DEVELOPMENTS IN FEED FORMULATIONS, FEEDING PRACTICES AND CULTURE TECHNIQUES FOR MARINE SHRIMP LARVAE

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ABSTRACT

This paper reviews topics related to shrimp hatchery nutrition and zootechnics (managing captive or domestic animals) with special emphasis on practical diets and techniques that can significantly help shrimp hatchery operators to optimize daily protocols. Optimized techniques for the production and use of Artemia have been communicated extensively through workshops and publications (e.g. FAO Technical Paper by Laven and Sorgeloos 1996), but in reality these techniques are being applied much less strictly in shrimp hatcheries than in fish hatcheries. For shrimp there is still scope for improvement in the field of cyst selection, hatching, hygiene and enrichment. On the other hand, artificial diets are becoming more efficient in Artemia replacement, and some insights are given into the role of improved feed digestibility and feed processing technology. In another section, the effect of nutrition on egg quality, postlarvae (PL) quality and broodstock quality is illustrated with examples taken from recent publications. And finally, an overview is provided for developments in recirculation systems and intensive larviculture practices.

HATCHERY FEEDS

Artemia

Nutritional features

Composition

Any data on proximate composition of Artemia have a rather relative value, as a more detailed analysis reveals considerable fluctuations with strain, harvest season, batch and life stage. A comprehensive overview of proximate compositions found in literature is presented by Dhont and Van Stappen (2003). Lipids are undoubtedly the best documented dietary component of Artemia since the identification in the late 1970s and early 1980s of a linkage between variations in nutritional value when using different geographical sources of Artemia for shrimp, prawn, lobster and crab species that could be linked to differences in lipid composition (Léger et al. 1986). Studies revealed that the concentration of the essential fatty acid 20:5 n-3 (eicosapentaenoic acid, EPA) in Artemia nauplii determined its nutritional value for larvae of various marine fishes and crustaceans (Léger et al. 1986). Levels of this essential fatty acid vary tremendously from strain to strain and even from batch to batch. In addition, another essential fatty acid, docosahexaenoic acid (DHA: 22:6 n-3), is almost lacking in Artemia. In view of these fatty acid deficiencies in Artemia, appropriate techniques have been developed for improving the lipid profile of deficient Artemia strains (see Enrichment section below).

Although protein levels and amino acid profiles in Artemia may show fluctuations between strains and life stages, these fluctuations are generally much less pronounced than for the lipid fraction. The presence of low molecular weight peptides and free amino acids in nauplii (Garcia-Ortega et al. 2001), together with their autolytic capacity and high solubility, account for the easy digestion of the proteins by young larvae.

Most data on vitamins relate to Artemia franciscana (Table 1). An account of differences between strains could only be found for various derivates of ascorbic acid (AA) in cysts in Merchie et al. (1995). They observed considerable differences in ascorbic acid 2 sulfate (AAS) concentrations (296-517 µg/g DW expressed as AA) when comparing 10 different strains. Furthermore, they provided further evidence for the complete conversion of the ascorbic acid 2 sulfate form to free ascorbic acid in the developing larvae.

Enrichment

Bio-encapsulation, also called Artemia enrichment or boosting, is widely applied in marine fish hatcheries for enhancing the nutritional value of Artemia with essential fatty acids and vitamins. This practice is much less common in shrimp larviculture, even though the benefit of feeding enriched Artemia nauplii to shrimp postlarvae (PL) has also been documented repeatedly. For example, Immanuel et al.
Characteristics of Artemia cysts and nauplii

Table 1. Vitamin levels obtained by enrichment (µg/g DW).

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>ref.</th>
<th>unenriched nauplii</th>
<th>24h enriched nauplii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td></td>
<td>692</td>
<td>3100</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td></td>
<td>1000 - 12000</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiamine</td>
<td></td>
<td>7.5</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td></td>
<td>20 - 40</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td></td>
<td>47.3</td>
<td>38.0</td>
</tr>
<tr>
<td>Niacin</td>
<td></td>
<td>187</td>
<td>202</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td></td>
<td>86</td>
<td>81</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td></td>
<td>9.0</td>
<td>6.7</td>
</tr>
<tr>
<td>Biotin</td>
<td></td>
<td>3.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Folate</td>
<td></td>
<td>18.4</td>
<td>12.2</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td></td>
<td>3.5</td>
<td>3.9</td>
</tr>
</tbody>
</table>

a: Merchie et al. 1995
b: Mæland et al. 2000
c: Olsen 1999

(2007) fed P. monodon PL from PL_{15} till PL_{40} with unenriched and enriched Artemia, and obtained the highest weight gain and specific growth rate when feeding Artemia enriched with HUFA. Another study by Martin et al. (2006) reported increased survival rates in Farfantepenaeus paulensis fed Artemia enriched with n-3 HUFA.

For optimal enrichment, nauplii should be transferred or exposed to the enrichment medium as soon as possible before first feeding, so that they begin feeding immediately after opening of the alimentary tract (second Instar stage, Instar II). As a result, the increase in nauplius size during enrichment can be minimized. After 24-hour enrichment Great Salt Lake (GSL) nauplii will reach about 870µm in length, and after 48-hour enrichment about 1010µm. Most frequently used enrichment products are the so-called self-emulsifying concentrates (SELCOs). Enriched nauplii are harvested after 12 or 24h and thoroughly rinsed using analogous procedures as after hatching (Léger et al. 1987).

In their first stage of development, Artemia nauplii do not feed but consume their own energy reserves (Benijts et al. 1976). At the high water temperatures used during cyst incubation, freshly-hatched Artemia nauplii develop into the second larval stage within 6 to 10h. It is important to feed first-instar nauplii (Instar I) rather than starved Instar II metanauplii, which are transparent and less visible. Moreover, Instar II are about 50% larger in length and swim faster than first instars. As a result they are more difficult to catch and less acceptable as prey for those larvae with a critically small mouth size. Additionally, Instar II metanauplii have a lower individual dry weight (DW) and energy content than Instar I nauplii (Garcia-Ortega et al. 1998), reducing the energy uptake per unit of hunting effort for the predator (Léger et al. 1986). All this will be reflected in reduced larval growth in the face of increased Artemia cyst consumption: for instance, 20 to 30% more cysts are needed to compensate for the reduced wet weight biomass of starved metanauplii.

The nutritional effectiveness of any food organism is first determined by its ingestibility, which is determined by its size and shape. Many shrimp hatchery operators feed heat-killed or frozen Artemia Instar I to late zoea stages and early mysis. This immobilization of Artemia prevents the development into a larger Instar stage that would be difficult to ingest. In Vietnam, umbrella stage Artemia - harvested from the Artemia hatching tank after only 12 h - are sometimes fed to P. monodon from mysis 1 to postlarvae 2. Umbrella stage Artemia are smaller than Artemia nauplii and have a higher energy content (Lavens and Sorgeloos 1996). Naupliar size can also vary greatly from one geographical source of Artemia to another (Vanhaecke and Sorgeloos 1980). In the example given in Figure 2, it can be observed that after 24h cyst incubation nauplii from High 5 cysts are significantly smaller than nauplii from GSL cysts, making High 5 nauplii excellent live prey for early shrimp larvae with a small mouth size, such as late zoea and early mysis stages of marine shrimp. Additionally, High 5 Instar I nauplii contain more energy and nutrients than GSL nauplii, because of a significant increase in individual dry weight (2.55 to 2.85 µg per High 5 type Instar I nauplii versus 2.20 to 2.40 µg per GSL-type Instar I nauplii).

High 5 cysts are selected Artemia cysts (INVE) that have been submitted to proprietary processing steps to acquire specific beneficial characteristics such as exceptionally good separation, synchronous hatching and continuous Vibrio suppression during hatching.
Figure 1. Hatching rate and Instar I/II-ratio during the course of the hatching process for cysts from North-America (GSL), China (CN) and Eurasia (High 5).
Hygiene is another important issue in *Artemia* hatching. At high cyst densities and high incubation temperatures during hatching, bacterial development (e.g. on the released glycerol) can be considerable and hatching waters may become turbid, which may also result in reduced hatching yields (Lavens and Sorgeloos 1996). Therefore, it is recommended to apply routinely a disinfection procedure by using established commercial products, hence reducing the risk of pathogen transfer to the shrimp tanks and producing more and stronger *Artemia* nauplii.

**Shell-free Artemia**

Shell-free *Artemia* are dehydrated decapsulated *Artemia* cysts marketed as a dry off-the-shelf product. Shell-free particles sink fast and are, therefore, not suitable for mysis stages. However, with a particle size of 250 µm, it is an excellent feed for benthic postlarvae and can be used for partial or total replacement of *Artemia* nauplii (Stael et al. 1995; Ribeiro and Jones 1998). Shell-free *Artemia* retains its soluble nutrients and therefore ensures good survival.

Ribeiro and Jones (1998) obtained similar growth and survival of *P. indicus* postlarvae fed shell-free *Artemia* than with PL fed live *Artemia* nauplii, and better results than PL fed artificial diets. Wouters and Van Horenbeeck (2003a) reported excellent results of intensive Pacific white shrimp (*Litopenaeus vannamei*) culture in raceway tanks from PL<sub>10</sub> to PL<sub>30</sub>, fed shell-free *Artemia* as a sole diet: 89% and 98% survival rates were obtained at stocking densities of respectively 25,000 and 45,000 PL/m<sup>2</sup>.

In a trial at the Thai research facilities of INVE Technologies NV (unpublished data), *P. monodon* shrimp fed shell-free *Artemia* as a sole diet from PL<sub>5</sub> to PL<sub>15</sub> yielded similar survival rate, weight and length than shrimp that were fed live *Artemia* nauplii supplemented with a dry diet (3:1), and significantly better results than the control treatment that received 100% dry artificial feeds (Table 2).

![GSL](image1)

![High 5](image2)

*Figure 2. Evolution of average individual nauplius size (length in µm) during the early development of Artemia from North-America (GSL) and Eurasia (High 5).*
Artificial Feeds

Artificial diets will certainly gain in popularity in the near future because they reduce the problems and risks involved in the production of live food. Quite a few products are already commercially available for some aquatic species, but their nutritional composition, digestibility and physical performance, especially regarding suspension in the water column and leaching, need to be further optimized before they can completely replace live food in shrimp hatcheries. The application of micro-particulate diets may also depend on the goal which each hatchery seeks to achieve, i.e. the production of high numbers of PL (high densities and survival rates) or the production of high-quality fry (strong, resistant to stress and diseases). In any case, it is the cost-effectiveness of the different feeding strategies that will determine the preferential application of inert diets or live food organisms. It is likely that this may differ considerably from region to region, and between industrial and backyard hatcheries.

Feed digestibility

Shrimp nutrition involves an understanding of the behavioral, mechanical and physiological processes of feeding in the target larval or PL stage (Fegan and Wouters 2004). One of the key considerations is the development of the gut structure and function. Larval crustaceans have a simple gut structure that gradually becomes more complex. The physiology of the gut and gut enzymes also change and, since transit times may be quite short, designing a nutritious, easily-digestible diet is still a challenge (Kumlu and Jones 1995). This is particularly important in early PL stages, when the increased consumption of Artemia is becoming a high cost in the hatchery operation. It has been demonstrated with different penaeid species that enzyme secretion is specifically limited in PL (Brito et al. 2000, 2001; Ribeiro and Jones 1998), which often are unable to digest sufficient amounts of full-length proteins and longer peptides present in the feed. Moreover, artificial diets generally contain 90% or more dry matter, compared to only 10% in Artemia nauplii, which renders dry particles more difficult to digest than live food.

One area of investigation that has received little attention, especially when compared to the agrifeed industry, is the supplementation of artificial feeds with exogenous enzymes. Some studies with digestive enzymes have failed to demonstrate beneficial effects or have resulted in poor growth rates. The difficulty is to select or engineer enzymes that are active at relatively low temperatures, with an optimal functioning at the pH levels typical for the gut environment. Next, low-temperature feed processing technology is required to prevent denaturation of the applied thermolabile enzymes. Some examples of cold processing are microencapsulation for

Table 2. Shrimp hatchery output (INVE Technologies NV, unpublished data) from P. monodon PL fed shell-free Artemia compared to live Artemia nauplii and/or dry artificial feeds (AF). CSI represents a cumulative survival index of PL submitted to a salinity stress test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival (%)</th>
<th>Dry Weight (µg)</th>
<th>Length (cm)</th>
<th>CSI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean StDev</td>
<td>Mean StDev</td>
<td>Mean StDev</td>
<td>Mean StDev</td>
</tr>
<tr>
<td>Shell-free Artemia</td>
<td>44.5 8.7</td>
<td>489 87</td>
<td>1.02 0.08</td>
<td>137 18</td>
</tr>
<tr>
<td>Live Artemia + AF</td>
<td>45.5 13.6</td>
<td>488 13</td>
<td>1.01 0.07</td>
<td>112 5</td>
</tr>
<tr>
<td>AF</td>
<td>32.1 7.3</td>
<td>344 36</td>
<td>0.94 0.05</td>
<td>91 22</td>
</tr>
</tbody>
</table>

Figure 3. As this example of a store in Vietnam shows, many commercial diets for larval shrimp are already available.
the production of FRIPPAK® larval feeds (Jones et al. 1987; Sangha et al. 2000), as well as Refractance Window Drying (RWD™) for flakes (Wouters et al. 2003a, 2003c). Finally, feeds supplemented with enzymes need extra protection from nutrient leaching, as partial pre-digestion of raw materials will increase the proportion of water-soluble short peptides and amino acids. Similarly, an increased inclusion of fish hydrolysates and amino acids in the feed formula needs to be countered with adequate feed technology to prevent losses of solubles through leaching. For larval shrimp stages, microencapsulation is the best way to prevent excessive leaching, provided that easily digestible proteins and polymers are selected for cross-binding. The same applies for the selection of binders used in microbound particles for PL.

A recent publication in the field of enzyme technology (Sirvas-Cornejo et al. 2007) refers to the use of microcapsules based on commercial diets (FRIPPAK #2 CD) that have been supplemented with microbial digestive enzymes. The authors demonstrated the ability of the enzyme mixture to assist in the digestion of the protein available in the diet as well as in the walls of the capsules. Another approach is to select only highly-digestible ingredients for feed formulation, such as fresh marine ingredients (Wouters et al. 2003b): the ingredients are neither dried nor milled into a meal, but are included as fresh or fresh-frozen material directly into the processing line. Some researchers (Ezquerra and García-Carreño 1997; Lemos and Nunes 2008) have established in-vitro methods and in-vivo tests with enzymes and correlated protein digestibility of feed ingredients and/or commercial feeds with protein hydrolysis and shrimp growth in *L. vannamei*. These examples indicate that enzyme technology combined with advanced feed technology may offer potential benefits for the development of highly-digestible shrimp feeds, needed by the shrimp farmers to cope with increasing economic pressures.

The secretion of digestive enzymes in *L. vannamei* PL was studied by Gaxiola et al. (2006) after feeding diets with different protein sources (marine animal protein vs. vegetable protein). The results showed a more stable and higher enzyme production in PL that received marine animal protein, apparently as a result of stronger hydrolysis of the dietary protein and a higher concentration of free amino acids available for protein synthesis and muscular tissue generation.

**Artemia substitution**

*Artemia* replacement levels applied by commercial hatcheries are slowly but steadily increasing year after year, in part because of the progressive availability of improved diets as well as the fluctuating cost of *Artemia* cysts.

Table 3 summarizes recent findings on the performance of experimental and commercial hatchery feeds. In general, the use of microbound feeds results in lower survival as well as growth when fed at levels of 40-50% or higher. However, because of their lower cost compared to *Artemia*, a partial replacement can result in considerable savings during mysis and early postlarval stages. Results of commercial-scale trials performed by INVE Technologies and feedback from customers indicate that the economical benefit of using *Artemia* replacement levels above 65% does not justify the increased risk of culture failure, although this largely depends on the experience of the local hatchery staff. Not surprisingly, researchers have also tried to replace *Artemia* with other live food, such as nematodes (Focken et al. 2006) and cladocerans (Martín et al. 2006). This suggests that there is still much research room in the field of larval nutrition before diets can consistently guarantee high survival and optimal PL quality.

In late postlarval stages, however, total *Artemia* replacement is possible and has been common practice for several years. The use of shell-free *Artemia* - as mentioned earlier – has gained interest, as it allows application of feeding regimes without live food as of PL4-5. Microbound particles – i.e. crumbled extruded pellets – and flakes are, however, the most widely applied feeds in hatcheries and nurseries for feeding late PL. The challenge for the feed producers is to develop particles with desirable physical characteristics and controlled nutrient loss. Shrimp PL and juveniles are slow feeders that break up feed particles into smaller particles, some of which will be lost in the water. Also, particles with low water stability will disintegrate after several hours. Ultimately, this leads to water pollution and a reduction of the nutritional value of the feed. One way to prevent this problem is to increase feeding frequency from four

![Figure 4. Significant levels of Artemia replacement with commercial diets is possible and cost-effective, but more research is needed.](image-url)
Table 3. The effect of Artemia replacement. The use of microbound feeds decreases survival or growth when fed at levels of 40-50% or higher. In controlled laboratory conditions, however, the results are generally better than in commercial hatcheries.

<table>
<thead>
<tr>
<th>Species</th>
<th>Diet</th>
<th>Artemia replacement (%)</th>
<th>Larval stages</th>
<th>Result compared to Artemia control</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penaeus monodon</td>
<td>Crumbled experimental microbound diet</td>
<td>100</td>
<td>Z-PL</td>
<td>Similar survival but lower growth</td>
<td>Kanazawa et al. 1982</td>
</tr>
<tr>
<td>P. monodon</td>
<td>Crumbled experimental microbound diet</td>
<td>100</td>
<td>Z-PL</td>
<td>Similar survival and growth</td>
<td>Kanazawa 1985</td>
</tr>
<tr>
<td>P. monodon</td>
<td>Microencapsulated diet FRIPPAK</td>
<td>100</td>
<td>Z-PL</td>
<td>Similar survival and growth</td>
<td>Jones et al. 1989</td>
</tr>
<tr>
<td>Litopenaeus vannamei</td>
<td>Microencapsulated diet FRIPPAK</td>
<td>70-100</td>
<td>Z-PL</td>
<td>80% survival compared to 90% survival in live food control (commercial scale)</td>
<td>Jones et al. 1997</td>
</tr>
<tr>
<td>L. vannamei</td>
<td>Crumbled experimental microbound diets</td>
<td>100%</td>
<td>M</td>
<td>Reduced development in experiment 1; reduced growth in experiment 2; similar survival, growth and development in experiment 3</td>
<td>D’Abramo et al. 2006</td>
</tr>
<tr>
<td>L. vannamei</td>
<td>Crumbled microbound diets Microfeast</td>
<td>25, 50, 75, 100</td>
<td>M-PL</td>
<td>Decreased growth rates at 50, 75 and 100% and decreased survival at 100%</td>
<td>Samocha et al. 1999</td>
</tr>
<tr>
<td>Litopenaeus setiferus</td>
<td>Crumbled experimental microbound diets</td>
<td>40, 60, 100</td>
<td>Z-M</td>
<td>Decreased survival, growth, development and stress resistance (but similar survival at 40 and 60% in the presence of algae)</td>
<td>Gallardo et al. 2002</td>
</tr>
<tr>
<td>P. monodon</td>
<td>Microencapsulated diet FRIPPAK® FRESH</td>
<td>100</td>
<td>Z-PL</td>
<td>Increased survival, growth and development (one single dose of live algae in Zoea1)</td>
<td>Wouters et al. 2003b</td>
</tr>
<tr>
<td>P. monodon</td>
<td>Crumbled microbound diet FRIPPAK® FLAKE</td>
<td>40, 100</td>
<td>PL</td>
<td>Lower survival, similar (100%) or improved (40%) growth</td>
<td>Wouters et al. 2003c</td>
</tr>
<tr>
<td>L. vannamei</td>
<td>Crumbled microbound diet FRIPPAK® RW+</td>
<td>100</td>
<td>PL</td>
<td>Similar survival and growth in trial 1, lower survival and higher growth in trial 2 (98% survival in Artemia Shell-free control)</td>
<td>Wouters et al. 2003a</td>
</tr>
<tr>
<td>Farfantepenaeus aztecs</td>
<td>Liquid feeds EpifeedTM and LiqualifeTM</td>
<td>50, 100</td>
<td>M-PL</td>
<td>Decreased survival (except LiqualifeTM at 50%), growth and stress resistance</td>
<td>Robinson et al. 2005</td>
</tr>
<tr>
<td>Fenneropenaeus chinensis</td>
<td>Crumbled experimental microbound diets</td>
<td>100</td>
<td>M-PL</td>
<td>Reduced growth and development</td>
<td>Wang and Mai 2006</td>
</tr>
</tbody>
</table>
to six rations or more, and consequently reduce the quantity per ration. Another way is to improve the stability with binders such as carboxymethylcellulose, alginate and gum products, gluten, and others (Tan and Dominy 1997). These binders will also affect the texture of the feed particles. Cruz-Suárez et al. (2000) demonstrated that kelp meal increased stability but also increased the water retention capacity of shrimp pellets, making them softer, and thus more palatable.

**NUTRITION AND QUALITY**

**Broodstock nutrition and offspring quality**

Research into shrimp broodstock nutrition is a key element in the development of domesticated and genetically selected stocks for aquaculture. The development of genetically improved lines of shrimp may benefit from a simultaneous development of feeds specifically tailored to individual strain requirements (Fegan and Wouters 2004), and on the other hand, genetic selection could also aim at producing strains that can better utilize diets with for example vegetable protein sources. Wouters et al. (2001) reviewed the literature on shrimp broodstock diets and commented on the lack of insight on the subject. Very little research has been conducted on formulated diets as the sole diet, most being conducted with fresh feeds, either alone or in combination with formulated diets or dietary supplements.

During maturation, nutrient reserves, mainly from the hepatopancreas, are mobilized to support ovarian and testicular maturation. Tissue reserves can be depleted rapidly so that the diet becomes the most important contributor of nutrients to the maturing egg (Harrison 1990). This is particularly true when eyestalk ablation is used to accelerate maturation. Poor nutrient supply during maturation affects vitellogenesis and may result in lesser quantity of nutrients being transferred to oocytes. Spawns from such broodstock yield eggs with low nutrient reserves, and these eggs yield poor quality larvae.

Broodstock nutrition status and egg quality parameters have proven to be valuable tools for the prediction of larval quality. Common quality evaluation parameters are:

- For evaluation of ovarian maturation: size and location of the gonads, gonad pigmentation.
- To assess egg quality: egg diameter, hatching rate and biochemical composition (nutrient reserves).
- To assess larval quality: body length, metamorphosis to the next larval stage, survival, biochemical composition, nauplii color and phototaxism.

The following examples illustrate the effect of broodstock nutrition on egg quality.

Recent studies at the TAES maturation facilities in Texas (Gandy et al. 2007) demonstrated how larval survival rates were influenced by the dietary regime of the parental stock. The replacement of bloodworms with frozen adult enriched *Artemia* (*Artemia* biomass) resulted in higher hatching percentages and increased larval survival rates of *Farfantepenaeus aztecus* shrimp. These results confirm the earlier findings of Naessens et al. (1997) and Wouters et al. (1999) with *L. vannamei*.

During maturation, the level of triacylglycerides (TAG, the principal energy source) in the ovaries increases as TAG is incorporated into the egg during vitellogenesis. Interestingly, Racotta et al. (2003) and Palacios et al. (1999) demonstrated that the quality of larvae (larval survival) is related to the TAG and carotenoid content of the eggs.

Several authors have reported that nauplii pigmentation and nauplii viability can be significantly improved by the addition of carotenoids (such as astaxanthin) to broodstock diets. Spirulina (Regunathan and Wesley 2006) and paprika (Wyban et al. 1997), for example, have been proven to be effective carotenoid sources.

Egg hatching rates and larval metamorphosis rates have been correlated with the levels of alpha tocopherol (vitamin E) and vitamin C in the broodstock diet (Alava et al. 1993; Chamberlain 1988).

**Larval nutrition and PL quality**

It is generally believed that optimal larval quality is of primary importance to obtain maximum yields during larval and postlarval culture, as well as during stocking in ponds for further growout (Alvarez et al. 2004). The results available from published reports establish a link between PL quality and subsequent PL performance in small-scale experimental settings (i.e. grow-out in aquaria or plastic containers), but hard evidence to prove a prolonged effect during growout in ponds is difficult to find (Lee 2005).

Most PL quality tests commonly applied in shrimp hatcheries rely on a combination of parameters (Fegan 1992; Wickins and Lee 2002), such as:

- Nutritional condition (e.g. diet, gut content, nutrient composition).
- Behavior (e.g. swimming and feeding behavior).
- Morphology (e.g. gill development, deformities, rostral spine count, tail or muscle appearance/size).
- Hatchery performance (e.g. hatchery survival rate, PL size and size variation).
- Health status (e.g. fouling, necrosis, absence of pathogens).
- Stress tests performance (e.g. osmotic and formalin stress survival).

Alvarez et al. (2004) showed with *L. vannamei* that “early indicators,” such as stress tests with zoea larvae and percent metamorphosis to zoea, can be useful predictive indicators of larval performance during culture to later stages, and they also found a correlation between survival data of an osmotic stress test (18 g/L) done at stage PL1 and survival up to stage PL20. When Alvarez et al. (2004) grouped larval batches according to survival from an osmotic stress test at PL1, several biochemical traits were associated to a better survival at low salinities, such as higher concentrations of TAG and fatty acids. In another study by this team (Palacios et al. 2004), survival to an osmotic stress test was higher and gill area was larger in PL20 fed *Artemia* nauplii enriched with medium huFA levels (from treatments with low, medium and high huFA concentrations), probably as a result of an increased DHA content and a higher DHA/EPA ratio in the diet and in the tissues of the shrimp fed this diet. This is in line with several studies reporting higher larval survival to salinity stress tests in PL offered a diet containing high levels of HUFA (Tackaert et al. 1992; Rees et al. 1994; Kontara et al. 1997).

Another nutrient that has been linked to increased PL quality is vitamin C. Work on the early postlarval stages of *L. vannamei* has shown that dietary levels of at least 40 mg/kg (Merchie et al. 1997) can increase resistance to salinity stress, and Merchie et al. (1998) demonstrated a drop in osmotic stress mortality in *P. monodon* PL after raising the vitamin C content in the feed from 100 to 3400 mg/kg.

**Grow-out feeding and broodstock quality**

Gaxiola et al. (2006) reported on a series of trials conducted in Mexico on domesticated *L. vannamei* shrimp. The nutrition of the animals was controlled from PL stage of the F0 generation until the broodstock of the F1 generation and their offspring. The goal was to investigate the adaptation of shrimp to a certain diet and the capacity to pass that trait on to the next generation. The diets offered differed in protein level and protein source (marine animal protein vs. vegetable protein). They found that PL and juveniles fed high protein levels from marine protein sources produced broodstock with acceptable fecundity and good egg quality. Broodstock that were obtained from PL raised on vegetable protein sources exhibited low spawning performance, even though these spawners received a balanced broodstock diet during the maturation phase (*Artemia* biomass, bloodworm, squid, mussel and broodstock pellet). Finally, when shrimp were fed low quality PL feeds and low quality grow-out feeds (low protein level, vegetable protein sources), the resulting females did not spawn viable eggs at all (Maldonado 2005).

**RECIRCULATING AND INTENSIVE LARVICULTURE SYSTEMS**

Recirculating aquaculture systems (RAS) are increasingly being used in fish hatcheries, not only to save heating costs, but especially to guarantee more stable water quality conditions, which apparently cause less stress on the animals and result in better larviculture performance. For shrimp larviculture, however, the use of RAS is a relatively new field, even though the management of water quality and microbial communities would be potentially powerful tools to improve the larval output. In the typically used traditional culture systems (TCS), water quality can rapidly degrade which leads to increased mortalities and a higher incidence of diseases. In addition, the development of RAS for larval rearing is a prerequisite for the intensification of larviculture (higher stocking densities) and for rendering hatchery production more profitable.

Gandy (2004) evaluated RAS for the production of *F. azteca* PL. Water was re-used during six larval rearing cycles, and in the latest trials – after implementing a biofilter to exert better nitrite reduction – the PL survival in RAS was similar or higher than that in control tanks, and also length, weight and stress tolerance were similar. The author recommended complete cleaning and sterilization of the larval rearing system and startup of a new biofilter for each larval cycle. Further research is
planned to deal with increasing nitrate levels during continuous re-use of culture water, possibly through the optimization of nitrate-utilization by algae.

Ghanekar (2005) reported preliminary results with RAS for *P. monodon* larviculture in India. The survival in the larval phase (N-PL₂) was 66%, and survival in the PL phase (PL₂-PL₁₁) was 86%. The larval development rate, however, was reduced, and the system therefore requires further improvements. Thach et al. (2005) also applied a RAS for commercial-scale *P. monodon* larviculture in Vietnam, with stocking densities of 250 larvae/L. The first eight days after stocking, a batch culture system was applied and larvae were fed FRIPPAK Fresh and LANSY-Shrimp larval feeds (INVE Aquaculture) as well as *Chaetoceros* algae. From day 9 onward, recirculation rates of 300 %/day were applied, which was increased to 400%/day when decreased water quality was observed. PL₁₁ survival rates ranged from 20% to 80%, with half of the batches yielding 60% or more. The farmer claimed to be able to sell the resulting PL at much better prices than regular PL, due to a better quality score, better survival during grow-out, and lower incidence of WSSV infection.

Ongoing research at the CENAIM-ESPOL Foundation in Ecuador by one of the present authors (Cobo and co-workers) investigates the effect of stocking density, feeding strategy and water recirculation rate on the survival and growth of *L. vannamei* larvae in RAS. Their RAS included a UV filter, sedimentation base, protein skimmer and biofilter. Tested stocking densities were as high as 750 to 2000 larvae/L, and water recirculation rates varied between 250 and 1000 %/day. Dry weight, length, larval development rate and biomass were not affected by stocking densities, but survival rates were significantly reduced at densities of 1500 and 2000 larvae/L. In a repeat trial, however, the RAS yielded PL₂ survival rates of 60% at 1000 larvae/L and 39% at 2000 larvae/L, applying water recirculation rates of 500%/day. Higher water flow yielded poor survival rates. The reported survival of 60% at a density of 1000 larvae/L (this is approximately five times higher than the density applied in TCS) is comparable or only slightly less than the typical survival rates obtained in traditional systems. Moreover, the RAS were able to maintain acceptable ammonia and nitrite levels. Additional repeat trials were done at stocking densities of 1000 larvae/L. Survival rates N₂-PL₂ averaged 50% (+/- 7%), dry weights were slightly reduced, but the total biomass was over five times higher than biomass production in TCS. Final ammonia, nitrite, nitrate and total solid levels were lower in RAS than those measured in TCS. No differences in bacterial numbers were detected.

In conclusion, we believe that RAS and intensive systems have shown their effectiveness in maximizing biomass and controlling water quality, but sometimes with a reduction in other production parameters (growth, larval development or survival). Further developments are needed to tackle these shortcomings and to ensure consistent outputs at commercial level. The present achievements indicate that these practices hold promise in producing better quality PL, reducing operating costs, and increasing biosecurity.

**LITERATURE CITED**


Wouters et al. 2006. Successful culture of larvae of *Litopenaeus vannamei* fed a microbound diet exclusively from either stage PZ₂ or M₁ to PL₁. Aquaculture 261:1356-1362.


