Quantifying Gregarine Infestation of *Penaeus vannamei* on a Commercial Shrimp Farm and Some Attempts at Treatment

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Introduction

During the first months of 1990, many shrimp farms in Ecuador experienced a significant decline in production due to increased mortality. Investigations began to determine the cause and biological studies have continued (Espinoza et al. 1991; ESPOL 1991).

Various gregarines *(Nematopsis* spp.) are known to reside in the digestive tract of cultured shrimp (Johnson 1989; Jimenez 1992). These common parasites became the focus of our investigation. A relatively rapid diagnostic method was needed to determine the incidence and severity of the infestation within each pond population. Feed trials were also conducted in an attempt to control the parasite.

Materials and Methods

1. Counting the Parasites

Most production ponds are stocked with juvenile shrimp transferred from larval rearing ponds. On the night of their transfer, a minimum of 20-30 juvenile shrimp were randomly selected and 25 were examined to determine the incidence and severity of *Nematopsis* spp. infection from each pond. If this was left until the next day, the parasite count was always lower because the animal had excreted most of the intestinal contents. Therefore only freshly killed shrimp recently taken from a pond were used for analysis.

Each animal was split at the abdominal-cephalothorax junction and its gut was separated by forceps from the posterior portion of the hepatopancreas and placed on a microscope slide. The condition of the gut was recorded as empty, partial or full. A 22 mm x 22 mm coverslip was used to scrape the gut contents onto the slide and then to cover them, dispersed in one or two drops of distilled water. This scraping is very important as *Nematopsis* spp. tends to attach to the epithelial lining. Before counting, a few light taps on the coverslip facilitated distribution and accurate counts. The empty gut was placed under another coverslip on the same slide and examined for any remaining parasites. A microscope (x100 magnification) was used to count the parasites under both coverslips on the slide.

For larger animals with full guts, the volume of material was so great that it became impossible to distinguish the contents of the intestine due to the concentration of material under the coverslip. In this case, the procedure was to scrape the maximum volume of gut contents onto the slide, allowing enough light to pass so that an accurate count could be made across the whole slide. The anterior portion of the midgut was scraped first and, for a large shrimp with a full gut, the posterior contents were discarded without being analyzed. In shrimp, *Nematopsis* spp. are found in the trophozoite stage, which varies in shape from the early oval-shaped form to the larger finger-shaped form.

2. Attempts to Control Infestation

An attempt was made to control *Nematopsis* spp. in larval and production ponds by using medicated feeds. Feeding trials were completed in 0.5-ha larval rearing ponds stocked at 1.2-1.6 million per hectare. Twelve ponds were stocked in April 1990. Three were used as controls and received a 30% protein feed without medication. The other three groups of three ponds were each fed a medicated feed. All feeds used in the trials were made by the same company, Molinos Champion S.A., Guayaquil, Ecuador. The first group received a 22% protein feed with an antibiotic (a potentiated sulfa drug mixture); the second, an antigregarine feed (active ingredient and dosage not disclosed by the feed company); and the third received one week of antibiotic feed and one week of antigregarine feed, alternating each week until the time for transfer. Feed was given on a daily basis at a rate of 5% estimated biomass initially, decreasing to 2% in the pond as the shrimp grew, adjusted on a weekly basis.

The shrimp were transferred after 30-40 days of growth in the larval rearing

ponds and examined according to the above procedure. When a production pond was fully stocked, sometimes receiving juveniles from four larval ponds, five shrimp were sampled every week from each pond during the 15-week production cycle. Deli Shrimp has 26 ponds on this farm.

Results and Discussion

The feeding trial results are summarized in Table 1. One pond among the control set had excellent survival (99%) and few *Nematopsis* spp. (3%) were stocked with wild larvae which accounted for 16% of the total number of juveniles from the control ponds. It appears that the source of the seed is an important factor for the health of juveniles. This same relationship, high survival with low infestation rate, was seen in the production ponds as well.

Eight production ponds were stocked between 17 April and 11 May in 1990. Although only duplicate ponds were studied, interesting results were seen (Table 2). The two ponds that showed the lowest incidence of infestation and the lowest parasite burdens were those stocked with wild larvae. The variations in the data suggest that there may be other factors involved. Bacterial (*Vibrio*) levels were not measured during the trials and could be an important factor.

Considering the short span of time that ingested material remains in the shrimp gut, it may be necessary to use higher concentrations of antigregarine agents for effective control. The dosages that have proven effective in chickens or fish may not apply to invertebrates like shrimp.

In subsequent cage studies, it was observed that if infested shrimp have no contact with the pond bottom, they will eventually clear themselves of infestation. This has also been seen in aquarium studies. Such arrangements are not, however, practical management tools in commercial production.

Conclusion

There is an inverse relationship between the severity of infestation of shrimp with *Nematopsis* spp. and their survival. The medicated feeds used here did not alleviate the infestation nor improve survival, but more work is needed. The source of seed appears to be important because wild postlarvae performed better (higher survival, lower infection) than those from the laboratory. Practical diagnostic methods and other shrimp parasites need to be further standardized for comparative studies. Studies on the absorptive capabilities of the shrimp gut would also be useful for determining appropriate levels of antigregarine or antibacterial agents.

Table 1. Survival, percentage infestation with *Nematopsis* spp. and parasite burdens in shrimp (*Penaeus vannamei*) from larval rearing ponds after treatments with different medicated feeds.

Feed medication	No. of ponds sampled	Shrimp survival (%)	Shrimp with *Nematopsis* spp. (%)	Average no. of parasites per shrimp
1.Antibiotic	3	52	82	35
2.Antigregarine	2[a]	66	90	83
3.Alternating 1,2	3	73	69	46
4.None (Control)	3	75	47	29

[a]One replicate was discarded because the shrimp from this pond had empty guts.

Table 2. Survival, percentage infestation with *Nematopsis* spp. and parasite burdens in shrimp (*Penaeus vannamei*) from production ponds after treatments with different medicated feeds. The data are from duplicate ponds.

Feed medication	Shrimp survival (%)	Shrimp with *Nematopsis* spp. (%)	Average no. of parasites per shrimp	Origin of stocked juveniles
Alternating one week antibiotic feed with one week antigregarine feed	90 74	83 37	45 4	laboratory wild
Rotating one week antibiotic feed, one week control non-medicated feed, one week antibacterial feed	47 32	74 95	34 106	laboratory laboratory

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