

Right place, wrong time: mixture toxicity effects of pathogens and HABs on bivalve recruitment.

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Anthropogenic activities such as global shipping and aquaculture have spread many pathogens and harmful algae to such an extent that they are considered cosmopolitan species. Combined with coastal eutrophication and overfishing, this has led to a dramatic increase in the size, frequency and severity of harmful algal blooms (HABs) across the globe. The resulting food-web disruption, biodiversity loss and socio-economic costs are unprecedented. Through hypoxia, physical agitation, starvation and the production of potent marine toxins, HABs may lead to mass mortalities of marine life at all trophic levels. Yet, despite many years of research, the nutrient fluxes and phytoplankton dynamics that cause a harmful algal bloom - as well as the full extent of their environmental impact - is not fully understood. If we are to safeguard our marine ecosystems and all of its ecosystem services, this knowledge needs to become available for the successful management of HABs.

Nearly all wild and cultured bivalve populations experience periodical episodes of mass mortality. Rather than a single stressor, these dramatic events are often caused by an interplay of abiotic and biotic pressures. These episodes could become more frequent as pathogens and harmful algal blooms (HABs) - both long known to contribute considerably to these events - are expected to benefit from climate change. Moreover, it was recently discovered that pathogens like *Vibrio* spp. benefit from the copious amounts of organic carbon released by HAB species. Regardless of their co-occurrence, to date science has failed to address the potential for mixture toxicity/adverse effects between both stressors. Specifically, nothing is known on these potential effects on the earliest, most sensitive life stages of bivalves. In this study we addressed the question whether this negligence leads to a substantial underestimation of the effect of harmful algae on bivalve recruitment. More specifically, we studied whether potentially harmful dinoflagellates and *Vibrio* spp. may synergistically affect the development, viability and immunological resilience of blue mussel (*Mytilus edulis*) larvae.

Fertilized eggs of *M. edulis* were exposed to environmentally relevant concentrations of six dinoflagellates (*Alexandrium minutum*, *A. ostenfeldii*, *Karenia mikimotoi*, *Protoceratium reticulatum*, *Prorocentrum cordatum*, *P. lima* and *P. micans*) or a known pathogen (*Vibrio coralliilyticus* or *V. splendidus*) or both. After 5 days of exposure, the larval development, larval viability and phenoloxidase innate immune response of the exposed mussel larvae were measured. Significant adverse effects were found for all algae (except *P. cordatum*) and *V. splendidus*. The phenoloxidase immune response was also found to react in a species-dependent manner to the presence of both the algae and a pathogen. Yet, regardless of their individual effects, no mixture toxicity/adverse effect was observed. This can be attributed to a difference in timing of each stressors impact: dinoflagellates were found to produce their adverse effect sooner rather than later - often completing their effect within the first 48 hours of the test. The pathogen *V. splendidus*, on the other hand, only became toxic when the larvae started to feed at the end of the five 5 days exposure period. Understanding the effects of algae, might be sufficient to successfully manage the effects of HABs on bivalve recruitment.

To safeguard bivalve stocks, however, similar studies should still be undertaken on adult bivalves which filter both the toxic phytoplankton and bacteria alike.