Research Paper

# The Increase of Fungal Shrimp Disease *Fusarium solani* in Response to Water Quality

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**Water pollution may increase the abundance of viruses, bacteria, filamentous fungi, algae and protozoa that parasite on freshwater organisms causing an effect. Over the past few decades, various species of pathogenic fungi have been reported to cause numerous diseases in different countries. This study aim to examine the relationship between the increase of fungal diseases (caused by *Fusarium solani*) and water quality through comparison of the morphological characteristics between prawn shrimp (*Penaeus monodon*) among two regions (polluted; Jazan Sea *vs.* unpolluted; Farsan Island). We collected samples from two different regions and measured weight (g), body length (mm), and antenna length (mm) as well as reported color and black spot. We also isolate fungi from 10 sample for each study region to diagnose fungal disease of *Fusarium solani*. We finally measured parameters of water quality including PH, temperature, $O_2$ (mg/l), total dissolved solid (mg/l), total organic carbon (mg/l), dissolved organic carbon (mg/l) and biodegradable dissolved organic carbon %. Results show a significant variation in the morphological characteristics of prawn shrimp (*Penaeus monodon*) between the two study regions in terms of shape, color, weight (g), body length (mm) and antennae length (mm) as well as variation in water quality components. A relationship was found between water quality and the increased incidence of fungal parasite *Fusarium solani* of prawn shrimp (*Fusarium solani*). Good water quality was observed on Farasan Island, associated with species uninfected by *Fusarium solani*. In contrast, the Jazan Sea, where most species were found to be infected with *Fusarium solani*, was associated with poor water quality, affecting morphological characteristics. Therefore, we aim to establish a theoretical basis for early prevention and control of fungal diseases in shrimp associated with water quality.**

**Key words: Pollution, disease, brawn shrimp, *Fusarium solani*, phenogram, parasite, fungi, prawn shrimp, *Penaeus monodon***

Water pollution is known to affect the diversity and composition patterns of aquatic fungal communities[1-5]. Water pollution can increase the abundance of viruses, bacteria, filamentous fungi, algae, and protozoa that parasitize freshwater organisms, causing significant impacts[6]. Over the past few decades, various pathogenic fungi have been reported to cause numerous diseases in different countries[7]. More recently, an increase in fungal shrimp diseases has been observed as a result of the high-density greenhouse model. With the rapid growth of shrimp species, parasitic fungal diseases are also increasing dramatically. This problem can pose a threat to shrimp and human health, as well as significant economic losses annually[8]. However, fungi are among the least studied groups of aquatic microorganisms due to the inherent difficulties in culturing and sequencing their Deoxyribonucleic Acid (DNA)[9].

Recently, several studies have shown that shrimp are susceptible to several diseases[10-12], including leprosy, a disease caused by a group of protozoa, all belonging to the Microsporidia family[13]. A recent study showed that the body of most brown shrimp (*Farfantepenaeus aztecus*) in the Jazan Sea (Saudi Arabia) are infected with the fungus *Fusarium*

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*solani* (*F. solani*) in response to water contamination, which may cause a fungal disease that affects growth, reproduction, species diversity, behaviour, and mortality rates. Another study revealed that the fleshy part of the shrimp's body changes color from pure white to opaque white as a result of water contamination with *F. solani*. Females, which are white, turn blue-black due to a fungal infection of the pigment cells in the shell. Some of this pigment is dispersed throughout the body by scratching or even during molting[14]. Black Spot Disease (BSD) is a fungal disease associated with poor water quality, which in turn leads to an increase in organic matter and an increase in the number of fungi invading the shrimp body, especially in areas of wounds or abrasions[15]. In farmed pacific white shrimp (Penaeus vannamei), BSD was later identified as *F. solani*, causing a mass mortality, reported in China in 2020[16].

This study aimed to investigate the relationship between the increase of fungal diseases (caused by *F. solani*) and water quality; pH, temperature, $O_2$, Total Dissolved Solid (TDS), Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC), and Biodegradable Dissolved Organic Carbon (BDOC), by comparing morphological characteristics; weight (g), body length (mm), antenna length (mm), shape, and color of prawn shrimp (*Penaeus monodon*) between two regions (polluted; Jazan Sea *vs.* unpolluted; Farasan Island). All study areas were located along the Red Sea in Saudi Arabia, Jazan and Farasan regions (fig. 1). The Jazan Sea is characterized by poor water quality and contains bioaccumulation of heavy metals and hazardous compounds, which increases the risk of heavy metal pollution of the marine ecosystem[17], and affects the pH, chemical oxygen demand, etc. of the marine ecosystem and plants[18], which may increase the growth of fungal communities[19]. On the other hand, Farasan Island is known for its pristine natural environment and high species diversity within small population centres associated with low population density and human activities. We also aimed to clarify whether prawn shrimp in the other study area (Farasan Island) was also invaded by the fungal parasite *F. solani*.

## MATERIAL AND METHODS

### Prawn shrimp collection:

We have conducted a scientific trip to two different areas in the Red Sea (Farasan Island and Jazan Sea; fig. 1). We collected samples using a fishing net in cooperation with fishermen. We collected 100 samples from Farasan Island, and 100 samples of prawn shrimp (*P. monodon*) from Jazan Sea and placed them directly in icebox to keep samples safe from accumulation and temperature. They were preserved and taken to the laboratory to be examine based on their morphological properties.


**Fig. 1: Study location in Saudi Arabia: Farasan Island and Jazan Sea**

**Morphological measurement:**

In the laboratory, we measured weight (g) using the electronic balance (the regular electronical weight measurement device). We then measured body length (mm) and antenna length (mm) using the stylus (ruler). Other morphological characteristics, such as shape, color, and black spot were also reported.

**Fungi isolation:**

Fungi were extracted from 10 samples of prawn shrimp (*P. monodon*) from each study area, starting from the body area where fungi grew, including the head, black spots, gills, and rarely the abdomen, using small tweezers. These were placed on sterile microscope slides with Safranine O staining. We marked each slide to identify the study area containing the parasites/fungi. We identified the fungal species extracted under a microscope. This protocol was used in a previous related study.

**Water quality:**

We measured temperature with a thermometer, oxygen concentration ($O_2$ mg/l) with a YSI Pro20i dissolved oxygen meter (607132), pH and TDS with an electronic water quality tester, DOC with a TOC analyser. We measured BDOC (total BDOC) by adding 200 ml (0.85°) of water through a 2 mm filter over a glass container. 2 ml (0.0085°) of native bacteria were added to the water. We sampled 40 ml (0.17°) of water and separated it into two subsamples, each with a volume of 20 ml (0.085°). The sample was measured using a Dohrman 80 total carbon analyser and stored in the dark at 20° (68°F) for 4 w. DOC concentration was calculated from the average of the four readings to provide the most accurate results. BDOC concentration was measured in parts per million.

## RESULTS AND DISCUSSION

We found that shrimp collected from the Jazan Sea displayed black coloration in various parts of their bodies (black spot), such as the head and tail (fig. 2). These black parts were specifically used for tissue extraction (fungi isolation) where we diagnosed the presence of a dangerous fungal disease (*F. solani*) that infects shrimp (fig. 3). Prawn shrimp (*P. monodon*) collected from Farasan Island were distinguished by their bright color and lack of black spot and fungal disease (fig. 3).

Parameter of water quality significantly differ between the two study regions (Jazan Sea *vs.* Farasn Island). The six parameters; PH; 6.8 *vs.* 4.3, temperatures; 24 *vs.* 22, and organic materials; 0.28 *vs.* 1.7; DOC, TOC, and BDOC were significantly higher in the Jazan Sea, whereas $O_2$ was observed to have lower values in Jazan Sea. TDS was relatively similar between the two regions 1.13 mg/l in Farsan Island and 1.26 in Jazan Sea (Table 1). In general, components of water quality in Farasan Island were extremely better than Jazan Sea (fig. 4).

**Fig. 2: Prawn Shrimp (P. monodon) collected from Jazan sea**

**Fig. 3: Prawn Shrimp (*P. monodon*) collected from Farasn island**

**TABLE 1: PARAMETERS OF WATER QUALITY COMPONENTS; $O_2$ (mg/l), TEMPERATURE, BDOC %, PH, TDS (mg/l), TOC (mg/l), DOC (mg/l) AS A COMPARISON BETWEEN FARASAN ISLAND AND JAZAN SEA**

| DOC (mg/l) | TOC (mg/l) | TDS (mg/l) | PH | BDOC % | Temp. | $O_2$ (mg/l) | Region |
|---|---|---|---|---|---|---|---|
| 9.36 | 1.21 | 1.13 | 4.3 | 9.28 | 22 | 4.7 | Farasan Island |
| 15.84 | 4.39 | 1.26 | 6.8 | 14.7 | 24 | 1.8 | Jazan Sea |

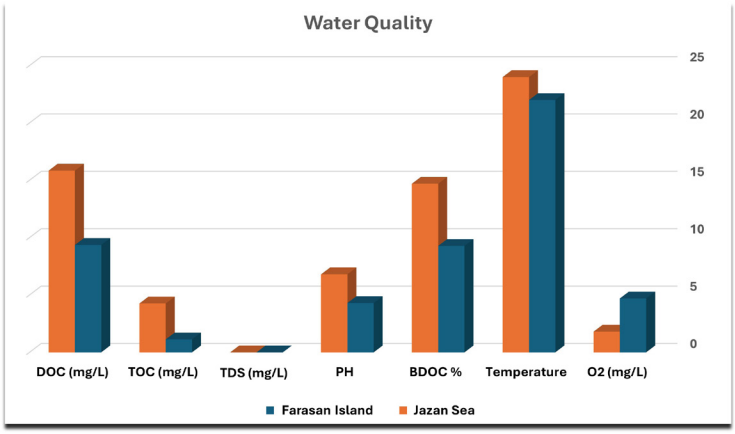


**Fig. 4: Comparison of $O_2$ (mg/l), Temperature, PH, TDS (mg/l), TOC (mg/l), DOC (mg/l) and BDOC %, between prawn shrimp (*P. monodon*) collected from Jazan Sea and Farasn Island**
**Note: (■): Farasn Island and (■): Jazan Sea**

The results showed significant variation in the morphological characteristics of prawn shrimp species between Jazan Sea and Farasan Island, as we noticed that all three water quality parameters: weight (g), body length, and antenna length (mm) showed higher values for prawn shrimp species collected from Farasan Island than those species in Jazan Sea (Table 2 and fig. 5).

The color of prawn shrimp (*P. monodon*) appears to vary between the two study areas. Farasan Island does not have any dark prawn shrimp (*P. monodon*) or a dark spot of their body, such as the head, abdomen, or tail. All species exhibited the full percentage of light shrimp color (100 % light shrimp color and 0 % dark shrimp color; fig. 3 and fig. 6). In comparison, Jazan Sea shrimp has 35 % light shrimp color and 65 % dark shrimp color (black spot over the body; fig. 2 and fig. 6).

**TABLE 2: COMPARING MORPHOLOGICAL FACTORS OF 20 PRAWN SHRIMP (*P. monodon*) USED FOR TISSUE EXTRACTION AND PARASITE DIAGNOSE; 10 BRAWN SHRIMP WERE COLLECTED FROM JAZAN SEA AND OTHER 10 FROM FARASN ISLAND**

| Color | Weight (g) | Antenna length (mm) | Body length (mm) | Region |
|---|---|---|---|---|
| Light prawn | 37.4673 | 13 | 9.7 | Farasan Island |
| Light prawn | 28.9872 | 12.8 | 10.8 | Farasan Island |
| Light prawn | 35.8973 | 15 | 11.3 | Farasan Island |
| Light prawn | 33.21 | 14.7 | 12.6 | Farasan Island |
| Light prawn | 39.378 | 17 | 10 | Farasan Island |
| Light prawn | 32.441 | 8 | 14.8 | Farasan Island |
| Light prawn | 26.7652 | 16.4 | 9.3 | Farasan Island |
| Light prawn | 37.8398 | 11 | 9.4 | Farasan Island |

| | | | | |
|---|---|---|---|---|
| Light prawn | 32.7653 | 14 | 11.6 | Farasan Island |
| Light prawn | 34.9883 | 16 | 10.7 | Farasan Island |
| Light prawn | 33.7365 | 10.4 | 5.9 | Jazan Sea |
| Dark prawn | 28.773 | 8.2 | 10 | Jazan Sea |
| Light prawn | 29.6781 | 11.7 | 8.5 | Jazan Sea |
| Dark prawn | 25.811 | t | 5.4 | Jazan Sea |
| Light prawn | 24.7925 | 9.5 | 7.4 | Jazan Sea |
| Light prawn | 25.9562 | 3.7 | 5.2 | Jazan Sea |
| Dark prawn | 25.17 | 7.8 | 8.2 | Jazan Sea |
| Dark prawn | 23.2111 | 5.9 | 7.1 | Jazan Sea |
| Light prawn | 27.378 | 10.6 | 9 | Jazan Sea |
| Dark prawn | 32.441 | 8.9 | 6.8 | Jazan Sea |

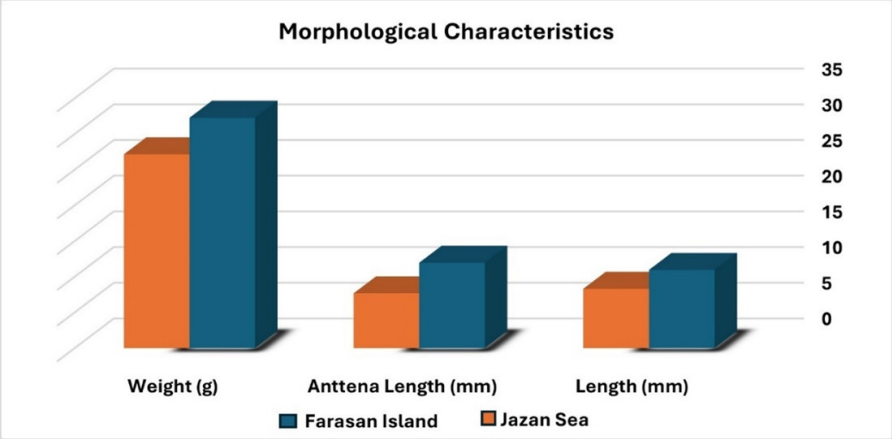


**Fig. 5: Comparison of weight (g) and the length of body and antenna (mm) between prawn shrimp (*P. monodon*) collected from Jazan Sea and Farasn Island**
**Note: (■): Farasn Island and (■): Jazan Sea**

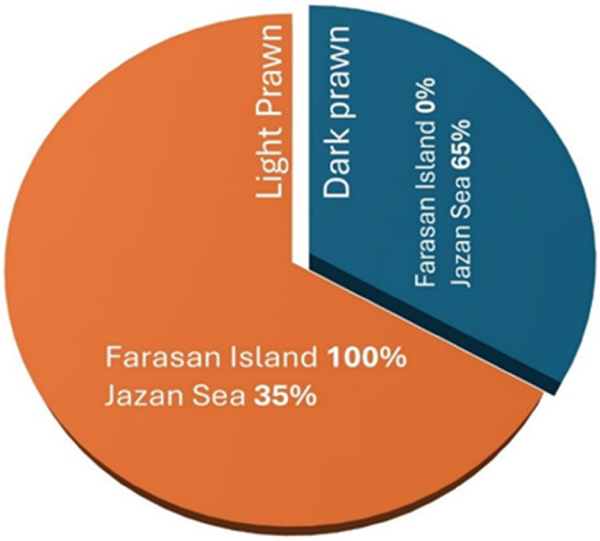


**Fig. 6: Color ratio of prawn shrimp (*P. monodon*) between two regions: Jazan Sea *vs.* Farasan Island**

A strong relationship was found between water quality components and the increased incidence of shrimp fungal disease caused by *F. solani*. The water quality of the Jazan Sea is characterized by high concentrations of organic matter in terms of temperature, pH, TOC, DOC, BDOC, and $O_2$ (fig. 4). These conditions may provide a suitable environment for the growth of *F. solani*, which invades most shimp species. This species may be highly susceptible to pollution in the Jazan Sea, which contains bioaccumulation of heavy metals and hazardous compounds that increase fungal populations[19]. Therefore, most shrimp species in Jazan are affected by BSD, one of the most common diseases associated with *F. solani*[16]. In most studies, tissues were isolated from black spot, and the presence of *F. solani* was identified reinforces previous study on the fact that shrimp with black edges or a black head and tail were relatively affected by *F. solani*[20-22]. 65 % of the dark shrimp color was recorded in shrimp species in Jazan (fig. 2 and fig. 6) and 0 % in Farasan Island where water quality components were free of pollution (Table 1), indicating the presence of non-black spot brown shrimp species without change in morphological characteristics.

The morphological characteristics of the brown shrimp varied significantly between the two study areas in terms of shape, color, weight, body length, and antennal length (fig. 5), and were associated with water quality. The Farasan prawn shrimp (*P. monodon*) had significantly long body, long antennal, and high weight, which were associated with excellent water quality and the presence of non-fungal parasites (Table 1). Therefore, the body size of prawn shrimp (*P. monodon*) appears to be normal, compared to the Jazan shrimp (fig. 2 and fig. 3), where water components, such as high pH, temperature, or low oxygen, reduce body length, antennal length, and weight (fig. 5). We did not observe any wounds or scratches, even during the molting process.

These findings may reveal the impact of water pollution on freshwater communities through fungal diseases. Water quality pollution can cause significant mortality in these organisms, affecting species decline, growth, diversity, abundance, behaviour, and fragility. Thus, there is6 a close link between water quality and the increase in fungal diseases affecting prawn shrimp (*P. monodon*), which alter the morphological characteristics of prawn shrimp (*P. monodon*). These mechanisms pose a significant threat to human health as consumers of infected shrimp. Humans are at risk of accumulating dangerous concentrations of contaminants in their bodies as a result of their consumption of seafood, in addition to causing acute, lethal, or non-lethal harm to organisms, such as impaired growth and reproduction or accumulation in fatty tissues, causing abnormal growth and reproduction. This problem could also gradually impact the economy if it reaches epidemic levels among freshwater communities and the marine ecosystem. Therefore, we aim to establish a theoretical basis for the early prevention and control of fungal shrimp diseases. The upcoming study is expected to provide insights into preventing or mitigating shrimp disease factors.

**Author's contribution:**

The experiments, including laboratory work, data collection, statistical analyses, and manuscript writing, were conducted by Sultan Areshi at the Biology Department of Jizan University.

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**Conflict of interests:**

The authors declared no conflict of interests.

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