Performance of Early Juvenile Giant River Prawns (*Macrobrachium rosenbergii*) Fed Fish, Soybean, Shrimp and Four Insect Based Diets While Under Low Temperature Stress

Malcolm L. McCallum
School of Agriculture and Applied Sciences, Langston University, Langston, Oklahoma 73050

Samad D. Weston
Department of Agriculture and Natural Resources, Langston University, Langston, Oklahoma 73050

Yonathan Tilahun
School of Agriculture and Applied Sciences, Langston University, Langston, Oklahoma 73050

Abstract: Current guidelines for *Macrobrachium rosenbergii* advise introduction into outdoor ponds when water is > 16º C. However, little is known about possible interactions between diet quality and temperature tolerance. We examined use of seven rations for prawns raised at suboptimal temperatures. Juvenile prawns readily consumed all seven rations presented. Growth and survivorship were best on shrimp- and silkworm-based rations; whereas, the poorest growth was on fishmeal- and Black Soldier Fly larvae meal (BSFLM)-based rations. Soybean-, cricket-, mealworm-, and fishmeal-based diets had similar survivorship. Mortality occurred within 24 hr after each feeding of the BSFLM-based diet, possibly related to antimicrobial compounds produced by BSFL. Prawns raised at lower temperatures may require better quality feed compared to guidelines based on previous studies at ideal temperatures. Producers must determine whether it is better to stock prawns at higher rates or feed more expensive rations to manage survivorship of juvenile at lower temperatures.

Introduction

Ectotherms face continuous challenges imparted by interactions between the kinetics of their physiology and the quality of their diets. The rates at which stress response, pathogen defense, digestion, and growth ensue are directly tied to ambient temperature. This is a problem for outdoor production of the Giant River Prawn (*Macrobrachium rosenbergii*) in temperate regions. This is a tropical species of significant commercial importance that is native to rivers of the East Asian continent and surrounding Islands (New 1990; New 2002; New 2005). Temperatures of 26 – 31ºC (Sabdifer and Smith 1985) or 25 - 32ºC (Zimmermann 1998) are considered ideal for growth (New 1990; Satapornvanit 2006). The critical thermal minima for this species when acclimated to temperatures of 20 - 30ºC are 10 – 16.24ºC for postlarvae and 10.5 – 16.98ºC for juveniles (Herrera et al. 1998; Manush et al. 2004). Food consumption is significantly reduced when temperatures fall below 25ºC (Newman et al. 1982). Prawns start to manifest a physiologically compromised state with acute or slow changes in temperature below 15ºC and are severely compromised with acute drop from 20ºC to 10ºC (Chung et al. 2012). Prawns raised for seven days under exposure to *Lactococcus garvieae* at 20ºC demonstrate low phagocytic activity, tolerance to ammonia and salinity (Cheng et al. 2003) and impeded immunological responses due to a sudden drop in temperature from their thermal ideal (28ºC) to 22ºC (Chang et al. 2015). Mortality is substantially elevated as temperatures fall below 12ºC (Satapornvanit 2006). Urea-N excretion is minimal at 17ºC and rises with temperature (Chen and Kou 1996). Growth nearly doubles for every 5ºC increase in temperature from 20 to 36ºC, without an appreciable change in conversion ratio (Faranfarmaian and Moore 1978). However, growth is more erratic at lower temperatures (Whangchai et al. 2007). Guidelines suggest cultivation temperatures should be above 16ºC (Herrera et al. 1998; New 2002). However, observations in research settings generally demonstrate higher survivorship than actually observed in commercial settings (Tidwell et al. 2005).

Diets resulting in higher survival and growth also tend to impart better responses to stressors (Racotta et al. 2003). Research with prawns has related temperature stress effects on neuroendocrine responses (Chang et al. 2015), oxygen consumption (Farmanfarmaian and Moore 1978; Chen and Kou 1996; Niu et al. 2003; Manush et al. 2004), nitrogen excretion (Chen and Kou 1996), pH and ion balance (Cheng et al. 2003), food consumption (Niu et al. 2003), growth (Farmanfarmaian and Moore 1978; Niu et al. 2003), and survivorship (Herrera et al. 1998; Chung et al. 2012; Manush et al. 2004). Additional nutritional studies with the *M. rosenbergii* are numerous; however, little work has approached the currently growing interest in more sustainable fish meal replacements such as insect meal.

Oklahoma has never had a prawn industry in the state despite apparent suitability of its climate and unique aquaculture infrastructure characteristics (McCallum and Tilahun [In Press]). Guidelines for earliest dates to stock ponds in Oklahoma are lacking. We set up a laboratory experiment comparing performance of juvenile freshwater prawns fed seven different diets comprising corn meal and selected protein feedstuffs. The study was conducted at temperatures comparable to those observed in surface waters in Oklahoma in late April through early May to help formulate guidelines for outdoor pond culture of prawns for Oklahoma producers and to test potential interactions between temperature, stress and diet. We hypothesized that prawns would grow slower at cooler temperatures and have lower survivorship than typically expected at higher temperatures, and that prawns may perform better on arthropod- and crustacean-based protein sources than on soybean or fishmeal diets. We predicted that growth and survivorship would be higher when fed arthropod- and crustacean-based protein sources than on fishmeal or soybean meal.

Materials and Methods

Prawns were obtained from Stickfin's Fish Farm (St. Augustine, Florida) and housed communally for one week after arrival in aerated 40-L aquaria containing moderately hard synthetic freshwater made with reagent grade chemicals (U.S. E.P.A. 2002). Following that week, 105 prawns were individually housed in 125 ml Pyrex Erlenmeyer flasks containing 100 ml of synthetic freshwater. An inverted Pyrex 25 ml flask was inserted into the neck of each larger flask to prevent contamination from airborne particulate. Each prawn was placed in a weigh dish and excess water removed using a transfer pipette and then weighed on an electronic analytical balance to the nearest 0.001 g. Next, each prawn was then placed in its flask where it resided for the duration of the study (28 days).

Water quality was monitored weekly and water was changed if it became cloudy or otherwise fouled. The pH (7.4 – 7.8), hardness (160 – 180 $CaCO_3$/L), and alkalinity (57 – 64 $CaCO_3$/L) never exceeded the typical range for moderately hard synthetic freshwater (U.S. E.P.A. 2002). Temperature was maintained at 21ºC throughout the study.

We obtained commercial fish meal and soybean meal from a local feed store (Stillwater Milling Company, Stillwater, Oklahoma). Dried silkworm pupae (*Bombyx mori*), mealworm larvae (*Tenbrio molitor*), "river" shrimp from Chubby Mealworms (Las Vegas, Nevada) and dried crickets (*Acheta domesticus*) came from Fluker Farms (Port Allen, Louisiana). Dried black soldier fly larvae (*Hermetia illucens*) came from Enviroflight, LLC (Yellow Springs, Ohio). Feedstuffs were ground and mixed with a household coffee grinder. Rations were balanced on a 35% protein basis (Table 1; New 2002; although even higher protein levels are effective [Millikin et al. 1980]) using bakery grade yellow corn meal (Quaker Oats Company, Chicago, Illinois). Prawns (n_{total} = 105 prawns, n = 15 prawns/treatment) were fed one of each ration (n = 7 treatments) *ad libitum* throughout the study, although the quantity varied according to bodyweight. Guidelines suggest prawns should be fed 10-20% of the total bodyweight (New 2002), but this is based on bulk weights, not individual measures. Because of this, it was impractical to feed such small quantities at the onset of the study. Each prawn received 15% of its last measured bodyweight. If food remained after 24 hr, we would reduce the quantity by 10% as fed on the next feeding. If food was consumed after 24 hr, we increased the feeding rate by 10% as fed, and added the additional quantity to the flask. Food was delivered by sprinkling the dried meal on the water surface.

If food settled on the bottom began to mold, we siphoned it off with a transfer pipette for disposal.

Daily observations of molting and mortality were recorded. At the end of the study, all remaining prawns were weighed. Growth data were tested for normality using an Anderson-Darling test. We compared the weight gain between treatments using a one-way ANOVA with a Tukey means comparison test. Survivorship and molting frequency was compared between groups using Chi Square. We used an alpha = 0.05 to assess significance.

Results

In all cases, prawns readily consumed food when presented and could be seen feeding on remaining food particles until the next feeding. None of the prawns consumed 100% of the food offered after 24 hrs, with obvious remnants of food typically remaining for days. Prawns did not perform equally well on all rations. Growth ($F_{6, 104} = 4.69$, $P < 0.001$) varied among treatments (Table 2, 3). Growth data were not normally distributed ($A^2 = 16.56$, $P < 0.001$) so we transformed these data using the normalize function in MiniTab 13.0. Prawns gained weight better on shrimp (-0.060, -1.708) and silkworm pupae (-0.040, -1.688) than on fishmeal. They also gained better on shrimp (-0.303, -1.9506) and silkworm pupae (-0.283, -1.931) than on black soldier fly larvae. No other significant differences in growth were observed.

There were marginal differences in survivorship ($\chi^2 = 10.81$, df = 6, P = 0.094) among treatments. Prawns survived equally well when fed fish meal, soybean meal, ground mealworms and ground crickets ($\chi^2 = 4.42$, df

Table 1. Nutritional breakdown on an as fed basis of the eight ingredients used to formulate diets for juvenile freshwater prawns (*Macrobrachium roosenbergii*).

	Fish meal	Soybean meal	Shrimp meal	Cricket meal	Black Soldier Fly meal	Silkworm pupae meal	Mealworm meal	Corn meal
DM %	90.12	91.26	92.40	93.0	95.7	94.0	94.8	88.0
Protein %	70	40	50	64	37	45	53.6	7.4
Fat %	14.82	18.48	13.13	19.0*	14.2*	29%	29.2	1.8
Gross energy MJ/KG DM	21.9	19.7	19.1	21.8*	23.8	25.8**	24.4**	13.8
Crude Fiber %	2.22%	7.10%	1.2%	8.5	7.0%	6%	18.5	7.4%

*Finke 2002

**http://feedipedia.org

Table 2. Performance of juvenile Giant Freshwater Prawns (*Macrobrachium rosenbergii*) on seven diets at the lower threshold of thermal tolerance.

Protein source	Starting BM (g) Mean (SE)	Ending BM* (g) Mean (SE)	Growth* (g) Mean (SE)	Molts (n)	Survivorship (%)
Fish meal	0.020 (0.002)	0.022 (0.002)	0.002 (0.001)	4	6.7
Soybean meal	0.020 (0.002)	0.028 (0.004)	0.008 (0.004)	4	33
Shrimp	0.025 (0.002)	0.039 (0.004)	0.015 (0.003)	8	86.7
Silkworm	0.018 (0.001)	0.033 (0.006)	0.016 (0.006)	10	66
Mealworm	0.030 (0.002)	0.035 (0.003)	0.014 (0.009)	6	40
Cricket	0.023 (0.002)	0.025 (0.003)	0.003 (0.001)	13	40
Black Soldier Fly	0.026 (0.002)	0.027 (0.002)	0.0003 (0.0003)	3	6.7
Overall	0.023 (0.001)	0.030 (0.001)	0.008 (0.002)	48	42

*Ending body mass (BM) and growth are the averages for those that survived to the end of the study; whereas, starting BM includes the entire starting population.

Table 3. ANOVA table for the response of growth to different foods.

Source	DF	SS	MS	F	P
Food type	6	15.778	2.630	4.69	< 0.001
Error	98	54.997	0.561		
Total	104	70.775			

= 4, P = 0.352). They performed just as well when fed ground shrimp or silkworm pupae (χ^2 = 0.196, df = 1, P = 0.158). Prawns had higher survivorship when fed shrimp meal or silkworm pupae meal than when fed black soldier fly or fish meal (χ^2 =10.84, df = 3, P = 0.013). There were no observed differences in molting frequency (χ^2 = 5.64, df = 6, P = 0.464) among treatments.

Within 24 hours of first feeding black soldier fly larvae to prawns, 27% died; whereas, the other feeds had 93 – 100% survivorship after 24-hr (Total 24-hr survivorship on other feeds = 97.8%). Mortality rose to 40% by the fifth day when fed the Black Soldier Fly larvae meal-based ration. After seven days, remaining food particles (mostly corn meal) were removed from all flasks, and new feed introduced. Then, 24-hr after being again fed the Black Soldier Fly larvae meal-based ration, another 47% of prawns died, with a total mortality of 93.3% in this treatment. The single remaining prawn survived until the end of the study, but its growth was negligible.

Discussion

Temperature and nutritional stress manifest in physiological trade-offs for ectotherms, and our results provide evidence that when raised at suboptimal temperatures freshwater prawns appear to become more sensitive to food quality compared to previous studies performed at ideal temperatures. Wild prawns feed on aquatic invertebrates, detritus and algae (Balaz and Ross 1976). Previous studies on nutrition of prawns were conducted within the thermal optima for this species and largely determine fishmeal and soybean meal to be adequate protein feeds (Hasanuzzaman et al. 2009; Gupta et al. 2007; Koshio et al. 1992) although reports as low as 11% survivorship on fishmeal are known (Kumlu 1999). Addition of shrimp oil to a balanced diet doubled the final biomass of prawns (Sandifer and Joseph 1976). Further, post-larvae fed entirely on *Artemia* nauplii perform well (Barros and Valenti 2003; New 2002). In general, prawns performed acceptably on proven protein sources (shrimp meal, soybean meal) but growth and survivorship on less suitable feeds (fishmeal, Black Soldier Fly larvae meal) was less impressive. Arginine, one of three key amino acids thought to be important to prawns (e.g., Methionine, Lysine [D'Ambramo and Sheen 1994]) is generally more abundant in soybean and shrimp meals than fish meal (Watts 1968); however, amino acid requirements have been difficult to elucidate (Reed and D'Abramo 1989).

Differences in survivorship and growth may be explained by changes in feeding behavior, more efficient digestion, and more effective stress responses when temperatures are warm compared to suboptimal. The time prawns spend feeding is known to decline with temperature (Niu et al. 2003). Consequently, when at optimal temperatures, prawns may increase consumption sufficiently to overcome the minor inadequacies of amino acid composition. However, when housed at sub-optimal temperatures, feeding behavior is suppressed and they cannot fully accommodate for reduced limiting nutrient supply, leading to poorer performance. Further work is needed to elucidate amino acid needs of prawns (Mukhopadhyay et al. 2003; D'Ambramo and Sheen 1994) so that informed supplementation with imperfect feeds is possible.

Physiological processes in all ectotherms are tied to the ambient temperature (Wilmer et al. 2004; Manush et al. 2004; Manush et al. 2006), including digestion in prawns (Kumlu 1995; Newman et al. 1982). Each of these ingredients require different residency times and enzyme assemblages to digest and absorb, and each enzyme operates in an ideal temperature range. It is likely that digestive enzyme activity in prawns was suppressed by lower temperatures, leading to inefficient digestion of less perfect feeds. Further, as a kinetically controlled biochemical process, production of non-essential amino acids is altered at lower temperatures. Combined with feeding behavior, digestion and biochemical processes could explain why prawns fed fish, soybean and shrimp meal perform well at optimal temperatures; whereas, survival and growth is much reduced at cooler temperatures.

Unlike other ingredients in this study, freeze dried black soldier fly larvae appear unsuitable for feeding prawns, at least at suboptimal temperatures. Significant mortality occurred within 24 hours of feeding fly meal to the prawns. Black soldier flies are known to harbor compounds with antimicrobial properties (i.e., defensin-like peptide4, a 40 amino acid AMP; Elhag et al. 2017; Park et al. 2015) sufficiently powerful to suppress growth of *Escherichia coli*, *Salmonella* spp., antibiotic resistant *Staphylococcus aureus*, and gram positive *Pseudomonas aeruginosa* (Lalander et al. 2015; Liu et al. 2008). Their excretions are also known to inhibit growth in the larvae of other dipterids (Bradley and Sheppard 1984). This insect has been fed successfully to poultry (Cullere et al. 2016; Elwert et al. 2010; Dluokun 2000), livestock (Veldkamp and Bosch 2015; Veldkamp et al. 2012), dogs and cats (Bosch et al. 2014), and fish (Shakil Rana et al. 2015; Tran et al. 2015; Sealey et al. 2011; St-Hilaire et al. 2007).

Previous studies of black soldier fly larvae meal for decapod diets exist, though not for *M. roosenbergii*. Growth of juvenile white shrimp (*Litopenaeus vannamei*) was increasingly suppressed as black soldier fly larvae meal became a higher proportion of the diet (Cummins et al. 2017). Weight gain, final body weight, feed conversion ratio, and specific growth rate of shrimp became less acceptable as the dietary component of black soldier fly larvae meal increased. Survivorship of these shrimp was much higher than in our study (~91% vs. 6.7%). Other than the species involved, the primary differences in these two studies is that ours involved much younger/ smaller animals (initial prawn BW = 0.023 g +/- 0.001 vs. initial shrimp BW = 1.24 +/- 0.01 g), diets with a much larger component of black soldier fly meal, smaller housing (100 ml vs. 110 L), individualized housing (vs. communal), and we maintained a lower temperature (21ºC vs 29.5ºC). These differences provide several avenues for the vastly different survivorship levels. The younger prawns may have less developed functional immune/stress response systems. Stress and immune responses are known to undergo ontogenetic changes as an organism grows (Manning and Turner 1976). The larger soldier fly component in the feed should deliver a larger dose of antimicrobial chemicals. The larger housing and resultant larger water volume used with white shrimp may be sufficient to dilute feed-borne compounds in the water column to non-toxic levels. The differences in temperature may also be a significant factor. Prawns were already under thermal and potentially nutritional stress. These two stressors are known to participate in a trade-off system that provides added risk to additional stressors (Padmanabha et al. 2011; Cotter et al. 2011; Karasov et al. 2007). Considering that as an ectotherm, all physiological processes are bound to thermal optima (Wilmer et al. 2004), the stress response of prawns may be compromised by low temperature making them more susceptible to chemical stressors like those produced by soldier fly larvae. A confounding element to these results is that we did not provide perches for molting behavior. This may have provided across-the-board higher mortality (McCallum et al. 2018).

Black soldier fly has also been proposed for use in human diets (Wang and Shelomi 2017; Dossey and Morales-Ramos 2016; van Huis et al. 2015; Boland et al. 2013). The recent findings above suggest that incorporation of black soldier fly meal into the human food chain should be done with great consideration. The compounds black soldier fly larvae produce could lead to new strains of microbes afflicting humans and animals that are highly resistant to antibiotics. Further, there has been no testing to determine if these compounds have long-term health effects for the organisms ingesting them, or if these compounds could be transferred from food animals to humans. Until such studies are undertaken, it seems prudent to avoid using this protein source for humans or animals intended for human consumption. Other insects in this study do not appear to hold such risks. Thus far, we have found little evidence that the scientific community has considered the risks associated with antimicrobial compounds in black soldier flies. It has been more focused on heavy metal accumulation in the larvae, microbial decontamination prior to processing, and food allergies (Rouge and Barre 2017; Wang and Shelomi 2017).

Fish, shrimp, and soybean meals are among the most commonly used protein sources in animal feeds, including prawns (Hasanuzzaman et al. 2009; Koshio et al. 1992). Fish and shrimp meal continue to be sourced from wild fisheries and are increasingly expensive (Carter and Hauler 2000), thus discounting the sustainability of aquaculture operations (Love et al. 2014; Gatlin et al. 2007). Further, they can harbor contaminants from the wild environment (Costa 2007; Dorea 2006; Hardy 2002; Galindo-Reyes et al. 1999). Using soybean meal eliminates dependence on wild stocks for protein and reduces the contaminant problem, but brings an array of other environmental issues connected to crop farming such as pesticide use (van Meter et al. 2018; Mitsch et al. 2001; Pimentel et al. 1993). Soybean meal also contains phytoestrogens (Coward et al. 1993), which may adversely impact invertebrate reproductive potential, growth and development (Jefferson et al. 2005; Ryokkynen and Kukkonen 2006; McCallum et al. 2013). Hence, there are good reasons to desire alternatives to these two products, especially in aquaculture and aquaponics, the latter of which prides itself on being sustainable (Forchino et al. 2017; Konig et al. 2016; Goddek et al. 2015). If the freshwater prawn industry is positioned as a sustainable alternative to wild-caught shrimp, it can carve a larger place in the market and likely draw higher prices. Abandoning less-sustainable feed ingredients is an important step to reaching this goal (Sánchez-Muros et al. 2014). Although in its infancy, carefully selected and prepared insect meals may serve this purpose (Rumpold and Schlüter 2013).

The performance of prawns at temperatures similar to those in April – May in Oklahoma suggest that if producers intend to stock ponds that early, they will need to feed very high quality feeds or stock ponds at higher levels than typically recommended to ensure a harvestable product. Alternatively, producers could cover small ponds with greenhouse structures to extend the season (Pillai et al. 1999), restrict production to indoor recirculating or aquaponics facilities, or wait until later in the year when water temperatures are more suitable to stock ponds with juvenile prawns.

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