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BREEDING FOR DISEASE RESISTANCE IN AUSTRALIAN SHRIMP: HOW DO WE GET THERE?

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SUMMARY

Shrimp farming is a highly valuable aquaculture industry globally. Domesticated and selectively bred stocks of *Litopenaeus vannamei* are farmed throughout Asia and South America, however, selective breeding in Australian farmed shrimp (*Penaeus monodon*) is currently severely underutilised. Disease is the biggest threat to shrimp production globally and selective breeding is thought to be a more effective long term disease management strategy. Breeding resistant shrimp has been accomplished for very few diseases using laboratory disease challenge tests, sib-selection and conventional breeding methodologies. Genomic selection offers the potential to significantly advance shrimp selective breeding particularly for complex traits like disease resistance through increased accuracy and selection intensity. In Australia, a breeding program is currently underway developing and applying new and improved methods for selection for disease resistance in shrimp.

INTRODUCTION

Selective breeding plays an important role in increasing farming productivity and helping to meet the increasing global demand for animal protein. Aquaculture is the fastest growing primary production industry, yet less than 10% of world aquaculture production is based on selectively bred and genetically improved stocks (Gjedrem *et al.* 2012). Within the global aquaculture industry, farming of penaeid shrimp is a highly valuable sector, with most production taking place in Asia and South America using the species *Litopenaeus vannamei* (Pacific White Shrimp). Domesticated specific pathogen free (SPF) and recently selectively bred populations have been developed for this species, largely in response to the widespread disease problems the industry has faced and the catastrophic losses that result when a disease manifests in a new region (Lightner 2005). However, in the Australian shrimp farming context, the major species farmed is *Penaeus monodon* (Black Tiger Shrimp) and production is based nearly exclusively on unimproved seed derived from wild caught broodstock (although there are smaller scale domestication and breeding programs currently being developed).

Disease is perhaps the most significant issue for shrimp production globally (Stentiford *et al.* 2012) and until recently Australia has been fortunate to remain free of the major pathogens that have resulted in catastrophic production losses in Asia and Latin America. Over the last decade losses due to disease are thought to have cost the industry at least $20bn (Shinn 2016). For example, White Spot Syndrome Virus (WSSV) is estimated to have cost at least $8bn, however, some estimates make it closer to $15bn since its emergence in South East Asia in the early 1990's (Lightner *et al.* 2012). Acute Hepatopancreatic Necrosis Disease (AHPND), a more recent disease impacting shrimp farming, is estimated to cause losses in production in the Thai shrimp industry alone between $1.7 and $2bn annually (Shinn 2016).

In December 2016 the first outbreak of WSSV was detected in Australia in South East Queensland and has had a significant immediate impact on production, brought about uncertain consequences for future production in the area, as well as having ramifications to seafood products in Australia more broadly. Additionally, an AHPND-like disease was detected in 2 Australian

shrimp farms in early 2016, which was found to be caused from a similar acting, but different pathogenic strain of bacteria than that found in Asia (Nick Moody, CSIRO pers. comm.). These examples highlight how exotic diseases pose a great threat to Australian shrimp farming; however, Australian farms are also often exposed to endemic pathogens, such as gill-associated virus (GAV), that have a less devastating, but nonetheless persistent impact on production (Munro *et al.* 2011). This is because these viruses are highly prevalent in wild and farmed stocks; prevalence of GAV for example approaches 100% in some cases of *P. monodon* populations (Walker & Winton 2010).

As shrimp lack an adaptive immune system, common disease management strategies such as vaccination are not an option for shrimp. The most common management strategy used in regions where highly pathogenic diseases are present is the use of specific pathogen free (SPF) stocks that are tested and certified free of major disease causing pathogens. Whilst not selected for resistance or tolerance to the pathogen, SPF shrimp have allowed the industry to operate in regions where pathogens are present through the stocking of "clean" shrimp into ponds. However, SPF shrimp are still naïve to infection with massive losses due to disease continuing to occur and there is evidence they perform poorly in the presence of disease compared to wild stocks (Moss *et al.* 2001). Improving disease resistance through selective breeding is seen to be a more sustainable, long term strategy for the industry and as a result instigation of selective breeding programs for shrimp that capitalise on additive genetic variability in disease tolerance within farmed populations are underway.

MEASURING DISEASE RESISTANCE

The ability to accurately and reliably measure a trait under selection is core to any breeding program. For shrimp disease, measuring resistance is largely based on survival, either on-farm during grow out, or in laboratory challenge tests. Laboratory challenges tests are most commonly used because inoculation of the pathogen and environmental conditions can be more easily controlled. Challenge methods in shrimp include; injection of the pathogen into abdominal muscle, ingestion of infected material and waterborne exposure. Breeding programs that utilize disease challenge tests to measure disease resistance are based on family selection. Here a subset of progeny from a family are removed from the core breeding nucleus facilities and disease challenged. Family survival estimates are then calculated after a specified amount of time post inoculation and families are ranked on their survival performance. Families to perpetuate into the breeding program are then selected based on the family's performance. This approach means the breeding candidates themselves are never exposed to the disease, but rather chosen based on the estimated breeding values (EBV) of their disease challenged sibs (i.e. sib selection). This allows breeding companies to not only improve disease tolerance through accumulation of additive genetic variability, but practice SPF management strategies. One disadvantage of the approach, however, is that family selection only utilises the between-family genetic variance within a population and ignores 50% of the available genetic variance that is represented within-family. This, coupled with the phenotypic performance of the selected candidate having never been evaluated can lead to inaccuracies in EBV, reduced selection intensity, and therefore can lower the genetic gains realised.

Another characteristic of shrimp disease challenge tests is that resistance is often only measured as a single trait, survival. However, survivorship is complex, can be influenced by many non-disease related factors and may not manifest predominantly, or entirely through survivorship, instead causing issues with growth or deformities (e.g. runt deformity syndrome caused by Infectious Hypodermal Hematopoietic Necrosis Virus (Lightner 1999)). Therefore, alternative methods such as measuring viral load, or presence of disease associated genetic markers, may be useful in evaluating disease resistance.

A large assumption made when using controlled challenge tests in breeding programs is that resistance measured during challenge testing accurately reflects resistance under grow out farm

conditions. This is largely untested for shrimp breeding programs. If there are differences then significant genotype-by-environment (GxE) interactions may be occurring which will reduce the efficiency of selection and genetic gains realised. The only known correlation published on this issue in shrimp was a phenotypic correlation between TSV challenge survival and commercial pond survival in *L. vannamei* (Moss *et al.* 2005). Here moderate positive correlations were reported (0.55 and 0.68), however, phenotypic correlations are insubstantial as there is no inclusion of the genetic effects; this information is still lacking in shrimp.

LESSONS FROM OVERSEAS GENETIC IMPROVEMENT PROGRAMS

There are few published studies that have investigated the quantitative genetics of disease resistance in shrimp. However, information on the implementation and success of disease resistance traits being incorporated into breeding programs is variable and very limited. Nearly all work has been carried out on *L. vannamei* and the most well-known success story in shrimp has been selecting *L. vannamei* for resistance against Taura Syndrome Virus (TSV). This trait has been incorporated in several breeding programs (Cock *et al.* 2009), as it has high phenotypic variation (14.6 - 93.8%) and genetic variance is moderate to high; heritability estimates across the different breeding programs range between 0.2 – 0.4 (Argue *et al.* 2002, Odegard *et al.* 2011). Response to selection has also been very good, with survival rates shown to increase by at least 18.4% per generation (Argue *et al.* 2002, White *et al.* 2002). Unfortunately, TSV disease resistance was found to be negatively correlated with growth (Argue *et al.* 2002), therefore both growth and resistance to TSV were incorporated in the breeding programs as separate traits selected for in individual breeding lines (Argue *et al.* 2002, Odegard *et al.* 2011). Despite this impediment, selecting for TSV resistance has been so successful that TSV resistant shrimp are widely used throughout the shrimp farming industry and TSV is no longer considered a major threat to production.

Conversely, breeding for resistance to WSSV has had limited success. This can in part be due to the highly virulent nature of this virus and very small genetic variation often observed both under field and controlled challenge conditions (>90 % mortality is commonly found). Estimates of heritability for resistance to WSSV under controlled challenge conditions were found to be <0.1 (Gitterle *et al.* 2005). Similar to TSV, resistance to WSSV was also negatively correlated (- 0.55 & - 0.64) with harvest weight (Gitterle *et al.* 2005). More recently, however, there have been reports of significant improvement of resistance to WSSV: For example 3 families of *L. vannamei* from a Panamanian breeding program had significantly higher survival compared to the unselected "Kona" shrimp breeding line (Cuellar-Anjel *et al.* 2011). It is difficult to get a full appreciation of how successful breeding for resistance to WSSV has been, most likely due to the commercial sensitivities of genetically improved stocks; however, this virus continues to be a major problem for shrimp farming worldwide which would suggest breeding for improved resistance has had little success so far.

OPPORTUNITIES FOR AUSTRALIAN SHRIMP FARMING

Australia has been somewhat fortunate that until recently it has been free of many of the highly virulent and devastating diseases that have occurred in overseas shrimp farms. The only known example in Australia whereby a breeding program has directly incorporated disease testing was via viral screening of wild and domesticated *P. monodon* broodstock to identify individuals with natural high GAV loads that were then removed from the spawning group (Coman *et al.* 2013). It is unknown how effective this strategy was in reducing the impact of GAV on production and there is no evidence that the approach leads to significant accumulation of advantageous additive genetic variance for GAV tolerance. Moving forward, GAV will likely continue to be an important virus affecting Australian shrimp farms, as this virus is highly prevalent in the wild and in shrimp farms.

Conventional methods of quantitative genetics used so far for shrimp breeding programs, while successful at improving growth rate, have been less effective for improving disease resistance as evidenced by an absence of resistant strains to most virulent diseases. Possibly this lack of progress is a consequence of the selection models used (i.e. sib selection) and/or laboratory challenge tests which don't accurately estimate disease additive genetic variation as it manifests itself on-farm under complex environmental interactions. Genomic selection, however, offers the potential to increase the accuracy and selection intensity of complex traits like disease resistance (Castillo-Juarez *et al.* 2015), along with more readily accessible integration of on-farm performance. This is because genomic selection allows individual phenotype data from both laboratory and on-farm performance trials to be linked with predictive genome-wide markers which can then be applied to select unchallenged individuals through genotyping only (i.e. thereby maintaining SPF status in the breeding nucleus). Genomic selection under this model would increase genetic gain as it utilizes both between and within-family variance and is able to estimate individual EBVs to use for selection of breeding candidates. Furthermore, the identification of SNPs associated with disease resistance may also be applied through quantitative trait loci (QTL) and marker assisted selection. All of this combined should allow for greater accuracy of genetic merit estimates, increased selection intensity and hence genetic gains for disease resistance traits (Castillo-Juarez *et al.* 2015). Developing and applying these new technologies are currently underway for *P. monodon* in a developing breeding program in Australia.

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