

# Considerations for *Litopenaeus vannamei* Reared in Inland Low Salinity Waters

D. Allen Davis<sup>1</sup>, Imad. P. Saoud, William J. McGraw, and David. B. Rouse

<sup>1</sup>Department of Fisheries and Allied Aquacultures  
Swingle Hall 203, Auburn University  
Auburn, AL 36849-5419. Tel. (334) 8449312/8449306. Fax. (334) 8449208.  
Email ddavis@acesag.auburn.edu

## ABSTRACT

Shrimp culture in inland low-salinity well water is a growing industry in many regions of the world, including the southern United States of America. Although shrimp culture techniques in waters of marine origin are well established, they are not necessarily applicable to shrimp culture in low salinity well water. Inland well waters tend to vary among each other in salinity and ion profiles. If shrimp culture using inland well water is to develop further, we must systematically deal with production problems which include 1) identification of suitable water sources 2) development of acclimation and stocking procedures 3) identify specific nutritional requirements for low salinity environments. This paper summarizes work that we have done to a) assist in the development of acclimation procedures for *Litopenaeus vannamei* postlarvae (PL), b) evaluate a variety of well water sources for their culture potential and identify mineral imbalances that may be correlated to poor survival and growth c) provide baseline data for the identification of nutritional manipulations that may mitigate the variable survival that has plagued low salinity production systems. Results suggest that PL survival in native seawater is affected by an interaction between PL age and water salinity. In native seawater, ten day old PL exhibited greater survival at salinities greater than 4 ppt than at salinities of 2 ppt and lower. Fifteen and twenty-day old PL survived in salinities as low as 1 ppt. Survival of PL in well water varied among water sources, and was affected by the concentration of various ions such as potassium (K<sup>+</sup>), magnesium (Mg<sup>2+</sup>), and sulphate (SO<sub>4</sub><sup>2-</sup>), as well as PL age. Based on current data, the supplementation of potassium to well water sources “low” in potassium will result in increased growth and survival. Based on our limited knowledge of the interaction of salinity and nutritional requirements the supplementation of potassium, magnesium and sodium chloride as well as selected amino acids and potentially lipids could mediate some of the problems encountered when rearing marine shrimp in low salinity production situations. Current data suggests that nutritional and/or environmental manipulations are technically feasible and probably are the most economically viable solutions to facilitate better production in many of the low salinity farms.

## INTRODUCTION

The culture of marine shrimp primarily occurs in near-coastal areas using waters of estuarine and oceanic origin. Due to variety of social, economic and environmental issues the inland culture of marine shrimp has been expanding in a number of countries. In the United States, farmers in Alabama, Arizona, Florida, Illinois, Indiana, Michigan, Mississippi, South Carolina and Texas have harvested *Litopenaeus vannamei* raised in low salinity well water. Despite the apparent success of a few farms, culturing shrimp in low-salinity inland waters has resulted in a wide variety of unique problems that must be addressed.

*L. vannamei* is the most commonly cultured shrimp in the western hemisphere (Rosenberry, 2000) and inhabits and/or has been cultured in coastal waters ranging in salinity from 1 ppt to 40 ppt (Bray *et al.*, 1994). Because of the ready availability of post-larvae (PL), and this specie's tolerance to low salinity, it is currently grown in inland waters ranging in salinity from 28.3 ppt (Smith & Lawrence, 1990) to 0.5 ppt (Samocha *et al.*, 2001). Commercial production of marine shrimp in US inland low salinity waters has been occurring for about 15 years. Many of the problems seen on low salinity facilities are typical of any aquaculture operation, yet there are also a variety of problems that seem to be associated with the unique rearing environment.

### ACCLIMATION AND/OR TOLERANCE TO LOW SALINITY ENVIRONMENTS.

Post larvae (PL) are purchased from commercial hatcheries and are generally shipped in water with salinity above 15 ppt. Consequently, the first problem for the farmer using inland low-salinity well water is to acclimate the PL to ambient salinities (and ionic profiles) before stocking them into grow out facilities. Although acclimation procedures in brackish seawater and related problems are described in the literature (Aquacop 1991; Villalon 1991; Kumulu & Jones 1995; Samocha *et al.*, 1998; Tsuzuki *et al.*, 2000), they do not tackle issues related to non-marine waters. Moreover, since the rule of constancy of composition of seawater does not apply to inland well water, results of acclimation studies using seawater cannot be extrapolated to include shrimp survival in non-oceanic waters. Penaeid PL are not tolerant to large salinity fluctuations when very young. They also appear to lose tolerance as juveniles or adults. The age of tolerance to wide salinity fluctuation for most penaeid post larvae is between PL<sub>10</sub> and PL<sub>40</sub> (Mair, 1980; Cawthorne *et al.*, 1983; Kumulu & Jones, 1995; Rosas *et al.*, 1999 ; Tsuzuki *et al.*, 2000), thus limiting the period a farmer can hold shrimp before acclimating them to low salinity well waters. Also, since inland well waters vary in ionic profiles, the tolerance of various ages of shrimp PL to these waters might also vary.

Although a number of farmers have been "successful" at acclimating PL to the low salinity conditions on the farms, quite often survival to early juvenile is variable and may be due to acclimation techniques. Consequently, the first protocol to establish is one for salinity acclimation. To evaluate the tolerance of *L. vannamei* PL to low salinity environments a series of acclimation studies and biological assays were conducted using either artificial sea

salt or waters from various low salinity inland water sources. Post larval shrimp, *L. vannamei*, were obtained from commercial hatcheries producing high health shrimp. Upon receipt of 8-d old PL (PL<sub>8</sub>) the shrimp were acclimated over a period of approximately two hours to artificial seawater (Instant Ocean) at 28 °C and 24 ppt and maintained in a 220-L (1.1 x 0.8 x 0.25 m) polyethylene tank connected to a biological filter. The PL were fed artemia (200 per shrimp) and a commercial feed, “PL redi-reserve” manufactured by Ziegler Bros Inc. (Gardner, PA, USA) at 25-50% body weight.

Experimental waters were collected from various wells across the Southeastern United States (See Table 1 for ionic profiles) and reconstituted seawater was prepared using Instant Ocean salt mix. Acclimation tests were conducted in a series of white cylindrical containers (594 cm<sup>2</sup>, 30 cm deep) containing 2 L of water at 28 °C and 24 ppt and aerated with an air diffuser. Shrimp in the containers were offered PL-redi-reserve three times daily and artemia twice daily. Each battery of four replicate containers was supplied with filtered fresh water from a separate water tank using a submersible pump and irrigation-style flow restrictors. Salinity reduction rate was adjusted by changing the flow restrictors and reducing the water volume in the containers back to 2 L every hour. Three types of acclimation experiments were conducted in order to 1) test various salinity endpoints (0, 1, 2, 4, 8, and 12 ppt) to determine the lower limits of salinity tolerance of three ages of PL (10, 15 and 20 days) at a constant rate of reduction 2) determine if the rate of salinity reduction influenced survival at each of the three age classes 3) determine the response of the three age classes to various inland well water sources. Survival was determined for each treatment 48 hr after the initiation of the acclimation study.

Table 1. Ionic profile of various inland well waters from the southern USA. Numbers are concentration in mg.L<sup>-1</sup>. Sources are from West Alabama <sup>(A)</sup>, Australia <sup>(AU)</sup>, south Florida <sup>(F)</sup>, East Mississippi <sup>(M)</sup> and West Texas <sup>(T)</sup>.

Ions	Instant Ocean	C <sup>A</sup>	HC <sup>A</sup>	JS <sup>A</sup>	L <sup>A</sup>	LC <sup>A</sup>	KM <sup>A</sup>	NH <sup>A</sup>	O <sup>A</sup>	R <sup>A</sup>	TC <sup>A</sup>	W <sup>A</sup>
Salinity	4.0	6.1	0.7	16.3	7.3	3.8	3.7	0.9	6.0	4.5	3.4	3.4
Na	1316.3	2483.1	219.1	5732.6	2504	1480.9	1316.2	364.1	2394.7	1957	1395.1	1122.5
Ca	42.4	174.29	3.02	104.7	540	29.8	330.5	5.24	109.06	156.87	55	281.6
Mg	132.26	30.29	0.35	76.7	63.6	6.7	14.5	0.96	28.63	28.24	12.4	53.9
K	47.32	16.45	2.2	34.4	15.4	8.5	10.1	3.48	15.4	12.21	9.5	7.9
P	0.33	0	0	0.4	0.6	0	0	0	0.15	0	0	0
Fe	0	0	0	80	1.2	0	0.2	0	0	0	0	0
Mn	0	0.1	0	0.1	0.1	0	3.3	0	0	0.03	0	0.2
Zn	0	0.01	0	0	0.1	0	0.1	0	0	0	0	0.4
B	0.73	1.1	0.29	7.3	0.4	1.5	0.1	0.41	1.28	1.38	1.1	1
Mo	0	0	0	0	0	0	0.5	0	0	0.03	0.4	0
Al	0	0	0	0.1	0.9	0	0.4	0	0	0	0	0
Ba	0.02	0.76	0.01	2.3	1.4	0.2	0.4	0.02	0.7	1.39	0.3	7
Cr	0	0	0	0	0.1	0	0	0	0	0	0	0
Cl	1922.1	3589	332.9*	9807	5873	2129	2489	553.8*	3859	3192.5*	1949	2514
SO <sub>4</sub> <sup>-</sup>	262.3	0	.	0	12.1	0	0	.	6.05	.	0	0

(0) indicates below detectable limits.

(.) is for values that have not been estimated.

(\*) is for values that have been calculated mathematically.

Table 1. Continued.

Ions	Instant Ocean	P <sup>M</sup>	FL <sup>F</sup>	WT <sup>T</sup>	LA <sup>T</sup>	FO <sup>A</sup>	RT <sup>A</sup>	WC <sup>A</sup>	HBOI <sup>F</sup>	B <sup>AU</sup>
Salinity	4.0	0.79	2.2	8.9	3.5	5.1	2.9	23.5	1.0	19.6
Na	1316.3	299.8	987.2	2799.8	1015.5	1990.3	1164.6	9021.1	290	5140
Ca	42.4	3.8	51.5	697.8	147.1	124.3	44.5	250.8	54	772
Mg	132.26	0.7	60.6	455.3	100	28.6	10.4	126.5	53	948
K	47.32	3.2	35.3	63.9	17.8	12	8.7	38.2	15	30
P	0.33	0.1	0	4.1	1.1	0	0	0.9	0	10.9
Fe	0	0	0.3	7.3	1.2	3.3	0	5.4	0	0.5
Mn	0	0	0	0	0.1	0.2	0	0.3	0	0
Zn	0	0	0	0.1	0	0.1	0.1	0.1	0	0
B	0.73	0.4	0.9	1.6	4.3	0.9	0.9	12.6	0.25	0.26
Mo	0	0	1.2	0.2	0	0.4	0.7	0.5	0	0
Al	0	0	0	1.5	0.3	0	0	0	0	0
Ba	0.02	0	0	0	0.1	0.7	0.3	5.8	0.023	0
Co	0	0	0	0	0	1.8	0	2.2	0	0
Cl	1922.1	450	1070	2769	1230	3200.6*	1689	14007.3*	450	12380
SO <sub>4</sub> <sup>2-</sup>	262.3	0	806	3617	1127	.	0	.	140	265

(0) indicates below detectable limits.

(.) is for values that have not been estimated.

(\*) is for values that have been calculated mathematically.

Two trials using different groups of shrimp were used to evaluate the influence of age on the tolerance of the shrimp to reach a given salinity endpoint. Results were similar for both trials (Tables 2 and 3) and a significant interaction between age of PL and salinity endpoint was observed. Survival after 48 hours ranged from 0 – 50% for PL<sub>10</sub> acclimated to 2 ppt and below. These values were significantly lower than those observed for PL exposed to 4 ppt and above which had survival rates of 80 – 100%. Survivals of PL<sub>15</sub> and PL<sub>20</sub> were lower for shrimp exposed to 0 ppt (0 – 8%) than for those in which the salinity was reduced to 1 ppt and above (82 – 100%). There were no differences in survivals among age groups acclimated to salinities higher than 4 ppt.

Table 2. Mean survival (%) of 10-, 15- and 20-day old postlarval *Litopenaeus vannamei*, 24 and 48 hours after reaching the target salinity endpoint (Trial 1). Values within a column having a \* are significantly different from the control (12 ppt), using Dunnett's t-test ( $P < 0.05$ ).

Salinity	10-d		15-d		20-d	
	24 h	48 h	24 h	48 h	24 h	48 h
0	8*	0*	77*	7*	42*	8*
1	13*	5*	98	90	87	92
2	97	50*	100	85	100	87
4	100	92	100	95	100	90
8	100	82	100	93	100	85
12	100	80	100	95	100	83
PSE <sup>a</sup>	3.8	6.0	3.7	4.5	9.9	5.2

<sup>a</sup> Pooled Standard Error

Table 3. Mean survival (%) of 10-, 15- and 20-day old postlarval *Litopenaeus vannamei*, 24 and 48 hours after reaching the target salinity endpoint (Trial 2). Values within a column having a \* are significantly different from the control (12 ppt), using Dunnett's t-test ( $P < 0.05$ ).

Salinity	10-d		15-d		20-d	
	24 h	48 h	24 h	48 h	24 h	48 h
0	50*	0*	0*	0*	0*	0*
1	57*	35*	100	88	97	82
2	70*	45*	100	95	100	90
4	100	100	100	93	100	100
8	100	92	100	90	100	93
12	100	100	100	95	98	95
PSE <sup>a</sup>	6.9	7.0	-	4.7	1.5	4.3

<sup>a</sup> Pooled Standard Error

Assuming that marine water is optimal for shrimp culture, results of the present work clearly indicate an effect of age on salinity tolerance and indicates that *L. vannamei* younger than PL<sub>15</sub> should not be acclimated to salinities lower than 4 ppt. However, PL<sub>15</sub> and older can be acclimated down to 1 ppt. Since the acclimation of the shrimp was done under similar rates of acclimation one could conclude that the acclimation procedure was too fast. Consequently, a second experiment was designed to evaluate the effects of rate of salinity reduction. The data from this experiment is presented in Table 4. Results indicate that salinity end point and acclimation rate had no effect on survival of PL<sub>15</sub> and PL<sub>20</sub>. Significant differences between treatments were found as early as 24 hours after acclimation with PL<sub>10</sub>. However, acclimation rate did not significantly influence survival whereas salinity end point did. Survival of PL<sub>10</sub> acclimated to 4 ppt was significantly higher than that of PL<sub>10</sub> acclimated to 1 ppt, irrespective of the rate of acclimation.

Table 4. Mean survival (%) of 10-, 15- and 20-day old post larval *Litopenaeus vannamei*, 24 and 48 hours after reaching the two target salinity endpoints using three rates of acclimation. Difference in percent survival due to the main effects and their interactions are indicated at the bottom of the table (nsd = no significant difference).

Salinity	Rate	10-d		15-d		20-d	
		24h	48h	24h	48h	24h	48h
1	High	40	23	100	88	98	87
	Medium	57	42	100	92	100	83
	Low	65	43	100	92	100	88
4	High	97	77	100	93	100	93
	Medium	98	77	100	98	100	88
	Low	98	83	100	88	100	92
P value	Salinity	0.0001	0.0001	nsd	nsd	nsd	nsd
	Rate	0.2080	0.2481	nsd	nsd	nsd	nsd
	Interaction	0.3795	0.4392	nsd	nsd	nsd	nsd

Based on the above mentioned results it would appear that age has a more pronounced impact on the shrimps' ability to acclimate to low salinity. Although the data suggests that a salinity reduction of 46% per hour is feasible, it should be noted that the present work was conducted in controlled laboratory settings and low stocking densities of shrimp. The authors suggest an acclimation rate of 4 ppt hr<sup>-1</sup> down to a salinity of 4 ppt and a reduction rate of 1 ppt hr<sup>-1</sup> thereafter as a more conservative approach to salinity acclimation. This conservative acclimation rate was used in subsequent experiments to test the various low salinity well water sources.

Data from a series of biological assays with various well water sources are presented in Table 5 and the ionic composition of the various waters are presented in Table 1. Forty-eight hour survival of *L. vannamei* postlarvae in inland saline well water varied with PL age and water source. Survival in eight of the 15 waters tested was different from the control at PL<sub>10</sub> while survival in only seven and five waters were different from the controls at PL<sub>15</sub> and PL<sub>20</sub>, respectively. Some waters were not suitable for shrimp acclimation at all PL



stages tested.

Table 5. Mean survival (%) of *Litopenaeus vannamei* in various inland saline well waters compared to survival in an artificial seawater control, 48 hours after initiating acclimation.

	PL <sub>10</sub>	PL <sub>15</sub>	PL <sub>20</sub>
Experiment 1.			
Average Wt. (mg)	3.85	8.42	.75
L (7.3 ppt)	48.3*	51.7*	90.0
C (5.6 ppt)	83.3	70.0	90.0
R (4.5 ppt)	75.0	75.0	91.7
Control (4ppt)	80.0	85.0	93.3
Experiment 2.			
Average Wt. (mg)	4.77	5.34	25.01
LC (3.8 ppt)	63.3	55.0*	73.3
JS (16.3 ppt)	0.0*	0.0*	0.0*
Control (4ppt)	73.3 <sup>a</sup>	97.8	85.0
Experiment 3.			
Average Wt. (mg)	1.03	6.89	11.82
P (0.7 ppt)	6.7*	45.0*	36.7*
TC (3.4 ppt)	0.0*	28.3*	28.3*
FL (2.2 ppt)	95.0	98.3	98.3
WT (8.9 ppt)	65.0*	91.7	100.0
LA (3.5 ppt)	81.7	95.0	95.0
KM (3.7 ppt)	0.0*	41.7*	50.0*
Control 4ppt	93.3	100.0	100.0
Experiment 4.			
Average Wt. (mg)	2.98	8.77	19.48
O (6.0 ppt)	83	98	88
NH (0.9 ppt)	43*	87	55*
HC (0.7 ppt)	25*	65*	N/A
Control (4ppt)	83	88	92
Experiment 5.			
Average Wt. (mg)	2.15	7.17	14.54
W (4.3)	90	98.3	96.7
Control (4ppt)	92.5	98.3	95.0

<sup>a</sup> = significantly different from other controls at PL10.

\* Indicates that the value is different from the control.

The two lowest salinities were those of HC and P, both being less than 1 ppt. Survival in these two waters was significantly lower than in the controls. The highest salinity was that of JS at 16.3 ppt, yet total mortality resulted when *L. vannamei* PLs were maintained in JS water. No correlation was found between PL survival and inland well water salinity. Excellent survival was observed in the FL water at 2.2 ppt while low survival was observed in the TC and KM waters at 3.4 ppt and 3.7 ppt, respectively.

To evaluate the influence of ionic composition of the waters on PL survival, correlation analyses were conducted using the collected data. Post-larval survival was correlated to potassium ( $K^+$ ) concentration at all PL ages tested (Tables 6 and 7). Weaker correlations were also found between PL survival and magnesium ( $Mg^{2+}$ ), manganese ( $Mn^{2+}$ ) and sulfate ( $SO_4^{2-}$ ).

Table 6. Regression models describing the stepwise selection of ions possibly responsible for *Litopenaeus vannamei* PL survival at various ages.

PL <sub>10</sub>					
Variable	Parameter Estimate	Standard Error	Type II Sum of Squares	F	Prob>F
Intercept	-0.7244	0.6456	4.4893	1.26	0.2663
Mg	-0.4302	0.0444	334.6681	93.85	0.0001
K	1.3813	0.1311	395.9223	111.03	0.0001
P	-2.7568	1.8309	8.0845	2.27	0.1374
Fe	6.7255	1.5637	65.9666	18.50	0.0001
Mo	-21.8925	2.0247	416.9027	116.92	0.0001
Ba	5.8310	0.7086	241.4294	67.71	0.0001
SO4	0.0206	0.0015	631.8574	177.20	0.0001

PL <sub>15</sub>					
Variable	Parameter Estimate	Standard Error	Type II Sum of Squares	F	Prob>F
Intercept	11.6377	0.7497	1598.9288	240.95	0.0001
K	0.0927	0.0176	184.1333	27.75	0.0001
Mn	-1.0847	0.4220	43.8432	6.61	0.0125
Cl	-0.0011	0.0003	77.3536	11.66	0.0011

PL <sub>20</sub>					
Variable	Parameter Estimate	Standard Error	Type II Sum of Squares	F	Prob>F
Intercept	5.1099	0.7601	252.5802	45.19	0.0001
K	0.1699	0.0190	449.1629	80.36	0.0001
B	1.5203	0.3185	127.3314	22.78	0.0001
Al	-2.3100	0.7764	49.4836	8.85	0.0041
Ba	3.4319	0.6964	135.7456	24.29	0.0001

ΣPL					
Variable	Parameter Estimate	Standard Error	Type II Sum of Squares	F	Prob>F
Intercept	5.9754	0.5248	968.4080	129.66	0.0001
Mg	-0.2847	0.0299	679.1963	90.93	0.0001
K	0.8452	0.0754	937.3465	125.50	0.0001
B	-1.5345	0.3824	120.2627	16.10	0.0001
Mo	-17.7638	1.8929	657.7885	88.07	0.0001
Al	2.9663	1.3110	38.2353	5.12	0.0247
Ba	4.5949	0.6140	418.3574	56.01	0.0001
SO4	0.0232	0.0024	711.4510	95.25	0.0001

Table 7. Pearson's correlation coefficient between *Litopenaeus vannamei* PL survival and various ions in the waters tested.

	PL <sub>10</sub>		PL <sub>15</sub>		PL <sub>20</sub>		All ages	
	Pearson's	P >  R	Pearson's	P >  R	Pearson's	P >  R	Pearson's	P >  R
Na	0.332	0.004	-0.211	0.075	0.344	0.004	0.174	0.011
Ca	-0.043	0.717	-0.216	0.069	0.112	0.362	-0.039	0.572
Mg	0.328	0.005	0.344	0.003	0.465	<0.001	0.351	<0.001
K	0.557	<0.001	0.458	<0.001	0.601	<0.001	0.510	<0.001
P	0.268	0.023	0.151	0.207	0.297	0.014	0.231	0.001
Fe	0.063	0.598	0.124	0.299	0.242	0.046	0.127	0.065
Mn	-0.428	<0.001	-0.407	<0.001	-0.281	0.021	-0.350	<0.001
Zn	-0.251	0.033	-0.0343	0.003	-0.077	0.530	-0.207	0.003
B	0.359	0.002	0.189	0.113	0.325	0.007	0.283	<0.001
Mo	-0.038	0.749	-0.085	0.476	-0.034	0.782	-0.045	0.516
Al	-0.068	0.572	-0.109	0.365	0.107	0.385	-0.023	0.744
Ba	0.024	0.842	-0.391	<0.001	-0.006	0.961	-0.092	0.182
Cl	0.176	0.139	-0.294	0.012	0.139	0.257	0.035	0.611
SO <sub>4</sub>	0.213	0.073	0.309	0.008	0.367	0.002	0.269	<0.001
Ca + Mg	0.089	0.453	-0.033	0.781	0.258	0.034	0.102	0.140
Na + K	0.343	0.003	-0.198	0.094	0.358	0.003	0.185	0.007
Na + K + Cl	0.260	0.027	-0.268	0.023	0.245	0.044	0.104	0.132

The present work confirms reports that *L. vannamei* can be acclimated to some inland, low salinity well waters. The fact that survival was higher in some waters than in others underscores the need for water suitability evaluations. All saline well waters are not equal, even if they originate from the same aquifer but at different locations. The C water and the TC water are from wells that are less than 3 km apart and drilled into the same aquifer, yet they have different salinities and ionic compositions. Furthermore, PL acclimated much better to the C water than to the TC water and juveniles survived and grew better in the former than in the latter.

Based on these results, the ionic composition of a saline water appears to be more important than salinity with regards to its effect on shrimp survival and growth. Cawthorne *et al.* (1983) demonstrated that single salt solutions (NaCl) were not suitable for shrimp culture at any salinity, although in seawater, the ions most important in osmoregulation are chloride and sodium (Castille & Lawrence, 1981; Ferraris *et al.*, 1986; Parado-Esteva *et al.*, 1987). Results of the present study as well as ongoing research suggest that, in the presence of adequate salinity, potassium was the ion most correlated with PL survival. Similar results were found by Adrian Collins (personal communication) working with shrimp osmoregulation in Australia as well as Fielder *et al.* (2001) working with the snapper *Pagrus auratus*. These results could be due to the fact that Cl<sup>-</sup> and Na<sup>+</sup> concentrations in all the waters tested were at levels suitable for shrimp survival, but potassium was not. Preliminary studies involving supplementation of K<sup>+</sup> ions to some of the waters that were unsuitable for shrimp culture, presented favorable results. When shrimp were maintained in waters in which K<sup>+</sup> was increased to equal its concentration in 4 ppt seawater, PL survival increased from < 50% to more than 85%. Other ions also found to be correlated with survival (Mg<sup>2+</sup> and SO<sub>4</sub><sup>2-</sup>) were reported as important in osmoregulation by Mantel and Farmer (1983).

#### **LONG-TERM SURVIVAL IN WELL WATERS.**

To evaluate the effects of selected water sources on the growth of shrimp, a 4 week growth trial was initiated under laboratory conditions. One week before the start of the growth trial, four recirculating systems consisting of four 144-L (0.6 x 0.6 x 0.4 m) polyethylene tanks each, were filled with saline water. Three systems were filled with saline well water and one with reconstituted seawater at 4 ppt. Water from each system flowed through a common drain into a sump tank where it was circulated through a trickling biological filter and returned to the tanks. Temperature was maintained at 29 ± 1 C. The saline well waters were chosen depending on the results of the acclimation experiment. One water exhibited very good survival (> 90 %; W; 4.3 ppt) during the acclimation trial, one exhibited intermediate survival (70 - 90 %; C; 6.1 ppt) and one exhibited low survival (< 30 %; TC; 3.4 ppt). Groups of 15 shrimp were randomly removed from holding facilities, weighed to the nearest 0.01 g and transferred to the research tanks. The shrimp were fed a 35% protein shrimp feed (Rangen Inc.) four times daily at a rate that assumes a weekly growth of 1 g per week and 1.75:1 FCR. Shrimp in each tank were counted twice a week and their feed ration was adjusted accordingly. Four weeks after stocking, the shrimp from each tank were counted and weighed.

Results of the 4-week growth trial are presented in Table 8 . Juvenile shrimp survival in the W well water was similar to survival in the control but significantly higher than survival in the C or TC well waters. Juvenile survival in the C water was greater than in TC water but less than in the control. Percent growth was significantly different among all treatments, being highest in the W well water. Percent growth is not reported for the TC water because the final mean weight of the shrimp was lower than the initial mean weight due to mortality of the larger shrimp within that treatment. Survival and growth of juveniles in the various waters followed the same trend as survival of PL in the same waters during the acclimation study (Fig. 1). Waters that were found suitable for PL acclimation were also suitable for juvenile survival and growth under laboratory conditions. Similar results were observed by Cawthorne *et al.* (1983) working with *Penaeus monodon* and by Harpaz & Karplus (1991) working with *P. semisulcatus*. Consequently, a stepwise assessment of water suitability, starting with a 48 hr bioassay using PL, would eliminate marginal waters and allow further efforts to be directed at promising waters.

Table 8. Average initial and final weights, percent growth and percent survival of individual shrimp in the various waters tested. Values (means of four replicates) within a column with similar superscripts are not significantly different from each other.

	Initial weight (g)	Final weight (g)	% Survival
W (4.3 ppt)	1.36 <sup>a</sup>	3.75 <sup>a</sup>	78.3 <sup>a</sup>
C (5.6 ppt)	1.34 <sup>a</sup>	2.64 <sup>b</sup>	43.3 <sup>b</sup>
TC (3.4 ppt)	1.35 <sup>a</sup>	0.62 <sup>c</sup>	5.0 <sup>c</sup>
Control (4 ppt)	1.35 <sup>a</sup>	3.19 <sup>a,b</sup>	80.0 <sup>a</sup>
Pooled Standard Error	0.02	0.24	4.14

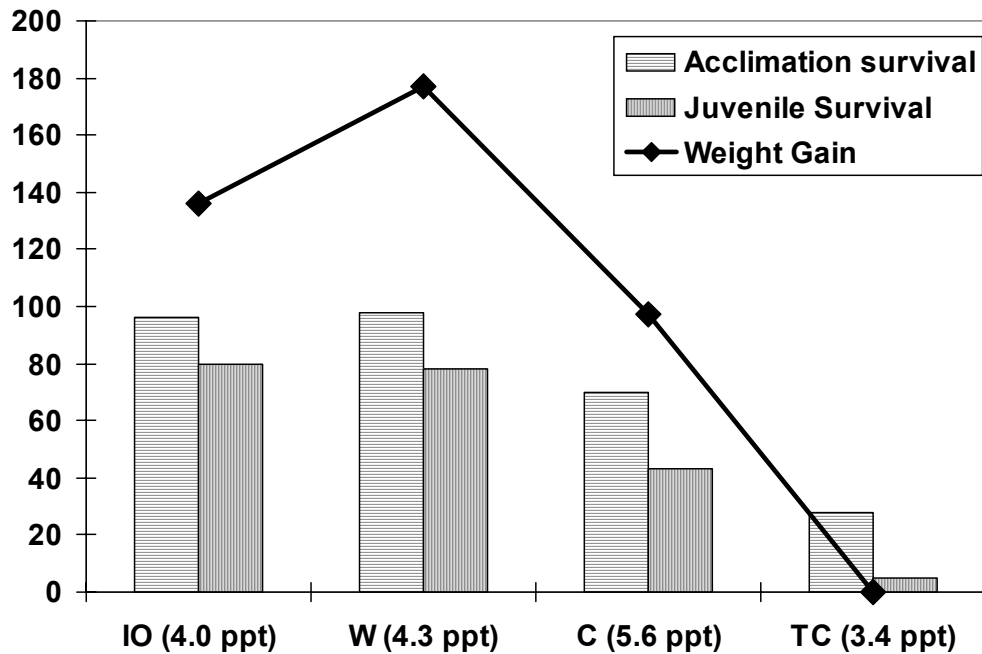


Figure 1.-Survival and growth of *Litopenaeus vannamei* juveniles and survival of PL15 *L. vannamei* in various inland well waters from the West Alabama

### POTENTIAL OF DIETARY NUTRIENT MANIPULATIONS.

The ionic composition of a saline water appears to be more important than salinity with regards to its effect on shrimp survival and growth. This is probably due to the fact that most of these waters contain adequate levels of sodium and chloride to meet the shrimps physiological requirement. However, other ions are not at sufficient levels in the water or possibly the diet to meet physiological requirements. Quite often inland shrimp farmers complain of a slow die-off of shrimp. They report that shrimp are easily stressed by handling, temperature and low dissolved oxygen levels of the water. The farmers often observe lethargic shrimp along the sides of nursery tanks and ponds, or stressed shrimp even after gentle handling. Stress is often characterized by a whitening of the tail, cramping and possibly death. A probable reason for this response would be ionic imbalances and nutrient reserves caused by this unique environment.

Since aquatic animals can obtain minerals from both ambient water and feed, dietary supplements of selected minerals could facilitate better survival and growth of shrimp held in low salinity conditions. Davis *et al.* (1990) evaluated the response of juvenile shrimp offered a semi-purified diet with or without a mineral supplement. This research was conducted in outdoor tanks at a salinity of 10 ppt and indicated that if the mineral pre-mix

was removed growth was reduced by 11.8%. Thus, confirming an influence of dietary minerals on growth, even in the presence of natural foods. When the shrimp were held at 35 ppt and the mineral pre-mix was removed a growth depression was also observed (7.2%) but it was considerably less than that at the lower salinity. This makes sense, as it is well accepted that the dietary mineral requirements of aquatic animals are strongly influenced by the levels of minerals found in the water.

If one looks at the mineral profile of most low salinity well waters as compared to the profiles of low salinity water of oceanic origin (see Table 1) you find that the levels of potassium and magnesium are much lower in the low salinity well water source. Hence, one would suspect that dietary supplements may be required. Marine species reared in seawater do not require dietary sources of magnesium and potassium, whereas freshwater species reared in freshwater do (for a review see Davis & Gatlin, 1996). Consequently, these minerals may be low in marine shrimp feeds that are fed to marine shrimp reared in low salinity water. Shiau & Hsieh (2001) also noted that *P. monodon* appears to require a dietary source of potassium when reared in brackish water but may not when reared in full strength seawater.

In addition to K and Mg being of potential importance, Gatlin *et al.* (1992) reported that the addition of NaCl to diets for red drum, a euryhaline fish, held at low salinity resulted in an increase in growth. Given this information, the supplementation of K, Mg and NaCl to practical diets is recommended and research into the influence of minerals on growth and survival in low salinity water is warranted.

Protein, energy and fatty acids could also play an important role in low salinity feeds, by influencing the ability of shrimp to cope with low salinity environment via their influence on osmoregulation. Robertson *et al.* (1993) found an effect of diet protein level on growth of shrimp maintained at various salinities. Moreover, Schoffeniels (1970) found that some amino acids (AA) such as Proline, Glycine and Alanine are involved in intracellular osmotic regulation of penaeid shrimp. Marangos *et al.* (1989) also observed that the non-essential free amino acids of *P. japonicus* muscle were mobilized for osmoregulation. Consequently, the addition of ingredients rich in these AA to the diet or the inclusion of these AA in the crystalline form might increase survival and growth of shrimp reared in inland low salinity well waters.

Free amino acids and minerals clearly play an important role in osmoregulation but there is also a general energy expenditure that is required to maintain ionic pumps for ion exchange and other energy costs associated with osmoregulation. Carbohydrates and lipids can play a direct or indirect role in providing energy for osmoregulation and influencing the permeability of cellular membranes. It has been demonstrated that blood glucose levels fall when shrimp are exposed to extreme salinities (Spaargaren & Haefner, 1987, Lignot *et al.*, 1999). Hence, the energy content of the diet particularly with respect to carbohydrates could impact the availability of energy and the ability of the shrimp to maintain itself.

Osmoregulation is a membrane mediated process for which enzymatic pathways (e.g. Na<sup>+</sup> -



K<sup>+</sup>-ATPase pumps) and membrane permeability influence osmotic balance. Fatty acids and phospholipids are major components of biological membranes and have been found to be influenced by environmental parameters such as salinity and temperature (Castell, 1979). Consequently, the type of lipids available for membrane synthesis may also influence the ability of an animal to respond to low salinity conditions.

Inland low salinity shrimp culture is now a *fait accompli*. In the USA, shrimp grown in inland groundwater have been harvested and sold in Texas, Alabama, Florida and even Illinois and Indiana. Unfortunately, not all water sources are suitable and some produce highly variable results; hence, water suitability for shrimp culture is being tested at various institutions and is the initial step in developing any farm. For those waters that appear suitable for culturing shrimp, there appears to be an interaction between physiological requirements for nutrients and the mineral profile of the water. Consequently, dietary modifications of minerals, amino acids and lipids need to be evaluated for their impact on growth, survival and stress resistance.

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