Aquaculture 416–417 (2013) 78–84

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Estimation of genetic parameters for survival to multiple isolates of Taura syndrome virus in a selected population of Pacific white shrimp *Penaeus* (*Litopenaeus*) *vannamei*

Dustin R. Moss [a,b,*], Shaun M. Moss [a], Jeffrey M. Lotz [c]

[a] Oceanic Institute, 41-202 Kalanianaole Hwy., Waimanalo, HI 96795, USA
[b] Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, HI 96822, USA
[c] Department of Coastal Sciences, University of Southern Mississippi, Gulf Coast Research Laboratory, PO Box 7000, Ocean Springs, MS 39566, USA

ARTICLE INFO

Article history:
Received 26 April 2012
Received in revised form 15 July 2013
Accepted 22 July 2013
Available online 27 July 2013

Keywords:
Shrimp
Genetic correlations
Heritability
Taura syndrome
Penaeus vannamei

ABSTRACT

Taura syndrome virus (TSV) is an economically important pathogen of the Pacific white shrimp, *Penaeus* (*Litopenaeus*) *vannamei*. To date, >40 unique TSV isolates have been identified and phylogenetic analyses of these isolates have revealed four distinct genetic groups named according to their geographic origin: Americas, Belize, South East Asia, and Venezuela. Although there is evidence that virulence varies among different TSV isolates, little is known about how shrimp survival is correlated among isolates (i.e. genetic correlations). In addition, estimates of genetic correlation between TSV survival and other commercially important traits are limited. 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). A total of 180 full-sib families were challenged with TSV: 130 families challenged with Americas and Belize group isolates and 50 families challenged with isolates from all four genetic groups. In addition, 100 of these families were tested for growout performance in a recirculating aquaculture system (RAS) at intensive stocking densities (>230 shrimp/m^2). All families were from a shrimp line selected for TSV resistance and growth over multiple generations. Genetic correlations for survival among TSV isolates were positive and of moderate to high magnitude (r_G = 0.35–0.99). Genetic correlations for TSV survival and RAS growth were all negative, but of low magnitude (r_G = −0.07 to −0.29). Correlations between TSV survival and RAS survival varied from slightly negative to moderately positive. These results indicate that breeding for survival to any one of the four TSV isolates evaluated in this study should, in general, improve survival to the other isolates. Results also suggest that there are no significant costs associated with selection for TSV resistance relative to growout performance.

1. 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 (Food and Agriculture Organization, 2011). TSV was first identified in Ecuador in 1992 (Hasson et al., 1995; Lightner, 1995) and has since spread to all major shrimp farming regions of the Americas and Asia (Hasson et al., 1999; Tang and Lightner, 2005; Tu et al., 1999; Yu and Song, 2000). TSV is highly virulent and TSV-associated mortalities in unselected, naïve populations of *P. vannamei* can range from 40 to 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 (Argue et al., 2002; Bienfang and Sweeney, 1999; Clifford and Preston, 2006; Wyban, 1999); 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 (Argue et al., 2002; Fjalestad et al., 1997; Gitterle, 1999; White et al., 2002), including the establishment of some selectively bred stocks which exhibit >80% survival to TSV in *per os* laboratory challenges (Moss et al., 2011; Srisuvan et al., 2006; Wyban, 2000).

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

* Corresponding author at: Oceanic Institute, 41-202 Kalanianaole Hwy., Waimanalo, HI 96795, USA. Tel.: +1 808 259 3185; fax: +1 808 259 9762.
E-mail address: dmoss@oceanicinstitute.org (D.R. Moss).

0044-8486/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquaculture.2013.07.037

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 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 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).

2. Materials and methods

2.1. 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 BZ01 challenge 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).

2.2. 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, 1000 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 difference 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

Table 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

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 (Hennig et al., 2004; White et al., 2002) 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 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., 2002). Shrimp were fed to satiation with a commercial, pelleted diet for the remainder of the trial. To prevent (or reduce) cannibalism, tanks were checked every 3–6 h 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.

2.3. 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 2 and 3. Components were estimated using a multivariate mixed linear animal model and is written as:

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

where 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); $a \sim (0, A\sigma^2_a)$ is the vector of additive genetic values; $e \sim (0, I\sigma^2_e)$ 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 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

Table 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)	1701	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)	1236	77.7 ± 14.5	43–100
11[a,b,c,d]	BZ01	50 (2)	1236	79.4 ± 14.5	35–100
11[a,b,c,d]	TH01	50 (2)	1236	88.8 ± 9.9	63–100
11[a,b,c,d]	VE05	50 (2)	1236	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
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 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	8809	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.

were conducted using GenStat version 15 (VSN International, Hemel Hempstead, UK).

3. 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 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 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 4).

Genetic correlations between survival to USTX95 and BZ01 (bivariate model) were similar across generations (Table 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 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 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). 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/m^2 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 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 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.

4. 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 strongly suggest 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 come 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 (Hennig et al., 2004; White et al., 2002). 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)

Table 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).

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

* $p \leq 0.05$.
** $p \leq 0.005$.

Table 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).

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**

* $p \leq 0.05$.
** $p \leq 0.005$.

Table 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).

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	–	–

* $p \leq 0.05$.

reported a survival of 0% for Kona shrimp exposed to BZ01 and Côté et al. (2008) reported survival ranging from 10 to 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 ($r_G = 0.35 \pm 0.23$) 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 2). This likely contributed to large correlation SEs (Table 4) 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; Srisuvan et al., 2006; Tang and Lightner, 2005); 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 to −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 (1997) conducted four experiments and found a general trend of lower TSV survival with increased size/age for *P. vannamei* ranging from 2 g 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. (2005a) 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 (Argue et al., 2002; Cock et al., 2009; Gitterle, 1999). This is attributed, in part, to high phenotypic/genotypic variation in shrimp survival to TSV (Moss and Moss, 2009), which allows for a large 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. (2005a) 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. (2005b) 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.

5. 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.

Acknowledgments

This study was funded by Grant No. 2002-38808-01345 from the U.S. Department of Agriculture/CSREES. We thank Jeff Lotz and Verlee Breland at Gulf Coast Research Laboratory for their assistance with viral challenges. We also thank the staff of Oceanic Institute's Shrimp Department for the technical support.

References

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.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.

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

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. Journal of General Virology 78, 313–319.

Cao, Z., Wang, S.Y., Breland, V., Moore, A.-M., Lotz, J.M., 2010. Taura syndrome virus loads in *Litopenaeus vannamei* hemolymph following infection are related to differential mortality. Diseases of Aquatic Organisms 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., 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.

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.

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

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.

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. Diseases of Aquatic Organisms 64, 91–98.

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

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.

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

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

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

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.

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. Diseases of Aquatic Organisms 23, 115–126.

Hasson, K.W., Lightner, D.V., Mohney, L.L., Redman, R.M., Poulos, B.T., Mari, J., Bonami, J.R., 1999. 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.

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. Global Aquaculture 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.

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.

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., 1999. The penaeid shrimp viruses TSV, IHHNV, WSSV, and YHV: current status in the Americas, available diagnostic methods and management strategies. Journal of Applied Aquaculture 9 (2), 27–52.

Lotz, J.M., 1997. Effect of host size on virulence of Taura syndrome virus (TSV) to the marine shrimp *Penaeus vannamei* (Crustacea: Penaeidae). Diseases of Aquatic Organisms 30, 45–51.

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. Journal of Genetic Virology 83, 915–926.

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, pp. 425–445.

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

OIE, 2012. OIE International Aquatic Animal Health Code, 15th edition. Office International des Epizooties, Paris, France, p. 1.3.1.

Otoshi, C.A., Tang, L.R., Dagdagan, D., Holl, C.M., Tallamy, C., Moss, D.R., Arce, S.M., Moss, S.M., 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., 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., McIlwain, S., Lotz, J.M., 1997. Susceptibility to Taura syndrome virus of some penaeid shrimp species native to the Gulf of Mexico and the southeastern United States. Journal of Invertebrate Pathology 69, 165–176.

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, pp. 62–104.

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. Diseases of Aquatic Organisms 71, 1–10.

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

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 Research 112, 69–76.

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

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. Diseases of Aquatic Organisms 38, 159–161.

United States Marine Shrimp Farming Program, 2010. FY09 Progress Report, I & II. Cooperative State Research, Education and Extension Service, US Department of Agriculture. p. II-11, III-8.

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*). Developmental and Comparative Immunology 35, 241–246.

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*. Journal of the World Aquaculture Society 33 (2), 341–348.

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

Wyban, J.A., 2000. Breeding for fast growth and virus resistance. Global Aquaculture Advocate 3 (6), 32–33.

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*. Journal of the World Aquaculture Society 24, 39–45.

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