Seasonal Variation of Acetylcholinesterase Activity as a Biomarker in Brain Tissue of *Capoeta umbla* in Pülümür Stream

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**A B S T R A C T**

This study was carried out between March 2015 and February 2016 with the aim of determining the seasonal variation of acetylcholinesterase (AChE) activity in brain tissue of *Capoeta umbla* (Heckel, 1843) caught from Pülümür Stream (Tunceli). The seasonal fluctuations in the AChE activity of *C. umbla* which caught between the 39°6' 19''N - 39°36' 56''E (A) and 39°11' 36''N-39°41' 36''E (B) coordinates of Pülümür Stream, were studied over a period of one year. The enzyme activity was higher in spring and summer than in autumn and winter. In addition, the minimum value for the AChE activity was determined in the winter season and the maximum value was determined in the spring.

**Keywords:** Acetylcholinesterase activity, *C. umbla*, Pülümür stream, seasonal variation

**INTRODUCTION**

Acetylcholinesterase (AChE) is a significant neurotransmitter which regulate the nerve impulse transmission in cholinergic synapses of organocholinergic system in fish. Level of the enzyme activity is the indicator of the physiological state of the nervous system. Especially high enzyme activity is occurred in central nervous system of fish first of all in its brain (Chuiko et al. 1997). The AChE enzyme inhibition causes the accumulation of acetylcholine, causing continuous and excessive stimulation of nerve/muscle fibres, leading to tetany, paralysis and ultimate death (Kirby et al. 2000; Forget et al. 2003). The AChE activity, inhibited by the action of pesticides and other contaminants, is also considered as a potential biomarker in monitoring environmental pollution (Robillard et al. 2003). In addition to pollution, seasonal changes are known to be an important factor affecting biomarker activity (Barda et al. 2014) In fact; interpretation of biomarker data is difficult because natural fluctuations of environmental factors during the year are probably to influence enzymatic activity. Therefore, changed levels of a particular enzymatic biomarker may merely reflect natural variation in the annual physiological cycle of a species rather than exposure to chemical pollution (Robillard et al. 2003). The enzymatic activity responses can also be affected by seasonal variations of both environmental factors and metabolic activities. From this point of
view, the understanding of natural changes of biomarkers can be useful for interpretation of field results and to differentiate the onset of biological disturbance from the natural variability (Bocchetti and Regoli 2006; Barda et al. 2014).

Fish, which they are relatively easy to identify numbers, biodiversity and behaviour are valuable bioindicators. They are less susceptible to natural micro-environmental changes from lower organisms, making them suitable for assessment of regional and macro environmental changes (Gadzala-Kopciuch et al. 2004). In this study, *C. umbla* lived in Püllumür Stream, was preferred because of its easy sampling and economic importance.

The results of a one year-long study on the AChE activities in *C. umbla* collected from Püllumür Stream were evaluated and the seasonal changes of AChE activities were reported.

**Materials and Methods**

**Location of sampling site**

Püllumür Stream is one of the major water sources of the Tunceli. It originates from the Püllumür district of Tunceli province and merges with Munzur Stream in Tunceli province centre to form Uzunçayır Dam Lake. This stream has a surface area of 569 ha and a discharge of 662 m$^3$/s (Yeşil 2017). This work carried out between the coordinates A (39°06’ 19’’N- 39°36’ 56’’E) and B (39°11’ 36’’N – 39°41’ 36’’E) of the Püllumür Stream (Tunceli) (Figure 1).

**Figure1. Location (A to B) of the sampling area (URL: 1).**

**Environmental factors**

In situ measurements of physical variables, water temperature (Temp), dissolved oxygen (DO) and pH values were conducted using a YSI multi-probe field meter.

**Fish sampling and preparation**

The fish samples were caught by the electroshock device from Püllumür Stream monthly. Fifteen fishes were caught for each season, including 5 fish per month. Totally, 60 fishes were used in the study. Average weight and length measurements of seasonally collected fish are given in Table 1. Fishes were brought to the Laboratory of Fisheries Faculty of Munzur University as alive with air-reinforced tanks. They were anesthetized with benzocaine then total length and total body weight of each fish were measured. It was autopsied and brain tissues of fish were taken immediately. Tissue were stored at -20 ºC until analysis.

**Table1. Total length and weight of seasonally caught fish.**

<table>
<thead>
<tr>
<th>Season</th>
<th>n (number of fish)</th>
<th>Total Length (cm)</th>
<th>Weigh (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>15</td>
<td>25.12±1.20</td>
<td>170.54±35.03</td>
</tr>
<tr>
<td>Summer</td>
<td>15</td>
<td>25.08±1.99</td>
<td>152.81±29.80</td>
</tr>
<tr>
<td>Autumn</td>
<td>15</td>
<td>23.80±1.00</td>
<td>142.68±29.60</td>
</tr>
<tr>
<td>Winter</td>
<td>15</td>
<td>24.12±0.50</td>
<td>144.92±17.40</td>
</tr>
</tbody>
</table>

**Acetylcholinesterase (AChE) activity analysis procedure**

Brain tissue samples were dried on a drying paper, weighed and homogenized by dilution with 0.05 M sodium-phosphate buffer pH 7.4, containing 1/10 of 0.25 M sucrose. It was then centrifuged at 3500 rpm at 4°C for 15 minutes and supernatants were removed. The AChE activity was determined.
according to the method of Ellman et al. (1961) at a wavelength of 412 nm, and run time of 5 min. Tissue protein values were detected according to Lowry et al. (1951) to determine specific enzyme activity.

**Statistical analysis**

Statistical analysis of the data obtained at the trial was made using the SPSS 24.0 statistical program. The results are given as median ± standard error. The data obtained were tested by one-way analysis of variance (Oneway-ANOVA).

**Results**

**Environmental factors**

Seasonal variations in the environmental parameters (water temperature, dissolved oxygen and pH) measured at sampling station from March 2015 till February 2016, are given in the Table 2. Water temperature has changed in proportion to the seasonal change. In the Pülümür Stream, elevations in temperature from 2°C up to 19°C were recorded during the study period. The dissolved oxygen did not show any significant difference between seasons. The pH value of water is lower than other seasons in summer.

<table>
<thead>
<tr>
<th>Table 2. Seasonal mean temperature (Temp.), dissolved oxygen (DO) and pH values.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seasons</td>
</tr>
<tr>
<td>Temp. ( °C)</td>
</tr>
<tr>
<td>DO mg/L</td>
</tr>
<tr>
<td>pH</td>
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</tbody>
</table>

<sup>a,b,c</sup> The difference between the values indicated by different upper symbols on the same line is statistically significant (p<0.05).

**Acetylcholinesterase activity**

The AChE activity of brain in *C. umbra* showed different among seasons (Table 3). The mean the AChE activities fluctuated between 0.014 to 0.54 U/mg protein. An effect on the enzyme activity of dissolved oxygen and pH values has not been determined. However, in *C. umbra* the activity of the AChE increased during the spring and summer along with elevations in water temperature, but in late summer, autumn and winter decrease of temperature appeared reduction in activity. In the spring and summer seasons the AChE activity of the fishes was similar (p<0.05) higher autumn and winter (p<0.05). And the difference between seasons was statistically significant. The AChE activity of fish in autumn is higher than winter, lower than other seasons (p<0.05). Furthermore, for the study period, the lowest enzyme activity was detected winter (January-February) while the highest enzyme activity was detected in spring (April).

<table>
<thead>
<tr>
<th>Table 3. Seasonal variations in the AChE activity in brain of <em>C. umbra</em> (mean ± SD).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seasons</td>
</tr>
<tr>
<td>Spring</td>
</tr>
<tr>
<td>Summer</td>
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<tr>
<td>Autumn</td>
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<td>Winter</td>
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</tbody>
</table>

<sup>a,b,c</sup> The difference between the values indicated by different top symbols in the same column is statistically significant (p<0.05).

**Discussion**

Enzymatic activity responses may also be affected by seasonal changes in environmental factors, in terms of natural changes in biomarkers. In this respect, understanding of the natural changes of biomarkers can be useful for interpretation (Bocchetti and Regoli 2006). The AChE is an enzyme that has been widely used as an important biomarker of exposure to organophosphorous (OP) and carbamate (CB) insecticide in environmental monitoring (Fulton and Key 2001). Once the natural fluctuations of such parameters have been defined, any significant change beyond the normal range can be used as a marker of contaminant intake in the organism (Ricciardi et al. 2006).

The seasonal impact on the AChE activity has been reported by other authors. Bueno-Krawczyk et al. (2015), Astyanax bifasciatus followed the seasonal change of the AChE activity in brain and muscle tissue and found the lowest activity in winter. The AChE activity of *Mytilus* sp. from Baltic Sea showed significant seasonal differences with maximum activities in summer and minimum activities in winter (Pfeifer et al. 2005). A similar result was reported by Cikciokoglu Yildirim et al. (2014); they detected that the AChE activity of *C. umbra* caught from the Uzunçayır Dam Lake was considerably higher in the spring compared to autumn in the gill and liver. Findings from the present study indicate that the AChE activity of *C. umbra* captured in spring and summer higher than that of fish captured in the autumn and winter (Figure 2). This result is compatible with the findings of other studies.
Fish are particularly susceptible to environmental temperature changes because they are poikilothermic species. For this reason, temperature is defined as one of the most important abiotic factors for fish, as it can potentially affect all metabolic, physiological and ecological aspects and behavioural trends of the life cycle of fish (Almeida et al. 2015). Most enzymatic activities in poikilothermic species change with the temperature of their environment. In fact, the level of the AChE activity does not directly depend on environment temperature, but depend on the physiological activity, which is tightly correlated with water temperature (Forget et al. 2003). Bocquené and Galgani (1998) claimed that temperature is the most important regulatory factor on the natural changes in the AChE activity. Because temperature can affect both contaminant concentrations and physiological activity of fish (Kopecka et al. 2006). In some studies, the AChE activity has been reported to increase with increasing water temperature. It has been reported by the Hogan (1970) that the increase in water temperature caused an increase in the AChE activity of Lepomis macrochirus. Seasonal variation of the AChE activity in relation to temperature and salinity was observed in Mytilus sp. in the Baltic Sea and significant differences were detected (Pfeifer et al. 2005). In this study, the water temperatures at Pülmür Stream rise up to 19°C in summer and decreased to 2°C in winter. For the study period, the lowest enzyme activity was detected winter while the highest enzyme activities were detected in spring and summer. For this reason, the AChE activity in fish is thought to follow seasonal changes in water temperature.

It has been suggested that the natural changes in AChE activity are not directly related to the age, sex, or reproductive period of the organism (Bocquené and Galgani 1998). However, in our study, the highest the AChE activity of brain in Perca fluviatilis has the highest the AChE activity of brain in April–May which the reproductive period.

The AChE fluctuations of C. umbra in Pülmür Stream are revealed by this study our results show that there had been significant seasonal variability in the AChE activity measured over a 12-month study period. Differences in enzyme activity may be attributed to changes in water temperature at the Pülmür Stream.

References


Figure 2. Seasonal fluctuations in the AChE activity in brain of C. umbra.

Perca fluviatilis has the highest the AChE activity of brain in April–May which the reproductive period.


