CHAPTER 3. THERMAL LETHAL STUDIES

3.1. Introduction

Temperature is considered one of the controlling factors of the aquatic environment and has pervasive influences on the biology, physiology and ecology of aquatic organisms. Water temperature has directly affected and determined the limit for life, the rate of growth and metabolic ratio and influences the activity of organisms (Elliot, 1995). The extreme fluctuations in temperature by means of natural or man-made have significant effects on aquatic populations (Matthews, 1987). A variety of human activities can affect the thermal requirements of aquatic organisms including discharge of heated effluent from nuclear and thermal power stations and other industries (Brooker, 1981). Information on thermal tolerance studies appears to be reliable tools for measuring physiological stress and adaptation of aquatic organisms (Kowalski et al., 1978; Paladino et al., 1980). The relatively narrow range of temperatures of the waters inhabited by aquatic organisms is defined as the species thermal niche (Magnuson et al., 1979). Aquatic animals can only survive over certain range of thermal niche, and outside the lower and upper incipient lethal temperature it will eventually die (Wotton, 1990). The lethal temperatures were also found to limit the distribution of fish species. For instance, the goldfish can tolerate a temperature range from 0 to 40°C (Fry, 1971). This is in strong contrast to some Antarctic fish species that will die at temperatures above 5°C (MacDonald et al., 1987). Species respond positively or negatively in terms of
the thermal preference area and make necessary adjustments to stress by
widening or narrowing their distribution.

In the natural environment, the organism can experience temperature
variations that are above the lethal temperatures, which may be immediately
lethal or lethal over an extended period (Brett, 1956). Among different
environmental factors, temperature fluctuations in the aquatic environment due
to heated effluent discharge from power plants have prompted a growing
number of laboratory studies. While the lethal response is recognized as the
most important one of the critical thermal responses, sub-lethal responses are
also significant (Cairns, 1972). The thermal preference of aquatic organisms is
a complex subject with several interacting factors in field observation (Elliot,
1981). Field observations of thermal preference are often difficult to interpret
due to confounding variables such as food availability, competition and
predation, and presence of thermal refugia. Therefore, the exact determination
of thermal preference is routinely done in the laboratory where preference can
be examined under closely controlled conditions, and it can help to identify a
habitat suitable for a species in terms of water temperature. There is a close
relationship between ambient temperature and the survival of organisms.
Organisms can adapt within certain limits of temperature tolerance to live in
relatively constant or fluctuating thermal conditions. The poikilothermic
organisms make themselves compensate for environmental temperature
variations through acclimatization mechanisms (Crossins and Bowlers, 1987).
Proper conservation and management of aquatic animals requires detailed
knowledge of its temperature requirements and thermal protection standards to protect and restore populations in aquatic environments (McCullough, 1999).

The lethal and critical temperatures of the species that have been previously acclimated allow studying the adaptation and tolerance capacity of the organisms. The purpose of this present experiment is to study the upper thermal tolerance of test species acclimated at different temperatures and to establish the lethal temperature and critical temperature maximum of the species.
3.2. Materials and Methods

The marine fin fish, *Mugil cephalus*, *Aplocheilus lineatus* and shrimp *Penaeus monodon* were selected for the present study. The taxonomical positions of these organisms are as follows.

3.2.1. Taxonomy of Experimental Animals

### 3.2.1.1. Taxonomical Position of *Penaeus monodon*

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Animalia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
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<tr>
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<td>Order</td>
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<tr>
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<td>Penaeus</td>
</tr>
<tr>
<td>Species</td>
<td><em>monodon</em></td>
</tr>
</tbody>
</table>

### 3.2.1.2. Taxonomical Position of *Mugil cephalus* (Linnaeus, 1758)

<table>
<thead>
<tr>
<th>Kingdom</th>
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<tbody>
<tr>
<td>Phylum</td>
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<tr>
<td>Subphylum</td>
<td>Vertebrata</td>
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<tr>
<td>Class</td>
<td>Osteichthyes</td>
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<td>Subclass</td>
<td>Actinopterygii</td>
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<td>Family</td>
<td>Mugilidae</td>
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<td>Order</td>
<td>Perciformes</td>
</tr>
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<td>Genus</td>
<td><em>Mugil</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>cephalus</em></td>
</tr>
</tbody>
</table>
3.2.1.3. Taxonomical Position of *Aplocheilus lineatus* (Valenciennes, 1846)

Kingdom
Phylum
Subphylum
Class
Subclass
Family
Order
Genus
Species
Animalia
Chordata
Vertebrata
Osteichthyes
Actinopterygii
Aplocheilidae
Cyprinodontiformes
*Aplocheilus*
*Lineatus*

3.2.2. Collection and Maintenance of Experimental Organisms

The fin fish, *M. cephalus* (3.776±0.862g) and *A. lineatus* (3.224±0.767g) and shrimp, *P. mondon* (1.646±0.798g) were collected from Kudankulam (Lat 8° 15’ N; Long 77° 50’E) and Thoothukudi coastal areas (Lat 8° 47’N; Long 78° 08’E). Then they were transported in oxygen-saturated polythene bags to the laboratory and were acclimated to laboratory water temperature (26±0.5°C) for two weeks. The salinity and pH of the water were maintained at 35±0.78 ppt and 8.0±0.36, respectively. They were fed twice a day with a formulated diet at 5% of their body weight. The food contained approximately 40% protein.

3.2.3. Acclimation of Test Organisms in Different Elevated Temperature

The fish and shrimps were introduced into different temperature water baths, namely, 26, 31 and 36°C for acclimatization. Acclimation temperature
for each set of experiments was attained by progressively increasing the temperature at 1-2°C per day to the required temperature. No mortality of the test species occurred during acclimatization period. During the acclimation period, the animals were fed twice daily with a formulated diet (approximately 40% protein content) at a rate of 5% of their body weight. Fifty percent of water was replaced on alternate days by adding water of the same temperature, without causing much stress to the animals. After achieving desired acclimated temperature, they were reared for two weeks following the method of Anandhakrishnan and Kutty (1974).

3.2.4. Critical Thermal Maximum (CT max) Study Experiment

After acclimating at desired experimental temperature, the organisms were starved for 24 hours before the commencement of the experiment (Beamish, 1964; Brett, 1964). Aquarium tanks of 35 litres capacity fitted with heating elements were used for the lethal studies. The experimental aquarium tank’s water temperature was progressively increased to the desired water temperature using 1000 W immersion heaters controlled by an electronic digital thermostat to maintain the specific experimental temperature constant (Plate 7). All the tanks were well aerated. Ten animals of uniform weight were maintained in each experimental tank, and the experiment set-up was in triplicate for each set of critical thermal study experiments. Then the temperature was increased at a constant rate of 1°C/10 minutes from the acclimation temperature until the loss of equilibrium response was observed in
Plate 7. Experimental Setup for Thermal Studies
organisms and the results were recorded as the critical thermal maximum (CTM) (Hernandez and Buckle, 1998).

3.2.5. Lethal Study Experiment

Ten animals of uniform size and weight were abruptly transferred to preheated water bath of specific temperature, to record the response of the organisms over a given time period of 96 hours (Richardson et al., 1994). The organisms were starved for 24 hours before the experiments (Brett, 1964). The temperature lethal to each animal type is determined by observing the cessation of the activity of shrimp or opercular movements of fish for each experimental temperature (Richardson et al., 1994). The temperature at which the animals showed 50% mortality was recorded in minutes as time to death from the initial temperature. A regression graph was drawn between the time for 50% mortality (minutes) and elevated temperature in the three acclimated temperature. From the regression graph the Median Lethal Temperature (MLT) ($LT_{50}$) was calculated. The Upper Lethal Temperature (ULT) was also recorded from the experiments (Tsuchida, 1995).

3.2.6. Statistical Analysis

Statistical analysis of ULT, MLT and CT max was carried out using one-way analysis of variance (ANOVA). Duncan’s multiple post hoc mean comparison analysis was performed to define the relationship between acclimation and test parameters (Zar, 1996).
3.3. Results

The present study was attempted to observe and understand the survival and resistance of organism to elevated temperature from selected acclimated temperatures of 26, 31 and 36°C. The Critical Thermal Maximum temperature and Upper Lethal Temperature were found to increase, along with increase in temperature, in all experimental organisms. The Median Lethal Temperature ($LT_{50}$) also showed the same trend in all the experiments. In median lethal temperature ($LT_{50}$), *M. cephalus* and *A. lineatus* showed no significant difference between them but *P. monodon* showed significant difference (Table 3.3). In the Critical Thermal Maximum and Upper Lethal Temperature studies, all three species showed significant difference (Table 3.1 and 3.2). The percentage of difference between acclimation temperature and Critical Thermal Maximum and Upper Lethal Temperature showed significant difference ($P<0.05$) in all three experimental species. However, there was no significant difference between *M. cephalus* and *A. lineatus* in the percentage of difference from acclimation temperature and Median Lethal Temperature ($LT_{50}$) but *P. monodon* showed significant difference than the other two species (Table 3.4).

3.3.1. Effect of Temperature on Upper Lethal Temperature (ULT) of *Penaeus monodon*

The Upper Lethal Temperature of *P. monodon* showed a marked increase with increase in acclimated temperature. As reported in table 3.1 the organisms acclimated to 26, 31 and 36 °C temperatures had Upper Lethal Temperature of
39.7, 42.7 and 45.7°C respectively (Figure 3.1). The low (26°C) and high (36°C) acclimated temperatures showed a noticeable difference of 6.0°C in Upper Lethal Temperature. In other acclimated temperatures, the ULT difference was 3.0 and 3.0°C between 26 vs. 31°C and 31 vs. 36°C respectively. Results are statistically significant for all three acclimated temperatures (P<0.05). The percentage of difference between acclimation temperature and ULT showed an increasing trend with increase of acclimation temperature. *P. monodon* acclimated at 26, 31 and 36°C showing 25.88, 29.99 and 33.17% difference in acclimation temperature, respectively. It was observed that there was significant difference between all three acclimation temperatures (Table 3.4).

### 3.3.2. Effect of Temperature on Upper Lethal Temperature (ULT) of *Mugil cephalus*

*M. cephalus* showed an increasing trend of Upper Lethal Temperature with increase of acclimated temperature. The Upper Lethal Temperature was 43.3, 45.7 and 47.7°C, respectively, for organisms acclimated at 26, 31 and 36°C (Table 3.1 and Figure 3.1). The Upper Lethal Temperature (ULT) difference between 26 vs. 36, 26 vs.31 and 31 vs. 36°C were 4.4, 2.4 and 2.0°C, respectively. The organisms acclimated at 31°C did not show much significant difference at 26 and 36°C. The organisms acclimated at 26 and 36°C, however, showed significant difference (P<0.05). The organisms showed an increasing trend of percentage difference from acclimation along with increasing acclimation temperature (Table 3.4). Significantly high value of percentage of
Table 3.1. Effect of temperature on Upper Lethal Temperature (ULT) of *Penaeus monodon, Mugil cephalus* and *Aplocheilus lineatus* exposed to three different elevated temperatures. Values are means with ±SE (n=3) and followed by a different letter (column wise) are statistically different (P<0.05). Different letters followed by species names (row wise) are statistically different (P<0.05)

<table>
<thead>
<tr>
<th>Acclimation temperature (°C)</th>
<th><em>Penaeus monodon</em> (°C)&lt;sup&gt;c&lt;/sup&gt;</th>
<th><em>Mugil cephalus</em> (°C)&lt;sup&gt;a&lt;/sup&gt;</th>
<th><em>Aplocheilus lineatus</em> (°C)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>39.7±0.333&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.3±0.333&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.3±0.333&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>31</td>
<td>42.7±0.333&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.7±0.333&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>44.7±0.333&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>36</td>
<td>45.7±0.333&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.7±0.882&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.7±0.333&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Figure 3.1. Temperature effect on Upper Lethal Temperature (ULT) of *Penaeus monodon, Mugil cephalus* and *Aplocheilus lineatus* exposed to three different elevated temperatures
difference (27.86%) was observed at 36°C, rather than the other acclimation temperatures of 26 (16.67%) and 31°C (22.22%).

3.3.3. Effect of Temperature on Upper Lethal Temperature (ULT) of *Aplocheilus lineatus*

The Upper Lethal Temperature increased with the increase in acclimation temperature in *A. lineatus*. Results revealed that the organisms acclimated at 26, 31 and 36°C temperature had Median Lethal Temperatures (LT$_{50}$) of 42.3, 44.7 and 46.7°C, respectively (Table 3.1 and Figure 3.1). The organisms acclimated at low (26°C) and high (36°C) temperature showed a difference of 4.4°C in the Upper Lethal Temperature. The difference of Upper Lethal Temperature between 26 vs. 31 and 31 vs. 36 was 2.4 and 2.0°C, respectively. The 31 and 36°C groups did not show any significant difference. The animals acclimated at 26°C had a statistically noticeable difference when compared with 31 and 36°C acclimated animals (P<0.05). The increasing trend of percentages of difference from acclimation temperature was observed with increase in acclimation temperature (Table 3.4). A significantly high value of 30.48% difference from acclimation temperature was observed at 36°C and low value of 19.08% at 26°C.

3.3.4. Effect of Temperature on Critical Thermal Maximum (CT$_{\text{max}}$) of *Penaeus monodon*

The critical thermal maximum of *P. monodon* exhibited an increasing trend along with increase in acclimation temperature. The test organisms acclimated at 26°C showed a CT$_{\text{max}}$ of 38.3, whereas the 31 and 36°C acclimated organisms had 42.0 and 44.7°C respectively (Table 3.2 and Figure 113).
A significantly high CT max temperature was observed at the 36°C acclimated group followed by 31 and 26°C acclimated organisms. All three acclimated temperature groups were significantly different between them (P<0.05). The CT max temperature difference between low (26°C) and high (36°C) acclimated temperature was 6.4°C. The CT max temperature difference between 26 vs. 31 and 31 vs. 36 was 6.4°C. The CT max temperature difference between 26 vs. 31 and 31 vs. 36 acclimated temperatures was 3.7 and 2.7°C, respectively. The increasing trend of percentage of difference from acclimation temperature was observed with increasing acclimation temperature. Results showed a significant difference between low and high temperature groups (Table 3.4). There was no statistical difference in the percentage of difference from acclimation temperature observed between 26 (29.49%) and 31°C (31.81%) and between 31 (31.81%) and 36°C (35.93%).

3.3.5. Effect of Temperature on Critical Thermal Maximum (CTmax) of Mugil cephalus

*M. cephalus* showed an increase of CT max temperature in response to increase of acclimated temperature. Table 3.2 showed that the organisms acclimated at 26, 31 and 36°C had mean CT max temperature of 42.3, 44.7 and 47.0°C, respectively (Figure 3.2). The difference in CT max temperature between low (26°C) and high (36°C) acclimated temperature was 4.7°C. The difference of CT max between 26 vs. 31 and 31 vs. 36 was 2.4 and 2.3°C, respectively. Statistically, the low (26°C) and high (36°C) temperatures had a significant difference (P<0.05). There was no significant difference between 31 vs. 36 and 26 vs. 31°C acclimated temperature. The increasing trend of
Table 3.2. Effect of temperature on Critical Thermal Maximum (CT max.) of *Penaeus monodon*, *Mugil cephalus* and *Aplocheilus lineatus* exposed to three different elevated temperatures. Values are means with ±SE (n=3) and followed by a different letter (column wise) are statistically different (P<0.05). Different letters followed by species names (row wise) are statistically different (P<0.05)

<table>
<thead>
<tr>
<th>Acclimation temperature (°C)</th>
<th><em>Penaeus monodon</em> (°C)</th>
<th><em>Mugil cephalus</em> (°C)</th>
<th><em>Aplocheilus lineatus</em> (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>38.3±0.333^c</td>
<td>42.3±0.882^b</td>
<td>41.3±0.333^b</td>
</tr>
<tr>
<td>31</td>
<td>42.0±0.577^b</td>
<td>44.7±0.333^ab</td>
<td>43.7±0.667^ab</td>
</tr>
<tr>
<td>36</td>
<td>44.7±0.333^a</td>
<td>47.0±0.577^a</td>
<td>45.7±0.667^a</td>
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</tbody>
</table>

Figure 3.2. Temperature effect on Critical Thermal Maximum (CT max.) of *Penaeus monodon*, *Mugil cephalus* and *Aplocheilus lineatus* exposed to three different elevated temperatures
percentage of difference from acclimation temperature was observed with increase of acclimation temperature (Table 3.4). There was no statistical difference in percentage of difference from acclimation temperature observed between 31 (24.74%) and 36°C (29.60%). The organisms acclimated at 26°C had a low value of 19.08% difference from acclimation temperature, which was significant compared to the other two acclimation temperatures (P<0.05).

3.3.6. Effect of Temperature on Critical Thermal Maximum (CTmax) of *Aplocheilus lineatus*

The CT max temperature of *A. lineatus* showed an increasing trend along with increase in acclimated temperature. Table 3.2 shows that the low (26°C), median (31°C) and high (36°C) acclimated temperatures had a CT max temperature of 41.3, 43.7 and 45.7°C, respectively (Figure 3.2). The difference in CT max temperature between 26°C and 36°C was 4.4°C. The difference of CT max temperature between the acclimated temperature of 26 vs. 31 and 31 vs. 36 was 2.4 and 2.0°C, respectively. The 26 and 36°C temperature acclimated *A. lineatus* showed high significant difference in CT max temperature (P<0.05). It was observed that there was no significant difference between 26 vs. 31 and 31 vs. 36°C. The increasing trend of percentage difference from acclimation temperature was observed with increasing acclimation temperature (Table 3.4). A significantly high value of 33.17% difference from acclimation temperature was observed at 36°C and low a value of 21.57% at 26°C. Results were significant in all three experimental species (P<0.05).
3.3.7. Effect of Temperature on Median Lethal Temperature (LT\textsubscript{50}) of *Penaeus monodon*

The Median Lethal Temperature (LT\textsubscript{50}) of *P. monodon* showed a detectable increase with increasing of acclimated temperature. The organisms acclimated at 26°C temperature had Median Lethal Temperature (LT\textsubscript{50}) of 31.3°C, whereas the organisms acclimated at 31 and 36°C temperature showed 33.3 and 38.3°C, respectively (Table 3.3 and 3.12). The difference in Median Lethal Temperature (LT\textsubscript{50}) between low (26°C) and high (36°C) acclimated temperature was 7.0°C. The difference between 26 vs. 31 and 31 vs. 36°C acclimated temperature had Median Lethal Temperature (LT\textsubscript{50}) of 2.0 and 5.0°C, respectively. It was observed that the Median Lethal Temperature (LT\textsubscript{50}) increased significantly with increase of acclimated temperature (P<0.05). The regression trends in all three acclimated temperatures indicated a strong relationship between acclimation temperature and Median Lethal Temperature (LT\textsubscript{50}) (Figure 3.3, 3.4 and 3.5). The organism showed an increased pattern of percentage difference from acclimation along with increasing acclimation temperature (Table 3.4). A high value of percentage of difference (59.67%) was observed at 31°C, and the same in other acclimation temperatures was 26°C (51.65%) and 36°C (55.58%). Statistically, there was no significant difference between 26 and 31°C and also between 31 and 36°C.

3.3.8. Effect of Temperature on Median Lethal Temperature (LT\textsubscript{50}) of *Mugil cephalus*

*M. cephalus* showed a marked increase of LT\textsubscript{50} with the increase in acclimation temperature. Table 3.3 shows that the Median Lethal Temperature
Figure 3.3. The regression graph of median lethal temperature (LT$_{50}$) of *Penaeus monodon* exposed to different elevated temperature from acclimated temperature of 26°C

![Graph 3.3](image)

\[y = -0.0016x + 40.547\]

\[R^2 = 0.9869\]

Figure 3.4. The regression graph of median lethal temperature (LT$_{50}$) of *Penaeus monodon* exposed to different elevated temperature from acclimated temperature of 31°C

![Graph 3.4](image)

\[y = -0.0017x + 43.138\]

\[R^2 = 0.9979\]

Figure 3.5. The regression graph of median lethal temperature (LT$_{50}$) of *Penaeus monodon* exposed to different elevated temperature from acclimated temperature of 36°C

![Graph 3.5](image)

\[y = -0.0014x + 46.357\]

\[R^2 = 0.9846\]
(LT$_{50}$) of 26, 31 and 36°C temperature acclimated organisms was 34.0, 35.0 and 37.3°C, respectively (Figure 3.12). The low (26°C) and high (36°C) acclimated temperature group had a difference in Median Lethal Temperature (LT$_{50}$) of 3.3°C. The Median Lethal Temperature (LT$_{50}$) difference between 26 vs. 31°C and 31 vs. 36°C was 1.0 and 3.3°C, respectively. The regression too indicated that Median Lethal Temperature (LT$_{50}$) values depend on acclimation temperature and the results were significant (Figure 3.9, 3.10 and 3.11). A similar trend was observed with increase in acclimation temperature (Table 3.4). There was no statistical difference in the percentage of difference from acclimation temperature was observed between 31 (53.57%) and 36°C (59.10%). The organisms acclimated at 26°C had low value of 42.47% difference from acclimation temperature which was significant when compared to other experimental acclimation temperatures.

### 3.3.9. Effect of Temperature on Median Lethal Temperature (LT$_{50}$) of *Aplocheilus lineatus*

The Median Lethal Temperature (LT$_{50}$) values of *A. lineatus* presented in Table 3.3 showed that the LT$_{50}$ of 26, 31 and 36°C acclimated organisms were 33.7, 35.0 and 38.0°C temperature, respectively (Figure 3.12). The low (26°C) and high (36°C) acclimated temperature had a difference in Median Lethal Temperature (LT$_{50}$) which was 4.3°C. The 36°C temperature acclimated organism showed high Median Lethal Temperature (LT$_{50}$), which was statistically significant than the other two acclimation temperatures. There was no significant difference found between 26 and 31°C acclimation temperatures. The regression analysis also revealed that the LT$_{50}$ values depend on all three
Figure 3.6. The regression graph of median lethal temperature (LT$_{50}$) of *Aplocheilus limneatus* exposed to different elevated temperature from acclimated temperature of 26°C

\[ y = -0.0016x + 42.96 \]
\[ R^2 = 0.9445 \]

Figure 3.7. The regression graph of median lethal temperature (LT$_{50}$) of *Aplocheilus limneatus* exposed to different elevated temperature from acclimated temperature of 31°C

\[ y = -0.0016x + 44.256 \]
\[ R^2 = 0.9561 \]

Figure 3.8. The regression graph of median lethal temperature (LT$_{50}$) of *Aplocheilus limneatus* exposed to different elevated temperature from acclimated temperature of 36°C

\[ y = -0.0015x + 46.622 \]
\[ R^2 = 0.9852 \]
Figure 3.9. The regression graph of median lethal temperature (LT$_{50}$) of *Mugil cephalus* exposed to different elevated temperature from acclimated temperature of 26°C

\[ y = -0.0018x + 44.402 \]

\[ R^2 = 0.9796 \]

![Temperature vs. Time Graph](image)

Figure 3.10. The regression graph of median lethal temperature (LT$_{50}$) of *Mugil cephalus* exposed to different elevated temperature from acclimated temperature of 31°C

\[ y = -0.0018x + 45.408 \]

\[ R^2 = 0.9736 \]

![Temperature vs. Time Graph](image)

Figure 3.11. The regression graph of median lethal temperature (LT$_{50}$) of *Mugil cephalus* exposed to different elevated temperature from acclimated temperature of 36°C

\[ y = -0.0019x + 48.223 \]

\[ R^2 = 0.9925 \]

![Temperature vs. Time Graph](image)
Table 3.3. Effect of temperature on median lethal temperature (LT$_{50}$) of *Penaeus monodon*, *Mugil cephalus* and *Aplocheilus lineatus* exposed to three different elevated temperatures. Values are means with ±SE (n=3) and followed by a different letter (column wise) are statistically different (P<0.05). Different letters followed by species names (row wise) are statistically different (P<0.05)

<table>
<thead>
<tr>
<th>Acclimation temperature (°C)</th>
<th><em>Penaeus monodon</em> (°C)$^b$</th>
<th><em>Mugil cephalus</em> (°C)$^a$</th>
<th><em>Aplocheilus lineatus</em> (°C)$^a$</th>
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<td>38.0±0.577$^a$</td>
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</tbody>
</table>

Figure 3.12. Temperature effect on median lethal temperature (LT$_{50}$) of *Penaeus monodon*, *Mugil cephalus* and *Aplocheilus lineatus* exposed to three different elevated temperatures
Table 3.4. Effect of temperature on the % of difference from acclimation temperature of *Penaeus monodon*, *Mugil cephalus* and *Apocheilus lineatus* exposed to three different elevated temperatures. Values are means with ±SE (n=3) and followed by a different letter (column wise) are statistically different (P<0.05). Different letters followed by species names (row wise) are statistically different (P<0.05)

<table>
<thead>
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<th>Acclimation temperature (°C)</th>
<th>% of difference from acclimation temperature</th>
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<tbody>
<tr>
<td></td>
<td>Between 26°C acclimation temperature and Upper Lethal Temperature (ULT)</td>
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<td></td>
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<td><em>Mugil cephalus</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td><em>Apocheilus lineatus</em>&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>25.88±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.67±0.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.08±0.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.49±0.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.08±2.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.57±0.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>51.65±2.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>31</td>
<td>29.99±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.22±0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.74±0.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.81±1.59&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>24.74±0.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.33±1.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59.67±1.25&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>36</td>
<td>33.17±0.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.86±2.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.48±0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.93±0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.60±1.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.17±1.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.58±1.80&lt;sup&gt;a&lt;/sup&gt;</td>
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acclimation temperatures (Figure 3.6, 3.7 and 3.8). Organisms acclimated at 26, 31 and 36°C showed 43.56, 53.47 and 56.74% difference from acclimation temperature, respectively (Table 3.4). It is observed that there was no significant difference between 31 and 36°C. The low acclimation temperature (26°C) showed significant difference in percentage of difference from acclimation temperature than the other two acclimation temperatures.
3.4. Discussion

Every aquatic organism has a range of temperature that it can tolerate, beyond which thermal stress is created. Any drastic temperature change may produce a significant disturbance in the normal activities and survival of organisms (Beitinger et al., 2000). In the present study, *M. cephalus* showed a higher value of Upper Lethal Temperature and Critical thermal Maximum than the other tested species (*P. monodon* and *A. lineatus*). In all three tested species, the ULT, CT max showed an increasing trend along with increasing in acclimation temperatures in the order of *M. cephalus* > *A. lineatus* > *P. monodon*. But in the case of Median Lethal Temperatures, the *P. monodon* showed higher values than other species in the order of *P. monodon* > *M. cephalus* > *A. lineatus*. Critical thermal maximum (CT max) has been shown to have a direct effect on the physiology and metabolism of aquatic organisms (Chatterjee et al., 2004). The studies of the critical thermal maximum in the aquatic organisms are used as an indicator of adaptation and physiological stress (Paladino et al., 1980). The critical temperatures have also been used to compare the limits of tolerance of the species (Barrionuevo and Fernandes, 1995). When aquatic organisms entered temperatures outside the upper thermal limit it could be lethal, and survival depends on the time of exposure to the temperature (Elliot, 1981). Inside the polygon of temperature response of the poikilotherm, the critical thermal maximum defines the resistance of the species (Fry, 1947; Brett, 1956). Cox (1974) reported that the critical thermal maximum (CT max) is the arithmetic mean of the collective thermal points at which the locomotors’
activity becomes disorganized, and the animal loses its ability to escape from a condition that will promptly lead to its death. The pattern of result has been the same in the present study. The fish and crustaceans lost control of their ability to swim, as well as control over their physiological activities at higher temperature. At the extremely high temperatures, the protein gets denatured, and recovery becomes impossible; and such kind of denaturation does not occur at low temperatures. This shows that higher temperatures are more detrimental than lower temperatures (Kasim, 2002)

In the present investigation, it was found that the experimental species have their own individual critical, median and lethal temperatures with different acclimation temperatures. It further revealed that the lethal and critical temperature values of these organisms were species-specific (Smith and Scott, 1975; Currie et al., 1998). These results have been extensively used as a comparative method for quantifying the difference in thermal tolerance between different species. Because the capacity of animals to acclimate to different temperature regimes differs among species, it indicates that the degree of eurythermality of the organisms may be correlated with the thermal stability of the environment. The resistance of animals to increased temperatures may be considered as criterion-specific to species that are more resistant to environmental temperature variations (Sukumaran et al., 2002a). The knowledge of the thermal biology of species based on the preferred and lethal temperatures is very important in order to understand the capacity of the species to adapt to the thermal changes occurring in its habitat (Hernandez et
The median lethal temperature (LT$_{50}$) value gives an idea of the impact of temperature on organisms. Organisms with low LT$_{50}$ values show less lethal effects than those with high LT$_{50}$ temperatures. Organisms acclimated at the higher temperature indicated that continuous exposure approaching the median lethal temperature (LT$_{50}$) increased its sensitivity to high temperature. Similar findings were reported in Prawn (Hernandez et al., 1996; Manush et al., 2004) and fish (Chatterjee, 2004). The result confirms that the thermal tolerance is largely dependent on organisms’ prior thermal exposure history or acclimation. As a result, typical seasonal acclimation in the aquatic environment allows species to be more tolerant to higher temperatures in summer than in winter (Bavelhimer and Bennett, 2000). The aquatic organisms that live within their thermal tolerance limits may compensate extreme variations of seasonal temperatures in aquatic environment by previous acclimation processes (Prosser, 1973). However, the unexpected temperature changes may affect the resistance of the organisms (Becker et al., 1977). Due to wide fluctuations of temperature, some species die out in areas where they were once found or colonize new area where the water temperature favours them (Hernandez and Buckle, 1997). Species respond to temperature changes in the aquatic system in terms of the thermal preference with the necessary adjustments to the impact of temperature by widening or reducing their distribution. The physiological performance and bioenergetics of ectotherms are highly dependent on the ambient temperature of the aquatic system (Rodnick et al., 2004). Fieldmeth et
al. (1974) reported that an increased scope of thermal tolerance allows fish to maintain its physiological functions over a wider range of temperatures when exposed to fluctuating temperatures. The duration of temperature exposure depends on the acuteness of the lethal nature of temperature and the capacity of the species to resist or tolerate temperature (Kasim, 1982). Knowledge of the relationship between the constant or fluctuating acclimation temperature and Critical Thermal maximum, Upper Lethal Temperature and Median Lethal Temperature ($LT_{50}$) further helps us understand the biology, distribution and ability to adapt to thermal changes that occur in the natural habitat where the anomalous temperature variations over the preference zone exposes the species to thermal lethal limits.