2011-12-01

The Economics of Partial Artemia Replacement Using Two Commercially Available Feeds in the Diets of Litopenaeus vannamei from Z3/M1 – PL10

Monique A. Giguere
University of Miami, m.luna0226@gmail.com

UNIVERSITY OF MIAMI

THE ECONOMICS OF PARTIAL *ARTEMIA* REPLACEMENT USING TWO COMMERCIALLY AVAILABLE FEEDS IN THE DIETS OF *LITOPENAEUS VANNAMEI* FROM Z3/M1 – PL10

By

Monique A. Giguere

A THESIS

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Master of Science

Coral Gables, Florida

December 2011

UNIVERSITY OF MIAMI

A thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science

THE ECONOMICS OF PARTIAL *ARTEMIA* REPLACEMENT USING TWO COMMERCIALLY AVAILABLE FEEDS IN THE DIETS OF *LITOPENAEUS VANNAMEI* FROM Z3/M1 – PL10

Monique A. Giguere

Approved:

_____	_____
Daniel Benetti, Ph.D.	Terri A. Scandura, Ph.D.
Professor of Marine Affairs and Policy	Dean of the Graduate School
_____	_____
Maria Estevanez, M.A., M.B.A	Jorge Arturo-Suarez, Ph.D.
Senior Lecturer	Research Scientist
Marine Affairs and Policy	CENIACUA, Colombia

GIGUERE, MONIQUE A. (M.S., Marine Affairs and Policy)

The Economics of Partial *Artemia* Replacement Using Two Commercially Available Feeds in the Diets of *Litopenaeus vannamei* from Z3/M1 – PL10.

(December 2011)

Abstract of a thesis at the University of Miami.

Thesis supervised by Daniel Benetti, Ph.D.
No. of pages in text. (34)

The purpose of this study was twofold: 1) to identify commercially available feeds that could serve as suitable replacements for newly hatched *Artemia* in the diets of *L. vannamei* from Z3/M1 to PL10 without significantly affecting survival, final length and weight, and quality of the larvae and 2) to identify an ideal substitution rate between live *Artemia* and a replacement feed that maximizes feed and labor costs savings, survival, and PL quality. In Experiment 1, two commercially available *Artemia* replacement feeds, Zeigler EZ Artemia and Bernaqua Vitellus, were administered according to manufacturer's guidelines in order to identify which feed served as a more suitable replacement diet. In Experiment 2, the more successful feed from Experiment 1 was administered in three different co-feeding strategies, in which the inert feed replaced a certain percentage of live *Artemia*. Mean percent survival was not significantly different between the Control, EZ Artemia, and Vitellus treatment groups in Experiment 1 ($P<0.05$). Both the EZ Artemia and Vitellus treatments yielded significantly different final mean lengths (mm) and weights (mg) from the Control group. The Vitellus feed results for all performance factors (mean percent survival, final length (mm), final weight (mg), and percent stress test mortality) were not significantly different than those of the EZ Artemia treatment,

despite receiving no *Artemia* during the culture period, while the EZ Artemia treatment received 75% *Artemia* from PL5-PL10. For these reasons, the Vitellus feed was selected as the more successful feed in Experiment 1. In Experiment 2, there was no significant difference between the four treatment groups (Control, V50, V100/50, and V100/75) for mean percent survival and percent stress test mortality ($P<0.05$). The V100/50 and V100/75 treatments' mean final lengths (mm) and weights (mg) were significantly different than those of the Control treatment. There were no significant differences between the V50, V100/50, and V100/75 treatments for any of the observed performance factors. These results indicate that the maximum substitution rate of Vitellus for *Artemia* in this experiment (the V100/75 treatment) was successful in replacing 84.33% of newly hatched *Artemia* in the larval culture of *L. vannamei* from Z3/M1-PL10 without resulting in significantly different survival and stress test mortalities compared to the Control group. Feeding schedules such as V100/75 treatment help streamline production efforts in commercial operations and result in increased production cost savings when compared to other replacement feeding schedules that begin in the early mysis stages. The V100/75 feeding schedule influences variable feed and labor costs the greatest because farmers are able to delay the culturing of *Artemia* an additional 7 days (until PL5) from what is typically performed in larviculture facilities.

ACKNOWLEDGEMENTS

I would like to thank Guillermo Jaramillo, M.A., R&D Manager, Harris B. Wright, Ph.D., Geneticist, Eduardo Figueras Gil, Production Manager, and Krista Hillman, R&D Biologist, from Shrimp Improvement Systems LLC for supplying the facility for these experiments and for their technical assistance with this study. I thank Daniel Benetti, Ph.D, Maria Luisa Estevanez, M.A., M.B.A., and Jorge Arturo-Suárez, Ph.D. for their guidance and help to improve the earlier drafts of this manuscript. I also thank Pascal Boisot, Aquaculture Research Engineer, InVivo NSA, and Bernaqua for supplying the experimental Vitellus feed used in this study.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AA	Ascorbic acid
AAS	Ascorbic acid 2-sulphate
ANOVA	Analysis of variance
DHA	Docosahexaenoic acid
EDTA	Ethylenediaminetetraacetic acid
EPA	Eicosapentaenoic acid
GSL	Great Salt Lake
HPV	Hepatopancreatic parvo-like virus
HSD	Tukey's Honestly Significant Difference
HUFAs	Highly unsaturated fatty acids
IPNV	Infectious pancreatic necrosis virus
M	Mysis
NPG	Nauplii per gram
PL	Postlarvae(al)
SBF	San Francisco Bay
SPF	Specific pathogen free
V100/50	Experiment 2 treatment group in which larvae receive 100% Vitellus from Z3/M1-PL4 and then 50% *Artemia* 50% Vitellus from PL5-PL10
V100/75	Experiment 2 treatment group in which larvae receive 100% Vitellus from Z3/M1-PL4 and then 25% *Artemia* 75% Vitellus from PL5-PL10
V50	Experiment 2 treatment group in which larvae receive 50% *Artemia* and 50% Vitellus from Z3/M1-PL10
WSSV	White spot syndrome virus
Z	Zoea

1. CHAPTER 1: INTRODUCTION: A SUMMARY OF PROBLEMS ASSOCIATED WITH *ARTEMIA* PRODUCTION AND ITS USE IN AQUACULTURE

1.1. Dependence on a singular source for *Artemia* supply

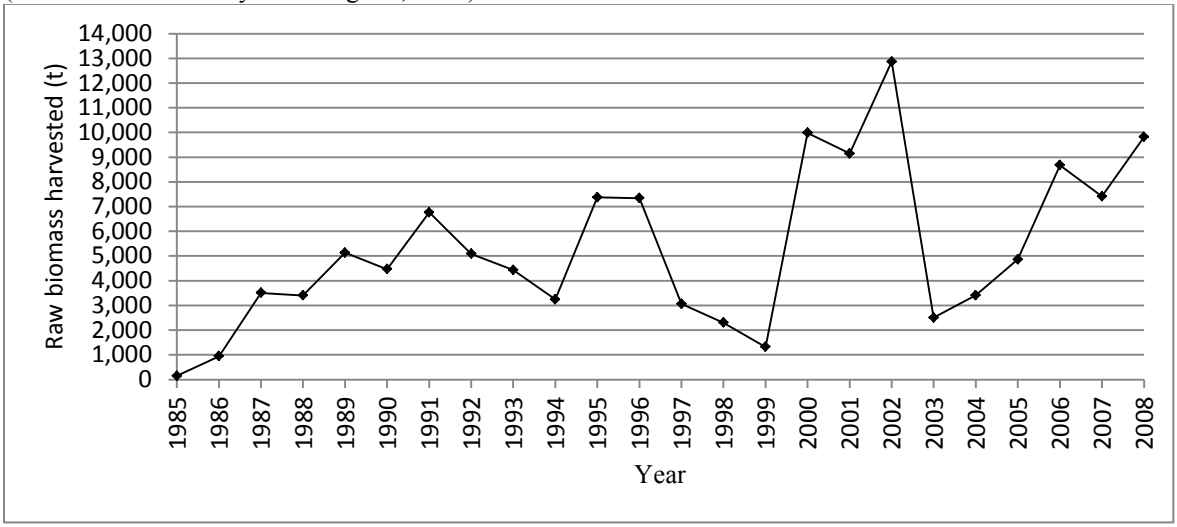
The demand for *Artemia* for use in aquaculture operations has increased dramatically with the growth of the aquaculture industry over the last 35 years. Potential problems with reliance on *Artemia* as a main component of larviculture diets is well documented in literature as far back as the early 1960s (Sorgeloos, 1976).

The Great Salt Lake (GSL) in Utah, USA has been the primary source of *Artemia* cysts to the world market since the development of the aquaculture industry. Research on the historical and present use of *Artemia* in aquaculture indicate that over 90% of the world's commercial harvest of *Artemia* cysts have been derived from GSL (Dhont & Stappen, 2003; Lavens & Sorgeloos, 2000b; Treece, 2000). Such dependence on a singular source for a product that is crucial to the larviculture of numerous marine species has, and will continue to be, detrimental to the stability of aquaculture operations worldwide.

The potential for *Artemia* supply to act as a bottleneck to the further expansion of the industry was repeatedly highlighted at international conferences in the 1960s as cyst prices continued to soar and hatching quality became more and more unreliable (Bengston, Leger, & Sorgeloos, 1991; Sorgeloos, 1976). Sorgeloos (1980) indicated that the amount of cysts needed to produce one million nauplii could range from 4-50g, a 90% difference in hatching rate. By the late 1980s, the search for potential alternatives to the use of live *Artemia* was crucial, an effort still ongoing today (Dhont & Sorgeloos, 2002).

Unpredictable harvests from GSL (Figure 1.1) are caused by several factors including changing weather patterns and lake salinity due to El Nino events (Sorgeloos, Dhert, & Candreva, 2001), low food availability due to decreases in primary production, decreases in the harvest season due to bad weather, contamination of cyst streaks with dying biomass, and cyst buoyancy problems due to low salinity levels (Lavens & Sorgeloos, 2000b).

Figure 1.1. Historical *Artemia* cyst harvests (tons of raw biomass) from GSL, Utah, USA (Great Salt Lake Ecosystem Program, 2010).



Conditions such as the above mentioned led to highly variable harvests in the mid-late 90s (Martín et al., 2006), with final harvest weights ranging from 2680-6640 metric tons of raw wet cysts in a given year (Lavens & Sorgeloos, 2000b). Cyst prices remained highly variable throughout the early 90s ranging from 20-100 dollars/kg depending on concentration of essential fatty acids. In 1997, over 1,500 metric tons of cysts were required for hatcheries, with 80-85% of these sales going to shrimp hatcheries, mainly in China and Southeast Asia (Dhont & Sorgeloos, 2002; Stappen, 1996). To date, no other source of *Artemia* cysts has been discovered with the production power of GSL. Smaller quantities of cysts are available from various saltworks and man-managed ponds

worldwide (Dhont & Sorgeloos, 2002; Triantaphyllidis, Abatzopoulos, & Sorgeloos, 1998); however, these operations are cost/market driven and their profitability highly dependent on the production of GSL in a given year (Lavens & Sorgeloos, 2000b). No stable future supply of *Artemia* cysts can be guaranteed at this time due to a lack of information on the ecology of new production sites (Dhont & Sorgeloos, 2002; Lenz, 1987).

For over twenty years, studies have highlighted the need for artificial diets to replace *Artemia* (Jones, Kanazawa, & Abdel Rahman, 1979); however, progress in the development and optimization of these feeds have been slow at best. While these diets have certainly improved in terms of their nutritional composition, digestibility, and stability in the water, further work must be done to identify how efficiently these feeds can be substituted for *Artemia* (Wouters, Cobo, Dhont, & Wille, 2009). Shi-Yen Shiau (1998) highlighted the increasing importance of artificial diets as the intensities of aquaculture operations increase. In the end, as always, it comes down to a bottom line of whether these artificial diets present a cost-effective alternative to *Artemia*. If these diets can be utilized in a way that promotes ease of use and results in economic savings, then perhaps we may see a decrease in demand for traditional live feed items such as *Artemia*.

1.2. Nutritional deficiencies, variable nutrient composition, and hatching quality between strains of *Artemia*

It is well documented that the nutritional composition of *Artemia* differs largely between and among strains regardless of harvest batch or season (Dhont & Sorgeloos, 2002; Lavens & Sorgeloos, 2000a; Martín et al., 2006; Sorgeloos, 1980; Stappen, 1996) (Table 1.1).

Table 1.1. Compositional variation between strains of *Artemia* in the naupliiar stage (Dhont & Stappen, 2003).

Source	Protein (%)	Lipid (%)	Carbohydrate (%)	Ash (%)	Fiber (%)	Dry weight (μg)
GSL, USA	41.6-61.9	14.4-23.1	3.6-10.6	7.1-9.5	5.9	1.65-2.31
PR China	47.3	12	-	21.4	-	3.09
France	55.7	12.4	-	15.4	-	2.7-3.1
SFB, USA	41.9-59.2	15.9-27.2	11.2	8.17	-	1.45-2.87

Fatty acid concentration is one of the most researched biochemical components of *Artemia* (Bengston, 2003; Leger, Bengston, Sorgeloos, Simpson, & Beck, 1987). Six fatty acids have been found to make up 80% of total fatty acids in *Artemia*: palmitic acid (16:0), palmitoleic acid (16:1n-7), oleic acid (18:1n-9), linoleic acid (18:2n-6), linolenic acid (18:3n-3), and eicosapentaenoic acid (EPA) (20:5n-3) (Leger, Simpson, & Sorgeloos, 1986). Variations in the concentrations of these fatty acids between strains of *Artemia* is summarized in Table 1.2. While both fatty acid and amino acid concentrations vary between and within strains of *Artemia*, amino acid fluctuations tend to be much lower (Wouters et al., 2009). The concentration of n-3 series fatty acids, particularly EPA and docosahexaenoic acid (DHA) (22:6n-3), are of paramount importance when considering the nutritional quality of an *Artemia* strain (Dhont & Sorgeloos, 2002; Treece, 2000). EPA maintains flexibility/permeability of biomembranes and is linked to increased larval survival, while DHA is essential for the production of neural, retinal, and brain tissue and is associated with improved larval quality and growth (Dhont & Sorgeloos, 2002; Sorgeloos et al., 2001). Research highlights the importance of maintaining a DHA/EPA ratio greater than 2 for promoting pigmentation, growth, and stress resistance (Dhont & Sorgeloos, 2002), a value which can only be attained through enrichment due to low concentrations of DHA in *Artemia* nauplii. The variation in concentration of fatty acids has been found to be highly dependent on the diet fed

(Schauer, Johns, Olney, & Simpson, 1980) and the environmental conditions encountered by the parent *Artemia* (Dhont & Sorgeloos, 2002; Stappen, 1996).

Table 1.2. Variation in fatty acid content between strains of *Artemia* in the naupliiar stage (Dhont & Stappen, 2003).

Source	Palmitic acid (16:0)	Palmitoleic acid (16:1n-7)	Oleic acid (18:1 n-9)	Linoleic acid (18:2 n-6)	Linolenic acid (18:3n-3)	Eicosapen-taenoic acid (20:5n-3)	Docosahex-aenoic acid (22:6n-3)
GSL, USA	13.2-19.4	4.1-7.4	20.3-34.8	5.7-10.1	28.6-40.0	3.5-8.9	trace
SFB, USA	3.6	0.1	6.1	1.8	8.2	0.6	-
PR China	12.6	6.7	17.8	11	3.6-39.3	1.4-7.5	0.0-0.4
Urmia, Iran	-	-	-	-	-	2.7	0.4
A.parthenogenetica	15.7	1.6	23.7	12.2	6.8	3.5-14.7	0.0-0.4
Madagascar	18.6	20.2	21.2	7.4	6.25	24.5	0
A. persimilis,Arg	14.4	9.5	17.9	8	16.7	0	0.3
A. tibetiana, China	23.6	2.4	40.4	6.2	trace	44.7	0.2

Other compounds also found to vary between strains of *Artemia* include pigments (canthaxanthin), ascorbic acid 2-sulphate (AAS) (converted completely to ascorbic acid (AA) upon hatching), minerals, trace elements, and the total amount of free amino acids (Dhont & Stappen, 2003; Sorgeloos, Coutteau, Dhert, Merchie, & Lavens, 1998; Wouters et al., 2009). Insufficient levels of AA result in poor feed conversion, reduced molting frequency/incomplete molting, impaired collagen synthesis, decreased wound healing, and melanized lesions under the exoskeleton (aka black death syndrome) in penaeid shrimp (Pedrazzoli et al., 1998). Supplemental AA has been found to be beneficial to skeletal development, growth, survival, immunoactivity, and resistance to stress and toxicants (Sorgeloos et al., 1998).

Newly hatched *Artemia* are nutritionally deficient in lipids, essential fatty acids (particularly EPA and DHA) (Sorgeloos et al., 2001), vitamins, and anti-oxidants (Dhont & Sorgeloos, 2002; Treece, 2000). While these essential fatty acids and other nutrients are present in *Artemia*, they are in sub-optimal concentrations. These nutrients can be

boosted in *Artemia* through enrichment, however this represents an additional cost to a feed that is already expensive to purchase and culture. Additionally, the stability of certain enrichment ingredients, particularly DHA, is difficult to guarantee due to its inherent catabolism upon ingestion by the *Artemia* (Merchie, 1996; Sorgeloos et al., 2001). An easier solution would be to incorporate the nutrients required by the cultured species into an inert diet that maintains its nutrient profile until ingested by the larvae.

Hatching quality is another factor that is highly variable between strains and batches of *Artemia* (Bengston et al., 1991; Sorgeloos, Lavens, Leger, Tackaert, & Versichele, 1986; Wouters et al., 2009). Hatching quality of cysts is determined by several factors including hatching efficiency (nauplii/g cysts), hatching synchrony (time elapsed between first and last cysts hatched), and hatching percentage (hatching cysts/100 cysts) (Dhont & Sorgeloos, 2002). Variation between these factors, in addition to higher levels of highly unsaturated fatty acids (HUFAs), account for the major price differences between strains of *Artemia* (Prescott & Michotte, 1980). A minimum nutritional value, hatching synchrony of less than 7 hours, and a hatching efficiency greater than 200,000 nauplii per gram (NPG) are all crucial factors that aquaculturists scrutinize during their selection of an *Artemia* strain (Sorgeloos et al., 2001). Other important factors to consider when selecting strains are size and energy content at intended time of feeding (Dhont & Sorgeloos, 2002).

1.3. *Artemia* as a vector for disease

There has been an increasing amount of research in recent years on the capability of *Artemia* to act as a possible vector or host of certain pathogens (Zhang et al., 2010).

Artemia have been found to act as reservoirs and/or mechanical carriers for several bacterium and viruses including infectious pancreatic necrosis virus (IPNV), nodavirus, *Vibrio*, *Staphylococcus*, *Erwinia*, *Micrococcus*, and *Bacillus* (Briggs, n.d.; Sudhakaran, Yoganandhan, Ishaq Ahmed, & Sahul Hameed, 2006).

During the breaking stage of *Artemia* cysts during hatching, glycerol is released, offering an ideal culture medium for *Vibrio* (Sorgeloos et al., 2001) and potential infection for larvae after feeding. One study found that white spot syndrome virus (WSSV) was capable of infecting *Artemia* at four different life stages (nauplii, metanauplii, pseudoadults, and adults) and becoming a carrier of the virus (Zhang et al., 2010). Conversely, other studies have concluded that *Artemia* do not act as carriers or reservoirs for WSSV in *Penaeus indicus* (Hameed et al., 2002).

The incidence of hepatopancreatic parvo-like virus (HPV) in larval stages of penaeid shrimp is high. Mortality ranges from 50-100% in 4-8 weeks in juvenile *P. merguiensis*, with stunted growth occurring later in adult life (Sivakumar, Sarathi, Venkatesan, Sivaraj, & Hameed, 2009). A study by Sivakumar et al. (2009) proved that horizontal transmission of HPV from *Artemia* to healthy *Penaeus monodon* is possible. *Artemia* were also found to act as reservoirs or mechanical carriers of *Macrobrachium rosenbergii* nodavirus and extra small virus to the freshwater prawn *Macrobrachium rosenbergi* (Sudhakaran et al., 2006).

1.4. The need for economic evaluation of *Artemia* production vs. the use of replacement diets

Very little research has been focused on the economics of individual aspects of aquaculture operations. Broad definitions of the variable and fixed costs of facilities are

outlined in literature when calculating the operational costs of facilities (Lipton & Harrell, 2004; Urban Jr. & Pruder, 1991). Very little is discussed, however, about the variation in cost of contribution of certain aspects of production costs, such as those that arise from the production and use of *Artemia*. Pillay and Kutty (2005a) highlight the need for aquaculture scientists to incorporate economics into their experimental designs in order for their findings to be more easily integrated into commercial applications.

Variable costs can be divided into the subcategories of variable production costs and variable labor costs which are defined in Table 1.3.

Table 1.3. Variable production and labor costs of an aquaculture operation (Pillay & Kutty, 2005a).

Variable production costs	*Variable labor cost*
Water fees	Wages in cash and kind
Farm preparation and maintenance	Board and lodging provided
Purchase of animals	Number of man-hours/day and number of man-days/month
Purchase of feed/feed ingredients	
Purchase of chemicals, drugs, and fertilizers	
Purchase of electricity and fuel	
Purchase of shipping materials/containers	
Freight and transportation costs	
Adjustments for inventory changes in feeds and other major production materials	

These costs are undoubtedly site specific and vary significantly depending on intensity of production. Feed costs typically account 36-50% of variable production costs (Moss & Leung, 2006; Pillay & Kutty, 2005b; Quintero & Roy, 2010; Sorgeloos, 2000; Valenti & Tidwell, 2006) and up to 62-68% of total production costs on intensive farms (Tacon, Nates, & McNeil, 2006). In addition to influencing variable labor costs, the production of live feeds such as *Artemia* influence several aspects of variable production costs, including: farm preparation and maintenance, water fees, purchase of feed, purchase of chemicals, and the purchase of electricity and fuel. Whereas an inert feed

only influences the singular aspect of purchase of feeds in variable production costs. Variation in the nutrient content of *Artemia* nauplii and fluctuations in cyst prices also lead to increasing variation in unit costs of production. A preliminary cost analysis of these factors could help managers more clearly evaluate potential cost savings in their feeding practices. One such analysis is performed on the data from this study in Chapter 4.

In addition to influencing variable cost, the uncertainty of the ability for *Artemia* cyst production to keep up with the growth of the aquaculture industry can be added to the long list of risks associated with the industry. Risk in aquaculture is greater than in any other form of animal husbandry (Pillay & Kutty, 2005a). The use of *Artemia* replacement diets would streamline feed management practices and provide a means of limiting or reducing the risk associated with live feeds production if further improvement of these feeds yields increased substitution rates.

1.5. Current progress with *Artemia* replacement diets

Factors such as the above mentioned have motivated researchers and farmers to evaluate possible alternatives to live feeds. *Artemia* replacement diets are manufactured in both dry and wet forms. Important factors to consider when selecting dry or wet replacement diets are adequate nutritional content, stability in the water column, particle size and distribution, digestibility, minimal leaching of nutrients, and the capacity to be stored over long periods of time (Wouters et al., 2009; Zelaya, Davis, & Rouse, 2007).

The majority of *Artemia* replacement studies recommend a 50% or less substitution rate of *Artemia* for an inert diet while still maintaining comparable survival,

growth/metamorphose rates, and response to stress tests (Gamboa-Delgado & Le Vay, 2009; Jones, Kamarudin, & Le Vay, 1993; Robinson, Samocha, Fox, Gandy, & McKee, 2005; Zelaya et al., 2007). Some studies have had more success with *Artemia* substitution than those above. Microbound diets have been completely substituted for live *Artemia* nauplii with the freshwater prawn *M. rosenbergii* and achieved growth and survival rates that were 80% of what was typically seen with live feeds (Kovalenko, D'Abramo, Ohs, & Buddington, 2002). D'Abramo et al. (2006) performed an experiment using the same microbound diet in Kovalenko's experiment with *L. vannamei* and also found the diet was able to serve as an effective substitute for *Artemia* nauplii from the M1 to PL1 stages. The success of these two experiments is attributed to the consistent nutrient composition of the microbound diet and its high moisture form (D'Abramo, Perez, Sangha, & Puello-Cruz, 2006).

A study by Gamboa-Delgado and Le Vay (2009) indicated that *L. vannamei* larvae co-fed diets of *Artemia* and inert feed showed significantly higher survival than larvae fed either the *Artemia* or inert feed alone, with growth rates from the 25 and 50% replacement diets being comparable to growth observed with *Artemia* alone. Additionally, larvae reared on co-fed diets metamorphosed to the PL1 stage faster. Another notable result from this study was that in all co-fed treatments, carbon contributions from *Artemia* to the growth of the mysis and early PL stages were much higher than expected, considering the proportions of feed provided. This higher incorporation of nutrients from *Artemia* is thought to be partially due to the higher digestibility of live food items compared to inert feeds (Gamboa-Delgado & Le Vay, 2009).

Many studies report that shrimp fed diets with partial replacement of *Artemia* by inert diets had less weight gain (Robinson et al., 2005) and shorter lengths (Robinson et al., 2005; Samocha, Matsumoto, Jones, & Torano, 1999) than those shrimp fed only *Artemia*. In contrast, Calderon et al. (2004) reported no significant difference in rostro-caudal length at PL3 between treatments fed liquid replacement diets and those fed with only live feeds. Additionally, some studies have shown a greater assimilation and/or ingestion of inert diets in the presence of live food items (Gamboa-Delgado & Le Vay, 2009).

Zelaya et al. (2007) reported similar growth rates in four treatment groups: (1) commercial dry feed plus algal paste, (2) commercial feed, algal paste, and *Artemia* every day, (3) commercial feed, algal paste, and *Artemia* every other day, and (4) not supplemented; relied on natural occurring algae and commercial feed. However, treatments supplemented with *Artemia* (2 and 3) had higher weights at the end of the experiment compared to treatments 1 and 4. This most likely represents a higher incorporation of the commercial feed due to the presence of *Artemia*, as seen in Gamboa-Delgado and Le Vay's study, or increased feeding on both the commercial and live feed due to the higher digestibility of the *Artemia*. Interestingly, there were no significant differences found in Zelaya's study between diets 3 and 4 in which *Artemia* were fed every day and every other day, respectively.

The success of *Artemia* replacement diets lies in the development of a middle ground, that is, an ideal substitution rate for marketed replacement feeds that provides maximum nutritional benefit to the species while still resulting in economic savings for the producer. The primary goal of this study is to identify one such ideal substitution rate.

While there is still much debate over the efficacy of live feeds replacement, there are some undoubtable benefits:

- Reduction of primary larviculture feeds cost
- Improved disease control/reduction
- Decreased personnel, electricity, and equipment necessary for culture of *Artemia*
- Customization of replacement feeds to contain a nutrient profile specific to the culture species
- Potential for replacement feeds to be administered using auto-feeders, leading to reduced employment hours necessary for feeding, and increased control over the frequency and duration of feeding

2. CHAPTER 2: MATERIALS AND METHODS

2.1. Experimental goals and design

The purpose of this study was twofold: 1) to identify commercially available feeds that could serve as suitable replacements for *Artemia* in the diets of *L. vannamei* from Z3/M1 to PL10 without significantly affecting survival, final length and weight, and quality of the larvae and 2) to identify an ideal substitution rate between live *Artemia* and a replacement feed that maximizes feed and labor costs savings, survival, and PL quality. In Experiment 1, two commercially available *Artemia* replacement diets, Zeigler EZ Artemia and Bernaqua Vitellus, were administered according to manufacturer's guidelines in order to identify which feed served as a more suitable replacement diet. In Experiment 2, the more successful feed from Experiment 1 was administered in three different co-feeding strategies, in which the inert feed replaced a certain percentage of live *Artemia* as illustrated in Figure 2.1.

Figure 2.1. *Artemia* and replacement feed protocols for Experiments 1 and 2.

	Treatment	Z3/M1 to PL4	PL5 to PL10
Experiment 1	Control	100% *Artemia*	
	EZ Artemia	100% EZ Artemia	25% EZ Artemia/75% *Artemia*
	Vitellus	100% Vitellus	
Experiment 2	Control	100% *Artemia*	
	V50	50% Vitellus/50% *Artemia*	
	V100/50	100% Vitellus	50% Vitellus/50% *Artemia*
	V100/75	100% Vitellus	75% Vitellus/25% *Artemia*

2.2. Experimental animals and facilities

This study was conducted at Shrimp Improvement Systems LLC, Tavernier, Florida. Nauplii used in Experiments 1 and 2 were acquired from a mass spawn of 9 and

25 females of specific pathogen free (SPF) broodstock *L. vannamei*, respectively. PL stages indicated correlate with culture days rather than observed changes in ontogenetic development. Cylindrical 200L tanks were used in both experiments. Before stocking, each tank was prepared with a weighted air line, heater, and elastic plastic cover. Fish Mate F14 auto-feeders were added later to each tank in order to administer dry feeds overnight once larvae metamorphosed to PL. Tanks were filled with 100L seawater at 28°C and treated with 0.5g vitamin C, 1.5g ethylenediaminetetraacetic acid (EDTA), 1.0g probiotic, and 0.3mL Treflan prior to receiving nauplii. Nauplii were collected from the hatching crypt and concentrated in a bucket of known volume. Nauplii were vigorously mixed by hand, sampled with a 5mL Hensen-Stemple pipet, and counted to obtain the density of the collection bucket. A sample from the collection bucket was taken and observed under a microscope to check for spinal/oral deformities and the overall quality of the nauplii. Prepared tanks were then stocked with 15,000 nauplii/tank. After stocking, the temperature in each tank was gradually increased to 30°C. Both tank numbers and treatment types were randomly drawn to assign treatment groups. Tank volumes were increased to 150L and 190L at Z2 and Z3, respectively, and tank temperatures increased to 33°C from N1-PL1.

2.3. Feeds and feeding schedules

Zeigler EZ Artemia (particle sizes: 50-200µm and 300-500µm) and Bernaqua Vitellus (particle sizes: 50-150µm and 150-400µm) were the two *Artemia* replacement feeds used in this study. A proximate analysis of each product is shown in Table 2.1. EZ Artemia is a liquid microencapsulated synthetic *Aremtia* replacement feed designed to

mimic the color, taste, texture, and nutritional value of *Artemia* nauplii. Vitellus is a dry *Artemia* replacement feed composed of extracted yolk platelets from *Artemia* cysts. Both feeds can be fed directly into the tank. Mackay Marine Grade A *Artemia* cysts sourced from GSL were used in both experiments. *Artemia* cysts were decapsulated, thoroughly rinsed after hatching, and stored at 18°C until use. New batches of *Artemia* were hatched once every 24 hours. Dead *Artemia* (killed using a short heat sock treatment) were offered to appropriate treatments to facilitate prey capture and decrease competition for algae (Bengston et al., 1991) from Z3/M1 until M3/PL, after which live *Artemia* were offered. *Artemia* culture batches were counted daily by diluting a 1mL sample from the culture tank in 9mL seawater, removing a 1mL sample from the dilution, and adding Lugol's solution to immobilize the *Artemia*. The number of *Artemia*/mL of the dilution was counted and the following equation used to determine the concentration of *Artemia* in the culture tank: [# *Artemia* in count][10,000]= # *Artemia*/L in culture tank. Shrimp larvae were fed 3 times daily at 07:00, 12:00, and 17:00, with the first two doses supplying 16.67% of the total feeding amount each, and the last feeding contributing the remaining 66.67%.

In addition to live *Artemia* or a replacement feed, all treatments in both experiments received *Thalassiosira weissflogii* from N5-PL2, Zeigler EZ Larva (at three different particle sizes: 10-50µm, 10-100µm, and 100-250µm) from Z1-PL5, Zeigler AP100 (100-150µm) from M3/PL-PL3, Mackay MP3 (150-250µm) from PL4-PL6, and Mackay MP4 (250-400µm) from PL7-PL10. Algae cells/mL were calculated in each tank on a daily basis using a haemacytometer in order to stabilize algae concentrations between treatments. In the event that algae became too clumped in floc to count, algae

concentrations were adjusted by sight between tanks. All feeds including dead *Artemia* were filtered through a 200μm mesh before feeding until larvae metamorphosed to PL.

Table 2.1. Particle size and proximate analysis of experimental feeds used in Experiments 1 and 2.

Experimental feed	Particle size[a] (μm)	Crude protein[b] (%)	Crude fat[b] (%)	Crude fiber[c] (%)	Ash[c] (%)	Moisture[c] (%)
Zeigler EZ Larva	10-50, 10-100, 100-250	11.0	6.0	1.0	-	70.0
Zeigler EZ Artemia	50-200, 300-500	14.0	4.5	-	10.0	73.0
Bernaqua Vitellus	50-150, 150-400	50.0	14.0	3.5	10.0	8.0
Zeigler Larval AP 100	100-150	50.0	12.0	2.5	10.0	12.0
Mackay MP3 and MP4	150-250, 250-450	50.0	16.0	3.0	8.0	10.0

[a] Particle size as stated by manufacturer

[b] Guaranteed minimum by manufacturer

[c] Guaranteed maximum by manufacturer

Feeding schedules for all feeds except *Artemia* and *Artemia* replacement feeds are summarized in Table 2.2. These feeds remained constant for all treatments in Experiments 1 and 2. Feeding schedules for *Artemia* and *Artemia* replacement feeds in Experiment 1 (Table 2.3) were administered based on Juarez et al. (2010) *Artemia* feeding regime and manufacturer's guidelines for individual replacement feeds, respectively. The primary goal of Experiment 1 was to determine which feed served as a more suitable replacement for *Artemia* when used according to manufacturer recommendations. Treatment groups in Experiment 2 (Table 2.4), Control, V50, V100/50, and V100/75, offered decreasing amounts of *Artemia*, respectively, with V100/75 only receiving 25% live *Artemia* from PL5 to PL10. The goal of this experiment was to pinpoint an ideal substitution rate of Vitellus for traditional *Artemia*. The vast majority of *Artemia* replacement studies conducted apply replacement percentages over

the entire culture period (for example: 50% replacement from M1 – termination of the study). This experiment was developed to test whether *L. vannamei* larvae can be delayed feeding *Artemia* until PL5 in order to maximize economic savings while still attaining comparable growth, survival, and larval quality compared to alternative replacement feeding schedules.

2.4.Tank maintenance and daily monitoring

Daily larvae checks were performed by removing a 900mL sample from each tank and observing larvae for swimming behavior, gut fullness, signs of deformity, and density in the sample. Temperature, salinity, and dissolved oxygen parameters were recorded daily. Larvae counts to estimate populations between treatment tanks were performed at Z3 and M3 stages in both experiments.

Tank maintenance included the removal of sludge from the top of tank, air lines, and heaters, clearing salt from air lines, siphoning the bottom of tanks, and performing water exchanges. Siphoning was only performed when the bottom of tanks was significantly fouled. *L. vannamei* tend to spend more time towards the bottom of their tanks as they progress through the PL stages, therefore repeated siphoning can negatively impact the survival of a treatment group, and skew survival results. In order to minimize mortalities during siphoning, the removed water from each tank was swirled and allowed to settle in a bucket. The bottom of the settled bucket was then re-siphoned quickly and the majority of water and captured larvae returned to the culture tank. 50% water exchanges were performed after M1 when necessary using a filter fitted with a 400μm mesh to prevent larvae from being removed from the tanks during the process.

Table 2.2. Feeding protocol for all feeds except live *Artemia* and *Artemia* replacement feeds for all treatment groups in Experiments 1 and 2.

Culture Day	Life stage	Zeigler EZ Larva 10-50µm	Zeigler EZ Larva 10-100µm	Zeigler EZ Larva 100-250µm	Zeigler Larval AP100 100-150µm	Mackay Marine MP3 100-250µm	Mackay Marine MP4 250-450µm
1	N2						
2	N5						
3	Z1	0.11*					
4	Z2	0.34					
5	Z3	0.45					
6	Z3/M1	0.34	0.59				
7	M2		1.35				
8	M3		1.80				
9	M3/PL		0.34	1.80	0.17*		
10	PL1			2.36	0.25*		
11	PL2			2.70	0.50*		
12	PL3			2.93	0.75*		
13	PL4			3.24		0.80*	
14	PL5			3.60		1.0*	
15	PL6					1.0*	
16	PL7						1.50*
17	PL8						2.00*
18	PL9						2.25*
19	PL10						2.25

* represents an end of day feeding only

All feeds filtered through a 200µm mesh until PL

Table 2.3. Feeding protocol for *Artemia* and *Artemia* replacement feeds for treatment groups in Experiment 1.

Culture Day	Life stage	Control (1,000s/tank)		EZ Artemia (mL/tank), (1,000/tank)			Vitellus (g/tank)	
		Dead *Artemia*	Live *Artemia*	EZ Artemia (50-200µm)	EZ Artemia (50-200µm)	Live *Artemia*	Vitellus (50-150µm)	Vitellus (150-400µm)
1	N2							
2	N5							
3	Z1							
4	Z2							
5	Z3							
6	Z3/M1	150*		0.90*			0.60*	
7	M2	315		1.70			1.26	
8	M3	375		1.80			1.50	
9	M3/PL	210	140.0	2.10			0.50	1.18
10	PL1		450.0	2.40				1.80
11	PL2		600.0	3.00				2.40
12	PL3		750.0		3.80			3.00
13	PL4		855.0		4.40			3.42
14	PL5		960.0		2.30	720.0		3.84
15	PL6		1050.0		2.60	787.5		4.2
16	PL7		1140.0		2.70	855.0		4.56
17	PL8		1140.0		3.30	855.0		4.56
18	PL9		1140.0		3.80	855.0		4.56
19	PL10		1140.0		3.90	855.0		4.56

* represents an end of day feeding only

All feeds filtered through a 200µm mesh until PL

Table 2.4. Feeding protocol for *Artemia* and *Artemia* replacement feeds for treatment groups in Experiment 2.

Culture Day	Life stage	Control (1,000s/tank)		V50 (g/tank), (1,000/tank)				V100/50 (g/tank), (1,000/tank)			V100/75 (g/tank), (1,000/tank)		
		Dead *Artemia*	Live *Artemia*	Vitellus (50-150µm)	Vitellus (150-400µm)	Dead *Artemia*	Live *Artemia*	Vitellus (50-150µm)	Vitellus (150-400µm)	Live *Artemia*	Vitellus (50-150µm)	Vitellus (150-400µm)	Live *Artemia*
1	N2												
2	N5												
3	Z1												
4	Z2												
5	Z3												
6	Z3/M1	150.0*		0.38*		75.0*		0.75*			0.75*		
7	M2	315.0		0.79		157.5		1.575			1.575		
8	M3	375.0		0.94		187.5		1.875			1.875		
9	M3/PL	210.0	140.0	0.32	0.74	105.0	105.0	0.63	1.47		0.63	1.47	
10	PL1		450.0		1.13		225.0		2.25			2.25	
11	PL2		600.0		1.50		300.0		3.00			3.00	
12	PL3		750.0		1.88		375.0		3.75			3.75	
13	PL4		855.0		2.14		427.5		4.28			4.28	
14	PL5		960.0		2.40		480.0		2.40	480.0		3.60	240.0
15	PL6		1050.0		2.63		525.0		2.63	525.0		3.94	262.5
16	PL7		1140.0		2.85		570.0		2.85	570.0		4.28	285.0
17	PL8		1140.0		2.85		570.0		2.85	570.0		4.28	285.0
18	PL9		1140.0		2.85		570.0		2.85	570.0		4.28	285.0
19	PL10		1140.0		2.85		570.0		2.85	570.0		4.28	285.0

* represents an end of day feeding only
All feeds filtered through a 200µm mesh until PL.

After water exchanges (100L), 0.5g vitamin C, 1.5g EDTA, 1.0g probiotic were added to each tank to minimize stress and neutralize heavy metals. While in use, auto-feeders were checked every morning to ensure that feed was delivered to the tank overnight. In the event of an auto-feeder failure, un-dropped feed slots were given to the tanks in the morning in 30 minute intervals.

2.5.Termination of experiments and data collection

In batches of 4-6, water levels in tanks were lowered down to 50L using a filter fitted with a 400μm mesh. Each tank was then harvested into labeled 19L buckets fitted with 500μm side mesh screens and air lines. The salinity stress test was performed first with 150 larvae from each treatment. Larvae were counted into beakers filled with 2L seawater. To begin the test, larvae were collected in a fine mesh net and transferred to prepared beakers of 2L freshwater for 30 minutes. After the test was completed, animals were transferred back to a 2L beaker of seawater for 30 minutes to reacclimate. The number of dead larvae was then recorded for each group. Larvae were considered dead after showing no response to mechanical stimuli. Total larvae in each tank were then counted.

To calculate survival for each tank, animals were transferred to a bucket containing 10L seawater. Water was stirred vigorously by hand to randomize larvae in the bucket and a 50mL sample was taken immediately after and counted. This process was repeated 3 times per tank.

Larvae where then collected in a mesh net and shaken vigorously to remove excess water. A sample from the center of the ball of larvae (the moistest) was weighed and counted. The rest of the ball of larvae was then transferred to a beaker of water zeroed on a scale and weighed. The weight per animal of each tank was then calculated using the following formula: [weight of sample]/[number of animals in sample] = weight per animal.

Larvae where then transferred to plastic containers with lids and frozen. 150 larvae per treatment were measure within one week to the nearest millimeter using a dissecting microscope.

2.6.Statistical analyses

Data from both experiments (mean survival, final length, final weight, and stress test mortality) were analyzed using one-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) to identify statistical differences between treatment means at a 95% confidence level. Cochran's test was used to check variance homogeneity at a 95% confidence level. Survival and stress test mortality data were converted to arcsine prior to performing ANOVAs. All tests were performed using Statgraphics Centurion XVI.I software (StatPoint Technologies, Inc.).

3. CHAPTER 3: RESULTS

3.1. Experiment 1

Statistical analysis results for Experiment 1 are summarized in Table 3.1. Mean percent survival was not significantly different between the Control, EZ Artemia, and Vitellus treatment groups. Both the EZ Artemia and Vitellus treatments yielded significantly different final mean lengths (mm) and weights (mg) from the Control group. Mean percent stress test mortality was significantly different between the Control and Vitellus treatments. For these reasons, the Vitellus feed was determined to be more the successful *Artemia* replacement feed from Experiment 1, and was selected for use in Experiment 2.

Table 3.1. Mean percent survival, length, weight, and percent mortality after stress test for treatment groups in Experiment 1.

Treatment	Count	Survival (%)	Length (mm)	Weight (mg)	Stress Test Mortality (%)
Control	4	43.333 ± 12.104^{a}	10.328 ± 0.671^{a}	6.627 ± 1.361^{a}	6.833 ± 3.416^{a}
EZ Artemia	4	44.556 ± 12.010^{a}	8.775 ± 0.638^{b}	3.861 ± 0.590^{b}	$13.667 \pm 4.018^{a,b}$
Vitellus	4	41.222 ± 5.020^{a}	8.075 ± 0.791^{b}	3.369 ± 0.982^{b}	24.833 ± 11.793^{b}

Mean ± SD. Means not sharing a common superscript are statistically different at $P<0.05$

3.2. Experiment 2

Statistical analysis results for Experiment 2 are summarized in Table 3.2. There was no significant difference between the four treatment groups (Control, V50, V100/50, and V100/75) for mean percent survival and percent stress test mortality. The V100/50 and V100/75 treatments' mean final lengths (mm) and weights (mg) were significantly different than those of the Control treatment. There were no significant differences between the V50, V100/50, and V100/75 treatments for any of the observed performance

factors (mean percent survival, final length (mm), final weight (mg), and percent stress test mortality).

Table 3.2. Mean percent survival, length, weight, and percent mortality after stress test for treatment groups in Experiment 2.

Treatment	Count	Survival (%)	Length (mm)	Weight (mg)	Stress Test Mortality (%)
Control	3	71.989 ± 6.248^{a}	10.430 ± 0.223^{a}	7.315 ± 1.081^{a}	8.667 ± 3.771^{a}
V50	3	72.411 ± 0.967^{a}	$9.828 \pm 0.569^{a,b}$	$5.940 \pm 1.198^{a,b}$	10.500 ± 5.030^{a}
V100/50	3	66.922 ± 7.005^{a}	9.347 ± 0.625^{b}	4.308 ± 1.069^{b}	16.000 ± 6.325^{a}
V100/75	3	70.089 ± 5.311^{a}	8.960 ± 0.297^{b}	4.043 ± 0.734^{b}	14.000 ± 4.037^{a}

Mean ± SD. Means not sharing a common superscript are statistically different at $P<0.05$

These results indicate that the maximum substitution rate of Vitellus for *Artemia* in this experiment (the V100/75 treatment) was successful in replacing *Artemia* in the diet of *L. vannamei* from Z3/M1 to PL10.

4. CHAPTER 4: DISCUSSION

Survival is one of the most important criteria scrutinized in penaeid larviculture production. Mean percent survival results from both Experiments 1 and 2 indicate that complete or partial replacement of *Artemia* using either EZ Artemia or Vitellus yields survival percentages not significantly different than those of control groups. Vitellus (V100/75 treatment) was found to effectively replace 84.33% of newly hatched *Artemia* in the larval culture of *L. vannamei* from Z3/M1-PL10 without resulting in significantly different survival and stress test mortalities compared to the Control group. Additionally, the V100/75 treatment's results were not statistically different than those of the V50 treatment, while only using 31.33% of the *Artemia* used in the V50 diet, indicating the potential for feed and labor cost savings by delaying the feeding of *Artemia* until PL5. The 84.33% substitution of Vitellus for newly hatched *Artemia* (V100/75) represents a much higher substitution rate than other *Artemia* replacement studies that begin their substitution during the mysis stages. Waiting to administer *Artemia* until PL5 significantly reduces both variable feed and labor costs for the producer.

The results of this study agree with the findings of Gamboa-Delgado and Le Vay (2009) that a 50% or less substitution rate of *Artemia* for a replacement diet must be used in order to attain similar survival, final lengths, weights, and response to stress tests as treatment groups receiving 100% *Artemia*. Similarly, the results of the V100/50 and V100/75 treatments agree with Robinson (2005) and Samocha (1999) that shrimp fed inert diets partially replacing *Artemia* had less weight gain and shorter lengths than treatments fed exclusively *Artemia*. However, as previously mentioned, final lengths and weights are secondary considerations to good survival and response to stress tests in the

commercial production of PLs. By these standards, the results seen in the V100/50 and V100/75 treatments are satisfactory, especially in light of the potential economic savings that could result from the use of such feeding strategies.

Despite the fact that both Vitellus and EZ Artemia feeds showed no significant differences in survival when compared to the Control treatment; the Vitellus feed produced more satisfactory results as an *Artemia* replacement feed. Feed particle sizes are more consistent in all sizes of Vitellus than in the EZ Artemia feeds. Filtering the 50-200µm EZ Artemia through a 200µm mesh frequently resulted in feed particles left behind. In Experiment 1, the Vitellus feed results for all performance factors were not significantly different than those of the EZ Artemia treatment, despite receiving no *Artemia* during the culture period, while the EZ Artemia treatment received 75% *Artemia* from PL5-PL10. This could be due to the increased digestibility of the *Artemia* yolk platelets that the Vitellus feed is composed of and/or increased availability of the feed in the water column due to its neutral buoyancy. Vitellus also has significantly higher percent crude protein (50%) and crude fat (14%) contents than EZ Artemia at 14% and 4.5%, respectively.

The key part of this study was the identification of an ideal substitution rate of a replacement feed for newly hatched *Artemia.* The V100/75 treatment represents a feeding schedule that is easy to adopt in commercial applications while yielding variable feed and labor cost savings for the producer. This feeding schedule should be distinguished from compensatory growth, as larvae are not being starved, but rather delayed in the feeding of only the live *Artemia* portion of their diet. Feeding schedules such as V100/50 and V100/75 help streamline production efforts in commercial operations and result in

increased production cost savings when compared to other replacement feeding schedules that begin in the early mysis stages. The V100/75 feeding schedule influences variable feed and labor costs the greatest because farmers are able to delay the culturing of *Artemia* an additional 7 days (until PL5) from what is typically performed in larviculture facilities.

As mentioned previously, there is a need for analysis of the impact *Artemia* replacement feeds could potentially have on variable feed and labor costs. One such preliminary analysis has been conducted using the feeding schedules for treatment groups in Experiment 2. Feed costs for *Thalassiosira weissflogii*, Zeigler EZ Larva, Zeigler Larval AP 100, and Mackay Marine MP3 and MP4 were not included in this analysis, as their contributions to the feed cost is constant between treatments.

As seen in Table 4.1, the total price of feeds/treatment as well as the percentage of *Artemia* used in each treatment decreases from the Control, to V50, to V100/50, to V100/75. In this example, the minimum cost of *Artemia*/*Artemia* replacement feed needed to raise 1 million *L. vannamei* from Z3/M1 to PL10 was $148.78 (V100/75), a $25.97 difference in cost from the Control group. An even larger difference in cost can be seen in Table 4.2 in which labor cost to culture *Artemia* is summarized. By utilizing a feeding schedule such as the V100/50 or V100/75 where the feeding of *Artemia* is delayed until PL5, the cost to produce *Artemia* over the production period can be lowered from $525.00 to $245.00. The cumulative feed and labor costs by treatment are given in Table 4.3. In this case, a 56.27% difference in total feed and labor cost exists between the Control and V100/75 treatments. The use of dry replacement feeds such as Vitellus have the potential to influence labor cost beyond what is summarized in these tables, as they

are capable of being placed in auto-feeders rather than being hand-fed like live or liquid feeds.

This analysis shows the clear advantage of utilizing a feeding schedule that combines *Artemia* replacement with a delayed feeding of live *Artemia* versus an *Artemia* replacement feeding schedule that begins at Z3/M1. While these estimates will vary significantly by country and culture intensity, they provide a clear view of the advantages of exploring alternative *Artemia* replacement feeding schedules. Further studies on the use of feeds such as Vitellus in live feeds replacement should include the exploration of alternative feed management strategies. The continued development of high intensity penaeid farms and hatcheries alike needs to be unimpeded by the potential shortage of *Artemia*. While the supply of *Artemia* to world aquaculture markets may not be as precarious today as it was in recent years, the continued use of high levels of live feeds in larval diets still represents an economic bottleneck to the commercial culture of many marine species.

Table 4.1. Analysis of feed cost for *Artemia* and *Artemia* replacement feeds for treatment groups in Experiment 2 (per million *L. vannamei* larvae).

Treatment	Type of feed	Particle size (μm)	Amount of feed needed (million *Artemia*), (kg/million larvae)	Price of feed (USD/kg)	Price of feed needed/million larvae (USD)	Total price of feeds/treatment (USD)
Control	Dead/live *Artemia*	-	3.4950	$50.00	$174.75	$174.75
V50	Vitellus small	50-150μm	0.1610	$41.19	$6.63	$159.35
	Vitellus medium	150-400μm	1.5865	$41.19	$65.35	
	Dead/live *Artemia*	-	1.7475	$50.00	$87.38	
V100/50	Vitellus small	50-150μm	0.3220	$41.19	$13.26	$153.61
	Vitellus medium	150-400μm	2.0780	$41.19	$85.59	
	Live *Artemia*	-	1.0950	$50.00	$54.75	
V100/75	Vitellus small	50-150μm	0.3220	$41.19	$13.26	$148.78
	Vitellus medium	150-400μm	2.6255	$41.19	$108.14	
	Live *Artemia*	-	0.5475	$50.00	$27.38	

Prices do not include shipping costs

Artemia prices for Grade A Mackay Marine Artemia (200,000 NPG)

Table 4.2. Analysis of labor cost to culture *Artemia* for treatment groups in Experiment 2.

Treatment	# of *Artemia* culture days	Labor cost/day (3.5hrs/day at $10.00/hr)	Labor cost to culture *Artemia*
Control	15	$35.00	$525.00
V50	15	$35.00	$525.00
V100/50	7	$35.00	$245.00
V100/75	7	$35.00	$245.00

Assuming 3.5 hours of labor/day to set up, decapsulate, hatch, and rinse *Artemia*

Number of culture days includes 1 day of labor before the first day of *Artemia* feeding to prepare initial *Artemia* culture

Table 4.3. Total feed and labor costs for treatment groups in Experiment 2 per million *L. vannamei* larvae.

Treatment	Total price of feeds/treatment/million larvae (USD)	Labor cost to culture *Artemia*	Total feed and labor costs
Control	$174.75	$525.00	$699.75
V50	$159.35	$525.00	$684.35
V100/50	$153.61	$245.00	$398.61
V100/75	$148.78	$245.00	$393.78

WORKS CITED

Bengston, D. A. (2003). Status of marine aquaculture in relation to live prey: Past, present and future. In J. G. Stottrup, & L. A. McEvoy (Eds.), *Live feeds in marine aquaculture* (pp. 1-16). Malden, MA: Blackwell Science.

Bengston, D. A., Leger, P., & Sorgeloos, P. (1991). Use of *Artemia* as a food source for aquaculture. In R. A. Browne, P. Sorgeloos & C. N. A. Trotman (Eds.), *Artemia biology* (pp. 255-285). Boca Raton, FL: CRC Press.

Briggs, M. (n.d.). *Penaeus vannamei (boone, 1931).* Retrieved 11/14, 2010, from http://www.fao.org/fishery/culturedspecies/Litopenaeus_vannamei/en

Calderon, F. R. O., Imai, D. M., Arce, S. M., Argue, B. J., & Moss, S. M. (2004). Performace of larval and postlarval pacific white shrimp, *Litopenaeus vannamei* (Boone), fed two commercial liquid diet supplements. *Journal of Applied Aquaculture, 15*(3-4), 21-35. doi:10.1300/J028v15n03_02

DAbramo, L. R., Perez, E. I., Sangha, R., & Puello-Cruz, A. (2006). Successful culture of larvae of *Litopenaeus vannamei* fed a microbound formulated diet exclusively from either stage PZ2 or M1 to PL1. *Aquaculture, 261*(4), 1356-1362. doi:10.1016/j.aquaculture.2006.09.020

Dhont, J., & Sorgeloos, P. (2002). Applications of *Artemia*. In T. J. Abatzopoulos, J. A. Beardmore, J. S. Clegg & P. Sorgeloos (Eds.), *Artemia: Basic and applied biology* (pp. 251-277). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Dhont, J., & Stappen, G. V. (2003). Biology, tank production and nutritional value of *Artemia*. In J. G. Stottrup, & L. A. McEvoy (Eds.), *Live feeds in marine aquaculture* (pp. 65-121). Malden, MA: Blackwell Science.

Gamboa-Delgado, J., & Le Vay, L. (2009). *Artemia* replacement in co-feeding regimes for mysis and postlarval stages of *Litopenaeus vannamei*: Nutritional contribution of inert diets to tissue growth as indicated by natural carbon stable isotopes. *Aquaculture, 297*(1-4), 128-135. doi:10.1016/j.aquaculture.2009.09.009

Great Salt Lake Ecosystem Program. (2010). *Historical brine shrimp harvest data.* Retrieved October 2, 2011, from http://wildlife.utah.gov/gsl/harvest/historic_harvest_data.php

Hameed, A. S. S., Murthi, B. L. M., Rasheed, M., Sathish, S., Yoganandhan, K., Murugan, V., & Jayaraman, K. (2002). An investigation of *Artemia* as a possible vector for white spot syndrome virus (WSSV) transmission to *Penaeus indicus*. *Aquaculture, 204*(1-2), 1-10. doi:10.1016/S0044-8486(01)00655-X

Jones, D. A., Kamarudin, M. S., & Le Vay, L. (1993). The potential for replacement of live feeds in larval culture. *Journal of the World Aquaculture Society, 24*(2), 199-210. doi:10.1111/j.1749-7345.1993.tb00009.x

Jones, D. A., Kanazawa, A., & Abdel Rahman, S. (1979). Studies on the presentation of artificial diets for rearing the larvae of *Penaeus japonicus* Bate. *Aquaculture, 17*(1), 33-43. doi:10.1016/0044-8486(79)90136-4

Juarez, L. M., Moss, S. M., & Figueras, E. (2010). Maturation and larval rearing of the pacific white shrimp, *Penaeus vannamei*. In V. Alday-Sanz (Ed.), *The shrimp book* (pp. 305-352). Nottingham, United Kingdom: Nottingham University Press.

Kovalenko, E. E., D'Abramo, L. R., Ohs, C. L., & Buddington, R. K. (2002). A successful microbound diet for the larval culture of freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture, 210*(1-4), 385-395. doi:10.1016/S0044-8486(02)00127-8

Lavens, P., & Sorgeloos, P. (2000a). Experiences on importance of diet for shrimp postlarval quality. *Aquaculture, 191*(1-3), 169-176. doi:10.1016/S0044-8486(00)00426-9

Lavens, P., & Sorgeloos, P. (2000b). The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. *Aquaculture, 181*(3-4), 397-403. doi:10.1016/S0044-8486(99)00233-1

Leger, P., Bengston, D. A., Sorgeloos, P., Simpson, K. L., & Beck, A. D. (1987). The nutritional value of *Artemia*: A review. In P. Sorgeloos, D. A. Bengston, W. Decleir & E. Jaspers (Eds.), *Artemia research and its applications: Proceedings of the second international symposium on the brine shrimp Artemia* (pp. 357-372). Wetteren, Belgium: Universa Press.

Leger, P., Simpson, K. L., & Sorgeloos, P. (1986). The use and nutritional value of *Artemia* as a food source. *Oceanography and Marine Biology, 24*, 521-623.

Lenz, P. H. (1987). Ecological studies on *Artemia*: A review. In P. Sorgeloos, D. A. Bengston, W. Decleir & E. Jaspers (Eds.), *Artemia research and its applications: Proceedings of the second international symposium on the brine shrimp Artemia* (pp. 3). Wetteren, Belgium: Universa Press.

Lipton, D., & Harrell, R. (2004). *Figuring production costs in finfish aquaculture.* No. UM-SG-MAP-90-02).Maryland Sea Grant Extension. Retrieved from http://www.mdsg.umd.edu/programs/extension/aquaculture/finfish/factsheets/FF4/

Martín, L., Arenal, A., Fajardo, J., Pimentel, E., Hidalgo, L., Pacheco, M., . . . Santiesteban, D. (2006). Complete and partial replacement of *Artemia* nauplii by *Moina micrura* during early postlarval culture of white shrimp (*Litopenaeus schmitti*). *Aquaculture Nutrition, 12*(2), 89-96. doi:10.1111/j.1365-2095.2006.00383.x

Merchie, G. (1996). Use of nauplii and meta-nauplii. In P. Lavens, & P. Sorgeloos (Eds.), *Manual on the production and use of live food for aquaculture* (pp. 137-163). Rome, Italy: Food and Agriculture Organization of the United Nations.

Moss, S. M., & Leung, P. (2006). Comparative cost of shrimp production: Earthen ponds versus recirculating aquaculture systems. In P. Leung, & C. Engle (Eds.), *Shrimp culture: Economics, market, and trade* (pp. 291-300). Ames, IA: Blackwell Publishing.

Pedrazzoli, A., Molina, C., Montoya, N., Townsend, S., Leon-Hing, A., Parades, Y., & Calderon, J. (1998). Recent advances on nutrition research of *Penaeus vannamei* in Ecuador. *Reviews in Fisheries Science, 6*(1-2), 143-151.

Pillay, T. V. R., & Kutty, M. N. (2005a). Economics and financing of aquaculture. In *Aquaculture principles and practices* (2nd ed., pp. 284-303). Ames, IA: Blackwell Publishing.

Pillay, T. V. R., & Kutty, M. N. (2005b). Shrimps and prawns. In *Aquaculture principles and practices* (2nd ed., pp. 473-509). Ames, IA: Blackwell Publishing.

Prescott, S. R., & Michotte, J. P. (1980). The economics of brine shrimp as a larval food. *Fish Farming International, 7*(No. 1), 11-12.

Quintero, H. E., & Roy, L. A. (2010). Practical feed management in semi-intensive systems for shrimp culture. In V. Alday-Sanz (Ed.), *The shrimp book* (pp. 443-453). Nottingham, United Kingdom: Nottingham University Press.

Robinson, C. B., Samocha, T. M., Fox, J. M., Gandy, R. L., & McKee, D. A. (2005). The use of inert artificial commercial food sources as replacements of traditional live food items in the culture of larval shrimp, *Farfantepenaeus aztecus*. *Aquaculture, 245*(1-4), 135-147. doi:10.1016/j.aquaculture.2004.11.051

Samocha, T. M., Matsumoto, T., Jones, E. R., & Torano, M. (1999). Use of artificial diets to reduce *Artemia* nauplii requirements for production of *Litopenaeus vannamei* postlarvae. *Israeli Journal of Aquaculture - Bamidgeh, 51*(4), 157-168.

Schauer, P. S., Johns, M. D., Olney, C. E., & Simpson, K. L. (1980). International study on *Artemia* IX. lipid level, energy content and fatty acid composition of the cysts and newly hatched nauplii from five geographical strains of *Artemia* . In G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers (Eds.), *The brine shrimp Artemia: Proceedings of the international symposium on the brine shrimp Artemia salina* (pp. 3). Wetteren, Belgium: Universa Press.

Shiau, S. (1998). Nutrient requirements of penaeid shrimps. *Aquaculture, 164*(1-4), 77-93.

Sivakumar, V. K., Sarathi, M., Venkatesan, C., Sivaraj, A., & Hameed, A. S. S. (2009). Experimental exposure of *Artemia* to hepatopancreatic parvo-like virus and subsequent transmission to post-larvae of *Penaeus monodon*. *Journal of Invertebrate Pathology, 102*(3), 191-195. doi:10.1016/j.jip.2009.08.001

Sorgeloos, P. (1976). The brine shrimp, *Artemia salina*: A bottleneck in mariculture? *FAO Technical Conference on Aquaculture,* Kyoto, Japan. 321-324.

Sorgeloos, P. (1980). The use of the brine shrimp *Artemia* in aquaculture. In *The brine shrimp Artemia* (pp. 25-46). Wetteren, Belgium: Universa Press.

Sorgeloos, P. (2000). Challenges and opportunities for aquaculture research and development in the next century with special emphasis on feed development. *International Aquafeed,* (Directory and Buyers' Guide), 4-7.

Sorgeloos, P., Coutteau, P., Dhert, P., Merchie, G., & Lavens, P. (1998). Use of brine shrimp, *Artemia* spp., in larval crustacean nutrition: A review. *Reviews in Fisheries Science, 6*(1-2), 55-68.

Sorgeloos, P., Dhert, P., & Candreva, P. (2001). Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. *Aquaculture, 200*(1-2), 147-159. doi:10.1016/S0044-8486(01)00698-6

Sorgeloos, P., Lavens, P., Leger, P., Tackaert, W., & Versichele, D. (1986). *Manual for the culture and use of brine shrimp Artemia in aquaculture*. Ghent, Belgium: State University of Ghent, Faculty of Agriculture.

Stappen, G. V. (1996). Introduction, biology and ecology of *Artemia*. In P. Lavens, & P. Sorgeloos (Eds.), *Manual on the production and use of live food for aquaculture* (pp. 79-106). Rome, Italy: Food and Agriculture Organization of the United Nations.

Sudhakaran, R., Yoganandhan, K., Ishaq Ahmed, V. P., & Sahul Hameed, A. S. (2006). *Artemia* as a possible vector for macrobrachium rosenbergii nodavirus (MrNV) and extra small virus transmission (XSV) to *Macrobrachium rosenbergii* post-larvae. *Diseases of Aquatic Organisms, 70*(1-2), 161-166.

Tacon, A. G. J., Nates, S. F., & McNeil, R. J. (2006). Overview of farming systems for marine shrimp with particular reference to feeds and feeding. In P. Leung, & C. Engle (Eds.), *Shrimp culture: Economics, market, and trade* (pp. 301-314). Ames, IA: Blackwell Publishing.

Treece, G. D. (2000). *Artemia production for marine larval fish culture.* No. 702. Southern Regional Aquaculture Center.

Triantaphyllidis, G. V., Abatzopoulos, T. J., & Sorgeloos, P. (1998). Review of the biogeography of the genus *Artemia* (Crustacea, Anostraca). *Journal of Biogeography, 25*(2), 213-226.

Urban Jr., E. R., & Pruder, G. D. (1991). A method of economic comparisons for aquaculture diet development. *Aquaculture, 99*(1-2), 127-142. doi:10.1016/0044-8486(91)90293-G

Valenti, W. C., & Tidwell, J. H. (2006). Economics and management of freshwater prawn culture in western hemisphere. In P. Leung, & C. Engle (Eds.), *Shrimp culture: Economics, market, and trade* (pp. 261-276). Ames, IA: Blackwell Publishing.

Wouters, R., Cobo, M. L., Dhont, J., & Wille, M. (2009). Developments in feed formulations, feeding practices and culture techniques for marine shrimp larvae. *The Rising Tide, Proceedings of the Special Session on Sustainable Shrimp Farming,* The World Aquaculture Society, Baton Rouge, LA. 79-91.

Zelaya, O., Davis, D. A., & Rouse, D. B. (2007). The influence of *Artemia* and algal supplements during the nursery phase of rearing pacific white shrimp, *Litopenaeus vannamei*. *Journal of the World Aquaculture Society, 38*(4), 486-496. doi:10.1111/j.1749-7345.2007.00122.x

Zhang, J. S., Dong, S. L., Dong, Y. W., Tian, X. L., Cao, Y. C., Li, Z. J., & Yan, D. C. (2010). Assessment of the role of brine shrimp *Artemia* in white spot syndrome virus (WSSV) transmission. *Veterinary Research Communications, 34*(1), 25-32. doi:10.1007/s11259-009-9329-x