

**EFFECT OF PH, TEMPERATURE AND SOME POLLUTANTS ON FISH
DIGESTIVE ENZYMES IN NATURE AND EXPERIMENTS**

Kuz'mina VV
I.D.Papanin Institute for Biology of Inland Waters
Russian Academy of Sciences, 152742 Borok, Yaroslavl
Phone:(085)2253845 Fax:(085) 2253845
e-mail:vkuz'minav@ibiw.yaroslavl.su

Golovanova I L

Effect of pH, temperature and some pollutants on fish digestive enzymes in experiments

It is known, that temperature, pH and different modifiers may change the digestive enzyme activities (Ugolev & Kuz'mina,1993). At the same time it was demonstrated that the temperature influences the effect of various modifiers as well as pH-function of fish digestive enzymes. The degree of the effect depends on the species of fish, chemical structure of modifiers, medium temperature etc. (Kuz'mina & Nevalenny,1983; Kuz'mina,1984,1987; Golovanova et al, 1994). However, in our previous works an integral investigation of the above factors was not made. The present work is aimed at the study of the effect of temperature, pH and some pollutants (dichlorvos, naphthalene and cadmium) on proteolytic, amylolytic and sucrase activities of intestinal mucosa in some species of freshwater fish,namely, bream, *Abramis brama*, zander, *Stizostedion lucioperca*, perch, *Perca fluviatilis*, pike, *Esox lucius* and blue bream, *Abramis ballerus* in vitro experiments. Total proteolytic activity was estimated according to an increase of tyrosine by the modified method of Anson (1938), total amylolytic and sucrase activities by the modified method of Nelson (Ugolev & Iezuitova,1969). The casein (10 g/l), starch (18 g/l) and saccharose (50 mM) were used as substrates. For preparation of the homogenates the Ringer solution (pH 5.0, 7.4 and 8.5) without phosphorus ions was used. Preincubation of homogenates with CdSO₄ (50 mg/l of total cadmium) was carried out during 60 min at 0°, 10°, 20° and 37 °C, at the above pH values.

The data on mono-, bi- and polyfactor effects of pH, temperature and cadmium testify that in the most of cases the cadmium (but not dichlorvos and naphthalene) effects only slightly the proteolytic activity (Table 1). The increase of pH from 7.4 to 8.5 causes the growth of enzyme activity in bream at all temperature values, in zander and perch at 10° - 37 °C only. The decrease of pH from 7.4 to 5.0 as a rule significantly diminishes the enzyme activity especially in zander and perch in all temperature ranges. In the presence of cadmium this effect is became stronger at pH 5.0 and is reduced at pH 8.5. The level of intestinal mucosa proteolytic activity grows significantly with temperature in all fish species. However a degree of the increase of enzyme activity in the temperature range from 0° to 37 °C is different in various pH zones due to various Q₁₀ values. In bream it is larger at pH 7.4 and in zander and perch at pH 8.5. At the same time both the temperature and the cadmium are less effective then the temperature itself. Thus the change of temperature from 20 °C to 0 °C at pH 7.4 decreases proteolytic activity in 5.4, 4.0 and 3.3-fold in bream, zander and perch correspondingly. The change of pH from 7.4 to 5.0 at temperature 20 °C decreases significantly the enzyme activity (6.6 and 3.4 - fold) in zander

and perch only. The most changes are observed under polyfactor effects of pH 5.0, temperature 0 °C and cadmium. In this case the proteolytic activity is reduced 7.7, 8.8 and 7.3-fold in bream, zander and perch accordingly. In alkaline zone of pH this influence is approximately two times as less.

Table 1.

Effect of temperature, pH and cadmium on intestinal mucosa proteolytic activity in some fish species, $\mu\text{mol} \times \text{g}^{-1} \times \text{min}^{-1}$, N=5-8

Temperature, °C	Cadmium concentration, mg Cd/l	pH 5.0	pH 7.4	pH 8.5
Bream				
0	0	0.08-0.01	0.10-0.05	0.28-0.05
	50	0.07-0.04	0.07-0.02	0.17-0.01
10	0	0.24-0.07	0.28-0.04	0.63-0.24
	50	0.21-0.03	0.18-0.06	0.26-0.13
20	0	0.46-0.12	0.54-0.06	1.20-0.55
	50	0.30-0.07	0.48-0.10	0.92-0.14
37	0	1.32-0.30	4.05-0.82	3.30-2.52
	50	0.89-0.15	3.00-0.76	2.53-2.13
Zander				
0	0	0.42-0.09	0.87-0.12	0.86-0.11
	50	0.39-0.10	0.63-0.17	0.77-0.11
10	0	0.45-0.18	1.73-0.29	2.11-0.35
	50	0.41-0.12	1.68-0.30	2.07-0.28
20	0	0.52-0.16	3.44-0.83	4.50-0.53
	50	0.63-0.16	3.25-0.50	3.67-0.70
37	0	1.08-0.26	6.66-0.94	0.29-0.46
	50	1.07-0.21	6.32-0.36	9.66-0.74
Perch				
0	0	0.20-0.11	0.82-0.22	0.52-0.21
	50	0.37-0.08	0.45-0.19	0.59-0.16
10	0	0.54-0.07	1.15-0.16	1.93-0.19
	50	0.78-0.11	1.22-0.25	1.67-0.14
20	0	0.79-0.16	2.70-0.22	3.11-0.19
	50	0.91-0.19	2.15-0.31	1.89-0.18
37	0	1.59-0.27	7.89-0.47	8.07-0.74
	50	1.93-0.24	5.96-0.61	6.48-0.78

The effects of pH, temperature and cadmium (not dichlorvos or naphtalene) on total amyolytic activity are observed (Table 2). The enzyme activities are 2-4 times lower at 0 °C then at 20 °C and pH 5.0 then at pH 7.4 in all fish species. Cadmium significantly decreases enzyme activity in bream at pH 7.4, in zander and perch at pH 8.5. The simultaneous changes in temperature (20° → 0 °C) and (7.4 → 5.0) reduce the total amyolytic activity 7 times in bream and 3 times in zander and perch. The above enzyme activity is 3-7 times as low in fish studied under polyfactor effects of pH 5.0, 0 °C and cadmium then at pH 7.4, 20 °C, lack of cadmium. But these changes are due to bifactor effect of temperature and pH mainly. Mono-, bi- and polyfactor effects of pH, temperature and cadmium on succharase activity of intestinal mucosa in bream and zander are similar to those of total amyolytic activity, but are weaker. The same regularities of all enzymes studied were observed in pike and blue bream.

Table 2.

Effect of temperature, pH and cadmium on intestinal mucosa total amyolytic activity in some fish species, $\mu\text{mol} \times \text{g}^{-1} \times \text{min}^{-1}$, N=5-8

Temperature, °C	Cadmium concentration, mg Cd/l	pH 5.0	pH 7.4	pH 8.5
Bream				
0	0	1.38 ± 0.09	2.55 ± 0.17	2.12 ± 0.07
	50	1.30 ± 0.15	2.37 ± 0.20	2.28 ± 0.22
10	0	1.61 ± 0.16	4.60 ± 0.21	3.68 ± 0.33
	50	1.59 ± 0.15	3.57 ± 0.29	3.24 ± 0.23
20	0	2.51 ± 0.26	9.62 ± 0.63	7.10 ± 0.58
	50	2.52 ± 0.25	8.00 ± 0.59	6.34 ± 0.40
Zander				
0	0	0.20 ± 0.01	0.34 ± 0.03	0.35 ± 0.03
	50	0.17 ± 0.01	0.32 ± 0.03	0.28 ± 0.04
10	0	0.22 ± 0.02	0.43 ± 0.03	0.49 ± 0.04
	50	0.21 ± 0.02	0.42 ± 0.05	0.38 ± 0.01
20	0	0.30 ± 0.02	0.62 ± 0.05	0.65 ± 0.05
	50	0.28 ± 0.02	0.59 ± 0.07	0.49 ± 0.06
Perch				
0	0	1.42 ± 0.07	1.91 ± 0.02	2.18 ± 0.11
	50	1.46 ± 0.02	1.89 ± 0.06	2.08 ± 0.04
10	0	1.67 ± 0.03	2.81 ± 0.07	3.31 ± 0.09
	50	1.69 ± 0.02	2.75 ± 0.10	3.13 ± 0.12
20	0	2.09 ± 0.06	3.67 ± 0.15	4.52 ± 0.14
	50	1.91 ± 0.06	3.40 ± 0.05	3.89 ± 0.11

Effect of pH and seasonal temperature on perch enzyme activity in nature.

Intestine enzyme activity of perch from 5 acidic and 1 neutral lakes has been investigated using two methodical approaches. In the first case the enzyme activity is determined by a standard method (for 1 g of enzyme active preparation). In the second case the enzyme activity is calculated for real intestine mucosa and intestine content mass. In all seasons especially in spring and autumn the size and mass of perch from neutral lake are higher than in acidic lakes due to the presence of large fish specimens. In all seasons except for the winter the total proteolytic activity of intestinal mucosa in perch from neutral lake is 1.8-3.4 times higher than in acidic lakes (Table 3). Total amyolytic activity in perch from the neutral lake is significantly higher than in acidic lakes in spring and autumn, the sucrase activity - only in spring ($P < 0.05$). The effect of cadmium on the enzyme activities studied hasn't been found in perch from both neutral and acidic lakes. The differences observed are probably conditioned by the largest differences of fish feed intensity estimated as ratio of intestine content mass to empty intestine mass in this period (1.95 ± 0.9 and 0.34 ± 0.06 correspondently). The spectrum of food objects and feeding intensity dynamic differ too. In the neutral lake the predators prevail over benthophages, in acidic lakes were only benthophages. The maximum feeding activity in the first case are observed in spring, in the second case in summer (Komov & Zhgareva, 1995). The differences of real enzyme activity (in all mucosa and all intestine content) between perches from neutral and acidic lakes became still more considerable as compared with those estimated by a standard method. Thus, the acidification of lakes causes changes in perch type of feeding and intestine enzyme activity.

Table 3.

Enzyme activity of intestinal mucosa in perch from neutral and acidic lakes, $\mu\text{mol} \times \text{g}^{-1} \times \text{min}^{-1}$, N=5-10

Season temperature,	Lake pH	Cadmium concentration, mg/l	Total proteolytic activity	Total amylolytic activity	Sucrase activity
Spring 10 °C	7.0	0	6.18 ± 1.23	3.49 ± 0.20	0.64 ± 0.06
		50	5.74 ± 1.14	2.91 ± 0.21	0.53 ± 0.06
	4.5	0	2.06 ± 0.56	2.56 ± 0.17	0.38 ± 0.04
		50	1.84 ± 0.55	2.28 ± 0.18	0.25 ± 0.04
Summer 20 °C	7.0	0	3.98 ± 1.87	2.12 ± 0.17	0.25 ± 0.04
		50	3.29 ± 1.03	2.77 ± 0.39	0.26 ± 0.07
	4.5	0	2.13 ± 0.28	2.11 ± 0.16	0.35 ± 0.11
		50	2.19 ± 0.39	2.14 ± 0.16	0.30 ± 0.13
Autumn 10 °C	7.0	0	2.53 ± 1.12	3.44 ± 0.26	0.32 ± 0.09
		50	2.04 ± 0.84	2.87 ± 0.17	0.30 ± 0.08
	4.5	0	0.83 ± 0.26	2.46 ± 0.17	0.40 ± 0.05
		50	0.74 ± 0.14	2.18 ± 0.19	0.42 ± 0.06

So, the monoeffect of temperature or pH on digestive enzymes is shown. But the enzyme activities to a larger extent are influenced by a complex effect of these factors in all fish studied in nature and experiment. In the most of cases cadmium didn't cause any changes in enzyme activity.

References

- Golovanova, IL, Chuiko, GM & Pavlov, DF 1994 Effects of cadmium, naphthalene and DDVP on gut carbohydrases activity in bream (*Abramis brama L.*) and mozambique tilapia (*Oreochromis mossambicus Peters.*) Bull. Environ. Contam. Toxicol. V.52, N 3, pp.338-345.
- Komov, VT & Zhigareva, NN 1995 The perch feeding in acidified lakes of the Darwin National Reserve the Volga region. Biological resources of the white sea and inland waters of the European north. Abstr. Inter. conf. 19-23 November, 1995. Petrozavodsk, pp.138-139 (in Russian)
- Kuz'mina, VV 1994 Effect of the temperature on pH-function of fish intestine phosphatase. Voprosy ichthyol. V.24, N 1, pp.151-157 (in Russian).
- Kuz'mina, VV 1987 Regulatory properties of enzymes involved in membrane digestion in fishes. Zh. obsch. biol. N 6, pp.828-837 (in Russian).
- Kuz'mina, VV & Nevalenniy, AN 1983 Effect of H⁺ concentration on carbohydrate activity of fish digestive tract. Voprosy ichthyol. V.23, N 3, pp.481-490 (in Russian)