

Supplementary Information

Diel and tidal $p\text{CO}_2$ x DO fluctuations provide physiological refuge to early life stages of a coastal forage fish

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Table S1: *Experiments one and two* - Statistical results from linear mixed effects models determining if static $p\text{CO}_2$, static DO or the interaction of these two factors significantly impacted survival and growth of *M. menidia* early life stages. p values in bold denote significant differences.

Response trait	Fixed effects	χ^2	d.f.	p value
Embryo survival	$p\text{CO}_2$	8.20	6	0.182
	DO	84.79	6	<0.001
	$p\text{CO}_2 \times \text{DO}$	19.84	4	<0.001
Larval survival	$p\text{CO}_2$	8.90	6	0.179
	DO	123.48	6	<0.001
	$p\text{CO}_2 \times \text{DO}$	7.05	4	0.133
Hatch length	$p\text{CO}_2$	8.00	6	0.238
	DO	191.98	6	<0.001
	$p\text{CO}_2 \times \text{DO}$	5.77	4	0.217
Growth rate	$p\text{CO}_2$	2.80	6	0.833
	DO	99.67	6	<0.001
	$p\text{CO}_2 \times \text{DO}$	2.79	4	0.593

Table S2: *Experiments three and four* - Statistical results from linear models determining if mean $p\text{CO}_2$ -DO level (control $p\text{CO}_2$ -normoxic, intermediate $p\text{CO}_2$ -reduced DO, extreme $p\text{CO}_2$ -hypoxic) significantly impacted survival and growth of *M. menidia* early life stages in static treatments only. p values in bold denote significant differences.

Experiment	Response trait	F	d.f.	p value
Three	Embryo survival	212.77	2, 11	<0.001
	Larval survival	49.91	2, 11	<0.001
	Hatch length	31.56	1, 7	0.001
	Growth rate	6.34	1, 6	0.045
Four	Embryo survival	6.87	2, 11	0.012
	Larval survival	50.90	2, 12	<0.001
	Hatch length	40.23	2, 8	<0.001
	Growth rate	9.76	1, 6	0.026

Table S3: Experiments three and four - Statistical results from linear models determining if mean $p\text{CO}_2$ -DO level (intermediate $p\text{CO}_2$ -reduced DO or extreme $p\text{CO}_2$ -hypoxic), cycling pattern (static, small diel fluctuation, large diel fluctuation or tidal fluctuation) or the interaction of these two factors significantly impacted survival and growth of *M. menidia* offspring. Only cycling pattern was tested in the intermediate $p\text{CO}_2$ -reduced DO level in experiment three due to complete larval mortality in the extreme $p\text{CO}_2$ -hypoxic treatments. *p* values in bold denote significant differences.

Experiment	Response trait	Factor	F	d.f.	<i>p</i> value
Three	Embryo survival	Level	100.587	1, 31	<0.001
		Cycling Pattern	27.528	3, 31	<0.001
		Level x Cycling Pattern	26.221	3, 31	<0.001
	Larval survival	Cycling Pattern	3.4726	3, 15	0.043
	Hatch length	Level	20.452	1, 22	<0.001
		Cycling Pattern	1.418	3, 22	0.264
		Level x Cycling Pattern	0.655	1, 22	0.427
	Growth rate	Cycling Pattern	2.887	3, 7	0.112
Four	Embryo survival	Level	1.393	1, 31	0.247
		Cycling Pattern	8.245	3, 31	<0.001
		Level x Cycling Pattern	5.769	3, 31	0.038
	Larval survival	Level	18.924	1, 32	<0.001
		Cycling Pattern	71.562	3, 32	<0.001
		Level x Cycling Pattern	20.617	3, 32	<0.001
	Hatch length	Level	34.054	1, 28	<0.001
		Cycling Pattern	11.963	3, 28	<0.001
		Level x Cycling Pattern	10.088	3, 28	<0.001
	Growth rate	Level	4.087	1, 23	0.055
		Cycling Pattern	7.393	3, 23	0.001
		Level x Cycling Pattern	11.359	2, 23	<0.001

Table S4: Spawning ripe *M. menidia* collected from Mumford Cove, CT to fertilise embryos used in static and fluctuating $p\text{CO}_2 \times \text{DO}$ experiments. Adult lengths are shown as mean total lengths (TL, cm) $\pm$ standard deviation.

Experiment	Collection date	Fertilisation date	Number of female spawners	Female lengths	Number of male spawners	Male lengths
1	05/08/2017	05/09/2017	21	9.3 ± 1.3	39	8.1 ± 1.1
2	06/08/2017	06/09/2017	28	11.0 ± 0.9	23	8.9 ± 1.2
3	14/05/2018	16/05/2018	12	9.0 ± 0.8	32	7.7 ± 1.0
4	14/06/2018	15/06/2018	22	9.6 ± 1.4	29	8.3 ± 0.8

Table S5: Seawater parameters from experiments one and two. Means $\pm$ SD are listed for pH_{NIST} , dissolved oxygen (DO), partial pressure of CO_2 ($p\text{CO}_2$), total alkalinity (A_{T}), temperature, and salinity.

Exp.	Treatment	Measured pH_{NIST}	Measured DO (mg L^{-1})	$p\text{CO}_2^*$ (μatm)	A_{T} ($\mu\text{mol kg}^{-1}$)	Temperature ($^{\circ}\text{C}$)	Salinity (psu)
One	Control $p\text{CO}_2$ -Normoxic	8.18 ± 0.02	7.8 ± 0.1	370 ± 1	2001 ± 6	24.7 ± 0.4	30 ± 0
	Control $p\text{CO}_2$ -Reduced DO	8.12 ± 0.04	4.1 ± 0.4	439 ± 2	1996 ± 7	24.6 ± 0.4	30 ± 0
	Control $p\text{CO}_2$ -Hypoxic	8.15 ± 0.05	2.7 ± 0.4	400 ± 1	2004 ± 3	24.9 ± 0.3	30 ± 0
	Intermediate $p\text{CO}_2$ -Normoxic	7.56 ± 0.10	7.7 ± 0.1	1826 ± 7	2005 ± 8	24.5 ± 0.3	30 ± 0
	Intermediate $p\text{CO}_2$ -Reduced DO	7.46 ± 0.06	4.0 ± 0.4	2338 ± 5	2002 ± 4	24.7 ± 0.4	30 ± 0
	Intermediate $p\text{CO}_2$ -Hypoxic	7.48 ± 0.08	2.7 ± 0.4	2189 ± 4	2010 ± 3	24.2 ± 0.5	30 ± 0
	Extreme $p\text{CO}_2$ -Normoxic	7.19 ± 0.07	7.7 ± 0.1	4368 ± 19	1998 ± 9	24.0 ± 0.6	30 ± 0
	Extreme $p\text{CO}_2$ -Reduced DO	7.22 ± 0.03	4.1 ± 0.3	4119 ± 17	2003 ± 8	24.2 ± 0.5	30 ± 0
	Extreme $p\text{CO}_2$ -Hypoxic	7.20 ± 0.05	2.5 ± 0.3	4337 ± 36	2014 ± 17	24.3 ± 0.4	30 ± 0
Two	Control $p\text{CO}_2$ -Normoxic	8.17 ± 0.08	7.8 ± 0.1	385 ± 2	2062 ± 11	24.4 ± 0.3	30 ± 0
	Control $p\text{CO}_2$ -Reduced DO	8.07 ± 0.09	4.2 ± 0.3	505 ± 1	2046 ± 4	24.4 ± 0.4	30 ± 0
	Control $p\text{CO}_2$ -Hypoxic	8.06 ± 0.09	3.1 ± 0.5	520 ± 5	2050 ± 19	24.0 ± 0.4	30 ± 0
	Intermediate $p\text{CO}_2$ -Normoxic	7.50 ± 0.07	7.8 ± 0.1	2173 ± 22	2060 ± 21	24.7 ± 0.3	30 ± 0
	Intermediate $p\text{CO}_2$ -Reduced DO	7.50 ± 0.05	4.1 ± 0.4	2157 ± 12	2055 ± 12	24.4 ± 0.3	30 ± 0
	Intermediate $p\text{CO}_2$ -Hypoxic	7.50 ± 0.05	3.0 ± 0.3	2151 ± 19	2039 ± 17	24.1 ± 0.4	30 ± 0
	Extreme $p\text{CO}_2$ -Normoxic	7.19 ± 0.11	7.7 ± 0.1	4539 ± 55	2064 ± 25	24.4 ± 0.4	30 ± 0
	Extreme $p\text{CO}_2$ -Reduced DO	7.19 ± 0.07	4.1 ± 0.4	4512 ± 20	2060 ± 9	24.4 ± 0.4	30 ± 0
	Extreme $p\text{CO}_2$ -Hypoxic	7.19 ± 0.06	3.0 ± 0.3	4473 ± 64	2053 ± 29	24.2 ± 0.5	30 ± 0

* $p\text{CO}_2$ was calculated from pH_{NIST} and A_{T} using CO2SYS.

Table S6 - Seawater parameters from experiment three. Means $\pm$ SD are listed for daily mean, minimum and maximum of pH_{NIST}, dissolved oxygen (DO) and partial pressure of CO₂ (*p*CO₂). Values for non-varying parameters are listed as mean $\pm$ SD over the experiment.

Level	Cycling Pattern		Measured pH _{NIST}	Measured DO (mg L ⁻¹)	<i>p</i> CO ₂ * (μatm)	<i>A</i> _T (μmol kg ⁻¹)	Temperature (°C)	Salinity (psu)
Control <i>p</i> CO ₂ -Normoxic	Static	Mean	8.13 $\pm$ 0.03	7.6 $\pm$ 0.2	425 $\pm$ 1			
		Min.	-	-	-	1998 $\pm$ 6	23.9 $\pm$ 0.2	30 $\pm$ 0
		Max.	-	-	-			
Intermediate <i>p</i> CO ₂ -Reduced DO	Static	Mean	7.45 $\pm$ 0.04	3.7 $\pm$ 0.5	2351 $\pm$ 6			
		Min.	-	-	-	1999 $\pm$ 5	24.6 $\pm$ 0.2	31 $\pm$ 0
		Max.	-	-	-			
	Small Diel Fluctuation	Mean	7.48 $\pm$ 0.19	3.9 $\pm$ 1.3	2147 $\pm$ 5			
		Min.	7.13 $\pm$ 0.11	2.3 $\pm$ 0.4	1166 $\pm$ 3	1983 $\pm$ 4	23.8 $\pm$ 0.2	31 $\pm$ 0
		Max.	7.73 $\pm$ 0.02	6.0 $\pm$ 0.3	4953 $\pm$ 11			
	Large Diel Fluctuation	Mean	7.48 $\pm$ 0.35	3.8 $\pm$ 1.4	2163 $\pm$ 11			
		Min.	6.84 $\pm$ 0.06	1.4 $\pm$ 0.3	521 $\pm$ 2	2004 $\pm$ 23	24.0 $\pm$ 0.2	31 $\pm$ 1
		Max.	8.05 $\pm$ 0.02	6.2 $\pm$ 0.2	9926 $\pm$ 59			
	Tidal Fluctuation	Mean	7.44 $\pm$ 0.32	4.3 $\pm$ 1.3	2426 $\pm$ 42			
		Min.	6.90 $\pm$ 0.07	1.6 $\pm$ 0.3	699 $\pm$ 13	2015 $\pm$ 22	23.9 $\pm$ 0.2	31 $\pm$ 1
		Max.	7.94 $\pm$ 0.02	6.1 $\pm$ 0.4	8667 $\pm$ 142			
Extreme <i>p</i> CO ₂ -Hypoxic	Static	Mean	7.18 $\pm$ 0.07	2.4 $\pm$ 0.6	4447 $\pm$ 39			
		Min.	-	-	-	1988 $\pm$ 5	24.1 $\pm$ 0.2	31 $\pm$ 1
		Max.	-	-	-			
	Small Diel Fluctuation	Mean	7.26 $\pm$ 0.20	3.1 $\pm$ 0.8	3705 $\pm$ 39			
		Min.	6.85 $\pm$ 0.08	1.9 $\pm$ 0.2	1872 $\pm$ 21	1983 $\pm$ 9	23.9 $\pm$ 0.2	31 $\pm$ 1
		Max.	7.54 $\pm$ 0.03	4.2 $\pm$ 0.2	9590 $\pm$ 97			
	Large Diel Fluctuation	Mean	7.18 $\pm$ 0.33	3.3 $\pm$ 1.6	4409 $\pm$ 13			
		Min.	6.63 $\pm$ 0.07	1.1 $\pm$ 0.2	1058 $\pm$ 3	1986 $\pm$ 6	24.0 $\pm$ 0.2	31 $\pm$ 0
		Max.	7.77 $\pm$ 0.02	5.3 $\pm$ 0.2	15970 $\pm$ 48			
	Tidal Fluctuation	Mean	7.12 $\pm$ 0.32	3.4 $\pm$ 1.1	5177 $\pm$ 129			
		Min.	6.61 $\pm$ 0.07	1.6 $\pm$ 0.5	1349 $\pm$ 36	2008 $\pm$ 38	24.2 $\pm$ 0.2	31 $\pm$ 1
		Max.	7.68 $\pm$ 0.09	5.0 $\pm$ 0.5	17013 $\pm$ 417			

**p*CO₂ was calculated from pH_{NIST} and *A*_T using CO2SYS.

Table S7 - *Seawater parameters from experiment four.* Means $\pm$ SD are listed for daily mean, minimum and maximum of pH_{NIST}, dissolved oxygen (DO) and partial pressure of CO₂ (*p*CO₂). Values for non-varying parameters are listed as mean $\pm$ SD over the experiment.

Level	Cycling Pattern		Measured pH _{NIST}	Measured DO (mg L ⁻¹)	<i>p</i> CO ₂ * (μatm)	<i>A</i> _T (μmol kg ⁻¹)	Temperature (°C)	Salinity (psu)
Control <i>p</i> CO ₂ -Normoxic	Static	Mean	8.17 $\pm$ 0.07	7.5 $\pm$ 0.2	379 $\pm$ 2			
		Min.	-	-	-	2025 $\pm$ 10	23.7 $\pm$ 0.4	32 $\pm$ 1
		Max.	-	-	-			
Intermediate <i>p</i> CO ₂ -Reduced DO	Static	Mean	7.45 $\pm$ 0.04	4.2 $\pm$ 0.4	2337 $\pm$ 19			
		Min.	-	-	-	2032 $\pm$ 5	23.8 $\pm$ 0.5	32 $\pm$ 1
		Max.	-	-	-			
	Small Diel Fluctuation	Mean	7.53 $\pm$ 0.17	5.7 $\pm$ 0.7	1931 $\pm$ 2			
		Min.	7.34 $\pm$ 0.09	4.0 $\pm$ 0.6	876 $\pm$ 1	2020 $\pm$ 3	23.5 $\pm$ 0.5	31 $\pm$ 0
		Max.	7.85 $\pm$ 0.04	6.6 $\pm$ 0.5	3059 $\pm$ 5			
	Large Diel Fluctuation	Mean	7.49 $\pm$ 0.31	5.5 $\pm$ 0.9	2119 $\pm$ 2			
		Min.	6.89 $\pm$ 0.11	3.0 $\pm$ 0.2	747 $\pm$ 1	2030 $\pm$ 2	23.6 $\pm$ 0.4	32 $\pm$ 0
		Max.	7.91 $\pm$ 0.03	6.1 $\pm$ 0.3	8810 $\pm$ 8			
	Tidal Fluctuation	Mean	7.40 $\pm$ 0.27	5.4 $\pm$ 0.8	2672 $\pm$ 3			
		Min.	6.87 $\pm$ 0.04	3.2 $\pm$ 0.4	948 $\pm$ 1	2024 $\pm$ 2	23.6 $\pm$ 0.6	31 $\pm$ 0
		Max.	7.82 $\pm$ 0.06	6.6 $\pm$ 0.3	9277 $\pm$ 10			
	Static	Mean	7.18 $\pm$ 0.05	3.3 $\pm$ 0.2	4489 $\pm$ 6			
		Min.	-	-	-	2028 $\pm$ 3	23.6 $\pm$ 0.5	31 $\pm$ 0
		Max.	-	-	-			
Extreme <i>p</i> CO ₂ -Hypoxic	Small Diel Fluctuation	Mean	7.25 $\pm$ 0.14	4.3 $\pm$ 0.5	3783 $\pm$ 7			
		Min.	7.02 $\pm$ 0.06	3.0 $\pm$ 0.2	2341 $\pm$ 5	2016 $\pm$ 7	23.7 $\pm$ 0.6	32 $\pm$ 1
		Max.	7.45 $\pm$ 0.03	5.2 $\pm$ 0.3	6490 $\pm$ 11			
	Large Diel Fluctuation	Mean	7.29 $\pm$ 0.27	4.4 $\pm$ 1.2	3478 $\pm$ 24			
		Min.	6.74 $\pm$ 0.04	2.6 $\pm$ 0.3	1258 $\pm$ 10	2027 $\pm$ 1	23.7 $\pm$ 0.5	31 $\pm$ 1
		Max.	7.71 $\pm$ 0.03	5.7 $\pm$ 0.2	12624 $\pm$ 79			
	Tidal Fluctuation	Mean	7.18 $\pm$ 0.25	4.5 $\pm$ 1.0	4425 $\pm$ 30			
		Min.	6.77 $\pm$ 0.04	2.7 $\pm$ 0.3	1515 $\pm$ 10	2010 $\pm$ 13	23.9 $\pm$ 0.5	31 $\pm$ 0
		Max.	7.63 $\pm$ 0.04	5.9 $\pm$ 0.3	11658 $\pm$ 78			

**p*CO₂ was calculated from pH_{NIST} and *A*_T using CO2SYS.