

Supplementary material

Effects of high $p\text{CO}_2$ on early life development of pelagic spawning marine fish

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Table S1. Generalised linear mixed-effects (GLME) analysis for multiple descriptive parameters of *Solea senegalensis*, *Diplodus sargus* and *Argyrosomus regius* eggs and larvae, from two batches (A and B), maintained at three experimental $p\text{CO}_2$ conditions (control, medium and high)

P-values in bold indicate statistically significant differences to control $p\text{CO}_2$ condition and Batch A ($P < 0.01$)

Variable	LME coefficient	s.e.	d.f.	<i>t</i>	<i>P</i>
<i>Solea senegalensis</i>					
Hatching rate					
Intercept	-0.02	0.014	6	-1.429	0.203
Treatment Medium	-0.113	0.02	6	-5.609	0.001
Treatment High	-0.136	0.02	6	-6.748	0.0005
Batch B	0.0254	0.02	6	0.2554	0.2554
Batch B × Treatment Medium	-0.06	0.028	6	-2.111	0.079
Batch B × Treatment High	-0.031	0.028	6	-1.096	0.315
Survival rate					
Intercept	0.37	0.09	6	4.121	0.006
Treatment Medium	-0.396	0.161	6	-2.459	0.049
Treatment High	-0.79	0.217	6	-3.635	0.011
Batch B	-0.989	0.258	6	-3.838	0.009
Batch B × Treatment Medium	0.977	0.977	6	3.053	0.022
Batch B × Treatment High	1.419	0.349	6	4.059	0.01
Growth rate					
Intercept	0.148	0.004	12	35.434	<0.0001
Treatment Medium	-0.019	0.006	12	-3.203	0.01
Treatment High	0.0004	0.006	12	0.072	0.943
Batch B	-0.01	0.006	12	-1.778	0.101
Batch B × Treatment Medium	0.023	0.008	12	2.755	0.017
Batch B × Treatment High	0.0003	0.008	12	0.042	0.967
Dry weight					
Intercept	4.073	0.103	6	39.328	<0.0001
Treatment Medium	0.223	0.133	6	1.683	0.143
Treatment High	0.319	0.128	6	2.498	0.047
Batch B	-0.285	0.172	6	0.149	0.149
Batch B × Treatment Medium	-0.333	-0.333	6	-1.359	0.223
Batch B × Treatment High	-0.322	-0.322	6	-1.381	0.216
Egg diameter					
Intercept	1.004	0.006	12	172.643	<0.0001
Treatment Medium	0.006	0.008	12	0.794	0.443
Treatment High	0.008	0.008	12	1.201	0.328
Batch B	0.007	0.008	12	0.81	0.434
Batch B × Treatment Medium	0	0.012	12	0.04	0.969
Batch B × Treatment High	-0.005	0.012	12	-0.421	0.681
<i>Diplodus sargus</i>					
Hatching rate					
Intercept	-0.133	0.019	6	-6.952	0.0004
Treatment Medium	-0.148	0.028	6	-5.313	0.002
Treatment High	-0.223	0.028	6	-7.931	0.0002
Batch B	0.238	0.026	6	8.977	0.0001
Batch B × Treatment Medium	0.106	0.038	6	2.792	0.031
Batch B × Treatment High	-0.217	0.039	6	-5.52	0.002
Survival rate					
Intercept	-1.488	0.373	6	-3.984	0.007
Treatment Medium	0.847	0.528	6	1.604	0.159
Treatment High	0.465	0.528	6	0.881	0.412
Batch B	0.946	0.528	6	1.791	0.123
Batch B × Treatment Medium	-1.693	0.747	6	-2.267	0.064
Batch B × Treatment High	-1.478	0.747	6	-1.979	0.095

Variable	LME coefficient	s.e.	d.f.	<i>t</i>	<i>P</i>
Growth rate					
Intercept	0.062	0.006	12	9.629	< 0.0001
Treatment Medium	-0.019	0.009	12	-2.045	0.063
Treatment High	-0.027	0.009	12	-2.923	0.013
Batch B	-0.017	0.009	12	-1.825	0.093
Batch B × Treatment Medium	-0.014	0.013	12	-1.072	0.305
Batch B × Treatment High	-0.018	0.013	12	-1.391	0.189
Dry weight					
Intercept	3.962	0.009	6	40.597	< 0.0001
Treatment Medium	-0.267	0.16	6	-1.661	0.148
Treatment High	-0.516	0.19	6	-2.712	0.035
Batch B	0.241	0.124	6	1.939	0.101
Batch B × Treatment Medium	0.556	0.187	6	2.972	0.025
Batch B × Treatment High	0.521	0.219	6	2.378	0.055
Egg diameter					
Intercept	0.977	0.004	12	246.447	< 0.0001
Treatment Medium	0.006	0.006	12	1.13	0.26
Treatment High	0.009	0.006	12	1.748	0.082
Batch B	0.002	0.006	12	0.404	0.685
Batch B × Treatment Medium	-0.009	0.008	12	-1.223	0.223
Batch B × Treatment High	-0.005	0.008	12	-0.685	0.494
<i>Argyrosomus regius</i>					
Hatching rate					
Intercept	-0.151	0.039	6	-3.926	0.008
Treatment Medium	-0.22	0.067	6	-3.587	0.011
Treatment High	-0.368	0.038	6	-5.455	0.002
Batch B	0.447	0.046	6	9.807	0.0001
Batch B × Treatment Medium	0.098	0.071	6	1.364	0.222
Batch B × Treatment High	-0.033	0.081	6	-0.414	0.693
Survival rate					
Intercept	-0.686	0.24	6	-2.858	0.029
Treatment Medium	-1.032	0.398	6	-2.589	0.041
Treatment High	-0.199	0.347	6	-0.574	0.587
Batch B	0.15	0.337	6	0.446	0.671
Batch B × Treatment Medium	0.224	0.539	6	0.415	0.692
Batch B × Treatment High	-0.739	0.511	6	-1.445	0.198
Growth rate					
Intercept	0.009	0.013	12	0.696	0.499
Treatment Medium	0.098	0.018	12	5.322	0.0002
Treatment High	0.011	0.018	12	0.574	0.576
Batch B	0.09	0.018	12	4.89	0.0004
Batch B × Treatment Medium	-0.096	0.026	12	-3.668	0.003
Batch B × Treatment High	-0.042	0.026	12	-1.618	0.132
Dry weight					
Intercept	4.083	0.126	6	2.986	< 0.0001
Treatment Medium	-0.015	0.18	6	-0.085	0.935
Treatment High	0.047	0.175	6	0.269	0.797
Batch B	0.014	0.1775	6	0.08	0.939
Batch B × Treatment Medium	0.103	0.247	6	0.419	0.689
Batch B × Treatment High	0.293	0.232	6	1.264	0.253
Egg diameter					
Intercept	1.283	0.01	12	121.756	< 0.0001
Treatment Medium	-0.011	0.015	12	-0.765	0.459
Treatment High	0.027	0.015	12	1.845	0.09
Batch B	0.009	0.015	12	0.622	0.546
Batch B × Treatment Medium	0.054	0.021	12	2.584	0.024
Batch B × Treatment High	-0.007	0.021	12	-0.327	0.749

Table S2. Generalised linear mixed-effects (GLME) analysis for morphometric traits of *Solea senegalensis*, *Diplodus sargus* and *Argyrosomus regius* larvae at 0 days post-hatching, from two batches (A and B), maintained at three experimental $p\text{CO}_2$ conditions (control, medium and high)

P-values in bold indicate statistically significant differences to control $p\text{CO}_2$ condition and Batch A ($P < 0.05$)

Variable	LME coefficient	s.e.	d.f.	<i>t</i>	<i>P</i>
<i>Solea senegalensis</i>					
Standard Length					
Intercept	2.019	0.02	12	100.097	< 0.0001
Treatment Medium	0.015	0.028	12	0.512	0.618
Treatment High	0.021	0.028	12	0.732	0.478
Batch B	0.095	0.028	12	3.348	0.006
Batch B × Treatment Medium	0.01	0.04	12	0.256	0.802
Batch B × Treatment High	-0.009	0.04	12	-0.217	0.832
Yolk sac area					
Intercept	0.86	0.041	12	21.065	< 0.0001
Treatment Medium	-0.137	0.058	12	-2.371	0.035
Treatment High	-0.107	0.058	12	-1.857	0.038
Batch B	-0.051	0.058	12	-0.878	0.397
Batch B × Treatment Medium	0.052	0.082	12	0.637	0.536
Batch B × Treatment High	-0.04	0.082	12	-0.494	0.63
Muscle Height					
Intercept	0.166	0.003	12	62.022	< 0.0001
Treatment Medium	0.009	0.004	12	2.538	0.012
Treatment High	0.006	0.004	12	1.56	0.012
Batch B	-0.007	0.004	12	-1.789	0.075
Batch B × Treatment Medium	0.002	0.005	12	0.343	0.732
Batch B × Treatment High	0.003	0.005	12	0.611	0.542
<i>Diplodus sargus</i>					
Standard Length					
Intercept	2.855	0.035	12	82.058	< 0.0001
Treatment Medium	0.039	0.049	12	0.796	0.427
Treatment High	0	0.049	12	0	0.89
Batch B	0.151	0.049	12	3.061	0.002
Batch B × Treatment Medium	0.205	0.069	12	1.174	0.242
Batch B × Treatment High	0.082	0.069	12	2.954	0.004
Yolk sac area					
Intercept	0.484	0.021	12	22.997	< 0.0001
Treatment Medium	0.003	0.029	12	0.11	0.915
Treatment High	-0.003	0.029	12	-0.11	0.915
Batch B	0.062	0.029	12	2.1	0.058
Batch B × Treatment Medium	-0.076	-0.076	12	-1.806	0.096
Batch B × Treatment High	-0.058	-0.058	12	-1.38	0.193
Muscle Height					
Intercept	0.215	0.003	12	65.438	< 0.0001
Treatment Medium	-0.005	0.005	12	-1.01	0.332
Treatment High	0.0005	0.005	12	0.086	0.933
Batch B	0.004	0.005	12	0.458	0.458
Batch B × Treatment Medium	0.022	0.007	12	3.404	0.005
Batch B × Treatment High	0.009	0.007	12	1.342	0.204
<i>Argyrosomus regius</i>					
Standard Length					
Intercept	2.863	0.067	12	42.778	< 0.0001
Treatment Medium	-0.518	0.095	12	-6.335	< 0.0001
Treatment High	0.054	0.095	12	0.572	0.578
Batch B	-0.599	0.094	12	-6.335	< 0.0001
Batch B × Treatment Medium	0.689	0.134	12	5.123	0.0002
Batch B × Treatment High	0.061	0.134	12	0.457	0.656

Variable	LME coefficient	s.e.	d.f.	<i>t</i>	<i>P</i>
Yolk sac area					
Intercept	1.182	0.052	12	22.892	< 0.0001
Treatment Medium	-0.593	0.073	12	-8.122	< 0.0001
Treatment High	-0.232	0.073	12	-3.174	0.008
Batch B	-0.434	0.073	12	-5.942	< 0.0001
Batch B × Treatment Medium	0.543	0.103	12	5.256	0.0002
Batch B × Treatment High	0.063	0.103	12	0.614	0.55
Muscle Height					
Intercept	0.247	0.005	12	50.582	< 0.0001
Treatment Medium	-0.051	0.007	12	-7.41	< 0.0001
Treatment High	0.006	0.007	12	0.908	0.382
Batch B	-0.046	0.007	12	-6.696	< 0.0001
Batch B × Treatment Medium	0.062	0.009	12	6.434	< 0.0001
Batch B × Treatment High	0.0006	0.009	12	0.058	0.955

Table S3. Generalised linear mixed-effects (GLME) analysis for morphometric traits of *Solea senegalensis*, *Diplodus sargus* and *Argyrosomus regius* larvae at 3 days post-hatching, from two batches (A and B), maintained at three experimental $p\text{CO}_2$ conditions (control, medium and high)

P-values in bold indicate statistically significant differences to control $p\text{CO}_2$ condition and Batch A ($P < 0.05$)

Variable	LME coefficient	s.e.	d.f.	<i>t</i>	<i>P</i>
<i>Solea senegalensis</i>					
Standard Length					
Intercept	3.139	0.03	12	104.969	<0.0001
Treatment Medium	0.027	0.042	12	0.641	0.534
Treatment High	-0.137	0.042	12	-3.243	0.007
Batch B	0.05	0.042	12	1.185	0.259
Batch B × Treatment Medium	0.017	0.06	12	0.29	0.777
Batch B × Treatment High	0.191	0.06	12	3.202	0.008
Muscle Height					
Intercept	0.215	0.003	12	81.27	<0.0001
Treatment Medium	0.008	0.004	12	2.183	0.03
Treatment High	-0.002	0.004	12	-0.597	0.441
Batch B	-0.004	0.004	12	0.231	0.231
Batch B × Treatment Medium	-0.005	0.005	12	-1.04	0.3
Batch B × Treatment High	0.006	0.005	12	1.128	0.261
<i>Diplodus sargus</i>					
Standard Length					
Intercept	3.437	0.064	12	53.33	<0.0001
Treatment Medium	-0.142	0.091	12	-1.563	0.144
Treatment High	-0.253	0.091	12	-2.78	0.017
Batch B	0.002	0.091	12	0.978	0.978
Batch B × Treatment Medium	-0.047	0.129	12	-0.364	0.722
Batch B × Treatment High	0.038	0.129	12	0.298	0.771
Muscle Height					
Intercept	0.225	0.003	12	74.125	<0.0001
Treatment Medium	-0.003	0.004	12	-0.736	0.462
Treatment High	0.005	0.004	12	1.256	0.211
Batch B	-0.001	0.004	12	-0.279	0.781
Batch B × Treatment Medium	0	0.006	12	-0.581	0.562
Batch B × Treatment High	-0.003	0.006	12	-0.011	0.991
<i>Argyrosomus regius</i>					
Standard Length					
Intercept	2.948	0.06	12	48.923	<0.0001
Treatment Medium	0.119	0.085	12	1.396	0.188
Treatment High	0.147	0.085	12	1.729	0.109
Batch B	0.093	0.085	12	0.295	0.295
Batch B × Treatment Medium	0.002	0.002	12	0.015	0.988
Batch B × Treatment High	0.093	-0.282	12	-2.342	0.037
Muscle Height					
Intercept	0.252	0.003	12	74.481	<0.0001
Treatment Medium	0.0002	0.005	12	0.042	0.967
Treatment High	0.018	0.005	12	3.714	0.0003
Batch B	0.005	0.005	12	0.304	0.304
Batch B × Treatment Medium	0.008	0.007	12	1.217	0.225
Batch B × Treatment High	-0.014	0.007	12	-2.055	0.041

Table S4. Generalised linear mixed-effects (GLME) analysis for otolith morphometrics of *Solea senegalensis*, *Diplodus sargus* and *Argyrosomus regius* larvae at 3 days post-hatching, from two batches (A and B), maintained at three experimental $p\text{CO}_2$ conditions (control, medium and high)

P-values in bold indicate statistically significant differences to control $p\text{CO}_2$ condition and Batch A ($P < 0.05$)

Variable	LME coefficient	s.e.	d.f.	<i>t</i>	<i>P</i>
<i>Solea senegalensis</i>					
Area					
Intercept	446.75	12.12	90	36.847	<0.0001
Treatment Medium	14.42	17.15	90	1.016	0.315
Treatment High	52.45	17.15	90	3.059	0.003
Batch B	99.33	17.15	90	5.793	<0.0001
Batch B × Treatment Medium	-72.72	24.25	90	-2.999	0.003
Batch B × Treatment High	-74.3	24.25	90	-3.064	0.004
Perimeter					
Intercept	79.597	1.101	90	72.3	<0.0001
Treatment Medium	1.621	1.557	90	1.041	0.301
Treatment High	4.64	1.557	90	2.98	0.004
Batch B	8.663	1.557	90	5.564	<0.0001
Batch B × Treatment Medium	-5.777	2.202	90	-2.624	0.01
Batch B × Treatment High	-6.18	2.202	90	-2.807	0.006
Width					
Intercept	23.341	0.461	90	50.6	<0.0001
Treatment Medium	0.519	0.652	90	0.796	0.428
Treatment High	1.165	0.652	90	1.786	0.077
Batch B	2.529	0.652	90	3.877	0.0002
Batch B × Treatment Medium	-2.421	0.923	90	-2.625	0.01
Batch B × Treatment High	-2.298	0.923	90	-2.491	0.015
<i>Diplodus sargus</i>					
Area					
Intercept	400.8	13.57	90	29.537	<0.0001
Treatment Medium	46.476	19.19	90	2.422	0.017
Treatment High	46.476	19.19	90	2.422	0.017
Batch B	0.242	19.19	90	0.013	0.99
Batch B × Treatment Medium	-53.079	27.139	90	-1.956	0.054
Batch B × Treatment High	-17.167	27.139	90	-0.633	0.529
Perimeter					
Intercept	75.492	1.176	90	64.198	<0.0001
Treatment Medium	3.902	1.663	90	2.346	0.021
Treatment High	3.902	1.663	90	2.346	0.021
Batch B	-0.36	1.663	90	-0.216	0.829
Batch B × Treatment Medium	-4.225	2.352	90	-1.796	0.076
Batch B × Treatment High	-1.134	2.352	90	-0.482	0.631
Width					
Intercept	22.461	0.429	90	52.323	<0.0001
Treatment Medium	1.196	0.607	90	1.97	0.052
Treatment High	1.196	0.607	90	1.97	0.052
Batch B	0.413	0.607	90	0.681	0.498
Batch B × Treatment Medium	-2.009	0.858	90	-2.34	0.021
Batch B × Treatment High	-0.825	0.858	90	-0.961	0.339
<i>Argyrosomus regius</i>					
Area					
Intercept	420.06	17.76	86	23.652	<0.0001
Treatment Medium	-14.7	25.56	86	-0.575	0.5667
Treatment High	66.66	25.12	86	2.654	0.009
Batch B	29.34	26.64	86	1.101	0.274
Batch B × Treatment Medium	-52.14	36.92	86	-1.412	0.161
Batch B × Treatment High	-47.46	36.61	86	-1.296	0.198

Variable	LME coefficient	s.e.	d.f.	<i>t</i>	<i>P</i>
Perimeter					
Intercept	76.93	1.752	86	43.92	< 0.0001
Treatment Medium	-0.606	2.521	86	-0.241	0.81
Treatment High	6.889	2.477	86	2.781	0.007
Batch B	3.33	2.627	86	1.267	0.208
Batch B × Treatment Medium	-5.973	3.641	86	-1.64	0.126
Batch B × Treatment High	-5.579	3.611	86	-1.545	0.105
Width					
Intercept	22.995	0.585	86	39.283	< 0.0001
Treatment Medium	-0.507	0.842	86	-0.602	0.548
Treatment High	1.689	0.828	86	2.041	0.044
Batch B	0.35	0.878	86	0.399	0.691
Batch B × Treatment Medium	-0.777	1.217	86	-0.382	0.525
Batch B × Treatment High	-0.461	1.207	86	-0.638	0.703