

Research



Cite this article: Jarrold MD, Munday PL. 2019 Diel CO₂ cycles and parental effects have similar benefits to growth of a coral reef fish under ocean acidification. *Biol. Lett.* **15**: 20180724.
<http://dx.doi.org/10.1098/rsbl.2018.0724>

Received: 16 October 2018
Accepted: 14 January 2019

Subject Areas:

environmental science, evolution

Keywords:

parental effects, phenotypic plasticity, acclimation, pH variability, daily fluctuations, coral reefs

Author for correspondence:

Michael D. Jarrold
e-mail: michael.jarrold@my.jcu.edu.au

Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.4381721>.

Global change biology

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

Michael D. Jarrold^{1,2} and Philip L. Munday²

¹College of Science and Engineering, and ²ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Queensland 4811, Australia

MDJ, 0000-0003-4224-9683

Parental effects have been shown to buffer the negative effects of within-generation exposure to ocean acidification (OA) conditions on the offspring of shallow water marine organisms. However, it remains unknown if parental effects will be impacted by the presence of diel CO₂ cycles that are prevalent in many shallow water marine habitats. Here, we examined the effects that parental exposure to stable elevated (1000 µatm) and diel-cycling elevated (1000 ± 300 µatm) CO₂ had on the survival and growth of juvenile coral reef anemonefish, *Amphiprion melanopus*. Juvenile survival was unaffected by within-generation exposure to either elevated CO₂ treatment but was significantly increased (8%) by parental exposure to diel-cycling elevated CO₂. Within-generation exposure to stable elevated CO₂ caused a significant reduction in juvenile growth (10.7–18.5%); however, there was no effect of elevated CO₂ on growth when diel CO₂ cycles were present. Parental exposure to stable elevated CO₂ also ameliorated the negative effects of elevated CO₂ on juvenile growth, and parental exposure to diel CO₂ cycles did not alter the effects of diel CO₂ cycles on juveniles. Our results demonstrate that within-generation exposure to diel-cycling elevated CO₂ and parental exposure to stable elevated CO₂ had similar outcomes on juvenile condition. This study illustrates the importance of considering natural CO₂ cycles when predicting the long-term impacts of OA on marine ecosystems.

1. Background

Parental effects occur when the environmental conditions experienced by the mother and/or father alter the phenotype of their offspring [1–3]. Thus, parental effects represent a form of phenotypic plasticity that spans generations. Parental effects have the potential to increase offspring fitness, although they can also act to reduce offspring performance [4]. Consequently, parental effects have gained interest as a potential mechanism that may assist marine organisms to persist in the face of ongoing rapid climate change and ocean acidification (OA) [5,6]. Many experimental studies have shown that within-generation exposure to OA conditions predicted to occur by the end of the century can negatively impact a range of traits (including survival, development and growth) in early life-stages of various marine taxa [7]. However, in some instances, early life-stage tolerance to OA is increased when parents experience similar conditions to their offspring [8–10].

Many ecologically and economically important marine species live in shallow water coastal habitats. These habitats can experience substantial natural fluctuations in CO₂ on a variety of temporal scales [11]. Despite this, most OA experiments to date on shallow water species have used stable elevated CO₂ levels consistent with open ocean environments and thus may have limited ecological relevance [12]. Perhaps the most well-known CO₂ fluctuations are those that occur over a 24 h period, primarily driven by the net effects of photosynthesis

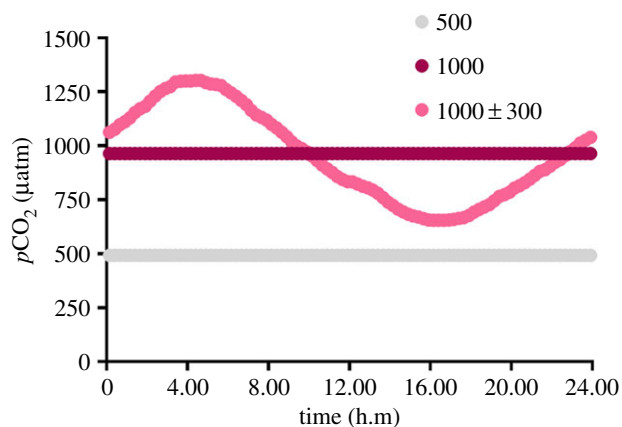


Figure 1. Mean daily $p\text{CO}_2$ profiles for the 500, 1000 and 1000 ± 300 μatm CO_2 treatments. (Online version in colour.)

Table 1. Experimental seawater parameters. Values are means ± 1 s.d. for mean, min and max pH (total scale) and $p\text{CO}_2$. Means ± 1 s.d. for total alkalinity (TA), temperature and salinity are also shown.

parameter	$p\text{CO}_2$ treatment (μatm)		
	500	1000	1000 ± 300
mean pH_T	7.99 ± 0.03	7.75 ± 0.03	7.76 ± 0.09
min. pH_T	—	—	7.63 ± 0.06
max. pH_T	—	—	7.88 ± 0.03
mean $p\text{CO}_2$	493 ± 24	964 ± 50	957 ± 233
min. $p\text{CO}_2$	—	—	681 ± 63
max. $p\text{CO}_2$	—	—	1270 ± 127
TA ($\mu\text{mol kg}^{-1}$)	2495 ± 155	2494 ± 150	2458 ± 129
temperature ($^\circ\text{C}$)	28.5 ± 0.07	28.5 ± 0.1	28.5 ± 0.09
salinity (‰)	35.4 ± 0.7	35.4 ± 0.7	35.4 ± 0.8

and respiration over a day–night cycle [13]. These diel CO_2 cycles occur in many shallow water ecosystems, including coral reefs, and are expected to increase in magnitude with ongoing OA owing to the change in seawater buffering capacity as the ocean absorbs more CO_2 [14,15]. Recent studies accounting for diel CO_2 cycles have demonstrated that they can either alleviate or intensify the within-generation responses of marine organisms to OA [16–20]. However, it is unknown if diel CO_2 cycles will alter the outcome of parental effects on offspring traits, which limits our ability to accurately predict how marine organisms will respond to OA in the long term.

The influence that parental effects have on offspring fitness is related to how predictable variations in environmental conditions are over time [1]. Consequently, the outcome of parental effects in the presence of diel CO_2 cycles may differ from those expressed in a stable CO_2 environment. To test this, we conditioned adult pairs of coral reef anemonefish, *Amphiprion melanopus*, to control (500 μatm), stable elevated (1000 μatm) and diel-cycling elevated (1000 \pm 300 μatm) CO_2 (figure 1 and table 1). Juveniles from control parents were reared in all CO_2 treatments, whereas juveniles from the two elevated CO_2 treatments were reared in the same conditions as their parents. Comparisons between treatments allowed us to determine the within-generation effects of stable elevated

and diel-cycling elevated CO_2 on juvenile growth and survival, and how these responses were modified by parental exposure to either stable elevated or diel-cycling elevated CO_2 .

2. Material and methods

(a) Parental conditioning

Adult breeding pairs of the cinnamon anemone fish, *Amphiprion melanopus*, were collected from the Bramble and Trunk Reef region of the Great Barrier Reef in September 2016. Diel CO_2 cycles on shallow coral reefs in the region have been shown to range between ± 50 and 200 μatm [21,22], although a threefold amplification is expected to occur by the year 2100 [14]. Breeding pairs were housed in 60 l aquaria and maintained at temperature conditions matching the collection location (22.5 $^\circ\text{C}$ winter–28.5 $^\circ\text{C}$ summer) for 1 year prior to the experiment. Pairs were assigned to the control (500 μatm), stable elevated (1000 μatm) and diel-cycling elevated (1000 \pm 300 μatm) CO_2 treatments at the end of August 2017 (control CO_2 = 6 pairs, stable elevated CO_2 = 3 pairs and diel-cycling elevated CO_2 = 4 pairs). This allowed pairs to be conditioned in their CO_2 treatments for three months before the start of the breeding season in December 2017. Two pairs in the diel-cycling elevated CO_2 treatment failed to rear enough offspring through to hatching. Therefore, at the beginning of March, three control pairs (pair # 2, 3 and 26), from which offspring had been obtained, were transferred to diel-cycling elevated CO_2 . These pairs were allowed to produce two clutches before a clutch was taken for the experiment. Breeding pairs were provided with half of a terracotta pot as a shelter and a spawning site. Temperatures were increased from winter temperatures of 22.5 $^\circ\text{C}$ at a rate of 0.5 $^\circ\text{C}$ per week until the summer breeding temperature of 28.5 $^\circ\text{C}$ was reached in the first week of December 2017 and maintained for the rest of the experiment. A total of 22 clutches were sourced from all pairs between January and May 2018.

(b) Larval and juvenile rearing

Breeding pairs were checked daily for the presence of eggs. On the night of hatching, pots were removed from the 60 l breeding tanks and transferred to an aerated 100 l larval rearing aquarium. During the 12-day pelagic larval stage that occurs immediately after hatching, larvae from pairs reared under diel-cycling elevated CO_2 were reared under stable CO_2 conditions (1000 μatm) to represent the more stable pelagic ocean environments they occupy. Larvae from control and stable elevated CO_2 pairs were reared under the same CO_2 conditions as their parents. Larvae were reared following standard protocols (electronic supplementary material).

At 12 days post hatch (dph), settlement stage fish (identified by the transition to dark coloration) were randomly transferred into 40 l juvenile rearing tanks (5–12 fish per tank). Juveniles from control CO_2 pairs were split into control (control–control), stable elevated (control–stable) and diel-cycling elevated (control–cycling) CO_2 conditions. Juveniles from stable elevated and diel-cycling elevated CO_2 pairs were only reared in the same conditions as their parents (stable–stable and cycling–cycling). CO_2 treatments were duplicated and juveniles from each clutch were split between duplicate tanks (i.e. one tank per replicate CO_2 treatment). In some cases, there were insufficient larvae to stock duplicate tanks and so another clutch from that pair was used, with juveniles being placed into a tank on the other replicate CO_2 treatment. Juveniles were reared for 28 days. They were fed a combination of freshly hatched *Artemia* nauplii (100 ml from a stock of 1.5 l in which one teaspoon of cysts were hatched) and weaning fish feed (0.05 g) daily for the first two dph. From 3–7 dph, they were fed once daily (0.05 g) on the weaning feed.

From 8–28 dph, juveniles were fed twice daily (0.05 g each time) on the weaning feed. At the end of the rearing period, juveniles were euthanized with clove oil anaesthetic. The number of fish remaining in each tank was recorded. Individuals were then blotted dry, weighed (nearest mg) on an analytical balance (AX224, Sartorius, Bradford, USA) and photographed in a lateral position next to a ruler. Standard length (SL) to the nearest 0.1 mm was estimated for each fish from the digital photographs using ImageJ software (<http://rsb.info.nih.gov/ij/>).

For full details on the experimental system and seawater manipulation, please refer to the electronic supplementary material.

(c) Statistical analyses

The effect of CO₂ treatment on juvenile survival was tested using a general linearized model fitted with a binomial distribution, which was weighted to the number of fish initially stocked into each tank. The effects of CO₂ treatment on wet weight and SL were tested using linear mixed effects models. Pair, clutch and tank were included as random effects, with tank nested within clutch nested within a pair. The number of fish remaining in a tank at the end of the experiment was included as a covariate to account for density-dependent effects. Additive models were used (i.e. number + CO₂ treatment) based on Akaike information criterion. Pairwise comparisons were made between the control–control and other CO₂ treatments based on the linear model summary outputs. All analyses were conducted using R v. 3.4.0. [23] using the ‘lme4’ and ‘nlme’ packages.

3. Results

Juvenile survival ranged from 82.2% to 96.7% in the control–stable and cycling–cycling treatments, respectively, and was significantly affected by CO₂ treatment (figure 2a, $\chi^2 = 17.68$, d.f. = 4, $p = 0.001$). Survival in the control–control treatment ($88.7 \pm 3.6\%$, mean \pm s.e.) was significantly lower than in the cycling–cycling treatment ($96.7 \pm 2.2\%$) ($z = 2.09$, $p = 0.037$). Juvenile wet weight and SL were also significantly affected by CO₂ treatment (figure 2b,c, min. $F_{4,31} = 3.48$, $p = 0.019$). Juvenile exposure to stable elevated CO₂ (control–stable) caused an 18.5% and 10.7% reduction in weight wet and SL, respectively, compared to the control–control treatment (max. $t = -2.08$, $p = 0.045$). However, there were no significant differences between the control–control and other treatment groups (min. $t = -2.19$, $p = 0.051$), indicating that both diel CO₂ cycles and parental exposure to elevated CO₂ restored growth to the same level as control. The number of fish remaining in a tank at the end of the experiment had a significant effect on wet weight and SL (min. $F_{4,31} = 5.97$, $p = 0.020$). Positive relationships between the number of fish in each tank and wet weight/SL were observed (min. $F_{1,454} = 17.59$, $R^2 = 0.037$, $p < 0.0001$). The full model summary outputs are available in the electronic supplementary material.

4. Discussion

Our findings show that both parental effects and diel CO₂ cycles can significantly modify the growth of a coral reef fish under OA conditions. Parental exposure to stable elevated CO₂ (1000 μ atm) alleviated the negative effect of high CO₂ on the growth of juvenile *A. melanopus*. This is consistent with past work on the same species [8] and the Atlantic silver-side, *Menidia menidia* [9]. In contrast to juvenile fish reared at stable elevated CO₂, no negative effect on growth was observed

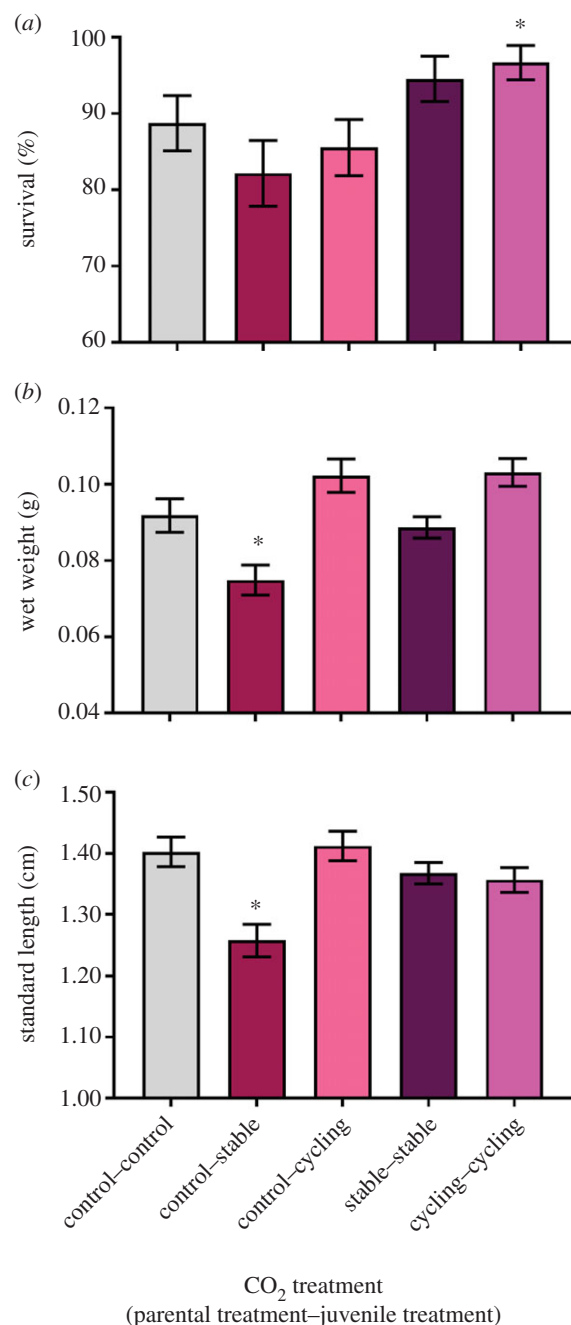


Figure 2. Effects of within-generation and parental exposure to stable elevated and diel-cycling elevated CO₂ on (a) survival, (b) wet weight and (c) SL of juvenile cinnamon anemone fish, *Amphiprion melanopus*. Bars represent means \pm s.e. Asterisks (*) denote a significant difference compared to the control–control treatment. (Online version in colour.)

in juveniles that experienced within-generation exposure to diel-cycling elevated CO₂ (1000 \pm 300 μ atm). Diel CO₂ cycles were also shown to alleviate the negative effect of elevated CO₂ on the growth of pink salmon, *Oncorhynchus gorbuscha*, larvae [17]. Importantly, the beneficial effect of diel CO₂ cycles under elevated CO₂ on the growth of juvenile fish in our study was unchanged by parental exposure to the same treatment, suggesting that a diel-cycling regime is predictable enough to prevent negative parental effects from occurring.

Our work demonstrates that parental exposure to stable elevated CO₂ and within-generation exposure to diel-cycling elevated CO₂ both alleviate the negative effect of elevated CO₂ on juvenile growth of a marine fish. One possible explanation for this is that energetic costs for juvenile fish living under elevated CO₂ are reduced when their parents have

experienced the same CO₂ conditions and when diel CO₂ cycles are present. Consistent with this hypothesis, a previous study on *A. melanopus* showed that parental exposure restored juvenile resting metabolic rates and size under elevated CO₂, demonstrating that the cost of living was lower if parents had experienced the same conditions [8]. Possible mechanisms for the restoration of metabolic rates across generations are epigenetic modification of gene expression [24] or the inheritance of acclimated mitochondria from mothers [25], as has been observed in marine fish exposed to elevated temperature. Finally, it is important to mention that improved juvenile growth, caused by altered metabolic rates/energy re-allocation, could have negative consequences later in life as a result of trade-offs between traits [26,27].

The physiological mechanisms responsible for the beneficial effect of diel CO₂ cycles on the growth of juvenile fishes under elevated CO₂ are uncertain. However, there is evidence to suggest that acclimation of metabolic rates may be responsible. For example, in addition to alleviating the negative effects of elevated CO₂ on growth, a diel-cycling CO₂ regime also restored maximal metabolic rates of *O. gorbuscha* larvae to levels seen in control fish [17]. Alterations in metabolic rates under elevated CO₂ are thought to be linked to the costs associated with defending acid–base status [28]. Under elevated CO₂ fishes actively increase intracellular and extracellular HCO₃⁻ concentrations to prevent plasma and tissue acidosis, a process that takes place over a time-scale of hours to days depending on the level of acidification experienced [29]. Consequently, the presence of diel CO₂ cycles could mean that elevated CO₂ levels are not experienced for long

enough to allow complete acid–base regulation to occur. This could potentially result in more energy being available for other processes and warrants further investigation.

This study shows that parental effects are not required to improve juvenile fish growth under elevated CO₂ if diel CO₂ cycles are present. Thus, our research adds to a growing body of literature that highlights the importance of incorporating natural CO₂ variability in OA experiments to accurately predict the responses of shallow water coastal marine species to rising CO₂ levels [16–20]. Further work is needed to determine how parental exposure modifies the responses of other marine organisms to diel-cycling elevated CO₂, especially those that are more sensitive to OA than most fishes, as a more challenging parental environment might lead to negative outcomes for offspring fitness in some species [4].

Ethics. This research was carried out following approval from the James Cook University animal ethics committee (permit no. A2210).

Data accessibility. Tropical Research Data Hub: <http://.doi.org/10.25903/5c0f3a323a749> [30].

Authors' contributions. M.D.J. and P.L.M. designed the experiment. M.D.J. carried out the experiment, data collection and analysis. M.D.J. wrote the manuscript with input from P.L.M. Both authors agree to be held accountable for the content herein and approve the final version of the manuscript.

Funding. This project was funded by the ARC Centre of Excellence for Coral Reefs Studies and an ARC Future Fellowship awarded to P.L.M.

Competing interests. We declare we have no competing interests.

Acknowledgements. We thank Ben Lawes, Simon Wever and Andrew Thompson for their technical support with the aquarium systems at JCU. We also thank Blake Spady, Donald Warren, Bridie Allan, Daniel Roberts and Leela Chakravarti for assistance with fish collection.

References

- Burgess SC, Marshall DJ. 2014 Adaptive parental effects: the importance of estimating environmental predictability and offspring fitness appropriately. *Oikos* **123**, 769–776. (doi:10.1111/oik.01235)
- Lane A, Campanati C, Dupont S, Thiyagarajan V. 2015 Trans-generational responses to low pH depend on parental gender in a calcifying tubeworm. *Sci. Rep.* **5**, 10847. (doi:10.1038/srep10847)
- Salinas S, Munch SB. 2012 Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. *Ecol. Lett.* **15**, 159–163. (doi:10.1111/j.1461-0248.2011.01721.x)
- Marshall DJ, Uller T. 2007 When is a maternal effect adaptive? *Oikos* **116**, 1957–1963. (doi:10.1111/j.2007.0030-1299.16203.x)
- Munday PL. 2014 Transgenerational acclimation of fishes to climate change and ocean acidification. *F1000 Prime Rep.* **6**, 99. (doi:10.12703/P6-99)
- Ross PM, Parker L, Byrne M. 2016 Transgenerational responses of molluscs and echinoderms to changing ocean conditions. *ICES J. Mar. Sci.* **73**, 537–539. (doi:10.1093/icesjms/fsv254)
- Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS, Duarte CM, Gattuso JP. 2013 Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Glob. Chang. Biol.* **19**, 1884–1896. (doi:10.1111/gcb.12179)
- Miller GM, Watson S-A, Donelson JM, McCormick MI, Munday PL. 2012 Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. *Nat. Clim. Chang.* **2**, 858–861. (doi:10.1038/nclimate1599)
- Murray C, Malvezzi A, Gobler C, Baumann H. 2014 Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Mar. Ecol. Prog. Ser.* **504**, 1–11. (doi:10.3354/meps10791)
- Parker LM, O'Connor WA, Raftos DA, Pörtner H, Ross PM. 2015 Persistence of positive carryover effects in the oyster, *Saccostrea glomerata*, following transgenerational exposure to ocean acidification. *PLoS ONE* **10**, e0132276. (doi:10.1371/journal.pone.0132276)
- Duarte CM, Hendriks IE, Moore TS, Olsen YS, Steckbauer A, Ramajo L, Carstensen J, Trotter JA, McCulloch M. 2013 Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuar. Coasts* **36**, 221–236. (doi:10.1007/s12237-013-9594-3)
- Wahl M, Saderne V, Sawall Y. 2016 How good are we at assessing the impact of ocean acidification in coastal systems? Limitations, omissions and strengths of commonly used experimental approaches with special emphasis on the neglected role of fluctuations. *Mar. Freshw. Res.* **67**, 25–36. (doi:10.1071/MF14154)
- Falter JL, Lowe RJ, Zhang Z, McCulloch M. 2013 Physical and biological controls on the carbonate chemistry of coral reef waters: effects of metabolism, wave forcing, sea level, and geomorphology. *PLoS ONE* **8**, e53303. (doi:10.1371/journal.pone.0053303)
- Shaw EC, McNeil BI, Tilbrook B, Matear R, Bates ML. 2013 Anthropogenic changes to seawater buffer capacity combined with natural reef metabolism induce extreme future coral reef CO₂ conditions. *Glob. Chang. Biol.* **19**, 1632–1641. (doi:10.1111/gcb.12154)
- Pacella SR, Brown CA, Waldbusser GG, Labiosa RG, Hales B. 2018 Seagrass habitat metabolism increases short-term extremes and long-term offset of CO₂ under future ocean acidification. *Proc. Natl Acad. Sci. USA* **115**, 3870–3875. (doi:10.1073/pnas.1703445115)
- Jarrold MD, Humphrey C, McCormick MI, Munday PL. 2017 Diel CO₂ cycles reduce severity of behavioural abnormalities in coral reef fish under ocean acidification. *Sci. Rep.* **7**, 10153. (doi:10.1038/s41598-017-10378-y)
- Ou M *et al.* 2015 Responses of pink salmon to CO₂-induced aquatic acidification. *Nat. Clim. Chang.* **5**, 950–955. (doi:10.1038/NCLIMATE2694)

18. Cornwall CE, Hepburn CD, McGraw CM, Currie KI, Pilditch CA, Hunter KA, Boyd PW, Hurd CL. 2013 Diurnal fluctuations in seawater pH influence the response of a calcifying macroalga to ocean acidification. *Proc. R. Soc. B* **280**, 20132201. (doi:10.1098/rspb.2013.2201)
19. Wahl M, Schneider Covachá S, Saderne V, Hiebenthal C, Müller JD, Pansch C, Sawall Y. 2018 Macroalgae may mitigate ocean acidification effects on mussel calcification by increasing pH and its fluctuations. *Limnol. Oceanogr.* **63**, 3–21. (doi:10.1002/lno.10608)
20. Mangan S, Urbina MA, Findlay HS, Wilson RW, Lewis C. 2017 Fluctuating seawater pH/pCO₂ regimes are more energetically expensive than static pH/pCO₂ levels in the mussel *Mytilus edulis*. *Proc. R. Soc. B* **284**, 20171642. (doi:10.1098/rspb.2017.1642)
21. Albright R, Langdon C, Anthony KRN. 2013 Dynamics of seawater carbonate chemistry, production, and calcification of a coral reef flat, Central Great Barrier Reef. *Biogeosciences* **10**, 6747–6758. (doi:10.5194/bg-10-6747-2013)
22. Kline DI *et al.* 2015 Six month *in situ* high-resolution carbonate chemistry and temperature study on a coral reef flat reveals asynchronous pH and temperature anomalies. *PLoS ONE* **10**, e0127648. (doi:10.1371/journal.pone.0127648)
23. R Core Team. R. 2017 *A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See <https://www.R-project.org/>.
24. Ryu T, Veilleux HD, Donelson JM, Munday PL, Ravasi T. 2018 The epigenetic landscape of transgenerational acclimation to ocean warming. *Nat. Clim. Chang.* **8**, 504–509. (doi:10.1038/s41558-018-0159-0)
25. Shama LNS, Strobel A, Mark FC, Wegner KM. 2014 Transgenerational plasticity in marine sticklebacks: maternal effects mediate impacts of a warming ocean. *Funct. Ecol.* **28**, 1482–1493. (doi:10.1111/1365-2435.12280)
26. Marshall DJ, Morgan SG. 2011 Ecological and evolutionary consequences of linked life-history stages in the sea. *Curr. Biol.* **21**, R718–R725. (doi:10.1016/j.cub.2011.08.022)
27. Pechenik JA. 2006 Larval experience and latent effects—metamorphosis is not a new beginning. *Integr. Comp. Biol.* **46**, 323–333. (doi:10.1093/icb/ijc028)
28. Pörtner HO, Knust R. 2007 Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* **315**, 95–98. (doi:10.1126/science.1135471)
29. Heuer RM, Grosell M. 2014 Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *AJP Regul. Integr. Comp. Physiol.* **307**, R1061–R1084. (doi:10.1152/ajpregu.00064.2014)
30. Jarrold M. 2018 Data from: Diel CO₂ cycles and parental effects have similar benefits to growth of a coral reef fish under ocean acidification. Tropical Research Data Hub. (doi:10.25903/5c0f3a323a749)