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Necrotizing hepatopancreatitis (NHP) infected *Penaeus vannamei* female broodstock: Effect on reproductive parameters, nauplii and larvae quality

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Abstract

Necrotizing hepatopancreatitis (NHP) is a disease of cultured *Penaeus vannamei* caused by a gram-negative intracellular bacterium rickettsia-like organism. NHP was first reported in Texas in 1985, as being responsible for mortalities in shrimp ponds in Central and South America. The growth and proliferation of NHP within the hepatopancreatic epithelial cells is associated with anorexia, lethargy, abdominal muscle atrophy, softened exoskeleton, growth retardation and mortalities ranging from 20% to 95%.

Although NHP can cause high mortalities in commercial ponds, it has been considered a problem only in broodstock ponds and maturation laboratories due to an increase in broodstock mortalities in Colombia. In order to evaluate additional negative effects at this level, we investigated the effect of NHP infection upon the reproductive behavior of females and the effect of maternal infection on nauplii and larval quality.

Broodstock from NHP affected ponds were transferred to a maturation laboratory. After copula, females were transferred to individual spawning tanks. After spawning, females were sacrificed for histopathology and PCR analysis, while the eggs were placed in individual tanks for hatching. Females were classified into three groups according to their histopathological findings: NHP negative ($n=23$) had no NHP lesions, positive for NHP with lesions grade 1 ($n=20$) and NHP positive females with lesions grade 2 ($n=12$). For each group, we analyzed the number of eggs, hatching percentage and number of nauplii per female. Our results show that females with NHP lesions grade 2 presented a significant reduction in the number of eggs and nauplii ($P<0.05$) as compared with NHP negative females and females grade 1 NHP disease. No significant differences were found in hatching percentage among the three groups.

Triglyceride levels in nauplii II were significantly higher in NHP negative females than in females with grade 2 NHP ($P<0.05$), whereas there was no difference between NHP negative and grade 1 females. No significant differences were found for glucose and cholesterol levels in nauplii among the three groups.

No differences were found in survival at zoea I, mysis I, PL-1 and PL-10 for the three groups. However, after a salinity stress test, the PL-10 survival decreased significantly in larvae from NHP positive females. Also a significant decrease in PL-10 length was also observed in the progeny of these females (grades 1 and 2).

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This study demonstrated that NHP disease in female shrimp spawners affects both maturation and larviculture causing a decrease in the number of eggs and nauplii per female, a reduction in the levels of triglyceride in nauplii II and decreasing larvae growth and resistance to osmotic stress test at PL-10 stage.

Keywords: *Penaeus vannamei*; Necrotizing hepatopancreatitis; Broodstock; Larvae quality; Triglyceride

1. Introduction

Necrotizing hepatopancreatitis (NHP) is a disease of penaeid shrimp caused by a gram-negative intracellular rickettsial-like bacteria (NHPB), which target the tubular epithelial cells of the hepatopancreas (HP). Clinical signs of NHP disease include reduced feed intake, empty guts, soft shells, flaccid bodies, black or darkened gills, lethargy and marked atrophy of the hepatopancreas. Mortalities can be severe ranging from 25% to 95% in severely affected ponds (Loy et al., 1996). NHP was originally identified in 1990 in cultured *Penaeus vannamei* in Texas (USA) (Frelier et al., 1992) and has now been reported in several locations throughout the Western Hemisphere including Peru, Ecuador, Venezuela, Brazil, Nicaragua, Panama, Costa Rica and Mexico (Loy et al., 1996; Aguirre-Guzmán and Ascencio-Valle, 2000). In Colombia, NHP disease does not cause mortalities in commercial ponds, but affects broodstock rearing ponds and shrimp in maturation laboratories, resulting in loses and decrease nauplii availability (Brinez et al., 2003).

As NHP mainly damage shrimp hepatopancreas, it might impair its function on lipid transfer and storage. Transfer of lipids from the hepatopancreas to the ovary via the haemolymph during ovarian maturation has been demonstrated in *Penaeus japonicus* females (Teshima et al., 1989). Penaeid species present an increase of total lipid concentration in the ovaries and, in most species, a concomitant decrease of the total lipid level in the hepatopancreas can be observed. It was therefore thought that the hepatopancreas was the origin of the lipids accumulated in the ovaries (Wouters et al., 2001). However, evidence shows that a majority of the accumulated ovarian lipids originate from the diet and not from de novo lipid synthesis, which is too low to significantly contribute to the increase in ovarian lipids (Wouters et al., 1999).

Considering that NHP severely affects the hepatopancreas and markedly reduces feed intake, it might interfere with ovarian lipids contents by decreasing lipid storage and transfer and by diminishing lipid ingestion through the diet. As hatching, nauplii development and metamorphosis to zoea require large amounts of energy that are transferred from the female. It has been postulated that eggs with more metabolic reserves have a greater probability of producing nauplii that will survive to zoea stages (Lavens and Sorgeloos, 1991; Ouellet et al., 1992). To determine whether NHP disease in the female could modify their fertility, the present study analyzes the reproductive parameters, nauplii biochemical composition and larval growth, survival and PL10 resistance to a salinity stress test in females with and without NHP disease.

2. Materials and methods

2.1. Animals and maturation

Adult shrimp *P. vannamei* (>35 g) were selected from a broodstock raising pond with confirmed NHP infection by histopathological analysis of symptomatic animals and final pond survival of 12.5% during the broodstock culture stage. Survivors were stocked in a maturation laboratory. After an acclimation period, 1000 females were subjected to unilateral eyestalk ablation. Females were stocked with males (1:1.35 sex ratio) in black fiberglass maturation tanks at a density of 6 animals m^2. Water was exchanged daily (300%), temperature was maintained at 28 °C and salinity kept at 35 ppt. Diet consisted of 50% squid, 15% polychaetes, 30% Artemia and 5% commercial formula, divided into four equal daily rations that were adjusted to equal a total daily supply equivalent to 25% live wet weight.

2.2. Experimental design and evaluation of reproductive parameters

In order to minimize variation due to spawning in different days, only females that spawned at a given day and (included animals with clinical signs of NHP infection) were analyzed. Two different assays were performed: in the first experiment, reproductive parameters were evaluated in 31 copulated females, while in the second experiment, reproductive parameters, as well as nauplii and larvae quality were determined in 24 spawns.

Copulated females were transferred to individual spawning tanks of 250 l, filled with filtered seawater (30 ppt salinity and 32 °C). After spawning, each female was sacrificed for histopathology and PCR diagnosis of NHP.