DIGESTIVE ENZYMES AS AN INDICATOR

OF FEEDING ECOLOGY OF WILD FISH

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Introduction

The comparison of the activities of some enzymes involved in cavital and membrane-linked digestion in various fish species has demonstrated the correlation between the type of feeding and the level of the enzymatic activity. Carnivorous fish have as a rule high protease activity while omnivorous and herbivorous fish demonstrates high carbohydrase activity (Ugolev & Kuz'mina, 1993a, 1994). In recent years it has been established a relationship between the properties of fish digestive enzymes and environmental temperature which determined by many conditions in particular by temperature range of fish feeding (Ugolev & Kuz'mina, 1993b). The aim of this investigation is to study the correlation between food spectrum or biochemical food composition, motive feeding activity, as well as temperature conditions of fish feeding and a number of their enzyme characteristics.

For the purpose of revealing an indicator of fish feeding ecology 37 species of sea and freshwater cartilaginous, sturgeons and bony fishes were studied. It was investigated two basic ichthyocenoses, namely the Black Sea fish community and the Volga river fish community. Both the sea and freshwater fish species belong to ecological groups of different feeding mode - ichthyophages, planktophages, benthophages, phytoplankto-benthophages, phyto-benthophages, euryphages, plankto-ichthyophages and ichthyo-benthophages (Svetovidov, 1964; Poddubny & Gallat, 1995).

Results

Effect of food spectrum and biochemical food composition. Different species of fish show insignificant variation in the proteinases activity (Fig.1). Proteolytic activity in cartilaginous varies from 1.1+0.5 μmol/min in benthophage buckler skate to 3.2+0.9 μmol/min in ichthyophage spiny dogfish. At the same time the variation of enzyme activity in marine teleosts had a greater range with a minimal level found in benthophage ichthyophage flounder (0.5 μmol/min) and maximal one found in plankto-ichthyophage Black Sea Scad (4.5 μmol/min). For most of the studied fishes the values were 2.0-4.0 μmol/min (red mullet, whiting, black umber, high-body pickerel, gray mullet, gilheat, rocking, barfish, bluefish) while for there benthophage species (flounder, comber, ratan goby) it less than 2.0 μmol/min and for another three ichthyophages (scorpion, Black Sea scad, toad goby) over 4.0 μmol/min. The total proteolytic activity of intestinal mucosa in freshwater fish was higher. In teleost it changed from 2.2+0.5 μmol/min for benthophages carp and roach to 6.0+0.8 μmol/min for ichthyophage pike. In the most benthophage and ichthyophage - benthophage species the activity level varied from 3.0 to 5.0 μmol/min. In sturgeons the total proteolytic activity was rather high too (3.9+0.5 μmol/min for sturgeon and 6.1+0.7 μmol/min for sterlet).

The activity of carbohydrases varied considerably in different species especially teleosts, depending on their food. In particular in marine teleosts the amylolytic activity varied from 0.7+0.2 μmol/min for ichthyophage whiting to 4.1+0.5 μmol/min for phyto-benthophage gilthead. However the relationship between carbohydrases activity and the type of feeding was not strongly pronounced in this fish group. The amylolytic activity in the intestinal mucosa of freshwater teleosts varied more considerably: from 0.2+0.1 μmol/min for ichthyophage pike to 12+0.2 μmol/min for phyto-benthophage crucian-carp (Fig.2). It is interesting that the acivity level rose gradually in the folloing order: ichthyophages → ichthyophages-benthophages → benthophages → phyto-benthophages.

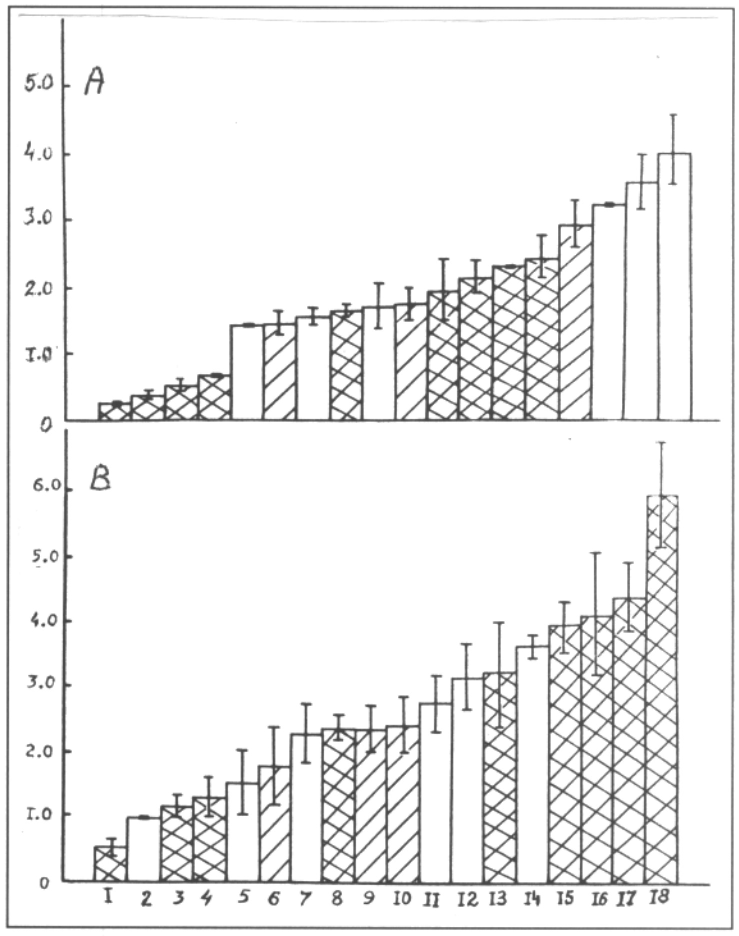

Figure 1. Total amilolytic (A) and total proteolytic activity in some species in the Black Sea fish.
Vertical, enzyme activity, μmol × g^{-1} × min^{-1}, A: 1-spiny dogfish, 2-buckler - skate, 3-stingray, 4-rockling, 5-whiting, 6-gray mullet, 7-comber, 8-bluefish, 9-Black Sea scad, 10-black umber, 11-gilthead, 12-high-body pickerel, 13-red mullet, 14-barfish, 15-toad goby, 16-ratan goby, 17-scorpion, 18-flounder. B: 1-flounder, 2-comber, 3-stingray, 4-buckler skate, 5-ratan goby, 6-spiny dogfish, 7-red mullet, 8-whiting, 9-black umber, 10-high-body pickerel, 11-gray mullet, 12-gilthead, 13-rockling, 14-barfish, 15-bluefish, 16-scorpion, 17-toad goby.

The most interspecies differences were found for protease activity carbohydrase activity ratio (stingray - 0.4, buckler skate - 2.1, spiny dogfish - 6.5; sturgeon - 0.9, sterlet - 2.2). In some marine teleost phyto-benthophage and benthophages this index varied from 0.16 to 0.89; in the other (benthophages, plankto-ichthyophages and bentho-ichthyophages) this index changed from 1.3 to 2.3. In ichthyophages the ratio of protease/carbohydrase activity was close to 3.5. However the most differences were demonstrated for freshwater teleosts. Thus, in this fish group protease/carbohydrase ratio increased in the following order: roach -0.5, bream -1.3, perch -3.5, burbot - 5.3, pike -30. As it is seen, this index in the ichthyophage pike is 60 - fold higher than in the phytoplankto-benthophage roach. Analyses of α-amylase activity have shown the range of variability in different species, with the maximum activity (carp) 41.4 mg/min exceeding by 70-100 times the minimal activity (sander and pike), 0.9+0.1 and 1.2+0.2 mg/min (Ugolev & Kuz'mina, 1994). The range of variability of disaccharidases activity is several fold lower: the maximum are 22.5-fold higher than minimum for sucrase (the white bream - pike) and 4.1-fold higher for maltase (crucian carp - pike). Thus, the most significant changes in the level of the enzymatic activity effected by the feeding spectrum have been demonstrated for enzymes realizing of the initial stages of carbohydrate hydrolysis.

In a number of cases the type of feeding correlates with regulatory properties of enzymes. In particular, tributyrin causes the inhibition of carbohydrase activity in benthophages (up to 60%) and stimulation of one in ichthyophages and ichthyophage-benthophages (up to +120%). The same agent may cause the opposite effect for alkaline phosphatase in the same fish species (Fig.3). As the indicator of fish feeding intensity, the comparison of the same enzyme activity under standard temperature (for example 20ºC) as well as the coefficient of enzyme activity variation may be used. Most values of enzyme activity and least values of variation coefficient testify about good feeding condition and high feeding intensity of fish population (Kuz'mina, 1980).

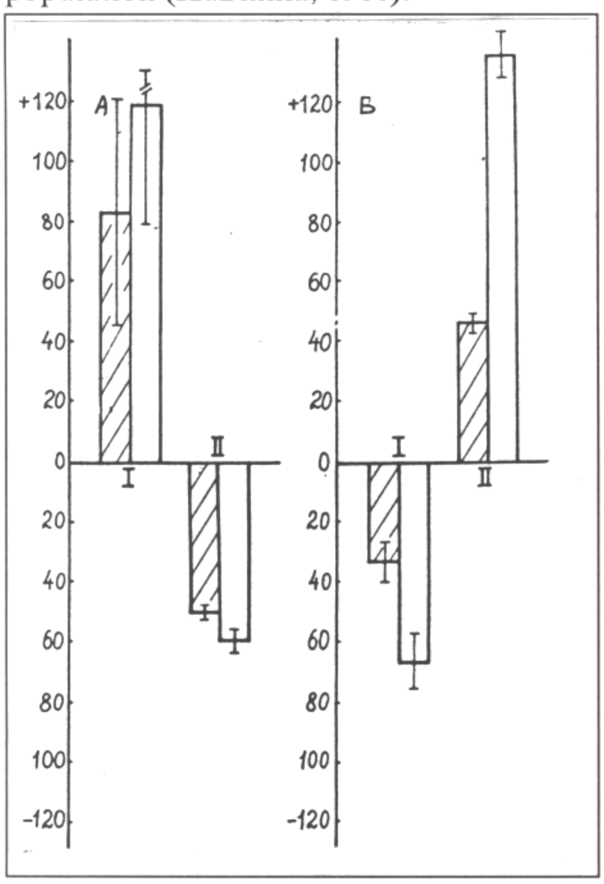

Figure 3. Effect of tributyrin on intestinal mucosa sucrase (I) and alkaline phosphatase (II) activity in burbot (A) and bream (B).
Vertical, stimulation (+) or inhibition (-), % of control, light columns, 0ºC, hatched columns, 20ºC.

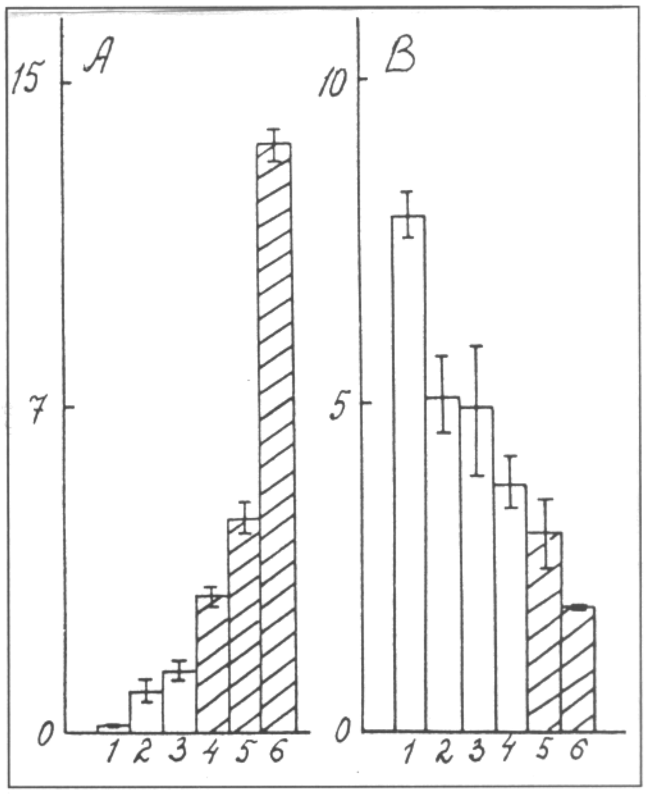

Figure 2. Total amylolytic (A) and total proteolytic (B) activities of intestinal mucosa in some freshwater fish. Vertical, enzyme activity, μmol × g^{-1} × min^{-1}, A: 1-pike, 2-burbot, 3-perch, 4-bream, 5-roach, 6-crucian carp. B: 1-pike, 2-asp, 3-burbot, 4-sheatfish, 5-bream, 6-carp.

Motive feeding activity. The study of α-amylase activity in the blood in some freshwater species shows that in some fish its level is lower than 1 (burbot - 0.30+0.05, safrefish - 0.33+0.03, bream - 0.52+0.05, blue bream - 0.69+0.12, white bream - 0.73+0.15, crucian carp 0.81+0.15, roach - 0.98+0.16 mg × ml^{-1} × min^{-1}, in other fish is higher than 1 (pike 1.13+0.32, pike-perh 1.18+0.14, ruff - 1.33+0.23, ide - 1.42+0.42, perch - 1.49+0.10 mg × ml^{-1} × min^{-1}. The most interspecies differences were found for the ratio blood α-amylase activity/intestine mucosa α-amylase activity. This index is 0.33 for blue bream, 0.57 for safrefish, 0.72 for bream, 1.24 for burbot, 3.79 for perch, 5.57 for pike perch and 6.20 for pike. As it can be seen the fishes with less motive feeding activity have low values of this index, while fishes with more motive feeding activity have high values of the index.

Temperature condition of fish feeding. The comparison of some characteristics of various hydrolases in fish with different type of feeding shows that some of them may be indicator of feeding behaviour. The most differences of temperature characteristics were revealed for enzyme realizing the initial stages of carbohydrate and protein degradation. In particular α-amylase temperature optimum is 40 ^{0}C for benthophages and planktophages and 30 ^{0}C for ichthyophages and ichthyophages-benthophages. The relative enzyme activity in the zone of low and physiological temperatures in the former is 10-30% of the maximal activity, in the latter is 50-90% of the maximal one (Fig.4). The temperature optimum of enzymes hydrolizing various proteins lies in the zone of high temperature (50-60 ^{0}C). However the relative enzyme activity values of proteolysis in zone low and physiological temperatures in typical and facultative predators significantly differs from those of typical bethophages and planctophages. In the former group the relative activity of enzyme realizing initial stages of proteolysis (pepsin) is 50-90% from maximal activity, in the latter group the same index is 5-20% only. One more important characteristic is the apparent energy of activation of some enzymes. It was established that E_{act} values of α-amylase differed in predatory and non predatory fish (Fig.5). In particular, in benthophages and planktophages E_{act} values changed from 9.4 kcal/mol (blue bream) to 11.3 kcal/mol (bream) in the temperature zone 10-30 ^{0}C, while in typical and facultative predators it changed from 1.5 kcal/mol (pike) to 3.6 kcal/mol (perch) in summer. It is interesting that this indicator in the zone 0-10 ^{0}C changes from 9.1 kcal/mol (bream) to 11.4 kcal/mol in fishes of the former group and from 2.2 kcal/mol (pike perch) to 6.7 kcal/mol (pike) in fishes of the latter group in winter. E_{act} values of proteases realizing

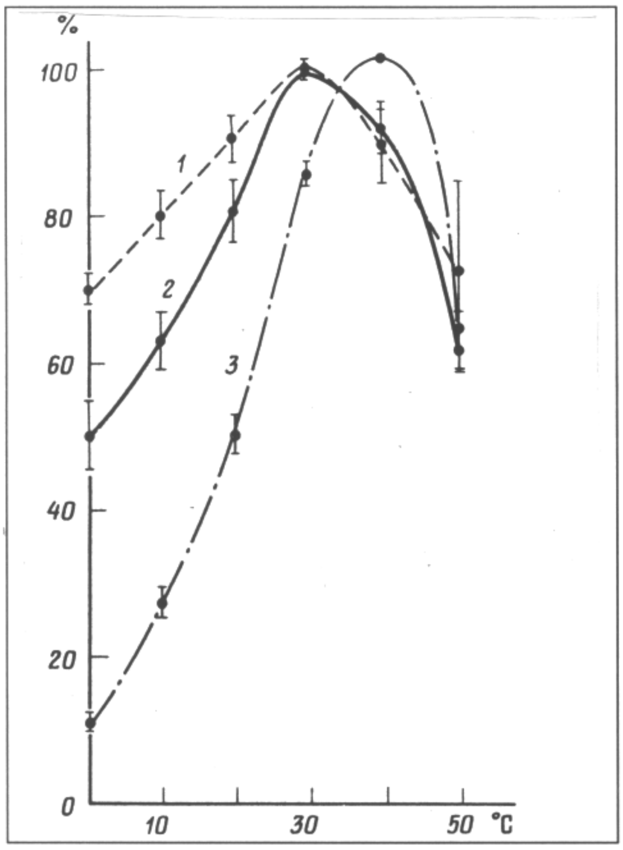

Figure 4. Effect of temperature on α-amylase activity of intestinal mucosa in pike-perch (1), safrefish (2) and bream (3).
Ordinate, enzyme activity, % of maximal one. Abscissa, temperature, ^{0}C.

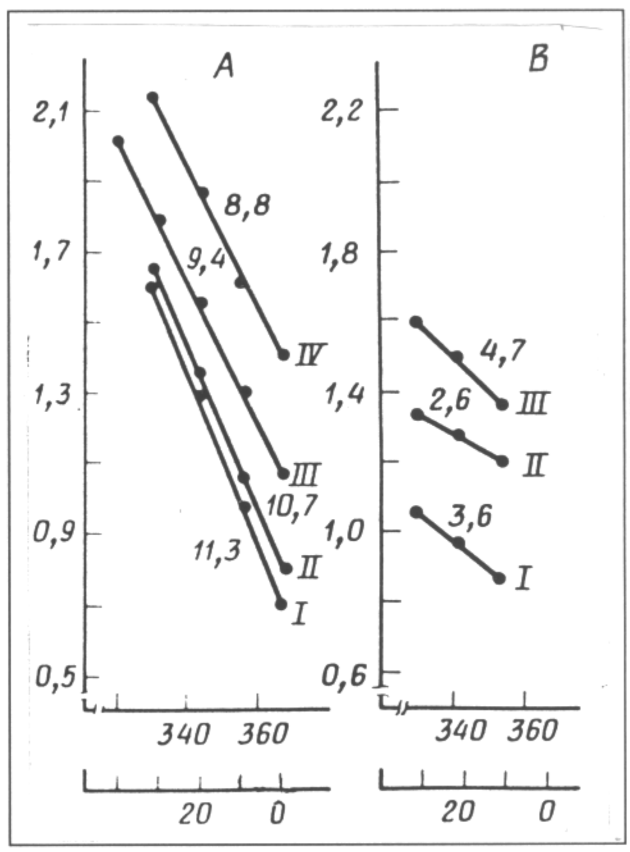

Figure 5. Arrhenius plots for intestinal mucosa α-amylase in some freshwater fish.
Ordinate, lg (V × 10^2) where V is a rate of enzymatic reaction. Abscissa, 1/T × 10^5 is value inversely proportional to absolute temperature (bottom line is temperature, ^{0}C).

initial stages of proteolysis differ more significantly. For pepsin of most predators the physiological temperature zone is characterised by very low values of E_{act} 1.1-1.5 kcal/mol, while for trypsin of most benthophages and planktophages the same temperature zone is characterised by high values of E_{act} closed 10 kcal/mol. Hence, low E_{act} values of these enzymes testify to a predator type of fish feeding but high E_{act} values testify to a non predatory type of fish feeding. Some information about it may be received using K_m and V values too. Under the same conditions the enzymes of predators have less K_m values than those of non predators. K_m values of sucrase are 28.6 mM and 14.8 mM in pike but 45.7 mM and 24.1 mM under 0^0 and 20 ^{0}C correspondinly. The same regularity is revealed for intestinal mucosa alkaline phosphatase in different fish species with similar type of feeding. It is known, that interspecies differences of V values are similar to those of enzyme activity (Ugolev & Kuz'mina, 1993a,b).

Thus, many characteristics of fish digestive enzymes are fine adapted to the spectrum, biochemical food composition, motive feeding activity and feeding intensity as well as to environment temperature of active exogenic fish feeding. This phenomenon is observed for various taxonomic groups of marine and freshwater fish during all its history life. In spite of intraspecies variability in some of them mean values are effective indicators of feeding ecology of wild fish. Composition of different enzyme characteristics let get more complete information about this important problem.

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