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Effect of Frozen Zooplankton Feed on Growth and Reproductive Performance of Crayfish (*Procambarus clarkii*)

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The objectives of this study were to compare the effects of frozen zooplankton as feeds on the growth and reproductive performance in crayfish *Procambarus clarkii*. Four trial feeds such as commercial feed (CF), two frozen Fairy shrimps; *Branchinella thailandensis* (FB), *Streptocephalus sirindhornae* (FS), and frozen water flea *Moina macrocopa* (FM) were investigated. Completely Randomized Design (CDR) (with 6 replications was used for the 90 day experiments. Average initial weight of crayfish was 5.80±0.17 g. Fecundity and growth performance of the crayfish were significantly different (P<0.05). Crayfish fed with FS had the highest fecundity and Gonadosomatic Index (GSI) values, followed by FB, CF and FM as feeds (with fecundity values were 696.42±84.77, 568.19±71.45, 372.52±88.60, and 305.06±62.95 eggs/female, respectively). GSI were 21.30±5.30 ,15.97±4.95 ,12.38±4.53, and 10.46±4.64 %, respectively. Growth performance of crayfish fed with FB had the highest specific growth rates followed by CF, FS and FM as feeds (with 0.98±0.17 ,0.93±0.23, 0.93±0.11, and 0.76±0.13 % day^{-1}, respectively). This research suggests that the frozen Fairy shrimp can be used in crayfish culture to improve reproductive and growth performance.

Keywords: Crayfish, fecundity, growth, Gonadosomatic Index (GSI).

Introduction

The red swamp crayfish (*Procambarus clarkii*) is a native to the southeastern United States. This crayfish has become established worldwide through accidental and intentional introductions by humans (Quan *et al*., 2014). The North American crayfish is one of the most widely introduced freshwater species in the world, especially due to its high economic importance as a shellfish delicacy (Loureiro *et al*., 2015). This crayfish is also popular in the live ornamental aqua trade market. This species is strikingly red in color that caused its commercial advertisement as freshwater "lobster" for aquaria (Simon

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et al., 2005). As yet, in Thailand the crayfish *P. clarkii* is popular as an ornamental invertebrate attraction for this hobby. It is gaining economic importance for ornamental aquaculture.

Growth and reproduction are two important characteristics expressing fitness and adaptation of a species to its environment. A profitable harvest from the field or from aquaculture can be expected from fast growth and successful reproduction (Guan and Wiles, 1999). We used the crayfish *P. clarkii* to improve these endpoints by the experimental evaluation of different feeds.

For example, live feeds supply necessary nutrients for crayfish development and can contribute digestive enzymes from food organisms that aid in digestion (Barros and Valenti, 2003; Dahms *et al*., 2007a). Gutiérrez-Yurrita *et al*. (1998) reported that both immature and adult size-classes of *P. clarkii* in the Doñana National Park ingested mainly plants and organic detritus, but a higher proportion of animal food originates from zooplanktonic cladocerans, insect larvae (mayflies, chironomids), snails, and microcrustaceans with the exception of ostracodes (Paloma *et al*., 2004). Frozen feed or live feed, e.g., *Moina* sp., *Artemia* spp. and fairy shrimp can be used as live-food based nutrition for aquaculture organisms. The possibility of using fairy shrimps as feed for giant freshwater prawn culture was suggested by Velu and Munuswamy (2008) and Sriphutorn and Sanoamuang (2011). Alive adult fairy shrimp (*Streptocephalus sirindhornae*) improve the growth and enhancement of the carotenoid content in freshwater prawn. They can be used as suitable feeds for aquatic animals such as ornamental fish and giant freshwater prawn (Sornsupharp *et al*., 2013). The nutritional importance of fairy shrimps in terms of protein and as a carotenoid source for fish and prawn has been highlighted because of their high individual biomass, high reproductive rate, and rapid growth (Dararat *et al*., 2012). Sornsupharp *et al*. (2015) studied the digestibility of dried fairy shrimp (*S. sirindhornae*) as a feed ingredient for flower-horn fish and found that dried fairy shrimp meal is a suitable food for smaller sized fish. Success in aquaculture is based on various criteria, where selection of suitable feeds becomes increasingly important (Velu and Munuswamy, 2007). Sornsupharp and Sornsupharp (2016) used frozen fairy shrimp *Branchinella thailandensis* as a carotenoid source for crayfish. They found that growth performance of crayfish were not significant compared to commercial food, frozen midge and frozen cladocerans. So, if live food should not be available frozen food should be used instead of live food in aquaculture.

Objectives: We were investigating the effect of frozen zooplankton as feed on reproductive and growth performance of the red swamp crayfish *P. clarkii*.

Materials and methods

Experimental trials

Crayfish (*P. clarkia*) were purchased form an ornamental aqua shop in Buriram Province, Thailand. The crayfish were acclimatized to laboratory conditions at the Department of Fisheries, Buriram Rajabhat University. Complete Randomized Design (CRD) experiments were performed for 4 different feeds: ornamental commercial shrimp pellet feed (CF); two fairy shrimp species as frozen food: *B. thailandensis* (FB) and *S. sirindhornae* (FS), and frozen water fleas, *Moina macrocopa* (FM). The crayfish were fed with these different food sources for 90 days in 40 L PE tanks. Crayfish (n=72) with an initial weight of 5.80±0.17 g were thus fed in four treatments with six replicates each. Water siphoning was exchanged every day (10%) to remove remaining feces from the previous day. After water exchange, live feed was offered at 10% of total crayfish body weight at 8:00 h and 16:00 h. At the end of the v life history parameters and weight of crayfish was determined, as: growth rate, weight gain, specific growth rate, survival rate, GSI, and fecundity.

Experimental data

Growth and reproductive performance were recorded during the 90 days trial. At the end of the experiment, the wet weight of crayfish was measured according to Chettri *et al.* (2007) and Carmona-Osalde *et al.* (2004). GSI and fecundity was calculated following Xu *et al.* (2010) and Peruzza *et al.* (2015).

Weight Gain (%) = [(final weight – initial weight)/initial weight] x 100

Specific Growth Rate; SGR (%/day) = [($\log_e$ final weight – $\log_e$ initial weight)/time] X 100

Survival Rate (%) = [(initial number – final number)/initial number] X 100

GSI = (weight of the gonad/total body weight) X 100

Fecundity = (weight of the gonad X egg number of weight of gonad sample)/ weight of sample gonad.

Statistical analysis

All data were subjected to statistical analysis using one-way analysis of variance. Significant differences between means were evaluated by Duncan Multiple Range Test (Steel and Torrie, 1980). Differences were determined as being significant at the 95 % level ($P< 0.05$).

Results

Growth performances

Growth performances of crayfish fed with different feeds in terms of weight gain and specific growth rate are shown in table 1 ($P<0.05$). The weight gain of the crayfish fed with FB was higher than those fed with CF, FS and FM (as 8.27±2.02, 7.67±2.69, 7.55±1.11, and 5.98±0.85 g, respectively). The specific growth rate of the crayfish fed with FB, CF, FS and FM were 0.98±0.17 ,0.93±0.23, 0.93±0.11, and 0.76±0.13 % day^{-1}, respectively. However, survival rates of crayfish fed with different feeds were not significantly different ($P>0.05$). The equal survival rate of crayfish fed with CF, FS, FB were 78±27.27 % and FM was 67±29.81 %.

Table 1. Growth performance of crayfish fed with different feeds after 90 days.

Growth performance	**Feeds**			
	CF	FB	FS	FM
Initial weight (g)	5.73±0.34^{a}	5.70±0.65^{a}	5.70±0.45^{a}	6.07±0.61^{a}
Final weight (g)	13.40±2.83^{a}	13.97±2.30^{a}	13.26±1.14^{a}	12.05±0.51^{a}
Weight gain (g)	7.67±2.69^{ab}	8.27±2.02^{a}	7.55±1.11^{ab}	5.98±0.85^{b}
SGR (% day^{-1})	0.93±0.23^{ab}	0.98±0.17^{a}	0.93±0.11^{ab}	0.76±0.13^{b}
Survival rate (%)	78±27.27^{a}	78±27.27^{a}	78±27.27^{a}	67±29.81^{a}

Values are means ± standard deviations, n=18. Mean values in rows followed by different superscript are significantly different ($P<0.05$)

Reproductive performance

The GSI and fecundity of the crayfish fed with different feeds are presented in Table 2. Significant differences ($P<0.05$) of GSI and fecundity contents were observed between the treatments. The GSI of crayfish fed with FS were higher than those fed with FB, CM and FM (showing 21.30±5.30, 15.97±4.95, 12.38±4.53 and 10.46±4.64 %, respectively. The crayfish fed with FS had the highest mean fecundity values as 696.42±84.77 eggs female^{-1}. Mean fecundity values of crayfish fed with FB, CF, FM were 568.19±71.45, 372.52±88.60, and 305.06±62.95 eggs female^{-1}, respectively.

Table 2. GSI (%) and fecundity (eggs female^{-1}) of crayfish fed with different feeds after 90 days.

Reproductive performance	Feed			
	CF	FB	FS	FM
GSI	12.38±4.53^{b}	15.97±4.95^{ab}	21.30±5.30^{a}	10.46±4.64^{b}
Fecundity	372.52±88.60^{ab}	568.19±71.45^{ab}	696.42±84.77^{a}	305.06±62.95^{b}

Values are means ± standard deviations, n=8. Mean values in rows followed by different superscript are significantly different ($P<0.05$)

Discussion

The most frequent items (Paloma *et al*., 2004) found in the stomach of red swamp crayfish (*P. clarkii*) were fresh macrophytes, detritus, and sediment grains. More generally and depending on the available food items *P. clarkii* positively selects for insect larvae (mayflies, chironomids), snails, and microcrustaceans except for ostracods in the field. In rice fields this crayfish selects detritus and plants, but invertebrates formed the largest fraction of their diet (Correia, 2003). In the above study, crayfish fed with fairy shrimp demonstrates a good growth performance in terms of weight gain and specific growth rate are higher than with commercial feeds and *M. macrocopa*. In the field *P. clarkii* shows omnivorous feeding with green plants and insects (Smart *et al*., 2002). Zooplankton is the most stimulatory of the natural dietary items tested for crayfish (Kreider and Watt, 1998). Although crayfish preferred natural or fresh feed over compound feed in a study of Jover and co-workers, the optimum nutrient levels for *P. clarkii* were 22-26% crude protein, 6% lipid, and 36-41% carbohydrate (Jover *et al*., 1999).

Live feeds are favorable for marine and freshwater hatcheries because they are readily ingested, easily digested, and do affect water quality to a lesser extend (Munuswamy *et al*., 1997; Dahms *et al*., 2007b; Dahms *et al*., 2011). Predominant live feeds in aquaculture are *Artemia* nauplii (Barros and Valenti, 2003), *M. micrura* (Mart ń *et al*., 2006), *M. mongolica* (He *et al*., 2001), fairy shrimp *S. dichotomus* (Velu *et al*., 2003), and *S. sirindhornae* (Sriphuthon and Sanoamuang, 2011). Frozen organisms instead of live food are a worthwhile alternative and suitable for many farmers. Frozen *Artemia* naupii can use in shrimp hatcheries and blood worm (chironomids) can be used in the ornamental fish farms (Chimsung, 2014).

As for shrimps, maturation diets are provided by fresh feeds. They generally comprise suitable digestible protein, essential fatty acids, cholesterol, and chemo-attractant properties which are found in natural food sources in a shrimp habitat. Fresh feeds typically include polychaete worms, mollusks and crustaceans that have been extensively used for shrimp brood stocks (Chimsung, 2014). Palmer *et al*. (2014) demonstrated that fresh polychaetes had high nutrient components, in particular high total lipid (DHA) and this is important for the maturation of shrimps. Fresh natural food is one of three major factors that have an impact on the maturation of ovary and spawning in shrimps (Salarzadez, 2014). A fresh food maturation diet with an adequate HUFA composition is of importance for brood stock nutrition in black tiger shrimp. For example, squid, polychaete, oyster, and pork affect the spawning frequency and fecundity of female shrimp (Hoa *et al*., 2009). Likewise, Memon *et al*.

(2012) demonstrated that fresh squid diet is behaviorally preferred by banana shrimp over polychaete and cockle due to its higher influence on increasing spermatophore quality. The results of the present study shows that crayfish fed with fairy shrimp had a higher GSI and fecundity than when fed with commercial feeds.

In conclusion, the present study found that crayfish fed with frozen *B. thailandensis* had the highest growth and reproductive performance. Frozen fairy shrimp can thus be used as feed to improve growth and reproductive performance in the crayfish *P. clarkii*.

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