Presently Atmospheric CO­2 levels are over 400 ppm Dunn *et al.* (2020) well above preindustrial levels of approximately 275 ppm(Macfarling *et al.*, 2006). The world’s oceans absorb as much as one third of annual anthropogenic CO2 (Doney *et al.*, 2009), causing an increase in oceanic pCO2. The increased pCO2  drives a decrease in pH, causing oceanic pH to decline from its pre-industrial revolution level of 8.2 to a current average below 8.1, in a process termed ocean acidification (OA) (Caldeira and Wickett, 2003)

Initial studies of the impact of OA on marine organisms focused largely on challenges faced by calcifying organisms (Fabry *et al.*, 2008). However more recently there has been a number of studies that examine how OA impacts the physiology of an array of organisms. Changes in pH have been shown to impact respiratory physiology (Miller, 1985; Bridges, 1995; Widdicombe and Spicer, 2008; Seibel, 2016). Negative impacts to respiratory physiology make it more difficult to obtain oxygen from the environment and may limit aerobic energy production. This has been shown in crab, squid, fish, and sipunculids (Pörtner *et al.*, 1998; Langenbuch and Portner, 2002; Metzger *et al.*, 2007; Rosa and Seibel, 2008; Munday *et al.*, 2009; Walther *et al.*, 2009).

Studies within cephalopods have shown various, and sometimes conflicting, responses to hypercapnia and the resulting low pH. At environmentally relevant ranges of 700-1700 μatm adult Cuttlefish *Sepia officinalis* show no aerobic metabolic response (Gutowska *et al.*, 2008) whereas embryonic *S. officinalis* showed an increase in routine metabolism (Rosa *et al.*, 2013). In squid, the vertically migrating epipelagic squid *Dosidicus gigas* showed metabolic depression (Rosa and Seibel, 2008) whereas the near-shore reef squid *Sepioteuthis lessoniana* showed no change in metabolism. The benthic octopus *Octopus rubescens* had a short term (24 hour) increase in metabolism which then returned to pre-exposure levels (Onthank *et al.*, 2020).

In studies where P­CO2 is pushed above 2,000 μatm there is a continued variation in response. Cuttlefish increase calcification of their cuttlebone (Gutowska *et al.*, 2008){Gutowska 2010}, whereas squid statoliths are malformed and more porous (Kaplan *et al.*, 2013). Above 4,000 μatm squid continue to show a negative aerobic metabolic response (Hu *et al.*, 2014)

The majority of studies which examine the impact of OA on cephalopods has focused primarily on cuttlefish and squid. As of this writing there has only been one other study we are aware of that explored how OA effects octopus and no studies on bathyl occurring species of octopus . Here, we examine the impact of OA on the Smoothskin octopus *Muusoctopus leioderma* (Berry, 1911). This species can be found in the Northern Pacific from the Sea of Okhotsk off Siberia to California. They are reported to live on muddy or silty bottoms at bathyal depths ranging from 250-1400m, with greatest frequency of occurrence between 450-650 m {Conners et al., 2016}, but have been previously been reported as shallow as 70m {Hochberg 1998}. Recently a population has been found at depths reachable by SCUBA (10-15m) in Burrows Bay, Skagit County, Washington State, USA. Morphological and genetic data were used to confirm the species identity of this population as *Muusoctopus leioderma* (Onthank unpublished data)*.*

As part of a marine environmental physiology course at the Rosario Beach Marine Lab (RBML) *Muusoctopus leioderma* were collected and held at 1000 or 1800 μatm for 24 hours and 7 days. Routine metabolic rates and critical partial pressure (P­crit) were recorded for each treatment. The Salish Sea, where the RBML is located, is a unique location for OA studies as CO2-rich water from the California Undercurrent wells up into this shallow basin producing persistent hypercapnic conditions (Murray *et al.*). The Pco2 regularly reaches 1000 μatm (Onthank *et al.*). Habitats such as the Salish Sea will experience accentuated acidification due to local hypoxia and eutrophication {cai et al.2011; Melzner et al. 2013}. The goal of this study was to examine the impacts on hypercapnia on aerobic metabolism of a bathyal associated species of benthic octopus. We hypothesized that because this species likely encounters hypercapnic waters historically at the depths where it is found, and in the location where this population was collected, they likely have adapted mechanisms to maintain their aerobic metabolic physiology, even when faced with exceptionally high PCO2.

**Methods**

*Seawater pH Measurement*

This research was carried out at the Rosario Beach Marine Laboratory in Anacortes Washington. Water samples were taken at the octopus collection site in Burrows Bay. Samples were taken at depth where the octopus were collected via SCUBA. A 50 ml High-density polyethylene sample container filled with air was opened at depth and filled with a water sample, excludeing all air bubbles. A screw top lid was used to cap the sample. All water samples were immediated transported to the RBML where pH on the total scale (pHT) was measured using the *m*-cresol purple spectrophotometric method {Dickson et al. 2007} within 3 h. Akalinity was determined by open-cell titration {Dickson et al. 2007}, and alkalinity values were calculated from titration data using the secarb package in R {Gattuso et al. 2015}. The resulting measured alkalinity and pH were used to calculate the PCO2.

*Octopus Collection*

Twenty (need to confirm this number) *Muusoctopus leioderma* (mass = 2.5- 70.0 g) were collected in summer of 2021 from Burrows Bay, Skagit County, Washington State, USA by SCUBA. Octopuses were found on the sediment bottom during night dives. Individuals were placed in plastic resealable bags for transport to RMBL. At RMBL octopuses were placed in holding aquaria with sediment that had previously been collected from Burrows Bay. The holding aquaria were supplied with unmodified seawater directly form the lab seawater system which uses seawater pumped from Rosario Bay.

*PCO2 Level Selection*

As discussed above, the Salish Sea has persistent hypercapnic conditions that reach 1000 μatm CO2 (Murray *et al.*) and in the future may increase by an additional 800 μatm {Barry et al. 2010; Cai et al. 2011; Melzner et al. 2013; Bianucci et al. 2018}. At the time of octopus collection, water samples from Burrows Bay were approximately 1000 μatm, which is consistent with values recorded previously by Onthank et. al. (Seibel *et al.*). The selected PCO2 range allowed us to examine whether the Burrows Bay population that regularly experience 1000 μatm CO2 would be impacted by the predicted increased CO2 levels resulting in 1800 μatm CO2+ locally.

*Hypercapnia Exposure*

After being held in aquaria for at least 24 hours, octopus were transferred to treatment tanks. Each tank (113.5 L) was made using an insulated cooler with an overhead window in the lid to allow observation of octopus. The tank system included a chiller to maintain temperature, and a venture injector to keep tank water oxygenated. Each tank had a slow constant water exchange from the lab sea water system which flowed fresh seawater into the tank at ~100 mL min-1 and drained from an overflow port. This prevented building up waste in each tank with out having to do large, full-tank water changes. Temperature and PCO2 of the each tank was controlled with a custom pH-stat system which received temperature input form a PT-100 temperature probe and pH input from a single junction glass pH electrode. The pH electrodes were calibrated daily using pH calculated via *m-*cresol purple spectrographic method {Dickeson et al. 2007}. Temperature probes were calibrated using an alcohol thermometer. To modify pCO2 pure gaseous CO2 was slowly bubbled in to the tank by a solenoid controlled via the pH-stat. Temperature was controlled by power on/off a chiller controlled by the same pH-stat.

In addition to pH measurements, the total alkalinity (AT) was measured weekly using a modified open cell titration based on Dickson et al. {2007}. Off gassing time was increased from 6 to 10 minutes with vigorous stirring via a stir bar and motor. Titrations were verified against a standard of known alkalinity. The alkalinity values were calculated from titration data using the seacarb package in R {Gattuso et al. 2015} The measured alkalinity and desired PCO2 were used to calculate pH setpoints and tank pH set points were updated weekly.

After at least 24 hours acclimating in holding aquaria, octopus were transferred to treatment tanks. One octopus was placed in a treatment tank and held at either 1000 μatm or 1800μatm for 24 hours and then its routine metabolic rate (RMR) and oxygen critical partial pressure (Pcrit) were measured (method desribed below) and it was then returned to the same treatment tank and held for additional 6 days, for a total of 7 days exposure to its treatment PCO2. Routine metabolic rate and Pcrit were then measured again after the 7th day of exposure. During treatment feeding was done by placing purple shore crabs in the treatment tank after the first 24 hour RMR data collection and removed 24 hours prior to RMR and Pcrit measurements on day 7.

*Routine Metabolic Rate Measurement*

Routine metabolic rates were measured after fasting for 24 h. Octopus were placed in 1 L (Do we need to include 120 ml and 25 ml data I.e. was data so crap we had to toss) flow through water-jacketed respirometers. The same type of pH-stat system that was used in the treatment tanks was used to adjust the pH of the seawater for the flow through respirometry. PyroScience Firesting or Presense O2 flow-through optode cells and robust temperature probes were palced on the incurrent and excurrent channel of each respirometer. A peristaltic pump was used to cycle water through the system. Flow rates for each respirometer were measured at the start and end of each respirometry run by measuring output of water mL for one minute. Octopuses were placed in the respirometers for 24 h and aerobic metabolic rates were measured throughout; (did we trim 3 h data?) After RMR and Pcrit experiments were completed, the octopuses were removed and oxygen consumption was measured in the empty respirometer to determine back-ground respiration, which was typically 5% or less of octopus respiration. After background respiration was measured, inflow and outflow optodes were connected immediately in series to evaluated drift. No drift was detectable. RMR was calculated from raw oxygen data using the resp.pyro and resp.presens function in the OTools package in R (<https://github.com/KirtOnthank/OTools>).

*Pcrit Measurement*

Following 24 h RMR measurements, the respirometer was closed by connecting the inflow of the respirometer to the outflow. Oxygen concentration in the respirometer was allowed to fall to at least 3% of atmospheric satruaton. Pcrit was determined from aerobic metabolic rate (R) as function of oxygen pressure (PO2­) by using the Pcrit α-method described in Seibel et al. (Seibel *et al.*). We used the calc pcrit function in the respirometry package in R (<https://CRAN.R-project.org/package=respirometry>).

*Statistical Analysis*

The effects of

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