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Advances of the Artemia Project: Improvement of white shrimp *Penaeus vannamei* production through feeding strategies based on live and formulated feed

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Abstract

The results of two series of activities executed within the framework of an ongoing cooperation project between Ecuador and Belgium are documented.

Firstly a new quantitative method for the evaluation of postlarval shrimp is described. Following the operational procedure, the postlarvae were separated into different classes according to their ability to resist a constant water current. The qualified shrimp were then submitted to an osmotic stress situation or a nursery rearing period of three weeks duration. It was found that good resistance to the water current corresponded to good resistance to osmotic shock. A similar positive relation could be demonstrated between resistance to the water current and the nursery performance in terms of growth but not in terms of survival.

In a second group of investigations larvae of *Penaeus vannamei* were reared through the zoea and mysis stages and harvested as 7 to 8 day postlarvae. The larval feeding was based on diet of algae, *Artemia* and formulated diets, supplemented with rotifer (*Brachionus plicatilis*) from the second zoea stage onward. Rotifer feeding shortens the combined duration of the zoea and mysis stage with almost one day. High HUFA levels of the rotifers administered during pre-postlarval stages tend to increase the moulting frequency during the later postlarval phase.

Resumen

Los resultados de dos series de actividades experimentales ejecutados en el Proyecto Artemia, dentro del marco de Cooperación entre los gobiernos de Ecuador y Bélgica son documentados.

En primer lugar, un nuevo método cuantitativo para la evaluación de post-larvas de camarón es descrito. Seguido del procedimiento operacional, las post-larvas son separadas en diferentes clases de acuerdo a su habilidad de resistir una corriente de agua constante. El camarón clasificado es luego sometido a una situación de stress osmótico o a una simulación de cultivo a nivel de precriadero por el periodo de tres semanas. Se observó que aquellas post-larvas que presentaron buena resistencia a la corriente de agua, correspondieron a una buena resistencia a los cambios osmóticos. Una relacion positiva similar puede ser demostrada entre la resistencia a una corriente de agua y el desarrollo en precriaderos en terminos de crecimiento, más no en términos de supervivencia.

En el segundo grupo de investigaciones, larvas de *Penaeus vannamei* fueron cultivadas desde los estadios de zoea y mysis hasta su cosecha como post-larvas de 7 u 8 días. La alimentación de las larvas fue realizada en base a una dieta con algas, *Artemia* y dietas formuladas, suplementadas con rotíferos (*Brachionus plicatilis*) desde el segundo estadío de zoea en adelante. La alimentación con rotíferos acorta la duración combinada de estadío zoea y mysis en un solo día. Altos niveles de HUFA de los rotíferos proporcionados durante los estadios de pre-post-larva tendieron a incrementar la frecuencia de muda durante la última fase de post-larva.

Introduction

An ongoing cooperation project between Ecuador and Belgium aims at the production of *Artemia* products and their optimized use in combination with other live feeds and formulated diets in specific phases of the shrimp culture cycle. The project also foresees the establishment of a National Reference Center that deals with the quality control of Artemia products, with training and technical assistance. For practical purposes the project is referred to as "the Artemia Project".

The Ecuadorian counterparts involved in the project are the Fundación de Investigaciones de los Recursos Bioacuáticos (FIRBA), the Cámara de Productores de Camarón (CPC) and the Escuela Superior Politécnica del Litoral (ESPOL). The contribution of the latter institution is realized via its research station CENAIM (Centro Nacional

contribution to the project is made available by the Belgian Administration for Development Cooperation (BADC). The project's activities are scientifically coordinated by the Laboratory of Aquaculture and Artemia Reference Center (University of Ghent, Belgium).

The presence of both public and private counterparts indicates that the project's activities are situated on two different levels: one is applied research, the other is practical application. The first objective is the production of *Artemia* and the logical next is the application of Artemia products and other diets in shrimp feeding regimes.

This report overviews the work related to the evaluation of postlarval quality and the use of specific live feeds in larval and postlaval rearing of *P. vannamei*.

Larval Quality

From the hatchery manager's point of view the success of a larval rearing cycle is often evaluated in terms of survival and growth of the animals. When it comes to accurately estimating the potential for future successful pond production, the subject becomes much more delicate. It has indeed been demonstrated by Rees *et al.* (1992) that neither size nor growth rate are reliable criteria for postlarval quality.

A variety of larval quality indicators and consequent assessment methods have been described and are reviewed by Fegan (1992) and Wilkenfeld (1992). Common practice is to evaluate the physiological condition of the larvae by determining their ability to withstand stressing conditions induced by temperature, osmotic shocks or certain chemicals (Tackaert *et al.*, 1989; Bauman and Jamandre, 1990; Durán Gómez *et al.*, 1991). Morphological characteristics such as e.g. muscle size in the sixth abdominal segment, gill development and overall pigmentation are also claimed as valid criteria for the evaluation of postlarval quality (Bauman and Scura, 1990). It is agreed that these features indeed provide information on the animals' quality or condition at the time of determination, but a possible significant relationship with the posterior performance in the growout ponds is still being questioned (Samocha and Lawrence, 1992). Moreover, most quality evaluations are executed under a variety of experimental conditions and a large number of them apply subjective criteria which are difficult to quantify (e.g. pigmentation, larval activity).

In this study the development of a quantitative method was initiated. The procedure selected here is based on an evaluation method practiced in Ecuador (own observations) and in South East Asia (Anonymous, 1991): the postlarval shrimp are submitted to a centripetal water current that is manually created in a bucket. It is believed that good postlarvae have the strength to swim into the current and move towards the vertical wall of the bucket. Poor quality animals however will concentrate in the center of the bucket's bottom.

Materials and Methods

In our method, the centripetal current -the turmoil in the bucket- is replaced by a linear waterflow in a PVC-tube. The complete set-up (Fig. 1) consists of two distinct parts: the first one is a container with an overflow pipe (header tank). The tank is equipped with an outflow tube that feeds the second part of the set-up: a horizontal pipe of one meter length and an inner diameter of 4.2 cm. At both ends the pipe is equipped with an upward bend (90 degrees); the one nearest to the header tank being further elongated with a Y-shaped piece. Once a waterflow is established in the system, a sample of about 100 postlarvae is introduced through one leg of the Y-piece. The animals that are carried with the waterflow and leave the tube at the opposite end are collected on a submerged screen.

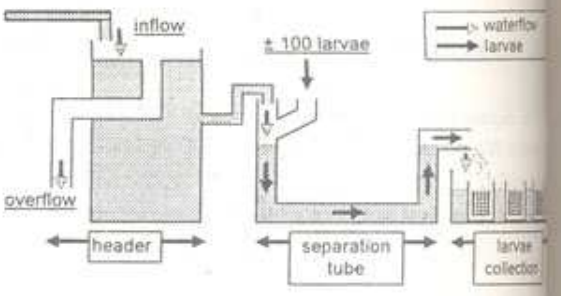


Figure 1: Schematic diagram of the set-up and the operation of the apparatus for classifying postlarval shrimp according to their ability to withstand a constant water current.

After a number of preliminary runs, it was judged to select a waterflow of 7 cm.s^{-1}. Such current was found adequate to separate PL-10 postlarvae within a reasonably short period of time into different classes related to their resistance to the flow (Water Flow Resistance Classes: WRC's).

Upon separation of the larvae it was attempted to relate flow resistance with osmotic stress resistance as well as with growth and survival in a laboratory scale nursery experiment.

The sensitivity to osmotic stress was measured following the method described by Rees et al. (1993). For each WRC three samples of ten animals are transferred with a small volume of culture water into plastic beakers. At one cm above the bottom a small window is cut out of the vertical wall of the recipients; this opening is covered with a 500 micrometer screen. At the start of the test the culture water is evacuated through this screen by a quick tilting of the beakers which are then submerged in a bath containing fresh water at ambient temperature (around 25 °C). From then onward every five minutes the postlarvae are individually prodded with a needle and the number of immobile animals is recorded. The Osmotic Stress Sensitivity Index (OSSI) is calculated from the data obtained during the first 30 minutes of the experiment (Fig. 3).

Growth tests for the different WRC's were executed in 50 l aquaria (4 replicates per treatment). The tanks were stocked with PL-10 postlarvae at densities of 3 animals per liter. For a subsequent period of three weeks the postlarvae were fed the 600-800 micrometer fraction of a grower commercial grow-out pellet (30 % protein content) at a rate of 30% per day; the food was administered in three daily rations. The feedings were adjusted weekly according to individual wet weight measurements. Uneaten food, most feces and possible dead animals were removed once a day before the first feeding. The experiment was run at ambient temperature with á daily water exchange of 30%.

Upon termination of the experiment, survival and growth of the juvenile shrimp (individual dry and

weight) were determined for each tank taking into account all harvested animals.

Results

The classification of postlarvae into different WRC's proved to be reproducible. A number of consecutive runs with animals of the same larval culture tank (commercial hatchery) revealed similar patterns. Fig. 2 shows the case in which the animals were separated in 3 different lots according to the time during which they resist the water current: lot 1 groups those animals that left the tube within the first 2 minutes; the postlarvae from the second lot remained in the tube for at least 2 minutes but not more than 6 minutes. The animals in the last group never left the tube during the 6 minutes that the experiment lasted.

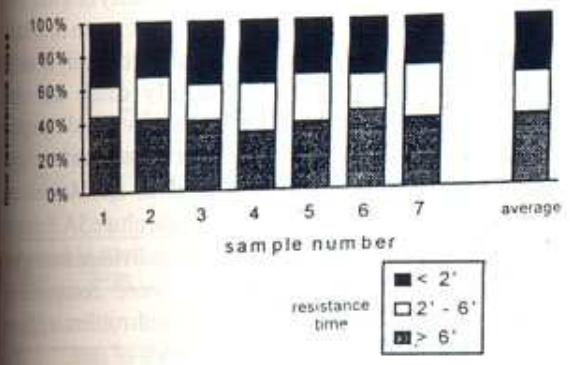
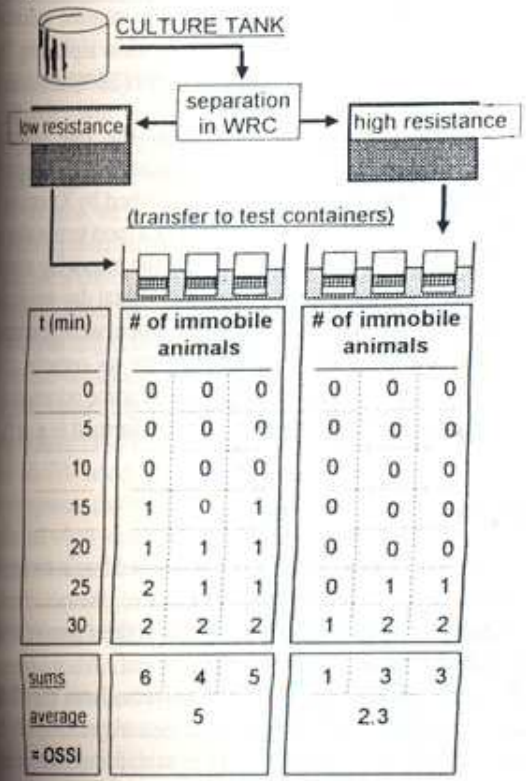


Figure 2: Flow resistance of a batch of PL10 larvae: individual results of seven replicate separation runs and corresponding average value.



t (min)	# of immobile animals			# of immobile animals		
0	0	0	0	0	0	0
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	1	0	1	0	0	0
20	1	1	1	0	0	0
25	2	1	1	0	1	1
30	2	2	2	1	2	2
sums	6	4	5	1	3	3
average	5			2.3		
= OSSI						

Figure 3. Schematic diagram of the execution of the osmotic stress test and subsequent calculation of the osmotic stress sensitivity index: typical example for two WRC's.

When animals from the different WRC's were subjected to the osmotic stress test a remarkable relation was revealed: good resistance to osmotic stress clearly corresponds to good resistance to the water current (see also Fig. 3).

The nursery culture experiment indicates a similar positive correlation between flow resistance and growth of the postlarvae. For this test a new batch of animals was separated into 2 classes: class 1 groups what could be called the weak animals that were washed out of the tube within 2 minutes. The strong animals of class 2 remained in the tube during 6 minutes (OSSI-values for the different classes were respectively 5 and 2.3). Upon termination of the bio-assay no significant difference in survival could be detected between both WRC's (83 and 82%). Larval growth, measured in terms of individual dry and wet weight showed 17% resp. 19% significantly different figures: the animals resisting best osmotic stress and water flow grew to the biggest size.

Discussion

It has been shown that the same larval culture run produces animals with very different resistance to osmotic stress. This probably explains the often wide variation of the OSSI's within one tank i.e. when a mixture of animals is evaluated (own observations). Such variation is also likely to impair the outcome of nursery experiments where one aims at the establishment of a possible direct link between resistance to osmotic stress and the production potential of a batch of postlarvae.

From this work there can be concluded that the physiological condition of postlarvae, measured in terms of osmotic stress resistance, correlates with physical performance which in the present case is the ability to withstand a water current.

The applied method is non-destructive and permits to subject the qualified animals to subsequent evaluations such as e.g. survival and growth in nursery systems. Consequently it offers a better possibility to elucidate the still unanswered question whether or not good postlarval resistance to osmotic stress guarantees superior production results in the remaining phases of the shrimp rearing cycle. Our preliminary results in this respect indicate that such relationship may indeed exist and is primarily expressed by the growth rate rather than by the survival.

The developed apparatus is simple to construct, the procedure is easy to standardize and the result is quantitative. As such, the method meets the requirements of a practical postlarval qualification tool. The application on industrial scale may provide both the seller and the buyer of postlarvae with more accurate information than does the outcome of the osmotic stress test.

Larval feeding

A next group of experiments aimed at the improvement of the larval rearing through the manipulation of feeding strategies. More specifically, the effects of adding rotifers to the feeding regime of *P. vannamei* was studied.

Because of size limitations, the feeding of *Artemia* nauplii in larval shrimp rearing is not initiated before the late zoea or early mysis stages. Offering the smaller sized rotifers to the first zoea stages would therefore favor relative energy intakes and consequently enhance larval development and growth (Léger *et al.*, 1986). Léger (1988) positions rotifers in a live food based feeding regime (Fig. 4) and their appropriate dimensions to fill up the size gap between algae and *Artemia* nauplii is illustrated by Thai practices where introducing *Brachionus plicatilis* in the feeding regime of larval *P. monodon* reduces the dependence on algae and *Artemia* (Kongkeo, 1991). Rotifers also facilitate the early introduction of high quality protein of animal origin in the larval diet and the predator may take advantage of the prey's set of digestive enzymes to better utilize simultaneously administered feeds (Jones *et al.*, 1991).

Due to their non-selective filter feeding behaviour and high metabolic rates the rotifers can very rapidly be enriched with a whole range of active components that are then passed on to the predator via the food chain: essential long chain poly unsaturated fatty acids (Léger *et al.*, 1989), vitamins (Merchie et al., *1993)* , prophylactics and antibiotics (Léger, 1988) and probionts (Gatesoupe, 1993).

A number of these advantages are recognized by Chinese shrimp growers who found out that better survival is obtained in the larval culture of *P. orientalis* when using rotifers as the main food during Z2-Z3 stages (Liu Fengqi, 1993). In this particular case the rotifers are collected from fertilized outdoor earthen ponds.

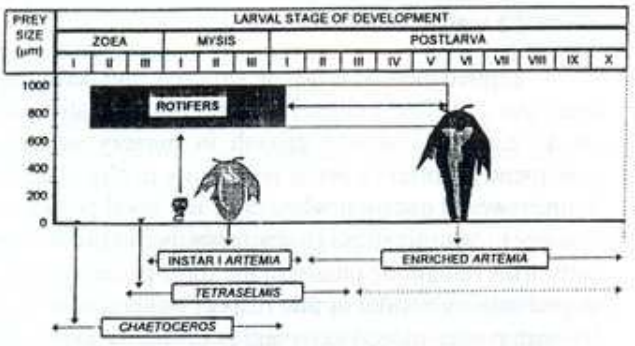


Figure 4: The use of algae, rotifers and *Artemia* in a feeding regime for shrimp larvae (modified from Léger , 1988).

Materials and methods

For the presented experimental purposes a rotifer culture method was established in which the animals were fed either fresh baker's yeast or Culture Selco (Inve Aquaculture SA, Belgium). The latter product is a commercially available, formulated rotifer culture diet with a high HUFA content. The culture method was based upon the recommendations of Lavens etal. (1992) and was successfully applied under Ecuadorian conditions in 10 liter buckets as well as in both cylindroconical and flat-bottomed tanks of 25, 100 and 500 liter. The rotifers belonged to the S-strain of *Brachionus plicatilis* of which a starter culture was made available by the Laboratory for Aquaculture and Artemia Reference Center (University of Ghent, Belgium).

Fatty acid profiles of the cultured rotifers were not determined. Applying standard culture conditions, Lavens et al. (1992) report levels of 5.4, 4.4 and 15.6 $mg.g^{-1}$ rotifer dry matter of EPA, DHA and (n-3) HUFA, respectively. Rotifers grown on conventional culture diets (baker's yeast and unicellular algae) only contain 4 $mg.g^{-1}$ dry matter of (n-3) HUFA and not more than 0.2 $mg.g^{-1}$ of DHA (Komis *et al.*, 1991).

Shrimp nauplii were obtained from shrimp spawning stations using wild-caught gravid females. Third stage nauplii were reared in 250 liter tanks until the moult to Z2 was completed. Then, the animals were transferred to U-shaped experimental tanks with a capacity of 4 liter. From this point onward the feeding with rotifers can be initiated, as preliminary observations proved the capacity of second stage zoea larvae to successfully catch and ingest reproducing female *Brachionus*. The larval feeding regimes in the various trials were based on a combination of algae (*Chaetoceros gracilis* and *Tetraselmis* sp.), *Artemia* and formulated diets (Frippak, Inve Aquaculture SA, Belgium). According to the objective of each individual feeding trial the experimental treatments were composed by supplementing this control regime with rotifers at different densities and during different periods of time.

In the U-shaped tanks the culture water was aerated through a perforated PVC-tube positioned on the tank's bottom. Each container was equipped with a filter system via which about 90% of the culture water was changed daily. During the execution of this routine procedure possible sediments were removed by slow siphoning. The set-up allowed for temperature control (28 °C in all trials).

During the course of a trial the rate of larval development was monitored and quantified by means of the Larval Stage Index (LSI) as described by Kanazawa et al. (1985) and Teshima *et al.* (1983). Upon termination of an experiment (usually PL7-PL8) the surviving animals were individually counted, the individual dry weight and the length of the sixth abdominal segment were determined and the animals were submitted to an osmotic stress situation as previously described. On some occasions also intermediate measurements were performed (e.g. at PL2-PL3).

Results

The first larval rearing trials learned that in the absence of other prey organisms (e.g. *Artemia*), the rate of rotifer consumption directly correlates with their density as well as with the larval stage of development. When *Artemia* is offered simultaneously with rotifers (from the Z3-M1 stages onward), the former is ingested preferentially but not exclusively (Table 1). This mixed ingestion of both small and large preys was observed within the experimented range of larval stages (Z3-PL5).

Table 1.- Larval stage related rotifer consumption in experimental cultures of *P. vannamei* under different conditions of prey density.

Larval stage	Initial prey density		Rotifer consumption		Observations
	rotifers (rot./ml)	*Artemia* (art./ml)	measured (rot/larvae/5h)	calculated (rot/larvae/d)	
Z2	2	0	19.0	91	rotifer limitation
	4	0	23.3	112	
	8	0	59.3	285	
Z3	4	0	59.5	286	
	8	0	73.3	352	
	16	0	132.0	634	
M1	6	0	78.5	377	
	6	0.7	25.6	123	
	12	0	115.0	552	
	24	0	150.1	720	
M1-M2	6	0.7	27.7	133	
M2-M3	6	1.5	47.3	227	

Table 2: Daily evolution of the larval stage index (LSI) of *P. vannamei* in cultures fed a diet of *Chaetoceros* (CH), freshly hatched *Artemia* nauplii and different levels of rotifers.

Days of Culture	Larval Stage	Larval feeding schedule							
		CH	Artemia	Rotifers					
		(10^3 cells.ml^{-1})	(art.ml^{-1}.d^{-1})	(rot.ml^{-1}.d^{-1})	LSI	(rot.ml^{-1}.d^{-1})	LSI	(rot.ml^{-1}.d^{-1})	LSI
1	Z2	80	2	1,25	2	2,5	2	5	2
2	Z2-Z3	80	2.55	8	2.66	16	2.57	32	2.55
3	Z3	80	3	20	3	40	3	80	3
4	Z3-M1	80	3.2	23	3.1	46	3	92	3.2
5	Z3-M1	80	4	18	4	36	3.81	72	4
6	M1	80	-	6	-	12	-	24	-
7	M1-M2	80	5.6	6	5.8	6	5.8	6	5.6

In a next feeding trial it was observed that the calculated LSI index was independent from the daily amounts of rotifers offered (Table 2). Therefore, in all subsequent experiments with combined rotifer and *Artemia* feeding the rotifers were administered following the feeding schedule presented in Table 3. For the Z2 and Z3 stages the schedule is composed as to provide the minimal number of rotifers without limitations to be expected. During the first two mysis stages the Z3-regime remains unchanged and is then followed by an increase during M3. The data presented in table 4 illustrate the impact of prolonged rotifer feeding (up to three vs. five days old postlarvae) and the effect of the HUFA level in the rotifers. The rate of larval development during the mysis stages is clearly positively affected when rotifers are included in the feeding schedule: the larval stage index indicates that rotifer feeding shortens the period prior to the first postlarval stage with nearly one day (this conclusion could be drawn fromall the experiments carried out so far). The results suggest that this advantage is already reached by the mid-mysis fase. An elevated HUFA content in the administered otifers apparently further accelerates larval development.

Larval and postlarval growth (measured in terms of individual dry weight and total length) also improve in rotifer fed treatments. When the rotifer feeding is extended up to five day old postlarvae (regardless of the type of rotifers used), the impact becomes even more pronounced in the animals' dry weight, but total length is not further affected. The influence of the HUFA content of the rotifers is primarily reflected in the recorded values of individual dry weight. To a lesser extend this trend isalso noticeable in the figures of total postlarval length.

The sensitivity to osmotic stress decreases when baker's yeast fed rotifers (low HUFA levels) are offered. On the contrary, when the HUFA input is increased via Culture Selco fed rotifers, the low OSSI values indicate a better resistance to osmotic shocks.

Table 3: Rotifer and *Artemia* feeding Table for experimental culture of *P.vannamei* larvae.

Larval stage	Algae feeding (10^3cells/ml)		Rotifer feeding (rot./ml)				Artemia feeding (art./ml)			
	Chaetoceros	*Tetraselmis*	8 am	4 pm	10 pm	total	8 am	4 pm	10 pm	total
Z2	100	10	4	4	8	16	-	-	-	-
Z2-Z3	100	10	4	4	8	16	-	-	-	-
Z3	100	15	6	6	12	24	-	-	-	-
Z3-M1	50	20	6	6	12	24	0.075	0.075	0.15	0.3
M1	50	20	6	6	12	24	0.125	0.125	0.25	0.5
M1-M2	50	20	6	6	12	24	0.2	0.2	0.4	0.8
M2	50	10	6	6	12	24	0.3	0.3	0.6	1.2
M2-M3	50	10	8	8	16	32	0.3	0.3	0.6	1.2
M3	50	10	8	8	16	32	0.375	0.375	0.75	1.5
M3-PL1	50	10	8	8	16	32	0.375	0.375	0.75	1.5
PL1-PL2	50	10	(6)	(6)	(12)	(24)	0.5 to 1.5	0.5 to 1.5	1 to 3	2 to 6
PL3 to PL5	/	/	(6)	(6)	(12)	(24)	0.5 to 1.5	0.5 to 1.5	1 to 3	2 to 6
PL6...	/	/	0	0	0	0	2 ...	2 ...	4 ...	8 ...

Table 4: Larval stage index, individual dry weight, total length and osmotic stress sensitivity index of *P. vannamei* larvae and postlarvae fed algae and *Artemia* (treatment 1, control treatment) or algae and *Artemia* supplemented with rotifers (treatments 2 through 5). The rotifers were fed until the M3-PL1 stage (treatments 2 and 4) or until the PL4-PL5 stage (treatments 3 and 5). Rotifers with a low HUFA-content were obtained from baker's yeast fed cultures and administered to treatments 2 and 3. Rotifers rich in HUFA's were obtained from Culture Selco fed cultures and administered to treatments 4 and 5.

		Stage	Treatment				
			1 Con	2 RBs	3 RBl	4 RCs	5 RCl
Larval stage index (LSI)		Z3-M1	3.907	3.963	3.950	3.860	3.943
		M1-M2	4.030	4.967	4.900	4.567	4.600
		M3-PL1	5.900	6.633	6.730	6.633	6.567
Individual dry weight	(mg)	PL2-PL3	200.0	221.1	230.0	237.0	235.0
		PL7-PL8	355.4	404.5	447.2	472.8	553.3
Length of sixth abdominal segment	(mm)	PL7-PL8	1619.6	1683.3	1688.0	1715.9	1718.6
Osmotic stress sensitivity index (OSSI)		PL7-PL8	1.200	1.833	1.504	0.933	0.621

Discussion

It is observed that rotifer feeding during the zoea and mysis stages markedly accelerates the larval development. This is expressed by significantly speeding up the metamorphosis to the postlarval stage withalmost one day. The differences in length of the sixth abdominal segment in PL-7 show that during the postlarval stages the animals continue to moult faster since at this age every new moult in *P. vannamei* postlarvae results roughly in a 50 mm increase of this biometric (Van Horenbeeck, unpublished data). Our results indicate a tendency that high HUFA levels in the larval diet shorten even more the inter-moulting time. Extending the rotifer feeding till five days following the metamorphosis however does not affect the postlarval moulting speed, suggesting that the nutritional input during the pre-postlarval phase is of prime importance for future development. This confirms the findings of Léger et al. (1985) who were able to draw similar conclusions upon studying the effect of high HUFA diets during zoea and mysis stages.

A prolonged administration of rotifers increases the postlarval dry weight. Since total length (and consequently the moulting stage) is not affected by such practice, the overall result is expressed by less slender postlarvae. Following criteria applied by Asian farmers this may imply postlarvae of inferior quality (Anonymous, 1991).

The sensitivity to osmotic stress tends to decrease when rotifers with high HUFA levels are offered to the larvae. Such reaction is noticed even when rotifer feeding is discontinued at the time of metamorphosis which again confirms the importance of HUFA-rich diets during the early larval stages (Léger *et al.*, 1985). The importance of these dietary components to improve stress resistance is documented for marine shrimp and fish species (Tackaert et al., 1989, Dhert *et al.*, 1992). It should however be beared in mind that in our experiments the stress tests were executed simultaneously for all treatments. Consequently, as discussed earlier, the obtained results refer to animals of the same age but of different moulting stages and therefore do not allow to conclude whether increased stress resistance is related to the advanced stage of development or to the HUFA content or the administered food itself.

Acknowledgments

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References

Anonymous, 1991. Farmers' criteria for choosing quality shrimp fry. Excerpts from Aquaculture TECH TALK, 1989, Feedpro Corporation, 509 Camba St., San Nicolas, Manila, Philippines. In: Sorgeloos, P. (ed.). Larviculture and Artemia Newsletter. State University of Ghent, Faculty of Agricultural Sciences. Laboratory of Aquaculture and Artemia Reference Center, Ghent, Belgium. Number 20, June 1991 pp 29-30.

Bauman, R., G.,H., and Jamandre, D., 1990. A practical method for determining quality of *Penaeus monodon* (Fabricius) fry for stocking in grow out ponds. In: New, M.B., de Saram, H., and Singh, T. (eds.). Proceedings of the Aquatech conference on Technical and Economic aspects of shrimp farming. Publ. INFOFISH. Kuala Lumpur.

Bauman, R., G., H., and Scura, E., 1990. Determining the quality of *Penaeus monodon* postlarvae. In: Book of Abstracts. World Aquaculture 1990, June 10-14. Halifax, Nova Scotia, Canada.

Dhert, Ph., Lavens, P., and Sorgeloos, P., 1992. State of the art of Asian seabass *Lates calcarifer* larviculture. Journal of the World Aquaculture Society, 23(4), 317-329.

Duran Gomez, R., Rodruez, J.M., and Morales, J., 1991. Stress-tests: a practical tool to control postlarval shrimp quality. In: Lavens, P., Sorgeloos, P., Jaspers, E., and Ollevier, F. (Eds.). Larvi '91 - Fish and Crustacean Larviculture Symposium. European Aquaculture Society, Special Publication Nº 15, Gent, Belgium.

Fegan, D. F. 1992. Recent developments and issues in the penaeid shrimp hatchery industry. In: Wyban, J., (Ed.). Proceedings of the Special Session on Shrimp Farming. World Aquaculture Society, Baton Rouge, LA, U.S.A.

Gatesoupe, F. J. 1991. *Bacillus* sp. spores: a new tool against early bacterial infection in turbot larvae, *Scophthalmus maximus*. In: Lavens, P., Sorgeloos, P., Jaspers, E., and Ollevier, F. (Eds.). Larvi '91 - Fish and Crustacean Larviculture Symposium. European Aquaculture Society, Special Publication Nº 15, Gent, Belgium.

Jones, D. A., Kamarudin, M. S., and Le Vay. 1991. The potential for replacement of live feeds in larval culture. In: Lavens, P., Sorgeloos, P., Jaspers, E., and Ollevier, F. (Eds.). Larvi '91 - Fish and Crustacean Larviculture Symposium. European Aquaculture Society, Special Publication Nº 15, Gent, Belgium

Kanazawa, A., Teshima, S., and Sakamoto, M., 1985. Effects of dietary lipids, fatty acids, and phospholipids on growth and survival of prawn (*Penaeus japonicus*) larvae. Aquaculture, 50, 39-49.

Komis, A., Candreva, P., Franicevic, V., Moreau, V., Van Ballaer, E., Léger, Ph., and Sorgeloos, P., 1991. Successful application of a new combined culture and enrichment diet for the mass cultivation of the rotifer *Brachionus plicatilis* at commercial hatchery scale in Monaco, Yugoslavia, France and Thailand. In: Lavens, P., Sorgeloos, P., Jaspers, E., and Ollevier, F. (Eds.). Larvi '91 - Fish and Crustacean Larviculture Symposium. European Aquaculture Society, Special Publication Nº 15, Gent, Belgium.

Kongkeo, H. 1991. An overview of live feeds production system design in Thailand. In: Fuchs, W., and Main, K. L., (eds.). 1991. Rotifer and Microalgae Culture Systems. Proceedings of a U.S. - Asia Workshop. Honolulu, HI, Januari 28 - 31, 1991, pp 175-186.

Lavens, P., Dhert, P., Merchie, G., Stael, M., Devresse, B., and Sorgeloos, P., 1992. A standard procedure for the mass production on an artificial diet of rotifers with a high nutritional quality for marine fish larvae. In: Book of Abstracts, Third Asian Fisheries Forum, Singapore, October 26-30,1992. Asian Fisheries Society, Philippines.

Léger, P., Bieber, G.F., and Sorgeloos, P., 1985. International study on Artemia. XXXIII. Promising results in the rearing of *Penaeus stylirostris* using a prepared diet as algal substitute and for Artemia enrichment. Journal of the World Mariculture Society 16: 354-367.

Léger, P., Bengtson, D. A., Simpson, K.L., and Sorgeloos, P. 1986. The use and nutritional value of Artemia as a food source. Oceanography and Marine Biology: An Annual Review 24:521-623.

Léger, P. 1988. Optimized feeding regimes in shrimp hatcheries. Seminar presented at the opening ceremony of the bilateral Ecuadorian Belgian Artemia project, Guayaquil, Ecuador, March 25, 1988.

Léger, P., Grymonpré, D., Van Ballaer, E., and Sorgeloos, P. 1989. Advances in the enrichment of rotifers and Artemia as food sources in marine larviculture. In: Aquaculture Europe '89. Short communications. Abstracts. European Aquaculture Society Special Publication, number 10. Bredene, Belgium.

Léger, P., Verpraet, R., Nelis, H. J., De Leenheer A.P., and Sorgeloos, P. 1990. Live food mediated drug delivery as a tool for disease treatment in larviculture. 1. The enrichment of sulfadrugs in rotifers and Artemia nauplii. A case study. In: Book of Abstracts. World Aquaculture 1990, June 10-14. Halifax, Nova Scotia, Canada.

Liu Fengqi, 1991. Larviculture of Chinese white shrimp *Penaeus orientalis* in the People's Republic of China. In: Lavens, P., Sorgeloos, P., Jaspers, E., and Ollevier, F. (Eds.). Larvi '91 - Fish and Crustacean Larviculture

Symposium. European Aquaculture Society, Special Publication Nº 15, Gent, Belgium.

Merchie G., Lavens, P., Radull, J., Nelis, H., Sorgeloos, P., and A. De Leenheer. 1993.

Effect of feeding vitamin C-enriched live food on the hatchery production of *Macrobrachium rosenbergii*. In: Book of Abstracts. World Aquaculture 1993, May 26-28. Torremolinos, Spain.

Rees, J.F., Curé, K., Pyiatiratitivorakul, S., Sorgeloos, P., and Menasveta, P. 1994. Highly unsaturated fatty acid requirements of *Penaeus monodon* postlarvae: an experimental approach based on *Artemia* enrichment. Aquaculture,

Samocha, T. M., and Lawrence, A. L. 1992. Shrimp nursery systems and management. In: Wyban, J., (Ed. . roceedings of the Special Session on Shrimp Farm , World Aquaculture Society, Baton Rouge, LA, U.S.A.

Tackaert, W., Abelin, P., Dhert, P., and Sorgeloos, P. 1989. Stress resistance in postlarval penaeid shrimp reared under different feeding procedures. Journal of the World Aquaculture Society 20(1): 74A.

Teshima, S., Kanazawa, A., and Sasada, H., 1983. Nutritional value of dietary cholesterol and other sterols to larval prawn, *Penaeus japonicus* Bate. Aquaculture, 31, 159-167.

Wilkenfeld, J. S. 1992. Commercial hatchery status report: an industry panel viewpoint. In: Wyban, J., (Ed.). Proceedings of the Special Session on Shrimp Farming. World Aquaculture Society, Baton Rouge, LA, U.S.A.