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Reproductive behavioural differences between wild-caught and pond-reared *Penaeus monodon* prawn broodstock

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ABSTRACT

Ongoing problems exist with the commercial scale domestication of *Penaeus monodon*. One of the major issues, in terms of reproductive performance, is the low egg hatch rate of eggs from these captive bred prawns. The current study investigated the related issue of mating success. Time lapse video observations were conducted to compare the mating behaviour of pond-reared (domesticated) and wild-caught prawn *P. monodon* broodstock.

Mating success of the pond-reared prawns was found to be low relative to wild-caught. It was determined that both male and female prawns contributed to this low mating rate suggesting both genders were impacted negatively by the domestication process. The causative factors for the low mating success are yet to be determined, however external physical abnormalities and lack of sexual maturity did not appear to play a role. The most notable behavioural difference between wild-caught and domesticated prawns was a reduced level of pursuit behaviour by domesticated males. This and other behavioural differences are discussed in relation to an increasing body of evidence that male prawns respond to sex pheromones produced by receptive females and that males detect these chemical signals in part, via their second antennal flagella. Accordingly we hypothesise that pond-reared (domesticated) females may have a reduced ability to produce or release sex pheromones and males, a reduced ability to detect them when compared to their wild-caught counterparts.

1. Introduction

A number of programs have been initiated to domesticate (closing the life cycle in captivity on a commercial scale) the black tiger shrimp (*Penaeus monodon*), an important aquaculture species (Bierne et al., 2000; Chamberlain, 2003; Chung et al., 2011; Coman et al., 2009; Jones and Lai, 2003; Macbeth et al., 2007; Wyban, 2007a,b). Closing the life cycle of this species could relieve the industry's current dependence on wild-caught broodstock and assist development of specific pathogen free (SPF) broodstock and genetically improved culture lines. Without full domestication, diseases are commonly introduced onto farms via the wild-caught broodstock. These diseases include viral pathogens which are believed to be the major factor responsible for the dramatic worldwide decline in *P. monodon* production since 1997 (Fegan, 2002; Spann and Lester, 1997; Tanticharoen et al., 2008). To date however, most *P. monodon* hatchery operators prefer to use wild-caught broodstock because domesticated broodstock show relatively poor reproductive performance. Reproductive criteria where domesticated broodstock are inferior include: lower spawning rates, delayed spawning, lower egg hatch rates and poorer larval survival.

Many factors have been shown to contribute to poor egg hatch rates in domesticated stock (Hansford and Marsden, 1995; Wouter et al., 2001) with much attention focused on underlying egg and sperm quality issues (Coman et al., 2006). Potential differences in mating behaviour between wild and domesticated stock has received relatively little attention despite being the important first step in the process of egg fertilization.

Mating in *P. monodon* and other 'closed thelycum' penaeid species, occurs at night between a post-moult female and an intermoult male (Primavera, 1985). Wild-caught *P. monodon* have been shown to mate successfully in captivity when exposed to a range of tank shapes and environmental conditions (Primavera, 1985; Wouter et al., 2001). Evidence that mating has been successful includes a non-zero egg hatch rate. Where, however, zero hatch rates are recorded, and no further observations are made, it is difficult to isolate the causative agent(s) which may include sperm and egg quality and/ or failure to mate. For *P. monodon*, direct evidence of mating success can be found by visual examination of the thelycum of the moulted female to confirm the presence of spermatophores (Makinouchi and Hirata, 1995). This practice is typically carried out on the morning after the female

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moults but this data is rarely recorded as the inspection is a stressful process for the newly moulted female. Perhaps the strongest evidence that low mating success contributes to low egg fertility in domesticated *P.monodon* is that artificial insemination (Laxminarayana and Muthu, 1984) can increase egg hatch rate significantly (Kenway et al., 2006), validating that, under these circumstances, egg and sperm quality are not always the causative agents.

Gender contribution to the reduced egg hatch rate in domesticated *P. monodon* was addressed in a study conducted by Coman et al. (2006). After comparing hatch rates from reciprocal matings (wild-caught males or wild-caught females are replaced with domesticated counterparts) they reported that the domesticated females, but not males, caused an increase in the number of spawnings that did not hatch. Thus from their study we know that domestication compromised female fertility, however the question remains: was it due to a reduced ability to attract males and mate, or issues related to egg quality?

In an earlier study Menasveta et al. (1993) also used reciprocal matings to isolate male and female effect on fertilization rates of domesticated and wild-caught *P. monodon* and showed that both males and females contributed equally to the 45% decline in egg fertilization rate that occurred when either. Mating success, however, was not recorded in their study, so its contribution to the reduction in egg fertilization remains unknown.

Thus the current study was designed to clarify whether differences exist in mating behaviour and hence mating success, between wild-caught and domesticated broodstock and whether this may contribute significantly to reported poor hatch rate of eggs from domesticated *P. monodon*. To this end, we monitored the behaviour of pond-reared vs wild-caught *P. monodon* males and females under laboratory conditions using time lapse video recordings. Observations were recorded of the events and stages at which alteration or interruption of the mating process occurred in the domesticated broodstock relative to the wild-caught. In addition, we reciprocally crossed wild-caught and domesticated males and females to determine the male and female contribution to any observed behavioural differences related to a decline in mating success.

2. Material and methods

2.1. Experimental prawns

Prawns used in the experiments came from two sources: (i) third generation pond-reared (domesticated) prawns (D) and (ii) wild-caught sexually-mature prawns (W) collected from near shore waters, North Queensland, Australia. The D stock consisted of 30 males and 30 females that were 14 months old. Individuals were harvested from a 200 m^2 plastic lined pond at the Bribie Island Research Centre (BIARC) located in southern Queensland. Prawns were reared at an average density of 4 m^2 and were fed twice daily on a diet consisting of high protein pellets (Higashimaru- *Marsupenaeus japonicus* diet) with a twice weekly supplement of fresh-frozen mullet and squid. For the W treatment group, 25 females and 20 males of unknown age were captured from fishing grounds off Cairns in north Queensland and air freighted to BIARC in southern Queensland. The founding stock for the domesticated line came originally from the same spawning grounds as the W stock.

The average size for a pond-reared male and female prawn was 78.4 ± 1.2 grams and 94.6 ± 2.0 grams, respectively, and 84.2 ± 1.8 grams and 105.6 ± 0.9 grams, respectively for wild-caught individuals.

2.2. Holding facilities

Prior to transfer to holding tanks, prawns were physically examined for any abnormalities (including in external genitalia and antennule damage). Prawns were then eye tagged for individual identification, weighed and moult staged assessed according to Promwikorn et al. (2004). After any prawns with damaged antennae were rejected, remaining individuals were transferred to a holding tank and held for a seven day acclimation period at a density of 2 per m^2. After acclimation, pre-moult females were transferred to a small (3 m × 2 m) holding tank (Tank A) in a temperature and light controlled room. Approximately six inter-moult males (W and D lines) were also transferred to Tank A. Water temperature in holding tanks was maintained at 28 °C with a 150% water exchange conducted daily and individuals fed a diet of fresh frozen squid or mussel on alternative days to excess twice daily.

2.3. Observation tanks

Three time lapse video surveillance cameras (Sony) were mounted above three circular observation tanks (diameter of 1.5 m 1.2 m depth). Each afternoon tanks were filled with filtered (20 μm), preheated seawater (28 °C) to a depth of 1 metre. No water exchange occurred and the room was heated to 28 °C to maintain water temperature. A single air stone in each tank released a fine stream of bubbles that maintained O_2 levels at 8 mg/L without disturbing the water surface or distorting the video image. Lighting was supplied by red bulbs positioned 1 metre above each tank. Observation tanks were cleaned and refilled daily.

2.4. Observations

At 18:00 h the 3 pre-moult females most likely to moult were transferred from Tank A to the 3 observation tanks with two inter-moult males (see Table 1) and one inter-moult female (of the same origin as the pre-moult female) for overnight video surveillance. The following morning males and inter-moult females were returned to their respective tanks. Any moulted female from the observation tank was returned to the acclimation tank. If a female moulted in the observation tank, the video cassette was given a confidential coding, to enable subsequent viewing and assessment of behavioural criteria to be made without prior knowledge of the origin (W or D) of the female.

2.5. Behaviour classification

Various pre-mating, mating and post-mating male and female behaviours were recorded from the video films and a brief description of these are given in Table 2. As no interactions were observed between the intermoult female and other prawns in the viewing tank, her data are not included.

If two males pursued the moulted female, only the behaviour of most active male (most time moving) was recorded.

Distances of males from the female were estimated in terms of prawn body lengths with one body length being 20 cm.

‘Male moving’ was defined as the percentage of time (40 min post moult), the prawn was not stationery. ‘Stationery’ was when the outline of the carapace remained unmoved for more than 5 s. The stationery time was then totalled and deducted from the 40 min to give the ‘time moving’, which was then converted to a percentage.

Table 1
Pairings of male and female prawns placed in observation tank for videoing.

Origin of prawn	Females (1 pre-moult and 1 inter-moult)	Males (2 inter-moult)
	W	W
	W	D
	D	D
	D	W