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# Epinephrine Enhances Virulence-Associated Traits of *Vibrio alginolyticus* and Increases Mortality in *Sciaenops ocellatus* (Linnaeus, 1766)

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## ABSTRACT

Stress hormones released during aquaculture handling and environmental disturbances can influence host–pathogen interactions, yet their effects on marine bacterial pathogens remain largely unexplored. This study examined how the stress hormone epinephrine affects the virulence of *Vibrio alginolyticus* isolated from diseased red drum (*Sciaenops ocellatus*). Ten isolates were exposed to epinephrine concentrations ranging from 0 to 200 μM, and changes in bacterial motility, biofilm formation and extracellular enzyme activities were evaluated. In vivo pathogenicity was assessed by experimentally challenging red drum with epinephrine-treated and untreated *V. alginolyticus* strains. Epinephrine exposure significantly enhanced bacterial motility, biofilm formation and the activities of lipase, phospholipase, haemolysin and caseinase ($p < 0.05$). Fish challenged with epinephrine-treated bacteria exhibited higher cumulative mortality (60%–100%) compared to those exposed to untreated strains (40%–60%). These findings indicate that exposure to epinephrine enhances the virulence of *V. alginolyticus*, although the underlying regulatory mechanisms remain to be elucidated. This study provides new evidence linking host stress physiology with bacterial pathogenicity in marine aquaculture and underscores the importance of stress management to prevent stress-associated vibriosis outbreaks.

## 1 | Introduction

Aquaculture of *Sciaenops ocellatus* (Linnaeus 1766), commonly known as red drum, has gained increasing attention in recent years, particularly in Asia, including Vietnam, where it has become an increasingly important marine aquaculture species (Linh et al. 2022; Li et al. 2023; Jiang et al. 2024). As a high-value species for both commercial production and recreational fisheries, red drum has become an important component of coastal aquaculture. In Vietnam, it is also among the marine fish species prioritized for aquaculture development by the national government (Government of Vietnam 2021).

However, disease outbreaks remain a major constraint to sustainable production. Among bacterial diseases, vibriosis caused by pathogenic *Vibrio* species has been associated with substantial economic losses in marine fish aquaculture, including red drum (Mohamad et al. 2019; Linh et al. 2022; Shafiee et al. 2024; Zhou et al. 2024). *Vibrio alginolyticus* is one of the *Vibrio* species that is commonly detected in seafood in Asia and is recognized as an opportunistic pathogen of aquatic animals and humans (Cao et al. 2024; Sun et al. 2024). In fish, infection with this bacterium can cause darkened skin, scale loss, haemorrhages around the mouth, fin bases, abdomen and anal region, along with fin erosion, ascites and high mortality (El-Sayed et al. 2021). Recent reports indicate

that *V. alginolyticus* infections have become an increasing threat to red drum aquaculture in Vietnam (Yen et al. 2021; Linh et al. 2022).

The occurrence and severity of bacterial diseases in aquaculture are closely linked to host physiological stress. Handling, transport, high stocking density and environmental fluctuations can activate the stress response in fish, leading to the release of catecholamines such as epinephrine, norepinephrine and dopamine, which regulate immune and metabolic processes (Guo and Dixon 2021; Tort and Balasch 2022). Although these hormones are essential for maintaining host homeostasis, they may also influence host–pathogen interactions. In fish, catecholamine-mediated stress responses have been associated with altered susceptibility to *Vibrio* infection (Khansari et al. 2017) and bacterial physiology and virulence (Boukerb et al. 2021). At the same time, catecholamines can function as inter-kingdom signalling molecules that modulate bacterial growth, physiology and virulence-associated traits, as initially demonstrated in enteric Gram-negative bacteria (Lyte and Ernst 1992) and later reported in aquatic bacterial pathogens, including *Vibrio* spp., *Aeromonas hydrophila* and *Yersinia ruckeri* (Nakano et al. 2007; Yang et al. 2014; Gao et al. 2019; Torabi Delshad et al. 2019).

Previous studies have shown that catecholamines can enhance siderophore production, swimming motility, biofilm formation, chemotaxis, exopolysaccharide synthesis and the expression of genes related to flagellar assembly in several aquatic pathogens, including Vibrio harveyi (Yang et al. 2014), *Vibrio campbellii* (Weigert Muñoz et al. 2022), *Vibrio parahaemolyticus* (Nakano et al. 2007) *Y. ruckeri* (Torabi Delshad et al. 2019) and *A. hydrophila* (Gao et al. 2019).

However, catecholamine responses appear to be species-specific. For example, the stimulatory effect of norepinephrine observed in *V. parahaemolyticus* was not reproduced in *Vibrio vulnificus*, indicating that responses in one pathogenic aquatic *Vibrio* species cannot necessarily be extrapolated to another (Nakano et al. 2007). Despite the increasing importance of *V. alginolyticus* in red drum aquaculture, it remains unclear whether epinephrine can alter the virulence-associated phenotypes of *V. alginolyticus* isolates from diseased red drum and whether such changes translate into increased mortality during experimental infection.

Therefore, this study aimed to investigate the influence of the stress hormone epinephrine on the virulence of *V. alginolyticus* in red drum. The effects of epinephrine were examined both in vitro by assessing bacterial motility, biofilm formation and lytic enzyme production (lipase, phospholipase, haemolysin and caseinase) and in vivo in red drum (*S. ocellatus*) through experimental challenge assays.

## 2 | Materials and Methods

### 2.1 | Source of *V. alginolyticus*

Ten *V. alginolyticus* isolates were obtained from a natural outbreak of haemorrhagic disease in red drum (*S. ocellatus*) cultured in Hue City, Central Vietnam. Preliminary identification was performed using MALDI–TOF mass spectrometry (matrix-assisted laser desorption/ionization–time of flight) at the University of Sydney, Australia, and species identity was subsequently confirmed by whole-genome sequencing (WGS). As all isolates originated from the same outbreak, they may represent closely related strains; therefore, the isolates were treated as independent bacterial isolates rather than confirmed genetically distinct strains.

Briefly, genomic DNA was extracted using the Quick-DNA Miniprep Plus Kit (Cat. #D4086, Zymo Research, USA) following the manufacturer's instructions. Sequencing was conducted on an Illumina MiSeq platform, achieving 60–100× average coverage. Species identification was conducted by referenced alignment using BWA MEM (Li 2013, with reads mapped against a combined reference database comprising publicly available genomes of *Vibrio* species corresponding to those identified by MALDI–TOF analysis of isolates obtained from diseased red drum. The WGS data were generated solely for species confirmation and were not used for comparative genomic analyses. Therefore, the sequence data have not yet been deposited in a public repository.

Bacterial stocks were preserved at −80°C in 15% (v/v) glycerol and recovered on thiosulphate–citrate–bile salts–sucrose agar (TCBS; HiMedia, India) prior to experimentation. A single colony was subcultured on tryptone soya agar (TSA; HiMedia, India) and subsequently grown in tryptone soya broth (TSB; HiMedia, India) containing 2% NaCl at 28°C under constant shaking (180 rpm). Cell density was determined spectrophotometrically at 600 nm ($OD_{600} = 1.0 \approx 1.2 \times 10^9$ CFU $mL^{-1}$, unpublished data).

### 2.2 | Epinephrine Preparation

Epinephrine (Epi—Sigma-Aldrich, Singapore) was dissolved in 0.1 N HCl to prepare a 10 mM stock solution. The stock solution was subsequently filtered through a 0.2 μm Millex hydrophobic PTFE syringe filter (Merck, Germany) and stored at −20°C until use. Working concentrations of 25, 50, 100 and 200 μM were freshly prepared for each experiment. Epinephrine concentrations used in this study were selected on the basis of concentrations previously employed to investigate catecholamine-mediated regulation of bacterial virulence-associated traits in aquatic pathogens (Nakano et al. 2007; Yang et al. 2014; Gao et al. 2019). These concentrations have been shown to influence bacterial behaviour, including motility, biofilm formation and other virulence-associated traits, and were therefore considered appropriate for examining dose-dependent responses to epinephrine in *V. alginolyticus*. They were selected as experimental concentrations rather than to reproduce physiological circulating levels in fish.

### 2.3 | Experimental Fish

Red drum (*S. ocellatus*) weighing 5–7 g were purchased from a commercial hatchery in Hue, Vietnam.

Fish were confirmed *Vibrio*-free by the Hue Sub-Department of Animal Health based on clinical examination and routine bacteriological culture prior to transfer. Fish were acclimated

for 2 weeks in a 1000 L tank containing 20 ppt seawater under continuous aeration at 28°C ± 2°C. Groups of 20 fish were randomly distributed into nine 80 L tanks with independent aeration and water flow and maintained for 1 additional week prior to the experimental challenge. Fish were fed twice daily to satiation using a commercial diet (De Heus, Vietnam) and starved for 24 h prior to experimental challenge to standardize physiological conditions and minimize handling-related stress (Macchia et al. 2022).

Water quality parameters were maintained within optimal ranges: dissolved oxygen (DO) > 5 mg $L^{-1}$, pH 7.3–7.8, total ammonia ($NH_4^+$) < 2 mg $L^{-1}$, nitrite ($NO_2^-$) < 1 mg $L^{-1}$ and nitrate ($NO_3^-$) < 80 mg $L^{-1}$, as measured using a commercial kit (Sera, Vietnam).

## 2.4 | In Vitro Effects of Epinephrine on *V. alginolyticus* Virulence

The effects of epinephrine on the virulence traits of *V. alginolyticus* were evaluated following Yang et al. (2014) with minor modifications. Ten *V. alginolyticus* isolates were examined to evaluate the effects of epinephrine on virulence-related traits, including swimming motility, biofilm formation and extracellular enzyme activities. For each isolate and treatment (0, 25, 50, 100 and 200 μM epinephrine), three independent biological replicates were performed, each based on a separate overnight culture. Each biological replicate was measured in triplicate (technical replicates), and mean values were used for statistical analysis ($n = 3$ biological replicates per isolate per treatment).

### 2.4.1 | Motility Assay

Soft agar plates were prepared by using TSB supplemented with 2% NaCl and 0.3% agar (w/v) (HiMedia, India). After autoclaving and cooling to 55°C, epinephrine was added to achieve final concentrations of 0 (control), 25, 50, 100 and 200 μM, and 20 mL of medium was poured into sterile 90-mm Petri dishes. Plates were allowed to solidify at room temperature before use.

Overnight cultures of *V. alginolyticus* were adjusted to $OD_{600}$ = 0.5, and 5 μL of bacterial suspension was inoculated onto the centre of each plate. Plates were incubated at 28°C for 24 h, after which the diameter of the swimming halo was measured as an indicator of motility. For each isolate, motility in epinephrine-treated plates was compared with that in control plates without epinephrine. No external control strains were included in this assay.

### 2.4.2 | Biofilm Formation

Biofilm formation was quantified using the crystal violet microtitre plate method following Mitsuwan et al. (2025). An overnight culture of *V. alginolyticus* was diluted in TSB + 2% NaCl to achieve an optical density at 600 nm ($OD_{600}$) of 0.1, corresponding to approximately $1.0 \times 10^8$ CFU mL−1 based on a previously established $OD_{600}$–CFU standard curve. Epinephrine was added to reach final concentrations of 0, 25, 50, 100 and 200 μM. The treatment without epinephrine (0 μM) served as the negative control. Aliquots (200 μL) of each culture were dispensed into triplicate wells of a 96-well flat-bottom plate and incubated at 28°C for 24 h without shaking. After incubation, wells were gently washed twice with sterile saline to remove non-adherent cells, fixed with 150 μL of 99% methanol for 20 min, air-dried, and stained with 1% crystal violet for 15 min. Excess dye was rinsed off, and bound stain was solubilized in 150 μL of 95% ethanol. The absorbance was measured at 570 nm using a Tecan Infinite Pro 200 plate reader (Tecan, Austria).

Because the objective of this experiment was to determine whether epinephrine affects biofilm formation, a positive control strain was not included. The negative control (0 μM) was used as the baseline to compare the relative increase in biofilm biomass across treatments.

### 2.4.3 | Extracellular Enzyme Activity Assays

Extracellular enzyme activities of *V. alginolyticus* were all determined on TSA plates supplemented with epinephrine and specific substrates following Bunpa et al. (2016) with minor modifications.

For the lipase assay, autoclaved TSA (at 55°C) was thoroughly mixed with 1% Tween 80 (Xilong, China) and epinephrine at concentrations of 25, 50, 100 and 200 μM before being poured into Petri dishes. An overnight culture of *V. alginolyticus* in TSB with 2% NaCl was diluted to an OD of 0.5 at 600 nm, and then a 5 μL drop of this diluted culture was placed on the epinephrine-supplemented TSA plates containing 2% NaCl and 1% Tween 80 (Xilong, China). The plates were then incubated for 2 days at 28°C, after which the diameter of each opalescent zone of each colony was measured.

Other enzyme assays were conducted similarly. For phospholipase activity, 1% egg yolk emulsion (Sigma-Aldrich, Australia) was added to TSA with 2% NaCl. For caseinase activity, 4% skim milk powder (A2, Australia) was added, and for haemolysin activity, 5% defibrinated sheep blood (Oxoid, Australia) was added to TSA with 2% NaCl. Opalescent or clearing zones surrounding colonies were then measured after the 2-day incubation at 28°C. Control plates without epinephrine supplementation were included.

For each enzyme assay, groups refer to the different epinephrine treatment concentrations, and enzyme activity was expressed as the mean ratio of the opalescent or clearing zone diameter to the colony diameter for each epinephrine concentration and isolate.

## 2.5 | In Vivo Effects of Epinephrine on *V. alginolyticus* Virulence in Red Drum

### 2.5.1 | Bacterial Preparation

Four *V. alginolyticus* isolates were used for the in vivo challenge experiment. Two isolates (S1 and S2) exhibiting the highest extracellular enzyme activities in the in vitro assays were selected

as representative highly virulent isolates, whereas two isolates (S3 and S4) exhibiting comparatively lower enzyme activities were selected as less virulent representatives.

Each isolate was cultured in TSB plus 2% NaCl with or without epinephrine (100 μM) and incubated at 28°C, 140 rpm for 24 h to mid-log phase. Cultures were centrifuged (3500 rpm, 30 min, 4°C) and resuspended in sterile saline (0.85%). Optical density ($OD_{600} = 1.0$) corresponded to $1 \times 10^9$ CFU $mL^{-1}$, based on prior calibration using viable plate counts. The concentration of 100 μM epinephrine was selected for the in vivo challenge experiment because it consistently induced significant increases in motility, biofilm formation and extracellular enzyme activities across the tested isolates during the in vitro assays.

The bacterial inoculum used for challenge ($1.2 \times 10^6$ CFU $mL^{-1}$) was prepared by serial dilution, and viable counts were confirmed using the Miles and Misra method (Miles et al. 1938). This challenge dose was determined in a preliminary pilot experiment conducted in the absence of epinephrine to induce approximately 50% mortality in red drum under the experimental conditions and was subsequently applied uniformly to all isolates and treatments.

#### 2.5.2 | Experimental Challenge

Fish were challenged by immersion exposure. For each treatment, three replicate tanks containing 20 fish per tank were exposed to either epinephrine-treated or untreated *V. alginolyticus* suspensions at a final concentration of $1.2 \times 10^6$ CFU $mL^{-1}$ for 30 min in 10-L aerated tanks. Following exposure, fish were returned to their respective experimental tanks supplied with continuous aeration and flow-through seawater and monitored for 14 days. Control fish were exposed to sterile saline only.

Dead and moribund fish were removed twice daily. Kidney samples were cultured on TCBS agar for pathogen re-isolation and identification using API 20E (bioMérieux, France). Mortality data were analysed using Kaplan–Meier survival analysis.

**2.5.2.1 | Statistical Analysis.** Data analysis was processed using the Statistical Package for the IBM SPSS software version 23.0. Normal distribution and homogeneity of variance were verified by using Shapiro–Wilk and Levene's tests before performing ANOVA. For in vitro assays, each *V. alginolyticus* isolate was treated as an independent biological unit. Differences among epinephrine treatments were analysed using one-way ANOVA followed by Tukey's post hoc test. Enzyme activities (zone/colony ratios) were log-transformed when necessary to meet assumptions.

For the in vivo challenge experiment, survival data from individual fish were used to generate Kaplan–Meier survival curves, whereas each treatment was conducted in three independent replicate tanks to ensure reproducibility of the challenge results. Differences in survival distributions among treatments were evaluated using the log-rank test. Differences were considered significant at $p < 0.05$.

## 3 | Results

### 3.1 | In Vitro Effects of Epinephrine on *V. alginolyticus* Virulence

#### 3.1.1 | Swimming Motility

Exposure to epinephrine significantly enhanced the swimming motility of *V. alginolyticus* ($p < 0.05$; Figure 1). Representative images of the motility assay are provided in Figure S1. Compared with the untreated control, all epinephrine-treated groups exhibited significantly larger motility halos. The greatest increase was observed at 50 μM epinephrine (31 ± 0.37 mm). Motility decreased slightly at 100 and 200 μM but remained significantly higher than in the untreated control.

Among the 10 isolates tested, S1 and S2 consistently displayed higher motility responses than the remaining isolates across all treatments. Therefore, the pooled mean response of all isolates is presented together with the individual responses of S1 and S2 to illustrate isolate-specific variation. Epinephrine increased swimming motility, with maximal stimulation occurring at 50 μM, after which motility gradually declined at higher concentrations but remained significantly greater than in the untreated control.

#### 3.1.2 | Biofilm Formation

Exposure to epinephrine resulted in greater biofilm production by *V. alginolyticus* compared with the untreated control (0 μM) ($p < 0.05$; Figure 2; Figure S2). Biofilm biomass ($OD_{570}$) with increasing epinephrine concentration, with the highest values observed at 200 μM. Significant increases in biofilm formation were detected at 100 and 200 μM compared with the control, whereas the 25 and 50 μM treatments produced only slight, non-significant increases ($p > 0.05$). The stimulatory effect of epinephrine on biofilm formation was generally consistent across the 10 tested isolates. No distinct biofilm formation pattern was observed for isolates S1 and S2 compared with the remaining isolates. These results indicate that epinephrine enhances biofilm formation in *V. alginolyticus*, particularly at higher concentrations.

#### 3.1.3 | Extracellular Enzyme Activities

Epinephrine enhanced the production of extracellular enzymes, including lipase, phospholipase, haemolysin and caseinase, with significant increases observed at concentrations of 50–200 μM ($p < 0.05$; Figure 3).

For lipase and phospholipase activities, opalescent zone diameters were significantly larger at 50–200 μM epinephrine than in the untreated control ($p < 0.05$), with the greatest activity recorded at 100 μM ($p < 0.05$). No significant difference was detected between the 25 μM treatment and the control ($p > 0.05$).

Similarly, caseinase and haemolysin activities were significantly elevated at 50–200 μM epinephrine relative to the untreated control ($p < 0.05$), with peak activity observed at 100 μM. The

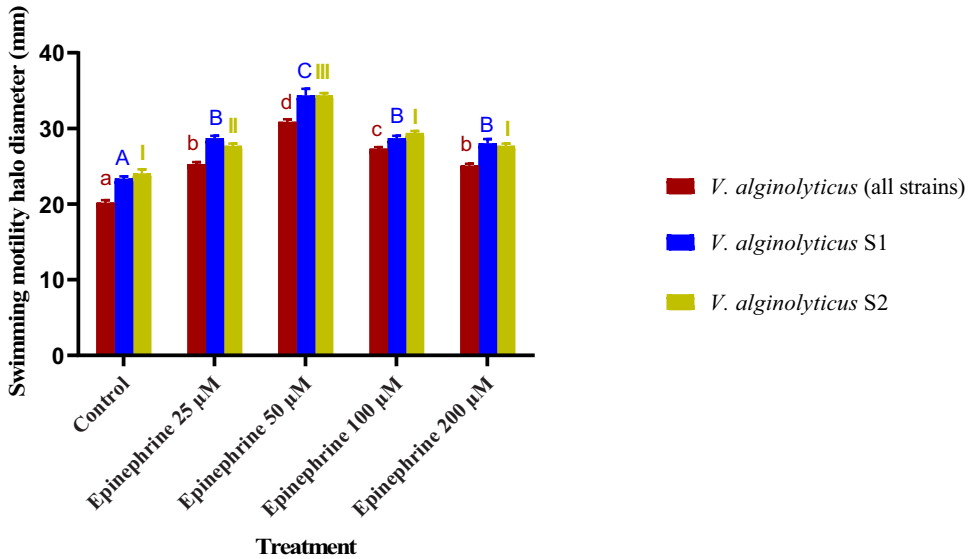


**FIGURE 1** | Effect of epinephrine on the swimming motility of *Vibrio alginolyticus*. Swimming motility was assessed by measuring the diameter of swimming halos after 24 h of incubation. Values are presented as means ± SE. Data for 'all strains' represent the mean of 10 independent *V. alginolyticus* isolates, each tested in three independent biological replicates. Data for two highly motile isolates (S1 and S2) are shown separately. Lowercase letters (a–d) indicate significant differences among epinephrine treatments for all strains ($p < 0.05$). Uppercase letters (A–C) indicate significant differences among epinephrine treatments within isolate S1, whereas Roman numerals (I–III) indicate significant differences among epinephrine treatments within isolate S2 ($p < 0.05$). No statistical comparisons were performed between isolates.

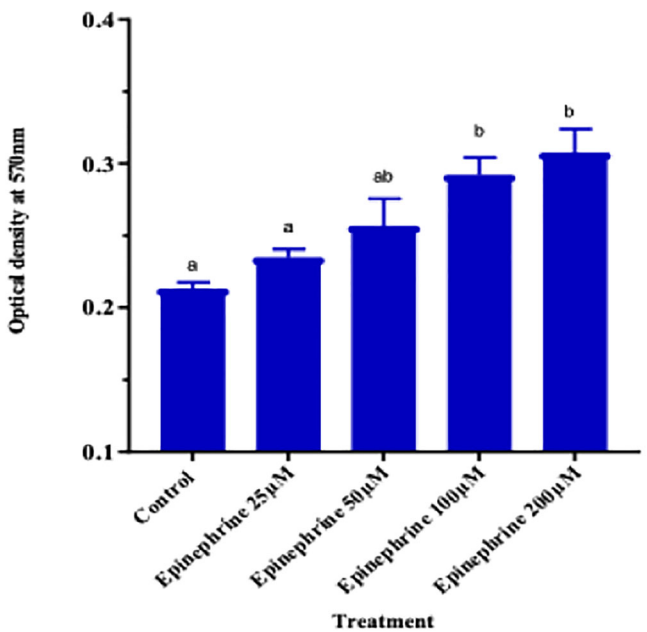


**FIGURE 2** | Effect of epinephrine on biofilm formation of *Vibrio alginolyticus*. Biofilm biomass was quantified using the crystal violet assay and expressed as absorbance at 570 nm. Values are presented as means ± SE. Data represent the mean of 10 independent *V. alginolyticus* isolates, each tested in three independent biological replicates. Different letters denote statistically significant differences among epinephrine treatments ($p < 0.05$).

25 µM treatment did not differ significantly from the control. The highest enzyme activity was observed at 100 µM. These findings demonstrate that epinephrine stimulates the production of multiple virulence-associated enzymes in *V. alginolyticus*, with the strongest response occurring at 100 µM.

### 3.2 | Effect of Epinephrine Exposure on Virulence of *V. alginolyticus* in Red Drum

Fish challenged with epinephrine-treated *V. alginolyticus* isolates exhibited significantly higher cumulative mortality and lower survival probabilities than fish challenged with the corresponding untreated isolates (Kaplan–Meier analysis, $p < 0.05$; Figure 4).

For the highly virulent isolates S1 and S2, epinephrine pre-treatment markedly increased pathogenicity, resulting in final mortalities of 80%–100%, whereas fish challenged with untreated bacteria exhibited mortalities of approximately 40%–60%. The strongest effect was observed for isolate S1, in which survival declined to nearly zero following epinephrine exposure.

A similar but less pronounced trend was observed for the less virulent isolates S3 and S4. Fish challenged with epinephrine-treated bacteria showed final mortalities of approximately 60%–70%, compared with 45%–50% in the corresponding untreated groups. No mortality occurred in the negative control group throughout the experimental period. This trend was consistently observed for both highly virulent (S1 and S2) and less virulent (S3 and S4) isolates, indicating a negative effect of epinephrine treatment on fish survival following bacterial challenge.

Following bacterial challenge, infected fish exhibited typical clinical signs of vibriosis, including scale loss and frayed caudal fin, abdominal distension with mucous ascitic fluid and haemorrhagic lesions in internal organs, particularly the liver, kidney and spleen (Figure 5). During the experimental infection, bacteria

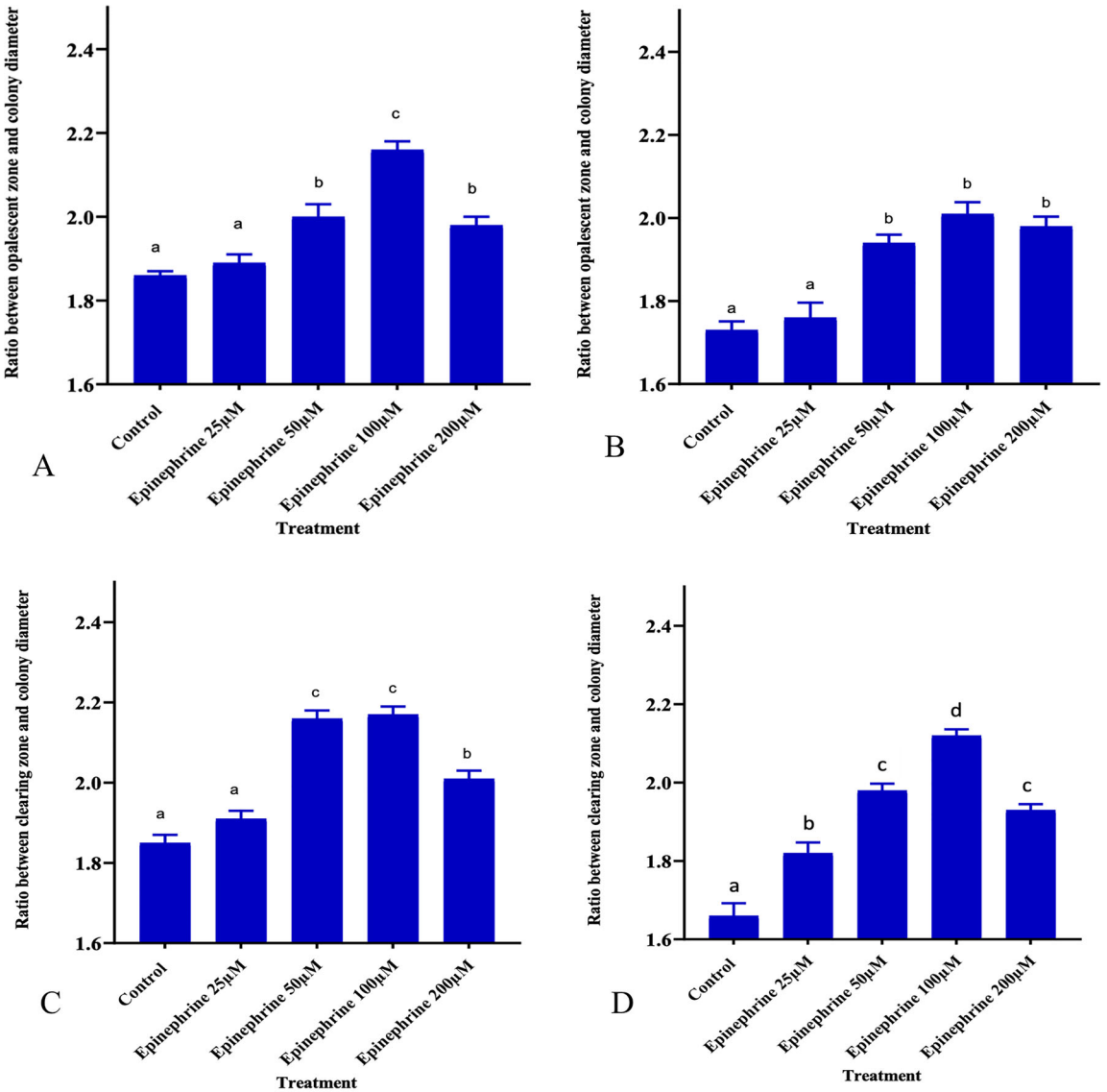


**FIGURE 3** | Effect of epinephrine on extracellular enzyme activities of *Vibrio alginolyticus*. (A) Lipase, (B) phospholipase, (C) caseinase and (D) haemolysin activities in response to increasing concentrations of epinephrine. Enzyme activity was expressed as the ratio of the opalescent (lipase and phospholipase) or clearing zone (caseinase and haemolysin) diameter to colony diameter. Values are presented as means ± SE. Data represent the mean of 10 independent *V. alginolyticus* isolates, each tested in three independent biological replicates. Different letters indicate statistically significant differences among epinephrine treatments ($p < 0.05$).

were re-isolated from the kidney of moribund fish on TCBS agar. Representative colonies showing morphology consistent with *V. alginolyticus* were purified and identified using the API 20E system (bioMérieux, France). The biochemical profiles of the re-isolated strains matched those of the original inoculum, confirming successful re-isolation of the challenge bacterium. No bacteria were recovered from the surviving fish or from the negative control group at the end of the experiment.

## 4 | Discussion

The present study demonstrated that epinephrine enhances several phenotypes associated with virulence in *V. alginolyticus*, including swimming motility, biofilm formation, extracellular enzyme production and pathogenicity in *S. ocellatus*. Similar responses have been described in other aquatic bacterial pathogens, including *V. harveyi, A. hydrophila* and *Y. ruckeri*, supporting the concept that host-derived catecholamines function not only as stress hormones but also as inter-kingdom signalling molecules capable of altering bacterial behaviour during infection (Yang et al. 2014; Gao et al. 2019; Torabi Delshad et al. 2019; Boujnane et al. 2024). Together, these findings suggest that *V. alginolyticus* is also able to respond to host neuroendocrine signals in ways that favour successful host colonization and disease progression.

The simultaneous enhancement of these traits suggests that epinephrine does not act on individual virulence factors in isolation but instead influences broader regulatory systems involved in bacterial adaptation to the host environment. In several Gram-negative bacteria, catecholamines are perceived through

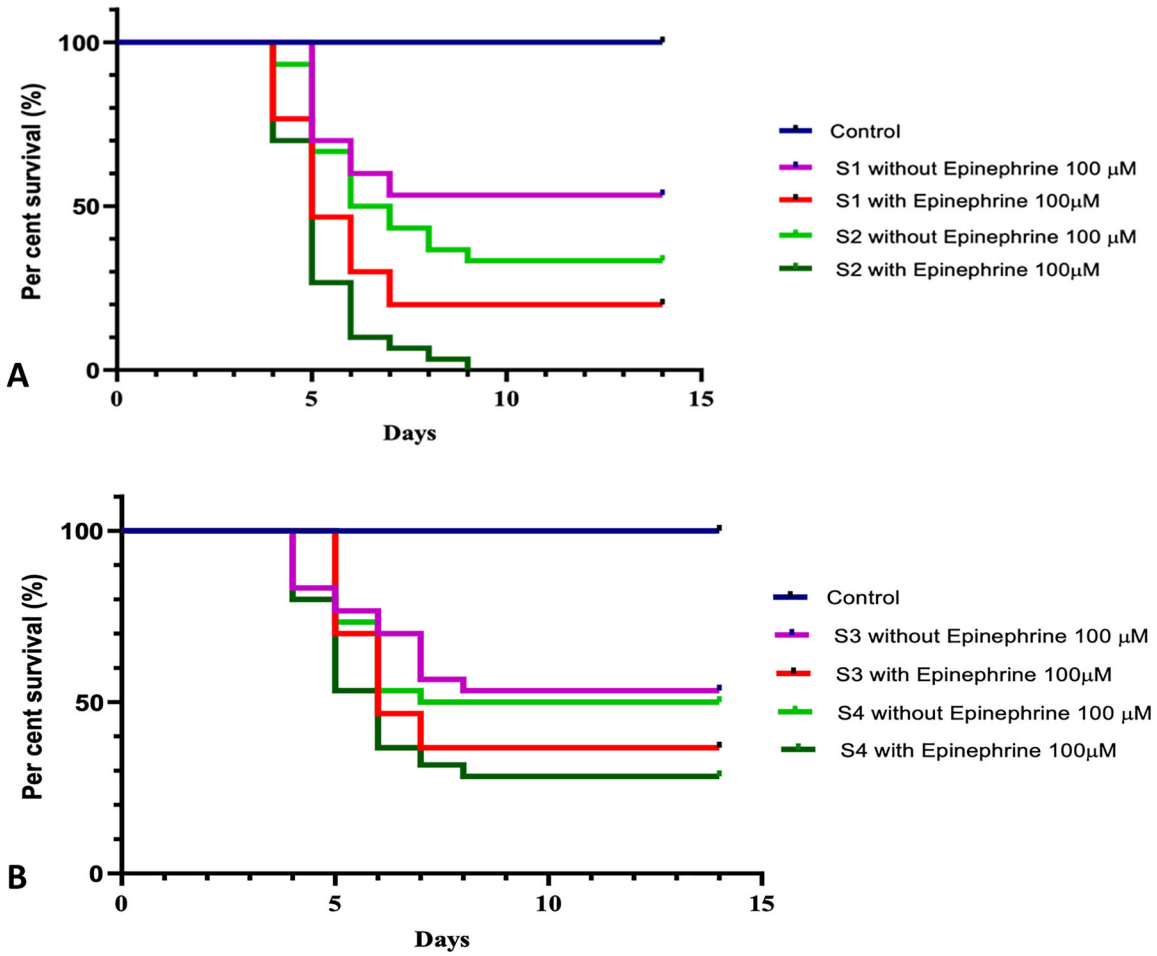


**FIGURE 4** | Effect of epinephrine exposure on the survival of *Sciaenops ocellatus* challenged with *Vibrio alginolyticus*. Kaplan–Meier survival curves showing cumulative survival of fish challenged by immersion with untreated or epinephrine-treated bacteria ($1.2 \times 10^6$ CFU $mL^{-1}$). (A) Highly virulent isolates (S1 and S2). (B) Less virulent isolates (S3 and S4). Each treatment consisted of three replicate tanks containing 20 fish per tank. Survival was monitored for 14 days post-challenge. The negative control group maintained 100% survival throughout the experiment.

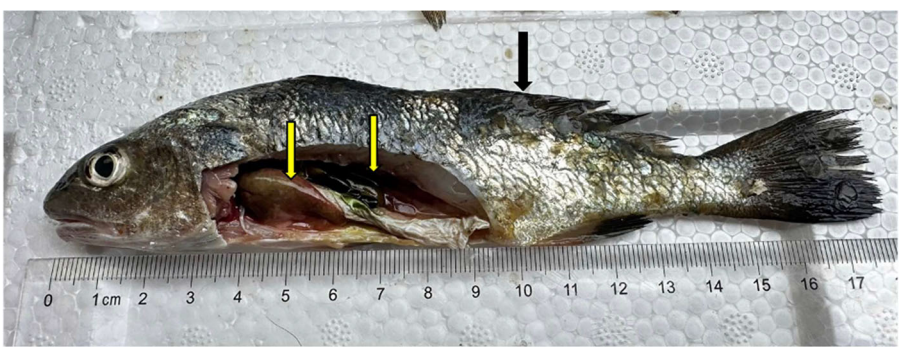


**FIGURE 5** | Representative clinical signs observed in *Sciaenops ocellatus* experimentally infected with *Vibrio alginolyticus*. Infected fish showing scale loss (black arrows), frayed caudal fin and abdominal distension with mucous ascitic fluid and marked haemorrhagic lesions in internal organs, including the liver, kidney and spleen (yellow arrows).

adrenergic sensing pathways such as the QseBC two-component regulatory system, which integrates environmental signals with the expression of genes involved in flagellar synthesis, chemotaxis, quorum sensing and secretion of virulence factors (Bansal et al. 2007; Boukerb et al. 2021; Schreiber et al. 2025).

Although homologous signalling pathways have not yet been experimentally confirmed in *V. alginolyticus*, the simultaneous enhancement of multiple virulence-related phenotypes is more consistent with the involvement of a global regulatory mechanism than with independent regulation of individual traits. This

interpretation agrees with previous observations showing that catecholamines stimulate flagellar gene expression and bacterial motility in both aquatic and enteric pathogens (Bansal et al. 2007; Gao et al. 2019).

The phenotypic changes observed following epinephrine exposure are likely to increase bacterial fitness throughout the infection process. Enhanced swimming motility may improve access to susceptible host tissues, biofilm formation supports persistence following colonization, and elevated extracellular enzyme production may accelerate tissue destruction and nutrient acquisition. Acting at successive stages of infection, these responses together provide a plausible explanation for the increased mortality observed in *S. ocellatus*. Similar hormone-mediated increases in virulence have also been described in *A. hydrophila, V. harveyi* and *Y. ruckeri* (Yang et al. 2014; Gao et al. 2019; Torabi Delshad et al. 2019; Boujnane et al. 2024). Among these responses, the increase in biofilm formation deserves particular attention because biofilm development is closely linked to quorum-sensing regulation in *Vibrio* species.

The enhanced biofilm formation observed after epinephrine exposure may also reflect interactions between host-derived catecholamines and quorum-sensing pathways. In *Vibrio* species, quorum sensing coordinates collective behaviours through signalling molecules and transcriptional regulators such as LuxR, which control biofilm development, motility, secretion systems and other traits associated with host adaptation (van Kessel et al. 2020).

Similar regulatory mechanisms have also been implicated in biofilm formation and surface colonization in *V. parahaemolyticus* (Zhang et al. 2021). Although direct evidence is currently lacking for *V. alginolyticus*, these observations raise the possibility that epinephrine interacts with quorum-sensing pathways to promote biofilm formation and other virulence-associated functions. Whether this mechanism operates in *V. alginolyticus* remains unknown and deserves further investigation at the molecular level.

Fish exposed to epinephrine-treated *V. alginolyticus* exhibited significantly higher mortality than those infected with untreated bacteria. This increase in mortality indicates that the effects of epinephrine extend beyond measurable changes in bacterial phenotype and ultimately influence disease outcome in the host. These findings demonstrate that the in vitro responses have biologically relevant consequences during infection and suggest that host-derived catecholamines can enhance the pathogenic potential of *V. alginolyticus*. Similar increases in pathogenicity following catecholamine exposure have been reported for other aquatic bacterial pathogens, including *A. hydrophila* and *Y. ruckeri* (Torabi Delshad et al. 2019; Gao et al. 2019), suggesting that responsiveness to host-derived catecholamines may be a conserved feature shared by diverse aquatic bacterial pathogens.

From an aquaculture perspective, these findings have important practical implications. Disease outbreaks under intensive farming conditions are commonly attributed to stress-induced suppression of host immune function. However, our findings imply that host stress may simultaneously enhance pathogen virulence through neuroendocrine signalling. Fish exposed to common husbandry stressors, including handling, transport, high stocking density, temperature fluctuations and deteriorating water quality, experience rapid release of catecholamines through activation of the hypothalamic–pituitary–interrenal (HPI) axis, resulting in the rapid release of catecholamines such as epinephrine and norepinephrine (Barton and Iwama 1991; Guo and Dixon 2021; Tort and Balasch 2022). These observations imply that stressful farming conditions may favour disease development through two parallel processes: Weakening host defences while simultaneously increasing bacterial virulence. This dual response offers a broader explanation for why routine husbandry practices such as handling, transport or deteriorating water quality frequently precede outbreaks of vibriosis.

Our study focused on phenotypic responses to epinephrine, whereas the underlying molecular mechanisms remain unresolved. The proposed involvement of adrenergic sensing and quorum-sensing pathways is based on phenotypic observations and evidence from other bacterial species rather than direct experimental verification in *V. alginolyticus*. Combining transcriptomic analyses with targeted gene-expression studies and functional genetics will be essential to determine how epinephrine regulates virulence in *V. alginolyticus*. Such studies would provide direct evidence for the regulatory pathways inferred from the present phenotypic observations.

### Author Contributions

**Nguyen Duc Quynh Anh**: conceptualization, investigation, methodology, formal analysis, writing – original draft, data curation, resources. **Tran Quang Khanh Van**: investigation, validation, resources, formal analysis. **Nguyen Nam Quang**: investigation, resources, formal analysis. **Tran Nguyen Ngoc**: investigation, validation, formal analysis, resources, data curation. **Nguyen Thi Xuan Hong**: investigation, writing – original draft, formal analysis, resources. **Nguyen Thi Hue Linh**: investigation, writing – original draft, resources, formal analysis. **Nguyen Ngoc Phuoc**: conceptualization, funding acquisition, writing – original draft, writing – review and editing, methodology, validation, formal analysis, data curation, supervision, project administration, software, visualization. **Ruth N. Zadoks**: conceptualization, writing – original draft, writing – review and editing, methodology, supervision, validation. **Carola Venturini**: conceptualization, writing – original draft, writing – review and editing, methodology. **Francisca Samsing Pedrals**: conceptualization, writing – original draft, writing – review and editing, data curation, formal analysis.

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### Ethics Statement

All experimental procedures involving fish were approved by the Animal Ethics Committee of Hue University, Vietnam (Approval No. HUVN0054) and were conducted in accordance with institutional and international guidelines for the care and use of animals in research.

## Consent

All authors have read and approved the final version of the manuscript and consent to its submission.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.

**Figure S1**. Representative images of swimming motility assays using *Vibrio alginolyticus* isolate S1, selected as a representative highly motile isolate, following exposure to 0, 25, 50, 100 and 200 μM epinephrine (A–E, respectively). Swimming halo diameters increased after epinephrine exposure, with the largest halo observed at 50 μM. Motility declined slightly at higher concentrations (100–200 μM) but remained greater than that of the untreated control, consistent with the quantitative data presented in Figure 1. **Figure S2**. Representative crystal violet-stained microplate showing biofilm formation after washing and air-drying. Columns 1–5 correspond to *Vibrio alginolyticus* isolate S1 exposed to 0, 25, 50, 100 and 200 μM epinephrine, respectively, whereas Columns 6–10 correspond to isolate S2 exposed to 200, 100, 50, 25 and 0 μM epinephrine, respectively. Rows represent replicate wells. The image is representative of the quantitative biofilm measurements presented in Figure 2.