Energy metabolism and survival of the juvenile recruits of the American lobster (Homarus americanus) exposed to a gradient of elevated seawater \(pCO_2\)

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\textbf{A B S T R A C T}

The transition from the last pelagic larval stage to the first benthic juvenile stage in the complex life cycle of marine invertebrates, such as the American lobster Homarus americanus, a species of high economic importance, represents a delicate phase in these species development. Under future elevated \(pCO_2\) conditions, ocean acidification and other elevated \(pCO_2\) events can negatively affect crustaceans. This said their effects on the benthic settlement phase are virtually unknown. This study aimed to identify the effects of elevated seawater \(pCO_2\) on stage V American lobsters exposed to seven \(pCO_2\) levels. The survival, development time, metabolic and feeding rates, carapace composition, and energy metabolism enzyme function were investigated. Results suggested an increase in mortality, slower development and an increase in aerobic capacity with increasing \(pCO_2\). Our study points to potential reduction in juvenile recruitment success as seawater \(pCO_2\) increases, thus foreshadowing important socio-economic repercussions for the lobster fisheries and industry.

\textbf{1. Introduction}

Up to 85\% of all benthic marine species possess complex life cycles with distinct larval stages that precede a metamorphosis to the juvenile phase, eventually leading to the adult phase (Pechenik, 1999). Relevant examples exist across multiple phyla, such as molluscs, echinoderms, and decapod crustaceans (Ekkstrom et al., 2015). Commercially important crustacean species, namely shrimps, crabs and lobsters, have complex life cycles that include several developmental stages that are associated to distinct habitats and characterised by distinctive mor-phological, physiological, and behavioural traits (Charmantier et al., 1991; Factor, 1995; Spicer and Eriksson, 2003; Spicer and Gaston, 1999). Among these crustaceans, the American lobster, Homarus americanus (H. Milne Edwards, 1837), possesses three pelagic larval stages followed by an intermediate post-larval stage that eventually settle on the benthos. This marks the success of juvenile recruitment (Incze and Wahle, 1997). The metamorphic moult to the post-larval stage (stage IV) represents a pivotal transition between the larval pelagic phase and the juvenile benthic phase (Factor, 1995; Wahle and Steneck, 1991; Wahle, 2003). This metamorphosis is critical for the stage IV post-larvae to settle and moult successfully to the first juvenile phase (stage V), which is considered as the recruitment stage (Incze et al., 1997). The first developmental stages following lobster recruitment have naturally high mortality rates and are known to represent a bottleneck in the lobsters’ life cycle (Wahle et al., 1991). Following this sensitive phase, the resulting abundance of successful benthic recruits is a fair predictor of the ultimate contribution to upcoming adult populations per year class. This can help define the future viability of natural populations and the sustainability of stocks, particularly when under harvesting pressure (Steneck and Wilson, 2001; Wahle et al., 1991).

With the lobster landed value’s progressive growth to over $ 1.2 billion just in 2016 in Atlantic Canada according to Fisheries and Oceans Canada (http://www.dfo-mpo.gc.ca/stats/commercial/sea-maritimes-eng.htm), and landings reaching record highs above 40 000
millions tonnes in the USA in the early 2000's (Wahle et al., 2011), the Canadian and American lobster industries prioritize the protection of this resource after seeing record-breaking population declines in the early 2000's in some regions along the coast of both countries (Comeau et al., 2004; Stenneck and Wilson, 2001).

Since benthic recruitment begins with the successful settlement of stage IV post-larvae, the release of individuals starting at this developmental stage has become a popular method in an attempt to increase recruitment rates and artificially enhance future stock abundances (e.g. Bannister and Addison, 1998; Castro et al., 2001; Comeau et al., 2004). Within the context of a rapidly changing and often degrading environment, as the consequence of human activities in coastal areas, the viability of natural populations and harvested stock may however be under threat in some areas (Cheung et al., 2010). While the biological impacts of global warming are already relatively well known on the American lobster (e.g. Chiasson et al., 2015; Drinkwater et al., 2005), the investigation of the potential impacts of OA and other elevated pCO2 conditions on this species is still in its beginning phase (e.g. Keppel et al., 2012; Arnegård et al., 2013; Waller et al., 2016, McLean et al., 2018). Understanding the impacts of ocean acidification (OA) and other extreme elevated pCO2 events (e.g. extreme coastal events and leakages from carbon capture storage (CCS) systems) (IPCC, 2014) on the critical early life stages of the American lobster is of fundamental importance (Waller et al., 2016), particularly for the survival of post-larval (stage IV) individuals released into the wild for stock enhancement (Addison and Bannister, 2014).

Ocean acidification is the result of anthropogenic atmospheric CO2 uptake since the beginning of the industrial revolution, leading to an increase in seawater pCO2 and [HCO3-], and a lowering in seawater pH and [CO3^2-] (Zeebe and Wolf-Gladrow, 2001; IPCC, 2014). According to the IPCC (2014) under RCP 8.5 climate scenario, an increase in atmospheric CO2 to approx. 500 μatm by 2050 and 1000 μatm by 2100 will correspond to a drop in the open ocean pH from the current 8.1 to 7.95 and 7.75 respectively (Pörtner et al., 2014). In coastal areas, natural fluctuations already reach levels beyond the average global predicted values for seawater pH and pCO2 (Duarte et al., 2013; Hoffman et al., 2010), as the result of a number of processes, such as coastal influx of freshwater and human-induced coastal eutrophication that causes high respiration in the water column and in the benthic communities (Waldbusser and Salisbury, 2013).

Meanwhile, the potential construction of CCS in our oceans as an attempt to slow down the negative impacts of climate change (Blackford et al., 2009; Blackford et al., 2015a,b) may represent a local driver for seawater pCO2 increases to up to 9000 μatm due to accidental fissures caused by technical or geological accidents in these ocean-based systems. Marine benthic organisms could be significantly affected (Blackford et al., 2008; Blackford et al., 2009; Blackford et al., 2014; Donohue et al., 2012; Small et al., 2016; Widdicombe et al., 2015) since oceanic currents and stratification can easily trap and transport water masses rich in CO2 released by a CCS leak (Phelps et al., 2015), making the construction of these facilities a serious risk and an additional threat to marine biodiversity and ecosystem functioning (Blackford et al., 2015a,b; Christen et al., 2013). The recommendations and risk factors resulting from trial CCS facilities in North-Eastern Atlantic waters and land-based ones (e.g. Saskatchewan, Canada) will determine the future of CCS in North American and its adjacent deep ocean floors (https://www.saskpower.com/power-future/infrastructure-projects/carbon-capture-and-storage/boundary-dam-carbon-capture-project). Therefore, the future increase and intensification of elevated pCO2 events in coastal areas could have important repercussions for the survival of marine species with complex life cycles whose pCO2 sensitivity may vary with developmental stage, potentially creating a window of significant vulnerability if a CO2-rich water mass were to reach a species during a sensitive life stage.

Our aim was to investigate the effects of the exposure of post-larval (stage IV) of the American lobster to a seven-level gradient of elevated seawater pCO2 on the life history and physiological traits of the next life stage: stage V, the first juvenile stage. Seawater pCO2 conditions tested here ranged from current to future seawater pCO2 scenarios occurring in coastal areas and estuaries, as well as future OA conditions, and CCS leakages. Post-larval stage IV individuals of the American lobster were raised in different pCO2 levels in order to investigate the implications of pre-exposure throughout the moult process on the stage V juveniles. Survival, development, structure, metabolism, feeding rates, and energetics of the juvenile lobsters were measured along the experimental pCO2 gradient. In addition, we derived vertical profiles from in situ physical and chemical parameters (i.e. temperature, pH, and salinity) where the berried female lobsters had been collected for our study (Shediac station, NB, Canada). The vertical profiles improved our understanding of the natural pCO2 conditions experienced by lobsters throughout their life cycle.

2. Materials and methods

2.1. Physical and chemical characterisation of lobster habitat

Vertical physical and chemical profiles of female lobster habitat parameters (i.e. temperature, pH, and salinity) were measured directly from nine daily in situ seawater samples over in Shediac station (NB, Canada) using a Conductivity-Temperature-Depth (CTD) oceanographic sensor. Seawater samples were collected in 500 mL borosilicate bottles, poisoned with a saturated solution of HgCl2 and stored before being analyzed, following standard operating procedures described in Dickson et al. (2007). Stored samples were analyzed within three months of collection. The dissolved inorganic carbon (DIC) was de-terminated using gas extraction from an acidified sample with a coulo-metric quantification of the CO2 released (Johnson et al., 1985). The total alkalinity (TA) was determined by open-cell potentiometric titration with a five-point method (Haradsson et al., 1997). Certified Re-ference Material supplied by Professor Andrew Dickson, Scripps In-stitution of Oceanography, San Diego, USA, was analyzed in duplicate every 20 samples for accuracy. CTD-pH measurements were calibrated against pH values that were calculated from DIC and TA measurements from water samples. Since only nine water samples per profile were collected, the relationship between total alkalinity and salinity was calculated for each year between 2012 and 2016 in order to get total alkalinity profiles. The pCO2 and saturation states were then calculated using pH and total alkalinity.

2.2. Specimen collection, transport and maintenance

Lobstermen from the Maritimes Fishermen’s Union captured egg-bearing female lobsters (H. americanus; H. Milne Edwards, 1837) using benthic lobster traps off the coast of the Acardian peninsula (NB, Canada) in the Baie des Chaleurs of the Northumberland Strait (47°46′47″N 64°42′49″W) in May 2016. Ovigerous females were held on land in highly aerated 500 L tanks supplied with mechanically and biologically filtered seawater from the Baie des Chaleurs (T = 20 °C, pH = 8.0, salinity = 28) at the Hornorus Inc.-Coastal Zones Research Institute (Shediac, NB). Hatched individuals were transferred to 20 L kreissels containing recirculated mechanically and biologically filtered seawater and fed frozen Artemia (Hikari, Kyorin Co. Ltd, Kanschai City, Japan) twice daily. Larvae were reared communally to the stage IV. Immediately after moulting to stage IV, 2000 stage IV post-larval lobsters (considered 0 days old for this experiment) were transported by car in aerated coolers (Coleman 48-Quart Cooler, Brampton, ON, Canada) to Fisheries and Oceans Canada’s Biological Station laboratory in Saint Andrews (NB, Canada) within 6 h. Seawater conditions were monitored during transport to be maintained as stable as possible around culturing conditions.
In order to test the impacts of post-larval exposure to elevated pCO$_2$ levels on the life history and physiology of juvenile lobsters, post-larval (stage IV) lobsters were exposed to a gradient of current and future pCO$_2$ scenarios. The latter were chosen based on current global ocean conditions (400 μatm) to predicted pCO$_2$ values between now and the end of the century (600, 800, 1000 μatm, IPCC, 2014), ecologically relevant coastal pCO$_2$ fluctuations (1200 μatm, Waldbuscher and Salisbury, 2013), and potential trends achieved from industrial acci-dents involving carbon capture storage (CCS) leakages (2000 and 3000 μatm, Rastelli et al., 2016).

Upon arrival, specimens were transferred carefully but rapidly to individual basket-like containers (3.5” in diameter and 3” in height, Net Pots, Canadian Wholesale Hypotronics, Elie, MN, Canada) that were rafted at the water surface of 500 L tanks (1 m diameter x 0.70 m depth). The dissolved oxygen content, pH, and temperature inside and outside the pots were monitored with test specimens in preliminary trials. As parameters were comparable inside the lobster containers and in the surrounding surface seawater in the experimental tanks, we confirmed that pH and other environmental parameters were not affected by lobster respiration. Post-larval stage IV lobsters were split into the 24 CO$_2$-enriched tanks (four true replicate 500 L tanks per pCO$_2$ level were used), and four tanks that represented the control treatment, with one individual per container, corresponding to 15 indiv. per 500 L tank, and to 60 indiv. per pCO$_2$ condition.

Feeding was converted to blocks of herring, Clupea harengus, to facilitate food management, and because of the higher nutritional value of fish relative to Artemia. Following the transfer into the pCO$_2$ treatments, the lobsters were given 72 h to adjust to the new container, diet, and treatments. Dead individuals were replaced within the 72 h period before starting any experimental measurements or observations. The lobsters that replaced dead specimens had between 24 and 48 h less exposure time than the lobsters that survived the 72 h acclimation time, which we do not believe had a major impact on results obtained throughout the 40 d exposure period. The moult to the juvenile phase (stage V) of each lobster determined the end of the post-larval inter-moult period, and the end of the exposure period per individual.

Incoming water was supplied from Brandy Cove in the Passamaquoddy Bay (NB, Canada), passed through a series of sand (20 μm) and a UVe filters, heated at 18 °C to mimic the optimal temperature for growth and survival of juvenile lobsters from Baie des Chaleurs (Daoud et al., 2014), and bubbled with ambient air. The treated water was re-oxygenated as it entered two air-bubbled header tanks, responsible for filling each experimental tank at 2 L min$^{-1}$. Experimental tanks were also equipped with an air bubbler to maintain oxygen saturation, and a water pump (Maxi-Jet 400, Marineland Aquarium Products, Cincinnati, OH, USA) to facilitate water mixing and water chemistry homogeneity throughout the tanks and the lobster containers. Daily measurements of salinity (see next section for daily measurements) remained relatively constant throughout the experiment at 31.5 (± 0.02) on average.

The pCO$_2$ treatments were maintained using a pH regulation system (IKS, AquaStar, Karlsbad, Germany) equipped with a glass electrode per tank to measure pH every 5 min. From the measured pH in each tank, the IKS system individually released CO$_2$ gas into the seawater, main-taining the desired pH level for all six conditions tested in this study. IKS pH levels were calibrated daily from independent pH measurements made in each of the tanks (see next section for daily measurements).

### 2.3. Experimental design and pCO$_2$ manipulation system

<table>
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<th>pCO$_2$ (μatm)</th>
<th>n</th>
<th>111</th>
<th>112</th>
<th>116</th>
<th>106</th>
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<td>18.10 ± 0.12</td>
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<td>600</td>
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<td>800</td>
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<td>18.12 ± 0.089</td>
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<td>1000</td>
<td>18.10 ± 0.12</td>
<td>18.12 ± 0.089</td>
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<td>2000</td>
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**Table 1**

Mean (± SE) measured and calculated (indicated with an asterisk *) seawater physico-chemical parameters in control and experimental conditions over the course of the experiment. The number of daily measurements: n, temperature: T; salinity: Sal; dissolved oxygen, p in the total scale: pH; CO$_2$: partial pressure: pCO$_2$; bicarbonate ion concentration: HCO$_3$; carbonate ion concentration: CO$_3^{2-}$; total dissolved inorganic carbon: DIC; total alkalinity: TA; saturation state of seawater with respect to aragonite: Ω$_{ara}$; saturation state of seawater with respect to calcite: Ω$_{cal}$.
2.5. Determination of survivorship and development periods

Individual lobsters were checked daily to record mortalities and evidence of a stage change (e.g. shed carapace). Dead individuals were removed immediately to avoid bacterial accumulation and contamination in the tanks. The starting moult date presumed for stage IV lobsters was the same day as their arrival. The intermoult period (IP) to the stage V moult was then determined in number of days using the following formula:

\[ IP = M_{IV} - M_{V} \]  

Where \( M_{IV} \) is the moulting date at stage IV and \( M_{V} \) is the moulting date at stage V observed in the experimental tanks.

2.6. Determination of feeding rates and routine metabolic rates

Routine metabolic rates (RMR), defined here as oxygen consumption rates during rest activity when lobsters are mostly immobile and not disrupted by light, noise, or physical disturbances, were used as proxies of metabolism for the juvenile lobsters at stage V across \( pCO_2 \) condition. Feeding rates (FR) and RMR were determined for each \( pCO_2 \) condition in eight (i.e. two per tank) freshly moulted stage V individuals, which moulted the same day, per seawater condition.

In order to determine FR, individuals were not fed for 24 h and then fed pre-weighed and seawater-soaked blocks of herring as their food. Non-ingested food was removed and immediately weighed after 1 h in the containers with each selected individual in order to calculate the FR per individual as mg \( h^{-1} \) wet body mass \( g^{-1} \) as follows:

\[ FR = \frac{\Delta H}{WBM \times 24 \times t} \]  

Where \( H \) is the mass (mg) of consumed herring, \( WBM \) is the wet body mass (g) for each individual stage V juvenile lobster, and \( t \) is the elapsed time during food consumption.

Hereafter, the same individuals were deprived of food again for 24 h to prevent effects of specific dynamic action on the metabolic rate measurements (Speakman and McQueenie, 1996). Following this procedure, single lobster individuals from each tank were carefully placed in 16 mL borosilicate glass vials (Vials w/Cap 1.5 drams, VWR International Ltd, Ville Mont-Royal, QC, Canada). Each vial was sealed with mesh and a rubber band in order to prevent the lobster from escaping, while allowing for sufficient water flow in the vial during a 12 h period. During this time, the lobster could adjust to the vial, thus reducing stress from the handling and introduction to the new environment. One control vial per \( pCO_2 \) condition was used to investigate the potential microbial respiration in the seawater. These contained seawater from the tested tank and were manipulated identically to all other vials. Following the 12 h adjustment period, the mesh was replaced by lids in order to seal the vials with the appropriate seawater \( pCO_2 \) as in the corresponding experimental tanks. The vials containing both juveniles and the blank samples were moved using an 18 °C water bath to an infrared-illuminated, temperature-controlled room at 18 °C. Each vial was equipped with a stirring rod that was isolated from the lobster by a mesh to ensure homogenous oxygen concentrations throughout the vials once sealed shut. All sealed vials were kept in water basins over magnetic stirrer plates (Mix 15, 2 Mag AG, Munich, Germany) to acti-vate stirrers to mix water within chambers.

Preliminary trials showed linear oxygen consumption above 70 % \( O_2 \) saturation, and RMR measurements were stopped before reaching such limit. \( O_2 \) concentration (\( \mu L \cdot L^{-1} \)) were measured using a non-invasive fiber-optic system (Fibox 4, PreSens, Regensburg, Germany) composed of an optical fiber, a temperature probe and reactive oxygen sensor spots glued inside the vials and calibrated according to the manufacturer instructions with 0 and 100 % buffers. Measurements were recorded at the beginning and end of the incubation period as the oxygen consumption has been proved linear.

RMR (\( \mu mol \ O_2 \ h^{-1} \)) for individual stage V juvenile lobsters was calculated following Eq. (2) and corrected by the blank control vials to remove oxygen consumption due to microbial activity.

\[ RMR_{vial} = \frac{\Delta O_2 \times V}{\Delta t} \]  

Where RMR\(_{vial} \) is the oxygen consumption inside the vial, \( \Delta O_2 \) is the difference between the initial and final \([O_2] \) (\( \mu mol \ O_2 \ h^{-1} \)), \( V \) is the volume of the vial (L), and \( \Delta t \) is the incubation time (h) for each individual stage V juvenile lobster.

And after each measure, the lobster was photographed using a microscope (M80, Leica Microsystems GmbH, Wetzlar, Germany) at x7.5 magnification with a picture acquisition system (IC80 HD, Leica Microsystems GmbH) for morphometric measurements, blotted dry with wipes (KimWipe, Kimtech Science, Brampton, ON, Canada) and weighed to obtain the wet body mass. Specimens were then dissected using non-metal tools (White Plastic Tweezers, Swiss Precision Instrument Inc., Garden Grove, CA, USA) into two sections (cephalothorax and claws, and abdomen and telson), which were frozen and individually stored at -80 °C using liquid nitrogen to preserve the carapace and tissues to measure the mineral contents and enzyme activities in further analyses.

2.7. Determination of morphometrics

To investigate effect of elevated \( pCO_2 \) on growth and body proportions, the sampled specimens used for FR and RMR measurements were photographed and analyzed using ImageJ Software (ImageJ 1.45s, National Institute of Health, Madison, WI, USA). Seven morphological characteristic lengths were measured (see S2 in Supplementary Materials). The rostrum length (1), starting from behind the eye to the tip of the rostrum structure, the dominant claw's pollex (2), starting from the joint to the tip of the structure, and the dactylus (3), starting from the joint to the tip of the structure. The thorax length (4), from the junction to the abdomen to the junction with the rostrum, the abdomen length (5), starting from the junction of the first segment to the thorax to the tip of the last segment that joins with the telson. Finally, the telson length (6), starting at the junction with the abdomen to the tip of the structure was measured. The total lobster length was calculated from adding the measured lengths of structures 4, 5, and 6. The mea-surements of cephalothorax and abdomen lengths were also used in order to determine ratio changes of these two sections across \( pCO_2 \) le-vels.

2.8. Determination of carapace mineral content

The effects of low \( pCO_2 \) levels on carapace mineral content were determined using the whole cephalothorax carapace of all stage V ju-venile lobsters at all \( pCO_2 \) conditions. Chemical analyses on the car-apace of the stage V juvenile lobsters were performed at the Laboratoire de Chimie Marine et Spectrométrie de Masse at the Institut des Sciences de la Mer de Rimouski (ISMER) at the University of Quebec in Rimouski (Rimouski, Canada). Previously frozen cephalothorax carapace samples were removed from the rest of the upper body using plastic dissection tools (White Plastic Tweezers, Swiss Precision Instrument Inc.) to avoid element contamination from metal tools and freeze dried (T = −50 °C) for 12 h to remove any residual moisture (Freeze Freeze Dry Systems, Labconco, Kansas City, MO, USA). Samples were then weighed on a high precision microbalance (MX5 Analytical Micro-balance, Mettler Toledo). Hereafter, they were digested in a mixture of pure nitric acid and hydrogen peroxide (375 mL: 125 mL) (TraceSelect grade, Sigma Aldrich, St. Louis, MO, USA) at room temperature for 24 h and after short periods warming in a water bath. Samples diluted in ultrapure water...
Fig. 1. Time series of physical and chemical seawater vertical profiles over the months of May to November, during which the highest abundance of post-larval (stage IV) and juvenile (stage V) American lobsters occur in the wild in this region. The vertical profiles of salinity (sal), and temperature (T °C) were obtained from in situ CTD-measurements, while the pH in the total scale (pH$_T$), CO$_2$ partial pressure: pCO$_2$ (μatm), saturation state of seawater with respect to calcite (Ω$_{cal}$) and aragonite (Ω$_{ara}$) were calculated using total alkalinity (TA), dissolved inorganic carbon (DIC), and pH measurements from 2013 (top) and 2015 (bottom) seawater samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
water were analyzed by inductively coupled plasma (ICP) interfaced to a quadrupole mass spectrometer (MS - ICP-MS, Agilent 7500c with micro flow nebulizer, Agilent Technologies, Santa Clara, CA, USA) equipped with an autosampler (ASX 520, Teledyne CETAC, Omaha, NE, USA). Element signals were acquired for 200 msec per mass, and three acquisitions were realized. Element quantification ([Sr$^{2+}$], [Ca$^{2+}$], [Mg$^{2+}$], [Na$^+$], and [K$^+$]) was performed in normal mode with a ten-point external calibration using multi-element reference material (Multi-Element 5, Sigma Aldrich, St. Louis, MO, USA) with a concentration range between 0.10 and 50 for [Sr$^{2+}$] and 1.00 and 500 ng mL$^{-1}$ for other elements. Performances of the method and instrument stability were assessed repeatedly by the analysis of a quality control solution of known metal concentration during the course of analysis coupled with a procedural blank. Control of the system, acquisition and data processing were carried out with the Agilent ChemStation software (Agilent Technologies).

2.9. Assays of energy metabolism enzymes

Frozen abdomen tissues were separated from the carapace in a polystyrene petri-dish, before being weighed and mixed in two 2 mL microtubes over ice with one parts tissue and 50 parts potassium phosphate buffer (100 mM, pH 7.0 for lactate dehydrogenase (LDH) and 8.5 for the electron transport system (ETS)). Tissues were homogenized in the respective microtubes over ice using a mechanical homogenizer made of rubber that was cleaned with alcohol, rinsed with distilled water, and dried between each sample measurement. After being cen-trifuged at 10000 g for 5 min at 4 °C, aliquots were transferred to clean microtubes for each individual to quantify ETS and LDH activity. Duplicate wells for the diluted homogenate of each individual lobster were mixed in a polystyrene well plate (96-Well Assay Microplate, Fisher Scientific) with reaction solutions for the LDH and ETS and passed through a well plate reader (2104 EnVision Multilabel Plate Reader, Perkin Elmer Inc, Waltham, MA, USA) providing the reaction slopes of the solution absorbance (at 340 and 490 nm, respectively). All well readings were performed at 18 °C to mimic seawater experimental conditions. LDH and ETS enzyme activities (U mg protein$^{-1}$) were calculated with the extinction coefficient of 6.22 mL cm$^{-1}$ µmol$^{-1}$ for NADH and 15.9 mL cm$^{-1}$ µmol$^{-1}$ INT. Protein content was determined for each tissue dilution using the Bicinchoninic Acid Protein Assay Method (Krohn et al., 1985) with BSA for a standard curve. Further, the effects of increasing pCO$_2$ level on the organisation of the energy metabolism pathways was examined through the ETS:LDH ratio, outlining the amplitudes of metabolic remodelling.

2.10. Statistical analyses

A best-fit curve approach was used to determine the effect of exposure to increasing pCO$_2$ levels on the life history and physiological traits of stage V juvenile lobsters. Each trait was tested under linear, logarithmic, two-order polynomial, and three-order polynomial regressions in order to determine the regression type that provided the best representation of the trends observed. Regressions were drawn using the recorded data from all individual lobsters used in the experiment for the survival and IP, and from all sampled individuals for the FR, RMR, morphology, carapace mineral content, ETS, LDH, and ETS:LDH.

Data were first tested for the assumption of normal distribution with a Shapiro-Wilk test, and then verified for variance homogeneity using Levene’s test. Once assumptions were met using a global model that included all measured variables throughout the experimental period, the variable “tank”, representing the four replicate tanks for each individual lobster, was tested in the complete model as a random factor, and removed in all cases because it was never found to be significant ($P > 0.05$). Three significant covariates ($P < 0.05$) were included in final models for most analyses to take into account differences between individuals and scientific methods: (1) wet body mass was used as a covariate for FR, RMR, and mineral quantification, (2) total body length was used as a covariate for all morphological trait lengths except for the total body length and the cephalothorax-abdomen ratio, (3) temperature recorded during the O$_2$ measurements was used as a covariate for RMR. Out of the statistically different models ($P > 0.05$), the one with the best AIC and R$^2$ was selected as the final best-fit curve for each measured trait.

3. Results

3.1. In situ physical and chemical profiles of lobster habitat

The physical and chemical environment in which post-larval American lobsters are present in nature, between May and November, ranges from the surface to the seabed throughout the water column (Factor, 1995; Spicer and Eriksson, 2003; Wahle, 2003; Wahle et al., 1991). The vertical profiles of temperature, salinity, pH, pCO$_2$, and seawater saturation states with respect to aragonite and calcite (Ω),

<table>
<thead>
<tr>
<th>Year</th>
<th>Depth (m)</th>
<th>Sal</th>
<th>T °C</th>
<th>pH$_T$</th>
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</table>
indicate that the environment in which stage IV post-larvae evolve is varying, and highly fluctuating (Fig. 1, Fig. 2) in time and depth.

3.2. Survivorship and development periods

The effects of exposure to the seven-level elevated pCO2 gradient on mean lobster survivorship are presented in Fig. 3 and summarised in Table 2. All mortalities recorded were a result of mortality during or immediately following the moult process of stage IV to stage V. Stage V lobster juvenile's mean survival was highest at the 600 μatm pCO2 level and lowest at the 1200 μatm pCO2 level (60.52 ± 8.04 and 39.02 ± 8.08 %, respectively, see Table 2). Mean survival decreased significantly with increasing pCO2 (F 2, 264 = -2.043, P = 0.041), which was best described by a linear regression (Fig. 3).

Stage V intermoult period (IP) (mean ± SE, Fig. 2, Table 2) was found to be the shortest at the 400 μatm pCO2 level, and increased significantly with increasing pCO2 (F 1, 269 = 9.928, P = 0.002, R2 = 0.037, Adjusted-R2 = 0.033), which was best described by a linear regression (Fig. 4).

3.3. Feeding rates and routine metabolic rates

The mean feeding rates (FR) of stage V lobsters (Mean ± SE, Fig. 5, Table 2) was significantly affected across the pCO2 gradient investigated (F 1, 35 = 3.376, P = 0.046, R2 = 0.170, Adjusted-R2 = 0.120), having the lowest rates at the 800 μatm pCO2 level and highest at the 2000 μatm pCO2 level: 74.93 ± 24.50, 241.19 ± 40.33 mg g−1 h−1, respectively. The relationship between seawater pCO2 and FR was best described by a second order polynomial regression (Fig. 5). In more detail, FR first increased with increasing seawater pCO2 between 400 and 1000 μatm followed by a plateau between 1000 and 2000 μatm, and finally FR decreased between 2000 and 3000 μatm.

Mean routine metabolic rates of stage V juvenile lobsters (see figure S1 in Supplementary Materials, Table 2) ranged between 1.0647 ± 0.185 μmol O2 h−1 under control conditions and 0.615 ± 0.141 μmol O2 h−1 at 3000 μatm of seawater pCO2. However, no significant effect of seawater pCO2 on this variable was detected (F 1, 47 = 1.580, P = 0.215, R2 = 0.166, Adjusted-R2 = 0.109).

3.4. Morphometrics

Mean morphological trait of the juvenile lobsters showed significant effects of pCO2 level for the abdomen length, decreasing with pCO2 level (F 1, 45 = 7.605, P = 0.00851, R2 = 0.820, Adjusted-R2 = 0.811), for cephalothorax length (CL), increasing steadily with pCO2 level (F 1, 45 = 6.446, P = 0.0148, R2 = 0.512, Adjusted-R2 = 0.489), and for telson length (TL), decreasing with pCO2 level (F 1, 45 = 0.133, P = 0.00271, R2 = 0.802, Adjusted-R2 = 0.854). These relationships were each best described by a linear regression (Fig. 6). Stage V lobster juvenile mean cephalothorax-abdomen length ratio (CL:AL) increased with increasing pCO2 level (F 1, 45 = 6.916, P = 0.0117, R2 = 0.136, Adjusted-R2 = 0.116), which was best described by a linear regression (Fig. 6).

3.5. Carapace mineral content

Due to sample contamination at the 3000 μatm pCO2 level during transportation, enzyme activities were not measured at this level. Of the mineral component tests, only [Mg2+] increased significantly with increasing pCO2 (F 1, 35 = 4.611, P = 0.040, R2 = 0.174, Adjusted-R2 = 0.123), which was best described by a linear regression (Fig. 7). Stage V lobsters’ mean [Mg2+] were lowest at the 800 μatm pCO2 level and reached a maximum at the 2000 μatm pCO2 level (Table 2).
addition, there were no significant differences in carapace \([\text{Sr}^{2+}], [\text{Ca}^{2+}], [\text{Na}^+], \text{and [K}^+]\) quantifications, nor for the \([\text{Ca}^{2+}]; [\text{Mg}^{2+}]\) at the different \(p\text{CO}_2\) tested.

3.6. Electron transport system (ETS), lactate dehydrogenase (LDH), and ETS:LDH

Due to sample contamination at the 3000 \(\mu\text{atm} \text{pCO}_2\) level during transportation, enzyme activities were not measured at this level. Stage V lobster mean ETS activity was the lowest at the 400 \(\mu\text{atm} \text{pCO}_2\) level and highest at the 1200 \(\mu\text{atm} \text{pCO}_2\) level (18.3 \(\pm\) 1.05, 29.8 \(\pm\) 3.93 U mg protein\(^{-1}\), respectively). The ETS activity increased significantly with increasing \(p\text{CO}_2\) level (\(F_{1,36} = 19.488, P < 0.001, R^2 = 0.351\), Adjusted-\(R^2 = 0.333\), Fig. 8). Stage V lobster juvenile mean LDH activity was the highest at the 400 \(\mu\text{atm} \text{pCO}_2\) level and lowest at the 2000 \(\mu\text{atm} \text{pCO}_2\) level (26.1 \(\pm\) 1.28, 21.1 \(\pm\) 1.22 U mg protein\(^{-1}\), respectively). The LDH activity decreased significantly with increasing \(p\text{CO}_2\) levels (\(F_{1,36} = 7.219, P = 0.0109, R^2 = 0.167\), Adjusted-\(R^2 = 0.144\), Fig. 6). ETS:LDH was the smallest at the control \(p\text{CO}_2\) level at 0.718 \(\pm\) 0.07 (see Table 2), and highest at the highest \(p\text{CO}_2\) level measured at 1.36 \(\pm\) 0.05, increasing significantly with \(p\text{CO}_2\) level (\(F_{1,36} = 59.809, P < 0.001, R^2 = 0.624\), Adjusted-\(R^2 = 0.614\), Fig. 8).

4. Discussion

Life history and physiological responses of stage V juvenile American lobsters, an ecologically and economically important marine species with a complex life cycle, was examined for the first time under a seven-level \(p\text{CO}_2\) gradient ranging from 400 to 3000 \(\mu\text{atm} \text{pCO}_2\) (pH: 8.1–7.1). The negative impacts of the exposure to increasing seawater \(p\text{CO}_2\) on life history and physiological traits of juvenile lobsters are largely linear. In addition, the relationships between increasing \(p\text{CO}_2\) level and survival, development, and morphology appear to be ex-plained by the observed increase in mitochondrial content and changes in feeding rates (FR) under elevated \(p\text{CO}_2\) conditions. Our discussion focuses on the potential impact of ocean acidification (OA), extreme \(p\text{CO}_2\) events and potential leakages from carbon capture storages (CCS) on future benthic recruitment of the American lobster, and the con-sequent implications on the viability of the connected fishing industry.

4.1. Impact of increasing seawater \(p\text{CO}_2\) level on life history traits

Juvenile lobster survival decreased by 24% with increasing \(p\text{CO}_2\) level. Reduced juvenile survival is a common occurrence among early life stages of crustaceans exposed to multiple global change drivers including elevated \(p\text{CO}_2\) levels: e.g. the European lobster, \(H. \text{gammarus}\), at 1100 and 9000 \(\mu\text{atm}\) (Small et al., 2016), the porcelain crab, \(P. \text{cinctipes}\), at 1400 \(\mu\text{atm}\) (Carter et al., 2013; Ceballos-Osuna et al., 2013), the edible crab, \(C. \text{pagurus}\), at 7.9 and 7.06 pH levels (Metzger et al., 2011).
increased mortality risks do to exposure to abiotic fluctuations, increasing the mismatches between favourable environmental conditions and suitable substratum for recruitment, and predation.

While the total length of the juveniles remained unaffected by the exposure to elevated $p$CO$_2$, the proportions of measured morphological traits differed significantly, although slightly, with increasing seawater $p$CO$_2$. Similarly, the carapace size of the juvenile blue king crab, *P. platypus*, was affected by exposure to OA seawater $p$CO$_2$ levels (1600 $\mu$atm), displaying slightly smaller carapace size at specific developmental stages (Long et al., 2017). Another study on six-month old juvenile American lobsters indicated that total length decreased after 90–120 days exposure to 2000 $\mu$atm $p$CO$_2$ conditions (McLean et al., 2018). In comparison, the juvenile lobsters from this study experienced proportional length alterations, but perhaps because the lobsters were exposed for the duration of the entire post-larval stage, total length was not yet affected.

The thorax is the region where critical structures are located, such as the respiratory and circulatory systems, the primary digestive system, the central nervous system, as well as the reproductive organs (Bliss, 1983). Because the thorax holds a major part of these funda-mental systems and organs, it is an important region for respiration and gas exchange functions that help maintain acid-base balance, for di-gestion, reproduction, and nervous system functions that are also im-perative for the species survival.

Furthermore, the abdomen and telson are important structures for swimming and manoeuvrability (Factor, 1995). A reduction in the length of the latter two structures relative to enlarging the thorax may represent a trade-off in morphological pro-portions, perhaps in order to increase the gas-exchange capacity by enlarging the area responsible for maintaining adequate respiratory and cardiovascular functions, and thus enabling a more effective main-tenance of internal homeostasis (Bliss, 1983). In fact, the juveniles of this study were able to maintain unchanged metabolic rates across the entire $p$CO$_2$ gradient tested, which may be helped by the enlarged thorax relative to the abdomen and telson at high $p$CO$_2$: levels: see also section below on metabolic rates. Besides the compensatory effects of the enlarged thorax, shortening the abdomen and telson could have negative functional repercussions (e.g. on predator evasion) from an ecological point of view. If these patterns persist into adulthood, a shortened abdomen may also have important economical implication for the lobster fisheries. It will be interesting in the future to investigate this subject further, since there is no evidence to support or oppose the possibility that $p$CO$_2$ effects on morphology might persist or be reverted throughout development and into adulthood.

The mineral composition of the carapace of the juveniles did not drastically change (Fig. 5), as only [Mg$^{2+}$] content linearly increased with exposure to increasing $p$CO$_2$. Small et al. (2010) also reported an increase in carapace [Mg$^{2+}$] in 3000 and 20 000 $\mu$atm $p$CO$_2$ conditions in the chelae of adult velvet swimming crabs, *Necora puber*, and in 1200 $\mu$atm for European lobster larvae (Arnold et al., 2009). It appears that such an increase in carapace [Mg$^{2+}$] might increase the proportion of more soluble polymorphs that are high in Mg content, potentially subjecting juvenile lobsters to preferential carapace dissolution under future elevated $p$CO$_2$ (Ries, 2011). Furthermore, this could lead to negative impacts on carapace structure and hardness, but also ventilation, food acquisition, mobility and defense of juveniles of the *H. americanus*. In contrast with our results, the European lobster exposure to $p$CO$_2$: levels above has been associated with a decrease in carapace [Mg$^{2+}$] in both larvae and juveniles in $p$CO$_2$: conditions above 1000 $\mu$atm (Agnalt et al., 2013; Small et al., 2016). Variability found in the mineralogical responses to elevated $p$CO$_2$: further support the idea that species-specific differences exist for the impacts to future OA and CCS leakages in phylogenetically closely related species (e.g. Calosi et al., 2013).

Altogether, carapace structure and mineralisation are not strongly affected by the exposure to elevated $p$CO$_2$: conditions and the body proportion favouring the maintenance of the carapace length relative to the abdomen length may be expected to enable the maintenance of
adequate respiratory and cardiovascular functions, and thus appropriate metabolic rates. However, the significant decrease in survival and extension of the IP along with the increase in $p\text{CO}_2$, both considering OA and CCS scenarios, show patterns of vulnerability in life history traits apparently being explained by underlying physiological impacts on cellular metabolism and energetics.

4.2. Impact of increasing seawater $p\text{CO}_2$ on metabolic rates, feeding rates, and energy metabolism

Routine metabolic rates (RMR) were maintained across the seven-level seawater $p\text{CO}_2$ gradient tested. The ability to maintain oxygen consumption rates under elevated $p\text{CO}_2$ was also reported, for example, in larvae of the American lobster subjected to 750 μatm (Waller et al., 2016), in larvae and juveniles of the European lobster exposed to 1100 μatm $p\text{CO}_2$ (Small et al., 2015), six-month-old juveniles of the European lobster exposed to 1100 μatm (Small et al., 2016), and juvenile porcelain crabs, Petrolisthes cinctipes subjected to 7.6 pH levels (Carter et al., 2013) when exposed to future OA conditions. In the present study, this ability does not seem to explain any costs associated with elevated $p\text{CO}_2$ and it is possible that the increase in cephalothorax length enabled the lobsters to maintain its respiratory capacity across $p\text{CO}_2$ conditions. Differently from RMR, the impacts of increasing sea-water $p\text{CO}_2$ on feeding rates (FR) vary across the gradient tested. In italy, FR increased with increasing $p\text{CO}_2$, a response previously observed in juvenile American lobsters under exposure to end-century $p\text{CO}_2$ scenarios (Waller et al., 2016). However, under $p\text{CO}_2$ levels mimicking CCS leakages, the current study reported a decrease in FR towards control conditions, an effect also reported for the juveniles of the European lobster in CCS conditions (Small et al., 2016). Here, reduced FR under CCS conditions either suggests that nutritional requirements for growth are satisfied at this rate, or that the ingestive ability of juvenile lobsters cannot increase under extreme $p\text{CO}_2$ conditions and there is an excess production of waste. Overall, elevated $p\text{CO}_2$ involves limited effects on RMR and FR. This suggests that the potential costs of $p\text{CO}_2$ appear at a higher level of biological complexity that was not explored in this study.

Juvenile lobster exposure to increasing $p\text{CO}_2$ levels elicited a rela-tively strong positive linear response on the enzymatic activity in the mitochondrial electron transport system (ETS) and a slightly weaker negative linear response on the lactate dehydrogenase (LDH) activity. In marine species, ETS activity can be used as a proxy for aerobic respiration related to the energy budget of an organism, and LDH as an enzyme related to anaerobic glycolytic capacity (Tonn et al., 2016). Thus, the significant increase in ETS and decrease in LDH observed here with increasing $p\text{CO}_2$ are indicative of a change in the energy metabolism apparatus in OA and CCS conditions. This is even clearer when
looking at the stronger positive linear response derived from the ETS:LDH, which further suggests a organisation of the energy metabolism that favours aerobic capacity over anaerobic capacity during the stage-long acclimation of juvenile lobsters to elevated pCO2 levels.

The metabolic organisation could be a result of a mito-hormetic response in stressful conditions (Yun and Finkel, 2014) that can be brought on by differential gene expression at elevated pCO2 levels (Ristow and Schmeisser, 2014; Schulz et al., 2007). In such conditions, mitochondrial oxidative stress can trigger cytosolic signalling pathways that culminate in the multiplication and increment of mitochondrial content in order to meet the energy requirement imposed by the ex-posure to elevated pCO2 (Valero, 2014). This hypothesis however re-quires further validation via the examination on the impacts of elevated pCO2 levels on oxidative stress: production rate of ROS and markers of oxidative stress as products of peroxidation of lipids, carbonylation of proteins or oxidation of nucleotides (8-oxoguanosine). Additionally, these measurements should be conducted with parallel tests of mtDNA content, which is expected to increase as a response to oxidative stress.

In a study that examined the effects of an end-century OA pCO2 level (710 μatm) on the European lobster’s larval stages (Rato et al., 2017), LDH and ETS activity displayed no significant differences throughout development. However, DNA damage was significantly higher in stage III larvae that were exposed to high pCO2, corresponding to increased ROS levels in OA conditions. Under elevated pCO2 conditions, DNA damage resulting from elevated ROS levels were also found in the decapod Littopenaeus vannamei (Wang et al., 2009), and reduced anti-oxidant levels were reported in crustacean copepods Calanus sinicus (Zhang et al., 2016). Although it was not measured in the American lobster juveniles in this study, oxidative stress and DNA damage could partly explain the lower survival rates at elevated pCO2 conditions.

The increase in the ETS activity in the juvenile lobsters in this study may indicate a higher metabolic stress that would trigger stronger responses leading to metabolic remodelling. However, the impact of these responses on energy use within the juvenile lobsters under elevated pCO2 levels are unknown and cannot be explained by RMR. Together with negative impacts on the life history traits with increasing seawater pCO2 levels, the mito-hormetic response can likely maintain essential physiological functions for repair and maintenance with increasing pCO2 levels. Unfortunately, beyond intermediate pCO2 levels used in this study, this response may not be efficient, making the most elevated pCO2 levels lethal for the majority of stage V juvenile lobsters.

4.3. Integrating laboratory biological responses to in situ lobsters

habitat physical and chemical profiles

The vertical profiles throughout 2012 and 2015 (Fig. 8), and more specifically between the months of May and November (Fig. 7), indicate that stage IV lobster post-larvae encounter higher pCO2 values as they naturally swim to the benthos in order to settle for juvenile recruitment. Following the post-larval settlement stage, it is apparent that newly moulted juvenile lobsters in the benthos may be subject to pCO2 values that are currently more already elevated than the IPCC pCO2 predic-tions for 2050 in the open ocean (2014, RCP 2.6, RCP 4.5, RCP 6.0). It is possible that the effects of the first levels of the pCO2 used in this study are less noticeable on the life history and physiology of the specimens because such pCO2 values (between 400 and 800 μatm) are already experienced at this life-stage in the wild. Therefore, the individuals may be already adapted to these pCO2 levels at the post-larval and juvenile phase in development. The pCO2 and saturation state (Ωsat and Ωcalc) values become biologically harsher for the crustacean with depth in the water column with respect to the acid-base regulation capacity (Whiteley, 2011), and consequently, on the energy budget and meta-bolism (Carter et al., 2013). In the future, the potential effects of low pH/elevated pCO2 levels will increase with time for the American lobsters, as elevated pCO2 levels become a prominent chemical threat.

4.4. Conclusion

In conclusion, increasing seawater pCO2 has mostly negative implications on juvenile American lobsters, severely decreasing survival rates. Aerobic metabolism capacity increases considerably, but the resulting efficiency of the energy metabolism does not appear to support the costs imposed by exposure to elevated pCO2 levels. Other impacts of elevated pCO2 levels include slower development, altered feeding-behaviour, and a significant transformation of the body proportions and carapace mineral content. These are likely compensatory effects to at-tempt to sustain fundamental mechanisms necessary for survival.

Relative to OA, CCS leakage implications on juvenile lobsters display the most serious biological threats, foreshadowing a high-risk potential on early lobster life history if CCS systems were to be constructed in the North-West Atlantic shores linked to American lobster habitats. By preventing the successful completion of consecutive developmental phase (Byrne, 2011), the negative impacts of stage-long exposure to increasing seawater pCO2 on juvenile lobster survival may threaten the species recruitment success over time. Thus, the implication of the exposure to OA, CCS leakages, and other low pH drivers on lobster juveniles may negatively reduce future population abundances along American and Canadian shores especially in coastal environments experiencing drastic drops in pH in the water column. By incorporating the potential of low pH impacts on American lobster recruitment, it may be possible to better predict population variability and pH vul-nerability hot-spots along the North-West Atlantic coast. This could significantly improve the future plans for stock management projects and better prepare local fishermen for the future of this crucial Can-dian crustacean.

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Conflicts of interest

The authors confirmed that there are no competing interests.

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