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## **Age determination of fire salamander (*Salamandra salamandra* L.)**

### **by skeletochronology**

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**Date and place:** 15.7.2008., Department of Biology, Faculty of Science, University of Zagreb

During the period from March 4<sup>th</sup> until April 23<sup>rd</sup> 2008 we studied 24 specimens of fire salamander (*Salamandra salamandra* L.) caught in the Park Maksimir in Zagreb. We removed third toe of the hind limb and released animals at the site. The purpose of the study was to determine an average age, age of sexual maturity of fire salamander on still unknown research site in the park Maksimir. Also, we wanted to compare methods for making histological sections and establish reliable procedure for age determination by skeletochronology. We compared two methods of tissue cutting, in paraffin blocks and with frozen samples in cryostat. We also compared two types of dyeing, dyeing with hemalaun eosin and Ehrlich hematoxylin. Results showed that the oldest individual was 19 years old and the youngest was 6 years old. Sexual maturity is reached by the age of 4 to 6 years. The best method for making histological sections approved to be cutting samples on cryostat and dyeing with Ehrlich hematoxylin. For statistically correct age determination of population this method should be provided on bigger sample, with same percentage of both sexes during the whole year. With our analysis we demonstrate that skeletochronology is a good method for aging fire salamander, and that is applicable on fingers so there is no need to sacrifice animals.

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**Određivanje starosti pjegavog daždevnjaka (*Salamandra salamandra* L.)  
metodom skeletokronologije**

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Na području parka Maksimir u Zagrebu, u razdoblju od 4. 3. do 23. 4.2008. godine uhvaćene su 24 jedinke pjegavog daždevnjaka (*Salamandra salamandra* L.). Svakoj od njih je odrezan treći prst stražnje noge, a životinje su odmah puštene. Cilj istraživanja je bio odrediti starost jedinki i vrijeme postizanja spolne zrelosti pjegavih daždevnjaka na dosad još neistraženom području parka Maksimir. Osim toga, željelo se usporediti metode prilikom izrade histoloških preparata i dobiti pouzdan postupak za određivanje starosti metodom skeletokronologije. Usporedile su se metode rezanja parafinskih blokova i metoda rezanja tkiva u kriostatu. Također su se usporedila dva načina bojenja, hemalaun eozinom i Ehrlichovim hematoksilinom. Rezultati istraživanja su pokazali da je najstarija jedinka imala 19 godina, a najmlađa 6 godina. Spolna zrelost jedinki nastupa u dobi od 4 do 6 godina života. Najbolja metoda za izradu histoloških preparata utvrdilo se da je bila rezanje tkiva na kriostatu te bojenje Ehrlichovim hematoksilinom. Za statistički ispravnu procjenu dobne strukture populacije potrebno je metodu primijeniti na većem broju uzoraka, uzorkovati životinje različite veličine i pritom obuhvatiti jedinke oba spola. Ovim radom se pokazalo kako je skeletokronologija dobra metoda za određivanje individualne starosti jedinki pjegavog daždevnjaka koja se može primjenjivati na člancima prstiju i na taj način spriječiti žrtvovanje jedinki.