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Relation of Allozyme Genotype to Survivorship of Juvenile Bream, *Abramis brama* L., Acutely Exposed to DDVP, an Organophosphorus Pesticide

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Recently the anthropogenic pollution of aquatic environment became an important factor influencing state and adaptive potential of the natural fish populations. As a one of possible mechanism of such influence is a variable resistance of the fish genetic variants to toxic stress. Under the pressure of pollutants more tolerant genotypes will have preference in surviving comparing to less tolerant ones. As a result, the tolerant genetic variants will be accumulated in the population while the sensitive ones will be eliminated. Consequently, these will lead to changes in population genetic structure, decreasing its heterogeneity and adaptive potential.

It is well known that allozyme variants are essentially the genetically determined products of the allelic genes. Several authors have stated the necessity to consider such variants in the biomonitoring of aquatic environment (Gyllensten and Ryman 1985; Chagnon and Guttman 1989). For this purpose it is necessary to accumulate more knowledge revealing the relationships between the genetic variants of the different allozyme loci and survivorship in different fish species subjected to pollution stress. Recently several investigators have shown that certain genotypes of some mosquitofish allozyme loci (isocitrate dehydrogenase-2, glucose phosphate isomerase-2, malate dehydrogenase, mannosephosphate isomerase and fumarate hydratase) are related somehow to time-to-death of the fish acutely exposed to some heavy metals in the laboratory (Chagnon and Guttman 1989; Newman et al. 1989; Diamond et al. 1989, 1991). However, we do not know of any papers concerning differential survivorship of fish genotypes exposed to as important environmental pollutants as organophosphorus pesticides (OP).

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The present study was designed with the aim to test in the laboratory experiment if the survivorship of bream (*Abramis brama* L.) exposed to organophosphorus pesticides DDVP relates to the differences in the genotypes at the peroxidase (Po) locus.

MATERIALS AND METHODS

The juvenile bream (*Abramis brama* L.) for this study have been obtained by the direct individual breeding of the adult fish caught during spawning period (May) in the Volga Bay of the Rybinsk reservoir (Russia, 57° N, 36° E). The eggs obtained from the fish caught in the wild were fertilized in the incubation vessels with fresh water. After that the allozyme genotypes of the heart muscle peroxidase (Po; EC 1.11.1.7) of each parent fish were determined using polyacrylamide gel electrophoretic method (Slynko 1992). It was shown previously that bream Po locus is polymorphic, has three alleles presented with six allozyme genotypes and those alleles have been labeled as Po 79, Po 100 and Po 116 according to increasing anodal migration (Slynko 1992). Since the genotype 116/116 is quite rare (frequency of about 5 %) such fish have not been caught in sufficient amount and have not been tested. To establish the survivorship of the "clear" alleles separately only the progeny of parents having the same homozygous genotypes 79/79 and 100/100 were incubated further and used in the study. The eggs obtained from the heterozygous parents were discarded.

The eggs were incubated in the separate chambers of incubation apparatus with flowing water from Rybinsk reservoir and under similar conditions. The temperature and oxygen regimes were controlled during all incubation period. The fry were transferred to Institute experimental ponds after they turned to the exogenous feeding. Each genotype was kept in the separate pond. The fry were fed on the natural food during all summer time. The procedure was described in more detail elsewhere (Slynko et al. 1992). One month prior to the experiment the fry (age about 6 mon) were transferred to the laboratory. The fry were maintained in the fiberglass holding tanks with flowing natural water at 19±1° C, provided with constant aeration, a 12L:12D photoperiod, and were fed *ad libitum* once daily with artificial trout pellets. At the beginning of the toxicity tests the fish sizes were: body length 74.3±0.6 mm (mean±S.E.) and weight 6.13±0.18 g for genotype 79/79, and 61.9±0.4 mm and 3.62±0.10 g for genotype 100/100, respectively.

Chemical grade DDVP (dichlorvos; 2,2,-dichlorovinyl

dimethyl phosphate, CAS #62737, 97.5 % active ingredient) obtained from the Volgograd pesticide producing factory (Volgograd, Russia) was used. DDVP nominal concentrations ranging from 11 to 31 mg/l were tested. DDVP was predissolved in acetone, dissolved in distilled water and introduced into each aquaria in the volume necessary to achieve required toxicant concentration. Final carrier solvent concentration in the water of any aquaria did not exceed 0.01 % (v/v).

The fish were exposed to DDVP for 120 hr using static-renewal protocol (U.S. Environmental Protection Agency 1975). The water was replaced once daily. Well water with Ca^{++} content 40 mg/L, dissolved oxygen content 7.8 to 9.0 mg/L, pH 8.1 to 8.3 and temperature 19 ± 1^{o} C was used in the control and for the preparation of test solutions. Experiments were carried out in 40-L Plexiglass aquaria. Fish were not fed during the exposure period. Groups of 6 fish of every genotype were exposed simultaneously in the same aquaria. To distinguish the genetic variants during testing the fish with genotype 79/79 were marked by cutting out the upper rays of the tail fin. To prevent disturbances from the stress the procedure was carried out 2 wk prior the experiment. Mortality of the each genotype was registered and dead fish were removed from an aquaria every 24 hr during the entire exposure period. Fish were considered dead if they showed no signs of ventilation or response to repeated prodding.

The toxicity tests have been performed in two replicates. The LC_{50} values and their standard errors (S.E.) were calculated using method of Frumin et al. (1990) that allows the calculation these parameters based on data from one effect concentration only. The mortality data from all effect concentrations for each exposure time were used to calculate the means LC_{50}'s and their S.E's for every genotype tested. The average parameters were calculated in accordance to rules of estimating the general mean and its standard error from several independent means with its errors (Lakin 1973).

RESULTS AND DISCUSSION

No mortality occurred in the control during all testing period. The data on mortalities in fish exposed to DDVP are shown in Table 1. There was no mortality among genotype 79/79 fish during first 48 hr of exposure in all concentrations tested. Moreover, the fish of this genotype exposed to concentrations less than 21 mg/L survived all exposure period. As for the bream of genotype 100/100 17 to 33 % of the fish died after 24 hr of exposure to DDVP in the concentrations ranged

Table 1. Mortalities of the bream genotypes during acute exposure to DDVP (% of total fish number)

Exposure time, hr	DDVP concentrations, mg/L																			
	11	14	17	19	21	23	25	27	29	31	11	14	17	19	21	23	25	27	29	31
	Genotype 79/79										Genotype 100/100									
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	17	17	33
48	0	0	0	0	0	0	0	0	0	0	0	0	17	17	17	17	33	33	67	83
72	0	0	0	0	0	0	17	17	33	33	0	17	33	33	50	67	67	83	83	100
96	0	0	0	0	0	0	17	33	50	83	17	33	50	67	83	83	100	100	100	-
120	0	0	0	0	17	17	50	67	100	100	33	67	83	83	100	100	-	-	-	-

Mean values for two replicates are presented; N=6 fish/assay

from 25 to 31 mg/L. After 48 hr of exposure the mortalities in the fish of this genotype were noticed in all concentrations tested, except 11 and 14 mg/L where fish survived this period. In the fish of both genotypes the mortalities increased with an exposure duration. However, during all exposure period the genotype 100/100 fish exhibited higher mortalities than 79/79 genotypes fish when data from equal concentrations were compared. At the end of exposure period (120 hr) the mortalities exceeding 33 % were observed in the genotype 100/100 fish in all exposure variants and all fish died in the concentrations higher than 19 mg/L. In the genotype 79/79 fish the mortality at that time reached 100 % only in the highest concentrations, 29 and 31 mg/L. Thus, the results demonstrate that the bream of the genotype 79/79 exhibited higher survivorship than the genotype 100/100 under the acute DDVP action.

Mean LC_{50} values obtained for both tested bream genotypes are given in Table 2.

Table 2. Mean LC_{50} values (mg/L) of the bream genotypes acutely exposed to DDVP.

Exposure time, hr	Genotype			
	N	79/79	N	100/100
24	-	-	4	33.05 ± 0.82^{a}
48	-	-	8	26.18 ± 0.52^{b}
72	4	32.52 ± 0.81^{a}	8	21.11 ± 0.46^{c}
96	4	$28.88 \pm 0.77^{a,b}$	6	16.66 ± 0.52^{d}
120	4	26.11 ± 0.73^{b}	4	13.77 ± 0.53^{e}

Means ± S.E. are presented; N - number of effect concentrations used for calculation of mean LC_{50} and S.E. values; similar letter superscripts indicate statistically insignificant differences ($p>0.05$).

Since genotype 79/79 fish did not exhibit mortalities during first 2 d of exposure to DDVP, the 24-hr and 48-hr LC_{50} values have not been calculated. The statistical analysis has shown that 72-, 96- and 120-hr LC_{50} values for the bream of genotype 79/79 were significantly ($p<0.05$) higher than the respective values for genotype 100/100. Revealed differences ranged from 1.54 to 1.90 times between two genotypes

and have increased with exposure duration. Based on these results we suggest that with further increase of exposure time the differences between LC_{50} values of two tested genotypes may become more pronounced.

DDVP used in the present study was chosen as a model OP possessing a set of properties typical for this chemical class. Therefore revealed differences in the resistance of the tested bream genotypes to DDVP suggest that the selection of the more resistant fish of genotype 79/79 may occur in the natural bream populations under the OP pollution pressure. There are several examples of differential survivorship of fish allozyme genotypes exposed to chemicals belonging to other, than OP, classes. For instance, the mosquitofish genotypes at the glucose phosphate isomerase-2 locus differ in resistance to cadmium and copper (Chagnon and Guttman 1989).

If the genotypes have been marked using the allozymes it seems more likely that the genotype differential survival may be based on various susceptibilities of respective allozymes to toxicants. However, it has been shown that in vivo OP may either inhibit or activate Po activity in fish (Grishchenko et al. 1976). This indicates that Po is not specific target for OP. That is why in the present study sensitivity of Po to DDVP seems unlikely to contribute to differential survival of bream genotypes. At the same time, OPs are well known specific inhibitors of another enzyme, the acetylcholinesterase (AChE). Anti-AChE effects of OP play a great role in their toxicity for fish (Coppage 1975; Kozlovskaya et al. 1985). Fish brain AChE is a polymorphic enzyme and its isoenzymes have different kinetic properties (Baldwin and Hochachka 1970). There are evidences that the sensitivities of the fish brain AChE isoenzymes to OP are different (Lim et al. 1971; Kozlovskaya and Flerov 1981). We suggest that Po and AChE loci in bream may be interconnected. Consequently, if the sensitivities of the AChE isoenzymes to OP are different this may explain different susceptibilities of the tested genotypes to DDVP toxic action.

However, one may suggest another explanation of the different survivorship of bream genotypes. We found previously that differences in the uptake and depuration rates play a great role in the interspecific differences in resistance of fish to acute DDVP action (Chuiko 1988). Same mechanisms may contribute to the revealed variabilities in the tolerance of tested genotypes to DDVP. Further investigations are urgently needed to find precise mechanisms responsible for the various resistance of different fish genotypes to OP.

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REFERENCES

Baldwin J, Hochachka PW (1970) Functional significance of isoenzymes in thermal acclimatization. Acetylcholinesterase from the trout brain. Biochem J 116:833-883

Chagnon NL, Guttman SI (1989) Differential survivorship of allozyme genotypes in mosquitofish populations exposed to copper or cadmium. Environ Toxicol Chem 8:319-326

Chuiko GM (1988) Various resistance mechanisms of carp (*Cyprinus carpio* L.) and perch (*Perca fluviatilis* L.) to DDVF organophosphorus compounds. In: Ryans RC (ed.) Fate and effects of pollutants on aquatic organisms and ecosystems: proceedings of USA-USSR symposium, Athens, Georgia, October 19-21, 1987. EPA/600/9-88/001, US EPA Environmental Research Laboratory-Athens, Athens, Georgia

Coppage DL (1975) Organophosphate pesticides: specific level of brain AChE inhibition related to death in sheepshead minnows. Trans Amer Fish Soc 101:534-536

Diamond SA, Newman MC, Mulvey M, Guttman SI (1991) Allozyme genotype and time-to-death of mosquitofish, Gambusia holbrooki, during acute inorganic mercury exposure: a comparison of populations. Aquat Toxicol 21:119-134

Diamond SA, Newman MC, Mulvey M, Dixon PM, Martinson D (1989) Allozyme genotype and time to death of mosquitofish, *Gambusia affinis* (Baird and Girard), during acute exposure to inorganic mercury. Environ Toxicol Chem 8:613-622

Frumin GT, Chuiko GM, Pavlov DF, Menzykova OV (1992) New rapid method to evaluate the median effect concentrations of xenobiotics in hydrobionts. Bull Environ Contam Toxicol 49:361-367

Grishchenko L, Verkhovsky A, Trondina G (1976) Effect of benzophosphate (phosalon) on fish organism. Exper Water Toxicol (USSR) 6:196-203 (in Russian)

Gyllensten U, Ryman N (1985) Pollution biomonitoring programs and the genetic structure of indicator Species. Ambio 14:29-31

Kozlovskaya VI, Flerov BA (1981) Organophosphorus pesticides and their hazards for aquatic animals. In: Butorin NV, Flerov BA, Lukiyanenko VI, Umorin PP (eds) Theoretical aspects of aquatic toxicology.

Nauka, Leningrad (in Russian)
Kozlovskaya VI, Chuiko GM (1985) Chlorophos-induced changes in the perch (*Perca fluviatilis* L.) brain acetylcholinesterase. Hydrob Z (USSR) 21:49-53 (in Russian)
Lakin GP (1973) Biometria. Vysshaya Shkola Publ, Moscow (in Russian)
Lim R, Davis GA, Agranoff BW (1971) Electrophoretic studies on solubilized proteins of goldfish brain. Brain Res 25:121-131
Newman MC, Diamond SA, Mulvey M, Dixon Ph (1989) Allozyme genotype and time to death of mosquitofish, *Gambusia affinis* (Biard and Girard) during acute toxicant exposure: a comparison of arsenate and inorganic mercury. Aquat Toxicol 15:141-156
Slynko Yu V (1992) Muscle isoenzymes polymorphism in Cyprinides of USSR. II. Peroxydase (PO, E.C.1.11.1.7) In: Biol Inland Waters Inform Bull No 66, Nauka, Leningrad (in Russian)
Slynko Yu V, Gerassimov Yu V, Gretchanov IG (1991) Morphological description and frequency of aberrant specimens among yearling bream (*Abramis brama* L.) different genotypes at the heart muscle peroxidase locus. Voprosy Ikhtiologii (Russia) 31:1010-1014 (in Russian)
U S Environmental Protection Agency (1975) Methods for acute toxicity test with fish, macroinvertebrates, and amphibians. EPA-660/3-75-009 Corvalis, Oregon, USA