Effects of Gradual and Acute Changes in Salinity on the Moisture, Salt, and Free Amino Acid Content in the Tail Muscle of Brown Shrimp (*Penaeus aztecus*)

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The effects of both gradual and acute changes in growth salinity on tissue moisture, salt, and free amino acid content of brown shrimp (*Penaeus aztecus*) were investigated. To keep the internal osmotic pressure in balance with the environment, shrimp immediately responded to increases in salinity by a loss in moisture and to decreases in salinity by a gain in moisture. The tissue salt content was also rapidly adjusted to salinity changes. Increases and decreases in salinity resulted in increases and decreases in salt content, respectively. The response in the free amino acid content to changes in salinity was similar to that of salt but markedly delayed. However, once the free amino acid content started to contribute to the osmotic balance, the tissue moisture content reverted back to normal levels. If changes in salinity are performed prior to harvesting, it is important to allow shrimp a long acclimation period in order to achieve maximum flavor enhancement at a maximum yield.

In earlier research, McCoid et al. (1984) showed that the environmental growth salinity will directly affect the concentration of free amino acids in penaeid shrimp. Free amino acids, which are present in shrimp tissue as osmoregulators, have also been shown to be major contributors to the flavor of seafoods (Hashimoto, 1965; Jones, 1969; Nair and Bose, 1965; Simudu and Hujita, 1954; Thompson et al., 1980). McCoid et al. (1984) postulated that the production of more flavorful cultured shrimp would be possible through manipulation of pond salinity. However, in addition to free amino acids, both inorganic ions and tissue moisture content can play important roles during osmoregulation in aquatic animals. In response to an increase in salinity, the animal must increase its intracellular ionic strength by an increase in osmoeffectors or a reduction in tissue moisture content. The converse is true during reductions in environmental salinity (Lockwood, 1962; Bursey and Lane, 1971).

The purpose of this research was to determine the relationship between the free amino acid concentration, salt, and moisture content in shrimp subjected to both gradual and acute changes in environmental salinity.

EXPERIMENTAL PROCEDURES

Preparation and Maintenance of Aquariums. Six 190-L fiberglass aquariums were prepared with dechlorinated tap water and Aquarium Systems Instant Ocean. Aeration to each aquarium was provided by two Fritz Mighty III pumps. A 5-cm layer of dolomite, topped with a 2.5-cm layer of course gravel covered the bottom of each aquarium. Desired temperatures were maintained by 200-W Ybo submersible heaters. Water quality was monitored daily for temperature, ammonia (Aquarium Systems Sea Tests ammonia kit), pH (Corning Model 10 pH meter), dissolved oxygen (YSI Model 57 oxygen meter), and salinity (AC hand-held refractometer).

Effect of Environmental Salinity on Moisture, Salt, and Free Amino Acid Content in Shrimp. Brown shrimp (*Penaeus aztecus*), harvested in Corpus Christi Bay, were transported live in ice chests to the Texas A&M University Research and Extension Center, Corpus Christi. Upon arrival, the shrimp were transferred to prepared aquariums and subjected to both gradual and acute salinity changes.

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Gradual Salinity Changes. Since the water in Corpus Christi Bay had a salinity of 39 ppt at the time of harvest and the water in the aquariums was kept at 31 ppt, the sea water for transportation was slowly diluted over a period of 5 h by dechlorinated tap water. Twenty-five shrimp were then transferred from the sea water into each of the six aquariums kept under heavy aeration. The mean length and weight of the animals were 9.97 ± 0.86 cm and 8.49 ± 2.10 g, respectively.

Following a 72-h acclimation period, six shrimp, one from each tank, were removed and the tail meat was analyzed for moisture, chloride, and free amino acid nitrogen (FAA-N) content. The salinity of two aquariums was subsequently adjusted to 30 ppt while the salinity of the remaining four aquariums was changed to 10 and 50 ppt, two aquariums at each salinity. Salinity changes were performed at a rate of 2 ppt/h with a maximum of 10 ppt/day, using appropriate amounts of either dechlorinated tap water or a concentrated solution of Instant Ocean salt. Samples (two shrimp from each aquarium) were taken 24 h after final salinities had been reached and at 48-h intervals thereafter for a period of 9 days.

Acute Salinity Changes. In order to determine the effect of acute salinity changes on the moisture, chloride, and free amino acid content of penaeid shrimp, 28 brown shrimp were transferred from seawater at 27 ppt salinity and 26 °C to each of six aquariums maintained at 25 ppt and 25.5 °C. Since salinity and temperature changes were minimal during this part of the research, the shrimp were stocked directly into the aquariums. Mean length and weight of the shrimp were 8.91 ± 0.35 cm and 6.09 ± 0.88 g, respectively.

Following a 24-h acclimation period, the tail meat of two shrimp randomly selected from each aquarium was analyzed for moisture, chloride, and free amino acid nitrogen content. Salinities were subsequently changed to 10 ppt in three aquaria and to 50 ppt in the remaining three aquaria. The changes were performed acutely with appropriate volumes of dechlorinated tap water or concentrated Instant Ocean salt. Two shrimp from each aquarium were removed 1, 2, 4, 8, 12, 24, and 48 h after the salinity had been changed and analyzed for moisture, chloride, and free amino acid nitrogen content.

Chemical Methods. After sampling, the shrimp were deheaded, peeled, and deveined and the tail meat was analyzed for moisture, chloride, and free amino acid nitrogen. Samples were a composite of shrimp removed from each aquarium kept at the same salinity, and each analysis

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Table I. Moisture Content (%) of Brown Shrimp Acclimated to Different Salinities[a]

salinity, ppt	time,[b] days 1	3	5	7	9	av
10	76.85[a]	77.87[a]	78.72[a]	77.64[a]	78.98[a]	78.01[a] ± 0.86
30	76.73[a]	76.06[b]	76.18[b]	76.82[a]	78.04[ab]	76.77[b] ± 0.79
50	74.04[b]	74.54[c]	75.40[b]	77.33[a]	76.76[b]	75.67[c] ± 1.41

[a] Means within each column having the same letter are not significantly different ($p \le 0.05$). [b] After final salinity had been reached.

Table II. Chloride Content (as % NaCl) of Brown Shrimp Acclimated to Different Salinities[a]

	time,[b] days									
	1		3		5		7		9	
salinity, ppt	W[c]	D[d]	W	D	W	D	W	D	W	D
10	0.288[a]	1.234[a]	0.424[a]	1.918[a]	0.311[a]	1.471[a]	0.311[a]	1.389[a]	0.340[a]	1.581[a]
30	0.369[a]	1.591[a]	0.409[a]	1.703[a]	0.420[a]	1.765[a]	0.384[a]	1.659[a]	0.383[a]	1.743[a]
50	0.545[b]	2.100[b]	0.599[b]	2.192[a]	0.685[b]	3.045[b]	0.741[b]	3.266[b]	0.539[b]	2.315[b]

[a] Means within each column having the same letter are not significantly different ($p \le 0.05$). [b] After final salinity had been reached. [c] On wet-weight basis. [d] On dry-weight basis.

Table III. Free Amino Acid Nitrogen Content (mM AAN/100 g) of Brown Shrimp Acclimated to Different Salinities[a]

	time,[b] days									
	1		3		5		7		9	
salinity, ppt	W[c]	D[d]	W	D	W	D	W	D	W	D
10	17.57[a]	75.88[a]	18.34[a]	83.09[a]	20.13[a]	99.75[a]	19.96[a]	89.28[a]	20.71[a]	98.55[a]
30	20.46[a]	87.97[ab]	22.87[b]	95.44[a]	23.83[a]	100.61[a]	23.51[b]	101.43[a]	22.60[a]	102.87[a]
50	26.05[b]	100.29[b]	31.73[c]	124.61[b]	31.06[b]	123.50[b]	32.67[c]	144.08[b]	29.96[b]	128.92[b]

[a] Means within each column having the same letter are not significantly different ($p \le 0.05$). [b] After final salinity had been reached. [c] On wet-weight basis. [d] On dry-weight basis.

was performed in duplicate. Moisture and chloride contents were determined according to the AOAC (1980) and the amino acid nitrogen concentration according to a modification of the copper procedure of Spies and Chambers (1951) as described by Cobb et al. (1973).

Statistical Analysis. All data were statistically analyzed by using analysis of variance (ANOVA) at the data processing Center of Texas A&M University. Data that showed significant differences were further analyzed by Duncan's multiple-range test or the Student's t-test.

RESULTS AND DISCUSSION

Gradual Changes in Salinity. *Moisture Content.* Table I shows the effect of environmental salinity on the moisture content of brown shrimp. The shrimp used for this part of the study had been subjected to gradual changes in salinity with sampling commencing 1 day after final salinity had been reached.

Even after gradual adaptation and 24-h acclimation, environmental salinity had a marked effect on the moisture content of brown shrimp. In general, shrimp subjected to low salinity had a high moisture content while shrimp subjected to high salinity had a low moisture content. Shrimp that had been gradually changed from 30 to 10 ppt had the highest moisture content throughout the entire sampling period. However, the moisture content of these shrimp was only significantly different from the moisture content of shrimp kept at 30 ppt at days 3 and 5 after the final salinity had been reached. The moisture content of shrimp exposed to 50 ppt as compared to 30 ppt was significantly lower through the third day after the salinity changes had been completed. With the exception of day 7, the moisture content of shrimp subjected to 10 ppt was significantly higher than in shrimp subjected to 50 ppt throughout the entire sampling period.

The data indicate that moisture content plays an important role in the osmoregulatory mechanism of penaeid shrimp. Even after gradual adaptations and a 1-day acclimation at the new salinities, shrimp subjected to a dilution of the environment had a statistically significant higher moisture content than shrimp subjected to an osmotically more concentrated environment. However, the role of moisture during osmoregulation may be temporal since during prolonged acclimation the moisture content appeared to gradually revert back to normal levels independent of the salinity to which the shrimp had been exposed.

Chloride Content. The concentrations of chloride ions, reported as percent sodium chloride, in brown shrimp adapted to different salinities are shown in Table II. In order to compensate for potential variations in the moisture content, the sodium chloride concentrations are reported both on a wet- and dry-weight basis.

As is evident from Table II, the tissue chloride concentration in shrimp increased with increasing salinities and decreased with decreasing salinities. This is in agreement with earlier reports (McFarland and Lee, 1963; Bursey and Lane, 1971). The response of tissue chloride content however appeared to be much more dramatic toward salinities above 30 ppt than toward salinities below 30 ppt. This is best exemplified by the analyses of variance that indicated no significant difference in the chloride content between shrimp subjected to 10 and 30 ppt, while between shrimp subjected to 50 ppt and shrimp kept at 30 ppt there was a consistently significant difference throughout the testing period. Within each sampling group, variation in the chloride content with time after final salinity had been reached was also most dramatic in the tissue of shrimp exposed to 50 ppt. The highest concentration of sodium chloride was found in shrimp that had been kept for 7 days at 50 ppt and contained 0.74% NaCl.

Free Amino Acid Content. Similar to the chloride concentration, the tissue free amino acid concentration was also demonstrated to be salinity dependent. High environmental salinity resulted in a high free amino acid content while low environmental salinity gave a low free

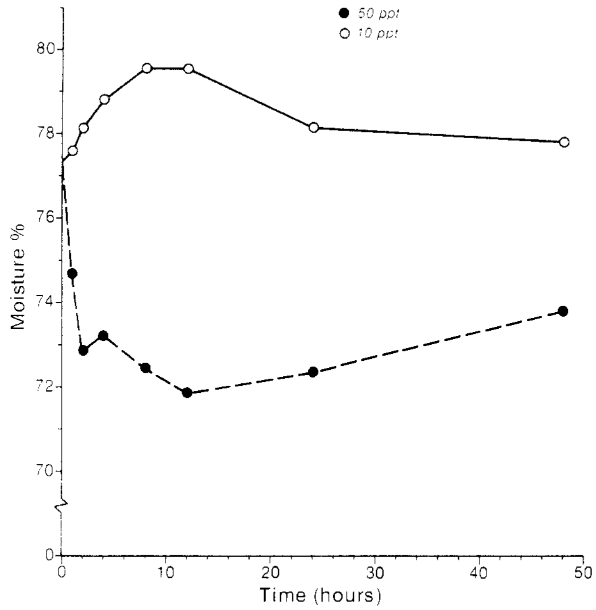

Figure 1. Effect of acute salinity changes on the moisture content of *P. aztecus*. Original salinity 25 ppt.

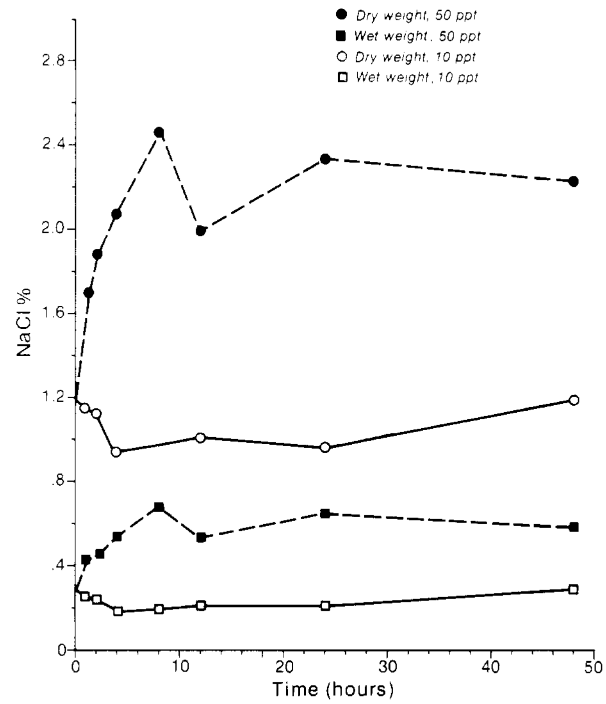

Figure 2. Effect of salinity changes on the chloride content of *P. aztecus*. Original salinity 25 ppt.

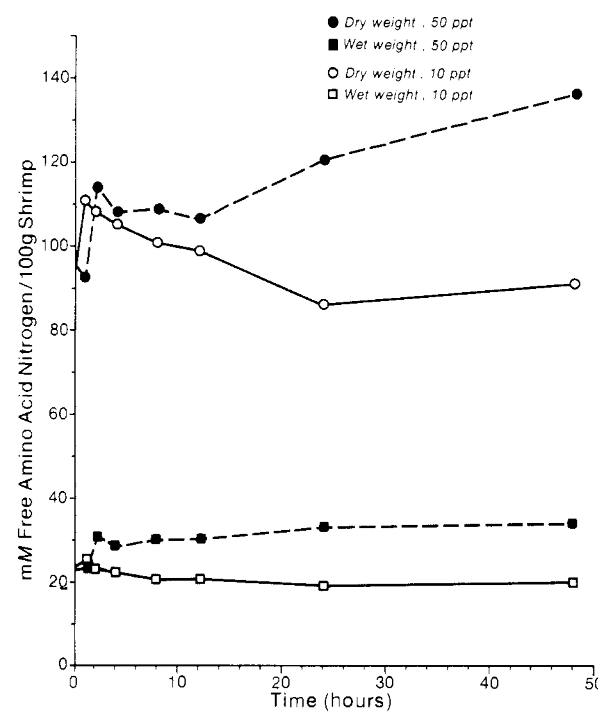

Figure 3. Effect of acute salinity changes on the free amino acid concentration of *P. aztecus*. Original salinity 25 ppt.

amino acid content. These observations are consistent with previous reports on *Penaeus vannamei* (McCoid et al., 1984) and *Penaeus japonicus* (Sameshima and Shimamura, 1980). Table III shows the effect of environmental salinity on the free amino acid content in *P. aztecus* exposed to different salinities. Although the free amino acid concentrations of shrimp subjected to 10 ppt were lower than that in shrimp held at 30 ppt, the differences were not consistently statistically significant. However, for every sampling period, the free amino acid content was significantly higher in the tissues of shrimp subjected to 50 ppt than in shrimp held at 30 ppt or shrimp subjected to 10 ppt. Within each salinity group, the free amino acid content in shrimp held at 50 ppt showed the strongest variation with time after final salinity had been reached. From day 1 onward, there was a gradual increase in the free amino acid concentration until the seventh day after which the concentration dropped. It is noteworthy that, on the seventh day, the shrimp acclimated to 50 ppt had maximum moisture content, maximum salt content, and also maximum free amino acid content.

The average free amino acid nitrogen concentration in the tissue of *P. aztecus* over a period of 9 days after being gradually acclimated to 50 ppt salinity was 30.29 mM/100 g with a standard deviation of 2.57. This is in close agreement with McCoid et al. (1984), who reported an average free amino acid nitrogen concentration in *P. vannamei* acclimated to 50 ppt of 28.71 mM/100 g, indicating no species difference with regard to the role that free amino acids play during osmoregulation in penaeid shrimp.

Acute Changes in Salinity. Changes in moisture, chloride (as percent NaCl), and free amino acid nitrogen content in the tail muscle tissue of brown shrimp subjected to acute changes in salinity from 25 to 50 and 10 ppt are shown in Figures 1–3, respectively.

Moisture Content. Within 1 h after the environmental salinity had been acutely changed, there was a statistically significant ($p < 0.05$) difference in the moisture content between the shrimp changed to 10 ppt as compared to the shrimp changed to 50 ppt. The shrimp exposed to dilution of the environment absorbed moisture while shrimp subjected to an increase in salinity lost moisture. This process continued through the first 12 h after the salinities had been changed at which time the difference had reached a maximum of over 7 percentage points. Visual observations indicated that the shrimp during this period were under heavy stress, especially the group subjected to an increase in salinity. After the initial 12-h period, the trend reversed itself as shrimp kept at 10 ppt appeared to expel moisture while shrimp kept in 50 ppt reabsorbed part of the moisture lost during the first 12 h.

The data indicate that tissue moisture plays an important part in the osmoregulatory mechanism of shrimp especially during the early stages following changes in the environmental salinity. Immediate response toward hyper- or hypoosmotic shocks are changes in tissue hydration presumably through both passive or active mechanisms allowing for the equilibration of external and internal osmotic concentrations.

Chloride Content. The effects of acute salinity changes on the tissue chloride content (reported as percent NaCl) of brown shrimp are shown in Figure 2. As was the case after gradual changes in salinity, the tissue chloride concentration increased with increasing salinity and decreased with decreasing salinity. Analysis of variance indicated a statistically significant difference ($p < 0.05$) in the chloride content in tail muscle of shrimp changed to 10 ppt as compared to 50 ppt even at 1 h after the change. The maximum difference in chloride content between shrimp changed to 10 ppt as compared to 50 ppt occurred after 8 h when shrimp held at 50 ppt contained approximately 0.5 percentage point more salt than shrimp held at 10 ppt. Beyond 8 h after the changes in salinity, the tissue chloride content appeared to have stabilized, even showing a slight reversal in the trend that was apparent immediately after the changes in salinity.

Siebers et al. (1972) stated that the osmotic pressure of the extracellular fluids in the crab *Carcinus maenas* could almost be totally accounted for by inorganic ions. Sodium and chloride ions have also been shown to be the major contributors to the osmotic pressure exerted by the hemolymph in crustaceans (Schoffeniels and Gilles, 1970). Castille and Lawrence (1981) further showed that, in shrimp, Na^+ and Cl^- are maintained at higher concentrations in the hemolymph than in ambient seawater at low salinities and at lower concentrations at high salinities. Our study showed a rapid response in tissue chloride with changes in environmental salinity. These rapid reponses indicate that inorganic ions contribute to the osmotic pressure balance in brown shrimp.

Free Amino Acid Content. Figure 3 shows the effect of environmental salinity on the free amino acid concentration in shrimp subjected to acute changes in salinity. Calculated on a wet-weight basis, there was a statistically significant difference in the free amino acid content of shrimp changed from 25 to 10 and 50 ppt at 2 h after the change in salinity. This difference remained throughout the entire testing period. However, with the exception of some variation within the first 2 h, calculated on a dry-weight basis there was no significant difference in the free amino acid concentration between the two sets of shrimp during the first 12 h after the change in salinity. After 12 h, the concentration of free amino acids as calculated on a dry-weight basis changed dramatically. Shrimp subjected to 50 ppt showed an increase in free amino acids while shrimp subjected to 10 ppt showed a marked decrease.

The data indicate that free amino acids contribute to the osmotic balance in shrimp subjected to different salinities. However, due to an apparent delay in free amino acid synthesis, these compounds do not contribute much to the regulation of osmotic pressures the first 12 h after the salinities have been changed. During this very early period after changes in salinity, inter- and intracellular fluids seem to be kept in balance with extracellular fluids through regulation of the tissue moisture content.

CONCLUSIONS

Both processing characteristics and eating quality of shrimp are affected by the environmental salinity of the water in which the animals are harvested. In order to keep the internal osmotic pressure in balance with the external osmotic pressure, shrimp subjected to an increase in salinity will immediately lose moisture while shrimp subjected to a dilution of the environment will gain moisture. On a wet-weight basis, the concentration of osmoregulators thus appears to change with changes in salinity. The tissue chloride content will also respond quickly to changes in salinity. In absolute terms, the chloride content will increase with increasing salinity and decrease with decreasing salinity. The contribution of free amino acids to cellular osmotic regulation was significant but dramatically delayed. As best exemplified during acute changes in salinity, it took 12 h before the concentration of free amino acids responded to a salinity change. During the initial 12 h, the moisture content thus appeared to be the balancing force between external and internal osmolarity. However, after 12 h the free amino acid content began to respond to the changes in salinity and the moisture content reverted back toward normal levels.

As pointed out by McCoid et al. (1984), due to the flavor characteristics of free amino acids, shrimp grown at high salinities are no doubt more flavorful than shrimp grown at low salinities. The additional salt content resulting from growth at high salinities will also enhance the flavor effect. However, if this technology is to be used during culturing of penaeid shrimp, a long acclimation period is needed in order to realize the full benefits of flavor enhancement. If shrimp are harvested too soon after a salinity manipulation, the yield will be low due to a low moisture content. If on the other hand the shrimp are allowed to acclimate completely, a product of maximum flavor can be harvested at maximum yeild.

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