TAURA SYNDROME VIRUS RESISTANCE IN PACIFIC WHITE SHRIMP, *PENAEUS* (*LITOPENAEUS*) *VANNAMEI*: ESTIMATION OF GENETIC PARAMETERS RELEVENT TO SELECTIVE BREEDING

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By

Dustin R. Moss

Dissertation Committee:

Jinzeng Yang, Chairperson
James Brewbaker
Yuanan Lu
Shaun Moss
Clyde Tamaru

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ABSTRACT

Taura syndrome virus (TSV) is an economically important pathogen of Pacific white shrimp, *Penaeus* (*Litopenaeus*) *vannamei*. TSV is highly virulent and TSV-associated mortalities in unselected, naïve populations of *P. vannamei* range from 40-95%. TSV-associated crops losses are estimated to be >$1 billion USD. Selective breeding of *P. vannamei* for TSV resistance began in the mid-1990s and several breeding programs have developed lines of shrimp which exhibit varying degrees of TSV resistance. Despite long-standing breeding efforts, several important aspects of TSV resistance have yet to be properly studied. In this dissertation, I conducted four studies to address key knowledge gaps in breeding *P. vannamei* for TSV resistance.

The first study investigated the effects of inbreeding on TSV resistance. Inbreeding was found to have moderate to severe effects on TSV survival and the effects of inbreeding appeared worsen as environmental quality decreased. The second study focused on the estimation of genetic correlations for shrimp survival to a genetically, diverse suite of TSV isolates and the estimation of genetic correlations between TSV survival and growout performance traits. Genetic correlations for shrimp survival among TSV isolates were generally high, suggesting that breeding for resistance to a single TSV isolate should result in increased resistance to other tested isolates. Genetic correlations among TSV survival and growout performance traits were generally low and suggest that there are no major impediments to simultaneous genetic improvement of these traits. The objective of the third study was to estimate correlations and heritability for TSV survival in two size-classes (2.5 g and 8.0 g) of shrimp. Heritabilities were similar between size-classes and genetic and phenotypic correlations were high. These results show that the common practice of challenging small juveniles (1-3 g) to TSV and using this data to select for TSV resistance is likely effective in improving farm survival during TSV epizootics. The final study investigated the effects of viral load on TSV survival. Survivors were found to generally have lower viral loads compared to moribund shrimp. This finding suggests that immune mechanism(s) that suppress viral load are important to a shrimp's ability to survive TSV infection.

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CHAPTER 1: LITERATURE REVIEW

Shrimp belonging to the family Penaeidae include commercially important species inhabiting tropical and sub-tropical waters around the world (Bailey-Brock and Moss 1992). These shrimp are cultured worldwide and generate significant foreign exchange for the major shrimp-farming countries in Asia and the Americas. According to the Food and Agriculture Organization of the United Nations (FAO), an estimated 3.5 million metric tons (MT) of farmed penaeid shrimp were produced in 2009 with an estimated value greater than $14.6 billion (FAO 2011). Despite the economic importance of farmed shrimp, the global shrimp farming industry has been slow to adopt genetic improvement strategies that are prevalent in more mature meat-producing industries, such as the poultry and swine industries. This has resulted in production inefficiencies and reduced profits for shrimp farmers.

Historically, farmers have relied on the capture of wild shrimp to stock their ponds (Lightner et al. 2009). Wild shrimp are caught as postlarvae or broodstock. Postlarvae are stocked directly into ponds, whereas broodstock are spawned in captivity to produce postlarvae. Wild-caught shrimp pose a serious risk to the industry because they may be carriers of virulent pathogens which can be spread, both horizontally and vertically, throughout a shrimp culture facility or a shrimp farming region. Pandemics caused by viruses, particularly Taura syndrome virus (TSV) and White spot syndrome virus (WSSV), have resulted in significant economic losses in Asia and the Americas, and these losses can be attributed, in part, to the use of infected, wild-caught shrimp (Lightner 2003, Lightner et al. 2009).

Another significant disadvantage of culturing wild-caught shrimp is the inability of the farmer to benefit from selective breeding or other genetic improvement strategies. Selective breeding of terrestrial animals has resulted in dramatic improvements in growth, feed conversion efficiency, and reproductive performance over successive generations. For example, the chickens that we eat today grow twice as fast on half the amount of feed as the chickens of 50 years ago (Boyle 2001), and this improvement is due, in large part, to the selective breeding practices of poultry breeders. However, benefits accrued from the genetic improvement of shrimp lag far behind those realized in

more mature meat-producing industries, despite the fact that many penaeid shrimp possess characteristics amenable to selective breeding, including high fecundity, a relatively short generation time (9-12 months), and ease of captive reproduction. Selective breeding offers tremendous opportunity for increased production and profitability to shrimp farmers by improving commercially important traits. As the economic benefits of selective breeding become more compelling, it is likely that the global shrimp farming industry will invest significant resources in selective breeding programs to produce genetically superior stocks.

Genetic Improvement Programs for Penaeid Shrimp

Over the past several years, there has been an increasing trend among shrimp farmers to stock their ponds with postlarvae produced from captive broodstock in an effort to minimize the negative impacts of disease (Crocos and Moss 2006). The disease status of captive broodstock can be controlled, to a significant extent, using Specific Pathogen Free (SPF) shrimp in conjunction with a comprehensive biosecurity strategy designed to minimize the introduction and spread of pathogens in a maturation/hatchery facility (Lotz 1997a, Lightner 2003, Lightner et al. 2009). SPF shrimp are free of specified pathogens and SPF status is contingent on the level of biosecurity where the shrimp are maintained (Moss et al. 2003). Currently, only SPF populations of *P. vannamei* are commercially available on a large scale, and this factor has contributed to *P. vannamei* usurping *P. monodon* as the most commonly cultured shrimp species in the world. In 2000, an estimated 630,984 MT of farmed *P. monodon* were produced globally, whereas only 145,387 MT of farmed *P. vannamei* were produced during the same year (FAO 2011). However, in 2009, farmed *P. vannamei* production increased to 2,327,534 MT and this represents a 15-fold increase over 10 years. During the same period, farmed production of *P. monodon* only increased slightly to 769,219 MT. Historically, shrimp farmers in the Americas have cultured *P. vannamei*, so this dramatic species shift has occurred primarily in Asia where more *P. vannamei* are now produced than in the Western Hemisphere (FAO 2011).

Following the initial development of SPF populations of *P. vannamei* in the early 1990s (Wyban et al. 1993), a number of research and commercial shrimp breeding

programs were established, mostly in the Western Hemisphere. These programs generated basic information about the quantitative genetics of penaeid shrimp, including heritability (h^2) estimates, estimates of phenotypic and genetic variance, phenotypic and genetic correlations, and genotype × environment interactions. Importantly, these programs provided evidence that selective breeding of shrimp can be effective in improving commercially important traits. Currently, there are shrimp breeding programs in the Americas, Asia, Australia, New Caledonia, Madagascar, and the Middle East (Rosenberry 2006, Clifford and Preston 2006).

The primary traits of interest for shrimp breeders are growth and resistance to viral pathogens (Clifford and Preston 2006). Shrimp breeders can use several different selection strategies to improve these traits, including individual (mass) and family selection. Individual selection is based on an individual's own phenotype or performance, and individuals are either culled or selected based on their phenotype relative to the population mean. Family selection is based on mean family performance and can be divided into between-family selection and within-family selection. Between-family selection relies on a comparison of family means where entire families are either culled or selected based on mean family performance. Within-family selection is based on the ranking of an individual's performance within each family, and individuals are either culled or selected based on their relationship to their own family mean (see Tave 1993 and Gjedrem and Thodesen 2005 for a detailed discussion on different selection strategies).

The most appropriate selection strategy depends on a number of factors, including the h^2 of the trait under selection and the degree of environmental variance. Individual selection typically is used to improve traits with high h^2, whereas family selection is used to improve traits with low h^2 or when there are uncontrollable sources of environmental variance. Sib selection, a form of between-family selection, is used when shrimp have to be sacrificed in order to measure the trait of interest (e.g. tail to body weight ratio). This form of selection is particularly useful in SPF breeding programs designed to improve disease resistance. With this approach, the decision to cull or select a particular family is based on the phenotypic performance of shrimp that are exposed to the pathogen of

interest in a disease-challenge test. Unexposed, SPF siblings from the best performing families are then used to propagate the next generation.

Individual selection can result in rapid genetic gains in a short period of time for those traits with high h^2. In addition, the cost required to implement a breeding program based on individual selection is relatively low compared to family-based selection programs. However, a significant disadvantage in using individual selection is the potential for inbreeding because the genetic relationships among the breeding population typically are unknown. Inbreeding results from the mating of individuals who are related by ancestry and can cause a reduction in heterozygosity within a population (Falconer and Mackay 1996). As the level of inbreeding accumulates in a population, inbreeding depression can occur resulting in a reduction in mean phenotypic performance of certain traits (typically fitness-related traits like survival and fecundity). Rapid accumulation of inbreeding can be prevented in individual selection programs by keeping a large number of broodstock in the breeding population (~ 50 pairs of broodstock per generation, Bentsen and Olesen 2002). Inbreeding can be controlled using family selection because a breeder can avoid mating closely related individuals (e.g. brothers and sisters or first cousins). This can be accomplished by keeping families physically separated or by using markers to differentiate among families, and by maintaining accurate pedigree records. In many shrimp breeding programs, visible implant elastomer (VIE) tags are used as markers to identify families (Godin et al. 1996). These tags are made of a non-toxic, colored elastomer which is injected into the shrimp's connective tissue just under the endocuticle and is readable through the exoskeleton. Molecular markers, such as microsatellites, can also be used to determine parentage (Jerry et al. 2004). Microsatellites are short, non-coding DNA sequences which are repeated many times throughout an organism's genome, and these markers are particularly effective for parentage testing because they are co-dominant, highly polymorphic, and inherited in a Mendelian fashion. Because the use of molecular markers precludes the need for separate rearing areas or the use of physical tags, high selection intensities can be attained, resulting in more rapid genetic gains. However, the use of molecular makers requires the application of technologies typically unavailable to most shrimp farmers (e.g. Polymerase Chain Reaction), and may be cost prohibitive.

Currently, there are a number of shrimp breeding programs that use family selection and physical tags to improve growth and disease resistance. More sophisticated breeding programs are beginning to emerge which rely on the computation of estimated breeding values (EBVs) using computer software for the integration and analysis of pedigree and performance data (e.g. REML analysis and BLUP estimation of EBVs, Castillo-Juárez at al. 2006, and see Gjerde 2005 for more information about BLUP and other methods used to estimate breeding values). As indicated previously, only SPF populations of *P. vannamei* are commercially available on a large scale, so it has been the species of choice for most commercial breeding programs, particularly in the Western Hemisphere. There are ongoing breeding programs for other penaeid species, including *Penaeus* (*Fenneropenaeus*) *chinensis* (Wang et al. 2006), *Penaeus* (*Litopenaeus*) *stylirostris* (Goyard et al. 1999), *Penaeus* (*Marsupenaeus*) *japonicus* (Coman et al. 2004), and *P. monodon* (Coman and Preston 2008), although most of these programs are research-oriented or are at early stages of commercial development.

Selection for Disease Resistance

Shrimp farmers have suffered significant economic losses over the past 20 years due to various diseases (Lightner 2003, Flegel 2006) and these losses have catalyzed fundamental changes in the way shrimp aquaculture is practiced (Moss et al. 2001, Lightner et al. 2009). Biosecurity protocols are now common among industry stakeholders (Boyd 2006) and there are continuing efforts to develop disease-resistant shrimp (Clifford and Preston 2006). To date, shrimp breeders have focused most of their efforts on developing families of shrimp with enhanced resistance to TSV and WSSV (Argue et al. 2002, Kong et al. 2003, Jiang et al. 2004, Gitterle et al. 2005a) because these two viruses have had the greatest economic impact on the shrimp farming industry (Lightner 2003). A noteworthy exception was reported by Tang et al. (2000) who provided unequivocal evidence that a line of *P. stylirostris* was selectively bred for complete resistance to Infectious hypodermal and hematopoietic virus (IHHNV). Their results indicated that IHHNV did not replicate in postlarval or juvenile *P. stylirostris*, although the genetic basis for IHHNV resistance was not reported. (Note: throughout this

document, the words “resistant” and “resistance” are used as general terms to refer to a shrimp’s ability to survive a viral infection.)

Selective breeding for TSV resistance began in the mid-1990s in response to a TSV epizootic that devastated populations of *P. vannamei* in Ecuador and the subsequent spread of TSV throughout the Americas. TSV is a single-stranded RNA virus belonging to the family *Dicistroviridae* (Bonami et al. 1997) and can infect juvenile shrimp within two to four weeks after stocking into nurseries or growout ponds. Cumulative mortalities of unselected shrimp in TSV-infected ponds were reported to be as high as 80 – 90% (Brock et al. 1997, Lightner et al. 1998). Revenue losses from TSV in 1993 were estimated to be $400 million in Ecuador alone (Lightner 1999), and this virus has since spread to and impacted major shrimp farming regions in Asia (Tu et al. 1999, Phalitakul et al. 2006).

Breeding programs designed to enhance TSV resistance have generated valuable information about the quantitative genetics of disease resistance in shrimp and have highlighted some challenges associated with trying to improve resistance through selection. Unlike growth, h^2 estimates for TSV resistance are considered low to moderate ($h^2 \leq 0.25$). Argue et al. (1999) reported h^2 estimates for TSV resistance in successive generations of *P. vannamei* with estimates ranging from -0.04 ± 0.01 (SE) to 0.31 ± 0.07. Fjalestad et al. (1997) reported a mean h^2 estimate of 0.22 ± 0.09 for TSV resistance in *P. vannamei*, and Argue et al. (2002) reported a half-sib h^2 estimate of 0.19 ± 0.08 and a realized h^2 estimate of 0.28 ± 0.14 for one generation of selection in the same species.

Despite low to moderate h^2 for TSV resistance, significant improvements in this trait have been made through selection. Argue et al. (2002) reported an 18.4% increase in TSV survival after one generation of selection in a population of *P. vannamei*, compared with an unselected control population. Fjalestad et al. (1997) reported a selection response of 12.4% (expressed as the relative increase in TSV survival per generation) for the same species. White et al. (2002) reported an absolute increase in mean TSV survival from 24% to 37% among selected *P. vannamei* families over a 3-year period. In addition, there was an increase in survival from 65% to 100% among the best performing families during the same time period. Gitterle (1999) reported that, after an initial TSV outbreak in Columbia, pond survival typically was about 45%. However, survival returned to pre-

TSV levels of about 80% after just three generations of intense mass selection (selection of survivors from infected ponds).

The ability to improve TSV resistance by selection (despite low to moderate h^2) is attributed, in part, to high phenotypic/genotypic variation in TSV survival. This variation allows for a larger selection differential (and higher selection intensity) which increases the selection response (Falconer and Mackay 1996). Argue et al. (2002) reported that TSV survival ranged from 15% to 94% among 80 *P. vannamei* families exposed to TSV in a *per os* laboratory-challenge test. Similarly, White et al. (2002) reported that TSV survival ranged from 0% to 100% among 176 families. Although large variations in TSV survival have been observed among populations of *P. vannamei* families, the magnitude of this variation can decline as selection progresses. For example, while mean family survival increased from 44% to 84% after five generations of selection for TSV resistance among a population of *P. vannamei* families at Oceanic Institute (OI, Waimanalo, Hawaii, USA), the coefficient of variation (CV) for TSV survival decreased from 43.3% and 13.6% (Moss et al 2011). This reduction in variability is expected as selection progresses and will result in progressively lower selection responses (Falconer and Mackay 1996).

In addition to developing lines of shrimp with enhanced resistance to TSV, shrimp breeders have explored the possibility of selecting shrimp for WSSV resistance. These efforts were in response to the introduction and spread of this virus throughout the Americas in the mid- to late-1990s (Rosenberry 1999, 2000). WSSV initially was identified in Taiwan in 1992 (Chou et al. 1995) and spread rapidly throughout Asia (Inouye et al. 1994, Wongteerasupaya et al. 1995, Flegel and Alday-Sanz 1998). WSSV first appeared in the U.S. in 1995 and was identified in many shrimp farming regions of the Americas by 1999 (Lightner 1996a, Lightner 1999, Jory and Dixon 1999). WSSV is a double-stranded DNA virus belonging to the family Nimaviridae (Escobedo-Bonilla et al. 2008) and cumulative mortalities of shrimp in WSSV-infected ponds were reported to exceed 90% (Lightner 1999, Gitterle et al. 2005a).

Heritability estimates for WSSV resistance typically are lower than those reported for TSV. Published h^2 estimates for WSSV resistance in *P. vannamei* range from 0.00 to 0.21, and most estimates are <0.1 (Gitterle et al. 2005a, 2006a, 2006b). Not surprisingly,

only small improvements in WSSV resistance have been made through selection. Gitterle et al. (2005a) reported a mean selection response of only 2.8% after one generation of selection for WSSV resistance in a population of *P. vannamei*. In addition, after two generations of selection, a different population of *P. vannamei* exhibited no increase in WSSV survival. Mean survival for generations 0, 1, and 2 was 11.6%, 8.8%, and 7.9%, respectively. More recently, Gitterle (2006a) reported that WSSV survival for *P. vannamei* ranged from 3.1% to 33.3%. Survival varied by shrimp line, generation, and challenge method, but survival typically was <10%. Perez et al. (2005) challenged *P. vannamei* juveniles from three breeding programs with WSSV. Two of the programs used mass selection to enhance WSSV resistance (i.e. juveniles came from broodstock that were selected from WSSV-infected ponds with cumulative mortalities > 95%), whereas the other was a domestication program where shrimp were not exposed to WSSV. Interestingly, there was no significant difference in survival after WSSV exposure between the mass selected shrimp and the unselected shrimp. Mean survival (± SD) ranged from 1.7 ± 1.7% to 3.3 ± 3.3%. Efforts to improve WSSV resistance in the Chinese fleshy prawn, *P. chinensis*, have been more encouraging. Kong et al. (2003) reported that shrimp survival increased from 0-10% to 0-30% after three years of mass selection from WSSV-infected ponds. Furthermore, survival of shrimp selected for five generations ranged from 12-45%, compared to <1% for unselected shrimp when reared as cohorts in WSSV-infected ponds.

Penaeid shrimp, like many other aquaculture species, are highly fecund and, as a result, very few broodstock are required to produce sufficient numbers of offspring for the next generation. This characteristic, coupled with selective pressures (both natural and artificial), can lead to genetic bottlenecks making Penaeid shrimp populations susceptible to inbreeding, especially if pedigree records are unavailable or incomplete (Newkirk 1978). Additionally, most shrimp breeding programs were initiated from a narrow genetic base (Clifford and Preston 2006) making them even more susceptible to inbreeding. Despite the susceptibility of captive shrimp populations to inbreeding and the significant impacts of viral pathogens on global shrimp production, only one study to date has examined the effects of inbreeding on shrimp survival after exposure to viral pathogens. Moss et al. (2007) reported IBD estimates (reduction in phenotype per 10%

increase in inbreeding) for *P. vannamei* ranging from moderate (-8.3% for TSV isolate USHI94 and -11.1% for TSV isolate USTX95) to severe (-31.4% for TSV isolate BZ01 and -38.7% for WSSV), although not all estimates were statistically significant. Additional details of this study are presented in Chapter 2. See Tang et al (2000) for additional information on TSV isolates.

Disease resistance in shrimp may be negatively or positively correlated with other commercially important traits. Argue et al. (2002) reported a negative genetic correlation between mean family weight and mean family survival to TSV for one generation of *P. vannamei* (r_G = -0.46 ± 0.18 SE). Similarly, researchers from CENIACUA reported that harvest weight of *P. vannamei* was negatively correlated with WSSV survival in laboratory challenge tests, with genetic correlations (± SE) ranging from -0.31 ± 0.51 to -0.94 ± 0.64 (CENIACUA and AKVAFORSK 2002, Gitterle et al. 2005a). These results suggest that pleiotropic genes (genes that directly affect two or more traits) may be responsible for the observed trade-off between disease resistance and shrimp growth. Alternatively, the correlated responses may have resulted from other factors including sampling error, the genetic makeup of the population under study (including founder effects), and environmental correlations. Environmental correlations are caused by un-analyzed variation among or within ponds or laboratory conditions, resulting from variable feed supply, population density, temperature, or other factors that affect shrimp performance. Additional research is needed to investigate the genetic basis of these relationships so that more effective breeding strategies can be developed, if disease resistance is targeted for selection.

It would be advantageous for shrimp breeders to realize collateral benefits of multiple disease resistance when selecting shrimp for resistance to a single pathogen. However, available data do not support this outcome for TSV and WSSV resistance. Researchers from the Waddell Mariculture Center (Charleston, South Carolina, USA) and OI injected juvenile *P. vannamei* from selected families with TSV and WSSV in individual bioassays and found no significant phenotypic correlation in family survival between these two pathogens (r_P = 0.02, Moss et al. 2005). A similar observation was made by a commercial broodstock supplier in Hawaii who reported that family survival to TSV and WSSV from laboratory challenges were not correlated (Wyban 2000). Jiang

et al. (2004) found only a slight positive correlation in mean family survival to TSV and WSSV in a population of *P. vannamei* ($r_P = 0.29$). These results suggest that there is strong genotype × virus (i.e. environment) interaction for disease resistance (at least for TSV and WSSV) and that the development of disease-specific, resistant shrimp lines is warranted.

Shrimp breeders can employ several strategies to enhance disease resistance in shrimp. The least expensive approach is mass selection of survivors from infected ponds. This approach may be effective in improving TSV survival if shrimp are cultured under non-biosecure conditions, but it is inappropriate for an SPF breeding program (Lotz 1997a). For example, Colombian shrimp farmers bred survivors from TSV infected ponds and, after five generations of selection, pond survival increased from 10% to 70% (Gitterle 1999). However, these shrimp were carriers of TSV and when they were co-cultured with uninfected shrimp, massive mortalities occurred (Flegel 2001). Although mass selection may have limited benefits in enhancing TSV resistance under non-biosecure conditions, this approach was not effective in improving WSSV resistance (Pérez et al. 2005). This is not surprising since mass selection strategies are better suited for traits with moderate to high heritability (Falconer and MacKay 1996).

Shrimp breeders can improve disease resistance more effectively by exposing representative shrimp from different families to viable virus (either through feeding of infected tissue or by injection of a homogenate containing infected tissue) under controlled, laboratory conditions and estimating family survival after a specified amount of time, usually days to weeks (Prior et al. 2002, White et al. 2002). This information is then used to determine which families should contribute to the next generation of shrimp. As indicated previously, sib selection typically is used in SPF breeding programs designed to improve disease resistance. With this approach, the decision to cull or select a particular family is based on mean family survival after a disease-challenge test, and unexposed, SPF siblings are then used to propagate the next generation of shrimp from those families that are selected.

There are a number of concerns associated with using laboratory challenges to assess disease resistance in shrimp and these concerns have been discussed in detail elsewhere (Moss et al. 2005, Gitterle et al. 2006a, Gitterle et al. 2006b). Briefly, major

concerns include: 1) shrimp survival under artificial, laboratory conditions may not be predictive of survival in outdoor, commercial ponds; 2) shrimp survival may differ among labs and/or between challenges because of differences in challenge- test protocols (e.g. mode of exposure, age/size of shrimp at challenge, salinity and/or other water quality parameters, etc.); and 3) in *per os* challenges, exposed shrimp do not ingest equal doses of infected tissue and this results in differential rates of infection within and among families. More research is needed to optimize and standardize disease-challenge protocols so that greater genetic gains can be made to enhance disease resistance.

A number of other viruses and prokaryotes continue to negatively impact the global shrimp farming industry. However, these pathogens have received little or no attention from shrimp breeders to date. This may be due to the fact that breeding shrimp for disease resistance is a costly and lengthy process, and is only justified if a pathogen has a significant economic impact, there are no cost-effective measures to prevent or treat infection, and if there is additive genetic variation in resistance to the pathogen under selection (i.e. the trait is heritable). Additionally, reliable disease-challenge protocols must be developed for the pathogen under selection if a family-based breeding program is to be used to enhance disease resistance. Finally, it is important to note that each additional trait added to a selection program will inevitably lead to slower progress (i.e. smaller selection response per generation) for all selected traits, even if the traits are positively correlated. This restriction puts an upper limit on the number of traits that can be reasonably selected for in a given shrimp line.

Taura Syndrome Virus

TSV is a non-enveloped, icosahedral virus containing a single-stranded, positive-sense RNA genome of 10,205 nucleotides (Bonami et al. 1997; Mari et al. 2002). The genome is comprised of two large open reading frames (ORFs). ORF1 contains sequence motifs for non-structural proteins (i.e. helicase, protease, and RNA-dependent RNA polymerase), whereas ORF2 contains sequences for structural proteins, including three capsid proteins (Bonami et al. 1997; Mari et al. 1998; Robles-Sikisaka et al. 2001; Mari et al. 2002). TSV is classified to the genus *Cripavirus* within the family *Dicistroviridae* (superfamily of picornavirus; Mayo 2002a; Mayo 2002b).

As is common in RNA viruses, TSV is prune to mutation due to a lack of proofreading enzymes (Holland et al. 1982). In fact, a comparison of 40 TSV isolates collected at different times and/or from different locations, based on the deduced amino acid sequence of a highly variable region of the capsid-2 protein, identified 31 unique sequences (Tang and Lightner 2005). A phylogenetic analysis of these isolates revealed three distinct genetic groups named according to their geographic origins: Americas, Belize, and Southeast Asia. A more recent analysis, including newly collected isolates, identified a fourth genetic group of TSV originating from Venezuela (Côté et al. 2008).

P. vannamei is the principle host for TSV. TSV can infect all sizes/ages of *P. vannamei*; however, infections are typically seen in juveniles (<5 g) about 14-40 days after stocking into nursery tanks or growout ponds (Lightner 1995; Lightner 1999). Mortalities due to TSV in naïve or unselected *P. vannamei* populations have ranged from 40-95% (Lightner 1999). Several Western hemisphere penaeids (*P. stylirostris*, *P. setiferus*, and *P. schmitti*) can be infected, with infections sometimes resulting in disease and mortality (Brock et al. 1997; Overstreet et al. 1997). In contrast, other Western hemisphere species (*P. aztecus* and *P. duorarum*), as well as economically important Eastern hemisphere species (*P. chinensis*, *P. monodon*, and *P. japonicas*) have been experimentally infected with TSV, but do not display signs of disease (Brock et al. 1997; Overstreet et al. 1997; Flegel 2006).

TSV was first identified in Ecuador in 1992 (Lightner 1995; Hasson et al. 1995) and has since spread to all major shrimp farming regions of the Americas and Asia (Hasson et al. 1999a; Tu et al. 1999; Yu and Song 2000; Tang and Lightner 2005). The value of TSV-associated crops losses in the Americas between 1992 and 1995 was estimated at $1 billion USD (Lightner 1995). While no published estimates of TSV-associated crop losses from 1996 to present are available, periodic (if not continual) outbreaks throughout the Americas since 1995 and the spread of TSV to Asia (Tang and Lightner 2005) have undoubtedly had an enormous economic impact on the shrimp farming industry.

Taura syndrome (the disease caused by TSV) has three distinct phases: acute, transition, and chronic (Brock et al. 1997; Hasson et al. 1999b). The acute phase is rapid in individual shrimp (typically <24 hr), but can last for several days in an infected

population (Brock et al. 1997; Hasson et al. 1999b). During this phase, shrimp are lethargic and not feeding. Moribund shrimp display expansion of red chromatophores throughout the body surface, especially in the uropods (Lightner 1995). In the transition phase, shrimp often display melanized cuticular lesions that developed during the acute phase (Lightner 1995; Hasson et al. 1999b). Shrimp that successfully resolve these lesions and survive the next molting cycle typically appear normal. TSV-associated mortality most often occurs during the acute phase, likely due to osmotic failure resulting from widespread destruction of the cuticular epithelium (Lightner and Redman 2010). Mortality may also occur in transition phase and is likely associated with osmotic failure and/or infections by opportunistic bacteria (Lightner 1996b; Brock 1997). Shrimp in the chronic phase typically remain infected for life (Lightner 1995). These shrimp appear normal, but may be less tolerant to stress and, importantly, may pass the virus to progeny by vertical transmission (Hasson et al. 1999a, 1999b; OIE 2009a).

A variety of methods are available to diagnose TSV infection, including routine histology, *in situ* hybridization with cDNA specific probes, antibody-based methods, and reverse transcription PCR (RT-PCR; OIE 2009b; Lightner and Redman 2010). However, OIE (2009b) recommends RT-PCR for disease surveillance and screening purposes.

CHAPTER 2: EFFECTS OF INBREEDING ON GROWTH, GROWOUT SURVIVAL, AND SURVIVAL TO VIRAL PATHOGENS

Introduction

As the global shrimp farming industry matures, the use of domesticated, genetically improved shrimp stocks will become more common. In fact, numerous selective breeding programs for penaeid shrimp have been initiated over the last decade (Clifford 1998; Bienfang and Sweeney 1999; CENIACUA 1999; Goyard et al. 1999; Wyban 2000; Argue et al. 2002; Clifford et al. 2003; Moss et al. 2005; Clifford and Preston 2006). Penaeid shrimp, like most aquaculture species, are highly fecund and, as a result, very few broodstock are required to produce sufficient numbers of offspring for the next generation. This characteristic, coupled with selective pressures (both natural and artificial), can lead to genetic bottlenecks making shrimp populations susceptible to inbreeding, especially if pedigree records are unavailable or incomplete (Newkirk 1978). Additionally, most shrimp breeding programs were initiated from a narrow genetic base (Clifford and Preston 2006) making them even more susceptible to inbreeding. Breeders have recognized the risks associated with inbreeding and most attempt to minimize those risks through careful management of their breeding stocks (Clifford and Preston 2006). Some breeding companies have also developed germplasm protection strategies based on inbreeding (Doyle et al. 2006). Thus, knowing the severity to which inbreeding affects economically valuable traits is essential when designing an efficient and effective shrimp breeding program or germplasm protection strategy.

Inbreeding can be defined as the mating of individuals that are related by ancestry and results in a reduction of heterozygosity within a population (Falconer and Mackay 1996). The inbreeding coefficient (*F*) is a measure of inbreeding and can be defined as both the probability that two alleles at any given locus are identical by descent (alleles are descendents from a single ancestor) and the probable proportion of an individual's loci containing genes that are identical by descent (Falconer and Mackay 1996; Bourdon 1997). Inbreeding depression is the effect of inbreeding measured as the reduction in mean phenotypic performance with increasing levels of inbreeding within a population (Falconer and Mackay 1996; Lynch and Walsh 1998). Inbreeding depression typically is

seen in fitness-related traits (e.g. survival and various reproductive traits) and has been well documented in many agricultural plants and animals, as well as many laboratory animals (for review see Falconer and Mackay 1996 and Lynch and Walsh 1998). Inbreeding depression has also been reported for variety of aquaculture species, including channel catfish (Bondari and Dunham 1987), scallops (Ibarra 1995), rainbow trout (Pante et al. 2001), Atlantic salmon (Rye and Mao 1998), and Pacific oysters (Evans et al. 2004).

Despite the potential negative effects of inbreeding and the use of inbreeding as a germplasm protection mechanism, little is known about inbreeding depression in penaeid shrimp, including the Pacific white shrimp, *Penaeus* (*Litopenaeus*) *vannamei*, which is the most economically important cultured penaeid species worldwide (Moss 2004). De Donato et al. (2005) reported anecdotal evidence of an association between inbreeding and increased deformities, as well as reduced growth and FCR, during the growout of *P. vannamei*. However, these negative effects may have resulted from the presence of Infectious hypodermal and hematopoitic necrosis virus (IHHNV) on the farm. Deformities and reduced growth are consistent with Runt deformity syndrome caused by IHHNV (Kalagayan et al. 1991). Inbreeding has also been associated with reduced hatchery and growout performance in other penaeid species including *P.* (*Litopenaeus*) *stylirostris* (Bierne et al. 2000; Goyard et al. 2002) and *P.* (*Marsupenaeus*) *japonicus* (Sbordini et al. 1986, 1987; Keys et al. 2004). However, none of these studies clearly demonstrated the effects of inbreeding on shrimp performance.

The objective of this study was to investigate the effects of inbreeding on growth and survival of *P. vannamei* under a variety of growout conditions and during laboratory exposure to viral pathogens (survival only). This was accomplished through a retrospective analysis of family performance data from the Oceanic Institute's (OI) shrimp breeding program.

Materials and Methods

Study population

In 1991, OI initiated a selective breeding program for *P. vannamei* as part of the U.S. Marine Shrimp Farming Program (USMSFP). There are complete pedigree records

for the breeding population and it is comprised of eight founder populations collected from the wild at different geographic locations between 1989 and 2000. Since its inception, shrimp in the breeding program have been free of all pathogens listed by the USMSFP (2010), including those pathogens that are International Office of Epizootics (OIE) notifiable (OIE 2009a). The breeding population has been exposed to many different selection and management regimes during its history. The population has been artificially selected for growth since its inception and a portion of the population has been selected for resistance (or survivability) to Taura syndrome virus (TSV) since 1995. Selection for TSV resistance has been based on between-family selection, whereas individual, between-family, and within-family selection regimes have been used to improve growth.

Typically, 40 to 160 families were produced at OI each year (one generation/year) and, after evaluation, about 40 families were chosen as broodstock to produce the next generation. The population was separated into two lines (groups of shrimp produced and evaluated at different times) with each line consisting of 20-80 families per generation. Shrimp lines originated from the same founder populations and germplasm (typically in the form of broodstock) was moved between lines in most generations to maintain pedigree connectedness and to manage inbreeding (goal of <1% increase/generation). Selection response for growth and TSV survival were measured periodically, but not for every generation. Selection response estimates for growth have ranged from 3.1% to 25.0% per generation (Fjalestad et al. 1997; Argue and Alcivar-Warren 2000, Argue et al. 2002) and selection response estimates for TSV survival have ranged from 12.4% to 18.4% (Fjalestad et al. 1997; Argue et al. 2002). For a more detailed description of the founder stocks and the breeding program see Wyban et al. (1993), Carr et al. (1997), and Argue et al. (2002).

Performance evaluations

Data used for the retrospective analysis of family performance were generated during a 9-year period (1998-2006) of shrimp research and included growout and viral-challenge trials. The mean inbreeding level of the study population increased from 5% to 9% over this period. Prior to the start of the performance trials, shrimp from each family

(i.e. offspring from a unique dam-sire combination produced by artificial insemination; Arce et al. 2000) were tagged using a visible implant elastomer (Godin et al. 1995), and with each family received a unique tag code. At the termination of each trial, shrimp were identified (by family) from tag codes, counted, and individually weighed (growout trials only).

Growout trials were conducted in either a flow-through, earthen-bottom pond (EP) or concrete, recirculating raceways (RR; see Table 2-1 for a summary of growout trial parameters). Fifteen growout trials were conducted during the study period. A total of 583 families were evaluated and 77,546 and 99,389 individual shrimp performance records were collected for growth and survival, respectively.

Viral-challenge trials were conducted under laboratory conditions at the University of Arizona (Tucson, AZ, USA), the Gulf Coast Research Laboratory (Ocean Springs, MS, USA), or the Waddell Mariculture Center (Bluffton, SC, USA). Shrimp were challenged to three genetically distinct isolates of TSV (HI94, TX95, and BZ01) and one isolate of White spot syndrome virus (WSSV; see Table 2-2 for a summary of viral challenge trial parameters). Isolates HI94 and TX95 belong to the Americas Group of TSV (TSV-AG), whereas BZ01 is a member of the Belize Group (TSV-BG). For a description of TSV phylogenetics see Tang and Lightner (2005). Seventeen viral-challenge trials were conducted during the study period: 14 trials for TSV-AG, 2 for TSV-BG, and 1 for WSSV. The number of families challenged was 375 for TSV-AG, 120 for TSV-BG, and 10 for WSSV. A total of 14,898, 2,485, and 89 individual shrimp survival records were collected for TSV-AG, TSV-BG, and WSSV, respectively. TSV-challenge protocols were based on those described by White et al. (2002) and WSSV challenge protocols were similar to those described by Prior et al. (2003). See Table 2-3 for summary statistics on shrimp performance.

Data analysis

Effects of inbreeding on mean family performance were analyzed for 11 traits: growth (RR and EP combined), RR growth, EP growth, growout survival (RR and EP combined), RR survival, EP survival, TSV-AG survival (HI94 and TX95 combined),

HI94 survival, TX95 survival, TSV-BG (BZ01), and WSSV survival. The significance and magnitude of inbreeding effects were determined using the following linear model:

$$Y_{ij} = \mu + CG_i + bF_j + e_{ij}, \quad (1)$$

where Y_{ij} is the trait mean for the *j*th family in the *i*th contemporary group (CG); μ is the overall trait mean; CG_i is the fixed effect of the *i*th CG; *F* is the inbreeding coefficient of the *j*th family; *b* (the statistic of interest) is the coefficient for the regression of Y_{ij} on F_j; and e_{ij} is random error. The random error term in this model includes within-CG environmental (e.g. replicate tank effects) and genetic/family effects (i.e. differences in family performance unrelated to inbreeding) which could not be isolated with this analysis given the data structure. For this study, a CG was defined as a group of shrimp families produced at approximately the same time (within a 10-d period) and evaluated in the same growout or viral-challenge trial. Contemporary groups generally included all families available at the time of the trial (20 to 80 families). On occasion, some families were not challenged due to limited numbers of shrimp (resulting from poor hatchery or nursery survival) or limited tank space (disease challenge trials). For the latter scenario, the families to be evaluated were chosen at random. Thus, each CG was an unbiased sample of the available families at the time of the trial. Inbreeding coefficients were calculated using Lineage 1.06 software (Cornell University, Ithaca, NY, USA). All analyses were performed using SPSS 14.0 software (SPSS Inc., Chicago, IL, USA) and significance level was set at $\alpha = 0.05$ for all analyses.

Inbreeding depression (IBD), expressed as the percent change in phenotype per 10% increase in *F*, was calculated for all traits with the following equation:

$$\text{IBD} = [(b \times 0.1) / a)] \times 100, \quad (2)$$

where *b* and *a* are the regression coefficient and y-intercept from the regression of Y_{ij} on F_j (see equation 1), respectively.

The effect of environmental quality on IBD for survival was estimated by regressing IBD estimates on the environmental values of the performance trial

environments (Doyle et al 2006), where the environmental values are quantitative estimates of environmental quality. Lacking any direct measures of environmental quality, the predicted performance of non-inbred genotypes (the Y-intercept of the regression of Y_{ij} on F_j) was used as an estimate of the environmental value of the trial environments (Falconer and Mackay 1996). From the available data, it was possible to estimate a total of six environmental values in this way (data from combined analyses for growout survival and TSV-AG were excluded).

Results

Regression coefficients for growth in the RR, EP, and combined analyses were all negative and significant (Table 2-4). However, estimates of IBD on growth were relatively low (<4% reduction in growth per 10% inbreeding). Regression coefficients for growout survival were either slightly negative (EP analysis) or slightly positive (RR and combined analyses) and none were significant (Table 2-4). Consequently, IBD estimates for growout survival were essentially zero.

Regression coefficients for TX95 and TSV-AG combined analyses were negative and significant, whereas the regression coefficient for HI94 was negative but approaching significance ($p = 0.09$; Table 2-5). Estimates of IBD on survival to TSV-AG isolates were moderate and ranged from -8.3% (HI94) to -11.1% (TX95). Regression coefficients for the TSV-BG and WSSV analyses were both strongly negative, but neither was significant. Estimates of IBD on survival to TSV-BG and WSSV were -31.4% and -38.7%, respectively.

There was a significant linear relationship between IBD and environmental value ($b = 6.4$; $p = 0.01$; $r^2 = 0.84$; Fig. 2-1). For environments with high environmental values (non-inbred survival >70%) IBD was low to moderate (<12% reduction in phenotype). However, IBD appeared to worsen (but not all IBD estimates were significant) as the environmental value declined (i.e. as the mean survival of non-inbred genotypes progressively decreased).

Discussion

In the present study, inbreeding had a small but significant effect on growth in the RR, EP, and combined analyses. IBD estimates ranged from -2.6% (RR) to -3.9% (EP) and are similar to IBD estimates reported for other penaeid species. Keys et al. (2004) estimated IBD to be -3.3% when comparing inbred and outbred populations of *P. japonicus*, although this estimate was not significant. Bierne et al. (2000) found a significant positive correlation between microsatellite tri-locus heterozygosity and growth rate in a population of *P. stylirostris* and reported a mean IBD of 5% when comparing growth of single-locus heterozygotes and homozygotes. IBD estimates for shrimp are similar to those reported for other aquaculture species: -0.8 to -6.1% for rainbow trout (body weight; Gjerde et al. 1983; Su et al. 1996; Pante et al. 2001), -0.6 to -2.6% for Atlantic salmon (body weight; Rye and Mao 1998), -2.3% for catarina scallops (length; Ibarra et al. 1995), and -8.8% for Pacific oysters (body weight; Evans et al. 2004).

Survival is considered a fitness trait and IBD typically affects these types of traits more than traits like growth (Falconer and MacKay 1996). However, in the present study, growout survival was unaffected by inbreeding (IBD estimates ranged from -0.1 to 0.5%). Keys et al. (2004) estimated IBD on survival (from 30-day postlarvae (PL30) to PL156) of *P. japonicus* to be -3.4%, with survival of younger shrimp (PL30-PL80) being most affected by inbreeding. This is consistent with recent reports about oysters which indicate that IBD on oyster survival occurs primarily at less than 3 months of age (Launey and Hedgecock 2001; Bierne et al. 1998). In the present study, shrimp were stocked in growout trials at about PL60 (~70 days old), so it is possible that any inbreeding effects on growout survival occurred prior to stocking. Furthermore, previous research at OI indicates that there is a significant inbreeding effect on survival at early life stages (unpublished data); IBD estimates were -13% for hatch rate and -11% for hatchery survival (nauplius to PL10).

Effects of inbreeding on survival after pathogen exposure has been reported for oysters (Frierman and Andrews 1976), wild fish (Arkush et al. 2002; Giese and Hedrick 2003) and cultured fish (Hollebecq 1994; Shapira et al. 2005). However, this study provides the first estimates of IBD on survival after pathogen exposure for penaeid shrimp. Estimates of IBD on survival to TSV-AG were moderate and ranged from -8.3%

(HI94; regression coefficient not significant, $p = 0.09$) to -11.1% (TX95; regression coefficient significant, $p = 0.00$). Estimates of IBD on survival to TSV-BG (-31.4%) and WSSV (-38.7%) were high, but neither of the regression coefficients were significant. The lack of statistical significance for the HI94, TSV-BG, and WSSV analyses was likely due to low power ($\beta = 0.4$, 0.3, and 0.1, respectively), resulting from the high variability in survival among families (within CG) and the relatively small sample size for these traits.

Direct selection can offset (at least partially) inbreeding depression in a breeding program. Conversely, inbreeding depression can decrease selection response. We did not estimate selection response for any of the 11 traits as part of this study. However, selection responses (per generation) of 3.1% to 25.0% for growth (Fjalestad et al. 1997; Argue and Alcivar-Warren 2000, Argue et al. 2002) and 12.4% to 18.4% for TSV survival (Fjalestad et al. 1997; Argue et al. 2002) have been reported for this breeding population. Selection response for WSSV survival is likely to be low given the extremely low heritability for this trait (≤ 0.07; Gitterle et al. 2005a). These results suggest that strictly minimizing inbreeding may be necessary in shrimp breeding programs selecting for TSV or WSSV resistance, whereas substantial gains for growth can likely be achieved through selection even at low to moderate levels of inbreeding ($F < 0.2$). However, it is probably prudent for breeding programs to manage inbreeding irrespective of the traits under selection.

In the present study, IBD appeared to be sensitive to environmental quality, when environmental value (i.e. the predicted survival of non-inbred genotypes in a performance trial environment) was used as a quantitative estimate of environmental quality. IBD for survival became increasingly more severe as environmental quality declined (or the environment became more stressful). However, there were two potential problems with this analysis. First, non-significant IBD estimates were used, so the present regression may not properly estimate the "true" relationship between IBD and environmental quality. Second, the genetic constitution of the CGs were not the same and this may have biased (increased or decreased) the environmental value estimates, especially for WSSV and TSV-BG survival which were only evaluated for one or two CGs (narrow genetic pool). Despite these problems, the strong environmental sensitivity seen in this study

should not be discounted. Population genetics theory suggests that inbred populations may be more susceptible to environmental stress due to reduced genetic variability (Frankham 1995). Increased IBD in stressful environments has been reported for many plants (Schemeske 1983; Schmitt and Ehrnhardt 1990; Wolfe 1993) and animals (Jiménez et al. 1994; Keller et al. 2001), including Drosophila sp. (Miller 1994; Dahlgaard and Loeschcke 1997; Bijsma 1999). However, this phenomenon is not universal (Waller 1984; Johnston 1992; Norman et al. 1995). To our knowledge, this is the first study to investigate the relationship between IBD and environmental quality in shrimp and this line of research should be explored further.

Hatchery operators often buy shrimp broodstock from a few sources and attempt to use these stocks and their descendents as breeders for multiple generations. Significant declines in performance are often reported after two generations and this may be caused, in part, by inbreeding. As a crude germplasm protection strategy, broodstock suppliers generally provide very limited genetic diversity to the hatchery operator, so that inbreeding will accumulate rapidly if the hatchery operator attempts to breed the stocks for more than one generation. This forces the hatchery operator to purchase broodstock annually. Results from this study suggest that this strategy may be successful, since the effect of IBD on shrimp survival to TSV and WSSV appears to be severe and both viruses are present in most major shrimp regions. Of course, the successfulness of this strategy will depend on the breeding strategy of the hatchery operator (number spawners per generation, outcrossing of lines, etc), the degree of relatedness among the broodstock sold to the hatchery, and possibly environmental quality.

The mean inbreeding level of the study population was low (<10%) throughout the study period and few families had inbreeding levels greater than 20%. As a result, IBD at moderate to high levels of inbreeding may differ from the IBD estimates obtained in this study, although IBD is typically linear (Falconer and MacKay 1996). The rate of inbreeding accumulation in the study population was low (<1%/generation) and IBD may be more severe at higher inbreeding accumulation rates (Gjerde et al. 1983). Inbreeding accumulation in well managed breeding programs will likely be similar to the rate observed in this study. However, inbreeding may accumulate much faster at commercial hatcheries, where the mating of unpedigreed stocks is common.

In summary, inbreeding had a small but significant effect on growth. Growout survival was not affected by inbreeding, but inbreeding effects could have occurred prior to stocking (< 1-2 g). IBD on survival to TSV-AG was moderate (8-12%), whereas IBD on survival to TSV-BG and WSSV appeared to be more severe (>30%). However, the regression coefficients for TSV-BG and WSSV were not significant. IBD appeared to be sensitive to environmental quality, with IBD becoming more severe as environmental quality declined. Clearly, more research is needed to evaluate the effects of inbreeding on shrimp under diverse stock management strategies and growout conditions.

Table 2-1. Summary of growout trial parameters. EP = earthen pond; RR = recirculating raceway.

System	Size (m^2)	Water exchange (%/day)	Salinity (ppt)	Feed (% protein)	Stocking density (shrimp/m^2)	Stocking Wt (g)	Harvest Wt (g)
EP	337	25-100	2-34	35-45	80-202	1-3	15-25
RR	58-75	< 1	25-35	35	100-302	1-3	15-25

Table 2-2. Summary of viral challenge trial parameters. TSV = Taura syndrome virus; WSSV = White spot syndrome virus. Tanks used for TSV exposure contained shrimp from multiple families, whereas vessels used for WSSV exposure contained individual shrimp.

Virus	Exposure Method	Initial Wt (g)	Duration (d)	Tank Volume (L)
TSV	*per os*	2-6	14-21	2,000-4,000
WSSV	Injection	~1	7	0.26

Table 2-3. Summary statistics for performance evaluations. CG = contemporary group; RR = recirculating raceway; EP = earthen pond; TSV = Taura syndrome virus; WSSV = White spot syndrome virus.

Trait	Mean family performance ± SD	Range of CG means
RR growth (g/wk)	1.45 ± 0.21	1.29 – 1.89
EP growth (g/wk)	1.28 ± 0.16	1.08 – 1.41
RR survival (%)	77.06 ± 15.54	67.80 – 86.18
EP survival – EP (%)	82.00 ± 10.70	69.84 – 88.08
TSV-HI94 survival (%)	57.12 ± 28.42	32.81 – 83.91
TSV-TX95 survival (%)	50.21 ± 23.29	38.25 – 77.81
TSV-BZ01 survival (%)	38.83 ± 22.52	35.88 – 44.51
WSSV survival (%)	18.03 ± 16.62	N/A

Table 2-4. Results from regression of mean family growth and growout survival on inbreeding coefficient (*F*) and inbreeding depression (IBD) expressed as percent change in phenotype per 10% increase in *F*. RR = recirculating raceway; EP = earthen pond; *b* = regression coefficient; *a* = y-intercept; and N = number of family means used in the analyses. Numbers in parentheses in the "System" column are the number of contemporary groups. Numbers in parentheses in the "N" column are the number of families evaluated (some families were evaluated in both systems). *P*-values refer to the significance of *b*.

Trait	System	N	# shrimp	*b* ± SE	*p*	*a*	IBD	Mean *F* ± SD
Growth (g/wk)	RR (9)	380	54,477	-0.37 ± 0.17	0.03	1.45	-2.6	0.079 ± 0.047
	EP (6)	295	23,069	-0.43 ± 0.19	0.02	1.08	-3.9	0.062 ± 0.044
	Combined (15)	675 (583)	77,546	-0.40 ± 0.13	0.00	1.06	-3.6	0.071 ± 0.047
Survival (%)	RR (9)	380	70,581	4.34 ± 11.26	0.70	83.14	0.5	0.079 ± 0.047
	EP (6)	295	28,808	-1.11 ± 11.86	0.93	78.60	-0.1	0.062 ± 0.044
	Combined (15)	675 (583)	99,389	2.03 ± 8.19	0.80	78.42	0.3	0.071 ± 0.047

Table 2-5. Results from regressions of mean family survival to viral pathogens on inbreeding coefficient (*F*) and inbreeding depression (IBD) expressed as percent change in phenotype per 10% increase in *F*. TSV = Taura syndrome virus; AG = Americas group; BG = Belize group; WSSV = White spot syndrome virus; *b* = regression coefficient; *a* = y-intercept; and N = number of family means used in the analyses. Numbers in parentheses in the "System" column are the number of contemporary groups. Numbers in parentheses in the "N" column are the number of families challenged (some families were challenged against both TSV isolates). *P*-values refer to the significance of *b*.

Trait	Isolate	N	# Shrimp	*b* ± SE	*p*	*a*	IBD	Mean $\underline{F}$ ± SD
TSV-AG (%)	HI94 (8)	178	4,694	-59.54 ± 35.61	0.09	71.99	-8.3	0.079 ± 0.069
	TX95 (6)	293	10,204	-90.79 ± 30.80	0.00	81.86	-11.1	0.061 ± 0.034
	Combined (14)	471 (375)	14,898	-71.04 ± 22.99	0.00	80.41	-8.8	0.076 ± 0.078
TSV-BG (%)	BZ01 (2)	120	2,485	-173.21 ± 118.18	0.15	55.17	-31.4	0.063 ± 0.017
WSSV (%)	N/A (1)	10	89	-85.69 ± 202.28	0.68	22.17	-38.7	0.048 ± 0.028

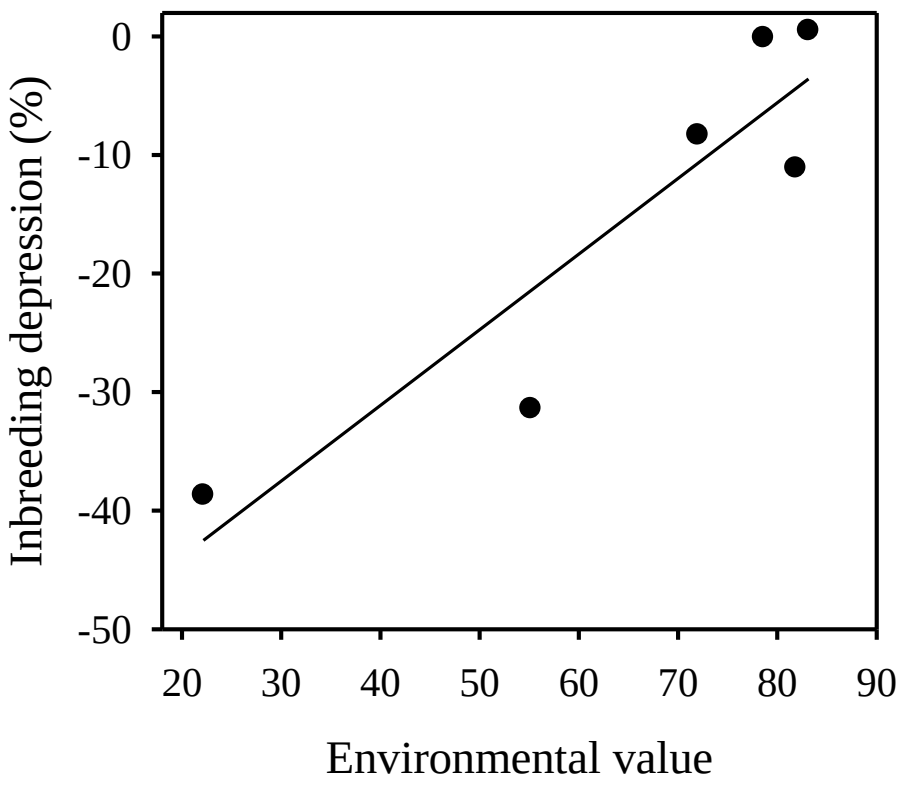

Figure 2-1. The effect of environmental quality on inbreeding depression for survival. The environmental value (Y-intercept of the regression of Y_{ij} on F_j) is the predicted survival of non-inbred genotypes ($F = 0$) in a performance trial environment and was used as a quantitative measure of environmental quality. Inbreeding depression (IBD) is expressed as the percent reduction in phenotype (survival) per 10% increase in F.

CHAPTER 3: ESTIMATION OF GENETIC PARAMETERS FOR SHRIMP SURVIVAL TO MULTIPLE ISOLATES OF TAURA SYNDROME VIRUS

Introduction

Taura syndrome virus (TSV) is an economically important pathogen of Pacific white shrimp, *Penaeus* (*Litopenaeus*) *vannamei*, which is the most commonly cultured shrimp species worldwide (FAO 2011). TSV was first identified in Ecuador in 1992 (Lightner et al. 1995; Hasson et al. 1995) and has since spread to all major shrimp farming regions of the Americas and Asia (Hasson et al. 1999a; Tu et al. 1999; Yu and Song 2000; Tang and Lightner 2005). TSV is highly virulent and TSV-associated mortalities in unselected, naïve populations of *P. vannamei* can range from 40-95% (Lightner 1999).

Selective breeding of *P. vannamei* for TSV resistance began in the mid-1990s and several research and commercial breeding programs have developed lines of shrimp which exhibit varying degrees of TSV resistance (Bienfang and Sweeney 1999; Wyban 1999; Argue et al. 2002; Clifford and Preston 2006). [Note: the terms "resistant" and "resistance" have been adopted by many stakeholders in the shrimp farming industry to refer to a shrimp's ability to survive viral exposure.] Although heritability for TSV resistance is considered low to moderate, significant improvements in TSV resistance have been made (Fjalestad et al. 1997; Gitterle 1999; Argue et al. 2002; White et al. 2002), including the establishment of some selectively bred stocks which exhibit >80% survival to TSV in *per os* laboratory challenges (Wyban 2000; Srisuvan et al. 2006; Moss et al. 2011).

TSV has a single-stranded, positive-sense RNA genome comprised of two open reading frames (ORFs), with ORF1 coding for non-structural proteins (helicase, protease, and RNA polymerase) and ORF2 coding for structural proteins, including three capsid proteins (Bonami et al. 1997; Mari et al. 2002). As is common with RNA viruses, TSV is prone to mutation due to a lack of proofreading enzymes (Holland et al. 1982). A comparison of 40 TSV isolates, based on the deduced amino acid sequence of a highly variable region of the capsid-2 protein, identified 31 unique sequences (Tang and Lightner 2005). Phylogenetic analysis of these sequences revealed three distinct genetic

groups of TSV named according to their geographic origin: Americas, Belize, and South East Asia. A more recent analysis, including newly collected isolates, identified a fourth genetic group originating from Venezuela (Côté et al. 2008).

Shrimp survival after TSV exposure has been documented for only a few isolates, so the virulence of most isolates is unknown. However, there is evidence that virulence varies among isolates. Erickson et al. (2005) conducted four *per os* laboratory challenges, using isolates BLZ02 (Belize group) and USHI94 (Americas group), and found that survival of Kona shrimp (an unselected, reference population of *P. vannamei*; see Hennig et al. 2004) was lower when the shrimp were exposed to the Belize-group isolate. Shrimp survival to BLZ02 ranged from 0 to 35%, whereas shrimp survival to USHI94 ranged from 20 to 70%. Similar results were reported by Tang and Lightner (2005) for Kona shrimp exposed to USHI94 and a different Belize-group isolate (BZ01). Srisuvan et al. (2006) reported survival for two populations of *P. vannamei* (Kona shrimp and a population selected for TSV resistance) when exposed to isolates USHI94, BZ01, TH04 (South East Asia group), and VE05 (Venezuela group). Survival patterns for Kona shrimp and the selected population were similar with survival to USHI94 being the highest for both populations (21.5% and 100%, respectively). Survival to VE05 (11.4% and 95.3%) was the next highest, followed by TH04 (5.3% and 82.7%), and BZ01 (0.0% and 77.5%).

While there is evidence that virulence varies among TSV isolates at the shrimp population level, little is known about how within-population variation for survival is correlated across TSV isolates (i.e. phenotypic and genetic correlations). Moss et al. (2005) reported a positive phenotypic correlation ($r_P = 0.52$) for survival among families (n = 80) of selectively bred *P. vannamei* exposed to two TSV isolates (USHI94 and BLZ02). However, additional estimates of phenotypic or genetic correlations for survival to multiple TSV isolates have yet to be reported.

Estimates of phenotypic and genetic correlations between TSV survival and other commercially important traits are also limited. Argue et al. (2002) found no correlation between pond survival and survival to TSV isolate USTX95 (Americas group) in *P. vannamei*, but did find a negative genetic correlation ($r_G = -0.46$) between growth and TSV survival. More recently, Moss et al. (2005) reported a negative correlation (r_P = -

0.15) between harvest weight and TSV survival (USHI94 or USTX95) for the same *P. vannamei* population.

For selective breeding programs to operate effectively, information about genetic correlations between important traits is needed to properly define selection goals and optimize selection/breeding protocols. Specifically with regard to breeding for TSV resistance, it is unknown if survival to multiple TSV isolates are unique traits or represent a single survival trait. Furthermore, it is unknown how isolate-specific TSV survival is related to other commercially important traits. The objectives of this study were to (1) estimate genetic correlations for shrimp survival to a genetically diverse suite of TSV isolates and (2) estimate genetic correlations between isolate-specific TSV survival and growout performance traits (i.e. growth and growout survival).

Materials and Methods

Breeding population

Shrimp for this study came from Oceanic Institute's (OI; Waimanalo, HI, USA) selective breeding program. There are complete pedigree records for the breeding population and it is comprised of eight founder populations of *P. vannamei* collected from the wild at different geographic locations within the natural range of this species. Since the inception of the breeding program, shrimp have been specific pathogen free (SPF) for all pathogens listed by the US Marine Shrimp Farming Program (for most current list, see USMSFP 2010), including those pathogens that are International Office of Epizootics (OIE) notifiable (OIE 2012).

The breeding population has been artificially selected for growth for 14 generations and a portion of the population has also been selected for TSV resistance (or survivability) for the last 10 generations. Each year, 40-160 families were produced (one generation/year) and, after evaluation, about 40 families were chosen as broodstock to produce the next generation. The population was separated into two lines with each line consisting of 20-80 families per generation. One line, referred to as Growth Line, was primarily selected for growth and the other line, referred to as TSV Line, was selected for a combination of TSV resistance and growth. Founder populations were the same for both lines and germplasm (typically in the form of broodstock) was moved between lines

periodically to maintain pedigree connectedness and to manage inbreeding (goal of <1% per generation).

Selection for TSV resistance was based on shrimp survival during laboratory, *per os* challenges. For early generations (1-6), TSV challenges and selection decisions were based on single-isolate challenges using either USHI94 or USTX95. In later generations (7-11), several multi-isolate challenges were conducted and selection decisions incorporated BZ01challenge data when available. This was done because phenotypic variability was highest for BZ01 and allowed for increased selection intensity. Growth evaluations were originally conducted (generations 1-8) in an earthen pond at stocking densities <100 shrimp/m^2. However, growout evaluations of the later three generations were conducted in a recirculating aquaculture system (RAS) at super-intensive stocking densities (>235 shrimp/m^2). For further details on OI's founder stocks and breeding program see Wyban et al. (1993), Carr et al. (1997), and Argue et al. (2002).

Production and evaluation of shrimp families

Performance data for 180 full-sib families (offspring of 177 sires and 175 dams) were used for this study and represent generations 7 (G7), 9 (G9), and 11 (G11) of the TSV Line. These generations were chosen because families within each generation were challenged with multiple isolates of TSV (allows for estimation of genetic covariance using multi-trait animal model), challenges for two isolates (USTX95 and BZ01) were conducted in multiple generations (allows for estimation of genetic covariance both within and across generations), and TSV evaluations (both within and across generations) were conducted at the same disease-challenge facility. Families in G9 and G11 were also evaluated for growout performance in RAS. It should be noted that families in G8 and G10 were challenged and selected for TSV resistance. However, only a single TSV isolate was used (USHI94) and challenges were conducted at a different facility than that used for G7, G9, and G11. Although performance data from G8 and G10 were excluded from this study, pedigree information from these generations was used.

Families for each generation were produced over 5-9 d using artificial insemination (Arce et al. 2000). Mated females were placed in individual tanks for spawning. After hatching, ~15,000 nauplii from each family were randomly selected and

transferred to family-specific, 100-L larval rearing tanks. Shrimp hatchery techniques, similar to those described by Wyban and Sweeney (1991), were used for rearing shrimp to 10-day postlarvae (PL-10).

After larval rearing, 1,000 PL-10 were randomly selected from each family and stocked into family-specific, 500-L nursery tanks. Nursery tanks were connected to a common recirculation system to minimize water quality and temperature differences among tanks. When shrimp reached 1-2 g wet weight, randomly selected juveniles (300-500) from each family were tagged with a fluorescent elastomer (Godin et al. 1996). Each family received a unique tag code and, after tagging, shrimp were returned to their respective nursery tanks until all families were tagged (≤ 5 d). Nursery tanks were then harvested and shrimp were counted and examined for tag quality. Shrimp with poor tags were discarded. The remaining tagged shrimp from each family were batch-weighed and transferred to evaluation facilities.

Tagged juveniles from all families were transferred (within 1-wk of tag checking) to Gulf Coast Research Laboratory (Ocean Springs, MS, USA) for a TSV challenge. Shrimp (~2.5 g) were stocked into replicate 4000-L tanks with each tank receiving representatives from all families. For all challenges, one (G7 and G11) or two (G9) of the replicate tanks were used as a negative control. For G11, 12 SPF Kona shrimp were also added to each challenge tank. Kona shrimp were used as a positive control population (White et al. 2002; Hennig et al. 2004) and were the same age/size as G11 shrimp. For G7 and G9, shrimp in each challenge tank were exposed to isolate USTX95 (Americas group) or BZ01 (Belize group). For G11, shrimp in each challenge tank were exposed to one of four TSV isolates (Table 3-1): USTX95, BZ01, TH04 (South East Asia group), and VE05 (Venezuela group). These isolates were chosen because they are genetically diverse and represent each genetic group of TSV. For details on TSV phylogenetics see Tang and Lightner (2005) and Côté et al. (2008).

After stocking, shrimp were allowed to recover for 3-5 d prior to the start of the challenge. Mortalities during the recovery period were minimal (<1%) and all mortalities were identified to family and deducted from stocking numbers. Challenge procedures were similar to those of White et al. (2002). Briefly, challenges commenced (day-0) when shrimp (mean weight of 2-3 g) were fed infected shrimp tissue at a rate of 3% of

tank biomass (Argue et al., 1999). Shrimp were fed to satiation with a commercial, pelleted diet for the remainder of the trial. To mitigate cannibalism, tanks were checked every 3-6 hr to remove dead/moribund shrimp. Challenges were terminated and survival was assessed on day-21. Water quality parameters were ~15 ppt, 26-27 C, ≥6 mg/L dissolved oxygen (DO), and <0.25 ppm NH_3-N for all challenges. Mortalities/moribund shrimp from all challenges, as well as challenge survivors from G7, were tested for TSV using RT-PCR.

Shrimp from G9 and G11 families were also evaluated for growth and growout survival in a 75-m^2 RAS raceway at OI. See Otoshi et al. (2007; 2009) for system description and general management procedures. Water quality parameters were 32-35 ppt, 26-32 C, 3.1-9.0 mg/L DO (typically 4.5-6.0 mg/L), <3 ppm NH_3-N, and <5 ppm NO_2-N. For each generation, tagged juveniles from each family (134-326 shrimp per family) were stocked into the raceway immediately after tagging checking (see above). Untagged shrimp (mixture of juveniles from available families) were also stocked in the raceway to achieve desired stocking densities. After 81-88 d, shrimp were harvested and all tagged shrimp were individually weighed. Since tracking of individual shrimp during growout was impossible, family stocking weights were used to estimate growth of individual shrimp. RAS growth (g/d) was calculated as (individual harvest weight – family stocking weight) / days of culture.

Data analysis

Data used for the estimation of (co)variance components for the random effects of TSV survival and RAS growout performance traits are presented in Tables 3-2 and 3-3. Components were estimated using a multivariate mixed linear animal model and is written as:

$$y = Xb + Za + e,$$

where $\underline{y}$ is a vector of observations (0 or 1 for TSV and RAS survival traits; g/d for growth) for two (bivariate model) or four (four-trait model) traits; b is the vector of fixed effects: sex (analyses of RAS growth data), tank (single-generation analyses), generation

(multi-generation or "combined" analyses), and generation × tank (combined analyses); $a \sim (0, A\sigma_a^2)$ is the vector of additive genetic values; $e \sim (0, I\sigma_e^2)$ is the vector of random errors; X and Z are known design matrices relating observations to levels of b and a, respectively; A is the additive genetic relationship matrix; and I is an identity matrix. The error covariance between the traits was set to zero because individual shrimp had phenotypes (i.e. survival or growth) for only one trait.

A four-trait model was used to estimate (co)variance components for/between TSV survival traits in G11. A series of bivariate models were used to estimate (co)variance components for/between survival to isolates USTX95 and BZ01, both within and across generations. A series of bivariate models was also used to estimate (co)variance components for/between TSV survival traits and RAS growth, both within and across generations. Lastly, a series of bivariate models was used to estimate (co)variance components for/between TSV survival traits and RAS survival, both within and across generations.

Genetic correlations between traits were estimated as $r_G = \sigma_{a(i,j)} / \sqrt{(\sigma^2_{a(i)} \times \sigma^2_{a(j)})}$, where $\sigma_{a(i,j)}$ is the additive genetic covariance for the ith and jth traits and $\sigma^2_{a(i)}$ and $\sigma^2_{a(j)}$ are the additive genetic variances. The statistical significance (H_0: $r_G = 0$; $\alpha = 0.05$) of genetic correlations was determined by testing z-scores against a large sample normal distribution (Kutner et al. 2005). Heritability was calculated as $h^2 = \sigma^2_{a(i)} / (\sigma^2_{a(i)} + \sigma^2_{e(i)})$, where $\sigma^2_{e(i)}$ is the error variance for the ith trait. All analyses and parameter estimations were conducted using GenStat version 15 (VSN International, Hemel Hempstead, UK).

Results

Mean family survival (mean of family-by-tank means ± SD) in TSV challenges increased from 47.6 ± 17.5% to 77.7 ± 14.5% for USTX95 and from 39.8 ± 19.9% to 79.4 ± 14.5% for BZ01 (Table 3-2). There was also a general trend of reduced phenotypic variability over time. In G11, mean family survival was high (≥77.7%) for all four isolates, with survival being the highest for VE05 and variability the lowest for TH01 in this generation. As expected, mean TSV survival of Kona shrimp was lower than for selected shrimp in G11. For Kona shrimp, survival was highest for USTX95 (29.2 ± 5.9), followed by VE05 (20.8 ± 5.9), TH04 (8.3 ± 11.8), and BZ01 (0.0).

Shrimp survival in negative control tanks was >99% for all challenges. All dead/moribund shrimp collected during challenges tested positive for TSV by RT-PCR. In addition, all challenge survivors from G7 were positive for TSV.

Genetic correlations (± SE) among TSV survival traits in G11 (four-trait model) were all positive and of moderate to high magnitude (0.35 ± 0.23 – 0.99 ± 0.26; Table 3-4). Only one correlation (BZ01-TH04) was not significantly different from zero. Heritabilities were low to moderate for the four TSV survival traits, with estimates ranging from 0.16 ± 0.04 for BZ01 to 0.33 ± 0.07 for TH04 (Table 3-4).

Genetic correlations between survival to USTX95 and BZ01 (bivariate model) were similar across generations (Table 3-5), ranging from 0.59 ± 0.25 to 0.87 ± 0.30. All correlations were positive, significantly different from zero, and in general agreement with the correlation estimated from the four-trait analysis of G11 data. Heritabilities for survival to USTX95 were similar across generations (ranging from 0.19 ± 0.05 to 0.26 ± 0.06; Table 3-5) and in close agreement with the estimate from the four-trait analysis of G11 data (0.26 ± 0.05). Heritabilities for survival to BZ01 were more variable and ranged from 0.24 ± 0.05 to 0.41 ± 0.07 (Table 3-5). The heritability for survival to BZ01 in G11 using the bivariate analysis (0.26 ± 0.06) was higher than the estimate from the four-trait analysis for G11 data (0.16 ± 0.04).

Mean family stocking and harvest weights were similar for G9 and G11 (Table 3-3). Mean family growth and survival were 0.21 ± 0.02 g/d and 70.0 ± 14.2% in G9, respectively. For G11, stocking density was 401 shrimp/m^2 compared to 237 shrimp/m2 in G9. Despite the increased stocking density, mean family growth (0.21 ± 0.02 g/d) was similar to that in G9 and survival was higher (80.2 ± 8.0) and less variable.

Genetic correlations between TSV survival traits and RAS growth (bivariate models) were all negative and of low magnitude (Table 3-6). Correlations between survival to BZ01 and RAS growth for G9 (-0.29 ± 0.17) and the combined analysis for G9 and G11 (-0.27 ± 0.13) were significantly different from zero. None of the other correlations between TSV survival traits and RAS growth were statistically significant. Genetic correlations between TSV survival traits and RAS survival (bivariate models) ranged from slightly negative (-0.13 ± 0.19) to moderately positive (0.30 ± 0.20), with none of the correlations being significantly different from zero (Table 3-6). Heritabilities

for RAS growth ranged from 0.43 ± 0.05 to 0.52 ± 0.06. Heritabilities for RAS survival were lower, ranging from 0.11 ± 0.03 to 0.21 ± 0.04.

Discussion

Survival of shrimp in negative control tanks was high (>99%) for all challenges. All dead/moribund shrimp collected during challenges tested positive for TSV. In total, these results provide strong evidence that challenge mortality can be attributed to TSV. All challenge survivors from G7 tested positive for TSV and this suggests that challenge procedures were sufficient to expose all shrimp in challenge tanks to TSV. Kona shrimp survival in G11 (29.2% for USTX95, 20.8% for VE05, 8.3% for TH04, and 0.0 for BZ01) provided further validation of challenge results, as isolate-specific TSV survival closely matched survivals previously reported for this population. Kona shrimp came from an SPF, unselected population of *P. vannamei* which is highly susceptible to TSV and exhibits consistently low survival in TSV challenges, irrespective of TSV isolate (White et al. 2002; Hennig et al. 2004). Due to these characteristics, Kona shrimp are often used as a control or reference population in TSV challenges of selected lines/families to validate challenge results (Hennig et al. 2004). Cao et al. (2010) reported survivals of 27% and 31% for USTX95 in two challenges using Kona shrimp. Srisuvan et al. (2006) reported Kona survivals of 11%, 5%, and 0% for isolates VE05, TH04, and BZ01, respectively. Similarly, Tang and Lightner (2005) reported a survival of 0% for Kona shrimp exposed to BZ01 and Côté et al. (2008) reported survival ranging from 10-40% for VE05.

Genetic correlations among TSV survival traits in G11 (four-trait model) were all positive and of moderate to high magnitude. Only the USTX95-BZ01 correlation (r_G = 0.35 ± 0.23) was not significantly different from zero, but this might be an artifact of limited data. For all TSV challenges, there were only two replicate challenge tanks per isolate and limited numbers of shrimp per family were evaluated in each challenge tank (see Table 3-2). This likely contributed to large correlation SEs and a lack of power for testing the statistical significance of some correlations.

Genetic correlations between survival to USTX95 and BZ01 (bivariate model) were consistent across generations. All correlations were significantly different from

zero and in general agreement with the correlation estimated from the four-trait analysis of G11 data. Moss et al. (2005) reported a correlation (r_P = 0.51) of similar magnitude for *P. vannamei* families challenged with TSV isolates USHI94 and BLZ02. However, additional estimates of phenotypic or genetic correlations for survival to multiple TSV isolates have yet to be reported. In fact, to our knowledge, data presented here include the first published genetic correlations for survival to genetically distinct isolates of a single viral pathogen in a marine invertebrate.

Several studies have demonstrated that virulence varies among TSV isolates (Erickson et al. 2005; Tang and Lightner 2005; Srisuvan et al. 2006); however, the reason(s) for this are unclear. Furthermore, it is unclear if variations in capsid-2 gene sequences used to characterize TSV isolates have any bearing on viral function (e.g. binding to host cells and recognition by host pattern recognition receptors). Thus, the four isolates used in this study may not be representative of all isolates within their respective genetic groups and, because of this, genetic correlations reported here may not be representative of correlations between other closely-related isolates. For example, the correlation between BZ01 (Belize Group) and TH04 (SE Asia group) may not be representative of correlations between other Belize Group and SE Asia group isolates. Furthermore, it cannot be assumed that genetic correlations between isolates within a genetic group will be high simply because they belong to the same genetic group. However, results from this study suggest that correlations between isolates will, at worst, not be correlated and, at best, be highly correlated.

Correlations among TSV survival traits in this study are higher than correlations for survival between TSV and other viral pathogens. Moss et al. (2005) found no significant correlation (r_P = 0.02) for family survival between TSV and White spot syndrome virus (WSSV; a DNA virus) in *P. vannamei*, and a commercial breeding program reported similar results (Wyban 2000). In addition, a negative correlation (r_P = -0.28) between mean family time of death for TSV and Yellowhead virus (YHV; a RNA virus) has been reported in *P. vannamei* (USMSFP 2010). The lack of strong correlations for survival between viral pathogens is not surprising, as immune mechanism(s)/pathway(s) or combinations of these, and their underlying genetic basis, likely differ among viral pathogens. For example, virus-binding proteins (VBPs) in

penaeid shrimp species can be virus/viral protein specific (Sritunyalucksana et al. 2013). VBPs are considered important for recognition of viral pathogens and activation of innate immune mechanisms; although, how the VBPs activate antiviral immune responses is still poorly understood (Sritunyalucksana et al. 2013; Tassanakajon 2013). Similarly, Veloso et al. (2011) found that the transcriptomic response to TSV and YHV differed in both TSV resistant and susceptible lines of *P. vannamei*.

Genetic correlations between TSV survival traits and RAS survival ranged from slightly negative (-0.13) to moderately positive (0.30) and none of the correlations were significantly different from zero. Similarly, Argue et al. (2002) found no correlation (r_G = 0.0) between pond survival and survival to USTX95 in *P. vannamei*. In addition to pathogen infection, growout survival can be influenced by a myriad of biotic and abiotic factors, such as primary productivity, water quality, feed inputs, weather, and management. Physiological response(s) to these factors, and their underlying genetic basis, are likely unrelated to antiviral immune responses.

Genetic correlations between TSV survival traits and RAS growth were all negative and of low magnitude (-0.07 – -0.29). Correlations between survival to BZ01 and RAS growth for G9 and the combined analysis of G9 and G11 were significantly different from zero, but none of the other correlations were significant. Argue et al. (2002) reported a negative correlation of moderate magnitude (r_G = -0.46) between growth and survival to USTX95 for *P. vannamei*. Moss et al. (2005) reported a weak but statistically significant negative correlation (r_P = -0.15) between harvest weight and TSV survival (USHI94 or USTX95) for the same population. Negative genetic correlations between TSV survival traits and growth suggest that the traits are influenced by common genes (i.e. pleiotropy). However, other causes of observed negative correlations between these traits have been suggested, such as sampling error, unaccounted for environmental correlations, and the genetic makeup of the shrimp population under study (Moss et al. 2005). Regardless, simultaneous selection for TSV resistance and growth has progressed well as evidenced by high TSV survival and good growth (at a high density) in G11. In addition, good growth of TSV-resistant stocks has been reported in other selected *P. vannamei* breeding programs (see Cock et al. 2009).

Mean weight of shrimp used in TSV challenges was ~2.5 g. Viral challenges commonly use shrimp of this size because (1) shrimp are large enough to tag for family identifications and (2) large numbers of shrimp can be held in laboratory challenge facilities (i.e. more animals per tank). Overstreet et al. (1997) found no significant differences in TSV survival for *P. vannamei* between <0.1and 5 g. In contrast, Lotz (1997b) conducted four experiments and found a general trend of lower TSV survival with increased size/age for *P. vannamei* ranging from 2g to 30 g; however, this trend was only statistically significant in two of the four experiments. It is unclear if genetic correlations between TSV survival traits or between TSV survival traits and RAS growout traits are size-specific (i.e. dependent on shrimp size during TSV challenge). However, Moss et al. (in prep) found a significant correlation ($r_P = 0.70$) for TSV survival (USTX95 isolate) in two size classes (2 g and 8 g) of *P. vannamei* representing 50 families.

Heritabilities for RAS growth ranged from 0.43 to 0.52, whereas heritabilities for RAS survival ranged from 0.11 to 0.21. These are consistent with previously reported heritabilities for these traits in *P. vannamei*. Castillo- Juárez et al. (2007) reported heritabilities for growth (i.e. body weight corrected for age) ranging from 0 to 0.52, with most estimates being >0.30. Gitterle et al. (2005b) reported heritabilities for growth and survival ranging from 0.01 to 0.54 and from 0.02 to 0.12, respectively. Similarly, Argue et al. (2002) reported heritabilities for survival ranging from -0.10 to 0.21.

Heritabilities for TSV survival traits (0.16 – 0.41) are similar to previously reported estimates. Argue et al. (2002) reported paternal half-sib, full-sib, and realized h^2 estimates (± SE) of 0.19 ± 0.08, 0.14 ± 0.05 and 0.28 ± 0.14, respectively. Similarly, Fjalestad et al. (1997) reported a maternal half-sib h^2 estimate of 0.22 ± 0.9. Despite low to moderate h^2, significant improvements in TSV survival have been made through selection (Gitterle 1999; Argue et al. 2002; Cock et al. 2009). This is attributed, in part, to high phenotypic/genotypic variation in shrimp survival to TSV (Moss and Moss 2009), which allows for a larger selection differential (and higher selection intensity) and subsequently increases selection response (Falconer and Mackay 1996). In this study, mean family survival (mean of family-by-tank means) in USTX95 and BZ01 challenges

increased considerably over the course of this study and there was a general trend of reduced phenotypic variability as selection progressed.

Separate hatchery and nursery rearing of full-sib families is common in shrimp breeding programs, since families within a cohort are generally produced over several days or weeks, physical tagging is not possible until shrimp reach ~1-g (Godin et al. 1996), and molecular genotyping (necessary if families are mixed at larval or early post-larval stages) of large numbers of shrimp is often cost prohibitive. Separate hatchery and nursery rearing of full-sib families was used in the present study and this, along with the breeding design (i.e. lack of half-sibs), did not allow for modeling/estimation of effects, other than additive genetic effects, common to full-sibs (c^2). If unaccounted for, c^2 can confound estimates of additive genetic effects and result in overestimation of genetic correlation and heritability (Falconer and Mackay 1996).

Castillo-Juárez et al. (2007) reported multiple c^2 estimates for *P. vannamei* harvest weight and found that, while most estimates were small (c^2 <0.1), not accounting for c^2 resulted in consistent overestimation of h^2. Gitterle et al. (2005b) reported similar c^2 estimates (0.07-0.09) for harvest weight but lower estimates (0.02-0.05) for survival of two selected lines of *P. vannamei*. However, these estimates are substantially higher than those reported from another *P. vannamei* breeding program (<0.01 for harvest weight and survival; Rocha et al. 2007). Furthermore, Gitterle et al. (2005a) reported that c^2 related to separate hatchery and nursery rearing of full-sib families were negligible for survival after exposure to WSSV.

As previously mentioned, heritability estimates from this study are in general agreement with published estimates for TSV survival and this suggests that c^2 effects were minimal in this study. In addition, age differences between families within a generation were minimal (5-9 d), hatchery and nursery rearing protocols were standardized across families, and nursery tanks were connected to a common recirculation system to minimize water quality and temperature difference between tanks. For these reasons, genetic correlations estimated in this study are likely close to the "true" parameters for this population.

Conclusions

Breeding for resistance to any of the four TSV isolates used in this study should, in general, improve resistance to the other isolates. However, correlated responses to selection may be lower than the primary selection response for some trait combinations, due to the moderate magnitude of genetic correlations. As an RNA virus, TSV is prone to mutation and mutations appear to have some effect on shrimp survival, as evidenced by imperfect genetic correlations among several isolates. Thus, a strategy to improve general TSV resistance by selecting for resistance to a genetically diverse suite of isolates may be prudent. Alternatively, selection for a highly virulent isolate and/or an isolate for which there is a lot of phenotypic variability for survival (i.e. an isolate for which intense selection and correlated responses to selection can be maximized) may be effective in improving general TSV resistance. Importantly, correlations among TSV survival traits and growout performance traits suggest that there are no major impediments to simultaneous genetic improvement of these traits.

Lastly, only four of the >40 TSV isolates were used in this study, so breeding protocols and selection goals based on generalizations from this study should be viewed with caution. As new virulent isolates are identified it would be prudent for breeders to challenge selected stocks against the new isolate(s) and determine if changes to selection procedures are warranted.

Table 3-1. Taura syndrome virus (TSV) isolates used in *per os* challenges of *P. vannamei*. Table was modified from Srisuvan et al. (2006).

TSV Isolate	Collection Location	Collection Year	GenBank Accession #
USTX95	Texas, USA	1995	-
BZ01	Belize	2001	AY590471
TH04	Thailand	2004	AY997025
VE05	Venezuela	2005	DQ212790

Table 3-2. TSV challenge data used for the estimation of genetic correlations and heritabilities: number of shrimp families, total shrimp, and mean family (±SD) survival by generation. Numbers in parentheses are the number of replicate challenge tanks. Superscripts refer to analyses for which each generation of data was used.

Generation	TSV Challenge				
	Isolate	# of families	Total shrimp	Survival (%)	Survival Range (%)
7[a]	USTX95	80 (2)	1,701	47.6 ± 17.5	0 – 88
7[a]	BZ01	80 (2)	1716	39.8 ± 19.9	0 – 88
9[a,c,d]	USTX95	50 (2)	955	75.0 ± 18.2	35 – 100
9[a,c,d]	BZ01	50 (2)	971	43.8 ± 25.9	0 – 85
11[a,b,c,d]	USTX95	50 (2)	1,236	77.7 ± 14.5	43 – 100
11[a,b,c,d]	BZ01	50 (2)	1,236	79.4 ± 14.5	35 – 100
11[a,b,c,d]	TH01	50 (2)	1,236	88.8 ± 9.9	63 – 100
11[a,b,c,d]	VE05	50 (2)	1,236	90.1 ± 10.7	53 – 100

[a] Bivariate analysis of USTX95 and BZ01 survival data.

[b] Four-trait analysis of TSV survival data.

[c] Bivariate analyses of TSV survival and RAS growth data.

[d] Bivariate analyses of TSV survival and RAS growout survival data.

Table 3-3. Growout data/performance in a recirculating aquaculture system (RAS) used for the estimation of genetic correlations and heritabilities: number of shrimp families, total shrimp, stocking density, mean family stocking weight (±SD), harvest weight (±SD), growth (±SD), and survival (±SD) by generation. Superscripts refer to analyses for which each generation of data was used and correspond to superscripts in Table 3-2.

Generation	RAS Growout						
	# of families	Total shrimp	Density (per m^2)	Stocking wt (g)	Harvest wt (g)	Growth (g/day)	Survival (%)
$9_{c,d}$	50	13,276	237	1.9 ± 1.5	19.1 ± 1.5	0.213 ± 0.018	70.0 ± 14.2
$11^{c,d}$	50	8,809	401	1.4 ± 0.2	20.2 ± 1.0	0.214 ± 0.011	80.2 ± 8.0

[c] Bivariate analyses of TSV survival and RAS growth data.

[d] Bivariate analyses of TSV survival and RAS growout survival data.

Table 3-4. Estimates of heritability (h^2 ± SE; on the diagonal) for four TSV survival traits (i.e. survival to four TSV isolates) and genetic correlations (r_G ± SE; below diagonal) among these traits for a selected population of *P. vannamei*. Estimates were calculated from variance components obtained from multivariate (4) animal model using a single generation of survival data (Generation 11, G11). * $p \leq 0.05$; ** $p \leq 0.005$.

Trait	USTX95	BZ01	TH04	VZ05
USTX95	0.26 ± 0.05			
BZ01	0.90 ± 0.31**	0.16 ± 0.04		
TH04	0.56 ± 0.25*	0.35 ± 0.23	0.33 ± 0.07	
VE05	0.87 ± 0.29**	0.99 ± 0.26**	0.50 ± 0.24*	0.27 ± 0.06

Table 3-5. Estimates of heritability (h^2 ± SE) for survival to two TSV isolates and genetic correlations (r_G ± SE) between these two traits for three generations in a selected population of *P. vannamei*. Estimates were calculated from variance components obtained from bivariate animal models using either a single generation of survival data or survival data from three generations (combined analysis). * $p \leq 0.05$; ** $p \leq 0.005$.

Generation	h^2		r_G
	USTX95	BZ01	
7	0.22 ± 0.04	0.24 ± 0.05	0.85 ± 0.25**
9	0.19 ± 0.05	0.41 ± 0.07	0.87 ± 0.30**
11	0.26 ± 0.06	0.26 ± 0.06	0.59 ± 0.25*
Combined	0.22 ± 0.03	0.32 ± 0.03	0.75 ± 0.15**

Table 3-6. Genetic correlations ($r_G \pm$ SE) among growout performance traits (growth and survival) in a recirculating aquaculture system (RAS) and TSV survival traits (i.e. survival to four TSV isolates) for two generations in a selected population of *P. vannamei*. Estimates were calculated from variance components obtained from bivariate animal models using either a single generation of survival data or survival data from both generations (combined analysis). * $p \leq 0.05$.

RAS Trait	Generation	r_G			
		USTX95	BZ01	TH04	VZ05
Growth	9	-0.26 ± 0.18	-0.29 ± 0.17*	--	--
	11	-0.07 ± 0.18	-0.24 ± 0.19	-0.27 ± 0.20	-0.09 ± 0.18
	Combined	-0.12 ± 0.13	-0.27 ± 0.13*	--	--
Survival	9	0.12 ± 0.19	0.24 ± 0.17	--	--
	11	0.30 ± 0.20	-0.11 ± 0.19	-0.12 ± 0.20	-0.13 ± 0.19
	Combined	0.19 ± 0.13	0.16 ± 0.12	--	--

CHAPTER 4: ESTIMATION OF GENETIC CORRELATION AND HERITABILITY FOR TAURA SYNDROME VIRUS RESISTANCE IN TWO SIZE CLASSES OF SHRIMP

Introduction

Taura syndrome virus (TSV) is an economically important pathogen of farmed penaeid shrimp. Taura syndrome (TS), the disease caused by TSV, was first identified in commercial shrimp farms located near the mouth of the Taura River (Gulf of Guayaquil, Ecuador) in 1992 (Lightner 1995; Hasson et al. 1995). Since then, the disease has spread to all major shrimp farming regions of the Americas and Asia (Hasson et al. 1999a; Tu et al. 1999; Yu and Song 2000; Tang and Lightner 2005). Several commercially important penaeid species (of American and Asian origins) can be naturally or experimentally infected with TSV (Brock et al. 1997; Overstreet et al. 1997; Flegel 2006). However, *Penaeus* (*Litopenaeus*) *vannamei*, the most economically important cultured shrimp species worldwide (FAO 2011), is the most severely affected by TS (i.e. highest mortality; Brock et al. 1997, Hasson et al. 1995, 1999b, Lightner 1996a, Lightner 1999, Overstreet, 1997). TS-associated mortalities >90% have been reported in naïve or unselected *P. vannamei* populations (Brock 1997; Brock et al. 1997; Lightner 1999).

In response to TSV epizootics in the Americas, a variety of strategies to mitigate crop loss from this pathogen were implemented in the Western Hemisphere, including the establishment of selective breeding programs to develop TSV-resistant shrimp (Moss et al. 2005). Selective breeding efforts began in the mid-1990s and have generally been successful, as significant improvements in TSV resistance (measured as survival to viral exposure) have been reported (Fjalestad et al. 1997; Bienfang and Sweeney 1999; Gitterle 1999; Wyban 1999; Wyban 2000; Argue et al. 2002; White et al. 2002; Clifford and Preston 2006; Moss et al. 2011).

Shrimp breeding programs commonly use laboratory *per os* challenges to assess TSV resistance. With this approach, shrimp from multiple families (i.e. dam-sire combinations) are exposed to TSV and family survival is measured after a specific

amount of time, usually 14-21 d (White et al. 2002). This information is then used to determine which families will be selected as breeders to produce the next generation.

Juvenile shrimp, 1-3 g wet weight, are commonly used in *per os* challenges for TSV (Argue et al. 2002; White et al. 2002; Tang and Lightner 2005; Srisuvan et al. 2006; Côté et al. 2008) and other viral pathogens (Tang et al. 2000; Dhar et al. 2002; Gitterle et al. 2005b, 2006b; Pérez et al. 2005). This size is preferred because shrimp are large enough to be tagged for family identification (minimum effective tagging size is ~1 g; Godin et al. 1996), but still small enough to allow for relatively large numbers of shrimp to be stocked in laboratory-scale challenge tanks.

TSV infections in farmed populations of *P. vannamei* are typically seen in juveniles (<5 g) about 14-40 days after stocking into nursery tanks or growout ponds; however, TSV can infect a wide range of sizes/ages (i.e. from postlarvae to adults) of this species (Lightner 1995; Lotz 1997; Overstreet 1997). There is a paucity of information on the effects of shrimp size on TSV virulence for *P. vannamei* and other penaeid species. Overstreet et al. (1997) found no effect of shrimp size on TSV survival in experiments with *P. vannamei* and *P. setiferus*, but results may have been confounded by genetic differences between experimental populations (within a species). Lotz (1997b) conducted four experiments using various size classes of *P. vannamei* ranging from 1-30 g and found a consistent trend of lower TSV survival with increasing shrimp size. However, this effect was only statically significant in two of the experiments. In contrast, higher survival with increased size/age has been reported for *P. vannamei* exposed to Baculovirus penaei (Leblanc and Overstreet 1990) and for *P. stylirostris* challenged with Infectious hypodermal and haematopoietic necrosis virus (IHHNV; Bell and Lightner 1987).

Despite limited evidence that virulence of TSV and other viral pathogens varies with shrimp size, genotype × age interactions for viral resistance have yet to be examined in shrimp. For selective breeding programs to operate effectively, estimates of quantitative genetic parameters, most notably heritability and genetic correlations, are needed to properly define selection goals and optimize selection/breeding protocols.

Specifically with regard to breeding for TSV resistance, it is unknown if a single viral challenge of small juveniles is the most effective protocol for evaluating TSV resistance in shrimp families. The objective of this study was to estimate the genetic correlation and heritability for TSV survival in two size classes of *P. vannamei*.

Materials and Methods

Shrimp for this study came from Oceanic Institute's (OI; Waimanalo, HI, USA) selective breeding program. There are complete pedigree records for the breeding population and it is comprised of eight founder populations of *P. vannamei* collected from the wild at different geographic locations within the natural range of this species. Since the inception of the breeding program, shrimp have been specific pathogen free (SPF) for all pathogens listed by the US Marine Shrimp Farming Program (for most current list, see USMSFP 2010), including those pathogens that are International Office of Epizootics (OIE) notifiable (OIE 2012).

The breeding population has been artificially selected for growth for 14 generations and a portion of the population (referred to as TSV Line) has also been selected for TSV resistance (or survivability) for the last 10 generations. Each year, 20-80 TSV Line families were produced (one generation/year) and, after evaluation, about 20 families were chosen as broodstock to produce the next generation. Selection for TSV resistance was based on shrimp survival during laboratory, *per os* challenges. Challenges typically used juveniles ranging in size from 1 to 3 g. For further details on OI's founder stocks and breeding program see Wyban et al. (1993), Carr et al. (1997), and Argue et al. (2002).

For this study, 50 full-sib families were produced from 50 sires and 50 dams representing 19 selected families from the previous generation. Mean inbreeding coefficient for the 50 families was 0.07 and ranged from 0.05 to 0.09. Families were produced over 5 d using artificial insemination (Arce et al. 2000). Mated females were placed in individual tanks for spawning. After hatching, ~15,000 nauplii from each family were randomly selected and transferred to family-specific, 100-L larval rearing

tanks. Shrimp hatchery techniques, similar to those described by Wyban and Sweeney (1991), were used for rearing shrimp to 10-d postlarvae (PL-10).

After larval rearing, 1,000 PL-10 were randomly selected from each family and stocked into family-specific, 500-L nursery tanks. Nursery tanks were connected to a common recirculation system to minimize water quality and temperature differences among tanks. When shrimp reached ~1 g wet weight, randomly selected juveniles (300-500) from each family were tagged with a fluorescent elastomer (Godin et al. 1996). Each family received a unique tag code and, after tagging, shrimp were returned to their respective nursery tanks until all families were tagged ($\leq$ 5 d). Nursery tanks were then harvested and shrimp were counted and examined for tag quality. Shrimp with poor tags were discarded. The remaining tagged shrimp from each family were used for performance evaluations (growout and TSV challenges). Shrimp for TSV challenges were randomly assigned to one of two experimental groups (challenge 1 and challenge 2) and held in separate tanks until transfer to disease-challenge facilities.

For challenge 1, tagged juveniles were transferred (within 1 wk of tag checking) to Gulf Coast Research Laboratory (GCRL; Ocean Springs, MS, USA) for a TSV challenge. Shrimp (mean weight = 2.5 g) from each family were stocked into four replicate 4000-L tanks (~12 shrimp/family/tank) with one tank serving as a negative control. In addition, 12 SPF Kona shrimp were added to each challenge tank. Kona shrimp were used as a positive control population (White et al. 2002; Hennig et al. 2004) and were the same age/size as TSV Line shrimp. Challenge procedures were similar to those of White et al. (2002). Briefly, challenges commenced (day-0) when shrimp were fed shrimp tissue infected with TSV (isolate USTX95; see Tang and Lightner 2005 and Côté et al. 2008 for details on TSV phylogenetics) at a rate of 3% of tank biomass (Argue et al. 1999). For the remainder of the challenge, shrimp were fed to satiation with a commercial, pelleted diet. To mitigate cannibalism, tanks were checked every 3-6 hr to remove dead/moribund shrimp. The challenge was terminated and survival assessed on day-21.

Shrimp for challenge 2 were transferred to GCRL one week after the completion of challenge 1. Protocols for challenge 2 were the same as for challenge 1, except that mean shrimp weight was 8.0 g and ~8 shrimp/family were stocked into each challenge tank. Larger shrimp size resulted in a reduction in the number of shrimp/tank for challenge 2, so that tank biomass was below the maximum tank biomass (established in previous trials) for maintaining acceptable water quality.

Water quality parameters for both challenges were ~15 ppt, 26-27 C, ≥6 mg/L dissolved oxygen (DO), and <0.25 ppm NH_3-N for all challenges. For each challenge, >20 dead/moribund shrimp were tested for TSV using RT-PCR to verify infection.

A summary of the data used for the estimation of (co)variance components for the random effects of TSV survival are presented in Table 4-1. Components were estimated using a bivariate, mixed linear animal model that is written as:

$$y = Xb + Za + e,$$

where y is a vector of survival observations (0 or 1); b is the vector of fixed effects (i.e. tank within challenge effects); $a \sim (0, A\sigma_a^2)$ is the vector of additive genetic values; $e \sim (0, I\sigma_e^2)$ is the vector of random errors; X and Z are known design matrices relating observations to levels of b and a, respectively; A is the additive genetic relationship matrix; and I is an identity matrix. The error covariance was set to zero because individual shrimp had phenotypes (i.e. survival) in only one challenge.

Genetic correlation for TSV survival between size classes (i.e. challenges) was estimated as $r_G = \sigma_{a(i,j)} / \sqrt{(\sigma^2_{a(i)} \times \sigma^2_{a(j)})}$, where $\sigma_{a(i,j)}$ is the additive genetic covariance for the ith and jth size classes and $\sigma^2_{a(i)}$ and $\sigma^2_{a(j)}$ are the additive genetic variances. Phenotypic correlation (r_P) for TSV survival was estimated as the correlation of family survivals (mean of family-by-tank survivals) for 2.5-g and 8.0-g size classes. Heritability for TSV survival was calculated as $h^2 = \sigma^2_{a(i)} / (\sigma^2_{a(i)} + \sigma^2_{e(i)})$, where $\sigma^2_{e(i)}$ is the error variance for the ith size class. All analyses and parameter estimations were conducted using GenStat version 15 (VSN International, Hemel Hempstead, UK).

Results and Discussion

Shrimp survival in negative control tanks was 100% for both challenges. All dead/moribund shrimp collected during challenges tested positive for TSV by RT-PCR. These results strongly suggest that environmental (i.e. random or non-modeled) effects among challenges were minimal and that challenge mortality can be attributed to TSV exposure. Kona shrimp survival (mean of tank means; ± SD) was 25.0 ± 8.3% and 20.8 ± 7.2% for 2.5-g and 8.0-g size classes, respectively (Table 4-1). Kona shrimp survival provided further validation of challenge results, as survivals closely matched TSV survivals previously reported for this population (White et al. 2002; Hennig et al. 2004; Cao et al. 2010). Kona shrimp came from an SPF, unselected population of *P. vannamei* which is highly susceptible to TSV and exhibits consistently low survival in TSV challenges. Due to these characteristics, Kona shrimp are often used as a control or reference population in TSV challenges of selected lines/families to validate challenge results (White et al. 2002; Hennig et al. 2004).

Mean family TSV survival (± SD) for the 2.5-g size class (challenge 1; 75.2 ± 14.0%) was higher than for the 8.0-g size class (challenge 2; 63.6 ± 15.1%), although family survivals were highly variable in both challenges (Table 4-1; Fig. 4-1). Similarly, Lotz (1997b) reported a trend of lower TSV survival with increasing shrimp size (1-30 g) in *P. vannamei*, but this effect was only statically significant in two of four experiments. Conversely, Overstreet et al. (1997) found no effect of shrimp size on TSV survival for *P. vannamei* and *P. setiferus*; however, only small shrimp (≤3 g) were tested and results may have been confounded by genetic differences between experimental populations within a species. A trend of higher host survival with increasing age/size has been reported for *P. vannamei* exposed to Baculovirus penaei (Leblanc and Overstreet 1990) and for *P. stylirostris* exposed to IHHNV (Bell and Lightner 1987).

The effect of host size on viral virulence has also been reported in fish. Hanson et al. (2004) reported increasing susceptibility of channel catfish, *Ictalurus punctatus*, to Channel catfish virus disease with age. Kasai et al. (1993) reported an opposite trend for rainbow trout, *Oncorhynchus mykiss*, exposed to infectious hematopoietic necrosis virus.

Phenotypic correlation for TSV survival between the size classes was $r_P = 0.82$ and was significantly different from zero ($p = 0.00$; Fig. 4-1). Genetic correlation for TSV survival was also high ($r_G = 0.87 \pm 0.31$; Table 4-2). These results show that TSV survival of 2.5-g and 8.0-g size classes in *P. vannamei* are either strongly related traits or the same trait (i.e. $r_G = 1$). TSV infections in farmed populations of *P. vannamei* are typically seen in juveniles (<5 g) about 14-40 days after stocking into nursery tanks or growout ponds; however, TSV can infect a wide range of sizes/ages of this species (Lightner 1995; Lotz 1997; Overstreet 1997). Only two size classes of shrimp were challenged in this study, so it's unknown how survival of shrimp ≤8 g is related to survival of larger size classes. However, results suggest that the common practice of challenging small juveniles (1-3 g) to TSV in *per os*, laboratory challenges and using these data to select for TSV resistance can be highly effective in improving farm survival of *P. vannamei* (at least up to 8 g) during TSV epizootics.

For both challenges, there were limited numbers of shrimp/family evaluated in each challenge tank and this likely contributed to the large SE for r_G. For challenge 1, ~12 shrimp/family were stocked into each challenge tank. Larger shrimp size in challenge 2 required a reduction to ~8 shrimp/family in each challenge tank, so that tank biomass was below the maximum tank biomass allowable (established in previous trials) for maintaining acceptable water quality. This demonstrates the inherent difficulty in evaluating disease resistance for large numbers of shrimp families in laboratory challenge facilities and why challenges are typically conducted using small juveniles (1-3 g; i.e. immediately after tagging for family identification).

Heritability for TSV survival was 0.23 ± 0.05 for the 2.5-g size class and 0.22 ± 0.06 for the 8.0-g size class (Table 4-2). These values are similar to previously reported heritabilities for TSV survival. Argue et al. (2002) reported paternal half-sib, full-sib, and realized h^2 estimates (± SE) of 0.19 ± 0.08, 0.14 ± 0.05 and 0.28 ± 0.14, respectively. Similarly, Fjalestad et al. (1997) reported a maternal half-sib h^2 estimate of 0.22 ± 0.9.

Shrimp breeding programs commonly employ separate hatchery and nursery rearing of full-sib families, since families within a cohort are generally produced over

several days or weeks, physical tagging is not possible until shrimp reach ~1-g (Godin et al. 1996), and molecular genotyping (necessary if families are mixed at early life stages) of large numbers of shrimp is often cost prohibitive. Separate hatchery and nursery rearing of full-sib families was used in the present study and this, along with the breeding design (i.e. lack of half-sibs), did not allow for modeling/estimation of effects, other than additive genetic effects, common to full-sibs (c^2). If unaccounted for, c^2 can confound estimates of additive genetic effects and result in overestimation of genetic correlation and heritability (Falconer and Mackay 1996). For *P. vannamei*, c^2 estimates have been reported for growth/harvest weight, growout survival, and White spot syndrome virus resistance (Gitterle et al. 2005a; Gitterle et al. 2005b; Castillo-Juárez et al. 2007; Rocha et al. 2007). These estimates are all low relative to corresponding estimates of additive genetic (co)variance and would not substantially alter estimates of heritability and genetic correlation if unaccounted for. As previously mentioned, heritability estimates from this study are in general agreement with published estimates for TSV survival and this suggests that c^2 effects were minimal in this study. In addition, age differences between families were small (≤5 d), hatchery and nursery rearing protocols were standardized across families, nursery tanks were connected to a common recirculation system to minimize water quality and temperature difference between tanks, and families were held in communal tanks prior to transfer to disease challenge facilities.

In conclusion, TSV survival for 2.5-g and 8.0-g size classes are highly related and may, in fact, be a single trait. Heritabilities for TSV survival were similar between size classes and genetic and phenotypic correlations were high ($r > 0.8$). These results, along with the fact that *P. vannamei* are commonly harvested at ≤20 g, suggest the common practice of challenging small juveniles (1-3 g) to TSV and using these data to select for TSV resistance is likely effective in improving farm survival of *P. vannamei* during TSV epizootics.

Table 4-1. TSV challenge data used for the estimation of genetic correlations and heritabilities for two size classes of *P. vannamei*: mean shrimp weight, number of shrimp families, total shrimp, mean family (±SD) survival, range of family survival, and mean survival (mean of tank means) for Kona control shrimp. Numbers in parentheses are the number of replicate challenge tanks.

Challenge	Weight (g)	# of families	Total shrimp	Survival (%)	Survival range (%)	Control survival (%)
1	2.5	50 (3)	1,890	75.2 ± 14.0	43 – 100	25.0 ± 8.3
2	8.0	50 (3)	1,155	63.6 ± 15.1	33 – 100	20.8 ± 7.2

Table 4-2. Estimates of heritability (h^2 ± SE; on the diagonal) and genetic correlation (r_G ± SE; below diagonal) for TSV survival in two size classes of selectively bred *P. vannamei*. Estimates were calculated from variance components obtained from a bivariate animal model using a single generation of survival data.

Size class	2.5 g	8.0 g
2.5 g	0.23 ± 0.05	
8.0 g	0.87 ± 0.31	0.22 ± 0.06

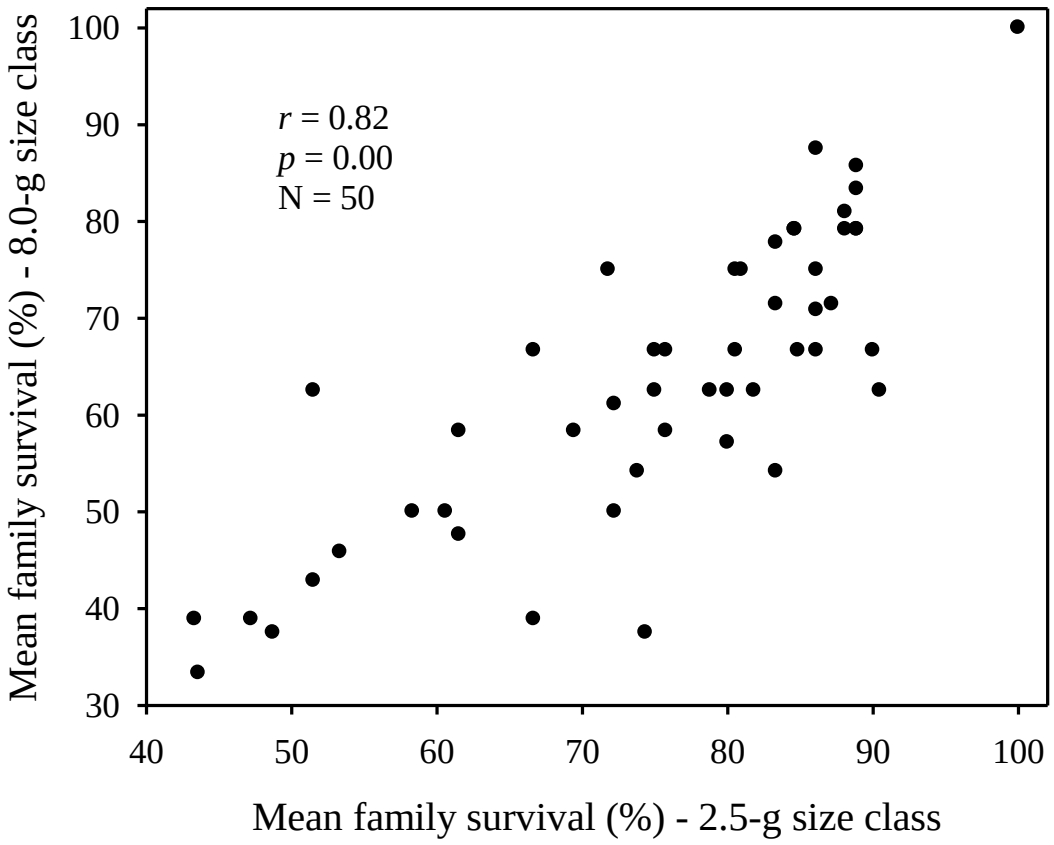

Figure 4-1. Relation of mean family survivals between two size classes of *P. vannamei* challenged with TSV.

CHAPTER 5: RELATIONSHIP BETWEEN VIRAL LOAD AND SURVIVAL TO TAURA SYNDROME VIRUS

Introduction

Taura syndrome virus (TSV) is an economically important pathogen of farmed penaeid shrimp. TSV was first identified in Ecuador in 1992 (Lightner et al. 1995; Hasson et al. 1995) and has since spread to all major shrimp farming regions of the Americas and Asia (Hasson et al. 1999a; Tu et al. 1999; Yu and Song 2000; Tang and Lightner 2005). Multiple penaeid species can be infected with TSV, with *Penaeus* (*Litopenaeus*) *vannamei*, the most economically important cultured shrimp species worldwide (FAO 2011), being the most severely affected by TSV (Brock et al. 1997, Hasson et al. 1995, 1999b, Lightner 1996a, Lightner 1999, Overstreet, 1997; Flegal 2006). TSV-associated mortalities ranging from 40-95% have been reported in naïve, unselected *P. vannamei* populations (Brock 1997; Brock et al. 1997; Lightner 1999).

TSV can infect all sizes/ages of *P. vannamei*; however, infections are typically seen in juveniles (<5 g) about 14-40 days after stocking into nursery tanks or growout ponds (Lightner 1995; Lightner 1999). TSV infection has three distinct phases: acute, transition, and chronic (Brock et al. 1997; Hasson et al. 1999b). The acute phase is rapid in individual shrimp (typically <24 hr), but can last for several days in an infected population (Brock et al. 1997; Hasson et al. 1999b). During this phase, shrimp exhibit lethargy and loss of appetite). Moribund shrimp display expansion of red chromatophores throughout the body surface, especially in the uropods (Lightner 1995). In the transition phase, shrimp often display melanized cuticular lesions that developed during the acute phase (Lightner 1995; Hasson et al. 1999b). Shrimp that successfully resolve these lesions and survive the next molting cycle typically appear normal (chronic phase). TSV-associated mortality most often occurs during the acute phase, likely due to osmotic failure resulting from widespread destruction of the cuticular epithelium (Lightner and Redman 2010). Mortality may also occur in transition phase and is likely associated with osmotic failure and/or infection by opportunistic bacteria (Lightner

1996b; Brock 1997). Shrimp in the chronic phase typically remain infected for life (Lightner 1995). These shrimp appear normal, but may be less tolerant to stress (Lotz et al. 2005) and, importantly, may pass the virus to progeny by vertical transmission (Hasson et al. 1999a, 1999b; OIE 2009a).

TSV is a non-enveloped, icosahedral virus containing a single-stranded, positive-sense RNA genome of 10,205 nucleotides (Bonami et al. 1997; Mari et al. 2002). The genome is comprised of two large open reading frames (ORFs). ORF1 contains sequence motifs for non-structural proteins (i.e. helicase, protease, and RNA-dependent RNA polymerase), whereas ORF2 contains sequences for structural proteins, including three capsid proteins (Bonami et al. 1997; Mari et al. 1998; Robles-Sikisaka et al. 2001; Mari et al. 2002). TSV belongs to the genus *Cripavirus* within the family *Dicistroviridae* (superfamily of picornavirus; Mayo 2002a; Mayo 2002b).

In response to TSV epizootics in the Americas, a variety of strategies to mitigate crop loss from this pathogen were implemented in the Western Hemisphere, including the establishment of selective breeding programs to develop TSV-resistant *P. vannamei* stocks (Moss et al. 2005). [Note: the terms "resistant" and "resistance" have been adopted by many stakeholders in the shrimp farming industry to refer to a shrimp's ability to survive viral exposure.] Selective breeding efforts began in the mid-1990s and have generally been successful, as significant improvements in TSV resistance (referred to as "TSV survival" in this chapter) have been reported (Fjalestad et al. 1997; Bienfang and Sweeney 1999; Gitterle 1999; Wyban 1999; Wyban 2000; Argue et al. 2002; White et al. 2002; Clifford and Preston 2006; Moss et al. 2011).

Despite improvements in TSV survival through selective breeding, little is known about the underlying immune mechanism(s) that allow some shrimp to survive infection or allow some families to have higher TSV survival. Research to date suggests that mechanisms that suppress viral load are important to a shrimp's ability to survive TSV infection (see Flegel and Sritunyalucksana 2011 for a review of shrimp responses to viral pathogens). Srisuvan et al. (2006) found that TSV copy number in selectively bred, TSV-resistant *P. vannamei* were significantly lower than for shrimp from a TSV

susceptible population when exposed to three different TSV isolates. Susceptible shrimp also had a higher incidence of necrosis of cuticular epithelial cells and lymphoid organ spheroids (signs of acute and chronic phases of TSV infection, respectively). Similarly, Cao et al. (2010) found that TSV copy number of moribund shrimp from 65 selected families of *P. vannamei* were ~10^3 times higher than for TSV challenge survivors from the same families. The authors also found a statistically significant relationship between TSV copy number and individual shrimp survival, but did not investigate whether the relationship was consistent among families. It is possible that individual shrimp and shrimp families differ in their ability to suppress viral load and/or tolerate certain TSV loads.

For selective breeding programs, selecting individuals/families based on their ability to suppress viral load is more desirable than selecting for tolerance of high viral loads, as the breeding population may progress towards complete TSV resistance (i.e. no infection). In addition, chronic stage survivors with high viral load may be an important disease vector in shrimp ponds (Lotz et al. 2003) and can be less tolerant to stress (Lotz et al. 2005). The objectives of this study were to investigate the relationship between viral load and TSV survival and to determine if the relationship is consistent among shrimp families.

Materials and Methods

Shrimp and TSV-challenge procedures

For details on the genetic background and production of shrimp families, as well as TSV-challenge procedures, see Chapter 4. Briefly, shrimp for this study came from Oceanic Institute's (Waimanalo, HI, USA) selective breeding program and were specific pathogen free (SPF) prior to TSV exposure (see USMSFP 2010 for SPF pathogen list). Shrimp were tagged with a fluorescent elastomer (Godin et al. 1996), with each family (unique dam-sire combination) receiving a unique tag code. Representative shrimp (mean weight = 2.5 g) from 50 families were stocked into four replicate 4000-L tanks (~12 shrimp/family/tank) with one tank serving as a negative control. In addition, 12

SPF Kona shrimp were added to each challenge tank and served as a positive control population (White et al. 2002; Hennig et al. 2004). The challenge commenced (day-0) when shrimp were fed shrimp tissue infected with TSV (isolate USTX95; see Tang and Lightner 2005 and Côté et al. 2008 for details on TSV phylogenetics) at a rate of 3% of tank biomass (Argue et al. 1999). For the remainder of the challenge, shrimp were fed to satiation with a commercial, pelleted diet. To minimize cannibalism, tanks were checked every 3-6 hr to remove dead/moribund shrimp. The challenge was terminated and shrimp survival assessed on day-21. Water quality parameters for challenge tanks were ~15 ppt, 26-27 C, ≥6 mg/L dissolved oxygen (DO).

Hemolymph collection was attempted for all moribund shrimp and for survivors from families with ≥3 moribund shrimp samples. Note: collection of hemolymph was not successful for all moribund and surviving shrimp. Moribund shrimp were identified as being listless and laying on their side. Dead shrimp were not sampled. Hemolymph has been shown to be suitable for quantifying TSV loads using real-time quantitative, reverse transcription-PCR (qRT-PCR; Poulos et al. 2008). A total of 53 moribund and 69 surviving shrimp were sampled. Twelve families were represented in the samples, with each family having 4-10 survivor samples and 3-6 moribund samples.

Quantification of TSV copy number

Quantification of TSV viral load (i.e. copy number; VL) in shrimp samples was conducted using qRT-PCR protocols described by Cao et al. (2010). Briefly, qRT-PCR was performed using TSV primers 101F/101R and a molecular beacon (MP) probe that were designed using the TSV 822 cDNA sequence reported by Lu et al. (2004; Genbank Accession# AF277675). Primers 101F (5'-ACT ACA ACG AGC CCA GAT TC-3') and 101R (5'-GCC ATA GGG TTC AGG GAT G-3') generate a 101 bp DNA fragment after amplification. The MB probe was synthesized and labeled with fluorescent dyes, 6-carboxyfluoroscein (FAM; fluorophore) on the 5' end and N-[4-(4-dimethylamino) phenylazo] benzoic acid (DABCYL; quencher) on the 3' end. The sequence of the MB probe was 5'-FAM-<u>CCA GCG</u> TTT GAT ACT AAC CGT GCT ATG <u>CGC TGG</u>-

DABCYL-3', with the underlined nucleotides forming the stem sequence and the loop region being complementary to a 21 nucleotides sequence in the fragment amplified by 101F/101R. All hemolymph samples were tested in duplicate. VL was quantified by comparing the threshold cycle value of samples against a standard curve generated using a dilution series of in vitro transcribed RNA (known copy number).

Data analysis

VL was $\log_{10}$ transformed prior to all analyses. Mean VL of moribund and surviving shrimp were compared across families using a paired *T*-test. Logistic regression was used to test the effects of VL, shrimp family, and VL × family interaction on the probability of morality (Y=1). The logistic regression model is written as:

$$P(Y=1) = 1 / [1 + e^{-(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3)}],$$

where P denotes probability; β_0 is the intercept, $\beta_{1\text{-}3}$ are regression coefficients for VL (x_1), family (x_2), and VL x family (x_3), respectively. Problems with model convergence due to small sample sizes (i.e. samples per family) and quasi-separation of data dictated the use of Firth's (1993) penalized maximum likelihood estimation (Heinze 1999). Statistical significance of regression coefficients was tested using likelihood ratio tests. For this model, β_2 tested the equality of intercepts (H_0: family-specific intercepts are equal) and β_3 tested parallelism of regression slopes (H_0: family-specific regression slopes are equal). TSV copy number at $P(Y = 1) = 0.50$ represents the VL at which an individual shrimp has a 50% of mortality and can be expanded to represent the VL at which 50% of all shrimp are expected to die (referred to here as VL_{50}). VL_{50} was calculated for each family using family-specific regression coefficients (see below). Correlations between family TSV survival and VL_{50}, mean VL of survivors, and mean VL of moribund shrimp were obtained using Pearson product-moment correlations. Analyses were performed using SAS software (Cary, NC, USA) and a significance level of $\alpha = 0.05$ was used for all tests.

Results and Discussion

Shrimp survival in the negative control tank was 100%. TSV survival of Kona shrimp was 25% and was similar to TSV survivals previously reported for this population (White et al. 2002; Hennig et al. 2004; Cao et al. 2010). Kona shrimp come from an SPF, unselected population of *P. vannamei* which is highly susceptible to TSV and exhibits consistently low survival in TSV challenges. Due to these characteristics, Kona shrimp are often used as a control or reference population in TSV challenges of selected lines/families to validate challenge results (White et al. 2002; Hennig et al. 2004). In total, these results provide strong evidence that challenge mortality can be attributed to TSV.

TSV survival for the 12 families sampled for VL quantification ranged from 43 to 89%. All shrimp sampled were infected with TSV, as determined by qRT-PCR. Most challenge survivors (52 of 69) had VL $<10^4$ TSV copies μl^{-1} hemolymph and six of these had VL <600 copies μl^{-1} (Fig. 5-1). Two survivors had uncharacteristically high VL ($>10^6$ TSV copies μl^{-1}). It is unclear if these shrimp were tolerating high VL (i.e. not dying) during the chronic phase of infection, were destined to survive but were still in the transition phase, or were still in the acute phase of infection. Shrimp mortality in TSV challenges of *P. vannamei* typically ceases between 10-14 d post-exposure (White et al. 2002; Srisuvan et al. 2006; Cao et al. 2010), as was the case in the present study. Thus, it is unlikely that shrimp in acute or transition phases remained on day-21 when the trial was terminated.

VL of moribund shrimp was highly variable, ranging from $10^{3.51}$ to $10^{8.54}$ copies μl^{-1} (Fig. 5-1). A majority of moribund shrimp (30 of 53) had VL $>10^6$ copies μl^{-1}, but 13 moribund shrimp had VL between $10^{3.51}$ - 10^4 copies μl^{-1}. It is possible that moribund shrimp with relatively low viral loads died for reasons other than TSV infection or were weak/immune compromised prior to TSV exposure causing them to be highly sensitive to TSV infection (i.e. die at relative low VL). These scenarios seem unlikely, especially the former, given the survival of negative and positive controls (see above) and the large number of moribund shrimp (13 of 53) with low VL. Inconsistencies associated with

sampling of moribund shrimp may have contributed to the observed variation in VL. Moribund shrimp were identified as being listless and laying on their side, but when an individual shrimp reached this state or how long it would have remained in a state of morbidity prior to death are unknown. Ideally, all moribund shrimp would be sampled at the same predetermined time point (e.g. the exact time of death or exact time when probability of mortality = 1), though this level of sampling precision is impossible.

Mean (mean of family means) VL of moribund and surviving shrimp were statistically different across families (t = 6.89, df = 11). Mean VL of moribund shrimp was $10^{6.04}$, with family VL ranging from $10^{3.78}$ to $10^{7.94}$ copies μl^{-1} (Table 5-1). Mean VL of survivors was lower with a mean of $10^{3.85}$ and family VL ranging from $10^{3.26}$ to $10^{4.91}$ copies μl^{-1} (Table 5-1). Cao et al. (2010) reported mean TSV VL of $10^{5.77}$ and $10^{8.70}$ copies μl^{-1} for moribund and surviving shrimp from 65 families of *P. vannamei*, respectively. VL for both groups was higher than for the present study; however, differences between mean VL for moribund shrimp and survivors were similar. Similarly, Srisuvan et al. (2006) found that shrimp from a TSV-resistant population of *P. vannamei* had 10^2-10^3 lower VL than Kona shrimp (TSV susceptible) when challenged with TSV. The observation of lower TSV VL for survivors compared to moribund shrimp is in general agreement with patterns reported for *P. vannamei* infected with White spot syndrome virus (WSSV; Huang et al. 2011) and Yellow head virus (Anantasomboon et al. 2008).

Correlations between family TSV survival and mean VL of survivors (r = -39; p = 0.21) and moribund shrimp (r = -27; p = 0.38) were not significant, although both regression coefficients were negative (Figs. 5-2, 5-3). Given the magnitude of regression coefficients and the small sample size of this study (12 families), information from a larger number of families (and perhaps more individuals per family) is needed to better define the relationship (or lack thereof) between family TSV survival and VL of moribund and/or surviving shrimp. Cao et al. (2010) found no difference in VL of moribund shrimp among family groups with low, medium, and high TSV survival, but did report a statistically significant, inverse relationship between VL of survivors and

mean TSV survival of family groups. A strong correlation between VL of survivors and family TSV survival would be beneficial to shrimp breeders, as family selection for TSV survival (a common practice of SPF breeding programs) would not only increase the portion of individuals surviving TSV infection, but would also result in survivors having lower viral loads. In others words, selection for increased TSV survival would also progress toward complete TSV resistance (i.e. no infection).

VL (χ^2 = 48.27, df = 1, p = 0.00) and shrimp family (χ^2 = 23.35, df = 11, p = 0.02) both had a significant effect on probability of mortality. The effect of VL × family was not significant (χ^2 = 18.89, df = 11, p = 0.06), which suggests that the relationship between VL and probability of mortality was similar across families. Cao et al (2010) also found a statistically significant relationship between TSV VL and individual shrimp survival, but did not investigate family or VL × family effects. Huang et al. (2011) reported differences in WSSV VL among four families of *P. vannamei*, but did not test the statistical significance of these differences.

Logistic regression curves were estimated for each family. All curves had a common slope, but each had a unique intercept (Fig. 5-4). TSV copy number at P(Y = 1) = 0.50 represents the VL at which 50% of all shrimp are expected to die (VL_{50}) and was calculated for each family from logistic regression curves (Table 5-1). If viral tolerance is simply defined as a shrimp's ability to withstand viral infection without dying and it is assumed that more tolerant individuals can withstand more severe infections without dying (i.e. higher VL), then VL_{50} is a logical estimator of viral tolerance at the family level. VL_{50} estimates ranged from $10^{3.81}$ to $10^{6.86}$ and show that families vary greatly in their ability to tolerate TSV infection. Surprisingly, family VL_{50} was not significantly correlated with mean family survival (Fig. 5-5; r = -0.15; p = 0.64). This, along with the observed strong effect of VL on probability of mortality, show that family TSV survival is primarily determined by the proportion of shrimp within a family that can maintain low VL, not by how tolerant a family is of TSV (as measured by VL_{50}). However, individual shrimp with high tolerance to TSV (as evidenced by high VL in a few survivors) may exist within a population.

Despite efforts to improve TSV survival through selective breeding, little is known about the underlying immune mechanism(s) that allow some shrimp to survive infection or allow some families to have higher TSV survival. Results from this study are in general agreement with past studies (Srisuvan et al. 2006; Anantasomboon et al. 2008; Cao et al. 2010; Huang et al. 2011) and suggest that mechanism(s) that suppress VL are important to a shrimp's ability to survive TSV infection. Tolerance of high VL appears possible in *P. vannamei*; however, the physiological capabilities that allow shrimp to tolerate high VL appear to be less common (only a few survivors with high loads were observed) and may be of little importance in determining TSV survival at the family level.

An epidemiological model of TSV showed that exposure of naïve shrimp to acutely infected, chronically infected, and dead shrimp are important transmission pathways for TSV (Lotz et al. 2003). While not specifically addressed in the epidemiological model, it seems logical to assume that VL of infected shrimp may increase transmission coefficients of these pathways and, subsequently, result in more secondary infections. Thus, selective breeding programs should have a goal to develop shrimp lines which have both higher TSV survival and lower VL (not higher TSV tolerance). Incorporating VL into a selection index with TSV survival, as proposed by Moss et al (2005), appears to be a valid approach to achieving this goal. However, basic quantitative genetic parameters (e.g. heritability) should be estimated and inconsistencies in VL sampling addressed prior to implementing a selection program based (or partially based) on TSV VL.

Table 5-1. Mean TSV viral load (VL; $\log_{10}$ copies μl^{-1} hemolymph) of surviving and moribund shrimp and VL_{50} (viral load at which 50% of all shrimp are expected to die) for families of *P. vannamei* challenge with TSV. Numbers in parentheses are the number of shrimp sampled.

Family	Mean TSV VL for survivors	Mean TSV VL for moribunds	VL_{50}
1	3.73 (5)	4.49 (4)	4.06
2	3.36 (6)	4.11 (5)	3.81
3	4.22 (5)	7.02 (6)	5.61
4	4.01 (5)	6.39 (5)	5.06
5	4.45 (6)	5.53 (4)	6.09
6	3.70 (6)	5.48 (5)	4.77
7	3.26 (6)	3.78 (3)	3.99
8	3.49 (4)	5.04 (4)	4.07
9	4.08 (5)	7.74 (5)	6.06
10	4.09 (6)	7.67 (5)	6.17
11	3.29 (10)	6.22 (4)	5.26
12	4.91 (5)	7.94 (3)	6.86
Mean	3.85 (69)	6.04 (53)	5.15

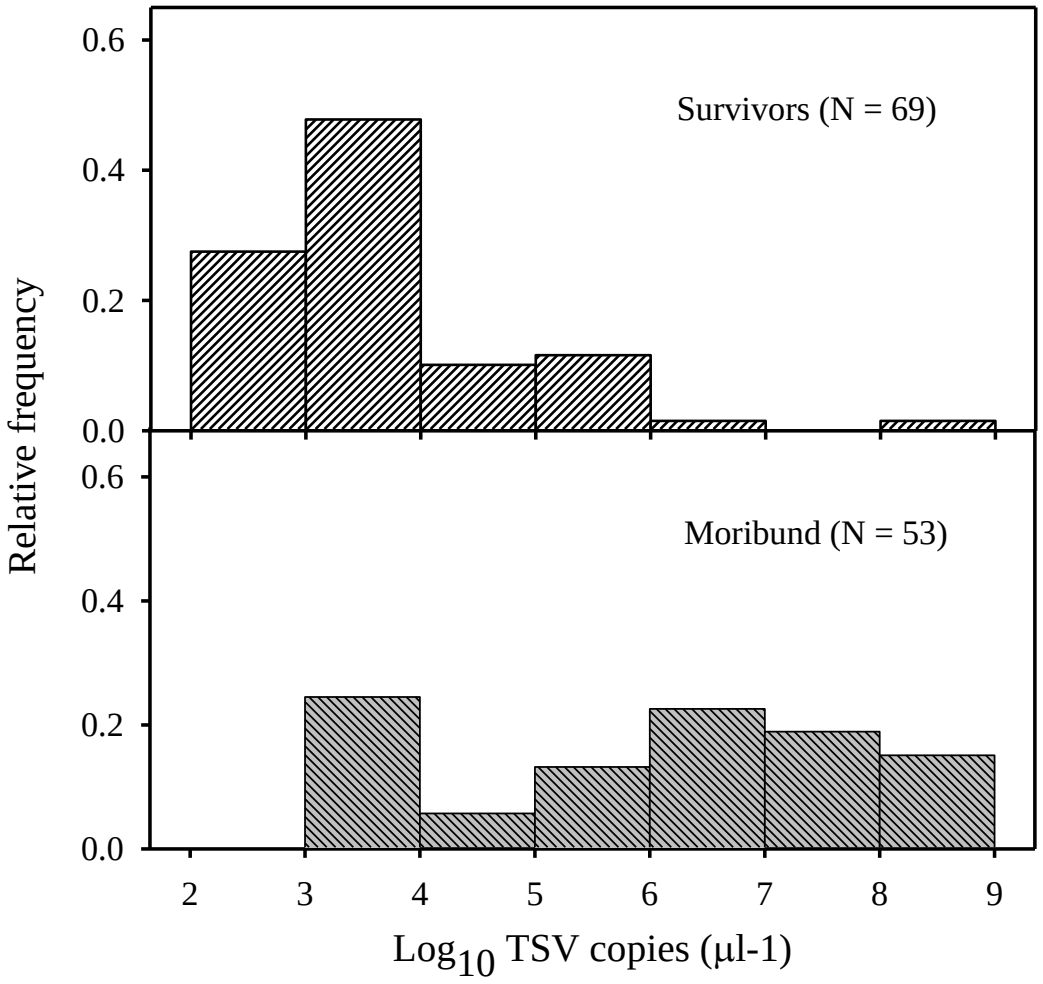

Figure 5-1. Relative frequency of TSV viral load (log_{10} TSV copies ul^{-1}) for survivors and moribund shrimp from a TSV challenge of *P. vannamei*.

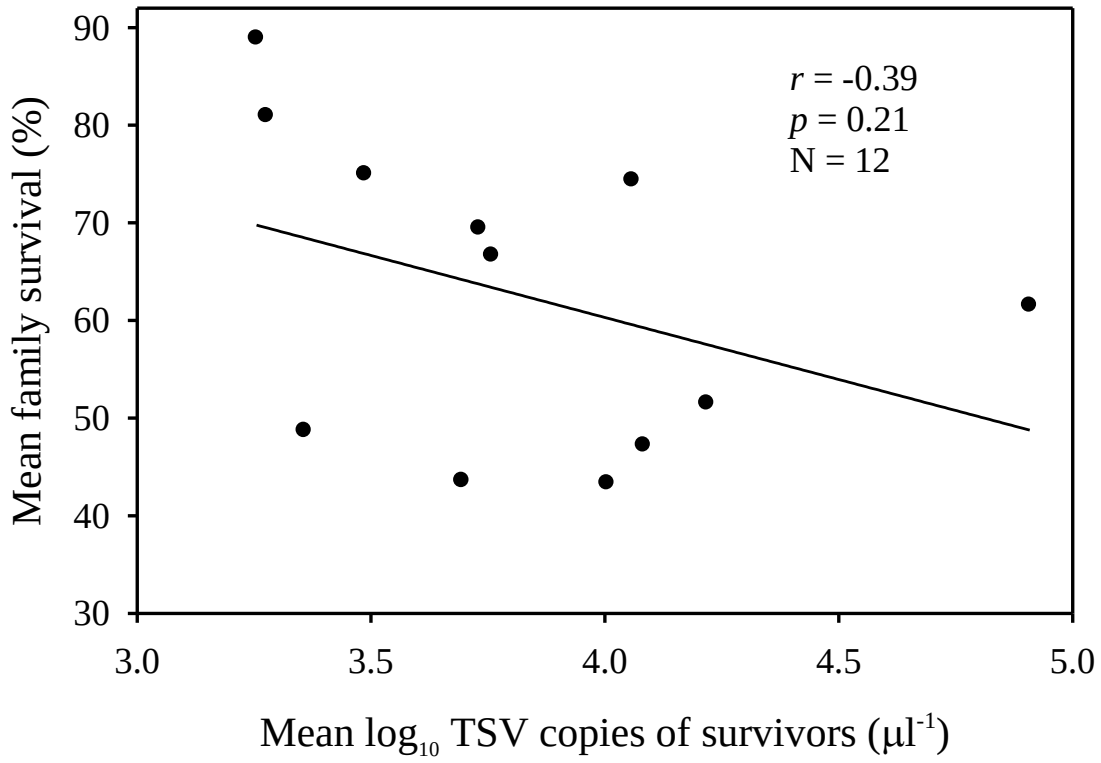

Figure 5-2. Relationship between mean viral load (log_{10} TSV copies ul^{-1}) of survivors and family survival (%) for 12 families of *P. vannamei* challenged with TSV. Best-fit line was used to show relation trend.

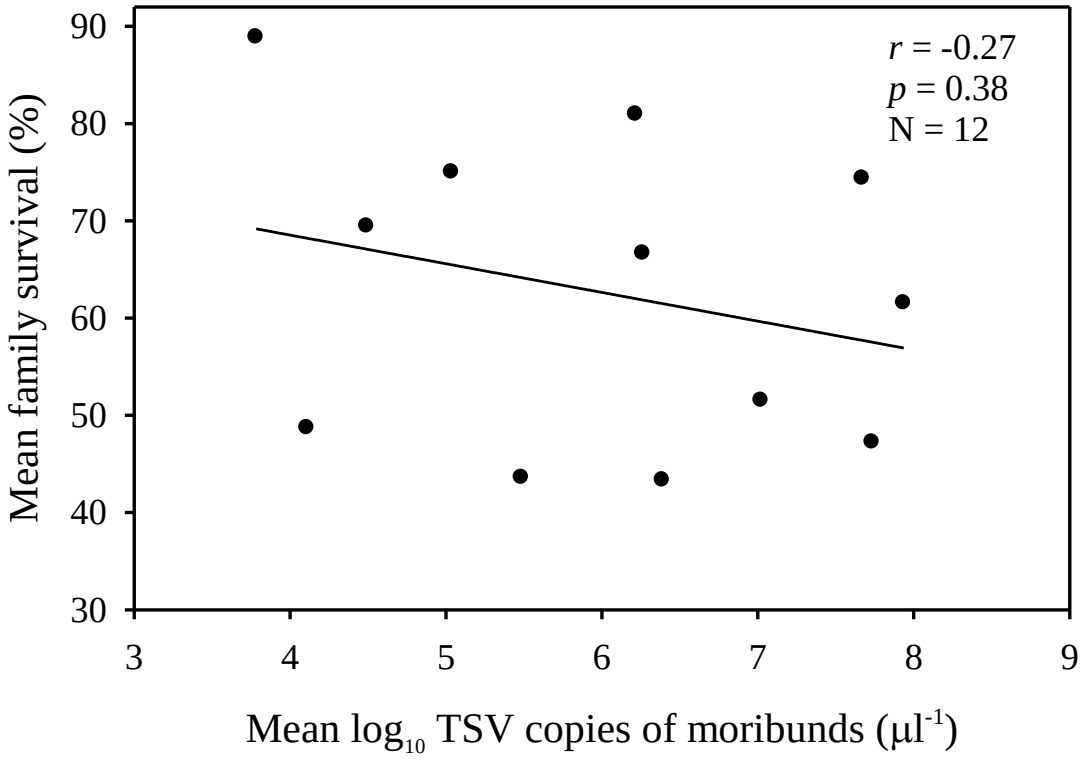

Figure 5-3. Relationship between mean viral load (log_{10} TSV copies ul^{-1}) of survivors and family survival (%) for 12 families of *P. vannamei* challenged with TSV. Best-fit line was used to show relation trend.

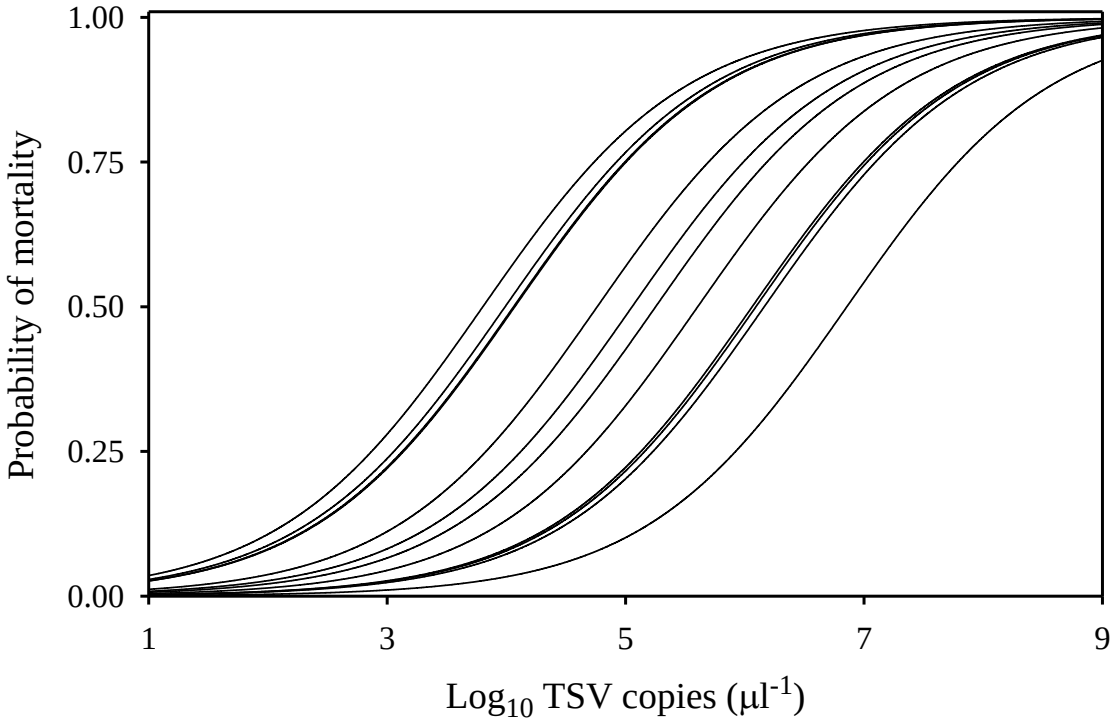

Figure 5-4. Logistic regression curves showing the effect of TSV viral load (log_{10} TSV copies ul^{-1}) on probability of death for 12 families of *P. vannamei* challenged with TSV.

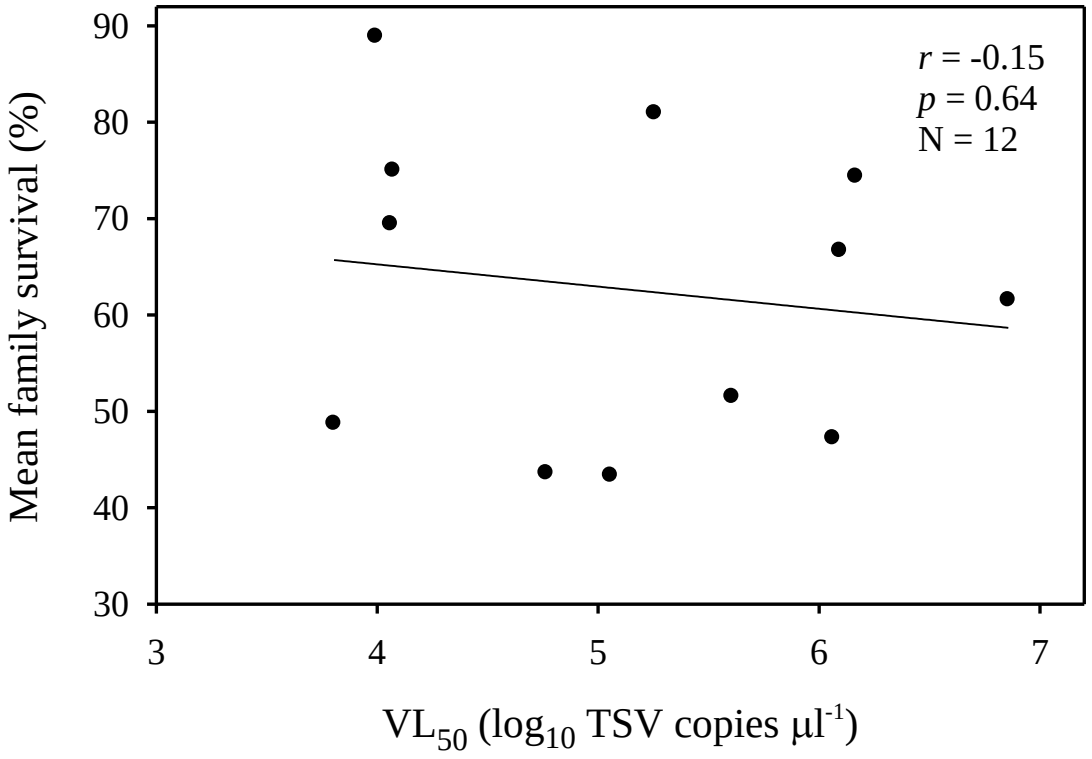

Figure 5-5. Relationship between VL_{50} (viral load at which 50% of all shrimp are expected to die) and family survival (%) for 12 families of *P. vannamei* challenged with TSV. Best-fit line was used to show relation trend.

CHAPTER 6: SUMMARY

Taura syndrome virus (TSV) is an economically important pathogen of Pacific white shrimp, *Penaeus* (*Litopenaeus*) *vannamei*. TSV is highly virulent and TSV-associated mortalities in unselected, naïve populations of *P. vannamei* range from 40-95%. TSV-associated crops losses are estimated to be >US$1 billion. Selective breeding of *P. vannamei* for TSV resistance began in the mid-1990s and several breeding programs have developed lines of shrimp which exhibit varying degrees of TSV resistance. Despite long-standing breeding efforts, several important aspects of TSV resistance have yet to be properly studied. In this dissertation, I conducted four studies to address key knowledge gaps in breeding *P. vannamei* for TSV resistance. Results from these studies should allow breeders to better define selection goals and optimize selection/breeding protocols.

The first study investigated the effects of inbreeding on shrimp performance in several environments, including laboratory challenges to three isolates of TSV, laboratory challenges to White spot syndrome virus (WSSV), and growout ponds and raceways at low and normal salinity. An analysis was conducted using 14 years of pedigree and nine years of shrimp performance data. Inbreeding depression (IBD) on survival to viral pathogens ranged from moderate (8.3% reduction per 10% inbreeding) to severe (38.7% reduction). Inbreeding had a small effect on growth (<4% reduction) but no effect on growout survival. The effects of inbreeding on survival appeared to be sensitive to environmental quality, as IBD was more severe in more stressful environments. These results suggest that moderate to high levels of inbreeding (>10%) should be avoided in shrimp breeding programs, especially when shrimp are reared under stressful conditions.

The second study focused on the estimation of genetic correlations for shrimp survival after exposure to a genetically, diverse suite of TSV isolates and the estimation of genetic correlations between TSV survival and growout performance traits. A total of 180 full-sib families were challenged with TSV and 100 of these families were also evaluated for growout performance. Genetic correlations for survival among TSV isolates were positive and of moderate to high magnitude (0.35 – 0.99). Genetic correlations for TSV survival and growth were all negative, but of low magnitude (-0.07

– -0.29). Correlations between TSV survival and growout survival varied from slightly negative to moderately positive (-0.12 – 0.30). These results indicate Breeding for resistance to any of the four TSV isolates used in this study should, in general, improve resistance to the other isolates. However, correlated responses to selection may be lower than the primary selection response for some trait combinations, due to the moderate magnitude of genetic correlations. As an RNA virus, TSV is prone to mutation and mutations appear to have some effect on shrimp survival, as evidenced by imperfect genetic correlations among several isolates. Thus, a strategy to improve general TSV resistance by selecting for resistance to a genetically diverse suite of isolates may be prudent. Alternatively, selection for a highly virulent isolate and/or an isolate for which there is a lot of phenotypic variability for survival (i.e. an isolate for which intense selection and correlated responses to selection can be maximized) may be effective in improving general TSV resistance. Importantly, correlations among TSV survival traits and growout performance traits suggest that there are no major impediments to simultaneous genetic improvement of these traits.

The objective of the third study was to estimate correlations and heritability for TSV survival in two size classes of shrimp. Shrimp from 50 full-sib families were challenged with TSV at ~2.5 g and then naïve shrimp from the same families were challenged at ~8.0-g. Heritabilities for TSV survival were similar between size classes (~0.2) and genetic and phenotypic correlations were high (>0.8). These results, along with the fact that *P. vannamei* are commonly harvested at ≤20 g, suggest the common practice of challenging small juveniles (1-3 g) to TSV and using these data to select for TSV resistance is likely effective in improving farm survival during TSV epizootics.

The objectives of the final study were to investigate the effects of viral load on TSV survival and to determine if these effects are consistent among shrimp families. Moribund shrimp and survivors from 12 shrimp families challenged with TSV were sampled. TSV viral load (VL) in samples was determined using qRT-PCR. VL was highly variable for both moribund shrimp and survivors. However, mean VL of moribund ($10^{6.04}$ copies μl^{-1} of hemolymph) and surviving shrimp ($10^{3.85}$ copies μl^{-1}) were statistically different across families. VL and shrimp family both had a significant effect on probability of shrimp mortality. The effect of VL × family was not significant,

which suggests that the relationship between VL and probability of mortality was similar across families. This finding suggests that immune mechanism(s) that suppress viral load are important to a shrimp's ability to survive TSV infection. The use of VL as a selection metric should allow breeders to develop shrimp lines that have both higher TSV survival and lower VL (not higher TSV tolerance).

LITERATURE CITED

Anantasomboon, G., Poonkhum, R., Sittidilokratna, N., Flegal, T.W. Withyachumnarnkul, B., 2008. Low viral loads and lymphoid organ spheroids are associated with yellow head virus (YHV) tolerance in whiteleg shrimp *Penaeus vannamei*. Development. Comparat. Immunol. 32, 613-626.

Arce, S.M., Moss S.M, Argue, B.J., 2000. Artificial insemination and spawning of Pacific white shrimp, *Litopenaeus vannamei*: implications for a selective breeding program. In: Tamaru, C.C-T., Tamaru, C.S., McVey, J.P., Ikuta K. (Eds.), Spawning and maturation of aquaculture species: Proceedings of the Twenty-Eighth US-Japan Natural Resources Aquaculture Panel, UJNR Technical Report No. 28, pp. 5-9.

Argue, B.J., Arce S. M., Lotz J.M., Moss S.M., 1999. Heritability for resistance to Taura syndrome virus in specific pathogen free *Litopenaeus vannamei*. World Aquaculture ’99 Book of Abstracts, Sydney, Australia, World Aquaculture Society, 28.

Argue, B.A., Alcivar-Warren, A., 2000. Genetics and breeding applied to the Penaeid shrimp farming industry. In: Bullis, R.A., Pruder, G.D. (Eds.), Controlled and Biosecure Production Systems: Evolution and Integration of Chicken and Shrimp Models. Proceedings of a Special Session on Integration of Shrimp and Chicken Models, World Aquaculture Society, Sydney, Australia. Oceanic Institute, Waimanalo, HI, USA, pp. 29-54.

Argue, B.A., Arce, S.M., Lotz, J.M., Moss, S.M., 2002. Selective breeding of Pacific white shrimp (*Litopenaeus vannamei*) for growth and resistance to Taura syndrome virus. Aquaculture 204, 447-460.

Arkush, K.D., Giese, A.R., Mendonca, H.L., McBride, A.M., Marty, G.D., Hedrick, P.W., 2002. Resistance to three pathogens in the endangered winter-run chinoock (*Oncorhynchus tshawtscha*): effects on inbreeding and major histocompatibility complex genotypes. Can. J. Fish. Aquat.Sci. 59(6), 966-975.

Bailey-Brock, J.H., Moss, S.M., 1992. Penaeid taxonomy, biology, and zoogeography. In: Fast, A.W., Lester L.J. (Eds.), Marine Shrimp Culture: Principles and Practices. Elsevier Science Publishers, New York, NY, USA, 9-27.

Bell, T.A., Lightner, D.V., 1987. IHHN disease of *Penaeus stylirostris*: effects of shrimp size on disease expression. J. Fish Dis. 10, 165-170.

Bentsen, H.B., Olesen, I., 2002. Designing aquaculture mass selection programs to avoid high inbreeding rates. Aquaculture 204, 349-359.

Bienfang, P.K., Sweeney, J.N., 1999. Animal health assurance drives CEATECH's breeding program. Glob. Aquac. Advocate 2(6), 72-73.

Bierne, N., Launey, S., Naciri-Graven, Y., Bonhomme, F., 1998. Early effect of inbreeding as revealed by microsatellite analysis on *Ostrea edulis* larvae. Genetics 148, 1893-1906.

Bierne, N., Beuzart, I., Vonau, V., Bonhomme, F., Bedier, E., AQUACOP, 2000. Microsatellite-associated heterosis in hatchery-propagated stocks of the shrimp *Penaeus stylirostris*. Aquaculture 184, 203-219.

Bijlsma, R., Bundgard, J., Van Putten, W.F., 1999. Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. J. Evol. Biol. 12, 1125-1137.

Bonami, J.R., Hasson, K.W., Mari, J., Poulos, B.T., Lightner, D.V., 1997. Taura syndrome of marine penaeid shrimp: characterization of the viral agent. J. Gen. Virol. 78, 313-319.

Bondari, K., Dunham, R.A., 1987. Effects of inbreeding on economic traits of channel catfish. Theor. Appl. Genet. 74, 1-9.

Bourdon, R.M., 1997. Understanding Animal Breeding, 2nd edn. Prentice Hall, New Jersey, USA. p. 341.

Boyd, C.E., 2006. Global shrimp OP survey results and recommendations. In: Boyd, C.E., Jory, D.E., Chamberlain, G.W. (Eds.), Operating Procedures for Shrimp Farming. Global Aquaculture Alliance, St. Louis, MO, USA, pp. 2-6.

Boyle, M.L. 2001. Chicken breeding and genetics. In: Bullis, R.A., Pruder, G.D. (Eds.), Controlled and Biosecure Production Systems: Evolution and Integration of Chicken and Shrimp Models, Proceedings of a Special Session on Integration of Shrimp and Chicken Models. World Aquaculture Society, Sydney, Australia, pp. 23-28.

Brock, J.A., 1997. Special topic review: Taura syndrome, a disease important to shrimp farms in the Americas. World J. of Microbiol. and Biotechnol. 13, 415-418.

Brock, J.A., Gose, R.B., Lightner, D.V., Hasson, K., 1997. Recent developments and an overview of Taura Syndrome of farmed shrimp in the Americas. In: Flegel, T.W., MacRae, I.H. (Eds.), Diseases in Asian Aquaculture III. Fish Health Section, Asian Fisheries Society, Manila, Philippines, pp. 275-284.

Cao, Z., Wang, S.Y., Breeland, V., Moore, A.-M., Lotz, J.M., 2010. Taura syndrome virus loads in *Litopenaeus vannamei* hemolymph following infection are related to differential mortality. Dis. Aquat. Org. 91, 97-103.

Carr, W.H., Fjalestad, K.T., Godin, D., Swingle, J., Sweeney, J.N., Gjedrem, T., 1997. Genetic variation in weight and survival in a population of specific pathogen-free shrimp, *Penaeus vannamei*. In: Flegel, T.W., MacRae, I.H. (Eds.), Diseases of Asian Aquaculture III. Fish Health Section, American Fisheries Society, Manila, Philippines, pp. 379-393.

Castillo-Juárez, H., Montaldo, H.H., Campos-Montes, G., 2006. Model shrimp breeding: program uses BLUP methodology. Glob. Aquac. Advocate 9(4), 52-53.

Castillo-Juárez, H., Casares, J.C.Q., Campos-Montes, G., Villela, C.C., Ortega, A.M., Montaldo, H.H., 2007. Heritability for body weight at harvest size in the Pacific white shrimp, *Penaeus* (*Litopenaeus*) *vannamei*, from a multi-environment experiment using univariate and multivariate animal models. Aquaculture 273, 42-49.

CENIACUA, 1999. Columbia's closed-cycle program for penaeid shrimp genetic selection and improvement. Glob. Aquac. Advocate 2(6), 71-83.

CENIACUA and AKVAFORSK, 2002. Selective breeding of *Litopenaeus vannamei* in Colombia. Panorama Acuicola 7(2), 30-31.

Chou, H.Y., Huang, C.Y., Wang, C.H., Chiang, H.C., Lo, C.F., 1995. Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. Dis. Aquat. Org. 23, 165-173.

Clifford, H.C., 1998. Super Shrimp: a domesticated line of *P. stylirostris* and viable alternative to *P. vannamei* culture in TSV-positive regions. World Aquaculture '98 Book of Abstracts, World Aquaculture Society, Las Vegas, NV, USA.

Clifford, H.C., Preston, N.P., 2006. Genetic Improvement. In: Boyd, C.E., Jory, D.E., Chamberlain, G.W. (Eds.), Operating Procedures for Shrimp Farming. Global Aquaculture Alliance, St. Louis, MO, USA, pp. 8-18.

Clifford, H.C., Scura, E.D., Jaramillo, G., 2003. Rapid response to a newly emerging viral pathogen using a field performance driven shrimp breeding program. World Aquaculture 2003 Book of Abstracts, Salvador, Brazil, World Aquaculture Society.

Cock, J., Gitterle, T., Salazar, M., Rye, M., 2009. Breeding for disease resistance in Penaeid shrimps. Aquaculture 286, 1-11.

Coman, G.J., Crocos, P.J., Preston, N.P., Fielder, D., 2004. The effects of density on the growth and survival of different families of juvenile *Penaeus japonicus* Bate. Aquaculture 229, 215-223.

Coman, G.J., Preston, N.J., 2008. Black tiger – domestication, selective breeding advance in Australia. Glob. Aquac. Advocate 11(1), 57-58.

Côté, Navarro, S., Tang, K.F.J., Noble, B., Lightner, D.V., 2008. Taura syndrome virus from Venezuela is a new genetic variant. Aquaculture 284, 62-67.

Crocos, P.J., Moss, S.M., 2006. Maturation. In: Boyd, C.E., Jory, D.E., Chamberlain, G.W. (Eds.), Operating Procedures for Shrimp Farming. Global Aquaculture Alliance, St. Louis, MO, USA, pp. 20-27.

Dahlgaard, J., Loeschcke, V., 1997. Effects of inbreeding to three life stages of *Drosophila buzzati* after embryos were exposed to a high temperature stress. Heredity 78, 410-416.

De Donato, M., Manrique, R., Ramirez, R., Mayer, L., Howell, C., 2005. Mass selection and inbreeding effects on a cultivated strain of *Penaeus* (*Litopenaeus*) *vannamei* in Venezuela. Aquaculture 247, 159-167.

Dhar, A.K., Lakshman, D.K., Amundsen, K., Robles-Sikisaka, R., Kaizar, K.N., Roy, S., Hasson, K.W. Allnutt, F.C.T., 2010. Characterization of a Taura syndrome virus isolate originating from the 2004 Texas epizootic in cultured shrimp. Arch. Virol. 155, 315-327.

Doyle, R.W., Moss, D.R., Moss, S.M., 2006. Shrimp copyright: inbreeding strategies effective against illegal copying of genetically improved shrimp. Glob. Aquac. Advocate 9(2), 76-79.

Erickson, H.S., Poulos, B.T., Tang, K.F.J., Bradley-Dunlop, D., Lightner, D.V., 2005. Taura syndrome virus from Belize represents a unique variant. Dis. Aquat. Org. 64, 91-98.

Escobedo-Bonilla, C.M., Alday-Sanz, V., Wille, M., Sorgeloos, P., Pensaert, M.B., Nauwynck, H.J., 2008. A review on the morphology, molecular characterization, morphogenesis and pathogenesis of White spot syndrome virus. J. Fish Dis. 31(1), 1-18.

Evans, F., Matson, S., Brake, J., Langdon, C., 2004. The effects of inbreeding on performance traits of adult Pacific oysters (*Crassostrea gigas*). Aquaculture 230, 89-98.

Falconer, D.S., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, 4th edn. Logman Group Essex, England, p. 58.

Firth, D., 1993. Bias reduction of maximum likelihood estimates. Biometrika 80, 27-38.

Fjalestad, K.T., Gjedrem, T., Carr, W.H., Sweeney, J.N., 1997. Final report: the shrimp breeding program. Selective breeding of *Penaeus vannamei*. Oceanic Institute, Waimanalo, HI, USA.

Flegel, T.W., Alday-Sanz, V., 1998. The crisis in Asian shrimp aquaculture: current status and future needs. J. Appl. Ichthyol. 14, 269-273.

Flegel, T.W., 2001. The shrimp response to viral pathogens. In: Browdy, C.L., Jory, D.E. (Eds.), The New Wave: Proceedings of the Special Session on Sustainable Shrimp Farming. World Aquaculture Society, Baton Rouge, LA, USA, pp. 254-278.

Flegel, T.W., 2006. Detection of major penaeid shrimp viruses in Asia, a historical perspective with emphasis on Thailand. Aquaculture 258, pp. 1-33.

Flegel, T.W., Sritunyalucksana, K., 2011. Shrimp molecular responses to viral pathogens. Mar. Biotechnol. 13, 587-607.

Food and Agriculture Organization, 2011. FAO yearbook, Fishery and Aquaculture Statistics, 2009. Rome, Italy, p.78.

Frankham, R., 1995. Conservation genetics. A. Rev. Ecol. Syst. 29, 305-327.

Frierman, E.M., Andrews, J.D., 1976. Occurrence of hematopoietic neoplasms in Virginia oysters (*Crassostrea virginica*). J. Nat. Cancer Inst. 56(2), 319-324.

Giese, A.R., Hedrick, P.W., 2003. Genetic variation and resistance to a bacterial infection in the endangered Gila topminnow. Anim. Conserv. 6(4), 369-377.

Gitterle, T., 1999. Evaluación de la resistencia de diferentes poblaciones del camarón marino *Litopenaeus vannamei* (Boone 1931) al Virus sel Sindrome del Taura (TSV) bajo condiciones controladas. Thesis work, Jorge Tadeo Lozano University, Bogota, Colombia.

Gitterle, T., Salte, R., Gjerde, B., Cock, J., Johansen, H., Salazar, M., Lozano, C., Rye, M., 2005a. Genetic (co)variation in resistance to White spot syndrome virus (WSSV) and harvest weight in *Penaeus* (*Litopenaeus*) *vannamei*. Aquaculture 246, 139-149.

Gitterle, T., Rye, M., Salte, R., Cock, J., Johansen, H., Lozano, C., Suárez, J.A., Gjerde, B., 2005b. Genetic (co)variation in harvest body weight and survival in *Penaeus* (*Litopenaeus) vannamei* under standard commercial conditions. Aquaculture 243, 83-92.

Gitterle, T., Gjerde, B., Cock, J., Salazar, M., Rye, M., Vidal, O., Lozano, C., Erazo, C., Salte, R., 2006a. Optimization of experimental infection protocols for the estimation of genetic parameters of resistance to White Spot Syndrome Virus (WSSV) in *Penaeus* (*Litopenaeus*) *vannamei*. Aquaculture 261, 501-509.

Gitterle, T., Ødegård, J., Gjerde, B., Rye, M., Salte, R., 2006b. Genetic parameters and accuracy of selection for resistance to White Spot Syndrome Virus (WSSV) in *Penaeus* (*Litopenaeus*) *vannamei* using different statistical models. Aquaculture 251, 210-218.

Gjerde, B., Gunnes, K., Gjedrem, T., 1983. Effects of inbreeding on survival and growth in rainbow trout. Aquaculture 34, 327-332.

Gjedrem, T., Thodesen, J., 2005. Selection. In: Gjedrem, T. (Ed.), Selection and Breeding Programs in Aquaculture. Springer, Dordrecht, The Netherlands, pp. 89-110.

Gjerde, B., 2005. Prediction of breeding values. In: Gjedrem, T. (Ed.), Selection and Breeding Programs in Aquaculture. Springer, Dordrecht, The Netherlands, pp. 197-227.

Godin, D.M., Carr, W.H., Hagino, G., Segura, F., Sweeney, J.N., Blankenship, L., 1996. Evaluation of a fluorescent elastomer internal tag in juvenile and adult shrimp *Penaeus vannamei*. Aquaculture 139, 243-248.

Goyard, E., Peignon, J-M., Vanaa, V., Dufour, R., Bedier, E., 1999. INFREMER's shrimp genetics program. Glob. Aquac. Advocate 2(6), 26-28.

Goyard, E., Patrois, J., Peignon, J.M., Vanaa, V., Dufour, R., Viallon, J., Bedier, E., 2002. Selection for better growth of *Penaeus stylirostris* in Tahiti and New Caledonia. Aquaculture 181, 215-223.

Hanson, L.A., Rudis, M.R., Petrie-Hanson, L., 2004. Susceptibility of channel catfish fry to Channel catfish virus (CCV) challenge increases with age. Dis. Aquat. Org. 62, 27-34.

Hasson, K.W., Lightner, D.V., Poulos, B.T., Redman, R.M., White, B.L., Brock, J.A., Bonami, J.R., 1995. Taura syndrome in *Penaeus vannamei*: demonstration of viral etiology. Dis. Aquat. Org. 23, 115-126.

Hasson, K.W., Lightner, D.V., Mohney, L.L., Redman, R.M., Poulos, B.T., Mari, J., Bonami, J.R., 1999a. The geographic distribution of Taura Syndrome Virus (TSV) in the Americas: determination by histology and in situ hybridization using TSV-specific cDNA probes. Aquaculture 171, 13-26.

Hasson, K.W., Lightner, D.V., Mohney, L.L., Redman, R.M., Poulos, B.T., White, B.L., 1999b. The Taura syndrome virus lesion development and disease cycle in the Pacific white shrimp, *Litopenaeus vannamei*. Dis. Aquat. Org. 36, 81-93.

Heinze, G., 2004. A comparative investigation of methods for logistic regression with separated or nearly separated data. Statist. Med. 25, 4216-4226.

Hennig, O.L., Keller, K., Rasmussen, L., Arce, S.M., Moss, S.M., White-Noble, B., Lightner, D.V., Breland, V., Lotz, J., 2004. Strain of reference shrimp aids researchers, farmers. Glob. Aquac. Advocate 7(6), 74.

Holland, J., Spindler, K., Horodyski, F., Grabau, E., Nichol, S., VandePol, S., 1982. Rapid evolution of RNA genomes. Science 215(4540), 1577-1585.

Hollebacq, M.G., Haffray, P., 1994. Genetic improvement of common carp *Cyprinus carpio* L.: state of the art. Bull. Fr. Peche Piscic. 333, 93-124.

Huang, Y.-C., Yin, Z.-X., Ai, H.-S., Huang, X.-D., Li, S.-D., 2011. Characterization of WSSV resistance in selected families of *Litopenaeus vannamei*. Aquaculture 311, 54-60.

Ibarra, A.M., Cruz, P., Romero, B.A., 1995. Effects of inbreeding on growth and survival of self-fertilized catarina scallop larvae, *Argopecten circularis*. Aquaculture 134, 37-47.

Inouye, K., Miwa, S., Oseko, N., Nakano, H., Kimura, T., Momoyama, K., Hiroaka, M., 1994. Mass mortality of cultured Kuruma shrimp *Penaeus japonicus* in Japan in 1993: electron microscopic evidence of causative virus. Fish Pathol. 29, 149-158.

Jerry, D.R., Preston, N.P., Crocos, P.J., Keys, S., Meadows, J.R.S., Li, Y., 2004. Parentage determination of Kuruma shrimp *Penaeus* (*Marsupenaeus*) *japonicus* (Bate) using microsatellite markers. Aquaculture 235, 237-247.

Jiang, D., Rocha, J.L., Ciobanu, D., Mileham, A., van der Steen, H., White-Noble, B.L., Lightner, D.V., 2004. Quantitative, molecular genetic selection for shrimp disease resistance. Glob. Aquac. Advocate 7(1), 52-55.

Jiménez, J.A., Hughes, K.A., Alaks, G., Graham, L., Lacy, R.C., 1994. An experimental study of inbreeding depression in a natural habitat. Science 266, 271-273.

Johnston, M.O., 1992. Effects of cross and self-fertilization on progeny fitness in *Lobelia cardinalis* and *L. siphilitica*. Evolution 46, 688-702.

Jory, D.E., Dixon, H.M., 1999. Shrimp white spot virus in the western hemisphere. Aquac. Magazine 25, 83-91.

Kalagayan, H., Godin, D., Kanna, R., Hagino, G, Sweeney, J., Wyban, J., Brock, J., 1991. IHHN virus as an etiological factor in Runt-deformity syndrome (RDS) of juvenile *Penaeus vannamei* cultured in Hawaii. J. World Aquac. Soc. 22(4), 235-243.

Kasai, K., Yonezawa, J., Ono, A., Hasegawa, A., Honma, T., 1993. Brood and size dependent variation in susceptibility of rainbow trout, *Orcorhynchus mykiss*, to artificial infection of infectious hematopoietic necrosis virus (IHNV). Fish Pathol. 28(1), 35-40.

Keller, L.F., Grant, P.R., Grant, B.R., Petren, K., 2002. Environmental conditions affect the magnitude of inbreeding depression in survival of Darwin's finches. Evolution 56, 1229-1239.

Keys, S.J., Crocos, P.J., Burridge, C.Y., Coman, G.J., Gerard, D.P., Preston, P.P., 2004. Comparaative growth and survival of inbred and outbred *Penaeus* (*marsupenaeus*) *japonicus*, reared under controlled environment conditions: indications of inbreeding depression. Aquaculture 241, 151-168.

Kong, J., Zhang, Q., Liu, P., Yue, Z., Wang, Q., 2003. Selective breeding raises WSSV resistance in fleshy prawns. Glob. Aquac. Advocate 6(2), 25-26.

Kutner, M.K., Nachtsheim, C.J., Neter, J., Li, W., 2005. Applied Linear Statistical Models, 5th edn. McGraw-Hill/Irwin, New York, New York, p. 85.

Launey, S., Hedgecock, D., 2001. High genetic load in the Pacific oyster *Crassostrea gigas*. Genetics 159, 255-265.

Leblanc, B.D., Overstreet, R.M., 1990. Prevalence of Baculovirus penaei in experimentally infected white shrimp (*Penaeus vannamei*) relative to age. Aquaculture. 3-4, 237-242.

Lightner, D.V., 1995. Taura syndrome: An economically important viral disease impacting shrimp farming industries of the Americas including the United States. Proceedings of the 99th annual meeting of the United States Animal Health Association, Reno, NV, USA, pp. 1-12.

Lightner, D.V., 1996a. Epizootiology, distribution and the impact on international trade of two penaeid shrimp viruses in the Americas. Revue Scientifique et Technique, Office International des Epizooties 15, 579-601.

Lightner, D.V. (ed.), 1996b. A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp. World Aquaculture Society, Baton Rouge, LA, USA. p. 304.

Lightner, D.V., 1999. The penaeid shrimp viruses TSV, IHHNV, WSSV, and YHV: current status in the Americas, available diagnostic methods and management strategies. J. Applied Aquac. 9(2), 27-52.

Lightner, D.V., 2003. Exclusion of specific pathogens for disease prevention in a penaeid shrimp biosecurity program. In: Lee, C. S., O'Bryen, P.J., Biosecurity in Aquaculture Production Systems: Exclusion of Pathogens and Other Undesirables. World Aquaculture Society, Baton Rouge, LA, USA, pp. 81-116.

Lightner, D.V., Redman, R.M., Poulos, B.T., Nunan, L.M., Mohney, L.H., Mari, J.L., Hasson, K.W., Pantoja, C.R., Nelson, K.T., Zhou, J.L., Wang, Q., Garza, J., White, B.L., 1998. Viral diseases of shrimp in the Americas: diagnosis, distribution, and control strategies. In: Jory, D.E. (Ed.), Proceedings of the First Latin American Shrimp Farming Congress, Panama City, Panama, pp. 1-10.

Lightner, D.V., Redman, R.M., Arce, S.M., Moss, S.M., 2009. Specific pathogen-free shrimp stocks in shrimp farming facilities as a novel method for disease control in crustaceans. In: Shumway, S.E., Rodrick, G.E. (Eds.), Shellfish safety and quality. Woodhead Publishing, Cambridge, England, pp. 384-416.

Lightner, D.V., Redman R.M., 2010. The global status of significant infectious diseases of farmed shrimp. Asian Fisheries Sci. 23, 383-426.

Lotz, J.M., 1997a. Disease control and pathogen status assurance in an SPF-based shrimp aquaculture industry, with particular reference to the United States. In: Flegel, T.W., MacRae, I.H. (Eds.), Diseases of Asian Aquaculture III. Fish Health Section, American Fisheries Society, Manila, Philippines, 243-254.

Lotz, J.M., 1997b. Effect of host size on virulence of Taura syndrome virus to the marine shrimp *Penaeus vannamei* (Crustacea: Penaeidae). Dis. Aquat. Org. 30, 45-51.

Lotz, J.M., Flowers, A.M., Breland, V., 2003. A model of Taura syndrome virus (TSV) epidemics in *Litopenaeus vannamei*. J. Invert. Path. 83, 168-176.

Lotz, J.M., Anton, L.S., Soto, M.A., 2005. Effect of chronic Taura syndrome virus infection on salinity tolerance of *Litopenaeus vannamei*. Dis. Aquat. Org. 65, 75-78.

Lu, Y., Wang, S.Y., Lotz, J.M., 2004. The use of differential display to isolate viral genomic sequence for rapid development of PCR-based detection methods, a test for Taura syndrome virus. J. Virol. Methods 121, 107-114.

Lynch, M., Walsh, B., 1998. Genetics and Analysis of Quantitative Traits. Sinauer Associates, Massachusetts, USA, pp. 251-274

Mari, J., Bonami, J.R., Lightner, D.V., 1998. Taura syndrome of penaeid shrimp: cloning of viral genomic fragments and development of gene probes. Dis. Aquat. Org., 33, 11-17.

Mari, J., Poulos, B.T., Lightner, D.V., Bonami, J.R., 2002. Shrimp taura syndrome virus: genomic characterization and similarity with members of the genus Cricket paralysis-like viruses. J. of Genetic Virol., 83, 915-926.

Mayo, M.A., 2002a. Virus taxonamy – Houston 2002. Arch. Virol., 147(8), 1655-1656.

Mayo, M.A., 2002b. A summary of taxonomic changes recently approved by ICTV. Arch. Virol., 147(5), 1071-1076.

Mayo, M.A., 2005. Changes to virus taxonomy 2004. Arch. Virol., 150, 189-198.

Miller, P.S. 1994. Is inbreeding depression more severe in a stressful environment? Zoo Biol. 13, 195-208.

Moss, D.R., Arce, S.M., Otoshi, C.A., Doyle, R.W., Moss, S.M., 2007. Effects of inbreeding on survival and growth of Pacific white shrimp *Litopenaeus vannamei*. Aquaculture, 27(S1), S30-S37.

Moss, D.R., Arce, S.M., Otoshi, C.A., Moss, S.M., 2011. Shrimp breeding for resistance to Taura syndrome virus. Glob. Aquac. Advocate, 14(4), 40-41.

Moss, S.M., 2004. White shrimp in Asia – Fad or the Future? Fishfarmer Magazine Jul/Aug, 20-21.

Moss, S.M., Arce, S.M., Argue, B.J., Otoshi, C.A., Calderon, F.R.O., Tacon, A.G.J., 2001. Greening of the blue revolution: efforts toward environmentally responsible shrimp culture. In: Browdy, C.L., Jory, D.E. (Eds.), The New Wave: Proceedings of the Special Session on Sustainable Shrimp Farming. World Aquaculture Society, Baton Rouge, LA, USA, pp. 1-19.

Moss, S.M., Arce, S.M., Moss, D.R., 2003. SPF defined: pathogen-free status of shrimp limited. Glob. Aquac. Advocate, 6(6), 86-87.

Moss, S.M., Doyle, R.W., Lightner, D.V., 2005. Breeding shrimp for disease resistance: challenges and opportunities for improvement. In: Walker, P., Lester, R., Bondad-Reantaso, M.G. (Eds.), Diseases of Asian Aquaculture V. Fish Health Section, American Fisheries Society, Manila, Philippines, pp. 379-393.

Moss, S.M., Moss, D.R., 2009. Selective breeding of penaeid shrimp. In: Shumway, S.E., Rodrick, G.E. (Eds.), Shellfish safety and quality. Woodhead Publishing, Cambridge, England, 425-445.

Newkirk, G.F., 1978. A discussion of possible sources of inbreeding in hatchery stock and associated problems. Proc. World Mariculture. Soc. 9, 93-100.

Norman, J.K., Sakai, A.K., Weller, S.G., Dawson, T.E., 1995. Inbreeding depression in morphological and physiological traits of *Schiedea lydgatei* (Caryophyllaceae) in two environments. Evolution 49, 297-306.

OIE, 2009a. OIE International Aquatic Animal Health Code, 12th edition. Office International des Epizooties. Paris, France, p. 265.

OIE, 2009b. Manual of diagnostic tests for aquatic animal diseases, 6th edition. Office International des Epizooties. Paris, France, p. 383.

OIE, 2012. OIE International Aquatic Animal Health Code, 15th edition. Office International des Epizooties. Paris, France, p. 1.3-1.

Otoshi, C., Tang, L., Dagdagan, D., Holl, C., Tallamy, C., Moss, D., Arce, S., Moss, S., 2007. Super-intensive growout of the Pacific white shrimp, *Penaeus* (*Litopenaeus*) *vannamei*: recent advances at the Oceanic Institute. In: Rakestraw, T., Douglas, L., Flick, G. (Eds.), Proceedings from the 6th International Conference on Recirculating Aquaculture. Virginia Polytechnic and State University, Blacksburg, VA, USA, pp. 1-5.

Otoshi, C.A., Tang, L.R., Moss, D.R., Arce, S.M., Holl, C.M., and Moss, S.M., 2009. Performance of Pacific white shrimp, *Penaeus (Litopenaeus) vannamei*, cultured in biosecure, super-intensive, recirculating aquaculture systems. In: Browdy, C.L., Jory, D.E. (Eds.), The Rising Tide, Proceedings of the Special Session on Sustainable Shrimp Farming. World Aquaculture Society, Baton Rouge, LA, USA. pp 165-175.

Overstreet, R.M., Lightner, D.V., Hasson, K.W., McIIwain, S., Lotz, J., 1997. Suseptability to TSV of some penaeid shrimp native to the Gulf of Mexico and southeast Atlantic Ocean. J. of Invert. Pathol. 69, 165-176.

Pante, M.J.R., Gjerde, B., McMillan, I., 2001. Effects of inbreeding on body weight at harvest in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 192, 201-211.

Pérez, F., Volckaert, F.A.M., Calderón, J., 2005. Pathogenicity of white spot syndrome virus on postlarvae and juveniles of *Penaeus* (*Litopenaeus*) *vannamei*. Aquaculture 250, 586-591.

Phalitakul, S., Wongtawatchai, J., Sarikaputi, M., Viseshakul, N., 2006. The molecular detection of Taura syndrome virus emerging with White spot syndrome virus in penaeid shrimps of Thailand. Aquaculture 260, 77-85.

Poulos, B.T., Noble B.W., Lightner, D.V., 2008. Comparison of Taura syndrome virus (TSV) detection methods during chronic-phase infection in *Penaeus vannamei*. Dis. Aquat. Org. 82, 179-185.

Prior, S., Browdy, C.L., Shepard, E.F., Laramore, R., Parnell, P.G., 2003. Controlled bioassay systems for determination of lethal infective dosage of tissue homogenates containing Taura syndrome or White spot syndrome virus. Dis. Aquat. Org. 54(2), 89-96.

Robles-Sikisaka, R., Garcia, D.K., Klimpel, K.R., Dhar, A.K., 2001. Nucleotide sequence of 3'-end of the genome of Taura syndrome virus of shrimp suggests that it is related to insect picornaviruses. Arch of Virol., 146, 941-952.

Rocha, J., Jiang, D., Kinghorn, B., van der Steen, H., 2007. Biosecure zero-exchange shrimp technology (BioZEST): A paradigm shift for the U.S. Industry, ATP Final Performance Report, Advanced Technology Program, US Department of Commerce, p.62-104.

Rosenberry, B., 1999. World Shrimp Farming 1999. Shrimp News International, San Diego, CA, USA.

Rosenberry, B., 2000. World Shrimp Farming 2000. Shrimp News International, San Diego, CA, USA.

Rosenberry, B., 2006. World Shrimp Farming 2006. Shrimp News International, San Diego, CA, USA.

Rye, M., Mao, I.L., 1998. Nonadditive genetic effects and inbreeding depression for body weight in Atlantic salmon (*Salmo salar* L.). Livest. Prod. Sci., 57, 15-22.

Sbordani, V., De Matthaeis, E., C.M. Sbordoni, La Rosa, G., Mattoccia, M., 1986. Bottleneck effects and the depression of genetic variability in hatchery stocks of *Penaeus japonicus* (Crustacea, Decapoda). Aquaculture 57, 239-251.

Sbordani, V., La Rosa, G., Sbordoni, C.M., De Matthaeis, E., 1987. Genetic changes in seven generations of hatchery stocks of the kuruma shrimp, *Penaeus japonicus* (Crustacea, Decapoda). Sel., Hybrid., and Genet. Eng. in Aquac. 1, 143-155.

Schemske, D.W., 1983. Breeding system and habitat effects in three neotropical *Costus* (Zingiberaceae). Evolution 37, 523-539.

Schmitt, J., Ehrhardt, D.W., 1990. Enhancement of inbreeding depression by dominance and suppression in *Impatiens copensis*. Evolution 44, 269-278.

Shapira, Y., Magen, Y., Zak, T., Kotler, M., Hulata, G., Levavi-Sivan, B., 2005. Differential resistance to koi herpes virus (KHV)/carp interstitial nephritis and gill necrosis virus (CNGV) among common carp (*Cyprinus carpio* L.) and crossbreds. Aquaculture 245, 1-11.

Srisuvan, T., Noble, B.L., Schofield, P.J., Lightner, D.V., 2006. Comparison of four Taura syndrome virus (TSV) isolates in oral challenge studies with *Litopenaeus vannamei* unselected and selected for resistance to TSV. Dis. Aquat. Org. 71, 1-10.

Sritunyalucksana, K., Utairungsee, T., Sirikharin, R., 2013. Reprint of: Virus-binding proteins and their roles in shrimp innate immunity. Fish & Shellfish Immunol. 34, 1018-1024.

Su, G-S., Liljedahl, L-E, Gall, G., 1996. Effects of inbreeding on growth and reproductive traits in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 142, 139-148.

Tang, K.F-J., Durand, S.V., White, B.L., Redman, R.M., Pantoja, C.R., Lightner, D.V., 2000. Postlarvae and juveniles of a selected line of *Penaeus stylirostris* are resistant to infectious hypodermal and hematopoietic necrosis virus infection. Aquaculture 190, 203-210.

Tang, F.J., Lightner, D.V, 2005. Phylogenetic analysis of Taura syndrome virus isolates collected between 1993 and 2004 and virulence comparison between two isolates representing different genetic variants. Virus Res. 112, 69-76.

Tassanakajon, A., 2013. Innate immune system of shrimp. Fish & Shellfish Immunol. 34, 953.

Tave, D., 1993. Genetics for fish hatchery managers, 2nd ed. Kluwer Academics, Massachusetts, USA.

Tu, C., Huang, H.T., Chuang, S.H., Hsu, J.P., Kuo, S.T., Li, N.J., Hsu, T.L., Li, M.C., Lin, S.Y., 1999. Taura syndrome in Pacific white shrimp *Penaeus vannamei* cultured in Taiwan. Dis. Aquat. Org. 38, 159-161.

United States Marine Shrimp Farming Program, 2010. FY09 Progress Report, vol. I & II. Cooperative State Research, Education and Extension Service, US Department of Agriculture, p. II-13.

Veloso, A., Warr, G.W., Browdy, C.L., Chapman, R.W., 2011. The transcriptomic response to viral infection of two strains of shrimp (*Litopenaeus vannamei*). Developm. Comp. Immuno. 35, 241-246.

Waller, D.M., 1984. Differences in fitness between seedlings derived from cleistogamous and chasmogamous flowers in *Impatiens copensis*. Evolution 38, 427-440.

Wang, Q., Kong, J., Li, J., Wang, W., 2006. Selective breeding – genetic improvements in fleshy shrimp continue in China. Glob. Aquac. Advocate, 9(6), 74-75.

White, B. V., Schofield, P.L., Poulos, B.T., Lightner, D.V., 2002. A laboratory challenge method for estimating Taura syndrome virus resistance in selected lines of Pacific white shrimp *Litopenaeus vannamei*. J. of the World Aquac. Soc. 33(2), 341-348.

Wolfe, L.M., 1993. Inbreeding depression in *Hydrophyllum appendiculatum*: role of maternal effects, crowding, and parental mating history. Evolution 47, 374-386.

Wongteerasupaya, C., Vickers, J.E., Sriurairatana, S., Nash, G.L., Akarajamorn, A., Boonsaeng, V., Panyim, S., Tassanakajon, A., Withyachumnarnkul, B., Flegel, T.W., 1995. A non-occluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn, *Penaeus monodon*', Dis. Aquat. Org. 21, 69-77.

Wyban, J.A., Sweeney, J.N., 1991. The Oceanic Institute shrimp farming manual: intensive shrimp production technology. The Oceanic Institute, Waimanalo, Hawaii, USA.

Wyban, J.A., Swingle, J.S., Sweeney, J.N., Pruder, G.D., 1993. Specific pathogen free *Penaeus vannamei*. J. of the World Aquac. Soc. 24, 39-45.

Wyban, J.A., 1999. Selective breeding for TSV-resistant shrimp. Glob. Aquac. Advocate 2(6), 30.

Wyban, J.A., 2000. Breeding for fast growth and virus resistance. Glob. Aquac. Advocate 3(6), 32-33.

Yu, C.-I., Song, Y.-L., 2000. Outbreaks of Taura syndrome in Pacific white shrimp *Penaeus vannamei* cultured in Taiwan. Fish Pathol. 35, 21-24.