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DEFINING ENVIRONMENTAL STRESS CONDITIONS THAT PRODUCE DIFFERENTIAL SURVIVAL IN BLACK TIGER SHRIMP, *PENAEUS MONODON*

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SUMMARY

Breeding for shrimp lines that are resilient to sudden environmental stressors requires accurate definition and measurement of stress traits, along with the additive genetic control of the trait. In a breeding program for *Penaeus monodon*, families that exhibit increased tolerance to acute salinity and ammonia changes are targets for selection; however, there is currently no information on how to best challenge shrimp to induce differential survival and measurement of environmental stress tolerance. This study developed challenge testing methodologies for acute salinity and ammonia tolerance in postlarval *P. monodon*. Results showed differential survival among postlarvae at a range of dose levels. By applying the stress methods developed in a commercial scenario to differentiate between individual families, rankings and selection of more resilient lines could be incorporated in selective breeding programs. Further, the potential to apply these measures as a stress resistance marker in a commercial scenario will be evaluated following the determination of trait heritability and by correlating larval stress performance rankings with grow-out performance.

INTRODUCTION

Domestication and selective breeding programs have led to significant production gains in shrimp farming globally. Whilst selection programs have focused on growth and disease as primary traits to improve, cultured shrimp are also exposed to a range of environmental stressors throughout their production cycle that can affect productivity and survival, either though inducing mortality events, or by decreased growth. Therefore, the ability to identify family lines and select for increased tolerance to sudden environmental stress events may be a desirable trait for future breeding programs. Currently there is no data on how best to conduct challenge tests for environmental stress tolerance in shrimp and whether such traits exhibit significant additive genetic variance (i.e. is heritable). This study focused on developing methods of testing the resilience of postlarvae (PL) to acute salinity and ammonia stress in a way that can be quickly and easily applied in a commercial hatchery prior to stocking into ponds. Following the challenge of sufficient numbers of families, the genetic and genomic basis of this resilience could be determined and may allow heritability to be determined and a genetic marker for stress tolerance to be developed and incorporated into selective breeding programs.

MATERIALS AND METHODS

Penaeus monodon broodstock were sourced from Northern Territory coastal waters and progeny spawned in a commercial hatchery at Flying Fish Point, Queensland. Broodstock maturation and spawning followed routine commercial procedures, with multiple females spawned in communal spawning tanks and the progeny of multiple families reared in communal greenwater rearing tanks. At PL stage 15, 10,000 PL were transported via air freight to Bribie Island Research Centre (BIRC), Woorim, Queensland for experimental testing. At BIRC, PL were stocked at a density of 2500 PL

in 5 tonne fibreglass tanks, held indoors with a 12 h day/night photoperiod, receiving 4L/m-1 of filtered 29 ±0.5°C seawater and fed on a diet of commercial flake and pellet.

A range of salinity and ammonia dose rates were tested at a range of PL ages from 15 to 42 (Table 1). Salinity doses were achieved by mixing required volumes of freshwater with undiluted seawater, termed 100% seawater. A calibrated YSI probe was used to ensure 100% seawater salinity levels were consistent over time (38±0.2 ppt). The required volume of 30% ammonia solution AR, NH_4OH (Chem-Supply) was added to the treatments to achieve the required dose, which was also confirmed with a titration kit (API).

Table 1. Salinity and ammonia treatment dose levels at each PL age.

PL age	Salinity rate (% of raw seawater)	PL age	Ammonia rate (mg^{-1})
15	0, 5, 10, 15, 20, 100	15	11; 21; 53; 214
25	0, 5, 10, 15, 100	22	32; 43; 53; 64
28	0, 5, 10, 15, 100	27	21; 27; 32; 43; 53
32	0, 5, 10, 15, 100	28	16; 21; 27
		34	11; 16; 21; 27; 32

Each treatment was performed in 9 L containers containing 2 L of water. The water temperature of both rearing tanks and experimental containers was 29 ±0.5°C. Treatment container temperature was maintained by placing the containers in a temperature controlled water bath. Treatment salinity or ammonia water parameters (Table 1) were set up prior to commencement of PL stress treatment in batches that were then distributed to each 2 L replicate to ensure consistency among replicates.

Approximately 30 ±10 PL were added to each control and treatment container without prior counting, to reduce handling stress. The total number of animals per treatment was calculated at the subsequent hourly time points when data was collected on the number of live and dead animals. The zero time post treatment was as the animals entered the water, any animals identified as dead in the first 30 s were considered dead prior to entering the experiment, i.e. dead in the rearing tanks, and were removed from the container and excluded from any analyses. An assessment of whether animals were alive or dead was made at 30 min intervals for the first 2 h of treatment then at hour intervals for the remainder of the 5 h experiment. Motile versus non-motile animals were separated by gently swirling the water; motile, live animals would swim and non-motile animals would settle in the centre of the container. The mortality of non-motile animals was then confirmed by gently disturbing them with forceps, if no movement was observed they were considered dead and were subsequently counted and removed from the container. The three control treatments were treated in the same manner with the same level of physical disturbance.

The length of dead PL were measured on 1 mm grid paper for each time point and then the survivors were measured at the end of the experiment.

RESULTS AND DISCUSSION

Suitable parameters for stress testing would be those that showed differential survival of postlarvae (PL), with the ultimate objective of differentiating families that are more or less tolerant to environmental stress. For the practical application of this test in a commercial environment, the ideal mortality curve would have a rate near 50% at 3 h, and a flattening out of mortality beyond. For example the salinity dose of 10% and 15% salinity at PL 32 (Figure 1A), was considered an optimal level (Table 2). While an ammonia rate between $21mg^{-1}$ and 27 mg^{-1} was recommended at PL 34 (Figure 1B & Table 2). While the range of dose rates and ages have not previously been tested, a dose rate of $20mg^{-1}$ on PL5 *P. monodon* has reportedly resulted in 53-55% mortality after 72 h (Pan *et al.* 2003).

The results of this study identified initial ranges that achieved the objective of determining suitable stress testing parameters with both salinity stress and ammonia stress for various PL ages (Table 2).

Table 2. Salinity and ammonia stress mortality at 3 h and recommendations on optimal parameters

PL age	Salinity dose level (%seawater)	Mortality (%) at 3 h	Optimal parameter recommendation	PL age	Ammonia dose level (mg^{-1})	Mortality (%) at 3 h	Optimal parameter recommendation
15	0	100	>20%	15	11	2	>21<53
	5	100			21	3	
	10	100			53	100	
	15	100			214	100	
	20	84					
25	0	100	≥15%	22	32	49	<32
	5	98			43	94	
	10	90			53	100	
	15	75			64	100	
28	5	94	10% or 15%	27	21	43	21
	10	62			27	96	
	15	39			32	100	
					43	99	
					53	100	
32	5	94	10% or 15%	28	16	1	16>21
	10	66			21	82	
	15	53			27	97	
	20	20					
				34	11	0	21>27
45	15	4			16	0	
	20	0			21	19	
					27	85	
					32	95	

T-tests showed a significant difference in mortality between all salinity dose levels at 3 h post-treatment ($P<0.05$), except for between 10% and 15% salinity dose levels ($P>0.05$). Significant differences in mortality was also observed between all ammonia dose levels at 3 h ($P<0.05$), except between 32 mg^{-1} and 16 mg^{-1} dose levels ($P>0.05$). This study determined that PL were more tolerant to lower salinity levels at later ages (Figure 2). T-tests revealed significant differences ($P<0.005$) between all ages at the 3 h time point, with the exception of PL 25 that did not differ significantly between PL 15 or PL 32 ($P>0.5$). At PL 15 a salinity ratio of 20% seawater: 80% freshwater resulted in 84% mortality at 3 h post-treatment, while at PL 32 the same salinity resulted in just 20% mortality at 3 h (Figure 3).

Previous studies have found that larger postlarval shrimp have a greater tolerance to salinity stress, often linked with the development of gills (Chong-Roles *et al.* 2014); however, the current study found that mortality was strongly linked to age rather than size (Figures 3 & 4).

This study found that there was no significant effect between PL size and mortality time for salinity or ammonia (ANOVA $P>0.05$); this was only tested at PL 25. The lack of relationship between mortality and size indicated that PL mortality was not simply removing fast or slow growing PL. Furthermore, environmental rearing effects among the PL were minimised as the batch

of PL tested underwent communal spawning and rearing where they were subjected to the same environmental conditions. Therefore, this study supports the hypothesis that there may be a significant genetic effect on resilience to environmental stress and subsequently further research should be directed into the genetic, genomic or physiological influences on resilience.

The genetic basis for this resilience as well as heritably may be elucidated in future studies by applying the methods developed in this study to discriminate between resistant and susceptible families. Genomic markers may also be developed that could then be utilised in a selective breeding program to establish more resilient lines, thereby leading to improved survival and production yields in shrimp farming.

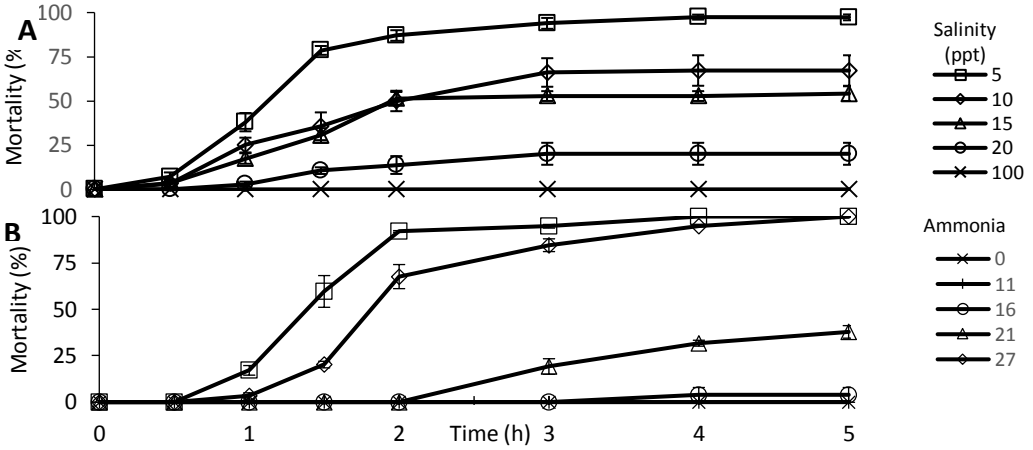

Figure 1. Example of *Penaeus monodon* mortality curves for salinity (A) and ammonia (B) challenge at postlarval ages PL 32 (A) PL 34 (B) (SEM bars).

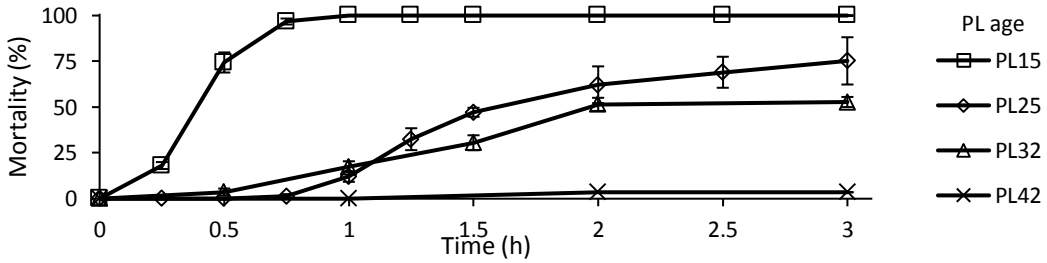

Figure 2. Effect of postlarval age on survival to 15% salinity for *Penaeus monodon* (SEM bars).

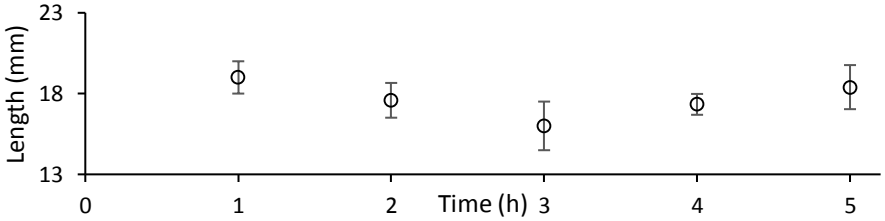

Figure 3. Length of dead postlarve following 16 mg^{-1} of ammonia at PL 25 (SEM bars).

REFERENCES

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