Mortality of freshwater-acclimated *Litopenaeus vannamei* associated with acclimation rate, habituation period, and ionic challenge

William J. McGraw¹, John Scarpa*

*Harbor Branch Oceanographic Institution, Inc., Aquaculture Division, 5600 US 1 North, Fort Pierce, FL 34946, USA*

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**Abstract**

The effect of acclimation time, habituation period, and final freshwater ion composition on the survival of freshwater-acclimated Pacific white shrimp *Litopenaeus vannamei* postlarvae was investigated. During each of three experiments, shrimp were acclimated from 30 ppt to freshwater (≤ 1 ppt TDS) utilizing various acclimation times (32, 40, 48, 72 h) with a constant or variable rate of salinity reduction. Shrimp were then held at the final acclimation salinity for 0, 1 or 2 days (habituation period) before being transferred to challenge ion treatment solutions. Ion treatment solutions derived from chloride-based chemicals were of the same total ion concentration, but either strongly monovalent or strongly divalent. An acclimation time of 72 h compared to 48 h, with no habituation period, increased shrimp survival by 27%. A 1-day habituation period compared to no habituation after 48-h acclimation also increased mean shrimp survivals by 27%. Decreasing acclimation time at higher salinities (i.e., 30 down to 1 ppt) and increasing acclimation time at lower salinities (≤ 1 ppt) allowed successful freshwater acclimation of shrimp within 32 h. This demonstrates a critical period for freshwater acclimation of marine shrimp at lower salinities. The longer freshwater acclimation time and habituation period probably allowed shrimp to equalize hemolymph ions before transfer to ionically unbalanced challenge solutions. The survival of freshwater-acclimated shrimp can be improved by either extending the acclimation time from 48 to 72 h or providing a habituation period of 2 days after 48-h acclimation. However, growth at these ion concentrations still needs to be verified.

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**Keywords:** *Litopenaeus vannamei*; Acclimation rate; Habituation period
1. Introduction

Marine shrimp culture located miles away from the ocean using saline or fresh well water is a new and fast growing sector of aquaculture in the United States (Ednoff, 2001). The emergence of inland shrimp farming can be traced to several factors, e.g., high cost of coastal land, environmental concerns, and the increasing demand for shrimp in the US (Hopkins et al., 1996). Pacific white shrimp Litopenaeus vannamei have been grown successfully in freshwater (650 ppm TDS) at Harbor Branch Oceanographic Institution (Van Wyk et al., 1999) and in low salinity water in other areas of the US (Samocha et al., 1998; Ednoff, 2001; Samocha et al., 2002).

Shrimp farmers using inland well water (< 5 ppt) have experienced increased survivals when shrimp are given a period of time in final acclimation water before transfer to ponds (Teichert-Coddington, Green Prairie Aquafarm, personal communication, 2000). The authors have observed acute mortality of freshwater-acclimated Pacific white shrimp L. vannamei postlarvae when transferring shrimp from acclimation tanks to various ion treatment solutions of the same or slightly different salinity. Shrimp would experience immediate, or within 24 h, loss of equilibrium and mortality. This mortality was thought to be caused by a faster than normal acclimation rate (>50% reduction in salinity per 8-h period; Van Wyk et al., 1999), an environmental ionic imbalance into which shrimp were transferred (McGraw and Scarpa, 2003), or immediate transfer of shrimp from acclimation solutions without a period of habituation.

Information concerning the necessary minimum ion requirements in freshwater (< 1000 ppm TDS) for marine shrimp culture and acceptable freshwater acclimation procedures is limited (Van Wyk et al., 1999; Anonymous, 2002; McGraw et al., 2002; McGraw and Scarpa, 2003). The ionic composition of well water varies greatly between locations and depth of wells (Feth, 1970). Therefore, understanding factors, such as salinity acclimation, that affect the survival of marine shrimp in freshwater are imperative to the advancement of the U.S. shrimp farming industry. The following experiments were designed to examine acclimation time of marine shrimp to freshwater, time spent in final acclimation solution before transfer (habituation period) to final solutions, and ionic composition of final waters, to understand how these factors affect initial survival.

2. Materials and methods

Approximately 5000 specific pathogen free (SPF) 9-day-old postlarvae (PL-9) Pacific white shrimp L. vannamei were received from an instate hatchery (Shrimp Improvement Systems, Islamorada, FL) on three separate dates for each of three experiments. Shrimp were maintained in 80-l aquariums with seawater (30 ppt) and fed a prepared diet (44% protein, Bonnie, Laramore and Hopkins, Ft. Pierce, FL) ad libitum three times per day until the age of PL-15 when acclimation to HBOI freshwater (280 ppm Cl⁻ or 7.9 mM) began. The general experimental design consisted of four different acclimation times (32, 40, 48 and 72 h) and 0, 1 or 2 days that shrimp spent at the final salinity (habituation period) before being transferred to one of four different experimental treatment solutions (strongly monovalent, strongly divalent, seawater, and HBOI freshwater). Habituation
described here refers to a period of time when salinity remains constant while shrimp are kept in acclimation water before being transferred to final solutions. Final treatment solutions of different total and individual ion concentrations were included to challenge shrimp exposed to the different acclimation times and habituation periods in order to understand how these factors contribute to shrimp mortality. The treatment solution of HBOI fresh water (same as acclimation water) was used as a control, while those with different individual and total ion compositions were included to represent the extremes that might be encountered from saline well water sources located in various areas and at different depths.

Shrimp were acclimated to HBOI freshwater (280 ppm Cl\(^-\)) at different rates using HBOI freshwater, which was also used as the final habituation solution. This was accomplished by placing approximately 1000 postlarvae in each of three 40-l aquaria containing 20 l of seawater (30 ppt) and adding HBOI freshwater, according to the experimental protocol, using an airline valve and tubing through siphon action. The volume of water in aquaria was reduced as necessary to maintain a constant rate of salinity reduction based on treatment design. Salinity was checked every hour with a refractometer and HBOI freshwater addition was adjusted as necessary to maintain the acclimation rate. When total ion concentrations in acclimation water decreased to < 1 ppt, chloride was measured using Hach DR/3 spectrophotometric methods (Hach, Loveland, CO) and used to indicate total ion concentration in order to maintain acclimation rate. Shrimp were fed ad libitum during acclimation, but not during the 24-h period in final treatment solutions. Temperature of acclimation water and treatment solutions were maintained between 26 and 27 °C throughout all experiments as temperature fluctuation is known to affect the success of acclimation of shrimp to freshwater (Tsuzuki et al., 2000).

After acclimation to HBOI freshwater, 10 shrimp from each acclimation treatment tank were placed in triplicate 4-l plastic containers, each containing 2 l of aerated treatment solution, either immediately (0-day habituation period), after 1 day, or after 2 days spent in HBOI freshwater. After 24 h, shrimp survival was checked using a probe response. All shrimp were then removed from treatment solutions and replaced with shrimp from the next habituation period (i.e., 0-day habituated shrimp replaced with 1-day habituated shrimp, etc.).

Final treatment waters were one of four solutions (Table 1): strongly monovalent (500 ppm TDS), strongly divalent (500 ppm TDS), full-strength seawater (30 ppt TDS), or HBOI freshwater (same as final habituation solution, 546 ppm TDS).

![Table 1](image1.png)

<table>
<thead>
<tr>
<th>Treatment solution</th>
<th>Na(^+) (ppm)</th>
<th>Ca(^{2+}) (ppm)</th>
<th>Mg(^{2+}) (ppm)</th>
<th>K(^+) (ppm)</th>
<th>Cl(^-) (ppm)</th>
<th>TDS(^a) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly monovalent</td>
<td>143 (6.2)</td>
<td>8 (0.1)</td>
<td>26 (1.1)</td>
<td>6 (0.15)</td>
<td>317 (8.9)</td>
<td>500</td>
</tr>
<tr>
<td>Strongly divalent</td>
<td>47 (2.0)</td>
<td>24 (0.6)</td>
<td>77 (3.2)</td>
<td>2 (0.05)</td>
<td>350 (9.9)</td>
<td>500</td>
</tr>
<tr>
<td>HBOI freshwater</td>
<td>181 (7.8)</td>
<td>44 (1.1)</td>
<td>31 (1.3)</td>
<td>10 (0.25)</td>
<td>280 (7.9)</td>
<td>546</td>
</tr>
<tr>
<td>Seawater</td>
<td>10,770</td>
<td>414</td>
<td>1,300</td>
<td>399</td>
<td>19,350</td>
<td>32,233</td>
</tr>
</tbody>
</table>

\(^a\) TDS = total dissolved solids, but does not include bicarbonate ion present in ion solutions, and other ion components found in seawater and HBOI freshwater.
Monovalent and divalent ion concentrations were derived from previous ion experiments (McGraw and Scarpa, 2002, 2003) that were designed to examine survivability and growth of shrimp in ionic solutions skewed toward the essential monovalent (Na\(^+\), K\(^+\)) or divalent (Ca\(^{2+}\), Mg\(^{2+}\)) ions involved in shrimp osmoregulation (Schmidt-Nielsen, 1990).

Monovalent and divalent treatment waters were created from two stock solutions: a monovalent solution containing Na\(^+\), K\(^+\), and Cl\(^-\) and a divalent solution containing Ca\(^{2+}\), Mg\(^{2+}\), and Cl\(^-\). Divalent ions (Ca\(^{2+}\), Mg\(^{2+}\)) and monovalent ions (Na\(^+\), K\(^+\)) were added in an approximate ratio of seawater (g of ion per kg of seawater: Cl\(^-\) = 19.35, Na\(^+\) = 10.77, Mg\(^{2+}\) = 1.30, Ca\(^{2+}\) = 0.414, K\(^+\) = 0.399; monovalent ratio = 27Na/1K; divalent ratio = 3.15Mg/1Ca). These treatment solutions were formulated using chloride-based chemicals (NaCl, CaCl\(_{2}\)·2H\(_2\)O, MgCl\(_{2}\)·6H\(_2\)O and KCl, Sigma, St. Louis, MO) added to de-ionized water. Monovalent and divalent solutions were then mixed together per volumetric proportion as 75% monovalent and 25% divalent (i.e., strongly monovalent) and vice versa for strongly divalent (Table 1). Magnesium carbonate and sodium bicarbonate were added to monovalent and divalent solutions to produce a total alkalinity of 50 mg/l. Mean pH for monovalent and divalent treatment waters was 7.8.

Seawater (30 ppt) and HBOI freshwater (control) were obtained from onsite wells. All ions in final treatment solutions, except Na\(^+\) and Cl\(^-\), were measured using Hach DR/3 spectrophotometric methods (Hach). Chloride was measured using a titration kit (Lamotte, Chestertown, MD, USA). Sodium concentrations were calculated. All measured ion concentrations were within 5% of the listed calculated values. Mean pH for seawater and freshwater was 7.6 and 8.2, respectively.

Three-way ANOVA and Student–Newman–Keuls (Lentner and Bishop, 1993) were used to compare survival of shrimp in treatment solutions against acclimation time, habituation period, and final ion treatment solution. Percentage survival data were transformed (arcsine \(\times\) square root) before statistical analyses using software (SuperAnova, Abacus Concepts, Berkeley, CA). Differences were considered significant if \(P < 0.05\). Survival of shrimp in acclimation aquaria (during acclimation and habituation period) was visually estimated and discussed but not used for statistical analysis.

2.1. Experiment I: 40-, 48- and 72-h acclimation rate

Shrimp were acclimated from seawater (30 ppt) to HBOI freshwater (280 ppm Cl\(^-\)) at a constant hourly reduction rate in 40, 48, and 72 h (i.e., 9.1, 7.6, and 5.2% reduction in salinity per hour). A three-way ANOVA (acclimation time, habituation period and ionic challenge) was used to compare treatment means of shrimp survival in ion treatment solutions.

2.2. Experiment II: 32-, 40- and 48-h acclimation rate with 8-h cessation

Shrimp were acclimated from seawater (30 ppt) to HBOI freshwater (280 ppm Cl\(^-\)) in 32, 40, and 48 h. Acclimation stopped at the beginning of hour 15 for all treatments and salinities were held constant for each treatment (5, 5.5, and 9 ppt, respectively) for an 8-h period before acclimation resumed. Acclimation rates were maintained at 12.0%, 11.4%, and
8.2% reduction in salinity per hour for the first 14 h and 18.5%, 11.2%, and 9.6% reduction per hour from hour 23 until the remaining time left in the 32-, 40-, and 48-h acclimation treatment periods, respectively. Cessation described here refers to a pause in salinity change before continuation of acclimation, while habituation (described above) refers to a period of time when salinity remains constant and shrimp are kept in acclimation water before being transferred to ion treatment solutions. A three-way ANOVA was used to compare treatment means of shrimp survival in ion treatment solutions.

2.3. Experiment III: 32- and 40-h variable acclimation rate and 48-h constant acclimation rate

Shrimp were acclimated from seawater (30 ppt) to freshwater (350–380 ppm or 10–10.7 mM Cl\(^-\)) at a constant rate during the first 24 h for two acclimation treatments (32 and 40 h). This resulted in a salinity reduction rate of 13.5% per hour (0.9 ppt TDS at the end of hour 24). The salinities for these two treatments were then reduced to HBOI freshwater (280 ppm Cl\(^-\)) in 8 or 16 h, which was associated with a reduction in salinity of 4.3% and 2.2% per hour. The total time for these acclimation treatments was 32 and 40 h. A standard 48-h acclimation with a constant rate of salinity reduction (50% reduction in salinity per 8 h) was also used as a single treatment for comparison. A three-way ANOVA was used to compare treatment means of shrimp survival in ion treatment solutions.

3. Results

3.1. Experiment I: 40-, 48- and 72-h acclimation rate

Acclimation time and habituation period did not affect 24-h shrimp survival (76–81% and 73–82%, respectively); however, final ion treatment solution significantly affected survival (Table 2). Strongly monovalent and HBOI freshwater treatment solutions produced overall survivals (85–88%) that were significantly higher compared to seawater and divalent treatments (70%).

A significant interaction occurred between acclimation time and habituation period. In the 48-h acclimation time, shrimp transferred immediately (0 habituation period) into treatment solutions had low survival (58%), whereas the 40- and 72-h acclimation times with no habituation period exhibited survivals (76%, 85%) similar to shrimp given a 1- or 2-day habituation period.

A significant interaction also occurred between acclimation time and final treatment solutions. For the divalent solution, the 40- and 48-h acclimation times had similar survivals (63%, 59%) while the 72-h acclimation time showed markedly increased shrimp survival (87%). The monovalent solution produced similar survivals for the 40- and 72-h acclimation time (92%, 90%), while the 48-h acclimation time had less survival (82%). The seawater treatment exhibited variable shrimp survival for all acclimation times (50–83%). The HBOI freshwater treatment solution produced higher shrimp survival (93%) with the 48-h acclimation rate compared to the 40- and 72-h rate (84%, 78%).
3.2. Experiment II: 32-, 40- and 48-h constant acclimation with 8-h cessation

The 32-h acclimation time with 8-h cessation produced >90% shrimp mortality during acclimation. The few remaining shrimp were pale and lethargic and were not placed in treatment solutions. The 40-h acclimation time with 8-h cessation produced approximately 20% mortality during acclimation, while no shrimp mortality was noted in the 48-h acclimation aquaria. Acclimation time and habituation period did not affect final shrimp survival (84–86% and 80–89%, respectively), however, final ion treatment solution significantly affected survival (Table 3). Mean survival for the monovalent ion treatment (94%) was significantly greater than the divalent or seawater treatment (81%, 79%), but not the freshwater control (86%).

A significant interaction occurred between habituation period and ion treatment solutions. The monovalent solution had similarly high survival (91–97%) among habituation times (0, 1, 2 days), whereas the divalent solution showed high survival (98%) with 2-day habituation and reduced survival (72%) at 0- and 1-day habituation. The seawater treatment produced the same survival (75%) between the 0- and 2-day habituation periods, but showed increased survival (88%) for 1-day habituation. The HBOI freshwater control produced the same survival for the 1- and 2-day habituation period (88%) while 0-day habituation exhibited 82%.

3.3. Experiment III: 32- and 40-h variable acclimation rate and 48-h constant acclimation rate

Acclimation time, habituation period, and final ion treatment did not significantly affect shrimp survival (Table 4). Shrimp survival varied from 84% to 91% among

Table 2
Mean survival (% ± S.E., n = 3) of PL-18 Pacific white shrimp *Litopenaeus vannamei* in final treatment solutions after 40-, 48-, and 72-h acclimation times and 0-, 1-, or 2-day habituation periods

<table>
<thead>
<tr>
<th>Ion treat solution</th>
<th>Acclimation time Mean survival (%)</th>
<th>Mean ion treat</th>
<th>0-day habituation 40 h 48 h 72 h</th>
<th>1-day habituation 40 h 48 h 72 h</th>
<th>2-day habituation 40 h 48 h 72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly monovalent</td>
<td>90 ± 10 73 ± 9 90 ± 6 97 ± 3 87 ± 3 90 ± 10 90 ± 6 87 ± 3 90 ± 6 88 ± 2a</td>
<td>0-day habituation 40 h 48 h 72 h</td>
<td>0-day habituation 40 h 48 h 72 h</td>
<td>0-day habituation 40 h 48 h 72 h</td>
<td></td>
</tr>
<tr>
<td>Strongly divalent</td>
<td>53 ± 9 17 ± 17 87 ± 8 57 ± 12 80 ± 10 83 ± 9 80 ± 10 80 ± 10 90 ± 10 70 ± 5b</td>
<td>1-day habituation 40 h 48 h 72 h</td>
<td>1-day habituation 40 h 48 h 72 h</td>
<td>1-day habituation 40 h 48 h 72 h</td>
<td></td>
</tr>
<tr>
<td>Seawater</td>
<td>83 ± 9 50 ± 21 77 ± 14 73 ± 3 80 ± 6 63 ± 12 57 ± 15 80 ± 6 70 ± 21 70 ± 4b</td>
<td>2-day habituation 40 h 48 h 72 h</td>
<td>2-day habituation 40 h 48 h 72 h</td>
<td>2-day habituation 40 h 48 h 72 h</td>
<td></td>
</tr>
<tr>
<td>Freshwater</td>
<td>77 ± 7 93 ± 3 87 ± 7 77 ± 9 93 ± 7 77 ± 12 97 ± 3 93 ± 7 70 ± 21 85 ± 3a</td>
<td>0-day habituation 40 h 48 h 72 h</td>
<td>0-day habituation 40 h 48 h 72 h</td>
<td>0-day habituation 40 h 48 h 72 h</td>
<td></td>
</tr>
</tbody>
</table>

Mean for accl. time: 78 ± 3 76 ± 4 81 ± 3

Mean for hab. period: 73 ± 5 80 ± 3 82 ± 3

Values followed by a different superscript are significantly different at *P*<0.05.

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final ion treatments. A significant interaction between acclimation time and habituation period demonstrated that as acclimation time increased from 32 to 48 h, shrimp survival for 0- and 2-day habituation increased from 86–89% to 84–94%, respectively. For 1-day habituation, survival decreased from 91% to 79% as acclimation time increased from 32 to 40 h and then increased to almost 90% for the 48-h acclimation time.

Table 3
Mean survival (% ± S.E., n = 3) of PL-18 Pacific white shrimp *Litopenaeus vannamei* after 40- and 48-h constant acclimation times with 8-h cessation (C*) and 0-, 1-, or 2-day habituation periods

<table>
<thead>
<tr>
<th>Ion treat solution</th>
<th>Acclimation time</th>
<th>Mean ion treat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 h (C)</td>
<td>48 h (C)</td>
</tr>
<tr>
<td></td>
<td>0-day habituation</td>
<td>1-day habituation</td>
</tr>
<tr>
<td>Strongly monovalent</td>
<td>100 ± 0</td>
<td>90 ± 10</td>
</tr>
<tr>
<td>Strongly divalent</td>
<td>67 ± 7</td>
<td>73 ± 7</td>
</tr>
<tr>
<td>Seawater</td>
<td>73 ± 15</td>
<td>77 ± 15</td>
</tr>
<tr>
<td>Freshwater</td>
<td>73 ± 9</td>
<td>90 ± 6</td>
</tr>
</tbody>
</table>

Mean for accl. time 86 ± 7 84 ± 5
Mean for hab. period 80 ± 7 86 ± 5 89 ± 6

Values followed by a different superscript are significant at P < 0.05.
*C* = 8-h cessation from hours 15 to 23 during acclimation from seawater (30 ppt) to freshwater (280 ppm Cl	extsuperscript{−}).
4. Discussion

4.1. Effect of acclimation time

Van Wyk et al. (1999) suggested postlarval shrimp should be acclimated from full strength seawater (35 ppt) to freshwater (300 ppm Cl\(^{-}\)) at a rate of 50% reduction in salinity per 8 h over a 48-h period. Although 48-h acclimation from seawater to freshwater produced acceptable survival for this study, immediate transfer of shrimp to a strongly divalent solution after freshwater acclimation showed markedly decreased survivals compared to transfer after 72-h acclimation. The 72-h acclimation treatment probably allowed greater equalization of ions between shrimp hemolymph and the surrounding acclimation medium, thereby reducing osmoregulatory stress in the shrimp that resulted in higher survival. This balance of ions between the environment and shrimp hemolymph provided by the extended acclimation time (Bursey and Lane, 1971; Lin et al., 2000) probably enabled shrimp to survive ion imbalance of divalent solutions of equal total ion concentration. This concurs with results of Pantastico and Oliveros (1980) who found that acclimation of *Peneaus monodon* (PL-20, 35, and 90) from 16 to 0 ppt conducted over a period of 3 days significantly increased survivals of all three PL ages compared to a 1- or 2-day acclimation period.

Acclimation times of 32 h (Experiment III) and 40 h (all exps) yielded similar survival to 72-h acclimation time after transfer to final treatment solutions. However, during the 32- and 40-h acclimation times, shrimp mortality was noted in the acclimation aquaria. Shrimp mortalities that occurred in acclimation aquaria were often consumed by cohorts, which made estimation of shrimp survival in aquaria difficult (survival in acclimation aquaria was not used in analysis). Osmoregulatory ability of crustaceans that inhabit environments with changing salinity varies between populations, cohorts, seasons and temperatures (Mantel and Farmer, 1983). Osmoregulatory ability has been suggested by Laramore et al. (2001) to be variable between and within shrimp cohorts. Salinity shock treatment is used in shrimp growout operations as a test before stocking shrimp into ponds to show the health status of shrimp, which have been recently received from hatcheries (Villalon, 1991). Therefore, it seems probable that some of the weaker shrimp within the 32- and 40-h acclimation aquaria succumbed to salinity stress leaving the “hardier” individuals for transfer into ion solutions, which showed similar survival to longer acclimation times (48 and 72 h) in all ion treatment solutions.

An extended acclimation period at very low salinities (280–380 ppm Cl\(^{-}\)) showed an obvious advantage in increased survival of shrimp for this study. This concurs with the study conducted by Pantastico and Oliveros (1980) who determined the critical period of freshwater acclimation of *P. monodon* from 16 ppt occurred at salinities <6 ppt with peak mortalities evident at salinities <2 ppt. Successful freshwater acclimation rates for *L. vannamei* suggested by Van Wyk et al. (1999) shows salinity reduction decreased from 2 ppt/h for the first 8 h down to 0.063 ppt/h for the last 8 h during a 48-h period, demonstrating the importance of an extended acclimation period at low salinities.
4.2. Effect of habituation period

Although not a statistically significant factor, a 1-day habituation period generally produced an increase in shrimp survival over immediate transfer to final treatment solutions. Survival increased 9–31\% in the divalent ion solution as habituation period increased from 0 to 2 days. Shrimp in the 48-h acclimation treatment showed an increase in survival up to 27\% for the 1- or 2-day habituation period over immediate transfer. Ions in the hemolymph of crustaceans have been previously shown to equalize to steady concentrations after 24 h. *P. monodon* transferred from 45 to 15 ppt needed 24 h until ionic and osmotic changes in the hemolymph ceased (Lin et al., 2000). *Farfantepenaeus duorarum* transferred from full strength seawater to salinities ranging from 7 to 28 ppt also needed 24 h to adjust ion concentrations to that of the changing medium (Bursey and Lane, 1971). Two species of crayfish (*Procambarus clarkii* and *P. zonangulus*), found in fresh and brackish water, experienced osmotic stability in hemolymph after 48 h when transferred from freshwater to a salinity of 20 ppt (Newsom and Davis, 1994).

Although shrimp given 2-day habituation showed an increase in survival when transferred to treatment solutions, other studies show longer-term changes to low-salinity environments. Shrimp kept in low-salinity water for several weeks are thought to have increased tolerance to low-salinity ion fluctuation possibly due to long-term physiological adaptation to hypotonic conditions. Permeability of crustacean gills to the external medium may also be modified in response to long-term acclimation to dilute media (Mantel and Farmer, 1983). Invertebrates collected from natural low-salinity environments were more tolerant of low-salinity test solutions while animals taken from seawater were more tolerant of high-salinity test waters (Stancyk and Shaffer, 1977; Kangas and Skoog, 1978).

4.3. Salinity change vs. ion change

The ionic make up of shrimp hemolymph is predominantly (88\%) sodium and chloride (Chen and Chen, 1996). Therefore, it was expected that the divalent solution would be more challenging to shrimp and produce increased mortality compared to the monovalent or the control solution (HBOI freshwater). Monovalent and HBOI freshwater have ion ratios similar to shrimp hemolymph. These treatment solutions would be expected to produce less osmotic stress in shrimp and have higher survivals than the strongly divalent solution, which they did.

This present study also demonstrated that shrimp acclimated to freshwater can be immediately transferred into full strength seawater with good survivals. Strongly divalent ion treatment solutions, which had similar salinity as the final acclimation water, appear to exhibit an equal or greater detrimental effect on shrimp survival as compared to a change in salinity of nearly 30 ppt. *L. vannamei* (>PL-15) are capable of tolerating a high rate of salinity reduction, e.g., from 24 to 1 ppt in about 5 h (McGraw et al., 2002). *Fenneropenaeus indicus* (PL 7–22) can survive an abrupt salinity change of up to 10 ppt, but transfer of shrimp from 30 to 5 and 10 ppt resulted in mass mortalities (Kumlu and Jones, 1995). Experience of the authors have
shown *L. vannamei* (PL 15–20) can survive a salinity change from 30 to 15 ppt with limited mortality but changes >20 ppt produced greatly increased mortality. However, it is evident from the present study that PL-15 shrimp can survive greater hypertonic salinity changes than hypotonic changes as shrimp transferred from freshwater to 30 ppt had good survival. This may be related to the increase in enzyme activity and reduced gill permeability that would be prevalent from freshwater acclimation (Siebers et al., 1982, 1983). Shrimp hemolymph is isotonic to external salinities at about 25 ppt (Castille and Lawrence, 1981; Parado-Estepa et al., 1987) so immediate transfer to higher salinities from low-salinity acclimation water would seem to benefit shrimp in returning hemolymph ion concentrations back to previous, less stressful levels before acclimation.

### 4.4. Importance of PL age and development

Gill development in relation to osmoregulatory ability in penaeids is correlated with PL age (PL-12 to PL-15). For the present study, all PLs used for experiments were purposefully the same age (PL-15) before acclimation began. Smaller animals have a larger surface to volume ratio and in general have higher uptake capacities to balance a larger salt loss per unit weight (Mantel and Farmer, 1983) during hypotonic conditions. Other work has shown the importance of PL age (PL 10–20) in relation to osmoregulation ability when acclimating shrimp from 24 ppt to a salinity of 1 ppt (McGraw et al., 2002). Shrimp at the PL15–20 stage showed significantly higher survival when exposed to low salinities (1 ppt) and higher rate of salinity reduction (47% per h) from 24 to 1 ppt. However, osmoregulatory ability in relation to age and individual ion manipulation by shrimp remains unclear. Vargas-Albores and Ochoa (1992) showed three different size groups (11.0–12.6, 12.7–14.3 and 14.4–16.0 cm) of *P. stylirostris* maintained constant Na\(^+\) concentrations in the hemolymph while K\(^+\) levels and osmolality were significantly different between size groups. All animals were taken from the same location. Larger animals tended to accumulate more K\(^+\) and possess greater osmolality. Potassium and Na\(^+\) tended to increase in a constant ratio with increasing hemolymph osmolality.

### 5. Conclusion

The present study demonstrated that mortality of freshwater acclimated PL-15 *L. vannamei* placed in solutions of various ion concentrations and salinities can be reduced by allowing an extended acclimation time from 48 to 72 h or by increasing time in acclimation water (habituation period) before transfer to strongly divalent solutions of the same salinity. Increasing habituation period did not show an increase in tolerance of shrimp to a change of 30 ppt total salinity as direct transfer of shrimp from freshwater to seawater resulted in high survivals. Solutions with greater monovalent compared to divalent ions showed significantly better survival after successful freshwater acclimation. Increasing acclimation time at lower salinities increased shrimp survival at shorter than recommended freshwater acclimation times (i.e. 48 h).
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