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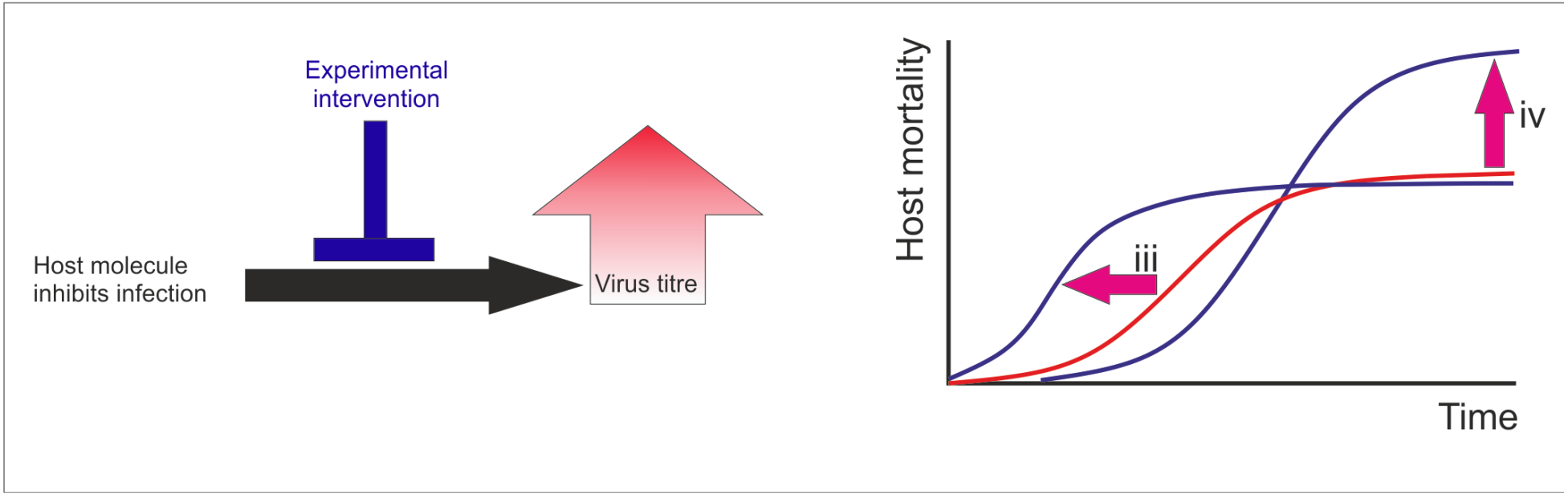
Abstract: The sustainable intensification of crustacean aquaculture, which is dominated by the farming of penaeid shrimp species, continues to be beset by viral disease outbreaks. Despite this, reports exist of differential susceptibility to viral infection between different shrimp species and populations, and between shrimp and other decapod crustaceans. These reports have, in part, provided the motivation to identify key mechanisms of antiviral resistance, or refractivity, in commercially-important species. Within the last decade these studies have created significant advances in our understanding of host virus interactions in decapod models. However, at the same time, the complexity of host virus interactions has presented significant challenges for interpretation of anti-viral immune responses. In this short review, recent progress in our understanding of the complexity of host virus interactions are considered, and challenges to the unequivocal identification of anti-viral immunity are highlighted. Special consideration is given to the advances in understanding being created by the use of RNA interference approaches. Based on the 'state of the art', it is concluded that the identification of effective intervention strategies for application at farm scale currently presents an unrealistic target for the aquaculture industry. Future technical developments necessary to support continued progress are also considered.

***Graphical Abstract**



Highlights

- Viral diseases of crustaceans are a significant impediment to global food security
- This review discusses the challenges of identifying anti-viral immunity
- Recent advances using RNA interference techniques are summarised
- Technological advances to support studies of host virus interaction are considered

Recent progress toward the identification of anti-viral immune mechanisms in decapod crustaceans

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Abstract

The sustainable intensification of crustacean aquaculture, which is dominated by the farming of penaeid shrimp species, continues to be beset by viral disease outbreaks. Despite this, reports exist of differential susceptibility to viral infection between different shrimp species and populations, and between shrimp and other decapod crustaceans. These reports have, in part, provided the motivation to identify key mechanisms of antiviral resistance, or refractivity, in commercially-important species. Within the last decade these studies have created significant advances in our understanding of host virus interactions in decapod models. However, at the same time, the complexity of host virus interactions has presented significant challenges for interpretation of anti-viral immune responses. In this short review, recent progress in our understanding of the complexity of host virus interactions are considered, and challenges to the unequivocal identification of anti-viral immunity are highlighted. Special consideration is given to the advances in understanding being created by the use of RNA interference approaches. Based on the 'state of the art', it is concluded that the identification of effective intervention strategies for application at farm scale currently presents an unrealistic target for the aquaculture industry. Future technical developments necessary to support continued progress are also considered.

Keywords

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Introduction

Infectious disease outbreaks represent a key limitation to the sustainable expansion of the aquaculture industry, necessary to meet the joint challenges of Global Food Security and poverty alleviation (Stentiford et al., 2012; Lafferty et al., 2015; Thitamadee et al. 2016). For example, losses to White Spot Syndrome Virus (WSSV), the causative agent of white spot disease (WSD) in decapod crustaceans, have been estimated to cost between $8 - 15 bn globally (Stentiford et al., 2012). More locally, individually outbreaks of disease can be truly devastating. For example: in 1996, in the Khulna region in Bangladesh, WSSV affected approximately 90% of *Penaeus monodon* shrimp farms resulting in a 20% decrease in production. As a consequence exports dropped from > 25k t to > 18k t in 1997–1998 (Nazul Alam et al. 2007).

Nonetheless, evidence exists that some species of decapod crustacean, or indeed individuals within a population or species, demonstrate refractivity to infection with virus. For example, although WSSV has been classified by the World Organisation for Animal Health (OIE) as being 'infectious for all decapods', host susceptibility varies between different penaeid species (Wang et al. 1999; Wu & Muroga, 2004; Cuéllar-Anjel et al, 2012). Refractivity to WSSV has also been reported in wild crustaceans. In India, refractivity has been identified in the freshwater prawn *Macrobrachium rosenbergii* (Sahul Hameed et al., 2000) and four species of crab (Sahul Hameed et al., 2003). In the UK studies have determined the susceptibility of temperate water crustaceans to infection with WSSV and identified three categories of susceptibility to WSSV. In Type 1 hosts (e.g. the Chinese mitten crab *Eriocheir sinensis*) pathology to WSSV mimics that described for penaeids. Type 2 hosts (e.g. the langoustine *Nephrops norvegicus*) are susceptible to WSSV only by direct injection, whilst Type 3 hosts (e.g. the European shore crab *Carcinus maenas*) suffer low mortality in response to injection or oral exposure (Bateman et al,. 2012). Whilst presenting very interesting models of virus resistance or refractivity, these reports also emphasize the problem of

63 asymptomatic reservoirs of infection, both in the wild and in culture, which will likely prove
64 impossible to control in shrimp producing countries. Ultimately there can be little or no prospect
65 for the global eradication of disease causing organisms within crustacean culture and, instead, we
66 must develop approaches to control or restrict their destructive impacts.

67 Within the last 10-15 years high resolution studies have identified an immunogenetic component
68 to virus refractivity. Insights in this field have largely been generated from dedicated studies of the
69 molecular interactions between virus and host, or from studies of the transcription of host and
70 virus genes (e.g. Zeng, 2013), studies of protein interactions (e.g. Ye et al., 2012a; Ye et al., 2012b),
71 to 'omic comparisons of the transcriptome of naïve and infected hosts or between susceptible and
72 refractive individuals (e.g. Veloso et al., 2011; Li et al. 2013; Sookruksawong et al., 2013; Xue et al.,
73 2013a; Zeng et al., 2013). Consecutive reviews of the field have done much to distil core
74 mechanistic insights of host-virus interaction (Flegel, 2009; Liu et al., 2009; Flegel and
75 Sritunyalucksana, 2011; Sritunyalucksana et al., 2012; Li and Xiang, 2013; Wang et al., 2014;
76 Shekhar and Ponniah, 2015; Verbruggen et al., 2016). Some reports have attributed anti-viral
77 resistance in penaeids to the expression of key genes or proteins (Luo et al., 2003; Wu et al., 2008;
78 Zhi et al., 2011), yet other reports have questioned some of these mechanisms, and their
79 particular significance to penaeids (Wu and Muroga, 2004; Hayes et al., 2010).

80

81 Herein, consideration is given to the challenge of identifying host anti-viral immune mechanisms
82 using studies of gene transcription or comparative transcriptomics as well as using recombinant
83 proteins or antibody inhibition studies. The potential for understanding host-mediated refractivity
84 to infection using RNA inhibition methods are considered before the future prospects for
85 mechanistic understanding of host responses to viral infection are highlighted.

86

The challenges of identifying anti-viral 'immunity' and 'resistance'

Philosophically, an initial challenge in this field arises from identifying appropriate definitions of 'resistance' or 'immunity' that can be consistently agreed by the research community. For many viruses, the ultimate outcome of infection is the death of the host. In these cases, should changes in gene transcription or phenotype of the host during the viral infection cycle necessarily be regarded as 'immunity', or simply viewed as the interaction of a host and pathogen? Alternatively, 'immunity' has sometimes been identified as a delay in the onset of mortality (e.g. Visetnan et al., 2014; Peepim et al., 2016). Unquestionably, a delay in the infection cycle of a virus and the associated tissue pathology and infection outcome, is of mechanistic interest. However, if the ultimate outcome is 100% mortality, should this be considered as evidence of 'immunity' or 'resistance'? Perhaps, with our current level of understanding, it would be more appropriate to refer to 'refractivity' to viral infection?

Many reports of anti-viral refractivity in decapod crustaceans have been published, from studies of changes in gene transcription of single genes or using comparative transcriptomics (e.g. Li et al. 2013; Zeng, 2013). This represents a series of challenges to interpretation. Firstly, it is widely accepted that the transcription of a gene, whilst indicating the potential for a change in phenotype, does not necessarily result in the expression of a mature protein that has an effect. As discussed previously (Smith et al., 2003), it is essential that gene transcription studies are supported by evidence of functional change in the phenotype of the infected individuals. In the case of viral infection, it is often difficult to discriminate secondary effects of virus pathology, including tissue degeneration and opportunistic secondary infection, which might affect the expression of key genes or proteins (e.g. heat shock proteins, Danwattananusorn et al., 2011; antioxidant enzymes, Hung et al., 2014; or antimicrobial peptides – contrast the findings of Antony et al., 2011 with those of Hipolito et al. 2014), but which may only be an indirect result of the

initial viral infection. Indeed, the work of Goncalves et al. (2014) has emphasised the importance of discriminating between host gene expression constituting an effective immune response and that associated with end stage mortality. Future studies may need to adopt a more refined approach to the pooling of gene transcription data sets, for example: analysing samples according to the time to mortality – rather than simply comparing control with inoculated hosts at fixed time points.

The motivation to identify an immune host also rather ignores the role of the virus in causing disease and the potential complication of viral co-infection (e.g. Tang et al., 2003), defective interfering particles (DIPs) (Fenner et al., 1974), or non-infectious viral sequences within the host genome (Tang and Lightner, 2006; reviewed in Flegel and Sritunyalucksana, 2011), in mediating infection outcome from any inoculation. Studies of the interactions of insect hosts with viruses offer many insights of the potential complexity that ultimately mediate infection outcome. For example: the effects of heterologous viral interactions have been reported in mosquito cell cultures by Burivong et al. (2004). These authors recorded three separate observations. First they demonstrated that the number of mosquito C6/36 cells infected with *Aedes albopictus* denosvirus (AalDNV) decreased as the cell line was serially passaged, from an initial infection rate of 92 % to a final rate of ca. 20 % after 10 passages. Second they also demonstrated that cells persistently infected with AalDNV did not show increased levels of infection in response to super-infection with AalDNV. These first two observations were explained in terms of the production of viral defective interfering particles (DIPs) during replication. DIPs are formed as a result of replication errors within the host cell and result in genome deletions within the replicated viral genomes (Huang and Baltimore, 1970). Deletions in genome length mean that these particles can be replicated more quickly than the wild-type virus and ultimately lead to a low level of fluctuating wild-type viremia (Frank, 2000), a situation which has been reported in penaeid shrimp (Tsai et al.,

1999; Flegel et al., 2004). The third key observation recorded by Burivong et al. (2004) was that C6/36 cells persistently infected with AalDNV were more resistant to super-infection with Dengue virus (DEN-2) than naive cells. The production of DEN-2 viral particles, detected using a monoclonal antibody for the DEN-2 virus envelope protein 4G2, was initially delayed in persistently infected C6/36 cells (Burivong et al., 2004). A suggested explanation for this third observation was that the presence of a persistent infection blocked any virally-triggered apoptosis (Burivong et al., 2004). However, it could also be caused by the negative competition for replication resources between the two viruses or between the viruses and DIPs (Fenner et al., 1974).

For decapod crustaceans as well, evidence is growing of the real complexity of interactions between hosts and viruses (see review of Flegel & Sritunyalucksana, 2011). It is the case that many viruses circumvent, or manipulate, host gene expression to promote viral replication (e.g. Liu et al 2007; Wang et al., 2011; Zuo et al. 2011; Wang et al., 2013a; Qiu et al., 2014; Wang et al., 2014). The complexity of host virus interactions, and our current limited understanding of them, has led some authors to complex conclusions in order to argue a case for host immunity. As one example, Ye et al. (2012) have reported that the VP466 peptide expressed by WSSV forms a complex with the Rab GTPase of *Penaeus japonicus*. This complex mediates the reorganisation of the host cell cytoskeleton to promote phagocytosis of the virus. Ye et al. (2012) concluded that the host exploited a virus protein to 'initiate host immunity'. A more parsimonious explanation might be that the one role of WSSV VP466 is to facilitate cellular entry by binding with the host Rab GTPase as a prerequisite for viral replication within host tissues.

The outcomes of recombinant peptide or antibody binding studies also require careful consideration. As described, there is evidence that viruses circumvent or manipulate host gene expression to facilitate host cell entry or replication. In the example provided in Figure 1, viruses

might gain entry to host cells through binding with extracellular (A) or host cell-surface-expressed peptides or molecules (B). There is evidence in the literature of viruses binding to host lectins (e.g. Zhao et al., 2009; Chen et al., 2013) and host GTPases (e.g. Ren et al., 2012) to facilitate host cell entry. However, the challenge of recombinant peptide studies is that these recombinants may not be completely functional *in vivo* (blue peptides in C-E). Injection of incompletely-functional recombinant peptides in these situations may limit viral entry, or viral titre, and may prevent virally-mediated mortality of the host. However, this does not necessarily mean that the native protein is part of an anti-viral immune mechanism (e.g. Zhao et al., 2009; Havanapan et al., 2014), it may simply represent a host protein that is exploited by the virus to gain entry to the cell as a necessity for replication. Similarly, antibody binding studies of either the cell surface (F, H) or extracellular receptor (G) or virus (I), in isolation, do not prove that the native host peptide is responsible for an antiviral response, it may reflect a host peptide or pathway that is manipulated by the virus to facilitate the infection cycle. Ultimately, recombinant and antibody methods can be used as a tool to understand the mechanism of host virus interaction but, without supporting studies, it is difficult to unequivocally attribute this to host anti-viral immune response (Sritunyalucksana et al., 2012).

Recent advances using RNA interference approaches

The RNA interference (RNAi) pathway degrades mRNAs or inhibits their translation (Hannon, 2002). In general, microRNA (miRNA) achieves the silencing of gene transcription by binding to mRNA and preventing translation, whilst short interfering RNA (siRNA) identifies mRNA to be degraded through the action of endonucleases. Both types of interfering RNA are classed as small RNAs (sRNA) and act in association with Argonaute (Huang and Zhang, 2012a) at RNA-induced

183 silencing complexes (RISCs). Experimentally, siRNA are introduced into the host or cell line as
184 longer double-stranded RNA which is then cleaved by the protein Dicer (Su et al., 2008).

185 RNA inhibition studies in crustacean virus models have been prosecuted for over ten years, with
186 early studies demonstrating that both long sequences of viral dsRNAs and non-sequence specific
187 dsRNAs could inhibit viral replication in decapod species. For example: Robalino *et al*. (2004)
188 demonstrated that non-specific dsRNA transcribed from a duck immunoglobulin sequence could
189 reduce mortality caused by either Taura Syndrome Virus (TSV) or WSSV in *Penaeus vannamei*.
190 Robalino *et al*. (2004) concluded that the non-specific dsRNA induced a 'general anti-viral
191 mechanism'.

192 The use of sequence-specific dsRNA has since proved to be a powerful tool to test theories of host
193 virus interactions and the roles of key host proteins (Figure 2). Theoretically, if the hypothesis is
194 that a particular host peptide or protein facilitates viral uptake or viral replication, then inhibition
195 of that host molecule through RNAi should cause a reduced viral titre or delay in host mortality
196 after virus inoculation (Figure 2, panel A). This has been reported for a good number of studies
197 (e.g. Labreuche et al., 2009; Shi et al., 2012; Qiu et al., 2014; Xue et al., 2013b; Wen et al., 2014;
198 Peepim et al., 2016). However, other interpretations of experimental interventions remain difficult
199 to resolve. For example, Wang et al. (2015) compared the titre of WSSV after dsRNA inhibition of
200 *LvTAB2* in *Penaeus vannamei;* in *Drosophila* TAB2 is recognised as a key intermediate of the IMD
201 pathway. Wang et al. (2015) demonstrated a consistent reduction in WSSV copies, compared to
202 the PBS control, after dsRNA knockdown of TAB2 (their Figure 9). This would be consistent with an
203 interpretation that TAB2 facilitates infection in *P. vannamei* (see Figure 2, panel A). However,
204 Wang et al. (2015) concluded that *LvTAB2* may have important roles to play in 'shrimp innate
205 immunity'.

206 Alternatively, if the hypothesis is that a particular host molecule prevents or inhibits viral uptake
207 or replication, then inhibition of that host molecule through RNAi should lead to an increase in
208 viral titre, or a faster onset of virus-induced mortality (Figure 2, panel B). Again, a number of
209 studies have presented data in accordance with this view (see Table 1). Of interest are the high
210 number of studies that identify the role of host apoptosis in controlling infection outcome,
211 particularly the important role played by initiator and effector caspases. A full review of the role of
212 pro- and anti-apoptotic pathways in mediating viral outcome is beyond the scope of this short
213 review and already have been extensively and recently reviewed, more generally (Benedict et al,.
214 2002; Irusta et al., 2004; Amara and Mercer, 2015), and specifically for decapod virus interactions
215 (Molthathong et al., 2008; Hirono et al., 2011; Leu et al., 2013; Shekhar and Ponniah, 2015; Xu et
216 al. (2014); Verbruggen et al., 2016). The potential role of virally-derived miRNAs in regulating host
217 cell apoptosis is considered further below.

218 Evidence for the role of an anti-viral host RNAi pathway was perhaps first identified by Tirasophon
219 *et al*. (2005). Using sequence-specific long (> 100bp) dsRNA, Tirasophon *et al*. (2005)
220 demonstrated that fragments coding for the Yellow Head Virus (YHV) helicase, protease and
221 polymerase genes inhibited YHV replication in *P. monodon* lymphoid cell cultures. More recently,
222 RNAi studies targeting host Dicer and Argonaute proteins (Table 1) have clearly demonstrated the
223 important role of a host RNAi mechanism in controlling aspects of the viral infection cycle that
224 may support refractivity to viral infection. Recent excellent reviews of our developing knowledge
225 of the anti-viral potential of host RNAi and non-coding miRNAs have been offered by Huang et al.
226 (2012), Labreuche & Warr (2013), Wang et al. (2013), Xu et al. (2014a), He et al. (2015) (see also
227 Kaewkascholkul et al. 2016). These articles highlight the diversity in response to viral infection;
228 diverse responses that may not fit the classical paradigm of receptor and effector arms of the
229 innate immune mechanism in decapod crustaceans (Hauton, 2012; Hauton et al., 2015).

Inevitably however, RNA inhibition does not operate solely in favour of the host. The important role of host cell apoptosis in mediating infection outcome has been identified above. Recently Huang et al. (2014) have reported the identification of 89 WSSV-expressed microRNAs from *in vivo* studies in *Penaeus japonicus*. Their data showed that at least one of these miRNAs (WSSV-miRNA-N24) could inhibit the expression of the *Penaeus japonicus* caspase 8 gene and prevent apoptosis of host cells that might otherwise have restricted viral replication. Liu et al. (2016) have also reported the expression profile of miRNAs of WSSV-infected *Penaeus chinensis*, providing exquisite insights into the differential expression of RNA inhibition pathways of both the host and virus in different tissues across the host. The rapid growth in number of reports of the interaction of virus and host sRNA has highlighted the significant analytical and bioinformatic challenges of data interpretation in decapod hosts, for which there are no fully assembled or annotated genomes. It will undoubtedly take further years for the field to mature and for the significance of these rapid developments to be rationalised and confirmed in a wider range of commercially important decapod crustaceans and for different virus genotypes.

Future prospects

Technological developments will continue to support advances in this field. Since 2012, significant progress has been made in the use of primary and secondary cell culture to support *in vitro* virus infection experiments. For penaeid species, good results have been secured using cell cultures established from the lymphoid organ (Jose et al., 2012; Li et al., 2014; Puthumana et al., 2015; Li et al., 2015). Efforts in this field should continue (Hauton, 2012). Firstly, it is important that the field develops similar progress with analogous tissues in the sub-order Pleocyemata, which includes those crabs and lobsters that are potential key reservoirs of virus in the wild. Secondly, and

253 ultimately, there is still a need to develop an immortal crustacean cell line with which to
254 standardise an *in vitro* model for laboratory application (Hauton, 2012).

255 Future progress in the assembly and annotation of model penaeid and other decapod genomes is
256 likely to require a combination of high throughput short reads (e.g. Illumina™) with low coverage
257 long reads (e.g. PacBio™) and conventional Sanger sequencing of complex repeat regions.
258 However, this investment of time and resource is essential. A fully assembled genome will prove
259 invaluable as a scaffold with which to compare evolutionary differences in the immune gene loci
260 of host species, strains and populations (e.g. Guethlein et al. 2015) that will support further
261 mechanistic insights of the immunogenetic component of host refractivity to virus infection.

262 To date, the use of CRISPR-Cas9 for gene editing (Doudna and Charpentier, 2014; Sternberg and
263 Doudna, 2015) has not been deployed in experimental shrimp models to elucidate the molecular
264 interactions between hosts and viruses. However, the ability to delete single genes and fragments
265 of genes will undoubtedly prove to be a powerful tool with which to understand the interactions
266 and silencing of sRNA in both hosts and viral pathogens.

267

268 In conclusion, within the past six years there has been a dramatic increase in our appreciation of
269 the complexity of host virus interactions within decapod crustaceans. As described, rapid advances
270 in our understanding of the roles of host- and virus-RNA interference via sRNAs, combined with
271 the rigorous application of RNA knockdown techniques to understand the function of host
272 immune-related genes, has created significant insight, but at the same time has identified the
273 complexity and intimacy of these interactions. It is clear that the field of decapod immunology is
274 developing from a classical paradigm of receptor and effector arms that detect an invading
275 pathogen and elicit potent antimicrobial, degranulation and inflammatory immune responses, to a

more refined view of the intricate host virus interactions that take place at the level of nucleotides, rather than proteins.

Whilst it is true that this field needs time for findings to mature and the significance of key datasets to be appreciated, it seems very likely that there will be no single key, or 'magic bullet', to the identification of anti-viral immunity in cultured or wild crustaceans. As such, the realisation of this complexity means that, in future studies, it will be paramount to present sufficient meta-data for any experiment (for example, the MISA guidelines argued in Hauton et al., 2015) so that any differences between replicated experiments conducted by different research teams can be identified and rationalised (Freedman et al., 2015; Baker, 2016).

Ultimately, with our present level of understanding of the complexity of host virus interaction, there seems little immediate prospect of identifying a single mechanism for disease intervention that will be effective, and cost effective, at farm scale. In the interim, it will be necessary to explore alternate approaches (e.g. biosecurity, environmental management) to minimise the incidence and impact of viral outbreaks within commercial operations. The implementation of effective management practices will provide time in which the technological developments described above can brought to bear on the problems of viral infection within crustacean aquaculture.

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558

559

560 **FIGURE LEGENDS**

561

562 **Figure 1**. Simplified schematic of recombinant protein expression and antibody binding studies,
563 and their limitations. In this example (A, B) the virus gains entry to the host cell through
564 subversion of either secreted or cell-surface expressed binding proteins (green symbols); these do
565 not constitute host immune responses. In C-E alternate scenarios are presented in which non-
566 functional recombinantly-expressed binding proteins (blue symbols) interfere with viral uptake,
567 whilst in F-I antibody (black symbols) methods are used to block or bind key peptides involved in
568 viral uptake. Scenarios C-I might all result in a reduction in viral titre, or a delay in the onset of
569 virally induced mortality, however none of them necessarily indicate a host anti-viral mechanism.

570

571 **Figure 2.** Schematic of the hypothesized outcomes of an RNAi experiment. In panel A the
572 expression of a host protein that facilitates viral uptake/infection is abrogated using RNA
573 inhibition. In this case the experimental outcome should be a reduction in viral replication/titre,
574 resulting in either a delay (i) or reduction (ii) in host mortality. In panel B, the expression of a host
575 protein that inhibits infection is abrogated, which should either precipitate (iii) or increase overall
576 host mortality (iv).

577

578

Tables

Table 1 Examples of studies using RNA inhibition in the decapod host to identify proteins that are involved in the inhibition of viral uptake or replication (see Figure 2, panel B).

Classification	Host	Virus	Molecule/molecule family	Pathway	Reference
Binding	*Penaeus japonicus*	WSSV	C type lectin - LdlrLec1	Carbohydrate binding	Hu et al. (2014)
	P. japonicus	WSSV	C type lectin - LdlrLec2	Carbohydrate binding	Hu et al. (2014)
Immune effector arm	*Penaeus monodon*	WSSV	Penaeidin 5 - PenmonPEN5	Antimicrobial peptide	Woramongkolchai et al. (2011)
	Penaeus vannamei	WSSV	Astakine - LvAST	Astakine, promotes haemopoesis. LvAST binds to WSSV VP37 and shrimp F_1-ATP synthase subunit	Liang et al. (2015)
	P. japonicus	WSSV	Thioester-containing proteins - TEP1 and TEP2	Effectors of the Jak/STAT signalling pathway	Ren et al. (2015)
Apoptosis	*P. japonicus*	WSSV	Effector caspase - *PjCasp*	Apoptotic pathway	Wang et al. (2008)
	P. japonicus	WSSV	*Pj Caspase* - containing 'fragment 3'	Apototic pathway	Zhi et al. (2011)
	P. chinensis (but RNAi experiment conducted in P. japonicus)	WSSV	Cathepsin C - *Fc-Cath C*	Protein degradation, proenzyme activator, apoptosis pathways	Wang et al. (2012)
	P. vannamei	WSSV	Translationally controlled tumor protein (TCTP)	Cell growth, cell cycle progression, and anti-apoptotic factor	Wu et al. (2013)
	P.vannamei	WSSV	Inhibitors of apoptosis - *LvIAP1*, *LvIAP3*	Inhibit caspases - apoptotic regulation	Wang et al. (2013b)
	Procambarus clarkii	WSSV	Prohibitin - PcPHB1	Apoptosis, aging, stress responses, cell proliferation, and immune regulation	Lan et al. (2013)
Host RNAi pathway	*P.monodon*	Gill associated	Dicer1 - Pm Dcr1	Endoribonuclease, which is responsible for cleavage of long dsRNA into siRNAs. Shrimp	Su et al. (2008)

	virus		RNAi pathway	
P .japonicus	WSSV	Dicer2	RNAi mediated inhibition	Huang and Zhang (2013)
P .japonicus	WSSV	Argonaute - Ago1A and Ago1B	RNAi mediated inhibition	Huang & Zhang (2012a)
P. japonicus	WSSV	Shrimp MicroRNA - miR7	RNAi mediated inhibition, targeting the 3'-UTR of WSSV early gene *wsv477*	Huang & Zhang (2012b)
P. japonicus	WSSV	Shrimp MicroRNA-965	RNAi mediated inhibition – targeting gene *wsv240*	Shu et al. (2016)

Figure

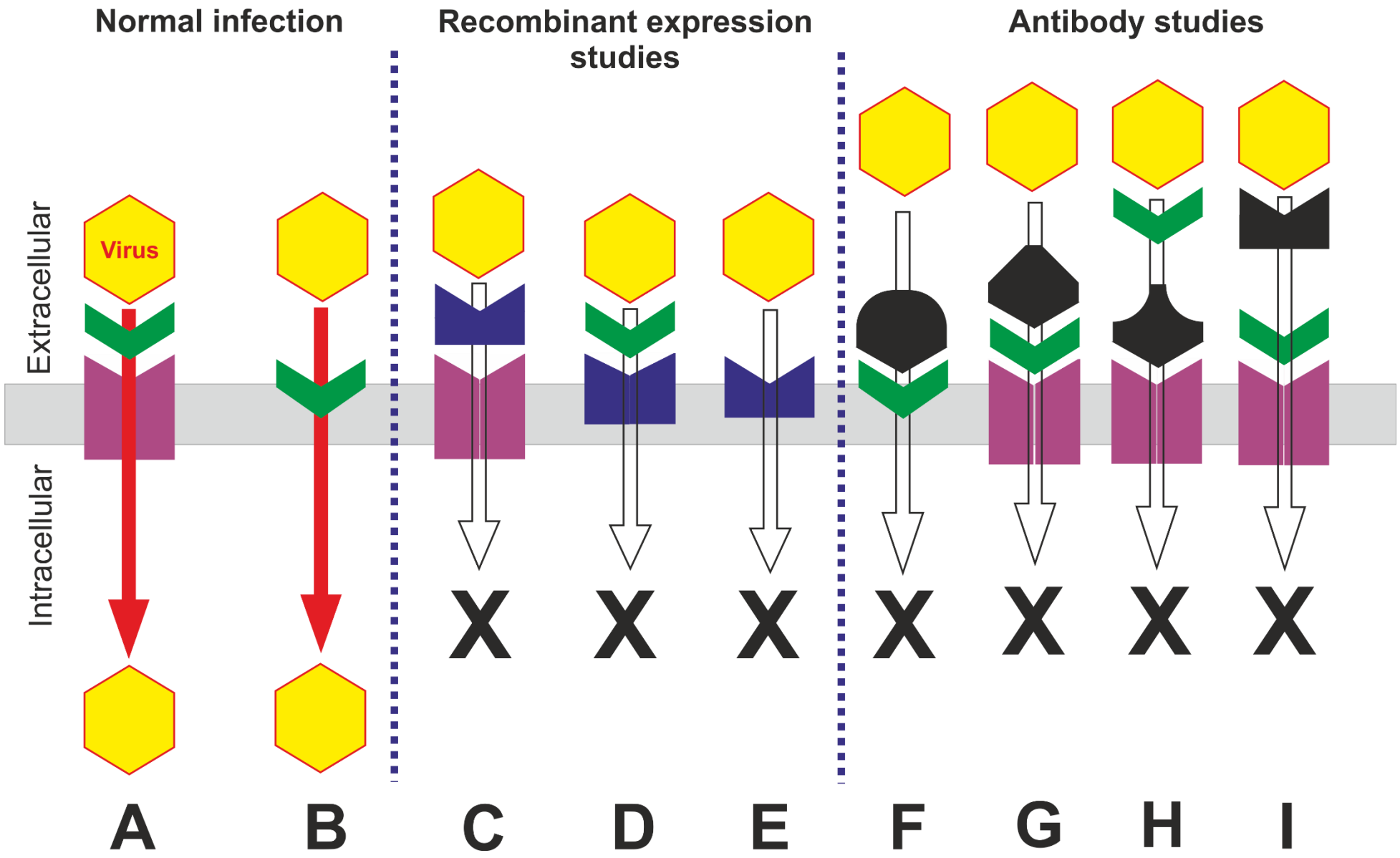
Normal infection
Recombinant expression studies
Antibody studies
Extracellular
Intracellular
Virus
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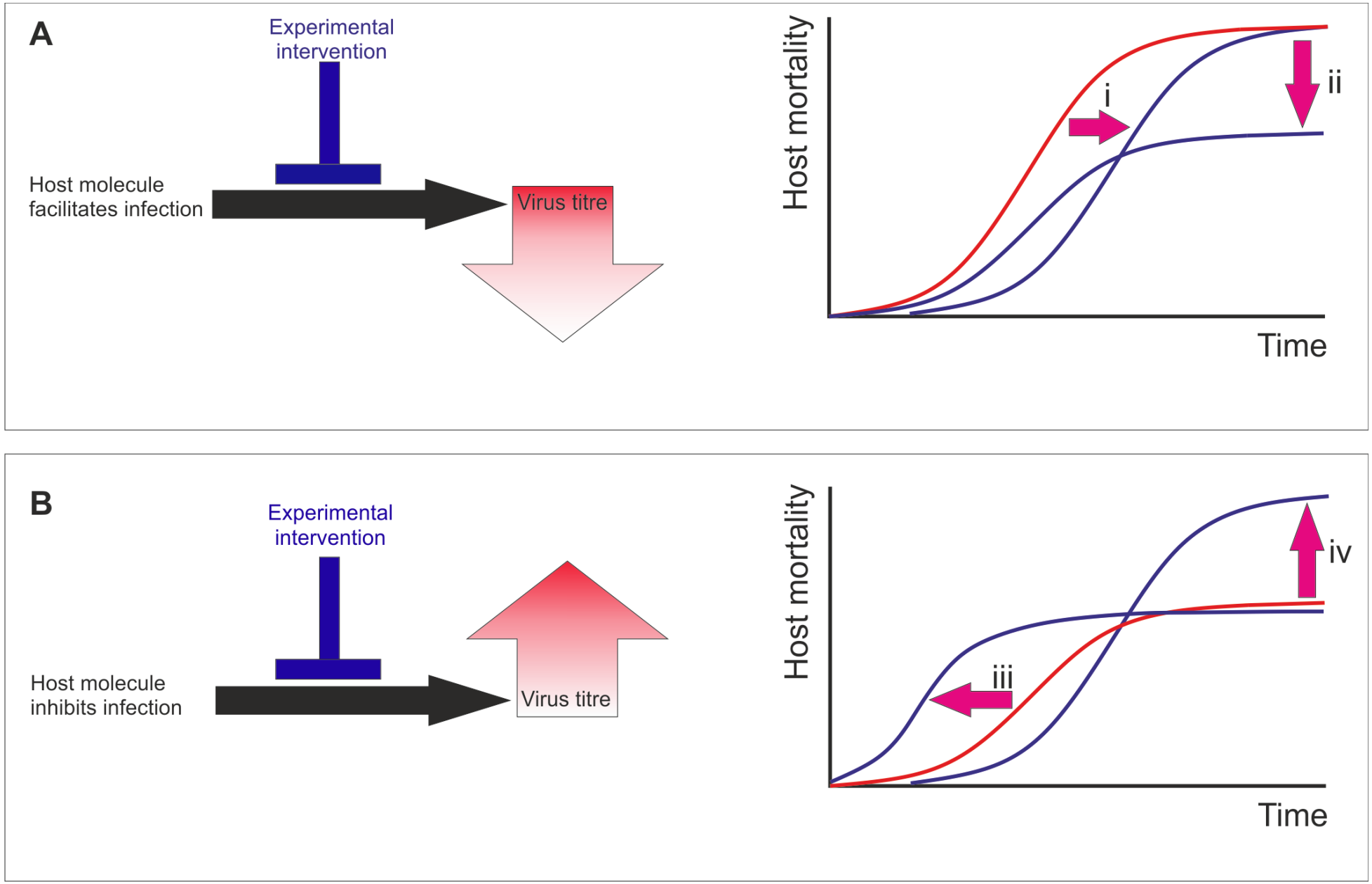
Figure


A
Experimental intervention
Host molecule facilitates infection
Virus titre
Host mortality
i
ii
Time
B
Experimental intervention
Host molecule inhibits infection
Virus titre
Host mortality
iii
iv
Time