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Effects of inbreeding on survival and growth of Pacific white shrimp *Penaeus* (*Litopenaeus*) *vannamei*

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Abstract

The objective of this study was to investigate the effects of inbreeding on the performance of Pacific white shrimp, *Penaeus* (*Litopenaeus*) *vannamei*, under various culture conditions through a retrospective analysis of family performance data. Fourteen years of pedigree and eight years of performance data from Oceanic Institute's selective breeding program were used in the analysis. During this period, shrimp performance was evaluated in growout trials (in pond and raceway systems), laboratory challenges to three isolates of Taura syndrome virus (TSV), and a laboratory challenge to White spot syndrome virus (WSSV). The effects of inbreeding on growth and survival were estimated by regressing family phenotypic means (adjusted for contemporary group effects) on inbreeding coefficients. During growout, inbreeding had a small but significant effect on growth (2.6 to 3.9% reduction per 10% inbreeding) but had no effect on survival. The effects of inbreeding on survival after exposure to viral pathogens ranged from moderate (8.3% reduction per 10% inbreeding) to severe (38.7% reduction), although not all effects were significant. Furthermore, the effects of inbreeding on survival appeared to be sensitive to environmental quality, as inbreeding depression was more severe in more stressful environments (smallest effect during growout trials and largest effect during exposure to WSSV). 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. In addition, the effects of inbreeding on survival appear to be significant enough to justify the use of inbreeding as a germplasm protection strategy (under certain scenarios) for genetic improvement programs.

Keywords: Shrimp; Growth; Survival; Genetics; Inbreeding; Penaeid; *Penaeus* (*Litopenaeus*) *vannamei*

1. 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

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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 a variety of aquaculture species, including channel catfish (Bondari and Dunham, 1987), scallops (Ibarra et al., 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* (Sbordani 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 Oceanic Institute's (OI) shrimp breeding program.

2. Materials and methods

2.1. 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 (2004), including those pathogens that are International Office of Epizootics (OIE) notifiable (OIE, 2002). 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, 40–160 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). The 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

Table 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

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).

2.2. 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., 1996), and each family received a unique tag code. At the termination of each trial, shrimp were identified (by family) from the 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 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 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, 2485, 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 3 for the summary statistics on shrimp performance.

Table 2
Summary of viral-challenge trial parameters

Virus	Exposure method	Initial wt (g)	Duration (days)	Tank volume (L)
TSV	*Per os*	2–6	14–21	2000–4000
WSSV	Injection	~1	7	0.26

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.

2.3. 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:

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

where Y_{ij} is the trait mean for the jth family in the ith contemporary group (CG); μ is the overall trait mean; CG_i is the fixed effect of the ith CG; F is the inbreeding coefficient of the jth 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)

Table 3
Summary statistics for performance evaluations

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 (%)	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

CG=contemporary group; RR=recirculating raceway; EP=earthen pond; TSV=Taura syndrome virus; WSSV=White spot syndrome virus.

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-day period) and evaluated in the same growout or viral-challenge trial. Contemporary groups generally included all families available at the time of the trial (20–80 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 a SPSS 14.0 software (SPSS Inc., Chicago, IL, USA) and the 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:

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

where b and a are the regression coefficient and y-intercept from the regression of Y_{ij} on F_j (see Eq. (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 the combined analyses for growout survival and TSV–AG were excluded).

3. Results

Regression coefficients for growth in the RR, EP, and combined analyses were all negative and significant (Table 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 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 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. 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 mean survival of non-inbred genotypes progressively decreased).

4. 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 the microsatellite tri-locus heterozygosity and growth rate in a population of *P. stylirostris* and reported a mean IBD of 5% when comparing the 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.,

Table 4
Results from regressions 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

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

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.

Table 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

Trait	Isolate	N	# shrimp	b±SE	P	a	IBD	Mean F±SD
TSV–AG (%)	HI94 (8)	178	4694	−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	2485	−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

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 "Isolate" 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.

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).

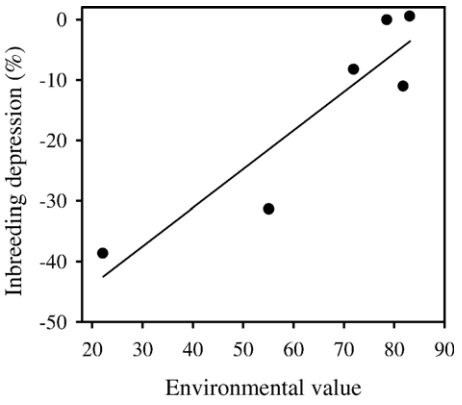

Fig. 1. The effect of environmental quality on inbreeding depression for survival. The environmental value (y-intercept of the regression of y_{ij} on F_j) is the predicted survival of non-inbred genotypes (F=0) in a performance trial environment and was used as a quantitative measure of environmental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

The 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 (Hollebacq and Haffray, 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 (β=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%–25.0% for growth (Fjalestad et al., 1997; Argue and Alcivar-Warren, 2000, Argue et al., 2002) and 12.4%–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., 2005). 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 the 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 (Schemske, 1983; Schmitt and Ehrhardt, 1990; Wolfe, 1993) and animals (Jiménez et al., 1994; Keller et al., 2002), including *Drosophila* sp. (Miller, 1994; Dahlgaard and Loeschcke, 1997; Bijlsma, 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 these 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 farming regions. Of course, the success 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 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 the environmental quality declined. Clearly, more research is needed to evaluate the effects of inbreeding on shrimp under diverse stock management strategies and growout conditions.

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