

Viral ecology comes of age

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Just over a quarter century ago, two teams of microbial ecologists observed an unusually large number of ‘virus-like particles’, or VLPs, in lake and marine environments – thousands to millions of times higher than previous findings (Bergh *et al.*, 1989; Proctor and Fuhrman, 1990). Subsequent observations confirmed this central finding and demonstrated that the majority of these VLPs were viruses resulting from the (largely) lysis of microbial cells. It follows then that viruses could have the *potential* to significantly influence the structure and function of natural ecosystems. Yet, given technical barriers to measurement, the viral ecologists – whose seminal work launched the field – had relatively few observations upon which to base *quantitative* estimates of viral effects. Instead, early efforts required extrapolation from a few observations of density, diversity and induced mortality to establish global estimates of impact (Suttle, 2005). With time, these extrapolations became paradigms (Breitbart, 2012). But now, nearly three decades after these initial findings, the viral ecology toolkit is transformed (Brum and Sullivan, 2015) and its paradigms are ripe for re-assessment.

Looking into our *Crystal Ball* reveals that by 2020, the viral ecology field will have shifted from predominantly ‘specialists’ to more broadly include ‘non-specialists’. This will occur both collaboratively, but also through long-standing protocol development efforts – enabling measurements of viruses and their influence to be routinely integrated into all studies of environmental microbiology. The confluence of advances in sequencing technology and community-supported bioinformatics platforms will make it feasible to more broadly measure microbial ecosystem properties. Engagement with models and modelers will help disentangle the ecologically relevant signals to better understand how microbial viruses impact ecosystems. The blueprint for the former is already playing out in the democratization of virus ecology specific community tools and database

development (Wommack *et al.*, 2011; Roux *et al.*, 2014; Bolduc *et al.*, 2016; Kindler *et al.*, 2016). Similarly, collective efforts to quantitatively model virus–microbe interactions at scales from molecules to ecosystems have provided the foundation for tighter integration between empiricists and modelers (Weitz, 2015). With this broader interest in the role viruses play in diverse microbial ecosystems and expanded resources from which to study these impacts, our *Crystal Ball* reveals the revision of major paradigms in viral ecology.

The first paradigm to be revised is that viruses are 10-fold greater in abundance than microbes. Already, re-analysis of decades of virus and microbial abundance data find that virus–host ratios vary widely and are not well-described by a 10:1 ratio (Knowles *et al.*, 2016; Parikka *et al.*, 2016; Wigington *et al.*, 2016). The interpretation, cause and consequence of this variability remains uncertain. Our *Crystal Ball* suggests that the field will move beyond enumerating ‘total viruses’ by developing and leveraging new technologies to resolve lineage-specific virus–host interactions at the community level. For double-stranded DNA phage of microbes at least, genomics is already being embraced as the foundation for viral taxonomy (Simmonds *et al.*, 2016) and elucidating viral populations in natural samples (Brum and Sullivan, 2015), as well as connecting viruses and hosts at community scales (Edwards *et al.*, 2016; Roux *et al.*, 2016). The picture is far less simple for giant dsDNA viruses (Wilhelm *et al.*, 2016), as well as ssDNA and RNA viruses, and our *Crystal Ball* remains cloudy here. Where possible, however, such lineage-specific virus–host measurements will (i) help elucidate mechanisms driving total virus-to-microbe-ratios, (ii) present microbial ecologists the opportunity to study viruses specific to focal microbes and (iii) inspire collaborations between theorists and empiricists that will guide experiments towards sampling schemes that will better inform ecosystem models.

The second paradigm to be revised is that viruses are ‘the most diverse biological entities’ on the planet. This paradigm has its roots in back-of-the-envelope calculations that the global ‘virosphere’ contains some 2 billion proteins – a number extrapolated from a handful of phage genomes (Rohwer, 2003). However, viruses may not be the most diverse biological entities at either the level of proteins or organisms. First, it seems that many proteins must repeatedly occur within virus genomes as

large 'collector's curves' from global ocean metagenomics datasets approach saturation at just a few million proteins (Ignacio-Espinoza *et al.*, 2013; Brum *et al.*, 2015). At present, sparse sampling limits extrapolation to estimate the size of the global virosphere to speculation. However, glimpses of the scope of diversity can be seen in comparing the few million ocean viral proteins observed (Ignacio-Espinoza *et al.*, 2013; Brum *et al.*, 2015) to the tens of millions of proteins observed for the prokaryotes in many of these same samples (Sunagawa *et al.*, 2015). Second, viruses appear less diverse at the organismal level. Though viral taxonomy is challenging, collectors curves from the *Tara* Oceans expedition suggest that the number of 'species' (95% average nucleotide identity populations approximate 'species', (Gregory *et al.*, in press)) in surface ocean viral communities, at least for double stranded DNA viruses, approach saturation at ~15k (Roux *et al.*, 2016), which is lower than the ~37k and >150k observed or estimated for prokaryotes (Sunagawa *et al.*, 2015) and eukaryotes (de Vargas *et al.*, 2015) respectively, in many of the same waters. A major challenge remains that we do not yet capture all viruses in such surveys, but at least for assemblable, double stranded DNA viruses the global 'catalog' is becoming clearer. Thus our *Crystal Ball* reveals that as experts across vast organismal scales and theoretical backgrounds increasingly collaborate to sample and interpret viral biodiversity, including environments beyond marine systems (Paez-Espino *et al.*, 2016), such efforts will ultimately reveal whether or not viral diversity rivals that of prokaryotes and eukaryotes.

The third paradigm to be revised is that viral lysis results in reducing cells from particulate organic matter to dissolved organic matter that is rapidly recycled. Briefly, the impact of this 'viral shunt' paradigm (Wilhelm and Suttle, 1999) is that viruses keep organic matter small, which would reduce both food supply to larger eukaryotes, including fish stocks, and carbon flux from the surface to deep oceans. A complementary scenario proposed over a decade ago is that sticky viral lysates could lead to aggregation that increases carbon flux to the deep sea (Weinbauer, 2004) – something akin to a 'viral shuttle'. Data is now emerging to directly test the viral shunt/shuttle paradigms. For example, genetic surveys and modeling outputs from the *Tara* Oceans expedition suggest that viruses, more so than any other prokaryotic or eukaryotic organisms surveyed, best predict global ocean carbon flux (Guidi *et al.*, 2016). Thus new questions are raised as to whether viruses are 'shunting' and/or 'shuttling' organic carbon when lysing their hosts, and how this might change across ecosystems? Our *Crystal Ball* suggests that new capabilities and analysis techniques will resolve such questions and better define ecosystem 'roles' for viruses.

In summary, our *Crystal Ball* suggests that viral ecology tools to studying viruses will become standard in the microbial ecology 'toolkit' and, in doing so, virus data will finally be at the scale so that viruses can be meaningfully integrated into predictive ecosystem models. This will help elucidate how viruses influence living systems – from humans to the global biosphere. Along the way the study of viral ecology will continue to identify wonders unimagined and, over time, reveal them as widespread reality.

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