Black gill disease of cage-cultured ornate rock lobster *Panulirus ornatus* in central Vietnam caused by *Fusarium* species

Nha, V.V.[1], Hoa, D.T.[2] and Khoa, L.V.[3]

1. Research Institute for Aquaculture No.3, 33 Dang Tat str., Nha Trang, Khanh Hoa, Vietnam, Email: lvkhoa@dah.gov.vn;
2. Nha Trang University, 02 Nguyen Dinh Chieu str., Nha Trang, Khanh Hoa, Vietnam;
3. Department of Animal Health, 15/78 Giai Phong str., Dong Da dist. Hanoi, Vietnam.

In Vietnam, cage-culture of ornate rock lobster *Panulirus ornatus* has been practiced for several decades in the central coastal region, especially in Phu Yen and Khanh Hoa provinces. In recent years the lobster industry has been contributing significantly to national commercial sector, yielding approximately US $ 100 million annually[15,17]. During 2003 – 2005, a research group from Research Institute for Aquaculture No. 3 investigated 272 lobster cages in Phu Yen, Khanh Hoa provinces and found that moribund and dying lobsters showed some gross clinical signs such as red body, blistered gills and black gills. Of these, black gills appeared to have caused mortality in 69.5% of cages out of the 272 cages studied. Mortalities were found mainly at the grow-out stages, thereby causing great losses for the industry[16]. Black gill disease was first reported in 1975 from American spiny lobster *Homarus americanus*[11]. In general, black gill condition in shrimp caused by Fusarium species initially produces generalized "gill discoloration" which gradually develops to "blackened gill" condition and eventually leading to death of affected individuals[6]. Other situations such as exposure to nitrite, ascorbic acid deficiency and infection by infectious hypodermal and haematopoietic necrosis virus, *Flexibacter* or fungus *Haliphthorus* are also known to produce black gill conditions in lobsters[3]. Previously, the disease has never been reported in lobster cultured in Vietnam, although it was known to occur in black tiger shrimp *Penaeus monodon*[9]. This paper describes black gill disease of ornate rock lobster, morphology of the fungus isolated and the pathological aspects of infected animals.

Materials and methods

Isolation and identification

Ornate rock lobster *P. ornatus* (30 to 220 g in body weight) showing gill discoloration from pale brown to black and/or wounded were collected from cages for examination. Small pieces of the gills were removed from these animals for observation under a light microscope.

Small pieces of the gill from a total of 97 diseased lobsters were washed in sterile sea water and each sample was inoculated onto a potatoes dextrose agar (PDA) petri disc with 2% NaCl and 1 g/L Streptomycin sulfate and 1 g/L ampicilline to avoiding bacterial contamination. To obtain a pure culture, a single spore culture was made 4 days after incubation at 30°C. The fungal isolate was inoculated onto PDA and incubated at 30°C for 4-10 days in the dark. Thirty conidia were selected randomly from the isolate and their sizes were measured to be calculated for the average size with standard deviations (SD). The fungus was identified according to Nelson *et al*., 1983[14].

Figure 1: Ornate rock lobster Panulirus ornatus with black gill disease collected from a farm of Khanh Hoa province, Vietnam in 2004.

Pathogenicity challenge

One strain NHT 01 recovered from a diseased lobster cultured at a farn in Van Ninh district, Khanh Hoa province in 2004 was selected from the isolates for an artificial experiment. Ten day old colonies of the fungal strain NTH 01 were used to harvest conidial suspension by adding 10 mL sterile seawater into each culture plate, and collecting the suspension. The fungal conidial suspension was calculated using haemocytometer, and adjusted to three concentrations of 8×10^3, 8×10^4 and 8×10^5 conidia/mL

Healthy lobsters with average weight of 40.2 ± 3.3 g/ were collected and kept in 180 L running seawater tanks for 7 days. Each tank contained 8 lobsters. Seawater was treated with 30 ppm chlorine and maintained at 28°C and pH 8.2. Five experimental tanks were set up for the purpose of experimental infection. Lobsters were injected intramuscularly at the second segment with 0.1 mL of conidial suspension. The control group was injected with 0.1 mL seawater into each lobster. Another group was kept in the same condition but without any injection. Tanks were aerated during the course of experiment. Moribund and fresh dead animals were sampled for observation and re-isolation of fungal elements. Mortality and abnormal behaviours of the experiment lobsters were also recorded.

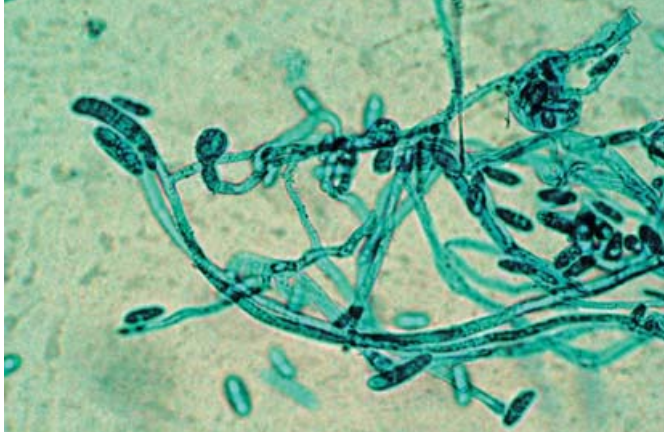

Figure 2: Fungal hyphae and conidia of an ornate rock lobster naturally infected Fusarium solani (cotton blue stain).

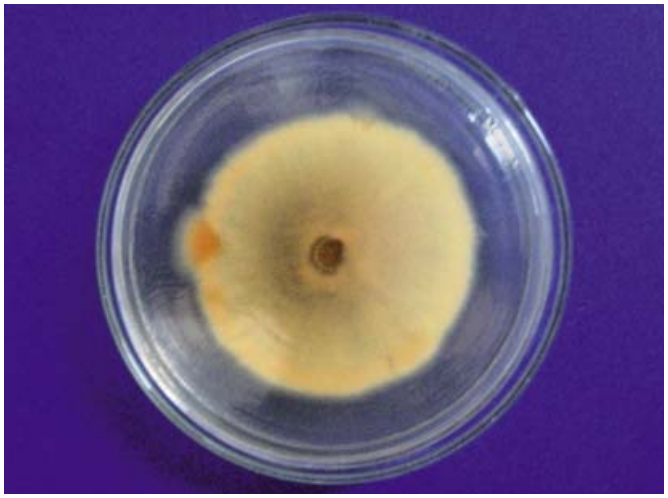

Figure 3: Surface of a 7 d-colony of Fusarium solani NHT 01 on PDA at 30C in the dark.

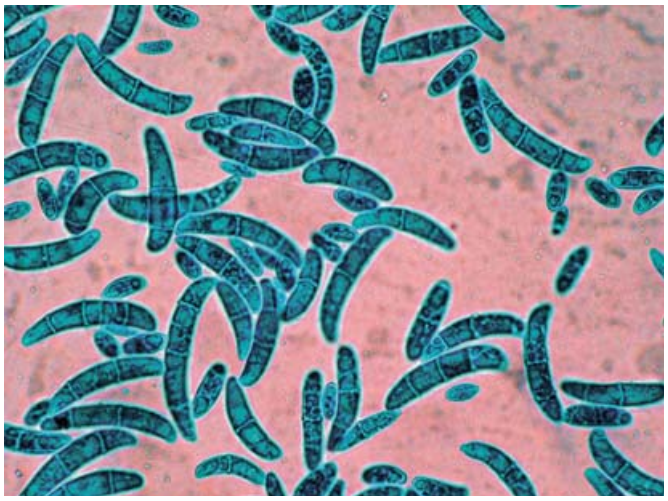

Figure 4: Conidia of Fusarium solani NHT 01 on PDA at 30C in the dark showing 1-4 septates (cotton blue stain).

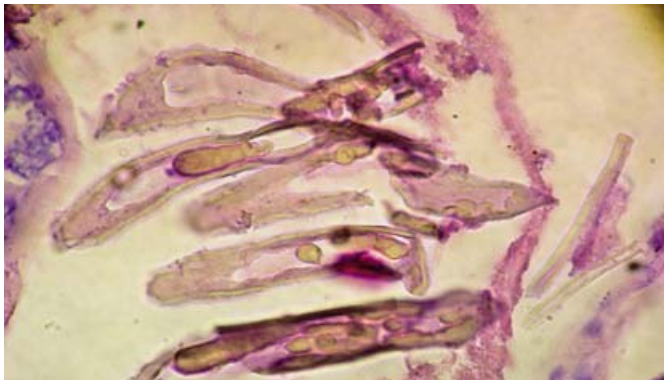

Figure 5: Fungal elements in the degenerative gills (H&E stain) of an artificially infected ornate rock lobster.

Histopathology

Gill samples from all of the 97 diseased lobsters with black gills were collected carefully and fixed in the Davidson solution for 12-36 h, then restored in 70% ethanol. Sampled tissues were embedded with paraffin, sectioned at 5 *µ*m, and stained with haematoxylin and eosin. Slides were observed under a light microscope (OLYMPUS CX31).

Results

Incidences

Based on field observation, lobsters with black gills became weak, lethargic, pale, had difficulty in respiration and were usually observed swimming near the water surface. In some cases, fouling by *Balanus* sp. and juvenile *Pteria* sp. were also observed on the shell. Gills became red brown to black. The lesions appeared to eventually destroy the gill filaments in the advanced stage of infection and spread out off the gills. Black spots due to formation of melanotic pigment were always observed in the gills of the infected lobsters (figure 1). Wet mounts of gill lesions showed the presence of invasive fungal mycelia and conidia (figure 2) in all diseased animals.

Septate mycelia of NTH 01 extending from the gill filaments and their conidia were clearly observed under a microscope. Ninety seven fungal isolates were recovered from total 97 infected lobsters (100%) with black gills.

Fungal isolation and identification

All the fungal strains recovered from the 97 diseased lobsters had similar character of conidial shapes and colony. Therefore, a strain NTH 01 was selected for further morphological observation in order to identify into species. The microscopic characteristics of the strain NHT 01 was described as follows:

Colonies on PDA at 30^{o}C were white to olive yellow or pale yellow to brownish yellow in aged cultures, 73.1 ± 0.8 mm after 7 days of inoculation (figure 3). Hyphae were septate and hyaline, 2.42 ± 0.41*µ*m in diameter. Conidiophores were elongated and monophialides forming microconidia in the aerial surface. Conidiophores were simple (non-branched) or branched monophialides. Microconidia were abundant, oval or ellipsoid, usually with one-cell, (11.6 ± 2.07 *µ*m) x (3.8 ± 0.8 *µ*m). Macroconidia were produced after 7 days of inoculation, usually abundant, subcylindric or slightly curved, 2 – 4 septates, predominantly 3-septate (24.7±1.9)*µ*m x (5.0±0.6) *µ*m (figure 4). Chlamydospores were formed on terminally lateral branches or intercalary and occasionally in chains or in pair. The fungus was identified as *Fusarium solani*.

Pathogenicity challenge

Ornate rock lobsters artificially infected with NTH 01 showed similar clinical signs to naturally infected animals. Cumulative mortality after 14 days were 57.1%, 72.4% and 77.1% in the 3 groups inoculated with conidial concentrations of 8 x 10^3, 8 x 10^4 and 8 x 10^5 conidia/mL, respectively. Control groups remained healthy, showed no mortality and no fungal elements in the gills during the course of experiment. Re-isolated fungus was morphologically similar to NTH 01.

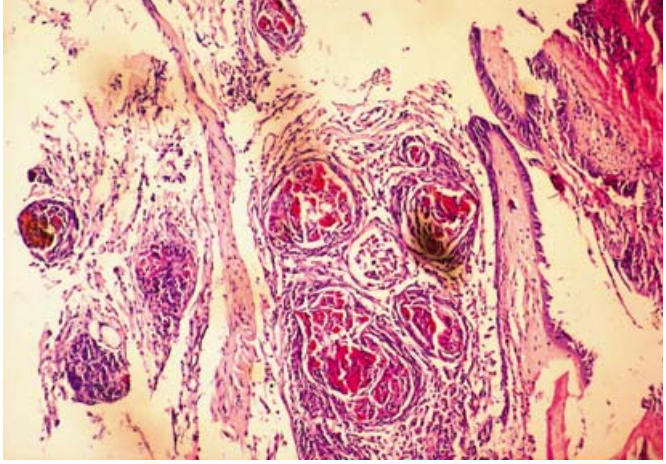

Figure 6: Fungal hyphae encapsulated by multiple layers of fusiform haematocytes (H&E stain) in the gill tissue of an artificially infected lobster.

Histological changes

Gill lesions of the artificial infected lobsters showed fungal mycelia inside the cuticle. Fungal elements in the degenerative gills were observed as threads in haematoxylin and eosin stained sections (Figure 5). Cross sections of the gill lesions showed that the fungal hyphae were encapsulated with multiple layers of fusiform haematocytes (figure 6).

Discussion

This study reported a first case of *F. solani* causing black gill disease in cage-cultured *Panulirus ornatus* in Vietnam. Attempts have made to recover 97 fungal strains from 97 ornate rock lobsters cultured in cages with black gill condition. The fungus was identified as *Fusarium solani* which is similar to a fungus reported from American lobster *Homarus americanus*.[11] *F. solani* is frequently isolated from American lobsters Homarus americanus[11], shrimp such as *Penaeus japonicus*[2] and *P. californiensis*[5], and sharks[1,12]. In Vietnam, several researchers have reported *Fusarium sp*. from *Panulirus sp*.[4] and shrimp[7], however, species identification had never been described until a study on black gill disease of *P. monodon* was made during 2001-2005[6]. Of which *F. incarnatum* was reported as a causative agent[9].

Pathogenesis and pathogenicity of *Fusarium* species to crustaceans have been known amongst fish pathologists for some time. In this study, *Fusarium solani* NTH 01 also showed high pathogenicity to healthy lobsters indicating that this is an important pathogen.

In addition, milky haemolymph syndrome was also known as the most problematic for spiny lobsters *Panulirus* sp. cultured in Vietnam with possible causative agent by Rickettsia-like bacteria[10]. Poor quality of water environment is usually accompanied with high mortality[13].

Finally, although lobsters are generally well equipped for the natural environment[18], unfavourable conditions such as polluted water, high density and overfeeding may lead to some disease problems. Good farm practices and better planning are highly recommended for successful lobster aquaculture.

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