

## Higher survival but smaller size of juvenile Dungeness crab (*Metacarcinus magister*) in high CO<sub>2</sub>

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### Supplemental Tables and Figures

**Supplemental Table 1:** Experiment seawater chemistry monthly means with standard deviations. Temperature (°C) is from the average of all 4 durafet pH sensors deployed in the experimental tanks. Salinity data are from periodic measurements taken of all 6 experimental tanks use a hand-held Orion salinity sensor. The ambient and high-CO<sub>2</sub> pH values are spectrophotometric pH averages from all treatment tanks. Alkalinity was calculated from the salinity values using the functional relationship in (Trigg *et al.*, 2019). The pCO<sub>2</sub> and calcite saturation state values were calculated using the SeaCarb R package (Gattuso *et al.*, 2021) with the spectrophotometric pH and calculated alkalinity values as carbonate system parameter inputs. (see supplemental figure 6 and supplemental raw data files for additional information.)

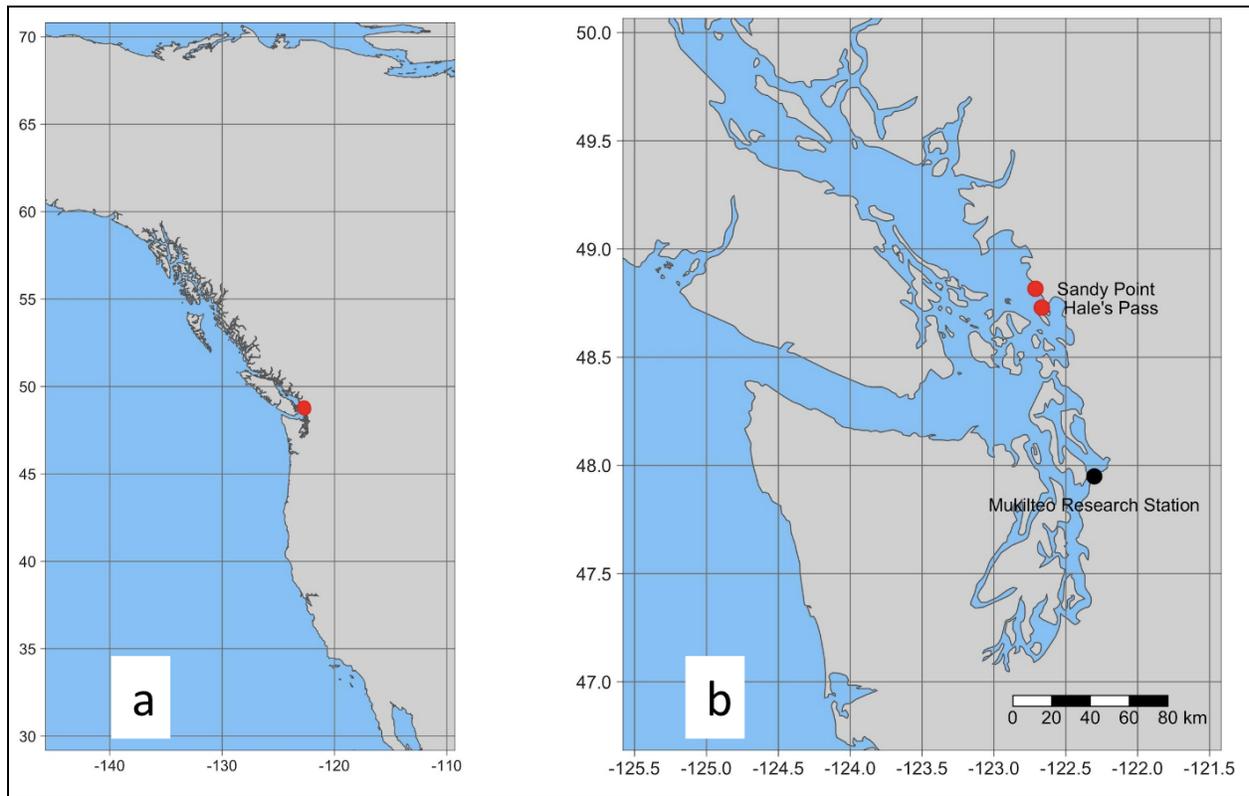
month	temperature	salinity	alkalinity	pH_amb	pH_high	pco2_amb	pco2_high	omega_amb	omega_high
August	12.52 (0.11)	29.99 (0)	2032.35 (0)	7.68 (0.04)	7.23 (0.04)	953.22 (107.78)	2716.05 (233.72)	1.3 (0.13)	0.5 (0.04)
September	12.83 (0.56)	30.27 (0.02)	2046.37 (0.95)	7.71 (0.09)	7.19 (0.05)	775.1 (113.01)	3131.5 (489.56)	1.64 (0.2)	0.46 (0.07)
October	12.38 (0.2)	30.79 (0)	2072.63 (0)	7.69 (0.02)	7.15 (0.06)	923.84 (15.33)	3527.46 (422.38)	1.39 (0.02)	0.41 (0.05)
November	11.44 (0.28)	30.46 (0.39)	2056.17 (19.73)	7.71 (0.03)	7.17 (0.09)	879.24 (20.77)	3208.95 (666.93)	1.37 (0.02)	0.43 (0.09)
December	10.39 (0.4)	30.42 (0.1)	2053.83 (5.22)	7.7 (0.08)	7.22 (0.15)	859.27 (31.83)	2878.78 (699.84)	1.34 (0.04)	0.5 (0.28)
January	9.23 (0.17)	30.08*	2036.97*	7.73*	7.21*	803.42*	2928.03*	1.33*	0.44*
February	8.45 (0.42)	29.75 (0.09)	2020.1 (4.76)	7.76 (0.02)	7.19 (0.08)	747.58 (45.34)	2977.28 (585.75)	1.33 (0.04)	0.39 (0.07)
March	8.39 (0.3)	29.5 (0)	2007.68 (0)	7.77 (0.03)	7.18 (0.09)	716.37 (43.27)	3048.15 (566.87)	1.36 (0.07)	0.37 (0.08)
April	9.14 (0.4)	29.39 (0.16)	2001.93 (7.96)	7.8 (0.04)	7.16 (0.06)	663.72 (54.2)	3173.15 (430.58)	1.5 (0.11)	0.36 (0.05)
May	9.55 (0.57)	29.82 (0.13)	2023.74 (6.42)	7.82 (0.01)	7.17 (0.09)	640.36 (6.81)	3142.06 (647.38)	1.6 (0.02)	0.4 (0.09)
June	11.13 (0.37)	29.49 (0.18)	2006.96 (9.12)	7.85 (0.02)	7.26 (0.21)	592.81 (32.75)	2741.84 (864.2)	1.78 (0.05)	0.56 (0.39)
July	11.75 (0.29)	29.6 (0)	2012.72 (0)	7.8 (0.01)	7.21 (0.16)	689.68 (12.47)	3012.12 (1069.51)	1.62 (0.02)	0.47 (0.17)
Grand_mean	9.99 (1.29)	29.84 (0.37)	2024.84 (18.4)	7.8 (0.05)	7.19 (0.12)	676.83 (81.62)	3054.62 (676.45)	1.55 (0.12)	0.43 (0.2)

\*No spectrophotometric pH or Orion salinity data were recorded for January because a U.S. Government shutdown prevented collection of these data. However, automated pH and salinity sensors operating in the experimental tanks during January indicate that interpolation by averaged data from December and February, as presented here, is a reasonable approximation.

**Table 2:** Sample sizes and p-values for analysis of the size of juvenile Dungeness crab reared in ambient and high CO<sub>2</sub>. Analysis compares the effect of CO<sub>2</sub> within a single life-stage. The “Size Diff” is the average size of crabs rear in ambient CO<sub>2</sub> minus the average size of crabs reared in high CO<sub>2</sub>. The “Percent Diff” is the percent difference between the ambient and high CO<sub>2</sub> measurement Significant p-values in bold.

Size Metric	Life-Stage	Ambient CO <sub>2</sub> Sample Size	High CO <sub>2</sub> Sample Size	Total Sample Size	Ambient CO <sub>2</sub> mean size	High CO <sub>2</sub> mean size	Size Diff	Size Units	Percent Diff	p-value
width	J1	51	66	117	4.9	4.89	0.01	mm	0.2	0.7972
width	J2	57	76	133	6.52	6.42	0.10	mm	1.6	0.1427
width	J3	50	70	120	8.64	8.22	0.41	mm	5.0	<b>0.0014</b>
width	J4	45	65	110	10.3	9.63	0.67	mm	7.0	<b>&lt;0.0001</b>
width	J5	39	56	95	12.84	12	0.84	mm	7.0	<b>&lt;0.0001</b>
width	J6	16	48	64	15.65	14.4	1.26	mm	8.7	<b>0.0030</b>
width	J7	4	26	30	17.85	16.61	1.24	mm	7.4	0.1551
length	J1	51	66	117	5.1	5.12	-0.03	mm	-0.5	0.5878
length	J2	57	75	132	6.01	6	0.01	mm	0.2	0.8406
length	J3	48	68	116	7.49	7.32	0.17	mm	2.4	0.1086
length	J4	45	65	110	8.78	8.32	0.46	mm	5.5	<b>&lt;0.0001</b>
length	J5	37	56	93	10.6	10.14	0.46	mm	4.5	<b>0.0077</b>
length	J6	14	46	60	12.74	11.87	0.87	mm	7.3	<b>0.0097</b>
length	J7	4	21	25	13.93	13.62	0.30	mm	2.2	0.6366
length/width	J1	51	66	117	1.04	1.05	-0.01	unitless	-0.7	0.3664
length/width	J2	57	75	132	0.92	0.94	-0.01	unitless	-1.4	<b>0.0288</b>
length/width	J3	48	68	116	0.87	0.89	-0.02	unitless	-1.9	0.0526
length/width	J4	45	65	110	0.85	0.86	-0.01	unitless	-1.3	<b>0.0344</b>
length/width	J5	37	55	92	0.82	0.85	-0.02	unitless	-2.9	<b>&lt;0.0001</b>
length/width	J6	14	46	60	0.81	0.83	-0.01	unitless	-1.5	0.0749
length/width	J7	4	21	25	0.78	0.81	-0.03	unitless	-4	<b>0.0206</b>
weight	J5	7	4	11	0.43	0.4	0.02	g	5.7	0.7336
weight	J6	13	29	42	0.73	0.63	0.10	g	15.3	0.0745
weight	J7	2	17	19	0.95	0.89	0.06	g	6.4	0.6964
weight/width	J5	7	3	10	0.03	0.04	-0.01	g/mm	-13.1	0.4060
weight/width	J6	13	28	41	0.05	0.04	0.0	g/mm	8.8	0.1901
weight/width	J7	2	17	19	0.05	0.05	0.0	g/mm	1.9	0.8634

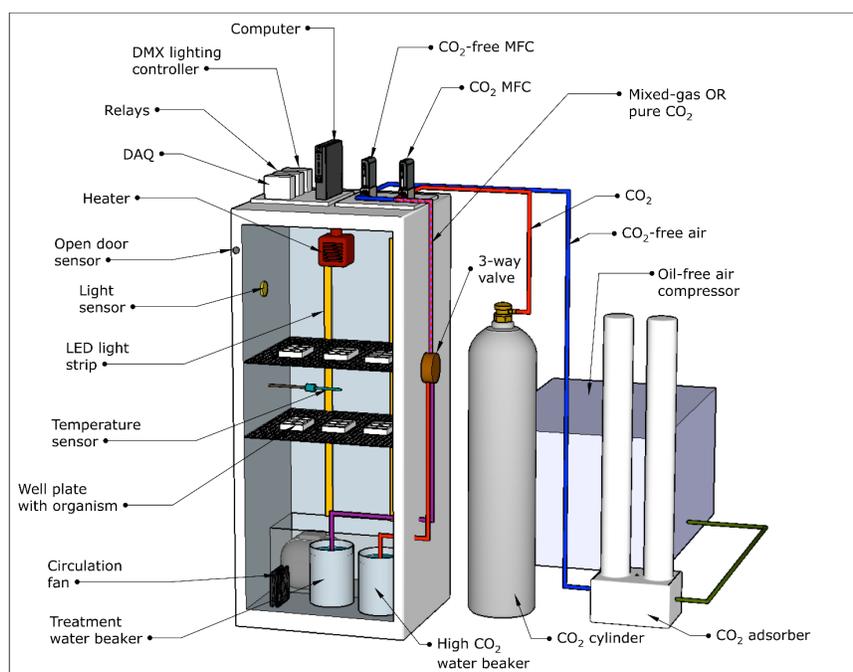
## Supplemental Figures



**Supplemental Figure 1:** Maps showing the location of Dungeness crab megalope collection sites and research station where experiments were conducted at the scale of the West Coast of North America (a) and at the scale of the Salish Sea (b). The two collection locations were Hale's Pass (48.72923 -122.667) and Sandy Point (48.81622 -122.711), which are ~10km apart. The Hale's Pass light trap site is ~150m from shore and the Sandy Point site is ~250m from shore.



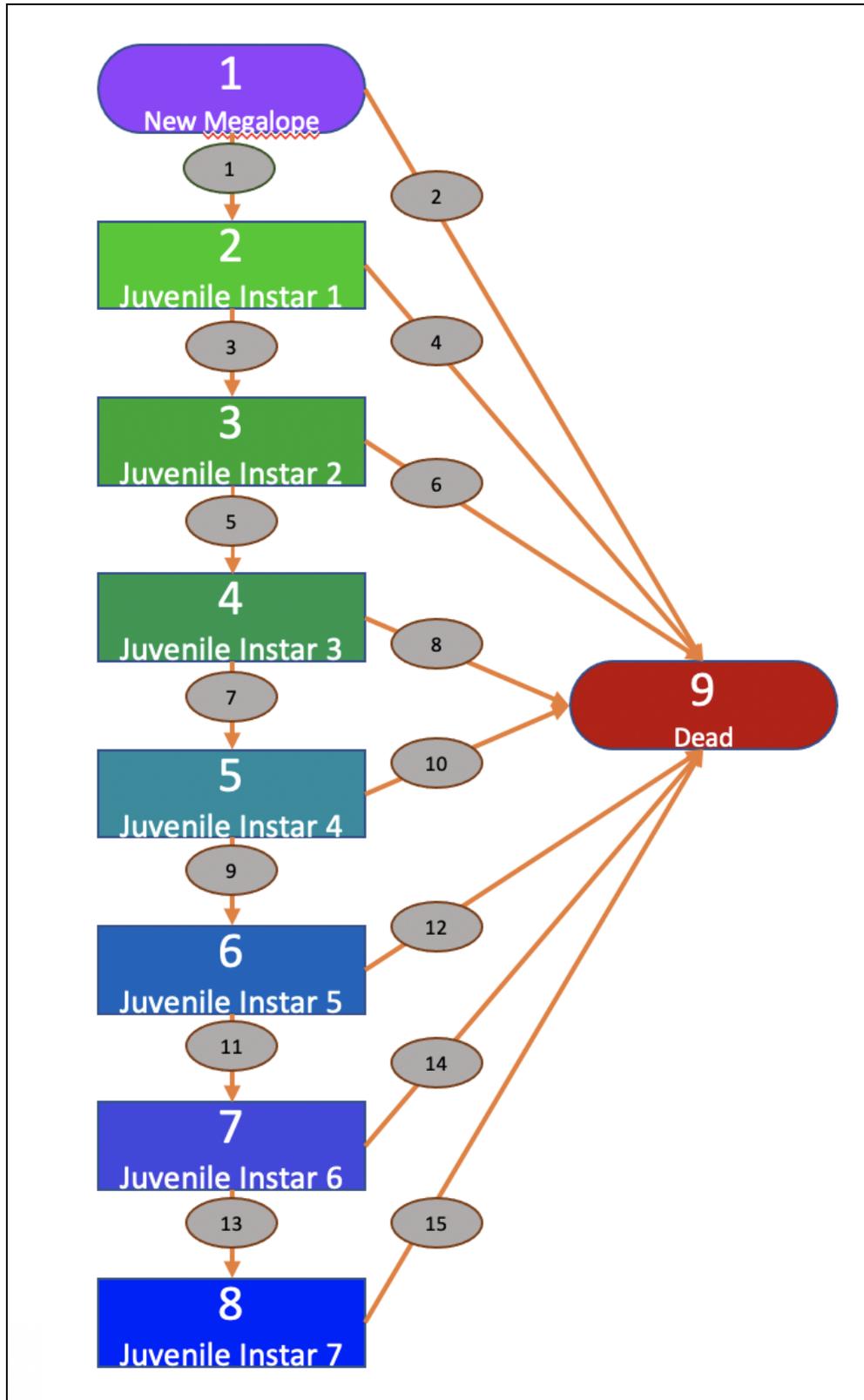
**Supplemental Figure 2:** Light trap used to collect megalope. (photo provided by Nicholas T. Jefferson, biologist with the Lummi Nation Department of Natural Resources).



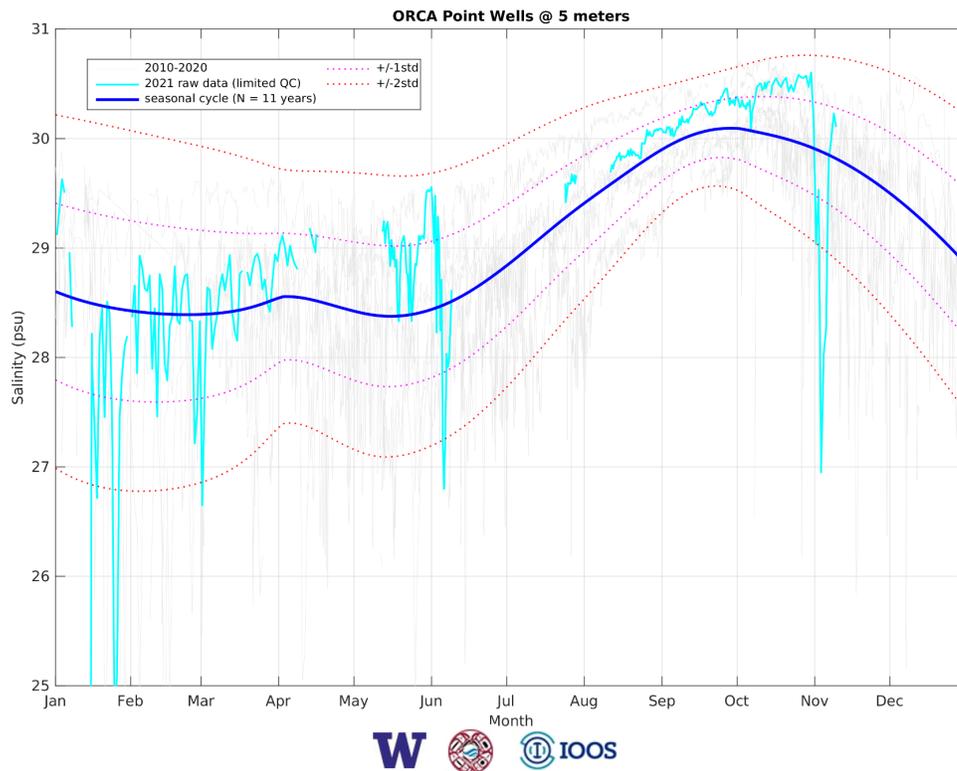
**Supplemental Figure 3:** Simplified schematic of CO<sub>2</sub>-chamber with door removed. The base unit is a small (311 L) refrigerator. The temperature, CO<sub>2</sub> concentration and lighting are all controlled via a custom Labview<sup>®</sup> program running on a small Windows<sup>®</sup> platform computer. For temperature control, a high precision silicon temperature probe provides input with output vis USB relays controlling the refrigerator compressor for cooling and a PTC electric heater with fan for heating. The concentration of CO<sub>2</sub> in the chamber is controlled with two mass flow controllers. One mass flow controller regulates the stream of CO<sub>2</sub>-free air from a CO<sub>2</sub>-adsorber. The second mass flow controller regulates the flow of CO<sub>2</sub> from a compressed gas cylinder. To produce treatment water for initial distribution into well plates or to maintain the CO<sub>2</sub> concentration in a chamber at target conditions, mixed-gas at the target CO<sub>2</sub> concentration (e.g. 400 ppm or 2100 ppm) is bubbled into a beaker of seawater. Bubbling in water maintains high humidity in the chamber, which reduces evaporation in the well plates. Although most bubbling uses mixed gas at the target concentration, immediately after opening and closing the door, pure CO<sub>2</sub> is briefly bubbled in the chamber to rapidly return the chamber from the ambient CO<sub>2</sub> concentration experienced when the door opens (~400ppm) to the target concentration. To prevent adding too much CO<sub>2</sub> to water being equilibrated for well plates, the temporary high CO<sub>2</sub>-stream is diverted by a 3-way valve to a second beaker of water. To control lighting, a DMX controller is used to regulate the intensity and color of four LED strips (two LED strips attached to the door are not shown in the schematic.) The LED strips are 4-color (rbgw), which allows production of any color frequency distribution in the visible spectrum. A light sensor in the chamber validates whether lights come on as programmed. Sensor inputs to the computer are routed through a Labjack<sup>®</sup> DAQ. The labview program provides an interface for controlling set points, a mechanism for viewing and archiving chamber data and the ability to set alarm thresholds for text message chamber condition updates.



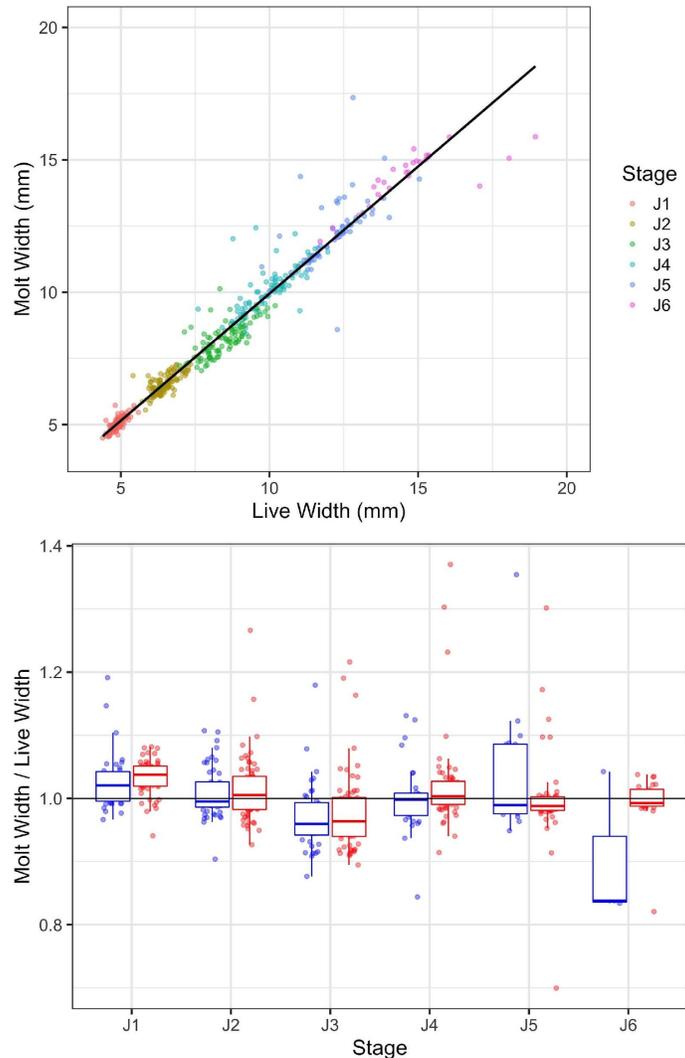
**Supplemental Figure 4:** Mesh jar used for rearing juvenile Dungeness crab in a CO<sub>2</sub> exposure experiment (“crabitat cups”, left panel) and multiple crabitat cups in a 1.2m diameter treatment tank (right panel).



**Supplemental Figure 5:** Transition diagram showing the 9 states and 15 transitions for multi-state hazard model.



**Supplemental Figure 6:** Salinity climatology data from the Point Wells buoy in Puget Sound (47° 45.67' N, 122° 23.83' W), approximately 25km from the Mukilteo Research Station intakes. The plot was downloaded from the ORCA Buoy web site ([https://nwem.apl.washington.edu/prod\\_PS\\_PWells.shtml](https://nwem.apl.washington.edu/prod_PS_PWells.shtml)) on December 8, 2021. The data show the seasonal decline in salinity that typically occurs around January.



**Supplemental Figure 7:** The upper panel shows the relationship between the carapace width of live Dungeness crab at a particular juvenile stage and the width of the discarded carapace after the crab molted out of that stage into the next. Each point is an individual crab at a particular life-stage and the black inline is the linear regression through all points. The lower panel shows the discarded molt to live crab carapace ratio at each life-stage for crab reared in ambient CO<sub>2</sub> (blue) and high CO<sub>2</sub> (red). A molt-to-live ratio of one would indicate that the discarded carapace was exactly the same size as the carapace on the live crab before it was discarded. The points are individual crabs at a particular life-stage with the bar indicating the median, the box encompassing the 25<sup>th</sup> and 75<sup>th</sup> percentiles and the whisker showing 1.5 times the inter-quartile range. There was no significant difference in the discard to live width ratio with regard to CO<sub>2</sub> treatment, but there was a significant difference among the stages (Supplemental Figure #Figure 6 lower panel; for model  $\text{discard/live} = \text{treatment} + \text{stage}$ ,  $p_{\text{treatment}} = 0.32$ ,  $p_{\text{stage}} < 0.01$  for all pairwise comparisons). The length of the delay between molting and the analysis of the discarded exoskeleton width had no effect on the discard/live width ratio, suggesting that the age of a discarded exoskeleton did not affect its size.