Vol. 3: 187–193, 1987 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published December 31

Estimation of the enzymes LDH, GOT and GPT in plasma of grey mullet *Mugil auratus* and their significance in liver intoxication

M. Krajnović-Ozretić, B. Ozretić

Rudjer Bošković Institute, Center for Marine Research, YU-52210 Rovinj, Yugoslavia

ABSTRACT: Activities of lactic dehydrogenase (LDH), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were measured in blood plasma and some tissues of grey mullet *Mugil auratus* Risso. After determination of optimal assay conditions, cardiac sampling for LDH estimation was recommended. Tissue distribution of enzymers and their origin in plasma were described, in particular the isozyme forms of LDH. Plasma levels of all 3 enzymes showed a significant increase following treatment with CCl_4.

INTRODUCTION

The measurement of plasma enzyme activity is a helpful diagnostic tool in mammalian pathological, toxicological and general clinical testing. Recently there have been some attempts to utilize these techniques in aquatic toxicology studies (Racicot et al. 1975, D'Apollonia & Anderson 1980, Mehrle & Mayer 1980, Wieser & Hinterleitner 1980, Casillas et al. 1983, Lockhart & Metner 1984, Casillas & Ames 1986, Krajnović-Ozretić & Ozretić 1988). As with mammals, it has been shown that after functional damage to tissues and organs of fish, some specific cellular enzymes leak into blood plasma where they have been detected (Bouck 1966, Bouck et al. 1975). In mammalian toxicology, the identification of altered plasma enzyme patterns can be used to evaluate the functional status of damaged organ(s) or tissue(s). In principle it would be very attractive to use the same approach with fish, but there are difficulties. Due to the wide – seasonally changing – homeostatic regulation in poikilothermic fish and to their species-specific differences and interspecific variability (Wedemeyer & McLeay 1981, Miller et al. 1983), in most cases the correlation between the physiological state and the biochemical response of fish is still not sufficiently clear (Mehrle & Mayer 1980).

Our preliminary measurements have indicated that the direct application to grey mullet of routine methods derived from mammalian studies and human clinical testing, or from other fish species, can lead to wrong interpretation of the actual enzyme activity in the plasma. Thus, this study attempted to optimise assay conditions for the measurements of lactic dehydrogenase (LDH), glutamate exaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) in grey mullet. It was also our intention to examine whether the presence of these cellular enzymes in plasma of a marine fish could be used as indicators of liver intoxication. LDH, GOT and GPT were selected as important cellular metabolic enzymes. Carbon tetrachloride (CCl_4) was used as a model hepatotoxic agent which has been widely applied to induce acute liver intoxication in several vertebrate species (Curtis et al. 1972, Raisfeld 1974) including rainbow trout *Salmo gairdneri* (Pfeifer et al. 1977, D'Apollonia & Anderson 1980) and English sole *Parophrys vetulus* (Casillas et al. 1983).

MATERIALS AND METHODS

Adult grey mullet *Mugil auratus* Risso, average weight 191 ± 25 g, were used as test animals. Three groups of about 15 to 20 fish were distributed into 250 l aereated basins with a continuous flow of seawater (salinity 37.2 ± 0.4 ‰, 20 ± 0.5 °C). Mullet were acclimated to aquarium conditions for 2 wk. They were fed once daily to satiation and remaining food was removed. No selection or identification of sexes was

0177-5103/87/0003/0187/$ 03.00

made. Acute liver injury was experimentally induced in 2 groups of 18 mullet using high CCl_4 doses. Each fish was injected intraperitoneally (i.p.) with a single dose of 1 or 2 ml CCl_4 kg^{-1} body weight. At selected intervals blood (0.3 to 0.5 ml) was sampled by cardiac puncture from 6 specimens. Blood sampling and i.p. CCl_4 injections were performed without the use of anaesthetics. Blood was sampled by caudal or by heart puncture. In both cases, after taking 1 to 1.5 ml of blood $100g^{-1}$ wet weight, all fish regularly survived and readily recovered. Heparinized syringes and vials were used to avoid coagulation. Blood was kept on ice and plasma was immediately separated with a refrigerated centrifuge (10 min at 2000 × g). Hemolysed samples were excluded.

The activities of LDH (EC 1.1.1.27), GOT (EC 2.6.1.1) and GPT (EC 2.6.1.2) were determined spectrophotometrically by measuring the oxidation rate of NADH (nicotinamide adenine dinucleotide, reduced) in a thermostated cuivette at 340 nm after incubation of samples with Na-pyruvate, L-aspartate and L-alanin, respectively (Bergmeyer & Bernt 1974a, b, c). The activity of the same enzymes was also measured in the extracts of liver, heart, white and red muscle, kidney and gill filaments. Samples were homogenised with a tissue grinder in 10 parts of a cold (+4 °C) 0.2 M Na-phosphate buffer (pH 7.4) with 20 % glycerol and 5 mM mercaptoethanol. The homogenates were centrifuged for 30 min at 17 000 × g, at 4 to 5 °C, and the supernatant immediately used for enzyme assay. For plasma, the enzyme activity was calculated in relation to the unit volume (ml) or to the unit weight (g) for the body tissues and was expressed as international Units (U). For LDH the Michaelis-Menten constants were calculated in relation to the substrate concentration.

Electrophoresis of LDH was carried out on cellulose acetate strips using 75 mM barbital buffer at pH 8.6 and the separation was achieved in 50 min at 200 V. The fractions were made visible by incubation with lactate, NAD (nicotinamide adenine dinucleotide), MTT (3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyl tetrazolium bromide) and phenazene-methosulphate (Burlina et al. 1979). All reagents were of the highest purity (Sigma).

RESULTS

Assay conditions

The optimal Na-pyruvate concentration for the measurement of LDH activity in plasma was estimated to be 0.8 mM at pH 6.5 and 25 °C (Fig. 1). No significant influence of substrate concentration or pH was shown for the activity of plasma GOT and GPT, while liver extracts did demonstrate specific maxima (Fig. 2). Two peaks were determined in relation to α-ketoglutarate, one at lower concentrations of 0.5 and 1 mM for GOT and GPT, respectively and a single broader peak at 15 mM α-ketoglutarate for both enzymes (Fig. 2A). The maximum activity for GOT was found between 50 and 150 mM L-aspartate and for GPT a sharp peak was determined at 50 mM L-alanin (Fig. 2B). The optimal pH range appeared to be between 6.6 and 8.8 (Fig. 2C). On the basis of these results and other data (not shown)

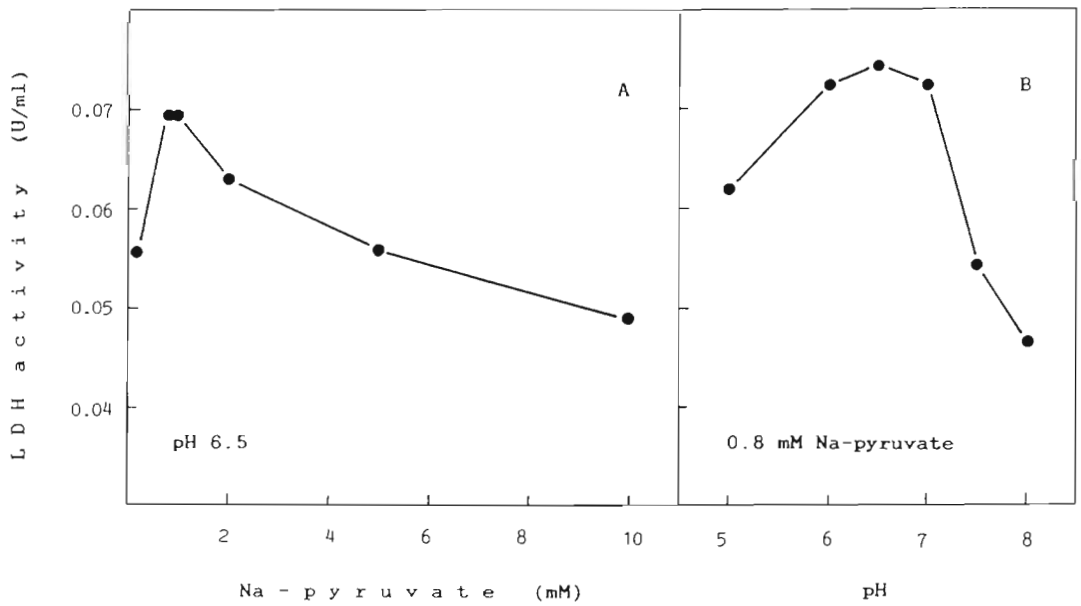

Fig. 1 *Mugil auratus*. LDH activity in cardiac-sampled plasma. (A) Optimal substrate concentration; (B) pH measured at 25° C

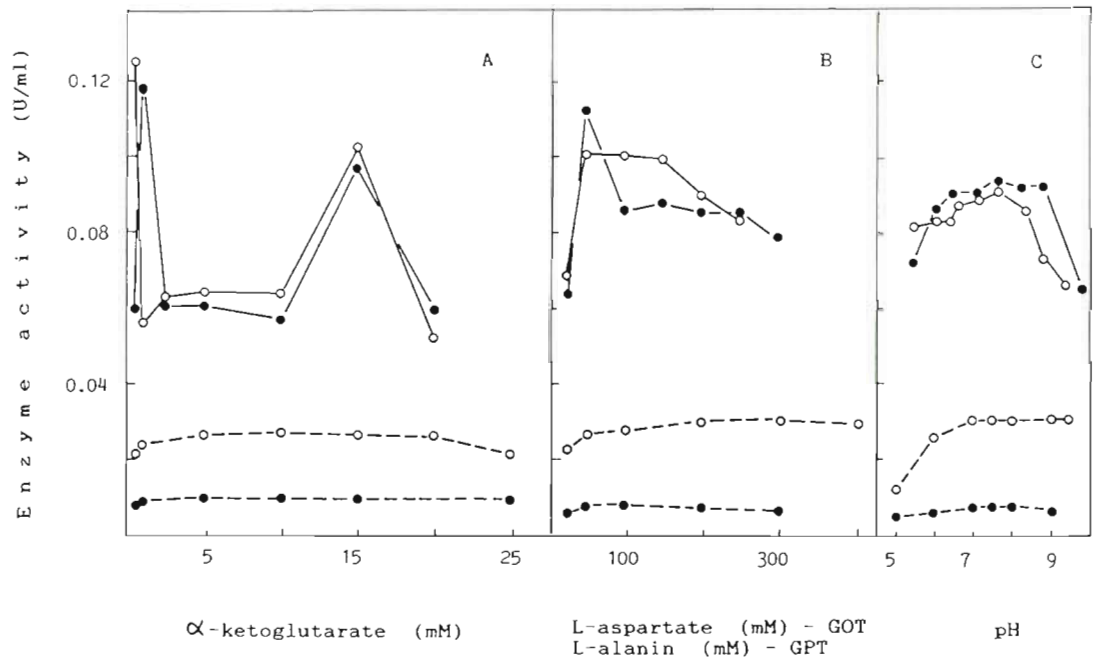

Fig. 2. *Mugil auratus*. (○) GOT and (●) GPT activity in (- - -) cardiac-sampled plasma and (——) liver extracts; (A and B) optimal substrate concentration; (C) pH measured at 25° C

the optimal assay conditions for the measurement of LDH, GOT and GPT in grey mullet were established (Table 1) and adopted for routine measurements.

Table 1 Optimal assay conditions adopted for routine measurement of LDH, GOT and GPT in mullet plasma

	LDH	GOT	GPT
pH	6.5	7.8	7.8
Phosphate buffer• (mM)	50	100	100
2-NADH (mM)	0.18	0.15	0.15
L-aspartate (mM)	–	200	–
L-alanin (mM)	–	–	50
α-ketoglutarate (mM)	–	15	15
MDH• (U ml^{-1})	–	7.5	–
LDH• (U ml^{-1})	–	3.7	3.7
Na-pyruvate (mM)	0.8	–	–
Temperature (°C)	25	25	25

• Data not shown

To assess the specific contribution of some organs and tissues to the plasma enzyme pattern the activity of LDH, GOT and GPT was measured, and the average values are compared in Table 2. Highest activity and total content of LDH were found in the white muscle tissue (926 U g^{-1} and 92 % of total activity). Although the specific activity of GOT in the white muscle was moderate (6.7 U g^{-1}), owing to its largest biomass (48 % of body weight) the total content of GOT was the highest (52.8 %) in relation to other tissues. The activity of GOT was higher in kidney, liver, red muscle and heart (16, 17, 25 and 37 U g^{-1}), but their total GOT was not more than 23 % of the whole body content. For GPT, as in other vertebrates, the liver of mullet was characterised by the highest specific activity (19.5 U g^{-1}) and by the highest content of the whole body GPT (52.7 %). The activity of all 3 enzymes was the lowest in plasma, for GPT very near to the detection limit.

Blood sampling

It was observed that within various samples the activity of LDH was very variable (Table 3). In plasma obtained by cardiac puncture the activity was lower and statistically more stable (coefficient of variation, CV = 23 %). On the other hand, in plasma sampled by caudal puncture the mean LDH activity was higher but fluctuated over a range of 2 orders of magnitude (CV = 91 %). The activity of GOT and GPT was not affected by the blood origin but, evidently because of the minimal detectable activity, the variability of GPT in plasma was extremely large (CV = 162 and 214 %).

LDH characterization

Due to the observed variability it was pertinent to analyse some characteristics of LDH from various samples. The electrophoretic separation showed that

Table 2. *Mugil auratus*. Distribution of LDH, GOT and GPT in mullet tissues (U g^{-1} wet wt) and in cardiac-sampled plasma (U ml^{-1}). Mean values and standard deviations (±SD) calculated from 10 samples. Values in % are the estimation of the relative wet weight and enzyme activity distribution in single tissues in relation to the whole body weight and activity

	%	LDH		GOT		GPT	
	wet wt	U g^{-1}	%	U g^{-1}	%	U g^{-1}	%
White muscle	48	926	92	6.7	52.8	0.15	10.8
		(84)		(1.1)		(0.22)	
Red muscle	4	417	3.5	25	16.4	2.6	15.6
		(76)		(2.9)		(1.1)	
Liver	1.8	8	0.03	17	5.0	19.5	52.7
		(6)		(4.5)		(2.8	
Kidney	0.25	69	0.04	16	0.7	5	2
		(23)		(2.5)		(1.2)	
Heart	0.08	482	0.08	37	0.5	0.4	0.02
		(21)		(1.1)		(0.2)	
Gill filaments	0.45	81	0.08	3.8	0.3	0.7	0.5
		(12)		(0.7)		(0.2)	
Blood plasma	3	0.07	<0.001	0.01	<0.01	0.001	<0.01
		(0.02)		(0.004)		(0.001)	

Table 3. *Mugil auratus*. LDH, GOT and GPT activity (U l^{-1}) in plasma of grey mullet sampled by cardiac and caudal punction. n: number of samples; $\bar{x}$: arithmetic mean; SD: standard deviation; CV: coefficient of variability (%)

	Cardiac sampling				Caudal sampling			
	n	$\bar{x}$ (SD)	Range	CV	n	$\bar{x}$ (SD)	Range	CV
LDH	46	70 (16)	47–111	23	33	2100 (1900)	300–11000	91
GOT	30	11 (4)	7–20	36	28	12 (3)	7–18	25
GPT	36	0.8 (1.3)	0–3.4	162	28	0.7 (1.5)	0–3.5	214

LDH in grey mullet tissue is present in at least 3 isozyme forms (Fig. 3). According to the results of Markert & Faulhaber (1965) and to the LDH_{1-5} nomenclature proposed by Wieme (1974), it was noticed that in mullet heart, red muscle and kidney extracts the highest concentration was found for the LDH_1 isozyme fraction while the LDH_5 fraction was predominant in the liver and particularly in the white muscle extracts. In plasma from the cardiac-sampled blood 2 weak LDH_1 and LDH_5 bands were detected. In plasma of the caudal sampled blood the slow-moving LDH_5 band was very consistent and a rather faint LDH_1 band was found ahead on the anodic side, resembling the white muscle isozyme pattern.

The substrate saturation test and the heat denaturation test (Fig. 4A, B), as well as the calculated Michaelis-Menten constants (Table 4), indicated that the nature of LDH in cardiac-sampled blood was different from that originating from caudal puncture. The latter was similar to the LDH found in the white muscle extracts.

Effect of CCl_4

The activity changes of LDH, GOT and GPT in plasma of mullet injected with CCl_4 are presented in Fig. 5. When a single dose of 1 ml CCl_4 kg^{-1} was applied, the maximal activity for all 3 enzymes in plasma was measured 6 h after the injection. Within 24 h the activity had fallen towards pre-injection levels. In the group injected with a single dose of 2 ml CCl_4 kg^{-1}, 12 h after injection the enzyme activity in plasma was increased by nearly 2 orders of magnitude and even up to 24 h, GOT and GPT were still increasing. LDH started to decrease ca 12 h post-injection. The electrophoretic separation indicated that the increased plasma LDH activity was generated by the presence of a substantial amount of LDH_5 isozyme fraction (Fig. 3B), the origin of which could be assigned to the very large LDH_5 pool from the white muscle.

DISCUSSION

It was observed that within various samples of plasma the LDH activity was very variable and it was realized that the activity levels as well as their variability were primarily related to the origin of the blood. All tests demonstrated a similarity of LDH between the samples from caudal plasma and the extracts from the white skeletal muscle (Fig. 4A, B and Table 4). In the

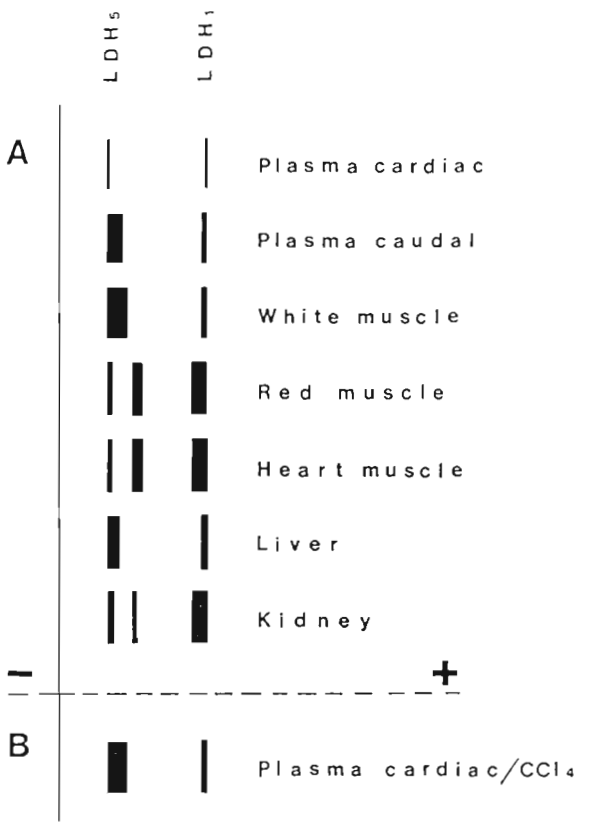

Fig. 3. *Mugil auratus*. (A) Electrophoretic isozyme fractions of LDH in plasma and tissue extracts; (B) LDH fractions in cardiac-sampled plasma from mullet treated with CCl_4

Table 4. *Mygil auratus*. Michaelis-Menten constants for LDH from various samples calculated in relation to Na-pyruvate concentration

Plasma caudal	0.237
Plasma cardiac	0.078
White muscle	0.174
Heart muscle	0.089
Liver	0.045
Kidney	0.048

case of caudal sampling, the average 30 times higher activity and the wide variability (Table 3) was probably derived from an induced 'leakage' of the enzyme from the extremely LDH-rich white muscle as a consequence of the physical injury produced by the penetration of the needle into the tissue structure. This was confirmed by the presence of a consistent LDH_5 isozyme band from muscle origin (Fig. 3). The activity of the cardiac-sampled plasma was always low, with small variations, and it very likely reflected the actual LDH concentration in the circulating blood. Thus it was confirmed that for an accurate evaluation of LDH in fish plasma, cardiac sampling should be used. This was clearly demonstrated in rainbow trout by Gaudet et al. (1975) and confirmed by Hille (1982), but other recent studies have not taken this limitation into account (Ikeda & Fujikata 1984, Lockhart & Metner 1984, Casillas & Ames 1986). The activity of GOT and GPT in

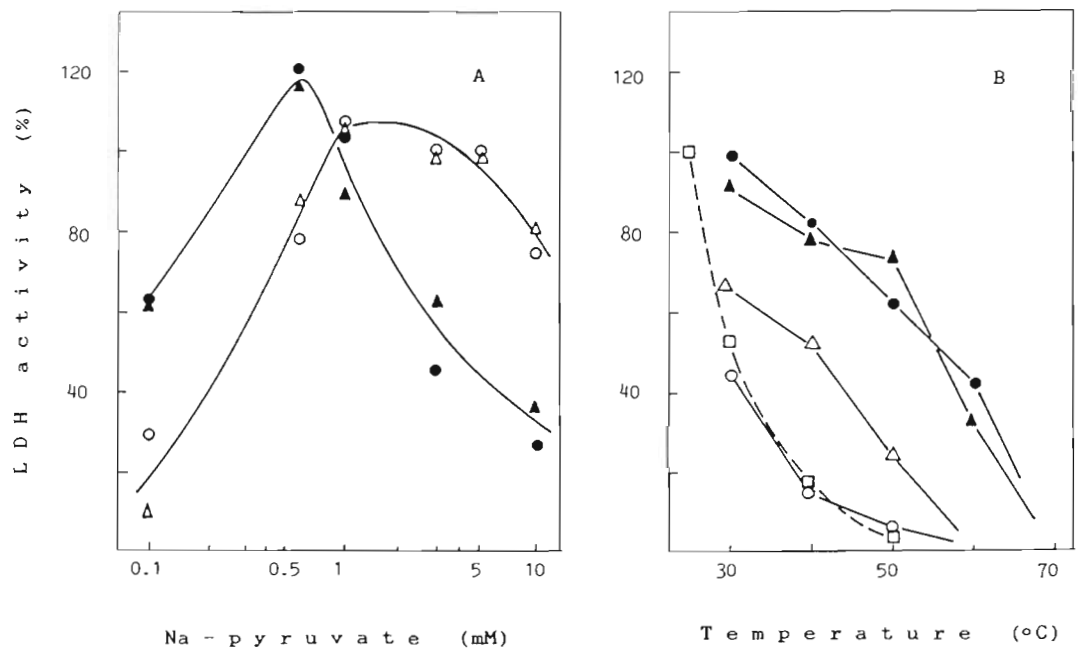

Fig. 4. *Mugil auratus*. (A) Substrate saturation curves, and (B) heat denaturation test for LDH measured in (●) cardiac- and (○) caudal-sampled plasma and in (△) white muscle and (▲) heart tissue extracts. (□) Heat sensitivity of LDH in cardiac-sampled plasma from mullets treated with CCl_4 is compared. Enzyme activity is expressed as (A) % of the values obtained with 0.8 mM Na-pyruvate, and (B) as % of the values measured at 25° C

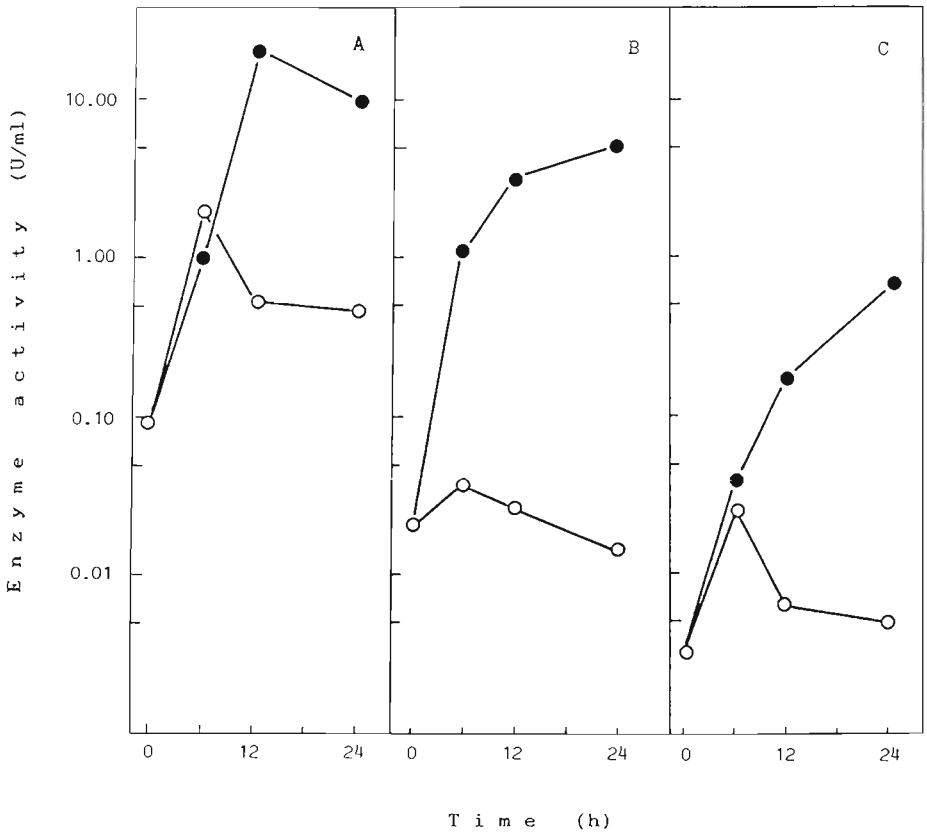

Fig. 5. *Mugil auratus*. Activity of (A) LDH, (B) GOT and (C) GPT in cardiac-sampled plasma from mullet after a single injection of (○) 1 ml and (●) 2 ml CCl_4 kg^{-1} wet wt

plasma were not affected by the blood sampling procedure.

Promptly after injection of CCl_4 the activity of all 3 enzymes in plasma of mullet increased (Fig. 5). Similar results were obtained by Racicot et al. (1975), Pfeifer et al. (1977) and Statham et al. (1978) for rainbow trout and by Casillas & Ames (1986) for English sole. In mammals the hepatotoxic effects of CCl_4 were not expressed as promptly as in fish and the resulting LDH > GOT > GPT pattern in plasma corresponded to the liver pattern (Schmidt & Schmidt 1975). In plasma of injected mullet a similar enzyme pattern to that in mammals was observed (LDH ≫ GOT ≥ GPT), but it did not correspond to the liver enzyme pattern (GPT ≥ GOT ≫ LDH; Table 2). According to Dando (1969) the activities of LDH in the liver of various fish species were very disparate, in the range of 10 to 10 000 U g^{-1} protein. Grey mullet, with a mean activity of 8 U g^{-1} wet weight or about 54 U g^{-1} protein, is evidently a fish species with rather low liver LDH, and owing to the limited liver biomass (1.4 to 2.0 % of the total body weight) the increased activity of LDH in plasma cannot be derived only from this organ. On the contrary, as indicated by Casillas & Ames (1986) for English sole, its origin can be considered to be a leak mainly from the LDH-rich white muscle tissue that, because of its large biomass and its high specific activity, participates as the largest LDH pool in the body of grey mullet (about 92 %). The muscular origin was also confirmed by the heat denaturation test (Fig. 4B) and by the isozyme fractionation of plasma from mullet treated with CCl_4 (Fig. 3B). In both cases the characteristcs of the increased LDH in plasma were similar to those found in the white muscle extracts. A suspected origin of LDH in plasma as a leakage from the LDH-rich red blood cells was not considered because hemolysed samples were not analysed.

The electrophoretic patterns of GOT from different mullet tissues, and the organ/tissue distribution of both GOT and GPT suggested the liver to be their origin (Krajnović-Ozretić & Ozretić 1987). As in mammals, GPT appeared to be a specific 'liver-guiding enzyme' that can be used as a sensitive indicator of hepatotoxic effects. However, the increased LDH activity in fish plasma cannot be considered as indicator of hepatotoxic disfunction.

LDH, GOT and GPT are cellular metabolic key enzymes with no evident function in vertebrate plasma. They were found in small concentrations in plasma, including fish plasma, derived probably from the regular physiological shedding of cells (Schmidt & Schmidt 1974). Therefore any detectable increase of their activity in plasma can be used as a reliable indicator of changed metabolic functions or structural damage at

the tissue level. Detection methods for these enzymes are simple, rapid and, what is important in fish physiology studies, can be carried out with small blood samples. The determination of the basic physicochemical characteristics and electrophoretic fractionation of the isozyme forms provides the opportunity to identify their origin and also permits estimation of the severity of injury at the tissue/cellular level, as was illustrated by Panteghini et al. (1984).

Acknowledgements. This work was financed by the Scientific Research Fund of S.R. Croatia and supported by the MED POL/FAO-UNEP program. The technical assistance of S. Dragic is gratefully acknowledged.

LITERATURE CITED

D'Apollonia, S., Anderson, P. D. (1980). Optimal assay conditions for serum and liver glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and sorbitol dehydrogenase from the rainbow trout *(Salmo gairdneri)*. Can. J. Fish. Aquat. Sci. 37: 163–169

Bergmeyer, H. U., Bernt, E. (1974a). Lactate-dehydrogenase, UV-assay with pyruvate and NADH. In: Bergmeyer, H. U. (ed.) Methods of enzymatic analysis, Vol 2. Academic Press, New York, p. 574–579

Bergmeyer, H. U., Bernt, E. (1974b). Glutamate-oxaloacetate transaminase, UV-assay, manual method. In: Bergmeyer, H. U. (ed.) Methods of enzymatic analysis, Vol. 2. Academic Press, New York, p. 727–733

Bergmeyer, H. U., Bernt, E. (1974c). Glutamate-pyruvate transaminase, UV-assay, manual method. In: Bergmeyer H. U. (ed.) Methods of enzymatic analysis, Vol. 2. Academic Press, New York, p. 752–758

Bouck, G. R. (1966). Changes in blood and muscle composition of rock bass *(Ambloplites rupestris)* as physiological criteria of stressful conditions. Ph. D. dissertation, Michigan State University, East Lansing, Michigan, USA

Bouck, G. R., Schneider, P. W., Jr., Jacobson, J., Ball, R. C. (1975). Characterization and subcellular localization of leucine aminonaphthylamidase (LAN) in rainbow trout *(Salmo gairdneri)*. J. Fish. Res. Bd Can. 32 (8): 1289–1295

Burlina, A., Zaninotto, M., Graziani, M. S., Rizzotti, P. (1979). The lactate dehydrogenase isoenzymes in the diagnosis of specific organ injury: an improved method of demonstration. In: Burlina, A., Galzingo (eds.) Clinical Enzymology Symposium 2: 369–376

Casillas, E., Ames, E. (1986). Hepatotoxic effects of CCl_4 on English sole *(Parophrys vetulus)*: possible indicators of liver dysfunction. Comp. Biochem. Physiol. 84C (2): 397–400

Casillas, E., Myers, M., Ames, E. (1983). Relationship of serum chemistry values to liver and kidney histophathology in English sole *(Parophrys vetulus)* after acute exposure to carbon tetrachloride. Aquat. Toxicol. 3: 61–78

Curtis, S. J., Moritz, M., Snodgrass, P. J. (1972). Serum enzymes derived from liver cells. I. The response to CCl_4 intoxication in rats. Gastroenterology 62: 84–92

Dando, P. R. (1969). Lactate metabolism in fish. J. mar. biol. Ass. U. K. 49: 209–223

Gaudet, M., Racicot, J. G., Leray, C. (1975). Enzyme activities of plasma and selected tissues in rainbow trout (*Salmo gairdneri* Richardson). J. Fish. Biol. 7: 505–512

Hille, S. (1982). A literature review of the blood chemistry of rainbow trout (*Salmo gairdneri* Rich.). J. Fish. Biol. 20: 535–569

Ikeda, Y., Fujikata, A. (1984). Variations of carp serum constituent levels with time for storage of whole blood. Bull. Jap. Soc. scient. Fish. 50 (8): 1337–1340

Krajnović-Ozretić, M., Ozretić, B. (1987). Specific alteration of transaminases in marine organisms under pollution impact. In: Vernberg, W. B., Calabrese, A., Thurberg, F. P., Vernberg, F. J. (eds.) Pollution physiology of estuarine organisms. Univ. S. Carolina Press, Columbia, p. 207–230

Krajnović-Ozretić, M., Ozretić, B. (1988). Toxic effects of phenol on grey mullet (*Mugil auratus* Risso). Bull. Environ. Contam. Toxicol. 40 (1)

Lockhart, W. L., Metner, D. A. (1984). Fish serum chemistry as a pathology tool. In: Cairns, V. W., Hodson, P. V., Nriagu, J. O. (eds.) Contaminant effects on fisheries. Willy, p. 73–85

Markert, C. L., Faulhaber, I. (1965). Lactate dehydrogenase izozyme patterns of fish. J. exp. Zool. 159: 319–332

Mehrle, P. M., Mayer, F. L. (1980). Clinical tests in aquatic toxicology: state of the art. Environ. Health Perspect. 34: 139–143

Miller III, W. R., Hendricks, A. C., Cairns, J., Jr. (1983). Normal range for diagnostically important hematological and blood chemistry characteristics of rainbow trout *(Salmo gairdneri)*. J. Fish. Res. Bd Can. 40 (4): 420–425

Panteghini, M., Malchiodi, A., Calarco, M., Bonora, R. (1984). Clinical and diagnostic significance of aspartate aminotransferase isoenzymes in sera of patients with liver diseases. J. clin. Chem. clin. Biochem. 22: 153–158

Pfeifer, K. F., Weber, L. J., Larson, R. E. (1977). Alanine aminotransferase (GPT) in rainbow trout: plasma enzyme levels as an index of liver damage. Proc. West. Pharmacol. Soc. 20: 431–437

Racicot, J. G., Gaudet, M., Leray, C. (1975). Blood and liver enzymes in rainbow trout (*Salmo gairdneri* Rich.) with emphasis on their diagnostic use: study of CCl_4 toxicity and a case of Aeromonas infection. J. Fish. Biol. 7: 825–835

Raisfeld, I. H. (1974). Models of liver injury: the effects of toxins on the liver. In: Becker, F. F. (ed.) The liver: normal and abnormal functions Part A. Marcel Dekker, New York, p. 203–223

Schmidt, E., Schmidt, F. W. (1974). The importance of enzymatic analysis in medicine. Principles. In: Bergmeyer, H. U. (ed.) Methods of enzymatic analysis, Vol. 1. Academic Press, New York, p. 6–14

Schmidt, E., Schmidt, F. W (1975). Enzyme patterns, Diagnostik 8: 427–432

Statham, C. N., Croft, W A., Lech, J. J. (1978). Uptake, distribution and effects of carbon tetrachloride in rainbow trout, *Salmo gairdneri*. Toxicol. appl. Pharmacol. 45: 131–140

Wedemeyer, G. A., McLeay, D. J. (1981). Some blood chemistry values for the rainbow trout, *Salmo gairdneri*. J. Fish. Res. Bd Can. 27 (6): 1162–1164

Wieme, R. J. (1974). Measurements after electrophoretic separation. In: Bergmeyer, H. U. (ed.) Methods of enzymatic analysis, Vol. 2. Academic Press, New York, p. 593–602

Wieser, W., Hinterleitner, S. (1980). Serum enzymes in rainbow trout as tools in the diagnosis of water quality. Bull. Environ. Contam. Toxicol. 25: 188–193

Responsible Subject Editor: Professor O. Kinne; accepted for printing on December 10, 1987