# Preliminary evalution of 5-aminolevulinic acid dehydratase in blood of lesser spotted dogfish (Sciliorhinus canicula L.) from the middle Adriatic

Preliminarni rezultati aktivnosti dehidrateze 5-aminolevulinske kiseline u krvi mačke bljedice (Scyliorhinus canicula L.) iz srednjeg Jadrana

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### INTRODUCTION

The activity of  $\delta$ -aminolevulinic acid dehydratase (ALA-D) catalyzes the formation of one molecule of porphobilinogen from two molecules of  $\delta$ -aminolevulinic acid.

ALA-D contains highly active tiol groups which easily bind heavy metals and inhibit enzyme activity (Shemim, 1976). It is well known that the activity of ALA-D in blood of humans is particularly depressed by the presence of lead in the blood (Bonsignore et al., 1956, Kuhnert et al., 1977). ALA-D activity is also depressed in the erythrocytes of fish under the influence of lead (Jackim, 1973; Hodson, 1976; Hodson et al., 1977). It was also shown that lead inhibited ALA-D in the erythrocytes of lesser spotted dogfish (Scyliorhinus canicula L.) (Beritić, et al., 1980, Tudor, 1980).

This paper is a preliminary evaluation of ALA-D activity in blood of Scyliorhinus canicula L. from the middle Adriatic. It may be an useful parameter for the biological monitoring of pollution by lead.

## MATERIALS AND METHODS

Lesser spotted dogfish specimens were trawled from the Split channel (43°26'N; 16°13'E). Alive fish were acclimated to aquarium condition for four weeks.

Blood of fish was collected by cardial puncture and Na-heparin used as anticoagulans (Hattingh, 1975).

ALA-D activity in blood of *S. canicula* was determined after Berlin and Schaller (1974) at 37°C. The amount of porphobilinogen produced in the incubation of enzymes was measured at 555 nm using Pay Unicam SP 600 spectrophotometer. Hematocrit was determined by microhematocrit Janetzki TH 11 centrifuge.

In ten repeated analyses the coefficient of variation of the method was  $2.28^{\circ}/_{\circ}$ .

ALA-D activity is expressed as  $\mu$ mol porphobilinogen/minute/1 erythrocytes.

# RESULTS

Effects of temperature and time of incubation on ALA-D activity was examined at 25 and 37°C. Enzyme activity was constant over 180 minutes (Fig. 1). However, ALA-D activity at 37° was about twice that at 25°C. ALA-D activity and hematocrit were examined at lo males and lo females of Scylior-hinus canicula.

Ranges, means, standard deviation, standard error and variability coefficient of ALA-D activity, as well as hematocrit and weight of tested fish are given in Table 1.

Table 1. ALA-D activity ( $\mu$ molPBG/min/1 erythrocytes), hematocrit (%) and weight of Scyliorhinus canicula L. from the middle Adriatic

	ALA-D (U/1)		Hem. (0/0)		Weight (g)	
	ී 10	♀ 10	රී 10	오 10	රී 10	오 10
Range	17,4—57,8	11,1—41,9	12,9—25,7	15,6—25,9	170—265	166—305
x	33,7	27,6	20,6	22,2	214	210
S	12,7	10,8	3,8	3,7	36	39
Sx	4.0	3,4	1.2	1,2	11	12
CV º/o	37.8	39,3	18,4	16,4	17	18

Event hough mean ALA-D activity in Scyliorhinus canicula females was somewhat lower than that in males the difference between them was not statistically significant (t = 1.15; P > 0.05). No statistically significant difference was found in mean hematocrit valeus (t = 0.96; P > 0.05) between examined males and females, either.

Regression relationship between ALA-D activity and fish weight log are given in Fig. 2. Negative correlation r=-0.658 between these two parameters was statistically significant at  $P \le 0.01$ .

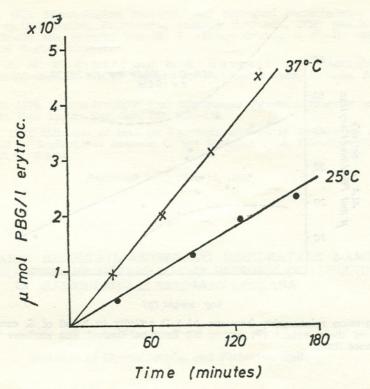


Fig. 1. Concentration of produced porphobilinogen as affacted by the incubation time of enzyme and substrate at  $25^{\circ}\text{C}$  and  $37^{\circ}\text{C}$  temperature

Weight of examined specimens an their hematocrit were not interdependent (males r = 0.07; females r = -0.13).

As shown by our results ALA-D activity of tested *S. canicula* specimens is likely to be dependent on the growth of fish, that is their age. After Jardas (1979) age composition of examined male and female *S. canicula* shows mainly specimens of the third year of age.

It has already been suggested (Tudor, 1980) that relative (%) ALA-D activity of individual S. canicula specimens and log of intraperitoneal lead level (ppm) are negatively correlated with the regression coefficient 29.8. Hodson et al. (1982) found that the inhibition of Salmo gairdneri due to the lead added to the water was independent of fish weight.

Owing to the very pronounced variability of ALA-D activity the biological monitoring of S. canicula should take care of taking the fish belonging to the same age groups.

### CONCLUSION

Negatively correlated ALA-D activity of S. canicula blood and log of fish weight are indicative of the fact that enzyme activity is likely to be dependent on fish age.

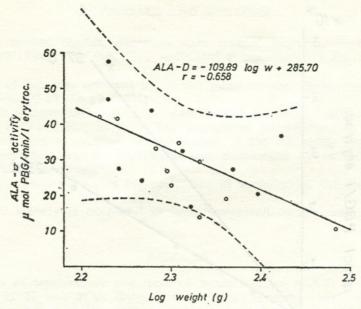


Fig. 2. Regresion relationship between ALA-D activity in blood of *S. canicula* and log of their weight (●) males, (○) females. Dashed line encloses 95% confidence limits

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# KRATKI SADRŽAJ

Aktivnost dehidrataze δ-aminolevulinske kiseline (D-DALK) u krvi mačke bljedice u negativnoj je korelaciji sa težinom riba. Prema ovim rezultatima aktivnost D-DALK vjerovatno je povezana sa starošću ispitanih primjeraka Scyliorhinus canicula.