Supporting Information. Spencer, L. H., Y. R. Venkataraman, R. Crim, S. Ryan, M. J. Horwith, and S. B. Roberts. 2020. Carryover effects of temperature and pCO$_2$ across multiple Olympia oyster populations. Ecological Applications.

Appendix S1

Data, R script analyses, gonad tissue images, and high-resolution supplementary figures are available on Figshare (Spencer et al. 2019).

Section S1: Recipe for Tris buffer (0.08 M, 28.0 PSU)

- 0.3603 mol of NaCl (J.T. Baker)
- 0.0106 mol of KCl (Fisher Scientific)
- 0.0293 mol MgSO4-(H2O)7 (Fisher Scientific)
- 0.0107 mol of CaCl2-2(H2O) (MP Biomedicals)
- 0.0401 HCl (J.T. Baker)
- 0.0799 mol of Tris base (Fisher Scientific)

Deionized water was added for a final volume of 1L

Section S2: Instruments used in experimental treatments in the hatchery

- Temperature treatment manipulation and monitoring:
  - Chilling experimental water: Teco Aquarium Chiller, Model TK-500
  - Continuous temperature measurements: Onset HOBO Water Temperature Data Loggers, Model U22-001
- **pCO$_2$ treatment manipulation and monitoring:**
  - pH monitoring: Durafet pH probes, Honeywell Model 51453503-505
  - pCO$_2$ injection control: Honeywell Dual Input Analytical Analyzer, Model 50003691-501
  - Temperature monitoring: HOBO Pendant Temperature Data Loggers, Model UA-002-64
- **Carbonate chemistry measurements:**
  - Temperature: Fisher Traceable Digital Thermometer, Model 15-077
  - Salinity: VWR Bench/Portable Conductivity Meter, Model 23226-505
  - pH: Mettler Toledo Combination pH Electrode, Model 11278-220
  - Total alkalinity: Mettler Toledo Excellence Titrator, Model T5 Rondolino
- Algal dosing: metering pump – Iwaki EZ Controller, Model EZCD1

Instruments used in field trial for continuous environmental data collection:
- pH: Honeywell Durafet II Electrode, in custom-built housing
- Salinity: via conductivity, Dataflow Systems Ltd. Odyssey Conductivity and Temperature Logger
- Dissolved Oxygen: Precision Measurement Engineering MiniDOT Logger
- Temperature: via dissolved oxygen probes
- Chlorophyll: Turner Designs Cyclops-7F Submersible Sensor with PME Cyclops-7 Data Loggers

Table S1: Carbonate chemistry parameters for three time points during the pCO₂ treatments, which are averages (± SE) from three replicate tanks per treatment. All parameters except for total alkalinity differed significantly between control/ambient (Amb.) and experimental/high (High.) tanks (One-way ANOVA). More details are available in Venkataraman, Spencer & Roberts, 2019.

Day	pH*** $F_{(1,16)} = 5838$, $p = 6.12e-22$		Total Alkalinity (µmol/kg) $F_{(1,16)} = 1.38$, $p = 0.257$		pCO₂ (µatm)*** $F_{(1,16)} = 235$, $p = 5.44e-11$		DIC (µmol/kg)* $F_{(1,16)} = 7.12$, $p = 0.0168$		$\Omega_{calcite}$*** $F_{(1,16)} = 529$, $p = 1.10e-13$		$\Omega_{aragonite}$*** $F_{(1,16)} = 527$, $p = 1.14e-13$	
	Amb.	High	Amb.	High	Amb.	High	Amb.	High.	Amb.	High	Amb.	High
5	7.82 ± 0.004	7.33 ± 0.002	2307.41 ± 25.45	2332.36 ± 31.05	747.51 ± 13.94	2481.23 ± 29.83	2233.41 ± 25.29	2408.51 ± 31.76	1.86 ± 0.02	0.62 ± 0.01	1.16 ± 0.012	0.58 ± 0.007
33	7.81 ± 0.005	7.31 ± 0.004	2747.00 ± 21.13	2917.60 ± 18.36	912.22 ± 12.69	3309.52 ± 7.22	2664.57 ± 19.99	3020.99 ± 17.99	2.23 ± 0.03	0.77 ± 0.02	1.40 ± 0.020	0.48 ± 0.014
48	7.82 ± 0.015	7.29 ± 0.004	2611.40 ± 31.01	2808.39 ± 12.24	863.47 ± 42.42	3343.89 ± 49.49	2533.28 ± 35.45	2920.52 ± 15.11	2.13 ± 0.06	0.68 ± 0.01	1.32 ± 0.035	0.42 ± 0.004

Section S3: Adult shell height

Oysters sampled for histology were also measured for shell height using digital calipers (mm), defined as the maximum distance from the umbo along the dorsal/ventral axis. Shell height was compared between treatments prior to and after pCO_2 exposure using two-way Analysis of Variance (ANOVA) for each cohort. Shell height was also compared among cohort using one-way ANOVA, excluding the younger O-2 cohort due to their smaller initial size.

Prior to pCO_2 treatments, adult shell height did not vary between temperatures treatments, but did among F1 cohorts, with D smaller than F (p=0.043). After pCO_2 treatment, D was smaller than both F (p=3.4e-6) and O-1 (p=3.5e-6). The O-1 cohort increased in size (p=0.019) in ambient pCO_2, but not in high pCO_2. No size differences among pre-pCO_2 and post-pCO_2 treatments were observed in the F, D or O-2 cohorts.
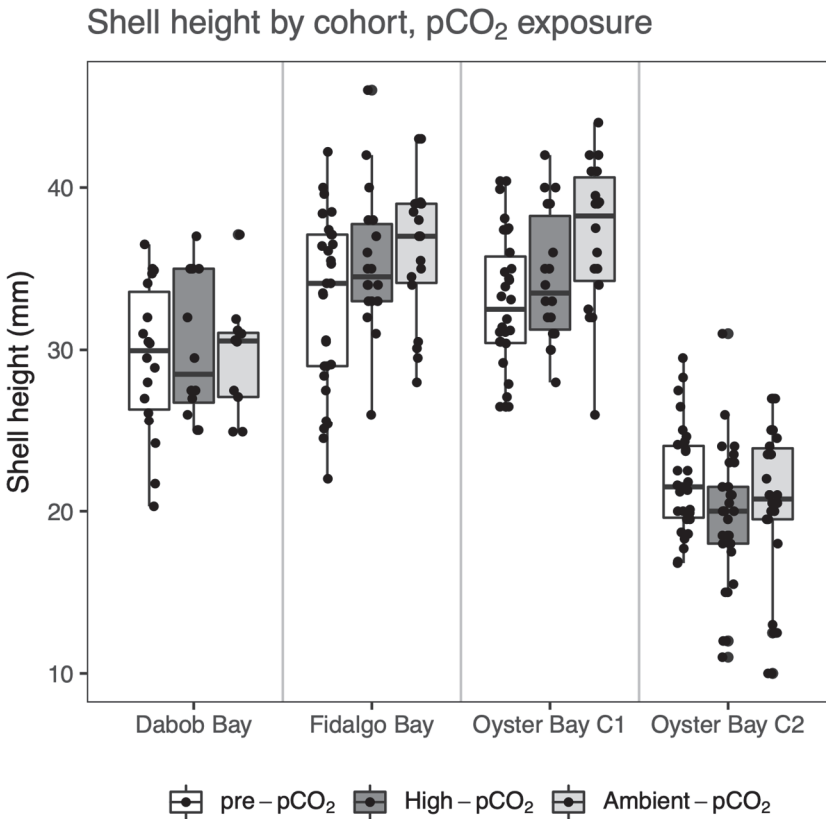

Figure S1: Adult shell height for each cohort, after temperature treatment but before pCO_2 treatment ("Pre-pCO_2"), and after 52 days in High-pCO_2 (3045±488 µatm, n=39), and Ambient-pCO_2 (7.82±0.02, n=39).

Table S2: Gonad stage designations, adapted from da Silva 2009		
Stage	Designation	Description
0	Inactive	Empty follicles, no presence of male or female gonad tissue.
1	Early gametogenesis	Gametes were mostly attached to the follicle wall. In the male developing line, spermatogonia and spermatocytes, very few spermatids; in the female developing line, mostly attached, developing ovogonia and early ovocytes.
2	Advanced gametogenesis	In the male developing line few spermatogonia, spermatocytes and spermatid balls were dominant, and few spermatozoa balls appeared in the follicle lumen; in the female developing line, few ovogonia present, ovocytes in vitellogenesis but attached were dominant, and ovocytes in post-vitellogenesis and located free in the follicle lumen less abundant.
3	Ripe	In both male and female developing lines, follicles contained mature gametes and sometimes a thin layer of primary germ cells. Abundant spermatozoa balls and mature ovocytes filled the follicle lumen, in male line and female line, respectively.
4	Spawned (full or partial), and/or resorbing	Gametes had been released, and follicles are dilated but lumen was empty, or contained residual mature gametes; residual oocytes of various sizes were sparsely distributed; residual spermatids were dissociated within follicle lumen. In some cases phagocytes were observed within follicles to re-absorb residual gametes. In many cases residual gametes of one sex remained, while developing gametes of the other sex were abundant.

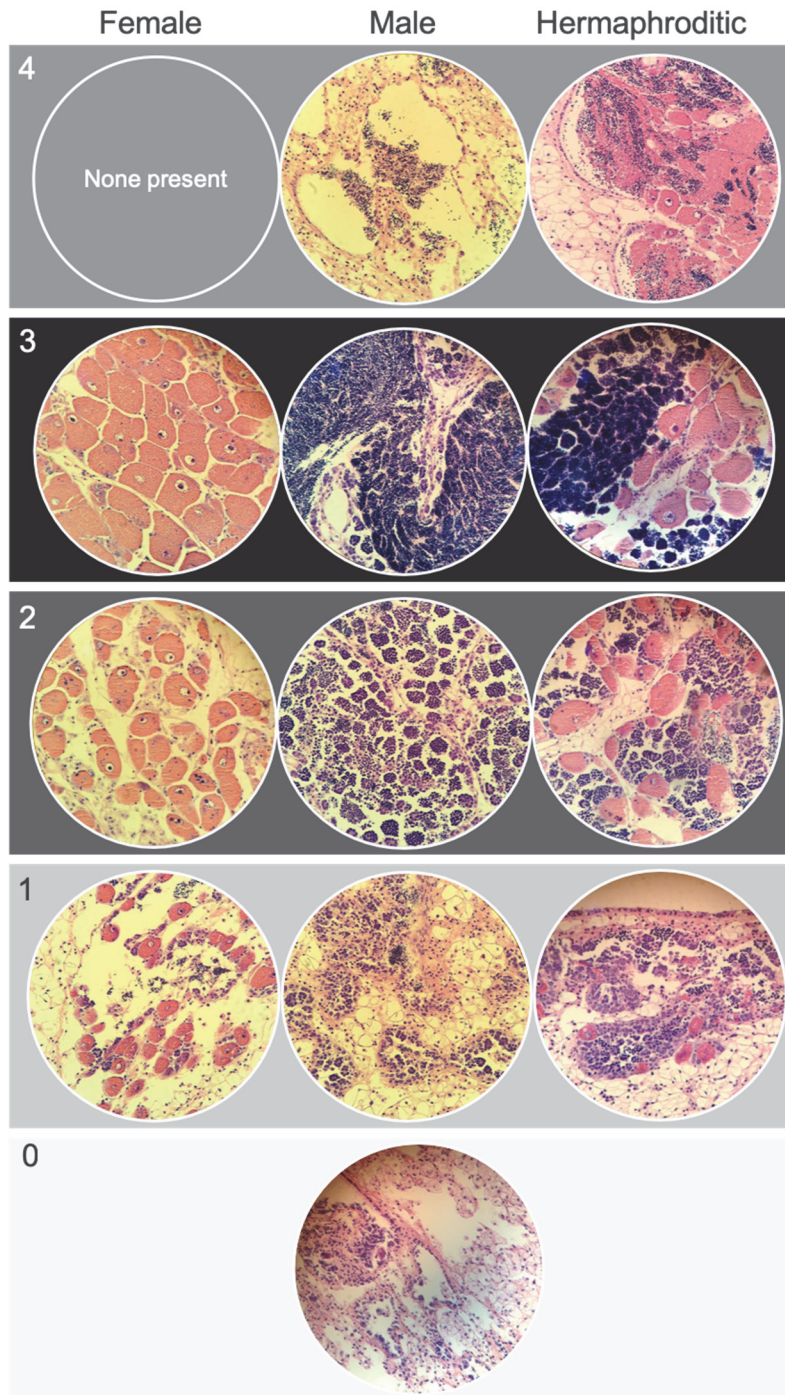| Table S3: Gonad sex designations, from da Silva 2009			
Sex (uncollapsed, from da Silva)	Sex, collapsed for statistical analyses	Designation	Description
F	F	Female	Follicles contain only female gonad material (any stage)
HPF	F	Hermaphroditic, predominantly female	Follicles contain predominantly female but also some male gonad material
H	H	Hermaphroditic	Follicles contain approximately half male and half female gonad material
HPM	M	Hermaphroditic, predominantly male	Follicles contain predominantly male but also some female gonad material
M	M	Male	Follicles contain only male gonad material (any stage)
I	I	Indeterminate	Follicles are empty, collapsed, or only undifferentiated gonia are visible

Figure S2: Examples of *Ostrea lurida* gonad stage designations. Stage 0 (no activity/sex differentiation); Stage 1 (early gametogenesis); Stage 2 (advanced gametogenesis); Stage 3 (late gametogenesis / ripe); Stage 4 (spawned and/or resorbing).

Section S4: Gonad sex and stage details and cohort traits

Of all sampled oysters, 53.4% were hermaphrodites, 8.3% contained only female gametes, and 31.1% contained only male gametes, and the remaining 7.2% were indeterminate. Across all treatments, gonad sex (collapsed for comparison) differed significantly among cohorts (χ^2=55.8, p=1.0e-4). Fifty percent of all O-1 oysters sampled were female or hermaphroditic-primarily female (HPF), while 33%, 24% and 11% of D, F, and O-2 were female or HPF. Male or hermaphroditic-primarily male oysters comprised 29%, 48%, 59% and 69% of O-1, D, F, and O-2 cohorts, respectively.
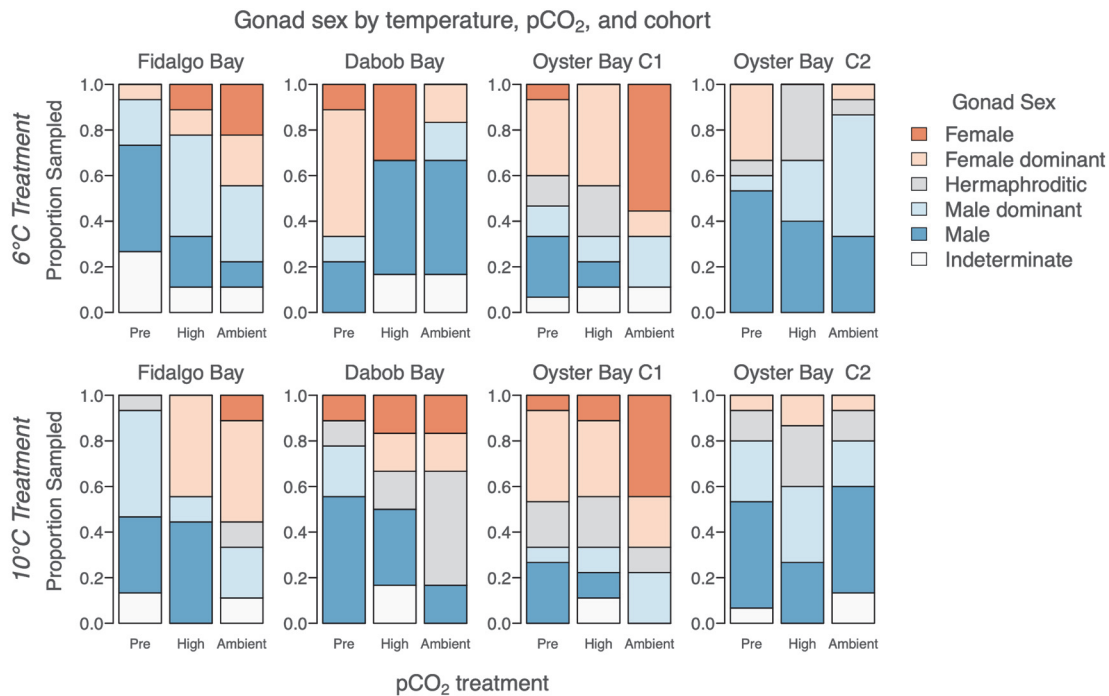

Figure S3: Gonad sex for each cohort, after temperature treatment but before pCO₂ treatment ("Pre"), and after 52 days in high pCO₂ (3045±488 µatm, n=39, "High"), and ambient pCO₂ (7.82±0.02, n=39, "Ambient"), separated by temperature treatment (6°C and 10°C).
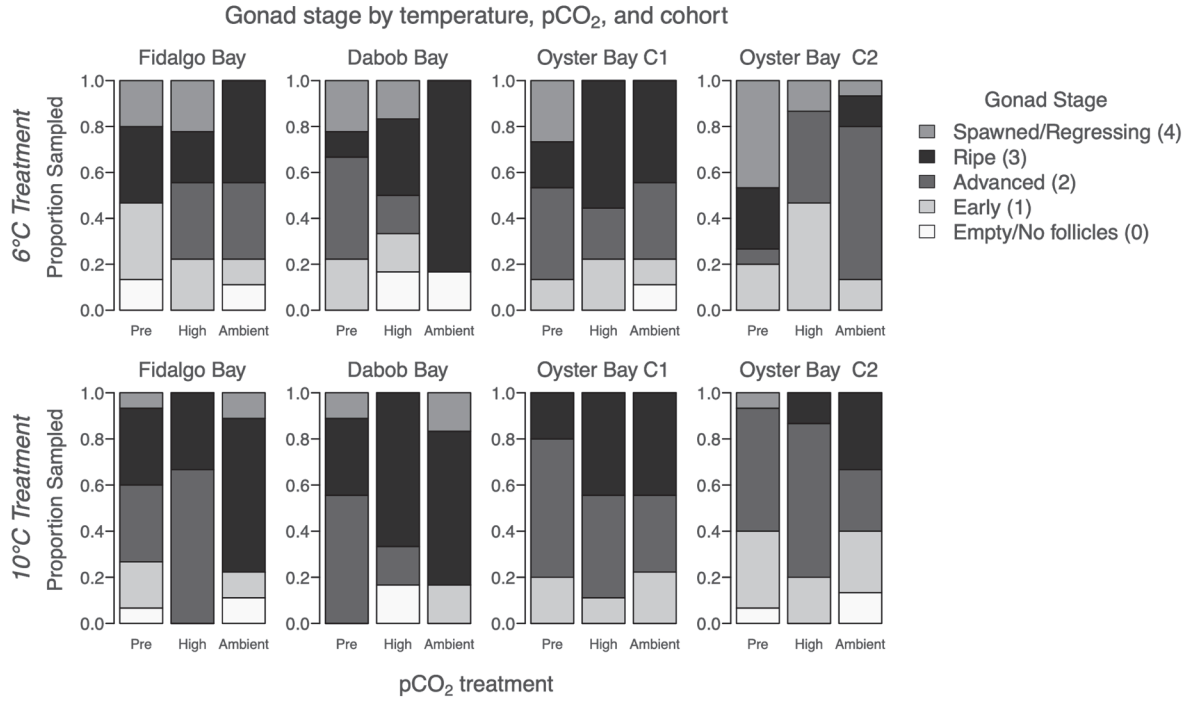
Figure S4: Gonad stage of the dominant sex for each cohort, after temperature treatment but before pCO₂ treatment ("Pre"), and after 52 days in high pCO₂ (3045±488 µatm, n=39, "High"), and ambient pCO₂ (7.82±0.02, n=39, "Ambient"), separated by temperature treatment (6°C and 10°C).

Table S4: Frequency of dominant gonad stages by cohort and pCO_2 exposure, separated by winter temperature treatment (6°C, 10°C). Results of Chi-Square between pCO_2 treatments are shown as p-values for dominant stage (Dom-stage), dominant sex (Dom-sex), Male stages (Male), and female stages (female). Across all cohorts in both 6°C and 10°C treatments, dominant stage and male stage differed by pCO_2 treatments. Stage differences were detected between pre-treatment (pre-pCO_2) and after treatment for ambient and high pCO_2 separately, and are indicated by superscripts A and B: A= pre-treatment and ambient pCO_2 differed; B=pre-treatment and high pCO_2 differed ($p<0.05$). Stage and sex comparisons in bold indicate that they were significantly different between temperature treatments, prior to pCO_2 treatments ("Pre" columns).

Dominant gonad stage for 6°C treatment, by cohort and pCO_2 exposure

Gonad Stage	Fidalgo Bay Pre pCO_2	Fidalgo Bay High pCO_2	Fidalgo Bay Amb pCO_2	Dabob Bay Pre pCO_2	Dabob Bay High pCO_2	Dabob Bay Amb pCO_2	Oyster Bay C1 Pre pCO_2	Oyster Bay C1 High pCO_2	Oyster Bay C1 Amb pCO_2	Oyster Bay C2 Pre pCO_2	Oyster Bay C2 High pCO_2	Oyster Bay C2 Amb pCO_2	All cohorts Pre pCO_2	All cohorts High pCO_2	All cohorts Amb pCO_2
													Dom-stage[A]: p=0.036 Dom-sex[A]: p=0.202 **Male[A,B]**: p=0.030 Female: p=0.219		
Spawned / Resorbing (4)	3	2	0	2	1	0	4	0	0	7	2	1	16	5	1
Ripe (3)	5	2	4	1	2	5	3	5	4	4	0	2	13	9	15
Advanced (2)	0	3	3	4	1	0	6	2	3	1	6	10	11	12	16
Early (1)	5	2	1	2	1	0	2	2	1	3	7	2	12	12	4
Empty Follicles (0)	2	0	1	0	1	1	0	0	1	0	0	0	2	1	3
Total Sampled	15	9	9	9	6	6	15	9	9	15	15	15	54	39	39

Dominant gonad stage for 10°C treatment, by cohort and pCO_2 exposure

Gonad Stage	Fidalgo Bay Pre pCO_2	Fidalgo Bay High pCO_2	Fidalgo Bay Amb pCO_2	Dabob Bay Pre pCO_2	Dabob Bay High pCO_2	Dabob Bay Amb pCO_2	Oyster Bay C1 Pre pCO_2	Oyster Bay C1 High pCO_2	Oyster Bay C1 Amb pCO_2	Oyster Bay C2 Pre pCO_2	Oyster Bay C2 High pCO_2	Oyster Bay C2 Amb pCO_2	All cohorts Pre pCO_2	All cohorts High pCO_2	All cohorts Amb pCO_2
													Dom-stage[A]: p=0.008 Dom-sex[A]: p=0.73 **Male[A]**: p=0.020 Female: p=1		
Spawned / Resorbing (4)	1	0	1	1	0	1	0	0	0	1	0	0	3	0	2
Ripe (3)	5	3	6	3	4	4	3	4	4	0	2	5	11	13	19
Advanced (2)	5	6	0	5	1	0	9	4	3	8	10	4	27	21	7
Early (1)	3	0	1	0	0	1	3	1	2	5	3	4	11	4	8
Empty Follicles (0)	1	0	1	0	1	0	0	0	0	1	0	2	2	1	3
Total Sampled	15	9	9	9	6	6	15	9	9	15	15	15	54	39	39

Section S5: Larval collection differences among cohorts

Total larvae collected differed by cohort ($F_{(3,8)}=15.3$, $p=0.001$). O-1 produced significantly more total larvae than F and O-2 ($p=0.0094$, $p=0.0014$, respectively), and D produced more total larvae compared to O-2 ($p=0.022$). Total larvae released by O-1, F, D, and O-2 was 10.1M, 3.6M, 2.7M and 2.1M, respectively. The same patterns were observed in average daily larvae released by cohort ($F_{(3,20)}=8.9$, $p=0.0009$). Date of first larval release differed by cohort ($F_{(3,8)}=15.1$, $p=0.0012$). Oyster Bay cohorts (O-1 and O-2) released larvae 9.9 days earlier than F and D cohorts on average. Larval pulse frequency differed by cohort ($F_{(3,8)}=9.8$, $p=0.0046$). On average, O-1, O-2, F, and D released larvae 6.4±2.3, 8.0±2.9, 3.8±1.9, and 2.8±1.0 days, respectively. The O-1 cohort released larvae more frequently than F ($p=0.017$), and O-2 more frequently than both F and D ($p=0.0066$, $p=0.043$, respectively).

Table S5: Timing and magnitude of larval production in 4 *Ostrea lurida* cohorts previously exposed to different winter temperatures (6°C and 10°C), then pCO₂ treatments ("Amb." is Ambient=841±85 µatm, pH 7.82±0.02, High=3045±488 µatm). Fidalgo Bay, Dabob Bay, and Oyster Bay are previously identified as genetically distinct populations (Heare et al, 2017; Heare et al., 2018). Two Oyster Bay cohorts were used (O-1, O-2), with O-2 being the offspring of O-1 and likely all siblings. For each metric, total ("Tot.") or mean ("Ave.") of all cohorts combined for each treatment is shown.

Cohort		*Fidalgo Bay (2 reps)*		*Dabob Bay*		*Oyster Bay - F1 (2 reps)*		*Oyster Bay - F2*		*All cohorts combined*	
pCO₂ treatment		Amb. pCO₂	High pCO₂	Amb. pCO₂	High pCO₂	Amb. pCO₂	High pCO₂	Amb. pCO₂	High pCO₂	Amb. pCO₂	High pCO₂
No. of broodstock	6°C	15/14	14/15	14	15	15/16	17/17	117	126	Tot: 191	Tot: 204
	10°C	15/14	14/14	9	16	17/17	15/15	115	111	Tot: 177	Tot: 185
Date of first release	6°C	142/139	156/145	163	133	134/137	140/141	139	137	Ave: 142	Ave: 142
	10°C	145/137	144/141	144	146	135/134	134/137	131	131	Ave: 138	Ave: 139
Date of max release	6°C	149/146	161/145	163	168	135/156	141/165	154	143	Ave: 151	Ave: 154
	10°C	145/137	144/144	151	147	135/152	151/137	140	143	Ave: 143	Ave: 144
Date of last release	6°C	173/149	173/191	187	173	170/170	168/168	173	161	Ave: 170	Ave: 172
	10°C	158/168	184/191	182	175	191/187	175/173	166	170	Ave: 175	Ave: 178
Ave. daily larvae released (x10³)	6°C	21/38	99/127	53	107	139/114	103/175	58	43	Ave: 79	Ave: 110
	10°C	139/84	58/56	117	101	192/107	133/111	36	57	Ave: 108	Ave: 88
Total larvae released (x10³)	6°C	127/150	591/892	105	959	697/1,482	719/1,397	518	389	Tot: 3.08M	Tot: 4.95M
	10°C	695/421	345/393	939	705	1,918/1,502	933/1,441	466	689	Tot: 5.9M	Tot: 4.5M
Total larvae released per broodstock (x10³)	6°C	2.4/3.0	11.7/16.5	2.5	21.3	12.9/25.7	11.7/22.8	2.0	1.4	Ave: 6.6	Ave: 13.5
	10°C	1.3/8.4	6.8/7.8	34.8	14.7	31.3/24.6	17.3/26.7	1.8	2.8	Ave: 18.8	Ave: 11.7
Maximum release (x10³)	6°C	111/140	247/308	105	356	462/484	250/809	133	131	Ave: 239	Ave: 350
	10°C	248/246	298/186	437	268	555/407	378/379	108	241	Ave: 333	Ave: 292
No. big release days (>10k)	6°C	2/1	4/4	1	4	3/8	5/5	6	5	Tot: 21	Tot: 27
	10°C	3/3	2/3	5	5	7/7	5/10	11	10	Tot: 36	Tot: 35

Section S6: Larval rearing methods and survival

Larval rearing methods
Larvae collected between May 19 and July 6 were separated by treatment and cohort and reared over 67 days from May 19 to July 25. For all culture tanks, seawater was heated to 18°C in a common 1,610-L recirculating reservoir (1610 L) using Aqua Logic digital temperature controllers (TR115SN), dosed with live algae cocktail via an Iwaki metering pump to achieve 100,000 cells/mL, and distributed to culture tanks. Larvae were grown in two connected 19-L flow-through tanks (19-L; 8-L/hr) with aerated, filtered seawater (1 µm) at 18°C. The two-tank larval system was used to cull dead larvae: water flowed from one 19-L tank where larvae were added but non-swimming larvae would remain ("mortality tank") to the next ("live tank"), carrying live, swimming larvae which were then contained on a 100 µm screen. Twice weekly, live larval tanks were screened into three size classes: 100 µm < X < 180 µm ("100 µm"), 180 µm < X < 224 µm ("180 µm"), >224 µm ("224 µm", which is when *O. lurida* larvae are near metamorphosis). Each size class was subsampled and counted, then the 100 µm and 180 µm classes returned to larval tanks. The number of live larvae returned to culture tanks informed stocking of newly released larvae. To maximize genetic diversity of offspring, newly spawned larvae ($\lesssim$ 50,000) were added to culture tanks continuously to a maximum stocking density of 200,000 larvae (~10 larva/mL) (PSRF pers. communication & FAO manual). The contents in the mortality buckets were collected during biweekly screenings on a 100 µm screen to count live and dead oysters, but live were not kept.

During the twice weekly screening days, larvae that were larger than 224 µm were moved to downwelling setting silos, separated by cohort, temperature and pCO_2 treatment. Setting tanks were 180 µm silos with 18°C filtered seawater (1 µm) dosed with live algae, which then flowed into each silo from 8-L/hr irrigation drippers. Pacific oyster shell fragments (224 - 450 µm) were sprinkled into each silo to cover the surface to provide a settlement substrate. Silos were cleaned with freshwater (18°C) several times per week. Live, metamorphosed oysters were counted on August 12 for survival rate from 224 um to post-metamorphosis ("post-set"), then transferred to 450 µm silos with ~17°C upwelling filtered seawater (5 µm) to continue rearing. Oysters were fed live algae using a gravity algae header tank, and rinsed 1-2 times per week with freshwater. On October 4, when oysters were between 13-20 weeks old, all groups were moved to screen pouches separated by cohort x temperature x pCO_2, affixed to the inside of shellfish cages, and hung in Clam Bay until June 2018.

		Fidalgo Bay (2 replicates)		Dabob Bay		Oyster Bay - F1 (2 replicates)		Oyster Bay - F2		All	
Table S6: Tank densities during offspring rearing.											
		Amb pCO2	High pCO$_2$	Amb pCO2	High pCO$_2$	Amb pCO2	High pCO$_2$	Amb pCO2	High pCO$_2$	Amb pCO2	High pCO$_2$
Cumulative larvae stocked	6°C	169,475	353,916	65,667	255,371	477,403	532,417	353,769	179,717	1,066,314	1,321,421
	10°C	319,277	271,016	283,858	310,875	937,920	660,451	324,166	237,790	1,865,221	1,480,132
Mean, larvae density (in 20L tank)	6°C	29,148 ± 33,256	54,265 ± 44,431	22,000 ± 22,036	43,293 ± 43,168	50,260 ± 51,338	58,662 ± 64,276	47,231 ± 38,553	31,877 ± 30,341	39,270 ± 40,579	46,777 ± 47,718
	10°C	40,150 ± 47,430	29,486 ± 30,781	49,584 ± 33,070	56,081 ± 49,214	88,962 ± 71,752	74,562 ± 72,312	53,588 ± 50,765	32,423 ± 41,853	60,478 ± 5,7472	48,774 ± 54,447
Median larvae density	6°C	18,013	42,783	19,117	26,160	33,797	32,608	45,667	20,800	26,397	28,192
	10°C	16,000	20,650	50,317	45,833	83,577	48,800	29,833	7,877	50,317	34,935
Total eyed larvae	6°C	11,119	11,780	2,496	10,686	11,931	6,029	22,186	9,735	47,732	38,230
	10°C	3,737	2,978	13,862	19,815	59,929 (split)	10,670	13,828	2,910	91,356	36,373
Total post set (singles)	6°C	1,503 (split)	670	501	834	124	192	356	334	2,484	2,030
	10°C	626	75	1311	1091	52	34	246	113	2,235	1,313
Juvenile shell height (mm)	6°C	9.0±2.7	8.4±3.5	6.5±1.9	4.9±2.3	11.2±3.5	11.0±3.4	11.0±3.7	7.5±3.0	-	-
	10°C	9.7±3.0	12.3±5.4	7.0±2.3	6.6±2.9	11.0±3.8	12.2±4.2	10.7±3.7	13.4±4.4	-	-
Juveniles deployed	6°C	240	257	240	240	85	77	122	90	677	664
Juveniles survived	6°C	60	159	72	81	32	45	22	4	186	289

Larval survival estimates

Larval survival was estimated from both twice-weekly larval counts and cumulative survival counts. Percent survival between biweekly larval counts was calculated by summing the number of live larvae in all size classes (100 μm, 180 μm, 224 μm), dividing by the number of live larvae restocked after the previous count, plus all new larvae added since. Cumulative percent survival from newly released larvae ("new larvae") to the near-metamorphosis stage ("eyed larvae"), and to post-metamorphosis ("post-set") were compared between treatments based on total number of new larvae stocked in culture tanks and eyed larvae in setting tanks over the larval rearing period. During larval rearing, culture tank densities were capped at 200,000 larvae (~10 larvae/mL), but ranged during the 67 day larval rearing period due to varying mortality and larval release timing. Daily tank densities were estimated from twice-weekly larval counts and number of new larvae added, then compared between temperature x pCO_2 treatments using a Kruskal-Wallis Test.

Biweekly larval survival, cumulative survival from new to eyed larvae, and survival from eyed larvae to post-set were compared among cohort x temperature x pCO_2 treatments using ANCOVA on fitted linear regression models. For biweekly percent survival, square-root arcsine transformation was applied, and biweekly tank density was included as a random effect. For cumulative survival models, mean stocking density and total larvae stocked in culture tanks were examined as candidate random effects with Pearson's correlation using `pairs` and `cor`. For post-set survival, cumulative eyed larvae stocked in setting tanks and percent survival to eyed larvae stage were also tested and survival data was log-transformed. Tank density factors that correlated significantly with cumulative survival were considered as random effects in full regression models alongside cohort, temperature and pCO_2. All models were optimized using stepwise deletion and selected based on AIC value, adjusted R-squared, and F-statistic.

Larval survival results

Larval survival between biweekly counts did not differ by pCO_2 or temperature, but did differ by cohort ($F_{(3,230)}=5.73$, $p=8.5e-4$). Pairwise tests indicate that O-1 survival was significantly lower than D ($p=3.8e-4$), O-2 ($p=5.4e-4$), and F ($p=0.019$). Mean biweekly survival of D, F, O-2, and O-1 cohorts was 62±22%, 59±24%, 55±24%, and 49±28%, respectively. Cumulative survival from new- to eyed-larvae was low across all treatments, and did not differ by parental temperature treatment ($F_{(1,14)}=2.3$, $p=0.15$), parental pCO_2 ($F_{(1,14)}= 1.9$, $p=0.19$), or cohort ($F_{(3,12)}= 1.4$, $p=0.29$) (Table 3). Cumulative survival from eyed larvae to post-set) ranged from 0.2% to 26.5% and differed by cohort ($F_{(3,11)}=3.8$, $p=0.04$). Pairwise tests revealed that this was influenced by low survival in the O-1 group and significance was not strong after removing O-1 ($F_{(2,9)}=4.1$, $p=0.06$). No survival differences through metamorphosis were detected between pCO_2 or temperature treatments.

Tank density prior to each biweekly screening was a significant factor influencing survival between bi-weekly counts ($F_{(1,230)}=10.4$, $p=0.0015$) and therefore was included as a random effect in the biweekly survival regression model. Mean stocking densities across the 67-day rearing period in O-1, D, O-2, and F were 76,500±71,100, 54,400±424000, 47,000±46,200, and 43,500±42,700, respectively. No random effects were retained in the cumulative survival from new- to eyed-larvae model. Total larvae stocked in larval culture tanks correlated with survival from eyed-larvae to post-set (i.e. through metamorphosis), and therefore was included as a random effect in the post-set survival model.

The number of days between first and last larval collection, and first and last eyed larvae varied by cohort, although this was not significant. Across treatments, eyed larvae were present soonest in F (14.5±2.5 days), followed by O-1 (16.5±1.75 days), O-2 (17.25±1.25 days), and lastly D (18.25±3 days) ($F_{(3,12)}=2.0$, p=0.16). The number of days between stocking the last batch of newly released larvae, and collecting the last eyed larvae were 22±5.8, 23.25±7.4, 29.5±4.7, and 32±4.8 for O-1, F, D, and O-2, respectively.

Table S7: Larval survival estimates by parental treatment and cohort.

		Fidalgo Bay		*Dabob Bay*		*Oyster Bay - F1*		*Oyster Bay - F2*		*All cohorts*	
		Amb pCO_2	High pCO_2	Amb pCO_2	High pCO_2	Amb pCO_2	High pCO_2	Amb pCO_2	High pCO_2	Amb pCO_2	High pCO_2
Average biweekly larval survival	6°C	56±25%	62±26%	69±18%	49±21%	44±23%	52±27%	60±24%	59±28%	56±24%	56±26%
	10°C	50±18%	51±26%	58±25%	70±18%	49±30%	46±29%	63±25%	55±21%	55±25%	56±25%
Cumulative survival to eyed larvae	6°C	8.5%	3.0%	5.1%	4.7%	2.7%	1.2%	6.7%	5.7%	5.7±2%	3.6±2%
	10°C	1.3%	1.4%	5.2%	6.6%	4.2%	0.7%	4.4%	1.0%	3.8±1%	2.4±3%
Cumulative survival, eyed larvae to post set	6°C	13.8%	5.9%	26.5%	9.3%	1.1%	3.6%	1.7%	3.5%	10.8 ±10%	5.6 ±2.7%
	10°C	18.5%	2.7%	9.7%	6.0%	0.2%	0.7%	1.9%	5.8%	7.6±8%	3.8±3%

Larval survival, by treatment and cohort

Section S7: Juvenile survival and environmental data

Associations among juvenile oyster survival and environmental summary statistics were explored to evaluate factors that best explain spatial variation in oyster survival. The mean and standard deviation of each environmental variable (temperature, dissolved oxygen, salinity, chlorophyll, and pH) during deployment were assessed independently by binomial generalized linear mixed models (glmm) using glmer from the lme4 package (vs. 1.1-19), and Wald tests with type II error. Significant single-factor variables were included in a full model, then backwards deletion was used to identify significant environmental factors in the most parsimonious model. Significant variables predicting juvenile survival included mean temperature, mean pH, and dissolved oxygen standard deviation. Figure S6 show proportion survival by each environmental summary statistic. See the "07_Juvenile-deployment.R " R code in the Spencer et al. 2019 for analysis details and results.
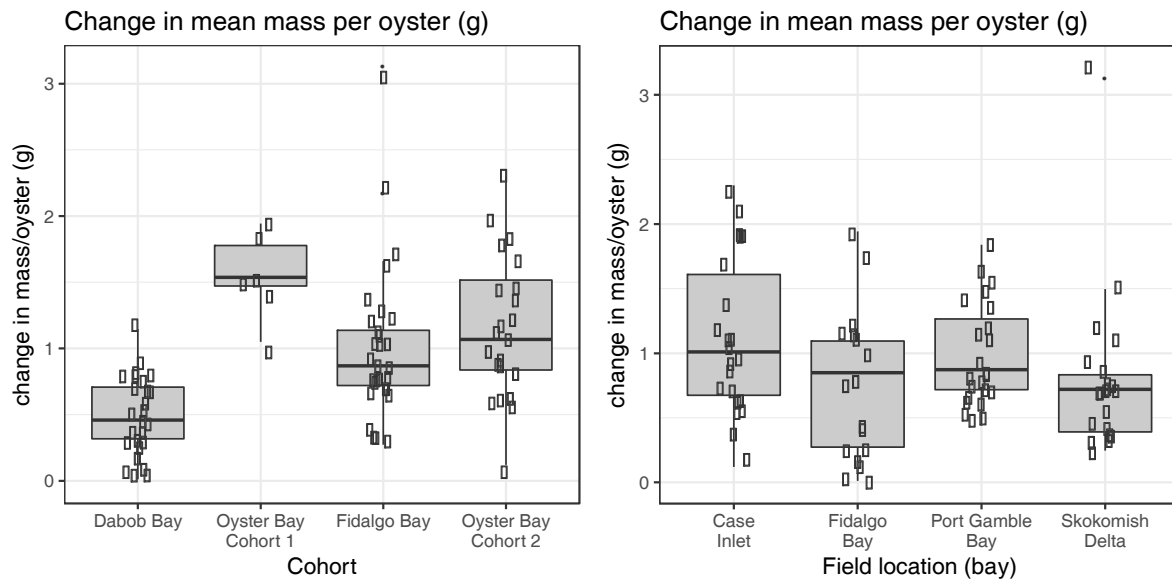

Figure S5: Juvenile mass change during field trial was significantly less in the Dabob Bay cohort than other 3 cohorts (left), and was significantly less in Fidalgo Bay than in Port Gamble Bay and Case Inlet (right). Mean mass / oyster represents the average final mass per oyster minus average initial mass within each deployment bag.
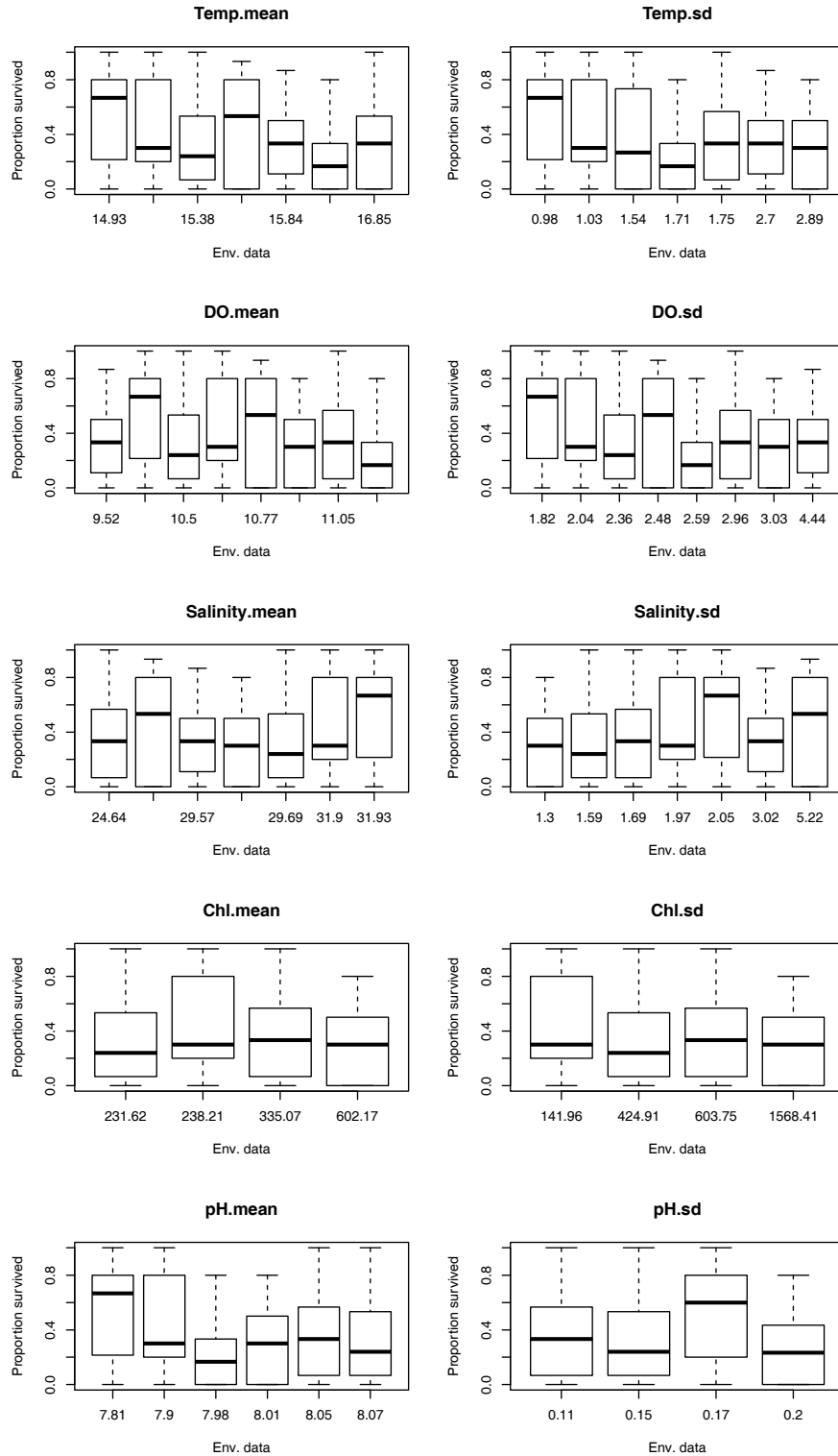
Figure S6: Juvenile proportion survival ~ environmental summary statistics. Model selection using backwards deletion indicates that survival was significantly related to mean temperature ("Temp.mean"), mean pH ("pH.mean"), and dissolved oxygen standard deviation ("DO.sd").

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