SHORT COMMUNICATION

Stability of some commonly measured blood-chemistry variables in juvenile salmonids exposed to a lethal dose of the anaesthetic MS-222

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Fish culturists and fish biologists make use of blood-chemistry indices for evaluation of fish stress responses, nutritional condition, reproductive state, tissue damage due to handling procedures and health status (McDonald & Milligan 1992; Wagner & Congleton 2004; Congleton & Wagner 2006). Stress-related changes in blood chemistry occur within seconds or minutes after fish are disturbed (Mazeaud & Mazeaud 1981; Gingerich & Drott 1989), so that precautions must be taken to ensure that blood-sampling procedures do not alter the indices of interest. Serial sampling of fish from the same rearing unit may cause progressive changes in some indices (Pickering, Pottinger & Christie 1982; Laidley & Leatherland 1988; Björnsson, Young, Lin, Deftos & Bern 1989); therefore, all fish needed for a sample are often captured at the same time and immediately anaesthetized (batch sampling). Relatively high concentrations (150–200 mg L$^{-1}$) of tricaine methanesulphonate (MS-222), which bring about an irreversible deep plane of anaesthesia within a few minutes, have been reported to be effective in preventing changes in plasma cortisol, a primary stress–response indicator (Davis, Parker & Suttle 1982; Barton, Schreck, Ewing, Hemmingsen & Patino 1985), and are recommended for prevention of stress-related changes in blood chemistry (Wedemeyer, Barton & McLeay 1990). Lower, sedating or immobilizing doses of anaesthetic may elicit rather than prevent stress responses (Strange & Schreck 1978; Smit, Hattingh & Burger 1979; Iwama, McGee & Pawluk 1989; Thomas & Robertson 1991).

Although a number of studies have described the physiological responses of fish to sedating and immobilizing doses of MS-222, only a few studies have reported on responses to higher, lethal concentrations of MS-222 or other anaesthetics. The few studies using higher concentrations of MS-222 (125 mg L$^{-1}$, Laidley & Leatherland 1988; 150 mg L$^{-1}$, Holloway, Keene, Noakes & Moccia 2004) have evaluated changes in blood chemistry at the time of induction of deep anaesthesia, 2–3 min after the initiation of exposure. No previous studies have determined the response of commonly measured blood-chemistry indices to longer (over 10 min) periods of exposure to higher ($\geq$ 150 mg L$^{-1}$) concentrations of MS-222. When fish are batch sampled, typically in groups of 10–20, collection of blood samples (and often of other tissues) and recording of length and mass data may require 20 min or longer, even with the participation of several trained personnel. Therefore, information on blood-chemistry responses to a frequently used and recommended sampling protocol – batch anaesthesia in 200 mg L$^{-1}$ MS-222 – is lacking. The objective of the present study was to determine the stability of 13 commonly employed blood-chemistry indices when juvenile Chinook salmon Oncorhynchus tshawytscha were held in a 200 mg L$^{-1}$ solution of buffered MS-222 for up to 35 min.

Underyearling spring-run Chinook salmon were obtained from Clearwater National Fish Hatchery, Ahsahka, ID, USA, and reared for 9 months in a 600 L circular tank supplied with 10–12 L min$^{-1}$ of
aerated well water at 11–12 °C. They were fed a semi-moist commercial salmon diet (Biodiet Grower, Bio-Products, Warrenton, OR, USA) at 75% of recommended full ration levels. A natural photoperiod was maintained with daylight-balanced artificial lights. Two trials were performed: at the time of the first trial, the fish averaged 146 mm in fork length and 33 g in mass; at the time of the second trial, the fish averaged 156 mm in length and 41 g in mass.

Thirty fish were dip netted from the rearing tank and placed in a bucket with 8 L of well water containing 200 mg L⁻¹ MS-222 and 240 mg L⁻¹ NaHCO₃. The fish lost equilibrium in less than 20 s and regular opercular movements ceased in less than 90 s (ventricular contractions continued, however, for a much longer period). At 2, 8, 14, 25 and 35 min intervals after placing the fish in the anaesthetic, six fish were removed and blood samples were withdrawn by caudal vessel puncture, entering from the ventral midline with a 20-gauge syringe needle. Blood samples (0.15–0.25 mL) were aspirated into a heparinized syringe, ejected into polypropylene centrifuge tubes and placed on ice for up to 15 min. Plasma was separated by centrifugation (5 min at 1200 g) and immediately frozen at −80 °C. This procedure was repeated for each of three tanks (the experimental unit) on two occasions (trials 1 and 2).

Before analysis, the six plasma samples taken at each time interval were divided into two subsets of three samples each, thawed and each subset was pooled by taking an equal volume (0.07–0.09 mL) from each of the three tubes. The 60 pooled samples (5 time intervals × 3 tanks × 2 pooled samples per time interval per tank × 2 trials) were analysed for glucose, total protein, cholesterol, triglyceride, total calcium, Na⁺, Cl⁻ and K⁺ concentrations, and for alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK) and alkaline phosphatase (AP) activities. Assays were performed at Gritman Medical Center, Moscow, ID, USA, using a Dimension model AR-1MT autoanalyzer (Dade Behring Newark, DE, USA) according to the guidelines for operation and standardization established by the Joint Commission for Accreditation of Health Care Organizations and by the College of American Pathologists. Dilution of subsamples was necessary so that ALT, AST and CK activities would fall within the recommended assay ranges (dilutions were 1:6 for ALT and AST, 1:27 for CK). Samples were analysed in sets of 10; samples from all sampling times were included in each set to minimize bias caused by interassay variation.

Data were analysed by repeated measures analysis of variance (ANOVA), with trial as a between-subjects factor and sampling time as the within-subjects factor. Values determined for the two pooled subsamples taken from the same group at the same time were averaged before analysis. The data did not require transformation before analysis; although enzyme activity data are often heteroscedastic, distributions are improved by pooling of samples, as was done in this study. The Greenhouse–Geisser factor ε was used to adjust P-values due to potential lack of sphericity (Kirk 1995). In addition, because tests were performed for 13 different blood-chemistry variables, the P-values required for significance were adjusted by the Holm procedure (Kirk 1995) to control the experiment-wise Type I error rate. The required P was estimated as <0.05 (13-ε)⁻¹, where r was the rank-order of the estimated ANOVA P-value for variable q when P-values for all 13 indices were ranked in ascending order from 0 to 12. Adjusted criteria for significance ranged from P<0.0038 (i.e. 0.05/13) for the variable with the lowest ANOVA P to P<0.05 (i.e. 0.05/1) for the variable with the highest ANOVA P.

For ALT and LDH data (ε factors > 0.7), the mean values for samples taken at the first (2 min) sampling interval were compared with the mean values for samples taken at 8, 14, 25 and 35 min by Dunnett’s test (two-tailed). For all other indices (because ε factors < 0.7), the mean values of samples taken at the first sampling interval were compared with the mean values for samples taken at the four later sampling intervals by paired t-tests, with the t-test P-values required for significance adjusted by the Holm procedure.

Values for glucose, calcium, potassium, LDH and CK increased over time in the blood of anaesthetized fish, as indicated by ANOVA P-values that met adjusted criteria for significance (Table 1). Although ANOVA P-values < 0.05 were estimated for ALT, AST, total protein and AP, the criteria for significance required by the Holm procedure were not met for these indices. Pair-wise comparison of means indicated, however, that ALT and AST means for 25 min samples were significantly higher than means for 2 min (initial) samples. For total protein, the difference between 2 and 25 min means approached but did not attain significance. Activities of AP varied over time, but did not trend upward or downward. Values for cholesterol, triglycerides, Na⁺ and Cl⁻ did not change significantly over time (Table 1).

An increase in plasma glucose after only 8 min of anaesthesia (26% higher than at 2 min) was unex-
Table 1 Results of ANOVA testing* to determine the effects of anaesthesia time on blood-chemistry values for juvenile Chinook salmon. Mean blood chemistry values (±SE) are shown for samples taken after 2, 8, 14, 25, or 35 min of anaesthesia in two trials†. Significant P-values‡ are italicized, as are also mean values for time intervals differing significantly from initial (2 min) sample means.

<table>
<thead>
<tr>
<th>Variable</th>
<th>ANOVA P</th>
<th>Time interval (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>ALT (IU L⁻¹)</td>
<td>0.018</td>
<td>33.8 ± 2.0</td>
</tr>
<tr>
<td>AST (IU L⁻¹)</td>
<td>0.032</td>
<td>347 ± 12</td>
</tr>
<tr>
<td>LDH (IU L⁻¹)</td>
<td>0.002</td>
<td>549 ± 67</td>
</tr>
<tr>
<td>CK (IU ml⁻¹ × 10⁻²)</td>
<td>0.001</td>
<td>5.33 ± 0.64</td>
</tr>
<tr>
<td>AP (IU L⁻¹)</td>
<td>0.025</td>
<td>51.1 ± 2.8</td>
</tr>
<tr>
<td>Glucose (mg dL⁻¹)</td>
<td>&lt; 0.001</td>
<td>64.0 ± 1.4</td>
</tr>
<tr>
<td>Total Protein (g dL⁻¹)</td>
<td>0.038</td>
<td>2.72 ± 0.07</td>
</tr>
<tr>
<td>Cholesterol (mg dL⁻¹)</td>
<td>0.088</td>
<td>195 ± 8</td>
</tr>
<tr>
<td>Triglyceride (mg dL⁻¹)</td>
<td>0.240</td>
<td>215 ± 9</td>
</tr>
<tr>
<td>Total calcium (mmol L⁻¹)</td>
<td>0.003</td>
<td>2.44 ± 0.07</td>
</tr>
<tr>
<td>Na⁺ (mmol L⁻¹)</td>
<td>0.350</td>
<td>160 ± 1</td>
</tr>
<tr>
<td>Cl⁻ (mmol L⁻¹)</td>
<td>0.270</td>
<td>128 ± 1</td>
</tr>
<tr>
<td>K⁺ (mmol L⁻¹)</td>
<td>0.005</td>
<td>3.18 ± 0.14</td>
</tr>
</tbody>
</table>

*Repeated measures ANOVA; P-values for the factor time (within-subjects factor) were adjusted by the Greenhouse-Geisser factor epsilon.
†The trial effect was significant only for ALT (mean values were approximately 15% higher in Trial 2); tank effects and all interactions were non-significant.
‡Criteria for significance were adjusted by the Holm procedure to account for multiple testing: the P-values required for significance ranged from 0.0038 for glucose to 0.05 for Na⁺.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase; AP, alkaline phosphatase.

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References


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