Adult krill survival and body condition under conditions of global change

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**Methods**

*Experimental system*

The experiment was conducted at the NOAA Northwest Fishery Science Center’s Mukilteo Research Station in Mukilteo, WA, USA in an experimental system designed to conduct studies on sensitivity of marine species to OA, warming, and ocean deoxygenation. The experimental system contains 13 replicates, each with entirely independent control over treatment conditions, and utilizes flowing seawater from Puget Sound filtered to 1 µm, UV sterilized, and maintained at 12°C. Prior to flow into each replicate, seawater was degassed. Each replicate then added carbon dioxide, oxygen, and nitrogen under real-time, data-driven feedback control system using LabView Software (version needed) with Honeywell Durafet III pH and Vernier optical dissolved oxygen probes and Omega thermistors. Flow through the aquaria that housed krill was controlled with a solenoid valve, and during periods of flow was approximately 53 L/hr.

*Treatments*

During the acclimation period (11 days), the experimental system was held at 11°C and water chemistry was not manipulated (i.e., conditions reflected that of intake water). After the acclimation period, replicates were assigned to treatments in a way that balanced percent survival across treatments, and the experimental system was brought to treatment conditions over 72 hours. The study included 3 scenario treatments with conditions that differed between day (13.5 hours) and night periods (7.5 hours), in an attempt to capture the different environmental conditions experienced by adult krill over the diel cycle: modern (N=3 replicates; “day” targets: T=11°C, pH=7.64, DO=5.6 mg O2/L; “night” targets: T=12°C, pH=7.73, DO=9.2 mg O2/L); heat wave (“day” targets: T=13°C, pH=7.64, DO=5.4 mg O2/L; “night” targets: T=14°C, pH=7.73, DO=8.8 mg O2/L); and future ocean (“day” targets: T=13°C, pH=7.50, DO=4.5 mg O2/L; “night” targets: T=14°C, pH=7.57, DO=8.8 mg O2/L). These treatments were based on the observational data presented in Reum et al 2014 and 2016 and model output presented in Khangaonkar et al 2019. The transition in chemistry between the two periods of the day was achieved over approximately 2 hr by quickly altering the chemistry of the water flowing into the krill aquaria and letting the water mass change as water with the new conditions replaced the old. During the first 3 days that the krill were held in the lab, their diel cycle was flipped so that feeding and data collection, which happened during their nighttime conditions, occurred during daylight hours. Flow into krill aquaria was stopped for 7 hours/day during night conditions to allow the krill to feed.

*Chemistry*

Probes in each replicate continuously recorded temperature, pH, and DO. Temperature probes were calibrated with a ThermoScientific Orion Star A322 temperature probe, pH probes with Tris buffer, and DO probes with a PreSens optical oxygen Fitbox4 sensor and oxygen sensor spots (SP-PSt3-NAU-D5-YOP). Salinity was measured daily in each replicate with Honeywell UDA (Toroidal Conductivity Monitor Model Q46CT). Each replicate was monitored weekly for pH, DO, and salinity. pH conditions in the aquaria of each replicate were monitored via spectrophotometry using an Ocean Optics USB 230 2000+ Fiber Optic Spectrometer and m-cresol purple indicator dye (Sigma Aldrich). DO conditions were monitored with the Presens systems, and salinity with ThermoScientific Orion Star A322. Discrete water samples for measurement of total alkalinity and DIC were taken X days after the beginning of the experiment and Y days after its end. These samples were analyzed at XXXX using standard operating procedures for all carbon chemistry analyses (Dickson et al 2007).

We used XXX and XXX measurements to estimate pCO2 with CO2sys version 2.1 [50], using the K1 and K2 constants from Lueker et al. [51], KHSO4 constant from Dickson [52], [B]T from Uppstrom [53], and the total pH scale.

*Collection*

Adult *Euphausia pacifica* krillwere collected from Puget Sound off of Mukilteo, WA (47.982569, -122.307623 +/- .25nm) on September 9 and 10, 2019 between 20:30-21:30. To capture krill, a 1 meter ring net with 333μm mesh was towed obliquely at 2-3 knots at an approximate depth of 25 m for 3 minutes. The contents of each tow were immediately transferred to a temperature-controlled, aerated holding tank and transported to the laboratory, where they were haphazardly sorted into the experimental system. The approximate time from collection to placement into the laboratory experimental system was 3-4 hours. No gravid females (based on ovary color) were included in the study (just 4 were sited in the collected krill).

*Husbandry*

Krill were held under acclimation conditions for 11-12 days and treatment conditions for 35-37 days. They were housed in 44L aquaria inside each replicate experimental treatment at an initial density of 80-85 krill per replicate (1.8 krill/L; ~ 3 g krill per replicate). Every day during the period of no flow in the aquaria, each replicate was fed 6.9 mL Instant Algae (Reed Mariculture Shellfish Diet 1800; approximately 13.8 billion cells). Every three days, each replicate was given 0.44 mL EZ Larvae (Zeigler 250-600 micron Larvae Concentrate). Every four days, each replicate was given approximately 44,000 freshly hatched *Artemia salina* (San Francisco Bay; density ≃ 1 nauplius/mL aquarium). EZ Larvae and *Artemia salina* were also added during the no-flow period. Every four days, each aquarium was cleaned during the period of water flow using a siphon and sponge.

*Survival and size*

Mortalities were assessed visually every Monday and Thursday in each replicate as was the presence or absence of molts. Incomplete carcasses collected during cleaning on those days that had the majority of the segmented abdomen intact were included in the mortality count; disconnected eye stalks, rostrums, and antennae were not included in the mortality estimates. At the end of the study, it was apparent that not all mortalities were captured by visual assessment as the number counted plus the number of survivors did not equal the number of krill at the beginning of the study. For each replicate, we determined the number of missing krill and added these animals as extra mortalities at the rate that matched the observed mortality rate, which was highest in the first three weeks of captivity.

For each morality over the course of the experiment and for all individuals collected at the end of the experiment, species identification was confirmed and telson length was measured using a Nikon microscope (SMZ715T) with Sony Camera (DFK 33UX226c) configured with Imaging Source IC Capture software (version 2.4.642.2631).

*Respirometry*

Respiration rate was measured at the level of the individual using PreSens optical oxygen Fitbox4 sensor and oxygen sensor spots (SP-PSt3-NAU-D5-YOP) in 22.5 mL borosilicate vials with polycone-lined phenolic caps. Each trial, which included 17 krill and 2 water-only blanks for measurement of background O2 change, was 2 hours long, with a 10 min acclimation period and measurements taken every 15 min. In order to assure that dissolved oxygen did not decrease below 70% saturation, we truncated all datasets at XXX minutes after initiation. We assumed that activity of the krill mixed water in the incubation vial and that any mechanical mixing within the vials would prevent measurement of resting metabolic rate. During a trial, incubation vials were placed in a water bath held between 11.4-12℃. Krill were starved prior to the trials for mean ± st. dev. = 28.5 ± 1.7 hr, and trials took place during nighttime conditions over the course of two days. All individuals whose respiration rate was measured were included in the samples collected for lipid analysis.

*Lipid analysis*

At the end of the study, samples (N=1-4/replicate) were collected for lipid analysis. In order to have enough tissue for sample analysis, krill from each replicate were pooled until ~100mg of tissue was collected (N≃3-5 krill). All krill for lipid analysis were collected during the “nighttime”, no-flow period and were starved for at least 24 hrs prior to lipid collection (mean ± st. dev. = X ± Y hr). Prior to sample collection, each krill was identified to species, photographed for measurement of telson length, rinsed in filtered seawater, patted dry with a Kim Wipe, and weighed. Each composite sample was frozen to -80⁰C immediately after collection.

To characterize the lipid profile of wild krill, 10 hours after collection on September 10, 2019, 4 ~100 mg samples of freshly caught krill (N≃3 krill per sample). These animals we held outside of the experimental system under temperature control and aeration, under starvation conditions.

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*Statistics*

**Results**

*Chemistry*

The experiment began with 4 replicate systems for each treatment, but, due to problems that occurred over the course of the experiment (faulty thermistors (N=2 replicates), pH probe failure (N=1 replicate), and a data feedback failure that led to high temperature (N=1)), it ended with 3 replicates in the modern treatment, 2 for the heat wave treatment, and 2 for the future ocean treatment. A storm event occurred between 18-19 October, which placed increased sediment and strain on system filters, and temporarily caused more variance in treatment conditions, resulting in the data feedback failure referenced above.

*Survival*

Discussion

Aguilera et al 2020

“This study showed a link between variations in pH and copepod traits and performance in the upwelling system of the Humboldt Eastern Boundary Upwelling system (EBUs). In particular, deleterious efects of low pH on EPR, egg size, and egg production efciency were evident afer independent statistical analyses of the observations. Moreover, the deleterious efects of low pH efects on EPR were mitigated by the availability of chlorophyll,

suggesting that the efects of pH on copepod performance in this system are modulated by food resource. Tese fndings have implications for our understanding of organismal response to OA.