Ghost Factors of Laboratory Carbonate Chemistry Are Haunting Our Experiments

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Abstract. For many historical and contemporary experimental studies in marine biology, seawater carbonate chemistry remains a ghost factor, an uncontrolled, unmeasured, and often dynamic variable affecting experimental organisms or the treatments to which investigators subject them. We highlight how environmental variability, such as seasonal upwelling and biological respiration, drive variation in seawater carbonate chemistry that can influence laboratory experiments in unintended ways and introduce a signal consistent with ocean acidification. As the impacts of carbonate chemistry on biochemical pathways that underlie life, growth, development, reproduction, and behavior become better understood, the hidden effects of this previously overlooked variable need to be acknowledged. Here we bring this emerging challenge to the attention of the wider community of experimental biologists who rely on access to organisms and water from marine and estuarine laboratories and who may benefit from explicit considerations of a growing literature on the pervasive effects of aquatic carbonate chemistry changes.

Introduction

The recognition of ocean acidification (OA) and associated changes in seawater carbonate chemistry as a threat to marine ecosystems has motivated more than a decade of experimental work aimed at elucidating the effects of changing carbon-
and can interact with changing carbonate chemistry. While we recognize the complexity imparted by a suite of interacting water chemistry variables, our purpose here is to bring attention to the issue of uncontrolled carbonate system variables as ghost factors affecting experimental marine research. We (the authors) have all experienced challenges controlling seawater carbonate chemistry in our laboratory experiments, even when biological response to OA is a primary objective of the research. Our goal is to encourage biologists and ecologists who are not already thinking about seawater carbonate chemistry to consider how this suite of variables could affect both current research and interpretation of past experiments, and to embrace this variability as an opportunity in future work. While our discussion mainly focuses on the marine systems in which we work, we note the broader relevance of this issue. Acidification resulting from human activities affects water types on the continuum from marine to estuarine and freshwater (Phillips et al., 2015).

**Drivers of Laboratory Carbonate Chemistry**

In coastal ecosystems, carbonate system chemistry can vary sharply across both space and time. Spatial variation can occur at the scale of millimeters, for example, in diffusive boundary layers (Noisette and Hurd, 2018) to the scale of kilometers or more, while temporal variation can occur over scales of minutes to decades (Takeshita et al., 2015). Two major processes (and many minor processes) contribute to this variation. First, biological processes can drive variation in coastal estuarine carbonate chemistry (Lowe et al., 2019). Photosynthesis (which consumes CO₂) and respiration (which produces CO₂) can cause large oscillations in carbonate system variables over the day-night cycle (e.g., Silbiger and Sorte, 2018). Similarly, seasonal algal blooms and the subsequent decay of algal biomass can cause sharp seasonal declines in pH (Wallace et al., 2014). Second, physical factors controlling the mixing or stratification of coastal waters, such as temperature and upwelling, bring low-pH, high-alkalinity (Xue and Cai, 2020). Importantly, these processes act to modify the expression of the global OA signal in coastal waters, often amplifying the extremes of exposure on top of the secular trend of CO₂ enrichment. Where measured, many, if not most, coastal ecosystems worldwide exhibit seasonal and finer-scale variations in carbonate chemistry that easily exceed the range of values associated with the change in surface open ocean conditions from the pre-industrial levels of 280 ppm to the present-day 415-ppm CO₂ in the atmosphere (Yu et al., 2011; Carstensen and Duarte, 2019).

For experimental biologists, the wide spatial and temporal variation in carbonate chemistry presents a key challenge in marine laboratories. Depending on the laboratory, the day, or even the time of day, one may be conducting studies that differ substantively in pH or other aspects of carbonate chemistry that cross thresholds for biological responses. Not knowing whether studies are conducted at opposite ends of hidden functional response curves or reaction norms can introduce uncertainties that dampen the ability to resolve dynamics of interest.

Unintended carbonate chemistry modifications can inadvertently be imposed on marine biological experiments that are conducted at field stations as a result of external and internal factors that affect seawater chemistry (Fig. 1). Most experiments conducted at coastal field stations involve a stationary intake pipe providing seawater to an entire laboratory. The pipe draws in whatever water flows by that place at that moment, independent of the physical and biological processes that have influenced the chemistry of that water. Once water is drawn into the seawater system, respiration continues and may even increase as a result of biofouling and temperature affects in the laboratory. Photosynthesis may or may not continue, depending on the setting. Often, seawater from the system is held in secondary containers for extended periods of time, potentially intensifying these effects. Thus, the mechanics of seawater delivery frequently cause seawater carbonate chemistry to deviate substantially from natural conditions. Except in dedicated OA experiments, the carbonate chemistry of the seawater that ends up in experimental chambers is rarely measured or adjusted. This is especially true when the treatment variable of interest is, for example, temperature, light, nutrients, or biological interactions, such as competition and predation. As a consequence, investigators may not be aware of seawater carbonate chemistry conditions that could influence experimental results and challenge replicability.

**Biological Context: Marine Laboratories Are Hotspots for Biological Discovery**

The main reason to conduct laboratory studies at field stations is for access to wild organisms as close to their natural context as possible—including the environmental variation they experience. For this reason, among many others, marine laboratories have always been, and remain, vital resources for all of biology, not just explicitly marine science. Biologists owe a deep and continuing debt to field stations for a staggering breadth of discovery, ranging from the basics of fertilization, development, and cytology in the nineteenth century, through the central discoveries of neurophysiology and cell physiology in the mid-twentieth century, to the cytoskeleton, molecular motors, and green fluorescent protein (GFP) in living memory—all
seemingly far removed from climate change and its biological consequences. Yet it is not hard to imagine how such research might be skewed by water quality, whether to alter outcomes or to impede replication.

Consider a story told about T. H. Morgan, whose fame as the leading *Drosophila* exponent only eclipsed his first reputation as an experimental embryologist. Like most embryologists in his time, Morgan worked primarily with marine animals. Among other topics, he studied fertilization in solitary ascidians, hermaphrodites that deploy self-incompatibility mechanisms of various efficacy. One day, he had before him several dishes, each containing both eggs and sperm dissected from one individual *Styela* (an ascidian). Into one dish he squirted juice from a lemon otherwise meant for his tea, with the effect that acidification overcame the block to selfing. Whether the lemon juice got into the watch-glass accidentally or on purpose varies in the telling (Morgan, 1942; Sturtevant, 2001; A. Whiteley [University of Washington], pers. comm. to GvD). The question implied is, how often have we, in effect, failed to notice that stray drop of lemon juice?
The story of Morgan’s lemon is a leaf taken from more than a century’s literature on fertilization biology that relies heavily on field station laboratory studies. It is hard to overstate the importance of fertilization biology to ecology, biogeography, and life-history evolution, in addition to its obvious relevance for development. It is also clear how quantitative traits like fertilization success, parthenogenesis, or hybridization rates, all of which are key determinants of incipient speciation barriers, might plausibly be sensitive to OA-like excursions or associated parameters. For a hypothetical example, the ctenophore *Beroë* is reported to be naturally polyspermic (Yatsu, 1912; Carré and Sardet, 1984). This observation, which is succeeded by highly credible descriptions of nuclear migration and electrical responses reported by skilled observers (Carré and Sardet, 1984; Goudeau and Goudeau, 1993), anecdotally seems difficult to reproduce (GvD, unpubl. data). Is the original observation doubtful? Almost certainly not. Instead, perhaps ctenophore egg physiology is sensitive to seawater chemistry such that only under exceptional circumstances (e.g., a marine lab emplaced hardly a mile from the abyss) do investigators have access to suitable water for these organisms to perform as they do in nature. Indeed, Yatsu’s (1912) paper, reporting this pioneering but almost-forgotten embryologist’s visit to the Stazione Zoologica in Naples to study cell division in *Beroë*, includes a pointed quote:

As to experimentation, I wish to lay especial stress upon the following points. Great care was taken to secure good water quite far from the shore. The water taken near the city of Naples was so polluted that it was unfit for use . . . This is the indispensable condition for ctenophore experiments. The high mortality in Driechs and Morgan’s work [the same lemon-wielding Morgan] seems to have been due to the neglect of this precaution. (Yatsu, 1912, pp. 1–2)

Another family of examples can be found in developmental plasticity, regeneration, and cloning by echinoderm larvae. These phenomena, which are linked mechanistically by indeterminacy of cell fate in echinoderm planktotrophic larvae, are profoundly significant to theories of life-history evolution and adaptation. Strathmann’s landmark demonstration that urchin plutei alter developmental investment in larval versus juvenile structures in response to food regime (Strathmann et al., 1992) has been repeated by many others (e.g., Heyland and Hodin, 2004), with clear parallels emerging in OA-inspired studies on development under acidification (Byrne et al., 2013). Similarly, larval regeneration and cloning are now well documented in planktotrophic larvae representing four echinoderm classes (Bosch et al., 1989; Jaekle, 1994; Balser, 1998; Vickery et al., 2002; Eaves and Palmer, 2003). Clones routinely arise at low frequency in lab cultures of larvae, without apparent induction (Vickery and McClintock, 2000; Eaves and Palmer, 2003; Allen et al., 2019). Since cloning appears prevalent in some natural plankton (notably, the Gulf Stream: Bosch et al., 2003; Knott et al., 2003), it seems implausible that cloning in lab culture is merely an artifact (e.g., an aberrant outcome of wound-induced regeneration). Yet a definite inducer remains elusive; some lab culture studies relate cloning frequency to food quality or abundance (Vickery and McClintock, 2000; Allen et al., 2019) or predator cues (Vaughn and Strathmann, 2008), but at least one study shows that OA-like conditions induce buds resembling incipient cloning events (Chan et al., 2013). That seawater chemistry demonstrably modifies responses, whether to mimic or suppress relevant physiological pathways, implies the need for inclusion in experimental studies.

**Flushing the Pipes: What We Can Do Moving Forward**

Despite the widespread and increasing understanding of the importance of OA on organisms and ecosystems, it remains underappreciated as an underlying environmental influence on biological processes. Both historically and currently, foundational work on development, physiology, and behavior of marine organisms is undertaken at marine laboratories where OA has been and is currently operating as a ghost factor in experiments. This underlying influence has changed over time, in part due to accelerating anthropogenic changes in climate and land use that affect the coastal and estuarine carbonate chemistry (Carstensen and Duarte, 2019) where marine laboratories are located. We encourage even biologists who are not actively pursuing OA research to account for the effects of carbonate chemistry in laboratory seawater systems. Seawater carbonate chemistry should be considered, measured, and controlled in laboratory studies as a critical, yet often overlooked, environmental factor. In closing, we offer a few constructive recommendations.

*Monitor, describe, or control lab water chemistry*

Awareness of carbonate chemistry as a factor should motivate marine laboratories and users to adopt new practices suitable to their aims. At the simple end of a spectrum, we urge monitoring and reporting key parameters for the sake of replication. Everyone already does this for temperature by using a cheap thermometer; increasingly affordable solutions include installing automated sensors (at the station level) or monitoring chemistry with benchtop salinity and pH probes (at the end-user level). Samples can be collected and analyzed in-house, following established best practices in Dickson et al. (2007); or, for more demanding needs, they can be sent for analysis by other laboratories.

Next, experimenters ought to consider intervening to limit unwanted variation. These interventions may involve only simple actions, such as providing aeration to equilibrate water to atmospheric CO2 concentrations or adjusting the timing of experiments to avoid predictable excursions in ambient conditions (e.g., avoiding seasonal blooms or estuarine input). More
focused applications might need to include carbonate chemistry variables as controlled factors in experimental design. We recognize, though, that measuring and controlling these variables can be both challenging and expensive. Depending on the setting, productive collaborations can be forged between experimentalists and carbonate chemists to reduce costs and transfer expertise. Such collaborations have allowed OA science to train a new generation of experimental biologists who are well versed in measuring and manipulating carbonate chemistry.

We need to think carefully about those not-so-obvious experimental conditions that can influence our results. For example, depending on their position in an experimental chamber, individuals may be exposed to millimeter-scale boundary layer effects that cause significant variation in carbonate chemistry. Other potentially insidious factors include the introduction of naive animals into treatment conditions (Suckling et al., 2014) and unintended variation in densities (or biomass) of organisms between treatments, which can affect respiration and cause changes in seawater alkalinity for calcifying organisms (Mos et al., 2016; Suckling et al., 2020).

Finally, any factor that shapes lab outcomes implies a priority to validate results from laboratory studies with relevant evidence from natural populations wherever possible—that is, establishing field cognates for observed effects. As always, this is a powerful adjunct to experimental research and lends confidence to interpretations.

**Critically evaluate earlier work for unknown variation in carbonate chemistry parameters**

Scientists give each other little credit for repeating classic results or replicating others’ work. However, the realization of a ghost factor in historical experiments implies that many experimental studies might have been either conducted near reaction norm extrema, resulting in misleadingly large effects, or subject to background variation along an unknown reaction norm, misleadingly obscuring real effects.

**Embrace the variation and put it to work**

The growing number of studies that intentionally address variability inherent in seawater carbonate chemistry (Murray et al., 2014; Kapsenberg and Hofmann, 2016) suggest the rich information that is contained in this hidden variation. Experimental science relies on willing subjects: some organisms readily take to laboratory environments, hence the heavy reliance on, say, famously robust sea urchins instead of more sensitive echinoderms in developmental biology. Yet laboratory intolerance might be a clue to the very responses that reveal causes at the physiological level (as in the Morgan anecdote) or that shape large-scale outcomes at the ecological level. More broadly, plasticity reflects an adaptive genome, and response variation reflects population- to clade-level genetic diversity. As we seek to explain, predict, and shape systems-level responses to environmental change, biologists may now be ready to cope with variability in both environmental parameters and physiological responses, rather than continuing to depend on the predictable uniformity of inbred lines, hyper-robust model organisms, and, ideally (or presumably), stable lab conditions.

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**Literature Cited**

Allen, J. D., E. L. Richardson, D. Deaker, A. Agüera, and M. Byrne. 2019. Larval cloning in the crown-of-thorns sea star, a keystone coral predator. *Mar. Ecol. Prog. Ser.* 609: 271–276.

Baber, E. J. 1998. Cloning by ophiuroid echinoderm larvae. *Biol. Bull.* 194: 187–193.

Bosch, L., R. B. Rivkin, and S. P. Alexander. 1989. Asexual reproduction by oceanic planktotrophic echinoderm larvae. *Nature* 337: 169.

Byrne, M., M. Lamare, D. Winter, S. A. Dworjanyn, and S. Uthicke. 2013. The stunting effect of a high CO2 ocean on calcification and development in sea urchin larvae, a synthesis from the tropics to the poles. *Philos. Trans. R. Soc. B Biol. Sci.* 368: 20120439.

Campbell, J. E., and J. W. Fourquarean. 2014. Ocean acidification out-weighs nutrient effects in structuring seagrass epiphyte communities. *J. Ecol.* 102: 730–737.

Carré, D., and C. Sardet. 1984. Fertilization and early development in *Beroe ovata*. *Dev. Biol.* 105: 188–195.

Carstensen, J., and C. M. Duarte. 2019. Drivers of pH variability in coastal ecosystems. *Environ. Sci. Technol.* 53: 4020–4029.

Chan, K. Y. K., D. Grünbaum, M. Arnberg, M. Thorndyke, and S. T. Dupont. 2013. Ocean acidification induces budding in larval sea urchins. *Mar. Biol.* 160: 2129–2135.

Clark, T. D., G. D. Raby, D. G. Roche, S. A. Binning, B. Speers-Roesch, F. Jutfelt, and J. Sundin. 2020. Ocean acidification does not impair the behaviour of coral reef fishes. *Nature* 577: 370–375.

Clements, J. C., and H. L. Hunt. 2015. Marine animal behaviour in a high CO2 ocean. *Mar. Ecol. Prog. Ser.* 536: 259–279.

Dickson, A. G., C. L. Sabine, and J. R. Christian. 2007. *Guide to Best Practices for Ocean CO2 Measurements*. PICES Spec. Publ. 3. North Pacific Marine Science Organization, Sidney, British Columbia, Canada.

Eaves, A. A., and A. R. Palmer. 2003. Reproduction: widespread cloning in echinoderm larvae. *Nature* 425: 146.

Fabry, V. J., B. A. Seibel, R. A. Feely, and J. C. Orr. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J. Mar. Sci.* 65: 414–432.
Frieder, C. A., S. L. Applebaum, T.-C. Francis Pan, and D. T. Manahan. 2018. Shifting balance of protein synthesis and degradation sets a threshold for larval growth under environmental stress. * Biol. Bull. 234:45–57.

Fu, F. X., A. O. Tatters, and D. A. Hutchins. 2012. Global change and the future of harmful algal blooms in the ocean. *Mar. Ecol. Prog. Ser.* 470:207–233.

Goudeau, M., and H. Goudeau. 1993. Successive electrical responses to insemination and concurrent sperm entries in the poly spermic egg of the ctenophore *Beroe ovata*. *Dev. Biol.* 156:537–551.

Harris, K. E., M. D. DeGrandpre, and B. Hales. 2013. *A. W. E. GALLOWAY*.

Heyland, A., and J. Hodin. 2004. Heterochronic developmental shift caused by thyroid hormone in larval sand dollars and its implications for phenotypic plasticity and the evolution of nonfeeding development. *Evolution* 58:524–538.

Ilyina, T., R. E. Zeebe, and P. G. Brewer. 2010. Future ocean increasingly transparent to low-frequency sound owing to carbon dioxide emissions. *Nat. Geosci.* 3:18–22.

Jaeckle, W. B. 1994. Multiple modes of asexual reproduction by tropical and subtropical sea star larvae: an unusual adaptation for genet dispersal and survival. *Biol. Bull.* 186:62–71.

Kapsenberg, L., and G. E. Hofmann. 2016. Ocean pH time-series and drivers of variability along the northern Channel Islands, California, USA. *Limnol. Oceanogr.* 61:953–968.

Knott, K. E., E. J. Balser, W. B. Jaeckle, and G. A. Wray. 2003. Identification of asteroid genera with species capable of larval cloning. *Biol. Bull.* 204:246–255.

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

Lowe, A. T., J. Bos, and J. Ruesink. 2019. Ecosystem metabolism drives pH variability and modulates long-term ocean acidification in the Northeast Pacific coastal ocean. *Sci. Rep.* 9:1–11.

Morgan, T. H. 1942. Do spermatozoa penetrate the membrane of self-inseminated eggs of *Ciona* and *Sytella*? *Biol. Bull.* 82:455–460.

Mos, B., M. Byrne, and S. A. Dworanjan. 2016. Biogenic acidification reduces sea urchin gonad growth and increases susceptibility of aquaculture to ocean acidification. *Mar. Environ. Res.* 113:39–48.

Murray, C. S., A. Malvezzi, C. J. Gobler, and H. Baumann. 2014. Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Mar. Ecol. Prog. Ser.* 504:1–11.

Nagelkerken, I., and P. L. Munday. 2016. Animal behaviour shapes the ecological effects of ocean acidification and warming: moving from individual to community-level responses. *Glob. Change Biol.* 22:974–989.

Noisette, F., and C. Hurd. 2018. Abiotic and biotic interactions in the diffusive boundary layer of kelp blades create a potential refuge from ocean acidification. *Funct. Ecol.* 32:1329–1342.

Padilla-Gamín, J. L., J. D. Gaitán-Espitia, M. W. Kelly, and G. E. Hofmann. 2016. Physiological plasticity and local adaptation to elevated pCO2 in calcareous algae: an ontogenetic and geographic approach. *Evol. Appl.* 9:1043–1053.

Phillips, J. C., G. A. McKinley, V. Bennington, H. A. Bootsma, D. J. Pilcher, R. W. Sterner, and N. R. Urban. 2015. The potential for CO2-induced acidification in freshwater: a Great Lakes case study. *Oceanography* 28:136–145.

Raven, J. A., C. J. Gobler, and P. J. Hansen. 2020. Dynamic CO2 and pH levels in coastal, estuarine, and inland waters: theoretical and observed effects on harmful algal blooms. *Harmful Algae* 91:101594.

Rossi, T., I. Nagelkerken, J. C. A. Pistevos, and S. D. Connell. 2016. Lost at sea: ocean acidification undermines larval fish orientation via altered hearing and marine soundscape modification. *Biol. Lett.* 12:20150937.

Sibigler, N. J., and C. J. B. Sorte. 2018. Biophysical feedbacks mediate carbonate chemistry in coastal ecosystems across spatiotemporal gradients. *Sci. Rep.* 8:796.

Strathmann, R. R., L. Fenaux, and M. F. Strathmann. 1992. Heterochronic developmental plasticity in larval sea urchins and its implications for evolution of nonfeeding larvae. *Evolution* 46:972–986.

Sturtevant, A. H. 2001. *Reminiscences of TH Morgan. Genetics* 159:1–5.

Suckling, C. C., M. S. Clark, L. S. Peck, and E. J. Cook. 2014. Experimental influence of pH on the early life-stages of sea urchins. I. Different rates of introduction give rise to different responses. *Invertebr. Reprod. Dev.* 58:148–159.

Suckling, C. C., M. V. Cazachur, J. E. Ellis, and A. J. Davies. 2020. European sea urchin somatic and gonadal responses to differing stocking densities and seawater flow rates: a case study for experimental design considerations. *J. Shellfish Res.* 39:159–171.

Takeshita, Y., C. A. Frieder, T. R. Martz, J. R. Ballard, R. A. Feely, S. Kram, S. Nam, M. O. Navarro, N. N. Price, and J. E. Smith. 2015. Including high-frequency variability in coastal ocean acidification projections. *Biogeosciences* 12:5853–5870.

Vaughn, D., and R. R. Strathmann. 2008. Predators induce cloning in echinoderm larvae. *Science* 319:1503–1503.

Vickery, M. S., and J. B. McClintock. 2000. Effects of food concentration and availability on the incidence of cloning in planktotrophic larvae of the sea star *Pisaster ochraceus*. *Biol. Bull.* 199:298–304.

Vickery, M. S., M. C. L. Vickery, and J. B. McClintock. 2002. Morphogenesis and organogenesis in the regenerating planktotrophic larvae of asteroids and echinoids. *Biol. Bull.* 203:121–133.

Wallace, R. B., H. Baumann, J. S. Grear, R. C. Aller, and C. J. Gobler. 2014. Coastal ocean acidification: the other eutrophication problem. *Estuar. Coast. Shelf Sci.* 148:1–13.

Xue, L., and W.-J. Cai. 2020. Total alkalinity minus dissolved inorganic carbon as a proxy for deciphering ocean acidification mechanisms. *Mar. Chem.* 222:103791.

Yatsu, N. 1912. Observations and experiments on the ctenophore egg. I. The structure of the egg and experiments on cell division. *J. Coll. Sci. Imp. Univ. Tokyo* 32:1–21.

Yu, P. C., P. G. Matson, T. R. Martz, and G. E. Hofmann. 2011. The ocean acidification seascape and its relationship to the performance of calcifying marine invertebrates: laboratory experiments on the development of urchin larvae framed by environmentally-relevant pCO2/pH. *J. Exp. Mar. Biol. Ecol.* 400:288–295.