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Quantifying the impact of temperature variation on birnavirus transmission dynamics in hard clams *Meretrix lusoria*

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Abstract

Susceptibility of hard clams *Meretrix lusoria* to birnavirus (BV) infections caused by temperature variations, from a mechanistic perspective, has rarely been explored. We used a deterministic susceptible–infectious–mortality (SIM) model to derive temperature-dependent key epidemiologic parameters based on data sets of viral infections in hard clams subjected to acute temperature changes. To parameterize seasonal pattern dependence, we estimated monthly based cumulative mortality and basic reproduction numbers (R_0) between 1997 and 2017 by way of statistical analysis. Two alternative disease control models were also proposed to assess status of controlled temperature-mediated BV infection by using, respectively, control reproduction number (R_C)-control line criterion and removal strategy-based control measure. We showed that based on R_C-control strategy, when temperatures ranged from 15 to 26.8°C, proportion of susceptible hard clams removed should be at least 0.22%. Based on removal-control strategy, we found that by limiting pond water temperature to 25–30°C, together with increased removal rates and periods to remove hard clams, it is better to remove hard clams from June and August to reduce both mortality rate and spread of BV. Our results can be used to monitor BV transmission potential in hard clams that will contribute to government control strategy to eradicate future BV epidemics.

KEYWORDS
birnavirus, control measures, disease transmission dynamics, hard clams, modelling, temperature

1 | INTRODUCTION

The hard clam, *Meretrix lusoria* (Bivalvia: Veneriade), which is mainly distributed in Japan, South Korea, China, Southeast Asia and the southwest coast of Taiwan, is an economically predominant bivalve. It was reported that in Taiwan in 2016 both its farming areas and stocking rates ranked first among all bivalve fisheries (FACOA, 2018). However, Ho (2005) and Hsieh, Wang, Huang, and Yeh (2017) reported that the farming of hard clams there has suffered from high mortality rates in spring and summer since 1969, while Deng, Chu, and Hsu (2017) state that, in recent years, mass mortality in farmed hard clams between June and September has been observed. The most likely causal factors for such mortality broadly cover infectious diseases, temperature variations, abrupt changes of salinity due to heavy rain, and industrial and pesticide contamination, high culture density and inappropriate feeding (Kou, Chen, & Lo, 1989; Kou, Lin, Chen, & Lo, 1984; Tseng, 1976; Yang, Lo, Huber, & Kou, 1978).

Viral infections in hard clams have been investigated by identifying strains, including the Birnaviridae and Reoviridae families—hard clam virus (HCV) from cultured hard clams (Chou, Li, & Lo, 1994; Lo, Hong, Huang, & Wang, 1988). Viruses, categorized as being in the Birnavirdae family, possess bisegmented and

double-stranded RNA (dsRNA) (Munang'andu, Mutoloki, & Evensen, 2016a). Aquabirnavirus, as a genus, belong to the Birnavirdae family, was birnaviruses that isolated from fish, molluscs and crustaceans (Munang'andu et al., 2016a). If it causes diseases in salmonid, aquabirnavirus is specifically referred to as an infectious pancreatic necrosis virus (IPNV); whereas, if it infects other marine organisms, it is called marine aquabirnavirus (MABV) (Munang'andu et al., 2016a). The MABV has been in vitro isolated from various bivalve mollusc species including the *M. lusoria* (Lo et al., 1988; Renault, 2011). Although Lo et al. (1988) isolated birnavirus (BV) from the gills of *M. lusoria* in Taiwan, information regarding virology and disease epidemics caused by BV among clam populations has received limited attention.

Several studies have indicated that environmental stressors, such as fluctuating water temperature, chemical pollutants and co-infection from other pathogens, have had significant effects on the pathogenicity of viruses (Farley, Banfield, Kasnic, & Foster, 1972; Hseu, Hsieh, & Huang, 2017; Liao, Yeh, & Chen, 2008; Montes, Durfort, & Garcia-Valero, 2001). Adverse effects of BV infections, in combination with environmental stressors, such as temperature variations on mortalities of hard clams have also been demonstrated experimentally (Chou, Chang, Lee, & Chiou, 1998; Chou et al., 1994). Consequently, evidence regarding their susceptibility to viral infections caused by environmental stressors in a mechanistic perspective should be urgently sought. Accordingly, we strongly recommend a study of exploring transmission dynamics of the BV disease among populations subjected to temperature change by adopting rigorous mathematical models designed to fill the knowledge gap.

The potential usefulness of mathematical modelling in predicting disease transmissions in aquatic animal populations needs both to be widely applied and to receive more attention (Anderson & May, 1979; Reno, 1998). Reno (1998) reported that by applying basic principles of epidemiological modelling to aquatic ecosystems, the spread of disease between fish farms has been successfully proven (Jonkers, Sharkey, Thrush, Turnbull, & Morgan, 2010; Taylor, Norman, Way, & Peeler, 2010). Notably, the susceptible–infectious–recovery (SIR) class of models has been a cornerstone epidemiological model that has been applied to simulate various disease epidemics, such as furunculosis and salmonid alpha virus in salmons (Alaliyat & Yndestad, 2015; Aldrin, Huseby, & Jansen, 2015; Anderson & May, 1979; Krkošek, 2010; Ögüt, LaPatra, & Reno, 2005; Salama & Murray, 2011). It has also been demonstrated that the simple SIR model could explain 92% of observed variations among susceptible, infected and removed (dead) populations during an experimental furunculosis epidemic (Ögüt, Reno, & Sampson, 2004). We modified the standard SIR structure to a BV disease-induced mortality-based susceptible–infectious–mortality (SIM) model in order to construct a mechanistic link between empirical data sets and theoretically epidemiological models. Effectiveness of control measures, such as environmental (temperature, oxygen and pollutions)-, host (species, age, density and immunity)- or pathogen (virulence and strain)-related factors, could also be determined based on simulations created in an SIR-modified SIM model (Ögüt, 2001).

Therefore, based on the constructed SIM model in this study, temperature-dependent epidemiological parameters of a BV disease could be obtained by applying data sets of viral infections. Annual cumulative mortalities of hard clams subjected to various culture temperatures could also be evaluated based on field investigations. The principal objective of this study was to estimate *in situ* epidemics of BV disease during outbreak seasons by applying developed mathematical models. We also aimed to provide a pragmatic strategy to contain BV disease transmission among clam populations in farmed ponds by utilizing a mechanistic approach.

2 | MATERIALS AND METHODS

2.1 | Study experimental data

Cumulative mortality data of BV-exposed hard clams were adopted from Chou et al. (1994) (Figure S1a,b and Tables S1, S2 and S4). Briefly, the aquatic BV was previously isolated from the gills of hard clams in a cultured farm near Tung-Shyr in southern Taiwan before undertaking infection experiments. The viruses were propagated in an eel ovary (EO) cell line, which was obtained from the ovary of a Japanese eel *Anguilla japonica* and cultured in Leibovitz's L-15 medium with added antibiotics at 26°C (Chen & Kou, 1981; Chou et al., 1994). The hard clams were acquired from a farm pond in the Lu-Kang Prefecture of Taiwan and cultured in sea water at 25 ± 1°C (Chou et al., 1994).

There were two kinds of experimental data obtained from five infection trials (trial I–V). The mortality in each trial was observed over a period of 3 weeks. Cumulative mortality of hard clams infected with the BV at different concentration is the first kind of the data obtained from trial I–II (Chou et al., 1994). For trial I, waterborne inoculations were performed by immersing 4-month-old clams (n = 30, mean weight 1.1 g) at BV concentrations of 10^4, 10^5 and 10^6, 50% tissue culture-infected dose (TCID50) ml^{-1} for 2 hr; subsequently, the virus-inoculated hard clams were reared in running sea water at 25 ± 1°C. Trial II was conducted by injecting BV at concentrations of $10^{2.7}$, $10^{3.7}$ and $10^{4.7}$ TCID50 0.05 ml^{-1} $clam^{-1}$ into 1-year-old hard clams (n = 20, mean weight 5.6 g). Clams without BV treatment were immersed or injected with supernatant from EO cell cultures without BV (Chou et al., 1994).

The other kind of experimental data adopted from Chou et al. (1994) was time-course cumulative mortality of hard clams subjected to temperature change. In order to examine the impact of acute temperature changes on the pathogenicity of BV in hard clams, mortality tests (trial III–V) were performed by infecting hard clams with BV before treatment with various culture scenarios involving temperature variations (Chou et al., 1994). Hard clams (mean weight 1.22 g) were cultured in running sea water at 25°C for 1 week and three temperature scenarios were conducted post-infection with 10^5 TCID50 ml^{-1} BV for 2 hr as follows, (a) trial III: temperature of sea water still at 25°C, (b) trial IV: temperature of sea water was transferred from culture temperatures of 25–33°C in 2 hr after immersion of 10^5 TCID50 ml^{-1} BV for 2 hr, and (c) trial V: temperature of sea water was transferred from 25 to 15°C in 2 hr.

For hard clams, the temperature range for survival varied from 3 to 39°C (Hseu et al., 2017). Hseu et al. (2017) discovered that they could grow faster in water temperatures from 25 to 32°C. On the other hand, for BV, Lo et al. (1988) found that BV replicated well at temperatures between 25 and 30°C. Although Chou et al. (1994) did not describe reasons for choosing temperatures between 15 and 33°C for treatments of BV infections, their choice appears to have been appropriate, since both hard clams and BV survived during the treatments.

2.2 | Study field data

In order to evaluate the impact of acute temperature changes on BV disease transmission in the hard clam populations, we adopted ambient and water temperatures as documented in the Taixi Township Quarterly samples of water temperatures between 2008 and 2016, as collated from an environmental monitoring report based on Yunlin Offshore Industrial Park (Taiwan EPA, 2017a, 2017b) (Figures S1b and S2). In order to establish the relationship between water and ambient temperatures, and to predict monthly water temperatures, between 1997 and 2017 in Taixi Township, daily ambient temperatures derived from an air quality monitoring site were obtained from the Environment Resource Database of the Environmental Protection Administration (Taiwan EPA, 2017b).

2.3 | Dose–response modelling

The Hill model, which has been used in pharmacology when the relationship between drug and drug effect is non-linear and saturable, can be applied to describe the relationship between the amount of pathogen and the probability of infection, illness or death (Brouwer, Weir, Eisenberg, Meza, & Eisenberg, 2017; Goutelle et al., 2008). Accordingly, the BV exposure concentration–cumulative mortality profiles were constructed by fitting a three-parameter Hill model to published data from infection experiments (Chou et al., 1994) (Figure S1b). The profiles were constructed to mechanistically quantify cumulative mortalities corresponding to specific viral titres. The relationship between BV exposure concentration and cumulative mortality can be expressed as Equation 1 (Table 1), where $L(C)$ is the cumulative mortality to a specific exposure concentration, C (TCID50 ml^{-1} or TCID50 ml^{-1} $clam^{-1}$) is the concentration of BV solution, L_{max} is the maximum cumulative mortality posed by BV infection, $LC50$ (TCID50 ml^{-1} or TCID50 ml^{-1} $clam^{-1}$) is the concentration causing 50% maximum cumulative mortality, and n is the fitted Hill coefficient in that $n = 1$ represents the Michaelis–Menten model, and $n > 1$ indicates that hard clams are ultrasensitive to pathogen toxicities.

2.4 | Susceptible–infectious–mortality (SIM) model

Based on the natural history of diseases, the process, which begins in a susceptible host, enters the subclinical stage, at which point, if the pathogens trigger onset, signs of disease will be seen. Finally, the infected individual will either recover, suffer disability or die (CDC, 2012). Since the experimental hard clams (susceptible) were supposedly mainly infected with BV through horizontal transmission (immersion), it was assumed that, being BV-infected, they would die (Chou et al., 1994). Therefore, a deterministic SIM model was employed to describe the population dynamics of BV-induced disease transmission in hard clam populations.

TABLE 1 Equations for concentration–response, susceptible–infectious–mortality (SIM), epidemiological and disease control models used in this study (see text for the symbol meanings)

Equations	
Concentration–response model	
$L(C) = \frac{L_{max} C^n}{LC50^n + C^n}$	(1)
Susceptible–infectious–mortality (SIM) model	
$\frac{dS}{dt} = -\beta SI$	(2)
$\frac{dI}{dt} = \beta SI - \alpha I$	(3)
$\frac{dM}{dt} = \alpha I$	(4)
Epidemiological model	
$R_0 = \frac{\beta}{\alpha}$	(5)
Disease control models	
R_C-control model	
$R_C = \frac{\beta(T)}{\alpha(T)} \times (1-q)$	(6)
Removal-control model	
$\frac{dS}{dt} = -\beta SI - (1-p)\gamma S$	(7)
$\frac{dI}{dt} = \beta SI - \alpha I - p\gamma I$	(8)
$\frac{dM}{dt} = \alpha I$	(9)
$\frac{dR}{dt} = (1-p)\gamma S + p\gamma I$	(10)

The population dynamics can then be divided into three compartments and described as Equations 2–4 (Table 1 and Figure S1b) where S, I and M, respectively, represent populations of hard clams in susceptible, infectious and mortality states, β is transmission rate (day^{-1}), and α is mortality rate (day^{-1}). In a closed population, the total number of individuals (N) would be equal to the summation of S, I and M. Because population dynamics are simulated in unit fractions, N equals 1.

The basic reproduction number (R_0), which is defined as the mean number of secondary cases generated by a primary case during an entire infectious period in an entirely susceptible population, may be estimated as Equation 5 (Anderson & May, 1991; Liao et al., 2006) (Table 1). Thus, R_0 is a measure of the potential for disease outbreaks and $R_0 < 1$ indicates that disease will disappear over time, whereas $R_0 > 1$ means there will be an epidemic.

To project the monthly cumulative mortality and R_0 of hard clams infected with BV in farms, the temperature-dependent cumulative mortality, β and α are constructed by fitting the regression models to their estimations at different temperatures. As a result, R_0 can be probabilistically determined in different temperatures by estimating the epidemiological parameters of temperature-dependent β and α with fitting.

2.5 | Disease control models

In order to provide containment strategies for the prevention and control of BV disease outbreaks in hard clam farm ponds, we proposed two control models based on the transmission pattern of BV, so as to break the chain of infections in populations, and to overcome impediments in the limited experimental data.

Due to the observed effects of temperature variations on both epidemics of BV disease and cumulative mortalities of BV-infected hard clams, we removed susceptible clams—along the lines of the containment strategy of quarantine—in order to contain disease outbreaks in differing culturing temperatures. Therefore, based on Equation 6 (Table 1) where q is the proportion of susceptible clams removed, a contour plot of control reproduction numbers (R_C) can be derived to provide a theoretical control measure for BV disease transmission in clam farms (Lipsitch et al., 2003). The R_C-control model may be implemented by simply applying cumulative mortality data. Thus, BV disease can be effectively controlled if R_C values are located in the region of $R_C < 1$.

Alternatively, in order to break the chain of virus transmission and contain a BV disease outbreak more effectively, we designed a more practical control strategy by adding a removal compartment (R) to the SIM model—that is the removal-control model—as expressed in Equations 7–10 (Figure 1 and Table 1) where γ was the removal rate and p the probability of infected population removed. Based on this model, the cumulative mortalities of BV-infected hard clams subjected to different culture water temperatures and lasting days of removal (D_L) at a certain removal rate γ can be predicted.

2.6 | Model calibration: cumulative mortalities of BV-infected hard clams

We applied Berkeley Madonna 8.0.1—developed by Robert Macey and George Oster of the University of California at Berkeley—to estimate epidemiological parameters of α and β by fitting the SIM model to the cumulative mortality data, as described in Equations 2–4. Hence, we adopted normalized root mean square error as $\mathrm{NRMSE}\,(\%) = \sqrt{\frac{\sum_{i=1}^{N}(P_i - O_i)^2}{N}} \times \frac{100\%}{\overline{O}}$ in order to assess performance of the SIM model and validate the simulation with the published data, where N denotes the number of observations, O_i is experimental data, P_i is the modelled result, and $\overline{O}$ is the mean of experimental data. NRMSE < 10% indicates that the simulation is excellent, >10% and <20% is good, >20% and <30% is fair, whereas >30% is poor (Martins et al., 2018).

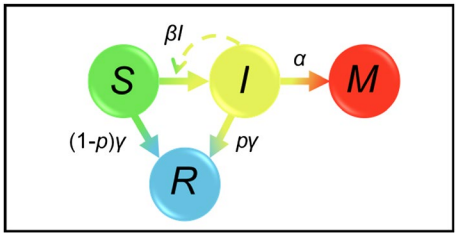

FIGURE 1 Schematic representing disease control strategy by adding a removal compartment (R) to susceptible–infectious–mortality (SIM) model [Colour figure can be viewed at wileyonlinelibrary.com]

2.7 | Uncertainty analysis

We employed TableCurve 2D package (version 5.01; AISN software) to perform model fitting and obtain the optimal statistic models. A Monte Carlo (MC) technique was performed with 10,000 iterations to obtain 2.5%- and 97.5%-tile and mean of R_0 estimates. The Crystal Ball software (version 2000.2; Decisioneering, Inc.) was used to implement the MC simulation.

3 | RESULTS

3.1 | Cumulative mortality

The cumulative mortality–BV dose (TCID50 ml^{-1} or TCID50 ml^{-1} $clam^{-1}$) relationship via exposure routes of immersion or injection is well described by a Hill-based dose–response profile (Figure 2a,b). The estimated *LC*50 of BV through immersion was 12,247 TCID50 ml^{-1} with n of 0.62 ($r^2 = .99$, $p < .001$), implying that BV could infect and cause mortalities of 50% of farmed clams via a waterborne route (horizontal transmission) (Figure 2a and Table S3). On the other hand, the *LC*50 estimate of BV via injection route was 3,809.8 TCID50 ml^{-1} $clam^{-1}$ with n of 0.35 ($r^2 = .99$, $p < .001$) (Figure 2b and Table S3). For the cumulative mortalities of hard clams posed by acute temperature changes post-horizontal transmission of BV disease through immersion, the SIM model-predicted cumulative mortality profiles fitted well to the published cumulative mortality data of hard clams posed by temperatures changing from 25 to 15°C, at 25°C, and from 25 to 33°C subsequent to 10^5 TCID50 ml^{-1} BV immersion, resulting in NRMSEs of 28.63, 17.60 and 9.32%, respectively (Figure 2c–e and Table S5).

3.2 | Temperature-dependent BV disease dynamics

The temperature-dependent epidemiological parameters of α, β and R_0 for hard clams infected with 10^5 TCID50 ml^{-1} were estimated by fitting a deterministic SIM model to the cumulative mortality data (Table S4). Results showed that both estimates of α and β were lowest and highest, respectively, among three kinds of treatments of acute temperature changes when culture temperature changed from 25 to 33°C post-virus immersion (Figure 3a,b and Table S5). Also, it was observed that cumulative mortalities of clams were below 0.5 at water temperatures from 15–31.72°C (Figure 3c and Table S6). However, there was an abrupt increase of cumulative mortality when the cultured temperature was higher than 31.72°C, suggesting that the cultured temperatures should be at least <31.72°C to prevent half of clams from mortality (Figure 3c and Table S6). Moreover, the R_0 values estimated probabilistically in three kinds

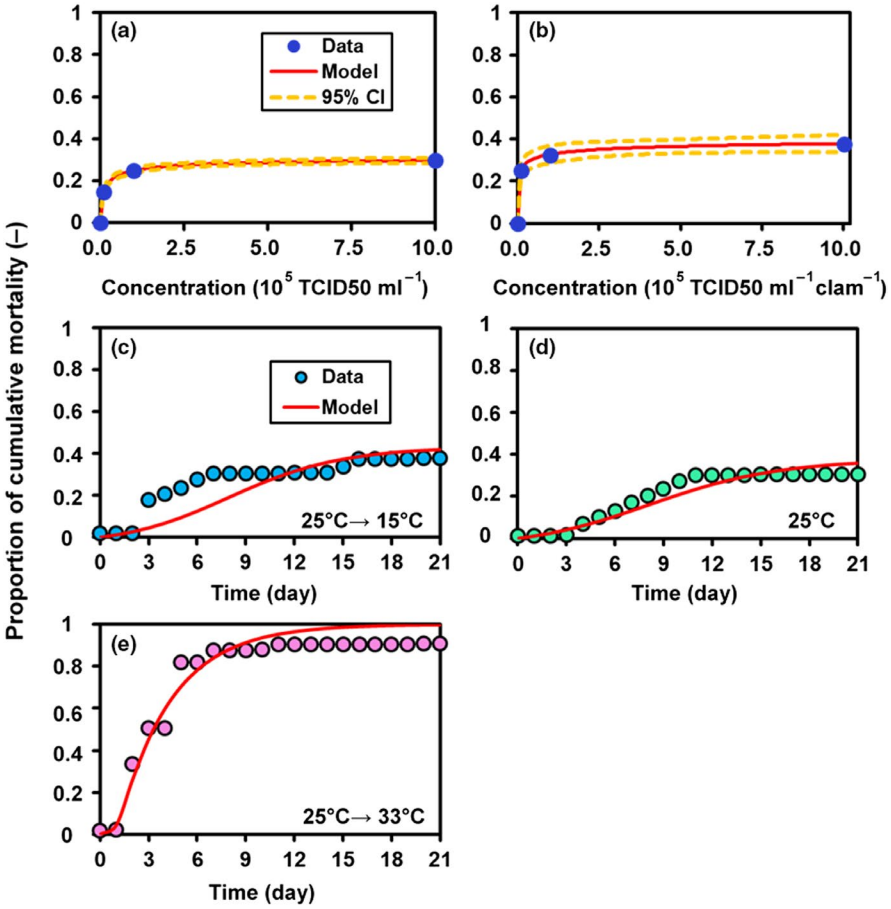

FIGURE 2 Proportion of cumulative mortality of hard clams infected with BV via (a) immersion at age of 4-month-old (n = 30) and (b) injection at age of 1-year-old (n = 20) at water temperature of 25°C. Fitted cumulative mortalities of hard clams subjected to water temperature (c) changed from 25 to 15°C, (d) at 25°C, and (e) changed from 25 to 33°C after virus infection [Colour figure can be viewed at wileyonlinelibrary.com]

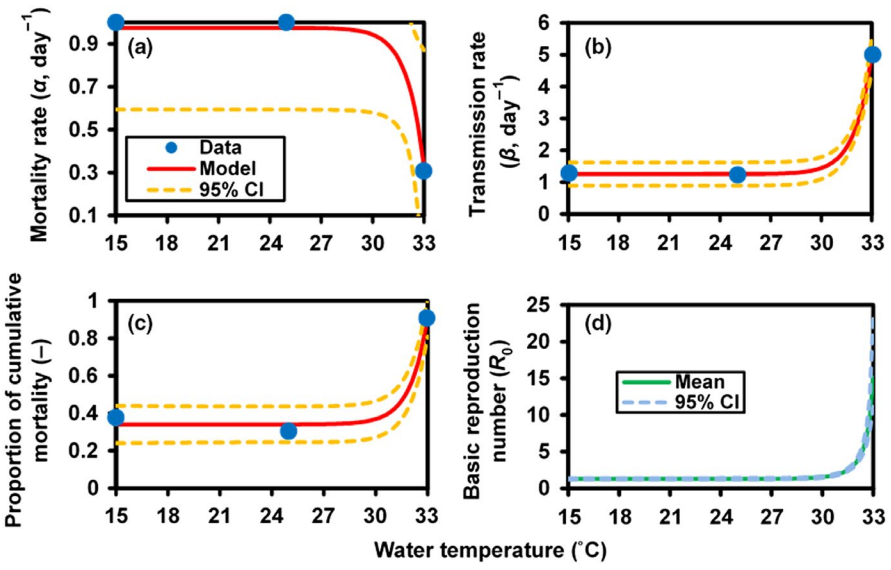

FIGURE 3 Temperature-dependent (a) mortality rate (α, day^{-1}), (b) transmission rate (β, day^{-1}), and (c) proportion of cumulative mortality in hard clams. (d) Estimation of temperature-dependent basic reproduction number (R_0) based on α and β estimations with uncertainties [Colour figure can be viewed at wileyonlinelibrary.com]

of temperature variations were highest when culture temperature changed from 25 to 33°C, followed by 25–15°C and at 25°C with estimates of 16.25 (95% CI: 11.13–23.36), 1.29 (1.20–1.39) and 1.29 (1.20–1.39) (Figure 3d), respectively, implicating that there were BV disease epidemics in any kinds of culture temperatures (R_0 > 1) with the occurrence of highest R_0 when hard clams were transferred from 25 to 33°C (Figure 3d and Table S5).

We estimated mean, 2.5% and 97.5% tile of water temperatures in farm ponds based on the collated average values of monthly ambient temperatures in Taixi Township by adopting the constructed relationship between ambient and water temperatures (Figure 4a, Figures S1c and S3). Results showed that there is an upward trend of average water temperatures during outbreak seasons of June, July and August with estimates of 28.9 (26.5–31.3), 29.9 (27.5–32.4) and

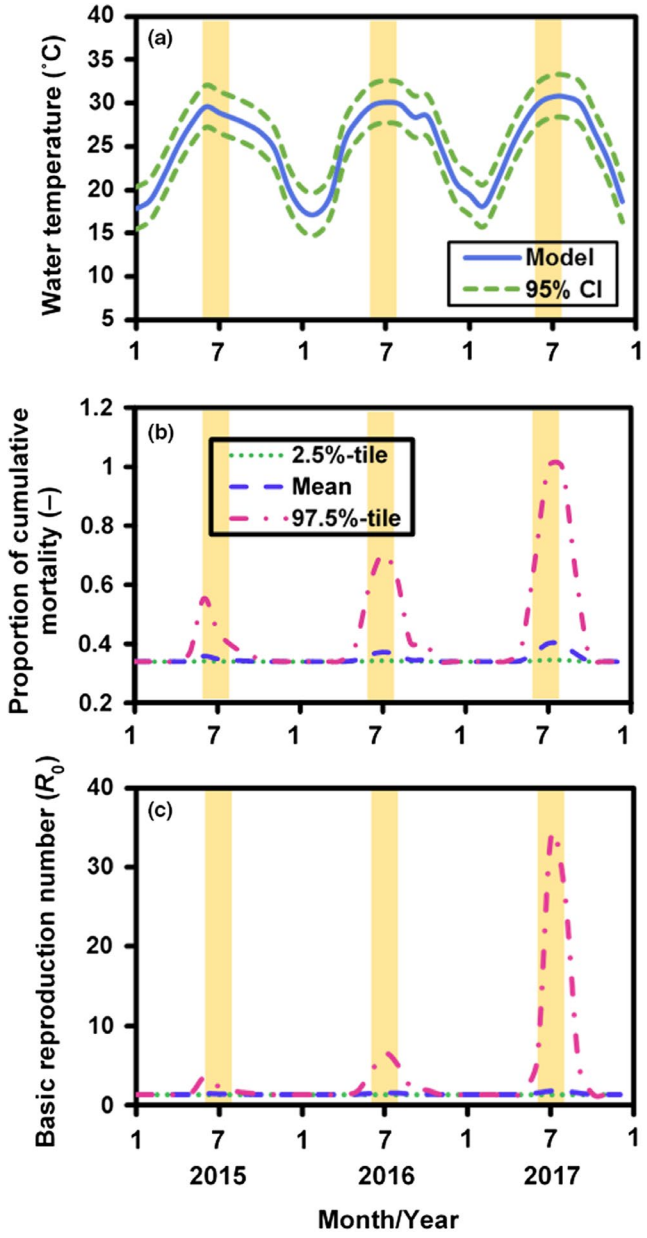

FIGURE 4 Estimation of (a) water temperature, (b) proportion of cumulative mortality, and (c) R_0 in clam farms in the period 2015–2017 based on monthly ambient temperature in Taixi Township. Yellow highlights indicate outbreak seasons of June–August [Colour figure can be viewed at wileyonlinelibrary.com]

30.5 (28.1–32.9)°C, respectively, in 2015, 2016 and 2017, respectively (Figure 4a).

The time-dependent cumulative mortality and R_0 were estimated by applying the constructed temperature-cumulative mortality and temperature-α and β profiles in Figure 3a,b,c and Table S6 (Figure 4b,c). It was found that cumulative mortalities estimated based on mean and 2.5% and 97.5% tile of water temperatures during outbreak seasons ranged from 0.34–0.46, 0.34–0.64 and 0.34–0.89 in 2015, 2016 and 2017, respectively (Figure 4b). In addition, estimated R_0 during outbreak seasons time-dependently increased with estimates ranging from 1.30–2.56, 1.31–5.19 and 1.33–22.04 in 2015, 2016 and 2017, respectively (Figure 4c).

In order to rigorously explore the BV disease transmission dynamics during outbreak seasons between 1997 and 2017, we estimated yearly ambient temperatures from June to August by applying the regression models (Figure 5a,d,g,j and Figure S1c and Table S7). Subsequently, both cumulative mortalities and R_0 between 1997 and 2017 were estimated by adopting the predicted values of water temperatures (Figure 5b,c,e,f,h,i,k,l). Results revealed that the average ambient temperatures during the outbreak seasons in Taixi Township were continuously increased between 1997 and 2017 with documented and estimated values of 27.95 and 29.65°C in 1997 and 2017, respectively (Figure 5a,d,g,j). However, cumulative mortalities during outbreak seasons evidently increased from 1997 to 2017 with estimations of 0.44 and 0.88 in 1997 and 2017, respectively (Figure 5b,e,h,k). Moreover, it was observed that a time-dependent increment and a sudden rise of R_0 during outbreak seasons from 1997 to 2017 and 2017, respectively. The R_0 estimates were 2.17 and 13.2 in 1997 and 2017, respectively (Figure 5c,f,i,l).

3.3 | BV disease control strategies

The contour plot illustrates a dependence of R_C on proportion of susceptible removed (q) and water temperatures (Figure 6). Results showed that the proportion of susceptible hard clams removed should be maintained at 0.22 to contain BV disease outbreak when temperatures ranged from 15 to 26.8°C (Figure 6). Moreover, the estimated proportions of clams removed should be at least 0.3, 0.4, 0.5 and 0.9 when water temperatures rise to 29.4, 30.4, 31 and 32.8°C, respectively, to achieve disease containment (R_C = 1) (Figure 6).

On the other hand, population dynamics can be predicted by the removal-control model with different removal rates (γ) and length of days to remove hard clams (D_L) at p = .5 (Figure 7 and Figure S4). The alternative removal-control strategy showed that the cumulative mortality could decrease from 0.42 at D_L = 0 to 0.15 at D_L = 7 days in the condition of γ = 0.1 when the water temperature was at 25°C (Figure 7a). However, the higher the water temperature, the less effective was the removal strategy for the containment of BV disease transmission among clam populations, with the cumulative mortalities only decreasing from 0.99 at D_L = 0 to 0.88 at D_L = 7 days when the water temperature was at 33°C (Figure 7a). Moreover, in the condition of γ = 0.5, the cumulative mortality was found to decrease from 0.42 at D_L = 0 to 0.04 at D_L = 7 days when the water temperature was at 25°C (Figure 7b). Similarly, at the higher water temperature of 33°C, the cumulative mortality was estimated to decrease from 0.99 at D_L = 0 to 0.58 at D_L = 7 days when γ = 0.5 (Figure 7b).

4 | DISCUSSION

Among all kinds of approaches for epidemiology to solve animal health problems—for example, biostatistics, animal health economics and risk analyses—mathematical modelling is the best way both to reveal the

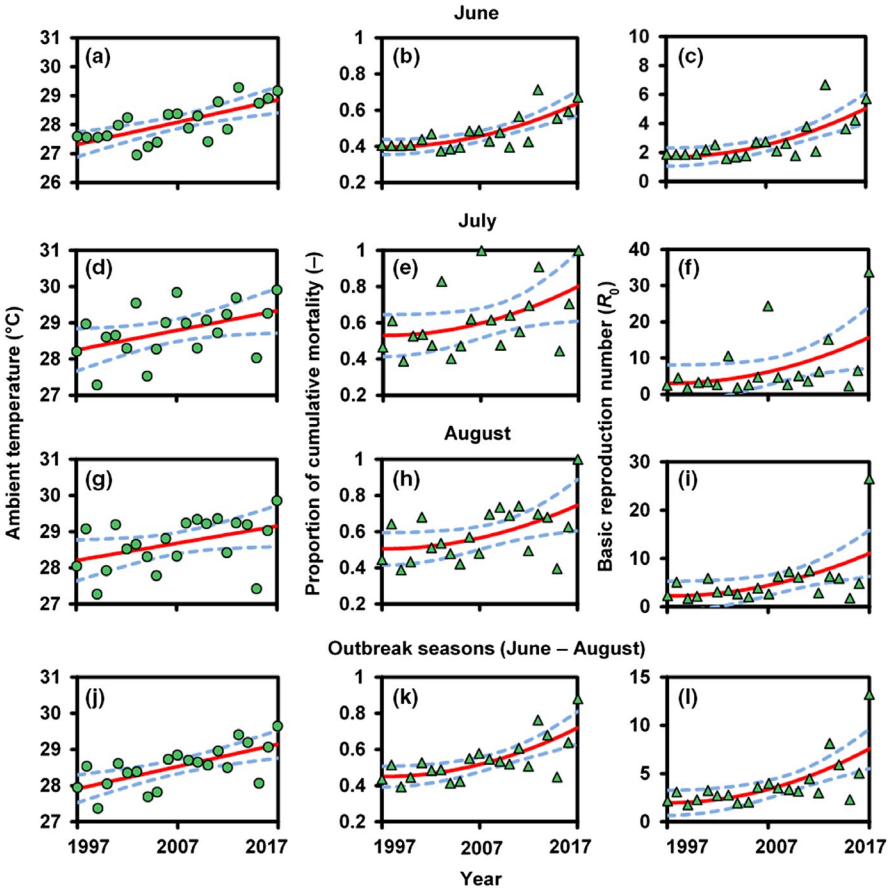

FIGURE 5 Regression of yearly (a, d, g, j) ambient temperature, (b, e, h, k) proportion of cumulative mortality, and (c, f, i, l) R_0 in hard clam population during June, July, August, and outbreak seasons (June–August) in the period 1997–2017. ●: measured data, ▲: predicted data, ——: model and - - -: 95% CI [Colour figure can be viewed at wileyonlinelibrary.com]

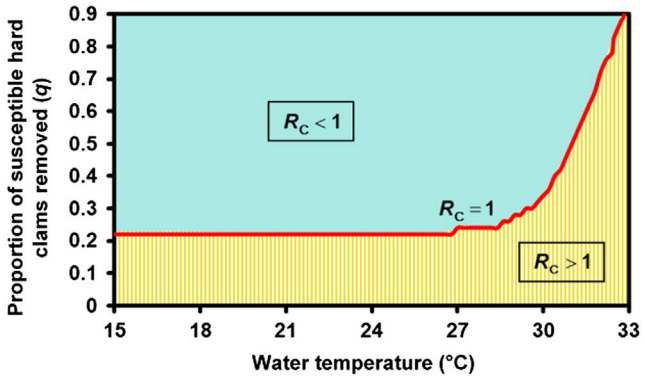

FIGURE 6 Contour plot of proportion of susceptible population removed and water temperature (°C)-dependent control lines of the control reproduction number (R_C) in that $R_C = \frac{\beta(T)}{\alpha(T)} \times (1-q)$ where β is transmission rate, α is mortality rate, T is water temperature, and q is proportion of susceptible hard clams removed [Colour figure can be viewed at wileyonlinelibrary.com]

processes that drive disease emergence and to assess the effectiveness of disease management policies (Peeler & Taylor, 2011; Powell & Hofmann, 2015). Powell and Hofmann (2015) divided the transmission models of molluscan diseases into three categories: (a) examining the effects of environmental changes on host population, (b) understanding the mechanisms triggering epidemics and (c) evaluating the influence of natural selection or genetic modification in disease development.

Suitable epidemic models can describe the dynamics of several diseases, provide insights into potential intervention strategies and help to make management decisions; also, they can help to assess both the consequences of disease and assist with planning and budgeting for its future control (Green, 2010; Murray, 2008). Similarly, since diseases can spread through waterborne pathogens, Bidegain, Powell, Klink, Ben-Horin, and Hofmann (2016) proposed disease dynamics models based on the containment of infectious particles in marine systems. Reno (1998) also adopted SIR-type modelling to simulate disease transmission in aquatic ecosystems, while a mechanistic approach has been successfully applied to predict the spread of viral diseases, such as an infectious pancreatic necrosis (IPN) in salmon farms, and herpesvirus in pilchards (Murray, 2006, 2009; Murray, O'Callaghan, & Jones, 2001; Ruane, Murray, Geoghegan, & Raynard, 2009).

For this study, we applied the SIM model to optimally simulate BV disease transmission in hard clams based on available information regarding BV disease-induced mortality. However, in the simple density-dependent model, reducing the susceptible population S was shown to be effective (Murray et al., 2001). We therefore developed the susceptible–infected–removed model, which shows how BV disease responds to interventions such as the reduction of transmission or increment of removal rate of infected or moribund clams. Modelling viral load-dependent cumulative mortalities of hard clams with the Hill-based model could also assist as a mechanistic tool to

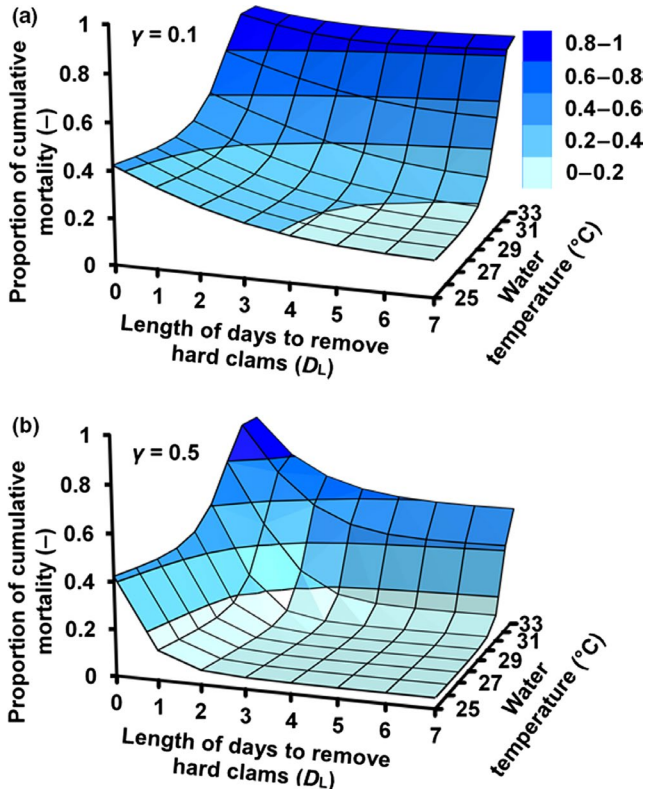

FIGURE 7 Surface plots showing cumulative mortality with respect to water temperature and length of days to remove hard clams (D_L) in scenarios of removal rates γs of (a) 0.1 and (b) 0.5, respectively, at probability of removing infectious population p = .5 [Colour figure can be viewed at wileyonlinelibrary.com]

predict cumulative mortalities of various clam farms when in situ viral titres of BV could be examined.

Water temperature is an environmental stressor that has significant effects on susceptibility of host populations and the virulence of viruses (Chou et al., 1994; Morley, 2010). Weber, Sturmer, Hoover, and Baker (2013) found that it both directly influences metabolisms of ectothermic organisms, such as hard clams, and that extreme temperatures can affect growth and reproduction, leading to poor health or death. We found that there is an observable time-dependent increase of ambient temperatures in the Taixi Township, in that the average temperature during the disease outbreak season of June to August increased from 27.95 to 29.65°C. Accordingly, based on model simulations, we estimated a significant increment of basic reproductive numbers of BV disease—from 2.17 to 13.2. With regard to the recorded temperature data sets, we also saw evidence to suggest that the proportion of cumulative mortalities surged from 0.44 to 0.88, demonstrating the significant impact of temperature increases on survival rates.

Moreover, Chen and Ho (2003) observed that the optimal rearing temperatures for hard clams in small (4.31 ± 0.27 mm) (mean ± SD), medium (11.86 ± 0.65 mm) and large sizes (20.63 ± 0.53 mm) were 25, 20 and 15–25°C with survival rates of 80.0%, 96.1% and 97.3%, respectively. However, when small clams were cultured at 30, 25 and 15°C, the average survival rates decreased to 18.6%, 80.0% and 77.0%, respectively, emphasizing the importance of temperature variations on survival (Chen & Ho, 2003). Consistent with the results of epidemiological modelling, we also demonstrated that temperature elevated from 25 to 33°C had the greatest mortality impact and the highest transmission rate and reproductive number of BV disease among the three infection scenarios. Hseu et al. (2017) also reported that hard clam mortality increases when short-term high temperatures are >40°C.

Morley (2010) also considered thermal pollution to be a stressor affecting molluscs during viral epidemics, while Chou et al. (1994) found water temperature to have a virulent effect on hard clams and McCowan et al. (2015) found that aquatic animals generally, and young organisms particularly, that had been infected with BV, died mostly during periods of high water temperatures. Notably, a study by Ho (2001) revealed that water temperature fluctuation, rather than temperature elevation, was more likely to contribute to higher mortality rates of hard clams. Finally, Dahl et al. (2011) found temperature to be a major influence on the development of infectious diseases in bivalve species. Dahl et al. (2011) found that temperature had a significant effect on the dynamics of the quahog parasite unknown (QPX) disease which led to higher mortality in the hard clam *Mercenaria mercenaria* population cultured at 13°C rather than at 21 and 27°C.

Raimondi, Wilson, Ambrose, Engle, and Minchinton (2002) reported that mass mortalities of the black abalone *Haliotis cracherodii*, caused by pathogens, were found to coincide with periods of warmer sea temperatures and El Niño Southern Oscillation (ENSO) events, while Fischlin et al. (2007) stated that an increase of 2–3°C above preindustrial temperature levels was expected to place between 20% and 30% of animal species at high risk of extinction. They also predicted major changes to the structures and functions of marine and other aquatic ecosystems. Other factors causing mass mortalities of hard clams in the ponds of southwest Taiwan have been documented, albeit in limited literature (Hsu, Liu, Chang, & Hsieh, 2016).

Because hard clams possess non-specific immune systems, including cell and humoral immunities, therefore during their breeding season, between June and September, they are at their most susceptible to infectious diseases (Bachére et al., 1995; Liu & Mai, 2003; Söderhäll & Cerenius, 1998; Wu et al., 2016). Similarly, higher nutrient resulting from overpopulated microalgae and competition from benthic communities in farm ponds also weaken immune systems, leading to high mortality levels (Ho, 2001). Hseu et al. (2017) reported temperature-dependent increments of cell immunity biomarkers, such as the total hemocyte count (THC), protein and glucose concentrations, and acid phosphatase activity in the haemolymph, implying that, in culture environments, the immunity responses of hard clams are closely associated with temperature variations.

Temperature control in relation to viral diseases is vital not only because of the effectiveness of a host's immune response, but also because of the ability of viruses to replicate themselves (Goodwin & Merry, 2011). Lo et al. (1988) demonstrated that the optimal temperatures for the multiplication of BV when isolated from hard clams are between 20 and 30°C, and they also reported that the optimum growth temperature for marine birnavirus (MABV), such as the yellowtail ascites virus (YAV) found in Japanese amberjack (*Seriola*

quinqueradiata), was 20°C, yet no occurrences of growth at 25°C were observed, either in vivo or in vitro (Sorimachi & Hara, 1985). Kitamura, Jung, and Suzuki (2000) observed that mass mortality of Japanese pearl oyster *Pinctada fucata* occurred in summer, whereas there was no isolation of MABV between June to September, although it increased after September. Similarly, Farley et al. (1972) found elevated levels of a herpes-type virus in oysters from thermally heated effluent water of a power station. Infectious pancreatic necrosis virus (IPNV), as another pathogen of *M. lusoria*, can survive for 10 days at 4°C in stream water, but it failed to replicate at 28°C (Roberts & Dobos, 1983; Tu, Spendlove, & Goede, 1975). Also, blue mussels (*Mytilus galloprovincialis*), which were positive for the aquabirnavirus (ABV), had detection rates of between 80% and 100%, particularly in July and August (Kitamura et al., 2007).

Farming management and culture conditions are closely associated with mass mortalities of hard clams in southwest Taiwan (Chen, 2003; Ho, 2001; Huang, Huang, Kuo, & Ding, 2004; Wang, Ou, Chiu, Hsu, & Hsieh, 2016; Weber et al., 2013). Contributing factors included farming density, quality of fingerlings, repeated use of virus-contaminated water and temperature change-associated weakened immunity of clams. Therefore, a pragmatic strategy aimed at effectively containing BV transmissions would be the prudent administration and measurements of water temperatures in ponds.

In the view of biosecurity, stocking equipment should be disinfected before clam farming. Reared clam larvae should be hatched from virus-free sperms and eggs. Filtration and irradiation of inflow water with ultraviolet (UV) light could reduce the probability of introducing virus to reared clams (Chu et al., 2019; Munang'andu et al., 2016a; Munang'andu, Mutoloki, & Evensen, 2016b). Implementation of biosecurity control measures and recording surveillance data during disease outbreaks are of great importance in preventing ABV transmission (Munang'andu et al., 2016a, 2016b). Thus, the utilization of sensors to inform farmers and automatically trigger temperature adjustments when they reached abnormal limits would constitute an effective real-time monitoring and controlling system (Dzulqornain, Al Rasyid, & Sukaridhoto, 2018). Hence, the Taiwan Fisheries Research Institute advocates a smart aquaculture system to replace traditional methodologies (Lin & Chang, 2017). Therefore, our transmission dynamics model could be incorporated with "cloud" systems and big data architecture in order to evaluate temperature change-associated disease dynamics and our proposed control strategies to combat BV disease outbreaks.

Overall, the current aquaculture technology, enhancing water exchange rate is the most common method for decreasing or retarding water temperature elevation in extreme weather. It can also reduce the pathogen particle concentration in farming water (Chen, 2003). In the recent field experience, part of farm water surface covered with solar panels can not only significantly retard water temperature elevation but also provide extra-electric power for farming equipment (Chu et al., 2019). However, based on our study results, it is useless to reduce R_C by less than 1 simply by regulating water temperatures. We have also shown that when temperatures are abnormally high during BV outbreak seasons—say 33°C—the removal rates of clams should be adjusted from 0.1 to 0.5. Therefore, to effect containment, their removal from infected ponds should be incorporated alongside temperature control. In addition, reducing stocking density can also decrease temperature stress by increasing the availability of food and oxygen for individual clams (Weber et al., 2013).

Culling susceptible hosts to prevent abalone population density from reaching the threshold of disease has proved successful (Culver & Kuris, 2000), similarly Pernet, Lupo, Bacher, and Whittington (2016) revealed that culling of infected individuals is a possible method of eradication; however, he found that to detect infected molluscs is neither feasible nor economic, which means that the restriction of movements of live animals and confinement rearing are possible ways to prevent disease transmissions. For clam farms, there is lacking technique to immediately distinguish infected clams from health individuals (Chu et al., 2019). Liao et al. (2008) demonstrated that using proper predator to remove certain part of infected population in farming pool was helpful to retard epidemic situation. Introducing economic-valued fish predator, for example blackhead seabream (*Acanthopagrus schlegelii*), might be helpful in easing epidemic situation and add some economic profit (Ho, 2001).

Although a number of prophylactic approaches have been developed for reducing losses from infectious diseases, conventional methods, such as disinfection and chemotherapeutants, with particular emphasis on the use of antibiotics, are ineffective for viral diseases (Subasinghe, 2009). Advanced applications, including herbal immunostimulants and vaccination, have also been employed to alleviate disease transmissions (Park, 2009). Similarly, Citarasu (2010) demonstrated that herbal extracts have the potential to inhibit, or block, the transcription of viruses in host cells and to boost their innate immunity. Modak, Sandino, Arata, Cárdenas-Jirón, and Torres (2010) found the ester filifolinyl senecionate to be a good antiviral therapy for IPN caused by BV, suggesting a possible strategy for controlling viral diseases in molluscs experiencing temperature variation. Moreover, given the unavailability of BV vaccines for aquaculture invertebrates, epidemiological approaches to eradicate farm pond diseases are crucial for improving health management (Munang'andu et al., 2016a; Renault & Novoa, 2004; Subasinghe, 2005).

Due to the limited information in record of water quality in Taiwan aquaculture, a limitation of this study has been a lack of water temperature data; however, this could be resolved by using a simple regression model to convert ambient temperatures to water temperatures, since several studies have investigated the relationship between air and water temperatures in aquatic systems as an alternative approach (Livingstone & Lotter, 1998; Mackey & Berrie, 1991). Therefore, the epidemiological parameters estimated in this study can be improved by adopting benthic water temperatures approximated to the habitats of hard clams.

Johnson (1984) considered stress, caused by a combination of temperature changes, heavy metals and ABV infections, to be a major factor in viral disease outbreaks among marine invertebrates, leading to mortalities in fish and bivalves. Thus, the mass mortalities of hard clams may be partly explained by such multiple stressors during cultivation (Chou et al., 1998, 1994; Chou,

Peng, Chang, Hsu, & Wu, 1999). Hence, we incorporated "variable temperature" as a key stressor into the modelling of BV disease dynamics in order to simulate cumulative mortality with a removal compartment in clam populations. Although this simulation provided useful information for the reduction of hard clam losses due to BV, the transmission model schema needs to be modified by including more compartmental systems appraised with more field and experimental data in order to facilitate containment of BV disease and exposures to multiple stressors.

This study used a mechanistic approach, based on experimental results and field data, in order to provide insights into BV disease dynamics and hard clam mortalities posed by temperature variations. The parsimonious framework model, alongside epidemiological concepts, could readily be adapted to in situ farm ponds in order to mitigate mass mortalities of hard clams due to changes of pond temperatures. Most importantly, the developed disease containment strategies, based on this model framework, should be effectively and efficiently coordinated, together with the current administration, to mitigate annual mass mortalities in farmed hard clam populations. We conclude, therefore, that the constructed model scheme could greatly benefit the sustainability and economics of farm ponds, given the limitation in vaccine development and other effective strategies. Furthermore, we suggest that the SIM modelling framework for predicting population dynamics and key epidemiological parameters, based on cumulative mortality data and field temperatures, might be widely adopted in order to prevent mass mortalities due to BV disease outbreaks and undesirable environmental stressors.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

1. Cumulative mortality data of BV-exposed hard clams were adopted from Chou et al. (1994).
2. Water temperatures between 2008 and 2016, as collated from an environmental monitoring report based on Yunlin Offshore Industrial Park (Taiwan EPA, 2017a, 2017b).

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SUPPORTING INFORMATION

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

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