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Health & Welfare  
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## Vibrio harveyi, a significant pathogen of maricultured species



3 August 2020 Dr. Xiao-Hua Zhang Dr. Xinxin He Dr. Brian Austin



Progress achieved in developing effective controls



*Vibrio harveyi* is a serious pathogen for many important maricultured species, causing a variety of lesions and diseases. Photo by Darryl Jory.

*Vibrio harveyi* is a member of the genus *Vibrio*, in the family *Vibrionaceae*, order *Vibrionales*, class *Gammaproteobacteria* and phylum *Proteobacteria*. It occurs naturally in marine habitats and has developed into a significant pathogen of wild and cultured marine fish and invertebrates (notably penaeid shrimp), especially in warm waters of Asia, southern Europe and South America.

This article – adapted and summarized from the [original publication](#) [Zhang, X., He, X. & Austin, B. *Vibrio harveyi*: a serious pathogen of fish and invertebrates in mariculture. Mar Life Sci Technol (2020)] – discusses *Vibrio harveyi* and its role as a serious pathogen to several maricultured species. For detailed information on the taxonomy; diseases; diagnosis; pathogenic mechanisms and disease controls for *V. harveyi*, refer to the original publication.

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Diseases and diagnosis

*V. harveyi* has been linked with disease in several warmwater fish and invertebrates (Table 1). It has been often associated with eye disease, for example in common snook, milkfish and other fish species. Without effective chemotherapy, the infected fish would inevitably become blind.

In fish, the pathogen has also been attributed with gastroenteritis, necrotizing enteritis, nodules on the opercula, scale drop and muscle necrosis, skin ulcers, tail rot and vasculitis in different fish species. With the latter infection, the fish lacked appetite, were lethargic and disoriented, and displayed necrotic subdermal cysts. Internally, meningitis, encephalitis, vasculitis, kidney necrosis and liver and kidney damage were recorded. Also, *V. harveyi* appears to exert great severity on/in immunosuppressed fish.

Zhang, Vibrio harveyi, Table 1a

Fish disease	Host	Range
Eye disease	Common snook ( <i>Centropomus undecimalis</i> ); Milkfish ( <i>Chanos chanos</i> ); Short sunfish ( <i>Mola mola</i> )	USA; Philippines; Spain
Gastroenteritis	Grouper ( <i>Epinephelus coioides</i> ); black sea bream, Japanese sea bass, yellowfin sea bream and red drum	Taiwan, China; Taiwan, China
Necrotizing enteritis	Summer flounder ( <i>Paralichthys dentatus</i> )	USA

Fish disease	Host	Range
Nodules on operculum	Tiger puffer (Takifugu rubripes)	Japan
Scale drop and muscle necrosis	Barramundi (Lates calcarifer); Hybrid grouper (Epinephelus fuscoguttatus x E. lanceolatus)	Vietnam; China
Skin ulcers	Sole (Solea senegalensis); Hybrid grouper (E. fuscoguttatus x E. lanceolatus)	Spain; China
Tail rot disease	Sea perch (Lateolabrax japonicus); Sea bream (Sparus aurata)	China; Malta

Table 1. Diseases of marine fish attributed to Vibrio harveyi. Modified from original to include only farmed species.

Zhang, Vibrio harveyi, Table 1b

Invertebrate disease	Host	Range
Acute hepatopancreatic necrosis disease	Whiteleg shrimp (Litopenaeus vannamei)	Malaysia
Bacterial white tail disease	Whiteleg shrimp (Litopenaeus vannamei)	China
Black shell disease	Black tiger shrimp (Penaeus monodon)	India
Bolitas negricans	Penaeid shrimp	Ecuador
Foot pustule disease	Abalone (Haliotis discus hannai)	China
Luminous vibriosis	Penaeid shrimp	Ecuador, Asia
Skin ulceration	Sea cucumber (Holothuria scabra)	Madagascar
White spot on the foot	Japanese abalone (Sulculus diversicolor)	Japan

Table 1. Diseases of marine invertebrates attributed to Vibrio harveyi. Modified from original to include only farmed species.

*V. harveyi* has been particularly troublesome in shrimp culture. The pathogen has been recovered from outbreaks of Bolitas negricans (Spanish = small balls; diseased shrimps contain balled epidermal tissue which can block the digestive tract) in Ecuador and luminous vibriosis (diseased animals can glow in the dark), and linked with black shell disease in black tiger shrimp (*Penaeus monodon*) in India and bacterial white tail disease in Pacific white shrimp (*Litopenaeus vannamei*) in China. With the latter disease, mass mortalities have been reported in which the affected shrimp displayed white or opaque lesions in the tail, which were attributed to muscle necrosis.

Together with *V. parahaemolyticus*, *V. owensii*, *V. campbellii* and *V. punensis*, *V. harveyi* has been reported to cause acute hepatopancreatic necrosis disease (AHPND) in Pacific white shrimp (*L. vannamei*) in Malaysian shrimp ponds. And its pathogenicity was confirmed in infectivity experiments.

Regarding diagnostic methods, modern molecular approaches have discriminating accuracy and include 16S rRNA gene sequencing, which has become the favored method of identifying isolates accurately and multilocus sequence analysis. Several polymerase chain reaction (PCR) tests have been developed to detect and diagnose *V. harveyi*, including a multiplex PCR, an enterobacterial repetitive intergenic consensus (ERIC)-PCR, and a specific recombinase polymerase amplification (RPA) designed specifically to recognize the *V. harveyi* toxR gene. There are also serological [study of serum and other bodily fluids] approaches available, including chemiluminescent-based dot blot test [a technique in molecular biology used to detect proteins].

Pathogenic mechanisms

Much of the research on pathogenicity has involved the use of laboratory cultures, some of which are of uncertain authenticity/identity. Therefore, there may be questions concerning whether or not the research has actually used genuine *V. harveyi* cultures, and the actual relevance of the data to the host. An initial conclusion may be reached that the pathogenicity mechanisms remain to be properly resolved. Nevertheless, infections and mortalities have been achieved using fish and cultures.

There are several known pathogenicity mechanisms and virulence factors for *V. harveyi*, including hemolysins, proteases, lipopolysaccharide (LPS), the capacity to bind iron, interaction with bacteriophages, biofilm formation, and quorum sensing. It is possible that pathogenicity reflects the interaction between two or more virulence factors functioning together or sequentially. Moreover, it is important to realize that the pathogenicity mechanisms of *V. harveyi* to fish and invertebrates may well be different.

Disease control

Multiple studies have addressed the spread of disease associated with *V. harveyi*, and initially, the use of antimicrobial compounds like Prefuran and oxytetracycline were successful. However, the concern over tissue residues and the development and spread of antibiotic resistance contributed to research in other areas of disease control, notably preventative rather curative.

Bacteriophage therapy

Some research has focused on bacteriophage therapy [curative use of bacteriophages – virus that infect and replicate within bacteria – to treat pathogenic bacterial infections] to control luminous vibriosis in shrimp aquaculture. For example, a cocktail of three bacteriophages isolated from shrimp farms in India, inhibited *V. harveyi* and resulted in greater survival of tiger shrimp larvae compared to controls.

Biological control

One example is the successful use of certain surface-associated bacteria recovered from sea cucumbers to control *V. harveyi* disease in brown-marbled grouper fingerlings. And for Pacific white shrimp, research has suggested that culture in biofloc can reduce hepatopancreas lesions attributed to *V. harveyi*.

## Dietary supplements

A wide range of dietary supplements have been proposed for the control of *V. harveyi*. Some have been commercialized, particularly in the Far East, for use with fish and shellfish. Other examples for use in fish include  $\beta$ -1,3-glucan, bovine lactoferrin, Chaga mushroom, the seed of the evergreen tree *Leucaena leucocephala*, garlic, ginger, green tea (*Camellia sinensis*), hawthorn (*Crataegus* sp.) extract, Japanese pepper tree (*Zanthoxylum piperitum*), kudzu (*Pueraria thunbergiana*), loquat tree (*Eriobotrya japonica*) and pericarp (*Zanthoxylum piperitum*). Immunomodulation and protection against challenge were common with the use of these dietary supplements.

Numerous dietary supplements have been researched for use with shrimp, including black nightshade (*Solanum nigrum*) extract and mangrove (*Rhizophora apiculata*) leaf extract. Probiotics have also been evaluated for control of *V. harveyi* infections, including *Bacillus coagulans*, marine red yeast (*Rhodotorula mucilaginosa*) and the marine bacterium *Pseudoalteromonas flavipulchra* JG1.

A common theme with the use of dietary supplements is evidence for improved growth performance, immunomodulation notably of cellular and innate immunity, and protection against challenge with *V. harveyi*. This raises the question concerning the reason(s) for the profound benefit of what is essentially the addition of a small quantity of dietary supplement. Could the reason reflect inadequacies in the formulation of diets or are these truly supplements, i.e., supplementing already adequate feed?

## Inhibition of quorum sensing

Quorum-sensing signal molecules may be involved in regulating virulence. For example, a protein of *Bacillus thuringiensis* interfered with quorum sensing in *V. harveyi* and could be considered for disease control strategies.

## Vaccines

Although vaccines are primarily used to control diseases of fish, some commercial products containing *V. harveyi* antigens have been marketed for the control of shrimp diseases in the Far East. But most of the research has been directed at the control of fish disease. There has been a dramatic improvement in the success of vaccines for the control of *V. harveyi* diseases, with research efforts that started with the traditional approach of inactivated whole-cell preparations progressing to purified subcellular components, and subsequently to the modern era of DNA vaccines.

Various types of vaccines have been developed for the control of *V. harveyi* diseases, including whole-cell vaccines, subunit vaccines, live vaccines and DNA vaccines. Although significant published research has been encouraging in terms of success of vaccines after challenge, it remains to be determined which vaccines could be produced at the price that the users would be prepared to pay.

## Perspectives

*Vibrio harveyi* is a serious pathogen for multiple species of marine fish and invertebrates particularly in the warm waters of Asia, southern Europe and South America. Although its virulence has been demonstrated with multiple isolates, there is a lack of clarity over the precise pathogenicity mechanisms, possibly reflecting differences between isolates as well as between fish and invertebrates, and overreliance on laboratory cultures, which are not necessarily reflective of the abilities of fresh isolates.

There has been much progress with the development of effective disease control strategies, including use of dietary supplements and vaccines. Clearly, further work is justified to better understand this serious pathogen of marine animals.

*References available from the original publication.*

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## Authors


-  Dr. Xiao-Hua Zhang

Dr. Xiao-Hua Zhang

Corresponding author  
MOE Key Laboratory of Marine Genetics and Breeding  
College of Marine Life Sciences

Ocean University of China  
Qingdao, 266003, China; and  
Laboratory for Marine Ecology and Environmental Science  
Qingdao National Laboratory for Marine Science and Technology  
Qingdao, 266237, China; and  
Frontiers Science Center for Deep Ocean Multispheres and Earth System  
Ocean University of China  
Qingdao, 266100, China

[110,99,46,117,100,101,46,99,117,111,64,103,110,97,104,122,104,120]

-  Dr. Xinxin He

Dr. Xinxin He





MOE Key Laboratory of Marine Genetics and Breeding  
College of Marine Life Sciences  
Ocean University of China  
Qingdao, 266003, China

-  Dr. Brian Austin

Dr. Brian Austin

Institute of Aquaculture  
University of Stirling  
Stirling, FK9 4LA, Scotland, UK

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