STRESS RESISTANCE AS A CRITERION TO EVALUATE QUALITY OF POSTLARVAL SHRIMP REARED UNDER DIFFERENT FEEDING PROCEDURES

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ABSTRACT
Crop failures in shrimp grow-out, caused by stocking poor quality fry, unable to adapt to the harsh pond environment is a dreaded phenomenon in shrimp farming. The objective of the present study is to evaluate the resistance of postlarval Penaeus monodon in a simple stress test as a measure of their quality and to establish a relationship between the results and the feeding strategies employed during larval and postlarval phase. For this Penaeus monodon postlarvae of different age and fed different live and artificial diets were exposed to decreased salinities. Although resistance generally improved with age of postlarvae, remarkable differences were found among the feeding treatments, reflecting the variable physiological condition of the shrimp fry fed these different diets. In all stages better resistance was observed in fry fed a nutritionally balancedω3-HUFA rich larval diet.

Resistance in early postlarval stages was dramatically improved by feeding a postlarval diet consisting of HUFA enriched Artemia. In later postlarval stages the effect of enrichment of Artemia seemed to be less pronounced.

This study is part of a program to develop species specific stress test procedures to evaluate resistance and ultimately quality of penaeid shrimp fry.

INTRODUCTION
Success of a shrimp farm depends, among other factors, on the quality and state of health of the postlarvae used for stocking the ponds. Despite adoption of acclimation techniques (Maugle, 1988), successful adaptation of the postlarvae to the harsh pond environment is not always obvious; it may indeed greatly vary with the source of the postlarvae - e.g. wild vs hatchery reared - as well as with the specific culturing techniques applied in the hatchery.
In that respect, more and more producers and buyers have become aware of the importance of the quality of the fry and are already applying visual criteria such as color, activity, shell cleanliness, gut content and muscle development, to evaluate the health condition of the shrimp. Although very valuable, these criteria however, do not allow to exactly quantify quality differences. Moreover, some of these criteria require a microscope which is not always available under field conditions.

The aim of the present study was to evaluate the resistance of postlarval shrimp in a simple stress test as a measure of their physiological condition and possibly their quality, and to establish a relationship between these results and the feeding regime applied during larval and postlarval culture.

In designing the stress test, it was kept in mind that, in addition to being 'useful' for research purposes, for example as a quantification of diet effectiveness, the test should also serve as a simple tool for the farmer to assess the quality of the shrimp prior to stocking.

Consequently, the stress test should meet the following criteria: (1) easy to standardize even under field conditions; (2) the test should allow simple quantification of the results and (3) the stress evaluation should be possible in a short time.

In this regard we decided to develop a test in which the postlarvae are subjected to a salinity shock. Resistance and consequently physiological condition of shrimp was assessed by measuring their survival after a constant time interval. Although, in the present study we were not able to correlate salinity shock resistance with performance in the ponds, we indeed assumed that survival of the shrimp under these stress conditions is function of their physiological condition.

We report here on the results of stress resistance of Penaeus monodon postlarvae reared under different feeding conditions.

MATERIALS AND METHODS

The culture set up at the Artemia Reference Center consisted of a series of 50 l bins. Nauplii VP. monodon shrimp larvae were stocked at a density of 100 animals per liter and further reared to 15 day old postlarvae. Salinity and temperature were maintained at 35 ppt and 28°C during the whole rearing period.

A detailed outline of the feeding treatments used is given in Table I. From Zoae 1 to PL 2 shrimp were fed two diet-combinations; the figure here one stands for

Chaetoceros followed by w3-HUFA-rich Artemia from mysis onwards; figure three stands for half Chaetoceros level supplemented with the w3-yeast Tosal and the artificial microparticulated diet 2M followed by w3 rich Artemia. From PL3 to PL15 shrimp were switched to 4 different postlarval diets:
- the AE groups stand for 24 hr Selco enriched G3S nauplii, thus fortified w3-HUFA
- the EG groups stand for freshly hatched G3S Artemia, thus containing low levels of w3-HUFA
- PRE stands for a commercial nursery feed
- and DEC for dry non hatching decapacitated cysts of G3S origin.

In order to detect physiological differences among shrimp of different age and fed different diets, 5 day, 10 day and 15 day old postlarvae were transferred from each feeding treatment to triplicate 1 L beakers containing two different salinity regimes: PL5 to 21 to 14 ppt, PL 10 to 14 and 7 ppt, PL15 to 14 and 0 ppt (see Figure 1). Survival was monitored after 30 and 60 minutes.

RESULTS AND DISCUSSION

Significant differences in survival were found between the larvae fed the different diets (see Table I). The survival of the larvae at PL 15 was always best in feeding regime 3 which was the most complete larval diet. The effect of postlarvae diet quality was less pronounced. Analyses of postlarval dry weight at stage PL5, PL10 and PL15 revealed few differences among the treatments (see Table III). The treatment which received the dry commercial feed however, performed significantly poorer at least in 10 and 15 day old postlarvae.

The 5 day old postlarvae were exposed to a salinity of 21 ppt, which in our experiments, corresponded to a decrease of 40% of the salinity level at which the animals were reared. We prefer to use this percentage denomination because it allows for better standardisation of salinity stress experiments with animals reared at different salinities. Statistical analysis of the survival results after 30 minutes exposure revealed no significant differences among feeding treatments (see Figure 2). Two-way ANOVA, in which the effect of larval diet and postlarval diet were compared separately, indicated that in the present test, survival of 5 day old postlarvae was not affected by the larval diet which the animals had received until PL2. As to the postlarval diet, those larvae fed enriched Artemia performed signi-
ificantly better than those fed the commercial diet. After 60 minutes shrimp fed enriched Artemia were significantly more resistant than all others. Two way ANOVA again indicated that differences in survival are affected by the postlarval diet rather than by the larval diet.

When the PLS were exposed to a salinity decrease of 60% or 14 ppt, very clear differences were observed among feeding treatments (see figure 3). Resistance of shrimp fed enriched Artemia was significantly better both after 30 and 60 minute exposure. In fact after 60 minutes exposure, mortality was complete in all treatments, but one exception: the diet of w3-HUFA enriched Artemia in combination with the w3-HUFA rich larval diet 3. Differences in survival after 30 exposure to stress were affected by the postlarval diet but also by the larval diet. Within the same postlarval treatment, larval diet 3 performed significantly better than larval diet 1. Or in other words the resistance of the PLS, especially when exposed to harsh conditions, is significantly improved when they have been fed w3-HUFA enriched diets.

Figure 4 shows the resistance of 10 day old postlarvae exposed to a salinity of 14 ppt. One way ANOVA revealed that the commercial diet combination with larval diet 1 performed significantly poorer than all other diets. Furthermore, it was shown that for a 30 minutes exposure enrichment did not significantly improve resistance of shrimp fed an Artemia nauplius diet. However, when exposed for 60 minutes resistance improved in shrimp which had received a diet of enriched Artemia or dry decapsulated cysts. Two way ANOVA again revealed that groups fed the w3-HUFA rich larval diet 3 were in better condition than those fed larval diet 1.

When exposed to a salinity decrease of 80% or 7ppt very pronounced differences were noted (see figure 5). Both after 30 and 60 minutes exposure, survival was again superior in the enriched Artemia treatments, especially when combined with the w3-HUFA rich larval diet 3. Remarkable is that the good performance at 14 ppt of shrimp fed decapsulated cysts is not found anymore when they are exposed to more drastic stress conditions. In fact, their mortality in 7 ppt was complete when they were exposed for 60 minutes.

From these results it appears, at least under mild stress conditions, that a diet of w3-HUFA poor Artemia, be it under the form of decapsulated cysts or nauplii are generally good to sustain resistance in 10 day old postlarvae provided of course that they have received a HUFA-rich larval diet. When the shrimp are exposed to more drastic conditions however, these diets seem to be far less adequate than the diet consisting of w3-HUFA enriched Artemia.

Exposure of 15 day old postlarvae to a salinity decrease of 60% revealed very good resistance in all feeding treatments both after 30 and 60 minutes (see Figure 6). It is clear that this mild condition was not really imposing a stress to the animals and therefore is not really suitable to detect differences in physiological condition.

We have therefore transferred the 15 day old postlarvae directly into freshwater (see results in Figure 7). When using a one way ANOVA, again no significant differences were detected after 30 minutes exposure. After 60 minutes, best resistance was obtained in shrimp fed dry decapsulated cysts, though not significantly different from the value obtained for shrimp fed GFS nauplii in combination with larval diet 1. In addition it was shown that under present conditions shrimp did not benefit from a diet of enriched Artemia. Two way ANOVA learned us that resistance in PL15 was still being affected by the larval diet. Within the same polysty larval treatment better resistance was again observed in shrimp which had received the HUFA rich larval diet 3.

Figure 8 represents resistance of PL5, PL10 and PL15 challenged during 30 minutes to the same salinity of 14 ppt or in other words a mild salinity stress. In most feeding treatments it appears that resistance is significantly improving as the postlarvae grow older. Remarkable however, is at least under these mild stress conditions, that shrimp which received enriched Artemia have already a quite elevated resistance at PL5 which equals or even surpasses resistance of PL10 fed other diets. Moreover, when they were fed with w3-HUFA rich larval diet 3 their resistance is only slightly inferior than that at PL15. This indicates that the beneficial effect of feeding HUFA rich diets is more pronounced at PL5 than at older PL stages. Practically this could also imply that the drastic mortalities, which are commonly observed when stocking early postlarvae, may possibly be alleviated by using shrimp which have been reared on an w3 rich larval diet combination with enriched Artemia.

In conclusion we can say that the present stress test may provide an interesting additional tool to evaluate diet effectiveness. The various feeding treatments used in our experiments resulted in few differences in growth, but indeed yielded significant differences in survival and especially stress resistance, reflecting the variable physiological condition of shrimp fry fed these diets. It is clear from this study that in addition to a postlarval diet consisting of enriched Artemia also a nutritionally balanced w3-HUFA rich larval diet should be used to maximize resistance and ultimately quality in hatchery.
reared shrimp. The variable physiological condition in shrimp postlarvae may indeed result in a differential performance under growth conditions. Furthermore, the superior stress resistance of postlarvae fed enriched Artemia might also be reflected in a better resistance to diseases. After all the role of dietary HUFA's in immunoreistance is very documented for many organisms (Di Luzio, 1972; Maeede and Mertin, 1976; Castell and Olivier, 1980).

Finally in view of the results obtained in this study with *P. monodon* we will further evaluate the present order to develop standardized species-specific procedures.

### REFERENCES

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### TABLE 1

FEEDING TREATMENTS USED IN REARING OF *Penaeus monodon* FROM ZOEAL(2) TO POSTLARVAL STAGE (PL) 15

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>STAGE OF <em>P. monodon</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Z1 - PL2</td>
<td>PL3 - PL15</td>
</tr>
<tr>
<td>AE1</td>
<td>CHAETOCEROS; AF ARTEMIA 24 HR SELCO ENRICHED GSL ARTEMIA</td>
</tr>
<tr>
<td>AE3</td>
<td>CHAETOCEROS; TOPAL; SM; AF ARTEMIA 24 HR SELCO ENRICHED GSL ARTEMIA</td>
</tr>
<tr>
<td>EG1</td>
<td>CHAETOCEROS; AF ARTEMIA NON ENRICHED GSL INSTAR 1 ARTEMIA</td>
</tr>
<tr>
<td>EG3</td>
<td>CHAETOCEROS; TOPAL; SM; AF ARTEMIA NON ENRICHED GSL INSTAR 1 ARTEMIA</td>
</tr>
<tr>
<td>PRE 1</td>
<td>CHAETOCEROS; AF ARTEMIA DRY COMMERCIAL DIET</td>
</tr>
<tr>
<td>PRE3</td>
<td>CHAETOCEROS; TOPAL; SM; AF ARTEMIA DRY COMMERCIAL DIET</td>
</tr>
<tr>
<td>DEC3</td>
<td>CHAETOCEROS; TOPAL; SM; AF ARTEMIA NON HATCHING DECAPSULATED CYSTS</td>
</tr>
</tbody>
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![Schematic outline of stress resistance experiments](image)

FIG. 1 Schematic outline of stress resistance experiments
FIGURE 2: STRESS RESISTANCE OF *P. monodon* PL5 at 21 ppt
(*= salinity decrease of 40%)

FIGURE 3: STRESS RESISTANCE OF *P. monodon* PL5 at 14 ppt
(*= salinity decrease of 60%)

FIGURE 4: STRESS RESISTANCE OF *P. monodon* PL10 at 14 ppt
(*= salinity decrease of 60%)

FIGURE 5: STRESS RESISTANCE OF *P. monodon* PL10 at 7 ppt
(*= salinity decrease of 80%)

TREATMENTS

% SURVIVAL

AE1, AE2, EG1, EG3, PRE1, PRE3, DEC3

TREATMENTS

% SURVIVAL

AE1, AE2, EG1, EG3, PRE1, PRE3, DEC3

TREATMENTS

% SURVIVAL

AE1, AE2, EG1, EG3, PRE1, PRE3, DEC3

TREATMENTS

% SURVIVAL

AE1, AE2, EG1, EG3, PRE1, PRE3, DEC3

TREATMENTS
FIGURE 6: STRESS RESISTANCE OF P. monodon PL15 at 14 ppt
(= salinity decrease of 60%)