Temporal and Spatial Variation of Plasma Biochemistry in Farmed Atlantic Salmon *Salmo salar* L. and Determination of Normal Ranges

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**Abstract**

Plasma biochemistry of farmed Atlantic salmon was compared spatially and temporally in the first year in the sea and normal ranges determined. Parameters varied significantly between fish from two farms and over time and were not normally distributed. In some cases these were correlated with time from stocking, fish size, water temperature and day length, but no single factor was dominant.

**Introduction**

Blood chemistry measures such as haematocrit, enzymes and electrolytes have been used in diagnosis and determination of health conditions in fish, including disease and environmental influences (Hille, 1982). Normal ranges in serum have been reported for Atlantic salmon by Sandes et al. (1988) and in rainbow trout by Hille (1982). However, many environmental and internal variables such as size of fish and season, photoperiod, water temperature and reproductive condition can affect blood chemistry (Folmar et al., 1992). Other variables include the method of obtaining samples, the anaesthetic used, handling of samples and level of stress (Canfield et al., 1994). The values for many biochemical measures in fishes are not normally distributed, with corresponding wide coefficients of variation (Miller et al., 1983). The determination of normal ranges can therefore be difficult and those quoted are often wide. Few studies have examined the seasonal variation in blood chemistry values and also differences with fish size. The present study examines temporal and spatial variation in plasma biochemistry in salmon in their first sea year. Sampling conditions and handling of samples were standardised to reduce variation in measurements. The variation with time, locality, fish size, water temperature and day length are examined. The health and feeding status of these fish were also followed to examine any likely impact on plasma biochemistry.

**Methods**

Salmon were sampled each month on two marine farms (IB in Lochaber and A in the Firth of Clyde) from stocking as smolts in April 1995 until March 1996. Stock numbers on each farm were c. 400,000 one seawinter salmon of a single year class, with an initial weight of 50 g, at a density of 17,000 per 16 m square cage. The husbandry conditions were maintained as similar as possible as were fish origin, commercial feed type and feeding regime.
Prior to the first daily feed ten fish were sampled on each sampling date by raising a section of the net. Fish were removed by handnet and killed immediately by a blow to the head and blood taken from the caudal vein with a syringe and needle and decanted into 2 ml lithium heparin containers. The fish was measured to fork length (mm below) and weight to nearest gramme. The sex was recorded and no fish was sexually mature or maturing, with the gonadosomatic index (GSI): weight g of gonad/fish wt x 100 less than 1% in all fish sampled. However, the sexes were not separated when analysing data. Samples were transported to the laboratory on ice within 4 hours of sampling and kept refrigerated overnight. Samples were centrifuged at 2000 rpm for 10 mins, frozen, and thawed 2 to 3 weeks’ later for analysis. Biochemical analysis was carried out, using a Vettest dry chemistry analyser used for veterinary investigation (Idexx Laboratories, Chalfont St. Peter, Bucks, UK), to determine indicators of impaired renal function, viz. albumin, globulin, total protein (TP), creatinine and urea, reduced hepatic function, viz. albumin, globulin, TP, alkaline phosphatase (ALKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and pancreatitis, viz. lipase and amylase. These analytes were measured as they are routinely used in veterinary medicine for diagnostic purposes and also in reference studies on fish biochemistry (Hille, 1982).

The health of the fish was assessed by monitoring mortalities daily, enumerating sea lice weekly (Copepoda: Caligidae) and taking bacteriological, virological and histopathological samples as necessary. Feed input was recorded as kg/pen/day and growth rate as specific growth (SGR) and food conversion rate (FCR) calculated monthly from feed input and sample weighing 100 fish per cage with an electronic balance. Data were tested

<table>
<thead>
<tr>
<th>Month</th>
<th>Albumin g/l</th>
<th>Globulin g/l</th>
<th>Total protein g/l</th>
<th>ALKP iu</th>
<th>ALT iu</th>
<th>AST iu</th>
<th>Creatinine µmol/l</th>
<th>Urea µmol/l</th>
<th>Lipase iu</th>
<th>Amylase iu</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>6.8-14.4</td>
<td>28-33</td>
<td>35.8-46.8</td>
<td>182-712</td>
<td>7.7-18.3</td>
<td>123-315</td>
<td>19-84</td>
<td>0.46-1.18</td>
<td>24-60</td>
<td></td>
</tr>
<tr>
<td>June</td>
<td>9.1-17.2</td>
<td>26-31</td>
<td>34.5-55.2</td>
<td>342-970</td>
<td>8.7-31.9</td>
<td>180-371</td>
<td>19-59</td>
<td>1.18-2.81</td>
<td>28-118</td>
<td>269-709</td>
</tr>
<tr>
<td>August</td>
<td>28-30</td>
<td>40.5-43.7</td>
<td>478-692</td>
<td>120-178</td>
<td>7.7-14.5</td>
<td>91-135</td>
<td>0.50-0.74</td>
<td>58-72</td>
<td>614-1082</td>
<td></td>
</tr>
<tr>
<td>September</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>October</td>
<td>39-51</td>
<td>63.0-85.6</td>
<td>387-775</td>
<td>4.3-10.3</td>
<td>289-557</td>
<td>30-43</td>
<td>1.43-2.07</td>
<td>83-128</td>
<td>666-906</td>
<td></td>
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<tr>
<td>November</td>
<td>22.3-33.2</td>
<td>31-53</td>
<td>49.1-87.0</td>
<td>213-845</td>
<td>5.8-8.4</td>
<td>308-610</td>
<td>14-118</td>
<td>0.40-2.06</td>
<td>70-178</td>
<td>496-936</td>
</tr>
<tr>
<td>December</td>
<td>16.3-24.2</td>
<td>29-46</td>
<td>46.7-69.5</td>
<td>353-1060</td>
<td>9.2-12.0</td>
<td>105-277</td>
<td>12-40</td>
<td>0.35-0.70</td>
<td>140-158</td>
<td>344-650</td>
</tr>
<tr>
<td>January</td>
<td>15.2-28.4</td>
<td>37-45</td>
<td>58.0-73.6</td>
<td>176-316</td>
<td>5.7-8.3</td>
<td>233-477</td>
<td>10-12</td>
<td>0.55-0.75</td>
<td>84-100</td>
<td>317-557</td>
</tr>
<tr>
<td>February</td>
<td>18.1-34.9</td>
<td>31-52</td>
<td>53.0-86.9</td>
<td>195-367</td>
<td>3.9-11.1</td>
<td>166-309</td>
<td>11-30</td>
<td>0.27-0.47</td>
<td>40-118</td>
<td>329-844</td>
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<tr>
<td>March</td>
<td>14.6-26.0</td>
<td>569-1071</td>
<td>176-1071</td>
<td>4-32</td>
<td>52-610</td>
<td>11-135</td>
<td>0.26-2.81</td>
<td>24-178</td>
<td>269-1082</td>
<td></td>
</tr>
<tr>
<td>Overall range</td>
<td>6.8-34.9</td>
<td>25-53</td>
<td>34.5-87.0</td>
<td>176-1071</td>
<td>4-32</td>
<td>52-610</td>
<td>11-135</td>
<td>0.26-2.81</td>
<td>24-178</td>
<td>269-1082</td>
</tr>
</tbody>
</table>

Table 1. Range in plasma biochemistry of salmon on two farms expressed as the range in 95% confidence limits.
Figure 1. Monthly plasma biochemistry parameters on two salmon farms, IB in open block, A in solid bar. Albumin, globulin, total protein, ALKP=alkaline phosphatase, ALT= alanine aminotransferase, AST= aspartate aminotransferase, lipase, amylase, creatinine and urea. 95% confidence limits are shown. Significant differences are indicated at P<0.05; S= significant differences between farms IB and A on a specific sampling month; I= significant difference between the current and last sampling date at farm IB and A= between sampling points for farm A.
for homogeneity of variances by Bartlett’s test and, where the frequency distribution was not normal, non parametric tests (Kruskal Wallis and Mann Whitney) were used to compare values between months and farms. The normal range was considered as data falling within the range of the 95% confidence limits of both farms combined. Individual blood measures were plotted against the constant variables (x): time (months from stocking), fish weight, water temperature, daylength, and Spearmans correlation calculated. Mean values between farms and between sampling dates were considered significantly different when $P<0.05$.

**Results**

*Albumin*

The range in confidence limits on both farms from May to March was 7 to 35 iu (Table 1). Albumin was significantly higher on both farms IB and A from November to February compared with the first four months at sea (Fig. 1). There was no significant difference in mean values between farms with the exception of February when albumin was higher in fish at IB (Fig. 1). Albumin was positively correlated with time and increasing fish weight in IB (weights shown in Fig. 2), negatively correlated with day length but was not correlated with temperature (Table 2).
Globulin
The range in globulin was 25 to 53 iu and mean values were significantly higher from November to February and also at farm IB compared with farm A during the winter. Globulin was only significantly positively correlated with time and weight of fish from farm IB (Table 2). Although there was no correlation with temperature, there was a significant inverse relationship with day length.

Total protein
The combined range in confidence limits of total protein was 35 to 87 iu. TP was significantly higher on both farms from November to February compared with May to August. TP was only significantly different between farms in January and February with higher values at IB. TP increased significantly with time (IB only) and weight of fish and was negatively correlated with day length, although there was no association with temperature.

**ALKP**
The range in ALKP was wide, from 176 to 1071 iu, with monthly fluctuations and no trend in values. There was no significant difference in ALKP between farms IB and A in any month. Given the fluctuations in ALKP it is not surprising that there was no correlation with time, fish weight, temperature or day length.

**ALT**
The annual range in ALT was 4 to 32 iu with a mean of 10 iu (Table 1, Fig. 1). Mean ALT was not significantly different between months, apart from June when it was significantly higher. There was no significant difference in ALT between farms IB and A in any month. ALT in fish from farm IB was negatively correlated with time and weight of fish. Although there was no relationship with temperature on both farms, ALT at farm IB was positively correlated with photoperiod. This may be coincidental as higher ALT may be primarily related to smaller fish size.

**AST.**
Values of AST were in the wide range 52 to 610 iu, with significant differences between months and also between farms IB and A. Consequently there were no significant correlations with time, fish size, temperature or photoperiod.

**Creatinine.**
The range in creatinine was wide, from 11 to 135 umol/l, and mean values were significantly different between months and between farms. There was no correlation with time, fish weight, temperature and photoperiod.
Urea
The range in urea was 0.26 to 2.81 mmol/l, with significantly higher values in June and July on both farms and also in November on farm IB. Urea in fish from farm IB was positively correlated with temperature and negatively correlated with fish weight.

Lipase
The range in lipase was wide, 24 to 178 iu. Lipase was significantly higher in November and December compared with other months and, from November, was also significantly higher at farm IB compared with A. However, there were no significant correlations with time, fish size, temperature or day length, perhaps due to these wide fluctuations.

Amylase
The range in amylase was wide, 269 to 1082 iu, with no significant differences between farms and between months, although there was a pattern of lower values in winter. Amylase was not correlated with time or fish weight but was positively correlated with temperature at farm IB.

Table 2. Spearman correlations of plasma biochemistry on time, fish weight, temperature and daylength. Significance as p<0.05. N=10 in all correlations.
Discussion

No consistent relationship of plasma biochemistry with season, fish weight, water temperature and photoperiod could be found in this study, although there was an indication that proteins and digestive enzyme amylase were lower at reduced water temperatures reflecting lower metabolic demand and reduced appetite. Sandnes et al. (1988) however found no significant seasonal variations in TP, albumin and the TP/albumin ratio in Atlantic salmon serum. However, results have been contradictory with high concentrations of TP in June and September (Sano, 1960) while Haider (1970) found highest levels in winter. Schlotfeldt (1975) found total protein correlated with temperatures with highest levels at the end of summer.

In other species, for example striped mullet and pinfish, serum chemistry varied with season (Folmar et al. 1992). Creatinine in rainbow trout varied monthly with no obvious rhythm (Sano, 1960). Blood sampled from the caudal vein rather than by cardiac puncture may show elevated creatinine kinase (Gaudet et al., 1975). Canfield et al. (1994) found elevations in creatine kinase in captive Australian snapper which were related to the problems in sampling and they suggested that the increases were due to damage to the skeletal muscle following repositioning of the needle.

Blood chemistry varied with month and to a lesser extent between farms, and these differences were frequently significant. In obtaining a range of normal values the extremes in levels have been accommodated, and in some parameters, this places restrictions on blood chemistry data in assessing changes in health status. However, the 95% confidence limits shown in Table 1 give an indication of the variation between individual fish. In most of these the limits are narrow with the exception of ALT and amylase where there was a wide natural variation between fishes.

Although sampling methods and handling were consistent to reduce variation in values, differences in husbandry, sea lice numbers and health status of fish may have affected plasma biochemistry. However, on the two farms considered here in the first year of the production cycle, no health problems were reported (bacteriological and histological samples were taken routinely) and there were no problems with inappetance. In comparing the health status of the fish on the two farms lice numbers were higher at farm IB (maximum number 5 cf. 1-2) but farm A had a burden of 1 to 2 Eubothrium crassum which were absent from fish at farm IB.

Blood chemistry values were not normally distributed and non parametric techniques had to be used for testing differences and correlations. Fish were sampled prior to feeding to reduce the variability due to sampling techniques and samples were stored on ice prior to laboratory analysis. The normal range in this study was calculated using the confidence limits of the sample mean but other measures have been used. Clinical chemistry evaluation in human medicine use a homoscedastic distribution of a mean plus or minus two standard deviations (Tietz, 1987). In crabs the normal range was also calculated as 2.2 times the standard deviation (Menon & Sivadas, 1967). However, in rainbow trout the blood chemistry parameters were not homoscedastic and
it was suggested that the normal ranges should be calculated using non-parametric statistics (Miller et al., 1983).

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References


