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The effects of adding microbial biomass to grow-out and maturation feeds on the reproductive performance of black tiger shrimp, *Penaeus monodon*

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ABSTRACT

A 40-day reproductive performance trial was conducted to assess the effect of targeted supplementation of *Penaeus monodon* broodstock grow-out and maturation diets with microbial biomass (MB). Over a seven month grow-out period, shrimp were fed a typical pelleted grow-out diet with or without 10% MB. Broodstock were then transferred to a maturation facility and a subset of animals from each grow-out diet fed on a typical fresh-frozen maturation diet that included a pellet ration with or without 30% MB (5.5% of total diet fed). At nine months of age, female broodstock were unilaterally eyestalk-ablated and reproductive assessments commenced. No significant difference in ovary maturation, hepatosomatic index, spawning and egg and nauplii production parameters was found between diet treatments ($p = >0.05$). However, females originating from control ponds displayed a higher gonadosomatic index at first spawn, whilst the percentage of eggs that hatched was lower in females fed a MB-inclusive maturation diet ($p = <0.05$). These results indicate that the inclusion of MB within broodstock grow-out and maturation diets (at the rates presented in this study) did not enhance reproductive performance of domesticated broodstock.

1. Introduction

To date, significant advances have been made in the domestication and genetic improvement of *Penaeus monodon*. These include superior growth and feed utilization (Glencross et al., 2013; Glencross et al., 2014), increased harvest yields (Preston et al., 2009) and increased viral tolerance (Sellars et al., 2015). Despite these improvements, the reproductive performance of domesticated broodstock remains inferior when compared with wild-caught broodstock (Arnold et al., 2013; Coman et al., 2006; Menasveta et al., 1993; Peixoto et al., 2005). As a result, progress in widespread commercial domestication of *P. monodon* has been slow, leaving most farming operations to rely on stocks produced from wild-caught broodstock. Continued reliance on the progeny of wild-caught broodstock is unfavourable as farmers risk the introduction of wild diseases and pathogens into the farming system, as well as precluding the opportunity for genetic improvements via selective breeding. Whilst reproductive performance in domesticated broodstock has been shown to improve over successive generations in captivity (Coman et al., 2013; Preston et al., 2009), there remains significant scope and economic merit to improving reproductive output and seedstock production from domesticated stocks (Arnold et al., 2013).

Broodstock nutrition is regarded as one of the primary factors that constrains the reproductive performance of domesticated penaeid shrimp (Arnold et al., 2013; Browdy, 1998; Coman et al., 2007a; Coman et al., 2007b; Emerenciano et al., 2013b). In general broodstock diets can be classified into two broad categories: 'grow-out diets' designed to facilitate rapid and sustained crop growth in juvenile shrimp; and 'broodstock conditioning and maturation diets' (henceforth referred to simply as maturation diets) designed to provide mature broodstock with the nutrients required for high larval output over successive spawnings. Inadequacies in broodstock diets are known to impact on the reproductive performance of broodstock either by negatively affecting egg formation and development (resulting in poor offspring viability) or through the stunting or inhibition of spawning activity (Clarke et al., 1990; Harrison, 1990; Wouters et al., 2001a; Wouters et al., 2001b). The nutritional status of females prior to maturation can have significant implications for subsequent performance. For example, Marsden et al. (1997) demonstrated that maturation diets fed to wild *P. monodon* broodstock after capture and during maturation significantly influenced spawning frequency and larval quality. However, these authors also noted that seasonal or individual variation, presumably reflected in large-part in the nutritional condition of the stocks, could not necessarily be eliminated by short-term dietary changes, and that the diet consumed in the period prior to maturation significantly influenced subsequent reproductive performance. This

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has led some authors to speculate that variation in diet prior to capture may explain the disparate reproductive performance between wild and domesticated broodstock (Arnold et al., 2013; Coman et al., 2006).

A particularly promising area of shrimp nutrition research is the use of concentrated microbial biomass (MB) aggregates, commonly referred to as biofloc. The growth promoting effects of MB on shrimp have been reported in a number of studies (Burford et al., 2004; Glencross et al., 2013; Glencross et al., 2014; Glencross et al., 2015; Kuhn et al., 2008; Kuhn et al., 2009). Nutritional studies have demonstrated that the growth enhancing effects of bioactive products derived from MB are critically dependent on meeting the overall nutritional demands of shrimp (Glencross et al., 2013; Glencross et al., 2014).

A number of studies have examined the effects of live MB on reproductive performance of farmed shrimp. Comparison of the reproductive performance showed that female *Farfantepenaeus duorarum* reared in a biofloc system had higher spawning activity than females reared in clear water (Emerenciano et al., 2013a). However, as demonstrated for growth rates in shrimp, the effects of MB on reproductive performance appear to be strongly co-dependent on other nutritional factors. For example, fresh food supplementation enhanced the reproductive performance of *Litopenaeus vannamei* reared under biofloc conditions (Emerenciano et al., 2013b).

Current operational and economic constraints within the commercial maturation and hatchery environment limit the use of bioflocs to the grow-out phase. Enclosed pre-conditioning and maturation facilities are not yet designed for bioflocs systems. This is, coupled with the incompatibility of animal management in 'opaque' biofloc systems, as in 'clear-water' systems broodstock must be visually assessed for ovary development and maturity daily. An alternative approach would be the inclusion of dried biofloc or bioactive products derived from MB within current commercial diets thereby allowing for broodstock supplementation across all stages of the lifecycle. The purpose of the current study was to assess the effects of including the MB derived bioactive product Novacq™ (Patent #2008201886), within the pelleted diets of domesticated *P. monodon*. Produced by manipulating marine bacteria and microalgae processes, Novacq™ has previously been demonstrated to improve growth, feed conversion efficiencies and viral tolerance within juvenile shrimp (Glencross et al., 2013; Glencross et al., 2014; Glencross et al., 2015; Sellars et al., 2015). The effect of MB (Novacq™) supplementation on reproductive performance during a 7 month grow-out phase and the subsequent 3 month maturation phase, which included a conditioning period (i.e., acclimation phase (21 days), preconditioning phase (28 days)) and reproductive performance period (40 days), was assessed.

2. Methodology

2.1. Stock origin and rearing

The experimental stocks used in this trial were from a fourth generation commercially domesticated *P. monodon* line maintained by Gold Coast Marine Aquaculture (GCMA). These stocks were spawned over two commercial spawning events in 2013, using the company's commercial maturation, hatchery and broodstock nursery protocols. Upon reaching PL15 (15 days post-metamorphosis from mysis to postlarval stage 1), juvenile shrimp were stocked into three earthen-lined grow-out ponds at a density of 2–3 shrimp/m^2 (Table 1). At this time, juvenile broodstock began receiving experimental grow-out diets. One group received the control diet that was a commercially-produced pellet that did not contain the MB ingredient, whilst the other group received a commercially-produced pellet formulated similarly to the control diet, but including 10% MB (10% of total diet; crude analysis of the two grow-out diets provided below: Table 2). Experimental grow-out pellets were produced by combining the MB ingredient with all experimental pellet raw-dry materials prior to pellet formulation. These dry materials were homogenization within a hammer mill (<1100 μm mesh), before being mixed with wet materials. The combined wet-dry ingredient mash was subsequent passed through the extruder (2 mm die) and cut to a length of 5 mm. In accordance with GCMA's commercial grow-out protocols, broodstock were fed their respective grow-out diets four times per day in preparation for maturation and spawning.

At 6 months of age (point of harvest), a representative sample of females (n = 25) from each pond was randomly sampled via cast net. Females were weighed before being returned to their pond of origin. When animals reached sexual maturity at approximately 7 months, a total of 80 male and 80 female domesticated broodstock from each respective grow-out treatment were randomly selected from the grow-out ponds via cast net. All shrimp were transported 2 h by road to CSIRO's research facility at the Bribie Island Research Centre (BIRC). Animals were stocked into a series of 10,000 L circular maturation tanks containing a fine layer of sand substrate (Crocos and Coman, 1997). Seawater flowed through the tanks at 4 L min^{-1} (57% water exchange per day) at an average salinity of 35 ± 1 ppt. During the acclimation period (3 weeks post stocking) water temperatures were ambient (≈27 °C), whilst water temperature was maintained at 27 °C during preconditioning and reproductive performance phases. Photoperiod was maintained at 14 h light:10 h dark with an artificial light system.

At BIRC, tanks were allocated a maturation diet as described in Fig. 1. Broodstock that had previously been reared on a control grow-out diet were either allocated to a control maturation diet (C + C) or switched to a MB-inclusive maturation diet (C + MB). Broodstock previously reared on a MB-inclusive grow-out diet were either allocated to a MB-inclusive maturation diet (MB + MB) or switched to a control maturation diet (MB + C). Control maturation diets consisted of a fresh-frozen invertebrate based maturation diet supplemented with a high-quality commercial broodstock maturation pellet. MB-inclusive maturation diets consisted of the same fresh-frozen invertebrate based diet supplemented with a high-quality broodstock maturation pellet that comprised of 30% MB prepared on a similar base as to the control maturation diet (a crude analysis of the two pelleted maturation diets is provided below in Table 2). Experimental maturation pellets were produced in the same fashion as growout pellets, by combining the MB ingredient with all experimental pellet raw-dry materials within a hammer mill (<1100 μm mesh), before subsequent mixing of dry-wet materials and extrusion (pressed through 3.5 mm die and cut to a length of 10–15 mm).

The total contribution of pelleted feeds within maturation diets was approximately 18.5%, with the remainder made up by fresh-frozen ingredients: artemia biomass (5.1% of total diet fed); bloodworm

Table 1
Stocking parameters for commercial grow-out ponds.

	Pond size (m^2)	No. shrimp stocked	Stocking density	Grow-out diet
Pond A	2000	6000	3 shrimp/m^2	MB pellet
Pond B	3000	5000	2 shrimp/m^2	MB pellet
Pond C	3000	5000	2 shrimp/m^2	Control pellet

Table 2
Proximate analysis of control and microbial biomass (MB) inclusive feeds used during broodstock grow-out and maturation.

Diet	Component	Control	MB
Grow-out	Protein	47.9	47.4
	Fat	7.9	8.4
	Moisture (air)	8.7	9.1
	Ash	9.0	12.6
	Crude fibre	2.6	3.0
Maturation	Protein	36.9	30.7
	Fat	8.5	8.3
	Moisture (air)	31.3	34.6
	Ash	9.3	9.9
	Crude fibre	0.4	2.1

All results are reported on a % weight per weight basis.