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Full Length Research Paper

Short-term response of flat tree oyster, *Isognomon alatus* to CO_2 acidified seawater in laboratory and field experiments

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Seawater changing chemistry has consequences on coastal ecosystems and their living resources. Future projections suggest the pH could drop ~0.2-0.3 pH units by the year 2100 under a business-as-usual (BAU) CO_2 emission scenario. Marine calcifying organisms such as corals, calcifying algae, crustaceans, mussels, oysters and clams are most likely to be impacted by ocean acidification. The *Isognomon alatus* (flat tree oyster) is an important species that can be negatively affected by the lowering of seawater pH. *Isognomon alatus* is an important food source, a substrate for other benthic organisms (e.g., stone crab, *Menippe mercenaria*) and contribute to nutrients recycling in coastal ecosystems. The study was conducted to test the impacts acidified seawater CO_2 on the growth of *I. alatus* under controlled laboratory conditions as well as field experiment. The *Isognomon alatus* lost weight and experienced negative growth rates of –0.56 ± 0.36 mg $g^{-1}day^{-1}$ under average pH values of 7.8 expected by the end of this century compared to a loss of –0.26 ± 0.23 mg $g^{-1}day^{-1}$ under ambient pH (value 8.1) conditions. In contrast, *I. alatus* incubated in a field experiment showed a gain in weight and positive growth of 3.30 ± 0.23 mg $g^{-1}day^{-1}$ despite exposure to pH levels (~7.4) during low tide significantly lower than those experienced in the laboratory. Overall, the results showed concern on the impacts of acidification flat tree oyster (Bivalvia:Isognomonidae). A decline of calcifying bivalves populations can impact coastal ecosystems function and indirectly affect the human beings that depend on them as a food source.

Key words: Ocean acidification, climate change, Isognomonidae, shell dissolution, bivalve's growth, estuarine.

INTRODUCTION

Anthropogenic carbon dioxide (CO_2) emission and the effect of its accumulation in the atmosphere and uptake by the oceans have raised severe concerns for its consequences to Earth's climate and oceanic ecosystems (IPCC, 2007; Andersson et al., 2008; Cole, 2013; IPCC, 2013; Bates et al., 2014). Atmospheric CO_2 has risen mostly due to the burning of fossil fuel from pre-industrial levels of about 280 ppm to current levels of 385 ppm (Caldeira and Wickett, 2005; Doney et al., 2015; IPCC, 2018, 2019). Atmospheric CO_2 concentration is expected to rise to ~750 ppm by the end of this century under a business-as-usual (BAU.) CO_2 emission scenario

(Schmittner et al., 2008; Cole, 2013; IPCC, 2014; Reith et al., 2019). The ocean acts as a natural carbon sink and absorbed about 25 – 30% of anthropogenic CO_2 (Sabine et al., 2004; Byrne et al., 2010; Byrne, 2011; Ryan et al., 2015). Consequently, the global oceanic surface pH of ~8.1 has declined by 0.1 units compared to pre-industrial pH values (~8.2) (Orr et al., 2005; IPCC, 2007, 2019). Numerical models predict a further drop in the ocean pH by 0.2 – 0.5 units by the year 2100 if CO_2 emissions from human activities continue to increase at present rates (IPCC, 2007; Brierley and Kingsford, 2009; Poloczanska et al., 2016).

Atmospheric carbon dioxide gas (CO_2) rapidly equilibrates with the concentration of CO_2 in the surface ocean (Kleypas et al., 2006; Byrne and Przeslawski, 2013; Fabricius et al., 2014; Doney et al., 2020). Therefore, an increase in atmospheric CO_2 raises the average surface seawater partial pressure of carbon dioxide (pCO_2) (Hofmann et al., 2010; Bijma et al., 2013). Increasing CO_2 dissolve in aqueous solution causes the pH to decrease and affects dissolved carbonaceous species in seawater (Doney et al., 2009; Zeebe, 2012; Pettit et al., 2013). Carbon dioxide gas (CO_2) dissolves in seawater to form carbonic acid, dissociating into protons and bicarbonate ions, thereby causing a decrease in seawater pH and carbonate ion concentrations (Orr et al., 2005; Feely et al., 2009; Orr et al., 2015; Baldry et al., 2020). This phenomenon is known as ocean acidification (McNeila and Matear, 2008; Doney et al., 2015; Melendez and Salisbury, 2017). A decrease in carbonate ion concentration (CO_3^{2-}) leads to decreased saturation states (Ω) of calcium carbonate ($CaCO_3$) minerals such as calcite ($\Omega_{calcite}$) and aragonite($\Omega_{aragonite}$), the two common crystalline components of marine organisms (Fabry et al., 2008; Hofmann et al., 2010; Olischläger and Wild, 2020). A reduction in saturation state (Ω) may suppress the rate of calcification, composition, and dissolution of the calcium carbonate ($CaCO_3$) of marine organisms such as foraminifera (Green et al., 1993; de Moel et al., 2009), corals (Langdon and Atkinson, 2005; Andersson and Gledhill, 2013; Fabricius et al., 2014; Kawahata et al., 2019). calcifying marine algae (El Haïkali et al., 2004; Robbins et al., 2009; Costa et al., 2019), coccolithophores (Riebesell et al., 2000; Dissard et al., 2009; Ridgwell et al., 2009; e Ramos et al., 2010), finfish (Ishimatsu et al., 2008; Lacoue-Labarthe et al., 2009), zooplankton (Wang et al., 2018; Campoy et al., 2020), echinoderms (Miles et al., 2007; McClintock et al. 2011; Ross et al., 2011), sea urchin (Miles et al., 2007; Emerson et al., 2017), and shellfish (Bamber, 1987; Bamber, 1990; Shirayama and Thornton, 2005; Bibby et al., 2008; Talmage and Gobler, 2009; Talmage and Gobler, 2010).

There is a great concern about the impact of ocean acidification on marine calcifying organisms (Gazeau et al., 2007; Comeau et al., 2009; Talmage and Gobler, 2009; Ross et al., 2011; Nguyen and Byrne, 2014; Bindoff et al., 2019). Populations of marine animals could respond negatively to the low pH resulting from rising CO_2 (Pörtner, 2008; Widdicombe and Spicer, 2008; Dupont and Thorndyke, 2009; Findlay et al., 2011) and marine invertebrates (Dupont and Thorndyke, 2009; Yu et al., 2011; Watson et al., 2012). Impact of ocean acidification on marine fauna includes reduced growth rates (Michaelidis et al., 2005; Berge et al., 2006; Gazeau et al., 2007), decreased reproductive success (Bibby et al., 2007; Kurihara et al., 2007; Kurihara et al., 2009; Ross et al., 2011; Olischläger and Wild, 2020) and shell dissolution (Bamber, 1990; Green et al., 1993; Shirayama and Thornton, 2005; Findlay et al., 2011). Another effect of seawater CO_2 acidification includes decreased metabolism (Michaelidis et al., 2005; Talmage and Gobler, 2010; Dissanayake, 2014; Liu et al., 2020), acidification of internal body fluids (Spicer et al., 2007), induced defenses (Bibby et al., 2007), increased susceptibility to infection (Holman et al. 2009; Mukherjee et al. 2013), shell thinning (de Moel et al., 2009), impairment of shell formation (Zhang et al., 2020), and impairment of immune function (Bibby et al., 2008; Mukherjee et al., 2013).

A few studies show marine organisms' short-term exposure to ocean acidification (Fabry et al., 2008; Dissard et al., 2009; Fabry et al., 2008; Talmage and Gobler, 2009). The blue mussel (*Mytilus edulis*) and Pacific oyster (*Crassostrea gigas*) calcification rates are estimated to decrease by 25 and10 %, respectively, following the end of the century, IPCC IS92a B.A.U.Scenario (~740 ppm in 2100) (Gazeau et al., 2007; IPCC, 2007, 2018). Ocean acidification impacts larval development of marine animals such as invertebrates (Kurihara and Shirayama, 2004; Dupont and Thorndyke, 2009), sea urchin (Kurihara and Shirayama, 2004; Martin et al., 2011), fish (Ishimatsu et al., 2008), sea snail (Onitsuka et al., 2014), amphipods (Egilsdottir et al., 2009), decapoda (Dissanayake and Ishimatsu, 2011; Curry, 2020), and gastropods (Bibby et al., 2007; Tagliarolo et al., 2013).

The increased pCO_2 (1500 – 2100 ppm, pH ~ 7.4) of seawater projected to occur by the year 2300 will severely impact the early development of *Crassostrea gigas* (Kurihara et al., 2007; Havenhand and Schlegel, 2009). There was no significant effect on the fertilization success of *Crassostrea gigas* and *Mytilus galloprovincialis* exposed to 2000 ppm CO_2 (~ pH 7.4) treatments

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(Kurihara et al., 2009). There was also no significant effect on the sperm swimming speed, sperm motility, and fertilization kinetics in a population of *Crassostrea gigas* under future ocean acidification levels (−0.35 pH unit change) (Havenhand and Schlegel, 2009).

Isognomon alatus (Gmelin, 1791) (Bivalvia: Isognomonidae), the flat tree oyster, is a sessile intertidal species that attach to hard substrata (Patrick, 1988; Saed et al., 2001; Wilk and Bieler, 2009). This species occurs from North America, Central Florida to Bermuda, the Bahamas, West Indies, Caribbean Central America, the northern coast of South America, and south Brazil (Thomas and Dangeubun, 1994; Abbott and Morris, 1995; Mikelsen and Bieler, 2008; Suarez-Ulloa et al., 2019). It grows on Red mangrove roots (e.g., *Rhizophora mangle*), tidal creek, rocks, and human-made structures (Siung, 1980; Patrick, 1988; Leal et al., 2019).

The study assessed a short time culture of *I. alatus* in manipulated seawater CO_2 concentrations (193 ppm, 390 ppm, and 766 ppm) and field experiment. Coastal ecosystems are most vulnerable to climate change stressors such as ocean acidification. The study's chief objective was to evaluate the impact of ocean acidification on the culture of *I. alatus* in 45 days experiment. The research seeks to understand the effect of lowering pH (~ 7.8 – 7.9) due to the manipulated levels of CO_2 concentration in seawater on the growth of *I. alatus* cultured in laboratory and field experiment with natural pH gradient (8.1 – 7.9) in Mangrove Bay Estuary, Bermuda. Knowledge of the impacts of ocean acidification on marine animals such *I. alatus* is critically important to understand the lowering of ocean pH and its consequences on coastal ecosystems. The short-term response of flat tree oyster is useful for predicting the impacts of climate change stressors such as seawater lowering pH on the bivalve's population to improve our understanding of their survival, mortality, and adaptations. The findings provide chemical, ecological knowledge about the flat tree oyster response to lowering pH to improve management of bivalves in mangrove system.

MATERIALS AND METHODS

Collection of *I. alatus*

Eighty-four specimens of the flat tree oyster, *Isognomon alatus* were randomly collected from attached rocks and mangroves at low tide in Mullet Bay Estuary (32° 22' 30''N, 64° 41' 35''W), St. George, Bermuda on 21st January 2009. Mullet Bay Estuary (an intertidal mudflat) is situated at the Northwestern extension of St Georges Harbour, south of St. Georges Island (Mackenzie et al., 1970; Zablocki et al., 2011). The species (Figure 1a) was first allowed to acclimatize (Figure 1b) to Naess laboratory conditions at the Bermuda Institute of Ocean Science (BIOS). The specimens were placed in a labeled Petri dish. They were then transferred into experimental tanks connected to a continuous seawater flow-through system with its source from Ferry Reach, North Atlantic Ocean, Sargasso Sea, Bermuda. The laboratory and field experiments were conducted from January 2009 to April 2009.

Laboratory experiment

Manipulation of CO_2 acidified seawater for the culture of I. alatus

A seawater flow through the system provided continuous flowing natural seawater into two head tanks (control and altered CO_2) placed at an elevated location above the experimental tanks (Figure 2). Tygon tubes (I.9 m) connected experimental tanks (n = 6) through head tanks, which then supplied continuous seawater at the same flow rate of 60 ml/m with gravitational force into individual tanks. Both head tanks initially had natural seawater (pH 8.1 – 8.2) flowing through them. Pure (100 %) carbon dioxide gas (CO_2) was bubbled into the natural seawater to alter the chemistry in one head tank. Three randomly selected control tanks (control, C1, C2, and C3; n = 3) were held under ambient seawater conditions, and other three tanks, the pH was altered by CO_2 bubbling. The altered pH in acidification treatment tanks (T1, T2, and T3, n = 3) was achieved by adjusting partial pressure of carbon dioxide (pCO_2) using various CO_2 air mixtures (T1 = 193 ppm, T2 = 390 ppm, and T3 = 766 ppm). CO_2 is measured in parts per million (ppm), while pCO_2 is measured parts per million per volume (ppmv). The concentration of CO_2 was measured with Carbon Coulometer. Flow rates of air and concentrated CO_2 gas were controlled at 12.0 ml/min from time to time using adjustable Agilent Flowmeter ADM1000.

Seawater samples were collected from the experimental tanks by filling 200 ml Kimax glass bottles to determine dissolved inorganic carbon (DIC) and total alkalinity (TA). The glass bottles were taped with Teflon tape before sampling to prevent atmospheric equilibration and provide good closure. Seawater samples were preserved with 0.1 ml of saturated mercuric chloride solution ($HgCl_2$) and stored at 4°C until analysis (Huang et al., 2012).

Total alkalinity is quantified with a Gran titration (Gran, 1952) but has been modified for an open-cell titration method quantified with nonlinear least-squares calculation (Dickson, 1981; Bradshaw and Brewer, 1988; Dickson et al., 2003). Dissolved inorganic carbon (DIC) is commonly measured by acidifying a known volume of water to release CO_2, which is then absorbed into an organic base solution and titrated with a coulometric detector (Johnson et al., 1987; Johnson et al., 1993; Cole, 2013) or which is quantified with an infrared detector (Goyet and Snover, 1993; Dickson and Goyet, 1994; DOE, 1994). Dissolved inorganic carbon (DIC) commonly consists of CO_3^{2}, HCO_3, and CO_2 (aq) (Andersson et al., 2008; Yan et al., 2020), is a principal constituent of seawater, and a good tracer of ocean acidification (Bates et al., 2014; Yan et al., 2020).

Total alkalinity (TA) was determined using an open-cell potentiometric acid titration with a precision of 0.04% (~0.8 µmoles kg^{-1}) (Dickson, 1981; Keeling, 1993; DOE, 1994; Bates et al., 1996). Dissolve inorganic carbon (DIC) was analyzed using an infrared AMICA DIC analyzer employing a Li-Cor 6262 NDIR analyzer with a precision of ~0.07% (~1.5 µmoles kg^{-1}) (http://www.bios.edu/Labs/co2lab/research/CO2_instrumentation.html) (Bates et al., 2012). Two junk samples and certified reference materials (CRMs) were run before the measurement of dissolved inorganic carbon and total alkalinity. Certified reference materials (CRMs) have been developed to control the quality of TA and DIC analysis (Dickson et al., 2003, 2007). The dissolved inorganic carbon (DIC) and total alkalinity (TA) were measured relative to certified reference material (CRM) (Dickson et al., 2007). The certified reference materials (CRMs) used in this study for DIC was 2011.39 µmoles kg^{-1} while TA was 2183.10 µmoles kg^{-1}. The DIC analysis accuracy was assessed with the difference between the measured CRM value and the certified CRM value (Huang et al., 2012). Correction is done only for dissolved inorganic carbon readings. The correction factor is obtained as CRM actual minus CRM input readings. The corrected value (thus CRM actual minus CRM input readings) was added to the value obtained for the

Figure 1. (a) Flat tree oyster, *Isognomon alatus* (Gmelin,1791).

Figure 1. (b) Acclimatization of *I. alatus* to Naess laboratory conditions at Bermuda Institute of Ocean Sciences, Bermuda.

Figure 2. A set up of manipulated CO_2 acidified seawater flow-through system at Bermuda Institute of Ocean Science. The experimental tanks (control tanks, n = 3 and acidification tanks, n = 3. Physicochemical parameters in the experimental tanks were measured using YSI 556 Multiple Parameter Handheld Sonde.

dissolved inorganic carbon (DIC) readings recorded by AMICA system to get the final DIC values.

Carbonate parameters such as partial pressure of carbon dioxide (pCO_2), dissolved carbon dioxide [CO_2], bicarbonate [HCO_3^-] and carbonate [CO_3^{2-}] concentrations, pH_{tot} (total H+ scale) were calculated at *in situ* temperature and salinity conditions based on total alkalinity (TA) and dissolved inorganic carbon (DIC) data (Jones et al., 2016) and then computed by adopting the first and second dissociation constants of carbonic acid (Mehrbach et al., 1973; Dickson and Millero, 1987) and calcite ($\Omega_{calcite}$) and aragonite ($\Omega_{aragonite}$) saturation using stoichiometric solubility products for the respective crystalline forms using CO2SYS software (Dickson and Millero, 1987; Orr et al., 2015).

Control tanks were under ambient seawater conditions with usual seawater carbonate chemistry (Table 1). Carbon dioxide gas (CO_2) was bubbled into one head tank to adjust pH; the initial adjusted pH ranged from 7.63 to 7.76. Each acidification treatment tank (T1 – T3) was connected to an appropriate carbon dioxide (CO_2) gas to alter carbonate chemistry (Table 1). However, seawater from Ferry Reach influence daily variation in water chemistry in the experimental tanks (Table 1). The daily changes in pH between the control tanks were pH = 8.1 – 8.2. While in the acidification tanks, adjustable pH was 7.8 – 7.9, about ~0.3 – 0.4 pH units, similar to the expected drop in ocean pH predictions by IPCC BAU IS92a scenario in the year 2100 (Caldeira and Wickett, 2003; Caldeira and Wickett 2005; IPCC, 2007; Findlay et al., 2011; IPCC, 2019). Calibrated YSI 556 Multiple Parameter Handheld Sonde (www.ysi.com) was used to measure physicochemical variables such as temperature, salinity dissolved oxygen concentration and saturation, and pH in the experimental tanks (Figure 2). In the experimental setup, physicochemicals were monitored for several days to ensure stable environmental conditions before transferring *I. alatus* into the tanks. Forty-two specimens of *I.alatus* were transferred and distributed randomly among control tanks (C1, C2, and C3) and acidification tanks (T1, T2, and T3). Each tank contains seven of *I.alatus* individually placed in labeled petri-dish. *I. alatus* shell dimensions (height, length, thickness, and buoyant weight) were determined at the start of the culture and biweekly to monitor shell growth dynamics.

Field experiment

I. alatus was cultured in Mangrove Bay Estuary (32°37'16"N, 64°41'38"W) (Figure 3a). The estuary is situated approximately 100 m east of the Bermuda Institute of Ocean Sciences (BIOS) on the northern shore of Ferry Reach, St. George's Parish, Bermuda

Table 1. Seawater chemistry of experimental tanks in the laboratory set up.

Set-up tanks	Carbonate chemistry				Ocean acidification indicators			Physicochemical	
	DIC (µmoles kg^{-1})	TA (µmoles kg^{-1})	pCO_2 (ppmv)	CO_2 (ppm)	pH	Calcite ($\Omega_{calcite}$)	Aragonite ($\Omega_{aragonite}$)	Temp (^oC)	Sal [PSU]
Control	2087.59	2380.77	357.2	Ambient	8.09	3.18	4.90	18.71	36.73
Acidification									
T1	2065.07	2395.42	306.4	193	8.15	3.55	5.47	18.80	36.84
T2	2087.07	2382.33	358.2	390	8.09	2.69	3.19	18.79	36.87
T3	2140.61	2382.78	464.5	766	7.99	2.69	4.24	18.84	36.85

CO_2 is measured in parts per million (ppm) while pCO_2 is measured parts per million per volume (ppmv). Acidification tanks (T1 – T3); DIC = Dissolved inorganic carbon, TA = total alkalinity; pCO_2 = Partial pressure of carbon dioxide; CO_2 = carbon dioxide. Saturation states of calcite = $\Omega_{calcite}$ and aragonite = $\Omega_{aragonite}$, physicochemical (Temp = temperature; Sal = salinity). PSU = Practical Salinity Unit. Manipulated CO_2 (ppm) = 193 (pre industrial), 358. 2 (current) and 766 (future projected level).

(Mackenzie, 1976). Mangrove Bay Estuary covers an area of 3,350 m^2, an average depth of 1.014017 m with thick strands of red (*Rhizophora mangle*) and black mangroves (*Avicennia nitida*) (Mackenzie, 1976) (Figure 3b). Three stations were randomly selected due to a natural gradient in CO_2 and pH along that transect in Mangrove Bay Estuary (Figure 3b) (Mackenzie, 1976). The stations are station A, inside the bay (32°22'16"N, 64°41'38"W), station B, middle of the bay (32°22'16"N, 64°41'39"W), and station C, the mouth of the bay (32°22'17"N, 64°41'40"W) (Figure 3b–c). Shell dimensions of 42 oysters were measured before placement in the stations (A, B, and C). Each tank contained oysters(n = 7 oysters/ tank). Each station (Figure 3c) is replicated (A1, A2, B1, B2, C1, and C2).

Physicochemical conditions and growth of *I. alatus*

The state of physicochemical in-laboratory and field culture characteristics such as seawater temperature (±0.1°C), salinity (±0.01 PSU), dissolved oxygen saturation and concentration (±0.01 mg L^{-1}), and pH (±0.01 units) were measured using calibrated YSI 556 Multiple Parameter Sonde.

Shell dimensions (height, length, thickness, buoyant weight, and growth) of *I. alatus* were measured before the experiment, at 2–3 weeks intervals, and at the end of the experiments. Shell height (maximum distance between the dorsal hinge and the ventral shell margins), shell length (maximum anterior-posterior distance) (Figure 4a), and thickness of *I. alatus* were measured to the nearest 0.1 mm using a Vernier caliper (Figure 4b).

Growth of *I. alatus* was calculated as either increase or decrease in buoyant weight using the buoyant weighing technique (Figure 4c).*Isognomon alatus* weight was obtained by weighing in a constant seawater temperature (21°C) throughout the experiment using an Adventurer Pro AV53 electronic balance scale (± 0.001 g) placed over a seawater bath. Flat tree oysters were suspended in the water bath from a hook underneath the electronic weighing scale (Figure 4c). A reference weight (stainless steel nut) was used by weighing before each measurement in the air (thus on the pan loader) and seawater to achieve constant air weight and buoyant weight. Growth rate (G) is defined as changes in the total weight of oysters. Growth rate (G) is expressed in per gram oyster per day (mg $g^{-1}day^{-1}$) and calculated using the formula:

$$G = [M_{t+1}-M_t]/ [M_t\times(T_{t+1}- T_t)] \quad (1)$$

Where, M_t and M_{t+1} are the oysters weight (g) at the beginning (T_t) and the end (T_{t+1}) of each growth interval.

Statistics

Growth of *I. alatus* in the laboratory (thus controls and acidified tanks) and field culture were subjected to student t-test and one-way analysis of variance (ANOVA), respectively using the Statistical Package for Social Sciences (SPSS) version 12.0 (Kinnear and Gray, 1999). The strength of the relationship between two shell morphometrics parameters established via power regression analysis using Excel Spread Sheet (Yadav, 2018).

Data archive

The research data are stored at the PANGAEA repository. The DOI: 10.1594/PANGAEA.921678.

RESULTS

Physicochemical conditions

Laboratory experiment

In experimental tanks, physicochemical conditions were not constant but fluctuated over the study period (Figure 5a–d). The average temperature progressively increased in the experimental tanks throughout the duration of the study, with occasional drops in temperature associated with

Figure 3. (a). A map of Mangrove Bay Estuary, Bermuda.

cold fronts moving over Bermuda. The seawater temperature in the tanks ranged from 16.5 to 20.7°C, with no significant differences between control and acidification tanks (Figure 5a). Salinity ranged from 36.3 to 36.8 (PS.U.) in control and acidification tanks (Figure 5b). Unexpectedly, from 11 to 18th March 2009, average salinity was slightly higher in acidification tanks than in control tanks due to reduced seawater flow rate and a higher evaporation rate because Tygon tubes that supplied seawater were partially blocked with organic particles. After cleaning the tubes, there was no observed difference in salinity in experimental tanks.

Dissolved oxygen concentration ranged from 4.8 to 6.5 mg L^{-1} in the control tanks and 4.8 to 6.6 mg L^{-1} in the acidification tanks (Figure 5c). Dissolved oxygen saturation (%) did not differ among the experimental tank and ranged from 63.4 to 88.8% (Figure 5d). Seawater pH ranged from 8.0 to 8.3 in the control tanks and 7.7 to 8.0 in the acidification tanks (Figure 5d). The control and acidification tanks maintained a relatively constant separation in pH levels (cultured at different levels of CO_2) throughout the experiment. There is no variation in the measured physicochemical variables in the experimental tanks such as temperature, salinity, dissolved oxygen, except pH (Figure 5a–c; Table 2). Average pH values (± standard deviation) in control tanks (8.10 ± 0.10) varied significantly ($p < 0.05$) from pH in acidification treatment tanks (7.80 ± 0.10) (Figure 5d;

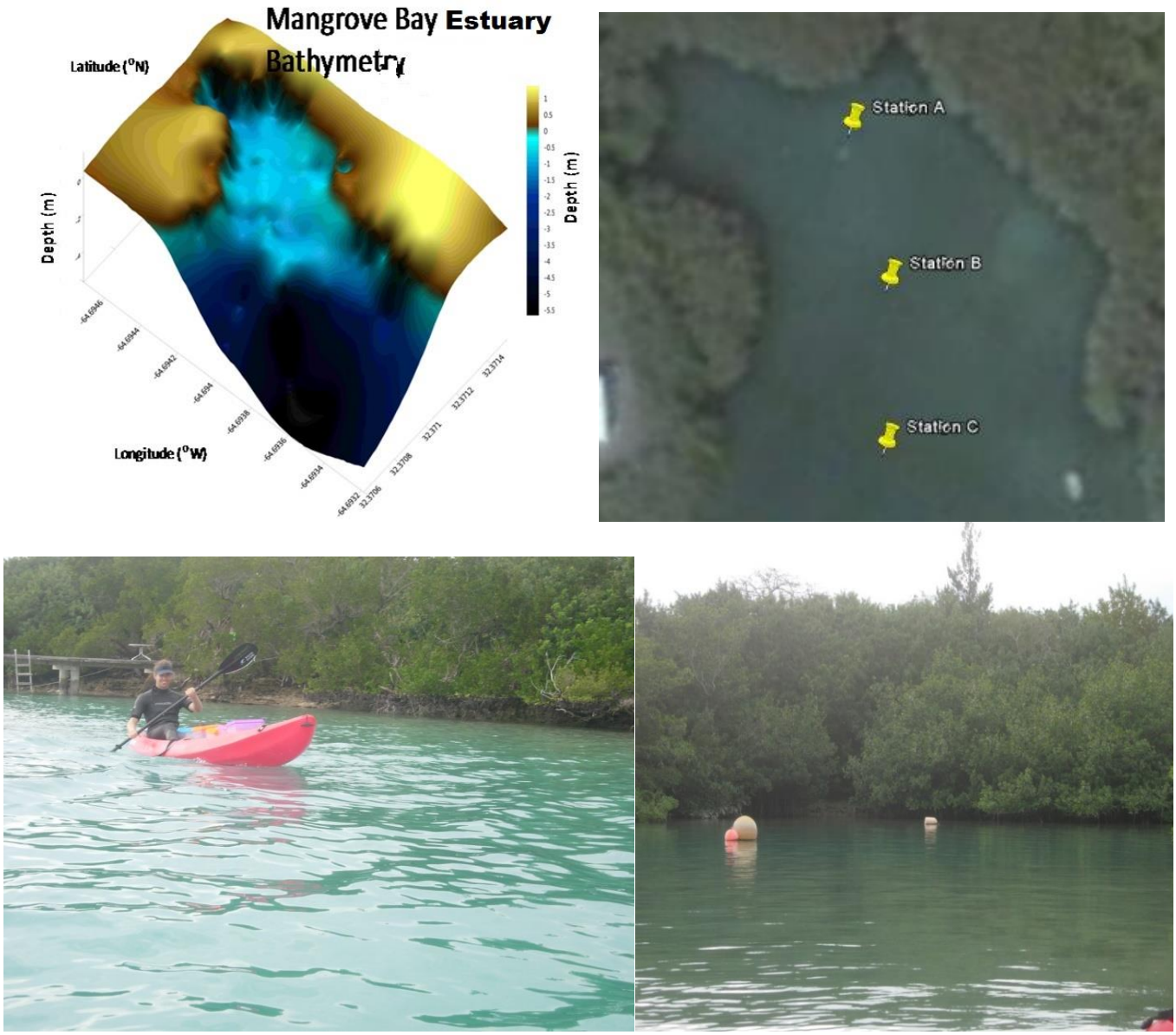


Figure 3. (b) Bathymetry map and the location of three-stations (A, B, and C) transect at Mangrove Bay Estuary, Bermuda.

Table 2).

Field experiment

Physicochemical conditions in Mangrove Bay Estuary are strongly related to tidal regime changes (Figure 6a–c; Table 3). The minimum temperature (16.74°C) and high salinity (36.8 PSU) were recorded at high tide. The maximum temperature (21.24°C) and low salinity (34.9 PSU) were recorded at low tide (Figure 6a–b). Dissolved oxygen concentrations varied from 5.3 to 7.9 mg L^{-1} (Figure 6c). The minimum dissolved oxygen concentration was recorded at low tide (Figure 6c). Ph ranged from 7.76 to 8.28; the minimum values were recorded at low tide and maximum pH was recorded at high tide (Figure 6d). There was no significant ($p > 0.05$, Table 3) variation in the measured physicochemical parameters among the stations (A, B, and C) in the Mangrove Bay Estuary, Bermuda.

Determination of shell dimensions and growth of *I. alatus*

Laboratory and field experiments

Isognomon alatus shell dimensions (height, length and

Figure 3. (c) The field culture of *I*. *alatus* at Mangrove Bay Estuary, Bermuda. Photos illustrate placement and retrieval of tanks from the estuary to determine shell dimensions of *I*. *alatus*.

buoyant weight) (Tables 4 and 5) differ in the laboratory and field experiments. The power regression analysis established an association (correlation of coefficient, R^2) between two measured shell dimension parameters (Tables 6 and 7). There was no change in thickness of *I*. *alatus* placed in the experimental tanks (Table 4 and 5). There was no change in the shell height, and shell length of *I*. *alatus* placed in the control tanks. In contrast, there is variation in shell height and length of *I*. *alatus* cultured in acidification treatment tanks (Figure 7a, b). There was a decrease in buoyant weight and negative growth of *I*. *alatus* in both control tanks and acidification treatment tanks (Figure 7c). The growth rate of *I*. *alatus* in the control and acidification tanks remained relatively constant over the entire growth period (Table 8).

However, there was a significant ($p< 0.05$) decreased

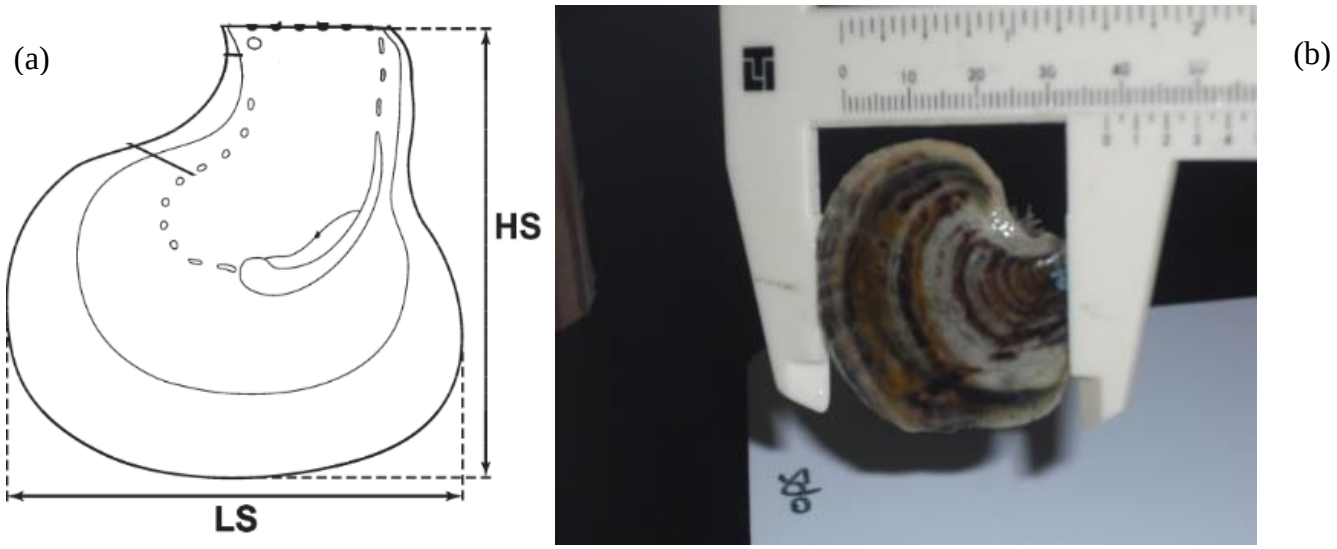
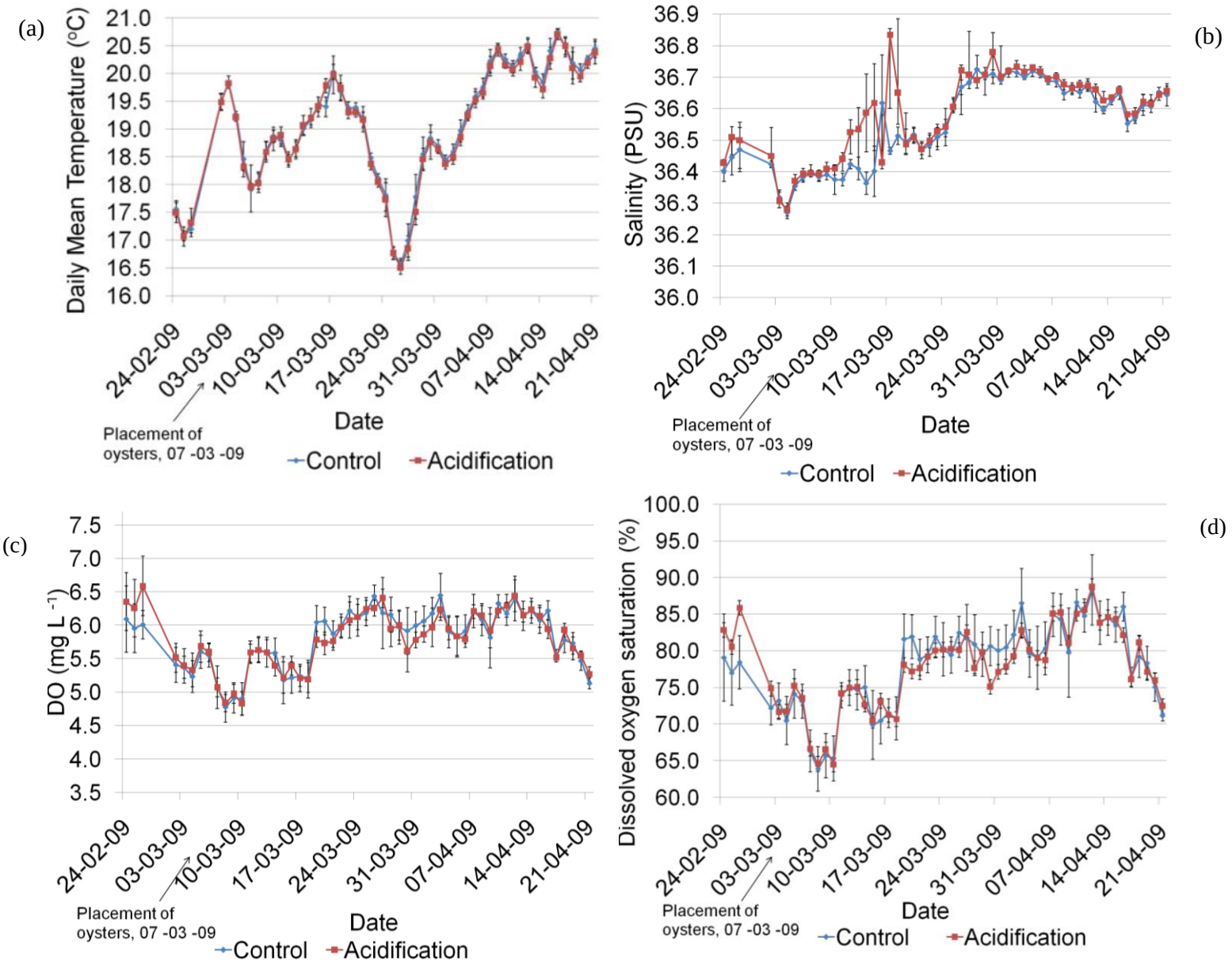


Figure 4. (a) Sketch of bivalve shell dimensions (thus shell length = LS and shell height= HS). (**b**) Determination of shell dimensions (height and length) of *Isognomon alatus* using Vernier caliper.

Figure 4. (c) Determination of shell weight of *Isognomon alatus* by buoyant weighing technique.

weight and negative growth of *I. alatus* cultured at the present pH (8.1 - 8.2) compared to those cultured at future low pH (7.8 - 7.9) (Table 8). In the first growth period, *I. alatus* in the control tank weight loss at a growth rate of -0.27 ± 0.03 mg $g^{-1}day^{-1}$ while those in acidification treatment tanks reduced weight at a growth rate -0.55 ± 0.40 mg $g^{-1}day^{-1}$. In the second growth period (24 days of exposure), *I. alatus* in the control tanks decreased in the growth rate of -0.25 ± 0.32 mg $g^{-1}day^{-1}$, whereas those in acidification tanks decreased -0.57 ± 0.59 mg $g^{-1}day^{-1}$. Overall, *I. alatus* cultured in control tanks reduced weight at the growth rate of -0.26 ± 0.23 mg $g^{-1}day^{-1}$ and those cultures in acidification tanks reduced the growth rate of -0.56 ± 0.36 mg $g^{-1}day^{-1}$ throughout the entire study period of 45 days (Figure 7a–c; Table 8).

There was no clear trend of changes in shell dimensions (height and length) (Figure 8a–b) and growth (Figure 8c) of *I. alatus* along the 3-station transect in Mangrove Bay Estuary, Bermuda. The initial growth of *I. alatus* determined after three weeks of placement at Mangrove Bay was higher than in the rest of the study period (Figure 8a–c). There was positive growth exclusive of some cases (growth period 3) in station C (Table 9). *I. alatus* cultured was slow in the field experiment but much slower at station C (Figure 8c).


(a)
Daily Mean Temperature (°C)
21.0
20.5
20.0
19.5
19.0
18.5
18.0
17.5
17.0
16.5
16.0
24-02-09
03-03-09
10-03-09
17-03-09
24-03-09
31-03-09
07-04-09
14-04-09
21-04-09
Date
Placement of oysters, 07 -03 -09
Control
Acidification
(b)
Salinity (PSU)
36.9
36.8
36.7
36.6
36.5
36.4
36.3
36.2
36.1
36.0
Date
Placement of oysters, 07 -03 -09
Control
Acidification
(c)
DO (mg L -1)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
Date
Placement of oysters, 07 -03 -09
Control
Acidification
(d)
Dissolved oxygen saturation (%)
100.0
95.0
90.0
85.0
80.0
75.0
70.0
65.0
60.0
Date
Placement of oysters, 07 -03 -09
Control
Acidification

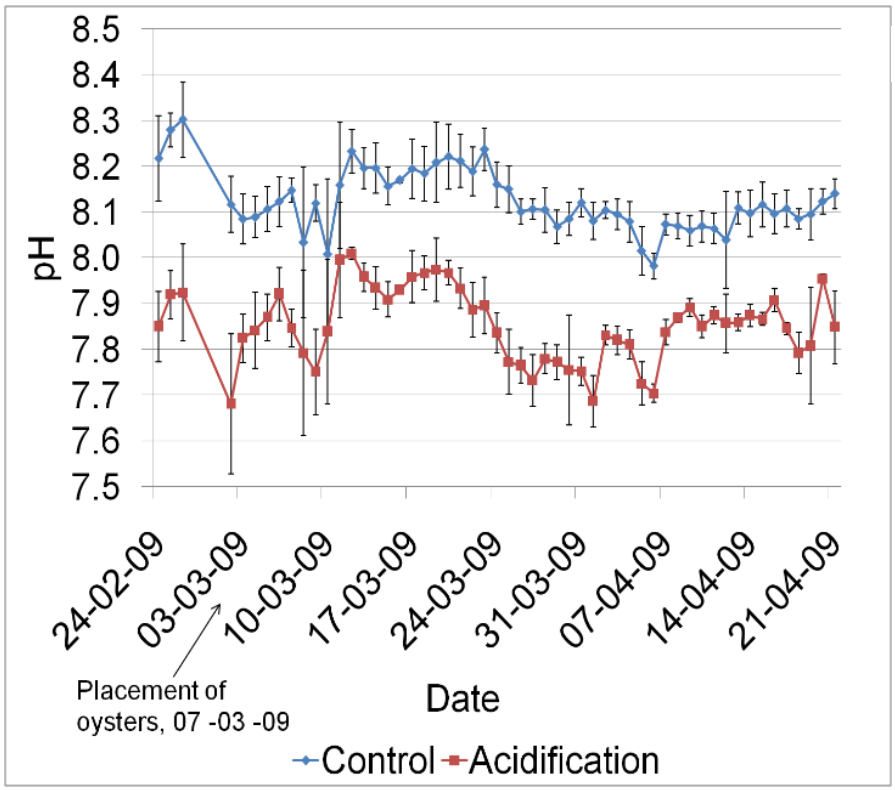


Figure 5. Average mean of daily physiochemical parameters: (a) temperature (°C), (b) salinity, (c) dissolved oxygen concentration (mg L^{-1}), dissolved oxygen saturation (%) (d), and (e) pH recorded in the experimental tanks. Error bars indicate the daily standard deviation from the mean of each parameter.

Table 2. Arithmetic mean (± **S.D.)** of physicochemical conditions in control and acidification tanks.

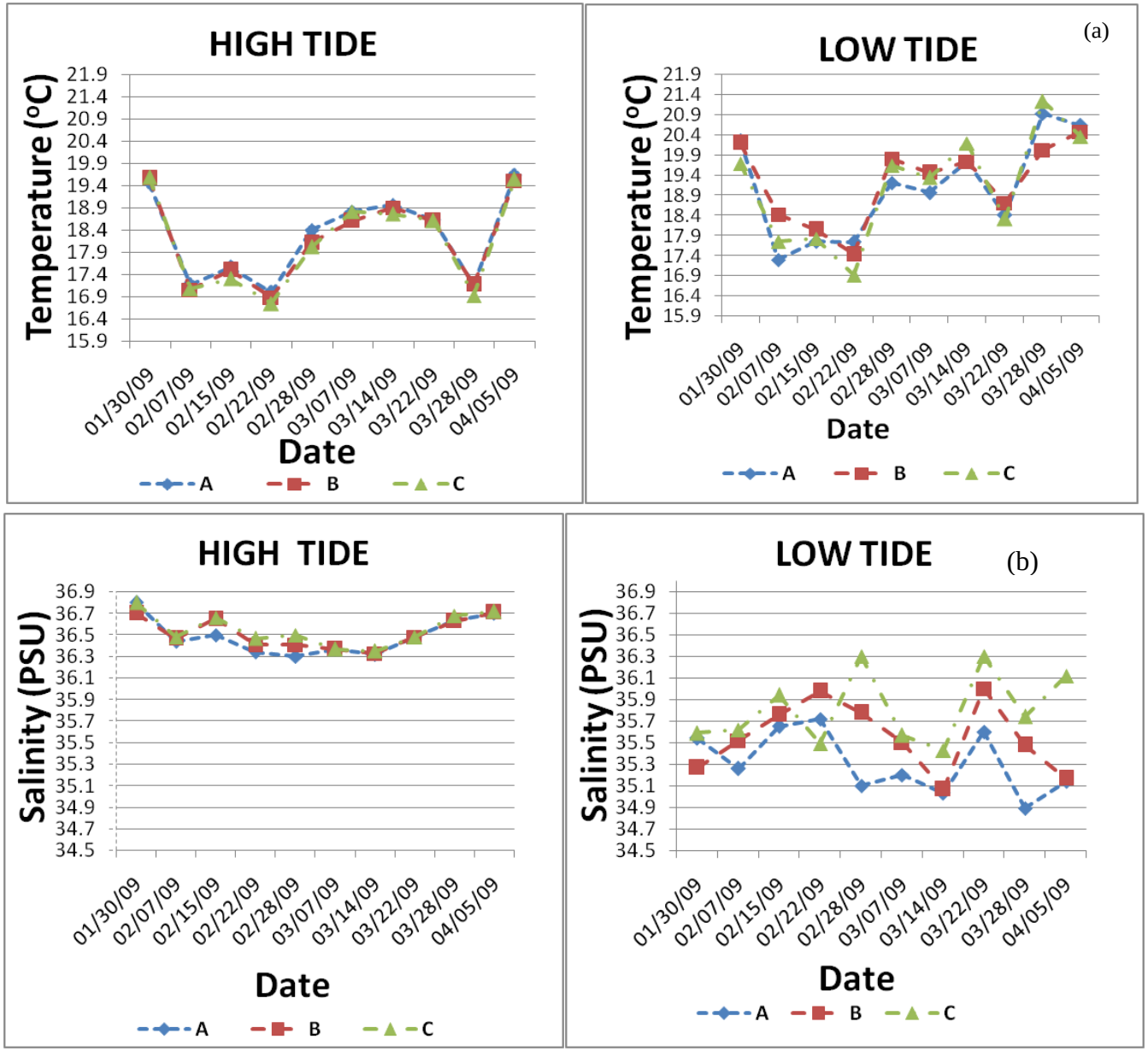
Physicochemical parameters (units)	Control	Acidification	F-value	P-value
Temperature [^{o}C]	19.39 ± 3.98	19.03 ± 1.09	0.88	> 0.05
Salinity [PSU]	36.59 ± 0.98	36.64 ± 1.12	0.68	> 0.05
DO [mgL^{-1}]	6.10 ± 1.89	7.30 ±1.90	0.96	> 0.05
DO [%]	79.04 ± 14.87	79.16 ± 21.30	0.54	> 0.05
pH	8.10 ± 0.10	7.80 ± 0.10	115.02	< 0.05

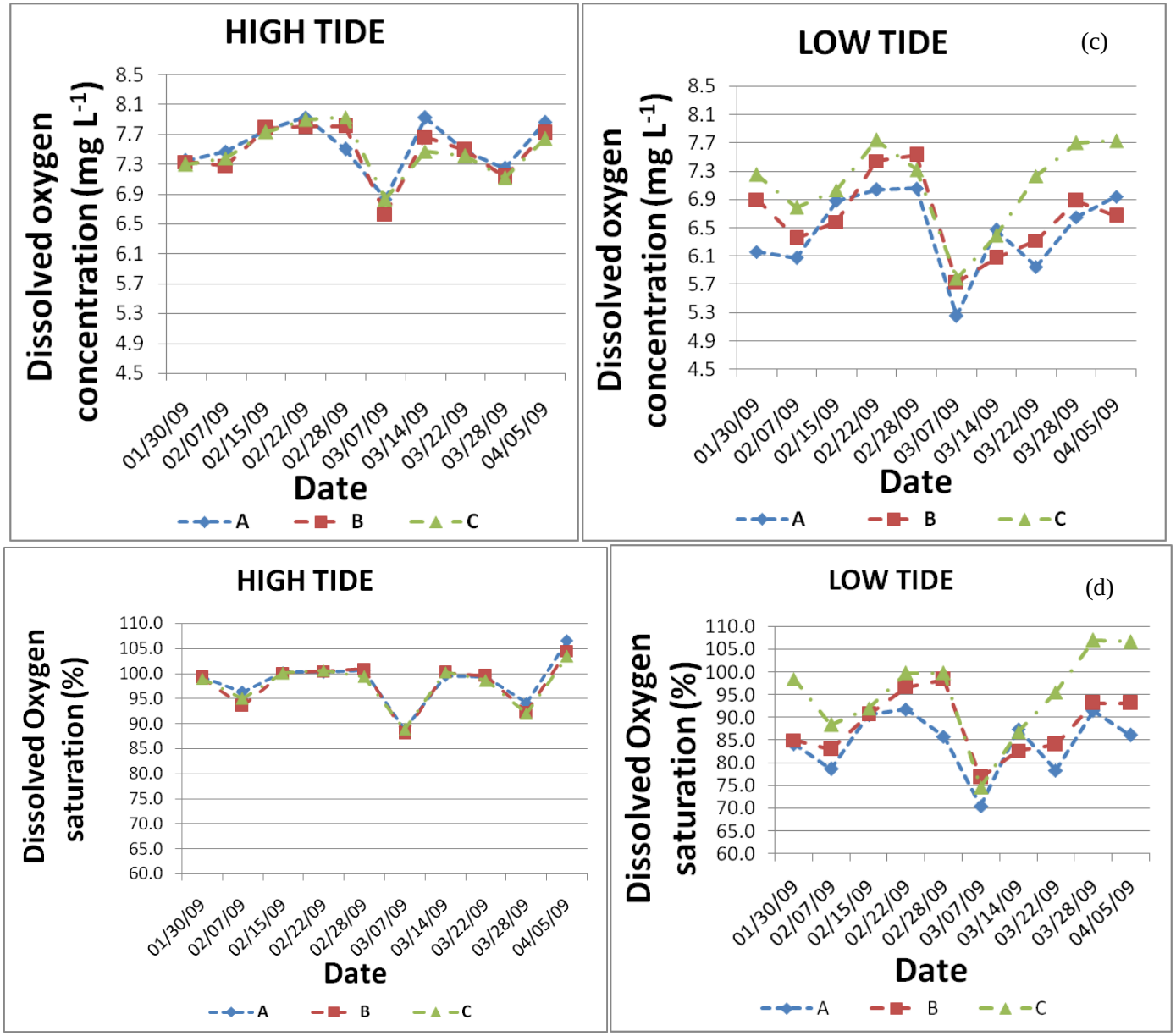
Mean ± S.D.; S.D. = Standard deviation, PSU = Practical Salinity Unit.

Table 3. Physicochemical parameters measured at Mangrove Bay Estuary, Bermuda.

Parameters (units)	Minimum	Maximum	Mean ± S.D.	F-value	P-value
Temperature [^{o}C]	16.74	21.24	18.65 ± 1.26	1.31	> 0.05
Salinity [PSU]	34.90	36.80	36.09 ± 0.55	3.94	> 0.05
DO [mg L^{-1}]	5.30	7.90	7.2 ± 0.51	0.76	> 0.05
DO [%]	70.50	107.0	94. 96 ± 7.18	1.46	> 0. 05
pH	7.76	8.28	8.28 ± 0.13	1.42	> 0.05

S.D. = Standard deviation; PSU = Practical Salinity Unit.


HIGH TIDE
Temperature (°C)
21.9
21.4
20.9
20.4
19.9
19.4
18.9
18.4
17.9
17.4
16.9
16.4
15.9
01/30/09
02/07/09
02/15/09
02/22/09
02/28/09
03/07/09
03/14/09
03/22/09
03/28/09
04/05/09
Date
A
B
C
(a)
LOW TIDE
Temperature (°C)
Date
A
B
C
HIGH TIDE
Salinity (PSU)
36.9
36.7
36.5
36.3
36.1
35.9
35.7
35.5
35.3
35.1
34.9
34.7
34.5
Date
A
B
C
LOW TIDE
(b)
Salinity (PSU)
Date
A
B
C


HIGH TIDE
Dissolved oxygen concentration (mg L-1)
8.5
8.1
7.7
7.3
6.9
6.5
6.1
5.7
5.3
4.9
4.5
01/30/09
02/07/09
02/15/09
02/22/09
02/28/09
03/07/09
03/14/09
03/22/09
03/28/09
04/05/09
Date
A
B
C
LOW TIDE
(c)
Dissolved oxygen concentration (mg L-1)
Date
A
B
C
HIGH TIDE
Dissolved Oxygen saturation (%)
110.0
105.0
100.0
95.0
90.0
85.0
80.0
75.0
70.0
65.0
60.0
Date
A
B
C
LOW TIDE
(d)
Dissolved Oxygen saturation (%)
Date
A
B
C

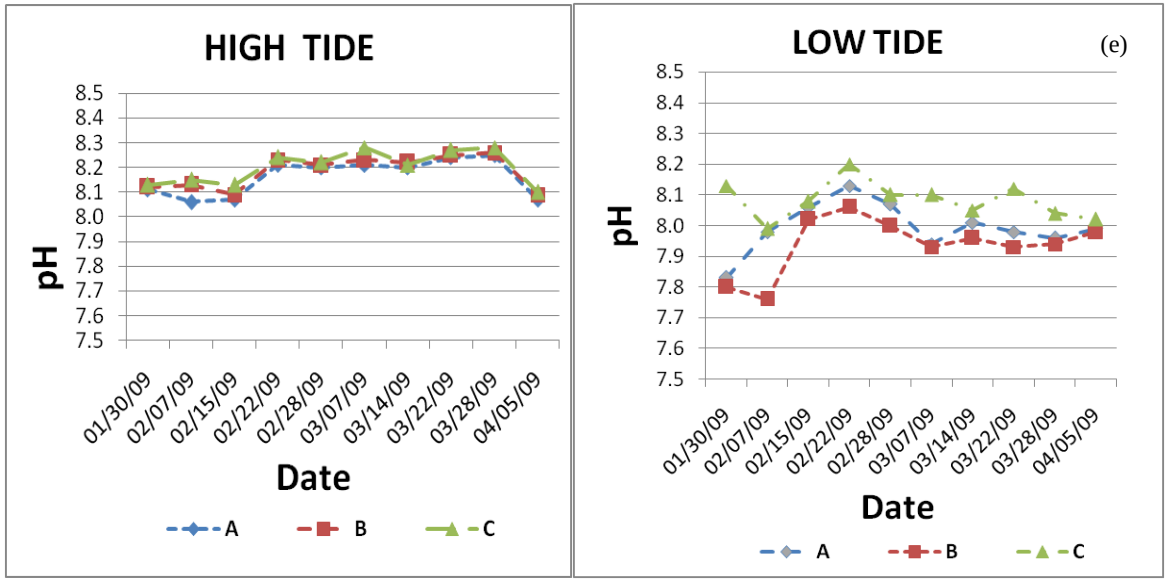


Figure 6. Weekly physicochemical parameters:(**a**) temperature (^{o}C), (**b**) salinity (PSU) and (**c**) dissolved oxygen concentration (mg L^{-1}), (**d**) dissolved oxygen saturation (%) and (e) pH recorded high and low tides along 3-stations (A, B, and C) transect at Mangrove Bay Estuary, Bermuda.

Table 4. Shell dimensions (mean ± S.D.) of *Isognomon alatus* in control and acidification tanks.

Shell dimensions	Control	Acidification
Initial buoyant weight (g)	0.838 ± 0.29	0.816 ± 0.38
Final buoyant weight (g)	0.829 ± 0.29	0.806 ± 0.39
Initial weight (g)	27.78 ± 3.64	28.44 ± 2.57
Final weight (g)	27.28 ± 3.64	27.75 ± 2.75
Initial height (mm)	27.08 ± 1.05	28. 44 ± 1.30
Final height (mm)	27.08 ± 1.05	27.72 ± 1.52
Initial length (mm)	25.82 ± 3.91	26.67 ± 3.33
Final length (mm)	25.82 ± 3.91	25.31 ± 3.54
Initial thickness (mm)	4.56 ± 0.67	4.36 ± 0.70
Final thickness (mm)	4.56 ± 0.67	4.37 ± 0.72

S.D. = Standard deviation.

Table 5. Shell dimensions (mean ± S.D.) of *Isognomon alatus* cultured along 3-stations (A, B, and C) transect at Mangrove Bay Estuary, Bermuda.

Shell dimensions	Stations		
	A	B	C
Initial buoyant weight (g)	1.30 ± 0.00	1.31 ± 0.22	1.53 ± 0.38
Final buoyant weight (g)	1.47 ± 0.10	1.36 ± 0.25	1.55 ± 0..34
Initial height (mm)	35.22 ± 0.50	32.72 ± 0.40	34.94 ± 1.09
Final height (mm)	34.29 ± 0.01	31.92 ± 1.12	33.93 ± 0.10
Initial length (mm)	32.50 ± 1.0	31.80 ± 1.90	34.30 ± 3.00
Final length (mm)	33.50 ± 0.5	33.30 ± 0.00	31.50 ± 0.90
*Thickness (period 2)	5.09 ± 0.88	5.18 ± 0.91	5.36 ± 1.08
*Final thickness (period 3)	5.08 ± 0.92	5.19 ± 0.95	5.32 ± 1.11

S.D. = Standard deviation.* Growth period 2 (09/03/09) and **Growth period 3 (05/04/09).

Table 6. Coefficient of correlation (R^2) for shell morphometric analysis of *Isognomon alatus* cultured in control and acidification tanks.

Shell morphometric relationships	Coefficient of correlation (R^2)			
	Control		Acidification	
Power regression between two shell morphometric variables	Before	After	Before	After
Buoyant weight (g)/length (mm)	0.269	0.267	0.746	0.740
Buoyant weight (g)/height (mm)	0.457	0.457	0.583	0.660
Buoyant weight (g)/thickness(mm)	0.565	0.565	0.811	0.810
Height (mm)/length (mm)	0.511	0.511	0.319	0.615
Height (mm) /thickness (mm)	0.402	0.402	0.537	0.362
Length (mm) /thickness (mm)	0.302	0.302	0.480	0.472

Average (± standard deviation) growth rate of *I. alatus* ranged from 0.26 ± 0.90 to 1.29 ± 0.77 mg $g^{-1}day^{-1}$ (station A), 0.33 ± 0.46 to 1.47 ± 0.57 mg $g^{-1}day^{-1}$ (station B) and -0.26 ± 2.01 to 0.56 ± 0.40 to mg $g^{-1}day^{-1}$ (station C) (Figure 8c; Table 9). Overall, a positive growth rate of *I. alatus* occurred in the field experiment; 0.55 ± 0.65 mg $g^{-1}day^{-1}$ (station A), 0.89 ± 0.43 mg $g^{-1}day^{-1}$ (station B) and 0.20 ± 0.70 mg $g^{-1}day^{-1}$ (station C) (Table 9). There was no significant ($p > 0.05$) (Table 9) variation in the growth rate (mg $g^{-1}day^{-1}$) of *I. alatus* among the stations (A, B, and C), although observation of negative growth rate for a replicate culture of *I. alatus* at station C (Figure 8c).

One oyster died in the acidification tank, while in the field experiment, mortality was three oysters from stations A and B.

DISCUSSION

Coastal areas such as estuarine are very dynamic ecosystems mostly affected by climate and environmental changes (Braga et al., 2020; Ramajo et al., 2020). Shellfish plays an essential ecological role in coastal ecosystems (Braga et al., 2020; Ramajo et al., 2020). Coasts and their marine biota are exposed to significant environmental heterogeneity due to natural drivers and anthropogenic stressors (Braga et al., 2020; Ramajo et al., 2020). The impact of anthropogenic ocean acidification on marine life is still unclear (Matoo et al., 2020; Yokoyama et al., 2020).

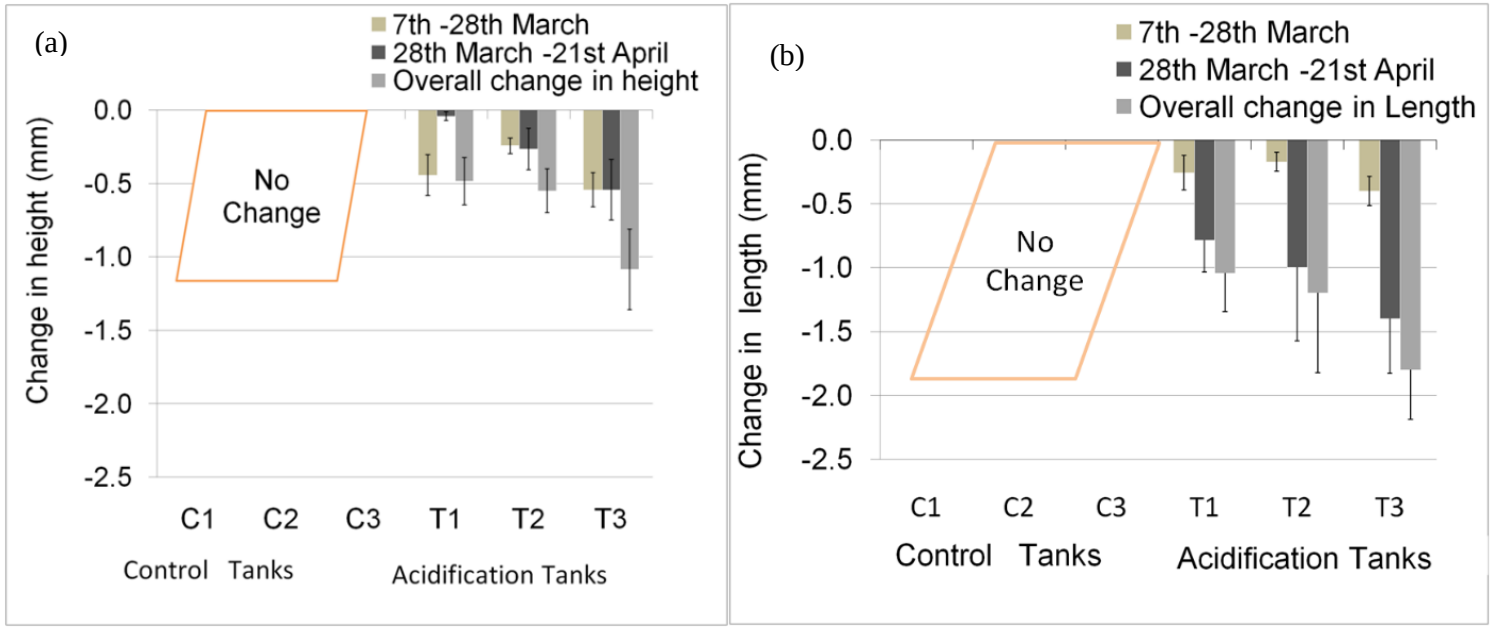
The seawater chemistry of the laboratory changes in pH due to manipulated CO_2 levels (Table 1). The measurement of physicochemical variables in experimental tanks in the laboratory except pH is typical of Atlantic coastal water conditions for that season (Figure 5a–d). The only variation is the pH in control and acidification tanks (Figure 5e) (Table 2). The average pH difference was between ~0.2 to 0.3 units, which corresponds to the lowering of surface ocean seawater pH anticipated in 2100 (Jokiel et al., 1978; Spencer, 1989; Herler and Dirnwöber, 2011).

The physicochemical parameters (Table 3) measured Mangrove Bay Estuary reflect variation in the tidal activity (Figure 6a–e). Many coastal estuarine bays exhibit natural variation in seawater pH due to diurnal changes in

Table 7. Coefficient of correlation (R^2) for shell morphometric analysis of *Isognomon alatus* cultured along 3-stations (A, B, and C) transect at Mangrove Bay Estuary, Bermuda.

Shell morphometric relationships	Coefficient of correlation (R^2)					
	Stations					
	A		B		C	
	Power regression between two shell morphometric variables					
	Initial	Final	Initial	Final	Initial	Final
Buoyant weight (g)/length (mm)	0.799	0.782	0.620	0.688	0.656	0.839
Buoyant weigh (g)/height (mm)	0.649	0.659	0.688	0.857	0.752	0.755
Buoyant weight (g)/thickness (mm)	N/A	0.542	N/A	0.673	N/A	0.865
Height (mm) /length (mm)	0.418	0.564	0.640	0.743	0.689	0.865
Height (mm)/thickness (mm)	N/A	0.226	N/A	0.441	N/A	0.573
Length (mm)/thickness (mm)	N/A	0.356	N/A	0.264	N/A	0.723

N/A = Not available.



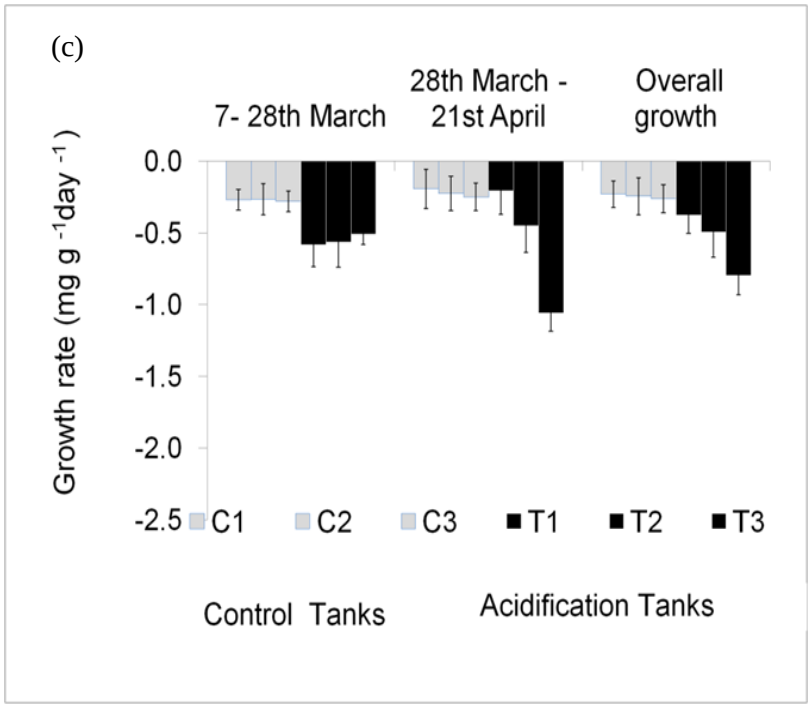


Figure 7.Changes in shell parameters: (**a**) height (mm) (**b**) length (mm) and (**c**) growth rate (mg $g^{-1}day^{-1}$) of *Isognomon alatus* over the 45 days experimental period. The period between sampling days were 21 and 24 days. Black bars are control tanks (C1 – C3), and white bars are Acidification tanks (T1 – T3). Error bars±SE, n = 7 except tank T3, in which one oyster died.

Table 8. Average (± S.D.) growth rate (mg $g^{-1}day^{-1}$) of *Isognomon alatus* cultured in control and acidification tanks.

Growth period	Culture date	Control	Acidification	Two-sample t-test
1	7 – 28 March, 2009	-0.27 ± 0.30	-0.55 ± 0.40	P < 0.05
2	28 March – 21 April, 2009	-0.25 ± 0.32	-0.57 ± 0.59	P < 0.05
	Overall growth	-0.26 ± 0.23	-0.56 ± 0.36	P < 0.05

the tidal regime and photosynthetic activity (Twilley et al., 1992; Yates et al., 2007). The temperature recorded (16.74 to 21.24°C) is typical of tropical coast weather from January to April. Water depth and volume may influence not only the estuarine water temperature but also season weather changes. During high tide, water volume in Mangrove Bay Estuary increases water depth with cooler temperatures. However, the estuarine water volume may decrease during low tide with a subsequent decrease in water depth, resulting in a shallow-water depth with much exposure to sunlight, then characterized by warmer temperatures. Salinity is maximum at high tide and minimum at low tide (Figure 6b). Tidal influence plays a critical role in estuarine salinity (La Peyre et al., 2016).There is no river flow, but groundwater sources may supply freshwater in Mangrove Bay Estuary. There is an increased flow of open seawater into the estuary during high tide, leading to increased salinity. Dissolve oxygen (concentration and saturation) (Figure 6c-d) and pH (Figure 6e) decreases with rising tidal with a minimum at high tide. At the same time, these parameters increase with the full surge with a maximum at low tide.

The loss of weight of *I*. *alatus* cultured in the laboratory suggests suppression in a stressful environment (Table 4). Food supply is the primary environmental factor that influences oyster growth and is affected by factors such as temperature, population density, and turbidity (Brown, 1986; Baillie and Grabowski, 2019; Campbell and Hall, 2019). The oyster growth rate is mainly regulated by food supply with temperature and salinity as secondary factors (Brown, 1988; La Peyre et al., 2016). Other possible environmental variables such as water temperature,

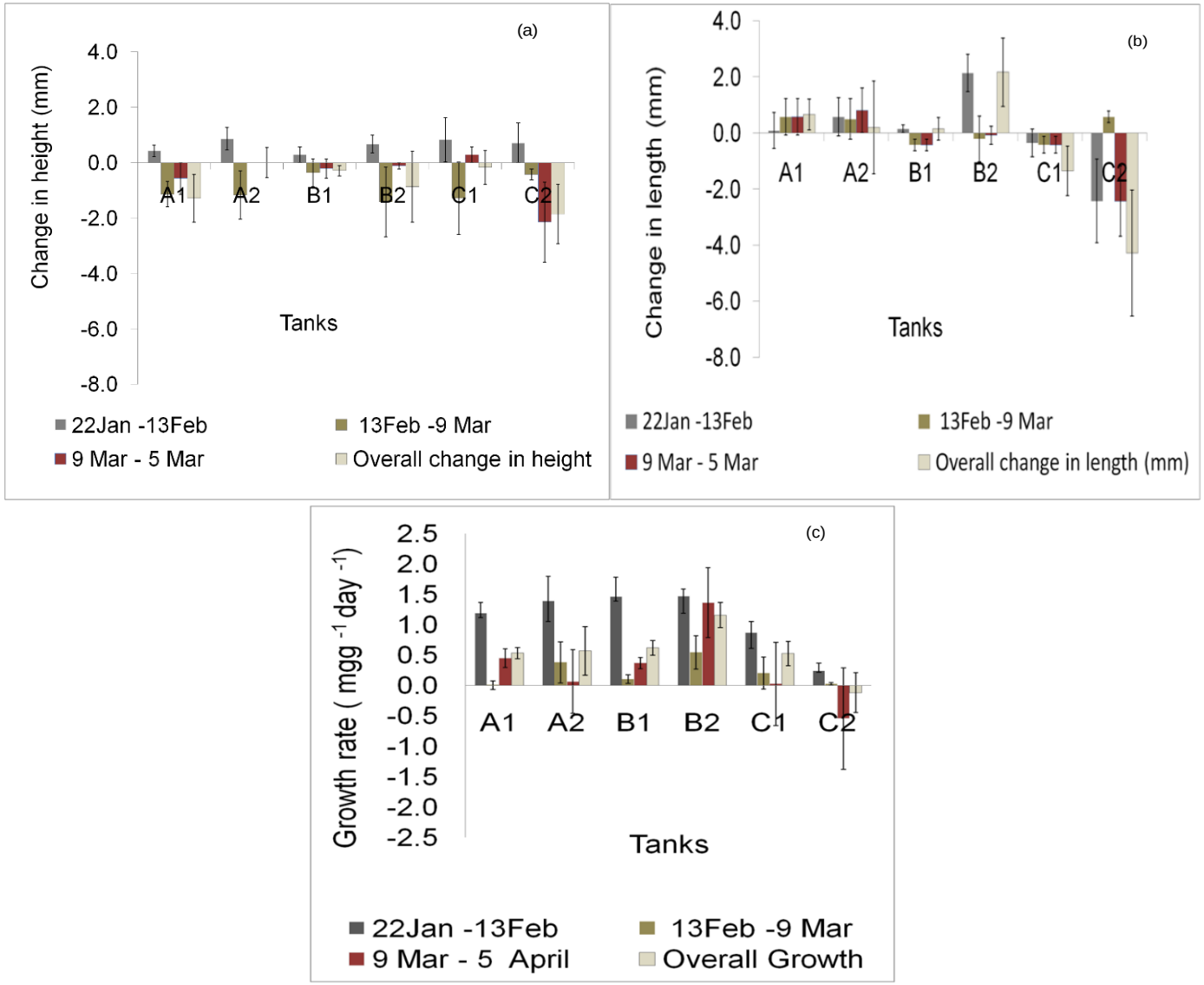


Figure 8. Changes in shell dimensions: (**a**) height (mm), (**b**) length (mm) and (**c**) growth rate (mg $g^{-1}day^{-1}$) of *Isognomon alatus* cultured in Mangrove Bay Estuary, Bermuda. Each station has a replicate (A1, A2, B1 B2, C1, C2). Error bars indicate standard errors.

Table 9. Average (± S.D.) growth rate (mg $g^{-1}day^{-1}$) of *Isognomon alatus* in the field cultured at Mangrove Bay Estuary, Bermuda.

Growth period	Culture date	A	B	C	One-way ANOVA
1	22 January – 3 March,2009	1.29 ± 0.77	1.47 ± 0.58	0.56 ± 0.40	P > 0.05
2	13 February – 9 March, 2009	0.19 ± 0.55	0.33 ± 0.46	0.11 ± 0.38	P > 0.05
3	9 March – 5 April,2009	0.26 ± 0.90	0.87 ± 0.88	-0.26 ± 2.01	P > 0.05
	Overall growth	0.55 ± 0.65	0.89 ± 0.43	0.20 ± 0.70	P > 0.05

S.D. = Standard deviation.

salinity, and oxygen are dynamic and may affect oysters' growth and survival (Dekshenieks et al., 1993; La Peyre et al., 2016; Dong et al., 2018). The shell dimensions (Table 5) of *I*. *alatus* cultured in the field culture reflect optimum conditions. The loss of weight of *I*. *alatus* in control tanks and acidification tanks (Figure 7a–c) could be due to a decrease in organic tissue due to insufficient food availability. There exists an association between two shell morphometric variables (Table 6 and 7). The drastic changes in shell dimensions (height, length, weight loss) (Table 4) and growth rate of *I*. *alatus* (Table 8) cultured in acidification tanks (Figure 7a–c) could be due to multiple environmental stressors including reduced metabolic activity, insufficient food source, and shell thinning in CO_2 acidified seawater (Figure 7a–c). Therefore, exposure of flat tree oysters to low pH could significantly decrease skeleton weight and damage the shell structure.

In this study, the shell length of *I*. *alatus* measured ranged from 27.08 to 28.44 mm in the laboratory while 31.92 to 35.22 mm in field culture. The shell height of *I*. *alatus* ranged from 25.82 to 27.72 mm in the laboratory but 31.80 to 34.30 mm in the field culture. The adult *I*. *alatus* ranged from 75 mm to 95 mm in shell length and 40 - 50 mm in shell height (Mikelsen and Bieler, 2008), although individuals as large as 90 mm in size have been encountered (Siung,1980).

In contrast, *I*. *alatus* showed a gain in weight and positive growth exclusive of growth period 3 (Table 9) in field culture could be due to favourable environmental conditions, despite exposure to low pH (~7.4) levels during low tide (Figure 8a–c). Therefore, *alatus* cultured in the natural field showed tolerance to low pH exposure during the low tide. The results demonstrated that flat tree oyster's response to future CO_2 levels is complex. Other environmental l factors such as food availability and diurnal variability of the tidal activity in an estuarine environment are also potential factors that can compound ocean acidification impacts on marine animals.

The weight loss and shell thinning of *I*. *alatus* observed in this study (Table 10) agree with the effects of lowering pH on marine animals when cultured in manipulated CO_2 acidified seawaters (Table 10) (Talmage and Gobler, 2010; Yu et al., 2011). The lowering of pH reduced the growth of *Mytilus edulis* (Berge et al., 2006). In pH 6.7, small mussels (11 mm mean length) showed low growth rates compared to control treatments, and large mussels (21 mm mean length) did not grow at all. A reduction in seawater pH was responsible for growth suppression, shell dissolution, tissue weight loss, and feeding activity suppression in three species of commercial bivalves (*Ostrea edulis*, *Crassostrea gigas,* and *Mytilus edulis*) (Bamber, 1990). A long-term lowering of seawater pH caused a reduction in haemolymph pH, buffered by the dissolution of the shell ($CaCO_3$), lower metabolic rate, and increased degradation of protein in mussel, *Mytilus galloprovincialis* (Michaelidis et al. 2005). Ocean acidification resulted in physiological stress in the adult mussel, *Mytilus chilensis* (Diaz et al., 2018).

There are biological consequences of changing ocean pH (Widdicombe and Spicer, 2008; Convey and Peck, 2019; Emanuel et al., 2020; Stokowski et al., 2020), withadverse effects on physiological processes in marine organisms (Fabry et al., 2008) and ecosystems structure and function (IPCC, 2007; Widdicombe and Spicer, 2008; Stokowski et al., 2020). There are detrimental effects of acidic waters on blue mussel, *Mytilus edulis* (Berge et al., 2006; Bibby et al., 2008) gastropoda, *Littorina Littorea* (Bibby et al., 2007), carpeted-shell clams, *Venerupis decussata* (Bamber, 1987; Bamber, 1990) through a rise in projected pCO_2 levels (Feely et al., 2009; IPCC, 2013; Doney et al., 2015). The calcification rates of the mussel (*Mytilus edulis*) and the Pacific oyster (*Crassostrea gigas)* declined with increasing pCO_2 (Berge et al., 2006; Gazeau et al., 2007). There was a decrease of 25 and 10% in mussel and oysters, respectively, at pCO_2 levels anticipated by the end of this century (~740 ppm, IPCC IS92a scenario) (Gazeau et al., 2007; IPCC, 2007, 2014, 2018). The reduction in seawater pH leads to growth suppression, shell dissolution, tissue weight loss, and feeding activity suppression of *Mytilus edulis* exposed to future seawater conditions (Bibby et al., 2008). The increased pCO_2 of seawater projected to occur by the year 2300 (pH 7.4) will severely impact the early development of *Crassostrea gigas* (Kurihara et al., 2007). However, there is no significant effect of 2000 ppm CO_2 (pH 7.4) treatments on the fertilization success of *Crassostrea gigas* and *Mytilus galloprovincialis* from Japan (Kurihara et al., 2009). There was no significant effect of future levels of ocean acidification (−0.35 pH unit change) on sperm swimming speed, sperm motility,

Table 10. Ocean acidification effect on culture of marine organisms.

Species	Cultured medium (pH, CO_2, and pCO_2 levels)	Duration	Effect	Source
Isognomon alatus (Gmelin, 1791) (Flat tree oyster)	7.8 – 7.9 (*Acidification-low pH; ~ Three levels of CO_2 = T1 = 193 ppm, T2 = 390 ppm, and T2 = 766 ppm) (Laboratory culture).	45 days	Reduced weight, shell thinning, and significant negative growth rate.	This study
	8.0 – 8.2 (Control-ambient seawater pH) (Laboratory culture)	45 days	Weight loss and significant negative growth rate.	
	7.76 – 8.28 but ~ 7.4 during low tide (Field culture)	45 days	Increase in weight and positive growth. rate	
Tritia reticula (Linnaeus, 1758) (Intertidal gastropod)	8.08 (Control; ambient seawater pH) 7.65 – 7.88 (Low pH; acidified with CO_2)	2 months	Shell repair, with a full repair rate observed in 75% of individuals.	Yokoyama et al. (2020)
Mytilus edulis (Linnaeus, 1758) (Blue mussel)	pCO_2 levels = ~ 400 vs. 800 µatm at control and elevated temperatures (10 vs. 15 °C)	-	Ocean acidification did not affect the metabolism of adult Mytilus *edulis*	Matoo et al. (2020)
Limacina helicina (Philipps,1774) (Plantonic sea snail)	7.78	30 days	28% decrease in calcification rate.	Comeau et al. (2009)
Crassostrea gigas (Thunberg, 1793) (Pacific oyster)	7.4	48 h	Inhibited larval development	Kurihara et al. (2007)
Mytilus edulis and *Crassostrea gigas*	740 ppm	2 h	A decrease in calcification 25% and 15%, respectively.	Gazeau et al. (2007)
Mytilus galloprovincialis (Lamarch, 1819) (Mediterranean mussel)	7.3	3 months	Significant decrease in growth rate.	Michealidis et al. (2005)

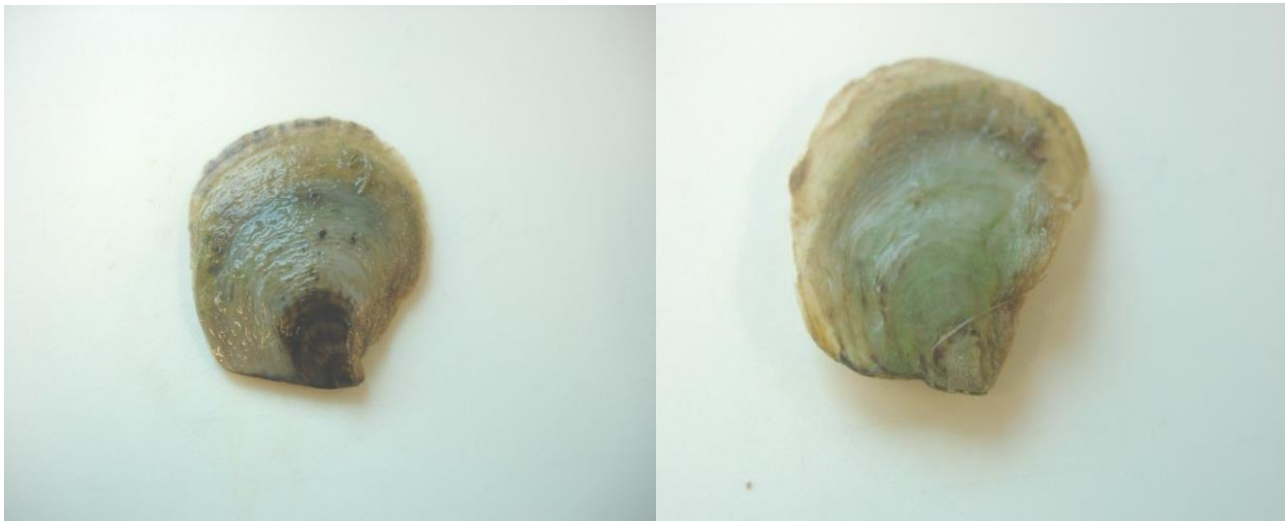
CO_2 = Carbon dioxide gas; pCO_2 = Partial pressure of carbon dioxide; acidification tanks (T1-T3).

and fertilization kinetics in a population of oyster, *Crassostrea gigas* (Havenhand and Schlegel, 2009). The acidified seawaters disrupt mangroves ecosystems and estuarine organisms; these will indirectly contribute to economic and societal implications.

Some marine fauna showed resilience to acidified water. When the gastropod, *Tritia reticulate* were cultured in high CO_2, it resulted in shell damage without physiological stress (Yokoyama et al., 2020). Individuals of *Tritia reticulate* were exposed to control ambient pH of 8.08 (control) and low pH scenarios (pH 7.65 and 7.88). After two months of exposure, all individuals showed shell repair, with a full repair rate observed in 75% of individuals (Yokoyama et al., 2020). Ocean acidification did not impact the metabolism and enzyme activities in the blue mussel, *Mytilus edulis*, although the temperature does (Matoo et al., 2020). The blue mussels (*Mytilus edulis*) showed changes in physiological filtration and cilia beat function when cultured increased dissolved carbon dioxide (Meseck et al., 2020). Estuarine is a complex dynamic system, and more than pH modulates the physiological flexibility of mussel, *Perumytilus purpuratus* populations (Ramajo et al., 2020).

I. alatus is a marine species with a potential for aquaculture to increase shellfish supply. The shell of *I. alatus* provides an area of the substrate for other species living in mangrove communities (for example stone crab, *Menippe mercenaria*, gastropod, *Batillaria minima,* and the Coffee bean snail, *Melampus coffeus*) (Patrick, 1988; Thomas and Dangeubun, 1994). The species plays a critical role in the uptake and recycling of nutrients

(a)



(b)

Figure 9.*Isognomon alatus* exposed to (a) ambient seawater pH (pH 8.1 – 7.9) and (b) altered seawater pH (pH 7.8 – 7.9), indicating shell thinning.

in the mangrove ecosystem due to their filter-feedings habits (Gutiérrez et al., 2003; Wilk and Bieler, 2009; Suarez-Ulloa et al., 2019). The species is also used to concentrate pollutants such as heavy metal concentration (e.g., Cu, Pb, Zn, Cd), thereby help to improve coastal water quality, especially in Venezuela, the Caribbean, and the Dominican Republic (Jaffe et al., 1998; Sbriz et al., 1998; Saed et al., 2001; Leal et al., 2019).

I. alatus is an essential food source for other marine organisms in mangrove estuaries and various sea birds (Gutiérrez et al., 2003; Suarez-Ulloa et al., 2019). The species act as a habitat for many small benthic bivalves (e.g., *Melampus coffeus*) that live on the shells and within the byssus threads that hold the oyster onto a substrate (Thomas and Dangeubun, 1994). The species act as ecosystem engineers to maintain coastal estuarine environment biodiversity, sediment stability, nutrient recycling, and safety (Thomas and Dangeubun, 1994; Emanuel et al., 2020). Ocean acidification will affect coastal ecosystems and their resources, such as mangrove bays that provide a substrate for marine benthic organisms (Zablocki et al., 2011; Linto et al., 2014; Leal et al., 2019). This study provides evidence that the lowering of seawater pH due to anthropogenic absorption of CO_2 projected to occur during this century may affect the shell dimensions and growth rate of *Isognomon alatus.* Environmental influence on flat tree oyster is critical for a better understanding of their response to lowering pH.

Conclusion

Flat tree oysters are important benthic fauna in the mangrove ecosystem, serving as a substrate for bottom-dwelling animals. The species are filter feeders supporting nutrient recycling and potential bioindicators for coastal pollution. Short-term bivalves culture in projected future pH conditions is critical to understand how the species will adapt to projected lowering pH due to increased CO_2 concentrations in seawater. *Isognomon alatus*, the flat tree oyster, exhibited much weight loss and a negative growth rate at pH values (7.8 – 7.9) expected by the year 2100 compared to growth rates at present ambient pH values (8.1 – 8.2) (Figure 9). The lowered pH effect (also known as ocean acidification) on the flat tree oyster includes weight loss, shell thinning, dissolution, and mortality. The weight loss and negative growth could be due to insufficient food availability in the experimental tanks. However, a higher decreased weight and negative growth of *I. alatus* cultured in acidification tanks are due to a disturbance system caused by the manipulated CO_2 in the seawater and the lack of nutritional food supply.

In contrast, *I. alatus* cultured along a natural variability of pH gradient due to tidal cycle in Mangrove Bay Estuary showed weight gain and positive growth, despite exposure to low pH (~7.4 value) during low tide. Overall, the results suggest that lowering pH could negatively impact shellfish, such as oysters, with potential dramatic coastal ecosystems changes in structure, function, and services. The study provides evidence of ocean acidification effect on flat tree oyster, commonly attached to mangroves in the coastal estuarine system with natural variation pH due to tidal activity. The findings are useful for modeling biogeochemical changes in coastal ecosystems. Further investigation on marine bivalves' long-term exposure to low pH and environmental controls is necessary to understand their growth under changing CO_2 seawater. Therefore, *I. alatus* under increased CO_2 conditions is critical for their conservation in projected climate change impacts on the coastal environment such as mangroves system and protecting life below water

(Sustainable Development Goal 14).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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