APPLICATION OF MICROSATELLITE MARKERS FOR ASSESSMENT OF FAMILY PERFORMANCE OF *Penaeus japonicus* WITHIN A COMMERCIAL POND

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SUMMARY
The domestication of the *Penaeus japonicus* prawn has opened this farming industry up to the possibility of genetic enhancement through selective breeding programs. To track pedigree in these commercial pond experiments, molecular tags must be used to ensure the animals can be sold at market. Thirty families were stocked into ponds and after six months animals representing the top 10% of the weight distribution were harvested. Investigation using a panel of eight microsatellite markers showed the consistent presence of eight high ranking families between the two ponds, implying little or no pond x family interaction. These families also yielded more harvested individuals and larger mean weights.
Keywords: *Penaeus japonicus*, microsatellite markers, pedigree, growth

INTRODUCTION
The Kuruma prawn, *Penaeus japonicus*, is endemic to the coastal marine waters of the Indo-west Pacific and is the basis for commercially important industries in south-western Europe, South-east Asia, and also in Australia, where the production of these prawns is worth over AU$15 million annually.

At present, many penaeid farmers rely on wild caught broodstock for production, but susceptibility to disease outbreak is leading the push towards prawn domestication, and in turn, stock enhancement through genetic improvement. In order to investigate any improvements that controlled breeding could bring to growth, in-pond survival and disease resistance, it is necessary to maintain reliable pedigree records.

Prawns shed their exoskeleton in order to grow, therefore it is not possible to use external tags to follow these crustaceans through their life-cycle. Internal markers such as elastomer dyes and passive transponders (Godin *et al.* 1996, Cacei *et al.* 1999, Jerry *et al.* 2001) are available, but these would render a commercial catch unsaleable, and so are not useful tools for a farmer wishing to monitor their breeding programs.

Previous aquaculture studies have shown that microsatellites are highly variable genetic markers that can be very informative when investigating fishery stock structures (O'Connell and Wright, 1997). This technique would allow progeny from various families to be stocked in communal ponds, and then to be retrospectively assigned to their family of origin. This pedigree analysis can be performed at any stage of grow out, provided the parental fingerprint is known or can be deduced from progeny data, and the markers used are suitably informative to discriminate between families. This study employed eight of these genetic tags to identify offspring from 30 maternal families raised

communally in commercial ponds and to then assess family performance by rankingthese according to weight.

MATERIAL AND METHODS

Microsatellite detection. After six months of grow out, animals were harvested at random with a cast net and weighed to the nearest gram. Pleopods were then collected and placed immediately on dry ice from those prawns which represented the heaviest 10% of the two commercial ponds. Total genomic DNA for microsatellite analysis was extracted from 40mg of prawn pleopod tissue, (shattered after freezing in liquid nitrogen) using a modified DNeasy™ 96 Tissue Kit (QIAGEN®) protocol, in which a shortened 2 hour incubation for proteinase K digestion was used. Microsatellite amplifications were performed in 96-well plates using an MJ Research PTC-200 thermocycler. Potential parents and progeny were genotyped at eight microsatellite loci (CSPJ002, 003, 005, 008, 010, 012, 014 and 015 Moore *et al* 1999). (PCR and sequencing conditions available from J. Meadows upon request). Gene profiles were analysed using GeneScan® 3.1 and Genotyper® 2.5 software.

Statistical analysis. Progeny were assigned to their maternal parent using the likelihood-based approach in CERVUS Version 2.0 (Marshall *et al.* 1998). The simulation module within CERVUS was first used to estimate the required critical differences in the likelihood ratio between the first and second most probable candidate maternal parent. The error rate was set at 1% for a pool of 30 maternal parents and 10 000 replications. Progeny were then assigned to a maternal parent by using the results of simulations in the parentage assignment module of CERVUS.

Families were ranked according to the number of progeny allocated. Due to sexual dimorphism in growth rates, male and female progeny were treated separately in analyses. Differences in mean weight of families within each pond were tested using analysis of variance. Effect of pond environment on mean family weight was tested using the following model in ASREML:

$$\mathbf{Wt}_{ijk} = \mathbf{mu} + \mathbf{family}_j + \mathbf{pond}_k + \mathbf{family.pond} + \mathbf{error}_{ijk}$$

where Wt_{ijk} is the weight of the *i*th individual in the *j*th family from the *k*th pond, *mu* is the population mean and $error_{ijk.}$ is the residual error.

RESULTS AND DISCUSSION

The eight microsatellite markers allowed 80% of progeny to be assigned to their maternal parent with greater than 80% confidence. It was not possible to genotype all 30 mothers, as some died before tissue samples could be obtained, and so it is possible that the individuals that could not be assigned to a family with any certainty, could have belonged to one of these 7 non-genotyped families. The presence of null alleles in the marker set may also have limited the success of progeny assignment.

After fingerprinting the progeny and assigning them into their family groups, it became apparent that there were differences in the number of progeny assigned to each family. However, generally the same eight families with the most individuals were ranked accordingly in both ponds.

There were no significant differences in mean weights between families for either the males or the females in Pond 25, but this was not the case in Pond 15. In Pond 15, the male weights followed the trend of Pond 25, but the females did not. It was shown that the families with the greater number of progeny tended to have the highest mean weight, but the large standard errors observed in the lower ranked families reduced the power of the analysis to detect statistical differences.

The experimental design only considered the family composition present in the top 10% of the pond weight distribution. There is therefore a sampling bias due to too few individuals represented in lower ranked families.

The ranking of the top eight families, for both males and females, was relatively consistent between the two pond samples, indicating that there was no significant pond effect on family ranking. That is, the different environments experienced by the families reared in each pond did not affect the position of their family ranking. Similar trends were seen in the males and females of each pond and so only the female data is presented (Table 1).

Table 1. Mean female weight (grams) ± S.D (number of individuals assigned to family in brackets) and relative family ranking based on number of progeny assigned for the top 10 families in the two commercial ponds

Family	Pond 15	Pond 25	Rank (Pond 15 and Pond 25)
E4LY70	21.7 ± 2.8 (22)	18.3 ± 3.1 (12)	1 & 6
E21LY119	20.4 ± 1.5 (20)	18.1 ± 1.9 (16)	2 & 4
E10NO#	21.6 ± 2.8 (17)	19.9 ± 3.5 (17)	3 & 2
E6RY207	20.4 ± 1.9 (17)	18.9 ± 3.1 (17)	4 & 3
E15LY74	20.6 ± 1.7 (13)	18.5 ± 1.7 (9)	5 & 8
E25LR35	19.3 ± 1.0 (10)	18.4 ± 2.3 (27)	6 & 1
E12LW12	21.2 ± 1.2 (9)	18.8 ± 1.5 (2)	7 & 17
E1LY75	19.9 ± 1.1 (9)	20.1 ± 2.6 (7)	8 & 12
E14LY71	20.1 ± 1.4 (8)	16.9 ± 0.1 (2)	9 & 18
E26?LB63	20.6 ± 2.6 (7)	18.2 ± 1.9 (8)	10 & 10

The commercial ponds were originally stocked with equal numbers of larvae from each family, so whilst it is possible that there was differential survival between families, the higher ranked families in both ponds tended to be composed of the higher weighted individuals, suggesting rank was attributed to weight and not survival.

Attaining high quality pedigree information is the rate-limiting step to designing selective breeding programs in this penaeid species. Kumura prawns are stocked into commercial ponds at post-larvae stage (~2 mm in size) and so are nearly impossible to physically tag, a process that is both economically unviable to perform and that would make the final crop unsaleable. Genetic tags allow the family composition of ponds to be estimated at any stage of growth and so can be used to watch for high performing families in terms of growth, in-pond survival and disease resistance. Importantly

though, it also enables the farmer to watch for inbreeding depression, and to minimise the deleterious effects associated with inbreeding in a highly prolific breeding species.

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