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Reply to Iglesias-Prieto et al.: Combined field and laboratory approaches for the study of coral calcification

Iglesias-Prieto et al. (1) present 24 h of continuous temperature, conductivity, and sea-level data recorded at a submarine spring in Mexico and argue that Crook et al. (2) attribute changes in coral calcification only to changes in aragonite saturation (Ω_{arag}) while ignoring these other parameters and their variability. On the contrary, Crook et al. (2, 3) conducted extensive monitoring of all these parameters over a 3-y period at both the spring (low pH) and control (high pH) sites (Fig. 1). Furthermore, corals were collected for the calcification study only from sites where mean temperature and salinity were closest to that of the control sites, while pH and aragonite saturation state were substantially different. These additional parameters have been considered critical to our choice of sites.

Despite the care with which our study was conducted and our results are reported, we acknowledge that it remains difficult to resolve the influence of different environmental factors on coral calcification in the field where multiple factors—all of which potentially influence calcification—covary. Indeed, in Crook et al. (2), we specifically state “One of the challenges posed by in situ field studies is that multiple environmental parameters may covary, making it difficult to resolve the influence of Ω_{arag} on calcification from that of other factors or to assess the extent to which the influence of Ω_{arag} may be modulated by other, covarying factors.” This is why combining laboratory manipulation experiments with field

work is so valuable. Specifically, in controlled experiments calcification responses to single variable manipulation can be accurately quantified. In our case, the experimental CO_2 manipulation result for *Porites astreoides* (4) is 100% consistent with our interpretation that field corals are responding to Ω_{arag} and not to temperature, salinity, or light.

To the best of our knowledge, no equivalent experimental data exist that show *P. astreoides* calcification declines with decreasing temperature or with freshening or changes in light in the absence of covariation in Ω_{arag} . Nor has there been any demonstration that the sensitivity of *P. astreoides* calcification to temperature, salinity, or light is equivalent to the changes in calcification that we measure. On the contrary, recent results from Carricart-Ganivet et al. (5) suggest that *P. astreoides* calcification increases with decreasing temperature. If the spring discharge is colder and this is driving the change in coral calcification, we would expect calcification to increase as pH decreases. This is clearly not the case.

Thus, in the absence of data to prove otherwise, our conclusions that *P. astreoides* calcification is responding to ocean acidification at this site and that calcification has not fully acclimated to chronic low saturation conditions still hold.

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- 1 Iglesias-Prieto R, Galindo-Martínez CT, Enriquez S, Carricart-Ganivet JP (2014) Attributing reductions in coral calcification to the saturation state of aragonite, comments on the effects of persistent natural acidification. *Proc Natl Acad Sci USA* 111:E300–E301.
- 2 Crook ED, Cohen AL, Rebolledo-Vieyra M, Hernandez L, Paytan A (2013) Reduced calcification and lack of acclimatization by coral colonies growing in areas of persistent natural acidification. *Proc Natl Acad Sci USA* 110(27):11044–11049.
- 3 Crook ED, Potts DC, Rebolledo-Vieyra M, Hernandez L, Paytan A (2012) Calcifying coral abundance near low-pH springs: Implications for future ocean acidification. *Coral Reefs* 31(1):239–245.
- 4 de Putron SJ, McCorkle DC, Cohen AL, Dillon AB (2011) The impact of seawater saturation state and bicarbonate ion concentration on calcification by new recruits of two Atlantic corals. *Coral Reefs* 30:321–328.
- 5 Carricart-Ganivet JP, Cabanillas-Terán N, Cruz-Ortega I, Blanchon P (2012) Sensitivity of calcification to thermal stress varies among genera of massive reef-building corals. *PLoS ONE* 7(3):e32859.

Author contributions: A.P. and E.D.C. designed research; A.P., E.D.C., A.L.C., Y.T., M.R.-V., and L.H. performed research; A.P., A.L.C., T.R.M., Y.T., M.R.-V., and L.H. contributed new reagents/analytic tools; A.P., E.D.C., A.L.C., T.R.M., Y.T., M.R.-V., and L.H. analyzed data; and A.P. and A.L.C. wrote the paper.

The authors declare no conflict of interest.

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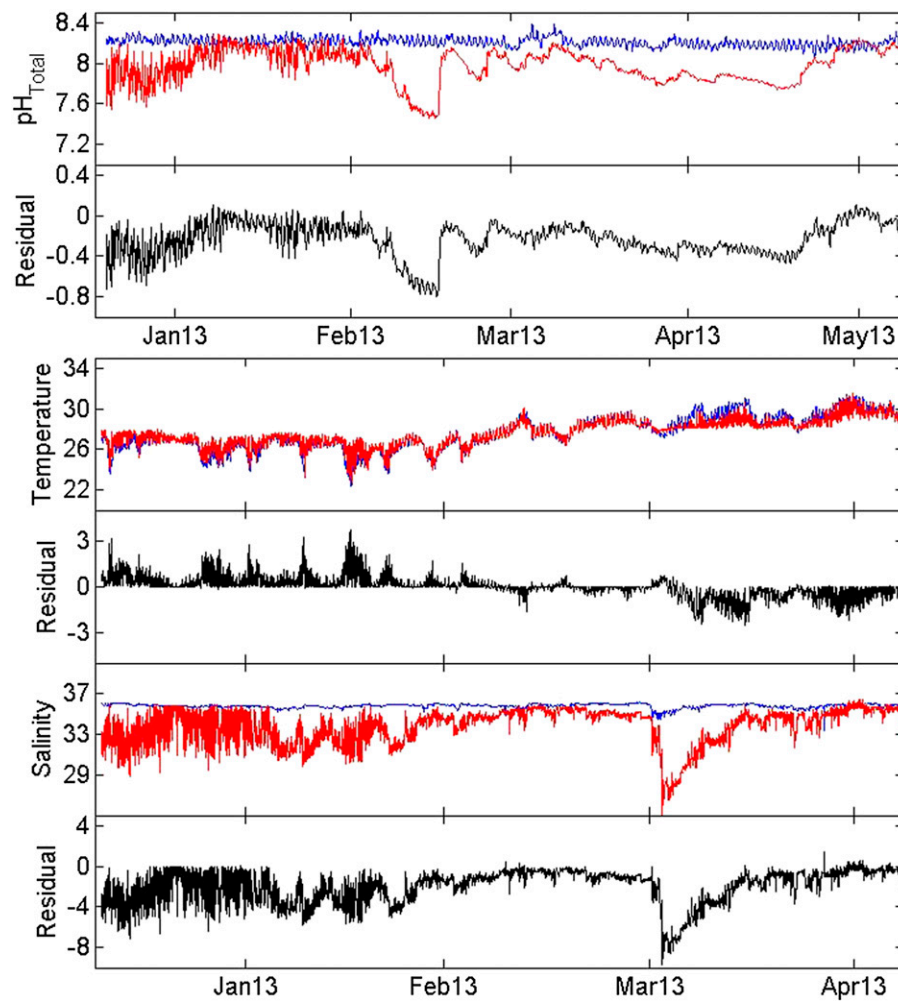


Fig. 1. pH, temperature, and salinity measurements at ojo Laja (red) and a nearby control site (blue) collected every hour over several months using in situ sensors. The temperature differences between the ojo and control are typically within 2 °C; sometimes the ojo is cooler and other times it is warmer. The salinity is >30, and pH is lower than ambient for more than 90% of the time. Using concurrent Ca, DIC, and Alk data collected frequently, the low pH values in the ojo are consistent with undersaturated conditions.