Effects of Salinity and Dissolved Oxygen Concentration on the Tail-Flip Speed and Physiologic Response of Whiteleg Shrimp, *Litopenaeus vannamei*

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Abstract: The swimming ability of shrimp is important for their survival and growth, which directly affects their avoidance of enemies and uncomfortable environment, search and capture of food, reproductive behavior, and distribution. The knowledge concerning the swimming ability of shrimp can be widely used in the conservation of fishery resources, improving capture efficiency and stock enhancement. As one of the edible marine organisms, *Litopenaeus vannamei* is a traditional fishery resource and an important economic aquaculture species in China. Dissolved oxygen (DO) concentration and salinity are considered to play crucial roles in the swimming ability of *L. vannamei*. The tail-flip speed ($S_{tf}$) of whiteleg shrimp *L. vannamei* (79.90 ± 0.41 mm, 5.76 ± 0.10 g) that were exposed to various salinities (20 ‰, 25 ‰, 30 ‰, 35 ‰, and 40 ‰) and DO concentrations (1.9, 3.8, 6.8, and 13.6 mg/L) was determined under laboratory conditions. Metabolite concentrations in the hemolymph, hepatopancreas, and abdominal muscles were measured before and after tail-flip fatigue to evaluate the physiologic effects of fatigue in *L. vannamei*. The results showed that salinity and DO significantly affected the $S_{tf}$ of *L. vannamei*. The $S_{tf}$ increased and subsequently decreased with the increase in salinity from 20 ‰ to 40 ‰. The relationship between $S_{tf}$ and salinity ($s$, ‰) can be expressed by the quadratic model as $S_{tf} = -0.2386s^2 + 15.528s - 145.12$, $R^2 = 0.9693$. The optimum salinity and corresponding maximum $S_{tf}$ were 32.54 ‰ and 107.52 cm/s, respectively. The $S_{tf}$ increased as the DO concentration increased from 1.9 mg/L to 13.6 mg/L. The relationship between $S_{tf}$ and DO (mg/L) can be expressed by the power model as $S_{tf} = 75.621 DO^{0.1753}$, $R^2 = 0.9981$. The different salinities and DO concentrations directly affected the physiology of the shrimp, inducing changes in hepatopancreas total protein, plasma total protein, abdominal muscle lactate, plasma lactate, plasma glucose, hepatopancreas glycogen, and abdominal muscle glycogen concentration. Fatigue from tail-flip led to severe loss of hepatopancreas glycogen under 20 ‰ salinity and plasma glucose under 25 ‰, 30 ‰, and 35 ‰ salinity. The triglyceride and lactate in the plasma concentration increased significantly in a range of salinities. In the DO concentration experiment, fatigue from tail-flip led to a severe loss of plasma glucose under 1.9 mg/L and 3.8 mg/L DO concentrations. The plasma lactate concentration increased significantly in all DO groups. The results suggested that the inappropriate salinity and DO significantly limited the tail-flip speed of shrimp, which was due to the accumulation of metabolites. The proper salinity and DO accelerated the elimination of metabolites, reduced the energy consumption of shrimp, and thus, improved the exercise ability of shrimp. This conclusion is of particular value in evaluating the swimming ability of shrimp and understanding its ecological processes to improve capture and rearing techniques.

Keywords: DO concentration; salinity; metabolite; swimming ability; whiteleg shrimp
1. Introduction

In cultured or natural waters, aquatic organisms face various environmental stresses, such as fluctuations in illumination, temperature, salinity, heavy metal, dissolved oxygen (DO), and food supply [1–5]. These environmental variables may also influence the behavior of aquatic animals living in such waters [3]. Furthermore, the exercise ability can reflect the physiologic level of aquatic organism; thus, the effects of environmental elements on the exercise ability can be used to determine the optimum habitat for animal [6].

Little research was performed concerning the effect of salinity on the exercise ability of penaeid shrimp. Yu et al. [6] found the critical swimming speed \( (U_{\text{crit}}) \) of \( L. \text{vannamei} \) is lower, which acclimates to 20‰. Zhang et al. [7] found that with the increase in salinity from 15‰ to 40‰, the swimming ability index of \( L. \text{vannamei} \) showed a trend of increasing first and then decreasing. Salinity stress causes large increases in the oxygen consumption of aquatic animals [8], and it would, therefore, reduce the animal’s scope for activity [9]. Some experimental studies on physiology found that osmotic stress might cause physiological changes. Under such conditions, shrimp may exercise three responses: (1) the increase in salinity caused the accumulation of plasma ions and large increases in plasma osmolality [9]; (2) the standard metabolic rate is greater in full-strength salinity than in near iso-osmotic salinity [9]. (3) the changes in salinity would significantly impair the heart rates and muscle functions. [6] These changes are directly related to a significant reduction in swimming ability.

Some research was performed on the effect of DO concentration on the exercise ability of fish, and little was relevant to penaeid shrimp [10–12]. Recent studies have shown that a deficient oxygen supply decreases energy metabolism or oxygen uptake. Thus, physiologic energy is reduced, provoking generalized metabolic depression [3,5,13–15].

The locomotion modes of shrimp include walking, tail-flip, and swimming [16]. As a favorable way of their defense against trawl gear and predators [17,18], \( L. \text{vannamei} \) have a rapid response of tail-flip escape. Long periods and high levels of tail-flip also occur within the trawl [19], which leads to exhaustion of the shrimp [20–22]. Shrimp are capable of tail-flip in the trawl mouth, and hence, their capture efficiency is dependent on tail-flip speed. Some researchers showed that the maximum tail-flip velocities of penaeid shrimp range from 0.6 m/s to 2.8 m/s [23,24], and the velocities of most species were less than 1 m/s [25]. However, a study on the effect of environmental stresses on tail-flip performance and physiological response of whiteleg shrimp \( L. \text{vannamei} \) has not been performed. This study determines the effects of salinity and DO concentration on the tail-flip speed \( (S_{\text{tf}}) \) and physiologic response of \( L. \text{vannamei} \) under laboratory conditions. These results would provide a scientific basis for determining the optimum habitat, improving the capture and stock enhancement, and assessing the exercise ability of penaeid shrimps.

2. Materials and Methods

2.1. Origin of Animals

The \( L. \text{vannamei} \) used in the current experiment were provided by Shazikou shrimp farm in Shandong Province, China. The shrimp were held in a 2 m³ recirculation tank for 30 d. During the acclimatizing period, the shrimp were fed with commercial pellets twice daily (Haima Brand, China), and before the experiment they were fasted for 24 h. The filtered seawater in the experimental tank was maintained at a salinity of 32.0 ± 1.0‰, a temperature range from 23.0 °C to 25.0 °C, and DO > 6 mg/L. Shrimps at the intermolt stage were used. The molt stage was distinguished based on exoskeleton hardness [26]. The body length (BL) and wet mass (WM) of the test shrimp are shown in Tables 1 and 2. BL was measured as the distance from the base of the eye notch to the posterior end of the telson.
Table 1. Tail-flip speed, distance and number of *L. vannamei* exposed to different salinity (means ± S.E. *n* = 10).

<table>
<thead>
<tr>
<th>Salinity (‰)</th>
<th>Body Length (cm)</th>
<th>Wet Mass (g)</th>
<th>Average SS (cm/s)</th>
<th>Average Distance of One Tail-Flip (cm)</th>
<th>Average Total Distance of Tail-Flips (cm)</th>
<th>Average Total Number of Tail-Flips</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>79.79 ± 1.34 a</td>
<td>5.71 ± 0.25 a</td>
<td>71.65 ± 4.83 b</td>
<td>29.27 ± 2.66 b</td>
<td>463.39 ± 70.56 b</td>
<td>15.86 ± 2.37 b</td>
</tr>
<tr>
<td>25</td>
<td>81.53 ± 1.36 a</td>
<td>6.55 ± 0.37 a</td>
<td>89.78 ± 3.58 ab</td>
<td>31.62 ± 2.27 a</td>
<td>674.10 ± 90.65 b</td>
<td>22.85 ± 2.72 b</td>
</tr>
<tr>
<td>30</td>
<td>80.33 ± 1.15 a</td>
<td>6.11 ± 0.27 a</td>
<td>118.31 ± 10.65 c</td>
<td>32.33 ± 2.71 a</td>
<td>865.86 ± 112.23 b</td>
<td>28.34 ± 2.90 b</td>
</tr>
<tr>
<td>35</td>
<td>79.10 ± 1.28 a</td>
<td>5.74 ± 0.28 a</td>
<td>107.01 ± 4.48 bc</td>
<td>32.02 ± 2.18 a</td>
<td>714.06 ± 93.24 bc</td>
<td>26.27 ± 2.52 b</td>
</tr>
<tr>
<td>40</td>
<td>81.14 ± 1.26 a</td>
<td>6.28 ± 0.29 a</td>
<td>93.29 ± 3.22 b</td>
<td>31.33 ± 2.09 a</td>
<td>534.14 ± 51.15 a</td>
<td>19.34 ± 2.057 a</td>
</tr>
</tbody>
</table>

Note: Values with different letters in the same column indicate significant differences from each other (*p* < 0.05).

Table 2. Tail-flip speed, distance, and number of *L. vannamei* exposed to different DO concentration (means ± S.E. *n* = 10).

<table>
<thead>
<tr>
<th>DO (mg/L)</th>
<th>Body Length (cm)</th>
<th>Wet Mass (g)</th>
<th>Average SS (cm/s)</th>
<th>Average Distance of One Tail-Flip (cm)</th>
<th>Average Total Distance of Tail-Flips (cm)</th>
<th>Average Total Number of Tail-Flips</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9</td>
<td>79.53 ± 0.98 a</td>
<td>5.15 ± 0.21 a</td>
<td>83.42 ± 2.19 a</td>
<td>25.20 ± 1.47 a</td>
<td>466.32 ± 31.19 a</td>
<td>19.89 ± 1.44 a</td>
</tr>
<tr>
<td>3.8</td>
<td>80.33 ± 1.15 a</td>
<td>5.82 ± 0.28 a</td>
<td>93.33 ± 1.82 b</td>
<td>27.42 ± 1.51 ab</td>
<td>585.31 ± 30.26 a</td>
<td>23.86 ± 1.20 ab</td>
</tr>
<tr>
<td>6.8</td>
<td>78.64 ± 0.81 a</td>
<td>5.22 ± 0.17 a</td>
<td>105.02 ± 3.14 c</td>
<td>31.09 ± 1.48 b</td>
<td>794.74 ± 64.27 b</td>
<td>27.26 ± 2.16 b</td>
</tr>
<tr>
<td>13.6</td>
<td>80.75 ± 1.05 a</td>
<td>5.83 ± 0.23 a</td>
<td>120.24 ± 4.47 d</td>
<td>31.60 ± 2.21 b</td>
<td>922.73 ± 69.09 b</td>
<td>33.15 ± 2.265 c</td>
</tr>
</tbody>
</table>

Note: Values with different letters in the same column indicate significant differences from each other (*p* < 0.05).

2.2. Experimental Apparatus

Tail-flip speed (SS) was measured in a glass flume (155 cm × 30 cm × 30 cm, L × W × H). For providing scale, the 5 cm × 5 cm grids were marked at the bottom of the flume. Above the glass flume of 240 cm, a video camera (25 frames/s) was set up for recording the tail-flip performance of the shrimp. The seawater in the flume was maintained at 23 °C.

2.3. Experimental Design

2.3.1. Salinity Experiment

Tests were conducted on five salinity groups (20‰, 25‰, 30‰, 35‰, and 40‰). During the acclimation period, 150 shrimps were divided equally into five tanks (S1, S2, S3, S4, and S5). The seawater salinity in tanks S1, S2, and S3 was adjusted to 20‰, 25‰, and 30‰, respectively, using artificial salt at a rate of 2–3‰ salinity reduction per day. The seawater salinity in tanks S4 and S5 was adjusted to 35‰ and 40‰, respectively, using artificial salt at a rate of 2–3‰ salinity increasing per day. After reaching the experimental salinity, the shrimps were acclimated for 5 d. Subsequently, 10 shrimps were used for the tail-flip speed test at each salinity level. Water in the flume was also adjusted to the corresponding salinity before the tail-flip speed test. The temperature and salinity were kept at 24.0 °C and DO > 6.0 mg/L.

2.3.2. DO Concentration Experiment

We established the effect of DO concentrations at four different oxygen levels (25%, 50%, 100%, and 200% of air saturation). During the experiment, actual DO concentration was 2.0 ± 0.3, 3.8 ± 0.4, 6.8 ± 0.7, 13.6 ± 2.1 mg/L, respectively. For DO acclimation, 120 shrimps were divided equally into four tanks (50 cm × 30 cm × 50 cm), where they were slowly acclimated (in decrements or increments of approximately 1–2 mg/L day). Water was changed with filtered (1 μm) and ultraviolet-sterilized seawater at a daily rate of 5% per tank volume. During the experiment, air, nitrogen, and pure oxygen were used to control the DO. A YSI-55 oxygen detector (YSI Incorporated, Yellow Springs, OH, USA) was used to measure the DO. After reaching the experimental DO, the shrimps were acclimated for 30 d. Subsequently, 10 shrimps were chosen for the tail-flip speed test at each DO level. The water in the flume was also adjusted to the corresponding DO before the tail-flip speed test. The temperature and salinity were kept at 24.6 °C and 33.2‰, respectively.
2.3.3. Tail-Flip Speed ($S_{tf}$)

One shrimp was used for each trial. Before the trial, the shrimp acclimated for 5 min in the experimental flume. During the testing, for inducing the tail-flip, the cephalothorax of the shrimp was tapped with a stick. The stimulus was repeated until the shrimp resettled on the bottom with no response. Then, the shrimp was immediately removed from the flume, and weighed to the nearest 0.01 g after drying with absorbent paper. The video was viewed using Potplayer software. The first and last frames of the tail-flip bout were superimposed with Photoshop software (Adobe) and the distance covered between the rostrums was measured using ImageTool software (UTHSCSA) as tail-flip distance ($d$, cm). Tail-flip time ($t$, s) was calculated as follows: $t = N/25$, where $N$ is the number of tail-flip frames. $S_{tf}$ ($v$, cm/s) was calculated as: $v = d/t$. Three to four tail-flip bouts were measured for each shrimp. The total number of tail-flips ($N_{tf}$) and the distance covered in each tail-flip ($d'$, cm) of each shrimp were measured. The total distance of tail-flips (cm) was calculated as: $N_{tf} \times d'$.

2.4. Tissue Collection and Biochemical Assessment

Ten similarly sized experimental shrimp that were not forced to exercise were taken as the control group. Post-exercise samples were withdrawn immediately after exhaustion of the shrimp. The shrimps were anesthetized in the tank with ice and water and the blood sampling was performed as quickly as possible so as to cause minimal disturbance to the shrimp. Approximately 200 µL hemolymph was collected from the ventral sinus at the base of the first abdominal segment using a 1 mL syringe containing 200 µL cooled anticoagulant solution [27]. The hemolymph was centrifuged at 200 × g for 10 min at 4 °C. The plasma was separated. The hepatopancreas and muscle in the first abdominal segment of the shrimp were dissected. All the samples were stored at −34 °C. Commercial kits were used for determining lactate, triglyceride, glucose, total protein, and glycogen (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu province, China).

2.5. Statistical Analysis

The differences in BL, body mass, $S_{tf}$, and metabolite concentrations in shrimps under different salinities and under different DO concentrations were analyzed using one-way ANOVA, and Duncan’s multiple comparison was used to test the differences among groups. The relationship between $S_{tf}$ and salinity, as well as DO concentration, was determined using curve estimation. Metabolite concentrations in the hepatopancreas, muscle, and plasma of the shrimp before and after tail-flip fatigue were analyzed using independent sample t-tests. Statistical analyses were performed using SPSS 21.0, and differences were considered significant when $p < 0.05$ for all analyses.

3. Results

3.1. $S_{tf}$

3.1.1. Effect of Salinity

The average $S_{tf}$, the average distance covered in each tail-flip, the average total distance of tail-flips and the average total number of tail-flips under different salinities are shown in Table 1. The average $S_{tf}$, the average total distance of tail-flips, and the average total number of tail-flips increased and then decreased with the increase in salinity from 20‰ to 40‰, whereas no significant differences were found for the average distance covered in each tail-flip. The average total distance of tail-flips and the average total number of tail-flips of shrimp at a salinity of 30‰ was significantly higher than those at 20‰ and 40‰ ($p < 0.05$). The average $S_{tf}$ at a salinity of 20‰ was significantly lower than those at 30‰, 35‰, and 40 ‰ ($p < 0.05$). The average $S_{tf}$ at a salinity of 30‰ was significantly higher than those at 20‰, 25‰, and 40‰ ($p < 0.05$). However, no significant differences existed in the average $S_{tf}$ of shrimps exposed to 25‰, 35‰, and 40‰ ($p > 0.05$). The relationship between average $S_{tf}$ and salinity (s) can be described by a quadratic model as $S_{tf} = -0.2386s^2 + 15.528s - 145.12$, $R^2 = 0.9693$ (Figure 1). The optimum salinity and corresponding maximum average
Sustainability 2022, 14, x FOR PEER REVIEW 6 of 14

Sustainability were 32.54% and 107.52 cm/s, respectively. The range of salinities within which the average \( S_{tf} \) was >90% of the maximum were estimated between 25.82% and 39.25%.

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** The relationship between tail-flip speed and salinity of *L. vannamei* (means ± S.E.).

3.1.2. Effect of DO

The average \( S_{tf} \), the average distance covered in each tail-flip, the average total distance of tail-flips, and the average total number of tail-flips under different DO concentrations are shown in Table 2. The average \( S_{tf} \), the average total distance of tail-flips, the average distance covered in each tail-flip, and the average total number of tail-flips decreased as DO decreased from 13.6 mg/L to 1.9 mg/L. The relationship between DO and average \( S_{tf} \) can be described by a power model as \( S_{tf} = 75.621 \text{ DO}^{0.1753} \), \( R^2 = 0.9981 \) (Figure 2). The BL and WM of the shrimp were not significantly different among the DO groups \((p > 0.05)\), whereas the average \( S_{tf} \) of shrimps in different DO concentrations were significantly different \((p < 0.05)\). When the shrimps were maintained in higher DO concentration group (approximately 13.6 mg/L), the average \( S_{tf} \) increased from 83.42 cm/s in hypoxia (approximately 1.9 mg/L) to 120.24 cm/s. The average distance covered in each tail-flip of shrimp at 6.8 and 13.6 mg/L was significantly higher than that of the shrimp at 1.9 mg/L \((p < 0.05)\). The average total distance of tail-flips of shrimp at 6.8 and 13.6 mg/L was significantly higher than those of the shrimp at 3.8 and 1.9 mg/L \((p < 0.05)\). The average total number of tail-flips of shrimp at 6.8 mg/L was significantly higher than that of the shrimp in 1.9 mg/L \((p < 0.05)\), and significantly lower than that of the shrimp at 13.6 mg/L \((p < 0.05)\).

![Figure 2](https://via.placeholder.com/150)

**Figure 2.** The relationship between tail-flip speed and DO concentration of *L. vannamei* (means ± S.E.).
### 3.2. Physiologic Response

Tables 3 and 4 show the physiologic responses in the hepatopancreas, muscle, and plasma of *L. vannamei* before (control) and after exercise fatigue in various salinities and DO concentrations.

**Table 3.** Physiological responses in plasma, hepatopancreas, and abdominal muscle of *L. vannamei* after tail-flip fatigue in different salinities.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Salinity (‰)</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.79 ± 0.13</td>
<td>2.64 ± 0.21</td>
<td>2.34 ± 0.20</td>
<td>3.04 ± 0.40</td>
<td>2.12 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>After exercise fatigue</td>
<td>1.63 ± 0.19</td>
<td>1.90 ± 0.20</td>
<td>1.55 ± 0.16</td>
<td>1.91 ± 0.22</td>
<td>2.11 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mmol L⁻¹)</td>
<td>0.07 ± 0.02</td>
<td>0.18 ± 0.02</td>
<td>0.16 ± 0.02</td>
<td>0.15 ± 0.01</td>
<td>0.18 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.24 ± 0.04</td>
<td>0.30 ± 0.04</td>
<td>0.31 ± 0.05</td>
<td>0.20 ± 0.03</td>
<td>0.25 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Total protein (mg mL⁻¹)</td>
<td>68.40 ± 7.99</td>
<td>116.91 ± 13.11</td>
<td>85.85 ± 10.43</td>
<td>98.95 ± 7.90</td>
<td>92.08 ± 15.60</td>
<td></td>
</tr>
<tr>
<td>Glycogen (mg g⁻¹)</td>
<td>0.42 ± 0.13*</td>
<td>0.59 ± 0.17**</td>
<td>0.23 ± 0.05*</td>
<td>0.44 ± 0.17*</td>
<td>0.61 ± 0.15**</td>
<td></td>
</tr>
<tr>
<td>Lactate (mmol L⁻¹)</td>
<td>1.32 ± 0.10*</td>
<td>1.19 ± 0.24</td>
<td>1.48 ± 0.44*</td>
<td>2.09 ± 0.31*</td>
<td>2.69 ± 0.19*</td>
<td></td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.23 ± 0.49*</td>
<td>6.22 ± 0.85*</td>
<td>3.58 ± 0.40*</td>
<td>2.94 ± 0.73*</td>
<td>3.85 ± 0.43*</td>
<td></td>
</tr>
<tr>
<td>After exercise fatigue</td>
<td>3.77 ± 0.19*</td>
<td>4.65 ± 0.40*</td>
<td>2.56 ± 0.30</td>
<td>3.13 ± 0.48*</td>
<td>4.95 ± 1.40*</td>
<td></td>
</tr>
<tr>
<td>Glycogen (mg g⁻¹)</td>
<td>151.28 ± 8.84</td>
<td>131.98 ± 5.35</td>
<td>110.33 ± 22.60</td>
<td>85.41 ± 7.07</td>
<td>164.82 ± 29.04</td>
<td></td>
</tr>
<tr>
<td>Lactate (mmol g⁻¹)</td>
<td>43.67 ± 5.83</td>
<td>32.01 ± 3.14</td>
<td>30.80 ± 8.23</td>
<td>40.70 ± 3.27</td>
<td>25.25 ± 12.06</td>
<td></td>
</tr>
<tr>
<td>Abdominal muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>42.52 ± 4.58*</td>
<td>22.00 ± 4.73</td>
<td>38.67 ± 3.71</td>
<td>42.02 ± 3.00</td>
<td>36.39 ± 9.18*</td>
<td></td>
</tr>
<tr>
<td>After exercise fatigue</td>
<td>133.69 ± 23.79</td>
<td>148.01 ± 16.15</td>
<td>140.10 ± 12.69</td>
<td>98.95 ± 2.93*</td>
<td>150.56 ± 14.78</td>
<td></td>
</tr>
<tr>
<td>Glycogen (mg g⁻¹)</td>
<td>70.84 ± 9.86*</td>
<td>100.81 ± 13.19</td>
<td>121.77 ± 14.44</td>
<td>108.05 ± 17.28</td>
<td>71.86 ± 7.30**</td>
<td></td>
</tr>
<tr>
<td>Lactate (mmol g⁻¹)</td>
<td>62.30 ± 12.66</td>
<td>86.43 ± 10.02</td>
<td>88.55 ± 12.99</td>
<td>84.07 ± 11.86</td>
<td>54.11 ± 4.07*</td>
<td></td>
</tr>
</tbody>
</table>

Note: Values are means ± SE. Values for the same control or fatigue group without the same letter are significantly different at the 0.05 probability level according to the ANOVA test and the same salinities with * are significantly different at the 0.05 probability level according to the independent-samples T test.

**Table 4.** Physiological responses in plasma, hepatopancreas, and abdominal muscle of *L. vannamei* after tail-flip fatigue in different DO concentrations.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>DO Concentration (mg L⁻¹)</th>
<th>1.9</th>
<th>3.8</th>
<th>6.8</th>
<th>13.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol L⁻¹)</td>
<td>2.03 ± 0.19*</td>
<td>2.04 ± 0.19*</td>
<td>2.30 ± 0.16*</td>
<td>1.52 ± 0.11*</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.19 ± 0.24*</td>
<td>1.87 ± 0.31*</td>
<td>1.68 ± 0.08*</td>
<td>0.93 ± 0.17*</td>
<td></td>
</tr>
<tr>
<td>After exercise fatigue</td>
<td>0.10 ± 0.02*</td>
<td>0.15 ± 0.01*</td>
<td>0.12 ± 0.01*</td>
<td>0.10 ± 0.02*</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mmol L⁻¹)</td>
<td>0.06 ± 0.02*</td>
<td>0.09 ± 0.04*</td>
<td>0.11 ± 0.02*</td>
<td>0.10 ± 0.03*</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>128.88 ± 9.34*</td>
<td>125.99 ± 10.02*</td>
<td>92.07 ± 9.85*</td>
<td>74.47 ± 10.73*</td>
<td></td>
</tr>
<tr>
<td>Total protein (mg mL⁻¹)</td>
<td>163.28 ± 5.29*</td>
<td>112.52 ± 10.47*</td>
<td>96.17 ± 9.52*</td>
<td>110.80 ± 16.98*</td>
<td></td>
</tr>
<tr>
<td>Glycogen (mg g⁻¹)</td>
<td>1.42 ± 0.32**</td>
<td>0.58 ± 0.12*</td>
<td>0.55 ± 0.22*</td>
<td>0.30 ± 0.11*</td>
<td></td>
</tr>
<tr>
<td>Lactate (mmol L⁻¹)</td>
<td>4.60 ± 0.52*</td>
<td>2.39 ± 0.12*</td>
<td>2.43 ± 0.42*</td>
<td>1.38 ± 0.23*</td>
<td></td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>3.44 ± 0.62*</td>
<td>4.41 ± 0.31*</td>
<td>2.90 ± 0.53*</td>
<td>3.09 ± 0.26*</td>
<td></td>
</tr>
<tr>
<td>Glycogen (mg g⁻¹)</td>
<td>4.36 ± 0.31*</td>
<td>4.27 ± 0.22*</td>
<td>3.02 ± 0.28*</td>
<td>3.20 ± 0.62*</td>
<td></td>
</tr>
<tr>
<td>Total protein (mg g⁻¹)</td>
<td>151.28 ± 24.95*</td>
<td>143.82 ± 20.37*</td>
<td>149.19 ± 18.56*</td>
<td>199.43 ± 41.16*</td>
<td></td>
</tr>
<tr>
<td>Lactate (mmol g⁻¹)</td>
<td>27.46 ± 12.79*</td>
<td>29.17 ± 5.05*</td>
<td>23.47 ± 4.00*</td>
<td>29.12 ± 9.81*</td>
<td></td>
</tr>
<tr>
<td>Abdominal muscle</td>
<td>35.64 ± 6.21*</td>
<td>31.02 ± 12.27*</td>
<td>25.74 ± 8.59*</td>
<td>22.39 ± 6.14*</td>
<td></td>
</tr>
<tr>
<td>Glycogen (mg g⁻¹)</td>
<td>7.68 ± 1.11*</td>
<td>8.67 ± 1.00*</td>
<td>3.11 ± 0.62*</td>
<td>1.02 ± 0.26*</td>
<td></td>
</tr>
<tr>
<td>Lactate (mmol g⁻¹)</td>
<td>151.17 ± 13.67*</td>
<td>155.92 ± 16.30*</td>
<td>164.03 ± 18.09*</td>
<td>132.27 ± 8.03*</td>
<td></td>
</tr>
</tbody>
</table>

Note: Values are means ± SE. Values for the same control or fatigue group without the same letter are significantly different at the 0.05 probability level according to the ANOVA test and the same DO concentrations with * are significantly different at the 0.05 probability level according to the independent-samples T test.
3.2.1. Effect of Salinity

The total protein concentrations in the plasma and abdominal muscle were not significantly different among the different salinity groups \((p > 0.05)\). After tail-flip fatigue, the plasma total protein concentrations increased and abdominal muscle total protein concentration decreased significantly at a salinity of 40‰ \((p < 0.05)\). The hepatopancreas total protein concentrations in the shrimp maintained at 35‰ salinity was lower than those in other salinities, whereas there were no significant differences in hepatopancreas total protein concentrations before and after exercise \((p > 0.05)\). The plasma triglyceride concentration increased after tail-flip fatigue, but significant differences only occurred at 20‰, 25‰, and 30‰ salinity \((p < 0.05)\). The salinity challenges had no significant effects on plasma lactate concentrations \((p > 0.05)\), whereas a significant increase in plasma lactate concentration followed tail-flip \((p < 0.05)\). The five salinity groups were not evidently different from each other in terms of hepatopancreas lactate concentrations \((p > 0.05)\). The abdominal muscle lactate concentrations of the shrimps at 30‰ and 35‰ were found to be significantly lower than that in the other salinity groups \((p < 0.05)\), whereas no significant difference was found in abdominal muscle lactate concentrations \((p > 0.05)\) after tail-flip fatigue. As the salinity increased, the hepatopancreas glycogen concentration in shrimp decreased and then increased. The abdominal muscle glycogen concentration in the shrimp at 40‰ salinity was much higher than those in the other salinity groups \((p < 0.05)\). After tail-flip fatigue, the plasma glucose concentration in shrimps at 25‰, 30‰, and 35‰ salinity decreased significantly \((p < 0.05)\). At 20‰ salinity, the hepatopancreas glycogen concentration was significantly lower than that in the control group. This trend was reversed in the abdominal muscle glycogen concentrations \((p < 0.05)\).

3.2.2. Effect of DO

In the DO experiments, the plasma total protein, plasma lactate, and abdominal muscle glycogen concentrations in shrimp increased as the DO concentration decreased from 13.6 mg/L to 1.9 mg/L. Plasma glucose concentration in the shrimps maintained at 13.6 mg/L was lower than those in the other DO groups. There were no significant differences in the total protein of hepatopancreas and abdominal muscle, plasma triglyceride, hepatopancreas lactate, abdominal muscle lactate, and hepatopancreas glycogen concentration \((p > 0.05)\). After tail-flip fatigue, the plasma total protein concentration in the shrimp maintained at 1.9 mg/L was significantly higher than that in the control group \((p < 0.05)\). Reductions were observed in the abdominal muscle total protein concentrations after fatigue at DO concentrations ranging from 1.9 mg/L to 13.6 mg/L. The trend was reversed in the abdominal muscle lactate concentrations, but the differences were not significant \((p > 0.05)\). After tail-flip fatigue, the plasma lactate concentrations significantly increased among the four DO groups \((p < 0.05)\), and significant decreases \((p < 0.05)\) in plasma glucose concentration occurred only at DO concentrations between 6.8 and 13.6 mg/L. No significant differences were found in plasma triglyceride, hepatopancreas lactate, hepatopancreas glycogen, and abdominal muscle glycogen concentrations \((p > 0.05)\).

4. Discussion

4.1. Effects of Salinity and DO on \(S_{tf}\)

4.1.1. Effect of Salinity

Tail-flip is used as a defensive mechanism to avoid danger and predators for shrimp \([28,29]\). Our results implied that salinity affected the continuous performing and the speed of tail-flip. The number of tail-flips produced by shrimp limited the bout distance produced. Relevant researches showed that the poor tail-flip performance in lower or higher salinity might be due to (1) the increase in standard metabolic rate; (2) the accumulation of plasma lactate \([9]\); and (3) the impairment of heart rates and muscle functions \([23,28–31]\).

In the theoretical “cost of osmoregulation”, the standard metabolic rate is greater in full-strength salinity than in near iso-osmotic salinity \([9]\). Hence, counting metabolic energy demands that allow the maintenance of homeostasis is necessary \([32]\). Acute increases of
salinity caused the accumulation of plasma ions and large increases in plasma osmolality, which are directly related to a significant reduction in activity [33–36]. In our study, shrimps were maintained at 30‰ salinity for a long time. Exposure to either diluted seawater (20‰ and 25‰ salinity) or concentrated seawater (35‰ and 40‰ salinity) both caused profound metabolic energy demands, which led to decreased tail-flip [6,7].

Disruption in blood oxygen transport or stress can be another reason for poor tail-flip performance in lower or higher salinity. In many euryhaline species, the changes in salinity lead to changes in routine oxygen uptake [36]. The decrease in oxygen intake could change the composition of the metabolic substrate of shrimp, thus, causing the accumulation of lactate, further affecting the muscle contractility. Our physiologic results also showed that the lactate concentrations of plasma and abdominal muscle in the shrimps maintained at lower or higher salinity were higher.

The reduction in tail-flip performance may also be a result of changes in heart rates. Claireaux et al. [4] found that when the salinity decreased from 30 ‰ to 26‰, the heart rate increased over the whole range of swimming speeds. The effect of salinity on the ability of the shrimp to maintain tail-flip performance was similar in other studies, wherein changes in salinity cause significant declines in $U_{crit}$ and the swimming ability index [6,7]. The responses in these studies are directly related to the reduction in integument and gill membrane permeability, or changes in plasma ion concentrations and muscle water content, which impair cardiac and skeletal muscle functions [6].

4.1.2. Effect of DO

The result of the DO experiment indicates an increase in tail-flip performance with the increase in DO concentration, which was similar with the result of other species [10,37]. The curves (Table 2; Figure 2) clearly indicate that tail-flip speed was markedly impaired at lower DO concentrations, and little improvement in shrimp performance was observed when the oxygen concentration was increased from 10 mg/L or 11 mg/L to well above the saturation value. Davis et al. [10] also found that variations in oxygen concentration above the saturation value had little or no effect on the activity of juvenile Pacific salmon Oncorhynchus spp.

Our results showed that the DO concentration affected not only the duration and the speed of tail-flip, but also the distance of one tail-flip. At the highest speeds, several factors may limit swimming performance, such as (1) the inability to supply enough oxygen to the gills and other tissues, (2) the accumulation of metabolic products, (3) the inadequate substrate, (4) the reduction in enzymatic processes [12], (5) ammonia build-up in the hemolymph, and (6) respiratory alkalosis [21,38]. These might significantly affect the muscle contraction velocity and power output and limit the functions of muscle for tail flipping. These results may imply that the ability of shrimp to present sufficient oxygen to their gills is a factor limiting maximum performance. Deficient oxygen supply decreases the energy supply for various physiologic activities [14,15,39,40], and hypoxia undoubtedly affects the fitness of aquatic animals. Hence, the metabolic cost of both cardiac and branchial pumps tends to place a restraint on the tail-flip performance of shrimp [12]. Moreover, the tachycardia observed during exercise in hypoxic water was more pronounced. In hypoxia, adjustments in shrimp respiration and circulation allow sustainable oxygen consumption, which may increase metabolic costs and lead to a decline in tail-flip performance.

4.2. Physiologic Response

4.2.1. Effect of Salinity

Shrimp maintained in different salinities possess a variety of distinct physiologic characteristics. The present experimental results showed that salinity significantly affected the concentration of carbohydrates in tissues. The glycogen concentrations in the hepatopancreas of shrimp maintained at 30‰ and 35‰ were lower than those in the other groups, whereas the abdominal muscle glycogen concentration in the shrimp maintained at 40‰ was much higher. The glucose concentration of crustaceans was found to increase
under stress conditions [22]. During stress, the carbohydrates could be a source of energy for shrimp [41,42]. Therefore, the high levels of glycogen may be due to the transport of glucose from the hemolymph to muscle and hepatopancreas in higher and lower salinities. On the other hand, our results also exhibited the increase in lactate in the hemolymph and muscle for shrimp maintained in unsuitable salinities, and the abdominal muscle lactate concentrations in the shrimps maintained at 30‰ and 35‰ were significantly lower. Tail-flip performance, as a short-term high-intensity anaerobic muscle work, must be limited by the ability of the muscle. The lactate accumulation in tissue significantly affected the skeletal muscle functions, leading to the decrease in tail-flip performance. The increase in abdominal muscle lactate concentrations under higher and lower salinities was usually a result of the reduction in permeability of integument and gill membrane because of the increasing osmotic pressure of the environment and the body fluids [43–45], which could lead to internal hypoxia. When the oxygen supply to the tissues was not meeting the aerobic demand, the lactate levels displayed as consequently elevated in the tissues [21,46]. The degree of lactate accumulation in tissue and salinity showed a polynomial relationship, which showed the same trend as the change in the tail-flip performance. This result suggested that lactate concentration can potentially function as reliable and rapid indicators to assess the tail-flip performance of shrimp in various salinities.

The results of exercise fatigue showed that the tail-flip fatigue of *L. vannamei* might be mainly due to the accumulation of hemolymph lactate rather than the consumption of metabolic substrates. Hence, after exercise fatigue, the plasma glucose concentration in shrimps at 25‰, 30‰, and 35‰ salinity decreased significantly and with no significant decrease in higher and lower salinities, whereas a significant increase in plasma lactate concentration followed tail-flip in all salinity groups. Lactate accumulation in the hemolymph can weaken the locomotory ability of crustaceans, [19,20,31,47–49]. Exercise fatigue caused a slight change in the muscle lactate concentration in *L. vannamei*. However, the plasma lactate concentration increased significantly, suggesting that the shrimp release lactate produced in the myotome into the circulation following anaerobic tail-flip [50]. The plasma triglyceride concentration increased after tail-flip fatigue in 20‰, 25‰, and 30‰ salinity. This result may be due to the water in blood loss after fatigue in all salinity groups. Escaped shrimps show lower water content in their blood, with concomitant blood solute increases [51,52].

Woo and Kelly [53] found that shrimps preferentially use carbohydrates and lipids when they are under iso-osmotic salinity. Under 20‰ salinity, the hepatopancreas glycogen concentrations decreased, and the abdominal muscle glycogen concentration increased after tail-flip fatigue. Glucosyl units were possibly mobilized and released into the muscle because the abdominal muscle glycogen levels increased, whereas the combined hepatopancreas glycogen showed a significant decline [21]. At 40‰ salinity, after tail-flip fatigue, the total plasma protein concentrations increased and the total abdominal muscle protein concentrations decreased. High-speed exercise would also lead to the loss of muscle protein. The current results show that the energy substrate of *L. vannamei* changes with salinity from a focused preference for proteins at 40‰ and carbohydrates at 20‰ to a mix of carbohydrates, lipids, and protein at the other salinity levels. Therefore, these changes in the metabolic substrate could be related to lower glycolysis and lipid catabolism under higher salinities and lower lipid and protein catabolism under lower salinities.

4.2.2. Effect of DO

In the DO experiment, the plasma total protein, plasma lactate, and abdominal muscle glycogen concentrations in the shrimp decreased as the DO concentration increased, and the concentrations of plasma glucose were lower in the higher DO concentration group than those in other groups. Relevant studies have shown that low dissolved oxygen would lead to the increase in lactate and total protein in plasma [21,22], when the oxygen supply was not meeting the aerobic demand, prawns obtained energy through anaerobic glycolysis leading to the increase in plasma lactate. The metabolic acidosis, which was caused by
the lactate accumulation in the plasma, significantly impaired cardiac and skeletal muscle functions, leading to the decrease in tail-flip performance [54]. On the other hand, the increase in hemocyanin indicated another strategy for shrimp in an anoxic environment. The increase in hemocyanin could increase the oxygen carrying capacity of the plasma. Harris and Andrews [22] also showed that *N. norvegicus* synthesizes hemocyanin when exposed to low oxygen concentration. However, under severe hypoxia, reduced hemocyanin occurs. Accordingly, no difference in total plasma protein was observed in the two lower 1.9 mg/L and 3.8 mg/L DO concentration groups.

Some studies also showed that for crustaceans, the glucose concentration in the plasma increases under stress conditions [22,42]. The current results suggest that glucose may be mobilized into muscle glycogen under lower DO concentrations. Hence, the muscle glycogen levels of *L. vannamei* at lower DO concentrations were higher than those of *L. vannamei* at higher DO concentrations in our study.

The effect of DO concentration on the average *S_{tf}* of shrimp could be determined by their muscular capabilities, an energy source that could be mobilized, and ability to remove metabolic products. In our study, the plasma glucose concentrations significantly decreased after tail-flip fatigue under higher and under normal DO concentrations, whereas no significant differences were found at two lower DO concentration groups. Thus, it can be seen that the shrimp has used anaerobic glycolysis to provide energy for tail-flip. However, some experiments also showed that glycolysis was not enhanced to compensate for the decline in aerobic metabolism under lower DO concentrations [35], which might be due to a reduction in the metabolic demand because of the weak tail-flip ability under lower DO groups. On the other hand, the current results also showed that the anaerobic glycolytic capacity was limited in a low pH due to the accumulation of plasma lactate in lower DO concentrations. Limits to capacity of the glycolytic may reportedly be set by the glycolytic enzymes’ sensitivity to inhibition at low pH [56–60].

The lactate accumulation in the plasma is undoubtedly related to anaerobic metabolism. The decrease in DO is associated with a significant increase in the plasma lactate concentrations, which results in a fall in plasma pH and limitation of the anaerobic glycolytic capacity. The degree of lactate accumulation in the plasma, which increased as the DO concentration decreased, marked the different ability of removal of lactate in different DO concentrations. This result could be another reason for affecting the tail-flip performance. Furthermore, the post-exercise plasma lactate level increased and DO concentration showed a significant functional relationship. These results suggest that plasma lactate concentration can potentially function as a reliable and rapid indicator to assess the tail-flip performance of shrimps in various environmental stresses. Further studies are necessary to better standardize this technique and evaluate its potential as a useful indicator of shrimp tail-flip.

5. Conclusions

Salinity and DO concentration significantly affected the tail-flip speed and physiologic response of whiteleg shrimp. The *S_{tf}* increased and subsequently decreased with the increase in salinity from 20‰ to 40‰. The optimum salinity and corresponding maximum *S_{tf}* were 32.54‰ and 107.52 cm/s, respectively. The *S_{tf}* increased as the DO concentration increased from 1.9 mg/L to 13.6 mg/L. The relationship between *S_{tf}* and DO (mg/L) can be expressed by the power model. The results of physiologic experiments suggested that the inappropriate salinity and DO significantly limited the tail-flip speed of shrimp, which was due to the accumulation of metabolites. The proper salinity and DO accelerated the elimination of metabolites, reduced the energy consumption of shrimp, and thus, improved the exercise ability of shrimp. This conclusion is of particular value in evaluating the swimming ability of shrimp and understanding its ecological processes to improve capture and rearing techniques.

**Author Contributions:** Experimental design, X.Z.; Writing—original draft, test operation and data processing, Y.D. and M.L.; Investigation, M.S. and Y.C.; Data curation, A.W.; Validation, J.D. and F.C.; Software, Z.Y. All authors have read and agreed to the published version of the manuscript.
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References


