SUPPLEMENTARY INFORMATION

Is the perceived resiliency of fish larvae to ocean acidification masking more subtle effects?

Edward C. Pope, Robert P. Ellis, Maria Scolamacchia, Jacob W. S. Scolding, Alexander Keay, Purazen Chingombe, Robin J. Shields, Rhiannon Wilcox, Douglas C. Speirs, Rod W. Wilson, Ceri Lewis and Kevin J. Flynn.

Table S1. Two-way ANOVA investigating the effect of pCO_2 and temperature on oil droplet volume in *D. labrax* larvae at 7 days (post-hatch).

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	0.0000005	1	0.0000005	$F_{1,8} = 0.4948$	P = 0.5017
***p* CO_2**	0.0000048	1	0.0000048	$F_{1,8} = 4.948$	P = 0.0568
Temperature	0.0000003	1	0.0000003	$F_{1,8} = 0.3196$	P = 0.5873
Residual	0.0000077	8	0.0000010		

Table S2. Two-way ANOVA investigating the effect of pCO_2 and temperature on the number of *D. labrax* larvae at the end of the study (42 days post-hatch).

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	6,120	1	6,120	$F_{1,8} = 0.6350$	P = 0.4485
***p* CO_2**	27,361	1	27,361	$F_{1,8} = 2.839$	P = 0.1305
Temperature	205,147	1	205,147	$F_{1,8} = 21.29$	P = 0.0017
Residual	77,101	8	9,638		

Table S3. Two-way ANOVA investigating the effect of pCO_2 and temperature on daily mortality (*Z)* in *D. labrax* larvae over 42 days.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	0.0007174	1	0.000717	$F_{1,8} = 6.207$	P = 0.0374
***p* CO_2**	0.0010520	1	0.001052	$F_{1,8} = 9.099$	P = 0.0166
Temperature	0.0026340	1	0.002634	$F_{1,8} = 22.79$	P = 0.0014
Residual	0.0009246	8	0.000116		

Table S4. Two-way ANOVA (matched by tank) investigating the effect of time and treatment (temperature/pCO_2 combinations) on *D. labrax* larvae dry weight over 42 days.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	10.15	9	1.13	$F_{9,24} = 1.12$	P = 0.3848
Time	395.5	3	131.8	$F_{3,24} = 131.3$	P < 0.0001
Treatment	7.07	3	2.36	$F_{3,8} = 2.94$	P = 0.0991
Subjects (matching)	6.42	8	0.80	$F_{8,24} = 0.80$	P = 0.6091
Residual	24.1	24	1.00		

Table S5. Two-way ANOVA investigating the effect of pCO_2 and temperature on specific growth rate (μ) of *D. labrax* larvae over 42 days.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	0.00000008	1	0.00000008	$F_{1,8} = 0.001608$	P = 0.9690
***p* CO_2**	0.00000008	1	0.00000008	$F_{1,8} = 0.001608$	P = 0.9690
Temperature	0.00020010	1	0.00020010	$F_{1,8} = 3.860$	P = 0.0850
Residual	0.00041470	8	0.00005183		

Table S6 Two-way ANOVA (matched by tank) investigating the effect of time and treatment (temperature/pCO_2 combinations) on *D. labrax* larvae total length over 42 days.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	25.68	9	2.853	$F_{9,24} = 1.826$	P = 0.1153
Time	379.80	3	126.6	$F_{\mathbf{3,24}} = 80.99$	P < 0.0001
Treatment	2.23	3	0.7436	$F_{3,8} = 0.5898$	P = 0.6387
Subjects (matching)	10.09	8	1.261	$F_{8,24} = 0.8065$	P = 0.6033
Residual	37.51	24	1.563		

Table S7 Two-way ANOVA (matched by tank) investigating the effect of time and treatment (temperature/pCO_2 combinations) on *D. labrax* larvae standard length over 42 days.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	2.083	9	0.2315	$F_{9,24} = 1.607$	P = 0.1694
Time	256.9	3	85.64	$F_{\mathbf{3,24}} = 594.6$	P < 0.0001
Treatment	3.805	3	1.268	$F_{3,8} = 5.798$	P = 0.0210
Subjects (matching)	1.75	8	0.2187	$F_{8,24} = 1.519$	P = 0.2029
Residual	3.457	24	0.144		

Table S8 Two-way ANOVA (matched by tank) investigating the effect of time and treatment (temperature/pCO_2 combinations) on *D. labrax* larvae pre-anal length over 42 days.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	0.971	9	0.1079	$F_{9,24} = 1.473$	P = 0.2140
Time	158.5	3	52.82	$F_{\mathbf{3,24}} = 721.2$	P < 0.0001
Treatment	1.615	3	0.5384	$F_{3,8} = 7.451$	P = 0.0105
Subjects (matching)	0.578	8	0.07225	$F_{8,24} = 0.9865$	P = 0.4705
Residual	1.758	24	0.07324		

Table S9. Two-way ANOVA (matched by tank) investigating the effect of time and treatment (temperature/pCO_2 combinations) on *D. labrax* larvae head height over 42 days.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	0.1602	9	0.018	$F_{9,24} = 3.123$	P = 0.0124
Time	12.76	3	4.252	$F_{3,24} = 746.3$	P < 0.0001
Treatment	0.3148	3	0.105	$F_{3,8} = 24.93$	P = 0.0002
Subjects (matching)	0.0337	8	0.004	$F_{8,24} = 0.7387$	P = 0.6572
Residual	0.1368	24	0.006		

Table S10. Two-way ANOVA (matched by tank) investigating the effect of time and treatment (temperature/pCO_2 combinations) on *D. labrax* eye diameter over 42 days.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	62002	9	6889	$F_{9,24} = 14.60$	P < 0.0001
Time	2105000	3	701659	$F_{3,24} = 1487$	P < 0.0001
Treatment	84598	3	28199	$F_{3,8} = 36.80$	P < 0.0001
Subjects (matching)	6130	8	766.3	$F_{8,24} = 1.624$	P = 0.1703
Residual	11326	24	471.9		

Table S11. Two-way ANOVA investigating the effect of time and treatment (temperature/pCO_2 combinations) on the C:N ratio of larval *D. labrax* over 42 days.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	0.2601	6	0.04334	$F_{6,23} = 7.852$	P = 0.0001
Time	0.1450	2	0.07251	$F_{2,23} = 13.14$	P = 0.0002
Treatment	0.1168	3	0.03894	$F_{3,23} = 7.055$	P = 0.0016
Residual	0.1270	23	0.00552		

Table S12. Two-way ANOVA investigating the effect of time (d28 and d42) and treatment (temperature/pCO_2 combinations) on the number of *A. salina* prey in the guts of larval *D. labrax*.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	8.319	3	2.773	$F_{3,13} = 5.613$	P = 0.0108
Time	1.64	1	1.64	$F_{1,13} = 3.320$	P = 0.0915
Treatment	1.277	3	0.4257	$F_{3,13} = 0.8617$	P = 0.4854
Residual	6.422	13	0.494		

Table S13. Two-way ANOVA (matched by tank) investigating the effect of prey type and pCO_2 regime on mean grazing rates of *D. labrax* larvae at 19°C. Mean values were calculated for each tank for the duration that each prey type was added to the tanks (rotifers $d_{2\text{-}d21}$, *A. salina* $d_{9\text{-}42}$). The variation in volumetric counts meant that occasionally a negative grazing level was calculated, such values were recorded as 0.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	21463	1	21463	$F_{1,4} = 0.4371$	P = 0.5447
Prey type	6469000	1	6469000	$F_{1,4} = 131.7$	P = 0.0003
Treatment	19001	1	19001	$F_{1,4} = 0.3009$	P = 0.6125
Subjects (matching)	252569	4	63142	$F4_{,4} = 1.286$	P = 0.4067
Residual	196431	4	49108		

Table S14. Two-way ANOVA investigating the effect of pCO_2 and temperature on wet weight of *D. labrax* after incubation for 67-69d post-hatch.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	25,220	1	25,220	$F_{1,56} = 5.301$	P = 0.0251
***p* CO_2**	34,995	1	34,995	$F_{1,56} = 7.356$	P = 0.0089
Temperature	272,127	1	272,127	$F_{1,56} = 57.20$	P < 0.0001
Residual	266,410	56	4,757		

Table S15. Two-way ANOVA investigating the effect of pCO_2 and temperature on total length of *D. labrax* after incubation for 67-69d post-hatch.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	27.6	1	27.6	$F_{1,56} = 3.966$	P = 0.0513
***p* CO_2**	9.9	1	9.9	$F_{1,56} = 1.426$	P = 0.2375
Temperature	452.0	1	452.0	$F_{1,56} = 64.86$	P < 0.0001
Residual	390.3	56	7.0		

Table S16. Two-way ANOVA investigating the effect of pCO_2 and temperature on routine metabolic rate of *D. labrax* after incubation for 67-69d post-hatch.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	13.77	1	13.77	$F_{1,56} = 1.307$	P = 0.2579
***p* CO_2**	1.32	1	1.32	$F_{1,56} = 0.1251$	P = 0.7249
Temperature	5.89	1	5.89	$F_{1,56} = 0.5584$	P = 0.4580
Residual	590.20	56	10.54		

Table S17. Two-way ANOVA investigating the effect of pCO_2 and temperature on active metabolic rate of *D. labrax* after incubation for 67-69d post-hatch.

	Sum of squares	Degrees of freedom	Mean square	$F_{DFn,DFd}$	P value
Interaction	13.7	1	13.7	$F_{1,56} = 0.645$	P = 0.4252
***p* CO_2**	44.3	1	44.26	$F_{1,56} = 2.085$	P = 0.1544
Temperature	106.9	1	106.9	$F_{1,56} = 5.036$	P = 0.0288
Residual	1,189.0	56	21.23		

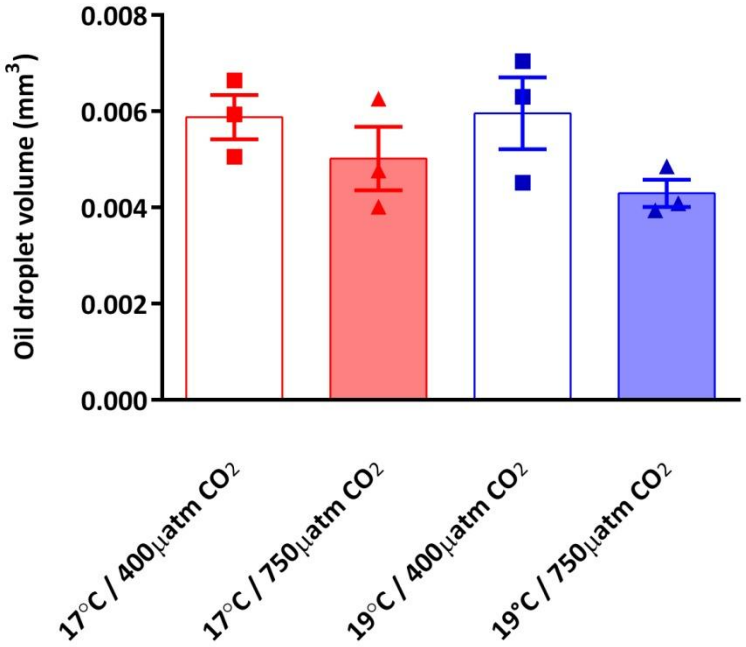

Fig. S1. Oil droplet volume in larval *D. labrax* after 7 days of incubation under experimental ocean acidification conditions. Data points are mean values for each experimental tank, column heights are means and error bars are ± 1 SEM.

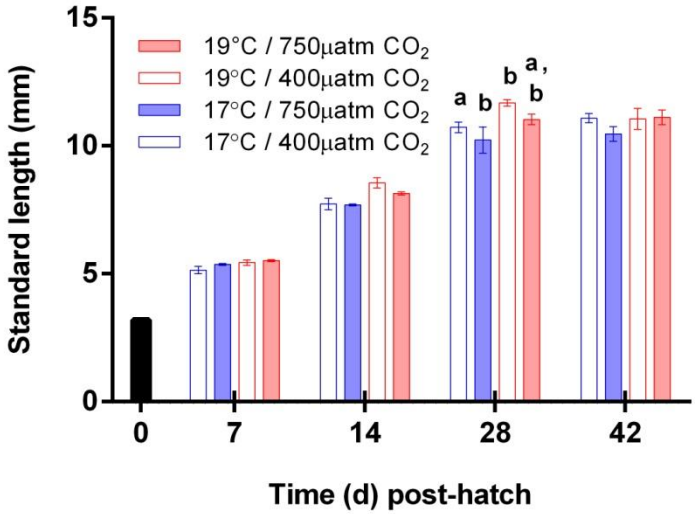

Fig. S2. Standard length of *D. labrax* incubated for 42 days under experimental ocean acidification conditions. Columns that do not share a letter at an individual time are significantly different (two-way ANOVA with Bonferroni post-test, P<0.05). Mean values ± 1 SEM, *N* = 3.

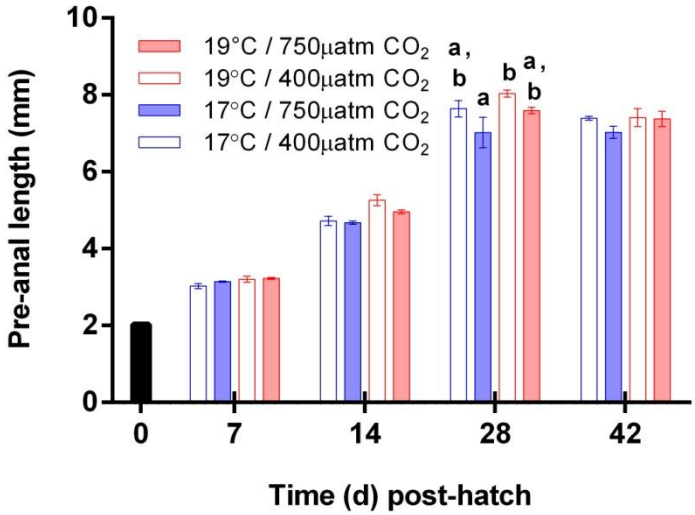

Fig. S3. Pre-anal length in *D. labrax* larvae incubated for 42 days under experimental ocean acidification conditions. Columns that do not share a letter at an individual time are significantly different (two-way ANOVA with Bonferroni post-test, P<0.05). Mean values ± 1 SEM, *N* = 3.

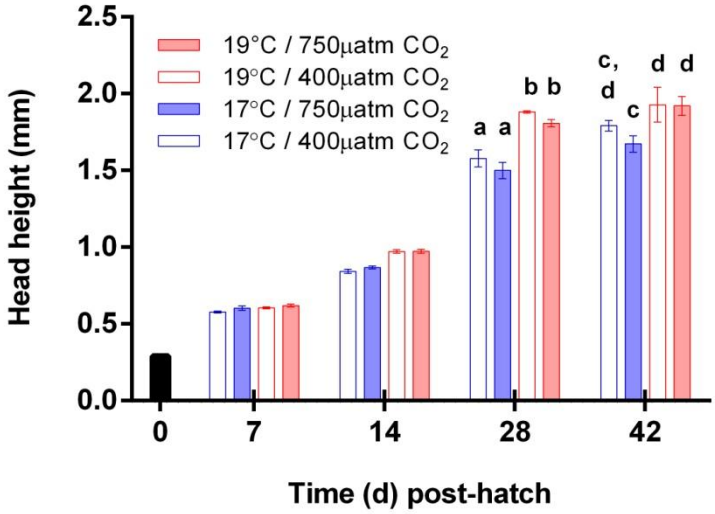

Fig. S4. Head height in *D. labrax* larvae incubated for 42 days under experimental ocean acidification conditions. Columns that do not share a letter at an individual time are significantly different (two-way ANOVA with Bonferroni post-test, P<0.05). Mean values ± 1 SEM,*N* = 3.

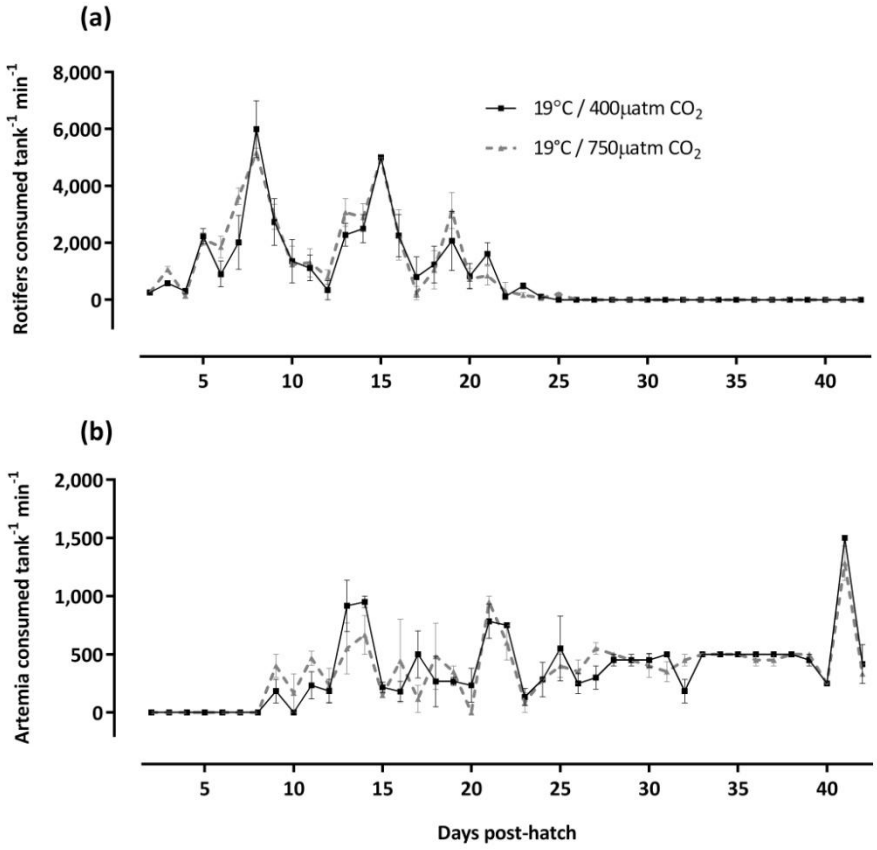

Fig. S5. Total grazing rates for tanks of *D. labrax* larvae incubated for 42 days at 19°C under two different pCO_2s and fed a regime of rotifers (A) moving on to *A. salina* (B). The variation in volumetric counts meant that occasionally a negative grazing level was calculated, such values were recorded as 0. Mean values ± 1 SEM, *N* = 3.

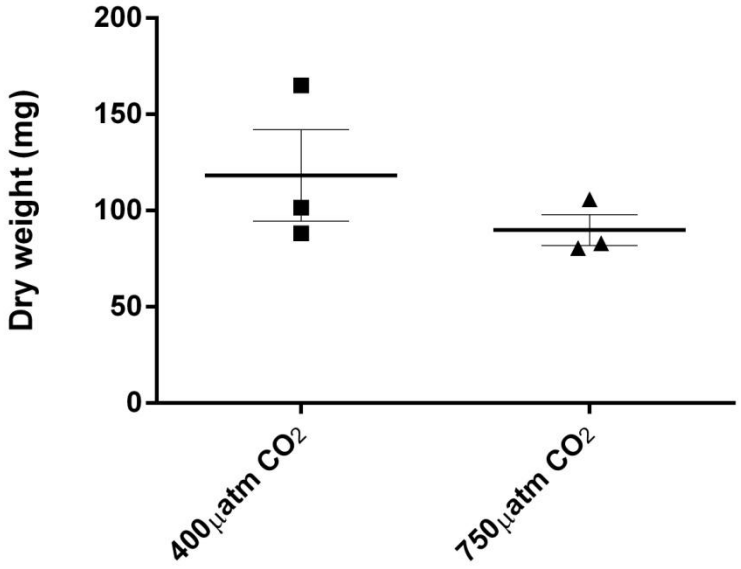

Fig. S6. Dry weight of *D. labrax* larvae incubated at 19°C for 78d under experimental ocean acidification conditions. Fish were transferred to aquaria on d49 for weaning. Lines = mean values ± 1SEM, *N* = 3.