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Supplement of

European sea bass, *Dicentrarchus labrax*, in a changing ocean

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SUPPLEMENTARY INFORMATION

European sea bass, *Dicentrarchus labrax*, in a changing ocean

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Table S1. Two-way ANOVA investigating the effect of $p\text{CO}_2$ 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
$p\text{CO}_2$	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 $p\text{CO}_2$ 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
$p\text{CO}_2$	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 $p\text{CO}_2$ 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
$p\text{CO}_2$	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/ $p\text{CO}_2$ 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 $p\text{CO}_2$ 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
$p\text{CO}_2$	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/ $p\text{CO}_2$ 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	$F_{3,24} = 80.99$	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/ $p\text{CO}_2$ 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	$F_{3,24} = 594.6$	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/ $p\text{CO}_2$ 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	$F_{3,24} = 721.2$	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/pCO₂ 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/pCO₂ 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/pCO₂ 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/pCO₂ 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 $p\text{CO}_2$ 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-d21}, *A. salina* d₉₋₄₂). 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	$F_{4,4} = 1.286$	P = 0.4067
Residual	196431	4	49108		

Table S14. Two-way ANOVA investigating the effect of $p\text{CO}_2$ 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
$p\text{CO}_2$	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 $p\text{CO}_2$ 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
$p\text{CO}_2$	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 $p\text{CO}_2$ 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	0.00776	1	0.00776	$F_{1,56} = 1.163$	P = 0.2855
$p\text{CO}_2$	0.00079	1	0.00079	$F_{1,56} = 0.1177$	P = 0.7328
Temperature	0.00370	1	0.00370	$F_{1,56} = 0.5551$	P = 0.4594
Residual	0.37350	56	0.00667		

Table S17. Two-way ANOVA investigating the effect of $p\text{CO}_2$ and temperature on maximal 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	0.00783	1	0.00783	$F_{1,56} = 0.5820$	$P = 0.4488$
$p\text{CO}_2$	0.03005	1	0.03005	$F_{1,56} = 2.234$	$P = 0.1406$
Temperature	0.06585	1	0.06585	$F_{1,56} = 4.896$	$P = 0.0310$
Residual	0.75320	56	0.01345		

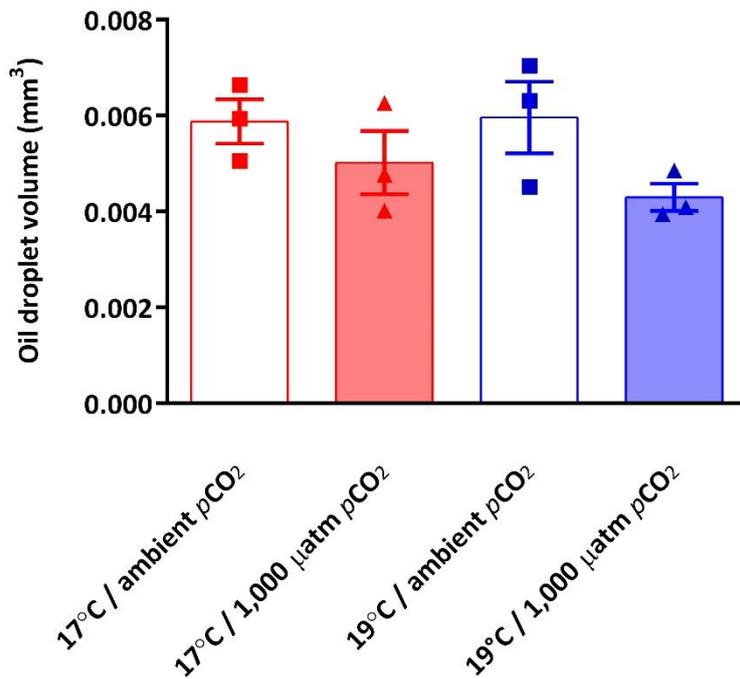


Fig. S1. Oil droplet volume in larval *D. labrax* after 7 days of incubation under experimental ocean acidification conditions. Data points are mean values for each experimental tank, column heights are means and error bars are ± 1 SEM. See Table 1 for details on each experimental condition.

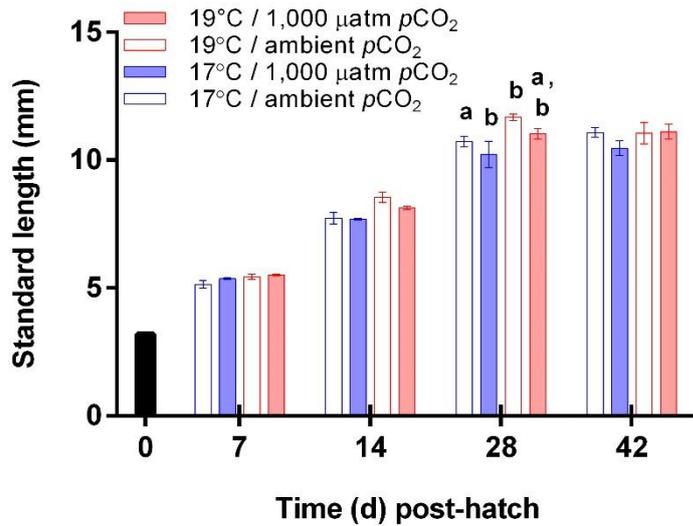


Fig. S2. Standard length of *D. labrax* incubated for 42 days under experimental ocean acidification conditions. Columns that do not share a letter at an individual time are significantly different (two-way ANOVA with Bonferroni post-test, $P < 0.05$). Mean values \pm 1 SEM, $N = 3$. See Table 1 for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

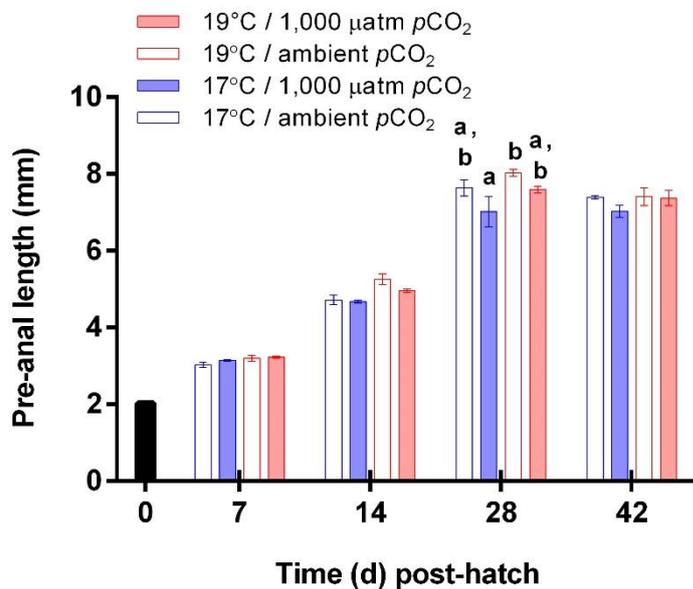


Fig. S3. Pre-anal length in *D. labrax* larvae incubated for 42 days under experimental ocean acidification conditions. Columns that do not share a letter at an individual time are significantly different (two-way ANOVA with Bonferroni post-test, $P < 0.05$). Mean values \pm 1 SEM, $N = 3$. See Table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

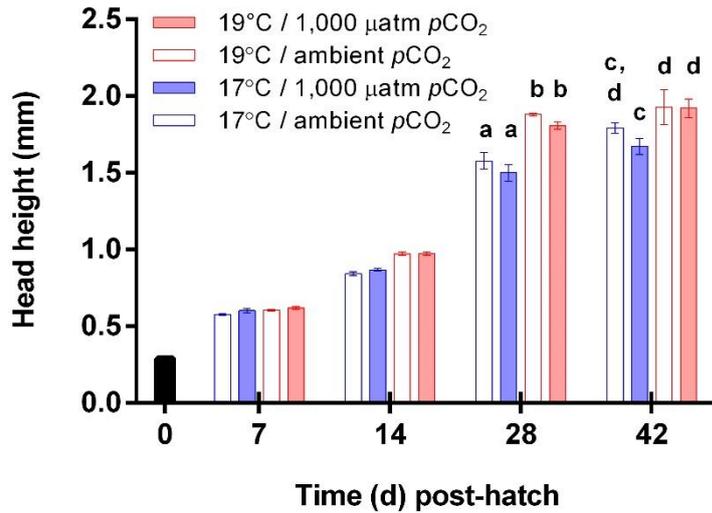


Fig. S4. Head height in *D. labrax* larvae incubated for 42 days under experimental ocean acidification conditions. Columns that do not share a letter at an individual time are significantly different (two-way ANOVA with Bonferroni post-test, $P < 0.05$). Mean values ± 1 SEM, $N = 3$. See Table 1 for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

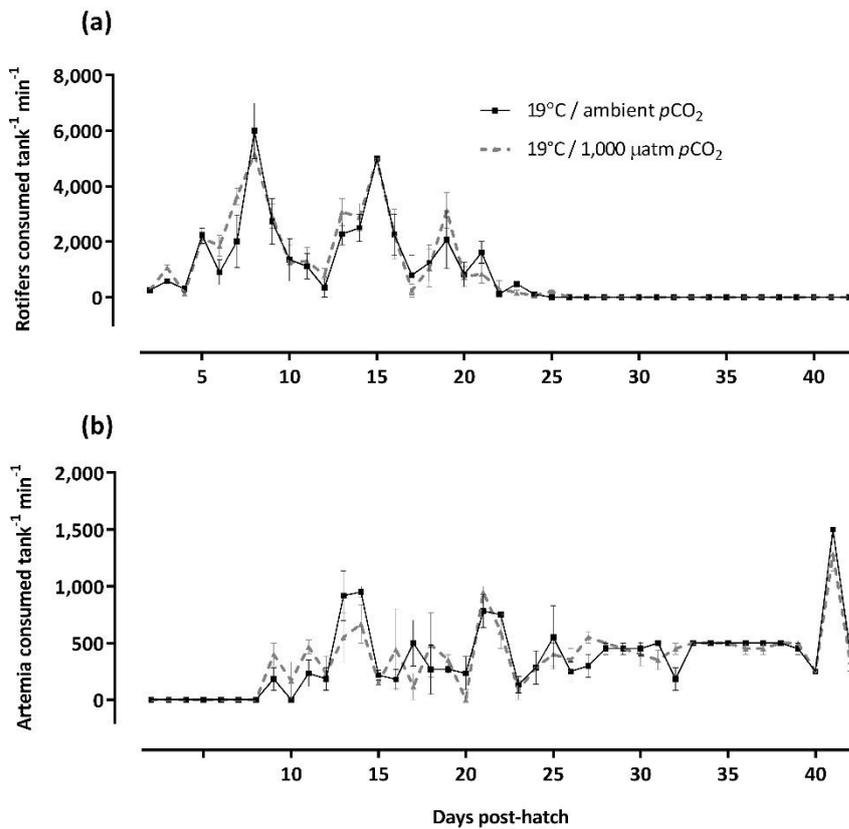


Fig. S5. Total grazing rates for tanks of *D. labrax* larvae incubated for 42 days at 19°C under two different pCO₂s and fed a regime of rotifers (A) moving on to *A. salina* (B). The variation in volumetric counts meant that occasionally a negative grazing level was calculated, such values were recorded as 0. Mean values ± 1 SEM, $N = 3$. See Table 1 for details on each experimental condition.

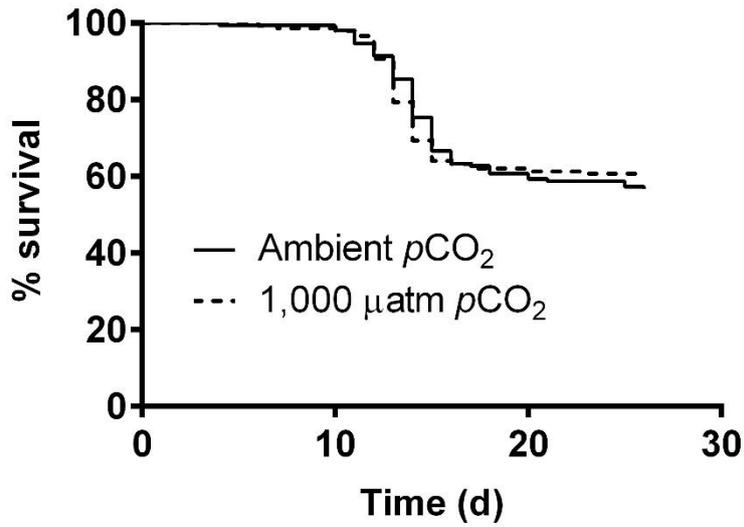


Fig. S6. Kaplan-Meier curves showing survival of *D. labrax* larvae in the weaning trial. The trial started at d49 post-hatch, with animals reared under either ambient or 1,000 μatm $p\text{CO}_2$ (both at 19 °C), weaned onto dry food over 7 days and then maintained for a further 19 days. See Table 1 for details on each experimental condition.

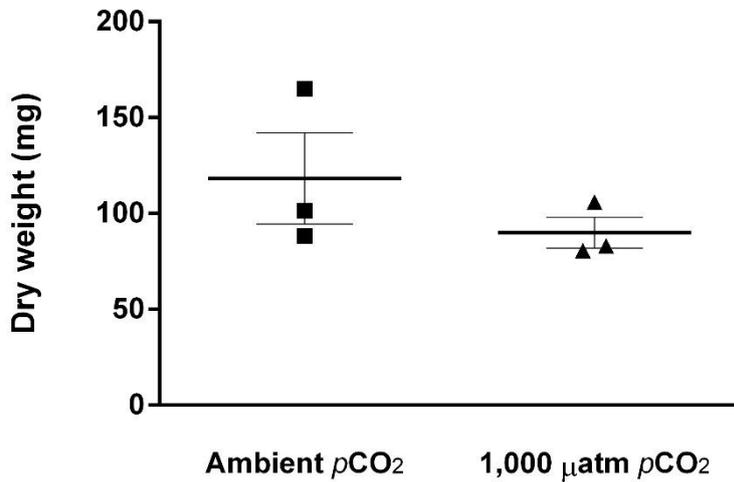


Fig. S7. Dry weight of *D. labrax* larvae incubated at 19°C for 78d under experimental ocean acidification conditions. Fish were transferred to aquaria on d49 for weaning. Lines = mean values \pm 1SEM, $N = 3$. See Table 1 for details on each experimental condition.

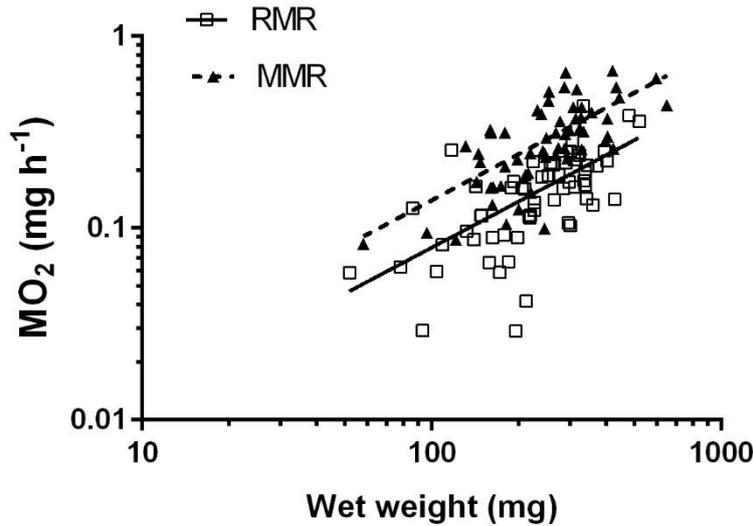


Fig. S8. Oxygen consumption vs. wet weight for juvenile (d₆₇₋₆₉ post-hatch) *D. labrax*. Regressions are power curves ($y = ax^b$) using an exponent value of 0.8. RMR = routine metabolic rate, MMR = maximal metabolic rate.

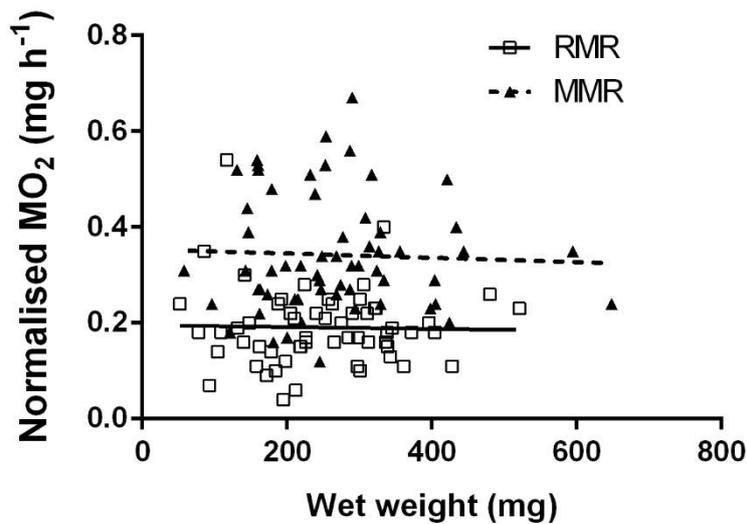


Fig. S9. Normalised (300 mg individual, scaling exponent of 0.8) oxygen consumption of juvenile (d₆₇₋₆₉ post-hatch) *D. labrax* vs. wet weight. RMR = routine metabolic rate, MMR = maximal metabolic rate. Linear regressions of both normalised RMR ($F_{1,58} = 0.03391$, $p = 0.8545$) and normalised MMR ($F_{1,58} = 0.09751$, $p = 0.7560$) do not have slopes significantly different from 0.