

EXPERT OPINION

Perspectives on deploying gene editing techniques to protect farmed invertebrates from pathogenic infection.

Chris Hauton¹

¹ School of Ocean and Earth Science, University of Southampton

Keywords: Better Management Practice, Gene editing, Immune receptors, Recirculating aquaculture

<https://doi.org/10.48045/001c.127248>

Bulletin of the European Association of Fish Pathologists

Vol. 45, Issue 1, 2025

The production of aquatic protein for human consumption is widely argued as playing a pivotal role in the provision of global food security. However, the sustainable intensification of aquaculture production faces key challenges from impacts of, for example, climate change and the incidence of pandemic diseases and syndromic disorders. Current initiatives to tackle these challenges rely on the dissemination of Better Management Practices to farmers, often based in remote locations. In the longer term our improved understanding of invertebrate immune systems, in particular pattern recognition receptors and pathways, will present positive solutions to disrupt host/pathogen interactions. Combining these insights with advances in bioscience tools, including gene editing, will provide novel avenues to reduce the impacts of disease in biosecure culture systems. Concerted research efforts between academic and industry scientists, supported by appropriate investment, should be directed toward overcoming challenges of implementing bioscience solutions to control diseases in order for the aquaculture industry to meet future global demand.

Introduction

The production of aquatic protein for human consumption is widely argued as playing a pivotal role in the provision of global food security. However, the sustainable intensification of aquaculture production faces key challenges from impacts of, for example, climate change and the incidence of pandemic diseases and syndromic disorders. Current initiatives to tackle these challenges rely on the dissemination of Better Management Practices to farmers, often based in remote locations.

In the long-term further investment in fundamental bioscience to improve understanding of invertebrate immune systems, in particular pattern recognition receptors and pathways, will present positive solutions to disrupt host/pathogen interactions. Species and even strain differences in the molecular architecture of the immune system are ultimately key to differences in the phenotype of immune susceptibility. Therefore, future research must be more sensitive also to species and even strain differences in host pathogen interaction, rather than assume that syntheses of immune interactomes established for superior taxonomic groups necessarily apply for the target

cultured species. Combining these insights with advances in bioscience tools, including gene editing, will provide novel avenues to reduce the impacts of disease in biosecure culture systems.

Ultimately, however, progress toward solutions to disease will need to be achieved across multiple fronts from farm management practices and sanitation through to the farm-scale deployment of disruptive technologies. The technical and cost challenges of farm deployment, combined with the ‘moving target’ of rapidly evolving existing and emergent pathogens, will mean that no single solution to the eradication of disease will apply for all cases and all crop species. Only with a holistic approach to disease management will the potential for aquaculture to meet the demand for global nutritional security be met.

Since the 1970s the aquaculture industry has witnessed prodigious global growth. In 2020, the UN Food and Agriculture Organisation (FAO) estimated that aquatic animal protein (fisheries and aquaculture) contributed 17% of total protein for human consumption (FAO 2020). In the same year, global aquaculture production reached a record 122.6 million tonnes, with a total value of USD 281.5 billion (FAO 2022). Farmed finfish contributed 47%, worth an estimated USD 146.1 billion, whilst the production of other farmed aquatic animal species contributed 14.4% from, mainly, bivalve molluscs (USD 29.8 billion), and 9% from crustaceans (USD 81.5 billion), with the remainder from other aquatic invertebrates and semi-aquatic species.

There is a growing acknowledgement of the role that aquaculture will play as the global population exceeds nine billion people. Moreover, a burgeoning ‘middle class’ is already driving a change in food consumption, with an increased preference for meat protein instead of traditional wheats and pulses (Stentiford et al. 2012). It is argued that aquaculture will make a significant contribution to global food security, and the importance of this contribution in countries currently suffering malnutrition or food insecurity (Garlock et al. 2022). Indeed, the consumption of aquatic foods (excluding algae) has increased at an annual rate of 3% p.a. compared with a global population growth of 1.6%; the annual per capita consumption of aquatic foods has increased from an average of 9.9kg in the 1960s to in excess of 20kg by 2019. The UN FAO predict that the aquaculture production of aquatic animals will reach 100 million tonnes by 2027 and 106 million tonnes by 2030 and that annual individual consumption of aquatic animal protein will rise to 21.4kg by 2030 (FAO 2022).

However, growth is unlikely to be evenly distributed across the globe. Aquaculture production is likely to remain dominated by Asia and it is to be expected that annual production will be routinely impacted by adverse weather conditions, or to outbreaks of existing or emergent pathogens, syndromic disorders, or other socioeconomic factors. Pathogenic infection remains a key limitation on aquatic food production; one that is exacerbated

through the intensification of production systems. Indeed, by some estimates, aquaculture production of some species (e.g. penaeid shrimp) in key countries has been static or erratic since 2017 (Anderson, Valderrama, and Jory 2019).

Estimates identify a US\$6 billion p.a. cost of aquatic animal diseases, with particular impacts being reported in defined sectors, such as shrimp farming (e.g. 40% of production being lost). In order for the aquaculture industry to deliver on the anticipated growth and contribution toward food security, it remains that significant interventions must be made in the production systems across multiple countries (Stentiford et al. 2017).

Technical solutions to the problems of disease have been developed for some diseases of finfish but, thus far, attention has focussed on application for high value crops within western aquaculture. For example, routine vaccination against vibriosis, enteric red mouth and furunculosis is practiced in European salmonid culture (e.g. Press and Lillehaug 1995). Such technological solutions for disease management of single diseases do not offer current prospect for lower value fish species and invertebrate taxa which are cultured predominantly across the globe in Low and Middle Income Countries (LMIC). To date, the best success for these crop species has resulted from the inception of Better Management Practices (BMPs) to improve pond and culture health. BMPs take a holistic view of the cultured crop and the culture environment; providing key advice on issues such as pond soil preparation, treatment of water after filling, maintenance of pond water quality, post-larval supply and quality, stock density, feeding regime, and monitoring during grow out. This advice is intended to reduce physiological stress within the culture stock during the growth cycle and has been shown to have positive outcome in reducing the incidence of disease outbreaks.

Currently therefore, significant improvements in aquaculture output are being achieved by raising farmer awareness of BMPs and through encouraging adherence to the advice (Wang et al. 2023). Efforts have been considerably supported by the release of mobile phone apps to assist remote farmers in culture practice at the pond-side (e.g. the Indian Council of Agriculture Research ICAR Central Institute of Brackishwater Aquaculture CIBA Vanami Shrimp app and Chingri Shrimp app) and also with the diagnosis and reporting of disease outbreaks (e.g. the ICAR National Bureau of Fish Genetic Resources NBFGR ReportFishDisease app). However, app development alone is not sufficient. The successful implementation of BMP through mobile phone apps works best when accompanied by farmer sensitization and extension workshops, which can upskill the work force, demonstrate the full utility of the app platform, and instil confidence in farmers using this technology at the pond side (e.g. Dickson et al. 2016).

Without doubt, BMPs will remain a key component of improved farm practice in the future. However, as the aquaculture sector strives to return to growth, particularly after the challenges of the coronavirus pandemic, there is a renewed sense that technological advances in the biosciences might be identified to directly meet the challenge of existing and emergent bacterial or viral diseases.

Historically, technology to disrupt the life cycle of disease-causing pathogens, particularly within shellfish aquaculture, has relied upon the administration of pre- or probiotics or the prophylactic administration of immunostimulants. As has been argued, many of these interventions are based on an incomplete understanding of the complexity of invertebrate immune systems (V. J. Smith, Brown, and Hauton 2003; Hauton and Smith 2007). It is surely an indication of their limited success that, globally, farmers are still challenged by pandemic disease outbreaks, none of which are effectively controlled through the addition of non-specific prophylactic treatments.

I argue that, rather than advocate for the continued development of non-specific immunostimulants, primers or probiotics, which – even if demonstrated to induce an immune response in laboratory settings – still seem unable to confer effective protection at aquaculture scale, might it be better to improve understanding of host/pathogen interaction and pathogen entry mechanisms in the first instance? If the existing host immune effector pathways offer no effective counter to pathogen multiplication, then it is surely not appropriate to pursue the repeated stimulation of that ineffective immune response. It must be more effective to prevent the pathogen gaining first entry into host tissues/host cells rather than to subsequently seek to disrupt pathogen replication or promote any non-specific and non-effective immune response.

In recent years, the identification of the nuanced complexity of host microbial receptors that bind and internalise invading pathogens might offer new mechanisms or pathways with which to disrupt pathogen entry. Variant receptors offer potential mechanisms to support exquisite sensitivity, specificity and, even phenotypes analogous to memory immunity in invertebrates (Criscitiello and de Figueiredo 2013; Armitage, Peuß, and Kurtz 2015; L. C. Smith and Lun 2017; Lv et al. 2020; Liberti et al. 2022; Buckle and Yoder 2022; Iwama and Moran 2023). As examples, Criscitiello and de Figueiredo (2013) and Iwama and Moran (2023) provide excellent reviews of the evolution of variant pattern recognition receptors PRRs within the wider invertebrates, including anti-viral Toll-like receptors and the RNA interference pathways, immunoglobulin superfamily peptides, arthropod Dscam receptors, gastropod FREP receptors, the *SpTransformer* Gene Family in gastropods, and IgSF Variable Domain Chitin-binding Proteins (VCBPs) in tunicates.

Exploiting this new understanding may identify ways to disrupt host pathogen interaction at the point of first contact. However, whilst significant advances in understanding have been made, there is still much more to do, particularly with a focus on disrupting pathogen entry.

Future research efforts should be sensitive to the evolutionary history of different cultivated species. For example, Bateman et al. (2012) have already reported that there is differential sensitivity to White Spot Syndrome Virus amongst the Decapoda. As a general observation, taxa within the Dendrobranchiata (true shrimps) exhibit greater susceptibility to infection with this pathogen compared to the Pleocyemata (crabs and crayfish). Literature summaries of the state of the art in our understanding of immune system complexity should provide species specific resolution (e.g. Qin et al. 2023) and should not merge theoretical models of immune pathways across phyla, based on the assumption of pathway conservation (for example: Zheng, Xu, and Liu 2019). Any future interventions will require resolution of host pathogen interactions will require verification at the level of the individual species, or even family line.

How can bioscientists exploit understanding host/pathogen interactions to disrupt the life cycle of disease-causing organisms? The development and optimisation of protocols to produce gene edited crops presents a significant breakthrough toward the goal of reducing pathogenic disease within aquaculture (Gratacap et al. 2019). The ability to edit key gene sequences provides two avenues for advance in the field. Firstly, the targeted deletion of key genes will allow the functions of those coded proteins to be explored *in vivo* in model species. This may apply to immune effector molecules but could equally apply to the removal of key immune receptor proteins from host species. Secondly, the targeted deletion of selected cell surface expressed or secreted immune receptor proteins could be used to subvert the pathogen life cycle at the point of host cell entry. The potential development of gene edited broodstock for aquaculture has already been presented, for fish (Blix et al. 2021; Gutási et al. 2023), for molluscs (Potts et al. 2021) and for crustaceans (Gonzalez-Duarte et al. 2021). Some early experiments have demonstrated exciting future prospects (Gui et al. 2016; Kumagai et al. 2017; Martin et al. 2016; Xu, Pham, and Neupane 2020; Molcho et al. 2022), and a small number of gene edited fish, including sea bream *Pagrus major*, tiger pufferfish *Takifugu rubripes* and olive flounder *Paralichthys olivaceus*, have been approved for human consumption (reviewed by Ledesma and Van Eenennaam 2024). Moreover, it is expected that pigs, gene -edited to resist porcine reproductive and respiratory syndrome, will soon be licenced in the USA and elsewhere (Cohen 2024).

Supporting such advances, is the development of updated legislation to permit this technology to be released, at scale, in the context of culture systems for food production. The recent proposals for the use of gene edited

crops, at least within the UK context, will permit the commercialisation of these technological solutions (Cohen 2024; The UK Gene Editing (Precision Breeding) Act 2023) and, in time, should help to reduce the costs associated with the initial development.

Nevertheless, significant hurdles remain. First and foremost, gene editing for disease resistance requires the identification of particular receptor or effector proteins that are key to a resistance phenotype in the host. Our knowledge of many host pathogen interactions in aquaculture is not yet sufficiently detailed that we can, with confidence, identify a single protein interaction or suite of proteins that are pivotal to the outcome of infection, such that they could be over-expressed or abrogated in a gene edited crop species. For many pathogens, especially those that are globally significant, our current best insights identify that multiple host pathogen interactions mediate entry and replication (e.g. Verbruggen et al. 2016), representing significant complexity from the perspective of gene editing. Continued progress will necessitate the sustained investment in fundamental basic science to understand the interaction of key hosts and their pathogens at a molecular scale.

The high cost of such technological innovation should not be underestimated as well. In the initial years of gene edited aquatic crops, the cost to grower and consumers will likely lead to this technology being prioritised for high value crops. This will not address the challenge of pandemic diseases in the wider world, particularly in LMIC countries. The development of gene edited stock will require improved systems for biosecurity and monitoring, including the use of indoor recirculating culture systems (Xiao et al. 2018; Ahmed and Turchini 2021) that can protect and maintain high value products whilst the technology for gene editing is developed and tested, and whilst costs remain high. As gene edited crops become validated and more widespread, then it is anticipated that the costs of growers will fall, permitting wider adoption.

Moreover, the challenges of practically implementing technology for the industry are great. It is one thing to propose the use of gene editing technology to understand host pathogen interactions in an experimental setting, it is a very different prospect to propose the creation of gene edited lines and the distribution at farm scale (Gonzalez-Duarte et al. 2021). These challenges are compounded for species with complex genomic structure (e.g. Zhang et al. 2019; Kawato et al. 2021) or in species that exhibit low genetic heritability that challenges the stability of a gene edited phenotype in subsequent breeding programmes (Cock et al. 2009; López-Ordaz et al. 2024).

Furthermore, the rapid co-evolutionary ‘arms race’ that takes place between host and pathogen, so neatly characterised in the ‘Red Queen Hypothesis’ (reviewed in Van Oosterhout 2021), will likely mean that any gene edited solution to infection will eventually be circumvented through the rapid evolution of the pathogen by mutation or horizontal gene transfer and

subsequent selection. Gene editing may fail by only buying time until a new strain or variant of the pathogen evolves, or until the emergence of a new pathogen that exploits a different entry mechanism.

Ultimately, we must anticipate that the successful deployment of gene edited crop lines will require a continuous pipeline from basic science, through to product verification and licencing, and then distribution to farming communities. This pipeline will need to be supported by the continuous improvement in the farm security and management practice toward the end goal of reducing losses to disease and achieving future food security.

Conclusions

We are at a pivotal time for the global aquaculture industry. Having demonstrated significant growth over multiple decades, the potential of the industry to contribute toward global food security is undeniable. However, significant challenges remain; challenges which – if not addressed – seriously threaten that potential to deliver. Farmer training and improved practice is buying time for the industry to develop technological solutions to the impacts of disease-causing organisms in culture environments. Herein I have argued briefly for a focus on the immune receptor arm of host immune systems as providing novel avenues for disrupting host pathogen interactions. Disruption at this point will subvert the pathogen life cycle at a point before significant replication can take place and will reduce the impact caused by, particularly, intracellular pathogens. Once host targets have been identified it will be possible to employ advances in gene editing technology to develop lines of crop species with improved resistance to key pathogenic organisms that can be used within a culture setting. Challenges to the implementation of gene editing have been discussed, including further investments in fundamental basic science to tease apart the intimate molecular interactions of particular species as hosts and their pathogens. Deploying such high-cost methods will require supporting technologies to improve biosecurity and monitoring of crop species and, so, development of culture technology will go hand in hand with advances in bioscience and with improvements to farm management practices.

Submitted: December 15, 2023 CET. Accepted: December 10, 2024 CET. Published: December 10, 2024 CET.



This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CCBY-4.0). View this license's legal deed at <http://creativecommons.org/licenses/by/4.0> and legal code at <http://creativecommons.org/licenses/by/4.0/legalcode> for more information.

REFERENCES

- Ahmed, N., and G. M. Turchini. 2021. "Recirculating Aquaculture Systems (RAS): Environmental Solution and Climate Change Adaptation." *Journal of Cleaner Production* 297:126604. <https://doi.org/10.1016/j.jclepro.2021.126604>.
- Anderson, J. L., D. Valderrama, and D. E. Jory. 2019. "GOAL 2019: 'Global Shrimp Production Review. Global Seafood Alliance.'" 2019. <https://www.globalseafood.org/advocate/goal-2019-global-shrimp-production-review/>.
- Armitage, S. A. O., R. Peuß, and J. Kurtz. 2015. "Dscam and Pancrustacean Immune Memory – A Review of the Evidence." *Developmental and Comparative Immunology* 48:315–23. <https://doi.org/10.1016/j.dci.2014.03.004>.
- Bateman, K. S., I. Tew, C. French, R. J. Hicks, P. Martin, J. Munro, and G. D. Stentiford. 2012. "Susceptibility to Infection and Pathogenicity of White Spot Disease (WSD) in Non-Model Crustacean Host Taxa from Temperate Regions." *Journal of Invertebrate Pathology* 110:340–51. <https://doi.org/10.1016/j.jip.2012.03.022>.
- Blix, T. B., R. A. Dalmo, A. Wargelius, and A. I. Myhr. 2021. "Genome Editing on Finfish: Current Status and Implications for Sustainability." *Reviews in Aquaculture* 13:2344–63. <https://doi.org/10.1111/raq.12571>.
- Buckle, K. M., and J. A. Yoder. 2022. "The Evolution of Innate Immune Receptors: Investigating the Diversity, Distribution, and Phylogeny of Immune Recognition across Eukaryotes." *Immunogenetics* 74:1–4. <https://doi.org/10.1007/s00251-021-01243-4>.
- Cock, J., T. Gitterle, M. Salazar, and M. Rye. 2009. "Breeding for Disease Resistance of Penaeid Shrimps." *Aquaculture* 286:1–11. <https://doi.org/10.1016/j.aquaculture.2008.09.011>.
- Cohen, J. 2024. "Meat from Gene-Edited Pigs Could Hit the Market." *Science* 383 (6686): 940–41. <https://doi.org/10.1126/science.ado9328>.
- Criscitiello, M. F., and P. de Figueiredo. 2013. "Fifty Shades of Immune Defense." *PLOS Pathogens* 9 (2): e1003110. <https://doi.org/10.1371/journal.ppat.1003110>.
- Dickson, M., N.-A. Ahmed, D. Kenawy, and F. Kruijsen. 2016. "Increasing Fish Farm Profitability through Aquaculture Best Management Practice Training in Egypt." *Aquaculture* 465:172–78. <https://doi.org/10.1016/j.aquaculture.2016.09.015>.
- FAO. 2020. "The State of World Fisheries and Aquaculture." Rome: FAO. <http://www.fao.org/documents/card/en/c/ca9229en>.
- . 2022. "The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation." Rome: FAO. <https://doi.org/10.4060/cc0461en>.
- Garlock, T., F. Asche, J. Anderson, A. Ceballos-Concha, D.C. Love, T.C. Osmundsen, and R.B.M. Pincinato. 2022. "Aquaculture: The Missing Contributor in the Food Security Agenda." *Global Food Security* 32:100620. <https://doi.org/10.1016/j.gfs.2022.100620>.
- Gonzalez-Duarte, R. J., W. Espinosa de Aquino, F. García-Carreño, and L. Rojo-Arreola. 2021. "The Hurdles of Delivery CRISPR-Cas9 Components for Gene Editing in Penaeid Shrimps." *Aquaculture Research* 52:5297–5306. <https://doi.org/10.1111/are.15398>.
- Gratacap, R. L., A. Wargelius, R. B. Edvardsen, and R. D. Houston. 2019. "Potential of Genome Editing to Improve Aquaculture Breeding and Production." *Trends in Genetics* 35:672–84. <https://doi.org/10.1016/j.tig.2019.06.006>.
- Gui, T., J. Zhang, F. Song, Y. Sun, S. Xie, K. Yu, and J. Xiang. 2016. "CRISPR/Cas9- Mediated Genome Editing and Mutagenesis of EcChi4 in Exopalaemon Carinicauda." *G3: Genes Genomes Genetics* 6:3757–64. <https://doi.org/10.1534/g3.116.034082>.

- Gutási, A., S. E. Hammer, M. El-Matbouli, and M. Saleh. 2023. "Review: Recent Applications of Gene Editing in Fish Species and Aquatic Medicine." *Animals* 13:1250. <https://doi.org/10.3390/ani13071250>.
- Hauton, C., and V. J. Smith. 2007. "Adaptive Immunity in Invertebrates: A Straw House without a Mechanistic Foundation." *BioEssays* 29:1138–46. <https://doi.org/10.1002/bies.20650>.
- Iwama, R. E., and Y. Moran. 2023. "Origins and Diversification of Animal Innate Immune Responses against Viral Infections." *Nature Ecology and Evolution* 7:182–93. <https://doi.org/10.1038/s41559-022-01951-4>.
- Kawato, S., K. Nishitsuji, A. Arimoto, K. Hisata, M. Kawamitsu, R. Nozaki, H. Kondo, et al. 2021. "Genome and Transcriptome Assemblies of the Kuruma Shrimp, *Marsupenaeus Japonicus*." *Genes, Genomes, Genetics* 11 (11): jkab268. <https://doi.org/10.1093/g3journal/jkab268>.
- Kumagai, H., T. Nakanishi, T. Matsuura, Y. Kato, and H. Watanabe. 2017. "CRISPR/Casmediated Knock-in via Non-Homologous End-Joining in the Crustacean *Daphnia Magna*." *PLoS One* 12:e0186112. <https://doi.org/10.1371/journal.pone.0186112>.
- Ledesma, A. V., and A. L. Van Eenennaam. 2024. "Global Status of Gene Edited Animals for Agricultural Applications." *The Veterinary Journal* 305:106142. <https://doi.org/10.1016/j.tvjl.2024.106142>.
- Liberti, A., O. Natarajan, C. G. F. Atkinson, and L. J. Dishaw. 2022. "Secreted Immunoglobulin Domain Effector Molecules of Invertebrates and Management of Gut Microbial Ecology." *Immunogenetics* 74:99–109. <https://doi.org/10.1007/s00251-021-01237-2>.
- López-Ordaz, R., A. Caballero-Zamora, J. M. Yáñez, L. Montoya-Rodríguez, B. Gómez-Gil, S. A. Soto Rodríguez, A. Martínez Ortega, J. C. Quintana-Casares, H. Castillo-Juárez, and G. R. Campos-Montes. 2024. "Genetic (Co)Variation for Resistance to White Spot Syndrome Virus, Acute Hepatopancreatic Necrosis, and Overall Pond Survival in *Litopenaeus Vannamei*." *Aquaculture International* 32:4885–99. <https://doi.org/10.1007/s10499-024-01406-6>.
- Lv, Z., L. Qiu, W. Wang, Z. Liu, Q. Liu, L. Wang, and L. Song. 2020. "The Members of the Highly Diverse Crassostrea Gigas Integrin Family Cooperate for the Generation of Various Immune Responses." *Frontiers Immunology* 11:1420. <https://doi.org/10.3389/fimmu.2020.01420>.
- Martin, A., J. M. Serano, E. Jarvis, H. S. Bruce, J. Wang, S. Ray, C. A. Barker, L. C. O'Connell, and N. H. Patel. 2016. "CRISPR/Cas9 Mutagenesis Reveals Versatile Roles of Hox Genes in Crustacean Limb Specification and Evolution." *Current Biology* 26:14–26. <https://doi.org/10.1016/j.cub.2015.11.021>.
- Molcho, J., R. Manor, M. Shamsian, G. Sudarshan, R. Ofir, D. Parker, S. Weil, et al. 2022. "On Genome Editing in Embryos and Cells of the Freshwater Prawn *Macrobrachium Rosenbergii*." *Aquaculture* 558 (2022): 738391. <https://doi.org/10.1016/j.aquaculture.2022.738391>.
- Potts, R. W. A., A. P. Gutierrez, C. S. Penaloza, T. Regan, T. P. Bean, and R. D. Houston. 2021. "Potential of Genomic Technologies to Improve Disease Resistance in Molluscan Aquaculture." *Philosophical Transactions of the Royal Society B* 376:20200168. <https://doi.org/10.1098/rstb.2020.0168>.
- Press, C. M., and A. Lillehaug. 1995. "Vaccination in European Salmonid Aquaculture: A Reviews of Practices and Prospects." *British Veterinary Journal* 151:45–69. [https://doi.org/10.1016/S0007-1935\(05\)80064-8](https://doi.org/10.1016/S0007-1935(05)80064-8).
- Qin, N., M. Li, H. Zhang, F. Li, X. Guo, M. Wu, Q. Zhang, T. Tang, and F. Liu. 2023. "Single von Willebrand Factor C-Domain Protein Confers Host Defense against White Spot Syndrome Virus by Functioning as a Pattern Recognition Receptor in *Macrobrachium Nipponense*." *International Journal of Biological Macromolecules* 241:124520. <https://doi.org/10.1016/j.ijbiomac.2023.124520>.

- Smith, L. C., and C. M. Lun. 2017. "The SpTransformer Gene Family (Formerly Sp185/333) in the Purple Sea Urchin and the Functional Diversity of the Anti-Pathogen rSpTransformer-E1 Protein." *Frontiers Immunology* 8:725. <https://doi.org/10.3389/fimmu.2017.00725>.
- Smith, V. J., J. H. Brown, and C. Hauton. 2003. "Immunostimulation in Crustaceans: Does It Protect against Infection?" *Fish and Shellfish Immunology* 15:71–90. [https://doi.org/10.1016/S1050-4648\(02\)00140-7](https://doi.org/10.1016/S1050-4648(02)00140-7).
- Stentiford, G. D., D. M. Neil, E. Peeler, J. D. Shields, H. J. Small, T. W. Flegel, J. Vlcek, et al. 2012. "Disease Will Limit Future Food Supply from the Global Crustacean Fishery and Aquaculture Sectors." *Journal of Invertebrate Pathology* 110:141–57. <https://doi.org/10.1016/j.jip.2012.03.013>.
- Stentiford, G. D., K. Sritunyaluksana, T. W. Flegel, B. A. P. Williams, B. Withyachumnarnkul, O. Itsathitphaisarn, and D. Bass. 2017. "New Paradigms to Help Solve the Global Aquaculture Disease Crisis." *PLoS Pathogens* 13:e1006160. <https://doi.org/10.1371/journal.ppat.1006160>.
- Van Oosterhout, C. 2021. "Mitigating the Threat of Emerging Infectious Diseases; a Coevolutionary Perspective." *Virulence* 12:1288–95. <https://doi.org/10.1080/21505594.2021.1920741>.
- Verbruggen, B., L. K. Bickley, R. van Aerle, K. S. Bateman, G. D. Stentiford, E. D. Santos, and C. R. Tyler. 2016. "Molecular Mechanisms of White Spot Syndrome Virus Infection and Perspectives on Treatments." *Viruses* 8:23. <https://doi.org/10.3390/v8010023>.
- Wang, Q. L., C. M. Rossignoli, E. B. Dompok, J. Su, S. A. Ali, M. Karim, and A. Gasparatos. 2023. "Sustainable Intensification of Small-Scale Aquaculture Production in Myanmar through Diversification and Better Management Practices." *Environmental Research Letters* 18:015002. <https://doi.org/10.1088/1748-9326/acab16>.
- Xiao, R., Y. Wei, D. An, D. Li, X. Ta, Y. Wu, and Q. Ren. 2018. "A Review on the Research Status and Development Trend of Equipment in Water Treatment Processes of Recirculating Aquaculture Systems." *Reviews in Aquaculture* 11:863–95. <https://doi.org/10.1111/raq.12270>.
- Xu, S., T. P. Pham, and S. Neupane. 2020. "Delivery Methods for CRISPR/Cas9 Gene Editing in Crustaceans." *Marine Life Science Technology* 2:1–5. <https://doi.org/10.1007/s42995-019-00011-4>.
- Zhang, X., J. Yuan, Y. Sun, S. Li, Y. Gao, Y. Yu, C. Liu, et al. 2019. "Penaeid Shrimp Genome Provides Insights into Benthic Adaptation and Frequent Molting." *Nature Communications* 10:356. <https://doi.org/10.1038/s41467-018-08197-4>.
- Zheng, S.-C., J.-Y. Xu, and H.-P. Liu. 2019. "Cellular Entry of White Spot Syndrome Virus and Antiviral Immunity Mediated by Cellular Receptors in Crustaceans." *Fish and Shellfish Immunology* 93:580–88. <https://doi.org/10.1016/j.fsi.2019.08.011>.