

**DEVELOPMENTAL CHANGES IN THE ACTIVITY OF MAJOR
INTESTINAL BRUSH BORDER ENZYMES IN THE WILD
JUVENILE NILE PERCH, *Lates niloticus* (Linnaeus, 1758)**

BY

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**A DISSERTATION SUBMITTED TO GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
AWARD OF A MASTER OF SCIENCE DEGREE IN
MOLECULAR BIOLOGY AND BIOTECHNOLOGY OF
MAKERERE UNIVERSITY**

MAY, 2011

DECLARATION

I **Charles Drago Kato** declare that the work presented here is original and has never been presented to any institution for any award

Signature

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DEDICATION

This work is dedicated to my lovely mother Mrs. Lovinsa Nalukwago and to my late father Mr. Yusuuf Bisaaso.

ACKNOWLEDGEMENTS

I wish to extend my gratitude first and foremost to the Almighty God for all his blessings

My sincere gratitude is extended to my supervisors: Dr. Edward Nyatia and Dr. Enock Matovu for the guidance they have always rendered to me when conducting this research and writing up the dissertation especially for their constructive criticisms and effort to make this work possible, I am forever grateful. My sincere appreciation is extended to Professor. Zehava Uni and Miss Ofra Kedar of The Hebrew University Jerusalem for all the guidance in carrying out the laboratory work and writing up this dissertation, I am very grateful. Special thanks go to the MSI-Nile perch project, the principal investigator: Dr. Justus Rutaisire and all the senior research scientists for the guidance and financial support, May the good Lord continue rewarding them abundantly.

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LIST OF ABBREVIATIONS AND ACRONYMS

BW	Body weight
EC	Enzyme commission
FAO	Food and Agriculture Organization
IW	Intestinal weight
LAP	Leucine-amino-peptidase
MSI	Millennium Science Initiative
OD	Optical density
Pc	Pyloric caeca
SI	Sucrase –isomaltase
U	Enzyme unit
γ -GT	Gamma-glutamyl-transferase

ABSTRACT

The Nile perch, *Lates niloticus* is a carnivorous fish and a potential candidate for aquaculture. The ability of fish to utilize ingested nutrients depends on availability of appropriate enzymes along the intestinal tract and can be used in the formulation of artificial diets.

The effect of fish size (total length) on the activity of three brush border enzymes was evaluated. Juvenile Nile perch at different stages of development were captured from the shores of Lake Victoria and the activity of three brush border enzymes in three intestinal sections (pyloric caeca, upper and lower intestine) in six different size groups evaluated. Two proteolytic enzymes, leucine aminopeptidase (LAP, EC 3.4.11.1), gamma glutamyl transferase (γ -GT, EC 2.3.2.2) and a carbohydrase (maltase, EC 3.2.1.20) were assayed.

All the three enzymes were influenced by the fish size, intestinal section and the interaction between the two factors ($p < 0.05$). The highest specific activity of LAP and maltase was observed in the upper intestine while that of γ -GT was highest in the lower intestine. The specific and relative total enzyme activities were significantly higher ($p < 0.05$) in the 11-15 and 16-20 size groups in all the tested enzymes. Total enzyme activity for all the enzymes increased with fish size. There was a significant correlation ($p < 0.05$) between the tested enzymes with the highest correlation between LAP and maltase, this correlation was highest in the pyloric caeca.

The results of the present study reveal that, the most critical stage in the nutrition of juvenile Nile perch occurs when the fish attains a total length of 11-20cm. This stage (11-20cm) necessitates intensification in the feeding regime through incorporation of high protein and carbohydrate rations. Presence of maltase indicates that this fish can utilize lower levels of carbohydrates in the diet.

CHAPTER ONE

INTERODUCTION

1.1 Background

The Nile perch, *Lates niloticus* is a carnivorous fish common to all major river basins like Nile, Niger, Chad, Senegal and Volta. The commonest place to find the Nile perch is in Lake Victoria where the species was introduced in 1962 (Hopson, 1972). The fish was introduced in Lake Victoria from its natural habitat in Lake Albert and Lake Turkana by the British (MacDougall, 2001). The British predicted that, Nile Perch would eat the small, non marketable fish in order to consolidate catches into a single marketable large fish (Kitchell *et al.*, 1997). The Nile perch can grow to attain a size up to 200kg with an average length of 85-100cm (FishBase, 2004). An explosive increase of Nile perch was reported for several areas of the lake in Uganda, Kenya and Tanzania around the early 1980's (Kitchell *et al.*, 1997). This explosion in the Nile perch resource made Lake Victoria the largest and economically most significant of the national fisheries (FAO, 1999). In 2004, a total of 80,492 metric tones of Nile perch valued at US \$242,504,000 were exported from East Africa (Ogutu-Ohwayo and Kirema-Mukasa, 2005). Records from Uganda fish processors and exporters association indicate that 37 metric tones valued at US \$ 143 million were exported from Uganda in 2005. For a long time the increase in Nile perch seemed to be a favorable development for the fish industry however, the average size of the fish captured and the catch rates have decreased dramatically (Ogutu-Ohwayo and Kirema-Mukasa, 2005). The over 350 endemic species of haplochromines have been depleted due to the indiscriminate feeding habits of the Nile perch, and as a

result the fish has become cannibalistic with the larger fish feasting on the smaller ones (Watsala, 1998).

The ability of fish to utilize ingested nutrients depends on availability of appropriate enzymes along the intestinal tract. Among the enzymes in the intestinal tract, brush border enzymes that are responsible for the final stages of digestion and absorption are very critical (Klein *et al.*, 1999). The activity of brush border enzymes has been found to be affected by age and dietary composition (Montagne *et al.*, 2002; Krogdahl and Bakke-McKellep, 2005). These plastic changes in enzymatic activities ensure that the fish does not produce large amounts of intestinal enzymes that would be wasted in the presence of low levels of enzyme substrate in the diet (Caviedes-Vidal *et al.*, 2000). Hence fish must alter the enzyme profiles in order to match the developmental and dietary changes during the different seasons. The effect of age on enzymatic activity has been shown in fish that undergo ontogenetic shifts in diet, to coincide with the fishes dietary changes (Moran and Clements, 2002; Drewe *et al.*, 2004). Some authors however, have demonstrated that at any time fish almost possess all the enzymes irrespective of the specific diet consumed (Harpaz and Uni, 1999; Chakrabarti *et al.*, 1995). Hence Chan *et al.* (2004) proposed that the activity of certain digestive enzymes in both carnivorous and herbivorous fish are influenced by phylogeny as compared to fish's natural diet. Leucine aminopeptidase (LAP) and gamma glutamyl transferase (γ -GT) are proteolytic enzymes that are responsible for the final break down of peptides and assimilation of amino acids respectively. The highest activity of LAP and γ -GT has been reported in the pyloric caeca, the intestinal

segment that is proposed to be involved in food digestion and absorption (Buddingston and Diamond, 1987). Carnivorous fish have been observed to exhibit a lower activity of disaccharide enzymes (Hakim *et al.*, 2006). Maltase is a disaccharide degrading enzyme involved in the breakdown of maltose that is produced during starch hydrolysis. Most herbivorous fish species have been observed to exhibit a significantly high activity of maltase in the upper intestinal segments indicating a clear proximal-distal gradient in enzymatic activity (Villanueva *et al.*, 1997).

1.2 Problem statement

The stress on the Nile perch resource has become a known fact that has been discussed in many regional forums. A number of approaches have been put in place to control over fishing and the capture of immature with insufficient scientific evidence. Such controlled fishing ventures have been unsuccessful and if nothing is done, Lake Victoria is in danger of becoming the world's largest pool of dead water (Baskin, 1994). To avert such undesirable situation, there is need to develop technologies to increase production of the Nile perch through aquaculture. However, culture of any candidate species cannot succeed without fully understanding the species feeding ecology and generating feeding technologies to aid its culture. Preliminary trials at Kajjansi Aquaculture Research and Development Centre have been unsuccessful in feeding artificial feeds to the Nile perch. The ability of fish to utilize nutrients depends on the synthesis of appropriate enzymes and the enzyme distribution along the gut lumen (Tengjaroenkul *et al.*, 2005). Among the enzymes in the gut lumen, intestinal brush border enzymes that are responsible for the final stages of digestion

and absorption are very critical. The activity of these intestinal brush border enzymes has been found to vary with developmental stage and this has been used as a major factor in formulation of fish diets for the different age/size groups. However for the Nile perch, no such data is available about the developmental changes in the activity of its intestinal brush border enzymes and how the enzyme activity varies across the intestinal segments. Absence of such data has hindered the development of artificial diets that would meet the nutrition requirements of the different fish size groups.

1.3 Justification/Significance

The Nile perch resource had made Lake Victoria the largest and economically most significant of the regional fisheries (FAO, 1999). However currently the average size of the fish captured and the catch rates have decreased dramatically (Ogutu-Ohwayo and Kirema-Mukasa, 2005). As a result scientists under the MSI-Nile perch project are designing strategies to breed the fish in captivity. This work cannot be successful without devising means to make the Nile perch accept artificial feeds. In other species, intestinal brush border enzymes have been indicated as a significant step in intestinal maturation and an important indicator of the ability of fish to utilize artificial diets. The current project aims establishing baseline data that would be used in the design of an appropriate feed that meets the nutritional requirements of each developmental stage. This work will also help understand the functional physiology of the different intestinal segments especially the pyloric caeca and the roles they play in nutrient digestion and absorption. Furthermore this work will provide baseline data about the digestive enzyme profiles in the wild type Nile perch that can be used to study the effect of different dietary rations in aquaculture.

1.4 Overall objective

To investigate the development changes in the activity of major intestinal brush border enzymes in the wild juvenile Nile perch, *Lates niloticus*.

1.5 Specific objectives

1. To establish changes in enzymatic activity of leucine aminopeptidase (LAP), γ -glutamyl transferase (γ -GT) and maltase among the different fish size groups.
2. To identify developmental changes in enzymatic activity of LAP, γ -GT and maltase along the intestinal tract
3. To establish if there is a correlation between the activities of LAP, γ -GT and maltase along the intestinal tract

1.6 Research questions

1. Are there differences in the enzymatic activities of LAP, γ -GT and maltase across the different fish size groups?
2. How does the activity of these brush border enzymes vary along the different intestinal segments?
3. Is there a correlation between the activities of these brush border enzymes along the different intestinal segments?

CHAPTER TWO

LITERATURE REVIEW

2.1 Fisheries in the Ugandan Economy

About twenty years after the introduction of the Nile perch, its numbers increased to such an extent that it became a dominant species, accounting up to 96.5% of the total catch by weight from Lake Victoria by 2001. This coincided with a growing demand for this fish on the regional and international markets that led to an increase in prices of Nile perch products which prompted fishers to increase their fishing effort (Balagadde, 2002; Bahiigwa and Keizire, 2003). The Nile perch led to an increase in total fishery and fishery related employment. Since the increase of the Nile perch resource, large factory fishing companies thrived to a great extent (Kitchell *et al.*, 1997). However, the introduction of the Nile perch has also caused serious ecological problems e.g. the richness and diversity of endemic species is rapidly declining. About 300 native species have already been driven to extinction due to the feeding patterns of the Nile perch (Schofield, 1999).

As a result of unsustainable fishing efforts, Nile perch catches decreased from 53.6kg boat-1 day-1 in 2000 to 24-32kg boat-1 day-1 in 2005 (Muhoozi *et al.*, 2005). This threat to the Nile perch export industry has prompted Uganda to invest in initiatives to stabilize the industry, and to promote aquaculture to boost production of Nile perch and tilapia.

2.2 General shape of Nile perch

The Nile perch has a sliver color with a blue tinge and a distinctive dark black eye with a bright yellow outer ring. Nile perch are usually seen around 2-4 kg, but have

been caught at sizes up to 200kg. They average around 85-100cm but can grow up to 193cm. Males are generally larger than females. The pre-opercle and pre-orbital bones have spines, with a large spine on the free edge of the operculum (FishBase, 2004). The Nile perch grows very fast during the first year. The growth rate then decreases during the second, third, fourth and fifth years (Acera, 1984).

2.3 Geographical range of the Nile perch

The Nile perch is commonly found in all the major river basins in Africa like the Nile, Chad, Niger, Senegal and Volta. The Nilotic population extends northwards into the the Mediterranean region and up to Lake Mariout situated in the Nile delta near Alexandria. Southwards the distribution includes parts of the Congo basin. The species thrive under lacustrine as well as riverine conditions and important populations exist in Lake Albert, Rudolph (Turkana) and Tana (Hopson, 1972). Around the 1950's the British introduced the Nile perch into Lake Victoria from its natural habitats (Albert and Turkana) and this is the most common area to find the Nile perch (MacDouquall, 2001).

2.4 Habitat

Nile perch can be found in many different types of fresh water but the species prefers warm, tropical waters where they grow to large sizes and occur in high densities. Adult Nile perch occupy all habitats in lakes and rivers (10-60m in depth) where there is enough oxygen with exception of rocks, swamps and the pelagic zone. Small juveniles are restricted to shallow waters near the shore (FishBase, 2004)

2.5 Feeding habits

A number of factors determine the type of prey ingested by the Nile perch these include, the feeding preference (Hagiwara *et al.*, 2007; Nunn *et al.*, 2007), prey availability, predator size, predator's catching efficiency, water temperature and turbidity (Moore and Moore, 1976). Although fish feeding is selective, it can vary according to food availability meaning that most fish species show extremely adaptable feeding habits, using items readily available in the environment (Azevedo, 1972).

Juvenile Nile perch feed on copepods, prawns in the genus *Caridina*, fish fry, small gastropods and bivalves. As the fish matures and moved to greater depths haplochromine cichlids constitute over 95% of their food consumption. Occasional items found in the Nile perch's diet include smaller fish in the genera *Barbus*, *Clarias*, *Haplochromis*, *Lates*, *Oreochromis* and *Xenoclarias*. Besides crustacean, Zooplankton, invertebrate preys include snails, clams and insects (odonate larvae, aquatic hemiptera, mayflies in the genus *Povilla*, and larvae of phantom midges. Fish in the genus *Rastrineobola* are very common in the diet in terms of occurrence, and are second to haplochromines (Acere, 1984). As Nile perch grow bigger, they take larger prey. Nile perch less than 80cm tend to feed on small fish than those greater than 80cm.

Katunzi *et al.* (2006) observed that zooplankton formed an important food source for Nile perch of less than 4cm and that fish above 5cm switched to midge larvae or shrimps depending on availability. Small Nile perch were mainly eaten by Nile perch

between 5 and 20cm while fish of >20cm mainly fed on dragonfly nymph. However the same authors reported that this ontogenetic shift in the diet of the juvenile Nile perch is seasonal and habitat related depending on the availability of the prey but noted that in most habitats, Nile perch between 5-30cm preferred shrimp almost exclusively.

2.6 Development of the intestinal tract

Body structures develop according to their importance and function (Sala *et al.*, 2005). In several species developmental modifications may be closely linked to ontogenetic changes in habitat and resource use (Ward-Campbell and Beamish, 2005). The development of the digestive system can be divided into three phases: the embryonic, larval and juvenile periods (Ferraris *et al.*, 1987). In different fishes, digestive segments are named in several ways in the literature. Some researchers divide the intestine into small and large (Clarke, 1980) while others pyloric caeca, anterior and posterior intestine (Zhu and Zhang, 1993).

Pyloric caeca in fish are sac shaped extensions, branching from the anterior intestine immediately after the pylorus of the stomach and their function is to increase the surface area for nutrient absorption (Buddington and Diamond, 1989). Pyloric caeca aid digestion and absorption of nutrients to the blood stream before passage of the food bolus to the intestine for further breakdown and absorption. They also neutralize the acid entering the intestine from the stomach which is supported by the absence of pyloric caeca in stomachless fish (Gawlicka *et al.*, 1995). Histologically, pyloric caeca are similar to the intestines and are lined by a simple columnar epithelium with

secretory cells. It is generally accepted that the presence of the pyloric caeca designate the transition from the larval to the juvenile period (Hamlin *et al.*, 2000).

The relative length of the intestines varies with diet; they are long in herbivorous and short in carnivorous (Roberts, 1989). In fish that feed on other fish, the intestines are straight or at mostly thrown, into loops. The highly folded intestinal mucosal aids in mixing the food with hepatic and pancreatic enzymes as well as with mucus secreted by goblet cells (Grau *et al.*, 1992). Neutral mucous compounds of the intestine participate in enzymatic food digestion, formation of food mass and absorption. The adult intestine and rectal goblet cells also contain a combination of neutral and acid mucus (Murray *et al.*, 1996). Dense inclusions in the rectal apical cytoplasm allow a further distinction between the intestine and rectum in both the adult and larval yellowtail flounder and suggest a function of intracellular rectal digestion of proteins that is especially important before gastric gland differentiation (Murray *et al.*, 1996). In the larval summer flounder, supranuclear inclusions are present in the cytoplasm of the anterior, medial, and posterior intestine although they are more prominent in the posterior intestine and function similarly in the pinocytic uptake of nutrients (Bisbal and Bengtson, 1995).

According to Zambonino Infante and Cahu (2001), the anterior intestine is a main site of extracellular proteolytic digestion during the larval period, due to its alkaline pH, and presence of pancreatic trypsin. The presence of brush border on the mucosal surface indicates active epithelial transport (Bisbal and Bengtson, 1995).

2.7 Fish dietary requirements

The dietary requirements of fish, during different stages of their life cycle, are determined by the functional morphology and development of the gut (Cahu and Zambonino Infante, 2001). The most important nutrients are proteins and lipids. Unlike many other vertebrate animals, fish generally rely more on lipids than on carbohydrates for their energy requirements and are not able to utilize carbohydrates efficiently (Deng *et al.*, 2000).

It has been shown that the simple morphological structure of the digestive tract correlates with a low production of enzymes (Dabrowski, 1979). Difficulties in rearing fish larvae on artificial diets may indicate a lower efficiency of digestion in some species (Kainz and Gollmann, 1980). The digestive enzymes introduced by live prey are therefore thought to aid digestive processes in the gut and play a role in activating the endogenous enzymes by cleaving the inactive zymogen forms (Kolkovski *et al.*, 1990). Thus, the lack of these enzymes in formulated feeds could partly explain the greater success of live feeds (Kolkovski *et al.*, 1990). Digestive capacity corresponds to the anatomical development of the digestive system which in turn is related to changes in habitat and diet during metamorphosis (Lovett and Felder, 1989). The absence of digestive capacity can be attributed to either physical or chemical characteristics that are not compatible with the enzymatic capacity of very young fish larvae. Digestive capacity may also be based upon the quality of food presented (Zambonino Infante and Cahu, 1994).

2.8 Intestinal brush border enzymes

The intestinal epithelium is considered to be the structure associated with the terminal digestion of luminal peptides in vertebrates. The use of enzymes in the brush border is widely accepted as an indicator of the intestinal function (Gawlicka *et al.*, 1995). Some authors correlate the presence of exo-proteases with the maturation of the intestinal enterocytes (Cahu and Zambonino-Infante, 1995; Gawlicka *et al.*, 1995). Intestinal peptide hydrolases are found in two main subcellular locations, the cytosol and the brush border membrane of enterocyte. Cytosolic enzymes are mainly di- and tri-peptidases located in the enterocyte cytosol. These cytosolic enzymes complete protein hydrolysis by reducing peptides to free amino acids. These enzymes are highly expressed in immature enterocyte during the first three weeks of life in larvae of temperate species, while they remain highly expressed in cold water species beyond this initial period (Zambonino-Infante and Cahu, 2001). With the maturation of the enterocyte, the activity of these cytosolic enzymes decreases concurrently with the development of several brush border enzymes (Cahu and Zambonino-Infante, 1995; Ma *et al.*, 2005). Brush border enzymes of fish like in other vertebrates are widely affected by age and the ingredients in the feed (Hakim *et al.*, 2006; Harpaz *et al.*, 2005a). Some herbivorous fish have been observed to exhibit a high activity of proteolytic enzymes than carnivorous fish (Fountoulaki *et al.*, 2005). This is a modification to maximize the efficiency of protein digestion when on diets that are very low in protein content

2.8.1 Leucine aminopeptidase (LAP)

Leucine aminopeptidase is an enzyme found anchored to the plasma membrane of a variety of cell types including the brush border of the intestine. In mammals it is one

of the major proteins of the intestinal microvillar membrane (Rawlings and Barratt, 1995). It is responsible for the surface digestion of the products of pancreatic proteases that are subsequently transported into the intestinal cells. LAP is a zinc dependent peptidase that catalyzes the removal of N terminal amino acids preferentially those that are neutral. Hakim *et al.* (2007) observed that, in carnivorous fish the activity of LAP is dependent on the intestinal section with a higher activity in the pyloric caeca, followed by the upper intestine and lowest in the lower intestine tract. However, in herbivorous fish the activity of LAP was not found to differ between the intestinal sections (Hakim *et al.*, 2006). This low activity of LAP in herbivorous fish suggests that LAP does not contribute to the digestive cascade in these species.

2.8.2 Gamma glutamyl transferase (γ -GT)

Gamma glutamyl transferase activity in the brush border membranes is an indicator of active amino acid transport across the intestinal membrane (Harpaz and Uni, 1999). It catalyzes the transfer of the γ -glutamyl group from γ -glutamyl compounds including glutamine to a wide variety of amino acid acceptors. In carnivorous fish a high activity of this enzyme has been reported across the different intestinal segments indicating that amino acid absorption occurs along the intestinal tract (Harpaz and Uni, 1999). The highest activity of γ -GT has been reported in the pyloric caeca (Hakim *et al.*, 2007). This high activity in the pyloric caeca has been found to have a positive influence on better food absorption in the Atlantic salmon. However Hakim *et al.* (2006) while working on the European sea bass reported a higher activity in the lower intestinal segment as compared to the pyloric caeca. Similar results were also

reported by Harpaz and Uni (1999) in the striped bass. Hakim et al. (2006) reported a high activity of γ -GT in fish that were fed diets low in protein as compared to fish fed on high protein diets.

2.8.3 Maltase

Maltase is a disaccharide degrading enzyme, it is required for the breakdown of maltose produced during the hydrolysis of starch. Maltase hydrolyses maltose into its component parts, i.e. two molecules of α -glucose. Maltase activity is produced by two enzymes maltase-glucoamylase (MGA; EC 3.2.1.20) contributing 20% and sucrase-isomaltase (SI; EC 3.2.1.48/10) contributing 80% (Nichols *et al.*, 1998). There is a proximal distal gradient in the activity of maltase, highest in the pyloric caeca, followed by the anterior intestine and lowest in the posterior intestine (Harpaz *et al.*, 2005; Hakin *et al.*, 2007). These authors proposed that this might be an effort to maximize carbohydrate utilization in diets deficient in the substrate. This would be due to the metabolic expense of producing large amounts of digestive enzymes by an animal ingesting low level of enzyme substrate (Caviedes-Vidal *et al.*, 2000). These authors also observed that carnivorous fish exhibit a lower activity of disaccharidase enzymes as compared to omnivorous and herbivorous fish. Hakim et al (2006) observed a significant high activity of maltase in fish that were fed on low carbohydrate diets compared to fish that fed a high carbohydrate diet. The author's argued that this might be an effort to increase the fish's ability to utilize carbohydrates on diets insufficient in carbohydrates.

CHAPTER THREE

3.0 Materials and methods

3.1 Study design

This was a cross-sectional study in which wild live juvenile Nile Perch at different stages of development were collected from Kigungu landing site on the shores of Lake Victoria (GPS: 36° 43'55"E, 0°03'36"N, and 1131m above sea level). Juvenile Nile perch were taken as fish with a total length ranging from 1-30cm (Katunzi *et al.*, 2006). The fish were captured by beach seining and immediately transferred to oxygenated tanks (2m×2m) containing lake water and taken to the laboratory where they were kept in the tank for 4-5 hours to attain a uniform physiological state. The fish were classified into six size groups of total length (1-5, 6-10, 11-15, 16-20, 21-25 and 26-30cm) based on the observation by Katunzi *et al.* (2006) that wild juvenile Nile perch undergo an ontogenetic dietary shift within the selected range of size groups

3.2 Sample size estimation

The sample size required for this study was calculated using GraphPad StatMate 2.00 as that with a significant level (α) of 0.05 and a 95% power of detecting a difference between means of 20.52, giving a required sample size of 10 in each size group

3.3 Sample handling and preparation

Ten (10) fish from each size group were randomly selected, sacrificed using a scalpel to sever the spine and their body weight measured. The abdomen was opened and the entire digestive tract removed, placed in falcon tubes and immediately stored in a -20°C freezer. The fish tissues were transported to Israel using thermal flasks

(Thermos EN12546) containing ice. Upon arrival the tissues were immediately transferred to a -20°C freezer until evaluation. All laboratory procedures were carried out in Professor Zehava's laboratory at the Department of Animal Science, Faculty of Agriculture, Food and Environmental Quality Sciences, Hebrew University Jerusalem.

The intestine was dissected, adipose tissue carefully cleaned off and the total intestinal weight measured using a Sartorius Bp1105 weighing balance. The intestinal tract was then divided into: the pyloric caeca, upper and lower intestine. In fish < 10cm the whole gut was taken since the intestine could not be easily dissected. The dissected sections were then weighed, placed in individually marked plastic tubes and immediately stored in a -20°C freezer and evaluated the following day.

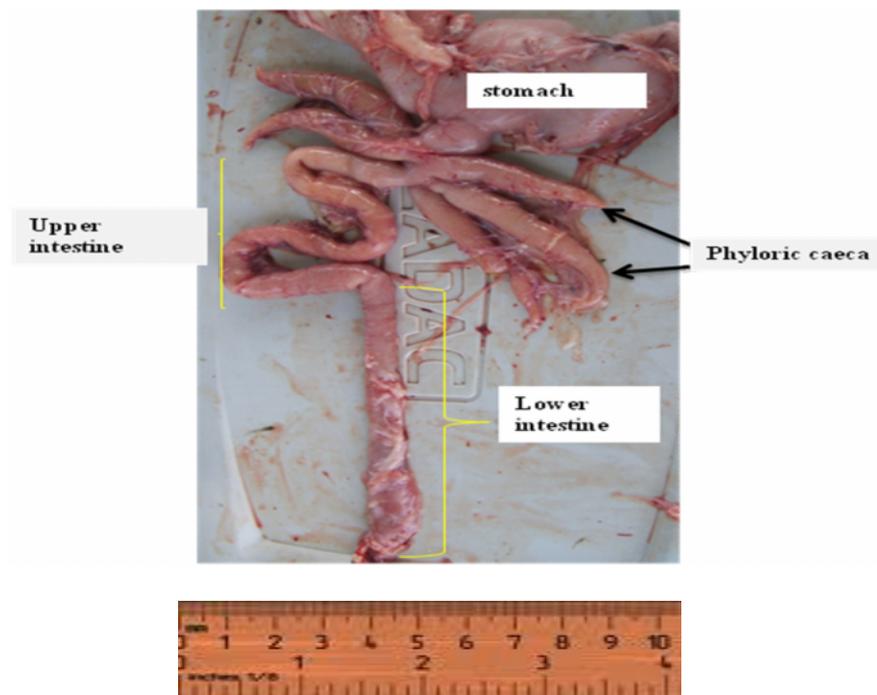


Fig.1. Intestinal tract of the Nile perch. Enzyme assays were done on the three intestinal segments: the pyloric caeca, the upper and lower intestine

3.4 Evaluation of brush border enzymes

Prior to evaluation, stored sections were briefly thawed and double distilled water added to make a dilution of 1:8. Zirkonium glass beads (Muhlmeier) were added to each tube and the mixture homogenized using a mini-Bead Beater (Bio-Spec) for 5 min. The homogenate was then centrifuged for 5 min at a speed of 4,000 rpm using an Eppendorf Centrifuge (5415R) placed in a cold room at 4°C. The extract was then used to assay the specific activity of LAP, γ -GT and maltase. Specific activity was calculated as the amount of enzyme required to release 1 μ mol of product per minute per milligram protein. Total enzyme activity was calculated as the product of the specific enzyme activity and total intestinal weight (IW). Relative total enzyme activity was calculated as the ratio between the total enzyme activity and fish body weight (BW).

3.4.1 Leucine aminopeptidase (EC 3.4.11.1) activity

The activity of leucine aminopeptidase (LAP) was measured using L-leucine-p-nitroanilide reagent (Randox Catalog.no LA 561) according to Harpaz and Uni (1999). The reagent (250 μ l) was added to 15 μ l of the homogenate on an ELISA plate and read at 1 min intervals for 15 min at 405 nm using a Tecan Sunrise micro-plate reader. One unit of activity was determined as the quantity required to release 1 μ mol L-leucine and p-nitroaniline per minute per milligram protein

3.4.2 Gamma glutamyl transferase (EC 2.3.2.2) activity

The activity of γ -GT was measured using a kit manufactured by Randox (Catalog.no GT 523) according to Harpaz and Uni (1999). Twenty microlitres of homogenate

sample was placed on an ELISA plate followed by 200 μ l of reagent. The plate was immediately read at 405nm at 1 min intervals for 15 min using a microplate reader (Tecan Sunrise). One unit of activity was determined as the quantity required to release 1 μ mol of 5-amino-2-nitrobenzoate per minute per milligram protein

3.4.3 Maltase (EC 3.2.1.20) activity

The hydrolysis of maltose by maltase was assayed according to the method of according to Harpaz and Uni (1999). Twenty (20 μ l) of sample extract were mixed with 20 μ l of substrate (0.056M maltose) on an ELISA plate. The plate was then incubated at 37°C for 45 min for glucose to form. The reaction was stopped by putting the plate on ice for 4 min. The presence of glucose as an indicator of enzymatic activity was then measured using the glucose oxidase reagent manufactured by Thermo electron corporation (catalog no. TR15103). After incubation, 2 μ l were taken from the reaction mixture and placed on an Elisa plate. Glucose oxidase reagent (300 μ l) was then added to all the wells and the plate incubated at 37°C for 10 min. After incubation, the plate was read using a Tecan Sunrise microplate reader at 500nm and compared to a glucose standard manufactured by Thermo Scientific (Lot V35674).

3.5 Total Protein determination

Protein concentration in the sample extracts was determined using a BIO-RAD kit (catalog no. 500-0113 and 500-0114). The protein concentration was measured by putting 5 μ l of sample extract on a microplate and adding 5 μ l of reagent A to all wells followed by 200 μ l of reagent B and incubated at room temperature for 10 minutes.

The OD was read at 750nm using a Tecan Sunrise microplate reader and compared with a standard (2mg/ml BSA standard catalog no. 500-0206).

3.6 Ethical issues

Since this study involved capture of premature fish and use of illegal nets, the necessary permission to capture the required samples and transport of fish tissues to Israel was sought from the Commissioner for Fisheries and National Council for Science and Technology through the MSI-Nile perch project. Captured fish that was not used in this study were immediately returned to the lake.

3.7 Data management and statistical analysis

Enzyme activity data was entered in Microsoft Office Excel and the specific enzyme activity, total enzyme activity and the relative total enzyme activity for the individual samples in the different size classes computed. Statistical analysis of enzyme activity was done using GraphPad 5.0 statistical package. The influence of the intestinal section, size group and their interaction was analyzed using a two-way ANOVA set at $P < 0.05$. Comparison between the enzymatic activity of the different intestinal segments in the same size group and that between the same segment among the different size groups was done using Tukey's Multiple Comparison Test set at $P < 0.05$. The correlation tests between the enzyme activities were done using Pearson correlation matrix set at a significant level of $P < 0.05$

CHAPTER FOUR

Results

4.1 Proteolytic enzymes

4.1.1 Leucine amino peptidase (LAP)

4.1.1.1 Specific enzyme activity

The results show that the two main factors, the fish size and intestinal section had a significant effect on the enzyme activity. The activity of LAP was influenced by the intestinal section ($p < 0.05$), the fish size ($p < 0.05$) and the interaction between the two factors ($F_{6, 92} = 3.37, p < 0.0048$). Comparison of the LAP activity in the same fish size group among the different intestinal sections using Tukey's Multiple Comparison Test revealed a significantly higher activity ($p < 0.05$) in the upper intestine of the 11-15 and 26-30 size groups as compared to the pyloric caeca and lower intestine. Comparison of the LAP activity in the same intestinal segment among the different size groups revealed a higher activity ($p < 0.05$) in the pyloric caeca of the 16-20 size group as compared to the 21-25 and 26-30 size groups. In the upper intestine, a higher activity ($p < 0.05$) was found in size group 11-15 and 26-30 as compared to size group 21-25. In the lower intestine, a higher activity ($p < 0.05$) was only found in the 16-20 size group as compared to the 26-30 size group (Fig. 2).

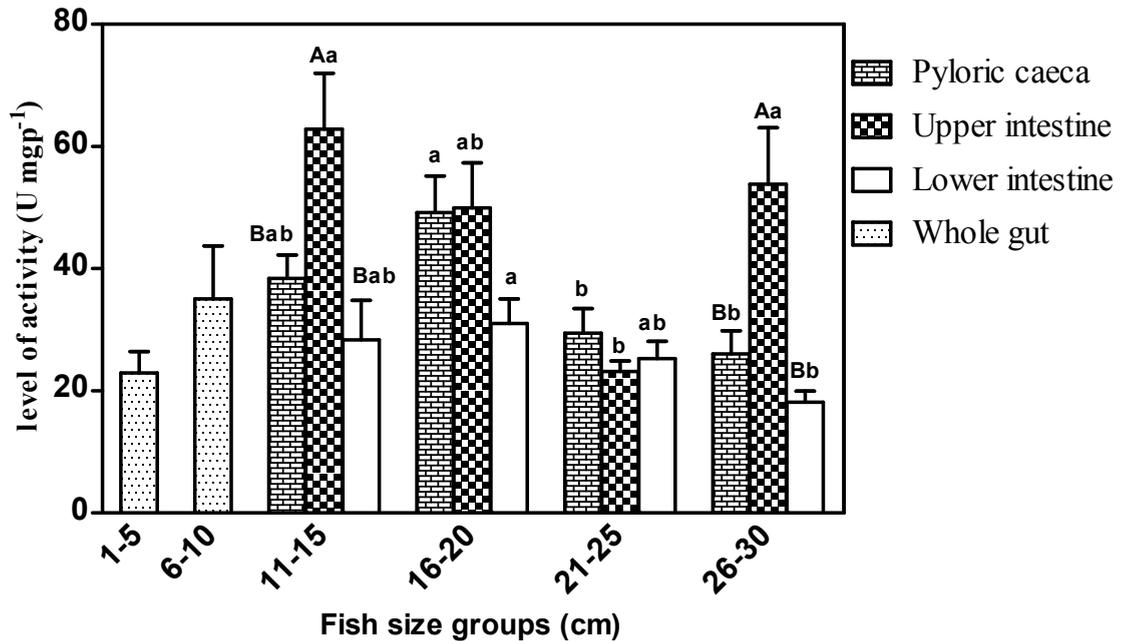


Fig.2. Specific activity of leucine aminopeptidase measured in the different intestinal sections of six different fish size groups. Capital letters above the columns represent the significant differences ($p < 0.05$) among intestinal sections in each size group. Small letters represent significant difference among the fish size groups within each segment.

4.1.1.2 Total enzyme activity of leucine aminopeptidase

The total enzyme activity of LAP was significantly influenced by the fish size ($p < 0.05$). Comparison of the LAP activity across the different size groups using Tukey's Multiple Comparison test revealed a significantly higher activity ($p < 0.05$) in size group 26-30 and a lower activity in size groups 1-5, 6-10 and 11-15 as compared to the other size groups (Fig. 3)

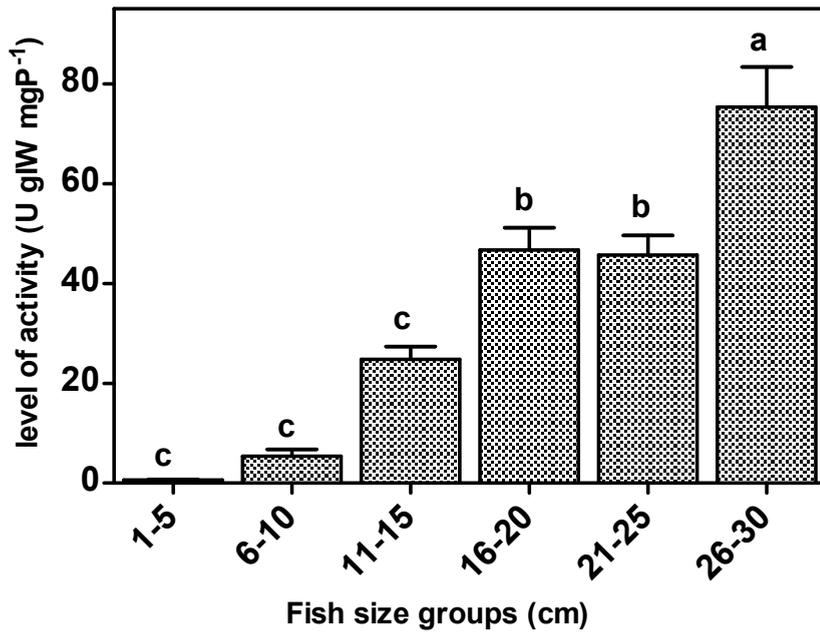


Fig.3. Total enzyme activity of leucine aminopeptidase among the different fish size groups. Letters above the columns represent significant differences ($p < 0.05$) among groups.

4.1.1.3 Relative total enzyme activity of LAP

The relative total enzyme activity of LAP was significantly influenced by the fish size ($p < 0.05$). Comparison of the LAP activity across the different size groups using Tukey's Multiple Comparison Test showed a significantly higher activity ($p < 0.05$) in the 16-20 size group as compared to the other groups and the lowest activity was revealed in size group 1-5 (Fig. 4).

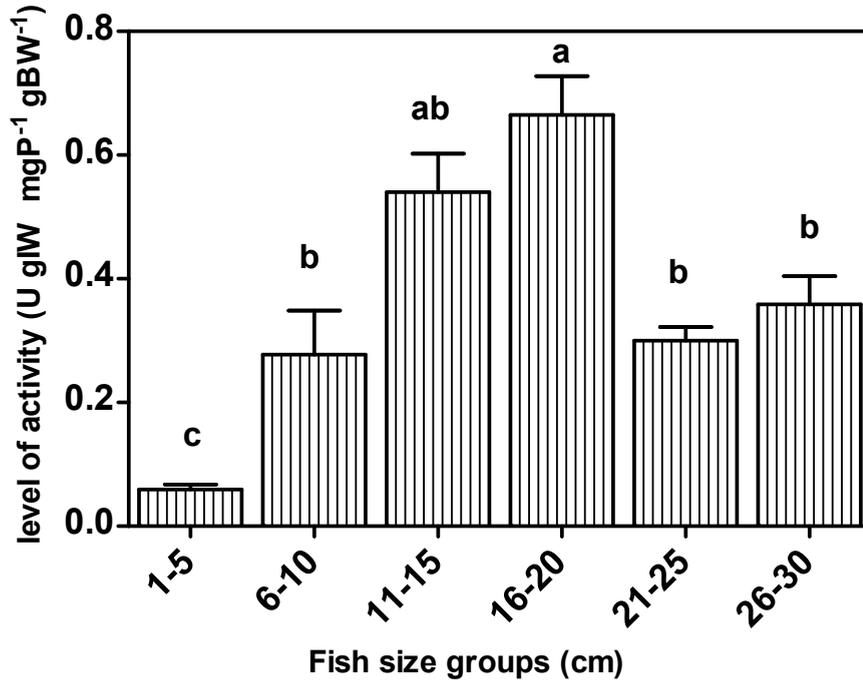


Fig.4. Relative total enzyme activity of leucine aminopeptidase among the different fish size groups. Letters above the columns represent significant differences ($p < 0.05$) among the size groups

4.1.2 Gamma glutamyl transferase (γ -GT)

4.1.2.1 Specific enzyme activity

The results show that the activity of γ -GT was influenced by the intestinal section ($p < 0.05$), the size of the fish ($p < 0.05$) and the interaction between the two factors ($p < 0.05$). Comparing the activity of γ -GT in the same fish size group among the different intestinal sections using Tukey's Multiple Comparison test revealed a significantly higher activity ($p < 0.05$) in the lower intestine of fish in the 21-25 and 26-30 size groups as compared to the upper intestine. Comparison of the activity of γ -GT in the same intestinal tract among the different size groups revealed a higher activity ($p < 0.05$) in the pyloric caeca of the 16-20 size group as compared to the 21-

25 and 26-30 size groups. In the upper section, a higher γ -GT activity ($p < 0.05$) was found in the 11-15 size group as compared to the 21-25 and 26-30 size groups. No significant difference was found in the activity of the enzymes in the lower intestine across the different size groups (Fig. 5).

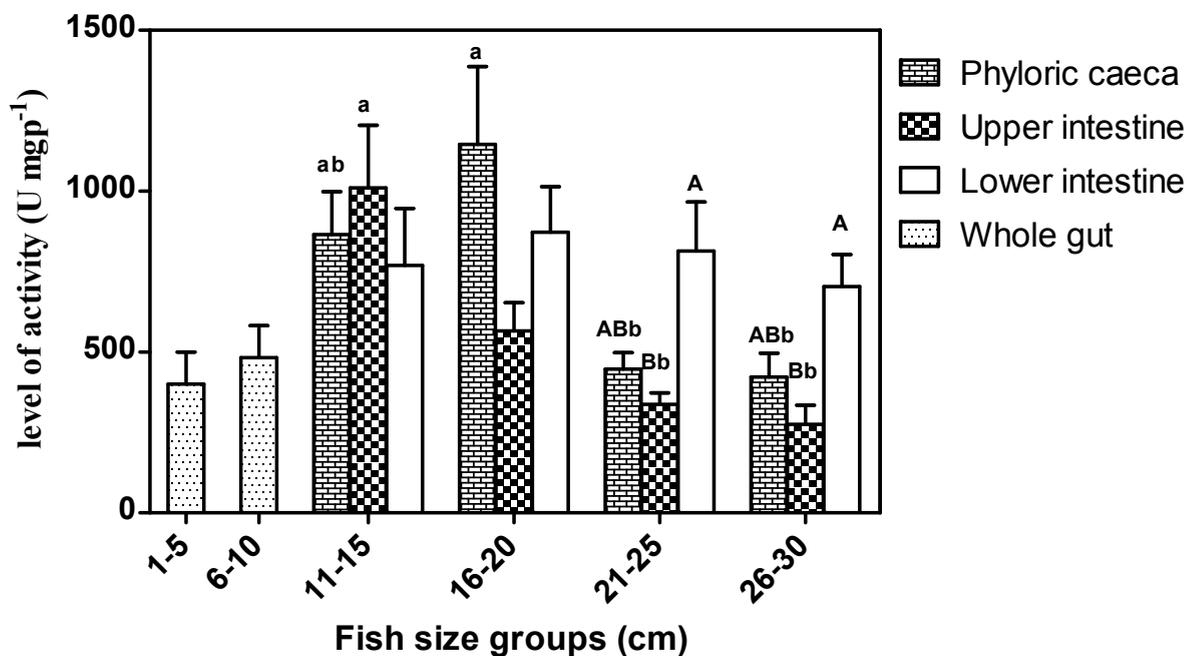


Fig.5. Specific activity of γ -glutamyl transferase measured in the different intestinal sections of six different fish size groups. Capital letters above the columns represent significant differences ($p < 0.05$) among intestinal sections in each size group. Small letters represent significant difference among the fish size groups within each segment.

4.1.2.2 Total enzyme activity of γ -GT

Total enzyme activity of γ -GT was influenced by the fish size ($p < 0.05$). Comparison of the γ -GT activity across the different size groups using Tukey's Multiple

Comparison test revealed a significantly lower activity ($p < 0.05$) in size groups 1-5, 6-10 and 11-15 as compared to the other size groups (Fig 6).

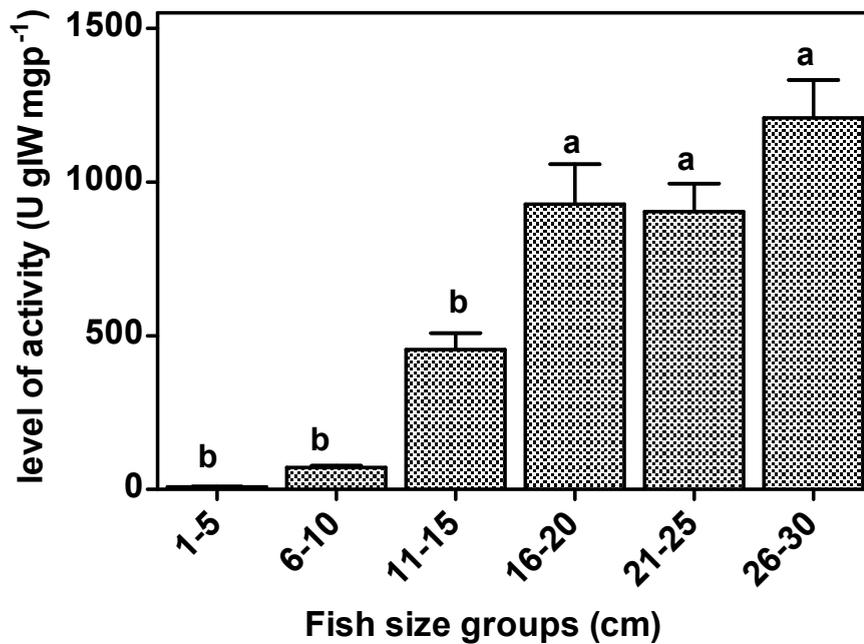


Fig.6. Total enzyme activity of γ -glutamyl transferase among the different fish size groups. Letters above the columns represent significant differences ($p < 0.05$) among groups

3.1.2.3 Relative total enzyme activity of γ -GT

The size of the fish revealed a significant influence ($p < 0.05$) on the relative total enzyme activity of γ -GT. Comparison of the γ -GT activity across the different size groups using Tukey's Multiple Comparison test revealed a higher activity ($p < 0.05$) in both the 11-15 and 16-20 size groups as compared to other size groups (Fig 7).

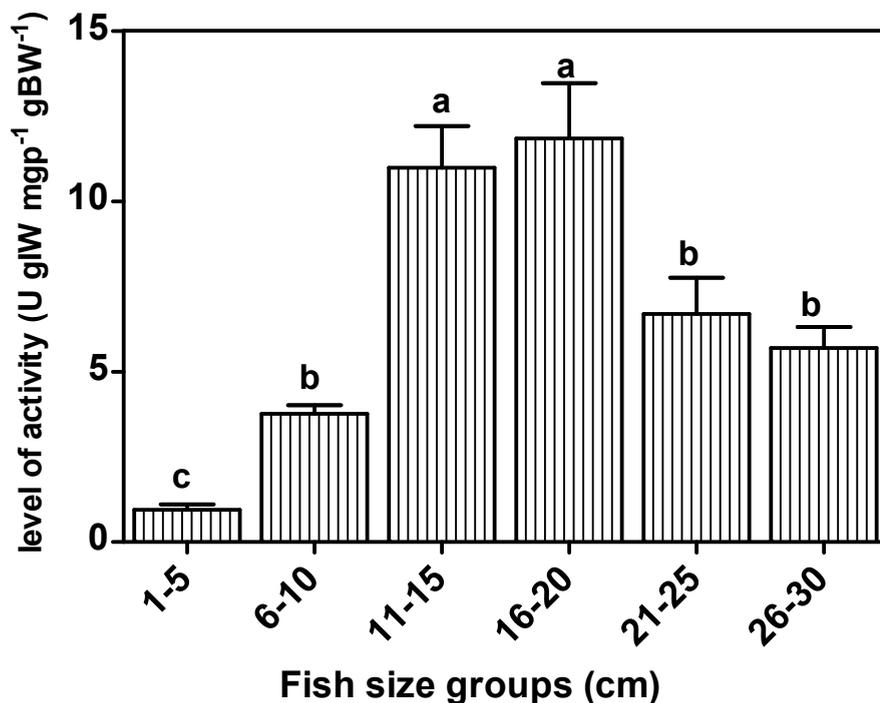


Fig.7. Relative total enzyme activity of gamma glutamyl transferase among the different fish size groups. Letters above the columns represent significant differences ($p < 0.05$) among the size groups.

4.2 Carbohydrases (Maltase)

4.2.1 Specific enzyme activity

The activity of maltase was influenced by the intestinal section ($p < 0.05$), the fish size ($p < 0.05$) and the interaction between the two factors ($p < 0.05$). Comparing the activity of maltase in the same fish size group among the different intestinal sections using Tukey's Multiple Comparison test revealed a significantly higher activity ($p < 0.05$) in the upper intestine of the 11-15 and 26-30 size groups. Comparison of the maltase activity in the same intestinal tract among the different size groups revealed a higher activity ($p < 0.05$) in the pyloric caeca of the 11-15 and 16-20 size groups. In the upper intestine, a higher activity ($p < 0.05$) was found in the 11-15 and 26-30 size

groups. In the lower intestine, a higher activity ($p < 0.05$) was revealed in the 11-15 size group (Fig 8)

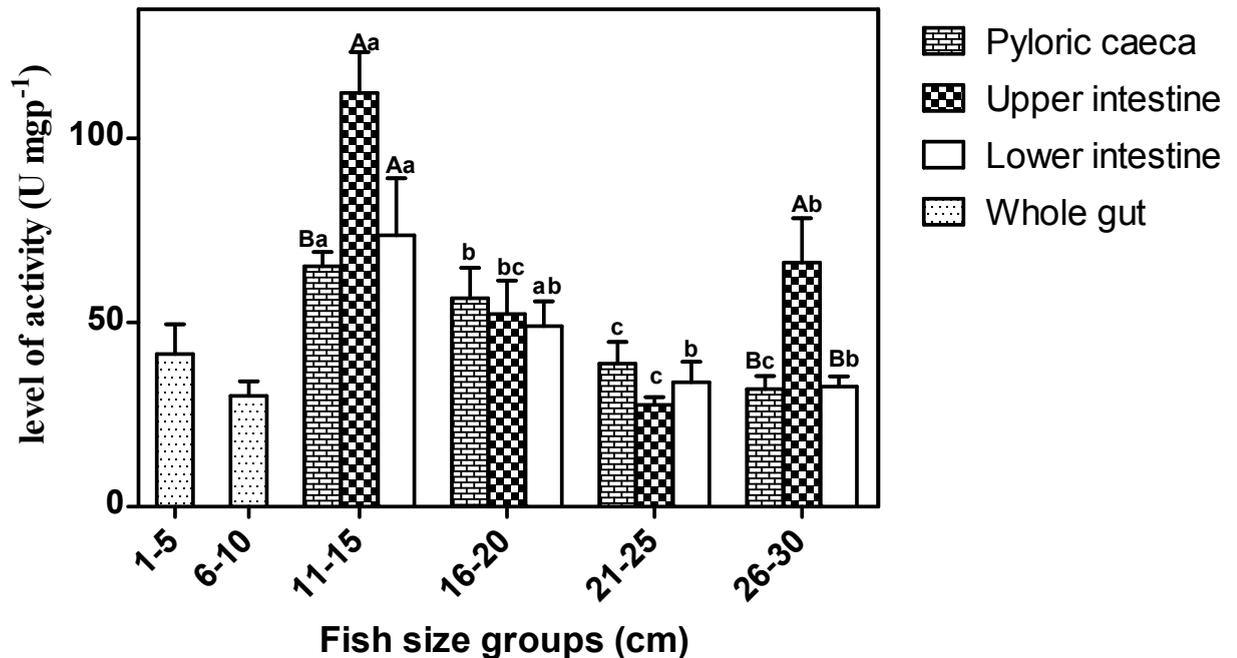


Fig.8. Specific activity of maltase measured in the different intestinal sections of six different fish size groups. Capital letters above the columns represent significant differences ($p < 0.05$) among intestinal sections in each size group. Small letters represent significant difference among the fish size groups within each segment.

4.2.1.2 Total enzyme activity of maltase

The total enzyme activity of maltase was influenced by the size of the fish ($p < 0.05$). Comparison of the activity of maltase across the different size groups using Tukey's Multiple Comparison Test revealed a significantly higher activity in the 26-30 size group ($p < 0.05$) as compared to the other size groups. The lowest activity was observed in size groups 1-5 and 6-10 (Fig. 9).

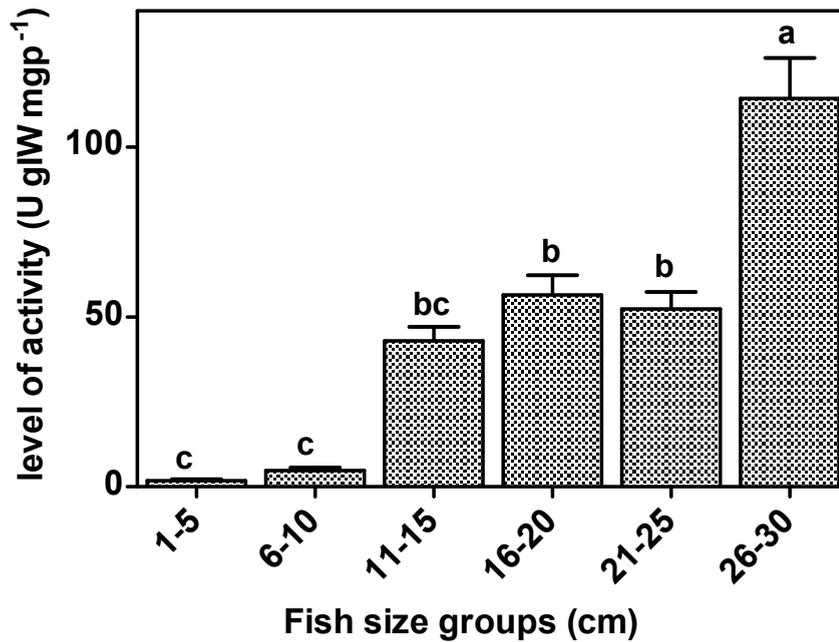


Fig.9. Total enzyme activity of maltase among the different fish size groups. Letters above the columns represent significant differences ($p < 0.05$) among groups

3.2.1.3 Relative total enzyme activity of maltase

Relative total enzyme activity of maltase was influenced by the fish size ($p < 0.0001$).

Comparison of the activity of maltase across the different size groups using Tukey's

Multiple Comparison test revealed a significantly high activity ($p < 0.05$) in the 11-15

and 16-20 size groups as compared to the other groups (Fig. 10).

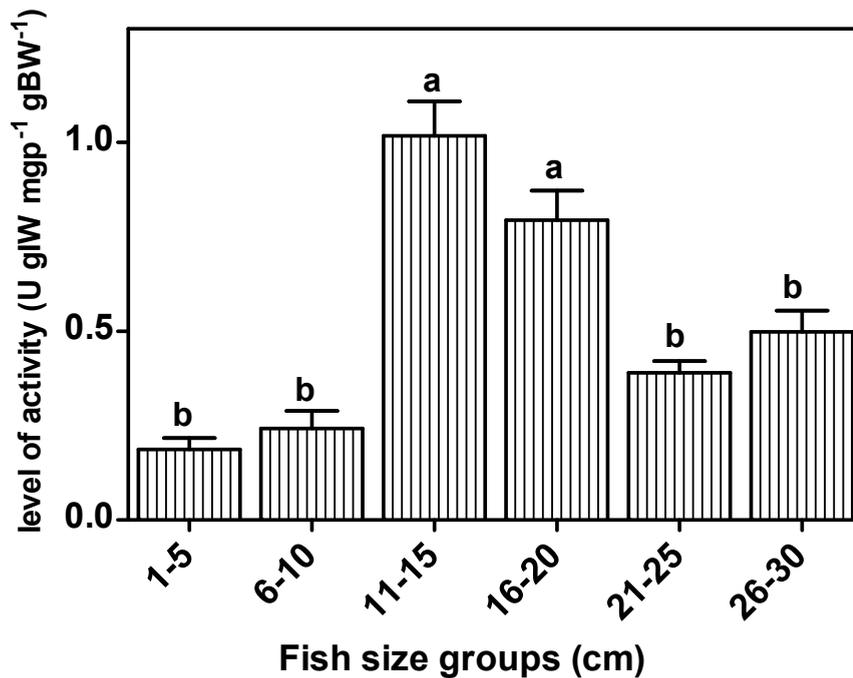


Fig.10. Relative total enzyme activity of maltase among the different fish size groups.

Letters above the columns represent significant differences ($p < 0.05$) among groups

4.3 Correlations between enzyme activities

Generally, the highest correlation was observed between LAP and maltase in all size groups, followed by that between γ -GT and maltase and lowest between LAP and γ -GT. The highest correlation between the tested enzymes was revealed in the pyloric caeca of all size groups as compared to the other intestinal segments (Table.1).

Table.1. Correlation between the Specific enzyme activities of brush border enzymes among the different fish size groups and intestinal segments

Size group (cm)	intestinal segment	LAP/ γ -GT	LAP/maltase	γ -GT /maltase
1-5	whole gut	0.820*	0.920*	0.910*
6-10	whole gut	0.930*	0.927*	0.930*
11-15	Pc	0.362	0.978*	0.323
	Upper	0.298	0.676*	0.610
	Lower	0.512	0.688*	0.862*
16-20	Pc	0.831*	0.914*	0.634
	Upper	0.632	0.824*	0.654
	Lower	0.369	0.845*	0.778*
21-25	Pc	0.790*	0.920*	0.273
	Upper	0.660	0.850*	0.570
	Lower	0.680	0.579	0.920*
26-30	Pc	0.954*	0.935*	0.900*
	Upper	0.234	0.342	0.965*
	Lower	0.321	0.314	0.600

Figures with asterisk indicate significant values ($p < 0.05$)

CHAPTER FIVE

DISCUSSION

The ability of fish to utilize a given diet depends on the activity of its enzymes along the digestive tract (Tengjaroenkul *et al.*, 2005). Digestive capacity of fish relates to the age/size and dietary composition (Lovett and Felder, 1989; Peres *et al.*, 1998). However, Chakrabarti *et al.* (1995), Harpaz & Uni (1999) demonstrated that at any time fish almost possess all the enzymes irrespective of the specific diet consumed. Similar results were observed in this study in that all tested enzymes were present in the different size groups of the wild juvenile Nile perch.

In this study it was revealed that the intestinal segment had a significant effect on all the tested enzymes. Harpaz *et al.* (2005a), Tibaldi *et al.* (2006) and Hakim *et al.* (2007) all reported the highest activity of LAP in the pyloric caeca. In this study the highest activity of LAP was revealed in the upper intestine. LAP is responsible for the surface digestion of the products of pancreatic proteases. The high activity of this enzyme in the upper intestine indicates the role of this intestinal section in protein digestion in the wild juvenile Nile perch. The highest activity of γ -glutamyl transferase revealed in the lower intestine is in agreement with earlier studies in the hybrid striped bass (Harpaz and Uni, 1999), the Asian sea bass (Harpaz *et al.*, 2005a) and the European sea bass (Tibaldi *et al.*, 2006) that found a high activity of γ -GT in the lower intestine. A higher activity of this enzyme in the lower intestine indicates that absorption of amino acids is elevated with movement along the gut and might be

an adaptation to efficiently absorb nutrients in order to compensate for the short intestinal tract in this fish.

The highest activity of maltase was observed in the upper intestine. A high activity of maltase has also been reported in other carnivorous fish like the Asian sea bass (Sabapathy and Teo, 1993; Harpaz *et al.*, 2005a) and in birds fed on a high protein, carbohydrate free diet (Sabat *et al.*, 1998). Maltase is a disaccharide degrading enzyme required for the breakdown of maltose produced during the hydrolysis of starch. Presence of this enzyme in a carnivorous fish on a natural diet probably suggests that the activity of these enzymes is genetically programmed and under the same trigger mechanism. Moreover there was a high correlation in the activity of these enzymes especially in the pyloric caeca confirming that this region is an extension of the intestine with an additive role in nutrient digestion and absorption (Horn, 1998).

The results revealed a significant influence of the fish size on the activity of all the tested brush border enzymes. There was a significantly higher specific enzyme activity in the 11-15 and 16-20 size groups. This age dependant change in specific enzyme activity, in which the young ones have a higher activity than the older ones has been observed in the European sea bass and Senegal sole (Zambonino Infante and Cahu, 1994; Martinez *et al.*, 1999; Ribeiro *et al.*, 1999 and Wang *et al.*, 2006). Kuz'mina (1996) also observed an abrupt increase in the activity of proteolytic enzymes in the carnivorous pike at an early age. Diet has been stated as one of the factors that influence the activity of digestive enzymes (Fernández *et al.*, 2001) especially in species that undergo an ontogenetic shift in diet (German *et al.*, 2004).

Different authors (Mkumbo & Ligtoet, 1992; Schofield, 1999; Ogutu-Ohwayo 2004 and Katunzi *et al.*, 2006) have reported the Nile perch to shifts from one diet to another. Katunzi *et al.* (2006) observed that in most habitats, juvenile Nile perch of < 5cm exclusively fed on zooplankton before shifting to midge larvae and shrimp. Above 10cm the main prey for Nile perch in most habitats was reported to be juvenile Nile perch (< 5cm) and *R. argenta*. In this study it was observed that the specific activity of both carbohydrase and proteolytic enzymes was significantly higher in the 11-15 and 16-20 size groups, this coincides with the change to a higher protein diet.

For all the tested enzymes, the total enzymatic activity increased with fish size. Studies have reported that, maturation of the intestinal tract and the relative increase in intestinal length increase the total capacity of the intestine to digest and assimilate nutrients (Ferraris *et al.*, 1987). The lower total enzyme activity in size groups 1-5 and 6-10 relates to immaturity of the digestive tract as compared to the larger size groups. This increase in enzyme activity with stage of development has been reported in other species (Kuz'mina, 1980; Kuz'mina, 1996; Kvale *et al.*, 2008 and Zouitena *et al.*, 2008)

The relative total enzyme activity followed the same trend as the specific enzyme activity in all the enzymes tested. It was highest in size groups 11-15 and 16-20 indicating that the best digestive capacity of the fish occurs around these size groups. The lower relative activity in the 1-5 and 6-10 size groups relates to the poorly

developed gut. A similar trend in relative total activity has been observed in other species (Kuz'mina, 1980 &1996)

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In this study the highest specific enzyme activity of LAP and maltase was observed in the upper intestine while that of γ -GT was observed in the lower intestine indicating the roles played by these sections in feed degradation and assimilation. This study further confirmed that the pyloric caeca is an extension of the intestinal tract involved in the digestion and absorption of nutrients. Total enzyme activity, an indicator of the intestinal capacity to digest increased with fish size, indicating that maturation of the intestinal tract and the relative increase in intestinal length increase the total capacity of the intestine to digest and assimilate nutrients

The highest specific enzyme activity and the relative total enzyme activity were observed in the 11-15cm and 16-20 cm size groups indicating that this stage has the highest capacity to digest and assimilate nutrients among the juveniles. This stage has also been shown to coincide with an ontogenetic dietary shift to a high protein diet in the wild. There was a correlation between all the tested enzymes indicating that production of these brush border enzymes is initiated under the same genetical trigger mechanism

6.2 Recommendations

The highest relative total enzyme activity, an indicator of the fish's capacity to digest and utilize nutrients was observed in fish between 11-20cm total lengths. This size group forms a critical stage in the formulation of artificial diets for this species. Hence necessitates intensification in the feeding regime that incorporates high protein

and carbohydrate rations that would match the enzyme activities. Presence of maltase in this species suggests that the juvenile Nile perch can utilize diets incorporating lower levels of carbohydrates as a cost effective venture to the aqua-culturist.

Since this study only provides insights in the dietary requirements of the juvenile Nile perch, studies are required for both the larval and adult stages to get a more comprehensive view of the nutrient requirements of the Nile perch at the different developmental stages. This is especially so for the larval stage that forms the most critical stage in aquaculture. This will identify the most suitable time to start feeding the fish and also indentify the stage to wean the fingerlings to artificial diets

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