Effect of salinity in survival, growth, and osmotic capacity of early juveniles of *Farfantepenaeus brasiliensis* (decapoda: penaeidae)

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Abstract

The effect of salinity in survival, growth and osmotic capacity of juvenile *Farfantepenaeus brasiliensis* was determined. Survival was not affected by salinities from 15 to 35\% during 96 h exposure periods. Shrimps exposed to 5 and 10\% were noticeably affected by salinity with a survival of 0 and 48\% after 96 h, respectively. Lethal salinity for 50\% of the individuals in 96 h (LS\textsubscript{50}, 96 h) at 28°C was 10\%. Growth rate (mg dw day \textsuperscript{-1}) was significantly higher at higher salinities. The isosmotic point of early juvenile *F. brasiliensis* was 794 mOSm kg \textsuperscript{-1}, at around 25\%. Results indicate that *F. brasiliensis* has low tolerance to low saline environments, and grows better in salinities greater than its isosmotic point. Osmotic capacity seemed to be related to biological characteristics that determine the environmental preferences and behavior, as well as the distribution of this shrimp species. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

Increased interest in the physiology of penaeid shrimp has derived from the aquaculture industry in many regions of the world. Many studies have focused on the biological aspects of several shrimp species, and increasing knowledge in this research field has allowed optimal conditions for their culture. For *Farfantepenaeus brasiliensis*, not much information of the biology is known, although it is distrib-
uted along the Florida coast in USA to the Bermudas and the Antilles, and along the Atlantic coast of South America to Rio Grande do Norte, Brazil (Sandoval, 1996).

In Mexico, *F. brasiliensis* has great economic importance in the fishery around the Peninsula of Yucatan. In this region, red shrimp are caught all year, mainly in the northwest of the Isla Contoy (Arreguín-Sánchez, 1981) and in a lesser amount in the Gulf of Mexico toward Cabo Catoche (Silva Neto et al., 1982).

Little is known about this species, the present information is mainly on its distribution and fishery. Biological and physiological aspects are virtually unknown; hence the need to do research that contributes to the knowledge of the environmental requirements of *F. brasiliensis* and to help establish a basis for its culture. To date, there are no studies that approach, in any integral way, the effects of salinity on the physiology of this shrimp species.

Postlarvae and juveniles are exposed to salinity, temperature, dissolved oxygen, and pH changes characteristic of coastal environments. Numerous investigations have demonstrated that when juveniles are exposed to environmental changes, there are modifications in the ionic and osmotic balance (Dall and Smith, 1981; Castille and Lawrence, 1981; Rosas et al., 1999a), respiratory activity (Dalla Via, 1986; Kutty et al., 1971; Rosas et al., 1999a; Chen and Nan, 1995), assimilation and growth (Chen et al., 1992; Ogle et al., 1992; Bray et al., 1994; Rosas et al., 1999b), and ammonia excretion (Spaargaren et al., 1982; Chen and Lai, 1993; Chen and Nan, 1995). These results have provided evidence that penaeid shrimp are well adapted to tolerate environmental fluctuations.

According to Castille and Lawrence (1981), and Claybrook (1983), the adaptive capacity of penaeid shrimps is specific and is determined by a number of evolutionary factors that have caused shrimp species to be distributed differently in the estuarine-marine gradient. *F. brasiliensis* have been associated with a high saline environment from north of Brazil to the Caribbean Sea (Scelzo and Zúñiga, 1987; Sandoval, 1996). For that reason, *F. brasiliensis* may be identified as have been *F. aztecu* and *F. duorarum* as limited euryhaline shrimp species (Zein-Eldin and Renaud, 1986; Wenner and Beatty, 1993). In that sense, (Venkataramiah et al., 1974) showed that such characteristic could be related with limitation on physiological mechanisms associated with the osmotic balance, which affect the energetic metabolism, the osmotic pressure, and finally the growth. On this base is possible to think that limited capacity for osmotic regulation of *F. brasiliensis* could be associated with the natural range distribution of this species.

Osmotic capacity (OC) is a useful indicator to measure the stress of shrimp exposed to a diversity of environmental factors, among them dissolved oxygen (Charmantier et al., 1994; Rosas et al., 1999a) and pollutants (Lignot et al., 1997). Unpublished data from studies in our laboratory have demonstrated that coupled to nitrogen excretion, hemolymph glucose, and hepatopancreas glycogen levels, OC allow us to determine optimum conditions for growth when shrimp are fed diets with different carbohydrate levels. The objective of this study was to determine the effect of salinity on growth, survival, and osmotic capacity of juvenile *F. brasiliensis*.
2. Material and methods

2.1. Origin of the animals

The postlarvae and early juveniles of Farfantepeneus brasiliensis used in the experiments were bred in the Group of Experimental Marine Biology, Faculty of Sciences, UNAM, in Ciudad del Carmen, Campeche, from spawners captured along the coasts of Quintana Roo, Mexico. Larvae from naupli I to postlarva 27 (PL_{27}, 27 days after the last metamorphic molt) were maintained in a fiberglass tank of 400 l capacity, at 37\(^{\circ}\)C salinity, constant aeration, and pH > 8. Larvae were fed a mixture of unincellular algae (diatoms and flagellates) and freshly hatched artemia nauplii to PL_{27} (Gallardo et al., 1995). At PL_{27}, shrimp were fed a mixture of artemia nauplii (25\%) and balanced food (75\%) with 50\% protein at 120\% of body weight.

2.2. Tolerance to abrupt salinity changes

To determine survival rate, shrimp at PL_{28} were transferred without previous acclimatization from rearing salinity (37\%\text{e}) to water at 5, 10, 15, 20, 25, 30, and 35\%\text{e}. Ten postlarvae in each salinity treatment (three replicates per treatment) were placed in 5 l plastic containers, and the postlarvae surviving after 96 h counted.

Water in each treatment was prepared the day before the experiments by diluting filtered (5 \(\mu\)m) and ultraviolet sterilized seawater with commercial purified water. The salinity was determined using an Atago refractometer with \(\pm 1\%\text{e}\) precision. Organisms were maintained under controlled experimental conditions of temperature (28\(^{\circ}\)C), photoperiod (12 h:12 h, light: dark) with lighting between 280 and 650 lux, and constant aeration. Shrimp were fed pellet food G-GAX (Gaxiola, 1994) with 50\% protein and with particle size between 210 and 300 \(\mu\)m at 20\% of body weight, and with freshly hatched artemia nauplii. Shrimp were fed two times daily at 1000 and 1800 throughout the experiments. Daily water exchange was 50\% in all the experimental tanks, with feces, sheds, and remaining food being withdrawn from all containers. The absence of movement and absence of response to touch were the criteria for death. Surviving shrimp were counted at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 h of exposure in each treatment. Dead shrimp were removed at time of each observation.

2.3. Osmotic pressure

Early juvenile F. brasiliensis (248±16 mg \text{ww}) were transferred directly from 37\%\text{e} to salinities of 5, 10, 15, 20, 25, 30, 35, and 40\%\text{e} at a temperature of 28\(^{\circ}\)C. After 2 h in these conditions, shrimp were dried on absorbent paper and samples of hemolymph (20 \(\mu\)L from one to three individuals) were taken using a hypodermic needle inserted in the heart (\(n\) between 7 and 17 samples). Osmotic pressure of three water samples in each treatment was also measured. To measure the osmotic pressure, a microosmometer (3 MO Plus, Advanced Instruments, Inc., Norwood, Massachusetts, USA) was used. Only shrimp in the intermolt stage were used for these determinations.
2.4. Growth and survival in different salinities

Before the experiments, a group of 40 individuals was randomly selected, dried on absorbent paper, and weighed on an OHAUS analytical balance (±0.0001 g) to obtain their initial wet weight. Thereafter, individuals were dried in a stove at 60°C for 24 h and were weighed to obtain the dry weight.

The effect of the salinity on growth and survival of shrimp was studied in animals acclimated to three salinities; 15, 25 and 35%. Sixteen PL were placed in 30L plastic tanks, which were used in each treatment (one replicate per treatment). Shrimp used in 15 and 25% salinities were acclimated through a device that supplies tap water to the experimental tanks until the required salinity was obtained (48 h). However, those treated at 35% were transferred directly from seawater of 37%.

Shrimp were maintained for 60 days at constant temperature (28°C), with a photoperiod of 12 h: 12 h, light: dark, and constant aeration. Shrimp were fed two times a day (pellets G-GAX, 50% protein) at 20% of body weight. Daily water exchange was 50% in all the experimental tanks. Feces, sheds, and the remaining food were eliminated every day during water exchange. Surviving shrimp were counted and temperature, pH, and dissolved oxygen were recorded on a daily basis. At the end of the experiment, shrimp were dried and weighed as described previously.

Differences in osmotic pressure, final weight, and growth rate between treatments were analyzed by one-way analysis of variance. When significant differences were found, Duncan’s Multiple Range test was used to identify significant differences between treatments. Differences are reported as statistically significant when $P < 0.05$ (Zar, 1984). The program STATISTICA 4.5 was used for statistic analysis.

3. Results

3.1. Tolerance to abrupt salinity changes

Survival of postlarval *F. brasiliensis* was not affected by salinities between 15 and 35% during 96 h exposure (Fig. 1). By contrast, shrimps at 10 and 5% were affected by salinity with survival of 48 and 0%, respectively, after 96 h. Lethal salinity $L_{50}$ for 96 h was 10% (Fig. 1).

3.2. Osmotic pressure (OP)

The isosmotic point of early juvenile *F. brasiliensis* (248±16 mg ww) was found around 25% (794 mOsm kg$^{-1}$), a salinity under which the animals were hyperosmotic. The highest OP was recorded in shrimp at 35% (872 mOsm kg$^{-1}$), and it was significantly different from the OP in 25%, 40%, 15–20–30%, 10% and 5% salinities ($P < 0.05$). The OP of shrimp at 35% was 2.3 times that at 5% (Table 1) ($P < 0.05$). There was found a significant linear relation between medium and hemolymph osmotic pressure with a slope value of 0.33 ($r^2 = 0.68$, $F = 12.80$, $P = 0.01$).

The osmoregulatory capacity (OC) of early juvenile *F. brasiliensis* varied with
salinity. The hypo-OC of shrimp at 40\% ( \approx 421 \text{ mOsm kg}^{-1} ) was 2.3 times greater than that in animals at 30 and 35\% (average of \approx 180 \text{ mOsm kg}^{-1} ) (P < 0.05). The lowest hyper-OC was observed in shrimp at 25\% (40 \text{ mOsm kg}^{-1} ), and increased with decreasing salinity until it reached its maximum value in shrimp at 10\% (287 \text{ mOsm kg}^{-1} ). The hyper-OC of animals at 5\% (251 \text{ mOsm kg}^{-1} ) was similar to that in shrimp at 15\% (246 \text{ mOsm kg}^{-1} ). Fig. 2 shown the OC for several penaeid species, comparing the OC in extreme salinities, we can separate \textit{F. brasilienensis} with the highest hypo-OC from \textit{F. chinensis} and \textit{L. stilyrostris}, when analyzing the hyper-OC, \textit{F. brasilienensis} and \textit{M. japonicus} have the lowest values and \textit{L. stylirostris} and \textit{L. setiferus} the highest values.

### 3.3. Growth and survival in different salinity treatments

During growth experiments, water temperature was maintained at 28±0.5°C, pH

<table>
<thead>
<tr>
<th>Salinity (%)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>F (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrimp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>7</td>
<td>11</td>
<td>17</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>380(^{(a)})</td>
<td>603(^{(a)})</td>
<td>733(^{(a)})</td>
<td>714(^{(a)})</td>
<td>833(^{(a)})</td>
<td>737(^{(a)})</td>
<td>872(^{(a)})</td>
<td>798(^{(3)})</td>
<td>179.4</td>
</tr>
<tr>
<td>S.E.</td>
<td>13.9</td>
<td>8.1</td>
<td>11.5</td>
<td>4.9</td>
<td>9.1</td>
<td>11.0</td>
<td>10.1</td>
<td>13.8</td>
<td></td>
</tr>
</tbody>
</table>

| Water        |   |    |    |    |    |    |    |    |      |
| n            | 3  | 3  | 3  | 3  | 3  | 3  | 3  | 3  |      |
| Mean         | 186 | 307 | 487 | 598 | 794 | 905 | 1071 | 1219 |      |
| S.E.         | 1.4 | 0.6 | 0.5 | 3.0 | 1.4 | 1.2 | 2.8 | 1.2 |      |

\(^{(a)}\) Different letters mean statistical differences (P < 0.05).
varied between 8.002 ± 0.106 in 15% e and 8.178 ± 0.100 in 35% e. Dissolved oxygen was kept close to the saturation level, varying from 6.95 ± 0.20 in 15% e to 6.28 ± 0.15 mgO₂ l⁻¹ in 35% e salinity. Final body weights and growth rates (mg dw day⁻¹) were significantly greater in shrimp maintained at 35% e (P < 0.05) (Table 2). Tissue water content was not affected by the different treatments. For 62 to 64 days, salinity did not affect the survival of shrimp maintained in any of the three experimental salinities (93.8% survival) (Table 2).

Table 2
Growth rate (mg day⁻¹), body water (%), and survival (%) of *F. brasiliensis* in different salinities. Mean ± S.E. *a*

<table>
<thead>
<tr>
<th>SALINITY %e</th>
<th>15</th>
<th>25</th>
<th>35</th>
<th>F (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial wet weight, mg</td>
<td>210 ± 1.1</td>
<td>210 ± 1.1</td>
<td>210 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Initial dry weight, mg</td>
<td>4.79 ± 0.2</td>
<td>4.79 ± 0.2</td>
<td>4.79 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Final wet weight, mg</td>
<td>500 ± 21b</td>
<td>462 ± 32b</td>
<td>568 ± 23a</td>
<td>4.25 (0.02)</td>
</tr>
<tr>
<td>Final dry weight, mg</td>
<td>127 ± 5b</td>
<td>122 ± 11b</td>
<td>151 ± 6a</td>
<td>4.46 (0.02)</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>7</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Days of culture</td>
<td>62</td>
<td>63</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Growth rate, mg dw day⁻¹</td>
<td>1.98 ± 0.09b</td>
<td>1.87 ± 0.18a</td>
<td>2.30 ± 0.09a</td>
<td>3.61 (0.04)</td>
</tr>
<tr>
<td>Body water, %</td>
<td>74.5 ± 0.4</td>
<td>73.7 ± 0.9</td>
<td>73.2 ± 0.06</td>
<td>1.27 (0.29)</td>
</tr>
<tr>
<td>Survival, %</td>
<td>93.8</td>
<td>93.8</td>
<td>93.8</td>
<td></td>
</tr>
</tbody>
</table>

*a* Different letters mean statistical differences (P < 0.05).
4. Discussion

Postlarval (PL) *F. brasiliensis* showed a wide range of tolerance to salinity changes, which closely corresponds with the adaptive mechanisms in penaeids that, at this stage of their life cycle, inhabit estuarine zones and are exposed to variable salinity. Postlarval *F. brasiliensis* grow in coastal lagoons or estuaries for 2 to 4 months before they migrate into the marine environment for reproduction (Sandoval, 1996).

Lethal salinity for 50% of the animals (LS<sub>50</sub>) varies among species and depends on the temperature and developmental stages of the organisms under study. Charmantier-Daures et al. (1988) found that in *Marsupenaeus japonicus* PL the SL decreased with increasing temperature, whereas in *Fenneropenaeus chinensis*, a relation between SL and temperature did not appear to be so direct, and PL was more resistant to salinity changes than other penaeid species.

The osmoregulatory capacity (OC) is a useful test to evaluate the physiological conditions of shrimp in cultivation and to detect the effects of sublethal stress (Charmantier et al., 1994). The OC has been related to temperature and developmental stages of shrimp (Charmantier-Daures et al., 1988), to the molting stage, and to variations in dissolved oxygen (Charmantier et al., 1994). According to these authors, a higher OC means a greater capacity to compensate for a salinity change, which may be reduced when the animals are in an adverse situation. The differences observed between OC obtained from literature and that obtained from *F. brasiliensis* experiments could indicate that *F. brasiliensis* and *M. japonicus* had a lower capacity to tolerate a diluted environment than observed in other species (Fig. 2).

The slope of the relation between medium and hemolymph osmotic pressure has been used to compare the OC among penaeid species (Castille and Lawrence, 1981; Parado-Estepa et al., 1987; Chen et al., 1992). When the slopes of this relation for different penaeid shrimp are compared (Table 3), shrimp of the genus *Litopenaeus* and *Fenneropenaeus* have the lowest values and those belonging to the genus *Farfan*tepenaeus have the highest slope values. As the deviation of the isosmotic line reflects the degree of regulation (slope = 0 means osmoregulator, slope = 1 means osmoconformer), organisms with high slope values have a poorer hyperosmotic regulation than those that have low values of the slope (Castille and Lawrence, 1981).

Analyzing the differences in the OC, we can separate the shrimp that inhabit more marine environments with greater hyporegulatory capacity (*F. brasiliensis, M. japonicus, F. aztecus, F. duorarum*) from those that inhabit more estuarine environments with greater hyperregulatory capacity (*L. stylirostris* and *L. setiferus*) (Fig. 2, Table 3). The
Table 3
Slopes of the linear relation between hemolymph and medium osmotic pressure in different penaeid species

<table>
<thead>
<tr>
<th>Species</th>
<th>Slope</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litopenaeus stylirostris</td>
<td>0.16</td>
<td>Castille and Lawrence (1981)</td>
</tr>
<tr>
<td>Fenneropenaeus chinensis</td>
<td>0.189</td>
<td>Chen et al. (1992)</td>
</tr>
<tr>
<td>Litopenaeus stylirostris</td>
<td>0.20</td>
<td>Charmantier and Aquacop (pers. comm.)</td>
</tr>
<tr>
<td>Litopenaeus vannamei</td>
<td>0.20</td>
<td>Castille and Lawrence (1981)</td>
</tr>
<tr>
<td>Litopenaeus setiferus</td>
<td>0.20</td>
<td>Rosas et al. (1999a)</td>
</tr>
<tr>
<td>Litopenaeus setiferus</td>
<td>0.23</td>
<td>Castille and Lawrence (1981)</td>
</tr>
<tr>
<td>Fenneropenaeus indicus</td>
<td>0.24</td>
<td>Parado-Estepa et al. (1987)</td>
</tr>
<tr>
<td>Farfantepenaeus brasiliensis</td>
<td>0.33</td>
<td>This paper</td>
</tr>
<tr>
<td>Farfantepenaeus aztecus</td>
<td>0.38</td>
<td>Castille and Lawrence (1981)</td>
</tr>
<tr>
<td>Farfantepenaeus duorarum</td>
<td>0.40</td>
<td>Castille and Lawrence (1981)</td>
</tr>
</tbody>
</table>

OC of penaeid shrimp can be related to their distribution according to salinity. (Scelzo and Zúñiga, 1987) found juvenile *F. brasiliensis* in high densities in water slightly hypersaline in the shallow Laguna La Restinga in Venezuela. Wenner and Beatty (1993) found in estuarine habitats in South Carolina that the distribution peaks of *L. setiferus* postlarvae coincide with a decrease in salinity in the area. In addition, the decrease in salinity contributes to the movement of *F. duorarum* out of the area and the decrease in salinity and temperature explain the low recruitment of this species into the estuarine system. Zein-Eldin and Renaud (1986), who analyzed the influence of environmental factors on the populations of *F. aztecus* and *L. setiferus* along the coast of Texas, have reported similar behaviors. Though both species are found distributed in a wide range of salinities, *L. setiferus* prefers lower salinities and stays near the coast to grow and reproduce, whereas *F. aztecus* prefers higher salinities, increases their burying activity in low salinities, and migrate to reproduce far away from the coast.

The isosmotic point of early juvenile *F. brasiliensis* (25%, 794 mOsm kg⁻¹) obtained in the present study is within the range of isosmotic point found for many penaeid species. For *M. japonicus* the isosmotic point is about 820 mOsm kg⁻¹ in postlarvae and 880 mOsm kg⁻¹ in adults, in *F. chinensis*, this point has been reported as 700 mOsm kg⁻¹ for postlarvae and 780 mOsm kg⁻¹ for adults (Charmantier-Daures et al., 1988). In *F. indicus* of 5–10 g, the isosmotic point was 780 mOsm kg⁻¹ (24%) (Parado-Estepa et al., 1987). Castille and Lawrence (1981) reported the isosmotic point in *F. aztecus* juveniles at 25.6%, in *F. duorarum* at 26.3%, in *L. setiferus* at 23.3%, in *L. stylirostris* at 24%, and in *L. vannamei* at 24.7%. In *Penaeus monodon*, the isosmotic point is at 26% (Ferraris et al., 1986).

The isosmotic point has been associated with optimum conditions for growth of penaeid shrimp. However, when comparing different isosmotic points reported and optimum salinity for growth in different shrimp species, it is possible to observe that not always does better growth coincide with the isosmotic point reported (Fig. 3). Some species grow better in salinities above their isosmotic point (*F. brasiliensis*, this work; *M. japonicus*, Liao, 1969, and *P. esculentus*, O’Brien, 1994), some have better growth in salinities near the isosmotic point (*F. indicus*, *P. monodon*, and *P. semisulcatus*, Raj and Raj, 1982), and a third group grow better in salinities below the isosmotic point (*F.
Fig. 3. Optimum growth salinities (bars) and isosmotic point (●) reported for several shrimp species. *F. brasiliensis* (this paper), *M. japonicus* (Liao, 1969), *P. monodon, F. indicus, P. semisulcatus* (Raj and Raj, 1982), *F. chinensis* (Chen et al., 1992), *F. aztecus* (Venkataramiah et al., 1974), *L. setiferus* (Rosas et al., 1999b), *L. vannamei* (Bray et al., 1994), *P. esculentus* (O’Brien, 1994).

The results obtained in our work provide evidence that *F. brasiliensis* is a species with limited capacity to tolerate low salinities and grows well in salinities greater than its isosmotic point. Results show that the isosmotic point is not strongly related to greater growth in this and some other penaeid shrimp. However, the osmotic capacity seems to have a direct relation with the biological characteristics of the species that determine environmental preferences and behavior, which in turn influence distribution.
in the estuarine ecosystem. The possibility of correlating more biological aspects of penaeid shrimp through evaluations of OC causes us to recommend the OC as the most adequate way of presenting osmotic pressure results in penaeid species.

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