Aquatic Ecology
Research Article

Introduction

Freshwaters such as rivers, streams and wetlands provide important habitats for nature conservation. They are also vital to human life and economic growth, recreation and transportation (Kinge et al. 2012). Anthropogenic activities strongly influence the natural status of almost all aquatic ecosystems. Physical, chemical and biological environmental pollutants can alter the quality of the recipient lakes, streams, rivers and coastal marine ecosystems (Gugliandolo et al. 2009). Among the pollutants, heavy metals may cause increasing ecological and global public health concerns. People’s exposure to heavy metals has risen dramatically as a result of an exponential increase in use in several industrial, agricultural, domestic and technological applications (Bradl 2002, Tchounwou et al. 2012). Environmental pollutants that threaten the ecosystems of living organisms can cause damage to the genetic material of organisms. As a result of environmental damage caused by environmental pollutants, exposed living beings or cells could be prone to a cascade of biological consequences: mutation, cancer, birth defects, diminished growth, abnormal development and reduced embryo, larval and adult survival of individuals or whole populations (Lee & Steinert 2003).

Blood parameters of reptiles may assist in evaluating physiological and health conditions...
of populations and may be used as an indicator in determining the environmental condition since these species are very susceptible to habitat changes \cite{Jacopson91, Raphael94, Dickinson02, Lopez-Olivera03}. The increased types and amounts of pollutants have led to develop several bioassay test systems in order to evaluate the genotoxic effects induced by these agents in living organism \cite{Zhelev13, Zhelev17, Pollo17, Salinas17}. Short-term tests are widely employed to monitor the genotoxicity of many chemicals to people, animals and plants. Among them, Micronucleus test (MN) can be used for the estimation of biological impacts of water pollutants and the resultant genotoxic damage in organisms inhabiting aquatic environments \cite{Bolognesi11, Hayashi11, Kousar12}. However, other nuclear abnormalities as a result of the genotoxic effect of pollutants, such as kidney-shaped nuclei (KS), lobed nuclei (LB), notched nuclei (NT) and blebbed nuclei (BL), were recently determined. Determination of the number of micronuclei together with other nuclear abnormalities formed in the cells of different species that are exposed to various environmental pollutants can be used to evaluate these genotoxic effects of pollutants \cite{Strunjak-Perovic10}. In recent years, nuclear abnormalities have been used with micronucleus to determine the effects of pollutants in various species \cite{Guilherme08, Napierska09, Strunjak-Perovic10, Pollo15, Pollo16}. The frequency of micronuclei and nuclear abnormalities caused by environmental pollutants were determined in Pelophylax ridibundus \cite{Pallas, 1771} from the Biga Stream (Canakkale, Turkey) by Çördük \textit{et al.} \cite{Corduk18}.

Turtles easily accumulate environmental contaminants in their habitats due to their long life spans and generalist diet, integrating a lifetime of exposure from multiple sources, interactions with sediments and ingestion of invertebrates or aquatic plants. Turtle populations can also persist in areas after other species have disappeared, partly because of their long lives and low generational turnover \cite{Meyer16}. Thus, they can be used in biomonitoring of genotoxic effects of environmental pollutants. \textit{Mauremys rivulata} \cite{Valenciennes, 1833} is listed in Appendix II of the Convention of European Wildlife and Natural Habitats of 1979. The species was reported as decreasing and endangered on Cyprus and decreasing and endangered on Cyprus and decreas-

The Biga Stream is one of the most important water bodies located in the Biga District, Canakkale, Turkey. It is 80 km long: originates from the Ida Mountains and flows into the Sea of Marmara; it has a wide catchment area. The stream has almost no economic contribution to the region but it is species rich \cite{Tosunoglu, 2016, 2017}. It is in danger because of excessive pollution due to the leather enterprises, agricultural areas and animal husbandry activities in the area. In this study, four collecting stations with a distance of about 20 km between them were selected along the Biga Stream (Fig. 1). The results of heavy metal analyses and information about stations as presented by Çördük \textit{et al.} \cite{Corduk18} are given in Table 1. Briefly, the second station had higher values of Al, Cu, Zn and Fe than the values obtained for the other stations. The fourth station had higher concentrations of heavy metals than the second station except Cu and Fe. Two stations (Stations 1 and 3) had low amounts of heavy metals and Station 1 had the lowest heavy metal level except for Fe \cite{Corduk18}.

**Collection of Animal Samples**

Specimens of \textit{M. rivulata} \textit{(N=10} from each station\textit{)} were captured along the Biga Stream in May 2014. Only healthy mature animals were studied and they were hand-picked, mostly from the water. Blood samples were obtained in the laboratory within one day of their capture. The samples were collected according to the guidelines of the university’s ethics committee \cite{2012/05-01}.

**Haematological analyses**

The red blood cell counts (RBC) and white blood cell counts (WBC) were carried out using a Neubauer haemocytometer, where standard Hayem’s solution for red blood cells and Turk’s solution for white blood cells were used as a diluting

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**Materials and Methods**

**Sampling area**

The Biga Stream is one of the most important water bodies located in the Biga District, Canakkale, Turkey. It is 80 km long: originates from the Ida Mountains and flows into the Sea of Marmara; it has a wide catchment area. The stream has almost no economic contribution to the region but it is species rich \cite{Tosunoglu, 2016, 2017}. It is in danger because of excessive pollution due to the leather enterprises, agricultural areas and animal husbandry activities in the area. In this study, four collecting stations with a distance of about 20 km between them were selected along the Biga Stream (Fig. 1). The results of heavy metal analyses and information about stations as presented by Çördük \textit{et al.} \cite{Corduk18} are given in Table 1. Briefly, the second station had higher values of Al, Cu, Zn and Fe than the values obtained for the other stations. The fourth station had higher concentrations of heavy metals than the second station except Cu and Fe. Two stations (Stations 1 and 3) had low amounts of heavy metals and Station 1 had the lowest heavy metal level except for Fe \cite{Corduk18}.

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**Haematological analyses**

The red blood cell counts (RBC) and white blood cell counts (WBC) were carried out using a Neubauer haemocytometer, where standard Hayem’s solution for red blood cells and Turk’s solution for white blood cells were used as a diluting
solution. The haematocrit (HCT) was determined using the micro-haematocrit method (TANYER 1985). The tubes were then spun in a micro-haematocrit centrifuge for 5 min at 12000 rpm and HCT was calculated with a total blood level divided by blood cell level. The haemoglobin concentration (HB) was measured using the Sahli method with a Sahli Haemoglobinometer (TANYER 1985). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated mathematically taking the above-mentioned results into consideration (TANYER 1985). Blood smears stained with Wright’s stain were used in measuring the blood cells. Cell measurements were performed under a microscope with an ocular micrometer. Forty erythrocytes were randomly chosen on each blood smear; cell lengths (EL) and widths (EW), together with the lengths (NL) and widths (NW) of their nuclei were measured in micrometers and then cell (ES) and nuclei sizes (NS) were computed with the formulas: EL·EW·π/4 and NL·NW·π/4, respectively. Cell photomicrographs were taken with an Olympus photomicroscope.

The descriptive statistics were obtained using the Statistical Package for the Social Sciences (SPSS Chicago, IL, USA), v. 10.0. The Mann-Whitney U test was used to compare differences between haematological parameters of the four studied sites.

Table 1. The information of each station and levels of heavy metals in the water samples taken from four stations of the Biga Stream

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</tr>
<tr>
<td>3</td>
<td>Buffer zone 40º05’59.46” N 27º10’08.19” E</td>
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<tr>
<td>4</td>
<td>Industrial zone 40º01’26.38” N 27º02’22.02” E</td>
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Measurement of Micronucleus and Nuclear Abnormalities

In this study, 1 ml of blood from the dorsal caudal vein of each specimen was taken for blood smears using a 5 ml syringe with needles with a diameter of 21mm (Ballard & Cheek 2003, Thrall et al. 2004). The peripheral blood smears for each sample were prepared on clean slides, after fixation in ethanol for 20 min; the slides were air-dried at room temperature, fixed with methanol for 15 min and then stained with Giemsa stain (10% v/v) (Josende et al. 2015). Three slides were prepared for each sample and a total of 1000 erythrocytes per slide were scored from each slide under an optic microscope (1000x magnification). The micronucleus was defined according to the criteria as follows: a) MN must not touch the main nuclei, b) MN should be smaller than one-third of the main nuclei) and c) MN must not be refractive and should be the same colour and intensity as the main nuclei (Heddle et al. 1976, Titenko-Holland et al. 1997, Fenech 2000). Nuclear abnormalities, such as kidney-shaped nuclei, lobed nuclei, notched nuclei and micronuclei (Fig. 2) were also determined and scored from the slides (Carrasco et al. 1990, Strunjak-Perovic et al. 2009, Josende et al. 2015).

The data were tested for normality using the Kolmogorov–Smirnov test. Since the data showed normal distribution, parametric tests were used to detect differences at the 0.05 level of significance. One-way analysis of variance (ANOVA) was used to evaluate significant differences in the mean values of different groups using SPSS, v. 20.0. The differences in total nuclear abnormalities between the four groups were compared using the Tukey’s test (p ≤ 0.05).

Results

Nuclear abnormalities such as binuclei, blebbed nuclei, kidney-shaped nuclei, lobed nuclei, notched nuclei and micronuclei (Fig. 2) were observed in erythrocytes from Mauremys rivulata from the Biga Stream.

We found no statistical difference among stations for haematological parameters of M. rivulata (Mann-Whitney U test, p>0.05; Table 2).

The frequencies of micronuclei and nuclear abnormalities in erythrocytes varied from 4.42% to 9.16% (Table 3). The frequency of total nuclear abnormalities was highest at Station 2 (9.16±0.02), followed by Station 4 (8.44±0.01). Station 1 and Station 3 had similar frequency: 4.84±0.01 and 4.49±0.01, respectively. Micronuclei were observed with low frequencies at all stations. The frequency of blebbed nuclei was higher than the other abnormalities at all station.
Table 2. Haematological values for *Mauremys rivulata* at each station. (N: Number of specimens, SD: Standard Deviation, RBC: Red Blood Cell Count, WBC: White Blood Cell Count, HB: Haemoglobin, HCT: Haematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration)

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Discussion

The genotoxicity of water resources may largely be based on heavy metals present in aquatic ecosystems (Salles et al. 2016). Heavy metals have toxic effects and can be accumulated in organisms (Barcellos et al. 1988, Topashka-Ancheva et al. 2003a, 2003b, Gajalakshmi & Ruban 2014). Many studies have reported that heavy metals affect reproductive, immune and nervous systems, behaviour and carcinogenesis in terrestrial species (Mazlia et al. 1989, Burger & Gochfeld 2000, Fair & Ricklefs 2002). Excessive exposure to some heavy metals during the developing stage
can change the level of gonadal steroid hormones, adrenal and thyroid hormones (Colborn 2002, Dawson 2000), which could be the cause of potentially serious effects on the survival of sensitive or vulnerable populations (Garcia-Fernandez et al. 2009, Lopez et al. 2010).

The concentrations of pollutants in the Biga Stream vary according to the pressures it is exposed to and are different at each station. These pollutants have genotoxic potential on genetic materials of organisms living in these habitats. Heavy metals are the most important source of inorganic pollutants in the water. The highest percentage of the total nuclear abnormalities and each kind of abnormality in erythrocytes of M. rivulata at Station 2 could be likely owing to the highest content of heavy metals as demonstrated by Çördük et al. (2018).

Based on our data, the amount of micronuclei alone was found to be insufficient to determine the genotoxic effect of the environment on M. rivulata. Similarly, Zúñiga-González et al. (2000, 2001) reported that turtles might not be good indicators of genotoxic exposure based on their results of MN test examining one specimen of Macrocelys temminckii (Troost in Harlan, 1835) and two specimens of Kinosternon subrubrum (Lacépède, 1788). Our results suggested that the other nuclear abnormalities should be used together with micronuclei to determine the levels of genetic damage in erythrocytes of M. rivulata. Guilherme et al. (2008) specified that due to its higher responsiveness, nuclear abnormalities assay represents an alternative to MN test, overcoming a possible lack of sensitivity related to the low MN frequency in wild fish.

When compared with the haematological values provided by previous studies (Pages et al. 1992, Metin et al. 2008, Yılmaz & Tosunoğlu 2010, Tosunoğlu et al. 2011), it was determined that the data we obtained for M. rivulata were similar.

Understanding the effects of adverse environmental conditions is important for managing the sustainability of ecosystems and maintaining species. Biomonitoring is a particularly useful tool for identifying pollutants affecting human and environmental health (Silva et al. 2003). Zapata et al. (2016) indicated that turtle species were often used as indicators of pollution levels but only a few reports assessed genotoxic effects through the application of the MN test or Comet Assay (CA) on erythrocytes of species inhabiting impacted environments. Meyers-Schöne et al. (1993) reported that Trachemys scripta (Schoepff, 1792) and Chelodina serpentina (L., 1758) may be useful as a bioindicator for genotoxic monitoring using CA.

In conclusion, the correlation between water pollutants and the percentage of the total nuclear abnormalities show that monitoring nuclear abnormalities in erythrocytes of Mauremys rivulata may provide baseline data for evaluating its role as a valuable biological marker for biomonitoring environmental pollutants.

Acknowledgements: This study was funded by the Scientific and Technological Research Council of Turkey (TUBITAK Project No. 113Z098).

References


Assessment of Nuclear Abnormalities in Erythrocytes of Balkan Pond Turtle Mauremys rivulata...


Received: 10.04.2018
Accepted: 30.07.2018