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Contribution to Special Issue: 'Towards a Broader Perspective on Ocean Acidification Research'

Comment

Calcium carbonate saturation state: on myths and this or that stories

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In a recent opinion article titled "The Omega Myth", Cyronak *et al*. provide a series of arguments as to why saturation state should not matter to marine calcifiers. In sections of their article, they highlight several aspects of our published work, and unfortunately appear to misinterpret the foundation for the kinetic–energetic hypothesis we have laid out previously. While we are in full agreement that omega sensitivity is not a substrate limitation issue, we more clearly detail below what a kinetic limitation means and why it is different from a substrate limitation. The kinetic argument we have previously presented highlights how the energetic cost of calcification increases with a decreasing saturation state (or omega). We then highlight several issues with a bicarbonate/proton flux model applied to newly developing marine bivalve larvae, and discuss how a bicarbonate/proton flux and omega-based sensitivity model do not have to be mutually exclusive. Our intent with this comment is to clarify the points raised by Cyronak *et al*. about our work, and help to move the thinking past dialectic debate towards a more synthetic view on ocean acidification impacts on marine calcifiers.

Keywords: biocalcification, bivalve larvae, ocean acidification, proton flux, saturation state.

The recent opinion piece "The Omega Myth" by Cyronak *et al.* (2016) calls to mind one of the best lessons in graduate school: "the answer to any dialectic - 'this or that' - question in complex systems is almost always YES". George Wilhelm Frederick Hegel acknowledged the powerful utility of dialectic questioning to lead to greater understanding through "hypothesis, antithesis, synthesis". Elegant in its simplicity and its lyrical feel, one hopes applying this adage to the study of global change and marine organisms will eventually lead us to higher levels of synthesis, or more simply the "YES". Dialectic debates, however, have an unfortunate proclivity for oversimplification, misunderstanding, and dogmatism; the "Omega Myth" piece by Cyronak *et al.* (2016) unfortunately slips into this trap. Myths are often based on real-world observations, magnified and dramatized by story-telling. One needs to look no further than Moby Dick, Jaws, or the Kraken to see how real-world phenomena provide the foundation for some of the best told stories of our oceans. In this instance, it is the story-telling by Cyronak *et al.* (2016), and others, that is creating "the myth" surrounding omega; not the published work we are aware of, and most certainly not ours. Here, we hope to bring back to light the kernel of truth that others have fictionalized, a basic and mechanistic omega sensitivity in bivalve larvae.

We must first clarify the foundation for our kinetic perspective on larval bivalve sensitivity to calcium carbonate saturation, denoted by the Greek omega, and defined as

$$\Omega = \frac{[Ca^{2+}][CO_3{}^{2-}]}{K'_{sp}}, \quad (1)$$

where the numerator is the product of the concentrations of calcium ($[Ca^{2+}]$) and carbonate ($[CO_3{}^{2-}]$) ions, and the denominator, K'_{sp}, is the apparent thermodynamic solubility product of the $CaCO_3$ mineral in question (typically aragonite in the studies considered here).

Cyronak *et al.* (2016) mistakenly interpret omega sensitivity of biocalcification as substrate limitation, specifically the lack of carbonate ions limits biocalcification. While such an inference is

understandable from Equation (1), this is not our description of omega sensitivity. Indeed, we will present arguments subsequently directly against such a limitation-based argument. While an omega value below 1 (for the calcium carbonate mineral in question) indicates that dissolution is *thermodynamically* favoured over precipitation, Cyronak *et al.* (2016) aptly point out that calcifiers use all forms of dissolved inorganic carbon (DIC) in producing calcium carbonate [through the use of carbonic anhydrase, see Roleda *et al.* (2012)], and this has been well documented in the literature for decades [as reviewed in McConnaughey and Gillikin (2008)] and measured in bivalve larvae as well by us (Waldbusser *et al.*, 2013). Dissolution (driven by omega in fact) is not likely to be important in developing bivalve larvae given the relatively slow, abiotic nature of dissolution, relative to the very rapid calcification event of initial shell formation. We argue that omega matters, owing to the rapid rate of calcification during the formation of the initial larval shell; omega gains relevance as a *kinetic* constraint, not a *thermodynamic* constraint. At the foundation of our kinetic argument is the rate law for calcium carbonate formation:

$$r = k(\Omega - 1)^n, \tag{2}$$

where r is the rate of calcium carbonate formation, k is the rate constant, Ω is the saturation state with respect to the form of calcium carbonate (aragonite in bivalve larvae), and n is the reaction order (assume to be 1 here). A keen reader will note the ostensible problem of this relationship as omega goes to unity, or lower. Are we suggesting such conditions preclude biocalcification? It is well documented that bivalves calcify faster than predicted from abiotic rates, and at times when ambient waters are thermodynamically unfavourable, where $\Omega < 1$ (Gazeau *et al.*, 2007; Waldbusser *et al.*, 2010, 2011). Our reply is an importance nuance to our argument, which is that the actual calcification rate observed cannot be predicted from this equation; rather, the equation describes the magnitude of the physiochemical kinetic barrier that biology must overcome to precipitate shell.

The elegance of this simple relationship between r, k, and Ω becomes apparent if we solve the equation for k, at the shell formation rates we documented previously from the two larval shell stages in Pacific oyster, *Crassostrea gigas*, larvae (Waldbusser *et al.*, 2013), for a range of Ω (Figure 1). During the initial shell formation event (called prodissoconch I) in many bivalve larvae, rapid calcification is mandated to complete the shell and allow attachment of the velum for swimming and feeding, before exhausting endogenous energy reserves [detailed in Waldbusser *et al.* (2013, 2015a, b) and references therein]. Emphasizing the need to form a complete shell, our hypothesis posits that the larvae must somehow elevate k to support the accelerated calcification rates as omega decreases, or elevate omega at the calcification site; either approach demands a biological energy subsidy to manipulate the physical chemistry. The energetic cost of such manipulation has been documented under more acidified conditions; wherein calcification or growth is diminished in many marine calcifiers, consistent with the less energy available for growth and more spent on maintenance processes (Kroeker *et al.*, 2010; Gattuso *et al.*, 2015). Our hypothesis is that maintaining accelerated rates under decreasing omega requires more energy per unit of calcium carbonate formed. Additional energy or lack thereof seems to modulate the response either through changes through ontogeny (Waldbusser *et al.*, 2010, 2013) or if more food is available in otherwise food-limiting conditions (Melzner *et al.*, 2011). However, developing bivalve larvae in the PDI stage does not typically have the luxury of slowing growth, or access to exogenous energy sources, and thus, their sensitivity to omega is acute.

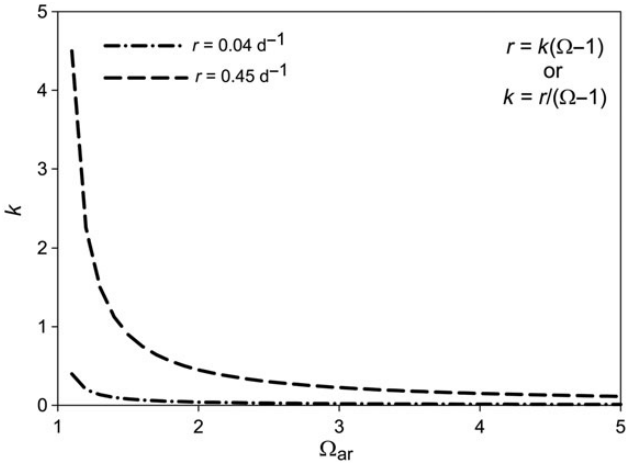

Figure 1. The computed rate constant (k) needed across a range of saturation states (Ω), at two representative calcification rates in Pacific oyster larvae, during the initial shell calcification known as prodissoconch I (PDI) (0.45 d^{-1}) and the rest of the larval shell known as prodissoconch II (0.04 d^{-1}). While this formulation does not permit calcification below the saturation horizon, it does describe the basic physical chemistry that organisms must contend with and overcome.

Where and how the organism spends energy to elevate k or omega at the calcification site is yet to be determined. We do, however, know the larger energetic cost for shell production is in protein synthesis, and not in pumping of the protons generated by calcium carbonate precipitation (Palmer, 1992; Cohen and Holcomb, 2009; Waldbusser *et al.*, 2013). Pan *et al.* (2015) recently provided more support to this argument by constraining the energetic expenses of acidification stress on sea urchin larvae and found that the greatest increase in energy spent under acidification stress was on protein synthesis, not on cross-membrane ion pumping. Alteration in the proteinaceous organic matrix that provides the framework for the calcium carbonate shell seems like one probable approach organisms can use to offset acidification stress. Additionally, the lower energetic investment in cross-membrane ion pumping, the exact mechanism for alleviating stress in a proton flux model, does not appear supported thus far by the energetic and growth relationships noted earlier. Ries (2011) proposed a physicochemical model of proton pumping in corals, and found in one species that a fixed proton gradient (between internal fluids and the external environment) model best explained the measured response. It is certainly possible that the limited up-regulation of cross-membrane ion pumps under acidification stress is due to the lack of capacity to do so, but more studies are needed along these lines to speak to the broader applicability. These observations and arguments do not preclude a proton flux stress/sensitivity in marine calcifiers; rather, they indicate that other physiological processes also appear at play to overcome this stress, and that the universal applicability/exclusivity of the proton flux model across taxa is uncertain.

Reiterating that while our omega sensitivity model is not framed in thermodynamics, but rather kinetics, we believe there is confusion arising from the fact that many of the chemical species in our kinetic model are defined in the oceans by the complex thermodynamics of carbonate chemistry. Specifically, here we refer to the

arguments centred on the $[HCO_3^-]/[H^+]$ construct. Efforts by our group and others (Jury *et al.*, 2010; Gazeau *et al.*, 2011; Waldbusser *et al.*, 2015a, b) to experimentally decouple carbonate system parameters in this debate are futile because the $[HCO_3^-]/[H^+]$ ratio is correlated with omega by the second dissociation constant of the carbonic acid system, which can be rearranged as

$$\frac{[HCO_3^-]}{H^+} = \frac{[CO_3^{2-}]}{K_2^*}, \quad (3)$$

where K_2^* is the second dissociation constant for the marine carbonate system. The rapid attainment of equilibrium for these acid–base reactions guarantees that carbonate ion concentration will always be simply proportional to the ratio of bicarbonate to proton concentrations, and it is easy to see from Equation (1) that this holds true for Ω as well in temperature- and salinity-controlled experimental systems. While it may seem like an academic argument, understanding what carbonate system parameter(s) matters most to various marine organisms is actually crucial to predicting ocean acidification (OA) impacts on marine ecosystems. In coastal zones, where many important calcium carbonate organisms reside, carbonate system parameters decouple because of changes in salinity, and thus alkalinity. For example, while the rapid global rise of atmospheric CO_2 has ensured a tight coupling of the present-day carbonate system parameters, in the geologic past, however, slower changes in CO_2 relative to ocean alkalinity resulted in a different response of carbonate system parameters to increasing CO_2 (Honisch *et al.*, 2012). Next, we will highlight five inductive lines of evidence of an omega-based sensitivity linked to a kinetic–energetic mechanism for marine calcifiers, and indicate the shortcomings of the competing proton flux/bicarbonate model where they appear. We present these arguments with the caveat that the relative importance of omega vs. $[HCO_3^-]/[H^+]$ may not always be mutually exclusive, nor does lack of applicability in one species invalidate its potential role in other species. Rather, ultimately we hope this discussion will lead to a more rational perspective across species, and to integrate these for a holistic organismal perspective within species.

First, in the formulation of the proton flux model (Jokiel, 2011), and subsequent arguments (Cyronak *et al.*, 2016; Thomsen *et al.* 2015), the authors correctly note that calcification lowers pH, as protons are generated when the basic carbonate ion is consumed into calcium carbonate. The subsequent removal of these protons from the calcification site is typically dependent on either active transport away from the calcification site (via proton pumps) or passive diffusion. In either case, mass balance requires those protons to be removed either from the fluids from which calcium carbonate is precipitate or the immediate surrounding water if pH is to be maintained or controlled at calcification surfaces. Diffusion is a generally slow process over larger time and space scales, which thus may seem to lead to proton accumulation in a larger organism. Developing bivalve (*C. gigas*) embryos are roughly 50–75 mm in diameter and have little ability to swim: they exist in low Reynolds number space, and thus, diffusion is the only way to exchange solutes. The diffusive boundary layer argument [as noted in Hurd *et al.* (2011)] has little applicability for most invertebrate larvae, as flow over a smooth surface is required to generate a diffusive boundary layer (DBL). Hurd *et al.* (2011), however, present DBL estimates for planktonic organisms, and these are all <1 mm in length, and at these spatial scales, diffusion is very rapid and effective in the transport of solutes. It is also critical to note that the original diffusion-based arguments were derived from oxygen fluxes in coral reefs and how these fluxes responded to changes in water flow over the reefs, thus altering diffusive boundary layers (Jokiel, 2011). What we stress is that the free diffusion coefficient for protons is roughly $10\times$ greater than that of other solutes, including oxygen, in marine waters (Li and Gregory, 1974), such that for the same boundary layer and reaction rate, the gradient of protons will be 10 times less steep than for other solutes (and thus build-up of protons less severe). Moreover, regarding the $[HCO_3^-]/[H^+]$ model, bicarbonate is consumed by calcification at the same rate at which protons are released; therefore, the need for excess protons to be exported is much less of a theoretical hurdle than that posed by importing bicarbonate into the organism. While internal transport of protons away from calcification sites may be important, direct measurements and modelling exercises should help to determine the scenarios under which this phenomenon may or may not matter. Given the large proton diffusion coefficient relative to other solutes and sub-millimetre diffusive layers in planktonic organisms existing in low Reynolds number conditions, we would caution against assuming that protons cannot diffuse away before becoming a problem for bivalve larvae. If the exterior environment increases the proton concentration, then this should reduce the flux; however, it will still be rapid, until there is no longer a gradient. So whether or not the proton flux will affect marine calcifiers, the only reasonable answer seems to be YES!

Second, an important component of the $[HCO_3^-]/[H^+]$ model is the role of bicarbonate in offsetting or buffering the proton accumulation (Cyronak *et al.*, 2016). Adding bicarbonate to seawater solution will only improve pH if conditions are below pH of $\sim$7.5 (or more exactly, the equivalence point of carbonic acid and carbonate ion concentration, achieved when $[H^+] = \sqrt{(K_1 \times K_2)}$, or when carbonate alkalinity and DIC are equivalent). While perhaps counterintuitive, increases in bicarbonate concentration when the system is at the alkalinity–DIC equivalence point noted above must be associated with an equivalent increase in both carbonic acid and carbonate ion concentrations, unless the solution is allowed to degas CO_2. Therefore, an increase in bicarbonate of a seawater-like fluid at pH $\sim$7.5 would mean that both P_{CO_2} and omega increase, while pH is stable!

The measurements by Thomsen *et al.* (2010) indicate the pH of the extrapallial (calcifying) fluids in their adult mussels to be slightly higher than 7.5, and DIC levels near seawater values, so adding bicarbonate will not increase pH in this case. It will, however, improve saturation state [as noted in Equation (3)]. In a series of measurements on other bivalves (including a different mussel species), Crenshaw (1972) found DIC concentrations $1.5–2\times$ seawater values, and pH between 7.3 and 7.4 in their calcifying fluids. Because of these high DIC concentrations, the resulting saturation states are above 2 in their calcifying fluids, even at these pH values. Bicarbonate accumulation in this case would *increase* pH, while omega is already in a favourable range. We have taken data from our experiments on mussel larvae (Waldbusser *et al.*, 2015a, b) to illustrate how, at least in this taxa and life history stage, a proton flux model linked to bicarbonate ion concentration is not very well supported. Again, acknowledging that Equation (3) thermodynamically binds the proton flux/bicarbonate ion model to saturation state, we show that if decomposed over a range of generally low pH (7.5–7.8), that omega explains $\sim$25% more variance in shell length than does bicarbonate (Figure 2). This enhanced explanatory power of omega increases to roughly 50% if the full range of data is used, but we

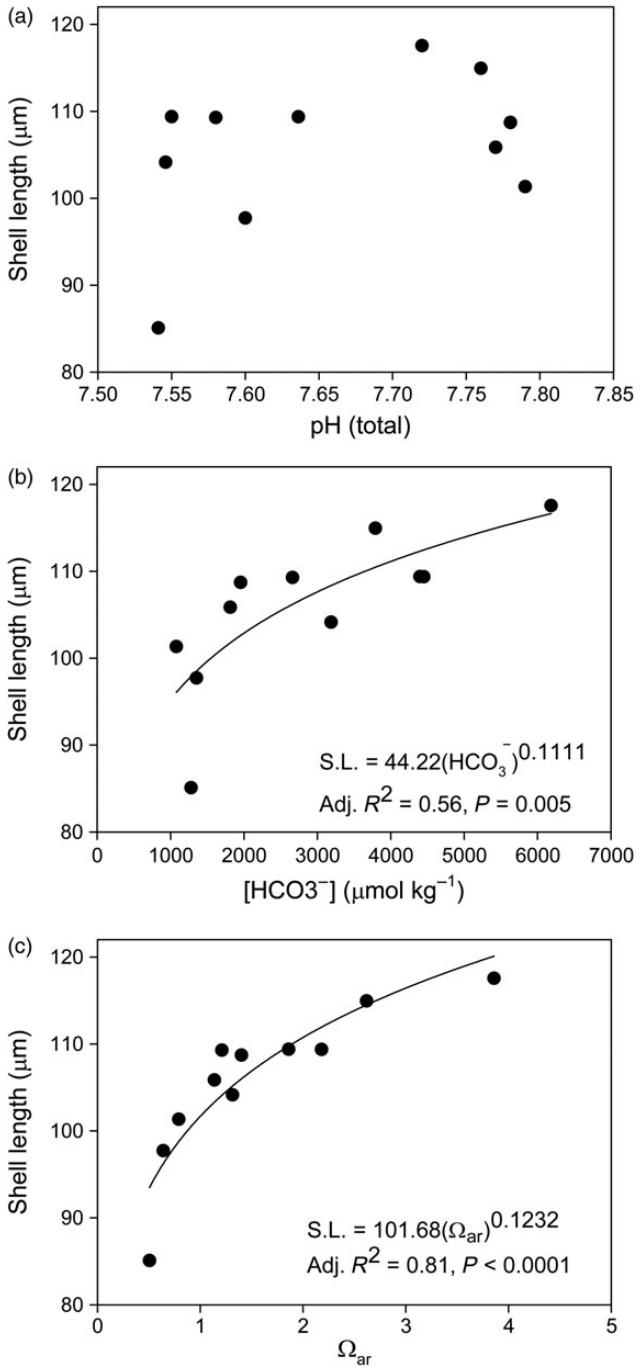

Figure 2. Data on shell length of 48-h-old *Mytilus galloprovincialis* and *Mytilus californianus* from Waldbusser *et al.* (2015a, b) plotted against (a) pH, (b) bicarbonate concentration, and (c) aragonite saturation state. Plotted are only data from pH conditions that fall between 7.5 and 7.8, pH conditions in which a proton flux-based sensitivity should be far more apparent. While bicarbonate concentration explains a significant amount of the variance in shell length across this range (supporting an increase in substrate benefit), omega explains roughly 25% more variance. Again, the thermodynamics prevents fully separating these variables. Our posit for an omega sensitivity does not preclude a bicarbonate accumulation mechanism by which omega would be increased.

chose to present the lower pH as this is when the greatest bicarbonate effect should manifest. So whether bicarbonate accumulation will be important or not to improving pH at calcification sites, we can answer YES, but only when the pH of calcifying fluids is below the critical values noted above.

Third, the previous arguments depend on the ability of marine calcifiers to isolate and control the chemistry in their calcifying fluid. Cyronak *et al.* (2016) point out that calcification occurs in isolated compartments within marine organisms, and thus environmental conditions are not identical with conditions within these calcification spaces (as has been well documented and reviewed elsewhere, and previously in our own work). The ability to isolate and control calcification fluid chemistry is not at all a generality across taxa or life stages within specific taxon. For instance, it appears that in adult bivalve mussels, there is little ability to regulate the calcifying fluids as P_{CO_2} levels increase in the surrounding waters (Thomsen *et al.*, 2010). The ability to isolate the calcifying fluids from the external environment also varies across bivalve taxa and life history stage (Crenshaw, 1972; Carriker, 1992; Waldbusser *et al.*, 2013). For bivalve larvae, it appears that the ability to isolate the calcification compartment from the external environment improves after the formation of the initial shell (Waldbusser *et al.*, 2013), but even adult bivalves are not always completely able to isolate these compartments [reviewed in Waldbusser *et al.* (2015b)]. Additionally, even in species thought to completely protect their calcified structures with soft tissue, such as corals, recent experimental work has highlighted that these calcification compartments may be more permeable to seawater than previously thought (Gagnon *et al.*, 2012; Tambutté *et al.*, 2012). So are calcification compartments in marine organisms exposed to, or isolated from, the external environment? We again answer a resounding YES!

Fourth, Cyronak *et al.* (2016) raise the spectre that perhaps shell dissolution is causing the responses we have recorded. We see clear sensitivity even when $\Omega > 1$, when dissolution would not be thermodynamically favoured. Dissolution does not explain the important observation of fully calcified, yet deformed larval shells recorded in our studies and others (Gazeau *et al.*, 2011; Thomsen *et al.*, 2015; Waldbusser *et al.*, 2015a, b). In fact, 2-d-old Pacific oyster larvae reared under corrosive conditions ($\Omega_{ar} \sim 0.5$) show severe deformities in a fully calcified shell (Waldbusser *et al.*, 2015a), rather than evidence of dissolution. Even after 4 d at saturation state of ~0.5, only very minor evidence of dissolution may be seen on the exterior of larval shells (Barton *et al.*, 2015, Figure 4). Furthermore, dissolution is typically abiotic and slow; Equation (2) can be rearranged to document dissolution as a function of saturation state by changing $(\Omega - 1)$ to $(1 - \Omega)$. Dissolution, in fact, is driven entirely by saturation state, so pH or bicarbonate concentrations will have no direct effect on dissolution; it is favoured or not in the definition of saturation state (Ω) and the rate is determined by Equation (2). Even still, would the predicted dissolution rate be able to explain the effects on the developing embryos of bivalve larvae we and others have noted? Over the course of 2 d, and during the course of the calcification event of the PDI shell, the mass balance simply cannot be satisfied by abiotic dissolution rates. It is well documented in slower calcifying organism that the interplay of dissolution and calcification will be far better balanced, and more easily tipped (and again saturation state is the driving variable for dissolution). We, therefore, contend that dissolution is likely trivial on the timescales of initial shell formation in many bivalve larvae, a day or less.

Finally, while we have used length as a proxy for calcification, the problem with shell length is that it assumes a constant relationship between shell extension, which results from organic matter addition to the shell (as periostracum), and shell thickening, which is, in fact,

the mineralization of the shell with calcium carbonate. Gaylord *et al.* (2011) found in California mussel larvae that shell area, shell thickness, and tissue mass did not all respond similarly to acidification after 8 d. Interestingly, shell thickness decreased under slightly elevated CO_2, but at higher levels did not change, whereas decreases in shell area were only significant at their highest CO_2 treatment. The largest effect they found was on tissue mass, and we would speculate that the increased demands of coping with the acidification stress are driving those responses, with dissolution likely only responsible for the differences in shell thickness. So does dissolution matter or not to marine calcifiers? We again answer YES, but it is very unlikely to be playing a major role on early shell development in bivalve larvae.

Finally, we note a striking pattern when looking at our data, or that of any other bivalve response to acidification experiments where enough data exist to document a pattern (Gazeau *et al.*, 2011; Thomsen *et al.*, 2015; Waldbusser *et al.*, 2015a, b): inflection points and threshold responses seen in measured variables almost always appear near the saturation horizon ($\Omega = 1$), and often above it. Importantly, we did just argue above that dissolution is probably of minor significance during the rapid calcification event of PDI shell formation, and we therefore believe, based on what would be predicted abiotic dissolution rates, that the omega sensitivity we see in developing bivalve larvae near (but not $=1$) is due to the energetic demand of rapid calcification in thermodynamically favourable, but kinetically challenging conditions. The ratio of $[HCO_3^-]/[H^+]$ is inextricably linked to omega, as noted above; we are, however, unaware of a mechanism that explains why the $[HCO_3^-]/[H^+]$ ratio would drive such an inflection point in larval bivalve responses. It is certainly worthy of further study to identify a mechanism that would force different species to share similar responses around a saturation state $= 1$, and perhaps these similar responses are for different reasons. So, we answer our final question whether omega or $[HCO_3^-]/[H^+]$ matters most to marine calcifiers with one final YES!

Our response to the "Omega Myth" may seem tongue in cheek; however, we believe Cyronak *et al.* (2016) fall squarely into the dialectic trap and in so doing miss the kinetic–energetic hypothesis for an omega sensitivity (Waldbusser *et al.*, 2013, 2015a, b). The vast diverse and beautiful array of marine calcifiers will likely prevent us from ever having a unified theory for calcification from ooids to otoliths, but some evident truths are present, and continued research will help further refine these and identify new hypotheses to test. To advance these truths, we must carefully define our questions; otherwise, we are left with the only rational answer to "this or that" questions, YES. The authors are correct in pointing out that an omega sensitivity linked to a substrate limitation is unfounded, and our work never laid claim to such a story. We want to be sure the omega myth based on dramatic licence is extinguished and our work is not misinterpreted. We also refrain from a naive approach to proclaim omega can explain the responses of all marine calcifiers, across all life history stages, as we have experimentally shown that ocean acidification can itself act as a multistressor on bivalve larvae (Waldbusser *et al.*, 2015b). We do strongly argue that the impact of omega (and not $[HCO_3^-]/[H^+]$) on the earliest life stages of marine bivalves is a major bottleneck for successful recruitment into adult populations, and that is one of the most imminent threats of ocean acidification to marine organisms. And therefore, as the Phoenix rises from the ashes for its rebirth, we argue the legend of (and mechanisms for) omega provides a greater understanding of how, in a more holistic sense, ocean acidification will impact marine calcifiers in an ever acidifying ocean.

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References

Barton, A., Waldbusser, G. G., Feely, R. A., Weisberg, S. B., Newton, J. A., Hales, B., Cudd, S., *et al.* 2015. Impacts of coastal acidification on the Pacific Northwest Shellfish Industry and adaptation strategies implemented in response. Oceanography, 28: 146–159.

Carriker, M. R. 1992. Prismatic shell formation in continuously isolated (*Mytilus edulis*) and periodically exposed (*Crassostrea virginica*) extrapallial spaces—Explicable by the same concept. American Malacological Bulletin, 9: 193–197.

Cohen, A. L., and Holcomb, M. 2009. Why corals care about ocean acidification: Uncovering the mechanism. Oceanography, 22: 118–127.

Crenshaw, M. A. 1972. Inorganic composition of molluscan extrapallial fluid. Biological Bulletin, 143: 506–512.

Cyronak, T., Schulz, K. G., and Jokiel, P. L. 2016. The Omega myth: What really drives lower calcification rates in an acidifying ocean. ICES Journal of Marine Science, 73: 558–562.

Gagnon, A. C., Adkins, J. F., and Erez, J. 2012. Seawater transport during coral biomineralization. Earth and Planetary Science Letters, 329–330: 150–161.

Gattuso, J. P., Magnan, A., Billé, R., Cheung, W. W. L., Howes, E. L., Joos, F., Allemand, D., *et al.* 2015. Contrasting futures for ocean and society from different anthropogenic CO_2 emissions scenarios. Science, 349. doi: 10.1126/science.aac4722.

Gaylord, B., Hill, T. M., Sanford, E., Lenz, E. A., Jacobs, L. A., Sato, K. N., Russell, A. D., *et al.* 2011. Functional impacts of ocean acidification in an ecologically critical foundation species. Journal of Experimental Biology, 214: 2586–2594.

Gazeau, F., Gattuso, J-P., Greaves, M., Elderfield, H., Peene, J., Heip, C. H. R., and Middelburg, J. J. 2011. Effect of carbonate chemistry alteration on the early embryonic development of the Pacific Oyster (*Crassostrea gigas*). PLoS ONE, 6: 23011–23018.

Gazeau, F., Quiblier, C., Jansen, J. M., Gattuso, J. P., Middelburg, J. J., and Heip, C. H. R. 2007. Impact of elevated CO_2 on shellfish calcification. Geophysical Research Letters, 34: L07603.

Honisch, B., Ridgwell, A., Schmidt, D. N., Thomas, E., Gibbs, S. J., Sluijs, A., Zeebe, R., *et al.* 2012. The geological record of ocean acidification. Science, 335: 1058–1063.

Hurd, C. L., Cornwall, C. E., Currie, K., Hepburn, C. D., McGraw, C. M., Hunter, K. A., and Boyd, P. W. 2011. Metabolically induced pH fluctuations by some coastal calcifiers exceed projected 22nd century ocean acidification: A mechanism for differential susceptibility? Global Change Biology, 17: 3254–3262.

Jokiel, P. L. 2011. Ocean acidification and control of reef coral calcification by boundary layer limitation of proton flux. Bulletin of Marine Science, 87: 639–657.

Jury, C. P., Whitehead, R. F., and Szmant, A. M. 2010. Effects of variations in carbonate chemistry on the calcification rates of *Madracis auretenra* (Madracis mirabilis sensu Wells, 1973): Bicarbonate concentrations best predict calcification rates. Global Change Biology, 16: 1632–1644.

Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecology Letters, 13: 1419–1434.

Li, Y. H., and Gregory, S. 1974. Diffusion of ions in sea water and in deep-sea sediments. Geochimica et Cosmochimica Acta, 38: 703–714.

McConnaughey, T. A., and Gillikin, D. P. 2008. Carbon isotopes in mollusk shell carbonates. Geo-Marine Letters, 28: 287–299.

Melzner, F., Stange, P., Trubenbach, K., Thomsen, J., Casties, I., Panknin, U., Gorb, S. N., *et al.* 2011. Food supply and seawater pCO(2) impact calcification and internal shell dissolution in the Blue Mussel *Mytilus edulis*. PLoS ONE, 6: e24223.

Palmer, A. R. 1992. Calcification in marine mollusks—How costly is it. Proceedings of the National Academy of Sciences of the United States of America, 89: 1379–1382.

Pan, T. C. F., Applebaum, S. L., and Manahan, D. T. 2015. Experimental ocean acidification alters the allocation of metabolic energy. Proceedings of the National Academy of Sciences, 112: 4696–4701.

Ries, J. B. 2011. A physicochemical framework for interpreting the biological calcification response to CO_2-induced ocean acidification. Geochimica et Cosmochimica Acta, 75: 4053–4064.

Roleda, M. Y., Boyd, P. W., and Hurd, C. L. 2012. Before ocean acidification: Calcifier chemistry lessons. Journal of Phycology, 48: 840–843.

Tambutté, E., Tambutté, S., Segonds, N., Zoccola, D., Venn, A., Erez, J., and Allemand, D. 2012. Calcein labelling and electrophysiology: Insights on coral tissue permeability and calcification. Proceedings of the Royal Society B: Biological Sciences, 279: 19–27.

Thomsen, J., Gutowska, M. A., Saphorster, J., Heinemann, A., Trubenbach, K., Fietzke, J., Hiebenthal, C., *et al.* 2010. Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat but are threatened by high levels of future acidification. Biogeosciences, 7: 3879–3891.

Thomsen, J., Haynert, K., Wegner, K. M., and Melzner, F. 2015. Impact of seawater carbonate chemistry on the calcification of marine bivalves. Biogeosciences Discuss, 12: 1543–1571.

Waldbusser, G. G., Bergschneider, H., and Green, M. A. 2010. Size-dependent pH effect on calcification in post-larval hard clam *Mercenaria* spp. Marine Ecological Progress Series, 417: 171–182.

Waldbusser, G. G., Brunner, E. L., Haley, B. A., Hales, B., Langdon, C. J., and Prahl, F. G. 2013. A developmental and energetic basis linking larval oyster shell formation to acidification sensitivity. Geophysical Research Letters, 40: 2171–2176.

Waldbusser, G. G., Hales, B., Langdon, C. J., Haley, B. A., Schrader, P., Brunner, E. L., Gray, M. W., *et al.* 2015a. Saturation-state sensitivity of marine bivalve larvae to ocean acidification. Nature Climate Change, 5: 273–280.

Waldbusser, G. G., Hales, B., Langdon, C. J., Haley, B. A., Schrader, P., Brunner, E. L., Gray, M. W., *et al.* 2015b. Ocean acidification has multiple modes of action on bivalve larvae. PLoS ONE, 10: e0128376.

Waldbusser, G. G., Voigt, E. P., Bergschneider, H., Green, M. A., and Newell, R. I. E. 2011. Biocalcification in the Eastern Oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. Estuaries and Coasts, 34: 221–231.

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