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Effect of Holding and Packing Conditions on Hemolymph Parameters of Freshwater Prawns, *Macrobrachium rosenbergii*, during Simulated Waterless Transport

Jamie G. Skudlarek, Shawn D. Coyle, Leigh Anne Bright[1], and James H. Tidwell

Aquaculture Research Center, Kentucky State University, 103 Athletic Road, Frankfort, Kentucky 40601, USA

Abstract

Preliminary market research has identified an unfulfilled market potential for live freshwater prawn, *Macrobrachium rosenbergii*, in urban areas of the USA and Canada. Truck transport is effective but requires shipment of large numbers of prawns to be economically feasible. Most retail markets are ill-equipped to hold large quantities for subsequent live sales. Air-freight of smaller quantities has potential but water weight limits its application. Shipping of live aquatic animals in waterless environments has been reported for some finfish and crustaceans. This project utilized biochemical characteristics of prawn hemolymph as measures of sublethal stress during simulated transport. Six trials were conducted to evaluate the effects of different variables involving preshipment holding conditions, acclimation prior to shipping, and conditions inside the shipping container. Trial 1 compared animals shipped in water with those in a non-aqueous environment. Trial 2 compared three chilling rates (slow chill, fast chill, and no chill). Trial 3 evaluated different holding protocols including fed versus unfed, reduced pH, increased water hardness, and salinity. Trial 4 evaluated the addition of ammonia scavengers to the transport containers. Trial 5 evaluated the use of carbon dioxide scavengers and an anesthetic, AQUI-S®. Trial 6 used the best results of Trials 2–5 in a combination of "Best Management Practices" (BMP) over extended time periods. Treatments had either three or four replications using Styrofoam boxes, each packed with six individually tagged prawns. Presoaked wood-shavings and ice packs were used to keep the boxes moist and cool. The boxes were then sealed in individual oxygenated plastic bags. Trials 1–5 were conducted for 16 h and Trial 6 had separate boxes which were opened at 16, 24, and 32 h. Baseline hemolymph samples were taken prior to packing and from prawn alive at the end of all trials. Hemolymph variables included pH, pO_2, pCO_2, HCO_3, tCO_2, calcium, ammonia, osmolality, glucose, lactate, total protein, magnesium, calcium, and potassium. Percent survival and weight loss were also measured. Results of Trial 1 indicated that compared to transport in water, non-aqueous environment significantly increased ($P \leq 0.05$) hemolymph levels of CO_2 and ammonia, and significantly reduced levels of oxygen. In Trial 2, survival in the Slow Chill treatment was significantly higher than in the No Chill treatment, although hemolymph characteristics were not impacted. In Trial 3 prawns held in tanks with added salt (17 ppt) had the highest survival and lowest hemolymph concentration of ammonia and partial pressure of CO_2. The ammonia scavengers in Trial 4 had no significant impact on survival or hemolymph variables. The CO_2 scavengers and anesthetic in Trial 5 had no statistically significant impact on survival. The BMP of Trial 6 consisted of, in the following order, holding in 17 ppt marine salt mix, slow chilling, anesthetic (AQUI-S) dip, and adding limewater ($Ca(OH)_2 + H_2O$) to the shipping box as a CO_2 scavenger. In Trial 6, after 32 h of simulated "waterless" transport, prawns in the BMP treatment had significantly higher survival (96%) than prawns in the Control treatment (58%). The BMP prawns also had significantly higher partial pressures of oxygen and lower partial pressures of CO_2 in the hemolymph.

Expansion of freshwater prawn, *Macrobrachium rosenbergii*, production in the southern USA is constrained by marketing limitations. Producers primarily rely on pond-side retail sales of fresh "on ice" product and/or wholesale marketing of processed frozen product to local restaurants and grocers. However, ethnic Asian markets pay a premium for live product

[1] Corresponding author.

compared to frozen or fresh on ice (Coyle et al. 2005a). Preliminary market research has identified an unfulfilled market potential of >50,000 kg/yr for live prawns in urban Asian communities of the USA and Canada. The estimated market demand in the greater New York area alone is approximately 500 kg/wk (Coyle et al. 2005a).

Previous research evaluated methods for transporting live prawns using live-haul tanks on trucks (Coyle et al. 2005a). In subsequent commercial-level verification trials, survival during transport was high but markets could not immediately absorb large quantities and survival during subsequent holding and retail display was poor. Consequently, buyers have indicated a desire for small volumes of live product to be delivered on a frequent basis to eliminate the need for long-term holding. Air-freight of smaller quantities of prawns has that potential but costs associated with "the water weight" limits its application. By air-shipping relatively small volumes of prawns without water (to reduce shipping costs), it may be possible to efficiently supply this market.

Shipping of live aquatic crustaceans in waterless environments has been commercially practiced for many years with crayfish, crabs, and lobsters (Chan 2001; Estrella 2002; Romaire et al. 2005) and with some species of marine shrimp, most notably the Kuruma prawn, *Penaeus japonicus* (Shigueno 1992; Goodrick et al. 1993). The basic principle underlying the technology of live transport of crustaceans out of water is temperature (cold)-induced anesthetization. This involves lowering of the water temperature to just above the lethal low temperature, which results in immobilization and reduced metabolic rate. Currently, this is not a commercial practice for *M. rosenbergii* based on poor survival when removed from water (New 1995; Kubaryk and Harper 2001; Salin 2005).

Previous studies on waterless transport of freshwater prawns measured only survival and weight loss as primary indicators of the effectiveness of variables being evaluated (Kubaryk and Harper 2001; Salin 2005). However, this approach does not provide information on sublethal stressors, which may be the primary contributors to subsequent mortality. In crustaceans, a number of hemolymph parameters have been measured in an effort to indicate stress. These include osmoregulatory capacity (Lignot et al. 1999), acid–base balance, and ion concentrations (Chen and Chen 2003), as well as ammonia, total protein, calcium, and magnesium (Ozbay and Riley 1999). Measurement of these physiological indicators could help focus efforts on the sublethal changes during waterless transport which have the greatest impacts on the animal's health and survival.

To ameliorate or prevent the negative effects of waterless transport, the manipulation of several factors in the steps prior to or during transport may be important. Kubaryk and Harper (2001) reported 15 C to be the optimal temperature for cold anesthetization of freshwater prawns in preparation for waterless transport. The rate of chilling to achieve this transport temperature may also affect transport stress and survival (Salin 2005). Anesthetics can be used to reduce the animal's metabolic rate, thereby reducing stress levels and metabolite production (i.e., ammonia) during handling and transport (Coyle et al. 2005b). Purging animals off feed prior to transport is commonly used in finfish (Jensen 1990). However, Kubaryk and Harper (2001) reported that market-size freshwater prawns fed during the preshipping holding period actually had better transport survival than those held without feeding.

The ability of the animal to osmoregulate can be negatively affected during waterless transport. Acclimation of the animals to their iso-osmotic salinity prior to waterless transport may reduce or delay the onset of stress (Sang and Fotedar 2004). The reported iso-osmotic point for *M. rosenbergii* is 17 ppt (Singh 1980). Ozbay and Riley (1999) stated that American lobsters, *Homarus americanus*, accumulate excess amounts of hemolymph CO_2 when exposed to air, but are able to maintain hemolymph acid–base balance by mobilizing $CaCO_3$ from their exoskeleton. Therefore, holding the animal in a buffering environment prior to packing might increase the animal's ability to maintain its internal acid–base balance.

The effects of different levels of pH in holding tanks on the acid–base balance, osmolality, and ion concentrations of the freshwater prawn have also been evaluated, and results suggest that preconditioning animals in a low pH media may be beneficial (Chen and Chen 2003).

Conditions inside the shipping container itself certainly influence survival of the animal during waterless transport. Ammonia accumulation is suspected of being a major stressor. Ammonia scavengers, such as zeolite, could potentially reduce the accumulation of total ammonia in the animal's body fluids by stripping ammonia from the environment inside the shipping container. However, Kubaryk and Harper (2001) reported that zeolite did not improve survival of the prawns shipped without water, although hemolymph ammonia levels were not measured. Kubaryk and Harper (2001) also suggested that carbon dioxide accumulation in the sealed containers could be a cause of mortality. Compounds such as calcium hydroxide and lithium hydroxide can tie up CO_2 and should be evaluated as a means of improving survival and extending transport times.

The overall goal of this research was to utilize the changes in hemolymph variables to monitor physiological changes during simulated waterless transport to develop a protocol for preconditioning, packing, and shipping live freshwater prawns. Each of the six separate trials addressed specific objectives. The objective of Trial 1 was to determine the impact of a non-aqueous environment on the animal's abilities to carry on basic metabolic functions. The objective of Trial 2 was to evaluate chilling rates prior to packing. The objective of Trial 3 was to evaluate different environmental parameters (pH, hardness, and salinity) to precondition the animals in the holding system prior to packing. The objective of Trial 4 was to evaluate adding ammonia scavengers to the transport container. The objective of Trial 5 was to evaluate adding CO_2 scavengers to the transport container and the use of the anesthetic AQUI-S®. The objective of Trial 6 was to evaluate the impact of combining the positive results from Trials 2–5 into a "Best Management Practices" (BMP) over longer transport periods.

Materials and Methods

Market-size mixed-sex freshwater prawns (mean $\pm$ SD $= 39.8 \pm 12.5$ g; size range $=$ 19.3–76.8 g) were obtained from a local Kentucky producer and held indoors in fiberglass raceways supplied with flow through reservoir water (ambient temperature 22 C) and aeration for $\geq$7 d before conducting experiments. The range of size of animals used is typical of farm-raised prawns in the region. Prawns were fed 2% of their body weight daily until 2 d before each trial, with exception of the "Fed" treatment prawns in Trial 3 which were fed up to the day of packing. Only prawns that appeared healthy with hardened exoskeletons were used for this research.

Water quality variables including temperature, dissolved oxygen, nitrite-nitrogen, total ammonia-nitrogen, and pH were measured daily in the holding tanks to ensure optimal holding conditions. Water temperature and dissolved oxygen were measured using an YSI Model 55 oxygen meter (YSI Industries, Yellow Springs, OH, USA). Total ammonia-nitrogen and nitrite-nitrogen were measured using a DREL 2000 spectrophotometer (Hach Company, Loveland, CO, USA); pH was measured with an electronic pH meter (pH pen, Fisher Scientific, Cincinnati, OH, USA). Unionized ammonia was calculated as a percentage of total ammonia based on temperature and pH according to Boyd (1979).

Protocol was similar in all six trials except the following. Treatments 1–5 were replicated in four Styrofoam containers ($30.5 \times 22.9 \times 20.3$ cm) packed with six prawns each. Trial 6 had three replicates for each time interval. Prior to packing, prawns were individually weighed and tagged. A single ice pack chilled in an ice bath to 2 C was placed in the bottom of each box to maintain temperature near 15 C. Untreated wood (poplar) shavings (Excelsior, Uline®, Waukegan, IL, USA) were soaked in water chilled to 10 C, then placed both below and atop the prawns. After packing, the boxes were sealed in individual plastic bags filled with approximately 20 L of pure oxygen which was injected through a small (1.3 cm) diameter hole drilled in the lid of each box.

All hemolymph samples were taken from the pericardial sinus (Lallier and Truchot 1989; Chen and Chen 2003) using a 1 mL syringe with a 25-gauge needle at room temperature. Approximately 0.30 mL of hemolymph was taken from each prawn and 0.10 mL was used immediately for measuring pH, pCO_2, pO_2, HCO_3, tCO_2, and K^+ using a VetStat blood gas analyzer (IDEXX Laboratories, Westbrook, ME, USA) as used by Ozbay and Riley (1999). The remaining portion of the hemolymph sample (0.20 mL) was mixed with a like amount of a buffered citrate anticoagulant (Cheng et al. 2003) and stored at 4 C for 24 h. It was then tested for glucose, lactate, ammonia, total protein, magnesium, and calcium using a VetTest analyzer (IDEXX Laboratories). Osmolality of the hemolymph was also measured with an osmometer by freezing-point depression method (Model 3320, Advanced Instruments, Inc., Norwood, MA, USA).

Baseline (0-h) values were taken from hemolymph samples of five prawns which were not packed or used in subsequent studies. Individual prawn weights were also recorded for each of these baseline samples. For trials 1–6, at 16 h postpacking, containers were opened and prawn conditions (live, moribund, or dead) and individual weights were recorded. Prawns were classified as live – if gill movement or heart beat was visible and leg movement could be enticed by probing, moribund – if gill movement or heart beat was visible and leg movement could not be enticed by probing, and dead – if there was no visible gill movement or heart beat. Hemolymph samples were taken from at least two live prawns per replicate box and analyzed using the same procedures described for the baseline samples. There appeared to be no difference in hemolymph coagulation between treatments. For Trial 6, there were also boxes opened at 24 and 32 h postpacking. Specific procedures for a specific objective are listed by trial.

Trial 1 compared animals submerged in water (Wet) to those not submerged in water (Dry). Prawns in both treatments were packed as previously described. For the Wet treatment, approximately 4 L of 15 C chilled aerated reservoir water was added so that all prawns were fully submerged. The Dry treatment boxes were packed in moist wood-shavings but without added water.

Trial 2 evaluated three chilling protocols prior to packing. The reported optimum temperature for cold anesthetization of freshwater prawns is 15 ± 1 C (Salin 2005). For the Slow Chill treatment (Slow Chill), 24 prawns were placed in a 380-L tank filled with aerated reservoir water from the holding tank with an initial temperature of 22 C. The tank was slowly chilled (3 C/h) with dechlorinated ice until the temperature of the water reached 15 C and then all prawns were removed from the water and packed. For the Fast Chill treatment (Fast Chill), 24 prawns (six at a time) were placed directly into the already chilled 380-L tank until the animals became motionless (10 sec), at which time they were removed and packed. The No Chill/Control treatment (No Chill) comprised packing of prawns directly removed from the 22 C holding tank.

Trial 3 evaluated the impact of different holding conditions during the 24-h period prior to packing. Fifty animals were evenly divided among five separate 380-L tanks filled with reservoir water (22 C) under the following test conditions: (a) the Control treatment (Control) prawns received no feed and no additions; (b) Fed treatment (Fed) prawns were fed at 2% per pound of body weight; (c) in the Salinity treatment (Salinity), the salinity was increased over 3 h to 17 ppt using a marine premix (Crystal Sea® Marine mix, Marine Enterprises International, Baltimore, MD, USA); (d) in the Low pH treatment (Low pH), pH was lowered from 8 to 5 over the course of 3 h by addition of glacial acetic acid; and (e) in the $CaCO_3$ treatment ($CaCO_3$), calcium carbonate was added to achieve a water hardness of 1000 mg/L.

Trial 4 evaluated three ammonia scavengers added to the transport containers. It was estimated that a prawn, with an average weight of 40 g, produces 0.3 mg of N/g/d (Wickins 1985), so six prawns would produce approximately 63 mg of total ammonia-nitrogen. On the basis of this estimate, in Treatment 1, 42 g

of zeolite (Zeolite) (Aquatic EcoSystems, Inc., Apopka, FL, USA) was placed in the bottom of each box. For Treatment 2, 42 g of activated carbon (Activated Carbon) was added to each box. For Treatment 3, 1.2 mL (according to label directions) of AmQuel Plus® (Kordon LLC, Hayward, CA, USA) was added to each box. In all treatments in Trial 4, 250 mL of water was placed in the bottom of the transport containers to increase contact with the ammonia scavengers.

Trial 5 evaluated the effect of adding carbon dioxide scavengers to the boxes. A commercial lithium hydroxide (LiOH) sheet (ExtendAir CO_2 Absorbent, Micropore Inc., Newark, DE, USA), originally designed for emergency use in submarines, was placed in the box on top of the overlaying wood-shavings. A second treatment evaluated limewater (1 g calcium hydroxide [$Ca(OH)_2$] per 100 mL of H_2O) added to four boxes at a rate of 250 mL per box. This trial also evaluated the use of an anesthetic, AQUI-S at 500 mg/L (AQUI-S New Zealand, Lower Hutt, New Zealand). Prawns in the anesthetic treatment (AQUI-S) were immersed in the anesthetic bath for 10 min and then immediately packed. AQUI-S is undergoing review by the US FDA and is presently not available for sale in the USA.

Trial 6 evaluated a combination of prepacking and packing variables based on the results from Trials 1–5. Some variables from previous trials were included if judged beneficial based on increased survival or a reduction in stress-related metabolites, even if not necessarily statistically significant. Variables combined into a BMP protocol included 17 ppt salinity (marine mix) in the holding tank, a slow chill rate, a prepacking anesthetic (AQUI-S), and the addition of limewater in the shipping box. For Trial 6, prawns were exposed to the anesthetic for 5 after chilling. This treatment combination was compared to a Control treatment of holding in 22 C reservoir water, no chilling, no anesthetic, and no added CO_2 scavenger. Twelve boxes of each of the two treatments (BMP versus Control) were packed to allow evaluation of three simulated transport durations (16, 24, and 32 h). After boxes were opened and samples taken, surviving prawns were placed in a recirculating aquaculture system (RAS) and monitored over a 24-h period to evaluate subsequent successful recovery.

Statistical analyses were conducted using Statistix version 6.0 (Analytical Software, Tallahassee, FL, USA). Differences were considered significant at $P \leq 0.05$ and highly significant at $P \leq 0.01$. For Trial 1, a two-sample t-test (Steel and Torrie 1980) was used to evaluate impacts of transport medium (Wet versus Dry). For Trials 2–5, ANOVA (Steel and Torrie 1980) was used to compare treatment means for chilling rates, holding conditions, ammonia scavengers, and carbon dioxide scavengers. If ANOVA indicated significant treatment differences, Fisher's least significant difference (LSD) test was used to separate means. For Trial 6, a series of two-sample t-tests were used to compare animals held under BMP versus Control at each specific holding time. All percentage and ratio data were arc sin square-root transformed prior to analysis (Zar 1984). However, data are presented in their untransformed form to facilitate interpretation.

Results

The VetStat blood gas analyzer and VetTest analyzer are designed for use with mammals and have not been validated for prawns. Therefore, the exact values reported may not be accurate and only trends may be useable. This equipment has been previously used to measure hemolymph of American lobster, *H. americanus*, when evaluating preshipment conditioning techniques also without validation (Ozbay and Riley 1999). In addition, other brands of "point-of-care" blood analyzers have been used in published research with decapods without validation (Cheng et al. 2003).

Water Quality

Water quality conditions in the holding tanks taken prior to conducting each trial (excluding Trial 3 which involved different holding conditions) averaged (±SD): water temperature, 22 ± 1 C; dissolved oxygen, 8.0 ± 1.0 mg/L;

total ammonia-nitrogen, 0.4 ± 0.2 mg/L; unionized ammonia-nitrogen, 0.05 ± 0.02 mg/L; nitrite-nitrogen, 0.5 ± 0.1 mg/L; pH, 8.0 ± 0.3; alkalinity, 92.4 ± 5.2 mg/L. These conditions are all considered acceptable for freshwater prawns (Boyd and Zimmermann 2000).

Trial 1

After 16 h of simulated transport, there was no significant difference in survival ($P \leq 0.05$) between the two treatments, which averaged 92% overall (Table 1). Prawns in the Dry treatment lost significantly more body weight (3%) compared to those in the Wet treatment (0.5%). Prawns in the Dry treatment had significantly greater hemolymph concentrations of ammonia, potassium, and glucose and partial pressure of carbon dioxide. Dry treatment prawns also had significantly greater levels of osmolality than those in the Wet treatment. All other parameters, including survival, pH, pO_2, HCO_3, tCO_2, lactate, total protein, magnesium, and calcium, were not significantly different.

TABLE 1. *The mean and standard deviation of hemolymph chemistries, survival, weight loss, osmolality, and sample baseline measured in Trial 1 (Wet versus Dry).*[1]

	Treatments		
Parameters	Baseline	Wet	Dry
Survival (%)	n/a	96.0 ± 9.0^a	88.0 ± 16.0^a
Weight loss (g)	n/a	0.5 ± 0.3^b	3.0 ± 0.6^a
pH	7.5 ± 0.0	7.5 ± 0.0^a	7.4 ± 0.2^a
NH_3 (μmol/L)	111 ± 41	128 ± 26^b	1164 ± 483^a
pO_2 (mmHg)	50.3 ± 6.7	52.5 ± 11.8^a	35.0 ± 15.9^a
pCO_2 (mmHg)	12.0 ± 1.8	36.8 ± 4.1^b	64.4 ± 15.9^a
HCO_3 (mmol/L)	9.0 ± 2.0	28.8 ± 3.0^a	36.3 ± 7.9^a
tCO_2 (mmol/L)	9.3 ± 1.2	29.9 ± 3.1^a	38.3 ± 7.8^a
K^+ (mmol/L)	5.0 ± 0.7	4.1 ± 0.3^b	8.2 ± 0.1^a
Ca^{++} (mg/dL)	8.1 ± 0.6	7.0 ± 2.8^a	11.5 ± 5.6^a
Lac (mmol/L)	0.6 ± 0.0[2]	$0.5 \pm 0.0^{a,2}$	3.4 ± 2.9^a
Mg^{++} (mg/dL)	2.6 ± 0.2	4.0 ± 1.0^a	5.5 ± 1.2^a
Glu (mg/dL)	11.8 ± 2.1	13.5 ± 3.1^b	24.3 ± 2.4^a
TP (g/dL)	5.8 ± 1.1	4.7 ± 0.9^a	5.0 ± 0.9^a
Os (mOsm/kg)	465.0 ± 4.1	467.5 ± 3.6^b	473.9 ± 2.9^a

n/a = not applicable; TP = total protein.

[1]Treatment means within a row followed by a different letter are significantly different ($P \leq 0.05$) by Student's two-sample *t*-test. Baseline values are presented for comparative purposes and were not included in statistical analyses.

[2]Missing or insufficient data.

Trial 2

Survival in the Slow Chill treatment (92%) was significantly greater than in the No Chill treatment (67%) (Table 2). Survival in the Fast Chill treatment was intermediate (75%). Weight loss during the 16-h study period was significantly less in the Fast Chill treatment than in the Slow Chill or No Chill treatments. Hemolymph pH was significantly greater in the No Chill treatment than in the Slow Chill treatment with the Fast Chill treatment being intermediate. There were no significant differences between treatments in any of the other measured hemolymph variables.

Trial 3

There were large differences in final survivals among treatments involving prepacking holding conditions (Table 3). Measured water quality conditions in the holding tanks are given in Table 4. After 16-h simulated waterless transport, survival was significantly greater in prawns held in 17 ppt salinity (Salinity) (79%) than in those held at elevated hardness ($CaCO_3$) (29%) or in the Fed treatment (50%). Hemolymph ammonia levels were significantly reduced in the Salinity treatment (768 μmol/L) compared to the $CaCO_3$ treatment (1315 μmol/L), Fed treatment (1430 μmol/L), or Unfed/Control (1183 μmol/L) but not significantly different from the Low pH treatment (842 μmol/L). Hemolymph pCO_2 levels were significantly lower in the Salinity treatment (36 mmHg) than in other treatments, which did not differ significantly from each other. Total carbon dioxide levels in the hemolymph were also significantly lower in the Salinity treatment (27 mmol/L) than in Unfed/Control (38 mmol/L), Fed (40 mmol/L), and Low pH (42 mmol/L) treatments, but not significantly different from the $CaCO_3$ treatment (32 mmol/L). Hemolymph glucose concentrations were significantly higher in the Unfed/Control and Low pH treatments (48 and 49 mg/dL, respectively) than in the $CaCO_3$ and Fed treatments (34 and 36 mg/dL, respectively). Total protein levels in the hemolymph were significantly higher in the Low pH

TABLE 2. *The mean and standard deviation of hemolymph chemistries, survival, weight loss, osmolality, and sample baseline measured in Trial 2 (Slow Chill versus Fast Chill versus No Chill).*[1]

Parameters	Treatments			
	Baseline	Slow chill	Fast chill	No chill
Survival (%)	n/a	92.0 ± 10.0^{a}	75.0 ± 9.0^{ab}	67.0 ± 14.0^{b}
Weight loss (g)	n/a	2.7 ± 0.3^{a}	1.9 ± 0.2^{b}	2.4 ± 0.2^{a}
pH	7.4 ± 0.1	7.5 ± 0.1^{b}	7.5 ± 0.0^{ab}	7.6 ± 0.0^{a}
NH_3 (μmol/L)	185 ± 10	905 ± 186^{a}	865 ± 85^{a}	927 ± 130^{a}
pO_2 (mmHg)	43.3 ± 4.0	24.0 ± 8.2^{a}	22.4 ± 1.4^{a}	17.5 ± 2.4^{a}
pCO_2 (mmHg)	16.5 ± 3.7	55.5 ± 10.7^{a}	46.4 ± 4.7^{a}	44.9 ± 9.1^{a}
HCO_3 (mmol/L)	10.6 ± 1.6	35.8 ± 4.3^{a}	35.5 ± 2.4^{a}	44.0 ± 4.0^{a}
tCO_2 (mmol/L)	11.2 ± 1.7	37.5 ± 4.5^{a}	36.9 ± 2.5^{a}	36.7 ± 4.3^{a}
K^+ (mmol/L)	5.6 ± 0.8	8.2 ± 1.0^{a}	7.2 ± 1.3^{a}	8.0 ± 1.0^{a}
Ca^{++} (mg/dL)	9.6 ± 2.4	21.5 ± 8.5^{a}	19.9 ± 2.2^{a}	19.4 ± 4.8^{a}
Lac (mmol/L)	0.6 ± 0.2	6.3 ± 4.4^{a}	2.5 ± 2.6^{a}	2.4 ± 0.9^{a}
Mg^{++} (mg/dL)	4.5 ± 1.5	5.9 ± 0.5^{a}	5.5 ± 0.9^{a}	5.4 ± 0.8^{a}
Glu (mg/dL)	20.0 ± 6.5	43.8 ± 13.3^{a}	37.5 ± 9.6^{a}	44.0 ± 23.2^{a}
TP (g/dL)	6.3 ± 1.6	5.9 ± 0.5^{a}	6.0 ± 1.2^{a}	5.1 ± 1.5^{a}
Os (mOsm/kg)	475.5 ± 4.4	481.9 ± 4.6^{a}	475.8 ± 4.5^{a}	479.1 ± 6.0^{a}

n/a = not applicable; TP = total protein.

[1]Treatment means within a row followed by a different letter are significantly different ($P \leq 0.05$) by Student's ANOVA. Baseline values are presented for comparative purposes only and were not included in statistical analyses.

and Fed treatments than in the Salinity and Unfed/Control treatments. Osmolality was significantly higher in the Salinity treatment than in any other treatment.

Trial 4

There was no significant difference in survival among the treatments in Trial 4, which averaged 81% overall (Table 5). Weight loss in the Zeolite treatment was significantly less than in the AmQuel plus, Activated Carbon, and Control treatments. Hemolymph calcium concentration was also significantly lower in the Zeolite treatment. Treatment variables had little impact on other measured hemolymph parameters.

Trial 5

Survival was mathematically higher in the AQUI-S (67%) and the Limewater (54%) treatments than the LiOH (38%) and Control (42%) treatments although differences were not statistically significant (Table 6). This was at least partially due to high within-treatment variation. Hemolymph ammonia concentration was significantly lower in the AQUI-S treatment than in the LiOH and Control treatments, but not significantly different than in the Limewater treatment. The level of lactate in hemolymph was significantly higher in the LiOH treatment (17.1 mmol/L) compared to AQUI-S, Limewater, and Control treatments (7.1, 9.4, and 8.1 mmol/L, respectively). Hemolymph calcium was significantly higher in the LiOH treatment. Total HCO_3 and tCO_2 were significantly lower in the LiOH treatment than in the AQUI-S, Limewater, and Control treatments. Hemolymph pCO_2 was significantly lower in the Limewater treatment (34.9 mmHg) than in the Control treatment (48.4 mmHg) and AQUI-S (45.5 mmHg) with the LiOH treatment being not significantly different from the other treatments.

Trial 6

In Trial 6, at 16-h postpacking there was no significant difference between treatments in terms of survival, which averaged >90% overall (Table 7). There was also no significant difference between the BMP and Control treatments in terms of weight loss or any of the hemolymph variables except osmolality which was significantly higher in the BMP treatment than in the Control. At 24-h postpacking,

TABLE 3. *The mean and standard deviation of hemolymph chemistries, survival, weight loss, osmolality, and sample baseline measured in Trial 3 (Low pH versus $CaCO_3$ versus Salinity versus Fed versus Control).*[1]

	Treatments					
Parameters	Baseline	Low pH	$CaCO_3$	Salinity	Fed	Control
Survival (%)	n/a	71.0 ± 8.0^{ab}	29.0 ± 21.0^{c}	79.0 ± 16.0^{a}	50.0 ± 27.0^{bc}	67.0 ± 13.0^{ab}
Weight loss (g)	n/a	2.1 ± 0.5^{a}	2.1 ± 0.1^{a}	1.9 ± 0.3^{a}	1.8 ± 0.3^{a}	1.8 ± 0.3^{a}
pH	7.4 ± 0.1	7.6 ± 0.1^{a}	7.4 ± 0.0^{a}	7.5 ± 0.1^{a}	7.5 ± 0.1^{a}	7.5 ± 0.1^{a}
NH_3 (μmol/L)	255 ± 45	842 ± 414^{bc}	1315 ± 131^{a}	768 ± 85^{c}	1430 ± 235^{a}	1183 ± 76^{ab}
pO_2 (mmHg)	44.0 ± 7.3	13.1 ± 0.9^{a}	$14.0 \pm 0.0^{a,2}$	21.4 ± 6.6^{a}	12.8 ± 0.4^{a}	15.0 ± 0.7^{a}
pCO_2 (mmHg)	11.5 ± 0.6	47.0 ± 4.4^{a}	$53.0 \pm 0.0^{a,2}$	35.9 ± 3.3^{b}	53.3 ± 7.9^{a}	52.0 ± 8.9^{a}
HCO_3 (mmol/L)	7.1 ± 1.1	40.5 ± 2.1^{a}	$30.3 \pm 0.0^{bc,2}$	25.9 ± 4.0^{c}	38.3 ± 2.7^{ab}	36.3 ± 5.0^{ab}
tCO_2 (mmol/L)	7.4 ± 1.1	41.9 ± 2.0^{a}	$32.0 \pm 0.0^{bc,2}$	27.0 ± 4.0^{c}	40.0 ± 2.6^{ab}	37.9 ± 4.9^{ab}
K^+ (mmol/L)	5.4 ± 0.8	8.7 ± 0.9^{a}	$0.0 \pm 0.0^{a,2}$	6.6 ± 1.0^{a}	$8.4 \pm 0.0^{a,2}$	8.7 ± 1.1^{a}
Ca^{++} (mg/dL)	8.2 ± 1.1	24.2 ± 3.7^{a}	20.2 ± 2.9^{a}	21.2 ± 1.6^{a}	27.6 ± 5.8^{a}	20.8 ± 4.1^{a}
Lac (mmol/L)	1.2 ± 0.3	2.9 ± 0.5^{a}	5.8 ± 5.4^{a}	6.7 ± 7.5^{a}	10.8 ± 4.4^{a}	7.4 ± 6.2^{a}
Mg^{++} (mg/dL)	2.3 ± 0.6	5.6 ± 0.5^{a}	5.4 ± 1.0^{a}	6.4 ± 0.3^{a}	7.0 ± 0.9^{a}	6.0 ± 1.1^{a}
Glu (mg/dL)	22.3 ± 5.1	48.9 ± 8.5^{a}	33.7 ± 5.7^{b}	37.0 ± 8.5^{ab}	36.3 ± 3.9^{b}	48.4 ± 10.5^{a}
TP (g/dL)	5.3 ± 1.0	5.7 ± 0.6^{a}	5.4 ± 0.8^{ab}	4.1 ± 0.7^{c}	5.8 ± 0.7^{a}	4.4 ± 1.0^{bc}
Os (mOsm/kg)	484.8 ± 9.6	464.8 ± 6.9^{c}	471.0 ± 6.5^{bc}	524.4 ± 10.8^{a}	482.8 ± 5.3^{b}	477.6 ± 4.5^{b}

n/a = not applicable; TP = total protein.

[1]Treatment means within a row followed by a different letter are significantly different ($P \leq 0.05$) by Student's ANOVA. Baseline values are presented for comparative purposes only and were not included in statistical analyses.

[2]Missing or insufficient data.

TABLE 4. *Water quality results of Trial 3 (Low pH versus $CaCO_3$ versus Salinity versus Fed versus Control) in tanks involving prepacking holding condition.*

	Holding tanks				
Parameters	Low pH	$CaCO_3$	Salinity	Fed	Control
Temperature (C)	20.3	20.1	20.3	21.7	20.3
DO (mg/L)	7.7	8.6	7.9	7.3	8.6
pH	5.0	7.74	8.0	7.8	8.0
Salinity (ppt)	0.20	0.20	16.9	0.20	0.20
Nitrite (mg/L)	0.115	0.085	0.127	0.346	0.189
Total ammonia (mg/L)	2.08	1.35	1.04	0.96	1.80
Unionized ammonia (mg/L)	0.000	0.027	0.041	0.026	0.070
Alkalinity (mg/L)	31	990	163	94	100
Total hardness (mg/L)	189	1126	2673	176	167

DO = dissolved oxygen.

survival was significantly higher in the BMP treatment (88%) than in the Control (58%) (Table 8). However, there were no significant differences in weight loss or any of the measured hemolymph variables. At 32-h postpacking, survival was again significantly higher in the BMP treatment (96%) than in the Control treatment (58%) (Table 9). Oxygen levels in the hemolymph of prawns packed under the BMP protocol (28 mmHg) were significantly higher than those in the Control (16 mmHg), while pCO_2, tCO_2, and HCO_3 were significantly lower in the BMP treatment than in the Control. Figure 1 illustrates survival and hemolymph concentrations of ammonia, carbon dioxide, and oxygen from animals from the two treatments at the end of three periods of simulated transport (16, 24, and 32 h).

At the end of Trial 6, all surviving prawns at 16, 24, and 32-h postpacking, including those from which hemolymph was drawn, were placed in an RAS to observe recovery and subsequent survival. After 16 h of simulated transport, Control prawns had a 68% recovery

TABLE 5. *The mean and standard deviation of hemolymph chemistries, survival, weight loss, osmolality, and sample baseline measured in Trial 4 (Zeolite versus Activated Carbon versus Amquel versus Control).*[1]

	Treatments				
Parameters	Baseline	Zeolite	Activated carbon	Amquel	Control
Survival (%)	n/a	83.0 ± 2.0^{a}	79.0 ± 8.0^{a}	79.0 ± 8.0^{a}	83.0 ± 13.0^{a}
Weight loss (g)	n/a	1.2 ± 0.1^{b}	1.6 ± 0.2^{a}	1.7 ± 0.3^{a}	1.9 ± 0.2^{a}
pH	7.3 ± 0.1	7.5 ± 0.0^{a}	7.5 ± 0.1^{a}	7.5 ± 0.0^{a}	7.5 ± 0.0^{a}
NH_3 (µmol/L)	212 ± 28	983 ± 260^{a}	1138 ± 198^{a}	1219 ± 162^{a}	1050 ± 102^{a}
pO_2 (mmHg)	45.5 ± 7.5	25.3 ± 8.7^{a}	14.9 ± 3.0^{a}	20.1 ± 8.0^{a}	18.9 ± 4.6^{a}
pCO_2 (mmHg)	16.5 ± 3.9	56.3 ± 3.5^{a}	56.1 ± 3.4^{a}	50.9 ± 5.2^{a}	52.6 ± 2.2^{a}
HCO_3 (mmol/L)	8.2 ± 0.9	38.5 ± 2.4^{a}	42.4 ± 3.5^{a}	37.9 ± 4.0^{a}	40.4 ± 0.2^{a}
tCO_2 (mmol/L)	8.6 ± 1.0	40.3 ± 2.4^{a}	44.2 ± 3.4^{a}	39.5 ± 4.1^{a}	42.0 ± 0.2^{a}
K^+ (mmol/L)	5.6 ± 1.3	8.1 ± 1.2^{a}	8.2 ± 1.1^{a}	7.6 ± 1.9^{a}	7.7 ± 0.3^{a}
Ca^{++} (mg/dL)	8.7 ± 1.0	18.5 ± 5.8^{b}	26.2 ± 1.9^{a}	24.1 ± 3.5^{a}	21.5 ± 1.4^{ab}
Lac (mmol/L)	1.4 ± 0.9	3.7 ± 4.2^{a}	6.8 ± 3.5^{a}	7.6 ± 6.1^{a}	2.2 ± 1.2^{a}
Mg^{++}(mg/dL)	1.2 ± 0.8	4.6 ± 0.6^{a}	5.7 ± 0.8^{a}	5.6 ± 1.2^{a}	5.3 ± 0.6^{a}
Glu (mg/dL)	12.6 ± 9.2	30.3 ± 4.8^{a}	45.5 ± 7.0^{a}	40.8 ± 17.9^{a}	43.5 ± 9.5^{a}
TP (g/dL)	5.2 ± 1.3	5.0 ± 0.7^{a}	4.4 ± 0.6^{a}	4.5 ± 0.5^{a}	4.9 ± 0.5^{a}
Os (mOsm/kg)	470.5 ± 13.8	471.9 ± 4.5^{a}	473.4 ± 2.2^{a}	474.5 ± 10.5^{a}	479.8 ± 6.0^{a}

n/a = not applicable; TP = total protein.

[1]Treatment means within a row followed by a different letter are significantly different ($P \leq 0.05$) by Student's ANOVA. Baseline values are presented for comparative purposes only and were not included in statistical analyses.

TABLE 6. *The mean and standard deviation of hemolymph chemistries, survival, weight loss, osmolality, and sample baseline measured in Trial 5 (AQUI-S® versus LiOH sheets versus Limewater versus Control).*[1]

	Treatments				
Parameters	Baseline	AQUI-S	LiOH	Limewater	Control
Survival (%)	n/a	67.0 ± 2.0^{a}	38.0 ± 21.0^{a}	54.0 ± 25.0^{a}	42.0 ± 10.0^{a}
Weight loss (g)	n/a	2.0 ± 0.3^{a}	1.8 ± 0.3^{a}	2.1 ± 0.7^{a}	2.2 ± 0.3^{a}
pH	7.5 ± 0.1	7.5 ± 0.0^{a}	7.3 ± 0.2^{a}	7.5 ± 0.1^{a}	7.5 ± 0.1^{a}
NH_3 (µmol/L)	854 ± 514	816 ± 302^{c}	1457 ± 243^{a}	1082 ± 189^{bc}	1221 ± 217^{ab}
pO_2 (mmHg)	41.3 ± 10.3	12.3 ± 2.5^{a}	22.8 ± 14.0^{a}	13.0 ± 3.0^{a}	13.3 ± 2.1^{a}
pCO_2 (mmHg)	15.0 ± 1.7	45.5 ± 4.1^{a}	41.5 ± 7.7^{ab}	34.9 ± 4.2^{b}	48.4 ± 5.9^{a}
HCO_3 (mmol/L)	9.4 ± 1.9	35.3 ± 5.3^{ab}	21.7 ± 8.5^{c}	27.5 ± 3.2^{bc}	37.8 ± 2.9^{a}
tCO_2 (mmol/L)	7.2 ± 5.2	36.7 ± 5.4^{ab}	23.0 ± 8.3^{c}	28.5 ± 3.2^{bc}	39.3 ± 2.7^{a}
K^+ (mmol/L)	4.2 ± 1.2	7.2 ± 0.8^{a}	$6.9 \pm 0.0^{a,2}$	7.9 ± 0.5^{a}	8.1 ± 4.8^{a}
Ca^{++} (mg/dL)	9.6 ± 1.4	22.3 ± 4.7^{b}	32.5 ± 4.8^{a}	26.0 ± 1.6^{b}	24.1 ± 3.9^{b}
Lac (mmol/L)	1.5 ± 0.8	7.1 ± 3.5^{b}	17.1 ± 6.9^{a}	9.4 ± 2.0^{b}	8.1 ± 4.8^{b}
Mg^{++} (mg/dL)	2.0 ± 0.6	5.2 ± 1.7^{a}	5.4 ± 0.9^{a}	5.7 ± 0.3^{a}	5.7 ± 1.0^{a}
Glu (mg/dL)	6.8 ± 2.1	48.1 ± 12.9^{a}	45.9 ± 13.9^{a}	47.0 ± 8.0^{a}	45.3 ± 15.4^{a}
TP (g/dL)	5.3 ± 1.1	4.5 ± 0.2^{a}	4.0 ± 1.0^{a}	5.0 ± 0.4^{a}	4.9 ± 0.8^{a}
Os (mOsm/kg)	453.0 ± 24.8	470.6 ± 6.5^{a}	485.5 ± 14.4^{a}	477.8 ± 7.4^{a}	472.5 ± 7.8^{a}

n/a = not applicable; TP = total protein.

[1]Treatment means within a row followed by a different letter are significantly different ($P \leq 0.05$) by Student's ANOVA. Baseline values are presented for comparative purposes only and were not included in statistical analyses.

[2]Missing or insufficient data.

rate while BMP prawns had 70% recovery rate. After 24 h, Control prawns had a 21% recovery rate while BMP prawns had 48% recovery rate. After 32 h, Control prawns had a 0% recovery rate while BMP prawns had 43% recovery rate. While subsequent recovery would not be important for immediate retail sales, it could be important if these techniques were utilized for other applications such as prolonged live display or broodstock shipping.

TABLE 7. *The mean and standard deviation of hemolymph chemistries, survival, weight loss, osmolality, and sample baseline measured in Trial 6 (BMP versus Control) unpacked at 16 h.*[1]

	Treatments		
Parameters	Baseline	BMP	Control
Survival (%)	n/a	96.0 ± 9.0[a]	92.0 ± 10.0[a]
Weight loss (g)	n/a	2.2 ± 0.5[a]	2.0 ± 0.4[a]
pH	7.5 ± 0.0	7.6 ± 0.0[a]	7.6 ± 0.0[a]
NH_3 (μmol/L)	372 ± 76	917 ± 225[a]	1060 ± 182[a]
pO_2 (mmHg)	42.0 ± 3.2	32.5 ± 8.7[a]	23.1 ± 8.4[a]
pCO_2 (mmHg)	11.3 ± 1.9	35.0 ± 5.8[a]	41.6 ± 2.0[a]
HCO_3 (mmol/L)	8.0 ± 1.6	28.7 ± 4.1[a]	33.9 ± 1.4[a]
tCO_2 (mmol/L)	8.4 ± 1.6	29.7 ± 4.3[a]	35.1 ± 1.4[a]
K^+ (mmol/L)	4.4 ± 0.8	6.2 ± 1.8[a]	7.2 ± 0.7[a]
Ca^{++} (mg/dL)	8.6 ± 1.1	15.5 ± 0.4[a]	17.4 ± 1.9[a]
Lac (mmol/L)	0.7 ± 0.2	1.7 ± 1.3[a]	2.5 ± 0.9[a]
Mg^{++} (mg/dL)	2.4 ± 0.6	5.2 ± 0.9[a]	5.5 ± 0.6[a]
Glu (mg/dL)	23.8 ± 3.9	26.1 ± 8.2[a]	26.1 ± 6.3[a]
TP (g/dL)	5.9 ± 0.4	4.5 ± 1.3[a]	4.9 ± 0.4[a]
Os (mOsm/kg)	460.5 ± 9.0	478.5 ± 5.3[a]	467.8 ± 5.3[b]

BMP = Best Management Practices; n/a = not applicable; TP = total protein.

[1]Treatment means within a row followed by a different letter are significantly different ($P \leq 0.05$) by Student's two-sample *t*-test. Baseline values are presented for comparative purposes only and were not included in statistical analyses.

TABLE 8. *The mean and standard deviation of hemolymph chemistries, survival, weight loss, osmolality, and sample baseline measured in Trial 6 (BMP versus Control) unpacked at 24 h.*[1]

	Treatments		
Parameters	Baseline	BMP	Control
Survival (%)	n/a	88.0 ± 16.0[a]	58.0 ± 17.0[b]
Weight loss (g)	n/a	2.6 ± 0.2[a]	2.3 ± 0.7[a]
pH	7.5 ± 0.0	7.6 ± 0.0[a]	7.5 ± 0.1[a]
NH_3 (μmol/L)	372 ± 76	1011 ± 204[a]	1167 ± 41[a]
pO_2 (mmHg)	42.0 ± 3.2	27.4 ± 9.6[a]	19.3 ± 9.0[a]
pCO_2 (mmHg)	11.3 ± 1.9	35.5 ± 8.2[a]	47.1 ± 8.8[a]
HCO_3 (mmol/L)	8.0 ± 1.6	28.8 ± 7.4[a]	35.6 ± 6.1[a]
tCO_2 (mmol/L)	8.4 ± 1.6	29.9 ± 7.6[a]	37.1 ± 6.2[a]
K^+ (mmol/L)	4.4 ± 0.8	7.0 ± 2.1[a]	8.0 ± 1.5[a]
Ca^{++} (mg/dL)	8.6 ± 1.1	17.0 ± 2.8[a]	19.9 ± 3.7[a]
Lac (mmol/L)	0.7 ± 0.2	2.1 ± 2.4[a]	5.3 ± 3.8[a]
Mg^{++} (mg/dL)	2.4 ± 0.6	4.6 ± 1.1[a]	5.8 ± 0.8[a]
Glu (mg/dL)	23.8 ± 3.9	37.0 ± 12.5[a]	28.5 ± 11.2[a]
TP (g/dL)	5.9 ± 0.4	4.6 ± 0.7[a]	4.7 ± 0.9[a]
Os (mOsm/kg)	460.5 ± 9.0	480.8 ± 11.0[a]	467.5 ± 13.8[a]

BMP = Best Management Practices; n/a = not applicable; TP = total protein.

[1]Treatment means within a row followed by a different letter are significantly different ($P \leq 0.05$) by Student's two-sample *t*-test. Baseline values are presented for comparative purposes only and were not included in statistical analyses.

Discussion

The results of this research demonstrate stress-induced changes in the hemolymph of freshwater prawns exposed to simulated conditions of waterless transport. The stress response is a series of coordinated physiological reactions increasing an organism's capacity to maintain homeostasis during physical or environmental stress. The general stress response induced by environmental changes, handling, and emersion associated with the procedures of collection and simulated waterless transport are common to all trials reported in this article and are evident by comparing baseline values to final values. However, as these physiological changes are basically common to all treatments, the data are discussed only in terms of treatment differences.

A number of studies have shown that various hemolymph components are useful as indicators of stress in decapod crustaceans. Hyperglycemia is a typical stress response to several environmental stressors including emersion, hypoxia, and cold shock (Kuo and Yang 1999; Durand et al. 2000). Increased hemolymph lactate concentrations have been reported following periods of exercise or emersion and indicate a switch to anaerobic metabolism (Johnson and Uglow 1985; Bergmann et al. 2001). Increased hemolymph ammonia following emersion occurs as elimination through the gills is impaired (Vermeer 1987; Hagerman et al. 1990; Paterson and Spanoghe 1997; Schmitt and Uglow 1997). Low pH in crustacean hemolymph has been reported following emersion as a result of respiratory and metabolic acidosis due to an increase of lactate and bicarbonate-carbonic acid during anaerobic metabolism (Hill et al. 1991; Bergmann et al. 2001). Changes in protein and amino acid content of crustacean hemolymph have been reported to occur following cold acclimation and changes in osmotic concentration (Chen et al. 1994; Issartel et al. 2005). Although the

TABLE 9. *The mean and standard deviation of hemolymph chemistries, survival, weight loss, osmolality, and sample baseline measured in Trial 6 (BMP versus Control) unpacked at 32 h.*[1]

Parameters	Treatments		
	Baseline	BMP	Control
Survival (%)	n/a	96.0 ± 9.0[a]	58.0 ± 22.0[b]
Weight loss (g)	n/a	2.4 ± 0.5[a]	1.9 ± 0.7[a]
pH	7.5 ± 0.0	7.6 ± 0.0[a]	7.6 ± 0.0[a]
NH_3 (µmol/L)	372 ± 76	1126 ± 110[a]	1150 ± 53[a]
pO_2 (mmHg)	42.0 ± 3.2	28.3 ± 6.0[a]	15.5 ± 5.4[b]
pCO_2 (mmHg)	11.3 ± 1.9	43.4 ± 5.3[b]	55.5 ± 5.4[a]
HCO_3 (mmol/L)	8.0 ± 1.6	37.5 ± 6.4[b]	50.5 ± 3.0[a]
tCO_2 (mmol/L)	8.4 ± 1.6	38.8 ± 6.6[b]	52.1 ± 3.1[a]
K^+ (mmol/L)	4.4 ± 0.8	7.9 ± 0.7[a]	8.5 ± 0.8[a]
Ca^{++} (mg/dL)	8.6 ± 1.1	16.0 ± 1.4[a]	19.4 ± 4.8[a]
Lac (mmol/L)	0.7 ± 0.2	1.4 ± 0.6[a]	4.3 ± 2.0[a]
Mg^{++} (mg/dL)	2.4 ± 0.6	4.8 ± 0.9[a]	5.9 ± 0.5[a]
Glu (mg/dL)	23.8 ± 3.9	36.0 ± 6.8[a]	39.0 ± 14.9[a]
TP (g/dL)	5.9 ± 0.4	4.3 ± 0.8[a]	4.4 ± 1.1[a]
Os (mOsm/kg)	460.5 ± 9.0	481.8 ± 7.6[a]	472.6 ± 6.7[a]

BMP = Best Management Practices; n/a = not applicable; TP = total protein.

[1]Treatment means within a row followed by a different letter are significantly different ($P \leq 0.05$) by Student's two-sample *t*-test. Baseline values are presented for comparative purposes only and were not included in statistical analyses.

analytical equipment used in these trials has not been validated for use with crustaceans, the trends and results are logical based on the previous research.

Results from Trial 1 indicate that the oxygen concentrations in the hemolymph of prawns in the Wet and Dry treatments were similar but there were large differences in hemolymph concentrations of ammonia and carbon dioxide. These data suggest that transport in a moist air environment appears to create more of an inability to diffuse waste products (such as ammonia and carbon dioxide) out of the hemolymph than an inability to absorb oxygen into the hemolymph.

Many crustaceans can tolerate fairly long periods of air exposure if the environment is kept moist and well oxygenated (Estrella 2002). Exposure to long-term hypoxia may lead to an increase in oxygen-carrying capacity in *M. rosenbergii* (Chen and Kou 1998). When prawns were held in water with low oxygen content, hemolymph pH and pCO_2 increased significantly in 6 h, but returned to normal values after 24 h (Cheng et al. 2003). The present study indicates no increase in hemolymph pH and a 33% increase in hemolymph pCO_2 in prawns held in air versus those held in water. Even more pronounced was the increase in ammonia content in the hemolymph. These findings are likely explained by the inability of the gills, which evolved to function (gas and excretory exchange) in water, to function efficiently in another media such as air (Petersen et al. 1974).

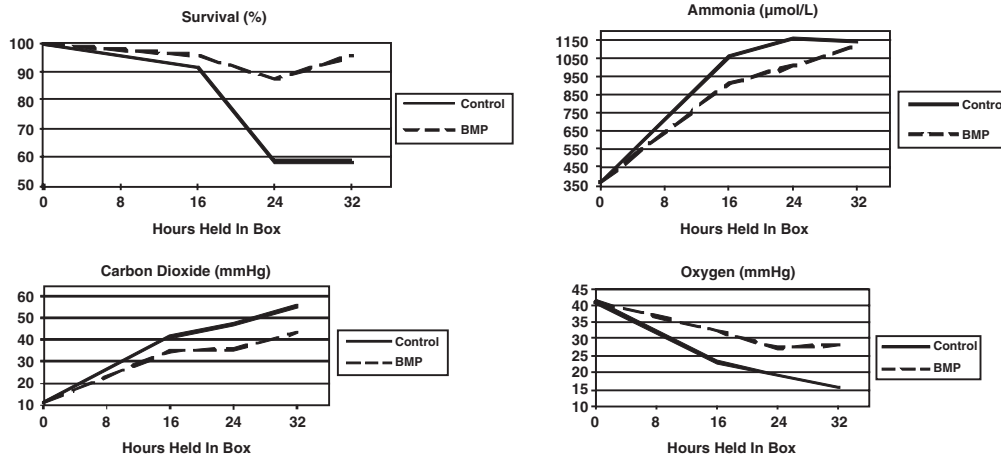

FIGURE 1. *The survival (%), and hemolymph concentrations of ammonia, carbon dioxide, and oxygen of BMP versus Control for all three packing times (16, 24, and 32 h) in Trial 6. BMP = Best Management Practices.*

The greater weight loss and increased osmolality of prawns held in air are probably due to dehydration. Much of the water/weight loss is likely explained by physical loss of water trapped within the brachial chamber during prolonged aerial exposure. However, disruption of normal physiological functions could also have played a role. Crayfish found in fresh or occasionally brackish waters are subject to constant ion loss and water gain because of the hypo-osmotic, hypo-ionic environment (McMahon 2002). Excess water is removed via renal organs, and ion loss is controlled or corrected by active ion uptake across permeable surfaces (gills) and re-uptake of ions from urine (McMahon 2002). Elevated potassium levels in the hemolymph of dry-transport prawns could be explained by the inability to perform these functions normally, that is, potassium is not being exchanged for sodium through branchial epithelial cells.

Chilling rate prior to packing had little effect on measured hemolymph variables but did have a relatively large impact on survival. Animals which were not chilled prior to packing had much lower survival rates (67%) than those which were gradually (>2 h) acclimated (Slow Chill: 92%) to the shipping temperature. Rapid changes in temperature can have a large impact on enzyme activity and function. The difference in survival, with no sublethal changes in hemolymph chemistry between treatments, would likely indicate a direct impact on cell function rather than representing a generalized stress condition which should have produced measurable changes in hemolymph parameters between treatments. Samet et al. (1996) found that long precooling periods in water prolonged the survival of the Kuruma prawn during 14 C air exposure, possibly allowing longer shipping times.

Results of Trial 3 manipulating the holding environment prior to packing appear to indicate that holding the animals at a salinity iso-osmotic with the normal hemolymph concentration is an advantageous conditioning step. Animals held at 17 ppt for 24 h prior to packing had the highest survival (79%), lowest hemolymph ammonia concentration (768 μmol/L), highest hemolymph oxygen concentration (21 mmHg), and lowest hemolymph pCO_2 concentration (36 mmHg). This likely indicates reduced metabolic demands to maintain homeostasis. This agrees with Sang and Fotedar (2004), who found that the western king shrimp, *Penaeus latisuleatus*, and brown tiger shrimp, *Penaeus esculentus*, expended less energy for osmoregulation at 30–34 ppt (their calculated iso-osmotic points). However, increasing the levels of calcium carbonate appeared to have a detrimental effect, resulting in the lowest survival (29%) and relatively high pCO_2 and ammonia in the hemolymph. This differs from the findings of Ozbay and Riley (1999), who reported that increasing calcium carbonate concentrations did not create large changes in hemolymph chemistries.

Ammonia scavengers added to shipping boxes in Trial 4 had little impact on prawn survival or hemolymph characteristics. This agrees with Kubaryk and Harper (2001), who found that the use of zeolite did not improve survival of prawns under simulated waterless shipping conditions. This would be consistent with an inability of prawn, when out of water, to eliminate waste products from the hemolymph into the box environment where it could be absorbed by the ammonia scavengers.

In Trial 5, the lowest hemolymph ammonia concentration and highest survival was in the prawns treated with the anesthetic, Aqui-S. Compared to the "Control," the use of limewater significantly lowered carbon dioxide levels in the prawn hemolymph. However, percent survival was not significantly different when compared to the "Control." The lowest survival rate occurred in the "LiOH" treatment (38%), which also had the highest level of ammonia in the hemolymph. The "LiOH" treatment had the highest level of hemolymph oxygen (23 mmHg) which could correlate with the significantly higher levels of calcium and lactate. McMahon (2002) states that an increase in hemolymph ions, particularly calcium, urate, and lactate, increases oxygen binding in freshwater crayfish.

The combination of pretransport holding conditions and transport conditions combined

into a BMP protocol did not increase survival at the end of 16 h of simulated transport. However, when simulated transport conditions were extended to 24 h, survival was higher in the BMP treatment (88%) than in the Control (58%). After 32 h of simulated transport, survival was much higher in the BMP treatment (96%) than in the Control (58%), which had lower concentrations of oxygen in the hemolymph and higher levels of carbon dioxide. Hemolymph levels of HCO_3 were also higher in the Control prawns, likely representing a response to buffer changes in critical hemolymph pH, which would tend to drop at higher CO_2 levels.

When the results of the three different holding times for Trial 6 (BMP versus Control) are combined into composite graphs (Fig. 1), it illustrates the periods when the most rapid and important changes were taking place over the 32-h period. Most mortality in the Control animals occurred between 16 and 24 h postpacking. The precursors to this mortality were major increases in hemolymph ammonia and carbon dioxide and decreases in oxygen concentrations during the first 16 h. The BMP protocol slowed the accumulation of ammonia and carbon dioxide and the depletion of oxygen, over the entire 32-h period (Fig. 1).

Conclusion

The high survival of the BMP treatment (96%) after 32 h indicates the potential for "just in time" delivery of live freshwater prawns into major metropolitan markets. These data indicate that there is a potential of shipping live freshwater prawns in a waterless environment for up to at least 32 h. However, based on relatively poor recovery (43% after 32 h) it is suggested that live product should be delivered and sold as soon as it is received to market. The study also demonstrates that proper procedures both prior to and during shipping are important to transport success. It should be emphasized that the prawns used in these experiments were in good condition and had been maintained in flow through 22 C water for $\geq$1 wk. These results could differ for recently harvested prawns. The procedures tested here in the BMP protocol should serve as a guideline for development of commercial procedures if marketing opportunities prove sufficiently promising. Additional research should evaluate higher biomass densities and longer durations of waterless transport to better determine potential of commercial application.

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