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Physiological feeding rates and cilia suppression in blue mussels (*Mytilus edulis)* with increased levels of dissolved carbon dioxide

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ABSTRACT

Gills of marine bivalves, the organs that mediate water flow for feeding and other physiological functions, are exposed to increasing levels of carbon dioxide (CO_2) in seawater, in response to ocean acidification (OA). We examined the effects of elevated dissolved CO_2 upon filtration and feeding behavior of the blue mussel, *Mytilus edulis,* under field conditions and in laboratory studies. We further investigated possible changes in cilia beat function in response to elevated dissolved CO_2. Physiological filtration and feeding variables measured; included clearance, filtration, organic ingestion, and assimilation rates and selection efficiency, which decreased with increasing CO_2. Absorption efficiency was not affected by dissolved CO_2. Cilia beat frequency declined in excised lateral cilia (lc) exposed to increasing CO_2 levels, which appears to account for decreased clearance rates observed in field and laboratory experiments. Our data suggest that under conditions of increased CO_2 blue mussels will experience changes in physiological filtration, feeding rates, and cilia beat function that could have consequences for fitness and performance.

1. Introduction

In molluscs, the gills are responsible for physiological processes including feeding, gas exchange, and reproduction (Carroll and Catapane, 2007; Frank et al., 2015). Cilia located on gill filaments create and control the current that allows water and particles to flow over the gills (Cranford et al., 2011) and are responsible for the capture and handling of food particles (Gallager, 1988; Strathmann and Leise, 1979; Ward et al., 1998; Ward and Shumway, 2004). Mussels contain three types of cilia including frontal cilia (fc), latero-frontal cilia (lfc), and lateral cilia (lc), and each has a specific morphology and function. The lateral cilia (lc) are responsible for creating a water current that facilitates gas exchange, food intake and waste removal (Carroll and Catapane, 2007); whereas, the frontal and latero-frontal cilia move particles along the gill for ingestion or rejection (Owen and McCrae, 1976; Ward and Shumway, 2004).

Regulation of cilia movement and pumping of water through the gills is directed by branchial nerve activity (Babak, 1913), and the blue mussel, *Mytilus edulis,* has been used as a model bivalve species to determine if branchial nerves contain cilioexcitatory fibers (Carroll and Catapane, 2007; Catapane et al., 1978; Chilvers and O'Callaghan, 2000; Jørgensen et al., 1990; Stefano et al., 1977). Previous laboratory studies found physiological evidence that neurotransmitters control lc with the drugs serotonin (5-HT) and dopamine (DA) shown to modify lc beat frequency (Aiello, 1970; Aiello and Guideri, 1964; Carroll and Catapane, 2007; Catapane et al., 1978; Riisgård et al., 2015). Addition of 5-HT resulted in stimulated adenylyl cyclase (AC) activity, which increased the level of cyclic adenosine monophosphate (cAMP), thus increasing ciliary beat frequency. Conversely introduction of DA inhibited AC activity and the production of cAMP, thereby reducing ciliary beat frequency (Murakami, 1987).

The physiological role of AC in generating cAMP in bivalves is unclear (Fabbri and Capuzzo, 2010; Schmid et al., 2007; Tresguerres et al., 2014); however, pathways for production of AC have been shown to be critical in glycogen breakdown, cilia beat frequency and activation, spawning induction, cardiac contractions, reproduction, and stress response (Fabbri and Capuzzo, 2010). Dysregulation of the AC system in blue mussels has been measured to document changes in lc activity under certain environmental conditions, including toxic algal blooms (Gainey Jr and Shumway, 1991), varying calcium concentrations (Murakami, 1987), copper and cadmium toxicity (Fraser et al., 2018; Sunila, 1981), and increased sulfide concentrations (Doeller, 1995). Production of AC is known to be an evolutionarily-conserved mechanism in mammals and non-mammals (i.e., bacteria, phytoplankton, sharks, boney fish, and corals (Chen et al., 2000; Gutowska et al., 2010; Tresguerres et al., 2014; Tresguerres et al., 2007); however, possible

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disruption of bivalve gill cilia beat frequency by the addition of CO_2 to seawater has not been determined (Tresguerres et al., 2014).

Increased atmospheric CO_2 has led to increased carbon dioxide absorption by the world's oceans (Feely et al., 2004). Data from Northeastern United States collected from the Gulf of Mexico to the Gulf of Maine showed a 2.5% increase in dissolved carbon dioxide in seawater from 2007 to 2015 (Wanninkhof et al., 2015). This increase in ocean acidification and potential effects upon marine organisms is a concern, especially for bivalves who have calcium carbonate shells (Dickinson et al., 2013; Gray et al., 2017; Waldbusser et al., 2015). Previous studies have found that dissolved CO_2 concentration was more important than pH or calcium-carbonate saturation in affecting feeding behavior (Vargas et al., 2015; Vargas et al., 2013; Waldbusser et al., 2015), suggesting that changes in ciliary activity may be responsible for the observed feeding changes. Surprisingly little research has addressed carbon dioxide effects upon cilia beat frequency in marine shellfish. If carbon dioxide is affecting cilia beat frequency, one would expect the response to be immediate, i.e., feeding rates should change rapidly in response to changing ρCO_2 in the environment. To test this hypothesis, we conducted short-term laboratory experiments and field measurements with blue mussels, *Mytilus edulis* to determine if increased CO_2 in the environment changed cilia beat rate. Three different experiments were conducted: (1) field measurements of physiological filtration and feeding rates under naturally-varying CO_2 concentrations, (2) laboratory measurements of physiological filtration and feeding rates at two different CO_2 concentrations (low and high), and (3) laboratory-measured beat frequency of cilia on excised mussel gills under a variety of dissolved CO_2 concentrations.

2. Methods

Adult *M. edulis* were collected from Milford, CT (41° 13′ 33.9096″ N, 72° 59′ 26.0046″ W) during May 2017 and May 2018. Field experiments were conducted in 2017, while laboratory experiments were performed in 2018. Cilia beat frequency experiments were done from 2017 to 2018.

2.1. Field experiment

Blue mussels (n = 40) were acclimated for 30 days to seawater from Milford Harbor, CT (41° 12′ 42.46″ N, 73° 3′ 7.75″ W) prior to field measurements. Field measurements were done on the Milford Laboratory dock. Prior to conducting the experiment, field samples for dissolved inorganic carbon (DIC) and pH were collected to measure daily fluctuations in the seawater carbonate chemistry and showed higher values during mid-afternoon and lowest values in the morning. On June 21, 2017, 16 blue mussels were selected haphazardly from the acclimated population, cleaned of encrusting organisms, and placed in biodeposition feeding chambers (described below) to measure filtration and feeding variables. Mussel size ranged from 30 to 38 mm (average = 33.79 ± 0.64 mm).

Based upon daily fluctuations in carbonate chemistry described above, filtration and feeding measurements were conducted twice, once when pH was high (16:00–18:30) and again when pH was low (06:00 to 08:30), using the biodeposition method described below. Carbonate chemistry was sampled every 15 min coincident with biodeposition method sampling. Salinity, temperature, and dissolved oxygen were measured in carbonate chemistry samples using a YSI probe (Model 556, Yellow Spring, OH, USA).

2.2. Laboratory experiment

Blue mussels (n = 30) were acclimated for 30 days to two different ρCO_2 treatment levels (875 ± 18 µatm and 2182 ± 71 µatm, Table 1) with water pumped from Milford Harbor, CT (41° 12′ 42.46″ N, 73° 3′ 7.75″ W) prior to measurement of filtration and feeding rates. The water in the laboratory exposure system was held in a holding tank before carbonate chemistry was manipulated. The CO_2 delivery system has compressed air first passed through a carbon dioxide absorber (Puregas, Broomfield, CO, USA) and then into mass flow controllers (Aalborg Instruments and Controls, Orangeburg, NY, USA), where it was mixed with controlled levels of research grade CO_2 in two PVC columns to produce ρCO_2- enriched water that flowed continuously into the experimental tanks. In addition, CO_2- enriched air was bubbled into each individual tank to maintain targeted CO_2. A full schematic of the system can be found in Perry et al. (2015).

Blue mussels were maintained at a constant temperature similar to the temperature of the field experiment (Table 1). On August 9, 2018, 16 blue mussels were selected haphazardly from each CO_2 treatment, cleaned of encrusting organisms, and placed in biodeposition feeding chambers (described below) to measure filtration and feeding rates. There were two biodeposition chamber systems used, one for each ρCO_2 treatment level. Mussel size ranged from 55.7 to 66.8 mm (average = 61.7 ± 1.3 mm).

Temperature and salinity were taken daily with a YSI probe (Model 556, Yellow Springs, OH, USA) during acclimation and biodeposition determinations. Daily pH and DIC were measured to quantify carbonate chemistry throughout the experiment (n = 15).

2.3. Physiological feeding rates

Blue mussel filtration and feeding rates, including clearance rate (CR), filtration rate (FR), rejection rate (RR), rejection proportion (% RP), selection efficiency (SE), organic ingestion rate (OIR), assimilation rate (AR), and assimilation efficiency (AE) were measured for during both field and laboratory experiments using the biodeposition method (Galimany et al., 2018b; Galimany et al., 2013; Galimany et al., 2017; Iglesias et al., 1998). For the laboratory experiment, two separate CO_2–treated header tanks dispensed water at a rate of 200 ml min^{-1} to 20 individual chambers (460 mm^2). For the field study only one header tank was used. Of the 20 chambers, 2 were used as controls (empty shells), and18 held blue mussels. To determine gut transit time (GTT) blue mussels were fed with a cultured, green alga *Tetraselmis chui* (PLY429), and time for feces color to change from brown to green was determined prior to starting the experiment. The collection of feces and pseudofeces was offset by the GTT and separately collected for each individual. Water from the header tank inflow and control chamber outflows for each treatment were sampled every 15 min for 1.5 h and filtered on pre-weighed, pre-combusted (450 °C for 4 h) GF/C filters. All filters were rinsed with isotonic ammonium formate and frozen until processing. Total weight and organic/inorganic fractions were calculated by drying filters in an oven at 60 °C to constant weight and then combusting at 450 °C for 4 h. Blue mussel shell length was recorded, and mussels were shucked immediately for dry tissue weight.

2.4. Standardization of physiological feeding rates

All physiological filtration and feeding variables were standardized for a 1 g dry tissue weight individual. Physiological rates were standardized using the following equation:

$$Y_w = (W_s/W_e)^b Y_e$$

where, Y_w was the weight-standardized physiological rate, Y_e was the experimentally measured rate, W_e was the dry body mass measured for each mussel, W_s was the standard weight (1.0 g) and b (0.60) was the power value that scales physiological rates to body weight for blue mussels (Bayne and Newell, 1983; Widdows et al., 1979; Widdows and Johnson, 1988).

Table 1
Values of mean pH (total seawater scale), temperature (°C), salinity, bicarbonate ion (HCO_3^-, μmol kg^{-1}) ρCO_2 (μatm), total (DIC) dissolved inorganic carbon (μmol kg^{-1}), $\Omega_{Calcite}$, and $\Omega_{aragonite}$ for laboratory and field experiments. Values represent means ± standard error. Asterisks indicate variables that were directly measured and not calculated using CO2SYS.

Experiment	Temp*	Salinity*	pH measured*	pH *in situ*	DIC	HCO_3^-	ρCO_2	Ω_{Ca}	Ω_{Ca}
Field									
Low ρCO_2	21.7 ± 0.1	24.6 ± 0.2	7.87 ± 0.02	7.85 ± 0.02	1688.55 ± 3.31	1585.67 ± 1.12	569 ± 21	2.18 ± 0.08	1.37 ± 0.05
High ρCO_2	20.5 ± 0.1	24.6 ± 0.2	7.66 ± 0.02	7.66 ± 0.02	1839.41 ± 7.70	1749.43 ± 8.00	976 ± 39	1.46 ± 0.05	0.92 ± 0.03
Lab									
Low ρCO_2	20.9 ± 0.2	25.4 ± 0.2	7.71 ± 0.01	7.70 ± 0.01	1848.34 ± 8.63	1753.58 ± 8.42	875 ± 18	1.70 ± 0.04	1.08 ± 0.03
High ρCO_2	20.9 ± 0.2	25.5 ± 0.2	7.34 ± 0.02	7.33 ± 0.02	1948.75 ± 8.20	1846.32 ± 7.58	2182 ± 71	0.78 ± 0.04	0.50 ± 0.03

2.5. LC cilia beat-rate measurements

Mussels used for cilia beat activity measurements ranged in size from 41.7 to 73.2 mm (average 59.2 ± 0.9 mm). Before removal of the gill, blue mussel health was checked by tactile stimulations. Only those mussels that offered resistance to being opened were used. Cilia were prepared for microscope observation of gill ciliary beating by removing the shell, the mantle, and the internal organs as described in Carroll and Catapane (2007). Excised gills were positioned in CO_2-enriched seawater (see above for enrichment of seawater), then viewed at 40x on an inverted Zeiss Microscope (Axio Observer Z1, Carl Zeiss Microscope, Germany).

We used a high-speed, all-digital video imaging system (Sisson-Ammons Video Analysis (SAVA), Ammons Engineering, Clio, MI, USA) to measure cilia beat frequency (Baxter and Minet, 2013; Navarrette et al., 2012; Sisson et al., 2003). Each gill was exposed sequentially to 2 different ρCO_2- treated water concentrations. Water was pumped over the excised gill in a flow-through chamber slide on the microscope stage using a peristaltic pump (Masterflex C/L Model 77120-52, Cole-Parmer Instrument Co., Vernon Hills, IL, USA), and after exposure lc beat frequency was recorded with SAVA. Then a water sample for DIC and pH was taken. Next the water in the slide was replaced with ρCO_2- enriched water for the next treatment which was allowed to circulate over the gill before cilia beats were measured again. This process was repeated for 30 mussels (n = 60 measurements). This ensured that each gill was exposed to two different levels of ρCO_2-enriched seawater and the change from high to low CO_2 or from low to high CO_2 was haphazardly done.

2.6. Carbonate chemistry sampling

All seawater for carbonate chemistry was collected in dark, polypropylene bottles (500 ml) and analyzed immediately for DIC on an Apollo SciTech DIC analyzer (Apollo SciTech, LLC, Newark, DE, USA). The DIC instrument was part of an international inter-laboratory comparison and measured within 0.5% of assigned values (Bockmon and Dickson, 2015). Colorimetric seawater pH was determined at 20 °C using a metacresol purple indicator dye (Sigma-Aldrich, St. Louise, MS, USA (Dickson and 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 for the calculation of ρCO_2 (μatm), bicarbonate ions (HCO_3^-), $\Omega_{Calcite}$, and $\Omega_{Aragonite}$ (Pierrot et al., 2006).

2.7. Statistics

The field, laboratory, and cilia experimental data were analyzed using Statgraphics Centurion (Statgraphics Technologies, Inc. The Plains, Virginia, USA). The rejection proportion, selection efficiency, and absorption efficiency data were transformed with arcsin (square root (%rejection proportion/100)) before running tests for normality (Zar, 2019). Data were first checked for normality (Shapiro-Wilk test $p > 0.05$), then differences in all filtration and feeding variables were compared with a T-test. Differences were considered significant if $p < 0.05$. For the cilia beat rate data, normality (Shapiro-Wilk test) and the constant variance test ($p > 0.05$) were met before regression analysis was used to determine if there was a significant relationship between cilia beat frequency and ρCO_2 and HCO_3^- concentration. The data means and standard errors were reported.

3. Results

3.1. Carbonate measurements

For the field experiment, the *in situ* pH in the afternoon was 7.85 ± 0.02 (low pCO_2) and in the morning 7.66 ± 0.02 (high pCO_2), with corresponding DIC concentrations of 1688.55 ± 3.31 μmol kg^{-1} and 1839.41 ± 7.70 μmol kg^{-1}, respectively (Table 1). This resulted in a calculated ρCO_2 of 569 ± 21 μatm in the afternoon and 976 ± 39 μatm in the morning. For the laboratory experiment, the low pCO_2 treatment had an *in situ* pH 7.70 ± 0.01, a DIC of 1848.34 ± 8.63 μmol kg^{-1}, and the high treatment measured 7.34 ± 0.02 and 1948.75 ± 8.20 μmol kg^{-1} for pH and DIC, respectively. The calculated ρCO_2 values were 875 ± 18 μatm and 2182 ± 71 μatm for the laboratory experiment. The rest of the environmental variables (T, S, HCO_3^-, $\Omega_{Calcite}$, $\Omega_{Aragonite}$,) are reported in Table 1. There was a significant difference ($p < 0.01$) between low and high CO_2 for both field and laboratory experiments.

3.2. Physiological feeding rates

For the field experiment, no difference in TSM, inorganic, and organic particles was observed between afternoon and morning conditions (Table 2, p = 0.88, 0.67, 0.10 respectively). A significant difference ($p < 0.01$) in CR was detected between low (afternoon) and high (morning) treatments (Table 2). CR was 28% lower in the high pCO_2 treatment compared to the low pCO_2 treatment. A corresponding reduction in FR ($p < 0.02$) was noted, with mussels in the low pCO_2 treatment clearing significantly more particles than those in the high pCO_2 treatment. The SE was significantly different (p = 0.02), indicating that mussels were selecting particles differently between treatments, with ingestion of 31% more organic particles by mussels in the low pCO_2 treatment as compared to the high treatment ($p < 0.01$). The low treatment had a significantly higher RR and RP than the high treatment ($p < 0.01$ and p = 0.04, respectively) The low treatment had a higher AR ($p < 0.01$) than the high pCO_2 treatment, but the AE did not differ between the two treatments (p = 0.07).

In the laboratory study, there was also no significant difference in TSM, inorganic, and organic particles between CO_2 treatments (p = 0.32, p = 0.17, p = 0.89, respectively) indicating that any differences between the low and high pCO_2 treatment were not caused by differences in particle loads (Table 2). The laboratory study observations were similar to those of the field study, with mussels with low pCO_2 having significantly higher CR (p = 0.01), FR (p = 0.02), OIR (p = 0.03), and AR (p = 0.04). In the laboratory study, CR was approximately 34% lower under high CO_2. There was a significant

Table 2
Feeding rate behavior for blue mussel under different CO_2 concentrations in the laboratory and the field. An * indicates a significant difference ($p < 0.01$) between treatment for each experiment. No statistical analysis was done between experiments just within experiments. TSM (total suspended material), inorganic, and organic reported in mg l^{-1}. RR was rejection rate (mg h^{-1}), RP was rejection proportion (%), SE was selection efficiency (a fraction), CR was clearance rate (l h^{-1}), FR was filtration rates (mg h^{-1}), OIR was organic ingestion rate (mg h^{-1}), AR was absorption rate (mg h^{-1}), and AE was absorption efficiency (a fraction). All rates were normalized to 1 g of dry tissue.

Experiment	TSM	Inorganic	Organic	CR	FR	SE	OIR	RR	RP	AR	AE
Field											
Low ρCO_2	12.04 ± 0.91	8.73 ± 0.36	3.16 ± 0.08	4.10 ± 1.13*	49.40 ± 4.07*	0.48 ± 0.01*	10.05 ± 0.86*	28.85 ± 2.30*	49.50 ± 2.37*	7.63 ± 0.67*	0.76 ± 0.01
High ρCO_2	12.12 ± 0.41	8.97 ± 0.42	3.12 ± 0.04	2.95 ± 0.17	35.76 ± 2.03	0.42 ± 0.02	6.89 ± 0.42	15.95 ± 1.21	44.38 ± 1.43	5.07 ± 0.35	0.73 ± 0.03
Laboratory											
Low ρCO_2	3.04 ± 0.38	1.51 ± 0.22	1.56 ± 0.10	1.10 ± 0.07*	3.35 ± 0.21*	0.62 ± 0.12*	1.57 ± 0.15*	0.70 ± 0.20	18.51 ± 3.89	1.08 ± 0.17*	0.69 ± 0.03
High ρCO_2	3.49 ± 0.24	1.93 ± 0.19	1.45 ± 0.23	0.73 ± 0.07	2.28 ± 0.08	0.24 ± 0.04	0.91 ± 0.12	0.54 ± 0.10	18.50 ± 3.9	0.57 ± 0.09	0.63 ± 0.03

difference in SE ($p = 0.04$), with more organic particles selected over inorganic particles based upon the OIR and AR. There was no significant difference in AE ($p = 0.23$).

3.3. Cilia beat frequency

LC beat frequency responded to changes in ρCO_2 concentration (Fig. 1). The calculated ρCO_2 also showed a significant correlation ($r^2 = 0.41$) with lc beat frequency decreasing as CO_2 concentrations increased ($p < 0.01$). The lc beat frequency showed a significant correlation ($r^2 = 0.32$) with HCO_3^- ($p < 0.01$; Fig. 1) with a decrease in cilia beat as calculated HCO_3^- concentrations increased.

4. Discussion

In the environment, feeding is known to be affected by food availability (Rahman et al., 2020; Tenore and Dunstan, 1973), temperature (Beukema et al., 2017; Jørgensen et al., 1990; Riisgård, 1988; Riisgård et al., 2011), salinity (Galimany et al., 2018a; Pourmozaffar et al., 2019), and oxygen concentration (Tang and Riisgård, 2018). Our results indicate that increases in ρCO_2 in the environment also affect feeding rates of marine bivalves. Field and laboratory trials demonstrated that increased dissolved CO_2 depressed filtration and feeding rates of blue mussels (Table 2). Observed changes in feeding rate may be related to two mechanisms revealed by our data: (1) changes in cilia activity, and (2) changes in particle selection. Both mechanisms may be contributing to lower feeding rates in sequence.

Changes in filtration and feeding rates of blue mussels under increased ρCO_2 have been reported previously and linked to changes in seawater viscosity (Riisgård, 1988; Riisgård and Larsen, 2007) as a result of temperature differences in trials (Melzner et al., 2011). In our study, temperature differences were not observed, suggesting that other mechanisms were controlling the observed reductions in filtration and feeding rates.

The decrease in CR in the experiments indicates that the volume of water being moved through blue mussels – referred to as pumping rate – was different between CO_2 treatments. Decreases in CR under OA have been observed for other marine bivalves (Melzner et al., 2011; Vargas et al., 2015; Vargas et al., 2013; Waldbusser et al., 2015), which is consistent with the results presented here. Water movement through the shell is a physical process mediated by the ciliary activity. In addition to the decreased CR in the whole mussel, we observed a decrease in cilia beat frequency in excised gills with increasing ρCO_2. All cells possess mechanisms to sense and respond to levels of ρCO_2 to maintain homeostatic acid/base balance, to adjust metabolism to environmental conditions, and to detect sensory stimuli (Fabbri and Capuzzo, 2010; Franzellitti and Fabbri, 2013; Melzner et al., 2020; Tresguerres et al., 2014). Marine mussels in particular live in rapidly-fluctuating habitats so they must have the ability to tolerate fluctuations, but when necessary also the mechanisms to respond quickly to environmental changes (Bayne and Newell, 1983; Bayne, 1976).

Much research has been applied to understanding physiological controls of ciliary activity in a wide range of organisms. The observed changes in ciliary activity could be a result of detection of ρCO_2 by the neuroendocrine system. The catecholaminergic system (CA) plays an important role in ciliary activity (Beiras and Widdows, 1995; Liu et al., 2018) and can modulate physiological activities through the neuroendocrine system involving G protein metabotropic receptors (GPCR). GPCR are classified into 4 broad classes, which include $G_{\alpha s}$ which activates adenylyl cyclase (AC) and $G_{\alpha i}$ that inhibits AC (Nelson et al., 2018; Pierce et al., 2002). In blue mussels, lc beating rate is controlled by reciprocal, serotonergic-dopaminergic innervations from the cerebral ganglion and visceral ganglia (Carroll and Catapane, 2007; Cochran et al., 2012; Mathieu et al., 2014). As ρCO_2 increases in the environment, there is a corresponding increase in external HCO_3^- and H^+ ions, which would be expected to increase cilia activity because AC

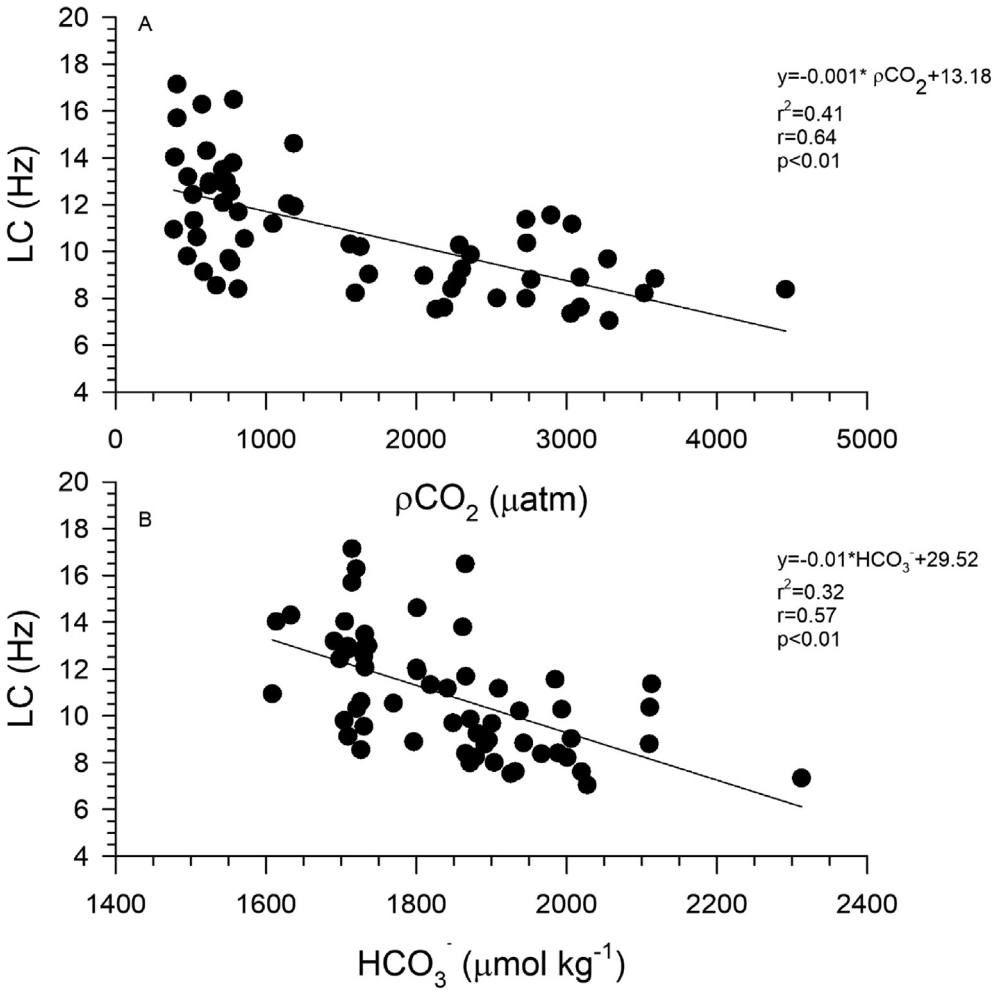

Fig. 1. Ciliary beat frequency of lateral cilia (lc) under different levels of pH, bicarbonate ions (HCO_3^-), and ρCO_2 (µatm).

is directly simulated by increases in HCO_3^- in marine bivalves (Fabbri and Capuzzo, 2010; Franzellitti and Fabbri, 2013; Tresguerres et al., 2014; Tresguerres et al., 2007). Instead, we observed decreasing ciliary activity with increased ρCO_2, and HCO_3^-. This suggests that enzymes within the $G_{\alpha i}$ may be activated. Gamma-aminobutyric acid (GABA) is an important $G_{\alpha i}$ that has been identified in marine bivalves. GABA receptors in bivalves inhibit AC by lowering levels of cAMP (Beaulieu and Gainetdinov, 2011; Neves et al., 2002) which in turn lowers cilia beat frequency (Bardales et al., 2011; Bonini and Nelson, 1988; Schmid et al., 2007). Previous research in marine organisms has demonstrated that maintenance of acid-base balance within the cells under increased CO_2 is accompanied by a reversal in proton gradient from the extra- to the intercellular, with HCO_3^- accumulating in the cell (Melzner et al., 2011; Pörtner et al., 2010; Thomsen and Melzner, 2010). GABA has a specific conductance for HCO_3^- (Clements and Hunt, 2015; Nilsson et al., 2012) and has been shown to function in both the cerebral ganglion and visceral ganglia as a $G_{\alpha i}$ (Cochran et al., 2012; Mathieu et al., 2014). The GABA receptors have already been implicated in OA effects upon other marine organisms, including bivalve settlement on acidic sediments (Clements et al., 2017), predator/prey relationships (Chivers et al., 2014), and olfactory function (Nilsson et al., 2012). The decrease in lc beat frequency suggests that increasing ρCO_2 in the external environment resulted in the activation of GABA receptors to maintain acid/base balance which, in turn, resulted in slower cilia beat frequency, lower pumping rate, and consequently reducing CR.

The suppression of cilia beat rate has been observed in blue mussels under a variety of environment conditions. For example, in blue mussels dopamine activation was shown to occur during exposure to toxic algae, resulting in reduced feeding rates and cilia beat rate (Gainey Jr and Shumway, 1991). High levels of manganese and calcium have also been reported to result in cilia cessation (Nelson et al., 2018; Stommel and Stephens, 1985). The inhibition of AC under increased ρCO_2 is of concern because of the vast array of roles it plays in bivalve physiology, including oogenesis, embryogenesis, development, hormone secretion, olfaction, cardiac contraction, smooth muscle function, and metabolism (Fabbri and Capuzzo, 2010). It is beyond the scope of this study to explore all the consequences of activating GABA receptors under OA; however, this research suggests that OA may be activating the $G_{\alpha i}$ family, therefore those pathways should be explored further. Enzymatic pathways activated under the $G_{\alpha i}$ family need to be further explored to understand how other physiological processes may be affected by OA.

Suppression of gill cilia beat rates and consequent lowering of CR and FR affect every aspect of mussel bioenergetics, as the individual's ability to extract trophic resources from the environment is degraded. This finding is fundamental to modeling and projecting changes in performance of bivalve mollusks in ecosystems experiencing elevated dissolved CO_2 from climate change, as well as changes related to eutrophication and freshwater discharge in estuarine environments. Other physiological and mechanistic changes attributable to CO_2 increase, including effects upon particle selection, which we also observed, must be considered within the context of lowered nutritional input. The changes in particle selection suggest that, in addition to neuroendocrine disruption, there were changes in the mucociliary transport system.

Changes in mucus production in bivalves is one of the primary reactions to chemical changes in the environment (Triebskorn et al., 1996; Wilbur and Saleuddin, 1983)

Our results found that SE for both field and laboratory studies decreased with increased ρCO_2, and in the field samples there was a significant difference in the rejection of particles, RR and RP (Table 2). Mucus serves many purposes in bivalves including lubrication and particle capture, transport, selection, and ingestion (Beninger and Dufour, 2000; Beninger et al., 1997; Beninger et al., 1993; Beninger and St-Jean, 1997). In bivalves, the physical process of ingesting or rejecting particles is correlated with the secretion of mucus (Jørgensen, 1996; Jørgensen et al., 1990). Our data suggest that there may be a changes in mucus production. Mucus production changes under elevated dissolved ρCO_2 have been observed in fish and snails (El-Gendy et al., 2019; Mota et al., 2020; Sveen et al., 2016). The addition of CO_2 to seawater can change seawater conductivity (Millero, 2000; Pawlowicz, 2010; Pawlowicz et al., 2010), which could cause modification of mucus production in bivalves and also seston particle surface properties. The physicochemical surface properties of particles (i.e., charge and wettability) are known to influence feeding selectivity of particles in blue mussels (Beninger et al., 1993; Rosa et al., 2017; Rosa et al., 2013; Ward et al., 1998). Nevertheless, the decreased SE and OIR presented here suggests that there might be changes in the mucociliary transport system and particle physiochemical properties that affect selection. Experiments with bivalves that select particles through hydrodynamics from cilia movement, such as the Eastern oyster, *Crassostrea virginica,* contrasted with those that rely mainly upon the mucocilary transport system could provide further insight into the possible role of changes in particle physiochemical properties with increased CO_2 (Rosa et al., 2017; Rosa et al., 2013; Ward et al., 1994) Further studies on mucus production and particle charge and wettability under increased CO_2 need to be performed.

Changes in feeding rates with changes in CO_2 in laboratory and field studies have been described in several bivalves species (Navarro et al., 2013; Vargas et al., 2015; Vargas et al., 2013). Our results are consistent with previous findings, with a decrease in CR as ρCO_2 increases. This study provides additional information on the potential mechanisms that may be changing feeding behavior. The data presented here implicate two mechanisms contributing to lowered feeding in the presence of elevated dissolved CO_2: (1) ciliary suppression lowers clearance and particle capture, and (2) mucociliary transport system changes the ability of the mussel to detect and sort nutritious particles from the seston. These effects have fundamental bioenergetic implications that must be considered when modeling and projecting bivalve and related ecosystem responses to ocean acidification.

CRediT authorship contribution statement

Shannon L. Meseck: Funding acquisition, Project administration, Supervision, Writing - original draft, Conceptualization, Formal analysis. **George Sennefelder:** Methodology, Resources, Conceptualization, Writing - review & editing. **Melissa Krisak:** Methodology, Writing - review & editing, Data curation. **Gary H. Wikfors:** Funding acquisition, Supervision, Writing - review & editing.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106675.

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