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# Heritability, Genetic Line and Inbreeding Effects on Resistance of Whiteleg Shrimp *Penaeus vannamei* Boone 1931 to Acute Hepatopancreatic Necrosis Disease (AHPND) in Mexico

**H. CASTILLO-JUÁREZ[1], H.H. MONTALDO[2,*], G.R. CAMPOS-MONTES[1], J.C. QUINTANA-CASARES[3], S.A. SOTO-RODRÍGUEZ[4], M. BETANCOURT-LOZANO[4], A. MARTÍNEZ-ORTEGA[3], R. LOZANO-OLVERA[4], B. GÓMEZ-GIL[4], A. CABALLERO-ZAMORA[1] and E.P. GALLAGA-MALDONADO[2]**

[1]Universidad Autónoma Metropolitana Unidad Xochimilco, CDMX, Mexico
[2]Universidad Nacional Autónoma de México CDMX, Mexico
[3]Maricultura del Pacıfico S.A. de C.V. Mazatlán, Sinaloa, Mexico
[4]Centro de Investigación en Alimentación y Desarrollo A.C. Unidad Mazatlán en Acuicultura y Manejo Ambiental Sinaloa, Mexico

## Abstract

The objective of this paper is to present preliminary results regarding heritability, genetic line differences and inbreeding effects for survival time in experimental challenges to acute hepatopancreatic necrosis disease (AHPND) in *Penaeus vannamei* Boone 1931. Here we present results of analyses conducted on data from a Resistance Line obtained from a merging of several Ecuadorian groups with a history of white-spot syndrome virus resistance, and a Growth Line with high genetic growth ability, obtained by selection in a Mexican hatchery. Family-identified animals from the two genetic lines and their crosses were inoculated by immersion in 2014, 2015 and 2016 using a *Vibrio parahaemolyticus* strain (M0904) AHPND+ obtained from a natural infection in Mexico. Heritabilities for survival time obtained using nested linear mixed models ranged from 9 to 18 %. Survival was greater for the Resistance Line compared to the Growth Line or the $F_1$ cross ($P < 0.02$). Our results point to the presence of additive genetic variation in both lines evaluated that may be exploited in breeding programmes to increase AHPND resistance.

[*]Corresponding author. E-mail address: montaldo@unam.mx

Additionally, our results support the idea that the Resistance Line is more resistant to AHPND than the Growth Line. Finally, comparisons between inbred and non-inbred animals suggest that the effect of inbreeding on AHPND resistance is small.

**Keywords:** acute hepatopancreatic necrosis disease, challenge tests, genetic resistance, heritability, *Penaeus*, shrimp

## Introduction

Acute hepatopancreatic necrosis disease (AHPND) is a bacterial disease in shrimp that has resulted in substantial economic losses in shrimp farms, causing high mortality rates, mainly in juvenile shrimp (Tran et al. 2013). Since its first outbreak in the People's Republic of China in 2009, the disease has been reported in Malaysia, Viet Nam, Thailand and Mexico (Hong et al. 2016). In Mexico, it has been recognized as a cause of large atypical mortality outbreaks in *Penaeus vannamei* Boone 1931 shrimp farms since 2013 (Nunan et al. 2014; Soto-Rodríguez et al. 2015).

The development and implementation of good sanitary management practices is crucial to the control of the disease (FAO 2013; Cock et al. 2015). Disease control in aquaculture animals can also be achieved by using genetic differences for disease resistance within each species (Cock et al. 2009; Ødegård et al. 2011; Yáñez et al. 2015). For superior results these strategies may be used in conjunction, in a manner similar to that long used in plants with the concept of integrated disease control (Moss et al. 2012; Russell 2013). An additional advantage of using genetic resistance as a control method for disease in aquaculture is a reduction in the use of biologicals, drugs and chemicals with the associated advantages related to environmental sustainability.

In shrimp production, a major factor that increases the practical importance of using genetic differences for disease control is the difficulty of implementing vaccination, because it is generally assumed that shrimp do not have the capacity to acquire immunological resistance (Cock et al. 2009), although this assumption has been questioned by Witteveldt (2006) and by Johnson et al. (2008). Shrimp, as with all crustaceans, do not produce antibodies, interferon or other acquired immune mechanisms common in vertebrates (Matsunaga and Rahman 1998; Cerenius and Söderhäll 2012). To consider the inclusion of disease resistance into the breeding objective, it is necessary to measure the genetic variation of the related traits (Yáñez et al. 2014). Therefore, for genetic improvement to AHPND resistance there is a need to estimate its heritability, since this is a key element in predicting the expected response to selection and evaluating the presence of genetic variability in different shrimp populations (Falconer and Mackay 1996). In addition to genetic selection within populations, crossbreeding is another common option used in animal breeding to take advantage of genetic differences. Information about the performance of genetic lines from different origins exposed to the pathogen is necessary to measure crossbreeding effects and the possibility of producing resistance lines derived from specific crossbreeding strategies (Gjedrem and Baranski 2010).

One practical option to detect genetic differences between selection candidates or populations in aquaculture for specific diseases is to use challenge tests based on survival time and survival rate to assess resistance (Ødegård et al. 2011; Gjedrem 2015). Here we present quantitative genetic analysis results of such testing in a selection nucleus of *P. vannamei* from a large shrimp hatchery in Mazatlán, Sinaloa in the northwest coast of Mexico (Maricultura del Pacífico). In this company, a shrimp line was selected for several generations for increasing growth rate at harvest size (130 days) and for general survival in the absence of any important disease outbreak (Castillo-Juárez et al. 2015).

A fundamental motivation for the development of these experimental challenges was the interest of the Mexican shrimp industry in testing possible sources of resistance to the white-spot syndrome virus (WSSV) and AHPND outbreaks that have caused serious losses in this industry. This was also related to mounting anecdotal but compelling evidence on the existence of a higher degree of genetic resistance to WSSV in commercially available breeding shrimp from Ecuador and other sources, using both field observations made by the Mexican shrimp industry and commercial challenge tests performed at the University of Arizona. This evidence coincided with a gradual recovery in productivity of the shrimp industry in Ecuador, after following a dramatic initial reduction caused by WSSV outbreaks. Since different selection procedures have been used in each country due to their different breeding and production conditions, it is important to evaluate and compare shrimp from Ecuador and Mexico in AHPND challenge tests. In 2013, the Asociación Nacional de Productores de Larva de Camarón (National Association of Shrimp Larvae Producers) brought shrimp of Ecuadorian origin assumed to have a higher resistance to WSSV to its quarantine unit facility. Some of these animals and their crosses were used in the Maricultura del Pacífico breeding programme to yield a new genetic line designed for resistance to AHPND and WSSV (Resistance Line). In 2014, Maricultura del Pacífico began activities in its disease challenge unit facility with the scientific guidance of the Universidad Autónoma Metropolitana – Xochimilco, the Universidad Nacional Autónoma de México and the Centro de Investigación en Alimentación y Desarrollo A.C. – Unidad Mazatlán (CIAD-Mazatlán), where AHPND and WSSV challenges were performed to compare the Resistance Line with the Mexican Growth Line and their crosses.

On the other hand, it has been suggested that relatively high inbreeding levels caused by the widespread mating of highly related (full-sib) animals from single commercial lines with a "genetic lock" may have had a major role in the recent outbreaks of AHPND and WSSV in many regions of the world (Doyle 2016). We find this hypothesis unlikely, because it relies on several interconnected processes, without actual direct evidence of its occurrence. Moreover, there is a lack of direct evidence from actual measurement data on genetic resistance in animals with different inbreeding levels for the specific diseases involved. Nonetheless, as an idea that has attracted interest, it is important to provide experimental evidence to support or discard it. The objective of this paper is to present preliminary results regarding heritability, genetic line differences, and inbreeding effects for survival time in experimental challenges to AHPND performed from 2014 to 2016 on the selection nucleus population of a large breeding company in Mexico.

Here we present results on differences between "purebred shrimp", from a merging of several Ecuadorian lines with a history of WSSV resistance (Resistance Line), and a Mexican line with high genetic growth ability, selected for many generations for growth and survival in the absence of any catastrophic disease outbreak (Growth Line).

## Material and Methods

### *Location and Population*

Experimental AHPND challenges were performed from 2014 to 2016 in a facility specially designed for this purpose by Maricultura del Pacífico. All the animals were inoculated by immersion method using a *Vibrio parahaemolyticus* strain (M0904) obtained from the pure bacterial strain collection from CIAD Mazatlan´s Bacteriology Laboratory. The M0904 strain was isolated from cultured shrimp affected with AHPND in northwestern Mexico (Soto-Rodríguez et al. 2015). Collection of dead and dying shrimp was made every hour. Some of these animals were used for histopathological studies to confirm the cause of death.

Challenge conditions varied across the years (Table 1). In 2014, two 30-litre aquaria per family and three batches with different family subsets were used in order to test all the families using this data structure. Six and seven tanks containing 1 000 litres of water were used in 2015 and 2016, respectively. Each tank was seeded with animals from all the families under study. Shrimp families were identified using plastic elastomers as in Castillo-Juárez et al. (2007). These studies included a control tank where animals were not challenged against AHPND.

**Table 1.** Averages and (standard deviations) for variables defining conditions of AHPND challenge tests of *Penaeus vannamei* in Sinaloa, Mexico.

| Year | Age (days) | Weight (g) | cfu.mL$^{-1}$ | Duration (h) | Number of families/ individuals per line | |
|---|---|---|---|---|---|---|
| | | | | | Resistance | Growth |
| 2014 | 55.4 (0.9) | 0.5 (0.3) | 1.95 x10$^{5}$ | 52 | 28/836 | 100/1 593 |
| 2015 | 75.8 (1.6) | 2.6 (0.9) | 3.16 x10$^{6}$ | 74 | 62/1 783 | 53/1 477 |
| 2016 | 83.1 (1.8) | 1.4 (0.7) | 1.09 x10$^{6}$ | 98 | 41/1 278 | 41/1 408 |

[1] cfu.mL$^{-1}$: Colony forming units.mL$^{-1}$ at inoculation (time = 0).

### *Heritability*

Estimates of heritability for survival time were obtained within genetic line (Resistance and Growth) and year. Linear mixed models with nested random sire/dam/progeny and fixed tank (or random aquarium in 2014) effects were applied. Sire, dam and error (progeny) variance components ($V_s$, $V_d$ and $V_e$, respectively), were estimated with restricted maximum likelihood methodology (Ødegård et al. 2011).

Phenotypic variance was defined as: $V_p = V_s + V_d + V_e$. Heritability was estimated from sire variance component as: $4V_s/V_p$, the dam variance component as: $4V_d/V_p$ and from the sire + dam component of variance as: $2(V_s + V_d)/V_p$ (Falconer and McKay 1996).

### *Comparison of Resistance and Growth Lines*

In 2014, the results presented here correspond to the survival pattern of the Resistance Line and $F_1$ Resistance Line x Growth Line cross (479 organisms from 16 families) included in the first batch, under similar contemporary conditions. In 2015 and 2016, purebred Resistance and Growth Line shrimp and several crosses were measured. The evolution of the survival of the two lines was compared using survival analysis with Kaplan-Meyer methodology (Miller 2011).

### *Inbreeding Effects on AHPND Resistance*

Inbreeding effects on AHPND resistance were evaluated using data from 2015 and 2016, where inbred animals were produced by mating full-sibs, thus, approximate inbreeding coefficients obtained were close to 25 %, but there were also families with several inbreeding levels. Therefore, inbreeding effects were studied by regression analysis using animals with different inbreeding levels under similar conditions. We used linear statistical models to simultaneously test for crossbreeding, inbreeding and non-genetic effects on survival times. We also compared inbred and non-inbred animals within genetic lines.

## Results

### *Heritability and Family Differences*

Preliminary heritability estimates for survival time after AHPND challenge presented by genetic line and by year, derived with different variance components obtained by simple sire/dam nested within-line models (Falconer and McKay 1996) are shown in Table 2. Mean survival times per family by year, within genetic line, are shown in Fig. 1 to 6. These figures demonstrate that there is significant variation between families within genetic lines.

**Table 2.** Heritability estimates (%) for survival time with simple linear statistical models from sire and dam variance components by line and year.

| | **Genetic Line** | | | | | |
|---|---|---|---|---|---|---|
| | **Resistance** | | | **Growth** | | |
| **Year** | **Sire component** | **Dam component** | **Sire + Dam components** | **Sire component** | **Dam component** | **Sire + Dam components** |
| 2014 | 28 | 1 | 15 | 0 | 18 | 9 |
| 2015 | 16 | 2 | 9 | 10 | 16 | 13 |
| 2016 | 22 | 7 | 15 | 23 | 12 | 18 |

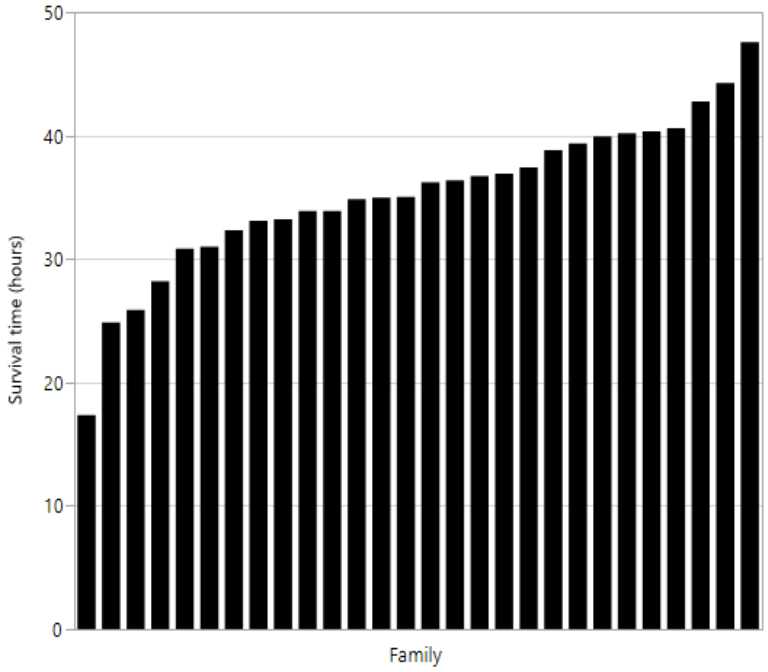


**Fig. 1.** Mean survival time by family for the Resistance Line in 2014 AHPND challenge test.

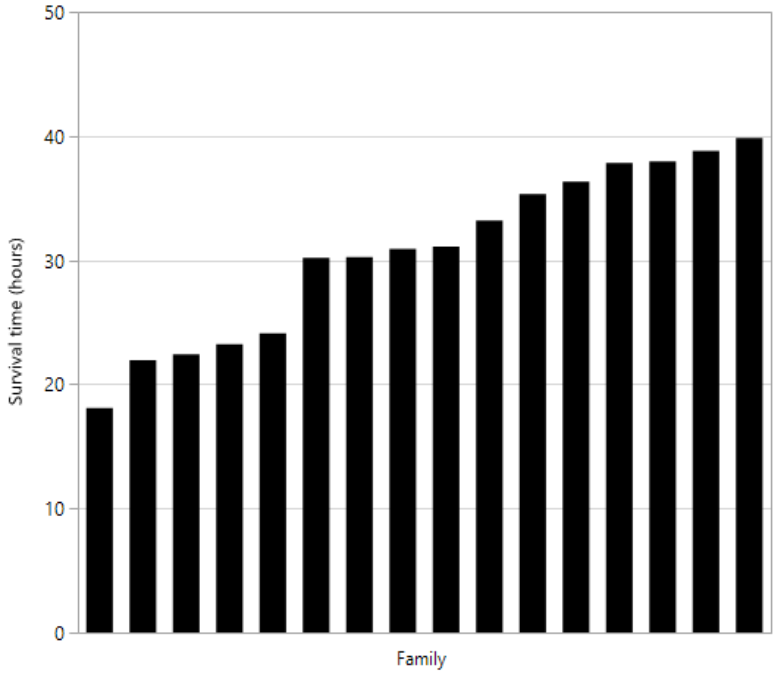


**Fig. 2.** Mean survival time by family for the $F_1$ Resistance x Growth cross in 2014 AHPND challenge test.

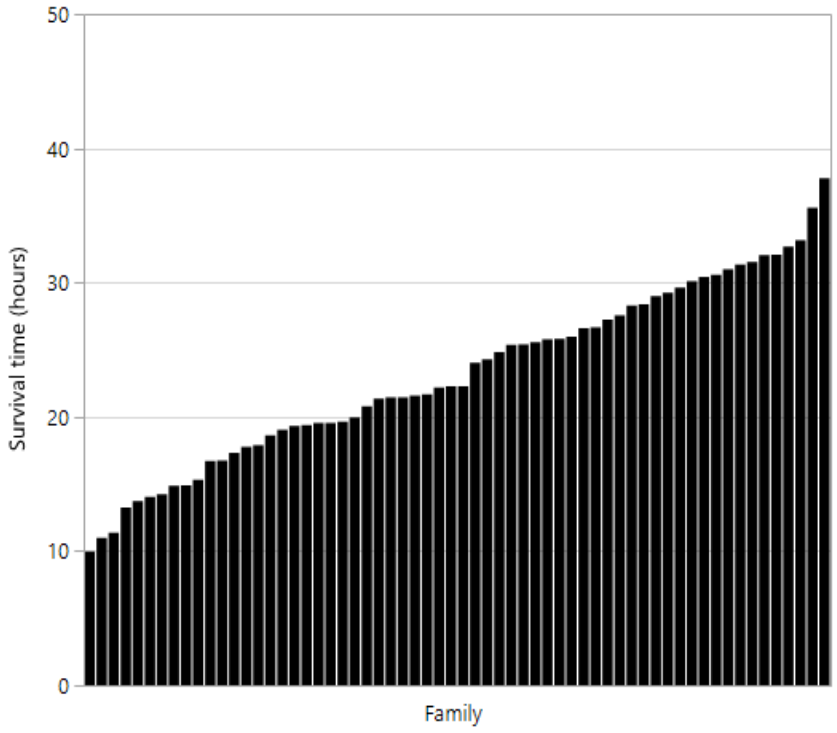


**Fig. 3.** Mean survival time by family for the Resistance Line in 2015 AHPND challenge test.

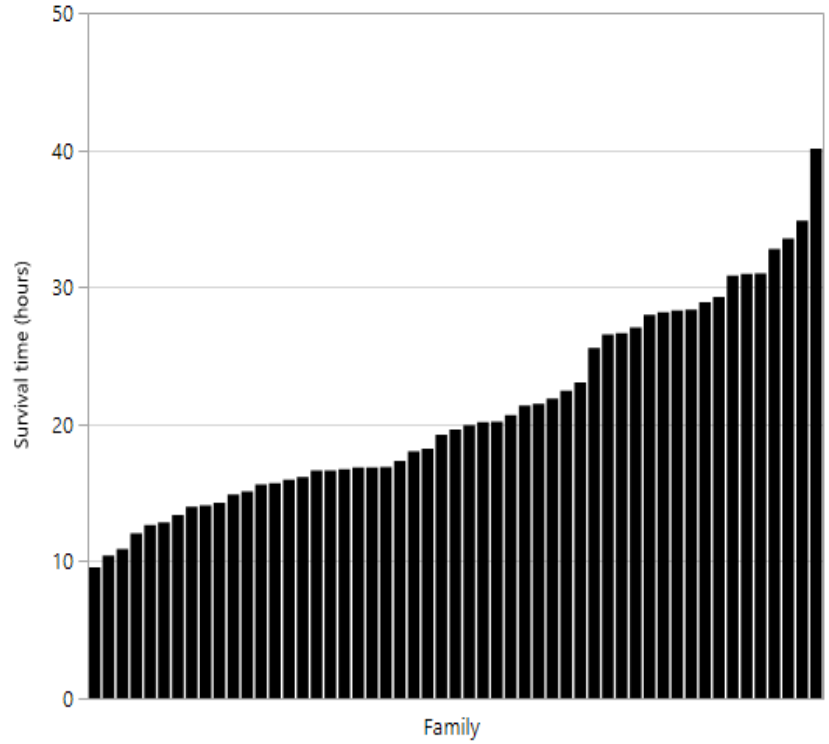


**Fig. 4.** Mean survival time by family for the Growth Line in 2015 AHPND challenge test.

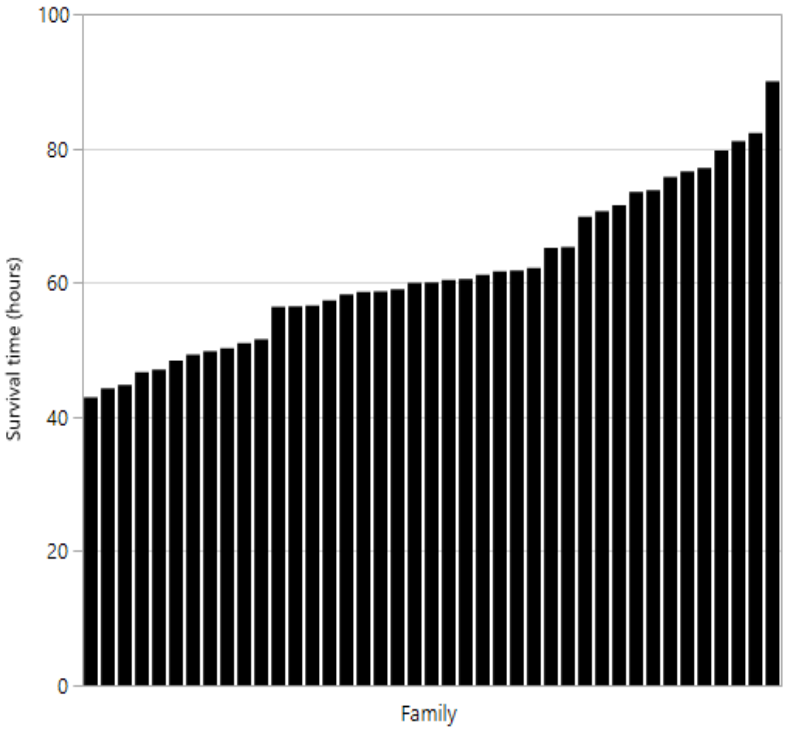


**Fig. 5.** Mean survival time by family for the Resistance Line in 2016 AHPND challenge test.

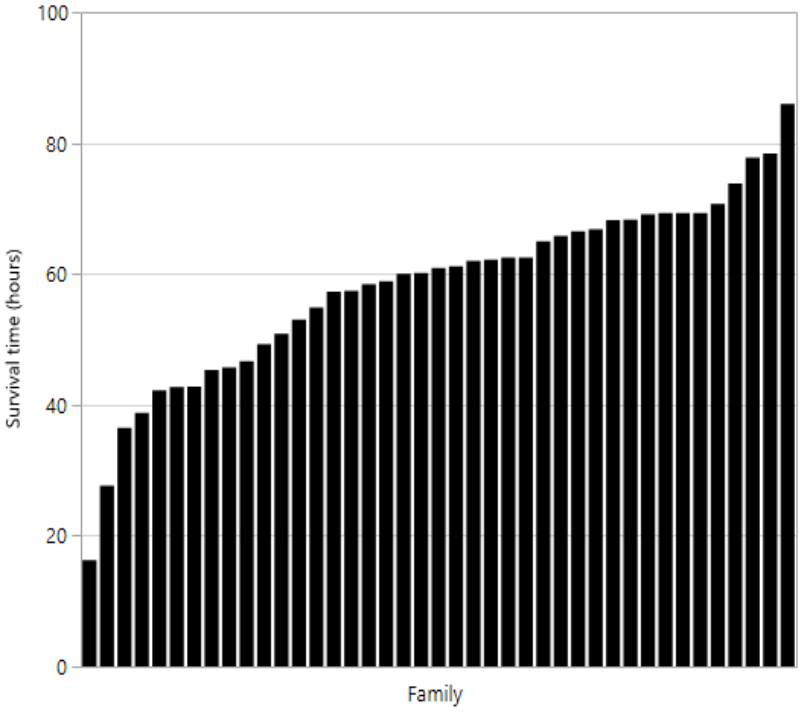


**Fig. 6.** Mean survival time by family for the Growth Line in 2016 AHPND challenge test.

### *Comparison of Purebred Genetic Lines for AHPND Resistance*

Differences were observed between the genetic lines across years (2014 to 2016) regarding resistance to AHPND, measured as survival time in hours after challenge. Figures 7, 8 and 9 show the evolution of survival for the Resistance Line versus the $F_1$ cross (Resistance Line x Growth Line) in 2014, and versus the Growth Line in 2015 and 2016. For 2014 data, mean survival times were 35.3 h for the Resistance Line and 30.7 h for the $F_1$ cross. The difference between genetic groups for survival trend (right-censored) was significant ($P < 0.001$). For 2015 data, mean survival times were 23.1 h for the Resistance Line and 21.0 h for the Growth Line. The difference between genetic lines for survival trend (right-censored) was significant ($P < 0.001$). In 2016, mean survival times were 61.7 h for the Resistance Line and 58.0 h for the Growth Line. The difference between genetic lines for survival trend (right-censored) was significant ($P < 0.02$).

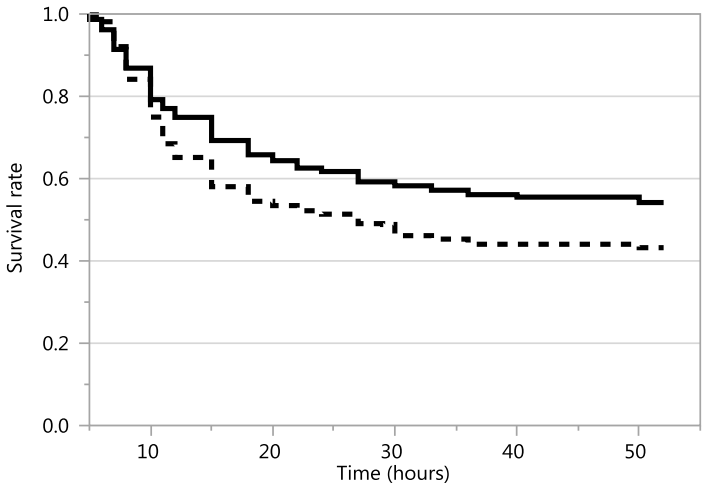


**Fig. 7.** Kaplan-Meyer survival plot for Resistance Line (solid line) and Resistance Line x Growth Line ($F_1$) cross (dotted line) in 2014 AHPND challenge test.

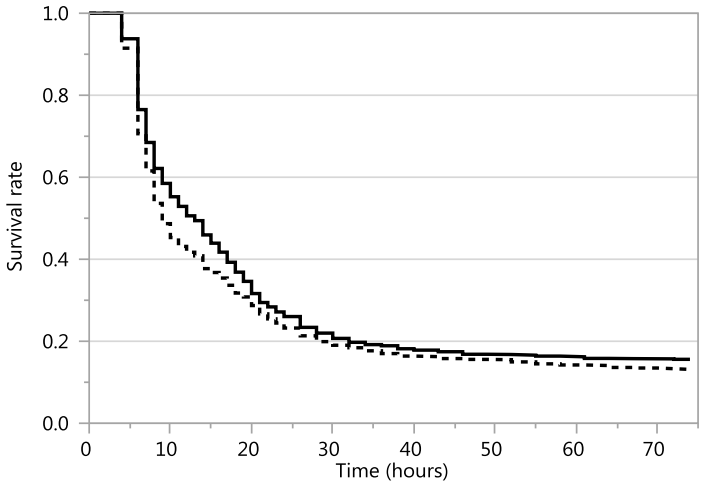


**Fig. 8.** Kaplan-Meyer survival plot for Resistance Line (solid line) and Growth Line (dotted line) in 2015 AHPND challenge test**.**

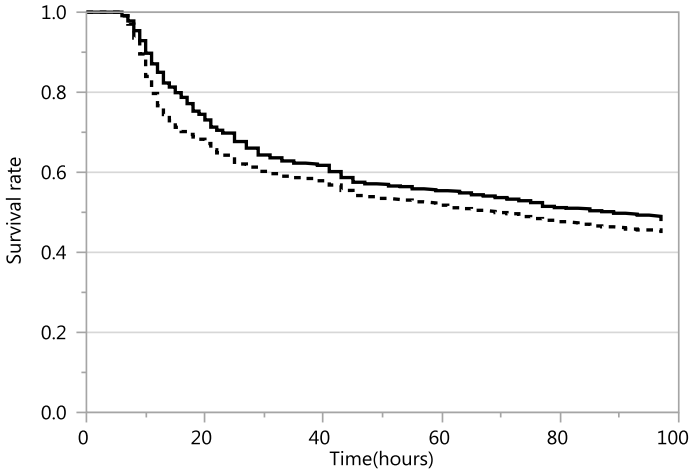


**Fig. 9.** Kaplan-Meyer survival plot for Resistance Line (solid line) and Growth Line (dotted line) in 2016 AHPND challenge test.

### *Inbreeding Effects on AHPND Resistance*

The results indicate no effects of inbreeding (F) on survival times to AHPND challenges with *P*-values > 0.10 and regression coefficient estimates of survival time on F close to zero (results not shown). This was also found in testing F effects on survival rates.

## Discussion

### *Heritability*

Combined sire + dam heritability estimates were, in general, statistically greater than zero, but tended to be lower than 20 %. There are no published studies regarding the heritability of resistance to AHPND to compare with our results. Selection (if genetic variation exists) can be used in breeding programmes to yield resistant lines to specific diseases (Cock et al. 2009), although, as Moss et al. (2005) suggest, heritability estimates under disease challenge conditions are not easily translated into practical commercial conditions.

Our results revealed important differences regarding the magnitude of the sire/dam variance components between lines, possibly due to data structure and/or to actual differences in genetic parameters between lines. In any event, our challenges found additive genetic variation for AHPND resistance, which may be used in shrimp breeding programmes. Greater survival times were observed for families from the Resistance Line when compared to the Growth Line or to the Growth x Resistance cross within year. These differences were mostly observed in the left half of the distributions, which involve the families with lower survival rates. This fact is consistent with the estimation of within-line genetic differences (heritability estimates).

It is important to bear in mind that genetic parameters for disease resistance traits may change across years and environments, since they depend on the interactions between the pathogens and their hosts (co-evolution), which are in general very dynamic processes (Ebert 1998), and favourable mutations can accumulate over years and introduce genetic variation (Cock et al. 2009). Hence, heritability estimation for disease resistance traits must be performed in each breeding cycle.

### *Comparison of Purebred Genetic Lines for AHPND Resistance*

To our knowledge, there are no published studies comparing genetic lines for resistance to AHPND challenges. Nonetheless, the differences between genetic lines in our AHPND challenges are consistent with those observed in commercial ponds in Mexico since 2013, where mortality rates have been clearly lower in the Ecuadorian-origin shrimp when AHPND and WSSV outbreaks have occurred. The higher resistance we observed in the Resistance Line is compatible with the hypothesis of a higher genetic resistance of the Ecuadorian breeding lines obtained by means of natural selection, because their breeding populations were maintained under WSSV (and probably other diseases) infection conditions for several generations. This disease challenge under natural conditions may have introduced selection pressure to these shrimp populations, leading them to develop resistance to other pathogens as well (Cock et al. 2015). Since the shrimp immune-like system is rather non-specific, the ability to succeed against one disease may also confer some protection against other diseases (Cock et al. 2009).

### *Inbreeding Effects on AHPND Resistance*

The results obtained in this study do not support a strong association between inbreeding and disease vulnerability in shrimp populations as suggested by Doyle (2016). On the other hand, experimental and theoretical evidence of the effect of inbreeding and genetic drift in small populations on general fitness and on disease resistance in arthropods is conflicting, indicating a complex picture in the presence of natural selection that points to the risk of providing overly general conclusions (Armbruster and Reed 2005; Facon et al. 2011; García-Dorado 2012; De los Ríos-Pérez et al. 2015).

## Conclusion

Our AHPND resistance challenge experiments performed from 2014 to 2016 in *P. vannamei* show that there is additive genetic variation in the Resistance Line and in the Growth Line that can be exploited in breeding programmes to increase AHPND resistance. The results presented here also support the idea that the Resistance Line formed from shrimp from Ecuador with a history of WSSV resistance is also more resistant to AHPND than the Mexican Growth Line. Finally, our experiments show that there is no effect of inbreeding on susceptibility to AHPND.

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