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ABSTRACT: Climate change and the associated environmental temperature fluctuations are contributing to increases in the frequency and severity of disease outbreaks in both wild and farmed aquatic species. This has a significant impact on biodiversity and also puts global food production systems, such as aquaculture, at risk. Most infections are the result of complex interactions between multiple pathogens, and understanding these interactions and their co-evolutionary mechanisms is crucial for developing effective diagnosis and control strategies. In this review, we discuss current knowledge on bacteria–bacteria, virus–virus, and bacterial and viral co-infections in aquaculture as well as their co-evolution in the context of global warming. We also propose a framework and different novel methods (e.g. advanced molecular tools such as digital PCR and next-generation sequencing) to (1) precisely identify overlooked co-infections, (2) gain an understanding of the co-infection dynamics and mechanisms by knowing species interactions, and (3) facilitate the development multi-pathogen preventive measures such as polyvalent vaccines. As aquaculture disease outbreaks are forecasted to increase both due to the intensification of practices to meet the protein

demand of the increasing global population and as a result of global warming, understanding and treating co-infections in aquatic species has important implications for global food security and the economy.

KEY WORDS: Host · Temperature · Climate change · Treatments · Fish · Shellfish · Disease outbreaks

1. INTRODUCTION

Both macro- and micro-parasites (or pathogens) are common in natural ecological communities, and most hosts are usually infected by multiple pathogenic species at the same time, a phenomenon known as co-infection (Kinnula et al. 2017). During co-infections, multiple pathogens are active in the same host, leading to a complex network of interactions. These interactions have the potential to alter disease dynamics, modify pathogen virulence, and influence the host's immune system.

Pathogen interactions can range from mutualistic, whereby pathogens mutually benefit each other resulting in synergetic interactions, to competitive (pathogenic species competing for resources and displaying negative effects on each other, also known as antagonistic interactions) (Mideo 2009, Telfer et al. 2010, Kotob et al. 2016). Synergistic co-infections can be particularly detrimental to the host, often resulting in high mortality rates. For instance, one pathogen can facilitate the invasion of another, potentially enhancing its virulence and even transferring virulence factors (de Lorgeril et al. 2018). Certain pathogens, such as bacteria, can exhibit cooperative behaviors (organisms working or acting together for common or mutual benefits); for example, towards the production of 'public goods' that assist in the invasion of other pathogens (Griffin et al. 2004). Additionally, the suppression or imbalance of the host immune system (immunosuppression) may facilitate the infection of secondary pathogens (Molina & Vilchez 2014, de Lorgeril et al. 2018). In some antagonistic interactions, the competition for host resources favors the selection and proliferation of the fittest pathogen, sometimes leading to proliferation of the most virulent pathogens (Mideo 2009, M. Sofonea et al. preprint doi/10.1101/258004). These interactions can, therefore, lead to altered pathogen composition, abundance, and interaction dynamics (i.e. modified host and pathogen interactions and pathogenicity) that differ from those observed in single infections (Read & Taylor 2001, Mideo 2009, Kotob et al. 2016).

Co-infections of aquatic animals by multiple pathogens are common, yet their investigation is often challenging due to the continual onslaught of existing and new

infectious agents (Lafferty et al. 2015, Flegel 2020). Disease outbreaks pose a significant problem in global aquaculture, with most aquatic diseases typically attributed to single etiological agents, such as specific bacteria or viruses (Kotob et al. 2016, de Lorgeril et al. 2018, English & Lima 2020). However, recent research is shedding light on the importance of diagnosing and understanding co-infections in aquatic animals to gain a better understanding of disease outbreaks (Petton et al. 2021, Wise et al. 2021). For instance, the increased juvenile Pacific oyster mortalities observed since 2008 have been linked to a polymicrobial infection (de Lorgeril et al. 2018, Petton et al. 2021). Oysters are first infected by ostreid herpesvirus infection (OsHV-1 µVar), which immunocompromises oysters by altering hemocyte physiology, facilitating secondary colonization by opportunistic bacterial pathogens, and resulting in oyster death (de Lorgeril et al. 2018). There is, therefore, an urgent need for a deeper understanding of how microorganisms interact to cause pathogenesis in the host, particularly considering how co-infection mechanisms may be exacerbated or modified by changing environmental conditions. This knowledge is crucial for disease control and prevention, effective aquaculture management, and the conservation of aquatic animal populations.

Seawater temperature increase is one of the main effects of climate change (Jyväsjärvi et al. 2015, Barbarossa et al. 2021) and can have profound effects on the biochemical, physiological, and behavioral processes of many organisms, including aquatic ectotherms (Volkoff & Rønnestad 2020, Deldicq et al. 2021). Warmer temperatures have been associated with decreased fitness, increased stress levels, and larki-depression in aquatic species, rendering them more susceptible to infections (Guo & Dixon 2021). Research has indicated that elevated temperatures can lead to increased disease outbreaks and fatalities among aquatic organisms, as higher temperatures can enhance the metabolism and, at times, the virulence of microorganisms (Karvonen et al. 2010, Kimes et al. 2012, Leung & Bates 2013, Reverter et al. 2020). The impacts of temperature increase on both the host's fitness and various pathogens have the potential to influence co-infection mechanisms and dynamics, although this area remains poorly understood. This review examines the implications of climate warming on bacterial and viral co-infections in aquaculture, including co-evolutionary dynamics, diagnosis methods, treatment options, and strategies for more sustainable disease management under climate change.

2. COMMON BACTERIAL AND VIRAL CO-INFECTIONS IN AQUACULTURE

2.1. Bacterial co-infections

Both natural and experimental bacterial co-infections have been reported in numerous aquatic species and have sometimes been suggested to be related to elevated water temperatures (**Table 1**) (Karlsen et al. 2014, Hjerde et al. 2015, Wise et al. 2021). For example, in striped mullet *Mugil cephalus*, co-infection with *Aeromonas hydrophila* and *Vibrio parahaemolyticus* was confirmed through biochemical tests, genome sequencing, and phylogenetic analysis. During the summer months, when poor water quality and elevated temperatures were observed at the fish farm, high mortality rates ranging from 75–85% were linked to these co-infections (El-Son et al. 2021). Similarly, striped catfish *Pangasianodon hypophthalmus* experience higher mortality rates (95%) when co-infected with *Edwarsiella larkiad* and *A. hydrophila* compared to single infections (80 and 10%, respectively) (Crumlish et al. 2010). Co-infected *P. hypophthalmus* with *E. larkiad* and *Flavobacterium columnare* also displayed higher mortalities (86.7–100%) than in single infections (80 and 3.3%, respectively) (Dong et al. 2015). These findings demonstrate that many bacterial co-infections can lead to significantly higher host mortalities compared to single-pathogen infections (Wise et al. 2021). However, antagonistic bacterial interactions resulting in lower host mortality have also been described, highlighting the complex nature of bacterial co-infections (Karlsen et al. 2014, Hjerde et al. 2015). Karlsen et al. (2014) observed that Atlantic salmon *Salmo salar* co-infected first with *Aliivibrio wodanis* and consequently by *Moritella viscosa* displayed lower mortalities than fish only infected by *M. viscosa*. They hypothesized that both bacteria may be competing for the same niche and that *A. Wodanis* may be able to outcompete *M. viscosa* growth by secreting toxins. Although both *M. viscosa* and *A. wodanis* are known etiological agents of winter ulcer disease, Karlsen et al. (2014) showed that co-infection prolonged the disease progression and pathogenesis. Low temperatures are a key factor of *M. viscosa* proliferation; however, the effect of temperature (i.e. increases or decreases) on the co-infection dynamics and the consequent effects on the hosts are not yet well elucidated. It is noteworthy that many bacterial pathogens can persist in close contact (e.g. surrounding environment, mucosa) of the host tissues for extended periods without causing harm. Therefore, sometimes opportunistic bacterial infections occur as secondary agents, with viruses or other pathogens (i.e. macro-parasites) acting as the primary

pathogens responsible for invading aquatic animals and allowing bacteria to enter via the creation of physical injuries or host immunosuppression (Barbosa Solomieu et al. 2015, de Lorgeril et al. 2018, Pękala-Safińska 2018, Nicholson et al. 2020, Ramírez-Paredes et al. 2021).

Bacterial co-infections are very common in aquatic farmed animals (Wise et al. 2021); however, as illustrated in the examples above, characterizing the different co-infection agents and understanding their interaction dynamics, including their different trajectories under different environmental conditions such as elevated temperature, is required to understand their effects on hosts and to allow design of effective treatment strategies.

2.2. Viral co-infections

Viral co-infections in aquatic animals are a poorly studied area of research; however, as with bacterial co-infections, evidence shows that viral co-infections can both lower and increase host mortality, highlighting the need to understand these co-infections on a case-to-case basis. *In vitro* experiments using monolayers of BF2 cells (a fibroblast-like cell that was isolated from the caudal trunk of 1 yr old bluegill, *Lepomis macrochirus*) pre-treated with supernatants of infected brown trout *Salmo trutta* revealed that infectious pancreatic necrosis virus (IPNV) infection exhibited antiviral activity against infectious hematopoietic necrosis virus (IHNV) due to the presence of interferon-like proteins (Saint-Jean & Pérez-Prieto 2007). *In vivo*, co-infection of *S. trutta* with equal infectious titers of IPNV and IHNV resulted in lower mortality (40%) compared to infection with either virus alone (65% for IPNV and 70–75% for IHNV) (Saint-Jean & Pérez-Prieto 2007). This protective effect may be attributed to the induction of an Mx gene, a marker of GTPases, in the kidney, liver, and spleen 3 d post-stimulation, which inhibits virus replication mediated by type I interferons (IFN-I) (Saint-Jean & Pérez-Prieto 2007). The impact of IPNV on the replication of IHNV and viral hemorrhagic septicemia virus (VHSV) was also evaluated in BF2 cells derived from bluegill *L. macrochirus*. The co-infection of IPNV and IHNV in these cells also resulted in a reduction in IHNV infectivity and the expression of IHNV viral antigens but had no effect on VHSV replication (Rodriguez et al. 2005). Similarly, Pakingking et al. (2004) examined the effects of non-lethal aquabirnavirus (ABV)–VHSV co-infection *in vitro* and *in vivo* in Japanese flounder *Paralichthys olivaceus*. *In vitro* assays using hirame natural embryo cells demonstrated that fish serum from ABV-infected cells exhibited antiviral activity against VHSV. *In vivo* results suggested that primary infection with a less virulent strain of ABV

decreased VHSV virulence through the induction of IFNs (Pakingking et al. 2004). Altogether, these studies show that viral co-infections in aquatic animals often result in viral interference, with one virus affecting the replication of another virus through competitive inhibition. However, in some cases, co-infecting viruses can co-exist (also known as accommodation) and can modify the virulence and, hence, disease severity (Okon et al. 2023).

For example, in shrimp *Litopenaeus vannamei*, viral co-infection with white spot syndrome virus (WSSV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV) resulted in 100% mortality, which was linked to the suppression of immune parameters such as phenoloxidase activity, superoxide dismutase, hemocyte counts, and decreased gene expression of prophenoloxidase and peroxinectin (Yeh et al. 2009). Similarly, mass mortalities of giant tiger prawn *Penaeus monodon* post-larvae were observed when infected with multiple viruses including monodon baculovirus (MVB), hepatopancreatic parvovirus (HPV), and WSSV (Manivannan et al. 2002). However, in some cases, shrimp naturally infected with multiple viruses (HPV, MVB, IHHNV, and WSSV) showed no mortalities but were reduced in size (Flegel et al. 2004). The tolerance of viral co-infections in shrimp, whereby they can coexist with viruses without exhibiting signs of disease, is still poorly understood. (Flegel 2009, 2020). Bonnichon et al. (2006) suggested that persistent viral infections like IHHNV may protect against more virulent viruses like WSSV in *L. vannamei.* The complexity of predicting the effects of co-infections on virulence and the selection of favored strains arises from the interplay of host and environmental factors on microorganism fitness as well as the potential role of co-evolutionary dynamics (Alizon & van Baalen 2008, Alizon et al. 2013).

Although some viral co-infections in reared aquatic animals have been characterized and some molecular mechanisms that may lead to synergetic or antagonist viral interactions have been described, the impact of exogenous parameters such as water temperature on viral co-infections remains unexplored. Many viral diseases in aquatic animals are tightly linked to increases in water temperature (e.g. cyprinid herpesvirus 3, CyHV-3; koi herpesvirus disease, KHV; and OsHV) (Bergmann & Kempter 2011, de Katnzow et al. 2016), which may mean that increases in temperature could lead to increases in the frequency and outcome of viral co-infections, but this topic requires further research.

2.3. Bacterial and viral co-infections and other co-infections

There are limited studies on bacterial and viral co-infections in fish and shellfish, but the available evidence suggests that co-infections with multiple pathogens often result in higher mortalities (i.e. virulence) compared to infections with a single pathogen. For instance, in laboratory experiments, tilapia (*Oreochormis niloticus* and *Oreochromis* spp.) infected with both tilapia lake virus (TiLV) and *A. hydrophila* had a mortality rate of 93%. By contrast, experimental infection with TiLV alone resulted in 34% mortality, and *A. hydrophila* alone caused 6.7% mortality (Nicholson et al. 2020). Co-infection between infectious spleen and kidney necrosis virus (ISKNV) and *Streptococcus agalactiae* has also been associated with high mortalities (>50%) in tilapia (Assis et al. 2017, Ramírez-Paredes et al. 2021). In Chinese perch *Siniperca chuatsi* culture ponds, co-infection with *A. hydrophila* and ISKNV was detected, and the study of interaction mechanisms revealed complex mixed antagonistic and synergistic effects. These effects involved the elevated expression of IRF1, Mx, Viperin, hepcidin, TNFα, and IL-1β mRNAs genes. Simultaneous inoculation with both pathogens resulted in increased host mortality (Liu et al. 2020). Accelerated mortalities have also been observed in whiteleg shrimp *L. vannamei* infected with WSSV, *V. parahaemolyticus*, and *V. anguillarum*. Particularly, when tripartite co-infection experiments were conducted, genes involved in the shrimp's innate immunity, such as prophenoloxidase 1 and 2 (ProPO), were down-regulated, while genes like LvMyD88 (myeloid differentiation factor 88, involved in the toll signaling activation pathway) and Lvakt (gene encoding AKT proteins and key component of the PI3K–AKT pathway, involved intracellular signaling during virus invasion) were up-regulated, suggesting that LvMyD88 and Lvakt may play a role in the shrimp immune response against viruses (Jang et al. 2014, Zhang et al. 2016).

In crayfish *Procambarus larkia*, experimental co-infection with WSSV and *Aeromonas veronii* also resulted in higher mortalities (100%) compared to *A. veronii* infection alone (70%) or WSSV infection alone (83.3%) (Yuan et al. 2021). Additionally, infection of Pacific oyster juveniles *Crassostrea gigas* with OsHV-1 μVar leads to an immune-compromised state that facilitates opportunistic bacterial colonization and pathogenicity, resulting in bacteremia and death (de Lorgeril et al. 2018). These findings highlight the detrimental impact of bacterial and viral co-infections on the health of fish and shellfish. However, in most cases, the mechanisms by which this is achieved (i.e. microorganism cooperation, sequential immunosuppression, etc.) are as yet extremely poorly understood.

In contrast to the previously mentioned examples, co-infection of *L. vannamei* with WSSV and *V. parahaemolyticus* resulted in lower mortality (83%) compared to WSSV infection alone (mortality of 97%). This suggests a potential competition between the pathogens, with *V. parahaemolyticus* inhibiting the replication of WSSV. However, immune gene expression in the gills of co-infected shrimp was higher than in the WSSV-infected group, indicating that the enhanced immune responses triggered by *V. parahaemolyticus* may contribute to the reduction in WSSV infection success (Pang et al. 2019).

Interestingly, Louhi et al. (2015) found that co-infection virulence of the bacterium *F. columnare* and the fluke *Diplostomum pseudospathaceum* in rainbow trout *Oncorhynchus mykiss* was not only associated with the identity of the co-infecting partners (i.e. species) but with their genotypes, which interacted differently and resulted in different virulence. Although most co-infections resulted in increased host mortalities, some reduced the fluke infection rate, suggesting that co-infections can drive the pathogen's fitness phenotypic variation.

Overall, the available literature highlights the complexity of co-infections, and that virulence evolution is probably largely shaped by the ecological and evolutionary interactions between co-infecting pathogens.

3. CO-EVOLUTIONARY IMPLICATIONS OF AQUACULTURE DISEASES UNDER CLIMATE WARMING

Co-evolutionary implications arise when 2 or more populations engage in long-term interactions, leading to reciprocal evolutionary change. This concept is often referred to as co-evolution. The Red Queen Hypothesis, proposed by Van Valen (1973), suggests that interacting species are in a continuous cycle of adaptation and evolution in response to each other. This idea finds strong support in host–parasite systems, whereby the host evolves mechanisms to evade the parasite, and the parasite counter-adapts to exploit the host (Kaltz & Shykoff 1998). In co-evolutionary dynamics, 2 main patterns can emerge: arms-race dynamic (ARD) and fluctuating selection dynamics (FSD). In ARD, both species accumulate adaptive mutations in directional evolution, constantly trying to outpace each other's adaptations. On the other hand, FSD promotes genetic variance and negative frequency-dependent selection, meaning that the fitness of a particular trait depends on its frequency in the population (Martiny et al. 2014, Strotz et al. 2018). In the context of pathogen–host interactions in aquaculture settings, understanding co-evolutionary implications is crucial for managing

disease outbreaks. By studying these dynamics, we can gain insights into the mechanisms underlying the evolution of virulence in pathogens and the evolution of host resistance. Additionally, co-evolutionary dynamics can shed light on the emergence of new strains or variants that can overcome existing host defenses, leading to disease outbreaks.

It is worth noting that co-evolutionary processes are complex and influenced by various factors, including genetic diversity, population size, ecological interactions, and environmental conditions. Therefore, studying co-evolution in pathogen–host systems requires a multidisciplinary approach that combines genetics, ecology, and evolutionary biology.

By understanding the co-evolutionary dynamics between pathogens and hosts, we can develop more effective strategies for disease prevention and control in aquaculture, such as implementing selective breeding programs to enhance host resistance or using management practices that disrupt the arms race between pathogens and hosts.

3.1. Within-host mixed-genotype interactions and consequences for disease severity and development

Studies have revealed that co-infection with multiple strains or genotypes of the same species is a common occurrence in bacterial and viral infections (Alizon & van Baalen 2008, Mideo 2009, Klafack et al. 2019, Leeks et al. 2019). Within-host mixed-genotype interactions can exhibit dynamics similar to those observed in co-infections between different species, involving competition for host resources and cooperation to evade the immune system (Alizon & van Baalen 2008, Mideo 2009). These interactions can lead to more severe infections and facilitate the development of antiviral resistance, enabling the pathogen to adapt to new hosts (Alizon & van Baalen 2008, Leeks et al. 2018).

The presence of mixed genotypes within hosts plays a significant role in driving co-evolutionary mechanisms, both in ARD and FSD (Strotz et al. 2018). Genetically distinct strains of parasites compete for host resources and exhibit cooperation or evasion strategies against the host's immune system, and these interactions have implications for the evolution of parasite and disease severity (Mideo 2009, Martiny et al. 2014, M. Sofonea et al. preprint doi:10.1101/258004).

These within-host mixed-genotype interactions contribute to the complexity of disease dynamics and have important implications for disease management. The presence of

multiple strains or genotypes can enhance the overall virulence of the infection and pose challenges for treatment strategies. Additionally, the co-existence of different genotypes can lead to the emergence of novel variants through genetic recombination or reassortment, further complicating disease control efforts.

A study by Delmotte et al. (2020) revealed that 2 distinct populations of OsHV-1 µVar infected different oyster families on French coasts (Atlantic and Mediterranean), indicating the presence of viral diversity and suggesting co-evolutionary interactions between the viruses and oyster populations. This highlights the importance of considering mixed-genotype co-infections in understanding disease development and severity (Mideo 2009, Sofonea et al. 2017). Similar processes have been studied in fish, where asymptomatic carp *Cyprinus carpio* can be infected by multiple haplotypes of CyHV-3 (Avarre et al. 2012).

In the case of CyHV-3, Gao et al. (2018) sequenced the genomes of 7 strains from different sites and observed 2 genetic clades (European and Asian), with evidence of inter-linage recombination, suggesting the existence of a third, unidentified lineage. Interestingly, the strains with the highest cell fitness *in vitro* were those with the longest cell passage and lowest virulence. Serial passages experiment of CyHV-3 in brain cells also showed that *in vitro* evolution of the virus resulted in a mixture of haplotypes, and the passage 78 isolate was less virulent than the original isolate or passage 99, indicating the potential for attenuation of viral strains (Klafack et al. 2019). Attenuated viruses elicit an immune response in vertebrates and can spread through large populations (Marsden et al. 1996, Ronen et al. 2003).

The presence of multiple viral genomes within cells or hosts can contribute to the maintenance of viral genetic diversity, and cooperation between different viral variants, such as immune evasion strategies, may play a role in virus–virus interactions and evolution (Sanjuán 2017). Viruses can generate de novo diversity rapidly, allowing them to adapt to new hosts and environments, especially in the presence of changing environmental conditions (Duffy et al. 2008).

Studying cell-to-cell viral transfer and understanding its implications for virus–virus interactions are areas that are still not well understood but hold promise for future research. Although viral replication in cell cultures is crucial for studying mixed-genotype co-infections, stable cell lines for invertebrate aquatic virology studies are limited (Vega-Heredia & Giffard-Mena 2021).

Understanding the dynamics of mixed-genotype co-infections and utilizing molecular tools offer valuable avenues for research. This approach would allow us to explore viral genetic diversity driven by mutation rates, which can contribute to managing drug resistance, immune escape, the emergence of new viruses, and the design of antiviral strategies in aquaculture co-infections.

3.2. Microbe–host horizontal gene transfer

Horizontal gene transfer (HGT) is a significant mechanism for the acquisition of novel genes and metabolic functions facilitating co-evolution among organisms (Boucher et al. 2003). In the context of viral infections in shrimp, IHHNV can persist silently in infected shrimp without causing visible signs of disease (Tang & Lightner 2006, Flegel 2009, 2020, Saksmerprome et al. 2011, Goic & Saleh 2012). Some shrimp species such as *Penaeus monodon*, *Litopenaeus vannamei*, and *L. stylirositris* have been observed to be resistant to IHHNV at certain stages of their life cycle (Tang & Lightner 2006, Saksmerprome et al. 2011, Flegel 2020).

One explanation for this resistance is that endogenous viral elements (EVEs) have been autonomously incorporated into the host genome. These EVEs are derived from the viral mRNA and act as a defense mechanism in shrimps, utilizing the RNA interference (iRNA) mechanism (Flegel 2009, 2020). According to Flegel's hypothesis, shrimp carrying protective EVEs would exhibit tolerance to lethal viruses and gain selective advantages over shrimp lacking such EVEs. This would result in positive selection for less virulent viral mutations and negative selection for more virulent ones. This could explain the high degree of tolerance to IHHNV observed in regions where both the virus and shrimp species are endemic (Flegel 2009, 2020).

If the shrimp EVE hypothesis is proven to be protective against viral diseases, it could have practical applications in breeding programs. The insertion of EVEs into specific genomic positions, analogous to natural genetic modification in shrimp, could be used to produce specific pathogen-free (SPF) stocks of shrimp or other organisms that exhibit tolerance to multiple viruses (Flegel 2009, 2020). However, it is necessary to fully understand the mechanisms and implications of EVEs in providing viral resistance and their potential applications in breeding programs.

3.3. Microbe–microbe HGT

The phylogenetic analysis of bacterial, archaeal, and eukaryotic genomes has provided evidence that a portion of genes in prokaryotic genomes have undergone horizontal transfer (Koonin et al. 2001). HGT is a well-known strategy employed by bacteria and other microbes to disseminate traits through the environment, enabling microbial cooperation and facilitating the acquisition of evolutionary novelties (Lee et al. 2022). HGT also plays a crucial role in driving microbial co-evolution and can even lead to the formation of hybrid organisms with enhanced fitness (Boto 2010, Power et al. 2021).

Studies on antibiotic resistance gene (ARG) transfer in aquaculture systems have demonstrated the occurrence of HGT. For example, research on *Vibrio parahaemolyticus* isolates and related bacterial species from shrimp farms revealed horizontal transfer of 278 genes between strains, with implications for antibiotic resistance, virulence, and metabolic fitness (Fu et al. 2022, Wang et al. 2022, Wanyan et al. 2023). HGT events were more frequent among closely related organisms or within habitats with similar environmental characteristics, such as high population densities where cells are nearby and capable of gene exchange (Kloesges et al. 2011, Fuchsman et al. 2017).

Various environmental factors, including nitrogen levels, pH, and temperature as well as microbial alpha diversity, mobile genetic elements, and the presence of opportunistic pathogens, have been implicated in the dissemination of ARGs in the gut of red swamp crayfish *Procambarus clarkii* (Wanyan et al. 2023). Furthermore, a positive correlation between heavy metal levels and florfenicol resistance was observed in the gut microbiomes of 3 fish species reared in aquaculture. In that study, 20 ARGs associated with antibiotic efflux, inactivation, target alteration, target protection, target replacement, and reduced antibiotic permeability were detected, and their spread was linked to physicochemical factors of the water (Wang et al. 2022). These findings highlight the importance of HGT in the dissemination of antibiotic resistance and the role of environmental factors in shaping the spread of ARGs in aquaculture settings. The development of effective strategies to mitigate the emergence and spread of antibiotic resistance in aquaculture systems is crucial. Thus, HGT is a significant mechanism for microbes to acquire new genes and traits, allowing them to adapt to their environment more effectively. Studies have shown that certain microbial communities, particularly those inhabiting anaerobic and high-temperature environments, have a higher propensity for HGT and gene sharing (Fuchsman et al. 2017). However, salinity does not seem to have a similar effect on gene transfer. While HGT is well-established as a mechanism for microbial evolution and co-evolution, its specific relevance to

host disease dynamics, particularly in the context of co-infections, deserves more attention (Boto 2010, Fuchsman et al. 2017).

The transfer of ARGs through HGT can have detrimental effects on co-infections and can pose challenges in the treatment of disease outbreaks in aquaculture. Similarly, the transfer of virulence factors via HGT can aggravate the severity of the disease outbreaks. It has been observed that warmer environments and laboratory settings exhibit higher rates of HGT, suggesting that global warming may potentially increase HGT rates (Fuchsman et al. 2017, Pallares-Vega et al. 2021).

4. IMPACT OF GLOBAL WARMING ON AQUACULTURE DISEASES AND CO-INFECTIONS

Temperature increases have profound effects on various micro- and macro-organisms, impacting biochemical, physiological, and behavioral processes (Vaumourin & Laine 2018). In the context of aquatic ecosystems, higher temperatures pose particular risks for ectothermic organisms, leading to heightened stress levels and compromised immune parameters (Harvell et al. 1999, Cascarano et al. 2021). These swelling temperature-induced stressors create favorable conditions for the occurrence and severity of co-infections.

The relationship between augmented temperatures and microbial dynamics has important implications for disease outbreaks and co-infections in both terrestrial and aquatic ecosystems. Studies have shown that elevated temperatures can lead to increased prokaryote metabolic and evolution rates (Smith et al. 2019) as well as higher antimicrobial resistance through HGT (MacFadden et al. 2018, Reverter et al. 2020) (**Fig. 1**). This is particularly notable in bacterial pathogens such as *Vibrio* species, which have shown increased abundance and prevalence in response to rising seawater temperatures (Vezzulli et al. 2012, 2016). Correspondingly, there has been a reported increase in *Vibrio* species infections in humans, attributed to the expanding geographic range of *Vibrio* due to temperature addition (Froelich & Daines 2020). See Table 1 for references.

Furthermore, experimental evidence has demonstrated higher mortalities in farmed aquaculture animals (oysters, carp) infected with bacterial and viral pathogens under warmer temperatures (Reverter et al. 2020, Combe et al. 2023). Given that the virus life cycle, including replication, is linked to the host's metabolism, temperature escalation is expected to affect host–virus interactions (Danovaro et al. 2011) like biochemical, physiological, and

behavioral processes in organisms, leading to increased stress and compromised immune systems in aquatic species, ultimately resulting in higher mortality rates of infected animals (Vaumourin & Laine 2018, Karvonen et al. 2021) (Fig. 1).

Higher temperatures and longer warmer periods enhance viral propagation within hosts, resulting in higher viral loads and transmission rates (Boyko et al. 2000, Amari et al. 2021). Warmer temperatures lead to increased opportunities for viral transmission among species that were previously geographically isolated (Jones 2020, Carlson et al. 2022, McKay, 2023). Notably, fluctuations and elevated water temperatures have been linked to reactivation and outbreaks of specific viruses such as CyHV-3 (St-Hilaire et al. 2005, Yuasa et al. 2008, Takahara et al. 2014) and OsHV-1 (de Kantzow et al. 2016, Prado-Alvarez et al. 2016, Delisle et al. 2018).

Global warming may lead to more disease outbreaks and co-infections in land and water ecosystems (Karvonen et al. 2010, Baker et al. 2022). Alterations in climatic conditions can disrupt ecological disease patterns, leading to the convergence of infections that would typically occur separately, ultimately resulting in co-infections and increased host mortality (Munson et al. 2008). For example, above-average winter temperatures have been associated with severe disease outbreaks involving co-infections between a bacterium, *Anaplasma phagocytophilum*, and a parasite, *Babesia divergens*, transmitted by ticks (Johnson et al. 2020), which is a well-known terrestrial disease. Similarly, co-infection of goldfish *Carassius auratus* by an ectoparasite, *Argulus* sp., and a bacterium, *Aeromonas hydrophila*, cause temperature-dependent mortalities, with the highest mortalities occurring at higher temperatures Shameena et al. (2021).

Temperature rise can also influence congener co-infection by facilitating the co-existence of multiple pathogen lineages, thereby altering the course of infection development (Fargues & Bon 2004). Co-infections play a crucial role in maintaining genetic variation in pathogens, potentially accelerating their adaptation to environmental changes and leading to the emergence of new genetic variants with variable traits (Vaumourin & Laine 2018). Recent studies have shown that elevated water temperatures (28°C) can enhance the expression of virulent genes in *A. hydrophila* infecting rohu fish *Labeo rohita* (Pattanayak et al. 2020).

Based on the presented evidence, to advance our understanding in this area, urgent research is needed to address the following questions: (1) How does global warming affect

the complex dynamics of inter and intra-specific co-infections? (2) What is the combined impact of elevated temperature and co-infections on disease severity and morbidity? (3) Does the temperature escalation favor the selection of more virulent pathogens? Investigating these aspects will provide valuable insights into the consequences of global warming on pathogen dynamics and the potential for increased virulence.

5. A FRAMEWORK TO STUDY CO-INFECTIONS IN AQUACULTURE

Co-infections have a significant impact on the severity and mortality rates of disease outbreaks in aquaculture. In this regard, we propose a framework to address 3 key knowledge gaps regarding co-infections in aquaculture: (1) Detection of co-infections in aquatic species and aquaculture settings, (2) Understanding the mechanisms and dynamics of co-infections, and (3) Developing effective treatments for co-infections (**Fig. 2**).

To tackle the first knowledge gap, the development and application of advanced diagnostic techniques, such as next-generation sequencing (NGS) and metagenomics or digital PCR (dPCR), can enable the simultaneous detection of multiple pathogens in a single sample. These approaches will provide a comprehensive view of the co-infection landscape in aquaculture systems.

To address the second knowledge gap, studies integrating ecological and epidemiological approaches are needed. Longitudinal monitoring of co-infection dynamics coupled with detailed ecological data on host–pathogen interactions and environmental factors can elucidate the mechanisms underlying co-infection patterns and their impacts on disease progression. Cell culture for laboratory experimentation and mathematical modeling will assist with this task.

Finally, addressing the third knowledge gap requires the development of targeted treatments for co-infections. This can involve the identification of key molecular pathways or host immune responses that can be modulated to mitigate the severity of co-infections. Additionally, the use of innovative treatment strategies, such as genetic manipulation, phage therapy, or combination therapies, should be explored to effectively combat co-infections in aquaculture.

By adopting this framework and leveraging novel methods and technologies, we can significantly advance our understanding of co-infections in aquaculture. This knowledge will

ultimately contribute to the development of effective strategies for disease management and prevention, ensuring the sustainability and productivity of aquaculture systems.

5.1. Detecting and understanding co-infections in aquaculture

The impact of global warming on pathogen interactions highlights the importance of promptly detecting co-infections in aquaculture disease management. To understand how microorganisms cooperate to induce pathogenesis in the host, various technologies are available.

5.2. dPCR

dPCR is a highly sensitive and accurate method for absolute quantification of DNA samples, eliminating the need for standard curves. This technique involves distributing DNA across multiple replicate reactions, enabling the use of Poisson statistics for precise quantification (Sedlak & Jerome 2013). By directly calculating the DNA molecule number from positive and negative reactions, dPCR provides absolute quantification and can determine the number of DNA copies per ml, particularly for low viral loads (Sedlak & Jerome 2013). Moreover, dPCR exhibits increased sensitivity and precision compared to traditional PCR assays or even multiplex PCR, making it capable of detecting mutant sequences that may be undetected by sequencing methods.

In the field of aquaculture, traditional microbiological diagnostics often have limitations in terms of precision and specificity, particularly for the detection of pathogens such as bacterial species and viral quasispecies. However, recent studies have demonstrated the potential of dPCR in aquaculture disease management. For example, the Naica System, a dPCR platform, was utilized for the absolute quantification of 5 bacterial species (*Moritella viscosa*, *Yersinia ruckeri*, *Flavobacterium psychrophilum*, *Listeria monocytogenes*, and *Desulfovibrio desulfuricans*) in environmental samples from salmonid aquaculture (Netzer et al. 2021). This technology eliminates the need for calibration curves and minimizes inaccuracies caused by variations in reaction efficiencies and the risk of cross-contamination (Netzer et al. 2021).

Additionally, a third-generation PCR technology digital droplet PCR (ddPCR) has been developed for simultaneous diagnosis of the bacterial pathogens *F. psychrophilum* and *Y. ruckeri* in water samples from land-based recirculation aquaculture system (RAS) used for *Salmo salar* production (Lewin et al. 2020). ddPCR demonstrated high sensitivity and

specificity in detecting both fish pathogens, including 4 subspecies, even at low concentrations in water samples (Lewin et al. 2020). This is a valuable tool for studying the evolution of pathogens such as CyHV-3 (Klafack et al. 2019).

5.3. Cell culture and NGS

In vitro experiments using cell cultures play a crucial role in studying co-infection in cultured aquatic animals and the evolution of pathogens. These experiments provide valuable insights into viral evolution, enabling researchers to unravel haplotype mixtures and understand variations within viral quasispecies (Klafack et al. 2019, Vega-Heredia & Giffard-Mena 2021). By conducting *in vitro* studies, it is possible to manipulate and control experimental conditions to observe the interactions between multiple pathogens and their hosts.

One interesting experiment was conducted with salmonid viral co-infection, where it was discovered that when 2 viruses infect salmon, one virus can affect the growth of the other virus: IHNV decreased substantially when IPNV was present. Only a small percentage of cells contained IHNV, while more cells contained IPNV. The order in which the viruses were introduced did not change the results (Alonso et al. 1999). Salmonid cell lines can produce interferon-like activity, an ability to 'interfere' with viral replication, in this particular example against IHNV but not against VHSV, potentially inducing an immune response by activating natural killer cells and macrophages, which makes also this cell line a useful model for studying IFN-induced cytokines against co-infection in salmonid fish viruses (Rodriguez et al. 2005).

Similarly, studies using cell lines infected with IPNV demonstrated restricted replication of VHSV, suggesting viral interference and providing insights into the blockage of viral RNA synthesis in the early stages of VHSV infection (Parreño et al. 2017). Also, the Grunt Fin (GF) cell line has been used to propagate nervous necrosis virus (NNV) and *Megalocytivirus* species (e.g. ISKNV), highlighting its potential for the production of a bivalent vaccine (Jitrakorn et al. 2020). Despite these significant findings, it is worth noting that stable host cell lines for the study of aquatic viruses remain limited (Vega-Heredia & Giffard-Mena 2021).

Advancements in genomics and NGS have transformed our understanding of co-infectious diseases in aquaculture, providing a powerful tool for identifying and characterizing pathogens and their interactions in aquatic environments. For example,

complete sequencing of the CyHV-3 genome has enabled the characterization of genetic variants and the study of the ecological and evolutionary aspects of mixed-genotype infections (Hammoumi et al. 2016). Knowledge of viral mutation rates, influenced by selective pressures, genetic drift, and recombination helps us comprehend immune escape, co-infection pathogenesis, intra-host genetic variations, and the emergence of new diseases (Sanjuán & Domingo-Calap 2016).

5.4. Phylogenetic approaches to study co-infection

Phylogenies, or evolutionary trees, are valuable tools for visualizing and analysing data and, depending on the research question, can assist in illustrating the relatedness between different species or strains, providing crucial insights into the identification of distinct genetic variants among pathogens, both within and among hosts. Notably, the application of phylogenetic analysis has revealed the presence of diverse CyHV-3 haplotypes within individual carp hosts, underscoring the genetic heterogeneity of the virus (Avarre et al. 2012). Furthermore, comprehensive genetic characterization coupled with phylogenetic and recombination analysis has shed light on the occurrence of potential inter-lineage recombination within the CyHV-3 strain, highlighting the existence of 2 genetic lineages (Gao et al. 2018).

In the context of co-infection in crayfish involving WSSV and *Aeromonas veronii*, a phylogenetic tree was constructed based on the amino acid sequences of 16S rRNA from bacteria species. Through this analysis, the bacterial strain LY-1, isolated from the crayfish gill, was identified as *A. veronii* (Yuan et al. 2021).

In prokaryotes, several evolutionary mechanisms such as HGT can also result in recombination and genetic variation. In this scenario, phylogenetic trees can help detect and identify similarities between the different variants, including the detection of individual genes that might have been transferred between strains (Koonin et al. 2001, Boucher et al. 2003, Rhodes et al. 2011). For instance, the complete genome sequence of *Vibrio harveyi* 345 was compared with 30 other *V. harveyi* strains, revealing evidence of gene exchange, including pathogenic and drug resistance genes, through HGT, which could contribute to pathogenicity and drug resistance (Deng et al. 2019).

Phylogenetic statistical methods provide a means to detect, quantify, and explain the clustering of co-infection diseases. By analyzing the evolutionary relationships and genetic similarities among pathogens, these methods can uncover patterns of co-infection and shed

light on the factors contributing to disease clustering and transmission dynamics. In our own experience for phylogenetic analyses, several tools should be used and compared, and as rules of thumb: 'the longer sequences, the better', 'the more genes, the better', and 'complete genomes are better'.

6. VACCINES AND PHAGE THERAPY FOR MANAGING CO-INFECTIONS IN AQUACULTURE

6.1. Vaccines

Fish vaccination has proven to be an effective strategy for preventing losses in fish farms, particularly in Northern Europe and North America (Sommerset et al. 2005, Sudheesh & Cain 2017). There are various methods of fish vaccination, including oral administration through feed (a large number of fish can be mass-vaccinated easily), immersion in a diluted vaccine suspension, and injection (Sommerset et al. 2005). One successful example of fish vaccination involves the use of a combined vaccine consisting of heat-inactivated KHV and formalin-inactivated *Aeromonas hydrophila* bacterium. This vaccine is administered orally in a volume of 3 ml, with a ratio of 2 parts KHV to one part *A. hydrophila* (Lusiastuti et al. 2020). This combined vaccine enhances the immune response in common carp *Cyprinus carpio* L. and koi *C. carpio* var. "*koi*" protecting against these pathogens. By utilizing oral vaccination methods, a large number of fish can be easily mass-vaccinated, making it a practical and efficient approach for disease prevention in aquaculture settings.

Several multivalent vaccines have been developed to target multiple pathogens in fish species, offering a convenient and effective approach to preventing co-infection diseases in aquaculture. One example is a multivalent vaccine against salmonid rickettsial septicaemia (SRS), infectious salmon anemia (ISA), IPNV, *Aeromonas salmonicida* (AS) and *Vibrio ordalii* (**Table 2**) (Tobar et al. 2015). This vaccine combines antigens from different pathogens into a single formulation, providing broad-spectrum protection against multiple diseases.

Similarly, another multiple vaccine targets *Vibrio anguillarum* and *V. ordalii* (Galindo-Villegas et al. 2013). By including antigens from both pathogens, this vaccine offers protection against multiple *Vibrio* species (Table 2), which cause significant disease in fish. In European seabass *Dicentrarchus labrax*, a long-term commercial bivalent vaccine has been developed against *V. anguillarum* and *Photobacterium damselae* subsp. *piscicida*, this

vaccine stimulates the production of specific antibodies for each pathogen, providing targeted protection against both pathogens and the fish (Spinos et al. 2017).

Furthermore, autogenous and commercial immersion vaccines (Table 2) have been developed for Danish rainbow trout *Oncorhynchus mykiss* to combat *Yersinia ruckeri* serotype 01, biotypes 1 and 2 (Yang et al. 2021). These vaccines, using local pathogen strains for immunization, provide protection and reduce the bacterial load in exposed fish, demonstrating their efficacy in disease control.

In hybrid tilapia (*Oreochromis mossambicus* × *O. niloticus*), a newly developed feed-based bivalent vaccine against *Streptococcus iniae* and *A. hydrophila* has shown significant and non-specific and specific immunological responses, leading to robust protection compared to the unvaccinated group (Monir et al. 2020). These examples highlight the effectiveness of multivalent vaccines in providing broad protection against multiple pathogens in different fish species. By combining antigens from various pathogens into a single vaccine formulation, these vaccines offer a practical solution for disease prevention in aquaculture and contribute to the overall health and well-being of farmed fish populations.

Vaccination in crustaceans has been a subject of debate, primarily because it was traditionally believed that crustaceans lacked adaptive immunity similar to vertebrates. However, recent research has challenged this notion and shed light on how the immune system of crustaceans responds to pathogens (Quintin et al. 2014, Chen-Fei et al. 2020). These findings suggest that crustaceans possess certain mechanisms for recognizing and responding to pathogens, although they may differ from the adaptive immunity observed in vertebrates. Evidence has shown that crustaceans can experience viral accommodation, whereby they tolerate multiple viral infections as persistent infections (Flegel et al. 2004, 2009, Flegel 2020). Crustaceans also can coexist with viruses and initiate responses to control viral replication and minimize the negative effects of infection. Furthermore, the presence of heritable EVEs in crustacean genomes indicates the long-standing interaction between crustaceans and viruses, suggesting a history of viral infections and the evolution of immune responses (Flegel 2020). Laboratory tests have shown that injecting or feeding crustaceans with double-stranded RNA (dsRNA) can inhibit co-infection of homologous viruses (Itsathitphaisarn et al. 2017, Flegel 2020). This indicates that dsRNA treatment can stimulate the immune system to mount antiviral responses, offering potential protection against viral co-infections in crustaceans. This immune stimulation could have important implications for their overall health and survival in the face of viral threats.

While the understanding of the immune response in crustaceans is still evolving, these studies highlight the potential for immunological responses and viral accommodation in crustaceans. Further research is needed to elucidate the specific mechanisms of crustacean immune responses and explore the possibility of developing vaccination strategies that can enhance their immune defences against viral infections.

6.2. Phage therapy

Phage therapy has reemerged as a promising alternative to antibiotics and vaccines for the treatment of bacterial infections, particularly in shrimps, which lack a specific immune response that can be effectively trained by vaccines (Culot et al. 2019, Li et al. 2019, Pirnay 2020). Phage cocktails, which consist of multiple phages targeting specific bacteria, have shown a synergistic effect by combining 2 or more phages against the same bacterium (Schmerer et al. 2014, Culot et al. 2019). Phage cocktails are designed to target different receptors of the same bacteria, thereby slowing down the development of bacterial resistance. This approach has been successful in combating bacterial infections in aquaculture farms. Phage libraries can be constructed and tested against pathogenic strains isolated from specific aquaculture farms, allowing for the development of tailored phage therapies (Culot et al. 2019). For instance, there is a phage cocktail available for combating *V. tubiashii* and *V. coralliitycis* infections in oyster aquaculture, developed by Intralytix (2016). Another example is BAFADOR, a phage-based therapy developed by Proteon Pharmaceuticals, which targets *Pseudomonas* spp. and *Aeromonas* spp. and is administered via immersion (Grzelak 2017, Culot et al. 2019). While multivalent options have been explored for certain fish species (Schmerer et al. 2014, Grzelak 2017, Culot et al. 2019), there is still an opportunity for developing phage therapies for other important species such as shrimp and mollusks. Further research and development efforts are needed to expand the application of phage therapy in aquaculture, including the exploration of multivalent phage cocktails that allow treating co-infections affecting shrimp, mollusks, and other species of interest.

7. FUTURE STRATEGIES TO MANAGE CO-INFECTIONS IN AQUACULTURE

Climate warming is projected to increase the impacts of bacterial and viral diseases in aquaculture globally, and it is expected that higher temperatures will exacerbate this threat by creating conditions more favorable for disease outbreaks. This poses risks to food security

and livelihoods in many regions that are reliant on aquaculture production. A better understanding of co-evolutionary dynamics, improved diagnostics, vaccines, and integrated management strategies will be key to sustainable disease control under climate change. Thus, we propose the following strategies as general rules to manage diseases in aquaculture: (1) selective breeding for disease resistance and thermotolerance (Carabaño et al. 2019); (2) improved biosecurity and sanitation on farms (FAO 2022); (3) use of immunostimulants, probiotics, and antivirals (Newaj-Fyzul & Austin 2015); (4) restricted antibiotic use policies and development of alternatives (Okeke et al. 2022); (5) climate-smart aquaculture practices like recirculating systems (Bergman et al. 2020, Ahmed & Turchini 2021); and (6) improving national and international cooperation for wildlife health as an essential component of global disease prevention, surveillance, control, and mitigation (Mackenzie & Jeggo 2019).

An ideal scenario involves having access to state-of-the-art technology and the ability to apply it practically in real-time disease detection methods. This entails utilizing platforms or wearable devices to swiftly identify and monitor disease occurrences or symptoms, providing early alerts for potential outbreaks, and tracking the spread of diseases and their virulence. Such systems can greatly benefit the health sector by promptly informing about the health situation in a specific area. This can be coupled with a register of the environmental characteristics, including sea water temperature, which can contribute to the creation of temperature models forecasting different diseases. To achieve real-time disease detection, the use of machine-learning algorithms for analyzing vast amounts of data is essential. Nevertheless, this task is challenging and complex, requiring advanced technologies, interdisciplinary collaboration, and the involvement of various stakeholders. Despite the challenges, adopting such methods presents numerous opportunities to enhance health outcomes and prevent diseases effectively. However, it is also important to take into consideration the wide diversity of aquaculture practices around the world, and the opportunities and limitations that each type of practice may offer. For example, closed, highly controlled systems that are not affected by environmental temperature may benefit from strategies aimed to prevent the entry of pathogens into the systems (i.e. water sterilizing technologies), whilst systems highly connected to the surrounding environments will need a multi-pronged approach to tackle both global warming and the increase of co-infections, such as those described above.

8. CONCLUSIONS

Co-infections in aquaculture pose a significant challenge, and improving our understanding of this phenomenon is crucial for effective disease management. Currently, co-infections are often overlooked and treated with unspecific approaches, leading to reduced efficacy and potential negative impacts on the aquaculture industry. Furthermore, the combination of disease outbreaks, indiscriminate drug use, and the looming threat of global warming exacerbates the urgency of addressing co-infections. To address these challenges, it is imperative to improve diagnostic methods that can identify multiple pathogens during infection outbreaks. This includes enhancing our knowledge of the interactions between pathogens and their co-evolutionary dynamics, which drive pathogen diversification and impact disease dynamics. Understanding the effects of rising water temperatures on co-infections is also vital, as higher temperatures can promote stronger interactions between pathogens, increase pathogenicity, and exacerbate the negative consequences on stressed and immune-compromized aquatic animals.

By reviewing the current evidence, we suggest that frequent increases in water temperatures can promote stronger interactions between pathogens and enhance pathogenicity at the individual level, which, combined with stressed and immune-compromized aquatic animals, may have devastating effects. According to the present review, we propose that the scientific community should consider (1) enhancing studies at the individual and cellular level of prevalent co-infective aquatic pathogens at multiple expanded temperatures, to start elucidating the co-infective dynamics at different swelling temperature regimes; (2) exploring the genetic interactions between bacteria–bacteria, bacteria–virus, and virus–virus during multiple infectious experiments; (3) implementing the use of technologies such as dPCR, NGS, and cell culture to explore phylogenetic approaches, to unravel the presence of new pathogens or variants; (4) the continued development of low-cost and effective vaccines and treatments (such as phage therapy) for multiple pathogens for cultured aquatic species.

By addressing these research priorities, we can advance our understanding of co-infections in aquaculture, develop improved diagnostic tools, and identify effective strategies for disease prevention and management. Such efforts are crucial for ensuring the sustainability and resilience of the aquaculture industry in the face of evolving pathogen dynamics and environmental challenges.

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1184 Table 1. Adaptive interactions and effects of high temperature on bacterial and viral pathogen co-infections in aquacultural species. NA: not

1185 analyzed; ND: no effect detected; S: synergistic; A: antagonistic

Host species	Co-infections	Type of adaptative interaction	Temperature effects low/high	Mortality rate (%) Monoinfection / co-infection	Immunity genes expressed	Co-infection method	Reference
	Bacterial co-infections						
Salmo salar	*Alivibrio wodanis* and *Moritella viscosa*	A	NA	NA	Genes encode bacteoriocins	*In vitro* mono and co-culture, sequencing, gene expression	Hjerde et al. (2015)
Salmo salar	*A. Wodanis* and *M. viscosa*	A	Increase mortality or virulence / ND	53 / 72	NA	Culture cytotoxicity assays, cell culture, and experimental infection	Karlsen et al. (2014)
Oreochromis niloticus L.	*Streptococcus agalactiae* and *Francisella noatunensis*	S	Increase mortality or virulence / ND	37.5 and 87.5 / 100	NA	Experimental infection, sequencing and MLST, and REP-PCR analysis	Assis et al. (2017)
Mugil cephalus	*Aeromonas hydrophila* and *Vibrio parahaemolyticus*	S	ND / increase mortality or virulence	NA / 75–87	NA	Water quality parameters, biochemical identification, sequencing, and phylogenetic analysis	El-Son et al. (2021)
	Bacterial and viral co-infections						
Oreochormis niloticus	*A. hydrophila* and tilapia lake virus (TiLV)	S	NA	6.7 and 34 / 93	NA	Biochemical identification, sequencing, experimental infection, histopathology	Nicholson et al. (2020)
Oreochormis spp.	*S. agalactiae* and spleen and kidney necrosis virus (ISKNV)	S	NA	NA	NA	Histopathology, electron microscopy, cell culture, and sequencing	Ramírez-Paredes et al. (2021)

Siniperca chuatsi	*A. hydrophila* and ISKNV	S	NA	22.9 and 38.1 / 81.9	IRF1, Mx, Viperin, HEPCIDIN, TNFα, IL-1β	Experimental infection, histopathology, gene expression	Liu et al. (2020)
Litopenaeus vannamei	*V. parahaemolyticus* and white spot syndrome virus (WSSV)	A	NA	97 / 83	ACP, AKP, POD, SOD, and LvECSIT	Experimental infection and gene expression	Pang et al. (2019)
Litopenaeus vannamei	*V. parahaemolyticus*, *V. anguillarum* and WSSV	S	NA	12.5 and 29.2 / 37.5 and 50	ProPO, LvMyD88, Lvakt	Experimental infection and gene expression	Jang et al. (2014)
Procambarus clarkii	*Aeromonas veronii* and WSSV	S	NA	70 and 83.3 /100	NA	Experimental infection, physiological, biochemical and histological identification, antibiotic susceptibility	Yuan et al. (2021)
Crassostrea gigas	Opportunistic bacteria and ostreid herpesvirus (OsHV-1 µVar)	S	NA	NA	Viperin, cGAS, IRF, TNF, SOCS2, CgBigdef2, Cg-PRP, Cg-EcSOD, among others	Experimental infection, *in situ* hybridization, transcriptome analyses	De Lorgeril et al. (2018)
	Viral co-infections						
Salmo trutta	Infectious pancreatic necrosis virus (IPNV) and infectious hematopoietic necrosis virus (IHNV)	A	NA	NA	Mx, IFN-I	Cell culture, cell cytotoxicity assay and gene expression	Saksmerprome et al. (2011)
Paralichthys olivaceus	Viral hemorrhagic septicemia virus (VHSV) and aquabirnavirus (ABV)	A	NA	90–100 and 0–45 / 0 and 40–80	Mx, IFNs	Experimental infection, cell culture and gene expression	Pakingking et al. (2004)
Litopenaeus vannamei	WSSV and infectious hypodermal and hematopoietic necrosis virus (IHHNV)	S	NA	NA	LGBP, ProPO, peroxinectin	Experimental infection and gene expression	Yeh et al. (2009)

Cyprinus carpio L.	CyHV-3 haplotypes	A	NA	90 / 18 and 28	NA	Experimental infection, cell culture, sequencing, digital PCR	Klafack et al. (2019)

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1189 Table 2. Commercially available vaccines (and treatments) against co-infections in aquacultural species. ND: not determined; NA: not analyzed;
1190 IPNV: infectious pancreatic necrosis virus; SRS: salmonid rickettsial septicemia; AS: *Aeromonas salmonicida*; Vo: *Vibrio ordalli*; ISA:
1191 infectious salmon anemia; KHV: koi herpes virus; IHNV-Sn1203: infectious hematopoietic necrosis virus, isolate Sn1203; IPNV-ChRtm213:
1192 IPNV, isolate ChRtm213

Host	Weight	Co-infection	Treatment	Administration	Duration of immunity	Reference
Vaccines						
Salmo salar	30 g	IPNV, SRS, AS, Vo, ISA	Blueguard	Intraperitoneal	NA	Tobar et al. (2015)
S. salar, *Oncorhynchus mykiss*, *O. kisutch* and *O. tschawytscha*	30 g	*Piscirichettsia salmonis* and IPNV	Blueguard	Intraperitoneal	NA	Tobar et al. (2015)
S. salar, *O. mykiss*, *O. kisutch*	30–50 g	SRS and ISA	Virbac-Centrovet polyvalent vaccine	Injection and Oral	NA	Tobar et al. (2015)
O. mykiss and *Dicentrarchus labrax*	ND	*Vibrio anguilarum* and *V. ordalii*	AQUAVAC *Vibrio* oral	Oral	Throughout the production cycle	Galindo-Villegas et al. (2013)
Oreochromis spp.	Minimum 10 g	*Streptococcus agalactiae* (serotype lb) and *S. iniae*	AQUAVAC STREP SA-SI	Intraperitoneal	At least 6 mo	MSD Animal Health (2022)
D. labrax	2.5 g	*V. anguillarum* biotype I and II and *Photobacterium damsela*	AQUAVAC *Vibrio pasteurella*	Intraperitoneal	NA	Spinos et al. (2017)

Oreochromis mossambicus × *O. niloticus*	5% body weight	*S. iniae* and *Aeromonas hydrophila*	Bivalent vaccine	Oral	NA	Monir et al. (2020)
Cyprinus carpio L. and *C. carpio* '*koi*'	5–10 g	*A. hydrophila* and KHV	Bivalent vaccine	Oral	NA	Lusiastuti et al. (2021)
O. mykiss	5 g	IHNV-Sn1203 and IPNV-ChRtm213	Bivalent vaccine	Intraperitoneal and Intramuscular	30–60 d	Xu et al. (2017)
S. trutta L.	2–7.5 g	IPNV and IHNV	DNA vaccine	Intramuscular	30 d	de las Heras et al. (2010)
O. mykiss	34 g	*A. salmonicida* subsp. *salmonicida, V. anguillarum, Yersinia ruckeri*	Pentavalent vaccine	Intraperitoneal	NA	Marana et al. (2019)
			Phage therapy			
All fish species	ND	*Pseudomonas* spp. and *Aeromonas* spp.	BAFADOR (Proteon Pharmaceuticals)	Feed additive for food or water bath	NA	Grzelak (2017)
Oysters	ND	*V. tubiashii* and *V. coralliitycs*	Intralytix (phage cocktail)	ND	NA	Intralytix (2016)

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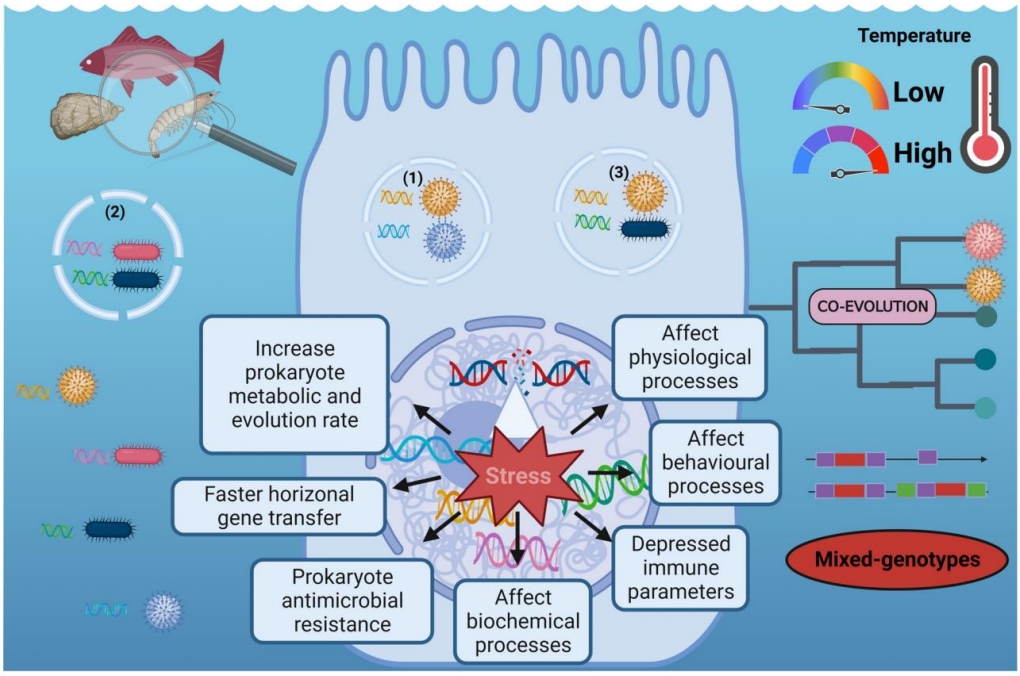

Fig. 1. The rise in extreme temperatures due to global warming is causing increased stress and physiological changes in aquatic species, compromising their immune systems and making them more susceptible to parasitic infections. The severity of viral and bacterial disease outbreaks is amplified in these conditions. Co-infections, where multiple pathogen agents can interact within the same host, can take 3 distinct forms: (1) co-infection by 2 different species of bacteria, (2) co-infection by 2 different species of viruses, or (3) co-infection by a virus and a bacterium. These interactions between mixed genotypes of pathogens and hosts can lead to the production of new variants, driving co-evolution. Understanding the complex interplay of bacterial and viral co-infections in aquaculture under global warming is crucial for mitigating the impact of disease on aquatic species. Created with BioRender

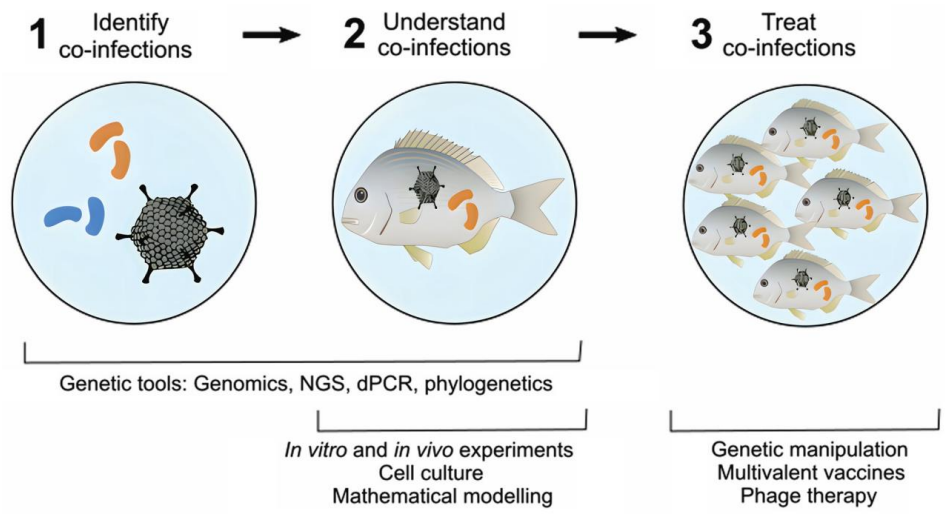

Fig. 2. Proposed research avenues and tools to advance the field of co-infections in aquaculture. NGS: next-generation sequencing; dPCR: digital PCR. Created with BioRender