

Experimental Transmission of Necrotizing Hepatopancreatitis Bacteria to Post-Larval *Litopenaeus Vannamei*

A.S. Leaflet R2256

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Summary and Implications

Day 15 specific pathogen free Post-Larval Pacific White Shrimp, *Litopenaeus vannamei*, were infected with Necrotizing Hepatopancreatitis bacteria (NHPB) by *per os* exposure. This is the first documented report of controlled post larval (PL) infection and mortality with NHPB.

Introduction

Necrotizing Hepatopancreatitis Bacteria is an obligate intracellular pathogen that causes serious economic losses throughout shrimp farms in the Americas. Specific challenge models for this disease currently do not exist. The goal of this study was to determine if post larvae could be infected, and to determine their utility in challenge models.

Materials and Methods

Specific pathogen free PL15s from the Kona line were received from the Oceanic Institute, Hawaii. After a 2 day acclimation period 200 post larvae were individually counted into 2 tanks of approximately 300L each. After a 24 hour fasting period, each tank was fed 3.0 g of either PCR positive infected or PCR negative SPF hepatopancreas tissue macerate. Challenge hepatopancreas tissue was negative for growth on TCBS agar after a 24 hour incubation at 37 C. PCR positive necrotizing hepatopancreatitis bacteria infected tissue was acquired from an NHP propagation tank stocked with 10-20 g shrimp that had been exposed to NHPB for a period of 31-40 days.

Results and Discussion

Survival rates from NHP challenge tanks ranged from 0-6% survival and from 70-86% in the SPF control tanks (Table 1) over a period of 21-25 days (Figure 1). NHPB diagnostic PCR (Lightner) was conducted during and after each study. The SPF negative controls remained PCR negative while moribund NHP challenged shrimp were positive at day 15 and upon termination of study. These experiments demonstrate that *Litopenaeus vannamei* post larvae are susceptible to infection and mortality with NHPB. Previously established challenge models for NHP have utilized adult shrimp exposed to infected material *per os* for disease challenge studies. These methods are costly and time consuming due to the cost of shrimp rearing and extended incubation period of the disease, therefore this experiment utilized post larvae to challenge

large numbers of individuals simultaneously. Such challenge techniques would be useful for determining differences in NHPB strain virulence and for evaluating large numbers of individuals or lines for genetic resistance.

Figure 1. Post larvae survival over time in days.

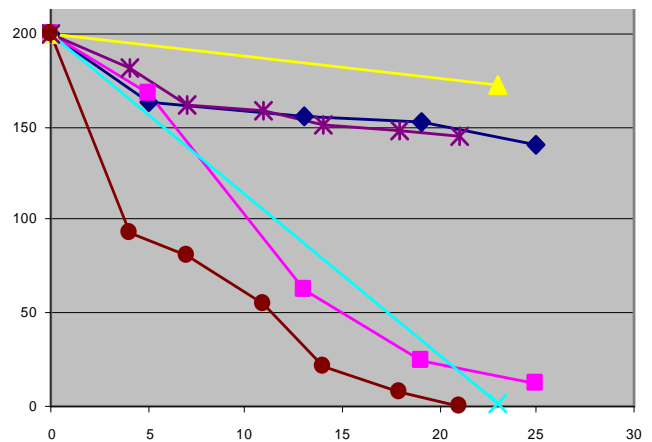


Table 1: Post larvae survival percentage in replicates

NHP 42 SPF control	72.5% survival
NHP 42 challenge	0% survival
NHP 37 SPF control	70% survival
NHP 37 challenge	6% survival
NHP 36 SPF control	86% survival
NHP 36 challenge	0.5% survival

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