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

Ayşegül PALA **Osman SERDAR***

Munzur University, Fisheries Faculty, 62000, Tunceli-Turkey

ABSTRACT

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 39̊ 06' 19"N- 39̊ 36' 56"E (A) and 39̊ 11' 36"N-39̊ 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

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*** SORUMLU YAZAR**

oserdar@munzur.edu.tr
Tel : +90 505 695 93 34

Pülümür Akarsuyu'ndaki *Capoeta umbla*'nın Beyin Dokusunda Bir Biyobelirteç Olarak Asetilkolinesteraz Aktivitesinin Mevsimsel Değişimi

Öz: Bu çalışma, Pülümür akarsuyundan (Tunceli) alınan *Capoeta umbla* (Heckel, 1843)'nın beyin dokusundaki asetilkolinesteraz (AChE) enzim aktivitesinin mevsimsel değişimini belirlemek amacıyla Mart 2015 ile Şubat 2016 tarihleri arasında gerçekleştirildi. Pülümür Akarsuyu'nun 39̊ 06' 19"N - 39̊ 36' 56"E (A) ve 39̊' 11' 36'N - 39̊ 41' 36"E (B) koordinatları arasında yakalanan *C.umbla*' nın AChE aktivitesindeki mevsimsel dalgalanmalar bir yıl boyunca incelendi. Enzim aktivitesi, ilkbahar ve yaz aylarında sonbahar ve kışa kıyasla daha yüksekti. Ayrıca, AChE aktivitesine ait minimum değer kışın, maksimum değer ise ilkbaharda tespit edildi.

Anahtar kelimeler: Asetilkolinesteraz (AChE) aktivitesi, *C. umbla*, Pülümür Akarsuyu, mevsimsel değişim

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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 Pülümür 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 Pülümür Stream were evaluated and the seasonal changes of AChE activities were reported.

Materials and Methods

Location of sampling site

Pülümür Stream is one of the major water sources of the Tunceli. It originates from the Pülümür 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 Pülümür Stream (Tunceli) (Figure 1).

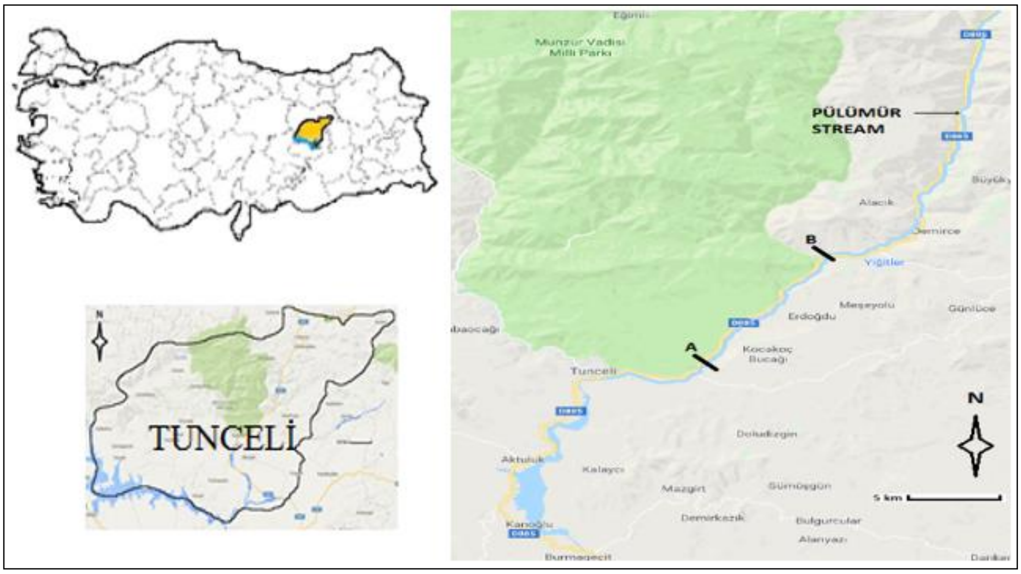

Figure1. Location (A to B) of the sampling area (URL: 1).

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 Pülümür 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.

	n (number of fish)	Total Length (cm)	Weigh (g)
Spring	15	25.12±1.20	170.54±35.03
Summer	15	25.08±1.99	152.81±29.80
Autumn	15	23.80±1.00	142.68±29.60
Winter	15	24.12±0.50	144.92±17.40

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 be 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 2. Seasonal mean temperature (Temp.), dissolved oxygen (DO) and pH values.

	Spring	Summer	Autumn	Winter
Temp. (˚C)	13.69 ± 2.40^{b}	18.68 ± 0.90^{c}	11.41 ± 2.10^{b}	2.55 ± 0.40^{a}
DO mg/L	9.08 ± 0.50^{a}	9.60 ± 0.70^{a}	8.59 ± 0.80^{a}	9.13 ± 0.80^{a}
pH	9.16 ± 0.40^{b}	7.11 ± 0.90^{a}	8.32 ± 0.30^{b}	8.46 ± 0.20^{b}

^{a,b,c} 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. umbla* 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. umbla* 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 them 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).

Table3. Seasonal variations in the AChE activity in brain of *C. umbla* (mean ± SD).

Seasons	AChE activity (U/mg protein)
Spring	0.54 ± 0.10^{c}
Summer	0.49 ± 0.05^{c}
Autumn	0.31 ± 0.45^{b}
Winter	0.04 ± 0.01^{a}

^{a,b,c} 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-Krawcayk 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 Cikcikoglu Yildirim et al. (2014); they detected that the AChE activity of *C. umbla* 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. *umbla* 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.

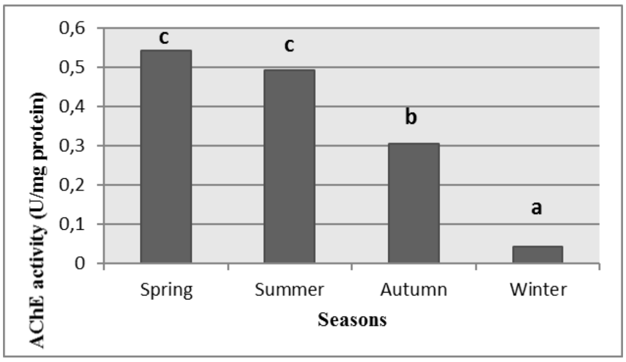

Figure 2. Seasonal fluctuations in the AChE activity in brain of *C. umbla*.

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 Pülümür Stream rise up to 19℃ in summer and decreased to 2℃ 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 *C.umbla* was occurred in April, which is their breeding season. This result is compliant with the finding reported by Chuiko and Kozlovskaya (1989). They declared P*erca fluviatilis* has the highest the AChE activity of brain in April-May which the reproductive period.

The AChE fluctuations of *C. umbla* in Pülümür 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 Pülümür Stream.

References

Almeida JR, Gravato C, Guilhermino L. 2015. Effects of temperature in juvenile seabass (*Dicentrarchus labrax* L.) biomarker responses and behaviour: implications for environmental monitoring. Estuaries and Coasts. 38(1):45-55. doi: 10.1007/s12237-014-9792-7

Barda I, Purina I, Rimsa E, Balode M. 2014. Seasonal dynamics of biomarkers in infaunal clam *Macoma balthica* from the Gulf of Riga (Baltic Sea). J Marine Syst. 129(2014): 150-156. doi: 10.1016/j.jmarsys.2013.05.006

Bocchetti R, Regoli F. 2006. Seasonal variability of oxidative biomarkers, lysosomal parameters, metallothioneins and peroxisomal enzymes in the Mediterranean mussel *Mytilus galloprovincialis* from Adriatic Sea. Chemosphere. 65(6): 913–921. doi: 10.1016/j.chemosphere.2006.03.049

Bocquené G, Carbamates Galgani F. 1998. Biological effects of contaminants: cholinesterase inhibition by organophosphate and carbamate compounds (No. 22). Copenhagen, Denmark: International Council for the Exploration of the Sea 19p

Bueno-Krawcayk ACD, Guiloski IC, Piancini LDS, Azevedo JC, Ramsdorf WA, Ide AH, Guimarães ATB, Cestari MM. Silva de Assis HC. 2015. Multibiomarker in fish to evaluate a river used to water public supply. Chemosphere. 135(2015): 257-264. doi: 10.1016/j.chemosphere.2015.04.064

Chuiko GM, Kozlovskaya VI. 1989. Seasonal fluctuations of brain acetylcholinesterase activity in perch (*Perca fluviatilis* L.). Physiology and Toxicology of Hydrobionts, Yaroslavl State University, Yaroslavl. p. 27-37.

Chuiko GM, Zhelnin Y, PoD'Gornaya VA. 1997. Seasonal fluctuations in brain acetylcholinesterase activity and soluble protein content in roach (*Rutilus rutilus* L.): a freshwater fish from northwest Russia. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology. 117(3): 251-257. doi: 10.1016/S0742-8413(97)00068-6

Cikcikoglu Yildirim N, Yildirim N, Danabas D, Danabas S. 2014. Use of acetylcholinesterase, glutathione S-transferase and cytochrome P450 1A1 in *Capoeta umbla* as biomarkers for monitoring of pollution in Uzuncayir Dam Lake (Tunceli, Turkey). Environmental toxicology and pharmacology. 37(3): 1169-1176. doi: 10.1016/j.etap.2014.04.001

Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical pharmacology. 7(2): 88-95. doi: 10.1016/0006-2952(61)90145-9

Forget J, Beliaeff B, Bocquene G. 2003. Acetylcholinesterase activity in copepods (*Tigriopus brevicornis*) from the Vilaine River estuary, France, as a biomarker of neurotoxic contaminants. Aquatic Toxicology. 62(3): 195-204. doi: 10.1016/S0166-445X(02)00084-X

Fulton MH, Key PB. 2001. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environmental Toxicology and Chemistry. 20(1): 37-45. doi: 10.1002/etc.5620200104

Gadzala-Kopciuch R, Berecka B, Bartoszewic J, Buszewski B. 2004. Some considerations about bioindicators in environmental monitoring. Polish Journal of Environmental Studies. 13(5): 453-462.

Hogan JW. 1970. Water temperature as a source of variation in specific activity of brain acetylcholinesterase of bluegills. Bulletin of Environmental Contamination and Toxicology. 5(4): 347-353.

Kirby MF, Morris S, Hurst M, Kirby SJ, Neall P, Tylor T, Fagg A. 2000. The use of cholinesterase activity in flounder (*Platichthys flesus*) muscle tissue as a biomarker of neurotoxic contamination in UK estuaries. Mar Pollut Bull. 40(9): 780-791. doi: 10.1016/S0025-326X(00)00069-2

Kopecka J, Lehtonen KK, Baršienė J, Broeg K, Vuorinen PJ, Gercken J, Pempkowiak J. 2006. Measurements of biomarker levels in flounder (*Platichthys flesus*) and blue mussel (*Mytilus trossulus*) from the Gulf of Gdańsk (southern Baltic). Mar Pollut Bull. 53(8-9): 406-421. doi: 10.1016/j.marpolbul.2006.03.008

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem. 193(1): 265-275.

Pfeifer S, Schiedek D, Dippner JW. 2005. Effect of temperature and salinity on acetylcholinesterase activity, a common pollution biomarker, in *Mytilus sp*. from the south-western Baltic Sea. J Exp Mar Biol Ecol. 320(1): 93-103. doi: 10.1016/j.jembe.2004.12.020

Ricciardi F, Binelli A, Provini A. 2006. Use of two biomarkers (CYP450 and acetylcholinesterase) in zebra mussel for the biomonitoring of Lake Maggiore (Northern Italy). Ecotox Environ Safe. 63(3): 406-412. doi: 10.1016/j.ecoenv.2005.02.007

Robillard S, Beauchamp G, Laulier M. 2003. The role of abiotic factors and pesticide levels on enzymatic activity in the freshwater mussel *Anodonta cygnea* at three different exposure sites. Comp Biochem Phys C. 135(1): 49-59. doi: 10.1016/S1532-0456(03)00049-8

URL:1.2018.https://www.google.com.tr/maps/@39.1667594,39.6417243,11.5z?hl=tr, 15.03.2018

Yeşil SÖ. 2017. Tunceli İli 2016 Yılı Çevre Durum Raporu, T.C. Tunceli Valiliği Çevre ve Şehircilik İl Müdürlüğü, 80 p. [in Turkish]