**The Application of Automatic Systems in Shrimp Production as Tools to Improve Feed Management**

by

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Abstract

Shrimp production has been one of the primary products of aquaculture due to its high value and product acceptance on the global market. The continued success of the shrimp industry will rely on improving feed management practices and reductions in labor costs. As feed is one of the main operating costs in shrimp aquaculture, much research effort has been put into developing cost-efficient practical diets but feeding protocols have typically received less attention. The repetitive nature of feed dispersion in aquaculture resulted in the integration of automatic feeders in shrimp production systems, which allows expansion of the number of meals without compromising labor costs. Hence, it was the overall objective of this doctoral research project to study and explore the potential for improvement of various feed management protocols for automatic feeders in shrimp production as well as evaluate overall role of automation in shrimp farming. In later stages, the project focused on exploring the potential of recently available passive acoustic feeding systems in determining potential dietary preferences among different commercial diets with various protein sources.

A 90-day outdoor pond production trial evaluated the development of standard feeding protocol's (SFP) for automatic feeding systems to maximize growth rates. Four treatments including: three fixed feeding treatments of 130, 145 and 160% of a SFP (SFP+30%, SFP+45%, SFP+60%, respectively) were offered to shrimp using automatic timer-feeders, and a fourth treatment utilized an on-demand AQ1 acoustic feeding system. In general, increased feed inputs resulted in higher production and best response was achieved with the AQ1 system which offered higher feed inputs resulting in larger shrimp and yields. Using cumulative data to date, a standard protocol for timer feeders (SPTF) was established and used in the second trial. This trial consisted of a 90-day pond production cycle which was conducted using the SPTF to evaluate shrimp production using different feeding schedules. Four treatments were utilized in this trial including: three fixed feeding treatments based on SPTF Day, SPTF Night and SPTF 115% 24hr were offered using automatic timer-feeders, while a fourth on demand treatment utilized AQ1 acoustic feeding system. A 11-wk growth trial was conducted in a parallel green-water semi-recirculating tank system that aimed at evaluated additional feeding protocols. Results for the pond trial further confirm higher yields with AQ1 acoustic feeding system and showed no statistical differences among timer feeder treatments, indicating no effect on time (day vs night vs 24 hrs) of feeding. Results in the tank trial indicated a relationship between growth response to increasing feed inputs and number of meals rather than feeding schedule alone. After two production cycles that validated the higher production efficiency of the acoustic system, all ponds were subsequently equipped with this technology and a third and last 90-day outdoor production trial was conducted to evaluate if shrimp preferred a specific protein source fed on demand. Four treatments consisted of a 35% crude protein commercial diet with different protein sources: all-plant, 8% poultry meal (PM), 8% fish meal (FM) and 12% FM. No statistical differences were observed in any of the main production parameters suggesting that shrimp did not clearly favor a particular diet when fed on demand. In conclusion, the research conducted throughout this doctorate provides further insight towards the establishment of effective feeding protocols for automatic feeders in shrimp production, and confirms that passive acoustic demand feeders are currently the most effective feed management tool in shrimp pond production. While validating acoustic feeders, we were also able to confirm that feed intake and growth were not compromised when shrimp were fed alternative protein based diets. This data contributes to the discussion of how can feed additives or physical properties of the diets can be used to further improve effectiveness of these feeders.

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"Quem quer passar além do Bojador,

Tem que passar além da dor.

Deus ao mar o perigo e o abismo deu,

Mas nele é que espelhou o céu."

Fernando Pessoa *in* Mensagem

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# CHAPTER I

# GENERAL INTRODUCTION

The continued growth of total human population and expansion of middle class in both established and emerging economies raises many challenges for the food production sector. Demand for high quality protein sources such as seafood products remains on the rise as most wild fisheries become either over or fully exploited (FAO, 2018). Under this scenario, aquaculture has increased considerably its role on the global seafood production stage throughout the last 60 years, and while currently providing about half of the global seafood available, its role is expected to continue increasing, eventually becoming the main source of seafood for human consumption.

While originally aquaculture was based in small outdoor ponds or pens with little to no human inputs, the necessity to increase productivity has pushed aquaculture into more intensive and complex systems. Drivers of increased production were initially simply expanding production area, followed by use of fertilization regiments, the introduction of complete feeds and more recently the use of supplemental aeration. These technological advances have all moved the industry to produce more product with a smaller on farm footprint. The development of complete feeds and nutrient requirement data to support them have been a primary driving forces within the industry. This is especially true for the main aquaculture species on a global scale such as salmon, shrimp, tilapia, trout, seabass and catfish.

Among aquaculture species raised for human consumption, penaeid shrimp are one of the main seafood commodities (Bondad-Reantaso, et al., 2012) due to its high market value and global acceptance. Among the different penaeid shrimp species, the Pacific white shrimp has been the overwhelming choice among farmers. This is due to its faster growth rate, safe high stocking densities, low salinity tolerance, lower protein requirements, possibility of breeding and domestication, relatively higher disease resistance (Bondad-Reantaso, et al., 2012; Cuzon, et al., 2004; Liu, et al., 2017), and overall capacity to grow in less than ideal conditions (Roy, et al., 2010). As in most other commercially important specie, the development of nutritionally sound complete feeds was one of the main drivers for expansion and intensification of shrimp aquaculture throughout the last two decades across the globe. In fact, as feed remains the most important operating cost and source of waste in shrimp aquaculture (Martinez-Cordova, et al., 2003; Tacon, Forster, 2003), there has been considerable research effort in continuous development and validation of nutritionally balanced and cost-effective shrimp diets. Consequently, most currently available commercial shrimp diets are regarded as adequate (Quintero, Roy, 2010). Yet, the development of efficient feed delivery protocols has often been overlooked. Understanding the cultured species natural feeding behavior is key towards effectively providing the nutrients necessary for optimal growth.

Shrimp are omnivorous benthic grazers (Cuzon, et al., 2004; Dall, et al., 1990; Varadharajan D., 2013)that favor slow and frequent ingestion of small food items. Moreover, shrimp externally masticate and break down food items before ingestion which enhances nutrient leaching concerns as surface area of each pellet that contacts water is higher than in animals that ingest entire pellets (e.g. salmon, catfish or tilapia). It is widely accepted that extending the period through which feed in contact with water reduces its nutritional value (Obaldo, 2006). Ullman, et al. (2019b) reported reduced growth in shrimp fed diets previously leached for over 0.5 hours. Traditional feeding practices for shrimp aquaculture had been set in 1 to 4 daily meals (Carvalho, Nunes, 2006), and while (Velasco, et al., 1999) did not observe improved growth when shrimp were fed above 4 daily meals, multiple authors reported improved shrimp growth with increasing number of daily meals (Carvalho, Nunes, 2006; Nunes, et al., 2019; Ullman, et al., 2019a; Ullman, et al., 2019c). However, many shrimp culture operations still rely on human labor to deliver every meal to each pond, hence increasing the number of rations can be economically unviable for many producers. This is particularly true in important production regions such as the Americas where labor costs are high in comparison to southeast Asia (Davis, 2018).

Successful intensive aquaculture in its totality can be extremely challenging but most of the daily tasks such as feeding and environmental (i.e. water) monitoring are ultimately simple and repetitive. Hence, they represent areas with great potential for automation and while integration of automatic systems in aquaculture is not a recent trend, the intensification of the sector has expanded both the integration of these systems as well as the development of new dedicated technologies. In shrimp farming, the adaptation of feed practices to automatic feeding systems may allow the farmer or manager to expand the number of daily rations for any given system and reduce labors costs. Carvalho, Nunes (2006); Ullman, et al. (2019a); Ullman, et al. (2019c) have reported enhanced growth performance when shrimp were fed multiple meals throughout the day.

Although automatic feeders such as timer feeders are a useful tool towards improving feed delivery efficiency, there are several other factors affecting feed management such as population size and fed intake estimations. Although population size estimations are usually challenging across the entire aquaculture sector, daily feeding rates for many aquaculture species can easily be adjusted by visual perception of feed intake. However, the benthic feeding nature of shrimp and common high turbidity in rearing units raises many challenges, as visual perception becomes practically impossible. Many shrimp farmers rely on predesigned feed tables based on historic data and production targets to establish daily feeding rates. In many cases, feed trays used to roughly estimated feed intake (Martinez-Cordova, et al., 1998) and adjust feeding protocols during the cycle, but this is a labor intensive procedure and can be economically impractical on a larger scale. This is valid for shrimp farms that disperse feed through hand-feeding or timer feeders, as both strategies assume that shrimp will be willing to consume the feed whenever it is offered.

In order to bypass visual limitations inherent to high turbidity in shrimp production systems, passive acoustic monitoring technology has been adapted to shrimp feeding through the development of passive acoustic demand feeders during the last decade (Bador, 2013; Silva, et al., 2019). This technology relies on capturing the signature clickling sound of shrimp mandibular resulting of feeding behavior (Peixoto, et al., 2020a; Peixoto, et al., 2020b; Silva, et al., 2019; Smith, Tabrett, 2013). Napaumpaipom, et al. (2013); Ullman, et al. (2019a); Ullman, et al. (2019c) were able to achieve better shrimp growth performance with passive acoustic demand feeders in comparison to hand-feeding and timer feeder feeding strategies. Although this is a fairly recent technology it has been validated within the industry since the early 2010's, but due to its impact on economic performance of farms, not much information regarding its practical application is widely available. Furthermore, the logistic and infrastructural effort inherent in conducting growth trials under production settings also results in limited information available in the literature.

In order to further understand the potential for improvement of feed management protocols for automatic feeders and how passive acoustic feeders are able to impact shrimp production both biologically (i.e. growth) and environmentally, three shrimp production projects were conducted. The first trial aimed at establishing a new standard feeding protocol for timer feeders in order to approximate it to passive acoustic feeder performance. The second trial aimed at applying the new protocol established during the previous trial while examining potential feeding schedule preferences of shrimp and compared each feeding strategy with passive acoustic feeders production output. As passive acoustic feeders were proven to be the most efficient feeding tool, a third and last production trial aimed at using this demand feeding technology to evaluate feed response to four commercial diets with different protein sources and inclusion levels. Hence, the main objectives of the research conducted throughout this doctoral degree were to: establish a new standard feeding protocol for timer-feeders in shrimp production that would approximate production performance to that of on demand passive acoustic feeders; and use automatic feeding systems to identify feeding preferences in shrimp, first by comparing feeding schedules (daytime, night time and 24h) and later on by using passive acoustic demand feeders to evaluate feed intake and growth performance of shrimp when offered different commercial diets with varying protein sources.

## 1. References

Bador, R., Blyth, P., Dodd, R., 2013. Acoustic control improves feeding productivity at shrimp farms. Global Aquaculture Advocate, 77-78.

Bondad-Reantaso, M.G., Subasinghe, R.P., Josupeit, H., Cai, J.N., Zhou, X.W., 2012. The role of crustacean fisheries and aquaculture in global food security: Past, present and future. J Invertebr Pathol. 110, 158-165.

Carvalho, E.A., Nunes, A.J., 2006. Effects of feeding frequency on feed leaching loss and grow-out patterns of the white shrimp *Litopenaeus vannamei* fed under a diurnal feeding regime in pond enclosures. Aquaculture. 252, 494-502.

Cuzon, G., Lawrence, A., Gaxiola, G., Rosas, C., Guillaume, J., 2004. Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. Aquaculture. 235, 513-551.

Dall, W., Hill, B.J., Rothlisberg, P.C., Staples, D.J., 1990. The Biology of the Penaeidae. Adv Mar Biol. 27, 1-461.

Davis, D.A., Ullman, C., Rhodes, M., Novriadi, R., Swanepoel, A., 2018. Automated Feeding Systems in Pond Production of Pacific White Shrimp. Global Aquaculture Advocate.

FAO, 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome.

Liu, G., Zhu, S.M., Liu, D.Z., Guo, X.S., Ye, Z.Y., 2017. Effects of stocking density of the white shrimp *Litopenaeus vannamei* (Boone) on immunities, antioxidant status, and resistance against Vibrio harveyi in a biofloc system. Fish Shellfish Immun. 67, 19-26.

Martinez-Cordova, L.R., Torres, A.C., Porchas-Cornejo, M.A., 2003. Dietary protein level and natural food management in the culture of blue (*Litopenaeus stylirostris*) and white shrimp (*Litopenaeus vannamei*) in microcosms. Aquaculture Nutrition. 9, 155-160.

Martinez-Cordova, L.R., Porchas-Cornejo, M.A., Villarreal-Colemnares, H., Calderon-Perez, J.A., Naranjo-Paramo, J., 1998. Evaluation of three feeding strategies on the culture of white shrimp *Penaeus vannamei* Boone 1931 in low water exchange ponds. Aquacultural Engineering. 17, 21-28.

Napaumpaipom, T., Chuchird, N., Taparhudee, W., 2013. Study on the Efficiency of Three Different Feeding Techniques in the Culture of Pacific White Shrimp (*Litopenaeus vannamei*). Journal of Fisheries and Environment. 37, 8-16.

Nunes, A.J.P., Sabry-Neto, H., da Silva, F.H.P., de Oliveira-Neto, A.R., Masagounder, K., 2019. Multiple feedings enhance the growth performance and feed efficiency of juvenile *Litopenaeus vannamei* when fed a low-fish meal amino acid-supplemented diet. Aquaculture International. 27, 337-347.

Obaldo, L.G., Masuda, R., 2006. Effect of diet size on feeding behavior and growth of pacific white shrimp, *Litopenaeus vannamei*. J. Appl. Aquaculture. 18, 101-110.

Peixoto, S., Soares, R., Davis, D.A., 2020a. An acoustic based approach to evaluate the effect of different diet lengths on feeding behavior of *Litopenaeus vannamei*. Aquacultural Engineering. 91.

Peixoto, S., Soares, R., Silva, J.F., Hamilton, S., Morey, A., Davis, D.A., 2020b. Acoustic activity of *Litopenaeus vannamei* fed pelleted and extruded diets. Aquaculture. 525.

Quintero, H.E., Roy, L.A., 2010. Practical Feed Management in Semi-Intensive Systems for Shrimp Culture. The Shrimp Book, edited by Alday-Sanz, V., 443-454. Nottingham, UK: Nottingham University Press. 443-453.

Roy, L.A., Davis, D.A., Saoud, I.P., Boyd, C.A., Pine, H.J., Boyd, C.E., 2010. Shrimp culture in inland low salinity waters. Reviews in Aquaculture. 2, 191-208.

Silva, J.F., Hamilton, S., Rocha, J.V., Borie, A., Travassos, P., Soares, R., Peixoto, S., 2019. Acoustic characterization of feeding activity of *Litopenaeus vannamei* in captivity. Aquaculture. 501, 76-81.

Smith, D.V., Tabrett, S., 2013. The use of passive acoustics to measure feed consumption by Penaeus monodon (giant tiger prawn) in cultured systems. Aquacultural Engineering. 57, 38-47.

Tacon, A.G.J., Forster, I.P., 2003. Aquafeeds and the environment: policy implications. Aquaculture. 226, 181-189.

Ullman, C., Rhodes, M.A., Davis, D.A., 2019a. Feed management and the use of automatic feeders in the pond production of Pacific white shrimp *Litopenaeus vannamei*. Aquaculture. 498, 44-49.

Ullman, C., Rhodes, M.A., Davis, D.A., 2019b. The effects of feed leaching on the growth of Pacific white shrimp *Litopenaeus vannamei* in a green-water tank system. Aquac. Res. 50, 3074-3077.

Ullman, C., Rhodes, M., Hanson, T., Cline, D., Davis, D.A., 2019c. Effects of Four Different Feeding Techniques on the Pond Culture of Pacific White Shrimp, *Litopenaeus vannamei*. Journal of the World Aquaculture Society. 50, 54-64.

Varadharajan D., P.N., 2013. Food and feeding habits of aquaculture candidate a potential crustacean Pacific white shrimp *Litopenaeus vannamei*. Aquac Res Development. 4, 1-5.

Velasco, M., Lawrence, A.L., Castille, F.L., 1999. Effect of variations in daily feeding frequency and ration size on growth of shrimp, *Litopenaeus vannamei* (Boone), in zero-water exchange culture tanks. Aquaculture. 179, 141-148.

Chapter II

# OPTIMIZING FEED AUTOMATION: IMPROVING TIMER-FEEDERS AND ON DEMAND SYSTEMS IN SEMI-INTENSIVE POND CULTURE OF SHRIMP *Litopenaeus vannamei*

Abstract

The continued success of shrimp farming will rely on improved feed management and reductions in labor costs. Shrimp are omnivorous, eating many small meals with limited stomach capacity for food storage. Hence, increased performance may be obtained by spreading feed through multiple meals. Initial work has demonstrated that moving from two feeding per day into multiple feeding systems increases growth rate and production. Further advances have been made with on-demand (satiation) feeding systems. The goal of this work was to continue the development of standard feeding protocol's (SFP) for automatic feeding systems to maximize growth rates in semi-intensive pond production of shrimp, *Litopenaeus vannamei*. For this work a 13-week pond production trial was performed in 16, 0.1 ha outdoors ponds, stocked at a 26 shrimp/m$^2$, and fed 1.5-mm 40% crude protein for the first four weeks, and 2.4-mm protein soy optimized feed (35% crude protein) for last nine weeks, both produced by Zeigler Inc.. Four treatments including: three fixed feeding treatments of 130, 145 and 160% of a SFP (SFP+30%, SFP+45%, SFP+60%, respectively) were offered using automatic timer-feeders, and a fourth treatment utilized on-demand AQ1 acoustic feeding system. No statistical differences were found between treatments for survival (ranging 75.2-81.4%) and FCR (ranging 0.96-1.11). In general, increased feed inputs resulted in higher production. The best growth response was with the AQ1 system which adjusted feed inputs in real time and offered higher feed inputs resulting in larger shrimp and yields. Based on results of this work and previous trials, standardized feeding protocols for automated systems can be developed

but to date, automated feedback systems which operate in real time outperform the standardized practices.

## 1. Introduction

Shrimp are one of the most popular seafoods. In aquaculture, *Litopenaeus vannamei* is the preferred shrimp species due to its culture characteristics and consumer acceptance. The continued success of shrimp farming will rely on intensification, improved feed management and reductions in labor costs. The cost of the feed is one of the most important variable costs, source of nutrients and consequently biological waste in shrimp production (Tacon*, et al.*, 2003). Commercially available shrimp feeds are generally adequate (Quintero*, et al.*, 2010), but proper application is essential for maximum economic and environmental improvements on aquaculture farms (Chatvijitkul*, et al.*, 2017; Van*, et al.*, 2017).

Shrimp are omnivorous benthic animals (Cuzon*, et al.*, 2004; Dall*, et al.*, 1990; Varadharajan D., 2013) with limited capacity to store food inside their digestive tract which results in slower continued ingestion of small quantities of feed. Several studies have shown enhanced growth performance for shrimp culture with multiple feedings throughout the day (Carvalho*, et al.*, 2006; Jescovitch*, et al.*, 2018; Ullman*, et al.*, 2019b; Ullman*, et al.*, 2019c). This was due to increased the availability of feeds but also the time that feed is in contact with water which is accepted to reduce its nutritional value (Obaldo*, et al.*, 2002). Ullman*, et al.* (2019a) reported reduced growth performance and higher FCR in shrimp feeds that were previously leeched for over 0.5 h before feeding. This confirms the hypothesis that the longer feed is in the water the lower the nutritional value hence indicating small quickly consumed meals are preferential due to improvements in growth and waste management. Nevertheless, offering multiple meals can be very labour intensive and economically impracticable in regions such as the Americas where labor cost is high in comparison to South East Asia which tends to use more feedings per day (Davis, 2018).

Contrary to many fish species, shrimp feeding behaviour does not allow visual perception of feed intake. Moreover, adequate estimations of population size and biomass are essential for proper feed management (NRC, 2011 #1) which is particularly complex in non-clear water systems such as ponds. Therefore, estimating or adjusting feed inputs to meet the intake demands of shrimp can be very challenging. Regardless, there are various strategies to manage feed inputs for shrimp production.

Quite often feed tables are used by farmers (Casillas-Hernandez*, et al.*, 2006). These are more often based on data from previous production cycle and serve as a reference for future cycles regardless of feed delivery system. Feed trays are one of the most common feed management strategies as they allow gross estimation of feed intake (Martinez-Cordova*, et al.*, 1998). Nevertheless, being a very high labour-intensive technique is a major setback (Bador, 2013; Davis, 2018; Ullman*, et al.*, 2019b). As a response to the necessity of the shrimp farming industry to improve its feed management protocols, some techniques and technologies have risen to address this issue.

Timer feeders are not a recent technology and are extensively used in various sectors and aquaculture production systems. These feeders enable the producer to increase the number of feedings without negatively impacting labor cost. Ullman*, et al.* (2019c) reported no significant improvements in production for ponds fed same increasing feed amount twice a day in contrast with ponds fed the same amount but fed six meals a day. This indicates that better productivity can be achieved by increasing both number of meals and feed inputs. In parallel, animal feeding activity is also an important tool in aquaculture. The most simple feeding feedback when culturing fish is visual observation, however, this will not work in shrimp ponds due to both the size of the animal and poor water visibility. Using a different approach, for the last decade on-demand acoustic feedback feeding systems have proven to be a reliable tool in shrimp farming (Silva*, et al.*, 2019). These feeding systems respond to the signature clicking noise produced by shrimp while feeding. Previous works by (Napaumpaipom*, et al.*, 2013) in high density, intensive systems and Jescovitch*, et al.* (2018) and Ullman*, et al.* (2019b); Ullman*, et al.* (2019c) in semi-intensive conditions have shown improvements in growth performance by application of acoustic demand-feeding system in comparison to hand-feeding and timer feeder techniques in semi-intensive systems. As a continuation, this study aims towards improving timer-feeder protocols by adjusting feed amount while comparing it to acoustic demand-feeding systems.

## 2. Material and methods

This trial was performed at Alabama Department of Conservation and Natural Resources, Claude Peteet Mariculture Center, Gulf Shores, Alabama. Pacific white shrimp *L. vannamei* larvae (2.3 mg) were obtained from Shrimp Improvement Systems (Islamorada, FL, USA), acclimated and nursed in a greenhouse system for 18 days. Juvenile shrimp (6 mg) were then stocked in outdoor ponds at a density of 26 shrimp/$m^2$. The production research was carried out in 16, 0.1 ha outdoor ponds over a 13 wk production period.

The ponds used through the growout period were approximately 0.1 ha in surface area (46 x 20 x 1.0 m) lined with 1.52 mm high-density polyethylene with a 25 cm layer of sandy-loam soil on the bottom. Ponds were filled with brackish water (10.8 – 12.9 g/L) from Intracoastal Canal between Mobile and Perdido Bay, Alabama, filtered through a 250 µm cloth mesh filter bag. Pond primary productivity was promoted by adding inorganic fertilizers (1687 mL of 32-0-0 and 303 mL 10-34-0 for 5.70 kg/ha of N and 1.03 kg/ha of P) to the ponds two weeks prior to stocking. The same fertilizing treatment was repeated for every pond one week after pond stocking as Secchi readings for all ponds were still approximate to the ponds total depth. To try to maintain dissolved oxygen (DO) above 3 mg/L, all ponds were supplied one 2-hp surface aerator (Aire-$O_2$, Aeration Industries International, Inc., Minneapolis, MN, USA) as the primary source of mechanical aeration and one 1-hp surface aerator (Aquarian, Air-O-Lator, Kansas City, MO, USA) for backup and/or additional aeration. No water exchange was performed throughout the trial.

### *2.1 Feed Management*

All ponds were offered the same two diets: 1.5-mm commercial diet (40% crude protein, 9% crude lipids) produced by Zeigler Inc. (Gardners, PA, USA) for the first four weeks, and 2.4-mm protein soy optimized feed (35% crude protein, 8% crude lipids) produced by Zeigler Inc. from the fourth week on, according to the treatments. Diet formulation for this experiment was the same as used by Ullman *et al*., (2019a). For evaluation of the potential for automation the four treatments used were a standard feeding protocol (SFP) + 30%, SFP + 45%, SFP + 60% and a passive acoustic feeding system (SF200 Sound feeding system, AQ1 Systems, Tasmania, Australia). SFP was calculated based on an expected weight gain of 1.3 g/wk, a feed conversion ratio (FCR) of 1.2, and a weekly mortality of 1.5% during growout period. The SFP used in this experiment was based on Davis*, et al.* (2006) which was developed to optimize growth and FCR when using two feedings per day, resulting in satisfactory results as reported by Sookying*, et al.* (2011b). It was also used as the reference for the development of a protocol for timer feeders with satisfactory results as well as reported by Sookying*, et al.* (2011a), Van*, et al.* (2017), Jescovitch*,* *et al.* (2018) and Ullman*, et al.* (2019b); Ullman*, et al.* (2019c). Each of the four replicates for each treatment was randomly assigned to a pond except for the AQ1 system treatment due to electricity constraints. All feeders used for SFP treatments were BioFeeder (BioFeeder SA, Guayaquil, Ecuador) timer-feeders, feeding once every 20 minutes from 0700 to 1900. Biofeeder feed management (e.g. set feed amount, turn on/off) was done remotely using the feeder's specific software. AQ1 feeding system fed *ad libitum* using a hydrophone with computer software to monitor feeding activity. All ponds under AQ1 system management were also equipped with an underwater DO sensor (placed approximately 10 cm off the pond bottom) and the system was set to only allow feeding when DO levels were above 4 mg/L. In all four ponds under AQ1 system treatment the main aerator was connected to the system so it could control aerator activity based on information provided by DO sensor. All ponds were hand-fed a SFP-based amount twice a day for the first 30 days after which BioFeeders were initiated. AQ1 system was started on the 34$^{th}$ day of pond production.

*2.2 Sampling and water quality*

After 17 days of pond culture, shrimp were sampled weekly through the remaining production cycle using a cast net (1.52 m radius and 0.96 cm mesh) to collect approximately 60 individuals per pond. Pond sampling enabled weight recording for growth assessment and inspection for general health. Ponds were monitored (DO, temperature, salinity, and pH) at least three times a day, at sunrise (0500-0530 h), afternoon (1400-1430 h) and sunset (1900-2000 h), using a YSI ProPlus meter (Yellow Springs Instrument Co., Yellow Springs, OH, USA) at the deepest point outside of the catch basin. Secchi disk readings were recorded once a week as total ammonia nitrogen (TAN) and chlorophyll *a* concentration were recorded twice a week. Water samples were taken in the morning at the surface and TAN was analysed with a high performance ammonia ion selective electrode (Thermo Fisher Scientific Inc., Waltham, MA, USA). Direct calibration of the electrode was conducted by preparing a serial dilution of a 100 +/- 1 mg/L ammonia standard (certified traceable to NIST standard reference material) to create three ammonia standards (0.1, 1.0 and 10.0 mg/L), calibration was performed prior to each week's analysis. Chlorophyll samples were taken once a week by filtering a water sample through glass fiber filters (47 mm diameter) using a vacuum pump. Filters were kept in plastic 35 mm film canisters and shipped to E.W Shell Fisheries Center at Auburn University. Analyses were performed according to standard analytical protocols for chlorophyll a by membrane filtration, acetone-methanol extraction of phytoplankton and spectroscopy (Eaton, *et al.*, 2005).

All AQ1 treatment ponds were provided a DO sensor with real-time oxygen information on those ponds. All sensors were cleaned twice daily to prevent fouling and misreading. Calibration was performed only once through the entire cycle. Due to equipment failure near the end of the cycle, one of the AQ1 treatment ponds had the DO sensor and automatic aeration disconnected and was fed *ad libitum* from 0700 to 1900.

*2.3 Harvest and shrimp value*

The ponds were harvested over three days at the end of the 13-week culture period. Ponds were partially drained and the night before harvest the level was reduced to about one third and aeration was provided using the surface aerator. On the day of harvest, the remaining water was drained and the shrimp were pumped out of the catch basin using a hydraulic fish pump equipped with a 25 cm diameter suction pipe (Aqua-Life pump, Magic Valley Heli-arc and Manufacturing, Twin-Falls, Idaho, USA). The pump was placed in the catch basin and shrimp were pumped, de-watered, and collected into a hauling truck. Shrimp were then rinsed, weighed in bulk, and 150 were randomly selected to measure individual weights and determine the size distribution. A subsample of these shrimp were collected and frozen for subsequent analysis. Whole body proximate with minerals analysis of the shrimp was performed by Midwest Laboratories (Omaha, NE, USA).

Shrimp prices used were the three year average (2014-2016) as reported by Urner Barry (Urner Barry, Toms River, NJ, USA) for Latin American Farmed white shrimp, whole. The partial value was calculated by subtracting the feed costs from the production value as calculated from the Urner Barry prices and the size distribution of shrimp produced. The feed prices were \$1.72/kg for the starter diet (40% CP, 9% CL) and \$1.09/kg for the grower diet (35% CP, 8% CL).

*2.4 Statistical analysis*

Statistical analysis of the growth data was conducted with SAS 9.4 (SAS Institute, Cary, NC, USA) to perform a one-way analysis of variance to determine significant difference (p-value < 0.05) among treatments, the assumptions for ANOVA were met. Student-Newman-Keuls multiple range test was used to determine differences among treatments. Effect of feed inputs in low DO occurrences was analyzed through a regression analysis.

3. Results

During this trial, main water quality parameters were kept within typical range for shrimp production (Boyd*, et al.*, 1992) (Table 1). To evaluate the effects of nutrient loading on oxygen demand the occurrences of DO reading below 2.5 were registered throughout the cycle. Figure1 shows the number of low DO occurrences for each pond identified by treatments. Regression analysis revealed that feed inputs affected biological oxygen demand (BOD) in the pond with most occurrences being registered at dawn. However, analysis did not show a linear correlation ($R^2$=0.0944) (Figure 1) between the number of low oxygen occurrences (<2.5 mg/L) in DO readings and the feed input for each pond.

Production data is summarized in Table 2 with final weights and yield generally following the level of feed input. The mean final individual weights of shrimp were significantly different between timer feeder treatments and AQ1 but not among timer feeder treatments. Weekly growth and yield were significantly different between the two treatments with lower feed inputs (SFP + 30% and SFP + 45%) and the highest feed input treatment (AQ1). There were no significant differences in survival (72.5-81.4%) or FCR (0.96-1.11) among treatments. Figures 3 and 4 present average treatments feed inputs and average individual weight throughout the production cycle. Feed inputs (kg/ha) were different among treatments, as shown in Table 2.

Results for feed input analysis are summarized in Figure 4. Data summarized in Figure 4 did not include data until day 17 due to lack of sampling although feed amount was adjusted on day 10 based on expected growth and survival. Combined analyses of data revealed increasing differences in size as previously indicated by Figures 2 and 3.

Proximate whole body composition analysis are summarized in Table 3. SFP + 60% produced shrimp had significantly lower ash% than SFP + 45% but no other statistical differences were found in any of the other parameter evaluated in whole body composition analysis.

Feed costs and economic value of shrimp produced is summarized in Table 2. Significant differences were found for all treatments in both feed inputs and feed cost. However, for shrimp value and partial income statistically significant differences were only found between both SPF + 30% and SFP + 45% in comparison to AQ1 treatment.

4. Discussion

Commercial shrimp feeds are considered nutritionally appropriate and are one of the primary operating costs of most farms. To ensure the investment in high quality feed is maximized it is important to focus on optimizing feeding protocols Shrimp have been traditionally fed 2 to 4 meals a day either by hand-dispersion or through the use of feed trays. However, shrimp can be described as grazers in that they have evolved to find small patches of food with high frequency indicating that feed frequency is an important driver of nutrient intake. Ullman*, et al.* (2019c) reported a significant increase in final weights of shrimp reared with 6 feeding/day as compared to those fed a similar amount of feed over two feedings per day. The use of automation to increase the number of feedings not only favors shrimp growth but also improves economic balance as labor requirement is reduced and feed efficiency is improved (Davis, 2018). Application of automatic feeders has shown many advantages in comparison to traditional methods. Within automatic feeders, on-demand acoustic feedback systems have shown improved performance over simpler timer-feeders (Jescovitch*, et al.*, 2018; Napaumpaipom*, et al.*, 2013; Ullman*, et al.*, 2019b; Ullman*, et al.*, 2018) and in some cased improved water quality has been reported.

During the entire production cycle water quality management aimed towards keeping dissolved oxygen levels above 3 mg/L. Given the variability between ponds as well as the variation in feed management it is difficult to draw conclusions from the water quality data. Jescovitch *et al.* (2018) reported increased levels of TAN associated with increased feed inputs using the AQ1 system. However, our feed loading was considerably higher than the previously mentioned study yet there were minimal differences in water quality. The lack of differences across feed input levels would indicate that we were within the processing capacity of the pond based ecosystem. Under our conditions, aeration was managed either using automated set points AQ1 system or through manual management. Although DO was closely managed, we counted the days for which DO dropped below 2.5 mg/l (Figure 1). This regression had a very weak fit ($R^2$ 0.0944) and further statistical tests showed no significant differences between treatments (p=0.2469) ultimately confirming that the ponds were able to process the nutrient load.

During the first 30 days the feeding program for all treatments was preprogramed following the previously described SFP which assumes estimates for the population as well as growth. Although this is not an optimized protocol it is assumed that primary productivity is considerable portion of nutrient intake and that feed inputs must be systematically increased to allow conditioning of the pond to the high feed loads. Also, as shrimp feed lower in food chain ponds primary productivity is more than likely one of the main sources of nutrients at this stage and uneaten feed will trigger phytoplankton growth as well (NRC, 2011). By evaluating feed inputs through the production cycle (Figure 2) and comparing this to the average individual growth (Figure 3) it is possible to discern some feeding differences. Between days 38 and 45 there was a substantial reduction in feed input for AQ1 feeding system. There are two possible interpretations of this: the small size of shrimp producing a minimal acoustic signal resulted in low feed inputs or primary productivity remains a sufficient food source for shrimp within that size class. As there were no differences in mean weights it would appear reduced feed inputs did not affect growth. From this point forward AQ1 feed inputs steadily increase up to day 59 where it peaked. From 50 days to the end of the production period, feed inputs were highest for the AQ1 treatments. Based on sample weight it was apparent that up to 45 days of culture the lowest level of feed input was acceptable. However, after this point SFP + 30% and SFP + 45% feed treatment resulted in smaller shrimp or a reduced growth rate. Shrimp fed using the SFP + 60% level maintained similar growth as the AQ1 system through day 73 after which it appeared that growth was reduced. This data leads us to believe it is possible to obtain high growth rate with lower feed inputs than AQ1 although at some point feed will become a limiting factor for growth. Regardless no differences in FCR among treatments were registered and reported values are more than acceptable throughout all treatments.

Shrimp were not sampled during the first week as representative samples were difficult to obtain with small shrimp in ponds. Hence, with the exception of the first few week of production the collected data can be used to develop a feed curve. To do this, final survivals are used to back calculate the estimated number of shrimp at any given time point and the percent body weight calculated. This data is presented in Figure 4 which does not include data from the first 17 days of production. This data can then be used as a recommended feed rate for shrimp produced under similar conditions.

Combined analyses or data also suggest that shrimp adjusted growth based on the amount of food with higher feed inputs resulting in larger shrimp. Supporting this conclusion is the fact that ponds fed SFP + 60% also registered numerically higher average survival. Also, although feed inputs were only differentiated from day 30 on (Figure 2), it is possible to identify larger individuals (Figure 3) in SFP + 60% ponds at the same time as feed inputs by percentage body weight (Figure 4) remain similar. This is likely a consequence of numerically higher survival (Table 2) in this treatment regardless of higher feed input and shrimp adjusting their growth to feed input as well. In short, combined analysis of data summarized in Figures 3 and 4 indicates that shrimp are able to adjust their growth based on feed availability it also suggests that there may be a threshold for feed input over which relative growth does not increase. Consequently, from an economical and water quality management perspective our data suggests that shrimp could have been be fed SFP + 30% until individual sizes reach about 18 grams (~day 50) and then feed inputs could be increased to SFP + 60% until the end of production. Increased feed inputs during the last two weeks of production, as was seen in the AQ1 system, was perhaps responsible for further increased shrimp size (Table 2).

Feed management and nutrient composition of the diet is known to influence proximate composition of the animal albeit shrimp seem to be less responsive than other animals. Significantly higher ash content of shrimp fed in SFP + 45% was observed compared to SFP + 60%. (Ullman, *et al.*, 2019b) has reported differences in several compounds between treatments, namely higher fat content for higher feed input treatments. However, in this research no differences were found in any components except for ash. In our work ash was significantly higher in shrimp reared on the SFP + 60% treatment as compared to those on the SFP + 60% feeding regime. Variation in ash content was not consistent across feed inputs; hence, it may simply be due to natural variation in the data or possibly small changes in macro minerals such as Ca and P.

## 5. Conclusion

The results of this study underline the results achieved in similar studies by Jescovitch, *et al.* (2018) and Ullman, *et al.* (2019b); Ullman, *et al.* (2019c), indicating that higher production and value of *L. vannamei* produced in semi-intensive pond culture can be achieved through application of on-demand acoustic feedback systems. This study also shows that it is possible to establish an efficient feeding protocol for timer feeders, thereby reducing the performance differences between the two technologies. Nevertheless, efficient use of timer feeders heavily relies on adequate feeding plans based on previous production cycles as well as post feeding observations. Poor estimations of survival, growth and feed response would likely have a negatively impact on growth, environmental conditions (water quality) and financial performance.

While the intrinsic nature of a feedback technology is to feed on demand in real time, it is virtually impossible for any timer feeder to be as efficient as a real-time passive feedback system. However, our results confirm that a standard feeding protocol can be developed for automated feeding system that will support the enhanced growth rates seen when using these systems. Thus, our results provide a degree of practical guidance for this level of technology. Increased product value may also offset the installation and running cost of any of these technologies. However, as reported by Ullman*, et al.* (2019c), it is not possible to accurately provide implementation costs due to a lack of linearity inherent in the facility and production setup.

6. References

Bador, R., Blyth, P., Dodd, R., 2013. Acoustic control improves feeding productivity at shrimp farms. Global Aquaculture Advocate, 77-78.

Boyd, C.E., Tucker, C.S., 1992. Water quality and pond soil analyses for aquaculture. Alabama Agricultural Experiment Station, Auburn University.

Carvalho, E.A., Nunes, A.J., 2006. Effects of feeding frequency on feed leaching loss and grow-out patterns of the white shrimp *Litopenaeus vannamei* fed under a diurnal feeding regime in pond enclosures. Aquaculture. 252, 494-502.

Casillas-Hernandez, R., Magallon-Barajas, F., Portillo-Clarck, G., Paez-Osuna, F., 2006. Nutrient mass balances in semi-intensive shrimp ponds from Sonora, Mexico using two feeding strategies: Trays and mechanical dispersal. Aquaculture. 258, 289-298.

Chatvijitkul, S., Boyd, C.E., Davis, D.A., McNevin, A.A., 2017. Embodied Resources in Fish and Shrimp Feeds. Journal of the World Aquaculture Society. 48, 7-19.

NRC, 2011. Nutrient requirements of fish and shrimp. National academies press. Washington, DC

Cuzon, G., Lawrence, A., Gaxiola, G., Rosas, C., Guillaume, J., 2004. Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. Aquaculture. 235, 513-551.

Dall, W., Hill, B.J., Rothlisberg, P.C., Staples, D.J., 1990. The Biology of the Penaeidae. Adv Mar Biol. 27, 1-461.

Davis, D.A., Amaya, E., Venero, J., Zelaya, O., Rouse, D.B., 2006. A case study on feed management to improving production and economic returns for the semi-intensive pond production of *Litopenaeus vannamei*, Avances en Nutrición Acuícola VIII. Memorias del Octavo Simposio Internacional de Nutrición Acuícola. Universidad Autonoma de Nuevo Monterrey, Nuevo León, México.

Davis, D.A., Ullman, C., Rhodes, M., Novriadi, R., Swanepoel, A., 2018. Automated Feeding Systems in Pond Production of Pacific White Shrimp. Global Aquaculture Advocate.

Eaton, A.D., Clesceri, L.S., Greenberg, A.E., Franson, M.A.H., 2005. Standard methods for the examination of water and wastewater. Journal of American Public Health Association. 21, 1600.

Jescovitch, L.N., Ullman, C., Rhodes, M., Davis, D.A., 2018. Effects of different feed management treatments on water quality for Pacific white shrimp *Litopenaeus vannamei*. Aquac. Res. 49, 526-531.

Martinez-Cordova, L.R., Porchas-Cornejo, M.A., Villarreal-Colemnares, H., Calderon-Perez, J.A., Naranjo-Paramo, J., 1998. Evaluation of three feeding strategies on the culture of white shrimp *Penaeus vannamei* Boone 1931 in low water exchange ponds. Aquacultural Engineering. 17, 21-28.

Napaumpaipom, T., Chuchird, N., Taparhudee, W., 2013. Study on the Efficiency of Three Different Feeding Techniques in the Culture of Pacific White Shrimp (*Litopenaeus vannamei*). Journal of Fisheries and Environment. 37, 8-16.

Obaldo, L.G., Divakaran, S., Tacon, A.G., 2002. Method for determining the physical stability of shrimp feeds in water. Aquac. Res. 33, 369-377.

Quintero, H.E., Roy, L.A., 2010. Practical Feed Management in Semi-Intensive Systems for Shrimp Culture. The Shrimp Book, edited by Alday-Sanz, V., 443-454. Nottingham, UK: Nottingham University Press. 443-453.

Silva, J.F., Hamilton, S., Rocha, J.V., Borie, A., Travassos, P., Soares, R., Peixoto, S., 2019. Acoustic characterization of feeding activity of *Litopenaeus vannamei* in captivity. Aquaculture. 501, 76-81.

Sookying, D., Davis, D.A., 2011a. Pond production of Pacific white shrimp (*Litopenaeus vannamei*) fed high levels of soybean meal in various combinations. Aquaculture. 319, 141-149.

Sookying, D., Silva, F.S.D., Davis, D.A., Hanson, T.R., 2011b. Effects of stocking density on the performance of Pacific white shrimp *Litopenaeus vannamei* cultured under pond and outdoor tank conditions using a high soybean meal diet. Aquaculture. 319, 232-239.

Tacon, A.G.J., Forster, I.P., 2003. Aquafeeds and the environment: policy implications. Aquaculture. 226, 181-189.

Ullman, C., Rhodes, M.A., Davis, D.A., 2019a. The effects of feed leaching on the growth of Pacific white shrimp *Litopenaeus vannamei* in a green-water tank system. Aquac. Res. 50, 3074-3077.

Ullman, C., Rhodes, M.A., Davis, D.A., 2019b. Feed management and the use of automatic feeders in the pond production of Pacific white shrimp *Litopenaeus vannamei*. Aquaculture. 498, 44-49.

Ullman, C., Rhodes, M., Hanson, T., Cline, D., Davis, D.A., 2019c. Effects of Four Different Feeding Techniques on the Pond Culture of Pacific White Shrimp, *Litopenaeus vannamei*. Journal of the World Aquaculture Society. 50, 54-64.

Van, T.P.T.H., Rhodes, M.A., Zhou, Y., Davis, D.A., 2017. Feed management for Pacific white shrimp Litopenaeus vannamei under semi-intensive conditions in tanks and ponds. Journal of Aquaculture Research. 48, 5346-5355.

Varadharajan D., P.N., 2013. Food and feeding habits of aquaculture candidate a potential crustacean Pacific white shrimp *Litopenaeus vannamei*. Aquac Res Development. 4, 1-5.

Table 1 - Summary of water quality parameters for the four treatments over the 13 wk culture period. Values are presented as mean ± standard deviation and maximum and minimum value are presented in parenthesis

| | SFP + 30% | SFP + 45% | SFP + 60% | AQ1 |
|---|---|---|---|---|
| Morning DO[a] (mg/L) | 3.81 ± 1.14<br>(1.65 , 9.90) | 3.95 ± 1.33<br>(0.82 , 13.81) | 3.66 ± 1.04<br>(1.77 , 7.93) | 3.66 ± 1.11<br>(0.78 , 7.02) |
| Afternoon DO[a] (mg/L) | 10.68 ± 2.78<br>(4.32 , 18.05) | 10.48 ± 2.81<br>(2.71 , 21.36) | 10.69 ± 2.73<br>(3.38 , 16.97) | 10.60 ± 2.99<br>(2.94 , 10.02) |
| Night DO[a] (mg/L) | 9.73 ± 2.70<br>(3.56 , 18.5) | 9.34 ± 2.97<br>(3.17 , 24.11) | 9.31 ± 2.69<br>(2.77 , 16.89) | 9.35 ± 3.04<br>(1.87 , 18.36) |
| Temperature (ºC) | 31.8 ± 1.7<br>(27.4 , 36.3) | 31.7 ± 1.6<br>(27.5 , 38.1) | 31.6 ± 1.7<br>(24.6 , 35.4) | 31.4 ± 1.6<br>(27.3 , 35.0) |
| pH | 8.48 ± 0.79<br>(6.81 , 10.01) | 8.45 ± 0.75<br>(6.8 , 9.81) | 8.39 ± 0.76<br>(6.87 , 9.87) | 8.33 ± 0.70<br>(6.95 , 10.18) |
| Salinity (g/L) | 9.27 ± 1.35<br>(7.13 , 12.09) | 10.71 ± 2.58<br>(7.73 , 11.41) | 9.68 ± 1.42<br>(6.72 , 12.36) | 10.28 ± 1.25<br>(8.03 , 12.88) |
| TAN[b] (mg/L) | 0.4 ± 0.7<br>(<0.001, 3.0) | 0.5 ± 1.0<br>(<0.001 , 4.0) | 0.6 ± 1.0<br>(<0.001 , 4.0) | 0.7 ± 1.9<br>(<0.0001, 6.0) |
| Chrolorophyll a (µg/L) | 307± 213<br>(3.7 , 990) | 363 ± 202<br>(71 , 745) | 396 ± 325<br>(25 , 1742) | 318 ± 203<br>(35 , 1044) |

[a] DO - Dissolved Oxygen

[b] TAN - Total Ammonia Nitrogen

Table 2 - Summary of Pacific white shrimp response to different feed management protocols

| Treatment | IndW (g) | Survival | Weekly Growth (g) | Yield (kg/ha) | Total Feed (kg/ha) | FCR | Feed Cost ($/ha) | Shrimp Value ($/ha) | Partial Income ($/ha) |
|---|---|---|---|---|---|---|---|---|---|
| SFP + 30% | 26.29[a] | 77.6 | 1.97[a] | 5,226[a] | 4,933[a] | 0.99 | 5,592[a] | 43,490[a] | 37,898[a] |
| SFP + 45% | 26.87[a] | 75.2 | 2.04[a] | 5,115[a] | 5,332[b] | 1.11 | 6,026[b] | 42,468[a] | 36,442[a] |
| SFP + 60% | 29.04[a] | 80.7 | 2.21[ab] | 6,128[ab] | 5,844[c] | 0.96 | 6,585[c] | 52,623[ab] | 46,039[ab] |
| AQ1 | 32.53[b] | 81.4 | 2.49[b] | 6,869[b] | 6,984[d] | 1.02 | 7,828[d] | 60,723[b] | 52,896[b] |
| *P*-value | 0.0096 | 0.9083 | 0.0091 | 0.0274 | <0.0001 | 0.7313 | <0.0001 | 0.0073 | 0.0164 |
| PSE | 1.18 | 6.52 | 0.093 | 39.62 | 5.07 | 0.097 | 55.3 | 3,362 | 3,380 |

[1]PSE: Pooled Standard Error

Table 3 - Means of whole body composition for each treatment as analysed by Midwest Laboratories (Omaha, NE, USA)

| Treatment | SFP + 30% | SFP + 45% | SFP + 60% | AQ1 | *P*-value | PSE |
|---|---|---|---|---|---|---|
| Moisture % | 74.9 | 75.0 | 75.2 | 74.1 | 0.5042 | 1.95 |
| Dry Matter % | 25.08 | 25.05 | 25.93 | 24.85 | 0.5042 | 1.95 |
| Protein % | 74.5 | 73.2 | 78.1 | 76.3 | 0.4336 | 8.79 |
| Fat % | 4.16 | 3.89 | 4.94 | 5.66 | 0.3793 | 2.75 |
| Ash % | $11.67^{ab}$ | $12.83^{a}$ | $10.29^{b}$ | $11.03^{ab}$ | 0.0250 | 2.06 |
| Sulfur % | 0.84 | 0.80 | 0.85 | 0.82 | 0.1278 | 0.04 |
| Phosphorus % | 1.62 | 1.61 | 1.43 | 1.57 | 0.2436 | 0.26 |
| Potassium % | 1.27 | 1.24 | 1.27 | 1.27 | 0.8351 | 0.09 |
| Magnesium % | 0.35 | 0.37 | 0.30 | 0.35 | 0.2459 | 0.09 |
| Calcium % | 3.62 | 4.10 | 3.09 | 3.69 | 0.3103 | 1.46 |
| Sodium % | 0.73 | 0.73 | 0.73 | 0.69 | 0.5147 | 0.08 |
| Iron (ppm) | 152.8 | 161.6 | 101.2 | 202.8 | 0.4222 | 173.41 |
| Manganese (ppm) | 7.6 | 7.1 | 3.6 | 6.6 | 0.3363 | 57.07 |
| Copper (ppm) | 137.5 | 136.0 | 125.8 | 136.8 | 0.0640 | 27.94 |
| Zinc (ppm) | 78.3 | 73.4 | 75.5 | 75.2 | 0.3439 | 6.18 |

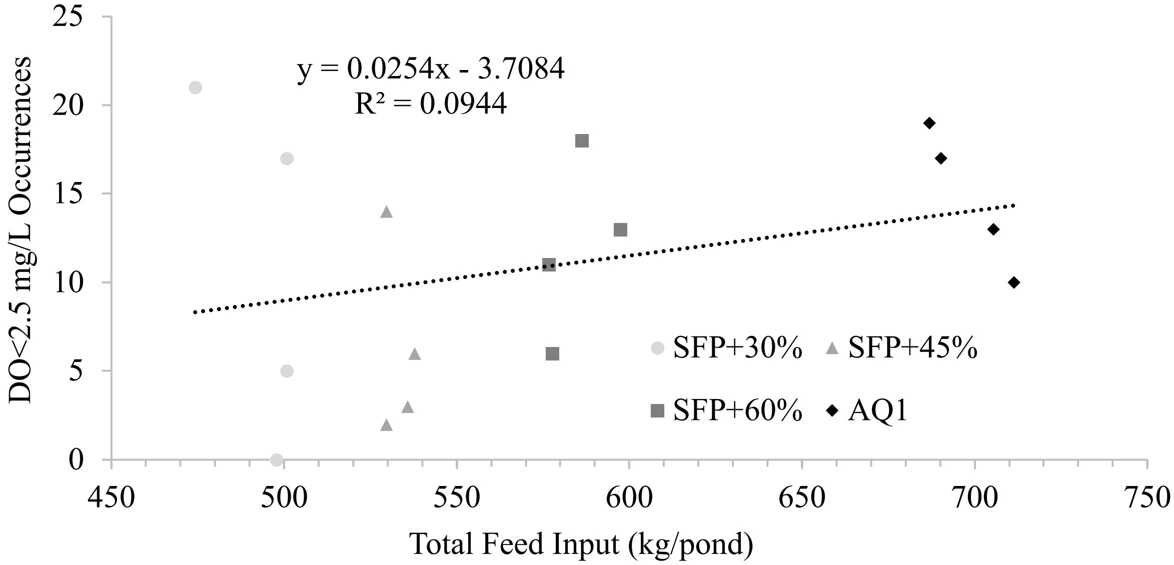


Figure 1 - Relationship between total low oxygen occurrences (< 2.5 mg/L) per treatment and total feed input.

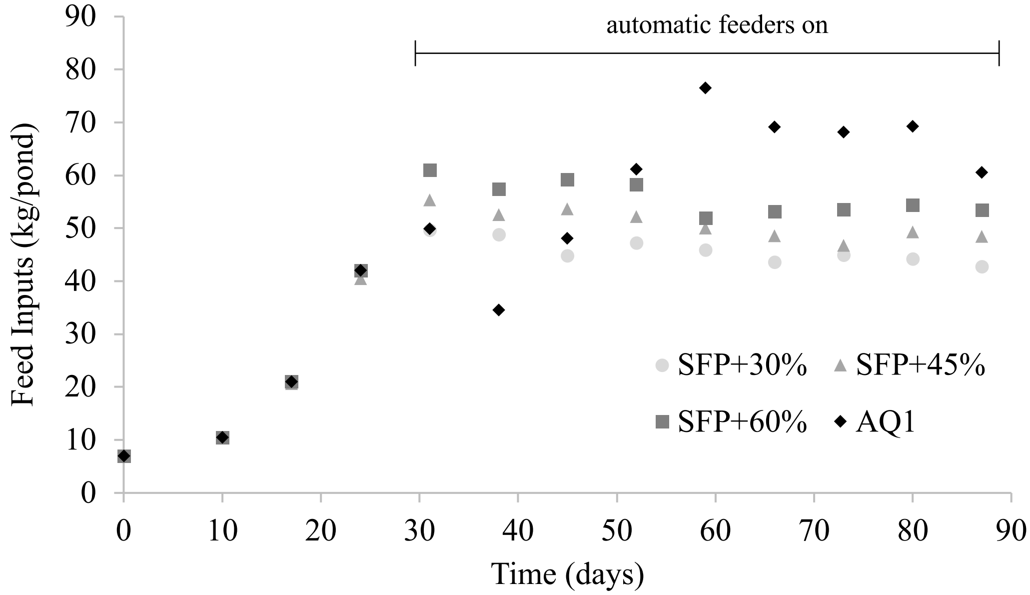


Figure 2 - Weekly feed inputs (kg/pond) through production cycle as average per treatment. Feed inputs were equivalent for the first 30-34 day. Timer feeders were initiated on day 30 and AQ1 feeders on day 34.

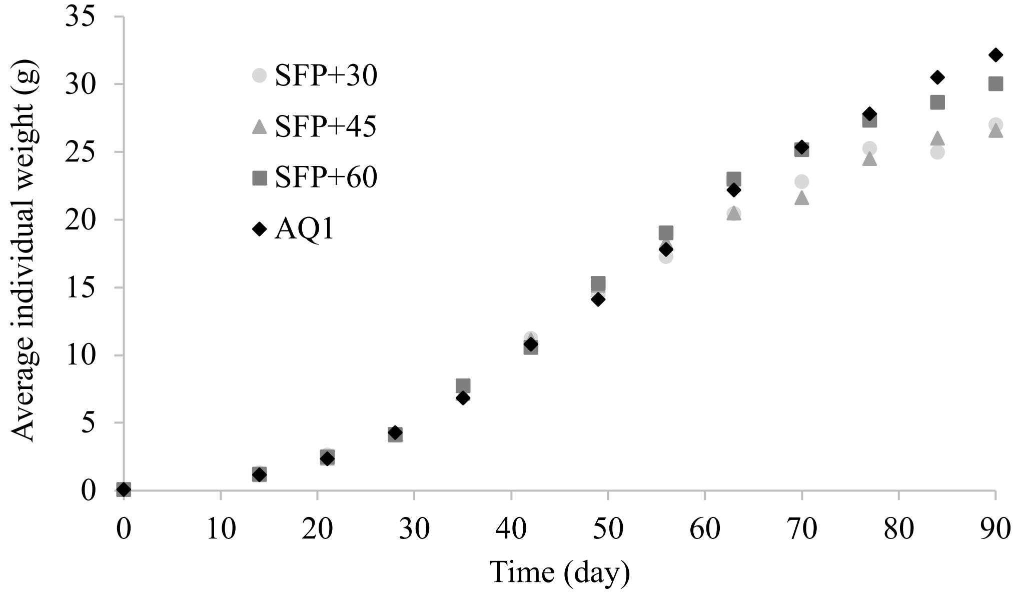


Figure 3 - Weekly average individual weight (g) as average per treatment.

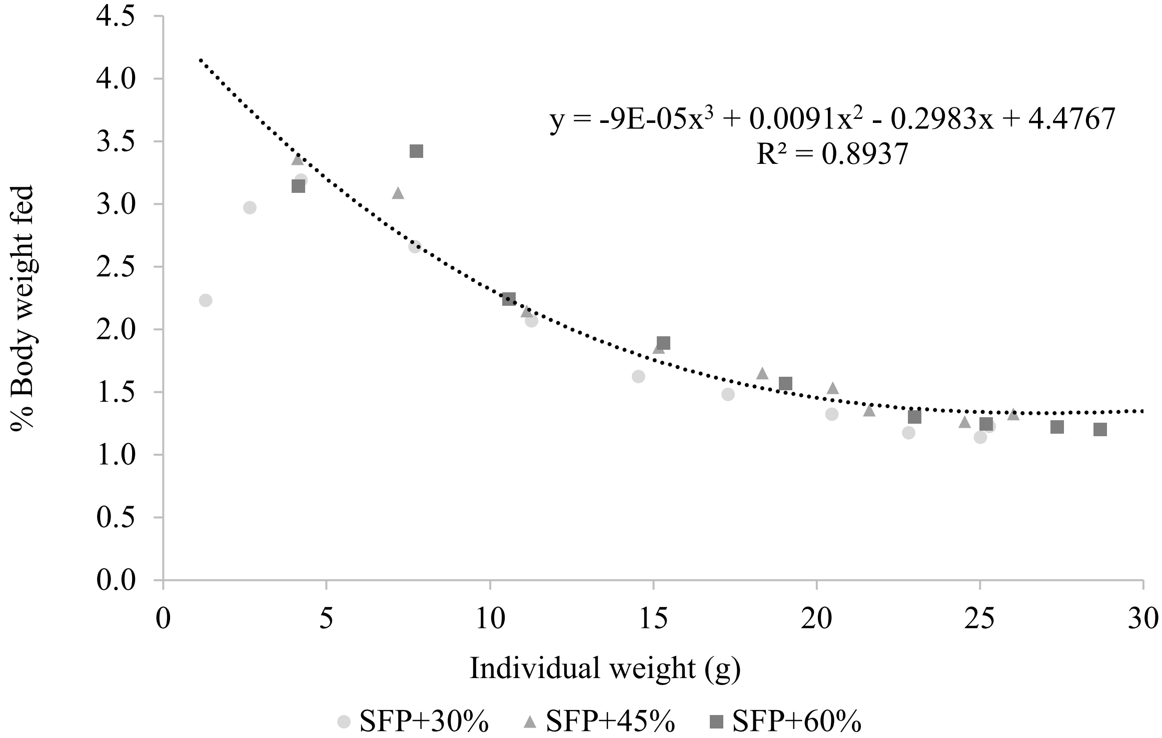


Figure 4 –Back calculated feed inputs expressed as percentage body weight for the various sizes of shrimp. Regression represents the results of pooled data.

Chapter III

# AUTOMATED FEEDING SYSTEMS FOR SHRIMP: EFFECTS OF FEEDING SCHEDULES AND PASSIVE FEEDBACK FEEDING SYSTEMS

Abstract

Shrimp aquaculture has been on a growing trend for the past four decades and its continued success will rely on efficient feeding protocols and reductions in labor cost. Various studies have demonstrated better growth and feed conversion of shrimp fed numerous meals compared to the traditional approach of offering 1 to 4 meals offered during the day. With the adoption of automatic feeding systems by the shrimp industry not only can more feedings be delivered but also the time of day when feed is delivered is less problematic. This opens the door to looking at various feed management strategies. The goal of this work was to continue the development of a standard feeding protocol for timer feeders (SPTF) to maximize growth rates and production efficiency in semi-intensive pond production of shrimp through manipulation of feeding schedules. For this work, a 13-week pond production trial was performed in 16, 0.1 ha outdoors ponds, stocked at 35 shrimp/m$^2$. Four treatments including: three fixed feeding treatments based on SPTF Day, SPTF Night and SPTF 115% 24hr were offered using automatic timer-feeders, and a fourth on demand treatment utilized AQ1 acoustic feeding system. To further evaluate different combinations of number of meals, feed quantities and time of feeding, a 11-week growth trial was conducted in 32, 800L circular tanks in a recirculating green-water system. Results for the pond trial further confirm higher yields with AQ1 acoustic feeding system and showed no statistical differences among timer feeder treatments. Results in the tank trial indicated a relationship between growth response to increasing feed inputs and number of meals rather than feeding schedule alone. We also observed

in the same trial a positive response on growth to more meals for identical feeding rates. Results confirm that increasing the number of feeding allows increased feed inputs and thus increased growth. The on demand feeding protocol using passive acoustics, resulted in the best overall shrimp performance.

1. Introduction

Shrimp are one of the most valuable commodities in the seafood trade industry. Since the early 2000's, *Litopenaeus vannamei* remains the preferred species for aquaculture owing to its excellent integration when employing a wide variety of different culture methods as well as market acceptance. As with most agriculture, shrimp farming success relies on increasing intensification and improved cost-efficiency. Artificial feeds are the main variable cost, source of nutrients and waste for feed based production systems (Martinez-Cordova*, et al.*, 2003; Tacon*, et al.*, 2003). Better feed management protocols can positively impact shrimp farms both environmentally and economically. Considerable research has been conducted in nutrition which has resulted in the availability of quality feeds. However, not as much research has been directed towards feed management techniques, such as improving feed delivery methods and feeding protocols.

Understanding feeding behavior is the basis for any improvement in feed delivery protocols. Shrimp are described as omnivorous benthic animals that favor frequent ingestion of small quantities of food. Multiple authors reported better growth when shrimp were fed multiple feedings during the day (Carvalho*, et al.*, 2006; Nunes*, et al.*, 2019; Ullman*, et al.*, 2019a; Ullman*, et al.*, 2019b). This is most likely due to shorter exposure to water which reduces nutrient leaching, even though shrimp have slower food consumption due to external mastication of most food items. Spreading daily feed inputs through small frequent meals is a preferred practice to mitigate this issue and improve feed delivery efficiency. While many farms still rely on hand-feeding 1 to 4 meals a day as a standard feed delivery protocol, the industry is gradually shifting towards more automated feed delivery methods. Works by (Ullman*, et al.*, 2019a); Ullman*, et al.* (2019b) observed improved growth when shrimp in semi-intensive ponds were fed identical feed rate through 6 meals with timer feeders by opposition with traditional bidaily hand-feeding. The same authors also reported in the same works that spreading the nutrient load through an extended period of time also allowed increases in feeding rate. In fact, while enabling farm managers to increase the number of meals with little to no drawback on labor costs, automatic feeders also allow for an extendsion the feeding schedule to periods of the day in which the farm would typically not be feeding.

Penaeid shrimp are often described as night feeders (Santos*, et al.*, 2016) yet (McTigue*, et al.*, 1989) found little evidence of feeding periodicity in juvenile *P. setiferus*. Wassenberg*, et al.* (1987) reported higher quantity of feed in the foregut of wild *P. esculentus* after sunset, and Reymond*, et al.* (1990) found growing *P. japonicus* gradually shifted towards night feeding. Many shrimp farmers report higher shrimp activity after sunset which suggests hence justifying the belief that penaeid shrimp may prefer feeding during nighttime. Yet, feeding through the night can be a challenging practice both logistically and environmentally. General unavailability of labor to work night shifts makes it hard for farms to have enough labor during those hours to ensure adequate feeding. Also, and more importantly, most semi-intensive shrimp ponds rely on natural productivity as the main source of oxygen in the system, hence increasing oxygen consumption as result of feeding increases the potential for oxygen depletion. This is even more so if the nutrient loading is not spread over multiple meals as well. Nevertheless, identifying potential preferences in feeding schedule can still be very useful for farms in which oxygen is not a limiting factor even when feeding during that period is not logistically possible.

While timer feeders are a very simple and straight forward tool, substantial effort was invested into monitoring shrimp behavior as a tool towards higher efficiency in shrimp production facilities for the past decade. Sound profile in culture ponds was first associated with *P. monodon* feeding activity (Smith*, et al.*, 2013) and more recently works by Napaumpaipom*, et al.* (2013), Ullman*, et al.* (2019a); Ullman*, et al.* (2019b) and Reis*, et al.* (2020) using commercially available passive acoustic feeding systems indicated better shrimp growth when compared to less technologically advanced solutions such as handfeeding or timer feeders.

Although higher technological solutions are available and proven to be very efficient, many producers are not yet ready to invest in the hardware, software and training that is required. Therefore, it is important to adapt timer feeder protocols to improve growth response. Ullman*, et al.* (2019a) reported better growth response for increasing feed inputs for timer feeders offering shrimp 6 meals a day. Reis*, et al.* (2020) indicated that it was possible to ever reduce the productivity gap between timer feeder protocols and passive acoustic systems by increasing feed inputs and the number of meals (32 meals a day). Although the various advantages of multiple meals are widely reported and understood, there is very limited information concerning any potential preferences for feeding schedule (i.e. day or night). A recent study by Nunes*, et al.* (2019) compared shrimp growth when fed manually and automatically but did not report differences within treatments that fed multiple meals automatically during the day compared to a similar treatment that fed around the clock.

Previous work Ullman *et al.* (2019) and Reis *et al.* (2020) evaluated feed inputs using timer feeders were optimized for 12 hr day. The objective of the present work was to further optimize feeding protocols by evaluating the efficacy of several combinations of feeding rates, schedules (i.e. daytime, nighttime and 24h) and number of meals under semi-intensive production conditions.

## 2. Material and Methods

This study was performed at the Alabama Department of Conservation and Natural Resources, Claude Peteet Mariculture Center, Gulf Shores, Alabama (Jannathulla*, et al.*). Pacific white shrimp (*L. vannamei*) larvae were obtained from American Penaeid (St. James City, FL, USA), acclimated and nursed in a greenhouse system for 14 days. Juvenile shrimp (0.03 g) were then stocked into 16 outdoor, 0.1 ha ponds at 35 shrimp/$m^2$ per square meter and stocked at 37 shrimp/tank in 32, 800 L tanks in green-water recirculation greenhouse system.

### *2.1 Outdoor Pond Trial*

Shrimp were offered two diets produced by Zeigler Bros. Inc (Garners, PA, USA): 1.5-mm commercial diet (40 percent crude protein, 9 percent crude lipids) for the first three weeks, and thereafter 2.4-mm 36 percent protein, 8 percent lipid fishmeal free extruded diet (Table 1). All treatments were fed the same amount of feed evenly distributed twice a day during the first 30 days of production. Thereafter the four feed management protocols were employed using automatic feeders.

Three protocols utilized feed applied daily using a timer based feeder (BioFeeder S.A., Guayaquil, Ecuador) which fed 34 meals evenly spread throughout the following schedules: Daytime (0700h-1900h), Nighttime (1900h-0700h) and 24h (Figure 1). Both day and nighttime feeding utilized Standard Protocol for Timer Feeders (SPTF) whereas for the 24 hr treatments feed inputs were increased by 15% from day 75 to 90. The SPTF (Figure1) was developed from previous data of growth cycles conducted and reported by Davis*, et al.* (2006). The SPTF assumed a feed conversion ratio (FCR) of 1.2 and weekly growth of 1.68 from day 30 to 45, 1.88 from day 46 to 60 and 2.08 from day 61 to day 90. Estimation of population was based on a 1.5 percent weekly mortality during the grow-out period.

A fourth treatment utilized on-demand passive acoustic feedback feeding system that integrates shrimp acoustic input through a hydrophone inside the pond and feeds based on acoustic response (AQ1 Feeder, AQ1 Systems Pty. Ltd., Tasmania, Australia). This system was initiated 30 days into the production cycle and was set to feed *ad libitum* up to a maximum of 16 kg per day (160 kg/ha/day) in order to minimize water quality degradation to critical levels. This system was also equipped with a dissolved oxygen (DO) sensor that stopped feeding and turned on aerators when below 3mg/L. Each treatment was replicated in four ponds.

Shrimp were sampled weekly from day 17 through the remaining weeks of the production stage using a cast net (1.52 m radius and 0.96 cm mesh) to collect approximately 60 individuals per pond. Pond sampling enabled growth assessment and inspection for general health. Ponds were monitored (DO, temperature, salinity, and pH) at least three times a day, at sunrise (5:00 to 5:30 a.m.), afternoon (2:00 to 2:30 p.m.) and sunset (7 to 8 p.m.), using a YSI ProPlus Meter (Yellow Springs Instrument Co., Yellow Spring, OH, USA). In order to try to maintain DO above 3 mg/L, all ponds were supplied with one 2-HP Aire-O2 (Aire-O2, Aeration Industries International, Inc., Minneapolis, MN, USA) as a main source of mechanical aeration and one 1-HP Air-O-Lator (Kansas City, MO, USA) for backup and/or supplemental aeration as needed.

Ponds were harvested over three days at the end of the 13-week culture period. Ponds were partially drained and the night before harvest the water level was reduced to about one third and aeration was provided using the surface aerator. On the day of harvest, the remaining water was drained and the shrimp were pumped out of the catch basin using a hydraulic fish pump equipped with a 25 cm diameter suction pipe (Aqua-Life pump, Magic Valley Heli-arc and Manufacturing, Twin-Falls, Idaho, USA). The pump was placed in the catch basin and shrimp were pumped, de-watered, and collected into a hauling truck. Shrimp were then rinsed, weighed in bulk, and 150 were randomly selected to measure individual weights and determine the size distribution. A subsample of these shrimp were collected and frozen for subsequent analysis. Whole body proximate with minerals analysis of the shrimp was performed by Midwest Laboratories (Omaha, NE, USA). The partial value was calculated by subtracting the feed costs from the production value as calculated from the Undercurrent News Portal for weeks 31 to 38 of 2019 and the size distribution of shrimp produced. The feed prices were $1.72/kg for the starter diet and $1.09/kg for the grower diet.

*2.2 Green-water Tank Trial*

A 11-week growth trial was performed in 32, 800 L tanks (0.8 $m^2$) in green-water recirculation greenhouse system, stocked at 30 shrimp/tank (3.55 ± 0.16 g, 37 shrimp/$m^2$). Water added to the system was pumped from a semi-intensive shrimp production pond for 2 hr per day at a rate of 8 L/min to provide a daily water exchange of 5% and a source of natural productivity from the pond.

Feeding protocols for the tanks mirrored those of the ponds study. Shrimp in all tanks were hand-fed the same feed amount four meals a day for the first 3 weeks after which a set of 8 treatments was designed to compare growth performance at different feeding schedules and feed levels. At this point, 24h belt-feeders (FIAP Belt-Feeder Pro 3kg 24h, FIAP GmbH, Ursensollen, Germany) were used to deliver feed. Five treatments were fed during daytime hours from 0700-

1900h with different number of equally spread feedings per day (4, 6 8 or 12 meals). One treatment was fed at night from 1900-0700h and two treatments fed a different daily ration over 24 hours. Tanks were fed a standard ration (SR) based on the SPTF that adjusted assuming a doubling of weight weekly until reaching 1.3g and a feed conversion ratio (FCR) of 1.2. The SR also assumed a weekly growth of 1.68 for weeks 4 and 5, 1.88 for weeks 6 and 7 and 2.08 from week 8 to 11. No adjustments were made for mortality. One treatment (SR 115 24h) gradually reached 115% at week 7 and the SR175 Day treatment only reached 115% of the SR during the last two weeks. All treatments were fed using belt feeders using lines of feed, except 4 meals per day treatments which were hand-fed.

Tanks were not sampled and feeding inputs were adjusted based on estimated growth predicted by the feeding protocol previously detailed. Tanks were monitored (DO, temperature, salinity, and pH) twice daily (7:00 to 7:30 a.m. and 3:00 to 3:30 p.m.). TAN was measured twice weekly with ion selective probe and nitrate and nitrite measured once weekly using Lamotte test kits.

*2.3 Statistical Analysis*

Statistical analysis of the growth data was conducted with SAS 9.4 (SAS Institute, Cary, NC, USA) to perform a one-way analysis of variance to determine significant difference (p-value $< 0.05$) among treatments in both trials. The assumptions for ANOVA were met. Student-Newman-Keuls multiple range test was used to determine differences among treatments. An additional linear regression test was conducted for select treatments SR160 in the tank trial to assess a potential linear correlation between the number of meals and average final individual weight. Contrast analysis on selected pairs was also conducted for four treatment pairs in the tank trial. Shrimp prices used in economic analysis were obtain from a personal communication in December 2020 for the various classes of whole fresh shrimp.

For the pond trial one replicate of AQ1 Systems treatment was eliminated from the data set due to electrical failure of the aeration system that ultimately led to nearly almost complete loss of shrimp in that pond. For the tank trial one replicate of the 4 Meals SR Day and one replicate of 6 Meals SR Day treatments was excluded due to human error in tank management. Exclusions are indicated in the respective tables.

## 3. Results

### *3.1 Outdoor Pond Trial*

During this trial, main water quality parameters were maintained within the typical range for shrimp production (Boyd*, et al.*, 1992) (Table 2). Parameters such as DO, temperature, pH and salinity were similar across treatments. TAN was numerically higher for AQ1 treatment which is likely the result of higher feed inputs. Identical results were reported by Jescovitch*, et al.* (2018) when comparing hand-feeding and timer feeder protocols with acoustic feedback feeders. Regardless, there is no clear indication this had any negative impact on shrimp growth.

The growth results are presented in Table 3. We observed a significant difference in weekly growth of the shrimp between those maintained on the AQ1 treatment (2.28 g/wk) and shrimp fed at night or on the 24hr treatment (1.91 and 1.89g/wk, respectively). However, the weekly growth of shrimp on the daytime feeding protocol was similar to shrimp maintained on the other 3 treatments (2.01 g/wk). This was also the case for final individual weights of shrimp maintained on the AQ1 treatment (29.65g) which was significantly higher than final mean weights of shrimp reared on either the night time and 24hr feeding schedule (24.81 and 24.56 g, respectively). Final mean weights of shrimp maintained on the Daytime treatment (26.13 g) was similar to those of the other 3 treatments There was a statistically higher feed input for AQ1 treatment ($P < 0.0001$): however, no significant differences in FCR ($p=0.8951$), which ranged from 0.99 to 1.03, were observed. Even though the final weight and weekly gain was similar for shrimp maintained on the AQ1 and daytime protocols the yield was significantly higher for shrimp maintained with AQ1 (800.63 kg/pond) which is likely due to the numerically higher survival and larger final weight of the shrimp. Nevertheless there was no significant difference in survival ($p=0.4123$) which ranged from 69.15 to 77.00%.

Average individual weights of the shrimp from weekly cast net sampling is presented in Figure 2. We did not find statistical differences among feed inputs for automatic feeders treatments, both nighttime and 24 h feeding treatments resulted in numerically lower total feed inputs as compared to the daytime treatment. Lower feed inputs were a consequence of skipping meals at night to avoid oxygen depletion beyond our mechanical aeration capacity. We also found that shrimp fed during nighttime and 24 hours grew slower (g/wk) hence resulting in smaller individuals than shrimp fed with AQ1. Since there seems to be little correlation between feeding schedule and growth it is reasonable to believe that the differences between AQ1 and both of those timer feeder treatments is related to the overall feed inputs. No statistical differences in proximate composition were found among shrimp reared on the various treatments (Table 4).

### *3.2 Green-water Tank Trial*

As in the pond trial described above, main water quality parameters were kept within typical range for shrimp production (Boyd*, et al.*, 1992) throughout the green-water tank trial (Table 2). Unlike in the outdoor ponds, the tanks used in this trial had continual aeration reducing the occurrence of low DO, and water was circulated through the system minimizing any water quality differences.

Shrimp growth throughout the trial is summarized in Table 5. Results indicate a positive response to increased number of meals more than a specific schedule. Regardless of the feeding schedule, increasing number of meals resulted in larger individuals (Figure 4). Contrast analysis of specific treatments (Table 5) showed statistically significant larger individuals and weight gain ($p$=0.04 and 0.04, respectively) when animals were fed 12 meals through 24h but feed inputs were increased from SR to SR 115%.

Regression analysis of average final individual weights revealed a positive response among treatments for any growth parameters being fed the same amount during daytime for increasing number of meals (p-value=0.005, $R^2$=0.4941). Data from this trial (Table 5) however indicates numerical differences with more meals corresponding to production of larger animals but no statistical differences were found.

## 4. Discussion

Commercially available feeds for shrimp production are nutritionally appropriate and account for the largest variable cost on a farm. Maximized return on feed investment is only possible through efficient feeding protocols. Shrimp have been traditionally offered 2 to 4 meals a day by hand-dispersion using feed tables or feed trays to manage inputs. These techniques are very human-labor dependent. However, it has been widely reported and accepted that shrimp growth is favored through regular intake of small quantities of feed (Carvalho*, et al.*, 2006; Napaumpaipom*, et al.*, 2013; Reis*, et al.*, 2020; Ullman*, et al.*, 2019a; Ullman*, et al.*, 2019b). Automatic feeders have been a useful tool to address this issue for they not only favor shrimp growth by increasing the number of meals but also positively effect economic balance by reducing or shifting labor requirements. Ullman *et al.* (2019a,b) reported significantly better growth response for shrimp fed similar amounts but fed 6 meals a day as compared with traditional two meals a day. In both trials, increasing the number of meals also allowed higher feed inputs. Within automatic feeders, on-demand passive acoustic feedback feeding systems are reported to outperform more basic timer-feeders (Napaumpaipom*, et al.*, 2013; Reis*, et al.*, 2020; Ullman*, et al.*, 2019a; Ullman*, et al.*, 2019b). However, Reis *et al* (2020) proposed that it is possible to reduce the performance gap between passive acoustic feedback systems to timer-feeders optimizing feed inputs for timer feeders. Following results reported by Reis*, et al.* (2020) suggesting that feed inputs gradually become a growth limiting factor. The standard feeding protocol for automatic feeders of the present study took that into consideration by gradually scaling up feed rates. Those same results were used as the basis for the establishment of feeding protocols (Figure 1) using in both trials reported in this publication (Figure 1).

Pond production results from this study (Table 3) are aligned with Napaumpaipom*, et al.* (2013), Ullman*, et al.* (2019a), Ullman*, et al.* (2019b), and Reis*, et al.* (2020) that reported overall higher productivity with utilization of the AQ1 System acoustic feedback system. Application of acoustic feeding system resulted in higher yields, and low FCR despite considerably higher feed inputs. Feed inputs, yield and FCR for the presented research are plotted in Figure 3 for another perspective of how the application passive feedback acoustic feeding systems results in feed inputs but also larger yields and low FCR, although not significantly lower. An important consequence of the application of AQ1 that also favored previously mentioned production parameters was the reasonably higher survival achieved in ponds in which this technology was deployed.

More than adequate growth parameters reported in this study for shrimp fed through timer-feeder-adjusted protocols further confirms that production efficiency can be improved through higher number of meals when daily rations are increased as well. This conclusion is supported by previous research (Ullman*, et al.*, 2019b) and the response of shrimp in the tanks trial where growth improved with the number of feedings. Nevertheless, no statistical differences were found among timer-feeder treatments that were fed identical feeding rates throughout opposite periods of the day (i.e. day or night) or even a slightly higher preplanned rate around the clock. Feeding increases respiratory activity of the animals and when oxygen is low feed inputs should be reduced or punctually eliminated to minimize respiration (i.e. oxygen consumption). Skipping meals is a common and appropriate management practice in pond aquaculture to avoid oxygen depletion. Exclusive night feeding increases the likelihood of necessity to make adjustments (i.e. reduce) in feed inputs as it is also when oxygen is naturally lowest due to algal communities shifting from photosynthesis to aerobic respiration. Low oxygen levels require the initiation of mechanical aeration hence in this work it is possible to associate lower feed inputs with higher electrical consumption for similar treatments (Table 3). Thus, limited aeration capacity in all ponds led to slight reductions in feed inputs as result of occasional skipping feedings during nighttime to avoid severe oxygen depletion that might compromise the crop. Consequent lower overall feed inputs unsurprisingly resulted in smaller animals, lower yield and weekly growth, but similar survival and FCR. None of these differences between timer feeder treatments in an outdoor pond setup were substantial enough to conclude that a specific feeding schedule (day vs night or 24 hr a day) favored shrimp growth. In fact, feeding during night-time required more frequent use of mechanical aeration as measured by electrical demand, it is likely that any potential benefits from feeding during this period is offset by electricity costs as well as higher risk of severe oxygen depletion.

The results of the green-water indoor tank trial validate the growth performance of shrimp in outdoor ponds report in this and other studies. Production parameters for the tank trials (Table 4) suggest better growth performance when feed inputs and number of meals are both increased. This is particularly evident when we look at the treatment that combined the most meals with the higher feed inputs (Figure 4). A regression analysis that compared all treatments fed SR 160 during daytime did find a positive response between the number of meals and growth performanc (p-value=0.005, $R^2$=0.4941). Furthermore, no significant differences were found among both treatments feeding the same amount in 12 meals throughout different schedules. However, contrast analysis did reveal better growth for the treatment feeding 12 meals in 24h with SR 115% by opposition to SR. Van*, et al.* (2017) and Roy*, et al.* (2012) reported similar growth for shrimp reared in green-water tanks and fed slight (10%) variations of a SPF with identical assumptions as the one presented in this publication.

Results of this experiment confirm that improvement in shrimp growth can be achieve though a combination of higher feed inputs and number of meals. The number of meals is closely related to daily feed amount as at a given number of meals there is a maximum feed rate that results in efficient feed delivery and shrimp growth. In order to increase the feeding rate beyond that point the number of meals must be increased as well. This conclusion falls in line with our earlier explanation as well as studies by Ullman*, et al.* (2019a); Ullman*, et al.* (2019b), and Reis*, et al.* (2020) under outdoor pond production conditions.

Contrary to the outdoor ponds, all tanks were provided constant aeration therefore eliminating its potential impact in growth as a limiting factor. For this green-water tank trial we did not find the feeding schedule to have a determining impact in shrimp growth which follows in line with our conclusions for the pond trial. Our conclusion also fall in line with results reported by Nunes, *et al.* (2019) who did not find differences for shrimp fed similar feed inputs through automatic feeders just during the day or both day and night. The results of this trial ultimately suggest that the number of meals and feed input were more impactful to animal growth than a specific feeding schedule. The increased necessity for mechanical aeration in treatments that fed during night-time translated in higher electricity costs urges caution when establishing feed plans that disperse meals during such period. In short, the utilization of automatic feeders has allowed faster growth resulting in shorter production cycles which ultimately results in higher yields. These trial results further validate widely reported low (< 1.5) feed conversion ratios (FCR's) for well managed feeding protocols for shrimp feed with automatic feeders in semi-intensive ponds across the board (Figure 3).

## 5. Conclusion

The results of the pond trial confirm and expand on previously published work that demonstrated appreciable increase in productivity and value of shrimp produced in semi-intensive outdoor ponds through application of on-demand acoustic feeding systems. While the intrinsic nature of a feedback technology is to feed on demand in real time, it is virtually impossible that any timer feeder will be as efficient as a real-time passive feedback system. Adjustments to feeding rates and number of meals in timer feeders were instrumental in reducing the performance gap between acoustics based and the timer feeder treatments in outdoor ponds. The results in both outdoor and indoor systems also lead us to conclude that while being described as night feeders in the while *L. vannamei* do not seem to have a preferred feeding schedule in captivity as long as environmental conditions and overall feed rates are appropriate. However, higher electric consumption associated with increased mechanical aeration in ponds fed during nigh time suggests that this practice must be carefully planned in order to prevent severe oxygen depletion that could compromise the crop.

The continuous development of very efficient technologies for shrimp production, from feeders to water quality sensors to farm management software is undeniable. Yet, no one individual technology or device is able to solve every production or feed management issue in semi-intensive shrimp aquaculture. Biometric, environmental and financial improvements are indeed within reach when feeding planning considers both quality records from previous cycles as well as frequent revision based on real-time survival, growth and feed response estimations.

6. References


Boyd, C.E., Tucker, C.S., 1992. Water quality and pond soil analyses for aquaculture. Alabama Agricultural Experiment Station, Auburn University.

Carvalho, E.A., Nunes, A.J., 2006. Effects of feeding frequency on feed leaching loss and grow-out patterns of the white shrimp *Litopenaeus vannamei* fed under a diurnal feeding regime in pond enclosures. Aquaculture. 252, 494-502.

Davis, D.A., Amaya, E., Venero, J., Zelaya, O., Rouse, D.B., 2006. A case study on feed management to improving production and economic returns for the semi-intensive pond production of *Litopenaeus vannamei*, Avances en Nutrición Acuícola VIII. Memorias del Octavo Simposio Internacional de Nutrición Acuícola. Universidad Autonoma de Nuevo Monterrey, Nuevo León, México.

Jannathulla, R., Rajaram, V., Kalanjiam, R., Ambasankar, K., Muralidhar, M., Dayal, J.S., 2019. Fishmeal availability in the scenarios of climate change: Inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources. Aquac. Res. 50, 3493-3506.

Jescovitch, L.N., Ullman, C., Rhodes, M., Davis, D.A., 2018. Effects of different feed management treatments on water quality for Pacific white shrimp *Litopenaeus vannamei*. Aquac. Res. 49, 526-531.

Martinez-Cordova, L.R., Torres, A.C., Porchas-Cornejo, M.A., 2003. Dietary protein level and natural food management in the culture of blue (*Litopenaeus stylirostris*) and white shrimp (*Litopenaeus vannamei*) in microcosms. Aquaculture Nutrition. 9, 155-160.

McTigue, T.A., Feller, R.J., 1989. Feeding of juvenile white shrimp Penaeus setiferus: periodic or continuous? Marine Ecology Progress Series. 52, 227-233.

Napaumpaipom, T., Chuchird, N., Taparhudee, W., 2013. Study on the Efficiency of Three Different Feeding Techniques in the Culture of Pacific White Shrimp (*Litopenaeus vannamei*). Journal of Fisheries and Environment. 37, 8-16.

Nunes, A.J.P., Sabry-Neto, H., da Silva, F.H.P., de Oliveira-Neto, A.R., Masagounder, K., 2019. Multiple feedings enhance the growth performance and feed efficiency of juvenile *Litopenaeus vannamei* when fed a low-fish meal amino acid-supplemented diet. Aquaculture International. 27, 337-347.

Reis, J., Novriadi, R., Swanepoel, A., Guo, J.P., Rhodes, M., Davis, D.A., 2020. Optimizing feed automation: improving timer-feeders and on demand systems in semi-intensive pond culture of shrimp *Litopenaeus vannamei*. Aquaculture. 519.

Reymond, H., Lagardere, J.P., 1990. Feeding Rhythms And Food Of *Penaeus japonicus* Bate (CRUSTACEA, Penaeidae) In Salt-Marsh Ponds - Role Of Halophilic Entomofauna. Aquaculture. 84, 125-143.

Roy, L.A., Davis, D.A., Whitis, G.N., 2012. Effect of feeding rate and pond primary productivity on growth of *Litopenaeus vannamei* reared in inland saline waters of West Alabama. North American Journal of Aquaculture. 74, 20-26.

Santos, A.D., Lopez-Olmeda, J.F., Sanchez-Vazquez, F.J., Fortes-Silva, R., 2016. Synchronization to light and mealtime of the circadian rhythms of self-feeding behavior and locomotor activity of white shrimps (*Litopenaeus vannamei*). Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology. 199, 54-61.

Smith, D.V., Tabrett, S., 2013. The use of passive acoustics to measure feed consumption by Penaeus monodon (giant tiger prawn) in cultured systems. Aquacultural Engineering. 57, 38-47.

Tacon, A.G.J., Forster, I.P., 2003. Aquafeeds and the environment: policy implications. Aquaculture. 226, 181-189.

Ullman, C., Rhodes, M.A., Davis, D.A., 2019a. Feed management and the use of automatic feeders in the pond production of Pacific white shrimp *Litopenaeus vannamei*. Aquaculture. 498, 44-49.

Ullman, C., Rhodes, M., Hanson, T., Cline, D., Davis, D.A., 2019b. Effects of Four Different Feeding Techniques on the Pond Culture of Pacific White Shrimp, *Litopenaeus vannamei*. Journal of the World Aquaculture Society. 50, 54-64.

Van, T.P.T.H., Rhodes, M.A., Zhou, Y., Davis, D.A., 2017. Feed management for Pacific white shrimp Litopenaeus vannamei under semi-intensive conditions in tanks and ponds. Journal of Aquaculture Research. 48, 5346-5355.

Wassenberg, T.J., Hill, B.J., 1987. Natural diet of the tiger prawns *Penaeus esculentus* and *P. semisulcatus* Australian Journal of Marine and Freshwater Research. 38, 169-182.

Table 1 – Formulation of the 2.4mm 35% protein extruded sinking feed used in both shrimp production trials produced by Zeigler Bros Inc. As the diets were produced commercially the sources are of ingredients and composition of the premixes are not reported.

| Ingredient | g/100g as is |
| --- | --- |
| Solvent Extracted Soybean Meal | 50.0 |
| Whole Wheat | 23.1 |
| Poultry-by Product Meal | 8.0 |
| Corn Gluten (60% protein) | 8.0 |
| Dicalcium phosphate | 3.13 |
| Fish Oil | 5.0 |
| Bentonite | 1.5 |
| Lecithin | 1.0 |
| Vitamin Premix | 0.12 |
| Mineral Premix | 0.12 |
| Stable C (35% activity) | 0.02 |
| Copper Sulfate | 0.01 |

Table 2 – Summary of water quality parameters observed over the 13-wk. growth trial in ponds and the 11-wk growth trial in green-water tanks. Values (n=4) are presented as mean ± standard deviation and maximum and minimum value are presented in parenthesis.

| | Pond production | | | | Green-water System |
|---|---|---|---|---|---|
| | SPTF Day | SPTF Night | SPTF 115 24H | AQ1 | |
| Morning DO[a] (mg/L) | 3.52 ± 1.22 (1.02 , 16.56) | 3.78 ± 1.11 (0.16 , 12.81) | 3.76 ± 0.89 (1.23 , 6.58) | 3.36 ± 0.97 (0.23 , 6.26) | |
| Afternoon DO[a] (mg/L) | 11.52 ± 2.57 (5.47 , 19.55) | 11.31 ± 2.33 (5.11 , 17.02) | 11.1 ± 2.44 (2.99 , 16.24) | 10.65 ± 2.35 (4.28 , 16.87) | |
| Night DO[a] (mg/L) | 9.75 ± 2.84 (2.04 , 18.39) | 10.28 ± 2.97 (2.51 , 17.14) | 9.27 ± 2.83 (1.29 , 16.89) | 9.48 ± 2.61 (3.07 , 16.48) | |
| Daily DO (mg/L) | | | | | 6.31 ± 0.86 (2.1 , 7.87) |
| Temperature (ºC) | 32 ± 1.64 (27 , 36.3) | 31.9 ± 1.56 (27.5 , 36.1) | 31.9 ± 1.57 (27.6 , 35.3) | 31.9 ± 1.5 (26.8 , 35.7) | 28.88 ± 1.50 (24.9 , 32.7) |
| pH | 8.3 ± 0.54 (6.78 , 9.39) | 8.3 ± 0.52 (6.55 , 9.31) | 8.24 ± 0.54 (6.74 , 9.24) | 8.21 ± 0.50 (6.73 , 9.33) | 7.75 ± 0.37 (6.76 , 8.54) |
| Salinity (g/L) | 14.39 ± 2.14 (11.15 , 22.37) | 14.77 ± 1.26 (12.15 , 20.18) | 15.55 ± 1.43 (12.2 , 23.27) | 15.55 ± 1.23 (13.5 , 21.45) | 17.20 ± 0.29 (14.81 , 17.91) |
| TAN[b] (mg/L) | 0.42 ± 0.96 (<0.001, 4.0) | 0.46 ± 1.11 (<0.001 , 5.0) | 0.83 ± 1.54 (<0.001 , 6.0) | 1.32 ± 2.27 (<0.0001, 10.0) | 0.05 ± 0.09 (0 , 0.3) |
| $NO_2$ | | | | | 0.33 ± 0.34 (0 , 0.99) |
| $NO_3$ | | | | | 8.8 ± 9.02 (4.4 , 35.2) |

[a]DO – Dissolved Oxygen
[b] TAN - Total Ammonia Nitrogen

Table 3 – Production results of *L. vannamei* reared in 0.1 ha production ponds over a 13-wk culture period. Nursed shrimp (0.05 g) were stocked at a density of 35 shrimp/m$^2$. Values within a column with different superscripts are significantly different based on Student-Newman-Keuls test.

| | Final weight (g) | Survival (%) | Weekly Gain (g/wk) | Yield (kg) | Feed Input (kg) | FCR | Feed Cost ($/ha) | Shrimp Value ($/ha) | Partial Income ($/ha) | Electric (kWh/ha) |
|---|---|---|---|---|---|---|---|---|---|---|
| SPTF Daytime | 26.13$^{ab}$ | 69.15 | 2.01$^{ab}$ | 625.38$^{b}$ | 641.67$^{b}$ | 1.03 | 7,495.7$^{b}$ | 32,268.4$^{b}$ | 25,354.3$^{b}$ | 21,060$^{bc}$ |
| SPTF Nighttime | 24.81$^{b}$ | 69.55 | 1.91$^{b}$ | 602.94$^{b}$ | 613.58$^{b}$ | 1.01 | 7303.9$^{b}$ | 27,283.9$^{b}$ | 20,582.3$^{b}$ | 26,730$^{a}$ |
| SPTF 115 24h | 24.56$^{b}$ | 71.77 | 1.89$^{b}$ | 615.90$^{b}$ | 617.71$^{b}$ | 0.99 | 7270.9$^{b}$ | 28,034.0$^{b}$ | 21,302.0$^{b}$ | 24,678$^{ab}$ |
| AQ1 System[1] | 29.65$^{a}$ | 77.00 | 2.28$^{a}$ | 800.63$^{a}$ | 790.10$^{a}$ | 0.99 | 8,826.3$^{a}$ | 39,624.8$^{a}$ | 31,446.6$^{a}$ | 18,320$^{c}$ |
| P-value | 0.0500 | 0.4123 | 0.0500 | 0.0057 | <0.0001 | 0.8951 | <0.0001 | 0.0053 | 0.0083 | 0.0025 |
| PSE[2] | 1.1200 | 3.2054 | 0.0862 | 30.8977 | 13.9822 | 0.0355 | 154.21 | 1884.1 | 1677.0 | 1155.8 |

[1]n=3
[2]PSE: Pooled Standard Error

Table 4 – Proximate composition[1] of *L. vannamei* stocked at a density of 35 shrimp/$m^2$ and reared in 0.1 ha production ponds over a 13-wk culture period using various feed management strategies. Values within a column with different superscripts are significantly different based on Student-Newman-Keuls test. No significant differences were observed among treatments.

| % dry matter | SPTF Daytime | SPTF Nighttime | SPTF 115 24h | AQ1 | *P*-value | PSE[2] |
|---|---|---|---|---|---|---|
| Dry Matter (as is) | 26.50 | 26.08 | 27.20 | 26.03 | 0.2833 | 0.420 |
| Protein | 74.75 | 74.80 | 73.88 | 75.07 | 0.7065 | 0.983 |
| Fat | 7.38 | 7.26 | 7.57 | 7.40 | 0.9407 | 0.55 |
| Ash | 9.98 | 10.33 | 11.03 | 11.03 | 0.5428 | 0.718 |
| Sulfur | 0.78 | 0.80 | 0.77 | 0.78 | 0.2420 | 0.014 |
| Phosphorus | 1.33 | 1.34 | 1.28 | 1.32 | 0.7098 | 0.047 |
| Potassium | 1.23 | 1.27 | 1.21 | 1.23 | 0.3609 | 0.025 |
| Magnesium | 0.27 | 0.28 | 0.29 | 0.30 | 0.6011 | 0.017 |
| Calcium | 2.68 | 2.71 | 2.53 | 2.81 | 0.8649 | 0.222 |
| Sodium | 0.77 | 0.73 | 0.71 | 0.69 | 0.8272 | 0.034 |
| Iron (ppm) | 87.33 | 80.38 | 80.40 | 92.03 | 0.9806 | 15.317 |
| Manganese (ppm) | 4.63 | 4.10 | 4.13 | 5.70 | 0.5801 | 21.106 |
| Copper (ppm) | 115.00 | 123.25 | 124.75 | 105.33 | 0.2486 | 10.175 |
| Zinc (ppm) | 64.98 | 65.90 | 63.60 | 62.97 | 0.3439 | 6.180 |

[1]analysed by Midwest Laboratories (Omaha, NE, USA)
[2]PSE: Pooled Standard Error

Table 5 – Performance of juvenile shrimp (3.55 ± 0.16 g) reared at 37 shrimp/$m^2$ over an 11-week culture period and offered feed at various rations. Mean values (n=4) within a column with different superscripts are significantly different based on Student-Newman-Keuls test.

| | Final Weight (g) | Survival (%) | Weight Gain (g/wk) | Final Biomass (g) | FCR[1] |
|---|---|---|---|---|---|
| 4 Meals SR Day[2] | 15.10$^{a}$ | 94.5$^{a}$ | 1.36$^{b}$ | 428.3$^{ab}$ | 1.35 |
| 6 Meals SR Day[2] | 16.00$^{a}$ | 78.9$^{b}$ | 1.44$^{a}$ | 379.3$^{a}$ | 1.52 |
| 8 Meals SR Day | 16.88$^{ab}$ | 86.7$^{ab}$ | 1.52$^{ab}$ | 438.8$^{ab}$ | 1.31 |
| 12 Meals SR Day | 16.82$^{ab}$ | 86.7$^{ab}$ | 1.52$^{ab}$ | 437.9$^{ab}$ | 1.32 |
| 6 Meals SR Night | 15.92$^{a}$ | 86.7$^{ab}$ | 1.44$^{a}$ | 414.2$^{ab}$ | 1.40 |
| 12 Meals SR 24h | 16.81$^{ab}$ | 86.7$^{ab}$ | 1.52$^{ab}$ | 436.6$^{ab}$ | 1.33 |
| 12 Meals SR 115 24h | 18.41$^{b}$ | 88.4$^{ab}$ | 1.67$^{a}$ | 485.8$^{b}$ | 1.33 |
| 6 Meals SR 115 Day | 16.76$^{ab}$ | 87.5$^{ab}$ | 1.51$^{ab}$ | 439.9$^{ab}$ | 1.34 |
| P-value | 0.0488 | 0.2001 | 0.0440 | 0.042 | 0.2562 |
| PSE[3] | 0.312 | 1.930 | 0.028 | 12.203 | 0.040 |

| Paired Contrast | | | (*P*-value) | | |
|---|---|---|---|---|---|
| 6 Meals SR: Day[2] vs Night | 0.914 | 0.077 | 0.909 | 0.201 | 0.178 |
| 6 Meals Day: SR vs SR115 | 0.264 | 0.825 | 0.272 | 0.307 | 0.464 |
| SR Day: 12 vs 24 hr | 0.987 | 0.995 | 1.000 | 0.958 | 0.976 |
| 12 Meals 24 hr: SR vs SR 115 | 0.039 | 0.672 | 0.037 | 0.057 | 0.783 |

[1]FCR: Feed Conversion Ratio
[2]n=3
[3]PSE: Pooled Standard Error

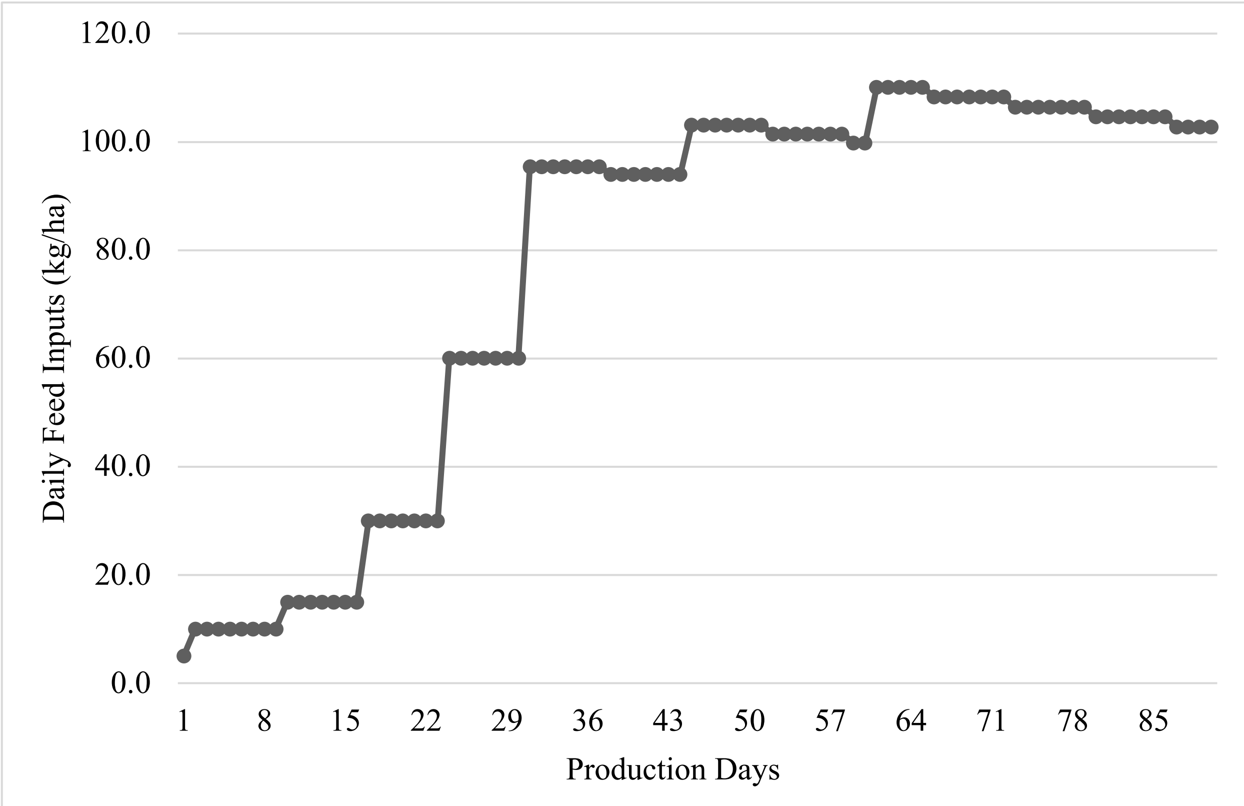


Figure 1 - Preplanned Daily Feed Inputs for SPTF in semi-intensive outdoor shrimp pond production throughout a 90 day culture period

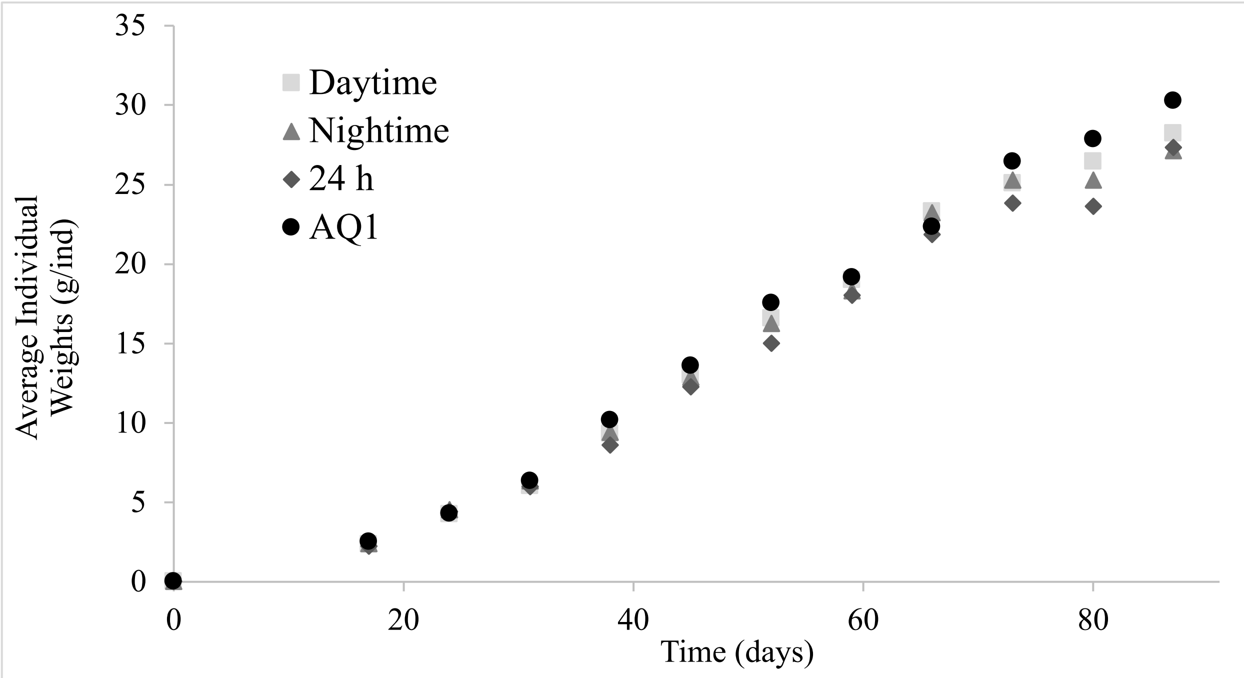

Figure 2 – Average individual weight of weekly sampled individuals (cast-net) per treatment through a 90 day semi-intensive outdoor shrimp pond production cycle

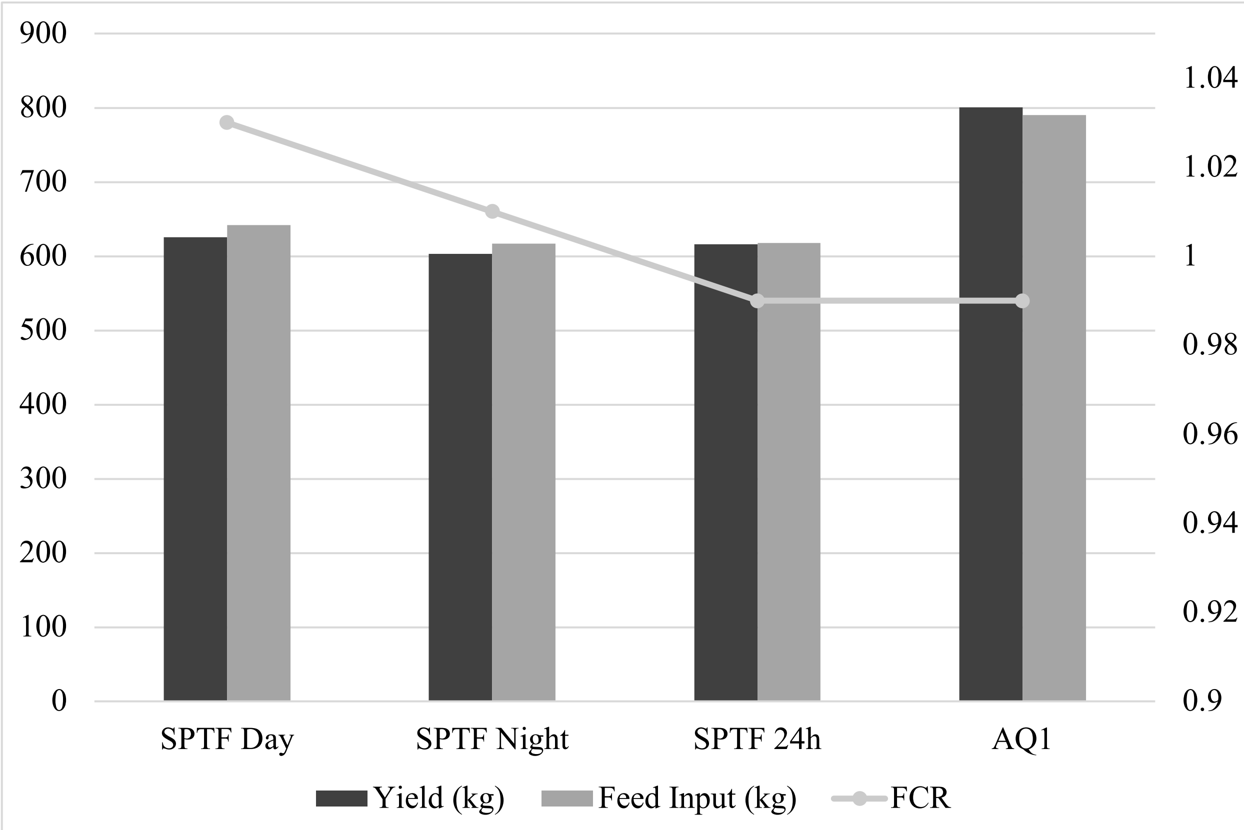

Figure 3 - Yield and cumulative feed input per treatment at the end of a 90 days semi-intensive outdoor shrimp pond production cycle

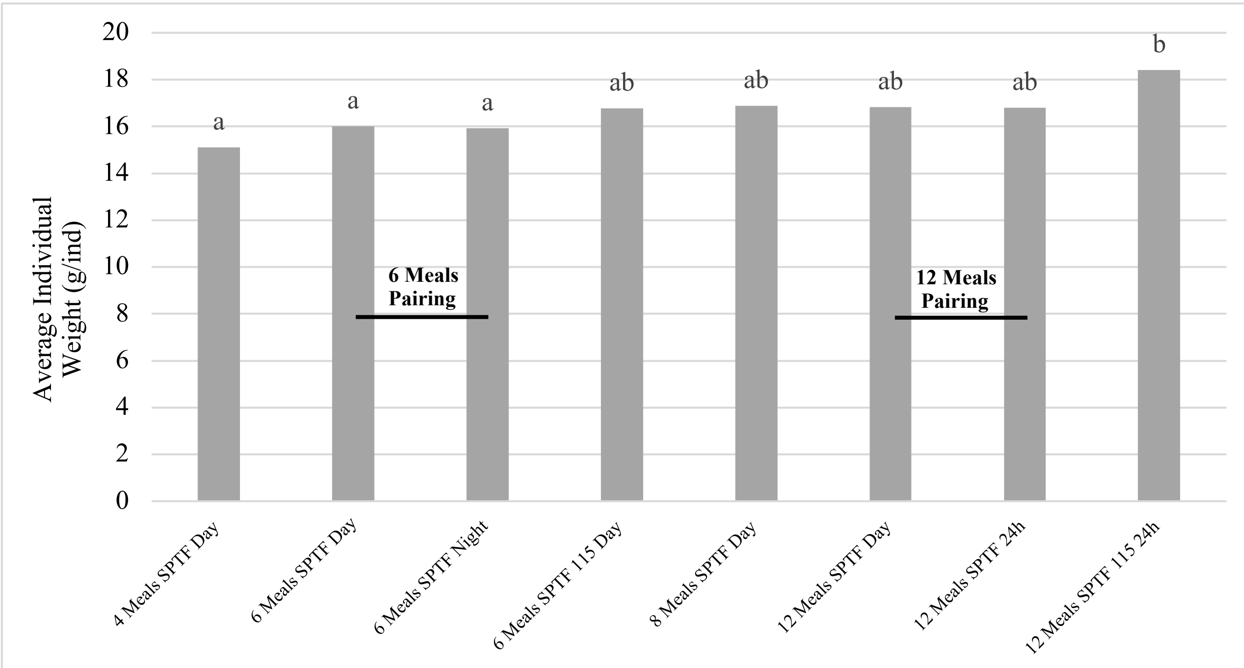

Figure 4 - Average final individual weight for indoor greenwater tank shrimp production cycle

CHAPTER IV

# PASSIVE ACOUSTIC FEEDERS AS A TOOL TO ASSESS FEED RESPONSE AND GROWTH IN SHRIMP POND PRODUCTION


Abstract

Shrimp production has been one of the most important sectors of aquaculture for the last few decades for both its market value and consumer acceptance. The majority of shrimp feeding protocols in typical production setups rely on a combination of feed trays and predetermined feed plans which do not account for real time consumption or feed preferences. However, for the last decade, development of passive acoustic monitoring has allowed a much more direct measurement of shrimp feed intake by capture and integration of clicking sounds produced by shrimp while eating. Integrating acoustic responses with automated feeding systems has allowed the development of on demand feeding for shrimp. Hence, this technology is a potential tool to help understand feed preferences when the feeding protocol is based on real time demand for feed rather than predetermined quantities. Building on previous research, the goal of this trial was to use passive feedback acoustic feeders as a tool to evaluate whether shrimp preferrences differ among commercial diets with different protein sources when given the option to eat as much as requested. A 13-wk trial was performed in 16, 0.1 ha outdoors ponds, stocked at 30 shrimp/$m^2$ and equipped with the AQ1 acoustic feeding system. At day 45 acoustic system was initiated and four treatments were assigned with a 35% crude protein commercial diet with different protein sources including all-plant, 8% poultry meal (PM), 8% fish meal (FM) and 12% FM. A second growth trial was conducted in a 20 tank (800L) outdoor recirculating system with similar density (35 shrimp/tank) and shrimp were offered a predetermined feeding rate. We did not observe statistical differences

in any of the main production parameters evaluated. Results of this study indicate that shrimp did not clearly prefer a particular diet. This suggests that, irrespective of ingredient matrix, a well-balanced feed will produce suitable growth even when shrimp are allowed to determine their feed intake. The use of acoustic feeders opens the door for nutrition research for which the shrimp are fed on demand.

1. Introduction

Shrimp production has been one of the most important sectors of aquaculture for the last few decades for both its market value and product acceptance. Intensification of production systems has been both a necessity and an instrument to increase shrimp productivity, for which one of the most important tools are artificial complete feeds. While fishmeal remains an important feed ingredient for the aquaculture industry, it is also one of the most expensive dietary components (NRC, 2011), particularly for marine species. However, the overall trends in fishmeal production since the late 1990's indicates a consistent decrease, result of reduction in capture (Shepherd*, et al.*, 2013), and this decreasing trend is expected to continue. Reduction in supply and increase in price has reduced the cost-effectiveness of fish meal and fish oil as feed ingredients which pushed the aquaculture industry towards identification of alternative ingredients (Tacon*, et al.*, 2008). Substantial research effort has been directed to study the potential of less costly, more sustainable alternatives for fishmeal in many species from plant-based sources (e.g. soy protein concentrate, corn protein concentrate, and distiller's dried grain with solubles) to terrestrial animal byproducts (e.g. poultry byproducts meal, feather meal, and blood meal).

Alternative ingredients such as soy-based products and poultry byproducts as ingredients for shrimp feeds have been extensively tested and validated in both smaller research systems

(Amaya*, et al.*, 2007; Galkanda-Arachchige*, et al.*, 2020; Guo*, et al.*, 2020; Guo*, et al.*, 2019; Ray*, et al.*, 2010; Samocha*, et al.*, 2004; Sookying*, et al.*, 2011b) systems as well as outdoor pond systems (Reis*, et al.*, 2020; Sookying*, et al.*, 2011a; Ullman*, et al.*, 2019a; Ullman*, et al.*, 2019b). As result, commercial feeds for the shrimp growout stage can have very low levels of fishmeal or even no inclusion whatsoever. Although it has been widely proven in research and production settings alike that excellent shrimp growth parameters can be achieved when feeds produced with very low levels of fishmeal are applied, many farmers remain skeptical that shrimp will consume such diets in comparison to diets with higher levels of fish meal. Contrary to many fish, shrimp feed on the bottom of the production system such as semi-intensive ponds. This further complicates monitoring of feed consumption in production systems.

Nutritionally sound complete feeds are paramount for optimal shrimp growth, but that is just one component of the equation of successful aquaculture production. In fact, development of adequate feeding strategies has often been overlooked (Tacon, 2013) as it does play a determining role in overall biological (i.e. animal growth), environmental and economical performance of any operation. Automatic timer feeders have been an important tool to improve growth performance of shrimp through higher number of meals and feeding rates, but during the last decade passive acoustic monitoring of shrimp feeding behavior allowed the development of highly efficient acoustic demand feeders (Bador, 2013). These feeders capture the clicking sound produced by shrimp mandibular activity during the external mastication process through a hydrophone placed inside the pond and disperse feed accordingly following estimation through a dedicated algorithm. Application of this technology in outdoor pond production has resulted in improved growth of shrimp when compared to timer feeders or more traditional practices such as handfeeding

(Jescovitch, *et al.*, 2018; Napaumpaipom, *et al.*, 2013; Reis, *et al.*, 2020; Ullman, *et al.*, 2019a; Ullman, *et al.*, 2019b).

As multiple demand feeding technologies were introduced in the aquaculture industry, it also became commonplace to use these tools not just as a feed delivery method but also as a tool to monitor animal behavior and potentially further improve the device, technology and/or algorithm. In fact, these feeders can also be used to compare feed dispersion of different feeds as a measure of feeding drive. While various authors have studied the application of passive acoustic feeding systems in shrimp production, there is not much work done in using this technology as a tool to evaluated diet preference. Therefore, it was the objective of this research project to use passive acoustic feeders in outdoor shrimp production ponds as a tool to identify any feed preference when shrimp were provided an option to eat on demand four different diets with varying protein sources and inclusion levels. A second trial in 20, 800L outdoor recirculating system was also conducted to identify potential differences in growth response when shrimp were subjected to same feeding regimen and tank management was uniform across rearing units.

## 2. Material and Methods

The outdoor pond trial was performed at the Alabama Department of Conservation and Natural Resources, Claude Peteet Mariculture Center (Gulf Shores, AL, USA), while the outdoor tank trial was performed at E.W Shell Fisheries Center (Auburn, AL, USA). Pacific white shrimp (*L. vannamei*) larvae were obtained from American Penaeid (St. James City, FL, USA), acclimated and nursed in a greenhouse system for 14 days. Juvenile shrimp (0.03 g) were then stocked into 16 outdoor, 0.1 ha ponds at 30 shrimp/$m^2$ per square meter, and juvenile shrimp (0.11g ± 0.02) were stocked in the tank trial were stocked at 35 shrimp/tank in 20, 800L tanks.

## 2.1 *Outdoor Pond Trial*

### *2.1.1 Feed Management*

Each pond was equipped with an on-demand passive acoustic feedback feeding system that integrates shrimp acoustic input through a hydrophone inside the pond and feeds based on acoustic response (AQ1 Feeder, AQ1 Systems Pty. Ltd., Tasmania, Australia). Each feeder was connected to a main controller on the levee with wireless connection to an office. For the first 17 days, all ponds were hand-fed a predetermined amount of the same 1.5-mm commercial diet (40% crude protein, 9% crude lipids) produced by Zeigler Bros. Inc. (ZBI, Gardners, Pa., USA). After that period, feeders were used and diets were changed to four 2.4mm commercial diets (35% protein, 8% lipids) with different ingredients as protein sources. The all plant (Soybean meal and corn protein based) diet served as a basal which was then modified to produce three other diets including 8% poultry meal (8% PM), 8% fish meal (8% FM) and 12% fish meal (12% FM) (Table 1). The acoustic system was initiated on the 44$^{th}$ day of the production cycle and each pond was fed on demand upto a maximum of 160 kg/ha/day All ponds were supplied with one 2-HP Aire-O2 (Aire-O2, Aeration Industries International, Inc., Minneapolis, MN, USA) as the main source of mechanical aeration and one 1-HP Air-O-Lator (Kansas City, MO, USA) for backup and/or supplemental aeration. Oxygen sensors connected to the feeding system were set to initiate mechanical aeration when DO readings fell below a 3 mg/L. While each feeder was calibrated before the initiation of the system, some feeders had to be re-calibrated within the first three weeks of utilization as they were allowing feeding up to 210 kg/ha/day.

*2.1.2 Sampling and Harvest*

Shrimp were sampled weekly from day 12 through the remaining weeks of the production stage using a cast net (1.52 m radius and 0.96 cm mesh) to collect approximately 60 individuals from each pond. Pond sampling enabled growth assessment and inspection for general health. Ponds were manually monitored for DO, temperature, salinity, and pH at least three times a day, at sunrise (5:00 to 5:30 a.m.), afternoon (2:00 to 2:30 p.m.) and sunset (7 to 8 p.m.), using a YSI ProPlus Meter (Yellow Springs Instrument Co., Yellow Spring, OH, USA). Total ammonia nitrogen was monitored once a week using a ion-selective electrode (Orion 4-Star Plus pH/ISE, Thermo Fisher Scientific, Waltham, MA, USA).

The ponds were harvested over three days at the end of the 13-week culture period. Ponds were partially drained and the night before harvest the water level was reduced to about one third and aeration was provided using the surface aerator. On the day of harvest, the remaining water was drained, and the shrimp were pumped out of the catch basin using a hydraulic fish pump equipped with a 25 cm diameter suction pipe (Aqua-Life pump, Magic Valley Heli-arc and Manufacturing, Twin-Falls, Idaho, USA). The pump was placed in the catch basin and shrimp were pumped, de-watered, and collected into a hauling truck. Shrimp were then rinsed, weighed in bulk, and 150 were randomly selected to measure individual weights and determine the size distribution. A subsample of these shrimp was collected and frozen for subsequent analysis. Whole body proximate with minerals analysis of the shrimp was performed by Midwest Laboratories (Omaha, NE, USA). The partial value was calculated by subtracting the feed costs from the production value as calculated from the Undercurrent News Portal for weeks 31 to 38 of 2019 and the size distribution of shrimp produced. The feed prices were $1.72/kg for the starter diet (40%

CP, 9% CL) and prices for grower diets were as follows: \$1.12/kg for All Plant, \$1.02/kg for 8% PM, \$1.19/kg for 8% FM, and \$1.22/kg for 12%FM.

*2.2 Green-water Tank Trial*

An 8-week growth trial was conducted at E.W. Shell Fisheries Center (Auburn, AL, USA), for which a 20 tank, 800L recirculating outdoor system was stocked at 35 shrimp/$m^2$ (0.11g ± 0.02). Tanks were not sampled to assess growth and feeding inputs were adjusted based on estimated growth predicted by a feeding protocol following similar assumptions as Davis*, et al.* (2006). Shrimp were fed the predetermined daily feeding rate in four meals throughout the day (0700, 1100, 1500, 1900). During the first week of the trial, the four commercial diets were crumbled to about 2-mm is size to be suitable for the shrimp feeding. Starting from the second week to the end of the experiment, shrimp were fed the 2.4-mm commercial diets. The system was monitored (DO, temperature, and salinity) twice a day (7:00 to 7:30 a.m. and 3:00 to 3:30 p.m.) using a YSI 650 multi-parameter instrument (YSI, Yellow Springs, OH, USA). pH was checked twice weekly using a waterproof pH Test 30 (Oakton instrument, Vernon Hills, IL, USA). While TAN and nitrite were measured twice a week with a YSI photometer 9500 kit (YSI, Yellow Springs, OH, USA.

2.3 *Statistical Analysis*

Statistical analysis of the growth data was conducted with SAS 9.4 (SAS Institute, Cary, NC, USA) to perform a one-way analysis of variance to determine significant difference (p-value < 0.05) among treatments in both trials. The assumptions for ANOVA were met. Student-Newman-Keuls multiple range test was used to determine differences among treatments. For the pond trial one replicate of the 8% FM diet treatment was removed from the data set due to electric failure of aeration that led to nearly complete loss of shrimp in that pond.

## 3. Result

### *3.1 Pond Trial*

Main water quality parameters were kept within the typical range for shrimp production (Boyd*, et al.*, 1992) (Table 2), and dissolved oxygen (DO), temperature, pH and salinity were similar across treatments. Jescovitch*, et al.* (2018) reported higher TAN when passive acoustic feeders were used in comparison with other feeding strategies, but there is no clear evidence the TAN levels throughout the production cycle of this experiment had any negative impact on shrimp growth.

Results for shrimp growth and production parameters are presented in Table 3. We did not observe statistically significant differences among treatments for any of the measured production parameters. Identical weekly growth rate is also corroborated by weekly sampling estimations (Figure 1) which show similar growth among all treatments throughout the production cycle. Total feed cost for the poultry meal diet treatments was significantly lower than the cost for the treatment feeding the higher inclusion level of fish meal, however this difference was not translated to statistical differences in any other economic indicators such as production cost, shrimp value or partial income.

Results of proximate whole body composition analysis are summarized in Table 4. No statistical differences were found among any treatment for any of the parameters tested. Results for tail muscle amino acid composition analysis are summarized in Table 4 as well. We observed significantly lower levels of valine in individuals fed all plant diet ($p=0.022$), but no other differences were observed for any other amino acid analyzed. Apparent protein retention for the population was calculated based on crude protein level in the diet per proximate analysis report (Table 1) and protein content of shrimp at the end of the production cycle per proximate analysis report (Table 4). No statistical differences were observed in apparent protein retention between treatments (p=0.6501), which overall ranged from 19.81% to 39.8%.

*3.2 Tank Trial*

As in the pond trial, main water quality parameters in the recirculating tank system were also kept within the typical range for shrimp production (Boyd*, et al.*, 1992) (Table 2). Both TAN and nitrite levels were also kept within acceptable range for shrimp production as well. This outdoor tank system was provided continuous aeration, reducing likelihood of low DO events, and circulation contributed to identical conditions in every rearing tank at any given time. There were no significant differences in any of the growth performance indicators measured including final weight, survivals, weight gain (WG), WG (%) or FCR between the shrimp fed with the four different protein sources diets.

4. Discussion

A continuous decrease in availability of fish meal and subsequent increase of price has perhaps made the identification and application of alternative protein sources for fish meal in feeds the main global priority in aquaculture nutrition for the last two decades. While many different alternative protein sources have been tested and validated as suitable for shrimp production, many farmers still believe that fish meal favors feed intake. The recent development and validation of passive acoustic monitoring feeding technology for shrimp production (Bador, 2013;

Napaumpaipom*, et al.*, 2013; Reis*, et al.*, 2020; Ullman*, et al.*, 2019a; Ullman*, et al.*, 2019b) opens a new door to evaluate food consumption in shrimp production conditions. One of these demand feeding technologies was applied in this experiment as a tool to evaluate feed response (as feed input) and growth of shrimp in outdoor pond conditions when offered diets with various protein sources.

Production results for the pond trial are within typical values for outdoor shrimp pond production. However, overall average final weights and FCR in this trial were respectively lower and higher than those reported by Reis*, et al.* (2020); Ullman*, et al.* (2019a) under similar pond production conditions when passive acoustic feeders were applied. During the first 44 days of the production cycle shrimp were offered the same ration, during which period animals cast net sampling indicated fairly uniform growth which was expected. Yet we also observed very similar average individual sizes as a result of weekly sampling for the grow-out period during acoustic feeders were used and different diets were offered (Figure 1). Therefore, average weekly growth rate (p= 0.7736) or final average weight (p=0.7604) were not significantly different among any of the treatments. Analysis of average daily feed inputs indicate a general increase and stabilization of growth during the last third of the production cycle. The reduction in shrimp growth observed in the ponds fed the 8% FM diet was associated with higher variation within treatment resulting of substantial algae crash in two of the ponds which compromised feeding for a few days, therefore dictating this lower datapoint. Other two treatments also experience slight reductions in growth during the same period which is likely related to temporary water quality degradation as well.

While acoustic feeders have become an ever-growing trend in shrimp pond production, one of the main concerns regarding its application is the increase in feeding rates during the latter stages of the production cycle which many farmers believe is mere overfeeding as FCRs tend to increase. Through back-calculation of population size (assuming constant weekly mortality rate) we were able to calculate average bi-weekly FCR throughout the cycle and did observe an increase during the last third of the cycle. While it is reasonable to believe larger animals do not grow as efficiently as smaller individuals, we do not presume this is the reason for such an increase in FCR and recommend caution in assuming inefficient dispersion by the feeder. This increase is probably better explained by a multitude of factors such as within treatment variation related to timing of molting cycles in each pond as well as general deterioration of water quality conditions that may compromise feeding efficiency during the later stages of the production cycle.

In short, in this outdoor pond production trial we were not able to establish any correlation between shrimp feeding activity as measured per feed input (kg/ha/day) and any specific diet which leads us to believe that when all diet were well balanced and feed was not a limiting factor, shrimp did not prefer a specific protein source. This interpretation of the results is further validated by the lack of difference in growth performance of shrimp fed these same diets in a green-water recirculating system.

Continuous expansion of the application of acoustic monitoring systems in shrimp aquaculture as well as integration of new data is likely to continue to be a gamechanger in the industry. In fact, there is great potential for the use of acoustic monitoring and feeding tools in shrimp nutrition research under both practical and laboratory conditions. Recent studies have used this technology to study acoustic and growth response to pelleted and extruded diets (Soares, 2021b), acoustic response to various pellet sizes (Peixoto*, et al.*, 2020) and the acoustic and growth response of shrimp to soy-based diets coated with various attractants (Soares, 2021a). As the industry continues moving towards higher efficiency systems, it is likely that more studies using this technology will continue providing insight with regard to feed formulation and processing that may further enhance both shrimp growth and feeding system efficiency.

## 5. Conclusions

The utilization of passive acoustic feeders and other passive acoustic monitoring technologies remains a useful instrument for identification of feed behavior patterns in shrimp. The results of the pond trial falls in line will previous research in shrimp nutrition that validated the use of alternative protein sources in commercial shrimp production diets. We were not able to establish a relationship between feed inputs and growth response for any of the treatments. Hence, we conclude that when feed is not a limiting factor and complete diets are nutritionally balanced shrimp do not seem to increase their feed intake. The absence of an enhanced growth response in a tank trial to any particular diet also used in the pond trial further supports this conclusion. While many farmers in various regions of the globe still believe shrimp to not like feeds with little to no fish meal, this study further confirms that more than acceptable shrimp growth can be achieved through nutritionally balanced commercial feeds produced with fish meal substitutes.

6. References


Amaya, E.A., Davis, D.A., Rouse, D.B., 2007. Replacement of fish meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*) reared under pond conditions. Aquaculture. 262, 393-401.

Bador, R., Blyth, P., Dodd, R., 2013. Acoustic control improves feeding productivity at shrimp farms. Global Aquaculture Advocate, 77-78.

Boyd, C.E., Tucker, C.S., 1992. Water quality and pond soil analyses for aquaculture. Alabama Agricultural Experiment Station, Auburn University.

Davis, D.A., Amaya, E., Venero, J., Zelaya, O., Rouse, D.B., 2006. A case study on feed management to improving production and economic returns for the semi-intensive pond production of *Litopenaeus vannamei*, Avances en Nutrición Acuícola VIII. Memorias del Octavo Simposio Internacional de Nutrición Acuícola. Universidad Autonoma de Nuevo Monterrey, Nuevo León, México.

Galkanda-Arachchige, H., Davis, D.A., 2020. Evaluation of differently processed soybean meal products as ingredients in practical diets for Pacific white shrimp *Litopenaeus vannamei*. Aquaculture Nutrition. 26, 287-295.

Guo, J.P., Huang, Y., Salze, G., Roy, L.A., Davis, D.A., 2020. Use of plant-based protein concentrates as replacement for fishmeal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*) reared under high stocking density and low salinity conditions. Aquaculture Nutrition. 26, 225-232.

Guo, J.P., Reis, J., Salze, G., Rhodes, M., Tilton, S., Davis, D.A., 2019. Using high protein distiller's dried grain product to replace corn protein concentrate and fishmeal in practical diets for the Pacific white shrimp *Litopenaeus vannamei*. Journal of the World Aquaculture Society. 50, 983-992.

Jescovitch, L.N., Ullman, C., Rhodes, M., Davis, D.A., 2018. Effects of different feed management treatments on water quality for Pacific white shrimp *Litopenaeus vannamei*. Aquac. Res. 49, 526-531.

N.R.C., 2011. Nutrient requirements of fish and shrimp. National academies press. Washington, DC.

Napaumpaipom, T., Chuchird, N., Taparhudee, W., 2013. Study on the Efficiency of Three Different Feeding Techniques in the Culture of Pacific White Shrimp (*Litopenaeus vannamei*). Journal of Fisheries and Environment. 37, 8-16.

Peixoto, S., Soares, R., Davis, D.A., 2020. An acoustic based approach to evaluate the effect of different diet lengths on feeding behavior of *Litopenaeus vannamei*. Aquacultural Engineering. 91.

Ray, A.J., Lewis, B.L., Browdy, C.L., Leffler, J.W., 2010. Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. Aquaculture. 299, 89-98.

Reis, J., Novriadi, R., Swanepoel, A., Guo, J.P., Rhodes, M., Davis, D.A., 2020. Optimizing feed automation: improving timer-feeders and on demand systems in semi-intensive pond culture of shrimp *Litopenaeus vannamei*. Aquaculture. 519.

Samocha, T.M., Davis, D.A., Saoud, I.P., DeBault, K., 2004. Substitution of fish meal by co-extruded soybean poultry by-product meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture. 231, 197-203.

Shepherd, C.J., Jackson, A.J., 2013. Global fishmeal and fish-oil supply: inputs, outputs and markets. J Fish Biol. 83, 1046-1066.

Soares, R., Peixoto, S., Davis, R. P., & Davis, D. A., 2021a. Feeding behavior and growth of *Litopenaeus vannamei* fed soybean-based diets with added feeding effectors. Aquaculture.

Soares, R., Peixoto, S., Galkanda-Arachchige, H. S., & Davis, D. A., 2021b. Growth performance and acoustic feeding behavior of two size classes of *Litopenaeus vannamei* fed pelleted and extruded diets. Aquaculture International, 1-17.

Sookying, D., Davis, D.A., 2011a. Pond production of Pacific white shrimp (*Litopenaeus vannamei*) fed high levels of soybean meal in various combinations. Aquaculture. 319, 141-149.

Sookying, D., Silva, F.S.D., Davis, D.A., Hanson, T.R., 2011b. Effects of stocking density on the performance of Pacific white shrimp *Litopenaeus vannamei* cultured under pond and outdoor tank conditions using a high soybean meal diet. Aquaculture. 319, 232-239.

Tacon, A.G., Jory, D., & Nunes, A., 2013. Shrimp feed management: issues and perspectives. in: Hasan, M.R.N., M.B. (Ed.), On-farm feeding and feed management in aquaculture. Food and Agriculture Organization of the United Nations, Rome, pp. 481-488.

Tacon, A.G.J., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture. 285, 146-158.

Ullman, C., Rhodes, M.A., Davis, D.A., 2019a. Feed management and the use of automatic feeders in the pond production of Pacific white shrimp *Litopenaeus vannamei*. Aquaculture. 498, 44-49.

Ullman, C., Rhodes, M., Hanson, T., Cline, D., Davis, D.A., 2019b. Effects of Four Different Feeding Techniques on the Pond Culture of Pacific White Shrimp, *Litopenaeus vannamei*. Journal of the World Aquaculture Society. 50, 54-64.

Table 1 – Formulation (g/kg) of each 2.4mm 35% CP extruded sinking feed with various protein sources used to assess shrimp growth in both outdoor pond and outdoor green-water recirculating semi-intensive shrimp production trials. Proximate analysis performed by Midwest Laboratories (Omaha, NE, USA) with results expressed as g/100g. PM: Poultry meal; FM: Fish Meal.

| | All Plant | 8% PM | 8% FM | 12% FM |
|---|---|---|---|---|
| Soybean Meal | 560.0 | 500.0 | 537.0 | 575.0 |
| Wheat | 191.0 | 231.0 | 219.0 | 216.0 |
| Menhaden Fish meal | 0.0 | 0.0 | 80.0 | 120.0 |
| Poultry-By Meal | 0.0 | 80.0 | 0.0 | 0.0 |
| Corn Gluten | 120.0 | 80.0 | 60.0 | 0.0 |
| Dicalcium Phosphate | 41.3 | 31.3 | 26.3 | 16.3 |
| Fish Oil - Topdress | 30.0 | 30.0 | 30.0 | 30.0 |
| Fish Oil - Mixer | 30.0 | 20.0 | 15.0 | 20.0 |
| Bentonite | 87.7 | 77.7 | 77.7 | 72.7 |
| Lecithin | 10.0 | 10.0 | 10.0 | 10.0 |
| Vitamin Premix[a] | 1.2 | 1.2 | 1.2 | 1.2 |
| Mineral Premix[a] | 1.2 | 1.2 | 1.2 | 1.2 |
| Stay C-35% active | 0.2 | 0.2 | 0.2 | 0.2 |
| Copper Sulfate | 0.1 | 0.1 | 0.1 | 0.1 |
| Proximate Composition (%) | | | | |
| Phosphorus | 1.47 | 1.28 | 1.41 | 1.32 |
| Crude Protein | 37.5 | 38.1 | 37.7 | 37.9 |
| Moisture | 8.99 | 9.62 | 8.44 | 9.41 |
| Crude Fat | 6.90 | 7.54 | 7.68 | 7.02 |
| Crude Fiber | 8.8 | 9.2 | 9.9 | 12.0 |
| Ash | 8.57 | 8.89 | 8.95 | 8.99 |

[a]Premixes are proprietary products therefore composition is not listed.

Table 2 - Summary of water quality parameters observed over the 13-wk. growth trial in ponds (n=4) and the 8-wk growth trial in green-water tanks (n=5). Values are presented as mean ± standard deviation, and maximum and minimum value are presented in parenthesis.

| | Outdoor ponds | | | | Green-water system |
|---|---|---|---|---|---|
| | All Plant | 8% Poultry | 8% FM | 12% FM | |
| Morning DO[a] (mg/L) | 4.20 ± 0.91 (1.02 , 7.42) | 4.19 ± 1.11 (0.65 , 9.16) | 4.21 ± 0.92 (1.35 , 8.25) | 4.04 ± 0.91 (0.82 , 8.19) | 8.90 ± 1.76 (5.80 , 12.09) |
| Afternoon DO[a] (mg/L) | 9.14 ± 1.99 (4.17 , 14.74) | 9.43 ± 2.20 (3.18 , 15.2) | 9.61 ± 2.37 (2.64 , 18.36) | 9.42 ± 2.13 (3.73 , 16.82) | 7.97 ± 1.95 (4.25 , 12.95) |
| Night DO[a] (mg/L) | 8.20 ± 2.11 (3.30 , 14.37) | 8.75 ± 2.59 (2.49 , 18.03) | 8.77 ± 2.61 (1.05 , 20.88) | 8.27 ± 2.42 (1.59 , 14.61) | |
| Temperature (ºC) | 30.95 ± 1.82 (25.8 , 35.2) | 31.18 ± 1.82 (25.2 , 35.3) | 31.08 ± 1.81 (25.9 , 35.2) | 31.29 ± 1.88 (25.9 , 36.3) | 29.09 ± 1.49 (24.1, 35.5) |
| pH | 8.31 ± 0.55 (7.35 , 9.59) | 8.34 ± 0.55 (7.29 , 9.85) | 8.36 ± 0.57 (7.33 , 9.98) | 8.30 ± 0.53 (7.13 , 9.68) | 8.12 ± 0.33 (7.7 , 8.8) |
| Salinity (g/L) | 7.01 ± 1.16 (2.83 , 10.78) | 6.96 ± 1.99 (2.67 , 16.01) | 7.37 ± 1.51 (2.67 , 12.91) | 7.36 ± 1.78 (4.26 , 11.89) | 6.03± 0.21 (5.5 , 6.5) |

| | | | | | |
|---|---|---|---|---|---|
| TAN[b] (mg/L) | 0.50 ± 1.36 (<0.001, 7.0) | 0.40 ± 1.08 (<0.001 , 5.0) | 0.39 ± 0.99 (<0.001 , 5.0) | 0.46 ± 1.26 (<0.0001, 7.0) | 0.17 ± 0.14 (0.01 , 0.51) |
| $NO_2$ (mg/L) | | | | | 0.02 ± 0.01 (<0.001 , 0.05) |

[a]DO - Dissolved Oxygen
[b]TAN - Total Ammonia Nitrogen

Table 3 – Production results of *L. vannamei* reared in 0.1 ha production ponds over a 13-wk culture period fed four different commercial diets (n=4). Nursed shrimp (0.03 g) were stocked at a density of 30 shrimp/$m^2$. Values within a column with different superscripts are significantly different based on Student- Newman-Keuls test

| Treatment | Growth (g/week) | Final mean weight (g) | Feed Input (Kg/ha) | Survival (%) | Yield (kg/ha) | FCR[1] | Electric Use (kWh/ha) | Production Cost ($/kg) | Feed Cost | Shrimp Value ($/ha) | Partial Income[2] ($/ha) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| All Plant | 1.64 | 21.02 | 7898 | 91.66 | 5355 | 1.54 | 17,348 | 1.75 | 8,975[ab] | 41,429 | 32,454 |
| 8% PM | 1.67 | 21.54 | 8084 | 88.03 | 5725 | 1.48 | 16,990 | 1.54 | 8,394[b] | 44,756 | 36,362 |
| 8% FM[3] | 1.72 | 22.59 | 7596 | 92.95 | 6276 | 1.21 | 18,523 | 1.46 | 9,155[ab] | 49,880 | 40,725 |
| 12% FM | 1.64 | 21.49 | 7631 | 80.92 | 5227 | 1.50 | 15,810 | 1.86 | 9,420[a] | 40,676 | 31,256 |
| P-value | 0.7736 | 0.7604 | 0.4918 | 0.7982 | 0.6070 | 0.6081 | 0.3544 | 0.5032 | 0.0253 | 0.5502 | 0.5325 |
| PSE[4] | 0.06 | 0.73 | 17.46 | 8.13 | 540.48 | 0.14 | 950.16 | 0.189 | 201.27 | 4454.11 | 4491.23 |

[1]FCR – Feed Conversion Ratio
[2]Partial Incomer – shrimp value minus feed cost
[3]n=3
[4]PSE: Pooled Standard Error

Table 4 – Means of whole body composition for each treatment, proximate and minerals as analysed by Midwest Laboratories (Omaha, NE, USA) and amino acid composition (presented as % of dry weight of muscle tissue) as analyzed by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA) with means separation through Student-Newman-Keuls test.

| | All Plant | 8% PM | 8% FM | 12% FM | *P*-value | PSE[1] |
|---|---|---|---|---|---|---|
| Proximate composition | | | | | | |
| Dry Matter (%) | 25.78 | 26.40 | 22.28 | 23.28 | 0.1070 | 0.012 |
| Protein (%) | 75.43 | 75.28 | 76.45 | 76.23 | 0.5782 | 0.702 |
| Fat (%) | 10.95 | 10.95 | 8.56 | 9.94 | 0.1976 | 0.841 |
| Fiber (%) | 6.60 | 6.73 | 7.28 | 7.43 | 0.3097 | 0.351 |
| Ash (%) | 11.05 | 11.08 | 12.08 | 11.35 | 0.0823 | 0.283 |
| Mineral content | | | | | | |
| Sulfur (%) | 0.74 | 0.75 | 0.74 | 0.76 | 0.5421 | 0.012 |
| Phosphorus (%) | 1.23 | 1.21 | 1.30 | 1.28 | 0.4893 | 0.046 |
| Potassium (%) | 1.20 | 1.20 | 1.23 | 1.21 | 0.8115 | 0.039 |
| Magnesium (%) | 0.28[ab] | 0.27[b] | 0.30[a] | 0.29[ab] | 0.0349 | 0.006 |
| Calcium (%) | 2.55 | 2.66 | 2.86 | 2.56 | 0.1269 | 0.093 |
| Sodium (%) | 0.66 | 0.65 | 0.70 | 0.64 | 0.5500 | 0.034 |
| Iron (ppm) | 118.1 | 108.4 | 81.03 | 114.68 | 0.8422 | 32.093 |
| Manganese (ppm) | 6.60 | 6.15 | 5.78 | 7.9 | 0.0591 | 0.512 |
| Copper (ppm) | 116 | 116.25 | 115 | 116.25 | 0.9960 | 4.232 |
| Zinc (ppm) | 59.60 | 60.88 | 61.35 | 63.53 | 0.0909 | 0.991 |
| Amino acids | | | | | | |
| Alanine (%) | 3.95 | 4.04 | 4.17 | 4.11 | 0.1133 | 0.060 |
| Arginine (%) | 4.97 | 5.04 | 5.22 | 5.03 | 0.4350 | 0.111 |
| Aspartic acid (%) | 6.10 | 6.22 | 6.26 | 6.19 | 0.6780 | 0.094 |
| Cysteine (%) | 0.51 | 0.54 | 0.54 | 0.50 | 0.6925 | 0.026 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Glutamic acid (%) | 9.13 | 9.44 | 9.39 | 9.27 | 0.0477 | 0.147 |
| Glycine (%) | 3.96 | 3.94 | 4.58 | 4.22 | 0.2082 | 0.225 |
| Histidine (%) | 1.25 | 1.29 | 1.28 | 1.27 | 0.9683 | 0.061 |
| Hydroxylysine (%) | 0.12 | 0.09 | 0.12 | 0.12 | 0.0864 | 0.009 |
| Hydroxyproline(%) | 0.24 | 0.29 | 0.25 | 0.26 | 0.3099 | 0.020 |
| Isoleucine (%) | 2.88 | 2.94 | 2.95 | 2.93 | 0.5693 | 0.038 |
| Lanthionine (%) | 0.06 | 0.05 | 0.06 | 0.07 | 0.3880 | 0.011 |
| Leucine (%) | 4.53 | 4.63 | 4.67 | 4.62 | 0.3350 | 0.051 |
| Lysine (%) | 4.32 | 4.41 | 4.46 | 4.43 | 0.4993 | 0.067 |
| Methionine (%) | 1.38 | 1.40 | 1.43 | 1.43 | 0.2292 | 0.022 |
| Ornithine (%) | 0.29 | 0.26 | 0.35 | 0.33 | 0.1141 | 0.026 |
| Phenylalanine (%) | 2.76 | 2.80 | 2.86 | 2.83 | 0.5690 | 0.048 |
| Proline (%) | 3.96 | 4.08 | 3.71 | 3.85 | 0.3466 | 0.1423 |
| Serine (%) | 1.73 | 1.92 | 1.85 | 1.91 | 0.4804 | 0.0940 |
| Taurine (%) | 0.40 | 0.41 | 0.40 | 0.40 | 0.9356 | 0.0140 |
| Threonine (%) | 2.19 | 2.23 | 2.23 | 2.31 | 0.1662 | 0.0365 |
| Tryptophan (%) | 0.66 | 0.68 | 0.69 | 0.70 | 0.2071 | 0.0148 |
| Tyrosine (%) | 2.16 | 2.22 | 2.24 | 2.24 | 0.1446 | 0.0271 |
| Valine (%) | 3.36[b] | 3.53[a] | 3.55[a] | 3.59[a] | 0.0221 | 0.0461 |
| *Total* | 60.89 | 62.49 | 63.28 | 62.60 | 0.2887 | 0.8536 |

[1]PSE: Pooled Standard Error

Table 5 – Production results of L. vannamei reared in outdoor green-water recirculating tank (800L) system over a 8-wk culture period fed four different commercial diets (n=5). Nursed shrimp (0.1 g) were stocked at a density of 35 shrimp/tank. Values within a column with different superscripts are significantly different based on Student- Newman-Keuls test

| Treatment | Final mean weight (g) | Growth (g/wk) | Weight gain (g/week) | Survival (%) | Biomass (g) | FCR |
|---|---|---|---|---|---|---|
| All Plant | 7.02 | 0.86 | 224.144 | 93.14 | 228.17 | 1.35 |
| 8% PM | 6.46 | 0.79 | 215.986 | 97.14 | 219.98 | 1.40 |
| 8% FM | 7.21 | 0.89 | 236.786 | 95.43 | 240.86 | 1.28 |
| 12% FM | 6.91 | 0.85 | 233.848 | 98.29 | 237.73 | 1.29 |
| P-value | 0.215 | 0.235 | 0.366 | 0.327 | 0.365 | 0.321 |
| PSE[1] | 0.246 | 0.06 | 8.925 | 8.13 | 8.905 | 0.051 |

[1]PSE: Pooled Standard Error

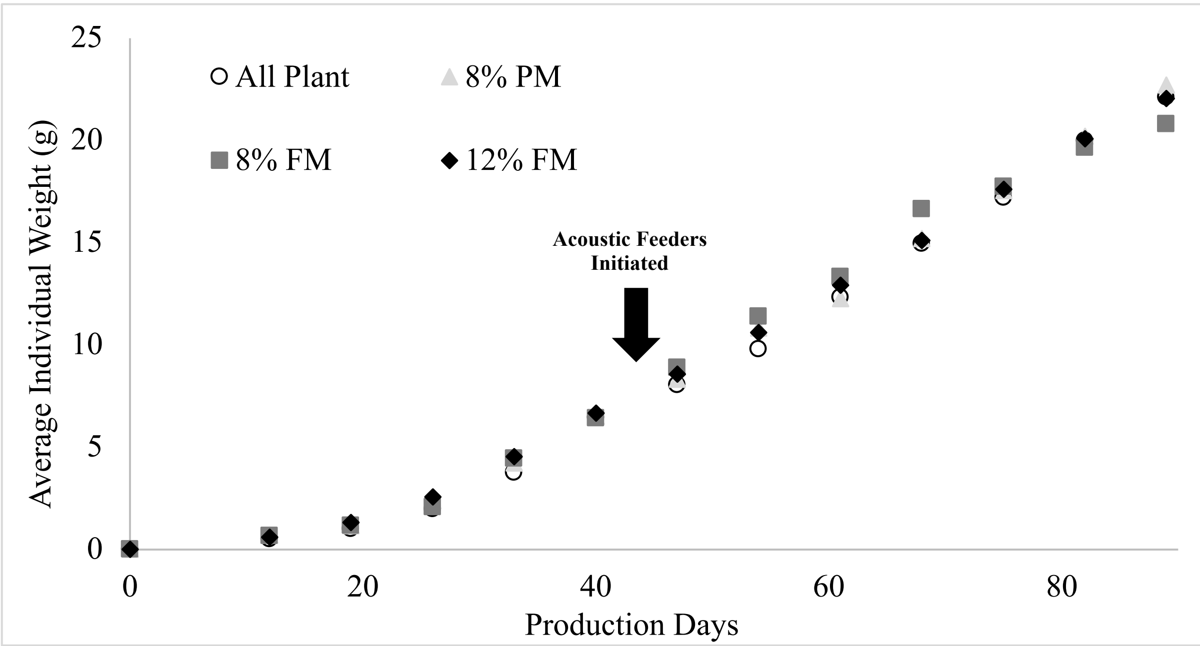


Figure 1 – Average individual weights of weekly sampled individuals (cast-net) per treatment through a 90 day semi-intensive outdoor pond shrimp production cycle

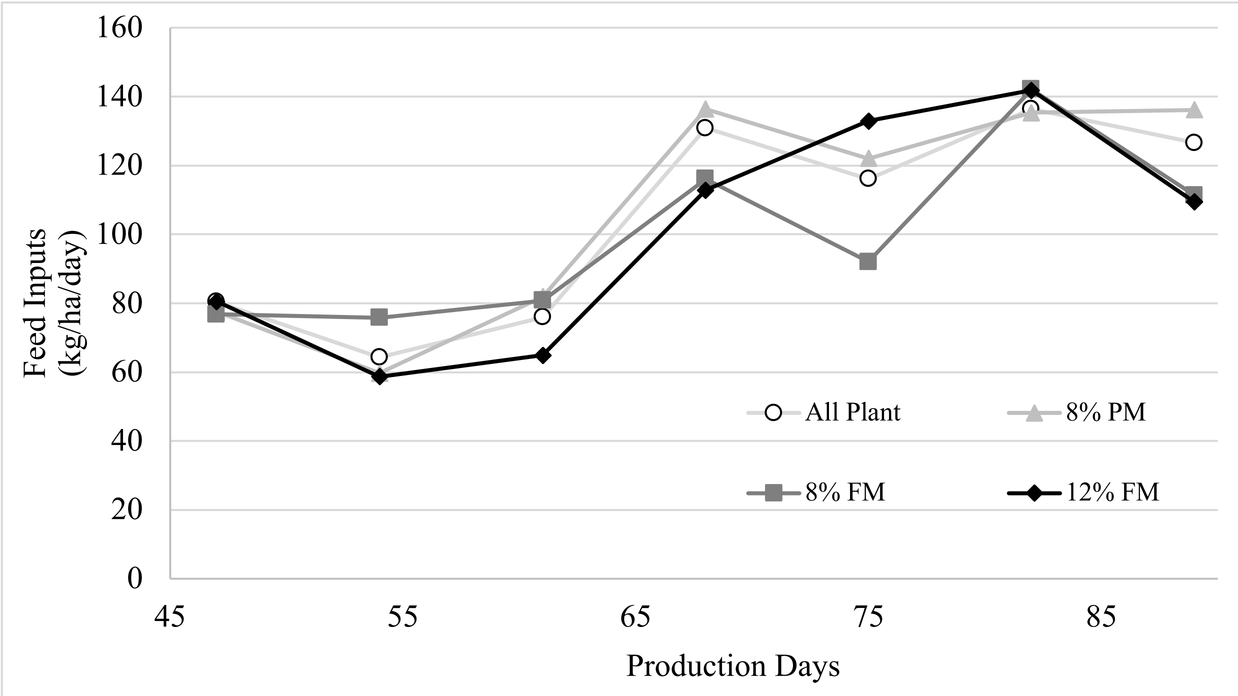


Figure 2 – Average daily feed inputs per treatment through a 90 day semi-intensive outdoor pond shrimp production cycle fed using passive acoustic feedback system (limited to 160 kg/ha/day)

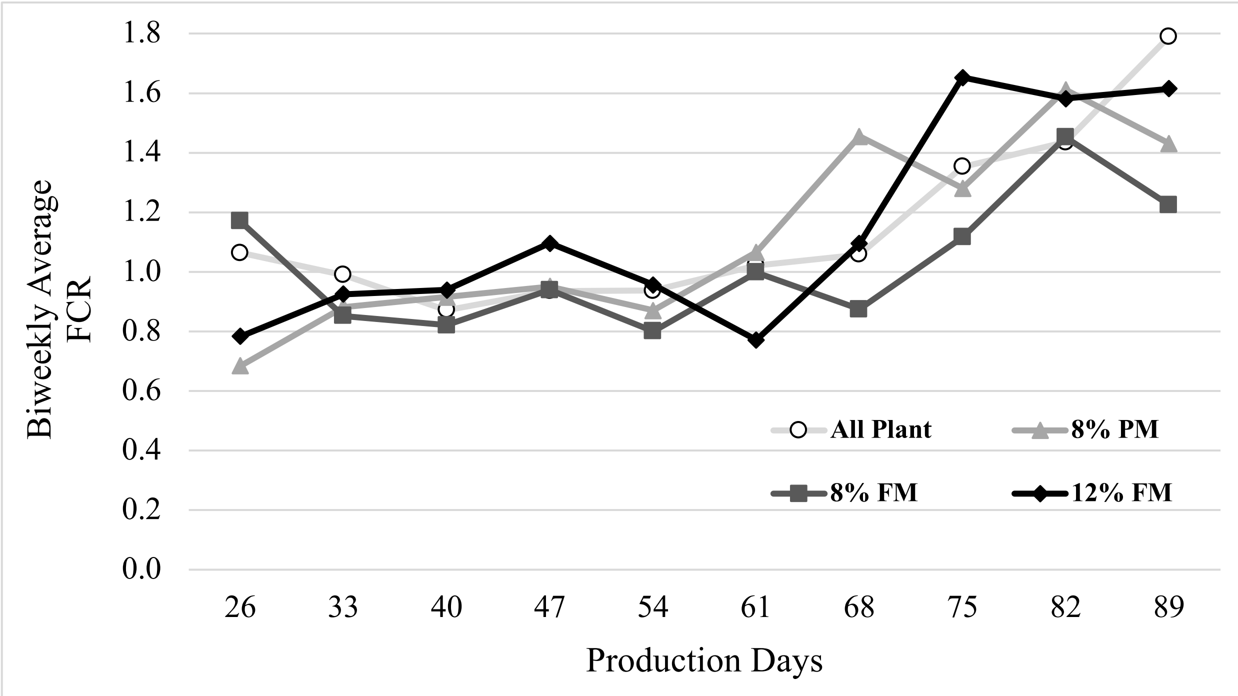


Figure 3 – Average biweekly FCR per treatment through a 90 day semi-intensive outdoor pond shrimp production cycle fed using passive acoustic feedback system (limited to 160 kg/ha/day). Population size was back-calculated based on initial and final population estimations and assuming uniform mortality throughout the cycle.

# CHAPTER V

# REVIEW OF THE UTILIZATION OF PASSIVE ACOUSTIC FEEDING TECHNOLOGY AND CONCLUSION

It is well understood that providing nutritionally balanced complete feeds through adequate feeding strategies are key to effectively meeting the daily nutrient requirements for each aquatic organism being cultured. Simultaneously, very effective feeding strategies have limited value if cultured animals are offered less than ideal feeds. Yet, if it is true that feed management in aquaculture can be very challenging on multiple levels, it is also true that many farmers have developed or integrated more or less sophisticated strategies to improve feeding efficiency. In fact, most successful farmers usually rely on a combination of techniques.

The natural feeding behavior of shrimp and common husbandry conditions raise specific challenges as visual perception of feed intake is disabled. Hence, feed management in shrimp production has mostly relied on feeding tables based on historic production data as well as weekly sub-sampling data and rough estimation of feed intake using feed trays which can also be used as a feed delivery platform. These practices are reactive management strategies that occur after the fact often resulting in wasted feed wastage and can be quite labor intensive.

As in many other industries that have traditionally relied on human labor to perform repetitive tasks, the aquaculture industry has also developed and adapted technology to improve productivity through automation of operations and reduce operating costs. The first steps towards automation and new technology is usually taken by large corporations with high investment capacity and later on by smaller farmers as less costly alternatives to each technology enter the market. During earlier stages of aquaculture expansion, it was common that pre-existing technologies would be merely adapted to aquaculture systems, of which timer feeders are a clear example. In the context of shrimp farming, this is a useful tool to achieve the high number of meals necessary to improve feeding efficiency as well as reducing labor costs (Davis, 2018). However, this approach to feed management still relies on a reactive approach as it is set on the assumption that shrimp will readily consume the predetermined feed amount dispersed during any meal.

Some of the most common demand feeders in aquaculture have used video recording and dedicated software to integrate algorithms associated with feed dispersion. Pinkiewicz*, et al.* (2011) developed a dedicated fish tracking software for cage farmed salmon which was able to monitor movement throughout the enclosure and could provide real-time feedback for welfare indicators, Rillahan*, et al.* (2009) evaluated Atlantic cod behavior in offshore aquaculture cages by using a combination of underwater cameras (during daytime) and ultrasonic transmitters. Coves*, et al.* (2006) monitored European sea bass triggering activity of a self-feeding system through a combination of pit-tagging and video recording and observation. While video systems are a common tools, they usually require dedicated software to analyze the footage and are only effective in low turbidity systems, therefore negating its introduction in shrimp farming.

Shrimp are typically raised in production systems that contain considerable levels of natural foods that contribute to nutrition but also produce a highly turbid environment. Compared to most cultured fish, shrimp are also relatively smaller animals, and would be more similar to fingerling production rather than larger food fish. The reduced size of shrimp, discontinuous growth and molting cycle make pit-tagging impractical. The answer to the constraints presented by this subsector of the industry has been to study an alternative that would not be precluded by suspended solids in the water. Acoustic profiling of aquatic animals and crustaceans in specific is not a new or even recent concept, however, its adaptation to aquaculture systems on a commercial scale is fairly recent. There are several approaches acoustic technology approaches but passive acoustic profiling of shrimp as a tool to develop demand feeders has been one of the primary trends in the sector for the last decade.

In the acoustic landscape of a shrimp production unit, the clicking sound parameters are key information to infer their feeding activity when fed commercial diets (Peixoto*, et al.*, 2020; Peixoto, 2020; Smith*, et al.*, 2013a; Smith*, et al.*, 2013b). These parameters are affected by many factors such as life stage of the animal and physicochemical properties of the feed (i.e. texture, size, manufacturing process, etc). Smith*, et al.* (2013a) did not observes differences in clicking profile of *P. monodon* fed commercial diet or squid, and Peixoto*, et al.* (2020) reported higher number of clicks when *L. vannamei* were fed longer diets but not the acoustic profile of each click. Silva*, et al.* (2019)reported faster consumption of feed pellets in large (35 g) shrimp as most clicks occurred faster after capture of food item in comparison to smaller size classes, although the number of clicks did not vary with size. While there are a few studies published on impact of diet in clicking profile, there is much fewer information on smaller size individuals (i.e. larvae, post-larvae and juvenile shrimp). Understanding the acoustic profile in smaller individuals may result in optimization of the feeding technology for earlier life stages as well. Yet, as mentioned in previous chapters of this dissertation, one of the main concerns for the application of this technology in production systems is sound interference caused by aeration, heavy rain, pumps, or other sources that may override the sound of feeding activity.

The two main limitations towards understanding the true impact of passive acoustic systems in shrimp farming are the overwhelming industry application that usually does share production data due to its economic implications, and the logistic and infrastructural challenges of conducting trials under production conditions directly comparable to commercial operations. Yet, some authors were able to carry out valuable research in production systems. Napaumpaipom*, et al.* (2013) conducted a 120-day shrimp (*L. vannamei*) production trial in 1ha ponds stocked at 75 shrimp/$m^2$ under three different feed management techniques including hand-feeding, automatic timer feeders and a passive acoustic feeding system (AQ1 Systems, Tasmania, Australia). While the acoustic system self-managed feeding rates, feed inputs for the other two treatments were adjusted based on feed consumption estimations using a feed tray. This study reported that application of acoustic feeders resulted in lower feed conversion ratios, larger shrimp, higher yields and growth rates. They also surmised that the increase feeding frequency improved water quality albeit limited data was presented.

A study by Ullman*, et al.* (2019b) compared the same feeding strategies in as the previous author in smaller 0.1ha ponds using a lower stocking density (17 shrimp/$m^2$). However in this study feeding rates for both hand-feeding and timer feeder were calculated based on a protocol reported by Davis*, et al.* (2006) and no feeding trays were used to assess feed consumption. Results obtained for this study followed previous conclusions by Napaumpaipom*, et al.* (2013). Ullman*, et al.* (2019b) also found that total feed inputs were considerably higher with the acoustic feedback system even with feed inputs being restricted to 120 kg/ha/day. In a feeding study in which the effect of acoustic feeders on water quality was assessed, Jescovitch*, et al.* (2018) found that higher nutrient loading (i.e. feeding rate) in ponds feed with a passive acoustic system resulted in higher total ammonia nitrogen (TAN) and nitrite during the last third of the 120 day production cycle. The author also pointed that although nitrogen pollution was higher in those ponds, the concentrations were still within the safety range for the culture of *L. vannamei*, and that spreading the nutrient load through more meals would allow higher feed inputs that ultimately increases the overall nutrient loading in the system.

As a consequence of more than adequate shrimp growth reported by Ullman*, et al.* (2019b), a subsequent study was conducted by Ullman*, et al.* (2019a) under identical conditions but on a shorter 90-day production cycle and under higher stocking density (38 shrimp/$m^2$). The authors were still able to achieve shrimp of commercial harvest size on a shorter culture cycle through application of acoustic feeders and optimizing protocols for timer feeders. In this study, acoustic feeders were allowed to feed up to 160 kg/ha/day and feed inputs were again significantly higher by comparison with the other feeding protocols. Subsequent studies to those conducted by Ullman*, et al.* (2019b) and Ullman*, et al.* (2019a) are the ones previously presented in this dissertation. The systematic increase in feeding rates allowed establishment of a new standard protocol for timer-feeders which was able to approximate overall production efficiency to that of passive acoustic feeders. The second study adopted his new protocol to evaluate schedule preferences (day, night and 24 hrs per day) in shrimp feeding in both ponds and tanks, and wasn't able to document improved performance to a specific schedule. However, night feeding in semi-intensive outdoor ponds favored oxygen depletion which resulted in increased aeration and reduced feeding when DO was low, therefore suggesting this would not be a recommended practice. The improvements in protocols for timer feeders in the first two studies were not able to achieve the fundamental higher efficiency of the passive acoustic feeding system. Given, that simple timer feeders are not likely to match a real time feeding program we conducted the final study using only passive acoustic feed management. This last trial was conducted to evaluate protein source preferences in commercially available feeds through the utilization of passive acoustic feeders. The absence of differences among treatments in this trial was yet further proof that when feed is not a limiting factor, shrimp do not prefer a specific protein source in the growth diets provided. This goes against the common belief of many farmers that remain skeptical regarding food consumption by shrimp when offered diets with very low or no inclusion of fish meal. Results of this trial are an important element in propelling the discussing of how can feed attractants and other additives or manipulation of the physical properties of feed can be employed as a vehicle to improve feeding efficiency by reducing the feed consumption time.

In conclusion, the integration of automatic systems in aquaculture has been one of the main trends in aquaculture for the last two decades but has been particularly evident with the expansion of passive acoustic feeders in shrimp production during the last decade. It is clear that technological development of dedicated devices and software, as well as integration of artificial intelligence systems will continue to be a pivotal driving factor of sustainable, highly efficient, intensive aquaculture. While continuous data collection from both new and already operating systems will more than likely be the main reason for performance optimization of automatic systems across the board, there are a few topics that we believe could play an important role in the evolution of passive acoustic technology in shrimp farming in the future. As these systems continue to make their mark on the industry, it is expected that more research will likely continue to be done in smaller systems. This will provide data regarding both physicochemical properties of feed as well as attractants and its expansion of acoustic profiling data on smaller individuals in order to optimize currently available or future acoustic feeding systems to a wider spectrum of shrimp life cycle stages and sizes.

1. References

Coves, D., Beauchaud, M., Attia, J., Dutto, G., Bouchut, C., Begout, M.L., 2006. Long-term monitoring of individual fish triggering activity on a self-feeding system: An example using European sea bass (*Dicentrarchus labrax*). Aquaculture. 253, 385-392.

Davis, D.A., Amaya, E., Venero, J., Zelaya, O., Rouse, D.B., 2006. A case study on feed management to improving production and economic returns for the semi-intensive pond production of *Litopenaeus vannamei*, Avances en Nutrición Acuícola VIII. Memorias del Octavo Simposio Internacional de Nutrición Acuícola. Universidad Autonoma de Nuevo Monterrey, Nuevo León, México.

Davis, D.A., Ullman, C., Rhodes, M., Novriadi, R., Swanepoel, A., 2018. Automated Feeding Systems in Pond Production of Pacific White Shrimp. Global Aquaculture Advocate.

Jescovitch, L.N., Ullman, C., Rhodes, M., Davis, D.A., 2018. Effects of different feed management treatments on water quality for Pacific white shrimp *Litopenaeus vannamei*. Aquac. Res. 49, 526-531.

Napaumpaipom, T., Chuchird, N., Taparhudee, W., 2013. Study on the Efficiency of Three Different Feeding Techniques in the Culture of Pacific White Shrimp (*Litopenaeus vannamei*). Journal of Fisheries and Environment. 37, 8-16.

Peixoto, S., Soares, R., Davis, D.A., 2020. An acoustic based approach to evaluate the effect of different diet lengths on feeding behavior of *Litopenaeus vannamei*. Aquacultural Engineering. 91.

Peixoto, S., Silva, J. F., Soares, R., Hamilton, S., Morey, A., Davis, D. A., 2020. The role of acoustics in shrimp feeding activity. in: Aquaculture America, H., Hawaii, USA (Ed.).

Pinkiewicz, T.H., Purser, G.J., Williams, R.N., 2011. A computer vision system to analyse the swimming behaviour of farmed fish in commercial aquaculture facilities: A case study using cage-held Atlantic salmon. Aquacultural Engineering. 45, 20-27.

Rillahan, C., Chambers, M., Howell, W.H., Watson, W.H., 2009. A self-contained system for observing and quantifying the behavior of Atlantic cod, *Gadus morhua*, in an offshore aquaculture cage. Aquaculture. 293, 49-56.

Silva, J.F., Hamilton, S., Rocha, J.V., Borie, A., Travassos, P., Soares, R., Peixoto, S., 2019. Acoustic characterization of feeding activity of *Litopenaeus vannamei* in captivity. Aquaculture. 501, 76-81.

Smith, D.V., Tabrett, S., 2013a. The use of passive acoustics to measure feed consumption by *Penaeus monodon* (giant tiger prawn) in cultured systems. Aquacultural Engineering. 57, 38-47.

Smith, D.V., Shahriar, M.S., 2013b. A context aware sound classifier applied to prawn feed monitoring and energy disaggregation. Knowl-Based Syst. 52, 21-31.

Ullman, C., Rhodes, M.A., Davis, D.A., 2019a. Feed management and the use of automatic feeders in the pond production of Pacific white shrimp *Litopenaeus vannamei*. Aquaculture. 498, 44-49.

Ullman, C., Rhodes, M., Hanson, T., Cline, D., Davis, D.A., 2019b. Effects of Four Different Feeding Techniques on the Pond Culture of Pacific White Shrimp, *Litopenaeus vannamei*. Journal of the World Aquaculture Society. 50, 54-64.

LITERATURE CITED


Amaya, E.A., Davis, D.A., Rouse, D.B., 2007. Replacement of fish meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*) reared under pond conditions. Aquaculture. 262, 393-401.

Bondad-Reantaso, M.G., Subasinghe, R.P., Josupeit, H., Cai, J.N., Zhou, X.W., 2012. The role of crustacean fisheries and aquaculture in global food security: Past, present and future. J Invertebr Pathol. 110, 158-165.

Boyd, C.E., Tucker, C.S., 1992. Water quality and pond soil analyses for aquaculture. Alabama Agricultural Experiment Station, Auburn University.

Carvalho, E.A., Nunes, A.J., 2006. Effects of feeding frequency on feed leaching loss and grow-out patterns of the white shrimp *Litopenaeus vannamei* fed under a diurnal feeding regime in pond enclosures. Aquaculture. 252, 494-502.

Casillas-Hernandez, R., Magallon-Barajas, F., Portillo-Clarck, G., Paez-Osuna, F., 2006. Nutrient mass balances in semi-intensive shrimp ponds from Sonora, Mexico using two feeding strategies: Trays and mechanical dispersal. Aquaculture. 258, 289-298.

Chatvijitkul, S., Boyd, C.E., Davis, D.A., McNevin, A.A., 2017. Embodied Resources in Fish and Shrimp Feeds. Journal of the World Aquaculture Society. 48, 7-19.

Coves, D., Beauchaud, M., Attia, J., Dutto, G., Bouchut, C., Begout, M.L., 2006. Long-term monitoring of individual fish triggering activity on a self-feeding system: An example using European sea bass (*Dicentrarchus labrax*). Aquaculture. 253, 385-392.

Cuzon, G., Lawrence, A., Gaxiola, G., Rosas, C., Guillaume, J., 2004. Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. Aquaculture. 235, 513-551.

Dall, W., Hill, B.J., Rothlisberg, P.C., Staples, D.J., 1990. The Biology of the Penaeidae. Adv Mar Biol. 27, 1-461.

Dall, W., Hill, B.J., Rothlisberg, P.C., Staples, D.J., 1990. The Biology of the Penaeidae. Adv Mar Biol. 27, 1-461.

Davis, D.A., Amaya, E., Venero, J., Zelaya, O., Rouse, D.B., 2006. A case study on feed management to improving production and economic returns for the semi-intensive pond production of *Litopenaeus vannamei*, Avances en Nutrición Acuícola VIII. Memorias del Octavo Simposio Internacional de Nutrición Acuícola. Universidad Autonoma de Nuevo Monterrey, Nuevo León, México.

Davis, D.A., Ullman, C., Rhodes, M., Novriadi, R., Swanepoel, A., 2018. Automated Feeding Systems in Pond Production of Pacific White Shrimp. Global Aquaculture Advocate.

Eaton, A.D., Clesceri, L.S., Greenberg, A.E., Franson, M.A.H., 2005. Standard methods for the examination of water and wastewater. Journal of American Public Health Association. 21, 1600.

FAO, 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome.

Galkanda-Arachchige, H., Davis, D.A., 2020. Evaluation of differently processed soybean meal products as ingredients in practical diets for Pacific white shrimp *Litopenaeus vannamei*. Aquaculture Nutrition. 26, 287-295.

Guo, J.P., Huang, Y., Salze, G., Roy, L.A., Davis, D.A., 2020. Use of plant-based protein concentrates as replacement for fishmeal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*) reared under high stocking density and low salinity conditions. Aquaculture Nutrition. 26, 225-232.

Guo, J.P., Reis, J., Salze, G., Rhodes, M., Tilton, S., Davis, D.A., 2019. Using high protein distiller's dried grain product to replace corn protein concentrate and fishmeal in practical diets for the Pacific white shrimp *Litopenaeus vannamei*. Journal of the World Aquaculture Society. 50, 983-992.

Jannathulla, R., Rajaram, V., Kalanjiam, R., Ambasankar, K., Muralidhar, M., Dayal, J.S., 2019. Fishmeal availability in the scenarios of climate change: Inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources. Aquac. Res. 50, 3493-3506.

Jescovitch, L.N., Ullman, C., Rhodes, M., Davis, D.A., 2018. Effects of different feed management treatments on water quality for Pacific white shrimp *Litopenaeus vannamei*. Aquac. Res. 49, 526-531.

Liu, G., Zhu, S.M., Liu, D.Z., Guo, X.S., Ye, Z.Y., 2017. Effects of stocking density of the white shrimp *Litopenaeus vannamei* (Boone) on immunities, antioxidant status, and resistance against *Vibrio harveyi* in a biofloc system. Fish Shellfish Immun. 67, 19-26.

Martinez-Cordova, L.R., Porchas-Cornejo, M.A., Villarreal-Colemnares, H., Calderon-Perez, J.A., Naranjo-Paramo, J., 1998. Evaluation of three feeding strategies on the culture of white shrimp *Penaeus vannamei* Boone 1931 in low water exchange ponds. Aquacultural Engineering. 17, 21-28.

Martinez-Cordova, L.R., Torres, A.C., Porchas-Cornejo, M.A., 2003. Dietary protein level and natural food management in the culture of blue (*Litopenaeus stylirostris*) and white shrimp (*Litopenaeus vannamei*) in microcosms. Aquaculture Nutrition. 9, 155-160.

McTigue, T.A., Feller, R.J., 1989. Feeding of juvenile white shrimp *Penaeus setiferus*: periodic or continuous? Marine Ecology Progress Series. 52, 227-233.

N.R.C., 2011. Nutrient requirements of fish and shrimp. National academies press. Washington, DC.

Napaumpaipom, T., Chuchird, N., Taparhudee, W., 2013. Study on the Efficiency of Three Different Feeding Techniques in the Culture of Pacific White Shrimp (*Litopenaeus vannamei*). Journal of Fisheries and Environment. 37, 8-16.

Nunes, A.J.P., Sabry-Neto, H., da Silva, F.H.P., de Oliveira-Neto, A.R., Masagounder, K., 2019. Multiple feedings enhance the growth performance and feed efficiency of juvenile *Litopenaeus vannamei* when fed a low-fish meal amino acid-supplemented diet. Aquaculture International. 27, 337-347.

Obaldo, L.G., Divakaran, S., Tacon, A.G., 2002. Method for determining the physical stability of shrimp feeds in water. Aquac. Res. 33, 369-377.

Peixoto, S., Silva, J. F., Soares, R., Hamilton, S., Morey, A., Davis, D. A., 2020. The role of acoustics in shrimp feeding activity. in: Aquaculture America, H., Hawaii, USA (Ed.).

Peixoto, S., Soares, R., Davis, D.A., 2020. An acoustic based approach to evaluate the effect of different diet lengths on feeding behavior of *Litopenaeus vannamei*. Aquacultural Engineering. 91.

Peixoto, S., Soares, R., Silva, J.F., Hamilton, S., Morey, A., Davis, D.A., 2020b. Acoustic activity of *Litopenaeus vannamei* fed pelleted and extruded diets. Aquaculture. 525.

Pinkiewicz, T.H., Purser, G.J., Williams, R.N., 2011. A computer vision system to analyse the swimming behaviour of farmed fish in commercial aquaculture facilities: A case study using cage-held Atlantic salmon. Aquacultural Engineering. 45, 20-27.

Quintero, H.E., Roy, L.A., 2010. Practical Feed Management in Semi-Intensive Systems for Shrimp Culture. The Shrimp Book, edited by Alday-Sanz, V., 443-454. Nottingham, UK: Nottingham University Press. 443-453.

Ray, A.J., Lewis, B.L., Browdy, C.L., Leffler, J.W., 2010. Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. Aquaculture. 299, 89-98.

Reis, J., Novriadi, R., Swanepoel, A., Guo, J.P., Rhodes, M., Davis, D.A., 2020. Optimizing feed automation: improving timer-feeders and on demand systems in semi-intensive pond culture of shrimp *Litopenaeus vannamei*. Aquaculture. 519.

Reymond, H., Lagardere, J.P., 1990. Feeding Rhythms And Food Of *Penaeus japonicus* Bate (CRUSTACEA, Penaeidae) In Salt-Marsh Ponds - Role Of Halophilic Entomofauna. Aquaculture. 84, 125-143.

Rillahan, C., Chambers, M., Howell, W.H., Watson, W.H., 2009. A self-contained system for observing and quantifying the behavior of Atlantic cod, *Gadus morhua*, in an offshore aquaculture cage. Aquaculture. 293, 49-56.

Roy, L.A., Davis, D.A., Saoud, I.P., Boyd, C.A., Pine, H.J., Boyd, C.E., 2010. Shrimp culture in inland low salinity waters. Reviews in Aquaculture. 2, 191-208.

Roy, L.A., Davis, D.A., Whitis, G.N., 2012. Effect of feeding rate and pond primary productivity on growth of *Litopenaeus vannamei* reared in inland saline waters of West Alabama. North American Journal of Aquaculture. 74, 20-26.

Samocha, T.M., Davis, D.A., Saoud, I.P., DeBault, K., 2004. Substitution of fish meal by co-extruded soybean poultry by-product meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture. 231, 197-203.

Santos, A.D., Lopez-Olmeda, J.F., Sanchez-Vazquez, F.J., Fortes-Silva, R., 2016. Synchronization to light and mealtime of the circadian rhythms of self-feeding behavior and locomotor activity of white shrimps (*Litopenaeus vannamei*). Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology. 199, 54-61.

Shepherd, C.J., Jackson, A.J., 2013. Global fishmeal and fish-oil supply: inputs, outputs and markets. J Fish Biol. 83, 1046-1066.

Silva, J.F., Hamilton, S., Rocha, J.V., Borie, A., Travassos, P., Soares, R., Peixoto, S., 2019. Acoustic characterization of feeding activity of *Litopenaeus vannamei* in captivity. Aquaculture. 501, 76-81.

Smith, D.V., Shahriar, M.S., 2013b. A context aware sound classifier applied to prawn feed monitoring and energy disaggregation. Knowl-Based Syst. 52, 21-31.

Smith, D.V., Tabrett, S., 2013a. The use of passive acoustics to measure feed consumption by *Penaeus monodon* (giant tiger prawn) in cultured systems. Aquacultural Engineering. 57, 38-47.

Soares, R., Peixoto, S., Davis, R. P., & Davis, D. A., 2021a. Feeding behavior and growth of *Litopenaeus vannamei* fed soybean-based diets with added feeding effectors. Aquaculture.

Soares, R., Peixoto, S., Galkanda-Arachchige, H. S., & Davis, D. A., 2021b. Growth performance and acoustic feeding behavior of two size classes of *Litopenaeus vannamei* fed pelleted and extruded diets. Aquaculture International, 1-17.

Sookying, D., Davis, D.A., 2011a. Pond production of Pacific white shrimp (*Litopenaeus vannamei*) fed high levels of soybean meal in various combinations. Aquaculture. 319, 141-149.

Sookying, D., Silva, F.S.D., Davis, D.A., Hanson, T.R., 2011b. Effects of stocking density on the performance of Pacific white shrimp *Litopenaeus vannamei* cultured under pond and outdoor tank conditions using a high soybean meal diet. Aquaculture. 319, 232-239.

Tacon, A.G., Jory, D., & Nunes, A., 2013. Shrimp feed management: issues and perspectives. in: Hasan, M.R.N., M.B. (Ed.), On-farm feeding and feed management in aquaculture. Food and Agriculture Organization of the United Nations, Rome, pp. 481-488.

Tacon, A.G.J., Forster, I.P., 2003. Aquafeeds and the environment: policy implications. Aquaculture. 226, 181-189.

Tacon, A.G.J., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture. 285, 146-158.

Ullman, C., Rhodes, M., Hanson, T., Cline, D., Davis, D.A., 2019b. Effects of Four Different Feeding Techniques on the Pond Culture of Pacific White Shrimp, *Litopenaeus vannamei*. Journal of the World Aquaculture Society. 50, 54-64.

Ullman, C., Rhodes, M.A., Davis, D.A., 2019a. Feed management and the use of automatic feeders in the pond production of Pacific white shrimp *Litopenaeus vannamei*. Aquaculture. 498, 44-49.

Ullman, C., Rhodes, M.A., Davis, D.A., 2019a. Feed management and the use of automatic feeders in the pond production of Pacific white shrimp *Litopenaeus vannamei*. Aquaculture. 498, 44-49.

Ullman, C., Rhodes, M.A., Davis, D.A., 2019a. The effects of feed leaching on the growth of Pacific white shrimp *Litopenaeus vannamei* in a green-water tank system. Aquac. Res. 50, 3074-3077.

Van, T.P.T.H., Rhodes, M.A., Zhou, Y., Davis, D.A., 2017. Feed management for Pacific white shrimp Litopenaeus vannamei under semi-intensive conditions in tanks and ponds. Journal of Aquaculture Research. 48, 5346-5355.

Varadharajan D., P.N., 2013. Food and feeding habits of aquaculture candidate a potential crustacean Pacific white shrimp *Litopenaeus vannamei*. Aquac Res Development. 4, 1-5.

Velasco, M., Lawrence, A.L., Castille, F.L., 1999. Effect of variations in daily feeding frequency and ration size on growth of shrimp, *Litopenaeus vannamei* (Boone), in zero-water exchange culture tanks. Aquaculture. 179, 141-148.

Wassenberg, T.J., Hill, B.J., 1987. Natural diet of the tiger prawns *Penaeus esculentus* and *P. semisulcatus* Australian Journal of Marine and Freshwater Research. 38, 169-182.