EFFECT OF TEMPERATURE ON THE DIGESTIVE ENZYMES OF CHANNEL CATFISH ICTALURUS PUNCTATUS (RAFINESQUE)

by

SHAFIUDDIN AHMED ASADI

B.Sc., Osmania University, India, 1955

B.V.Sc. & A.H., Osmania University, India, 1962

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industries

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1967

Approved by:

Major Professor

TABLE OF CONTENTS

PAGE

INTRODUCTION..... 1

LITERATURE REVIEW..... 1

Studies with Warm-Water Fishes..... 2

Studies with Cold-Water Fishes..... 11

METHODS AND MATERIALS..... 15

Peptic Activity..... 16

Extraction..... 16

Substrate..... 17

Method..... 17

Tryptic Activity..... 18

Extraction..... 18

Substrate..... 18

Method..... 19

Amylolytic Activity..... 19

Extraction..... 19

Substrate..... 19

Iodine Method..... 20

DNS Method..... 20

RESULTS..... 20

EXPERIMENT I..... 21

Peptic Activity..... 21

Tryptic Activity..... 24

Amylolytic Activity..... 26

EXPERIMENT II..... 34

Peptic Activity..... 34

Tryptic Activity..... 35

Amylolytic Activity..... 35

DISCUSSION..... 37

Peptic Activity..... 41

Tryptic Activity..... 42

Amylolytic Activity..... 44

Optimum Temperature..... 44

CONCLUSIONS..... 45

LITERATURE CITED..... 46

ACKNOWLEDGEMENTS..... 52

APPENDIX..... 53

INTRODUCTION

Fish farming is a rapidly growing industry. There are relatively few warmwater species of fish cultured for food. Since channel catfish are adapted to culturing and a principal species raised for sport fishing and commercial production, their management require detailed knowledge. Parman (1967) suggested the need for more knowledge of the ecological factors involved in maintaining stocks for impressive gains. He further wrote, the fish digest their food entirely through a set of powerful enzymes, and the gut may reflect the environment of fish. Though he was writing about the fishes of the sea it would also seem to be true for warmwater fishes. Underhill (1952) suggested a great deal of research was necessary to prove the following speculation. "It would appear there is a certain temperature at which freshwater fishes of the temperate zone digest their food at a maximum rate and attain a maximum growth and utilization".

It has long been suggested that environmental temperature affected fish activity, food consumption, utilization of food and hence the rate of growth of the individual. Little is known, however, regarding the mechanism of this effect.

The present studies were designed to determine the presence of various digestive enzymes and the affects of different temperatures on the secretion and/or activity of these enzymes.

LITERATURE REVIEW

Little experimental work has been reported on the digestive physiology of fishes, and in particular the channel catfish (Ictalurus punctatus,

Rafinesque).

Warm-water Fishes

Spallanzani (1783) studied digestion in fishes by filling tubes with flesh of fishes, and putting the tubes into the stomach of pikes, eels, barbles and carp. These were then recovered at intervals and observations on digestion were made. Sullivan (1907) reported a marked increase in volume and acidity of the gastric juice following feeding of elasmobranchs (Chondrichthyes). He found pancreatic extracts contained trypsin in an inactive form, which could be activated by extracts of the intestines. Lipase activity was also reported. Herwerden (1908), Vonk (1927 and 1929) and Bayliss (1935) reported acidity in teleost stomachs indicating digestive processes.

Riddle (1909) used Spallanzani's method to determine the influence of season and temperature on the rate of digestion in six species of cold-blooded vertebrates. The species studied included freshwater "dogfish" (Amia calva). The rate of digestion was measured in 150 individuals, using Mett tubes made from glass tubing, 2.5 millimeter in diameter, filled with goagulated fresh egg albumin. The tubes were inserted directly into the stomach. The animals were killed after periods ranging from 17 to 168 hours. The tubes were then recovered and the amount of digestion was estimated from the number of millimeters of albumin that had been digested from the tube. Riddle concluded that all animals studied were capable of digestion at all seasons provided they were held at a suitable temperature. A smaller amount of proteolytic ferment was secreted during the fasting (winter) period than during feeding (midsummer) period. He also concluded that temperature

markedly modified the rate of digestion and the minimum and maximum temperatures at which digestion was possible varied in different forms of cold-blooded vertebrates. Pepsin secretion decreased with low temperatures. High temperatures might also diminish secretion. It was also reported that temperature changes might affect not only the action of the ferment on the hydrolysis of food materials, but also affected factors conditioning the digestion rate such as the acidity of the secretion and the rate of absorption. Riddle suggested that effects of temperature on digestive processes must be considered under two headings: (1) accelerating action of increased temperature on chemical processes involved, and (2) retarding action of high or low temperatures due to production of smaller amounts of digestive enzymes or actual destruction of enzymes at extreme temperatures. Van Slyke and White (1911) used dogfish for protein digestion studies in the stomach and intestine. Chopped beef meal, boiled to coagulate the protein, was fed through a stomach tube. The authors found that during the first six hours a considerable amount of coagulated protein was dissolved and absorbed from the stomach. Little or no food passed into the intestines, since it contained only slightly more nitrogen matter than the intestines of starving dogfish. During the period from 6 to 12 hours after feeding protein passed, both digested and solid, into the intestine and the progressive hydrolysis of dissolved peptones continued. Pentapeptides, resulting from gastric digestion, were broken on an average to the tri-peptide stage in the intestine. After 24 hours 40 to 45% of the total nitrogen had disappeared and of that left, in both stomach and intestine, 65 to 85% was in solution and consisted of materials averaging midway between di- and tri-peptides. During the second 24 hours the disappearance of both soluble and insoluble protein

continued. By the end of the third day, solution and absorption of protein was practically complete.

Almy's (1926) studies indicated that there was a peptic enzyme acted in the presence of HCl in herring (Clupeidae). Dobreff (1927) found free HCl in the stomach of fasting elasmobranchs (Chondrichthyes). The amount was increased following feeding. Hathaway (1927) studied pumpkinseed (Lepomis gibbosus), bluegill (Lepomis marochirus), and largemouth black bass (Micropterus salmoides). His results indicated these fishes consumed about three times as much food per day at 20°C as at 10°C. Babkin and Bowie (1928) reported evidence for the presence of trypsin in liver and bile of Fundulus. Markus (1932) reported the rate of metabolism in largemouth bass (Micropterus salmoides) and smallmouth bass (M. dolomieui) was low at 4°C and increased up to 22°C. After this temperature was reached the rate of increase of metabolism decreased. Large bass did not take food voluntarily when temperature was below 10°C during the 90 days of the experiment. These workers also reported bass (Micropterus) did not take food readily unless the stomach was empty. Maloney (1949) reported a relationship between food consumption and temperature in bluegill (Lepomis marochirus). Bluegills subjected to eight-day periods at five different temperatures, ranging from 10°C to 30°C had an optimum temperature for feeding between 15° and 25°C. Similarly, Oscar and Day (1950), reported feeding was determined to a large extent by both oxygen supply and temperature, because both directly affected metabolism. They also indicated the usual temperature for different tropical species ranged from about 70 to 80°F. Probst's (1950) 32 years of research showed the yield of carp (Cyprinus carpio) ponds was positively correlated with average temperature during the growing period from May to September.

Gastric secretion in mammals, as reported by Babkin (1950), is controlled through a combination of nerves and hormonal paths, but little is known about the mechanism in fish. The effect of temperature on food consumption in fishes with special reference to the brown bullhead (Ictalurus nebulosus) was reported by Underhill (1952). Three fish were maintained at constant temperature of 20 ± 0.5°C. Others were held at constant temperatures of 15, 18, 22, and 28°C respectively for four weeks. The study included effects of temperature on food consumption, weight gains and utilization. Weight gain increased as the temperature was held at from 15° to 22°C and then decreased at high temperatures with the exception of one fish made greater gains at 28°C than 25°C. Underhill concluded the optimum temperature for the brown bullhead (Ictalurus nebulosus) for utilization of food and growth was close to 22°C and suggested the same temperature for bluegill (Lepomis marochirus). Maximum gain was obtained at 22°C although more food was consumed at 25°C. At 15°C little physical activity was recorded. Activity was greater the higher the temperature. Chen (1954) recommended that Tilapia mossambica should not be stocked unless the water temperature was 15°C. When water temperature rises to 32° or higher, feeding should be stopped, and feed should be supplied only in late afternoon when the water cools down. Sullivan (1954) reported both bony fish and selachians to have surface thermal receptors. According to him, impulses from ampullae and the lateral-line organs are poured into the central nervous system at a rate which was characteristic of the temperature of the environment. Further, he suggested the frequency of spontaneous movements was related to the equilibrium temperature that was greatest at the temperature ordinarily selected by the fish if placed in a temperature gradient. Gibson and Hirst (1955) found growth of

preadult guppies (Lebistes reticulatus) to be in the neighbourhood of 23 to 25°C with less growth at temperatures of 20, 30, and 32°C. Sarig (1956) reported that carp (Cyprinus carpio) stopped feeding and growth was retarded below 12°C. As the temperature approached zero some carp lost weight. Yashouv's (1956) work supported others in which feeding balanced supplementary foods developed carp without natural food. The extent of growth depended on external conditions such as temperatures, and water flow.

In feeding studies with one year old carp (Cyprinus carpio) on colored food, Maltzan (1957), found that time of passage through the alimentary canal varied from 18 hours at 10°C to 4½ hours at 26°C. He suggested that absorptive processes were influenced by rate of food passage through the alimentary canal. Rate of passage was slower in fish than in some mammals. Maltzan suggested much of the difference was doubtless associated with temperature, which has marked effect upon the rate of activity of the canal. Kinne (1960) reared F_1 generation of wild desert pup-fish Cyprinodon macularis baird and girard (Lepomis bumilis) at environments of 15, 20, 25, 30, and 35°C each. Growth, food intake, and food conversion were studied. He found growth rate depended largely on temperature. Fastest growth rate was observed at 30°C and decreased with decreases in temperature. Food intake of fish of the same age was maximum at 30°C. Food conversion was maximum at 20°C and decreased with increases in temperature. Optimum growth in older fish, as reported, was between 22°C and 26°C. Yashouv (1960) reported Tilapia nilotica died at 5°C while at 8°C they entered a cold stupor. Growth began at 13°C and at 19°C and higher temperatures spawning began. He reported T. falliaea was more sensitive to cold than T. nilotica. The effect of temperature on metabolic activities such as spawning was also reported by other workers.

Patience (1961) found temperature to be an important factor in spawning of the shiner (Luxilus cormitis). Spawning did not occur until the water temperature was 66 to 70°F. The minimum temperature at which fry of largemouth black bass (Micropterus salmoides) feed was near 15.9°C (Strawn, 1961). Growth rate at 15°C was slow, and increased with increase in temperature. Maximum growth occurred at 27.5 and 30°C while at 15°C eggs hatched and fry rose from the nest. Few fry fed at 15°C and most died of starvation.

Moss and Scott (1961) studied dissolved oxygen requirements of three species of fish found higher standard metabolic rates in channel catfish (Ictalurus punctatus) than in bluegill (Lepomis marochirus) at 25°C. Their studies at three different temperatures, 25, 30, and 35°C did not indicate significant differences between standard metabolic rates at the temperatures studied. Gyula (1962) measured rates of gastric digestion of largemouth black bass (Micropterus salmoides) at 5, 10, 20, and 25°C using X-ray techniques. He found the rate of digestion was more rapid at 5 to 10°C than at 20 and 25°C. Hickling (1962) wrote that fish have a wide range of enzymes associated with the wide range of food they consume. He further stated that environmental conditions also effect supplementary feeding since appetite increased as the temperature rose. He reported carp (Cyprinus carpio) may stop feeding at temperatures below 10°C and Tilapia melanopleura ceased to feed at about 13 to 15°C. In Tilapia feeding activity was high at 24 to 25°C. Hickling suggested variations in the rate of feeding at different temperatures might be associated with activity of the digestive enzymes. The products from ingested food broken down by the digestive enzymes of fish are absorbed through the gut into the tissue of fish. Feeding was most active and fish were hungry when the water was warm during July and August in temperate

climates. Hickling suggested that water temperature was important to all activities of living creatures. Growth of all fish slowed down to a standstill well above the freezing point of water. He stated that wide variations in seasonal temperature may result in a like seasonal variation in the rate of growth. Tiemeier, Deyoe and Wearden (1964), and Deyoe and Tiemeier (1966) have fed supplemental pelleted feed to channel catfish (Ictalurus punctatus). In these studies the rate of growth in channel catfish was indicated as being affected by various factors including temperature. Growth rate in channel catfish decreased with a marked drop in the water temperature. Fish during periods of low temperatures had feed in their digestive tracts but were apparently unable to utilize it efficiently. Better growth and conversion was obtained at higher temperatures and during extended periods of water temperature above 65°F. The authors recommended feeding of fish from April to September or when the water temperature was above 60°F. Other studied (Deyoe and Tiemeier, 1956) showed that channel catfish did not efficiently utilize feed when water temperature dropped in August. Marked changes in water temperature, particularly sharp drops, reduced both growth and feed conversion rates. Morris (1965) calculated accurately measured metabolic rate-temperature curves over the range of 7 to 27°C after acclimation to 12, 17, 22, and 27°C. He reported body size to be a significant factor in determining the metabolic rates at different intermediate temperatures. No size-related differences were found in acclimation abilities. Goronagase (1965) studied digestive enzymes in Tilapia mossambica and the effects of diet on their activity. Three groups of six fish each revealed that amylase activity on high carbohydrate diets was greatest in the intestine, less in the oesophagus and least in the stomach. The tryptic activity was

greatest in the intestine of fish on high protein diets. Ranade and Kewalramani (1966) studied the rate of food passage in the intestine of three species of fish, L. roluta, C. migala, and C. catla. The differences in concentration of proteolytic enzymes were slight. The relationship between the rate of food passage and the concentration of digestive enzyme was concluded to be of great significance in fish biology. Food items which pass rapidly through the intestine may be more efficiently utilized, because rapid passage of food was normally associated with high digestibility. High concentrations of the requisite enzyme were also reported under these conditions. Moreover, the opinion was expressed by these workers that a rapid passage might also lead to increase in the rate of feeding. The usefulness of different food items depends upon their digestibility.

Tiemeier, Deyoe and Wearden (1966) studied nutritional qualities of four diets fed to channel catfish (Ictalurus punctatus). Feed was supplied as a dry feed from June to October. Extreme high temperatures and sudden decreases in temperature were not encountered. The conversion rates for the diets, as reported, were more uniform than those obtained in 1964. Howard (1966) reported the period of incubation of channel catfish (Ictalurus punctatus) eggs to range from 5 to 10 days at temperatures between 70 and 80°F. Dupree and Sneed (1966) fed purified diets containing casein, wheat gluten and soybean protein to channel catfish fingerlings. Weight gain and feed conversion of fish fed diets that contained wheat gluten and soybean protein were greater when fed at a water temperature of 76°F than when fed at 69°F. However casein was utilized almost equally well at both temperatures. Energy requirements as reflected in weight gain were assumed to be greater at 69°F. Dendy, Varikul, Sumawidjaja and Potaros (1966) reported Tilapia mossambica

obtained during July and August contained an abundance of food. There were decreases in, or absence of, foodstuff in the digestive system of fish collected on September 16th and October 10, when the temperature was approximately 15.5°C.

In a study on the relationship between rate of feeding, rate of growth and rate of conversion in Tilapia mossambica and T. nilotica, Shell (1966) reported the rate of feeding and conversion rate depended on many factors including water temperature. General metabolic activities and absorption were increased by a rise in temperature. The same might also be concluded from the work of Wolmy (1966) who reported that rise in temperature stimulated the toxic action of ammonia. Ammonia at 50 mg per liter for a young carp (Cyprinus carpio) weighing 50 g was lethal in 30 minutes at 17°C. At 23°C it was lethal in only 13 minutes. Hepher (1966) also reported that fish growth and food chains are affected by a number of factors including temperature. In Taiwan the fish rearing season is reported to be from early April to mid November, when the temperature ranged from 25 to 33°C (Tang, 1966).

West (1966) studying channel catfish (Ictalurus punctatus) growth, food conversion and survival, maintained 22 groups of fry of 20 each at 21, 23, 25, 27, 29, 31, 33, 34, 35, and 36°C. He reported that 36°C was too high for survival, as all but three fish died at this temperature. Maximum feed efficiency was obtained at 28.9°C. This was slightly lower than the temperature of maximum growth rate, which was 29 to 30°C. Food consumption by catfish continued to increase at temperatures above the optimum though growth decreased. Food consumption per gram of body weight increased with age. Paloheim, and Dickie (1966) reviewed studies concerning the relation between

total metabolism, body weight and its dependence on food intake and temperature, in fishes. The temperature effects in the experiments were estimated from multiple regression analysis. The effect of temperature on general metabolic level was different from different temperature series. Nordlie (1966) conducted studies with the black bullhead (Ictalurus melas) to determine whether secretion of compensating peptic digestive capacities was influenced by thermal acclimation. Crude pepsinogen extracts from entire stomachs of two groups of fish were used. One group was acclimated to 12°C and the other group to 24°C for more than 60 days. The extract from the group of fish acclimated to 24°C showed higher peptic activity than that from 12°C acclimated fish. It was concluded that black bullhead probably have greatest digestive capacity during summer months. During the winter they most likely exist at reduced activity levels, on stored fat and glycogen. Other studies on the effect of temperature on other metabolic activities have been reported in various species of fish, (Swingle, 1966, Clemens, 1964) and indicate a significant temperature effect on metabolic activity.

Cold-water Fishes

Temperature effects on coldwater fishes have also been investigated. Titcomb (1920) reported that temperature plays an important role in the growth of trout (Salmo trutta). Trout grew better at 51°F than at 48°F. Belding (1928) pointed out the influence of temperature on fish life from the standpoint of environment, habits, and activities. Water temperature not only governed the distribution of fish by regulating their physiological activities, but also was intimately associated with their resistance to disease and to adverse conditions of environment such as reduced oxygen

supply and presence of toxic substances in the water. Belding concluded temperature was an essential factor in determining a suitable habitat for fish. Considerable variations in temperature tolerance between different species of fish was reported, and it was suggested that 70 to 80°F was suitable for apparent comfort of brook trout (Salvelinus fontinalis). It was also concluded that growth was governed by the abundance of food and the rate of metabolism of the fish. Trout fingerlings (Salvelinus fontinalis) grew more slowly in cold water than in warm water. Belding defined "optimum temperature" for existence of each species to be that of its greatest resistance to disease. A temperature above optimum lessens resistance to disease and increases death rate.

Embody (1931) found trout (Salvelinus fontinalis) were in great distress and refused to eat when a temperature of 83.3°F was reached. He did not record death losses at this temperature. Evidence of temporary physiological upset was noticed following a considerable drop in temperature. M'Gonigle (1932) found rate of respiration to increase in trout (Salmo trutta) with a rise in temperature to 71°F. It then became irregular but decreased in rate. Dawes (1930) concluded if a second meal was taken at a short interval after the first, the food left the stomach and passed down the alimentary tract more quickly. Pentelow (1939) reported optimum temperature for brown trout (Salmo trutta) of 50 to 60°F. Growth was found to increase as temperature increased from 38 to 50°F, reached a maximum between 50 to 60°F and declined when the temperature exceeded 60°F. Pentelow suggested that fish, like other organisms, need food to provide energy for vital processes such as respiration, digestion and excretion, and other activities. He stated that excess food

over metabolic needs is available for increases in body substances, i.e., growth. Trout in his studied ate more and grew more under warmer conditions (50 to 60°F) than when water was colder (38°F). Above 60°F, however, there was a marked decline in the efficiency of food conversion and since this was accompanied by no increase, and often a decrease, in appetite, the growth rate fell markedly. Between 40 to 50°F growth was found to be roughly proportional to the amount of food, but such a simple relationship was not observed at 50°F.

Investigations made on chinook salmon (Onchynchus tshayscha) between June 1935 and October 1937 were reported by Allen (1940). Growth in salmon was found to take place from early April until late October, but not during winter. Large seasonal variations were reported in the quantity of food found in the stomach of fish. It increased rapidly to a maximum early in the rapid growth period and then fell steadily during the remainder of the summer to a low value which continued during winter. When the temperature was below 7°C, young salmon remained quiet in a deep pool. When the temperature increased they were more active and were present in shallow water with a moderate current. Allen concluded that temperature probably determines whether growth takes place. The change during the summer from rapid to slow growth was suggested as being caused by higher water temperatures (15°C or above), other external causes or possibly by changes within the fish. A relationship between the condition of fish and their growth was also reported by Allen.

Wingfield (1940) studying the effects of environmental factors on growth of brown trout (Salmo trutta) suggested factors influencing growth may be divided into two groups - physico-chemical and biotic. In the first group

he included light, water temperature and chemical constituents of the medium to be most important. He also found the rapid increase during summer to be correlated with a large increase in food intake which reached a maximum about August and diminished during the winter. Brown (1946) grew two-year-old trout (Salmo trutta) under controlled environmental conditions in water of different temperatures. The efficiency of food utilization was low when the temperature was low and also when the activity was high. Food consumed by trout was maximum at 10 to 19°C and the activity of the fish was highest between 10 and 12°C. Maintenance requirements of trout of equal weight increased with temperature. Baldwin (1956) held brook trout (Salvelinus fontinalis) at six temperatures within the range of 3.5 to 21.0°C. Trout consumed the most food and made best growth at 13°C. Utilization of food consumed for growth declined with increased temperature.

Experiments of Allyn (1959) were designed to observe the effects of temperature upon chinook salmon (Onchynchus tshawyscha) during the period from the egg to the fingerling stage. Temperatures ranged from 34 to 74°F. There was no survival to the stage where vertebrae or fin rays could be counted for lots reared at temperatures below 39°F or above 62°F. The lower temperature threshold for normal development of sockeye salmon (Oncorhynchus nerka) eggs was established to be between 55 and 57.5°F. Johnson (1967) concluded the best growth of trout (Salmo trutta), may be expected to occur at temperatures between 55 and 65°F and some portion of the trout pond should offer water temperatures in this range for best production.

Various workers have studied the effect of temperature on, and optimum temperature for, production, spawning, effect on hormone secretion and hormone effects on various species of fish (Surber 1935, Yashouv 1960, Patience

1961, Clemen and Johnson 1964, Howard 1966, and Bolock and Labib 1966, etc.).

Information on the effects of water temperature on digestive activities of fish is needed because feeding activity and other metabolic activity appear to be temperature related. Channel catfish are becoming an important food fish, thus information of this nature is needed to develop sound management and cultural practices. The present studies conducted here were undertaken to investigate the effects of environmental water temperature on the digestive activity of channel catfish.

METHODS AND MATERIALS

Channel catfish used for these studies were obtained from Tuttle Creek Fisheries Research Laboratory. They were Age Class II fish which were moved to a holding tank in the laboratory. They were later transferred to a tank held at a constant temperature in the Department of Grain Science and Industries. For some studies fish were moved directly from the Tuttle Creek pond to the constant temperature tank. The glass tank, measuring $2\frac{1}{2}$ X $2\frac{1}{2}$ X $2\frac{1}{2}$ ft, had a capacity of about 100 gallons. The tank was filled with dechlorinated water, obtained from tap water passed through a dechlorinator and was not changed while the experiment was in progress. The temperature of the tank was controlled by passing a mixture of hot and cold water through a metallic coil and with a thermostatically controlled heating coil. An agitator was also used to aid in keeping the temperature uniform throughout the tank and to supply oxygen. The temperature was maintained within $\pm$ 0.5°C of the designated experimental temperature. All fish were kept for at least 14 hours at constant temperature before they were killed for study. Material collected from each fish was used to determine both stomach and intestinal

proteinase activity. Other fish were used to determine the intestinal amylolytic activity. A fish taken from an outside pond at a temperature of 2.2°C at 1.37 meter depth was used for the study of the pepsin and trypsin activity at this temperature.

The temperature of the glass laboratory tank was adjusted to 14.5°C and fish were transferred to the tank. After 14 hours fish were killed for the study of the proteolytic enzymes of the stomach and intestine. The temperature was then raised to higher constant temperatures. Care was taken to maintain the fish at each temperature level for a minimum of 14 hours before they were killed. Thus all fish were acclimated for 14 hours to the particular temperature being studied. Studies were conducted to determine effects of various temperatures on pepsin, trypsin, and amylase activities. Each experiment was also repeated, after raising the temperature gradually to about 37.7°C (100°F) holding it there for 12 hours and then bringing it down to some specific constant temperature. Thus temperature effects were studied with both increasing (Experiment I) and decreasing (Experiment II) temperatures.

Peptic Activity

Peptic activity was studied in fish taken from water maintained at 2.2, 14.5, 22.2, 23.9, 26.7, 29.4, and 33.3°C. Preliminary experiments were conducted to determine the optimum incubation, pH and temperature for pepsin activity.

Extraction

Entire stomach extracts used for studies on pepsin activities, were

excised immediately after killing fish. The stomachs were cleaned of contents and sliced at once with a blade and scalpel, as described by Elliott (1955). The stomachs were immediately frozen whenever delays in study were unavoidable. The sliced stomach tissue was extracted with 25 ml of glycine-HCl buffer (Gomori, 1955) for each gram of stomach. They were then homogenized for 5 minutes in a blender and stored in a refrigerator at 5°C for 24 hours. The stomach tissue and buffer was then centrifuged and the extract was diluted 1:10 before use.

Substrate

Denatured hemoglobin (Nutritional Biochemical Corporation) was used as the substrate for pepsin activity studies. A 2% solution was prepared in 0.2 M glycine-HCl buffer, and the pH was adjusted to 2.2.

Method

The method of Anson (1938) was used with phenol reagent (Folin and Ciocalteu, 1927), except that a spectrophotometer was used for optical density readings. The substrate solution (5 ml) and extract were placed in a water bath for 5 to 10 minutes to bring the temperature to 33°C. One ml of the enzyme extract was then added and incubated with the substrate. Trichloracetic acid (0.3 N, 10 ml) was added and the tubes were shaken vigorously to stop the reaction after both 5 and 10 minutes of incubation. Two sample tubes were used for each period of incubation. The reaction mixture was filtered through Whatman No. 3 filter paper. Five ml of the digestion filtrate was added rapidly to 10 ml of 0.5 N sodium hydroxide and 3 ml of the diluted phenol reagent. The mixtures were placed in a spectrophotometer and readings

taken at 570 mμ; water was used as a blank. Tests were conducted in duplicate, and a separate blank was run with each tube. The absorbancy of the developed color after correcting for the blank reading was used to estimate the activity of the enzyme.

Tryptic Activity

Tryptic activity was studied at the same temperature as peptic activity. Preliminary experiments were also conducted to find the optimum pH for incubation and the optimum temperature for studies of tryptic activity.

Extraction

The proteinase was extracted from the whole intestine as described for pepsin extracts, except for the use of a phosphate buffer of pH 6.9. The buffer was prepared as described by Gomori (1955).

Substrate

The hemoglobin substrate was prepared in a manner similar to that described previously (Anson, 1938). Eight ml of 1 N sodium hydroxide, 72 ml distilled water, 36 g urea, and 10 ml of 22% hemoglobin were mixed and the mixture was held for 30 to 60 minutes to denature the hemoglobin. The solution was then mixed with a solution containing 10 ml of 1 M potassium dihydrogen phosphate and 4 g of urea. The final pH was adjusted with potassium dihydrogen phosphate to 6.9, the optimum incubation pH found for tryptic activity of the channel catfish.

Method

The precedure used was similar to that for peptic activity. Because urea was in the substrate solution, it was necessary to wait 30 minutes after the addition of trichloracetic acid before filtering the digestion filtrate and the blank. The water-bath was adjusted to 55°C, which was found to be optimum for tryptic activity of channel catfish.

Amylolytic Activity

Amylase activity studies were conducted with extracts from fish held at 15.5, 21.1, 23.9, 26.7, and 27.7°C respectively. In vitro studies were conducted to determine optimum incubation temperature and pH for these studies.

Extraction

The amylase was extracted from intestine in distilled water using 100 ml for each gram of intestine as described by Noerloose (1965). Samples were obtained and treated as described for pepsin extraction. The supernant solution obtained by centrifuging was used without further dilution to determine the activity of amylase.

Substrate

Starch substrate was prepared as described by Oser (1965). For this 0.2 g of sodium chloride, 1.515 g of potassium dihydrogen phosphate and 1.98 g of sodium phosphate were dissolved in distilled water and the final solution was diluted to 500 ml. Two grams of soluble starch was added to 100 ml of the above buffer and boiled for 15 minutes with stirring. It was then cooled and the pH was adjusted to 4.8.

Iodine Method

Two ml of substrate solution was placed in each test tube. The test tube and the enzyme extract were then placed in a water bath adjusted to 55°C. When the enzyme extract and substrate reached the incubation temperature, one ml of enzyme extract was transferred to the substrate tube. The reaction was stopped after 5 or 10 minutes by addition of 1 ml of 1 N HCl. Then 01. ml of iodine solution (Ryzhova, 1965) was added and the contents were diluted with 10 ml of water. The absorbancy was read in a spectrophotometer at 540 mμ (Amador and Wacker, 1965) with water as reference. The percent absorbance after correcting for the blank was used in expressing the activity of the enzyme. A blank and duplicates were prepared for each test.

Dinitrosalicylate (DNS) Method

The starch substrate used in the above iodine method was also used in this test. The method is similar to the iodine method except the reaction was stopped with DNS reagent (Schwimmer, 1950), and the mixture was heated for 10 minutes in a boiling water-bath. A standard curve for reducing sugars was prepared by using different maltose concentrations.

RESULTS

Experiments were conducted to determine the optimum pH and incubation temperature for each enzyme studied. Two experiments using Age Class II fish were conducted for each of the enzymes studied for the effect of environmental temperatures on the enzyme secretion. Experiment I was designed to investigate the effects of increasing temperature, while experiment II conducted for decreasing temperature effects. The results of the experiments

involving stomach and intestinal extracts did not show any apparent difference (Tables 1-7 and Appendix Tables 1-7).

EXPERIMENT I

Peptic Activity

In vitro studies indicated optimum conditions for peptic activity were pH 2.2 and 33°C. The results of these studies are indicated in Figs. 1 & 2. Temperature and pH conditions which differed from these resulted in decreased activity.

The proteolytic activity of pepsin was studied using extracts obtained from fish held at 2.2, 14.5, 22.2, 23.9, 26.7, 29.4 and 33.3°C respectively. The fish used for studies at 2.2°C were obtained directly from an outside pond during January 1967. The results of studies on pepsin activities are shown in Table 1, and Appendix Table 1.

Table 1. Peptic Activity of Channel Catfish at Constant Environmental Temperatures - Expt. I.

Temperature Deg. C.	Activity			
	5 min.		10 min.	
	No. of Samples	Net Activity*	No. of Samples	Net Activity*
2.2	2	0.14	2	0.24
14.5	2	0.34	2	0.54
22.2	2	1.03	2	1.55
23.9	2	1.21	2	1.645
26.7	2	0.9	2	1.33
29.4	2	0.62	2	1.025
33.3	2	0.2	2	0.53

*(Average of two samples - Blank) P.C. absorbance.

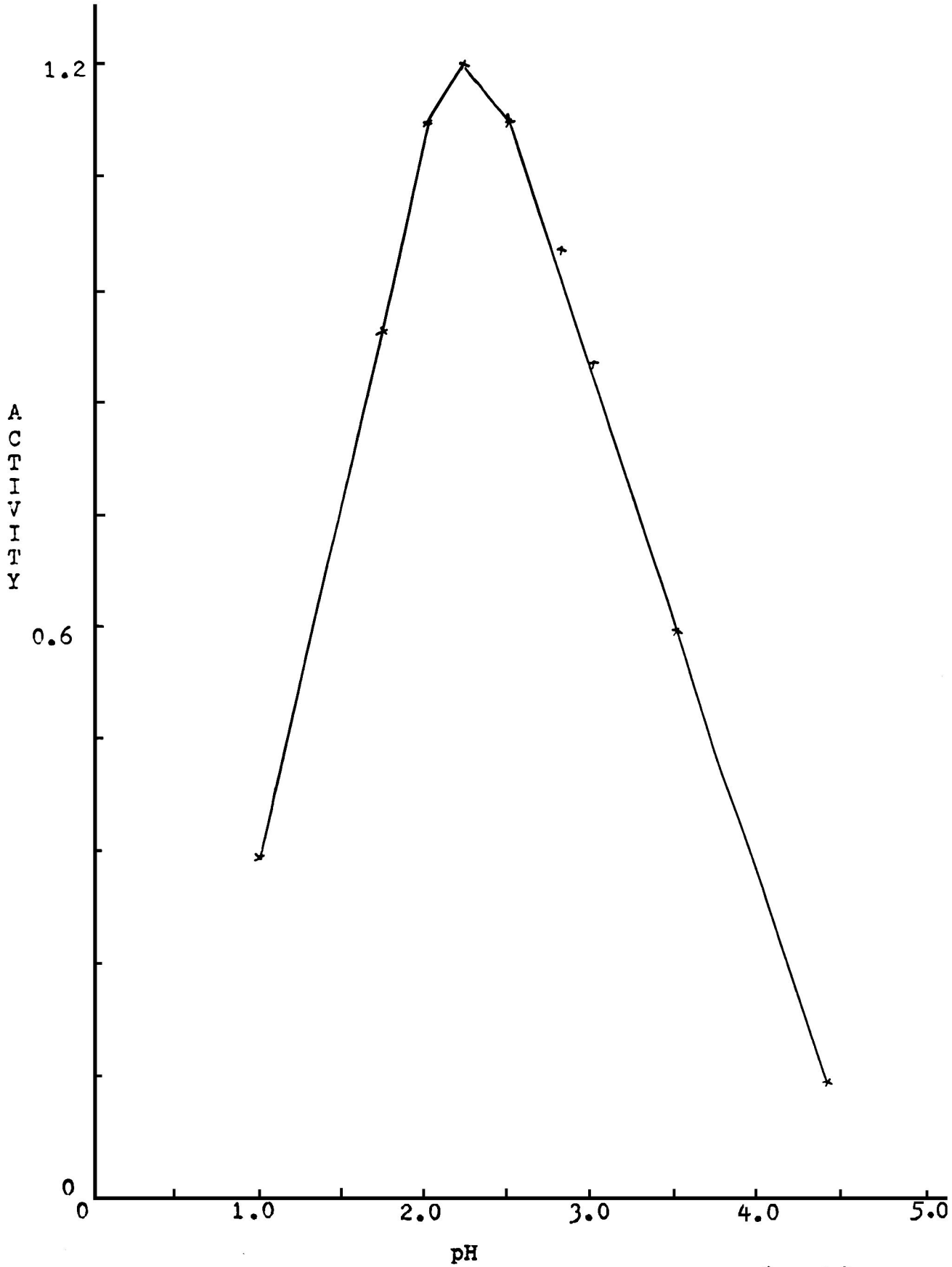

Figure 1 Optimum pH curve for the stomach extract (pepsin).

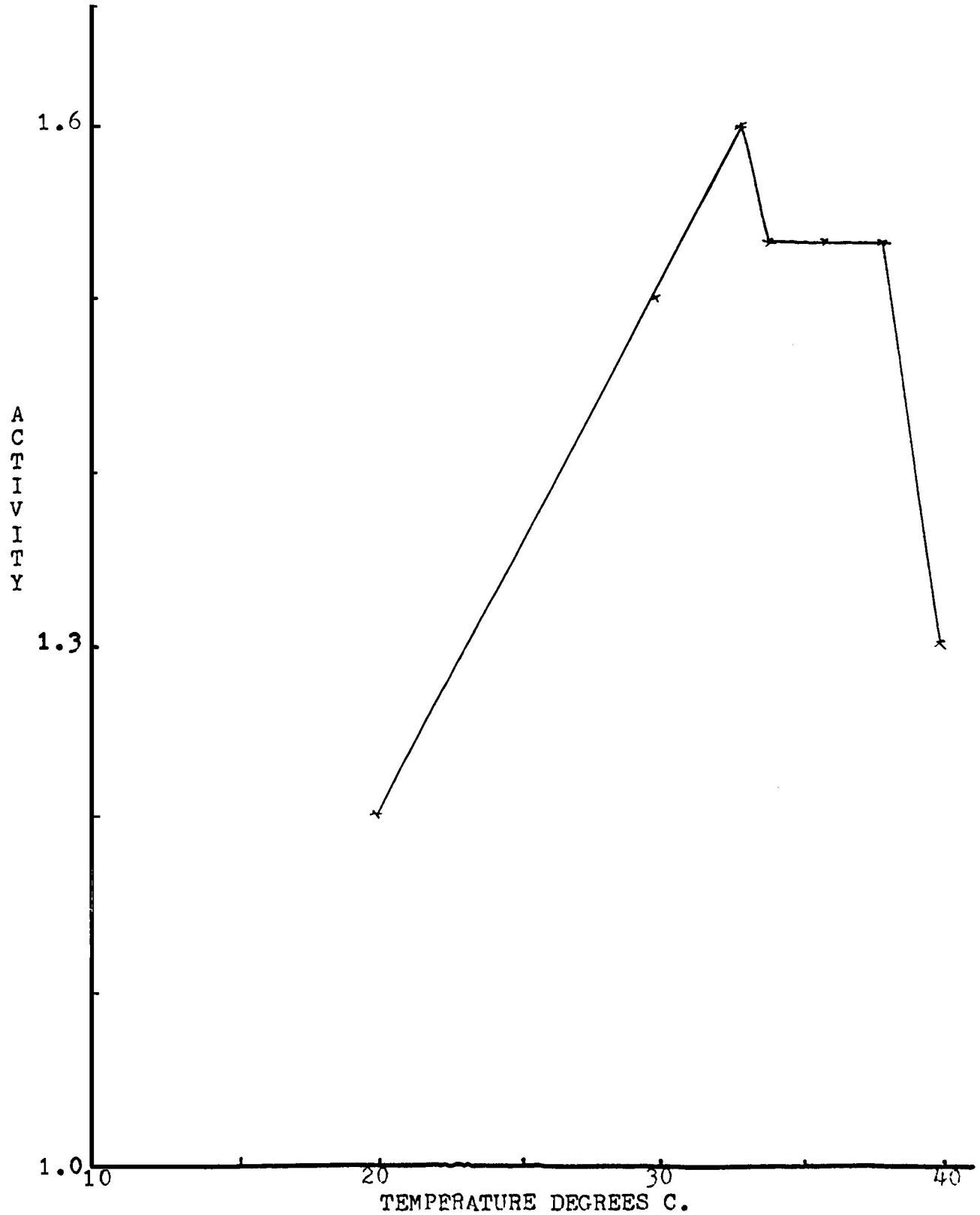

Figure 2 Optimum temperature curve of the stomach extract (pepsin).

After checking the tank for satisfactory performance of the control at 14.5°C seven fish were transferred to the aquarium. After about 14 hours at this temperature one fish was killed. The proteinase activity of the stomach extract was measured, as described above. The average activity of duplicate tests was 0.54 O.D. units, following 10 minutes reaction time. The fish were then fed with turkey liver, and after 14 hours one more fish was examined for activity. The activity appeared to have increased slightly and gave a reading of 0.55 O.D. units. Little food was found in the stomach. The remaining liver pieces were removed from the tank and in other tests fish were not fed. In subsequent tests fish were killed after being held at constant temperature settings of 22.2, 23.9, 26.7, 29.4, and 33.3°C respectively for minimum of 14 hours. The proteinase activity (Table 1, and Fig. 3) was increased to 23.9°C, above which the activity decreased. At temperatures above 26.6 to 29.4°C the fish appeared to be in a state of distress.

Tryptic Activity

The results of preliminary *in vitro* studies conducted to determine the optimum pH and temperature for trypsin activity measurements are indicated in Figs. 4 and 5. The optimum pH was found to be 6.8 to 6.9 and the optimum temperature near 55°C for tests on trypsin activity.

Extracts of whole intestine from fish used for pepsin studies were also used for estimation of trypsin activity. Enzyme activity was not observed at 2.2°C following 10 minutes of reaction time, but after 20 minutes slight activity (0.001 O.D. units) was noted (Table 2 and Appendix Table 2). At 14.5°C the reactivity was 0.001 O.D. units after 10 minutes and 0.002 O.D. units after 20 minutes. After feeding turkey liver to the fish at this

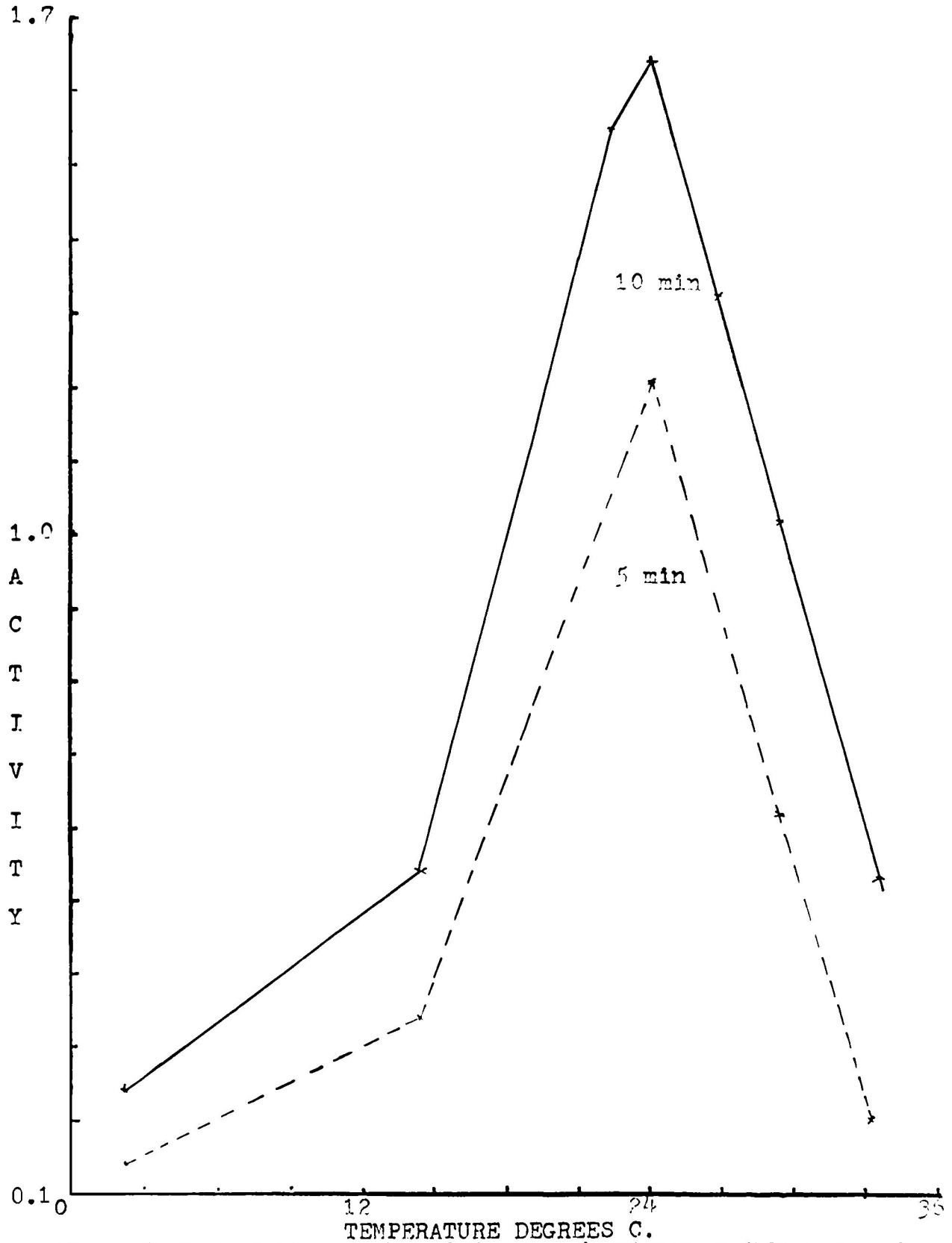

Figure 3 Proteolytic activity of the stomach extract at different environmental temperatures.

temperature a little increase in the activity was found, 0.005 O.D. units following 20 minutes of reaction time. The trypsin activity increased with increase in environmental temperature to 23.9°C and then decreased at temperatures above 23.9°C (Fig. 6). Significant activity was still present at 33.3°C.

Table 2. Proteolytic activity of the Intestinal Extract of the Channel Catfish at Constant Temperatures - Expt. I.

Temperature Deg. C	Activity			
	10 min.		20 min.	
	No. of Samples	Net Activity*	No. of Samples	Net Activity*
2.2	2	-	2	0.0010
14.5	2	0.001	2	0.0015
22.2	2	0.440	2	0.6400
23.9	2	1.550	2	1.6600
26.7	2	0.325	2	0.5100
29.4	2	0.120	2	0.2000
33.3	2	0.070	2	0.1200

*(Average of two samples - Blank) P.C. absorbance.

Amylolytic Activity

The optimum *in vitro* pH for amylase activity was 4.8 (Fig. 7) and the optimum temperature was 55°C (Fig. 8).

In studies on amylolytic activity, an initial water temperature of 15.5°C was used. Six fish were transferred to the tank and after about 14 hours a fish was killed. The temperature was then raised to the next level to be studied and after 14 hours at this temperature a fish was killed. The

same procedure was followed for each temperature studied, i.e., 21.1, 23.9, 26.7, and 29.4°C.

Whole intestinal extracts were used for amylase studies and the iodine method of Ryzhova (1965) was used for amylase activity measurements. The activity at 15.5°C was 0.02 and 0.07 O.D. units following 5 and 10 minutes reaction times respectively (Table 3 and Appendix Table 3). The activity increased to 0.05 and 0.1 O.D. units following 5 minutes reaction and 0.09 and 0.16 O.D. units following 10 minutes reaction time at 21.1, and 23.9°C respectively. It then dropped to 0.02 and 0.05 O.D. units at 26.6°C water temperature. The highest activity, 1.57 and 1.66 O.D. units after 5 and 10 minutes respectively, was found at 23.9°C (Table 3 and Fig. 9).

Table 3. Amylolytic activity of the intestinal extract of channel catfish at constant environmental temperatures. (Iodine Method) - Experiment I.

Temperature Deg. C.	Activity			
	5 min.		10 min.	
	No. of Samples	Net Activity*	No. of Samples	Net Activity*
15.5	2	0.020	2	0.070
21.1	2	0.050	2	0.090
23.9	2	0.100	2	0.160
26.7	2	0.025	2	0.050
27.7	2	0.024	2	0.050
29.4	2	0.030	2	0.035

*(Blank-Average of two samples) P.C. absorbance.

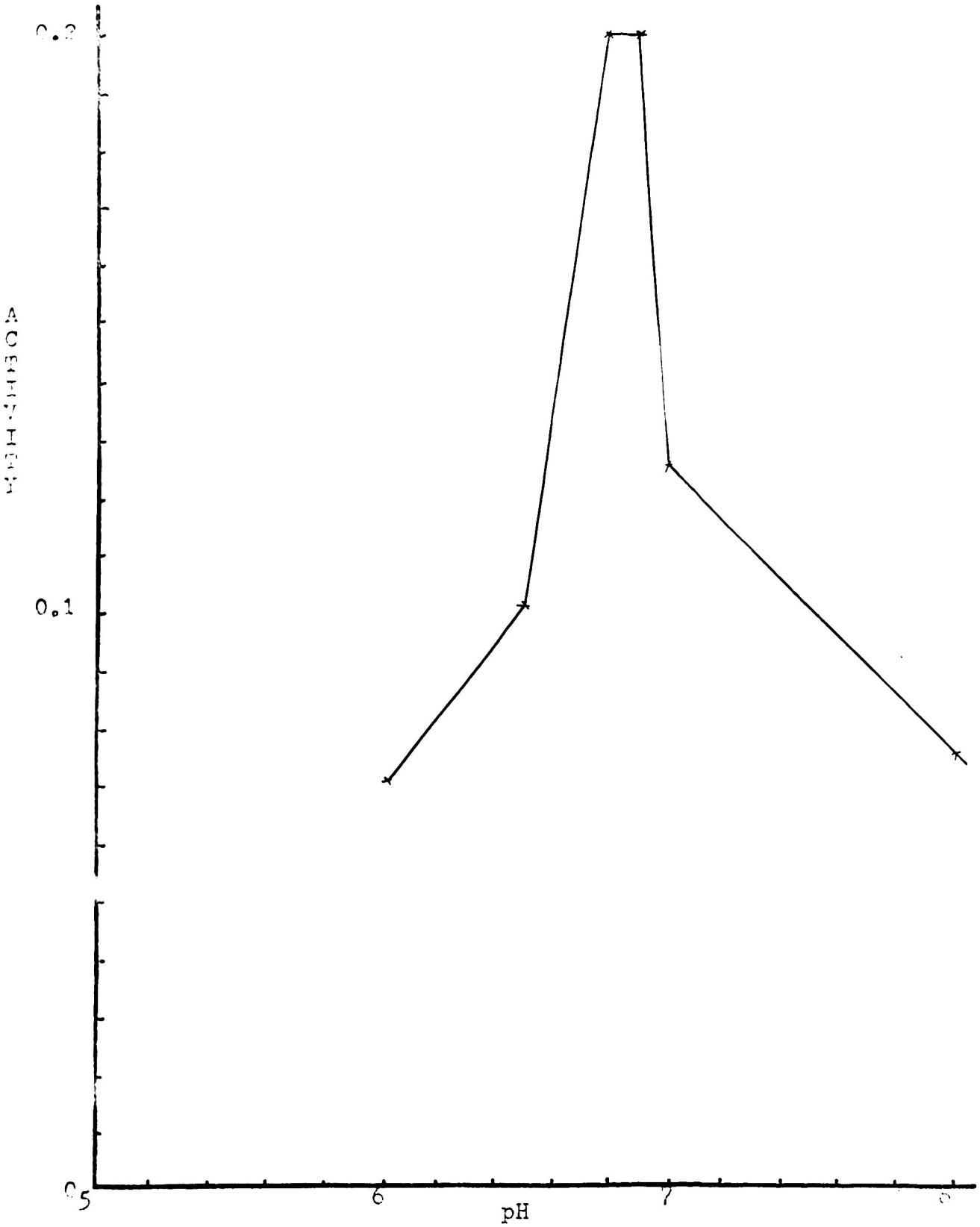

Figure 4 Optimum pH curve for the proteinase activity of the intestinal extract.

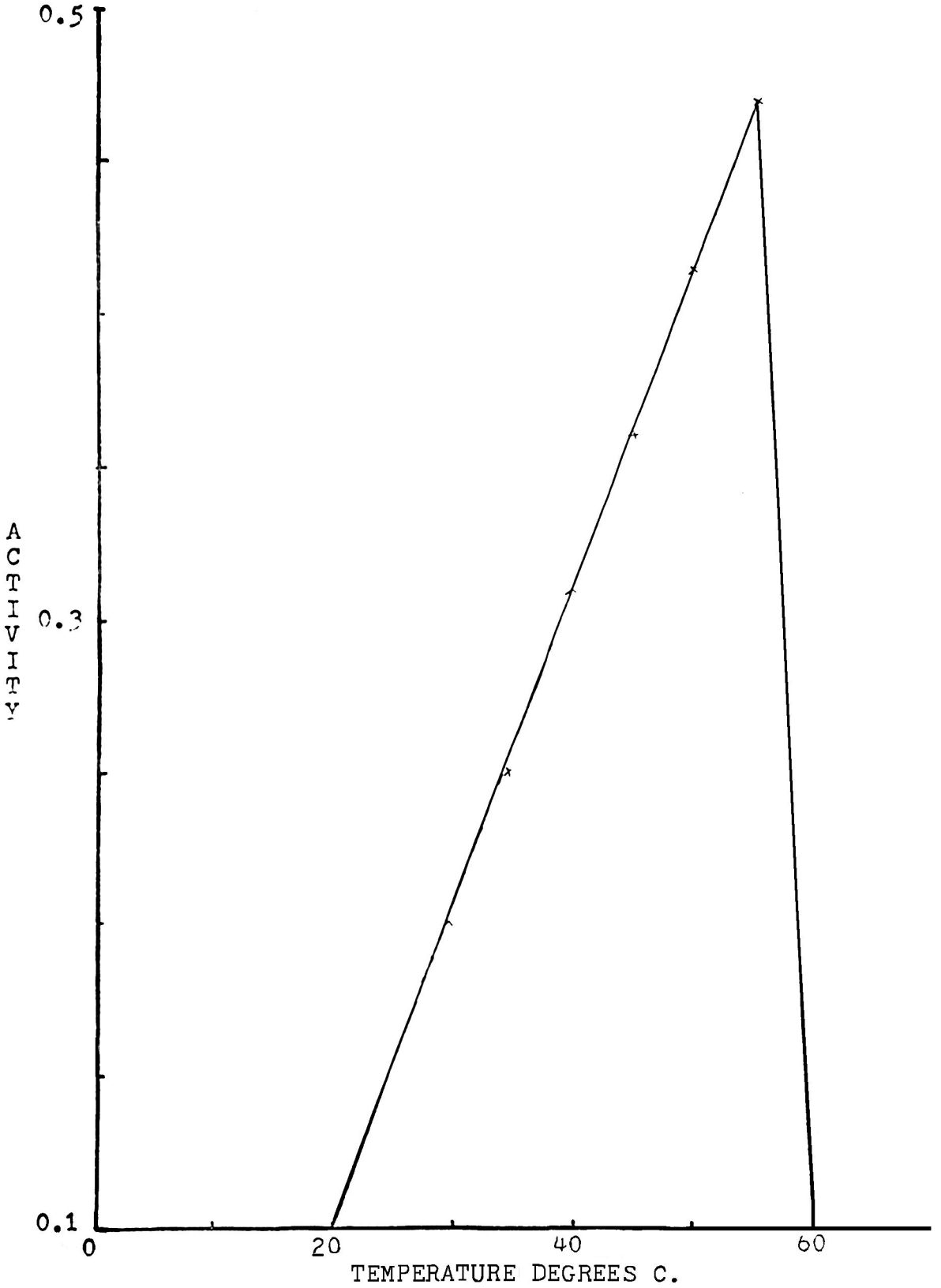

Figure 5 Optimum temperature curve for the proteinase activity of the intestinal extract.

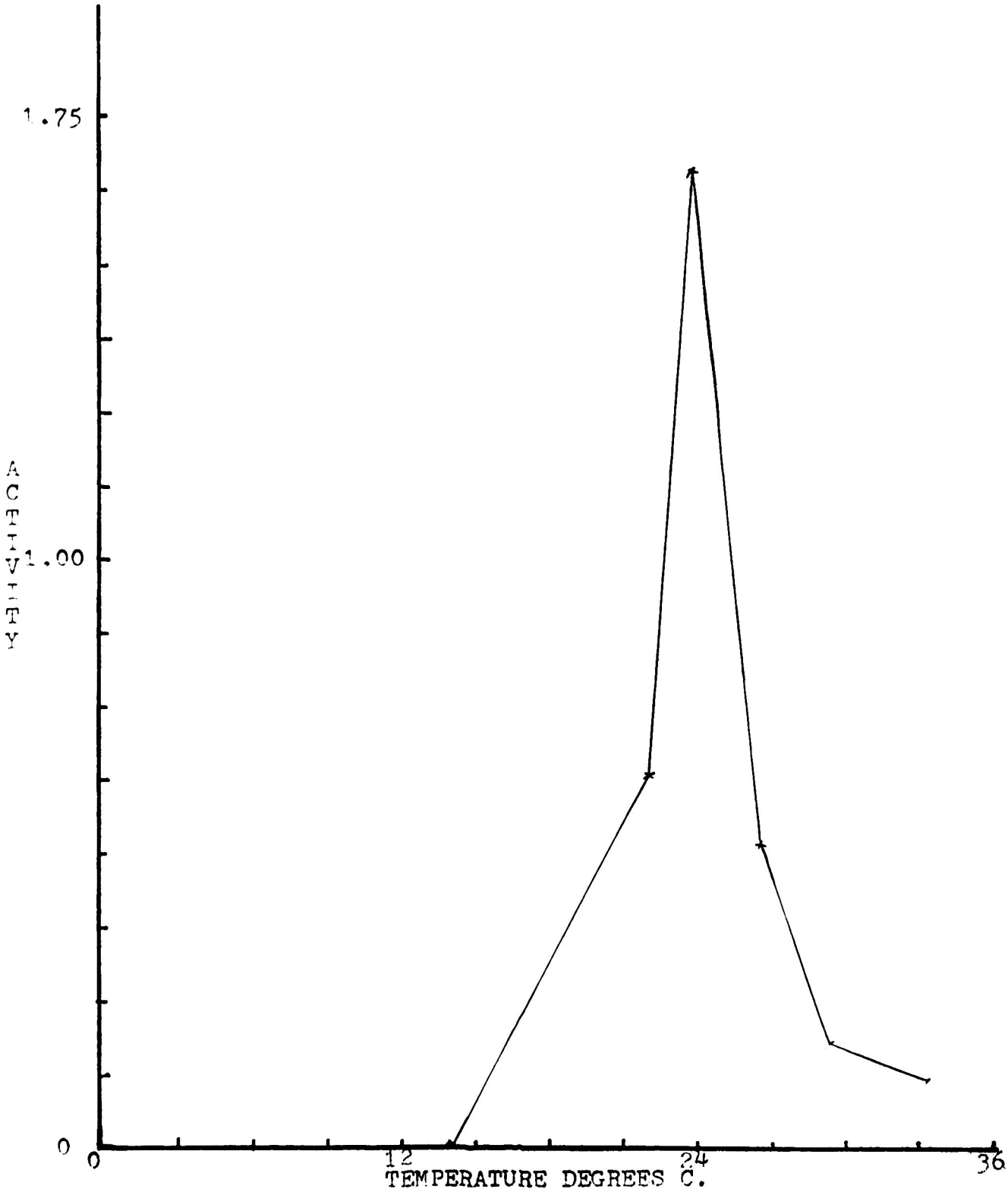

Figure 6 Proteolytic activity of the intestinal extract at different environmental temperatures.

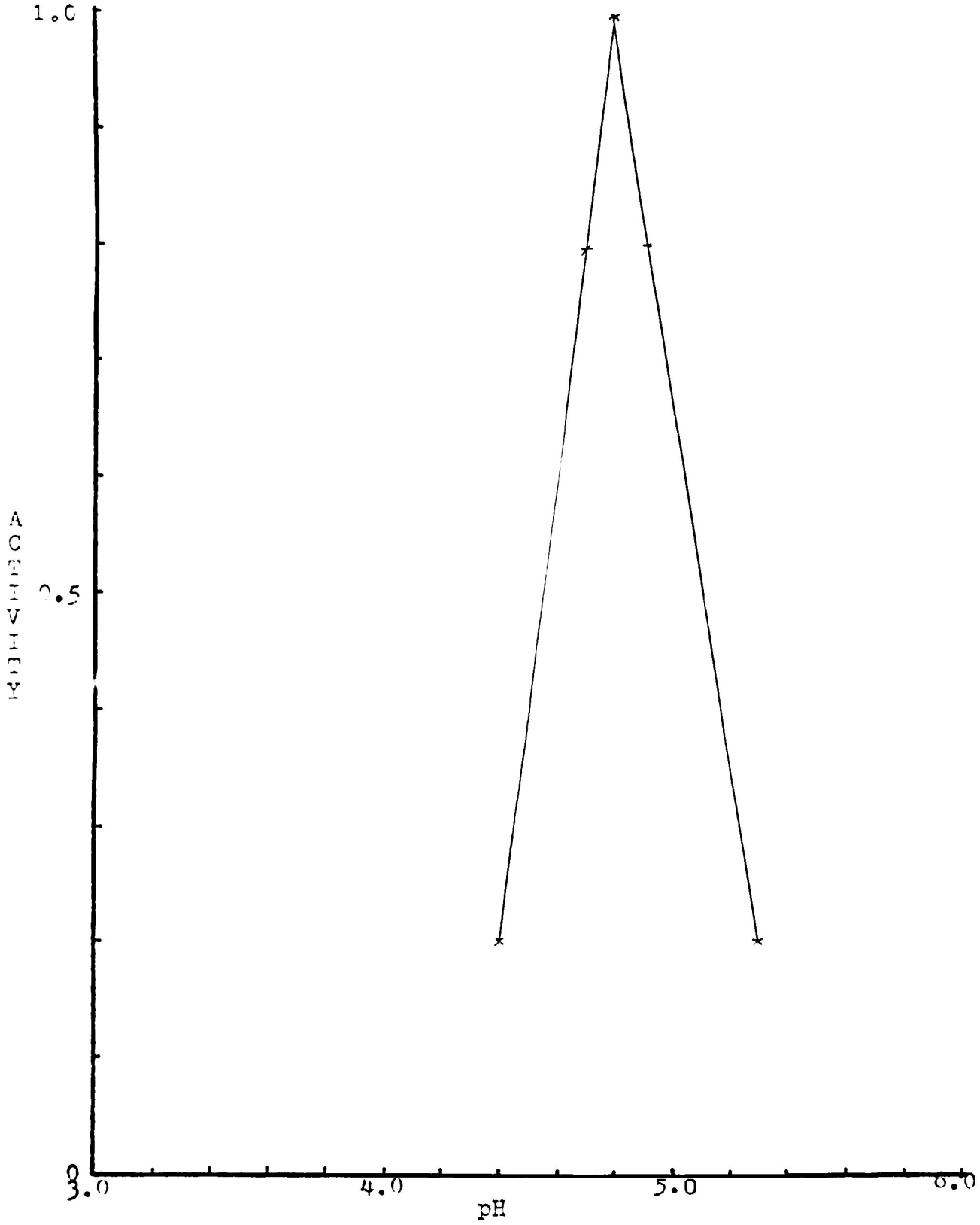

Figure 7 Optimum pH curve for the amylolytic activity of the intestinal extract.

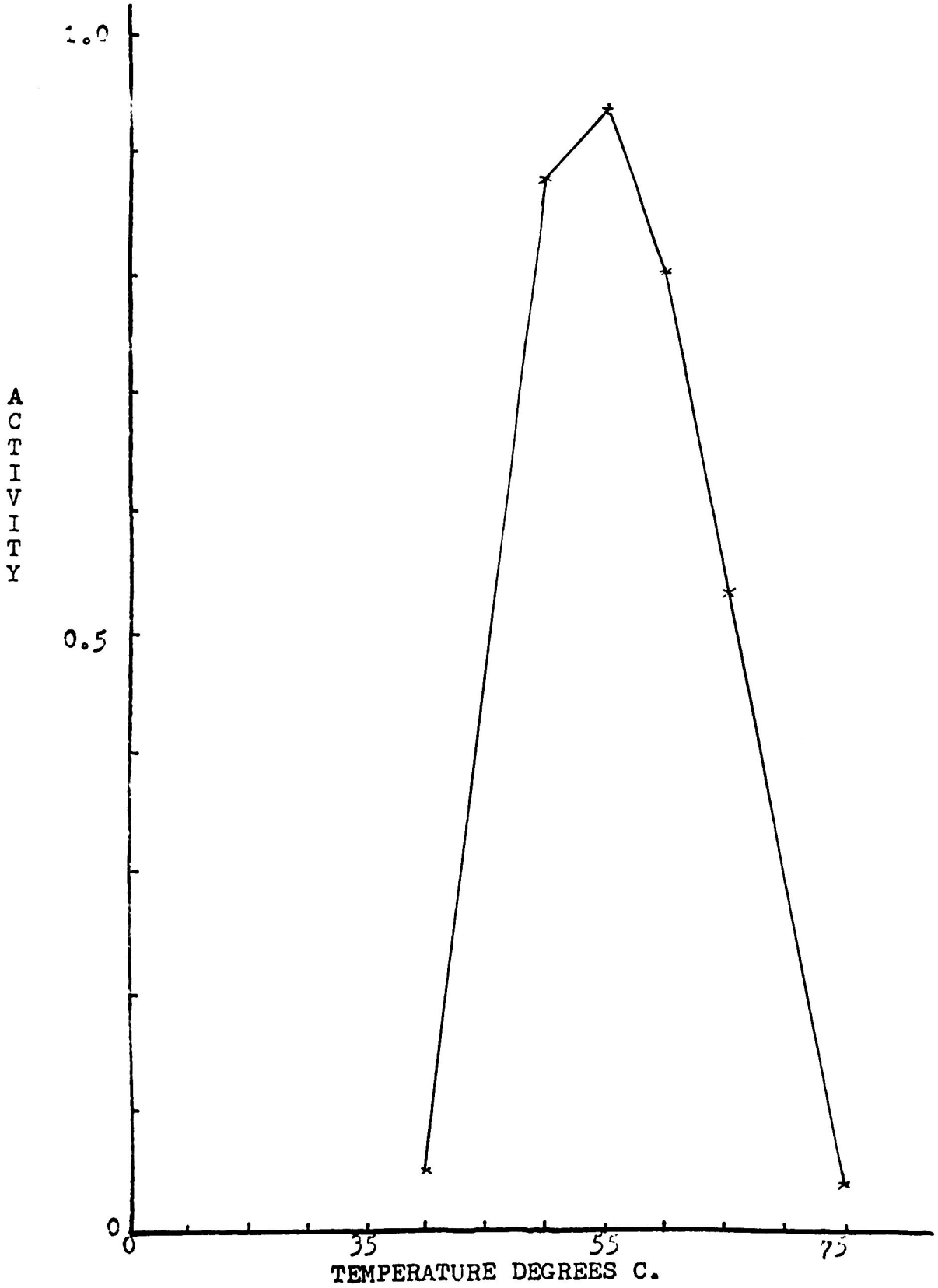

Figure 8 Optimum temperature curve for the amylolytic activity of the intestinal extract.

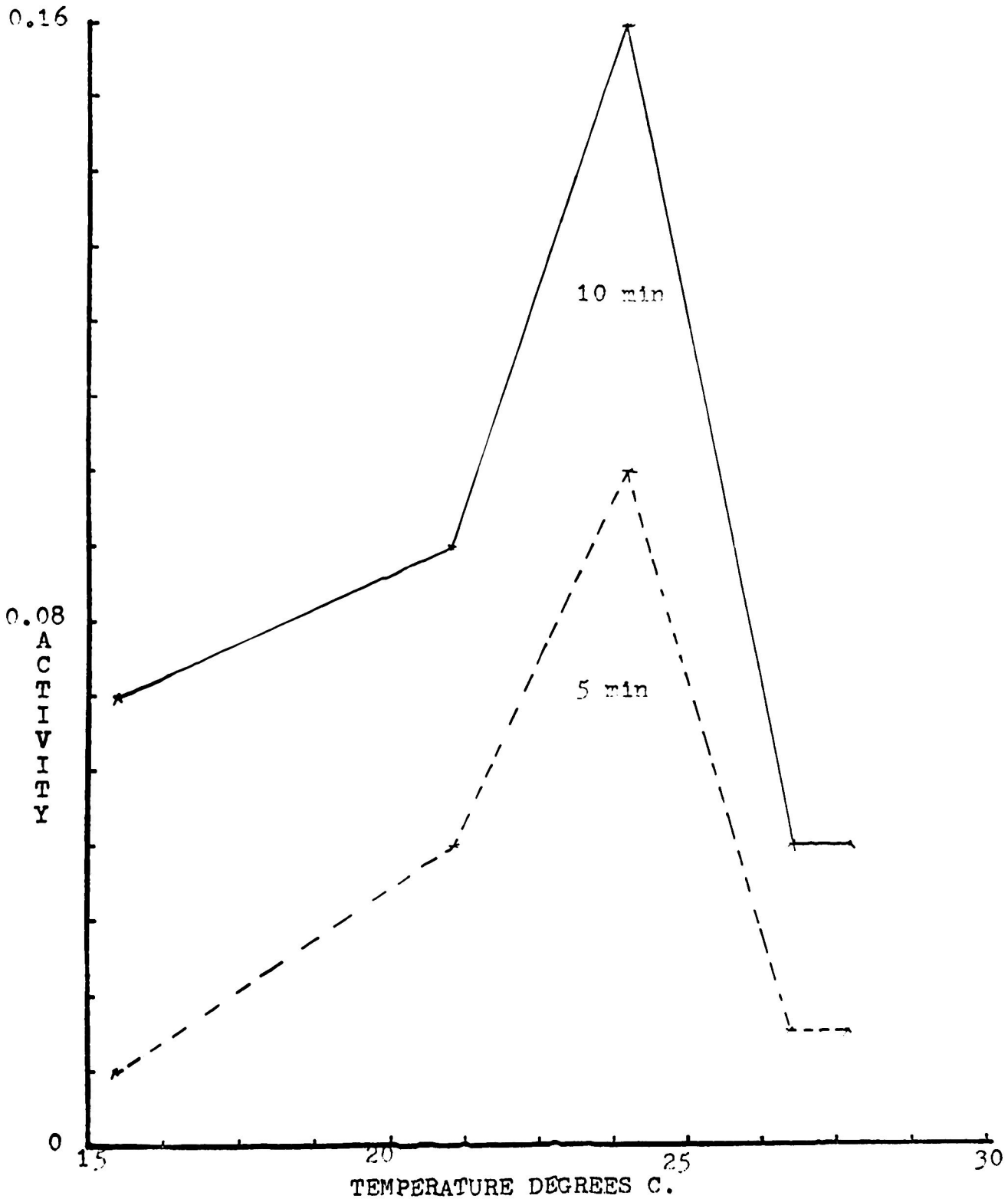

Figure 9 Amylolytic activity of the intestinal extract at different environmental temperatures.

EXPERIMENT II

Peptic Activity

The procedures for these studies were the same as were used for experiment I, except the fish were transferred to water at 14.5°C and the water temperature was then raised gradually, over about two days, to approximately 38°C. This temperature was maintained for about 12 hours, and was then decreased to 33.3°C. Fish enzymes were taken at this temperature after about 14 hours. Extraction procedures for proteinase activity were those followed in the first experiment. The temperature was then decreased to the other temperature levels, 29.4, 23.9, 22.2, 15.5, and 14.5°C, in the manner described previously. Fish were maintained for at least 14 hours at each constant temperature before being killed. Results similar to those obtained in experiment I were indicated (Table 4 and Appendix Table 4). The highest activity was again found at 23.9°C.

Table 4. Peptic activity of the channel catfish at constant environmental temperatures. - Experiment II.

Temperature Deg. C	Activity			
	5 min.		10 min.	
	No. of Samples	Net Activity*	No. of Samples	Net Activity*
14.5	2	0.345	2	0.53
15.5	2	0.350	2	0.57
22.2	2	1.028	2	1.54
23.9	2	1.200	2	1.66
26.7	2	0.902	2	1.33
29.4	2	0.625	2	1.03
33.3	2	0.190	2	0.54

*(Average of two samples - Blank) P.C. absorbance.

Tryptic Activity

Procedures similar to those employed in Experiment I were used and the intestine of the fish used for pepsin activity was used for tryptic studies. The results (Table 5 and Appendix Table 5) were again similar to those of Experiment I (Table 2). The highest activity was observed at 23.9°C.

Table 5. Proteolytic activity of the intestinal extracts of channel catfish at constant environmental temperatures - Experiment II.

Temperature Deg. C	Activity			
	10 min.		20 min.	
	No. of Samples	Net Activity*	No. of Samples	Net Activity*
14.5	2	-	2	0.0014
22.2	2	0.45	2	0.63
23.9	2	1.53	2	1.65
26.7	2	0.32	2	0.52
29.4	2	0.12	2	0.205
30.0	2	0.10	2	0.150
33.3	2	0.07	2	0.12

*(Average of two samples - Blank) P.C. absorbance.

Amylolytic Activity

Five fish were transferred to the thermostatically controlled tank at a water temperature of about 15.5°C and the temperature was raised gradually to 29.4°C. Care was taken to maintain experimental temperatures for 14 hours on the final setting before killing the fish. After testing at 29.4°C the temperature was then dropped to 26.7°C and after 14 hours a fish was

killed for studies on amylolytic activity. The results of this study did not agree with the results of the first study. The extract from the fish at 26.7°C was found to be more active than had been observed at 23.9°C. Another fish was killed at 26.7°C to determine if the previous result could be duplicated. The results of this test were similar to those of the first experiment which indicated less activity at 26.7°C than at 23.9°C.

Table 6. Amylolytic activity of the intestinal extract at constant environmental temperatures. (Iodine Method) - Experiment II.

Temperature Deg. C	Activity			
	5 min.		10 min.	
	No. of Samples	Net Activity*	No. of Samples	Net Activity*
15.5	2	0.025	2	0.08
21.1	2	0.045	2	0.08
23.9	2	0.1	2	0.162
26.7	2	0.028	2	0.051
27.7	2	0.025	2	0.051
29.4	2	0.025	2	0.035

*(Blank - Average of two samples) P.C. absorbance.

The studies were also repeated using the DNS method of Schwimmer (1950) for determining amylase activity. The same intestinal extracts were used for both methods. The results as indicated in Table 7 and Appendix Table 7 and Fig. 10, agree with the results in the first study. The enzymic activity was calculated from standard curve for reducing sugar (Fig. 11).

Table 7. Amylolytic activity of the intestinal extract of channel catfish at constant environmental temperatures - DNS Method.

Temperature Deg. C	Activity			
	5 min.		10 min.	
	No. of Samples	Net Activity*	No. of Samples	Net Activity*
15.5	2	225	2	315
21.1	2	300	2	480
23.9	2	480	2	690
26.7	2	150	2	200
27.7	2	140	2	195

* Average amount of reducing sugar of two samples, calculated from the standard maltose curve.

DISCUSSION

Constant efforts to produce human foods from animal sources at low cost lead to a continuing research for more suitable combinations of known nutrients in the most suitable environmental conditions. Channel catfish is an important species for sport fishing and commercial production in much of the United States. Its management requires detailed knowledge. One of the factors affecting the success of food fish is economical production to marketable size. A fish like other organisms needs food to provide energy for vital processes such as respiration, digestion, excretion, and other activities. Food above that necessary for metabolic needs is available for increasing the body substance, i.e., growth. Growth rate depends on utilization of food consumed. Because digestion rate is a function of the activity of enzymes present, a decreased enzymic activity must result in a decrease in

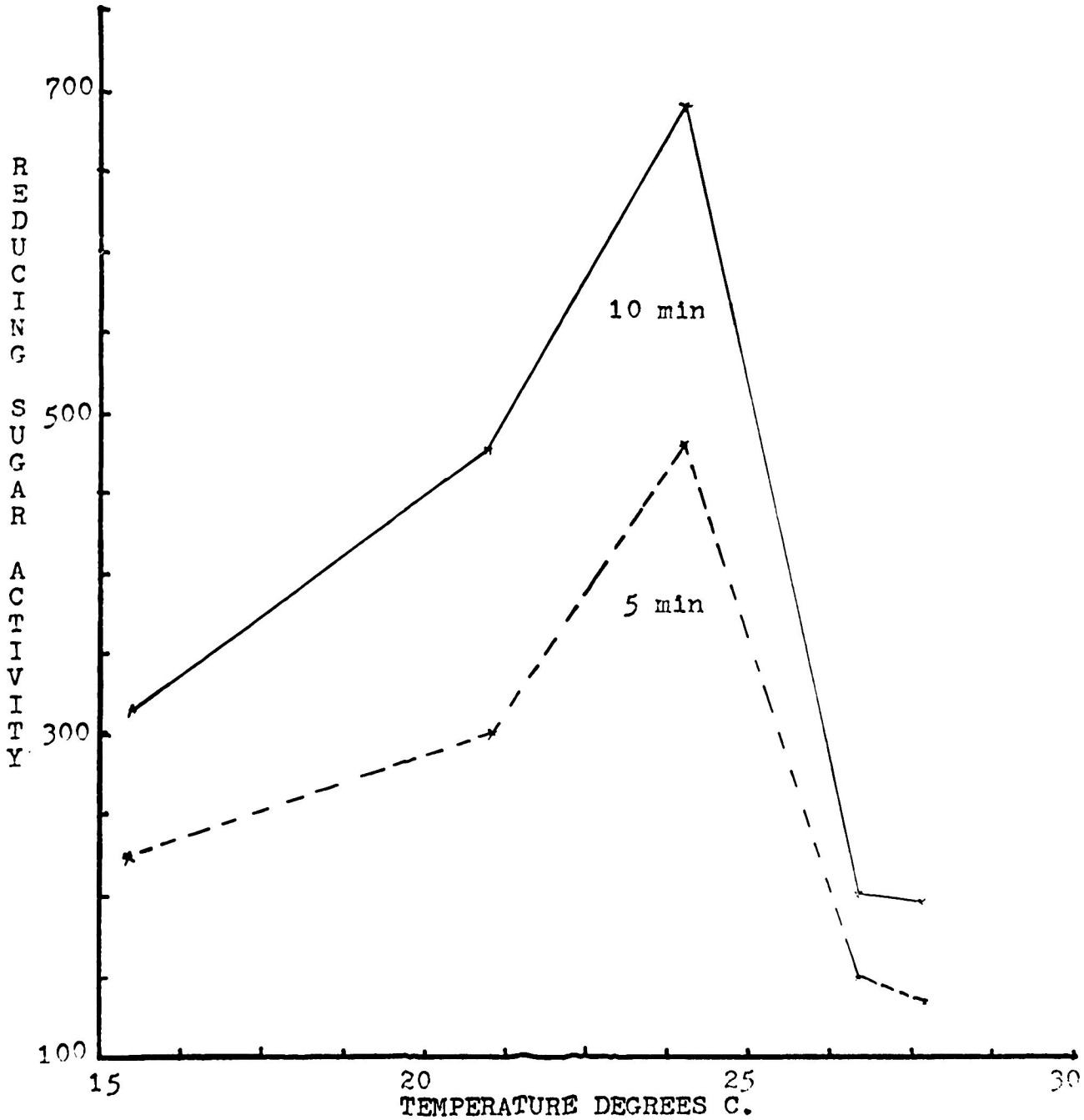

Figure 10 Amylolytic activity of the intestinal extract at different environmental temperatures-DNS method.

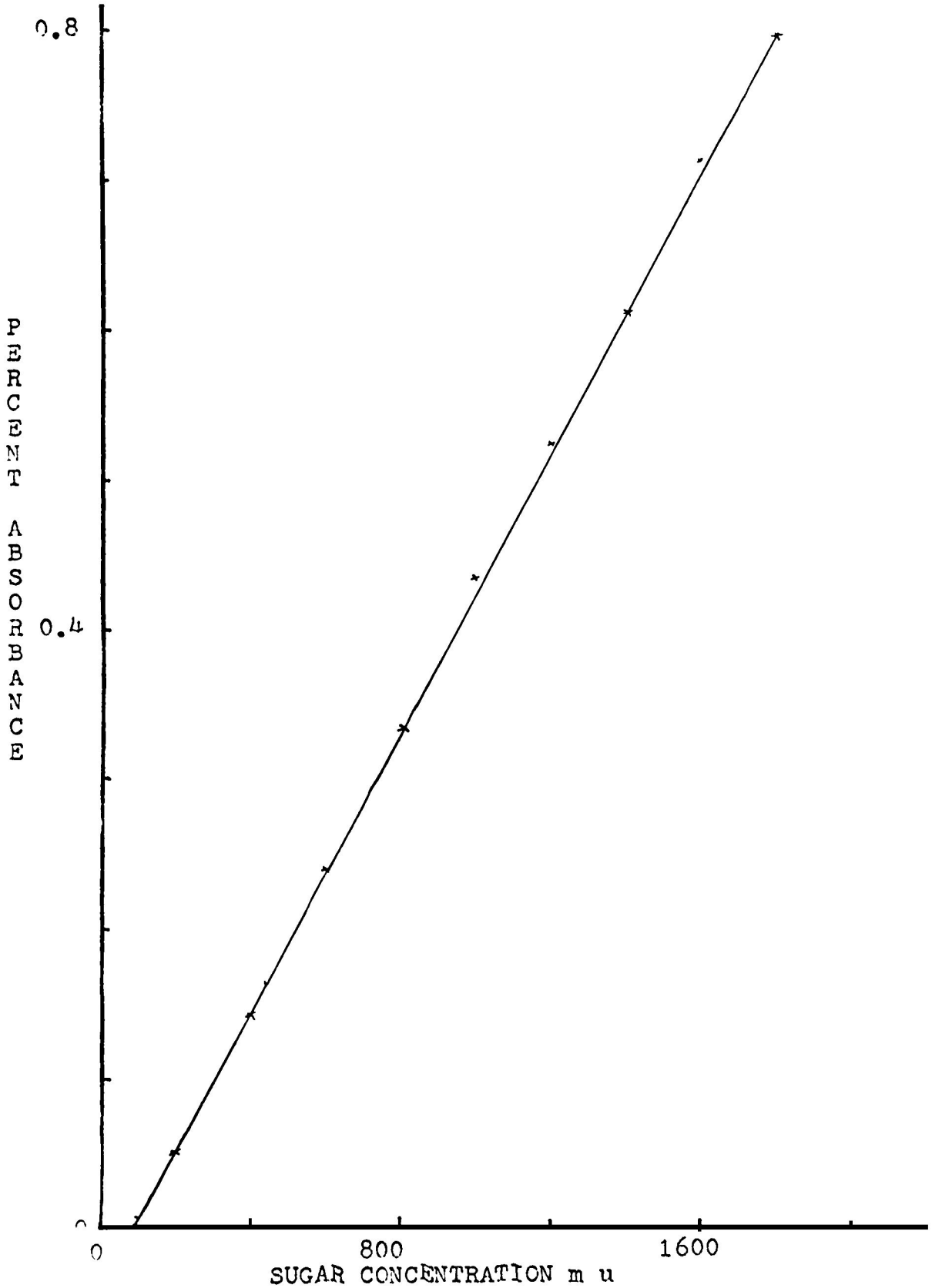

Figure 11 Standard curve for reducing sugar.

digestive power leading to lowered metabolic activity including growth. Parman (1967) stated that fish digest food entirely through activity of their own enzymes. Fish cannot depend on bacterial digestion because their digestive tract is small. Shell (1966) reported that fish fed at too high a rate waste much of the food and even though growth may be excellent the cost of production is excessive. He further reported that decaying uneaten food may bo detrimental to fish growth. If feeding rates are too low, approaching only the need for maintenance, growth is slow and conversion of feed to flesh is low. Bocause fish are not acclimated to feeding, feed supply may be wasted. Hickling (1962) pointed out that fish cannot grow (or even live at all) if they do not have a supply of the right quantity and quality of food. The ingested food, according to him, is broken down by digestive enzymes of fish, and the products are absorbed through the gut into the tissues of the fish. Hickling also mentioned many factors including water temperature which affected appetite, food intake, metabolism and growth. Belding (1928) maintained that a fish is a poikilothermal animal and its body temperature fluctuates with environment, being slightly above that of the surrounding medium. Experiments on a variety of fish by Clausen (1934) indicated that the body temperature of a fish is approximately the same as that of the surrounding water. Sullivan (1954) reported that fish have thermal receptors. Impulses from ampullae and lateral-line organs are transmitted to the central nervous system at a rate characteristic of the environment.

There is no controversy on the point that the temperature affects the growth and other activities of fish. These studies were planned to determine temperature effects on the digestion ability affording a maximum rate of growth.

In our studies all fish received the same treatment so it is felt that the fish were in similar condition at the time of study.

The results of two experiments do not indicate apparent differences when fish were taken from water which was increasing in temperature or from water in which temperature was decreasing.

Under the conditions we studied channel catfish acclimatized to temperatures in a range of from 2.2 to 33.3°C. This range might be wider but it is in general agreement with the report of Clausen (1934), Morris (1965), Nordlie (1966), Hepher (1966), Tang (1966), and others who have conducted studies on growth.

Peptic Activity

The data indicate pepsin secretion or activity at pond temperatures in the winter (2.2°C) to be very low and the secretion to increase with temperature increases up to 23.9°C. Secretion is much less at higher temperatures. The peptic activity at 2.2°C (pond temperature) was lowest and a continuous gradual rise in activity with temperature increases up to 15.5°C was found, followed by a sharp increase from 15.5 up to 23.9°C (Fig. 3). Above 23.9°C a sharp drop in activity occurred as the temperature was increased to 33.3°C, indicating decreased enzyme secretion. These results are in agreement with those of others. Riddle (1909) concluded pepsin secretion decreases with low temperature while at high temperatures it may also be diminished or destroyed. Riddle (1909), and Oscar and Day (1950) stated that the range of favorable environmental temperatures varies with species. Nordlie (1966) found higher peptic activity at 24°C than at 12°C in the brown bullhead (Ictalurus melas). Others have found that all metabolic activities increase

with increased environmental temperature. Markus (1932) reported that bass stopped feeding voluntarily below 10°C. This may be a response to a lack of digestive enzyme activity to break down food. Maloney (1949), Oscar and Day (1950), Underhill (1952), Kinne (1960), and others have found increased food consumption with increased environmental temperature, and decreased consumption above certain temperatures depending on species. Baldwin (1966) reported utilization of food for growth declined with increase in temperature above 13°C in trout. Effects of temperature on enzyme activity may also be related to growth rate. This is in agreement with reports showing increased growth with increased temperature. Studies by Tiemeier, Deyoe and Wearden (1964) indicated highest growth rates when water temperature was about 65 to 70°F and poor growth followed sudden temperature drops. Deyoe and Tiemeier (1965) obtained best feed conversion in July when water temperature ranged from 75 to 80°F. Channel catfish did not utilize the feed when water temperature dropped in August (62-73°F). Markus (1932) reported increases in weight in bass up to 22°C, after which the rate of increase diminished. Underhill (1952) reported that weight gain in the brown bullhead increased from 15°C up to 22°C and decreased thereafter. West's (1966) report also agrees in general with the increased growth in channel catfish due to increased temperature.

Tryptic Activity

Tryptic activity at 2.2°C was slight. Activity of trypsin was observed at 15.5°C but below this temperature trypsin secretion appeared to be negligible and hence there would be little digestive activity in intestine. Therefore, rate of feed passage into the intestine would probably decrease

and would not stimulate voluntary intake of more food. Blaxter, McGraham and Waiman (1956) reported that appetite for food fails at a particular distension of alimentary tract and Markus (1932) reported bass do not take food readily unless the stomach is empty. In Markus' studies largemouth black bass did not take food voluntarily at water temperature below 10°C. Strawn (1961) reported the minimum temperature at which bass fry feed was near 9 to 15°C. Sarig (1956) reported carp stop feeding below 12°C and Tilapia started growth and feeding at 13°C (Yashouv, 1960). As the temperature of water increased up to 23.9°C in our studies the trypsin activity also increased (Table 2, and Appendix Table 2). At 22.2°C to 23.9°C there was a sharp rise and then a sharp decrease in trypsin activity as the temperature was raised from 23.9 to 29.4°C (Fig. 6). A more gradual decrease then occurred up to 33.3°C. Teimeier, Deyoe and Wearden (1964) concluded better results of conversion and more growth were obtained in channel catfish at high water temperature when an extended period of water temperature above 65°F occurred. Results of the present study also agree with those of Dupree, and Sneed (1966), who obtained greater weight gain and better feed conversion in channel catfish at 76°F with wheat gluten and soybean proteins than at 69°F. Other reports also state that fish obtained more food and thus had increased growth in summer. Water temperatures are higher in summer than in winter, thus increaed feeding activity and growth may be influenced by effects on enzyme activities. Brown (1946), Maltzone (1957), and Ranade (1966) also reported increased growth rate with increased temperature. A decrease in growth rate at higher temperatures has also been reported by many workers. These effects appear to correspond with decreased activity observed for trypsin. Markus (1932) reported decreased weight gain above 22°C.

Amylolytic Activity

The result of experiment on amylolytic activity were in general agreement with the temperature effects on the other enzyme systems studied. Data in Tables 3 & 6, Appendix Tables 3 & 6 and Figs. 9 & 10 indicate that amylolytic activity increased from 15.5 to 23.9°C with a sharp drop in activity at 29.4°C.

Optimum Temperature

The optimum temperature for secretion of the three enzymes studied appears to be close to 23.9°C. West (1966) found an optimum temperature of 29°C for channel catfish growth. The difference may be due in part to age factors, as West used fry for his studies. Kinne (1960) obtained optimum growth rate at 30°C in wild desert pup-fish, Cryprinodon macularis, but reported the optimum growth in older fish to lie between 20 and 26°C. Brittan (1924) reported fishes could withstand with little difficulty exposure to a water temperature of 25°C, but at 30°C much distress was shown. Oscar and Day (1950) stated the usual temperature range for tropical species to be from about 70 to 80°F and Underhill (1952), studying the brown bullhead, a member of catfish family, obtained maximum weight gain at 22°C. He reported fish at 28°C lost weight.

Fish in these studies were sluggish and remained in one corner at temperatures below 18°C. Above 18°C they were more active and moved from one corner to the other. Activity increased with temperature. Above 26.7°C apparent uneasiness or distress was observed, with the fish moving up and down in the tank. These symptoms increased with further increase in temperature and disappeared on reducing the water temperature. These were only

observations, but when considered in conjunction with the analytical result, it seems that water temperatures above 23.9°C were less desirable for the channel catfish used in these studies.

CONCLUSIONS

Conclusions drawn from these studies may be summarized as follows:

1. The channel catfish appears to acclimatize to different temperatures within 14 hours within the range of 14.5 to 33.3°C.
2. The secretion of enzymes studied is affected by water temperature.
3. Pepsin secretion is very low at 2.2°C and the secretion increases with increase in temperature to 23.9°C, thereafter the secretion diminishes.
4. Trypsin secretion from 2.2 to 15.5°C is negligible and increases from 15.5 to 23.9°C after which it diminishes.
5. The amylolytic activity increases as temperature increases from 15.5 to 23.9°C and thereafter decreases suggesting decreased secretion of amylase.
6. The temperature range for pepsin secretion is wider than for trypsin and amylase.
7. Optimum temperature for secretion of the enzyme studied seems to be very close to 23.9°C.
8. Temperature above 23.9°C seems to be less desirable for channel catfish.

LITERATURE CITED

Allen, K. Radway. 1940. Biology of the early stages of salmon. J. Ani. Ecol. 9,1-23.

Allyn, Seymour. 1959. Effects of temperature upon the formation of vertebrae and finrays in young chinook salmon. Trans. Am. Fish. Soc. 88,58-69.

Almy, L. H. 1926. The role of the proteolytic enzymes in the decomposition of herring. J. Am. Chem. Soc. 48,2136-2146.

Amador, E., and W. E. C. Wacker. 1965. Methods in Biochemical Analysis. Vol. XIII, Ed. David Glick Pubd. Inter Science Publishers, John Wiley & Sons, N. Y. pp. 234-237.

Anson, M. L. 1938. The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. J. Gen. Physiol. 22(1),79-90.

Babkin, B. P. 1950. Secretory mechanisms of digestive glands. 2nd ed. Hoeber, N. Y.

_____, and D. J. Bowei. 1928. The digestive system and its function in Fundulus heteroclitus. Biol. Bull. 54,254-277.

Baldwin, N. S. 1956. Food consumption and growth of brook trout at different temperatures. Trans. Am. Fish. Soc. 86,323-328.

Bayliss, L. E. 1935. Digestion in plaice. J. Marine Biol. Asso. U.K. 20,73-91.

Belding, David L. 1928. Water temperature and fish life. Trans. Am. Fish. Soc. 58,98-105.

Blaxter, K. L., N. McC. Graham and F. W. Waiman. 1956. Some observations on the digestibility of food by sheep and on related problems. Brit. J. Nutri. 10(2),69-91.

Bolock, A. R. El. and Labib, W. 1966. Carp culture in the U.A.R. FAO I/E-5.

Britton, S. W. 1924. The effect of extreme temperatures on fishes. Am. J. Physiol. 67,411-421.

Brown, Margarate E. 1946. The growth of brown trout: The effect of temperature on the growth of two-year-old trout. J. Exptl. Biol. 22,145-155.

_____. 1957. "Experimental studies on growth. The relation between food and growth" in "Physiology of Fishes" Ed. Brown, M. E., Pub. Academic Press, Inc. N. Y. Vol. I, pp. 361-400.

Chaudhry, Hiralal. 1966. Breeding and selection of cultivated warm-water fishes in Asia and Far East. FAO: FR IV/R-3.

Chen, Tung Pai. 1954. The culture of Tilapia in rice paddies on Taiwan. Chinese-Am. Comm. R. Recon. Fish Series No. 2. Quoted from: Hickling, C. F. "Fish Culture" Faber & Faber (1962), pp. 125-182.

Clausen, Ralph G. 1934. Body temperature of fresh water fishes. Ecol. 15(2),139-144.

Clemen, H. P. and Jhonson. 1964. Specificity of the gonadal hydration factor in the pituitary of some fresh-water fishes. Copeia, 1964 (2):389-98.

Combs, Bobby D. 1965. Effects of temperature on the development of salmon. Prog. Fish-Culturist 27(3),134-137.

Currie, B. T. and Bull, H. B. 1951. Peptic hydrolysis of egg-albumin. II. Molecular weight distribution of peptides. J. Biol. Chem. 193,29.

Dawes, B. 1930. The absorption of fats and lipoids in plaice. J. Marine Biol. Asso. U.K. 17,75-102.

Dendy, J.S., V. Varikul, K. Sumawidjaja and M. Potaros. 1966. Production of Tilapia mossambica, plankton and benthos as parameters for evaluating nitrogen in pond fertilizers. FAO: FR, II/E-12.

Deyoe, C. W. and O. W. Tiemeier. 1965. Feeding channel catfish. Feedstuff: 37(1),53-54.

_____. 1966. Channel catfish feeding research. Feedstuff: 38(8),56.

Dobreff, M. 1927. Quoted from Brown, M. E. The Physiology of Fishes, Vol. I, Academic Press, N. Y. (1957) pp. 126-127.

Dupree, H. K. and K. E. Sneed. 1966. Response of channel catfish fingerlings to different levels of major nutrients in purified diets. Bur. Sports Fish. & Wildl., Tech. Paper 9,1-21.

Elliott, K. A. C. 1955. "Tissue slice technique," in "Methods in Enzymology", Vol. I, Ed. Colowick and Kapalan.

Embody, G. C. 1931. Concerning high water temperature and trout. Proc. Am. Fish. Soc. LI,1-4.

Folin, O. and V. Ciocalteu. 1927. On the tyrosine and trypsophane determination in proteins. J. Biol. Chem. 73,627-650.

Gibson, M. B. and B. Hirst. 1955. The effect of salinity and temperature on preadult growth of guppies. Copeia: 1955(3),241-243.

Gomori, G. 1955. "Preparation of buffers for use in enzyme studies," in "Methods of Enzymology", Vol. I, Ed. Colowick and Kapalan.

Goronagase. 1965. Z. Vergleich Physiol. 49(3),270-84. Quoted from: Physiology of digestion in Tilapia mossambica digestive enzymes and the effect of diet on their activity. Chem. Abst. 64(3), 3996d.

Gyula, Molnar. 1962. Relation between water temperature and gastric digestion of large mouth bass. Can. Fish. Res. Bd. J. 19, 1005-1012.

Hathaway, Edward S. 1927. The relation of temperature to the fishes. Ecol. 8,428-434.

Hepher, B. 1966. Some limiting factors affecting the dose of fertilizers added to fish ponds, with special reference to the Near East. FAO: FR: II/R-1.

Herwerden. 1908. Quoted from: Barrington, E. W. J. "Alimentary canal and digestion" in "Physiology of Fishes", Vol. I, Academic Press, N. Y. 1957. Ed. Brown, M. E. pp. 109-162.

Hickling, C. F. 1962. "Fish Culture" Publ. Faber & Faber, London. pp. 125-149.

Howard, P. Clemens. 1966. A review of selection and breeding in the culture of warm-water food fishes in North America. FAO: FR: IV/R-4.

Johnson, M. G. 1967. Summer temperature relations in stream-fed ponds in Southern Ontario. Prog. Fish Culturist, 29(1),21-26.

Kinne, Otto. 1960. Growth, food intake and food conversion in a euryplastic fish exposed to different temperatures and salinities. Physiol. Zool. 33(4),288-317.

Kramer, R. H. and L. L. Smith, Jr. 1960. First year growth of large mouth bass and some related ecological factors. Trans. Am. Fish. Soc. 89,222-223.

M'Gonigle. 1932. Some effects of temperature and their relation to fish culture. Trans. Am. Fish. Soc. 62,119-125.

Maloney, J. E. 1949. A study of food consumption of the bluegill to temperature. Master's Thesis. University of Minn. pp. 1-36.

Markus, Henry C. 1932 The extend to which temperature changes influence food consumption in large mouth bass. Trans. Am. Fish. Soc. 62,202-210

Maltzan, Grafin V. M. 1957. Zool. Zentr. Abst. 3,55,191-218. Quoted from: "The alimentary canal and digestion", in "Physiology of Fishes," Vol. I, Ed. Brown, M. E. pp. 109-162.

Moreloose, P. de. 1965. Pharm. Week Blad, 100(34),957-61. Quoted from: Chem. Abst. 63(13),18548 a.

Morris, Robert W. 1965. Thermal acclimation of metabolism of the yellow bullhead, Ictalurus natalis. Physiol. Zool. 38,219-227.

Moss, D. D. and D. C. Scott. 1961. Dissolved-oxygen requirements of three species of fish. Trans. Am. Fish. Soc. 90,377-393.

Nordlie, Frank G. 1966. Thermal acclimation and peptic digestive capacity in the black bullhead, Ictalurus melas. Am. Midland Natur. 75(2),416-424.

Oscar, L. C. and M. Day. 1950. Care of aquarium fishes. U. S. Dept. Int. Fish Leafl. 43,1-9.

Oser, B. L. 1965. Hawk's Physiological Chemistry. 14th ed. McGraw-Hill Book Co. N. Y. p. 517.

Paloheime, J. E. and L. M. Dickie. 1966. Food and growth of fishes II. Effects of food and temperature on the relation between metabolism and body weight. J. Fish. Res. Bd. Can. 23(6),869-908.

Parman, George. 1967. Food from the sea. Feedstuff. 39(5),66-69.

Patience, Kidd Nurnberger. 1961. Observation on the spawning temperature of Luxilus cormitis. Trans. Am. Fish. Soc. 61,215.

Pentelow, F. T. K. 1939. The relation between growth and food consumption in the brown trout. J. Expt. Biol. 16,446-473.

Probst, E. 1950. Quoted from: Hickling, C. F. "Fish Culture." Ed, Faber & Faber. London. pp. 44-45.

Ranade, S. S. and H. G. Kewalramani. 1966. Studies of the rate of food passage in the intestine of L. roluta, C. migala, and C. catla. FAO, FR: III/E-2.

Riddle, Oscar. 1909. The rate of digestion in cold-blooded vertebrates. The influence of season and temperature. Am. J. Physiol. 24,447-458.

Ryzhova, A. P. 1965. Quoted from Chem. Abst. 64(3),3900.

Sarig, S. 1956. The importance of pond fish culture in winter. Bamidgeh 8(5).

Schlamowitz, M. and L. U. Peterson. 1959. J. Bio. Chem. 234(12),3137-3145.

Schwimmer, S. 1950. Kinetics of malt alpha-amylase action. J. Biol. Chem. 186,181-193.

Shell, E. W. 1966. Relationship between rate of feeding, rate of growth and rate of conversion. FAO, FR: III/E-9.

Spallanzani. 1783. Quoted from Riddle, O. "The rate of digestion in cold blooded vertebrates - The influence of season and temperature." Am. J. Physiol. 24,447-458.

Strawn, Kirk. 1961. Growth of large mouth bass fry at various temperatures. Trans. Am. Fish. Soc. 90,334-335.

Sullivan, Charlotte M. 1954. Temperature reception and responses in fish. Can. J. Fish. Res. Bd. II,153-170.

Sullivan, M. X. 1907. The physiology of digestive tract of elasmobranchs. Bull. U. S. Bur. Fish. 27,1-27.

Surber, E. W. 1935. Production of bass fry. Prog. Fish Culturist. I-131 (8),1-7.

Swingle. 1966. Biological means of increasing productivity in ponds. FAO: FR: V/R-1.

Tang, Kun-An and Shing-Hsiang Chen. 1966. A survey of the legal pasture soils of milkfish ponds in Taiwan. FAO, FR: II/E-10.

Tiemeier, O. W., C. W. Deyoe and S. Wearden. 1964. Supplemental pellet feeding of channel catfish. Kansas Agr. Expt. Sta. Pro. Rept. 97.

_____. 1966. Experiments with supplemental feeds for channel catfish. FAO, FR: III/E-6.

Titcomb. 1920. Quoted from M'Gonigle (1932).

Underhill, J. C. 1952. The effect of temperature on food consumption in fishes, with special reference to the brown bullhead. Master's Thesis, Univ. Minn.

Van Slyke, D. D., and G. F. White. 1911. Digestion of protein in the stomach and intestine of dogfish. J. Biol. Chem. 9,209-217.

Vonk. 1927. 1929. Quoted from: Barrington, E.J.W. "Alimentary canal and digestion", in "Physiology of Fishes". Vol. 1, Academic Press. N. Y. 1957. pp. 109-162.

West, Boyce W. 1966. Growth, food-conversion, food consumption, and survival at various temperatures of channel catfish. M. S. Thesis. Univ. Ark.

Wingfield, C. A. 1940. The effects of certain environmental factors of the growth of brown trout. J. Exptl. Biol. 17,435-448.

Wolmy, Pawel. 1966. Fertilization of warm-water fish ponds in Europe. FAO: FR: II/R-7.

Yashouv, A. 1956. Problems in carp nutrition. Bamidgeh, 8(5),79-87.

Yashouv, A. 1960. Effect of low temperature on Tilapia nilotica and Tilapia galilaea. Bamidgeh: 12(3),62-66.

ACKNOWLEDGEMENTS

The writer wishes to take this time to express his appreciation to Dr. Charles W. Deyoe, major professor, for his guidance and suggestions during studies, research and preparation of this thesis.

The writer is also grateful to Dr. O. W. Tiemeier for providing the facilities and materials needed to carry out the work.

Funds to conduct the experiments were provided by Kansas Agricultural Experiment Station and the Bureau of Commercial Fisheries.

Appreciation is also expressed to Dr. M. M. MacMasters, for her valuable suggestions, not only in course work but also in private affairs, which assisted a lot in graduating M.S.

He sincerely thanks all professors in Osmania Veterinary College, Osmania University, Hyderabad, India, for encouragement particularly Mr. M. Imaduddin for his initiative to proceed abroad, and Dr. M. Waliullah and Dr. V. Krishna Reddy for their sincere suggestion to join the Department of Flour and Feed Milling Industries, KSU.

Also, the writer expresses grateful appreciation to his parents, Mr. & Mrs. G. Mohiuddin, and his brother, Mr. Vikaruddin A. Asadi and his family, for their encouragement and financial support.

Recognition is also accorded to the author's wife, Shahnaaz, for the efforts and encouragement during studies and for her help in preparation of this thesis.

A P P E N D I X

Appendix Table 1. Peptic activity of the channel catfish at different constant temperatures - Experiment I.

Temperature Deg. C	Percent absorbance 5 min.				Activity* 5 min.	Percent absorbance 10 min.				Activity* 10 min.
	1	2	Av†	Blank		1	2	Av†	Blank	
2.2	0.31	0.31	0.31	0.17	0.14	0.409	0.411	0.41	0.17	0.240
14.5	0.51	0.51	0.51	0.17	0.34	0.71	0.71	0.71	0.17	0.540
22.2	1.19	1.21	1.20	0.17	1.03	1.721	1.719	1.72	0.17	1.550
23.9	1.37	1.39	1.38	0.17	1.21	1.814	1.816	1.815	0.17	1.645
26.7	1.07	1.07	1.07	0.17	0.90	1.50	1.50	1.50	0.17	1.330
29.4	0.789	0.791	0.79	0.17	0.62	1.2	1.19	1.195	0.17	1.025
33.3	0.37	0.37	0.37	0.17	0.20	0.701	0.699	0.7	0.17	0.530

* Activity = (Average-Blank) P.C. absorbance

\+ Av† = Average of 1 & 2.

Appendix Table 2. Proteolytic activity of the intestinal extract of channel catfish at constant environmental temperatures - Experiment I.

Temperature Deg. C	Percent absorbance 10 min.				Activity* 10 min.	Percent absorbance 20 min.				Activity* 20 min.
	1	2	Av.+	Blank		1	2	Av.+	Blank	
2.2	0.17	0.17	0.17	0.17	—	0.171	0.171	0.171	0.17	0.001
14.5	0.172	0.17	0.171	0.17	0.001	0.171	0.172	0.1715	0.17	0.0015
22.2	0.609	0.611	0.61	0.17	0.440	0.81	0.81	0.81	0.17	0.640
23.9	1.72	1.72	1.72	0.17	1.550	1.83	1.83	1.83	0.17	1.660
26.7	0.49	0.5	0.495	0.17	0.325	0.68	0.68	0.68	0.17	0.510
29.4	0.289	0.291	0.29	0.17	0.120	0.371	0.369	0.37	0.17	0.20
33.3	0.24	0.24	0.24	0.17	0.070	0.289	0.291	0.29	0.17	0.12

* Activity = (Average-Blank) P.C. Absorbance.

\+ Av.+ = Average of 1 & 2.

\- Indicates no activity.

Appendix Table 3. Amylolytic activity of the intestinal extract of channel catfish - Experiment I.

Temperature Deg. C	Percent absorbance 5 min.				Activity* 5 min.	Percent absorbance 10 min.				Activity* 10 min.
	1	2	Av+	Blank		1	2	Av+	Blank	
15.5	0.47	0.45	0.46	0.48	0.02	0.41	0.41	0.41	0.48	0.07
21.1	0.43	0.43	0.43	0.48	0.05	0.391	0.389	0.39	0.48	0.09
23.9	0.381	0.379	0.38	0.48	0.1	0.32	0.32	0.32	0.48	0.16
26.4	0.454	0.456	0.455	0.48	0.025	0.43	0.43	0.43	0.48	0.05
27.7	0.455	0.457	0.456	0.48	0.024	0.43	0.43	0.43	0.48	0.05
29.4	0.45	0.45	0.45	0.48	0.03	0.44	0.45	0.445	0.48	0.035

* Activity = (Blank - Average) P.C. Absorbance.

\+ Av. = Average of 1 & 2.

Appendix Table 4. Peptic activity of channel catfish at different constant environmental temperatures - Experiment II.

Temperature Deg. C	Percent absorbance 5 min.				Activity* 5 min.	Percent absorbance 10 min.				Activity* 10 min.
	1	2	Av.+	Blank		1	2	Av.+	Blank	
14.5	0.52	0.51	0.515	0.17	0.345	0.71	0.699	0.70	0.17	0.53
15.5	0.52	0.52	0.52	0.17	0.35	0.74	0.74	0.74	0.17	0.57
22.2	1.2	1.196	1.198	0.17	1.028	1.71	1.71	1.71	0.17	1.54
23.9	1.37	1.37	1.37	0.17	1.20	1.83	1.83	1.83	0.17	1.66
26.7	1.073	1.071	1.072	0.17	0.902	1.49	1.51	1.50	0.17	1.33
29.4	0.8	0.79	0.795	0.17	0.625	1.20	1.20	1.20	0.17	1.03
33.3	0.365	0.367	0.36	0.17	0.19	0.71	0.71	0.71	0.17	0.54

* Activity = (Average-Blank) P.C. Absorbance.

\+ Av. = Average of 1 & 2.

Appendix Table 5. Proteolytic activity of the intestinal extract of channel catfish at constant environmental temperatures - Experiment II.

Temperature Deg. C	Percent absorbance 10 min.				Activity* 10 min.	Percent absorbance 20 min.				Activity* 20 min.
	1	2	Av.+	Blank		1	2	Av.+	Blank	
14.5	0.17	0.17	0.17	0.17	—	0.171	0.171	0.171	0.17	0.001
22.2	0.62	0.62	0.62	0.17	0.45	0.80	0.80	0.80	0.17	0.63
23.9	1.7	1.7	1.7	0.17	1.53	1.82	1.82	1.82	0.17	1.65
26.7	0.48	0.5	0.49	0.17	0.32	0.689	0.691	0.69	0.17	0.52
29.4	0.29	0.29	0.29	0.17	0.12	0.377	0.376	0.375	0.17	0.205
30.0	0.27	0.27	0.27	0.17	0.1	0.32	0.32	0.32	0.17	0.15
33.3	0.241	0.239	0.24	0.17	0.07	0.29	0.29	0.29	0.17	0.12

* Activity = (Average - Blank) P.C. Absorbance.

\+ Av. = Average of 1 & 2.

\- Indicates no activity.

Appendix Table 6. Amylolytic activity of the intestinal extract of channel catfish - Iodine method - Experiment II.

Temperature Deg. C	Percent absorbance 5 min.				Activity* 5 min.	Percent absorbance 10 min.				Activity* 10 min.
	1	2	Av.+	Blank		1	2	Av.+	Blank	
15.5	0.456	0.454	0.455	0.48	0.025	0.4	0.4	0.40	0.48	0.08
21.1	0.44	0.43	0.435	0.48	0.045	0.41	0.39	0.40	0.48	0.08
23.9	0.38	0.38	0.38	0.48	0.1	0.319	0.317	0.318	0.48	0.162
26.7	0.453	0.451	0.452	0.48	0.028	0.43	0.428	0.429	0.48	0.051
27.7	0.455	0.455	0.455	0.48	0.025	0.425	0.433	0.429	0.48	0.051
29.4	0.45	0.46	0.455	0.48	0.025	0.445	0.445	0.445	0.48	0.035

* Activity = (Blank - Average) P.C. Absorbance.

\+ Av. = Average of 1 & 2.

Appendix Table 7. Amylolytic activity of the intestinal extract of channel catfish at constant environmental temperatures - DNS method.

Temperature Deg. C	5 min. Percent absorbance 1	2	Av.+	Activity* 5 min. ug glucose	10 min. Percent absorption 1	2	Av.+	Activity* 10 min. ug glucose
15.5	0.07	0.07	0.07	225	0.116	0.114	0.115	315
21.1	0.1	0.1	0.1	300	0.19	0.19	0.19	480
23.9	0.19	0.19	0.19	480	0.28	0.28	0.28	690
26.7	0.034	0.036	0.035	150	0.051	0.049	0.05	200
27.7	0.035	0.035	0.035	140	0.048	0.05	0.049	195

* Activity taken as ug glucose formed, calculated from standard curve.

+ Average of 1 & 2.

EFFECT OF TEMPERATURE ON THE DIGESTIVE ENZYMES OF CHANNEL CATFISH *ICTALURUS PUNCTATUS* (RAFINESQUE)

by

SHAFIUDDIN AHMED ASADI

B.Sc., Osmania University, India, 1955

B.V.Sc. & A.H., Osmania University, India, 1962

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industries

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1967

Studies were conducted to determine the presence of various digestive enzymes and to investigate the effect of environmental water temperature on the digestive activity of channel catfish.

Age Class II channel catfish were used for these studies. Fish were transferred to a glass tank filled with dechlorinated water with a capacity of about 100 gallons. Temperature was controlled by passing mixtures of hot and cold water through a metallic coil and using a thermostatially controlled heating coil. An agitator was used to aid regulation of oxygen and temperature.

Two experiments were conducted for each of the enzymes studied with both increasing and decreasing temperatures. Peptic and tryptic activities were studied in fish taken from water maintained at 2.2, 14.5, 22.2, 23.9, 26.7, 29.4, and 33.3°C, and amylase activity studies were conducted with extracts from fish held at 15.5, 21.1, 23.9, 26.7, 27.7, 29.4°C. Preliminary experiments were also conducted to determine optimum pH and optimum temperature in vitro, for the enzymes studied. The methods of extraction and analysis are described.

The results indicate the presence of pepsin in stomach extracts and trypsin and amylase in intestinal and pancreatic extracts. The optimum in vitro pH found for pepsin, trypsin and amylase were 2.2, 6.8 to 6.9, and 4.8 respectively. The optimum temperature in vitro found for pepsin was 33°C. Trypsin and amylase activity was highest at 55°C.

Enzymes of channel catfish responded rapidly to changes in temperature between 14.5°C to 33.3°C. Secretion of the enzymes studied was affected by water temperature. The optimum environmental temperature for secretion of the enzymes studied was close to 23.9°C. Pepsin activity was found from 2.2°C

(fish pond temperature in winter) to 33.3°C, whereas trypsin secretion in considerable amounts appeared at 14.5°C. All enzymes studied were found to be secreted in increasing quantities up to 23.9°C. Secretion diminished at higher temperatures.