

From fish to frogs and beyond: Impact and host range of emergent ranaviruses

Stephen J. Price^{a,b}, Ellen Ariel^c, Alicia MacLaine^c, Gonçalo M. Rosa^{b,d}, Matthew J. Gray^e,
Jesse L. Brunner^f, Trenton W.J. Garner^{b,*}

^a UCL Genetics Institute, Gower Street, London WC1E 6BT, UK

^b Institute of Zoology, Zoological Society of London, Regent's Park, NW1 4RY London, UK

^c College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, 4811 QLD, Australia

^d Department of Biology, University of Nevada, Reno, NV 89557, USA

^e Center for Wildlife Health, University of Tennessee Institute of Agriculture, Knoxville, TN 37996, USA

^f School of Biological Sciences, Washington State University, Pullman, WA 99164, USA

ARTICLE INFO

Keywords:

Host range

Ranavirus

Emerging pathogen

Disease

ABSTRACT

Ranaviruses are pathogens of ectothermic vertebrates, including amphibians. We reviewed patterns of host range and virulence of ranaviruses in the context of virus genotype and postulate that patterns reflect significant variation in the historical and current host range of three groups of *Ranavirus*: FV3-like, CMTV-like and ATV-like ranaviruses. Our synthesis supports previous hypotheses about host range and jumps: FV3s are amphibian specialists, while ATVs are predominantly fish specialists that switched once to caudate amphibians. The most recent common ancestor of CMTV-like ranaviruses and FV3-like forms appears to have infected amphibians but CMTV-like ranaviruses may circulate in both amphibian and fish communities independently. While these hypotheses are speculative, we hope that ongoing efforts to describe ranavirus genetics, increased surveillance of host species and targeted experimental assays of susceptibility to infection and/or disease will facilitate better tests of the importance of hypothetical evolutionary drivers of ranavirus virulence and host range.

1. Background

Ranaviruses are large double-stranded DNA viruses of the family *Iridoviridae*, which infect amphibians, reptiles and fish (Duffus et al., 2015). They are considered important emergent pathogens and several lines of evidence point to humans playing a significant role in emergence: 1) disease outbreaks have occurred frequently in cultured amphibians and fish (Zhang et al., 2001), 2) ranavirus has been detected frequently infecting invasive populations of non-native species and traded animals in Europe, Asia and South America (Une et al., 2009; Sharifian-Fard et al., 2011; Soto-Azat et al., 2016) and 3) translocations by humans are thought to have facilitated range expansion on at least two continents (Picco and Collins, 2008; Price et al., 2016). The broad host range and role of people in emergence were key reasons behind the World Organisation for Animal Health's (OIE) decision to list ranaviruses as notifiable pathogens of amphibians (Schloegel et al., 2010) and fish (OIE, 2016). However, the ecological impacts of emergent ranaviruses on their ectothermic hosts, at the levels of individuals and populations, are highly variable. In some cases, emergent disease causes extensive mortality and drives host

populations into rapid demographic decline that can persist over multiple host generations. Alternatively, persistent disease dynamics may not be associated with observable population decline, and asymptomatic infections can also occur at high prevalence. Why host responses exhibit such variation, even within single host species is a subject of much debate and research effort.

Several ecological factors have been identified as correlates of mortality events. These factors can be broadly classified as: 1) age-dependent and host-specific susceptibility; 2) abiotic drivers, and; 3) host abundance and/or density. The difficulty with attribution is that many of these factors manifest coincidentally (Brunner et al., 2015). For example, outbreaks of *Epizootic haematopoietic necrosis virus* (EHNV) in red-finned perch (*Perca fluviatilis*) are age-specific and seasonal (Whittington et al., 2010), and mass mortality of adult common frogs may be linked to aggregation during breeding (Cunningham et al., 1996; Price et al., 2016). Attempting to completely disentangle the role of each factor is challenging due to interactions; temperature, for instance, can directly affect the outcome of ranavirus infections but also affects key aspects of the ecology of ectothermic vertebrates (Brunner et al., 2015).

* Corresponding author.

E-mail address: trent.garner@ioz.ac.uk (T.W.J. Garner).

What is less explored is how systematic and pairwise variation in ranavirus genomes correlates with patterns of epidemic disease dynamics. However, research on *Frog virus 3* (FV3) has illustrated how the disease process is regulated by viral gene expression, ranaviruses evolve rapidly in single host species populations, and they recombine (Abrams et al., 2013; Epstein and Storfer, 2016; Price, 2016; Claytor et al., 2017). These findings, combined with evidence of heritable variation in host immunity to ranaviruses (Teacher et al., 2009; Echaubard et al., 2016), suggest that the emergence of epidemic disease dynamics (or lack thereof) must be governed to some degree by the genetic tools available to emergent ranaviruses. The specifics of gene conservation, expression and virulence are covered elsewhere, including in articles in this special issue. Here we review reports of ranavirus infection and epidemics in North and Central America, the region where ranavirus has been studied most intensively, and Europe, a region where a recently described ranavirus lineage is the cause of emerging disease, in the context of the phylogenetic identity of the causative ranaviruses. Where possible, we explore the relationships between ecological conditions and viral identity and comment on relative host ranges.

For our purposes, we will use terminology based on the phylogenetic analyses published in Jancovich et al. (2015), which identified four to five distinct *Ranavirus* lineages, but we will focus on the three associated with amphibians (frequently termed “amphibian-like ranaviruses” but hereon referred to as amphibian-associated ranaviruses): FV3-like ranaviruses, their sister group the common midwife toad virus (CMTV)-like variants, and the more basal *Ambystoma tigrinum* virus (ATV)-like group. These first two are monophyletic groups but for the purposes of this article we expand the ATV-like group to include all fish-associated forms at the base of the amphibian-like ranavirus phylogeny (Ariel et al., 2016; Subramaniam et al., 2016) which results in a paraphyletic group (Fig. 1). While there is significant variation encapsulated within these lineages (Echaubard et al., 2014), the deeper divisions described by these groups likely represent evolutionary steps

that involved changes in host range germane to our topic (Jancovich et al., 2010; Abrams et al., 2013). As more complete ranavirus genomes become available, we expect that ranavirus taxonomy and systematics will undergo further revision.

2. FV3-like ranaviruses in American herpetofauna

Fifty years after the serendipitous discovery of FV3 in the United States, FV3-like ranaviruses continue to cause mortality across the planet in wild and captive amphibians, chelonians, fish and squamate reptiles (Granoff et al., 1965; Duffus et al., 2015). The early work on FV3 presaged several interesting aspects of the biology of this virus. First, while experiments with this and closely related ranaviruses were often lethal to larval, and to a lesser extent adult amphibians, some individuals survived with persistent, asymptomatic infections (Clark et al., 1968, 1969; Tweedell and Granoff, 1968; Wolf et al., 1968). Second, the first FV3-like ranaviruses were isolated from animals purchased from biological suppliers (Clark et al., 1969), although there are few details of their particular origins (see Granoff et al., 1965). Third, it became clear, at least from cell culture experiments, that FV3 and related viruses have very broad host ranges (Granoff et al., 1966; Clark et al., 1968). Each of these patterns has been upheld in the five decades since.

Ranaviruses have been detected in amphibians across the United States and Canada (Fig. 2; Duffus et al., 2015). The vast majority of ranavirus detections have been FV3-like ranaviruses associated with mortality events, especially in larval amphibians (Green et al., 2002; Miller et al., 2011; Duffus et al., 2015). North American FV3-like ranaviruses have thus developed a reputation for high virulence, which has been supported by laboratory infection experiments (e.g., Pearman and Garner, 2005; Schock et al., 2008; Echaubard et al., 2016), though the outcome of exposure varies a great deal with host phylogeny and life history correlates, and virus genotype (Hoverman et al., 2010, 2011). Whilst episodic and recurrent mass mortality events attributed

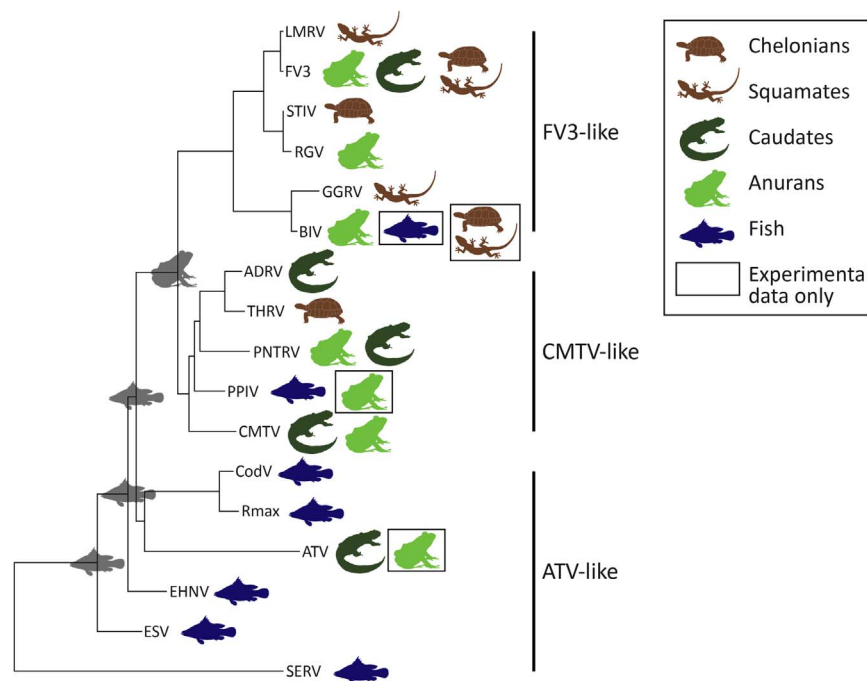


Fig. 1. Phylogenetic perspective on host range of ranaviruses in the context of broad virus type (*Frog virus* (FV)3-like, common midwife toad virus (CMTV)-like, *Ambystoma tigrinum* virus (ATV)-like). Putative ancestral hosts (fish or amphibian) are denoted by gray host images at nodes. Host ranges of individual isolates serve as a guide only and do not control for observer effort. Hosts are summarised based on membership of five higher order taxonomic groups: chelonian, squamate, caudate, anuran and fish. The overall topology of the tree follows Fig. 3 of Stohr et al. (2007), which was simplified by removing tips for clarity of presentation. Isolate abbreviations: SERV, short-finned eel ranavirus; ESV, European sheatfish virus; EHNV, Epizootic haematopoietic necrosis virus; ATV, *Ambystoma tigrinum* virus; Rmax, Ranavirus maximus; CodV, Cod iridovirus; CMTV, common midwife toad virus; PPIV, pike-perch iridovirus; PNTRV, Portuguese newt and toad ranavirus; THRV, *Testudo hermanni* ranavirus; ADRV, *Andrias davidianus* ranavirus; BIV, Bohle iridovirus; GGRV, German gecko ranavirus; RGV, *Rana grylio* iridovirus; STIV, Soft-shelled turtle iridovirus; FV3, *Frog virus 3*; LMRV, *Lacerta monticola* ranavirus.

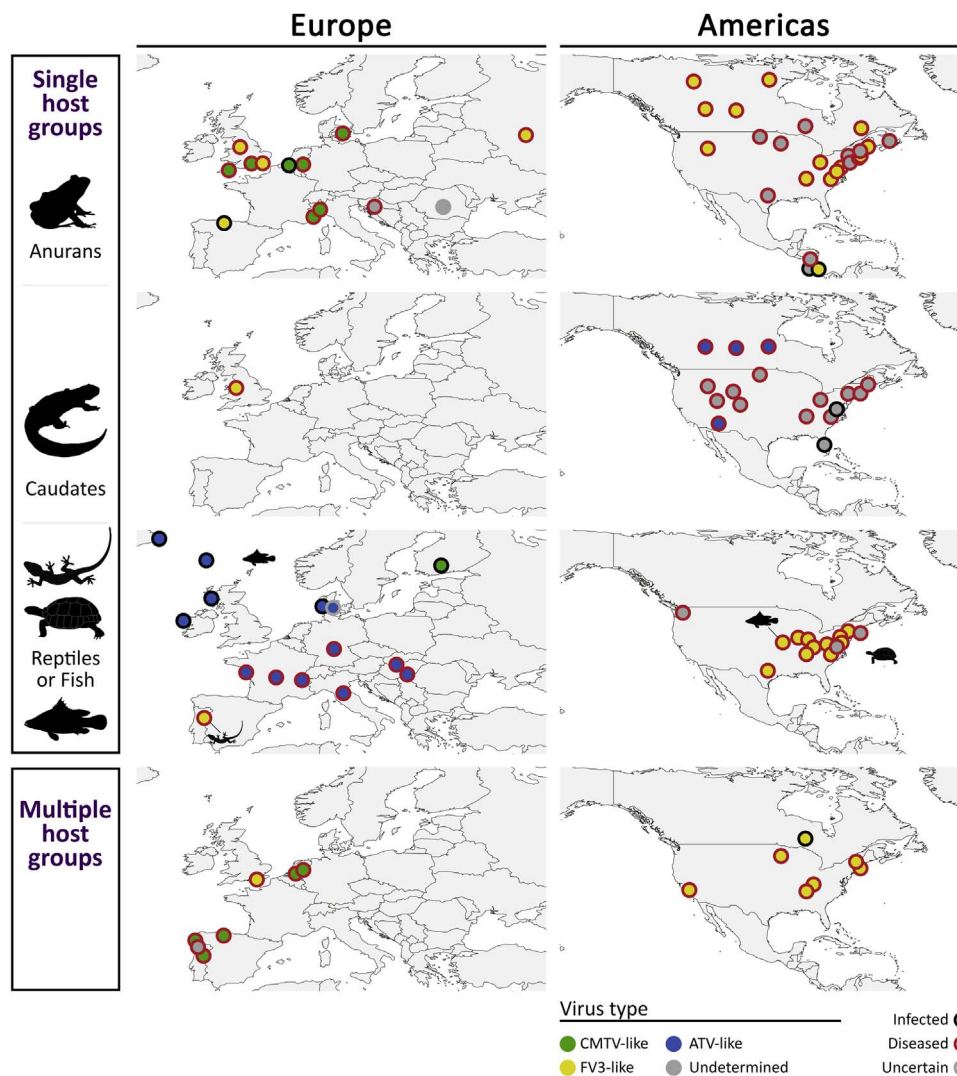


Fig. 2. Host range, distribution and impact of the major groups of amphibian-associated ranavirus in Europe and the Americas. Points mark approximate locations and sometimes represent multiple neighbouring incidents at a local scale which share the same attributes but could not be represented clearly as individual points at the continental scale used here. Datasets were generated by extending data tables published by Duffus et al. (2015).

to FV3-like ranaviruses have been noted in numerous amphibian species across North America and in all post-hatching life history stages, no clear case of population decline has been reported (Brunner et al., 2015). In addition, improved diagnostic methods and increased attention may explain the growing number of cases of FV3-like ranaviruses detected in wild populations in the absence of disease or notable mortality events (e.g., Crespi et al., 2015; O'Connor et al., 2016). Caution is warranted when interpreting an absence of reports of declines associated with ranavirosis, as lack of disease detection could be a consequence of sampling design (Gray et al., 2015). Outbreaks of ranavirosis and mortality events can last for less than two weeks and may go unobserved when monitoring is infrequent (Wheelwright et al., 2014; Hall et al., 2016). Few longitudinal studies at sites with recurring outbreaks have been conducted in North America (Brunner et al., 2015; Gray et al., 2015). Simulations indicate that local extinction of amphibian populations, especially isolated ones, is possible for both common and rare North American amphibian species (Earl and Gray, 2014; Earl et al., 2016).

Wild and captive chelonians and squamate reptiles infected with FV3-like viruses have also experienced lethal ranavirosis (Belzer and Seiber, 2011; Duffus et al., 2015; Kimble et al., 2015). Serological and PCR data in chelonians in North America reflect low incidence of infection which are short-lived because of high virulence (Johnson

et al., 2010; Allender et al., 2013a, 2013b) or because they are quickly cleared (Brunner et al., 2015). Ranavirus infections in chelonians are likely, at least initially, the result of spill over from amphibian mortality events (e.g., Belzer and Seiber, 2011). Similarly, ranavirus infection in wild North American fish attributable to an FV3-like variant are rare, and one appears to have arisen through intimate contact with infected amphibians (Mao et al., 1999; Waltzek et al., 2014). Overall it appears that FV3-like ranaviruses predominantly exploit amphibian hosts in North America, can spill over into other vertebrate classes (Brenes et al., 2014), but may not be sustained when spill-over occurs.

Evidence is starting to accrue that FV3-like ranaviruses infect an incredibly broad range of ectothermic hosts in the rest of the Americas (Fox et al., 2006; Whitfield et al., 2013; Stark et al., 2014). For example, surveys of adult, Costa Rican amphibians report relatively low prevalence (16.6%) across a wide range of amphibian species but no mortality events have been detected (Whitfield et al., 2013). Conversely, the only record from Nicaragua is of a mortality event affecting amphibian larvae of several species (Stark et al., 2014). Preliminary molecular data shows high sequence identity to FV3 (Whitfield et al., 2012), but for both countries it is still unclear whether FV3-like ranaviruses are endemic or introduced.

Experimental data and case studies of captive and cultured ectotherms indicate that what is observed in the wild represents a

subset of available North American hosts susceptible to infection (and in many cases lethal ranavirosis) caused by FV3-like ranaviruses (Johnson et al., 2007; Miller et al., 2007; Hoverman et al., 2011; Waltzek et al., 2014). A lack of monitoring or infrequent sampling are likely to bias data on host and geographic ranges of ranaviruses, but host ecology and environmental conditions may also limit opportunity for transmission between hosts. Experimental data has also indicated that FV3-like ranaviruses are not equivalent (Hoverman et al., 2011; Duffus et al., 2014a); they may develop non-overlapping host specificities though environmental conditions will sometimes overwhelm the signature of host specialisation (Duffus et al., 2014a; Echaubard et al., 2014; Brand et al., 2016).

3. ATV-like ranaviruses in North American ambystomatid salamanders

FV3-like ranaviruses share North America with ATV-like forms (Fig. 2), initially described as the cause of recurrent and annual mass mortality of larval tiger salamanders (Jancovich et al., 1997; Docherty et al., 2003). While these mortality events can be catastrophic, affecting an entire year class, ATV epidemics do not always lead to notable mortality (Greer et al., 2009) and ATV has not been clearly linked to population declines (Brunner et al., 2004). As with FV3-like viruses, host populations vary in their resistance or tolerance to ATV infection (Schock et al., 2009) and ATV strains differ in their virulence (Brunner and Collins, 2009).

Initial experimental data supported the hypothesis that ATV is a caudate amphibian specialist however subsequent studies have shown that some anurans are susceptible to infection and lethal disease (Jancovich et al., 2001; Schock et al., 2008). Still, ATV has yet to be isolated from any hosts other than ambystomatid salamanders in the western half of North America, where they have an apparently long evolutionary history (Jancovich et al., 2005; Storfer et al., 2007; Epstein and Storfer, 2016). ATVs have yet to be shown to infect reptile or fish hosts, although sister taxa within the ATV-like group are fish pathogens (see below and Fig. 1; Jancovich et al., 2015; Stöhr et al., 2015). As with FV3-like ranaviruses, it is highly unlikely that the overall host range of ATV-like ranaviruses has been described exhaustively, but it is likely that subsequent research will continue to support a much narrower host range for North American ATVs compared to their FV3-like neighbours.

4. European fish infections due to two *Ranavirus* lineages

For four decades ranaviruses have been described infecting both marine and freshwater European fishes (Fig. 2; Jensen et al., 1979; Ahne et al., 1989; Ariel et al., 2010). Recent sequencing efforts support the conclusion that European piscine ranaviruses fall into two lineages: CMTV-like and our expanded classification of the proposed ATV-like ranaviruses (Jancovich et al., 2015; Ariel et al., 2016; Holopainen et al., 2016; Subramaniam et al., 2016). Arguably the most notable Ranavirus affecting fish is EHN, individually listed as notifiable by the OIE and the European Union (Commission Directive 2008/53/EC). Although endemic in Australian redfin perch (*Perca fluviatilis*), EHN has yet to be detected in Europe despite active surveillance by European Member and Associated States. Short-finned eel ranavirus (SERV) is another ATV-like ranavirus that appears to originate from Australasia but was opportunistically isolated once in Europe from non-native and asymptomatic eels imported from New Zealand (Bovo et al., 1999; Jensen et al., 2009). Ranavirosis in Europe was first described in marine fishes infected with an ATV-like ranavirus (Jensen et al., 1979), and subsequent surveys of aqua-cultured marine fish identified a near-identical virus infecting clinically healthy turbot fry (*Scophthalmus maximus*) and lumpfish (*Cyclopterus lumpus*) (Ariel et al., 2010, 2016; Stagg et al., 2017).

Wild European freshwater fish have experienced lethal ranavirosis

attributable to ATV-like ranaviruses, the *European catfish virus* (ECV) and European sheatfish virus (ESV), which appear to be the same virus (Ahne et al., 1989; Marsh et al., 2002; Bigarre et al., 2008; Stöhr et al., 2015; Price, 2016). Although SERV has not been detected in wild or cultured European fish populations, experimental evidence showed that European freshwater species experience high levels of mortality when exposed to this ranavirus (Jensen et al., 2009), while North American bullheads (*Ameiurus nebulosus*) do not (Gobbo et al., 2010). Experimental studies also indicate that ECV has an even broader range of European fish hosts (Gobbo et al., 2010; Jensen et al., 2009, 2011). However common frogs (*Rana temporaria*) appear resistant to ECV, EHN and SERV despite susceptibility to infection and disease caused by ranaviruses from two other lineages circulating in Europe (Bayley et al., 2013; Price et al., 2014). Although data on susceptibility of reptilian and amphibian hosts to these ATV-like ranaviruses isolated from fish is limited, none of these basal forms have been identified from either of these host groups and have therefore been postulated to be fish specialists (Ariel et al., 2016). Jancovich et al. (2010) proposed two models for ancestral host use in the amphibian-associated ranaviruses. The first involved an ancestral fish host and multiple, subsequent jumps into amphibians whilst the alternative involved an ancestral amphibian host which jumped into fish. Our synthesis supports an ancestral fish host (Jancovich et al.'s first model) and the classification of the ATV-like ranaviruses as a group of specialised fish viruses with ATV itself representing a single case of a member of the ATV-like group jumping between ectothermic host classes with subsequent adaptation and specialisation to ambystomatid salamanders (Fig. 1). In contrast, pike-perch iridovirus (PPIV) appears less phylogenetically constrained in host range. Opportunistically isolated from asymptomatic pike-perch fingerlings (*Lucio perca*), experiments have shown PPIV can infect and in some cases cause disease in other freshwater fish, but can also infect and cause lethal disease in common frogs (Tapiovaara et al., 1998; Jensen et al., 2009, 2011; Bayley et al., 2013). Whole genome sequence analysis of PPIV indicates it is CMTV-like (Holopainen et al., 2016), a clade increasingly recognized as important pathogens of amphibians and reptiles (see below).

5. Invasive FV3-like ranaviruses specialized for British common frogs

Epidemic ranavirosis affecting European amphibians that was first detected in the UK in the late 1980s (Cunningham et al., 1996) was caused by FV3-like forms that may have emerged twice in mainland Great Britain (Price et al., 2016). Ranavirus has yet to be detected on any other British islands, or on mainland Ireland (Price et al., 2016). All evidence therefore points to FV3 being recently (several decades) invasive in Britain. Although the exact route of introduction is unknown, human population density best explains the pattern of range expansion exhibited by British ranaviruses, which is a strong indication that spread is in part due to human activities (Price et al., 2016). Several aquatic vertebrate species commonly introduced to garden ponds preceding and during the emergence of UK ranavirosis, including Asian newts, North American bullfrogs, ornamental goldfish and carp, have been hypothesized as potential vectors (e.g. Hyatt et al., 2000). However, koi carp and goldfish were experimentally resistant to infection with FV3-like ranaviruses, and a ranavirus isolated from diseased koi in Asia is phylogenetically distinct from the FV3-like ranaviruses found in Great Britain (Bang Jensen et al., 2011; George et al., 2015). Ranavirus infections have yet to be described for Asian newts leaving the North American bullfrog, and North America, as a likely source of UK FV3-like ranaviruses.

North American FV3-like ranaviruses are notable for their host promiscuity, but host range of invasive British FV3-like ranaviruses appears to be comparatively narrow. FV3-like ranaviruses are responsible for mass mortality that has caused persistent local population declines that pose a conservation threat to common frogs (*R. tempor-*

aria) in England (Teacher et al., 2010). However, to date, incidents of mass mortality have predominantly affected adult common frogs with records of disease and mortality affecting other species comparatively rare. In addition, surveys of other common frog life history stages at locations where disease persists in adults have not revealed infections other than in adults (Duffus et al., 2013). Field data indicate that infection and disease in other British amphibian species is absent, geographically constrained (e.g., a single point location for the non-native and invasive common midwife toad, *Alytes obstetricans*) or in the case of the common toad (*Bufo bufo*), rare and unlikely to lead to significant levels of mortality or host population declines (Duffus et al., 2014a, 2014b). The comparatively reduced susceptibility of common toads compared to common frogs has been confirmed experimentally (Duffus et al., 2014a). Analyses of citizen science records of common frog mortality events even suggest that the presence of toads within amphibian potential host assemblages may be of benefit to frogs; whilst toads increased the likelihood of the occurrence of outbreaks of ranavirosis, their presence reduced the probability of experiencing highly virulent outcomes (North et al., 2015).

6. Community impacts of CMTV-like viruses in European amphibians

The narrow host range exhibited by British FV3-like ranaviruses stands in sharp contrast to the recent observations of lethal ranavirosis across western and central continental Europe. In the last decade, cases affecting amphibians reported in six continental European countries were consistently associated with another *Ranavirus* lineage, the CMTV-like ranaviruses. In the most extreme cases, six anuran and caudate amphibian species - from six genera and representing the complete amphibian community assemblage - have experienced simultaneous mortality events affecting larvae, juveniles and adults that caused sudden, multispecies and persistent population declines (Price et al., 2014). Although not all recent emergences of severe CMTV-like ranavirosis on the continent have so comprehensively affected host communities (Fig. 2), collectively they represent an enormously broad amphibian host range and are consistently causing mass mortality affecting multiple life history stages (Price et al., 2014; Miaud et al., 2016; Rijks et al., 2016; Rosa et al., 2017). When host range is combined from reports of emergent and lethal CMTV-like ranaviruses on the European continent it encompasses 15 amphibian species in 10 genera, or nearly 20% of European amphibian biodiversity recognized by the IUCN, and a squamate reptile (Temple and Cox, 2009; Price et al., 2014; Miaud et al., 2016; Rijks et al., 2016; Rosa et al., 2017), which serves to reinforce the emerging view of CMTV-like viruses as important pathogens with extremely broad host ranges.

There is a strong likelihood that additional mortality events caused by CMTV-like ranaviruses are going unreported. Mortality of Danish *Pelophylax esculentus* involving hundreds of frogs likely only came to the attention of the academic community because of a targeted appeal through the media and local environmental organisations (Ariel et al., 2009). Amphibians from Italy and Switzerland, countries that have yet to report ranavirosis caused by CMTV-like variants affecting wild amphibians, collected for research objectives unrelated to infectious diseases developed severe ranavirosis and experienced mass-mortality in captivity (Holopainen et al., 2009; Stohr et al., 2013). Captive conditions may have affected the ability to tolerate infection in these cases but it is perhaps more likely that these mortality events in captivity are symptomatic of more widespread but as yet unobserved epidemic disease outbreaks in the wild due to CMTV-like viruses. Underreporting aside, the preponderance of evidence indicates that CMTV-like variants are invasive in Continental European amphibian communities.

Incidents of mass die-offs in Spain, France, Portugal and the Netherlands are all recent, and were previously unreported by amphibian monitoring programmes active before the die-offs occurred and

that detected the initial outbreaks. Phylogenetic analyses of CMTV-like ranaviruses responsible for these events consistently yield shallow clades exhibiting little or no variation among isolates derived from mixed host species assemblages and multiple sites within the respective regions (Price et al., 2014; Rijks et al., 2016; Rosa et al., 2017). These patterns are consistent with recent introduction events involving viruses with broad host range at time of introduction. The increased frequency of genes with evidence of positive selection in the genome of the type CMTV from Spain relative to other isolates of *Ranavirus* could also be interpreted as consistent with this model of invasion following recent introduction (Price, 2016). FV3-like ranaviruses are circulating on the Continent in both amphibians and reptiles and, in at least two systems, exhibit overlap with the distribution of CMTV-like ranaviruses, but are relatively rare (Price et al., 2014; Stöhr et al., 2015; Rosa et al., 2017). Assuming recent introduction of and invasion by CMTV-like variants, FV3-like ranaviruses may be undergoing replacement by invasive genotypes though recent studies suggest that overlapping range could result in 'mosaic' viruses generated by widespread recombination of two divergent genotypes (Price, 2016; Price et al., 2014; Rosa et al., 2017; Claytor et al., 2017). Given the phylogenetic relationship between CMTV-like variants affecting European herpetofauna and PPIV (Fig. 1), the recent emergence of CMTVs in European herpetofauna and the more historical evidence of asymptomatic infections in European freshwater fish species in the absence of observations of amphibian disease, it is possible that fish play an important role in the distribution of CMTV-like viruses affecting amphibians and reptiles in Europe.

7. Future directions

The list of locations and hosts from where ranavirus epidemics have been observed is growing but evidence of the age of the host-pathogen association is usually lacking. An exception is the Ambystoma-ATV system, where two lines of evidence support a very old association (Storfer et al., 2007; Epstein and Storfer, 2016). In long established systems, where host populations have engaged in a prolonged arms race with *Ranavirus*, examining host genomes for signatures of positive selection is likely to elucidate host defences which may in turn shed light on immune evasion and virulence evolution in the virus. In contrast, systems comprising asymptomatic infections occurring at low prevalence are somewhat counterintuitive to expectations of an evolutionary arms race (i.e. even where substitution rates are low, generation times of viruses are so short that they are expected to easily outpace their host), and may be explained by poor detection success and in other cases by a failure to identify the primary host. Ranaviruses are commonly held up as multi-host pathogens but transmission routes are generally poorly characterised in the wild and there have been few, if any, attempts to assess the relative quality of individual host species or quantify the role each plays in maintaining epidemics in the wild. We have outlined some basic geographic and host range patterns that suggest that FV3-like ranaviruses are predominantly amphibian generalists, ATV-like variants are fish specialists that in one case have host-switched to caudate amphibians, while CMTV-like ranaviruses affecting European herpetofauna may have the capacity to more routinely exploit the full breadth of the aquatic vertebrate community (Fig. 1). However, we postulate these relationships from an extremely limited evidence base and many questions remain as to how and when such systems can be maintained.

Even where active management and monitoring of populations precede observations of epidemic disease and patterns of viral diversity are suggestive of recent incursion, there is usually no direct and concrete evidence that ranaviruses were not circulating asymptotically in the affected hosts or some reservoir species prior to the onset of disease (Price et al., 2014; Rosa et al., 2017). Surveys of archived specimens might be useful in this respect but the weight of indirect evidence in some cases already points strongly to recent human

behaviour creating opportunities for host and geographic range expansion (Price et al., 2016). It's possible that this has enabled ranavirus to escape its host-pathogen coevolutionary history and left naïve populations woefully exposed to a novel or re-emerging pathogen. After all, human factors have affected the direction of ranavirus evolutionary trajectories in other ways: ranavirus contamination of aquaculture and trade appears to have presented suitable opportunities for the evolution of increased virulence as well as recombination of divergent virus types (Storfer et al., 2007; Hoverman et al., 2011; Price, 2016; Clayton et al., 2017). However, there are striking differences in, for example, the exploitation of the assumed naïve communities following incursions into Europe by FV3-like and CMTV-like viruses and it is clear that virus genotype – likely modulated through an interaction with host and environmental factors – must have played a significant role.

A paucity of data has previously limited comparative genomic studies of *Ranavirus* but the recent focus on these pathogens as important threats to diverse hosts has resulted in the application of next-generation sequencing technologies to resolve phylogenetic relationships, understand evolutionary processes, and reveal the contributions of both to the epidemiology of this group. Genome content (and perhaps also the means to manipulate it) varies among even closely related ranaviruses and this is likely to impact on key viral traits such as infectivity, virulence and host range (Price, 2016). In addition to pairwise variation, genome content also varies systematically with genome size moving in both directions. There remains uncertainty about relationships among viruses at the root of the amphibian-associated ranaviruses (Jancovich et al., 2015; Stöhr et al., 2015; Price, 2016) but it appears there has been two independent cases of genome reduction in the amphibian-associated ranaviruses; one on the terminal branch to ATV, and the other on the ancestral branch leading to the last common ancestor of FV3-like and CMTV-like ranaviruses. Genomes of the remaining ATV-like ranaviruses are approximately 7–20 kb larger than ATV itself and the other amphibian-associated ranaviruses. It is possible that this high-level genome reorganisation is mediated through the purging of duplicated genes or pseudogenes and may be associated with a shift in host range to include amphibians. Genome size can increase through the acquisition of new genes and each of the three *Ranavirus* groups we've focused on contains lineage-specific genes (Price, 2016). Ranaviruses acquire new genes through lateral gene transfer from micro-organisms and their hosts as well as gene duplication events (Filee, 2009). Perhaps unsurprisingly, some of these newly acquired genes have undergone recent positive selection, which might be associated with adaptation to new hosts following host jumps (Abrams et al., 2013). The genome of CMTV, isolated during recurrent epidemics in Spain that have resulted in collapse of amphibian communities, has also been subject to widespread positive selection (Price, 2016), further highlighting how scans for adaptive change might identify both virulence genes and lineages undergoing geographic and/or host range expansion.

Although functional annotation of ranavirus genomes is generally lacking (homology searches yield no functional information for approximately two-thirds of ranavirus open-reading frames), gene knockout methodologies continue to offer a promising approach to explore how variable loci contribute to key viral traits (Robert and Jancovich, 2016). It is also possible that detection of more subtle changes to ranavirus genomes, such as silent shifts in GC content at the third codon position (synonymous substitutions) could yield information about use of hosts or environments. Finally, experimental challenges of amphibians and reptiles with members of the *Iridoviridae* only previously associated with infection of invertebrates can also elicit infections (Weinmann et al., 2007; Marschang et al., 2016) which suggests that broadening the scope of comparative genomic studies to include viruses of other genera in the family (such as the invertebrate viruses as well as those genera that are assumed to infect fish only) may help identify genes and mechanisms of genome evolution which determine virulence and host range.

Acknowledgements

SJP and TWJG were supported by NERC grants NE/M000338/1, NE/M000591/1, and NE/M00080X/1. GMR was supported by a doctoral scholarship (SFRH/BD/69194/2010) from Fundação para a Ciência e a Tecnologia (FCT).

References

- Abrams, A.J., Cannatella, D.C., Hillis, D.M., Sawyer, S.L., 2013. Recent host-shifts in ranaviruses: signatures of positive selection in the viral genome. *J. Gen. Virol.* 94, 2082–2093. <http://dx.doi.org/10.1099/vir.0.052837-0>.
- Ahne, W., Schlotfeldt, H.J., Thomsen, L., 1989. Fish viruses: isolation of an icosahedral cytoplasmic deoxyribovirus from sheatfish (*Silurus glanis*). *Zent. Vet. Reihe B J. Vet. Med. Ser. B* 36, 333–336.
- Allender, M.C., Mitchell, M.A., McRuer, D., Christian, S., Byrd, J., 2013a. Prevalence, clinical signs, and natural history characteristics of frog virus 3-like infections in eastern box turtles (*Terrapene carolina carolina*). *Herpetol. Conserv. Biol.* 8, 308–320.
- Allender, M.C., Mitchell, M.A., Torres, T., Sekowska, J., Driskell, E.A., 2013b. Pathogenicity of Frog Virus 3-like virus in Red-eared Slider Turtles (*Trachemys scripta elegans*) at two environmental temperatures. *J. Comp. Pathol.* 149, 356–367. <http://dx.doi.org/10.1016/j.jcpa.2013.01.007>.
- Ariel, E., Kielgast, J., Svart, H.E., Larsen, K., Tapiovaara, H., Jensen, B.B., Holopainen, R., 2009. Ranavirus in wild edible frogs *Pelophylax kl. esculentus* in Denmark. *Dis. Aquat. Organ.* 85, 7–14. <http://dx.doi.org/10.3354/dao02060>.
- Ariel, E., Holopainen, R., Olesen, N.J., Tapiovaara, H., 2010. Comparative study of ranavirus isolates from cod (*Gadus morhua*) and turbot (*Psetta maxima*) with reference to other ranaviruses. *Arch. Virol.* 155, 1261–1271. <http://dx.doi.org/10.1007/s00705-010-0715-z>.
- Ariel, E., Steckler, N.K., Subramaniam, K., Olesen, N.J., Waltzek, T.B., 2016. Genomic sequencing of ranaviruses isolated from turbot (*Scophthalmus maximus*) and atlantic cod (*Gadus morhua*). *Genome Announc.* 4, <http://dx.doi.org/10.1128/genomeA.01393-16>.
- Bang Jensen, B., Reschova, S., Cinkova, K., Ariel, E., Vesely, T., 2011. Common carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) were not susceptible to challenge with ranavirus under certain conditions. *Bull. Eur. Assoc. Fish Pathol.* 31, 112–118.
- Bayley, A.E., Hill, B.J., Feist, S.W., 2013. Susceptibility of the European common frog *Rana temporaria* to a panel of ranavirus isolates from fish and amphibian hosts. *Dis. Aquat. Organ.* 103, 171–183. <http://dx.doi.org/10.3354/dao02574>.
- Belzer, W., Seiber, S., 2011. A natural history of *Ranavirus* in an eastern box turtle population. *Turt. Tortoise Newsl.* 15, 18–25.
- Bigarre, L., Cabon, J., Baud, M., Castric, J., 2008. Ranaviruses associated with high mortalities in catfish in France. *Bull. Eur. Assoc. Fish Pathol.* 28, 163–168.
- Bovo, G., Giacometti, G., Montesi, F., Cappellozza, E., Ormelli, S., 1999. Isolation of an irido-like viral agent from New Zealand eel. Presented at the Proceedings of the 9th International Conference of European Association of Fish Pathologists, Rhodes, p. 153.
- Brand, M.D., Hill, R.D., Brenes, R., Chaney, J.C., Wilkes, R.P., Grayfer, L., Miller, D.L., Gray, M.J., 2016. Water temperature affects susceptibility to *Ranavirus*. *EcoHealth* 13, 350–359. <http://dx.doi.org/10.1007/s10393-016-1120-1>.
- Brenes, R., Gray, M.J., Waltzek, T.B., Wilkes, R.P., Miller, D.L., 2014. Transmission of *Ranavirus* between ectothermic vertebrate hosts. *PLoS ONE* 9, e92476. <http://dx.doi.org/10.1371/journal.pone.0092476>.
- Brunner, J.L., Collins, J.P., 2009. Testing assumptions of the trade-off theory of the evolution of parasite virulence. *Evol. Ecol. Res.* 11, 1169–1188.
- Brunner, J.L., Schock, D.M., Davidson, E.W., Collins, J.P., 2004. Intraspecific reservoirs: complex life history and the persistence of a lethal ranavirus. *Ecology* 85, 560–566. <http://dx.doi.org/10.1890/02-0374>.
- Brunner, J.L., Storfer, A., Gray, M.J., Hoverman, J.T., 2015. *Ranavirus* ecology and evolution: from epidemiology to extinction. In: Gray, M.J., Chinchar, V.G. (Eds.), *Ranaviruses*. Springer International Publishing, 71–104. http://dx.doi.org/10.1007/978-3-319-13755-1_4.
- Clark, H.F., Brennan, J.C., Zeigel, R.F., Karzon, D.T., 1968. Isolation and characterization of viruses from the kidneys of *Rana pipiens* with renal adenocarcinoma before and after passage in the red eft (*Triturus viridescens*). *J. Virol.* 2, 629–640.
- Clark, H.F., Gray, C., Fabian, F., Zeigel, R., Karzon, D.T., 1969. Comparative studies of amphibian cytoplasmic virus strains isolated from the leopard frog, bullfrog, and newt. In: Mizell, M. (Ed.), *Biology of Amphibian Tumors, Recent Results in Cancer Research*. Springer Berlin Heidelberg, 310–326. http://dx.doi.org/10.1007/978-3-642-85791-1_26.
- Clayton, S.C., Subramaniam K., Chinchar, V.G., Gray, M.J., Miller, D.L., Salemi, M., Wisely, S.M., Waltzek, T.B., 2017. Evidence of genetic recombination between ranavirus strains in a captive American bullfrog (*Lithobates catesbeianus*) population. *Virology* (in press).
- Crespi, E.J., Rissler, L.J., Mattheus, N.M., Engbrecht, K., Duncan, S.I., Seaborn, T., Hall, E.M., Peterson, J.D., Brunner, J.L., 2015. Geophysiology of wood frogs: landscape patterns of prevalence of disease and circulating hormone concentrations across the eastern range. *Integr. Comp. Biol.* 55, 602–617. <http://dx.doi.org/10.1093/icb/icc096>.
- Cunningham, A.A., Langton, T.E.S., Bennett, P.M., Lewin, J.F., Drury, S.E.N., Gough,

- R.E., MacGregor, S.K., 1996. Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*). *Philos. Trans. R. Soc. Lond. Ser. B-Biol. Sci.* 351, 1539–1557. <http://dx.doi.org/10.1098/rstb.1996.0140>.
- Docherty, D.E., Meteyer, C.U., Wang, J., Mao, J., Case, S.T., Chinchar, V.G., 2003. Diagnostic and molecular evaluation of three iridovirus-associated salamander mortality events. *J. Wildl. Dis.* 39, 556–566. <http://dx.doi.org/10.7589/0090-3558-39.3.556>.
- Duffus, A.L.J., Nichols, R.A., Garner, T.W.J., 2013. Investigations into the life history stages of the common frog (*Rana temporaria*) affected by an amphibian ranavirus in the United Kingdom. *Herpetol. Rev.* 44, 260–263.
- Duffus, A.L.J., Nichols, R.A., Garner, T.W.J., 2014a. Experimental evidence in support of single host maintenance of a multihost pathogen. *Ecosphere* 5, 142. <http://dx.doi.org/10.1890/ES14-00074.1>.
- Duffus, A.L.J., Nichols, R.A., Garner, T.W.J., 2014b. Detection of a frog virus 3-like ranavirus in native and introduced amphibians in the United Kingdom in 2007 and 2008. *Herpetol. Rev.* 45, 608–610.
- Duffus, A.L.J., Waltzek, T.B., Stöhr, A.C., Allender, M.C., Gotesman, M., Whittington, R.J., Hick, P., Hines, M.K., Marschang, R.E., 2015. Distribution and host range of ranaviruses. In: Gray, M.J., Chinchar, V.G. (Eds.), *Ranaviruses*. Springer International Publishing, 9–57. http://dx.doi.org/10.1007/978-3-319-13755-1_2.
- Earl, J.E., Gray, M.J., 2014. Introduction of *Ranavirus* to isolated wood frog populations could cause local extinction. *EcoHealth* 11, 581–592. <http://dx.doi.org/10.1007/s10393-014-0950-y>.
- Earl, J.E., Chaney, J.C., Sutton, W.B., Lillard, C.E., Kouba, A.J., Langhorne, C., Krebs, J., Wilkes, R.P., Hill, R.D., Miller, D.L., Gray, M.J., 2016. *Ranavirus* could facilitate local extinction of rare amphibian species. *Oecologia* 182, 611–623. <http://dx.doi.org/10.1007/s00442-016-3682-6>.
- Echaubard, P., Leduc, J., Pauli, B., Chinchar, V.G., Robert, J., Lesbarrères, D., 2014. Environmental dependency of amphibian–ranavirus genotypic interactions: evolutionary perspectives on infectious diseases. *Evol. Appl.* 7, 723–733. <http://dx.doi.org/10.1111/eva.12169>.
- Echaubard, P., Pauli, B.D., Trudeau, V.L., Lesbarrères, D., 2016. Ranavirus infection in northern leopard frogs: the timing and number of exposures matter. *J. Zool.* 298, 30–36. <http://dx.doi.org/10.1111/jzo.12281>.
- Epstein, B., Storfer, A., 2016. Comparative genomics of an emerging amphibian virus. *G3 GenomesGenetics* 6 (1), 15–27. <http://dx.doi.org/10.1534/g3.115.023762>.
- Filee, J., 2009. Lateral gene transfer, lineage-specific gene expansion and the evolution of Nucleo Cytoplasmic Large DNA viruses. *J. Invertebr. Pathol.* 101, 169–171. <http://dx.doi.org/10.1016/j.jip.2009.03.010>.
- Fox, S.F., Greer, A.L., Torres-Cervantes, R., Collins, J.P., 2006. First case of ranavirus-associated morbidity and mortality in natural populations of the South American frog *Ateolagnathus patagonicus*. *Dis. Aquat. Organ.* 72, 87–92. <http://dx.doi.org/10.3354/dao072087>.
- George, M.R., John, K.R., Mansoor, M.M., Saravanakumar, R., Sundar, P., Pradeep, V., 2015. Isolation and characterization of a ranavirus from koi, *Cyprinus carpio* L., experiencing mass mortalities in India. *J. Fish Dis.* 38, 389–403. <http://dx.doi.org/10.1111/jfd.12246>.
- Gobbo, F., Cappellozza, E., Pastore, M.R., Bovo, G., 2010. Susceptibility of black bullhead *Ameiurus melas* to a panel of ranavirus isolates. *Dis. Aquat. Organ.* 90, 167–174. <http://dx.doi.org/10.3354/dao02218>.
- Granoff, A., Came, P.E., Rafferty, K.A.J., 1965. The isolation and properties of viruses from *Rana pipiens*: their possible relationship to the renal adenocarcinoma of the leopard frog. *Ann. N. Y. Acad. Sci.* 126, 237–255. <http://dx.doi.org/10.1111/j.1749-6632.1965.tb14278.x>.
- Granoff, A., Came, P.E., Breeze, D.C., 1966. Viruses and renal carcinoma of *Rana pipiens*. I. The isolation and properties of virus from normal and tumor tissue. *Virology* 29, 133–148. [http://dx.doi.org/10.1016/0042-6822\(66\)90203-0](http://dx.doi.org/10.1016/0042-6822(66)90203-0).
- Gray, M.J., Brunner, J.L., Earl, J.E., Ariel, E., 2015. Design and analysis of ranavirus studies: surveillance and assessing risk. In: Gray, M.J., Chinchar, V.G. (Eds.), *Ranaviruses*. Springer International Publishing, 209–240. http://dx.doi.org/10.1007/978-3-319-13755-1_8.
- Green, D.E., Converse, K.A., Schrader, A.K., 2002. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996–2001. *Ann. N. Y. Acad. Sci.* 969, 323–339.
- Greer, A.L., Brunner, J.L., Collins, J.P., 2009. Spatial and temporal patterns of *Ambystoma tigrinum virus* (ATV) prevalence in tiger salamanders *Ambystoma tigrinum nebulosum*. *Dis. Aquat. Organ.* 85, 1–6. <http://dx.doi.org/10.3354/dao02061>.
- Hall, E.M., Crespi, E.J., Goldberg, C.S., Brunner, J.L., 2016. Evaluating environmental DNA-based quantification of ranavirus infection in wood frog populations. *Mol. Ecol. Resour.* 16, 423–433. <http://dx.doi.org/10.1111/1755-0998.12461>.
- Holopainen, R., Ohlemeyer, S., Schütze, H., Bergmann, S.M., Tapiovaara, H., 2009. *Ranavirus* phylogeny and differentiation based on major capsid protein, DNA polymerase and neurofilament triplet H1-like protein genes. *Dis. Aquat. Organ.* 85, 81–91. <http://dx.doi.org/10.3354/dao02074>.
- Holopainen, R., Subramaniam, K., Steckler, N.K., Claytor, S.C., Ariel, E., Waltzek, T.B., 2016. Genome sequence of a ranavirus isolated from pike-perch *Sander lucioperca*. (e01295-16) *Genome Announc.* 4. <http://dx.doi.org/10.1128/genomeA.01295-16>.
- Hoverman, J.T., Gray, M.J., Miller, D.L., 2010. Anuran susceptibilities to ranaviruses: role of species identity, exposure route, and a novel virus isolate. *Dis. Aquat. Organ.* 89, 97–107. <http://dx.doi.org/10.3354/dao02200>.
- Hoverman, J.T., Gray, M.J., Haislip, N.A., Miller, D.L., 2011. Phylogeny, life history, and ecology contribute to differences in amphibian susceptibility to ranaviruses. *EcoHealth* 8, 301–319. <http://dx.doi.org/10.1007/s10393-011-0717-7>.
- Hyatt, A.D., Gould, A.R., Zupanovic, Z., Cunningham, A.A., Hengstberger, S., Whittington, R.J., Kattenbelt, J., Coupar, B.E.H., 2000. Comparative studies of piscine and amphibian iridoviruses. *Arch. Virol.* 145, 301–331. <http://dx.doi.org/10.1007/s007050050025>.
- Jancovich, J.K., Davidson, E.W., Morado, J.F., Jacobs, B.L., Collins, J.P., 1997. Isolation of a lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. *Dis. Aquat. Organ.* 31, 161–167. <http://dx.doi.org/10.3354/dao031161>.
- Jancovich, J.K., Davidson, E.W., Seiler, A., Jacobs, B.L., Collins, J.P., 2001. Transmission of the *Ambystoma tigrinum virus* to alternative hosts. *Dis. Aquat. Organ.* 46, 159–163. <http://dx.doi.org/10.3354/dao046159>.
- Jancovich, J.K., Davidson, E.W., Parameswaran, N., Mao, J., Chinchar, V.G., Collins, J.P., Jacobs, B.L., Storfer, A., 2005. Evidence for emergence of an amphibian iridoviral disease because of human-enhanced spread. *Mol. Ecol.* 14, 213–224. <http://dx.doi.org/10.1111/j.1365-294X.2004.02387.x>.
- Jancovich, J.K., Bremont, M., Touchman, J.W., Jacobs, B.L., 2010. Evidence for multiple recent host species shifts among the ranaviruses (Family Iridoviridae). *J. Virol.* 84, 2636–2647. <http://dx.doi.org/10.1128/JVI.01991-09>.
- Jancovich, J.K., Steckler, N.K., Waltzek, T.B., 2015. Ranavirus taxonomy and phylogeny. In: Gray, M.J., Chinchar, V.G. (Eds.), *Ranaviruses*. Springer International Publishing, 59–70. http://dx.doi.org/10.1007/978-3-319-13755-1_3.
- Jensen, B.B., Ersboll, A.K., Ariel, E., 2009. Susceptibility of pike *Esox lucius* to a panel of Ranavirus isolates. *Dis. Aquat. Organ.* 83, 169–179. <http://dx.doi.org/10.3354/dao02021>.
- Jensen, B.B., Holopainen, R., Tapiovaara, H., Ariel, E., 2011. Susceptibility of pike-perch *Sander lucioperca* to a panel of ranavirus isolates. *Aquaculture* 313, 24–30. <http://dx.doi.org/10.1016/j.aquaculture.2011.01.036>.
- Jensen, N.J., Bloch, B., Larsen, J.L., 1979. The ulcus-syndrome in cod (*Gadus morhua*). III. A preliminary virological report. *Nord. Vet. Med.* 31, 436–442.
- Johnson, A.J., Pessier, A.P., Jacobson, E.R., 2007. Experimental transmission and induction of ranaviral disease in Western Ornate box turtles (*Terrapene ornata ornata*) and red-eared sliders (*Trachemys scripta elegans*). *Vet. Pathol.* 44, 285–297. <http://dx.doi.org/10.1354/vp.44-3-285>.
- Johnson, A.J., Wendland, L., Norton, T.M., Belzer, B., Jacobson, E.R., 2010. Development and use of an indirect enzyme-linked immunosorbent assay for detection of iridovirus exposure in gopher tortoises (*Gopherus polyphemus*) and eastern box turtles (*Terrapene carolina carolina*). *Vet. Microbiol.* 142, 160–167.
- Kimble, S.J.A., Karna, A.K., Johnson, A.J., Hoverman, J.T., Williams, R.N., 2015. Mosquitoes as a potential vector of *Ranavirus* transmission in terrestrial turtles. *EcoHealth* 12, 334–338. <http://dx.doi.org/10.1007/s10393-014-0974-3>.
- Mao, J., Green, D.E., Fellers, G., Chinchar, V.G., 1999. Molecular characterization of iridoviruses isolated from sympatric amphibians and fish. *Virus Res.* 63, 45–52.
- Marschang, R.E., Stöhr, A.C., Papp, T., 2016. Repeated detection of an Invertebrate Iridovirus (IIV) in amphibians. *J. Herpetol. Med. Surg.* 26, 1–2. <http://dx.doi.org/10.5818/15-03-041.1>.
- Marsh, I.B., Whittington, R.J., O'Rourke, B., Hyatt, A.D., Chisholm, O., 2002. Rapid differentiation of Australian, European and American ranaviruses based on variation in major capsid protein gene sequence. *Mol. Cell. Probes* 16, 137–151. <http://dx.doi.org/10.1006/mcpr.2001.0400>.
- Miaud, C., Pozet, F., Gaudin, N.C.G., Martel, A., Pasmans, F., Labrut, S., 2016. *Ranavirus* causes mass die-offs of alpine amphibians in the southwestern alps (France). *J. Wildl. Dis.* <http://dx.doi.org/10.7589/2015-05-113>.
- Miller, D., Gray, M., Storfer, A., 2011. Ecopathology of ranaviruses infecting amphibians. *Viruses* 3, 2351–2373. <http://dx.doi.org/10.3390/v3112351>.
- Miller, D.L., Rajeev, S., Gray, M.J., Baldwin, C.A., 2007. Frog virus 3 infection, cultured American bullfrogs. *Emerg. Infect. Dis.* 13, 342–343.
- North, A.C., Hodgson, D.J., Price, S.J., Griffiths, A.G.F., 2015. Anthropogenic and ecological drivers of amphibian disease (Ranavirus). *PLoS ONE* 10, e0127037. <http://dx.doi.org/10.1371/journal.pone.0127037>.
- O'Connor, K.M., Rittenhouse, T.A.G., Brunner, J.L., 2016. Ranavirus is common in wood frog (*Lithobates sylvaticus*) tadpoles throughout Connecticut, USA. *Herpetol. Rev.* 47, 394–397.
- OIE, 2016. Aquatic Animal Health Code [WWW Document]. (<http://www.oie.int/en/international-standard-setting/aquatic-code/access-online/>) (Accessed 23 January 2017).
- Pearman, P.B., Garner, T.W.J., 2005. Susceptibility of Italian agile frog populations to an emerging strain of *Ranavirus* parallels population genetic diversity. *Ecol. Lett.* 8, 401–408. <http://dx.doi.org/10.1111/j.1461-0248.2005.00735.x>.
- Picco, A.M., Collins, J.P., 2008. Amphibian commerce as a likely source of pathogen pollution. *Conserv. Biol.* 22, 1582–1589. <http://dx.doi.org/10.1111/j.1523-1739.2008.01025.x>.
- Price, S.J., 2016. Comparative genomics of amphibian-like ranaviruses, nucleocytoplasmic large DNA viruses of poikilotherms. *Evol. Bioinform. Online* 11, 71–82. <http://dx.doi.org/10.4137/EBO.S33490>.
- Price, S.J., Garner, T.W.J., Nichols, R.A., Balloux, F., Ayres, C., Mora-Cabello de Alba, A., Bosch, J., 2014. Collapse of amphibian communities due to an introduced *Ranavirus*. *Curr. Biol.* 24, 2586–2591. <http://dx.doi.org/10.1016/j.cub.2014.09.028>.
- Price, S.J., Garner, T.W.J., Cunningham, A.A., Langton, T.E.S., Nichols, R.A., 2016. Reconstructing the emergence of a lethal infectious disease of wildlife supports a key role for spread through translocations by humans. *Proc. R. Soc. B* 283, 20160952. <http://dx.doi.org/10.1098/rspb.2016.0952>.
- Rijks, J.M., Saucedo, B., Sluijs, A.S., der, Wilkie, G.S., Asten, A.J.A.M., van, Broek, J., van den, Boonyarittichai, R., Stege, M., Sterren, F., van der, Martel, A., Pasmans, F., Hughes, J., Gröne, A., Beurden, S.J., van, Kik, M.J.L., 2016. Investigation of amphibian mortality events in wildlife reveals an on-going *Ranavirus* epidemic in the north of the Netherlands. *PLoS ONE* 11, e0157473. <http://dx.doi.org/10.1371/journal.pone.0157473>.

- Robert, J., Jancovich, J.K., 2016. Recombinant ranaviruses for studying evolution of host-pathogen interactions in ectothermic vertebrates. *Viruses* 8, E187. <http://dx.doi.org/10.3390/v8070187>.
- Rosa, G.M., Sabino-Pinto, J., Laurentino, T.G., Martel, A., Pasmans, F., Rebelo, R., Griffiths, R.A., Stöhr, A.C., Marschang, R.E., Price, S.J., Garner, T.W.J., Bosch, J., 2017. Impact of asynchronous emergence of two lethal pathogens on amphibian assemblages. *Sci. Rep.* 7, 43260. <http://dx.doi.org/10.1038/srep43260>.
- Schloegel, L.M., Daszak, P., Cunningham, A.A., Speare, R., Hill, B., 2010. Two amphibian diseases, chytridiomycosis and ranaviral disease, are now globally notifiable to the World Organization for Animal Health (OIE): an assessment. *Dis. Aquat. Organ.* 92, 101–108. <http://dx.doi.org/10.3354/dao02140>.
- Schock, D.M., Bollinger, T.K., Chinchar, V.G., Jancovich, J.K., Collins, J.P., 2008. Experimental evidence that amphibian ranaviruses are multi-host pathogens. *Copeia* 2008, 133–143. <http://dx.doi.org/10.1643/CP-06-134>.
- Schock, D.M., Bollinger, T.K., Collins, J.P., 2009. Mortality rates differ among amphibian populations exposed to three strains of a lethal ranavirus. *EcoHealth* 6, 438–448. <http://dx.doi.org/10.1007/s10393-010-0279-0>.
- Sharifian-Fard, M., Pasmans, F., Adriaenssens, C., Devisscher, S., Adriaens, T., Louette, G., Martel, A., 2011. Ranavirosis in invasive bullfrogs, Belgium. *Emerg. Infect. Dis.* 17, 2371–2372. <http://dx.doi.org/10.3201/eid1712.110236>.
- Soto-Azat, C., Peñañiel-Ricarte, A., Price, S.J., Sallaberry-Pincheira, N., García, M.P., Alvarado-Rybak, M., Cunningham, A.A., 2016. *Xenopus laevis* and emerging amphibian pathogens in Chile. *EcoHealth* 13, 775–783. <http://dx.doi.org/10.1007/s10393-016-1186-9>.
- Stagg, H.E.B., Guðmundsdóttir, S., Vendramin, N., Ruane, N., Sigurðardóttir, H., Christiansen, D.H., Cuenca Navarro, A., Petersen, P.E., Munro, E.S., Olesen, N.J., 2017. Isolation and characterisation of a new ranavirus isolated from lumpfish in the north Atlantic area. In: Proceedings of The 4th Ranavirus symposium, 7–10th of June, Budapest, Hungary.
- Stark, T., Laurijssens, C., Weterings, M., Sluijs, A.S., der, Martel, A., Pasmans, F., 2014. Death in the clouds: ranavirus associated mortality in assemblage of cloud forest amphibians in Nicaragua. *Acta Herpetol.* 9, 125–127. http://dx.doi.org/10.13128/Acta_Herpetol-13516.
- Stohr, A.C., Fleck, J., Mutschmann, F., Marschang, R.E., 2013. *Ranavirus* infection in a group of wild-caught Lake Urmia newts *Neurergus crocatus* imported from Iraq into Germany. *Dis. Aquat. Organ.* 103, 185–189. <http://dx.doi.org/10.3354/dao02556>.
- Stöhr, A.C., López-Bueno, A., Blahak, S., Caeiro, M.F., Rosa, G.M., Alves de Matos, A.P., Martel, A., Alejo, A., Marschang, R.E., 2015. Phylogeny and differentiation of reptilian and amphibian ranaviruses detected in Europe. *PLoS ONE* 10 (2), e0118633. <http://dx.doi.org/10.1371/journal.pone.0118633>.
- Storfer, A., Alfaro, M.E., Ridenhour, B.J., Jancovich, J.K., Mech, S.G., Parriss, M.J., Collins, J.P., 2007. Phylogenetic concordance analysis shows an emerging pathogen is novel and endemic. *Ecol. Lett.* 10, 1075–1083. <http://dx.doi.org/10.1111/j.1461-0248.2007.01102.x>.
- Subramaniam, K., Toffan, A., Cappellozza, E., Steckler, N.K., Olesen, N.J., Ariel, E., Waltzek, T.B., 2016. Genomic sequence of a *Ranavirus* isolated from short-finned eel (*Anguilla australis*). *Genome Announc.* 4. <http://dx.doi.org/10.1128/genomeA.00843-16>.
- Tapiovaara, H., Olesen, N.J., Lindén, J., Rimaila-Pärnänen, E., von Bonsdorff, C.H., 1998. Isolation of an iridovirus from pike-perch *Stizostedion lucioperca*. *Dis. Aquat. Organ.* 32, 185–193. <http://dx.doi.org/10.3354/dao032185>.
- Teacher, A.G.F., Garner, T.W.J., Nichols, R.A., 2009. Evidence for directional selection at a novel Major Histocompatibility Class I marker in wild common frogs (*Rana temporaria*) exposed to a viral pathogen (*Ranavirus*). *PLoS ONE* 42, e4616. <http://dx.doi.org/10.1371/journal.pone.0004616>.
- Teacher, A.G.F., Cunningham, A.A., Garner, T.W.J., 2010. Assessing the long-term population impact of *Ranavirus* infection in wild common frog populations. *Anim. Conserv.* 13, 514–522. <http://dx.doi.org/10.1111/j.1469-1795.2010.00373.x>.
- Temple, H.J., Cox, N.A., 2009. European Red List of Amphibians. Office for Official Publications of the European Communities, Luxembourg.
- Tweedell, K., Granoff, A., 1968. Viruses and renal carcinoma of *Rana pipiens*. V. Effect of *frog virus 3* on developing frog embryos and larvae. *J. Natl. Cancer Inst.* 40, 407–410.
- Une, Y., Sakuma, A., Matsueda, H., Nakai, K., Murakami, M., 2009. Ranavirus outbreak in North American bullfrogs (*Rana catesbeiana*), Japan, 2008. *Emerg. Infect. Dis.* 15, 1146–1147. <http://dx.doi.org/10.3201/eid1507.081636>.
- Waltzek, T.B., Miller, D.L., Gray, M.J., Drecktrah, B., Briggler, J.T., MacConnell, B., Hudson, C., Hopper, L., Friary, J., Yun, S.C., Malm, K.V., Weber, E.S., Hedrick, R.P., 2014. New disease records for hatchery-reared sturgeon. I. Expansion of *frog virus 3* host range into *Scaphirhynchus albus*. *Dis. Aquat. Organ.* 111, 219–227. <http://dx.doi.org/10.3354/dao02761>.
- Weinmann, N., Papp, T., Pedro Alves de Matos, A., Teifke, J.P., Marschang, R.E., 2007. Experimental infection of crickets (*Gryllus bimaculatus*) with an invertebrate iridovirus isolated from a high-casqued chameleon (*Chamaeleo hoehnelii*). *J. Vet. Diagn. Investig.* 19, 674–679. <http://dx.doi.org/10.1177/104063870701900609>.
- Wheelwright, N.T., Gray, M.J., Hill, R.D., Miller, D.L., 2014. Sudden mass die-off of a large population of wood frog (*Lithobates sylvaticus*) tadpoles in Maine, USA, likely due to *Ranavirus*. *Herpetol. Rev.* 45, 240–242.
- Whitfield, S.M., Donnelly, M.A., Geerdes, E., Kerby, J.L., 2012. *Ranavirus* infection in native amphibians at La Selva biological station, Costa Rica: the first report of *Ranavirus* in central America. *Herpetol. Rev.* 43, 425–427.
- Whitfield, S.M., Geerdes, E., Chacon, I., Ballester Rodriguez, E., Jimenez, R.R., Donnelly, M.A., Kerby, J.L., 2013. Infection and co-infection by the amphibian chytrid fungus and ranavirus in wild Costa Rican frogs. *Dis. Aquat. Organ.* 104, 173–178. <http://dx.doi.org/10.3354/dao02598>.
- Whittington, R.J., Becker, J.A., Dennis, M.M., 2010. Iridovirus infections in finfish – critical review with emphasis on ranaviruses. *J. Fish Dis.* 33, 95–122. <http://dx.doi.org/10.1111/j.1365-2761.2009.01110.x>.
- Wolf, K., Bullock, G.L., Dunbar, C.E., Quimby, M.C., 1968. Tadpole edema virus: a viscerotropic pathogen for anuran amphibians. *J. Infect. Dis.* 118, 253–262.
- Zhang, Q.Y., Xiao, F., Li, Z.Q., Gui, J.F., Mao, J.H., Chinchar, V.G., 2001. Characterization of an iridovirus from the cultured pig frog *Rana grylio* with lethal syndrome. *Dis. Aquat. Organ.* 48, 27–36. <http://dx.doi.org/10.3354/dao048027>.