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Evaluation of growth performance in a diallel cross of three strains of giant freshwater prawn (*Macrobrachium rosenbergii*) in Vietnam

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ABSTRACT

The giant freshwater prawn (*Macrobrachium rosenbergii*) is one of the most important crustacean species produced in inland aquaculture in many tropical and subtropical countries worldwide. The aim of the current study was to evaluate the growth performance of three strains of giant freshwater prawn that originated from geographically separated locations in a complete (3×3) diallel cross as a starting point for a stock improvement program for the industry in Vietnam. Crosses were established over two generations using two wild Vietnamese river populations (Dong Nai and Mekong) domesticated for the study and an introduced Hawaiian strain that had been in culture for many generations. Juveniles from nine strain combinations were produced using single-pair matings. Results after 15 weeks of grow-out in hapas showed that growth performance of the Hawaiian strain was best among the purebred strains and that certain cross combinations grew significantly faster than purebred strains. Mean body weights of specific cross combinations with Dong Nai or Mekong as dams and the introduced strain (Hawaiian) as sires were significantly heavier than those of purebred Dong Nai or Mekong strains. While males reached heavier mean weights than females, male variation among the strains was obscured by social factors that produced different frequencies of male morphotypes (blue claw, orange claw and small males). Results suggest presence of potentially valuable heterosis and possible impact of the direction of cross. From a practical viewpoint this could be exploited upon by either, including different forms of crossbreeding, or alternatively, by creating a composite population for future selection. Potential problems and challenges encountered during the trials are discussed.

1. Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii*) is one of the most important crustacean species produced in inland aquaculture in many tropical and subtropical countries worldwide. In 2004, world total farmed *M. rosenbergii* volume reached more than 194,000 tons, with an estimated market value that exceeded US$ 810 million, of which 99% was produced in Asia (FAO, 2004). This figure, however, is probably an underestimate because it excludes production from Vietnam. Production of giant freshwater prawn (GFP) from aquaculture in Vietnam was estimated at approximately only 3000 tons/year during the 90's, but this increased to more than 10,000 tons by 2002 (Phuong et al., 2006). GFP is now a high priority species for development in Vietnam with the annual aquaculture production target for the species by 2010 set at 60,000 tons reared in 32,000 ha (Government of Vietnam, 1999).

M. rosenbergii has been disseminated widely around the world and the species is now present in at least 43 countries (New, 2000) where it is used for both research and commercial culture purposes. Little attention however, has been paid to date to the genetic attributes of cultured stocks. Most broodstock used in culture were developed from the 'western' form of *M. rosenbergii* (*sensu* De Bruyn et al., 2004a) that were originally collected in Malaysia and then translocated to Hawaii. After many years of culturing essentially unimproved stocks, declines in productivity have become an increasing concern for many culture industries. A number of factors may contribute to productivity declines, including high levels of inbreeding due to sourcing broodstock directly from grow-out ponds, and selection of breeders based on their readiness to spawn that often involves early maturing, small females (Mather and de Bruyn, 2003). This latter practice is likely to cause an indirect, negative response on weight at final harvest.

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Surprisingly, while GFP aquaculture has expanded rapidly worldwide, genetic approaches to stock improvement of cultured lines have not been widely implemented. Thus, there is the need for systematic breeding programs in the future to improve economically important traits in this species.

Significant productivity advances have been achieved via selective breeding programs in aquaculture over the last 10 years, particularly in a number of finfish species where improvements of up to 10 to 20%/generation have been achieved, notably for Atlantic salmon *Salmo salar* (Gjedrem, 2000; Quinton et al., 2005), coho salmon *Oncorhynchus kisutch* (Hershberger et al., 1990; Neira et al., 2006), channel catfish *Ictalurus punctatus* (Dunham and Brummett, 1999), Nile tilapia *Oreochromis niloticus* (Bentsen et al., 1998; Ponzoni et al., 2005; Eknath et al., 2007) and Rohu carp *Labeo rohita* (Mahapatra et al., 2006). In contrast, selective breeding programs have been initiated on only a few commercially important crustacean species, notably in some marine penaeid prawn species (Hetzel et al., 2000; Argue et al., 2002; Goyard et al., 2002; Preston et al., 2004; De Donato et al., 2005; Gitterle et al., 2005a,b) and freshwater crayfish (Jones et al., 2000; Jerry et al., 2005). The few examples of selective breeding undertaken on crustacean species have, however, achieved a high response to selection for fast growth rate with genetic gains of around 10%/generation.

An alternative approach to improving the productivity of cultured stocks is via crossbreeding (intraspecific crossbreeding or interspecific hybridization) to exploit potential heterosis (hybrid vigour) in crossbred offspring. Hybrid lines are used widely in the horticulture and grains industries to increase yields of important crops (Lamb, 2000). While the approach has been trialed only sparingly in aquaculture, examples exist of hybrid crosses with superior performance to pure lines, e.g. common carp *Cyprinus carpio* (Bakos and Gorda, 1995; Hulata, 1995). Crossbreeding has been used to transfer favourable traits among strains (Fjalestad, 2005). This method, particular diallel crossing has also been employed to establish a genetically diverse synthetic base population prior to initiating a breeding program. This was the strategy employed in the GIFT project (Genetic Improvement of Farmed Tilapia) (Bentsen et al., 1998). Crossbreeding can be a relative simple and inexpensive method for improving local strains (Fjalestad, 2005), when compared with selective breeding. Selective breeding programs however, can provide significant economic benefit over the long term of operation. For example, approximately US$ 3 million is required annually to undertake the breeding program for Atlantic salmon in Norway, but the estimated annual profit from this program is US$ 45 million (Gjedrem, 1997). Similarly, the genetic improvement program for Nile tilapia has been highly beneficial with benefit:cost ratios ranging from 8.5 to 60 (Ponzoni et al., 2007). Initial high investment however, can be a major constraint on initiating and running new selective breeding programs in aquaculture. As a result, a crossbreeding approach may be beneficial for small aquaculture industries where resources are limited for selection programs, for example the approach adopted for Pacific blue shrimp *Penaeus* (*Litopenaeus*) *stylirostris* in New Caledonia (Goyard et al., 2008). Thus, which strategy is trialed, in part, may depend on the stage of development of individual aquaculture industries and the breeding objectives for specific cultured species.

Giant freshwater prawn is distributed naturally across south and south east Asia, from Pakistan in the west to southern Vietnam in the east, and the distribution includes northern Oceania and some western Pacific islands (New, 2002). While adult prawns live in freshwater environments, females need to move to estuarine areas as their larvae require brackish-water for early survival and development (Ismael and New, 2000). GFP larvae have only a limited potential capacity for marine dispersal (Mather and de Bruyn, 2003). Recent studies have documented genetic diversity in wild *M. rosenbergii* stocks across the species' extensive natural distribution and have suggested that variation is high and structured spatially among major river drainages (De Bruyn et al., 2004a,b, 2005; De Bruyn and Mather, 2007). Thus, the potential exists to exploit naturally high levels of diversity in wild *M. rosenbergii* stocks in breeding programs to develop improved lines for the culture industry.

Little is known however, about potential for hybrid vigour in intraspecific-crosses among strains of GFP collected from independent drainage basins. The current study developed two culture strains from wild populations of *M. rosenbergii* collected from two discrete freshwater drainages in southern Vietnam and set up a diallel cross with a third culture strain of GFP sourced from Hawaii ('Anuenue' strain) that came originally from Malaysia. The specific objectives were to compare relative performance of individuals from different genetic backgrounds, as well as the performance of all possible reciprocal crosses.

2. Materials and methods

2.1. Collection of wild GFP populations and broodstock conditioning

Wild *M. rosenbergii* populations were collected from two discrete drainage systems in Vietnam in November 2005 to form the founder stocks for development of new culture strains. The first population originated from the Dong Nai River and the second population was collected from the Mekong River. Because the Mekong River has an extensive geographical basin, juvenile prawns were collected from three geographically widespread sites in Vietnam to ensure that samples represented broad genetic variation present in the lower river basin. Sampled Dong Nai and Mekong individuals were stocked into separate hapas (4×8×1.5 m) suspended in the water column of a 2000 m^2 earthen pond. Air supply systems were installed in each hapa and operated during the night (9pm to 6am) to maintain dissolved oxygen concentrations. After 2 months, healthy adult prawns were chosen as broodstock and transferred for conditioning. Females and males from each strain were held in separate hapas for an additional 2 months for conditioning. Broodstock were fed twice daily with a 40% protein commercial prawn pellet and this was substituted with chopped beef liver or squid every 2 days (Daniels et al., 2000). The third strain, Hawaiian, originated from Malaysia and has been cultured outside south east Asia for more than 30 years (New, 2000). The Hawaiian strain was introduced to Vietnam in early 2005 as juveniles for on-farm trials in the Mekong Delta and collected as broodstock at the end of the trial from a local hatchery in An Giang province. Numbers of initial collections for each strain are presented in Table 1.

2.2. Mating design and family production

2.2.1. Generation 1 (G1)

The study was carried out at the National Breeding Center for Southern Freshwater Aquaculture that is part of the Research Institute for Aquaculture No. 2, Vietnam. Wild Dong Nai and Mekong and the newly introduced Hawaiian strain broodstock (G0) were used in

Table 1
Collection sites, date and number of individuals collected to established Vietnamese foundation strains

Strain initials	Collection sites	Geographical position	Date	Number
D	Dong Nai River	10°56′N 106°49′E	4 Nov. 2005	437 juveniles
M	Mekong River			
	Hong Ngu	10°48′N 105°20′E	7 Nov. 2005	86 adults
	My Tho	10°21′N 106°22′E	21 Nov. 2005	225 juveniles
	Chau Doc	10°42′N 105°07′E	1 Dec. 2005	50 adults
H	An Giang		5 Apr. 2006	47 breeders

Note: (i) Juvenile: sub-reproductive individual.
(ii) Adult: sexually mature individual.
(iii) Breeder: individuals selected as broodstock for breeding experiments.