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Pathology and pathogenesis of *Vibrio* infection in fish: A review

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ABSTRACT

Vibrio-associated ailments are important globally, not only among marine aquaculture systems but also among wild fish populations. Vibriosis leads to significant economic losses in fish farms. The disease is generally characterised by external skin lesions, haemorrhages, and septicaemia. Fish or shellfish of any age group are susceptible to the infection but, young animals are more prone. The infection starts with the crucial initial attachment of the bacterium to the host tissue, which is brought about by various virulence factors. This is followed by proliferation and invasion into the internal organ systems through blood circulation. However, the host defence systems provide barriers against the invasion through physical, cellular and chemical mechanisms. Nevertheless, environmental stress might tilt the balance of successful invasion and disease establishment. Therefore, basic knowledge on the pathology and pathogenesis of vibriosis, the virulence factors of the organism and the host defence mechanisms are important in the attempts to control the emergence of vibriosis. In this review, the current knowledge on pathology, histopathology, pathogenesis and virulence factors of *Vibrio* bacterium is discussed.

1. Introduction

Vibrio spp. are key pathogen in many aquaculture systems and are abundant in tropical and temperate marine environments (Ina-Salwany et al., 2019). They are part of the normal flora of the marine environment (Blancheton et al., 2013) and the intestine of many aquatic species (Egerton et al., 2018). Therefore, vibriosis is a major fish disease among many species of cultured and wild fish, leading to significant economic losses (Mohd Nor et al., 2019). Fish diseases attribute to economic loss by reduced sales due to mortalities, low production, and low farm-gate price due to low quality fish or by increased expenditure in disease management and control (Peterman and Posadas, 2019). In vibriosis endemic waters, the production cost of marine cage culture Asian seabass fish was estimated to increase by 7.8% of total cost due to various costs incurred by mortality, treatment, and diagnosis (Mohd Nor et al., 2019). Moreover, increased susceptibility of newly introduced young fish in grow-out systems than adult fish leads to high production cost in grow-out production (Ransangan et al., 2012).

Earlier, the term vibriosis was often used to describe the septicaemia caused by *Vibrio anguillarum*. Subsequent studies found that the disease is not always seen as systemic infection and therefore, a broader definition of vibriosis would be an infection caused by bacterium of *Vibrio* spp. (Edigius, 1987). Presently, eight genera have been identified within the family Vibrionaceae. They are *Aliivibrio*, *Echinimonas*, *Enterovibrio*, *Grimontia*, *Photobacterium*, *Salinivibrio*, *Vibrio* and *Thaumasiovibrio* (Sawabe et al., 2013; Amin et al., 2017). To date, the Vibrionaceae family has 172 validated species, which include *V. salmonicida, V. anguillarum, V. ordalli, V. harveyi, V. alginolyticus, V. vulnificus, V. parahaemolyticus, V. mimicus* and *Photobacterium damselae* subsp. *damselae* that cause significant impacts on cultured marine fish and shellfish species (Amalina et al., 2019; Mohamad et al., 2019a). Amongst the many *Vibrio* species, *V. harveyi*, *V. vulnificus*, *V. alginolyticus*, and *V. parahaemolyticus* are the frequently encountered fish pathogens that are associated with significant economic losses in aquaculture industry (Mohamad et al., 2019b; Deng et al., 2020).

Vibriosis is one of the oldest bacterial diseases, identified

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prominently among marine vertebrate and invertebrate around the world (Huang et al., 2017). Since the first *Vibrio* infection that was described in eels following *V.* (*Listonella*) *anguillarum* infection in 1909, many outbreaks have been reported in cultured fish species as well as in humans (Austin, 2010). Vibriosis has been reported in various life stages of fish; larval, juvenile, or adult fishes (Gauger et al., 2006; Mohi et al., 2010; Dong et al., 2017; Mohamad et al., 2019b).

The occurrence and distribution of *Vibrio* spp. are seasonal and temperature dependent. Currently, vibriosis has a worldwide distribution, in tropical (Dong et al., 2017; Amalina et al., 2019) as well as temperate regions (Macián et al., 2004; Gomez-Gil et al., 2007; Baker-Austin et al., 2013) especially in Asia, North America and Europe (Ina-Salwany et al., 2019). It has also been reported in waters with different salinities (Mohd-Aris et al., 2019a; Sumithra et al., 2019; Sony et al., 2021). Some *Vibrio* spp. show a better adaptation to freshwater (Fouz et al., 2010). Furthermore, *Vibrio* spp. are found more frequently in static water with high load of organic matter (Cheng et al., 2009). Moreover, infected molluscs and crustaceans are thought to play an important role in maintaining the bacterial count in the environment and the ability of the bacterium to survive and trigger infections in aquaculture systems (Miccoli et al., 2019).

Some *Vibrio* spp. are zoonotic in nature. Both *V. parahaemolyticus* and *V. vulnificus* are ingested through raw or under-cooked seafood, mainly oysters, and leads to septicaemia followed by soft tissue necrosis in extremities and in some cases death in humans (Oliver, 2005; Ralph and Currie, 2007; Huang et al., 2008). *V. vulnificus* is considered the more serious human pathogen with case fatality rate of 50% (Hackbusch et al., 2020). Biotype 1 and 3 are human pathogens. However, only biotype 2 is associated with fish diseases and occasionally infects humans (Mohamad et al., 2019a). *V. cholera* is a well-known human pathogen for many years. It causes fatal diarrhoeal illness through contaminated water and food. *V. cholera* O1 and O139 serogroups are mainly associated with human epidemics (Kanungo et al., 2022). *V. mimicus,* another human pathogen, causes gastroenteritis and diarrhoea in humans (Chitov et al., 2009). Although rarely, *V. harveyi* has also been reported as an opportunistic pathogen in human wound infections particularly contacted with seawater (Montánchez and Kaberdin, 2019).

With the increasing incidence of vibriosis among marine organisms that lead to severe disease outbreaks, attempts to control this infection is a major concern. In trying to formulate the biosecurity and disease control protocols, understanding of the disease development including pathology and pathogenesis is extremely important. This review summarizes the current knowledge on the pathology and pathogenesis of vibriosis in fish, particularly on the source of pathogen, route of entry, adherence and colonisation of the host tissue, virulence factors of the host, and the role of stress in establishment of *Vibrio* infections.

2. *Vibrio* infections in fish

Endemic *Vibrio* infections in fish could be seen where pathogenic bacteria are abundant and persistent yearlong. *Vibrio* species are naturally found in estuarine environments; therefore, they are prevalent in cultured marine fish and shellfish species (Amalina et al., 2019; Mohamad et al., 2019b; Sohn et al., 2019, 2021). Two-year investigation carried out by Liu et al. (2016) identified that prevalence of important *Vibrio* spp.; *V. harveyi*, *V. alginolyticus* and *V. parahaemolyticus* in large yellow croaker, *Pseudosciaena crocea*, in sea cage culture was 6.7–73.3% but none of the single species dominated the infection. In fact, fish aged below two years were more susceptible to *V. alginolyticus* while two- to three-year-old fish were more susceptible to *V. harveyi*. (Liu et al., 2016).

Pathogenic *Vibrio* could be isolated from healthy fish and pond sediments over a long period, and the seasonal abundance correlates with environmental and physico-chemical parameters of water (Mohamad et al., 2019b). Natural phenomena and climate also affect the abundance and infection of *Vibrios* (Liu et al., 2016). However, recently, the relationship between expansion of *Vibrio* endemic areas and global warming has been identified (Baker-Austin et al., 2013). Human *Vibrio* infections have shown an increase in temperate areas of Northern Sea like Baltic Sea (Baker-Austin et al., 2013) and German Bight (Hackbusch et al., 2020) with relation to fluctuations in sea surface temperature.

Intensive fish farming increases the risk of infections due to factors such as poor water quality, high density, and stress and *Vibrio* species has been reported to severely affect several commercial fish species (Ina-Salwany et al., 2019). Amongst the many *Vibrio* species, *V. harveyi*, *V. vulnificus*, *V. alginolyticus*, and *V. parahaemolyticus* are the frequently encountered fish pathogens (Mohamad et al., 2019c; Deng et al., 2020). Sea cage culture system is popular in rearing marine fish and many outbreaks of Vibrio infection has been reported (Table 1).

Co-infection is an active simultaneous or secondary infection caused by two or more pathogens in a host at the same time (Kotob et al., 2016). Co-infection of fish may cause by homologous pathogens such as bacterial (Mohamad et al., 2019b, 2019c; El-Son et al., 2021; Sony et al., 2021; Han et al., 2021), viral (Jin et al., 2022), or parasitic pathogens (Alarcón et al., 2016) or heterologous pathogens such as virus and bacteria (Amal et al., 2018), parasites and bacteria (Zhang et al., 2015), parasites and viruses (Ogut and Cavus, 2014), and fungus and bacteria (Cutuli et al., 2015).

Outcome of the co-infection is diverse because the interaction between two or more pathogen may lead to either increase load of both pathogens, one or more may be decreased or only one may increase while other decreases because the pathogens constantly compete each other for nutrients and predilection sites within the host (Kotob et al., 2016). Moreover, the disease progression and severity are varied due to synergistic or antagonistic effects of interacting pathogens. Synergistic effects can result from the immunosuppression induced by first pathogen facilitate the infection by subsequent pathogens leading to more severe disease (Kotob et al., 2016; Amal et al., 2018). In contrast, antagonistic effects occur when pathogens compete for nutrients and locations and some pathogens may change the site of second pathogen or modulate immune responses of host to hinder the second infection (Chen et al., 2013; Hjerde et al., 2015; Kotob et al., 2016).

Majority of experimental studies on fish pathogens focus on infection with a single bacterial species but, fish are exposed to multiple microorganisms in natural environment. Especially, open-net cage culture of marine food fish species is reared in natural brackish water bodies where fishes are concurrently interacting with many pathogens (Mohd Nor et al., 2019). Information on co-infection in fish is still a discoverable research area where many gaps of knowledge are prevailed (Kotob et al., 2016). Occurrence of multiple fish pathogens with *Vibrio* spp. in natural disease outbreaks have been discovered in recent studies (Dong et al., 2017; Abdelsalam et al., 2021; Sony et al., 2021; Han et al., 2021). However, some isolated bacteria did not induce clinical disease in the experimental setup despite the high doses used suggesting that they may act as opportunistic pathogens (Dong et al., 2017). Immunocompromised fish may become targets for *Vibrio* infections easily. Kang et al. (2022) reported a secondary infection of *V. splendidus* causing mortality in sea horses (*Syngnathus schlegeli* and *Hippocampus haema*) secondary to gas bubble disease, an important disease in sea horses.

Moreover, *Vibrio* spp. have been identified as secondary to some viral infections such as *V. salmonicida* and *V. carchariae* with infectious pancreatic necrosis virus, *Vibrio* spp. and *Photobacterium damselae* subsp. *damselae* with viral nervous necrosis virus and *V. harveyi* with marine birnavirus displaying synergetic interaction between pathogens (Kotob et al., 2016). Table 1 summarizes the *Vibrio* spp., affected fish species, culture system and country of some *Vibrio* infection outbreaks.

3. Pathology and clinical signs

Vibriosis displays a wide pathological manifestation, depending on the affected host species, bacterial strain, dose of infection, duration of the infection and environmental conditions. However, the common clinical signs of *Vibrio* infection in fish include lethargy, loss of appetite,

Table 1
Vibrio spp., affected fish species, and country of some *Vibrio* infection outbreaks.

Vibrio species	Affected fish species	Culture system	Country	Reference
V. harveyi	Indo-Pacific Sergeant (*Abudefduf vaigiensis*), Snubnose pompano (*Trachinotus blochii*)	Tank	India	Sony et al. (2021)
	Groupers (*Epinephelus fuscoguttatus*)	Sea cage	Malaysia	Mohd-Aris et al. (2019a)
	Hybrid grouper (*E. fuscoguttatus* × *E. lanceolatus*)	Sea cage	China	Zhu et al. (2017)
	Asian seabass (*Lates calcarifer*)	Sea cage	Malaysia	Ransangan et al. (2012)
	Large yellow croaker (*Pseudosciaena crocea*)	Sea cage	China	Liu et al. (2016)
	Arabian sturgeon (*Acanthurus sohal*)	Indoor aquarium	Egypt	Hashem and El-Barbary (2013)
	Sea bream (*Sparus aurata*)	Tank (sea water)	Malta	Haldar et al. (2010)
	Olive flounder (*P. olivaceus*), black rockfish (*Seastes schlegeli*), turbot (*Scophthalmus maximus*)	Sea cage	Korea	Won and Park (2008)
	Summer flounder (*Paralichthys dentatus*)	Tank (sea water)	Northeast of United States	Gauger et al. (2006)
V. alginolyticus	Cobia (*Rachycentron canadum*), Asian seabass (*L. calcarifer*)	Sea cage	India	Krupesha Sharma et al. (2013); Rameshkumar et al. (2014)
	Crimson snapper (*Lutjanus erythropterus*)	Sea cage	China	Cai et al. (2013a)
	Large yellow croaker (*P. crocea*)	Sea cage	China	Chen et al. (2008)
V. vulnificus	Brown-marbled grouper (*E. fuscoguttatus*)	Sea cage	Thailand	Hoihuan et al. (2021)
	Grass carp (*Ctenophayngodon idellus*)	Fresh water tank	China	Liu et al. (2019a)
	Tilapia (*Oreochromis niloticus*)	Sea cage	India	Sumithra et al. (2019)
V. parahaemolyticus	Sea bass, *Dicentrarchus labrax*	Sea cage	Tunisia	Khouadja et al. (2013)
V. mimicus	Yellow catfish (*Pelteobagrus fulvidraco*) southern catfish (*Silurus soldatovi meridionalis*), Zhengchuan catfish (*S. soldatovi meridionalis* ♂ × *S. asotus* ♀)	Fresh water tank	China	Geng et al. (2014)
V. ponticus	Golden pompano (*Trachinotus ovatus*)	Sea cage	China	Liu et al. (2018)
	Red rose snappers (*L. guttatus*)	Sea cage	Mexico	Gomez-Gil et al. (2007)
	Sea bream (*S. aurata*)	Sea cage	Spain	Macián et al. (2004)
Concurrent infections				
V. harveyi, *V. vulnificus*, *V. cholera*, *Photobacterium damselae* subsp. *damselae*	Asian seabass (*L. calcarifer*), snappers (*L. guttatus*), hybrid groupers (*Epinephelus* spp.)	Sea cage	Malaysia	Mohamad et al. (2019b)
V. harveyi, *V. alginolyticus*	Hybrid groupers (Camouflage grouper, *E. polyphekadion* × Tiger grouper, *E. fuscoguttatus*)	Sea cage	Malaysia	Mohamad et al. (2019c)
V. communis, *V. parahaemolyticus*, *V. alginolyticus*, *V. vulnificus*	Groupers (*Epinephelus* spp.)	Sea cage	Malaysia	Amalina et al. (2019)
V. harveyi, *V. parahaemolyticus*, *V. alginolyticus*	Large yellow croaker (*P. crocea)*	Sea cage	China	Liu et al. (2016)
V. parahaemolyticus + *Aeromonas hydrophila*	Striped mullet (*M. cephalus*)	Earthen pond	Egypt	El-Son et al. (2021)
V. parahaemolyticus, *P. damselae*, *Shewanella putrefaciens*	Asian seabass (*L. calcarifer*) Tilapia (*O. niloticus*) striped mullet (*M. cephalus*), Orange clownfish (*Amphitrion percula*)	Sea cage	India	Sony et al. (2021)
V. vulnificus, *P. damselae*, *S. putrefaciens*,	Asian seabass (*Lates calcarifer*), striped mullet (*M. cephalus*), green chromide (*Etroplus suratensis*)	Tank	India	Sony et al. (2021)
V. cholerae, *A. veronii*	Koi carp (*Cyprinus carpio* var. *koi*)	Tank	China	Han et al. (2021)
V. alginolyticus, *Aeromonas spp.*, *Enterococcus faecalis*	Nile tilapia (*O. niloticus*), African catfish (*Clarius gariepinus*)	Lake	Egypt	Abdelsalam et al. (2021)
V. harveyi, *V. tubiashii*, *Tenacibaculum litopenaei*, *Tenacibaculum sp. Cytophaga sp*	Asian seabass (*L. calcarifer*)	Cage	Vietnam	Dong et al. (2017)

skin and fin ulcerations with body discolouration (Mohamad et al., 2019a).

3.1. Gross pathology and clinical signs of vibriosis in fish

There are many literature that report the gross pathology of vibriosis in fish, either from experimental or natural infections. Experimental infections have been used extensively to study the virulence of different species and strains of *Vibrio* spp., the pathology and host response, and the effect of vaccination (Nehlah et al., 2016; Chin et al., 2019; Mohamad et al., 2019a; Devi et al., 2022). Although experimental infections can replicate major clinical signs and lesions that were observed in field cases, in situ behaviour of the same organism may differ in artificial setting than that of natural environment. Therefore, it is a necessity in experimental setup to mimic natural infection processes as far as possible to reproduce and examine the impact of natural infections (Le Roux, 2016). In experimental settings, marine hosts and other fish models were used to study vibriosis. The usage of fish models such as zebrafish and marine tilapia are seen as advantageous since they produce similar lesions as the natural hosts, cheaper and easier to be conducted (Runft et al., 2014; Zhang et al., 2016; Abu Nor et al., 2020).

Many different routes were used to introduce the pathogen or their purified products into fish. Intraperitoneal (Hashem and El-Barbary, 2013; Shen et al., 2017; Firmino et al., 2019; Soto-Rodriguez et al., 2019; Lozano-Olvera et al., 2020) and intramuscular (Dong et al., 2017; Marudhupandi et al., 2017; Zhu et al., 2017) routes are widely used. However, injection is not a natural route of infection. Nevertheless, skin abrasion (Shen et al., 2017; Chin et al., 2020), immersion (Martins et al., 2010; Shen et al., 2017; Sumithra et al., 2019) or skin patch (Fouz et al., 2010) methods are more suitable as they closely represent the natural course of infection. These routes allow the investigation on host-pathogen interactions and disease progress such as pathogen attachment, colonisation, route of penetration and associated tissue pathology. External gross lesions caused by vibriosis typically starts as skin petechiae, particularly at the abdominal area, fins and operculum. The lesion subsequently progresses into necrosis, either in the form of

erosion, ulceration, or rot, based on the severity or size of the necrosis.

Infection by the same *Vibrio* bacteria in different fish species resulted in various gross lesions and clinical signs owing to the difference in fish species, life stage, and virulence of bacterial species. However, infection in young fish is rapid with sudden and high number of deaths without visible clinical signs (Mohi et al., 2010; Marudhupandi et al., 2017). But adult fish develop acute or chronic disease display external skin lesions such as pigmentation and ulcers (Mohamad et al., 2019a). The clinical signs, mortality rates, and lesions observed following different routes of infection from selected reports are summarised in Table 2.

Intramuscular (IM) route of infection is commonly practised in young/small fish. Injections are commonly administered at the base of the caudal fin (Krupesha Sharma et al., 2013) or dorsolateral body wall (trunk) (Mohi et al., 2010; Dong et al., 2017; Zhu et al., 2017). Intramuscular injections of highly pathogenic *Vibrio* species can induce high mortality within 24 h or 48 h without developing any external lesions (Dong et al., 2017; Zhu et al., 2017; Sumithra et al., 2019). From 48 h onwards, surviving fish develops oedema and necrosis initially at the site of infection characterised by swelling, 'standing up' of scales and redness. Gradually, by day 3, these lesions extend to nearby musculature causing massive areas of scale drop and severe necrosis in skin and muscle tissue (Mohi et al., 2010; Dong et al., 2017; Marudhupandi et al., 2017; Zhu et al., 2017). By day 5, invasiveness of bacteria is evident by extensive tissue damage to internal organs such as spleen, liver, and kidney with chronic granulomatous lesions other than injection site after *V. harveyi* infection (Mohi et al., 2010). However, haemorrhages in liver, spleen, and brain (Dong et al., 2017), mottled appearance in liver and enlarged kidneys (Krupesha Sharma et al., 2013), exudative fluid-filled stomach and intestines (Sumithra et al., 2019) were also recorded after experimental IM injections.

Intraperitoneal (IP) injection of different *Vibrio* spp. resulted in per-acute to acute pathology mostly depending on the fish species, bacterial species, and concentration of the inoculum (Firmino et al., 2019; Soto-Rodriguez et al., 2019; Lozano-Olvera et al., 2020). It is evident that introduction of high bacterial load into the peritoneal cavity induces serious lesions in the viscera and internal organs (Lozano-Olvera et al., 2020). In addition to common signs of haemorrhagic and necrotic lesions on the skin, abdominal and anal swelling and ocular lesions were also seen after IP injection of *Vibrio* pathogens (Table 2). Swelling of abdomen is resulted by ascites or accumulation of intestinal effusions (Liu et al., 2018; Devi et al., 2022).

Challenge infections by immersion method . could reproduce skin necrosis and ulceration like those observed in natural infections in fish. Yanuhar et al. (2022) reported burn-like skin wounds circulated by a clear red margin and presence of mucous on the wound. Immersion infection could induce severe disease in some hosts. Martins et al. (2010) observed 100% mortality within 24 h after sea horses, *Hippocampus reidi* were immersed in *V. alginolyticus* (10^7 CFU/mL) culture for 15 min. They developed skin ulcers around the mouth, eye protrusion and distended abdomens during infection. Moreover, fresh skin abrasion could be severely necrosed and haemorrhagic after immersing with pathogenic *Vibrio* spp. (Chin et al., 2019). Chin et al. (2019) made artificial skin abrasion in Asian seabass, *L. calcarifer*, fingerlings (6.67 ± 1.8 g) by inflicting scales from the lateral body. After immersion with 10^7 CFU/mL of *V. harveyi*, fish showed severe and extensive skin necrosis and haemorrhages at the lateral body wall and observed 100% mortality by 120 h post infection.

Natural *Vibrio* infections are usually observed in summer season when the water temperature is between 25 and 29 °C (Mohi et al., 2010; Shen et al., 2017). Fingerlings or small-sized fish are most susceptible to *Vibrio* infections possibly due to low resistance to pathogens (Shen et al., 2017; Mohamad et al., 2019b). *Vibrio* infection in farm-grown fish may show high morbidity rate but low mortality rate (Mohi et al., 2010; Mohamad et al., 2019c; Sumithra et al., 2019). Main lesions in naturally infected fish are similar to those observed in experimental infections. These include various skin lesions that started as erosion and ulceration (surface necrosis), and subsequently extend to the underlying muscle layers. These lesions are usually accompanied by fin erosion, scale loss, exophthalmia, and distended abdomen (Martins et al., 2010; Hashem and El-Barbary et al., 2013; Liu et al., 2019a; Sumithra et al., 2019; Xie et al., 2020) (Fig. 1a and 1b). The necrosis usually involves severe tail erosion or complete loss of tail, jaw muscle necrosis and rotten-fins (Gauger et al., 2006; Haldar et al., 2010; Shen et al., 2017; Mohamad et al., 2019b, 2019c).

Internally, naturally infected fish show many haemorrhagic lesions in liver, kidney, intestines, viscera, and enlargement of visceral organs. Moreover, distended abdomen due to ascites in viscera or intestines is also common (Liu et al., 2014; Firmino et al., 2019; Sumithra et al., 2019). In some cases of vibriosis, nodular lesions characterised by granuloma formation were reported. This nodular lesion was seen in the branchial chamber of gills, as reported in one to three-year-old cultured and hatchery-bred tiger puffers, *Takifugu rubripes* in Japan following infection by *V. harveyi*. Mortality was low during late summer, but reached to 30% in autumn (Mohi et al., 2010). However, juvenile fishes might show different internal lesions. For example, (*V. harveyi*) infection in summer flounder, *P. dentatus* juveniles resulted in one-third of the fish population showing ascites and atresia coli, probably due to the infection that affects the organogenesis of juvenile fishes (Gauger et al., 2006). These are classical lesions of flounder infectious necrotising enteritis (FINE) disease following infection by *Vibrio*. Moreover, infected fish may not show both internal and external lesions at the same time. Amalina et al. (2019) reported that the 40% of the fish showed both external and internal lesions while 21% and 10% had only external lesions and internal lesions, respectively.

Generally, clinical signs observe in experimentally or naturally infected fish are not different. However, young, or small fish will develop per-acute disease where no apparent clinical signs appear before death (Zhu et al., 2017). First signs of infection often are loss of appetite, lethargy, irregular movements, oedema in abdomen, and sudden death (Liu et al., 2014; Soto-Rodriguez et al., 2019; Lozano-Olvera et al., 2020; Devi et al., 2022). Adult fish may develop acute or chronic stage of disease with behavioural changes like lethargy, anorexia, and various degree of skin ulcers (Hashem and El-Barbary, 2013).

Despite the route of infection, artificially infected fish show generalised physiological and behavioural changes after experimental challenge with Vibrios. Among them are lethargy, anorexia, loss of balance, irregular or sluggish movements, and swimming near the bottom of the tank (Liu et al., 2004, 2018; Marudhupandi et al., 2017; Yanuhar et al., 2022). Some fish show continuous spiralling movements and swimming in isolation or floating on the surface (Liu et al., 2018).

However, IP injection of pathogenic *Vibrio* species caused 90–100% mortality in different fish species within a short time period such as *V. harveyi* in white snook, *Centropomus viridis* within 10 h (Soto-Rodriguez et al., 2019), in sea bass, *D. labrax* within 48 h (Firmino et al., 2019), *V. ponticus* in white snook, *C. viridis* within 25 h and *V. cholerae* in Indian major carp, *L. rohita* within 84 h (Devi et al., 2022). However, *V. vulnificus*, *V. ponticus*, and *V. alginolyticus* caused 100% mortality within 5–10 days post infection (Liu et al., 2004, 2014, 2019a). Interestingly, same *V. harveyi* that caused per-acute disease in seabass showed very mild disease symptoms and 25% mortality in seabream (*Sparus aurata*) fingerlings (Firmino et al., 2019).

Onset time of clinical disease could be dose-dependent as well. For instance, Lozano-Olvera et al. (2020) challenged white snook, *C. viridis* with three different doses of *V. ponticus* and observed differences in initiation of clinical signs in each group. All fish injected with high dose, 3.1×10^6 of *V. ponticus* died within 25 h post-infection whereas fish received moderate dose, 2.9×10^5 showed 85% death rate within 60 h post-infection. Meanwhile, fish injected with low dose, 3.0×10^4 of *V. ponticus* showed 90% survival within 7-day experiment. High and moderate doses induced similar behavioural changes such as anorexia and lethargy and external lesions such as discolouration of dorsal fin and

Table 2
Routes of infection, *Vibrio* spp., fish species, fish weight and age, bacterial concentration, and clinical and pathological changes following experimental vibriosis.

Vibrio spp.	Fish species	Average body weight / size	Bacterial concentration / duration	Clinical signs and gross pathology	References
Intramuscular injection					
V. harveyi	Asian sea bass (*Lates calcarifer*)	21 g	1.0×10^4 – 2.5×10^6 CFU/fish	Inoculation with 10^6 CFU/mL *V. harveyi* leads to high mortality up to 100% within 24 h. All fish succumbed within this period did not show distinct clinical signs. Inoculation with 10^5 CFU/mL *V. harveyi* led to mortality between 50% and 80% within 72 h. Clinical signs included initially "stand-up" scales around the injection site (likely due to oedema), followed by spread the lesion and drop of scales. Severe muscle necrosis was apparent by 48–72 h. By day 3, severe muscle necrosis, scale drop and massive mortality were observed.	Dong et al. (2017)
V. harveyi	Grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*)	18 g	10^7 CFU/fish	High mortality within 48 h post infection without apparent signs. Surviving fish developed scale drop and small areas of muscle necrosis at injection site from 48 h onwards and severity of lesions progressed to massive scale drop and severe muscle necrosis with signs of lethargy and collapse.	Zhu et al. (2017)
V. harveyi	Tiger puffer (*Takifugu rubripes*)	10 g	1.0×10^8 CFU/fish	Infected fish showed anorexia, loss of balance loss, swim near bottom, and lethargy within 48 h. Inoculation site was swollen between 3 and 15 days, with 20% mortality, between day 3–6, injection site had foci with liquefactive or caseous necrosis of the lateral muscle.	Mohi et al. (2010)
V. alginolyticus	Asian sea bass (*L. calcarifer*)	40 g	1.0×10^3 CFU/fish	No external lesions, but brain, spleen, and gills were severely congested. Liver was mottled, while kidney was congested and enlarged within 7-day observation.	(Krupesha Sharma et al., 2013)
V. parahaemolyticus	Clownfish (*Amphiprion sebae*)	5.2 g	1.0×10^6 CFU/fish	Mortality was seen as early as day 2 at 20%, and reaches 100% mortality in 6 days. Clinical signs include irregular movement, lethargy, anorexia, red-margined skin ulcers, haemorrhages on the tail and base of the fins	Marudhupandi et al. (2017)
Intraperitoneal injection					
V. harveyi CAIM 1508	White snook (*Centropomus viridis*)	7.3 g	9.5×10^4 - 10^7 CFU/fish	Anorexia within few hours. At 3 h, irregular swimming pattern and rapid opercular movement were observed. Mortality started at 4 hpi. 100% mortality within 10 h.	Soto-Rodriguez et al. (2019)
V. harveyi	Arabian sturgeon (*Acanthurus sohal*)	100 g	10^4 - 10^6 CFU/fish	90% cumulative mortality (14 days). Infected fish showed lethargic, anorexic, superficial or deep haemorrhagic ulcers on the skin of body and head and loss of pigmentation with haemorrhagic dots	Hashem and El-Barbary (2013)
V. harveyi (Three strains)	Summer flounder (*Paralichthys dentatus*)	5 – 15 g	10^7 CFU/fish	Infected fish showed distended abdomen, reddened anal area and occasionally ventral body area, ascites, protruded intestines from the anus and blind intestinal sac.	Gauger et al. (2006)
V. harveyi	Seabream (*Sparus aurata*)	7.0 g	10^3 – 10^6 CFU/fish	Mortality of seabream ranged between 0% and 25% depending on the concentration of the inoculum. Those inoculated with 10^6 CFU showed 25% mortality within 2 – 4 days and without clinical signs, while those inoculated with 10^3 – 10^5 CFU showed very low to no mortality within 14 days.	Firmino et al. (2019)
	Sea bass (*Dicentrachus labrax*)	46.0 g		Mortality of sea bass ranged between 0% and 95% depending on the concentration of inoculum. Those inoculated with 10^6 CFU showed 95% mortality within 48 h, with external lesions of haemorrhages in the fins, mouth, operculum, and inflammation in the vent. Those inoculated with 10^5 CFU showed 40% mortality, while those inoculated with 10^3 - 10^4 showed no mortality.	
V. alginolyticus	Cobia (*Rachycentron canadum*)	10 g	1×10^4 – 10^8 CFU/fish	LD_{50} was 3.28×10^4 CFU/g fish weight. Lethargic, abdominal swelling (ascites), skin darkening, and ocular lesion. 100% mortality with 10^7 CFU/g fish weight within 7 days	Liu et al. (2004)
V. ponticus	Golden pompano (*Trachinotus ovatus*)	65 g	1×10–10^7 CFU/fish	Mortality ranged between 95% and 100% when infected by 10^6 – 10^7 CFU/fish within 9 – 10 days. About 50% mortality within 9 days when infected by 10^4 cfu/fish. Infected fish showed clinical signs of sluggish, floating in the water, swimming in isolation and spiralling. Lesions observed were skin and fins hyperaemia and ulceration in the skin, ocular hyperaemic and gut effusion	Liu et al. (2018)
	White snook (*C. viridis*)	6 g	3.0×10^4 CFU/g fish 2.9×10^5 CFU/g fish 3.1×10^6 CFU/g fish	At the dose of 10^6 CFU/g, 100% mortality within 25 hpi. Clinical signs started at 6 hpi. Lethargic, anorexic, isolated, unresponsive, skin discolouration, congested anal area, and haemorrhages on ventral body and fins. At 10^5 CFU/g dose, 83% mortality within 60 hpi. Starting at 23 hpi, infected fish showed similar signs. 95% survival at 10^4 CFU/g dose by 7 days. Infected fish	Lozano-Olvera et al. (2020)

(*continued on next page*)

Table 2 (*continued*)

Vibrio spp.	Fish species	Average body weight / size	Bacterial concentration / duration	Clinical signs and gross pathology	References
				showed lethargy and anorexia at 40 hpi and recovered later	
V. vulnificus	Grass carp (*Ctenopharyngodon idellus*) Zebrafish (*Danio rerio*)	10 g 0.5 g	$1.0 \times 10^3 - 10^7$ CFU/g fish	Mortality reached 100% within 5 days after infection at the dosage of 1.0×10^7 CFU/g for grass carp. LD_{50} was 7.53×10^3 CFU/g fish weight. External lesions observed in the grass carp were skin, gills, fins, and abdominal ulcerations and haemorrhages, anal swelling and hyperaemia, haemorrhages on muscles and viscera No clinical signs, external lesion, and death observed in the zebrafish.	Liu et al. (2019a)
V. cholerae non O1/ O139 serotype	Indian major carp (*L. rohita*)	23 g	5.2×10^5 CFU/fish	100% mortality within 84 h. Haemorrhages in ventral body and anus area, enlarged abdomen	Devi et al. (2022)
Immersion					
V. alginolyticus	Sea horse (*Hippocampus reidi*)	9 – 15 cm	1.0×10^7 CFU/mL for 15 min	100% mortality within 24 h. Infected fish showed mouth epithelial necrosis	Martins et al. (2010)
V. vulnificus	Eels (*Anguilla anguilla*)	8 – 10 g	8.0×10^5 CFU/mL - 8.0×10^7 CFU/mL for 60 min	First mortality observed between 1 and 4 days. Mortality rate was not mentioned. External lesions of haemorrhages, ulcerations around the fins, mouth, and anus between 7 and 72 h.	Valiente et al. (2008)
V. harveyi & *V. alginolyticus*	Humpback grouper (*cromileptes altivelis*)	10–15 cm	1.25×10^6 CFU/mL	Behavioural changes such as swirling, loss of balance, and settling in the pond bottom, weakness, reduced feed intake, skin wounds, ascites, and bulging out of eyes. Wound edges were reddened and mucous were observed on the wounds.	Yanuhar et al. (2022)
Intramuscular injection and immersion					
V. vulnificus	Tilapia (*Oreochromis niloticus*)	10–20 g	2.1×10^8 CFU/fish (intramuscular injection) 1.0×10^8 CFU/mL (immersion)	100% mortality between 24 and 48 h when intramuscularly infected with 1.0×10^8 CFU while 100% mortality observed between 3 and 5 dpi when immersed with 1.0×10^8 CFU/mL. Infected fish reduced feed intake, swim near the surface, showed imbalance and sudatory behaviour, skin pigmentation, exophthalmia, ascites, petechial haemorrhages on skin and fins, generalised internal haemorrhages, dark liver, enlarged spleen, yellowish exudate in gut and fragile kidneys	Sumithra et al. (2019)

congestion in anal area. However, these lesions started at 6 h and 23 h post-infection in high and moderate dose group, respectively. In contrast, low dose group developed mild clinical signs such as anorexia and lethargy within 40 h post-infection but they recovered later. Behavioural changes such as lethargy, balance loss, erratic movements and difficult breathing patterns may be shown due to the narcotic effect of acetylcholine esterase, neurotoxic extracellular product of *Vibrio* species, on the central nervous system of fish (Milton, 2006; Dias et al., 2016).

In natural infection, adult fish show low degree of mortality rate with development of skin-related lesions (Mohi et al., 2010; Sumithra et al., 2019). However, the progression of mortality can vary in different fish species. For instance, Sumithra et al. (2019) reported a 20% cumulative mortality within a one-week period in tilapia, *Oreochromis* spp. infected with *V. vulnificus* while Mohi et al. (2010) observed 30% mortality approximately during two-month period in tiger puffers, *T. rubripes,* after naturally infected with *V. harveyi*. However, natural mortality rate might be low but may reach high rate of between 80% and 100%. High rates of mortality were previously observed in freshwater catfish such as the yellow catfish, *P. fulvidraco*, southern catfish, *S. soldatovi meridionalis* and Zhengchuan catfish, *S. soldatovi meridionalis* ♂ × *S. asotus* ♀ that were infected by *V. mimicus* (Geng et al., 2014).

Pathogenic lesions and clinical signs in concurrent infections could leads to significant consequence by amplifying pathogenicity of each pathogen (Amal et al., 2018). Han et al. (2021) artificially infected koi carps, *C. carpio* as a single infection or co-infection with *Aeromonas veronii* and *Vibrio cholerae* and found out that *A. veronii*-infected group and co-infected group showed severe clinical signs observed at the naturally infected fish such as swollen and haemorrhagic anus and blood-tinged ascitic fluid in the stomach but *V. cholerae*-infected fish did not show any external or internal lesions except haemorrhages on the intestine wall. However, mortality results showed 100% mortality in suggested that both bacteria could be pathogenic to koi carp, *A. veronii* being more virulent than *V. cholerae*. Based on the results, authors suggest that *A. veronii* could be the primary pathogen and *V. cholerae* being an opportunistic pathogen in the mass mortality of koi carp farm. Recently, Abdelsalam et al. (2021) identified 60% mortality and septicaemic lesions in polyculture of Nile tilapia, *O. niloticus* and African catfish, *Clarias gariepinus* and isolated *Enterococcus faecalis*, *A. veronii*, *V. alginolyticus*, and *A. caviae* from moribund fish. following the intraperitoneal injection of separate bacteria in Nile tilapia (40–50 g) resulted different mortality rates, *E. faecalis* being the highest (80%) and *A. caviae* the lowest (30%). However, all isolates showed external and internal lesions like that of natural co-infection.

In general, through experimental infections, it was determined that vibriosis involves three stages of development; acute, subacute, and chronic. The early or acute infection leads to lethargy, abnormal swimming pattern and small dots of haemorrhage on the skin, particularly near the fins while mortality is low (Mohi et al., 2010; Shen et al., 2017). Subacute infection produces eye lesions with extensive skin ulcers and haemorrhages, while the internal organs appear severely congested with haemorrhages, an indication of septicaemia (Shen et al., 2017). The most obvious clinical sign is the high rate of mortality (Chin et al., 2019; Zhu et al., 2017). In chronic infection, infected fish show fin rots, occasional healing skin ulcers and distended abdomen with ascitic fluid, while the internal organs show chronic inflammation some times in the form of granuloma (Mohi et al., 2010). Cumulative mortality might be high (Gauger et al., 2006; Shen et al., 2017).

3.2. Histopathology of vibriosis and distribution of Vibrio spp

Knowledge on histopathology and lesion development have greatly

Fig. 1. Gross pathological changes of fish infected with *Vibrio* spp. (a) Scale drop and skin ulcer (arrow) at lateral abdomen of a hybrid grouper, *Epinephelus lanceolatus* × *E. fuscoguttatus* and (b) Scale drop and exophthalmia (arrow) in a red snapper, *Lutjanus* sp.

aided in explaining the pathogenesis of vibriosis in fish. Routine and special histological techniques such as the immunohistochemistry had allowed better understanding on the pathogen distribution in tissues following infection.

Once pathogen gains entry and establishes itself within the host, secretion of toxin and the responses by host contribute to lesions development (Ruwandeepika et al., 2012). This is a well-known concept in veterinary pathology, usually referred to as host-pathogen interactions. However, in vibriosis, the cellular and molecular mechanisms that are accountable for either the initiation of infection or the clearing of the pathogen are not fully understood. Moreover, the extent of incubation period and processes that lead to lesion development awaits further studies, especially when bacterial virulence, host species and environmental factors interact in the course of infection. Nevertheless, the host immune responses seem to significantly influence the pathogenesis of vibriosis (Gong et al., 2021). Evidences suggest that the initial lesions develop within the non-specific cellular components at the site of infection before the infection spread from the site of entrance to other internal organs (Mohi et al., 2010). In most cases, vibriosis starts with the development of external lesions that subsequently spreads to systemic disease and eventually death if left untreated (Dong et al., 2017; Lozano-Olvera et al., 2020).

The histological lesions of vibriosis have been studied in details, mostly in experimental infection. In general, it must be highlighted that vibriosis is a septicaemic disease where the pathogen and their toxins are actively circulating, resulting in lesions in many tissues. Hence, the pathogen is expected to be present in many tissues (Firmino et al., 2019; Sony et al., 2021). Theoretically, lesions are more severe and the pathogen is more frequently distributed in tissues that are adjacent to the initial site of infection or the route of entry. For examples, lesions may be severe in the skin, gills, and mucosal surfaces following natural infection (Shen et al., 2017; Xie et al., 2020) or experimental infection by

immersion (Valiente et al., 2008; Martins et al., 2010; Shen et al., 2017), severe skin and muscle necrosis following experimental intramuscular infection (Mohi et al., 2010; Dong et al., 2017; Zhu et al., 2017) or natural infection via abraded skin (Liu et al., 2016), and prominent ascites and peritonitis with inflammation of the serosa surfaces of visceral organs following experimental intraperitoneal infections (Liu et al., 2004; Gauger et al., 2006; Soto-Rodriguez et al., 2019; Devi et al., 2022; Lozano-Olvera et al., 2020).

Soto-Rodriguez et al. (2019) showed experimental intraperitoneal infection by *V. harveyi* strain CAIM 1508 or *V. ponticus* strain CAIM 1751 in pacific white snooks, *C. viridus* resulted in relatively similar lesions but with different disease progression. *Vibrio harveyi* strain CAIM 1508 caused per-acute septicaemia while *V. ponticus* strain CAIM 1751 caused acute vibriosis with moderate septicaemia. Localisation of the bacteria was evident by histopathological observation of bacterial colonies in the peritoneum, mesentery, liver, spleen, kidney, and pancreas. In fact, peritonitis, necrotic mesenteries, intravascular hemosiderosis and macrophage infiltrations in the abdominal cavity were also noted. The CAIM 1751 strain leads to the presence of bacterial colonies in the mesenteric capillaries, visceral connective tissue, and brain, apart from the above-mentioned organs (Soto-Rodriguez et al., 2019). The bacterial colonies are mostly observed in various tissue capillaries, as well as in the vascular walls (Peng et al., 2016), and are the hallmark histopathological lesions of septicaemia that suggest the hematogenous spread of the pathogen.

The ulcers on the skin, mouth, anus, and fins are usually characterised by areas of deep tissue excavation (Dong et al., 2017; Chin et al., 2019). Haemorrhage is usually prominent, while the surrounding muscle tissues are undergoing severe necrotic that is accompanied by oedema. Infiltration of fat cells may be seen intermixed with the necrotic muscles. Inflammatory cells are abundant and interspersed in the affected areas (Dong et al., 2017; Xie et al., 2020). Mohi et al. (2010) observed the initial neutrophil infiltration followed by the formation of suppurative foci, which subsequently developed into encapsulating granuloma surrounding the necrotic foci. Extracellular bacterial cells were reported to be present in close contact with the various cell types, most of which were necrotic (Valiente et al., 2008). A comparative study between naturally-infected and intramuscularly-infected seabass, *L. calcarifer* with *V. harveyi* reported similar histological changes that were characterised by remarkably severe muscle necrosis and infiltrations by abundant immune-related cells to the affected muscle tissues (Dong et al., 2017). When experimental infection was made intramuscularly, *V. harveyi* could be isolated from the muscle, spleen, and liver of moribund or dead juvenile hybrid groupers, *E. fuscoguttatus* × *E. lanceolatus*.

Histopathological changes are also reported in the gastrointestinal tract, mainly in the intestine, while those of the stomach are considerably scarce. In the stomach, loss of tubular glands of the gastric pits and engorged capillaries have been reported (Rameshkumar et al., 2014). Intraperitoneal injection of *V. ponticus* strain HAINUV01 into golden pompano, *T. ovatus* resulted in necrotic and degenerative changes to the intestines, liver, and spleen (Liu et al., 2018). Haemocyte infiltration, apoptosis of mucosal cells and sloughing off the villi were obvious in intestines. Congested intestine was also reported in tilapia, *O. niloticus* infected by *V. vulnificus* (Sumithra et al., 2019). In fact, enteritis and peritonitis are common clinical signs of FINE, a disease condition in flounders thought to be associated with *V. harveyi* (Lee et al., 2002; Gauger et al., 2006). Gauger et al. (2006) monitored summer flounders, *Paralichthys dentatus* specially for *V. harveyi* and found that 75% of randomly examined 102-days old healthy-looking or diseased larvae had mild to moderate enteritis. In infected summer flounders, gut-associated lymphoid tissue (GALT) at the junction of stomach and anterior intestine showed increased number of macrophages, lymphocytes and few heterophils. Mild to severe multifocal peritonitis were obvious at the adjacent peritoneal tissues with infiltration of inflammatory cells in the muscular layer extended up to the adjacent peritoneal mesentery tissue. Therefore, the serosa of stomach, intestine and colon had more necrotic foci than the respective mucosal surface. It was believed that the macrophages of GALT might have a role in mobilisation of the bacterium to these locations. Moreover, *V. harveyi* was more abundant in juvenile gut than larval gut (Gauger et al., 2006). It was possible that *Vibrio* spp. had just started to colonise those fish that appear healthy or without evidence of enteritis and peritonitis. Degeneration, necrosis, and desquamation of villous epithelium were observed in addition to the increased number of Goblet cells in the stomach of Asian seabass (*L. calcarifer*) following experimental infection with *V. alginolyticus* (Krupesha Sharma et al., 2013). Infection by *V. anguillarum* in the larvae of gnotobiotic seabass showed evidence of shedding of non-apoptotic enterocytes without any damage (Rekecki et al., 2013). Some of the shed cells showed active phagocytosis while intact bacterial cells were observed close to the enterocyte brush border. The intra-enterocytic lysosomes were detected with particles, suggesting ingestion of the bacterial particles, but no intact intracellular bacteria were observed. Further, the immunogold-positive tread-like structures, suggesting the outer membrane vesicles transporting virulent factors were also detected in the lumen. Rekecki et al. (2013) suggested that the phagocytic activity of exfoliated enterocytes in larval stage is a compensation for the lack of fully functional immune system of early age.

In the liver, noticeable histopathological changes included haemorrhage, lymphocytic infiltration, mild to severe hepatocellular degeneration, hepatocyte necrosis that appeared as cell lysis, and the presence of numerous melano-macrophage centres. Severe liver haemorrhage was reported in tilapia, *O. niloticus* that were infected with *V. vulnificus* (Sumithra et al., 2019). Hepatic fatty degeneration produces prominent halo in the hepatocytes that displaces the hepatocytes nuclei towards the periphery. This lesion was initially observed at the periphery and around the central vein from day 6 post infection, and progressed to form larger vacuoles in the hepatocytes that were located around blood vessels at day 9 post-infection (Peng et al., 2016). In some cases, the liver appeared grossly pale, likely due to severe fatty degeneration and less severe hepatic congestion as previously reported in a natural infection of *V. alginolyticus* in cage-cultured cobia, *R. canadum* (Rameshkumar et al., 2014). Vacuolar degeneration of the hepatocytes was also reported, along with hepatocyte necrosis and the presence of bacterial colonies in the liver. Toxins produced by *Vibrio* species are suggested to contribute to vacuole formation. Figueroa-Arredondo et al. (2001) observed that vacuoles were produced in Vero cells with the involvement of endoplasmic reticulum within four hours after exposure to toxins of non-O1 strains of *V. cholerae*. From our experience, hepatocytes necrosis and infiltrations of inflammatory cells tend to be more prominent when fishes were infected via the intraperitoneal route. These might be due to the direct contact of the intraperitoneally inoculated pathogen with the serosa surface of the liver. Findings of infection dynamics and different route of infection in zebrafish model following *V. anguillarum* infection supports this theory. Schmidt et al. (2017) challenged zebrafish intraperitoneally and immersion with 10^3 cells/fish and 1.25×10^9 cells/mL of *V. anguillarum*, respectively. Intraperitoneally infected fish showed movement of bacteria from hypodermis, peritoneal cavity, intestine, kidney, spleen, and liver within 24 h period whereas bath challenge resulted in localisation of bacteria on the epithelia, swim bladder, blood and finally at the spleen and kidney. Furthermore, semi quantitative estimation based on severity grade and degree of tissue change following a challenge with different concentration of *V. ponticus* in white snook, *C. viridus* showed that the highest concentration (3.1×10^6 CFU/g fish) of bacteria cause greater damage to liver and pancreas while lower concentrations (3.0×10^4 CFU/g fish) affect gastrointestinal and nervous system (Lozano-Olvera et al., 2020).

In the gills, many histopathological changes were reported, especially those in the secondary lamella. Possibly this is because the secondary lamella is an extremely delicate structure that is continuously exposed to the aquatic environment. The lesions include congestion,

haemorrhages, epithelium desquamation, secondary lamella aneurysm/telangiectasia, epithelial hyperplasia, and secondary lamellar fusion (Martins et al., 2010; Valiente et al., 2008; Firmino et al., 2019). Experimental infection of *V. vulnificus* bt2-serE in eels, *Anguilla anguilla* by immersion showed an immediate uptake of some bacteria into the blood capillaries of gills followed by the presence of extracellular bacterial cells within or around the blood vessels of other organs (Valiente et al., 2008).

Following experimental or natural infection, the pathogen could be isolated from the kidney. For example, *V.* (*Listonella*) *anguillarum* and *P. damselae* subsp. *piscicida* were successfully isolated from the anterior kidney and the spleen of European sea bass, *Dicentrarchus labrax* following an experimental infection (Mosca et al., 2014). Although the histopathological changes were unremarkable in the kidney, immunohistochemistry revealed positive reactions against *V. anguillarum* within the macrophage-like cells and the blood vessels in the spleen at 24-h post infection and in the head kidney and spleen at 72- h post infection. Rameshkumar et al. (2014) reported renal lesions that included acute glomerulonephritis. Enhanced expression of melano-macrophage centres, collapsed renal tubules and desquamation of tubular epithelial cells were also described in vibriosis (Dong et al., 2017). The lesions have been described in many species of fish including in seahorses, *H. kuda* infected by *V. harveyi* and *V. alginolyticus* (Xie et al., 2020). Similarly, Martins et al. (2010) observed glomerular and tubular degeneration, leucocyte infiltration, and necrotic foci in the kidneys of seahorses, *H. kuda* infected by *V. alginolyticus* infection.

Some *Vibrio* species are reported to persist and cause severe cellular damages in spleen (Valiente et al., 2008). Congestion, haemorrhage, hemosiderin deposition, presence of melano-macrophage centres, and necrosis are commonly seen in infected fish (Abdelsalam et al., 2021; Han et al., 2021). Bacterial virulence or co-current pathogens cause varied lesions (Han et al., 2021). In the brain, severe congestion and haemorrhage are usually prominent especially in the blood vessels at the cerebral cortex, meninges and adjacent to the ventricles. These lesions were reported to be more severe in naturally-infected fish (Dong et al., 2017). Histopathological changes in the heart are not frequently reported, but some studies observed pericardial haemorrhage in Asian seabass (Krupesha Sharma et al., 2013) and severe necrosis in heart of *C. viridis* (Soto-Rodriguez et al., 2019). In the spleen, white pulp was atrophied with splenic congestion and lymphopenia (Liu et al., 2018).

It is known that fish may succumb to infection without developing gross lesions. This is usually associated with peracute death due to septic shock (Amaro et al., 2015). In such instances, histopathological lesions are usually less prominent too, especially those that concern the circulatory disturbances. So, lesions such as haemorrhages and congestion are usually mild, while lesions suggestive of peracute injury such as cellular swelling, degeneration and necrosis may be seen. Common histopathological lesions in vibriosis in different tissues are shown in

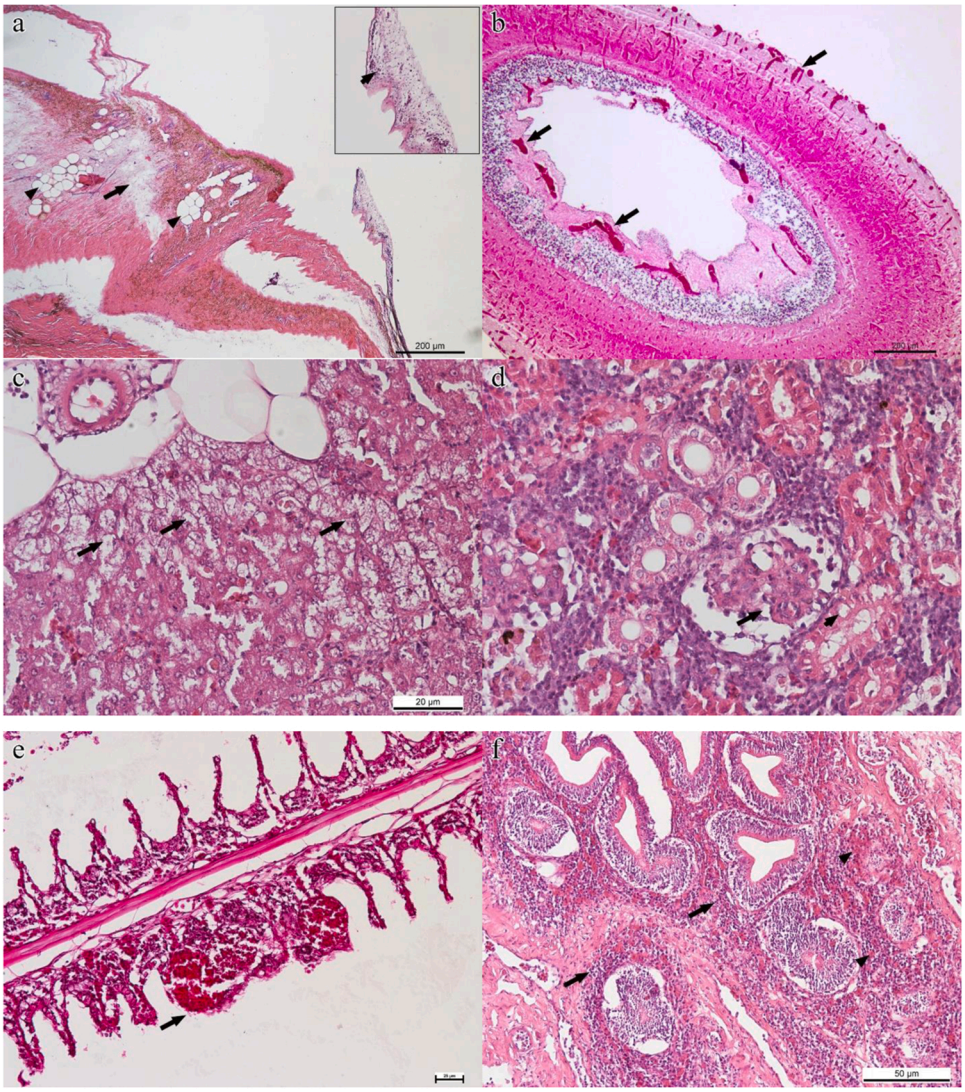

Fig. 2. Histopathological changes in the skin and muscle, brain, liver and kidney of hybrid grouper, *Epinephelus lanceolatus* × *E. fuscoguttatus* following infection with *Vibrio* spp. (a) Necrosis of muscle (long arrow) with fatty infiltration (arrowheads) with infiltration of inflammatory cells in the epidermis (short arrow). (b) Generalised vascular congestion (arrows) in the parenchyma and the meninges of brain. (c) A focal area of severe hepatocyte necrosis in the liver. (d) atrophied and shrunken glomerulus (long arrows) with glomerulitis. Tubular epithelial cells are usually necrotic or with vacuolar degeneration (short arrow). (e) Aneurism or telangiactasis seen in the secondary lamella of the gills (arrow). (f) Extensive enteritis by lymphocytes (arrows) and mast cells (arrowheads) in the intestine.

Fig. 2.

3.3. Use of haematology in vibriosis

Haematological parameters combined with routine diagnostic methods have been used in various fish studies and are a very useful tool in disease diagnosis, monitor as well as to gather essential information on physiological status of fish (Fazio, 2019). However, the interpretation of values is compromised by the scarcity of reference values and established normal data bases of many fish species (Currie et al., 2022). Recently, some researchers established normal reference values for important farmed and wild fish species but the diversity of intrinsic and extrinsic factors such as physiological (species, age, gender, life cycle stage, health, nutrition, stress), environmental (temperature, water quality, density, salinity, photoperiod), sampling protocols, and culture systems among many other factors have an impact on haematological parameters (Chen et al., 2004, 2022).

Common haematological parameters of fish include counts of total red blood cell (RBC), total white blood cells (WBC) and thrombocytes, haematocrit value (PCV), red blood cell indexes (MCV, MCH, MCHC), and haemoglobin concentration (Hb) (Campbell, 2004). Many studies used serum biochemistry and blood cell analysis to monitor changes in health status of fish. Among them, some studies challenged with *Vibrio* spp. (Chen et al., 2004; Gong et al., 2021), feeding fish with probiotic bacteria (Amenyogbe et al., 2022) or herbal products (Harikrishnan et al., 2012), and used immunostimulants (Esmaeili et al., 2021). Nevertheless, it is in utmost importance to study relationship between haematological parameters and histopathological outcome during infections in different fish species.

Differences in pathogenesis or severity of different bacterial species signifies changes in pathological lesions and blood chemistry parameters. For an example, Chen et al. (2004) showed that acute *V. vulnificus* infection in tilapia resulted in reduced appetite, liver haemorrhages and peritoneal adhesions but none of infected fish showed bacteraemia whereas *Streptococcus iniae* infected fish manifested listlessness, skin haemorrhages and bacteraemia after 24 h. Results of blood chemistry showed significantly lower plasma iron, calcium, and creatine kinase in both infections but non-significantly higher haematocrit, Na:K ratio, glucose, and total protein in *V. vulnificus* infected fish while lower values for same parameters except for glucose in *S. iniae* infected fish than control fish (Chen et al., 2004). Bacterial dose influences over cellular and humoral immune components of blood were examined in *V. vulnificus* infection. Gong et al. (2021) infected half tongue sole, *Cynolossus semilaevis*, with low (10^4), moderate (10^7) and high (10^{10}) doses of *V. vulnificus*. After 24 h post-infection, all three groups showed increased monocyte and decreased lymphocytes values in blood probably due to initial phagocytic activities. However, elevated thrombocyte counts and combined activity of neutrophils, monocytes and eosinophils, elevated level of serum total complement (CH50) and alkaline phosphatase were observed only in high dose group showing higher immune responses within initial three days. Similarly Valiente et al. (2008) reported a noticeable reduction of plasma haemoglobin and haematocrit values, increase in leucocyte number, and widespread haemorrhages in parallel to bacterial growth in liver, spleen, blood, mucous and gills of eel, *Anguilla anguilla*, fingerlings after infection with *V. vulnificus*.

Yellowfin sea bream, *Acanthopagrus latus*, fed diets supplemented with bovine lactoferrin, iron-binding glycoprotein, demonstrated increased total plasma protein, complement activity, and haematocrit and no differences in red and white blood cell counts and haemoglobin but protected by *V. harveyi* (high survival rate) challenged at 56-days after feeding trial (Esmaeili et al., 2021). Feeding kelp grouper, *Epinephelus bruneus*, with 1% and 2% *Pueraria thunbergiana* increased red blood cell count, white blood cell count, monocyte and lymphocyte count, haemoglobin, and haematocrite and showed low mortality rate when challenged with *V. harveyi* (Harikrishnan et al., 2012). In addition, abrupt changes in salinity affects the blood immune parameters and susceptibility to infection (Chen et al., 2018b). Orange spotted grouper, *E. coioides*, reared in 34% salinity at 27 °C showed decrease in total leucocyte count, lysozyme activity, respiratory burst, alternative complement pathway, and phagocytic activity when transferred to 6% salinity and higher mortality after challenged with *V. alginolyticus* (Chen et al., 2018b).

4. Pathogenesis

Just like pathogenesis of many other diseases, the pathogenesis of vibriosis involves the three major factors; the host, pathogen, and environment. In general, it should be emphasised that the host factor (different species of fish) and the pathogen factor (different species of *Vibrio* spp.) could significantly affect the outcome and severity of the disease. Establishment of infection by the pathogen that subsequently leads to disease development is actually a battle between the host and the pathogens. Pathogens, being the invader, certainly have ammunitions and techniques, known generally as virulence factors that give them advantages in trying to establish infection. The hosts, on the other hand, would be ready with efficient defence mechanisms, both innate and adaptive in trying to prevent establishment of infection. This battle is strongly influenced by the environmental factors, in the form of stress. In many instances, stressful environment favours establishment of infection. This section covers the survival of *Vibrio* spp. and their ability to colonise and infect the host, their entry into the host, and the host-pathogen interactions that ultimately lead to lesion development. Detailed discussions on the virulence factors of the pathogen and the role of environmental stress are covered in subsequent topics.

4.1. Survival of Vibrio spp. outside the host

The ability of pathogenic *Vibrio* spp. to survive outside the host is important in ensuring successful transmission of infection. A vast population of well-adapted commensal bacteria, including potential pathogens live on the mucosal surfaces of animals. Therefore, mucosal immune system of vertebrates develops memory to identify these commensals and pathogens, while some commensals evolve into less virulent pathogen over time to gain benefits from the host (Gomez et al., 2013).

It is well-known that members of the family Vibrionaceae are normal inhabitants of aquatic environments and animals. In general, they inhabit both the body surface and the intestine of aquatic animals (Zhang and Austin, 2005). Therefore, many *Vibrio* spp. are considered non-pathogenic to fish and they can be isolated mostly from the gills and skin of healthy fish (Sohn et al., 2019). They are also part of the normal flora in mussels and oysters (Destoumieux-Garzon et al., 2020). Nevertheless, they could act as opportunistic pathogens when the extrinsic factors of fish become unfavourable (Soto-Rodriguez et al., 2003). However, the phylogenetic analysis that compares the N-terminus of vibriolysin-like proteases of *Vibrio* spp. revealed that *V. vulnificus*, *V. mimicus*, *V. cholerae*, *V. parahaemolyticus* and *V. alginolyticus* are serious pathogens while *V. harveyi*, *V. campbellii* and *V. splendidus* are opportunistic pathogens (Huang et al., 2018). Thus, the costal marine environmental attributes such as the temperature, salinity, pH and nutrition often change with the season and influence the prevalence of *Vibrio*. In fact, the ability to withstand these changes is showed in genetic diversity of *Vibrio* organisms, particularly in the aquatic nutrient cycle. In general, survival and infectivity of *Vibrio* spp. are higher in brackish and marine waters compared to fresh water (Hassan et al., 2021). They uptake dissolved organic matter (Neogi et al., 2018), provide unsaturated essential fatty acids (Estupiñán et al., 2020), degrade toxic substances like aromatic hydrocarbons from marine sediments (Hedlund and Staley, 2001) and digest chitin, a polymer of N-acetylglucosamine that is produced in the aquatic environment from the cell walls of insects and crustaceans (Markov et al., 2015).

Being an opportunistic pathogen, continuous environmental persistence is important for *Vibrio* organisms. Mohamad et al. (2019b) found high *Vibrio* counts in the sediment of net-caged fish farm than in the water throughout a nine-month study period, indicating that the sediment is a probable reservoir of pathogenic *Vibrio* spp. Julie et al. (2010) reported similar observation with *V. alginolyticus* in French Atlantic water for a four-month period. Furthermore, population of non-virulent strains of *Vibrio* in the same environment might contribute to the increase in the virulence of pathogenic stains (Lemire et al., 2015). Similarly, Xue et al. (2017) suggested that the diversity of environmental bacterial populations and disease occurrence may be correlated. This is because high diversity of bacterial species and low number of pathogenic bacteria were observed in the recirculating aquaculture system (RAS) of non-diseased fish than that of the diseased fish. Furthermore, Kim and Lee (2017) demonstrated a correlation between bacterial counts of the fish fillets and the aquaculture water after administering pathogenic *Vibrio* spp. into the aquarium water. In addition, bacterial biofilms are the well-organised microcolonies of bacteria that attached to living or inert surfaces. The colonies are covered with a self-produced extracellular sheath called extra polymeric substances (EPS), which is resistant to antibodies and extracellular enzymes (Vinay et al., 2019). In fact, biofilms are more resistant to antimicrobials and disinfectants than the free-living forms (Elexson et al., 2013) and contribute significantly to the survival of pathogenic strains in the environment.

4.2. Adherence and colonisation

One of the first crucial steps in microbial pathogenesis is the attachment of bacterium to mucosal surfaces of the host. Successful attachment is a pre-requisite for subsequent colonisation and establishment, and the microbial cell-surface hydrophobicity determines the successful adhesion of *Vibrio* spp. to the host tissues (Won and Park, 2008). For example, pathogenic strains of *V. harveyi* have been shown to possess positive hydrophobicity following an in vitro pathogenicity experiment (Soto-Rodriguez et al., 2003; Won and Park, 2008). The mucous that covers the fish body, the epithelia of primary and secondary lamella of the gills and the gastro-intestinal tract with its complex compositions play roles in preventing establishment of pathogens onto the host surface. The mucous composition varies with the type of tissue, the species, sex and life stage of fish, and the stress level (Benhamed et al., 2014). It is secreted continuously, and functions to trap microorganisms before they could reach the epithelium. In fact, the mucin, glycolipids and glycoconjugates that are present in the mucous, facilitate the entrapment of bacteria to the mucous, reducing the chances of attachment of microorganisms to the host surface. Furthermore, adhesin, a chemical compound found on the bacterial cell wall is known to attach to the adhesin receptors in the mucous (Chen et al., 2008), while lectins, a sugar-binding protein in the bacterial cell wall, binds to the carbohydrate molecules (Acord et al., 2005) including N-acetylneuraminic acid, glucose, N-acetyl-glucosamine, N-acetyl-galactosamine, galactose and fucose of the skin mucous (Guardiola et al., 2014). These reduce the ability of pathogens, including *Vibrio* spp. to attach to the host surface. Moreover, silencing of Type II secretion system (T2SS) genes, the *secA, secD, secF, yajC, yidC* resulted in a positive relationship between T2SS and *V. alginolyticus* adhesion to mucosal membrane (Guo et al., 2017).

Other than carbohydrates, enzymes that are present in the mucous of fish epithelium play vital role in immune responses against bacterial attachment and colonisation. Lysozyme, protease, antiproteases, peroxidase, alkaline phosphatase and esterase have been identified in fish mucous (Guardiola et al., 2014). Similarly, Chen et al. (2008) detected more lysozymes and IgM in the skin mucous than in the gill or gut mucous and a decreased lysozyme activity of the host leads to higher susceptibility to *V. alginolyticus* infection. It is important to note that some *Vibrio* spp. survived the actions of fish mucous (Won and Park, 2008), suggesting that fish mucous probably acts as a nutritious medium for pathogenic *Vibrio*. For example, exposure to *V. anguillarum* resulted in increased protease activity, exopolysaccharide production, flagellar motility, biofilm formation and mRNA gene levels in the mucous of seabass, *D. labrax* larvae (Li et al., 2015), but the bacterium successfully adhered to the host surface and triggered an adaptive immune response with expression of gene activities.

It is worthwhile to note that the flagella of *Vibrio* are an essential virulent factor that are strongly associated with the attachment, colonisation, biofilm formation and invasion of microorganism into the host tissue (Kirov, 2003). Some *Vibrio* spp. have a polar flagellum, such as *V. harveyi* (Montánchez and Kaberdin, 2019), *V. anguillarum* (Larsen and Boesen, 2001) and *V. alginolyticus* (Wang et al., 2016), while *V. parahaemolyticus* (Kirov, 2003) and *V. mimicus* (Tercero-Alburo et al., 2014) have additional lateral flagella. In fact, the flagella motility of *V. harveyi* is considered an important factor in the successful adherence and colonisation onto the host surface (Montánchez and Kaberdin, 2019). On the other hand, since *V. parahaemolyticus* strains have two types of flagella; the sheathed polar flagellum that facilitates the initial movement towards the host, and the non-sheathed lateral flagella that helps the bacterium to swarm over the outer surface of host cells, colonisation and biofilm formation are more efficient in this *Vibrio* spp. (Kirov, 2003). Furthermore, flagellin is recognised by the TLR5 in the cell membrane and this ligand recruits MyD88, the cascade of signalling pathway, followed by the activation of transcription factors, which induces transcription of inflammatory cytokines by the host, like IFN-α and IL-6 (Kumar et al., 2009, 2019). The highly expressed membrane form-TLR5 after intraperitoneal injection of live *V. parahaemolyticus* in the kidneys and intestines of fish suggested the natural expression of the TLR5 induction by the flagella that cause tissue injuries (Wang et al., 2016).

Vibrio parahaemolyticus, *V. alginolytius*, *V. harveyi*, Gammaproteobacteria (a class within Vibrionaceae family) (ArunKumar et al., 2019), *V. mimicus* (Tercero-Alburo et al., 2014) and *V. anguillarum* (Grześkowiak et al., 2012) have been demonstrated with the ability of biofilm formation. The biofilms, which are matrix-enclosed, surface-associated communities of micro-organism, is a key factor for environmental survival and transmission of many bacteria. Therefore, biofilm formation is another important virulent factor in the colonisation of pathogenic *Vibrio* spp., and environmental temperature of 25°C is optimal for biofilm formation by pathogenic *V. parahemolyticus* strains (Song et al., 2017). Once a bacterium reaches and successfully attaches to a surface of the host, biofilm formation begins. The subsequent formation of microcolonies or biofilm is mediated by the movement and growth of the attached bacteria. For many bacterial species, biofilm formation is initiated by the flagella-mediated motility that enhances movement towards and along the surface. Furthermore, extracellular matrix components are required to produce mature biofilms that keep the biofilm attached to the surface for the subsequent surface colonisation (Yildiz and Visick, 2009).

The outer membrane proteins (OMPs) of *Vibrio* have an essential role in initial attachment of the bacterium to the host cells. They represent a large group of proteins called β-barrel proteins that are present in the outer membrane of Gram-negative bacterial cells (Duperthuy et al., 2011; Liu et al., 2015). Zhu et al. (2019b) reported a successful in-vitro adhesion of *V. harveyi* to grouper embryonic cells but showed inhibition of adhesion in the presence of anti-native OMP antibodies, particularly the TolC. Furthermore, *V. mimicus* could adhere to epithelioma papulosum cyprinid cells in-vitro when co-incubated with recombinant OmpU (Liu et al., 2015).

4.3. Route of entry

Vibrio infection commences with the initial contact of the bacterium with the host. This is followed by the pathogen invading the host tissues, overwhelming the host defences to colonise and multiply, and causing

damages to tissues of the host (Ruwandeepika et al., 2012). According to Mohamad et al. (2019a), the most recognised routes of infection by *Vibrio* spp. for the initial contact are through the mouth, the gills or the skin aberrations since *Vibrio* spp. are ubiquitous in aquatic environment (Fig. 3).

Fish intestinal mucosa is a single-cell layer and unlike mammals, it lacks the Payer's patches, M cells, immunoglobulin A and J-chain immunoglobulins (Rombout et al., 2011). The intestine has been identified as a portal of entry for *V. anguillarum* (Olsson et al., 1996; Liu et al., 2014) and *V. alginolyticus* (Chen et al., 2008). The bacterium initially positions itself in close contact with the cellular brush border prior to phagocytosis of the bacterium by the enterocytes.

Transmission electron microscopy (TEM) shows that the bacterial cells are engulfed by the macrophage-like or neutrophilic cells residing the intraepithelial space (Rekecki et al., 2013). Furthermore, up-regulation of b7r, the mononuclear phagocyte marker and TLR5 that is responsible in recognising bacterial flagellin, implied the bacterial entry through the intestinal mucosa (Liu et al., 2014). Moreover, Olsson et al. (1996) suggested that *V. anguillarum* enters the circulation through the intestines before it infects the visceral organs of orally-infected turbot (*Scopthalmus maximus*). Moreover, *V. alginolyticus* showed high affinity index (e_m/k_x) and high number of adhering bacteria to the gut mucous than the skin and gill in large yellow croaker (*Pseudosciaena crocea*) suggesting the entry through gut mucosa (Chen et al., 2008). However, there is lack of clarity in the mechanism of antigen uptake in the intestine although the enterocytes, M-cells and intraepithelial macrophages were suggested to play some role in antigen uptake (Parra et al., 2016). The bacteria could be translocated through intracellular or paracellular mechanisms, where endocytosis or penetration could be used by the former and relaxation of cellular junctions might involve the latter (Ringø et al., 2003).

Immunofluorescent-labelled *Vibrio* has been used to investigate the route of infection, localisation, and migration of bacteria inside the host (Le Roux, 2016). Possible route of entry through gills has been shown in eels, *Anguilla anguilla* following immersion exposure to immunofluorescent *V. vulnificus* Bt2- serE (Valiente et al., 2008). Bacterial cells that were attached to the gill epithelium either entered the capillaries immediately or multiplied in the gill tissue. However, the mechanism for the pathogen to cross the cellular barrier is unknown but there was no correlation between the infectious dose and the number of attached bacteria, suggesting that the number of binding receptors in the gills are constant. Kato et al. (2013) observed the same portal of entry when the gill epithelial cells of Japanese flounder, *P. olivaceus* took up inactivated *V. anguillarum* more frequently than the intestine or skin following an immersion with immune-reactive bacterin. The bacteria were observed in the epithelial cells of primary lamella of the gills and the cells that were found scattered in the adjacent connective tissue. This suggests internalisation of the inactivated pathogen through the gills. Nevertheless, this study may not completely reflect the field case of vibriosis, but enough to provide important clues regarding the route of entry, as inactivation of bacteria might destroy the adhesion property of the pathogen to the mucosal surface.

Apart from the mouth and gill, skin is another possible route of entry for *Vibrio* spp. into the host. Close associations of *V. harveyi, V. alginolyticus* and *V. parahaemolyticus* with superficial skin lesions or cuts, and the higher involvement of muscles than liver, spleen or kidney indicate the entrance of *Vibrio* spp. via disintegrated skin (Liu et al., 2016). In fact, experimentally, an enhanced *V. harveyi* infection was observed in

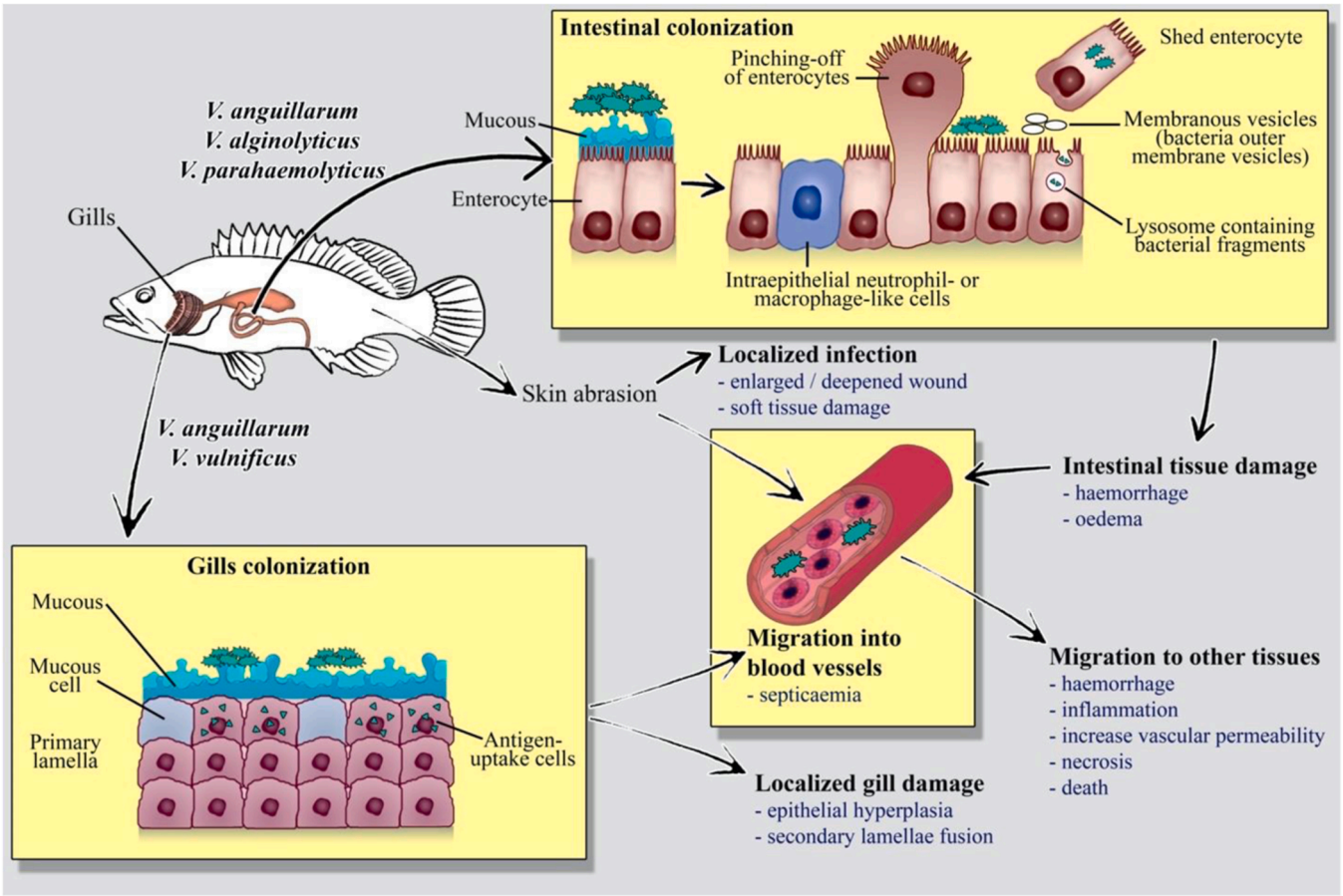

Fig. 3. Schematic drawing on the routes of entry of *Vibrio* spp. into the host. The most common routes of entry are the skin abrasion, mouth and gill, before the bacterium colonizes the local tissue and enters the blood circulation to cause systemic infection.

Asian seabass after skin abrasions compared to those without skin abrasions (Chin et al., 2020). Lindell et al. (2012) demonstrated the internalisation of *V. anguillarum* via skin epithelial cells through an in vitro study. Similarly, experimental infection of *V. harveyi* in European abalone, *Halotis tuberculata* revealed immediate bacterial colonisation in the gills and hypobranchial glands and presence of the organism in the haemolymph after 3 h, suggesting the portal of entry through the epithelial tissues of gill and hypobranchial gland area (Cardinaud et al., 2014).

4.4. Immuno-pathogenesis of Vibrio infections

Following a bacterial infection, the host immune system triggers the innate and adaptive immune responses to neutralise the invading bacteria. The initial adaptive immune response in tissues involves the infiltration of lymphocytes, the B- and T- cells (Magnadottir, 2010; Soto-Rodriguez et al., 2019; Lozano-Olvera et al., 2020), while granulation formation is an inflammatory response to a chronic infection (Manrique et al., 2015). However, granulations are also observed in some of the recent *Vibrio* infections (Mohi et al., 2010; Lozano-Olvera et al., 2020) involving the mesenteric tissue and liver following experimental intraperitoneal infection of juvenile white snook, *C. viridis* with *V. ponticus*. Pyogranulomatous lesions in branchial chambers, inner operculum, myocardium, liver, spleen and kidneys are observed in tiger puffer, *T. rubripes* following natural infection and following subsequent experimental intramuscular injection of *V. harveyi* (Mohi et al., 2010). Furthermore, activated CD4 + T-cells mediate the delayed type hypersensitivity reaction, leading to tissue destruction. The CD4 + T-cells produce cytokines, causing extensive accumulation of macrophages and formation of granuloma while localised concentrations of lysosomal enzymes in these granulomas cause extensive tissue necrosis. It is a known fact that liquefaction occurs from the proteases that are released by bacteria (Liu et al., 2019b) or proteases that are released by the host cells. In fact, liver damage may also be the result of poor elimination of the introduced proteases.

In general, inflammatory responses are crucial in host defence mechanisms following adherence and colonisation of bacterium. By having various inflammatory-inducing components, such as the lipopolysaccharide (LPS), peptidoglycan, lipopeptides, lipid-A associated proteins, flagellin, pilin, DNA and exotoxins (Heumann and Roger, 2002), Gram-negative bacteria seem to have a strategic tactical advantage over the host. The host typically response to these virulence factors by overproduction of inflammatory mediators that cause severe tissue damage and even death. This is termed as sepsis, which is well-studied in human but information is relatively scarce in terrestrial and marine animals (Hernández-Cabanyero et al., 2020; Faridon et al., 2021). Activation of macrophages by the cell-wall endotoxins of the bacteria results in the release of high levels of pro-inflammatory cytokines, which cause septic shock (Heumann and Roger, 2002). Similarly, cytokines are important in regulating pre-inflammatory responses in fish against pathogens and many PAMPs stimulate the release of cytokines from fish phagocytes (Sepulcre et al., 2016).

Recent advances in molecular techniques permit understanding of early immune responses in host. These include techniques such as cloning of cDNA sequences of immune-related genes to further understand their roles in bacterial infections, and the detection and measurement of genes of proinflammatory cytokines and chemokines such as the IL-6, CCL19, IL-1β, and IL-8 in various organs of fish infected by *Vibrio* spp. (Chen et al., 2018a). Presence of and changes in IL-6 gene expression in the head kidney, spleen, gills and liver following challenge by *V. anguillarum* were previously observed (Zhu et al., 2019a). In general, IL-6 was highly expressed in these organs after infection, suggesting enhanced phagocytosis and killing by monocyte-macrophage. Early response by upregulation of pro-inflammatory cytokines, the IL-1β and IL-6 and the chemokine IL-8 in the spleen and head kidney after an intraperitoneal infection by *Listonella anguillarum* (*V. anguillarum*) and *P. damselae* subsp. *piscicida* was confirmed in European seabass, *D. labra* although no obvious histological lesions was observed in these organs. These findings indicate efficient pro-inflammatory response against *Vibrio* infection (Mosca et al., 2014).

Among the 18 outer membrane proteins of *V. parahaemolyticus*, VP1667, a putative outer membrane protein and VP2369, a murein transglycosylase A upregulated genes are related to several innate immune components. These include COX2, TNF-α, IL-6, IL-8, IL-15, C3b, NF-KB, TLR-1 and TLR-3 (Peng et al., 2018). Moreover, VP2369 upregulates lysozyme and IL-21 while VP1667 upregulates INF-γ and IL-1β genes. These prove that the outer membrane proteins of *V. parahaemolyticus* are responsible for humoral immune responses and involve in stimulation of inflammatory reactions. The pro-inflammatory cytokines stimulate the acute phase responses and thereby, the production of APPs from the liver (Nam et al., 2012). Hepcidins, an important APPs, cause hypoferremia in bacterial infections, leading the depletion of iron-availability in the host environment (Mosca et al., 2014).

5. Virulence of *Vibrio* spp

Each step of the infection cycle is influenced by the bacterial virulence factors. They are genes, expressed by the bacteria that ultimately lead to injuries to the host tissues (Defoirdt, 2014). Pathogenic *Vibrio* spp. possess virulence factors, such as the membrane and secretory proteins, polysaccharide capsule, outer membrane components, siderophores and biofilm forming proteins (Ina-Salwany et al., 2019). Almost all these virulence factors are located superficially or secreted out to the surrounding environment. In fact, secretion pathways have been readily recognised in pathogenic bacteria, which transport the virulence factors to the outside environment of bacterial cells (Ruwandeepika et al., 2012). In this subtopic, important virulence factors of *Vibrio* spp. and their roles in disease development are discussed.

5.1. Proteases

Bacterial proteases, excreted to extracellular milieu cause severe tissue damage during infection and are considered as important determinants of virulence in *Vibrio* (Austin and Zhang, 2006). *Vibrio* spp. produce and secrete several extracellular proteases (ECPs), which include the metalloproteases, collagenases, serine proteases, cysteine proteases, gelatinases and caseinases (Ruwandeepika et al., 2012; Liu et al., 2019b). They are responsible for the proteolysis and haemolysis reactions seen in infected fish. For example, vibriolysin-like proteases (VPLs) are the principal factors in the pathogenicity of *V. vulnificus* and *V. parahaemolyticus*, while metalloproteases (VLP III and VLP VIII) of *V. vulnificus* and *V. parahaemolyticus* degrade type IV collagen in the vascular basement membrane (Huang et al., 2018).

Moreover, metalloproteases of *V. vulnificus* influence apoptosis (Lee et al., 2014) and increase vascular permeability (Park et al., 2014). Chang et al. (2005) revealed that extracellular metalloproteases of *V. vulnificus* initiate prothrombin activation and fibrinolysis. The rapid prothrombin activation is useful in the initial invasion of the bacterium since the generated fibrin leads to formation of thrombus in the vasculature. These fibrin clots trap the bacteria and prevent them from being phagocytosed. When the bacterial numbers increased and urged to break free from the fibrin clot, fibrinolysis activity of metalloproteases dissolves the material (Chang et al., 2005). Moreover, the proteolytic activity of metalloproteases destroys the collagen and the elastin, disintegrating the tissues. It has been shown that *V. harveyi* strain AP6 produces a metalloprotease of 35 kDa in size, and is able to digest the gelatine, fibronectin, and type IV collagen in vitro (Teo et al., 2003). Similarly, Liu et al. (2019b) demonstrated the presence of large number of characterised gelatinolytic proteases in several marine *Vibrio* spp. Most species produce proteases after 24 h and some of these proteases showed activity until after 60 h. This helps *Vibrio* to survive in

collagen-rich surroundings.

Some studies suggested that the serine protease is a major protease in vibriosis (Lee et al., 2002; Won and Park, 2008) while others reported the cysteine proteases as major protease (Liu et al., 1997; Lee et al., 1999). However, the haemolytic activity on fish or rabbit erythrocytes, protease activity on casein and cytotoxic activity on chinook salmon embryo and epithelioma papullosum cyprinid cells are strain-dependent. Similarly, siderophores are produced by all strains of *V. harveyi* and the ECPs of all except ATCC 14126 strain are inhibited by phenyl methanesulfonyl fluoride (PMSF), indicating that the type is serine protease. On the other hand, the ECPs of ATCC 14126 strain is inhibited by trans-epoxysuccinyl-L-leucylamido-(4-guanidino) butane (E-64) and thus, the enzyme is cysteine protease. Intraperitoneal injection of purified ECPs or serine protease (33-kDa) from three isolates of *V. harveyi* highlighted the virulence that resulted in gastroenteritis and death in groupers, *E. coioides* and red drum, *Sciaenops ocellatus* (Lee et al., 2002).

Siderophore, phospholipase and ECPs produced by *Vibrio* spp. significantly contribute to the severity of infection as positive correlations between them and mortality of brine shrimp, *Artemia franciscana* nauplii have been previously demonstrated (Soto-Rodriguez et al., 2003). Haemolysins of *Vibrio* causes haemolysis and cytotoxicity of red blood cells and gill cells, respectively (Bai et al., 2010). Lysis of erythrocytes could cause oxygen starvation in host cells leading to steatosis, mainly in liver (Peng et al., 2016). Taken together, these proteases and haemolysins of pathogenic *Vibrio* could be responsible for the circulation disorders associated with *Vibrio* infections such as lysis of red blood cell, rupture of vasculature causing haemorrhages, intravascular congestion, tissue destructions and septicaemia.

5.2. Lipopolysaccharide

Lipopolysaccharide (LPS) is an endotoxin of Gram-negative bacteria. It consists of three part; lipid A, polysaccharide and O-specific chain (Hang et al., 2013). It is recognised by the TLR5, TLR25, PTX3 and C1q receptors (Li et al., 2020) and is capable of inducing physiological, inflammatory, pathological and immunological responses. Immunologically, it stimulates cellular and humoral immune responses in fish by activating the macrophages, leucocytes, complement pathways and production of antibodies (Swain et al., 2008). The activated macrophages, in turn, produce cytokines such as IL-1, IL-6 and TNF-α that are involved in inflammatory reactions. As discussed before, these mediators are released to control the invading pathogens but excessive production could lead to septic shock and multiple organs failure (Fujihara et al., 2003).

LPS is also known to increase the plasma lysosomal level by activating the renal macrophages (Paulsen et al., 2001) and possibly the intestinal macrophages (Paulsen et al., 2003). Inducible nitric oxide synthases (iNOS) are key enzymes that are involved in generation of nitric oxide (NO) that is produced by respiratory burst to develop a cytotoxic environment (Torrecillas et al., 2017). Experimental intraperitoneal injection of LPS causes more production and accumulation of nitric oxide in the heart, plasma, gills, brain, muscle, liver and kidney of air-breathing catfish, *Clarias magur*. Higher expression of iNOS gene was observed in hepatic macrophages, proximal and distal tubules of kidney, atrial endocardium, neuroepithelial cells of gills and glial cells of brain following LPS treatment, indicating effective enhancement under infectious conditions (Choudhury et al., 2018). High immunoreactivity for iNOS was also detected in the intestinal mucosa and submucosa of European seabass, *D. labrax* (Torrecillas et al., 2017).

5.3. Outer membrane proteins

The outer membrane proteins (OMPs) of Gram-negative bacteria are key attributes in the bacterial pathogenicity. The OMPs play crucial roles particularly in bacterial adherence, nutrient uptake, and withstanding host defence mechanisms (Tang et al., 2017). They can trigger antibody production by the host since they act as epitopes on the surface (Pattipeilohy et al., 2019) thus, could be used to induce bactericidal neutralising antibodies that inhibit colonisation in the host (Cai et al., 2013a). Moreover, OMPs play a role in adaptation of *Vibrio* spp. to different salinities (Xu et al., 2004). Important information on OMPs of some *Vibrio* spp. are listed in Table 3.

5.4. Haemolysin and siderophores

All organisms, including bacteria, need iron to maintain cellular homoeostasis. Since free iron is absent in fish body, pathogenic bacteria obtain iron by secreting siderophores to chelate iron thus, capturing iron from outer membrane receptors or uptake as free or protein-bound form (Tong and Guo, 2009). Haemolysin is an exotoxin that helps in iron-acquisition by *Vibrio* spp. and is considered an important virulent factor (Ruwandeepika et al., 2010). Furthermore, gene analyses revealed that haemolysin-related genes from various *Vibrio* spp. can be considered as virulence genes. These include the *vhh* gene of *V. harveyi* (Ruwandeepika et al., 2010), *vmh* gene of *V. mimicus* (Geng et al., 2014), *vah* gene of *V. anguillarum* (Li et al., 2013) and *tlh* gene of *V. alginolyticus* (Jia et al., 2010). The pore-forming activity of haemolysin liberates iron-binding proteins such as haemoglobin, lactoferrin and transferrin from red blood cells and causes damage to polymorphonucleated cells, particularly the neutrophils and mast cells (Zhang and Austin, 2005). There are five haemolysin families that have been identified in *Vibrio* spp., which include thermostable direct haemolysin (TDH), thermolabile haemolysin (TLH) and δ-VPH of *V. parahaemolyticus*, HlyA of *V. cholera*, and novel haemolysin gene (*hlx*) family (Zhang and Austin, 2005; Jia et al., 2010). There are contradicting opinions regarding the involvement of haemolysin in the pathogenesis of vibriosis. Bai et al. (2010) demonstrated haemolytic and cytotoxic activities of the haemolysin of *V. harveyi* (VHH) on flounder erythrocytes and gill cell line (FG-9307). But some authors consider the haemolytic activity of *V. harveyi* as an insignificant factor in the pathogenicity of this bacterium (Soto-Rodriguez et al., 2003; Won and Park, 2008). In the red blood cells, haemolysin causes formation of tubular protrusions within 20 min of exposure, which then gradually increased in number and size of the pores. This ultimately leads to lysis of the red blood cells after 2 h of incubation. A haemolysin from *V. anguillarum* possesses phospholipase protein, which is encoded by *plp* gene and causes haemolysis by acting on phosphatidylcholine of the red blood cells (Li et al., 2013). In gill cell line, haemolysin causes cell death by caspase-dependent apoptosis pathway (Bai et al., 2010).

Sequelae of haemolysis is liberation of haemoglobin and production of hemosiderin, an iron-binding protein that is deposited mainly in melano-macrophage centres of fish. Therefore, large amount of hemosiderin deposits could be seen in the melano-macrophage centres in the liver, spleen and kidney following a chronic infection by *V. anguillarum* in plaice or flatfish (Agius and Roberts, 2003). However, this is a non-specific lesion that is also observed in many other chronic infections by other bacteria.

5.5. Type III secretion systems (T3SSs)

Almost all virulence-associated factors are found on or outside the bacterial cells. However, T3SSs are highly specialised injection apparatus of Gram-negative bacteria that inject bacterial proteins (effectors) directly into the cytoplasm of host cells through the cell membranes. T3SSs have been identified in *V. alginolyticus* (Zhao et al., 2010; Zhou et al., 2013), *V. harveyi* (Henke and Bassler, 2004) and *V. parahaemolyticus* (Broberg et al., 2010; Ham and Orth, 2012). The best studied T3SSs of *Vibrio* involved *V. parahaemolyticus* where T3SS1 was found in all tested environmental and clinical *V. parahaemolyticus* isolates, while T3SS2 was reported in clinical and some environmental isolates (Makino et al., 2003; Zhang and Orth, 2013). The T3SS1 is

Table 3
Animal species, methods and function of outer membrane proteins (OMPs) testing of *Vibrio* spp.

Vibrio spp.	Function	OMP/classification	MW	Tested host animal/method	Reference
V. harveyi	Adhesion	TolC	48.1 kDa	Grouper embryonic cells	Zhu et al. (2019b)
	Immunogenicity	TolC	48.1 kDa	Grouper (*Epinephelus fuscoguttatus*× *E. lanceolatus*)	Zhu et al. (2019b)
		OmpW	-	Humphead snapper (*Lutjanus sanguineus*)	Huang et al. (2019)
		OMP173	18.0 kDa	Flounders (*Paralichthys olivaceus*)	Yu et al. (2013)
		OMP 214 (OmpW)	25.0 kDa	Flounders (*Paralichthys olivaceus*)	
		OmpK	28.0 kDa	Orange-spotted grouper (*Epinephelus coioides*)	Ningqiu et al. (2008)
V. alginolyticus	Iron-regulation	OmpU	37.2 kDa	Antibody block assay	Lv et al. (2020)
		OmpA	36.01 kDa	Carp (*Cyprinus carpio*)	Xiong et al. (2010)
		OmpV	28.14 kDa		
		OmpU	36.28 kDa		
		VPA1435 (iron-regulated protein)	67.55 kDa		
		VA1631 (agglutination protein)	47.75 kDa		
	Osmoregulation	VA2212 (FA transport protein)	49.96 kDa	Carp (*Cyprinus carpio*)	Xiong et al. (2010)
		OmpV	28.14 kDa		
		OmpU	36.28 kDa		
	Immunogenicity	VA1061 (lipoprotein)	18.71 kDa	Carp (*Cyprinus carpio*)	Xiong et al. (2010)
		VPA0860 (FA transport protein)	47.75 kDa		
		OmpU	32.91 kDa	Crimson snapper (*Lutjanus erythropterus*)	Cai et al. (2013a)
		OmpW	23.47 kDa	Crimson snapper (*Lutjanus erythropterus*)	Cai et al. (2013b)
		OmpK	48.3 kDa	Asian seabass (*Lates calcarifer*)	Silvaraj et al. (2020)
		LamB	40–49 kDa	Zebra fish (*Danio rerio*)	Lun et al. (2014)
		OmpK, OmpW	23.0 kDa	Grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*)	Nehlah et al. (2016)
			31.0 kDa		
V. parahaemolyticus	Osmoregulation	OmpW	23.47 kDa	-	Xu et al. (2004)
		OmpV	28.15 kDa		
		elongation factor TU	43.15 kDa		
	Immunogenicity	VP1234	-	Mice	Gao et al. (2020)
		VP0802	51.86 kDa	Mice	Li et al. (2014)
		VP1667	~65.0 kDa	Zebra fish (*Danio rerio*)	Peng et al. (2018)
		VP2369	~45.0 kDa		
		OmpW	27 kDa	Large yellow croaker (*Pseudosciaena crocea*)	Mao et al. (2007a)
		OmpV	32 kDa		
		OmpK	33 kDa		
		OmpU	47 kDa		
		TolC	48 kDa		
		psuA, pvuA	78 kDa	Large yellow croaker (*Pseudosciaena crocea*)	Mao et al. (2007b)
			80 kDa		
		LptD	-	Mice	Zha et al. (2016)
		LamB	40–49 kDa	Zebra fish (*Danio reiro*)	Lun et al. (2014)
V. ichthyoenteri	Immunogenicity	OmpA	52.0 kDa	Flounders (*Paralichthys olivaceus*)	Tang et al. (2017)
		OmpT	47.0 kDa	Flounders (*Paralichthys olivaceus*)	Tang et al. (2019)
V. tubiashii	Iron-chealating	HutA	77 kDa	Mice	Beaubrun et al. (2011)
		Transport protein	78 kDa	Mice	
V. mimicus	Adhesion	OmpU	55.6 kDa	Epithelioma papulosum cyprinid (EPC) cells	Liu et al. (2015)
	Immunogenicity	LamB	40–49 kDa	Zebra fish (*Danio rerio*)	Lun et al. (2014)
V. splendidus	Attachment, Invasion	OmpU	30.0 kDa	Oyster (*Crassostrea gigas*)	Duperthuy et al. (2011)
V. vulnificus	Immunogenicity	OmpII-U-A	70.0 kDa	European eel (*Anguilla anguilla*)	He et al. (2020)
		OmpU	51 kDa	Japanese eel *(Anguilla japonica)*	Le et al. (2018)
		LamB	40–49 kDa	Zebra fish (*Danio rerio*)	Lun et al. (2014)

mainly involved in cytotoxicity that induces autophagy, rounding of cell and followed by cell lysis (Burdette et al., 2009; Broberg et al., 2010). On the other hand, the T3SS2 is associated with enterotoxicity (Akeda et al., 2011; Ham and Orth, 2012).

6. The role of stress in *Vibrio* infection

It is widely known that stress greatly influences the outcome of an infection. This have been studied in many diseases of terrestrial and aquatic animals. Among the common stressors encountered by cultured fish include transport, handling, over-crowding, malnutrition, fluctuations in water temperature, oxygen and salinity levels, and peripheral effects of exposure to infectious disease (Azila et al., 2017; Mariana et al., 2019; Chin et al., 2020). Host responses to stress are observable through gross or microscopic examinations of organs, particularly the gills, liver, skin, and components of the urogenital tract (Harper and Wolf, 2009). Lesions associated with stress are either non-specific or specific, depending on the types of stressors. This has been extensively described in previous study (Harper and Wolf, 2009). More importantly, stress influences the host's immune system. When the stressor is acute and short-termed, the fish immune response reveals an activating phase that enhances the innate responses. In contrast, when the stressor is chronic and long-termed, the immune response shows suppressive effects that increases the risk of infection (Tort, 2011).

Exposure to stressors was found to induce cortisol secretion in the plasma and skin mucus in a time-dependent manner. The high level of cortisol affects the lymphocytes by significantly lowering the number of Ig-positive lymphocytes (Espelid et al., 1996; Tort, 2011). The high cortisol level also increased the stress- and immune-related gene expression profiles. During stress, the expressions of pro-inflammatory and anti-inflammatory cytokines are greatly affected. Depending on species of fish, type of stressors and tissue, the expressions might be up-regulated or down-regulated (Khansari et al., 2018). Furthermore, modulation of the immune system is not only at tissue level, but also at cellular and biochemical levels. For example, fish from polluted sites tend to show depressed spleen somatic index. Similarly, differential blood cell counts reveal consistent increase in the phagocytes and decrease in thrombocytes of stressed fish, while the phagocytic activity is significantly depressed (Pulsford et al., 1994). It is important to note the synergistic effect of more than one stressor on fish leads to severely

reduced bactericidal and inflammatory activities, and significantly alters the blood-cell compositions (Rebl et al., 2020). It seems that stress causes the fish to compromise their ability to resist infections.

It must be emphasised that the environmental stressors do not only affect the fish, but also affect the *Vibrio* organisms. Qian et al. (2020) studied the responses of bacterial biofilm formation to stress following modified atmospheres from anaerobiosis to aerobiosis, and found that the ability of biofilm formation by *V. parahaemolyticus* was efficiently decreased during the physiological conversion from anaerobiosis to aerobiosis. This can be explained by down-regulation of expression of biofilm formation genes (*luxS*, *aphA*, *mshA*, *oxyR*, and *opaR*), EPS production genes (*cpsA*, *cpsC*, and *cpsR*), and virulence genes (*vopS*, *vopD1*, *vcrD1*, *vopP2*β, and *vcrD2*β) that can negatively affect the ability of *Vibrio* spp. to colonise the host (Qian et al., 2020). Several environmental stressors, including osmotic stress, ethanol, temperature shift and iron starvation have also been shown to affect the *Hfq* gene and subsequently the ability of *V. alginolyticus* to form biofilms (Liu et al., 2011).

However, through years of natural selection, *Vibrio* spp. such as *V. cholera* has adapted to overcome environmental stress, particularly the aquatic environment by using an array of genes. Furthermore, some *Vibrio* spp. have evolved from non-toxigenic environmental species to become pathogenic *Vibrio* through acquisition of virulence genes thus, capable of overcoming the stress of new host by infecting susceptible fish (Nurliyana et al., 2019). For example, the genome of *V. cholera* carries the genetic determinants that enable the bacterium to survive both aquatic environment and the host environment (Faruque et al., 2004). The host immune system is successfully evaded by altering their surface antigenicity and surface charge, expression of enzymes and effector--mediated modulation of adaptive immune response to enhance their chances of attachment and colonisation (Rueggeberg and Zhu, 2016). Similarly, *V. vulnificus* has successfully adapted to stressful environmental changes while living freely in seawater and upon colonisation of the host (Hulmann et al., 2003). In fact, studies have shown that exposure of *V. parahaemolyticus* to sublethal stress conditions in new host induces an increased ability to form biofilm (da Rosa et al., 2017). Therefore, biofilm production by clinical strains of *Vibrio* was found to be consistently higher than the environmental strains. The growth rate of clinical strains was not affected at pH 5.5, 7.5, and 8.5 as compared to the environmental strains, demonstrating a tolerance to acidic and alkaline conditions by the clinical strains (Cam and Brinkmeyer, 2019).

It is obvious that environmental stressors affect both the host and the agent. Therefore, the ability to reduce the effect of environmental stress either by the host or the bacterium eventually influences the outcome of infection. In general, *Vibrio* spp. have the capability to adapt to the environmental stress through upregulating selected genes that not only maintain their survival in the aquatic environment, but also upregulating the virulence genes to ensure the virulence. On the other hand, environmental stressors suppress immunity of the host, and often the host could not adapt to these stresses thus, enhancing susceptibility to infection. Therefore, environmental stress usually works in favour of initiating an infection and must be dealt-with by farmers.

7. Quorum sensing

Quorum sensing is an important mechanism that ensures survival of bacterial pathogens in the environment as well as progression of bacterial diseases. It is a cell-to-cell communication that allows bacteria to share information pertaining to cell density, metabolism, growth, virulence and responses to stress thus, enables the bacteria to adjust the gene expression accordingly (Rutherford and Bassler, 2012). Quorum sensing is considered important in the development of many bacterial diseases as it regulates crucial processes including antibiotic production, biofilm formation and virulence factor secretion (Rutherford and Bassler, 2012). Basically, the signalling of quorum sensing is mediated by small intracellularly-produced molecules, referred to as autoinducers (Verbeke et al., 2017). They are excreted out of microbial cells either passively or actively and are detected at threshold level after binding with cognate receptors, followed by a signal transduction cascade (Sharma et al., 2020). Water solubility and membrane permeability of autoinducers help in their free movement through the cell membrane to maintain a similar concentration inside and outside (Li and Zhao, 2020).

Hastings and Nealson (1977) introduced the quorum sensing concept when they discovered a positive correlation between *V. fishery* population density and bioluminescent photophores in Hawaiian bobtail squid, *Euprymna scolopes*. Since then, *Vibrio* has become a model organism for bacterial communication studies mainly due to the bioluminescent ability of several *Vibrio* spp. (Ball et al., 2017). Quorum sensing system of *Vibrio* spp. uses three signal transduction pathways with unique autoinducer molecule in each system. LuxM/LuxN system uses N-acyl homoserine lactones (*AI-1*), LuxS/LuxPQ system uses autoinducer 2 (*AI-2*) and CqsA/CqsS system uses cholera autoinducer 1 (*CAI-1*) (Ball et al., 2017; Chen et al., 2020). The autoinducers of each system follows a definitive pattern and the concentration differs with the bacterial growth phase. At low cell densities, autoinducer synthases *LuxM*, *LuxS* or *CqsA* produce low concentrations of signal molecules and the master regulator, the *LuxR* remains destabilised. In high cell densities, the signal molecules produced by *LuxM*, *LuxS* or *CqsA* are in high concentration and *AI-1*, *AI-2* and *CAI-1* bind to *LuxN*, *LuxPQ* and *CqsS* receptors, respectively. Thus, the *LuxU* is dephosphorylated and the phosphorylation of *LuxO* is suppressed. *LuxR* is activated and expressed in high levels and regulates group behaviour genes that resulted in production of siderophore, metalloprotease and other genes (Milton, 2006; Ball et al., 2017). It has been shown that quorum sensing enhances the viability of *V. cholerae* following environmental stress conditions by upregulating the expression of RpoS (Juoelsson et al., 2007).

It is worthwhile to mention that quorum sensing can be disrupted to control progression of a disease. This is termed as quorum quenching. Quorum quenching basically blocks quorum sensing signals between bacterial cells using enzymes that degrade (Sharma et al., 2020) or modify (Hong et al., 2012) autoinducers and reduce the virulence of *Vibrio* pathogens. So far, the two most widely studied quorum quenching enzymes are the lactonases and acylases, the enzymes that target the abovementioned N-acyl homoserine lactones. The concept of using quorum quenching enzymes to control bacterial diseases is attractive and promising, as this approach will unlikely lead to development of resistance (Defoirdt et al., 2008).

8. Application of pathology to control vibriosis in fish

The knowledge on pathology and pathogenicity of *Vibrio* spp. in aquatic animals has tremendously aid in developing control measures (Georgiadis et al., 2001). Traditionally, study of pathology of a certain disease largely revolves around understanding the epidemiologic triad that include the three most important factors; the host, agent, and environment. Imbalance interaction between these three factors would contribute to infection and a disease state to the host. Once important components of each of these factors are understood through research, control measures may be implemented. In many aquatic diseases including vibriosis, the agent and environment factors are generally difficult to control (Assefa and Abunna, 2018), thus study and application are lesser compared to those involving the host factors. Many research has been conducted mainly to address the host factors (Ina--Salwany et al., 2019). This topic addresses some of the application of pathology to control vibriosis based the epidemiologic triad factors, and focuses on the manipulation of the host factor using vaccines.

Due to the nature of aquatic environment, many factors are beyond control such as the water temperature, water current, inclement weather, water quality, exposure to wild aquatic animals, and presence of pathogens including *Vibrio* spp. (Assefa and Abunna, 2018; Hackbusch et al., 2020; Liu et al., 2016). This is especially true when fish are farmed in sea cages (Mohamad et al., 2019b; Nurliyana et al., 2019). Control of vibriosis related to the environment and pathogen factors

includes implementation of biosecurity to control human movement into and around fish farms. To control presence of pathogens in farming premises, chemical compounds are routinely used as disinfectants (Assefa and Abunna, 2018). Min et al. (2015) used monopersulfate compound against *V. harveyi* and found that 2.4 ppm concentration inhibits *V. harveyi* growth after one hour exposure and it decreased the movement but did not cause mortalities in *Litopenaeus vannami* within 7 days. Furthermore, through research, it is known that *Vibrio* spp. present in high concentration in fish with vibriosis. Thus, fish carcasses should be properly disposed. The practice of disposing dead fish into the water could potentially spread the disease to farm and wild aquatic animals.

Research to manipulate the host factors mainly revolves around the aim to improve the host's immune system with vaccine, or mediate competition with the pathogen using probiotics. In general, vaccines contain killed, attenuated, or parts of a particular pathogen that trigger a specific immune response of the host. Traditional vaccines such as inactivated or live attenuated vaccines and vaccine produced by genetic engineering such as subunit vaccines, DNA vaccines and live vector vaccines are being used in many aquaculture species in prevention of vibriosis (Ji et al., 2020).

Inactivated vaccines are produced by multiplication of bacteria in large quantities in culture media followed by inactivation through physical or chemical methods which kills entire bacteria to lose pathogenicity without compromising the antigenicity (Dadar et al., 2017). Inactivated vaccines were the earliest and still the commonest commercialised type of vaccines to be used in aquaculture industry. They are being used to control *Vibrio* infections in cultured fish species such as Atlantic salmon, salmonids, seabream, and European sea bass in Mediterranean and Asian region (Dadar et al., 2017; Matsuura et al., 2019; Miccoli et al., 2019; Ji et al., 2020). Researchers have widely used 0.2–1% formalin to inactivate bacterial cultures owing to the simple technique and easy storage (Dadar et al., 2017; Nguyen et al., 2017; Abu Nor et al., 2020). However, surface antigen composition of the bacterial cell wall can be destroyed to some extent by this method (Sun et al., 2020). Therefore, recently, some researchers used peptide-based killing method to preserve the complete surface antigen structures (Gu et al., 2021). However, killed vaccines are needed to be incorporated with an adjuvant and to be repeated to achieve the maximum effectivity (Tafalla et al., 2013). Paraffin oil and mineral oil have been used commonly in licensed *Vibrio* vaccines (Ji et al., 2020). Formalin- killed whole cell monovalent and bivalent *Vibrio* vaccines with booster doses showed great potential as killed vaccines (Aly et al., 2021; Abou-Okada et al., 2020; Mohamad et al., 2022). Inactivated whole cell vaccines can be administered intraperitoneally (Abu Nor et al., 2020; Aly et al., 2021; Diab et al., 2021;), intramuscularly (Gu et al., 2021) or by incorporating with feed (Mohamad et al., 2022), immersion (Diab et al., 2021).

Chemically or genetically weakened bacteria are used to develop live attenuated vaccines. Once inoculated into the body, mutated pathogen will grow and multiply within the body without causing actual disease. More importantly, unlike inactivated vaccine, attenuated pathogens stimuli host immune system as in the natural infection and generate long-term or even lifelong protection (Ji et al., 2020). Live attenuated vaccines induce cellular, humoral, and mucosal immunity because of the unchanged antigenic presentation. It can spread the vaccine strain in the population if vaccinated fish could spread the vaccine strain (Dadar et al., 2017; Mohd-Aris et al., 2019b).

Attenuation of bacteria is achieved through traditional methods or modern genetic modification techniques. Traditionally, virulent strains serially passage in specific antibiotics or on laboratory media to achieve random mutation. Modern advancement in molecular biology facilitates knock out of specific genes of wild bacterial strains. Basically, virulence-associated genes or metabolic pathway genes are altered by insertion, deletion, or disruption (Mohd-Aris et al., 2019b; Ji et al., 2020). Hu et al. (2012) developed a live attenuated vaccine against *V. harveyi* by serial passaging of virulent wild type *V. harveyi* strain T4D on increasing concentration of rifampicin to attenuate and obtained mutant *V. harveyi* T4DM. Mutant *V. harveyi* T4DM showed slow growth in iron-deplete medium, low capacity in haemolysis, and reduced resistance to killing by serum implying the reduce in virulence. Moreover, serum antibody titre were increased indicating highly effective protection against *V. harveyi* and *V. alginolyticus* in Japanese flounder (*Paralichthys olivaceus*). Many researchers employed genetic engineering such as overlap extension PCR technique and allelic exchange mutagenesis to develop attenuated *Vibrio* strains to be used as live attenuated vaccines (Mohd-Aris et al., 2019b). It has been reported that *hopPmaJ* mutant, Δ*claP* mutant, Δ*acfA* mutant, and Δ*sodB* mutant of *V. alginolyticus*, Δ*air1*Δalr2 mutant of *V. anguillarum* and protease deletion mutant of *V. harveyi* can be used as candidates for live attenuated *Vibrio* vaccines (Mohd-Aris et al., 2019a; Ji et al., 2020).

Live attenuated vaccines use less virulent or weakened strains to mimic natural infection and thereby induce both cellular and humoral immune responses. However, they have the risk of reverting the virulence and develop mild to serious disease if attenuated inadequately or when replicating in the fish and persist in the target host, they can be released into the environment (Chu et al., 2015; Mohd-Aris et al., 2019b). To overcome this, Chu et al. (2015) developed an inducible bacterial lysis system which was proved to be controlled by iron-limitation signals in *V. anguillarum* strain MVAV6203 by enhancing biosafety of live *V. anguillarum* strain MVAV6203 vaccine inducing similar levels of immune protection and 89.3% of relative percent survival in vaccinated zebrafish.

Genetic engineering has also been used to develop vibriosis vaccines other than conventional methods. Natural or synthetic immunogenetic material of a pathogen is introduced to a heterologous microorganism such as bacteria or yeast and use as a vaccine (Ji et al., 2020). Outer membrane proteins (OMPs) of *Vibrio* spp. have been widely used in experimental vaccine development (Table 3). Nehlah et al. (2016) introduced *OmpK* and *OmpW* of *V. alginolyticus* into pET32 Ek/LIC vector to express in *Escherichia coli*. Juvenile hybrid groupers vaccinated at day 0 and day 29 with OMP-expressing inactivated *E. coli* were challenged at day 28 and showed increased antibody production by both OPM vaccines. *OmpK*-expressed inactivated *E. coli* provided 100% survival.

Research on DNA vaccines also popular in fish vaccine studies as they induce both cellular and humoral immunity (Xu et al., 2019). DNA vaccines are being developed against common *Vibrio* pathogens such as *V. harveyi*, *V. parahaemolyticus*, *V. anguillarum*, *V. mimicus*, and *V. alginolyticus* and prepared commonly as intramuscularly injectable vaccines (Ji et al., 2020). Bacterial ghosts are a novel vaccine candidate in fish vaccines. Bacterial ghosts are non-living intact empty envelop of bacterial cells, particularly Gram-negative bacteria. Generally, the emptying of bacteria is achieved biologically using controlled expression of bacteriophage PhiX174 lysis gen E or chemically by sponge-like protocol (Zhu et al., 2022). Bacterial ghosts of *Vibrio* species such as *V. alginolyticus*, *V. mimicus*, *V. parahaemolyticus*, and *V. vulnificus* has been developed (Zhu et al., 2022). Bacterial ghosts can be used as a delivery system to DNA vaccines as well (Ji et al., 2020; Mohd-Aris et al., 2019a).

Probiotic, group of live microbes, use in aquaculture is trending as an alternative to antibiotic and has many benefits. Probiotics are known to mediate competition, exclusion, and displacement of pathogens in *Vibrio* (Chabrillón et al., 2005). Furthermore, probiotics also promote growth, improve and balance the microbial population, and provide antagonist action in the gut, and modulate and stimulate the immune system (Ina-Salwany et al., 2019). Amenyogbe et al. (2022) fed cobia, *Rachycentron canadum* with locally isolated *Bacillus* sp. RCS1 and *B. cereus* RCS3 in the concentration of 1×10^{10} CFU/mL and 1×10^{12} CFU/mL, respectively for 70 days before challenge intraperitoneally with 200 μl of 1×10^{9} copies/g *V. harveyi*. Haematological and biochemical indices of serum and relative percentage of survival rates of both probiotic spp. were significantly increased in treatment groups. Moreover, purified antagonist substances from probiotic bacteria have potential in treatments or control of vibriosis. Recently, Gao et al. (2017) demonstrated

the presence of anti-*Vibrio* substance, amicoumacin A, in the probiotic *Bacillus pumilus* and its activity in destruction of bacterial cell membranes causing cell death in *V. vulnificus* bacterial cells.

9. Conclusions and future recommendations

Vibrio bacteria, being commensal, opportunistic, or even primary pathogens, pose a great threat to the finfish and shellfish aquaculture production systems. The infection causes significant economic and social loss throughout the world. The existence and survival of obligate and non-obligate *Vibrio* organisms in the environment need to be explored to understand their pathogenicity. Virulent strains of *Vibrio* attach to the mucosal surfaces of host and proliferate using various cell surface factors. Many of these organisms would be inactivated or killed by the non-specific host defence mechanisms, however, the resistant and surviving bacterial cells penetrate the mucosal barrier and multiply in host to cause tissue damage. The proliferated bacteria are transported to systemic organs through blood resulting in septicaemia in the host. Excessive inflammatory reactions and immune responses by the host and the reactions of the various virulent factors such as proteases, LPS and extracellular proteins of the bacteria would lead the host to succumb to septicaemic shock and ultimate death. Although substantial advances in basic knowledge on disease progress and virulence have been made in past years, the evidence of novel adaptations like intracellular survivability shows that vibriosis is here to remain as an important bacterial infectious disease in aquatic animals. The modern approaches like genomics and transcriptome profiling give better understanding on pathogenesis of the various *Vibrio* spp. and the host immune responses. These understandings are helpful in rational approach to disease diagnosis as well as prevention and control measures.

Research studies on Vibriosis in fish has so far focused on understanding the pathogenesis and virulence of *Vibrio* spp. in different hosts, availability, and mechanisms of viability in the environment and development of control strategies. However, despite of many research and management approaches, adaptations, emergence of new strains and environment persistency as commensal are causes to high mortalities seen in aquaculture farms. Vibriosis control through vaccination is more effective and environmentally safe compared to use of antimicrobials. Fish vaccine development has evolved from inactivated or live attenuated to genetically engineered vaccines over past few decades. However, gaps in basic research on immune systems of different fish species has limited the development of species-specific vaccines. New generation gene technologies should be used more in developing highly effective vaccines.

CRediT authorship contribution statement

Tilusha Manchanayake: Writing – original draft, Writing – review & editing, Visualization. **Annas Salleh**: Conceptualization, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition. **Mohammad Noor Azmai Amal, Ina Salwany Md Yasin, Mohd Zamri-Saad**: Conceptualization, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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References

Abdelsalam, M., Ewiss, M.A.Z., Khalefa, H.S., Mahmoud, M.A., Elgendy, M.Y., Abdel-M 1 am, D.A., 2021. Coinfections of *Aeromonas* spp., *Enterococcus faecalis,* and *Vibrio alginolyticus* isolated from farmed Nile tilapia and African catfish in Egypt, with an emphasis on poor water quality. Microb. Pathog. 160, 105213 https://doi.org/10.1016/j.micpath.2021.105213.

Abou-Okada, M., Gendy, N.M., Elhelw, R., 2020. Effect of booster vaccination on immunoprotection in European seabass vaccinated against vibriosis. Aquac. Res. 00, 1–13. https://doi.org/10.1111/are.14930.

Abu Nor, N., Zamri-Saad, M., Md Yasin, I.S., Salleh, A., Mustaffa-Kamal, F., Matori, M.F., Azmai, M.N.A., 2020. Efficacy of whole cell inactivated *Vibrio harveyi* vaccine against vibriosis in a marine red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) model. Vaccines 8, 734. https://doi.org/10.3390/vaccines8040734.

Acord, J., Maskell, J., Sefton, A., 2005. A rapid microplate method for quantifying inhibition of bacterial adhesion to eukaryotic cells. J. Microbiol. Methods 60, 55–62. https://doi.org/10.1016/j.mimet.2004.08.011.

Agius, C., Roberts, R.J., 2003. Melano-macrophage centres and their role in fish pathology. J. Fish Dis. 26, 499–509. https://doi.org/10.1046/j.1365-2761.2003.00485.x.

Akeda, Y., Kodama, T., Saito, K., Iida, T., Oishi, K., Honda, T., 2011. Identification of the *Vibrio parahaemolyticus* type III secretion system 2-associated chaperone VocC for the T3SS2-specific effector VopC. FEMS Microbiol. Lett. 324, 156–164. https://doi.org/10.1111/j.1574-6968.2011.02399.x.

Alarcón, M., Thoen, E., Poppe, T.T., Bornø, G., Mohammad, S.N., Hansen, H., 2016. Co-infection of *Nucleospora cyclopteri* (Microsporidia) and *Kudoa islandica* (Myxozoa) in farmed lumpfish, *Cyclopterus lumpus* L., in Norway: a case report. J. Fish Dis. 39, 411–418. https://doi.org/10.1111/jfd.12372.

Aly, S.M., Elissa, A.E., EliBanna, N.I., Albutti, A., 2021. Efficiency of monovalent and polyvalent *Vibrio alginolyticus* and *Vibrio parahaemolyticus* vaccines on the immune response and protection in gilthead sea bream, *Sparus aurata* (L.) against vibriosis. Fish Shellfish Immunol. 111, 145–151. https://doi.org/10.1016/j.fsi.2020.10.011.

Amal, M.N.A., Koh, C.B., Nurliyana, M., Suhaiba, M., Nor-Amalina, Z., Santha, S., Diyana-Nadhirah, K.P., Yusof, M.T., Ina-Salwany, M.Y., Zamri-Saad, M., 2018. A case of natural co-infection of Tilapia Lake Virus and *Aeromonas veronii* in a Malaysian red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) farm experiencing high mortality. Aquaculture 485, 12–16. https://doi.org/10.1016/j.aquaculture.2017.11.019.

Amalina, N.Z., Santha, S., Zulperi, D., Amal, M.N.A., Yusof, M.T., Zamri-Saad, M., Ina-Salwany, M.Y., 2019. Prevalence, antimicrobial susceptibility and plasmid profiling of *Vibrio* spp. isolated from cultured groupers in Peninsular Malaysia. BMC Microbiol. 19, 1–15. https://doi.org/10.1186/s12866-019-1624-2.

Amaro, C., Sanjuán, E., Fouz, B., Pajuelo, D., Lee, C., Hor, L., Barrera, R., 2015. The fish pathogen *Vibrio vulnificus* biotype 2: epidemiology, phylogeny, and virulence factors involved in warm-water vibriosis. Microbiol. Spectr. 3 https://doi.org/10.1128/microbiolspec.VE-0005-2014.

Amenyogbe, E., Zhang, J.-d, Huang, J.-S., Chen, G., 2022. The efficiency of indigenous isolates *Bacillus* sp. RCS1 and *Bacillus cereus* RCS3 on growth performance, blood biochemical indices and resistance against *Vibrio harveyi* in cobia fish (*Rachycentron canadum*) juveniles. Aquac. Res. 25, 101214 https://doi.org/10.1016/j.aqrep.2022.101241.

Amin, A.K.M.R., Tanaka, M., Al-Saari, N., Feng, G., Mino, S., Ogura, Y., Hayashi, T., Meirelles, P.M., Thompson, F.L., Gomez-Gil, B., Sawabe, T., 2017. *Thaumasiovibrio occultus* gen. nov. sp. nov. and *Thaumasiovibrio subtropicus* sp. nov. within the family Vibrionaceae, isolated from coral reef seawater off Ishigaki Island, Japan. Syst. Appl. Microbiol. 40, 290–296. https://doi.org/10.1016/j.syapm.2017.04.003.

ArunKumar, M., LewisOscar, F., Thajuddin, N., Nithya, C., 2019. Isolation and screening of biofilm forming *Vibrio* spp. from fish sample around south east region of Tamil Nadu and Puducherry. Biocatal. Agric. Biotechnol. 17, 379–389. https://doi.org/10.1016/j.bcab.2018.12.017.

Assefa, A., Abunna, F., 2018. Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish, 10 pages Vet. Med. Int., 5432497. https://doi.org/10.1155/2018/5432497.

Austin, B., 2010. Vibrios as causal agents of zoonoses. Vet. Microbiol. 140, 310–317. https://doi.org/10.1016/j.vetmic.2009.03.015.

Austin, B., Zhang, X.H., 2006. *Vibrio harveyi*: a significant pathogen of marine vertebrates and invertebrates. Lett. Appl. Microbiol. 43, 119–124. https://doi.org/10.1111/j.1472-765X.2006.01989.x.

Azila, A., Ramli, R., Ridzuan, M.S.M., Murni, M., Hashim, S., Sudirwan, F., Abdullah, S. Z., Mansor, N.N., Amira, S., Zamri-Saad, M., Amal, M.N.A., 2017. The presence of *Vibrionaceae*, *Betanodavirus* and *Iridovirus* in marine cage-cultured fish: role of fish size, water physicochemical parameters and relationships among the pathogens. Aquac. Rep. 7, 57–65. https://doi.org/10.1016/j.aqrep.2017.06.001.

Bai, F., Sun, B., Woob, N.Y.S., Zhang, X., 2010. *Vibrio harveyi* hemolysin induces ultrastructural changes and apoptosis in flounder (*Paralichthys olivaceus*) cells.

Biochem. Biophys. Res. Commun. 395, 70–75. https://doi.org/10.1016/j.bbrc.2010.03.141.
Baker-Austin, C., Trinanes, J.A., Taylor, N.G.H., Hartnell, R., Siitonen, A., Martinez-Urtaza, J., 2013. Emerging *Vibrio* risk at high latitudes in response to ocean warming. Nat. Clim. Chang. 3, 73–77. https://doi.org/10.1038/nclimate1628.
Ball, A.S., Chaparian, R.R., van Kessel, J.C., 2017. Quorum sensing gene regulation by LuxR/HapR master regulators in *Vibrios*. J. Bacteriol. 199, 105–117. https://doi.org/10.1128/JB.00105-17.
Beaubrun, J.J., Gopinath, G., Kothary, H., Franco, A., Curtis, S.K., Eribo, B.E., Tall, B.D., 2011. Influence of iron-chelated growth conditions on outer membrane protein production and virulence of *Vibrio tubiashii*. Food Microbiol. 28, 1409–1413. https://doi.org/10.1016/j.fm.2011.04.003.
Benhamed, S., Guardiola, F.A., Mars, M., Esteban, M.A., 2014. Pathogen bacteria adhesion to skin mucus of fishes. Vet. Microbiol. 171, 1–12. https://doi.org/10.1016/j.vetmic.2014.03.008.
Blancheton, J.P., Attramadal, K.J.K., Michaud, L., Roque d'Orbcastel, E., Vadstein, O., 2013. Insight into bacterial population in aquaculture systems and its implication. Aquac. Eng. 53, 30–39. https://doi.org/10.1016/j.aquaeng.2012.11.009.
Broberg, C.A., Zhang, L., Gonzalez, H., Laskowski-Arce, M.A., Orth, K., 2010. A *Vibrio* effector protein is an inositol phosphatase and disrupts host cell membrane integrity. Science 329, 1660–1662. https://doi.org/10.1126/science.1192850.
Burdette, D.L., Seemann, J., Orth, K., 2009. Vibrio VopQ induces PI3- kinase-independent autophagy and antagonizes phagocytosis. Mol. Microbiol. 73, 639–649. https://doi.org/10.1111/j.1365-2958.2009.06798.x.
Cai, S.H., Lu, Y.S., Wu, Z.H., Jian, J.C., 2013a. Cloning, expression of *Vibrio alginolyticus* outer membrane protein-OmpU gene and its potential application as vaccine in crimson snapper, *Lutjanus erythropterus*. Biochem. J. Fish Dis. 36, 695–702. https://doi.org/10.1111/jfd.12036.
Cai, S.H., Lu, Y.S., Jian, J.C., Wabg, B., Huang, Y.C., Tang, J.F., Ding, Y., Wu, Z.H., 2013b. Protection against *Vibrio alginolyticus* in crimson snapper *Lutjanus erythropterus* immunized with a DNA vaccine containing the ompW gene. Dis. Aquat. Org. 106, 39–47. https://doi.org/10.3354/dao02617.
Cam, S., Brinkmeyer, R., 2019. The effect of temperature, pH and iron on biofilm formation by clinical versus environmental strains of *Vibrio vulnificus*. Foila Microbiol. 65, 557–566. https://doi.org/10.1007/s12223-019-00761-9.
Campbell, T.W., 2004. Hematology of lower vertebrates. In: 55th Annual meeting of the American College of Veterinary For. Pathol. (ACVP) and 39th Annual meeting of the American Society of Clinical Pathology (ASVCP). ACVP and ASVCP (Eds.). International Veterinary Information Service, Ithaca NY, Middleton WI, USA (1214–1104).
Cardinaud, M., Barbou, A., Capitaine, C., Bidault, A., Dujon, A.M., Moraga, D., Paillard, C., 2014. *Vibrio harveyi* adheres to and penetrates tissues of the European abalone *Haliotis tuberculate* within the first hour of contact. Appl. Environ. Microbiol. 80, 6328–6333. https://doi.org/10.1128/AEM.01036-14.
Chabrillón, M., Rico, R.M., Arijo, S., Diaz-Rosales, P., Balebona, M.C., Moriñigo, M.A., 2005. Interactions of microorganisms isolated from gilthead sea bream, Sparus aurata L., on *Vibrio harveyi*, a pathogen of farmed Senegalese sole, *Solea senegalensis* (Kaup). J. Fish Dis. 28, 531–537. https://doi.org/10.1111/j.1365-2761.2005.00657.x.
Chang, A.K., Kirn, H.Y., Park, J.E., Acharya, P., Park, I.S., Yoon, S.M., You, H.J., Hahm, K.-S., Park, J.K., Lee, J.S., 2005. *Vibrio vulnificus* secretes a broad-specificity metalloprotease capable of interfering with blood homeostasis through prothrombin activation and fibrinolysis. J. Bacteriol. 187, 6909–6916. https://doi.org/10.1128/JB.187.20.6909-6916.2005.
Chen, C.-Y., Wooster, G.A., Bowser, P.R., 2004. Comparative blood chemistry and histopathology of tilapia infected with *Vibrio vulnificus* or *Streptococcus iniae* or exposed to carbon tetrachloride, gentamicin, or copper sulfate. Aquaculture 239, 421–443. https://doi.org/10.1016/j.aquaculture.2004.05.033.
Chen, F., Lu, X.-J., Nie, L., Ning, Y.-J., Chen, J., 2018a. Molecular characterization of a CC motif chemokine 19-like gene in ayu (*Plecoglossus altivelis*) and its role in leukocyte trafficking. Fish Shellfish Immunol. 72, 301–308. https://doi.org/10.1016/j.fsi.2017.11.012.
Chen, J., Lu, Y., Emam, M., Zhang, H., Wang, H., 2020. Current advances in *Vibrio harveyi* quorum sensing as drug discovery targets. Eur. J. Med. Chem. 207, 112741 https://doi.org/10.1016/j.ejmech.2020.112741.
Chen, Q., Yan, Q., Wang, K., Zhuang, Z., Wang, X., 2008. Portal of entry for pathogenic *Vibrio alginolyticus* into large yellow croaker *Pseudosciaena crocea*, and characteristics of bacterial adhesion to mucus. Dis. Aquat. Org. 80, 181–188. https://doi.org/10.3354/dao01933.
Chen, Y., Huang, B., Huang, S., Yu, X., Li, Y., Song, W., Lu, F., 2013. Coinfection with *Clonorchis sinensis* modulates murine host response against *Trichinella spiralis* infection. Parasitol. Res. 112, 3167–3179. https://doi.org/10.1007/s00436-013-3493-1.
Chen, Y.-Y., Cheng, A.-C., Cheng, S.-A., Chen, J.-C., 2018b. Orange-spotted grouper *Epinephelus coioides* that have encountered low salinity stress have decreased cellular and humoral immune reactions and increased susceptibility to *Vibrio alginolyticus*. Fish Shellfish Immunol. 80, 392–396. https://doi.org/10.1016/j.fsi.2018.06.028.
Cheng, A., Cheng, S., Chen, Y., Chen, J., 2009. Effects of temperature change on the innate cellular and humoral immune responses of Orange-spotted grouper *Epinephelus coioides* and its susceptibility to *Vibrio alginolyticus*. Fish Shellfish Immunol. 26, 768–772. https://doi.org/10.1016/j.fsi.2009.03.011.
Chin, Y.K., Al-saari, N., Zulperi, Z., Mohd-Aris, A., Salleh, A., Silvaraj, S., Mohamand, A., Lee, J., Zamri-Saad, M., Ina-Salwany, M.D., 2019. Efficacy of bath vaccination with a live attenuated *Vibrio harveyi* against vibriosis in Asian seabass fingerling, *Lates calcarifer*. Aquac. Res. 51, 389–399. https://doi.org/10.1111/are.14386.
Chin, Y.K., Ina-Salwany, M.Y., Zamri-Saad, M., Amal, M.N.A., Mohamad, A., Lee, J.Y., Annas, S., Al-saari, N., 2020. Effects of skin abrasion in immersion challenge with *Vibrio harveyi* in Asian seabass *Lates calcarifer* fingerlings. Dis. Aquat. Org. 137, 167–173. https://doi.org/10.3354/dao03435.
Chitov, T., Kirikaew, P., Yungyune, P., Ruengprapan, N., Sontikun, K., 2009. An incidence of large foodborne outbreak associated with *Vibrio mimicus*. Eur. J. Clin. Microbiol. Infect. Dis. 28, 421–424.
Choudhury, M.G., Kumari, S., Das, K.B., Saha, N., 2018. Lipopolysaccharide causes NFκB-mediated induction of inducible nitric oxide synthase gene and more production of nitric oxide in air-breathing catfish, *Clarias magur* (Hamilton). Gene 658, 18–27. https://doi.org/10.1016/j.gene.2018.03.018.
Chu, T., Guan, L., Shang, P., Wang, Q., Xiao, J., Liu, Q., Zhang, Y., 2015. A controllable bacterial lysis system to enhance biological safety of live attenuated *Vibrio anguillarum* vaccine. Fish Shellfish Immunol. 45, 742–749. https://doi.org/10.1016/j.fsi.2015.05.030.
Currie, A.R., Cockerill, D., Diez-Padrisa, M., Haining, H., Henriquez, F.L., Quinn, B., 2022. Anemia in salmon aquaculture: Scotland as a case study. Aquaculture 546, 737313. https://doi.org/10.1016/j.aquaculture.2021.737313.
Cutuli, M.T., Gibello, A., Rodriguez-Bertos, A., Blanco, M.M., Villarroel, M., Giraldo, A., Guarro, J., 2015. Skin and subcutaneous mycoses in tilapia (*Oreochromis niloticus*) caused by *Fusarium oxysporum* in coinfection with *Aeromonas hydrophila*. Med. Mycol. Case Rep. 9, 7–11. https://doi.org/10.1016/j.mmcr.2015.06.002.
Dadar, M., Dhama, K., Vakharia, V.N., Hoseinifar, S.H., Karthik, K., Tiwari, R., Khandia, R., Munjal, A., Salgado-Miranda, C., Joshi, S.K., 2017. Advances in aquaculture vaccines against fish pathogens: global status and current trends. Rev. Fish. Sci. Aquac. 25, 184–217. https://doi.org/10.1080/23308249.2016.1261277.
Davis, B.J.K., Corrigan, A.E., Sun, Z., Atherly, E., DePaola, A., Curriero, F.C., 2021. A case-control analysis of traceback investigations for *Vibrio parahaemolyticus* infections (vibriosis) and pre-harvest environmental conditions in Washington State, 2013–2018. Sci. Total Environ. 752, 141650 https://doi.org/10.1016/j.scitotenv.2020.141650.
Defoirdt, T., 2014. Virulence mechanisms of bacterial aquaculture pathogens and anti-virulence therapy for aquaculture. Rev. Aquac. 6, 100–114. https://doi.org/10.1111/raq.12030.
Defoirdt, T., Boon, N., Sorgeloos, P., Verstraete, W., Bossier, P., 2008. Quorum sensing and quorum quenching in *Vibrio harveyi*: lessons learned from in vivo work. ISME J. 2, 19–26.
Deng, Y., Xu, L., Chen, H., Liu, S., Guo, Z., Cheng, C., Ma, H., Feng, J., 2020. Prevalence, virulence genes, and antimicrobial resistance of *Vibrio* species isolated from diseased marine fish in South China. Sci. Rep. 10, 14329. https://doi.org/10.1038/s41598-020-71288-0.
Destoumieux-Garzon, D., Canesi, L., Oyanedel, D., Travers, M.A., Charriere, G.M., Pruzzo, C., Vezzulli, L., 2020. *Vibrio*-bivalve interactions in health and disease. Environ. Microbiol. 22 (10), 4323–4341. https://doi.org/10.1111/1462-2920.15055.
Devi, M.S., Paria, P., Kumar, V., Parida, P.K., Maurye, P., Behera, B.K., Das, B.K., 2022. Molecular identification and pathogenicity study of virulent *Vibrio cholerae* non O1/O139 serotype associated with mortality of farmed *Labeo rohita* (Hamilton, 1822), in India. Aquaculture 547, 737529. https://doi.org/10.1016/j.aquaculture.2021.737529.
Diab, A.M., Khalil, R.H., Abu Leila, R.H.M., Abotaleb, M.M., Khallaf, M.A., Dawood, M.A.O., 2021. Cross-protection of *Listonella anguillarum* and *Vibrio alginolyticus* FKC bacterins to control vibriosis in European sea bass (*Dicentrarchus labrax*). Aquaculture 535, 736379. https://doi.org/10.1016/j.aquaculture.2021.736379.
Dias, M.K.R., Sampaio, L.S., Proietti-Junior, A.A., Yoshioka, E.T.O., Rodrigues, D.P., Rodriguez, A.F.R., Ribeiro, R.A., Faria, F.S.E.D.V., Ozório, R.O.A., Tavares-Dias, M., 2016. Lethal dose and clinical signs of *Aeromonas hydrophila* in *Arapaima gigas* (Arapaimidae), the giant fish from Amazon. Vet. Microbiol. 188, 12–15. https://doi.org/10.1016/j.vetmic.2016.04.001.
Dong, H.T., Taengphu, S., Sangsuriya, P., Charoensapsri, W., Phiwsaiya, K., Sornwatana, T., Khunrae, P., Rattanarojpong, T., Senapin, S., 2017. Recovery of *Vibrio harveyi* from scale drop and muscle necrosis disease in farmed barramundi, *Lates calcarifer* in Vietnam. Aquaculture 473, 89–96. https://doi.org/10.1016/j.aquaculture.2017.02.005.
Duperthuy, M., Schmitt, P., Garzón, E., Caro, A., Rosa, R.D., Le Roux, F., Lautrédou-Audouy, N., Got, P., Romestand, B., de Lorgeril, J., Kieffer-Jaquinod, S., Bachère, E., Destoumieux-Garzón, D., 2011. Use of OmpU porins for attachment and invasion of *Crassostrea gigas* immune cells by the oyster pathogen *Vibrio splendidus*. PNAS 108, 2993–2998. https://doi.org/10.1073/pnas.1015326108.
Edigius, E., 1987. Vibriosis: pathogenicity and pathology: a review. Aquaculture 67, 15–28.
Egerton, S., Culloty, S., Whooley, J., Stanton, C., Ross, R.P., 2018. The gut microbiota of marine fish. Front. Microbiol. 9, 873. https://doi.org/10.3389/fmicb.2018.00873.
Elexson, N., Afsah-Hejri, L., Rukayadi, Y., Soopna, P., Lee, H.Y., Tuan Zainazor, T.C., Nor Ainy, M., Nakaguchi, Y., Mitsuaki, N., Son, B., 2013. Effect of detergents as antibacterial agents on biofilm of antibiotics-resistant *Vibrio parahaemolyticus* isolates. Food Control 35, 378–385. https://doi.org/10.1016/j.foodcont.2013.07.020.
El-Son, M.A.M., Nofal, M.I., Abdel-Latif, H.M.R., 2021. Co-infection of *Aeromonas hydrophila* and *Vibrio parahaemolyticus* isolated from diseased farmed striped mullet (*Mugil cephalus*) in Manzala, Egypt – a case report. Aquaculture 530, 735738. https://doi.org/10.1016/j.aquaculture.2020.735738.
Esmaeili, A., Sotoudeh, E., Morshedi, V., Bagheri, D., Dorafshan, S., 2021. Effects of dietary supplementation of bovine lactoferrin on antioxidant status, immune response and disease resistance of yellowfin sea bream (*Acanthopagrus latus*) against

Vibrio harveyi. Fish Shellfish Immunol. 93, 917–923. https://doi.org/10.1016/j.fsi.2019.08.045.
Espelid, S., Lokken, G.B., Steiro, K., Bogwald, J., 1996. Effects of cortisol and stress on the immune system in Altantic salmon (*Salmo salar* L.). Fish Shellfish Immunol. 6, 95–110. https://doi.org/10.1006/fsim.1996.0011.
Estupiñán, M., Hernández, I., Saitua, E., Bilbao, M.E., Mendibil, I., Ferrer, J., Alonso-Sáez, L., 2020. Novel *Vibrio* spp. strains producing Omega-3 fatty acids isolated from coastal seawater. Mar. Drugs 18 (2), 99. https://doi.org/10.3390/md18020099.
Faridon, B.S., Zamri-Saad, M., Annas, S., 2021. Retrospective analysis of the incidence, aetiology and risk factors of sepsis based on post-mortem examination records in ruminants. Thai J. Vet. Med. 51, 285–291.
Faruque, S.M., Nair, G.B., Mekalanos, J.J., 2004. Genetics of stress adaptation and virulence in toxigenic *Vibrio cholerae*. DNA Cell Biol. 23, 723–741. https://doi.org/10.1089/dna.2004.23.723.
Fazio, F., 2019. Fish hematology analysis as an important tool of aquaculture: a review. Aquaculture 500, 237–242. https://doi.org/10.1016/j.aquaculture.2018.10.030.
Figueroa-Arredondo, P., Heuser, J.E., Akopyants, N.S., Morisaki, J.H., Giono-Cerezo, S., Enríquez-Rincón, F., Berg, D.E., 2001. Cell vacuolation caused by *Vibrio cholerae* hemolysin. Infect. Immun. 69, 1613–1624. https://doi.org/10.1128/IAI.69.3.1613-1624.2001.
Firmino, J., Furones, M.D., Andree, K.B., Sarasquete, C., Ortiz-Delgado, J.B., Asencio-Alcudia, G., Gisbert, E., 2019. Contrasting outcomes of *Vibrio harveyi* pathogenicity in gilthead seabream, *Sparus aurata* and European seabass, *Dicentrachus labrax*. Aquaculture 511, 734210. https://doi.org/10.1016/j.aquaculture.2019.734210.
Fouz, B., Liorens, A., Valiente, E., Amaro, C., 2010. A comparative epizootiologic study of the two fish-pathogenic serovars of *Vibrio vulnificus* biotype 2. J. Fish Dis. 33, 383–390. https://doi.org/10.1111/j.1365-2761.2009.01130.x.
Fujihara, M., Muroi, M., Tanamoto, K., Suzuki, T., Azuma, H., Ikeda, H., 2003. Molecular mechanisms of macrophage activation and deactivation by lipopolysaccharide: roles of the receptor complex. Pharmacol. Ther. 100, 171–194. https://doi.org/10.1016/j.pharmthera.2003.08.003.
Gao, X.-Y., Liu, Y., Miao, L.-L., Li, E.-W., Hou, T.-T., Liu, Z.-P., 2017. Mechanism of anti-*Vibrio* activity of marine probiotic strain *Bacillus pumilus* H2, and characterization of the active substance. AMB Express 7, 23.
Gao, Z., Ye, C., Zhou, L., Zhang, Y., Ge, Y., Chen, W., Pan, J., 2020. Evaluation of the β-barrel outer membrane protein VP1243 as a candidate antigen for a cross-protective vaccine against *Vibrio* infections. Microb. Pathog. 147, 104419 https://doi.org/10.1016/j.micpath.2020.104419.
Gauger, E., Smolowitz, R., Uhlinger, K., Casey, J., Gómez-Chiarri, M., 2006. *Vibrio harveyi* and other bacterial pathogens in cultured summer flounder, *Paralichthys dentatus*. Aquaculture 260, 10–20. https://doi.org/10.1016/j.aquaculture.2006.06.012.
Geng, Y., Liu, D., Han, S., Zhou, Y., Wang, K.Y., Huang, X.L., Chen, D.F., Peng, X., Lai, W. M., 2014. Outbreaks of vibriosis associated with *Vibrio mimicus* in freshwater catfish in China. Aquaculture 433, 82–84. https://doi.org/10.1016/j.aquaculture.2014.05.053.
Georgiadis, M.P., Gardner, I.A., Hedrick, R.P., 2001. The role of epidemiology in the prevention, diagnosis, and control of infectious diseases of fish. Prev. Vet. Med. 48, 287–302. https://doi.org/10.1016/S0167-5877(00)00202-6.
Gomez, D., Sunyer, J.O., Salinas, I., 2013. The mucosal immune system of fish: the evolution of tolerating commensals while fighting pathogens. Fish Shellfish Immunol. 35, 1729–1739. https://doi.org/10.1016/j.fsi.2013.09.032.
Gomez-Gil, B., Fajer-Avila, E., Garcia-Vargas, F., 2007. Vibrios of the spotted rose snapper *Lutjanus guttatus* Steindachner, 1869 from northwestern Mexico. J. Appl. Microbiol. 102, 1518–1526. https://doi.org/10.1111/j.1365-2672.2006.03199.x.
Gong, C., Hao, Y., Liu, Y., Zhao, Y., Liu, Y., Wang, G., He, Z., Liu, J., An, B., Zhang, Y., Yu, Q., Wang, Y., Hou, J., 2021. Immune response and intestinal microbial succession of half-smooth tongue sole (*Cynoglossus semilaevis*) infected with *Vibrio vulnificus*. Aquaculture 533, 736229. https://doi.org/10.1016/j.aquaculture.2020.736229.
Grześkowiak, Ł., Collado, M.C., Salminen, S, 2012. Evaluation of aggregation abilities between commensal fish bacteria and pathogens. Aquaculture 356–357, 412–414. https://doi.org/10.1016/j.aquaculture.2012.04.015.
Gu, Q.-q, Wang, G.-h, Li, N.-q, Hao, D.-f, Liu, H.-m, Wang, C.-b, Hu, Y.-h, Zhang, M., 2021. Evaluation of the efficacy of a novel *Vibrio vulnificus* vaccine based on antibacterial peptide inactivation in turbot, *Scophthalmus maximus*. Fish Shellfish Immunol. 118, 197–204. https://doi.org/10.1016/j.fsi.2021.09.008.
Guardiola, F.A., Cuesta, A., Abell, E., Meseguer, J., Esteban, M.A., 2014. Comparative analysis of the humoral immunity of skin mucus from several marine teleost fish. Fish Shellfish Immunol. 40, 24–31. https://doi.org/10.1016/j.fsi.2014.06.018.
Guo, L., Huang, L., Su, Y., Qin, Y., Zhao, L., Yan, Q., 2017. *sec*A, *sec*D, *sec*F, *yaj*C, and *yid*C contribute to the adhesion regulation of *Vibrio alginolyticus*. MicrobiologyOpen 7, e551. https://doi.org/10.1002/mbo3.551.
Hackbusch, S., Wichels, A., Gimenez, L., Döpke, H., Gerdts, G., 2020. Potentially human pathogenic *Vibrio* spp. in a coastal transect: occurrence and multiple virulence factors. Sci. Total Environ. 707, 136113 https://doi.org/10.1016/j.scitotenv.2019.136113.
Haldar, S., Maharajan, A., Chatterjee, S., Hunter, S.A., Chowdhury, N., Hinenoya, A., Asakura, M., Yamasaki, S., 2010. Identification of *Vibrio harveyi* as a causative bacterium for a tail rot disease of sea bream *Sparus aurata* from research hatchery in Malta. Microbiol. Res. 165, 639–648. https://doi.org/10.1016/j.micres.2009.12.001.
Ham, H., Orth, K., 2012. The role of type III secretion System 2 in *Vibrio parahaemolyticus* pathogenicity. J. Microbiol. 50, 719–725. https://doi.org/10.1007/s12275-012-2550-2.
Han, Z., Jiang, B., Hu, X., Lv, A., Chen, L., Guo, Y., 2021. Concurrent infections of *Aeromonas veronii* and *Vibrio cholerae* in koi carp (*Cyprinus carpio* var. koi). Aquaculture 535, 736395. https://doi.org/10.1016/j.aquaculture.2021.736395.
Hang, B.T.B., Milla, S., Gillardin, V., Phuong, N.T., Kestemont, P., 2013. In vivo effects of *Escherichia coli* lipopolysaccharide on regulation of immune response and protein expression in striped catfish (*Pangasianodon hypophthalmus*). Fish Shellfish Immunol. 34, 339–347. https://doi.org/10.1016/j.fsi.2012.11.025.
Harikrishnan, R., Kim, J.-S., Balasundaram, C., Heo, M.-S., 2012. Protection of *Vibrio harveyi* infection through dietary administration of *Pueraria thunbergiana* in kelp grouper, *Epinephelus bruneus*. Aquaculture 324–325, 27–32. https://doi.org/10.1016/j.aquaculture.2011.10.019.
Harper, C., Wolf, J.C., 2009. Morphologic effects of the stress response in fish. ILAR J. 50, 387–396. https://doi.org/10.1093/ilar.50.4.387.
Hashem, M., El-Barbary, M., 2013. *Vibrio harveyi* infection in Arabian sturgeon fish (*Acanthurus sohal)* of red sea at Hurghada, Egypt. Egypt. J. Aquat. Res. 39, 199–203. https://doi.org/10.1016/j.ejar.2013.10.006.
Hassan, M.A., Abd Allah, N.A., Mabrok, M., 2021. Inevitable impact of some environmental stressors on the frequency and pathogenicity of marine vibriosis. Aquaculture 536, 736447.
Hastings, J.W., Nealson, K.H., 1977. Bacterial bioluminescence. Annu. Rev. Microbiol. 31, 549–595. https://doi.org/10.1146/annurev.mi.31.100177.003001.
He, L., Wu, L., Lin, P., Zhai, S., Guo, S., Xiao, Y., Wan, Q., 2020. First expression and immunogenicity study of a novel trivalent outer membrane protein (OmpII-U-A) from *Aeromonas hydrophila*, *Vibrio vulnificus* and *Edwardsiella anguillarum*. Aquaculture 519, 734932. https://doi.org/10.1016/j.aquaculture.2020.734932.
Hedlund, B.P., Staley, J.T., 2001. *Vibrio cyclotrophicus* sp. nov., a polycyclic aromatic hydrocarbon (PAH)-degrading marine bacterium. Int. J. Syst. Evol. Microbiol. 51, 61–66. https://doi.org/10.1099/00207713-51-1-61.
Henke, J.M., Bassler, B.L., 2004. Quorum Sensing Regulates Type III Secretion in *Vibrio harveyi* and *Vibrio parahaemolyticus*. J. Bacteriol. 186, 3794–3805. https://doi.org/10.1128/JB.186.12.3794-3805.2004.
Hernández-Cabanyero, C., Sanjuán, E., Fouz, B., Pajuelo, D., Vallejos-Vidal, E., Reyes-López, F.E., Amaro, C., 2020. The effect of the environmental temperature on the adaptation to host in the zoonotic pathogen *Vibrio vulnificus*. Front. Microbiol. 11, 489.
Heumann, D., Roger, T., 2002. Initial responses to endotoxins and Gram-negative bacteria. Clin. Chim. Acta 323, 59–72. https://doi.org/10.1016/S0009-8981(02)00180-8.
Hjerde, E., Karisen, C., Sørum, H., Parkhill, J., Willassen, N.P., Thomson, N.R., 2015. Co-cultivation and transcriptome sequencing of two co-existing fish pathogens *Moritella viscosa* and *Aliivibrio wodanis*. BMC Genom. 16, 447. https://doi.org/10.1186/s12864-015-1669-z.
Hoihuan, A., Soonson, P., Bunlipatanon, P., Thawonsuwan, J., Tanasomwang, V., Areechon, N., Unajak, S., 2021. Molecular genotyping and phenotyping of *Vibrio vulnificus* isolated from diseased, brown-marbled grouper (*Epinephelus fuscoguttatus*) in Thailand with preliminary vaccine efficacy analysis. Aquaculture 545, 737188. https://doi.org/10.1016/j.aquaculture.2021.737188.
Hong, K.W., Koh, C.L., Sam, C.K., Yin, Y.F., Chan, K.G., 2012. Quorum quenching revisited - from signal decays to signalling confusion. Sensors 12, 4661–4696. https://doi.org/10.3390/s120404661.
Hu, Y.-H., Deng, T., Sun, B.-G., Sun, L., 2012. Development and efficacy of an attenuated *Vibrio harveyi* vaccine candidate with cross protectivity against *Vibrio alginolyticus*. Fish Shellfish Immunol. 32, 1155–1161. https://doi.org/10.1016/j.fsi.2012.03.032.
Huang, J.-F., Zeng, B.-O., Liu, D., Wu, R.-B., Zhang, J., Liao, B.-Q., He, H.-L., Bian, F., 2018. Classification and structural insight into vibriolysin-like proteases of *Vibrio* pathogenicity. Microb. Pathog. 117, 335–340. https://doi.org/10.1016/j.micpath.2018.03.002.
Huang, K.-C., Hsieh, P.-H., Huang, K.-C., Tsai, Y.-H., 2008. *Vibrio* necrotizing soft-tissue infection of the upper extremity: factors predictive of amputation and death. J. Infect. 57, 290–297. https://doi.org/10.1016/j.jinf.2008.07.009.
Huang, P., Cai, J., Yu, D., Tang, J., Lu, Y., Wu, Z., Huang, Y., Jian, J., 2019. An IL-6 gene in humphead snapper (*Lutjanus sanguineus*): identification, expression analysis and its adjuvant effects on *Vibrio harveyi* OmpW DNA. Fish Shellfish Immunol. 90, 546–555. https://doi.org/10.1016/j.fsi.2019.11.013.
Huang, W.S., Zhu, M.H., Chen, S., Wang, Z.X., Liang, Y., Huang, B., Nie, P., 2017. Molecular cloning and expression analysis of a fish specific interferon regulatory factor, IRF11, in Orange spotted grouper, *Epinephelus coioides*. Fish Shellfish Immunol. 60, 368–379. https://doi.org/10.1016/j.fsi.2016.12.007.
Hulmann, A., Rosche, T.M., Kong, I.-S., Hassan, H.M., Beam, D.M., Oliver, J.D., 2003. Rpos-dependent stress response and exoenzyme production in *Vibrio vulnificus*. Appl. Environ. Microbiol. 69 (10), 6114–6120. https://doi.org/10.1128/AEM.69.10.6114-6120.2003.
Ina-Salwany, M.Y., Al-saari, N., Mohamad, A., Mursidi, F.A., Mohd-Aris, A., Amal, M.N. A., Kasai, H., Mino, S., Sawabe, T., Zamri-Saad, M., 2019. Vibriosis in fish: a review on disease development and prevention. J. Aquat. Anim. Health 31, 3–22. https://doi.org/10.1002/aah.10045.
Ji, Q., Wang, S., Ma, J., Liu, Q., 2020. A review: progress in the development of fish *Vibrio* spp. vaccines. Immunol. Lett. 226, 46–54. https://doi.org/10.1016/j.imlet.2020.07.002.
Jia, A., Woo, N.Y.S., Zhang, X.-H., 2010. Expression, purification, and characterization of thermolabile hemolysin (TLH) from *Vibrio alginolyticus*. Dis. Aquat. Org. 90, 121–127. https://doi.org/10.3354/dao02225.
Jin, Y., Bergmann, S.M., Mai, Q., Yang, Y., Liu, W., Sun, D., Chen, Y., Yu, Y., Liu, Y., Cai, W., Dong, H., Li, H., Yu, H., Wu, Y., Lai, M., Zeng, W., 2022. Simultaneous isolation and identification of Largmouth Bass Virus and Rhabdovirus from

moribund largemouth bass (*Micropterus salmoides*). Viruses 14, 1643. https://doi.org/10.3390/v14081643.
Julie, D., Solen, L., Antoine, V., Jaufrey, C., Annick, D., Dominique, H.-H., 2010. Ecology of pathogenic and non-pathogenic *Vibrio parahaemolyticus* on the French Atlantic coast. Effects of temperature, salinity, turbidity and chlorophyll *a*. Environ. Microbiol. 12, 929–937. https://doi.org/10.1111/j.1462-2920.2009.02136.x.
Juoelsson, A., Kan, B., Zhu, J., 2007. Quorum sensing enhances the stress response in *Vibrio cholera*. Appl. Environ. Microbiol. 73, 3742–3746. https://doi.org/10.1128/AEM.02804-06.
Kang, G., Choi, W.-M., Joo, M.-S., Woo, W.-S., Park, C.-I., 2022. A case report of secondary infection by *Vibrio splendidus* associated with gas bubble disease in syngnathid fishes (*Syngnathus schlegeli* and *Hippocampus haema*). Fish Aquat. Sci. 25 (1), 40–48. https://doi.org/10.47853/FAS.2022.e5.
Kanungo, S., Azman, A.S., Ramamurthy, T., Deen, J., Dutta, S., 2022. Cholera. Lancet 399, 1429–1440. https://doi.org/10.1016/S0140-6736(22)00330-0.
Kato, G., Takano, T., Sakai, T., Matsuyama, T., Nakayasu, C., 2013. *Vibrio anguillarum* bacterin uptake via the gills of Japanese flounder and subsequent immune responses. Fish Shellfish Immunol. 35, 1591–1597. https://doi.org/10.1016/j.fsi.2013.09.007.
Khansari, A.R., Balasch, J.C., Vallejos-Vidal, E., Parra, D., Reyes-López, F.E., Tort, L., 2018. Comparative immune- and stress-related transcript response induced by air exposure and *Vibrio anguillarum* bacterin in rainbow trout (*Oncorhynchus mykiss*) and gilthead seabream (*Sparus aurata*) mucosal surfaces. Front. Immunol. 9, 856. https://doi.org/10.3389/fimmu.2018.00856.
Khouadja, S., Lamari, F., Bakhrouf, A., 2013. Characterization of *Vibrio parahaemolyticus* isolated from farmed sea bass (*Dicentrarchus labrax*) during disease outbreaks. Int. Aquac. Res. 5, 13. ⟨http://www.intaquares.com/content/5/1/13⟩.
Kim, J.Y., Lee, J.-L., 2017. Correlation of total bacterial and *Vibrio* spp. populations between fish and water in the aquaculture system. Front. Mar. Sci. 4, 147. https://doi.org/10.3389/fimmu.2018.00856.
Kirov, S.M., 2003. Bacteria that express lateral flagella enable dissection of the multifunctional roles of flagella in pathogenesis. FEMS Microbiol. Lett. 224, 151–159. https://doi.org/10.1016/S0378-1097(03)00445-2.
Kotob, M.H., Menanteau-Ledouble, S., Kumar, G., Abdelzaher, M., El-Matbouli, M., 2016. The impact of co-infections on fish: a review. Vet. Res. 47, 98. https://doi.org/10.1186/s13567-016-0383-4.
Krupesha Sharma, S.R., Rathore, G., Verma, D.K., Sadhu, N., Philipose, K.K., 2013. *Vibrio alginolyticus* infection in Asian seabass (*Lates calcarifer*, Bloch) reared in open sea floating cages in India. Aquac. Res. 44, 86–92. https://doi.org/10.1111/j.1365-2109.2011.03013.x.
Kumar, H., Kawai, T., Akira, S., 2009. Toll-like receptors and innate immunity. Biochem. Biophys. Res. Commun. 388, 621–625. https://doi.org/10.1016/j.bbrc.2009.08.062.
Kumar, V., Nguyen, D.V., Baruah, K., Bossier, P., 2019. Probing the mechanism of VPAHPND extracellular proteins toxicity purified from *Vibrio parahaemolyticus* AHPND strain in germ-free *Artemia* test system. Aquaculture 504, 414–419. https://doi.org/10.1016/j.aquaculture.2019.02.029.
Larsen, M.H., Boesen, H.T., 2001. Role of flagellum and chemotactic motility of *Vibrio anguillarum* for phagocytosis by and intracellular survival in fish macrophages. FEMS Microbiol. Lett. 203, 149–152.
Le, H., LiHua, D., JianJun, F., Peng, L., SongLin, G., 2018. Immunogenicity study of an expressed outer membrane protein U of *Vibrio vulnificus* in Japanese eel (*Anguilla japonica*). J. Appl. Microbiol. 125, 1642–1654. https://doi.org/10.1111/jam.14068.
Le Roux, F., 2016. Diagnosis of vibriosis in the era of genomics: lessons from invertebrates. Rev. Sci. Tec. OIE 35, 259–269. https://doi.org/10.20506/rst.35.1.2431.
Lee, K., Liu, P., Chuang, W., 2002. Pathogenesis of gastroenteritis caused by *Vibrio carchariae* in cultured marine fish. Mar. Biotechnol. 4, 267–277. https://doi.org/10.1007/s10126-002-0018-9.
Lee, K.K., Liu, P.C., Chen, Y.L., 1999. Electrophoretic characterization of a novel cysteine protease produced by *Vibrio harveyi*. Electrophoresis 20, 3343–3346.
Lee, M., Kim, J., Yang, Y.J., 2014. VvpM, an extracellular metalloprotease of *Vibrio vulnificus*, induces apoptotic death of human cells. J. Microbiol. 52, 1036–1043. https://doi.org/10.1007/s12275-014-4531-0.
Lemire, A., Goudene`ge1, D., Versigny, T., Petton, B., Calteau, A., Labreuche, Y., Le Roux, F., 2015. Populations, not clones, are the unit of *Vibrio* pathogenesis in naturally infected oysters. ISME J. 9, 1523–1531. https://doi.org/10.1038/ismej.2014.233.
Li, C., Ye, Z., Wen, L., Chen, R., Tian, L., Zhao, F., Pan, J., 2014. Identification of a novel vaccine candidate by immunogenic screening of *Vibrio parahaemolyticus* outer membrane proteins. Vaccine 32, 6115–6121. https://doi.org/10.1016/j.vaccine.2014.08.077.
Li, J., Zhao, X., 2020. Effects of quorum sensing on the biofilm formation and viable but non-culturable state. Food Res. Int. 137, 109742 https://doi.org/10.1016/j.foodres.2020.109742.
Li, L., Mou, X., Nelson, D.R., 2013. Characterization of Plp, a phosphatidylcholine specific phospholipase and hemolysin of *Vibrio anguillarum*. BMC Microbiol. 13, 271. https://doi.org/10.1186/1471-2180-13-271.
Li, X., Bossier, P., Dierckens, K., Laureau, S., Defoirdt, T., 2015. Impact of mucin, bile salts and cholesterol on the virulence of *Vibrio anguillarum* towards gnotobiotic sea bass (*Dicentrarchus labrax*) larvae. Vet. Microbiol. 175, 44–49. https://doi.org/10.1016/j.vetmic.2014.10.033.
Li, Y., Xia, P., Wu, J., Huang, A., Bu, G., Meng, F., Kong, F., Cao, X., Han, X., Yu, G., Pan, X., Yang, S., Zeng, X., Du, X., 2020. The potential sensing molecules and signal cascades for protecting teleost fishes against lipopolysaccharide. Fish Shellfish Immunol. 97, 235–247. https://doi.org/10.1016/j.fsi.2019.12.050.
Lindell, K., Fahlgren, A., Hjerde, E., Willassen, N.-P., Fällman, M., 2012. Lipopolysaccharide O-antigen prevents phagocytosis of *Vibrio anguillarum* by rainbow trout (*Oncorhynchus mykiss*) skin epithelial cells. PLoS One 7, e37678. https://doi.org/10.1371/journal.pone.0037678.
Liu, D., Wu, C., Wu, R., Huang, J., Liao, B., Lei, M., Zhang, Y., He, H., 2019b. Comprehensive analysis of the phylogeny and extracellular proteases in genus *Vibrio* strain. Microb. Pathog. 131, 1–8. https://doi.org/10.1016/j.micpath.2019.03.018.
Liu, H., Wang, Q., Liu, Q., 2011. Roles of Hfq in the stress adaptation and virulence in fish pathogen *Vibrio alginolyticus* and its potential application as a target for live attenuated vaccine. Appl. Microbiol. Biotechnol. 91, 353–364. https://doi.org/10.1007/s00253011-3286-3.
Liu, L., Ge, M., Zheng, X., Tao, Z., Zhou, S., Wang, G., 2016. Investigation of *Vibrio alginolyticus, V. harveyi,* and *V. parahaemolyticus* in large yellow croaker, *Pseudosciaena crocea* (Richardson) reared in Xiangshan Bay, China. Aquac. Rep. 3, 220–224. https://doi.org/10.1016/j.aqrep.2016.04.004.
Liu, P.C., Lee, K.K., Tu, C.C., Chen, S.N., 1997. Purification and characterization of a cysteine protease produced by pathogenic luminous *Vibrio harveyi*. Curr. Microbiol. 35, 32–39.
Liu, P.C., Lin, J.Y., Hsiao, P.T., Lee, K.K., 2004. Isolation and characterization of pathogenic *Vibrio alginolyticus* from diseased cobia *Rachycentron canadum*. J. Basic Microbiol. 44, 23–28. https://doi.org/10.1002/jobm.200310316.
Liu, R., Lian, Z., Hu, X., Lu, A., Sun, J., Chen, C., Liu, K., Song, Y., Yiksung, Y., 2019a. First report of *Vibrio vulnificus* infection in grass carp *Ctenopharyngodon idellus* in China. Aquaculture 499, 283–289. https://doi.org/10.1016/j.aquaculture.2018.09.051.
Liu, S., Li, E., Cai, Y., Wang, S., Ren, Z., Li, Q., Guo, W., Wu, Y., Zhou, Y., 2018. Isolation, identification and pathogenicity characterization of *Vibrio ponticus* from the golden pompano *Trachinotus ovatus*. Aquaculture 496, 285–290. https://doi.org/10.1016/j.aquaculture.2018.04.065.
Liu, X., Wu, H., Chang, X., Tang, Y., Liu, Q., Zhang, Y., 2014. Notable mucosal immune responses induced in the intestine of zebrafish (*Danio rerio*) bath-vaccinated with a live attenuated *Vibrio anguillarum* vaccine. Fish Shellfish Immunol. 40, 99–108. https://doi.org/10.1016/j.fsi.2014.06.030.
Liu, X., Gao, H., Xiao, N., Liu, Y., Li, J., Li, L., 2015. Outer membrane protein U (OmpU) mediates adhesion of *Vibrio mimicus* to host cells via two novel N-Terminal motifs. PLoS One 10, e0119026. https://doi.org/10.1371/journal.pone.0119026.
Lozano-Olvera, R., Abad-Rosales, S.M., Gomez-Gil, B., Soto-Rodriguez, S.A., 2020. Experimental infection of the white snook *Centropomus viridis* Lockington (1877) with *Vibrio ponticus*: Histopathological manifestations and screening for putative virulence genes. Aquaculture 528, 735599. https://doi.org/10.1016/j.aquaculture.2020.735599.
Lun, J., Xia, C., Yuan, C., Zhang, Y., Zhong, M., Huang, T., Hu, Z., 2014. The outer membrane protein, LamB (maltoporin), is a versatile vaccine candidate among the *Vibrio* species. Vaccine 32, 809–815. https://doi.org/10.1016/j.vaccine.2013.12.035.
Lv, T., Dai, F., Zhuang, Q., Zhao, X., Shao, Y., Guo, M., Lv, Z., Li, C., Zhang, W., 2020. Outer membrane protein OmpU is related to iron balance in *Vibrio alginolyticus*. Microbiol. Res. 230, 126350 https://doi.org/10.1016/j.micres.2019.126350.
Macián, M.C., Garay, E., Grimont, P.A., Pujalte, M.J., 2004. *Vibrio ponticus* sp. nov. a neighbour of *V. fluvialis-V. furnissii* clade, isolated from gilthead sea bream, mussels and seawater. Syst. Appl. Microbiol. 27, 535–540. https://doi.org/10.1078/0723202041748127.
Magnadottir, B., 2010. Immunological control of fish diseases. Mar. Biotechnol. 12, 361–379. https://doi.org/10.1007/s10126-010-9279-x.
Makino, K., Oshima, K., Kurokawa, K., Yokoyama, K., Uda, T., Tagomori, K., Iijima, Y., Najima, M., Nakano, M., Yamashita, A., et al., 2003. Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of *V. cholerae*. Lancet 361, 743–749. https://doi.org/10.1016/S0140-6736(03)12659-1.
Manrique, W.G., da Silva-Claudiano, G., de Castro, M.P., Petrillo, T.R., Figueiredo, M.A. P., de Andrade Belo, M.A., Quiroga Berdeal, M.I., Rodini de Moreaes, J.E., Ruas de Moraes, F., 2015. Expression of cellular components in granulomatous inflammatory response in *Piaractus mesopotamicus* model. PLoS One 10, e0121625. https://doi.org/10.1371/journal.pone.0121625.
Mao, Z., Yu, L., You, Z., Wei, Y., Liu, Y., 2007a. Cloning, expression and immunogenicity analysis of five outer membrane proteins of *Vibrio parahaemolyticus* zj2003. Fish Shellfish Immunol. 23, 567–575. https://doi.org/10.1016/j.fsi.2007.01.004.
Mao, Z., Yu, L., You, Z., Wei, Y., Liu, Y., 2007b. Expression and immunogenicity analysis of two iron-regulated outer membrane proteins of *Vibrio parahaemolyticus*. Acta Biochim. Biophys. Sin. 39, 763–769. https://doi.org/10.1111/j.1745-7270.2007.00339.x.
Mariana, S., Alfons, Badr, G., 2019. Impact of heat stress on the immune response of fish. J. Surv. Fish. Sci. 5, 149–159. https://doi.org/10.18331/SFS2019.5.2.14.
Markov, E.Y., Kulikalova, E.S., Urbanovich, L.Y., Vishnayakov, V.S., Balakhonov, S.V., 2015. Chitin and products of its hydrolysis in *Vibrio cholerae* ecology. Biochemistry 80, 1109–1116. https://doi.org/10.1134/S0006297915090023.
Martins, M.L., Mouriño, J.L.P., Fezer, G.F., Buglione Neto, C.C., Garcia, P., Silva, B.C., Jatobá, A., Vieira, F.N., 2010. Isolation and experimental infection with Vibrio alginolyticus in the sea horse, Hippocampus reidi Ginsburg, 1933 (Osteichthyes: Syngnathidae) in Brazil. Braz. J. Biol. 70, 205–209. https://doi.org/10.1590/S1519-69842010000100028.
Marudhupandi, T., Kumar, T.T.A., Prakash, S., Balamurugan, J., Dhayanithi, N.B., 2017. *Vibrio parahaemolyticus* a causative bacterium for tail rot disease in ornamental fish, *Amphiprion sebae*. Aquac. Rep. 8, 39–44. https://doi.org/10.1016/j.aqrep.2017.09.004.
Matsuura, Y., Terashima, S., Takano, T., Matsuyama, T., 2019. Current status of fish vaccines in Japan. Fish Shellfish Immunol. 95, 236–247. https://doi.org/10.1016/j.fsi.2019.09.031.

Miccoli, A., Saraceni, P.R., Scapigliati, G., 2019. Vaccines and immune protection of principal Mediterranean marine fish species. Fish Shellfish Immunol. 94, 800–809. https://doi.org/10.1016/j.fsi.2019.09.065.
Milton, D.L., 2006. Quorum sensing in vibrios: complexity for diversification. Int. J. Med. Microbiol. 296, 61–71. https://doi.org/10.1016/j.ijmm.2006.01.044.
Min, J.-R., Na, K., Chong, H., Jeong, S.-H., 2015. Bactericidal efficacy of a monopersulfate compound against *V. harveyi* and toxicity to *Litopenaeus vannamei*. Korean J. Fish Aquat. Sci. 48, 661–667. https://doi.org/10.5657/KFAS.2015.0661.
Mohamad, A., Mursidi, F.-A., Zamri-Saad, M., Amal, M.N.A., Salleh, A., Monir, M.S., Loqman, M., Hairudin, F., Al-saari, N., Ina-Salwany, M.Y., 2022. Laboratory and field assessments of oral *Vibrio* vaccine indicate the potential for protection against Vibriosis in cultured marine fishes. Animals 12, 133. https://doi.org/10.3390/ani12020133.
Mohamad, N., Amal, M.N.A., Ina Salwany, M.Y., Saad, M.Z., Nasruddin, N.S., Al-saari, N., Mino, S., Sawabe, T., 2019a. Vibriosis in cultured marine fishes: a review. Aquaculture 512, 734289. https://doi.org/10.1016/j.aquaculture.2019.734289.
Mohamad, N., Mustafa, M., Amal, M.N.A., Zamri-Saad, M., Ina Salwany, M.Y., AlSaari, N., 2019b. Environmental factors associated with the presence of Vibrionaceae in tropical cage-cultured marine fishes. J. Aquat. Anim. Health 31, 154–167. https://doi.org/10.1002/aah.10062.
Mohamad, N., Roseli, F.A.M., Azmai, M.N.A., Saad, M.Z., Ina Salwany, M.Y., Zulkiply, N. A., Nasruddin, N.S., 2019c. Natural concurrent infection of *Vibrio harveyi* and *V. alginolyticus* in cultured hybrid groupers in Malaysia. J. Aquat. Anim. Health 31, 88–96. https://doi.org/10.1002/aah.10055.
Mohd Nor, N., Yazid, S.H.M., Daud, H.M., Amal, M.N.A., Mohamad, N., 2019. Costs of management practices of Asian seabass (*Lates calcarifer* Bloch, 1790) cage culture in Malaysia using stochastic model that includes uncertainty in mortality. Aquaculture 510, 347–352. https://doi.org/10.1016/j.aquaculture.2019.04.042.
Mohd-Aris, A., Saad, M.Z., Daud, H.M., Yusof, M.T., Ina-Salwany, M.Y., 2019a. *Vibrio harveyi* protease deletion mutant as a live attenuated vaccine candidate against vibriosis and transcriptome profiling following vaccination for *Epinephelus fuscoguttatus*. Aquac. Int. 27, 125–140. https://doi.org/10.1007/s10499-018-0311-x.
Mohd-Aris, A., Muhamad-Sofie, M.H.N., Zamri-Saad, M., Daud, H.M., Ina-Salwany, M.Y., 2019b. Live vaccines against bacterial fish diseases: a review. Vet. World 12, 1806–1815. https://doi.org/10.14202/vetworld.2019.1806-1815.
Mohi, M.M., Kuratani, M., Miyazaki, T., Yoshida, T., 2010. Histopathological studies on *Vibrio harveyi* infected tiger puffer (*Takifugu rubripes*) (Temminck et Schlegel), cultured in Japan. J. Fish Dis. 33, 833–840. https://doi.org/10.1111/j.1365-2761.2010.01184.x.
Montánchez, I., Kaberdin, V.R., 2019. *Vibrio harveyi:* a brief survey of general characteristics and recent epidemiological traits associated with climate change. Mar. Environ. Res. 154, 104850 https://doi.org/10.1016/j.marenvres.2019.104850.
Mosca, F., Ciulli, S., Volpatti, D., Romanod, N., Volpe, E., Bulfon, C., Massimini, M., Caccia, E., Galeottc, M., Tiscar, P.G., 2014. Defensive response of European sea bass (*Dicentrarchus labrax*) against *Listonella anguillarum* or *Photobacterium damselae* subsp. *piscicida* experimental infection. Vet. Immunol. Immunopathol. 162, 83–95. https://doi.org/10.1016/j.
Nam, B.-H., Seo, J.-K., Go, H.-J., Lee, M.J., Kim, Y.-O., Kim, D.-G., Le, S.-J., Park, N.G., 2012. Purification and characterization of an antimicrobial histone H1-like protein and its gene from the testes of olive flounder, *Paralichthys olivaceus*. Fish Shellfish Immunol. 33, 92–98. https://doi.org/10.1016/j.fsi.2012.04.006.
Nehlah, R., Ina-Salwany, M.Y., Zulperi, Z., 2016. Antigenicity analysis and molecular characterization of two outer membrane proteins of *Vibrio alginolyticus* strain VA2 as vaccine candidates in tiger grouper culture. J. Biol. Sci. 16, 1–11. https://doi.org/10.3923/jbs.2016.1.11.
Neogi, S.B., Lara, R., Alam, M., Harder, J., Yamasaki, S., Colwell, R.R., 2018. Environmental and hydroclimatic factors influencing *Vibrio* populations in the estuarine zone of the Bengal delta. Environ. Monit. Assess. 190, 565. https://doi.org/10.1007/s10661-018-6925-7.
Nguyen, H.T., Nguyen, T.T.T., Tsai, M.-A., Ya-Zhen, E., Wang, P.-C., Chen, S.-C., 2017. A formalin-inactivated vaccine provides good protection against *Vibrio harveyi* infection in orange-spotted grouper (*Epinephelus coioides*). Fish Shellfish Immunol. 65, 118–126. https://doi.org/10.1016/j.fsi.2017.04.008.
Ningqiu, L., Junjie, B., Shuqin, W., Xiaozhe, F., Haihua, L., Xing, Y., Cunbin, S., 2008. An outer membrane protein, OmpK, is an effective vaccine candidate for *Vibrio harveyi* in Orange-spotted grouper (*Epinephelus coioides*). Fish Shellfish Immunol. 25, 829–833. https://doi.org/10.1016/j.fsi.2008.09.007.
Nurliyana, M., Amal, M.N.A., Zamri-Saad, M., Ina-Salwany, M.Y., 2019. Possible transmission routes of *Vibrio* spp. in tropical cage-cultured marine fishes. Lett. Appl. Microbiol. 68, 485–496. https://doi.org/10.1111/lam.13146.
Ogut, H., Cavus, N., 2014. A comparison of ectoparasite prevalence and occurrence of viral haemorrhagic septicemia virus (VHSV) in whiting *Merlangius merlangus euxinus*. Rev. Biol. Mar. Oceanogr. 49, 91–96. https://doi.org/10.4067/S0718-19572014000100010.
Oliver, J.D., 2005. Wound infections caused by *Vibrio vulnificus* and other marine bacteria. Epidemiol. Infect. 133, 383–391. https://doi.org/10.1017/S0950268805003894.
Olsson, J.C., Joborn, A., Westerdahl, A., Blomberg, L., Kjelleberg, S., Conway, P.L., 1996. Is the turbot, *Scophthalmus maximus* (L.), intestine a portal of entry for the fish pathogen *Vibrio anguillarum*? J. Fish Dis. 19, 225–234. https://doi.org/10.1111/j.1365-2761.1996.tb00129.x.
Park, J.E., Park, J.W., Lee, W., Lee, J.S., 2014. Pleiotropic effects of a *Vibrio* extracellular protease on the activation of contact system. Biochem. Biophys. Res. Commun. 450, 1099–1103. https://doi.org/10.1016/j.bbrc.2014.06.121.
Parra, D., Korytář, T., Takizawa, F., Sunyer, J.O., 2016. B cells and their role in the teleost gut. Dev. Comp. Immunol. 64, 150–166. https://doi.org/10.1016/j.dci.2016.03.013.
Pattipeilohy, M., Amin, M., Lestari, U., Suarsini, E., 2019. Assessment of the outer membrane protein profile of *Vibrio parahaemolyticus* using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis method. Drug Invent Today 12, 4.
Paulsen, S.M., Engstad, R.E., Robertsen, B., 2001. Enhanced lysozyme production in Atlantic salmon (*Salmo salar* L.) macrophages treated with yeast *β*-glucan and bacterial lipopolysaccharide. Fish Shellfish Immunol. 11, 23–37. https://doi.org/10.1006/fsim.2000.0291.
Paulsen, S.M., Lunde, H., Engstad, R.E., Robertsen, B., 2003. *In vivo* effects of β-glucan and LPS on regulation of lysozyme activity and mRNA expression in Atlantic salmon (*Salmo salar* L.). Fish Shellfish Immunol. 14, 39–54. https://doi.org/10.1006/fsim.2002.0416.
Peng, B., Lin, X.-P., Wang, S.-N., Yang, M.-J., Peng, X.-X., Li, H., 2018. Polyvalent protective immunogens identified from outer membrane proteins of *Vibrio parahaemolyticus* and their induced innate immune response. Fish Shellfish Immunol. 72, 104–110. https://doi.org/10.1016/j.fsi.2017.10.046.
Peng, W., Shi, Y., Li, G.-F., He, L.-G., Liang, Y.-S., Zhang, Y., Zhou, L.-B., Lin, H.-R., Lu, D.-Q., 2016. *Tetraodon nigroviridis*: a model of Vibrio parahaemolyticus infection. Fish Shellfish Immunol. 56, 388–396. https://doi.org/10.1016/j.fsi.2016.07.017.
Peterman, M.A., Posadas, B.C., 2019. Direct economic impact of fish diseases on the East Mississippi catfish industry. N. Am. J. Aquac. 81, 222–229. https://doi.org/10.1002/naaq.10090.
Pulsford, A.L., Lemaire-Gony, S., Farley, S., 1994. Effects of stress on the immune system of fish. In: Sutcliffe, D.W. (Ed.), Water Quality and Stress Indicators in Marine and Freshwater Systems: Linking Levels of Organisation. Freshwater Biological Association, Ambleside, UK, pp. 111–123.
Qian, H., Li, W., Guo, L., Tan, L., Liu, H., Wang, J., Pan, Y., Zhao, Y., 2020. Stress response of *Vibrio parahaemolyticus* and *Listeria monocytogenes* biofilms to different modified atmospheres. Front. Microbiol. 11, 23. https://doi.org/10.3389/fmicb.2020.00023.
Ralph, A., Currie, B.J., 2007. *Vibrio vulnificus* and *V. parahaemolyticus* necrotising fasciitis in fishermen visiting an estuarine tropical northern Australian location. J. Infect. 54, 111–114. https://doi.org/10.1016/j.jinf.2006.06.015.
Rameshkumar, P., Kalidas, C., Tamilmani, G., Sakthivel, M., Nazar, A.K.A., Maharshi, V. A., Rao, K.S., Gopakumar, G., 2014. Microbiological and histopathological investigations of *Vibrio alginolyticus* infection in cobia *Rachycentron canadum* (Linnaeus, 1766) cultured in sea cage. Indian J. Fish. 61, 124–127.
Ransangan, J., Lal, T.M., Al-Harbi, A.H., 2012. Characterization and experimental infection of *Vibrio harveyi* isolated from diseased Asian seabass (*Lates calcarifer*). Malays. J. Microbiol. 8, 104–115. https://doi.org/10.21161/mjm.03512.
Rebl, A., Korytar, T., Borchel, R., Strzelczyk, J.E., Goldammer, T., Verlein, M., 2020. The synergistic interaction of thermal stress coupled with overstocking strongly modulates the transcriptomic activity and immune capacity of rainbow trout (*Oncohynchus mykiss*). Sci. Rep. 10, 14913. https://doi.org/10.1038/s41598-020-71852-8.
Rekecki, A., Ringø, E., Olsen, R., Myklebust, R., Dierckens, K., Bergh, Ø., Laureau, S., Cornelissen, M., Ducatelle, R., Decostere, A., Bossier, P., Van den Broeck, W., 2013. Luminal uptake of *Vibrio* (Listonella) *anguillarum* by shed enterocytes – a novel early defence strategy in larval fish. J. Fish Dis. 36, 419–426. https://doi.org/10.1111/jfd.12009.
Ringø, E., Olsen, R.E., Mayhew, T.M., Myklebust, R., 2003. Electron microscopy of the intestinal microflora of fish. Aquaculture 227, 395–415. https://doi.org/10.1016/j.aquaculture.2003.05.001.
Rombout, J.H.W.M., Abelli, L., Picchietti, S., Scapigliati, G., Kiron, V., 2011. Teleost intestinal immunology. Fish Shellfish Immunol. 31, 616–626. https://doi.org/10.1016/j.fsi.2010.09.001.
da Rosa, J.V., Kaefer, K., da Conceicao, N.V., Conceicao, R.C.S., Timm, C.D., 2017. Biofilm formation by *Vibrio parahaemolyticus* from fish. Pesqui. Vet. Bras. 37, 339–345. https://doi.org/10.1590/s0100-736×2017000400007.
Rueggeberg, K.-G., Zhu, J., 2016. The *Vibrio cholera* stress response: an elaborate system geared toward overcoming host defences during infection. In: de Bruijn, F.J. (Ed.), Stress and Environmental Regulation of Gene Expression and Adaptation in Bacteria I & II. John Wiley & Sons. https://doi.org/10.1002/9781119004813.ch97.
Runft, D.L., Mitchell, K.C., Abuaita, B.H., Allen, J.P., Bajer, S., Ginsburg, K., Neely, M.N., Withey, J.H., 2014. Zebrafish as a natural host model for Vibrio cholerae colonization and transmission. Appl. Environ. Microbiol. 80, 1710–1717. https://doi.org/10.1128/AEM.03580-13.
Rutherford, S.T., Bassler, B.L., 2012. Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb. Perspect. Med. 2, a012427 https://doi.org/10.1101/cshperspect.a012427.
Ruwandeepika, H.A.D., Defoirdt, T., Bhowmick, P.P., Shekar, M., Bossier, P., Karunasagar, I., 2010. Presence of typical and atypical virulence genes in *Vibrio* isolates belonging to the *Harveyi* clade. J. Appl. Microbiol. 109, 888–899. https://doi.org/10.1111/j.1365-2672.2010.04715.x.
Ruwandeepika, H.A.D., Jayaweera, T.S.P., Bhowmick, P.P., Karunasagar, I., Bossier, P., Defoirdt, T., 2012. Pathogenesis, virulence factors and virulence regulation of vibrios belonging to the *Harveyi* clade. Rev. Aquac. 4, 59–74. https://doi.org/10.1111/j.1753-5131.2012.01061.x.
Sawabe, T., Ogura, Y., Matsumura, Y., Feng, G., Amin, A.K.M.R., Mino, S., Nakagawa, S., Sawabe, T., Kumar, R., Fukui, Y., Satomi, M., Matsushima, R., Thompson, F.L., Gomez-Gil, B., Christen, R., Maruyama, F., Kurokawa, K., Hayashi, T., 2013. Updating the *Vibrio* clades defined by multilocus sequence phylogeny: proposal of eight new clades, and the description of *Vibrio tritonius* sp. nov. Front. Microbiol. 4, 414. https://doi.org/10.3389/fmicb.2013.00414.

Schmidt, J.G., Korbut, R., Ohtani, M., Jørgensen, L.G., 2017. Zebrafish (*Danio rerio*) as a model to visualize infection dynamics of *Vibrio anguillarum* following intraperitoneal injection and bath exposure. Fish Shellfish Immunol. 67, 692–697. https://doi.org/10.1016/j.fsi.2017.06.052.

Sepulcre, M.P., Lopez_Castejon, G., Meseguer, J., Mulero, V., 2016. The activation of gilthead seabream professional phagocytes by different PAMPs underlines the behavioural diversity of the main innate immune cells of bony fish. Mol. Immunol. 44, 2009–2016. https://doi.org/10.1016/j.molimm.2006.09.022.

Sharma, A., Singh, P., Sarmah, B.K., Nandi, S.P., 2020. Quorum sensing: its role in microbial social networking. Res. Microbiol. 171, 159–164. https://doi.org/10.1016/j.resmic.2020.06.003.

Shen, G.M., Shi, C.Y., Fan, C., Jia, D., Wang, S.Q., Xie, G.S., Li, G.Y., Mo, Z.L., Huang, J., 2017. Isolation, identification and pathogenicity of *Vibrio harveyi*, the causal agent of skin ulcer disease in juvenile hybrid groupers *Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*. J. Fish Dis. 40, 1351–1362. https://doi.org/10.1111/jfd.12609.

Silvaraj, S., Yasin, I.S.M., Karim, M.M.A., Saad, M.Z., 2020. Elucidating the efficacy of vaccination against Vibriosis in *Lates calcarifer* using two recombinant protein vaccines containing the outer membrane protein K (r-OmpK) of *Vibrio alginolyticus* and the DNA Chaperone J (r-DnaJ) of *Vibrio harveyi*. Vaccines 8, 660. https://doi.org/10.3390/vaccines8040660.

Sohn, H., Kim, J., Jin, C., Lee, J., 2019. Identification of *Vibrio* species isolated from cultured olive flounder (*Paralichthys olivaceus*) in Jeju Island, South Korea. Fish. Aquat. Sci. 22, 14. https://doi.org/10.1186/s41240-019-0129-0.

Song, X., Ma, Y., Fu, J., Zhao, A., Guo, Z., Malakar, P.K., Pan, Y., Zhao, Y., 2017. Effect of temperature on pathogenic and non-pathogenic *Vibrio parahaemolyticus* biofilm formation. Food Control 73, 485–491. https://doi.org/10.1016/j.foodcont.2016.08.041.

Sony, M., Sumithra, T.G., Anusree, V.N., Amala, P.V., Reshma, K.J., Alex, S., Sanil, N.K., 2021. Antimicrobial resistance and virulence characteristics of *Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Vibrio harveyi* from natural disease outbreaks of marine/estuarine fishes. Aquaculture 539, 736608. https://doi.org/10.1016/j.aquaculture.2021.736608.

Soto-Rodriguez, S.A., Roque, A., Lizarraga-Partida, M.L., Guerra-Flores, A.L., Gomez-Gil, B., 2003. Virulence of luminous vibrios to *Artemia franciscana* nauplii. Dis. Aquat. Org. 53, 231–240. https://doi.org/10.3354/dao053231.

Soto-Rodriguez, S.A., Lozano-Olvera, R., Abad-Rosales, S.M., Martínez-Brown, J.M., Ibarra-Castro, L., 2019. Susceptibility of Pacific white snook *Centropomus viridis* to *Vibrio* species. Dis. Aquat. Org. 134, 189–195. https://doi.org/10.3354/dao03370.

Sumithra, T.G., Reshma, K.J., Anusree, V.N., Sayooj, P., Sharma, S.R.K., Suja, G., Amala, P.V., Joseph, S., Sanil, N.K., 2019. Pathological investigations of *Vibrio vulnificus* infection in genetically improved farmed tilapia (*Oreochromis niloticus* L.) cultured at a floating cage farm of India. Aquaculture 511, 734217. https://doi.org/10.1016/j.aquaculture.2019.734217.

Sun, X., Jin, P., Liu, Q., Wang, Q., Zhang, Y., Liu, X., 2020. A CpG-riched plasmid as vaccine adjuvant reduce antigen dose of an inactivated *Vibrio anguillarum* vaccine in turbot (*Scophthalmus maximus* L.). Fish Shellfish Immunol. 98 https://doi.org/10.1016/j.fsi.2020.01.031.

Swain, P., Nayak, S.K., Nanda, P.K., Dash, S., 2008. Biological effects of bacterial lipopolysaccharide (endotoxin) in fish: a review. Fish Shellfish Immunol. 25, 191–201. https://doi.org/10.1016/j.fsi.2008.04.009.

Tafalla, C., Bogwald, J., Dalmo, R.A., 2013. Adjuvants and immunostimulants in fish vaccines: current knowledge and future perspectives. Fish Shellfish Immunol. 35, 1740–1750. https://doi.org/10.1016/j.fsi.2013.02.029.

Tang, X., Wang, H., Liu, F., Sheng, X., Xing, J., Zhan, W., 2017. Outer membrane protein A: an immunogenic protein induces highly protective efficacy against *Vibrio ichthyoenteri*. Microb. Pathog. 113, 152–159. https://doi.org/10.1016/j.micpath.2017.10.043.

Tang, X., Wang, H., Liu, F., Sheng, X., Xing, J., Zhan, W., 2019. Recombinant outer membrane protein T (OmpT) of *Vibrio ichthyoenteri*, a potential vaccine candidate for flounder (*Paralichthys olivaceus*). Microb. Pathog. 126, 185–192. https://doi.org/10.1016/j.micpath.2018.11.001.

Teo, J.W.P., Zhang, L.-H., Poh, C.L., 2003. Cloning and characterization of a metalloprotease from *Vibrio harveyi* strain AP6. Gene 303, 147–156. https://doi.org/10.1016/S0378-1119(02)01151-4.

Tercero-Alburo, J.J., González-Márquez, H., Bonilla-González, E., Quiñones-Ramírez, E. I., Vázquez-Salinas, C., 2014. Identification of capsule, biofilm, lateral flagellum, and type IV pili in *Vibrio mimicus* strains. Microb. Pathog. 76, 77–83. https://doi.org/10.1016/j.micpath.2014.09.012.

Tong, Y., Guo, M., 2009. Bacterial heme-transport proteins and their heme-coordination modes. Arch. Biochem. Biophys. 481, 1–15.

Torrecillas, S., Caballero, M.J., Mompel, D., Montero, D., Zamorano, M.J., Robaina, L., Rivero-Ramírez, F., Karalazos, V., Kaushik, S., Izquierdo, M., 2017. Disease resistance and response against *Vibrio anguillarum* intestinal infection in European seabass (*Dicentrarchus labrax*) fed low fish meal and fish oil diets. Fish Shellfish Immunol. 67, 302–311. https://doi.org/10.1016/j.fsi.2017.06.022.

Tort, L., 2011. Stress and immune modulation in fish. Dev. Comp. Immunol. 35 (12), 1366–1375. https://doi.org/10.1016/j.dci.2011.07.002.

Valiente, E., Padrós, F., Lamas, J., Llorens, A., Amaro, C., 2008. Microbial and histopathological study of the vibriosis caused by *Vibrio vulnificus* serovar E in eels: the metalloprotease Vvp is not an essential lesional factor. Microb. Pathog. 45, 386–393. https://doi.org/10.1016/j.micpath.2008.09.001.

Verbeke, F., De Craemer, S., Debunne, N., Janssens, Y., Wynendaele, E., Van de Wiele, C., De Spiegeleer, B., 2017. Peptides as quorum sensing molecules: measurement techniques and obtained levels in-vitro and in-vivo. Front. Neurosci. 11, 183. https://doi.org/10.3389/fnins.2017.00183.

Vinay, T.N., Ray, A.K., Avunje, S., Thangaraj, S.K., Krishnappa, H., Viswanathan, B., Reddy, M.A., Vijayan, K.K., Patil, P.K., 2019. *Vibrio harveyi* biofilm as immunostimulant candidate for high-health pacific white shrimp, *Penaeus vannamei* farming. Fish Shellfish Immunol. 95, 498–505. https://doi.org/10.1016/j.fsi.2019.11.004.

Wang, Y.-D., Wang, Y.-H., Hui, C.-F., Chen, J.-Y., 2016. Transcriptome analysis of the effect of *Vibrio alginolyticus* infection on the innate immunity-related TLR5-mediated induction of cytokines in *Epinephelus lanceolatus*. Fish Shellfish Immunol. 52, 31–43. https://doi.org/10.1016/j.fsi.2016.03.013.

Won, K.M., Park, S.I., 2008. Pathogenicity of *Vibrio harveyi* to cultured marine fishes in Korea. Aquaculture 285, 8–13. https://doi.org/10.1016/j.aquaculture.2008.08.013.

Xie, J., Bu, L., Jin, S., Wang, X., Zhao, Q., Zhou, S., Xu, Y., 2020. Outbreak of vibriosis caused by *Vibrio harveyi* and *Vibrio alginolyticus* in farmed seahorse *Hippocampus kuda* in China. Aquaculture 523, 735168. https://doi.org/10.1016/j.aquaculture.2020.735168.

Xiong, X.-P., Zhang, B.-W., Yang, M.-J., Ye, M.-Z., Peng, X.-X., Li, H., 2010. Identification of vaccine candidates from differentially expressed outer membrane proteins of *Vibrio alginolyticus* in response to NaCl and iron limitation. Fish Shellfish Immunol. 29, 810–816. https://doi.org/10.1016/j.fsi.2010.07.027.

Xu, C., Ren, H., Wang, S., Peng, X., 2004. Proteomic analysis of salt-sensitive outer membrane proteins of *Vibrio parahaemolyticus*. Res. Microbiol. 155, 835–842. https://doi.org/10.1016/j.resmic.2004.07.001.

Xu, H., Xing, J., Tang, X., Sheng, X., Zhan, W., 2019. Immune response and protective effect against Vibrio anguillarum induced by DNA vaccine encoding Hsp33 protein. Microb. Pathog. 137, 103729 https://doi.org/10.1016/j.micpath.2019.103729.

Xue, S., Xu, W., Wei, J., Sun, J., 2017. Impact of environmental bacterial communities on fish health in marine recirculating aquaculture systems. Vet. Microbiol. 203, 34–39. https://doi.org/10.1016/j.vetmic.2017.01.034.

Yanuhar, U., Nurcahyo, H., Widiyanti, L., Junirahma, N.S., Caesar, N.R., Sukoso, S., 2022. *In vivo* test of *Vibrio alginolyticus* and *Vibrio harveyi* infection in the humpback grouper (*Cromileptes altivelis*) from East Java Indonesia. Vet. World 15, 1269–1282. https://doi.org/10.14202/vetworld.2022.1269-1282.

Yildiz, F.H., Visick, K.L., 2009. *Vibrio* biofilms: so much the same yet so different. Trends Microbiol. 17, 109–118. https://doi.org/10.1016/j.tim.2008.12.004.

Yu, L.-p, Hu, Y.-h, Sun, B.-g, Sun, L., 2013. Immunological study of the outer membrane proteins of *Vibrio harveyi*: insights that link immunoprotectivity to interference with bacterial infection. Fish Shellfish Immunol. 35, 1293–1300. https://doi.org/10.1016/j.fsi.2013.07.043.

Zha, Z., Li, C., Li, W., Ye, Z., Pan, J., 2016. LptD is a promising vaccine antigen and potential immunotherapeutic target for protection against *Vibrio* species infection. Sci. Rep. 6, 38577. https://doi.org/10.1038/srep38577.

Zhang, C., Li, D.L., Chi, C., Ling, F., Wang, G.X., 2015. *Dactylogyrus intermedius* parasitism enhances *Flavobacterium columnare* invasion and alters immune-related gene expression in *Carassius auratus*. Dis. Aquat. Org. 116, 11–21. https://doi.org/10.3354/dao02902.

Zhang, L., Orth, K., 2013. Virulence determinants for *Vibrio parahaemolyticus* infection. Curr. Opin. Microbiol. 16, 70–77. https://doi.org/10.1016/j.mib.2013.02.002.

Zhang, Q., Dong, X., Chen, B., Zhang, Y., Zu, Y., Li, W., 2016. Zebrafish as a useful model for zoonotic *Vibrio parahaemolyticus* pathogenicity in fish and human. Dev. Comp. Immunol. 55, 159–168. https://doi.org/10.1016/j.dci.2015.10.021.

Zhang, X.-H., Austin, B., 2005. Haemolysins in *Vibrio* species. J. Appl. Microbiol. 98, 1011–1019. https://doi.org/10.1111/j.1365-2672.2005.02583.x.

Zhao, Z., Chen, C., Hu, C.-Q., Ren, C.-H., Zhao, J.-J., Zhang, L.-P., Jiang, X., Luo, P., Wang, Q.-B., 2010. The type III secretion system of *Vibrio alginolyticus* induces rapid apoptosis, cell rounding and osmotic lysis of fish cells. Microbiology 156, 2864–2872. https://doi.org/10.1099/mic.0.040626-0.

Zhou, Z., Pang, H., Ding, Y., Cai, J., Huang, Y., Jian, J., Wu, Z., 2013. VscO, a putative T3SS chaperone escort of *Vibrio alginolyticus*, contributes to virulence in fish and is a target for vaccine development. Fish Shellfish Immunol. 35, 1523–1531. https://doi.org/10.1016/j.fsi.2013.08.017.

Zhu, K., Lu, X.-J., Lu, J.-F., Chen, J., 2019a. The interleukin-6 regulates the function of monocytes/macrophages (MO/MΦ) via the interleukin-6 receptor β in ayu (*Plecoglossus altivelis*). Fish Shellfish Immunol. 93, 191–199. https://doi.org/10.1016/j.fsi.2019.07.049.

Zhu, W., Wei, Y., Li, Z., Li, G., Han, F., Hao, L., Wu, J., Liu, X., Zhang, Y., 2022. Research progress on bacterial ghosts as novel fishery vaccines. Aquaculture 543, 737526. https://doi.org/10.1016/j.aquaculture.2021.737526.

Zhu, Z., Dong, C., Weng, S., He, J., 2019b. Identification of outer membrane protein TolC as the major adhesin and potential vaccine candidate for *Vibrio harveyi* in hybrid grouper, *Epinephelus fuscoguttatus* (♀) × *E. lanceolatus* (♂). Fish Shellfish Immunol. 86, 143–151. https://doi.org/10.1016/j.fsi.2018.11.037.

Zhu, Z.M., Dong, C.F., Weng, S.P., He, J.G., 2017. The high prevalence of pathogenic *Vibrio harveyi* with multiple antibiotic resistance in scale drop and muscle necrosis disease of the hybrid grouper, *Epinephelus fuscoguttatus* (♀) × *E. Lanceolatus* (♂), in China. J. Fish Dis. 41 (4), 589–601. https://doi.org/10.1111/jfd.12758.