

Adding paprika to *Penaeus vannamei* maturation diet improves nauplii quality

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Nauplii quality often declines in commercial systems following sustained nauplii production by *Penaeus vannamei*. Changes include a color loss in both mature broodstock ovaries and larval egg yolk, increased larval deformities, reduced larval feeding rates and reduced survival to larval stage zoea II (Z-II). Experiments were conducted to study the effects of addition of paprika to *Penaeus vannamei* (Pacific whiteleg shrimp) maturation diet under simulated commercial conditions. In one experiment, a group of broodstock afflicted by pigment deficiency syndrome (PDS), as evidenced by a decline in survival to ZII from 75% to 25%, was used. Food-grade paprika was added to the maturation diet. Four weeks after the paprika addition, nauplii quality improved significantly ($p < 0.01$) with a mean survival to Z-II of 83%. In a follow-up experiment conducted with PDS broodstock, Z-II survival was determined before and after paprika addition to diets fed to both eyestalk ablated and unablated females. During the first four weeks following paprika addition, Z-II survival continued to decline; however, by week 10, Z-II survival improved significantly ($p < 0.01$) for larvae from both ablated and unablated females. Throughout experiment 2, there were no significant differences in nauplii quality or Z-II survival between ablated and unablated females within treatment periods. These experiments indicate that paprika is a simple (inexpensive, readily available) maturation diet additive that may supply some essential nutrient(s) needed for good sustainable quality of nauplii production. In commercial trials in Ecuador where paprika has become a dietary ingredient, no evidence of PDS has appeared during six months of high level production of nauplii.

Introduction

Worldwide production of farmed-raised shrimp experienced exponential growth during the 1980s. Production increased from less than 70,000 tonnes in 1980 to over 730,000 tonnes by 1991.⁽⁹⁾ Virtually all of this tremendous industry growth was based on the use of wild-caught animals. Seedstock was either gathered directly from the sea as postlarvae or produced in hatcheries from wild-caught broodstock. To date, seed production based on sustained reproduction of marine shrimp has not been a principle goal or concern in the commercial industry. Growing interest in the use of certified pathogen-free stocks and selective breeding of marine shrimp⁽¹¹⁾ will require efficient, controlled reproduction systems.

Our long term objective was to develop a high performance nauplii production system using pond-reared, specific pathogen free (SPF) *Penaeus vannamei* broodstock. A system was designed as a commercial prototype so that transfer to commercial users could be easily achieved. Over an 8-year period of study the nauplii production system was continuously refined with the goal of devel-

oping a highly efficient system suitable for commercial applications.⁽¹⁰⁾

A high quality maturation diet is an essential requirement for successful captive production of nauplii.⁽⁶⁾ To date, most nauplii production systems rely on fresh and frozen natural feeds such as mussels, krill, squid and polychaete worms. The diet originally used in our system consisted of a combination of frozen squid and frozen blood worms (*Glycera dibranchiata*) and yielded excellent reproductive performance.⁽¹⁰⁾ Under sustained conditions of high-level nauplii production, however, a general decline in nauplii quality as indicated by declining larval performance was observed. This decline was associated with a loss of pigmentation (bleaching) in the ovaries of mature females and in the egg yolk. We hypothesized that declining larval quality was caused by reduced pigment levels in the yolk of developing shrimp embryos resulting from the loss of ovarian pigmentation and termed the condition pigment deficiency syndrome (PDS).

If PDS was caused by a dietary pigment deficiency, then supplementation of the maturation diet with a simple, inexpensive pigment might enhance larval performance.

Table 1. Operating parameters for the Oceanic Institute nauplii production systems (from Wyban and Sweeney¹⁰)

Stocking density	80 animals per tank (5.5/m ²)
Sex ratio	35 females:4 males
Water exchange	
1830 to 1520 h	13.3 L/min (9.4%/h)
1530 to 1830 h	4.0 L/min (2.8%/h)
Total exchange	200%/day
Temperature	27.5°C to 28.5°C
Dissolved oxygen range	4 to 5 ppm
Photoperiod	13 hours light, 11 hours dark
Feed	4 times/day/tank
0900 h	200 g bloodworms
1130 h	200 g squid
1400 h	200 g bloodworms
2000 h	500 g squid
% biomass	9% bloodworms, 16% squid

Paprika, a source of high levels of certain pigments was suggested as a possible supplement. Paprika had already been found to be an effective, inexpensive pigment source for juvenile American lobsters *Homarus americanus*.⁽⁴⁾ An initial experiment was conducted to determine if supplementation of maturation diet with food-grade paprika could reduce PDS in our nauplii production system. Following encouraging results, a second experiment was

conducted to test again whether paprika could reduce or prevent PDS. Another part of the follow-up study was to evaluate the effects of the paprika-supplemented diet and female eyestalk ablation on nauplii quality.

Materials and Methods

The experiments were conducted at the Oceanic Institute (OI) in Waimanalo, Hawaii, USA. Specific pathogen free (SPF) broodstock *P. vannamei* produced in earthen ponds in the OI shrimp quarantine facility in Kona, Hawaii, were used for all experiments. Broodstock were F2 generation SPF and were approximately 1-year old. Female and male broodstock were approximately 50 g and 43 g each, respectively.

A detailed description of the OI nauplii production system is presented in Wyban and Sweeney.⁽¹⁰⁾ For these experiments, 80 broodstock shrimp (35 females and 45 males) were stocked into 4.3 m diameter maturation tanks (8500 L volume each). Operating parameters used in the maturation system are listed in Table 1. Four times per day broodstock were fed a diet that consisted of alternating frozen squid (California Frozen Foods, Monterey) cut into 0.5 cm cubes and frozen bloodworms (*Glycera dibranchiata*) from Eastern Bait, Hancock, Maine, cut into 7 cm lengths. Paprika supplementation was achieved by marinating the daily chopped squid ration overnight in a refrigerator (8°C) with approximately 2% (2 g paprika per 100 g squid) food-grade paprika (ToneÆs, Des Moines, Iowa).

In experiment 1, all females were unilaterally eyestalk ablated. Paprika supplementation began 12 weeks after ablation and continued for 14 weeks. In experiment 2, a second group of broodstock were used. A total of 35

females were used, 10 were unilaterally ablated while 25 were left unablated. Paprika addition began 10 weeks after ablation and continued for 12 weeks. Larval quality was monitored for 9 weeks following ablation. Larvae from ablated and unablated females were reared separately and survival to Z-II for both groups was recorded separately. This experiment was designed to determine whether diet and/or ablation contributed to PDS.

Larval rearing was conducted in the Oceanic Institute indoor shrimp hatchery and similar procedures were followed in both experiments. Each day, a mixture of nauplii from several spawning tanks was collected. Pooled nauplii were rinsed in filtered seawater.

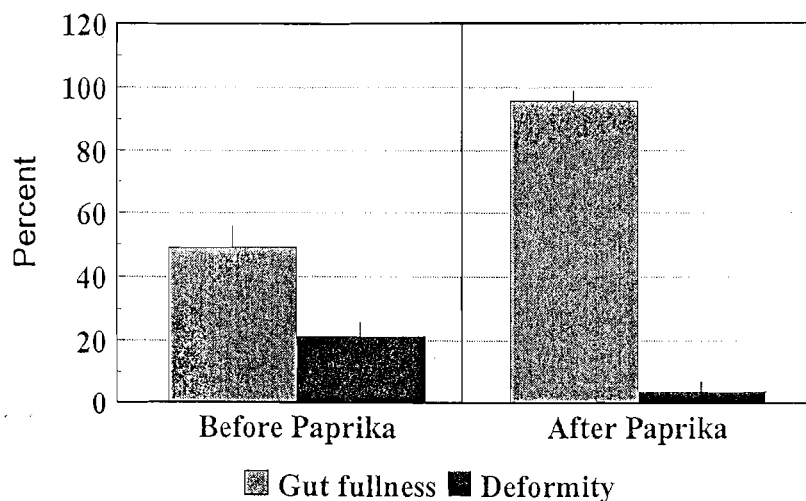


Figure 1. Gut fullness and deformities of larval stage zoea I comparing before and after paprika addition to maturation diet in experiment 1. Bar heights are means and vertical lines on top of bars are standard error of means (SEM).

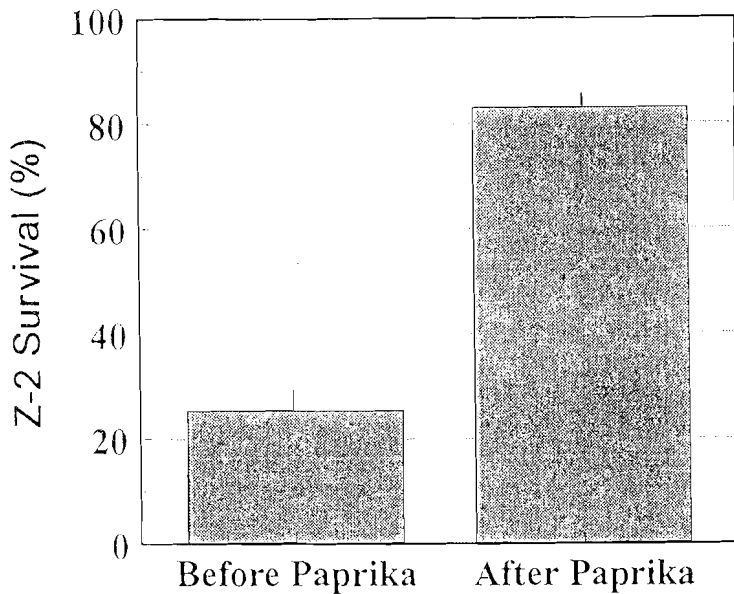


Figure 2. Survival to Z-II comparing before and after paprika addition to maturation diet in experiment 1. Bar heights are means and vertical lines on top of bars are standard error of means (SEM).

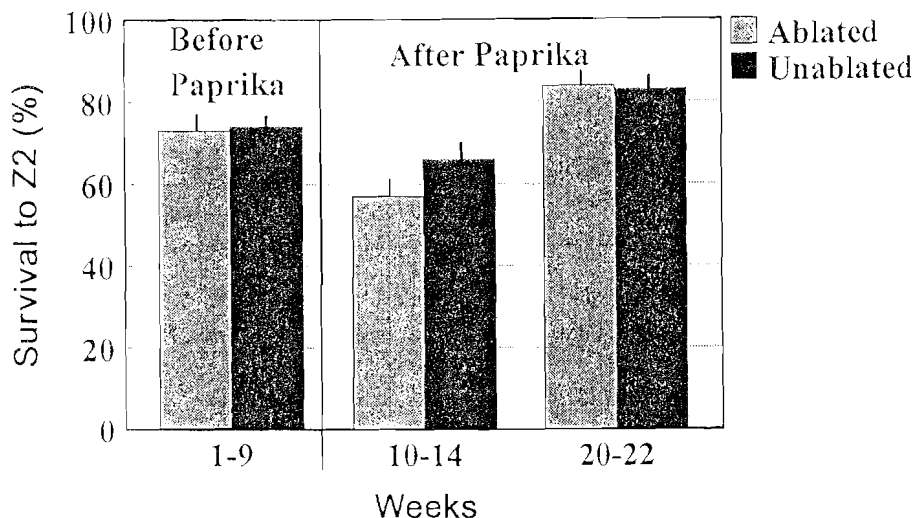


Figure 3. Survival to Z-II in experiment 2 comparing larvae from ablated and unablated females before and after addition of paprika to diet. X-axis scale is weeks after eyestalk ablation. Bar heights are means and vertical lines on top of bars are standard errors of means (SEM).

ter (1 μm \rightarrow 0.5 μm \rightarrow 0.2 μm filters) to eliminate debris and bacteria. One hundred nauplii were stocked in plastic beakers containing 1 liter of 0.2 μm filtered seawater (33 ppt) containing 10 ppm EDTA. In experiment 2, three separate beakers (replicates) were used daily for each treatment. Water temperature in the beakers ranged from 27 to 31°C. Gentle aeration was provided using small airstones. Cultured *Chaetoceros gracilis* was added to each beaker daily to maintain a concentration of 150,000 cells.mL.

Stocked nauplii were examined microscopically. In experiment 1, percent nauplii deformity, including type of deformity and severity, was recorded. The visual degree of pigmentation in the yolk and appendages was also recorded. The number of zoea I with full guts and deformities were counted and recorded as percentages. Survival to Z-II was determined and recorded for each beaker. In experiment 2, larval quality was monitored by recording survival to Z-II.

Results

In experiment 1, four weeks after ablation, a significant decline in larval quality was observed. In Z-I larvae, 52% has empty guts and 21% exhibited moderate to severe deformities, mostly in the tail (Fig. 1). Survival to Z-II had declined to 25% (Fig. 2). These observations led to the search for a dietary supplement that could reverse this condition (PDS).

Four weeks after the addition of paprika to the maturation diet, larval quality significantly improved. Percentage of larvae with full guts increased from 49% before paprika to 96% after the addition of dietary paprika ($p < 0.01$). Percentage of

Table 2. Commercial-scale nauplii production trial in Ecuador using paprika diet (from Garriques and Wyban⁽⁵⁾).

Parameter	
Number of females	266
Duration (weeks)	26
Total nauplii produced	589 million
Total spawns	3,326
Nauplii per female	2.2 million
Nauplii per month	91 million
Nauplii per day	3.4 million
Spawns per day	20
Spawning rate (% females/day)	7.5%
Nauplii per spawn	158,380

deformed larvae declined from 21% to 4% after paprika supplementation ($p < 0.01$) (Fig. 1). Percent survival to Z-II improved from 25% to 83% after paprika supplementation ($p < 0.01$) (Fig. 2).

No significant differences in larval quality were observed between ablated and unablated females throughout experiment 2 (Fig. 2). Through week 9, no decline in Z-II survival was observed. Survival in ablated females was 72.9% compared to 73.8% from non-ablated females. During weeks 10 to 14 (after ablation), survival to Z-II declined in both treatments (ablated and non-ablated) to an average of 61% ($p < 0.01$). By week 19 (10 weeks after the provision of paprika) survival to Z-II significantly increased ($p < 0.01$) and had returned to a mean of 84% (both treatments).

Discussion

Sustained production of high quality larvae is essential for advanced mariculture systems. While the combination of squid and bloodworms is sufficient to produce high levels of nauplii production in *P. vannamei*, this diet appears to lack some critical nutritional factor(s). Their absence resulted in PDS which was characterized by low feeding rates in Z-I larvae, high levels of Z-I deformities and low survival to Z-II. Addition of paprika to the broodstock diet reversed PDS and restored larval quality and survival to normally encountered levels. Paprika supplementation also restored some coloration to mature ovaries that appeared bleached out when PDS was observed.

Scott et al.⁽⁸⁾ listed paprika as containing 275 mg/kg xanthophyll compared to levels ranging from 6,000 mg/kg (marigold) to 20 mg/kg (yellow corn) found in other natural sources. Latscha⁽⁷⁾ reported that paprika contains the pigments capxanthin and capsolue and stated that it is not known whether these pigments can be

transformed by crustaceans. D'Abramo et al.⁽⁴⁾ reported that paprika principally contained the pigment compounds beta-carotene, beta-cryptoxanthin, capsanthin and capsorubin. They found that a paprika-supplemented purified diet fed to juvenile American lobsters (*Homarus americanus*) resulted in increased tissue levels of astaxanthin and suggested a biosynthetic pathway whereby paprika carotenoids are converted to astaxanthin. Our work suggest that, like *H. americanus*, *P. vannamei* may be capable of transforming the carotenoids in paprika to astaxanthin. Interestingly, the effects of this transformation became apparent after a lag-time of several weeks. In both experiments, at least 4 weeks were needed after addition of dietary paprika before the PDS symptoms were reversed.

Based on the results of these experiments, paprika was adopted as a standard ingredient in our shrimp maturation diet. Since adopting this approach, no problems with PDS have been observed during more than four years of continuous maturation work. In a commercial trial of High Health *P. vannamei* broodstock conducted in Ecuador, the paprika-supplemented diet yielded excellent and sustained nauplii production. About 600 pond-reared High Health broodstock *P. vannamei* from Hawaii produced over 589 million nauplii over a 6-month period (Table 2). Excellent larval rearing success was achieved with these animals and no sign of PDS was observed throughout the trials.⁽⁵⁾ These studies demonstrate that paprika is a simple-to-use, inexpensive additive for *P. vannamei* maturation diet and prevents or reverses PDS under conditions of sustained nauplii production.

This research project was funded by the U.S. Department of Agriculture through grant no. CSRS-88-3880503320 to the Oceanic Institute.

Notes and References

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