

## Juvenile Dungeness crab foraging behavior and lipid composition is altered more by food quantity than seawater pH in a multi-stressor experiment

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### ABSTRACT

Increases in atmospheric, anthropogenic carbon are driving reductions in seawater pH, a process referred to as ocean acidification. Reduced seawater pH can influence behavior of marine animals, but little is currently known about how juvenile crustaceans will respond. We conducted lab experiments to improve our understanding of the consequences of pH exposure and food quantity on juvenile Dungeness crab (*Metacarcinus magister*, (Dana, 1852)) behavior and nutritional condition. To understand the foraging and pH sensing behavior of juvenile crab, and how this interacts with their nutritional status, we exposed recently settled second instar juveniles to either ambient pH or reduced pH for 42-d, crossed with either a 'maintenance'- or low-quantity 'challenge' diet treatment. After the experimental exposure period, we introduced crab into foraging and sensing pH behavior experiments. In the foraging experiment, we placed crab in a behavior arena with unidirectional flow, where we measured the food discovery time and time allocation of activities in 300-s trials for all individual crab. Food quantity and pH exposure influenced both the speed with which juvenile crab identified and allocation of activities but there was no interactive effect of experimental factors. For our pH sensing experiment, we used a two-current flume plumbed with both ambient and reduced pH seawater. This flow-through flume provided a choice between the pH treatment waters and allowed us to measure the amount of time individuals spent on either side of the arena in 300-s trials. There was no effect of prior diet or pH exposure on the amount of time juvenile crab spent in either seawater pH condition. In addition to the behavior trials, we evaluated crab nutritional condition by quantifying the total lipid content of whole-body tissues and fatty acid profile composition of juvenile crab fed either the maintenance or low-quantity diet during the experimental pH exposure period. The proportional fatty acid profiles differed for crab based on their diet and pH exposure, with no interactive effects. However, we did not detect differences in the concentrations of key summary categories of fatty acids (e.g., saturated, mono-unsaturated, or polyunsaturated) based on pH exposure. Our results indicate that reduced food availability has a greater impact on juvenile Dungeness crab foraging behavior and nutritional condition than reduced seawater pH exposure representing the 0.3 pH unit decrease predicted by 2100.

### 1. Introduction

Rapidly increasing concentrations of anthropogenic CO<sub>2</sub> have been linked to atmospheric and ocean warming as well as ocean acidification (IPCC, 2022). The reduction in seawater pH and shifts in carbon chemistry associated with the increased absorption of atmospheric CO<sub>2</sub>

has been termed anthropogenic ocean acidification (OA; Caldeira and Wickett, 2003). Since the industrial revolution, atmospheric CO<sub>2</sub> has increased ~40%, and the world's oceans have absorbed approximately one third of anthropogenically produced CO<sub>2</sub>, resulting in an average decline of 0.1 pH units (IPCC, 2022). It is increasingly well understood that climate change is affecting individual organismal responses to

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abiotic and biotic factors, which are leading to large-scale changes to populations, communities, and ecosystems in the marine environment (e.g., Gattuso et al., 2015; Gaylord et al., 2015; Hoegh-Guldberg and Bruno, 2010).

Considerable focus has been devoted to highly calcified organisms and revealed negative, positive, and mixed impacts of OA (Hendriks et al., 2010; Kroeker et al., 2013). However, crustaceans, which produce a CaCO<sub>3</sub>-reinforced outer cuticle, are often capable of acclimating to pH changes over relatively short time periods (Whiteley, 2011). Some crustacean physiological responses include alterations in O<sub>2</sub> uptake rates (Egilsdottir et al., 2009), extracellular acid-base balance (Spicer et al., 2007), and hemocyte physiology (Meseck et al., 2016). Crustacean physiological responses can additionally impact behaviors. Some examples of crustacean behaviors for which pH influence have been investigated include sensing and foraging (de la Haye, 2012; Wu et al., 2017), righting activity (Zittier et al., 2013), swimming (Dissanayake and Ishimatsu, 2011), feeding (Appelhans et al., 2012; Saba et al., 2012; Schram et al., 2017), and predator-prey interactions (de la Haye et al., 2011).

To date, most studies of crustaceans have focused on species living in highly variable temperate intertidal zones, which may influence species-specific responses and how individual responses could cascade to higher trophic levels (Gaylord et al., 2015). Recent research aimed at modeling of the sensitivity of organisms important to fisheries in the California Current System (CCS) has identified early life stages of Dungeness crab (*Metacarcinus magister* (Dana, 1852)), including new recruits, as being sensitive to anthropogenic CO<sub>2</sub>, due to the timing of larval recruitment and seasonal upwelling (Bednarek et al., 2020; Berger et al., 2021; Busch and McElhany, 2016). However, our limited understanding of the OA effects on *M. magister* are primarily based on larval and adult stages with many fewer studies including juveniles, which may be vulnerable to sublethal effects of OA that could increase the risk of predation during this potentially sensitive life history stage (Busch and McElhany, 2016). One recent effort striving to improve our understanding of Dungeness crab population responses found a trade-off between improved survival rates and smaller body sizes of juvenile Dungeness crab exposed to reduced seawater pH (McElhany et al., 2022).

Adult and juvenile Dungeness crab inhabit multiple stressor hotspots where exposure of Dungeness crab populations to reduced pH and dissolved oxygen or elevated temperature is predicted to increase (Berger et al., 2021). There is some evidence that epibenthic predators, including *M. magister*, may experience the most dramatic effects of future OA (Marshall et al., 2017). Indirect effects have been identified as being an important driver of predicted declines in *M. magister*, primarily because of a reduction in prey and prey quality (Busch and McElhany, 2016). Some of this reduction in prey may be driven by exposure to reduced pH but is likely to be more impacted by hypoxic conditions often associated with upwelling regions (Marshall et al., 2017). An increased exposure to pH variability, higher amplitude with low predictability associated with ocean acidification, in the Pacific has been associated with increased oyster shell dissolution (Bednarek et al., 2022). Despite increased dissolution, multiple bivalves, important prey for crab (Thomas et al., 2020), have demonstrated increased resistance to predation with exposure to reduced pH, resulting in potentially reduced food availability with increased acidification (Lemasson and Knights, 2021). The relationship between potential reductions in calcareous prey items and increased physiological stress associated with exposure to elevated pCO<sub>2</sub> is an important gap in knowledge that has not been directly addressed to date (McElhany et al., 2022).

Behavior can be one way that organisms can buffer or mitigate potential impacts on nutritional condition and can be influenced by abiotic environmental cues. Such behavioral pathways can include settlement timing and habitat selection, feeding, or antipredator behaviors through biochemical and physiological pathways such as information disruption, elevated metabolic load, and avoidance of altered environments (Wang and Wang, 2020). A medium-term exposure to OA can be metabolically

expensive (Pörtner et al., 2004). For example, animals will make up for lost energy either physiologically, by reducing their metabolic rate to preserve energy (Rion and Kawecki, 2007), or behaviorally, by putting greater efforts into obtaining food (Hughes and Seed, 1995). Under challenging environmental conditions, crabs may dedicate more time to foraging activities, such as handling and consumption (Liu et al., 2019; Sun et al., 2015). Behavioral responses can also be altered as a result of a reduced ability to gather environmental information involved in decision making, including risk-taking, foraging behaviors, and avoidance of unfavorable locations (Dodd et al., 2015; Maboloc et al., 2020; Watson et al., 2014; Wu et al., 2017).

Another consequence of environmental stressors is the impaired or altered trophic efficiency of consumers. Higher quantities and quality of food may help mitigate the stress of OA on a consumer (e.g., Cominassi et al., 2020; Hettlinger et al., 2013; Thomsen et al., 2013; Towle et al., 2015). For example, reduced seawater pH has been shown to alter production of fatty acids (FA) and the transfer of essential FA to consumers (Bermúdez et al., 2016; Cripps et al., 2016; Díaz-Gil et al., 2015; Rossoll et al., 2012). FA play multiple roles in organismal physiology at the molecular level and in addition to their roles as organismal condition indicators, can also be used as trophic biomarkers (Dalsgaard et al., 2003). FA can be classified based on their degree of saturation with double bonds and common classifications include FA no double bonds (saturated FA = SAFA), one double bond (monounsaturated FA = MUFA), or more than three double bonds (polyunsaturated FA = PUFA). Because multiple PUFA, including eicosapentaenoic acid (20:5 $\omega$ -3, EPA) and docosahexanoic acid (22:6 $\omega$ -3, DHA) can serve anti-inflammatory roles, measurement of the relative abundance of FA over time can be indicative of organismal stress and immune responses to reduced seawater pH (reviewed by Ericson et al., 2019). The PUFA arachidonic acid (ARA, 20:4 $\omega$ -6) alternately can play a role in inflammation pathways and biosynthesis of prostaglandins in many organisms, including humans (Di Costanzo et al., 2019). Trigg et al. (2019) identified multiple lipids and lipid classes impacted by exposure to low pH.

To test the interaction of food quantity and ocean acidification, we ran a multi-stressor experiment for juvenile *M. magister*, in which we manipulated diet quantity (2 levels) and seawater pH (2 levels), as a conditioning experiment to prepare crab for later behavioral tests. Diet levels were chosen to reflect food availability sufficient to support growth and incorporation of common fatty acid trophic markers in a higher quantity ('maintenance' diet) and a calorically poor, challenge diet ('low quantity' diet). Previous work has demonstrated that we can reliably begin to see diet FA markers in consumer tissues after 6-weeks (Thomas et al., 2020). As a result of differing amounts of clam tissue included in diets, the maintenance diets contained higher concentrations of total lipid and long chain polyunsaturated fatty acids relative to the low-quantity diets. Seawater pH was allowed to vary naturally through time but for the reduced-pH treatment, CO<sub>2</sub> was added intermittently to maintain a constant offset of 0.3 pH units below ambient seawater conditions. After the 42-d conditioning, we performed behavioral trials with individual crab where we measured food discovery time and the time allocation of foraging activities, to test the hypothesis that crab from the ambient pH treatment would find food faster. In a flume choice experiment, we hypothesized that juvenile crab spend more time in the ambient pH water, regardless of pH exposure during the conditioning period.

## 2. Materials and methods

### 2.1. Juvenile crab collection

All 2020 sample collection and laboratory research detailed here adhered to all the University of Oregon's 2020 COVID safety protocols. On 12 June 2020, we collected 600 megalopae from a regularly monitored light trap in the Charleston Marina, Charleston, OR, USA (Miller and Shanks, 2004). They were fed raw chopped clam meat ad libitum

every two days (Pacific razor clams (*Siliqua patula*), purchased locally at Chuck's Seafood, Charleston, OR, USA). Settled juvenile crab were maintained in an ambient flow-through seawater table as a common cohort until 22 July 2020, at which point crab were individually haphazardly allocated to experimental chambers. We randomly assigned 48 fully intact juvenile crab with carapace width of similar size ( $1.33 \pm 0.10$  cm, mean  $\pm$  SD,  $N = 48$ ) individually to 2.7-L flow-through experimental tanks on 22 July 2020, and maintained them in experimental pH conditions for 42-d (22 July –7 September 2020) on formulated pelleted diets (Fig. 1A, B). To provide some environmental complexity for the crab, each experimental chamber was provided with a 5-cm long PVC tube section (2.54 cm diameter) and to control for changes in day length during the experimental period, crab were maintained on a 12-h light:12-h dark cycle. Crab were acclimated to the experimental chambers for 1 week prior to the start of the experimental pH treatment exposure, when an additional subset of the original juvenile crab cohort was collected for initial tissue and behavior analyses.

2.2. Exposure experiment set up and seawater chemistry

Experimental chambers ( $20 \times 12.5 \times 11$  cm), consisting of solid hard plastic containers with clear, transparent lids, contained one juvenile crab, to prevent cohort cannibalism, and were maintained in a flow-through water table with ambient temperature seawater. Each replicate container was fitted with an airtight lid fitted with a single pass-through valve for gravity-fed flow-through seawater delivery from one of four mixing tanks, where seawater pH was adjusted by bubbling either air or an air-CO<sub>2</sub> mixture (Fig. 1B). Two mixing tanks were used to adjust the pH for each of the two treatment levels (four tanks total) with randomized experimental chamber location relative to the mixing tanks to reduce the impacts of pseudoreplication. This means that chambers housing each crab receiving water from the same mixing tank are more interdependent with each other than with chambers receiving water from the other mixing tanks (Cornwall and Hurd, 2015). Crab maintained in each experimental chamber were then evaluated after 42-d for behavior (Fig. 1C) and whole-body nutritional content.

To avoid artifacts resulting from local fluctuations in atmospheric CO<sub>2</sub> due to traffic volume on the road adjacent to our experimental facility all air bubbled into mixing tanks was first CO<sub>2</sub>-scrubbed by passing through a chamber filled with Sofnolime® CO<sub>2</sub> absorbent (soda lime). Air-CO<sub>2</sub> gas mixtures were made and maintained using a multitube gas

proportioning rotameter (OMEGA engineering, FL-2GP-45G-04G). The rotameter was plumbed to a CO<sub>2</sub> gas cylinder containing pure CO<sub>2</sub> and a Super Luft SL-65 high pressure aquarium air pump, which pushed air through the CO<sub>2</sub> scrubbing chamber before passing to the rotameter. Mixing tanks were designated as either ambient (no CO<sub>2</sub> manipulation, intermittently bubbled with CO<sub>2</sub>-scrubbed air) or reduced pH offset (bubbled with air-CO<sub>2</sub> mixtures to maintain a pH offset of 0.3 units below ambient, based on IPCC predictions (IPCC, 2022). Target pH levels were maintained with Honeywell Universal Dual Analyzers (UDAs) fitted with Honeywell Durafet pH probes (Kapsenberg et al., 2017), calibrated with certified Tris buffer (Dickson et al., 2007) provided by A. Dickson at Scripps Institute of Oceanography, University of California San Diego. Reduced pH offset programming was checked a minimum of once every 12 h to ensure appropriate pH levels were maintained throughout the experimental period. Our experimental period included two seasonal upwelling episodes, accompanied by a transient return to more typical pH levels for this time of year (pH ~7.8) at the beginning of August 2020. Seasonal upwelling regularly occurs along the US West Coast because of California Current dynamics (IPCC, 2022). Frequent upwelling events results in spatial and temporal variability in seawater pH. Moreover, the lab is situated in the outer South Slough estuary where lab seawater intake schedules are timed to move seawater into lab holding tanks before distribution to campus research labs (Galloway et al., 2020). Previously published pH data for the outer South Slough environment indicate that at the sensor location, seawater pH varied around 7.8–8.0, with transient low pH events approaching pH 7.4 (Bednaršek et al., 2022).

All seawater carbon chemistry samples were collected from experimental chambers housing crab. Seawater carbon chemistry for subsets of experimental chambers were monitored five days a week using bottle samples, primarily for spectrophotometric pH measurements (Fig. 2A, Fig. S1A), and YSI (YSI Professional Quatro with pH/ORP, DO, Conductivity/Salinity, and temperature probes) observations of salinity, temperature, and % dissolved oxygen (Fig. S1B–D, Fig. 2). Subsets of replicates were regularly monitored to ensure appropriate seawater chemistry was maintained and for regular calculation of seawater pH treatment offset from ambient (Fig. 2B). Additional daily seawater samples collected adjacent to the seawater intake for the lab at OIMB are included for reference and to document the degree to which experimental replicates represented simultaneous environmental conditions. Representative chambers from each of the four treatments were

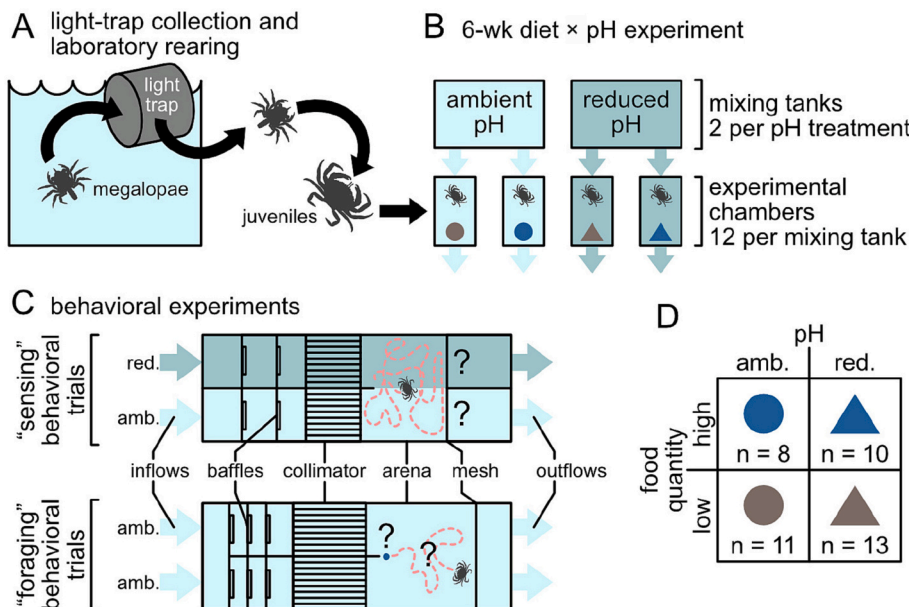
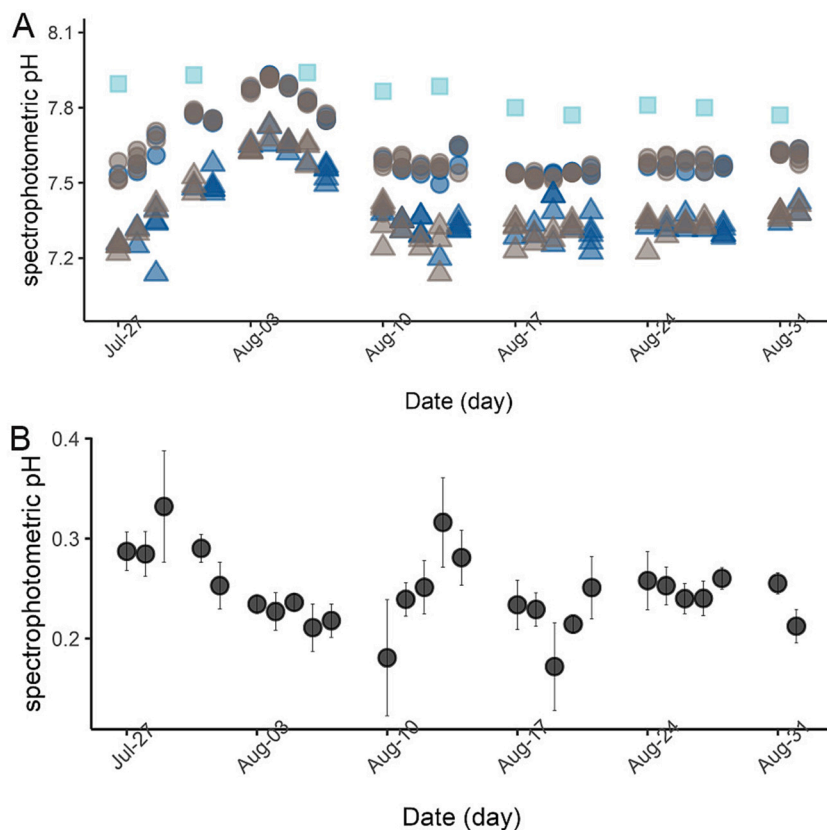


Fig. 1. Simplified schematic of experimental methods and workflow. A) Dungeness crab megalopae were collected using a light trap and then maintained in a flow-through water table to allow them to settle prior to B) assignment to experimental treatments in which food quantity and pH were manipulated. Pseudoreplicated experimental units housing a single crab were haphazardly arranged across the seawater table (simplified here for clarity). C) Crab behavior was evaluated for foraging and sensing behavioral trials. D) Experimental treatments are represented by two different colors (diet, maintenance (MT) = blue, low quantity (LQ) = grey) and shapes (pH; circle = ambient, triangle = reduced pH). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 2.** Time-series of replicate crab containers and in situ seawater pH. Seawater bottle samples were collected twice weekly from each experimental replicate and the results of A) spectrophotometric pH analysis of samples collected from a subset of experimental replicates and an environmental reference sample (intake) and B) Mean daily pH offset (difference between the control/ambient pH and the reduced seawater pH treatment) for spectrophotometric pH data. Data points represent the mean difference between all spectrophotometric pH samples collected each day ( $N = 3-5$  in each treatment, varied due to random order of sample analysis for each given day of the week). Our target offset was 0.3 pH units. Symbol colour code: light blue squares = in situ reference samples, blue = maintenance, grey = low food quantity; symbol shape key: circles = ambient pH, triangles = reduced pH. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

haphazardly chosen for analysis each day, resulting in two bottle samples and YSI measurements per week per replicate. Additional salinity, temperature, and pH measurements were made haphazardly between regular bottle sample collections to spot check and ensure consistent water chemistry levels. The pH of the header tanks was regularly monitored but we report the seawater chemistry of replicates and not header tanks because this is more representative of the conditions experienced by the juvenile crab.

Following bottle sample collection, bottles were maintained in a cool dark location and were analyzed within 10–15 min of collection. For spectrophotometric pH measurements, an ultraviolet-visible miniature spectrophotometer (Ocean Optics Flame-S-Vis-NIR-ES) equipped with a 10-cm pathlength cuvette holder (Ocean Optics, CUV-UV-10) was temperature controlled at 20 °C with a recirculating water bath (Thermo Neslab RTE-210). Spectrophotometer data was recorded using the Ocean Optics OceanView spectroscopy software with graphical user interface. Sample pH was determined on the total hydrogen scale ( $\text{pH}_T$ ) following the addition of *m*-cresol purple pH sensitive dye (SOP 6b, Dickson et al., 2007). Seawater total alkalinity (TA) were determined by open cell potentiometric automatic titrator (Mettler-Toledo T5) equipped with a pH probe (Mettler-Toledo, DGI115) (Dickson et al., 2007). Seawater samples were siphoned into a 250-mL jacketed beaker plumbed to water the RTE-210 water bath to maintain a constant titration temperature of 20 °C. Titrant volumes were recorded in real time using the Mettler-Toledo LabX® software package and subsequently used to calculate seawater TA (SOP 3b, Dickson et al., 2007). To ensure appropriate quality control, seawater samples were collected and checked by evaluating measurement deviations between replicate samples in addition to measurement comparisons to certified reference materials provided by A. Dickson.

We employed a  $2 \times 2$  factorial experimental design (pH  $\times$  diet) to investigate the combined effects of reduced seawater pH (ambient or reduced pH) and diet quality (LQ = Low quantity or MT = Maintenance)

on juvenile Dungeness crab (LQ-reduced pH,  $N = 13$ ; LQ-ambient pH,  $N = 11$ ; MT-reduced pH,  $N = 10$ ; MT-ambient pH,  $N = 8$  in MT, treatment size changed over the course of the experimental period due to mortality). Mortality over the experimental period was extremely low, a total of 6 out of 48 crabs expired with at least one crab from each treatment. All juvenile crab were fed every other day and experimental chambers were monitored daily for molting activity and fecal pellet removal. All molted carapaces were promptly removed to be counted and measured. Juvenile crab molted a maximum of one time during our experiment. We found no differences in the time it took for the first molt to occur (Table S2). We did not track where each crab was in its molt cycle at the termination of the experimental period because most crab only molted once all around the same time-period at the beginning of the experimental period.

### 2.3. Diet preparation

During this exposure period, crab were maintained on one of two controlled diets adapted from the pelletized controlled feeding experiments (Thomas et al., 2020). Based on the growth rate and previously quantified levels of dietary fatty acid incorporation in juvenile crab tissue, we used a previously defined pelletized clam diet formulation (Thomas et al., 2020) as the designated ‘maintenance’ diet in the present study. Additionally, we modeled our maintenance diet on the preparations outlined by Thomas et al. (2020) because their results demonstrated ecologically relevant lipid and fatty acid profiles despite utilization of a formulated diet that did not include foraging energetic demands such as shell breaking. Our ‘low-quantity’ challenge diet was made using the same techniques as the maintenance diet but with  $\frac{1}{4}$  (by weight) of the clam tissue as was used to create the maintenance diet pellets. The low-quantity diet was designed based on lab observations in which we determined that crabs could survive on the formulated calorie-restricted diet and fed with the same frequency as the maintenance diet.

To generate these formulated diets, we first lyophilized locally obtained clam meat, which was then homogenized into a fine powder using an Oster® food processor. The food pellets were made by suspending the same mass of dry components in an alginate solution at a 10% w/v concentration as described by Thomas et al. (2020). The maintenance food pellets were made using homogenized clam meat as the entirety of the 10% w/v dry component. In contrast, the low-quantity food pellets had 75% of the clam material replaced by cellulose (i.e., 2.5% w/v clam powder and 7.5% w/v cellulose, AlphaCel Non-Nutritive Bulk, MP Biomedicals), a nutritionally deficient filler material that is not digestible by crab. The crab were fed gelatinized pellets every other day for the first 21-d and then fed every day for the last 21-d. Each crab received 14–16 g wet weight of pellets on each feeding day. To ensure there was no interference in control of seawater pH by the breakdown products of uneaten food, individual experimental tanks were cleaned daily, and leftover food was removed prior to the next feeding. Due to some limited mortality prior (6 crab across all treatments) to the termination of the experimental period, we had a relatively low sample size and did not have a balanced number of individuals in each diet-pH treatment (LQ-reduced pH,  $N = 13$ ; LQ-ambient pH,  $N = 11$ ; MT-reduced pH,  $N = 10$ ; MT-ambient pH,  $N = 8$ ). We used the food pellets prepared as described for experimental behavior trials to evaluate crab foraging and sensing behaviors.

#### 2.4. Behavioral experiments

For behavioral trials (Fig. 1C), we constructed separate flumes; one flume with two seawater sources, ambient or reduced seawater pH, for the pH “Sensing” behavior trials, and one flow-through flume with a single source of seawater for the food “Foraging” trials. The flume designs were based on previously published plans (Jutfelt et al., 2017), and are briefly described here. Both flumes were constructed with plexi-glass and assembled using acrylic cement (Fig. S2). For the foraging experiment, the flow rate was  $\sim 0.5$  cm per second (Fig. S2A), and for the sensing experiment it was  $\sim 1.8$  cm per second (Fig. S2B). Crab behavior in foraging and sensing trials were recorded using an iPhone 11 camera mounted above the flume arena. Low, but balanced, symmetrical lighting was provided above the arena by two fluorescent lamps mounted above the flume to allow filming without disrupting crab behavior.

For food foraging trials, one 189 L seawater reservoir provided either ambient or reduced pH seawater, depending on crab exposure treatment, to the flume and behavior arena (Fig. S2A). All foraging trials were performed in the same pH environment in which they had been maintained for the 42-d experimental period. We used three new, previously unused food-grade plastic trash cans, thoroughly rinsed with freshwater prior to and following each behavior trial, as seawater reservoirs of pre-treated seawater, siphoned from the same seawater system used to adjust pH for the experimental set-up, for flow-through behavioral flumes. For trials requiring the reduced seawater pH, CO<sub>2</sub> was bubbled into the water reservoir as needed until it was stable at the same seawater pH as the pH in the reduced pH flow through tanks housing the crab for the experimental exposure. For the foraging behavior experimental trials, a fresh, maintenance-treatment food pellet was placed  $\sim 15$  cm upstream from the crab, in the flume (Fig. S2A).

A single crab was placed underneath a perforated plastic cup, downstream from the food for five minutes to acclimate to the flume and the cue. After acclimation we began the video recording and released the crab from the cup. The observer backed up at least 3 m, and the trial ran for 300-s. After 300-s, the observer ended the recording, and the crab was placed back in their experimental tank. The tank was then emptied and flushed for 5 min to make sure there were no leftover cues from the food pellet and crab. This was repeated for all 45 crab (one crab was accidentally trialed twice; we use data from the first trial only). The timing of the foraging trials was determined in a pilot project conducted in Summer 2019 and was sufficient for crab for display foraging

behavior if they were going to forage (Hannah Hayes pers. comm).

We analyzed the behavioral trial videos in the Behavioral Observation Research Interactive Software program (BORIS, Friard and Gamba, 2016), and classified the behaviors as total time spent either handling (actively eating/putting food in mouth), moving (all 8, non-chelate legs moving), or resting (no movement). To minimize potential observer bias, we had two people independently scored behavior recordings. Periodic checks were performed to ensure that each viewer scored behaviors consistently. There were no markers in videos or file naming schemes to indicate the treatment to which the crab in the video belonged. We documented the time it took the crab to find and contact the food pellet. To compare the time spent handling, moving, and resting behaviors, we fit a set of Dirichlet regression models to the data, including diet, pH, and their interaction as potential predictors using the R package ‘DirichletReg’ (Maier, 2021). We used the “alternative” modeling strategy and, for simplicity, only used predictors to model the expected values (i.e., the precision model was intercept only). Because mixing tanks were shared among individual crab within each pH treatment (two mixing tanks per pH), experimental units could be pseudoreplicated. Unfortunately, to our knowledge DirichletReg and similar frequentist packages for Dirichlet regression do not facilitate random effects models that could account for mixing tank effects. As an alternative, we compared the time spent on different foraging activities between tanks within pH treatments, using Wilcoxon rank sum test with the expectation that tank effects would arise in finding differences. We found no significant differences and moved forward with a usual, fixed-effects Dirichlet regression (Table S1). Values of 0 and 1 in our data were accounted for by the package, which applied the following transformation:

$$p_{trans} = \frac{p(N-1) + \frac{1}{c}}{N} \quad (1)$$

Where  $p$  is the untransformed proportion of the time a single crab was observed doing a particular behavior,  $N$  is the total number of observations ( $N_{crab} = 45$ ), and  $C$  is the number of categories (i.e. behaviors,  $C = 3$ ) (Maier, 2021). We compared the candidate models using Akaike’s Information Criterion corrected for small samples (AICc) and calculated their respective AIC weights (Burnham and Anderson, 1998).

To compare food-finding times among treatments, we fit a series of mixed-effects Cox proportional hazards models using combinations of diet, pH, and their interaction as predictors and mixing tank as a random effect to account for potential pseudoreplication (package *coxme*, Therneau, 2022). A survival analysis approach was appropriate, as we could treat finding food as a “death” event and because some crab never found the food pellet during the trial period (data were right censored). We compared models using AICc and weights.

For the pH sensing experiments, two separate 121 L reservoirs provided either ambient or reduced pH seawater to one side of the flume (Fig. S2B). Water was treated as described for foraging trials to maintain appropriate ambient or reduced pH conditions based on crab experimental exposure. Flow rates from the two seawater reservoirs were balanced to ensure equal flow rates and avoid changing behavior based on flow. Acceptable pH sensing trials required that the two different pH flows in the flume must be distinct. A dye test was performed every time the trash cans were filled or refilled to ensure only 2–5 cm of mixing happened on the midline (Jutfelt et al., 2017). To initiate the trial, the observer began the video recording, removed a crab from their experimental tank, and placed it in the middle of the flume. The observer backed away at least 3 m and let the trial run for 300-s, after which, the crab was returned to its experimental tank. The timing of the sensing trials was determined in a pilot project conducted in Summer 2019 and reflected the short-term environmental sensing-based choices (Steven Manos pers. comm). The flume was then allowed to run for five minutes to flush any previous cues left behind by the last crab. We repeated the trial for all 45 crab. We then analyzed the videos in BORIS (Friard and Gamba, 2016) and classified the behaviors as total time spent on the

ambient side or on the reduced pH side. We fit a series of mixed-effects beta regression models to the time spent in the ambient-pH flow as proportions (0–1), using diet, pH, and their interaction as potential predictors (glmmTMB, Brooks et al., 2022). Only the conditional model was fit; we assumed precision to be constant among treatments (i.e., the precision model was intercept only). To account for 0 or 1 values in our data, we transformed the data using Eq. (1). We compared the candidate models using AICc and weights.

In addition to the behavioral aspects central to our study, pH and diet may represent additional stressors that may influence the energetics of the crab. Accordingly, we assessed time to molt and proportional growth ((final carapace width - initial) / initial) using a mixed-effects survival analysis approach (coxme in package coxme, (Therneau, 2022) and ANOVA approach (lmer in package lme4, (Bates et al., 2023), respectively with a series of models including diet, pH, and their interactions, with mixing tank as a random effect.

## 2.5. Nutritional quality

To evaluate organismal condition of crab and nutritional quality of diets we evaluated the lipid composition (fatty acids) of whole body and muscle tissues of crab and prepared diets respectively. For evaluation of prepared food pellets, we collected a subset of prepared pellets from each batch of maintenance and low-quantity food pellets. All tissues for total lipid and fatty acid analysis were frozen at  $-80^{\circ}\text{C}$  within the month before being freeze dried for a minimum of 48 h. To ensure all tissues were dry. To prepare freeze dried tissues for lipid extraction, tissues were ground to fine powder using a stainless-steel mortar and pestle. Immediately following tissue homogenization, a subset of the tissues was weighed and immediately digested in chloroform for  $\sim 12$  h. sealed under nitrogen. Total lipid extraction and subsequent derivatization of fatty acid methyl esters (FAME) was performed using a modification of the method described by (Taipale et al., 2016) and briefly summarized here. Following initial digestion in chloroform, a known amount of unmethylated nonadecanoic acid (C19) was added as an internal standard and lipids were extracted using a 2:1:0.75 solution of chloroform: methanol: 0.9% NaCl solution. This solution was then sonicated, vortexed, and centrifuged so that the organic layer could be removed and evaporated to dryness under a steady stream of nitrogen gas. The organic phase was then resuspended in toluene and 1% sulfuric acid-methanol solution for 90 min at  $90^{\circ}\text{C}$  to transesterify FAME. Solutions were then cooled and  $\text{KHCO}_3$  was added to neutralize the acidic solution. Once neutralized, hexane was added to the solution, vortexed and centrifuged to separate FAME in the hexane solution, which was removed and concentrated to 1.5 mL in glass vials for gas chromatography.

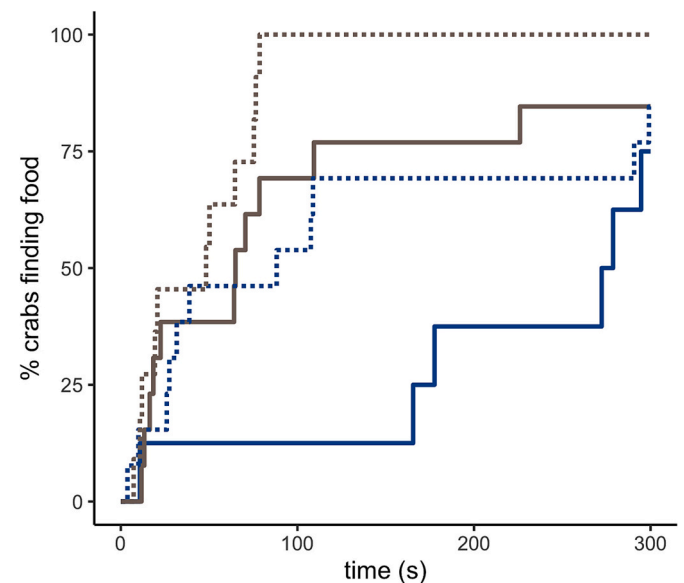
FAME were analyzed with a gas chromatograph mass spectrometer (GC-MS, Shimadzu, Model QP2020), fitted with a DB-23 column ( $30 \times 0.25 \text{ mm} \times 0.15 \mu\text{m}$ , Agilent, Santa Clara, CA, USA), using helium as the carrier gas. To ensure sufficient separation between FA peaks we utilized a heating program modified from Taipale et al. (2016) and described by Thomas et al. (2020). Individual FA were identified using relative retention times of a FAME standard (GLC 566C, Nu-Chek Prep, Elysian, MN, USA) and specific ions and quantified using integration of the major ion peaks with (Taipale et al., 2016) using Shimadzu Lab Solutions software. Following identification and quantification, individual FA peak areas for all FA identified were converted to proportions, representing the % contribution of all FA identified in each sample and those representing  $\geq 0.5\%$  of all FA were included for subsequent analysis ( $N = 29$  FA). Proportion data were compared and visualized using R (version 4.1.3, R Development Core Team, 2022). To ascertain differences in FA profile composition, we first created a Euclidean distance matrix and used PERMDISP (betadisper in vegan package; Oksanen et al., 2022) to determine whether significant permutational analysis of variance (PERMANOVA, 9999 permutations, adonis in package; Oksanen et al., 2022) results were due to differences in dispersion or location.

Comparisons were made based on pH exposure or diet treatment, with mixing tank as a blocking factor to account for pseudoreplicated mixing tanks ('strata', vegan package; Oksanen et al., 2022). To further evaluate diet quality, we compared categorical summaries of FA representing all saturated FA (SAFA, sum of all FA with zero double bonds), mono-unsaturated FA (MUFA, sum of all FA with one double bond), and polyunsaturated FA (PUFA, sum of all FA with three or more double bonds, regardless of carbon chain length) Summary FA of clams were compared using a Welch two sample *t*-test. We attempted to account for potential pseudoreplication in crab comparisons using a mixed-effects ANOVA approach with a series of models including diet, pH, their interactions, and mixing tank as a random effect (lmer in package lme4, (Bates et al., 2023) but we found that inclusion of mixing tank as a random effect over complicated the model and could not be run. Therefore, we analyzed summary FA with an ANOVA and post-hoc Tukey test when significant ANOVA differences were detected. The same statistical analyses were performed for specific essential long chain essential PUFA (sum of all FA with three or more double bond), including arachidonic acid (ARA,  $20:4\omega-6$ ), eicosapentaenoic acid (EPA,  $20:5\omega-3$ ), and docosahexanoic acid (DHA,  $22:6\omega-3$ ). Specific essential FA were selected for additional analysis because of their biological and physiological importance as biomarkers of organismal condition (reviewed by Ericson et al., 2019). All statistical analyses were performed in R and  $\alpha < 0.05$  were considered significant and follow a uniform colour shape scheme in figures (Fig. 1D).

## 3. Results

### 3.1. Foraging

Low-quantity-fed, reduced-pH crab were the fastest to find the food pellet in foraging trials, taking  $42 \pm 9$  s (mean  $\pm$  SE, including only crab that found the pellet, Fig. 3). Low-quantity, ambient-pH crab took  $63 \pm 18$  s and maintenance, reduced-pH crab took  $94 \pm 29$  s. Maintenance, ambient-pH crab took the longest to find the pellet, taking  $200 \pm 38$  s.



**Fig. 3.** Crab foraging behavior results. Percentage of crab finding the food pellet during 300-s Foraging behavioral trials. The best mixed-effects Cox proportional hazards model for these data included diet and pH Treatments (but not their interaction) as predictors (Table 1). Experimental treatments are represented by two different colors (food quantity, maintenance = blue, low quantity = grey) and line (pH; solid = ambient, dashed = reduced pH). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

All crab in the low-quantity, reduced-pH treatment combination found the pellet during the observation period; two crab each for all other treatment combinations did not find the pellet. The best candidate Cox proportional hazards model for food-finding time included diet and pH as predictors (Table 1) and agreed with the general pattern observed. The hazard ratio of finding food for low-quantity-fed crab relative to maintenance-fed ones was 2.6, and for reduced-pH crab relative to ambient-pH ones the hazard ratio was 2.1.

For foraging activities, maintenance-fed crab spent the greatest portion of the 300-s observation period crawling (ambient pH:  $45 \pm 10\%$ , reduced pH:  $42 \pm 7\%$ , mean SE, Fig. 4), approximately a third of the time resting (i.e., stationary, ambient:  $36 \pm 9\%$ , reduced:  $36 \pm 8\%$ ), the remaining time handling the food pellet (ambient:  $20 \pm 12\%$ , reduced:  $22 \pm 7\%$ ). In contrast, the low-quantity-fed crab spent the majority of the observation period handling the food pellet (ambient:  $67 \pm 10\%$ , reduced:  $85 \pm 3\%$ ), with smaller proportions of the time spent crawling (ambient:  $18 \pm 6\%$ , reduced:  $11 \pm 3\%$ ) and resting (ambient:  $15 \pm 6\%$ , reduced:  $3 \pm 1\%$ ). Time allocations were qualitatively similar between pH treatments within diet treatment. The best candidate Dirichlet regression model for crab foraging behavior included only diet as a predictor (Table 2) with predictions qualitatively similar to the observed patterns. The second-best candidate model included diet and pH as predictors, but this model was only about a third as probable to be the best model relative to the diet-only model (Table 2).

### 3.2. Sensing

Of the 300-s observation period, the majority and similar portions of time were spent in the ambient-pH flow of the flume for maintenance-fed, ambient-pH ( $70 \pm 10\%$ , mean  $\pm$  SE, Fig. S3), maintenance, reduced-pH ( $66 \pm 10\%$ ), and low-quantity, ambient-pH crab ( $68 \pm 11\%$ ). Low-quantity-fed, reduced-pH crab appeared to spend more similar portions of time between the ambient- and reduced-pH flows ( $42 \pm 10\%$ ,  $58 \pm 10\%$ , resp.). However, the best candidate beta regression model for crab pH-sensing was the null model, though the diet-only model had a  $\Delta$ AICc of 0.59 and the pH-only model had a  $\Delta$ AICc value  $<2$  (Table 3). The null model was 1.34 times more probable than the next best candidate model (diet only), given the models compared and the data (Table 3). The diet-only model predicted a slightly greater proportion of time spent in the ambient-pH flow for high-quantity-fed crab relative to low-quantity ones (Fig. S3).

### 3.3. Molting and growth

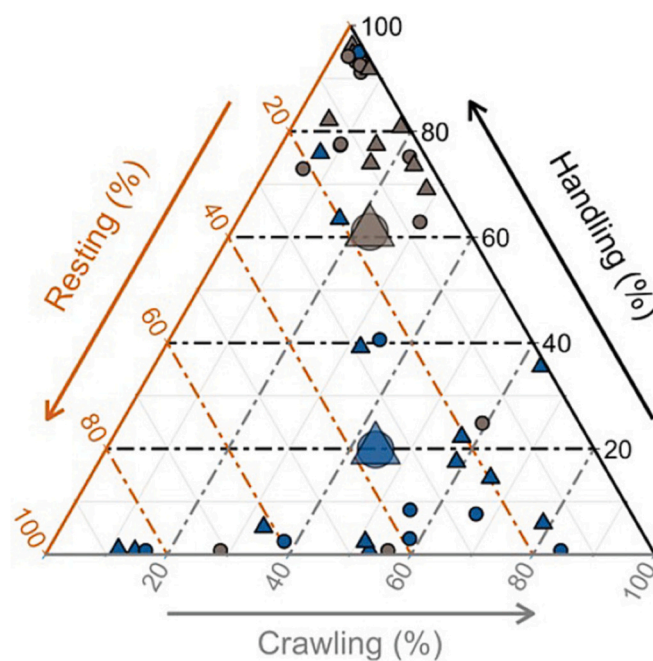
At most, crab molted once during the experimental period. In descending order, time to molt was  $13 \pm 1$  d (mean  $\pm$  SE) for maintenance-fed, reduced-pH,  $10 \pm 1$  d for maintenance, ambient-pH,  $9 \pm 1$  d for low-quantity, reduced-pH, and  $9 \pm 1$  d for low-quantity, ambient-pH crab. The best mixed-effects Cox proportional hazards model was the null model, with the diet-only model being second best ( $\Delta$ AICc = 1.90, Table S2).

Most crab, having molted, grew during the experimental period. The

**Table 1**

Corrected Akaike's Information Criteria (AICc) for mixed-effects Cox proportional hazards models of crab foraging time to find food.  $\Delta$ AICc is the difference between a model's AICc and the lowest AICc of the candidate model set. The AICc weights are given as *w*.

| Model   | df | Log-likelihood | AICc  | $\Delta$ AICc | <i>w</i> |
|---|----|----------------|-------|---------------|----------|
| Food found ~ Diet + pH                          | 2  | -117.704       | 239.7 | -             | 0.573    |
| Food found ~ Diet + pH + Diet: pH (interaction) | 3  | -117.601       | 241.8 | 2.09          | 0.201    |
| Food found ~ Diet only                          | 2  | -118.397       | 242.4 | 2.66          | 0.151    |
| Food found ~ pH only                            | 1  | -121.398       | 244.9 | 5.18          | 0.043    |
| Food found ~1 (null)                            | 0  | -122.243       | 245.5 | 5.80          | 0.032    |



**Fig. 4.** Crab sensing behavior results. Ternary plot of portions of time spent on different activities during the Foraging behavioral trials. Small points are data from individual crab in this study and large points are predictions from the best Dirichlet regression model (diet treatment only – note that pH treatment is not a factor in the model). Experimental treatments are represented by two different colors (food quantity, maintenance = blue, low quantity = grey) and shapes (pH; circle = ambient, triangle = reduced pH). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 2**

Corrected Akaike's Information Criteria (AICc) for Dirichlet regressions of crab foraging activities.  $\Delta$ AICc is the difference between a model's AICc and the lowest AICc of the candidate model set. The AICc weights are given as *w*.

| Model   | df | Log-likelihood | AICc   | $\Delta$ AICc | <i>w</i> |
|---|----|----------------|--------|---------------|----------|
| Foraging ~ Diet only                          | 5  | 62.1           | -112.7 | -             | 0.731    |
| Foraging ~ Diet + pH                          | 7  | 63.77          | -110.5 | 2.15          | 0.249    |
| Foraging ~ Diet + pH + Diet: pH (interaction) | 9  | 64.29          | -105.4 | 7.24          | 0.020    |
| Foraging ~1 (null)                            | 3  | 45.99          | -85.4  | 27.28         | <0.001   |
| Foraging ~ pH only                            | 5  | 46.23          | -80.9  | 31.75         | <0.001   |

**Table 3**

Corrected Akaike's Information Criteria (AICc) for mixed-effects beta regression models of crab sensing.  $\Delta$ AICc is the difference between a model's AICc and the lowest AICc of the candidate model set. The AICc weights are given as *w*.

| Model                                       | df | Log-likelihood | AICc  | $\Delta$ AICc | <i>w</i> |
|---|----|----------------|-------|---------------|----------|
| Sensing ~1 (null)                           | 3  | 12.332         | -18.1 | -             | 0.392    |
| Sensing ~ Diet only                         | 4  | 13.244         | -17.5 | 0.59          | 0.292    |
| Sensing ~ pH only                           | 4  | 12.566         | -16.1 | 1.95          | 0.148    |
| Sensing ~ Diet + pH                         | 5  | 13.607         | -15.7 | 2.40          | 0.118    |
| Sensing ~ Diet + pH + Diet:pH (interaction) | 6  | 14.093         | -14.0 | 4.10          | 0.050    |

non-molting crab did not grow. One maintenance-fed, reduced-pH crab decreased in size. Average growth for crab that did molt, in descending order, was  $30 \pm 3\%$  ( $\pm$ SE) for low-quantity-fed, reduced-pH,  $30 \pm 4\%$  for maintenance, ambient-pH,  $25 \pm 4\%$  for maintenance, reduced-pH, and  $20 \pm 4\%$  for low-quantity, ambient-pH crab. Because mixed-

effects ANOVA showed singular fits due to a small estimated mixing tank random effect, we also performed an analogous set of fixed-effects ANOVA omitting the random effect. The null models for both sets were the best, with the second-best models approximately half as likely as their respective null models (Table S3). In the fixed-effect set, all models except one (diet and pH, no interaction) had  $\Delta AICc < 2$ , suggesting near-equivocal support and limited effects of diet and pH (Table S3).

### 3.4. Nutritional assessment

The total lipid content of diets (clam tissue) for maintenance and low-quantity diets differed significantly (Fig. 5A, Welch two sample *t*-test,  $t = 2.77$ ,  $df = 11.86$ ,  $p = 0.02$ ). Following the experimental period, the total lipids of crab differed based on diet treatment but not pH exposure (Fig. 5B, Table 4). Despite a difference in total lipid based on diet designation, the only differences were based on comparisons between initial crab and crab maintained on diets (Tukey test, initial-MT  $p = 0.02$ , initial-LQ  $p < 0.001$ ). There were no significant differences in the total lipids of crab maintained on high vs. low quality diets despite a trend for lower total lipids in crab fed the low-quantity diet (Fig. 5B, Tukey test, MT-LQ  $p = 0.08$ ).

The proportional multivariate FA profiles of clam muscle tissue fed to juvenile crab ( $N = 29$  FA, Table S4), representing profile composition, differed based on diet quality (Fig. 6A, PERMANOVA<sub>clam</sub>,  $df_{clam} = 1$ ,  $F_{model_{clam}} = 20.59$ ,  $R^2_{clam} = 0.63$ ,  $p_{clam} = 0.003$ ). There was no difference in the clam diet data dispersion (PERMDISP<sub>clam</sub>,  $p = 0.84$ ). There was some variability in FA composition of clam muscle diets but there was greater dispersion of FA profiles between diet treatments (Fig. 6A). At the end of the experiment the FA profiles ( $N = 29$  FA, Table S5) of juvenile crab differed based on diet (PERMANOVA<sub>crab</sub>,  $df_{crab} = 1$ ,  $F_{model_{crab}} = 27.61$ ,  $R^2_{crab} = 0.32$ ,  $p_{crab} = 0.001$ ) and pH ( $df_{crab} = 2$ ,  $F_{model_{crab}} = 3.44$ ,  $R^2_{crab} = 0.08$ ,  $p_{crab} = 0.0001$ ; Fig. 6B) and there was no interactive effect ( $df_{crab} = 1$ ,  $F_{model_{crab}} = 0.26$ ,  $R^2_{crab} = 0.003$ ,  $p_{crab} = 0.84$ ). There was no difference in the crab data dispersion (PERMDISP<sub>crab</sub>,  $p = 0.23$ ). The FA profiles of the crab fed the low-quantity diet were more like each other, with more overlap in FA profiles of crab collected from the initial cohort and fed the maintenance diet (Fig. 6B).

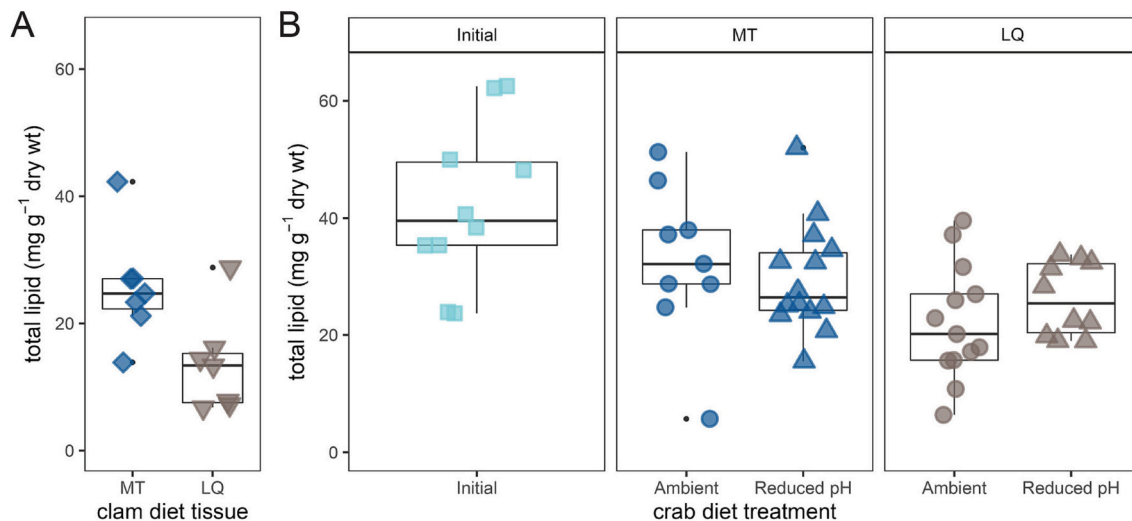
Despite differences in overall FA composition of maintenance and low-quantity diet treatments due to pH exposure, we found no

**Table 4**

Results of 2-way analysis of variance (ANOVA) for total lipids and summary FA from crab whole-body tissues collected at the beginning of the experimental period (initial) and after the 42-d exposure to experimental diet and pH treatments. Significant differences ( $p < 0.05$ ) are indicated (\*).

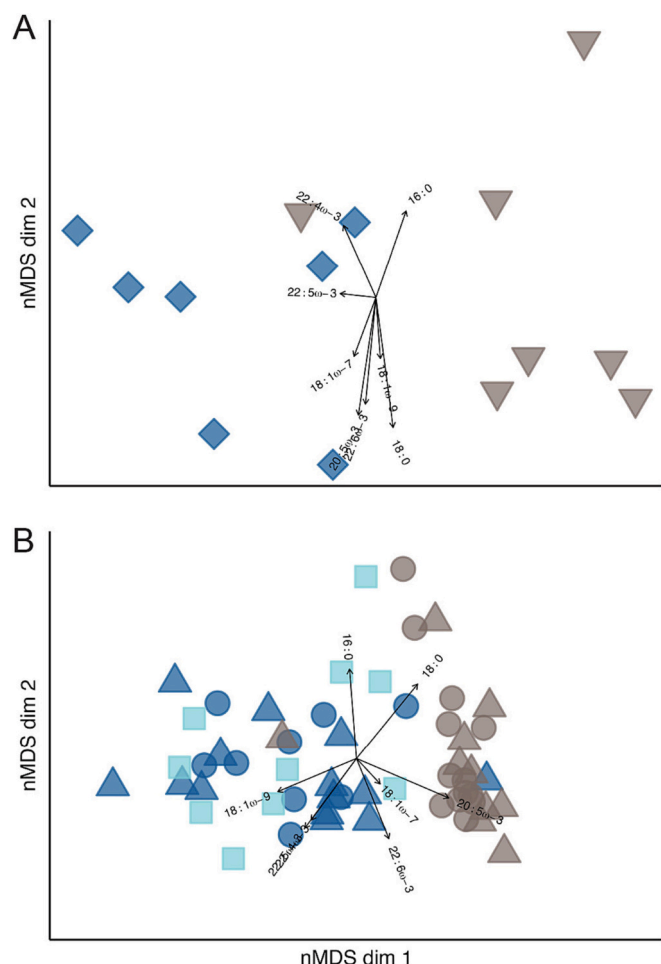
| Source   | df | Sum of squares | F      | p        |
|--|----|----------------|--------|----------|
| <i>Total lipid</i>                                       |    |                |        |          |
| Diet   | 2  | 2319           | 10.26  | <0.001*  |
| pH   | 1  | 5              | 0.04   | 0.84     |
| Diet: pH   | 1  | 131            | 1.16   | 0.29     |
| <i>SAFA</i>  |    |                |        |          |
| Diet   | 2  | 67.72          | 12.169 | <0.001*  |
| pH   | 1  | 1.53           | 0.55   | 0.462    |
| Diet: pH   | 1  | 0.66           | 0.239  | 0.627    |
| <i>MUFA</i>  |    |                |        |          |
| Diet   | 2  | 215.9          | 12.174 | <0.001*  |
| pH   | 1  | 2              | 0.222  | 0.639    |
| Diet: pH   | 1  | 1.5            | 0.168  | 0.684    |
| <i>PUFA</i>  |    |                |        |          |
| Diet   | 2  | 210.9          | 9.5    | <0.001*  |
| pH   | 1  | 4              | 0.36   | 0.550963 |
| Diet: pH   | 1  | 1.7            | 0.154  | 0.696097 |
| <i>arachidonic acid (20:4<math>\omega</math>-6)</i>      |    |                |        |          |
| Diet   | 2  | 0.702          | 32.491 | <0.001*  |
| pH   | 1  | 0.0044         | 0.405  | 0.527    |
| Diet: pH   | 1  | 0.0127         | 1.178  | 0.283    |
| <i>eicosapentaenoic acid (20:5<math>\omega</math>-3)</i> |    |                |        |          |
| Diet   | 2  | 33.69          | 42.007 | <0.001*  |
| pH   | 1  | 0.12           | 0.309  | 0.581    |
| Diet: pH   | 1  | 0.78           | 1.952  | 0.168    |
| <i>docosahexaenoic acid (22:6<math>\omega</math>-3)</i>  |    |                |        |          |
| Diet   | 2  | 21.057         | 19.341 | <0.001*  |
| pH   | 1  | 0.067          | 0.123  | 0.728    |
| Diet: pH   | 1  | 0.887          | 1.63   | 0.207    |

differences in the concentrations of summary FA or selected essential FA (Table 4). There were differences in the concentrations of SAFA, MUFA, and PUFA between initial crab and those maintained in the experimental



**Fig. 5.** Crab whole-body lipid content. Boxplots of the total lipids recorded in A) maintenance (MT) and low-quantity (LQ) clam diets fed to crab for the duration of the 42-d experimental period and B) total lipids of experimentally fed crab. Solid horizontal lines = median, boxes = 1st and 3rd quartiles, whiskers = full range of data, filled small black circles = outliers, colored circles and triangles represent individual data points overlaid on boxplots. Experimental treatments are represented by two different colors (food quantity, maintenance = blue, low quantity = grey) and shapes (pH; circle = ambient, triangle = reduced pH). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)





**Fig. 6.** Comparisons of diet and crab fatty acid composition. Non-metric multidimensional scaling (nMDS) plot of proportional multivariate fatty acid profiles (compositional data, FA included represent a minimum of  $\geq 0.5\%$ ) of: A) clam muscle tissue prepared as dried food pellets; and B) juvenile whole-body tissue ( $N = 29$  FA). Vector overlays represent those FA that contributed  $>5\%$  of total identified FA in at least 1 treatment group). Experimental treatments are represented by two different colors (food quantity, maintenance = blue, low quantity = grey) and shapes (pH; circle = ambient, triangle = reduced pH). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

treatments but there were no differences based on consumption of either the maintenance or low-quantity diet (Table 4, Fig. S4B, D, F). The FA concentration of clam diet tissue was not significantly different for SAFA (Welch,  $df = 10.82$ ,  $t = 1.84$ ,  $p = 0.09$ ) or MUFA (Welch,  $df = 10.59$ ,  $t = 2.10$ ,  $p = 0.06$ ) but did differ for PUFA concentrations (Welch,  $df = 9.94$ ,  $t = 2.29$ ,  $p = 0.05$ , Fig. SA, C, E). Despite a lack in differences in many of the concentration of summary FA groups, there were differences in the concentrations of essential FA arachidonic acid (ARA, Welch,  $df = 8.68$ ,  $t = 3.63$ ,  $p = 0.01$ ), eicosapentaenoic acid (EPA, Welch,  $df = 9.78$ ,  $t = 3.55$ ,  $p = 0.01$ ), and docosahexaenoic acid (DHA, Welch,  $df = 8.68$ ,  $t = 3.63$ ,  $p = 0.01$ , Fig. 7A, C, E). We also detected differences in crab whole body tissue based on diet (Table 4, Fig. 7B, D, F). Initial crab and those fed each diet had different concentrations of ARA, EPA, and DHA (Fig. 7B, D, F). For ARA, EPA, and DHA, the concentrations of these essential FA were lower in initial crab tissues and following the experimental period, reflected concentrations more similar to those in the food items consumed (Fig. 7B, D, F).

## 4. Discussion

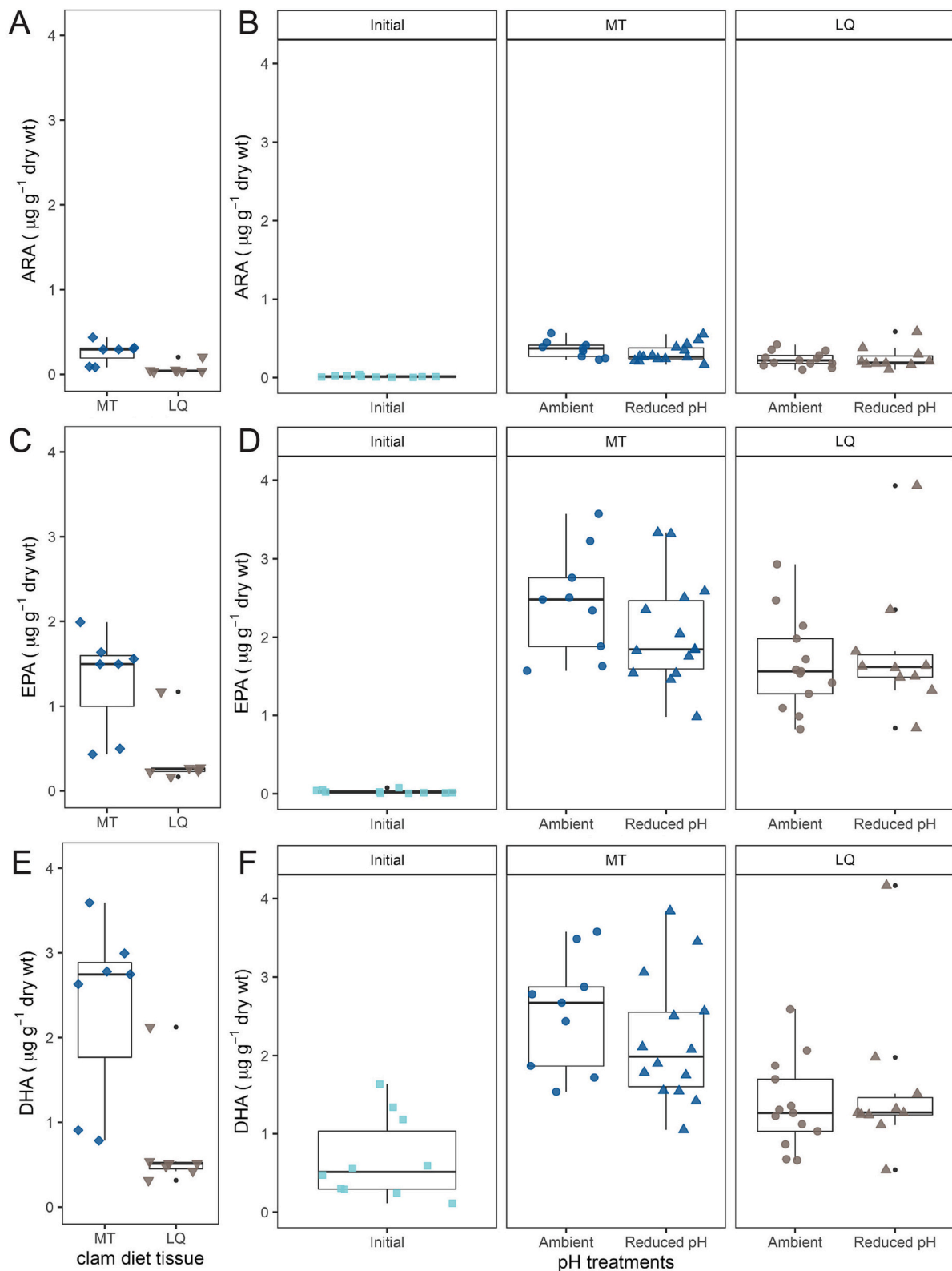
Over the course of the 42-d experimental period, juvenile crab maintained on either a maintenance or low-quantity diet in seawater at ambient or a reduced pH treatment exhibited shifts in behavioral responses and lipid composition. Crab fed a maintenance diet of clam muscle tissue took longer to find food items than crab maintained on a low-quantity diet, consisting of  $\frac{1}{4}$  of the clam muscle tissue mass per unit food item, and subsequently lower lipid content. Despite these differences in foraging behaviors based on diet, we found no differences in crab preference for seawater pH in our ‘sensing’ behavior trials. However, diet did significantly influence the proportional FA profiles of whole-body crab tissues. Despite finding combinations of diet and pH exposure influences on crab behavior and nutritional condition, we observed limited interactive effects of exposure to reduced pH and food availability.

### 4.1. Foraging

We found that pH and diet had an additive effect on the food-finding time, with diet being the most important factor. The fastest food-finding times were recorded for crab that received our low-quantity diet and were acclimated to reduced seawater pH, with all the crab in this treatment finding the food pellet during the trial. Alternately, the slower crab were maintained on the maintenance diet at ambient pH. Crab fed the low-quantity diet spent most of the behavior trials handling the food pellets (61%) compared to the crab fed the maintenance diet (~20%). Instead of targeting food handling during the trials, crab fed the maintenance diet spent a greater proportion of their time crawling or resting. When animals are starved or nutritionally limited, previous studies have demonstrated that they are faster when presented with food and take more risks to eat compared to satiated individuals (Mayntz et al., 2005; Wahle, 1992). Our results suggest that juvenile Dungeness crab fed a lower quality diet will prioritize foraging and food handling behaviors rather than exploratory behaviors, a result that was augmented following exposure to reduced seawater pH.

Crab acclimated to the reduced pH treatment were faster than crab acclimated to an ambient pH. High  $pCO_2$  conditions can cause shifts in acid-base regulation (Whiteley, 2011), which can be metabolically expensive using 2.8–40% of the organisms’ total energy expenditure to do active transport associated with acid-base balance and ion regulation (Leong and Manahan, 1997; Pannevis and Houlihan, 1992; Pörtner et al., 2004). This higher potential energy expenditure may therefore increase the importance of a sufficiently nutrient-rich diet. The exposure to reduced pH may have required more energy and may explain why the crab subjected to a reduced pH found food faster. Even though the differences in lipid/FA composition were not significantly different, the behavioral differences observed here may be physiologically significant, because changes in behavior are one of the first things to change, while shifts in lipid/FA content generally take more time (Galloway and Budge, 2020). Dungeness crab have demonstrated an ability to tolerate lower salinity than preferred through potential diet compensation following periods of starvation (Curtis and McGaw, 2012). This type of selective behavioral tolerance under different non-ideal salinity regimes could translate into the similar response we document with the juvenile Dungeness crab fed a low-quantity diet and exposed to reduced pH. Yet, this increase in foraging behavior is not universal for all stimuli when experiencing food restrictions. While starved Dungeness crab in this same experiment also spent more time searching for food, other multiple stressor studies have not identified a shift in foraging behavior. For instance, when reduced pH was combined with seawater warming, no changes in foraging behavior of the Japanese stone crab were detected (Wu et al., 2017).

There was no statistical effect of pH on the foraging behaviors we identified (e.g., food handling, resting, crawling), but there was a non-significant trend that is consistent with our food-finding time results.



**Fig. 7.** Essential fatty acid concentrations. Boxplots of selected essential FA concentrations including A) arachidonic acid (ARA, 20:4 $\omega$ -6) in clam tissue and B) ARA in crab maintained on experimental diets where initial crab differed from those fed the maintenance (MT) diet (Tukey,  $p < 0.001$ ) and low-quantity (LQ) diet ( $p < 0.0001$ ) and MT and LQ differed ( $p = 0.01$ ). C) Eicosapentaenoic acid (EPA, 20:5 $\omega$ -3) in clam tissue and D) EPA in crab maintained on experimental diets where initial crab differed from those fed the MT ( $p < 0.001$ ) and LQ diet ( $p < 0.0001$ ) as well as MT and LQ differed ( $p = 0.03$ ). E) Docosahexanoic acid (DHA, 22:6 $\omega$ -3) in clam tissue and F) DHA in crab maintained on experimental diets where initial crab differed from those fed the MT ( $p < 0.001$ ) and LQ diet ( $p = 0.02$ ) as well as MT and LQ differed ( $p < 0.001$ ). Solid horizontal lines = median, boxes = 1st and 3rd quartiles, whiskers = full range of data, filled small black circles = outliers, colored circles and triangles represent individual data points overlaid on boxplots. Experimental treatments are represented by two different colors (food quantity, maintenance = blue, low quantity = grey) and shapes (pH; circle = ambient, triangle = reduced pH). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Crab from the reduced pH group prioritized foraging; they spent more time handling the food, while the ambient pH group spent much of their time crawling and resting. It is possible that if our exposure period were longer, we may have seen a greater effect of pH, reflecting the results found from the food-finding time. Recent decapod sensitivity thresholds have been identified, consisting of an exposure to a pH of 7.75 for 60 days (Bednaršek et al., 2021). Because of the regular upwelling exposure within the California Current System in the nearshore and estuarine environments inhabited by Dungeness crab, it is likely that individuals may be exposed to these conditions seasonally. To better understand how food availability and pH influence feeding behavior, future studies could include an evaluation of consumption rates in addition to foraging and food handling time.

#### 4.2. Sensing

We found there was no difference between any of the treatments in the response of juvenile Dungeness crab to acute exposure to ambient or reduced seawater pH. This may indicate that Dungeness crab developing in the California Current System have evolved with and adapted to the fluctuating pH, augmented by seasonal upwelling, of this coastal environment and within the estuaries. Species that are more sensitive to shifts in seawater pH have demonstrated avoidance behavior to reduce exposure to reduced pH when presented with a stratified seawater pH environment (Gravinese et al., 2019; Maboloc et al., 2020). The lack of a pH effect in this experiment may indicate that prior pH exposure and continued internal ionic balance control is not sufficient motivation for crab to move away from or towards differing bodies of water over the time frame tested. This may suggest that juvenile Dungeness crab inhabiting coastal habitats along the US Oregon coast are more tolerant of a wider range of pH. Based on the variability associated with estuarine nursery habitats, like the one from which the larval crab from the present study were collected, this tolerance of a wide range in salinity, pH, and carbon chemistry may be a necessary adaptation for success (Cai et al., 2021). It is also possible that it may take longer for Dungeness crab to locomote to new locations to ameliorate exposure to unfavorable conditions. For instance, Dungeness crab tracked in situ migrated to deeper depths to avoid stressful salinity and temperature exposure following relatively short exposure (<20 min), demonstrating avoidance behavior for unfavorable conditions when the opportunity is presented (Curtis and McGaw, 2012).

#### 4.3. Nutritional quality

The total lipid and FA composition of crab maintained on experimental diets shifted over the 42-d experimental period, resulting in increased dissimilarity between initial crab maintained ad libitum on raw clam tissue and crabs fed formulated diets, with additional shifts in FA profile composition depending on pH exposure. Previous work suggests that crab biochemical and FA profile composition vary following food restriction (Wen et al., 2006). The biggest differences in total lipid and FA were between initial juvenile crab tissue samples and crab maintained in the experimental set-up despite greater lipid content of maintenance food pellets. The pooled population, from which initial crab samples were collected, were fed raw clam muscle tissue, however, since this was a pooled cohort, some cannibalism likely occurred, giving the initial crab an additional potential nutritional resource. Cannibalism is well documented within Crustacea and can occur within individuals in the same size class as well as those in smaller size classes or life history stages (Bleakley, 2018) and cannibalism has been documented in juvenile Dungeness crab even when alternate prey was available (Fernandez, 1999; Galloway et al., 2017). Initial crab may have consumed a mixed diet (clam muscle and crab cohort members), thus explaining some of the differences in total lipid and SAFA, MUFA, and PUFA concentrations between initial crab and those maintained in diet treatments. Mixed diets can improve lipid reserves and provide a more compressive range

in fatty acids, including essential FA and their precursors in more ideal ratios and thus improve physiological performance (D'Souza and Loneragan, 1999).

In the present study, diet did not ameliorate of the effects of reduced pH levels consistent with predicted ocean acidification as observed in other analyses. Previous studies have highlighted the role food availability has played in a reduction in or resistance to the impacts of ocean acidification conditions on physiological trade-offs, including on calcification, and metabolic rates (Pansch et al., 2014; Ramajo et al., 2016; Thomsen et al., 2013). Other studies have identified that while food availability or quality can offset some impacts of reduced pH, it may augment the negative impacts on other traits such as growth (Brown et al., 2018; Cole et al., 2016) or ecological processes (Brown et al., 2020). In contrast, our results do not show a marked impact on tissue lipid content or FA concentration due to reduced pH exposure in 2nd instar juvenile Dungeness crab. It is possible that the experimental period was insufficient to push crab past the theorized sensitivity threshold for decapods (60 d at pH 7.75) (Bednaršek et al., 2021). It is also possible that we would have seen a greater impact on nutritional quality if crab in the experiment foraged on shelled diet items, which would presumably require additional energetic demands. The ARA, EPA, and DHA concentration patterns mimic those of the crab following the experimental period suggests that they were not sufficiently stressed and the duration of the experimental period was sufficient for crab to reflect the quantity available. McElhany et al. (2022) found that juvenile Dungeness crab tolerated seawater pH 7.2 for an extended period, providing further evidence to support their tolerance of reduced seawater pH, likely tied to the affinity of crab in this life history stage for coastal estuarine habitats. Alternatively, allowing natural temporal variation in the ambient and reduced pH treatments provided intermittent pH refuges like those juvenile crab may experience in the nearshore habitats of the California Current System (Kapsenberg and Cyronak, 2019). Our results highlight the importance of including variation in experimental design (Kapsenberg and Cyronak, 2019) to simulate in situ conditions. Within this natural environmental variation experienced by juvenile Dungeness crab, there is also a variation in food availability, and despite reducing the amount of clam muscle in the low-quantity food pellets by  $\frac{3}{4}$ , we may not have had sufficient difference between the maintenance and low-quantity treatments to offset the metabolic demands on crab during the summer upwelling season.

#### 4.4. Conclusions

The comprehensive 2016 report from the West Coast Ocean Acidification & Hypoxia Science Panel recently identified OA as a major stressor to coastal resilience with ecological and economic consequences for the West Coast (Chan et al., 2016). To date, oyster fisheries on the West Coast have been well studied with respect to organismal responses to OA, making robust models and predictions more feasible (Barton et al., 2015). However, the effects of OA on Oregon's critically important commercial and recreational *M. magister* fishery are incomplete or unknown, potentially weakening model predictions. The Oregon Dungeness crab fishery is comprised of over 350 vessels on which between 3 and 33 million lbs. has been landed for an ex-vessel value range of \$33–74 million in the past 10 years (ODCC, 2022). Most research to date on the ecology of *M. magister* has focused on the adult (e.g., ~4-year-old) and pre-settlement larval (e.g., megalopae) life stages (Rasmuson, 2013). *M. magister* settlement dynamics, post-settlement competition, resource assimilation and sources of mortality in subadults are still largely a mystery (Galloway et al., 2017; Thomas et al., 2020). Indeed, a lack of understanding about the factors that affect subadult survival is a knowledge gap for managers of the commercial and sport Oregon crab fishery. Even though we did not observe any detrimental effects from ocean acidification when exposed to changes in a medium-term period, this may not be the case for the organisms in lower trophic levels. Researchers have predicted that some communities will experience a

decline in species richness due to ocean acidification and others will replace/dominate over them as CO<sub>2</sub> levels continue to rise (Sunday et al., 2017). If OA affects the ample food supply in the estuaries, there may be important indirect effects of reduced pH on Dungeness crab through changes in the availability or quality of their prey in the wild.

### CRedit authorship contribution statement

**Julie B. Schram:** Conceptualization, Methodology, Investigation, Visualization, Writing – original draft, Writing – review & editing, Project administration. **Hannah G. Hayes:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Erica Street:** Methodology, Writing – review & editing. **Natalie Thompson:** Methodology, Writing – review & editing. **Reyn M. Yoshioka:** Methodology, Writing – original draft, Visualization, Writing – review & editing. **Aaron W.E. Galloway:** Conceptualization, Writing – review & editing, Supervision.

### Declaration of Competing Interest

The authors declare that there are no known competing financial or personal interests that could have the appearance of influencing the data, results, or interpretations reported in this manuscript.

### Data availability

Data will be made available on request.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jembe.2023.151897>.

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