Comparative efficacy of MS 222 and Benzocaine in combating stress during simulated transport of *Puntius filamentosus* (Valenciennes)
7.1. Introduction

In medical usage, stress is the metabolic response of an animal to a stressor. According to Selye (1950) stress is the sum of all the physiological responses by which an animal tries to maintain or re-establish normal metabolism in the face of a physical or chemical force. Stress is categorised as acute or chronic and severe or mild. The degree to which stress affects any particular fish is determined largely by the severity of the stress, its duration and the health of the fish. Common stressors found in aquaculture practice are decrease in water level (Fryer, 1975; Thomas and Robertson, 1991), catching the fish using nets (Barton et al., 1980), increased stocking density (Barton et al., 1985), water temperature change (Barton and Peter 1982), artificial and natural reproduction (Hlavova 1992; Luskova and Lusk 1995a, b; Svobodova et al., 1997), fish handling (Wurts, 1995), etc.

It is well established that fish are severely stressed by capture and handling (Billard et al., 1981; Barton and Iwama, 1991; Pickering, 1992; Wendelaar Bonga, 1997) and that the stress response is characterised by disturbances in biochemistry and physiology, which may appear within seconds and can persist for hours or days (Mazeaud et al., 1977). The perturbations resulting from stress are often classified as primary, secondary or tertiary, depending on when they are elicited, and the mechanism involved. Primary responses include rapid changes in plasma levels of catecholamines and corticosteroids, and are generally considered as adaptive responses because they enable the animal to cope with stressful conditions imposed upon them. However, stress responses may get pushed beyond their normal limits leading to a cascade of detrimental secondary and tertiary effects (Mazeaud et al., 1977). Stress has been demonstrated to affect energy metabolism and growth rates, suppress the immune response, inhibit various reproductive processes (Barton and Iwama, 1991; Wendelaar Bonga, 1997; Pankhurst and Van Der Kraak, 1991).
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1997), and influence post-mortem flesh quality (Wells et al., 1986; Watabe et al., 1991; Lowe et al., 1993). Capture, handling, crowding, confinement, transport and anaesthesia are the components of aquaculture and laboratory practices that can cause stress in fish. Various biochemical and haematological indices are used to indicate and assess the effect of stressors in fish. Indices used are concentration of glucose, cortisol, lactate, ammonia and chlorides in blood plasma, haematocrit value and mean corpuscular volume, relative weight of spleen, etc. (Thomas, 1990; Palikova and Svobodova, 1995; Spurny and Mares, 1997).

In order to fully assess the suitability of a species for export, a range of stress indices should be measured in response to common fish handling practices. Fish respond to stress with acute increases in plasma levels of the catecholamines, adrenaline and noradrenaline, as well as slower but more sustained increases in plasma levels of the corticosteroid cortisol (Sumpter, 1997). Increases in catecholamine and corticosteroid levels generally reflect in the by increase in plasma levels of glucose as these hormones have the ability of mobilising glucose (Barton and Iwama, 1991; Wendelaar Bonga, 1997). Concentrations of cortisol and glucose are considered as the most important stress indicators in fish (Svobodova et al., 1999), which usually reflects the severity and duration of the stress (Donaldson, 1981; Barton and Iwama, 1991). A short-term intensive stress leads to a large increase in the cortisol concentration followed by a slow decrease (Barton et al., 1980). The concentration of glucose also follows a similar course but with certain delay (Barry et al., 1993; Carragher and Ress, 1994). Plasma lactate levels also increase typically in stressed fish, particularly if any aspect of the stressor results in increased activity, or a decrease in oxygen availability (Thomas et al., 1999). To date, most of the information on the stress response of fish is derived from studies on salmonids (Sumpter, 1997) and a range of temperate, non-salmonid species (Pankhurst and Sharples, 1992; Vijayan et al., 1993; Sunyer et al., 1995; Waring et al., 1996; Barnett and Pankhurst, 1998). Stress investigations have also been conducted on high latitude species (Ryan,
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1995; Lowe and Wells, 1996), but excepting a few, (Pankhurst et al., 1997) there is very little information on the stress response of tropical species.

Anaesthesia is a biological state induced by an external agent, which results in partial or complete loss of sensation or loss of voluntary neuromotor control through chemical or non chemical means (Summerfelt and Smith, 1990). In fisheries research and assessment as well as in aquaculture operations, anaesthetics are commonly used to minimise stress in fish and to reduce the physical injury during various handling procedures. (Cho and Heath, 2000). A wide array of anaesthetics are being used in aquaculture/ fisheries and the choice of anaesthetic generally depend on several considerations, like 1) availability; 2) cost effectiveness; 3) ease of use; 4) nature of study; 5) safety of the user. Commercial harvesting techniques and handling procedures for ornamental fishes did not involve the use of any chemical anaesthetics till recently. However with the increase in concern for fish health and product quality, potential use of anaesthetics had to be increased to reduce the fish stress resulted by the intensive handling procedures. Loss of sensation is important for the reduction of stress during the various procedures such as fish handling, transportation, blood sampling and surgery (Brown, 1993; Burka et al., 1997).

The ability of Tricaine methanesulphonate (MS 222) and Benzocaine to suppress normal stress response potentially is being tested here by comparing the blood parameters following sampling via cardiac puncture. Tricaine methanesulphonate (MS 222) is the most commonly used anaesthetic for fish and is the only registered anaesthetic in North America (Marking and Meyer, 1985). The effect of MS 222 during anaesthesia have been investigated on a variety of blood parameters including Cholesterol (Wedemeyer 1970), electrolytes (Houston et al., 1971), glucose and lactate (Savio et al., 1977), haematocrit (Reinitz and Rix, 1977), cortisol (Strange and Schreck, 1978; Barton and Peter, 1982; Iwama et al., 1989), gases (Iwama et al., 1989), amino acids (Morales, et al., 1990) and lipids.
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(Harrington *et al.*, 1991). Iwama *et al.*, (1989) are of the opinion that the administration of MS 222 may act as stressor under certain circumstances. This information is critical for fisheries researchers utilising MS 222 in experiments; particularly those investigating stress related parameters, where such effects may potentially alter the results of the research. Large number of studies have demonstrated the efficiency of Benzocaine and have suggested it as an effective anaesthetic to reduce the stress in fishes (Ferreira *et al.*, 1979; Barham *et al.*, 1979; Ferreira *et al.*, 1984a, b; Allen, 1988; Barham and Schoonbee, 1990; Gilderhus *et al.*, 1991; Allen *et al.*, 1994, and Gomes *et al.*, 2001a, b). Ferreira *et al.* (1979) compared the anaesthetic potency of Benzocaine with that of MS 222 at concentrations of 50, 80, and 100 mg/l in *Cyprinus carpio* and *Sarotherodon mossambicus*. The results indicated that Benzocaine hydrochloride was a more effective anaesthetic than MS 222 at the concentrations applied.

The objectives of the present study is to compare efficacy of MS 222 and Benzocaine to minimise the stress during packing and transportation of ornamental fish and to compare the physiological effects of fish exposed to MS 222 and Benzocaine following handling stress. Indian tiger barb was used as a model animal for the experiment. Physiological effects in Indian tiger barbs due to different dosages of MS 222 and Benzocaine were estimated and compared. This research provides necessary data for assessing the potential of MS 222 and Benzocaine as sedative to reduce the stress response in Indian tiger barb during handling and transportation procedures. In this study, basal plasma cortisol, glucose and lactate levels were determined from wild Indian tiger barb and taken as an index of stress.

7.2. **Materials and Methods**

7.2.1. **Experimental fish**

Mature adults of Indian tiger barb, *Puntius filamentosus* were used for the experiment. The fishes were collected from the Chalakudy river and
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Transported to the laboratory. Fish length and weight averaged at 12.5 cm and 12g, respectively. Prior to experiment, the fishes were acclimatised to the laboratory conditions for 7 days in large FRP tanks with 1000 litre capacity with a constant aerated flow of water. Fish were fed two times a day with commercial pellets. Feeding was stopped 2 days before the commencement of the experiment. Water temperatures during acclimatisation period was about 27 ± 1°C, pH -7.2 -7.6 and dissolved oxygen - 7-8 mg/L.

### 7.2.2. Experimental design

Low density polyethylene (LDPE) cover with 16cm width and 60cm length was used as packing material. 24 bags of identical size and dimension were taken and filled with 1 litre aerated water. These bags were divided into eight groups containing three replicates in each group. Each bag is filled with 6 numbers of Indian tiger barb (*Puntius filamentosus*) having a length ranging from 120 to 130 mm. The bags were oxygenated with equal amount of pure medical oxygen. The upper end of the bags was tied immediately after filling the oxygen. All the bags were wrapped with newspapers and put in five ply corrugated master cartons (60 x 40 x 55cm size) with thermocool lining inside, sealed and kept at 22 ± 1°C for up to 48 hours. Simultaneously another batch of experiment was done and kept at 28 ± 1°C to compare the effect of temperature on the fish. Each bag was sampled one by one at an interval of 6 hours. The same experiment was repeated with two commercial anaesthetics, MS 222 (Tricaine methanesulphonate) at a concentration of 40mg/l and Benzocaine with 20 mg/l at both 22 ± 1°C and 28 ± 1°C. The groups of fish containing bags without anaesthetics were treated as control.

### 7.2.3. Collection of blood

The blood from the fishes were collected as per the method of Stoskopf (1993). Blood samples extracted by cardiac puncture were frozen to -25 °C until analysis. Samples were centrifuged at 4500 rpm at 4°C for 7 minutes.
in a cooling centrifuge and supernatant was taken for the analysis. Blood samples taken from the fishes within 3 minutes of capture and just before packing were used as control and provided the base line values of the blood parameters studied.

7.2.4. Estimation of Plasma Cortisol

The plasma cortisol was estimated as per the method of Barry et al. (1993) using ELISA technique.

Plasma cortisol values were estimated (in µg/dl) from the standard curve.

7.2.5. Estimation of Plasma Glucose

The plasma glucose was estimated as per the Enzymatic colorimetric method Kunst et al. (1984).

Glucose concentration (mg/dl) = Absorbance of Sample A / (Absorbance of Standard A x Concentration of the standard)

7.2.6. Estimation of Plasma Lactate

The plasma lactate was estimated as per the Colorimetric method of Shimojo et al. (1989)

Lactate concentration (mg/dl) = Absorbance of Sample A / (Absorbance of Standard A x Concentration of the standard)

7.2.7. Statistical analysis

Statistical analysis (Three-way ANOVA) was done to find out whether there was any significant difference between different treatments, temperature and time periods.
7.3. Results

Mean plasma cortisol levels in wild fish sampled within 3 minutes of capture were low (18.41μg/dl) but increased markedly to 81.3μg/dl during capture, handling, and transportation to the laboratory. After acclimatisation in the rearing tank, the plasma cortisol was found to get almost stabilised to the initial level of 18.72μg/dl. Similarly, mean glucose level in the wild fish just after capture was 71mg/dl, but increased up to 178mg/dl by the time it was brought to the laboratory. Lactate level (21.08mg/dl) increased from 21.08mg/dl at the beginning of the experiment to 36.87mg/dl on reaching the laboratory. After acclimatisation, plasma glucose and lactate were found to have reduced to the base levels of 72.75mg/dl and 20.87mg/dl respectively (Table 7.1).

7.3.1. Plasma Cortisol

The pattern of elevation in the plasma cortisol level was similar in both anaesthetised and control groups. The concentration of cortisol was found to be extremely high in control groups when compared to that of anaesthetised fish at both the temperatures. Elevation was found to be least in fishes anaesthetised with MS 222. Mean plasma cortisol levels in fish treated with MS 222 at 22 ± 1°C was 64.15μg/dl within the first 6 hours, declined and almost reached the base line with in 48 hours, gradual and then by decreased till the end of experiment except for a shoot up (37.26 ± 2.98μg/dl) in 36th hour (Fig.7.1). Plasma cortisol levels increased to 70.52μg/dl at the 6th hour in Benzocaine treated fishes, then gradually reduced to 33.1 ± 2.91μg/dl in 30 hours and increased to 38.63 ± 5.09μg/dl by the 36th hour. At the end of the experiment the plasma cortisol level was 20.29 ± 2.1μg/dl. In the control group, highest cortisol (78.4 ± 4.04μg/dl) elevation was noticed at 6th hour, which declined to 23.51 by the end of the experiment with an increase of 47.29 ± 3.13μg/dl at 36th hour.
Table 7.1: Plasma parameters of *Puntius filamentosus* during collection, transport and acclimatisation periods

<table>
<thead>
<tr>
<th>S.No</th>
<th>Plasma Parameters</th>
<th>After Capture (Mean ± S.D.)</th>
<th>After transport (Mean ± S.D.)</th>
<th>After acclimatisation (Mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cortisol</td>
<td>18.41 ± 3.73</td>
<td>81.3 ± 3.94</td>
<td>18.72 ± 3.46</td>
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<tr>
<td>2</td>
<td>Glucose</td>
<td>71.3 ± 5.03</td>
<td>178.0 ± 6.56</td>
<td>72.75 ± 5.02</td>
</tr>
<tr>
<td>3</td>
<td>Lactate</td>
<td>21.08 ± 3.92</td>
<td>36.87 ± 5.02</td>
<td>20.87 ± 2.91</td>
</tr>
</tbody>
</table>
Fig. 7.1: Changes in the plasma cortisol in *Puntius filamentosus* during the simulated transport at 22 ±1°C.

Fig. 7.2: Changes in the plasma cortisol in *Puntius filamentosus* during the simulated transport at 28 ±1°C.
Experiments conducted at 28 ± 1°C temperatures also exhibited an increasing trend in cortisol level with time (Fig.7.2). Mean cortisol concentrations at the 6th hour, i.e., the peak values were 115.66 ± 4.19, 72.24 ± 5.96 and 90.58 ± 4.11μg/dl for control, MS 222 and Benzocaine treated samples respectively. After that, a gradual reduction was noticed in the level of cortisol in all the samples including control. The variations noticed were a slight increase in mean cortisol level in MS 222 treated fishes by 36th hour (39.66μg/dl), an increase to 41.57μg/dl in 36hour and a further decrease to 21.82μg/dl in 48 hours in Benzocaine treated fishes. Plasma cortisol in control group almost stabilised in 30 and 36 hours and arrived at 24.72 ± 2.38μg/dl in 48hours.

Statistical analysis showed that there was significant difference in plasma cortisol levels between experimental periods. (p<0.01). Six, 12 and 18 hours showed significantly higher values than all others. (Table.7.1). But between these three periods the difference was not significant. There was significant difference in cortisol levels between temperatures 22 ± 1°C and 28 ± 1°C (p<0.01). 28 ± 1°C showed significantly higher values than temperature 22 ± 1°C. There was significant difference in cortisol levels between the anaesthetised and control groups (p<0.01). Control showed significantly higher values than MS 222 and Benzocaine treated groups. But between the anaesthetised groups the difference was not significant.

7.3.2. Plasma Glucose

Concentration of plasma glucose level in Indian tiger barb showed a gradual increase up to 36 hours of experiment and a slow decline thereafter. Highest glucose level was found in control groups. Among the anaesthetised groups lowest glucose concentration was recorded in MS 222 treated fishes. In animals treated with MS 222 and maintained at a temperature of 22 ± 1°C, the mean glucose level at 6 hours of experiment was 76mg/dl (Fig.7.4). This increased to 122mg/dl and 138mg/dl in the 12th and 18th hours respectively. Steady decrease in glucose levels were
Fig. 7.3: Changes in the plasma glucose in *Puntius filamentosus* during the simulated transport at 22 ±1°C

Fig. 7.4: Changes in the plasma glucose in *Puntius filamentosus* during the simulated transport at 28 ±1°C
Resource abundance and survival of indigenous ornamental fishes of Central Kerala with emphasis on handling and packing stress in *Puntius filamentosus* (Valenciennes) observed from 24th hour onwards and reached 71mg/dl at 48 hours. After 36 hours the glucose level almost stabilised and approached the base line values. Blood samples taken from the Benzocaine treated fishes also exhibited the same pattern as found in the MS 222 treated fish samples. The mean glucose level of Benzocaine treated samples at 6 hours was 83mg/dl, which increased to 152mg/dl by 12 hours; thereafter it declined gradually and reached 79mg/dl by the end of the experiment. In control group, a sudden elevation of glucose from 6th hour to 12th hour was observed (i.e., from 98mg/dl to 158mg/dl). But afterwards it stabilised by 18 hours and then declined to 86 mg/dl in 48 hours.

The plasma glucose level in Indian tiger barb maintained at 28 ± 1°C was slightly high in MS 222 treated groups (Fig. 7.5). At 6 hours the glucose level increased to 81mg/l, which continued and reached a peak in 16 hours (147mg/dl). After that, the glucose level started declining, got stabilised by 36 hours and ended at a value of 110mg/dl in 48 hours. In Benzocaine treated groups the glucose level increased rapidly from 6 hours (87mg/dl) to 12 hours (150mg/dl) and reached its maximum (168mg/dl) in 36 hours. Glucose levels did not exhibit much change in the 24th and 30th hours but reduced to 125 mg/dl by the end of the experiment. Mean glucose level in the 6th hour in control group was 98mg/dl. A sudden increase was noticed at 12th hour (168mg/dl), which continued to reach a peak in 18 hours (182 mg/dl), then showed a slow decline and reached 134mg/dl in 48 hours. The glucose levels did not change too much in 36 and 42 hours in control group.

Significant difference could be seen in glucose concentrations between the experimental periods (p<0.01). Eighteenth hour showed significantly higher values than all the rest (Table 7.2). Between temperatures there was significant difference in glucose levels (p<0.01). 28 ± 1°C showed significantly higher values than 22 ± 1°C. Significant difference could be seen between the control and anaesthetised treatment groups (p<0.01). Control groups showed significantly higher values compared to MS 222 and

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Benzocaine treated groups. But between the anaesthetised groups the difference was not significant.

7.3.3. Plasma lactate

Plasma lactate levels also exhibited an increasing trend with increase in time and incubation up to 36 hours of experiment, after which a decrease in lactate level was noticed with further increase in time. Plasma lactate levels in the anaesthetised groups were comparatively lower than unanaesthetised fish samples. Within the anaesthetised groups MS 222 treated groups showed lower lactate levels. In animals treated with MS 222 and maintained at 22 ± 1°C, the mean plasma lactate level was 21.25 ± 2.08mg/dl in 6 hours (Fig 7.5). However this slowly increased and reached a maximum of 31.12 ± 4.11mg/dl in 36 hours and declined to 23.05 ± 1.69 mg/dl by 48 hours. The lactate level increased to 24.6 ± 3.48mg/dl at 6 hours in Benzocaine treated fish samples. Highest lactate level (43.15 ± 2.6 mg/dl) was recorded in 36 hours, which decreased rapidly to 40.53 ± 2.26 mg/dl in 42 hours and dropped to 28.15 ± 2.64mg/dl by the end of the experiment. In control group a sudden increase in lactate levels were monitored after 6 hours (28.18mg/dl) and since then, a rapid increase was noted up to 36 hours of experiment. Highest concentrations were attained in 30-42 hours time period with a peak of 51.04 ± 3.59mg/dl in 36th hour, which reduced to 38.26 ± 2.09mg/dl in 48 hours.

In the experiment at 28 ± 1°C also the plasma lactate level showed a similar trend as found in the experiment conducted at low temperature, but the amount of lactate in the plasma was found to be higher at this temperature (Fig.7.6). Lowest lactate levels were found at 6th hour and highest were noticed in 36 hours for both anaesthetic treated and control groups. The highest lactate concentrations were obtained during 36th hour i.e., 54.12 ± 4.1, 66.6 ± 4.22 and 71.92 ± 5.04mg/dl respectively for samples treated with MS 222, Benzocaine and control.
Fig. 7.5: Changes in the plasma lactate in *Puntius filamentosus* during the simulated transport at $22 \pm 1^\circ C$.

Fig. 7.6: Changes in the plasma lactate in *Puntius filamentosus* during the simulated transport at $28 \pm 1^\circ C$. 

Table. 7.2: ANOVA of changes in plasma cortisol in *Puntius filamentosus* during the simulated transport at different temperatures and treatments

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>ms</th>
<th>F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>28086.6565</td>
<td>47</td>
<td>597.5884</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between periods</td>
<td>22490.2358</td>
<td>7</td>
<td>3212.891</td>
<td>52.5161</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Between temperatures</td>
<td>984.9126</td>
<td>1</td>
<td>984.9126</td>
<td>16.0988</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Between treatments</td>
<td>2347.8904</td>
<td>2</td>
<td>1173.940</td>
<td>19.1886</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>2263.6277</td>
<td>37</td>
<td>61.1791</td>
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</table>

Table. 7.3: ANOVA of changes in plasma glucose in *Puntius filamentosus* during the simulated transport at different temperatures and treatments

<table>
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<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>ms</th>
<th>F</th>
<th>Significance</th>
</tr>
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<tr>
<td>Total</td>
<td>42664.4977</td>
<td>47</td>
<td>907.7553</td>
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<td></td>
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<tr>
<td>Between periods</td>
<td>27869.8866</td>
<td>7</td>
<td>3981.4124</td>
<td>60.4363</td>
<td>p&lt;0.001</td>
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<tr>
<td>Between temperature</td>
<td>5468.4468</td>
<td>1</td>
<td>5468.4468</td>
<td>83.0089</td>
<td>p&lt;0.001</td>
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<tr>
<td>Between treatments</td>
<td>6888.6852</td>
<td>2</td>
<td>3444.3426</td>
<td>52.2838</td>
<td>p&lt;0.001</td>
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<tr>
<td>Error</td>
<td>2437.4792</td>
<td>37</td>
<td>65.8778</td>
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</table>

Table. 7.4: ANOVA of changes in plasma lactate in *Puntius filamentosus* during the simulated transport at different temperatures and treatments

<table>
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<tr>
<th>Source</th>
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<th>ms</th>
<th>F</th>
<th>Significance</th>
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<tr>
<td>Total</td>
<td>10514.622</td>
<td>47</td>
<td>223.7154</td>
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<td></td>
</tr>
<tr>
<td>Between periods</td>
<td>3786.2140</td>
<td>7</td>
<td>540.8877</td>
<td>16.6857</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Between temperatures</td>
<td>3557.5659</td>
<td>1</td>
<td>3557.566</td>
<td>109.7467</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Between treatments</td>
<td>1971.4443</td>
<td>2</td>
<td>985.7222</td>
<td>30.4084</td>
<td>p&lt;0.001</td>
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<tr>
<td>Error</td>
<td>1199.3979</td>
<td>37</td>
<td>32.4162</td>
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</tbody>
</table>

SS - Sum of squares
ms - Mean squares
df - degree of freedom
F - Variance ratio
There was significant difference in lactate concentration between the experimental periods \(p<0.01\). Thirty sixth hour gave significantly higher values compared to all others (Table 7.3). Between 24\(^{th}\), 30\(^{th}\), 42\(^{nd}\) and 48\(^{th}\) hours, the difference was not significant. Significantly lower values were observed in period 1. Between temperatures there was significant difference in lactate levels \(p<0.01\). The temperature 28 ± 1°C showed significantly higher values than 22 ± 1°C. Significant difference was observed between the control and anaesthetised treatment groups \(p<0.01\). Control groups showed significantly higher values followed by Benzocaine and MS 222 treated groups. But between the anaesthetised groups the difference was not significant.

7.4. Discussion

Plasma cortisol, glucose and lactate levels in wild Indian tiger barb, \(Puntius filamentosus\) sampled within 3 minutes of capture were regarded as base line data for these parameters in unstressed fish. The significant increase in the levels of these biochemical constituents during the initial transportation of fish to the laboratory indicated that commercial capture and handling was highly stressful. Acclimatisation of fishes in one week reduced the values of blood parameters to a considerable level. A further elevation in plasma cortisol glucose, and lactate levels during the experimental hours and reduction at the end as noticed in the present study also strengthen the previous statement. The reduced levels of these parameters at 7 days of post transportation experiment point out that the animals were able to recover from the stress it had, during the handling and transporting periods.

So far there is no published information on the blood parameters of Indian tiger barb \(P. filamentosus\) with respect to handling and transportation stress. Plasma cortisol at higher levels is most likely to be a reflection of fish being stressed at the time of handling and packing procedure. Studies have shown that plasma cortisol profile can vary for a single species.
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depending on severity and duration of the stress and the experimental procedures used (Pickering and Pottinger, 1989, Barton and Iwama 1991, Punkhurst and Sharples, 1992). In the present study, following a stress response the barbs were found to exhibit slow return of cortisol to the basal levels within 48 hours of transport.

Plasma cortisol levels increase after external disturbances and its predominant role in stress response is generally recognised (Wendelaar Bonga, 1997). Specker and Schreck (1980) found elevated levels of plasma cortisol after transporting smolting coho salmon *Oncorhynchus kisutch* at densities of 12 - 120 kg m⁻³ for 4-12 hours, and concluded that stress was more intense during loading and the first few hours of transportation. In the present study, elevation of plasma cortisol was observed from the 6th hour onwards. Plasma cortisol recovery profiles can vary between species and even strain, and tend to be related to the severity and type of stress (Pickering and Pottinger, 1989; Barton and Iwama, 1991). Plasma cortisol levels returned to resting levels, 1 hour after net confinement in young-of-the-year-striped-bass (Young and Cech, 1993), approx. 4 hours after 30 seconds emersion stress in brown trout *Salmo trutta* and rainbow trout (Pickering and Pottinger, 1989), 48 hours in Atlantic salmon *Salmo salar*, 24 hours in the flounder *Platichthys flesus* and turbot *Scophthalmus maximus* after capture and 9 min net confinement (Waring et al., 1992, 1996), approx. 8 hours for brown trout and approx. 24 hours for rainbow trout after handling and 1 hour confinement (Pickering and Pottinger, 1989), 24 hours after capture from the wild in rainbow trout (Pankhurst and Dedual, 1994), 24 hours in domesticated brown trout following capture and handling (Pickering *et al.*, 1982), 24 hours in the sea raven after air exposure and chasing (Vijayan and Moon, 1994), and 48 hours in snapper after capture from the wild and transport to the laboratory (Pankhurst and Sharples, 1992). In general terms, these studies indicate that recovery from an acute stress usually takes place within 48 hours, and even less if the stress is of short duration (minutes). In contrast, chronic stress such as long term confinement can...
result in significantly elevated plasma cortisol levels for periods of up to 4 weeks before acclimation occurs (Pickering and Pottinger, 1989). In the present experiment the reduced rate of elevation of levels of cortisol elevation in all fishes transported at 22 ± 1°C initially, when compared with that at 28 ± 1°C indicated that the activity of corticosteroid hormones is temperature dependent and greatly diminished at low temperature transport.

Transportation procedures have been shown to increase blood glucose levels in several fish species. The elevated blood glucose in *Puntius filamentosus* returned to baseline levels 48 h after transportation. Other studies have also demonstrated the transient effect of transportation on blood glucose titers (Barton et al., 1980; Carmichael et al., 1984; Robertson et al., 1987). This metabolic disturbance may have been induced by catecholamines, i.e., hormones secreted as primary effects of stressors (Mazeaud and Mazeaud 1981) and sustained by increased levels of cortisol (Mommsen et al., 1999) that possibly was secreted during transportation.

The higher plasma glucose concentration over confinement stress may be the result of several factors, including glycogenolysis, gluconeogenesis and/or decreased clearance of glucose from the circulation (Vijayan et al., 1997). Laidley and Leatherland (1988) reported that significant hyperglycaemia could be evident within 16-32 min after stress in fish. Transport induced hyperglycaemia has been reported for small mouth bass *Micropterus dolomieu* (Carmichael et al., 1983), in largemouth bass *M. salmoides* (Carmichael, 1984), as well as in red drum *Sciaenops ocellatus* (Robertson et al., 1988). Such stress related increase of plasma glucose might have happened during the present experimental periods also. Van-Raaij et al. (1996) state that hyperglycaemia in such cases is probably a result of liver glycogenolysis stimulated by catecholamins and of stimulation of gluconeogenesis by cortisol during recovery. The increased plasma glucose levels during acute handling stress could potentially be due to the action of cortisol. (Barton and Iwama, 1991). The glucose
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Concentrations in blood plasma of fishes treated with anaesthetics was significantly lower when compared to that of the control group. This is important, from the point of view of preventing the depletion of energy reserves of fish and also reducing the overall stress due to the handling and transportation procedures.

Plasma lactate concentration is found to increases immediately after stress, primarily because of muscle glycolysis (Milligran and Girard, 1993; Wood and Perry, 1985), and this substrate may be used for glucose production and/or to combat glycogen chronic confinement stress in the liver of stressed fish (Vijayan and Moon, 1992; Vijayan et al., 1994). The return of plasma lactate concentration to unstressed levels at 24 hours from the increased levels at 2 hours post confinement was observed in *Oriochromis mossambicus* (Vijayan et al., 1997). The authors argued that this might be due to the enhancement of hepatic activity of lactate by the stressed fish. In the present study plasma lactate levels increased gradually and reduced to the base levels at 22 ± 1°C within 48 hours of experiment. In plasma lactate deposition, an elevated level is being continued even after 48 hours at 28 ± 1°C. These results are not in agreement with the studies in teleosts (Wood and Perry, 1985) and in rainbow trout (Vijayan and Moon, 1992).

Consistent changes in secondary stress responses (plasma lactate and glucose levels) were observed during handling and transport. Lactate increases in blood occur when insufficient oxygen is available for aerobic cell metabolism. This could be due to reduced ventilation, circulation or after heavy physical exercise (Houston *et al.*, 1971; Iwama *et al.*, 1989; Olsen *et al.*, 1995). Thomas *et al.* (1999) stated that aerobic conditions caused by the stress are known to result in breakdown of muscle glycogen and lactate out of which some of the lactate is released in to circulation. During the experimental periods, the oxygen levels were monitored at definite intervals, and were found to reduce with time. The consistency in the increase observed in lactate levels may be an effect of this fluctuation in the oxygen level during transport. However, fasting of the fish (36hours)
prior to transport would have affected the normal plasma glucose and lactate levels in the fish.

It is apparent that while comparing the blood parameters in Indian tiger barb there are remarkable variations in the primary and secondary stress indicators and their responses with time. Unlike other parameters, mean cortisol levels declined from elevated levels towards the base line in all samples. But glucose and lactate levels displayed an increasing trend. A rapid increase and slow decline in plasma glucose was monitored in all the samples. A slow but steady increase in the lactate levels from the base line was apparent in all samples up to 36 hours followed by a decline in the next 12 hours. No decrease from the base line was observed in any of the parameters in any stage of the experiment.

In conclusion the results obtained from the present study demonstrate that MS 222 and Benzocaine can be used as effective anaesthetic agents for commercial transportation of P. filamentosus for 48 hours. Application of these anaesthetics can reduce the stress as is evident from the study of blood parameters of Puntius filamentosus. The effect of stress in terms of cortisol, glucose and lactate levels of the fish was significantly reduced by the sedation with MS 222 and Benzocaine than the unanaesthetised control group. Of the temperatures studied, 22 ± 1°C was found to be ideal for transporting P. filamentosus, as both anaesthetics were very efficient at this temperature. The levels of plasma cortisol, glucose and lactate were higher at 28 ±1°C. When the anaesthetic potency of MS 222 was compared with that of Benzocaine, it was found that MS 222 was more efficient than the other since it produced lower elevation in the blood parameters of P. filamentosus.