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*Review Article*

# Milky Disease: A Review of Discovery, Pathogens, and Detection Methods in Crabs

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As an important economic crustacean in China, the crab has been a popular focus in aquaculture in recent years due to its high production and economic value. The epidemic of milky disease has severely damaged the crab farming industry, resulting in heavy economic losses and hindering the sustainable development of high-quality crab farming. The disease is characterized by severe emulsification of all tissues and the presence of large amounts of opaque, milky fluid in the body, with a high infection rate and high mortality. Determining the cause of the disease, its life cycle, transmission routes, and detection methods will help to provide direction for the prevention and control of crab milky disease. This article aims to review the discovery, main pathogens, and pathologies of milky disease, along with the currently established detection methods. We have also attempt to classify the control measures for the prevention and control of milky disease in crabs.

## 1. Introduction

*Crustacea*, a subphylum of phylum *Arthropoda* is consist of economically significant species distributed worldwide. According to 2022 fishery statistics, the total production of crustaceans in 2021 reached 6,032,032,862 tons in China, accounting for 11.55% of the total production of fish farming (2022 China Fishery Statistical Yearbook). In addition, crab aquaculture has become a major pillar of the industry. However, due to intensive farming and environmental degradation on farms, diseases have been one of the main constrains to the sustainable development of the industry [1–4]. Milky disease is one of several serious diseases that has erupted on farms cultivating *Eriocheir sinensis* located in Panjin, Liaoning Province, Northeastern China. The outbreak of milky disease had seriously jeopardized the development of the aquaculture industry and resulted in huge economic losses to the aquaculture industry [5, 6].

Recent studies have shown that a variety of pathogens, including *Candida oleophila* [6], *Vibrio alginolyticus* [7], *Microsporidium* [8], *Hematodinium* sp. [9], and *Metschnikowia bicuspidate* [10], are capable of causing milky disease in crabs. The aim of this paper is to review the current literatures on the discovery, main pathogens, established detection methods, and treatment modalities of milky disease.

## 2. The Discovery of Milky Disease in Crabs

In recent years, the consumption of crabs has risen, leading to increased economic benefits for crab farming. This surge in demand has led to an expansion of the scale and density of the farms, resulting in harsher environmental conditions and a higher prevalence of disease in the crabs. Milky disease, also known as "yellow water disease" and "emulsification disease," is a fulminant epidemic characterized by a high infection rate, widespread distribution, and high mortality. The main symptoms of this disease include accumulation of a milky fluid, weakened vitality, decreased appetite, and increased mortality (Figure 1). This is one of the most serious diseases affecting crab farming, causing significant economic losses, and prompting farmers to take extensive precautions.

(a) (b)

Figure 1: Comparison of Chinese mitten crab with milky disease and healthy Chinese mitten crab: (a) Chinese mitten crab with milky disease and (b) healthy Chinese mitten crab.

Table 1: Chronology of milky disease outbreaks in crabs in China.

| Year first reported | Location | Reference |
|---|---|---|
| 2002–2004 | Ningbo and Zhoushan, Zhejiang Province, China | [13] |
| 2001, 2004–2005 | Zhoushan, Zhejiang Province, China | [6, 11] |
| 2014 | Tianjin–Hebei Urban Agglomeration | [9] |
| 2018 | Panjin, Liaoning Province, China | [4] |
| 2020 | Tianjin, China | [12] |

The first case of milky disease was reported in the city of Zhoushan in 2001 [6]. In China, the provinces of Zhejiang, Jiangsu, Shandong, and Liaoning have been the areas most frequently affected by milky disease in recent years. In 2001, Xu et al. [6] reported that the incidence of milky disease in *Portunus trituberculatus* in Zhoushan City, Zhejiang Province, was 30%, with the mortality rate at 100%. From 2004 to 2005, the incidence of milky disease in cultured *P. trituberculatus* in Zhoushan City ranges from 20% to 50%, with an average mortality rate of 20%. However, in severe cases, the mortality rate can reach 60%–70% [11]. Wang et al. [9] reported an outbreak of milky disease in the urban agglomeration of Tianjin–Hebei in 2004 with *Hematodinium* sp. as the culprit agent. This was accompanied with a high incidence (70%–80%) and mortality (90%). In 2019, Bao et al. [4] identified *Metschnikowia bicuspidata* as the pathogen responsible for a similar outbreak in Panjin, wherein the mortality rate exceeded 20%. Xu et al. [12] demonstrated that Chinese mitten crabs infected with milky disease were introduced from Panjin to Tianjin between April and May 2020, resulting in a morbidity rate of 90% and a mortality rate of over 50%. This caused the massive morbidity and mortality of Chinese mitten crabs in the local area (Table 1).

## 3. Main Pathogens of Milky Disease in Crabs

Studies have shown that although the symptoms of crab milky disease are similar in different regions (Table 1), the pathogens are different. *C. oleophila* [6], *V. alginolyticus*, *Pseudomonas putida* [14], *Hematodinium* sp. [15], *Ameson portunus* [9], and a combination of *Candida lusitaniae* and *V. alginolyticus* [7, 13] are all known to potentially infect *P. trituberculatus* with this disease. Milky disease in giant mud crab (*Scylla serrata*) is caused by the pathogen *Hematodinium* sp. [16]. Two other major pathogens, *Hepatospora eriocheir* [8] and *M. bicuspidata* [10] are responsible for the onset of milky disease in *E. sinensis*. The disease is caused by four types of pathogens: yeast, *Vibrio*, *Hematodinium* sp., and microsporidia. A full description of each major pathogen is given below.

*3.1. Yeast.* Yeasts are heterotrophic facultative anaerobic bacteria. Their reproductive modes can be divided into budding fission or ascospore formation. The life cycle is divided into three categories: haploid, diploid, and monodiploid. The cell form is spherical, oval, and so on.

Yeasts are widespread in nature and can infect most aquatic economic species. Studies have shown that a number of fungi are susceptible to crustaceans, including *Pichia* [6], *Cryptococcus* [17], *Metschnikowia*, and *Candida* [6]. Stentiford et al. [18] isolated yeast for the first time from diseased crabs infected with *Hematodinium* sp. in 2003. Although the species of yeast has not yet been identified, this is the first report of yeast infection of crabs [18]. In the same year, Xu et al. [6] reported a case of milky disease in *P. trituberculatus* caused by *Pseudofilamentous* infection and identified the pathogen for the first time. Shi et al. [19] and Xu et al. [20] carried out a series of studies on the disease and showed that

*Pseudohyphomyces* could not only cause milky disease in *P. trituberculatus* but also infected other species of crabs, such as *Portunus sanguinolentus* and *Charybdis japonica*, by artificial infection.

In addition, *M. bicuspidata* was thought to be another causative agent of milky disease in *P. trituberculatus* [21]. In 2019, an outbreak of a disease with the same symptoms as the milky disease in *P. trituberculatus* was reported in Panjin, Liaoning Province. However, unlike *P. trituberculatus*, for which the cause of milky disease is still controversial, the cause of milky disease in Chinese mitten crabs is very clear. The pathogen of milky disease was identified as *M. bicuspidata* in Panjin, Tianjin, Liaoning, and other regions [4, 10, 12].

*M. bicuspidata*, belonging to *Metschnikowia*, is a conditionally pathogenic yeast first discovered in *Daphnia*. Its pathogenicity is largely determined by its relationship with the host, the environmental conditions, and the host environment. Infection with *M. bicuspidata* in its known hosts, *M. rosenbergii*, *P. trituberculatus*, *E. sinensis*, *Oncorhyncus tshawytscha*, *Daphnia*, and *Artemia*, results in reduced vitality and other symptoms, such as loss of feet, slow movement, and discharge of a milky fluid when the gill lid ruptures, bright yellow color of the hepatopancreas, and disarray of the gill filaments [22]. The incidence rate has been observed to negatively correlate with water temperature, with higher incidence at low temperature [4, 6, 23]. Furthermore, cases have been reported through waterborne transmission, cannibalism, and contact between hosts via the food chain [23–25].

*3.2. Vibrio*. Vibrio, a major pathogen belonging to the *Proteobacteria*, *Vibrionales*, is commonly found in natural waters. Vibrio is a Gram-negative bacterium with a short, curved form, a flagellated, mobile tail, and no buds or capsules. The adaptation temperature of Vibrio is in the range of 10–35°C, and the optimal temperature is approximately 28°C. There are many pathogenic factors of Vibrio, mainly including adhesion, extracellular products, the outer membrane protein, and the iron uptake system. Different pathogens play different roles at different times when they infect the host.

The pathogenic pathway of Vibrio mainly involves adhesion and invasion of host tissues. It can take up a large amount of nutrients from the host, while its secretion of lipopolysaccharides, phospholipases, proteases, and hemolysins can damage host tissues and organs [26, 27]. Notable Vibrio species include *Vibrio anguillarum*, *V. alginolyticus*, *Vibrio vulnificus*, and *Vibrio parahaemolyticus*, which are the common causes of bacterial infections in aquatic animals.*V. alginolyticus* is a Gram-negative, halophilic, conditional pathogenic bacterium that can infect fish, shrimp, shellfish, crabs, and other aquaculture animals. When environmental conditions are unfavorable and the immunity of the cultured animals is compromised, the infection rate of *V. alginolyticus* is increased, especially when the water temperature is between 25 and 32°C [28]. Wang et al. [13] proposed that the mixed infection of *C. lusitaniae* and *V. alginolyticus* was responsible for the outbreak of milky disease in Ningbo and Zhoushan, with *V. alginolyticus* being the primary etiological agent responsible for mortality in this species. This was further confirmed by Zhao et al. [7], Liu et al. [29], and Jin et al. [30], who also showed that *V. alginolyticus* was the causative agent of milky disease in *P. trituberculatus*. In addition, other species of Vibrio may be responsible for milky disease in crabs. Wang et al. [14] demonstrated that *P. putida* was the microorganism causing milky disease in *P. trituberculatus* from Zhoushan, Zhejiang Province.

*3.3. Hematodinium sp. Hematodinium* sp. is a common parasitic dinoflagellate pathogen [31] belonging to *Sarcomastigophora* and *Syndinida*, known to cause disease in marine crustaceans and reported to have a wide geographical distribution. The life cycle of the species of Syndinida to which *Hematodinium* sp. belongs usually includes at least three stages: multinucleate plasmodial stage, trophont, and dinospore. Due to the wide variety of host species and life histories, the morphology of *Hematodinium* sp. in different hosts is quite different. For example, the diameter of unicellular trophozoites ranges from 9 to 15 $\mu$m, and their size is close to that of host blood cells, while filamentous trophozoites and polynuclear sporophytes are more than three to five times the size of host blood cells [32]. The main mode and route of transmission of *Hematodinium* sp. has not been conclusively established.

In recent years, researchers have tried to verify whether *Hematodinium* sp. is transmitted by cannibalism, but the results of different experiments are very different. Walker et al. [33] found in a feeding experiment that American blue crabs could be successfully infected with *Hematodinium* sp. Li et al. [34] then infected 120 American blue crabs using the same feeding method, but only two crabs were successfully infected. Some scientists have demonstrated that cannibalism is not the main mode of transmission of *Hematodinium* sp. [35]. Li et al. [36] found that *Hematodinium* sp. spores can survive in the aquatic environment for up to 7 days, suggesting that the spores may invade the host body through the damaged site or during the host's vulnerable molting period, thereby spreading.

The high prevalence, extent of infection, and severe consequences of *Hematodinium* sp. have attracted considerable interest from the scientific and industrial communities by hydraulically [37] and vertically [38] spreading. Chatton et al. [39] first reported that *Hematodinium* sp. induced pathology in *Carcinus maenas*, followed by other reports of infection in crustaceans, such as *Callinectes sapidus* [40], *Cancer Borealis* [41], *Chionoecetes bairdi* [42], *Pandalus Platyceeros* [43], *Nephrops norvegicus* [44], and *Chionoecetes opilio* [45]. In 2006, Xu et al. [46, 47] first reported a *Hematodinium* sp. infection in Zhoushan City, China, causing milky disease in *Exopalaemon carinicauda*. This finding was later confirmed by Xu et al. [16], suggested that *Hematodinium* sp. was highly pathogenic, and causes widespread mortality in host animals with a seasonal prevalence [48].

Epidemics with different characteristics present no differences at different stages and seasons between host species [49]. It was shown that host, age, sex, and molt were all associated with the prevalence of *Hematodinium* sp. [50–52]. Four lifecycle forms of *Hematodinium* sp. have been identified in the

hemolysis of diseased crabs, including filamentous trophont, nonnucleated trophont, trophont clusters, and multinucleated sporonts [32].

*3.4. Microsporidium*. *Microsporidium*, belonging to the taxon *Microsportidia*, is unicellular eukaryotic parasites of many different species. Mature spores are round or oval in shape and contain polar tubes, also known as polar filaments. Most microsporidia parasitize mainly insects, while only a few microsporidia parasitize aquatic animals [53]. Most of these species are pathogenic and mainly infect economically important crustaceans such as shrimps and crabs, causing significant economic losses in aquaculture.

Researchers have therefore made microsporidia a research priority. Most microsporidia infecting crustaceans can cause hypertrophy of host cells and produce cystic structures such as xenomas at the parasite site. The life cycle is an important basis for the classification of microsporidia, as well as for the prevention and control of microsporidia. All microsporidia life cycles include four developmental stages: sporoplasmodium, merogony, sporogony, and spore. The life cycles of different microsporidia also appear to differ. Most microsporidia infecting vertebrates complete reproduction and usually enter the host by direct oral infection; most microsporidia infecting invertebrates have complicated life histories, and their reproduction must be completed by one or more intermediate hosts. There is no strict host specificity for microsporidia, especially in aquatic invertebrates, which can change hosts along the food chain. Microsporidia differ from other organisms in that they have a unique mechanism for infecting host cells. The pathways of spore invasion into host cells are active invasion and endocytosis. There are three modes of transmission, horizontal, vertical, and mixed [54, 55].

Wang and Gu [56] observed that *Endoreticulatus eriocheir* caused infection of the hepatopancreas in *E. sinensis* and investigated its morphology, pathology, and epidemiology. They concluded that this microsporidian should be attributed to the genus *Enterocytozoon*. However, Stentiford et al. [8] argued that *E. eriocheir* infected with *E. sinensis* bears little resemblance to the genus *Enterocytozoonidae*. Wang et al. [57] further showed that after *A. portunus* was inserted into the muscle of *P. trituberculatus*, its transparency gradually decreased with infection time until muscular leucorrhoea was observed.

Therefore, milky disease caused by different pathogens in crabs has similar clinical symptoms, anatomical features, and pathological characteristics. The crab carcasses of *P. trituberculatus* infected by *C. oleophila* [6], *P. putida* [14], *V. alginolyticus* [14, 29], *Hematodinium* sp. [46, 47], and *Microsporidium* [9, 58]; *E. sinensis* infected by *M. bicuspidate* [10, 12] and *Microsporidium* [57]; and *S. serrata* infected by *Hematodinium* sp. [16, 59] all show wasting, white muscle turbidity, atrophy, reduced vitality, swollen tarsal joints, reduced or no feeding, slow reaction; milky fluid in the lids and feet, edematous muscles, dark, tan, or black gills, empty intestines; homogeneous and pink muscles, noncoagulated hemolymph, and denatured and disordered liver cells. Small and irregular myocardial fibers dissolved and liquefied muscle fibers.

Although the clinical symptoms of various pathogens may be similar, simply classifying and naming these diseases are not a rigorous and scientific approach. The interaction between external pathogens, the environment, and the host can lead to various diseases in farmed crabs. To avoid the occurrence of phenomena such as "same name, different disease"; "same disease, different name"; "one symptom, different diseases"; and "one disease, different symptoms," the pathogenic mechanisms, life cycles, and transmission routes of each pathogen must be studied.

## 4. Detection Methods of Milky Disease

The pathogens that cause milky disease (e.g., fungi and Vibrios) are difficult to identify from their size and shape. Therefore, early, timely, accurate detection, and diagnosis are extremely important for the scientific prevention and treatment of diseases in farmed crabs in aquaculture. The diagnosis and detection of the pathogenic agent of milky disease have always been an important part of the prevention and control of this disease. To date, the detection methods for milky disease mainly include pathogenic tests, serological tests, and molecular biology tests.

*4.1. Pathogenic Testing*. Currently, there are two main methods for detecting the pathogen of milky diseasein crabs: microbial culture and microscopic examination. *M. bicuspidata* was isolated from the focal tissue and incubated in three types of media, brain–heart infusion broth (BHI), bengal red plate, and Vibrio selective agar (thiosulfate citrate bile salts sucrose agar, TCBS), at an incubation temperature of 28°C for 48 hr before the appearance of round, opaque, creamy white colonies with smooth edges and moist bodies [12]. After 36 hr of cultivation on nutriment agar (NA) and yeast extract peptone dextrose agar(YPD), 1–3 mm round and raised white colonies were formed [10]. The colonies on the red plates of Bengal had transparent cream–white edges with circular shapes [13].

The morphology, size, and disposition of the various pathogens of milky disease can be visually observed by microscopic examination. By H&E staining, the muscles, gills, and hepatopancreas of crabs infected by *M. bicuspidate* were observed. The most severe lesions were found in the muscle tissue, with atrophy following colonization of muscle fibers and clusters of organisms in isolated areas [10]. Microscopic examination revealed that a large number of pathogenic bacteria were observed in the hepatopancreas, muscle, and other parts of *P. trituberculatus* after *V. alginolyticus* infection [13]. When the crabs were infected with *Hematodinium* sp., the milky white fluid was taken for microscopy smear examination, and it was observed that the parasites were abundant in hepatopancreas, heart, gill, and other tissues [47]. The degree of destruction of the hepatopancreas varies with the time of infection and may be an indicator site for early diagnosis of the disease [5]. By H&E staining, eosinophilic granules were observed in the nucleus of hepatopancreas ducts of diseased crabs infected with *H. eriocheir* and the hypertrophic and dark granules observed by Masson staining were *E. sinensis* microsporidia [57]. H&E staining also showed that the muscle

bundles of *P. trituberculatus* infected with *Hematodinium* sp. were disorganized and dispersed. The spaces between the filaments and muscle bundles were filled with microsporidia, and protozoa parasites were also found in the gills, stomach, and intestine [9].

Although the pathogen culture method is the "gold standard" for detecting pathogenic microorganisms, it is not suitable for routine use in mainstream institutions due to its slow growth of microorganisms, complex culture processes, and long consumption times. In comparison, microscopic examination is simpler and faster but requires expensive equipment and technical experts. Additionally, typical pathological symptoms can only be observed when the symptoms are severe and difficult to detect in the early stages of infection.

*4.2. Serological Testing.* Serological and immunological methods are commonly used to detect pathogens. Jin developed an ELISA indirect rapid detection method and an indirect fluorescence antibody rapid diagnostic technology [30], both of which could specifically, sensitively, and efficiently diagnose milky disease caused by *V. alginolyticus*. Xie established indirect fluorescent antibody detection technology combined with polymerase chain reaction (PCR) for the detection of *Hematodinium* sp., which had a 77.8% positive rate and a 100% compliance rate [60]. This method was characterized by high sensitivity and high specificity, which provided a scientific basis for the subsequent detection of pathogens of *P. trituberculatus*. No serological detection method has been reported for *M. bicuspidata*.

Although serological and immunological detection methods are more sensitive than pathogenic detection methods, their use is limited by their slow detection rate and susceptibility to contamination.

*4.3. Molecular Biological Testing.* In contrast to classical pathogen isolation and serological testing, nucleic acid detection techniques in molecular biology have many advantages, such as a wide variety of sample sources, independence from sample processing and shape changes, independence from biological samples denaturation, and the ability to safely detect pathogenic bacteria after inactivation. Sequencing and PCR and its derivatives are the most commonly used molecular biology methods for pathogen identification.

PCR is currently the main method used to detect milky disease pathogens in crabs, offering the advantages of simplicity, high specificity, and the ability to identify species by sequencing [61–63]. Bao et al. [4], Ma et al. [10], and Xu et al. [12] confirmed that *M. bicuspidata* was the main pathogen causing milky disease in *E. sinensis* by extracting DNA from diseased crab tissues, amplifying sequences of different genes (18S ribosome DNA (rDNA), 26S rDNA, and ITS genes) and constructing phylogenetic trees. Wang et al. [14] amplified 16S rRNA of infected *P. trituberculatus* and identified the pathogen as *P. putida*. For the detection of *Hematodinium* sp., the causative agent of milky disease of *P. trituberculatus*, Shi et al. [11, 59] established a PCR detection method by designing a pair of specific primers based on ITS1 and 18S rDNA gene sequences. Li et al. [32], Xu et al. [47], and Wu et al. [48], using PCR amplification with specific primers for *Hematodinium* sp., combined with molecular sequencing, hemolymph detection, and genetic analysis, demonstrated that *Hematodinium* sp. was the major pathogen for *S. serrata* and *P. trituberculatus*.

Although PCR-based assays can be used to characterize pathogens, they cannot be used to quantify them. Therefore, many researchers have improved PCR technology and developed new PCR detection techniques such as immune PCR, nested PCR, and real-time quantitative PCR. Ding et al. [3] amplified a 931 bp fragment by nested PCR, which was confirmed to be 18S rDNA of *H. eriocheir* by comparison. They concluded that nested PCR was more sensitive than standard PCR and could avoid nonspecific amplification of similar size. Bao et al. [64] established a nested PCR assay for the specific detection of *M. bicuspidate* infecting *E. sinensis*, and the sensitivity and positivity rate were both higher than the large subunit ribosomal RNA gene and internal transcribed spacer PCRs. Chen et al. [65] established the SYBR Green I real-time quantitative PCR detection method using the SSR rRNA gene of microsporidia infected with milky disease in *P. trituberculatus* as the detection target, and the detection rate of microsporidia was 82.35%, compared with 64.71% for nested PCR. Liu et al. [66] reported that the detection limit of conventional PCR for *H. eriocheir* was $10^2$ copy/$\mu$L, while that of quantitative PCR was $10^1$ copy/$\mu$L, suggesting that quantitative PCR was more sensitive. Quantitative PCR can avoid PCR contamination and has high specificity, but its application is limited by expensive instrumentation and inability to meet the detection requirements of POCT.

Nucleic acid hybridization can also be used to detect pathogens in the laboratory. Ding et al. [3] designed specific primers and established an in situ hybridization detection method for *H. eriocheir*. This method had a low false positive rate but had some disadvantages, such as being time consuming and having a complicated operation process.

In the field of nucleic acid detection, the development of PCR strategy is well-established and has led to a number of derived techniques, such as PCR-RFLP (restriction fragment length polymorphism), PCR-SSCP (single-strand conformation polymorphism), qPCR, and dPCR. All of these techniques require equipment with different temperatures, which is time consuming. Therefore, with the demand for more portable and faster testing equipment and techniques, isothermal amplification technologies, which have more room for development, are receiving increasing attention [67]. Among the nucleic acid detection techniques for pathogen identification, strand displacement amplification (SDA) [68], loop-mediated isothermal amplification (LAMP) [69], nuclear acid sequence-based amplification (NASBA) [70], helicase-dependent isothermal DNA amplification (HDA) [71], rolling circle amplification (RCA) [72, 73], and recombinase polymerase amplification (RPA) [74] are currently used in the fields of animal husbandry, veterinary medicine, food science, and laboratory medicine. It is anticipated that these isothermal amplification techniques will also play an increasingly important role in the detection of aquatic pathogens.

## 5. Prevention and Treatment Measures and Recommendations

With the development of modern fisheries and the expansion of crab farming, the incidence of disease has increased, resulting in enormous losses to the aquaculture industry. Therefore, disease control should be a high priority for the farming industry. Therefore, disease control should be a high priority for the farming industry. In crab farming, the quality of the crab species, environmental conditions, level of farming supervision, disease resistance of the crab breed, and prevention measures are all closely related to the occurrence of the disease. Therefore, the crab farming industry should focus on the prevention of crab disease.

The quality of crab germplasm is key to the sustainable development of crab production. To improve the disease resistance of farmed crabs, we should standardize the techniques of healthy crab farming, control stocking densities, provide adequate bait, improve the farming environment, prohibit the abuse of fishery drugs, and avoid the use of antibiotics. During crab farming, any abnormalities must be thoroughly analyzed, accurately diagnosed, and appropriately treated. To date, there are no specific medicines for aquatic pathogens, such as fungi, bacteria, and parasites. We must adhere to the principle of "prevention first, comprehensive treatment" and take active and effective measures to control the onset and spread of diseases.

## 6. Outlook

In recent years, the crab farming industry has been severely affected by multiple outbreaks of milky disease in crabs, resulting in a sustained decline in its economic efficiency and limiting the pace of development. Although the pathogen has been identified, there is a clear lack of technical support for disease management and outbreak prevention.

Milky disease caused by bacteria, yeast, and parasites has similar clinical symptoms and is often seen as a symptom of many diseases. Further research into transmission routes, epidemiological models, life histories, and invasion mechanisms of these pathogens is needed to effectively control this disease in crustaceans. Effective drugs against the various pathogens need to be developed, as well as preventive measures to improve the immunity of the crabs and their husbandry. These steps will provide a theoretical basis and technical guarantee for the successful management of milky disease in crabs.

## Data Availability

The underlying data supporting the results of your study can be found, including, where applicable, hyperlinks to publicly archived datasets analyzed or generated during the study.

## Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Authors' Contributions

Hua Wei and Xiaodong Li contributed equally to this work. They were involved in conceptualizing the project and providing foundation. Hua Wei reviewed the literature and revised the paper. Yabing Tan wrote the manuscript. Sihan Zhou, Xinyi Cen, Xinran Wang, Yingying Zhao, and Yingdon Li contributed to the revisions of the article. All authors approved the final version of the manuscript for publication.

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