Supplementary material

Diel CO2 cycles and parental effects have similar benefits to growth of a coral reef fish under ocean acidification

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Larval rearing protocol

On the night of hatching, pots with egg clutches were removed from the 60 L breeding tanks and transferred to an aerated 100 L larval rearing aquarium. Larvae were fed rotifers (*Brachionus* sp.) at 100 individuals’ mL-1 each morning for the first 5 days. On these days 5 mL of non-viable *Nannochloropsis* algal paste was also added to the tanks to feed the rotifers. During these first 5 days aquariums had no water flow during the day (8am-4pm) and were then slowly flushed with filtered seawater each night. This daily cycle ensured that larvae could feed *ad libitum* throughout daylight hours and that any unconsumed food was removed each night. Half a teaspoon worth of freshly hatched *Artemia* naupli were added from days 3-12. A commercial weaning fish feed (INVE Aquaculture Nutrition Wean-S 200-400 µm) was added from day 10. A summer light cycle of 13 h of light/11 h of dark was simulated with fluorescent lights.

The larval rearing system comprised of two independent 8000 L recirculating seawater systems (one for control CO2 (500 μatm) and one for stable-elevated CO2 (1000 μatm))

A pH control system (AT Control, Aqua Medic, Germany) dosed CO2 into 3000 L sumps to achieve the desired pH level for each CO2 treatment. Each sump fed three 100 L larval rearing aquarium and another experimental room. pHNBS was measured daily using a pH meter (InLab Expert Pro electrode and Seven2Go Pro meter, Mettler Toledo, Switzerland), which was calibrated weekly using standard NBS buffers (InLab Solutions, Mettler Toledo, Switzerland). Temperature was recorded daily with a digital thermometer (Comark C26, Norfolk, UK). Salinity readings were taken weekly using a conductivity sensor (HQ15d; Hach, USA). Total alkalinity was measured weekly using Gran Titration (Metrohm 888 Titrando Titrator Metrohm AG, Switzerland) and certified reference material from Dr. A. G. Dickson (Scripps Institution of Oceanography). *p*CO2 values were calculated as a function of pHNBS, temperature and salinity using CO2SYS (Pierrot *et al*., 2006) employing constants from Mehrbach *et al* (1973) refit by Dickson & Millero (1987) and the KHSO4 dissociation constant from Dickson (1990). Mean values for each of these seawater parameters are presented in Supplementary Table 1.

**Table S1.** Experimental Seawater parameters for the larval rearing systems. Values are means ± 1 S.D. for pH (NBS scale) and *p*CO2. Means ± 1 S.D. for total alkalinity (TA), temperature and salinity are also shown

|  |  |
| --- | --- |
|   | *p*CO2 treatment (μatm) |
| Parameter  | 450 | 1000 |
| pHNBS | 8.15 ± 0.01 | 7.75 ± 0.03 |
| *p*CO2 | 452 ± 23 | 1023 ± 72 |
| TA (μmol Kg-1) | 2369 ± 96 | 2377 ± 96 |
| Temperature (°C) | 28.5 ± 0.08 | 28.5 ± 0.1 |
| Salinity (‰) | 36.1 ± 0.5 | 35.8 ± 0.6 |

Adult and juvenile experimental system

The adult and juvenile rearing system was a 14300 L recirculating seawater system. Briefly, the system consisted of an external 3,700 L sump tank connected to a bio-filter, protein skimmer, UV steriliser and a 1000 L algal bio-remediation tank. The external sump supplied water to six separate 1,600 L recirculating systems (two per CO2 treatment) that comprised of one 1000 L sump tank, eleven 40 L juvenile rearing tanks (only 4-7 tanks per system were used) and three 60 L breeding pair tanks, contained within a temperature-controlled room. Water was supplied to the internal sumps at a rate of approximately 1,600 L *per* day allowing for a complete exchange with the external sump. Holding tanks were supplied with water at a rate of 1 L min-1. Both the internal sumps and holding tanks were aerated with ambient air.

Elevated *p*CO2 treatments were achieved by dosing the 1000 L internal sumps with CO2. This was controlled by solenoid valves (M-Ventil Standard, Aqua Medic, Germany) connected to a pH control system (Aqua Medic AT Control System, Aqua Medic, Germany) with laboratory grade pH electrodes (Neptune Systems, USA). The Aqua Medic AT Control System has a curve function which allowed us to create fluctuating *p*CO2 profiles. For the stable *p*CO2 treatments pHNBS was measured daily using a pH meter (InLab Expert Pro electrode and Seven2Go Pro meter, Mettler Toledo, Switzerland). pH profiles in the fluctuating *p*CO2 treatments were recorded using a pH datalogger (Model 850060, Sper Scientific, USA) set to take a reading every 15 min. The pH logger was calibrated when required and checked each day by comparing the logger pH against the probe used for daily measurements. Seawater pH on the total hydrogen ion concentration scale (total scale, pHT) was measured each week with a spectrophotometer following standard operating procedures (Dickson *et al*., 2007) using the indicator dye meta/*m*-cresol purple (mCP) (*m*-cresol purple sodium salt 99%, non-purified, Acros Organic). Daily and fluctuating pHNBS measurements were converted to pHT based on the offset between weekly pHT and pHNBS measurements. Temperature was recorded daily with a digital thermometer (Comark C26, Norfolk, UK). Salinity readings were taken weekly using a conductivity sensor (HQ15d; Hach, USA). Total alkalinity was measured weekly using Gran Titration (Metrohm 888 Titrando Titrator Metrohm AG, Switzerland) and certified reference material from Dr. A. G. Dickson (Scripps Institution of Oceanography). All seawater parameters were measured in one randomly chosen tank on each system either daily or weekly. *p*CO2 values were calculated as a function of pHT, temperature and salinity using CO2SYS (Pierrot *et al*., 2006) employing constants from Mehrbach *et al* (1973) refit by Dickson & Millero (1987) and the KHSO4 dissociation constant from Dickson (1990). Mean values for each of these seawater parameters are presented in Table 1.

Summary outputs for statistical models

Code = CO2 treatment (1 = control-control, 2 = control-stable, 3 = control-cycling, 4 = stable-stable and 5 = cycling-cycling)

Generalized liner model for survival data

mod1.lmer = glm(prop ~ code, family=binomial(), weights=start, data=mdata)

Analysis of Deviance Table (Type II tests)

Response: prop

 LR Chisq Df Pr(>Chisq)

code 17.681 4 0.001424 \*\*

Call:

glm(formula = prop ~ code, family = binomial(), data = mdata,

 weights = start)

Deviance Residuals:

 Min 1Q Median 3Q Max

-1.9907 -0.6267 0.7133 1.0026 1.8623

Coefficients:

 Estimate Std. Error z value Pr(>|z|)

(Intercept) 2.0565 0.3202 6.422 1.34e-10 \*\*\*

code2 -0.5076 0.4170 -1.217 0.2234

code3 -0.2767 0.4313 -0.641 0.5212

code4 0.9088 0.5593 1.625 0.1042

code5 1.2577 0.6014 2.092 0.0365 \*

Linear mixed-effects model for wet weight data

mod.lme=lme(weight ~ number+code, random=~1|pair/clutch/tank, data=mdata)

Anova table

 numDF denDF F-value p-value

(Intercept) 1 398 23.737138 <.0001

number 1 31 5.974822 0.0204

code 4 31 5.958873 0.0011

Linear mixed-effects model fit by REML

 Data: mdata

 AIC BIC logLik

 -1685.667 -1644.575 852.8336

Random effects:

 Formula: ~1 | pair

 (Intercept)

StdDev: 9.009689e-07

 Formula: ~1 | clutch %in% pair

 (Intercept)

StdDev: 0.008605626

 Formula: ~1 | tank %in% clutch %in% pair

 (Intercept) Residual

StdDev: 0.007071878 0.03416387

Fixed effects: weight ~ number + code

 Value Std.Error DF t-value p-value

(Intercept) 0.06286930 0.012904003 398 4.872077 0.0000

number 0.00389445 0.001593250 33 2.444345 0.0200

code2 -0.01593713 0.006120182 33 -2.604029 0.0137

code3 0.01066288 0.006043913 33 1.764235 0.0869

code4 -0.01291397 0.008554117 8 -1.509679 0.1696

code5 0.00343256 0.007692755 11 0.446207 0.6641

Linear mixed-effects model for standard length data

mod2.lme = lme(SL ~ number+code, random=~1|pair/clutch/tank, data=mdata)

Anova table

 numDF denDF F-value p-value

(Intercept) 1 398 262.41060 <.0001

number 1 31 10.20851 0.0032

code 4 31 5.64022 0.0016

Linear mixed-effects model fit by REML

 Data: mdata

 AIC BIC logLik

 -103.5063 -62.41386 61.75317

Random effects:

 Formula: ~1 | pair

 (Intercept)

StdDev: 1.09065e-05

 Formula: ~1 | clutch %in% pair

 (Intercept)

StdDev: 0.04202022

 Formula: ~1 | tank %in% clutch %in% pair

 (Intercept) Residual

StdDev: 0.05054846 0.1975316

Fixed effects: SL ~ number + code

 Value Std.Error DF t-value p-value

(Intercept) 1.1847928 0.07313946 398 16.199093 0.0000

number 0.0287817 0.00900816 33 3.195076 0.0031

code2 -0.1351236 0.03747446 33 -3.605753 0.0010

code3 0.0133366 0.03705697 33 0.359896 0.7212

code4 -0.1052847 0.04868740 8 -2.162463 0.0625

code5 -0.0967030 0.04414011 11 -2.190819 0.0509

References

1. Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM. 1973 Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol. Oceanogr*. 18, 897–907. doi: 0.4319/lo.1973.18.6.0897

2. Dickson AG, Millero FJ. 1987 A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Res. Part A Oceanogr. Res. Pap*. **34**, 1733–43.

3. Dickson AG. 1990 Standard potential of the reaction: AgCl(s) + 1/2H2(g) = Ag(s) + HCl(aq), and and the standard acidity constant of the ion HSO4− in synthetic sea water from 273.15 to 318.15 K. *J. Chem. Thermodyn*. **22**, 113–27.