Effectively control of viral disease outbreak in shrimp culture system by selective predation

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Abstract:

Due to the limited understanding of the characteristics of predator-pathogen-prey interactions, few attempts to use selective predation for controlling diseases in prey populations have been successful. The global pandemic of white spot syndrome (WSS), caused by white spot syndrome virus (WSSV), causes devastating economic losses in farmed shrimp production. Currently, there is no effective control for WSS. Here, we determined the transmission dynamics of WSSV and the feeding ability and selectivity of fish on healthy, infected and dead shrimp by experiments and mathematical modeling. Accordingly, we developed a novel and convenient shrimp cultural ecosystem, which that effectively prevented WSS outbreaks, by introducing aquaculture fish species. This provides a scheme for developing control strategies for viral diseases with high transmission rate.

35 Predators prefer to eat infected preys in nature, and it is predicted that selective
36 predation on infected individuals can reduce the prevalence of diseases in prey
37 population (*1, 2*). The effectiveness of selective predation on disease prevention is
38 determined by the interplay of several factors (*3, 4*). Without a comprehensive
39 understanding of characters of predator, pathogen and prey, there are few successful
40 attempts of using selective predation to control disease in prey population, especially
41 diseases with high transmission rate (*5-7*).

42

43 Aquaculture is the third largest source of animal protein that accounts for 17% protein
44 consumed by the global population, and shrimps are the global aquaculture species (*8*).
45 However, white spot syndrome (WSS), caused by white spot syndrome virus (WSSV),
46 led to huge economic losses to the global shrimp aquaculture industry (*9*). Currently,
47 there is no effective prevention and control strategies for WSS, though several methods
48 have been tested in farmed shrimp system (*10-13*). WSS pandemic mainly occurs
49 depending on the sequential transmission of WSSV from healthy shrimps of consuming
50 WSSV-infected shrimps to other healthy shrimps (*14, 15*), lacking a predator to remove
51 the infected individuals from the high density single-species culture system (**fig. S1**).
52 The prevalence of diseases in prey population is positively correlated with disease
53 transmission rate, while negatively correlated with predation pressure and predator
54 selectivity (*16, 17*). Thus, to develop a shrimp culture system which controls WSSV
55 outbreak by selective predation, we studied WSSV transmission dynamic in the Pacific

56 white shrimp (*Litopenaeus vanmamei*) population and elucidated the feeding ability
57 and selectivity of diverse species of aquaculture fishes on shrimps.

58

59 To unravel the transmission dynamic of WSS, we carried out the studies on the
60 relationship among the body weight of one initial WSSV-infected shrimp, number of
61 deaths, and death time distribution. One piece of WSSV-infected dead shrimp infected
62 several healthy shrimps with the same bodyweight via ingestion. The infected number
63 of shrimps in groups of 1.98g, 6.13g, and 7.95g of average body weight were 57.3, 64.7
64 and 71.3, respectively. (**fig S2 and table S1**). This suggests the basic reproduction
65 number (R_0) of WSSV increases with the body weight of WSSV-infected dead shrimps.
66 Time to death was consistent across the three groups of body weight for WSSV-infected
67 shrimps, with the majority on the third to sixth day and the peak number of deaths on
68 the fourth and fifth days (**Figs. 1A and S3, and tables S2 to S4**). A mathematical model
69 (Model 1) was developed to describe the transmission dynamic of WSS (**Figs. 1A and**
70 **1B**, **supplementary text**). In addition, the changes of live and dead shrimp numbers
71 during WSSV transmission were determined by artificial infection experiments. The
72 number of live shrimps began to decrease after 2 days of WSSV infection, and
73 drastically decreased after 4 days of WSSV infection (**Fig. 1C**). The dynamic changes
74 of healthy, infected, and dead shrimps could be expressed by a mathematical model
75 (Model 2) (**figs. S4 and S5**, **supplementary text**). Model 2 predicts that it is possible
76 to cut off the transmission route of WSSV by removing infected and dead shrimps, but

77 the time window of prevention is about 2 days. Thus, the fish used as predator has to
78 ingest infected and dead shrimps promptly and continuously.

79

80 To identify the fish species for controlling WSSV transmission, we examined the
81 feeding ability and selectivity of diverse aquaculture fish species that ingest Pacific
82 white shrimps, including grass carp (*Ctenopharyngodon idella*), African sharptooth
83 catfish (*Clarias gariepinus*) (hereafter catfish), red drum (*Sciaenops ocellatus*), and
84 brown-marbled grouper (*Epinephelus fuscoguttatus*). The brown-marbled grouper did
85 not ingest dead shrimp continuously, and its daily dead shrimp ingestion rate is low
86 (**table S5**). The daily dead shrimp ingestion rates of grass carp, catfish and red drum is
87 8.26%, 4.99%, and 11.63% respectively (**tables S6 to S8**). The daily healthy shrimp
88 ingestion rate of grass carp, catfish and red drum is 2.09%, 1.01% and 6.04%
89 respectively, indicating the relatively high healthy shrimp ingestion rate of red drum
90 (**tables S9 to S11**). Grass carp and catfish have high feeding selectivity of dead shrimps
91 over infected and healthy shrimps (**Figs. 2A and S6, and tables S12 and S13**).
92 Additionally, grass carp and catfish move quickly and swallow infected and dead
93 shrimps completely. This suggests grass carp and catfish have high feeding selectivity
94 and ability, which can cut off the WSSV transmission route in which the healthy
95 shrimps ingest infected dead shrimps.

96

97 The capacity and specification of fish were determined for effective control of WSS.
98 Firstly, we identified the minimum body weight of co-cultured grass carp and catfish.
99 Four experimental ponds were set up, in which 600 healthy and 3 WSSV-infected
100 shrimps with body weight of 5 g were cultured with one grass carp of different body
101 weights. After 13 days in culture, the ponds co-cultured with one grass carps weighted
102 0.3 kg, 0.5 kg, 1 kg, 1.5 kg showed 0, 0, 82.4%, 79.4% of shrimp survival rate,
103 respectively (**Fig. 2B and table S14**). This indicates that the minimum body weight of
104 grass carp for effective control of WSS is 1 kg. Furthermore, experiments showed co-
105 culturing of catfish of body weight greater than 0.5 kg could effectively control the
106 WSS transmission in the shrimp population. (**fig. S7 and table S15**). Secondly, we
107 determined the capacity of grass carp for WSS prevention in shrimp populations of
108 different shrimp body weights based on experimental results. In a 10 m^2 pond with 750
109 shrimps, one 1 kg grass carp could control 70 pieces of 2.5 g WSSV-infected shrimps,
110 50 pieces of 5.0 g WSSV-infected shrimps, or 30 pieces of 7.8 g WSSV-infected
111 shrimps, which is consistent with the result of mathematical simulation (Model 3) (**Fig.
112 2C and table S16, supplementary text**). This suggests the capacity of grass carp for
113 control of WSS is negatively correlated with the body weight of shrimps. Thus,
114 releasing fishes in the early stages of shrimp production should improve their power for
115 controlling WSS. Lastly, the minimum stocking quantity of grass carp was determined
116 for effectively controlling WSS. We released 45-, 150-, 225-, 300-, 450-, 600- and 750-
117 grass-carps/hm^2, of bodyweight of 1 kg~1.25 kg in the 40 ponds (13.57 hm^2) at a
118 demonstration farm (**Figs. 2D and S8, and table S17**). When grass carp stocking

119 quantities were greater than or equal to 300-grass-carp/hm^2, the success rate of WSS
120 control was 100%.
121
122 We next translated the knowledge gleaned from experiments to an applied technology
123 scale. Firstly, the effectiveness of controlling WSSV transmission by co-culturing
124 shrimps with grass carp was tested in two experimental zones at a farm in Maoming,
125 Guangdong province, China (Farm 1) in 2011 (**Fig. 3A**). In the 18 ponds (6.03hm^2) of
126 zone A, we cultured 9x10^5/hm^2 of shrimps for 20 days, and then introduced 317–
127 450/hm^2 of grass carps with average body weight of 1 kg. We did not observe the WSS
128 outbreak in 17 ponds and harvested with 7,332 ± 2,059 kg/hm^2 in 110 days of culture
129 (**Fig. 3B and table S18**). One pond was unsuccessful due to pathogenic bacterium
130 (vibrio) infection. Shrimps were cultured without grass carps in 28 ponds (11.30hm^2)
131 of zone B. A total of 20 ponds in zone B had WSS outbreak, resulting in an average
132 yield of 1,844 ± 2,304 kg/hm^2. In 2012, we switched zones A and B, cultured shrimps
133 with grass carps in zone B but without fishes in zone A (**Fig. 3B and table S18**). In all
134 ponds of zone B, we did not observe the WSS outbreak and harvested with 8,587 ±
135 1,655 kg/hm^2 in 110 days of culture. Whereas, the average yield of zone A is 1,953 ±
136 2,188 kg/hm^2 due to the WSS outbreak occurred in 12 of 18 ponds. Secondly, to
137 evaluate the effectiveness of controlling WSSV transmission by co-culturing shrimps
138 with catfish, two experimental zones were designed at a farm in Qinzhou, Guangxi
139 province, China (Farm 2) in 2011 (**Fig. 3C**). In zone A, we cultured 7.5x10^5/hm^2 of
140 shrimps in 38 ponds (21.20hm^2) for 10 days, and then introduced 525–750/hm^2 of

141 catfish with average body weight of 0.5kg. Shrimps were cultured in 57 ponds
142 (67.00hm^2) without fish in zone B. In zone A, we did not observe the WSS outbreak in
143 all the ponds and harvested with 8730 ± 1187 kg/hm^2 in 110 days of culture (**Fig. 3E**
144 **and table S19**). However, the WSS outbreak occurred in 53 ponds of zone B, resulting
145 in an average yield of 450 ± 1420 kg/hm^2. In 2012, we split zone B into zone B1 and
146 B2. Shrimps were cultured with catfish in 38 ponds of zone A and 25 ponds (27.00 hm^2)
147 of zone B1, while shrimps were cultured without fish in 32 ponds (40.00 hm^2) of zone
148 B2 (**Fig. 3D**). We did not see a WSS outbreak and harvested with 9628 ± 1471 kg/hm^2
149 in zone A and 6375 ± 1000 kg/hm^2 in zone B1. Nevertheless, the WSS outbreak
150 occurred in 29 of 32 ponds of zone B2, resulting in the average yield of 500 ± 1900
151 kg/hm^2 (**Fig. 3E and table S19**). Moreover, releasing grass carp and/or catfish
152 effectively controlled WSS outbreak and substantially increased the shrimp production
153 at Farm 1 from 2013 to 2019 (**Fig. 3F and data S1**).

154

155 Theory predicted that selective predation on infected individuals can prevent outbreaks
156 of diseases in prey population (*1, 18*). By elucidating the transmission dynamics of
157 WSSV, we developed a cultural system to control disease outbreak in shrimp
158 population by restoring the interaction of predator and prey. In addition, the species,
159 body weight, and density of co-cultured fishes were determined by resolving the
160 feeding ability and selectivity of fishes on shrimps through experiments. This, to our
161 knowledge, is the first artificial system that successfully controls viral disease with high
162 transmission rate by selective predation. Furthermore, the use of aquaculture fish as

predator results in production of shrimps and fishes simultaneously in the system. It highlights the significance and provides a scheme of unraveling the characters of predator, pathogen, and prey in developing disease control strategies by selective predation.

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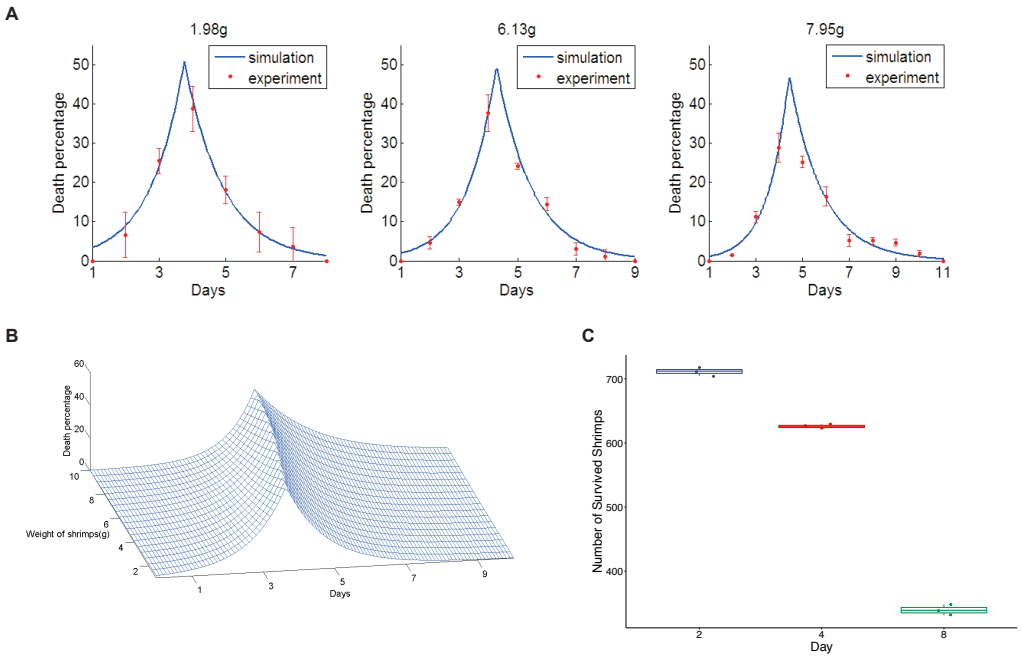

Fig. 1. Transmission dynamics of WSSV. (A) Daily death percentage of shrimp populations with initial WSSV-infected shrimps of different body weights. The red points with error bars are the results from experiments, and the solid blue lines are the results of model 1. **(B)** The illustration shows the death percentages of infected shrimps with different body weight each day. We estimate the parameters in the mode l and draw the above 3D surface to show the relationship between death percentage concerning shrimp weights and time. For all weights of shrimps, the death percentage rises at the beginning and then drops. The peak time for death is around the fourth day. **(C)** The relationship between the number of survived shrimps and days after healthy shrimps were co-cultured with WSSV-infected shrimps. Means and standard errors are shown.

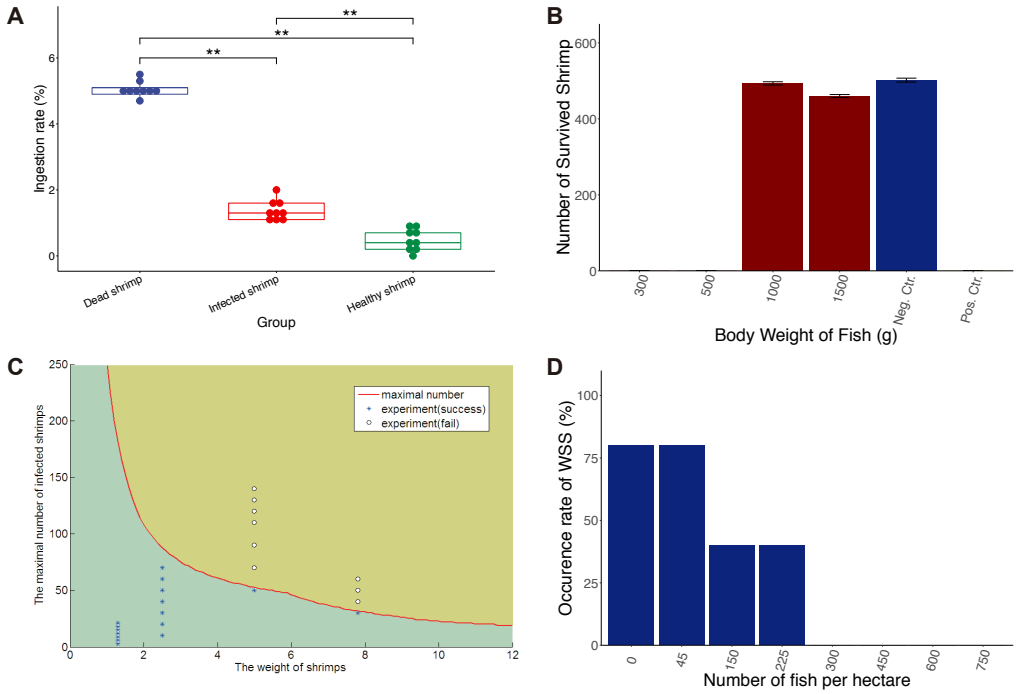

Fig. 2. Specification of grass carp for control of WSSV transmission. (A) Feeding selectivity of grass carp on dead, infected (endopod and exopod removed), and healthy shrimps. The diseased shrimps which infected WSSV would have reduction of activity and die within one day. The activity of shrimps reduced after the endopod and exopod removed. Thus, the endopod and exopod removed shrimps were used to resemble WSSV-infected shrimps. Grass carp ingested significantly more dead shrimps than infected (endopod and exopod removed) and healthy shrimps. **(B)** The effect of the different body weights of grass carps on the control of WSS outbreak. Means and standard errors are shown. **(C)** Capacity of 1kg grass carp for controlling WSS. Blue asterisks represent the number of infected shrimps successfully controlled by one 1-kg grass carp, while clear circles represent the number of infected shrimps that failed to be controlled by one 1-kg grass carp. The red line is simulated highest value of one 1-kg fish that can control the number of infected shrimps with different body weights of infected shrimps based on the model 3. **(D)** The relationship of the number of co-culturing grass carp and the occurrence rate of WSS. More than 300 grass carps of about 1.0kg per hectare can completely control the outbreaks of WSS, but less than 225 grass carp cannot fully control outbreaks of WSS.

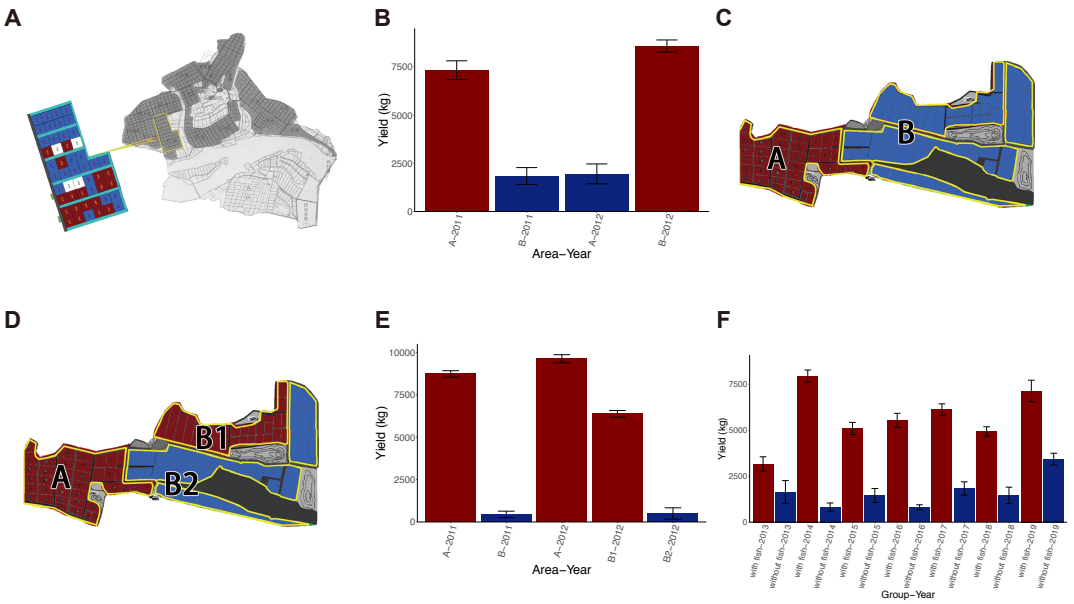

Fig. 3. Control of WSS in shrimp production by fish. (A) The design of field study for control of WSS using grass carp. The satellite map of the farm at Maoming, Guangdong Province, China (Farm 1) is shown. The 46 experimental ponds were divided into zone A (red) and zone B (blue). In 2011, shrimps were cultured with grass carps in the ponds in area A, while shrimps were cultured without fishes in the ponds in area B. In 2012, shrimps were co-cultured with grass carp in area B but without fishes in area A. **(B)** Total yield of shrimp production in ponds with (red) or without grass carp (blue) at Farm 1. Means and standard errors are shown. **(C)** The design of field study for control of WSS using catfish in 2011. The satellite map of the farm in Qinzhou, Guangxi province, China (Farm 2) is shown. The 95 experimental ponds were divided into zone A (red) and zone B (blue). Shrimps were cultured with catfishes in the ponds in area A, while shrimps were cultured without fishes in the ponds in area B. **(D)** The design of field study for control of WSS using catfish in 2012. Shrimps were continued to be cultured with catfishes in the ponds in area A, while area B was divided into two: shrimps were cultured with catfishes in the ponds in area B1, and shrimps were cultured without fishes in the ponds in area B2. **(E)** Total yield of shrimp production in ponds with (red) or without catfish (blue) at Farm 2. Means and standard errors are shown. **(F)** Total yield of shrimp production in ponds with (red) or without fishes (blue) at Farm 1 from 2013 to 2019. Means and standard errors are shown.