produced, acts as a metabolic sensor regulating ATP production and maintaining acid-base balance by sensing bicarbonate ions [68]. As CO_2 increases in seawater, the observed decrease in CR suggests that dopamine receptors may be activated, slowing cilia beat and decreasing the production of AC. In contrast to the OA response, CR in this study increased with temperature. The observed response is consistent with research that suggests that serotonin levels increase at higher temperatures, resulting in more water movement (higher CR) [69]. Further research on how these receptors respond to environmental change would provide confirming insight into the mechanisms by which this species may adapt to climate change.

In addition to the feeding functions CR/OIR, AE also can be altered according to CO_2 and temperature conditions to maintain nutritional requirements necessary for growth. In bivalves, a trade-off between food ingestion and AE allows energy intake to be maintained through a continuum between two strategies: rate (high OIR, relatively low AE) *versus* yield (low OIR, high AE) [70]. With respect to CO_2, it appears that sea scallops were not able to increase AE when OIR was lower and *vice versa*. This behavior was also observed for *M. gallo-provincialis*, which decreased AE with increasing CO_2 [71]. With respect to temperature, as OIR increased, a decrease in AE resulted in a constant AR for all temperatures but for 6.1˚C, which may be because this is approaching the lower temperature range at which Atlantic sea scallops actively feed [72]. With respect to the interaction between CO_2 enrichment and temperature, at low CO_2 enrichment, an inverse U shape response for AE was observed, but as CO_2 increased, the inverse U shape collapsed (Table 3). With low CO_2 enrichment, AE was higher at 7.4˚C than 6.1˚C, but this increase in AE was delayed until 9.4˚C at the highest CO_2 enrichment. This suggests that the thermal performance of AE for sea scallops may be reduce with OA. Even though it was beyond the scope of this manuscript, a plausible explanation for this observed change in AE is that the combined effect of CO_2 and temperature may be

changing activity of digestive enzymes for sea scallops. Under OA, there was an observed change in metabolic substrate from carbohydrates to protein for *Chiamys farreri* [73] and *M. coruscus* [74,75], resulting in changes in digestive enzymes to protein catabolism [73–75]. Digestive enzymes have also been observed to change under different temperatures in terms of how they process lipids, fatty acids, and proteins [75,76], with protein catabolism occurring near the temperature tolerance thresholds of an organism [77]. For *M. coruscus*, the combined effect of OA and temperature had a more important effect on changing digestive enzymes than when each was changed singularly [75]. Even though this experiment did not investigate digestive enzyme activity, the ER and O:N ratio may provide insight into whether or not metabolic processes may have changed from carbohydrate to protein metabolism.

In the present study, the interaction between CO_2 enrichment and temperature in the ER data suggests that temperature does not affect ammonia excretion for sea scallops the same way at the three CO_2 enrichment levels. ER measures the waste product derived from protein catabolism through nitrogen and oxidative reactions [78,79]. As a result of this interaction, O:N ratio that is calculated from ER also showed an interaction between CO_2 and temperature. In the Atlantic sea scallop, under normal diet and temperature, the O:N ratio has been reported to vary from 12 to 20, with pure protein catabolism at an O:N ratio of 8 [17]. At low-CO_2 enrichment and temperatures of 7.4 and 9.4˚C, the O:N ratios were >10 and significantly different than the other two temperatures (Table 3) suggesting that protein catabolism was minor at these temperatures; however, at 6.1˚C and 13.1˚C the O:N ratio was < 9. Pectinid in general have lower O:N ratios than other bivalves because they favor protein as a source of energy [54]. Pectinid are able to swim with 60% of the energy demands coming from protein degradation [80], partially explaining the lower O:N ratio relative to other bivalve species (*M. edulis* are considered stressed when O:N ratios are lower than 30 with tissue growth periods around 50; [81]). The food (TSM, PIM, and POM, S1 Fig) was similar among temperatures suggesting that diet was most likely not causing the low O:N ratios at 6.1˚C and 13.1˚C and indicating that more protein catabolism was likely occurring. At medium- and high-CO_2 enrichment, the O:N ratios were consistently similar to each other and near or <10 regardless of the temperature, further suggesting that protein catabolism was occurring continuously. Lower O:N ratios with increased CO_2 have been observed for *Spisula solidissima* [20], *M. edulis* [82], and *Tegillarca granosa* [78]. For oxidative metabolism, 1 g of protein requires 0.94 L of oxygen, but lipids and carbohydrates require 2.04 L for the same mass of lipids. Furthermore, when lipids and carbohydrates are used as energy sources, CO_2 is produced in larger quantities, which may not be favorable in a high-CO_2 environment. Under high ρCO_2 conditions protein catabolism provides a pool of amino acids that contribute to intracellular osmoregulation in bivalves [83], and produces ammonia waste that can be used to buffer intracellular pH [84]. By favoring proteins, the energy yield is 23.5 kJ g^{-1} as compared to 36.4 kJ g^{-1} and 17.2 kJ g^{-1} for lipids and carbohydrates, respectively [85]. The interaction between AE, ER, and O:N suggests that sea scallop may be changing metabolic substrates and digestive enzyme activity to higher protein catabolism under OA. The shift to protein metabolism may help with energy shortage in a short period of time, but the long term effects of increased protein metabolism at higher CO_2 should be further investigated, including how swimming ability may be impaired, as sea scallops rely upon protein degradation to swim.

Concerning growth markers, this 8-week experiment showed different results between shell and tissue measurements. Significant results attributable to OA were visible only on shell height and DSW, but not in DTW. This observation appears counterintuitive, as, under typical environmental conditions, shell weight is less variable than tissue weight that changes with reproductive and/or somatic tissues growth or weight loss when trophic conditions are poor; however, with sufficient food, as in this study, the decrease in growth may be attributed to CO_2

enrichment. Net shell growth being a combination of $CaCO_3$ deposition and dissolution, the high CO_2 that induces direct (increased dissolution) and indirect (less energy available; [86]) effects contribute to reduced shell growth. In comparison, tissue growth is altered only by the OA effect on bioenergetics, which explains why the effect on shell may be observed before the effect on soft tissues. Nevertheless, at a longer time scale, soft tissues can be expected to be also affected by OA as described for *C. gigas* [87] and *Spisula solidissima* [20].

Sea scallop habitat from the Georges Bank (GB) to the Mid-Atlantic Bight (MAB) has been experiencing rapid warming [12–14] and acidification [8,9], resulting in more days during which sea scallops are exposed to higher temperature and enriched CO_2 water. The MAB has higher temperatures, with less acidification, while GB has lower temperatures with higher acidification. Recent SFG modeling of sea scallop suggests that the MAB population may be more susceptible to temperature variations than the GB populations [3]. The results presented here concur with these findings that increased temperature may be a major driver of sea scallop growth in the MAB; however, these results suggest that CO_2 concentrations should also be considered. Incorporating the results of this study into individual based models such as those relying on dynamic energy budget theory, or scope for growth models can help address how scallop population dynamics may change over time under climate change.

The effects of temperature and OA upon calcification rate, survival, extrapallial fluid carbonate chemistry, and respiration rates in sea scallops were recently reported [88]. Some of our findings presented here are not consistent with those of reported [88] which may be attributable at least partially to experimental conditions and sampling regime that led to high mortality and no growth of individuals exposed to a calcite saturation index less than 2.5 and temperatures between 6–12˚C. Their results also found no difference in respiration with temperature, which is contrary to the results here and previous research with sea scallops [53,54]. We did not observe any differences in survival between CO_2 treatments or with temperature change (only 1 individual died within the first 2 weeks of this experiment) and our results showed net growth (Table 4), similar to what has been observed in the field [52]. Overall, although age and time exposure to temperature and OA treatments may contribute, differences in the results presented here and those of Cameron et al. [88] may be attributable in large part to differences in the trophic environment at which sea scallops have been exposed to. Our scallops had water flow rates 10x higher than their treatments, were continuously fed at a higher quantity of phytoplankton (food was not limiting)- which raises the question about the effect of food quantity/quality on sea scallops responses-, were exposed to OA and temperatures for a shorter durations, and this study used juveniles, not adults. Even though there are stark differences between our results and those of Cameron et al. [88], there was a consistency with DSW decreasing with increasing CO_2. This suggests that even with short-term exposures between 805 μatm and 1168 μatm, shell properties may change quickly under OA conditions.

Changes in bivalve feeding have been shown to vary with the food quality and quantity [89,90], and with changes in salinity [91]. For this experiment, changes in salinity were minor (Fig 1, 30.29 to 31.40) and food was *ad libitum*; however, we acknowledge that any slight variation may have some effect on physiological feeding measurements. Even though the seawater delivered in the experimental tanks was supplemented with a controlled amount of algae, variations in TSM or in the microalgal composition of the raw seawater may have affected the feeding behavior of scallops. Nevertheless, the effect of TSM would be limited, as this variable was different only one day (third physiological rate measurement at the temperature of 7.4˚C). Even with these limitations, the data provide useful physiological information about how sea scallops respond to the changing environments in which they live.

This study presents some of the first results on Atlantic sea scallop physiological responses to increased CO_2 under different temperatures during a season. Our results suggest that

Atlantic sea scallops bioenergetics will be affected by OA levels projected for the next century with lower energy intake. To what extent this change will affect growth and reproduction at a full life cycle and what could be the consequences for population dynamics and ecosystem remains to be defined. Bioenergetics models such as those based upon dynamic energy budget theory could provide insight into long-term prospects for this highly valuable fishery when coupled with accurate regional models of carbonate chemistry conditions.

Supporting information

S1 Fig. Boxplots of (A) total suspended matter (TSM, mg L^{-1}), (B) particulate inorganic matter (PIM, mg L^{-1}), and (C) particulate organic matter (POM, mg L^{-1}) measured during biodeposition. The temperature was not controlled and varied naturally with the season with temperature measuring 6.1˚C on 12/18/2019, 7.4˚C on 12/2/2019, 9.4˚C on 11/19/2022, and 13.1˚C on 11/6/2019. Different homogeneous groups ($p<0.05$) are indicated by lower case letters using a Kruskal-Wallis test.
(TIF)

S2 Fig. Equation fitting of physiological rates: clearance rate (CR; A, n = 60), respiration rate (RR; B, N = 74) and excretion rate (ER; C, n = 78) for sea scallops, *Placopecten magellanicus*. Rates for sea scallop were standardized at 12˚C. Lines were fitted from $Y_e = aW^b$, with Y_e the temperature corrected physiological rate and W the dry tissue weight (mg) for sea scallop. The power equation and r^2 are reported.
(TIF)

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