

# Effect of two maturation diet combinations on reproductive performance of domesticated *Penaeus monodon*

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## Abstract

The reproductive performance of tank-reared, 2nd generation domesticated *Penaeus monodon* was compared when fed on two experimental maturation diet combinations; a control diet (CD) containing a treatment portion of 32.5% squid (*Photololigo* sp.) and 32.5% bivalves (*Plebidonax* sp.); and a shrimp-supplemented diet (SSD) containing a treatment portion of 21.6% squid (*Photololigo* sp.), 21.6% bivalves (*Plebidonax* sp.) and 21.6% shrimp (sexually mature *Penaeus* sp. and *Metapenaeus* sp.). The remaining portion of both diets consisted of 5% polychaetes and 30% commercial shrimp pellets. Broodstock were fed on the diets from approximately 10 months of age until commencement of reproductive assessment at 11 months, and until completion of the assessment when females had completed two moult periods post-ablation. No significant difference in growth, survival, ovarian maturation, spawning and egg production was found between diet treatments ( $P > 0.05$ ). However, the percentage of spawnings that hatched (mean  $\pm$  standard error) (CD  $77.5 \pm 6.7\%$ ; SSD  $41.2 \pm 8.6\%$ ) ( $P < 0.01$ ), egg fertility rates per spawning (CD  $60.2 \pm 6.1\%$ ; SSD  $34.4 \pm 8.4\%$ ) ( $P < 0.05$ ), hatch rates per spawning (including both unhatched and hatched spawnings) (CD  $23.3 \pm 4.2\%$ ; SSD  $5.2 \pm 1.7\%$ ) ( $P < 0.01$ ) and nauplii per spawning ( $\times 10^3$ ) (CD  $41 \pm 9$ ; SSD  $6 \pm 2$ ) ( $P < 0.01$ ) were significantly lower for broodstock fed the SSD than the CD. No difference in spermatophore weight or sperm quantity was found between diet treatments ( $P > 0.05$ ). These results indicated that partial replacement of squid and bivalves with sexually mature shrimp in the maturation diet of tank-domesticated *P. monodon* had a negative effect on egg fertility and hatching. Furthermore, these results highlight the large effect that the final maturation diet can have on reproductive output of domesticated *P. monodon* broodstock.

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## 1. Introduction

Despite the long farming history of the giant tiger shrimp, *Penaeus monodon*, virtually all commercial stocks are still produced from wild-caught broodstock (Clifford and Preston, 2001; Moss and Crocos, 2001).

Progressing from using wild broodstock to domesticated stocks could alleviate problems of broodstock shortages, enable selection of stocks with genetically superior attributes, and allow more control over pathogens in the farming system (Gjedrem and Fimland, 1995; Primavera, 1997; Browdy, 1998; Crocos et al., 1999). Considerable efforts have been made towards domesticating *P. monodon* over the past two decades (e.g. Millamena et al., 1986; Menasveta et al., 1993; Pratoomchat et al., 1993; Makinouchi and Hirata, 1995; Hall et al., 2003; Coman et al., 2005, 2006). However, difficulties in

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controlling disease and providing appropriate nutrition and environmental conditions for rearing broodstock have contributed to the absence of commercial-scale breeding programs for *P. monodon*.

The development of suitable broodstock maturation diets and feeding regimes is critical for domestication of any species. In penaeids, the nutritional requirements for reproduction are far less understood than the requirements for the grow-out phase of production, largely due to the greater complexity of physiological processes involved in reproduction, and the demanding requirements and expense of conducting reproductive trials to evaluate broodstock diets (Wouters et al., 2001a). Considerable efforts have been made to understand nutrient requirements and develop artificial (semi-purified) diets for maturation of several penaeid species (e.g. Millamena et al., 1986; Bray and Lawrence, 1990a,b; Cahu et al., 1994; Xu et al., 1994; Marsden et al., 1997; Wouters et al., 2001b). However, despite these efforts, successful broodstock maturation still typically relies on nutrition derived from the provision of a variety of natural food organisms, such as squid, annelid worms (polychaetes), bivalves (mussels, clams, and oysters), crustaceans (shrimp, crab, krill, enriched *Artemia*) and fish, as a large portion of the diet (Primavera, 1983; Menasveta et al., 1993; Cavalli et al., 1997; Browdy, 1998; Wouters et al., 2001a).

Maturation diets consisting of different combinations of natural marine organisms generally produce high reproductive performance from wild *P. monodon* broodstock (e.g. Primavera and Caballero, 1992; Menasveta et al., 1993; Hansford and Marsden, 1995). However, the reproductive outputs of domesticated *P. monodon* stocks have commonly been inferior to the wild stocks when fed on similar diets based wholly or largely on combinations of natural marine organisms (Millamena et al., 1986; Menasveta et al., 1993, 1994; Makinouchi and Hirata, 1995; Coman et al., 2005, 2006). In many instances, the higher performances of the wild stocks may be due to a higher uptake of key nutrients required for reproduction while in the natural environment prior to being brought into captivity. In contrast, the domesticated stocks must obtain all nutrients required for reproduction from the diet provided. Any deficiencies or inappropriate balances of these nutrients in the diet would be apparent in lower reproductive performances. For this reason, it is important to know the contribution that all components of the diet have towards the observed reproductive performance when developing maturation diets for domesticated broodstock.

Crustacean tissues have been a key component of maturation diets for several penaeid species (e.g. Crocos

and Kerr, 1986; Menasveta et al., 1993; Cavalli et al., 1997; Crocos and Coman, 1997; Peixoto et al., 2004; Preston et al., 2004). The inclusion of fresh-frozen shrimp within these maturation diets has provided a nutritional source to replace a variety of crustacean prey items occurring in the natural diet of the penaeid broodstock (Rothlisberg, 1998). Furthermore, the inclusion of sexually mature shrimp within these maturation diets may have non-nutritional benefits by providing a source of reproductive hormones that contribute to the endocrinological cycle (Wouters et al., 2001a). However, due to the risks of disease transmission, feeding of crustacean tissues to penaeid broodstock is seen as a risk to biosecure broodstock production and considered unsuitable for commercial application (Wouters et al., 2001a). For this reason, shrimp and other crustacean tissues have been removed from the diet of tank-domesticated *P. monodon* stocks at CSIRO Marine and Atmospheric Research (CMAR) since 1999. Importantly, it is not known whether the removal of this crustacean component from the maturation diet has contributed to the moderate to low hatching rates found in these tank-domesticated *P. monodon* stocks in recent years (Coman et al., 2005, 2006).

The current study evaluated the reproductive performance of tank-reared, 2nd generation domesticated *P. monodon* when fed on two experimental maturation diets; a control diet (CD) containing a treatment portion of 32.5% squid (*Photololigo* sp.) and 32.5% bivalves (*Plebidonax* sp.); and a shrimp-supplemented diet (SSD) containing a treatment portion of 21.6% squid (*Photololigo* sp.), 21.6% bivalves (*Plebidonax* sp.) and 21.6% shrimp (sexually mature *Penaeus* sp. and *Metapenaeus* sp.). The effect of partial replacement of squid and bivalve with shrimp on reproductive performance of the broodstock was evaluated in order to identify whether the shrimp ingredient contains key nutrients that influence reproductive output.

## 2. Methods

### 2.1. Source and rearing of stocks

The experimental stocks were 2nd generation *P. monodon* reared in tanks at CMAR, which originated from founder stocks collected from a population off the coast of Weipa (12°48', 141°32'), Gulf of Carpentaria, Queensland, Australia. The stocks were comprised of seven families, which were each reared to PL15 (15 d post-metamorphosis from mysis to post-larval stage 1) in three replicate 100 L tanks (0.6 m dia.). From PL16 to PL30, the families were reared

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