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Successful culture of larvae of *Litopenaeus vannamei* fed a microbound formulated diet exclusively from either stage PZ2 or M1 to PL1

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Abstract

In three separate experiments, harpaticoid copepods *Tisbe monozota* (alive and dead) and a microparticulate microbound diet were evaluated as alternatives to live *Artemia* nauplii as food, beginning at either stage PZ2 or M1, in the larval culture of *Litopenaeus vannamei*. Larvae were cultured in 2 L round bottom flasks at a density of 150 L^{-1} (Experiment 1) and 100 L^{-1} (Experiments 2 and 3) at 28 °C, 35‰ salinity and 12:12 LD photoperiod, and fed 4×/day^{-1}. Larvae were initially fed a mixture of phytoplankton to stages PZ2 or M1 and then fed either live *Artemia*, live or dead copepods, or a microparticulate microbound diet. The experiments were terminated and all larvae were harvested when more than 80% of larvae had molted to postlarvae 1 (PL1) within any flask representing any of the treatments. The comparative value of the different diets and feeding regimes was determined by mean survival, mean dry weight and total length of individual larva, and percentage of surviving larvae that were PL1. Trypsin activity of samples of larvae from each treatment was also determined. The microparticulate microbound diet effectively served as a complete substitute for *Artemia* nauplii when fed beginning at stage M1. When fed at the beginning of the PZ2 stage, survival was comparable to that of larvae fed *Artemia,* but mean dry weight, mean total length, and percent of surviving larvae that were PL1 generally were significantly less. Responses to the feeding of copepods, whether fed dead or live, as a substitute were generally significantly less than those of larvae fed either the *Artemia* nauplii or the microparticulate diet. Values of trypsin activity (10^{-5} IU/μg^{-1} dry weight) corresponded to the relative proportions of the different larval stages within a treatment, with higher activity being characteristic of early stages. Previously demonstrated successful results with another species of crustacean suggest that the microparticulate microbound diet has characteristics that should be effective in the culture of the carnivorous stages of other crustacean and fish larvae that are currently fed live *Artemia* nauplii.

Keywords: Larviculture; *Litopenaeus vannamei*; Formulated; Microbound diet

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1. Introduction

The need for live food at some or all stages of larval culture of fish and crustacean species continues to be a drawback to additional and more predictable production of species globally. The proportionately high cost of labor associated with the production of live food, such as rotifers and newly hatched *Artemia* nauplii, is confounded by the challenge that the food will be nutritionally complete. In addition, the availability and cost of *Artemia* cysts fluctuates and contributes to an undesired lack of predictability in production costs. As a result, during the past four decades, a considerable amount of research effort has been devoted to the development of formulated diets that can either partially or completely substitute for live food at all, or at least certain, stages of larval development.

The chronic overall lack of success may reside in the seemingly insurmountable task of creating a formulated diet that has all the characteristics that are considered ideal for performance of a larval diet. Some of the desirable traits tend to be mutually exclusive, so some compromise is inevitably necessary. The pursuit of successful larval diets may require a redirection founded in simplicity and compromise coupled with the surrender of untested biases and exceptional goals (D'Abramo, 2002).

Formulated diets that have been prepared and tested for larval culture commonly fall under the categories of microencapsulated, microcoated, or microbound (Tucker, 1998). Early encouraging studies (Jones et al., 1987) suggested that potential value of microencapsulated feeds as substitutes for *Artemia,* but the success use of these formulated diets was never realized in the absence of a live food supplement. When a microencapsulated diet (Frippak®) was evaluated as a complete substitute for microalgae in the culture of larval *Litopenaeus vannamei* during stages PZ1 to PZ3 survival and total length were appreciably lower that what was achieved with live food (Sangha et al., 2000). The Frippack® diet was also evaluated as a complete substitute for algae and *Artemia* in the larval culture of *Penaeus japonicus* (LeVay et al., 1993). Survival was significantly lower and time required to reach stages M1 (algae fed) and PL1 (*Artemia* fed) was significantly higher. Most of the success realized, generally measured by growth, survival, and practicality, has been realized with microbound diets. Growth and survival of larvae of *P. japonicus* fed a carrageenan-based microbound diet from protozoea stage 2 (PZ2) were similar to that of larvae fed live diatoms and live *Artemia* nauplii (Bautista et al., 1989). Teshima et al. (1993) created successful carrageenan bound microbound diets with casein as the primary protein source for nutritional studies with larval *P. japonicus*. A high moisture, microbound diet successfully served as a complete substitute for live *Artemia* nauplii for the culture of larvae of the freshwater prawn *Macrobrachium rosenbergii*, from stage IV to PL (Kovalenko et al., 2002). Growth and survival were 80% of what was achieved with the feeding of the live food. This diet has also been prepared and proved effective in a low moisture (10%) form. The success of this diet was principally attributed to its high water stability and presumed high digestibility because gut retention times for carnivorous larvae are comparatively rapid. Therefore, this diet may presumably represent the foundation to use and refine for larval culture of other species at stages when carnivorous feeding is initiated during development.

The objective of this study was to determine whether the microbound formulated diet that proved successful as a complete substitute for *Artemia* in the larval culture of *M. rosenbergii* could be similarly effective in the laboratory culture of larvae of *L. vannamei*, from either stage PZ2 or PZ3 to PL1. In addition, the possible dietary-dependent effect on trypsin activity of the larval tissue was investigated.

2. Materials and methods

2.1. Animals, culture system and experimental design

The study consisted of three independent experimental trials, each trial consisting of the same dietary treatments (Table 1). The microbound microparticulate diet was fed as an exclusive dietary source, from stage PZ2 or M1 to PL1, substituting for either copepods or *Artemia* nauplii, or a combination of mixed microalgae and copepods or *Artemia* nauplii. For each trial, the experimental *L. vannamei* larvae were collected from a group of approximately 20,000 hatched from a single female obtained from Maricultura del Pacifico hatchery in Los Pozos, Sinaloa, México. The nauplii were transported to the Nutrition and Larviculture Laboratory of Centro de Investigación en Alimentación y Desarrollo (CIAD) Mazatlán Unit (Mexico) where culture of live food organisms and the trials were conducted. All nauplii were acclimatized to experimental conditions of 28 °C, filtered (Hytex 5 μm cartridge) 35‰ seawater that was gently aerated (one bubble per second). Nauplii (N5) were stocked at either 150 L^{-1} (Experiment 1) or 100 L^{-1} (Experiments 2 and 3) into 2 L round bottom flasks that contained the filtered seawater and were suspended in a 28 °C water bath under a 12/12 L:D photoperiod. Each dietary treatment consisted of five replicates (flasks) and larvae from each of the 5 replicates were examined to identify larval stage throughout the duration of the

Table 1
Experimental dietary treatments (feeding regimes) consisting of different combinations of a mixture of microalgae, live or dead natural food, or a formulated microbound, microparticulate diet fed at different stages in the larval culture of *L. vannamei*

Stage	Treatments					
	D1	D2	D3	D4	D5	D6
N5	Not fed	Not fed	Not fed	Not fed	Not fed	Not fed
PZ1	MA	MA	MA	MA	MA	MA
PZ2	MA	2 *Artemia* ml^{-1}	MA	2 cops ml^{-1}	MA	AD 8 mg ml^{-1}
PZ3	MA	2 *Artemia* ml^{-1}	MA	2 cops ml^{-1}	MA	AD 8 mg ml^{-1}
M 1	5 *Artemia* ml^{-1}	5 *Artemia* ml^{-1}	5 cops ml^{-1}	5 cops ml^{-1}	AD 12 mg ml^{-1}	AD 12 mg ml^{-1}
M 2	7 *Artemia* ml^{-1}	7 Artemia ml^{-1}	7 cops ml^{-1}	7 cops ml^{-1}	AD 12 mg ml^{-1}	AD 12 mg ml^{-1}
M 3	10 *Artemia* ml^{-1}	10 *Artemia* ml^{-1}	10 cops.ml^{-1}	10 cops ml^{-1}	AD 16 mg ml^{-1}	AD 16 mg ml^{-1}
PL 1	10 *Artemia* ml^{-1}	10 *Artemia* ml^{-1}	10 copsml^{-1}	10 cops ml^{-1}	AD 16 mg ml^{-1}	AD 16 mg ml^{-1}

cops: harpacticoid copepodites of *Tisbe monozota*.
MA: Mixed microalgae (50 cells $\mu 1^{-1}$ of *C. muelleri* (70%)+*I. galbana* (30%).
AD: Microbound microparticulate diet.

experiment. Molting stages were determined using a binocular microscope and were based on the descriptions of Tabb et al. (1972). All nauplii assigned to the different dietary treatments were initially fed a diet of mixed algae (*Chaetoceros muelleri* and *Isochrysis galbana* in a 70:30 ratio, respectively) at 50 cells μL^{-1} up to stage PZ2. All microalgal species were grown at CIAD-Mazatlán in a semi-continuous culture within 20 L glass containers at 20 °C, 35‰ salinity, and 24 h light. Cell density was measured using a haemocytometer (Brightline, 0.100 mm deep).

After molting to PZ2, the six different dietary treatment regimes, consisting of different, stage-dependent sequences of the feeding of either mixed microalgae, copepods, newly hatched *Artemia* nauplii, or a microbound microparticulate diet (Table 1) began. The *Artemia* nauplii (Great Salt Lake brand) were hatched daily for each feeding. A culture of the copepod *Tisbe monozota* was maintained under continuous culture conditions (27 ± 1 °C, 35‰ salinity, and 12 h:12 h L:D photoperiod). Protein and lipid contents (dry weight) of *T. monozota* grown under similar conditions were 67.5% and 7.5%, respectively, and the levels of eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids were 0.13% and 0.7%, respectively (Puello-Cruz et al., 2004). An unknown number of organisms was inoculated into a 300 L tank and fed once a day on a standard diet of a 3:1:1 mixture of microalgae consisting of *Tetraselmis suecica*, *C. muelleri*, and *I. galbana,* respectively, at a density of 320 cells L^{-1}. Information concerning ingredient composition, preparation, and proximate composition of the microbound microparticulate diet (62–65% moisture content) is presented in Kovalenko et al. (2002). The proximate composition, expressed on a dry weight basis, is 46.1% crude protein, 37.4% crude lipid, 5.6% ash, and the nitrogen free extract (by difference) is 10.9%. The crude protein, lipid, and ash contents (dry weight) of *Artemia* nauplii of equivalent state of development have been reported to be 53.8%, 16.2% and 11.1%, respectively (García-Ortega et al., 1998). The diet was air dried under a laboratory hood for approximately 20 h to a moisture content of 10%, sifted to the desired size (250–450 μ) through a sieve of appropriate mesh, and stored frozen at −20 °C. Prior to every feeding, a portion of the formulated diet was removed from storage, hydrated and fed as a suspension using micropipettes (Finnpipette Labsystems, Helsinki, Finland) to produce a concentration that ranged from 8 to 16 mg ml^{-1}, increasing through the later stages of development. For dietary treatments D4 and D5, the copepods were fed either dead (trial one) or alive (trials 2 and 3). All the different, stage-dependent diets were fed to the larvae 4× per day (08.00, 12.00, 16.00 and 20.00 h).

2.2. Data collection

During the course of an experimental trial, stages of larvae within the culture flasks were identified visually. When changes were noted, approximately 3 larvae were removed from a culture flask for confirmation and then returned. When a treatment was presumed to contain a majority of PLs, as identified by a change in swimming behavior, 10 shrimp were removed from each of the replicates of that treatment for confirmation of stage. If more than 80% of larvae had molted to postlarvae 1 (PL1) within any flask (replicate) representing any of the treatments, then the experimental trial was terminated. At that time, generally 9 days, all larvae were harvested from each of the culture flasks and mean survival, dry weight, total length and percent of surviving larvae that were PL were determined. Survival (%) was determined by counting all surviving larvae,

dividing by the total number that was initially stocked (200 or 300), and multiplying by 100. To determine mean individual dry weight (mg larva^{-1}) for each treatment ten larvae were randomly removed from each replicate, washed briefly in distilled water, placed into pre-weighed aluminum foil cups, dried in a laboratory oven at 60 °C for 24 h, and then weighed using a Mettler MT5 microbalance (Mettler Instrument Corp., P.O. Hightstown, N.J. 08520, Switzerland). This process was repeated until equivalent dry weights were achieved for consecutive weightings. Individual mean length (mm) of larvae for each treatment was determined by random removal of 10 larvae from each replicate. While positioned under a binocular microscope, each larva was measured from the tip of the rostrum to the tip of the telson using an electronic digital caliper.

Ten larvae were randomly removed from each of 5 replicate flasks of each treatment, homogenized in 1000 μl of Tris–HC1 buffer (pH 8.1), and centrifuged at 12,000 rpm for 3 min. Trypsin-like activity in the supernatant fraction was determined using *N*-α-*p*-toluenesulphonyl-L-arginine methyl ester (TAME) as a substrate (Rick, 1974), adapting the method of LeVay et al. (1993). A mixture of 100 μl of 10 mM substrate solution and 800 μl of Tris–HCl buffer (pH 8.1) was prepared in a 1.5 ml Eppendorf tube and equilibrated at 25 °C. Then 200 μl of supernatant sample was added and mixed. This reaction mixture was transferred to a 1 cm path length quartz cuvette placed into a thermostatically controlled (25 °C) holder. Change in absorbance at 247 nm over 3 min was measured in a Hewlett Packard 8453 Diode Array Spectrophotometer. Activity was expressed in IU (international units) equivalent to 1 μmol of substrate hydrolyzed in 1 min (Rick, 1974). Tissue trypsin content was calculated and activity was expressed as IU $\times 10^{-5}$ μg^{-1} dry weight.

2.3. Statistical analysis

For each of the three experimental trials, a one way ANOVA using the general linear model procedure (PROC GLM) was conducted for each of the response variables to determine whether significant differences existed among treatments. Survival and percentage of PL1 variables were subjected to the arcsine square root transformation prior to analysis. If significance was found, then Fisher′s Protected LSD procedure was used for mean separation to determine which treatments were significantly different from one another. For all comparisons, $P<0.05$ was accepted as the significance level.

3. Results

3.1. Experiment 1

Mean dry weight, total length, and survival were not significantly different between comparable dietary treatments in which exclusive feeding of either newly hatched *Artemia* nauplii (D1 and D2) or the microbound microparticulate diet (D5 and D6) exclusively was initiated at either larval stage M1 or PZ2 (Table 2). Almost 75% of the surviving larvae fed the microbound diet exclusively from stage PZ2 had metamorphosed to the PL1 stage at the termination of the experiment. This percentage was significantly higher than that of the comparable dietary treatment in which live *Artemia* nauplii were fed. When the microbound diet was fed from stage M1, the percentage of PL among the surviving larvae was significantly lower than that of larvae fed the *Artemia* nauplii. When either copepods or the microbound diet was fed from M1 (D3 vs. D5) the response variables were not significantly different. When these same diets were fed starting at the PZ2 stage, the microbound diet yielded significantly higher values for all responses except survival.

When the feeding of dead copepods instead of *Artemia* nauplii began at the PZ2 stage (D2 vs. D4), the mean total length of larvae was significantly less. When feeding of dead copepods instead of *Artemia* nauplii began at the M1 stage (D1 vs. D3), no significant difference between mean dry weight or mean total length was observed. Survival of larvae and percent of surviving larvae that had metamorphosed to P1 were significantly lower.

3.2. Experiment 2

Substitution of live copepods for *Artemia* nauplii was not as effective as what was achieved with dead

Table 2
Mean dry weight, total length, survival, and percent of total surviving larvae that were PL1 for the different dietary treatments in Experiment 1

Dietary treatment	Weight (μg)	Total length (mm)	Survival (%)	% PL1
D1	86.4 ± 24.2^{a}	$4.74\pm0.42^{a,b}$	$64.8\pm6.8^{a,b}$	46.5 ± 22.2^{b}
D2	$70.0\pm7.9^{a,b}$	$4.66\pm0.27^{a,b}$	$46.0\pm21.1^{a,b}$	41.9 ± 22.0^{b}
D3	$72.0\pm26.3^{a,b}$	$4.36\pm0.54^{b,c}$	41.2 ± 24.9^{b}	8.8 ± 15.5^{c}
D4	58.4 ± 12.6^{b}	4.16 ± 0.27^{c}	41.2 ± 20.6^{b}	7.8 ± 13.6^{c}
D5	$68.0\pm12.7^{a,b}$	$4.70\pm0.33^{a,b}$	$53.5\pm23.4^{a,b}$	15.3 ± 14.8^{c}
D6	85.0 ± 13.6^{a}	5.12 ± 0.22^{a}	66.0 ± 9.0^{a}	74.4 ± 13.0^{a}

Superscripted letters denote Mean±SD, five replicates per treatment. Means not sharing a common superscript are statistically different at $P<0.05$.

Table 3
Mean dry weight, total length, survival, and percent of total surviving larvae that were PL1 for the different dietary treatments in Experiment 2

Dietary treatment	Weight (μg)	Total length (mm)	Survival (%)	% PL1
D1	113.2±4.6[a]	5.56±0.13[a]	70.1±4.1[a]	74.3±3.3[a,b]
D2	106.0±7.1[a,b]	5.36±0.15[a,b]	71.3±6.1[a]	65.9±4.5[b]
D3	56.4±22.6[d]	4.26±0.24[c]	62.5±10[7a,b]	1.5±2.3[d]
D4	45.2±5.6[d]	4.06±0.23[c]	46.8±5.6[c]	0.0[d]
D5	95.2±10.4[b,c]	5.48±0.16[a]	71.7±9.2[a]	82.3±5.5[a]
D6	85.4±14.1[c]	5.12±0.26[b]	54.0±13.0[b,c]	49.2±13.9[c]

Superscripted letters denote Mean±SD, five replicates per treatment. Means not sharing a common superscript are statistically different at $P<0.05$.

copepods in Experiment 1. Mean dry weight, total length, survival, and percent of surviving larvae that were PL1 of larvae fed copepods instead of *Artemia* nauplii, from either stage PZ2 (D2 vs. D4) or stage M1 (D1 vs. D3), were significantly less with one exception (survival, D1 vs. D3). Substitution of the feeding of *Artemia* nauplii with the microbound diet from stage M1 (D1 vs. D5) yielded no significant differences in mean total length, survival, and percent of surviving larvae that had developed to the PL1 stage. Mean dry weight was significantly less. Mean weight, survival, and percent of surviving larvae that achieved the PL1 stage for larvae fed the microbound diet from the PZ2 stage were significantly less than those fed *Artemia* nauplii beginning at the same stage (D2 vs. D6). Feeding of the microbound diet yielded larval responses, except for survival, that were significantly greater than those of larvae that were fed live copepods in comparative dietary treatments (D3 vs. D5; D4 vs. D6) (Table 3).

Table 4
Mean dry weight, total length, survival, and percent of total surviving larvae that were PL1 for the different dietary treatments in Experiment 3

Dietary treatment	Weight (μg)	Total length (mm)	Survival (%)	% PL1
D1	90.0±11.1[b]	5.24±0.26[a,b]	61.1±15.1[a,b]	60.1±6.8[b]
D2	106.0±7.1[a]	5.46±0.21[a]	59.5±17.5[a,b]	63.4±7.8[a,b]
D3	56.4±22.6[c]	4.20±0.06[d]	57.5±7.4[a,b]	0.0[d]
D4	45.2±5.6[c]	4.02±0.07[d]	48.0±5.4[b]	0.0[d]
D5	95.2±10.4[a,b]	5.22±0.19[b]	62.0±18.5[a,b]	71.1±6.5[a]
D6	89.4±8.0[b]	4.94±0.06[b]	69.1±11.3[a]	39.0±14.1[c]

Superscripted letters denote Mean±SD, five replicates per treatment. Means not sharing a common superscript are statistically different at $P<0.05$.

Table 5
Mean trypsin activity (IU 10^{-5} μg^{-1} dry weight) of the tissue of larvae of *L. vannamei* under different feeding regimes

Experiment			
	1	2	3
Dietary treatment			
D1	15.7±11.8[c]	15.9±2.4[b]	16.5±3.3[b]
D2	25.4±4.7[b,c]	15.3±3.5[b]	12.6±2.6[b]
D3	42.0±31.3[a,b]	53.6±16.2[a]	41.9±15.6[a]
D4	46.7±11.5[a]	51.5±5.4[a]	42.9±10.3[a]
D5	31.3±15.1[a,b,c]	16.9±5.5[b]	17.8±1.2[b]
D6	25.7±3.0[b,c]	23.4±3.6[b]	19.7±3.2[b]

3.3. Experiment 3

When either the *Artemia* nauplii or the microbound diet was fed from stage M1, mean dry weight, total length, and survival were not significantly different. The percent of surviving larvae that had metamorphosed to PL1 was significantly higher for those larvae fed the microbound diet exclusively. When larvae were fed *Artemia* nauplii from stage PZ2 (D2), mean weight, length, and percent of surviving larvae that were PL1 were significantly higher than those observed for larvae fed the microbound diet exclusively (D6). Larvae fed either *Artemia* or the microbound diet, from either stage PZ2 or M1, had significantly higher mean dry weight, total length and percent of those surviving larvae that reached stage PL1 than larvae fed live copepods from comparative stages (D1vs. D3; D2 vs. D4; D3 vs. D5; D4 vs. D6). Survival was not significantly different between the comparable dietary treatments, except for one comparison (Table 4).

3.4. Trypsin activity

Trypsin activity values for larvae collected at the termination of each experiment for each of the dietary treatments are presented in Table 5. Mean values ranged from 12.6 to 53.6×10^{-5} IU μg^{-1} dry weight. Reduction of trypsin activity corresponded to an increasing proportion of individuals that were stage PL1 that were within each of the treatment groups as influenced by the quality of the dietary regimes.

4. Discussion

The microparticulate microbound diet served as a complete and effective substitute for newly hatched *Artemia* nauplii when fed from M1 to PL1 during the larval culture of *L. vannamei*. In 11 of the 12 possible comparisons of the four response variables (dry weight,

total length, survival, and percent of surviving larvae that were PL1) spanning the three independent experiments, the microbound dietary treatment performed as well as or significantly better than the *Artemia* nauplii dietary treatment. The dry form of the microbound diet used in this study demonstrated good intrinsic water stability and was maintained in the water column with gentle aeration. Exclusive feeding of the microbound diet instead of live *Artemia* nauplii, from as early as the PZ2 stage, yielded equivocal results, only demonstrating comparable value in Experiment 1. Although survival was generally similar, the lack of comparable efficacy may have resulted from particle size being too large for the PZ2 stage. Copepods, whether fed as dead or alive from stage PZ2 or M1, were not comparable substitutes for *Artemia* nauplii or the microbound diet across all response variables. When live copepods were fed, they were observed to remain at the bottom of the flask and the gentle aeration was insufficient to maintain them in the water column. The presumed lack of access of food may have been responsible for the poor performance of larvae in that dietary treatment and may explain why the response to the feeding of dead copepods in Experiment 1 was comparatively much better.

The decrease in trypsin activity relative to the proportion of larvae in the PL1 stage is supported by the previously reported results of Lovett and Felder (1990) for *Penaeus setiferus*, Kumlu and Jones (1995) for *P. indicus*, and Puello-Cruz et al. (2002) for *L. vannamei* who found that trypsin activity of larvae fed live food was highest in the zoel stages and progressively decreased through the mysid stages and to PL1. A similar trend was observed for larvae of *P. japonicus* fed live food (LeVay et al., 1993). The comparative stage-dependent change in trypsin activity during the larval cycle appears to be an ontogenic response that is unaffected by the feeding of the formulated diet used in the current study versus a live diet. However, as stated by Puello-Cruz et al. (2002), the levels of trypsin activity in the PZ2 stage of *L vannamei* are higher than levels of the same larval stage of other crustacean species. This characteristic, in conjunction with providing the appropriate sized particle, could be the foundation for achieving success in the feeding of the microbound diet exclusively from the PZ2 stage.

The utility of the microbound diet as a complete and nutritionally acceptable substitute for *Artemia* nauplii in the larval culture of *L. vannamei* supports the success previously achieved in the larval culture of another crustacean species. The high quality performance of the diet (in a high moisture form) was originally demonstrated for the larval culture of the freshwater prawn *Macrobrachium rosenbergii* from stage IV to PL (Kovalenko et al., 2002). The consistent nutrient composition of the microbound diet, whether fed in a moist or dry form, is the foundation for reliability in obtaining comparable growth within the duration of each larval cycle. The ingredient composition of the diet can be easily modified to address unique nutrient requirements. The high level of lipid and the corresponding fatty acid profile that is characteristic of the egg yolk ingredient can be reduced through extraction. This action would permit better control over the lipid and fatty acid content of the diet. The capacity to modify formulation and nutrient content of the diet is the foundation for its potential use as an effective substitute for live food for the larval culture of carnivorous stages of other species of crustaceans as well as fish. The laboratory successes that have been realized need to advance to the verification stage through evaluation under conditions of large-scale commercial production.

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